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Simultaneous Estimation of Paracetamol, Chlorzoxazone and Diclofenac Sodium in Pharmaceutical Formulation by a Novel HPLC Method

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Abstract

A rapid and sensitive high performance liquid chromatography method for determination of paracetamol, chlorzoxazone and diclofenac sodium has been developed. The chromatography system used a reversed phase C_8 column with UV- Vis detection at 280 nm. Mobile phase consisted of acetonitrile – 0.05 M ammonium dihydrogen ortho phosphate (60:40 v/v) (pH adjusted to 4.06 using 10% ortho phosphoric acid) at a flow rate of 1.5 ml/min using diazepam as internal standard (I.S.). The calibration curve was linear in the concentration range of 26-130 µg/ml for paracetamol, 20-100 µg/ml for chlorzoxazone and 4-20 µg/ml for diclofenac sodium. The lower limit of detection was found to be 6.51 µg, 4.97 µg, 0.84 µg for paracetamol, chlorzoxazone, diclofenac sodium respectively.

Keywords: Paracetamol, chlorzoxazone, diclofenac sodium, HPLC analysis

Introduction

Paracetamol (*p*-hydroxy acetanilide) is a compound with analgesic and antipyretic properties. It is much safer than aspirin in terms of gastric irritation, ulceration and bleeding [1,2]. Diclofenac sodium [2-[(2,6- dichloropheny)] amino] benzene acetic acid monosodium salt] is a compound with potent anti-inflammatory property. It affords quick relief of pain and wound edema [3,4]. Chlorzoxazone (5-chloro-2(3H)-benzoxazolone) is a compound with skeletal muscle relaxant property. It is used to decrease muscle tone and tension and thus to relieve spasm and pain associated with musculoskeletal disorders [5,6]. H.L. Rao *et al.* developed a method for simultaneous estimation of paracetamol and diclofenac sodium in pharmaceutical preparations [7]. S.S. Zarapkar *et al.* developed a reverse phase HPLC method for the simultaneous estimation of paracetamol and chlorzoxazone [8].

The objective of the present work was to develop and validate the rapid and sensitive high-performance liquid chromatography (HPLC) method for simultaneous determination of paracetamol, chlorzoxazone and diclofenac sodium in tablets.

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Materials and Methods

Drugs :

paracetamol,diclofenac sodium,chlorzoxazone-Trade drug product(Voldex MR)

Chemicals and solvents:

Ammonium dihydrogen ortho-phosphate was purchased from S.D. Fine Chemicals Ltd., India. Acetonitrile, methanol and water of HPLC grade were purchased from Qualigens Fine Chemicals, India. The gift samples of the drug were received from Martine and Brown pharmaceuticals (Hisar), India. Nylon syringe membrane filters (0.2 μm) were purchased from Sartoris, Germany.

HPLC system:

The HPLC system consisted of a delivery pump (Water 600 pump controller), a reversed phase analytical column C_8 (250 × 4.6 mm) 5 μm (Kromasil), a Rheodyne sample injector with a 20 μl loop volume and a variable wavelength (UV-Vis) detector (waters 2487 Dual Absorbance Detector).

Chromatographic conditions:

The mobile phase consisted of acetonitrile – 0.05 M ammonium dihydrogen ortho phosphate (60:40 v/v) (pH adjusted to 4.06 using 10% ortho phosphoric acid). The solution was filtered through a 0.2 μm membrane filters. The eluent was monitored with a UV-Vis detector set at 280 nm with a flow rate of 1.5 ml/min. Mobile phase was stirred on a magnetic stirrer during the HPLC run.

Standard solution and calibration curve:

A standard stock solution of paracetamol (1300 μg/ml), chlorozoxazone (1000 μg/ml), diclofenac sodium (200 μg/ml) and diazepam (1000 μg/ml, I.S.) were prepared in methanol. Subsequent dilutions were made in mobile phase to give the concentrations 26, 52, 65, 104 and 130 μg/ml for paracetamol; 20, 40, 50, 80, 100 μg/ml for chlorozoxazone and 4, 8, 10, 16, 20 μg/ml for diclofenac sodium.. The calibration curve was obtained by plotting the ratio of peak area of drug/I.S. (20 μg/ml) versus concentration.

Assay:

Twenty tablets were weighed accurately and finely powdered. The powder equivalent to 325 mg of paracetamol, 250 mg of chlorozoxazone and 50 mg of diclofenac sodium was weighed accurately and dissolved in 250 ml methanol (HPLC). The solution was filtered through 0.2 μm membrane filter paper. Four ml of the resulting solution was mixed with one ml of I.S. and was further diluted to 50 ml to get a solution having a concentration of 104 μg/ml of paracetamol, 80 μg/ml of chlorzoxazone, 16 μg/ ml of diclofenac sodium and 20 μg/ml of internal standard (I.S.). Twenty μl of this solution was injected in triplicate under the specified conditions. The peak area ratio (drug/I.S) obtained were related to slops and intercepts from the calibration data to calculate concentration of the drugs (Table 1).

Table 1. Results of HPLC assay

Paracetamol		Chlorzoxazone		Diclofenac sodium	
Amt. Claimed (mg/tablet)	Amt. found (mg/tablet)	Amt. Claimed (mg/tablet)	Amt. found (mg/tablet)	Amt. Claimed (mg/tablet)	Amt. found (mg/tablet)
325	324.92 325.01 325.32	250	250.11 249.93 250.40	50	49.92 50.21 50.10
Mean	325.08		250.14		50.07
%RSD	0.035		0.042		0.286

Validation of the assay:

To study the accuracy, reproducibility and precision, recovery experiments were carried out. The recovery of the added standard was studied at three different levels. To an aliquot of the analyzed formulation a known concentration of standard solution was added. The content of paracetamol, chlorzoxazone and diclofenac sodium was determined (Table 2). The linearity of the standard curve was confirmed by plotting the peak area ratio of drug/I.S. versus concentration. Linear regression analysis was performed to calculate the slope, the intercept and the correlation coefficient (r^2) of the calibration curve (Table 3).

Table 2. Results of recovery studies

	Paracetamol			Chlorzoxazone			Diclofenac sodium		
Amount added (mg)	5	10	15	5	10	15	5	10	15
Amount found (mg)	330.20	334.96	340.03	255.12	259.87	264.98	54.97	60.12	65.01
Percentage Recovery	100.06	99.988	100.00	100.04	99.95	99.99	99.94	100.2	100.01
Mean	100.01			99.993			100.05		

Table 3. Linear regression data for calibration curve

Drugs	Paracetamol	Chlorzoxazone	Diclofenac sodium
Concentration range (μg/ml)	26-130	20-100	4-20
Slope	0.0476	0.1071	0.0635
Intercept	0.026	0.0905	0.0072
R^2	0.9987	0.9986	0.9982

Results and Discussion

Figure 1 shows typical chromatograms of three drugs with internal standard. As per USP-XXIII, system suitability tests were carried out on freshly prepared standard stock solutions of drugs (Table 4). The calibration curve was linear in the range of 26-130 μg/ml for paracetamol, 20-100 μg/ml for chlorzoxazone and 4-20 μg/ml for diclofenac sodium. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 6.51 μg, 16.20 μg/ml for paracetamol; 4.97 μg, 27.68 μg/ml for chlorzoxazone and 0.84 μg, 2.82 μg/ml for diclofenac sodium.

Table 4. System suitability Parameters

Parameters	Paracetamol	Chlorzoxazone	Diclofenac sodium
Calibration range (μg/ml)	26-130	20-100	4-20
Theoretical plates	1427	3686	2371
Tailing factor	1	1	1
LOD	6.51	4.97	0.84
LOQ	16.20	27.68	2.82

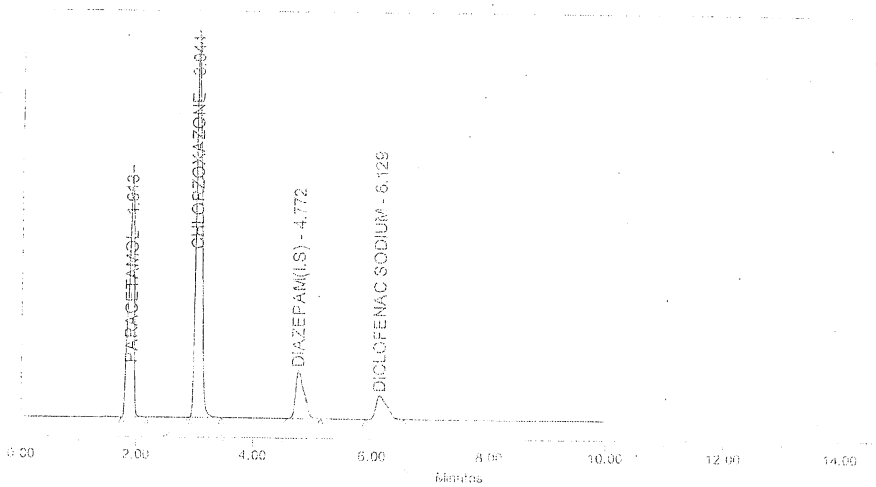

Figure 1. Chromatogram of Paracetamol, Chlorzoxazone and Diclofenac sodium with internal standard (Diazepam)

Conclusion

In conclusion, our method is rapid, sensitive, reproducible and well suited to the simultaneous determination of paracetamol, chlorzoxazone and diclofenac sodium by internal standard method.

References

Goodman and Gilman's(1992),The Pharmacological Basis of Therapeutics, Eighth edn., Volume I, Macmillan Publishing Company, Singapore 656-657.

Bailey L.C, Remington(1995), The Science and Practice of Pharmacy, Nineteenth edition, Volume II, Mack Publishing Company, Pennsylvania 1208.

British Pharmacopocia(2003), HMSO, Volume II, London 582-583.

Bailey L.C., Remington(1995), The Science and Practice of Pharmacy, Nineteenth edition, Volume II, Mack Publishing Company, Pennsylvania 1211.

The Merck Index(2001),, Thirteenth edition, Merck and Co. Inc. USA 379-380.

Bailey L.C., Remington(1995), The Science and Practice of Pharmacy, Nineteenth edition, Volume II, Mack Publishing Company, Pennsylvania 1033.

Rao H.L., Arora A.R. and Rao P. G. (1991), *Indian Drugs* 28 (6), 285-286.

Zarapkar S.S. and. Dhanvate A.A (1995), Simultaneous determination of chlorzoxazone and paracetamol from its pharmaceutical preparations using high performance liquid chromatography. *Indian Drugs* 32 (8), 405-408.

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