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Development and validation of HPTLC method for simultaneous quantification of Paracetamol, Phenylephrine hydrochloride, Nimesulide, Cetrizine and Caffeine in bulk and pharmaceutical dosage form

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ABSTRACT

A simple and accurate densitometric method has been optimized, developed and validated for the estimation of drugs in bulk and combined dosage form. The Chromatographic analysis was carried out on Merck aluminum plates precoated with silica gel 60 F_{254} was stationary phase. The optimized mobile phase was toluene: ethyl acetate: methanol: formic acid (16:2:4:0.8, v/v/v/v). The chamber of 20 cm × 20 cm was used and saturation time was 20 minutes. The retardation factor for Paracetamol, Phenylephrine hydrochloride, Nimesulide, Cetrizine and Caffeine was found to be 0.37, 0.09, 0.70, 0.27 and 0.51, respectively. Chromatographic analysis was carried out at 212 nm. The validation was done as per ICH Q2 (R1) Guideline. Linearity range for Paracetamol, Nimesulide, Cetrizine and Caffeine was found to be 200-1400 ng band^{-1} and that of Phenylephrine hydrochloride, was found to be 100-1400 ng band^{-1}. The proposed method was found to be significant for estimation of drug in bulk and combined dosage form.

Key words: Paracetamol, Phenylephrine Hydrochloride, Caffeine, Nimesulide, Cetirizine, HPTLC, Validation.

INTRODUCTION

High Performance Thin-Layer Chromatography is the most simple separation technique today available to the analyst. It can simultaneously handle several samples even of divergent nature and composition and analyses at a time [1]. HPTLC is a visual technique where the “densitogram” is “visible”. This gives us additional information and confidence in the results [2].

Phenylephrine hydrochloride(PHE) chemically is (1R)-1-(3hydroxy-phenyl)-2-(methylamino) ethanol hydrochloride (Figure 1.1). It is used as a nonspecific sympathomimetic which stimulates postsynaptic alpha receptor cause vasoconstriction, systolic and diastolic pressure.

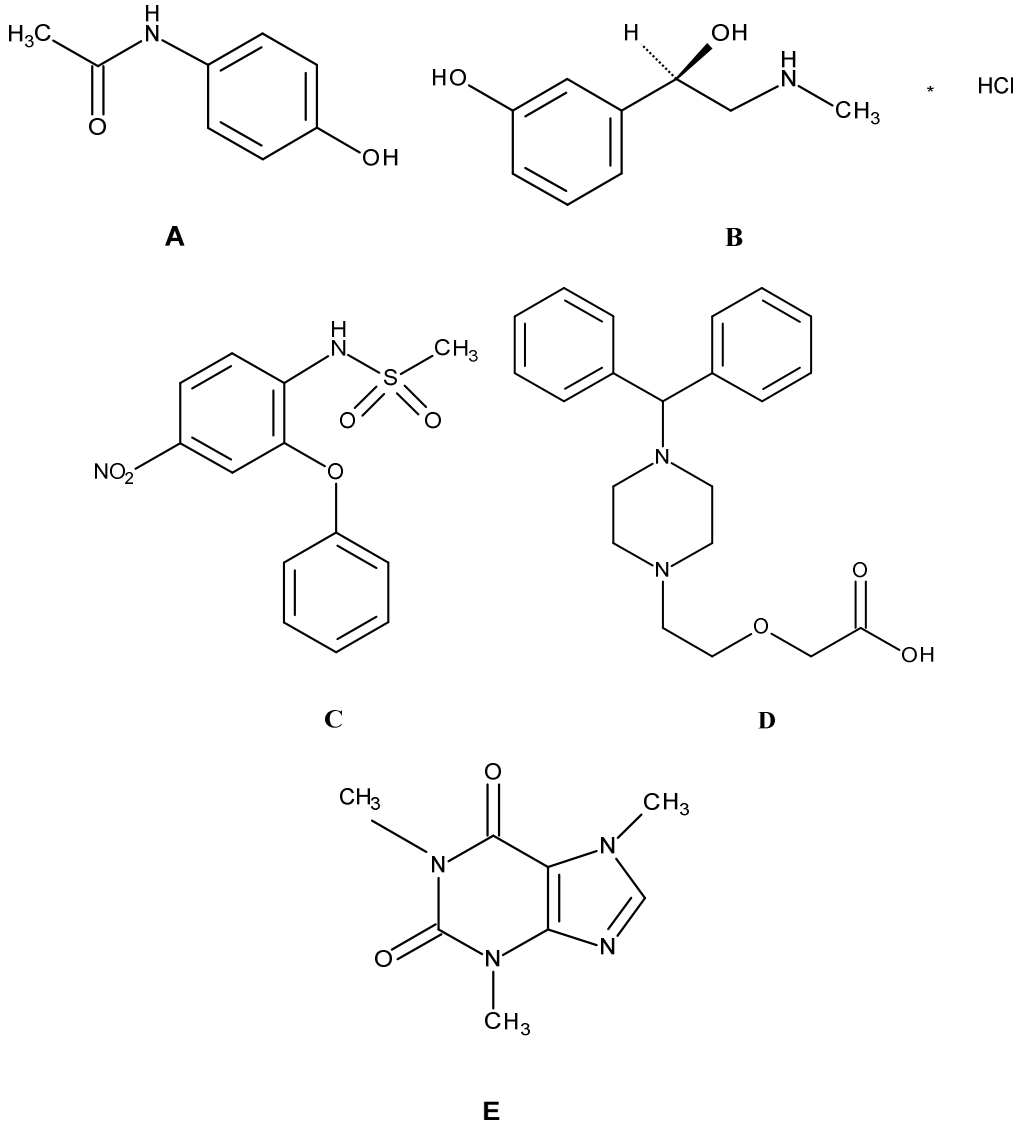

Figure No.1.1: Structures of (A) Paracetamol, (B) Phenylephrine hydrochloride, (C) Nimesulide, (D) Cetrizine and (E) Caffeine

PHE exhibit absorption maxima at 273nm [3-4]. Chemically Paracetamol (PARA) is N-(4-hydroxyphenyl) acetamide; commonly known as acetaminophen (Figure 1.1). PARA exhibit absorption maxima at 257 nm. It is used as analgesic and antipyretic. Nimesulide (NIM) is *N*-(4-Nitro-2-phenoxyphenyl) methane sulphonamide (Figure 1.1). NIM exhibit absorption maxima at 277 nm. Nimesulide is COX-2 selective, non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties [5-7]. Cetirizine hydrochloride (CET), chemically, [2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl] ethoxy] acetic acid. It is a non-sedative antihistamine, used in the treatment of seasonal hay fever, rhinitis, running nose, control sneezing of allergic origin (Figure 1.1) [8]. Caffeine (CAF) chemically is 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione. CAF is used as CNS stimulant, mild diuretic and respiratory stimulant. It is often combined with analgesics to treat migraine and other headache types [9] (Figure 1.1).

Methods for paracetamol and its combinations in pharmaceuticals or in biological fluids have been reported. Paracetamol has been determined in combination with other drugs using UV-spectrophotometry [10], thin-layer chromatography [11] (TLC), high-performance liquid chromatography [12] (HPLC) in pharmaceutical formulations. An HPLC method for phenylepherine in combination with ibuprofen has been reported [13]. Caffeine has been analyzed by a analytical methods such as spectrophotometry [14] and HPLC [15].

Cetirizine has been reported for analytical methods such as HPLC for estimations in formulations [16] and spectrophotometry [17]. Nimesulide the other analyte of multidrug combination has been quantified by analytical methods such as HPLC [18] and UV spectrophotometry [19].

Literature survey reveals that, individual analytical methods are reported but no HPTLC method is available for simultaneous quantification of the selected drugs in bulk and pharmaceutical dosage form, hence the study was undertaken.

MATERIALS AND METHODS

2.1. Instrumentation

The bands of standard and samples were applied with CAMAG High Performance Thin Layer Chromatography System (HPTLC) (Muttenz, Switzerland) Linomat V sample applicator using 100 µL CAMAG syringe. Chromatographic separation was achieved on 20 cm × 20 cm aluminum plates precoated with silica gel 60 F_{254}. The plates were washed with methanol and activated at 110°C for 5 min before use. After chromatographic development plates were dried with current of air and densitometric scanning was performed in reflectance/absorbance mode at 212nm using CAMAG TLC scanner III operated by winCATS software version 1.4.4. Slit dimension was 5 × 0.45 mm and scanning speed was 10 mm s^{-1}.

2.2 Chemicals and reagents

In HPTLC method development, toluene, ethyl acetate, dichloromehane, ammonia, methanol and formic acid of AR grade were used (Merck, India). Standard drugs were obtained as gift sample from Emcure Pharmaceutical Limited, Pune, Centaur Pharmaceuticals Private Limited, Pune and Necsunim Flu n' Cold tablets formulation was procured from local market.

2.3 Chromatographic conditions

Chromatographic separation was carried out by linear ascending method in 20 cm × 20 cm twin trough glass chamber (CAMAG) previously saturated with mobile phase for 20 min. The optimized mobile phase for estimation is toluene: ethyl acetate: methanol: formic acid (16:2:4:1, v/v/v/v).

2.4 Method Validation [20]

Linearity Limit of Detection and Quantification

Linearity was evaluated by applying minimum five concentration six replicates to HPTLC plate PARA, NIM, CET, CAF in the range of 200-1400 ng $band^{-1}$ and PHE 100-1400 ng $band^{-1}$. Calibration curve of peak area versus concentration was plotted.

Analysis of a marketed formulation

Powdered tablet equivalent to PARA 32.5 mg, PHE 5 mg, NIM 10 mg and CET 25 mg CAF 5 mg was transferred into a 100 mL volumetric flask and volume was made by methanol. The solution was sonicated for 20 min and filtered. Suitable volume was applied to get the concentration of 325 ng $band^{-1}$, 100 ng $band^{-1}$, 50 ng $band^{-1}$ 250 ng $band^{-1}$ and 50 ng $band^{-1}$ for PARA, NIM, PHE, CET and CAF, respectively. The analysis was performed in triplicate.

Precision

Repeatability studies and intermediate precision were performed at concentrations 600 ng $band^{-1}$, 1000 ng $band^{-1}$, 800 ng $band^{-1}$, 1000 ng $band^{-1}$ and 800 ng $band^{-1}$ for PARA, PHE, NIM, CET and CAF, respectively in single concentration with six replicates.

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for PARA, PHE, NIM, CET and CAF in sample was confirmed by comparing the R_f and spectra of the spots with that of standards.

Accuracy

The accuracy of the method was evaluated by standard addition method. Samples of PARA, PHE, NIM, CET and CAF were spiked with 80, 100 and 120 % of standard PARA, PHE, NIM, CET and CAF.

Robustness

For Mobile phase composition (± 0.1 mL), amount of mobile phase (± 0.5 %), time of activation 5 min (± 2 min), time from application to development (+ 20 min), time from development to scanning (+ 20 min) concentration of PARA, PHE, NIM, CET and CAF were 1000 ng $band^{-1}$, 800 ng $band^{-1}$, 600 ng $band^{-1}$, 1200 ng $band^{-1}$ and 400 ng $band^{-1}$, respectively.

RESULTS AND DISCUSSION

3.1 Optimization of chromatographic conditions

Different mobile phases containing solvents of different polarities in different ratios like toluene, hexane, ethanol, methanol, ammonia, dichloromethane and ethyl acetate has been tried. The solvent system containing toluene: ethyl acetate: methanol: formic acid (16:2:4:0.8, v/v/v/v) was selected as it gave the desired R_f values and resolution. The developing chamber was saturated with mobile phase for 20 min and development distance was 80 mm. The retardation factor for PARA, PHE, NIM, CET and CAF was found 0.34±0.02, 0.09±0.02, 0.77±0.02, 0.27±0.02 and 0.45±0.02, respectively (Figure3.1.2). Wavelength selected for the analysis was 212 nm which has given maximum absorbance for the PARA, PHE, NIM, CET and CAF (Figure 3.1.1).

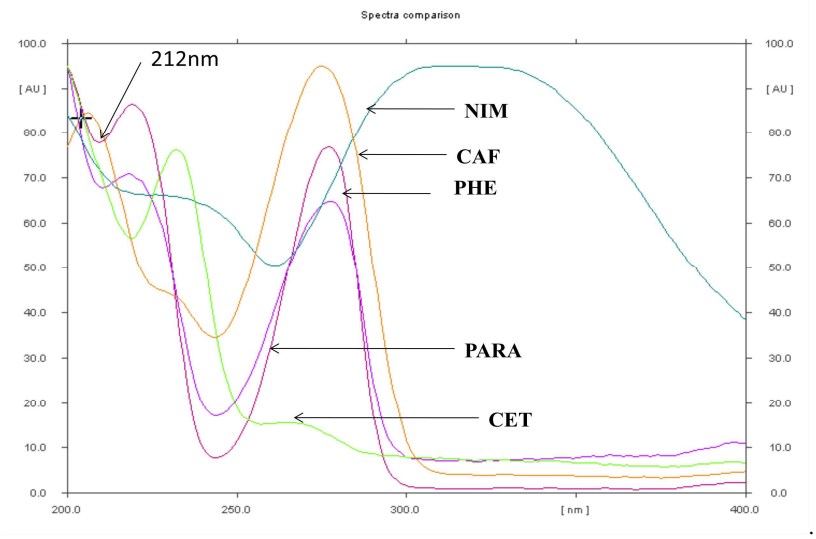

Figure 3.1.1: Overlay UV spectrum of selected drugs

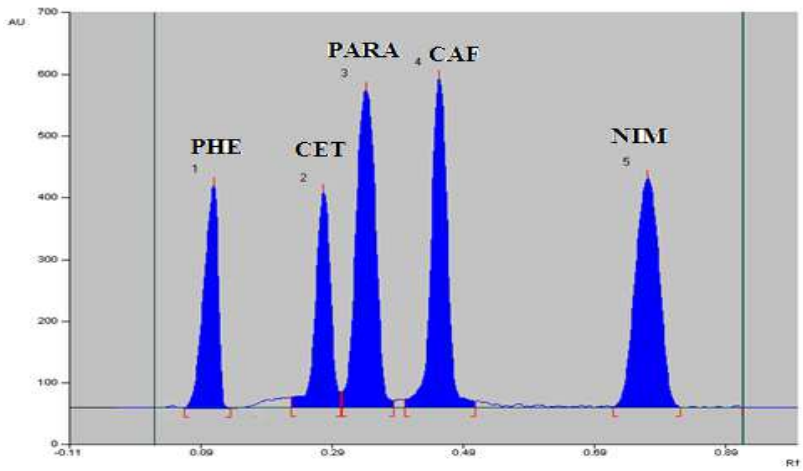

Figure 3.1.2: Densitogram of marketed formulation scanned at 212 nm

3.2 Method Validation

Linearity

A good linear relationship was observed for all five drugs (Figure 3.2.1, Figure 3.2.2, Figure 3.2.3, Figure 3.2.4 and Figure 3.2.5) Linearity range for PARA, NIM, CET and CAF was found in the range to be 200-1400 ng band^{-1} and that of PHE was found to be 100-1400 ng band^{-1} (Table 3.1).

Table 3.1: Linear regression data for calibration curves of PARA, PHE, NIM, CET and CAF. (n=6)

Parameters	PARA	PHE	NIM	CET	CAF
Linearity range (ng band^{-1})	200-1400	100-1400	200-1400	200-1400	200-1400
r^2	0.999	0.999	0.999	0.999	0.999
Slope	2.952	4.341	4.350	4.512	8.268
Intercept	8.933	87.59	37.45	1266	1293
LOD (ng band^{-1})	39.04	27.75	35.76	45.03	41.07
LOQ (ng band^{-1})	118.32	84.09	108.39	136.48	124.45
Sy.x^b	34.59	42.78	45.61	79.96	81.94

n- No of Replicates, LOD- Limit of Detection, LOQ- Limit of Quantification, b- Standard Deviation of Residuals from Line, r^2-Square of Correlation Coefficient.

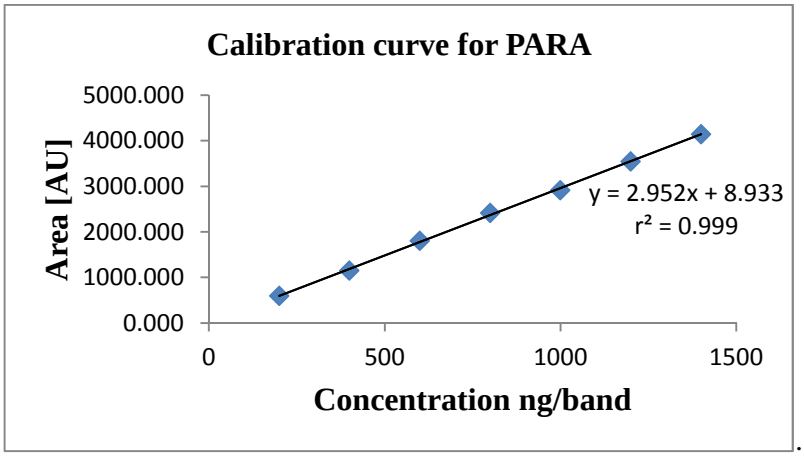

Figure 3.2.1: Calibration curve for PARA

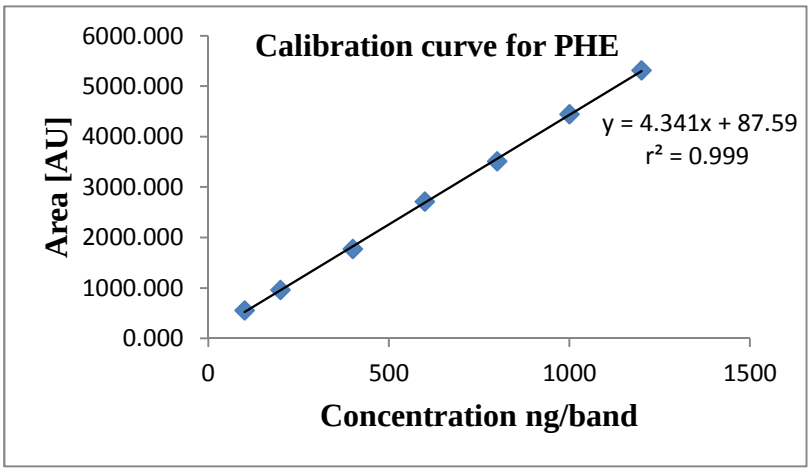

Figure 3.2.2: Calibration curve for PHE

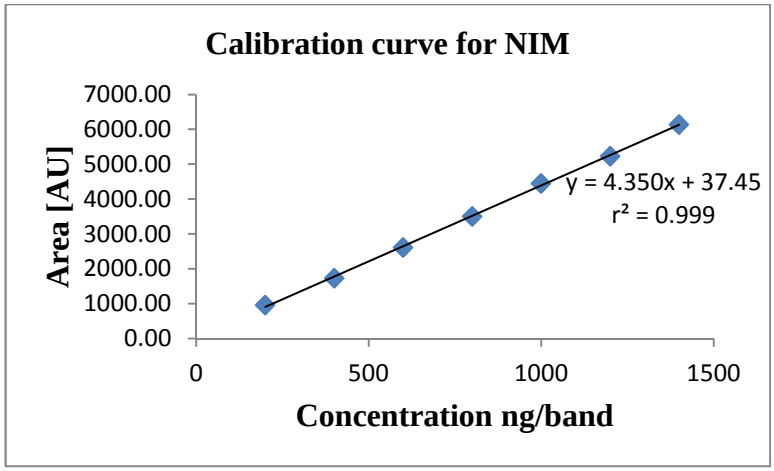

Figure 3.2.3: Calibration curve for NIM

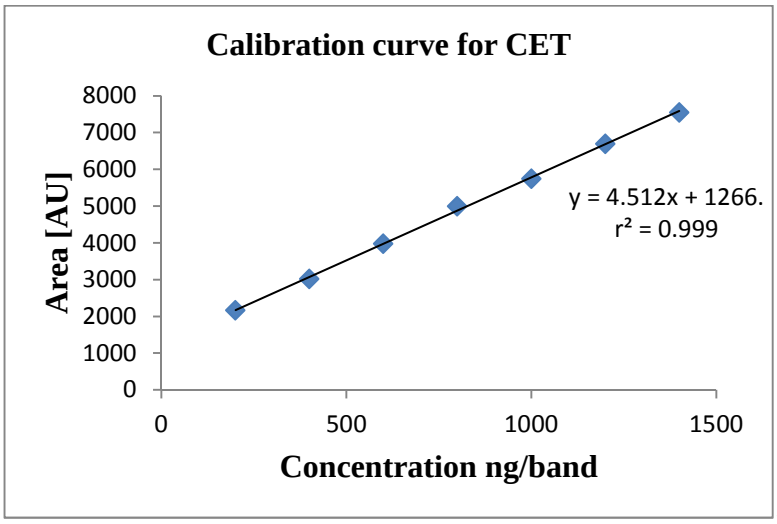

Figure 3.2.4: Calibration curve for CET

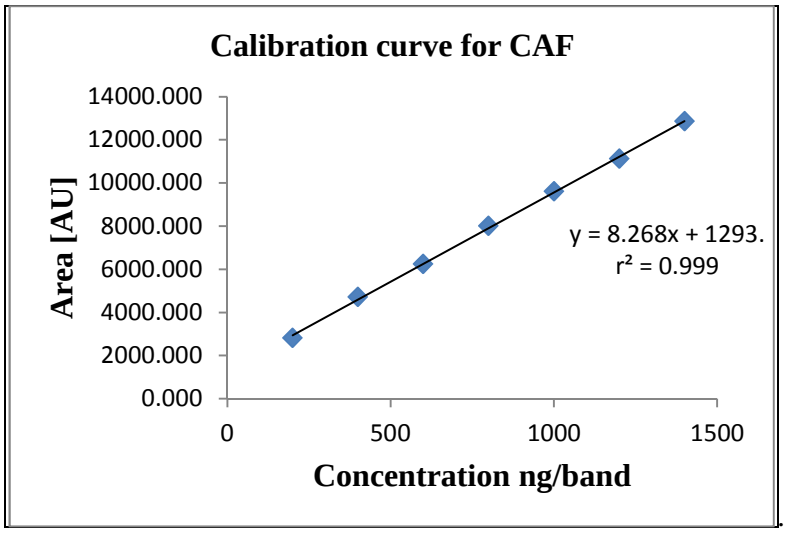

Figure 3.2.5: Calibration curve for CAF

Precision:

The developed method was found to be precise, with % RSD values for repeatability and intermediate precision studies < 2 as recommended by ICH Q2 (R1) guideline (Table 3.2).

Table 3.2: Results of Intra and inter day precision study (n=6)

Drug	Repeatability % RSD	Intermediate precision % RSD
PARA (600ng)	1.37	1.58
PHE (1000ng)	1.22	1.57
NIM (800ng)	1.37	1.23
CET (1000ng)	1.48	1.02
CAF (800ng)	1.56	1.43

n- Number of Replicates, RSD- Relative Standard Deviation

Robustness:
The % RSD of peak areas was calculated for each parameter and was found to be less than 2. % RSD of the study < 2 indicated that the method is robust (Table 3.3).

Table 3.3: Results of robustness (n=6)

Drug	Mobile phase composition (± 0.1 mL) (1000ng band^{-1})	Amount of mobile phase (± 5 %) (800ng band^{-1})	Time of activation 5 minute (± 2 min) (600ng band^{-1})
PARA	1.45	1.41	1.49
PHE	1.18	1.08	1.07
NIM	0.97	0.90	0.96
CET	0.88	0.87	0.86
CAF	0.81	1.09	0.95

n- Number of Replicates, RSD- Relative Standard Deviation

Specificity:
The peak purity of PARA, PHE, NIM, CET and CAF were assessed by comparing their respective spectra at the peak start (S), peak apex (M), and peak end (E) positions of the spot. A good correlation ($r \leq 0.998$) was obtained between the standard and sample spectra of PARA, PHE, NIM, CET and CAF indicating that peaks are pure and method is specific.

Recovery studies
Recovery was carried out at three levels i.e. multiple level recovery studies. The recovery of the method was evaluated by standard addition method. Sample concentration 200 ng band^{-1} of PARA, PHE, NIM, CET and CAF were spiked with 80, 100 and 120 % of standard PARA, PHE, NIM, CET and CAF (Table 3.4).

Table 3.4: Results of recovery studies (n=3)

Drug (Original concentration)	Amount taken (ng band^{-1})	Amount added (ng band^{-1})	Total amount present (ng band^{-1})	Amount Recovered (ng band^{-1})	% Recovery	% RSD
PARA 200 ng	200	160	360	1052.61	99.71	1.19
	200	200	400	1180.59	99.65	1.79
	200	240	440	1272.65	100.09	1.89
PHE 200 ng	200	160	360	1730.67	100.14	1.33
	200	200	400	1935.84	100.18	1.61
	200	240	440	2116.20	100.05	0.89
NIM 200 ng	200	160	360	1736.36	100.09	1.90
	200	200	400	1934.30	99.86	0.89
	200	240	440	2119.37	99.91	1.24
CET 200 ng	200	160	360	3896.14	99.95	1.20
	200	200	400	4279.16	100.09	1.46
	200	240	440	4705.09	99.99	1.56
CAF 200 ng	200	160	360	5411.87	100	1.27
	200	200	400	5851.88	100	1.79
	200	240	440	6435.60	100.02	1.10

n- Number of replicates, RSD- Relative Standard Deviation

Assay
The developed and validated method was used to estimate the content of all five drugs in the marketed formulation. It was found to contain 100.49 % (w/w) of PARA, 101 % (w/w) of PHE, 100.54 % (w/w) of NIM, 99 % (w/w) of CET and 99 % (w/w) of CAF.

CONCLUSION

Results obtained were statistically validated and were found to be reproducible. These methods can be applied for routine analysis of formulation used in the study.

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