QUALITATIVE EVALUATION OF *NAVA* AND *PURANA PIPPALI* AND ASSESSMENT OF ANTIASTHMATIC ACTIVITY INVIVO

A thesis submitted to

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Subject: Dravyaguna Vigyan

Under the Faculty of Ayurveda

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Under the Guidance of

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Month and Year: August 2015

CERTIFICATE

This is to certify that the thesis entitled "Qualitative evaluation of *nava* and *purana pippali* and assessment of antiasthmatic activity invivo" which is being submitted herewith for the award of the Degree of Vidyavachaspati (Ph.D.) in Dravyaguna Vigyan of Tilak Maharashtra Vidyapeeth, Pune is the result of original research work completed by Vd. Rajendra H.M. under my supervision and guidance. To the best of my knowledge and belief the work incorporated in this thesis has not formed the basis for the award of any Degree or similar title of this or any other University or examining body upon him.

Research Guide: Dr.Meenal D.Lad

Place: Pune
Date: 28/08/2015

DECLARATION

I hereby declare that the thesis entitled "Qualitative evaluation of *nava* and *purana Pippali* and assessment of antiasthmatic activity invivo" completed and written by me has not previously formed the basis for the award of any Degree or other similar title upon me of this or any other Vidyapeeth or examining body.

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Place: Pune
Date: 28/08/2015

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CONTENTS

ABBREVIATIONS

C. S.	Charaka Samhita
S. S.	Sushruta Samhita
A. H.	Asthanga Hridaya
CS. Su.	Charaka Samhita Sutrasthana
CS. Vi.	Charaka Samhita Vimanasthana
CS. Ci.	Charaka Samhita Chikitsasthana
CS. Ka.	Charaka Samhita Kalpasthana
CS. Si.	Charaka Samhita Siddhisthana
SS. Su.	Sushruta Smhita Sutrasthana
SS. Ci.	Sushruta Smhita Chikitsasthana
SS.U.	Sushruta Smhita Uttaratantra
AH. Su.	Ashtanga Hridaya Sutrasthana
AH. Ci.	Ashtanga Hridaya Chikitsasthana
AS. Ci.	Ashtanga Sangraha Chikitsasthana
HS	Harita Samhita
KS	Kashyapa Samhita
SG	Sharngadhara Samhita
CD	Chakradatta
BP. Ci.	Bhavaprakasha Chikitsasthana
GN	Gadanigraha
RM	Rajamarttanda

KK	Kalyanakaraka
VM	Vrindamadhava
VD	Vaidyamanorama
VS	Vangasena
ध.नि	Dhanvantari Nighantuh
सो.नि	Sodhala Nighantu
रा.नि	Raja Nighantu
मा.द्र	Madhava Dravyaguna
म.नि	Madanapala Nighantu
कै.नि	Kaiyadeva Nighantu,
भा.नि	Bhavaprakasha Nighantu
नि.आ	Nighantu Adarsha
प्रि.नि	Priya Nighantuh
हृ.नि	Hrdayadipika Nighantu
भा.दी	Bhanoji Deekshit Vyakhya
श.क.द्रु	Shabdakalpadruma
HPTLC	High Performance Thin Layer Chromatography
UV	Ultra Violet
R_f	Retention factor

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Chapter 1

Introduction

Ayurveda, the ancient traditional system of medicine mentions various concepts, which is needed to explore and revalidate them through scientific parameters for better understanding and thereby extending its scope of utility. Among these, one of the concepts is mentioned in *Sharngadhara Samhita* as:

नवान्येव हि योज्यानि द्रव्याण्यखिलकर्मसु।

विना विडङ्गकृष्णाभ्यां गुडधान्याज्यमाक्षिकै:॥ (शा.पूर्वखण्ड-१/४४)

All the plant drugs are to be used in *Nava*/fresh form only except few drugs like *Vidanga, Krishna, Guda, Dhanya, Ajya, Makshika,* should be used as *Purana*/old form. *Pippali* which is the synonym of *krishna* is one among these drugs which should be used as *Purana*/ old form only.

विडङ्गादि द्रव्यं विना तेन विडङ्ग प्रभृतिकं पुरातनं गुणकरमिति तात्पर्यार्थ:॥ (आढमल्ल टीका)

Adhamalla's Dipika, commentary on *Sharngadhara Samhita* mentions that *Vidanga, Pippali* etc. drugs if used in old form will be of good Quality/Potent.

पुरातनत्वं संवत्सरादुपरि भवति। (आढमल्ल टीका)

The time of these drugs which are to be used in old form is mentioned by *Adhamalla* as: *Pippali* and other drugs must be used one year old.

Hence quality of a drug is given much importance in order to achieve its therapeutic efficacy.

So *Dravya*/drug is considered as second important factor next to *Vaidya* in *Chikitsa Chatushpada*/four aspects of treatment, which are responsible for the cure of diseases, provided they have requisite qualities like its abundance, suitability, available in various forms and should possess all the properties[1].

Acharya Charaka mentions the Qualities of Ideal Drug is that which cures a disease and provides health is called as *Bhesaja*. Basing upon its importance, *Bhesaja* and *Chikitsa* are denoted with same nomenclature and synonyms[2]. In another context, *Acharya Charaka* mentions that the drug should be examined with respect to its nature, qualities, specific action etc[3]. Hence by these references it emphasizes that drug should be selected of good quality in order to achieve maximum therapeutic efficiency.

Hence there is necessary to revalidate the concept through scientific parameters by evaluating the Qualities of *Nava Pippali* (Fresh form) and *Purana Pippali* (Old form). Evaluation of a drug ensures the identity of a drug and determines the quality and purity of drug.

Though in practice the drug *Pippali* is used, the period of collection and time of usage means to determine between *Nava* and *Purana*. The absence of any particular component may interfere with the therapeutic efficacy of drug. Quantifying of the phytoconstituents may indicate the necessity of the concentration of particular phytoconstituents in producing said therapeutic effect.

Hence by this study it helps in identifying the presence or absence of phytoconstituents and its concentration in *Nava* and *Purana Pippali*. Study can also help in determining whether the supplied raw material is *Nava* or *Purana* and assures safety, quality and efficacy.

पिप्पली रेचनी हन्ति कासश्वासोदर ज्वरान्।

कुष्ठ प्रमेह गुल्मार्श: प्लीहशूलाममारूतान्॥ (भा.प्र)

पिप्पली ज्वरहा वृष्या स्निग्धोष्णा कटूतिक्तका।

दीपनी मारूत्श्वासकास श्लेष्मक्षयापहा॥ (रा.नि)

As per the classical literatures, *Pippali* is widely used in *Shwasa* and maximum formulations used in this disease contain *Pippali* as one of the ingredient, which indicates its importance in alleviating the disease.

Shwasa can be considered as Bronchial Asthma as one of the entity under *Shwasa***.**

Among several respiratory diseases affecting human, bronchial asthma is the most common disabling syndrome. The morbidity and mortality of the disease is increasing and making it a global concern. The increasing global prevalence of asthma, the larger burden it now imposes on patients, and the high health care costs have led to extensive research into its mechanisms and treatments. Asthma is one of the most common chronic diseases globally and currently affects approximately 300 million people worldwide. In developing countries where the prevalence of asthma had been much lower, there is a rising prevalence, which is associated with increased urbanization[4].

Hence Antiasthmatic activity is chosen on Animal models i.e., In vivo study, in order to evaluate the effect of *Nava* and *Purana Pippali* on Bronchial Asthma.

As Biological evaluation of plant drugs is useful to determine pharmacological activity, potency and toxicity and moreover this is an important evaluation for drugs because by their biological effects, this evaluation will conclude the effect[5].

Chapter 2

Aims and Objectives

1. Comparative evaluation of *Nava* and *Purana Pippali* by Physico-chemical and Phyto-chemical analysis.

2. To evaluate the Antiasthmatic activity of *Nava* and *Purana Pippali* by Invivo study.

Chapter 3

Review of Literature

Drug Review/*Pippali* – Ayurvedic literature

***Pippali* in Vedic Period:**

Pippali is frequently mentioned in Vedic literature. In Atharavaveda *Pippali* is mentioned as Rasayana, Ksipta Bhesaji, Atividdha Bhesaji and Vatikrta Bhesaji. Sayana quotes that it is useful in the treatment of Dhanurvata, Aksepaka etc., Vatavyadhies. Hindu mythology reveals that *Pippali* has its origin during Samudramathana along with Amrta. One context from Jaiminiya Bramana delineate that the son of saint Vasistha consumed *Pippali* to attain health and wealth (Jai.Br.3/149). In Koushika dharmasutra (10/16 & 16/38) *Pippali* and Sarsapakhanda are advocated for administration to neonates along with other herbs. This process is claimed to be Medhya. It is enumerated among the Bhesaja Gana of Atharva Parisista (32/1/4). According to Kesava Paddhati (26/33-40) it is indicated for Vata vikaras. All these references indicate that *Pippali* is a very old drug known to Indians for a long time and its antiquity goes beyond 2000-3000 yrs.

Synonyms:

Pippali: It gives protection from diseases.

Nagavalleedala: Leaves resembles to Piper betel leaves.

Magadhee: More grows in Magadha-South Bihar.

Vaidehee: Grows more in Northern Bihar.

Capala: Pungent in nature.

Upakulya: Grows near damp region.

Shoundee: Fruit resembles to tiny elephant trunk.

Kana: Having granules or fruits having granules like structure.

Teekshna tandula: Seeds are pungent in taste.

Krishna: Fruit is black in colour.

Kola: Fruit appears as a solidified rod consisting of different small round particles.

Usana, Krikara, katubija, Korangi, Tikta tandula, Shyama, Dantaphala, Magadodbhava, Smrtyahva.

Table 1: Classification on *Pippali*

Sr.	Name of Samhita/Nighantuh	Gana/Varga
1	Charaka Samhita	Kasahara, Hikkanigrahana,Shirovirecana, Vamana, Truptighna, Dipaniya, Shulaprashamana.
2	Sushruta Samhita	Tryushana, Pippalyadi, Urdhvabhagahara, Shirovirecana.
3	Ashtanga Sangraha	Pippalyadi
4	Bhavaprakasha Nighantuh	Haritakyadi
5	Dhanvantari Nighantuh	Shatapushpadi
6	Raja Nighantuh	Pippalyadi
7	Kaiyadeva Nighantuh	Oshadi
8	Madhava Dravyaguna	Vividhousadhi
9	Hrdayadipaka Nighantuh	Catushpada
10	Priya Nighantuh	Pippalyadi

Rasapanchaka:

Rasa: Katu

Guna: Laghu, Snigdha, Teekshna.

Vipaka: Madhura.

Virya: Anushnasheeta.

Doshakarma: Vatakapha shamaka.

Green or fresh fruit is Guru Guna, Madhura Rasa, Sheeta Veerya and Vatakaphakara-Pittahara.

Karma:

Kasahara, Shwasahara, Hikka nigrahana, Kshayahara, Rasayana, Deepana, Medhya, Mutrala, Truptighna, Shula prashamana, Mridu virechana, Yakrituttejaka, Pleehavridhi hara, Krimighna, Vrishya, Shirovirecana, Kushtaghna, Jwaraghna, Balya, Vishamajwara pratibandhaka.

Rogaghnata:

Kasa, Shwasa, Hikka, Aruchi, Agnimandhya, Ajeerna, Vibandha, Gulma, Udara, Arshas, Yakrit vikara, Pleeha vriddhi, Krimi, Pandu, Amavata, Kshaya, Rajayakshma, Rajorodha, Kushta, Jwara.

Prayojya anga: Phala and Mula.

Matra: Churna 1-3g.

Charaka and Sushruta have extensively quoted *Pippali* among the Dasaimani group and Ganas respectively. It is quite interesting to note that Vagbhata did not mention it in any of his Vargas (AH.Su-15). However he used it in the therapeutics extensively. Moreover he also happened to quote *Pippali* dvaya three times (AH.Ci.3/133;Ci.8/45 & Ci.9/105) against Susruta who quoted only once about *Pippali* dvaya (SS.Ci.37/36). It appears Caraka did not describe *Pippali* dvaya. But he mentioned about *GajaPippali*. Caraka described Vardhamana *Pippali* Rasayana. Caraka mentioned that *Pippali* should not be used in excessive quantities. However it may be used as Rasayana. In this context Cakrapani clarifies that the restriction is limited in diet and not for medicinal usage (CS.Vi.1/12-13). Vagbhata indicate *Pippali* specifically for Pleeha rogas.

Pippali is used as Aaharayogi Varga (CS.Su.27).

The fruits of *Pippali* were used as condiment (S.Su.46/221) and leaves as potherb (S.Su.46/262).

Three substances not to be used in excess:

Of all the substances, one should not resort too much to the three drugs viz. *Pippali*, Kshara and Lavana.

Justification for not using *Pippali* in excess:

Pippali inspite of their pungent taste are sweet in vipaka, heavy, neither too unctuous nor too hot, deliquescent and useful as medicine when administered afresh. Depending upon the frequency of use, they are both useful and harmful. When properly used they produce good results instantaneously, otherwise, they are responsible for the accumulation of doshas. When continuously used in large dose, they aggravate kapha owing to their heaviness and deliquenscent property; they aggravate pitta owing to their hot property. They do not alleviate

vata because they are not adequately unctuous or hot. They intensify the action of drugs to which they are added. Therefore, *Pippali* should not be used in excess.

***Pippali* – Rasayana Drug:**

The fruits of *Pippali* and Amalaki are ground for preparing a powder which is further impregnated with amalaki juice. It is mixed with sugar, honey and ghee. This recipe is given (licked) with milk to a person requiring use of rasayana. As a result of administration of rasayana properly, even the old person becomes like young.

***Pippali* Rasayana:** (CS.Ci.1-3/32-35)

a) Person desirous of availing the benefits of rasayana effects should take *Pippali* in numbers of five, seven, eight or ten with honey and ghee for a year. There is also another course where the use in terms of number of fruits is gradually increased such as three *Pippali* fruits should be taken in morning, after meal and before meal. These fruits should first be impregnated with alkali of palasha and then fried in ghee.
b) These should be taken (in the morning) with honey by those who want rasayana effect particularly in order to alleviate cough, wasting, pthisis, dyspnoea, hiccup, throat disorders, haemorrhoids, disorders of grahani, anaemia, intermittent fever, disorders of voice, chronic rhinitis, swelling, abdominal lump and vatabalasaka ailments.

This kind of provision of rasayana has been made by Caraka in order to achieve both types of objectives of medicine that is protective or preventive and curative, through oral administration of drug *Pippali*. As rasayana drug, *Pippali* has been prescribed for getting results of promotive therapy and simultaneously its applications as clinical measure has been indicated in various diseases.

***Pippali* Vardhamana Rasyana:**

On the first day ten *Pippali* fruits should be taken with milk. From the second day, onwards upto the tenth day ten fruits of *Pippali* should be decreased gradually in the same order till it comes back to ten (on the nineteenth day). After the drug is digested the person should take sastika rice with ghee extracted from milk. Thus, the use of *Pippali* is total number of one thousand prescribed for rasayana palasa kalpa for rasayana effects of *Pippali* based promotive therapy.

The *Pippali* fruits should be taken by the person with high strength in the form of paste, by those with medium strength in that of decoction and by those with low strength in the form of powder keeping the dosas and diseases in view.

The initial use of ten *Pippali* fruits is superior, that of six ones is medium and that of three fruits is inferior. These numbers are also applicable according to the degree of strength of the patient.

The rasayana use of *Pippali* is bulk promoting, beneficial for voice, increases life span, alleviates splenomegaly, sustains age and promotes intellect.

***Pippali* – Potential drug:**

As a rasayana drug, *Pippali* has its important place in the field of Indian Medicine. The potential of *Pippali* fruits is widely utilized in therapeutics which makes it a valuable indigenous drug in common use of medicinal practice. The drug covers wide range in clinical management of many diseases through common application in different modes, forms and pharmaceutics. It is a component of a prominent triad known as Trikatu (Comprising Shunti, Marica and *Pippali*) which is much used in clinical practice as well as pharmaceutical preparations of many compounds. Simultaneously *Pippali* is also component of Pancakola and Sadushana which are also frequently used in indigenous medicine.

Kinds and Varieties:

The fruits of *Pippali* as crude drug (in trade) appear to be derived from two or more species, including one which is Indonesian. Indian Long Pepper is a product either of Piper longum Linn or Piper prepuloides, while the Indonesian or Java Long Pepper imported from Malaysia is Piper retrofractum. The products of these species are used for the same purposes, though they vary in their effectiveness. Indian Long Pepper is mostly procured from the wild plants grown in some particular regions of its availability in more or less quantity. Some other relevents species include Piper sylvaticum Roxb.

As per Raja Nigantuh, four types.

a. *Pippali*: It is Piper longum Linn. which is found in Magadha etc., places.
b. Gaja*Pippali*: It is Piper chaba Hunter which is considered as fruit of Chavika plant, found in Bengal, Assam.
c. Saimhali: It is Piper retrofractum Vahl., which is imported from Srilanka, Singapore.
d. Vana*Pippali*: It is Piper sylvaticum Roxb. or Piper peepuloides roxb. which is found in forests of Bengal, Assam.

In crude drug market, there are two types of *Pippali* sold and procured for catering the requirement of drug, under the current names of raw material of chhoti pipal (small) and Badhi pipal (large) which are indigenous and imported respectively, for practical purpose of drug utilization.

Amayika Prayoga (Therapeutic Uses):

Jwara:

1. In fever, use of *Pippali*, triphala, curd, buttermilk, panchagavya ghrita and milk is efficacious (CS.Ci.3.303).
2. One should also use *Pippali* Vardhamana with diet of swastika with milk and ghee (CS.Ci.1.3.36-40).
3. Boiled milk, sugar, *Pippali*, honey and ghee – these should be churned together and taken. These Panchasara is useful in malarial fever, wasting due to chest-wound, consumption, dyspnoea and heart disease (SS.U.39.254-255).
4. Decoction of *Pippali* made in four times water should be taken (AS.Ci.2.41).
5. In case of constipation, gruel prepared of barley with *Pippali* and amalaka and fried with ghee should be given. It helps excretion of impurities and pathogenic material (AH.Ci.1.31).
6. *Pippali* mixed with honey alleviates cough, dyspnoea, fever, splenomegaly and hiccough. It is particularly recommended for children (BP.Ci.1.377,820).
7. Water boiled with *Pippali* is free from sliminess, stimulates digestion and alleviates disorders caused by vata and kapha, splenomegaly and fever (VM.1.136).
8. *Pippali* mixed with jaggery is prescribed in cough, indigestion, anorexia, asthma, anaemia, worms, chronic fever and deficient digestive power (VM.1.206).
9. One who takes goat's or cow's milk mixed with *Pippali* powder and honey definitely becomes free from heart disease, cough and irregular fever (RM.21.15).
10. In fever, *Pippali* should be given with equal quantity of triphala, while in cough and asthma, it should be given with honey and ghee (BP.Ci.1.378).
11. Pippalyadi Ghrita (Vangasena.jwara.74).

Atisara:

1. By taking *Pippali* with honey, buttermilk with citraka and by eating tender fruits of bilva one becomes free from bowel disorders (CS.Ci.19.113).
2. By using fine powder of *Pippali* or marica, dysentery even if chronic is destroyed (AH.Ci.9.40).

Visucika:

1. Paste of *Pippali* mixed with that of sunthi should be taken with hot water (SS.U.56.18).
2. One should take *Pippali*, ajamoda and ksavaka or *Pippali* and danti in equal quantity or paste of *Pippali* with danti along with the juice of kosataki (SS.U.56.17).

Arshas:

1. The use of buttermilk kept in a vessel anointed internally with the paste of *Pippali*, *Pippali*mula, cavya, citraka, vidanga, sunthi and haritaki is wholesome (SS.Ci.6.13).
2. *Pippali* in increasing doses beginning with ten and tila 20 g should be taken with milk. It promotes strength of the body as well as digestive fire (AH.Ci.8.62).
3. Haritaki fried with ghee and mixed with jaggery and *Pippali* or trivrut and danti should be taken. It acts as carminative (VM.5.9).

Kasa:

1. Pippalyadi Ghrita (CS.Ci.18.36-38).
2. Pippalyadi lehya (CS.Ci.18.135-137).
3. Paste of *Pippali* 10 g fried in oil and mixed with sugarcandy should be dissolved in kulatha water and taken. It alleviates cough caused by kapha (AS.Ci.4.65).
4. One should take *Pippali* mixed with rocksalt with warm water or sunthi mixed with sugar along with curd-water or *Pippali* powder with curd (AH.Ci.3.16).
5. Milk boiled with amalaka powder and added with ghee should be taken or *Pippali* should be used by the method of rasayana (AH.Ci.3.78).
6. Ghee cooked with *Pippali* and jaggery along with goat's milk is useful (AH.Ci.3.164).
7. Intake of *Pippali* with honey is useful in cough (BP.Ci.12.34).
8. *Pippali* kept in mouth with malayavaca, yavani and betel leaf checks dry cough (BP.Ci.1.335).

Hikka and Swasa:

1. Ghee cooked with purgatives checks hiccough immediately. Similarly the juices of amalaki and kapittha mixed with *Pippali* and honey (CS.Ci.17.135).
2. Old ghee, *Pippali*, kulatha, meat-soup of wild animals, wine, sour gruel, hingu, juice of matulunga, honey, draksha, amalaka and bilva-these are useful in asthma and hiccough (SS.U.51.46).
3. Root of ankota mixed with *Pippali*, salt, oil and ghee controls asthma immediately (KK.16.13).
4. Powder of *Pippali*, amalaka and sunthi mixed with honey and sugar should be given frequently. It checks hiccough and asthma (VM.12.6).
5. *Pippali* mixed with peacock's feather and taken with honey controls hiccough (RM.11.4).
6. *Pippali* mixed with sugar and taken as snuff checks hiccough (GN.2.11.50).

7. Pills made of *Pippali* and sunthi with rocksalt, honey and jiggery is kept in mouth in night. It alleviates asthma, wasting and cough caused by chest-wound (HS.3.12.34).
8. *Pippali* taken with honey in morning alleviates cough, asthma, anorexia, and wasting (VD.3.20).
9. In case of curable asthma, one should take *Pippali*, sunthi and rocksalt with honey in morning (VD.3.24).

Swarabheda:

1. *Pippali* and haritaki or sharp wine should be taken (CS.Ci.26.281).

Kshaya and Shosa:

1. Churned drink prepared of equal quantity of sugar, *Pippali*, oil, ghee and honey with double parched grain-flour promotes dhatus (CS.SU.23.25).
2. Boiled milk added with sugar, *Pippali* powder, ghee and honey should be taken. It alleviates cough and fever (CS.Ci.11.79).
3. Sitopaladi churna (CS.Ci.8.103).
4. The linctus prepared of *Pippali*, draksa and sarkara mixed with honey and oil alleviates wasting. Similarly acts the same of *Pippali*, asvagandha and sarkara with honey and ghee (VM.10.9).
5. One who takes powder of *Pippali* and triphala with honey at the time of food becomes free from consumption, dyspnoea, aggravation of kapha, fever and chronic coryza (RM.11.3).

Adhmana:

1. Narayana churna which consists of *Pippali*, trivrut and sugar (BP.Ci.24.95).

Udara:

1. Satpala Ghrita or *Pippali* or haritaki with jiggery and also group of alkali and aristas is useful (CS.Ci.13.78).
2. One thousand *Pippali* fruits impregnated with snuhi latex should be consumed gradually (SS.Ci.14.10).
3. *Pippali*-Vardhamana as prescribed in rasayana should be used (SS.Ci.14.10).
4. In kaphaja udara, one should use *Pippali* with hot water (GN.2.32.49).
5. *Pippali* is the best remedy for pleeha vriddhi (AH.U.40.48).
6. Alkali of pearl-oyster or *Pippali* should be used with milk for alleviation of enlarged spleen (VM.37.44).
7. Intake of *Pippali* powder mixed with lauhabhasma with milk alleviates enlargement of spleen (GN.2.32.145).

8. *Pippali* impregnated with alkaline water of palasha alleviates gulma and splenomegaly and improves digestive power (VM.37.46).

Gulma:

1. Intake of wine mixed with *Pippali*, *Pippali*mula, jeeraka, citraka and rocksalt destroys gulma even if severe (VM.30.31).

Shula:

1. Combination of *Pippali* and sunthi is the remedy for colic caused by kapha (SS.U.42.110).
2. *Pippali* Ghrita is effective in parinamashula (VM.27.18-19).
3. Powder consisting of *Pippali*, haritaki and lauhabhasma mixed with honey and sugar should be taken. It relieves severe pain immediately (VM.27.11).

Chardi:

1. *Pippali* taken with ghee, honey and sugar checks vomiting (SS.U.49.32).

Trisna:

1. One should keep *Pippali* in mouth and then take the churned drink mixed with sugar (CS.Ci.22.53).

Amlapitta:

1. *Pippali* with profuse honey should be taken (VM.53.17).
2. The patient should take in morning ghee cooked with *Pippali* decoction and paste and added with profuse honey (VM.53.22).
3. Sweet bolus made of equal quantity of jiggery, *Pippali* and haritaki should be used. It pacifies pitta and kapha and stimulates digestive fire (VM.53.29).
4. *Pippali* Ghrita mixed with honey (CD.52.53).

Sheetapitta:

1. One should use *Pippali*-Vardhamana or lasuna ao madhuka mixed with sugar or amalaka mixed with jiggery (VM.52.3).

Shotha:

1. Powder consisting of jaggery, *Pippali* and shunti alleviates oedema, indigestion, colic and dysuria (VM.39.10).
2. One should use *Pippali* with milk (CD.39.16).

Vatarakta:

1. *Pippali* Vardhamana is useful (SS.Ci.5.12).

Urustambha:

1. The use of *Pippali* Vardhamana with honey or jiggery is recommended (VM.24.13).

2. By taking the paste of *Pippali*, *Pippali*mula and bhallataka with honey one becomes free from urustambha (VM.24.10).
3. One should take *Pippali* or shunti with urine or dashamula decoction (CD.24.3).

Vatavyadhi:

1. Gridhrasi: Powder of *Pippali* should be taken with cow's urine and castor oil (BP.Ci.24.139).
2. Hanugraha: *Pippali* and fresh ginger should be chewed frequently and spitten followed by mouth-wash with hot water (BP.Ci.24.27).

Mukharoga:

1. Adhimamsa: *Pippali* mixed with honey should be used as gargle (SS.Ci.22.25).
2. Dantashula: *Pippali* mixed with honey and ghee should be kept in mouth. It is excellent remedy for toothache (VM.58.17).

Netraroga:

1. Timira: Nayanasukha vartti consisting of *Pippali* one part and haritaki two parts pounded with water alleviates defects of vision, pterygium, cataract.
2. Naktandhya: Liver of iguana is split open, filled with *Pippali* and cooked on fire. By using this as collyrium night blindness is alleviated (SS.U.17.24).
3. Pishtaka: When the fruit of brhati is maturing paste of *Pippali* is put there in. After sometime it is takenout and mixed with srotanjana and used as collyrium (SS.U.11.14) and Pippalyadi Gudikanjana is used (CS.Ci.30.150).

Shukradosha:

1. Use of *Pippali*, amalaki, lauha, triphala and bhallataka as rasayana alleviates disorders of semen caused by kapha (CS.Ci.30.150).

Streeroga:

1. Yoni dosha: Use of *Pippali*, lauhabhasma and haritaki with honey is efficacious (CS.Ci.30.84).
2. Antarvatni cikitsa: Jaundice during this period is treated with *Pippali*, ankota root, juice of horse's faeces and buffalo's curd (KS.P.300).
3. Sutika roga: During puerperium, if impurity is still there, powder of *Pippali*, *Pippali*mula, gaja*Pippali*, citraka and shunti should be given with hot jaggery-water. This should be continued for 2-3 days until impurity of blood is removed (SS.Sa.10.16).
4. Garbhanirodha: The woman who takes powder of *Pippali*, vidanga, tankana, all in equal quantity, with milk does not conceive (BP.Ci.70.33).

Rasayana:

1. *Pippali* rasayana (CS.Ci.1.3.32-40).
2. *Pippali* and amalaka powder are impregnated with amalaka juice and then mixed with sugar, honey and ghee. By taking it with milk even the old becomes like young (AH.U.40.27).
3. Ghee cooked with *Pippali* and milk alleviates all diseases (VS.7).

Raktapitta:

1. *Pippali* impregnated with vasa juice seven times and taken with honey (CD.9.29).

Kamala:

1. Vidanga or *Pippali* should be used as navana or anjana (GN.2.7.52).

Gandamala:

1. *Pippali* Vardhamana is useful (GN.4.1.44).

Dantodgamana:

1. For dentition gum should be rubbed with *Pippali* powder mixed with honey (GN.6.11.33).

Karnashula:

1. *Pippali* is put in a cotton-pouch and kept for a while on heated charcoal. The ear is fumigated with the smoke so coming out. It relieves pain (GN.3.2.71).

Ajeerna:

1. *Pippali* mixed with jiggery should be taken (SG.2.7.24).

Urdhvajatrugata roga:

1. *Pippali* taken with decoction of dashamula or triphala alleviates supraclavicular diseases (CK.323).

***Pippali* in various therapeutic formulations:**

Curna:

Asvagandhadi churna, Eladi curna, katphaladi curna, Kalyanaka curna, Drakshadi curna, Dusivisadi curna, Punarnavadya curna, Pancakola curna, Balacaturbhadra curna, Bilvamuladi curna, Visvadya curna, Sitopaladi curna, Haridradi curna, Talisadi curna, Hingwashtaka curna, Avipatti curna.

Avaleha-paka:

Narikela khanda, Brahma rasayana, Vasavaleha, Vasaharitaki avaleha, saubhagyasunthi paka, Agastyaharitaki avaleha, Drakshadi lehyam, Cyavanaprasham.

Ghrita:

Amritadi Ghrita, Astamangala Ghrita, Indukanta Ghrita, Kamadeva Ghrita, kumarakalyanaka Ghrita, Dasamulasatpala Ghrita, Dadimadya Ghrita, Dhanvantara Ghrita, Patoladya Ghrita, Pancakola Ghrita, Brihat Shatavari Ghrita, Brihat Asvagandha Ghrita, Mahakhadiraka Ghrita, Mahatriphala Ghrita, Sarasvata Ghrita, Sukumara Ghrita, Pippalyadi Ghrita.

Rasayogas:

Agnitundi vati, Agnimukha rasa, Ajirnari rasa, Antravrddhinasaka rasa, Abhrakadi vati, Arsakuthara rasa, Anandabhairava rasa, Kanakasundara rasa, Kaphaketu rasa, Kasturibhairava rasa, Jalodarari rasa, Dantodbhedagadantaka rasa, Nagavallabha rasa, Brahmi vati, Mritasanjivana rasa, Shirashuladri vajra rasa, Svacchandabhairava rasa, Hingulesvara rasa.

Vati:

Sarpagandhaghana vati, Sansamani vati, Suvarnamuktadi vati, Apatantrakanasini vati, Akarakarabhadi gutika, Eladi vati, Gorocanadi gutika, Vidalavanadi gutika, Maricyadi gutika, Mahalaksmivilasa vati.

Guggulu:

Amrita guggulu, Triphala guggulu, Kancanara guggulu, Kaishora guggulu, Gokshuradi guggulu, Yogaraja guggulu, Mahayogaraja guggulu, Saptavimsitaka guggulu, Simhanada guggulu.

Asava-Arishta:

Pippalyasava, Vidangarishta, Sarasvatarishta, Candanasava, Rohitakarishta, Kanakasava, Khadirarishta, Draksharishta, Asvagandharishta, Dashamularishta, Kumaryasava, Punarnavasava.

Lauha:

Candanadi lauha, Pippalyadi lauha, Raktapittantaka lauha.

Kwatha:

Citrakadi kwatha, Trptighna kwatha, Devadarvadi kwatha, Pippalyadi kwatha, Bharngyadi kwatha, Bhunimbadi kwatha, Maharasnadi kwatha, Vyoshadi kwatha, Elakanadi kwatham, Dashamulakatutraya kwatham, Dhanadanayanadi kwatha.

Anjana-Netra vartti:

Candrodayavartti, Candrakalanjana, Candraprabha vartti, Maricyadi curnanjana.

Nasya:

Pinasahara nasya, Madhukasaradi nasya.

Taila:

Kacchuradi taila, kasisadi taila, Pippalyadi taila, Vasacandanadi taila, Ksara taila, Nagaradi taila, Arimedadi taila, Bala taila.

Drug Review/*Pippali* – Modern literature

Vernacular names:

Sanskrit: *Pippali*, Tikshnatandula, Magadhi.

English: Dried catkins, Long Pepper.

Hindi: Pimpli, Pipal, Pipli.

Bengali: Pipli, Pepul.

Gujarati: Pipara, Pipli.

Marathi: Pimpli.

Telugu: Pippallu.

Tamil: Tippali.

Malayalam: Tippli.

Kannada: Hippali.

Punjabi: Pipal.

Oriya: Baihehi, Krykola.

Malay: Lada.

Sind: Fildray.

Tulu: Ippali.

Arab: Darfilfil.

Burma: Peikchin, Peikkhyen.

Canarese: Hippali, Tippali.

Chinese: Pi Po.

Deccan: Pipplie.

French: Poivre long.

German: Langer Pfeffer.

Greek: Peperi makron.

Italian: Pepe lungo.

Java: Chabijawa.

Lepcha: Kantin.

Mexican: Tlathancuaye.

Nepal: Pipal, Popal.

Persian: Filfilidaraz, Filfildray.

Portuguese: Pimenta longa.

Santal: Ralli.

Sinhalese: Tippili.

Spanish: Pimentera larga.

Urdu: Pipul.

Systemic Position:

Classification by Bentham and Hooker:

Kingdom – Plantae.

Class – Dicotyledons.

Division – Monochlamydeae or Incompletae.

Series – Micrembryae.

Family – Piperaceae.

Genus – Piper.

Species – longum.

Classification of Hutchinson:

Kingdom – Plantae.

Phylum – Angiospermae.

Sub phylum – Dicotyledons.

Division – Herbaceae.

Order – Piperales.

Family – Piperaceae.

Genus – Piper.

Species – longum.

Piperaceae:

Herbs or shrubs often with swollen nodes, usually aromatic. Leaves alternate, opposite or whorled, often gland dotted; stipules 0 or 2, connate, or adnate to the petiole. Flowers minute hermaphrodite or unisexual, in axillary or terminal catkin-like spikes subtended by a peltate bract. Perianth 0. Stamens 2-6 (rarely 7-8), hypogynous; anthers often jointed on the filaments, the cells sometimes confluent; dehiscence longitudinal.

Ovary of 3-4 carpels with many ovules; less commonly ovary 1-celled with 1 ovule; ovules orthotropous; stigmas distinct on the free carpels or ovary lobes or terminal on the undivided ovary, occasionally solitary, sessile, terminal simple or penicillate. Fruit small, indehiscent in

the 1-celled species or of cocci or follicles in the many-carpelled species. Seeds globose, ovoid or oblong; testa thin; albumen copious, floury; embryo enclosed in an amniotic cavity at the end of the albumen remote from the hilum; cotyledons minute or obsolete; radical superior.

Genera 7. Species 1150. Aromatic, stimulant, sialogogue. The three alkaloids jaboridine, piperine and piperovatine have been isolated from various species of piper. Jaboridine from Piper reticulatum Linn., Piperine from P.chaba Hunter, P.clusii C.DC., P.longum Linn., P.nigrum Linn. and Piperovatine from P.ovatum Vahl.

Piper longum Linn.

This is a glabrous undershrub/climber with erect or subscandent nodose stem and slender branches.Rootstock erect, thick, jointed, branched, stems numerous, 0.6-0.9 m., ascending or prostate, much branched , stout, cylindrical, thickened above nodes, finely pubescent. Leaves simple, alternate, stipulate and petiolate or nearly sessile according to their position on the plant, numerous, 6.3-9 cm.,long, 3-5 cm.wide, lower ones broadly ovate, very cordate with broad rounded lobes at base; upper ones oblong-oval, cordate at base, all subacute, entire, glabrous, thin, bullate with reticulate venation sunk above and raised beneath, dark green and shining above, pale and dull beneath; petiole of lower leaves 5-7.5 cm., stout, of upper leaves very short or none; stipules about 1.3 cm., membranous, lanceolate, obtuse, soon falling. Inflorescence and Flowers: Flowers unisexual, dioecious, minute, sessile, bracteate, without perianth very densely packed in spikate inflorescence, the male and female on separate thickness.

Spikes solitary, pedunculate, male larger and slender, 2.5 to 7.5 cm, bracts narrow, female spikes1.3-2.5 cm.long and 4-5 mm.diameter;bracts circular, flat, peltate; stamens 2; stigmas 3 or 4, short, spreading, persistent. Fruits: Small about 2.5 mm. in diameter, greyish green or nearly blackish when ripe and are partially sunk in the fleshy axis of the spike. The fruiting spikes are 2.5 to 3 cm. long and 2.5 to 3.5 mm. thick, erect, blunt, ovoid-oblong.

Flowering and Fruiting season:

Plant bears flowers during rainy season and fruiting afterwards, in autumn months.

Flower morphology:

Flower: Minute, naked, sessile.

Bracteate: Bract of the male flower narrow, orbicular. Bract of the female flower circular, flat, peltate. Unisexual, Incomplete, colour: Green at first, turning yellow later.

Perianth: Zero.

Androecium:

Number of stamens: 2-3.

Form-Free; Nature of filament: short, broad. Fixation of anther: Anthers often jointed on the filaments. Anther: 2-Celled, separated below; Dehiscence of anther: Longitudinal.

Gynoecium:

Number of carpels: One (Monocarpellary).

Position of ovary: Ovary sunk in and more or less confluent with the thick rachis.

Number of loculi: One (Monolocular).

Number of ovules in each loculus: Solitary, Orthotropous. Placentation: Erect; Nature of style: Short. Nature of Stigma: 3 or 4, short, spreading, persistent.

Seed:

Type: Adherent to the endocarp, globose, ovoid or oblong, testa thin.

Albuminous: Albumen floury.

Number of cotyledons: Two (Dicotyledonous).

Nature of Embryo: Embryo in a cavity remote from the hilum; radical superior.

Nature of Cotyledons: Minute or absolute.

Fruit drug: In transaction of the fruiting spikes are seen one seeded fruitlets, arranged in a circle on the main axis. The pericarp of the fruit has zones of Epicarp, Mesocarp and endocarp. Secretory cells are present in the outer parts of epicarp and round and oval type cells of sclerenchyma. Mesocarp has thin walled collapsed parenchymatous cells. Epicarp is waxy and filled dark brown contents. Sometimes the outer end of endocarp forms a dome like structure covering a few cells of endosperm and embryo. The major portion of the fruit under endocarp consists of perisperm, the cells of which are stocked with starch grains.

Habitat: *Pippali* plant is indigenous to North-Eastern and Southern India and Ceylon and cultivated in Eastern Bengal. It occurs in hotter parts of India from Central Himalayas to Assam, Khasi and Mikir hills, lower hills of Bengal and evergreen forests of Western Ghats from konkan to Travancore. Long pepper as sold in India appears to be derived from two or three species, including one which is Indonesian.

Indian Long pepper is a product either of Piper longum or Piper peepuloides, while the Indonesian or Java Long pepper imported from Malaysia is from Piper retrofractum. The product of these species is used for the same purposes, though they vary in their effectiveness. Indian Long pepper is mostly derived from the wild plants, the main sources of supply being Assam, West Bengal, Nepal and Uttar Pradesh. Small quantities are also available from evergreen forests of Kerala, West Bengal and certain parts of Andhra Pradesh.

Chemical Constituents: The plant contains essential oil consisting of long-chain hydrocarbons, mono and sesquiterpenes, caryophyllene being the main product. Other constituents are piperine, piplartine, piperlongumine, piperlonguminine and its dihydro-derivative, pipernonaline, piperundecalidine, pipercide and guineensine, sesamin, dieudesmin, β-sitosterol and dihydrostigmasterol. Four aristolactams (cepharanone B, aristolactam AII, piperlactam A and piperlactam B) and five 4, 5 – dioxoaporphines (cepharadione A, cepharadione B, norcepharadione B, piperadione (2 – hydroxy-1-methoxy-6-methyl-4H-dibenzo quinoline-4, 5-(6H)-dione), its 6-demethyl derivative and aminoacids, dehydropipernonaline from the fruit and tetrahydropiperine from the plant have been isolated. Two alkaloids Piper longumine and Piper longuminine characterized as N-(3,4,5-trimethoxy cinnamoyl)-piperidin-2-one and isobutylamide of piperic acid respectively(stem and roots); n-hexadecane, n-heptadecane, n-octadecane, terpinolene, zingiberene, p-cymene, p-methoxy acetaphenone, traces of dihydrocarveol, phenylethyl alcohol and two sesquiterpenes (essential oil from the dried fruit); piperine, piplartine, triacantane, dihydro-stigmasterol, an unidentified steroid, reducing sugars, glycosides, sesamin and methyl 3,4,5-trimethoxycinnamate (roots); major alkaloid piperine 4-5%and sesamin (stem and fruits); sesquiterpene hydrocarbon, caryophyllene, a sesquiterpene alcohol, carbonyl compound (essential oil); N-isobutyldeca-trans-2-trans-4-dienamide, piperine, piplartine, and a lignin d-sesamin, two piperidine alkaloids-piperundecalidine (fruit); sylvatin, sesamin and diaeudesmin (seed). Resin, volatile oil, starch, gum, fatty oil, inorganic matter.The dried fruit (on steam-distillation) yields 0.7% of an essential oil with spicy odour resembling that of pepper and ginger oil.

Parts Used: Dried unripe fruits or fruiting spikes and Roots.

Actions and Uses:

Dried spikes are acrid, vermifuge, mildly thermogenic, stomachic, aphrodisiac, carminative, expectorant, febrifuge, tonic, laxative, digestive,emollient and antiseptic. They are useful in anorexia, dyspepsia, vomiting, flatulent colic, diarrhea, cholera, dysentery, asthma, bronchitis, coryza, hiccough, consumption, gastric disorders, epilepsy, insomnia, fever, gonorrhea, haemorrhoids, gout and lumbago.

The fruits are used after childbirth to check postpartum haemorrhage, as a cholagogue in bile duct and gall bladder obstruction. Unripe fruit is used as an alternative and tonic. A decoction of immature fruits and roots is used in chronic bronchitis, cough and cold; also used in palsy, gout, rheumatism and lumbago.

The root is pungent; heating, stomachic, laxative, anthelmintic, carminative; improves the appetite; useful in bronchitis, abdominal pains, diseases of the spleen, tumours, ascites; causes biliousness. The unripe fruit is sweetish; cooling; useful in biliousness. The ripe fruit is sweetish, pungent; heating, stomachic, aphrodisiac, alterative, laxative, antidiarrhoeic, antidysenteric; useful in ʿvataʾ and ʿkaphaʾ, asthma, bronchitis, abdominal complaints, fevers, leucoderma, urinary discharges, tumours, piles, diseases of the spleen, pains, inflammations, leprosy, insomnia, jaundice, hiccough, tuberculous glands; increases biliousness (Ayurveda).

The root and fruit are used in palsy, gout, lumbago. The fruit has a bitter, hot, sharp, taste; carminative, tonic to the liver, stomachic, emmenagogue, abortifacient,, aphrodisiac, haematinic, diuretic, digestive; general tonic; useful in inflammation of the liver, pain in the joints, lumbago, snake-bite, scorpion-sting, night blindness (Unani).

In Travancore, an infusion of the root is prescribed after parturition, with the view of causing the expulsion of the placenta. It appears to partake, in a minor degree, of the stimulant properties of the fruit.

As an alternative tonic, long pepper is recommended for use in a peculiar manner. An infusion of three long peppers is to be taken with honey on the first day, then for ten successive days the dose is to be increased by three peppers every day, so that on the tenth day the patient will take thirty at one dose. Then the dose is to be gradually reduced by three daily, and finally the medicine is to be omitted.

Thus administered, it is said to act as a valuable alterative tonic in paraplegia, chronic cough, enlargements of the spleen and other abdominal viscera. Long pepper enters into the composition of several irritating snuffs; boiled with ginger, mustard oil, butter milk and curds it forms a liniment used in sciatica and paralysis. The dried immature fruit and the root in the form of decoction were extensively used in acute and chronic bronchitis attended with cough and was found to give gradual relief in all such cases.

The fruits are used as spice and also in pickles and preserves. They have a pungent pepper-like taste and produce salivation and numbness of the mouth.

Old long pepper is more efficacious in medicine than fresh article. Powdered long pepper administered with honey will relieve cough, cold, asthma, hoarseness and hiccup. For catarrh and hoarseness a mixture of long pepper, long pepper root, black pepper and ginger in equal parts is a useful combination. A compound powder consisting of the same ingredients and in equal parts and called *Chaturushana Churnam* is useful in colic and flatulence besides cough and coryza. It was tested and found successful.

For diseases of the Respiratory system, Vaidyas & Hakims use an extract prepared by boiling together 4 seers of Adhatoda leaves, 1 seer of white sugar, 16 *tolas* each of long pepper and ghee to the consistence of an extract and adding, when cool 1 seer of honey and mixing well. Dose is 1 to 2 *tolas*. A compound powder consisting of long pepper, ginger, black pepper, cinnamon and caraway in equal parts is a good expectorant; and infusion made of 10 peppers with honey makes a good expectorant. A powder called *Sringyadi Churna* consisting of *karkatashringi*, atis, long pepper and *Nagaramotha*, made into a linctus with honey is useful especially for coughs among children. In dry cough a compound powder made up of equal parts of long pepper, round zedoary, ginger, root of Clerodendron siphonanthus, *karkatashringi*, and raisins, is a very useful remedy given in doses of 30 grains with honey. In catarrhal fever with difficulty of breathing, a powder made of equal parts of *karkatashringi*, bark of Myrica sapida and long pepper is given with honey.

Unani physicians recommend a pill for asthma; it is made of filaments of Calotropis gigantea 2 parts, long pepper and rocksalt 1 part each. Pills are of the size of a jangli bor; dose is one such pill thrice daily. For bronchitis a pill of the same size but made up of various other ingredients viz.-black pepper, long pepper, borax, *karkatashringi*, cloves, alum, *bharangi*, *harka chilka*, dry ginger and *nimak Lahori*, all equal parts is recommended in Ilaj-ul-Gurba. Two such pills to be taken at bed time. As a valuable alterative tonic in paraplegia, asthma, chronic bronchitis, chronic cough, enlargements of the spleen and other abdominal viscera etc, it is used thus-An infusion of three long peppers is taken with honey or sugar on the first day, then for ten successive days the dose is increased by 3 peppers every day, so that on the 10^{th} day the patient takes 30 at one dose.

Then the dose is gradually reduced by 3 daily so as to finally omit the medicine. In rheumatism, roasted aments are beaten up with honey; they are also given powdered with black pepper and rocksalt (in the proportion of 2, 3 & 1 part respectively) in half *tola* doses for colic. A compound powder consisting of equal parts of long pepper, emblic and chebulic myrobalans and *Saindhava* salt, is a good digestive in doses of half to one drachm. In catarrh and bronchitis, a compound powder known as, cough powder is generally in use; it is prepared thus-Take of black pepper, ajowan, long pepper, rock salt, black salt and borax each 1 tola and Adhatoda leaves 40 *tolas*; put them all in a small pot, close the mouth carefully and put the pot over fire for a while till the ingredients within are completely burnt. Use the burnt powder 2 to 6 grains mixed with honey.

A fermented decoction called *Pippali Arista*, used in asthma, cough, anorexia, piles etc., is composed of long popper, *lodhra*, black pepper, grapes and Cissampelos pareira. Dose is ½

to 2 tolas twice a day. With black pepper, long pepper is used in the preparation of irritating snuffs for using in coma and drowsiness e.g., take of black pepper, long pepper, seeds of Moringa pterygosperma and ginger equal parts, powder the ingredients and rub them together with the juice of the root of Agati grandiflora. This preparation is used as a snuff in coma and drowsiness.

For indigestion, chronic and painful dyspepsia, dilatation of the stomach and chronic gastritis, a compound powder known as *Bhaskara Lavanam* is much in use; it is made up of long pepper, root of long pepper, coriander, nigella seeds, *saindhava lavana, vida lavana,* Cinnamon leaves, *talisa patra, nagakesara,* 2 palas each; pepper, omum, dry ginger and Rumex vasicarius, 1 pala each; cinnamon and cardamom seeds 6½ palas each; pomegranate fruit rind 4 palas, black salt 5 palas and samudra lavana, varieties of rock salt 8 palas all well powdered, mixed and sifted through cloth, the dose ½ to 1½ drs., or even 1 tola, twice a day with the first bolus of rice and butter milk. Another powder generally taken along with this, in case of dyspepsia and containing 8 ingredients and called *Ashta churnam* is made of equal quantities of black pepper, long pepper, dry ginger, omum, *Saindhava* salt, cumin seeds, nigella seeds and asafoetida.

Dose is 20 to 40 grains twice or thrice a day before meals. A compound powder of 5 pungents named *Pancha Kola Churnam* and consisting of long pepper, long pepper root, dry ginger, stem of pepper plant and *chitraka* is a good appetizer useful in dyspepsia, cough, flatulence and enlarged spleen. This was tried and found efficient. Dose is 10 to 30 grains twice a day. As rubefacient, oil containg it and ginger is applied in sciatica and paraplegia, as for instance the *Astakatvara Taila* recommended by Chakradatta, which consists of ginger and long pepper each 16 tolas, mustard oil 4 seers, butter milk 32 seers, curdled milk 4 seers, boiled together in the usual way.

This oil is rubbed externally in sciatica and paraplegia. Both fruit and root are much prescribed in palsy, gout, rheumatism, lumbago etc. Fruit is given to women after parturition to check haemorrhage and to ward off fever. As vermifuge it is one of the best remedies for colic in children. Fruit is used to some extent as a spice. Root is much used as a stimulant remedy and spice. The drug is also used in snake bite and scorpion sting.

Therapeutic uses:

The drug is very much considered useful for consumption. The study conducted on the drug *Pippali* has shown antitubercular activity in the active constituents derived from the plant drug. Piperine isolated from the drug possesses anticolic and analeptic potentialities.

The drug has a peculiar odour and a pungent bitter taste producing numbness on the tongue. The fruits are used as spice and also in pickles and preserves. They have a pungent pepper like taste and produce salivation and numbness of the mouth.

The fruits as well as roots, known as *Pippali* and *Pippali*mula respectively, are attributed with numerous medicinal uses and may be used for diseases of respiratory tract viz., cough, bronchitis, asthma and other allied ailments. It is used as counter-irritant and analgesic when applied locally for muscular pains and inflammations. A snuff in coma and drowsiness is used and internally as carminative; as sedative in insomnia and epilepsy. It is given as general tonic and haematinic. As cholagogue in obstruction of bile duct and gall bladder it is taken. It is used as an emmenagogue and abortifacient, and for miscellaneous purpose as antihelmintic and in dysentery and leprosy.

The drug *Pippali* is a prominent drug of Indian Medicine and it is most common and highly valuable medicine finding clinical, pharmaceutical and therapeutical uses in early classical texts of ancient medical system and presently the role of *Pippali* as an effective and potential drug predominantly continues in medical practice carrying support of experimental studies and multi- disciplinary investigations.

Pippali is chiefly an esteemed drug in cough, hiccough and asthma, bronchitis, pulmonary tuberculosis and allied diseases of respiratory system. It is specifically useful in chronic fever. *Pippali* belongs to valuable Rasayana group of drugs.

Therapeutically, the drug *Pippali* covers large number of clinical managements where *Pippali* is employed various forms, modes and formulations in addition to a single drug as well as a component of Trikatu (comprising Shunti, Marica and *Pippali*), trio-pungent drugs group occupying significant role in the therapeutics of indigenous system of medicine.

Pippali acts as Rasayana and its use as Vardhamana *Pippali* is well appreciated for the purpose of rasayana. The drug *Pippali* is administered for treatment of several diseases. It is frequently used in liver disorders, splenic enlargement, anaemia, haemorrhoids, worms, dyspepsia, anorexia, loss of appetite, constipation, abdominal colic, gout, rheumatism, urinary complaints, dysmenorrhoea, chronic fever, seminal disorders and general debility.

The use of *Pippali* in the mode Yogavahi (synergistic or potentiating way) may be preferred. The prolonged and excess use of single or individual drug may produce some adverse effects as cautioned by Caraka. Besides as a major drug, *Pippali* is commonly used as a spice.

Recent work on the fruit of Piper longum has shown the presence of the alkaloids Piperine (4-5%) and Piplartine, and two new liquid alkaloids, one of which is designated as alkaloid A.

This is closely related to Pellitorine producing marked salivation, numbness and a tingling sensation of mucous membranes of the mouth. Alkaloid A showed significant Invitro antitubercular activity against Mycobacterium tuberculosis H-37 Rv strain; It inhibited the growth of the bacillus in 20µg. /ml. concentrations. Sesamin, dihydrostigmasterol and a new sterol, Piplasterol are also present.

The fruits as well as the roots are attributed with numerous medicinal uses and may be used for diseases of respiratory tract viz., cough, bronchitis, asthma etc., as counter-irritant and analgesic when applied locally for muscular pains and inflammation, as snuff in coma and drowsiness and internally as carminative, as sedative in insomnia and epilepsy; as general tonic and haematinic, as cholagogue in obstruction of bile duct and gall bladder, as an emmenagogue and abortifacient; and for miscellaneous purposes as anthelmintic and in dysentery and leprosy.

Alcoholic extracts of the dry fruits and aqueous extracts of the leaves showed activity against Micrococcus pyogenes var.aureus and Escherichia coli. Ether extract of the fruits showed larvicidal properties.

Pharmacology:

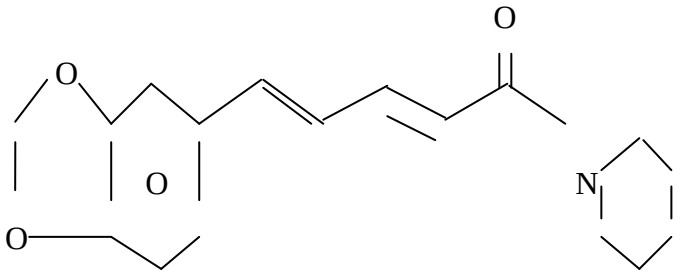
Antibacterial, anti-inflammatory, insecticidal, antimalarial, CNS stimulant, antitubercular, antihelmintic, hypoglycaemic, antispasmodic, cough suppressor, antigiardial, immune stimulatory, hepatoprotective, analeptic, antinarcotic, antiulcerogenic.

Toxicology:

LD_{50} value of Piperine in mice was 750-800 mg/Kg,P.O.

Chemistry: Alkaloids (Piperine), resin (Chavicin) and aromatic oil. Actions: Carminative and laxative. Therapeutics: Indigestion and constipation. Dose: Decoction (50-100 ml), Powder (1-3 g).

Structure of Piperine:



Antiprotozoal activity: Piper longum fruits have shown activity against experimental Giardia lamblia infection in mice at doses ranging from 250-900 mg/kg. Piper longum demonstrated efficacy against caecal amoebiasis (Entamoeba histolytica) in rats.

Piperine:

Gastroprotective activity: In a pharmacological study, piperine was found to protect against gastric ulceration; it also inhibited gastric acidity and pepsin A activity.

Propagation and Cultivation:

Piper longum is cultivated on a large scale in limestone soil, 450-600 m. below the Cherrapunji region which receives very heavy rains from the end of March to the middle of September and where the relative humidity is high. Long pepper is cultivated mainly by layering of mature branches or by suckers planted at the beginning of the rainy season. The vines are well manured with cowdung cake and start bearing three to four years after planting. The spikes are harvested in January, while still green and unripe, as they are most pungent at this stage. They are dried in the sun when they turn grey. The yield increases from 560 kg.per hectare in the first year to 1,680 kg.per hectare in the third. After the third year, the vines become less productive and should be replaced.

Plant regeneration from Callus ultures of Piper longum was achieved through organogenesis. Invitro grown shoots were used as explants for callus injunction. Competent callus was initiated around the nodal ring of tissue using Murashige and Skoog's medium supplemented with 1.0 mg/1 α-napthaleneacetic acid and 0.2 mg/1 N^6-benzyladenine. Optimum growth regulator concentrations for shoot induction and shoot elongation were found to be 0.5 mg/1 indole-3-acetic acid with 1.5 mg/1 benzyladenine and 0.1 mg/1 indole-3-acetic acid with 0.2 mg/1 benzyladenine respectively. Elongated shoots were rooted on half strength Murashige and Skoog's medium having 0.1 mg/1 indole-3-acetic acid. The rooted plantlets were successfully established in soil.

Morphogenetic potential of root, leaf, node and internode explants of P.longun have been reported. The highest number of shoot buds was produced on root explants followed by node, internode and leaf explants. Benzyladenine was suitable for shoot induction and its optimum concentration is 1-2 mg/l.

Market samples of *Pippali*:

In market we will get two types of *Pippali*. one is small in size and another of big size. Small sized *Pippali* is from India only, which comes from Assam-Bengal, also from wild and cultivation. Large sized *Pippali* is imported from Singapore, Srilanka.

Small sized *Pippali*'s amentum is 2.5 cms to 3.75 cms or 1 inch to 1.5 inch length, straight, blackish green in colour and shining. It looks like unripened fruit. Large sized *Pippali* length and breadth is more than small *Pippali* and blackish red in colour but when washed in water becomes dark red. There will be no odour in fresh *Pippali* but while drying one type of odour is produced. On taste causes tingling sensation with salivation.

Collection and Preservation: *Pippali* is stored in air tight bottles and placed in a dry place. While collecting, only ripened fruits should be collected and before preserving it should be dried properly.

Veeryakalavadhi (Potency): 2 years.

Substitutes and Adulterants:

The fruiting spikes of Piper longum are often adulterated with other Piper species like Piper peepuloides Roxb., Piper retrofractum Vahl and Piper betle Linn., the roots of Piper longum are adulterated with its stem pieces.

Macroscopic features of Fruits:

Fruit greenish-black to black, cylindrical, 2.5 to 5 cm long and 0.4 to 1 cm.thick, consisting of minute sessile fruits, arranged around an axis; surface rough and composite; broken surface shows a central axis and 6 to 12 fruitlets arranged around an axis; taste, pungent producing numbness on the tongue; odour, aromatic.

Microscopic features of Fruits:

Catkin shows 6 to 12 fruits, arranged in circle on a central axis, each having an outer epidermal layer of irregular cells filled with deep brown content and covered externally with a thick cuticle; Mesocarp consists of larger cells, usually collapsed, irregular in shape and thin walled; a number of stone cells in singles or in groups present; endocarp and seed coat fused to form a deep zone, outer layer of this zone composed of thin-walled cells and colourless, inner layer composed of tangentially elongated cells, having reddish brown content; most of endocarp filled with starch grains, round to oval measuring 3 to 8μ in dia.

Powder:

Deep moss green shows fragments of parenchyma, oval to elongated stone cells, oil globules and round to oval, starch grains, measuring 3 to 8μ in dia.

Identity, Purity and strength:

Foreign matter – Not more than 2 percent.

Total ash - Not more than 7 percent.

Acid insoluble ash - Not more than 0.5 percent.

Alcohol soluble extractive – Not less than 5 percent.

Water soluble extractive – Not less than 7 percent.

Thin Layer Chromatography:

T.L.C. of alcoholic extract of the drug on silica gel 'G' plate using Toluene:Ethylacetate (90:10) as mobile phase. Under U.V. (366nm) six fluorescent zones are visible at Rf. 0.15, 0.26, 0.34, 0.39, 0.50 and 0.80. On exposure to Iodine vapour seven spots appear at Rf. 0.04, 0.15, 0.26, 0.34, 0.39, 0.50 and 0.93 (all yellow). On spraying with Vanillin-Sulphuric acid reagent and heating the plate at 105° for ten minutes five spots appear at Rf. 0.04, 0.22, 0.35, 0.43 and 0.82. On spraying with Dragendorff reagent three spots appear at Rf. 0.15, 0.26 and 0.34 (all orange).

Organoleptic characters of the dried female spike:

Shape – Cylindrical, ovoid-oblong, slightly tapering or blunt.

Size – Length: 2.5-4 cms, diameter: 5-7 mm.

Fruit:

Type – Drupe, indehiscent.

Shape – Ovoid.

Size – Diameter about 2.5 mm.

Arrangement – Many fruits are closely packed on the axis and partially sunk in the solid fleshy spike.

Colour – Blackish green.

Odour – Aromatic.

Taste – Pungent.

Parameters for quality control:

Piperine (alkaloid): 1-2%

Foreign organic matter: <2%

Total ash: 6.95

Acid insoluble ash: 3.74

Water soluble ash: 1.39

Piperine is usually considered as Marker as well as therapeutic compound. There is similarity in this molecule of other Piperaceae member like black pepper. But each of them can be differentiated through chromatographic techniques.

Table 2: Histochemical Tests

Sl no.	Reagents used	Observation
a.	Conc.HNO_3	Kernel grayish yellow, ground tissue yellow, endocarp brown, stone cells yellowish brown.
b.	H_2SO_4	Endocarp dark brown, kernel grey, ground tissue light brown.
c.	Hcl	Ground tissue yellow, endocarp brown, kernel light yellow and stone cells brownish yellow.
d.	Ferric chloride	Ground tissue yellowish brown, kernel yellowish grey and endocarp brown.
e.	Iodine	Entire tissue in general and kernel in particular except the endocarp gets bluish blacks.

Isolation of Piperine:

Piperine is isolated from unripe fruit (Black Pepper) and Kernel of the ripe fruit (White Pepper) of Piper nigrum, from the fruit of ashanti (Piper clusii), from long pepper (Piper longum), seeds of Cubeba censii, Piper fainechotti and Piper chaba. The Piperine content of black pepper varies from 6 to 9%.

Finely powdered 20 g of black pepper is extracted with 300 ml 95% ethanol in a soxhlet extractor for 2 h. The solution is filtered and concentrated in vacuum on a water bath at 60°c. 20 ml of alcoholic potassium hydroxide is added to the filtrate residue and after it while decanted from the insoluble residue. The alcoholic solution is left overnight, whereupon yellow coloured needle shaped crystals are deposited. The yield of Piperine is 0.3 g. Melting point: 125-126°C.

Review of Previous Research works

Research works carried out on *Pippali* not referred to *Nava* and *Purana Pippali* specifically. Research works carried on Piper longum Linn are:

Overview for various aspects of the health benefits of Piper longum Linn. fruit.

Kumar S, Kamboj J, Suman, Sharma S.

J Acupunct Meridian Stud.2011 Jun; 4(2):134-40.Review.

Antioxidant activity of combined ethanolic extract of Eclipta alba and Piper longum Linn.

Ramesh V, Hari R, Pandian S, Arumugam G.

J Complement Integr Med. 2011 Dec 7; 8.

Studies on the neuroprotective role of Piper longum in C6 glioma induced rats.

Subramanian U, Poongavanam S, Vanisree AJ.

Invest New Drugs. 2010 Oct; 28(5):615-23.

Protective effect of Piper longum Linn on monosodium glutamate induced oxidative stress in rats.

Thomas M, Sujatha KS, George S.

Indian J Exp Biol.2009 Mar; 47(3):186-92.

HPLC assisted chemo biological standardization of alpha-glucosidase-I enzyme inhibitory constituents from Piper longum Linn-An Indian Medicinal plant.

Pullela SV, Tiwari AK, Vanka US, Vummenthula A, Tatipaka HB, Dasari KR, Khan IA, Janaswamy MR.

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Antifertility activity of Piper longum Linn in female rats.

Lakshmi V, Kumar R, Agarwal SK, Dhar JD.
Nat Prod Res.2006 Mar; 20(3):235-9.

Immunomodulatory and antitumour activity of Piper Longum Linn and Piperine.
Sunila ES, Kuttan G.
J Ethnopharmacol. 2004 Feb; 90(2-3): 339-46.

Effect of Piper longum Linn, Zingiber officinalis Linn and Ferula species on gastric ulceration and secretion in rats.
Agrawal AK, Rao CV, Sairam K, Joshi VK, Goel RK.
Indian J Exp Biol.2000 Oct; 38(10): 994-8.

A rapid method for isolation of Piperine from the fruits of Piper nigrum Linn.
Kanaki N, Dave M, Padh H, Rajani M.
J Nat Med. 2008 Jul; 62(3); 281-3.

Hypolipidemic effects of a new Piperine derivative GB-N from Piper longum in high-fat diet-fed rats.
Bao L, Bai S, Borijihan G.
Pharm Biol.2012 Aug; 50(8):962-7.

A clinical trial of *Pippali* with special reference to Abheshaja.
Pathak M, Vyas H, Vyas MK.
Ayu.2010 Oct; 31(4):442-6.

Mollusicidal activity of Piper cubeba Linn, Piper longum Linn and Tribulus terrestris Linn and their combinations against snail Indoplanorbis exustus Desh.
Pandey JK, Singh DK.
Indian J Exp Biol. 2009 Aug; 47(8):643-8.

Piper longum Linn extract inhibits TNF-alpha-induced expression of cell adhesion molecules by inhibiting NF-kappa B activation and microsomal lipid peroxidation.
Singh N, kumar S, Singh P, Raj HG, Prasad AK, Parmar VS, Ghosh B.
Phytomedicine.2008 Apr; 15(4):284-91.

Anti-inflammatory activity of Piperine.
Mujumdar AM, Dhuley JN, Deshmukh VK, Raman PH, Naik SR.
Jpn J Med Sci Biol.1990 Jun; 43(3):95-100.
Phytomedicine.2008 Apr; 15(4):284-91.

Disease Review - Bronchial Asthma

Bronchial Asthma is a chronic airway disorder which can affect people of all age groups. According to the global initiative for asthma (GINA), asthma is defined as a chronic inflammatory disorder of airways which is associated with airway hyper-responsiveness. It leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night or early morning. The increasing global prevalence of asthma, the large burden it now imposes on patients and the high health care costs have led to extensive research into its mechanisms and treatments.

Prevalence: Asthma is one of the most common chronic diseases globally and currently affects approximately 300 million people worldwide. In developing countries where the prevalence of asthma had been much lower, there is a rising prevalence, which is associated with increased urbanization. Asthma can present at any age, with a peak age of 3 years.

In childhood, twice as many males as females are asthmatic, but by adulthood the sex ratio has equalized. The severity of asthma does not vary significantly within a given patient; those with mild asthma rarely progress to more severe disease, whereas those with severe asthma usually have severe disease at the onset.

Major risk factors for asthma deaths are poorly controlled disease with frequent use of bronchodilator inhalers, lack of corticosteroid therapy and previous admissions to hospital with near-fatal asthma.

Etiology: Asthma is a heterogeneous disease with interplay between genetic and environmental factors. Several risk factors have been implicated.

Risk factors and Triggers involved in Asthma:

Endogenous factors:

Genetic predisposition

Atopy

Airway hyper responsiveness

Gender

Environmental factors:

Indoor allergens

Outdoor allergens

Occupational sensitizers

Passive smoking

Respiratory infections

Triggers:

Allergens

Upper respiratory tract viral infections

Exercise and hyper ventilation

Cold air

Sulfur dioxide and irritant gases

Drugs (β-blockers, Aspirin)

Stress

Irritants (Household sprays, paint fumes).

Pathogenesis:

Asthma is associated with a specific chronic inflammation of the mucosa of the lower airways. One of the main aims of treatment is to reduce this inflammation.

Pathology:

The airway mucosa is infiltrated with activated eosinophils and T lymphocytes and there is activation of mucosal mast cells. A characteristic finding is thickening of the basement membrane due to sub epithelial collagen deposition. The epithelium is often shed or friable, with reduced attachments to the airway wall and increased numbers of epithelial cells in the lumen.

Another common finding in fatal asthma is occlusion of the airway lumen by a mucous plug, which is comprised of mucous glycol proteins secreted from goblet cells and plasma proteins from leaky bronchial vessels. There is also vasodilatation and increased number of blood vessels. Direct observation by bronchoscopy indicates that the airways may be narrowed, erythematous and edematous.

Inflammation:

There is inflammation in the respiratory mucosa from the trachea to terminal bronchioles, but with predominance in the bronchi. There is good evidence that the specific pattern of airway inflammation in asthma is associated with airway hyper responsiveness, the physiologic abnormality of asthma, which is correlated with variable airflow obstruction. Many inflammatory cells are known to be involved in asthma with no key cell that is predominant.

Mast cells: Mast cells are important in initiating the acute bronchoconstrictor responses to allergens and several other indirectly acting stimuli such as exercise and hyperventilation, as well as fog. Activated mast cells are found at the airway surface in asthma patients and also in the airway smooth muscle layer. Mast cells are activated by allergens through an IgE-

dependent mechanism and binding of specific IgE to mast cells renders them more sensitive to activation. Mast cells release several bronchoconstrictor mediators, including histamine, prostaglandin D_2 and cysteinyl leukotrienes, but also several cytokines, chemokines, growth factors and neutrophins.

Macrophages and dendritic cells: Macrophages may traffic into the airways in asthma and may be activated by allergens via low affinity IgE receptors. Macrophages have the capacity to initiate a type of inflammatory response via the release of a certain pattern of cytokines. Dendritic cells take up allergens, process them to peptides and migrate to local lymph nodes.

Eosinophils: Eosinophil infiltration is a characteristic feature of asthmatic airways. Allergen inhalation results in a marked increase in activated eosinophils in the airways at the time of the late reaction.

Inflammatory Mediators: Many different mediators have been implicated in asthma and they may have a variety of effects on the airways that could account for the pathological features of asthma.

Inflammatory cells: Mast cells, Eosinophils, Basophils, Neutrophils, Platelets.

Structural cells: Epithelial cells, Smooth muscle cells, Endothelial cells, Fibroblasts, Nerves.

Mediators: Histamine, Leukotrienes, Prostanoids, Kinins, Adenoline, Endothelins, Nitric oxide, Cytokines, Chemokines.

Effects: Bronchospasm, Plasma exudation, Mucus secretion, Structural changes.

Asthma Triggers:

Several stimuli trigger airway narrowing, wheezing and dyspnea in asthmatic patients. Allergens, Virus infections, Pharmacological agents, Exercise, Physical factors, Food, Air pollution, Occupational factors, Hormonal factors, Gastro esophageal Reflux, Stress.

Clinical features:

The characteristic symptoms of asthma are wheezing, dyspnea and coughing, which are variable, both spontaneously and with therapy. Symptoms may be worse at night and patients typically awake in the early morning hours.

There is increased mucus production in some patients, with typically tenacious mucus that is difficult to expectorate. Typical physical signs are inspiratory and to a greater extent expiratory, rhonchi throughout the chest and those may be hyper inflation.

Differential diagnosis:

Many other conditions can cause symptoms similar to those of asthma. In children, other upper airway diseases such as allergic rhinitis and sinusitis should be considered as well as other causes of airway obstruction including: foreign body aspiration, tracheal

stenosis or laryngotracheomalacia, vascular rings, enlarged lymph nodes or neck masses. In adults, chronic obstructive pulmonary disease, congestive heart failure, airway masses, as well as drug-induced coughing due to ACE inhibitors should be considered. In both populations vocal cord dysfunction may present similarly.

Aims of Asthma Therapy:

1. Minimal chronic symptoms, including nocturnal.
2. Minimal exacerbations.
3. No emergency visits.
4. Minimal use of a required β_2–agonist.
5. No limitations on activities, including exercises.
6. Peak expiratory flow circadian variation<20%.
7. Normal PEF.
8. Minimal adverse effects from medicine.

Management:

The most effective treatment for asthma is identifying triggers, such as cigarette smoke, pets, or aspirin, and eliminating exposure to them. If trigger avoidance is insufficient, the use of medication is recommended. Pharmaceutical drugs are selected based on, among other things, the severity of illness and the frequency of symptoms. Specific medications for asthma are broadly classified into fast-acting and long-acting categories.

Bronchodilators are recommended for short-term relief of symptoms. In those with occasional attacks, no other medication is needed. If mild persistent disease is present (more than two attacks a week), low-dose inhaled corticosteroids or alternatively, an oral leukotriene antagonist or a mast cell stabilizer is recommended. For those who have daily attacks, a higher dose of inhaled corticosteroids is used. In a moderate or severe exacerbation, oral corticosteroids are added to these treatments.

Medications:

Medications used to treat asthma are divided into two general classes: quick-relief medications used to treat acute symptoms; and long-term control medications used to prevent further exacerbation.

Short term control: Short-acting beta$_2$-adrenoceptor agonists (SABA), such as salbutamol are the first line treatment for asthma symptoms. They are recommended before exercise in those with exercise induced symptoms.

Anticholinergic medications, such as ipratropium bromide, provide additional benefit when used in combination with SABA in those with moderate or severe symptoms. Anticholinergic bronchodilators can also be used if a person cannot tolerate a SABA. Older, less selective adrenergic agonists, such as inhaled epinephrine, have similar efficacy to SABAs. They are however not recommended due to concerns regarding excessive cardiac stimulation.

Long–term control: Corticosteroids are generally considered the most effective treatment available for long-term control. Inhaled forms such as beclomethasone are usually used except in the case of severe persistent disease, in which oral corticosteroids may be needed. It is usually recommended that inhaled formulations be used once or twice daily, depending on the severity of symptoms.

Long-acting beta-adrenoceptor agonists (LABA) such as salmeterol and formoterol can improve asthma control, at least in adults, when given in combination with inhaled corticosteroids. In children this benefit is uncertain. When used without steroids they increase the risk of severe side-effects and even with corticosteroids they may slightly increase the risk.

Leukotriene antagonists (such as montelukast and zafirlukast) may be used in addition to inhaled corticosteroids, typically also in conjunction with LABA. Evidence is insufficient to support use in acute exacerbations. In children they appear to be of little benefit when added to inhale steroids. In those under five years of age, they were the preferred add-on therapy after inhaled corticosteroids by the British Thoracic Society in 2009.

Mast cell stabilizers (such as cromolyn sodium) are another non-preferred alternative to corticosteroids.

Chapter 4

Botanical Identity

Latin name: Piper longum Linn.

Systemic position:

Kingdom – Plantae.

Phylum – Angiospermae.

Sub phylum – Dicotyledons.

Division – Herbaceae.

Order – Piperales.

Family: Piperaceae

Genus – Piper.

Species – longum.

Morphology:

A slender aromatic climber with perennial woody roots. Stems creeping below and climbing on supports, roots clasping at nodes which help to get attached to the host trees.

Leaves alternate, simple, stipulate, numerous, petiolate or nearly sessile according to their position on the plant, ovate-cordate, apex acute to acuminate, margin entire.

Lower leaves 0.5-7.5 cms often rounded ovate, basal lobes equal, like betel leaves, long petioled. Petiole is 2.5-7.15 cms, stout. Upper leaves sessile, much narrower with often often unequal basal lobes. Stipules about 1.3 cms, membranous, lanceolate, obtuse, soon falling.

Inflorescence and Flowers: Flowers unisexual, dioecious, minute, sessile, bracteates, without perianth very densely packed in spikate inflorescence, the male and female on separate thickness.

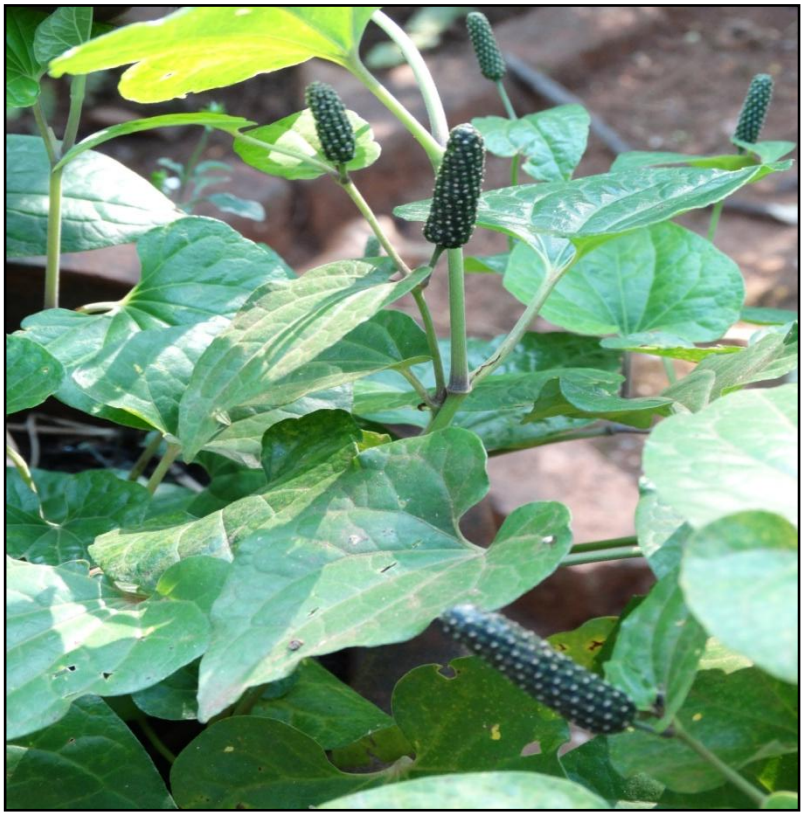
Fruits are berry spikes, cylindrical pedunculate, oblong, male larger and slender, red colour when ripened and becomes black colour on dried.

Male spikes 2.5-7.5 cms long, greenish yellow, glabrous, fleshy, cylindrical.

Female spikes 1.25-2 cms long, erect, yellow.

Fig.1: Piper longum Linn./*Pippali* plant

Fig.2: Piper longum Linn./*Pippali* fruit



Macroscopic and Microscopic examination:

Medicinal plant drugs are identified as per Macroscopic and Microscopic characteristic features. An examination to determine these characteristic features is the first and foremost step towards establishing the identity and the degree of purity of such plant drugs, and should be carried out before any further tests are undertaken.

Macroscopic/Organoleptic Evaluation of *Pippali* fruits

Colour: Black

Taste: Pungent

Odour: Sharp

Texture: Rough

Fracture: Hard

Size: 2.5 to 3 cm. long and 2.5 to 3.5 mm. thick

Shape: Erect blunt, ovoid-oblong.

Fig.3: Macroscopy of *Pippali* sample fruits

Fig.4: Macroscopy of *Pippali* sample fruits

Powder Microscopy of *Pippali* fruits:

Macroscopic:

Moss Green in colour; fine powder with aromatic odour, taste is pungent producing numbness on the tongue.

Microscopic:

A few mg of *Pippali* fruit powder was taken and warmed with chloral hydrate over water bath, washed, and mounted a small portion in glycerine; treated a few mg with iodine in potassium iodide solution and mounted in glycerine; treated a few mg of powder with solution of phloroglucinol, allowed to dry, added a few drops of hydrochloric acid and mounted in glycerine and observed the following characteristics in the different mounts.

Fragments of thin walled, polygonal parenchyma cells; plenty of endosperm cells occurs in various shapes and sizes; a few fragments of thick walled lignified different shape and sizes of stone cells with wide lumen, a few fragments of pointed unicellular and multicellular trichome, a few fragments of perisperm embedded with aleurone grains and oil globule, a few yellowish brown content cells, numerous simple, oval to rounded starch grains measuring upto 8µ in diameter.

Fig.5: Trichome in Powder microscopy of *Pippali* fruit.

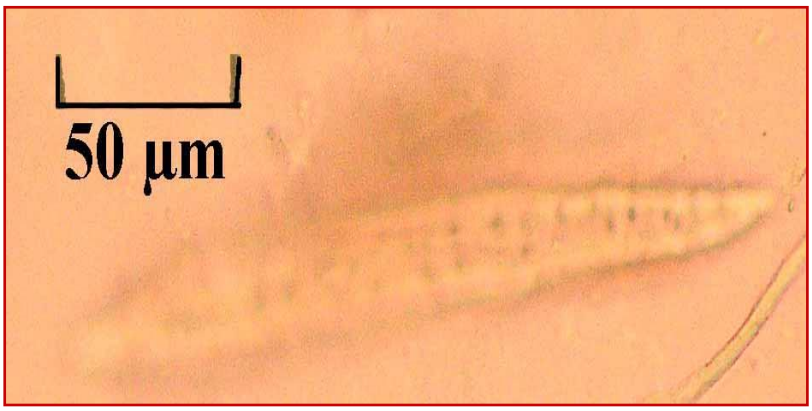
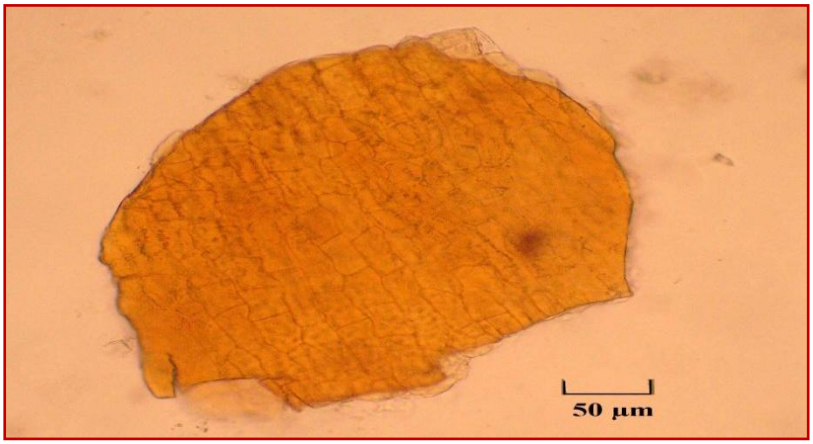


Fig.6: Perisperm in Powder microscopy of *Pippali* fruit.



Chapter 5

MATERIALS

Collection of plant material:

Piper longum Linn. fresh fruits were collected from three different places of its habitat:

Sample 1: Collected from Bihar.

Sample 2: Collected from Assam.

Sample 3: Collected from Kerala.

All the samples were identified and authenticated by Agharkar Research Institute, Pune.

Nava Pippali: Freshly collected fruits of *Pippali*.

Sample 1 fresh fruits were named as N1, sample 2 was named as N2 and sample 3 was named as N3. All the samples were dried in shade.

Purana Pippali: Freshly collected fruits of *Pippali* preserved for one year at room temperature.

Sample 1 old fruits were named as P1, sample 2 was named as P2 and sample 3 was named as P3.

Sample No.	***Nava Pippali***	***Purana Pippali***
Sample 1	N1	P1
Sample 2	N2	P2
Sample 3	N3	P3

All these samples were powdered, passed through sieve size of 44 number and packed in self sealed polythene bags after labeling. All the samples were kept at room temperature.

Laboratory equipments:

Electronic Balance

Hot air Oven

Muffle Furnace

Shaker Machine

Water-bath

Digital P^H meter

Clevenger apparatus

CAMAG HPTLC

PerkinElmer's LAMBDA 25 UV-Visible Spectrophotometer

Silica crucible

Histamine Chamber

Nebulizer

Incubator

Digital light Microscope

Invivo Study:

Institutional Animal Ethics Committee approved the experimental protocol of Piper longum Linn. fresh fruit (*Nava Pippali*) with reference no. IAEC/XXXI/SRU/232/2012 and of one year old fruit (*Purana Pippali*) with reference no. IAEC/XXXVI/SRU/328/2013. The pharmacological work was carried out as per norms of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Chapter 6

Methodology

Qualitative evaluation of ***Nava Pippali*** and ***Purana Pippali*** was performed by Physico-chemical and Phyto-chemical analysis.

Physico-chemical analysis was performed by the following procedures:

1. Determination of Moisture content.
2. Determination of Ash.
   (a) Total ash
   (b) Acid insoluble ash
3. Determination of extractives.
   (a) Water soluble extractive.
   (b) Methanol soluble extractive.
4. Determination of P^H.
5. Determination of Volatile oil.
6. Chromatographic analysis by High Performance Thin Layer Chromatography (HPTLC).
7. Quantitative estimation of Piperine.
8. Spectrophotometry by UV-Visible Spectrophotometer.

Phyto-chemical analysis was performed to detect the following Phytoconstituents:

1. Test for Alkaloids by Dragendroff's reagent.
2. Test for Tannins by Ferric Chloride test.
3. Test for Saponins.
4. Tests for Steroids and Terpenoids by Salkowski reaction.
5. Test for Carbohydrates by Molish's test.
6. Test for Glycosides by Benedict's test.
7. Test for Flavonoids by Shinoda test.
8. Test for Proteins by Biuret reagent.

Study type: Observational.

I. Physico-chemical analysis:

Determination of Moisture content:

The moisture content or loss on drying was determined by taking 2g of accurately weighed *Nava Pippali/Purana Pippali*, in a dried and previously weighed petri-dish. It was spread evenly and dried in an oven 110^0C till constant weight. The weight of *Pippali* sample after drying was noted and loss on drying was calculated. The percentage was calculated on the basis of air-dried *Nava Pippali/Purana Pippali* sample[6].

Determination of ash:

a. Determination of total ash:

The ash value of *Nava Pippali/Purana Pippali* was determined by incinerating about 2 g of accurately weighed *Nava Pippali/Purana Pippali* in a tarred silica crucible at a temperature not exceeding 450°C till constant weight, and then it is cooled and weighed. The percentage of ash was calculated with reference to air-dried *Nava Pippali/Purana Pippali* sample[6].

b. Determination of acid insoluble ash:

The ash obtained from the total ash content of *Nava Pippali/Purana Pippali*, was boiled for five minutes with 25 ml of dilute hydrochloric acid (2 N), the insoluble matter of *Pippali* sample was collected on an ashless filter paper, washed with hot water and ignited to constant weight. The percentage of acid insoluble ash was calculated with reference to air-dried *Nava Pippali/Purana Pippali* sample[6].

Determination of extractives:

a. Determination of water soluble extractive:

About 5g accurately weighed *Nava Pippali/Purana Pippali* sample was macerated with 100 ml of distilled water in a closed flask for twenty-four hours, shaking intermittently for first six hours and allowed to stand for eighteen hours. Then the *Pippali* sample was filtered, taking precaution against loss of solvent and 20 ml of the filtrate *Pippali* sample was evaporated to dryness in a previously weighed dried evaporating dish. Then the *Pippali* sample was dried over water-bath and then at 110^0C in hot air oven, to constant weight and weight was noted down. From the weight of the residue the percentage of water-soluble extractive of *Nava Pippali/Purana Pippali* was calculated[7].

b. Determination of methanol soluble extractive:

Methanol soluble extractive value of *Nava Pippali/Purana Pippali* was determined by same procedure as described in water soluble extractive value by taking methanol instead of water.

Determination of P^H Value:

A 5% w/v aqueous extract of *Nava Pippali/Purana Pippali* was prepared, filtered and the pH of the filtrate *Pippali* sample was noted with the help of digital P^H meter.

Determination of Volatile oil:

25 gm of coarsely powdered *Nava Pippali/Purana Pippali* sample was extracted with about 500 ml water in Clevenger's apparatus for about 3 hours. Volatile oil obtained was taken in diethyl ether and dried using anhydrous sodium sulphate[7].

HPTLC procedure:

Chromatographic analysis of *Nava Pippali/Purana Pippali* was performed on precoated aluminium TLC plates, silica gel F_{254} (E. Merck, Darmstadt, Germany) using a CAMAG (Muttenz, Switzerland) Linomat 5 sample applicator. Plates were prewashed with methanol, dried, and activated for 30 min at 110°C with the plates being placed between two sheets of glass to prevent deformation of the aluminium during heating. The samples of *Nava Pippali/Purana Pippali* were spotted in bands of 8 mm width, with a 100 µl syringe (Hamilton, Bonaduz, Switzerland). The rate of sample application was constant at 0.1 µl/s. Ascending development of the plate with a migration distance of about 70 mm, was performed using Toluene: Ethyl acetate (9.3:0.7 v/v) as the mobile phase in a CAMAG twin trough chamber, previously saturated with mobile phase for 20 min. The plates were dried in a current of air with the help of a hair dryer. Densitometric scanning was performed with a CAMAG TLC Scanner 4 at 254 and 366 nm, operated by winCATS software version 1.4.6. The source of radiation utilized was a deuterium and tungsten lamp emitting a continuous spectrum between 200 and 700 nm. The slit dimension was 6 × 0.20 mm and the scanning speed was 20 mm/s with 100 µm/step data resolution[8].

Further derivatization was done by spraying anisaldehyde-sulphuric acid reagent and the plate was observed in visible light.

Anisaldehyde-sulphuric acid reagent: 5 ml anisaldehyde mixed with 50 ml glacial acetic acid then 1 ml H_2SO_4 solution added.

Chromatographic analysis by High Performance Thin Layer Chromatography (HPTLC):

Sample preparation for HPTLC:

5% Methanolic extract of *Nava Pippali/Purana Pippali* was used for HPTLC study.

Standard preparation:

10 mg of standard piperine accurately weighed, was dissolved in 10 ml of methanol.

HPTLC procedure:

Chromatographic analysis of *Nava Pippali/Purana Pippali* was performed on precoated aluminium TLC plates, silica gel F_{254} (E. Merck, Darmstadt, Germany) using a CAMAG (Muttenz, Switzerland) Linomat 5 sample applicator. Plates were prewashed with methanol, dried, and activated for 30 min at 110°C with the plates being placed between two sheets of glass to prevent deformation of the aluminium during heating. The samples of *Nava Pippali/Purana Pippali* were spotted in bands of 8 mm width, with a 100 µl syringe (Hamilton, Bonaduz, Switzerland). The rate of sample application was constant at 0.1 µl/s. Ascending development of the plate with a migration distance of about 70 mm, was performed using Toluene: Ethyl acetate (4.5:5.5 v/v) as the mobile phase in a CAMAG twin trough chamber, previously saturated with mobile phase for 20 min. The plates were dried in a current of air with the help of a hair dryer. Densitometric scanning was performed with a CAMAG TLC Scanner 4 at 254 nm, operated by winCATS software version 1.4.6. The source of radiation utilized was a deuterium and tungsten lamp emitting a continuous spectrum between 200 and 700 nm. The slit dimension was 6 × 0.20 mm and the scanning speed was 20 mm/s with 100 µm/step data resolution[9].

Piperine quantification:

Spots possibly corresponding to piperine i.e. at about R_f 0.56 were scanned and their spectra were compared to that of piperine. Area of peak at 254 nm for spots having matching spectra was compared with area of piperine spot and piperine content of *Nava Pippali/Purana Pippali* was expressed as % w/w.

Spectrophotometry by UV- Visible Spectrophotometer:

Suitable dilutions of methanolic extract of *Nava Pippali/Purana Pippali* were scanned in190-1100 nm range using a PerkinElmer's LAMBDA 25 UV-Visible Spectrophotometer. The source of radiation utilized was a deuterium and tungsten lamp emitting a continuous spectrum. The scan was performed at speed of 240 nm/min with slit width of 1 nm with a unit data interval. Peaks were detected at a threshold of 0.01 and listed in a table[10].

II. Phyto-chemical analysis:

The methanolic and water extracts of *Nava Pippali/Purana Pippali* were subjected to preliminary phytochemicals testing.

Tests for Alkaloids

Draggendorff's reagent: The *Nava Pippali/Purana Pippali* sample was treated with few drops of dilute 2 N HCl and 0.5 ml Draggendorff's reagent. Brown precipitate was obtained[11].

Tests for Tannins

To aqueous extract of *Nava Pippali/Purana Pippali* samples, dilute solution of ferric chloride was added, blue colour was obtained, which changed to olive green by the addition of more amount of ferric chloride.

Test for Saponins

To an aqueous solution of *Nava Pippali/Purana Pippali* samples, solution of lead acetate was added; formation of white precipitate indicated the presence of saponins.

Tests for Steroids and Terpenoids

Salkowski reaction: To 2 ml of extract of *Nava Pippali/Purana Pippali* samples, 2 ml chloroform and 2 ml concentrate H_2SO_4 were added and shaken well. Chloroform layer appeared red and acid layer developed to greenish yellow fluorescence.

Tests for Carbohydrates

Alcohol and water extracts of *Nava Pippali/Purana Pippali* samples were tested for presence of carbohydrates.

Fehling's test: Each 1 ml Fehling's A & B solutions was mixed; boiled for 1 min and added equal volume of solution of *Nava Pippali/Purana Pippali* samples. Then Heated in boiling water bath for 5-10 min. First a yellow, then brick red precipitate was observed[11].

Test for Glycosides

Benedict's test: About 5 ml of Benedict's reagent was taken in a test tube and a few drops of solution of *Nava Pippali/Purana Pippali* sample was added and boiled. Formation of precipitate indicated presence of glycosides[11].

Test for Protein

100 mg of *Nava Pippali/Purana Pippali* sample was extracted with 10 ml of 80% of methanol and centrifuged at 2000 rpm for 10 min, then supernatant was discarded and residue was mixed with 5 ml distilled water. 5 ml of 10% tricholoroacetic acid was then added and heated on water bath for 30 min for digestion and centrifuged at 2000 rpm for 10 min. Residue was taken with distilled water and centrifuged at 2000 rpm for 10 min. Supernatant was discarded and again residue was taken in 2 ml distilled water. 5 ml of Biuret reagent was added to few drops of this suspension and centrifuged at 2000 rpm for 10 min. after 30 minutes. Supernatant was subjected to spectral scanning in 400-700 nm range using a

PerkinElmer Lamda25 UV-Visible spectrometer. Higher absorbance at 539 nm as compared to that of Biuret reagent confirmed the presence of proteins.

Biuret reagent: 0.425 gm Potassium sodium tartarate + 1.5 gm Copper sulphate pentahydrate + 0.25 gm Potassium iodide + about 50 ml water + 0.4 gm Sodium hydroxide → Volume made to 100 ml with water.

Test for Flavonoids

Shinoda test: To the methanol extract of *Nava Pippali/Purana Pippali* samples, magnesium turning and dil.HCl was added, there was no formation of red color which indicated the absence of flavonoids.

Invivo study of *Nava* and *Purana Pippali:*

Acute oral toxicity of *Nava Pippali/Purana Pippali*:

Acute oral toxicity study of *Nava Pippali/Purana Pippali* was performed according to the OECD test guideline 423-Acute Toxic Class Method. Young healthy adult Sprague Dawley female rats weighing between 160-180g body weights were divided into two groups of 3 animals/group[12]. Animals were housed individually in a well ventilated polypropylene cage. A 12-h light/12-h dark artificial photoperiod was maintained. Room temperature 22°C (±3°) and relative humidity 50–70% were maintained in the room. Animals had free access to pelleted feed (Nutrilab rodent, Tetragon Chemie Pvt Ltd., India) and Reverse osmosis (Rios, USA) purified water *ad libitum*. Animals kept in their cages for 5 days prior to dosing for acclimatization to the laboratory conditions. Prior to *Nava Pippali/Purana Pippali* sample administration animals were fasted for overnight and then 3- 4hrs post administration of test sample. This experiment was conducted with step wise procedure. The test sample was administered once orally via gastric intubation at a dose level of 2000 mg/kg body weight. Lethality and abnormal clinical signs were observed on the day of dosing of test sample and thereafter for 14 days. Body weights were recorded just prior to dosing and thereafter once in a week till completion of the experiment. Gross pathological changes were also observed at the end of experiment[13].

Antiasthamatic activity of *Nava Pippali/Purana Pippali*: Studied by two models.

- **Histamine induced bronchospasm.**
- **Invitro Mast Cell Degranulation by Compound 48/80.**

Research Design: Informal Experimental Research Design.

Study type: Before and after with Control design.

Histamine induced bronchospasm of *Nava Pippali/Purana Pippali*:

Animal husbandry

Young adult male Dunkin Hartley Guinea pigs (400-600 g b. wt) were used for the study. Animals were housed individually in polypropylene cages in a well-ventilated room (air cycles: 15/min; recycle ratio: 70:30) under an ambient temperature of 22±3°C and 40–65% relative humidity, with a 12-h light/dark artificial light cycle. They were provided with fresh lucerene and purified water *ad libitum*. Animals were acclimatized at least for 7 days to the laboratory conditions prior to initiation of the experiment[14].

Experiment design and treatment

Following acclimatization, animals were grouped into four (4 animals / group) and fasted overnight.

Group I	:	Positive control
Group II	:	Standard control (Salbutamol)
Group III	:	*Nava Pippali/Purana Pippali* (Low dose)
Group IV	:	*Nava Pippali/Purana Pippali* (High dose)

Prior to drug administration, each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol. The preconvulsion dyspnoea time (PCD) was noted for each animal. PCD is the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsion. As soon as preconvulsive dyspnoea commenced, animals were removed from the chamber and placed in fresh air to recover from dyspnoea for 24 h. The time for preconvulsive dyspnoea was recorded as basal value. After 24 h, animals of Group I received Normal saline and Group II received Salbutamol per oral. Animals of Group III and Group IV were treated with aqueous extract of *Nava Pippali/Purana Pippali* at low dose and high dose per oral. The experimental animals were again subjected to histamine aerosol later at an interval of 1, 4 and 24 h to determine PCD[15]. The protection offered by the treatment was calculated using the formula:

Percentage Protection = (1-T1/T2) x 100

Where,

T1 = Mean of PCD before administration of test drugs.

T2 = Mean of PCD after administration of test drugs at1h, 4h and 24 hrs.

Parameter for assessment: The time taken from aerosol exposure to the onset of dyspnoea.

End Point: Preconvulsion dyspnoea (PCD) [17].

Invitro Mast Cell Degranulation by Compound 48/80:

Experiment design and treatment

Test Drug: *Nava Pippali/Purana Pippali*

The overnight fasted male Wistar albino rats (180-220 g b. wt) were sacrificed with excess dose of anesthetic ether and the abdomen was cut open to expose the intestine. Pieces of mesentery with connecting lobes of fat and blood vessels were rapidly dissected out and cut into small pieces and placed in a beaker containing Ringer Locke solution for 30 min. Different dilutions of *Nava Pippali/Purana Pippali* samples (10, 30, 100, 300, 1000 µg/ml) were prepared in Ringer Locke solution. Then the tissues were incubated with Compound 48/80 (0.8 µg/ml) for a period of 30 min. The pieces of mesentery were then placed on a clean slide. Excess fatty layers and adhering tissues were carefully removed. The trimmed tissue was placed in 4% formaldehyde solution containing 0.1% O-Toludine blue for 20–30 min and the tissue was then de-stained with acetone and xylene (two changes each) for 5 min. Three pieces of mesentery were used for each concentration of the test sample[18].

The stained mesentery pieces were examined under a digital light microscope at 100 x magnification and 100 mast cells were counted, starting from the left hand side of the field and then proceeding clockwise. The number of intact, fragmented or disrupted mast cells was counted. A mast cell was considered disrupted if four or five granules were observed around the mast cells. The percentage of fragmented or disrupted and intact mast cells was calculated[19].

Statistical analysis:

The results were reported as mean ± SEM and analyzed for statistical significance using one-way analysis of variance (ANOVA) followed by Dunnett's '*t*'-test, for individual comparison of test samples with that of control. The analysis was carried out using Graph Pad Prism 4.0 Version.

Chapter 7

Observations and Results

Nava Pippali

Physico-chemical analysis:

Table 3: Loss on drying (% w/w)

Sr. No.	Samples	Values
1	N1	9.848
2	N2	9.978
3	N3	11.71

Table 4: Total ash (% w/w)

Sr. No.	Samples	Values
1	N1	4.94
2	N2	4.67
3	N3	4.74

Table 5: Acid insoluble ash (% w/w)

Sr. No.	Samples	Values
1	N1	1.15
2	N2	1.23
3	N3	0.95

Table 6: Water soluble extractive (% w/w)

Sr. No.	Samples	Values
1	N1	11.29
2	N2	16.07
3	N3	12.89

Table 7: Methanol soluble extractive (% w/w)

Sr. No.	Samples	Values
1	N1	7.63
2	N2	12.5
3	N3	9.44

Table 8: P^H (5 % aqueous extract) at RT

Sr. No.	Samples	Values
1	N1	6.72
2	N2	6.31
3	N3	5.90

Table 9: Volatile oil content (% w/w)

Sr. No.	Samples	Values
1	N1	1.0
2	N2	1.2
3	N3	0.80

Table 10: Quantitative estimation of Piperine content (% w/w)

Sr. No.	Samples	Values
1	N1	0.87
2	N2	Nil
3	N3	Nil

Table 11: Phyto-chemical analysis

Tests	Inference of Samples		
	N1	N2	N3
Alkaloids	Positive	Positive	Positive
Carbohydrates	Positive	Positive	Positive
Proteins	Positive	Positive	Positive
Flavonoids	Negative	Negative	Negative
Sterols and Terpenoids	Positive	Positive	Positive
Saponins	Positive	Positive	Positive
Glycosides	Positive	Positive	Positive
Tannins	Positive	Positive	Positive

Purana Pippali

Physico-chemical analysis:

Table 12: Loss on drying (% w/w)

Sr. No.	Samples	Values
1	P1	11.14
2	P2	12.46
3	P3	12.41

Table 13: Total ash (% w/w)

Sr. No.	Samples	Values
1	P1	3.48
2	P2	4.21
3	P3	3.76

Table 14: Acid insoluble ash (% w/w)

Sr. No.	Samples	Values
1	P1	0.61
2	P2	0.44
3	P3	0.65

Table 15: Water soluble extractive (% w/w)

Sr. No.	Samples	Values
1	P1	9.90
2	P2	14.30
3	P3	9.64

Table 16: Methanol soluble extractive (% w/w)

Sr. No.	Samples	Values
1	P1	7.76
2	P2	11.5
3	P3	7.88

Table 17: P^H (5 % aqueous extract) at RT

Sr. No.	Samples	Values
1	P1	5.93
2	P2	5.32
3	P3	5.49

Table 18: Volatile oil content (% w/w)

Sr. No.	Samples	Values
1	P1	1.0
2	P2	1.2
3	P3	1.2

Table 19: Quantitative estimation of Piperine content (% w/w)

Sr. No.	Samples	Values
1	P1	0.95
2	P2	Nil
3	P3	Nil

Table 20: Phyto-chemical analysis

Tests	Inference of Samples		
	P1	P2	P3
Alkaloids	Positive	Positive	Positive
Carbohydrates	Positive	Positive	Positive
Proteins	Positive	Positive	Positive
Flavonoids	Negative	Negative	Negative
Sterols and Terpenoids	Positive	Positive	Positive
Saponins	Positive	Positive	Positive
Glycosides	Positive	Positive	Positive
Tannins	Positive	Positive	Positive

Invivo Study

Nava Pippali

Acute Oral toxicity:

Table 21: Body weight of experimental animals fed by Control vehicle & *Nava Pippali*

Group	Treatment	Body weight (g)					
		-1 day	-1 hr before *Nava Pippali* administration Day 1	6h on Day 1	Day 2	Day 7	Day 14
I	Control (vehicle)	162.33±0.88	157.33±2.96	165.67±2.19	172.00±0.58	187.67±2.73	203.33±2.03
II	*Nava Pippali* (2000mg/kg b.wt.)	162.33±1.86	154.00±1.86	166.67±2.19	176.67±2.67	187.00±2.31	198.67±2.96

Values are expressed in mean ± SEM; n=3

Fig.8: Body weight of the experimental animals fed by Control vehicle & *Nava Pippali*

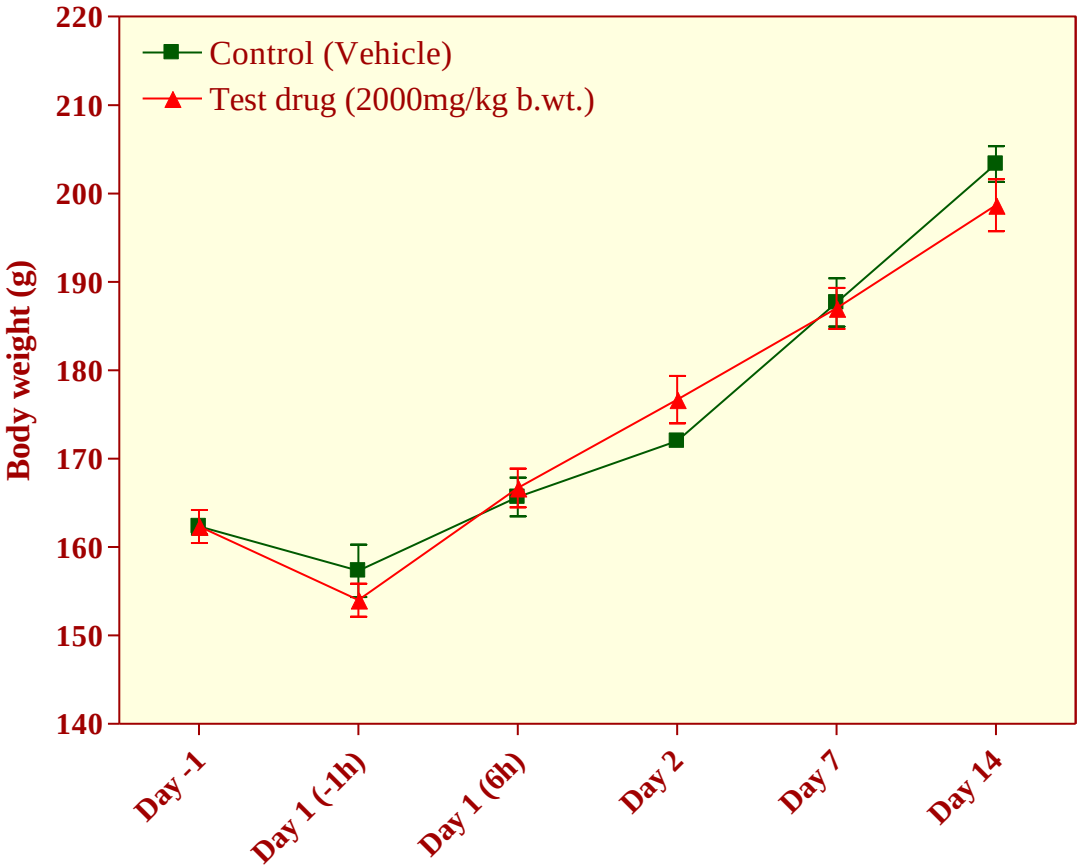


Table 22: Clinical Observation of individual animals fed by Control vehicle & Nava Pippali

Animal ID		**H**				**B**				**CL**			
Treatment (dose/route)		**Control (Vehicle)**											
Time (min / hr)		**30-35**	**60-65**	**120-125**	**240-245**	**30-35**	**60-65**	**120-125**	**240-245**	**30-35**	**60-65**	**120-125**	**240-245**
Lethality		X	X	X	X	X	X	X	X	X	X	X	X
Convulsion		X	X	X	X	X	X	X	X	X	X	X	X
Tremor		X	X	X	X	X	X	X	X	X	X	X	X
Straub tail		X	X	X	X	X	X	X	X	X	X	X	X
Sedation	*#1*	X	X	X	X	X	X	X	X	X	X	X	X
	#2	X	X	X	X	X	X	X	X	X	X	X	X
	#3	X	X	X	X	X	X	X	X	X	X	X	X
Excitation	*#1*	X	X	X	X	X	X	X	X	X	X	X	X
	#2	X	X	X	X	X	X	X	X	X	X	X	X
	#3	X	X	X	X	X	X	X	X	X	X	X	X
Abnormal gait (rolling)		X	X	X	X	X	X	X	X	X	X	X	X
Abnormal gait (tiptoe)		X	X	X	X	X	X	X	X	X	X	X	X
Jumps		X	X	X	X	X	X	X	X	X	X	X	X
Motor coordination		X	X	X	X	X	X	X	X	X	X	X	X
Loss of balance		X	X	X	X	X	X	X	X	X	X	X	X
Fore paw treading		X	X	X	X	X	X	X	X	X	X	X	X
Writhes		X	X	X	X	X	X	X	X	X	X	X	X
Piloerection		X	X	X	X	X	X	X	X	X	X	X	X

Animal ID	**H**				**B**				**CL**			
Treatment (dose/route)	**Control (Vehicle)**											
Time (min / hr)	**30-35**	**60-65**	**120-125**	**240-245**	**30-35**	**60-65**	**120-125**	**240-245**	**30-35**	**60-65**	**120-125**	**240-245**
Stereotypies (chewing)	X	X	X	X	X	X	X	X	X	X	X	X
Stereotypies (Head movements)	X	X	X	X	X	X	X	X	X	X	X	X
Head twitches	X	X	X	X	X	X	X	X	X	X	X	X
Scratching	X	X	X	X	X	X	X	X	X	X	X	X
Respiration	X	X	X	X	X	X	X	X	X	X	X	X
Aggressiveness	X	X	X	X	X	X	X	X	X	X	X	X
Fear	X	X	X	X	X	X	X	X	X	X	X	X
Reactivity to touch	X	X	X	X	X	X	X	X	X	X	X	X

Muscle tone	X	X	X	X	X	X	X	X	X	X	X	X
Loss of righting Reflex	X	X	X	X	X	X	X	X	X	X	X	X
Ptosis	X	X	X	X	X	X	X	X	X	X	X	X
Exophthalmos	X	X	X	X	X	X	X	X	X	X	X	X
Loss of grasping	X	X	X	X	X	X	X	X	X	X	X	X
Akinesia	X	X	X	X	X	X	X	X	X	X	X	X
Catalepsy	X	X	X	X	X	X	X	X	X	X	X	X
Loss of traction	X	X	X	X	X	X	X	X	X	X	X	X
Loss of corneal reflex	X	X	X	X	X	X	X	X	X	X	X	X
Analgesia	X	X	X	X	X	X	X	X	X	X	X	X
Defecation	X	X	X	X	X	X	X	X	X	X	X	X
Salivation	X	X	X	X	X	X	X	X	X	X	X	X
Lacrimation	X	X	X	X	X	X	X	X	X	X	X	X
Others:	X	X	X	X	X	X	X	X	X	X	X	X

Animal ID		**H**				**B**				**CL**			
Treatment (dose/route)		***Nava Pippali* - 2000mg/kg b.wt.**											
Time (min / hr)		**30-35**	**60-65**	**120-125**	**240-245**	**30-35**	**60-65**	**120-125**	**240-245**	**30-35**	**60-65**	**120-125**	**240-245**
Lethality		X	X	X	X	X	X	X	X	X	X	X	X
Convulsion		X	X	X	X	X	X	X	X	X	X	X	X
Tremor		X	X	X	X	X	X	X	X	X	X	X	X
Straub tail		X	X	X	X	X	X	X	X	X	X	X	X
Sedation	*#1*	X	X	X	X	X	X	X	X	X	X	X	X
	#2	X	X	X	X	X	X	X	X	X	X	X	X
	#3	X	X	X	X	X	X	X	X	X	X	X	X
Excitation	*#1*	X	X	X	X	X	X	X	X	X	X	X	X
	#2	X	X	X	X	X	X	X	X	X	X	X	X
	#3	X	X	X	X	X	X	X	X	X	X	X	X
Abnormal gait (rolling)		X	X	X	X	X	X	X	X	X	X	X	X
Abnormal gait (tiptoe)		X	X	X	X	X	X	X	X	X	X	X	X
Jumps		X	X	X	X	X	X	X	X	X	X	X	X
Motor coordination		X	X	X	X	X	X	X	X	X	X	X	X
Loss of balance		X	X	X	X	X	X	X	X	X	X	X	X
Fore paw treading		X	X	X	X	X	X	X	X	X	X	X	X
Writhes		X	X	X	X	X	X	X	X	X	X	X	X
Piloerection		X	X	X	X	X	X	X	X	X	X	X	X

Animal ID	**H**				**B**				**CL**			
Treatment (dose/route)	***Nava Pippali* - 2000mg/kg b.wt.**											
Time (min / hr)	**30-35**	**60-65**	**120-125**	**240-245**	**30-35**	**60-65**	**120-125**	**240-245**	**30-35**	**60-65**	**120-125**	**240-245**
Stereotypies (chewing)	X	X	X	X	X	X	X	X	X	X	X	X
Stereotypies (Head movements)	X	X	X	X	X	X	X	X	X	X	X	X
Head twitches	X	X	X	X	X	X	X	X	X	X	X	X
Scratching	X	X	X	X	X	X	X	X	X	X	X	X
Respiration	X	X	X	X	X	X	X	X	X	X	X	X
Aggressiveness	X	X	X	X	X	X	X	X	X	X	X	X
Fear	X	X	X	X	X	X	X	X	X	X	X	X
Reactivity to touch	X	X	X	X	X	X	X	X	X	X	X	X
Muscle tone	X	X	X	X	X	X	X	X	X	X	X	X
Loss of righting Reflex	X	X	X	X	X	X	X	X	X	X	X	X
Ptosis	X	X	X	X	X	X	X	X	X	X	X	X
Exophthalmos	X	X	X	X	X	X	X	X	X	X	X	X
Loss of grasping	X	X	X	X	X	X	X	X	X	X	X	X
Akinesia	X	X	X	X	X	X	X	X	X	X	X	X
Catalepsy	X	X	X	X	X	X	X	X	X	X	X	X
Loss of traction	X	X	X	X	X	X	X	X	X	X	X	X
Loss of corneal reflex	X	X	X	X	X	X	X	X	X	X	X	X
Analgesia	X	X	X	X	X	X	X	X	X	X	X	X
Defecation	X	X	X	X	X	X	X	X	X	X	X	X
Salivation	X	X	X	X	X	X	X	X	X	X	X	X
Lacrimation	X	X	X	X	X	X	X	X	X	X	X	X
Others:	X	X	X	X	X	X	X	X	X	X	X	X

X: Absent

Result:

- There were no treatment related deaths, abnormal clinical signs, remarkable body weight changes or gross pathological changes were observed in all the experimental animals fed by Control vehicle & *Nava Pippali*.
- From the above results, LD_{50} of *Nava Pippali* was found to be greater than 2000mg/kg b.wt. Hence, the test drug falls in the “category-5” or “unclassified” in accordance to the Globally Harmonised System of classification of chemicals.

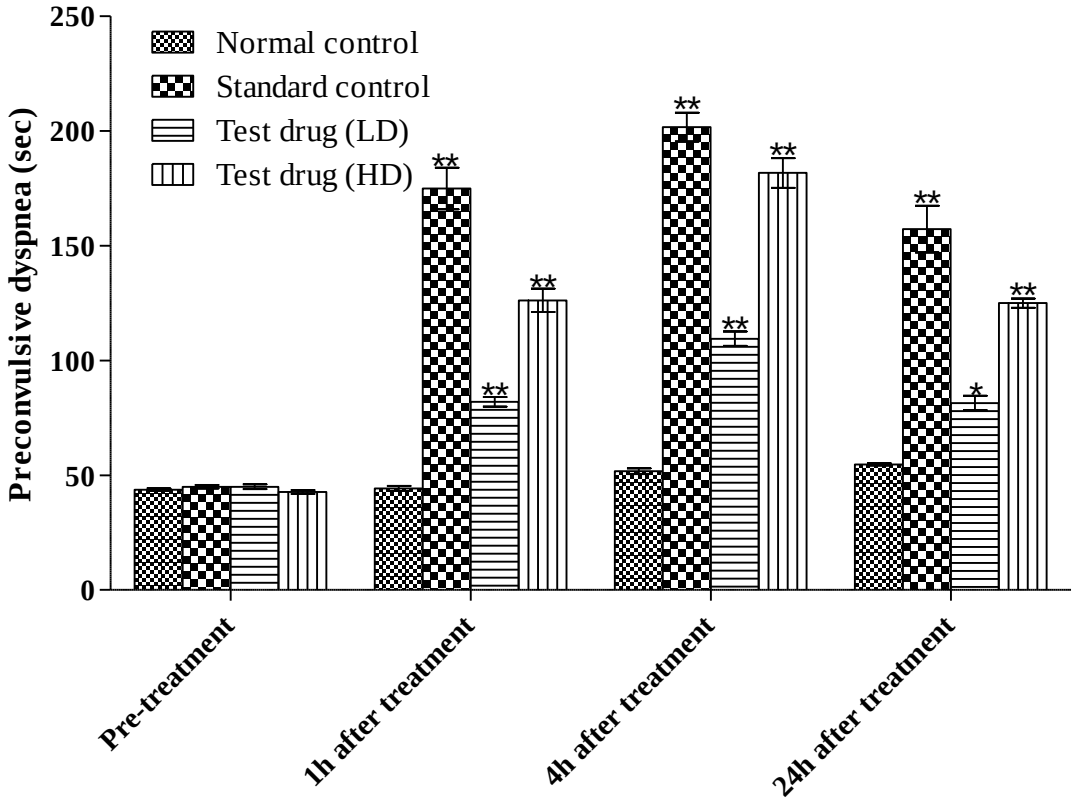
Antiasthamatic activity

Histamine induced bronchospasm:

Table 23: Effect of *Nava Pippali* on histamine induced bronchospasm

Group	Treatment	Preconvulsive dyspnea (sec)			
		Before treatment	After treatment		
			1h	4h	24h
I	Normal control	43.75±0.48	44.25±1.11	51.75±1.25	54.75±0.48
II	Standard control (Salbutamol)	45.00±0.82	175.00±9.08**	201.75±6.29**	157.25±10.13**
III	*Nava Pippali* (200 mg/kg)	45. 00±1.08	82.00±2.04**	109.50±3.12**	81.50±3.12*
IV	*Nava Pippali* (400 mg/kg)	42.75±0.85	126.25±5.11**	181.75±6.46**	125.00±2.04**

Fig.9: Effect of *Nava Pippali* on histamine induced bronchospasm



The results are expressed in mean ± SEM (n =4); Statistical analysis was done using prism 4.0 Version, Unpaired t test, and p values positive control vs test drug ** (0.01); Using graph pad prism.

Table 24: Percentage Protection of *Nava Pippali* against histamine induced bronchospasm

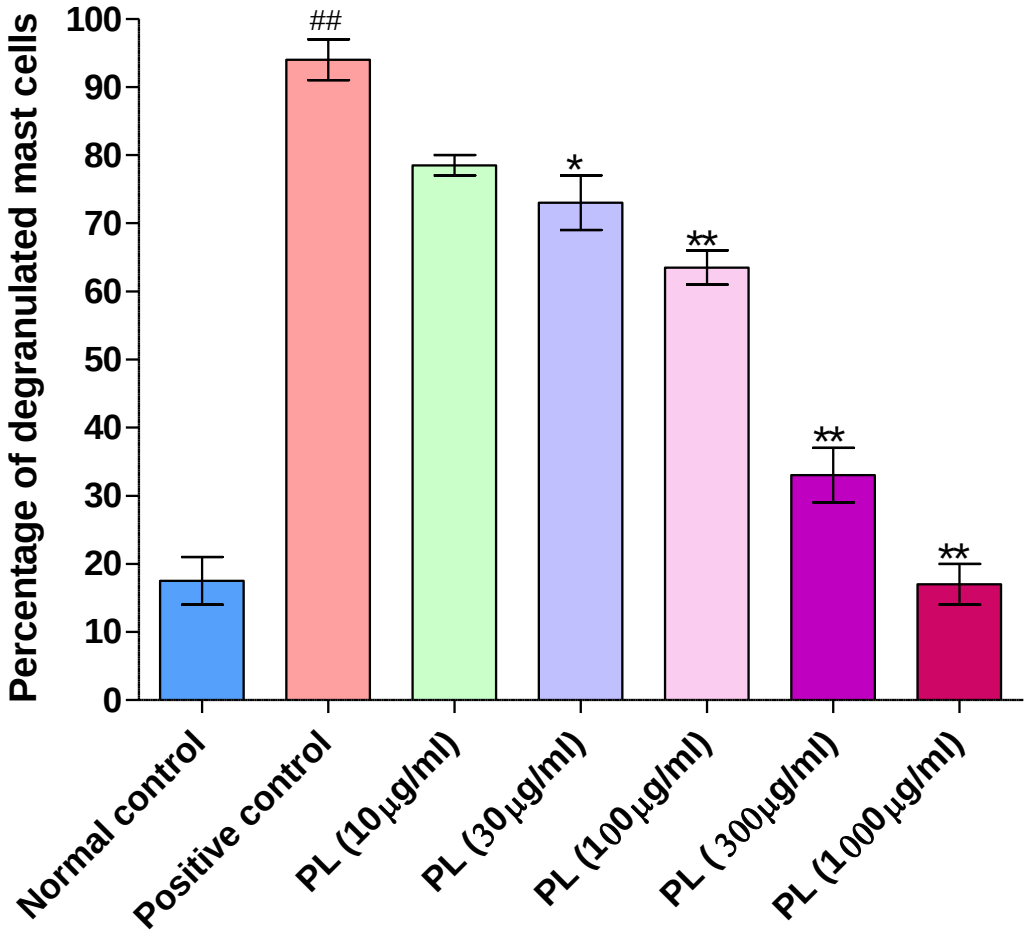
Group	Treatment	% Protection		
		1h	4h	24h
II	Standard control (Salbutamol)	74.12±1.10	77.67±0.30	71.03±1.92
III	*Nava Pippali* (200 mg/kg)	45.01±2.06	58.85±1.09	44.44±3.24
IV	*Nava Pippali* (400 mg/kg)	66.03±1.00	76.37±1.11	65.74±1.25

Table 25: Effect of *Nava Pippali* on Compound 48/80 induced Mast Cell Degranulation

S No	Concentration (µg/ml)	% Degranulation
1	Normal control	17.50±3.50
2	Positive control (0)	94.00±3.00
3	PL-10	78.50±1.50
4	PL-30	73.00±4.00
5	PL-100	63.50±2.50
6	PL-300	37.00±4.00
7	PL-1000	17.00±3.00

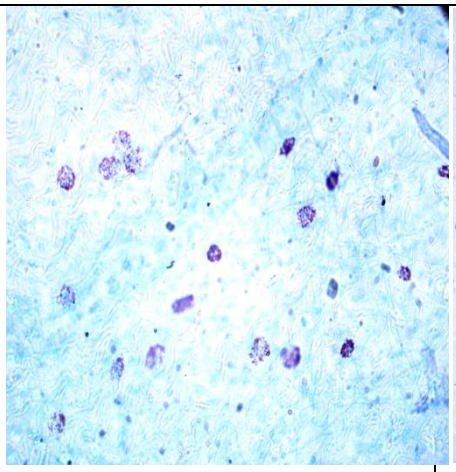
n=3/group; i.e. experiments were performed in triplicates.
PL - Piper longum Linn. fruit (*Nava Pippali*)

Fig.10: Effect of *Nava Pippali* on Compound 48/80 induced Mast Cell Degranulation

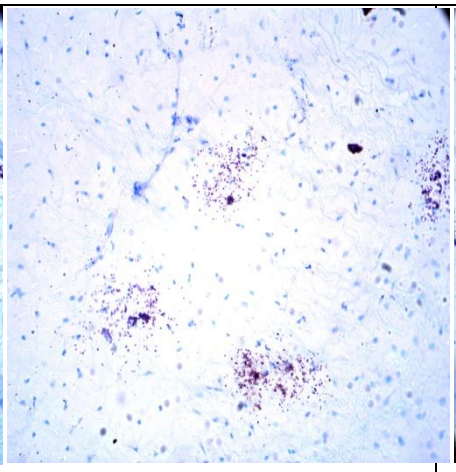


Each bar represents mean ± SEM (n=3). **p < 0.001as compared to positive control group.

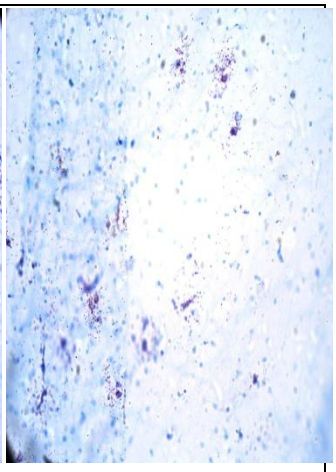
Fig.11: Mast Cell stabilization potential of *Nava Pippali*



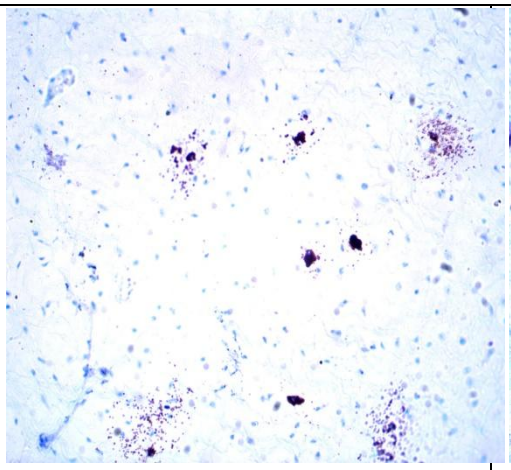
Normal Cells



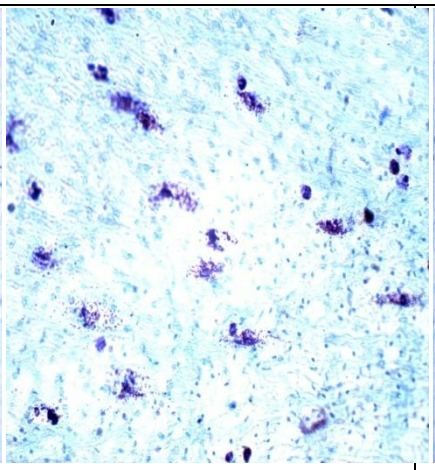
C 48/80 + Vehicle



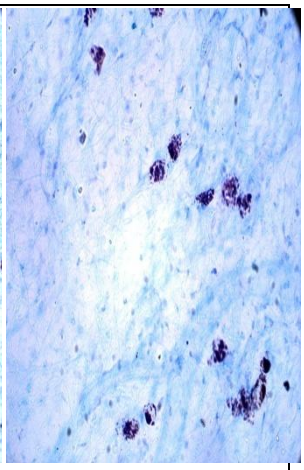
C 48/80 + PL 10µg/ml



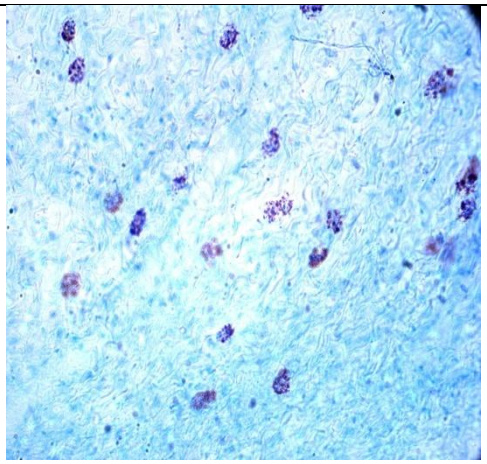
C 48/80 + PL 30 µg/ml



C 48/80 + PL 100 µg/ml



C 48/80 + PL 300 µg/ml



C 48/80 + PL 1000 µg/ml

Purana Pippali

Acute Oral toxicity:

Table 26: Individual animal body weight fed by *Purana Pippali*

Step/Dose	Animal number	Body weight (g)		
		Day 0	Day 7	Day 14
I *Purana Pippali* 2000 mg/kg b.wt.	1	165.2	175.0	188.6
	2	157.0	174.2	183.0
	3	158.6	180.4	192.2
II *Purana Pippali* 2000 mg/kg b.wt.	4	168.2	172.6	190.2
	5	174.6	186.2	197.8
	6	168.0	177.2	189.4

Table 27: Clinical observation of individual animals fed by *Purana Pippali*

Step/ Dose (mg/kg b.w.)	Animal number	Observation Days																	
		30 min	1 hr	2 hr	4 hr	1	2	3	4	5	6	7	8	9	10	11	12	13	14
I *Purana Pippali* 2000	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
II *Purana Pippali* 2000	4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	5	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	6	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N-Normal

Table 28: Individual animals gross pathological observation after fed with Purana Pippali

Step/Dose	Animal Number	Organs	Observations
I *Purana Pippali* 2000mg/kg b.wt.	1	Skin, eyes, brain, lungs, heart, liver, kidney, adrenals, spleen and sex glands	No abnormality detected
	2		No abnormality detected
	3		No abnormality detected
II *Purana Pippali* 2000mg/kg b.wt.	4		No abnormality detected
	5		No abnormality detected
	6		No abnormality detected

Result:

- There were no treatment related mortality, abnormal clinical signs or remarkable body weight changes were observed in experimental animals fed by *Purana Pippali*.
- No gross pathological observation was recorded in all the experimental animals.
- From the above tested conditions, LD_{50} of *Purana Pippali* was greater than 2000mg/kg body weight classified under GHS hazard category 5.

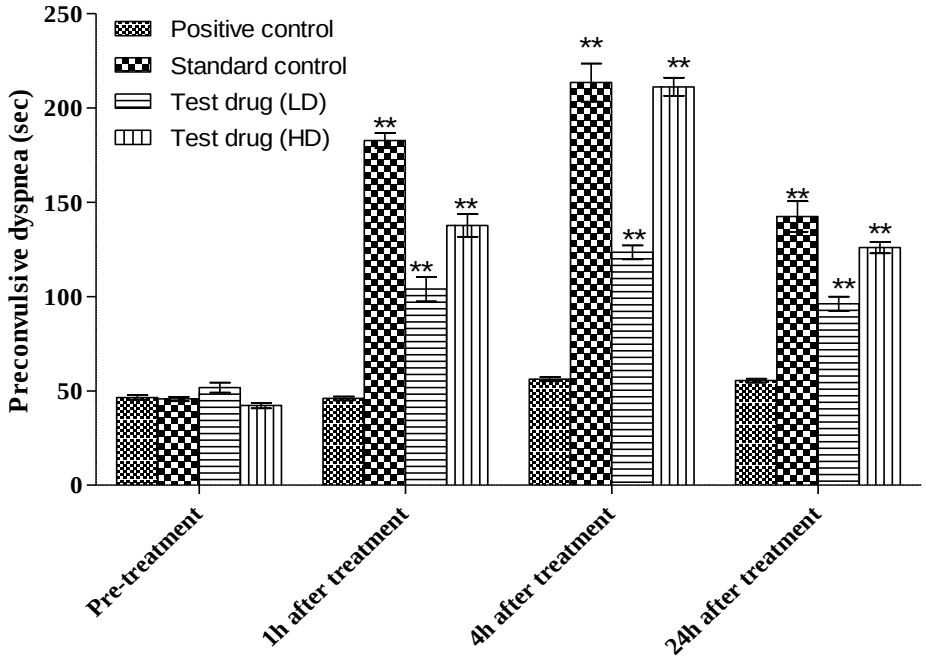
Antiasthamatic activity

Histamine induced bronchospasm:

Table 29: Effect of *Purana Pippali* on histamine induced bronchospasm

Group	Treatment	Preconvulsive dyspnea (sec)			
		Before treatment	After treatment		
			1h	4h	24h
I	Positive control	46.50±1.19	46.00±1.08	56.25±1.11	55.50±1.04
II	Standard control (Salbutamol)	45.75±1.18	182.75±4.01	213.50±10.14	142.50±8.11
III	*Purana Pippali* (200 mg/kg)	51.75±2.66	104.00±6.49	123.50±3.71	96.25±3.73
IV	*Purana Pippali* (400 mg/kg)	42.25±1.44	137.75±5.98	211.75±64.59	126.00±3.03

Fig.12: Effect of *Purana Pippali* on histamine induced bronchospasm



The results are expressed in mean ± SEM (n =4); Statistical analysis was done using prism 4.0 Version, Unpaired t test, and p values positive control vs test drug ** (0.01); Using graph pad prism.

Table 30: Percentage Protection of *Purana Pippali* against histamine induced bronchospasm

Group	Treatment	% Protection		
		1h	4h	24h
II	Standard control (Salbutamol)	74.97±0.30	78.41±2.44	67.71±2.42
III	*Purana Pippali* (200 mg/kg)	49.29±5.11	57.87±3.03	46.24±1.87
IV	*Purana Pippali* (400 mg/kg)	69.16±1.60	80.20±1.02	66.42±1.33

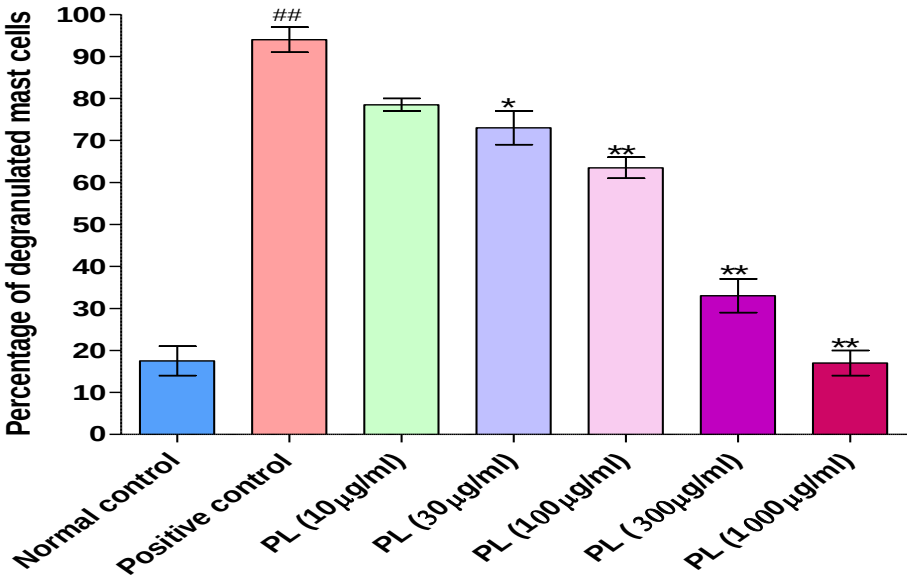
In vitro Mast Cell Degranulation by Compound 48/80:

Table 31:Effect of *Purana Pippali* on Compound 48/80 induced Mast Cell Degranulation

S No	Concentration (µg/ml)	% Degranulation
1	Normal control	12.33±1.20
2	Positive control (0)	96.67±0.88
3	PL-10	76.33±0.88
4	PL-30	70.33±2.73
5	PL-100	49.67±3.76
6	PL-300	33.67±1.86
7	PL-1000	14.67±0.33

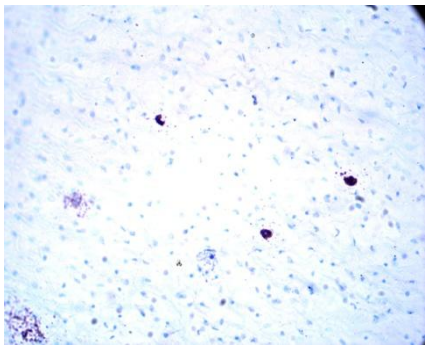
PL-Piper longum Linn.fruit (*Purana Pippali*)

Fig.13: Effect of *Purana Pippali* on Compound 48/80 induced Mast Cell Degranulation

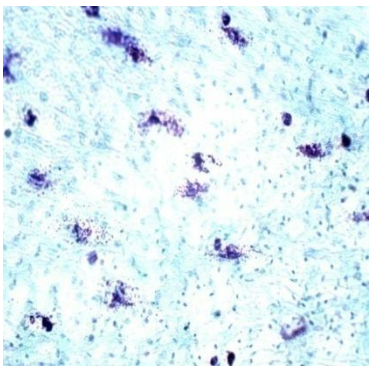


Each bar represents mean ± SEM (n=3). **p < 0.001as compared to positive control group.

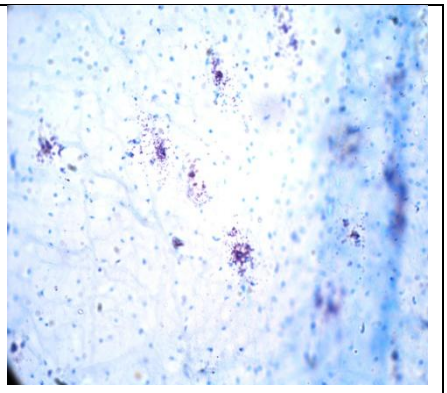
Fig.14: Mast Cell stabilization potential of *Purana Pippali*



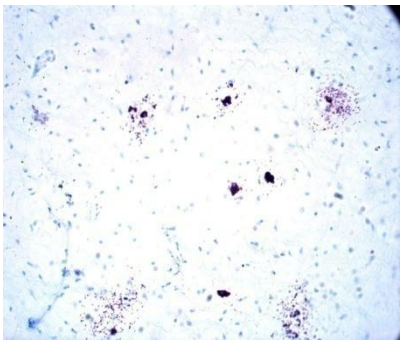
Normal Cells



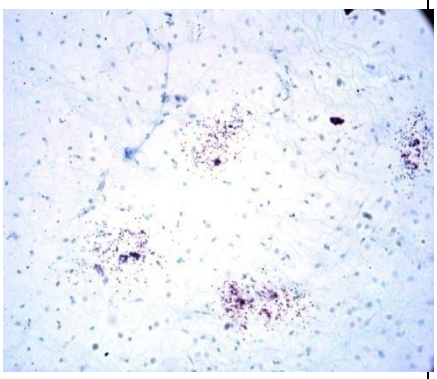
C 48/80 + Vehicle



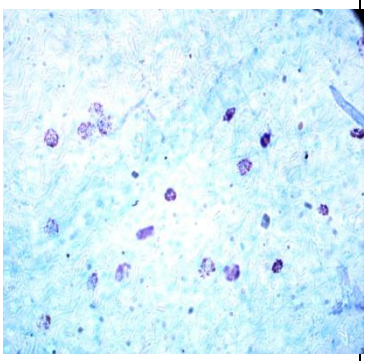
C 48/80 + PL 10µg/ml



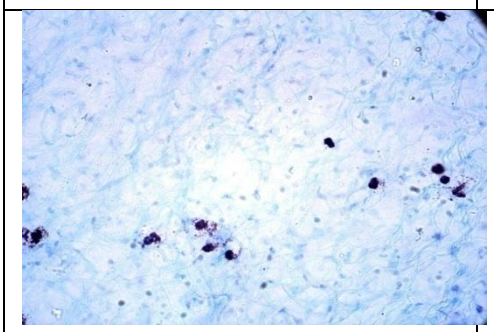
C 48/80 + PL 30 µg/ml



C 48/80 + PL 100 µg/ml



C 48/80 + PL 300 µg/ml



C 48/80 + PL 1000 µg/ml

Chapter 8

Discussion and Conclusion

Physico-chemical parameters:

Determination of loss on drying:

Loss on drying of P1, P2 and P3 *Pippali* samples (11.14, 12.46 and 12.41 % w/w) were more when compared with loss on drying of N1, N2 and N3 *Pippali* samples (9.848, 9.978 and 11.71% w/w).

Determination of total ash:

Total ash of P1, P2 and P3 *Pippali* samples (3.48, 4.21 and 3.76 % w/w) were less when compared with total ash of N1, N2 and N3 *Pippali* samples (4.94, 4.67 and 4.74 % w/w).

Determination of acid insoluble ash:

Acid insoluble ash of P1, P2 and P3 *Pippali* samples (0.61, 0.44 and 0.65 % w/w) were less when compared with acid insoluble ash of N1, N2 and N3 *Pippali* samples (1.15, 1.23 and 0.95 % w/w).

Determination of water soluble extractive:

Water soluble extractive values of P1, P2 and P3 *Pippali* samples (9.90, 14.30 and 9.64 % w/w) were less when compared with water soluble extractive values of N1, N2 and N3 *Pippali* samples (11.29, 16.07 and 12.89 % w/w).

Determination of methanol soluble extractive:

Methanol soluble extractive value of P1 *Pippali* sample (7.76%) was more when compared with methanol soluble extractive of N1 sample (7.63%) while methanol soluble extractive values of P2 and P3 *Pippali* samples were less (11.5 and 7.88 % w/w) when compared with that of N2 and N3 *Pippali* samples (12.5 and 9.44 % w/w).

Determination of P^H:

P^H of P1, P2 and P3 *Pippali* samples (5.93, 5.32 and 5.49) were slightly acidic when compared with P^H of N1, N2 and N3 *Pippali* samples (6.72, 6.31 and 5.90).

Determinaion of Volatile oil:

There was no change in the Volatile oil content of P1 (1.0%) and N1 (1.0%) *Pippali* samples, P2 (1.2%) and N2 (1.2%) *Pippali* samples. Volatile oil content of P3 *Pippali* sample (1.2%) was more when compared with that of N3 sample (0.80%).

HPTLC Profile:

Number of Peaks/Polyvalent Phytoconstituents was more in *Purana Pippali* when compared with *Nava Pippali* that is P1 *Pippali* sample has 9 peaks with corresponding ascending order of R_f values start from 0.11 to 0.99 in which highest concentration of the phytoconstituents was found to be 37.51% and its corresponding R_f value was found to be 0.68 respectively.

P2 sample has 7 peaks with R_f values from 0.20 to 0.99 in which highest concentration of the phytoconstituents was found to be 56.12% with R_f value 0.85.

P3 sample has 7 peaks with R_f values from 0.18 to 1.00 in which highest concentration of the phytoconstituents was found to be 50.72% with R_f value 0.82.

N1 *Pippali* sample has 7 peaks with corresponding ascending order of R_f values start from 0.25 to 0.94 in which highest concentration of the phytoconstituents was found to be 26.01% and its corresponding R_f value was found to be 0.61 respectively.

N2 sample has 5 peaks with R_f values from 0.26 to 0.88 in which highest concentration of the phytoconstituents was found to be 61.01% with R_f value 0.88.

N3 sample has 4 peaks with R_f values from 0.36 to 0.87 in which highest concentration of the phytoconstituents was found to be 67.29% with R_f value 0.87.

HPTLC Profile of Volatile oil:

Number of Peaks was more in *Purana Pippali* when compared with *Nava Pippali* that is P1 *Pippali* sample has 11 peaks with corresponding ascending order of R_f values start from 0.27 to 0.97 in which highest concentration of the phytoconstituents was found to be 17.03% and its corresponding R_f value was found to be 0.27 respectively.

P2 sample has 7 peaks with R_f values from 0.32 to 0.94 in which highest concentration of the phytoconstituents was found to be 41.11% with R_f value 0.32.

P3 sample has 7 peaks with R_f values from 0.12 to 0.97 in which highest concentration of the phytoconstituents was found to be 44.55% with R_f value 0.46.

N1 *Pippali* sample has 9 peaks with corresponding ascending order of R_f values start from 0.12 to 0.98 in which highest concentration of the phytoconstituents was found to be 19.86% and its corresponding R_f value was found to be 0.12 respectively.

N2 sample has 5 peaks with R_f values from 0.14 to 0.98 in which highest concentration of the phytoconstituents was found to be 35.85% with R_f value 0.14.

N3 sample has 6 peaks with R_f values from 0.18 to 0.99 in which highest concentration of the phytoconstituents was found to be 30.86% with R_f value 0.33.

Quantitative estimation of Piperine:

The R_f value of piperine was found to be 0.57. Sample N1 exhibited a spot at R_f 0.58, the spectra of which matches with that of piperine. The piperine content in the sample, on comparison of area of this spot with that of standard, was found to be 0.87% w/w.

Sample N2 though exhibited a spot at R_f 0.53, its spectra did not match with that of piperine.

The R_f value of piperine was found to be 0.63. The sample N3 revealed a spot at R_f 0.61, but its spectra was different from that of piperine.

The R_f value of piperine was found to be 0.52. Sample P1 exhibited a spot at R_f 0.55, the spectra of which matches with that of Piperine. The piperine content in the sample, on comparison of area of this spot with that of standard, was found to be 0.95% w/w.

Sample P2 though exhibited a spot at R_f 0.50, its spectra did not match with that of piperine.

The sample P3 revealed a spot at R_f 0.47, but its spectra was different from that of piperine.

Hence Piperine content of P1 *Pippali* sample (0.95% w/w) was more than N1 *Pippali* sample (0.87% w/w).

UV- Visible Spectroscopic analysis:

2.5% Methanol extracts of N1 *Pippali* sample showed 50 peaks at wavelength range of 664.6 nm to 197.6 nm, N2 sample has 55 peaks at wavelength from 664.4 to 194.0 nm and 1% methanol extract of N3 sample has 51 peaks at wavelength from 664.5 to 191.9 nm.

1% Methanol extracts of P1 *Pippali* sample showed 52 peaks with wavelength range of 663.5 nm to 211.1 nm, P2 sample has 40 peaks with wavelength from 664.1 to 208.0 nm and P3 sample has 24 peaks with wavelength from 664.5 to 218.0 nm.

Qualitative tests:

Test for Alkaloids was Positive for P1, P2 and P3 *Pippali* samples and also positive for N1, N2 and N3 *Pippali* samples.

Test for Carbohydrates was Positive for P1, P2 and P3 *Pippali* samples and also positive for N1, N2 and N3 *Pippali* samples.

Test for Proteins was Positive for all the *Nava* and *Purana Pippali* samples.

Test for Flavonoids was Negative for all the *Nava* and *Purana Pippali* samples.

Test for Sterols and Terpenoids were Positive for all the *Nava* and *Purana Pippali* samples.

Test for Saponins was Positive for all the *Nava* and *Purana Pippali* samples.

Test for Glycosides was Positive for all the *Nava* and *Purana Pippali* samples.

Test for Tannins was Positive for all the *Nava* and *Purana Pippali* samples.

Hence by Physico-chemical analysis it is noted that Quantitative changes are observed in *Purana Pippali*, which indicates the identity, quality and purity of drug.

By Phyto-chemical analysis it is noted that Qualitative tests are found to be positive in *Purana Pippali* also, which indicates the stability of active compounds present in it which is required for the therapeutic efficacy of drug.

Invivo Study

Acute oral toxicity: Acute oral toxicity study of *Nava* and *Purana Pippali* was performed according to the OECD test guideline 423-Acute Toxic Class Method.

There were no treatment related deaths, abnormal clinical signs, remarkable body weight changes or gross pathological changes were observed in all the experimental animals.

From the results, LD_{50} of *Nava Pippali* and *Purana Pippali* were found to be greater than 2000mg/kg b.wt. Hence, the test drugs falls in the "category-5" or "unclassified" in accordance to the Globally Harmonized System of classification of chemicals.

Antiasthmatic activity

To evaluate the efficacy of antiasthmatic property of a drug, evaluation of bronchodilator and mast cell stabilizing activity is used as pharmacodynamic parameter.

Histamine induced bronchospasm:

In Normal control, Preconvulsion dyspnea (PCD) was observed in 43sec before treatment and after treatment PCD was observed in 44sec, 51sec and 54sec at an interval of 1h, 4h and 24h of histamine aerosol exposure respectively.

In Standard control, Preconvulsion dyspnea (PCD) was observed in 45 sec before treatment and after treatment PCD was observed in 175sec, 201sec and 157sec at an interval of 1h, 4h and 24h of histamine aerosol exposure respectively.

In *Nava Pippali* at low dose of 200 mg/kg, Preconvulsion dyspnea (PCD) was observed in 45 sec before treatment and after treatment PCD was observed in 82sec, 109sec and 81sec at an interval of 1h, 4h and 24h of histamine aerosol exposure respectively.

In *Nava Pippali* at high dose of 400 mg/kg, Preconvulsion dyspnea (PCD) was observed in 42 sec before treatment and after treatment PCD was observed in 126sec, 181sec and 125sec at an interval of 1h, 4h and 24h of histamine aerosol exposure respectively.

In *Purana Pippali* at low dose of 200 mg/kg, Preconvulsion dyspnea (PCD) was observed in 51 sec before treatment and after treatment PCD was observed in 104sec, 123sec and 96sec at an interval of 1h, 4h and 24h of histamine aerosol exposure respectively.

In *Purana Pippali* at high dose of 400 mg/kg, Preconvulsion dyspnea (PCD) was observed in 42 sec before treatment and after treatment PCD was observed in 137sec, 211sec and 126sec at an interval of 1h, 4h and 24h of histamine aerosol exposure respectively.

Hence Preconvulsion dyspnea (PCD) time was found to be significantly increased in *Purana Pippali* when compared to *Nava Pippali* at an interval of 4h, which confirmed the bronchodilator activity.
In Standard control, the protection offered was 74%, 77% and 71% at an interval of 1h, 4h and 24h of histamine aerosol exposure respectively.

In *Nava Pippali* at low dose of 200 mg/kg, the protection offered was 45%, 58% and 44% at an interval of 1h, 4h and 24h of histamine aerosol exposure respectively.

In *Nava Pippali* at high dose of 400 mg/kg, the protection offered was 66%, 76% and 65% at an interval of 1h, 4h and 24h of histamine aerosol exposure respectively.

In *Purana Pippali* at low dose of 200 mg/kg, the protection offered was 49%, 57% and 46% at an interval of 1h, 4h and 24h of histamine aerosol exposure respectively.

In *Purana Pippali* at high dose of 400 mg/kg, the protection offered was 69%, 80% and 66% at an interval of 1h, 4h and 24h of histamine aerosol exposure respectively.

In this study, *Purana Pippali* showed maximum protection when compared to *Nava Pippali* at an interval of 4h, thereby protecting the animals to a significant extent from the development of asphyxia produced by histamine aerosol confirming that it has bronchodilator activity.

In vitro Mast Cell Degranulation by Compound 48/80:

In Normal control, mast cell degranulation was found to be 17%.

In Positive control, Compound 48/80, a known mast cell degranulating agent produced significant rat mesenteric mast cell degranulation which was 94%.

Nava Pippali produced significant reduction in the Compound 48/80 induced mast cell degranulation in a dose-dependent manner. The % inhibition of mast cell degranulation of *Nava Pippali* at concentration of 10µg/ml, was found to be 78%, at concentration of 30µg/ml, mast cell degranulation was found to be 73%, at 100µg/ml, mast cell degranulation was

found to be 63%, at 300µg/ml, mast cell degranulation was found to be 37% and at concentration of 1000µg/ml, mast cell degranulation was found to be 17% respectively.

In Positive control, Compound 48/80, a known mast cell degranulating agent produced significant rat mesenteric mast cell degranulation which was 97%.

Purana Pippali produced significant reduction in the Compound 48/80 induced mast cell degranulation in a dose-dependent manner. The % inhibition of mast cell degranulation of *Purana Pippali* at concentration of 10µg/ml, was found to be 76%, at concentration of 30µg/ml, mast cell degranulation was found to be 70%, at 100µg/ml, mast cell degranulation was found to be 49%, at 300µg/ml, mast cell degranulation was found to be 33% and at concentration of 1000µg/ml, mast cell degranulation was found to be 14% respectively.

In this study, *Purana Pippali* significantly protects compound 48/80 induced degranulation of mast cell when compared to *Nava Pippali*, which is responsible for decreasing airway inflammation by preventing release of various inflammatory mediators.

Conclusion

By Physico-chemical and Phyto-chemical analysis it is noted that Quantitative and Qualitative changes are observed in *Purana Pippali,* which indicates the identity, quality and purity of drug required for the therapeutic effect.

Purana Pippali is found to be more effective than *Nava Pippali* in antiasthmatic activity (Histamine induced bronchospasm) as it showed increased Preconvulsion dyspnea time and maximum protection against histamine aerosol exposure.

Purana Pippali is found to be more effective than *Nava Pippali* in antiasthmatic activity (Invitro mast cell degranulation by compound 48/80) as it showed significant protection against compound 48/80 induced mast cell degranulation by stabilizing it.

Though in practice the drug *Pippali* is used, the period of collection and time of usage means to determine between *Nava* and *Purana*. Hence *Purana Pippali* is to be used for better efficacy.

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पिप्पली क्षिप्तभेषज्यूतातिविद्धभेषजी।

तां देवाः समकल्पचन्नियं जीवितवा अलम्।।

पिप्पल्यः समवदन्तायतीर्जननादधि।

चं जीवमश्नवामहै न स रिष्यति पुरुषः।।

असुरास्त्वा न्यखनन् देवास्त्वोदवपन् पुनः।

वातीकृतस्य भेषजीमथो क्षिप्तस्य भेषजीम्।। (शौनक)

पिप्पली एतत्संज्ञा कणाद्यपरपर्याया ओषधिः।

हस्तिपिप्पल्यादि जातिभेदभिन्नः सर्वाः पिप्पल्यः।। (सायणः)

पिप्पली पर्यायः

१. उपकुल्या – उपकोलती। कुलसंस्त्याने बन्धुषु।उपगता कुल्याम् इति वा।। (भा.दी)

२. ऊषणा – ऊषति ऊष रुजायाम्।। (भा.दी)

३. कणा – कणाः सन्त्यस्याः इति ।। (नि.आ)

४. कृष्णा – कृष्णो वर्णोऽस्त्यस्याः इति ।। (भा.दी)

५. चपला – चपलः उष्णो रसो गुणोऽस्याः,तद्योगात् चपला इति।। (नि.आ)

६. तीक्ष्णतण्डुल – तीक्ष्णास्तण्डुला यस्याः।। (श.क.द्रु)

७. पिप्पली - पिपर्त्ति इति, पृ पालनपूरणयोः। पलति आतुरम् इति पिप्पली ।। (नि.आ)

८. मागधी – मगधेषु भवा इति। (भा.दी)

९. वैदेही – विदेहेषु भवा इति। प्रायो विदेहायां वीरभूमौ।। (भा.दी)

हरितक्यादि वर्ग- भा.नि

पिप्पली मागधी कृष्णा वैदेही चपला कणा ।

उपकुल्योषणा शौण्डी कोला स्यात्तीक्ष्णतण्डुला ॥

पिप्पली दीपना वृष्या स्वादुपाका रसायनी ।

अनुष्णा कटुका स्निग्धा वातश्लेष्महरी लघुः ॥

पिप्पली रेचनी हन्ति श्वासकासोदरज्वरान् ।

कुष्ठप्रमेहगुल्मार्शः प्लीहशूलाममारुतान् ॥

आद्रा कफप्रदा स्निग्धा शीतला मधुरा गुरुः ।

पित्तप्रशमनी सा तु शुष्का पित्तप्रकोपिणी ॥

पिप्पली मधुसंयुक्ता मेदः कफविनाशिनी ।

श्वासकासज्वरहरी वृष्या मेध्याऽग्निवर्द्धिनी ॥

जीर्णज्वरेऽग्निमान्द्ये च शस्यते गुडपिप्पली ।

कासाजीर्णारुचिश्वासहृत्पाण्डुकृमिरोगनुत् ।

द्विगुणः पिप्पलीचूर्णाद् गुडोऽत्र भिषजां मतः।। (भा.नि)

त्रिकटु लक्षणगुणाः

विश्वोपकुल्या मरिचं त्रयं त्रिकटु कथ्यते।।

कटुत्रिकं तु त्रिकटु त्र्यूषणं व्योष उच्यते ॥

त्र्यूषणं दीपनं हन्ति श्वासकासत्वगामयान् ।

गुल्ममेहकफस्थौल्यमेदःश्लीपदपीनसान् ॥ (भा.नि)

चतुरूषणस्य लक्षणगुणाः

त्र्यूषणं सकणामूलं कथितं चतुरूषणम्।

व्योषस्येव गुणाः प्रोक्ता अधिकाश्चतुरूषणे।। (भा.नि)

पञ्चकोलस्य लक्षणगुणाः

पिप्पली पिप्पलीमूलं चव्यचित्रकनागरैः ।

पञ्चभिः कोलमात्रं यत्पञ्चकोलं तदुच्यते ॥

पञ्चकोलं रसे पाके कटुकं रुचिकृन्मतम् ।

तीक्ष्णोष्णं पाचनं श्रेष्ठं दीपनं कफवातनुत् ।

गुल्मप्लीहोदरानाहशूलघ्नं पित्तकोपनम् ॥ (भा.नि)

षडूषणस्य लक्षणगुणाः

पञ्चकोलं समरिचं षडूषणमुदाहृतम् ।

पञ्चकोलगुणं तत्तु रूक्षमुष्णं विषापहम्।। (भा.नि)

ओषधि वर्ग - कै.नि

पिप्पली मागधी शौण्डी वैदेही चपला कणा।

कृष्णोपकुल्या मागधी श्यामाह्वा तीक्ष्णतण्डुला।।

पिप्पल्यार्द्रा हिमा गुर्वी स्वद्वी स्निग्धा कफप्रदा।

शुष्का लघुः स्वादुपाका स्निग्धानुष्णा रसे कटुः।।

कफवातहरा रुच्या सरा वृष्या रसायनी।

दीपनि पाचनी हृद्या पित्तला श्वासकासनुत्।।

निहन्ति कफगुल्मार्शो मेह प्लीह ज्वरोदरान्।

तीक्ष्णोष्णभावात् श्लेष्मघ्नी तस्माच्चैवाग्निदीपनी।।

शैत्यप्रसादमाधुर्यात् पित्तं हन्ति च पिप्पली।

औष्ण्यात् सरत्वात् पाकाच्च वातस्याप्यनुलोमनी।। (कै.नि)

पिप्पली शुण्ठि मरिचैर्व्योषं त्रिकटुकं कटु।

कटुत्रयं त्र्यूषणञ्च सग्रन्थि चतुरूषणम्।।

त्र्यूषणं दीपनं हन्यात् स्थौल्यगुल्मत्वगामयान्।

मेदोमेह कफ श्वासकासश्लीपद पीनसान्।। (कै.नि)

मागधी मागधीमूलं चविका विश्वचित्रकैः।

पञ्चकोलं समरिचं षडूषणमुदाहृतम्।।

पञ्चकोलं रसे पाके कटुकं दीपनं जयेत्।

गुल्मप्लीहोदरानाह कफ शूलानिलारुचीः।। (कै.नि)

पिप्पल्यादि वर्ग - रा.नि

पिप्पली कृकरा शौण्डी चपला मागधी कणा।

कटुबीजा च कोरङ्गी वैदेही तिक्ततण्डुला।।

श्यामा दन्तफला कृष्णा कोला च मगधोद्भवा।

उष्णा चौपकुल्या च स्मृत्याह्वा तीक्ष्णतण्डुला।।

पिप्पली ज्वरहा वृष्या स्निग्धोष्णा कटुतिक्तका।

दीप्पनी मारुतश्वासकास श्लेष्मक्षयापहा।। (रा.नि)

शतपुष्पादि वर्ग - सो.नि

पिप्पल्यां मागधी कृष्णा चपला तीक्ष्णतण्डुला।

उपकुल्या कणा श्यामा कोला शौण्डि तथोषणा।।

नीलिनी पिप्पली माद्री त्रयं कृष्णेति गीयते।

पथ्या धात्री गुडूचीनाममृता परिकीर्तिता।।

पिप्पली मरिचं शुंडी त्रयमेतद् विमिश्रितम्।

त्रिकटु त्र्यूषणं व्योषं कटुत्रिकमुदाहृतम्।।

पिप्पली पिप्पलीमूलं चव्यचित्रकनागरैः।

एकत्र मिश्रितेरेभिः पञ्चकोलमुच्यते।।

पिप्पल्यार्द्रा गुरुस्निग्धा श्लेष्मला स्वादुशीतला।

सा शुष्का कटुका वृष्या स्निग्धोष्णा दीपनी सरा।।

स्वादुपाकानिल श्लेष्म श्वासकासापहा लघुः।

पित्ताविरोधिनी रुच्या यकृत्प्लीहोदरापहृत्।। (सो.नि)

विविधौषधि वर्ग - मा.द्र

पिप्पली मारुत श्लेष्मकासश्वासान् विनाशयेत्।

श्लेष्मला स्वादुशीतार्द्रा गुर्वी स्निग्धा च पिप्पली।।

सा शुष्का विपरीता तु स्निग्धा वृष्या रसे कटुः।

स्वादुपाकाऽनिल श्लेष्म श्वासकासापहा सरा।। (मा.द्र)

श्रेष्ठ वर्ग :

गव्यं क्षीरं घृतं श्रेष्ठं सैन्धवं लवणेषु च।

धात्रीदाडिममम्लेषु पिप्पली नागरं कटौ।। (मा.द्र)

पिप्पल्यादि वर्ग - प्रि.नि

पिप्पली कटुवल्ली स्यात् कणा तु कणवत्फला।

फलं मूलञ्च कटुकं शूलहृत् दीपनं परम्।।

कफवातविकारेषु कासादिषु च शस्यते।

वर्धमानप्रयोगेन पिप्पली तु रसायने।।

वैदेही तु विदेहराज्यविजने जाता तथोत्पादिता।

चानीतान्यसमुद्रवसतिभ्यो मागधे मागधी।

कट्वी चापि रसे विपाकमधुरा वीर्ये कवोष्णा मता।

शस्ता वातकफोत्थरोगनिवहे संदीपनी पिप्पली।। (प्रि.नि)

शतपुष्पादि वर्ग - ध.नि

पिप्पली मागधी कृष्णा चपला तीक्ष्ण तण्डुला।

उपकुल्या कणा श्यामा कोला शौण्डी तथोषणा।

पिप्पली कटुका स्वादुर्हिमा स्निग्धा त्रिदोषजित्।

तृड्ज्वरोदरजन्त्वामनाशिनी च रसायनी।। (ध.नि)

चतुष्पादवर्ग - हृ.नि

कृष्णा कणोपकुल्या वैदेही पिप्पली चपला।

मगधा मागध्यन्या गजकृष्णा श्रेयसी गजाह्वा च।। (हृ.नि)

पिप्पली कल्प :

अथ प्रिये! प्रवक्ष्यामि पिप्पलीनां रसायनम्।

पिप्पली शोधयेत्पूर्वं किंशुकक्षारवारिणा।।

सप्तधा च ततः कुर्यात्तासां च घृतभर्जनम्।

ततस्सेवेत शुद्धाङ्गः पञ्चाष्टौ दश सप्रधा।।

वर्षमेकं तु मध्वाज्यै रोगास्तस्य न सन्ति च।

वलीपलितनिर्मुक्तो जीवेच्च शरदश्शतम्।।

प्रातः प्राग्भोजनात्पश्चाद्भक्षयेत्त्रिपिप्पलीः।

वर्षा व्याधिं जरां हन्ति शतायुष्यमवाप्नुयात्।।

एकद्वित्रिक्रमेणैव वर्धयत् दशवासरम्।

ह्रासयेत् पिप्पलीस्तद्वह्रासवृद्धी पुनः पुनः।।

गोक्षीरेण युतं यावत्पिप्पलीनां सहस्रकम्।

तावत्सेवेत शुद्धाङ्गे जरारोगविवर्जितः।।

अजाक्षीरेण संपेष्य पिबेद्युक्ता बलाधिका।

क्षीरशृता मध्यफला पूर्ववत् ह्रासवृद्धयः।।

यावत्सहस्रद्वितयं तावत्सेव्यं रसायनम्।

कासश्वासक्षयाः पाण्डुप्लीहशोणितमारुताः।।

मेहार्शोग्रहणीशोफ हिध्मवमि गलग्रहाः।

रसायनेन पिप्पल्या नश्यन्ति विषमज्वराः।।

जीवेद्वर्षशतं पूर्णं वलीपलितवर्जितः।। (आनन्दकन्दः)

कथिता कृष्णोपकुल्या शोण्डी कृष्णा च मागधी चपला।

कालोषणा कणवला विष्यन्दफलेति पिप्पली बाष्पी।। (अभिधानमञ्जरी)

कृष्णोपकुल्या वैदेही मागधी चपला कणा।

ऊषणा पिप्पली शौण्डी कोला.....।। (अमरकोश)

श्लेष्मला मधुरा चार्द्रा गुर्वी स्निग्धा च पिप्पली।

सा शुष्का कफवातघ्नी कटूष्णा वृष्यसम्मता।। (च.सू. २७)

तेषां गुर्वी स्वादुशीता पिप्पल्यार्द्रा कफावहा।

शुष्का कफानिलघ्नी सा वृष्या पित्ताविरोधिनी।। (सु.सू.४६)

श्लेष्मला स्वादुशीतार्द्रा गुर्वी स्निग्धा च पिप्पली।

सा शुष्का विपरीतातः स्निग्धा वृष्या रसे कटुः।।

स्वादुपाकानिलश्लेष्मश्वासकासापहासरा।

न तामत्युपयुञ्जीत रसायनविधिं विना।। (अ.हृ.सू.१३)

पिप्पली रसायनः

पञ्चाष्टौ सप्तदश वा पिप्पलीर्मधुसर्पिषा।

रसायन गुणान्वेषी समामेकां प्रयोजयेत्।।

तिस्त्रास्तिस्त्रस्तु पूर्वाह्णे भुक्त्वाऽग्रे भोजनस्य च।

पिप्पल्यः किंशुकक्षार भाविता घृतभर्जिताः।

प्रयोज्या मधुसंमिश्रा रसायनगुणैर्षिणा।।

जेतुं कासं क्षयं शोषं श्वासं हिक्कां गलामयान्।

अर्शांसि ग्रहणीदोषं पाण्डुतां विषमज्वरम्।।

वैस्वर्यं पीनसं शोफे गुल्मं वातबलासकम्।। (च.चि. १-३/३२-३५)

कृष्णाधात्रीफलरजः स्वरसेन सुभावितम्।

शर्करामधुसर्पिर्भिर्लीढ्वा वा योऽनुपयः पिबेत्।।

स नरोऽशीति वर्षोऽमि युवेव परिहृष्यति।। (अ.हृ. उ. ४०-२७)

पिप्पली वर्धमान रसायनः

क्रमवृद्ध्या दशाहानि दशपैप्पलिकं दिनम्।

वर्धयेत् पयसा सार्धं तथैवापनयेत् पुनः।।

जीर्णे शीर्णे च भुञ्जीत षष्टिकं क्षीरसर्पिषा।

पिप्पलीनां सहस्त्रस्य प्रयोगोऽयं रसायनम्।

पिष्टास्ता वलिभिः सेव्याः श्रिता मध्यबलैर्नरैः।

चूर्णाकृता ह्रस्वबलैर्योज्या दोषामयान् प्रति।।

दशपैप्पलिकः श्रेष्ठो मध्यमः षट् प्रकीर्तितः।।

प्रयोगो यस्त्रिपर्यन्तः स कनीयान् स चावलैः।।

बृंहणं स्वर्यमायुष्यं प्लीहोदर विनाशनम्।

वयसः स्थापनं मेध्यं पिप्पलीनां रसायनम्।। (च.चि. १-३/३६-४०)

अथ खलु त्रीणि द्रव्याणिनात्युपयुञ्जीताधिकमन्येभ्यो द्रव्येभ्यः

तद्यथा – पिप्पली, क्षारः, लवणमिति।। (च.वि. १/१५)

पिप्पल्यो हि कटुकाः सत्यो मधुरविपाका गुर्व्यो

नात्यर्थं स्निग्धोष्णाः प्रक्लेदिन्यो भेषजाभिमताश्च

ताः सद्यः, शुभाशुभकारिण्यो भवन्ति;

आपातभद्राः, प्रयोगसमसाद् गुण्यात्;

दोषसञ्चयानुबन्धाः- सततमुपयुज्यमाना हि

गुरु प्रक्लेदित्वान् श्लेष्माणंउत्क्लेशयन्ति, औष्ण्यात् पित्तं,

न च वात प्रशमनायोपकल्पन्तेऽल्पस्नेहोष्णभावात्;

योगवाहिन्यस्तु खलु भवन्ति, तस्मात् पिप्पलीर्नात्युपयुञ्जीता।। (च.वि. १/१६)

नवान्येव हि योज्यानि द्रव्याण्यखिलकर्मसु।

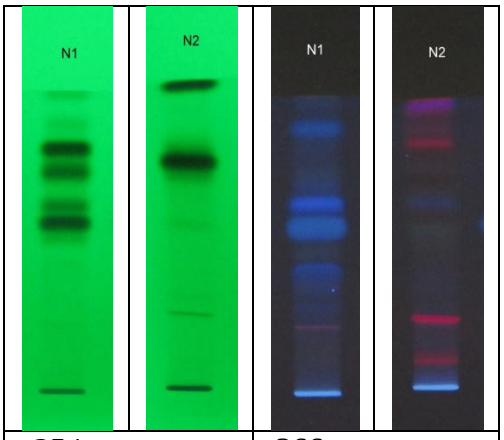
विना विडङ्गकृष्णाभ्यां गुडधान्याज्यमाक्षिकैः।। (शा.पूर्वखण्ड. १/४४)

विडङ्गादि द्रव्य विना तेन विडङ्ग प्रभृतिकं पुरातनं गुणकरमिति तात्पर्यार्थः।।

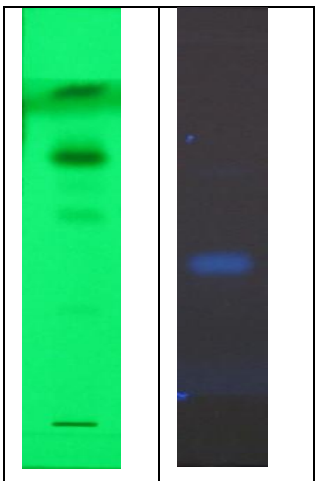
पुरातनत्वं संवत्सरादुपरि भवति। (आढमल्ल टीका)

Nava Pippali

Chromatogram of Methanol extracts of *Pippali*

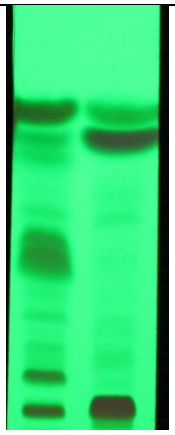
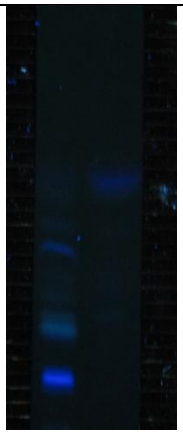


254 nm	366 nm
Chromatogram of N1 & N2	



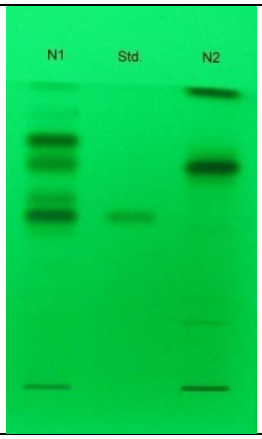
254 nm	366 nm
Chromatogram of N3	

Chromatogram of Volatile oil of *Pippali*

		
254 nm	366 nm	After spraying with Anisaldehyde-H_2SO_4 reagent
Chromatogram of Volatile oil (N1 & N2)		

		
254 nm	366 nm	After spraying with Anisaldehyde sulphuric acid reagent
Chromatogram of Volatile oil (N3)		

Chromatogram of Methanol extracts of *Pippali* (254 nm) along with Piperine standard

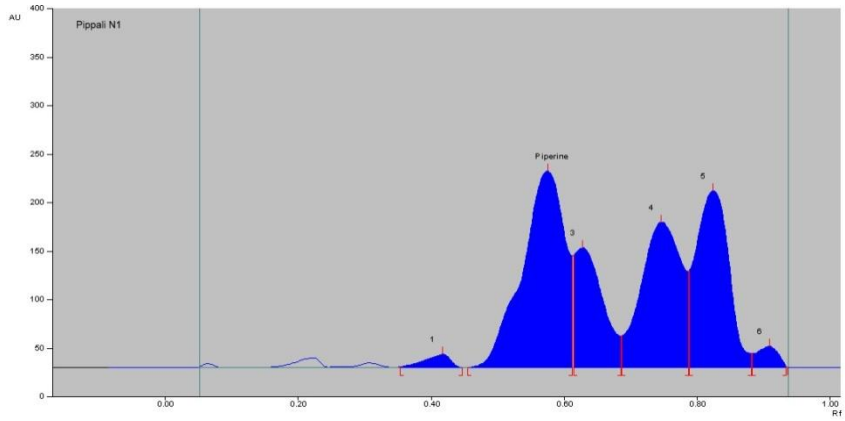


Chromatogram of N1 & N2 with Piperine

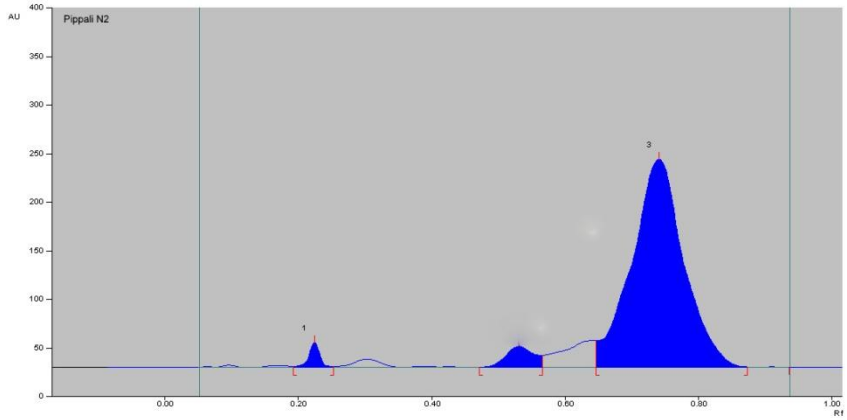
Chromatogram of N3 with Piperine

Densitograms (254 nm) of *Pippali* Methanol extracts

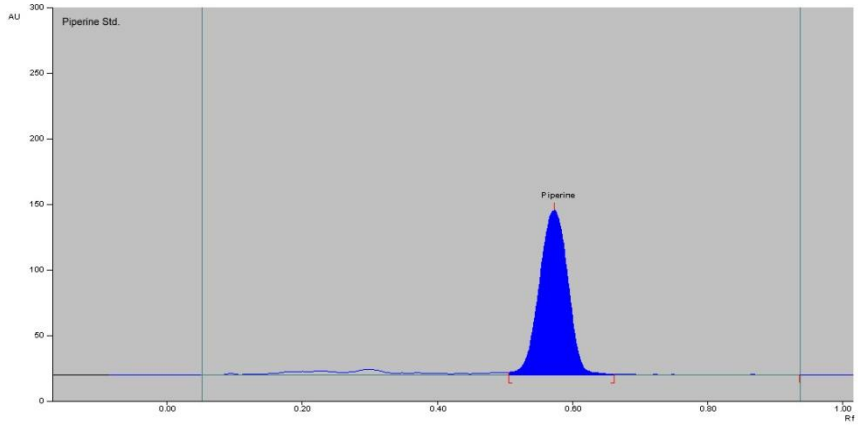
N1 Densitogram:



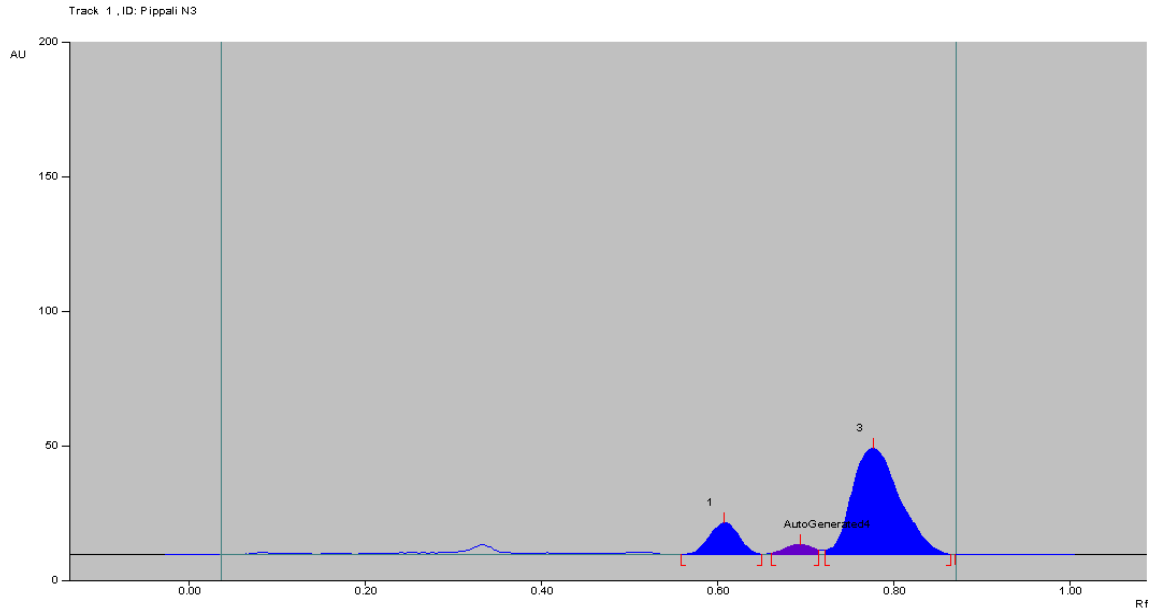
N2 Densitogram:

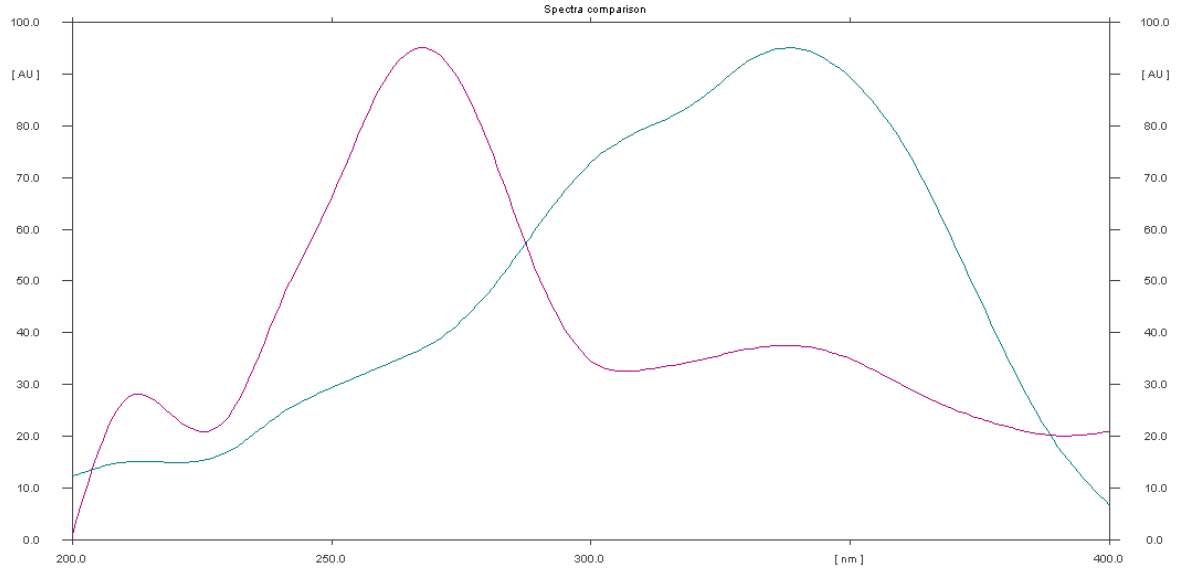


Piperine Densitogram:

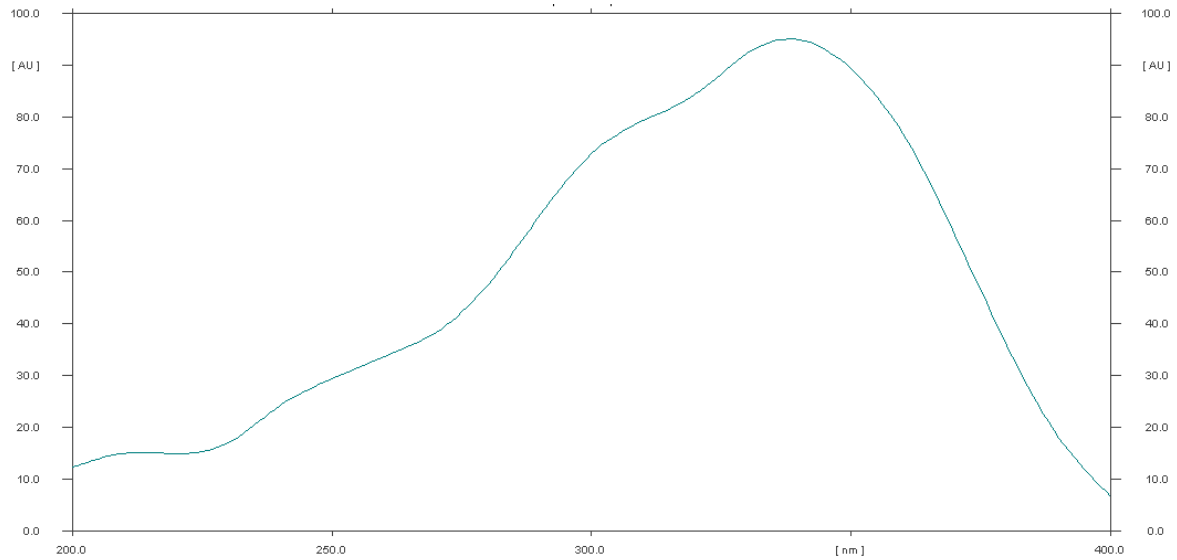


N3 Densitogram:





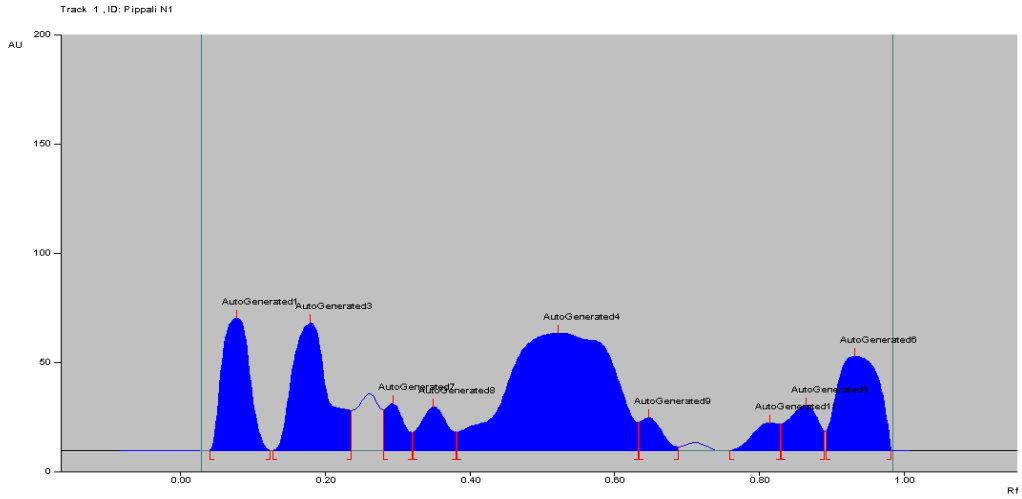
Comparative Spectra of Piperine & compound at R_f 0.61 of *Pippali* N3



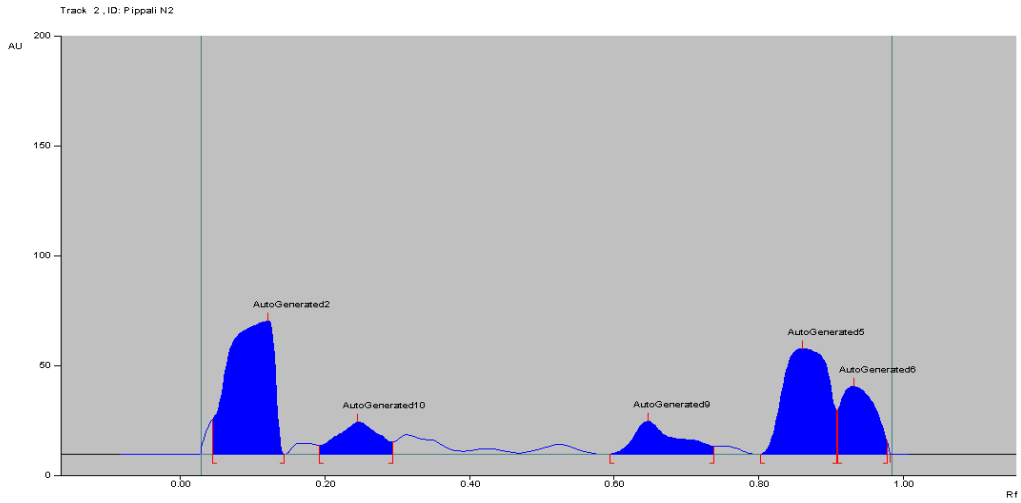
Spectra of Piperine

Densitograms (at 254 nm) of *Pippali* Volatile oil

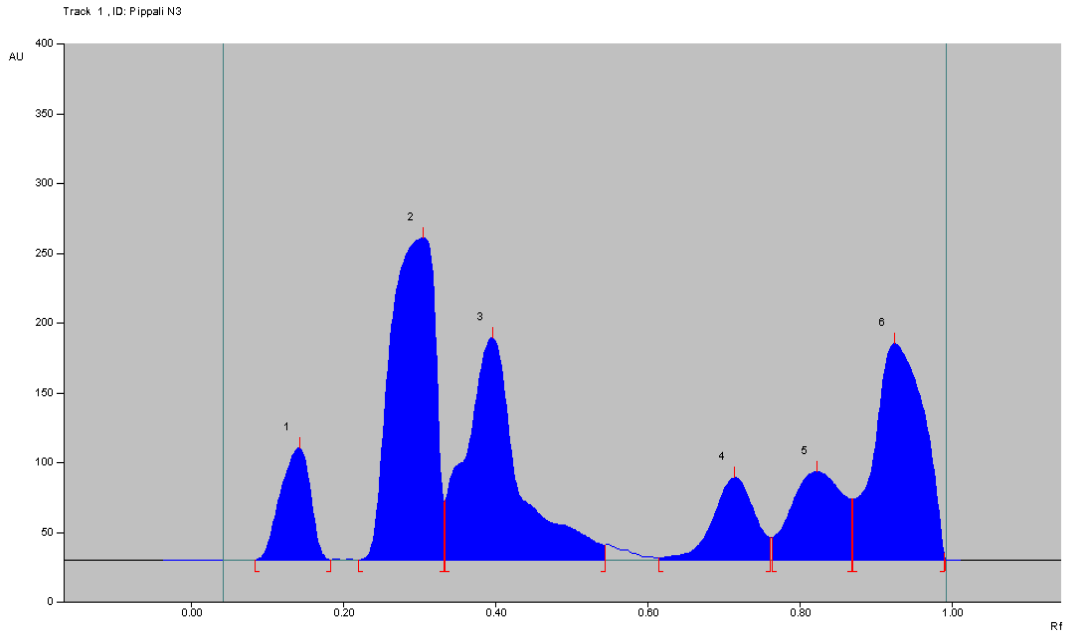
N1 Densitogram:



N2 Densitogram:



N3 Densitogram:



Analysis Report

SOP document	
Validated	Design
Description :	
Analysis	Pippali N1 and N2.cna
Created/used by	RMD Research and Development Center Tuesday, August 28, 2012 5:05:22 PM
Current user	RMD Research and Development Center

Stationary phase

Executed by	RMD Research and Development Center Tuesday, August 28, 2012 5:03:26 PM
Plate size (X x Y)	10.0 x 10.0 cm
Material	HPTLC plates silica gel 60 F 254
Manufacturer	E. MERCK KGaA
Batch	
GLP code	
Pre-washing	No
Modification	No

Definitions - Screening

Executed by RMD Research and Development Center Tuesday, August 28, 2012 5:03:47 PM

Samples

Pippali N2

Pippali N1

Sample application - CAMAG Linomat 5

Instrument	CAMAG Linomat 5
Executed by	RMD Research and Development Center Tuesday, August 28, 2012 4:54:31 PM

Linomat 5 application parameters

Spray gas :	Inert gas
Sample solvent type :	Methanol
Dosage speed :	150 nl/s
Predosage volume :	0.2 ul

Sequence

Syringe size:	100 µl
Number of tracks:	2
Application position Y :	10.0 mm
Band length :	9.0 mm

Detection - CAMAG TLC Scanner

Information

Application position	10.0 mm
Solvent front position	69.0 mm

Instrument

Instrument	CAMAG TLC Scanner
Executed by	RMD Research and Development Center Tuesday, August 28, 2012 4:56:27 PM
Number of tracks	2
Position of first track X	11.0 mm
Distance between tracks	15.6 mm
Scan start pos. Y	5.0 mm
Scan end pos. Y	70.0 mm
Slit dimensions	6.00 x 0.90 mm, Macro
Optimize optical system	Light
Scanning speed:	20 mm/s
Data resolution:	100 µm/step

Measurement Table

Wavelength	254
Lamp	D2
Measurement Type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector mode	Automatic
PM high voltage	264 V

Detector properties

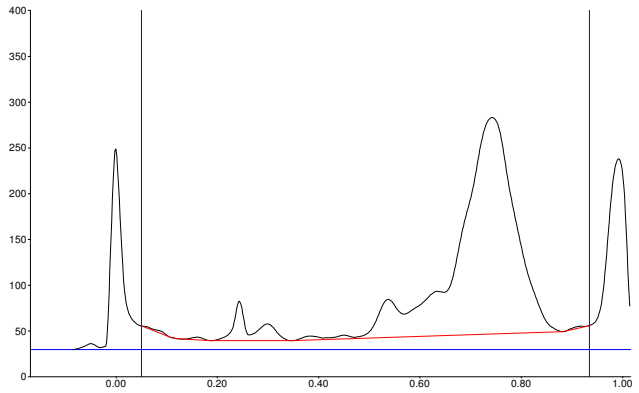
Y-position for 0 adjust	5.0 mm
Track # for 0 adjust	0
Analog Offset	10%
Sensitivity	Automatic (27)

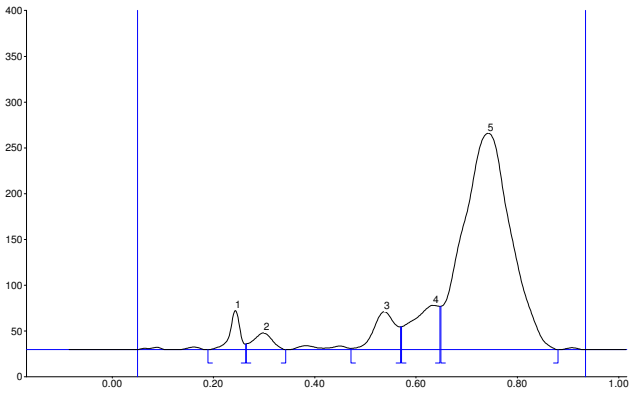
Integration

Properties

Data filtering	Savitsky-Golay 7
Baseline correction	Lowest Slope
Peak threshold min. slope	5
Peak threshold min. height	10 AU
Peak threshold min. area	50
Peak threshold max. height	990 AU
Track start position	13.1 mm
Track end position	65.4 mm
Display scaling	Automatic

Track 1, ID: Pippali N2





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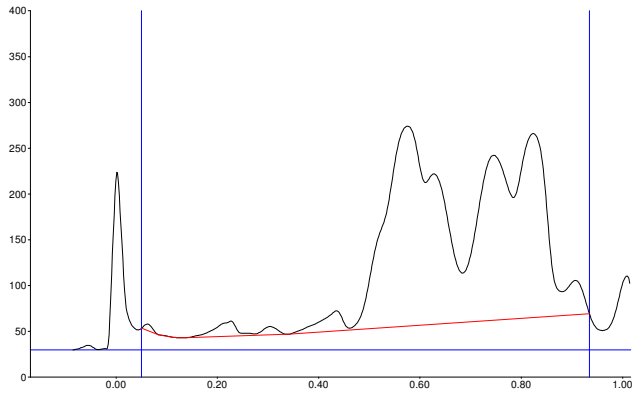
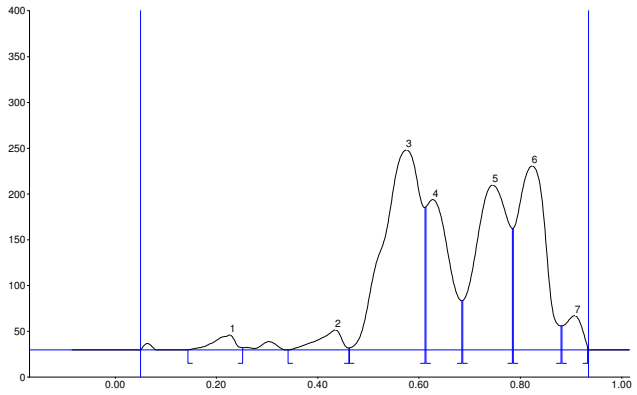
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.19	0.0	0.24	42.8	11.06	0.26	6.1	583.6	3.02	unknown *
2	0.27	6.4	0.30	18.3	4.72	0.34	0.0	462.1	2.39	unknown *
3	0.47	1.4	0.54	41.5	10.71	0.57	24.8	1238.5	6.41	unknown *
4	0.57	25.0	0.64	48.4	12.50	0.65	47.3	1763.6	9.13	unknown *
5	0.65	47.4	0.74	236.3	61.01	0.88	0.0	15275.4	79.05	unknown *

Track 2, ID: Pippali N1





Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.14	0.4	0.23	16.5	1.97	0.25	2.7	481.1	1.43	unknown *
2	0.34	0.0	0.44	22.0	2.62	0.46	2.2	691.1	2.05	unknown *
3	0.46	2.3	0.58	218.4	26.01	0.61	155.6	10945.3	32.53	unknown *
4	0.62	156.2	0.63	164.2	19.56	0.69	53.8	5125.1	15.23	unknown *
5	0.69	54.0	0.75	180.2	21.46	0.79	132.6	7932.3	23.58	unknown *
6	0.79	132.9	0.83	200.9	23.92	0.88	26.3	7614.2	22.63	unknown *
7	0.88	26.3	0.91	37.4	4.46	0.94	3.0	857.2	2.55	unknown *

Analysis Report

SOP document	
Validated	Design
Description :	Toluene+Ethyl acetate (4.5:5.5)
Analysis	Pippali N3.cna
Created/used by	RMD Research and Development Center Tuesday, October 16, 2012 2:37:40 PM
Current user	RMD Research and Development Center

Stationary phase

Executed by	RMD Research and Development Center Tuesday, October 16, 2012 2:35:19 PM
Plate size (X x Y)	5.0 x 10.0 cm
Material	HPTLC plates silica gel 60 F 254
Manufacturer	E. MERCK KGaA
Batch	
GLP code	
Pre-washing	No
Modification	No

Definitions - Screening

Executed by	RMD Research and Development Center Tuesday, October 16, 2012 2:34:54 PM

Samples

Pippali N3

Sample application - CAMAG Linomat 5

Instrument	CAMAG Linomat 5
Executed by	RMD Research and Development Center Tuesday, October 16, 2012 2:37:40 PM

Linomat 5 application parameters

Spray gas :	Inert gas
Sample solvent type :	Methanol
Dosage speed :	150 nl/s
Predosage volume :	0.2 ul

Sequence

Syringe size:	100 µl
Number of tracks:	3
Application position Y :	10.0 mm
Band length :	8.0 mm

Detection - CAMAG TLC Scanner

Information

Application position	10.0 mm
Solvent front position	83.5 mm

Instrument

	CAMAG TLC Scanner
Executed by	RMD Research and Development Center Tuesday, October 16, 2012 2:35:47 PM
Number of tracks	1
Position of first track X	12.0 mm
Distance between tracks	13.0 mm
Scan start pos. Y	8.0 mm
Scan end pos. Y	84.0 mm
Slit dimensions	6.00 x 0.10 mm, Micro
Optimize optical system	Light
Scanning speed:	20 mm/s
Data resolution:	100 µm/step

Measurement Table

Wavelength	254
Lamp	D2
Measurement Type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector mode	Automatic
PM high voltage	295 V

Integration

Properties

Data filtering	Savitsky-Golay 7
Baseline correction	Lowest Slope
Peak threshold min. slope	5
Peak threshold min. height	2 AU
Peak threshold min. area	10
Peak threshold max. height	990 AU
Track start position	13.1 mm
Track end position	74.1 mm
Display scaling	Automatic

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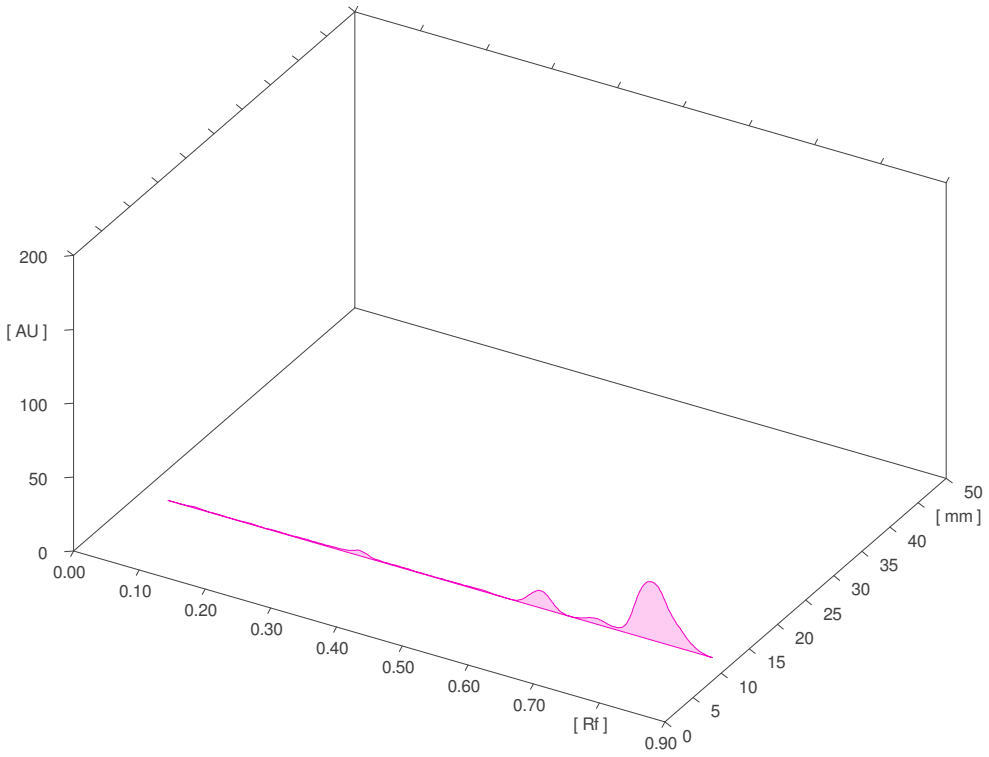
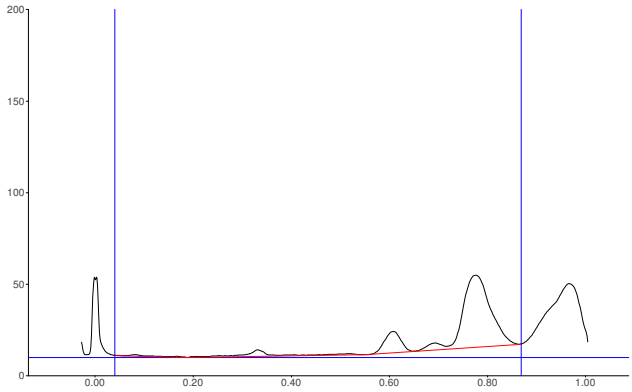
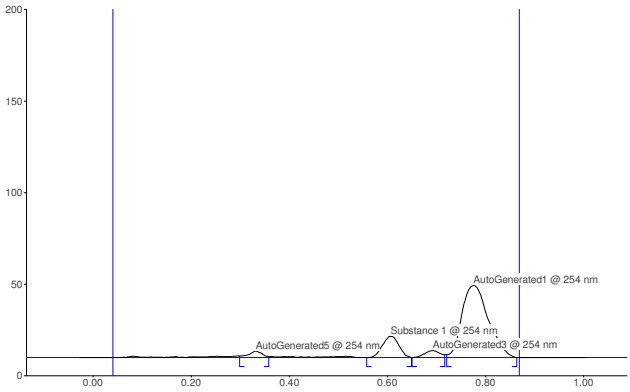
All tracks at WavelengthSc4



Track 1, ID: Pippali N3





Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.30	0.9	0.33	3.6	6.14	0.36	0.7	85.7	3.65	AutoGenerated5
2	0.56	0.0	0.61	11.7	19.97	0.65	0.2	336.8	14.34	Substance 1
3	0.65	0.1	0.69	3.9	6.60	0.72	1.6	106.0	4.51	AutoGenerated3
4	0.72	1.7	0.78	39.4	67.29	0.87	0.0	1820.4	77.50	AutoGenerated1

User : RMD Research and Development Center
Tuesday, October 16, 2012 2:37:45 PM

Analysis Report

SOP document	
Validated	Design
Description :	Toluene+Ethyl acetate (4.5:5.5)
Analysis	Pippali N3-Piperine estimation.cna
Created/used by	RMD Research and Development Center Tuesday, October 16, 2012 2:26:36 PM
Current user	RMD Research and Development Center

Stationary phase

Executed by	RMD Research and Development Center Tuesday, October 16, 2012 2:12:34 PM
Plate size (X x Y)	5.0 x 10.0 cm
Material	HPTLC plates silica gel 60 F 254
Manufacturer	E. MERCK KGaA
Batch	
GLP code	
Pre-washing	No
Modification	No

Definitions - Quantification

Executed by	RMD Research and Development Center Tuesday, October 16, 2012 2:11:53 PM

Calibration parameters

Calibration mode	Single level
Statistics mode	CV
Evaluation mode	Peak Height & Area

Samples

Sample ID: Pippali N3

Sample ID: Piperine standard

Standards absolute

Standard level1

Substance	Amount/fraction
Piperine	0.0100 mg
AutoGenerated1	0.0100 mg
AutoGenerated3	0.0100 mg
AutoGenerated5	0.0100 mg
Substance 1	0.0100 mg

Sample application - CAMAG Linomat 5

Instrument	CAMAG Linomat 5
Executed by	RMD Research and Development Center Tuesday, October 16, 2012 11:12:44 AM

Linomat 5 application parameters

Spray gas :	Inert gas
Sample solvent type :	Methanol
Dosage speed :	150 nl/s
Predosage volume :	0.2 ul

Sequence

Syringe size:	100 µl
Number of tracks:	3
Application position Y :	10.0 mm
Band length :	8.0 mm

Detection - CAMAG TLC Scanner

Information

Application position	10.0 mm
Solvent front position	83.5 mm

Instrument	CAMAG TLC Scanner
Executed by	RMD Research and Development Center Tuesday, October 16, 2012 2:13:03 PM
Number of tracks	2
Position of first track X	12.0 mm
Distance between tracks	13.0 mm
Scan start pos. Y	8.0 mm
Scan end pos. Y	84.0 mm
Slit dimensions	6.00 x 0.10 mm, Micro
Optimize optical system	Light
Scanning speed:	20 mm/s
Data resolution:	100 µm/step

Measurement Table

Wavelength	254
Lamp	D2
Measurement Type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector mode	Automatic
PM high voltage	295 V

Integration

Properties

Data filtering	Savitsky-Golay 7
Baseline correction	Lowest Slope
Peak threshold min. slope	5
Peak threshold min. height	2 AU
Peak threshold min. area	10
Peak threshold max. height	990 AU
Track start position	13.1 mm
Track end position	74.1 mm
Display scaling	Automatic

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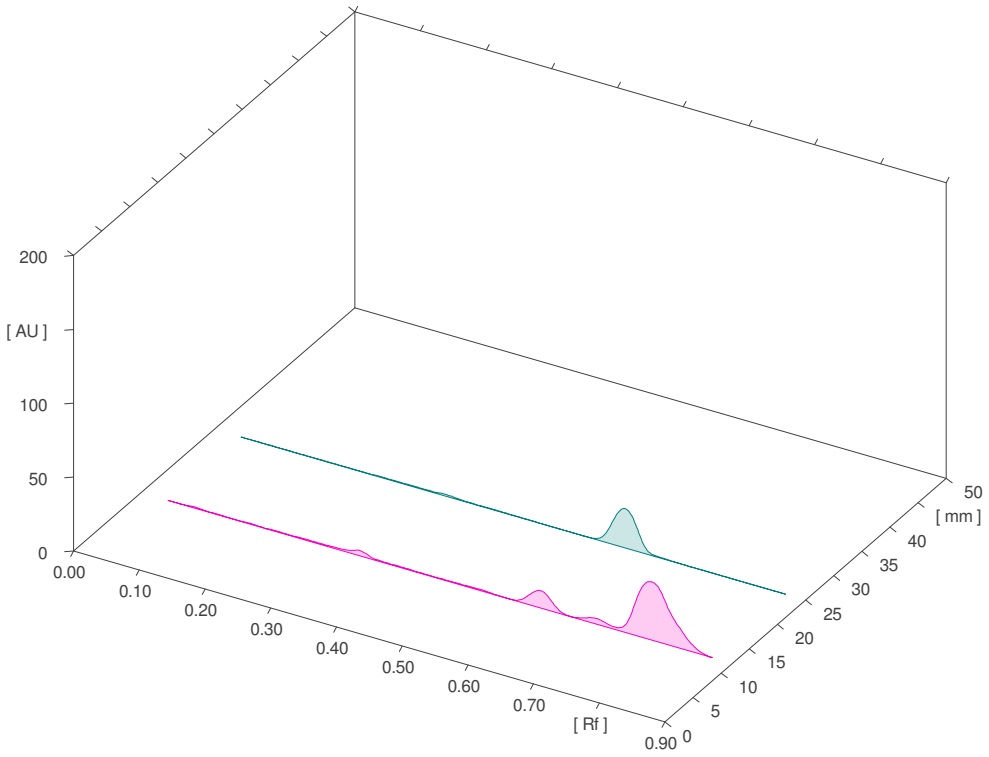
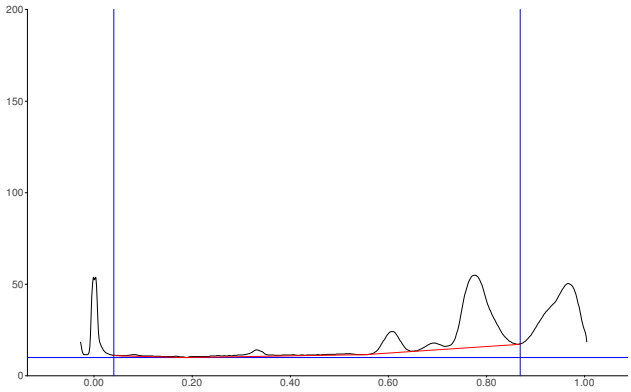
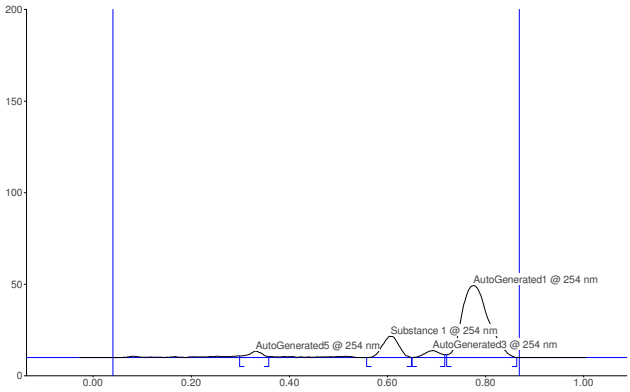
All tracks at WavelengthSc4



Track 1, ID: Pippali N3





Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.30	0.9	0.33	3.6	6.14	0.36	0.7	85.7	3.65	AutoGenerated5
2	0.56	0.0	0.61	11.7	19.97	0.65	0.2	336.8	14.34	Substance 1
3	0.65	0.1	0.69	3.9	6.60	0.72	1.6	106.0	4.51	AutoGenerated3
4	0.72	1.7	0.78	39.4	67.29	0.87	0.0	1820.4	77.50	AutoGenerated1

User : RMD Research and Development Center
Tuesday, October 16, 2012 2:26:39 PM

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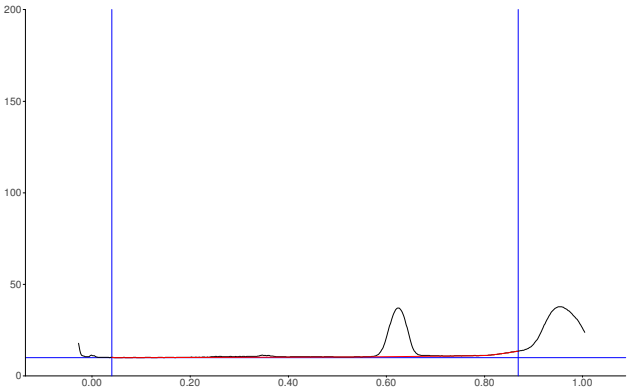
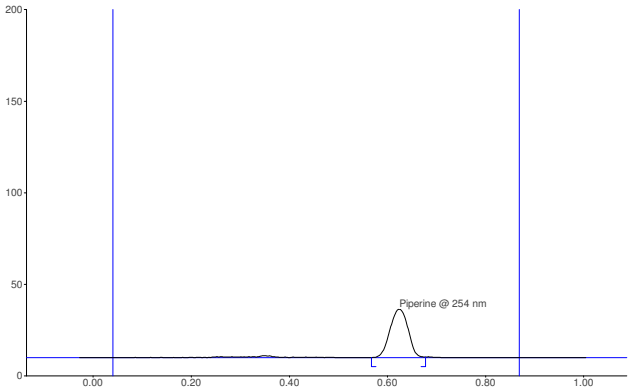
Track 2, ID: Piperine standard





Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.57	0.1	0.63	26.5	100.00	0.68	0.4	841.7	100.00	Piperine

User : RMD Research and Development Center
Tuesday, October 16, 2012 2:26:39 PM

Analysis Report

SOP document	
Validated	Design
Description :	
Analysis	Piperine estimation in Pippali N1 and N2.cna
Created/used by	RMD Research and Development Center Tuesday, August 28, 2012 5:22:40 PM
Current user	RMD Research and Development Center

Stationary phase

Executed by	RMD Research and Development Center
Plate size (X x Y)	10.0 x 10.0 cm
Material	HPTLC plates silica gel 60 F 254
Manufacturer	E. MERCK KGaA
Batch	
GLP code	
Pre-washing	No
Modification	No

Definitions - Quantification

Executed by RMD Research and Development Center Tuesday, August 28, 2012 5:13:11 PM

Calibration parameters

Calibration mode	Single level
Statistics mode	CV
Evaluation mode	Peak Height & Area

Samples

Sample ID: Pippali N1

Sample ID: Piperine STD

Sample ID: Pippali N2

Substance name	Rf	Window size	Deviation
Piperine	0.55	2.8 mm	10.00 %

Standards absolute

Standard level1

Substance	Amount/fraction
Piperine	2.0000 µg

Sample application - CAMAG Linomat 5

Instrument	CAMAG Linomat 5
Executed by	RMD Research and Development Center Tuesday, August 28, 2012 5:09:55 PM

Linomat 5 application parameters

Spray gas :	Inert gas
Sample solvent type :	Methanol
Dosage speed :	150 nl/s
Predosage volume :	0.2 ul

Sequence

Syringe size:	100 µl
Number of tracks:	3
Application position Y :	10.0 mm
Band length :	9.0 mm

Detection - CAMAG TLC Scanner

Information

Application position	10.0 mm
Solvent front position	69.0 mm

Instrument	CAMAG TLC Scanner
Executed by	RMD Research and Development Center Tuesday, August 28, 2012 5:13:53 PM
Number of tracks	3
Position of first track X	11.0 mm
Distance between tracks	15.6 mm
Scan start pos. Y	5.0 mm
Scan end pos. Y	70.0 mm
Slit dimensions	6.00 x 0.90 mm, Macro
Optimize optical system	Light
Scanning speed:	20 mm/s
Data resolution:	100 µm/step

Measurement Table

Wavelength	254
Lamp	D2
Measurement Type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector mode	Automatic
PM high voltage	264 V

Detector properties

Y-position for 0 adjust	5.0 mm
Track # for 0 adjust	0
Analog Offset	10%
Sensitivity	Automatic (27)

Integration

Properties

Data filtering	Savitsky-Golay 7
Baseline correction	Lowest Slope
Peak threshold min. slope	5
Peak threshold min. height	10 AU
Peak threshold min. area	50
Peak threshold max. height	990 AU
Track start position	13.1 mm
Track end position	65.4 mm
Display scaling	Automatic

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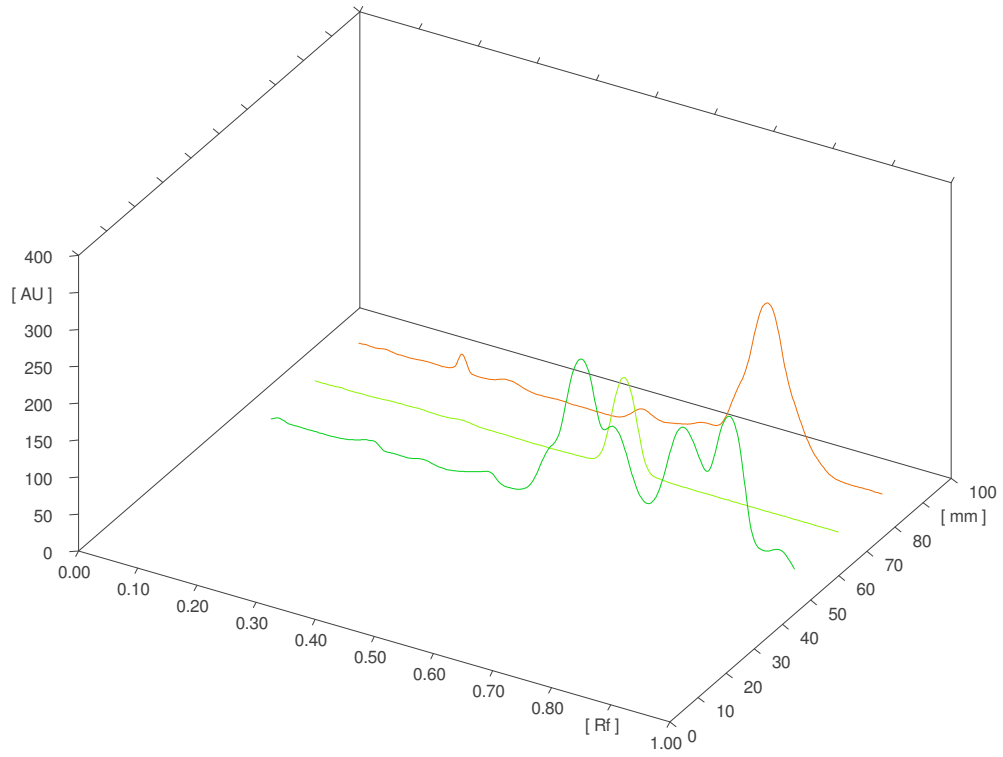
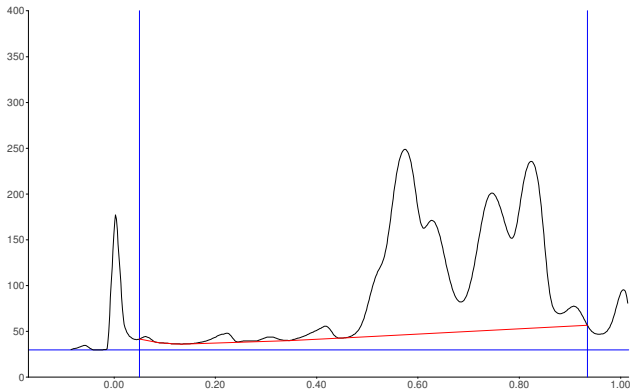
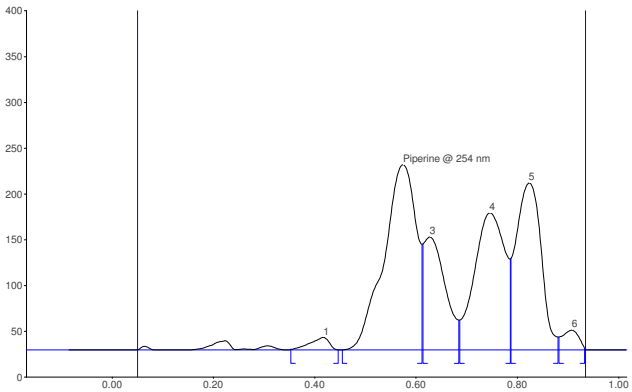
All tracks at WavelengthSc4



Track 1, ID: Pippali N1





Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.35	0.8	0.42	13.8	1.99	0.45	0.0	359.9	1.40	unknown *
2	0.46	0.2	0.58	202.4	29.19	0.61	115.4	8849.3	34.39	Piperine
3	0.62	115.7	0.63	123.4	17.80	0.69	32.7	3642.2	14.16	unknown *
4	0.69	32.8	0.75	149.8	21.60	0.79	99.4	6212.5	24.15	unknown *
5	0.79	100.2	0.83	182.4	26.29	0.88	14.2	6177.5	24.01	unknown *
6	0.88	14.3	0.91	21.7	3.13	0.94	1.7	487.5	1.89	unknown *

User : RMD Research and Development Center
Tuesday, August 28, 2012 5:22:42 PM

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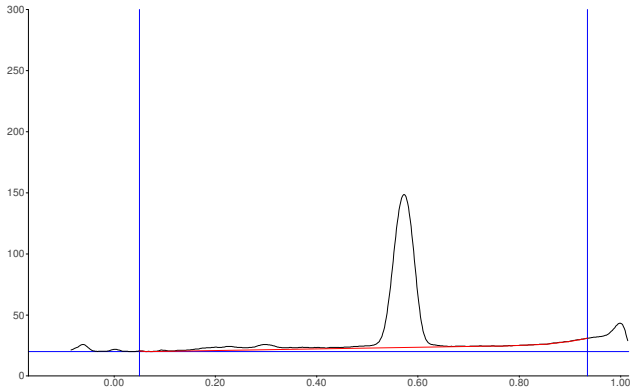
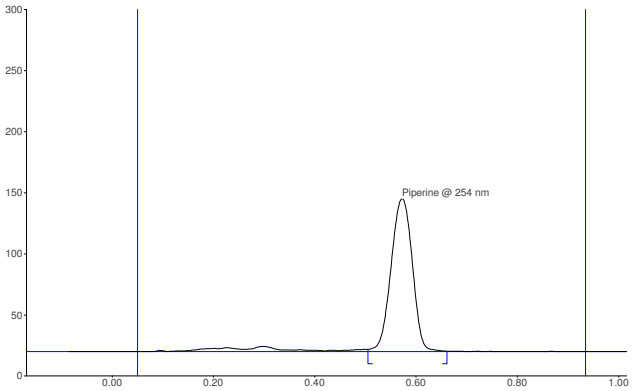
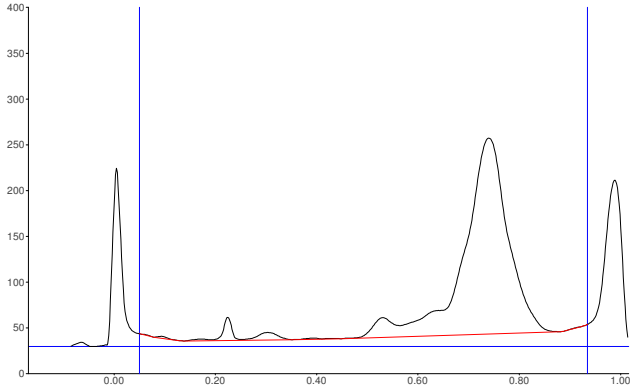
Track 2, ID: Piperine STD

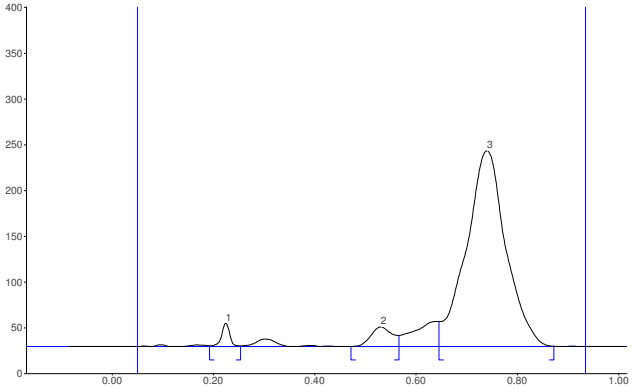




Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.51	2.0	0.57	125.4	100.00	0.66	0.4	3624.5	100.00	Piperine

Track 3, ID: Pippali N2





Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.19	0.9	0.23	25.3	9.72	0.25	0.9	287.8	2.33	unknown *
2	0.47	0.2	0.53	21.6	8.27	0.57	12.2	635.5	5.15	unknown *
3	0.65	27.3	0.74	213.9	82.01	0.87	0.4	11422.9	92.52	unknown *

Spectrum scan

Executed by	RMD Research and Development Center Tuesday, August 28, 2012 5:22:35 PM
Mode	All detected peaks
Slit dimensions	6.00 x 0.30 mm, Micro
Optimize optical system	Resolution
Scanning speed	100 nm/s
Data resolution	10 nm/step
Reference spectrum, pos X	5.0 mm
Reference spectrum, pos Y	5.0 mm

User : RMD Research and Development Center
Tuesday, August 28, 2012 5:22:42 PM

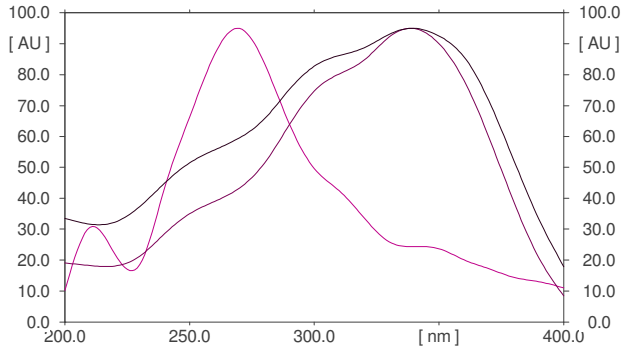
Measurement Table

Lamp	D2
Start wavelength	200 nm
End wavelength	400 nm
Measurement type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector Mode	Automatic

Detector properties

Y-position for 0 adjust	0.0 mm
Track # for 0 adjust	0
Analog Offset	10%
Sensitivity	Automatic (3)

Piperine on all Tracks



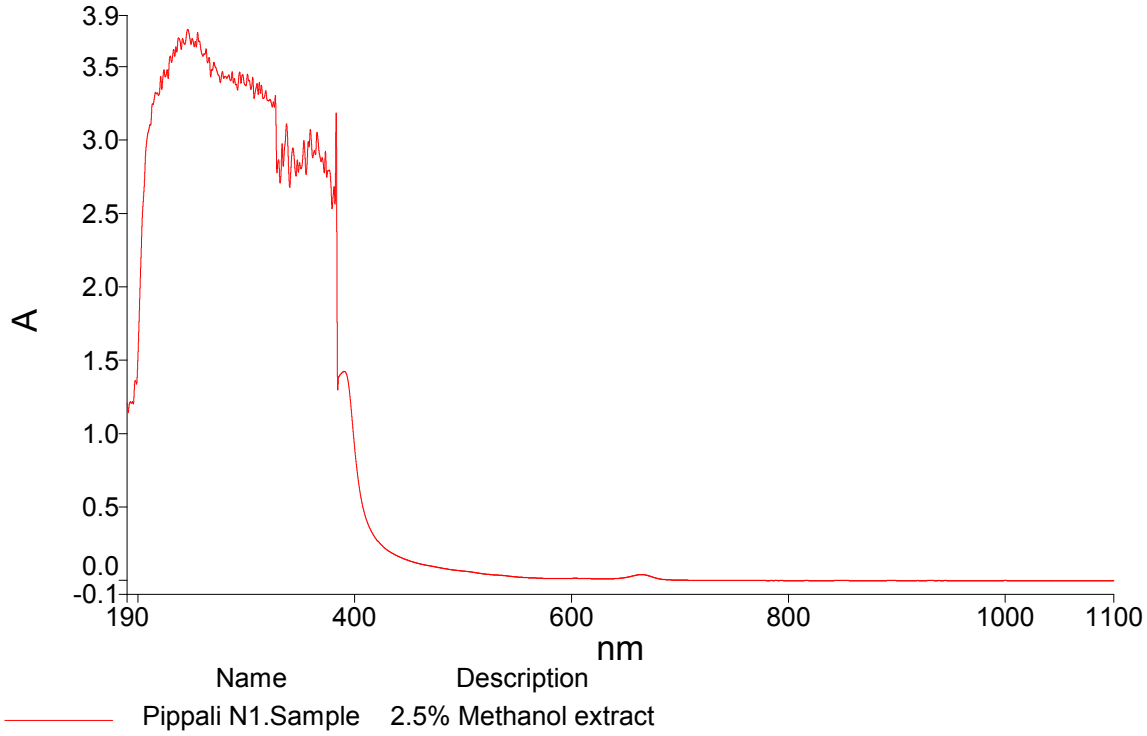
T	Rf	Substance	Max. @
1	0.58 Rf	Piperine	339 nm
2	0.57 Rf	Piperine	339 nm
3	0.53 Rf		269 nm

Analyst	RMD Research and Development Center
Date	Tuesday, August 28, 2012 12:51 PM

Sample	
Setting	Value
Filename	Pippali N1.Sample
Creation Date	8/6/2012 3:05:51 PM
Analyst	rmd
X-Axis Units	nm
X-Axis start value	1100
X-Axis end value	190
Data interval	-1
Number of points	911
Y-Axis Units	A
Description	2.5% Methanol extract

Instrument	
Setting	Value
Instrument Model	Lambda 25
Software Revision	PerkinElmer UV WinLab 6.0.3.0730 / Lambda25 1.27
Scan Speed (nm/min)	240
Slit Width (nm)	1
Lamps	Both on
Cycles	1
Accessory	None
Lamp change at (nm)	326

Pippali N1



Sample ID	Pippali N1.Sample
Description	2.5% Methanol extract
Threshold	0.01
Abscissa Range	1100 - 190 nm
Display Options	Peaks Listed by position

	Position (nm)	Intensity	Type
1	664.6	0.04155	Peak
2	390.4	1.426	Peak
3	382.9	3.186	Peak
4	380.9	2.686	Peak

PerkinElmer
precisely

	Position (nm)	Intensity	Type
5	376.6	2.798	Peak
6	373.0	2.927	Peak
7	370.0	2.883	Peak
8	365.2	3.054	Peak
9	363.0	2.932	Peak
10	359.0	3.074	Peak
11	357.1	2.993	Peak
12	353.1	2.998	Peak
13	349.1	2.849	Peak
14	347.0	2.867	Peak
15	342.9	2.948	Peak
16	337.1	3.113	Peak
17	333.0	2.977	Peak
18	329.7	2.867	Peak
19	326.8	3.306	Peak
20	325.0	3.263	Peak
21	317.9	3.338	Peak
22	314.0	3.375	Peak
23	312.0	3.395	Peak
24	309.9	3.387	Peak
25	305.9	3.430	Peak
26	301.1	3.452	Peak
27	296.5	3.448	Peak
28	293.9	3.466	Peak
29	289.0	3.422	Peak
30	287.0	3.467	Peak
31	283.9	3.446	Peak
32	280.5	3.436	Peak
33	278.0	3.469	Peak

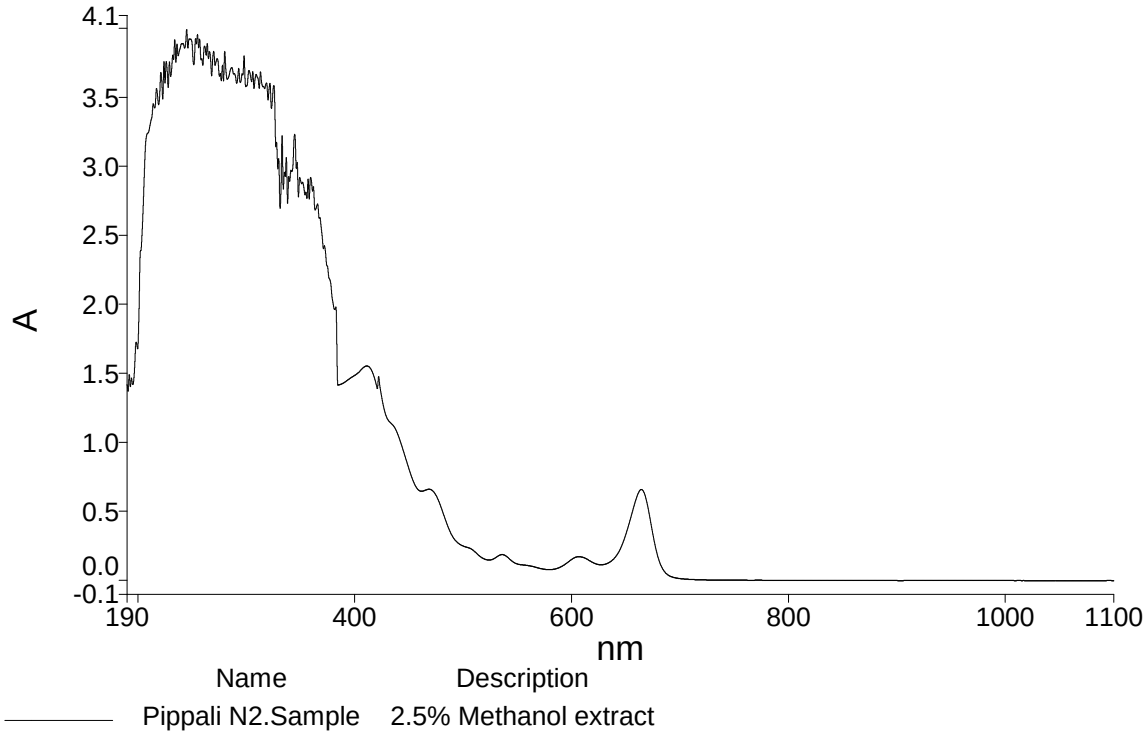
	Position (nm)	Intensity	Type
34	270.1	3.533	Peak
35	265.8	3.566	Peak
36	262.9	3.624	Peak
37	255.1	3.736	Peak
38	253.0	3.674	Peak
39	250.1	3.693	Peak
40	246.0	3.756	Peak
41	241.2	3.694	Peak
42	237.4	3.700	Peak
43	235.1	3.637	Peak
44	233.0	3.620	Peak
45	229.9	3.574	Peak
46	226.9	3.481	Peak
47	224.0	3.481	Peak
48	221.0	3.436	Peak
49	216.2	3.327	Peak
50	197.6	1.363	Peak

Analyst	RMD Research and Development Center
Date	Tuesday, August 28, 2012 12:46 PM

Sample	
Setting	**Value**
Filename	Pippali N2.Sample
Creation Date	8/6/2012 3:11:33 PM
Analyst	rmd
X-Axis Units	nm
X-Axis start value	1100
X-Axis end value	190
Data interval	-1
Number of points	911
Y-Axis Units	A
Description	2.5% Methanol extract

Instrument	
Setting	**Value**
Instrument Model	Lambda 25
Software Revision	PerkinElmer UV WinLab 6.0.3.0730 / Lambda25 1.27
Scan Speed (nm/min)	240
Slit Width (nm)	1
Lamps	Both on
Cycles	1
Accessory	None
Lamp change at (nm)	326

Pippali N2



Sample ID	Pippali N2.Sample
Description	2.5% Methanol extract
Threshold	0.01
Abscissa Range	1100 - 190 nm
Display Options	Peaks Listed by position

	Position (nm)	Intensity	Type
1	664.4	0.6602	Peak
2	606.8	0.1736	Peak
3	535.9	0.1883	Peak
4	468.6	0.6622	Peak

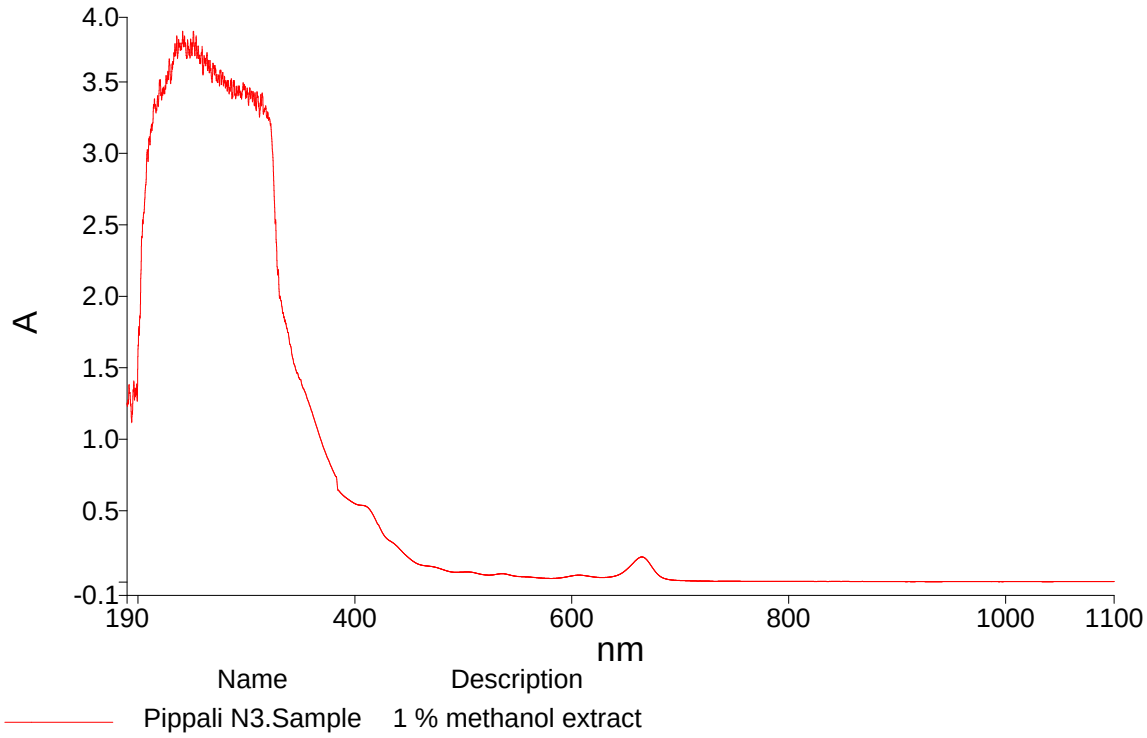
PerkinElmer precisely

	Position (nm)	Intensity	Type
5	422.0	1.479	Peak
6	411.1	1.555	Peak
7	372.0	2.427	Peak
8	365.7	2.729	Peak
9	361.9	2.855	Peak
10	359.3	2.921	Peak
11	357.0	2.911	Peak
12	354.9	2.815	Peak
13	349.2	2.922	Peak
14	346.8	3.031	Peak
15	344.6	3.234	Peak
16	339.2	2.930	Peak
17	336.9	3.066	Peak
18	335.1	2.959	Peak
19	333.0	3.223	Peak
20	329.9	3.059	Peak
21	325.4	3.587	Peak
22	321.8	3.603	Peak
23	318.6	3.606	Peak
24	313.1	3.687	Peak
25	309.1	3.668	Peak
26	306.0	3.688	Peak
27	302.4	3.697	Peak
28	298.0	3.802	Peak
29	293.0	3.711	Peak
30	286.6	3.717	Peak
31	280.1	3.834	Peak
32	278.0	3.736	Peak
33	275.9	3.676	Peak

	Position (nm)	Intensity	Type
34	273.0	3.782	Peak
35	269.9	3.835	Peak
36	266.0	3.833	Peak
37	264.0	3.891	Peak
38	261.4	3.872	Peak
39	256.9	3.924	Peak
40	255.0	3.957	Peak
41	253.2	3.927	Peak
42	248.7	3.924	Peak
43	245.0	3.994	Peak
44	241.7	3.891	Peak
45	236.0	3.889	Peak
46	234.0	3.921	Peak
47	232.1	3.807	Peak
48	229.1	3.758	Peak
49	226.1	3.761	Peak
50	224.0	3.760	Peak
51	221.2	3.682	Peak
52	217.2	3.567	Peak
53	214.1	3.455	Peak
54	198.3	1.728	Peak
55	194.0	1.467	Peak

Pippali N3

Raw Spectra



Peak Table

Sample ID	Pippali N3.Sample
Description	1 % methanol extract
Threshold	0.050
Abscissa Range	1100 - 190 nm
Display Options	Peaks Listed by position

	Position (nm)	Intensity	Type
1	664.5	0.1775	Peak
2	317.8	3.334	Peak
3	314.5	3.424	Peak
4	313.4	3.414	Peak

PerkinElmer precisely

Printed by: rmd

Template: SCAN WITH PEAK TABLE

Print Date and Time: October 14, 2012 11:33:28 India Standard Time

	Position (nm)	Intensity	Type
5	309.8	3.434	Peak
6	308.0	3.425	Peak
7	306.2	3.435	Peak
8	304.4	3.437	Peak
9	303.0	3.459	Peak
10	300.7	3.458	Peak
11	297.0	3.495	Peak
12	293.5	3.473	Peak
13	292.4	3.473	Peak
14	290.7	3.471	Peak
15	288.0	3.511	Peak
16	286.1	3.523	Peak
17	282.7	3.512	Peak
18	279.9	3.541	Peak
19	278.1	3.567	Peak
20	275.8	3.583	Peak
21	274.4	3.552	Peak
22	270.8	3.616	Peak
23	269.4	3.632	Peak
24	266.5	3.650	Peak
25	264.9	3.652	Peak
26	263.7	3.706	Peak
27	261.0	3.686	Peak
28	259.2	3.750	Peak
29	255.4	3.751	Peak
30	253.7	3.824	Peak
31	251.4	3.856	Peak
32	248.6	3.801	Peak
33	245.8	3.797	Peak

	Position (nm)	Intensity	Type
34	243.0	3.823	Peak
35	241.2	3.856	Peak
36	237.6	3.799	Peak
37	235.9	3.824	Peak
38	234.2	3.769	Peak
39	229.0	3.662	Peak
40	227.6	3.576	Peak
41	226.1	3.545	Peak
42	220.8	3.517	Peak
43	218.1	3.404	Peak
44	215.3	3.383	Peak
45	210.5	3.118	Peak
46	208.7	3.027	Peak
47	200.8	1.791	Peak
48	199.0	1.388	Peak
49	197.3	1.360	Peak
50	196.2	1.409	Peak
51	191.9	1.385	Peak

Analyst	rmd
Date	Sunday, October 14, 2012 11:14 AM

Sample	
Setting	Value
Filename	Pippali N3.Sample
Creation Date	10/2/2012 12:45:28 PM
Analyst	rmd
X-Axis Units	nm
X-Axis start value	1100
X-Axis end value	190
Data interval	-0.1
Number of points	9101
Y-Axis Units	A
Description	1 % methanol extract

Instrument	
Setting	Value
Instrument Model	Lambda 25
Software Revision	PerkinElmer UV WinLab 6.0.3.0730 / Lambda25 1.27
Scan Speed (nm/min)	240
Slit Width (nm)	1
Lamps	Both on
Cycles	1
Accessory	None
Lamp change at (nm)	326

Analysis Report

SOP document	
Validated	Design
Description :	
Analysis	Pippali Volatile oil.cna
Created/used by	RMD Research and Development Center Tuesday, August 28, 2012 5:28:32 PM
Current user	RMD Research and Development Center

Stationary phase

Executed by	RMD Research and Development Center Tuesday, August 28, 2012 5:25:44 PM
Plate size (X x Y)	7.0 x 10.0 cm
Material	HPTLC plates silica gel 60 F 254
Manufacturer	E. MERCK KGaA
Batch	
GLP code	
Pre-washing	No
Modification	No

Definitions - Screening

Executed by RMD Research and Development Center Tuesday, August 28, 2012 5:26:31 PM

Samples

Pippali N1

Pippali N2

Sample application - CAMAG Linomat 5

Instrument	CAMAG Linomat 5
Executed by	RMD Research and Development Center Tuesday, August 28, 2012 5:26:34 PM

Linomat 5 application parameters

Spray gas :	Inert gas
Sample solvent type :	User defined
Dosage speed :	330 nl/s
Predosage volume :	0.2 ul

Sequence

Syringe size:	100 µl
Number of tracks:	2
Application position Y :	10.0 mm
Band length :	9.0 mm

Detection - CAMAG TLC Scanner

Information

Application position	10.0 mm
Solvent front position	70.5 mm

Instrument

	CAMAG TLC Scanner
Executed by	RMD Research and Development Center Tuesday, August 28, 2012 5:26:37 PM
Number of tracks	2
Position of first track X	10.2 mm
Distance between tracks	16.6 mm
Scan start pos. Y	5.0 mm
Scan end pos. Y	71.0 mm
Slit dimensions	6.00 x 0.40 mm, Macro
Optimize optical system	Light
Scanning speed:	20 mm/s
Data resolution:	100 µm/step

Integration

Properties

Data filtering	Savitsky-Golay 7
Baseline correction	Lowest Slope
Peak threshold min. slope	5
Peak threshold min. height	10 AU
Peak threshold min. area	50
Peak threshold max. height	990 AU
Track start position	11.8 mm
Track end position	69.6 mm
Display scaling	Automatic

Measurement Table

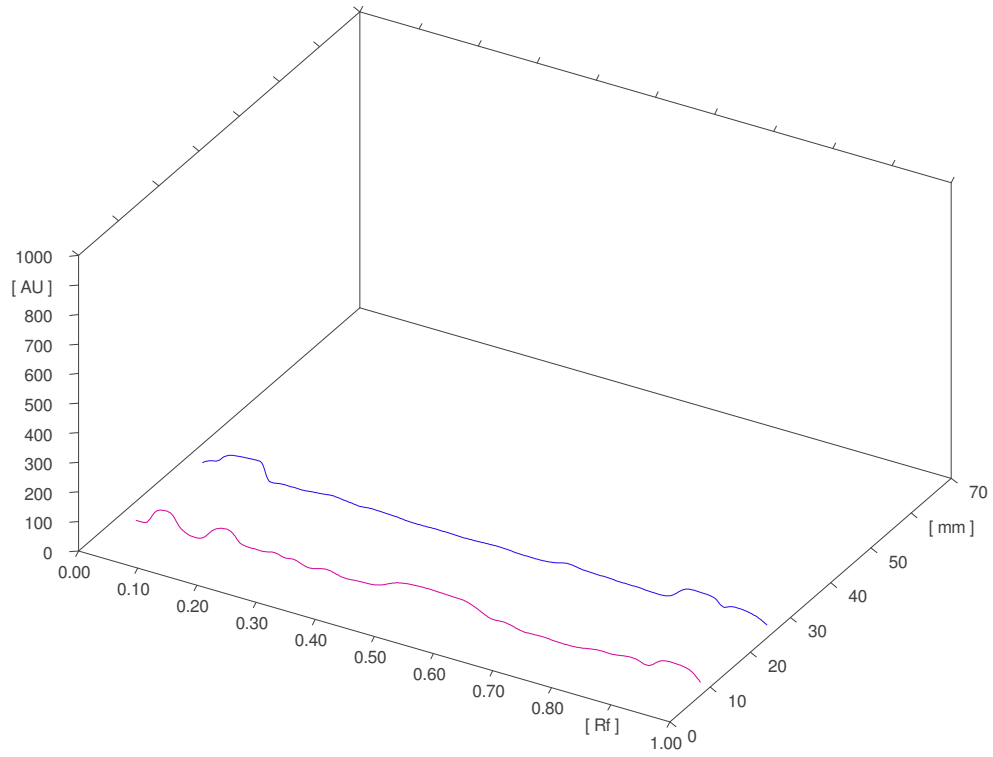
Wavelength	250
Lamp	D2
Measurement Type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector mode	Automatic
PM high voltage	192 V

Detector properties

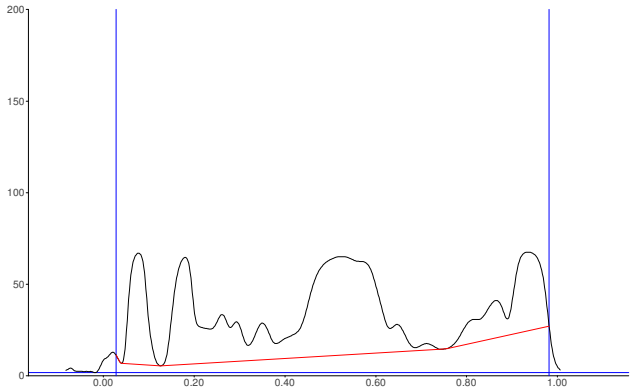
Y-position for 0 adjust	5.0 mm
Track # for 0 adjust	0
Analog Offset	10%
Sensitivity	Automatic (91)

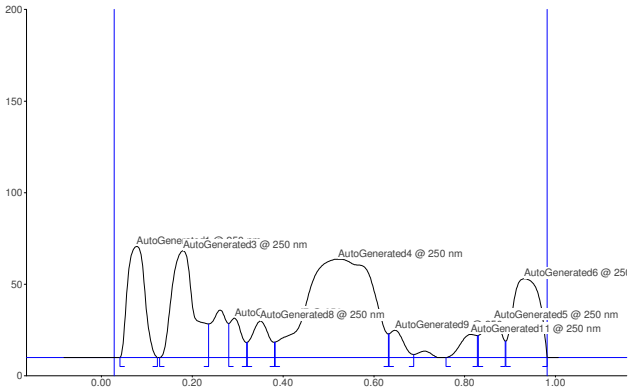
winCATS Planar Chromatography Manager

All tracks at WavelengthSc4



Track 1, ID: Pippali N1

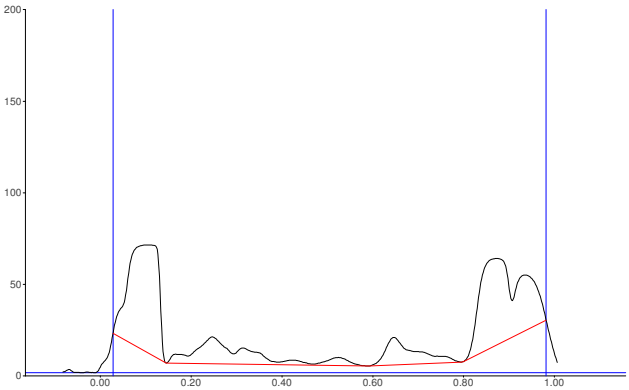


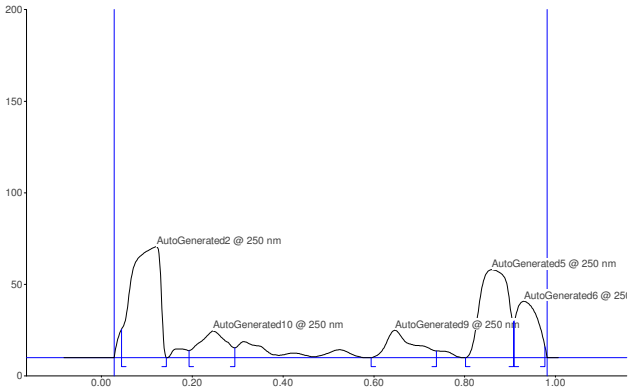


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.04	0.1	0.08	60.7	19.86	0.12	0.1	1600.3	12.34	AutoGenerated1
2	0.13	0.1	0.18	58.3	19.08	0.24	18.4	2024.0	15.60	AutoGenerated3
3	0.28	18.6	0.29	21.5	7.05	0.32	8.3	407.4	3.14	AutoGenerated7
4	0.32	8.4	0.35	20.0	6.55	0.38	8.4	520.0	4.01	AutoGenerated8
5	0.38	8.5	0.52	53.8	17.59	0.63	13.0	5444.5	41.97	AutoGenerated4
6	0.63	13.1	0.65	15.0	4.90	0.69	1.7	316.1	2.44	AutoGenerated9
7	0.76	0.0	0.81	12.7	4.17	0.83	12.0	319.8	2.47	AutoGenerated11
8	0.83	12.1	0.87	20.4	6.69	0.89	9.0	593.8	4.58	AutoGenerated5
9	0.89	9.0	0.93	43.1	14.11	0.98	0.0	1745.6	13.46	AutoGenerated6

User : RMD Research and Development Center
Tuesday, August 28, 2012 5:29:08 PM

Track 2, ID: Pippali N2





Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.04	15.6	0.12	60.7	35.85	0.14	0.0	2655.2	38.13	AutoGenerated2
2	0.19	4.0	0.25	14.6	8.63	0.29	5.5	588.8	8.46	AutoGenerated10
3	0.60	0.0	0.65	15.0	8.87	0.74	3.8	629.0	9.03	AutoGenerated9
4	0.80	0.0	0.86	48.2	28.44	0.91	19.8	2080.2	29.87	AutoGenerated5
5	0.91	20.3	0.93	30.8	18.21	0.98	6.0	1010.3	14.51	AutoGenerated6

Analysis Report

SOP document	
Validated	Design
Description :	
Analysis	Pippali N3 Volatile oil.cna
Created/used by	RMD Research and Development Center Tuesday, October 16, 2012 2:56:08 PM
Current user	RMD Research and Development Center

Stationary phase

Executed by	RMD Research and Development Center Tuesday, October 02, 2012 11:03:29 AM
Plate size (X x Y)	3.0 x 10.0 cm
Material	HPTLC plates silica gel 60 F 254
Manufacturer	E. MERCK KGaA
Batch	
GLP code	
Pre-washing	No
Modification	No

Definitions - Screening

Executed by	RMD Research and Development Center Tuesday, October 16, 2012 2:55:12 PM

Samples

Pippali N3-Volatile oil

Sample application - CAMAG Linomat 5

Instrument	CAMAG Linomat 5
Executed by	RMD Research and Development Center Tuesday, October 02, 2012 11:48:59 AM

Linomat 5 application parameters

Spray gas :	Inert gas
Sample solvent type :	User defined
Dosage speed :	330 nl/s
Predosage volume :	0.2 ul

Sequence

Syringe size:	100 µl
Number of tracks:	1
Application position Y :	10.0 mm
Band length :	10.0 mm

Detection - CAMAG TLC Scanner

Information

Application position	10.2 mm
Solvent front position	71.2 mm

Instrument

	CAMAG TLC Scanner
Executed by	RMD Research and Development Center Tuesday, October 02, 2012 2:07:02 PM
Number of tracks	1
Position of first track X	14.7 mm
Distance between tracks	10.2 mm
Scan start pos. Y	8.0 mm
Scan end pos. Y	72.0 mm
Slit dimensions	6.00 x 0.45 mm, Micro
Optimize optical system	Light
Scanning speed:	20 mm/s
Data resolution:	100 µm/step

Integration

Properties

Data filtering	Savitsky-Golay 7
Baseline correction	Lowest Slope
Peak threshold min. slope	5
Peak threshold min. height	10 AU
Peak threshold min. area	50
Peak threshold max. height	990 AU
Track start position	12.8 mm
Track end position	70.8 mm
Display scaling	Automatic

Measurement Table

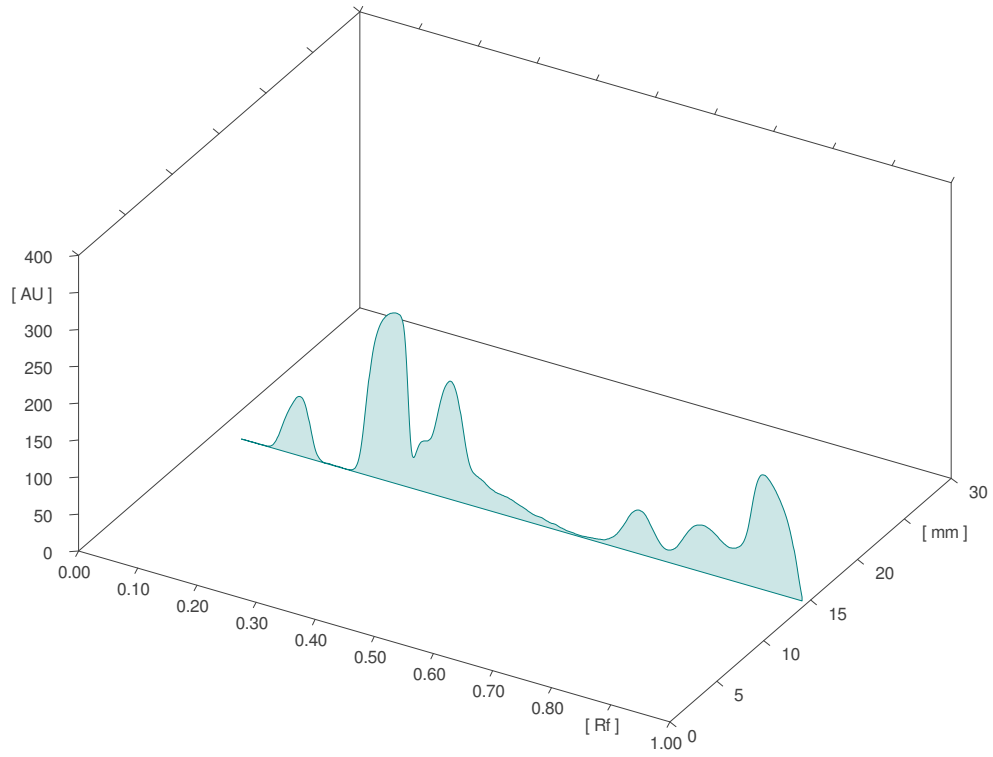
Wavelength	250
Lamp	D2
Measurement Type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector mode	Automatic
PM high voltage	157 V

Detector properties

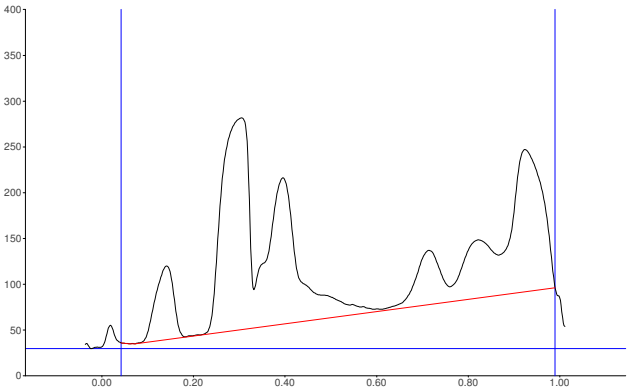
Y-position for 0 adjust	5.0 mm
Track # for 0 adjust	0
Analog Offset	10%
Sensitivity	Automatic (153)

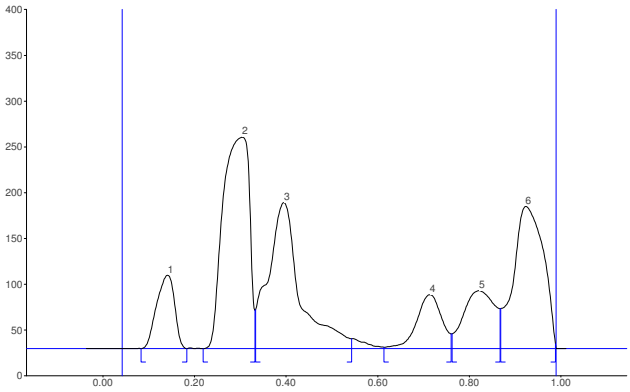
winCATS Planar Chromatography Manager

All tracks at WavelengthSc4



Track 1, ID: Pippali N3-Volatile oil



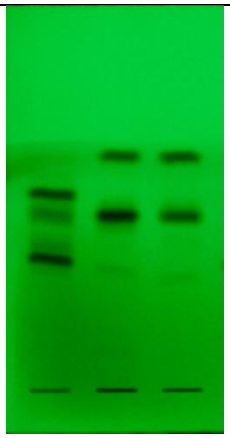


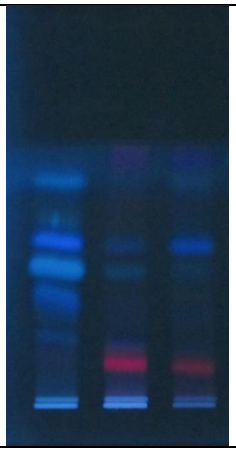
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.08	0.3	0.14	80.3	10.73	0.18	0.2	2220.4	6.93	unknown *
2	0.22	0.1	0.30	230.9	30.86	0.33	41.7	9474.5	29.58	unknown *
3	0.33	42.8	0.40	159.5	21.31	0.54	10.9	7815.3	24.40	unknown *
4	0.61	1.7	0.71	59.0	7.89	0.76	16.3	2282.5	7.13	unknown *
5	0.76	16.4	0.82	63.4	8.47	0.87	43.8	3048.0	9.52	unknown *
6	0.87	43.8	0.92	155.2	20.74	0.99	5.2	7191.6	22.45	unknown *

User : RMD Research and Development Center
Tuesday, October 16, 2012 2:56:11 PM

Purana Pippali

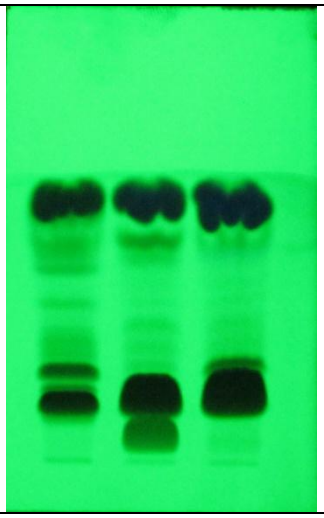
Chromatogram of Methanol extracts of *Pippali*

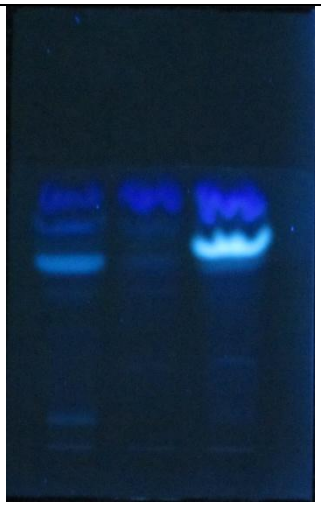




254 nm	366 nm
Chromatogram of P1,P2,P3	

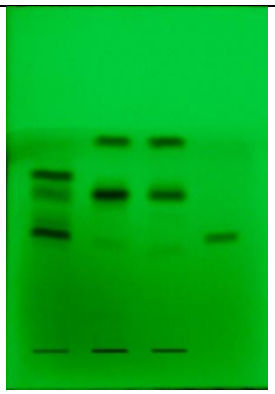
Chromatogram of Volatile oil of *Pippali*





254 nm	366 nm	After spraying with Anisaldehyde sulphuric acid reagent
Chromatogram of Volatile oil (P1,P2,P3)		

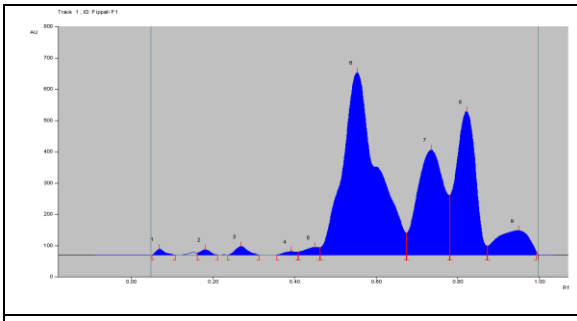
Chromatogram of Methanol extracts of *Pippali* (254 nm) along with Piperine standard



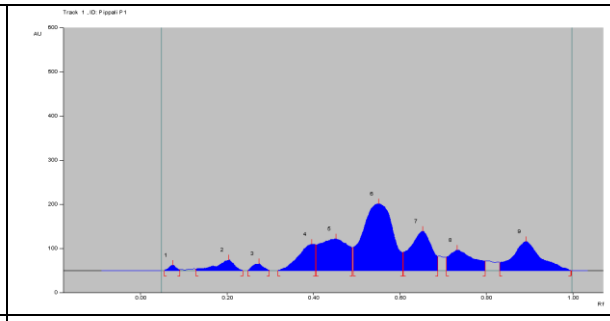
Chromatogram of P1,P2,P3 with Piperine

Densitograms of *Pippali* Methanol extracts

P1 Densitograms :

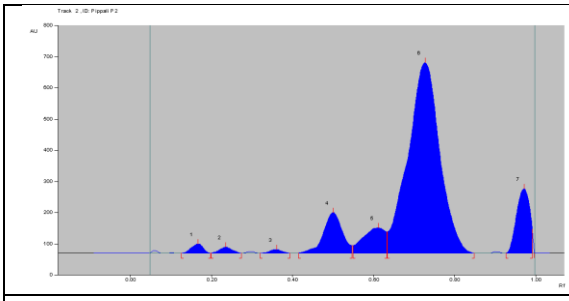


254 nm

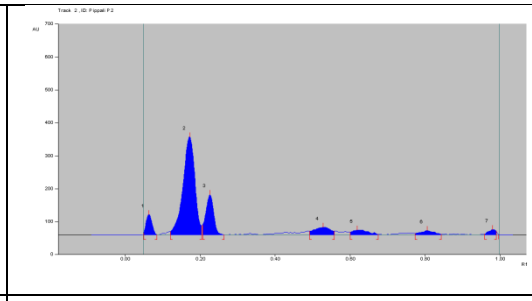


366 nm

P2 Densitograms :

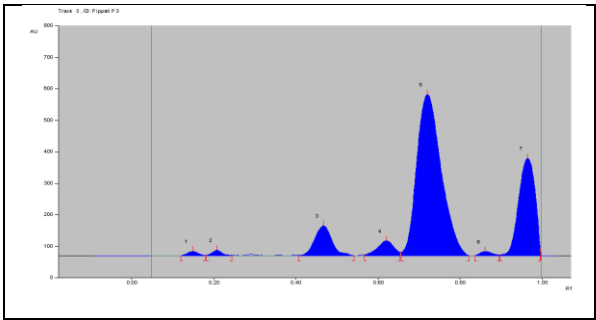
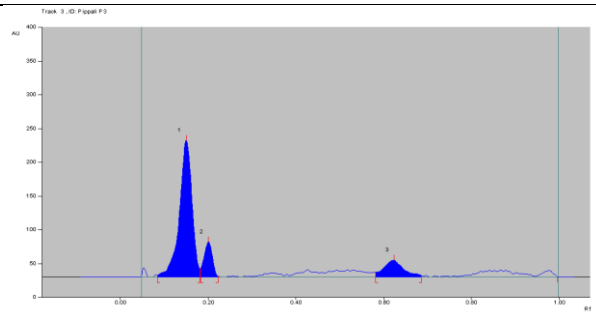


254 nm

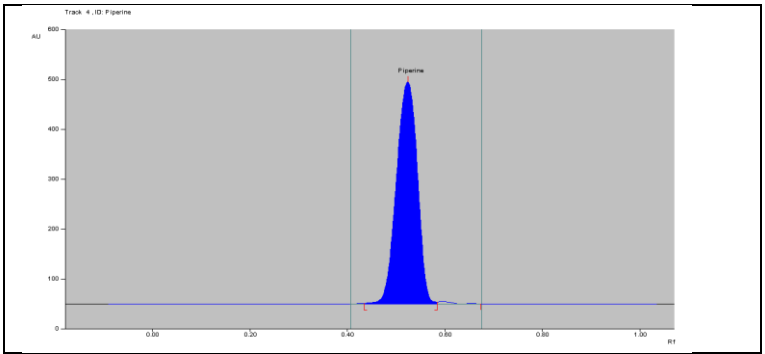


366 nm

P3 Densitograms :

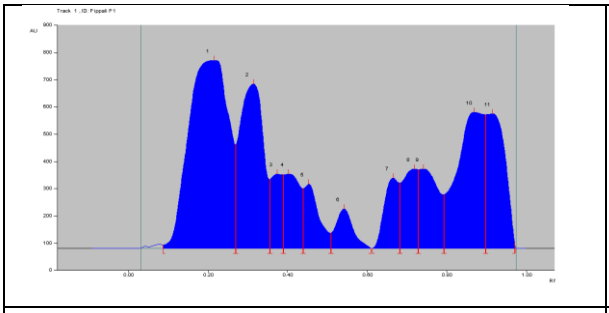
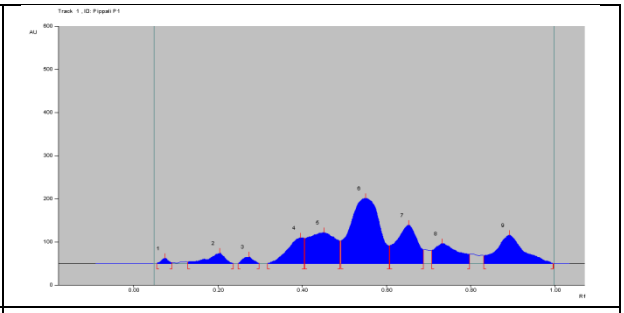
	
254 nm	366 nm

Piperine densitogram:

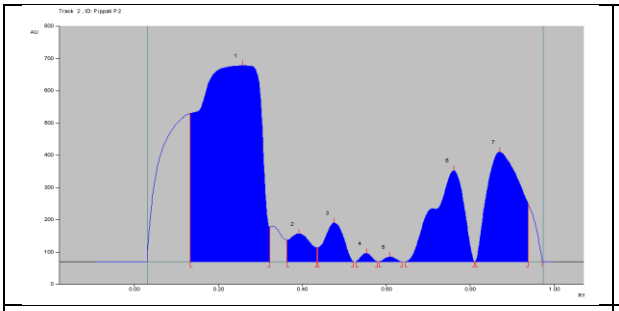
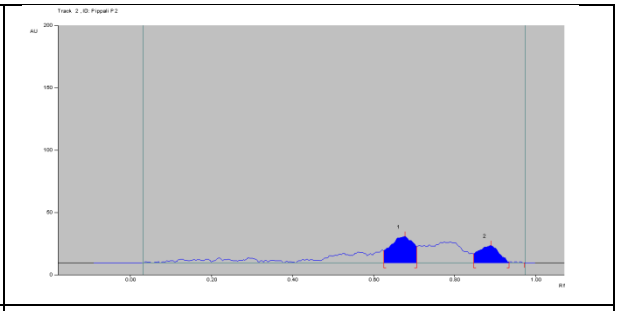

254 nm

Densitograms of Pippali volatile oil

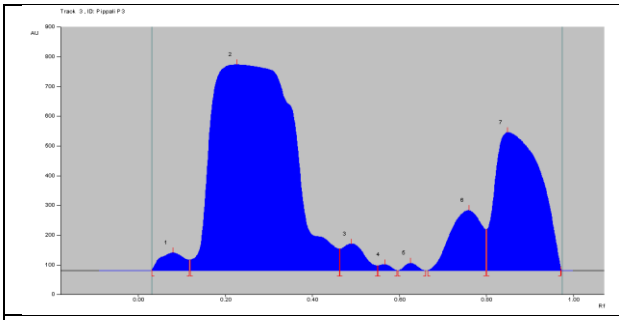
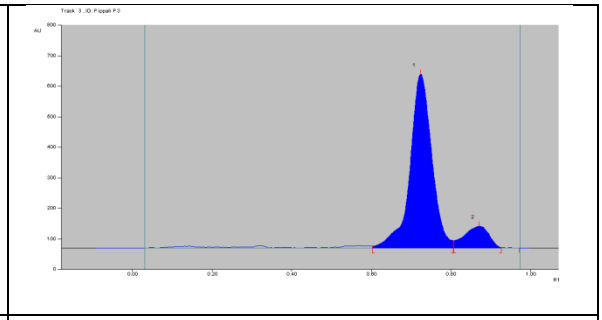
P1 Densitograms:

	
254 nm	366 nm

P2 Densitograms:

	
254 nm	366 nm

P3 Densitograms:

	
254 nm	366 nm

Analysis Report

SOP document	
Validated	Design
Description :	Toluene:Ethyl acetate (4.5:5.5)
Analysis	Pippali P1, P2, P3 profile.cna
Created/used by	RMD Research & Development Center Thursday, October 24, 2013 10:06:27 AM
Current user	RMD Research & Development Center

Stationary phase

Executed by	RMD Research & Development Center Wednesday, October 23, 2013 3:46:46 PM
Plate size (X x Y)	6.0 x 10.0 cm
Material	HPTLC plates silica gel 60 F 254
Manufacturer	E. MERCK KGaA
Batch	
GLP code	
Pre-washing	No
Modification	No

Definitions - Screening

Executed by	RMD Research & Development Center Wednesday, October 23, 2013 3:46:46 PM

Samples

Pippali P1
Pippali P2
Pippali P3

Sample application - CAMAG Linomat 5

Instrument	CAMAG Linomat 5
Executed by	RMD Research & Development Center Wednesday, October 23, 2013 3:47:19 PM

Linomat 5 application parameters

Spray gas :	Inert gas
Sample solvent type :	Methanol
Dosage speed :	150 nl/s
Predosage volume :	0.2 ul

Sequence

Syringe size:	100 µl
Number of tracks:	3
Application position Y :	10.0 mm
Band length :	9.0 mm

No.	Appl. position	Appl. volume	Vial #	Sample ID	Active
1	12.0 mm	8.0 µl	1	Pippali P1	Yes
2	27.3 mm	8.0 µl	2	Pippali P2	Yes
3	42.6 mm	8.0 µl	3	Pippali P3	Yes

Detection - CAMAG TLC Scanner

Information

Application position	10.0 mm
Solvent front position	66.0 mm

Instrument

	CAMAG TLC Scanner
Executed by	RMD Research & Development Center Wednesday, October 23, 2013 3:54:17 PM
Number of tracks	3
Position of first track X	11.0 mm
Distance between tracks	15.3 mm
Scan start pos. Y	5.0 mm
Scan end pos. Y	68.0 mm
Slit dimensions	6.00 x 0.90 mm, Macro
Optimize optical system	Light
Scanning speed:	20 mm/s
Data resolution:	100 µm/step

Integration

Properties

Data filtering	Savitsky-Golay 7
Baseline correction	Lowest Slope
Peak threshold min. slope	5
Peak threshold min. height	10 AU
Peak threshold min. area	50
Peak threshold max. height	990 AU
Track start position	12.7 mm
Track end position	65.9 mm
Display scaling	Automatic

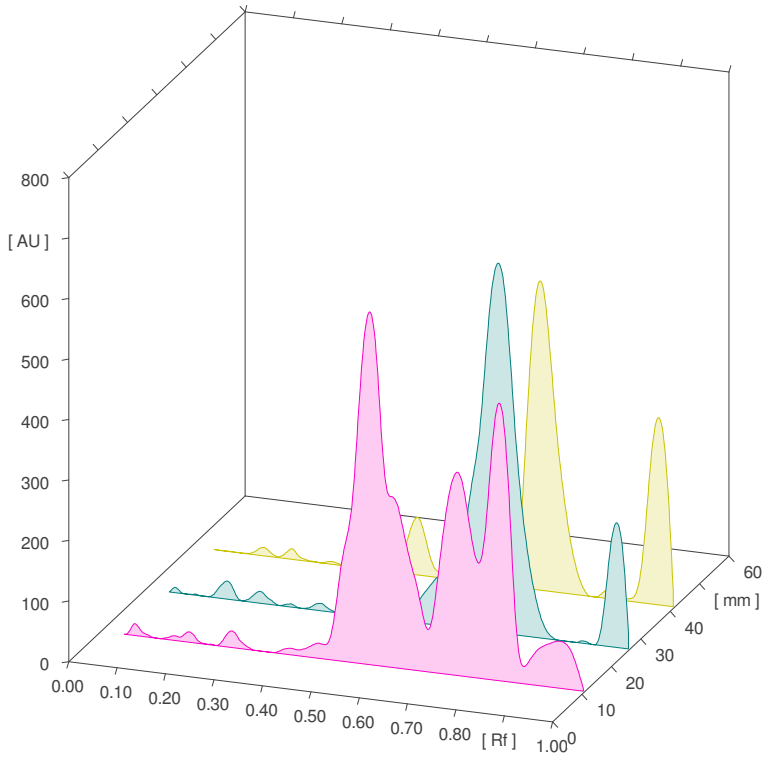
Measurement Table

Wavelength	254
Lamp	D2
Measurement Type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector mode	Automatic
PM high voltage	255 V

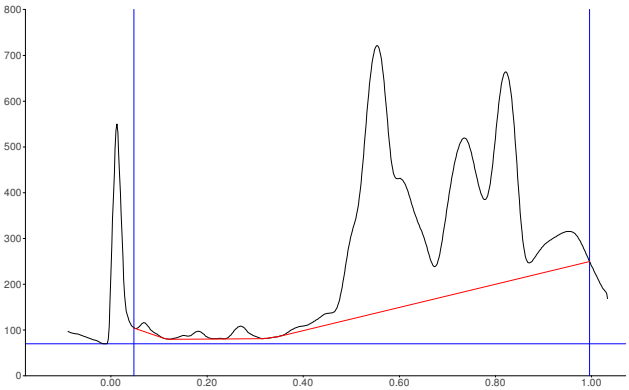
Detector properties

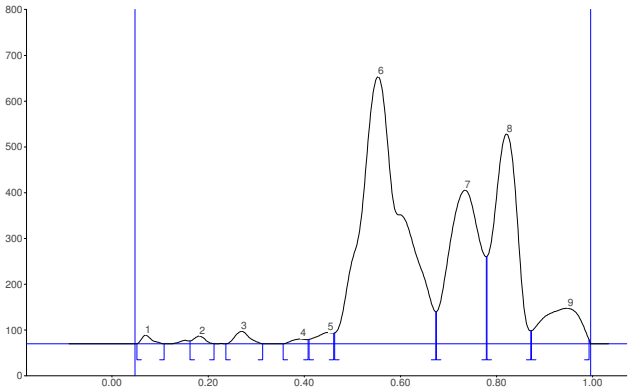
Y-position for 0 adjust	5.0 mm
Track # for 0 adjust	0
Analog Offset	10%
Sensitivity	Automatic (183)

All tracks at WavelengthSc4



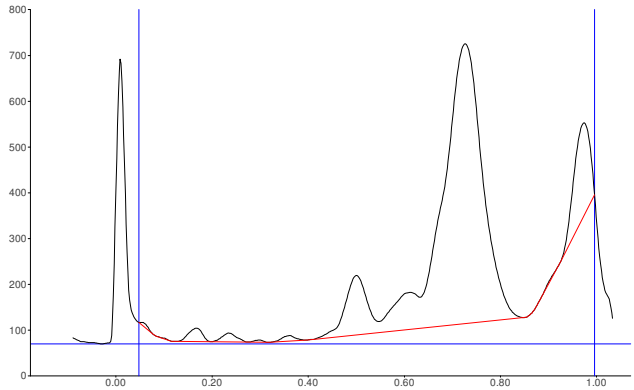
Track 1, ID: Pippali P1

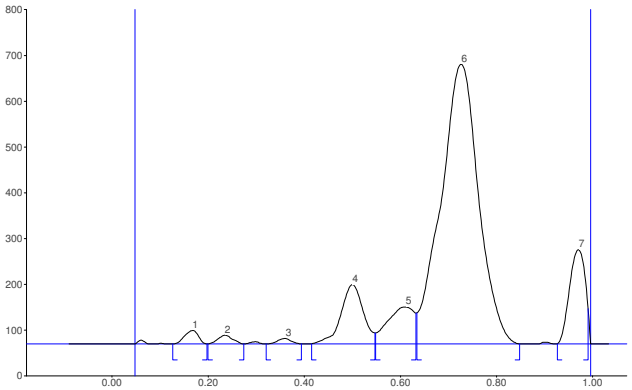




Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.05	0.2	0.07	19.2	1.23	0.11	0.0	260.6	0.40	unknown *
2	0.16	6.4	0.18	17.1	1.10	0.21	0.7	269.7	0.41	unknown *
3	0.24	0.1	0.27	27.4	1.76	0.31	0.1	537.9	0.82	unknown *
4	0.36	0.3	0.39	10.9	0.70	0.41	9.7	222.3	0.34	unknown *
5	0.41	9.9	0.45	25.1	1.62	0.46	23.7	573.4	0.88	unknown *
6	0.46	24.0	0.56	583.2	37.51	0.68	70.4	31556.7	48.21	unknown *
7	0.68	70.5	0.74	335.8	21.60	0.78	190.3	13906.3	21.25	unknown *
8	0.78	191.1	0.82	458.3	29.48	0.87	28.8	14167.6	21.65	unknown *
9	0.88	29.0	0.95	77.7	4.99	0.99	9.5	3958.1	6.05	unknown *

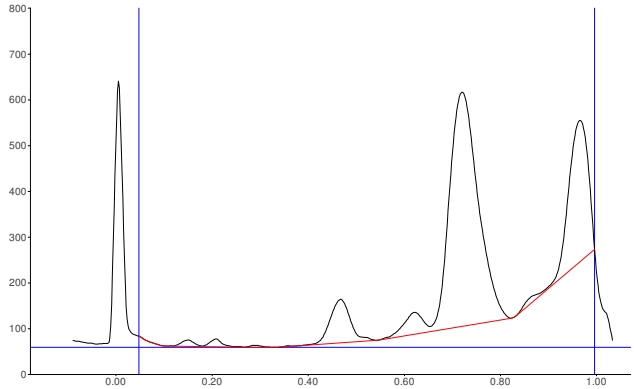
Track 2, ID: Pippali P2

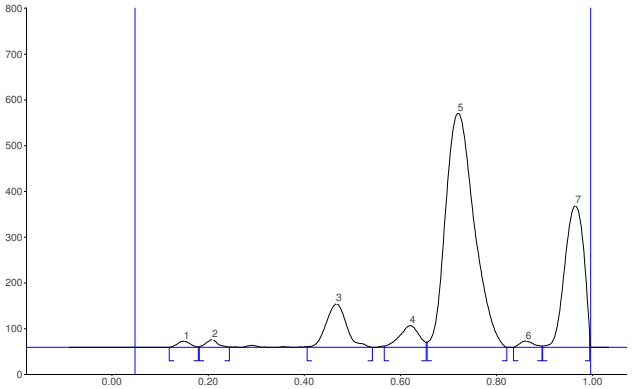




Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.13	0.1	0.17	29.5	2.71	0.20	0.3	554.7	1.28	unknown *
2	0.20	0.3	0.24	19.4	1.78	0.28	0.2	384.5	0.89	unknown *
3	0.32	0.0	0.36	12.2	1.12	0.39	0.3	225.8	0.52	unknown *
4	0.42	0.6	0.50	129.9	11.93	0.55	24.0	3923.5	9.05	unknown *
5	0.55	24.1	0.61	80.7	7.42	0.63	67.7	2907.0	6.71	unknown *
6	0.64	68.0	0.73	611.0	56.12	0.85	0.2	30976.5	71.48	unknown *
7	0.93	0.4	0.97	206.0	18.92	0.99	76.9	4364.7	10.07	unknown *

Track 3, ID: Pippali P3





Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.12	0.5	0.15	13.9	1.38	0.18	1.2	241.0	0.74	unknown *
2	0.18	1.5	0.21	17.4	1.72	0.24	0.9	275.8	0.85	unknown *
3	0.41	1.5	0.47	95.1	9.44	0.54	0.1	2606.0	8.04	unknown *
4	0.57	3.1	0.62	47.5	4.72	0.66	11.2	1252.3	3.86	unknown *
5	0.66	11.4	0.72	511.4	50.72	0.82	0.2	19783.0	61.00	unknown *
6	0.84	0.3	0.86	13.6	1.34	0.90	3.3	264.5	0.82	unknown *
7	0.90	3.4	0.97	309.4	30.68	1.00	26.1	8006.6	24.69	unknown *

Measurement Table

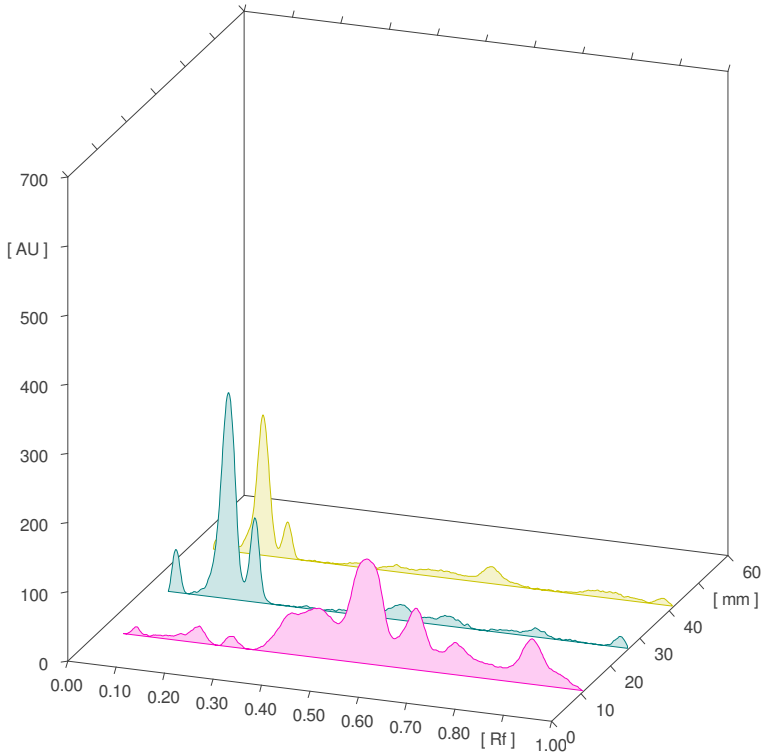
Wavelength	366
Lamp	Hg
Measurement Type	Remission
Measurement Mode	Fluorescence
Optical filter	K400
Detector mode	Automatic
PM high voltage	255 V

User : RMD Research & Development Center
Sunday, October 27, 2013 11:30:07 AM

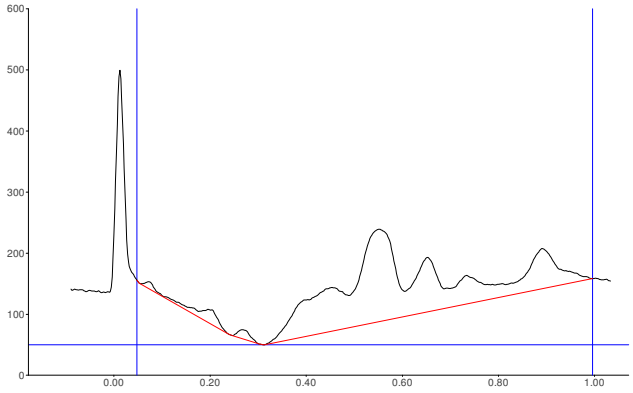
Detector properties

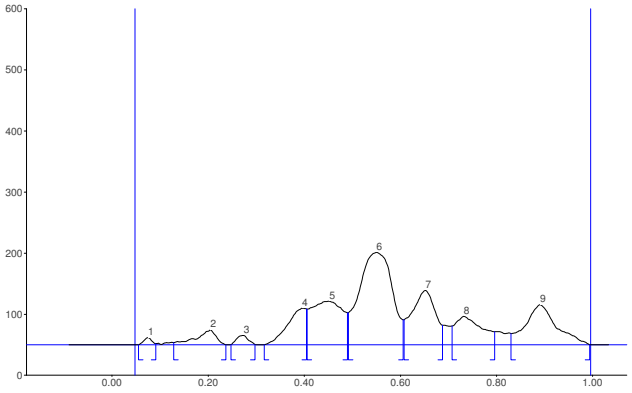
Y-position for 0 adjust	5.0 mm
Track # for 0 adjust	0
Analog Offset	10%
Sensitivity	Automatic (183)

All tracks at WavelengthSc4



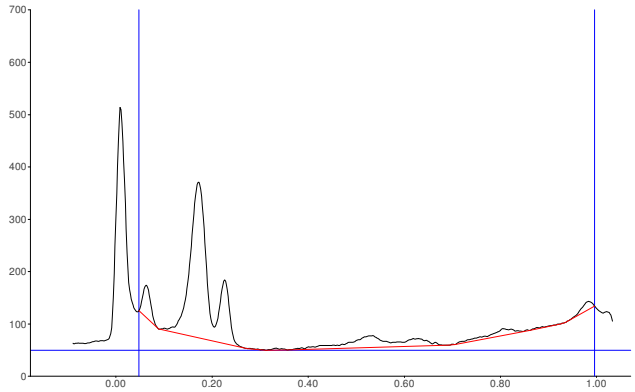
Track 1, ID: Pippali P1

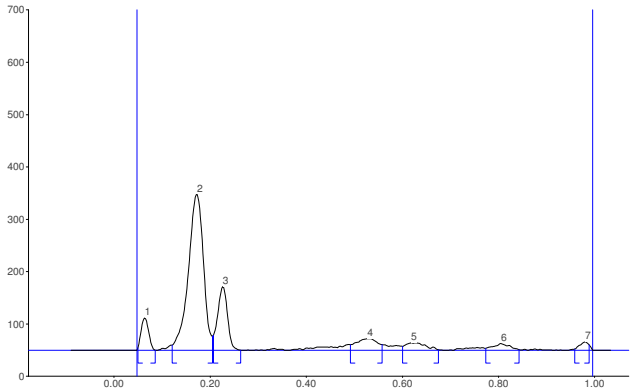




Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.06	0.3	0.08	12.0	2.24	0.09	2.2	131.1	0.67	unknown *
2	0.13	3.7	0.21	24.6	4.59	0.24	0.1	659.1	3.35	unknown *
3	0.25	0.6	0.28	15.5	2.88	0.30	0.7	249.5	1.27	unknown *
4	0.32	0.4	0.40	60.2	11.21	0.41	59.1	1513.3	7.69	unknown *
5	0.41	58.7	0.45	71.6	13.31	0.49	52.7	3079.4	15.64	unknown *
6	0.49	52.9	0.55	151.5	28.18	0.61	41.0	6750.2	34.28	unknown *
7	0.61	41.2	0.65	89.4	16.62	0.69	32.3	2831.0	14.38	unknown *
8	0.71	31.0	0.73	46.7	8.69	0.80	21.6	1712.1	8.70	unknown *
9	0.83	18.7	0.89	66.0	12.28	1.00	0.4	2764.7	14.04	unknown *

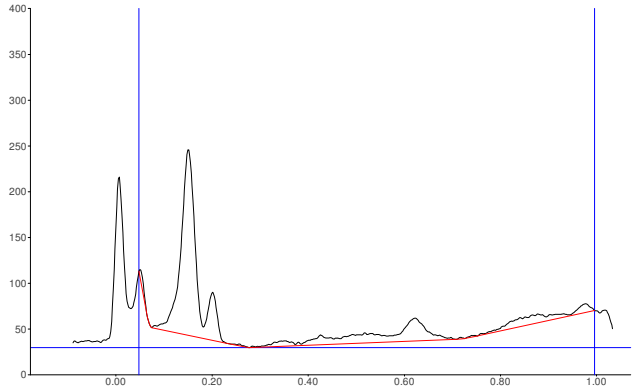
Track 2, ID: Pippali P2

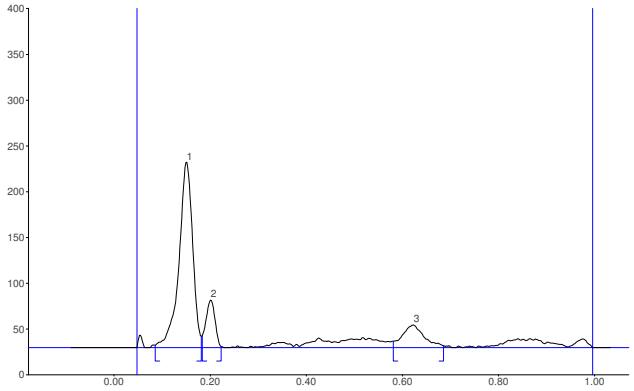




Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.05	5.4	0.06	61.9	11.29	0.09	0.5	636.3	6.38	unknown *
2	0.12	10.2	0.17	297.8	54.33	0.21	27.2	6135.5	61.54	unknown *
3	0.21	29.2	0.23	121.8	22.23	0.26	0.5	1638.3	16.43	unknown *
4	0.49	11.0	0.53	22.6	4.12	0.56	11.2	652.3	6.54	unknown *
5	0.60	7.9	0.62	14.7	2.69	0.68	1.2	410.5	4.12	unknown *
6	0.77	3.8	0.81	13.2	2.40	0.84	1.8	300.9	3.02	unknown *
7	0.96	2.8	0.98	16.1	2.94	0.99	10.7	196.5	1.97	unknown *

Track 3, ID: Pippali P3





Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.09	2.7	0.15	202.5	72.41	0.18	12.4	3775.2	73.21	unknown *
2	0.18	13.1	0.20	52.3	18.72	0.22	1.1	641.8	12.45	unknown *
3	0.58	7.2	0.63	24.8	8.87	0.69	2.2	739.5	14.34	unknown *

Documentation

Executed by RMD Research & Development Center Thursday, October 24, 2013 10:01:38 AM

Image Document

Executed by RMD Research & Development Center Thursday, October 24, 2013 10:01:35 AM

Child image
Image name Chromatogram at 254 nm

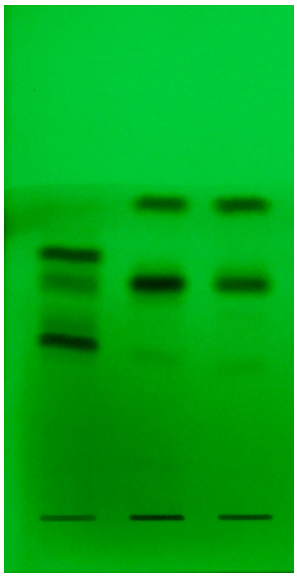
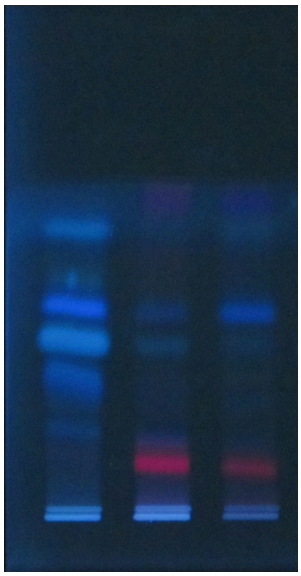


Image Document

Executed by RMD Research & Development Center Thursday, October 24,2013 10:01:38 AM

Child image
Image name Chromatogram at 366 nm



Analysis Report

SOP document	
Validated	Design
Description :	Toluene:Ethyl acetate (4.5:5.5)
Analysis	Piperine estimation in Pippali P1, P2, P3.cna
Created/used by	RMD Research & Development Center Sunday, October 27, 2013 11:28:59 AM
Current user	RMD Research & Development Center

Stationary phase

Executed by	RMD Research & Development Center Thursday, October 24, 2013 9:56:54 AM
Plate size (X x Y)	7.0 x 10.0 cm
Material	HPTLC plates silica gel 60 F 254
Manufacturer	E. MERCK KGaA
Batch	
GLP code	
Pre-washing	No
Modification	No

Definitions - Screening

Executed by: RMD Research & Development Center Thursday, October 24, 2013 9:57:17 AM

Samples

Pippali P1
Pippali P2
Pippali P3
Piperine

Substance name	Rf	Window size
Piperine	0.54	8.500

Sample application - CAMAG Linomat 5

Instrument	CAMAG Linomat 5
Executed by	RMD Research & Development Center Wednesday, October 23, 2013 2:17:26 PM

Linomat 5 application parameters

Spray gas :	Inert gas
Sample solvent type :	Methanol
Dosage speed :	150 nl/s
Predosage volume :	0.2 ul

Sequence

Syringe size:	100 µl
Number of tracks:	4
Application position Y :	10.0 mm
Band length :	9.0 mm

No.	Appl. position	Appl. volume	Vial #	Sample ID	Active
>1	12.0 mm	8.0 µl	1	Pippali P1	Yes
>2	27.3 mm	8.0 µl	2	Pippali P2	Yes

>3	42.6 mm	8.0 µl	3	Pippali P3	Yes
>4	57.9 mm	4.0 µl	4	Piperine	Yes

Detection - CAMAG TLC Scanner

Information

Application position	10.0 mm
Solvent front position	66.0 mm

Instrument

	CAMAG TLC Scanner
Executed by	RMD Research & Development Center Thursday, October 24, 2013 9:56:54 AM
Number of tracks	4
Position of first track X	11.0 mm
Distance between tracks	15.3 mm
Scan start pos. Y	5.0 mm
Scan end pos. Y	68.0 mm
Slit dimensions	6.00 x 0.90 mm, Macro
Optimize optical system	Light
Scanning speed:	20 mm/s
Data resolution:	100 µm/step

Measurement Table

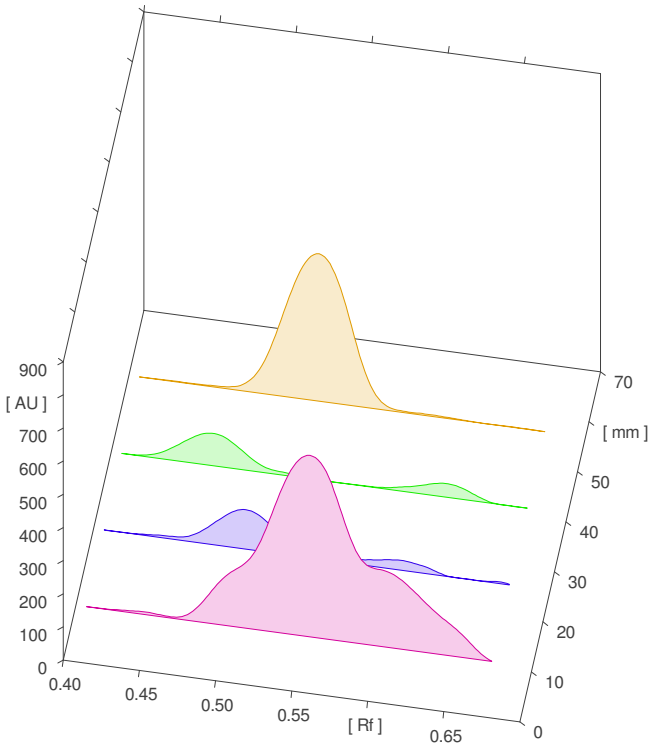
Wavelength	254
Lamp	D2
Measurement Type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector mode	Automatic
PM high voltage	270 V

Integration

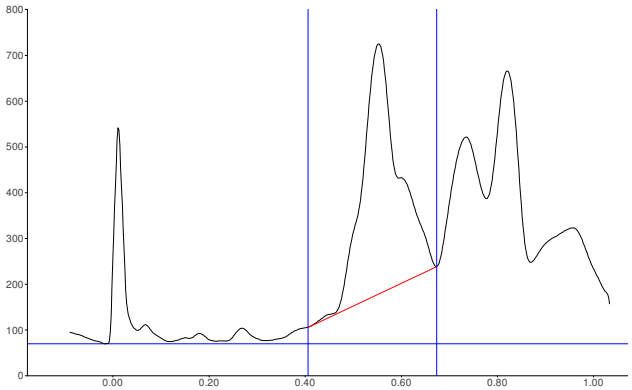
Properties

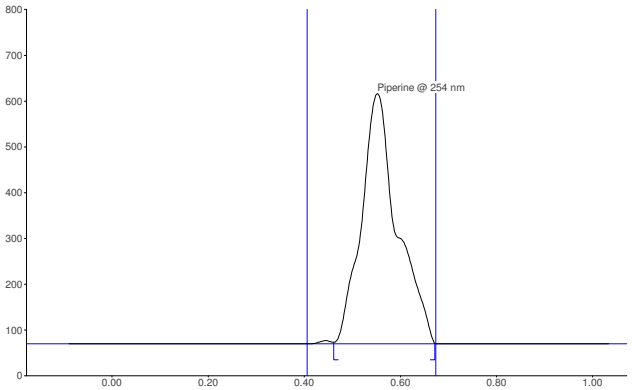
Data filtering	Savitsky-Golay 7
Baseline correction	Lowest Slope
Peak threshold min. slope	5
Peak threshold min. height	10 AU
Peak threshold min. area	50
Peak threshold max. height	990 AU
Track start position	32.8 mm
Track end position	47.8 mm
Display scaling	Automatic

All tracks at WavelengthSc4



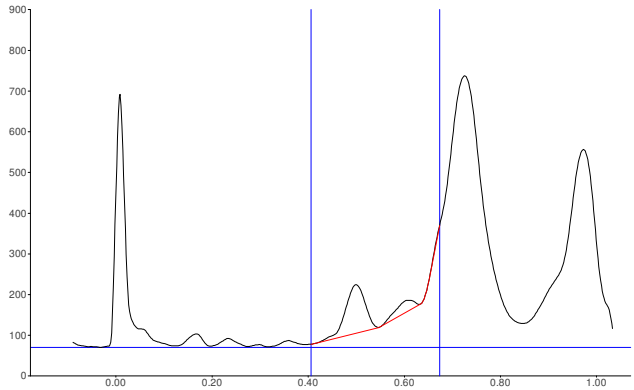
Track 1, ID: Pippali P1

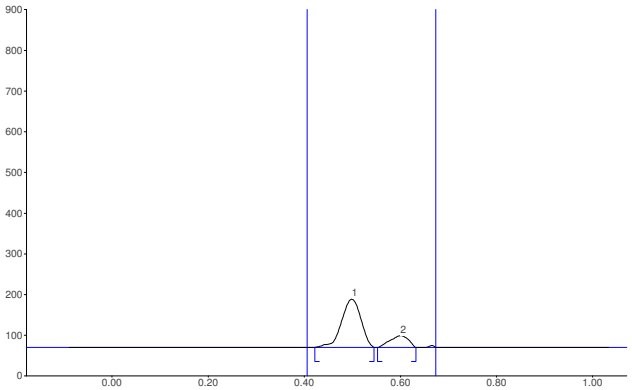




Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.46	3.5	0.55	546.7	100.00	0.67	1.2	26475.5	100.00	Piperine

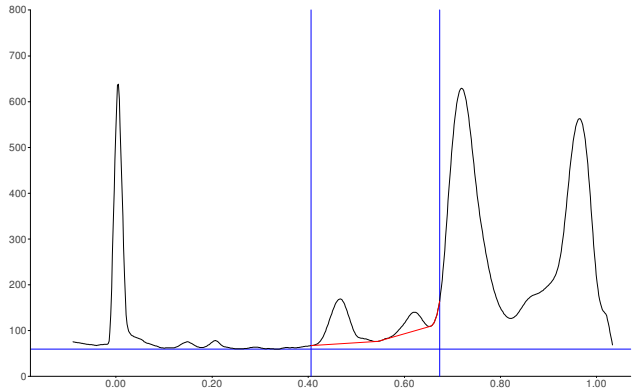
Track 2, ID: Pippali P2

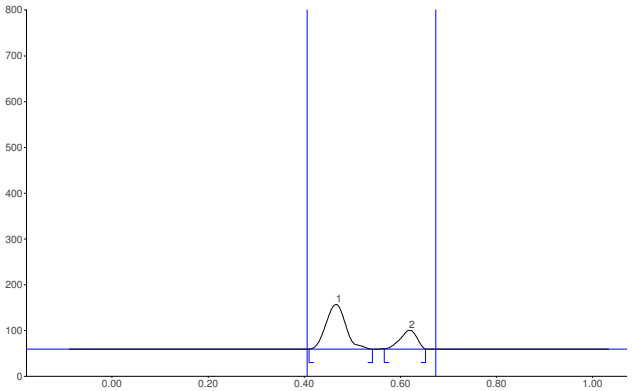




Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.42	0.6	0.50	119.0	80.22	0.55	0.7	3136.1	80.98	unknown *
2	0.55	0.2	0.60	29.3	19.78	0.63	0.2	736.6	19.02	unknown *

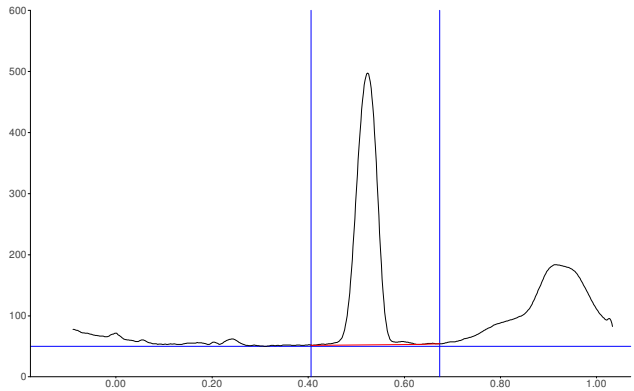
Track 3, ID: Pippali P3

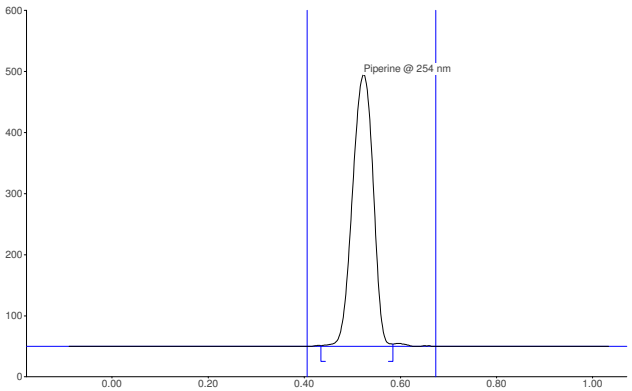




Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.41	0.3	0.47	97.9	70.30	0.54	0.0	2635.8	73.55	unknown *
2	0.57	1.0	0.62	41.4	29.70	0.65	0.4	947.9	26.45	unknown *

Track 4, ID: Piperine





Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.44	1.5	0.52	445.2	100.00	0.59	3.9	12456.0	100.00	Piperine

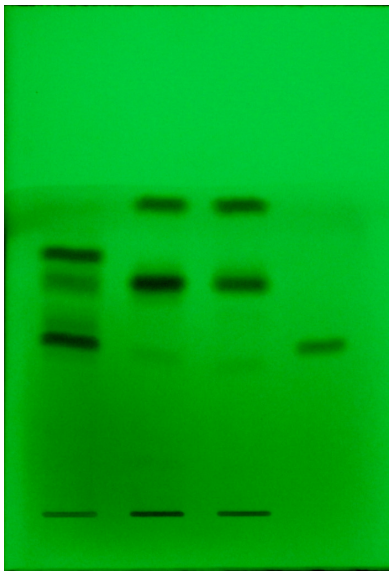
Documentation

Executed by RMD Research & Development Center Thursday, October 24, 2013 9:37:06 AM

Image Document

Executed by RMD Research & Development Center Thursday, October 24, 2013 9:37:05 AM

Image name Chromatogram at 254 nm



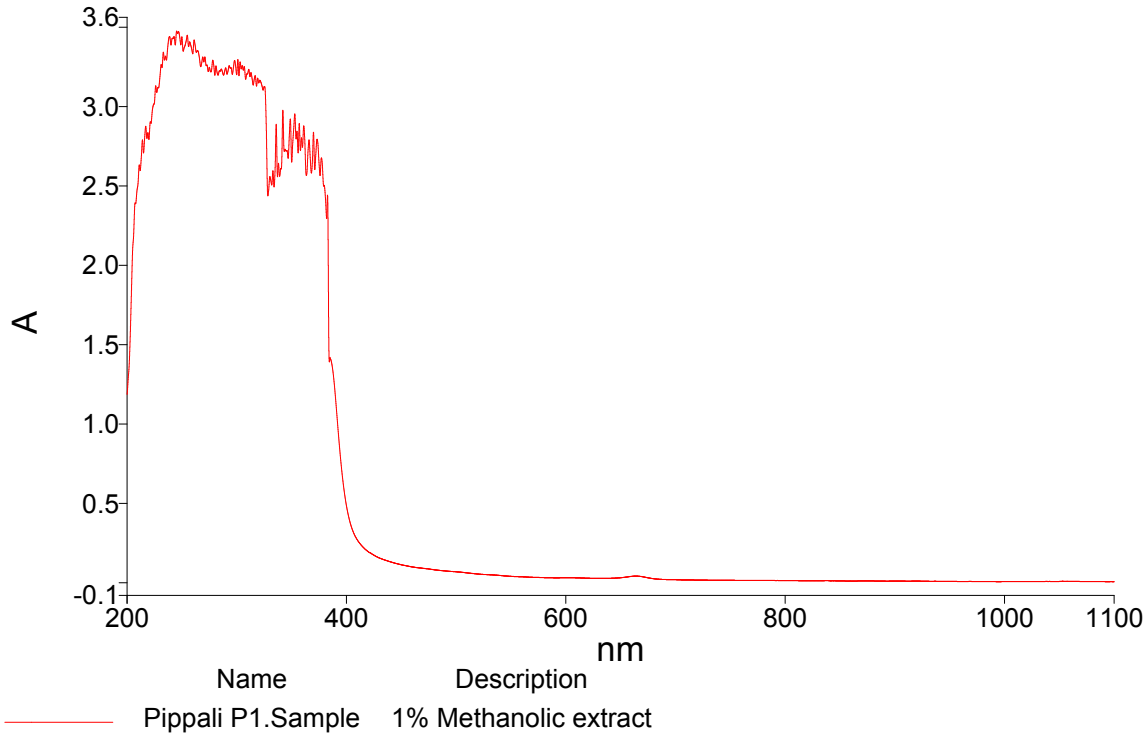
Created by RMD Research & Development Center on Thursday, October 24, 2013 9:37:05 AM

Analyst	RMD Research and Development Center
Date	Thursday, October 24, 2013 1:36 PM

Sample	
Setting	Value
Filename	Pippali P1.Sample
Creation Date	10/24/2013 12:33:54 PM
Analyst	rmd
X-Axis Units	nm
X-Axis start value	1100
X-Axis end value	200
Data interval	-1
Number of points	901
Y-Axis Units	A
Description	

Instrument	
Setting	Value
Instrument Model	Lambda 25
Software Revision	PerkinElmer UV WinLab 6.0.3.0730 / Lambda25 1.27
Scan Speed (nm/min)	240
Slit Width (nm)	1
Lamps	Both on
Cycles	1
Accessory	None
Lamp change at (nm)	326

Pippali P1



Sample ID	Pippali P1.Sample
Description	1% Methanolic extract
Threshold	0.01
Abscissa Range	1100 - 200 nm
Display Options	Peaks Listed by position

	Position (nm)	Intensity	Type
1	663.5	0.04306	Peak
2	382.8	2.443	Peak
3	377.2	2.677	Peak
4	373.3	2.796	Peak

PerkinElmer
precisely

	Position (nm)	Intensity	Type
5	370.0	2.840	Peak
6	365.9	2.791	Peak
7	361.1	2.879	Peak
8	359.0	2.805	Peak
9	357.0	2.895	Peak
10	354.9	2.845	Peak
11	352.9	2.955	Peak
12	348.8	2.922	Peak
13	342.0	2.978	Peak
14	338.0	2.644	Peak
15	336.0	2.889	Peak
16	333.0	2.597	Peak
17	330.2	2.559	Peak
18	325.4	3.127	Peak
19	321.1	3.167	Peak
20	319.1	3.180	Peak
21	316.8	3.195	Peak
22	313.0	3.214	Peak
23	310.9	3.235	Peak
24	306.9	3.252	Peak
25	305.0	3.258	Peak
26	303.1	3.282	Peak
27	301.0	3.297	Peak
28	298.0	3.291	Peak
29	293.1	3.258	Peak
30	289.9	3.244	Peak
31	286.0	3.237	Peak
32	281.1	3.257	Peak
33	278.1	3.293	Peak

	Position (nm)	Intensity	Type
34	275.0	3.248	Peak
35	270.9	3.313	Peak
36	269.0	3.313	Peak
37	261.1	3.420	Peak
38	257.1	3.408	Peak
39	254.9	3.450	Peak
40	250.0	3.440	Peak
41	246.9	3.473	Peak
42	245.2	3.476	Peak
43	242.8	3.441	Peak
44	238.7	3.442	Peak
45	235.0	3.318	Peak
46	233.1	3.343	Peak
47	231.1	3.265	Peak
48	226.1	3.131	Peak
49	219.0	2.836	Peak
50	217.0	2.876	Peak
51	214.0	2.791	Peak
52	211.1	2.633	Peak

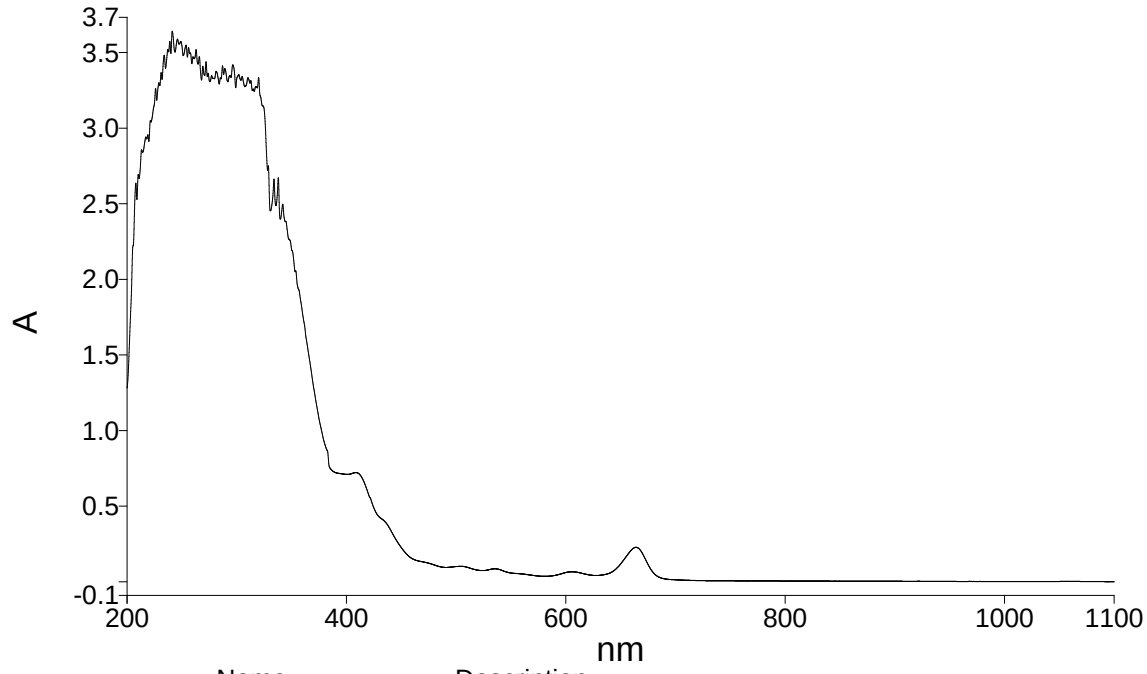
Analyst	RMD Research and Development Center
Date	Thursday, October 24, 2013 1:35 PM

Sample	
Setting	**Value**
Filename	Pippali P2.Sample
Creation Date	10/24/2013 12:23:25 PM
Analyst	rmd
X-Axis Units	nm
X-Axis start value	1100
X-Axis end value	200
Data interval	-1
Number of points	901
Y-Axis Units	A
Description	1% Methanolic extract

Instrument	
Setting	**Value**
Instrument Model	Lambda 25
Software Revision	PerkinElmer UV WinLab 6.0.3.0730 / Lambda25 1.27
Scan Speed (nm/min)	240
Slit Width (nm)	1
Lamps	Both on
Cycles	1
Accessory	None
Lamp change at (nm)	326

Pippali P2

Raw Spectra



Name	Description
Pippali P2.Sample	1% Methanolic extract

Peak Table

Sample ID	Pippali P2.Sample
Description	1% Methanolic extract
Threshold	0.01
Abscissa Range	1100 - 200 nm
Display Options	Peaks Listed by position

	Position (nm)	Intensity	Type
1	664.1	0.2288	Peak
2	606.0	0.06720	Peak
3	535.3	0.08689	Peak
4	408.6	0.7232	Peak

PerkinElmer precisely

Printed by:	rmd
Template:	SCAN WITH PEAK TABLE
Print Date and Time:	October 24, 2013 15:32:01 India Standard Time

	Position (nm)	Intensity	Type
5	341.9	2.498	Peak
6	337.9	2.673	Peak
7	334.0	2.665	Peak
8	328.8	2.752	Peak
9	319.9	3.335	Peak
10	312.9	3.315	Peak
11	310.2	3.334	Peak
12	305.0	3.343	Peak
13	301.8	3.354	Peak
14	296.4	3.419	Peak
15	289.1	3.394	Peak
16	287.0	3.413	Peak
17	281.3	3.377	Peak
18	277.1	3.347	Peak
19	273.9	3.364	Peak
20	272.0	3.440	Peak
21	269.1	3.410	Peak
22	265.9	3.474	Peak
23	262.9	3.520	Peak
24	260.1	3.473	Peak
25	256.0	3.533	Peak
26	253.8	3.550	Peak
27	248.9	3.575	Peak
28	246.0	3.590	Peak
29	241.2	3.643	Peak
30	239.0	3.575	Peak
31	237.1	3.522	Peak
32	233.9	3.483	Peak
33	231.1	3.368	Peak

	Position (nm)	Intensity	Type
34	229.1	3.301	Peak
35	226.0	3.264	Peak
36	219.0	2.957	Peak
37	217.1	2.942	Peak
38	213.2	2.858	Peak
39	210.2	2.695	Peak
40	208.0	2.638	Peak

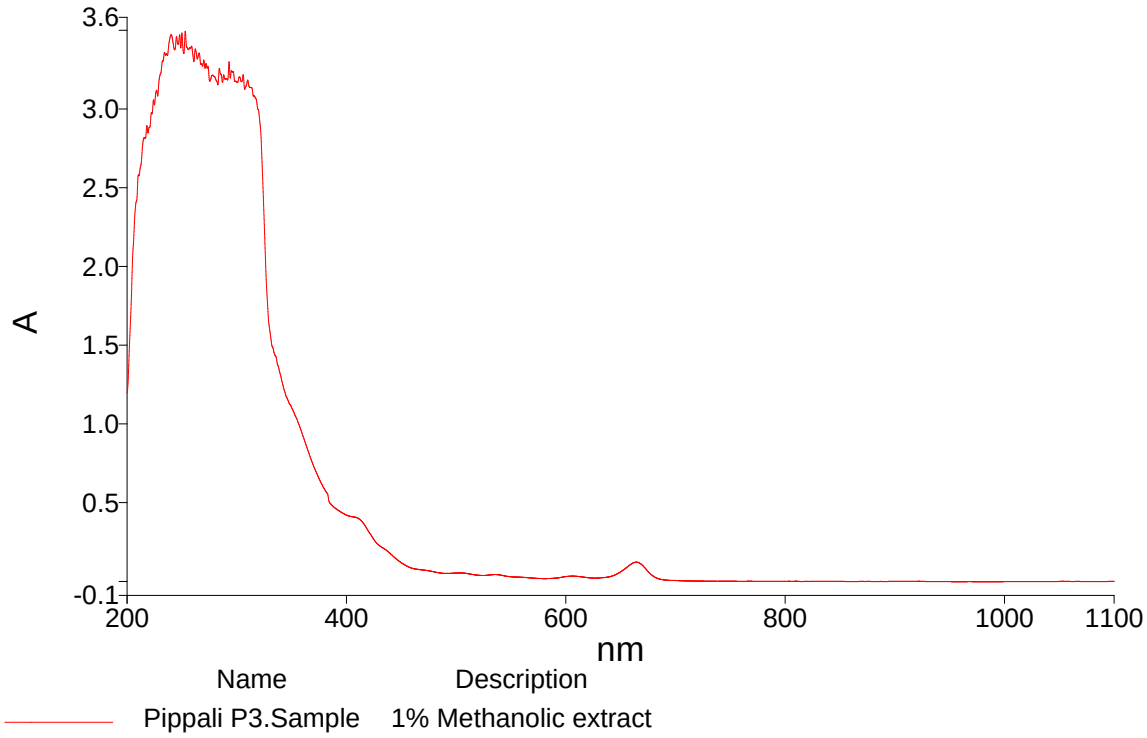
Analyst	RMD Research and Development Center
Date	Thursday, October 24, 2013 1:35 PM

Sample	
Setting	**Value**
Filename	Pippali P3.Sample
Creation Date	10/24/2013 12:28:21 PM
Analyst	rmd
X-Axis Units	nm
X-Axis start value	1100
X-Axis end value	200
Data interval	-1
Number of points	901
Y-Axis Units	A
Description	1% Methanolic extract

Instrument	
Setting	**Value**
Instrument Model	Lambda 25
Software Revision	PerkinElmer UV WinLab 6.0.3.0730 / Lambda25 1.27
Scan Speed (nm/min)	240
Slit Width (nm)	1
Lamps	Both on
Cycles	1
Accessory	None
Lamp change at (nm)	326

Pippali P3

Raw Spectra



Peak Table

Sample ID	Pippali P3.Sample
Description	1% Methanolic extract
Threshold	0.01
Abscissa Range	1100 - 200 nm
Display Options	Peaks Listed by position

	Position (nm)	Intensity	Type
1	664.5	0.1232	Peak
2	606.3	0.03456	Peak
3	310.0	3.185	Peak
4	305.9	3.219	Peak

PerkinElmer precisely

Printed by: rmd

Template: SCAN WITH PEAK TABLE

Print Date and Time: October 24, 2013 15:32:57 India Standard Time

	Position (nm)	Intensity	Type
5	302.5	3.204	Peak
6	295.2	3.245	Peak
7	293.0	3.301	Peak
8	288.1	3.218	Peak
9	284.1	3.259	Peak
10	278.0	3.216	Peak
11	272.0	3.290	Peak
12	270.0	3.313	Peak
13	265.7	3.356	Peak
14	262.2	3.382	Peak
15	258.8	3.400	Peak
16	253.1	3.496	Peak
17	250.0	3.484	Peak
18	248.0	3.473	Peak
19	245.1	3.462	Peak
20	240.2	3.473	Peak
21	234.1	3.361	Peak
22	226.9	3.120	Peak
23	224.1	3.059	Peak
24	218.0	2.896	Peak

Analysis Report

SOP document	
Validated	Design
Description :	Toluene:ethyl acetate (9.3:0.7)
Analysis	Pippali Volatile oil P1, P2, P3.cna
Created/used by	RMD Research & Development Center Sunday, October 27, 2013 11:56:28 AM
Current user	RMD Research & Development Center

Stationary phase

Executed by	RMD Research & Development Center Saturday, October 26, 2013 9:21:24 AM
Plate size (X x Y)	6.0 x 10.0 cm
Material	HPTLC plates silica gel 60 F 254
Manufacturer	E. MERCK KGaA
Batch	
GLP code	
Pre-washing	No
Modification	No

Definitions - Screening

Executed by	RMD Research & Development Center Saturday, October 26, 2013 9:21:40 AM

Samples

Pippali P1
Pippali P2
Pippali P3

Sample application - CAMAG Linomat 5

Instrument	CAMAG Linomat 5
Executed by	RMD Research & Development Center Saturday, October 26, 2013 9:42:43 AM

Linomat 5 application parameters

Spray gas :	Inert gas
Sample solvent type :	User defined
Dosage speed :	300 nl/s
Predosage volume :	0.2 ul

Sequence

Syringe size:	100 µl
Number of tracks:	3
Application position Y :	10.0 mm
Band length :	9.0 mm

Detection - CAMAG TLC Scanner

Information

Application position	10.0 mm
Solvent front position	66.0 mm

Instrument	CAMAG TLC Scanner
Executed by	RMD Research & Development Center Saturday, October 26, 2013 10:07:26 AM
Number of tracks	3
Position of first track X	14.0 mm
Distance between tracks	16.0 mm
Scan start pos. Y	5.0 mm
Scan end pos. Y	66.0 mm
Slit dimensions	6.00 x 0.90 mm, Macro
Optimize optical system	Light
Scanning speed:	20 mm/s
Data resolution:	100 µm/step

Integration

Properties

Data filtering	Savitsky-Golay 7
Baseline correction	Lowest Slope
Peak threshold min. slope	5
Peak threshold min. height	10 AU
Peak threshold min. area	50
Peak threshold max. height	990 AU
Track start position	11.8 mm
Track end position	64.6 mm
Display scaling	Automatic

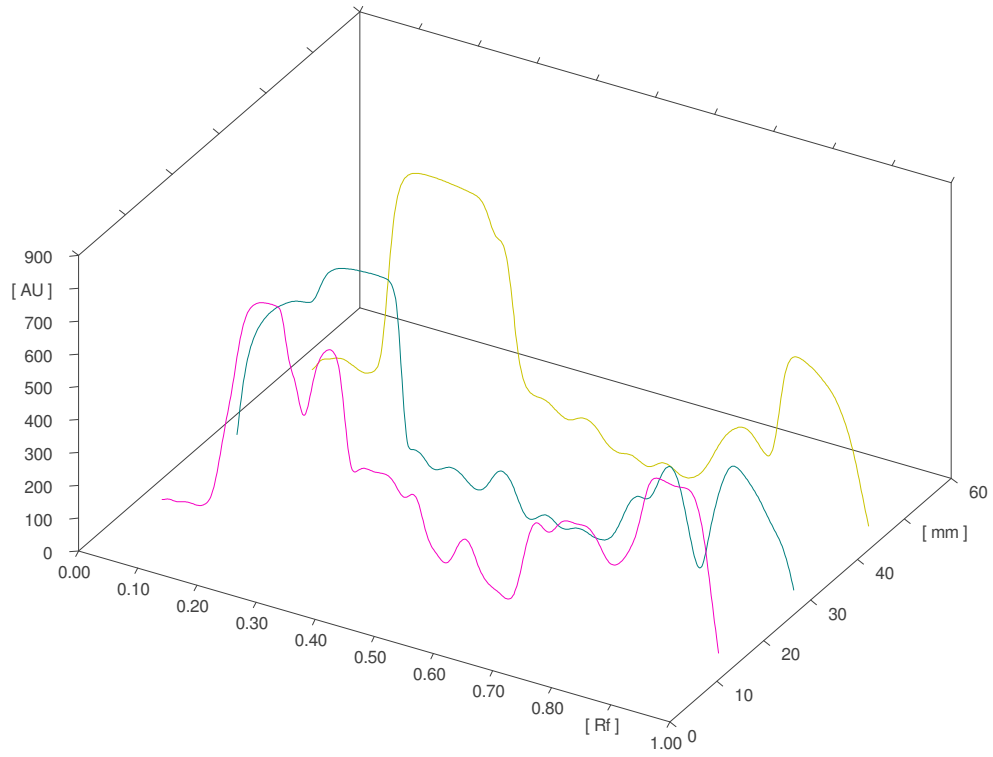
Measurement Table

Wavelength	254
Lamp	D2
Measurement Type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector mode	Automatic
PM high voltage	246 V

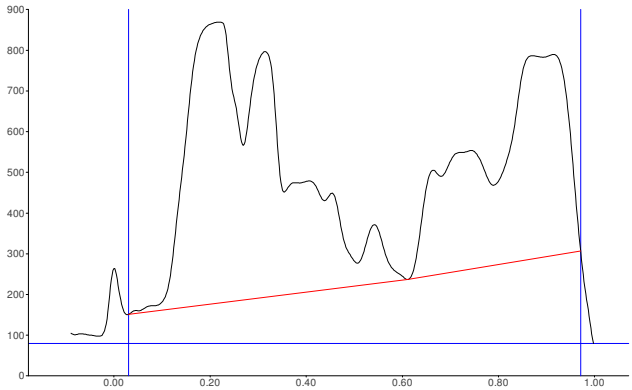
Detector properties

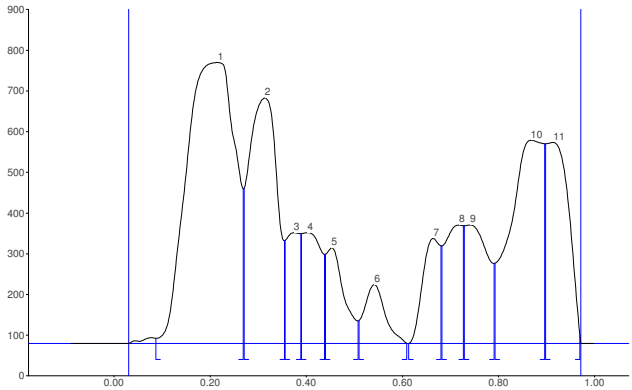
Y-position for 0 adjust	5.0 mm
Track # for 0 adjust	0
Analog Offset	10%
Sensitivity	Automatic (171)

All tracks at WavelengthSc4



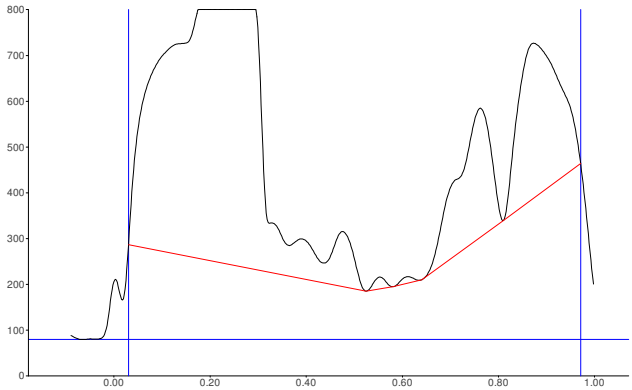
Track 1, ID: Pippali P1

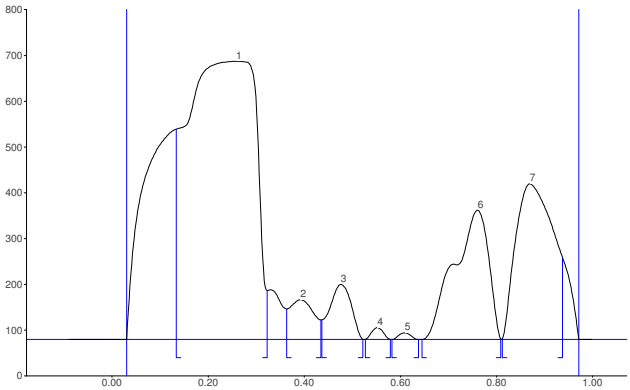




Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.09	13.1	0.22	690.4	17.03	0.27	379.8	45190.1	30.04	unknown *
2	0.27	381.7	0.31	603.3	14.89	0.36	252.5	23229.6	15.44	unknown *
3	0.36	253.5	0.38	272.6	6.72	0.39	270.0	5086.1	3.38	unknown *
4	0.39	270.1	0.40	272.5	6.72	0.44	219.1	7171.8	4.77	unknown *
5	0.44	219.5	0.45	235.3	5.80	0.51	55.7	5747.0	3.82	unknown *
6	0.51	56.7	0.54	144.9	3.57	0.61	0.5	4044.5	2.69	unknown *
7	0.61	0.0	0.67	258.1	6.37	0.68	239.9	6096.7	4.05	unknown *
8	0.68	239.9	0.72	291.2	7.18	0.73	289.4	7115.6	4.73	unknown *
9	0.73	289.5	0.74	291.4	7.19	0.79	196.9	9220.0	6.13	unknown *
10	0.79	197.5	0.87	499.5	12.32	0.90	490.9	22446.4	14.92	unknown *
11	0.90	491.0	0.92	494.2	12.19	0.97	21.2	15068.2	10.02	unknown *

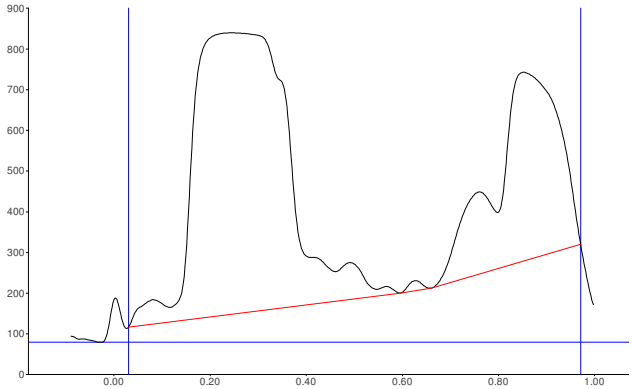
Track 2, ID: Pippali P2

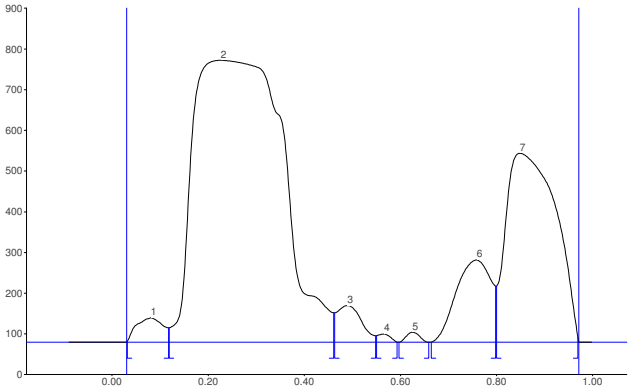




Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.13	459.7	0.26	607.7	41.11	0.32	107.0	56556.8	60.27	unknown *
2	0.36	66.7	0.39	86.8	5.87	0.44	43.1	2887.5	3.08	unknown *
3	0.44	43.3	0.48	120.5	8.15	0.52	0.6	3512.0	3.74	unknown *
4	0.53	0.5	0.55	26.1	1.76	0.58	0.3	408.3	0.44	unknown *
5	0.58	0.0	0.61	14.5	0.98	0.64	0.1	236.4	0.25	unknown *
6	0.65	0.2	0.76	282.6	19.11	0.81	1.1	13148.2	14.01	unknown *
7	0.81	1.9	0.87	340.0	23.00	0.94	180.0	17085.6	18.21	unknown *

Track 3, ID: Pippali P3





Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.03	2.8	0.08	59.9	3.85	0.12	35.7	2161.7	1.65	unknown *
2	0.12	35.8	0.23	692.7	44.55	0.46	72.6	84297.6	64.23	unknown *
3	0.46	72.6	0.49	90.4	5.81	0.55	16.1	3011.2	2.29	unknown *
4	0.55	16.3	0.57	20.5	1.32	0.59	0.5	348.3	0.27	unknown *
5	0.60	0.0	0.63	24.7	1.59	0.66	0.1	440.3	0.34	unknown *
6	0.67	0.4	0.76	202.2	13.00	0.80	138.1	9483.8	7.23	unknown *
7	0.80	138.1	0.85	464.5	29.87	0.97	13.2	31504.2	24.00	unknown *

Measurement Table

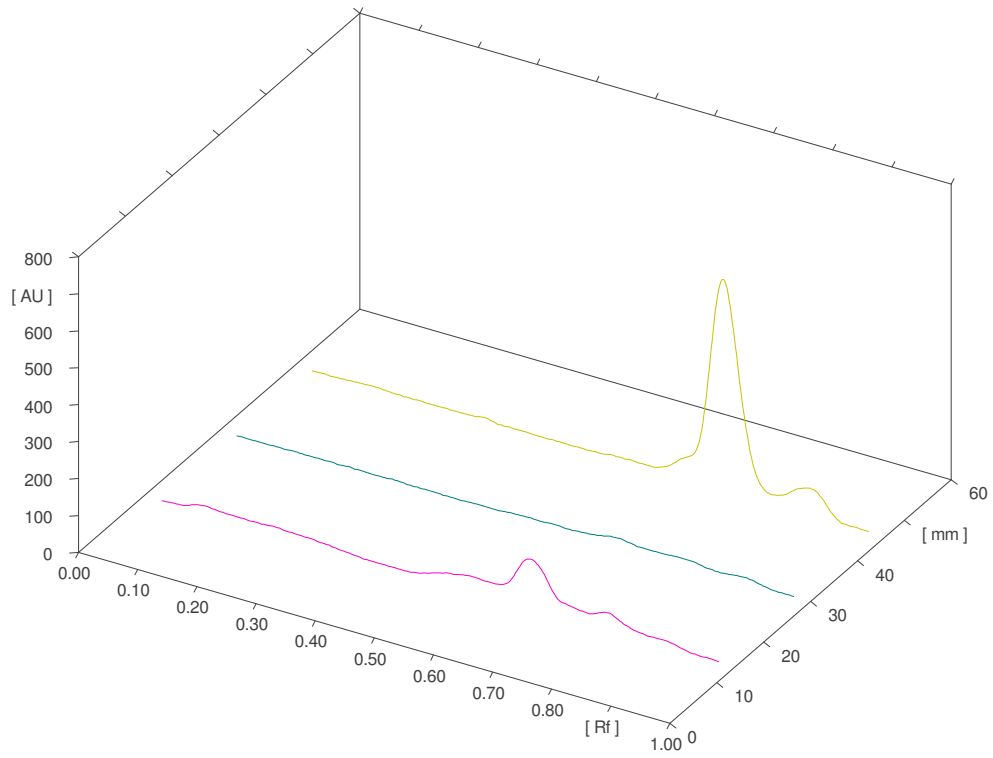
Wavelength	366
Lamp	Hg
Measurement Type	Remission
Measurement Mode	Fluorescence
Optical filter	K400
Detector mode	Automatic
PM high voltage	246 V

User : RMD Research & Development Center
Sunday, October 27, 2013 11:56:31 AM

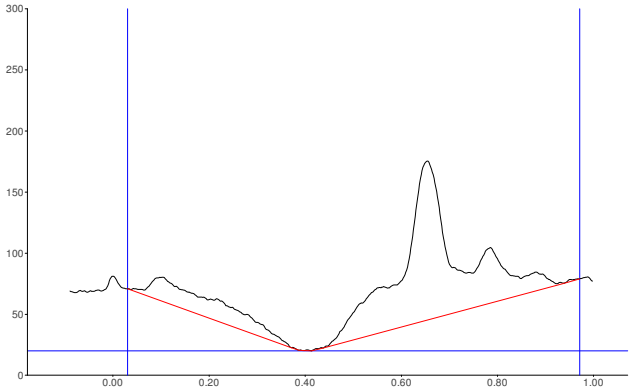
Detector properties

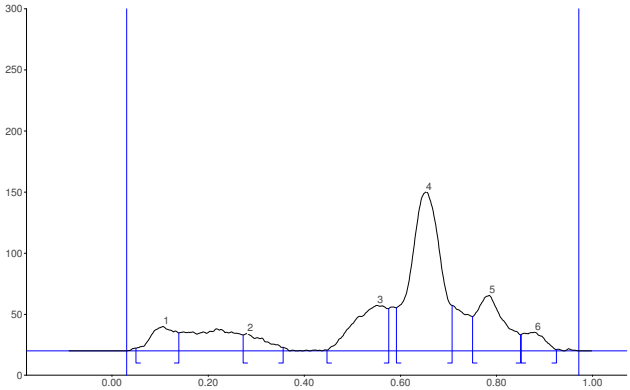
Y-position for 0 adjust	5.0 mm
Track # for 0 adjust	0
Analog Offset	10%
Sensitivity	Automatic (171)

All tracks at WavelengthSc4



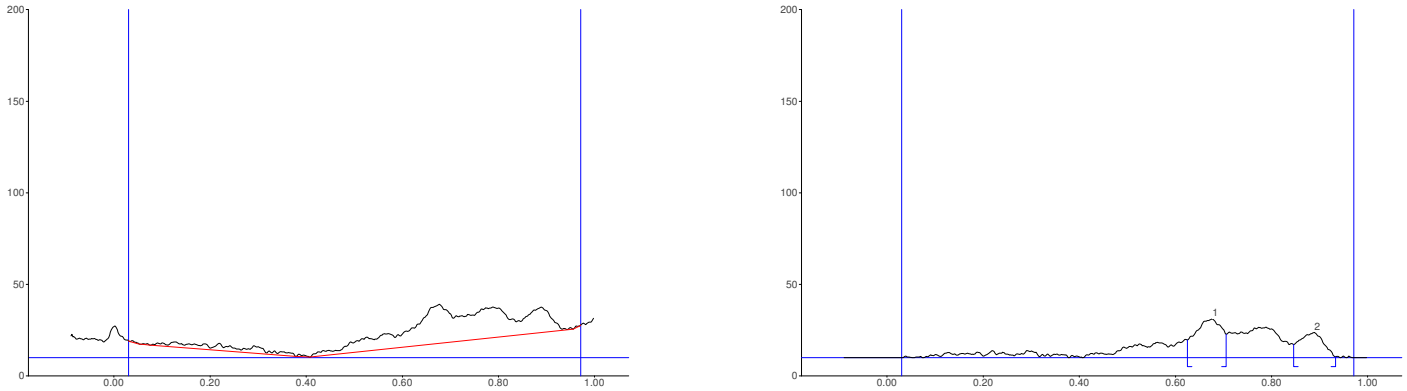
Track 1, ID: Pippali P1





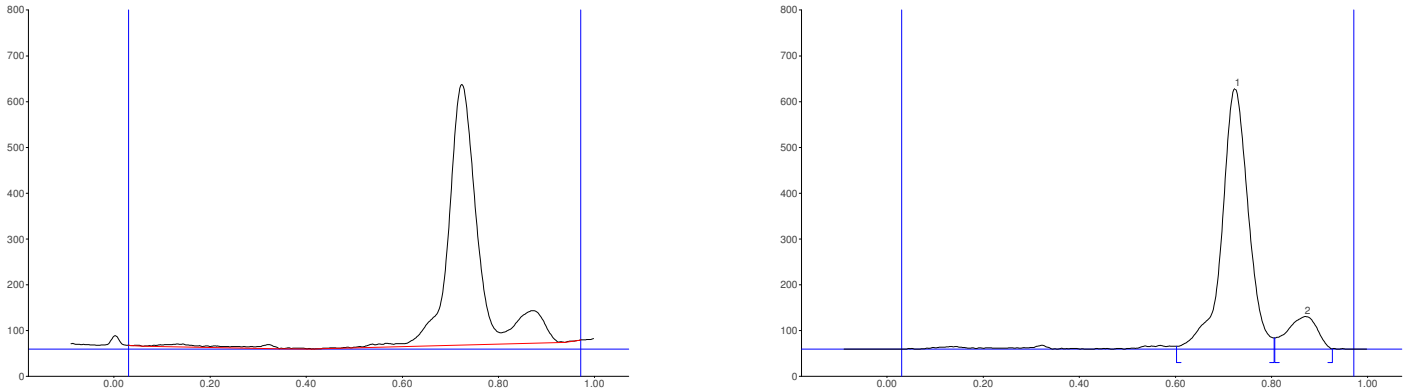
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.05	2.3	0.11	20.1	7.64	0.14	14.8	663.3	6.53	unknown *
2	0.27	13.1	0.28	14.2	5.41	0.36	3.0	421.9	4.15	unknown *
3	0.45	0.6	0.55	37.2	14.18	0.58	34.7	1697.0	16.70	unknown *
4	0.59	35.4	0.66	130.2	49.56	0.71	37.3	5230.6	51.49	unknown *
5	0.75	28.1	0.79	45.6	17.35	0.85	13.3	1697.6	16.71	unknown *
6	0.85	13.6	0.88	15.4	5.86	0.93	1.1	449.0	4.42	unknown *

Track 2, ID: Pippali P2



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.63	9.8	0.68	21.3	60.65	0.71	12.8	764.1	62.99	unknown *
2	0.85	7.2	0.89	13.8	39.35	0.94	0.6	448.9	37.01	unknown *

Track 3, ID: Pippali P3



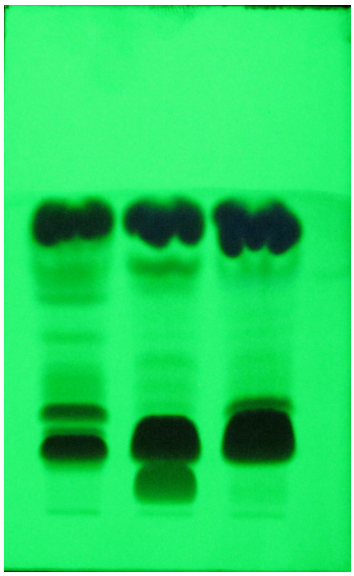
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.60	6.5	0.73	569.2	88.85	0.81	24.6	20381.2	87.61	unknown *
2	0.81	24.7	0.87	71.4	11.15	0.93	1.0	2882.8	12.39	unknown *

Documentation

Executed by	RMD Research & Development Center Saturday, October 26, 2013 10:23:24 AM

Image Document

Executed by	RMD Research & Development Center Saturday, October 26, 2013 10:23:12 AM
Image name	Chromatogram at 254 nm

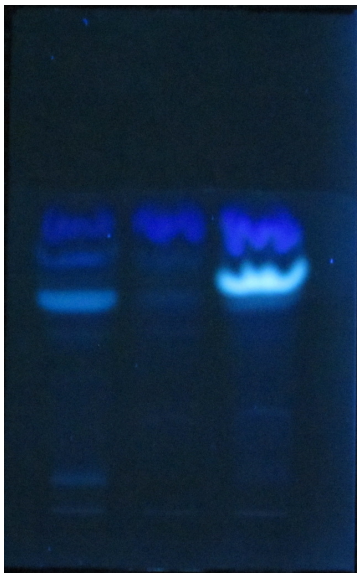


Created by RMD Research & Development Center on Saturday, October 26, 2013 10:23:12 AM

Image Document

Executed by RMD Research & Development Center Saturday, October 26, 2013 10:23:19 AM

Image name Chromatogram at 366 nm



Created by RMD Research & Development Center on Saturday, October 26, 2013 10:23:19 AM

Image Document

Executed by RMD Research & Development Center Saturday, October 26, 2013 10:23:23 AM

Image name Sprayed with Anisaldehyde-sulphuric acid reagent

Created by RMD Research & Development Center on Saturday, October 26, 2013 10:23:23 AM