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SIMULTANEOUS ESTIMATION OF PARACETAMOL, CHLORZOXAZONE AND DICLOFENAC POTASSIUM IN PHARMACEUTICAL FORMULATION BY A RP HPLC METHOD

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ABSTRACT

A fast and reliable high performance liquid chromatography method for determination of paracetamol, chlorzaxozone and diclofenac potassium has been developed. The chromatographic method was standardised using a reverse phase C18 column with UV-VIS detector at 254 nm. Mobile phase consisted of methanol-0.01M monobasic sodium phosphate with equal volume of 0.01M ortho phosphoric acid (70:30) (p^H adjusted to 2.5 ± 0.2 using 10% orthophosphoric acid) at a flow rate **1 ml/min**. The method was validated and produced accurate and precise result for these drugs.

KEY WORDS

Paracetamol, Chlorzoxazone, diclofenac potassium

INTRODUCTION

Paracetamol (N-Acetyl-p-aminophenol) is used in the Symptomatic management of pain and fever [1,2].

It is much safer than other non-steroidal anti-inflammatory drugs [3].Diclofenac potassium [2-[2,6-Dichlrophenyl]amino] benzeneacetic acid monopotassium salt] is a nonsteroidal anti inflammatory drug used for a variety of painful and inflammatory condition [4,5] .Chlorzoxazone (5-chlor-2(3H) benzoxazolone) is a skeletal muscle relaxant principally used for relieving painful muscle spasms occurring in musculoskeletal and neuromuscular disorders [6,7,8]. Zarapkar *et al*. developed a reverse phase HPLC method for the simultaneous estimation of paracetamol and chlorzoxazone [9].Beaulieu *et al*. reported a reverse phase method of estimation of diclofenac sodium in formulation [10].The objective of the present investigation was to establish and validate the fast and sensitive high performance liquid chromatography (HPLC) method for simultaneous determination of paracetamol, chlorzoxazone and diclofenac potassium in tablets.

Materials and Methods

Drugs used

Paracetamol, Diclofenac Potassium and Chlorzoxazone (Inhouse Reference Standard)

Chemicals and solvents

Sodium Phosphate Monobasic, Methanol, Ortho Phosphoric Acid, MilliQ water. All chemicals and solvents used were of GR/HPLC grade of Rankem, India Limited

HPLC System

The HPLC system consisted of a solvent delivery module Agilent 1100 Series Isocratic pump equipped with 20 **µl** loop and G1365B Multi Wavelength Detector. Integration was achieved by using the

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software Chemstation. Separation was carried out on a Zorbax, (150×4.6mm) 5µm C-18 Column.

Chromatographic Condition

The mobile phase was prepared by mixing solvents, Methanol Buffer (70:30) v/v ratio. The Buffer consists of equal volume of 0.01 (M) ortho Phosphoric Acid and 0.01 (M) Monobasic Sodium Phosphate, pH adjusted to (2.5±0.2) with orthophosphoric acid. The prepared mobile phase was filtered through a Millipore 0.45 **µm** membrane filter and ultrasonically degassed prior to use. Methanol and Water in the ratio of 70:30 (v/v) was used as diluent throughout the experiment. The detection wavelength was set at 254 nm. The elution was done at a flow rate of 1.0 ml/min under ambient condition.

Standard Solution and Calibration Curve

A standard stock solution of Paracetamol (1000 mcg/ml), Diclofenac Potassium (120 mcg/ml) and Chlorzoxazone (1000 mcg/ml) were prepared in diluent. Subsequent dilutions were made in diluent to prepare the concentrations 40,42,44,46,48 and 50 mcg/ml for Paracetamol; 10,12,14,16,18 and 20 mcg/ml for Diclofenac Potassium and 45,48,51,54,57 and 60 mcg/ml for Chlorzoxazone. The calibration curve was done by plotting peak area against sample concentration for each ingredient.

Assay

Twenty tablets were finely powdered and weighed accurately in the elctronic balance (model Metler Toledo AG285). The powder equivalent to 325 mg of paracetamol, 250 mg of chlorozoxazone and 50 mg of diclofenac potassium was weighed accurately and dissolved in 250 ml methanol (HPLC **Grade**). The solution was filtered through 0.45 µm Millex-HV syringe driven membrane filter unit. Further appropriate dilutions have been made to get concentration of 50µg/ml of paracetamol, 60 µg/ml of chlorzoxazone, 20-µg/ ml of diclofenac potassium. Twenty µl of this solution was injected in triplicate under the specified conditions. The peak areas obtained were related to **slopes** and intercepts from the calibration data to calculate concentration of the drugs (Table 1).

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RESULTS

Table 1.
Result of HPLC Assay

Paracetamol		Chlorzoxazone		Diclofenac Potassium	
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	324.52 323.46 324.65	250	250.4 249.8 250.2	50	49.54 50.25 49.56
Mean	324.21		250.13		49.78
RSD	0.002		0.001		0.008

Validation of Assay

To validate the developed method accuracy, reproducibility and recovery experiments were carried out. The recovery of the known amount of added standard was studied at three levels. To an aliquot of the analyzed formulation a known concentration of standard solution was added. The content of paracetamol, chlorzoxazone and diclofenac potassium was determined (Table 2).

Table 2.
Result of Recovery Studies

	Paracetamol			Chlorzoxazone			Diclofenac Potassium		
Amount added (mg)	10	20	30	10	20	30	10	20	30
Amount found (mg)	334.95	345.58	354.12	260.88	268.45	279.36	58.92	69.39	78.22
Percentage Recovery	98.92	100.50	99.90	98.56	100.92	99.35	99.02	98.45	99.33
Mean	99.77			99.61			98.33		

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Table 3.
Linear regression data for calibration curve

Drugs	Paracetamol	Diclofenac Potassium	Chlorzoxazone
Concentration range (mcg/ml)	40-50	10-20	45-60
Slope	32.018	12.637	2.8281
Intercept	9.66	-1.6329	1.8233
R^2	0.9999	0.9998	0.9983

RESULTS AND DISCUSSION

Figure 1 shows typical chromatograms of Paracetamol, Chlorzoxazone and Diclofenac Potassium drugs. 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 40-50 µg/ml for paracetamol, 45-60 µg/ml for chlorzoxazone and 10-20 µg/ml for diclofenac **potassium**. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.279 µg, 12.34 µg/ml for paracetamol; 3.17 µg, 18.53 µg/ml for diclofenac potassium and 0.73 µg, 12.35 µg/ml for chlorzoxazone.

Table 4.
System Suitability Parameters

Drugs	Paracetamol	Diclofenac Potassium	Chlorzoxazone
Calibration range (mcg/ml)	40-50	10-20	45-60
Theoretical Plate	1520	2252	2960
Tailing Factor	1	1	1
LOD	0.279	3.17	0.73
LOQ	12.34	18.53	12.35

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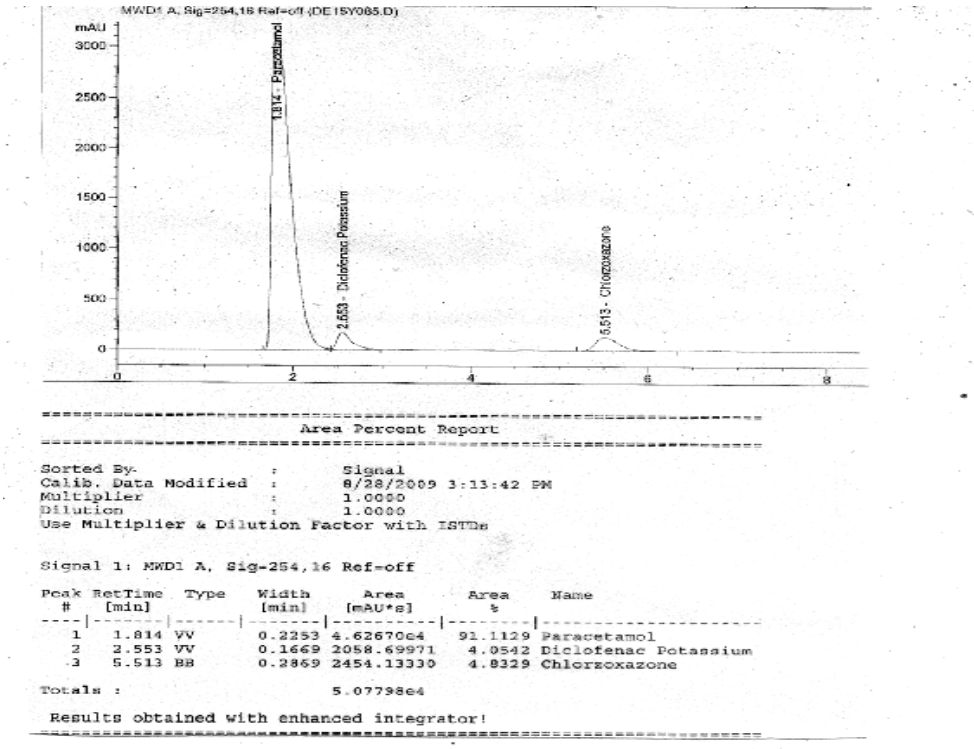

Area Percent Report

Sorted By : Signal
Calib. Data Modified : 8/28/2009 3:13:42 PM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: MWD1 A, Sig=254,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	1.814	VV	0.2253	4.62670e4	91.1129	Paracetamol
2	2.553	VV	0.1669	2058.69971	4.0542	Diclofenac Potassium
3	5.513	BB	0.2869	2454.13330	4.8329	Chlorzoxazone

Totals : 5.07798e4

Results obtained with enhanced integrator!

Fig 1.
Chromatogram of Paracetamol, Chlorzoxazone and Diclofenac Potassium

CONCLUSION

This is fast, reproducible, accurate method for simultaneous determination of Paracetamol, Diclofenac Potassium and Chlorzoxazone from various dosages forms.

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