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HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHIC DETERMINATION OF PARACETAMOL, DICLOFENAC, AND CAFFEINEIN COMBINED TABLET DOSAGE FORM

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Keywords:

Paracetamol, Diclofenac, Caffeine, Tablet dosage form, HPTLC

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ABSTRACT: A new simple High-Performance Thin Layer Chromato-graphic (HPTLC) method for determining Paracetamol, Diclofenac and Caffeine in combined tablet dosage form has been developed and validated. The mobile phase selected was Ethyl acetate: Methanol: ammonia (8: 2:0.1 v/v/v) with UV detection at 263 nm. The retention factor for Paracetamol, Diclofenac, and caffeine was found to be 0.639 ± 0.018, 0.267 ± 0.026, and 0.447 ± 0.026, respectively. The method was validated with respect to linearity, accuracy, precision and robustness as per the International Conference on Harmonization (ICH) guidelines. Results were found to be linear in the concentration range of 1000-6000 ng/band for Paracetamol 200-1200 ng/band for Diclofenac and 100-600 ng/band for caffeine, respectively. The method has been successfully applied to analyze drugs in pharmaceutical formulation. The % assay (Mean ± S.D.) was found to be 98.64 ± 0.184 for paracetamol, 97.17± 1.074 for Diclofenac and 98.27± 0.86 caffeine. The method can be used for routine analysis of these drugs in combined tablet dosage forms in quality-control laboratories.

INTRODUCTION: Paracetamol [PCM] is an analgesic and antipyretic drug that is used to relieve mild-to-moderate pain and fever temporarily. Paracetamol, also known as acetaminophen, is used to treat fever and mild to moderate pain. Paracetamol is N-acetyl-para-aminophenol [1]. Diclofenac is an anti-inflammatory drug used to relieve pain, swelling (inflammation), and joint stiffness caused by arthritis. This medication is known as a nonsteroidal anti-inflammatory drug (NSAID). Diclofenac [DCF] is 2-[2-(2,6-dichloroanilino) phenyl] acetic acid. Diclofenac Sodium is the sodium salt form of diclofenac, a nonsteroidal anti-inflammatory drug (NSAID) with analgesic, antipyretic and anti-inflammatory activity [2].

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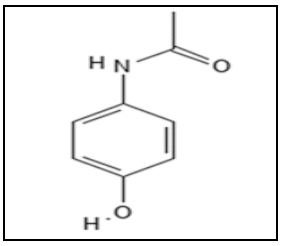
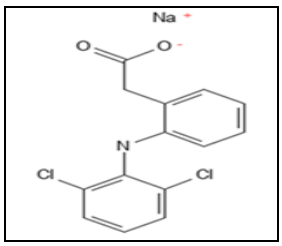
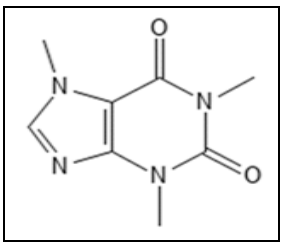
Caffeine [CAF] is 1,3,7-Trimethylpurine-2,6-dione, a central nervous system (CNS) stimulant of the methylxanthine class. It is a cognitive enhancer, increasing alertness and attentional performance [3]. The chemical structure of Paracetamol, Diclofenac, and Caffeine is presented in **Fig. 1.**



Paracetamol



Diclofenac Sodium



Caffeine

FIG. 1: CHEMICAL STRUCTURE OF DICLOFENAC SODIUM, PARACETAMOL AND CAFFEINE

The literature survey reveals High-Performance Liquid Chromatographic (HPLC) methods for the determination of PCM and DCF in combination with other drugs by HPLC [4-6]. Stress degradation study by HPLC for determination of PCM in combination with other drugs [7]. Spectrophotometric methods for estimating PCM and DCF as a single drug in dosage form have also been reported [8-10]. No reports were found for the simultaneous estimation of the paracetamol, diclofenac and caffeine in combined dosage form by the HPTLC method. This paper describes a simple, accurate, sensitive, and validated HPTLC method for the simultaneous quantification of these compounds as bulk drugs and in tablet dosage forms. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [11].

MATERIALS AND METHODS:

Reagents and Chemicals: Analytically pure samples of Paracetamol, Diclofenac Sodium and caffeine were kindly supplied by Emcure Pharma Pvt. Ltd. (Pune, India) and Cipla Pvt. Ltd. (Kurkumbh, India) respectively.

Toluene and Methanol (AR grade) were obtained from Thomas Baker Pvt Ltd (Mumbai, India). Ethyl acetate was obtained from LobaChemie Pvt Ltd. (Mumbai, India) and developed for the method. The pharmaceutical dosage form used in this study was PARASYL-DC (Krosyl pharmaceutical Pvt. Ltd., Vadodara, India) labeled to contain 500 mg of Paracetamo l,50 mg of Diclofenac and Caffeine 30 mgwere procured from the local market.

EXPERIMENTAL:

Instrumentation and Chromatographic Conditions: The samples were spotted in the form of bands of a width of 6 mm with space between bands of 10 mm, with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminum plate 60 F_{254} (10×10) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions were 5mm × 0.45mm and a scanning speed of 20 mm/sec was employed. The linear ascending development was carried out in a 10cm × 10cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using Ethyl acetate: Methanol: Ammonia (8: 2: 0.1 v/v/v) as a mobile phase. The optimized chamber saturation time for the mobile phase was 20 min. The length of the chromatogram run was 9 cm and the development time was approximately 15 min. TLC plates were dried in a current of air with the help of a hair drier.

Densitometric scanning was performed on a CAMAG thin-layer chromatography scanner at 263 nm for all developments operated by the Vision CATS software version. The radiation source was a deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm.

Preparation of Standard Stock Solutions: A standard stock solution of paracetamol was prepared by dissolving 10 mg of the drug in 10 mL of methanol to get a concentration of 1 ng/µL of paracetamol. Caffeine was prepared by dissolving 10 mg of the drug in 10 mL of methanol to get a concentration of 1 mg/mL from which 1 mL was further diluted to 10 mL to get a stock solution of 100 ng/µL of caffeine.

The standard stock solution of Diclofenac was prepared by dissolving 10 mg of the drug in 10 mL of methanol to get a concentration of 1 mg/mL from which 2 mL was further diluted to 10 mL to get a stock solution of 200 ng/µL of Diclofenac.

Selection of Detection Wavelength: After chromatographic development bands were scanned over the 200-400 nm range, and the spectra were overlain. It was observed that all three drugs showed considerable absorbance at 263 nm. So, 263 nm was selected as the wavelength for detection as shown in **Fig. 2, 3, and 4.**

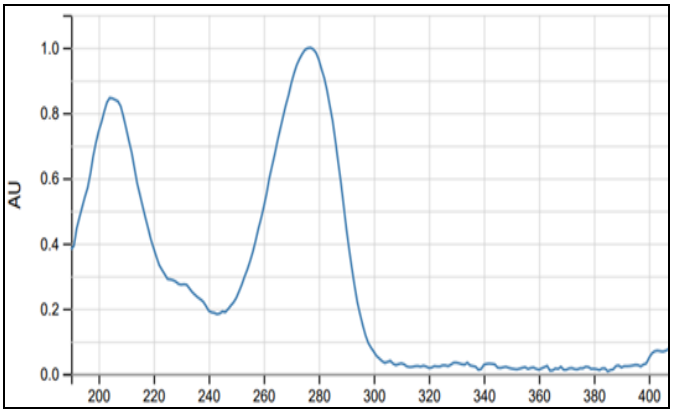


FIG. 2: SPECTRA OF CAFFEINE AT 278 NM

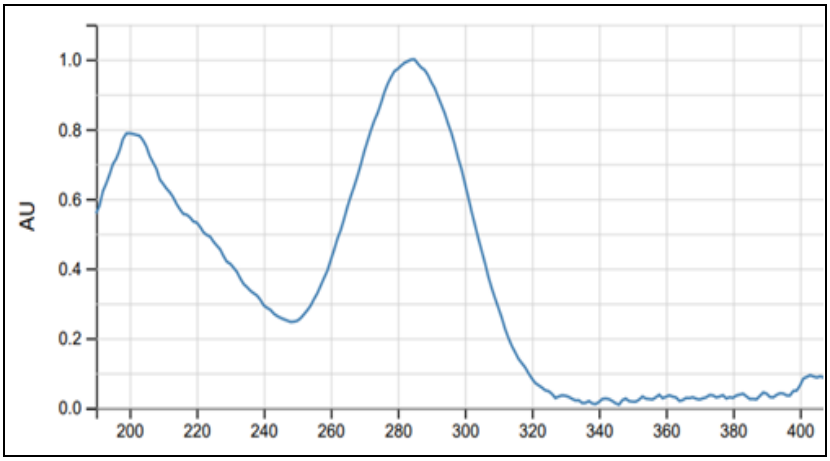


FIG. 3: SPECTRA OF DICLOFENAC SODIUM AT 283 NM

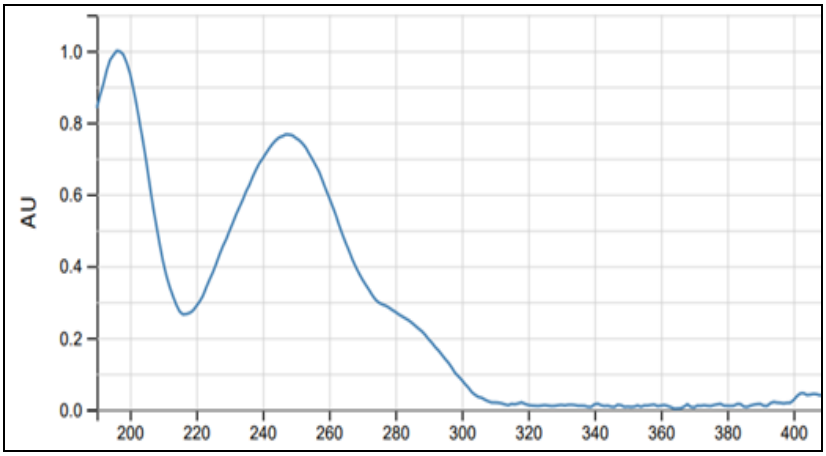


FIG. 4: SPECTRA OF PARACETAMOL AT 248 NM

Method Validation: The method was validated for linearity, accuracy, intra-day, inter-day precision, and robustness by following ICH guidelines [21].

Preparation of Calibration Curve: The standard stock solutions of paracetamol (1000 ng/µL), Diclofenac Sodium (200 ng/µL), and caffeine (100 ng/µL each) were applied by over-spotting on a TLC plate in the range of 1, 2, 3, 4, 5 and 6µL respectively with the help of CAMAG 100 µL sample syringe, using Linomat 5 sample applicator. The plate was developed and scanned under the above-established chromatographic conditions. Each standard in six replicates was analyzed and peak areas were recorded. Calibration curves of Paracetamol, Diclofenac and Caffeine were plotted separately of peak area vs respective concentration of Paracetamol, Diclofenac and Caffeine.

Precision: A Set of three different concentrations in three replicates of mixed standard solutions were prepared. All the solutions were analyzed on the same day to record any intraday variations in the results. For Inter day variation study, three different concentrations of the mixed standard solutions in the linearity range were analyzed on three consecutive days.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): LOD and LOQ for both drugs were calculated by using the values of slopes and intercepts of the calibration curves.

Robustness Studies: In the robustness study, the influence of small, deliberate variations of the analytical parameters on the peak area of the drugs was examined. Factors varied were mobile phase saturation (± 10%), composition (± 2%), time from application to development (0, 10, 20, and 30 min) and from development to scanning (0, 30, 60 and 90 min) development distance (± 10 %), One factor at a time was changed to estimate the effect. The method's robustness was checked at a concentration level of 2000 ng/ band for paracetamol, 600 ng/band for Diclofenac Sodium, and 200 ng/ band for caffeine. The results are given in **Table 1**

TABLE 1: ROBUSTNESS DATA IN TERMS OF PEAK AREA (% RSD)

Sr. no.	Parameter	(% RSD) *		
		Paracetamol	Diclofenac Sodium	Caffeine
1	Mobile phase composition	1.060	0.597	0.125
2	Mobile phase saturation	0.819	0.396	0.664
3	Time from application to development	0.544	0.638	0.307
4	Development to scanning	0.649	0.397	0.476
5	Development distance	1.651	0.199	0.488

*Average of three determinations.

Recovery Studies: To ascertain the accuracy of the proposed method recovery studies were carried out by standard addition method, as per ICH guidelines. The accuracy of the method was estimated by spiking the drug standard in a pre-determined laboratory mixture solution at concentration levels of 50%, 100% and 150% and determined as percent recovery studies. The results of recovery studies were expressed as percent recovery and are shown in **Table 2.**

TABLE 2: RECOVERY STUDIES OF PARACETAMOL, DICLOFENAC SODIUM AND CAFFEINE

Drug	Amount taken (ng/band)	Amount added (ng/band)	Total amount found (ng/band)	% Recovery	% RSD[a]
Paracetamol	2000	1000	2932.93	100.7	0.303
	2000	2000	3938.50	100.4	0.380
	2000	3000	4887.37	100.2	1.439
Diclofenac	200	100	294.01	100.3	0.346
	200	200	394.50	101.4	0.470
	200	300	489.95	100.5	0.282
Caffeine	120	60	175.82	100.5	1.046
	120	120	236.44	100.4	0.861
	120	180	292.06	100.6	0.178

[an] Average of three determinations.

RESULTS AND DISCUSSION: Different mobile phases containing various ratios of Ammonia, Toluene, Ethyl acetate, Methanol, Chloroform and Glacial acetic acid were examined (data not shown). Finally, the mobile phase containing Ethyl acetate: Methanol: Ammonia (8: 2: 0.1 v/v/v) was selected as optimal for obtaining well-defined and resolved peaks.

The optimum wavelength for detection and quantitation used was 263 nm. The retention factors for Diclofenac, Caffeine and Paracetamol were found to be 0.267±0.026, 0.447±0.026 and 0.623±0.018, respectively. A representative dendrogram of a mixed standard solution of Paracetamol diclofenac and caffeine is shown in **Fig. 5.**

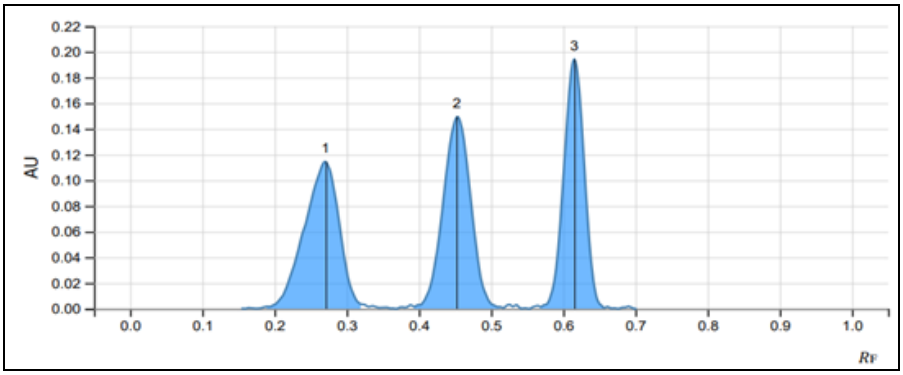


FIG. 5: REPRESENTATIVE CHROMATOGRAM OF THE MIXED STANDARD SOLUTION OF DICLOFENAC SODIUM (R_f = 0.267 ± 0.026), Caffeine (R_f = 0.447 ± 0.026), and Paracetamol (R_f = 0.623 ± 0.018).

Straight-line calibration graphs were obtained for Paracetamol, Diclofenac and Caffeine in the concentration range 1000-6000 ng/band, 200-1200 ng/band, and 100-600 ng/band with high correlation coefficient > 0.991. The proposed method was also evaluated for bulk drugs.

The % assay (Mean ± S.D.) was found to be 98.64± 0.184 for paracetamol, 97.17± 1.074 for Diclofenac, and 98.27± 0.863 for caffeine. The robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes in the operational parameters (% RSD < 2). The recovery study results for paracetamol ranged from 97.74 to 98.46 % with % RSD values ranging from 0.144to 0.885. For Diclofenac the recovery results ranged from 97.99 to 98.62 % with % RSD values ranging from 0.408to 0.967, and for caffeine the recovery study results ranged from 97.35 to 98.51 % with % RSD values ranging from 0.107to 0. 802. The method was found to be accurate and precise, as indicated by recovery studies, as recoveries were close to 100 % and % RSD not more than 2. Intra-day variation, as RSD (%), was found to be in the range of 0.235–0.439 for Paracetamol, 0.386–0.722 for Diclofenac and 0.203 – 0.836 for caffeine. Inter-day variation, as RSD (%) was found to be in the range of 0.111–1.387 for Paracetamol, 0.140–1.375 for Diclofenac and 0.065-0.485 for Caffeine. The summary of validation parameters of the proposed method is given in **Table 3.**

TABLE 3: SUMMARY OF VALIDATION PARAMETERS OF THE PROPOSED METHOD

Parameters	Paracetamol	Diclofenac	Caffeine
Linearity range (ng/band)	1000-500	200-1200	100-600
Correlation coefficient (r)	0.995	0.993	0.991
LOD[a] (µg/ml)	27.54	64.27	16.54
LOQ[b] (µg/ml)	83.46	94.76	44.13
Accuracy (% Recovery)	97.74 -98.46	97.99–98.62	97.35 - 98.51
Precision (% RSD) [c]			
Intraday (n[d] = 3)	0.23-0.43	0.38-0.72	0.20-0.83
Inter day (n = 3)	0.11-1.38	0.14-1.36	0.065-0.48

[a]LOD = Limit of detection. [b]LOQ = Limit of quantitation. [c]RSD = Relative standard deviation. Dn = Number of determinations.

CONCLUSION: The validated HPTLC method employed here proved to be simple, fast, accurate, precise, and robust, thus can be used for routine analysis of Paracetamol, Diclofenac, and Caffeine in combined tablet dosage form.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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