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Research Article

SIMULTANEOUS DETERMINATION OF 2-CHLORO METHYL PROPIONATE, 1,4-DI-BROMO BUTANE AND PARA ANISIC ALDEHYDE IN MEBEVERINE HYDROCHLORIDE API BY GAS CHROMATOGRAPHY-MASS SPECTROMETRIC WITH SELECTED-ION-MONITORING MODE

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ABSTRACT

Objective: To develop an accurate, precise and linear gas chromatography-mass spectrometric (GC-MS) selected-ion-monitoring (SIM) method for quantitative estimation of 2-chloro methyl propionate (2-CMP), 1,4-dibromo butane and para anisic aldehyde (PAA) as an genotoxic impurities in mebeverine HCl API (MEB) at ppm level and validated as per International Council of Harmonization (ICH) guidelines.

Methods: This method used in SIM mode mass selective detection was developed and validated for the trace level analysis of three impurities. All these three impurities are simultaneously determined by a GC-MS method using VF-624 Capillary column (60 m×0.32 mm×1.80 µm) with Helium as carrier gas and a flow rate of 2.0 mL/minutes. Chromatographic separation of 2-CMP, 1,4-DBB, and PAA was achieved in 7.91, 13.69, 18.45 minutes and m/z values were 63, 55, 135 on SIM mode.

Results: The method was linear for 2-CMP, 1,4-DBB and PAA in mebeverine HCl 1.90 µg/ml to 7.5 µg/ml, respectively. The coefficient of correlation (r^2) for the 2-CMP, 1,4-DBB and PAA was better than 0.999. The limit of detection obtained was 0.28, 0.35, 0.22 µg/ml and the limit of quantification (LOQ) obtained was 0.85, 1.06, 0.66 µg/ml. The method was fully validated, complying Food and Drug Administration, ICH and European Medicines Agency guidelines. Furthermore, verified precision, accuracy, LOQ precision, LOQ accuracy, ruggedness, and robustness.

Conclusion: The proposed method is specific, accurate, precise, linear, rugged and robust for the determination of the three genotoxic impurities in API of mebeverine HCl, and hence, is of wide applicability in pharmaceutical industries.

Keywords: 2-chloro methyl propionate, 1,4-dibromo butane, Para anisic aldehyde, Mebeverine HCl, Gas chromatography-mass spectrometric, Method development, Method validation.

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INTRODUCTION

Mebeverine HCl (Fig. 1) is an antimuscarinic. The IUPAC name was 4-[ethyl-[1-(4-methoxyphenyl) propan-2-yl] amino] butyl 3,4-dimethoxybenzoate; hydrochloride with molecular formula $C_{25}H_{36}ClNO_5$. It belongs to a group of compounds called musculotropic antispasmodics. These compounds act directly on the gut muscles at the cellular level to relax them. This relieves painful muscle spasms of the gut without affecting its normal motility. Mebeverine is used to relieve symptoms of irritable bowel syndrome and related intestinal disorders that are the result of spasms in the intestinal muscles. These include colicky abdominal pain and cramps, diarrhea alternating with constipation and flatulence. Mebeverine is also an inhibitor of calcium-depot replenishment. Therefore, it has a dual mode of action which normalizes the small bowel motility. It was first registered in 1965 and is marketed as Colofac, Duspatal, Colotal, Colospa, Mebeverine, Rudakol, Boots IBS relieve, Fomac, Mebecon and Duspatalin by Abbott Laboratories. British Pharmacopoeia described a non-aqueous titrimetric method for determination of MEB in the pure form [1,2].

According to current regulatory guidelines, it is important that the genotoxic impurities potentially damage the DNA at very low-level exposure. Genotoxic substances are the chemicals that harm an organism by damaging its genetic material. There are three primary effects that genotoxins can have on organisms by effecting their genetic information. Genotoxins can be carcinogens or mutagens or teratogens. Potential impurities most likely arise during synthesis, purification, and storage should be identified. As per USFDA guidelines regarding the limits of genotoxic impurities, a maximum of 1.5 µg per a day is the exposure limit [3,4].

Three genotoxic impurities, 2-chloro methyl propionate (2-CMP), 1,4-dibromo butane (1,4-DBB), and para anisic aldehyde (PAA) (Fig. 2) may present in the API of mebeverine HCl. An approach based on GC-MS is feasible within limits of time, ease of application, sensitivity, and cost. Despite the importance of the issue, no method is so far reported for the simultaneous determination of these impurities in API of mebeverine HCl [5,6].

METHODS

Chemicals and reagents

2-CMP, 1, 4-DBB and PAA were purchased from Sigma-Aldrich. Mebeverine hydrochloride was purchased from a local research laboratory. High-performance liquid chromatography grade ethyl acetate was purchased from MERCK. Water was purified by a Millipore-Q academic water purification system. All other chemicals and reagents used for the experiments were of analytical grade.

Instrumentations and conditions

The system consists of an GC-MS-QP 2010 plus (Shimadzu) with electron ionization probe. System control and data analysis were processed with GC-MS solutions software. Chromatography was perfumed on a VF-624 ms capillary column (60 m×0.32 mm×1.80 µm).

The GC oven temperature program utilized an initial temperature of 100°C and an initial holding time of 5.0 minutes, and then increased at 20°C/minutes to 200°C. The final temperature was held for 10.0 minutes. The injection temperature is 225°C. Helium gas was used as the carrier gas with a flow rate of 2.0 ml/minute and purge flow is 1.0 ml/minute. An injection volume with 1.0 µl.

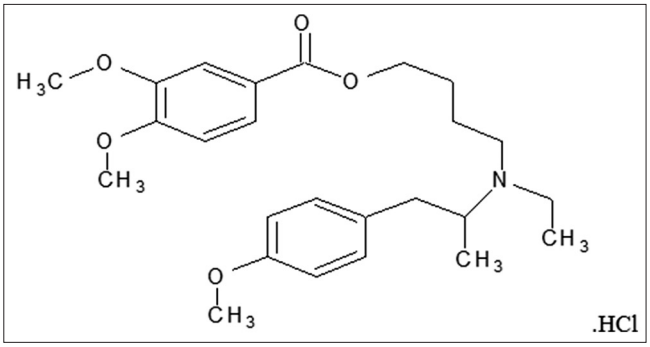

Fig. 1: Structure of mebeverine hydrochloride

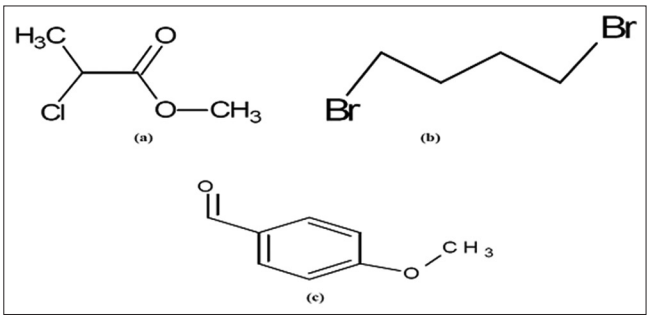

Fig. 2: Structure of (a) 2-chloro methyl propionate, (b) 1,4-dibromo butane, (c) Para anisic aldehyde

Selected-ion-monitoring (SIM) at unit resolution was employed to monitor the transitions of the prorogated forms of 2-CMP at m/z 63, 1, 4-DBB at m/z 55 and PAA at m/z 135 in the SIM mode. Optimized MS conditions were described as follows: GC-MS interface temperature with 250°C, ion source temperature is 260°C, solvent cut time with 6.0 minutes, detector voltage with 0.92 kv.

Preparation of solutions

Preparation of sample solution

Ethyl acetate was used as the diluent for the standard and sample solution preparation. Mebeverine HCl sample was prepared by weighing 10.0±0.005 g into a 20 ml volumetric flask and diluted to volume with diluents at a concentration of approximately 400 mg/ml. Sonicate for 10 minutes. Then filter this solution, filtrate was used for the analysis.

Preparation of standard solution

2-CMP, 1,4-DBB and PAA reference standard stock solution were prepared in diluent at a concentration of approximately 4.0 µg/ml each. The working standard solution of 2-CMP, 1,4-DBB and PAA were prepared by pipetting 0.75 ml from standard stock solution into 50 ml volumetric flask and diluted to volume with diluent at a concentration of approximately 1.5 µg/ml. The above Standard stock and working solution were stored at room temperature until use. The standard solution of each genotoxic impurity was prepared at 3.75 µg/ml with respect to sample concentration (400 mg/ml).

RESULTS AND DISCUSSION

Method development

This method development was implemented following Quality-by-Design principles including diluent selection, column screening, and column temperature determination. Method development samples were prepared using each of individual reference standard of mebeverine HCl, 2-CMP, 1,4-DBB and PAA.

Diluent selection

This method development was started with the selection of diluent that was suitable for dissolving 2-CMP, 1,4-DBB and PAA, but mebeverine HCl should not be dissolved. Because the sample solution is not passes through the mass ion source, 2-CMP, 1,4-DBB and PAA are soluble in methanol, ethyl acetate, and ethanol. While sample was in-soluble in ethyl acetate. Therefore, the diluent for 2-CMP, 1,4-DBB and PAA should be ethyl acetate.

Column screening

Column selection for chromatographic analysis was also an important step in method development. This study utilized a chromatographic basic rule "like attracts like" and focused on the polarity matching among column Stationary Phase and Mobile Phase. In this study, three columns, namely, VF-1 ms (30 m×0.32 mm×0.45 µm), VF-624 ms (60 m×0.32 mm×1.8 µm), and ZB-5 ms (30 m×0.25 mm×0.25 µm) for evaluated for column screening. The chromatographic parameters were first optimized to achieve good retention, high resolution and better peak shapes for the 2-CMP, 1,4-DBB and PAA in mebeverine HCl.

In the method development experiment, The VF-624 ms eluted three sharp peaks with minimal peak tailing for 2-CMP at retention time about 7.91 minutes, 1,4-DBB at about 13.69 minutes and PAA at about 18.45 minutes. It demonstrated that VF-624 column closely matched the 2-CMP, 1,4-DBB and PAA. The chromatogram obtained from the VF-624 ms column screening preliminarily concluded that this column was appropriate and meet the method requirement.

However, an additional column screening was continued for the purpose of developing more useful methods for future troubleshooting. The second column evaluated was the VF-1 ms (30 m×0.32 mm×0.45 µm). In this study, the VF-1 ms column could separate 2-CMP, 1,4-DBB and PAA with good peak symmetry. However, peak area was decreased by 30%, possibly due to the difference of particle sizes. Therefore, VF-1 ms column was not matched to these three genotoxic impurities.

The third column studied was ZB-5 ms (30 m×0.25 mm×0.25 µm) column. In this study, the ZB-5 ms column could not separate the peeks of 2-CMP, 1,4-DBB and PAA. Therefore, ZB-5 ms column was also not matched to these three genotoxic impurities.

Based on the above optimized methods for column screening, the results proved that the VF-624 ms (60 m×0.32 mm×1.8 µm) column afforded the best retention and separation of all three genotoxic impurities in mebeverine HCl. Hence, the VF-624 ms column was selected for further study.

Column temperature determination

Two column temperatures were evaluated during method development, namely, initial was same as 100°C, and final temperature is 200°C and 250°C. The determination was carried out based on a visual check of chromatogram and comparison of peak areas. In general, higher temperature has proven effective for improving the overall chromatographic performance, but the column temperature of 250°C eluted components faster and decreased the resolution of three impurity peaks in mebeverine HCl. When using the 200°C temperature, the peak separation is good and resolution is good. Hence, the column temperature of 200°C was determined for further study.

Mass spectral analysis

Based on the retention time obtained from the standard injection, solvent cut time and MS acquisition time were decided. As per the analysis conducted by GC-MS and the retention time of 2-CMP, 1, 4-DBB and PAA was in between 7.0 to 8.0 minutes, 13.0 to 14.0 minutes and 18.0 to 19.0 minutes, respectively. Hence, the solvent cut time was kept at 0.0 to 6.0 minutes. The three compounds were identified using the reference spectra (NIST) and m/z values for the SIM mode were finalized as 63 for 2-CMP, 55 for 1,4-DBB and 135 for PAA. The spectrum of the analytes, 2-CMP, 1,4-DBB and PAA, match to the reference spectra of NIST. The mass chromatogram and mass spectra of 2-CMP, 1,4-DBB and PAA are shown in Fig. 3.

Method validation

The proposed method was validated for specificity, linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ), LOQ precision and accuracy, ruggedness and robustness as per International Council of Harmonization method validated guidelines [7-9].

Specificity

The mebeverine HCl API sample was spiked with 2-CMP, 1,4-DBB and PAA, and sample was chromatographed to examine interference of any of the genotoxic impurity peaks with each other. The retention time for standard 2-CMP is 7.91 minutes, 1,4-DBB is 13.69 minutes, and PAA is 18.45 minutes. The chromatograms are shown in Fig. 4.

Repeatability

The precision of the method was evaluated at a single level. Repeatability was checked by calculating the percentage of relative standard deviation (%RSD) of six replicate determinations by injecting six freshly prepared solutions containing 1.5 µg/ml each of the mixture of impurities on the same day. As reported in Table 1, %RSD values were lower than 10.0% for the three impurities. This is confirmed an adequate precision of the developed method. The %RSD chromatograms of three impurities are shown in Fig. 5.

Linearity

The linearity of 2-CMP, 1,4-DBB and PAA genotoxic impurities were satisfactorily demonstrated with a five-point calibration graph

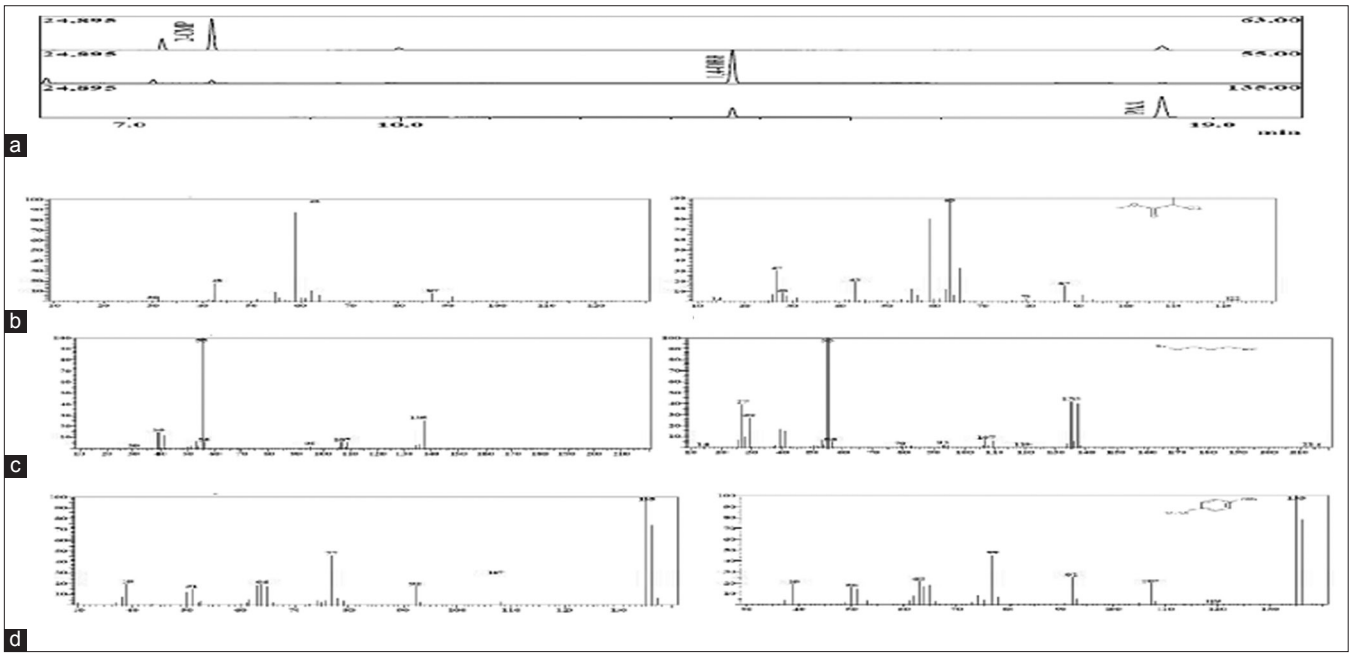

Fig. 3: (a) Mass chromatogram in SIM mode, (b-d) mass spectrums and reference mass spectrums (NIST) of 2-chloro methyl propionate, 1,4-dibromo butane and para anisic aldehyde

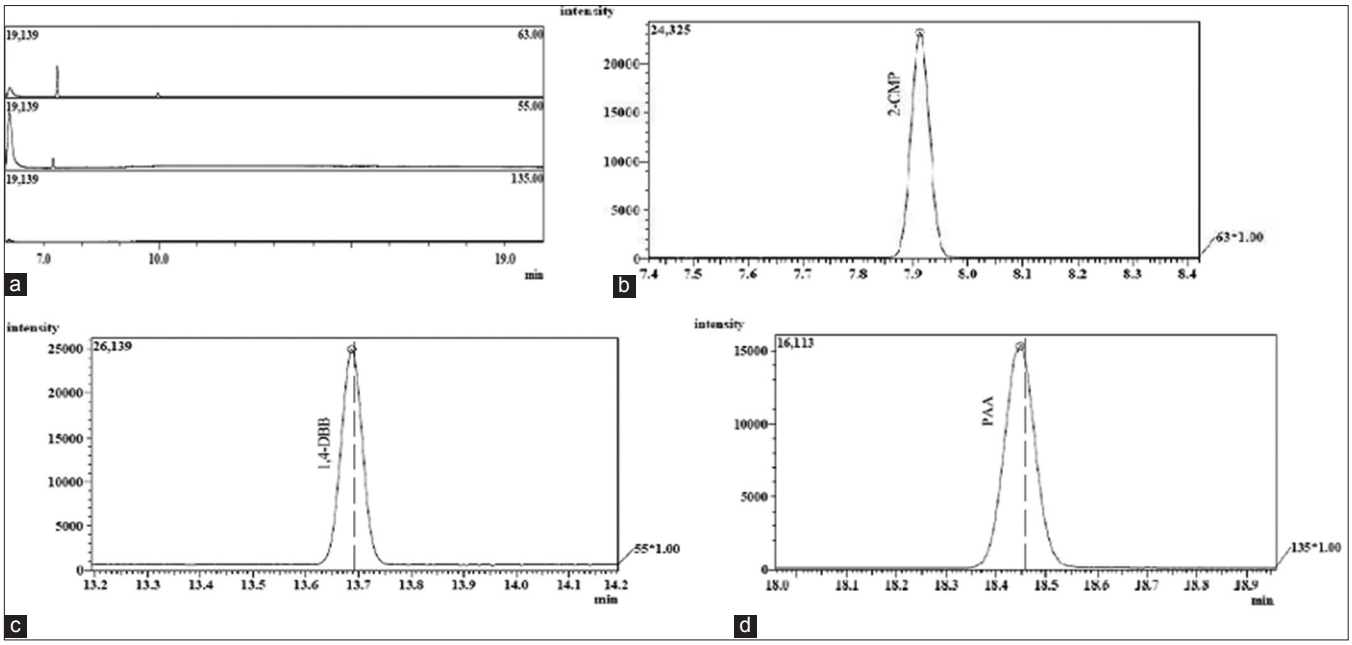

Fig. 4: Specificity chromatograms of (a) blank, (b) 2-chloro methyl propionate, (c) 1,4-dibromo butane, (d) para anisic aldehyde

Table 1: Repeatability data for 2-CMP, 1,4-DBB and PAA

Serial number	2-CMP	1,4-DBB	PAA
1	47996	5885	54733
2	47887	59321	54660
3	46185	58268	54906
4	49822	62922	56880
5	49488	62414	57709
6	49594	62860	58227
Average area	48495	60945	56186
Standard deviation	1408	2033	1615
% of RSD	2.90	3.34	2.87

2-CMP: 2-chloro methyl propionate, 1,4-DBB: 1,4-dibromo butane, PAA: Para anisic aldehyde, RSD: Relative standard deviation

between 1.9 and 7.5 µg/ml with respect to a sample concentration of 400 mg ml. The calibration curves were produced by plotting the average of triplicate genotoxic impurities injections against the concentration expressed in µg/ml. The slope, intercept, and correlation coefficient values were derived from linear least squares regression analysis. The correlation coefficient obtained in each case was >0.99. The corresponding linearity data and graphs are presented in Table 2 and Fig. 6. The results indicated that an excellent correlation existed between the peak areas and the concentrations of impurities.

Accuracy

Weighed accurately 10.0 g of the mebeverine HCl API into three different 25 ml of volumetric flasks and spiked with 50%, 100% and

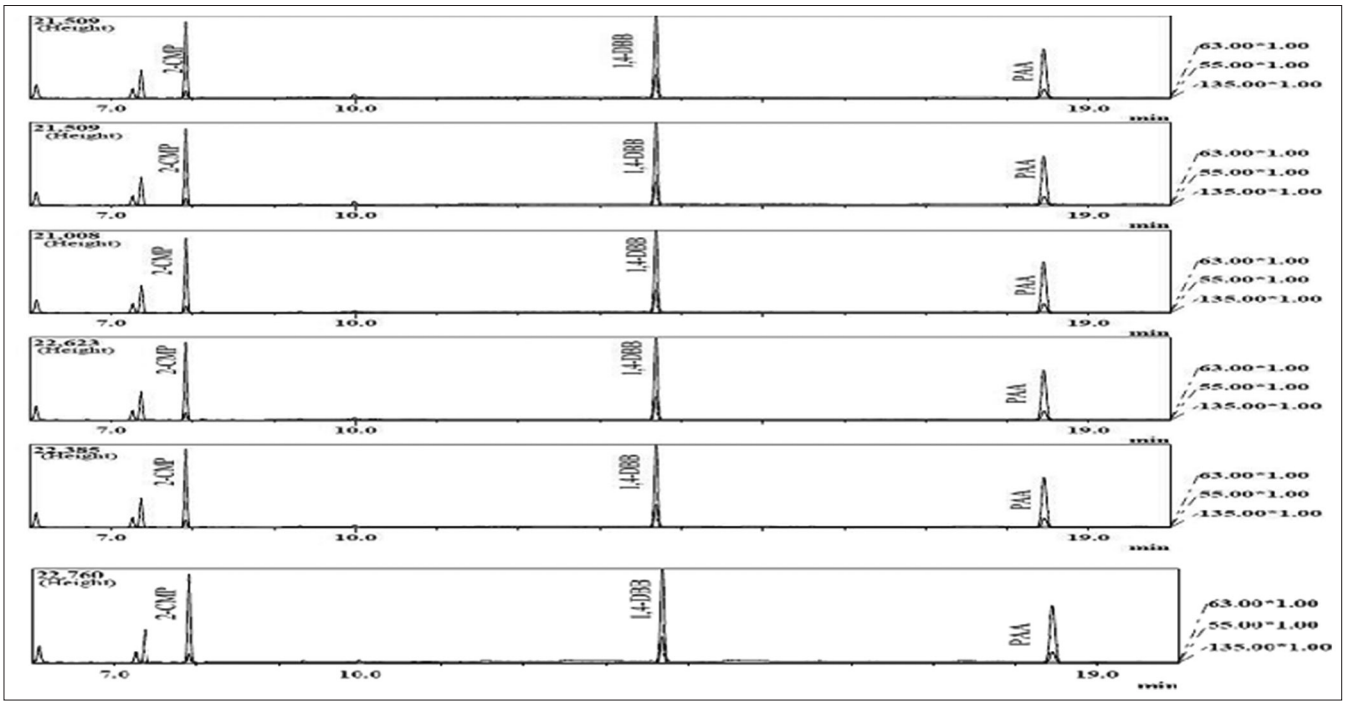

Fig. 5: Typical % relative standard deviation chromatogram of precision for 2-chloro methyl propionate, 1,4-dibromo butane and para anisic aldehyde

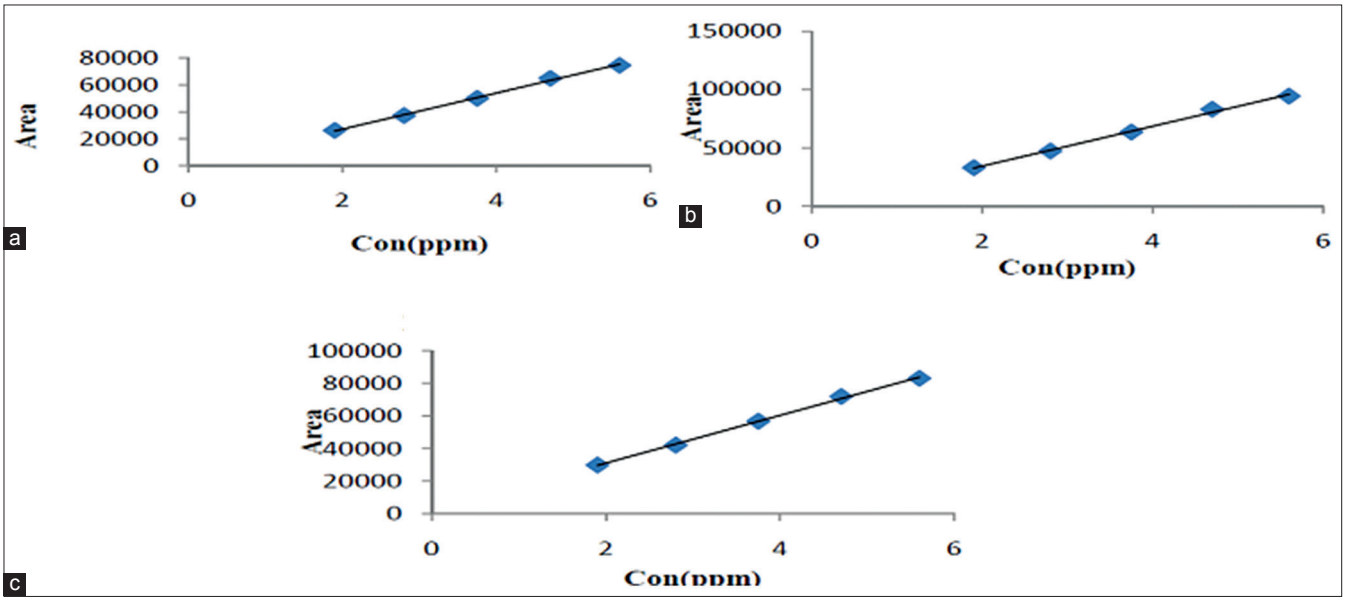

Fig. 6: Linearity graphs for (a) 2-chloro methyl propionate, (b) 1,4-dibromo butane and, (c) para anisic aldehyde

150% standard solutions of 2-CMP, 1,4-DBB and PAA. Added 20 ml of diluents, mixed well then made up with the same diluents, then filtered and the filtrate was used for injection. Standards of the three impurities and three spiked samples at 50%, 100% and 150% levels in triplicate are injected. From accuracy data, the % recovery of 2-CMP, 1, 4-DBB and PAA was found within the limits (100±15%). The results indicate that the method has an acceptable level of accuracy. The recovery data is presented in Table 3.

LOD and LOQ

The LOD and LOQ were calculated by instrumental and statistical methods. For the instrumental method, LOD is determined as the lowest amount to detect, and LOQ is the lowest amount to quantify, by the detector. Further LOD and LOQ values were established using calibration curve method. Standard solutions ranging from 1.9 to 7.5 µg/ml for three analytes were injected into the system for performing LOD and LOQ prediction study. Based on the concentrations obtained from slope and intercept of the prediction activity, LOD and LOQ precision activity performed. LOD values for 2-CMP, 1,4- DBB and PAA were 0.28, 0.35 and 0.22 µg/ml, respectively. LOQ values for 2-CMP, 1, 4-DBB and PAA were 0.85, 1.06, and 0.66 µg/ ml, respectively. Prepare the standard three impurities 2-CMP, 1, 4-DBB and PAA solutions at LOD and LOQ concentrations. The corresponding linearity data graphs at LOD and LOQ concentration are presented in Table 4 and Figs. 7 and 8.

LOQ precision

Prepare the standard 2-CMP, 1,4-DBB and PAA solutions at LOQ concentration (0.85, 1.06 and 0.66 µg/ml) and injected in six replicates. The %RSD (n=6) values obtained for the average area of 2-CMP, 1,4- DBB and PAA are 21238, 27371 and 27938. The acceptance criteria of %RSD for Three impurities are not more than 10%. The LOQ precision data and chromatograms of LOD and LOQ are shown in Table 5 and Fig. 9

LOQ accuracy

Weighed accurately 10.0 g of the Mebeverine HCl API into three different 25 ml of volumetric flasks and spiked with LOQ level three standard solutions of 2-CMP, 1, 4-DBB and PAA, add 10 ml of diluents mix well then makeup with the same diluents. Filter the solution take the filtrate for injection. Then, inject in triplicate. From accuracy data at LOQ level, the % recovery of 2-CMP, 1,4-DBB and PAA were found within the limits (100%±15%). The results are presented in Table 6.

Ruggedness

The ruggedness of the method was evaluated by performing the sample analysis in six replicates using different analyst on different days, and

Table 2: Linearity data for 2-CMP, 1,4-DBB and PAA

Concentration (µg/ml)	Area of 2-CMP	Area of 1,4-DBB	Area of PAA
1.9	26219	33046	29876
2.8	37286	47371	42078
3.75	50018	63675	56753
5.6	64869	83143	71923
7.5	74580	94506	83057
Correlation coefficient (r^2)	0.999	0.999	0.998

2-CMP: 2-chloro methyl propionate, 1,4-DBB: 1,4-dibromo butane, PAA: Para anisic aldehyde

Table 3: Accuracy data for 2-CMP, 1,4-DBB and PAA

% Accuracy	Average area of 2-CMP	Average area of 1,4-DBB	Average area of PAA
STD solution (n=3)	63988	88605	74394
50 % level (n=3)	33828	45931	35231
% of recovery	105.73	103.67	94.71
100 % level (n=3)	69114	92299	80861
% of recovery	108.01	104.17	108.69
150 % level (n=3)	91087	123477	117285
% of recovery	94.90	92.90	105.10

2-CMP: 2-chloro methyl propionate, 1,4-DBB: 1,4-dibromo butane, PAA: Para anisic aldehyde

Table 4: Linearity graph data for 2-CMP, 1,4-DBB and PAA at LOQ concentration

Concentration (µg/ml)	Area of 2-CMP	Area of 1,4-DBB	Area of PAA
1.9	26219	33046	29876
2.8	37286	47371	42078
3.75	50018	63675	56753
5.6	64869	83143	71923
7.5	74580	94506	83057
Correlation coefficient (r^2)	0.993	0.996	0.992
Slope	13372	17073	14652
STEYX	1135	1805	971
LOD	0.28 µg/ml	0.35 µg/ml	0.22 µg/ml
LOQ	0.85 µg/ml	1.06 µg/ml	0.66 µg/ml

2-CMP: 2-chloro methyl propionate, 1,4-DBB: 1,4-dibromo butane, PAA: Para anisic aldehyde, LOD: Limit of detection, LOQ: Limit of quantification

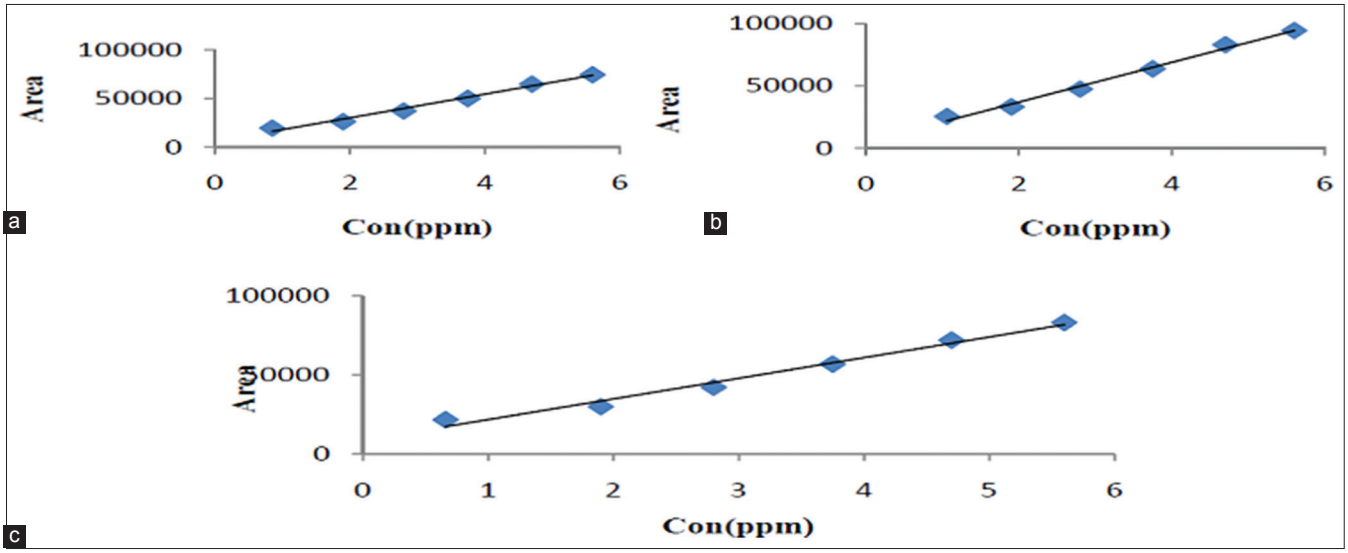

Fig. 7: Linearity graphs for (a) 2-2-chloro methyl propionate, (b)1,4-dibromo butane and (c) para anisic aldehyde at limit of quantification

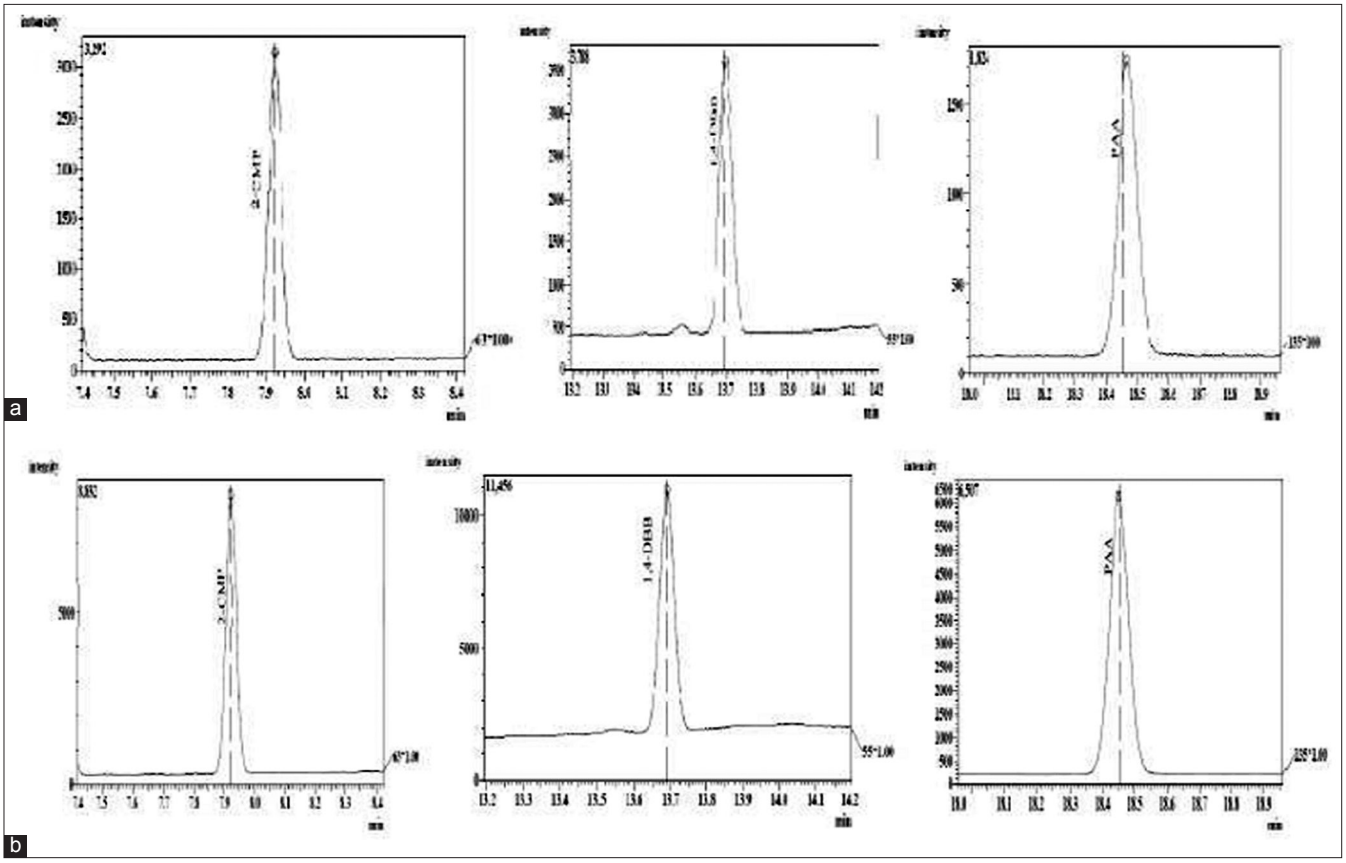

Fig. 8: (a) Limit of detection (b) limit of quantification chromatograms for 2-2-chloro methyl propionate, 1,4-dibromo butane and para anisic aldehyde

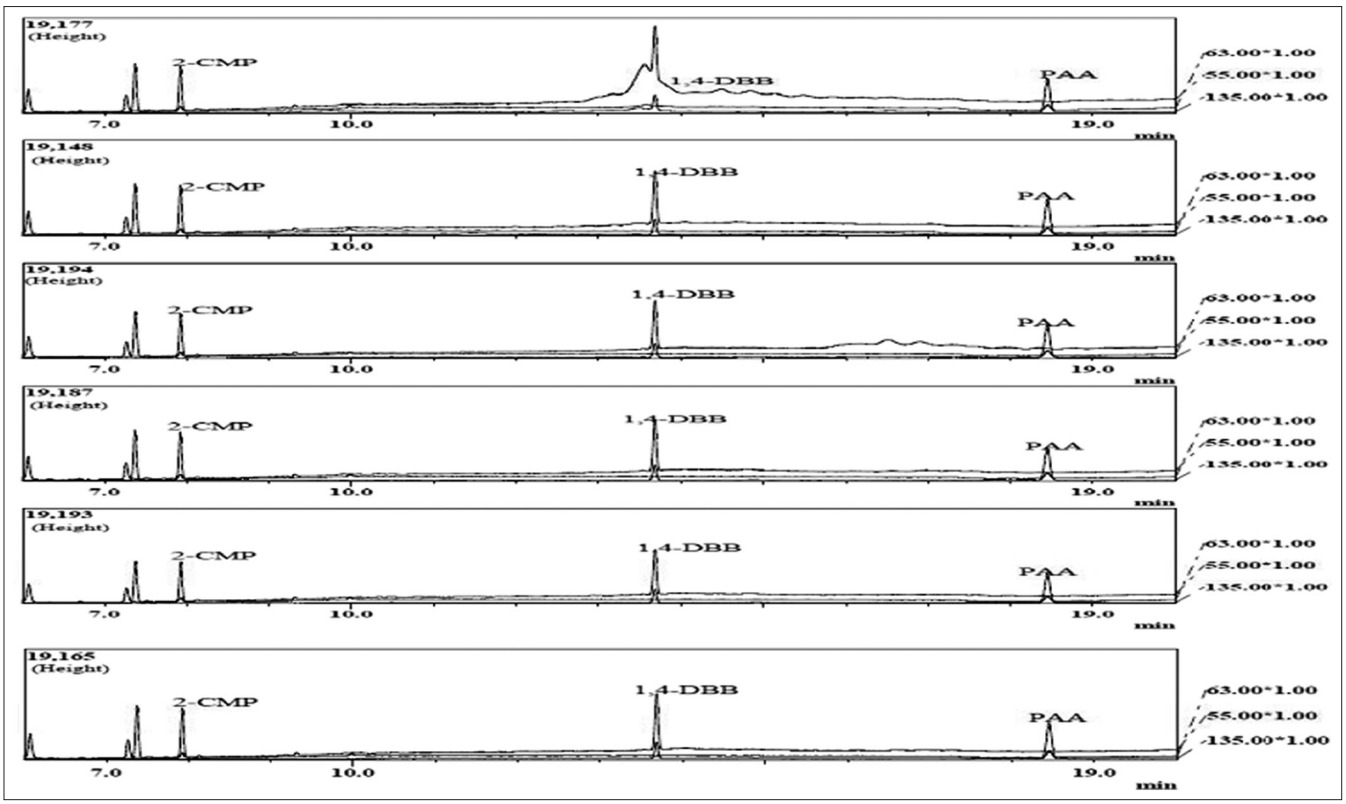

Fig. 9: Limit of quantification precision chromatograms for 2-chloro methyl propionate, 1,4-dibromo butane and para anisic aldehyde

Table 5: LOQ precision data for 2-CMP, 1,4-DBB and PAA

Serial number	Area of 2-CMP	Area of 1,4-DBB	Area of PAA
1	21270	26379	28653
2	22837	29682	30133
3	21054	27123	28168
4	21702	28564	28514
5	19569	25429	25289
6	20993	27046	26869
Average area	21238	27371	27938
Standard deviation	1063	1529	1666
% of RSD	5.01	5.59	5.96

2-CMP: 2-chloro methyl propionate, 1,4-DBB: 1,4-dibromo butane, PAA: Para anisic aldehyde, LOD: Limit of detection, LOQ: Limit of quantification, RSD: Relative standard deviation

Table 6: LOQ-accuracy data for 2-CMP, 1,4-DBB and PAA

Accuracy %	Average area of 2-CMP	Average area of 1,4-DBB	Average area of PAA
Standard solution	21238	27371	27938
LOQ level (n=3)	21720	27693	25545
% of recovery	102.27	101.18	91.43

2-CMP: 2-chloro methyl propionate, 1,4-DBB: 1,4-dibromo butane, PAA: Para anisic aldehyde, LOQ: Limit of quantification

Table 7: Ruggedness data for 2-CMP, 1,4-DBB and PAA

Name of impurity	Day 1 (RSD %)			Day 2 (RSD %)			Analyst 1 (RSD %)	Analyst 2 (RSD %)
	Analyst 1	Analyst 2	Analyst 1 and 2	Analyst 1	Analyst 2	Analyst 1 and 2	Day 1 and 2	Day 1 and 2
2-CMP	2.78	2.41	2.48	2.16	2.25	2.10	2.56	2.45
1,4-DBB	2.09	1.92	1.93	2.80	2.21	2.44	2.47	2.30
PAA	2.15	2.01	2.00	2.15	2.19	2.07	2.06	2.04

2-CMP: 2-chloro methyl propionate, 1,4-DBB: 1,4-dibromo butane, PAA: Para anisic aldehyde, RSD: Relative standard deviation

Table 8: Robustness data for 2-CMP, 1,4-DBB and PAA

Parameter	2-CMP		1,4-DBB		PAA	
	Average area (n=6)	RSD %	Average area (n=6)	RSD %	Average area (n=6)	RSD %
Flow rate (ml/min)						
1.8	41431	2.16	61913	2.58	55428	2.22
2.0	46001	2.04	62384	2.07	56033	2.24
2.2	46547	2.20	62686	2.28	57071	2.10
Column temp (°C)						
195	45614	2.25	61201	2.19	55096	2.60
200	45928	2.34	61196	2.83	55942	2.13
205	46357	2.31	61961	2.46	55815	2.58

the results are summarized as shown in Table 7. The %RSD values of less than 10.0% for 2-CMP, 1, 4-DBB and PAA content indicate that the method adopted is rugged.

Robustness

The robustness of the method was examined by replicate injections (n=6) of 1.5 µg/ml of three standard solutions with slight modifications on the chromatographic parameters (flow rate and column oven temperature). To study the effect of flow rate on the resolution, the flow rate of mobile phase was altered by ±0.2 ml/minute (1.8-2.2 ml minute from 2.0 ml/minute). The effect of column oven temperature on resolution was studied at 195°C and 200°C instead of 205°C. The RSD (%) obtained after changing the retention time and peak area was calculated, it should be not more than 10%. In conclusion, variations in all the studied parameters had no significant effects on retention time or peak area, and the developed method proved to be robust for 2-CMP, 1, 4-DBB and PAA quantifications. The data of robustness is following Table 8.

CONCLUSION

A GC-MS at SIM mode method was developed and validated that allows a simple and accurate quantification of 2-CMP, 1,4-DBB and PAA simultaneously at a very low concentration levels. It is a simple, selective and sensitive method using inexpensive reagents. The Precision, Linearity, Accuracy, LOD and LOQ values were observed to be well within the set of acceptance criteria. The described method is highly reliable technique for the quantification of genotoxic impurities in the Mebeverine HCl. This method is useful in Pharmaceutical industries and formulation analysis.

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