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Original Article

Estimation of Ursolic Acid in Methanolic Extract of *Momordica dioica* by HPTLC Method

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ABSTRACT

Background: A number of nonsteroidal anti-inflammatory medications have been shown to lessen pain and inflammation. Unfortunately, there are numerous side effects connected with their use. There are, however, medicinal plants that have anti-inflammatory therapeutic effects with little to no side effects. *Momordica dioica* is a herb that belongs to the Cucurbitaceae family. There are numerous phytoconstituents in it. Ursolic acid is one of them and a pentacyclic terpenoid that is found in nature and has a number of therapeutic benefits. The current study looked at Ursolic Acid's activities (UA), a secondary plant metabolite, for its anti-inflammatory properties in the *Momordica dioica* plant. The current study's objective was to create and validate an HPTLC method that was quick, accurate, precise, and specific for determining ursolic acid from *Momordica dioica* herbal extract. **Methodology/Conclusion/Significance:** For quick analysis of Ursolic acid determination, The High Performance Thin Layer Chromatography (HPTLC) was established and confirmed. On an aluminium HPTLC plate (60 F_{254}) coated with precoated silica gel with formic acid, ethyl acetate, and toluene (7:3:0,1), chromatographic separation was accomplished. The detection process was carried out at 530 nm. Ursolic acid's R_f value was discovered to be 0.795%. In the 400ng/band concentration range, linearity for ursolic acid was detected. The limits of detection and quantitation for ursolic acid were observed to be 0.04 ng/band and 0.14 ng/band, respectively. The mean % recovery of rosmarinic acid was (0.54). The method's specificity, robustness, linearity, precision, and accuracy have all been validated in compliance with ICH standards. The created method can be used to evaluate the regularity of ursolic acid in herbal extracts.

Keywords: Ursolic acid, Herbal Extract, *Momordica dioica*, HPTLC, Chromatography, Methanol, R_f value, Inflammation.

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INTRODUCTION

As a form of complementary medicine, medicinal plants and their secondary metabolites are increasingly used to treat disease. The medicinal plants contain a variety of potent phytoconstituents. Since a few decades ago, there has been a demand for nutraceutical formulations, and there has been a desire to guarantee the effectiveness, and the safety of herbal medicines. Chromatographic technology is a key tool for qualitative and quantitative analysis, and One tool for ensuring the reliability is the evaluation of photochemical.

The HPTLC is a sophisticated method for calculating chemical markers and biomarkers. The use of synthetic drugs puts the world's health at risk by raising the likelihood that consumers will develop diseases like cancer, diabetes, and neurodegenerative disorders. There is an immediate need to develop medicines using natural herbs as a solution for this. Indigenous medicines reduce the harmful effects of synthetic drugs, offering a favourable solution to the global health concern.[1]

Utilization steroids and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), such as azathioprine, is one of the treatment options for these inflammatory disorders. All of these medications have a variety of side effects that limit their continued use and muddle treatment options.[2-5] In order to treat inflammatory disorders, Discovering non-toxic, safe anti-inflammatory medications made from plants is a necessity.[6]

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A cucurbitaceous, evergreen climbing creeper, *Momordica dioica* (also referred to as kakrol or spiny gourd). It has a wide geographic distribution in Bangladesh and India and is indigenous to Asia. Over thousands of years, it has been used as a vegetable with significant nutritional value as well as a preventive and curative agent for a variety of diseases.[7]

Momordica dioica is a herb in the Cucurbitaceae family that climbs dioeciously. There are numerous phytoconstituents in it. *Momordica dioica* contains small amounts of alkaloids, steroids, triterpenes, flavonoids, glycosides, saponin, and flavonoids.[1] Despite of having its roots in the Indo-Malayan region, this genus now grows in South Asia, Polynesia, Tropical Africa, Bangladesh, Sri Lanka, Myanmar, China, and Japan.[8,9]

Ursolic acid, a pentacyclic terpenoid (3-hydroxy-urs-12-ene-28-oic acid, UA, Figure 1), is used medicinally in a number of ways. ursolic acid, Normal locations for a secondary plant metabolite include the fruit skin, stem bark, or leaves. As a component of herbal extracts used in folk medicine, this compound has been used for Despite having been known to have health-improving qualities for generations. Researchers have lately turned to this source of knowledge gathered over generations in order to naturally occurring biologically active substances.[10,13]

Aside from its many other medicinal benefits, UA has anti-inflammatory properties as well as a protective effect on the liver, brain, kidneys, and lungs. A review of the literature found that a validated simple, rapid, precise, and accurate HPTLC method was used to evaluate how much ursolic acid is present in the herbal extract for its anti-inflammatory activity.[14-16]

MATERIALS AND METHODS

Chemicals and reagents

Yarrow Chem Products, Mumbai, provided the standard ursolic acid. Analytical-grade chemicals and reagents were used all around.

Instrumentation

HPTLC system was utilized as instrument which included a Camag Linomat-v semiautomatic sample applicator, twin trough development chamber, Camag 100l syringe, Camag TLC Scanner-3, and WinCAT software version 1.s 254.3.6336.

Collection and preparation of plant material

The fruits of *Momordica dioica* were collected from Local market. The matured fruits were selected and cut into pieces. They were dried at room temperature and then grinded into fine powder.

Extraction of plant material

Macceration method of extraction was used. Ground fruits powder (100 g) were extracted with Methanol (250 mL) for two days. The extract was prepared.

Preparation of standard solution

A precisely measured 1 mg of ursolic acid was transferred to a 1 mL Effendrop and dissolved at a concentration of 1000 ug/mL in methanol.

Preparation of sample solution

For analysis of herbal Extracted Powder of *Momordica dioica*. An Extracted powder sample weighing exactly 10 mg of *Momordica dioica* and add it to 1 mL of Effendrop. After being sonicated for 30 min, the solution was filtered employing Syringe filter paper.

Optimization of mobile phase

Since the mobile phase is a crucial component of chromatographic methods, optimising the solvent system for effective extraction is the first step in developing a successful method. a method for determining the amount of ursolic acid in a formulation that produces a compact spot with significant value. Different combinations and ratios of the mobile phase were studied in order to optimise it. Toluene, ethyl acetate, and formic acid (7:3:0.1) was used, and the results a clear, sharp peak for ursolic acid, as shown in Figure 2. Densitogram of Ursolic acid in *Momordica dioica* extract was shown in Figure 3.

Preparation of dipping reagent

1% Anisaldehyde Sulphuric acid reagent (Add 0.5 mL Anisaldehyde in 50 mL of acetic acid and 1 mL of concentrated sulphuric acid) was used as a dipping reagent. And activated at 110°C after drying. Then scanned at 530 nm.

Selection of wavelength

The Wavelength selected was 530 nm as shown in Figure 4.

Chromatographic conditions

On a pre-coated silica gel aluminium plate60 F254, the sample was spotted in the shape of a 6mm band using a camag microlite syringe. The mobile phase was constituted of toluene, ethyl acetate, and formic acid (7:3:0.1v/v). At room temperature, the ideal mobile phase twin trough development chamber, saturation time was 20 min. with the aid of an air dryer, the developed TLC plate

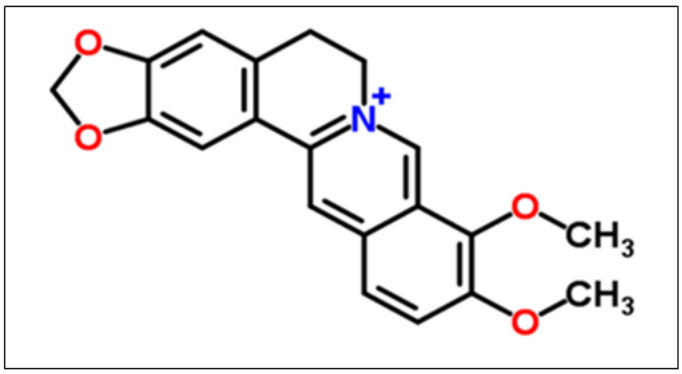


Figure 1: Structure of Ursolic acid.

was dried, dippied in 1% Anisaldehyde Sulphuric acid reagent and activated at 110°C and scanned at 530 nm. Overlapping of ursolic acid and momordica dioca extract shown in Figure 5.

Method validation

Linearity

Calibration curves were plotted to measure the linearity. a standard stock solution with a 200–1200ng/band concentration. By measuring peak areas against each band concentration, the calibration curve was created.[15,16]

Precision

In order to determine the instrumental precision, six times with the exact similar concentration of ursolic acid (400ng/band) were used. In order to estimate the continuity of methanol, the intra-day and inter-day precision were calculated, as well as the SD and % RSD values.

Accuracy

The pre-analyzed samples received 80, 100, and 120% of the standard as a spike. Three replicates of each level were used in the developed method to analyse ursolic acid and the powdered herbal extract, SD and %RSD values were computed.

LOD and LOQ

The equation Y= -14.13 + 0.4521X was used to calculate the Detection Limit (LOD) and Limit Qualification (LOQ).

LOD and LOQ are, respectively, 0.04 and 0.14.

Robustness

Robustness was evaluated using 400ng/band by making small deliberate change in saturation time (±5%) of mobile phase, the amount of phase (±1). The effect of various deliberate changes was studied on retention factor and peak area& height, the SD and % RSD was calculated.[17,18]

RESULTS AND DISCUSSION

In order to quantify, a wavelength of 530 nm was used. Ursolic acid's R_f value has been 0.79 following development with the mobile phase toluene, ethyl acetate, and formic acid (7:3:0.1v/v). Running of ursolic acid with momordica extract phases shown in Figure 6.

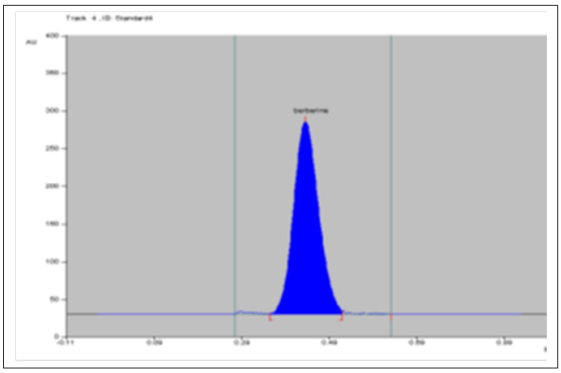
Linearity

Linearity is the property of an analytical procedure that allows test results to be, either directly or through a mathematical transformation, proportional to the analyte concentration within a predetermined range.[19]

Over the range of 100-600 ng/band, a fine linear relation between the amount and the response (peak area) was found. The calibration plot's linear regression data showed that the correction coefficients (r) were 0.9975. Figure 7 Ursolic acid's regression equation was determined to be Y= -14.13 + 0.4521X.

Precision

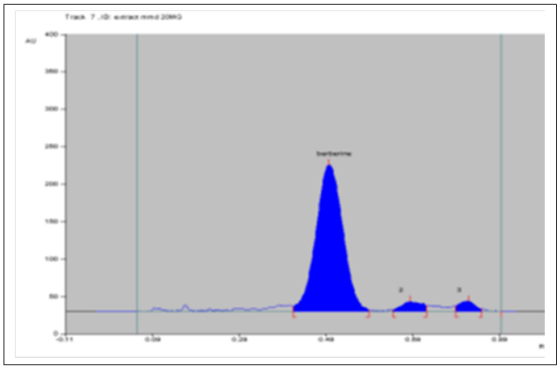
Instrumental precision was tested by repeatedly scanning the very same band of ursolic acid (400 ng/band) with a scanner (n = 6), and the results were portrayed as SD and %RSD, which as shown in Table 1, was discovered to be less than 3%.[2] In six replicate injections of standard sample solutions, the interday intraday precision studies response factor of standard compounds and %RSD were computed. Table 1 shows the findings.[1,20,21] 3D Spectra for Ursolic acid with *Momrdica dioica* Extract shown in Figure 8.



Track 4, ID: Standard4

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.35 Rf	0.5 AU	0.44 Rf	255.5 AU	100.00 %	0.52 Rf	4.5 AU	12173.7 AU	100.00 %	berberine

Figure 2: Typical Densitogram of Ursolic acid (R_f 0.79).



Track 7, ID: extract mmd 20MG

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.41 Rf	6.7 AU	0.50 Rf	195.9 AU	87.95 %	0.59 Rf	1.5 AU	10101.9 AU	91.50 %	berberine
2	0.64 Rf	2.1 AU	0.68 Rf	13.3 AU	5.95 %	0.72 Rf	7.9 AU	514.7 AU	4.66 %	unknown *
3	0.79 Rf	7.2 AU	0.82 Rf	13.6 AU	6.10 %	0.85 Rf	3.1 AU	424.0 AU	3.84 %	unknown *

Figure 3: Densitogram of Ursolic acid in *Momordica dioica* extract.

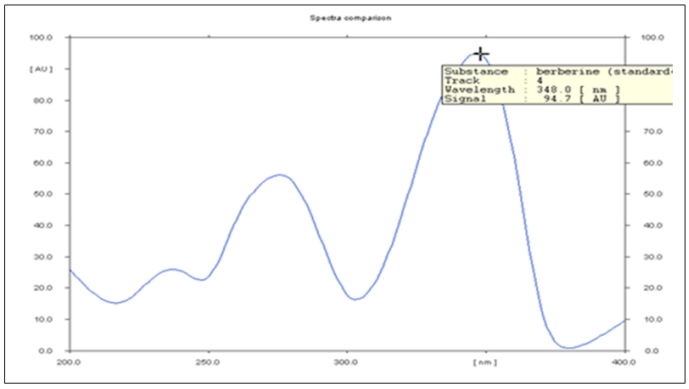


Figure 4: Selection of wavelength.

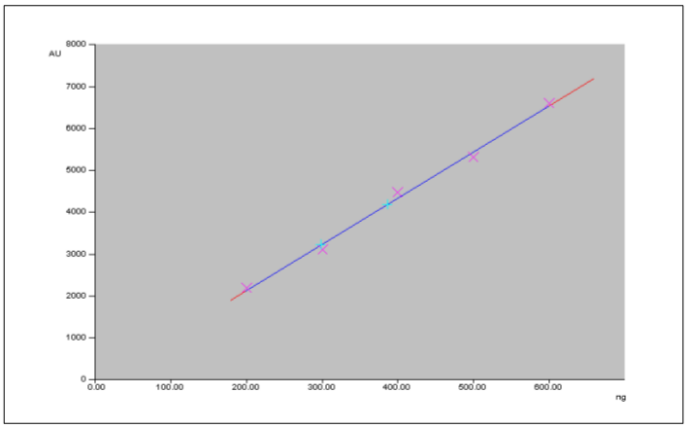
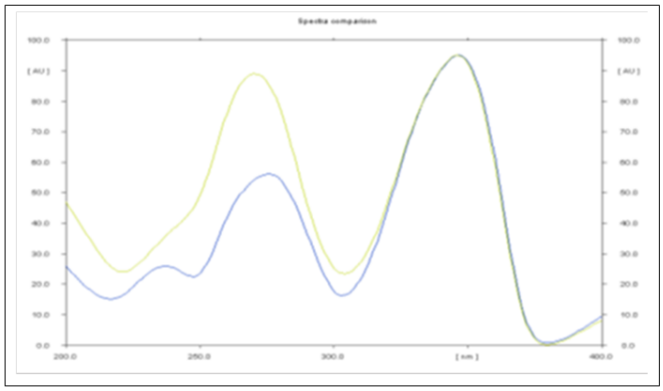


Figure 7: Linearity graph of Ursolic acid.



Track	Rf	Assigned Substance	Max. Signal	Display	r(s,m)	r(m,e)
1	0.41	berberine	42 AU @ 200 nm	☐	0.999999	0.999970
2	0.41	berberine	114 AU @ 346 nm	☐	0.999973	0.999954
3	0.42	berberine	203 AU @ 346 nm	☐	0.999981	0.999924
4	0.43	berberine	270 AU @ 347 nm	☑	0.999930	0.999917
5	0.45	berberine	293 AU @ 347 nm	☐	0.999925	0.999893
6	0.45	berberine	357 AU @ 347 nm	☐	0.999466	0.997366
7	0.49	berberine	211 AU @ 346 nm	☑	0.999631	0.998688

Figure 5: Overlay spectra ursolic acid+mmed extract.

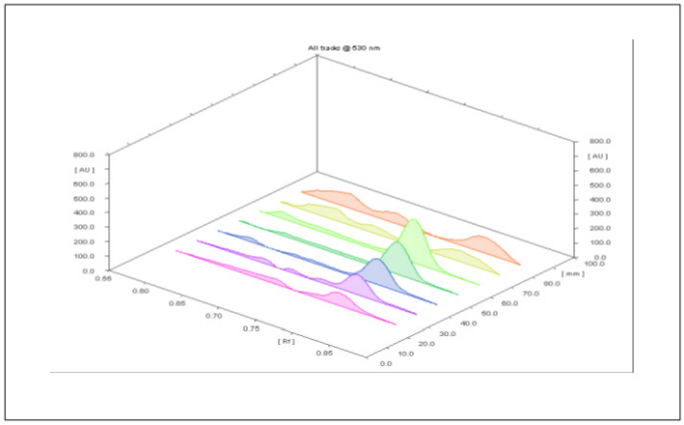


Figure 8: 3D Spectra for Ursolic acid + *Momrdica dioica* Extract.

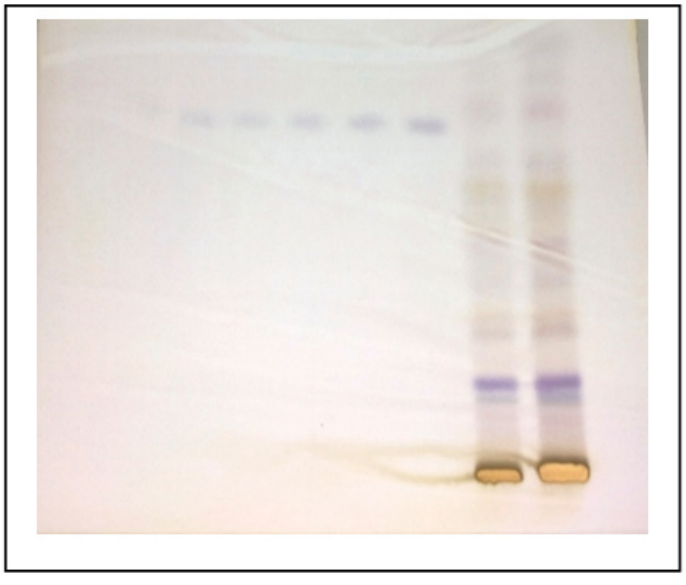


Figure 6: TLC Plate of Ursolic acid + *Momordica dioica* Extract.

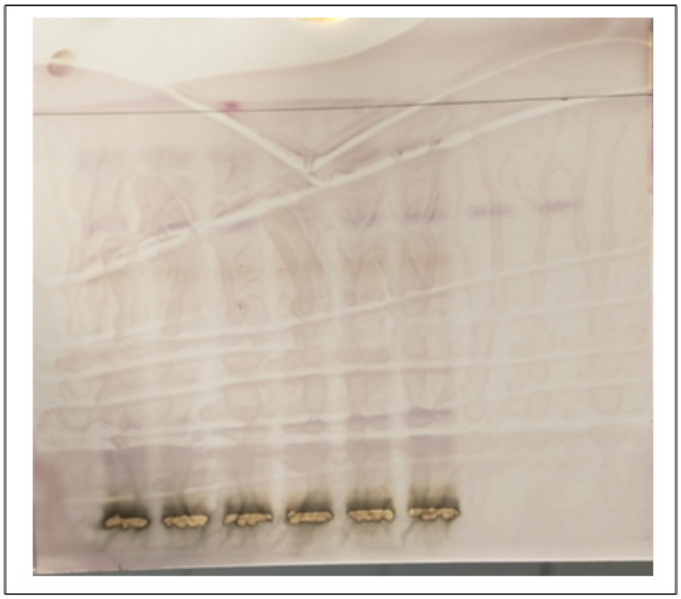


Figure 9: TLC Plate of Ursolic acid.

Table 1: Statistical evaluation for system precision.

Sl. No.	System precision
	Peak area
Mean	3073.6
S. D	44.4
RSD.	0.01

Table 2: Statistical validation for recovery study.

Level of recovery	% Recovery	S. D (±)	R. S. D
80%	56.16%	29.9	0.0055
100%	95.2%	33.5	0.0050
120%	105%	1.41	0.0002

Table 3: Result of robustness.

Factor	Level	Area	R_f
Saturation time			
15 min	-5	4460	0.75
20 min	0	4462	0.79
25 min	+5	4468	0.82
	S. D± R. S. D		± 0.
Total mobile phase	level	Area	R_f
9.1 mL	- 1	4216	0.72
10.1 mL	0	4462	0.79
11.1 mL	+1	4321	0.85
	S. D± R. S. D		1 ± 0.

Table 4: Summary of validation parameters.

Sl. No	Parameters	Ursolic acid
1	Coefficient of determination (r^2)	0.9975
2	Linearity range (ng)	100-600ng/band
3	LOD (ng)	0.04
4	LOQ (ng)	0.14
5	Linearity Equation	Y= -14.13 + 0.4521X

Accuracy

A method's accuracy can be measured by how closely test results correspond to the analyte's true value (10). By using analytical procedure studies, it was decided. It was loaded up with 80, 100, and 120% of the recommended amounts of ursolic acid. The mixtures underwent a triplicate analysis. Results are displayed in Table 2.

Limits of detection and quantification

The developed HPTLC method's Limit of Detection (LOD) and Limit of Quantification (LOQ) were established by injecting progressively lower concentrations of the standard solutions. The LOD and LOQ of Ursolic acid calculated 0.64 (ng/Band) and 1.94 (ng/Band) respectively. Running phase of ursolic acid shown in Figure 9.

Robustness

By slightly altering the chromatographic conditions, the method's robustness was assessed. (Mobile phase volume, Saturation time). It was noted that the chromatograms did not significantly change Table 3. Validation parameter summary shown in Table 4.

CONCLUSION

The determination of the amount of the active component in *Momordica dioica's* herbal extract Ursolic acid has been identified and quantified using HPTLC method. The mean % recovery of rosmarinic acid was (0.54). For the detection of ursolic acid in herbal formulations, a quick, focused, sensitive, dependable, and robust HPTLC method has been created and validated. ICH guidelines were successfully followed in the method's validation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MEMD: Methanolic extract of *Momordica dioica*; **NSAIDs:** Non-Steroidal Anti-Inflammatory Drugs; **UA:** Ursolic Acis; **TLC:** Thin layer chromatography; **SD:** Standard deviation; **RSD:** Relative Standard deviation; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification.

SUMMARY

The current study looked at ursolic acid's activities (UA), a secondary plant metabolite, for its anti-inflammatory properties in the *Momordica dioica* plant. The current study's objective was to create and validate an HPTLC method that was quick, accurate, precise, and specific for determining ursolic acid from *Momordica dioica* herbal extract. For quick analysis of Ursolic acid determination, The High performance thin layer chromatography (HPTLC) was established and confirmed. The method's specificity, robustness, linearity, precision, and accuracy have all been validated in compliance with ICH standards. The created method can be used to evaluate the regularity of ursolic acid in herbal extracts.

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