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Research Article

LIQUID CHROMATOGRAPHY TANDEM-MASS SPECTROMETRY METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ANALYSIS OF PARACETAMOL, GUAIFENESIN, PHENYLEPHRINE HYDROCHLORIDE, CHLORPHENIRAMINE MALEATE, AND AMBROXOL HYDROCHLORIDE IN BULK AND IN TABLET DOSAGE FORM

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ABSTRACT

Objective: The objective is to study liquid chromatography tandem-mass spectrometry (LC/MS/MS) method for simultaneous quantification of paracetamol (PCM), guaifenesin (GUA), phenylephrine hydrochloride (PE), chlorpheniramine maleate (CPM), and ambroxol hydrochloride (AMB) in tablet dosage form developed and validated as per the International Conference on Harmonization Q2 (R1) guideline.

Methods: The chromatograms were developed using a gradient mobile phase of WATER:methanol. Flow rate used was to 0.3 ml/min. Quantitation was performed using multiple reaction monitoring (MRM) mode to study parent to product ion transition, for paracetamol. (m/z 152.0 ≥ 110.0), guaifenesin (m/z 199.0 ≥163.0), phenylephrine hydrochloride (m/z 168.0≥ 150.0), chlorpheniramine maleate (m/z 275.0 ≥ 230.0) and ambroxol hydrochloride (m/z 379.0 ≥ 263.8).

Results: The retention times were found to be 1.76, 1.81, 1.90, 2.10, and 2.33 min for PCM, GUA, PE, CPM, and AMB, respectively. The linearity of the method was found to be in the concentration range of 10–200 ng/ml for PCM, GUA, PE, CPM, and AMB. Percentage relative standard deviation values for repeatability and intermediate precision studies were below 2%.

Conclusion: Developed method was found to be robust, precise, accurate, rapid and can be used to analyze fixed-dose tablet formulation used in the study.

Keywords: Liquid chromatography tandem-mass spectrometry, Method development, validation, International Conference on Harmonization Q2 (R1).

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INTRODUCTION

Paracetamol (PCM) [1,2], an analgesic and antipyretic agent (Fig. 1), is 4-hydroxyacetanilide. Literature survey revealed that spectrophotometric [3], high-performance thin-layer chromatography (HPTLC) [4-6], and HPLC [7-11] methods have been reported for PCM individually or in combination with other drug/s.

Chemically, guaifenesin (GUA) [12,13] an antipruritic, anti-allergic, histamine H_1 antagonist (Fig. 2) is 3-(2-methoxyphenoxy) propan-1, 2-diol. Literature survey revealed that spectrophotometric [14-17], HPTLC [18], and HPLC [19-24] analytical methods have been reported for GUA individually or in combination with other drug/s.

Phenylephrine hydrochloride (PE) [25,26], a sympathomimetic agent (Fig. 3), is (R)-1-(3-hydroxyphenyl)-2-methylamino ethanol hydrochloride. Literature survey revealed that spectrophotometric [27], HPTLC [28], and HPLC [29-33] techniques have been reported for PE individually or in combination with other drug/s.

Chlorpheniramine maleate (CPM) [34,35], an antihistaminic and antiallergics (Fig. 4), is (S)-3-(4-chlorophenyl)-*N, N*-dimethyl-3-(-3 pyridin-2-yl) propan-1-amine maleate. Spectrophotometric [36,37], HPTLC [38], and HPLC [39-44] analytical methods have been reported for CPM individually or in combination with other drug/s.

Ambroxol hydrochloride (AMB) [45], a mucolytic agent (Fig. 5), is trans-4-[(2-amino-3, 5-dibromo benzyl) amino] cyclohexanol hydrochloride. Literature survey showed spectrophotometric [46,47], HPTLC [48,49], and HPLC [50-54] methods of analysis for AMB individually or in combination with other drug/s.

A detailed survey of analytical literature for the estimation of these drugs revealed that there was no liquid chromatography tandem-mass spectrometry (LC/MS/MS) method available for simultaneous estimation of these drugs in combined tablet form. Hence, the present research study was undertaken.

METHODS

Pharmaceutical grade PCM, CPM, AMB, GUA, and PE were received as gift sample from Centaur Pharmaceuticals Ltd. and Emcure Pharmaceuticals Ltd., Pune, India. Drug formulation used in the study was Solvin Cold (Ipca Laboratories Ltd., Mumbai, India) containing PCM 500 mg, GUA 100 mg, AMB 30 mg, PE 10 mg, and CPM 2 mg, purchased from the local market. HPLC grade chemicals and reagents were used for LC/MS/MS analyses.

Instrumentation and chromatographic conditions

The LC/MS/MS system consisted of a HPLC system-1260 infinity with auto-injector (20 µl). Agilent MS 6460 triple quadruple MS was used. The software used was Agilent mass hunter workstation data acquisition with version 1.18.03. The column used was Poroshell 120 EC – C18 (4.6 × 50 mm 2.7 µm) of Thermo Technologies Corporation, Japan.

Mass spectrometry acquisitions were as follows, Ions polarity: Positive ion model, ion source type: Atmospheric pressure electrospray

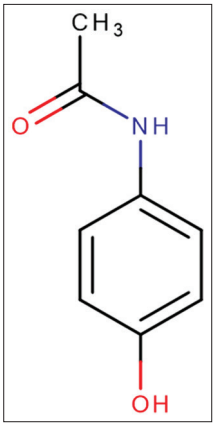

Fig. 1: Chemical structure of paracetamol

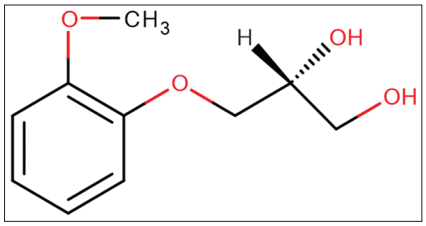

Fig. 2: Chemical structure of guaifenesin

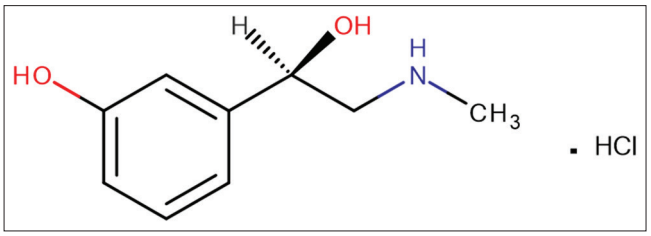

Fig. 3: Chemical structure of phenylephrine hydrochloride

ionization, capillary voltage (kv): 3.00, con voltage (v), gas temperature: 250 (°C), gas flow: 11 (l/min), nebulizer: 45 (psi), sheath gas heater: 300 (°C), sheath gas flow: 9 (l/min), and nozzle voltage: 500 (ev).

Preparation of standard solution

Standard stock solution: An accurately weighed quantity (10 mg) of PCM, GUA, PE, CPM, and AMB were transferred to 10.0 ml volumetric flask, separately. The mixture of methanol:water (80:20, v/v) was used as a diluent; all the drugs were dissolved and diluted to the mark to get final concentration 1000 mg/ml. These stock solutions were suitably diluted and used for further study.

Preparation of sample solutions

Twenty tablets were weighed accurately; average weight was calculated followed by fine powdering. Tablet powder equivalent to 1 mg of CPM was accurately weighed and transferred to a 100 ml volumetric flask. Diluent (70 ml) was added, sonicated for 30 min. Volume was made up to the mark with the diluent. The solution was filtered through Whatman no.1 filter paper, suitably diluted, and used in the study.

Method development

All drugs were prepared in methanol:water (80:20 v/v) as it was found to be a common solvent. A concentration of 10 ng/ml of all standards was used and injected onto the column for the selection of mobile phase. Water and methanol in different ratios were used in isocratic mode, but the results were found to be non-reproducible. The gradient system was also tried with the same mobile phase. Other chromatographic conditions, especially the composition of the mobile phase, were optimized through several trials

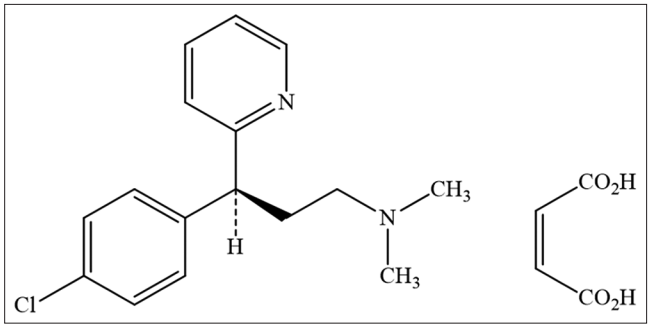

Fig. 4: Chemical structure of chlorpheniramine maleate

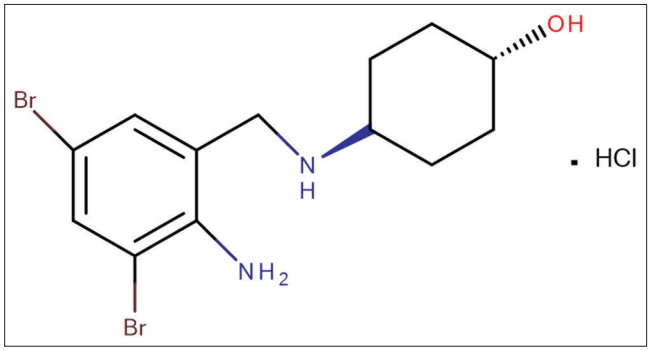

Fig. 5: Chemical structure of ambroxol hydrochloride

to achieve symmetric peak shape, linear response for concentration, and selective multiple reaction monitoring (MRM) transition.

Validation

Validation of the optimized LC/MS/MS method was carried out as per the International Conference on Harmonization Q2 (R1) guideline [55].

Study of linearity range

The standard stock solution (1000 mg/ml) containing PCM, GUA, PE, CPM, and AMB was used to prepare serial dilutions in a range of 10–200 ng/ml and injected onto the column and chromatographed through optimized chromatographic conditions. This study was repeated 6 times.

Limit of detection (LOD) and limit of quantification (LOQ)

To estimate the LOD and LOQ, standard deviation (SD) of y-intercept and slopes of calibration curves were used.

Precision

The precision of the method was confirmed by intra- and inter-day precision. Intraday studies were performed 6 times on the same day at a concentration of 100 ng/ml for all drugs. The interday precision of the method was checked by repeating analysis at a concentration of 100 ng/ml for 3 successive days. The percent relative SD (% RSD) was taken as a measure of precision.

Robustness

Robustness of the method was checked by making small but deliberates changes in the optimized chromatographic conditions, and the results were examined. The effect of change in flow rate and mobile phase ratio on peak areas was observed. The solution containing 100 ng/ml of all drug was injected (in triplicate) into sample injector of LC-MS/MS. The retention time and % RSD of peak areas were calculated for each parameter.

Specificity

To check the specificity of the LC/MS/MS method, the drugs were studied in MRM. The blank, standard, and sample solutions of PCM,

GUA, PE, CPM, and AMB were injected in the system, and the RT values for the respective drugs were observed.

Recovery study

Accuracy was evaluated through percentage recoveries of known amount of mixture of PCM, GUA, PE, CPM, and AMB, added to the solution of formulation. For recovery study, sample stock solution from tablet formulation was prepared. To the above prepared solution, 80, 100, and 120% of the standard drug solutions were spiked. Dilutions were prepared, and recovery studies were performed. The percentage ratios between the recovered and expected concentrations were estimated in triplicate.

Analysis of marketed formulation

The drug content of marketed tablet dosage form was determined 6 times using proposed method. Tablet powder equivalent to 1 mg of CPM was accurately weighed and transferred to a 100 ml volumetric flask. Around 70 ml of methanol was added, and the solution was sonicated for 30 min. Volume was made up to the mark with the methanol. The solution was filtered through Whatman no.1 filter paper and further diluted to get a concentration of 10, 50, and 150 ng/ml for CPM, PE, and AMB and 25 and 125 ng/ml for GUA and PCM used in the study. The percentage content of each drug was determined.

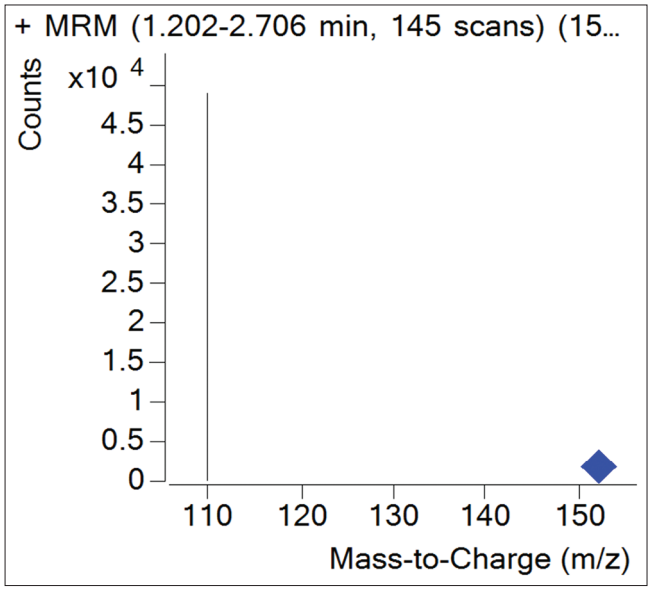

Fig. 6: Parent ion for paracetamol

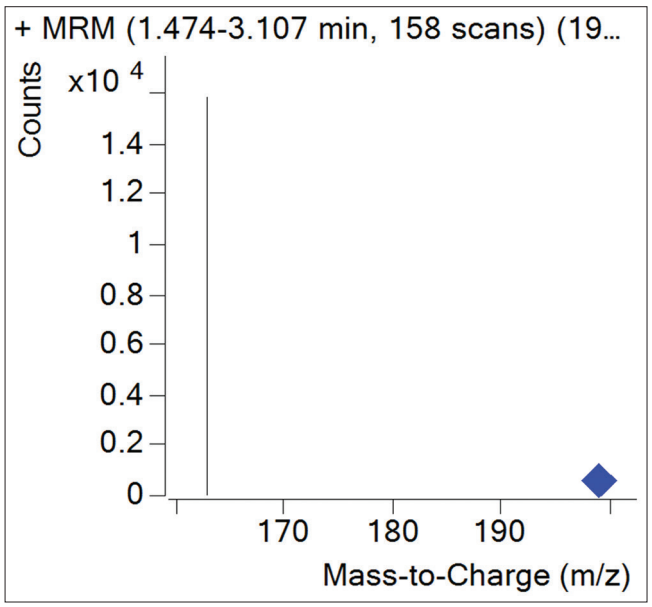

Fig. 7: Parent ion for guaifenesin

RESULTS AND DISCUSSION

LC/MS/MS detection

The parent and product ion were optimized by injecting a 10 ng/ml standard solution of five drugs in positive polarity mode. The intensity was much higher in the positive mode due to protonation at m/z 152.0 (PCM), 199.0 (GUA), 168.0 (PE), 275.0 (CPM), and 379.0 (AMB) in Q1MS. Full spectra for drugs are given in Figs. 6-10.

The most abundant product ion at m/z 110.0 (PCM), 163.0 (GUA), 150.0 (PE), 230.0 (CPM), and 263.8 (AMB) was observed by applying collision energy of 10, 5, 7, 10, and 15 eV, respectively. The MRM parameters were suitably optimized to obtained consistent and adequate response of analytes. Quantitation was performed using MRM mode to study parent to product ion transition for PCM (m/z 152.0≥110.0), GUA (m/z 199.0≥163.0), PE (m/z 168.0≥150.0), CPM (m/z 275.0≥230.0), and AMB (m/z 379.0≥263.8). Quantitation was done on the basis of major product ions. The product ion spectrum of PCM was due to the fragmentation by loss of neutral ketene (CH2=C=O) molecule. This results in the formation of major product ion at m/z 110.0. In GUA, loss of two water molecules results in the formation of major product ion at m/z163.0, and in PE, loss of one water molecule results in the formation of major product ion at m/z150.0. The product ion spectrum of CPM was due to loss of dimethyl amine (CH3-NH-CH3) to give product ion at m/z 230.0. For ambroxol, loss of 4 amino cyclohexanol (C6H13NO) results in the formation of major product ion at m/z 263.8.

Table 1: Gradient mobile phase

Time (min.)	Water (%)	Methanol (%)
2.00	60	40
4.00	90	10
5.00	0	100
5.01	60	40
9.00	60	40

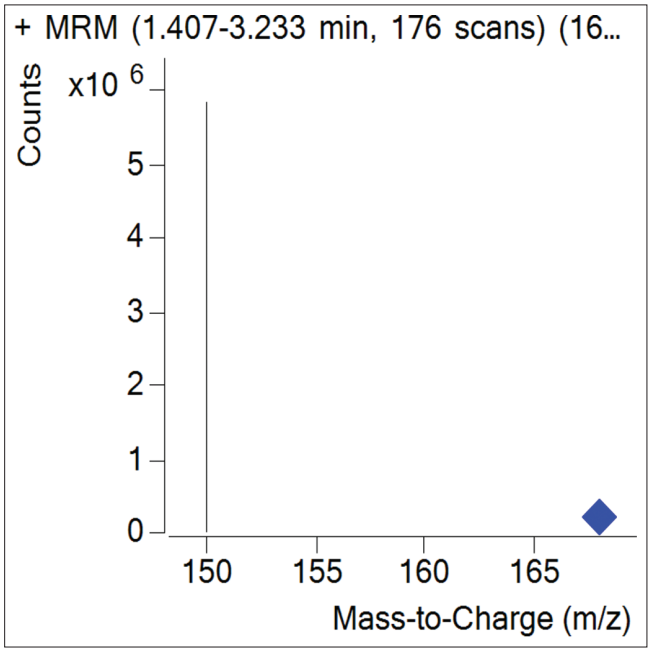

Fig. 8: Parent ion for phenylephrine

Table 2: Linear regression data for the calibration curves (n=6)

Parameters	PCM	GUA	PE	CPM	AMB
Linearity range (ng/ml)	10–200	10–200	10–200	10–200	10–200
r^2	0.999	0.999	0.999	0.999	0.999
Slope	12196.1	44.30	110586	1305282	188087
Intercept	–16493	76.82	804963.2	10520503	836191.9
Confidence limit of slope[a]	11916.9–12475.3	43.3–45.2	108865.3–112307.4	8223662–12817344	184822.9–191352.4
Confidence limit of intercept[a]	–45297.9–12311.6	–19.1–172.7	627398–982528.4	1283021–1327544	499355.6–1173028
$S_{y.x}$[b]	11666.3	38.85	71916	930249.8	136422.9

[a]95% confidence limit, [b]$S_{y.x}$ - Standard deviation of residuals from line. PCM: Paracetamol, GUA: Guaifenesin, CPM: Chlorpheniramine maleate, AMB: Ambroxol hydrochloride, PE: Phenylephrine hydrochloride

Table 3: LOD and LOQ of PCM, GUA, PE, CPM, and AMB (n=6)

Drug	LOD (ng/ml)	LOQ (ng/ml)
PCM	3.15	9.56
GUA	2.89	8.76
PE	2.14	6.50
CPM	2.35	7.12
AMB	2.39	7.25

PCM: Paracetamol, GUA: Guaifenesin, CPM: Chlorpheniramine maleate, AMB: Ambroxol hydrochloride, LOD: Limit of detection, LOQ: Limit of quantification, PE: Phenylephrine hydrochloride

Table 4: Intra- and inter-day precision of the LC/MS/MS method for PCM, GUA, PE, CPM, and AMB (n=6)

Drug	Concentration ng/ml	Intraday precision % RSD	Interday precision % RSD
PCM	100	0.487	0.561
GUA	100	0.506	0.618
PE	100	0.806	0.854
CPM	100	0.337	0.434
AMB	100	0.540	0.723

PCM: Paracetamol, GUA: Guaifenesin, CPM: Chlorpheniramine maleate, AMB: Ambroxol hydrochloride, % RSD: Percentage relative Standard deviation, LC/MS/MS: Liquid chromatography tandem-mass spectrometry, PE: Phenylephrine hydrochloride

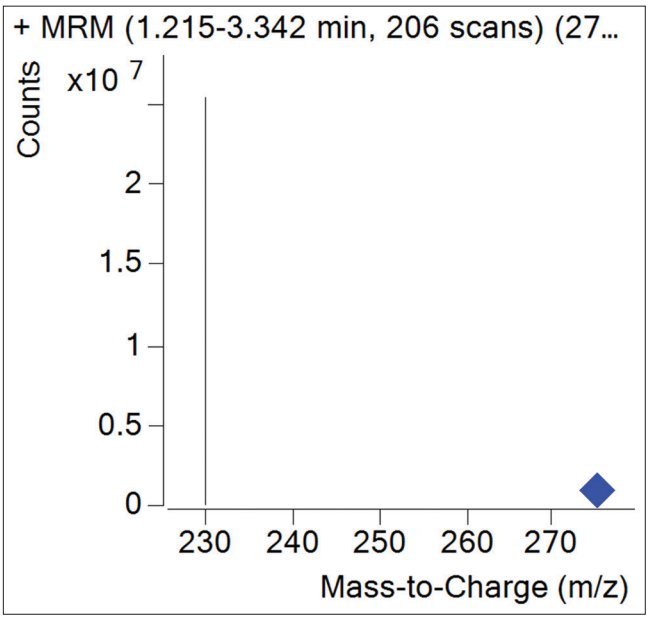

Fig. 9: Parent ion for chlorpheniramine

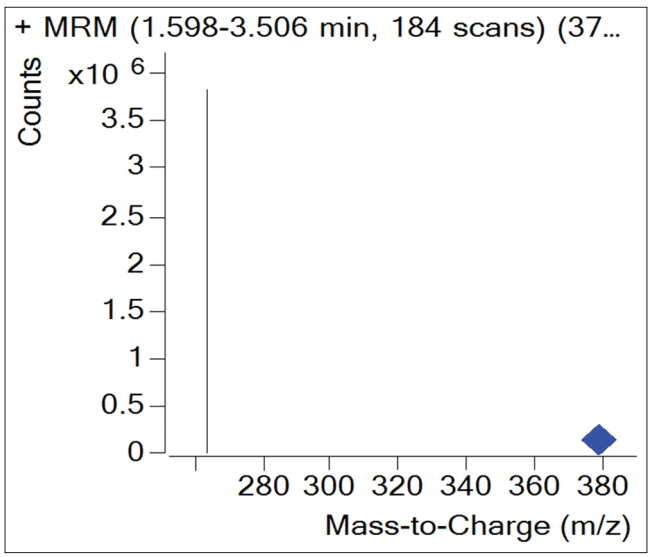

Fig. 10: Parent ion for ambroxol

Method development

The following gradient mobile phase of water:methanol was used for analysis (Table 1).

Flow rate was adjusted to 0.3 ml/min. The retention times were found to be 1.76, 1.81, 1.90, 2.10, and 2.33 min for PCM, GUA, PE, CPM, and AMB, respectively.

Preparation of standard solution

An accurately weighed quantity (10 mg) of PCM, GUA, PE, CPM, and AMB was transferred separately to 10.0 ml volumetric flasks and dissolved in methanol:water (80:20, v/v). Drugs were diluted to the mark to get 1000 mg/ml concentration for each standard. These stock solutions were further diluted suitably and used in further study. Following are the representative chromatograms of total ion (Fig. 11) and standard drugs (Figs. 12-16) in MRM mode.

Method validation

Validation of the optimized LC/MS/MS method was carried out with respect to the following parameters:

Linearity range

To construct the calibration curves by plotting the peak areas versus their corresponding concentrations, the mixed standard drug solutions were injected onto the column in the range of 10–200 ng/ml. The study was repeated 6 times and the mean peak area was considered for the construction of calibration curve. The results were linear over the concentration range of 10–200 ng/ml for PCM, GUA, PE, CPM, and AMB

Table 5: Robustness study of PCM, GUA, PE, CPM, and AMB (n=3, 100 ng/ml)

Parameter varied	Mobile phase composition (±0.1 ml)		Flow rate (0.3±0.01 ml/min)	
Drugs	**SD of peak area**	**% RSD**	**SD of peak area**	**% RSD**
PCM	4550.2	0.37	3952.8	0.32
GUA	32.44	0.72	23.51	0.52
PE	91521.8	0.78	52535.3	0.44
CPM	211848	0.15	194300.1	0.13
AMB	69433.0	0.35	46409.8	0.23

PCM: Paracetamol, GUA: Guaifenesin, CPM: Chlorpheniramine maleate, AMB: Ambroxol hydrochloride, % RSD: Percentage relative standard deviation, SD: Standard deviation, PE: Phenylephrine hydrochloride

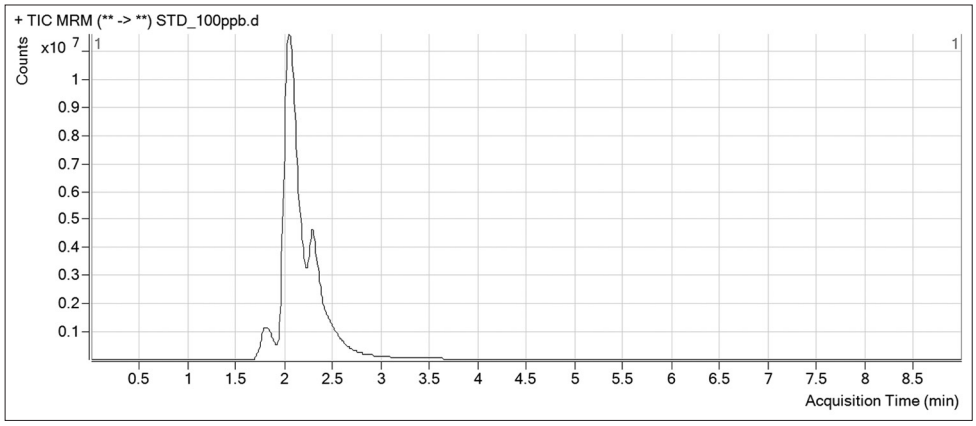

Fig. 11: Representative chromatogram of a mixture of standard drugs

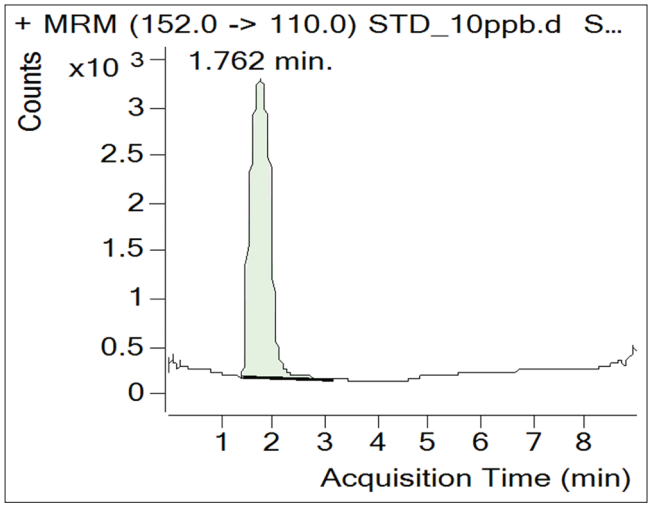

Fig. 12: Representative chromatogram of standard drug paracetamol

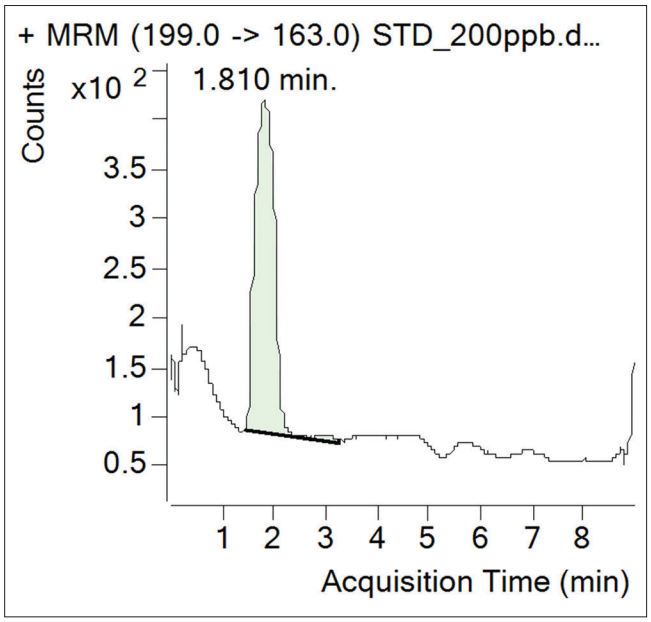

Fig. 13: Representative chromatogram of standard drug guaifenesin

(Table 2).

LOD and LOQ

Based on the SD of response of the calibration curve, LOD and LOQ were determined using formula 3.3 σ/S and 10 σ/S, respectively, where σ is the SD of the response (y-intercept) and S is the slope of the linearity plot (Table 3).

Precision

The developed method was found to be precise, with % RSD values for repeatability and intermediate precision studies (Table 4) below 2%.

Robustness

LC/MS/MS method was found to be robust as % RSD values found to be lower than 2% (Table 5).

Specificity

To check the specificity of the LC/MS/MS method, the blank, standard, and sample solutions were injected into the system and the respective retention times were observed. The study showed that there is no interference observed.

Table 6: Recovery study of PCM, PHE, CPM, GUA, and AMB (n=6)

Original concentration (mg)	Amount taken (ng)	Amount added (ng)	Total amount present (ng)	Amount Recovered (ng)	% Recovery	% RSD
PCM 500	25	20	45	44.82	99.60	0.597
	25	25	50	50.13	100.27	0.360
	25	30	55	55.16	100.29	0.555
GUA 100	25	20	45	44.89	99.75	0.897
	25	25	50	50.40	100.80	0.925
	25	30	55	55.19	100.34	0.709
AMB 30	25	20	45	44.93	99.84	0.988
	25	25	50	50.35	100.7	0.623
	25	30	55	54.68	99.43	0.963
PE 10	25	20	45	44.98	99.96	0.966
	25	25	50	50.25	100.50	0.934
	25	30	55	55.06	100.12	0.950
CPM 2	25	20	45	45.30	100.67	0.980
	25	25	50	50.01	100.02	0.705
	25	30	55	54.75	99.55	0.722

PCM: Paracetamol, GUA: Guaifenesin, CPM: Chlorpheniramine maleate, AMB: Ambroxol hydrochloride, % RSD: Percentage relative standard deviation, PE: Phenylephrine hydrochloride

Table 7: Analysis of marketed formulations (n=6)

Drug	Label claim (mg)	Amount found (mg)	Drug content (%)	% RSD
PCM	500	500.2	100.04	0.68
GUA	100	100.2	100.20	0.44
PE	10	10.009	100.09	0.56
CPM	2	2.003	100.15	0.61
AMB	30	29.99	99.97	0.54

PCM: Paracetamol, GUA: Guaifenesin, CPM: Chlorpheniramine maleate, AMB: Ambroxol hydrochloride, % RSD: Percentage relative standard deviation, PE: Phenylephrine hydrochloride

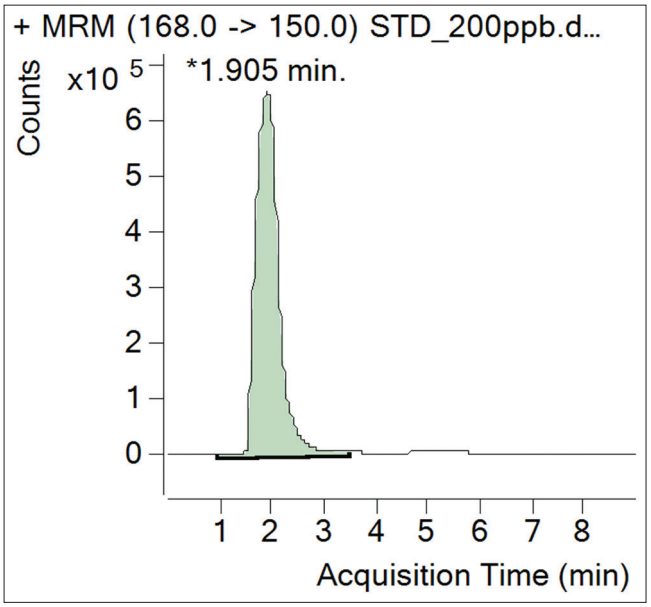

Fig. 14: Representative chromatogram of standard drug phenylephrine

Accuracy

Developed method found to be accurate as percentage recovery was between 98 and 102% (Table 6).

Analysis of marketed formulation

Five peaks at m/z 152.0 PCM, 199.0 GUA, 168.0 PE, 275.0 CPM, and 379.0 AMB were observed in the in Q1MS full spectra of the drug

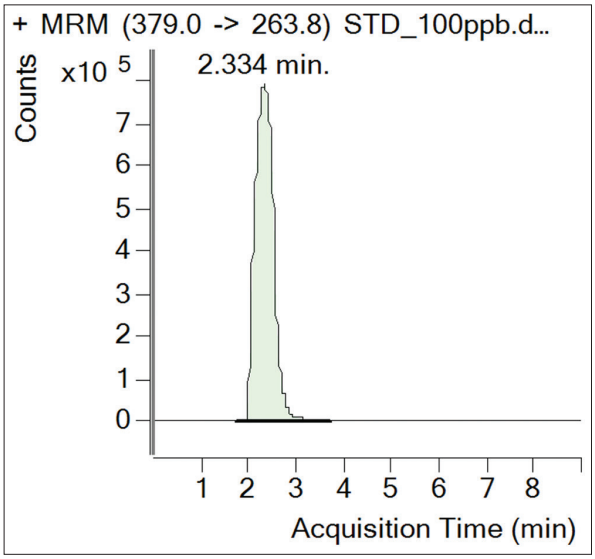

Fig. 16: Representative chromatogram of standard drug ambroxol

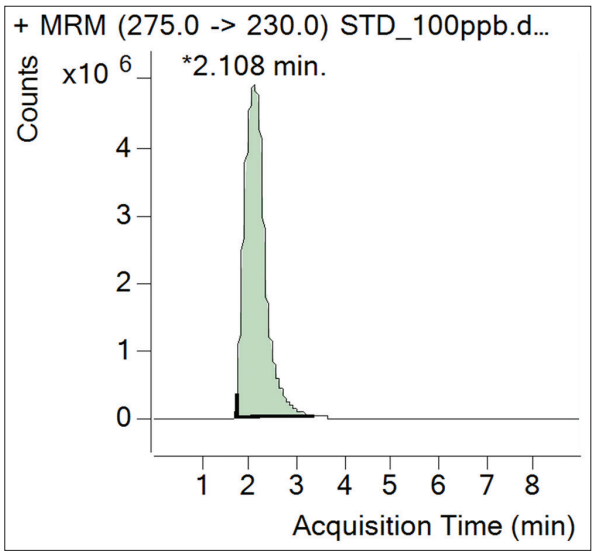

Fig. 15: Representative chromatogram of standard drug chlorpheniramine

samples extracted from tablet. The content of PCM, GUA, PE, CPM, and AMB was found to be 100.04, 100.20, 100.09, 100.15, and 99.99%, respectively. The low % RSD value indicated the suitability of the developed method for routine analysis of PCM, GUA, PHE, CPM, and AMB in pharmaceutical dosage form used in the study by LC/MS/MS technique (Table 7).

CONCLUSION

In the present research work, an attempt has been made to develop and validate new, precise, accurate, and robust LC/MS/MS method for simultaneous quantification of PCM, guaifenesin, PE, CPM, and AMB in the tablet formulation.

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AUTHORS' CONTRIBUTION

All authors have equal contribution in bringing out this article.

CONFLICTS OF INTEREST

The authors have no conflict of interest.

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