



Research Article

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Development and validation of HPTLC method for simultaneous estimation of Ambroxol hydrochloride, Phenylephrine hydrochloride, Chlorpheniramine maleate, Paracetamol and Guaiphenesin in pharmaceutical formulation

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ABSTRACT

A simple, sensitive, accurate, precise and robust high performance thin layer chromatographic method for simultaneous quantification of Ambroxol hydrochloride, Phenylephrine hydrochloride, Chlorpheniramine maleate, Paracetamol and Guaiphenesin in tablet dosage form has been developed and validated. Chromatographic separation was carried out on Merck precoated aluminium plates with silica gel 60 F₂₅₄ as the stationary phase. It is a double development method in which first optimized mobile phase used was ethyl acetate for Paracetamol and Guaiphenesin. Second mobile phase used was toluene: methanol: glacial acetic acid (1.4:8.3:0.3, v/v/v) for Ambroxol hydrochloride, Phenylephrine hydrochloride, Chlorpheniramine maleate. Detection wavelength was carried out at 277nm in reflectance/absorbance mode. The retardation factors were found to be 0.82±0.02 for Ambroxol hydrochloride, 0.67±0.02 for Phenylephrine hydrochloride, 0.12±0.02 for Chlorpheniramine maleate, 0.63±0.02 for Paracetamol and 0.47±0.02 for Guaiphenesin. Developed method was validated as per ICH Q2 (R1) guideline. Linearity range for AMB was found to be 1000-10000 ng band⁻¹, for PHE 200-2000 ng band⁻¹, for CPM 100-1000 ng band⁻¹, for PARA 100-1000 ng band⁻¹ and that of GUA 500-3000 ng band⁻¹. The tablet dosage form was found to contain 99.41% (w/w) AMB, 99.77% (w/w) PHE, 100.37% (w/w) CPM, 100.98% (w/w) PARA and 100.44% (w/w) GUA.

Key words: Ambroxol hydrochloride, Phenylephrine hydrochloride, Chlorpheniramine maleate, Paracetamol, Guaiphenesin, HPTLC, Validation.

INTRODUCTION

Ambroxol hydrochloride (AMB)^[1], a mucolytic agent is trans-4-[[[(2-Amino-3,5-dibromophenyl)-methyl] - amino]] - cyclohexanol hydrochloride (Figure 1). Literature survey revealed that some chromatographic methods has been reported for AMB either individually or in combination with other drugs^[2,3,4].

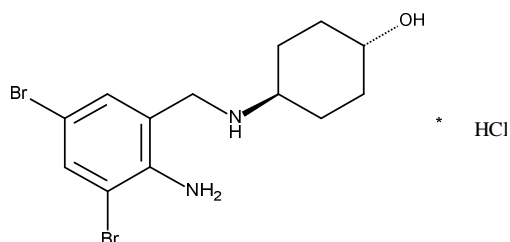


Figure 1: Chemical structure of Ambroxol hydrochloride

Phenylephrine hydrochloride (PHE)^[5] is sympathomimetic drug used to treat nasal decongestion. Chemically, it is (R)-1-(3-hydroxyphenyl)-2-methylaminoethanol hydrochloride (Figure 2). Literature survey revealed that spectrophotometric^[6], chromatographic^[4] methods has been reported for PHE individually or in combination with other drugs.

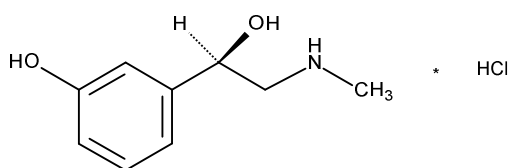


Figure 2: Chemical structure of Phenylephrine hydrochloride

Chlorpheniramine maleate (CPM)^[7] is used to relieve symptoms of allergy, fever and common cold. CPM is (S)-3-(4-chlorophenyl)-N,N-dimethyl-3-(pyridin-2-yl)propan-1-amine maleate (Figure 3). Literature survey reveals that UV^[8], HPLC^[9], HPTLC^[10] methods has been reported for CPM.

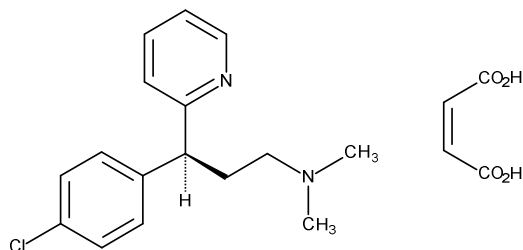


Figure 3: Chemical structure of Chlorpheniramine maleate

Paracetamol (PARA) is analgesic and antipyretic drug used for the relief of fever, headaches and other minor aches and pains.^[11] Paracetamol is N-(4-hydroxyphenyl) acetamide (Figure 4). Literature survey reveals that UV^[12], HPLC^[13], HPTLC^[14] methods have been reported for paracetamol.

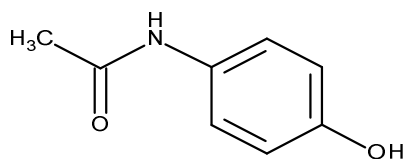


Figure 4: Chemical structure of Paracetamol

Antiallergic agent Guaiphenesin (GUA)^[4] is (+)-3-(2-methoxyphenoxy)-propane-1,2-diol (Figure 5). Literature survey revealed that UV^[15], HPLC^[16], HPTLC^[17] methods has been reported for Guaiphenesin.

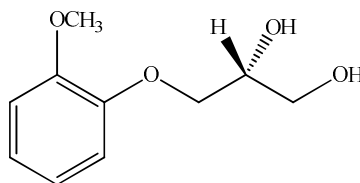


Figure 5: Chemical structure of Guaiphenesin

Literature survey revealed that individual analytical methods are available for selected drugs but densitometric method for simultaneous quantification is not available and hence study was undertaken.

EXPERIMENTAL SECTION

Chemical and reagents

Ambroxol hydrochloride was obtained as a gift sample from Emcure Pharmaceuticals Limited, Pune. Phenylephrine hydrochloride and chlorpheniramine maleate was supplied as a gift sample by Centaur Pharmaceutical Private Limited, Pune. Guaiphenesin was kindly supplied as a gift sample by Okasa Pharma Private Limited, Satara. Pharmaceutical grade of paracetamol was supplied as a gift samples of by Jain Pharmaceutical Limited, Pune. The formulation used for the analysis was Febrex CCF, Indoco Remedies, Aurangabad. Procured from local market and labeled to contain 325 mg PARA, 100 mg GUA, 30mg AMB, 10 mg PHE, 2 mg CPM. All chemicals and reagents used for the study were of analytical grade and procured from Merck Specialties Private Limited, Mumbai. Aluminum plates precoated with silica gel 60 F₂₅₄ were procured from E. Merck Darmstadt, Germany.

2.2 Instrumentation

The CAMAG HPTLC system consist of Camag 100 μ L syringe, Camag twin trough glass chamber, Camag TLC scanner III, Camag Linomat V sample applicator. The plates were washed with methanol and activated at 110 °C for 5 min before use. The bands applied were 6 mm wide and 5 mm apart and application rate was 0.1 μ Ls⁻¹

HPTLC conditions:

Chromatographic separation was carried out by double development linear ascending method in 20 × 10 cm twin trough glass chamber (CAMAG) previously saturated with the first mobile phase ethyl acetate for 20 min for PARA and GUA. Mobile phase used for each development was 20 mL with 80 mm solvent front. After chromatographic development plates were dried with the current of air and densitometric scanning was performed in reflectance/absorbance mode at 277 nm 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. For AMB, PHE and CPM the mobile phase used was toluene: methanol: glacial acetic acid (1.4: 8.3: 0.3, v/v/v) for 20 min. Mobile phase used for each development was 20 mL with 80 mm solvent front.

Preparation of standard solution

The standard stock solution was prepared 1000 μ g mL⁻¹ for AMB, PHE, CPM, PARA and GUA by dissolving 10 mg of standard drug in 10 mL methanol separately in volumetric flask to get 1000 μ g mL⁻¹.

2.3 Validation

The developed method was validated as per ICH Q2 (R1) guideline^[18] for linearity, range, precision, accuracy, specificity, LOD and LOQ.

Linearity and range

Linearity was evaluated by applying minimum five concentration six replicates to HPTLC plate for AMB in the range of 1000-10000 ng band⁻¹, PHE 200-2000 ng band⁻¹, CPM 100-1000 ng band⁻¹, PARA 100-1000 ng band⁻¹ and GUA 500-3000 ng band⁻¹. Calibration curve of peak area versus concentration was plotted.

Precision

Precision of the method was analyzed by intra and inter-day variation studies. To study intra-day variation each level of precision was investigated by single concentration with six replicates of AMB, PHE, CPM, PARA and GUA. Precision of AMB was studied at 4000 ng band⁻¹, PHE at 1000 ng band⁻¹, CPM at 600 ng band⁻¹, PARA at

400 ng band⁻¹ and GUA at 1500 ng band⁻¹. To study inter-day precision study, single concentration with six replicates of above mentioned drug concentration were analyzed on three successive days.

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were calculated to determine sensitivity as $3.3\sigma/S$ and $10\sigma/S$, respectively, where σ is the residual deviation of the response (y- intercept) and S is the slope of the linearity plot.

Specificity

In specificity studies, AMB, PHE, CPM, PARA and GUA standard solutions and the marketed sample solutions were applied on a HPTLC plate. The plate was developed in the respective mobile phase and scanned as mentioned above. The peak purity of AMB, PHE, CPM, PARA and GUA was assessed by comparing the UV spectra of drugs at peak start, peak apex and peak end positions of the band i.e., r (start, middle) and r (middle, end).

Accuracy

The accuracy of the method was evaluated by standard addition method. Samples of AMB, PHE, CPM, PARA and GUA were spiked with 80, 100 and 120 % of standard AMB, PHE, CPM, PARA and GUA.

Robustness

Robustness was studied by carrying out small, deliberate changes in analytical conditions. The analytical conditions varied were mobile phase combination (± 0.1 mL), amount of mobile phase ($\pm 5\%$), time from band application to chromatographic development and time from chromatography to scanning (+ 10 min). One factor was varied at a time to study the variation. The robustness of the proposed HPTLC method was studied six times at concentration of 2000 ng band⁻¹ for AMB, 400 ng band⁻¹ for PHE, 200 ng band⁻¹ for CPM, 200 ng band⁻¹ for PARA and 1000 ng band⁻¹ for GUA as it lies within the range of linearity. The standard deviation of peak areas and % relative standard deviation (% RSD) were calculated for each variable factor.

Solution stability

Solution stability of the standard solution 2000 ng band⁻¹ for AMB, 400 ng band⁻¹ for PHE, 200 ng band⁻¹ for CPM, 200 ng band⁻¹ for PARA and 1000 ng band⁻¹ for GUA was studied at an interval of 6h up to 48h. When stored at room temperature and estimated by comparing peak areas at each time interval against freshly prepared standard solution.

2.4 Analysis of formulation

The average weight of tablet is 709.2 mg equivalent (AMB 30 mg, PHE 10 mg, CPM 2 mg, PARA 325 mg and GUA 100 mg) was transferred into a 25 mL volumetric flask containing 25 mL methanol. The solution was sonicated for 20 min. followed by filtration to get concentration of 1200 $\mu\text{g mL}^{-1}$ of AMB, 400 $\mu\text{g mL}^{-1}$ of PHE 80 $\mu\text{g mL}^{-1}$ of CPM and from the resulting solution 0.5 mL diluted in 10 mL volumetric flask with methanol. The solution was sonicated for 10 min and filtered to get concentration of 2000 $\mu\text{g mL}^{-1}$ of PARA and 650 $\mu\text{g mL}^{-1}$ of GUA. suitable volume was applied to an HPTLC plate and developed in respective optimized mobile phases. This solution was used for the recovery study and assay with suitable dilutions and volume applications.

RESULTS AND DISCUSSION

3.1 Selection of detection wavelength:

After chromatographic development bands were scanned in the range of 200 to 400 nm and spectra were overlain. AMB, PHE, CPM, PARA and GUA showed considerable absorbance at 277 nm and hence was selected for densitometric analysis. (Figure 6).

3.2 Optimization of chromatographic conditions

The mobile phase was optimized to get desired R_f value range [0.1 - 0.9] with a resolution of > 1.5 . It was achieved by trying different mobile phases containing different polarity solvents in different ratios like toluene, *n*-hexane, dichloromethane, ethanol, methanol, water and ethyl acetate. It was observed that five drugs does not move in a single mobile phase hence double development strategy was applied. In which the first mobile phase was ethyl acetate for PARA and GUA. Both drugs showed desired R_f values and resolution. Second solvent system containing toluene: methanol: glacial acetic acid (1.4: 8.3: 0.3, v/v/v) was selected as it gave reproducible results for AMB,

PHE and CPM. The development chamber was saturated with respective mobile phases for 20 min and development distance was 80 mm. The retardation factor for AMB, PHE, CPM, PARA and GUA was found to be 0.82 ± 0.02 , 0.67 ± 0.02 , 0.12 ± 0.02 , 0.63 ± 0.02 and 0.47 ± 0.02 , respectively (Figure 7).

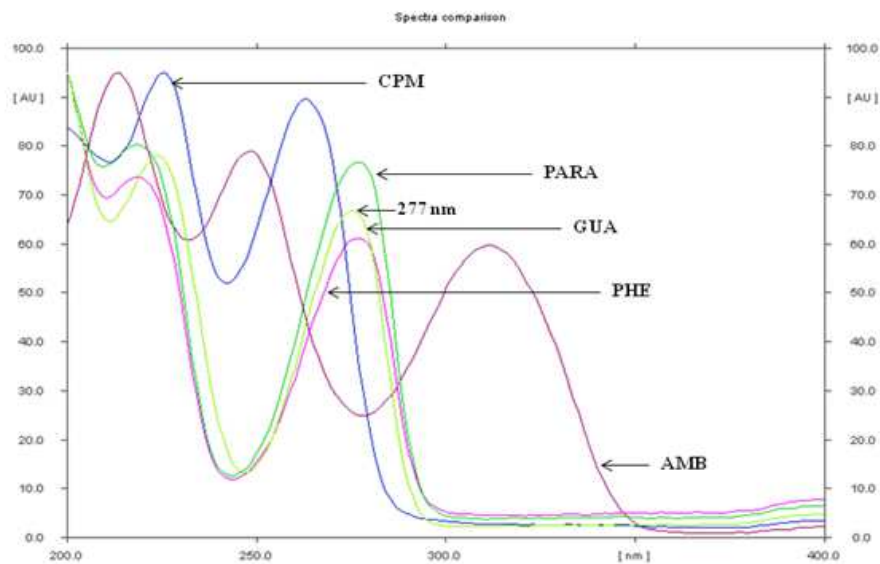


Figure 6: Overlain UV spectrum of AMB, PHE, CPM, GUA and PARA

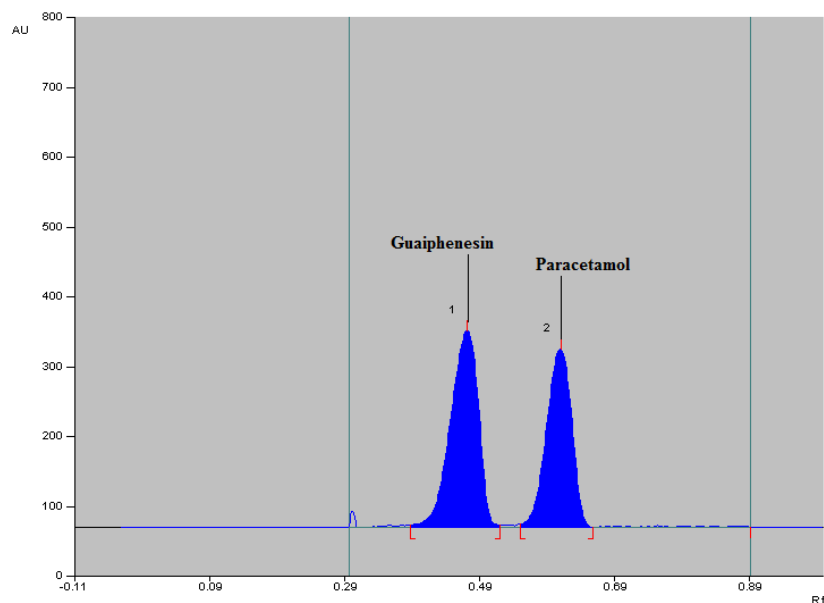


Figure 7: (A) Densitogram of Paracetamol and Guaiphenesin

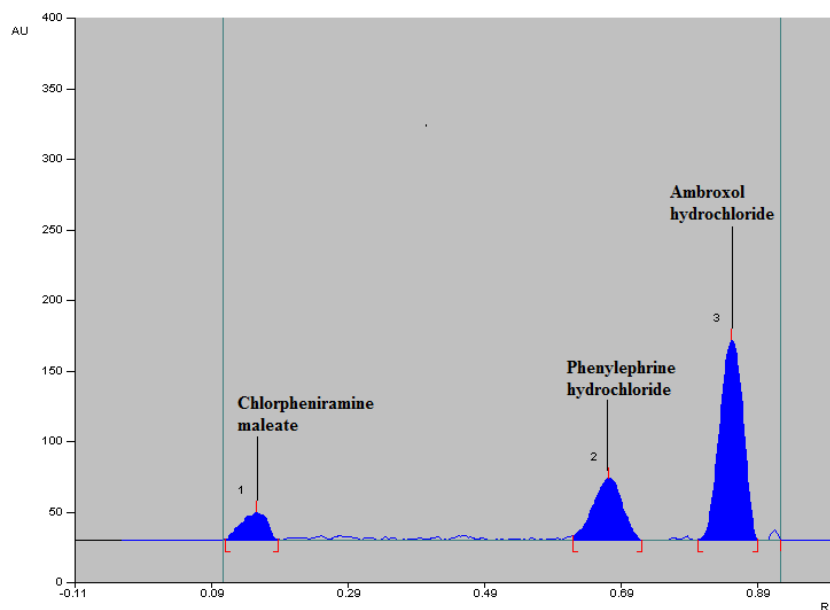


Figure 7: (B) Densitogram of Ambroxol hydrochloride, Phenylephrine hydrochloride, Chlorpheniramine maleate

3.3 Validation of the method

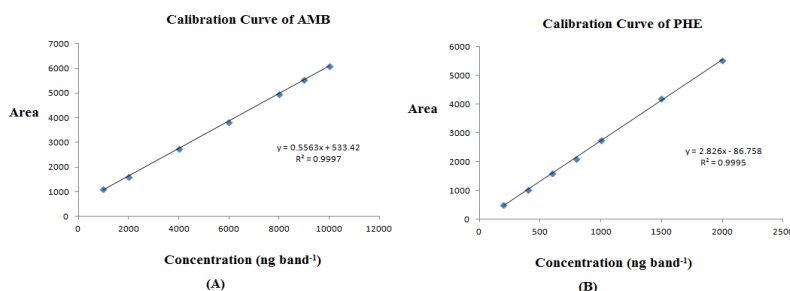
Linearity and range

Result showed that standard drug concentration and peak areas were found to be linear in the range of 1000-10000 ng band⁻¹ for AMB, 200-2000 ng band⁻¹ for PHE, 100-1000 ng band⁻¹ for CPM, 100-1000 ng band⁻¹ for PARA and 500-3000 ng band⁻¹ for GUA. A good linear relationship was observed for all five drugs (Figure 8).

Table 1 Linear regression data for calibration curves of AMB, PHE, CPM, PARA and GUA. (n=6)

Parameters	AMB	PHE	CPM	PARA	GUA
Linearity range (ng band ⁻¹)	1000-10000	200-2000	100-1000	100-1000	500-3000
r ²	0.9997	0.9995	0.9998	0.9994	0.9994
Slope	0.556	2.826	8.043	8.100	2.909
Intercept	533.42	86.75	160.47	223.13	1196.7
LOD (ng band ⁻¹)	206.04	49.95	18.71	32.57	92.95
LOQ (ng band ⁻¹)	624.36	151.38	56.70	98.71	281.67
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.



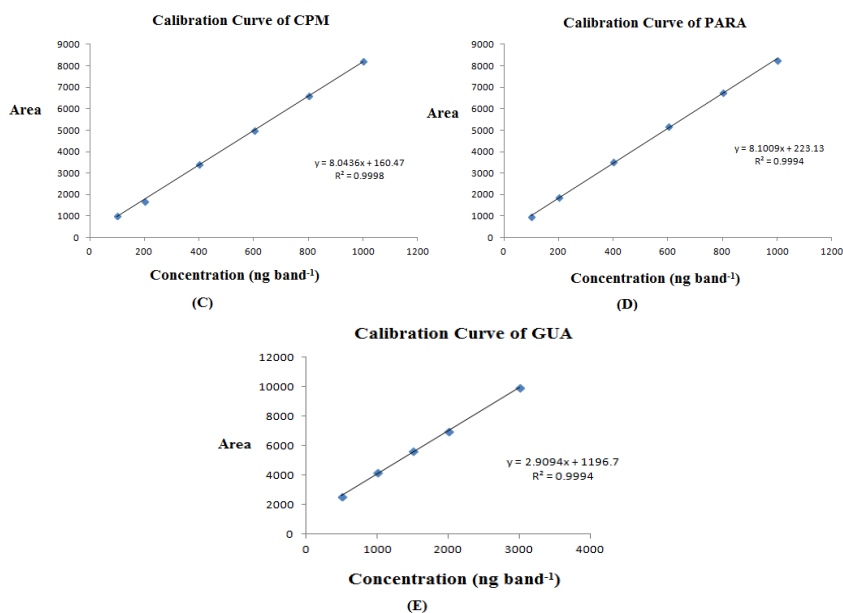


Figure 8: Calibration curve of (A) Ambroxol hydrochloride (B) Phenylephrine hydrochloride (C) Chlorpheniramine maleate (D) Paracetamol and (E) Guaiphenesin

Sensitivity

Result (Table 1) showed that the method is sensitive for the analysis of selected drugs.

Specificity

The peak purity for AMB, PHE, CPM, PARA and GUA was assessed by comparing UV spectrum acquired at the start (S), apex (M), and end (E) of the peak obtained from the scanning of band, that is, $r(S, M) = 0.998, 0.998$ and $r(M, E) = 0.998, 0.998$, respectively. Peak purity data showed that peaks obtained for AMB, PHE, CPM, PARA and GUA were pure and method is specific.

Precision

Both intra and inter-day precision studies showed % RSD < 2, as recommended by ICH guidelines, indicating good precision (Table 2).

Table 2 Results of Intra and inter day precision study (n=6)

Drug	Concentration in ng band ⁻¹	Intra day % RSD ^a	Inter day % RSD ^a
AMB	4000	1.180	1.385
PHE	1000	1.020	1.260
CPM	600	1.450	1.468
PARA	400	1.710	1.536
GUA	1500	1.255	1.127

n- no of replicates, RSD- relative standard deviation.

Accuracy

Recovery study indicating reliability of the proposed densitometric method for simultaneous estimation of AMB, PHE, CPM, PARA and GUA in the marketed formulation used in the study (Table 3).

Table 3 Results of recovery studies (n=6)

Drug	Amount taken ng band ⁻¹	Amount added ng band ⁻¹	Total amount present ng band ⁻¹	Amount Recovered ng band ⁻¹	% Recovery	% RSD
AMB	2000	1600	3600	3606.68	100.18	1.67
	2000	2000	4000	3993.21	99.83	1.32
	2000	2400	4400	4396.52	99.92	1.11
PHE	400	320	720	721.07	100.14	1.17
	400	400	800	801.75	100.21	1.29
	400	480	880	880.13	100.00	0.79
CPM	200	160	360	360.12	101.79	0.95
	200	200	400	400.15	100.38	0.97
	200	240	440	440.23	101.75	0.97
PARA	200	160	360	3321.83	100.25	1.54
	200	200	400	3712.26	100.00	1.67
	200	240	440	4088.12	100.01	1.11
GUA	1000	800	1800	1806.26	100.34	0.93
	1000	1000	2000	2000.23	100.01	1.01
	1000	1200	2200	2200.98	100.44	1.21

n- no of replicates, RSD- relative standard deviation.

Robustness studies

Robustness of the proposed densitometric method showed that peak areas of interest remained unaffected by small but deliberate changes in operational parameters (% RSD < 2) indicating robustness of the method (Table 4).

Table 4 Results of robustness studies (n=6)

Parameter	% RSD of peak area				
	AMB	PHE	CPM	PARA	GUA
Mobile phase composition ($\pm 0.1\text{mL}$)	1.163	0.829	1.614	0.815	0.581
Amount of mobile phase ($\pm 5\%$)	1.165	0.788	1.583	0.954	0.818
Time from application to development (+ 10min)	1.107	0.642	1.533	0.971	0.855
Time from development to scanning (+ 10min)	1.294	0.791	1.688	0.955	0.821

(AMB – 9000 ng band⁻¹, PHE – 1500 ng band⁻¹, CPM – 1000 ng band⁻¹, PARA – 100 ng band⁻¹, GUA – 500 ng band⁻¹). *n*- no of replicates, RSD- relative standard deviation.

Solution stability

Stability of standard solution of AMB, PHE, CPM, PARA and GUA were assessed at room temperature for 48h. The % RSD < 2 indicates that the solution were stable for 48h. at room temperature.

3.4 Analysis of marketed formulation

Developed densitometric method was applied to the selected marketed formulation. (Febrex CCF[®]) found to contain 99.41% of AMB, 99.77% of PHE, 100.37% of CPM, 100.98% of PARA and 100.44% of GUA.

CONCLUSION

The developed and validated HPTLC method is new, simple, accurate, fast, precise and robust and thus can be used for simultaneous quantification of Ambroxol hydrochloride, Phenylephrine hydrochloride, Chlorpheniramine maleate, Paracetamol and Guaiphenesin in selected pharmaceutical formulation.

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