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SIMULTANEOUS DETERMINATION OF HESPERIDIN METHYL CHALCONE, HESPERIDIN AND ASCORBIC ACID IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Three precise, sensitive and selective UV Spectrophotometric methods were developed and validated for the simultaneous estimation of hesperidin methyl chalcone, hesperidin and ascorbic acid in pharmaceutical dosage form. Method A was Simultaneous estimation by solving matrix using Cramer's rule. In this method analytical wavelength selected for estimation of HMC, HP and AA were 348, 298 and 261 nm. The absorptivity values were calculated at each wavelength for each drug and amount of drugs in dosage form was calculated by solving the matrix using cramer's rule. Method B was first derivative zero crossing point. In this method analytical wavelength selected for estimation of HMC, HP and AA were 328.2 nm (ZCP for HP and AA), 340.2 nm (ZCP for HMC and AA) and 283.9 nm (ZCP for HMC and HP) respectively. Method C was double divisor ratio derivative. In this method spectra of ternary mixture was divided by standard spectrum of other two drugs. For estimation of HMC, spectra of ternary mixture was divided by 18 µg/ml HP + 24 µg/ml AA, for HP, spectra of ternary mixture was divided by 9 µg/ml HMC + 36 µg/ml AA and for estimation of AA ternary mixture spectra was divided by 6 µg/ml HMC + 18 µg/ml HP. All developed methods were validated according to ICH guidelines. Precision of all methods were calculated as intraday and interday precision and

reported in terms of % RSD value, which was found to be less than 2% for all three proposed methods. Accuracy of all methods was reported in terms of % recovery, which was found to be in range of 95-105 %. All three developed methods were successfully applied for the simultaneous estimation of hesperidin methyl chalcone, hesperidin and ascorbic acid in pharmaceutical dosage form.

KEYWORDS: Hesperidin, Ascorbic acid, Spectroscopy, Cramer's rule, Double divisor ratio derivative.

INTRODUCTION

Hesperidin (HP; Figure 1) is a flavanone glycoside consisting of the flavone hesperitin bound to the disaccharide rutinose. The sugar cause hesperidin to be more soluble than hesperitin. Hesperidin methyl chalcone (HMC; figure 2) is also a flavone glycoside. Hesperidin and hesperidin methyl chalcone are naturally found in the peels and pulp of citrus fruits like oranges, lemons, grapefruits. These bioflavonoids function synergistically with vitamin C(Ascorbic acid, AA; figure 3) in regard to maintain healthy capillaries, to help to form collagen in connective tissue, to help to heal wounds, and to support a healthy immune system.[1-2]

Tablets (Peridin- C) are used in hot flashes associated with menopause and to improve the capillary strength.

Literature review suggests that methods have been reported for estimation of HMC by HPLC,[3] estimation of HP in human plasma by flourimetry,[4] HPTLC,[5] estimation of HP in combination with diosmin by spectroscopy[6] and HPLC,[7] Stability indicating RP-HPLC method for HP and diosmin in combination,[8] estimation of HP and naringin by HPLC,[9] simultaneous estimation of HP, diosmin and eriocitrin by HPLC,[10] Fast HPLC method for Rutin, truxerutin, diosmin and HP in food supplements.[11] Estimation of AA by HPLC,[12] estimation of AA and calcium pentothanate by RP-HPLC,[13] estimation of Ascorbic Acid and Gallic Acid in Phyllanthus Emblica by HPLC,[14] spectroscopic and RP-HPLC methods for AA in fruit juice and human plasma.[15] spectroscopic estimation of AA with rutin.[16]

The present work aimed to develop three Spectrophotometric methods for simultaneous determination of Hesperidin, Hesperidin methyl chalcone and Ascorbic acid in pharmaceutical dosage form.

MATERIALS AND METHODS

Materials

HP and HMC standard were purchased from Sigma Aldrich. Standard AA was purchased from RFCL limited.

Pharmaceutical dosage form Peridin-C tablets (Beutlich Pharmaceuticals) was labeled as ascorbic acid 200 mg, hesperidin complex 150 mg and hesperidin methyl chalcone 50 mg. Tablets were purchased online from amazon.

Reagents

Methanol (Analytical grade) was used as solvent in all three methods.

Instruments

A shimadzu 1700 double beam UV Visible spectrophotometer connected with UV PROBE 2.1 software was used for all measurements. Absorbance of all solutions were recorded in 1 cm quartz cell over 200-400 nm range. All samples were accurately weighed on electronic analytical balance (A×120, shimadzu).

Standard solutions

HMC standard solution (1000µg/ml): 25 mg of standard HMC was weighed accurately and transferred to 25 ml volumetric flask. 10 ml methanol was added to the flask and the solution was sonicated for 5 minutes. Volume up to the mark was made up with the methanol.

HP Standard solution (1000 µg/ml): 25 mg of standard HP was weighed accurately and transferred to 25 ml volumetric flask. 10 ml methanol was added to the flask and the solution was sonicated for 5 minutes. Volume up to the mark was made up with the methanol.

AA standard solution (1000 µg/ml): 25 mg of standard AA was weighed accurately and transferred to 25 ml volumetric flask. 10 ml methanol was added to the flask and the solution was sonicated for 5 minutes. Volume up to the mark was made up with the methanol.

From the standard solutions of HMC, HP and AA the working standard solutions of 100µg/ml were prepared by diluting 10 ml of each standard solution up to 100ml with methanol.

Spectrophotometric methods

Method A: Simultaneous estimation by solving matrix using Cramer's rule[17]

An absorbance of mixture at each wavelength is the combined absorbance of all the drugs. By applying the Beer-Lambert's equation A=abc where, A= absorbance of sample, a=absorptivity, b=path length and c=concentration of sample the absorbance of mixture at each wavelength is derived as follows:

a_1 Cx+ b_1 Cy+ c_1 Cz = A1 at λ1 (Analytical wavelength of HMC)

a_2 Cx+ b_2 Cy+ c_2 Cz = A2 at λ2 (Analytical wavelength of HP)

a_3 Cx+ b_3 Cy+ c_3 Cz = A3 at λ3 (Analytical wavelength of AA.)

A1, A2 and A3 are the absorptions of the mixture of the drugs at λ1, λ2 and λ3 respectively where Cx, Cy and Cz indicates the concentration of HMC, HP and A.A. In the above matrix, a1, a2 and a3 are the absorptivity of HMC, b1, b2 and b3 are the absorptivity of HP and c1, c2 and c3 are the absorptivity of AA at λ1, λ2 and λ3 respectively.

- **Determination of analytical wavelength**

From the working standard solutions of HMC, HP and AA 5 µg/ml HMC 15 µg/ml HMC and 20 µg/ml AA were prepared and scanned over 200-400 nm range. Analytical wavelength selected for HP HMC and AA were 348 nm, 298 nm and 261 nm respectively (Figure 4).

Absorptivity of all the drugs at all the wavelengths was determined from the calibration curves and above equations is derived as follows:

144.5Cx + 46.77Cy + 0.375Cz =A1 ..... (λ1)

132.12Cx + 138.13Cy + 12.18Cz =A2……. (λ2)

100.35Cx + 98.60Cy + 317.05Cz =A3……. (λ3)

Above equations have three unknowns, so the above 3 equations were drawn into matrix form and then cramer's rule was applied to solve the matrix. So the unknown concentration of each drug was found.

Method B: First derivative zero crossing point (ZCP)

In this method absorption spectra of HMC, HP and AA were transformed to first order derivative spectra with delta lambda 8 and scaling factor 100. The absorbance at 328.2 nm (Zero crossing point for HP and AA) of the first derivative spectra of the mixture containing HMC, HP and AA were measured for the estimation of HMC (Figure 5.1). Similarly the

absorbance at 340.2 nm (ZCP for HMC and AA) and 283.9 nm (ZCP for HMC and HP) were measured for estimation of HP and AA respectively. (Figure 5.2)

Method C: Double divisor ratio derivative

Major parameters that affect ratio derivative spectrophotometry are selection of analytical wavelength, divisor concentration, delta lambda and scaling factor. For selection of divisor concentration various concentration of HMC + HP, HP + AA and HMC + AA were tested. Among them 18 µg/ml HP + 24 µg/ml AA, 9 µg/ml HMC + 36 µg/ml AA and 6 µg/ml HMC + 18 µg/ml gave the best result for estimation of HMC , HP and AA respectively in terms of best correlation coefficient values. Analytical wavelength selected were 305.5 nm, 273.9 nm and 255.4 nm for estimation of HMC, HP and AA respectively (Figure 6.1, 6.2 and 6.3).

Validation of developed method[18]

The developed methods were validated according to International conference on Harmonization (ICH) guidelines Q2 [R1]. Validation of proposed methods was performed in terms of linearity, precision, accuracy, Limit of detection (LOD) and Limit of quantification (LOQ).

Linearity

From working standard solutions of HMC, HP and AA 5-9 µg/ml HMC, 15-27 µg/ml HP and 20-40 µg/ml AA solutions were prepared. Regression equation (Y=mx+C), correlation coefficient, standard error of slope, 95% confidence interval of slope, standard error of intercept, 95 % confidence interval of intercept were calculated for each method.

Precision

Intraday and interday precision of developed methods was measured in terms of %RSD. All the methods were repeated 3 times in a day for intra-day and on 3 different days for inter-day precision. The average % RSD of intra-day and inter-day measurements for determination of all the drugs was found to be less than 2 for all three methods.

Accuracy

Accuracy of method was assessed by recovery study from formulation at three level of standard addition (50%, 100% and 150%) in triplicate. % recovery within 95-105 % with low standard deviation justified the accuracy of developed method.

LOD and LOQ

LOD and LOQ of developed methods were calculated from the equations given by ICH guidelines.

LOD= 3.3 *σ/S

LOQ=10*σ/S

Where, σ= standard deviation of intercept

S=slope of calibration curve

Application of developed method to analyze formulation

20 tablets were weighed accurately and crushed finely. From crushed tablet powder amount equivalent to 50 mg HMC, 150 HP and 200mg AA was weighed accurately, transferred to 250 ml volumetric flask and dissolved in methanol with 10 minutes of sonication. Prepared solution was filtered using what man filter paper grade 1. Appropriate dilutions were made sample solution was subjected to analysis by all three developed methods. (Table 1)

RESULT AND DISCUSSION

Method A: Simultaneous estimation by solving matrix using Cramer's rule

Beer's law was obeyed in range of 5-10 µg/ml for HMC, 15-30 µg/ml for HP, 20-40 µg/ml for AA. It showed 0.9977, 0.9957 and 0.9986 r^2 values for HMC, HP and AA respectively, indicates good linearity. Intraday and interday precision values were indicated as %RSD and %RSD below 2 showed good precision of developed method. Low LOD and LOQ values indicate sensitivity of proposed method. . Accuracy of method was investigated by means of recovery study. Results obtained in range of 95-105 % shows good accuracy of developed method. All validation parameters were shown in table 2.1. Recovery study data are shown in Table 3.

Method B: First derivative zero crossing point (ZCP)

In this method linearity range selected was 5-9 µg/ml for HMC, 15-27 µg/ml for HP and 20-36 µg/ml for AA. R^2 value near to 1 indicates good linearity. All validation parameters and recovery data are shown in table 2.2 and 3 respectively.

Method C: Double divisor ratio derivative

Linearity range selected was same as method B. Proposed method was validated according to ICH guidelines. Table 2.3 shows all validation parameters for this method. Recovery study data are shown in table 3.

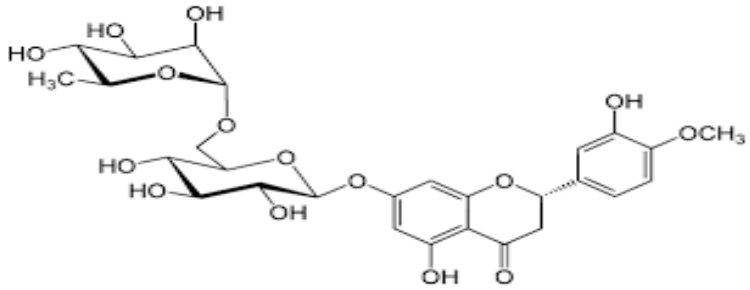

Figure 1: Chemical structure of Hesperidin methyl chalcone (HMC)

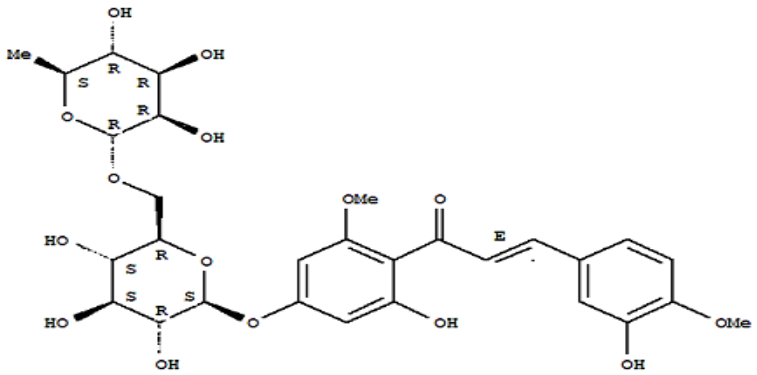

Figure 2: Chemical structure of Hesperidin (HP)

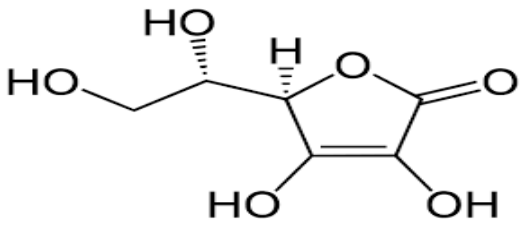

Figure 3: Chemical structure of Ascorbic acid (AA)

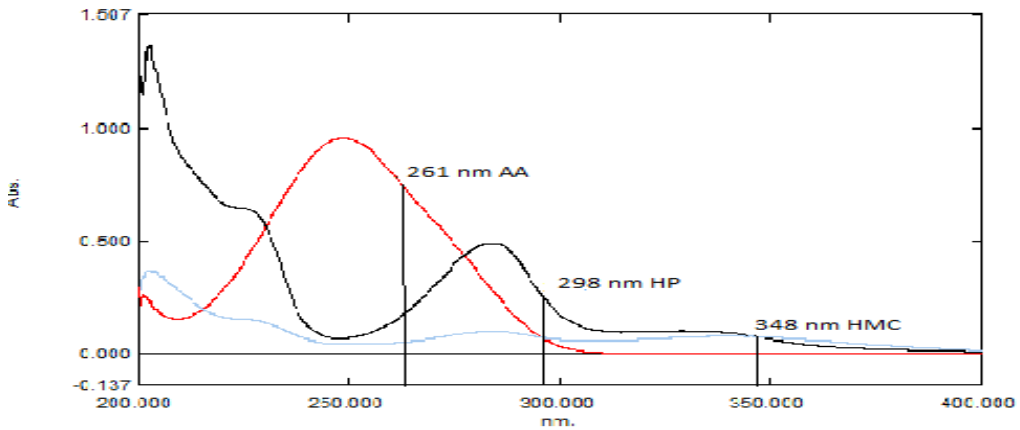

Figure 4: Selected analytical wavelength for HMC (5µg/ml Blue), HP (15 µg/ml Black), and AA (20µg/ml Red) (METHOD A)

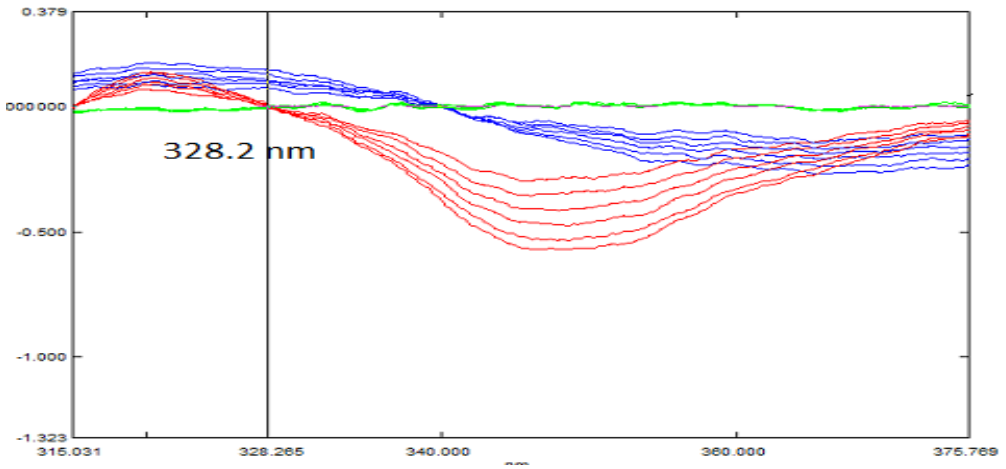

Figure 5.1: First order derivative overlay spectra of HMC (ZCP for HP and AA) (METHOD B)

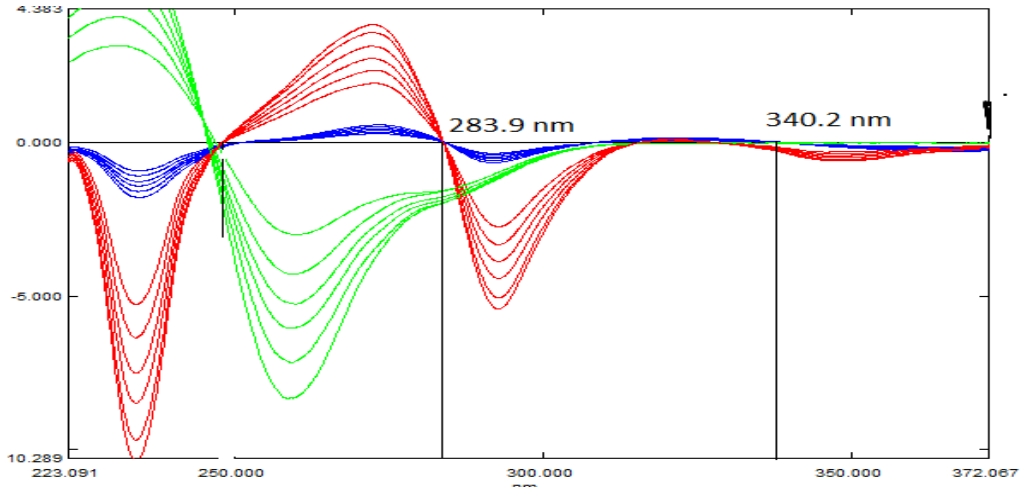

Figure 5.2: First order derivative overlay spectra of HP (ZCP for HMC and AA) and AA (ZCP for HMC and HP) (METHOD)

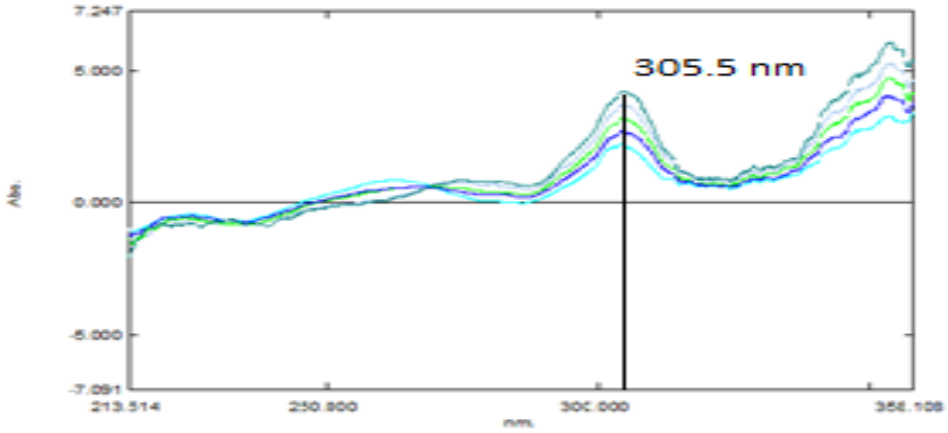

Figure 6.1: Ratio first order derivative spectra of HMC (5-9 µg/ml) (METHOD C)

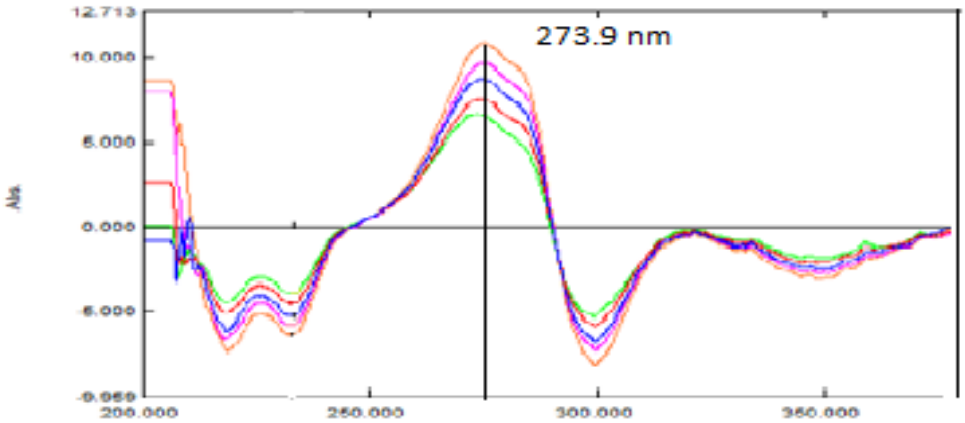

Figure 6.2: Ratio first order derivative spectra of HP (15-27 µg/ml) (METHOD C)

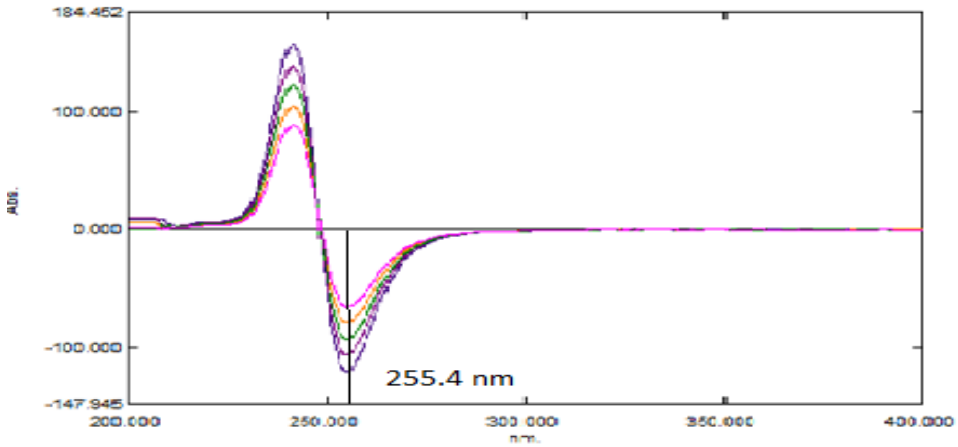

Figure 6.3: Ratio first order derivative spectra of AA (20-36 µg/ml) (METHOD C)

Table 1: Assay determination of HMC, HP and AA IN FORMULATION by developed methods

Formulation : Peridin C tablets			
Labelled claim : HMC:HP:AA (50mg:150mg:200mg)			
Method	**HMC**	**HP**	**AA**
A	95.5±0.691	97.37±1.310	102.80 ±0.921
B	96.34±0.841	96.58±0.937	100.79±1.389
C	95.75±0.147	96.37±0.629	101.10±0.198

Table 2.1: Summary of validation parameter for Method-A

METHOD A			
PARAMETER	**HMC**	**HP**	**AA**
Analytical wavelength (nm)	348	298	261
Linearity range(µg/ml)	5-10	15-30	20-40
Correlation coefficient	0.9977	0.9957	0.9986
Slope	0.0147	0.0134	0.0281
Std. error on slope	0.0003	0.0004	0.0005

Confidence interval (95%) on slope	0.0137 to 0.0156	0.0122 to 0.0146	0.0266to 0.0296
Intercept	-0.0018	0.0081	0.1014
Std. error on intercept	0.0026	0.0105	0.01644
Confidence interval (95%) on intercept	-0.0093 to 0.0056	-0.0200 to 0.0363	0.05575 to 0.1470
Intraday precision (%RSD)	0.9480	0.4750	0.538
Interday precision (%RSD)	1.0027	0.5285	0.419
LOD(µg/ml)	0.5030	0.7379	2.169
LOQ(µg/ml)	1.5243	2.2359	6.575

Table 2.2: Summary of validation parameter for Method-B

METHOD B			
PARAMETER	**HMC**	**HP**	**AA**
Analytical wavelength (nm)	328.9	340.2	283.9
Linearity range(µg/ml)	5-9	15-27	20-36
Correlation coefficient	0.9902	0.9987	0.9964
Slope	0.0108	-0.0151	0.0120
Std. error on slope	0.0006	0.0003	0.0004
Confidence interval (95%) on slope	0.0088 to 0.0128	-0.0161 to -0.0141	-0.0134 to -0.0107
Intercept	0.0014	0.0210	-2.198
Std. error on intercept	0.0044	0.0067	0.0120
Confidence interval (95%) on intercept	-0.0127 to 0.0155	0.0004 to 0.0425	-2.2360 to -2.1600
Intraday precision (%RSD)	1.2918	-0.6590	-0.3683
Interday precision (%RSD)	1.6795	-0.5637	-0.3539
LOD(µg/ml)	0.1584	0.9743	5.3175
LOQ(µg/ml)	0.4801	2.9525	16.1138

Table 2.3: Summary of Validation parameter for Method-C

METHOD C			
PARAMETER	**HMC**	**HP**	**AA**
Analytical wavelength (nm)	305	273.9	255.4
Linearity range(µg/ml)	5-9	15-27	20-36
Correlation coefficient	0.9999	0.9967	0.9996
Slope	0.5054	0.3198	-2.8140
Std. error on slope	0.0032	0.0106	0.0033
Confidence interval (95%) on slope	0.4949 to 0.5159	0.2859 to 0.3538	-2.9190 to -2.7080
Intercept	-0.3896	1.390	2.4160
Std. error on intercept	0.0204	0.2287	0.9461
Confidence interval (95%) on intercept	-0.4644 to -0.3148	0.6626 to 2.1180	-0.5948 to 5.4260
Intraday precision (%RSD)	0.8948	0.4729	-0.2153
Interday precision (%RSD)	0.7345	0.5314	-0.1448
LOD(µg/ml)	0.5266	1.2307	0.5474
LOQ(µg/ml)	1.5957	3.7296	1.6588

Table 3: Recovery study of HMC, HP and AA by developed methods

Method	% Spiking	C actual (µg/ml)			C added (µg/ml)			C recover* (µg/ml)			% recovery		
		HMC	HP	AA	HMC	HP	AA	HMC	HP	AA	HMC	HP	AA
A	50	4	12	16	2	6	8	1.94	5.81	8.09	97.00±0.75	96.83±0.12	101.12±0.94
	100	4	12	16	4	12	16	3.96	11.5	16.21	99.00±1.91	95.83±0.91	101.31±0.75
	150	4	12	16	6	18	24	5.87	17.33	24.61	97.83±0.98	96.27±1.50	102.54±1.01
B	50	4	12	16	2	6	8	1.91	5.79	8.18	95.50±0.13	96.50±0.19	102.25±0.86
	100	4	12	16	4	12	16	3.84	11.48	16.18	96.00±1.92	95.66±0.67	101.13±0.48
	150	4	12	16	6	18	24	5.79	17.5	24.45	96.50±1.63	97.22±0.43	101.88±1.09
C	50	4	12	16	2	6	8	1.93	5.88	8.05	96.50±0.93	98.00±1.29	100.63±1.15
	100	4	12	16	4	12	16	3.81	11.91	16.19	95.25±0.48	99.25±0.91	101.19±0.59
	150	4	12	16	6	18	24	5.89	17.85	24.19	98.17±0.76	99.16±0.28	100.79±1.03

CONCLUSION

Three UV spectrophotometric methods were successfully developed for simultaneous estimation of Hesperidin methyl chalcone, Hesperidin and ascorbic acid in their combined dosage form. All developed methods were validated according to ICH guidelines. Developed methods were successfully applied to pharmaceutical formulation for simultaneous estimation of HMC, HP and AA.

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