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Development and validation of HPLC method for ximenynic acid in semisolid dosage formulation

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Abstract

A novel 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). A Phenomenex Luna Column 5 (4.6 x 250 mm) was used for the analysis, using acetonitrile as the mobile phase, sodium dihydrogen phosphate monohydrate as the stationary phase, and 229 nm detection at 30 degrees Celsius. In spite of the presence of additional compounds, their presence had no effect on the detection of XMA in SDF. As part of validating this HPLC technique, a number of tests were performed to evaluate its specificity, linearity, accuracy, precision, and durability. International Conference on Harmonization standards were used to determine whether the method was adequate (ICH). Because it is simple to implement and produces reproducible results, the presented HPLC approach has the potential to be utilized in industry to standardize herbs and Phytomedicines. 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; HPLC; Validation

1. Introduction

Ayurvedic practitioners know that in order to get the therapeutic effects they want, it takes a combination of many plants. If a variety of plants are combined in a certain method, the resulting medication will be more effective and safer to use. About 31.4% of people in industrialized societies, 42% to 69% of Americans, 71% of Canadians, and 90% of Britons use them as dietary supplements or natural health products (vitamins, minerals, amino acids, essential fatty acids, herbal products, traditional Chinese medicines, homoeopathic medicines, and probiotics) to treat things like removing disease-causing agents, avoiding side effects, and getting a high quality of life [1] . Including scientific evidence has the potential to halt the production of substandard herbal products and ensure their proper use [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. Employ high-performance liquid chromatography (HPLC) for precise and rapid chemical determination [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 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

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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 HPLC 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.

We still need a defined HPLC technique for identifying XMA in semisolid dose forms. The purpose of this research, then, was to learn more about the semisolid XMA dosage form now in use. 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).

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

The HPLC instrumentation was a Waters 2695 Alliance system, from the company's Alliance line of products. It was coupled to a C18 column that measured 4.6 mm by 250 mm and included a 5 µ particle size, as well as a 2996 PDA detector and a 2489 UV/Visible detector. The sample loop for the previously used auto sampler injection system was 20 µl. The isocratic process in a pH 2.5 Sodium dihydrogen phosphate monohydrate buffer (solvent-A) and an acetonitrile mobile phase took 45 minutes to complete (solvent-B). The mobile phase flow rate was held constant throughout the experiment at 1 mL/min. All columns were preheated for 30 minutes before the first injection. While the column was maintained at 30 ˚C throughout the experiment, the sample temperature was kept constant at 10°C. After adjusting the PDA's wavelength to maximize the response from a single peak at 229 nm, the chromatogram was captured. Standard XMA was determined by comparing the retention durations and spectra of the sample solution to those of the reference standard. The research was conducted at 25 ˚C and 10 ˚C, or room temperature [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 0.1% 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 utilized for the sample [13].

2.5. Validation of XMA HPLC assay

The XMA test's RP-HPLC technology was tested and validated in accordance with the ICH Harmonized Tripartite Guidelines. Its precision, consistency, linearity, specificity, LOD, LOQ, robustness, and other characteristics were evaluated.

2.5.1. Specificity

The specificity of the method was evaluated by determining the peak purity of an XMA sample using a diode array detector and the Waters empower software. The investigation's findings were characterized by the purity angle, the purity threshold, and the purity flag.

2.5.2. System specificity

Six separate XMA tests were conducted at 50 ppm to evaluate the performance of the system. For XMA to be considered suitable, the relative standard deviation (% RSD) between peak area and retention times must be less than 2%.

2.5.3. Linearity

Standard curves for both compounds were constructed using a concentration range of 25-100 ppm by plotting the peak area vs. XMA concentration according to the Beer-Lambert law. Calibration curves were generated for each standard concentration range using weighted linear regression analysis with a weight of 1/x. This allowed us to get the highest quality outcomes. Critical investigation was conducted [14].

2.5.4. Accuracy

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].

2.5.5. 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.

2.5.6. Robustness

The robustness of an analytical technique is measured by its continued success after being subjected to small but carefully manipulated changes in its input parameters. This attribute is more proof that the technique is effective when used properly. Small, calculated adjustments were made to the chromatographic conditions in order to gauge their stability [16].

3. Results

The optimal mobile phase composition for the HPLC method has been determined via a series of experiments. Solution polarity, temperature, and mobile phase pH were all factors that were evaluated. Peaks that are almost similar and have a large amount of separation from each other and the standards were obtained using the suggested procedure, indicating that this was the most effective approach (Figure 1). The scanning was done at a wavelength of 229 nm because it was found to give the same results for every analyte and bring out the best in each one.

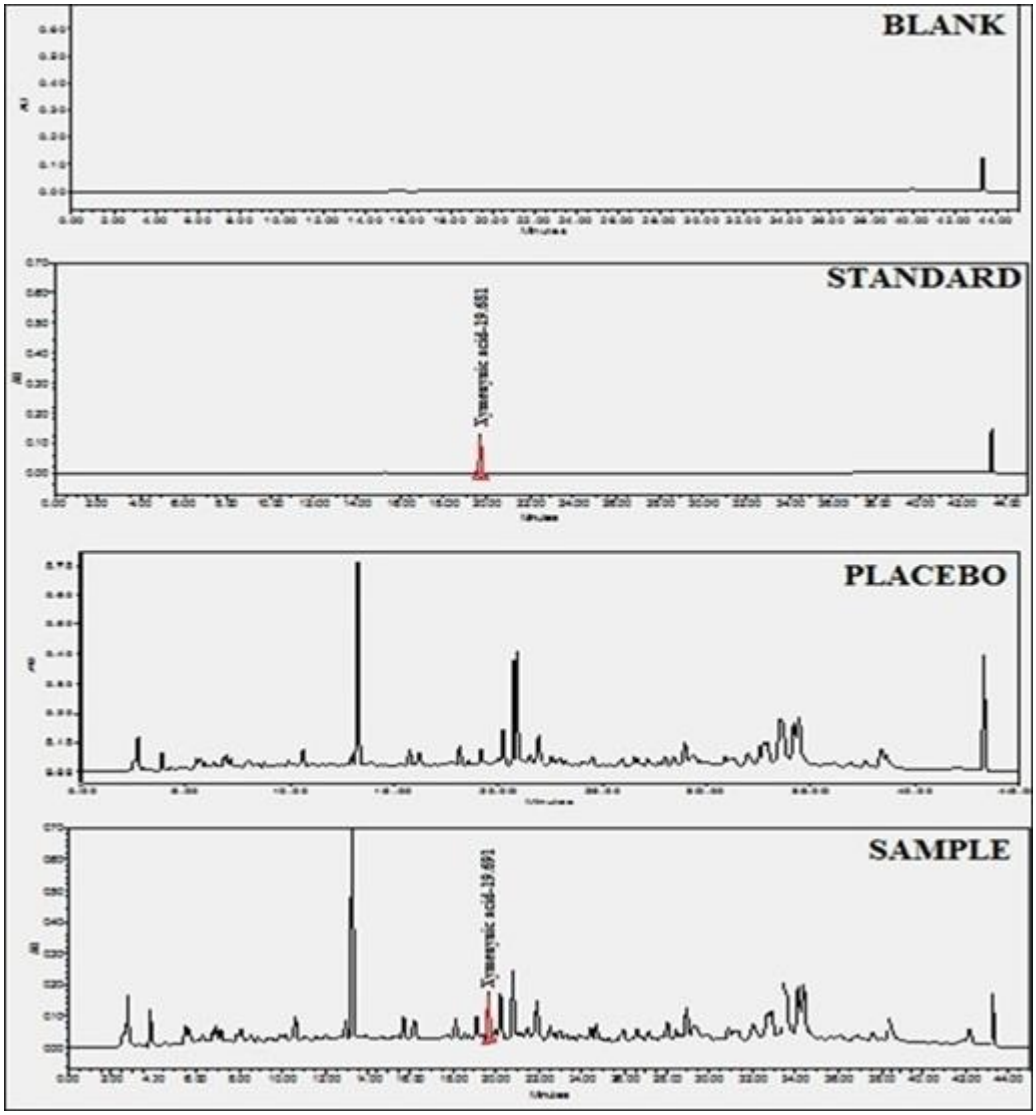

Figure 1 Chromatograms for Blank, Standard, Placebo and Sample

Calculated correlation values of 0.9994 between 50% and 150% of the standard concentration for XMA were satisfactory. Check Table 2 if you need a refresher. The normative graphs are shown in Figure 2, which may be seen online.

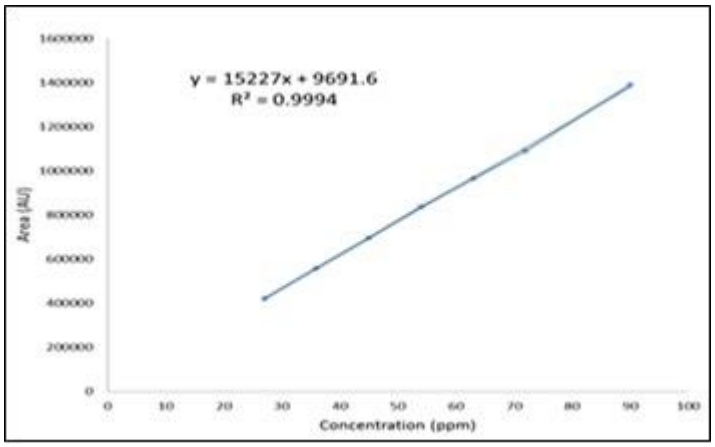

Figure 2 Linearity graph of XMA

Table 2 Correlation coefficients for XMA

% Level	Conc. of Xymenynic acid (ppm)	Average Peak area of Xymenynic acid
50	27	421521
60	36	559354
80	45	694931
100	54	838964
120	63	964837
140	72	1091298
150	90	1389812
r2		0.9994
Slope of Regression line		15227

Data on the system, the technique, and the intermediate precision are provided when a sample is applied and the peak area is scanned. Percentages of the relative standard deviation are provided (RSD). The relative standard deviation (RSD) of the xymenynic acid measurement technique was determined to be 0.4%. The XMA method was found to have an overall RSD of just 0.57 %. Results from two Xymenynic acid analyzers were compared, and the RSD for intermediate precision was determined to be 0.4%. (Table 3) The proposed technique was determined to be effective while evaluating the system, the method, and the intermediate precision values. We reached this conclusion after analyzing the system, the approach, and the intermediate degrees of accuracy. We based our findings on the %RSD figures. In order to determine the degree of purity of each peak, the beginning, middle, and end spectra of the standard and the extract were compared. Table 4 displays the values for the purity angle and purity threshold.

Table 3 Method Precision and Intermediate Precision for Xymenynic acid

Name of Analyte	Sr. No.	Method precision	Intermediate Precision
Xymenynic acid	1	97.0	96.2
	2	96.0	96.5
	3	97.1	96.2
	4	96.0	96.0
	5	97.0	95.6
	6	96.0	95.9
	Average	96.5	96.1
	% RSD	0.57	0.31
	Overall % RSD	0.4	

The suggested technique was strengthened after strength testing was conducted. When comparing the peak area for each analyte with each parameter, the RSD was less than 2%. When the numbers were crunched, this became apparent. Table 5 shows that the method's % RSD has steadily decreased over time, suggesting that it has gotten more dependable.

Table 4 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

Table 5 Robustness parameter for XMA

Robustness parameter		% RSD	Peak tailing	Theoretical plates	Remark
Xymenynic acid					
Wavelength (nm)	228	0.37	1.06	137439	Pass
	229	0.33	1.06	176732	Pass
	230	0.29	1.07	136039	Pass
Temperature (˚C)	25	0.55	1.05	174647	Pass
	30	0.33	1.06	176732	Pass
	35	0.36	1.05	184365	Pass
Flow (mL/min)	0.9	0.33	1.06	180143	Pass
	1.0	0.33	1.06	176732	Pass
	1.1	0.21	1.06	177132	Pass

Finding XMA's dynamic range required determining its accuracy at 50%, 100%, and 150% of the concentration of the reference standard. The findings are summarized in Table 6.

Table 6 Range of XMA

Injection No.	Peak area at 50% level	Peak area at 100% level	Peak area at 150% level
1	421139	840921	1385999
2	419907	837006	1393625
3	421903	836860	1388181
Average	420983	838262	1389269
% RSD	0.24	0.27	0.28

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 7 for more details.

Table 7 % Recovery of XMA

Analyte	Recovery level	% Recovery	Average % Recovery
Xymenynic acid	80% - 1	99.82	99.76
	80% - 2	100.47	
	80% - 3	98.99	
	100% - 1	100.09	100.10
	100% - 2	100.53	
	100% - 3	99.70	
	120% - 1	100.29	100.06
	120% - 2	100.36	
	120% - 3	99.54	

4. Discussion

Studies demonstrate that at the present time, there is no HPLC technique for determining whether or not S. album Linn Extract 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 HPLC 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

This study has led to the development of a linear, precise, accurate, specific, system-suitable, and powerful analytical strategy for XMA based on RP-HPLC-UV-DAD. The proposed approach for detecting XMA is not only novel, but also rapid, easy, and sensitive enough to fulfil the requirements of the ICH.

Compliance with ethical standards

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Disclosure of conflict of interest

We declare no conflict of interest.

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