Research Paper :

Method development and validation for the simultaneous estimation of aceclofenac, paracetamol and chlorzozazone in pharmaceutical dosage forms by high performance thin layer chromatography

T. VENKATESH KARTHIKEYAN, V. VAITHYALINGAM, G. YUVARAJ, A. RAVI KUMAR AND **R.K. NEMA**

See end of the article for authors' affiliations

Correspondence to:

R.K. NEMA
Rishiraj College of
Pharmacy, INDORE (M.P.)
INDIA

ABSTRACT

A simple, rapid, sensitive and highly precise High Performance Thin Layer Chromatographic Method has been developed for the estimation of Paracetamol, Aceclofenac and Chlorzoxazone in tablets. HPTLC was performed on CAMAG LINOMAT IV, TLC Scanner Version 3.20, using toluene, ethyl acetate and glacial acetic acid (17.5:10:0.5 v/v) as mobile phase. The Chromatogram was developed in CAMAG twin trough glass containing mobile phase. The TLC plates were scanned at 271 nm in schimadzu dual wavelength scanner, and R_f value of Paracetamol, Aceclofenac and Chlorzoxazone was found to be 0.12, 0.29 and 0.72, respectively. The linearity of Paracetamol, Aceclofenac and Chlorzoxazone shows a correlation coefficient of 0.9995, 0.9991, and 0.9997, respectively. The proposed method was validated by determining sensitivity, accuracy, precision and system suitability parameters.

Aceclofenac {2[(2,6-dichlorophenyl)amino]benzoic acid carboxymethyl ester} is an analgesic and non-steroidal anti-inflammatory drug. 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. Chlorzoxazone (5-chloro-2(3H)-benzoxazolone) is a compound with skeletal muscle relaxant property. It is used to decrease muscle tone and tension and used to relieve spasm and pain associated with musculoskeletal disorders. Aceclofenac is official in B.P[1], paracetamol in B.P and I.P[2,3] and chlorzoxazone in U.S.P[4]. B.P. suggests a potentiometric assay method for aceclofenac in bulk drugs. The I.P. and B.P. both suggest titrimetric and UV spectrophotometric assay method for paracetamol in bulk and tablet formulations. Literature survey revealed that high performance liquid chromatography spectrofluorimetric[5], calorimetric6, densitometric[7] and (HPLC)[8,9] methods have been reported for the estimation of aceclofenac in pharmaceutical dosage forms. A spectrophotometric method[10] has been reported for the simultaneous estimation of three drugs in formulation. This prompted us to develop and validate HPTLC method for the simultaneous estimation of Paracetamol, Aceclofenac and Chlorzoxazone in tablets.

MATERIALS AND METHODS
Instruments used:

CAMAGLINOMAT IV (Schimadzu Dual Wavelength Scanner), Silica HPTLC Plate, CAMAG Sample Applicator, CAMAG twin trough glass chamber, Hamilton Syringe-2.5µl, CAMAG TLC Scanner Version 3.20.

Chemicals and reagents:

Toluene, Ethyl acetate, Glacial acetic acid (HPLC grade from E-Merck).

Mobile phase:

Mixed 35ml of Toluene with 10 ml of ethyl acetate and then 1ml of glacial acetic acid is added to get the required mobile phase.

Chromatographic conditions:

Stationary phase : silica gel G_f 254
Mobile phase : Toluene+ethyl acetate+ glacial acetic acid 17.5:10:0.5 (v/v)
Lamp : deuterium
Wave length : 271nm
Migration distance : 70mm
Bandwidth : 3mm
Distance between : 10mm
the tracks

Varying quantities of the stock solution was suitably diluted with methanol to obtain the concentration of 100-500 µg/ml for Paracetamol, 100-500 µg/ml for Chlorzoxazone and 20-100 µg/ml for Aceclofenac. The

solution is then spotted on the TLC plates by using automatic application device. Chromatographic plate is then developed in a saturated twin trough chamber containing the mobile phase. After development the plates were scanned at 271 nm and the peak areas were measured. Calibration curve was constructed by plotting concentration against peak area.

Market sample analysis:

Twenty tablets were weighed and crushed to finely powdered material. Aliquot quantity of powder was accurately weighed and transferred to a 100ml volumetric flask and dissolved in methanol, and made up to 100ml with methanol. From this solution, further dilutions were made in methanol to get the required concentration. The solution is then spotted on the TLC plates by using automatic application device. The Chromatographic plate is then developed in a saturated twin trough chamber containing the mobile phase. After development the plates were scanned at 271nm and the peak areas were measured. The amount of Paracetamol, Aceclofenac and Chlorzoxazone were obtained from the calibration curve.

Recovery studies:

It was performed to assess the accuracy of the analytical method. The recovery experiments were carried out in triplicate by adding a known amount of drug to pre-analyzed sample and the percentage recovery was calculated.

RESULTS AND DISCUSSION

The retention factor (R_f) of Paracetamol, Aceclofenac and Chlorzoxazone were found to be 0.12, 0.29 and 0.72, respectively. The linearity range were found to be 100-500 µg/ml for Paracetamol, 100-500 µg/ml for Chlorzoxazone and 20-100 µg/ml for Aceclofenac. Correlation coefficient greater than 0.9991 for all the drugs indicates good linearity between concentration and peak area. The variance of ruggedness less than 0.1697 for HPTLC proves the suitability of the proposed method. The percentage recovery indicates the proposed method is highly accurate. The densitogram and the values pertaining to evaluation are given in the Table 1, 2, 3, 4, 5, 6 and 7 and Fig. 1, 2, 3, 4 and 5.

The proposed method was found to be simple, precise, rapid and sensitive for routine quantitative determination. The amount of drug recovered by the above methods was in good agreement with the label claim and the good percentage recovery in HPTLC indicates the reproducibility of the proposed method.
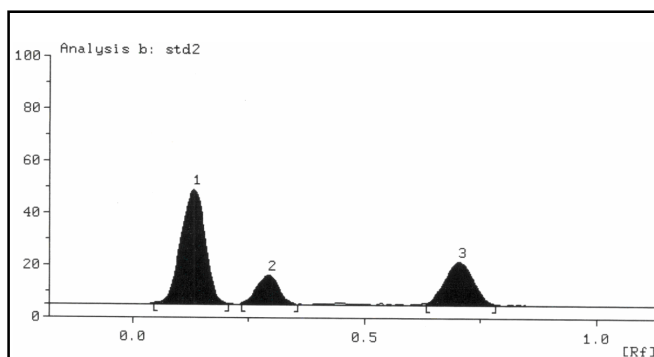
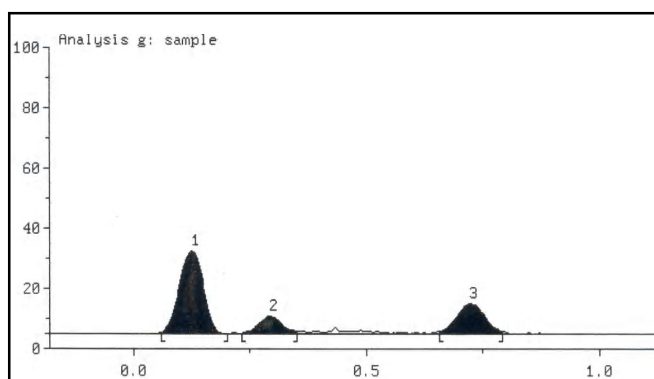**Fig. 1 :   Standard chromatogram of Paracetamol, Aceclofenac and Chlorzoxazone (HPTLC)**

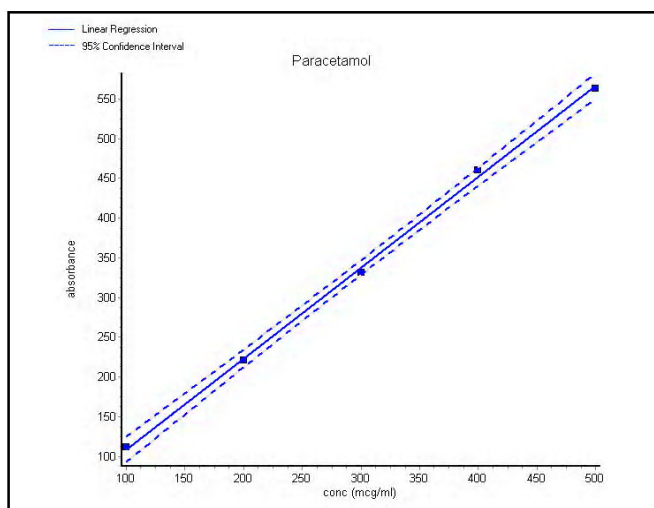**Fig. 2 :   Sample chromatogram of Paracetamol, Aceclofenac and Chlorzoxazone (HPTLC)**

Fig. 3 :   Linearity graph of Paracetamol (HPTLC)

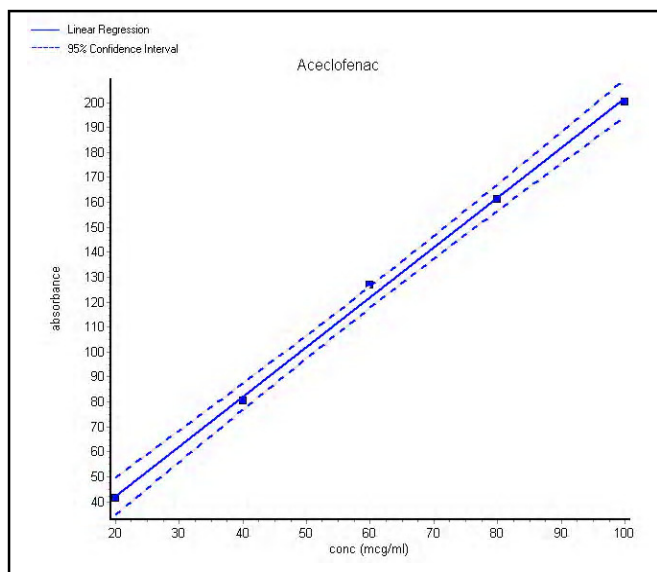| Table 1 : Linearity and range of Aceclofenac (HPTLC)		
Sr. No.	Concentration µg/ml	Peak area
1.	20	41.3
2.	40	80.5
3.	60	126.7
4.	80	161.3
5.	100	200.4

Fig. 4 :   Linearity graph of Aceclofenac (HPTLC)
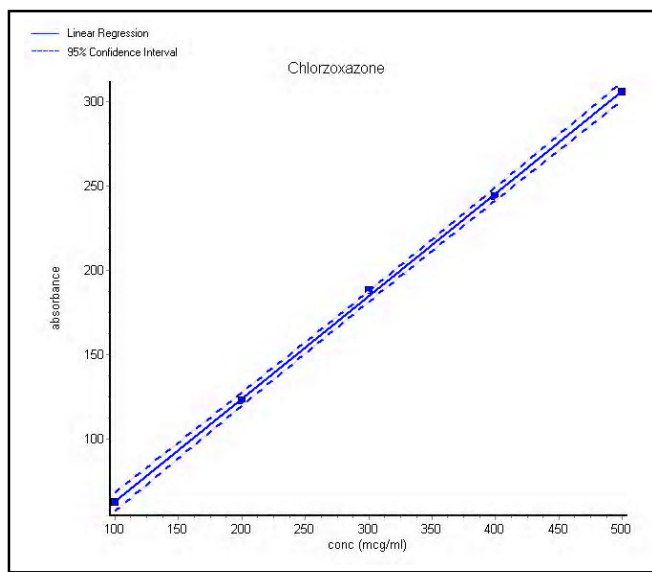
Fig. 5 :   Linearity graph of Chlorzoxazone (HPTLC)

Table 2 : Llinearity and range of Paracetamol (HPTLC)		
Sr. No.	Concentration μg/ml	Peak area
1.	100	111.3
2.	200	220.8
3.	300	331.6
4.	400	460.1
5.	500	563.2

Table 3 : Linearity and range of Chlorzoxazone (HPTLC)		
Sr. No.	Concentration μg/ml	Peak area
1.	100	62.2
2.	200	122.5
3.	300	187.7
4.	400	243.6
5.	500	305.9

Table 4 : Estimation of Paracetamol, Aceclofenac and Chlorzoxazone (HPTLC)					
Sr. No.	Name of the drug	Label claim (mg/tablet)	Amount found (mg/tablet)	Standard deviation	Relative standard deviation
1.	Aceclofenac	100	102.1286		
2.		100	101.8918	0.537229	0.52820
3.		100	101.1025		
1.	Paracetamol	500	501.9841		
2.		500	502.702	0.679907	0.13526
3.		500	503.3051		
1.	Chlorzoxazone	500	495.9923		
2.		500	495.7259	0.704765	0.14201
3.		500	497.0578		

Table 5 : Recovery of Paracetamol, Aceclofenac and Chlorzoxazone (HPTLC)					
Sr. No.	Drug	Amount of drug in sample (mg)	Amount of drug added (mg)	Amount recovered (mg)	Percent recovery
1.	Aceclofenac	102.1286	10	9.8655	98.6587
2.		101.8918	10	9.9445	99.4451
3.		101.1025	10	10.0234	100.2344
1.	Paracetamol	501.9481	50	49.6067	99.2135
2.		502.702	50	49.7575	99.5150
3.		503.3051	50	49.9083	99.8166
1.	Chlorzoxazone	495.992	50	49.5459	99.0919
2.		495.7529	50	50.0787	100.1574
3.		497.0578	50	49.5459	99.0919

Table 6 : LOD and LOQ (HPTLC)				
Sr. No.	Parameter	Aceclofenac	Paracetamol	Chlorzoxazone
1.	LOD	4µg/ml	8 µg/ml	17 µg/ml
2.	LOQ	15 µg/ml	29 µg/ml	57µg/ml

Table 7 : System Suitability Parameters (HPTLC)				
Sr. No.	Parameter	Paracetamol	Aceclofenac	Chlorzoxazone
1.	R_f	0.12	0.29	0.72
2.	Asymmetry factor	1.07	1.02	1.12
3.	Theoretical plates/meter	962	1956	4365
4.	Linearity	100-500 µg/ml	20-100 µg/ml	100-500 µg/ml

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Authors' affiliations:
T. VENKATESH KARTHIKEYAN AND V. VAITHYALINGAM, Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, CHENNAI (T.N.) INDIA
G. YUVARAJ, Department of Medical Chemistry, J.S.S. University, OOTACAMUND (T.N.) INDIA
A. RAVI KUMAR, Department of Pharmacognosy, Bapatala College of Pharmacy, BAPATLA (A.P.) INDIA

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