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Original Article

Simultaneous spectrophotometric estimation of Camylofin Dihydrochloride and Diclofenac Potassium in tablet dosage form

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ABSTRACT

The accurate, precise, sensitive and economical spectrophotometric method was developed and validated for simultaneous estimation of Camylofin Dihydrochloride and Diclofenac Potassium tablet dosage form. The method employed for UV estimation was simultaneous equation method. The method employs 248 nm as λ1 and 280 nm as λ2 for formation of equations. Camylofin Dihydrochloride and Diclofenac Potassium obeys Beer's law in the concentration range 5-80 µg/mL (r^2 = 0.998) and 5-80 µg/mL (r^2 = 0.997). The mean recovery for Camylofin Dihydrochloride and Diclofenac Potassium, were found to be 99.652 ±0.2129% and 99.80 ±0.0.00307% respectively. The developed method were validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values. Thus, the proposed methods were successfully applied for simultaneous determination of Camylofin Dihydrochloride and Diclofenac Potassium, routine analytical work.

1. INTRODUCTION

Analytical chemistry seeks improved means of measuring the chemical composition of natural and artificial materials. The discipline of analytical chemistry consists of qualitative and quantitative analysis. Analytical chemistry seeks improved means of measuring the chemical composition of natural and artificial materials [1-3]**.** Camylofin Dihydrochloride is a chemically, 3-methylbutyl 2-[2(diethylamino)ethylamino]-2-phenylacetate [4,5] and Diclofenac Potassium is, potassium; 2-[2-(2,6-dichloroanilino)phenyl]acetate [6,7].

Camylofin Dihydrochloride and Diclofenac Potassium combination is used in treatment of antiinflammatory diseases. Several methods are available in the literature for the determination of Camylofin Dihydrochloride i.e RPHPLC [8-10], GC [11,12] and Ultra Violet Visible spectroscopy [13] and Diclofenac Potassium i.e RP-HPLC [14,15,16,18,19,20,21,23] and Ultra Violet Visible spectroscopy [22] . Most of these methods are for the determination of Camylofin Dihydrochloride and Diclofenac Potassium or in combination with other drug and literature survey revealed that very few methods are reported for determination of both the drugs in pharmaceutical formulations in combination. Therefore it was thought worthwhile to develop simple, precise and robust analytical method for the same.

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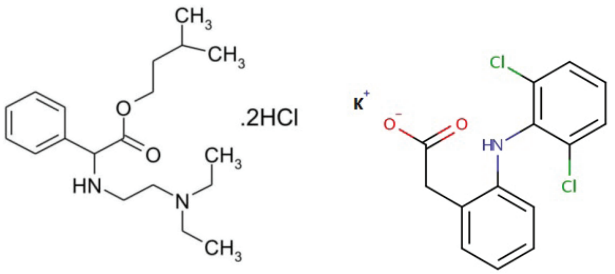

Camylofin Dihydrochloride Diclofenac Pottasium

Fig. 1. Chemical structure of Camylofin Dihydrochloride and Diclofenac Potassium[4,7]

2. EXPERIMENTAL

2.1 Chemicals and reagents

The bulk drugs of Camylofin Dihydrochloride (CMD) and Diclofenac Potassium (DFP) were obtained as a gift samples from Khandelwal Laboratories Pvt. Ltd. Uttarakhand. All the solvents and reagents used were of HPLC and analytical grade respectively. HPLC grade methanol, water, toluene, acetone were obtained from Merck Chemicals, India. Combination of Camylofin Dihydrochloride (CMD) and Diclofenac Potassium (DFP) tablets of brand name ANASPAS, manufactured by Khandelwal Laboratories Pvt. Ltd. Uttarakhand and Marketed by Abbott Healthcare Pvt. Ltd. Mumbai was purchased from local pharmacy.

2.2 Instrumentation

The proposed work was carried out on a Shimadzu UV-visible spectrophotometer (model UV-1800 series), which possesses a double beam double detector configuration with a1 cm quartz matched cell equipped with UV photo diode array detector, with 1 cm quartz cell and slit width 1.0 nm and Sansui-vibra DJ-150S-S electronic balance were used for spectrophotometric and weighing purposes respectively.

2.3 Selection of solvents

On the basis of solubility study methanol was selected as the solvent for dissolving CMD and DFP.

2.4 Preparation of standard stock solutions of CMD and DFP

2.4.1 CMD standard solution

An accurately weighed quantity of CMD (10 mg) was taken in 10 mL volumetric flask and dissolved in methanol (10 mL) with the help of ultrasonication for about 10 min. Then the volume was made up to the mark using methanol to get CMD standard stock solution (1 mg/mL).

2.4.2 DFP working standard solution

An accurately weighed quantity of DFP (10 mg) was taken in 10 mL volumetric flask and dissolved in methanol (10 mL) with the help of ultrasonication for about 10 min. Then the volume was made up to the mark using methanol to get Diclofenac Potassium standard stock solution (1 mg/mL).

2.4.3 CMD stock solution

CMD standard stock solution (5 mL) was diluted to 50 mL using 65% v/v methanol to get working standard solution (100 µg/mL).

2.4.4 DFP working stock solution

Diclofenac Potassium standard stock solution (5 mL) was diluted to 50 mL using 65% v/v methanol to get working standard solution (100 µg / mL).

2.5 Determination of λ max of individual component

An appropriate aliquot portion of CMD (0.40 mL) and DFP (0.30 mL) were transferred to two separate 10 mL volumetric flasks, the volume was made up to the mark using 65 %v/v methanol to obtain final concentration. Drug solutions were scanned separately between 200 nm to 400 nm.

2.6 Overlay spectra of CMD and DFP

The overlain spectrum of both drugs was recorded (**Fig. 2)** and two wavelengths 248 nm (λ max of CMD) and 280 nm (λ max of DFP) were selected for further study.

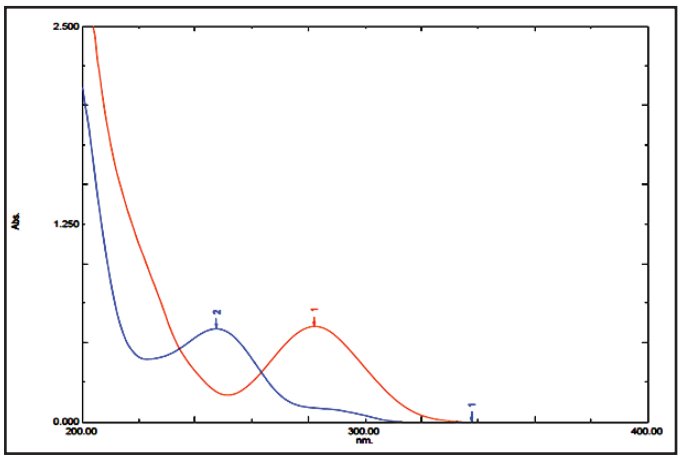

Fig. 2. Overlay Spectra of CMD and DFP

2.7 Linearity study for CMD

Accurately measured aliquot portions of working standard solutions of CMD were transferred to separate 10 mL volumetric flasks. The volume was made up to the mark using 65% v/v methanol to obtain concentrations (5 - 80 µg/mL). Absorbance of these solutions was measured at 248 nm, (Table 4). Calibration curve was plotted as absorbance verses concentration as shown in Fig.3.

2.8 Linearity study for DFP

Accurately measured aliquot portions of working standard solutions of DFP were transferred to seven separate 10 mL volumetric flasks. The volume was made up to the mark using 65% v/v methanol to obtain concentrations (5-80 µg/mL). Absorbance of these solutions was measured at 280 nm, The

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results are reported in the Table 4**.** Calibration curve was plotted as absorbance verses concentration as shown in Fig 4.

Table 1. Regression and optical characteristics of CMD and DEP

Parameters	Value For CMD	Value For DEP
Beer's law limit (µg/mL)	5-80	5-80
Correlation Coefficient (r)	0.014	0.021
Slope	0.0142	0.0128
Intercept	0.01749	0.0883

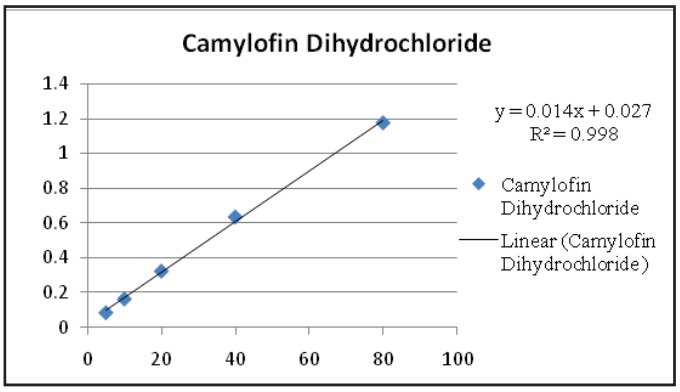

Fig. 3. Calibration curve of CMD at 248 nm wavelength

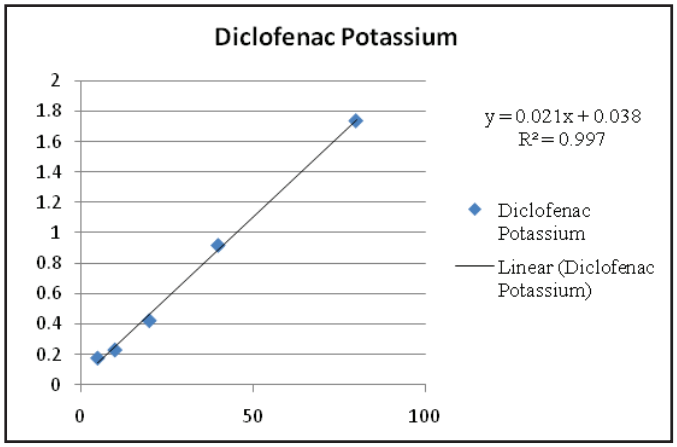

Fig. 4. Calibration curve of DFP at 280 nm wavelength

2.9 Estimation of laboratory mixture by proposed method

In order to see the feasibility of proposed method for simultaneous estimation of CMD and DFP in marketed pharmaceutical formulations, the method was first tried for estimation of drugs in standard laboratory mixture. Accurately weighed CMD (50 mg) and DFP (50 mg) were taken in 100 mL volumetric flask, dissolved in methanol with the help of ultrasonication for about 10 min and the volume was made up to mark using the same. Appropriate aliquot portion (1 mL) was transferred to 10 mL volumetric flask and further diluted using 65% v/v methanol to get CMD (50 **µg**/ mL) and DFP (50 **µ**g/ mL). The absorbance was recorded at 248 nm and 280 nm against solvent as blank.

Amount of each drug was estimated using following equations,

$$C_x = \frac{A_2 \times ay_1 - A_1 \times ay_2}{ax_2\, ay_1 - ax_1\, ay_2}$$

$$C_y = \frac{A_1 \times ax_2 - A_2 \times ax_1}{ax_2\, ay_1 - ax_1\, ay_2}$$

Where;

A_1 and A_2 are the absorbance of diluted mixture at λ_1 and λ_2

C_x and C_y are the concentration of X and Y respectively

ax_1 and ax_2 are absorptivities of X at λ_1 and λ_2 respectively

ay_1 and ay_2 are absorptivities of Y at λ_1 and λ_2 respectively

The results are reported in (Table No:2)

Table 2. Results of estimation of CMD and DFP standard laboratory mixture.

Analyte	%Concentration estimated (Mean±S.D.)	%R.S.D.
CMD	99.652±0.2129*	0.00527
DFP	99.80±0.52371*	0.00307

*Average of five determinations; R.S.D. = Relative Standard Deviation

2.10 Application of the proposed method for estimation of drugs in tablets

Twenty 'ANASPAS' tablets containing CMD (50 mg) and DFP (50 mg) were weighed and ground to fine powder. A quantity of sample equivalent to CMD (10 mg) and DFP (10 mg) was transferred into 100 mL volumetric flask containing methanol (70mL), sonicated for 15 min and the volume was made up to the mark and filtered through Whatman filter paper (No. 45). This solution was (1 mL) transferred to 10 mL volumetric flaks, dissolved and volume was adjusted to the mark. The relative absorbance of the solutions was measured at 304 nm and 285 nm against blank. The concentrations of two drugs in sample were determined by using simultaneous equations**.** The results are reported in the (**Table 3).**

Table 3. Results of estimation of CMD and DFP in tablets.

Analyte	Label claim (mg/tab)	% Label claim estimated (Mean ± S.D.)	%R.S.D.
CMD	40	99.30 ±0.57212	0.52371
DFP	30	99.80 ±0.64191	0.38796

*Average of five determinations; S.D. =Standard Deviation

3. RESULTS AND DISCUSSION

3.1 Validation of proposed method

The Proposed method was validated as per the ICH guidelines [25].

3.1.1 Accuracy [Recovery Study]: Accuracy of proposed method was ascertained on the basis of recovery study performed by standard addition method. A known amount of standard drug

solutions were added to the tablet powder to make final concentrations in the range of 80%, 100% and 120% and re-analyzed it by the proposed method. The absorbance recorded and the % recoveries were calculated using formula.

% Recovery = [A – B/ C] × 10

Where,

A = Total amount of drug estimated

B = Amount of drug found on reanalyzed basis

C = Amount of Pure drug added

The results are reported in (Table 4).

Table 4. Recovery study

Tablet: ANASPAS			Average Weight of Tablet = 554 mg					
Sr. No.	**Quantity Tablet Powder Taken (mg)**	**Percentage %**	**Amount of Pure Drug Added (mg)**		**Total Amount of Drug Recovered (mg)** ± S.D. (n = 3)		**% of Drug Recovered (n = 3)**	
			CMD	**DFP**	**CMD**	**DFP**	**CMD**	**DFP**
1.	110	80	45	45	55.02 ±0.173	55.01±0.143	100.03	100.01
2.	110	100	50	50	60.00±0.369	59.94 ±0.463	100	99.90
3.	110	120	55	55	65.95±0.525	64.93±0.656	101.46	99.89
						Mean	100.49	99.93
						SD	0.8344	0.0665
						% RSD	0.0083	0.0006

3.1.2 Precision: Precision was determined as intra-day and inter-day variations. Intra-day precision was determined by analyzing CMD (39, 40, and 41 µg/mL) and DFP (39, 40, and 41 µg/mL) for three times on the same day. Inter-day precision was determined by analyzing the same concentration of solutions for three different days over a period of week. The results are reported in Table 5.

Table 5. Precision study

Drug	**Conc. [µg/mL]**	**Intra-day Amount Found**		**Inter-day Amount Found**	
		Mean ± S.D [*n* = 3]	**% R.S.D.**	**Mean ± S.D. [*n* = 3]**	**% R.S.D.**
CMD	39	39.22 ± 0.3752	0.3791	39.45 ± 0.1230	0.1298
	40	40.00 ± 0.4552	0.4591	40.00 ± 0.8231	0.8298
	41	41.89 ± 0.2808	0.2811	41.16 ± 0.577	0.5779
DFP	39	39.28 ± 0.8420	0.0849	39.89 ± 0.3247	0.3239
	40	39.60 ± 0.3251	0.3271	39.60 ± 0.3264	0.3256
	41	41.78 ± 0.4725	0.4739	41.59 ± 0.8707	0.8791

3.1.3 Ruggedness: Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by two different analyst using same operational and environmental conditions. The results are reported in Table 6.

Table 6. Ruggedness study

	CMD 50 µg/mL		**DFS 50 µg/mL**	
	Amount Found in µg/mL Mean ± S.D. (n = 3)	**% RSD**	**Amount Found in µg/mL Mean ± S.D.(n = 3)**	**% RSD**
Analyst-I	50.02 ±0.6789	0.6687	50.03 ± 0.4564	0.4510
Analyst-II	50.08 ±0.5789	0.5732	49.98 ± 0.8165	0.8129
Day-I	49.95 ±0.3294	0.2451	49.92 ± 0.1953	0.1965
Day-II	49.92 ±0.0470	0.3694	49.88 ± 0.0891	0.7140

LOD: Limit of detection of CMD and DFP were found to be 0.06526 **µg** and 0.07313 **µg** respectively.

LOQ: Limit of quantization of CMD and DFP were found to be 0.08233 **µg** and 0.09769 **µg** respectively.

4. CONCLUSION

The UV-Spectrophotometric and RP-HPLC methods were developed and validated as per ICH guidelines for quantification of Camylofin Dihydrochloride and Diclofenac Potassium in tablet formulation. From the studies it can be concluded that, the analysis of combined dosage tablet formulation can be successfully performed by the UV-spectrophotometric simultaneous equation method. Drugs were analyzed on Shimadzu 1800 UV-visible spectrophotometer, equipped

with photo diode array (PDA) detector, with 1 cm quartz cell and slit width 1.0 nm. Use of 65% aqueous methanol for every final dilution made method more economic. RP-HPLC technique can also be successfully used for the estimation of the Camylofin Dihydrochloride and Diclofenac Potassium in their combined dosage tablet formulation. Chromatographic experimentations were performed using Systronics HPLC system equipped with 8600 HPLC pump and dual wavelength UV-VIS detector, data acquisition and processing was performed using Chemitochrom automation system software. The method was conducted using an isocratic reverse phase techniques. The mobile phase was prepared freshly filtered through 0.45 µm membrane filter (Millipore, USA) and sonicated for 30 min before use in order to degas the mobile phase. A C18 RP-Purosphere column (5 µm, 4.6mm* 250 mm), Germany was used for analysis.

The UV-Spectrophotometric and RP-HPLC methods have been evaluated for the linearity, accuracy, precision and robustness in order to ascertain the suitability of the analytical method. The method was also applied to marketed samples. It has been proved that both UV-Spectrophotometric and RP-HPLC method was selective, linear and precise. Both the methods are simple, reproducible, economical and rapid also. These methods may be adopted for routine quality control analysis of these two drugs in combined dosage form. Further it would be interesting to apply these methods for biological samples containing these drugs.

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