ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Simultaneous HPTLC Determination of Paracetamol and Aceclofenac in Tablet Dosage Form.

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ABSTRACT

A normal-phase simple, rapid and precise high performance thin – layer chromatographic method has been developed for simultaneous quantitative determination of Paracetamol and Aceclofenac in a tablet dosage form. The analysis was performed on Silica gel $60F_{254}$ on aluminum plates with acetonitrile – tolune – acetic acid, 6 : 4 : 0.1 v/v as a mobile phase. Detection and quantitation were performed densitometrically at wavelength 270nm. The developed method was validated for linearity, precision, solution stability, accuracy and robustness parameters. The linearity of Paracetamol and Aceclofenac were in the range of 375-1125 µg/mL and 75-225µg/mL respectively. The correlation coefficient of Paracetamol and Aceclofenac were observed 0.9996 and 0.9990 respectively. Accuracy was checked by performing recovery studies from the pharmaceutical preparation. The average was found to be 99.28 ± 1.72% for Paracetamol and 99.82 ± 1.18% for Aceclofenac. The proposed HPTLC method was found to be accurate , precise and rapid for the simultaneous determination of Paracetamol and Aceclofenac in tablet dosage form.

Keywords: Paracetamol, Aceclofeanc, HPTLC

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ISSN: 0975-8585

INTRODUCTION

Paracetamol (PCT) is chemically N (4- hydroxyl phenyl) acetamide and is used as analgesic and anti-pyretic agent . It is well known analgesic drug which is very effective to the treatment for relief pain and fever in adults and children. It has molecular formula $C_8H_9NO_2$ and molecular weight 151.16. It has a narrow therapeutic index- the therapeutic dose is close to the toxic dose. Aceclofenac which has molecular formula $C_{16}H_{13}Cl_2NO_4$, molecular weight 354.19. Aceclofenac (ACF) ,{ [2-(2',6'- dichlorophenyl) amino] phenyl acetoxyacetic acid } is a new phenyl acetic acid [1] derivative with potent analgesic and anti-inflammatory properties with improved gastric tolerance. Literature survey revealed that various methods have been reported for the simultaneous determination of Paracetamol and Aceclofenac in pharmaceutical formulations, viz, UV spectrophotometry [2-3], reverse phase HPLC[4-7] stability indicating, , HPTLC [8-13] . In this paper I have reported HPTLC method for the simultaneous determination of PCT and ACF in tablet dosage form. Aim of present work was to develop simple , economical , rapid , precise and accurate method for simultaneous determination of PCT and ACF. The key advantage of developed HPTLC method is that several samples can run using a small quantity of mobile phase. The proposed method was validated as per ICH guidelines.

EXPERIMENTAL

Working standards and chemicals:

PCT and ACF working standard were obtained from Glenmark (Mumbai, India). Tablet containing PCT (500mg) and ACF (100mg) were obtained from Unichem (Mumbai, India), AR grade methanol and acetonitrile were purchased from Merck India.

Instrumentation and Chromatographic condition

The samples were spotted in the form of bands of width 5 mm with a desaga 100 µL sample syringe on silica gel precoated Al plate 60 F_{254} , with 200 µm thickness . These bands were applied with the help of Desaga AS 30- sample applicator at a distance of 10mm from X axis and 15mm from Y axis at the edge of the HPTLC plate with the speed of 150nl/sec for methanol.

The plates were prewashed by methanol and activated at 110^0C for 5min prior to chromatography. The space between two bands was kept at 10mm. The slit dimension was kept at 4 x 3 mm and 4.0mm/s scanning speed was employed. The monochromator bandwidth was set at 10nm, each track was scanned thrice and base line correction was used. The mobile phase composed of acetonitrile : toluene : glacial acetic acid in the volume ratio 6 : 4 : 0.1 v/v/v.

Linear ascending development was carried out in a twin trough glass chamber saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 30min at room temperature (25^0c ± 2) at relative humidity of 55% ± 5. TLC plates were dried in current of air with the help of air dryer. Detection and quantification was performed in the absorbance mode using Desaga TLC scanner with Pro-Quant software. During the method development the spots on the TLC plate were visualized in a UV chamber equipped with a UV lamp at wavelength 254nm. The developed TLC plate was scanned between 200nm and 400nm wavelength using CD- 60 Densitometer. The wavelength selected for further quantification was 270nm.

Preparation of standard solution:

75mg of PCT and 15 mg of ACF was accurately weighed and transferred to a 50mL standard flask. It was dissolved in a methanol and then diluted up to the mark with methanol.

The concentration of the solution obtained was 1500µg/mL for Paracetamol and 300µg/mL for Aceclofenac (solution A). 5 mL of this solution A was diluted to 10mL in a volumetric flask with methanol. The concentration of the solution obtained was 750µg/mL for PCT and 150µg/mL for ACF.

Preparation of Sample solution:

Twenty tablets were weighed and the average weight was calculated. These tablets were powdered and a weight equivalent to one tablet was taken in a 100mL volumetric flask and dissolved in minimum amount of methanol and was sonicated for about 30 minutes then diluted upto the mark with methanol. This solution was filtered through syringe filter. Then 15 mL of the sample solution was taken in a 50mL dilution flask and was diluted upto the mark with methanol (solution A). From this stock solution 100% level sample solution was prepared by diluting 5 mL of solution A to 10 mL with methanol.

Validation of the Method:

The method was validated for linearity, precision, accuracy , specificity and solution stability.

Standard plots were constructed for both PCT and ACF in the range of 75-1125µg/mL. The experiment was repeated thrice on the same day and additionally on two consecutive days to determine intra- and inter-day precision, respectively.

The intermediate precision (ruggedness) of the method was determined by repeating the experiment on two different instruments. Accuracy was determined by recovery studies. It was carried out by standard addition method by spiking 10%, 20% and 30% of the standard drugs in the 100 % sample solution. Three determinations were performed at each level.

Further specificity of the method was tested by study of the resolution factor of the drug peaks from nearest resolving peaks. Robustness of the method was carried out by small changes in the mobile phase composition (± 0.1 mL for each component) and the effects on the results were studied. Time from spotting to chromatography and from chromatography to scanning was varied by ± 15 minutes.

Analysis of Marked formulation:

The developed method can be applied in determination of PCT and ACF in a tablet AroffPlus (Unichem) which is marketed oral solid dosage formulation. To determine the contents of Paracetamol and Aceclofenac (label claim : 500mg PCT and 100mg ACF per tablet), the contents of the tablet were emptied and weighed. The drug from the powder was extracted with 10mL of methanol. To ensure complete extraction of the analytes, it was sonicated for 30 min. The resulting solution was allowed to settle for about an hour and the supernatant was suitably diluted to give desired concentration. 10µL of the solution was applied on TLC plate followed by development, visualization and scanned.

The analysis was repeated in triplicate. The possibility of excipients interference in the analysis was studied.

RESULTS AND DISCUSSIONS

Optimization of the chromatographic conditions

In order to develop a normal phase HPTLC method for the determination of Paracetamol and Aceclofenac in combined dosage form the chromatographic conditions were optimized. For better separation and resolution, the mixtures of different solvents of varying polarity were tried.

The different compositions of mobile phases were changed for getting better separation of analytes. Initially, chloroform –ethyl acetate 4 : 6 (v/v) and acetonitrile, toluene 5 : 5 (v/v) were used. The best results were obtained by the use of acetonitrile, toluene and glacial acetic acid in the ratio of (6 : 4 : 0.1v/v/v). This mobile phase showed good resolution and separation of Paracetamol and Aceclofenac peak from other formulation components or excipients tested as seen in fig 1.

Densitometric scanning of all the tracks showed compound with Rf value 0.47 ± 0.03 for Aceclofenac and 0.62 ± 0.04 for Paracetamol. As the separation was takes place in a short time period the proposed method is quicker as compare to reported method.

ISSN: 0975-8585

Table 1: Optimized chromatographic conditions

Parameters	Chromatographic conditions
Development chamber	Twin trough chamber
Stationary phase	Silica gel
Mobile Phase	Acetonitrile : Toluene : Acetic acid (6 : 4 : 0.1v/v/v)
Chamber saturation	15 min
Sample applicator	AS 30 - SAMPLE APPLICATOR
Band	8mm
Space	12mm
Scanning speed	20mm/sec
Development distance	8 cm
Drying of plate	Room temperature
Densitometric scanner	CD 60 - DENSITOMETER / SCANNER
Lamp	Deuterium
Wavelength	270 nm
Volume	10µl

Method Validation:

Linearity and Range

Linearity was observed over the concentration range of 375 - 1125µg/mL for PCT and 75 - 225µg/mL for ACF (Table 2) . The linearity was confirmed by the high value of the correlation coefficients of r^2= 0.9996 for PCT and 0.9990 for ACF

Table 2. Linear regression data

Drug	Linearity range	Correlation coefficient (r^2)	Slope	Intercept
Paracetamol	375-1125 µg/mL	0.9996	3.430	-6.383
Aceclofenac	75-225 µg/mL	0.9990	7.946	-13.73

Precision

The developed method was validated for system precision and method precision.

The precision study of the proposed method gave the results in the prescribed limits of relative standard deviation. This is less than 2 % for both analytes. The low value of RSD showed that the proposed method was reliable and reproducible.

Table 3 Precision study for Paracetamol and Aceclofenac

Obs No	Paracetamol		Aceclofenac	
	Peak Area	% Assay	Peak Area	% Assay
1	2521	101.61	1101	101.66
2	2512	101.25	1081	99.82
3	2507	101.05	1072	98.98
4	2499	100.73	1067	98.52
5	2442	98.43	1055	97.41
6	2474	99.72	1076	99.35
	Mean	100.46	Mean	99.29
	S.D	1.187	S.D	1.298
	%R.S.D	1.182	%R.S.D	1.307

ISSN: 0975-8585

Solution stability:

Stability of a sample solution was checked by using sample prepared in the precision study.

The sample solution was stored at room temperature for 24 hrs then it was withdrawn at the intervals of 2 hr, 4 hrs , 12 hrs and then applied on the chromatographic plate. stored at room temperature for 24 hours, withdrawn at the intervals of 2hrs, 4 hrs, 12 hrs and 24 hrs and then applied on the chromatoplate.

After development , the chromatogram was evaluated for additional spots if any. There was no indication of compound instability in the sample solution. The results shows that the solutions were stable for 24 hrs at room temperature.

Specificity:

An investigation specificity was conducted during the validation of identification tests, the determination of impurities and the assay. Demonstration of specificity requires that there should not be any interference of impurities and excipients. In practice this was done by taking the chromatogram of sample solution and the assay result was unaffected by the extraneous material. It has been found that there was no interference of the diluents , placebo at the Rf value of the analytes.

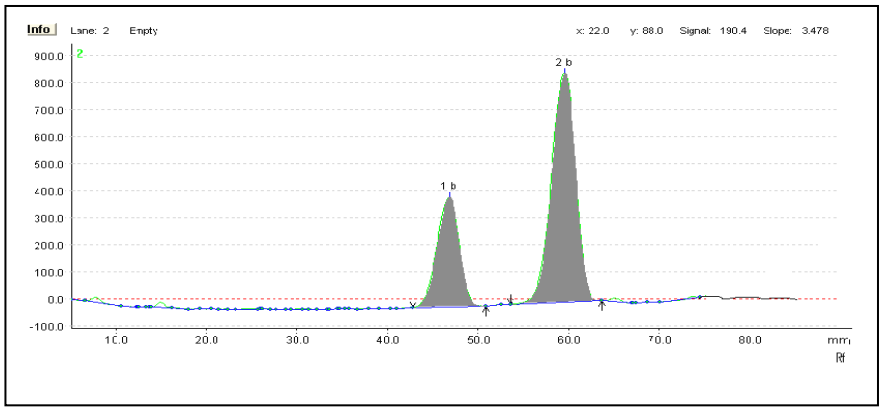

Fig 1: Typical HPTLC chromatogram 1) Aceclofenac 2) Paracetamol

System Suitability Test:

A system suitability test should be carried out to see if the HPTLC system is performing properly. System suitability tests were carried out as per the USP to confirm the suitability and the reproducibility of the system. The experiment was carried out using 100% level mixed standard solution of PCT and ACF. This solution was spotted five times on the chromatographic plate under the optimized chromatographic conditions. % RSD of the peak area shows that (Table 4) the proposed method was suitable for the system.

Table 4 System suitability for Paracetamol and Aceclofenac

Obs No	100% Level			
	Paracetamol		Aceclofenac	
	Peak area	Rf value	Peak area	Rf value
1	2588	0.64	1139	0.49
2	2499	0.62	1102	0.46
3	2451	0.63	1086	0.48
4	2459	0.62	1070	0.49
5	2412	0.61	1020	0.45
Mean	2481	0.62	1083	0.47
S.D	66.92	0.0102	39.098	0.016
% RSD	2.70	1.63	3.61	3.43

ISSN: 0975-8585

Accuracy (Recovery Experiment)

The accuracy of the method was determined by the standard addition method at three different levels. The sample solution of 100% level was considered as a zero level and 10% , 20% and 30% of the standard drug of PCT and ACF were added respectively. Each determination was performed in triplicates. The accuracy was then calculated as the percentage of the standard drug recovered by the recovery study. Mean recoveries for PCT and ACF from the sample solution are shown in Table 5 and 6. The results are within the acceptance limit and hence the method is accurate.

Table 5 : % Recovery of PCT

Amount of Paracetamol in ppm								
Sr.No	% Added	Original amount	Added amount	Total amount	Mean (n = 5)	% Recovery	S.D	% RSD
1	10	500	50.40	550.40	549.31	99.87	0.3881	0.3886
2	20	500	100.33	600.33	591.02	98.50	0.4922	0.4996
3	30	500	150.66	650.66	644.16	99.10	0.645	0.651

Table 6: % Recovery of ACF

Amount of Aceclofenac in ppm								
Sr.No	% Added	Original amount	Added amount	Total amount	Mean (n = 5)	% Recovery	S.D	% RSD
1	10	100	10.36	110.36	110.12	100.11	0.8592	0.8582
2	20	100	20.33	120.33	119.33	99.44	0.4777	0.4902
3	30	100	30.16	130.16	128.87	99.12	0.7884	0.7953

CONCLUSION

The HPTLC method for the determination of Paracetamol and Aceclofenac from their tablet dosage form was found to be accurate , precise, specific and rapid. The results of the recovery studies show the high degree of accuracy of the proposed method. The advantage of the proposed method is that it require less time and cost effective method. Solvent consumption during the analysis is less. Therefore the proposed method can be applied successfully in routine analysis.

ACKNOWLEDGEMENT

The author are thankful to Department of Chemistry, Mithibai College and Glenmark Pharmaceutical India limited for their support and for providing the free gift samples of working standards.

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