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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

The combination of Diosmin and Hesperidin can be used as capillary stabilizing and antihemorrhoidal agent. A rapid, simple, precise and cost effective and reversed-phase high performance liquid chromatography (RP-HPLC) method has been developed and validated for the simultaneous determination of Diosmin and Hesperidin in pharmaceutical formulations. Separation of both Diosmin and Hesperidin was achieved within 8 min with required resolution. Chromatographic separation was achieved on a waters symmetry C18 (100mm x 4.6mm) 2.6µm using a mobile phase consisting of 0.1% ortho phosphoric acid and methanol in the ratio of 60:40 at a flow rate of 1.0 ml/min. The detection was made 280 nm at nm and the retention time of Diosmin and Hesperidin were 6.049 and 8.560 minutes respectively.

Key-Words: High performance liquid chromatography, Diosmin, Hesperidin

Introduction

Flavonoids are a group of polyphenolic compounds with healthrelated properties. They are potent antioxidants, free radical scavengers [1] and metal chelators; they inhibit lipid peroxidation [2] and exhibit various physiological activities [3, 4], including anti inflammatory, anti-allergic, anti-carcinogenic, antihypertensive and anti-arthritic activities [5]. Due to the importance of flavonoids as contributors of beneficial health effects of citrus fruit, determination of such compounds occurring in citrus fruits play an important role in many areas of science. Lime juice is characterized by the presence of significant amounts of the flavanones, hesperidin and eriocitrin. Lime juice is also quite rich in flavones: diosmin has been recognized as one of the main flavonoid components of this juice [6]. Several sample preparation techniques such as hydrolysis [7], filtration/dilution [8], liquid extraction [9], ultrasound-assistedextraction [10] and solid phase extraction using molecularly imprinted polymers [11] were developed to allow HPLC-based determination. Reverse-phase high-performance liquid chromatography combined with different detectors is the commonly used analytical method for separation and identification of flavonoids [12–18].

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Diosmin is chemically 5-Hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7- [(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-[[(2*R*,3*R*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one), (mol wt. 608.545) and used as capillary stabilizing agent and antihemorrhoidal agent. Hesperidin is chemically (2*S*)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-[[(2*R*,3*R*,4*R*,5*R*,6*S*)-3,4,trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxy-2,3-dihydrochromen-4-one, (mol wt. 610.57) and it is also used as capillary stabilizing agent and antihemorrhoidal agent. The objective of present study was to develop and validate a new, sensitive, stable, accurate, precise, rugged , highly specific and system suitable HPLC method for the simultaneous estimation of Diosmin and Hesperidin in its combined dosage form.

Material and Methods

Chemicals and stock solutions

Diosmin and d hesperidin were purchased from Aldrich Chemical Company and were checked for purity by determining their physical constants and by spectral and HPLC analyses. All solvents were of the HPLC grade and were purchased from Merck (Germany).

Instrument and operating conditions

All chromatographic analyses were performed using a Waters HPLC system (Waters AssociateInc.), equipped with: Waters 515 HPLC pump attached to a Model 680

Automated Gradient Controller; a Waters 2487 Dual λ Absorbance detector; a Waters 746 data module and Rheodyne 7725*i* injector.

Sample preparation

Tablet powder (680 mg) equivalent to 450mg of diosmin in 250 ml volumetric flask add 50ml of 0.5M NaOH and sonicate for 15mins. Keep the sample to attain RT and then make up the volume with diluent (0.01M Trisodium buffer pH 12.4 :MeOH.)(60:40) further dilute 25ml to 100ml with diluent (0.01M Trisodium buffer pH 12.4: MeOH.)(60:40).

Results and Discussion

The column efficiency for Hesperidin peak is not less than 4000 theoretical plates and Diosmin peak is not less than 8000. The USP tailing factor of Hesperidin peak is not more than 1.5 and for Diosmin peak is not more than 1.8. The % relative standard deviation of area counts for five replicate injections is not more than 2.0 % for Hesperidine and Diosmin peak. The retention time of Hesperidin is about 6 min and 8.5 min for Diosmin peak.

Specificity

Spiked samples of the known impurities was prepared at 1% specification level and injected. % difference between mean of two injections of control sample and of spiked sample was calculated. The % difference between the control sample to spiked sample should not be more than 1%.There should not be any interference of placebo on retention of main peak. Purity angle must be low than purity threshold for main peaks.

Linearity

The ability of the assay (within a given range) to obtain test results which are directly proportional to the concentration/amount of the analyte. Series of combined standard preparations of Diosmin and Hesperidin reference standards were prepared over a range of 70% to 130%; from the proposed standard concentration. A least square fit graph of the individual area counts against the concentration of Diosmin and Hesperidin was plotted and the correlation coefficient, slope and intercept reported..

The method was found to linear for Diosmin and Hesperidin over the concentration range of 26µg/ml to 80µg/ml for Hesperidin and 229 µg/ml to 582µg/ml for Diosmin with a correlation coefficient greater than 0.999.

Precision

The closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. % RSD of six replicate injection of standard should not be more than 2.0 %. The % RSD of 6 replicate injections was found to be 0.18 and 0.10 for Diosmin & Hesperidin respectively.

Accuracy

The closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found. The recovery experiment was performed by spiking the APIs of both drugs into the equivalent weight of placebo at 80%, 100% & 120% level. These solutions prepared in triplicate and injected them in duplicate. The mean recovery should be in the range of 98% to 102 %. The %recoveries at 80%, 100%, 120% level concentration showed % mean recovery as 99.64 for Diosmin and 99.15 for Hesperidin.

Stability

Sample solution prepared and observed at different time intervals till 24 hrs. The absolute difference between area count of nth time point injection and the area counts of initial time point injection should be NMT 2% for any two consecutive time points.

The analyte was stable in the diluents solution for 22 hours when stored at room temperature There was no significant change up to 22 hours and cumulative % RSD observed to be 0.28 and 1.85 for Diosmin and Hesperidin respectively.

Force Degradation Studies

Stress degradation study was carried to confirm that during stability study or through its shelf life, any degradation product if found would not interfere with the peaks of Diosmin and Hesperidin. Sample and placebo were separately treated under degradation studies. Hesperidin degraded in range of 10-30% in acid, alkali and oxidation conditions while Diosmin showed less degradation but peak purity passed for every condition supporting the specificity in favor of the method in worst situations.

Conclusion

Since introduction into clinical practice flavanoids are widely accepted to treat cardio venous insufficiency and other hemorrhagic disorders. Diosmin and Hesperidin are widely used bioflavonoid. In order to analyze these drugs simultaneously a sensitive and accurate method was required. These developed in-house analytical methods have been an attempt to move forward in pharmaceutical formulations and dosages forms. The developed analytical methods are simple, accurate, reliable, specific, linear, precise and suitable for the routine quality control and stability-indicating studies of Diosmin and Hesperidin. The reliability of the method confirmed after the validation of the developed method.

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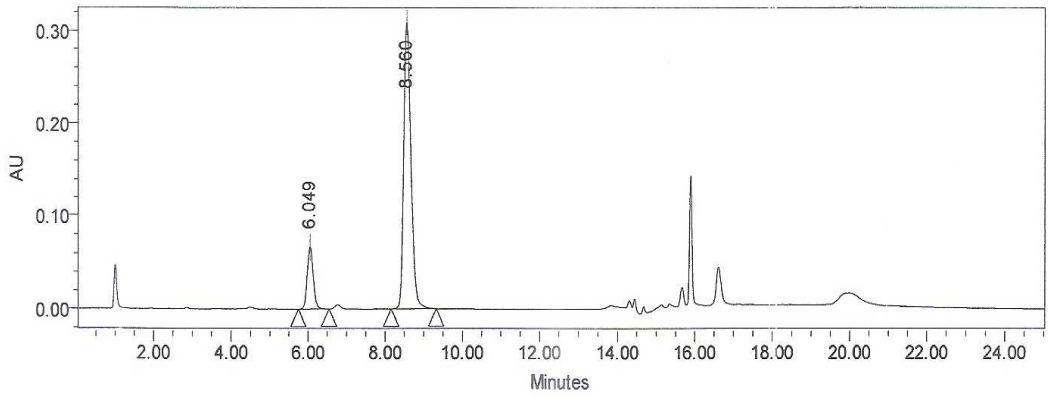

Fig. 1: System suitability

Table 1: System suitability

S.No.	Name	Retention time	Area (µV*sec)	USP Resolution	USP Tailing Factor	USP Plate Count
1	Hesperidin	6.049	723958	-	1.12	7127
2	Diosmin	8.560	3994952	7.93	1.32	10420

Table 2: Linearity plot of Diosmin

	Conc. (µg/ml)			
Linearity between	228.951	582.386	CC	0.99996

Table 3: Linearity plot of Hesperidin

	Conc. (µg/ml)			
Linearity between	26.880	68.186	CC	0.99998

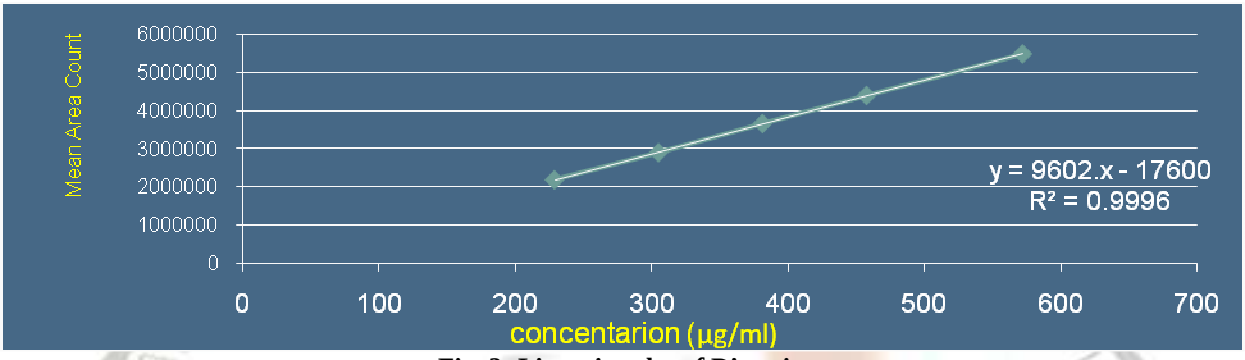

Fig. 2: Linearity plot of Diosmin

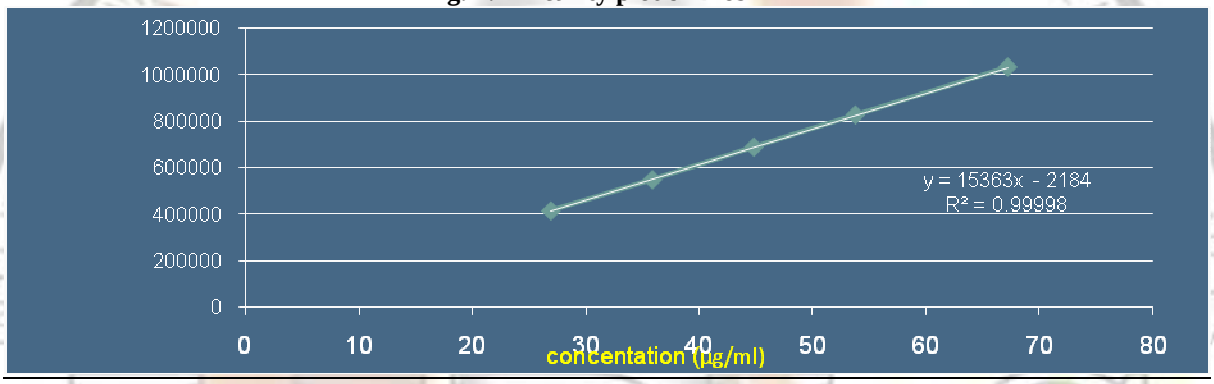

Fig. 3: Linearity plot of Hesperidin

Table 4: Std. area count

Injection	Std Area counts	
	Diosmin	Hesperidin
1	4004988	832162
2	4008295	832633
3	4014580	832608
4	4020288	832186
5	4019538	830848
6	4016831	831896
Mean	4013331	832185
SD	6984	828
%RSD	0.18	0.10

Table 5: Cumulative RSD

Time (min)	Diosmin		Hesperidin	
	Area Counts	Cumulative RSD (%)	Area Counts	Cumulative RSD (%)
0	4054402	-	691196	-
289	4053143	0.03	690016	0.18
484	4053098	0.04	689631	0.21
864	4052685	0.06	686585	0.58
844	4050689	0.13	683532	0.68
984	4049121	0.21	681345	1.11
1292	4043362	0.28	689083	1.85

Table 6: Degradation study of Diosmin

Sample	Area counts of Diosmin	Assay (mg/tab)	% Claim	% Degradation	Purity Angle	Purity Threshold
Acid Degradation (1N HCl/ 5ml/ 60mins/ 80°C/ Neutralised	4366599	456.4	101.4	1	0.238	1.028
Alkali Degradation (1NNaOH/5ml/30min80°C/Neutralisd)	4231830	442.4	98.3	4	0.314	1.038
Peroxide Degradation (0.5ml/ 30%H_2O_2)	4184835	438.5	98.2	5	0.298	1.041
Thermal Degradation (105°C,25.30 hours)	4565264	488.3	106.1	4	0.281	1.040

Table 7: Degradation study of Hesperidin

Sample	Area counts of Hesperidin	Assay (mg/tab)	% Claim	% Degradation	Purity Angle	Purity Threshold
Acid Degradation (1N HCl/ 5ml/ 60mins/ 80°C/ Neutralised)	863533	48.09	51.65	10	0.250	1.299
Alkali Degradation (1NNaOH/5ml/30min 80°C/Neutralisd	809021	43.83	88.5	15	0.364	1.354
Peroxide Degradation (0.5ml/ 30%H_2O_2)	688180	41.88	83.5	19	0.328	1.361
Thermal Degradation (105°C,25.30 hours)	838221	51.65	103.3	1	0.328	1.442