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Simultaneous Estimation and Validation of Paracetamol, Aceclofenac and Chlorzoxazone by HPLC in Pure and Pharmaceutical Dosage Form

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A simple, accurate, precise and reproducible high performance liquid chromatographic method has been developed for the simultaneous estimation of paracetamol, aceclofenac and chlorzoxazone in pharmaceutical dosage forms. A Phenomenex ODS C_{18} column (250 mm × 4.6 mm i.d., 3-5 mcm particle size) in gradient mode, with mobile phase acetonitrile and orthophosphoric acid (40:60) (v/v), the flow rate was 1 mL/min and effluent was monitored at 275 nm. The approximate retention time for paracetamol, aceclofenac and chlorzoxazone were 2.176, 4.003 and 5.513 min, respectively. The linearity for paracetamol, aceclofenac and chlorzoxazone was in the range of 10-100, 2-20 and 10-100 mcg/mL, respectively. Quantity found for paracetamol, aceclofenac and chlorzoxazone were 517.36, 103.16 and 517.76 mg, respectively. The percentage estimation of labeled claims of paracetamol, aceclofenac and chlorzoxazone from marketed tablet was found to be 103.47, 103.16 and 103.55, respectively. The method was validated in terms of accuracy, precision, specificity, ruggedness and robustness. The addition of known quantity of standard drugs in the pre-analyzed test solution and percentage recovery was calculated in each case carried out the recovery studies. The percentage recoveries obtained for paracetamol, aceclofenac and chlorzoxazone were found within the range of 97.27 -102.00 %. The proposed method is found to be accurate, precise, simple and rapid which can be used routinely for simultaneous estimation of proposed combination in tablet formulation.

Key Words: HPLC, Paracetamol, Aceclofenac, Chlorzoxazone.

INTRODUCTION

Paracetamol[1,2] is a non-opiate, non-salicylate analgesic and antipyretic. 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.

Aceclofenac[2] 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. Chemically, it is 2-[(2,6-dichloro phenyl-amino)phenyl]acetoxy-acetic acid. Its empirical formula is $C_{16}H_{13}Cl_2NO_4$ 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.

Chlorzoxazone[3] is 5-chloro-3*H*-benzooxazol-2-one. Its empirical formula $C_7H_4NO_2Cl$ and molecular weight 191.5. It inhibits antigen-induced bronchospasms and hence, used to treat asthma and allergic rhinitis. Chlorzoxazone is also a centrally acting agent for painful musculoskeletal conditions. It also has sedative property.

The literature survey[4-6] 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. There is no method has been reported for the estimation of paracetamol, aceclofenac and chlorzoxazone simultaneously. In the present investigation, an attempt was made to develop a simple and economical HPLC for the simultaneous estimation of paracetamol, aceclofenac and chlorzoxazone in pure and tablet dosage forms.

EXPERIMENTAL

High performance liquid chromatograph Shimadzu LC2010 series equipped with Quaternary constant flow pump, Auto injector with injection volume of 20 mcl. Photo diode Array detector and LC10 software, Phenomenex ODS C_{18} column (250 mm × 4.6 mm I.d., 3-5 mcm particle size) forms the stationary phase, a calibrated electronic single pan balance (Mettler AE 160) and certified reference standards of paracetamol, aceclofenac and chlorzoxazone were used. The drug samples were procured from the market. Tablets claim for paracetamol, aceclofenac and chlorzoxazone were 500, 100 and 500 mg/tablet, respectively. All chemicals and reagents used were of AR/HPLC grade. HPLC grade water was prepared by Millipore water purification system (Milli-Q) in the lab.

Preparation of standard solution: An accurately weighed quantity of paracetamol (50 mg), aceclofenac (20 mg) and chlorzoxazone (50 mg) were transferred to 100 mL volumetric flask, which was then dissolved and made up to volume with mobile phase. From the above stock solution 10, 20, 30, 40 and 50 mL of paracetamol and chlorzoxazone and 5, 10, 15, 20 and 25 mL of aceclofenac were transferred to 100 mL volumetric flasks which was then made up to volume with methanol to give final concentrations of 50, 100, 150, 200, 250 mcg/mL of paracetamol and chlorzoxazone; 10, 20, 30, 40, 50 mcg/mL of aceclofenac and standard chromatograms were recorded.

Optimized chromatographic conditions: HPLC analysis was performed by Gradient elution with flow rate of 1.0 mL/min. The mobile phase containing acetonitrile and orthophosphoric acid with pH adjusted to 7 with NaOH in the ratio (40:60) (v/v) were used to obtain well-resolved peaks. Injection volume of 20 mcl each, standard and sample solutions were injected into the column containing Stationary phase octyl decyl silane with particle size of 3-5 mcm. The detection wavelength and chromatographic run time was selected at 275 nm and 10 min, respectively.

Calibration curve: Linearity was assessed by injecting 20 µL of seven different standard concentrations obtained by diluting standard stock solution with mobile phase under optimized chromatographic conditions, which provides 10, 25, 30, 40, 50, 75, 100 mcg/mL of paracetamol and chlorzoxazone and 2, 5, 6, 8, 10, 15, 20 mcg/mL of aceclofenac. The chromatograms were recorded and using peak area of individual drugs *vs.* respective concentrations, linearity graph was plotted.

Sample preparation: 20 Tablets were weighed accurately and crushed to fine powder. An accurately weighed quantity of powder equivalent to 50 mg of paracetamol was then transferred to a 100 mL volumetric flask, sonicated for 15 min and made up to volume with mobile phase. Further the solution was filtered through Whatmann filter paper No. 4, which was used for determining the content of paracetamol, aceclofenac and chlorzoxazone simultaneously in conventional conditions.

Estimation method: With the optimized chromatographic conditions mentioned earlier, a steady base line was recorded. After the stabilization of baseline, successive aliquots of standard solutions, which have been prepared from stock solutions, containing varying concentrations were injected and chromatograms were recorded. The procedure was repeated using sample solutions having different concentrations which was prepared by diluting aliquot of sample stock solution to get final concentrations of 50, 100, 150, 200, 250 mcg/mL of paracetamol and chlorzoxazone; 10, 20, 30, 40, 50 mcg/mL of aceclofenac.

$$\text{Amount of drug in each tablet} = \frac{\text{Peak area of test compound} \times \text{std. dilution factor} \times \text{average weight of tablet}}{\text{Peak area of standard} \times \text{Sample dilution factor}}$$

RESULTS AND DISCUSSION

The method was chosen after several trials with acetonitrile and buffer in various proportions and at different pH values. A mobile phase consisting of acetonitrile and orthophosphoric acid in the ratio 40:60 (v/v) was selected to achieve maximum separation and sensitivity. The effects of flow rates in the range of 0.5-1.5 mL/min were examined where the flow rate of 1.0 mL/

min gave an optimal signal to noise ratio with a reasonable separation time. The overlain spectra of paracetamol, aceclofenac and chlorzoxazone in mobile phase showed isobestic point at 275 nm. Hence the detection wavelength was selected at 275 nm. Using reverse phase C_{18} column, the retention times of paracetamol, aceclofenac and chlorzoxazone were found to be 2.173, 4.015 and 5.511 min, respectively. The total time of analysis was less than 7 min. By plotting peak area against their respective concentrations showed that paracetamol and chlorzoxazone were found to be linear in the concentration range of 10-100 mcg/mL and aceclofenac at the concentration range of 2-20 mcg/mL. The corresponding values were depicted in Table-1.

TABLE-1
LINEARITY OF PARACETAMOL, ACECLOFENAC AND CHLORZOXAZONE

Concentration (mcg/mL)			Peak area		
Paracetamol	Aceclofenac	Chlorzoxazone	Paracetamol	Aceclofenac	Chlorzoxazone
10	2	10	202219	71481	270126
20	5	20	508188	182657	677069
30	6	30	615417	219285	822636
40	8	40	813424	293167	1082090
50	10	50	1014083	362279	1360625
75	15	75	1509047	545967	2020590
100	20	100	2001501	719581	2693821

The proposed method was successfully applied for the analysis of paracetamol, aceclofenac and chlorzoxazone labeled to contain paracetamol 500 mg, aceclofenac 100 mg and chlorzoxazone 500 mg as active substances). The results and statistical parameters were shown in Table-2. The low values of RSD (%) indicated high precision of the method. The precision of the proposed method was determined by assaying the standard solutions on the same day and on three different days over a period of two weeks as reproducibility and ruggedness, which were expressed in terms RSD (%). The intra-day and inter-day precision has been depicted in Tables 3 and 4, respectively. 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 three different levels to the pre-analyzed sample. Each level was repeated three times and the amount of drug found by the assay method, results and statistical parameters are reported in Table-5. Changing flow rate and detection wavelength from the optimized chromatographic conditions carried out robustness. Robustness also expressed in terms of RSD (%) was found to be less than 2 % and results were shown in Table-6. The results show that the method is precise, accurate and robust. Limit of detection (LOD) and limit of quantificaiton (LOQ) were determined from

TABLE-2
STATISTICAL VALIDATION

Drug	Label claim (mg/tab)	Amount estimated (mg/tab)*	Amount estimated* (%)	RSD (%)	SE
Paracetamol	500	517.36	103.47	0.75	1.75
Aceclofenac	100	103.16	103.16	0.74	0.34
Chlorzoxazone	500	517.76	103.55	0.40	0.98

*Mean of five determinations; RSD = Relative standard deviation;
SE = Standard error.

TABLE-3
PRECISION

Drug	Theoretical concentration (mcg/mL)	Intra-day measured concentration*	
		Amount estimated (mg/tab)	Percentage (%) RSD
Paracetamol	50	517.55	0.68
Aceclofenac	10	103.15	0.66
Chlorzoxazone	50	517.84	0.36

*Mean of six replicates of single concentration; RSD = Relative standard deviation.

TABLE-4
RUGGEDNESS

Drug	Theoretical concentration (mcg/mL)	Inter-day measured concentration*	
		Amount estimated (mg/tab)	Percentage (%) RSD
Paracetamol	50	512.19	0.61
Aceclofenac	10	104.73	0.28
Chlorzoxazone	50	515.54	0.61

Mean of six replicates of single concentration; RSD = Relative standard deviation.

TABLE-5
ACCURACY

Drug	Amount added (mg)	Amount recovered* (mg)	Recovery* (%)	Average recovery (%)	RSD (%)
Paracetamol 500 mg	25	24.38	97.54	98.99	1.52
	50	50.28	100.56		
	100	98.89	98.89		
Aceclofenac 100 mg	5	4.97	99.52	100.47	1.32
	20	19.98	99.91		
	40	40.80	102.00		
Chlorzoxazone 500 mg	25	24.34	97.39	97.39	0.13
	50	48.76	97.53		
	100	97.27	97.27		

*Mean of three determinations at each level.

TABLE-6
ROBUSTNESS

Drug	Theoretical concentration (mcg/mL)	Measured concentration*	
		Mean (mcg/mL)	Percentage RSD (%)
Paracetamol	500	522.62	1.35
Aceclofenac	100	105.63	1.50
Chlorzoxazone	500	523.35	1.85

*Mean of six replicates of single concentration; RSD = Relative standard deviation.

from linearity studies by using slope and standard deviation of three observations. The LOD was found to be 34.34 ng/mL for paracetamol, 6.86 ng/mL for aceclofenac and 34.35 ng/mL for chlorzoxazone, while LOQ values were found to 104.08, 20.81 and 104.09 ng/mL for paracetamol, aceclofenac and chlorzoxazone, respectively. Table-7 depicts limit of detection and limit of quantification.

TABLE-7
LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

Drug	Concentration (mcg/mL)	Mean peak area*	RSD (%)
Paracetamol	10	202219	0.04
	25	508788	
	30	615417	
Aceclofenac	2	71481	0.04
	5	182657	
	6	219285	
Chlorzoxazone	10	270126	0.04
	25	677069	
	30	822565	

*Mean of three replicates in each concentration; RSD = Relative standard deviation.

REFERENCES

1. Indian Pharmacopoeia, The Controller of Publications, New Delhi, India, edn.4, pp. 554-555 (1996).
2. British Pharmacopoeia, HMSO, London, edn. 15, Vol. 1, pp. 38-40, Vol. 2, pp. 1417-1418 (2003).
3. United States Pharmacopoeia, The United States Pharmacopoeia Convention, Inc., Rockville, edn. 25, pp. 410-411 (2002).
4. K.V. Gowda, D.S. Rajan, U. Mandal, P.S. Selvan, S. Solomon, A. Bose, A.K. Sarkar, T.K. Pal and T.K. Chattaraj, *Drug Dev. Ind. Pharm.*, **32**, 1219 (2006).
5. S.K. Cox, T. Hamner and J. Bartges, *J. Chromatogr. B*, **784**, 111 (2003).
6. M.K. Chouksey, A. Khandpur, N.K. Tyagi and G.N. Singh, *Indian Drugs*, **43**, 216 (2005).

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