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Difference spectrophotometric and RP-HPLC methods for simultaneous determination of aceclofenac and paracetamol in combined tablet dosage form

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ABSTRACT

A simple difference spectrophotometric method has been developed for the estimation of aceclofenac and paracetamol from pharmaceutical dosage form. The change in pH altered the absorption spectra of aceclofenac and paracetamol. Differential spectrum of paracetamol and aceclofenac in alkaline and acidic solution showed maximum absorbance at 267 nm and 246 nm and minimum at 232 nm and 276 nm respectively. Measurement of absorbance was carried out at zero-crossing wavelength 247 nm for paracetamol and 256.7nm for aceclofenac. Beer's law was valid in the concentration range of 1-20μg/ml for aceclofenac and 1-50 μg/ml for paracetamol. This method was validated for precision (n=6) and accuracy (100.15 ±0.761 %) and (99.73±0.436 %) respectively (n=3) at each level. The reverse phase high performance liquid chromatographic (RP-HPLC), has been developed and validated for the simultaneous estimation of paracetamol (PARA) and aceclofenac (ACE) in tablet dosage form. Waters C_{18} Octadecyl Silane (ODS) column (250 × 4.6 mm i. d., 5μ particle size) in isocratic mode was used. The mobile phase used was acetonitrile: 50 mM sodium dihydrogen phosphate (50:50) v/v, pH 3.0 ± 0.1 with orthophosphoric acid. The flow rate was 1.5 mL per minute and eluent was monitored at 276 nm. The retention times of PARA and ACE were found to be 1.92 min and 9.28 min respectively. The linearity for paracetamol and aceclofenac were in the range of 5 - 50μg /mL and 1.0 - 10μg /mL respectively (n=6). Percentage recoveries obtained for paracetamol and aceclofenac were 100.7% and 100.4 % respectively. The relative standard deviation was found to be 1.78% and 1.80 % for paracetamol and aceclofenac in triplicate analysis. Thus the proposed methods are precise, accurate, selective and rapid for simultaneous estimation of paracetamol and aceclofenac in tablet formulation.

KEYWORDS

Difference spectrophotometry;
Paracetamol;
Aceclofenac;
RP-HPLC.

INTRODUCTION

Paracetamol I is 4-hydroxyacetanilide also known as acetaminophen having analgesic and antipyretic activities. Paracetamol is official in IP[1], BP[2][2] and USP[3]. Aceclofenac is a novel non-steroidal anti-inflammatory drug, indicated for the treatment of pain and inflammation[4]. Chemically aceclofenac II is 2-[[2-[2-[(2, 6-dichlorophenyl) amino] phenyl] acetyl] oxy] acetic acid, which is a phenyl acetic acid derivative. Aceclofenac is official in BP[4]. Fixed-dose combination of 500 mg of paracetamol and 100 mg of aceclofenac is available as

tablet dosage form in the market and is used in treatment of pain and management of rheumatic arthritis, osteoarthritis and ankylosing spondylitis.

Literature survey reveals that there are UV and HPLC methods reported for the estimation of paracetamol in pharmaceutical formulations[8-17]. Literature survey revealed few spectrophotometric methods and RP-HPLC methods for the simultaneous estimation of paracetamol and aceclofenac[7,12]. Few bio-analytical HPLC methods were reported for the estimation of aceclofenac[18]. In the present study we have compared difference spectroscopy and RP-HPLC for estimation of paracetamol and aceclofenac simultaneously.

EXPERIMENTAL

Drugs and chemicals

Tablet formulations containing 100 mg of aceclofenac and 500 mg paracetamol were procured from the market. Acetonitrile HPLC grade was obtained from Merck India Ltd; HPLC grade water was prepared by Millipore water Purification system (Milli-Q) in the Lab., Sodium dihydrogen phosphate (AR grade) and Orthophosphoric acid (AR grade) were obtained from S.D. Fine chemicals and 0.1N NaOH, Methanol, and 0.1N HCl obtained from Qualigens, India.

Instruments

Shimadzu UV/VIS Spectrophotometer (Model 2450 Shimadzu, Japan)

High Performance Liquid Chromatograph, JASCO INTELLIGENT pump 980 equipped with INTELLIGENT UV VISIBLE DETECTOR UV–975, universal injector (Rheodyne) with injection volume of 20µL, BORWIN 1.5 VERSION software, Waters RP-C_{18} (250×4.6 mm), and 5µ particle size forms the stationary phase. Reference standards of paracetamol and aceclofenac were procured from IPCA Laboratories, Mumbai.

Chromatographic conditions

When several mobile phases were tried such as

1. Methanol: acetonitrile: water (80:10:10)
2. Acetonitrile: methanol (80:20).
3. Acetonitrile: methanol (70:30).

Finally the mobile phase selected for HPLC determination was acetonitrile: sodium dihydrogen phosphate buffer 50 mM (50:50) v/v; pH 3.0 ± 0.1 with orthophosphoric acid was found to be optimum. Mobile phase flow rate was 1.5 mL/min and eluent was monitored at 276 nm. Injection volume was 20µl.

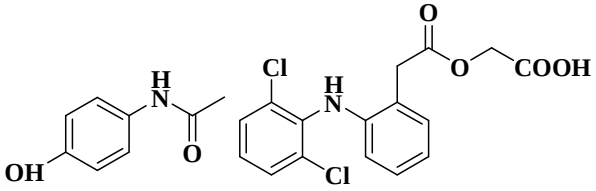
METHOD 1: Difference spectroscopy

1. Preparation of standard and sample solution

Pure 100mg of Paracetamol and Aceclofenac were weighed accurately and dissolved in 25 ml methanol and volume was made up to 100 ml in a volumetric flask (1mg/1ml). These solutions were further diluted with 0.1N NaOH and 0.1N HCl separately to give a working range of 1-50µg/mL of paracetamol and 1-20µg/mL aceclofenac.

2. Estimation of combined dosage form

Twenty tablets were weighed and powdered in a glass mortar and the powder equivalent to 100 mg of paracetamol along with 20 mg of aceclofenac was weighed accurately and transferred into a 100ml volumetric flask. The content was extracted with methanol and volume was made up to 100ml mark by distilled water. This solution was filtered through Whatman filter paper (0.45µ). These solutions were further diluted with 0.1N NaOH and 0.1N HCl separately. pH induced



1. Structure of aceclofenac 2. Structure of aceclofenac

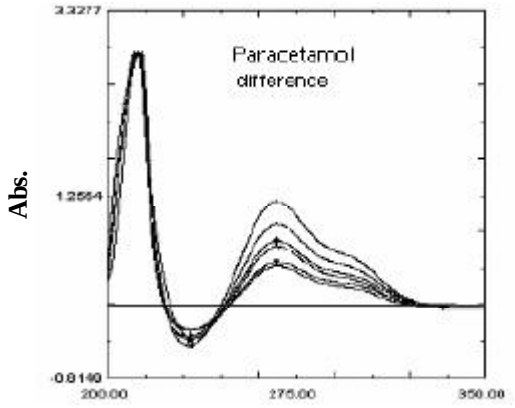


Figure 1: pH-induced difference spectrum of Paracetamol

spectra of Paracetamol is shown in figure 1. pH induced spectra of Aceclofenac is shown in figure 2.

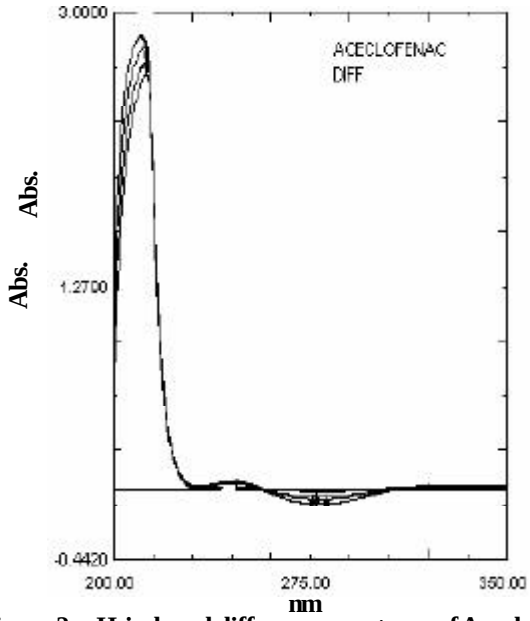


Figure 2: pH-induced difference spectrum of Aceclofenac

TABLE 1: Optimum conditions, optimum characteristic and statistical data of the regression equation

Parameters	Observation	
	Paracetamol	Aceclofenac
Zero-crossing wavelength	256.8 nm	247nm
Beer's law limit	1-50 (μg/ml)	1-20 (μg/ml)
Precision (% RSD)	0.459	0.481
Correlation coefficient	0.9995	0.9960
Regression equation(Y*)		
Slope(a)	0.06954	0.02064
Intercept (b)	- 0.0079	- 0.0069

RSD = Relative standard deviation

TABLE 2: Result of rggedness pameter sudies

Analyte Aceclofenac	Tablet anlysis		Recovery sudy	
	% Label claim estimated* (Mean±SD)	R.S.D	% Recovery estimated* (Mean±SD)	R.S.D
Intraday -Interday				
Day I				
Morning				
ACE	99.38 ± 1.19	1.2	101.46 ± 1.9	1.17
PARA	98.032 ± 1.8	1.23	99.95 ± 1.075	1.076
Evening				
ACE	99.41 ± 0.97	0.97	99.05 ± 1.6	1.6
PARA	99.19 ± 1.48	1.49	98.41 ± 0.94	0.96
Day II				
Morning				
ACE	99.66 ± 1.57	1.58	97.01 ± 0.34	0.35
PARA	98.93 ± 1.83	1.85	99.42 ± 0.23	0.23

Average of five determinations; SD stands for standard deviation; R.S.D stands for Relative Standard Deviation

Precision study

Precision was measured in terms of repeatability (n=6). The percentage relative standard deviation for paracetamol and aceclofenac was found to be 0.459 % and 0.481% respectively. The results of precision study is as shown in TABLE 1.

Ruggedness study

Ruggedness was ascertained by carrying out the analysis for interday variation, intraday variation and different analysts.

The results of interday variation, intraday variation are as shown in TABLE 2.

And the results of different analysts are as shown in TABLE 3.

Recovery study

To study the accuracy of the above method, recovery studies were carried out, known quantity of Paracetamol and Aceclofenac standard mixture was added to definite amount of pre analyzed formulation at three different levels 50, 100, and 150% (n=3)at each level. The results of recovery study is as shown in TABLE 4.

RESULTS AND DISCUSSION

Absorption spectral analysis of paracetamol and aceclofenac showed difference in absorbance between an adjacent maximum (267 nm and 246.8 nm) and minimum (232 nm and 276.0 nm) respectively. Measurement of absorbance was carried out at zero-crossing wavelength 247nm for paracetamol and 256.7nm for aceclofenac.Zero crossing differential Overlain spectra of Paracetamol and Aceclofenac is shown in figure 3.

A calibration curve was obtained at these two wavelengths for series of concentrations (n=6) in the range of 1-20μg/ml for aceclofenac and 1-50μg/ml for paracetamol. It was found to be linear and hence suitable for estimation of the drug. The slope, intercept, correlation coefficient are summarized in (TABLE 1).

To study the accuracy of the above method, recovery studies were carried out, known quantity of paracetamol and aceclofenac standard mixture was added to definite amount of pre analyzed formulation at three different level 50, 100, and 150 %(n=3) at each

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TABLE 3: Different analyst study

Sr.no.	Analyst	Weight of tablet powder (mg/ml)	Absorbance		% Label claim*	
			247nm	256.7nm	ACE	PARA
1	1	76.31	0.689	0.904	100.47	101.07
2	2	76.32	0.691	0.907	100.50	101.21
				Mean	100.48	101.14
				± S.D.	0.0224	0.0762
				C.V	.0.0222	0.0753

*Mean of three estimations

TABLE 4: Recovery studies of paracetamol and aceclofenac (n=3 at each level)

Level	Amount added (µg/ml)		Amount found(µg/ml)		% Recovery ±SD
	Paracetamol	Aceclofenac	Paracetamol	Aceclofenac	
50%	5	1	5.006	1.005	99.00± 0.734
100%	10	2	10.023	2.056	100.15 ± 0.456
150%	15	3	15.080	3.008	99.73 ± 0.638

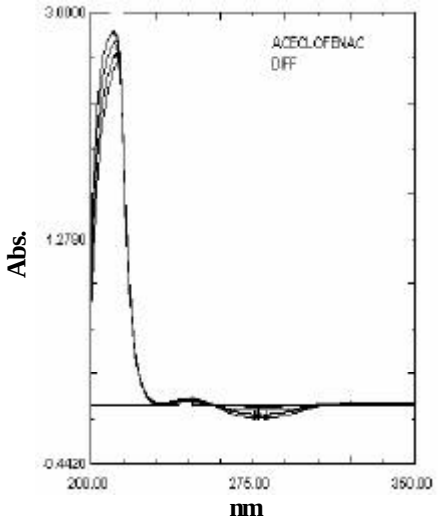

Figure 3: Zero crossing differential overlay spectrum of (a) Paracetamol (10µg/ml) and (b) Aceclofenac (10µg/ml)

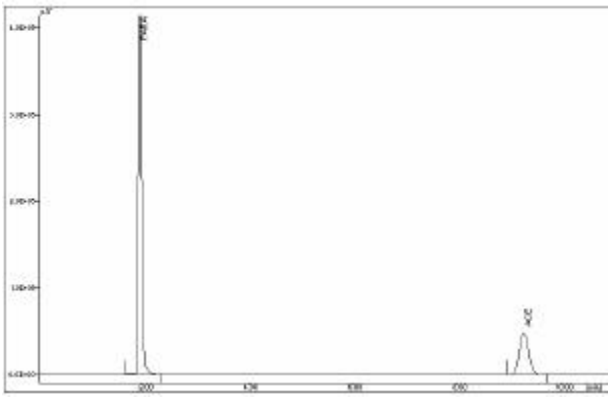

Figure 4:Typical chromatogram of paracetamol and aceclofenac

level. The total amount of paracetamol and aceclofenac determined using the proposed method was found to be (100.15 ±0.761%) and (99.73 ±0.436 %) respectively. The results are shown in TABLE 4.

Method II- RP-HPLC method

In the RP-HPLC method, separation and analysis Paracetamol and Aceclofenac were carried out in following condition:

Column specification: Princeton C18 column (250×4.6mm id) and 5µ particle size.

Moboile phase: The mobile phase used was acetonitrile: 50 mM sodium dihydrogen phosphate (50:50) v/v, pH 3.0±0.1 with orthophosphoric acid, filtered through 0.45µm membrane filter (degassed and sonicated).

Flow rate: flow rate is 1.5 mL per minute

Temperature: Ambient

Sample size: 20 µl

Detection wavelength: Detection of eluent was monitored at 276 nm.

Standard stock solution

Stock solutions of both the drugs aceclofenac and paracetamol were prepared by dissolving 100 mg of each drug separately in mobile phase to make volume of 100 mL. This gave 1000µg/mL of each drug.

Preparation of working standard

5 mL of the standard stock solution of paracetamol and 1 mL of the standard stock solution of aceclofenac was mixed and diluted to 100 mL with mobile phase (Solution I). The typical RP-HPLC chromatograph of Paracetamol And Aceclofenac is shown in figure 4.

Sample solution

Twenty tablets were weighed and powdered. Quantity equivalent to 500 mg of paracetamol and 100 mg of aceclofenac were taken in 100 mL volumetric flask containing mobile phase and kept for sonication for 10 min and final volume was made up to the mark with mobile phase (Solution A). The solution was filtered through 0.45 micron membrane filter paper and 1 mL of solution A was diluted to 100 mL with mobile phase to give Solution B. final concentration of 50µg/mL of paracetamol and 10µg/mL of aceclofenac.

Linearity and calibration

Aliquot of working standard solution (Solution 1) was taken in 10 mL volumetric flasks and diluted up to the mark with mobile phase such that the final concentration of paracetamol and aceclofenac were in the range of 5-50µg/mL and 1-10µg/mL respectively. Evaluations of two drugs were performed on PDA detector in wavelength range of 210-370 nm setting λmax of 276 nm. Peak area was recorded for all the peaks. The linearity of paracetamol and aceclofenac in the range of 5 - 50µg/mL and 1 - 10 µg/mL respectively with coefficient of correlation (r^2 = 0.9995) and (r^2 = 0.999) respectively (n=10) for each drug. The results are shown in TABLE 5.

TABLE 5 : Linearity and calibration for RP- HPLC method (n=10)

Sr. no.	Conc. (µg/ml) ACE	Peak area ACE	Conc. (µg/ml) PARA	Peak area PARA
1	1	186000.6	5	439950.9
2	2	287617.5	10	751166.3
3	3	384130.6	15	1153890
4	4	477692.5	20	1537559
5	5	585080.9	25	1856554
6	6	689885.1	30	2236823
7	7	782115.6	35	2570068
8	8	864467.5	40	2961017
9	9	1058034	50	3252350
10	10	1186938	60	3617167

Precision study

Precision of an analytical method is expressed as the S.D. and R.S.D. of the series of measurements. It was ascertained by replicate estimation of marketed formulation (n=5) are as shown in TABLE 6.

Assay

Standard and sample solutions (n = 6) were injected in to an injector of liquid chromatograph and peaks were recorded. From the average peak area ratio of paracetamol and aceclofenac the amount of drug samples (n = 6) were calculated. The values are given in TABLE 7.

Results

In replicate analysis (n = 6) of two drugs by the proposed method (RP-HPLC), the content of paracetamol and aceclofenac were found 494.45 mg/Tablet [Coefficient of variance, CV = 0.65] and 99.55 mg/Tablet [CV = 0.81] respectively. The values are given in TABLE 7.

Recovery study

To study the accuracy of the above method, recovery studies were carried out, known quantity of

TABLE 6: Results of % estimation of drugs in tablets (Precesion study) (n=5)

Brand Name: ZERODOL-P Average Wt. : 763.1mg									
Sr.No	Weight of tablet powder taken (mg/ml)	Weight of Standard (µg/ml)		Standard peak area		Sample peak area		% Estimated	
		ACE	PARA	ACE	PARA	ACE	PARA	ACE	PARA
1	76.30	10.01	50.0	1186937.5	3617166.66	1186958.3	369662.87	100.22	100.26
2	76.32					1186863.5	379663.13	100.11	100.14
3	76.30					1186953.32	389663.83	99.98	100.05
4	76.31					1186969.56	389665.11	100.05	100.26
5	76.32					1186943.63	369663.99	99.93	99.95
							Mean	100.06	100.13
							± S.D.	0.0665	0.1349
							C.V	0.0664	0.1347

*Average of Five determinations; SD stands for Standard Deviation, CV stands for coefficient of variance

TABLE 7: Assay of paracetamol and aceclofenac in tablets (ZERODOL-P)

Drug	Amount mg / tab		CV	Recovery
	Label claim	Found ± SD		(%)(n = 6)
Paracetamol	500	494.45	0.65	98.89
Aceclofenac	100	99.55	0.81	99.55

TABLE 8: Recovery study

Levels	Concentration of drug added in final solution (µg/mL)		Total % Recovery ± SD (n = 3 at each level)		Coefficient of Variance (CV)	
	PARA	ACE	PARA	ACE	PARA	ACE
80%	0.800	0.160	100.7±0.998	100.4±1.209	0.99	1.21
100%	1.000	0.200	99.50±0.549	100.68±0.392	0.55	0.39
120%	1.200	0.240	100.05±0.668	100.35±0.305	0.67	0.30

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TABLE 9: Robustness study (n=5)

Brand name: ZERODOL-P Flow rate:1ml average Wt. : 763.1mg									
Sr.no	Weight of tablet powder taken (mg/ml)	Weight of standard (µg/ml)		Standard peak area		Sample peak area		Retention time	
		ACE	PARA	ACE	PARA	ACE	PARA	ACE	PARA
1	76.30	10.01	50.0	2386937.5	5617166.66	2386958.3	569662.87	1.67	12.58
2	76.32					2386863.40	559663.13	1.67	12.56
3	76.30					2386953.12	55663.83	1.67	12.57
4	76.31					2386969.56	589665.11	1.68	12.58
5	76.32					2386943.63	569663.99	1.68	12.58

TABLE 10: Interday variation

Brand Name: ZERODOL-P (n=3) Average Wt. : 763.1mg									
Sr.no	Weight of tablet powder taken (mg/ml)	Weight of standard (mg/ml)		Standard peak area		Sample peak area		% Estimated	
		ACE	PARA	ACE	PARA	ACE	PARA	ACE	PARA
1	76.30	10.01	50.0	1186937.5	3617166.66	1186958.3	369662.87	100.22	100.26
2	76.32	10.02	50.01	1186945.3	3617166.23	1186863.5	379663.13	100.11	100.14
3	76.30	10.01	50.02	1186959.1	3617165.89	1186953.32	389663.83	99.98	100.05
							Mean	100.10	100.15
							± S.D.	0.0562	0.1248
							C.V	0.0564	0.1247

***Average of three determinations; SD stands for standard deviation, CV stands for coefficient of variance**

TABLE 11: Intraday variation

Brand Name: ZERODOL-P (n=3) Average Wt. : 763.1mg									
Sr.no	Weight of tablet powder taken (mg/ml)	Weight of standard (mg/ml)		Standard peak area		Sample peak area		% Estimated	
		ACE	PARA	ACE	PARA	ACE	PARA	ACE	PARA
1	76.30	10.01	50.0	1185937.5	3517166.66	1185958.3	369662.87	101.10	100.26
2	76.32	10.02	50.01	1185945.3	3517166.23	1185863.5	369663.13	101.40	100.14
3	76.30	10.01	50.02	1185959.1	3517165.89	1185953.32	369663.83	101.23	100.05
							Mean	101.24	100.22
							± S.D.	0.15044	0.10535
							C.V	0.1485	0.10538

***Average of three determinations; SD stands for Standard Deviation, CV stands for coefficient of variance**

paracetamol and aceclofenac standard mixture was added to definite amount of pre analyzed formulation at three different level 80, 100, and 120 %(n=3) at each level and contents were reanalyzed by the proposed method (RP-HPLC).The lower values of relative standard deviation (RSD) indicated that method is precise and accurate. The mean recoveries of paracetamol and aceclofenac were 100.7 % and 100.4 % respectively and showed no positive or negative interference of excipients in tablets. To ensure the reliability and practicability of the recovery studies were carried out.The values are shown in TABLE 8.

Method validation

1. Accuracy

Accuracy of the method was checked by recovery studies. The values are shown in TABLE 8. (n=3 at each level).

2. Precision

Precision of the method was studied by analysis of multiple samplings of homogeneous sample and expressed as CV. (n=5). The values are shown in TABLE 6.

3. Robustness

Robustness of the method was determined by making slight changes in chromatographic condition, like changing the flow rate from 1.5 ml/ min to 1ml/ min.(n=5). The values are shown in TABLE 9.

4. Ruggedness

Ruggedness was ascertained by carrying out the analysis for interday variation, intraday variation and different analysts.

The results of interday variation, intraday variation

TABLE 12: Different analyst

Sr.no	Weight of tablet powder taken (mg/ml)	Weight of standard (mg/ml)		Standard peak area		Sample peak area		% Label claim*	
		ACE	PARA	ACE	PARA	ACE	PARA	ACE	PARA
1	76.3	10.01	50.01	1185937.5	3517166.66	1185959.1	3517165.89	100.05	99.99
2	76.2	10.02	50.02	1185945.3	3517166.23	1185947.5	3517174.23	100.00	100.01
							Mean	100.02	100.00
							± S.D.	0.0251	0.0100
							C.V	0.0256	0.0102

TABLE 13: System suitability parameters

Sr. no	Parameters	Paracetamol	Aceclofenac
1	Number of theoretical plates(N)	7423.80	15966.12
2	Resolution (Rs)	0	38.48
3	Tailing factor (T)	1.02	1.10
4	Linearity range(µg/mL)	5 -50	1 -10
5	Limit of detection(µg/mL)	0.080	0.015
6	Limit of quantitation(µg/mL)	0.250	0.50
7	Capacity factor	202	1175
8	Selectivity factor	0	5.76
9	Asymmetry factor	1.13	0.95
10	Accuracy	100.7 %	100.4 %

TABLE 14: Comparative study between difference spectroscopic method and RP- HPLC method

Sr. no	Parameters	Difference spectroscopic method	RP-HPLC method
1	Solvent used	0.1 N NaoH, 0.1 N Hcl	Acetonitrile: sodium dihydrogen phosphate buffer 50 mM (50:50) v/v; pH 3.0 ±0.1 with orthophosphoric acid
2	Wavelength used	263.2 nm -paracetamol 256.7 nm -ceclofenac	276 nm
3	Linearity range	5 -50- Paracetamol 1 - 20 - Aceclofenac	5 - 50 - Paracetamol 1- 10 - Aceclofenac
4	Precision	0.459 % Paracetamol 0.481% -Aceclofenac	100.13%-Paracetamol 100.06%-Aceclofenac
5	Recovery	(100.15 ±0.761%) - Paracetamol (99.73 ±0.436 %) - Aceclofenac	100.7 %-Paracetamol 100.4 %-Aceclofenac
6	LOD(µg ml^{-1})	0.986- Paracetamol 0.296 -Aceclofenac	0.080 - Paracetamol 0.015 - ACeclofenac
7	LOQ(µg ml^{-1})	2.52 -Paracetamol 0.756 - Aceclofenac	0.250 - Paracetamol 0.50 - Aceclofenac

are as shown in TABLES 10 and 11. And the results of different analysts are as shown in TABLE 12.

System suitability test

As per USP 29/NF[3], system suitability tests were carried out on freshly prepared standard stock solutions of paracetamol and aceclofenac. 100µL of both drugs were injected in to the Chromatograph under the optimized chromatographic conditions and following parameters were studied to evaluate the suitability of the system Calibration range, Number of theoretical plates, Resolution, Retention time, Tailing factor and Limit of detection and Limit of quantification. The values of system suitability test were shown in TABLE 13.

RESULTS AND DISCUSSION

The TABLES 5-13 gives details of the findings of assay, recovery studies and system suitability parameters respectively. Since aceclofenac and paracetamol are polar in nature so reverse phase chromatography was chosen. C_{18} column was preferred over C_8 column for the separation of drug because sufficient resolution between the drugs (paracetamol and aceclofenac) peak was observed and as the carbon load is high in C_{18} as compaired to C_8, the retention and resolution will increased[6]. The mobile phase composing of acetonitrile: sodium dihydrogen phosphate buffer 50 mM (50:50) v/v; pH 3.0±0.1 with orthophosphoric acid showed good resolution peaks within a short run time. pKa of paracetamol is 9.5 and pKa of aceclofenac is 4.7 so at pH 3.0±0.1 Then aceclofenac remains in ionized and paracetamol remains in unionized form and its retention does not change much with pH. Detection was carried out at λmax 276 nm because at this wavelength both drugs are showing considerable (equal) absorbance and the peak height response of both the drugs was acceptable.

As per the ICH[5] current regulatory requirements resolution between the two components should be more than 3.0, tailing factor should be less than 2 and theoretical plates should be more than 2000. It is evident from system suitability study TABLE 13 that method developed for these two drugs in combination is passing the standards of regulatory requirements.

The method was found to be precise and accurate with low values of coefficient of variation which is shown in precision study in TABLE 6. Coefficient of variation

for paracetamol was found is 0.1347. And Coefficient of variation for aceclofenac was found is 0.0664.

TABLE 7 shows assay a result which indicates that method is precise and accurate. Hence it can be conveniently adopted for routine quality control analysis of the tablets.

The comparative study of these two methods (Difference spectroscopy and RP-HPLC method) is shown in TABLE 14.

CONCLUSION

The proposed first differential spectrophotometry method was capable of removing the absorbance of the interfering substances and it is simplest and most commonly employed technique for altering the spectral properties of the analyte is the adjustment of the pH by means of aqueous solutions of acid, alkali or buffers. Interferences from excipients, co-formulated drugs and also non-specific irrelevant absorption from formulation matrix since the absorbance did not change by altering the pH of medium.

And second the proposed RP-HPLC method was simple, rapid, and selective and economical.

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