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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ASSAY OF DIOSMIN AND HESPERIDIN IN COMBINED TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

A *rapid, simple, precise and accurate reverse phase high performance liquid chromatography (RP-HPLC) strategy has been developed and validated for the simultaneous estimation of Diosmin and Hesperidin in pharmaceutical formulations. Separation of both Diosmin and Hesperidin was accomplished inside 10 min with required resolution. Chromatographic partition was accomplished on a stainless steel section ODS-3(100×4.6mm) 3µ utilizing a portable stage comprising of blend of Methanol and Water in the proportion of 40:60 (v/v) at a flow rate of 1.0 ml/min. The discovery was made at 280 nm and the retention time of Hesperidin and Diosmin were 6.6 and 9.4 minutes individually. The developed analytical method was approved according to ICH rules. The proposed method is reproducible, robust, specific and appropriate for the concurrent quantitative examination of Diosmin and Hesperidin in combined tablet dosage forms.*

KEYWORDS: *High Performance Liquid Chromatography, Diosmin, Hesperidin.*

INTRODUCTION

Flavonoids are a gathering of polyphenolic mixes with medical advantage properties. They are intense cancer prevention agents, free radical foragers [1] and metal chelators; they hinder lipid peroxidation [2] and display different physiological exercises [3], including calming, hostile to unfavorably susceptible, against cancer-causing, antihypertensive and against joint exercises [4]. Because of the significance of flavonoids are donors of gainful wellbeing impacts of citrus organic product, assurance of such mixes happening in citrus natural products assume a critical part in numerous zones of science. Lime juice is portrayed by the nearness of huge amounts of the flavanones, Hesperidin and eriocitrin. Lime juice is likewise very rich in flavones: Diosmin has been perceived as one of the primary flavonoid segments of this juice [5]. A few example planning strategies, for example, hydrolysis, filtration/weakening, fluid extraction, ultrasound-assistedextraction [6] and strong stage extraction utilizing molecularly engraved polymers were created to permit HPLC-based assurance. Switch stage superior fluid chromatography joined with various finders is the usually utilized investigative technique for partition and distinguishing proof of flavonoids [7, 8].

Diosmin is artificially 5-Hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R, 6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl] oxychromen-4-one), (mol wt. 608.545) and utilized as slender balancing out specialist and antihemorrhoidal agent [9]. Hesperidin is synthetically (2S)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-[(2S,3R,4S,5S,6R)-3, 4,5-trihydroxy-6-[[(2R,3R,4R,5R,6S)-3,4,trihydroxy-6-methyloxan-2-yl] oxymethyl]oxan-2-yl]oxy-2,3-dihydrochromen-4-one, (mol wt. 610.57) and it is additionally utilized as slender balancing out specialist and anti hemorrhoidal operator. The goal of present investigation was to create and approve another, sensitive, accurate, exact, rough, profoundly particular and framework appropriate HPLC strategy for the concurrent estimation of Diosmin and Hesperidin in its joined measurements form [10-14].

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MATERIAL AND METHODS

Instrumentation:

SHIMADZU LC 2010/75 arrangement HPLC framework, furnished with auto-sampler, fitted with a 10 µl circle and PDA locator was utilized. The yield flag was observed and handled utilizing an Empower Software. The chromatographic segment utilized was a 100 mm x 4.6 mm, ODS-3 with 3µm particles.

Reagents and chemicals:

Diosmin and Hesperidin API's. Every one of the solvents were of HPLC grade, and different chemicals utilized were of analytical reagent (AR) review.

Chromatographic conditions:

The isocratic versatile stage comprised of blend of methanol : water (40:60 v/v) was circled through a stainless steel expository column at flow rate of 1.0 mL min-1. The factors PDA locator was set at 280 nm. Every one of the investigations was performed at column temperature was 30°C, and the volume of preparation injected into the column was 10µL.

Standard stock solutions:

Stock solutions of Diosmin and Hesperidin were prepared independently at concentrations of 2 mg/ml, utilizing 70% dimethylsulfoxide in methanol. The working standard was set up from above stock arrangements by diluting 6ml into 50mL with 30% dimethylsulfoxide in methanol.

Method Validation:

The proposed HPLC strategy was approved by the rules of International Conference on harmonization (ICH).

Specificity:

Spiked samples of the known concentrations were prepared at 1% determination level and infused. % difference between mean of two infusions of control test and of spiked sample was calculated. The % difference between the control test to spiked specimen ought not be more than 1%.There ought not be any interference of placebo on retention times of main peaks.

Linearity:

Linearity was studied by preparing standard solutions at six concentration levels from 25 to 150% of the objective analyte concentrations for Diosmin and Hesperidin individually. A least square fit diagram of the individual region counts against the concentration of Diosmin and Hesperidin was plotted and the correlation coefficient, slope and intercept reported.

Precision:

The closeness of two or more measurements to each other under the endorsed conditions. i.e. intraday and inter day precisions. The % RSD of six replicate injections of standard ought not to be more than 2.0 %.

Accuracy:

The closeness of agreement between the measured values to standard or known value. The %recovery was performed by spiking the APIs of the both drugs into the equivalent weight of placebo at 50%, 100% and 150% level. These solutions arranged in triplicate and injected them in duplicate. The mean recovery ought to be in the scope of 98% to102 %.

Robustness:

The robustness of the technique was dictated by little changes in the factors, for example, stream/flow rate rate (1.0± 0.1 mL min-1), column temperature (30°C± 5°C) and wavelength changes (280±2nm).The reliability of the method during the normal usage was checked by robustness.

Assay of Tablets:

Transfer 5 tablets into a clean, dry 250mL volumetric flask, add 70% DMSO in Methanol. Furthermore, blend the specimen on attractive stirrer plate with appropriate attractive bar for 45 minutes to disperse the tablets. Cool the solution to room temperature. Dilute to volume with 70% DMSO in Methanol and mix. Centrifuge the solution at 5000RPM for 10minutes.Dilute 3mL of clear supernatant solution to 100mL with 30% DMSO in Methanol and mix. Channel the solution through 0.45µ membrane filter. A reasonable volume (normally 10µl) of this solution was injected onto HPLC column, concentration of Diosmin and Hesperidin were obtained from the preconstructed calibration chart.

RESULTS AND DISCUSSION

Optimisation of chromatographic conditions:

In this paper we described a HPLC technique for simultaneous estimation of Diosmin and Hesperidin in tablets. Among various isocratic mobile phases examined, the utilization of an isocratic mobile phase of 40:60 (v/v) Methanol:Water was chosen on the premise of simplicity, better column recovery, and proper system suitability (hypothetical plate number, Tailing factor, Resolution, Capacity factor, k, and repeatability) for the analyte peak which consented to limits stipulated (table 1).

Method Validation:

The developed method was validated according to ICH guidelines regarding specificity, linearity, accuracy, precision and robustness.

Specificity:

Blank, Placebo and Standard working solutions were injected and compared, there is no peak found in the blank and placebo at the retention times times of these drugs, so the developed technique was specific and don't have any interference.

Linearity:

The information or data was subjected to statistical analysis utilizing a linear-regression model; regression equation and correlation coefficient were given in table 2. The outcomes have demonstrated good and having a broader range. The typical simultaneous chromatogram brought about a sharp, symmetrical, and well resolved peak at RT value of 6.606 min and 9.5 min for Hesperidin and Diosmin individually (fig.2, 3).

Precision:

The intraday precision (Repeatability) and inter day precision (Ruggedness) were performed and the %RSD was below 2%. The % RSD of 6 replicate injections of Diosmin & Hesperidin was shown at table (3).

Accuracy:

The % recoveries at 50%, 100%, 150% level focus demonstrated % mean recovery as 100.30 for Diosmin and 100.45 for Hesperidin. The outcomes appeared at table 4.

Robustness:

It demonstrated that their is no significant change in the retention time of analytes of Diosmin and Hespiridin by changing the flow rate (1.0± 0.10 mL min-1) and column temperature(30°±5°C). The % RSD estimations of retention time (tR, min), column temperature and wavelength changes were shown at table 5.

Assay of Tablets:

The developed and validated RP-HPLC technique was applied for the assay of Diosmin and Hesperidin in combined tablet dosage forms. Results were discovered as mean % recovery 100.3 and 100.2% for Diosmin and Hesperidin in tablets separately. The outcomes are appeared in table (6). It shows the effective use of developed method for the simultaneous determination of active substances.

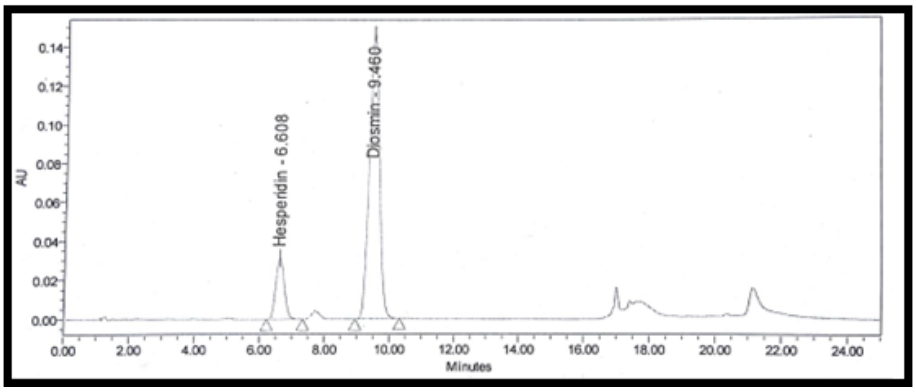

Fig. 1: Typical chromatogram of Diosmin and Hesperidin

Table No. 1: System suitability

S. No	Name	Retention time	Area (µV*sec)	EP Plate count	Tailing factor	Resolution
1.	Hesperidin	6.608	531768	2944	1.11	
2.	Diosmin	9.460	3139819	4332	1.27	5.28

Table No. 2: Linearity table of Diosmin and Hesperidin

% Level	Diosmin		Hesperidin	
	Concentration	Area	Concentration	Area
0	0	0	0	0
25	67.90	779295	7.52	132968
50	135.80	1535534	15.05	265067
75	203.71	2340858	22.57	399347
100	271.61	3081394	30.10	529318
125	339.51	3860045	37.62	664095
150	407.41	4645702	45.14	792772

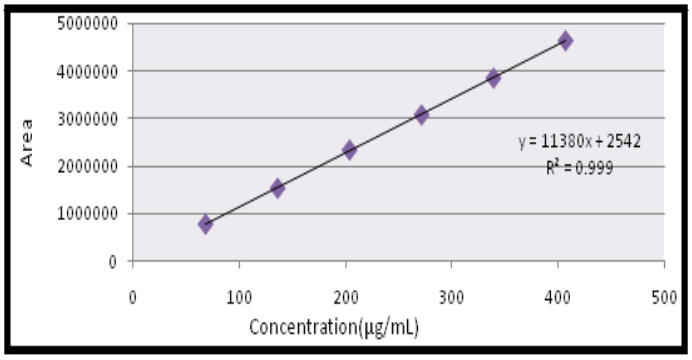

Fig. 2: Linearity plot of Diosmin

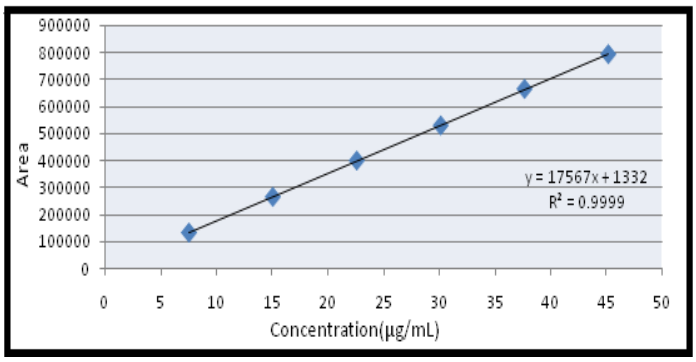

Fig. 3: Linearity plot of Hesperidin

Table No. 3: Precision table of Diosmin and Hesperidin

S. No.	Intraday Precision		Interday Precision	
	Diosmin	Hesperidin	Diosmin	Hesperidin
1	3187242	573729	3186108	572657
2	3209945	577546	3211713	577833
3	3207324	576626	3209225	576987
4	3218116	579919	3218825	579892
5	3168763	569585	3168120	569603
6	3229012	580864	3228046	580156
Mean	3203400	576378.2	3203673	576188
Std. Dev	8671.47	134.181	6887.393	0.017
%RSD	0.27	0.023	0.214	0.01

Table No. 4: Accuracy table of Diosmin and Hesperidin

%Level	Diosmin			Hesperidin		
	%Recovery	Mean % Recovery	% RSD	% Recovery	Mean % Recovery	% RSD
50%	100.78	100.56	0.12	100.75	100.68	0.07
	100.56			100.60		
	100.35			100.70		
100%	100.27	100.29	0.05	100.45	100.45	0.09
	100.35			100.55		
	100.25			100.37		
150%	100.05	100.08	0.09	100.45	100.47	0.05
	100.20			100.54		
	100.01			100.44		

Table No. 5: Robustness table of Diosmin and Hesperidin

S. No.	Robustness Parameters	%RSD	
		Diosmin	Hesperidin
1	Flow rate minus(0.9mL)	0.10	0.10
2	Flow rate plus(1.1mL)	0.11	0.12
3	Column temperature minus(25°(	0.10	0.20
4	Column temperature plus(30°C)	0.10	0.12
5	Wavelength minus(278nm)	0.21	0.20
6	Wavelength plus(282nm)	0.30	0.25

Table No. 6: Assay of tablets

S. No.	Diosmin	Hesperidin
1	100.30	100.90
2	99.65	101.10
3	99.85	100.80
4	100.45	100.60
5	100.79	101.00
6	100.80	101.10
Mean	100.30	100.90
Std. Dev	0.132065	0.194079
% RSD	0.17	0.20

CONCLUSION

This technique was revealed by the validation information, empowers accurate, specific, reproducible, robust and precise concurrent estimation of Diosmin and Hesperidin in pharmaceutical dosage forms. The technique was sufficiently delicate for quantitative estimation of the analytes in pharmaceutical preparations and would thus be able to be utilized for routine examination and quality control.

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