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DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROPHOTOMETRIC METHODS FOR THE ESTIMATION OF PARACETAMOL AND DICLOFENAC SODIUM IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Two simple, specific, sensitive, rapid, precise, accurate and economical UV Spectrophotometric methods viz. simultaneous equation method and Q-Absorbance ratio method have been developed and validated for the routine estimation of Paracetamol and Diclofenac Sodium in bulk and tablet dosage form. The absorption maxima of drugs were found to be at 247 nm for Paracetamol and 276 nm for Diclofenac sodium and iso-absorptive point at 265 nm in distilled water and these wavelengths were selected for the analysis. Paracetamol and Diclofenac sodium obeyed Beer- Lambert's law in the concentration range of 6-30 µg/ml at 247 nm and 276 nm respectively. Simultaneous equation method and Q-analysis method have been used in the estimation of both the drugs. Both the methods show good accuracy and precision which was validated statistically. These methods require no preliminary separation and can therefore be used for routine analysis of both drugs in quality control laboratories.

Keywords: Simultaneous equation method, Q-analysis method, Paracetamol, Diclofenac sodium, Distilled water, Validation.

INTRODUCTION

PCM is chemically N (4-hydroxyphenyl) acetamide. PCM is a non-opioid; non-salicylate analgesic [1]. It is commonly used in multi-ingredient preparation for migration, headache & antipyretic action. The mechanism of action of PCM is inhibition of cyclo-oxygenase (COX) [2]. It is a official in Indian pharmacopeia (IP), British pharmacopeia (BP), United state pharmacopeia (USP). Many methods like spectroscopy, HPLC & HPTLC method are reported for estimation of PCM [2]. Various hydrotropic agent have been used to enhance the aqueous solubility of large no. of drugs. E.g.-Sodium salicylate, Nicotinamide, Sodium ascorbate, Sodium citrate & other organic solvent such as urea, chloroform, diethyl form amide have been employed for solublization of poor water soluble drug to carry out spectrophotometer analysis [3]. The UV spectrophotometry analysis is often preferred in quality control testing & ordinary laboratory due to its broad availability & suitability [4]

DSC is chemically 2(2,6dichlorophenyl amino) benzene acetic acid sodium salt. Molecular formula $C_{14}H_{10}C_{l2}NNaO_2$. DSC is a potent, non-steroidal, anti-inflammatory drug (NSAID) used to treat inflammatory condition for e.g.-Acute injury. Different analytical methods have been employed for the quantification of DSC such as spectrophotometry, fluorimetry, FT-Raman spectroscopy, potentiometer, chromatography, voltammetry & polarography. Most of this faces certain problems such as the use of additional reagent, complex formation [5] Spectrophotometric & HPLC method have been reported for assay of DCS. DCS are extensively used for the treatment of inflammatory condition like osteoarthritis, rheumatoid, arthritis.

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The objective of this study was to develop & validate a simple & specific UV Spectrophotometric method for simultaneous determination of DSC & PCM in pure drug & marketed preparation & formulation [6-9].

MATERIALS AND METHODS

Materials

Paracetamol (Purity- 99.99%) and Diclofenac sodium (99.98%) samples were provided by CosmePharma, Goa. Tablets containing Paracetamol and Diclofenac sodium (50 tablets of each drug) were procured from local Pharmacy. Distilled water was used in the present study. UV-Visible spectrophotometer (Agilent, carry 60) with spectral band width of 0.1 nm and wavelength accuracy of + 0.5 nm with automatic wavelength correction was used to determine the absorbance.

Standard stock solution of Paracetamol and Diclofenac sodium were prepared by dissolving 100 mg Paracetamol and Diclofenac sodium in 100 ml of distilled water in 100 ml volumetric flask separately with vigorous shaking. From these stock solutions working standard solution having concentration 10 µg/ml of Paracetamol and 10 µg/ml of Diclofenac sodium were prepared by proper dilutions. They were scanned in the UV region 400-200 nm. The overlain spectrum (Figure 3) was obtained to determine the maximum absorbance and iso-absorptive point. These solutions were then further diluted to get various working solutions of each. Both the stock solutions were filtered through Whatman filter paper no. 41 [2].

Working solutions of Paracetamol and Diclofenac sodium

The calculated volumes of Paracetamol and Diclofenac sodium solutions were taken from the standard stock solutions and serial dilutions were prepared with distilled water containing 6, 12, 18, 24, 30 µg/ml of each, Paracetamol and Diclofenac sodium, alone and together [10-13].

Sample solutions

For the quantification of drugs from the marketed formulations, 20 tablets of Nofiva 500 mg, Agid pharmaceuticals ltd., containing Paracetamol and 20 tablets of Voveran-D 50 mg, Medreich ltd., containing Diclofenac sodium were weighed to calculate mean tablet weight of each drug. These tablets were crushed into powder. A weighed quantity of tablet powder equivalent to 50 mg of Paracetamol and 50 mg of Diclofenac sodium were transferred into 100 ml volumetric flask and diluted with distilled water, sonicated for 20 min. and made up to the volume with the same. Then the resulting solution was allowed to stand for 2 hrs. Filtered through Whatman filter paper no. 41 and the filtrate were suitably diluted to produce the desired concentration (6, 12, 18, 24, and 30 µg/ml) for both, Paracetamol and Diclofenac sodium, with distilled water. The absorbance of these solutions was taken at appropriate wavelengths and the values were put in the respective formulas to determine concentrations [14-18].

METHOD DEVELOPMENT

Estimation of Paracetamol and Diclofenac sodium from pharmaceutical formulation by simultaneous equation method

This method of analysis was based on the absorption of drugs (Paracetamol and Diclofenac sodium) at the wavelength maximum of the each other. Two wavelengths selected for the development of the simultaneous equations were 247 nm and 276 nm for Paracetamol and Diclofenac sodium respectively. The absorptivity values E (1%, 1cm) were determined for two drugs at all selected wavelengths [3].

The concentration of two drugs in mixture was calculated by using following equations.

$$C_{PCM} = \frac{A2ay1 - A1ay2}{ax2ay1 - ax1ay2} \quad (1)$$

$$C_{DCS} = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2} \quad (2)$$

Where, C_{PCM} and C_{DCS} are the concentrations of Paracetamol and Diclofenac Sodium respectively in mixture and in sample solutions. A1 and A2 are the absorbances of sample at 247 nm and 276 nm respectively. ax1 and ax2 are the absorptivity of PCM at 247 nm and 276 nm respectively. ay1and ay2 are the absorptivity of DCS at 276 nm and 247 nm respectively. Mixed standard solutions of DCS and PC in the ratio of 5:35, 10:30, 15:25, 20:20, 25:15, 30:10, and 35:5 µg/ml were prepared from standard solutions whose volume was made with distilled water and absorbance were measured at 247 nm and 276 nm.

Estimation of Paracetamol and Diclofenac sodium from pharmaceutical formulation by Q-Absorbance ratio method

Q-Absorbance method uses the ratio of absorbance at two selected wavelengths, one at iso-absorptive point and other being the λ max of one of the two drugs. The content of twenty tablets were accurately weighed and crushed into fine powdered. A quantity of powder equivalent to 50 mg of Paracetamol and 50 mg of Diclofenac sodium was transferred to 100 ml volumetric flask containing 60 ml distilled water, shaken manually for 20 min and the volume was made up to the mark and filtered through Whatman filter paper (no.41). The solution was further diluted with distilled water to give the concentration within Beer's Law range. Absorbance of this solution was measured at 247 nm and 265 nm respectively and concentrations of these two drugs in the tablet

formulation were calculated using equation (1) and equation (2) [4].

The concentration of two drugs in mixture was calculated by using following equations:

$$C_{PCM} = \frac{Qm-Qy}{Qx-Qy} \times \frac{A}{ax1} \quad (1)$$

$$C_{DCS} = \frac{Qm-Qx}{Qy-Qx} \times \frac{A}{ay1} \quad (2)$$

Where,
Qm = Absorbance of Sample at 247 nm/Absorbance of sample at 265 nm
Qy = E (1%, 1 cm) of PCM at 247 nm/ E (1%, 1 cm) of PCM a 265 nm
Qx = E (1%, 1 cm) of DCS at 247 nm/ E (1%, 1 cm) of DCS at 265 nm

'A', is the absorbance of mixture at 265 nm and ax1, ax2 and ay1, ay2 are E (1%, 1 cm) of PCM and DCS at 265 nm and 247 nm and Qm= A2/A1, Qy = ay2/ay1 and Qx = ax2/ax1.

VALIDATION OF METHOD

The proposed method of analysis for Paracetamol and Diclofenac sodium were validated as per the recommendations of ICH guidelines for the parameters like accuracy, linearity, precision, detection limit and quantitation limit [19].

Accuracy (Recovery studies)

To study the accuracy of proposed method, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). A known amount of Paracetamol and Diclofenac sodium were added separately to pre-analyzed powder and percent recoveries were calculated. The results of recoveries were satisfactory and are present in Table 4.

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 6-30 µg/ml for Paracetamol and Diclofenac sodium for simultaneous equation and Q-analysis. Accurately measured standard solution of Paracetamol and Diclofenac sodium were transferred to a series of 100 ml of volumetric flasks and diluted to the mark with distilled water for simultaneous equation method and Q-analysis method. The absorbance's of the solutions were measured at 247 nm and 276 nm against distilled water as blank for Simultaneous equation method and at 247 nm and 265 nm against distilled water as blank for Q-analysis method. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated.

Precision

The intraday and interday precision methods was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days 3 different concentrations of standard solutions of PCM and DCS for both methods [20].

Limit of detection and limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal –to-noise ratio (S/N) using the following equations designed by ICH guidelines.
$LOD = 3.3 \times \sigma/S$
$LOQ = 10 \times \sigma/S$
Where, σ = standard deviation of the intercept of calibration curve and S = slope of the calibration curve.

Analysis of PCM and DCS in synthetic mixture

Binary mixture was prepared for combination of both drug in ratio 6:4 (PCM and DCS). The absorbance was measured at 247 nm and 276 nm for simultaneous equation method and at 247 nm and 265 nm for Q-analysis method. The amounts of PCM and DCS present in sample solutions were determined by fitting the response into the equation for PCM and DCS. No interference of the excipients with absorbance of interest appeared; therefore proposed method is applicable for the routine simultaneous estimation of Paracetamol and Diclofenac sodium in mixture [5].

RESULTS AND DISCUSSION

The proposed method was validated as per ICH guidelines. The average percent drug estimated in tablet was 100.04% and 100.31% for PCM and DCS respectively indicating the accuracy of proposed analytical method. %RSD were found to be less than 2. Mean Standard Deviation for PCM and DCS was found to be 0.216 and 0.293 respectively. Mean standard error for PCM and DCS was found to be 0.308 and 0.250 respectively. Mean percent coefficient of variation for PCM and DCS was found to be 0.315% and 0.253% respectively. (Table 2). The low values of these statistical parameters validated the method. Mean percent recoveries for PCM and DCS were 101.08% and 101.40% respectively (Table 4). LOD and LOQ were found to be 0.154µg/ml and 0.481µg/ml for PCM and 0.335µg/ml and 0.915µg/ml for DCS respectively which indicate the method has required accuracy (Table 1). Interday and intraday precision studies showed that %RSD values <1% signifies the precision of the method. Thus the proposed analytical method can be successfully employed in the routine analysis of PCM and DCS containing dosage forms.

Fig 1. Structure of Paracetamol

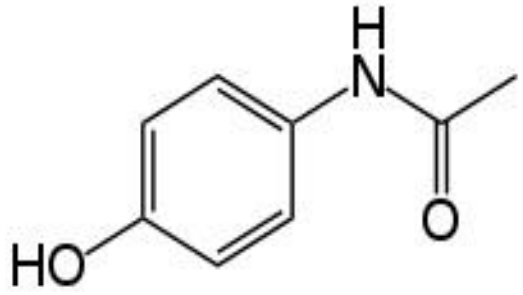

Fig 2. Structure of Diclofenac Sodium

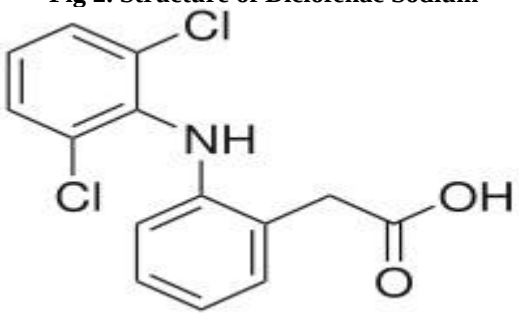

Fig 3. Overlain absorption spectra of Paracetamol (10 µg/ml) and Diclofenac sodium (10µg/ml)

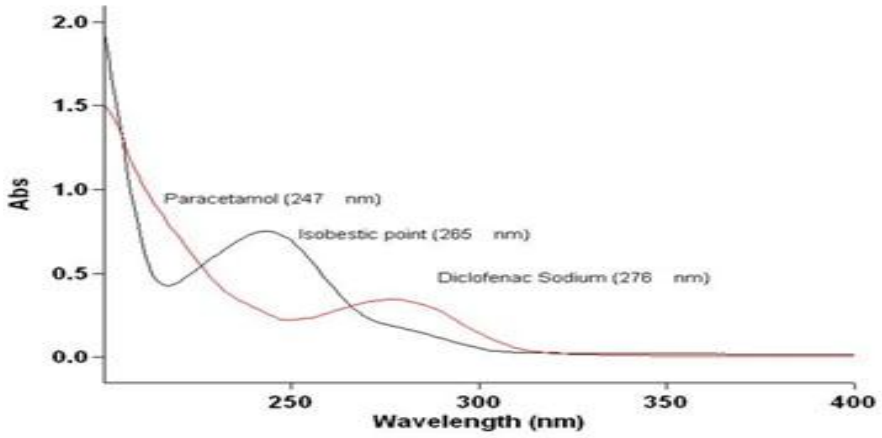

Table 1. Summary of validation parameters

Parameters	Paracetamol	Diclofenac sodium
Wavelength	247 nm	276 nm
Linearity range (µg/ml)	6-30	6-30
Correlation coefficient	0.997	0.998
Regression equation, Y=mx+c		
Slope	0.045	0.028
Intercept	0.008	0.024
LOD (µg/ml)	0.154	0.335
LOQ (µg/ml)	0.481	0.915

Table 2. Pharmaceutical formulation analysis

Parameters	Method- A		Method-B	
	PCM	DCS	PCM	DCS
Label claim (mg/tab)	500	50	500	50
Found (mg/tab)	499.90	50.02	499.94	49.98
Drug content*	100.01	100.65	100.07	99.97
±S.D.	0.209	0.281	0.223	0.305
%COV	0.142	0.135	0.489	0.372
SE	0.216	0.291	0.401	0.210

*Value for drug content (%) is the mean of five estimations

Method-A: Simultaneous equation method

Method-B: Q-analysis method

S.D.: Standard Deviation, COV: Coefficient of variance and SE: Standard error.

Table 3. Precision study

Drug	Concentration of drug (µg/ml)	Intraday precision Mean abs±%RSD(n=3)		Interday precision Mean abs±%RSD (n=3)	
		Method A	Method B	Method A	Method B
Paracetamol	6	0.351±0.645	0.457±0.523	0.356±1.891	0.396±1.098
	12	0.645±0.428	0.598±0.524	0.648±0.452	0.709±0.512
	18	0.701±0.313	0.801±0.296	0.703±0.402	0.756±0.441
Diclofenac sodium	6	0.305±1.120	0.411±1.109	0.307±1.642	0.326±1.564
	12	0.561±0.781	0.648±0.698	0.567±0.915	0.549±0.893
	18	0.697±0.792	0.573±0.687	0.699±0.725	0.710±0.746

Table 4. Recovery study

Drug	Level	Amount of sample (µg/ml)	Amount of standard spiked (µg/ml)	Total amount recovered (µg/ml)		Amount of standard recovered (µg/ml)+		% Recovery±RSD*	
				A	B	A	B	A	B
Paracetamol	80%	1.0	0.8	1.82	1.80	0.79	0.81	98.75	101.25
	100%	1.0	1.0	1.83	1.79	1.01	1.03	101	103
	120%	1.0	1.2	1.84	1.85	1.20	1.23	100	102.5
Diclofenac sodium	80%	1.0	0.8	1.81	1.02	0.81	0.82	101.25	102.5
	100%	1.0	1.0	1.80	1.84	1.02	1.01	102	101
	120%	1.0	1.2	1.78	1.81	1.21	1.21	100.83	100.83

*Average of six determinations, SD: Standard deviation, RSD: Relative standard deviation

1

CONCLUSION

Percent label claims are very close to 100 with low values of standard deviation, % coefficient of variation and standard error as it is evident from observation that there is good agreement between the amounts estimated and those claimed by manufacturers. Hence, it may be concluded that the proposed analytical method is new, simple, precise, rapid, eco-friendly and cost effective.

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