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A rapid HPTLC method to estimate ximenynic acid in semisolid dosage formulation

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Abstract

A novel HPTLC analytical technique and validation study were developed to determine the concentration of ximenynic acid (XMA) in semisolid dosage formulations (SDF) such as cream, gel, lotion, etc. The procedure makes use of reverse-phase high-performance liquid chromatography (HPLC). This study presents the first report of sensitive, selective, precise and robust HPTLC method, which has been developed and validated for quantification of the XMA from pharmaceutical formulation. The chromatographic development was carried out on HPTLC plates precoated with silica gel 60 F254 using a mixture of Toluene: Ethyl Acetate: Methanol: Formic acid (5:4:0.5:0.5 v/v/v/v) as mobile phase. Detection was carried out densitometrically at 254 nm. The Rf value of XMA were found at Rf about 0.3 ± 0.01. The method was validated as per ICH guideline with respect to linearity, accuracy, precision, robustness etc. The method is new, simple and economic for routine estimation of XMA in bulk, preformulation studies and pharmaceutical formulation to help the industries as well as researchers for their sensitive determination of XMA rapidly at low cost in routine analysis. Because XMA has the potential to be a game-changing method of treating ageing, its advancement may benefit the pharmaceutical and cosmetics sectors.

Keywords: Ximenynic acid; Skin Care Medicinal Formulation; HPTLC; Validation

1. Introduction

Herbal medicine plays an important role in the healthcare of many developing countries. The use of herbal products is increasing worldwide due to the distinct advantages. Nearly 80% of African and Asian population depends on traditional medicines for their primary healthcare. These medicines are readily available in the market from health food stores without prescriptions and have been widely used in India, China, USA, and have fairly good market all over the world [1]. Generally it is believed that the risk associated with herbal drugs is very less, but reports on serious reactions are indicating to the need for development of effective marker systems for identification of the individual components. Standardization, stability and quality control for herbal drugs are feasible, but difficult to accomplish. Further, the regulation of these drugs is not uniform across countries. There are variations in the methods used across medicine systems and countries in achieving stability and quality control [2]. Physicochemical features, biochemical tests, microbiological characteristics, and fingerprint profiles from HPLC and HPTLC are all useful markers for assessing the quality of Polyherbal formulations and for standardizing them [3].

Sandalwood, or *Santalum album* L. (Santaceae), is a ximenynic acid-releasing plant. Indian religious rituals and traditional Chinese medicine have both made use of it for centuries. Lots of santalbic acid, also known as ximenynic acid, may be found in sandalwood seed oil, along with trace levels of lauric, palmitic, and stearic acids. This acid is present in high concentrations in the oil as individual molecules [4]. Acid with the molecular formula $C_{11}H_{21}NO_3$. The fatty acid [5-7] ximenynic acid, commonly known as santalbic acid, is the conjugated enzyme form of the compound. This substance's

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chemical name is octadeca-11-trans-en-9-ynoic acid. Ximenynic acid is also known as santalbic acid. Antibacterial, antifungal, and anti-inflammatory properties have been shown in XMA in previous studies [8]. Numerous investigations in the scientific community have shown XMA's significance as an ingredient in many commercially successful cosmetics. It has been discovered to increase dermal and skin elasticity, develop the cell matrix, and fortify the body's defenses against free radicals and pollution. It works extremely effectively for both hair loss and cellulite [9].

There must be a stable analytical technique for separating the peaks of the API and any by-products, process contaminants, latent packing liquid, and excipients in medicinal creams and ointments. The purpose of analytical method validation is to guarantee reliable outcomes from various HTPLC analysis techniques. This is a very crucial phase in the process of designing novel dosage forms since it informs us about things like accuracy, linearity, detectability, and the limit of quantifiability. According to the International Conference on Harmonization (ICH) guidelines, "the purpose of validating an analytical technique is to find out whether it is good enough for its intended application." During the full process of creating a new medicine, validation data must now be supplied to the proper regulatory and regulatory agencies. Validation requirements for analytical methods often include guidelines from the USP and ICH.

However, in the current literatures HPTLC method was not reported for the analysis of Ximenynic acid in any semisolid dosage formulations. Looking at the advantages of HPTLC method, such as, is a cheap analytical tool because of it's less operating cost, high sample throughput and need for minimum sample clean-up. It was thought needful to develop HPTLC method for analysis of Ximenynic acid in semisolid dosage formulations. The standardization of herb mixes and the acceleration of the development of novel medications might benefit from confirming the chromatographic identification of XMA in semisolid dose form.

2. Material and methods

2.1. Chemicals and reagents

Sami lab India's regular ximenynic acid didn't require further purification since it was already very pure (99.12 %). Millipore, a French water treatment company, filtered and cleaned the water used to create this stock solution. Solvents, including methanol of analytical reagent quality, were purchased from Merck Ltd. (India). As stationary phases, aluminium plates precoated with silica gel $60F_{254}$ (20 x 20 cm, 0.2 mm thickness) (Merck) was used (Table 1).

Table 1 Standard and Semisolid Formulation used for validation studies

Sr. No	Name of Standard/Test	Batch No	Potency (%)
1	Ximenynic acid	SA/XA/03	99.12
2	Semisolid Dosage Formulation Placebo	NA	NA
3	Semisolid Dosage Formulation	NA	NA

2.2. Liquid chromatography conditions

A Camag HPTLC system comprising of Linomate V automatic sample applicator with Camag TLC Scanner 3 and Camag WinCAT software were used for detection and quantification of ximenynic acid in the formulations. The samples were spotted in the form of band of width 6 mm with CAMAG 100 µL syringe on precoated silica gel 60F254 aluminium plate (20 cm x 10 cm with 0.2 mm thickness) using Linomat 5 applicator CAMAG (Switzerland) fitted with a CAMAG 100 µL syringe. The volume applied on each track was 20 µL. The ascending development was carried out in the mobile phase Toluene: Ethyl Acetate: Methanol: Formic acid (5:4:0.5:0.5 v/v/v/v) in a CAMAG twin trough chamber (20 x10 cm). The optimized chamber saturation time for the mobile phase was 15 min at room temperature (25°C±2). The length of the chromatogram run was approximately 80 mm. The plates were developed and scanned within 10 min using densitometric scanner III in the absorbance mode at 254 nm for XMA. The source of radiation was deuterium lamp emitting a continuous radiation between 200 and 400 nm. The densitometric scanning was performed using CAMAG TLC scanner-3 (Switzerland) operated by win CATS software V 1.4.3.6336. The slit dimension was 5×0.45 mm with the scanning speed of 20 mm s-1. The amount of ximenynic acid present in the samples was evaluated by peak area with linear regression [10-12].

2.3. Preparation of the standard solution XMA

25 milligramme of XMA were put into a 500-milliliter volumetric flask. For 15 minutes, the mixture was sonicated in an ultrasonic water bath after 300 mL of the diluent (Methanol) was added. When the solution had cooled to the appropriate temperature, it was diluted.

2.4. Preparation of Sample Solution (For 10% w/w Cream)

One gramme of material, or 100 mg of XMA, was properly weighed before being added to a 200 ml volumetric flask. The volume was reduced by adding 60 mL of diluent, and then the mixture was heated in a water bath at 60°C for 30 minutes while being vigorously shook every so often. Next, the liquid was filtered via a 0.45 µ Teflon membrane. Fifty milliliters were prepared by diluting five milliliters of the resulting solution. The final product, at 50 ppm, was utilised for the sample [13].

2.5. Calibration curve and linearity

The acceptability of linearity data is often judged by examining the correlation coefficient and intercept of the linear regression line for the response versus concentration plot. The stock solution of ximenynic acid was diluted to five different concentrations between 50-150% of working concentration. The plate was developed and analyzed to generate calibration equation for quantification of ximenynic acid in samples. The curves showed coefficient of correlation ($r2$) ≥ 0.997.

2.6. Method Validations

Validation of the analytical method was done according to the International Conference on Harmonization guideline. The method was validated for specificity, Solution Stability, recovery, robustness and precision [14-15].

2.6.1. Specificity

Specificity was ascertained by applying 20 µL band of Standard, blank and sample solutions on the HPTLC Plates. The bands for ximenynic acid from sample solutions were confirmed by comparing the Rf and spectra of the bands to those of the standards. The peak purity of ximenynic acid was analyzed by comparing the spectra at three different levels, i.e. at peak start, peak apex and peak end positions of the spot.

2.6.2. Precision

Calibration curves for six sets of replicate measurements taken on the same day with varied levels of XMA were analyzed to determine the device's accuracy over the course of a single day. It was determined by measuring six sets of duplicate XMA samples at varying concentrations on separate days whether or not the results were consistent. The degree of accuracy was quantified by calculating the relative standard deviation, which is expressed as a percentage and often abbreviated as %RSD. Based on the results of six replicate tests performed at the reference concentration of 50 ppm in XMA solution, it was determined that an RSD of less than 2% represented an acceptable level of accuracy.

2.6.3. Solution Stability

The sample solution and standard solution were prepared as per the proposed method and subjected to stability study at room temperature for 6 h. The change in response of ximenynic acid in sample solution with respect to time is calculated as absolute percent difference against initial response.

2.6.4. Robustness

Table 2 Robustness parameter of Xymenynic acid

Sr No	Parameters	Working Parameter	- Changes	+ Changes
1	Saturation Time (minute)	15	14	16
2	Polar Solvent Volume (Formic acid)	0.5	0.4	0.6
3	Mobile Phase Volume	10	9	11

The volume of the mobile phase, polar solvent volume and saturation time was involved in this study. This parameter was changed as per Table 2. The effect of these changes on both the Rf values and peak areas were evaluated by calculating the relative standard deviations (RSD) for each parameter.

2.6.5. Accuracy (Recovery)

The reliability of the procedure was examined by taking three separate views of a standard XMA solution. Many experiments employed concentrations between 25 and 75 parts per million. We performed statistical analysis on the experimental data using the formula to determine how well the newly devised approach performed. Recovery rates between 90% and 100% are considered satisfactory [15-16].

3. Results

The HPTLC procedure was optimized with a view to develop a stability indicating assay method. Different compositions of the mobile phase for RP-HPTLC analysis were experimented with an objective to obtain high resolution and reproducible peaks. The required objective was achieved using Toluene: Ethyl Acetate: Methanol: Formic acid (5:4:0.5:0.5 v/v/v/v) mobile phase. It gave dense, compact and well separated spots of the drug. This mobile phase showed good resolution of ximenynic acid peak from the extract of *Santlum album*. The wavelength of 254 nm was found to be optimal for the highest sensitivity. Sharp and well defined peaks for the ximenynic acid were obtained at Rf 0.3± 0.02 when the chamber was saturated with mobile phase for 15 min at room temperature. The present method is quicker as the time needed for development of plate is reduced considerably to less than half an hour for chamber saturation and development of plate as compared to the previously reported method. The scanning wavelength selected was 254nm for ximenynic acid (Figure 1).

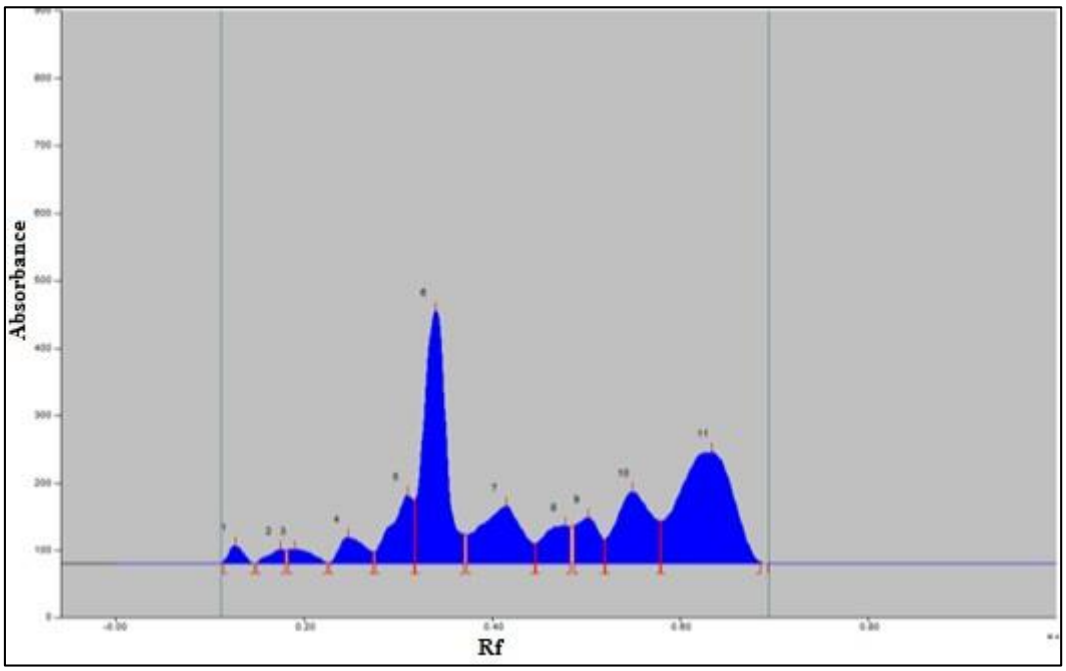

Figure 1 HPTLC chromatogram of Sample solution

3.1. Calibration curve and linearity

A calibration curve was constructed by plotting peak area against concentration of ximenynic acid (ppm). The results of Linearity are shown in Table 3. They confirm the linearity of the standard curves over the range studied (25-75 ppm). Linear regression of concentration versus peak area plots resulted in an average coefficient of determination (r2) greater than 0.9971. The average equation for calibration curves was y = 321.2x - 2830.4. The 3-D chromatographs of all calibration concentrations are shown in Figure 2 and 3 respectively.

Table 3 Correlation coefficients for XMA

Conc. of Xymenynic Acid (Ppm)	Average Peak Area of Xymenynic Acid
25	5563
33	7893
41	9953
50	12987
59	15876
66	18342
75	21673

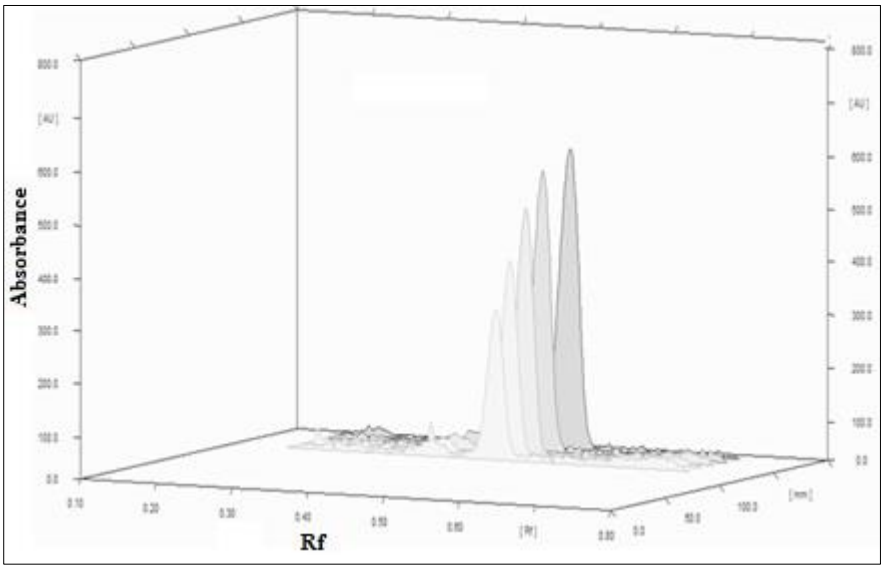

Figure 2 3D graph of calibration linearity of Xymenynic acid

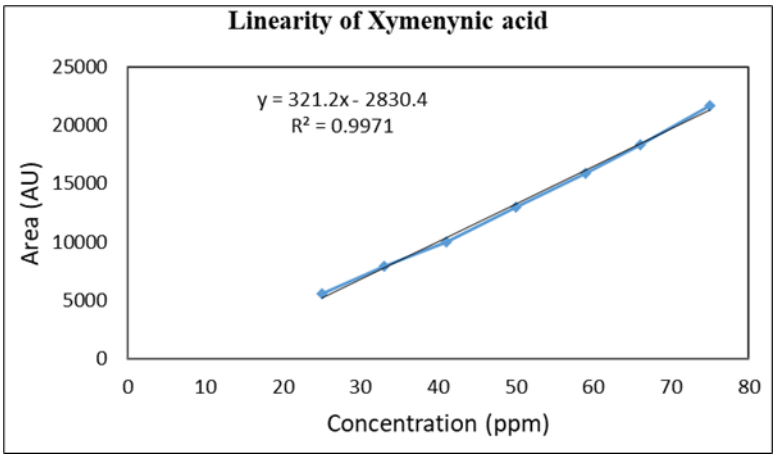

Figure 3 Linearity Graph for Xymenynic acid

System, Method and Intermediate precision of the developed method were expressed in terms of relative standard deviation (RSD) of the peak area. The results showed that the System, Method and intermediate variation of the results

at concentration of 50 ppm for ximenynic acid were within the acceptable range. The coefficients of variation for System, Method and Intermediate precision of the method were found to be less than 0.78 % (Table 5). The ximenynic acid was also analyzed by two different analysts within the same day and the results revealed that there is good intermediate precision between analysts (Table 4).

Table 4 Result of System Precision study for Xymenynic acid

Band Number	Peak Area of Xymenynic Acid
1	12987
2	12543
3	12654
4	12787
5	12861
Mean	12785
SD	161.61
% RSD	1.26

Table 5 Method Precision and Intermediate Precision for Xymenynic acid

Sr. No.	Assay (% W/W, Method Precision)	Assay (% W/W, Intermediate Precision)
1	99.00	100.00
2	98.12	99.32
3	99.43	99.10
4	99.89	100.20
5	98.20	99.50
6	100.19	100.12
Average	99.00	100.00
% RSD	0.87	0.52
Overall % RSD	0.78	

The results of spectral comparison for ximenynic acid were found to be specific at peak start (S), peak apex (M) and peak end (E), respectively. The closeness of peak purity values to 1 indicates that the spots were only attributed to a single compound. By comparing the Photos and Chromatograms of the Blank solution, Standard solution and sample solution, it was observed that no peak was co-eluted with the analyte band from Blank solution. Also purity of xymenynic acid in standard and sample solution was also observed as per Table 6.

Table 6 Specificity of XMA

Sr. No.	Test name	Analyte name	Purity flag	Specificity
1.	Xymenynic extract	Xymenynic acid	No	Specific
2.	Standard	Xymenynic acid	No	Specific
3.	Blank	No Peak	NA	NA

The results obtained in the new conditions (variation in composition mobile phase, polar solvent volume and saturation time) were in accordance with the original results as shown in Table 7, though the Rf varied very slightly (0.30± 0.1). The %RSD values for peak area was less than 1.0 indicating the highly robust nature of the developed method. The low RSD values indicate the robustness of the method.

Table 7 Result of Robustness study for Xymenynic acid

Robustness Parameter		% RSD	Rf	Peak Purity
Saturation Time (minute)	14	0.65	0.31	Pass
	15	0.12	0.30	Pass
	16	0.67	0.32	Pass
Polar Solvent Volume (Formic acid)	0.4	0.31	0.31	Pass
	0.5	0.81	0.30	Pass
	0.6	0.67	0.31	Pass
Mobile Phase Volume	9	0.63	0.31	Pass
	10	0.27	0.30	Pass
	11	0.96	0.29	Pass

The recovery study was completed with the use of standards added to the placebo solution at concentrations of 80%, 100%, and 120% of the working concentration. Between 99 and 102% of the original data was shown to be fully retrievable using XMA. Refer to Table 8 for more details.

Table 8 % Recovery of XMA

Analyte	Recovery level	% Recovery	Average % Recovery
Xymenynic acid	80% - 1	99.89	99.76
	80% - 2	100.28	
	80% - 3	99.12	
	100% - 1	99.20	99.73
	100% - 2	99.87	
	100% - 3	100.12	
	120% - 1	100.54	100.30
	120% - 2	99.59	
	120% - 3	100.76	

4. Discussion

Studies demonstrate that at the present time, there is no HPTLC technique for determining whether from semisolid dosage form contains XMA. Separation periods are too short, resolution is too low, solvent combinations are too intricate, and run times are too lengthy; these are all issues with the HPTLC procedures discussed so far for XMA. It also took a long time for these therapies to work. Furthermore, the results of using these techniques did not provide reliable information. The purpose of this research was to develop and evaluate a quick and easy method of measuring XMA. It was important that this be completed as soon as possible. Additionally, the time it takes for a cycle of chromatographic separation is often rather brief. Therefore, the validated analytical method may be used to check XMA's presence in drugs of any dosage.

5. Conclusion

Extensive literature survey revealed that there is **a** xymenynic acid is currently available for the treatment of Skin. As there is no official HPTLC method reported in major pharmacopeias like USP, EP, JP, BP and IP for determination of xymenynic acid in bulk and pharmaceutical formulation. This study has led to the development of a linear, precise, accurate, specific, system-suitable, and powerful analytical strategy for XMA based on HPTLC. The proposed approach for detecting XMA is not only novel, but also rapid, easy, and sensitive enough to fulfil the requirements of the ICH. The method can minimize the cost of reagents and time for analysis. It also utilized the merit of applying several sample spots on HPTLC plate, which maybe more advantageous for regulatory quality control laboratories especially to facilitate the post-marketing surveillance program. In addition, the method is inexpensive and not requires certain types of stationary phases. Thus, it can represent another good alternative for the already existing HPLC methods especially those using certain types of detectors which are not present in most of the laboratories.

Compliance with ethical standards

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Disclosure of conflict of interest

I declare no conflict of interest.

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