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Original Article

A Validated Stability-Indicating RP-HPLC Method for Quantification of Glycyrrhizic Acid and Piperine in Polyherbal Formulations

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Abstract

Glycyrrhizic acid and piperine are frequently used in conjunction with other therapies to treat a variety of disorders, although both medications have low water solubility and photosensitivity issues. The objective of this study is to develop a Reverse-phase High-Performance Liquid Chromatography (RP-HPLC) method, known for its selectivity, precision, sensitivity, and accuracy. This method is intended for the quantification of phytoconstituents in formulating polyherbal tablets as well as in certain Ayurvedic formulations. Glycyrrhizic acid and piperine were successfully separated by liquid chromatography using the phenomenex Luna C-18 column and an isocratic elution mode with a mobile phase made up of a combination of methanol and HPLC grade water. A photodiode array (PDA) detector was used to determine the retention times for glycyrrhizic acid and piperine, which were determined to be 2.06 minutes and 9.0 minutes and the method was found to be accurate (>95%) and precise (%RSD < 25) respectively. The method was established to be specific for the quantification of glycyrrhizic acid and piperine in in-house polyherbal tablets and some Ayurvedic formulations. Additionally, both phytoconstituents stress degradation studies were examined, and in the presence of degradation products, good drug peak separation was observed. Thus, glycyrrhizic acid and piperine may be regularly estimated *in vitro* and *in vivo* using this method.

Keywords: Glycyrrhizic acid; Piperine; RP-HPLC; Analytical method validation; Stability indicating; Polyherbal tablets.

1. Introduction

The use of herbal medicine is becoming increasingly significant in today's global health care systems [1]. Over the past few decades, great effort has been dedicated to developing appropriate and reliable analytical methods that certify the quality of herbal products in addition to improving and advancing their quality [2, 3]. Today, the most popular methods for evaluating and monitoring the quality of various herbal products are chromatographic and spectroscopic fingerprinting [4].

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Glycyrrhizic acid, derived from the root of *Glycyrrhiza Glabra*, is a well-established traditional herbal remedy with a rich history in Ayurvedic medicine. [5]. This phytocompound has many health benefits, including immunomodulation, anti-pyretic, anti-inflammatory, antiulcerous, gastroprotective, antiallergic, antioxidant and antiviral properties [6,7] **Figure 1(A).** The flavour of piperine, an alkaloid presents in the fruits and roots of *Piper longum* and *Piper nigrum*, is acrid [8]. In addition to being utilized as an insecticide, piperine is also employed as a bio-enhancer in some traditional medicines. A variety of therapeutic properties, including analgesic, antipyretic, anti-inflammatory, antioxidant, hepatoprotective, antithyroid, antihypertensive, anticancer, antiasthmatic, and CNS depressive activities, were demonstrated by recent pharmacological research on piperine [9, 10] **Figure 1(B).**

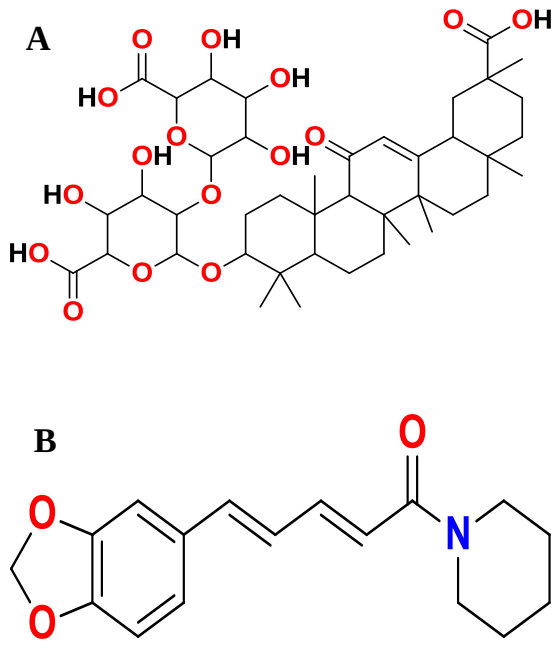


Figure 1. Chemical structure of (A) Glycyrrhizic acid and (B) Piperine.

For the individual estimation of glycyrrhizic acid and piperine, many analytical techniques are available, but none has been reported for a simple RP-HPLC technique for quantifying both phytoconstituents in polyherbal tablets and certain marketed formulations, regardless of what we have been taking. The reported methods, however, we're unable to achieve a simple and unique method along with analysis of the use of hazardous solvents, a lack of the use of green solvents, complicated mobile phases, and less accurate and repeatable HPLC processes [11-14].

The structural differences between Glycyrrhizic acid and Piperine necessitate tailored analysis conditions. Their distinct chemical compositions and functional groups demand unique analytical method to ensure accurate characterization and quantification. It is crucial to consider these variations in experimental design to obtain meaningful results when studying these compounds. Both compounds have been effectively quantified using identical analytical conditions due to their shared characteristics. Both compounds possess polar functional groups, allowing for their solubility in common mobile phases like methanol and water. Additionally, the compatibility of these compounds with HPLC simplifies the analytical method development, saving time and resources. The primary objective of this current research endeavour was to develop and validate a stability-indicating RP-HPLC technique. This technique was specifically designed to quantify Glycyrrhizic acid and piperine while adhering to the rigorous criteria established by the International Conference on Harmonization

(ICH). Additionally, this methodology was applied to analyze polyherbal tablets containing Glycyrrhizic acid and piperine, as well as various commercially available formulations. To ensure the robustness of the developed method, a range of stress or forced degradation studies were conducted. These encompassed diverse methodologies such as alkaline, acidic, thermal, oxidative, and sunlight-induced degradation. These investigations were essential to confirm the reliability and accuracy of the proposed approach.

2. Materials and Methods

2.1. Chemicals and reagents

Glycyrrhizic acid (95%) is provided by Otto chemicals Pvt Ltd, Mumbai and piperine (95%) is procured from Bio Med ingredients Pvt Ltd, Goa, India. HPLC grade methanol is purchased from Merck (Mumbai, India) respectively. Water (milli Q) was collected from KLE College of Pharmacy, Belagavi and marketed formulations containing glycyrrhizic acid and piperine were obtained from a local pharmacy.

2.2. Chromatography instrument and conditions

Shimadzu Agilent 1220 Infinity II instrument (LC-20AD, Japan) equipped with Quaternary pump with degasser (G7111A), auto-injector (G7129A), PDA detector (G7115A), and injecting value with 10 µL. Utilizing the software OpenLab CDS, the data interpretation and analysis were carried out. Chromatographic separation and analysis were conducted using a Phenomenex Luna C18 analytical column with specifications: C-18(2) 100, internal diameter of 250 mm and a length of 4.60 mm, and particle size of 5 m. This column is manufactured by Phenomenex Inc. Based in Canada. For glycyrrhizic acid and piperine, the optimal mobile phases were MeOH: Water (60:40) and MeOH: Water (70:30), respectively, and they were pumped through the column at a flow rate of 1 mL/min. Before usage, a PVDF filter membrane (0.45 m; Millex HV®, Millipore, USA) was used to ultrasonically degas the mobile phase. For the sample analysis, the injection volume was kept at 10 µL, and detection wavelengths of 251 nm for glycyrrhizic acid and 341 nm for piperine were used.

2.3. Primary stock and standard sample preparation

Separately, primary stock solutions of piperine and glycyrrhizic acid were prepared at a concentration of 1 mg/mL in methanol. The standard samples were then prepared by diluting the stock solutions with the mobile phase, resulting in concentrations ranging from 2 to 12 µg/mL for glycyrrhizic acid and 5 to 25 µg/mL for piperine. Before conducting the HPLC analysis, all standard solutions were stored in securely sealed volumetric flasks at a temperature of 4°C. [15, 16].

2.4. Method development

For saturating the column and enabling baseline correction, the mobile phase was pumped for 30 minutes. For each phytoconstituents, a standard calibration curve has been designed. The stock solutions were divided into several aliquots using diluents. Six injections of each concentration into the liquid chromatography system injected. At each time, the individual peak and retention times of both

phytoconstituents were meticulously recorded. Separate calibration curves were crafted for each phytoconstituents by merging the mean peak area plotted on the Y-axis with the corresponding concentration values on the X-axis. These calibration curves were subsequently employed to formulate regression equations [17, 18], which have been integrated into the formulation to ascertain the product's content accurately.

2.5. Preparation of polyherbal formulation

The preparation of polyherbal tablets directly compressible technique was used. These tablets contain ashwagandha, guduchi, pippali and licorice. The tablet containing 100 mg of each extract and total weight was about 500 mg. Composition of optimized polyherbal tablets was used to estimate of glycyrrhizic acid and piperine by HPLC method. As well as some marketed formulations are used for quantification of both phytoconstituents [19-21].

2.6. Analysis of some marketed Ayurvedic formulations

Precisely measured, 1 ml of the chosen formulations was placed into a 10 ml volumetric flask. Subsequently, the same procedure employed for the analysis of both the commercial and Ayurvedic formulations was applied to it. The HPLC analysis was used to quantify the parameter, and for glycyrrhizic acid Anuloma DS, U Prostone and for piperine Ayush Kwath, Haridra Khand and Sunarin polyherbal formulations were used.

2.7. Method validation

According to the criteria of the ICH standards, the method validation was carried-out [22-25].

2.7.1. Linearity

The preparation of different glycyrrhizic acid and piperine concentrations within the 2-12µg/ml and 5-25µ g/mL concentration ranges were used to test the method's linearity. Each solution was made in triplicates. To create the calibration curve, peak area was plotted against concentration. Results were obtained after three days of testing linearity across the same concentration range.

2.7.2. Sensitivity

The limits of detection (LOD) and quantification (LOQ) were calculated based on a calibration curve.

2.7.3. Precision

Precision was categorized into two parts. Intra-day (repeatability) and Inter-day (intermediate)

Intra-day (Repeatability): The standard solution of both the phytoconstituents was taken in three replicates and performed for three times in the same day on the standard solution of piperine having 4µg/ml, 5µg/ml, 6µg/ml concentration and 3µg/ml, 4µg/ml, 5µg/ml concentration for glycyrrhizic acid.

Inter-day (Intermediate): Three replicates of each phytoconstituents standard sample solution were used over the three consecutive days in the standard solution of piperine having 4µg/ml, 5µg/ml and 6µg/ml concentration and 3µg/ml, 4µg/ml and 5µg/ml concentration for glycyrrhizic acid.

2.7.4. System suitability

Peak area for preparing and glycyrrhizic acid was determined by taking six time reading of the performed test to prove the chromatographic system suitability.

2.7.5. Robustness

By including small chromatographic adjustments, the method's robustness was assessed. For glycyrrhizic acid change in wavelength (253nm and 249nm), flow rate (0.9 and 1.1 mL/min) and mobile phase ratio (62:38 and 58:42). For piperine change in wavelength (340nm and 344nm), flow rate (0.9 and 1.1 mL/min), change in mobile phase ratio (72:28 and 68:32).

2.7.6. Accuracy

The results of recovery experiments are based on the sample's % mean recovery at three distinct concentrations, such as 80, 100, and 120 % of piperine and glycyrrhizic acid. In triplicate, the samples were analysed, and percentage recoveries were reported.

2.7.7. Forced degradation studies

To handle phytoconstituent samples under stress-induced circumstances such oxidation, photo- and thermal degradation, acid and base hydrolysis, and intrusion of degraded products, forced drug degradation experiments have been carried out [26,27]. These tests assist in determining both the intrinsic stability of the therapeutic product's active components as well as their breakdown products.

2.7.7.1. Preparing of sample stock solution

To conduct stress degradation tests, the standard compound sample was precisely weighed to equal 10 mg of piperine and 10 mg of glycyrrhizic acid. It was then transferred to a volumetric flask with a capacity of 10 ml, 10 ml of diluent, and sonicated for 10 minutes to dissolve the samples.

2.7.7.2. Acid degradation (1 N HCL)

A 10 ml volumetric flask was filled with 1 ml of the sample stock solution, 1 ml of 1 N HCl, and sealed with paraffin and being heated in a water bath to 80 ° C for two hours. The flask was taken out and allowed to cool to room temperature. To neutralise the solution, added 1 N NaOH. Then, dilute the mixture to the desired volume using diluents. Solution was put into the system, and chromatograms were taken to assess the sample's stability.

2.7.7.3. Alkali degradation (1 N NaOH)

One millilitre of the sample stock solution was obtained and placed in a 10-ml volumetric flask. This flask was then sealed with paraffin and heated on a water bath at 80 °C for two hours. After being taken out of the water bath, the flask was allowed to cool to room temperature. To neutralise the solution, added 1 N HCL. Then, dilute the solution to the volume using diluents and mix. To evaluate the sample's stability, solution was introduced into the system, and chromatograms were obtained.

2.7.7.4. Oxidative degradation (30% H_2O_2)

A volumetric flask with a 10 ml capacity was filled with 1 ml of the sample stock solution, 1 ml of 30 % H2O2, and sealed with paraffin before being heated at 80 °C for two hours in a water bath. The flask was taken out of the water bath and allowed to cool at room temperature before being diluted and combined for volume. The stability of the sample was evaluated by injecting a solution into the instrument and recording the chromatograms.

2.7.7.5. Thermal degradation

Transfer 2 ml of (mobile phase) into a volumetric flask that has 2 ml of sample stock solution in it. The flask was paraffin-sealed, heated in a water bath for two hours at 80°C and the solution was cooled to room temperature. The solution was then combined after being diluted to 10 ml. To assess the sample's stability, solution was introduced into the apparatus, and chromatograms were obtained.

2.8. Statistical analysis

All the estimations were conducted in triplicate. The data acquired from this study underwent analysis using the method of relative standard deviation, and the outcomes were presented as the mean value accompanied by the standard deviation (SD).

3. Results and Discussion

3.1. Development and optimization of the method

The quantification of glycyrrhizic acid and piperine was accomplished using a new, reliable, and eco-friendly RP-HPLC technique. The UV spectra of both phytoconstituents in methanolic solution (10 µg/mL) were tested in the 200-400 nm range to determine the optimal wavelength. Glycyrrhizic acid and preparing both have the maximum UV absorption at 251 and 341 nm, respectively. Based on spectra of UV-absorption and the spectra acquired from the HPLC system coupled to the PDA detector, which indicates maximal absorbance, the wavelength for glycyrrhizic acid and piperine was selected. **Figure 2.**

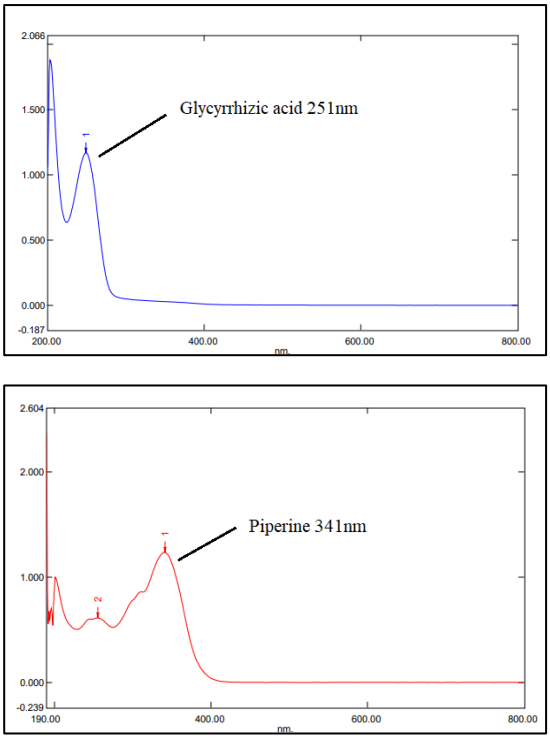


Figure 2. Maximum absorbance spectra of Glycyrrhizic acid and Piperine.

The composition of the mobile phase, flow rate and their pH, column oven temperature and wavelength detection, were changed to establish an ideal chromatographic process. Numerous experiments were undertaken using various mobile phase compositions, including methanol, acetonitrile, water, and combinations thereof, to produce distinct and well-resolved peaks. Both phytoconstituents were analysed at their respective λmax. Finally, the mobile phase composition containing MeoH: Water at ratio of 60:40 for glycyrrhizic acid and MeoH: Water at ratio of 70:30 for preparing and it was observed that a flow rate of 1 mL/min was the most suitable. The retention time was found to be 2.06 and 9.01 min for glycyrrhizic acid and piperine, respectively. It was evident from **Figure 3**. Both phytoconstituents showed excellent peak characteristics with minimal tailing.

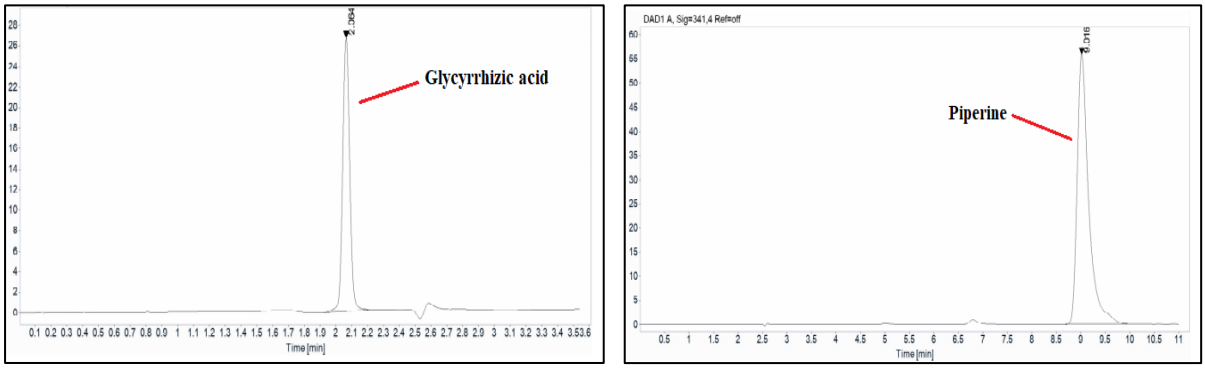


Figure 3. HPLC Chromatograms of Glycyrrhizic acid (RT =2.0 min) and Piperine (RT =9.0 min).

3.2. Method validation

3.2.1. System suitability

The validity and specificity of the developed method was ensured by the system suitability. An essential phase within the process of method development, this test evaluates the precision and consistency of the developed method for conducting chromatographic analysis. Parameters including peak area, retention time (tR), theoretical plates, and tailing factor were subjected to percent relative standard deviation (% RSD) calculations for both phytoconstituents. As outlined in **Table 1**, the % RSD values for peak area (<2), tailing factor (<2), and theoretical plates (N > 3000) all remained below the predefined acceptable thresholds.

3.2.2. Linearity

For both phytoconstituents, the calibration curve was linear over the test concentration range (2-12µg/ml) for glycyrrhizic acid and (5–25 µg/mL) for piperine. Regression study of both phytoconstituents with % RSD values less than 2.0 around the concentration ranges under investigation produced an average correlation coefficient of R2 = 0.999. **Table 2** and **Figure 4.**

Table 1. For the developed method of glycyrrhizic acid and piperine, system suitability study parameters (N=6).

Parameter	Glycyrrhizic acid		Piperine	
	Mean ± SD	% RSD	Mean ± SD	% RSD
TR (min)	2.05±0.04	1.9	9.01±0.003	0.03
Peak area	49.99±0.46	0.9	460.5±1.19	0.2
Plate count	9674.3±21.73	0.2	8650±10.01	0.1
Tailing factor	1.02±0.01	1.4	1.71±0.008	0.5

SD: Standard deviation, RSD: Relative standard deviation

Table 2. The developed method's linear regression data.

Parameters	Glycyrrhizic acid	Piperine
Linearity range (µg/ml)	2-12µg/ml	5-25µg/ml
Regression equation	Y= 75.40x-4.4186	Y= 683.06x-4.41
Slope	75.404	683.06
Intercept	4.4186	4.4186
Correlation coefficient (R^2)	0.9998	0.9991
LOD	0.020	0.105
LOQ	0.061	0.318

LOD- limit of detection, LOQ- limit of quantification

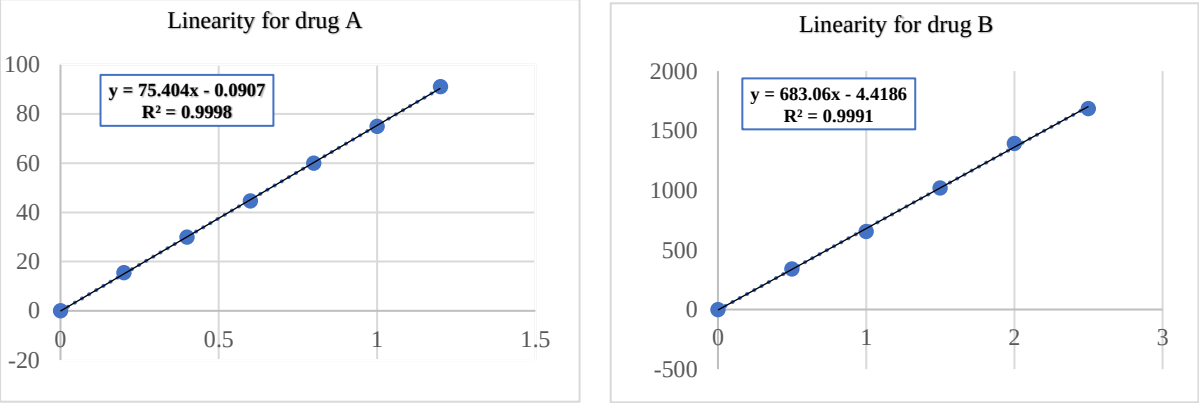


Figure 4. Linearity plots A. Glycyrrhizic acid and B. Piperine.

3.2.3. Limit of quantification (LOQ) and Limit of detection (LOD)

LOD and LOQ were determined as follows: LOD = 3.3 × δ/s and LOD = 10 × δ/s.

Where, δ: Standard deviation of Y-intercept, S: Mean slope of calibration curves

3.2.4. Precision

Inter-day and intra-day tests were performed to assess the precision. Glycyrrhizic acid (3, 4 and 5 g/ml) and piperine (4,5 and 6 g/ml) concentrations were examined for repeatability (intra-day precision) and intermediate precision (inter-day precision) on the same day at varying intervals and three subsequent days. The intra-day and inter-day precision % RSD values are less than 2 %, fulfilling the acceptance requirements and illustrative of the developed method's exceptional precision **Table 3.**

3.2.5. Robustness

For robustness, parameters for HPLC chromatography were slightly changed.

The findings confirmed the robustness of the developed HPLC technique by showing that the % RSD values (<2) and retention time are unchanged. 10 µg/mL was used as the analytes concentration for the current study **Table 4**.

Table 3. Precision data of Glycyrrhizic acid (1) and Piperine (2).

No.	Active content (µg/mL)	Intra-day (n=3)		Inter-day (n=3)	
		Mean± SD (µg/mL)	RSD (%)	Mean± SD (µg/mL)	RSD (%)
1	3	30.5±0.04	0.14	27.97±0.05	0.19
	4	40.18±0.43	1.06	37.22±0.68	1.84
	5	46.9±0.81	1.73	40.16±0.18	0.45
2	4	320.2±3.5	1.12	307.7±1.76	0.57
	5	392.69±0.98	0.25	374.6±1.58	0.42
	6	415.01±1.80	0.44	402.3±2.0	0.50

SD: Standard deviation, RSD: Relative standard deviation

Table 4. Evaluation of the developmental method's robustness for piperine and glycyrrhizic acid.

Comp.		Changes made	AUC ± SD.	RSD (%)
Glycyrrhizic acid	Flow rate (mL/min)	0.9	78.66±0.57	0.74
		1.1	83.16±0.22	0.27
	Wavelength (nm)	253	67.68±0.55	0.77
		249	69.27±0.45	0.65
	Mobile phase Ratio	62:38	57.2±0.28	0.49
		58:42	59.82±0.3	0.51
Piperine	Flow rate (mL/min)	0.9	940±0.18	0.02
		1.1	781.3±1.67	0.21
	Wavelength (nm)	340	774.4±2.78	0.36
		344	773.93±1.13	0.15
	Mobile phase Ratio	72:28	754.01±2.84	0.38
		68:42	915±4.63	0.51

AUC: Area under curve, RSD: Relative standard deviation, SD: Standard deviation

3.2.6. Accuracy

A previously examined reference solution containing glycyrrhizic acid and piperine (at a concentration of 4 g/mL) was enriched with precise amounts of composite samples at varying concentrations - specifically, low, medium, and high levels (corresponding to 50%, 100%, and 150% of a specified concentration). Subsequently, this enriched solution was subjected to analysis using an established methodology. Glycyrrhizic acid and preparing both had mean percentage recoveries that ranged from 98.83 to 101.2 % and 97.51 to 102.1 %, respectively **Table 5** showing the low % RSD and high recovery values, which point to the established method's good accuracy.

3.2.7. Forced-degradation studies

Table 6. Shows the results of a study on forced degradation. This study provides evidence for the relevancy of the developed method so there was sufficient separation between drug peak and their degradation product peak observation. **Figure 5** and **6**.

Table 5. Recovery studies of the developed method.

	Glycyrrhizic acid			Piperine		
Active content µg/mL	4			4		
Level (%)	50	100	150	50	100	150
Spiked Quantity µg/mL	6	8	10	6	8	10
Recovered Quantity µg/mL	5.9	7.9	10.1	5.85	7.9	10.2
Recover (%)	98.8	99.7	101.2	97.5	99.5	102.1

Table 6. Forced degradation data of glycyrrhizic acid and piperine**.**

Exposed stress degradation condition	% Drug degradation	
	Glycyrrhizic acid	Piperine
1 N HCL 80ºC, 2 h	38.4	32.6
1 N NaoH 80ºC, 2 h	9.25	8.23
30% H_2O_2 80ºC, 2 h	15.01	13.98
Thermal 80ºC, 2 h	5.4	3.9
Sunlight 6 h	29.0	60.51

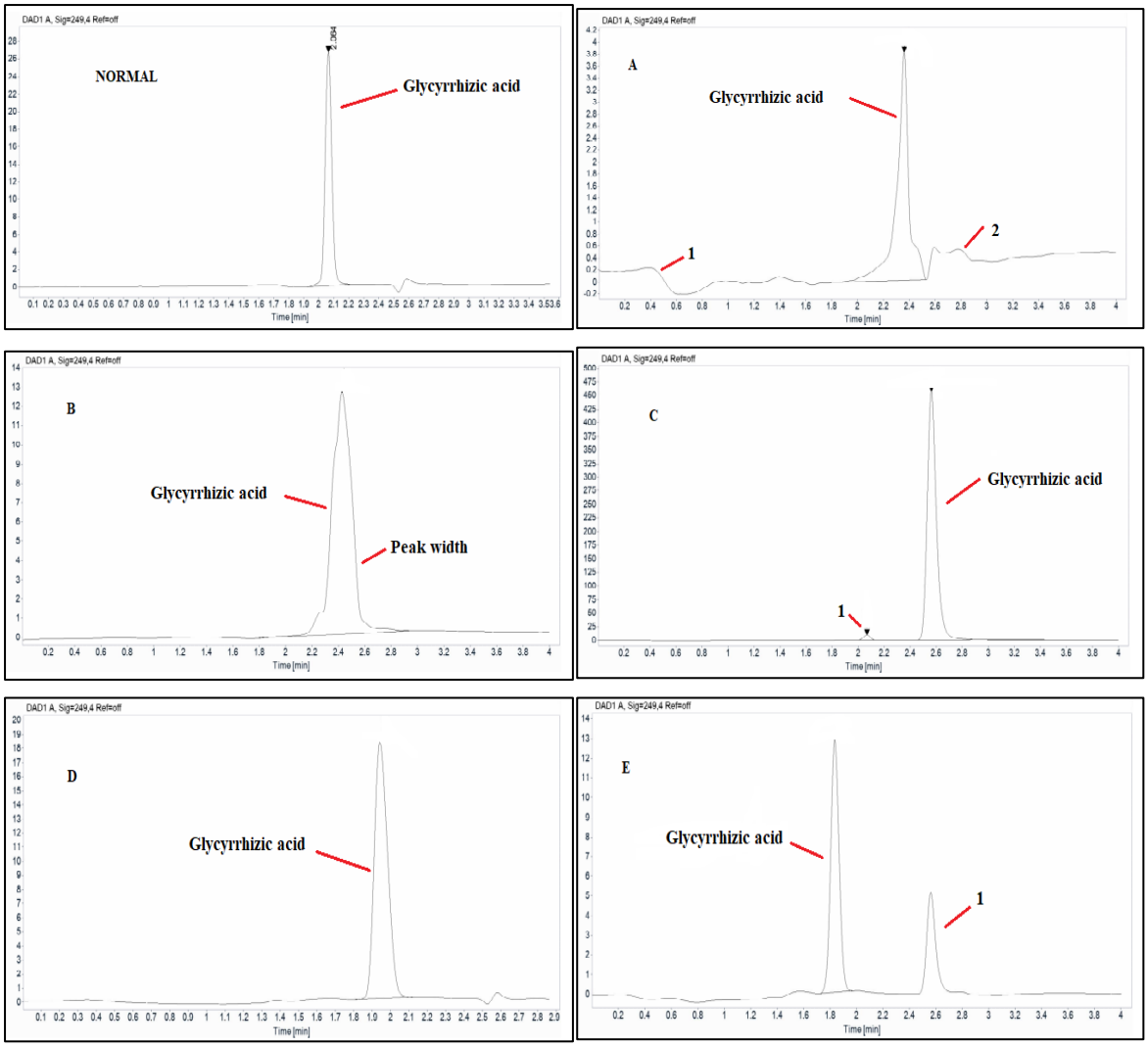


Figure 5. Glycyrrhizic acid in forced degradation study under Normal, Acid (A), Base (B), Oxidative (C), Thermal (D) and Photolytic (E) stress conditions.

3.3. Quantification of Glycyrrhizic acid and Piperine in Polyherbal formulations

By contrast, actual and observed phytoconstituent concentrations, the developed HPLC technique for the quantification of glycyrrhizic acid and piperine in in-house and certain marketed formulations was assessed for utilising known concentrations. **Table 7** and **Figure 7-9**.

Table 7. Quantitative data of Glycyrrhizic acid and Piperine.

Comp.	Brand name	Test concentration (µg/ml)	Amount estimated (µg/ml)	% Content
Glycyrrhizic acid	In-house	10	1.882	18.8
	Anuloma DS	10	0.373	3.73
	U Prostone	10	0.286	2.86
Piperine	In-house	10	0.380	3.80
	Ayush kwath	10	0.386	3.86
	Haridra Khand	10	0.0918	0.91
	Sunarin	10	0.075	0.75

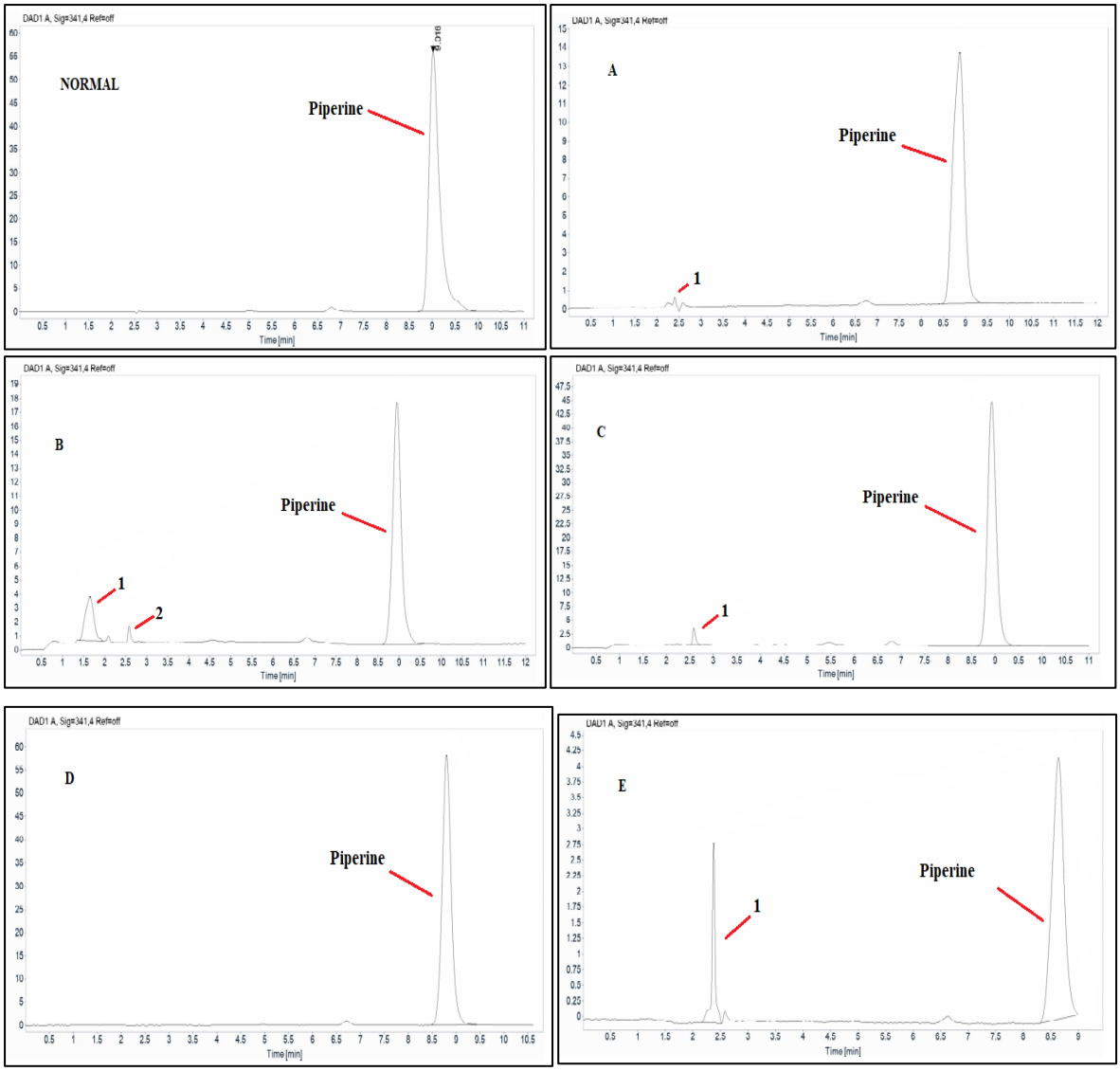


Figure 6. Piperine, in stress degradation study under Normal, Acid (A), Base (B), Oxidative (C), Thermal (D) and Photolytic (E) stress conditions.

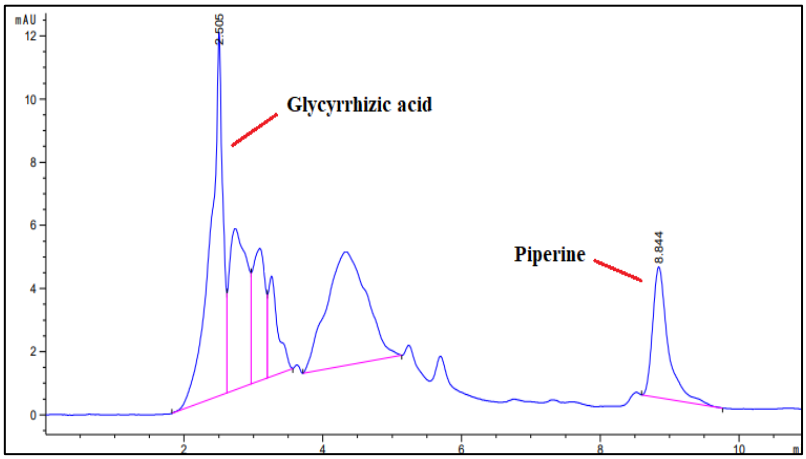
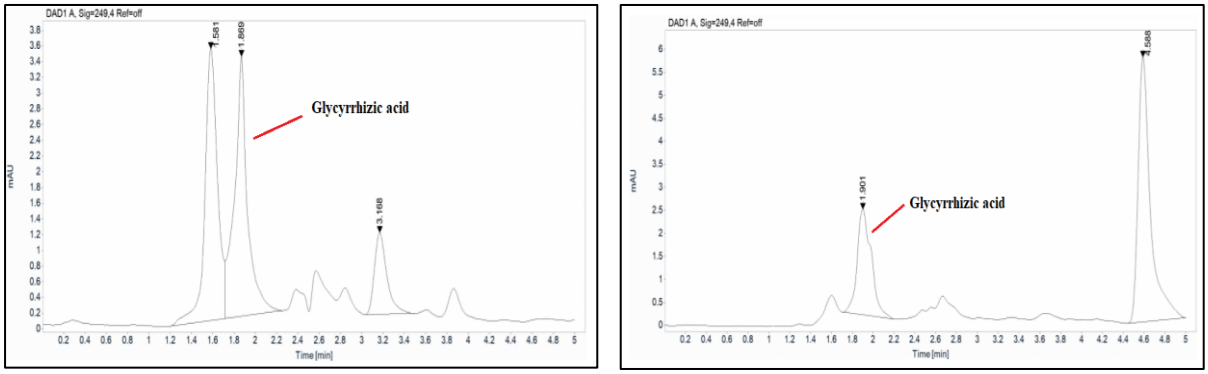
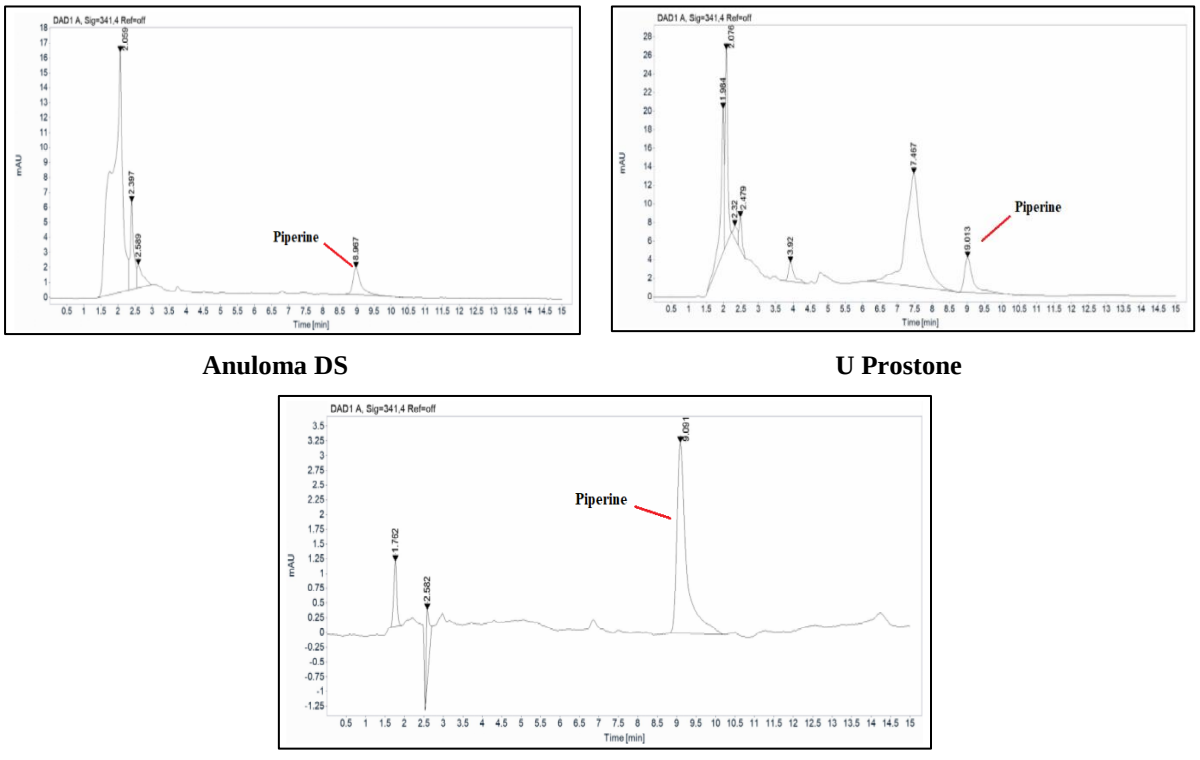


Figure 7. Chromatogram of in- house polyherbal tablet.



Anuloma DS **U Prostone**

Figure 8. Chromatogram of Marketed formulations of Glycyrrhizic acid (A. Anuloma DS and B. U Prostone).



Anuloma DS **U Prostone**

Sunarin

Figure 9. Chromatogram of Marketed formulations of Piperine (A. Ayush kwath, B. Haridra Khand and C. Sunarin).

3.4. Comparison with previously published HPLC methods

A comparative analysis was conducted between the recently developed HPLC method and previously published methods. This analysis encompassed numerous factors such as mobile phase ratios, mobile phase flow rate, wavelength selection, stability assessment, limits determination, and the practical applications of the HPLC methods. The comparison details were shown in **table 8** [28-36]. There is currently no single HPLC method

that can be used for a variety of analyses, such as the estimation of glycyrrhizic acid and piperine in prepared polyherbal formulation and some Ayurvedic-marketed products, as well as the evaluation of glycyrrhizic acid and piperine degradation behaviour using the same parameters of developed HPLC method. Compared to previously established techniques and existing literature related to glycyrrhizic acid and piperine, the present method demonstrates enhanced accuracy, affordability, and stability. This method utilizes methanol as the mobile phase and employs a water mixture (60:40 v/v for glycyrrhizic acid and 70:30 v/v for piperine), with a flow rate of 1 ml/minute and detection wavelengths set at 251 and 341 nm. Following the guidelines set forth by the International Council for Harmonisation (ICH) and staying within permissible limits, the developed method underwent thorough validation.

The devised approach offers a precise and straightforward means of quantifying glycyrrhizic acid and piperine within in-house formulations and Ayurvedic products available in the market. This underscores the sensitivity and reliability of the method.

Table 8. Comparison between previously published HPLC methods.

Sr. No	Mobile phase and flow rate	Wavelength (nm)	Limitations	Application	Ref.
Glycyrrhizic acid with other drugs					
1	Acetonitrile: aqueous acetic acid (60:40, V/V) and flow rate: 1.0 ml	254	Expensive	Determination of glycyrrhizic acid and liquiritin in licorice root	[28]
2	OPA (0.5%): acetonitrile (60:40, V/V) and flow rate: 1.0ml	254	Expensive and lack of stability study	Determining the glycyrrhizic acid content in licorice root	[29]
3	Methanol: diethyl ether: water: acetic acid (60:6:34:3, V/V) and flow rate: 1.0ml	248	More than 2 mobile phases used, expensive	Quantitative determination of glycyrrhizic acid and glycyrrhetinic acid in Fuzilizhong Pills	[30]
4	Acetonitrile: acetic acid (60:40, V/V) and flow rate: 1.0ml	252	Expensive	Simultaneous quantitative determination of glycyrrhizic acid and liquiritin in *Glycyrrhiza uralensis* extract	[31]
Piperine with other drugs					
1	Acetonitrile: water (0.05% acetic acid), (70:30, V/V) and flow rate: 1.0ml	342	Expensive and lack of stability study	Quantification of piperine in piper nigrum L.	[32]
2	Acetonitrile: water: acetic acid (60:39:0.5, V/V) and flow rate: 1.0ml	340	More than 2 mobile phases used, expensive	Estimation of piperine in piper nigrum L.	[33]
3	Acetonitrile: Methanol: water (65:35:5, V/V) and flow rate: 1.0ml	353	Expensive	Simultaneous Quantification of curcumin and piperine in a microparticle formulation Containing Curcuma longa and Piper nigrum	[34]
4	Acetonitrile: OPA (55:45, V/V) and flow rate: 1.0ml	330	Expensive	Simultaneous estimation of resveratrol and piperine in cubosome and human plasma	[35]
5	Acetonitrile: water (2% glacial acetic acid) and flow rate: 1.0 ml	346	Expensive	Simultaneous quantifications of quercetin and piperine in dual-drug loaded nanostructured lipid carriers	[36]

4. Conclusion

A novel stability indicating RP-HPLC method was deliberately developed for quantification of glycyrrhizic acid and piperine in prepared polyherbal tablets and some Ayurvedic formulations. The developed method was validated for several parameters that were within the acceptable ranges in accordance with ICH guidelines and found to be simple, rapid, more sensitive, and effective. For the analysis of glycyrrhizic acid and piperine, which are having distinct wavelengths, the developed method has been justified and made more affordable. With low LOD and LOQ values, the devised method showed high accuracy, precision, and linearity. Additionally, it showed clearly defined peaks with accurate quantification of both the compounds. A forced degradation study has unveiled that piperine and glycyrrhizic acid exhibit resistance to alkaline, oxidative, and thermal conditions. Nevertheless, it has been demonstrated that both compounds might undergo degradation when exposed to acidic and photolytic conditions.

As a result, this recently devised RP-HPLC method proves to be invaluable for the routine quantification of piperine and glycyrrhizic acid in diverse Ayurvedic and herbal formulations.

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Conflict of interest

The authors declare to have no conflict of interest.

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