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HIGH -PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE METHOD DEVELOPMENT OF URSOLIC ACID AND MOMETASON FUROATE: DEVELOPMENT AND VALIDATION

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ABSTRACT

The present work describes the development and validation of a high-performance liquid chromatography (HPLC) method for the quantitative estimation of Ursolic Acid and Mometasone Furoate in pharmaceutical dosage forms. Chromatographic optimization was performed using a C18 column and different mobile-phase compositions, flow rates, wavelengths, and column temperatures. The optimized chromatographic condition used methanol:water (60:40, v/v), a flow rate of 1.0 mL/min, detection at 296 nm, column temperature of 40 C, and a total run time of 5 min. The method was evaluated for linearity, system suitability, accuracy, precision, robustness, LOD, LOQ, and applicability to marketed formulations. Calibration data showed proportional increases in peak area across 10-60 ug/mL for Mometasone Furoate and 5-30 ug/mL for Ursolic Acid. System suitability data showed acceptable repeatability for retention time, theoretical plates, tailing factor, and resolution. Recovery values were close to 100% for both analytes, and intra- and interday %RSD values were below 1% in the supplied data. The method was further applied to Cystone and Yam Balance marketed preparations, where assay values were close to label claim. These findings indicate that the developed method is suitable for routine analysis, subject to verification of the noted source-data inconsistencies before journal submission.

KEYWORDS: HPLC; method development; analytical validation; Ursolic Acid; Mometasone Furoate; pharmaceutical dosage form; accuracy; precision; robustness.

1. INTRODUCTION

High-performance liquid chromatography is a standard analytical platform for the separation and quantification of active pharmaceutical ingredients in bulk drug substances and finished dosage forms. Method development typically involves systematic optimization of the stationary phase, mobile-phase composition, flow rate, detection wavelength, injection volume, and temperature to obtain adequate retention, peak symmetry, resolution, and run time. Analytical validation then evaluates whether the method is suitable for its intended purpose by assessing parameters such as specificity, linearity, accuracy, precision, sensitivity, robustness, and system suitability.

The objective of this study was to develop and validate an HPLC method for simultaneous quantitative analysis of Ursolic Acid and Mometasone Furoate in pharmaceutical dosage forms. The validation strategy followed the parameters described in the supplied study protocol, including linearity, system suitability, recovery-based accuracy, intraday and interday precision, robustness under deliberate chromatographic variation, LOD/LOQ estimation, and marketed formulation analysis.

2. MATERIALS AND METHODS

2.1 Chemicals, glassware, and instruments

Table 1: Chemicals used in the study.

S. No.	Chemical name	Company name
1	Ursolic Acid	Sigma-Aldrich
2	Mometasone Furoate	Sigma-Aldrich
3	Distilled water	Qualikem, HPLC grade
4	Octanol	CDH
5	Methanol	Merck
6	Chloroform	CDH
7	PBS buffer pH 7.2	Laboratory prepared
8	DMSO	Loba Chemie
9	Ethyl ether	Merck
10	Ethyl acetate	Loba Chemie
11	Hexane	Loba Chemie
12	Acetone	Merck
13	Ethanol	Merck

Table 2: Glassware used in the study.

S. No.	Glassware	Company name
1	Beakers (50 mL, 100 mL, 500 mL)	Borosil

S. No.	Glassware	Company name
2	Conical flask (100 mL)	Borosil
3	Glass rod	Borosil
4	Funnel	Borosil
5	Measuring cylinder	Borosil
6	Filtration assembly	Borosil
7	Pipette	Borosil

Table 3: Instruments used in the study.

S. No.	Instrument name	Company name
1	HPLC	Waters
2	Vacuum pump	LABPRO
3	Magnetic stirrer	INTLLAB
4	Ultrasonic bath sonicator	Lab Junction Ultrasonic Bath
5	Vortexer	Remi
7	Micropipette	Dragon Lab

2.2 Pre-formulation studies

Organoleptic properties of Ursolic Acid and Mometasone Furoate were evaluated by visual observation, including color, odor, appearance, and physical state. Qualitative solubility was assessed by transferring approximately 1 mg of each analyte into 10 mL test tubes and adding selected solvents. Melting point was determined using the open-capillary method in a Thiele tube. Functional group identification was performed by FTIR spectroscopy using KBr pellet preparation across the 4000-400 cm-1 range.

2.3 Chromatographic method development

The chromatographic method was developed on a Waters HPLC system equipped with a PDA detector and integrated using Empower software. A C18 column was used for separation. Several mobile-phase systems were screened. The optimized method used methanol:water (60:40, v/v), a flow rate of 1.0 mL/min, detection at 296 nm, an injection volume of 20 uL, a column temperature of 40 C, and a run time of 5 min. The source method section also reports screening over 200-400 nm using a photodiode array detector.

2.4 Standard and sample preparation

For Ursolic Acid, 10 mg was dissolved in 100 mL methanol to prepare a stock solution, followed by serial dilution to obtain calibration standards at 5, 10, 15, 20, 25, and 30 ug/mL. For Mometasone Furoate, 5 mg was dissolved in 5 mL methanol and sonicated for 15 min to obtain a 1 mg/mL stock solution; calibration standards were prepared at 10-60 ug/mL.

Marketed formulation samples were prepared in methanol, filtered, diluted, and injected into the HPLC system as described in the supplied protocol.

2.5 Validation parameters

The method was evaluated for linearity, system suitability, specificity, accuracy, precision, robustness, LOD, LOQ, and assay of marketed formulations. Accuracy was assessed by recovery studies at 80%, 100%, and 120% spiking levels. Precision was assessed as intraday and interday repeatability using replicate analyses. Robustness was evaluated by deliberate variation of mobile-phase composition and flow rate.

3. RESULTS

3.1 Pre-formulation characteristics

Table 4: Organoleptic properties of Ursolic Acid and Mometasone Furoate.

S. No.	Property	Ursolic Acid	Mometasone Furoate
1	Color	White	White
2	Odor	Odorless	Characteristic odor
3	Appearance	Solid powder	Solid powder
4	State	Solid	Solid

Table 5: Solubility study of Ursolic Acid.

Drug	Solvent	Observation/inference
Ursolic Acid	Water	Soluble
	Ethanol	Freely soluble
	Methanol	Soluble
	Chloroform	Soluble
	DMSO	Freely soluble

Table 6: Solubility study of Mometasone Furoate.

Drug	Solvent	Observation/inference
Mometasone Furoate	Water	Soluble
	Ethanol	Soluble
	Methanol	Freely soluble
	Chloroform	Slightly soluble
	DMSO	Freely soluble

Table 7. Melting point of Ursolic Acid and Mometasone Furoate.

S. No.	Drug	Observed	Reference range
1	Ursolic Acid	291 C	285-293 C
2	Mometasone Furoate	220 C	218-225 C

Both analytes were supplied as white solid powders. Ursolic Acid was recorded as odorless, whereas Mometasone Furoate was recorded as having a characteristic odor. The observed melting points were within the reported reference ranges in the supplied data.

3.2 FTIR characterization and chromatographic optimization

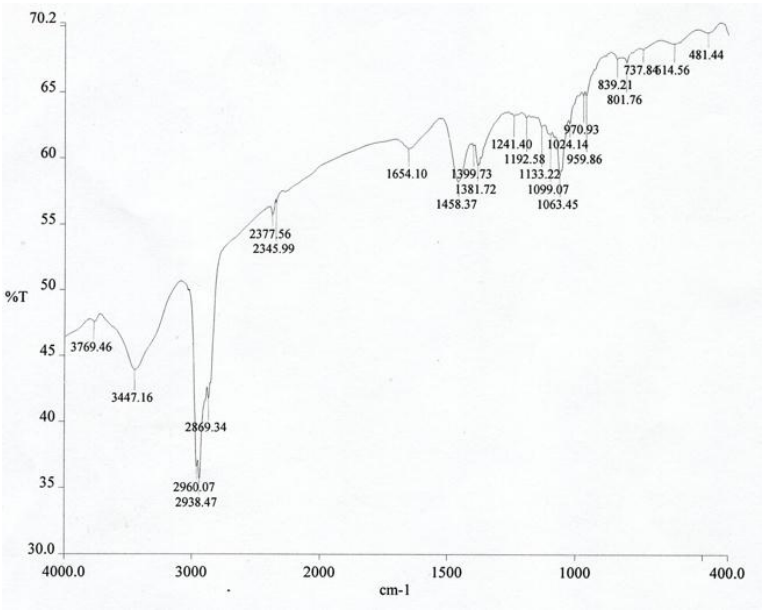

Figure 1: FTIR spectrum of Mometasone Furoate supplied in the source document.

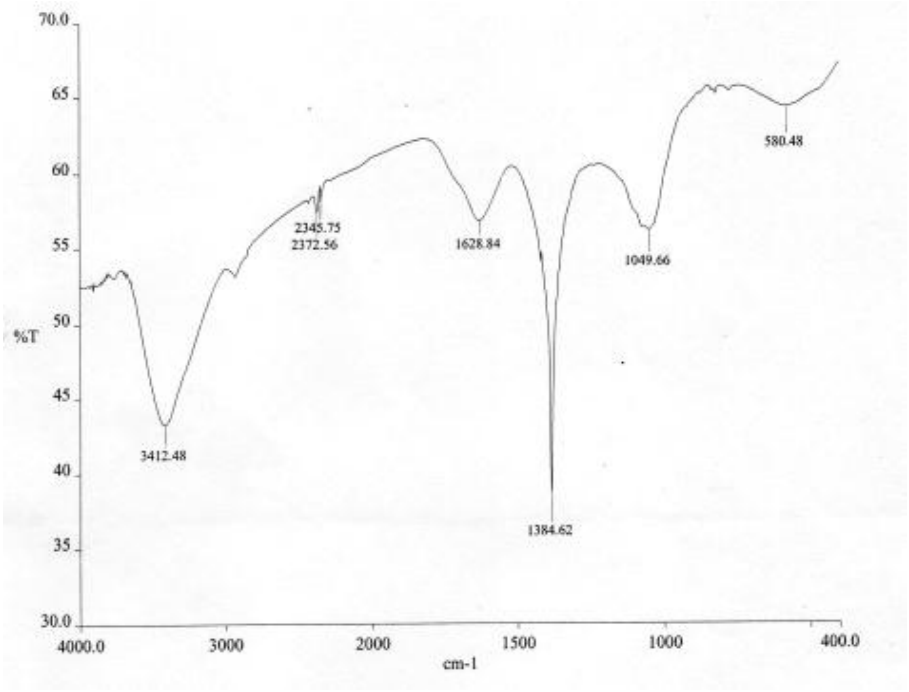

Figure 2: FTIR spectrum of Ursolic Acid supplied in the source document.

Table 8: Summary of chromatographic trials during optimization.

Trial no.	Mobile phase	Flow rate	Wavelength	Column temperature	Observation
1	Water:methanol (50:50)	1.0 mL/min	294 nm	35 C	Peak was properly eluted
2	Acetonitrile:water (70:30)	2.0 mL/min	298 nm	35 C	Separation occurred, but peak sharpness was not observed
3	Acetonitrile:water (60:40)	1.0 mL/min	296 nm	40 C	Tailing factor was high
4	Acetonitrile:water (50:50)	1.2 mL/min	298 nm	40 C	Tailing factor was high
5	Methanol:water (60:40)	1.0 mL/min	296 nm	40 C	Good separation was obtained and the condition was finalized

Among the screened chromatographic systems, methanol: water (60:40, v/v) at 1.0 mL/min and 296 nm gave the most acceptable separation in the supplied optimization record.

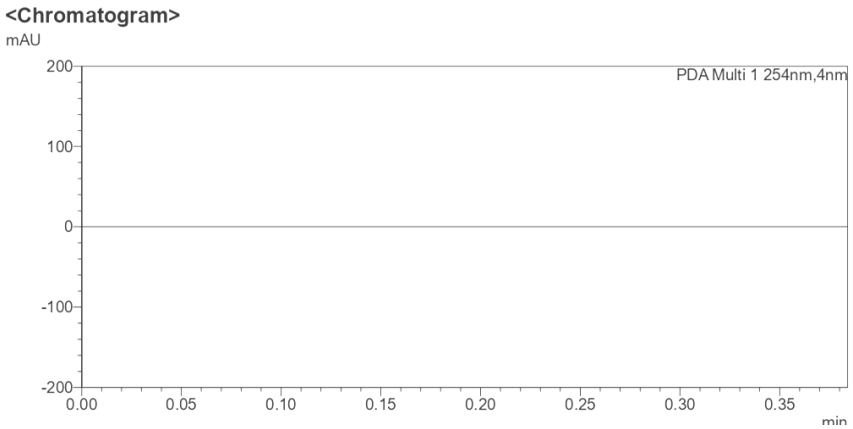

Figure 3. HPLC chromatogram of blank/mobile phase.

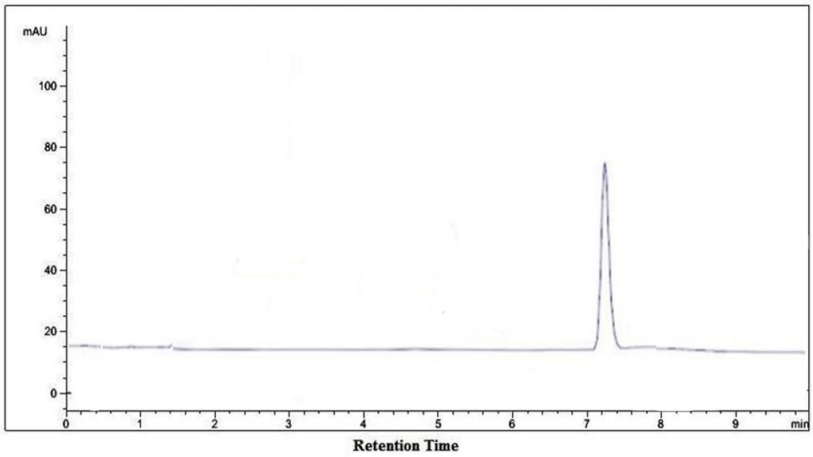

Figure 4: HPLC chromatogram of Ursolic Acid standard.

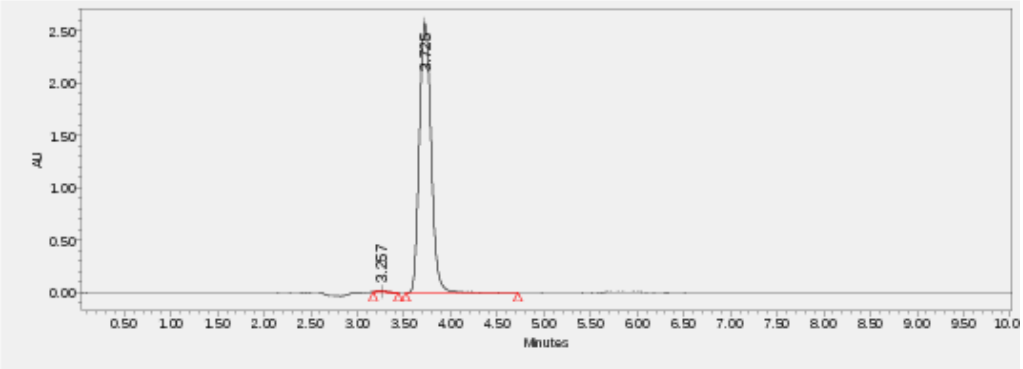

Figure 5. HPLC chromatogram of marketed preparation containing Ursolic Acid.

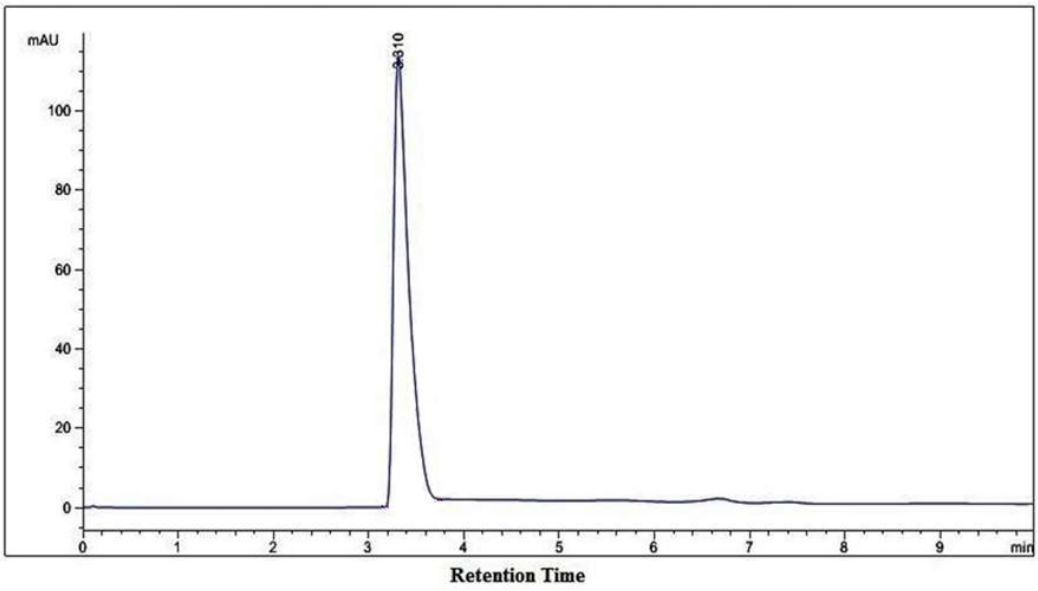

Figure 6. HPLC chromatogram of Mometasone Furoate standard.

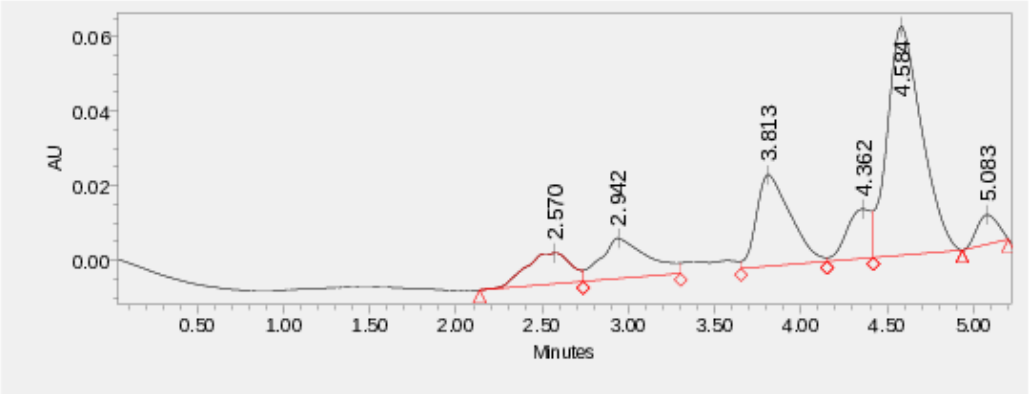

Figure 7. HPLC chromatogram of marketed preparation containing Mometasone Furoate.

3.3 Linearity

Table 9: Mometasone Furoate peak area against concentration.

S. No.	Concentration (ug/mL)	Mean peak area
1	10	493060
2	20	1902256
3	30	3563520
4	40	5298899
5	50	6910302
6	60	8245325

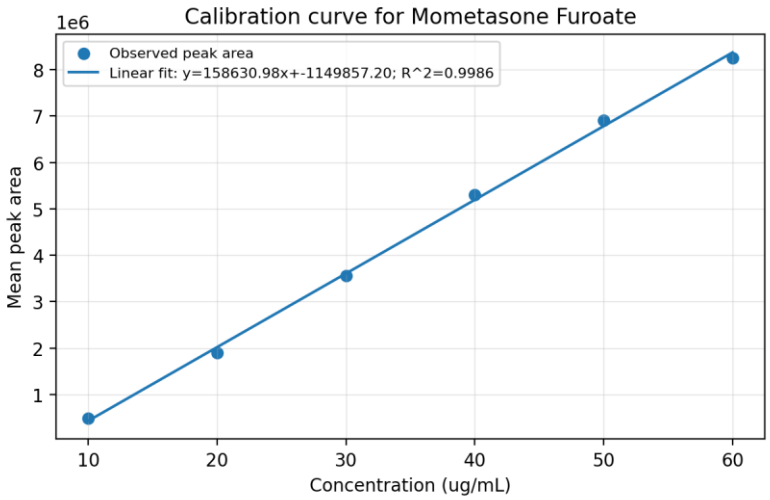

Figure 8. Regenerated calibration curve for Mometasone Furoate from tabulated data (R2 = 0.9986).

Table 10: Ursolic Acid peak area against concentration.

S. No.	Concentration (ug/mL)	Mean peak area
1	5	212850
2	10	785700
3	15	1441400
4	20	2164250
5	25	2757100
6	30	3528500

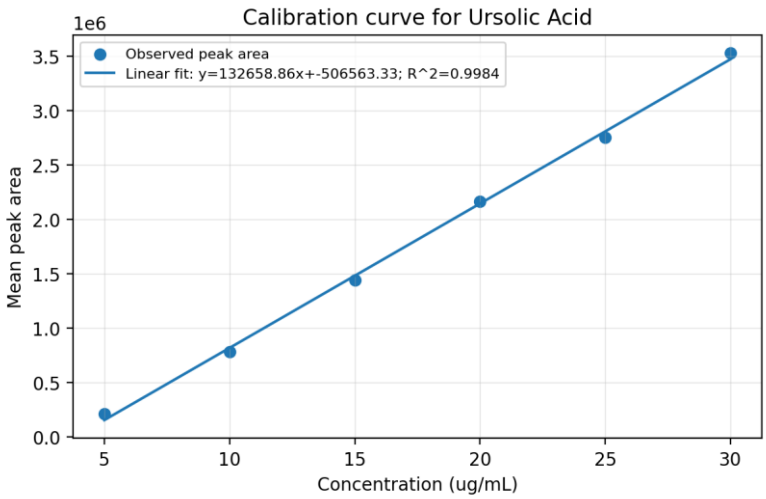

Figure 9: Regenerated calibration curve for Ursolic Acid from tabulated data (R2 = 0.9984).

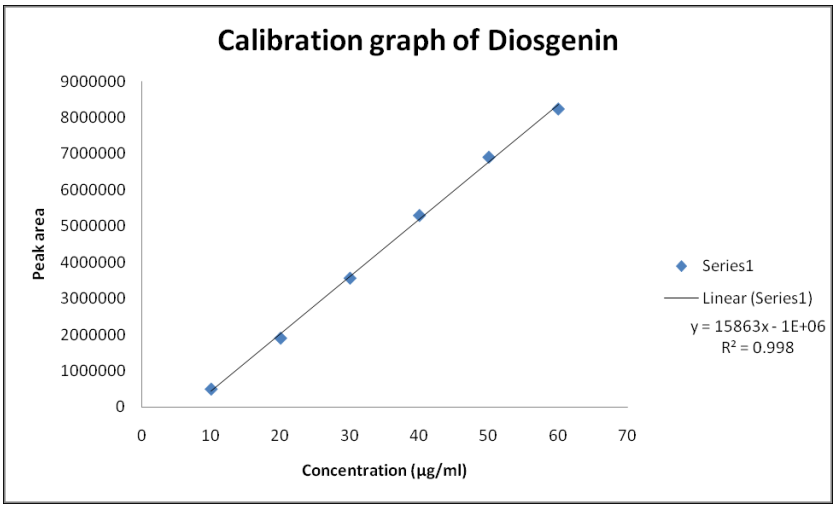

Figure 10: Original supplied calibration image corresponding to the Mometasone Furoate calibration table; the embedded source image label differs from the table label and should be corrected in the original dataset.

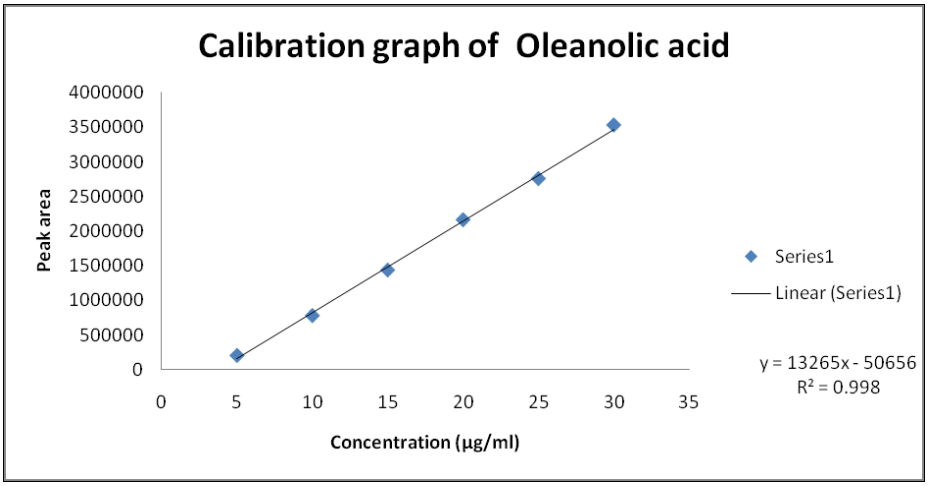

Figure 11: Original supplied calibration image corresponding to the Ursolic Acid calibration table; the embedded source image label differs from the table label and should be corrected in the original dataset.

Peak area increased with concentration for both analytes. Regression analysis of the supplied tabulated data gave R2 = 0.9986 for Mometasone Furoate and R2 = 0.9984 for Ursolic Acid. These values support linear detector response over the studied ranges.

3.4 System suitability

Table 11: System suitability data.

Drug	Parameter	Mean +/- SD (n=6)	%RSD
Mometasone Furoate	Retention time	3.2 +/- 0.0112	0.509
	Theoretical plates	24932.52 +/- 75.19	0.301
	Tailing factor	0.934 +/- 0.0067	0.717
	Resolution	2.026 +/- 0.0125	0.616
Ursolic Acid	Retention time	7.5 +/- 0.0307	0.204
	Theoretical plates	37764.51 +/- 153.19	0.551
	Tailing factor	0.8106 +/- 0.0081	0.999
	Resolution	4.166 +/- 0.0265	0.636

The system suitability results showed low %RSD values for retention time, theoretical plates, tailing factor, and resolution. The supplied data therefore indicate repeatable chromatographic performance under the optimized conditions.

3.5 Accuracy

Table 12: Accuracy data by recovery study.

Drug	Level (%)	Sample (ug/mL)	Std spiked (ug/mL)	Total (ug/mL)	Mean peak area +/- SD (n=3)	Found (ug/mL)	Mean recovery +/- SD (%)
Ursolic Acid	0	5	0	5	27850 +/- 1406.587	2.98	99.36 +/- 0.015
	80	5	2.4	7.4	114396 +/- 4567.657	5.431	100.46 +/- 0.202
	100	5	3.0	9.0	123706 +/- 1114.785	5.99	99.84 +/- 0.015
	120	5	3.6	8.6	194169 +/- 643.021	6.583	99.79 +/- 0.066
Mometasone Furoate	0	10	0	10	542184 +/- 32704.174	119.564	99.68 +/- 0.075
	80	10	6	16	1303901 +/- 59708.003	215.605	99.86 +/- 0.020
	100	10	9	19	1572134 +/- 13460.513	240.022	100.35 +/- 0.277
	120	10	12	22	1434372 +/- 6863.9153	263.568	99.89 +/- 0.058

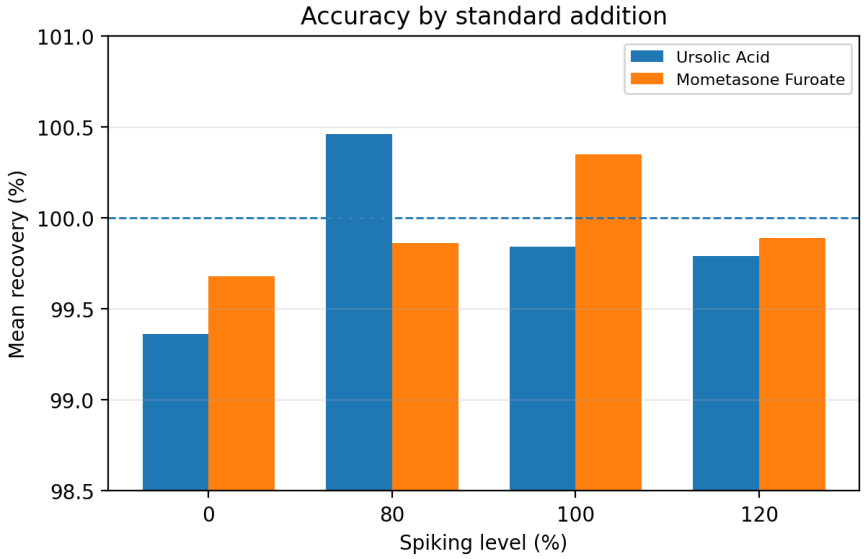

Figure 12. Recovery profile for Ursolic Acid and Mometasone Furoate.

Recovery values for both analytes were close to 100% across the assessed spiking levels. Because raw chromatograms and replicate-level values were not supplied, statistical comparison beyond the reported mean and SD values was not performed.

3.6 Precision

Table 13: Intraday precision data.

Drug	Concentration (ug/mL)	Mean peak area +/- SD (n=3)	%RSD
Ursolic Acid*	5	65246 +/- 434.7836	0.666
	10	103557 +/- 514.0205	0.496
	15	162823 +/- 527.3503	0.323
Average RSD			0.495
Mometasone Furoate	10	1192264 +/- 3338.237	0.279
	20	1476219 +/- 4747.698	0.321
	30	1751743 +/- 6255.65	0.357
Average RSD			0.319

Table 14: Interday precision data.

Drug	Concentration (ug/mL)	Mean peak area +/- SD (n=3)	%RSD
Ursolic Acid*	5	115179 +/- 486.4195	0.422
	10	148315 +/- 1051.752	0.709
	15	184807 +/- 1241.625	0.671
Average RSD			0.6
Mometasone Furoate	10	1192231 +/- 5475.735	0.459
	20	14842181 +/- 8841.038	0.595
	30	16585197 +/- 97139.66	0.585
Average RSD			0.546

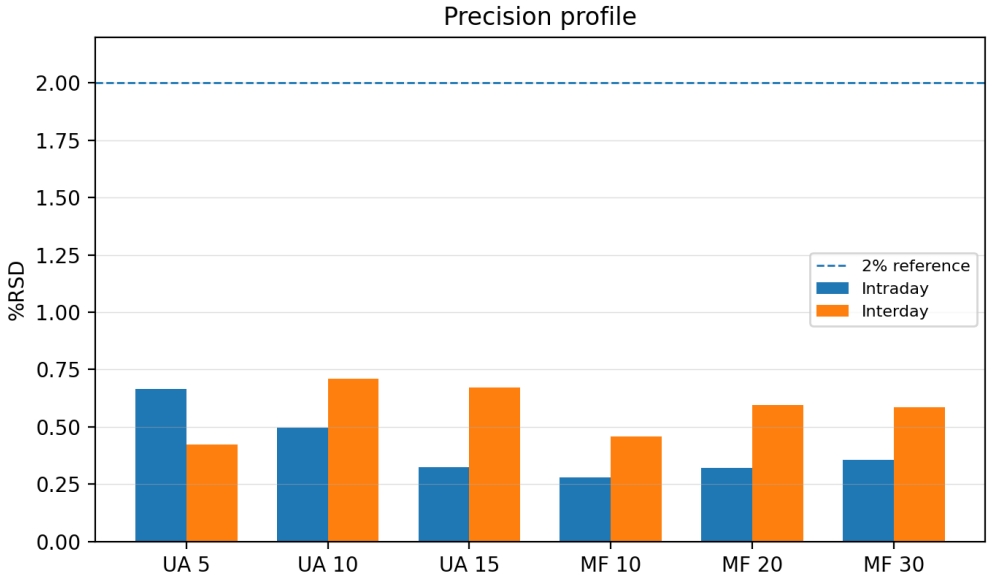

Figure 13: Intraday and interday precision expressed as %RSD.

Intraday and interday %RSD values were below 1% for all supplied concentration levels, indicating high repeatability in the reported dataset.

3.7 Robustness

Table 15: Robustness data for Ursolic Acid.

Parameter	Level	Mean peak area +/- SD (n=3)	%RSD	Rt +/- SD (n=3)	%RSD
Mobile phase (60:40 v/v)	80:8 v/v	67759 +/- 406.006	0.599	1.60 +/- 0.013	0.812
	90:12 v/v	68659 +/- 626.172	0.091	1.75 +/- 0.021	1.2
Flow rate (1.0 mL/min)	0.5 mL/min	69059 +/- 779.487	1.128	1.5 +/- 0.010	0.6
	1.2 mL/min	69287 +/- 514.304	0.742	1.96 +/- 0.019	0.969

Table 16: Robustness data for Mometasone Furoate.

Parameter	Level	Mean peak area +/- SD (n=3)	%RSD	Rt +/- SD (n=3)	%RSD
Mobile phase (60:40 v/v)	80:8 v/v	148265 +/- 2611.25	1.76	0.98 +/- 0.019	1.9
	90:12 v/v	147912 +/- 2266.005	1.56	1.02 +/- 0.017	1.6
Flow rate (1.0mL/min)	0.5 mL/min	148169 +/- 2874.236	1.93	1.15 +/- 0.009	0.782
	1.2 mL/min	147917 +/- 2730.084	1.84	1.20 +/- 0.018	1.5

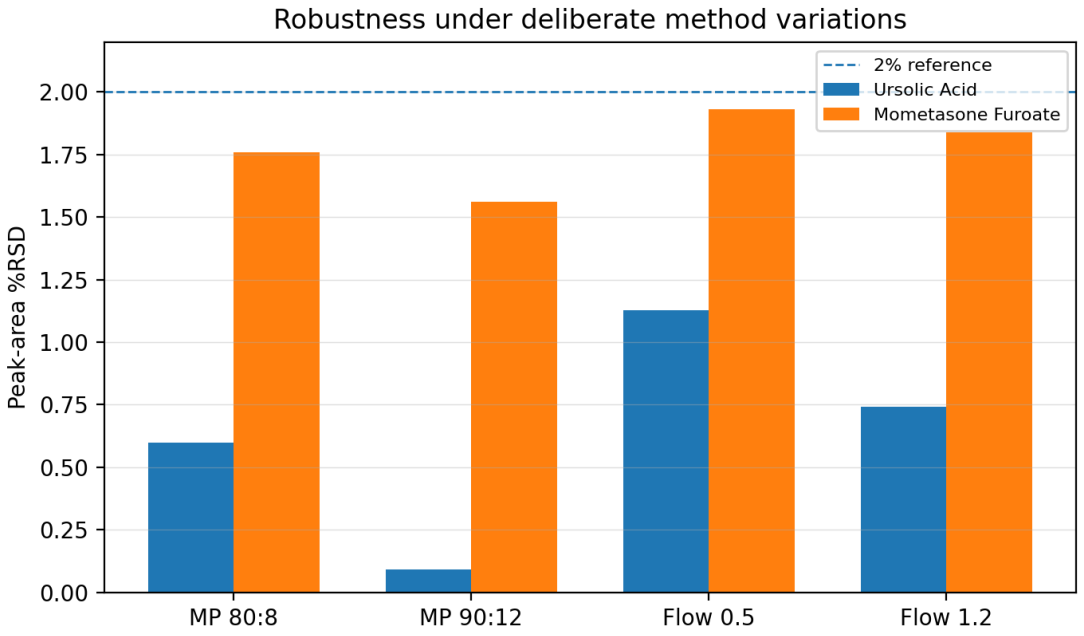

Figure 14: Peak-area %RSD under deliberate robustness variations.

Robustness testing under mobile-phase and flow-rate variations gave peak-area and retention-time %RSD values below 2% in the supplied data. This suggests that the method was not materially affected by the tested deliberate variations.

3.8 LOD and LOQ

Table 17: Limit of detection and limit of quantification.

Parameter	Ursolic Acid	Mometasone Furoate
LOD (ug/mL), n=5	12.09	0.647
LOQ (ug/mL), n=5	4.89	0.221

3.9 Analysis of marketed formulations

Table 18: Analysis of marketed preparation: Mometasone Furoate and Ursolic Acid.

Sample no.	Cystone peak area	Cystone assay	Yam Balance peak area	Yam Balance assay
1	1145419	98.80%	66862	99.28%
2	1253916	99.98%	68231	98.71%
3	1135354	99.90%	68449	100.60%
Mean	---	99.56%	---	99.53%
SD	---	0.006593937	---	0.00969484
%RSD	---	15098.71872	---	10266.286
SEM	---	0.003807	---	0.005597

Note. The %RSD values are reproduced from the supplied table but appear inconsistent with the reported assay means and SD values.

Table 19: Analysis of marketed formulations.

Marketed formulation	Amount taken (ug/mL)	Amount obtained mean +/- SD (ug/mL)	Analyte	% amount obtained mean +/- SD (n=5)
Cystone	5	4.95 +/- 0.030	Ursolic Acid	99.86 +/- 1.031
Yam Balance	15	14.90 +/- 0.042	Mometasone Furoate	99.06 +/- 0.642

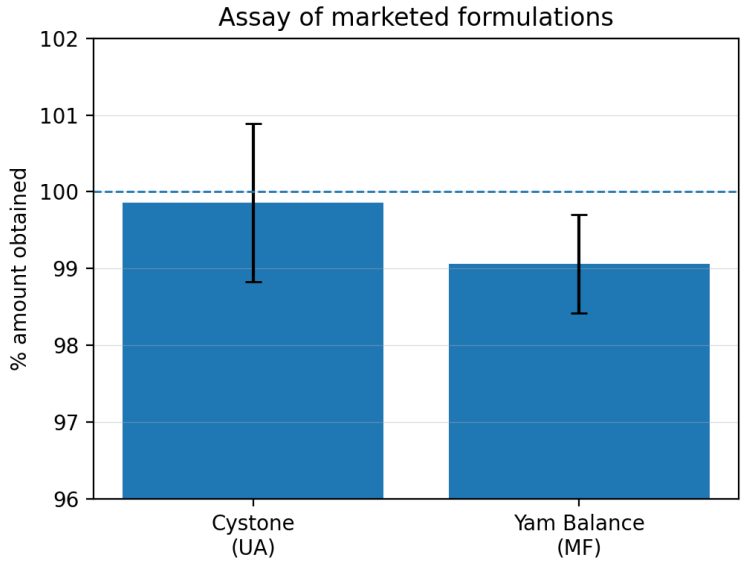

Figure 15: Assay of marketed formulations based on supplied mean +/- SD values.

The marketed formulation assay values were close to 100% in the supplied data, with Cystone showing 99.86 +/- 1.031% for Ursolic Acid and Yam Balance showing 99.06 +/- 0.642% for Mometasone Furoate.

4. DISCUSSION

The optimized RP-HPLC method used methanol:water (60:40, v/v) as the mobile phase and gave separated peaks for the two analytes within the stated run time. The reported retention time ranges indicated elution of Mometasone Furoate between approximately 3.0 and 3.5 min and Ursolic Acid between approximately 6.25 and 7.50 min. The system suitability data showed low variability in retention time and peak parameters, indicating stable chromatographic performance in repeated injections.

The calibration data demonstrated linear responses over the tested concentration ranges. The regenerated calibration plots from the supplied tabulated values produced high coefficients of determination, supporting the suitability of the method for quantitative estimation across the studied ranges. Accuracy values close to 100% and precision values below 1% RSD indicate acceptable trueness and repeatability in the supplied validation dataset. Robustness data also remained within the commonly used 2% RSD threshold under deliberate changes in mobile phase and flow rate.

Interpretation is limited by the absence of raw chromatograms, replicate injection tables, peak purity data, exact LOD/LOQ calculation worksheets, and inferential statistical analysis. In addition, the source dataset contains analyte-label inconsistencies in several images and precision tables. These issues do not prevent manuscript formatting, but they should be resolved before journal submission, regulatory use, or quality-control implementation.

5. CONCLUSION

A reversed-phase HPLC method was developed and validated for the quantitative analysis of Ursolic Acid and Mometasone Furoate in pharmaceutical dosage forms. The optimized method used a C18 column with methanol:water (60:40, v/v), a flow rate of 1.0 mL/min, detection at 296 nm, and a 5 min run time. Validation results from the supplied data indicate acceptable linearity, system suitability, recovery, precision, and robustness. The method was successfully applied to marketed formulations, with assay results close to the expected amount. Before external submission, the LOD/LOQ calculations, mislabeled calibration

figures, and inconsistent marketed-formulation %RSD values should be verified and corrected against the original experimental records.

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The following references are reproduced from the supplied source document and have not been independently verified here.

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