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Research Article

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Simultaneous spectrophotometric estimation of Paracetamol and Aceclofenac by second order derivative method in combined dosage form

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ABSTRACT

The objective of the study was to develop a simple, accurate, precise and rapid a UV spectrophotometric i.e. second order derivative method for the determination of paracetamol and aceclofenac in combined dosage form i.e. tablets by using methanol as a solvent. The method was further validated by ICH guidelines. The proposed second order derivative method involves the measurement of absorbance of one drug at zero crossing point of other; hence wavelengths 267 nm and 224 nm were selected for the estimation of paracetamol and aceclofenac respectively. The linearity of the proposed method was found in the concentration range of 1 to 12 µg /ml (r^2= 0.9984) for Paracetamol and 1 to 14 µg /ml (r^2= 0.9985) for aceclofenac respectively. The percentage mean recovery was found to be 99.580 % for paracetamol and 101.166 % for aceclofenac respectively. The method was also statistically validated for its linearity, accuracy and precision. Both intra and inter day variations showed less percentage (%) RSD values indicating high grade of precision of this method.

Keywords: UV spectrophotometric estimation, second order derivative method, paracetamol, aceclofenac, methanol

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INTRODUCTION

Paracetamol, is chemically N-(4-Hydroxyphenyl) acetamide. It is non-steroidal anti-inflammatory, analgesic and anti-pyretic drug. It is official in I.P. [1] and B.P. [2]

Aceclofenac, chemically {[[2-[(2,6-Dichlorophenyl)amino]phenyl]acetyl]oxy}Acetic acid. It is the non-steroidal anti-inflammatory, analgesic and anti-inflammatory drug. It is used in treatment of relief in variety of painful condition.

In literature survey reveals HPLC [3-6] and UV spectrophotometric method [6-8] for simultaneous determination of paracetamol and aceclofenac in combined dosage form.

EXPERIMENTAL SECTION

Instrument and reagents

Spectral scan was made on a Shimadzu UV-spectrophotometer, model 1800 (Shimadzu, Japan) with spectral band width of 0.5 nm with automatic wavelength corrections by using a pair of 10 mm quartz cells. All spectral measurements were done by using UV-Probe 2.42 software.

Reference standard of paracetamol and aceclofenac were obtained from reputed firm with certificate of analysis.

Preparation of standard drug solutions
10 mg standard paracetamol was weighed accurately and transferred to a 10 ml volumetric flask and sonicated with 5 ml methanol for 15 minutes. The volume was made up to the mark with methanol to give a stock solution of paracetamol of concentration 1000 µg /ml. From this solution, 1 ml of solution was pipetted out and transferred into 10 ml volumetric flask. The volume was made up to mark with methanol to give a working standard solution of concentration 100 µg/ml.

Similarly 10 mg standard aceclofenac was weighed accurately and transferred to a 10 ml volumetric flask and sonicated with 5 ml of distilled water for 15 minutes. The volume was made up to the mark with methanol to give a stock solution of methanol of concentration 1000 µg /ml. From this solution, 1 ml of solution was pipetted out and transferred into 10 ml volumetric flask. The volume was made up to mark with methanol to give a working standard solution of concentration 100 µg/ml.

Estimation from tablets
Twenty tablets were weighed accurately and average weight of each tablet was determined. Powder equivalent to 50 mg of paracetamol and 10 mg of aceclofenac was weighed and transferred in 100 ml of volumetric flask. A 30 ml of methanol added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with methanol to give concentration as 500 µg /ml of paracetamol and 100 µg/ml of aceclofenac respectively. For working sample solution 1 ml of such solution was diluted to 100 ml and such solution was used for analysis.

Method: Second order derivative method
(a) For Paracetamol
For the selection of analytical wavelength, 100 µg/ml solution of paracetamol was scanned in the spectrum mode from 350 nm to 200 nm by using methanol as blank. The second order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. From the spectrum, the amplitude of the second derivative spectrum was measured at 267 nm.

(b) For aceclofenac
For the selection of analytical wavelength, 100 µg/ml solution of aceclofenac was scanned in the spectrum mode from 350 nm to 224 nm by using methanol as blank. The second order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. From the spectrum, the amplitude of the second derivative spectrum was measured at 224 nm.

Preparation of calibration curves
Series of solutions containing 1 – 10 µg/ ml of paracetamol and 1 -10 µg/ ml of aceclofenac were used to determine linearity of the proposed method respectively. Solutions were scanned in the spectrum mode and absorbance spectra were converted to second order derivative spectra. The overlain spectra of paracetamol and aceclofenac were given in Fig. 1(a), 1(b) respectively.

After observing the overlain second order derivative spectra of paracetamol and aceclofenac, the zero crossing points of both drugs were selected for analysis of other drug. The first wave length selected was 267 nm, the zero crossing point of aceclofenac where paracetamol showed considerable absorbance. The second wavelength was 224 nm, the zero crossing point of paracetamol, where aceclofenac showed considerable absorbance. The calibration curves were plotted of amplitude against concentrations [Fig. 2 (a), 2(b)].

Fig. 1(a): Overlay spectra of second order derivative of paracetamol in the concentration range of 2 and 12 µg/ ml at 267 nm

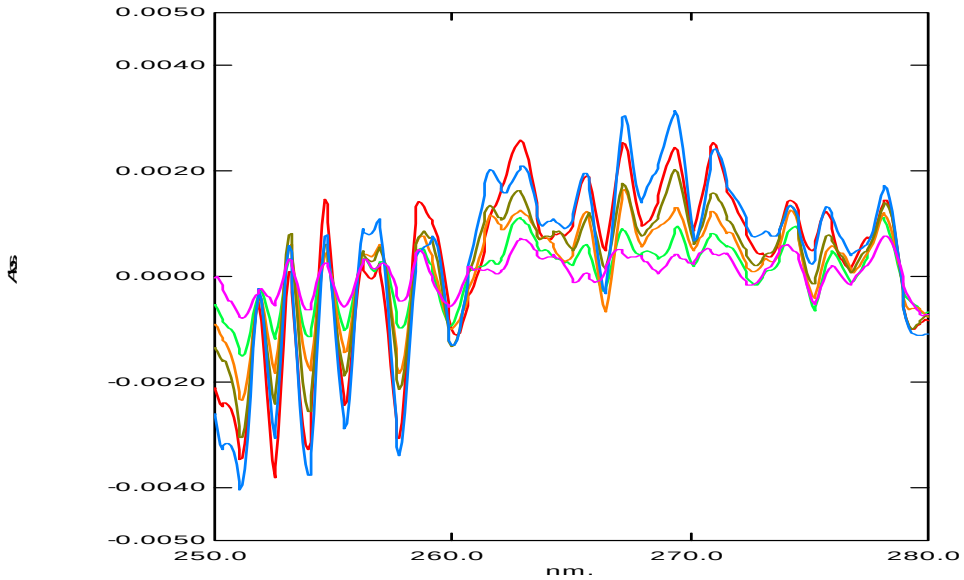


Fig. 1(b): Overlay spectra of second order derivative of aceclofenac in the concentration range of 2 and 14 µg/ ml at 224 nm

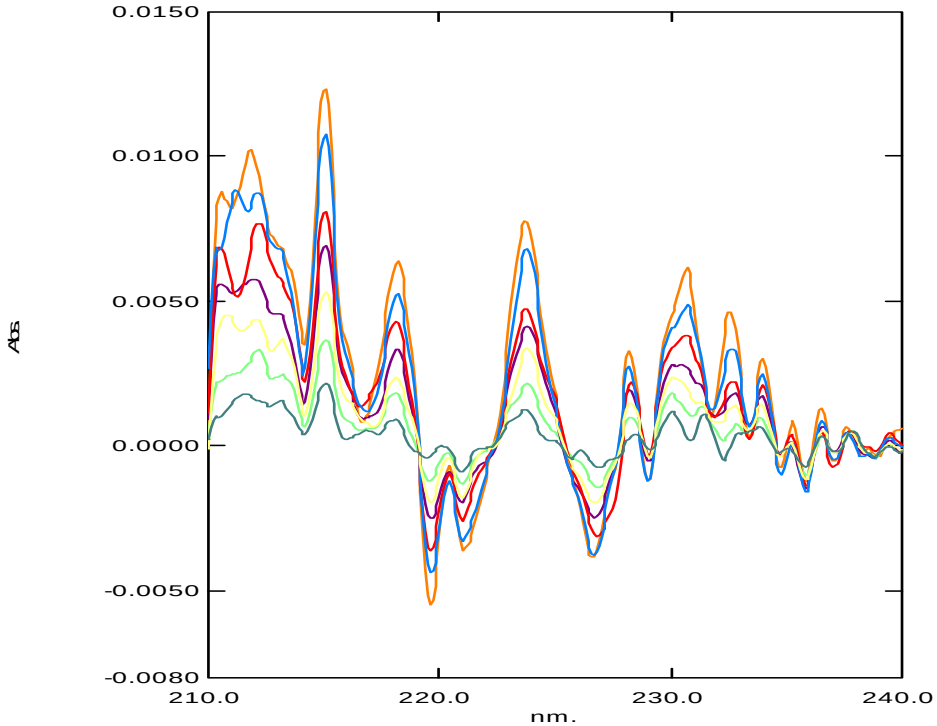


Fig.2 (a): Calibration curve of paracetamol in the concentration range of 2-10 µg/ml

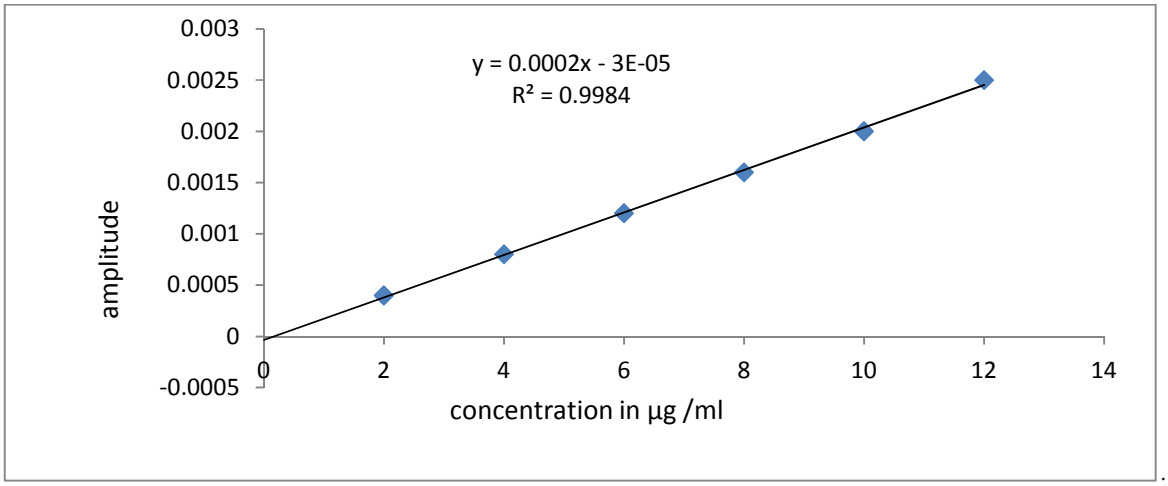
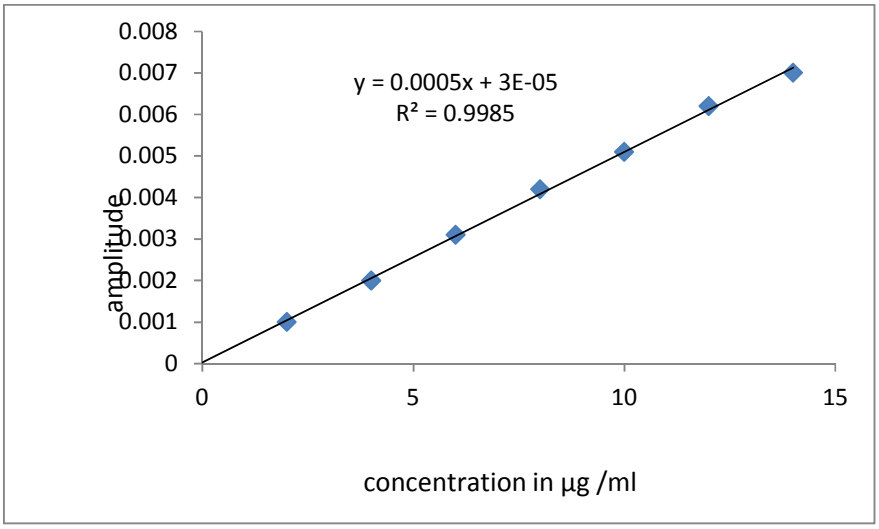


Fig.2 (b): **Calibration curve of aceclofenac in the concentration range of 2-14 µg/ml**



Results of the analysis are given in table 1.

Table 1: Values of results of optical and regression of drugs

Parameter	paracetamol	aceclofenac
Detection Wavelength (nm)	267	224
Beer Law Limits (µg/ml)	1-12	1-14
Correlation coefficient(r^2)	0.9998	0.9985
Regression equation (y=b+ac)		
Slope (a)	0.0002	0.0005
Intercept (b)	-0.00003	0.00003

Estimation from capsules

Powdered from twenty capsules were collected and weighed accurately and average weight of powder from each capsule was determined. Powder equivalent to 50 mg of paracetamol and 10 mg of aceclofenac was weighed and transferred in 100 ml of volumetric flask. A 30 ml of methanol was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with methanol to give concentration as 50 µg /ml of paracetamol and 10 µg /ml of aceclofenac respectively. A 10 ml of such solutions was diluted to 100 ml. It was scanned in the range of 200-350 nm against methanol. The absorbance spectra were converted to second order derivative spectra. Calculations were done as per the equations. The concentrations of paracetamol and aceclofenac

present in capsules were calculated by substituting the values of absorbance in linearity equations.

(a) For paracetamol Y = 0.0002x – 0.00005
(b) For aceclofenac Y = 0.0005x + 0.00005

Method Validation
These methods were validated according to ICH guidelines.

Accuracy
To ascertain the accuracy of proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). Percentage recovery for paracetamol and aceclofenac were 100.02 % to 100.15 and 100.03 % to 100.12 % respectively. (Table2).

Table 2: Statistical evaluation of the data subjected to accuracy

Level of % recovery	Amount present in µg/ml		Amount added in µg/ml		Amount found in µg/ml		% Recovery		Mean % recovery	
	PARA	ACE	PARA	ACE	PARA	ACE	PARA	ACE	PARA	ACE
80%	5	2	4	1.6	9.0054	3.603	100.06	100.11	100.13	100.03
	5	2	4	1.6	9.0108	3.602	100.12	100.07		
	5	2	4	1.6.	9.0189	3.607	100.21	99.93		
100%	5	2	5	2.0	9.9987	3.994	99.87	100.27	100.02	100.12
	5	2	5	2.0	10.009	4.003	100.09	100.17		
	5	2	5	2.0	10.011	4.004	100.11	99.92		
120%	5	2	6	2.4	11.027	4.411	100.25	99.85	100.15	100.11
	5	2	6	2.4	11.015	4.406	100.14	100.31		
	5	2	6	2.4	11.006	4.402	100.06	100.16		

PARA = Paracetamol, ACE= Aceclofenac

Linearity
The linearity of measurement was evaluated by analyzing different concentration of the standard solutions of paracetamol and aceclofenac. For both the drugs concentration range was found to be 1-12 µg/ml for paracetamol and 1-14 µg/ml for aceclofenac.

Precision
The method precision was established by carrying out the analysis of powder blend from capsules containing 50 mg of paracetamol and 10 mg of aceclofenac. The assay was carried out for the drugs by using proposed analytical method in six replicates. The values of relative standard deviation were 1.934 % for paracetamol and 1.329 % for aceclofenac in respectively indicating the sample repeatability of the method. The results obtained are tabulated in table 3**.**

Table 3: Statistical evaluation of the data subjected to method of precision

Sr. No.	Sample No.	% Assay	
		paracetamol	aceclofenac
1	1	100.0	100.0
2	2	97.5	102.0
3	3	101.0	102.0
4	4	98.0	99.00
5	5	102.5	102.0
6	6	98.5	102.0
Mean % assay		**99.58**	**101.16**
%R.S.D.		**1.934**	**1.329**

Intra-day precision was estimated by assaying tablets powder blend containing 50 mg of Paracetamol and 10 mg of aceclofenac. The assay was carried out for the drugs by using proposed analytical method in six replicates. The results were average for statistical evaluation.

Inter-day precision was estimated by assaying tablets powder blend containing 50 mg of paracetamol and 10 mg of aceclofenac for three consecutive days (i.e. 1st, 3rd and 5th days). The statistical validation data for intra and inter day

precision is summarized in table 4.

Table 4: Summary of validation parameter for intra-day and inter-day

Sr. No.	Parameters	Paracetamol	aceclofenac
1	Intra-day precision (N=3)amount found ± % R.S.D.	99.85% 1.205	92.75% 1.133
2	Inter-day precision (N=3)amount found ± % R.S.D.	98.88 1.259	96.75% 1.458

Both intra- day and inter-day precision variation found to be less in % RSD values. It indicates high degree of precision of the method.

RESULTS AND DISCUSSION

The developed second order derivative spectrophotometric method for simultaneous determination of paracetamol and aceclofenac in tablet formulation was found to be simple and convenient for the routine analysis of two drugs. The method is used to eliminate the spectral interference from one of the two drugs while estimating the other drug by selecting the zero crossing point on the derivative spectra of each drug as the selected wavelength. The proposed method is accurate, precise and reproducible. It is confirmed from validation data as given in tables 1 to 4. The % RSD was found to be less than 1, which indicates validity of method. Linearity was observed by linear regression equation method for paracetamol and aceclofenac in different concentration range. The correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity figure 2 (a) and 2 (b).

The assay results obtained by proposed method is shown in table 2 are in good agreement. Hence proposed method can be used for routine analysis of these two drugs in combined dosage form. Method is simple, accurate, precise, reliable, rapid, sensitive, reproducible and economical. It is validate as per ICH guidelines.

CONCLUSION

The proposed method is simple, precise, accurate and rapid for the determination of paracetamol and aceclofenac in combined dosage form. This method can be adopted as an alternative to the existing methods. It can be easily and conveniently adopted for routine quality control analysis.

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