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.

Keywords: Paracetamol, Aceclofenac, Chlorzoxazone, HPTLC, validation.

1. INTRODUCTION
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 & 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. & 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 \[5\], calorimetric \[6\], densitometric \[7\] and HPLC \[8,9\] methods have been reported for the estimation of aceclofenac in pharmaceutical dosage forms. A spectrophotometric method \[10\] have been reported for the simultaneous estimation of the three drugs in formulation. This prompted us to develop and validate HPTLC method for the simultaneous estimation of Paracetamol, Aceclofenac and Chlorzoxazone in tablets.

2. EXPERIMENTAL
2.1. MATERIALS AND METHOD
2.1.1. 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.

2.1.2. Chemicals and Reagents
Toluene – HPLC grade from E-Merck.
Ethyl acetate – HPLC grade from E-Merck.
Glacial acetic acid – HPLC grade from E-Merck.

2.1.3. 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.

2.1.4. Chromatographic Conditions
Stationary phase : silica gel G 
Mobile phase : Toluene + ethyl acetate + glacial acetic acid 17.5:10:0.5 (v/v)
Lamp : deuterium
Wavelength : 271nm
Migration distance : 70mm
Bandwidth : 3mm
Distance between the tracks: 10mm
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. 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.

2.2.1. 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 percentage recovery indicates the proposed method is highly accurate. The densitogram and the values pertaining to evaluation are given in the table 1-6 and figure 1-3.

2.2.2. 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.

3. RESULTS AND DISCUSSION
The retention factor (Rf) 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 1-6 and figure 1-3.

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<tr>
<th>Table No 1: Linearity and Range Of Aceclofenac</th>
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Figure No.2 Sample chromatogram of Paracetamol, Aceclofenac and Chloroxazone (HPTLC)
4. CONCLUSION

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.

5. REFERENCES


