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Method Development And Validation For The Simultaneous Estimation Of Paracetamol And Etodolac By Derivative UV Spectroscopic Method

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Abstract: A simple, novel, sensitive, precise and specific validated Spectrophotometric method was developed for simultaneous determination of Paracetamol and Etodolac in synthetic mixture and its dosage form. Methanol: Water (60:40) was selected as a common solvent for estimation of Paracetamol and Etodolac with λ_{max} at 247 nm and 280 nm respectively in methanol: water (60:40 v/v). Derivative method was selected for the estimation of both the drug simultaneously. The linearity was obtained in the concentration ranges of 5-25 µg/ml for Paracetamol and 2-18 µg/ml for Etodolac. The Zero Crossing Point (ZCP) of Paracetamol was 219.27 nm and Etodolac was 224.28 nm. The correlation coefficient was found to be 0.9994 and 0.9983 for Paracetamol and Etodolac respectively. The detection limit and quantification limit were found to be 0.34 and 1.02 µg/ml for Paracetamol and 0.37 and 1.11 µg/ml for Etodolac respectively. The method was validated as per the International Conference on Harmonization (ICH) guidelines.
Key Words: Paracetamol, Etodolac, Derivative Spectrophotometry.

Introduction:

Paracetamol (PCM), is chemically N-(4-hydroxyphenyl) acetamide [1, 2] (fig.1) [1]. It is a member of non-steroidal anti-inflammatory drugs (NSAIDs). PCM is official in Indian pharmacopoeia, British, European and United State pharmacopoeia [2-5]. Etodolac (ETD), is chemically 1,8-Diethyl-1,3,4,9-tetrahydropyrano (3,4-b)indole-1-acetic acid (fig.2)[6]. It is a member of non-steroidal anti-inflammatory drugs (NSAIDs). ETD is official in Indian pharmacopoeia, British, European and United State pharmacopoeia. A combination of 500 mg of Paracetamol and 400 mg of Etodolac is available commercially as tablet. This combination is used as analgesic and antipyretic in the treatment of Osteoarthritis.

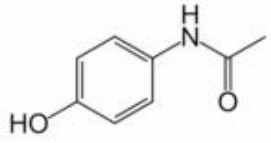

Fig.1: Structure of Paracetamol

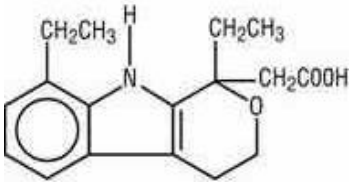

Fig.2: Structure of Etodolac

Literature survey revealed that many analytical methods are available for the determination of PCM [7-12] and ETD [13-17] individually and in combination with other drugs. However no method is available for the simultaneous estimation of these two drugs by UV-Derivative spectroscopic method. The multicomponent dosage form includes multiple entities and excipients; and posses considerable challenge to the analytical chemist during the development of assay procedure.

So, the aim of present study was to develop UV-derivative spectroscopic method and validate according to ICH guidelines [18].

Materials And Methods:

Instrument:

Instrument used was an UV-Visible double beam spectrophotometer, make: Shimadzu Corporation (Japan), Model UV-1800 with a bandwidth of 1.5 nm and a pair of 1 cm matched quartz cells. All weighing was done on analytical balance (Denver instrument, Germany). A sonicator (Electro quip Ultra sonicator, Texas) was used in the study. Calibrated glass wares were used throughout the work.

Chemicals:

Chemicals used were methanol (AR Grade, Sisco Chem Pvt Ltd, Andheri, Mumbai) and distilled water (Filtrate obtained through Distillation set). Marketed formulation containing Etodolac (400 mg) and Paracetamol (500 mg) (ETOGESIC-P, ETOFREE-P) was procured from the local pharmacy.

Method:

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. λ_{max} of both the drugs were 247 nm and 280 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.

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 (2-18 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 2-18 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

Application Of The Proposed Method For The Determination Of Paracetamol And Etodolac

Accurately weighed equivalent to 100 mg of PCM and 80 mg of ETD of tablet powder was transferred into 100 ml of volumetric flask, 20 ml of diluent was added to it and sonicated for 30 minutes and diluted up to the mark with diluent. The resulting solution was filtered and from the filtered solution aliquot was transferred to 10 ml volumetric flask and diluted with diluent to get a solution containing 10 µg/ml PCM and 8 µg/ml of ETD.

The absorbances of resulting solutions were measured at 224.28 nm and 219.27 nm. The concentration of PCM and ETD present in the sample solution was calculated by using the equation generated from calibration curve of respective drugs and % purity was calculated.

Result And Discussion:

The standard solutions of PCM and ETD were scanned separately in the UV range and First-order spectra for PCM and ETD were recorded which was shown in fig 3. The first order derivative absorption at 224.28 nm (zero cross point for ETD) was used for Paracetamol and 219.27 nm (zero cross point for PCM) was used for Etodolac. These two wavelengths can be employed for the determination of PCM and ETD without any interference from the other drug in their combined synthetic mixture.

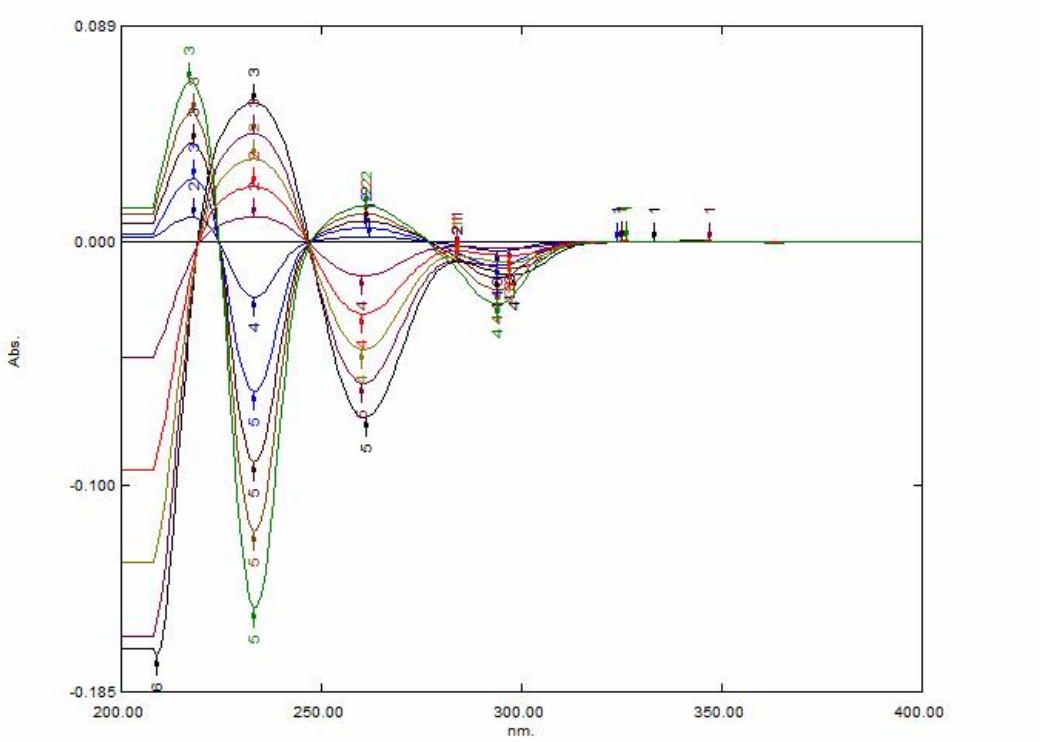

Fig. 3: Overlain of derivative spectrum (1st order) of PCM and ETD

Table 1: Statistical parameter of the calibration curve

Statistical Parameter	PCM	ETD
λ_{max}	247nm	280nm
Linearity range	5-25µg/ml	2-18µg/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 224.28 nm ± SD (n=3)**	**% RSD**	**Conc. (µg/ml)**	**Mean absorbance at 219.27 nm ± SD (n=3)**	**% RSD**
5	0.007	1.17	2	0.010	0.84
15	0.024	1.68	10	0.038	1.38
25	0.042	1.07	18	0.062	0.72

Table 3: Inter day precision data for PCM and ETD

PCM			ETD		
Conc. (µg/ml)	**Mean absorbance at 224.28 nm ± SD (n=3)**	**% RSD**	**Conc. (µg/ml)**	**Mean absorbance at 219.27 nm ± SD (n=3)**	**% RSD**
5	0.008	1.23	2	0.010	0.55
15	0.024	1.85	10	0.038	1.86
25	0.042	1.68	18	0.063	1.33

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	9.88	8.12	98.82±0.09	102.27±0.58
10	8	8	6.4	17.76	14.15	98.69±0.12	98.27±0.57
10	8	10	8	20.12	15.97	100.59±0.11	99.81±0.65
10	8	12	9.6	22.47	17.18	102.14±0.11	97.62±0.65

Table 5: Summary of validation parameters

Parameter	**PCM**	**ETD**
Linearity Range (µg/ml)	5-25	2-18
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)	 0.17-1.68 0.53-1.85	 0.35-1.63 0.55-1.86
Accuracy or Recovery (%)	98.69-102.14	97.62-102.27
LOD (µg/ml)	0.34	0.37
LOQ (µg/ml)	1.02	1.11

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	99.038 ± 0.2903	101.234 ± 0.2672
Etofree-P	500	400	98.076± 0.1459	100.539 ± 0.2672

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. 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 0.34 µg/ml and 1.02 µg/ml for PCM and 0.37µg/ml and 1.11 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.

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 97.62 % – 102.27 % 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 labelled 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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