Applications of HPTLC in Nutraceuticals: Review

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***Abstract*- High-Performance Thin Layer Chromatography (HPTLC) is an enhanced and automated method of thin-layer chromatography (TLC) that offers superior separation performance and detection limits and is frequently a great substitute for GC and HPLC. The study of phytochemicals and biological substances, the quantification of herbal drugs and active ingredients, the fingerprinting of formulations, and the detection of adulterants in formulations are all applications of HPTLC. HPTLC can be used to find forensically important compounds. The use of hyphens in HPTLC-MS, HPTLC-FTIR, and HPTLC-Scanning Diode Laser, among other advanced HPTLC-related techniques, has elevated HPTLC to the status of a powerful analytical tool. According to experts, HPTLC will be used in future combinatorial approaches and for the investigation of drug formulations, bulk medicines, and natural products will increase in the future. In this present review examination of nutraceuticals using HPTLC is noted for its consistency, purity profile, assay values, and precision and accuracy of results.**

***Keywords*: HPTLC, Nutraceuticals, TLC, chromatography.**

INTRODUCTION:

The process of learning about a sample by some type of chemical analysis can be used to summarize the science of analytical chemistry. Any solid, liquid, or gaseous compound may make up the sample being examined, and the analysis will produce data of some sort that will be relevant to the original query posed about the sample. Some information about the sample can be gleaned from the data obtained during the study. This information could be quantitative, qualitative, or both.

Nowadays, an analytical chemical analysis typically involves some kind of analytical apparatus that conducts the actual analysis, with computer software handling data processing and instrument control. In light of this, it is accurate to claim that analytical chemistry has become computerized.

Chemical analysis is a crucial part of a laboratory's ability to guarantee the regular, acceptable performance of analytical methods. Despite the significant number of significant published works on this topic, there is still variability in the approaches used because the validation of an analytical method depends on the method's particular purpose.

Additionally, this article offers a thorough analysis of the development of the HPTLC method, which can be used as a primer on analytical validation for real-world applications in both academic and industrial research.

In this article, we explore the many nutraceuticals that may be found in natural matrices and discuss the analytical methods that can be used to identify and/or analyze them. [1]

HPTLC

With better and more advanced separation efficiency and detection limits than thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC) is an improved and automated type of TLC. It is sometimes referred to as Flat-bed chromatography, High-Pressure Thin Layer Chromatography, or Planar Chromatography. It is a potent analytical technique that works well for both qualitative and quantitative problems [2, 3]. Depending on the type of adsorbents employed on the plates and the development solvent system, separation may be caused by partition, adsorption, or both phenomena.

Common HPTLC Analysis Methodologies

One of the most important steps for a qualitative and quantitative analysis is method development in thin-layer (planar) chromatography. A thorough literature review is always the first step in developing a new analytical process [4], providing important details regarding the physicochemical properties of the sample and its nature (structure, polarity, volatility, stability, and solubility). There are a lot of trial and error processes involved.

The following are the general steps required in developing an HPTLC method:

Basic Steps:

i. Selection of the stationary phase
ii. Mobile phase selection and optimization
iii. Sample Preparation and Application
iv. Chromatogram Development (separation)
v. Detection

Quantitation: HPTLC method validation for pharmaceutical analysis:

i. Specificity
ii. Linearity

iii. Range
iv. Accuracy
v. Precision
vi. Detection Limit, Quantitation Limit
vii.Robustness
Basic Steps

i. Selection of the stationary phase

The type of compounds to be separated should be taken into consideration while choosing the stationary phase during technique development [5]. HPTLC uses smaller plates (10*10 or 10*20 cm) with a noticeably shorter analysis duration (7–20 min) and development distance (usually 6 cm). HPTLC plates are employed for industrial pharmaceutical densitometric quantitative analysis because they offer better resolution, greater detection sensitivity, and superior in situ quantification [6].

ii. Mobile phase selection and optimization

The adsorbent substance utilized as the stationary phase, along with the physical and chemical characteristics of the analyte, are used to determine the mobile phase [7, 8].

iii. Sample Preparation and Application

A good solvent system is one that shifts all mixture constituents from their initial positions while adding nothing to the solvent front. Resolution of the interest peaks should occur between Rf 0.15 and 0.85. Eluent strength, a factor connected to the polarity of the mobile phase components, determines the elution power of the mobile phase. [9] The compound will elute more quickly (or spend less time in the stationary phase) the more nonpolar it is, and more slowly (or spend more time in the stationary phase) the more polar it is.

In order to create a test solution that can be applied directly to an HPTLC plate, a pharmaceutical preparation with a suitably high concentration of analyte is simply dissolved in a suitable solvent that will completely solubilize the analyte and leave excipients undissolved [10]. In order to get adequate resolution for quantification in HPTLC, applying the sample is undoubtedly the most important step [11]. The method used to apply samples depends on variables such the type of sample matrix, workload, and time limitations.

iv. Chromatogram Development (separation)

Even though the chromatogram creation stage is the most significant one in the HTLC process, crucial parameters are frequently missed [12]. Twin-trough chambers, also known as horizontal-development chambers, are used to generate HPTLC plates. The best repeatability is often found in saturated twin-trough chambers with filter paper installed. Twin-through chamber prevents humidity and solvent vapour preloading [13].

Detection—The quenching of fluorescence caused by UV radiation (often ranging from 200 to 400 nm) improves the detection of separated chemicals on the sorbent layers. Fluorescence quenching is a frequent name for this procedure.

Visualization at UV 254 nm

Phosphorescence quenching is how F254 should be characterised. In this case, after the source of excitation is removed, the fluorescence lasts only briefly. Though very brief, it lasts for more than ten seconds. Green fluorescence is produced when the fluorescent indicator F254 is activated by UV light at a wavelength of 254 nm [14]. The layer's emission is reduced by substances that absorb light at 254 nm, and the compound zones can be seen as a dark violet patch against a green background [15]. All compounds with conjugated double bonds produce this quenching. Under 254 nm, it is recommended to look for anthraglycosides, coumarins, flavonoids, propylphenols in essential oils, and some types of alkaloids including indole, isoquinoline, and quinoline alkaloids, among others [16].

Visualization at UV 366 nm

The best way to characterise F 366 is as fluorescence quenching. After the source of excitation is withdrawn in this case, the fluorescence disappears [14]. All anthraglycosides, coumarins, flavonoids, phenolcarboxylic acids, and several alkaloid types (Rauwolfia, Ipecacuanha alkaloids) exhibit this quenching [18].

Visualization at white light

By observing their natural colour in daylight (white light), a zone containing separated compounds can be identified [17].

Derivatisation

Derivatization is a procedural approach that, in order to facilitate chromatographic separations, principally alters the functionality of an analyte. Derivatization can be carried out by either submerging the plates in a suitable reagent or by misting the plates with the reagent [12]. Immersion is the recommended derivatization method for greater reliability.

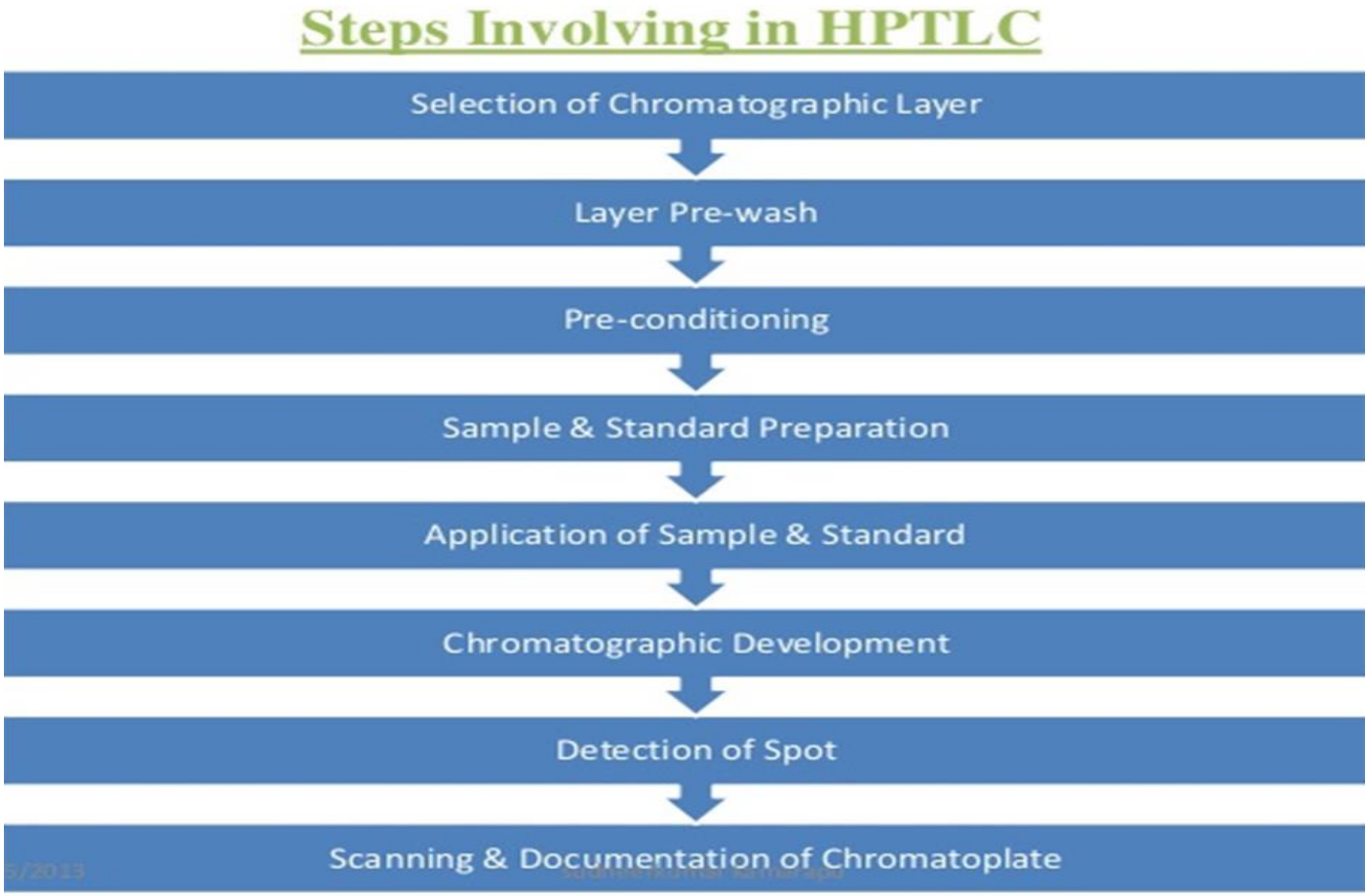

Figure 01: Steps involved in HPTLC [19]

General Applications

1. Pharmaceutical applications

- Quality control
- Content Uniformity Test (CUT)
- Identity- and purity checks
- Stability tests, etc.

2. Clinical applications

- Lipids
- Metabolism studies
- Drug screening
- Doping control, etc.

3. Cosmetics

- Identity of raw material
- Preservatives, coloring materials, etc.
- Screening for illegal substances, etc.

4. Herbal medicines and botanical dietary supplements

- Identification
- Stability tests
- Detection of adulteration
- Assay of marker compounds, etc.

5.Food and feed stuff

- Quality control
- Additives (*e.g.* vitamins)
- Stability tests (expiration), etc.

6. Industrial applications

- Process development and optimization
- Process monitoring
- Cleaning validation, etc.

7. Forensics

- Detection of document forgery

- Investigation of poisoning
- Dyestuff analyses, etc. [20]

NUTRACEUTICALS

Total overview

For a very long time, preventive medications for the treatment and prevention of diseases in humans and animals have been mostly derived from natural compounds, mostly from plants [21]. "Let food be your medicine and medicine be your food," Hippocrates (460–370 BC) said. Nowadays, there is a growing connection between food and pharmaceuticals. Thus, the combination of nutrition and pharmaceutics—two major factors in human wellness—was originally described as a nutraceutical 20 years ago [22]. So-called "functional foods" and "nutraceuticals" have received a lot of attention in research publications over the past 20 years. According to research on functional ingredients, there are good chances that these components will be used in food items, adding value for producers and improving consumer health [23].

According to Zeisel , nutraceuticals are dietary supplements that give a concentrated form of a hypothesized bioactive ingredient from a food, delivered in a non-food matrix, and used to enhance healthiness in dosages greater than those that might be received from regular foods. [24]

Functional foods are those that, when consistently taken, have health benefits that go beyond their nutritional value. The fundamental distinction between nutraceuticals and functional foods is how they are eaten, with nutraceuticals typically being taken as capsules, pills, or tablets. whereas regular foods are always ingested along with functional foods. Thus, food is called functional when a phytochemical is added to the food formulation. A nutraceutical is one that contains the same phytochemical and is contained in a capsule [25].

The presence of secondary metabolites (phytochemicals), which have been demonstrated to exert a wide range of biological functions, has been linked, at least in part, to the ability of some plant-derived diets to lower the risk of chronic diseases. When compared to pharmaceutical medications, these metabolites typically have modest potency as bioactive molecules, but because they are consumed consistently and in small amounts as part of the diet, they may have an obvious long-term physiological effect. Nutraceuticals may be predicted to have a positive impact on pathophysiological processes via a variety of biological pathways. These products are safe and well tolerated, however the heterogeneity of the research, contradictory outcomes, and/or poorly planned investigations make it difficult to evaluate the overall findings. However, they are frequently pricey, lack pharmaceutical-level production standard controls, and may not be effective [26]. In contrast, nutraceuticals are anticipated to be far safer and have fewer side effects than several medicines frequently given in the treatment of specific symptoms.

The composition and amounts of active elements in natural plants (as in any other natural source) change depending on the season, climate, temperature, humidity, soil, and a number of other factors. This presents another challenge in the manufacturing and consumption of nutraceuticals. Therefore, it is crucial to take into account the gathering, identifying, and maintenance of uniform quality, quantification, and standardization.

Therefore, the creation of cutting-edge analytical methodologies is essential to the study of nutraceuticals. It entails the discovery of new nutraceuticals, evaluation of their chemical composition and biological activity, quantification of the natural source, product development, dosage form quality control, etc.

Application of HPTLC in Nutraceuticals

1. Improved HPTLC Method for Determination of Curcuminoids from Curcuma longa

For the quantification of curcuminoids in Curcuma longa, a quick, improved, straightforward, and precise high performance thin layer chromatography approach has been devised. On precoated HPTLC LiChrosphere aluminium plates Si 60F254 with a mobile phase made up of chloroform-methanol (98:2 v/v), curcuminoids were separated. The investigation of densitometry was done in adsorption reflection mode at 366 nm. The method's accuracy and recovery were validated. In comparison to other plates, using LiChrosphere HPTLC plates resulted in greater resolution, repeatability, good selectivity, and compact band. The plant material that was obtained from the midhills had more curcuminoids.

The broadness of the spots issue is solved by the current HPTLC approach (using LiChrosphere plates). Prior to quantification, chromatographic separation allows for interference-free detection of low quantities of curcuminoids. The method is more appropriate for the routine analysis in a crop development plan due to the minimal solvent usage, offline procedure, and zero waiting time for the instrument setup. The use of HPTLC as an analytical method can be constrained by plate to plate variance and limit of quantification (as opposed to LC).This restriction can be circumvented, though, by including known concentrations in each chromatographic run along with the sample. Higher contents of curcuminiods (755.26ng, 533.16ng, 116.8ng, and 732.17ng, 714.40ng, and 132.8ng) of curcumin, demethoxycurcumin, and bis-demethoxycurcumin, respectively, in the midhills at 1,290m and 1,000m amsl of Himalaya were recorded, whereas, at higher (3,165m and 2,769m) altitudes and lower altitudes (350m and 800m), low contents of these curcuminiods were observed. To determine the impact of the age and stage of harvest on the curcuminoids contents in Curcuma longa, more research is required.

Si 60F254 HPTLC LiChrosphere aluminum plates are used in the suggested HPTLC method of analysis, which is quick, selective, and produces well-defined bands and repeatable peaks. The technique is shown to be cost-effective and can produce repeatable results for routine and large-scale analysis. [27]

2. Quantitative analysis of lycopene content in two commercially available tomato sauces: HPTLC-based quality check

Lycopene, the primary component of tomato edible products, was quantitatively analysed in two different brands (B1 and B2) of tomato-containing edible products (tomato sauces). Petroleum ether: toluene: water (5:5:0.5) was used as the mobile phase to construct the HPTLC profile. Lycopene Rf values were determined to be 0.94 in various tomato edible product brands. Comparing

the overall peak areas of lycopene with the corresponding peak areas of various formulations, the estimated lycopene concentrations for formulations B1 and B2 were 5.43 and 7.27 g mL-1, respectively. The current study justifies the use of HPTLC fingerprint profiles for determining the identification, purity, and quality of various tomato consumable items. It also generates data that could be helpful in developing standards for these goods. [28]

3. Development of a High-Performance Thin-Layer Chromatography Method for the Quantification of Alkyl Glycerolipids and Alkenyl Glycerolipids from Shark and Chimera Oils and Tissues

Alkyl lipids that have an ether bond at the sn-1 position of a glycerol backbone and alkenyl lipids that have a vinyl ether bond at the same location make up ether lipids. Either polar glycerophospholipids or neutral glycerolipids are forms of these ether glycerolipids. It is necessary to separate and quantify total alkyl and alkenyl glycerolipids from biological materials in order to identify any variations depending on tissue or physiopathological circumstances before examining the biological role of molecular species of ether glycerolipids. Using the separation of their corresponding alkyl and alkenyl glycerols, we describe the invention of the first high-performance thin-layer chromatography method for the quantification of total alkyl and alkenyl glycerolipids. This procedure begins with the reduction of all lipids after extraction, which converts neutral and polar ether glycerolipids into alkyl and alkenyl glycerols. Next, the lipids are appropriately purified, and the alkyl and alkenyl glycerols are developed in a linearly ascending manner on high-performance thin-layer chromatography plates before being stained, carbonised, and analyzed densitometrically. The quantification of alkyl and alkenyl glycerols in samples allowed for the direct determination of the amount of alkyl and alkenyl lipids contained in the samples. Calibration curves were generated using commercial alkyl and alkenyl glycerol standards. It's interesting that we discovered differing levels of these lipids in shark liver oil versus chimaera. In order to demonstrate the viability of this approach in various biological matrices (muscle, tumour), we quantified alkyl and alkenyl glycerolipids in periprostatic adipose tissues from human prostate cancer.

By separating alkyl and alkenyl glycerol, we were able to create a straightforward HPTLC method for quantifying total alkyl and alkenyl glycerolipids. Alkyl and alkenyl glycerolipids have been measured using this technique in adipose tissues and oils. We demonstrated that it could also be used in other matrices, like tumor and muscle tissues. It should be highlighted that adequate lipid concentrations are necessary, particularly for samples with little ether lipid content. [29]

4. A Greener HPTLC Approach for the Determination of beta-Carotene in Traditional and Ultrasound-Based Extracts of Different Fractions of Daucus carota (L.), Ipomea batatas (L.), and Commercial Formulation

Carotenes are yellow-orange pigments with the chemical formula C40H56 that are widely distributed in vegetable plants. The two main isomers that can be discovered in plants are - and -carotene [1]. The most common type of carotene in plants is called -carotene, and it is both a vital nutrient and a source of human vitamin A. Carotenes are a promising chemical for the pharmaceutical, food, and cosmetic industries due to their broad range of biological activity and advantages for animal health. The most recent studies on carotenes' biological and pharmacological effects were reviewed by Kim (2016) [3].

Ipomoea batatas L. Lam., also known as the sweet potato, is a plant whose roots have long been a useful source of food and energy for both people and animals. The sixth-most widespread food crop in the world is the sweet potato, which continues to be very valuable economically. The root of I. batatas contains dietary fiber, carbohydrates, vitamin A (as -carotene), vitamin B6, vitamin C, copper, manganese, potassium, and iron .

Due to the presence of phenolics, flavonoids, -carotene, anthocyanins, and derivatives of caffeoylquinic acid, the antioxidant capabilities of sweet potatoes have lately been examined. Numerous investigations have confirmed its use in medicine, notably given its antiviral and anti-diabetic properties .

Additionally, -carotene is found in different vegetable crops, dietary supplements, and commercial formulations. This can be done by itself or in combination with other phytopharmaceuticals using a variety of "high-performance thin-layer chromatography (HPTLC)" techniques.

Compared to other liquid chromatography-based techniques, greener HPTLC systems have a number of advantages . In order to determine -carotene in this study, the more environmentally friendly reversed-phase HPTLC method was utilized.

This study was conducted to develop and validate a quick, sensitive, and environmentally friendly reversed-phase HPTLC method for determining -carotene in TE and UBE of various fractions of carrots, sweet potatoes, and commercial formulations because there was no such method in the literature. The more environmentally friendly analytical method for measuring -carotene is sensitive, quick, accurate, precise, strong, and greener. The UBE of carrots, sweet potato, and marketed formulation A had significantly more -carotene than its TE. As a result, it is recommended that the UBE technique be used to extract -carotene from diverse carrot, sweet potato, and commercial formulation fractions. The calculated overall AGREE scale for the greener analytical methodology indicated the method for estimating carotene was very green. In comparison to amounts reported by UV and HPLC methods in the literature, the amount of -carotene found in various fractions of carrots and sweet potatoes in this investigation was significantly higher. As a result, the proposed analytical methodology and analysed fractions might be regarded as being superior to previously published UV and HPLC procedures for the determination of -carotene. These results indicate that a variety of food and pharmaceutical samples containing -carotene as an active medicinal ingredient can be determined using the more environmentally friendly reversed-phase HPTLC method. [30]

5. HPTLC Method for Determination of 20-Hydroxyecdysone in Sida rhombifolia L. and Dietary Supplements

A significant anabolic ecdysteroid that is found naturally is 20-hydroxyecdysone. The entire plant material of Sida rhombifolia L. was used to establish a straightforward thinlayer chromatography method to quantify 20-hydroxyecdysone in methanolic extract. The quantitative evaluation of dietary supplements used this strategy with effectiveness. Chloroform: methanol (8:2 v/v) was used as the developing solvent to separate the samples on glass TLC plates coated with silica gel 60F254. A reflectance/absorbance densitometric analysis of 20-hydroxyecdysone was carried out at 250 nm. The correlation coefficient for the calibration curve was

>0.999, and the calibration ranged from 200 to 1,000 ng spot. Additionally, six separate Sida species have their own distinct fingerprints seen on the HPTLC plate.
The temperate zone is where the Sida genus of plants are located. In this genus, there are thought to be 200 species . Sida rhombifolia L., commonly known as Mahabala in Ayurveda, is one of the species that is utilised as an anti-inflammatory and tonic . Ecdysone and 20-hydroxyecdysone (20E) were discovered during our earlier research on the methanolic extract of the entire plant of S. rhombifolia L. We also reported the discovery of new ecdysteroid glycosides.
HPTLC is the best tool for analyzing herbal drugs . Previously, Lafontetal reported on the HPTLC-MSMS of ecdysteroids .Ecdysteroids have also been examined in reverse phase and diol bonded separations . In this article, we offer a method for densitometric analysis-based quantification of 20E in S. rhombifolia L. extract and commercial preparations said to include 20E. Additionally, it has been discovered that some additional Sida species also contain ecdysterol compounds . Additionally, we are reporting here on the fingerprinting of a few Sida species using 20E as a marker chemical.
The suggested method is a quick and easy substitute for quantitatively determining 20E in S. rhombifolia methanolic extract. This technique can be used to ensure the quality of relevant extracts and market samples. [31]

6. Quantitative determination of rutin by High-Performance Thin-Layer Chromatography

High-performance thin-layer chromatography (HPTLC) was used to identify rutin and quantify it. This process was developed and might be suggested for approval and use in the food, cosmetics, and pharmaceutical industries. The ideal conditions for HPTLC of rutin with quantitative results interpretation on a personal computer were discovered experimentally and theoretically supported.
The method can be suggested for endorsement and application in the standardization of various dosage forms and medicinal plant raw materials containing rutin, as well as for production quality control in the food and cosmetics industries. [32]

7. Chromatographic fingerprint analysis of steroids in Aerva lanata L by HPTLC technique

Polpala [Aerva lanata (A. lanata) L.] is an important medicinal plant, found throughout tropical India as a common weed in fields and wasteland. Even now, wild collection of the species continues to be a source of raw drug in Ayurvedic preparations. Because of its popularity in folk medicine, A. lanata has become the subject of intense pharmacological and chemical studies for the last 30 years. Numerous studies have proven its versatile pharmacological activities: anthelmintic, demulcent[2], anti-inflammatory, diuretic, expectorant, hepatoprotective, nephroprotective, anti-diabetic activity, anti-hyperglycaemic activity in rats, microbial, cytotoxic, urolithiatic, hypoglycemic, anti- hyperlipidaemic, anti-parasitic and anti-helminthic activities.
In order to identify the bioactive compounds responsible for the above pharmacological activities phytochemical studies have been carried out by several workers with different kinds of bioactive compounds particularly alkaloids such as canthin-6-one and beta-carboline, aervine methylamine (10-methoxycanthin-(10-hydroxycanthin-6-one), aervoside 6-one(10-beta-Dglucopyranosyloxycanthin-6-one) and aervolanine (3-(6-methyoxy-beta-carbolin-1-yl) propionic acid) from leaves of A. lanata.
HPTLC is a valuable tool for reliable identification. It can provide chromatographic fingerprints that can be visualized and stored as electronic images. Many scientific documents are available on crude drug extracts, but promoting these herbal drugs in the international/national market is difficult due to lack of reproducible biological reports, selection of wrong plants, lack of data on the time, area of collection and identity of the botanical source.
HPTLC profile of steroids has been chosen here to reveal the diversity existing in A. lanata. Such fingerprinting is useful in differentiating the species from the adulterant and act as biochemical markers for this medicinally important plant in the pharma industry and plant systematic studies.
This work has shown that the chromatogram of the methanolic extract of A. lanata, which was obtained by HPTLC, contains 30 peaks. Therefore, it has been demonstrated that HPTLC fingerprinting is a linear, exact, and accurate method for herbal identification. It may also be utilised to further authenticate and characterize the medicinally significant plant. The manufacturer would benefit from the generated HPTLC fingerprints for quality assurance and standardization of herbal compositions. Such fingerprinting aids in separating the species from the adulterant and serves as a biochemical identifier for this crucial plant for the pharmaceutical industry and systematic research of plants. [33]

Conclusion

In this article, we review the basic steps and methodologies involved in HPTLC as well as applications in the analysis of nutraceuticals. HPTLC can be used to separate and purify a variety of compounds from natural and synthetic origins HPTLC is a versatile technique and is known for uniformity, purity profile, assay values, and precision and accuracy of results in the analysis of nutraceuticals. In this article, we explore the examples of nutraceuticals that may be found in natural matrices and discuss the analytical methods that can be used to identify and/or analyze them by using HPTLC.

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