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Analysis of Mother Tinctures with the help of High Performance Thin Layer Chromatography

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ABSTRACT

HPTLC is an analytical technique based on TLC, but with enhancements intended to increase the resolution of the compounds to be separated and to allow quantitative analysis of the compounds. It is a sophisticated and automated form of TLC. The procedure simultaneously processes the sample and standard that results in better analytical precision and accuracy at a faster pace. It allows several analyses to be done at the same time. HPTLC is a highly sophisticated equipment used for qualitative as well as quantitative analysis of drug ingredients i.e. glycosides, alkaloids etc. that are present in the tinctures and extracts. It is also used for developing in-house standardization of mother tinctures and developing analytical methods for testing mother tinctures. HPTLC method is very simple, powerful, rapid, reliable and cost effective with respect to the accuracy of the result based on both qualitative and quantitative analysis. For homoeopathic formulations, development of standard procedure through HPTLC is a new approach which may lead to proper standardization of different homoeopathic tinctures based on fingerprinting characteristics. This investigation shows that these particular characteristics may be used as standardization tool for homoeopathic tinctures more effectively and most accurately and is utmost essential which could enable the society in general to have quality homoeopathic formulations on one hand and to gain a momentum in homoeopathic medicine on the other. These types of findings, however, cannot rule out the need of further standardization and evaluation of various homoeopathic formulations but definitely it may lead to a new way in the development of standard procedures for different homoeopathic mother tinctures as well as various other formulations.[1]

Keywords: Standardization of homeopathic mother tinctures; Qualitative as well as quantitative analysis of drug ingredients.

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INTRODUCTION

The term "Chromatography", in Greek "Kromatous" meaning 'Colour' and "Graphos" meaning 'written' means colour writing. The credit for inventing chromatography goes to the Russian botanist, Mikhail Semyonovich Tsvet who used it for the first time in 1901 while he was researching on plant pigments. Later on, many scientists have developed several methods of chromatography following the basic principles.[1]

Chromatography is one of the most important laboratory technique in which the components of a mixture are separated on an adsorbent in order to analyze, identify, purify, and/or quantify the mixture or components. It is mostly implemented in science subjects such as chemistry and life-sciences, especially in biochemistry.[1]

Basic Principles

The main principle behind this technique is the differential adsorption of the various components of a mixture between two different phases i.e. mobile phase and stationary phase. Stationary phase refers to the support substance that are fix, whereas mobile phase is refer to the medium to which the mixture to be separated moves in a particular direction. The mixture gets separated by distributing its component molecules between the two phases. Distribution takes place on the bases of the structure, size, shape, charge or molecular weight.

1) Fixed or stationary phase- the adsorbent is termed as the stationary phase. For e.g. cellulose, silica gel, magnesium oxide.
2) Mobile or moving phase – the liquid in which the substance is dissolved is termed as mobile phase or eluent. For e.g. ether, benzene, alcohol. Their selection depends upon the relative solubility of the component of the mixture in them.[1]

Thus, in this, a liquid flows over a stationary solid phase carrying with it solutes that have varying degree of affinity for the stationary phase. Different rates of flows are thus produced for each solute and physical separation is achieved. Specific requirement for chromatographic tests of drugs including adsorbant and developing solvents are given in individual monographs. [4]

Uses of Chromatography in Homoeopathic Pharmaceutics:

Chromatography is useful in qualitative as well as quantitative analysis of drugs especially mother tinctures and lower potencies. Thin Layer Chromatography & Paper Chromatography are usually employed in the H.P.I assays and tests. [1]

Thin Layer Chromatography is a simple, quick and inexpensive procedure that gives quick answer as to how many components are there in a mixture. TLC is also used to support the

identity of a compound in a mixture when the Rf of a compound is compared with the Rf of a known compound. Rf is the retention factor or how far up a plate the compound travels. Rf values vary with the experimental conditions, and thus identification is best accomplished where an authentic specimen of the compound in question is used as reference substance. For this purpose, chromatograms are prepared by spotting on a thin layer adsorbant in a straight line parallel to the edge of the chromatographic plate, solutions of the substances to be identified, the authentic specimen, and a mixture of nearly equal amounts of the substance to be identified and authentic specimen. [4]

High Performance Thin Layer Chromatography (HPTLC) is a sophisticated and automated form of TLC. The procedure simultaneously processes the sample and standard that results in better analytical precision and accuracy at a faster pace. It allows several analyses to be done at the same time. [1]

Basic types of chromatography

A. Liquid Chromatography (L.C)

Mobile phase- Liquid

Specific methods:

a. **Adsorption Chromatography**- (Liquid-Solid Chromatography)

(i) Column- HPLC

(ii) Plane- TLC & HPTLC

b. **Partition Chromatography**- (Liquid- Liquid Chromatography)

(i) Column- HPLC

(ii) Plane- TLC & Paper Chromatography

c. **Ion- exchange Chromatography** (IEC)

d. **Size- exclusion Chromatography** (SEC)

B. Gas Chromatography (GC)

Mobile Phase- Gas

a. Gas- Solid Chromatography (GSC)

b. Gas- Liquid Chromatography (GLC)

C. Supercritical Fluid Chromatography (SFC)

Mobile Phase- Supercritical fluid.[1]

Advantages of Chromatography

Chromatography technique has many advantages over other techniques in analytical studies. It is very sensitive and reliable. The main advantage is that a complex mixture can be separated

accurately by using only a few micrograms of the sample. Also, separation takes less time as compared to other techniques. The other advantage is that equipment set-up is simple and easy. Because of these advantages, chromatography has become an important technique for clinical studies.[3]

Purpose of Selection of Topic

Of the many chromatographic methods presently available, thin-layer chromatography (TLC) is widely used for the rapid analysis of drugs and drug preparations. There are several reasons for this:

• The time required for the demonstration of most of the characteristic constituents of a drug by TlC is very short.[1]

• In addition to qualitative detection, TLC also provides semi-quantitative information on the major active constituents of a drug or drug preparation, thus enabling an assessment of drug quality. [1]

• TLC provides a chromatographic drug fingerprint. It is therefore suitable for monitoring the identity and purity of drugs and for detecting adulterations and substitutions.[1]

• With the aid of appropriate separation procedures, TLC can be used to analyze drug combinations and phytochemical preparations. [1]

TLC & HPTLC techniques are important analytical tools for micro-analytical separation and determination of natural products. They have the following advantages:

1. Simple to operate, economical, rapid, accurate (particularly HPTLC) [2]
2. Always available for use (percolated plates are commercially available) [2]
3. Method of detection does not place any restriction on choice of fluent, easily inspected.[2]
4. Neutral, basic, acidic or purely aqueous eluent can be employed. The whole chromatographic system is flexible. [(2)]
5. TLC & HPTLC can be exploited in the investigation and cultivation of medicinal plants.[2]
6. It is possible to run many samples of extracts from different chemical races simultaneously with authentic standards and high performance individuals can be recognized, selected and bred.[2]
7. This technique is also valuable for discovery of so-called chemical races.[2]
8. Also HPTLC is a major advancement of TLC principle requiring shorter time and better resolution. It enjoys a practical application status, as it combines the art of chromatography with quickness at a moderate cost.[2]

9. Also this method is very simple, powerful, rapid, reliable and cost effective with respect to the accuracy of the result based on both qualitative and quantitative analysis.[2]

Aim

To analyze different Mother Tinctures with the help of High Performance Thin Layer Chromatography.

Objectives:

1. To test the purity of the mother-tinctures to be tested w.r.t their components.
2. To analyze the number of components present in the given liquids.
3. To identify the given mother-tincture from the components separated on the chromatogram.

MATERIALS AND METHOD

A. **Chemicals and Materials**

Authentic parts of plant will be used to prepare the mother tincture as specified in HPI and other official pharmacopoeias. 2 mother tinctures will be studied namely Calendula and Thuja.

The solvents like 99.9% absolute ethanol, HPLC water, toluene, ethyl acetate, diethyl amine etc. based upon the different mobile and stationary phases specified for different mother tinctures will be used.

B**. Preparation of Standard Mother Tincture**

Standard mother tincture will be prepared as specified in HPI and other official Homoeopathic Pharmacopoeias and will be used in this investigation.

C. **Active Principle**

Main active principle present in the drug substance taken will be referred from the HPI and other official Pharmacopoeias.

D. **Standardization of Standard Mother Tincture-**

HPTLC unit from the Quality Assurance Department of Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune-18 will be used for quantitative evaluation. Suitable stationary phase and mobile phase will be used for different mother tinctures as specified in the reference books. The amount of active principle present will be calculated in the mother tincture.

E. **Standardization of the std. mother tincture by fingerprint method-**

Standardization of the mother tincture will be done by evaluating its fingerprint characteristics, using HPTLC method. Samples and standard will be applied simultaneously on the same TLC plate and studied. Rf Values of std. mother tincture will be matched with the help of its

characteristic spectra with that of other marketed samples. The amount of active principle will be calculated in individual mother tinctures. With this method we will compare all available mother tinctures with their market products and respective active principle will also quantify. Thus the method can be said to be standardized.

F. **Quantification of Active principle in Market Samples & Std. Mother Tincture-**

The amount of active principle will be calculated in Std. mother tincture and market samples and compared to validate the study.

RESULTS AND DISCUSSION

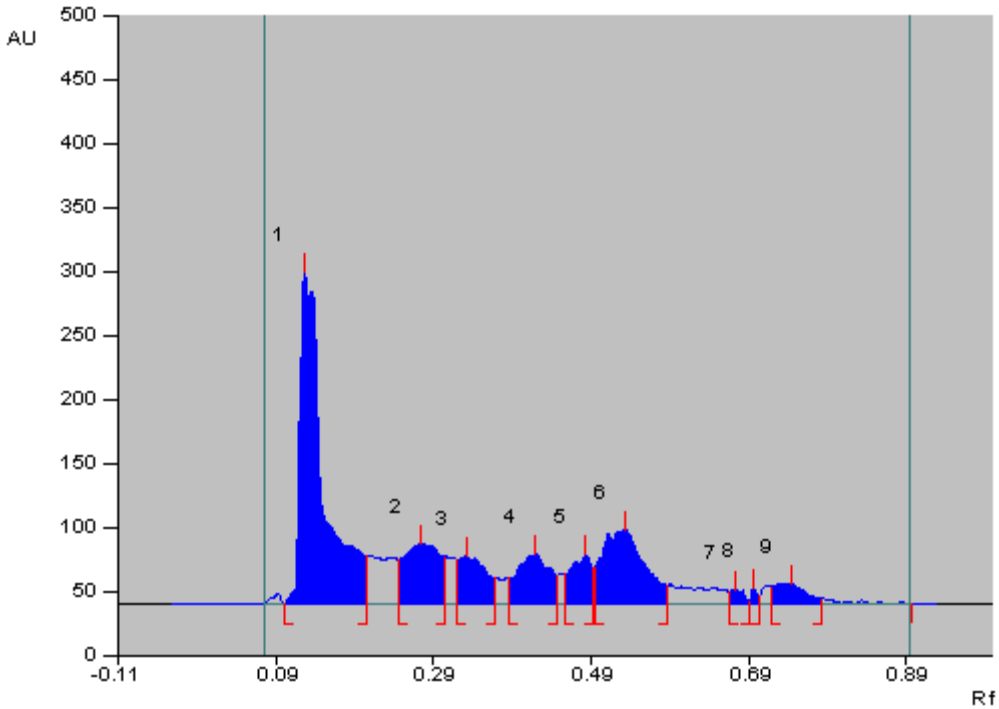
Calendula mother tincture prepared by taking calendula officinalis 700gm. (moist magma, solids100 gm., and plant moisture aprrox. 600ml) & strong alcohol 437ml. To make 1000 ml of tincture. [5,6]

254 nm CALENDULA

SATURATION TIME 15 MINS

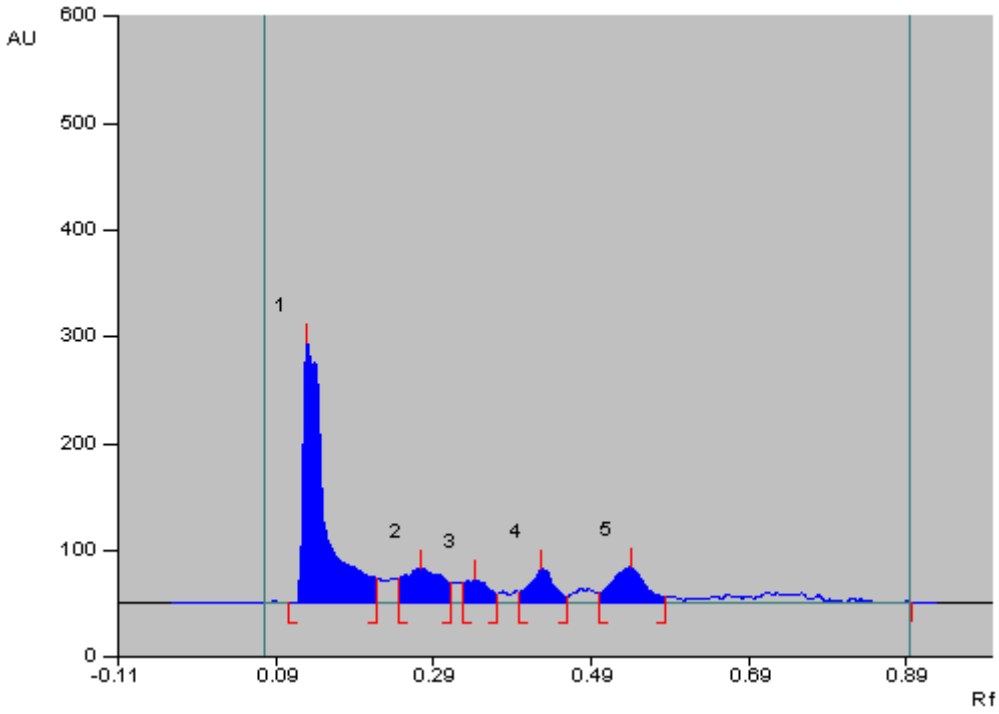
Mp-Ethyl acetate :formic acid: water 8:1:1(v/v)

Calendula densitogram- Sample-1



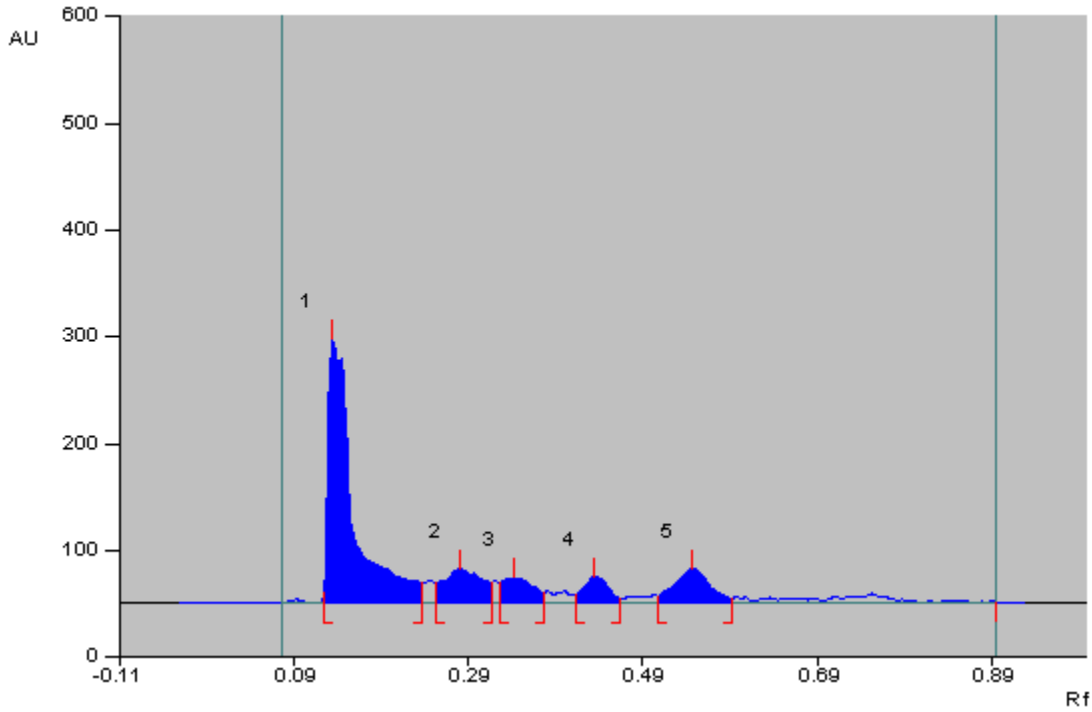
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.10 Rf	0.1 AU	0.13 Rf	258.6 AU	49.96 %	0.20 Rf	37.4 AU	6419.2 AU	43.59 %
2	0.25 Rf	34.6 AU	0.27 Rf	46.9 AU	9.05 %	0.30 Rf	36.7 AU	1761.0 AU	11.96 %
3	0.32 Rf	34.6 AU	0.33 Rf	37.7 AU	7.28 %	0.37 Rf	20.0 AU	1085.5 AU	7.37 %
4	0.39 Rf	19.9 AU	0.42 Rf	38.5 AU	7.45 %	0.45 Rf	22.4 AU	1310.3 AU	8.90 %
5	0.46 Rf	23.3 AU	0.48 Rf	38.4 AU	7.41 %	0.49 Rf	27.6 AU	808.0 AU	5.49 %
6	0.49 Rf	27.6 AU	0.53 Rf	58.1 AU	11.23 %	0.58 Rf	14.3 AU	2568.4 AU	17.44 %
7	0.66 Rf	9.3 AU	0.67 Rf	11.5 AU	2.22 %	0.69 Rf	0.0 AU	163.1 AU	1.11 %
8	0.69 Rf	2.2 AU	0.70 Rf	12.3 AU	2.37 %	0.70 Rf	6.9 AU	84.9 AU	0.58 %
9	0.72 Rf	13.2 AU	0.74 Rf	15.7 AU	3.04 %	0.78 Rf	3.8 AU	524.7 AU	3.56 %

Calendula densitogram- Sample-2



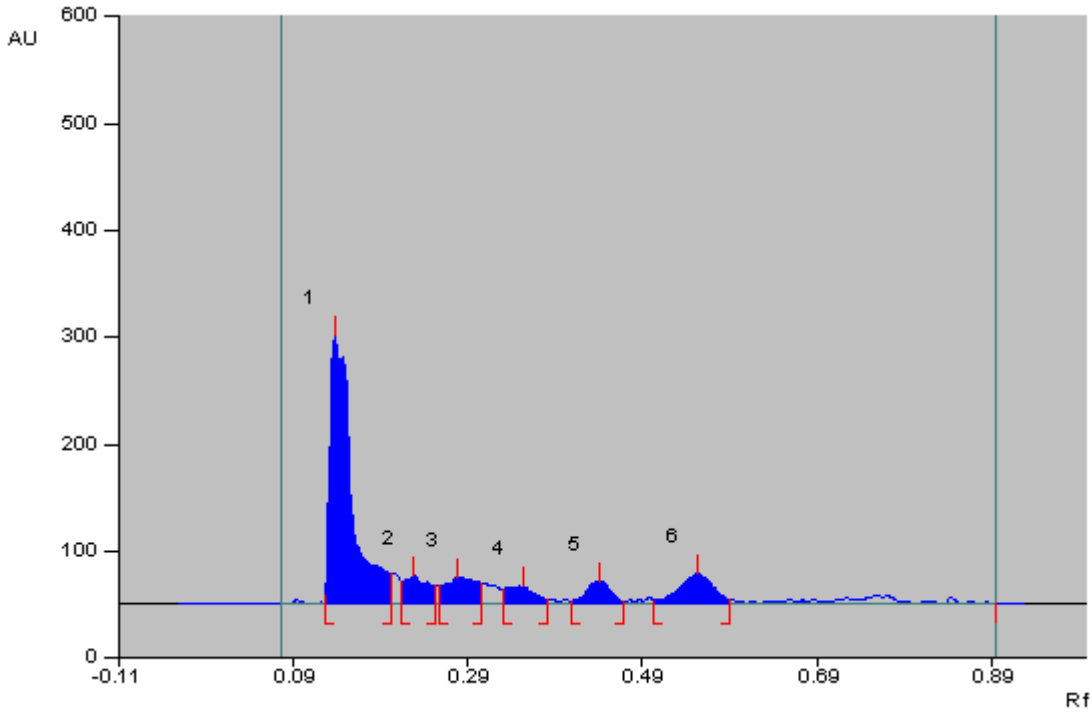
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.11 Rf	0.0 AU	0.13 Rf	244.2 AU	67.18 %	0.22 Rf	23.1 AU	5740.1 AU	60.28 %
2	0.25 Rf	23.1 AU	0.27 Rf	32.3 AU	8.89 %	0.31 Rf	18.8 AU	1284.4 AU	13.49 %
3	0.33 Rf	18.6 AU	0.34 Rf	22.0 AU	6.06 %	0.37 Rf	8.2 AU	544.9 AU	5.72 %
4	0.40 Rf	9.6 AU	0.43 Rf	31.7 AU	8.72 %	0.46 Rf	5.3 AU	799.7 AU	8.40 %
5	0.50 Rf	9.0 AU	0.54 Rf	33.3 AU	9.15 %	0.58 Rf	5.0 AU	1154.0 AU	12.12 %

Calendula densitogram- Sample-3



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.12 Rf	8.6 AU	0.13 Rf	248.1 AU	68.77 %	0.24 Rf	18.4 AU	5911.8 AU	63.03 %
2	0.25 Rf	19.1 AU	0.28 Rf	32.0 AU	8.86 %	0.32 Rf	19.0 AU	1138.2 AU	12.14 %
3	0.33 Rf	19.1 AU	0.34 Rf	23.5 AU	6.52 %	0.38 Rf	9.7 AU	691.5 AU	7.37 %
4	0.41 Rf	8.2 AU	0.43 Rf	24.5 AU	6.79 %	0.46 Rf	4.2 AU	571.0 AU	6.09 %
5	0.51 Rf	6.2 AU	0.55 Rf	32.7 AU	9.06 %	0.59 Rf	3.7 AU	1066.2 AU	11.37 %

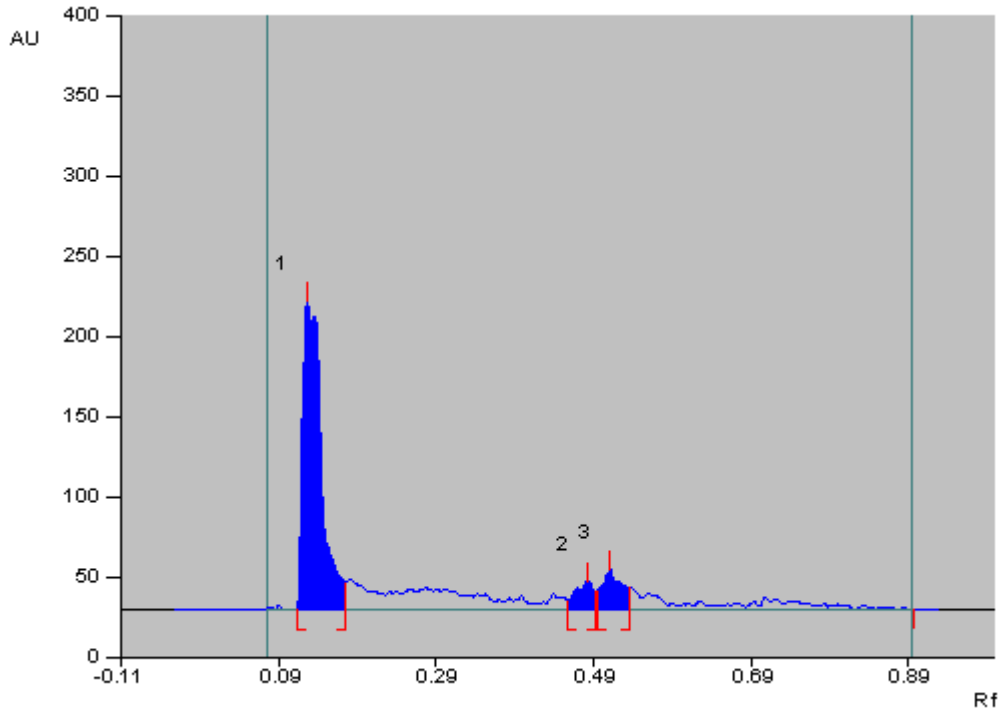
Calendula densitogram- Sample-4



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.13 Rf	7.1 AU	0.14 Rf	250.4 AU	68.27 %	0.20 Rf	28.0 AU	5251.6 AU	62.20 %
2	0.21 Rf	20.9 AU	0.23 Rf	26.6 AU	7.25 %	0.25 Rf	16.3 AU	597.6 AU	7.08 %
3	0.26 Rf	16.8 AU	0.28 Rf	24.3 AU	6.63 %	0.31 Rf	18.8 AU	768.4 AU	9.10 %
4	0.33 Rf	13.2 AU	0.35 Rf	16.1 AU	4.40 %	0.38 Rf	3.0 AU	443.6 AU	5.25 %
5	0.41 Rf	2.4 AU	0.44 Rf	21.1 AU	5.76 %	0.47 Rf	2.1 AU	488.2 AU	5.78 %
6	0.50 Rf	2.8 AU	0.55 Rf	28.2 AU	7.69 %	0.59 Rf	3.0 AU	893.3 AU	10.58 %

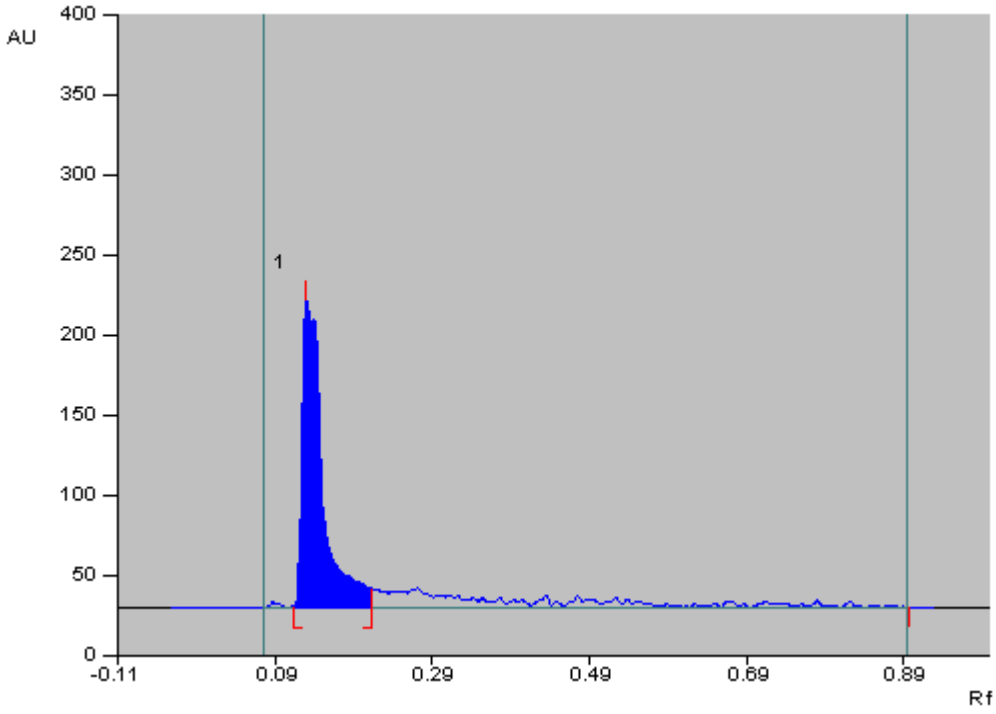
366 nm CALENDULA

Calendula densitogram- Sample-5



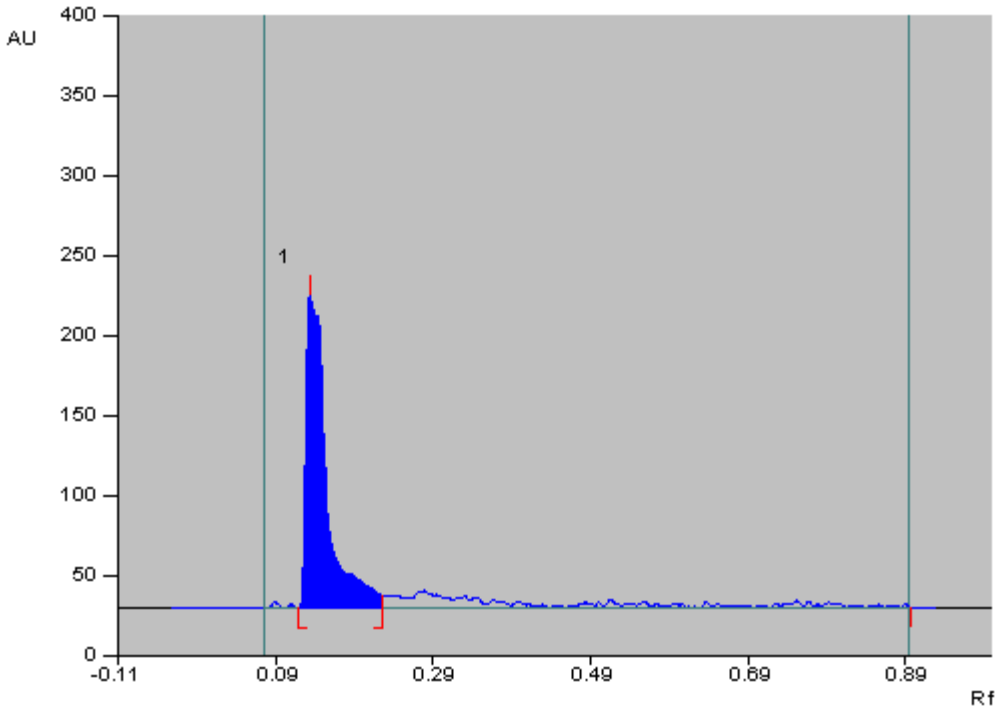
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.11 Rf	0.0 AU	0.13 Rf	192.3 AU	81.62 %	0.17 Rf	16.9 AU	3822.2 AU	81.81 %
2	0.46 Rf	5.6 AU	0.48 Rf	18.1 AU	7.67 %	0.49 Rf	11.0 AU	327.6 AU	7.01 %
3	0.49 Rf	11.4 AU	0.51 Rf	25.2 AU	10.71 %	0.53 Rf	13.6 AU	522.2 AU	11.18 %

Calendula densitogram- Sample-6



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.11 Rf	0.6 AU	0.13 Rf	193.1 AU	100.00 %	0.21 Rf	11.8 AU	4307.6 AU	100.00 %

Calendula densitogram- Sample-7



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.12 Rf	0.2 AU	0.13 Rf	196.4 AU	100.00 %	0.23 Rf	7.9 AU	4348.0 AU	100.00 %

Calendula mother-tincture

The scanning report as well as the fingerprint characters obtained after integration has been shown. From the results obtained after densitometric scanning, it was observed that the Std. MQ (D) of Calendula shows 3 peaks at Rf values 0.12, 0.24, 0.10.

The chromatogram of the mother tincture of calendula under study shows these 3 peaks at 0.12, 0.24, 0.10 respectively. Thus, from the chromatogram, rf values and peaks it is evident that the calendula mother tincture under study shows the same 3 components that are seen in the chromatogram of a standard calendula mother tincture.

Thus, we can conclude that the given calendula mother tincture, a market product is standard w.r.t its quality & quantity. Based on this approach our aim is to develop a standardized procedure to evaluate the mother tinctures for its accuracy, sensitivity and reproducibility. The

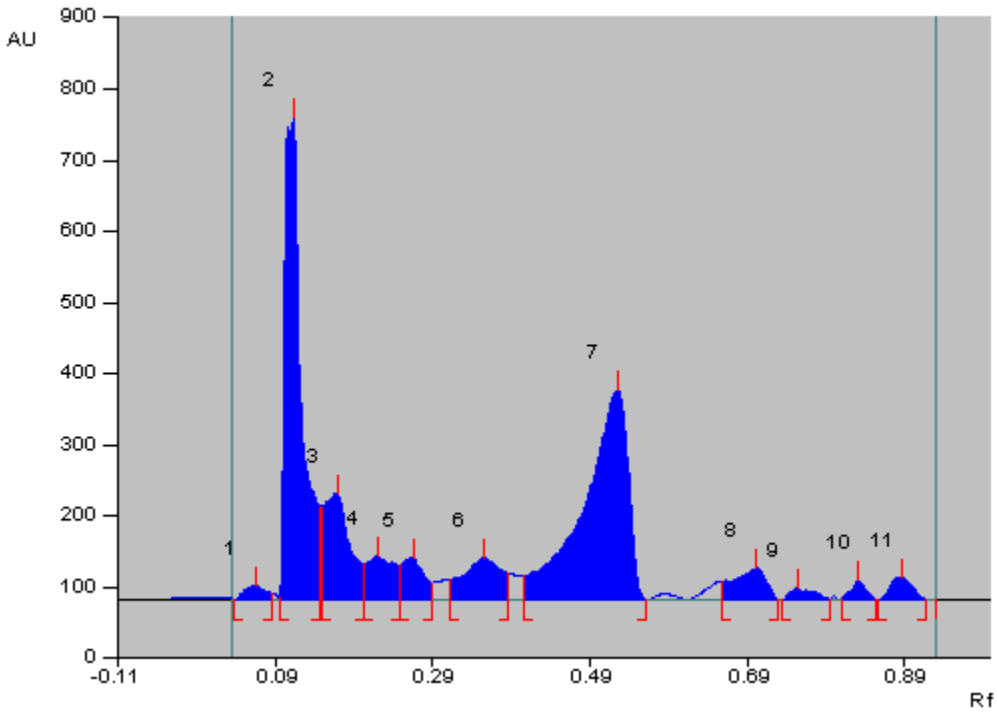
above HPTLC method is very simple, powerful, rapid, reliable and cost effective with respect to the accuracy of the result based on both qualitative and quantitative analysis.

254 nm THUJA

SATURATION TIME 15 MINS

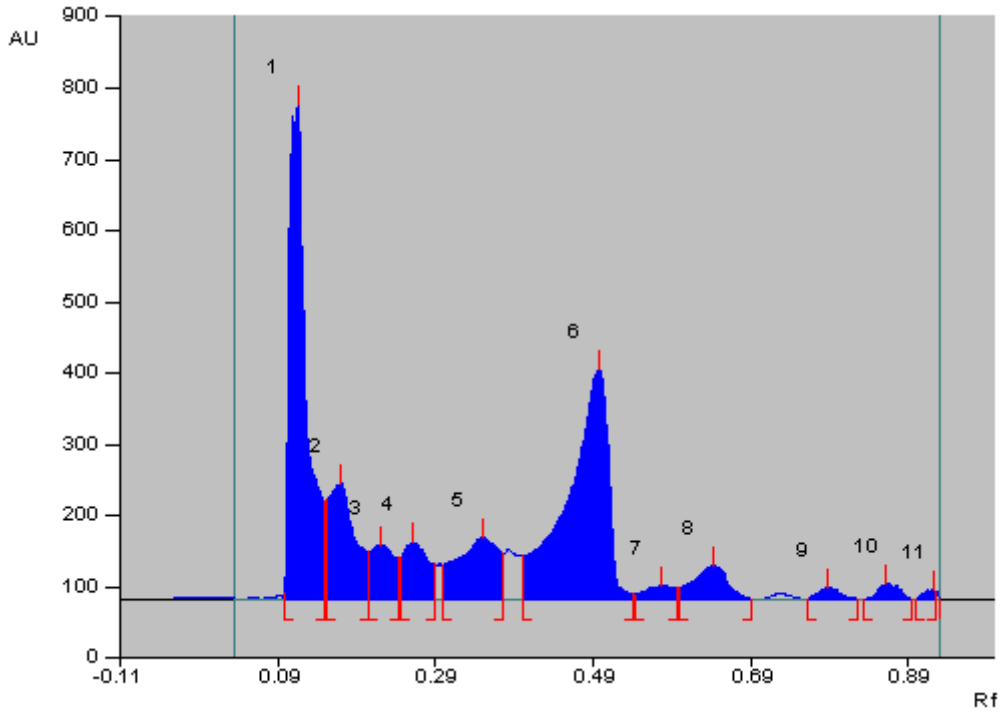
Mp:-chloroform:methanol 9:1

Thuja Densitogram- Sample 1



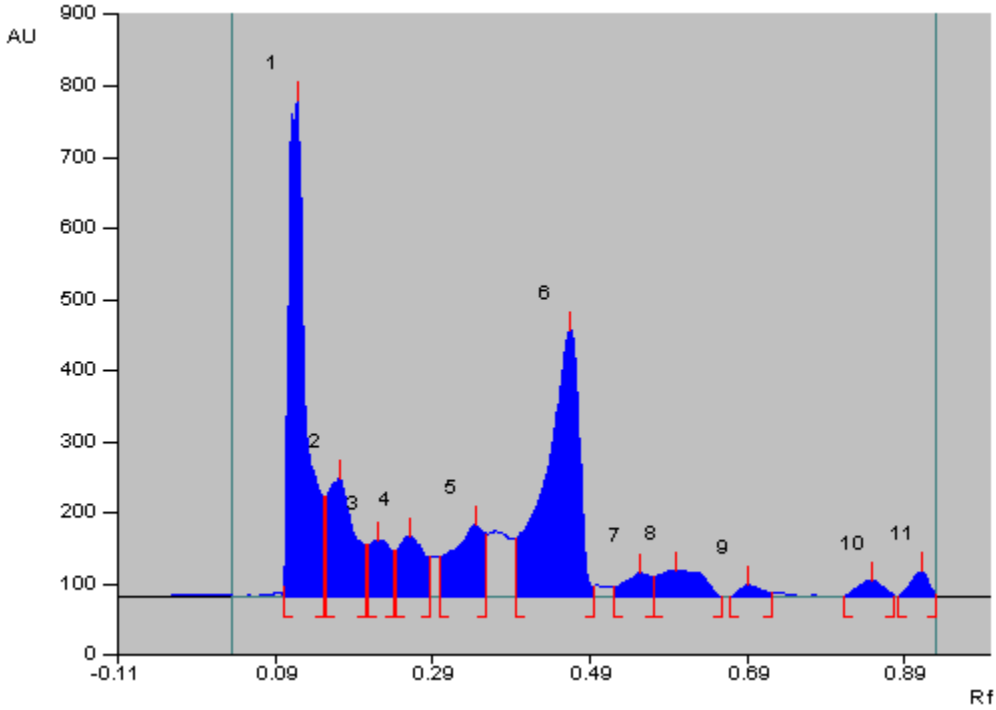
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	0.0 AU	0.06 Rf	19.7 AU	1.37 %	0.09 Rf	7.7 AU	434.2 AU	1.12 %
2	0.10 Rf	2.5 AU	0.11 Rf	677.4 AU	47.04 %	0.15 Rf	30.0 AU	12103.4 AU	31.24 %
3	0.15 Rf	130.1 AU	0.17 Rf	149.5 AU	10.38 %	0.20 Rf	50.0 AU	4124.9 AU	10.65 %
4	0.20 Rf	50.2 AU	0.22 Rf	60.8 AU	4.22 %	0.25 Rf	47.3 AU	1772.7 AU	4.58 %
5	0.25 Rf	47.7 AU	0.27 Rf	59.2 AU	4.11 %	0.29 Rf	23.8 AU	1327.5 AU	3.43 %
6	0.31 Rf	28.3 AU	0.36 Rf	58.3 AU	4.05 %	0.38 Rf	37.3 AU	2304.3 AU	5.95 %
7	0.41 Rf	32.8 AU	0.52 Rf	294.0 AU	20.42 %	0.56 Rf	0.1 AU	13634.8 AU	35.19 %
8	0.66 Rf	25.4 AU	0.70 Rf	43.8 AU	3.04 %	0.73 Rf	0.1 AU	1516.1 AU	3.91 %
9	0.73 Rf	0.6 AU	0.75 Rf	18.1 AU	1.25 %	0.79 Rf	0.6 AU	396.3 AU	1.02 %
10	0.81 Rf	0.5 AU	0.83 Rf	27.0 AU	1.88 %	0.85 Rf	0.5 AU	404.5 AU	1.04 %
11	0.86 Rf	0.3 AU	0.88 Rf	32.2 AU	2.24 %	0.92 Rf	0.4 AU	725.7 AU	1.87 %

Thuja Densitogram- Sample 2



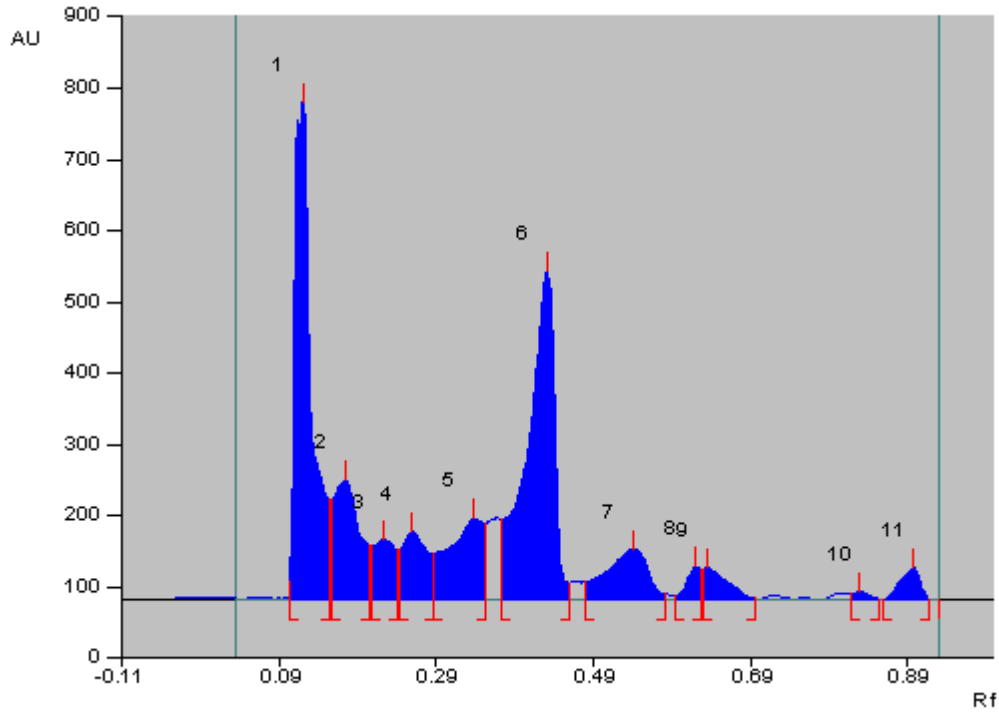
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.10 Rf	8.6 AU	0.12 Rf	695.9 AU	45.06 %	0.15 Rf	38.8 AU	12564.4 AU	29.95 %
2	0.15 Rf	139.8 AU	0.17 Rf	163.4 AU	10.58 %	0.20 Rf	35.4 AU	4606.2 AU	10.98 %
3	0.21 Rf	66.3 AU	0.22 Rf	76.9 AU	4.98 %	0.24 Rf	57.7 AU	1931.8 AU	4.61 %
4	0.24 Rf	58.1 AU	0.26 Rf	80.3 AU	5.20 %	0.29 Rf	49.7 AU	2145.8 AU	5.12 %
5	0.30 Rf	48.4 AU	0.35 Rf	87.4 AU	5.66 %	0.38 Rf	34.1 AU	3823.2 AU	9.11 %
6	0.40 Rf	61.4 AU	0.50 Rf	323.2 AU	20.92 %	0.54 Rf	7.2 AU	13517.3 AU	32.23 %
7	0.54 Rf	7.0 AU	0.57 Rf	18.9 AU	1.22 %	0.60 Rf	16.5 AU	590.6 AU	1.41 %
8	0.60 Rf	16.6 AU	0.64 Rf	47.4 AU	3.07 %	0.69 Rf	0.5 AU	1703.8 AU	4.06 %
9	0.76 Rf	0.9 AU	0.79 Rf	16.3 AU	1.06 %	0.82 Rf	0.1 AU	393.5 AU	0.94 %
10	0.83 Rf	0.2 AU	0.86 Rf	21.4 AU	1.39 %	0.89 Rf	0.3 AU	492.1 AU	1.17 %
11	0.90 Rf	0.2 AU	0.92 Rf	13.4 AU	0.87 %	0.92 Rf	12.1 AU	177.8 AU	0.42 %

Thuja Densitogram- Sample 3



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.10 Rf	14.1 AU	0.12 Rf	696.8 AU	42.26 %	0.15 Rf	40.6 AU	12449.9 AU	29.66 %
2	0.15 Rf	140.7 AU	0.17 Rf	165.5 AU	10.04 %	0.21 Rf	72.0 AU	4763.4 AU	11.35 %
3	0.21 Rf	72.1 AU	0.22 Rf	79.8 AU	4.84 %	0.24 Rf	33.4 AU	1856.2 AU	4.42 %
4	0.24 Rf	63.5 AU	0.26 Rf	85.3 AU	5.18 %	0.28 Rf	55.7 AU	2315.2 AU	5.52 %
5	0.30 Rf	56.4 AU	0.34 Rf	100.4 AU	6.09 %	0.36 Rf	38.4 AU	3461.2 AU	8.25 %
6	0.39 Rf	81.6 AU	0.46 Rf	374.2 AU	22.69 %	0.49 Rf	15.0 AU	12850.2 AU	30.61 %
7	0.52 Rf	13.7 AU	0.55 Rf	34.4 AU	2.09 %	0.57 Rf	28.4 AU	897.4 AU	2.14 %
8	0.57 Rf	28.6 AU	0.60 Rf	37.2 AU	2.25 %	0.66 Rf	0.1 AU	1740.9 AU	4.15 %
9	0.67 Rf	0.5 AU	0.69 Rf	16.4 AU	1.00 %	0.72 Rf	5.8 AU	373.1 AU	0.89 %
10	0.81 Rf	0.2 AU	0.85 Rf	23.4 AU	1.42 %	0.88 Rf	0.6 AU	609.1 AU	1.45 %
11	0.88 Rf	0.5 AU	0.91 Rf	35.3 AU	2.14 %	0.93 Rf	3.0 AU	660.1 AU	1.57 %

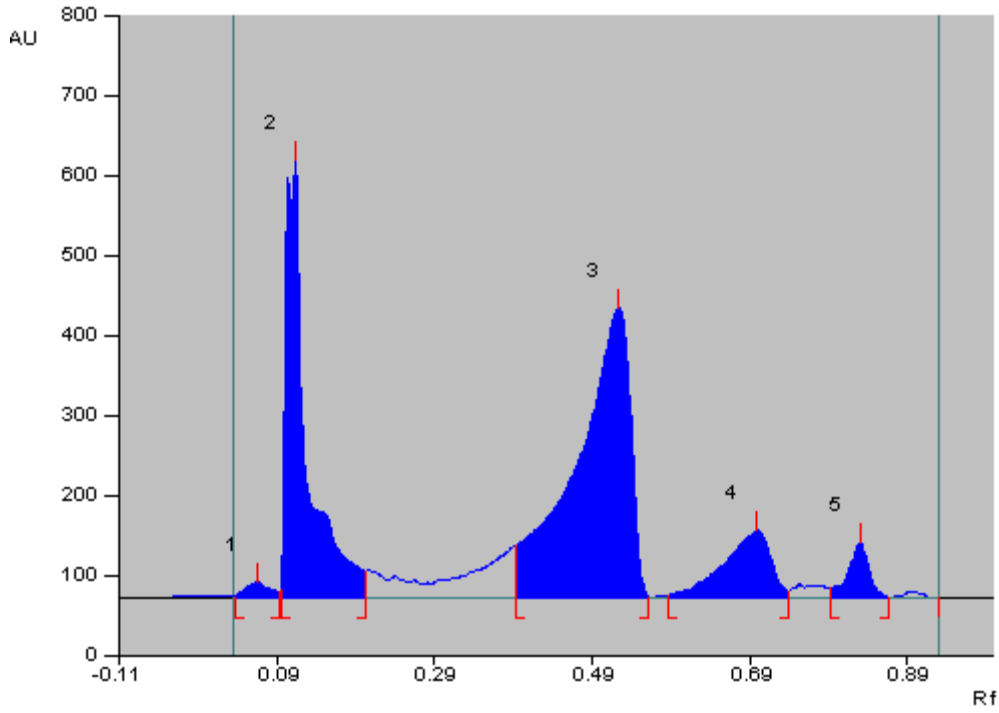
Thuja Densitogram- Sample 4



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.10 Rf	24.9 AU	0.12 Rf	698.0 AU	38.00 %	0.15 Rf	39.7 AU	12580.6 AU	27.88 %
2	0.16 Rf	139.7 AU	0.17 Rf	168.5 AU	9.17 %	0.21 Rf	75.8 AU	4725.6 AU	10.47 %
3	0.21 Rf	75.0 AU	0.22 Rf	83.5 AU	4.55 %	0.24 Rf	39.9 AU	1874.4 AU	4.15 %
4	0.24 Rf	70.2 AU	0.26 Rf	94.6 AU	5.15 %	0.28 Rf	34.1 AU	2546.3 AU	5.64 %
5	0.29 Rf	64.5 AU	0.34 Rf	114.2 AU	6.22 %	0.35 Rf	37.1 AU	4239.0 AU	9.39 %
6	0.37 Rf	113.1 AU	0.43 Rf	460.6 AU	25.07 %	0.46 Rf	23.5 AU	13129.0 AU	29.09 %
7	0.48 Rf	23.9 AU	0.54 Rf	70.1 AU	3.81 %	0.58 Rf	7.0 AU	3105.2 AU	6.88 %
8	0.59 Rf	5.9 AU	0.62 Rf	46.6 AU	2.54 %	0.63 Rf	42.9 AU	683.7 AU	1.51 %
9	0.63 Rf	42.9 AU	0.63 Rf	44.2 AU	2.41 %	0.69 Rf	1.4 AU	1149.0 AU	2.55 %
10	0.82 Rf	7.1 AU	0.83 Rf	12.4 AU	0.67 %	0.85 Rf	0.0 AU	176.5 AU	0.39 %
11	0.86 Rf	0.2 AU	0.90 Rf	44.2 AU	2.40 %	0.92 Rf	0.2 AU	920.1 AU	2.04 %

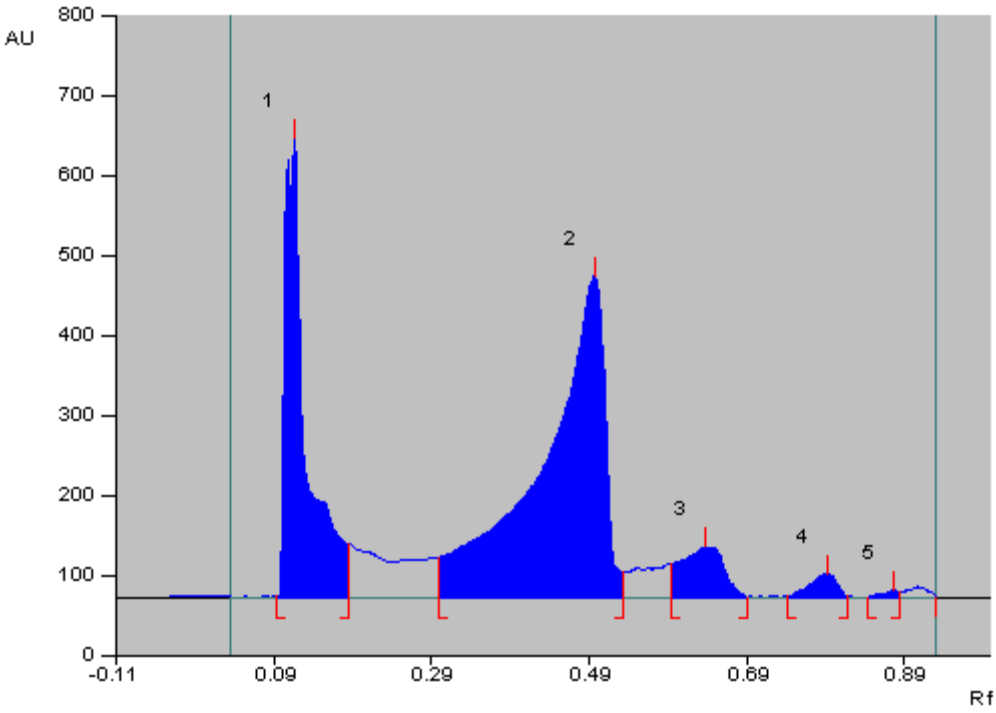
366 nm

Thuja Densitogram- Sample 5



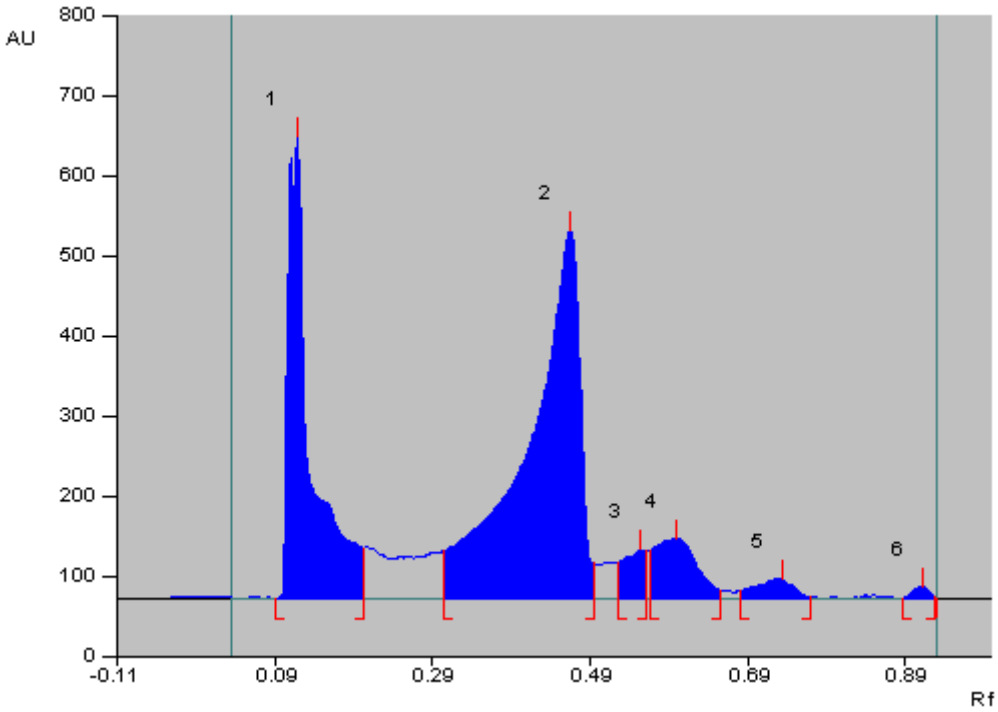
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	0.6 AU	0.07 Rf	19.6 AU	1.81 %	0.09 Rf	7.5 AU	495.7 AU	1.27 %
2	0.09 Rf	9.3 AU	0.11 Rf	546.6 AU	50.50 %	0.20 Rf	33.7 AU	12216.1 AU	31.32 %
3	0.39 Rf	64.0 AU	0.52 Rf	362.6 AU	33.50 %	0.56 Rf	0.5 AU	20546.4 AU	52.68 %
4	0.59 Rf	3.1 AU	0.70 Rf	84.4 AU	7.80 %	0.74 Rf	8.3 AU	4202.1 AU	10.77 %
5	0.79 Rf	12.2 AU	0.83 Rf	69.2 AU	6.40 %	0.87 Rf	0.1 AU	1545.0 AU	3.96 %

Thuja Densitogram- Sample 6



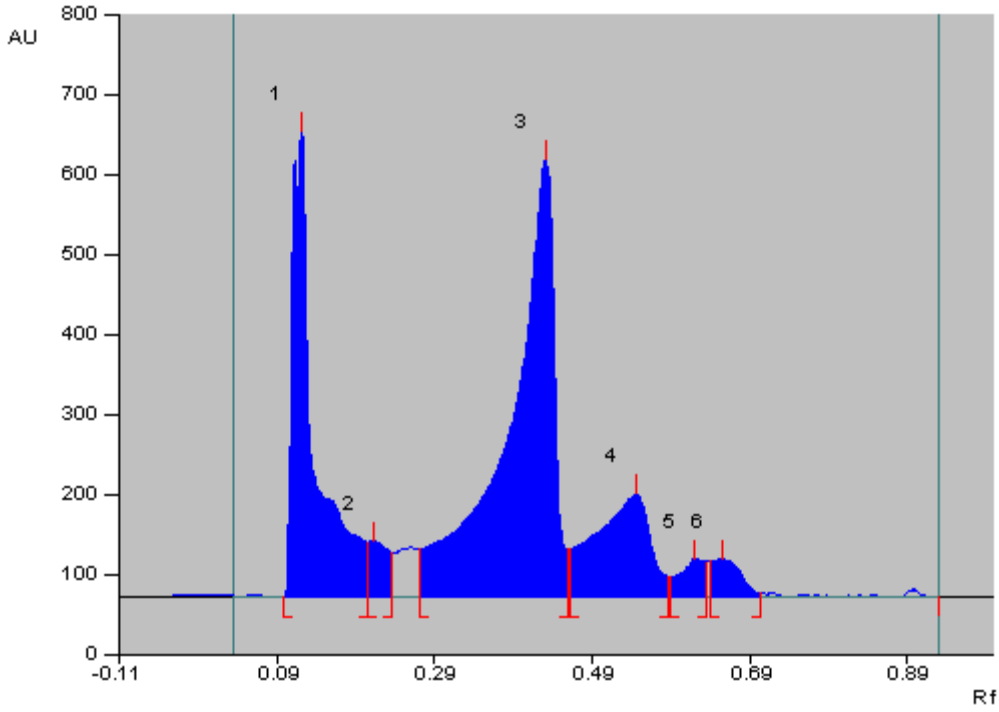
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.09 Rf	2.0 AU	0.12 Rf	575.3 AU	53.16 %	0.18 Rf	38.5 AU	12650.4 AU	29.73 %
2	0.30 Rf	50.5 AU	0.50 Rf	402.7 AU	37.21 %	0.53 Rf	32.8 AU	25967.5 AU	61.02 %
3	0.59 Rf	43.6 AU	0.64 Rf	63.9 AU	5.91 %	0.69 Rf	1.1 AU	2885.2 AU	6.78 %
4	0.74 Rf	2.0 AU	0.79 Rf	30.0 AU	2.77 %	0.82 Rf	1.6 AU	873.0 AU	2.05 %
5	0.84 Rf	0.7 AU	0.88 Rf	10.3 AU	0.95 %	0.88 Rf	7.1 AU	182.1 AU	0.43 %

Thuja Densitogram- Sample 7



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.09 Rf	0.8 AU	0.12 Rf	576.5 AU	47.55 %	0.20 Rf	33.3 AU	13491.3 AU	30.38 %
2	0.30 Rf	59.4 AU	0.46 Rf	459.1 AU	37.87 %	0.49 Rf	43.8 AU	24902.5 AU	56.07 %
3	0.53 Rf	45.8 AU	0.55 Rf	61.4 AU	5.06 %	0.56 Rf	30.2 AU	1404.2 AU	3.16 %
4	0.57 Rf	60.8 AU	0.60 Rf	75.6 AU	6.24 %	0.65 Rf	10.6 AU	3362.5 AU	7.57 %
5	0.68 Rf	10.0 AU	0.73 Rf	24.4 AU	2.01 %	0.77 Rf	1.4 AU	990.0 AU	2.23 %
6	0.89 Rf	0.7 AU	0.91 Rf	15.3 AU	1.26 %	0.93 Rf	3.7 AU	259.7 AU	0.58 %

Thuja Densitogram- Sample 8



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.10 Rf	0.6 AU	0.12 Rf	582.1 AU	40.91 %	0.20 Rf	38.4 AU	13782.4 AU	27.10 %
2	0.21 Rf	68.4 AU	0.21 Rf	69.6 AU	4.89 %	0.24 Rf	55.9 AU	1473.5 AU	2.90 %
3	0.27 Rf	59.6 AU	0.43 Rf	547.0 AU	38.44 %	0.46 Rf	59.4 AU	25542.8 AU	50.23 %
4	0.46 Rf	60.7 AU	0.54 Rf	129.3 AU	9.08 %	0.59 Rf	25.2 AU	7436.9 AU	14.62 %
5	0.59 Rf	24.7 AU	0.62 Rf	48.2 AU	3.38 %	0.63 Rf	43.7 AU	1226.5 AU	2.41 %
6	0.64 Rf	44.7 AU	0.65 Rf	46.8 AU	3.29 %	0.70 Rf	4.3 AU	1391.5 AU	2.74 %

Thuja mother tincture

Prepared by Thuja Occidental is in dry coarse powder 100gm, purified water 135 ml., and stron alcohol 885 ml to make 1000 ml of tincture. [5,7]

The scanning report as well as the fingerprint characters obtained after integration has been shown.

From the results obtained after densitometric scanning, it was observed that the Std. MQ (D) of Thuja shows 8 peaks at Rf values 0.05, 0.12, 0.22, 0.37, 0.47, 0.68, 0.84 & 0.93.

The chromatogram of the mother tincture of thuja under study shows only 2 of these 8 peaks at 0.37 & 0.68 respectively. Remaining peaks observed in the chromatogram of the mother tincture under study are different as compared to the standard thuja chromatogram.

Thus, we can conclude that the given thuja mother tincture, a market product is not standard w.r.t its quality & quantity and needs standardization. These 'extra' components could be contaminants, or they could be derived from leaves that were harvested prematurely, or the

manufacturers may have used a different thuja species or a different thuja variant than the standard.

Based on this approach our aim is to develop a standardized procedure to evaluate the mother tinctures for its accuracy, sensitivity and reproducibility. The above HPTLC method is very simple, powerful, rapid, reliable and cost effective with respect to the accuracy of the result based on both qualitative and quantitative analysis.

CONCLUSION

Thus, it can be seen from the above experiment, that analytical technique like chromatography, especially HPTLC, has tremendous scope in standardization & quality control of finished products. It not only helps to test the purity of the sample but also helps to quantify its components as well as identify them with the help of chromatogram. It is very simple, powerful, rapid, reliable and cost effective with respect to the accuracy of the result based on both qualitative and quantitative analysis.

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REFERENCES

1. Mandal & Mandal, Text Book of Homoeopathic Pharmacy, 3rd Edition,2012 Abstract- page no.-102, Purpose of selection- p.103 Introduction- page no. - 95-96
2. Kokate C. K., Purohit A. P., Gokhale S. B., Pharmacognosy, Nirali Prakashan: Pune, 51st Ed., Purpose of selection: p.7.25, 7.26
3. Hildebert Wagner, Sabine Bladt, Plant Drug Analysis A Thin Layer Chromatography Atlas Second Edition, , p.1
4. Dr. Sumit Goel, Art & Science of Homoeopathic Pharmacy, 1st Edition 2002, p-414-417

5. Homoeopathic Pharmacopoeia of India (HPI), Vol.I, 1st edition p-87, 199
6. Verma P.N. and Vaid I,Encyclopaedia of Homoeopathic Pharmacopoeia, BJain Publishers Pvt. Ltd.: New Delhi 3RD Edition2002, Vol.I p.612.
7. Verma P.N. and Vaid I,Encyclopaedia of Homoeopathic Pharmacopoeia, BJain Publishers Pvt. Ltd.: New Delhi 3RD Edition2002, Vol.III p.2390.