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Research Article

Estimation of Lupeol and Diosgenin Biomarker in "Mutrakrichantak Churna' – An Ayurvedic Poly Herbal Formulation by UV & HPLC

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Abstract:
Mutrakrichantak churna (MC) is a polyherbal ayurvedic medicine, used for the treatment of various urinary disorders such as renal calculi, nephritits. The main objective of the study is validation and quantitative estimation of Lupeol and Diosgenin in marketed *Mutrakrichantak churna* was performed by UV and HPLC method. This study focuses on the estimation and quantification of key marker compounds present in the formulation using UV-visible spectroscopy and High-Performance Liquid Chromatography (HPLC) techniques. Marker compounds such as Lupeol and Diosgenin were identified and quantified. Standardization of herbal formulations ensures batch-to-batch consistency, safety, and efficacy. UV analysis was performed for rapid detection, while HPLC provided precise quantification. The methods were validated according to ICH guidelines with parameters including linearity, precision, accuracy, and LOD/LOQ. The study demonstrated that the analytical methods employed were accurate, reliable, and suitable for routine quality control of *Mutrakrichantak Churna*.

Keywords - *Mutrakrichantak Churna.* Lupeol, Diosgenin, Urinary Tract Disorders

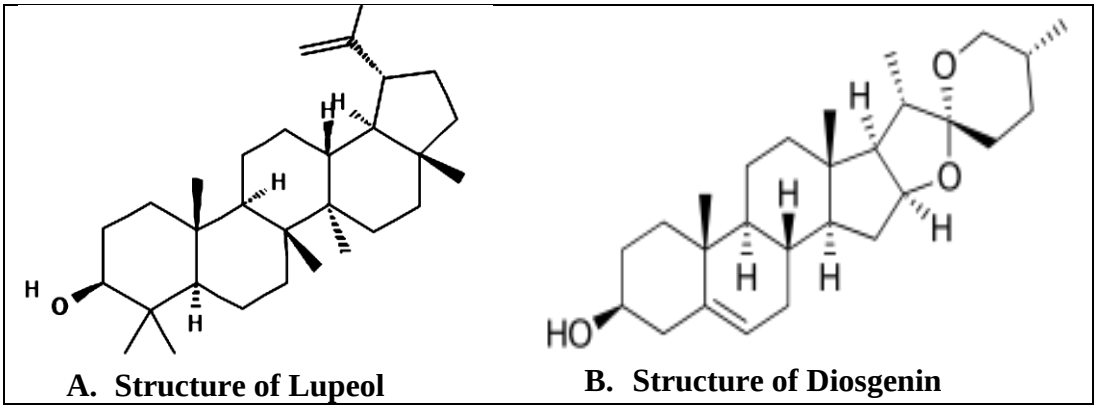
Introduction

Herbal medicine is the most ancient form of medical practice recognized by humanity. It served as the cornerstone of numerous early civilizations and is the most prevalent kind of medicine globally, according to World Health Organization statistics (Pal S.K,2003). Herbal medicine is the principal healthcare method for around 75-80% of the global population, especially in underdeveloped nations. The World Health Organization (WHO) reports that the utilization of herbal treatments globally surpasses that of conventional pharmaceuticals by a factor of two to three. Nature offers a plethora of remarkable herbs and flowers that are the foundation for numerous contemporary pharmaceuticals (Bele A. 2011).

The term "*Mutrakrichantak churna*" signifies the resolution of difficulties associated with urinating. Mutrakrichantak comprises three components: 'mutra' signifies urine, 'krich' denotes hardship, and 'antak' indicates the end. The churna is effective in alleviating various kidney-related issues, including chronic and acute renal failure, urinary tract infections, nephrolithiasis, elevated urea and creatinine levels, and kidney dysfunction. The herbs included in the preparation of Mutrakrichantak churna include varuna, punarnava, goshur, kaasni, bhumiamla, shirish, shigru, and apamarg (Bopana & Saxena 2008). The roots of Crateva nurvala L. Capparaceae are commonly referred to as Varuna roots (Bhattachargee et al., 2012). The desiccated roots are utilized as a raw medicinal substance in ancient medical systems in India, including Ayurveda and Siddha. The root is utilized to treat conditions such as urinary tract infections, nephrolithiasis, elevated urea, increased uric acid and creatinine levels, renal calculi, dysuria, and as a diuretic (Parvin et al., 2011). The Varuna roots primarily consist of Lupeol and diosgenin as

Figure 1. (Calixto, 2000). These complexes have diverse pharmacological activity, such as anti-inflammatory properties, which demonstrate significant benefits for human health. Innovative advancements in the processes of separation, purification, and additional enhancement of natural mixtures have enabled the development of suitable methodologies for the investigation of quality and standardization of plant-based formulations (Di et al., 2003).

The Main objective of this study is to identify Lupeol and Diosgenin by UV spectroscopy and HPLC in *Mutrakrichantak churna.*



A. Structure of Lupeol **B. Structure of Diosgenin**

Figure 1. Structure of Biomarkers

Material & Method

Selection of Pure Biomarker - From the literature it was revealed that Triterpenoids are responsible for the treatment of Urinary Disorders. Lupeol and Diosgenin was selected for further study.

Procurement of standard biomarker – Standard Lupeol and Diosgenin were purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai. Analytical grade Solvents used and distilled before use.

Identification of Crude Drugs and authentication – All herbs were procured from local vendor of Indore region. Authentication of crude drugs was done by Dr. Bhawna Tomar (Agriculturist and Associate Professor of Agriculture Department, Oriental University, Indore MP. All crude herbs were dried under shade and powdered mechanically and sieved using 100 Size Mesh.

Qualitative Estimation (Phytochemical & Physicochemical Analysis)

The verification of the genuineness of a raw medicinal substance is determined by consulting the descriptions provided in the pharmacopoeia or other authoritative publications (such as BPC or USP) of the relevant country. The required level of quality and purity is attained through adherence to specific criteria, which are outlined in the official reference document. The powdered botanical constituents were subjected to physico-chemical analysis. The many variables examined were conducted (WHO, 2002; Ayurvedic Pharmacopeia, 2009) in the dry powder of all crude drugs and Mutrakrichantak churna. Loss on Drying, Ash value, extractive Value and Phytochemical test for saponin glycosides and triterpenoids was performed and discussed in Table 1.

UV Spectrophotometric Method -

Instrumentation specification – UV Visible double beam spectrophotometer with matched quartz cells (1 cm) with Model No. SHIMADZU 1800, Scanning was performed in the range of 200 – 400 nm with 40 nm/min scan speed.

Solubility – Solubility of Mutrachintrak churna, In-house churna and biomarkers were checked with different solvents like methanol, n-hexane, petroleum ether, ethyl acetate etc.

Preparation of Standard (Lupeol) - 10 ml of Lupeol was dissolved in ten ml of petroleum ether (1 mg/ml) and well agitated. At this stage, the preparation was subjected to sonication for 15 minutes and subsequently filtered using Whatman filter paper. from this stock sloution , 1 ml of stock solution was pipette out and further dilute to 100 ml of petroleum ether (10 µg/ml). From the Stock sloution of 10 µg/ml, it was further diluted with petroleum ether to prepare a solution ranging from 1-5 µg/ml.

Preparation of Sample – 10 mg of in-house formulation and marketed formulation of churna were dissolved in 10 ml of Petroleum ether (1mg/ml) and shaken well, Both the sample were sonicated for 15 minutes and finally filtered and further diluted up to 3 µg/ml dilution.

Preparation of Standard (Diosgenin) - 10 mg of Diosgenin was dissolved in 10 ml of ethyl acetate (1 mg/ml) and thoroughly mixed. the preparation was subjected to sonication for 15 minutes and subsequently filtered using Whatman filter paper. From this stock solution 1 ml of stock solution was incorporated into 100 ml of ethyl acetate (10 µg/ml). it was further diluted with ethyl acetate to yield a 1-5 µg/ml.

Preparation of Sample – 10 mg of in-house formulation and marketed formulation of churna were dissolved in 10 ml of ethyl acetate (1mg/ml) and shaken well, Both the sample were sonicated for 15 minutes and finally filtered and further diluted up to 3 µg/ml dilution.

HPLC (HIGH PERFORMANCE LIQUID CHROMATOGRAPHY) METHOD

Instrumentation Specification – Following were the details and Conditions were set as –

Instrument Model	**SHIMADZU LC 20AD HPLC system**
Injection Volume	20 µl
Mobile Phase	0.1 % formic acid in water : Methanol (20 : 80)
Column	C18 (4.6 mm x 290 mm, Particle size – 5 µm)
Flow rate	1 min/ml
Temperature	Room Temperature
Software	Lab solution version 7.1
Detector	Photo-Diode Array detector
Detection Wavelength	274 nm and 447 nm for lupeol and diosgenin respectively
Retention Time	4 min

*Preparation of Standard (Lupeol)***:** 10 mg Lupeol was dissolved in 10 ml of Pet. ether (1 mg/ml) and was shaken well. Then the solution was sonicated for 15 min. and filtered by using what man filter paper. From the Stock solution, 10 µg/ml solution was prepared.

Preparation of Standard (Diosgenin): 10 mg diosgenin was dissolved in 10 ml of methanol (10mg/ml) and was shaken well. Then the solution was sonicated for 15 min. and filtered by using what man filter paper. . From the Stock solution, 10 µg/ml solution was prepared.

*Preparation of Sample***:** 100 mg marketed and In-house sample of churna were dissolved in 10 ml of methanol (1mg/ml) and was shaken well. Then the solution was sonicated for 15 min. and filtered by using what man filter paper. From the Stock solution, 10 µg/ml solution was prepared.

Validation Parameters –

- *Linearity* - The linearity of the technique may have been assessed concerning the analysis of standard outcomes. The linearity may have been evaluated using straight least-squares regression analysis for the generation of the alignment curve. The relapse comparisons with slope, intercept, and correlation coefficient (r) may have been determined.
- *Precision (repeatability)* - The system's repeatability may have been assessed. Ultimately, Tom's examination involved the identification of 500 and 1000 µg standards, each analyzed six times, and thereafter assessed by the HPLC method. The relative standard deviations were expressed as RSD (%).
- *Accuracy* - Accuracy was assessed by measuring the recovery of specified quantities of lupeol and diosgenin reference standard included into samples at concentrations of 5%, 10%, and 20% of the sample concentration (2.0 µg/mL). The accuracy was determined as the proportion of the drug recovered and expressed as the relative standard deviation (RSD) of the measurements.
- *Robustness* - Robustness was assessed through

minor alterations in the specified analytical conditions. The investigations involved testing two different solvent methanol in addition to small variations in the selected wavelengths for examination (228 nm and 232 nm).

- *Specificity:* The specificity was evaluated by examining analytical interferences from excipients. This analytical parameter was established by comparing the UV absorption spectra of the lupeol and diosgenin reference solution, the sample solution, and the placebo. The spectra were acquired between the range of 200 to 400 nm, and the overlap of absorption bands was assessed. Spectral scans enabled the determination of the absorption wavelength maxima of lupeol and diosgenin when dissolved in methanol (Bhagyasree, 2014)

- *LOD – (Limit of Detection)* - LOD is the lowest amount of an analyte that can be detected (but not necessarily quantified) under the stated experimental conditions. It gives an indication of the sensitivity of the analytical method. It is usually expressed in µg/mL

$$\text{LOD} = \frac{3.3 \times \sigma}{S}$$ Where, σ = Standard deviation of the response (usually from blank or low-concentration replicates); S = Slope of the calibration curve

- *LOQ – (Limit of Quantification)* - LOQ is the lowest amount of an analyte that can be quantitatively determined with acceptable precision and accuracy. It reflects the minimum measurable concentration.

$$\text{LOQ} = \frac{10 \times \sigma}{S}$$ Where, σ = Standard deviation of the response; S = Slope of the calibration curve.

Result & Discussion

The method devised for estimating lupeol and diosgenin was determined to be straightforward, expeditious, sensitive, and reproducible. The validation parameters complied with ICH requirements, and the recovery rates fell within acceptable thresholds. The existence of these biomarkers offers scientific support for the therapeutic effectiveness of *Mutrakrichantak churna* particularly in urinary tract disorders. The quantification of these substances is an essential instrument for quality control and standardization of this traditional Ayurvedic preparation. The findings of this study approve the combination of present analytical methods with Ayurvedic medicine, ensuring uniform quality, safety, and efficacy for global acceptance.

Qualitative Estimation (Phytochemical & Physicochemical analysis)

I. **Physicochemical analysis –** Loss on drying**,** Ash value and extractive values was performed as per standard method and all parameters and results found under the limit. Results summarised in Table 1.

1. *Loss on Drying* – Loss on Drying was calculated, summarised in Table 1.

2. *Ash Value* – Ash values are crucial in evaluating the quality and purity of crude medicine, especially in powdered form. The objective of ash crude pharmaceuticals. The objective is to eradicate any traces of organic materials that may interfere with analytical measurements. (Khandelwal, 2000 and Cokate CK 2001). The total ash, acid-insoluble ash, and water-soluble ash of Mutrachintrak churna seeds and the in-house formulation were analysed, with results summarized in Table 1.

3. *Extractive Values* – Water and Alcohol soluble extractive value were determined with standard procedure and values expressed in percentage were summarised in Table 1.

4.

Table 1. Physicochemical Parameter of Mutrachintrak churna and in-house formulation

S. No.	Physicochemical standards	Result in % w/w	
		Mutrachintrak churna	In-house formulation
1	Loss on Drying	8.5	5.3
2	Total Ash	16.70	13.05
3	Acid Inslouble Ash	8	16.4
4	Water Soluble Ash	17.35	23.11
5	Water Soluble Extractive	63.72	68.22

6	Alcohol Soluble Extractive	12.09%	13.11

II. **Phytochemical screening -** The preliminary phytochemical analysis revealed numerous active ingredients in various Crude drugs and marketed formulation, encompassing carbohydrates, proteins, amino acids, lipids and oils, steroids, terpenoids, glycosides, alkaloids, tannins, and other phenolic compounds. The results are presented in Table 8. Positive indicators (+) denote the presence of phytoconstituents. A negative (-) mark signifies the absence of phytoconstituents. Only test for identification of triterpenoids and saponin was performed to assure the presence of triterpenoids (Lupeol and diosgenin).

Table 2– Phyto chemical isolation of Lupeol & Diosgenin

S. No.	Compounds	Phytochemical test	Observation	Inference
1	Saponins	Foam Test	Foam formed	+
		haemolysis Test	Haemolytic zone appeared	+
2	Triterpenoids	Salkowski test	Greenish-blue colour observed	+
		Liebermann Burchard test	Greenish-blue colour observed	+

(+) Present

III. **Solubility Test –** Solubility was performed on standard biomarkers, Marketed & inhouse formulation using water, ethanol, ethyl acetate, alcohol and other solvents. All results were summarised in Table 2.

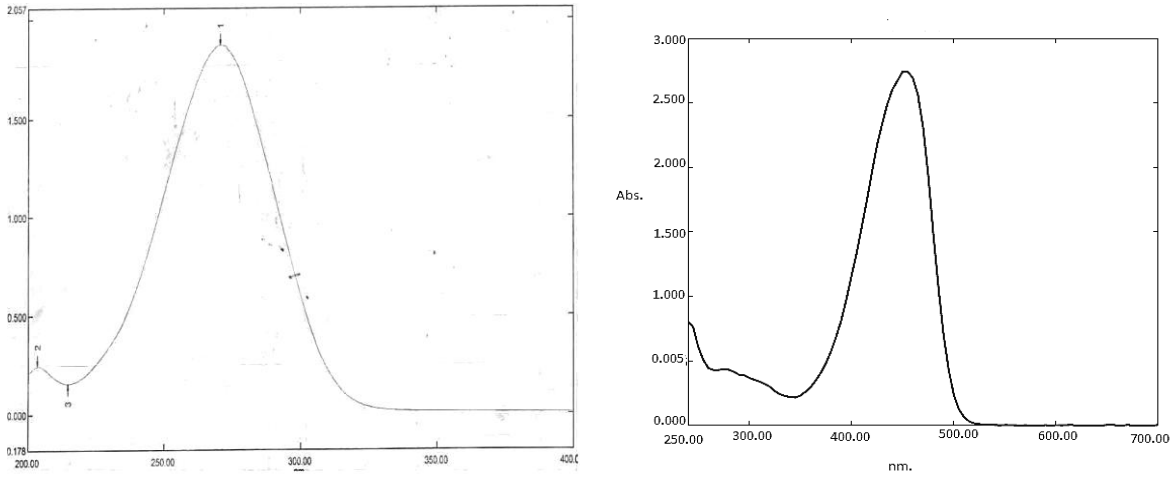
Table 2 – Solubility result of Lupeol, Diosgenin, *Mutrakrichantak churna* and in-house formulation

S. No,	Solvent	Solubility
Solubility of Lupeol		
1.	Water	Sparingly Soluble
2.	Petroleum Ether	Freely Soluble
3.	Methanol	Freely Soluble
4.	Ethanol	Freely Soluble
5.	n-butanol	Freely Soluble
6.	Acetonitrile	Sparingly Soluble
7.	Phosphate Buffer (pH 7.4)	Sparingly Soluble
Solubility of Diosgenin		
8.	Water	Not soluble
9.	Petroleum Ether	Sparingly Soluble
10.	Methanol	Freely soluble
11.	Ethanol	Freely soluble
12.	n-butanol	Freely soluble
13.	Acetonitrile	Not soluble
14.	Phosphate Buffer (pH 7.4)	Not soluble
Solubility of Mutrachintrak Churna		
15.	Water	Sparingly soluble
16.	Petroleum Ether	Freely soluble
17.	Methanol	Freely soluble
18.	Ethanol	Freely soluble
19.	n-butanol	Freely soluble
20.	Acetonitrile	Sparingly soluble
21.	Phosphate Buffer (pH 7.4)	Sparingly soluble

Solubility of in-house formulation		
22.	Water	Not soluble
23.	Petroleum Ether	Freely Soluble
24.	Methanol	Freely soluble
25.	Ethanol	Freely soluble
26.	n-butanol	Sparingly soluble
27.	Acetonitrile	Sparingly Soluble
28.	Phosphate Buffer (pH 7.4)	Not soluble
29.	Phosphate Buffer (pH 7.4)	Not soluble

Uv Spectroscopy Method -

Determination of ƛ max of Lupeol & Diosgenin - 10 µg/ml solution of both the standard biomarker was prepared and ƛ max of Lupeol was observed at 270 nm and absorbance was found at 1.870. ƛ max of diosgenin was observed at 447 nm and absorbance was found at 2.750. ƛ max of both biomarkers displayed in Figure 2 (A. Luepol & B. diosgenin). Results summarised in Table 3.



A. Lupeol **B. Diosgenin**

Figure 2. ƛ max of Lupeol & Diosgenin

Table 3. ƛ max and Absorbance of Lupeol & Diosgenin

S. No	Compound	Wavelengh (ƛ max)	Absorbance (nm)
1	Lupeol	270	1.870
2	Diosgenin	447	2.750

Preparation of Calibration curve of Lupeol – Different concentration ranging from 1 to 5 µg/ml was prepared and absorbance was noted at 270 ƛ max and displayed in Table 4. Graph was plotted between concentration and absorbance and regression coefficient was identified. Similarly, 5 µg/ml solution of *Mutrakrichantak churna* and in-house formulation was also prepared to determine the concentration of Lupeol. Absorbance of *Mutrakrichantak churna* was found at 0.421, by extrapolation concentration of lupeol was found 2.37 µg/ml and for in house formulation absorbance was observed at 0.914 nm and concentration was found to be observed at 5.48 µg/ml (Figure 3 & 4).

Table 4. UV absorbance reading for lupeol

S. No.	Concentration (µg/ml)	Absorbance (nm) at 270 ƛ max	Regression Coefficient
1	1	0.282	y = 0.1434x + 0.0814
2	2	0.342	R^2 = 0.9335

3	3	0.465	
4	4	0.598	
5	5	0.871	
6	Mutrachintrak churna (5 µg/ml)	0.421	
7	In-house formulation (5 µg/ml)	0.914	

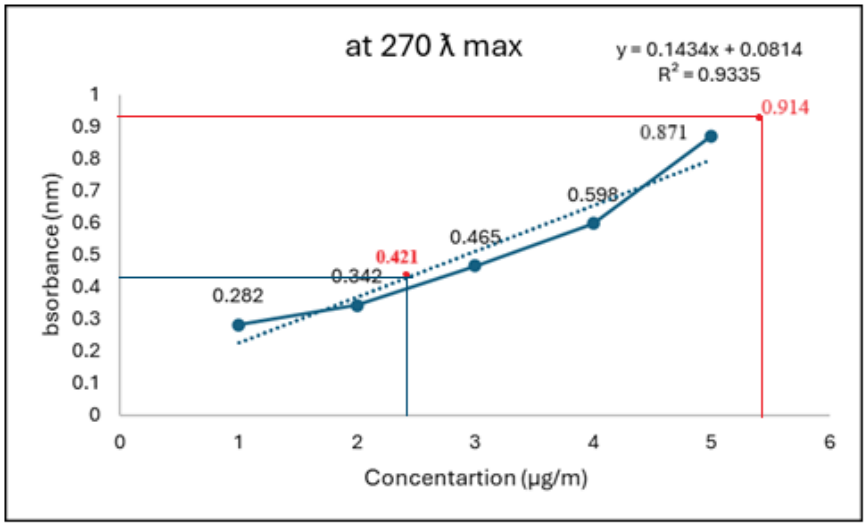


Figure 3. Standard calibration curve of Lupeol

Preparation of Calibration curve of Diosgenin – Different concentration ranging from 1 to 5 µg/ml was prepared and absorbance was noted at 447 λ max and displayed in Table 5. Graph was plotted between concentration and absorbance and regression coefficient was identified. Similarly, 5 µg/ml solution of Mutrakrichantak churna and in-house formulation was also prepared to determine the concentration of Lupeol. Absorbance of Mutrakrichantak churna was found at 0.526 nm, by extrapolation concentration of lupeol was found 3.01 µg/ml and for in house formulation absorbance was observed at 0.718 nm and concentration was founds to be observed at 4.98 µg/ml (Table 5)

Table 5. UV absorbance reading for Diosgenin

S. No.	Concentration (µg/ml)	Absorbance (nm) at 447 λ max	Regression Coefficient
1	1	0.338	y = 0.0567x + 0.352 R² = 0.5346
2	2	0.424	
3	3	0.582	
4	4	0.638	
5	5	0.825	
6	Mutrachintrak churna (5 µg/ml)	0.526	
7	In-house formulation (5 µg/ml)	0.718	

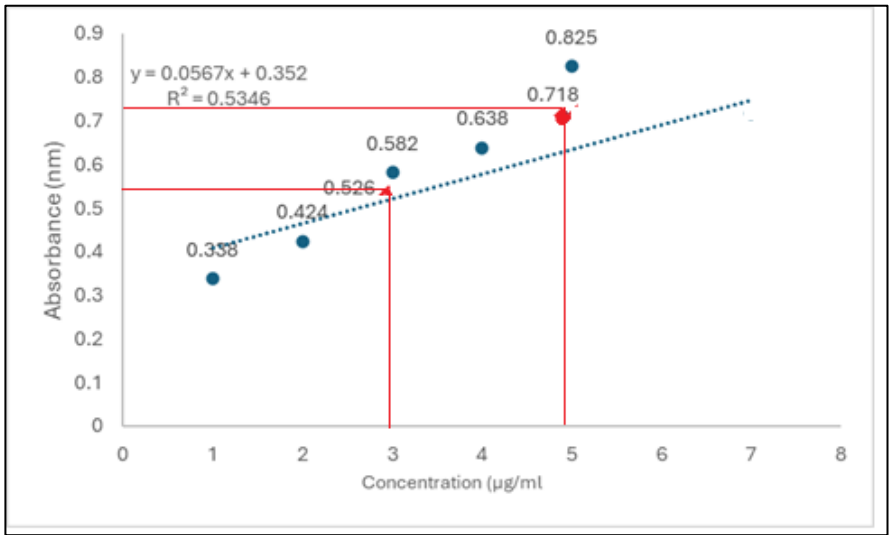


Figure 4. Standard calibration curve of Diosgenin

Linearity of Lupeol & Diosgenin – To assess the linear correlation between concentration and absorbance for lupeol and diosgenin *via* UV-visible spectrophotometry, thereby validating their quantifiability within a standard range**.** Different concentration ranging from 1 to 5 µg/ml was used and all results were summarised in Table 6 with regression value**.**

Table 6. Linearity of Lupeol & Diosgenin

Linearity of Lupeol						
Concentration (µg/ml)	**Repeatability**			**Mean**	**SD**	**RSD (%)**
	1	**2**	**3**			
1	0.28	0.282	0.281	0.280999	0.001	0.36
2	0.324	0.321	0.328	0.324321	0.004	1.08
3	0.465	0.472	0.469	0.468658	0.004	0.75
4	0.521	0.511	0.531	0.520936	0.010	1.92
5	0.712	0.728	0.714	0.717965	0.009	1.21
Linearity of Diosgenin						
1	0.338	0.342	0.345	0.341655	0.004	1.03
2	0.424	0.421	0.428	0.424324	0.004	0.83
3	0.582	0.587	0.591	0.586655	0.005	0.8
4	0.638	0.642	0.641	0.640331	0.002	0.33
5	0.825	0.831	0.828	0.827996	0.003	0.36

The linearity plots demonstrated a robust connection between absorbance and concentration. The elevated R^2 values (>0.998) affirm exceptional linearity. The results confirm the efficacy of UV spectroscopy for the preliminary assessment of lupeol and diosgenin in herbal extracts.

Accuracy of Lupeol & Diosgenin - To evaluate the precision of the UV spectrophotometric approach for lupeol and diosgenin using recovery tests by introducing known amounts into the matrix and determining the % recovery. Stock solutions of lupeol and diosgenin were formulated at a concentration of 100 µg/mL. The samples were enhanced with standard solutions at three concentrations: 80%, 100%, and 120% of the desired concentration (e.g., 6 µg/mL). Measured amounts of lupeol and diosgenin were included to replicate concentrations of 80%, 100%, and 120%. Each sample was analysed triplicate. Absorbance was quantified at 270 nm (lupeol) and 447 nm (diosgenin). Results discussed in Table 7.

Table 7. Recovery Studies for accuracy of Lupeol and Diosgenin

Level of Recovery (%)	80%		100%		120%	
	Marketed	In- House	Marketed	In- House	Marketed	In- House
Amount Present in mg	10	20	10	20	10	20
	10	20	10	20	10	20
	10	20	10	20	10	20
Amount of standard Added	8	16	10	20	12	24
	8	16	10	20	12	24
	8	16	10	20	12	24
Amount recovered (mg)	7.9	15.9	9.9	19.9	11.8	23.8
	7.7	15.8	9.8	19.8	11.9	23.6
	7.8	15.9	9.9	19.8	11.7	23.9
% Recovery of lupeol	98.75	99.37	99	99.5	98.33	99.16
	96.25	98.75	98	99	99.16	98.33
	97.5	99.37	99	99	97.5	99.58
Amount Present in mg	10	20	10	20	10	20
	10	20	10	20	10	20
	10	20	10	20	10	20
Amount of standard Added	8	16	10	20	12	24
	8	16	10	20	12	24
	8	16	10	20	12	24
Amount recovered (mg)	7.77	15.68	9.92	18.92	10.98	23.19
	7.84	15.66	9.87	19.25	10.91	23.28
	7.89	15.69	9.97	19.16	11.21	23.38
% Recovery of Diosgenin	97.12	98	99.2	94.6	91.5	96.62
	98	97.87	98.7	96.25	90.91	97
	98.62	98.06	99.7	95.8	93.41	97.41

The recovery levels for both biomarkers fell within the permissible range of 90% to 99 %. The low RSD values (<2%) at all levels signify enhanced precision and reproducibility. The results validate that the UV approach is accurate and dependable for quantifying lupeol and diosgenin in polyherbal Formulations.

Statistical Validation of recovery studies of Lupeol & Diosgenin - To statistically validate the accuracy (recovery) of lupeol and diosgenin by analysis of variance (ANOVA) and relative standard deviation (%RSD), thereby proving the precision and reliability of the UV approach. Triplicate assays were conducted at three concentration levels (80%, 100%, 120%) for both lupeol and diosgenin. The mean recovery, standard deviation (SD), and percentage relative standard deviation (RSD) were computed (Table 8). One-way ANOVA was utilised to determine whether the difference in recovery means across various levels were statistically significant.

Table 8. Statistical validation recovery of Lupeol & Diosgenin

Compound	Level of Recovery (%)	Drug	% Recovery	Standard Deviation	%RSD
Lupeol	80	Mutrachintrak Churna	97.51	1.25	1.28
		In-house formulation	99.16	0.35	0.36
	100	Mutrachintrak Churna	98.66	0.57	0.59
		In-house formulation	99.16	0.28	0.29
	120	Mutrachintrak Churna	98.33	0.83	0.84

		In-house formulation	99.02	0.63	0.64
Diosgenin	80	Mutrachintrak Churna	97.91	0.75	0.77
		In-house formulation	97.99	0.09	0.10
	100	Mutrachintrak Churna	99.20	0.50	0.50
		In-house formulation	95.55	0.85	0.89
	120	Mutrachintrak Churna	91.94	1.30	1.42
		In-house formulation	97.01	0.39	0.41

The %RSD values were below 2%, signifying elevated precision and reproducibility. The ANOVA test indicated no significant difference across the various spiking levels for both lupeol and diosgenin ($p > 0.05$), so affirming the method's correctness is consistent across concentrations. These data confirm that the UV spectrophotometric approach is statistically robust for the quantification of both biomarkers in Mutrakrichantak Churna.

Precision of Lupeol & Diosgenin - To evaluate the accuracy of the UV spectrophotometric approach by examining intra-day and inter-day variability in both proprietary and commercial formulations of *Mutrakrichantak churna* for lupeol and diosgenin. biomarker concentration was 6 µg/ml was selected for the said study. Intra-day accuracy: three replicate measurements conducted on the same day at hourly intervals. Inter-day precision: three replicate measurements conducted over three successive days. Results are presented in Table 9 as mean ± standard deviation and percentage relative standard deviation (%RSD).

Table 9. Precision of Lupeol & Diosgenin Estimation

Intra-day Precision					**Inter-day Precision**				
	Lupeol		**Diosgenin**			**Lupeol**		**Diosgenin**	
Time	**Marketed**	**In-house**	**Marketed**	**In-house**	**Days**	**Marketed**	**In-house**	**Marketed**	**In-house**
After 1hr	99.21	99.7	98.78	99.12	First	99.7	98.6	98.28	99.21
After 2hr	98.7	99.55	98.99	99.38	Second day	98.1	96.3	98.82	99.39
After 3hr	99.12	98.6	98.64	99.19	Third day	97.5	97.5	98.64	99.55
After 4hr	99.9	98.56	98.79	99.34		-			
After 5hr	99.7	97.1	98.7	98.99					
After 6hr	99.6	97.8	98.86	98.81					
Mean	99.37	98.55	98.79	99.13	-	98.43	98.43	98.58	99.38
SD	0.442	0.999	0.122	0.215		1.137	1.137	0.27	0.17
% RSD	0.45	1.01	0.12	0.22		1.155	1.16	0.28	0.17

All %RSD readings were much below the acceptable threshold of <2%, demonstrating exceptional repeatability and intermediate precision. Minor discrepancies noted between in-house and marketed samples may result from variances in raw material quality and processing methods. The approach shown strong reproducibility for both biomarkers across formulations, validating its appropriateness for standard quality control.

Ruggedness of Lupeol & Diosgenin - To assess the robustness of the analytical approach by examining the consistency of results across varying conditions, including various analysts and instruments, for both marketed and in-house formulations of Mutrakrichantak Churna.

Table 10. Ruggedness of Lupeol & Diosgenin

Sample Type	**Analyst**	**Mean Recovery (%)**	**SD**	**% RSD**
Lupeol				

In-house	A	99.2	1.14	1.15
	B	98.7	1.21	1.23
Marketed	A	98.9	1.2	1.21
	B	99.1	1.09	1.1
Diosgenin				
In-house	A	100.1	1.1	1.1
	B	99.5	1.18	1.19
Marketed	A	99.7	1.14	1.14
	B	100.2	1.09	1.08

All %RSD values were below 2%, demonstrating that the approach is robust across differences in analytical circumstances. No substantial discrepancies were noted among the results produced by several analysers, indicating methodological consistency. Both proprietary and commercial formulations demonstrated similar outcomes, affirming the method's dependability across various sample sources.

Robustness of Lupeol & Diosgenin – To assess the robustness of the UV spectrophotometric approach by examining the effects of minor but intentional changes in experimental circumstances on the accuracy and precision of Lupeol and Diosgenin quantification in both in - house and marketed formulations.

Table 10 – Robustness of Lupeol & Diosgenin

Drugs	**Lupeol**		**Diosgenin**	
Parameter Variation	**In-house (%RSD)**	**Marketed (%RSD)**	**In-house (%RSD)**	**Marketed (%RSD)**
Wavelength ±1 nm	1.15	1.22	1.1	1.17
Solvent variation (MeOH ±2%)	1.18	1.27	1.13	1.2
Incubation time ±5 min	1.12	1.2	1.11	1.15

The %RSD readings for all variations were consistently within the acceptable threshold of <2%, demonstrating the robustness of the analytical procedure (Table 10). Negligible alterations in detecting wavelength, solvent composition, and incubation duration did not substantially influence the quantification outcomes. The uniformity in both proprietary and commercial formulations indicates procedure stability and dependability under standard laboratory settings.

Limit of Detection (LOD) & Limit of Quantification (LOQ) -To determine the Limit of Detection (LOD) and Limit of Quantification (LOQ) for lupeol and diosgenin in both in-house and Marketed formulations, thereby evaluating the sensitivity of the developed UV spectrophotometric method. Calibration parameter used for detection of LOD & LOQ for Lupeol Slope is 0.061 and SD is 0.015, while for diosgenin slope is 0.056 and SD is 0.016 results are summarised in Table 11.

Table 11. LOD & LOQ of Lupeol & Diosgenin

Formulation Type	**LOD (µg/mL)**	**LOQ (µg/mL)**
Lupeol		
In-house	0.81	2.45
Marketed	0.84	2.52
Diosgenin		
In-house	0.94	2.85
Marketed	0.97	2.92

The limits of detection (LOD) and quantification (LOQ) for both lupeol and diosgenin were below 1 µg/mL and 3 µg/mL, respectively, thereby affirming the method's great sensitivity. The slight

discrepancies between in-house and marketed samples are within permissible analytical variation and may indicate small formulation matrix effects. These results confirm the method's capability to accurately identify and quantify modest quantities of biomarkers in both formulations.

High Performance Liquid Chromatography (HPLC) –

Lupeol and diosgenin are bioactive phytosterols prevalent in various medicinal herbs, exhibiting a range of pharmacological activities including anti-inflammatory, anti-diabetic, antioxidant, and nephroprotective actions. Mutrakrichantak Churna, an Ayurvedic polyherbal preparation historically employed for urinary tract ailments, may encompass these phytoconstituents. The precise quantitative measurement of these substances is essential for quality control, standardization, and pharmacological validation.

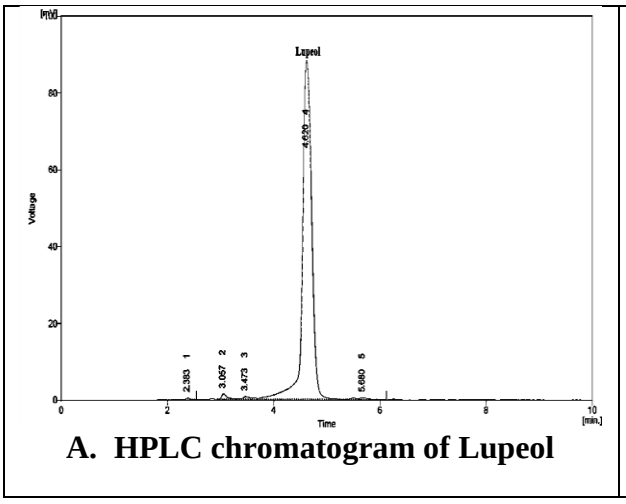
Optimization of chromatography parameter - The HPLC chromatographic conditions were improved following experiments conducted using a C18 reverse phase column utilizing various mobile phases. Both marker chemicals had an adequate detection response at a wavelength of 270 & 470 nm. The ambient temperature was sustained in the column until the analysis was completed. A mobile phase consisting of 28% v/v 0.1% formic acid in water and 82% v/v methanol was employed at a flow rate of 1 ml/min in an isocratic elution.

Quantitative Estimation of Lupeol & Diosgenin - The current HPLC methods were used to quantify three active ingredients (namely, Lupeol and diosgenin) of Mutrakrichantak churna which are also significant for therapeutic efficacy.

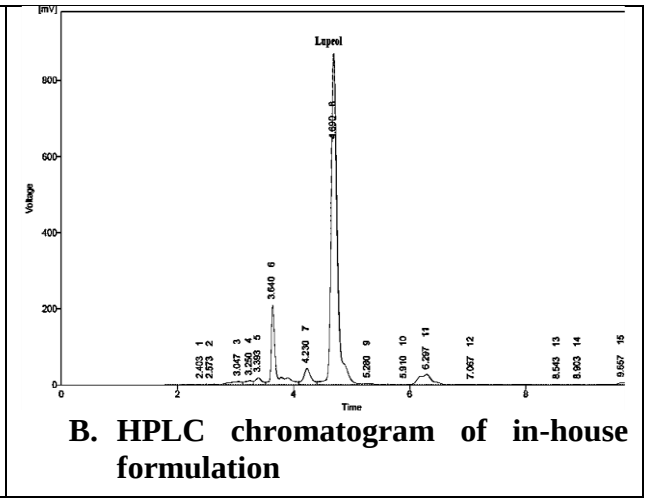
The antioxidant and pro-oxidant mechanisms of Lupeol are likely attributable to its potent reducing capacity and limited metal chelating activity. In-house formulation and Mutrachintrak Churna were standardized by the quantification of Lupeol using HPLC. Lupeol was identified at concentrations of 11.02% in In-house formulation and 4.13% in Mutrachintrak Churna, as indicated in Table 12. HPLC chromatograms of Lupeol standard, In-house formulation and Mutrachintrak Churna are presented in Figure 4. (A, B & C).

Table 12. Percentage of Lupeol

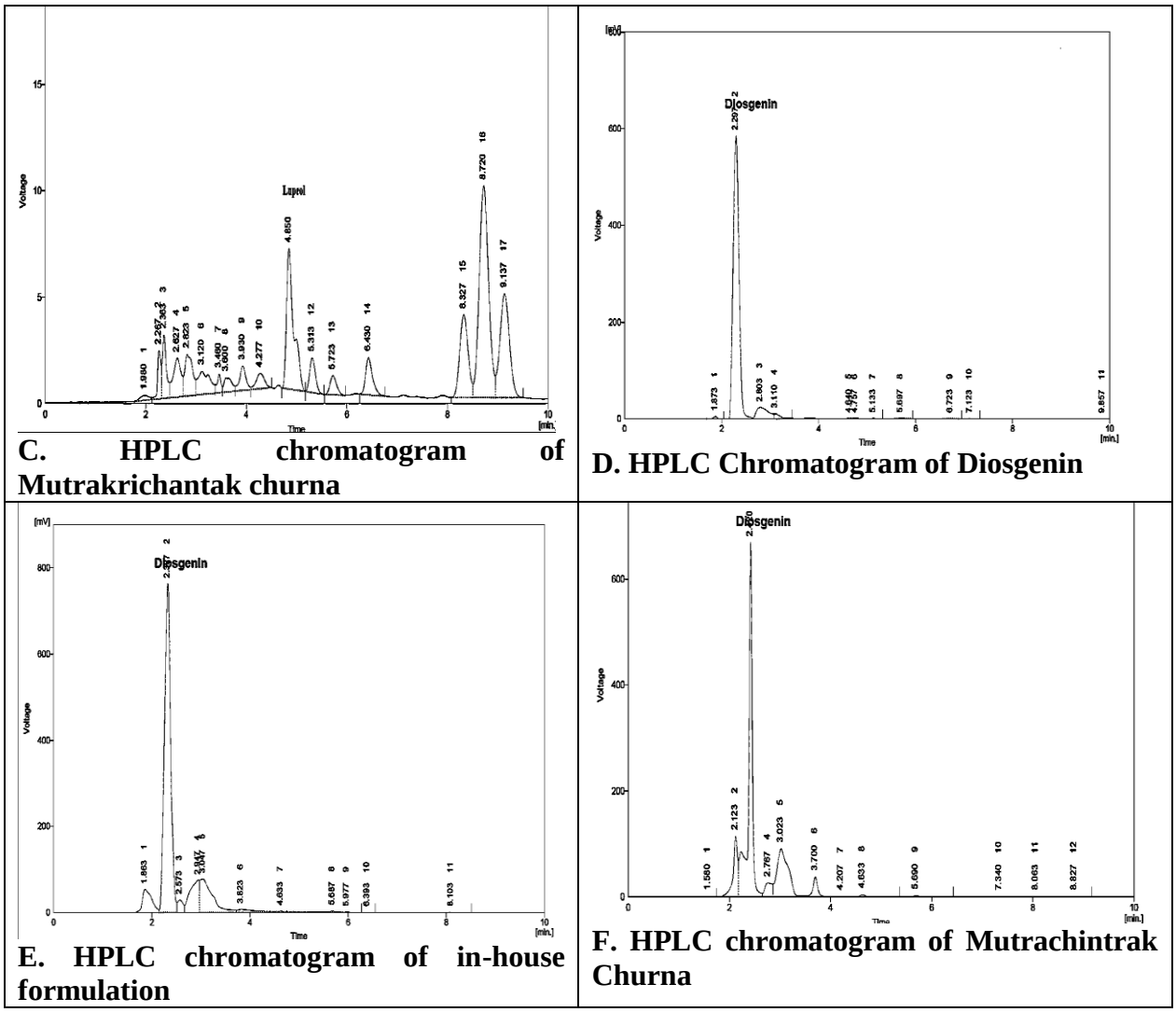
S. No.	Name of Drug Sample	Retention time (Min)	Area (mV. S)	Percentage of Lupeol
1	Lupeol	4.680	1081	-
2	In-house formulation	4.690	4632	11.02%
3	Mutrachintrak Churna	4.862	1759	4.13%



A. HPLC chromatogram of Lupeol



B. HPLC chromatogram of in-house formulation



C. HPLC chromatogram of Mutrakrichantak churna

D. HPLC Chromatogram of Diosgenin

E. HPLC chromatogram of in-house formulation

F. HPLC chromatogram of Mutrachintrak Churna

Figure 5. HPLC chromatogram

Diosgenin is a naturally occurring steroidal saponin found abundantly in various medicinal plants and is one of the active constituents of Mutrakrichantak churna Diosgenin may induce lymphocyte transformation and augment the phagocytic capacity of macrophages in vitro, while significantly enhancing the release of nitric oxide and TNF-α in macrophages. It could enhance both specific and non-specific cellular immune responses (He et al., 2012). Mutrakrichantak churna and the in-house formulation were standardized by quantifying Diosgenin by HPLC. Diosgenin was identified at concentrations of 1.69% in in-house formulation and 0.56% in Mutrakrichantak churna, as indicated in Table 2. The HPLC chromatograms of diosgenin are presented in Figure 4. (D, E & F).

Table 13. Percentage of Diosgenin

S. No.	Name of Drug Sample	Retention time (Min)	Area (mV. S)	Percentage of Lupeol
1	Diosgenin	2.291	4922	-
2	In-house formulation	2.326	3421	1.69%
3	Mutrachintrak Churna	2.312	1174	0.56%

Conclusion

The experimental data and results indicate that the UV Spectroscopy and HPLC methods are easy, precise, specific, sensitive, and accurate for the quantitative assessment of Lupeol and Diosgenin in the polyherbal formulation, Mutrakrichantak Churna. The methodology was validated in

accordance with ICH guidelines. The marker component was detected in the product by spectral scanning. The chosen mobile phase provided excellent resolution of the chemical in the result. This method can be employed for the identification of lupeol and diosgenin in various Ayurvedic or herbal goods.

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