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Research Article

Detection and Estimation of *alpha*-Amyrin, *beta*-Sitosterol, Lupeol, and *n*-Triacontane in Two Medicinal Plants by High Performance Thin Layer Chromatography

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A normal phase high performance thin layer chromatography (HPTLC) method has been developed and validated for simultaneous estimation of four components, *namely, alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane from two medicinally important plants, *Leptadenia reticulata* Wight & Arn. and *Pluchea lanceolata* (DC.) CB. Clarke. In Ayurveda, both plants have been reported to possess immunomodulatory activity. Chromatographic separation of the four components from the methanolic extracts of whole plant powders of *Leptadenia reticulata* Wight & Arn. and *Pluchea lanceolata* (DC.) CB. Clarke. was performed on TLC aluminium plates precoated with silica gel $60F_{254}$ using a suitable mobile phase. The densitometric scanning was done after derivatization at λ = 580 nm for α-amyrin, β-sitosterol, and lupeol, and at 366 nm for *n*-triacontane. The developed HPTLC method has been validated and used for simultaneous quantitation of the four components from the methanolic extracts of whole plant powders of *Leptadenia reticulata* Wight & Arn. and *Pluchea lanceolata* (DC.) CB. Clarke. The developed HPTLC method is simple, rapid, and precise and can be used for routine quality control.

1. Introduction

Herbal medicines have been used since ages to treat various ailments. Ayurveda is an Indian traditional system of medicine used since ancient times. It has a huge list of herbs used in various forms for treatment of different disease conditions. Owing to the medicinal properties attributed to herbal drugs, it is necessary to maintain their quality and purity, thereby justifying their acceptability in modern system of medicine. Standardisation of these herbal drugs is a challenge to the entire scientific fraternity. However, due to lack of suitable quality control and quality assurance standards for herbal drugs, it becomes difficult to ensure uniformity of their composition which in turn affects the efficacy of their final products. Analytical tools are important for qualitative, semiquantitative, and quantitative phytochemical analysis of herbal drugs and formulations. Chromatographic techniques such as high performance liquid chromatography (HPLC), high performance thin layer chromatography (HPTLC), and gas chromatography (GC) are used to efficiently determine the quality of the herbs by developing fingerprints and estimation of biomarkers. Among the wide choice of chromatographic techniques, HPTLC is a simple, fast, and accurate technique for use, making it advantageous over others for quick assessment of a number of samples simultaneously [1]. In the present research work, HPTLC method has been developed as a quality control tool for whole plant powder of *L. reticulata* and *P. lanceolata*.

L. reticulata belonging to family Asclepiadaceae commonly known as "Jivanti" has been reported to possess antitumour and anticancer activity [2]. *P. lanceolata* belonging to family Asteraceae commonly known as "Rasna" is an important xerophytic medicinal herb. It is traditionally used for dyspepsia, bronchitis, and rheumatoid arthritis [3, 4]. In Ayurveda both plants have been reported to be used as a "Rasayana" for immunomodulatory activity [5]. Previous chemical studies of *L. reticulata* showed the presence of flavonoids, triterpenes, and steroids. It is also a rich source

of biologically active cardiac and pregnane glycosides [2]. *P. lanceolata* contains high amounts of medicinally important secondary metabolites, namely, quercetin, *beta*-sitosterol, triterpenoids, and so forth [6, 7].

Among all phytochemicals, *beta*-sitosterol is a main phytosterol found in many plants. It has been reported to show anti-inflammatory, antineoplastic, antipyretic, and immunomodulating activity [8]. Also triterpenoids are among commonly present secondary metabolites in plants. *alpha*-Amyrin, a pentacyclic triterpenoid, has been reported to show anti-inflammatory properties. Lupeol has been reported to show anticarcinogenic and antitumour activity [9].

n-Triacontane has been reported to be present in number of plants including *L. reticulata*. *n*-Triacontane has also been reported to be tested for biological activity such as antibacterial, antidiabetic, and antitumor activity [10–12].

Some of the analytical methods for qualitative analysis of *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane from other plant samples are discussed herewith. RP-HPTLC separation of twelve compounds including *alpha*-amyrin, lupeol, and *beta*-sitosterol from *Brassica oleracea* L., *Solanum lycopersicum* L., *Rosmarinus officinalis* L., *Salvia officinalis* L., and *Quercus robur* L. was carried out [9]. Another normal phase HPTLC technique was also reported for separation and determination of *alpha*-amyrin and lupeol from *Brassica oleracea* L. leaf extracts. Visual estimation after derivatization using anisaldehyde-sulphuric acid was carried out [13]. A method comprising capillary GC was used to accurately quantitate *alpha*-amyrin, *beta*-sitosterol, and lupeol from aerial part of *J. anselliana* as reported [14]. A gas chromatography-mass spectrometry (GC-MS) method was used for analysing compounds in *Salvia bicolour Desf.* extract. Among various compounds, lupeol and *beta*-sitosterol were determined [15]. A method was reported to study the chemical constituents of the essential oil of *Laggera pterodonta* (DC.) Sch. Bip. using GC-MS [16]. Percentage of *n*-triacontane in essential oil of *Laggera pterodonta* (DC.) Sch. Bip. was evaluated.

However, no method was applied for quantifying the presence of *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane simultaneously from *L. reticulata* and *P. lanceolata*.

Hence, in present research work, a simple, rapid, precise, and accurate HPTLC method has been developed and validated using International Conference on Harmonization (ICH) guidelines for simultaneous determination and quantification of *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane form dried whole plant powder of *L. reticulata*. and *P. lanceolata*.

2. Experimental Methods

2.1. Experimental Reagents. The solvents, acetonitrile (purity 99.9%), petroleum ether (purity 99.8%), ethyl acetate (purity 99.0%), methanol (purity 98.9%), and chloroform (purity 99.9%) were obtained from E. Merck (India). The precoated TLC $60F_{254}$ plates were obtained from E. Merck (India).

2.2. Reference Standards. Reference standards *alpha*-amyrin (purity 99.3%), *beta*-sitosterol (purity 99.8%), lupeol (purity 99.7%), and *n*-triacontane (purity 98.9%) were procured from Sigma-Aldrich Chemie GmbH (Aldrich Division, Steinbeim, Germany).

2.3. Plant Materials. Whole plant of *L. reticulata* was collected from Bhumel village, district of Nadiad, Gujarat. The plant material was authenticated from Agarkar Research Institute, Pune, India (Voucher no. WP-090). Whole plant of *P. lanceolata* was collected from Sonamukhi Nagar, Jodhpur, India. Herbarium of *P. lanceolata* was authenticated from Botanical Survey of India, Pune, India (Certificate no. BSI/WRC/Tech./2012/79).

The duplicate herbaria of both the plants were prepared and are preserved in Ramnarain Ruia College, Matunga, India, for future reference. Both plant materials were washed with water to remove soil particles, dried in shade, finely powdered and then sieved through BSS mesh size 85, and stored in an airtight container at room temperature (25 ± 2°C).

2.4. Preparation of Solutions

2.4.1. Preparation of Stock Solution of alpha-Amyrin, beta-Sitosterol, Lupeol, and n-Triacontane. About 10.0 mg of *alpha*-amyrin was accurately weighed and transferred to 10.0 mL volumetric flask. 5.0 mL of methanol solution was added into the volumetric flask and sonicated in an ultrasonic bath (Model: TRANS-O-SONIC, Frequency: 50 Hz) for 5 minutes for complete dissolution of *alpha*-amyrin. The volume was then made up to the mark with methanol. A stock solution of *alpha*-amyrin with concentration of 1000 μg/mL was prepared. Similarly stock solutions of *beta*-sitosterol and lupeol were prepared. Stock solution of *n*-triacontane was prepared by taking accurately weighed 100 mg *n*-triacontane in a 10.0 mL volumetric flask and initially dissolving it in 5.0 mL chloroform as described above. The volume in the standard flask was then made up to the mark with methanol. A stock solution of *n*-triacontane with concentration of 10,000 μg/mL was prepared.

2.4.2. Preparation of Working Standard Solution of alpha-Amyrin (50.0 μg/mL), beta-Sitosterol (50.0 μg/mL), Lupeol (50.0 μg/mL), and n-Triacontane (500.0 μg/mL). 0.50 mL of above stock solution of each standard was then transferred to 10.0 mL volumetric flask and the contents of volumetric flask were diluted up to 10.0 mL by methanol to obtain mixture four standards of *alpha*-amyrin, *beta*-sitosterol, lupeol with concentration 50.0 μg/mL each, and standard of *n*-triacontane with concentration 500.0 μg/mL.

2.4.3. Preparation of Sample Solution. About 1.0 g of dried powder of whole plant of *L. reticulata* was accurately weighed and transferred to a 100 mL stoppered conical flask. 25.0 mL of methanol : chloroform (1 : 1, v/v) was added to it and the flask was sonicated in an ultrasonic bath for 15 minutes. The flask was then shaken at 50 rpm, on a conical flask

shaker overnight at room temperature (25 ± 2°C). Sample was filtered through Whatman filter paper no. 1. The filtrate was then finally filtered using 0.45 μm nylon filters (Millipore), collected in a beaker, and then evaporated on a hot water bath. The final volume was then made up to 10 mL with methanol : chloroform (1 : 1, v/v) in a 10 mL volumetric flask. The same procedure was followed for preparation of extract of whole plant of *P. lanceolata*.

2.4.4. Prederivatization Reagent. The standards and sample solution were applied in the form of a band on TLC plate. This plate was subjected to prederivatization by iodine. Iodine vapours were generated by heating iodine crystals in a closed stoppered flat bottom conical flask. The plate was then exposed to the iodine vapour in a dark enclosed chamber, for 10 minutes. After that, the plate was removed from the chamber and heated on Camag TLC plate heater at 100°C for 10 minutes, till the excessive iodine was removed. This prederivatized plate was finally used for development.

2.4.5. Preparation of Mobile Phase. The mobile phase used in the present research work for simultaneous quantification of *alpha*-amyrin, *beta*-sitosterol, and lupeol was prepared by mixing petroleum ether, ethyl acetate, and acetonitrile in the volume ratio of 8.2 : 1.8 : 0.1. During development of each plate, a fresh mobile phase was prepared.

2.4.6. Postderivatization Reagent. Anisaldehyde-sulphuric acid reagent was used as postderivatizing reagent. Anisaldehyde-sulphuric acid reagent was prepared by taking 10 mL of sulphuric acid, which was added to an ice cooled mixture of methanol (170.0 mL) and glacial acetic acid (20.0 mL). Further, 1.0 mL of anisaldehyde solution was added to the above mixture of methanol, glacial acetic acid, and sulphuric acid. The solvent mixture was thoroughly mixed by shaking and used as per requirement.

2.4.7. Chromatography. The Chromatography was performed on 20 cm × 10 cm TLC aluminum precoated silica gel $60F_{254}$ plate, with 200 μm layer thickness (E. Merck, Mumbai, India). Standard and sample solutions were applied to the plates as 8 mm bands, 6 mm apart from each other and 10 mm from bottom edge of the plate, under a continuous supply of nitrogen by means of a Camag Linomat V TLC sample applicator with a 100 μL syringe (Hamilton, Bonaduz, Switzerland). After the application, prederivatization was performed by exposing the plate to iodine vapour for 10 minutes. The prederivatized plate was developed vertically ascending in a twin-trough glass chamber (Camag, Switzerland) saturated with mobile phase comprising petroleum ether : ethyl acetate : acetonitrile (8.2 : 1.2 : 0.1 v/v/v). The optimized chamber saturation time for the mobile phase was 20 minutes at room temperature (25 ± 2°C). The chromatographic run length was 90 mm from the bottom edge of the plate. After development, the plate was air dried for complete removal of mobile phase and derivatized by dipping the developed plate in anisaldehyde-sulphuric acid reagent for 2 seconds. The plate was then air-dried for complete removal of anisaldehyde-sulphuric acid and heated at 110°C for 10 minutes. Densitometric scanning was then performed at λ = 580 nm for *alpha*-amyrin, *beta*-sitosterol, and lupeol in reflectance/absorbance mode and λ = 366 nm for *n*-triacontane using Camag TLC scanner 4 with winCATS software version 1.4.6. The slit dimension used was 6.0 × 0.45 mm (micro) with scanning speed of 20 mm/sec, throughout the analysis.

2.5. Method Validation

2.5.1. Linear Working Range of alpha-Amyrin, beta-Sitosterol, Lupeol, and n-Triacontane. Determination of linear dynamic range concentration of *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane was done by applying 2 μL, 4 μL, 6 μL, 8 μL, 10 μL, 12 μL, 14 μL, 16 μL, 18 μL, 20 μL, and 22 μL on TLC plate of working standard containing *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane.

The peak areas obtained from densitograms for each applied concentration of *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane were noted.

The calibration curves of all four standards were obtained by plotting graphs of mean peak areas of each standard versus corresponding concentration (Figure 1). The results, listed in Table 1, show that within the concentration range indicated, there was a good correlation between mean peak area and concentration of standards.

2.5.2. Limit of Detection (LOD) and Limit of Quantification (LOQ). The limit of detection (LOD) is defined as a peak, whose signal-to-noise (S/N) ratio is 3 : 1. The limit of quantification (LOQ) is defined as a peak, whose signal-to-noise (S/N) ratio is 10 : 1. The results are listed in Table 1.

2.5.3. System Suitability. System suitability was carried out to verify that resolution and reproducibility of the system were acceptable for the analysis. System suitability test was carried out by applying 6 μL standard solutions of *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane on TLC plate in six replicates under specified chromatographic conditions. The chromatograms were recorded. The values of percent relative standard deviations of peak area and retention factor of standards were taken as an indicator of system suitability. Since the values of percent relative standard deviations of peak area were found to be less than 2 and peaks were well-resolved, the method was suitable for analysis.

2.5.4. Specificity. The specificity of the proposed HPTLC method was ascertained by comparing visible chromatograms of *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane standards with those found in the sample. The chromatograms were compared by overlay. Good correlation was observed between chromatograms obtained from *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane standards and samples at all R_f (*alpha*-amyrin—R_f 0.68, *beta*-sitosterol—R_f 0.48, lupeol—R_f 0.61, and *n*-triacontane—R_f 0.91) values, respectively.

Table 1: Method validation data for simultaneous quantification of *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane from dried whole plant powder of *Leptadenia reticulata* (Retz.) Wight & Arn. and *Pluchea lanceolata* (DC.) CB. Clarke.

Parameters	Results			
	alpha-Amyrin	*beta*-Sitosterol	Lupeol	*n*-Triacontane
Linear range (μg/band)	0.1–1.0	0.1–1.0	0.1–1.0	1.0–10.0
Correlation coefficient (r)	0.999	0.999	0.999	0.999
LOD (μg/band)	0.03	0.03	0.03	0.30
LOQ (μg/band)	0.10	0.10	0.10	1.00
Stability of standard solution	Stable for minimum 48 hours	Stable for minimum 48 hours	Stable for minimum 48 hours	Stable for minimum 48 hours
System suitability (% R.S.D.)	Less than 2	Less than 2	Less than 2	Less than 2
	Leptadenia reticulata (Retz.) Wight & Arn.			
Repeatability-% R.S.D. range (n = 3) (on the same day)	0.93 ± 0.01	0.99 ± 0.06	1.07 ± 0.06	1.23 ± 0.06
Intermediate precision % R.S.D range (n = 9) (percent R.S.D. for three successive days)	0.94 ± 0.04	0.99 ± 0.06	1.03 ± 0.10	0.93 ± 0.04
Assay (mg/2 g)	0.99 ± 0.01	1.18 ± 0.02	0.62 ± 0.06	0.65 ± 0.06
Percent recovery	99.01 ± 0.07	98.14 ± 0.04	98.60 ± 0.01	99.46 ± 0.05
	Pluchea lanceolata (DC.) CB. Clarke.			
Repeatability-% R.S.D. range (n = 3) (on the same day)	1.00 ± 0.05	0.95 ± 0.02	1.00 ± 0.03	N.D.
Intermediate precision % R.S.D range (n = 9) (percent R.S.D. for three successive days)	0.96 ± 0.05	0.97 ± 0.06	0.98 ± 0.05	N.D.
Assay (mg/2 g)	0.72 ± 0.03	1.61 ± 0.06	0.17 ± 0.01	N.D.
Percent recovery	99.46 ± 0.01	99.25 ± 0.02	99.81 ± 0.05	N.D.

*Note: N.D.: not detected.

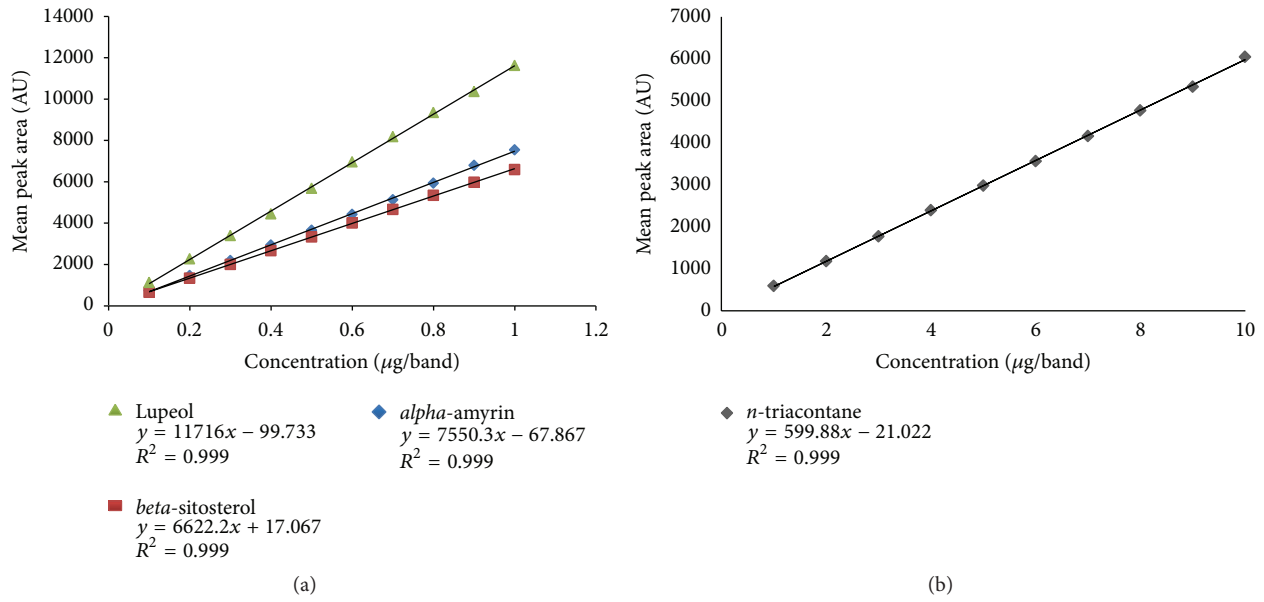

Figure 1: The figure represents a simultaneous plot of mean peak area v/s concentration of *alpha*-amyrin, *beta*-sitosterol, lupeol and *n*-triacontane standards, respectively.

2.5.5. Precision. The method was validated in terms of repeatability and intermediate precision.

The repeatability was evaluated in triplicates by applying extract of both plant materials on TLC plate on the same day, under the specified chromatographic conditions. The peak areas of *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane were recorded and assessed.

The intermediate precision of the method was evaluated by analyzing the sample solution in triplicate on three different days, under the specified chromatographic conditions. The peak areas of *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane were recorded and assessed.

The precision results were expressed as percentage relative standard deviations of peak areas of *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane. The results, listed in Table 1, indicate that the proposed method is precise and reproducible.

2.5.6. Standard Stability. The stabilities of standard *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane solution were determined by comparing the peak areas of the standard solution, and the stock solution was observed at different time intervals by spotting 6 μL of working standard solution on TLC plate for a period of minimum 48 hrs at room temperature. The results showed that the peak areas of *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane almost remained unchanged (values of percent relative standard deviations were less than 2) over a period of 48 hrs, and no significant degradation was observed within the given period. Thus, the standard solutions of *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane were stable for a minimum of 48 hrs.

2.5.7. Assay. The developed and validated HPTLC method was used for quantification of *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane from the extract of dried whole plant powder of *L. reticulata* and *P. lanceolata*. 4.0 μL of extract of both plant materials was applied as bands on the same TLC plate. The plate was developed and scanned under the specified chromatographic conditions. The chromatograms were recorded. To check the repeatability of the method, assay experiment was repeated seven times and the values of mean standard deviation (S.D.) and percent relative standard deviation (%R.S.D.) were calculated. The results of assay experiment are shown in Table 1. The amounts of *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane present in each sample solution were determined from the calibration curve, by using the peak areas of *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane in the sample.

2.5.8. Accuracy. The accuracy of the method was established by performing recovery experiment by using standard addition method at three different levels. To accurately weighed, about 1.0 g of dried whole plant powder of *L. reticulata*, known amounts of standards *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane were added and extracted. Each of the three different levels containing sample solution and standard was applied in seven replicates on the same plate. The plate was then developed and scanned under the specified chromatographic conditions, as described earlier. The *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane contents were quantified by the proposed method and the percentage recovery was calculated. The same procedure was repeated for dried whole plant powder of *P. lanceolata*. except the addition of *n*-triacontane. The percent recovery values are shown in Table 1.

3. Results and Discussion

During HPTLC analysis, several different mobile phases were tried for separation of *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane from other phytochemicals present in whole plant powder of *L. reticulata*. Good separation was achieved with the mobile phase comprising pet-ether: ethyl acetate: acetonitrile in the volume ratio of 8 : 2 : 0.1 along with prederivatization with iodine. Since, the phytochemicals, *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane showed no UV and visible sensitivity on plate, the plate was postderivatized further with anisaldehyde-sulphuric acid reagent. The R_f values for *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane were 0.60, 0.48, 0.69, and 0.91, respectively. Figure 2 shows typical HPTLC chromatograms of standard *alpha*-amyrin, standard *beta*-sitosterol, standard lupeol, and standard *n*-triacontane in extracts of dried whole plant powder of *L. reticulata* and dried whole plant powder of *P. lanceolata*.

Literature survey revealed that some of the related methods were reviewed. A qualitative normal phase HPTLC method was reported [13] for separating and determining *alpha*-amyrin and lupeol from *Brassica oleracea* L. leaf extracts. HPTLC silica gel $60F_{254}$ plates were used as stationary phase with the mobile phase as *n*-hexane-ethyl acetate in a volume ratio of 5 : 1. Postderivatization was carried out using anisaldehyde-sulphuric acid reagent. Identification of *alpha*-amyrin and lupeol was carried out by visual comparison of the colour of *alpha*-amyrin and lupeol after derivatization. The reported method was unable to resolve the isomeric compounds *alpha*-amyrin and lupeol, because there was no significant difference in their R_f values. In a reported method [9] chromatographic separation of twelve compounds including *alpha*-amyrin, lupeol, and *beta*-sitosterol from *Brassica oleracea* L., *Solanum lycopersicum* L., *Rosmarinus officinalis* L., *Salvia officinalis* L., and *Quercus robur* L. was studied. The study described a combination of two RP-HPTLC methods for a qualitative determination of twelve phytochemicals (*alpha*-amyrin, *beta*-amyrin, delta-amyrin, lupeol, lupenone, lupeol acetate, cycloartenol, cycloartenol acetate, ursolic acid, oleanolic acid, stigmasterol, and *beta*-sitosterol) and evaluation of their presence in different plant extracts. In the study, RP-HPTLC was used to analyse the phytochemicals. Experiment was performed on RP-HPTLC plates, using the combination of two mobile phases to isolate compounds; these were further identified using RP-HPLC method. The reported methods were only used for qualitative screening and identification of these compounds. Also capillary GC was used for quantitation of *alpha*-amyrin, *beta*-sitosterol, and lupeol from aerial part of *J. anselliana*. Solid phase extraction

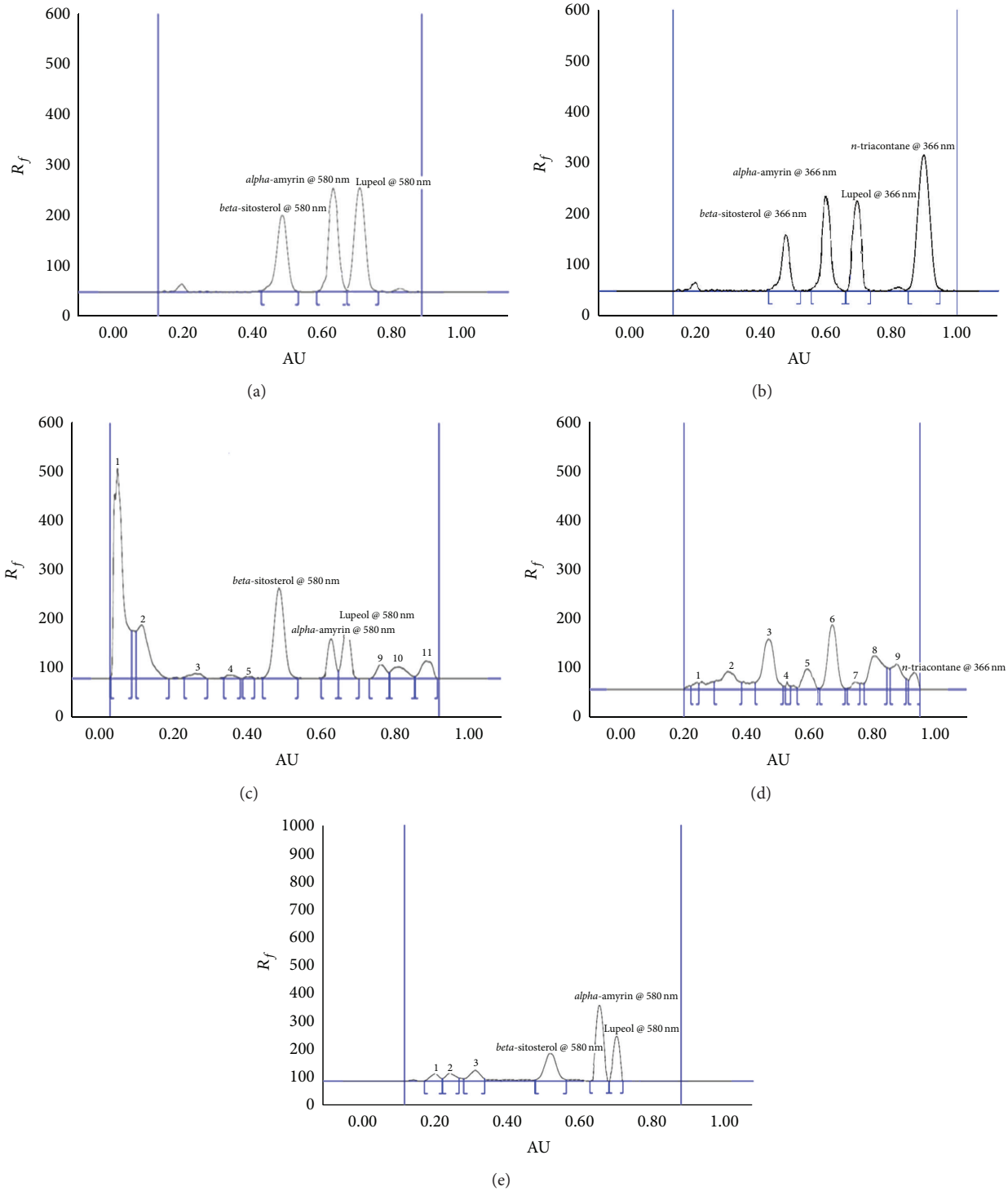

Figure 2: (a) and (b) represent HPTLC chromatogram of standard obtained at $\lambda = 580$ nm and $\lambda = 366$ nm. (c) and (d) represent HPTLC chromatogram of sample *L. reticulata* obtained at $\lambda = 580$ nm and $\lambda = 366$ nm. (e) represents HPTLC chromatogram of sample *P. lanceoata* obtained at $\lambda = 580$ nm.

was used to remove the matrix [15]. A GC/MS method was reported for analysing compounds in *Salvia bicolour Desf.* extract. *beta*-Sitosterol and lupeol were detected. The retention time observed was 41.04 mins for *beta*-sitosterol and 41.5 mins for lupeol [14]. Both methods were time consuming. Another method has been reported [16] to study the chemical constituents of the essential oil of Laggera pterodonta (DC.) Sch. Bip. using GC-MS. *n*-Triacontane was

detected using Helium as carrier gas and injection done at elevated temperature of 250°C. The flow rate of 1.61 mL/min was used. Retention time observed for *n*-triacontane was 30.217. *n*-Triacontane was found to be 43.18% approximately. No HPTLC method has been reported for quantification of *n*-triacontane from whole plant powder of *L. reticulata* and *P. lanceolata*.

Therefore, in the present research work, in order to standardize the plants with these markers, a precise and accurate HPTLC method for simultaneous estimation of *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane from the extract of whole plant powder of *L. reticulata* and *P. lanceolata* was developed. The present developed method is advantageous compared to the above reported methods as it uses a simple prederivatization technique of iodination which resolved the isomeric compounds, *alpha*-amyrin and lupeol. Also, alkanes are generally high molecular weight compounds and difficult to be analysed on TLC quantitatively. *n*-Triacontane which is an alkane has also been quantified. The mobile phase comprising petroleum ether: ethyl acetate: acetonitrile in the volume ratio of 8.2 : 1.8 : 0.1 helped in resolving the phytoconstituents without the interference from sample matrix, with the development time of less than 10 minutes which helped in reduction of analysis time. Finally the developed plates were derivatized by anisaldehyde-sulphuric acid reagent. The developed method was validated following ICH guidelines criteria and is economical, simple, and rapid which can be easily performed at any laboratory conditions with specified parameters of HPTLC.

4. Conclusion

The developed HPTLC technique is simple, precise, specific, and accurate, which can be used for the routine quality control analysis and simultaneous quantitative determination of *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane from the whole plant powder of *L. reticulata* and *P. lanceolata*. The method can be applied to effectively quantitate the presence of *alpha*-amyrin, *beta*-sitosterol, lupeol, and *n*-triacontane in other samples as well.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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