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Simultaneous Estimation of Aceclofenac and Paracetamol by HPTLC in Pure and Pharmaceutical Dosage Form

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A simple, accurate, precise and reproducible high performance thin layer chromatographic method has been developed for the simultaneous estimation of paracetamol and aceclofenac in pharmaceutical dosage forms. It was performed on TLC plate precoated with silica gel $60F_{254}$ as stationary phase using mobile phase comprising of toluene:isopropyl alcohol:ammonia (20:20:3, v/v/v) and the detection was carried out by UV detection at 254 nm showing R_f value of 4.8 for aceclofenac and 11.8 for paracetamol. The percentage estimation of labeled claims of aceclofenac and paracetamol from marketed tablet was found to be 99.84 and 99.72, respectively. The method was validated in terms of accuracy, precision, specificity and ruggedness. Linearity was observed between 60-140 µg/mL for aceclofenac and 460-540 µg/mL for paracetamol. The recovery studies were carried out by adding known quantity of standard drugs in the pre-analysed test solution and percentage recovery calculated in each case. The percentage recovery studies for paracetamol and aceclofenac were found within the range of 98.60-99.32 %. The proposed method was found to be accurate, precise, simple and rapid could be used for routine analysis.

Key Words: HPTLC, Paracetamol, Aceclofenac.

INTRODUCTION

Aceclofenac[1-7] (ACE) is an orally administered phenyl acetic acid derivative with effects on a variety of inflammatory mediators. It is from the class of non-steroidal antiinflammatory drug (NSAID). Chemically it is 2-[(2,6-dichloro phenylamino)phenyl]acetoxy-acetic acid. Its empirical formula is $C_{16}H_{13}NO_4Cl_2$ and molecular weight is 354.2. The mode of action of aceclofenac is largely based on the inhibition of prostaglandin synthesis. Aceclofenac is a potent inhibitor of the enzyme cyclo-oxygenase, which is involved in the production of prostaglandins.

Paracetamol[1-8] (PCM) is a non-opiate, non-salicylate analgesic and antipyretic drug. Chemically, paracetamol is 4-hydroxyl acetanilide or N-(4-hydroxy phenyl)acetamide. Its empirical formula is $C_8H_9NO_2$ and molecular weight is 151.2. It acts by inhibiting prostaglandin synthetase centrally. Specifically, it is a potent inhibitor of cyclo-oxygenase in the CNS.

The earlier literature survey[9-15] reveals the analytical methods like UV, HPLC and HPTLC are available for determination of these drugs individually and other combinations in pharmaceuticals and biological preparations. No method has been reported for the estimation of ACE and PCM simultaneously. In the present investigation an attempt was made to develop a simple and economical HPTLC with greater precision, accuracy and sensitivity for the simultaneous estimation of ACE and PCM in pure and tablet dosage forms.

EXPERIMENTAL

All chemicals and reagents used were of AR/HPLC grade silica gel precoated aluminum plates with thickness of 200 µm, E-Merck, Germany were used as a stationary phase with the instrument CAMAG-HPTLC system comprising of CAMAG LINMAT IV sample applicator, CAMAG TLC scanner III with CATS software and CAMAG twin trough chamber with stainless steel lids. Pure samples of ACE and PCM were obtained as a gift samples from Aeon Pharmaceuticals.

Preparation of standard solution: An accurately weighed quantity of 125 mg of paracetamol (working standard) and 25 mg of aceclofenac (working standard) were dissolved in methanol make up to 25 mL to obtain a stock solution of 5000 µg/mL of paracetamol and 1000 µg/mL of aceclofenac.

Chromatographic conditions: Various solvent systems were evaluated to arrive at an optimum resolution of the two drugs. The solvent system consisting of toluene, isopropyl alcohol and ammonia (20:20:3, v/v/v) gave dense, compact and well separated spots of the drugs from the mixture. The chamber was saturated for 10 min. Sample was applied at a constant rate of 0.16 µL/s having scan speed of 10 mm/s with 16 mm band width and the samples were separated by ascending technique. The chamber was maintained at 20 ± 0.5ºC temperature and 50-60 % relative humidity. The plate was scanned at 254 nm.

Calibration curve: ACE and PCM solutions ranging from 60-140 and 460/540 µg/mL were applied on TLC plate by automatic sample applicator. The plates were developed, dried and densiometrically scanned at 254 nm. Peak height and area were recorded for each concentration and curves (peak area *vs.* concentration) were constructed.

Sample preparation: To determine the content of aceclofenac and paracetamol simultaneously in conventional tablets (Label claim: 100 mg of aceclofenac and 500 mg of paracetamol per tablet), 20 tablets were weighed, their mean weight determined. They were finely powdered and powder equivalent to 100 mg of aceclofenac and 500 mg of paracetamol was weighed. Then equivalent weight of the powder was transferred into a 100 mL volumetric flask containing 50 mL of methanol. Sonicated for 0.5 h and diluted to 100 mL with methanol. Then the solution filtered through Whatmman filter paper No. 42. The filtered solution was then used for the estimation.

Estimation method: The sample solution was spotted on the chromplate with the help of Linomat IV spotting system. The chromplate was developed in a twin trough chamber containing the mobile phase. The chromatograms were recorded and R_f values were determined for paracetamol and aceclofenac. The amount of drug present was calculated by comparing the peak area values of standard with that of sample as follows:

$$\text{Amount of drug each tablet} = \frac{\text{Peak area of test}}{\text{Peak area of standard}} \times \frac{\text{Standard dilution factor}}{\text{Sample dilution factor}} \times \text{Average weight of tablet}$$

RESULTS AND DISCUSSION

Various solvent systems were evaluated to arrive at an optimum resolution of the two drugs. The solvent system consisting of toluene, isopropyl alcohol and ammonia (20:20:3, v/v/v) gave dense, compact and well separated spots of the drugs from the mixture The R_f values were found to be 4.8 and 11.8 for aceclofenac and paracetamol, respectively. Both the peaks were symmetrical in nature and low value of tailing was observed when plates were scanned at 254 nm. The plot of peak areas *vs.* concentration of aceclofenac and paracetamol were found to be linear in the concentration range 60-140 and 460-540 µg/mL, respectively. The corresponding correlation values are given in Table-1.

TABLE-1
LINEARITY STUDIES

Drug	Linearity range (µg)	Coefficient of correlation	Slope	Y-Intercept
Aceclofenac	60-140	0.9983	264.200	12.200
Paracetamol	460-540	0.9992	78.346	0.1695

The proposed method was successfully applied to the analysis of paracetamol and aceclofenac in tablet (labeled to contain paracetamol 500 mg and aceclofenac 100 mg as active substances). The results and statistical parameters are shown in Table-2. The low values of % RSD indicated high precision of the method. The precision of the method was demonstrated by repeatability studies. The precision of the proposed method was determined by assaying the standard solutions on the same day and on 3 different days over a period of 2 weeks and expressed as % RSD. The intra-day and inter-day precision have been depicted in Table-3. To confirm the accuracy of the proposed method, recovery experiments were carried out by standard addition technique by adding a known amount of standard at 3 different levels to the pre-analyzed sample. Each level was repeated 3 times and the amount of drug found by the assay method, results and statistical parameters are reported in Table-4. The results show that the method is precise and accurate.

TABLE-2
ESTIMATION OF ACECLOFENAC AND PARACETAMOL

Drug	Label claim (mg/tab)	Amount estimated*	Amount estimated* (%)	RSD (%)	SE
Aceclofenac	100	99.84 ± 0.16	99.84 ± 0.16	0.16	0.07
Paracetamol	500	498.63 ± 0.82	99.72 ± 0.03	0.16	0.37

*Mean and ± standard deviation for 5 determinations
RSD: Relative standard deviation, SE: Standard error

TABLE-3
INTRA-DAY AND INTER-DAY PRECISION OF PARACETAMOL AND ACECLOFENAC

Drug	Spiked concentration (µg/mL)	Intra-day measured concentration*		Inter-day measured concentration*	
		Mean (µg/mL)	RSD (%)	Mean (µg/mL)	RSD (%)
Paracetamol	480	480.26	0.19	480.05	0.16
	500	500.24	0.15	500.42	0.14
Aceclofenac	80	80.15	0.91	80.41	0.91
	100	99.93	0.79	100.40	0.80

*Mean of 5 different standards for each concentration
RSD: Relative standard deviation

TABLE-4
RECOVERY OF PARACETAMOL AND ACECLOFENAC

Label claim (mg/tablet)	Amount added (mg)	Amount recovered* (mg)	Recovery* (%)	Average recovery (%)	RSD (%)
Paracetamol (500 mg)	25	24.75 ± 0.15	98.98	99.32	0.49
	50	49.60 ± 0.37	99.20		
	75	74.79 ± 0.10	99.72		
Aceclofenac (100 mg)	5	5.01 ± 0.07	100.66	98.60	0.86
	10	9.77 ± 0.15	97.70		
	15	14.62 ± 0.16	97.46		

*Mean and standard deviation for 3 determinations

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