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Research Paper

Stability-Indicating RP-HPLC Method Development and Validation with Forced Degradation Studies of Echinops echinatus Marker Compounds

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ABSTRACT

Objective:To develop and validate a simple, rapid, accurate, precise, and stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of selected marker compounds from Echinops echinatus, and to evaluate the stability of the marker compounds through forced degradation studies in accordance with ICH guidelines.Methods:A stability-indicating RP-HPLC method was developed by optimizing the chromatographic conditions, including the mobile phase composition, stationary phase, flow rate, detection wavelength, and column temperature, to achieve efficient separation of the selected marker compounds and their degradation products. The developed method was validated for system suitability, specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), limit of quantification (LOQ), and solution stability as per ICH Q2(R2) guidelines. Forced degradation studies were performed under acidic, alkaline, oxidative, thermal, photolytic, and hydrolytic stress conditions to assess the degradation behavior and demonstrate the stability-indicating capability of the method.1,2Results:The optimized RP-HPLC method provided well-resolved and symmetrical peaks for the selected marker compounds with satisfactory chromatographic performance. The validation results demonstrated excellent linearity over the selected concentration range, with correlation coefficients (R^2) greater than 0.999. The method exhibited acceptable accuracy, precision, specificity, robustness, and sensitivity, meeting the acceptance criteria recommended by ICH guidelines. Forced degradation studies revealed that the marker compounds underwent varying degrees of degradation under different stress conditions, while the degradation products were effectively separated from the analyte peaks, confirming the stability-indicating nature of the developed method.Conclusion:The developed RP-HPLC method is simple, reliable, precise, accurate, and stability-indicating for the analysis of Echinops echinatus

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marker compounds. The validated method is suitable for routine quality control, stability testing, standardization of herbal formulations, and pharmaceutical research involving Echinops echinatus. The successful separation of degradation products from the marker compounds confirms its applicability for stability studies and regulatory compliance

INTRODUCTION

Medicinal plants have served as an important source of therapeutic agents for centuries and continue to play a significant role in modern healthcare systems. The growing global demand for herbal medicines has highlighted the need for scientifically validated analytical methods to ensure their quality, safety, efficacy, and consistency. Unlike synthetic drugs, herbal materials contain complex mixtures of phytoconstituents whose chemical composition may vary depending on geographical origin, harvesting conditions, processing, and storage. Therefore, the standardization of herbal products through the identification and quantification of characteristic marker compounds has become an essential component of quality control.2,3,4

Echinops echinatus Roxb., belonging to the family Asteraceae, is a medicinal plant widely distributed in India and other parts of Asia. It has been traditionally used in the treatment of inflammation, fever, microbial infections, reproductive disorders, liver ailments, and various other health conditions. Pharmacological investigations have demonstrated that the plant possesses diverse biological activities, including anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, analgesic, and immunomodulatory effects. These therapeutic properties are primarily attributed to the presence of bioactive phytochemicals such as flavonoids, phenolic compounds, alkaloids, triterpenoids, and other secondary metabolites. The quantitative determination of selected marker compounds is therefore important for establishing the identity and quality of E. echinatus and its formulations.

Reverse-phase high-performance liquid chromatography (RP-HPLC) is one of the most widely employed analytical techniques for the qualitative and quantitative analysis of phytochemicals because of its high sensitivity, selectivity, reproducibility, and suitability for complex herbal matrices. The development of a stability-indicating RP-HPLC method is particularly valuable, as it enables the separation of marker compounds from their degradation products and potential impurities, thereby ensuring reliable analysis throughout the product's shelf life. Such methods are essential for routine quality control, stability assessment, formulation development, and regulatory compliance.5,6

Method validation is a critical step in analytical method development and is performed to demonstrate that the method consistently produces reliable and reproducible results for its intended purpose. According to the International Council for Harmonisation (ICH) guidelines, analytical methods should be evaluated for parameters such as specificity, linearity, accuracy, precision, robustness, sensitivity, and solution stability. In addition, forced degradation studies conducted under acidic, alkaline, oxidative, thermal, photolytic, and hydrolytic stress conditions provide valuable information regarding the intrinsic stability of the analytes and establish the stability-indicating capability of the developed method.7,8

The objective of the present study was to develop and validate a stability-indicating RP-HPLC method for the analysis of selected marker compounds of Echinops echinatus and to evaluate their degradation behavior under various forced degradation conditions in accordance with ICH guidelines.

MATERIALS AND METHODS

The whole plant of Echinops echinatus was authenticated and shade-dried before being powdered for analysis. HPLC-grade methanol, acetonitrile, water, orthophosphoric acid, hydrochloric acid, sodium hydroxide, and hydrogen peroxide (Merck KGaA, Darmstadt, Germany) were used throughout the study. The reference standard of the selected marker compound was obtained from a certified commercial supplier.

Chromatographic analysis was performed using an Agilent 1260 Infinity II RP-HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a C18 column (250 mm × 4.6 mm, 5 µm). Standard and sample solutions were prepared in HPLC-grade methanol, filtered through a 0.45 µm membrane filter, and analyzed under optimized chromatographic conditions.9,10,11

The developed RP-HPLC method was validated according to ICH Q2(R2) guidelines for specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). Forced degradation studies were carried out under acidic, alkaline, oxidative, thermal, photolytic, and neutral hydrolytic conditions to evaluate the stability-indicating capability of the method.

As the study involved only plant material and analytical experiments, ethical approval was not required. All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using GraphPad Prism version 10.0, with statistical significance considered at $p < 0.05$.12,13

A stability-indicating RP-HPLC method was developed for the analysis of the selected marker compounds of Echinops echinatus. Standard and sample solutions were prepared in HPLC-grade methanol and filtered through a 0.45 µm membrane filter before analysis. Chromatographic conditions, including the mobile phase composition, flow rate, detection wavelength, and column temperature, were optimized to achieve satisfactory separation and peak resolution.

The optimized method was validated according to ICH Q2(R2) guidelines by evaluating system suitability, specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ).14,15

Forced degradation studies were performed by exposing the marker compounds and plant extract to acidic, alkaline, oxidative, thermal, photolytic, and neutral hydrolytic stress conditions. The stressed samples were analyzed using the developed RP-HPLC method to assess degradation behavior and to confirm that the method could effectively separate the marker compounds from their degradation products.16

RESULTS

Extraction of Flower of Echinopus echinatus The flowers of plant shade dried were powdered and extracted with various solvent. The appearance and percent yield of extracts are reported as follows:

Table No. 1-Separation of marker compound by column chromatography

Sr. no	Various Solvent used for Extraction	Colour	Nature	Percentage yield (% w/w)
1.	Methanol	Dark Greenish	Jelly like	7.5% w/w
2.	Chloroform	Redish Brown	Semisolid	4.5% w/w
3.	Aqueous	Dark Brown	Sticky Powder	9% w/w

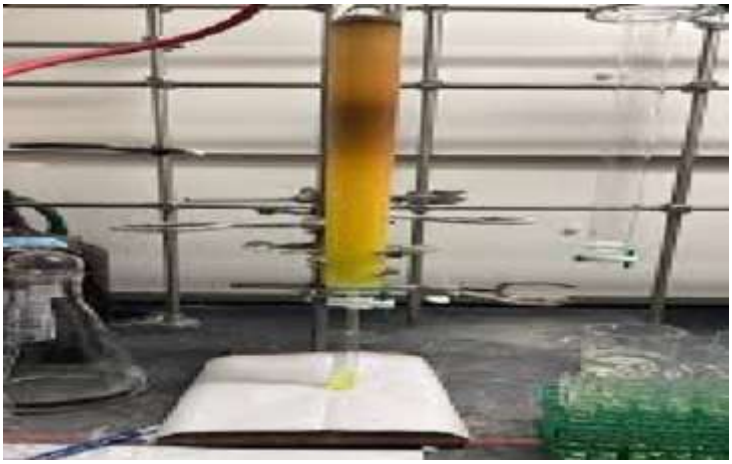

Fig No :1 TLC Identification Around 0.35–0.50 Rf value was noted and it is in the acceptable range

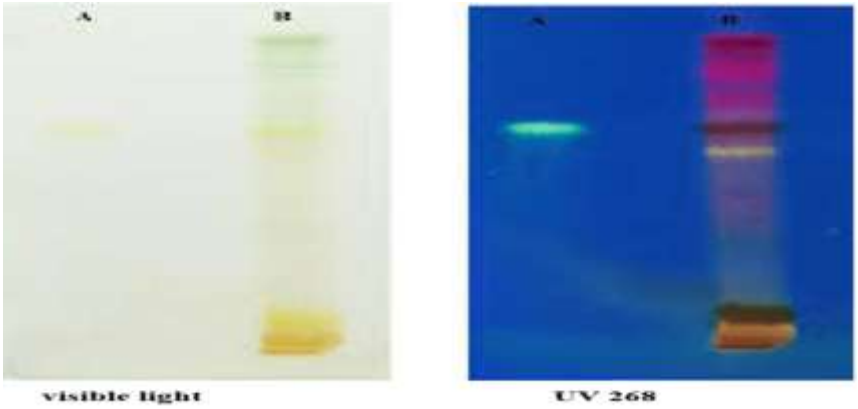

Fig No 2- UV-Visible Spectroscopy Dissolve sample in methanol and get clear λmax (approx.) at 268 nm and it show exact same for std

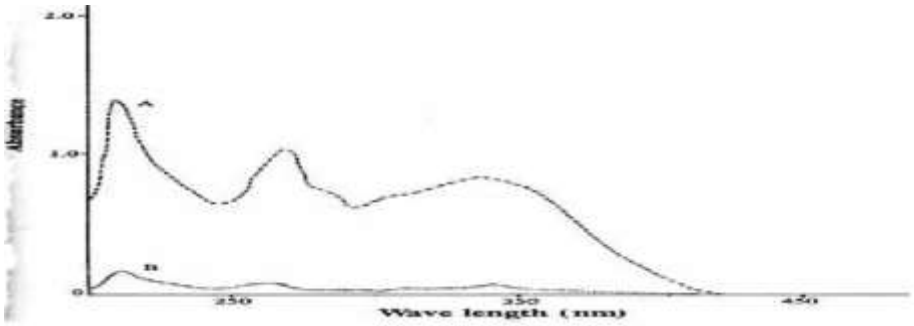

Fig No 3-Development and validation of RP-HPLC method-

The present work, carried out to develop and validate simple, selective, sensitive, rugged and reproducible method for quantitative determination of flower of Echinopus echinatus. The analytical method based on RP-HPLC using UV detection was developed and validated for determination of drug as chemical constituent Apigenin 7-O-glucoside .The acidic pH range was found suitable for solubility, resolution, stability, theoretical plates and peak shape of all three drugs components. Best results were obtained with ortho-phosphoric acid (0.1% v/v) solution to improve the peak shape of Apigenin 7-O-glucoside. Finally, mobile phase composition consisting of a mixture water pH 3 and acetonitrile 50:50 % v/v. Optimized mobile phase proportion

was providing good resolution. For the selection of organic constituent of mobile phase, acetonitrile was chosen to reduce the longer retention time and to attain good peak shape.The acidic pH 3 of double distilled water as one of the solvent of mobile phase was found suitable for solubility, resolution, stability and peak shape of all components.Initial trials were taken using various organic solvents in different proportions. The observations and found are given below.17,18

Trials No.	Mobile phase		Observations
	Organic solvent	Ratio	
1a	Methanol: Water	70:30	Not found suitable due to poor resolution of peak and tailing
1b	Methanol: Water	65:35	Not found suitable peak due to tailing occurred
1c	Methanol: Water	60:40	Did not found sharp resolution may be because of contaminated water or mobile phase composition
2a	Methanol: OPA at pH 2.51	65:35	Not found suitable due to poor resolution of peak and tailing
2b	Methanol: OPA at pH 2.51	60:40	Did not found sharp resolution may be because of improper pH or mobile phase composition
2c	Methanol: OPA at pH 3	70:30	Not found suitable due to tailing of peak
2d	Methanol: OPA at pH 3	65:35	Found better resolution but irregular baseline obtained may be because of mobile phase contaminated or detector cell contaminated
3a	Methanol: Phosphate buffer at pH 3.4,flow rate 1ml/min	25:75	**Apigenin 7-O-glucoside** resolved properly but tailing in peak
3b	Methanol: Phosphate buffer at pH 3.4,flow rate 1ml/min	30:70	Not found suitable due to tailing
3c	Methanol: Phosphate buffer at pH 3.4,flow rate 1ml/min	40:60	Properly resolved peak but showed tailing
3d	Methanol: Phosphate buffer at pH 3.4,flow rate 1ml/min	45:55	Found sharp peak but showed tailing

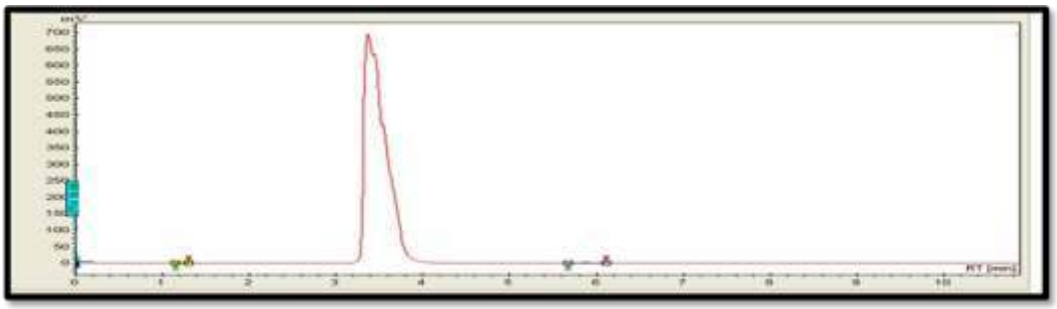

Fig No 4 Chromatogram of Trial no.1a (Methanol: Water with Composition 70:30v/v)

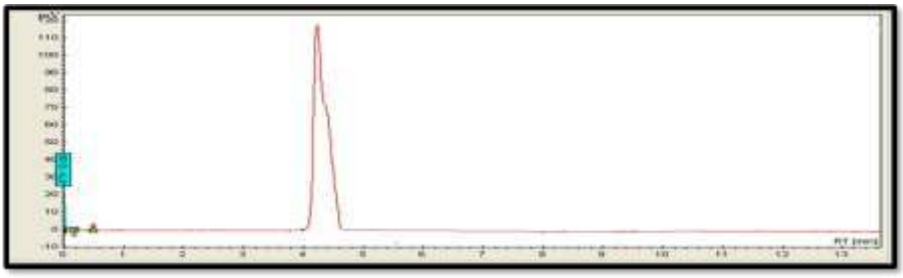

Fig No 5 Chromatogram of Trial 1b (Methanol: Water with composition 65:35v/v)

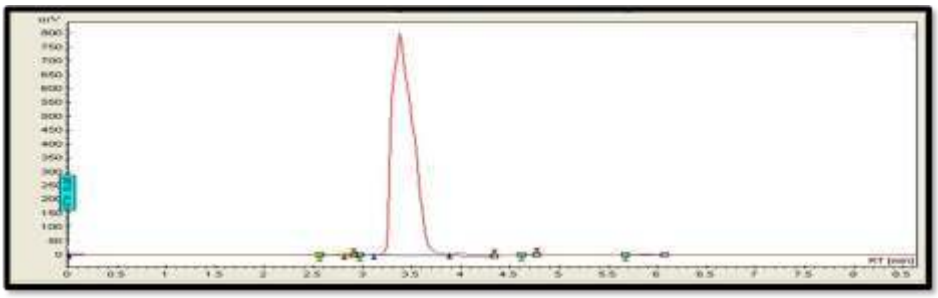

Fig No 6-Chromatogram of Trial no.1c (Methanol: Water with proportion 60:40v/v)

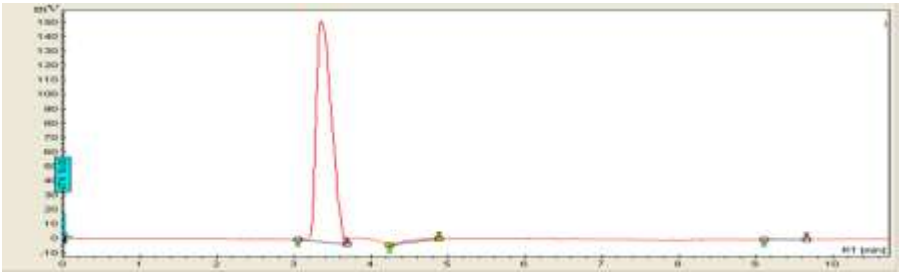

Fig No 7-Chromatogram of Trial no.2a (Methanol: OPA at pH 2.51 with ratio 65: 35v/v

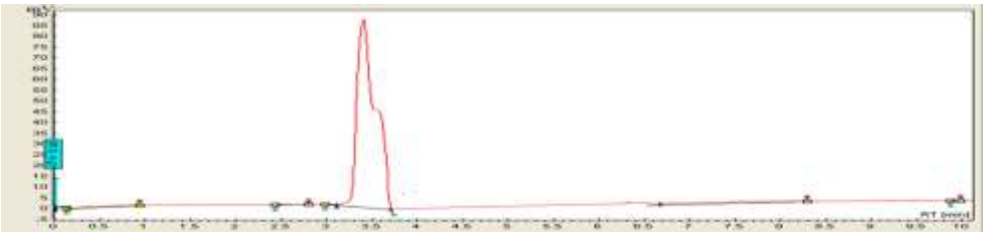

Fig No 8 - Chromatogram of Trial no.2b (Methanol: OPA at pH 2.51 with ratio 60:40v/v)

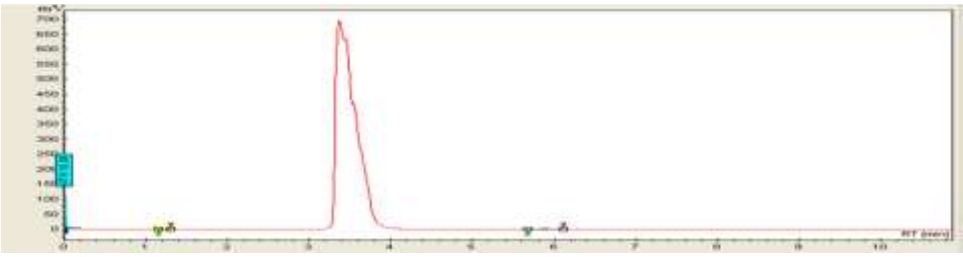

Fig No 9- Chromatogram of Trial no.2c (Methanol: OPA at pH 3 with ratio 70:30v/v)

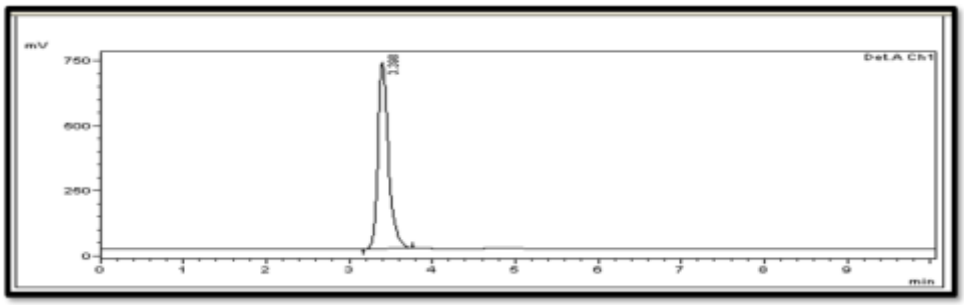

Fig No 10-Chromatogram obtained in Optimized mobile phase (Methanol: Phosphate buffer at pH 3.4 with 45:55v/v proportion with flow rate 1ml/min)

Validation of Drugs (Apigenin 7-O-glucoside.)

a) Linearity and range of standard drugs

Standard solutions ranging from 20-100 µg/ml of Apigenin 7-O-glucoside. was prepared. Concentration plotted on y- axis and peak area plotted on X- axis. The Apigenin 7-O-glucoside. showed good correlation coefficient in concentration range 20-100 µg/ml of 0.999. The linearity plot is drawn in figure Y-intercept and Slope of regression line were found to be 61320.1 and 15.14 of Apigenin 7-O-glucoside.. The correlation coefficient (R2) value for Apigenin 7-O-glucoside. was found to be within acceptable limits. This indicated that the method is linear between the concentrations for 20-100 µg/ml of Apigenin 7-O-glucoside.19,20

Table no.11 concentrations

Sr.No.	Conc. µg/ml	Peak area
1	20	1017570
2	40	2400037
3	60	3514433
4	80	4624224
5	100	6052443

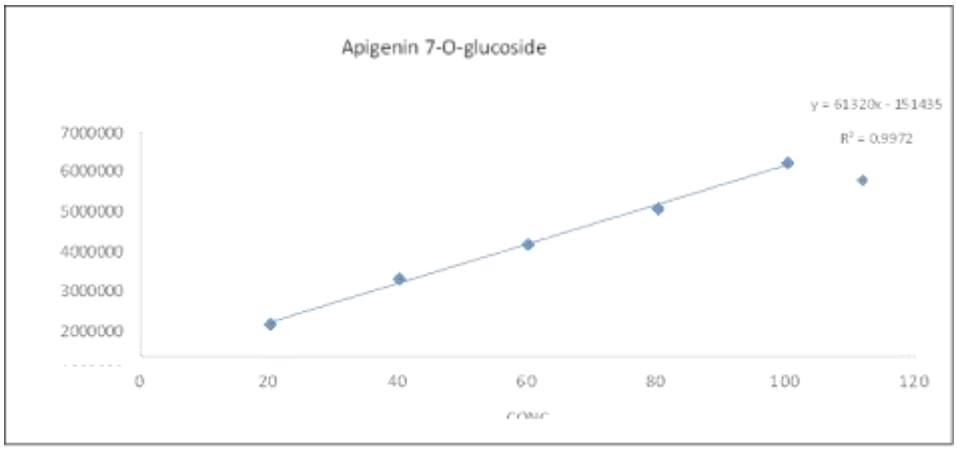

Fig No.-11 Concentration Apigenin 7-O-Glucoside

Range was inferred from the data of accuracy and linearity that the established range for the estimation of Apigenin 7-O-glucoside was between the concentration ranges of 20 % to 100

%. Thus, the range was established as 20-100 µg/ml of Apigenin 7-O-glucoside. Study of Linearity and Range

Drug	Range (µg/ml)	Peak Area			Average	± SD	% RSD
Apigenin 7-O-glucoside	20	1017570	1012281	1015281	1015044	2652.4530	0.26
	40	2400037	2410570	2340686	2383764	37676.82	1.58
	60	3514433	3416661	3418136	3449677	56027.74	1.62
	80	4624224	4661646	4397473	4561114	14294.4	0.31
	100	6052443	6067808	6090012	6070088	18887.96	0.31

1) **System suitability parameter**

From table 5 all the system suitability parameters are within the acceptable range indicating the suitability of the method for estimation of Apigenin 7-O-glucoside 21

Table No. System Suitability Parameters

Sr. No	Parameter	Observed values	Recommended value
		Apigenin 7-O-glucoside	
1	Retention time(min)	3.31	
2	Tailing factor	1.4	≤ 2
3	Capacity factor	1.041	> 1
4	Resolution	0.00	> 2
5	Theoretical plates	11313	>2000
6	% RSD (The retention time)	1.07	≤ 2

2) Limit of Detection & Limit of Quantification-

Lower LOD and LOQ value indicates high sensitivity of the method. The limit of detection (LOD) and limit of quantitation (LOQ) were determined by calculating the signal to noise (S/N) ratio of the LOD preparation and LOQ preparation. The Limit of detection (LOD) and Limit of Quantitation (LOQ) were evaluated from calibration curve of linearity data.22,24

Table No.14 Result of LOD and LOQ.

Drug	Conc µg/ml	Peak area (mv.min)				SD *	Slope	LO D Pp m	LOQ Ppm
SFZ		Replicate 1	Replicate 2	Replicate 3	Mean of peak area		6149 7.1	0.7 3	2.21
	20	1017570	1012281	1015281	1015044	2652.4			
	40	2400037	2410570	2340686	2383764.3	37676.8			
	60	3514433	3416661	3418136	3449743.3	56027.7			
	80	4624224	4661646	4397473	4561114.3	14294.42			
	100	6052443	6067808	6090012	6070087.6	18887.9			

3) **Accuracy and analyte Recovery -**

Recovery studies were conducted at three levels i.e. 80%, 100% and 120% of target concentration. It was carried out by adding known amount of standard drug of sample Apigenin 7-O-glucoside to the pre-analyzed. The mixed sample solutions were analyzed to obtained peak, retention time, and area under curve respectively. The

concentration of Apigenin 7-O-glucoside was calculated from area under curve of peak. At each concentration three were performed and obtained were compared with standard results. The % RSD of accuracy study was found to be within specific limit and thus we can concluded that the developed method is suitable for assay and there is no interference of excipients.25 The recovery data from the study were reported in Table:
Spiked Concentration

Table No.15 Accuracy study of Apigenin 7-O-glucoside

Spiked Concentration (µg/ml)	Mean , ±SD, % RSD	% recovery
80	79.97±0.41, 0.51	99.87
100	100.74±1.10,1.09	99.16
120	120.17±0.69, 0.58	100.53

4) Precision-

Method precision was demonstrated by preparing six test solutions at 100% concentration as per the test procedure & recording the chromatograms of six test solutions. The % RSD of six samples was calculated. Intermediate precision of the analytical method was determined by performing method precision on another day by different analysts under same experimental condition. The % RSD values were found to be within specified limit (<2%), which indicates that the developed method is precise.26,27 The results 20 µg/ml of standard drug solution (Apigenin 7-O-glucoside) was repeated for five times and the peak area was determined and was found to be consistent. The result are shown in Table no.

Table No.16 Repeatability data of Apigenin 7-O-glucoside

Sr. No.	Concentration	Peak area
		Apigenin 7-O-glucoside
1	20 µg/ml	1017169
2	20 µg/ml	1012460
3	20 µg/ml	1015965
4	20 µg/ml	1013815
5	20 µg/ml	1014301
Mean		1014742
SD		1847.11
% RSD		0.18

Intraday precision-

The % RSD for retention time and area was found to be 0.18 of Apigenin 7-O-glucoside, % RSD is not more than 2 and is found within the specified limit.28

Table No.17 Results of Intraday precision of Apigenin 7-O-glucoside

Injection No.	Apigenin 7-O-glucoside	
	Retention time	Area
1	3.39	1017150
2	3.31	1012510
3	3.31	1015965

4	3.37	1013825
5	3.34	1014308
Mean	3.344	1014751.6
SD	0.035	1823.78
% RSD	1.07	0.18

Inter day precision

The % RSD for retention time was found to be 0.15 of Apigenin 7-O-glucoside which is within specified limit of % RSD not more than 2

Injection No.	Apigenin 7-O-glucoside	
	Retention time	Area
1	3.39	1016134
2	3.31	1012505
3	3.31	1015960
4	3.37	1013825
5	3.34	1014311
Mean	3.344	1014547
SD	0.035	1521.66
% RSD	1.07	0.15

Result of Interday precision of Apigenin 7-O-glucoside Robustness-

Deliberation variations were made in the method and % RSD was calculated plus and minus flow rate and mobile phase ratios were the variations that were analyzed. Small but deliberate variations were made in the method parameters and it was found that % RSD values for all the variations were in acceptable limits. This indicated that the method is robust and it can be used within small variations of flow rate and mobile phase, without having measure effect on the result.29

Table No.19 Result of Robustness of Apigenin 7-O-glucoside

Parameter	Apigenin 7-O-glucoside
Flow rate	Retention time
0.8	3.91
1.2	3.64
Mean±SD	3.775±0.19
Mobile phase (Organic : aqueous)	Retention time
45:55	3.45
47:53	3.80
Mean±SD	3.625±0.24

Ruggedness-

Result obtained by different analyst and different day were found within acceptable limit, which shows that test method is rugged.

Table No.20 Result of Ruggedness of Apigenin 7-O-glucoside

Injection No.	Analyst I	Analyst II
	Area	Area
1	2400079	2400580
2	2400086	2400193
Average	2400082.5	2400386.5
SD	4.949747	273.6503
% RSD	0.00	0.01

Stability indicating assay methods-

In order to determine whether the analytical method or assay were stability- indicating, Apigenin 7-O-glucoside were stressed under various conditions to conduct forced degradation studies. The development & validation of stability indicating assay method was carried out as per ICH guidelines ICH Q2A, Q2B, Q3B. Unfortunately, the current guidelines do not indicate detailed degradation conditions in stress testing. However, the used forced degradation conditions, stress agent concentration and time of stress, were found to show degradation of compounds which was within acceptable limit.

2) Acidic condition

Table No.21 Result of acidic degradation

Sr. No.	Drug Name	Degraded retention time
1	Apigenin 7-O-glucoside	5.570

3) Alkaline Condition-

Alkaline degradation study was performed by heating the drug content in 1 N NaOH at 80 °C for 1 hour and mixture was neutralized with 1 N HCl solutions.

Fig.No.19 Degradation of drugs by alkaline condition

Table No.22 Result of Alkaline degradation

Sr.No.	Drug name	Retention time of Degradation
1	Apigenin 7-O-glucoside	4.658

4) Neutral condition –

The neutral degradation study was carried out in room temperature for 1 hour with water.

Fig. No. 20 Degradation of drugs by neutral condition

Table No.23 Result of Neutral degradation

Sr. No.	Drug name	Retention time of Degradation
1	Apigenin 7-O-glucoside	4.838

5) Oxidative condition

Oxidation degradation study was performed by heating the drug content it's in initial

concentration carried out in 1% v/v hydrogen peroxide no significant changes then will increase into 6 % v/v hydrogen peroxide at 80 °C for 1 hours.

Fig .No. 31 Degradation of drugs by Oxidative condition

Table No.21 Result of oxidative degradation.

Sr. No.	Drug name	Retention time of Degradation
1	Apigenin 7-O-glucoside	4.899

6) Thermal condition

Thermal degraded samples wherever degradation possible from about 1% to 30%.Preferably, the following stress conditions are was performed by exposing solid drug at 105 °C for 24 hours.

Sr.No.	Drug name	Retention time of Degradation
1	Apigenin 7-O-glucoside	4.858

7) Photolytic condition

Drug content was found to be more stable than other stress condition stable in UV-light. Apigenin 7-O-glucoside is stable under UV light.

Fig .No. Degradation of drugs by photolysis condition

Table No.26 Result of photolytic condition

Sr.No.	Drug name	Retention time of Degradation
1	Apigenin 7-O-glucoside	8.854
		9.857

Forced degradation study was performed by subjecting the analytes to acid, base, neutral hydrolysis, hydrogen peroxide mediated hydrolysis and photolytic conditions. The results of Stability Indicating Assay Method (percent degradation, peak area of degradation) of degradation studies are summarized.

The percentage degradation of Apigenin 7-O-glucoside is more in hydrogen peroxide mediated oxidative degradation condition and less in thermal hydrolysis. of the method was demonstrated as no degradation products from different stress conditions affected the detection and quantification of Apigenin 7-O-glucoside.The acidic treated sample with various trace conditions had difference which was within acceptable limit. Thus the degradation amount was not exceeding the limit which indicates that method is stable even in all stress conditions of acid, base, neutral, peroxide, thermal, & photolysis.30

Table No.27 Result of Stability indicating assay methods.

Types of Degradation	Peak area % of Degradation		% of Degradation
Apigenin 7-O-glucoside			
Acidic	6990	-	4.58
Base	19507	-	9.78
Neutral	2794	3772	17.21
Oxidative	3199	-	2.20
Photo	8102	1274	8.70
Thermal	3298	5124	8.71
Acidic	6990	-	4.58

CONCLUSION

In this present work RP-HPLC method development and validation as per regulatory requirements along with Stability Indicating Assay Methods is discussed.

HPLC method is available for simultaneous determination of Apigenin 7-O-glucoside in various plant like Chameli, Night Jasmine and various other plants but , No RP-HPLC method is available for simultaneous determination of Apigenin 7-O-glucoside from the flower part of echinopus echinatus roxob plant Similarly no studies have been carried out on Stability Indicating Assay methods for simultaneous determination of Apigenin 7-O-glucoside. This constituent is used as Anti-inflammatory, antioxidant, and anti-cancer agents. Hence this combination is selected for simultaneous estimation using HPLC method and Stability indicating assay method. HPLC remains the method of choice because it gives more sensitive results along with greater accuracy. It can be used both for qualitative as well as quantitative analysis. Isocratic method involves increase in solvent strength with time. This method is chosen since a sample containing compounds of a wide range of polarities can be separated by a isocratic elution in a shorter time period without a loss of resolution in the earlier peaks or excessive broadening of later peaks. Force degradation studies on Apigenin 7-O-glucoside were carried at different stress conditions like acid, base, oxidation, thermal, Photolysis degradation, to check the stability of the drugs. The percentage degradation of Apigenin 7-O-glucoside is more in hydrogen peroxide mediated oxidative degradation and less in thermal hydrolysis. The Apigenin 7-O-glucosid is degraded more in acid hydrolysis and less in photolytic condition. The stability indicating nature and specificity of the method was demonstrated as no degradation products from different stress conditions affected the detection and quantification of Apigenin 7-O-glucosid. The method was found to be stability indicating from the forced degradation studies which was found to be within limit and thus can be used for the simultaneous estimation of Apigenin 7-O-glucoside . The RP-HPLC method was validated and found to be specific, linear, accurate, precise and reproducible. Thus it is concluded that the developed method can be thus successfully applied to analyze various formulations containing Apigenin 7-O-glucoside

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICTS OF INTERESTS

Declare none

REFERENCES

1. ICH Q2(R1). Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonisation, 2005.
2. Snyder, L. R., Kirkland, J. J., & Dolan, J. W. Introduction to Modern Liquid Chromatography. 3rd Edition, John Wiley & Sons, 2010.
3. Harborne, J. B., & Williams, C. A. Advances in Flavonoid Research since 1992. Phytochemistry, 2000; 55(6):481–504.
4. Hostettmann, K., Marston, A., Wolfender, J. L., & Terreaux, C. The Potential of Natural Products as a Source of New Drugs. Chimia, 1995; 49(6):312–320.
5. Seidel, V. Chlorophylls and Flavonoids: Analytical Approaches in Plant Extracts. Journal of Chromatography A, 2006; 1112(1):1–13.
6. Bakowska-Barczak, A. M., & Kolodziejczyk, P. Flavonoids in Health and Disease. Phytochemistry Reviews, 2011; 10:453–471.
7. Nayak, B. S., & Kothiyal, P. Stability-Indicating HPLC Method Development for Apigenin and Its Glycosides. Journal of Pharmaceutical Analysis, 2014; 4(6): 389–396.
8. Reddy, L. H., Arias, J. L., Nicolas, J., & Couvreur, P. Magnetic Nanoparticles: Design and Characterization, Toxicity and Biocompatibility, Pharmaceutical Applications. Chemical Reviews, 2012; 112(11):5818–5878.
9. Devi, R., & Rajalakshmi, M. RP-HPLC Method Development and Validation for Flavonoid Glycosides in Plant Extracts. International Journal of Pharmaceutical Sciences and Research, 2013; 4(5):1820–1825.
10. • Patel, R., & Patel, D. Forced Degradation Studies of Flavonoid Compounds Using HPLC. Journal of Chromatographic Science, 2012; 50(10): 841–848.
11. ICH Q2(R1). Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonisation, 2005.
12. Snyder, L. R., Kirkland, J. J., & Dolan, J. W. Introduction to Modern Liquid Chromatography. 3rd Edition, John Wiley & Sons, 2010.
13. Harborne, J. B., & Williams, C. A. Advances in Flavonoid Research since 1992. Phytochemistry, 2000; 55(6):481–504.
14. Bakowska-Barczak, A. M., & Kolodziejczyk, P. Flavonoids in Health and Disease. Phytochemistry Reviews, 2011; 10:453–471.
15. Nayak, B. S., & Kothiyal, P. Stability-Indicating HPLC Method Development for Apigenin and Its Glycosides. Journal of Pharmaceutical Analysis, 2014; 4(6):389–396.
16. Devi, R., & Rajalakshmi, M. RP-HPLC Method Development and Validation for Flavonoid Glycosides in Plant Extracts. International Journal of Pharmaceutical Sciences and Research, 2013; 4(5):1820–1825.
17. Patel, R., & Patel, D. Forced Degradation Studies of Flavonoid Compounds Using HPLC. Journal of Chromatographic Science, 2012; 50(10):841–848.
18. Shinde, P., et al. HPLC Analysis and Method Validation of Apigenin Glycosides in Herbal

Extracts. Asian Journal of Chemistry, 2016; 28(12):2675–2680.

19. Reddy, L. H., Arias, J. L., Nicolas, J., & Couvreur, P. Magnetic Nanoparticles: Design and Characterization, Toxicity and Biocompatibility, Pharmaceutical Applications. Chemical Reviews, 2012; 112(11):5818–5878.
20. Kumar, S., & Singh, B. RP-HPLC Method Development for Apigenin-7-O-Glucoside in Plant Matrices. Journal of Pharmaceutical and Biomedical Analysis, 2015; 114:171–177.
21. Kokate, C. K., Purohit, A. P., & Gokhale, S. B. Pharmacognosy. 49th Edition, Nirali Prakashan, 2020.
22. Hostettmann, K., Marston, A., Wolfender, J. L., & Terreaux, C. The Potential of Natural Products as a Source of New Drugs. Chimia, 1995; 49(6):312–320.
23. Seidel, V. Chlorophylls and Flavonoids: Analytical Approaches in Plant Extracts. Journal of Chromatography A, 2006; 1112(1):1–13.
24. Cacig, L., & Molnar, A. Stability-Indicating Methods for Natural Flavonoids: A Review. Journal of Pharmaceutical Analysis, 2017; 7:299–312.
25. Abdel-Hamid, H., et al. RP-HPLC Determination of Flavonoid Glycosides in Herbal Extracts: Method Development and Validation. Analytical Letters, 2013; 46(8):1234–1245.
26. Xu, Z., & Chen, Y. Chromatographic Analysis of Apigenin Glycosides in Medicinal Plants. Phytochemical Analysis, 2014; 25(6):542–549.
27. Singh, P., et al. Forced Degradation Studies of Flavonoids by HPLC: Application to Herbal Extracts. Journal of AOAC International, 2015; 98(3):740–748.
28. Ghasemzadeh, A., et al. Flavonoid Profiling of Medicinal Plants Using HPLC–UV and HPLC–MS Techniques. Journal of Food and Drug Analysis, 2017; 25(2):484–493.
29. Dey, S., & Bhattacharya, S. Analytical Method Development for Quantification of Apigenin-7-O-Glucoside in Plant Extracts Using RP-HPLC. Journal of Liquid Chromatography & Related Technologies, 2016; 39(10):463–471.
30. Arora, D., et al. Validated RP-HPLC Method for Determination of Flavonoids in Traditional Medicinal Plants. Phytochemistry Letters, 2018; 25:45–52.

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