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Simultaneous spectrophotometric estimation of aceclofenac and thiocolchicoside by first 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. first order derivative method for the determination of aceclofenac and thiocolchicoside in combined dosage form i.e. tablets by using methanol as a solvent. The method was further validated by ICH guidelines. The proposed first order derivative method involves the measurement of absorbance of one drug at zero crossing point of other; hence wavelengths 259 nm and 353.6 nm were selected for the estimation of aceclofenac and thiocolchicoside respectively. The linearity of the proposed method was found in the concentration range of 1 to 14 µg /ml (r^2= 0.9995) for aceclofenac and 1 to 14 µg /ml (r^2= 0.9999) for thiocolchicoside respectively. The percentage mean recovery was found to be 100.52 % for aceclofenac and 100.72% for thiocolchicoside and 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, first order derivative method, thiocolchicoside, aceclofenac, methanol

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INTRODUCTION

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.

Thiocolchicoside, a semi synthetic derivative of naturally occurring compound of colchicoside from the seeds of various species of colchicum antumnale (autumn crocus, meadow saffron, Gloriosa upuba), chemically,*N*-[(7*S*)-3-(β-D-Glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide It is centrally acting muscles relaxant and it also show analgesic activity. It is used in treatment of muscular pain and gout.

In literature survey reveals, UPLC [1], HPTLC [2], HPLC [3] and UV spectrophotometric methods [3,4] for simultaneous determination of thiocolchicoside and acelofenac in combined dosage form.

MATERIALS AND METHODS

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 aceclofenac and thiocolchicoside were obtained from reputed firm with certificate of analysis.

Preparation of standard drug solutions

A 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.

Similarly 10 mg standard thiocolchicoside 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 thiocolchicoside 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 100 mg of aceclofenac and 4 mg of thiocolchicoside 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 1000 µg/ml of aceclofenac and 40 µg /ml of thiocolchicoside respectively. For working sample solution 1 ml of such solution was diluted to 100 ml and such solution was used for analysis.

Experimental

Method: first order derivative method

(a) For aceclofenac

For the selection of analytical wavelength, 100 µg/ml solution of aceclofenac was scanned in the spectrum mode from 350 nm to 200 nm by using methanol as blank. The first order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. From the spectrum, the amplitude of the first derivative spectrum was measured at 259 nm.

(b) For thiocolchicoside

For the selection of analytical wavelength, 100 µg/ml solution of thiocolchicoside was scanned in the spectrum mode from 350 nm to 200 nm by using methanol as blank. The first order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. From the spectrum, the amplitude of the first derivative spectrum was measured at 353.6 nm.

Preparation of calibration curves

Series of solutions containing 1 -14 µg/ ml of aceclofenac and 1 – 14 µg/ ml of thiocolchicoside were used to determine linearity of the proposed method respectively. Solutions were scanned in the spectrum mode and absorbance spectra were converted to first order derivative spectra. The overlain spectra of aceclofenac and thiocolchicoside were given in Fig. 1(a), 1(b) respectively.

Fig. 1(a): Overlay spectra of first order derivative of aceclofenac in the concentration range of 2 and 14 µg/ ml at 259 nm

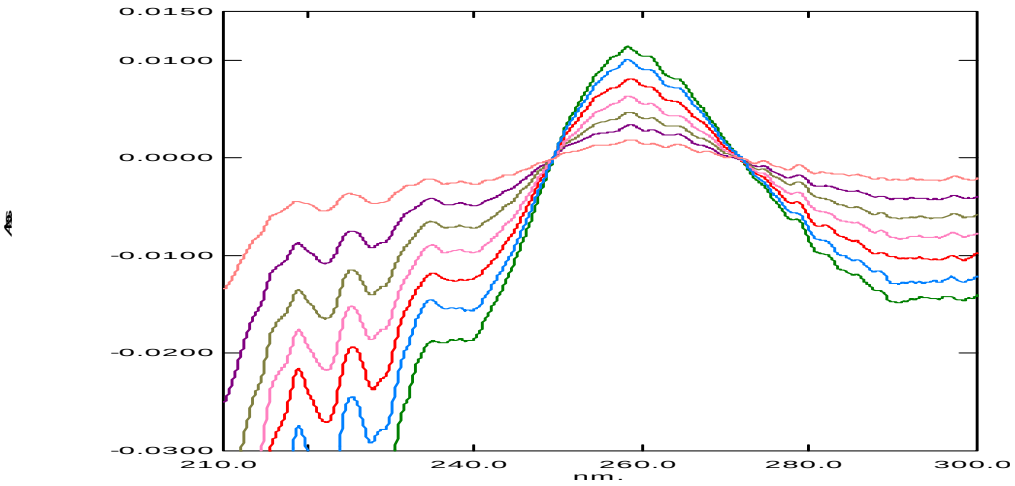
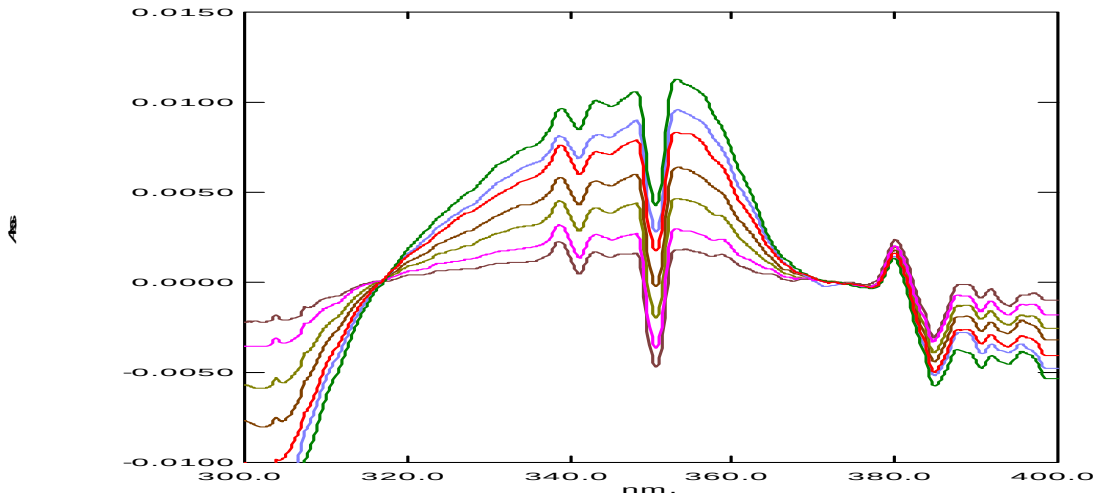


Fig. 1(b): Overlay spectra of first order derivative of thiocolchicoside in the concentration range of 2 and 14 µg/ ml at 353.6 nm



After observing the overlain first order derivative spectra of aceclofenac and thiocolchicoside, the zero crossing points of both drugs were selected for analysis of other drug. The first wave length selected was 253 nm, the zero crossing point of thiocolchicoside where aceclofenac showed considerable absorbance. The first wavelength was 353.6 nm, the zero crossing point of aceclofenac, where thiocolchicoside showed considerable absorbance. The calibration curves were plotted of amplitude against concentrations [Fig. 2 (a), 2(b)].

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

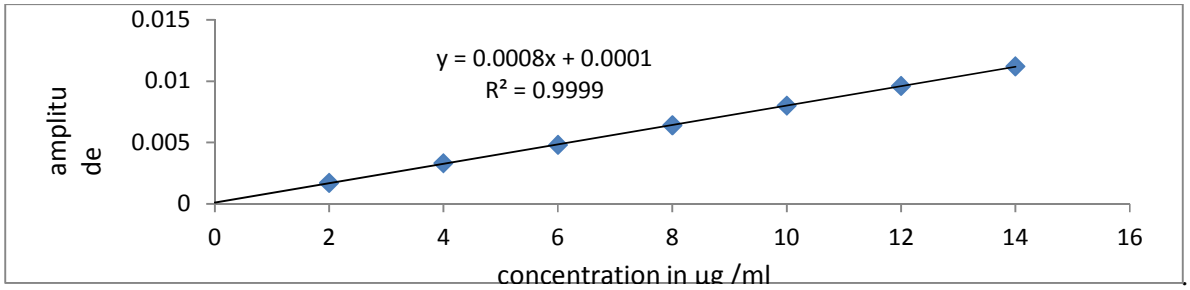
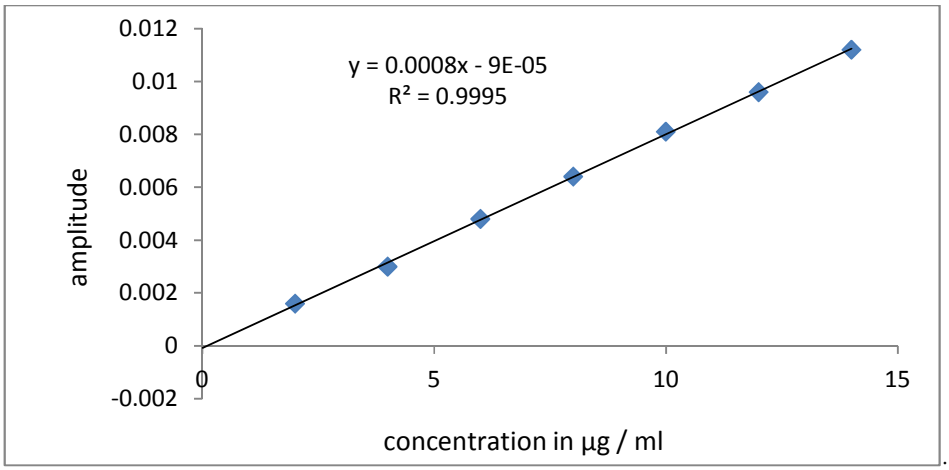


Fig.2 (b): Calibration curve of thiocolchicoside in the concentration range of 2-14 µg/ml



Results of the analysis are given in table 1.

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

Parameter	Aceclofenac	Thiocolchicoside
Detection Wavelength (nm)	259	353.6
Beer Law Limits (µg/ml)	1-14	1-14
Correlation coefficient(r^2)	0.9999	0.9995
Regression equation (y=b+ac)		
Slope (a)	0.0008	0.0008
Intercept (b)	0.0001	-0.00009

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 100 mg of aceclofenac and 4 mg of thiocolchicoside 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 40 µg /ml of thiocolchicoside and 1000 µg /ml of aceclofenac respectively. A 10 ml of such solutions was diluted to 100 ml. It was scanned in the range of 200-400 nm against methanol. The absorbance spectra were converted to first order derivative spectra. Calculations were done as per the equations. The concentrations of aceclofenac and thiocolchicoside present in capsules were calculated by substituting the values of absorbance in linearity equations.

(a) For aceclofenac Y = 0.0008x +0.0001
(b) For thiocolchiciside Y = 0.0008x - 0.00009

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 aceclofenac and thiocolchicoside were 99.82 % to 100.25 and 99.74 % to 100.27 % respectively. (Table2).

Linearity

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

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	
	THIO	ACE	THIO	ACE	THIO	ACE	THIO	ACE	THIO	ACE
80%	4	10	3.2	08	7.207	18.039	100.11	100.22	100.17	100.16
	4	10	3.2	08	7.211	18.030	100.16	100.17		
	4	10	3.2	08	7.217	18.043	100.24	100.09		
100%	4	10	4	10	8.0072	20.018	100.09	100.25	100.01	100.06
	4	10	4	10	7.979	20.022	99.74	100.11		
	4	10	4	10	8.016	19.964	100.21	99.82		
120%	4	10	4.8	12	8.818	22.052	100.27	100.24	100.18	100.20
	4	10	4.8	12	8.804	22.048	100.05	100.22		
	5	2	4.8	12	8.814	22.035	100.16	100.16		

THIO = thiocolchicoside, ACE= Aceclofenac

Precision

The method precision was established by carrying out the analysis of powder blend from capsules containing 4 mg of thiocolchicoside and 100 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 0.9961 % for thiocolchicoside and 0.6100 % 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	
		Thiocolchicoside	Aceclofenac
1	1	101.25	100.00
2	2	100.62	101.25
3	3	101.25	101.25
4	4	98.75	100.00
5	5	101.25	101.25
6	6	100.00	100.62
Mean % assay		100.52	100.72
%R.S.D.		0.9961	0.6100

Intra-day precision was estimated by assaying tablets powder blend containing 4 mg of thiocolchicoside and 100 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 4 mg of thiocolchicoside and 100 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	Thiocolchicoside	aceclofenac
1	Intra-day precision (N=3)amount found ± % R.S.D.	100.21% 0.9952	100.57% 0.9941
2	Inter-day precision (N=3)amount found ± % R.S.D.	98.81 0.9909	98.67% 0.8712

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 first order derivative spectrophotometric method for simultaneous determination of aceclofenac and thiocolchicoside 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 aceclofenac and thiocolchicoside 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 aceclofenac and thiocolchicoside 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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