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DEVELOPMENT AND VALIDATION OF NEW SIMPLE, SENSITIVE AND VALIDATED UV-SPECTROPHOTOMETRIC AND RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF PARACETAMOL AND ETODOLAC IN MARKETED FORMULATION

Ameen Shaikh[1]*, Gurdeep Singh[1], Neetesh K Jain[1], M.K.Gupta[1]

Department of Pharmacy, Oriental University, Indore, India

ABSTRACT

A simple, precise and highly selective analytical method was developed for simultaneous estimation of Paracetamol and Etodolac in tablet formulation. Estimation was carried out by multicomponent mode of analysis at selected wavelength of 256 and 286 nm for Paracetamol (PCM) and Etodolac (ETD) respectively in methanol: water (60:40). The method was validated in terms of linearity, accuracy (% Recovery), Precision (Interday, intraday, and reproducibility) and robustness. Both methods were linear (R^2 = 0.997-0.999 for UV method and 0.998 for RPLC method) and accurate (% recovery was 98.39-101.17%). The method was also obtained precise (% RSD <2 %) and robust. The linearity was obtained in the concentration ranges of 5.25 µg/ml for Paracetamol and 3-16 µg/ml for Etodolac. The method was validated as per international conference of Harmonization (ICH) guidelines.

Keywords: Paracetamol, Etodolac, UV, RP-HPLC, ICH.

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***Address for Correspondence**

Ameen Shaikh, PG Research Scholar, Dept.of Pharmacy, Oriental University, Indore

Email.id: shaikhameen@ymail.com

INTRODUCTION

Paracetamol is one of the most popular over the counter drugs. It has analgesic and anti-pyretic properties with weak anti-inflammatory activity and it is used in the symptomatic management of moderate pain and fever. It is available in different dosage froms: tablet, capsules, drops, elixirs, suspensions and suppositories[1]. The drug is official in Indian, BP, European and US pharmacopoeias[2-5].

Paracetamol is chemically N-(N-hydroxyphenyl) acetamide [6] (fig: 1) is a member of Non-Steroidal Anti-inflammatory Drugs (NSAIDs). Etodolac (ETD) is chemically 1,8- diethyl-1,3,4,9-tetrahydropyrano (3,4-b) indole-1- acetic acid (fig: 2) [7]. It is a member of Non-Steroidal Anti-inflammatory Drugs (NSAIDs). Etodolac is official in indian, British, European and United State Pharmacopoeias. A combination of 500 mg of paracetamol and 400 mg etodolac is available commercially in tablet. This combination is used as analgesic and anti-pyretic in the treatment of osteoarthritis.

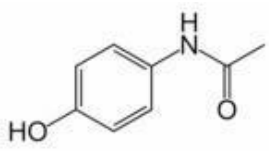

Figure 1: Structure of Paracetamol

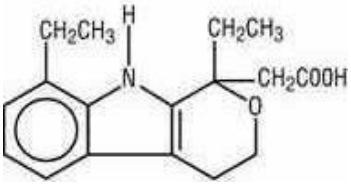

Figure 2: Structure of Etodolac

A comprehensive literature research reveals the lack of a spectrophotometric analytical method for simultaneous estimation of paracetamol [8-13] and etodolac [14-18] in pharmaceutical formulations. A successful attempt was made to develop accurate, precise and sensitive multi-component mode of analysis for estimation of both the drugs. The develop method is simple, rapid, selective, less expensive and less time consuming. The purpose of present study was to develop and validate spectrophotometric analytical methods for simultaneous estimation of paracetamol and etodolac in their combined tablet dosage from.

MATERIALS AND METHODS

Instrument: Instrument used was an UV-Visible double beam spectrophotometer Systronics-108 with a bandwidth of 1.5 nm and a pair of 1 cm matched quartz cells. The HPLC system consisted of a YL-9100 pump, a UV-visible detector, a Linhrocart C18 (250 x 4.60 nm), 5µm column, a Lichrocart, HPLC guard Cartridge and YL-Clarity software. All weighing was done on analytical balance (Denver instrument, Germany). A sonicator (Electroquip ultra sonicator, Texas) was used in the study. Calibrated glass wares were used throughout the work.

Chemicals: Chemicals used were methanol (Rankem Laboratories) and water (Merck Ltd., India). Marketed formulation containing Paracetamol 500 mg and Etodolac 400 mg (ETOGESIC-P) was procured from Burgeon Pharmaceuticals PVT LTD. Puducherry.

METHOD

Determination of λmax of Drugs:-

Standard solution (10g/ml) of pure Paracetamol and Etodolac was prepared. The pure drug solutions were scanned on UV spectrophotometer, which showed maximum absorbance at 256.0 and 286.0 for Paracetamol and Etodolac respectively.

Preparation of Standard Stock Solution:

10 mg of standard PCM and ETD were weighed separately and transferred to 100 ml separate volumetric flasks and dissolved in diluent (methanol: water in proportion of 60:40 (v/v)). The flasks were shaken and volumes were made up to mark with diluent to give a solution containing 100 µg/ml each of PCM and ETD.

Methodology:

The working standard solutions of PCM and ETD were prepared separately in diluent (methanol: water in proportion of 60:40 (v/v)) having concentration of 10 µg/ml. They were scanned in the wavelength range of 200- 400 nm against diluent methanol: water (60:40 v/v) as blank. λmax of both the drugs were 256 nm and 286 nm for Paracetamol and Etodolac respectively. The absorption spectra thus obtained were derivatised from first to fourth order. First order derivative spectrum was selected for the analysis of both the drugs. From the overlain spectra of both the drugs wavelengths selected for quantitation were 224.28 nm (zero cross point for ETD) for PCM and 219.27 nm (zero cross point for PCM) for ETD.

Validation of the Proposed Method:

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[19].

Linearity (Calibration Curve):

Appropriate aliquots from the standard stock solutions of PCM and ETD were used to prepare two different sets of dilutions: Series A, and B as follows. Series A consisted of different concentration of PCM (5-25 µg/ml). Appropriate aliquot from the stock solution of PCM (100 µg/ml) was pipette out in to a series of 10 ml volumetric flask and diluted with diluent to get final concentration in range of 5-25 µg/ml.

Series B consisted of varying concentrations of ETD (3-16 µg/ml). Appropriate aliquot of the stock solution of ETD (100 µg/ml) was transferred into a series of 10 ml volumetric flask and the volume was adjusted to the mark with diluent to get final concentration in range of 3-16 µg/ml. The calibration curves were constructed by plotting drug concentration versus the absorbance values of first derivative spectrum at 224.28 nm for PCM and 219.27 nm for ETD. The concentration of individual drugs present in the mixture was determined from the calibration curves in quantitation mode.

Precision:

The reproducibility of the proposed method was determined by performing the assay for the same day (intraday assay precision) and on three different days (inter day precision). Precision studies were performed by preparing nine determinations covering the specified range for the procedure (3 x 3 replicates for each concentration). Low % RSD shows that the method has good precision. The results of intraday and inter day precision were expressed in % RSD.

Accuracy:

The accuracy of the method was determined by calculating the recoveries of PCM and ETD by the standard addition method. Known amounts of standard solutions of PCM and ETD were added at 80 %, 100 % and 120 % level to prequantified sample solutions of PCM and ETD (10 µg/ml for PCM and 8 µg/ml for ETD). The amounts of PCM and ETD were estimated

by applying obtained observation values to the respective regression line. The results of accuracy were expressed in % Recovery.

Limit of Detection and Limit of Quantification:

The LOD and LOQ were separately determined based on the standard calibration curve. The residual standard deviation of y-intercept of regression lines may be used to calculate LOD and LOQ using following equations.

LOD = 3.3 * D/S

LOQ = 10 * D/S

Where, D = Standard deviation of the intercepts of regression line

S = Slope of the calibration curve

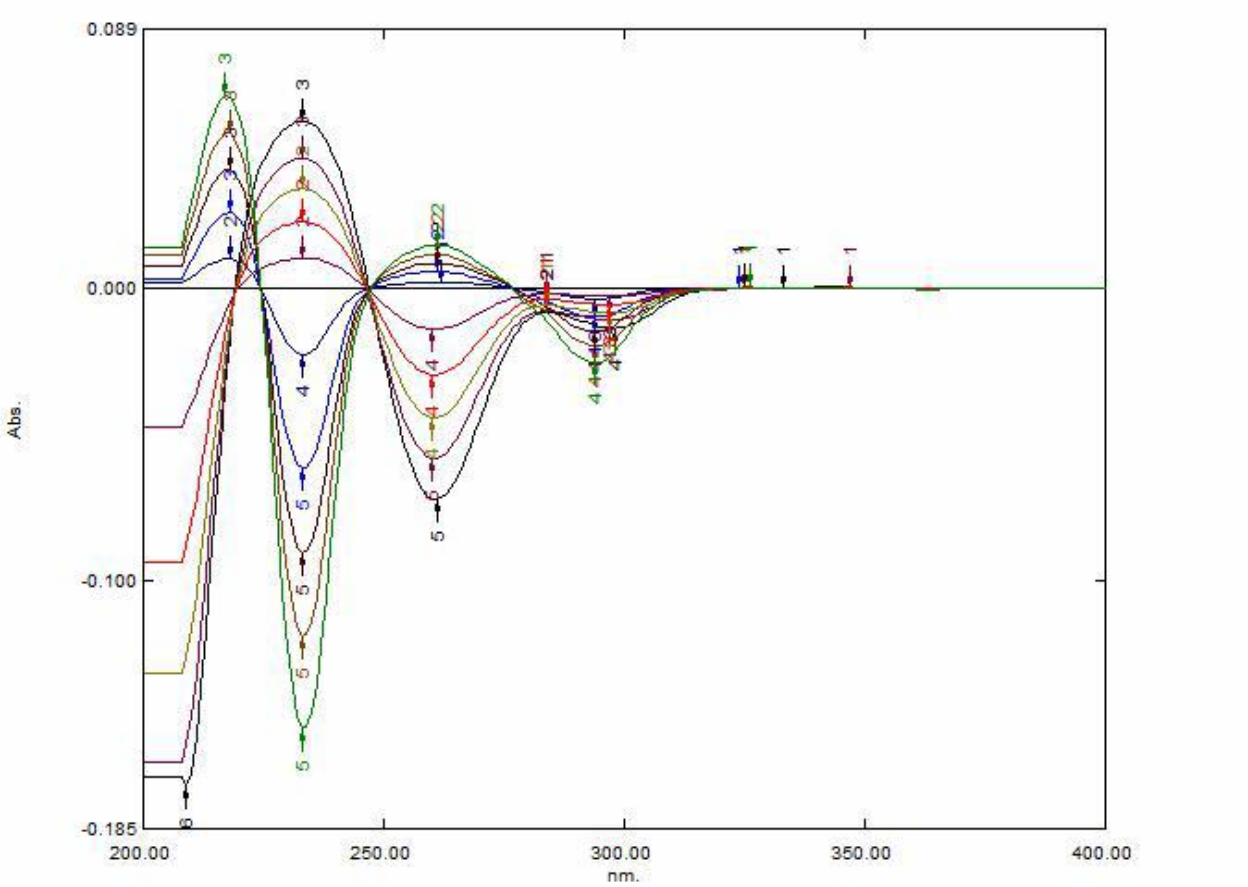

Figure 3: Overlain of derivative spectrum (1st order) of PCM and ETD

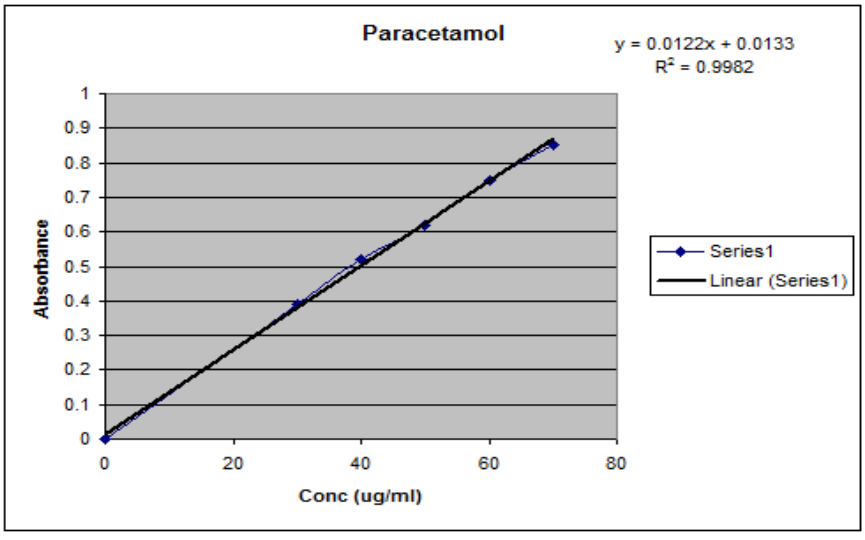

Figure 4: Standard Calibration Curve of Pure Paracetamol

Table 1: Statistical parameter of the calibration curve

Statistical Parameter	PCM	ETD
λmax	256nm	286nm
Linearity range	5-25µg/ml	3-16µg/ml
Linearity equation	y = 0.0017X-0.0012	y = 0.0033X+0.0043
Slope	0.0017	0.0032
Intercept	0.0011	0.0044
Standard deviation of slope	0.00004	0.000055
Standard deviation of intercept	0.00017	0.00036
Correlation co-efficient	0.9994	0.9983

Table 2: Intraday precision data for PCM and ETD

PCM			ETD		
Conc. (µg/ml)	Mean absorbance at 227.28 nm ± SD (n=3)	% RSD	Conc. (µg/ml)	Mean absorbance at 223.56 nm ± SD (n=3)	% RSD
5	0.006	1.14	2	0.009	0.89
15	0.023	1.49	10	0.035	1.24
25	0.039	1.01	18	0.058	0.73

Table 3: Inter day precision data for PCM and ETD

PCM			ETD		
Conc. (µg/ml)	Mean absorbance at 227.28 nm ± SD (n=3)	% RSD	Conc. (µg/ml)	Mean absorbance at 223.56 nm ± SD (n=3)	% RSD
5	0.007	1.23	2	0.010	0.65
15	0.024	1.81	10	0.036	1.67
25	0.042	1.58	18	0.056	1.67

Table 4: Accuracy data for PCM and ETD

Amt. of sample (µg/ml)		Amt. of std drug added(µg/ml)		Amt. recovered (µg/ml)		% Recovery ± SD	
PCM	ETD	PCM	ETD	PCM	ETD	PCM	ETD
10	8	0	0	9.87	8.19	98.52±0.08	101.27±0.47
10	8	8	6.5	16.96	13.45	98.39±0.12	98.76±0.45
10	8	10	8	19.53	15.57	100.21±0.13	98.89±0.63
10	8	12	9.7	21.47	16.17	101.14±0.13	97.72±0.57

Table 5: Summary of validation parameters

Parameter	PCM	ETD
Linearity Range (µg/ml)	5-25	3-16
Regression equation	y = 0.0017X-0.0012	y = 0.0033X+0.0043
correlation co-efficient	0.9994	0.9983
Precision (% RSD) Intraday (n=3) Interday (n=3)	 1.01-1.49 1.23-1.81	 0.73-1.24 0.65-1.67
Accuracy or Recovery (%)	98.39-101.14	97.72-101.27
LOD (µg/ml)	0.33	0.36
LOQ (µg/ml)	1.01	1.09

Table 6: Assay result of marketed formulation

Tablet	Label claim (mg/tablet)		Assay ± SD (% of label claim)	
	PCM	ETD	PCM	ETD
Etogesic-P	500	400	98.538 ± 0.2713	101.433 ± 0.2754

The calibration curves were constructed by plotting drug concentration versus the absorbance values of first derivative spectrum at 227.28 nm for PCM and 223.56 nm for ETD. Standard calibration curves for PCM and ETD were linear with Correlation coefficients (r^2) values in the range of 0.9994 and 0.9983 respectively at the selected wavelengths and the values were average of five readings. The Statistical parameter of the calibration curve was shown in table 1.

LOD and LOQ were found to be 0.33 µg/ml and 1.01 µg/ml for PCM and 0.36µg/ml and 1.09 µg/ml for ETD. Precision study showed co-efficient of variance (% CV) values less than 2 % for both PCM and ETD respectively. Result for the intra-day and inter-day precision was shown in table 2 and 3.

RESULT AND DISCUSSION

The accuracy of the method was confirmed by recovery studies from tablet at three different levels of 80 %, 100 %, 120 % recovery in the range of 98.39 % – 101.27 % justifies the accuracy of method. The results obtained from the recoveries of both drugs shown in table 4. The overall summary of all validation parameter was shown in table 5 which was carried out as per ICH guidelines. The results of marketed pharmaceutical dosage forms analysis of the combinations are shown in table 6 which showed good agreement with the labeled claim. There was no interference was observed from the presence of excipients in the amounts, which are commonly present in tablet dosage forms. From all the present work we can conclude that the proposed UV spectrometric method for quantitative determination of PCM and ETD in combined dosage form was found to be simple, rapid, precise, accurate and sensitive. The excipients of the commercial sample analyzed did not interfere in the analysis, which proved the specificity of the method for these formulations. The developed method was found to be more reproducible and sensitive.

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