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RESEARCH ARTICLE

Development of a Suitable HPLC Method for the Analysis of Impurities in Clorprenaline and Bromhexine Capsules and Identification of Unknown Impurities by 2D LC-Q-TOF MS

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Abstract: ***Background*:** Impurities may reduce antibacterial activity and affect clinical efficacy, However, there has been no report on the impurity of clorprenaline and bromhexine capsules.

***Objective*:** In order to determine the impurities in compound clorprenaline and bromhexine capsules.

***Methods*:** A new stability-indicating HPLC method was established. A Boston Green ODS column was used, and the UV detection was 225nm.

***Results*:** The established method was highly specific, sensitive, accurate, and suitable for routine quality control of clorprenaline and bromhexine capsules. The structures of unknown impurities were characterized by the MS/MS data.

***Conclusion*:** These results provide a sufficient basis for our subsequent study on the safety of Compound Clorprenaline and Bromhexine Capsules and also provide ideas for the impurity research of other compound preparations.

Keywords: Clorprenaline, bromhexine capsules, impurity, HPLC, validation, 2d lc-q-tof ms.

1. INTRODUCTION

Clorprenaline and bromhexine capsules are composed of chlorprenaline hydrochloride, decloxizine hydrochloride, and bromhexine hydrochloride (Fig. **1**). It is clinically used for the treatment of bronchial asthma and asthmatic bronchitis, and chronic bronchitis. Chlorprenaline hydrochloride is a β_2 adrenergic receptor agonist, decloxyzine hydrochloride is a histamine receptor H1 antagonist, and bromhexine hydrochloride is an expectorant [1].

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At present, it has been reported that a variety of detection methods were used to determine the content analysis of three principal components in the compound preparation. The national drug standard of China prescribes an HPLC method for the assay of three components [2]. Chlorprenaline hydrochloride, Chlorpromazine hydrochloride, and Bromhexine hydrochloride are listed in the legal standard [3-6]. However, there has been no report on the impurity of clorprenaline and bromhexine capsules. As we all know, impurities may reduce antibacterial activity and affect clinical efficacy, so it is necessary to characterize and control the impurities in drugs.

Various technologies are used to characterize unknown impurities [7-13]. In recent years, the combination of high-performance liquid chromatography and Q-TOF MS has

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become a widely used analytical technique for the separation and identification of drug impurities [14-18, 10]. UPLC-MS/MS has also been used for the separation and determination of drugs and their metabolites [19]. In addition, according to the requirements of ICH, the structure of impurities containing more than 0.1% should be identified to ensure the quality and safety of drugs [20].

The aim of this study was twofold: first, to develop an HPLC method for the quantitative analysis of unknown impurities with no requirements of specific impurity reference standards; second, to identify unknown impurities. As far as we know, this is the first paper to investigate impurities in compound clorprenaline and bromhexine capsules

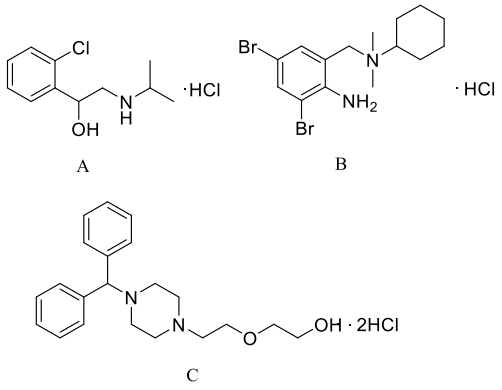


Fig. (1). The chemical structures of three principal components. A-Cloprenaline Hydrochloride; B- Bromhexine Hydrochloride; C-Decloxizine Hydrochloride.

2. EXPERIMENTAL METHOD

2.1. Materials

Compound Clorprenaline and Bromhexine Capsules (batch number: 20194946) used in this study were provided by Zhejiang Institute for Food and Drug Control (Hangzhou, China). Chlorprenaline hydrochloride reference Standards (99.8%), Bromhexine hydrochloride reference Standards(99.9%) and Decloxizine Hydrochloride reference Standards(99.1%) were supplied by National Institutes for Food and Drug Control.

HPLC grade phosphoric acid from Tedia (Fairfield, OH, USA); ammonium formate for mass spectrometry from Sigma-Aldrich (St. Louis, MI, USA); acetonitrile, formic acid and triethylamine from Merck (Darmstadt, Germany), sodium hydroxide (NaOH; analytical grade), and disodium hydrogen phosphate from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China); 30% hydrogen peroxide (H_2O_2; analytical grade) from Shanghai Lingfeng Chemical Reagent Co. Ltd. (Shanghai, China); hydrochloric acid (HCl; analytical grade) from Lanxi Yongli Chemical Industry Co. Ltd.(Jinhua, China). Ultrapure water was produced through a Millipore Milli-Q-Gradient purification system (Millipore, Bedford, MA, USA).

2.2. Instrumentation

2.3. HPLC Apparatus and Conditions

The methodology was studied using the Shimadzu LC-20AD liquid chromatography system, and impurities were identified using an Agilent liquid chromatography instrument. The first LC dimension included an Agilent 1260 liquid chromatograph system consisting of a quaternary pump, an autosampler, a column thermostat and a diode array detector (DAD). The second dimension included an Agilent 1290 high liquid chromatograph (California, America) system consisting of a binary pump and a diode array detector (DAD). The first- and second-dimension columns were connected by two six-position and six-port switching valves and were equipped with six stainless steel quantitative loops with a capacity of 100 μL, which acted as syringes for the second dimension. The detection wavelength was performed at 225 nm.

Chromatographic separation in the first dimension was carried out at 35°C using a Boston Green ODS analytical column (4.6 mm × 250 mm, 5 μm). The mobile phase consisted of (A) 0.5% triethylamine water solution（adjusted the pH to 3.0 with phosphoric acid） and (B) acetonitrile. The gradient conditions were: 0 min, 10% B; 10 min, 10% B (hold for 10 min); 30 min, 80% B (hold for 10 min); 40.1 min, 10% B (hold for 20 min). The flow rate was 1.00 ml/min, the injection volume was 10 μl, and the detection wavelength was performed at 225 nm. Chromatographic separation in the second dimension was carried out at 35 °C using a BOSTON GREEN ODS analytical column (4.6 mm ×150 mm, 5 μm). The mobile phase consisted of (A) 10 mM Ammonium acetate solution and (B) acetonitrile. The method uses isocratic elution with different elution ratios to analyze target compounds. The mobile phase flow rate was 1.0 mL/min.

2.4. Mass Spectrometric Conditions

Liquid chromatography was coupled with a hybrid quadrupole time-of-flight mass spectrometry (Model: 6538 Q-TOF) from Agilent Technologies Inc. (California, America). The mass spectrometry detector (MSD) was equipped with a dual-spray electrospray ionization (ESI) source, and the MS and MS2 spectra were recorded in positive mode. The samples were infused into the source chamber from the LC system through a T-junction with a splitting ratio of approximately 2:1. The ion source temperature was 350 °C, and the needle voltage was always set at 3500 V. Nitrogen was used as drying gas at a flow rate of 10 L/min. The collision energy was varied from 5 V to 40 V to maximize the ion current in the spectra.

Instrument control and data acquisition were performed with mass Hunter B.08.00 software from Agilent.

2.5. Sample Preparation

The blank excipient solution was prepared by dissolving known amounts of blank excipient in a mobile phase solution. Sample solutions were prepared by weighing the capsule contents of about 0.52 g that were dissolved in mobile phase solution, adjusted to 20.0 mL with a volumetric flask, and filtered. The stock solutions of Cloprenaline Hydrochloride, Bromhexine Hydrochloride, and Decloxizine Hydrochloride were 0.8 mg mL^{-1}, 1.6 mg mL^{-1} and 4.0 mg mL^{-1}, and prepared by dissolving in mobile phase solution. For the forced degradation solutions, about 0.52g of contents of capsules were subjected to the forced degradation conditions

indicated in the ICH guidelines. The selected stress conditions were as follows [21]: acidic degradation (1.0 mol L^{-1} HCl 1.0 mL, room temperature, 6h), Basic degradation (1.0 mol·L^{-1} NaOH 1.0 mL, room temperature, 6h), oxidation degradation (0.3% H_2O_2 1.0 mL, room temperature, 30 min), heat degradation (solid, 105 °C, 4 h), and Photodegradation (solid, 4500 lx ± 500 lx, 15 days). The acidic and alkaline stress samples were neutralized with 1.0 mol L^{-1} NaOH or 1.0 mol L^{-1} HCl solution before dilution, respectively. Finally, all degradation samples were diluted to a final nominal concentration of 4.0 mg mL^{-1} of Decloxizine Hydrochloride by the mobile phase.

3. RESULTS AND DISCUSSION

3.1. Development of HPLC Method for Separation of Impurities in Compound Clorprenaline and Bromhexine Capsules

3.1.1. Selection of the Initial Chromatographic Condition

At present, there is no specific test method for impurities in Compound Clorprenaline and Bromhexine Capsules. The determination method of Compound Clorprenaline and Bromhexine Capsules was chosen as the initial method for the determination of impurities. An ODS analytical column was used for content determination. The mobile phase consisted of a mixture of 1.0% triethylamine and methanol (45:55, v/v) (adjusted to pH 3.0 with phosphoric acid) at a flow rate of 1.0 mL·min^{-1}. The detection wavelength was 225 nm. Fig. (**2A**) shows the chromatogram of the sample solution. The impurities after the Decloxizine Hydrochloride peak were not completely separated, and impurity I of bromhexine hydrochloride in Chinese Pharmacopoeia and bromhexine hydrochloride were eluted simultaneously.

3.1.2. Optimization of Chromatographic Conditions

To develop a method for separation and detection of impurities, we tried to use the method of each component in the pharmacopoeia for the pre-experiment, but none were effective at detecting impurities. The final determination method was formed by optimizing the composition, proportion, and elution method of the mobile phase on the basis of chromatographic conditions for content determination. Acetonitrile was selected as the organic phase, and 0.5% triethylamine aqueous solution (adjusted pH to 3.0 with phosphoric acid) as the aqueous phase. Gradient elution and detection wavelength were 225 nm. Under the optimized chromatographic condition, the separation results of the sample solution were compared (Fig. **2B**). The main impurities in Compound Clorprenaline and Bromhexine Capsules were well separated.

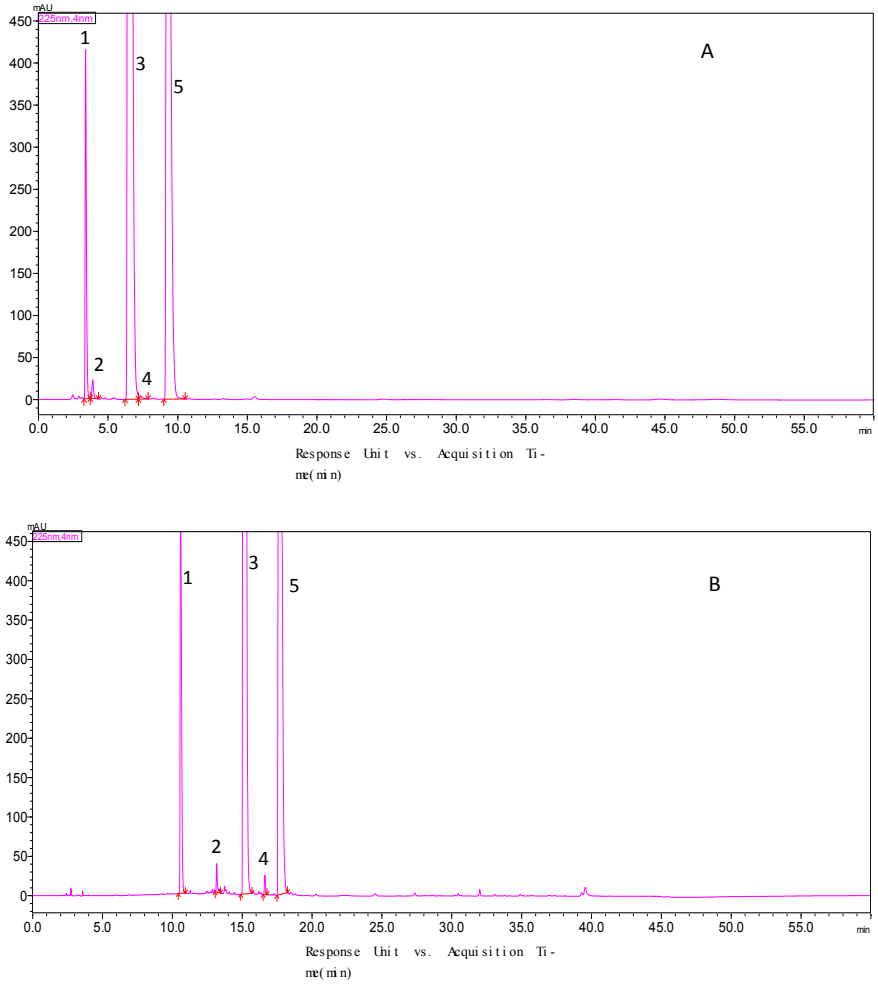


Fig. (2). Contd…

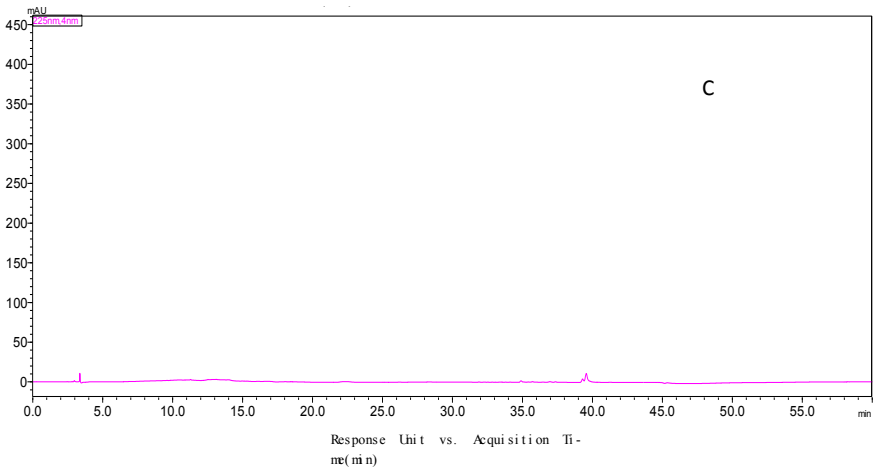


Fig. (2). Chromatogram of sample solution under different methods. (**A**) the initial condition; (**B**) the optimized condition; (**C**) Blank excipient solution. 1-Cloprenaline Hydrochloride; 2- Impurity 1; 3- Decloxizine Hydrochloride; 4- Impurity 2; 5- Bromhexine Hydrochloride.

Table 1. The results of the validation.

Statistical Parameter	Cloprenaline Hydrochloride		Decloxizine Hydrochloride		Bromhexine Hydrochloride	
Linearity	-		-		-	
Concentration range	1.77~88.62μg·mL^{-1}		8.51 ~ 425.40μg·mL^{-1}		3.35 ~ 167.28μg·mL^{-1}	
Regression equation	y = 4761.3 x + 2396.1		y = 12688 x + 45252		y = 17924 x + 2583.4	
Correlation coefficient (r)	0.9997		0.9998		1.0000	
SD[a] of concentration	5.6%		5.4%		4.6%	
Accuracy	Recovery (%)[b]	RSD (%)	Recovery (%)[b]	RSD (%)	Recovery (%)[b]	RSD (%)
Low	95.0	3.5	101.2	2.2	102.3	1.3
Medium	97.6	3.1	98.7	1.1	99.7	0.9
High	97.9	1.1	99.3	1.7	101.4	0.8
-	Concentration (μg·mL^{-1})	RSD (%)	Concentration (μg·mL^{-1})	RSD (%)	Concentration (μg·mL^{-1})	RSD (%)
LOQ[c]	0.82	4.3	0.60	3.1	0.63	4.6
LOD[c]	0.27	5.7	0.20	4.2	0.21	4.4

Note: [a]Standard deviation
[b] Three replicates for each level
[c] Six independent determinations.

3.2. Method Validation

The developed HPLC method for the determination of impurities in Compound Clorprenaline and Bromhexine Capsules was extensively validated concerning specificity, linearity, range, accuracy, LOD, LOQ, and robustness according to the ICH guidelines. The results of validation studies are given in Table **1**.

3.2.1. Specificity

The specificity of the current method was verified by forced degradation solution and blank excipient solution. The results showed that there was no interference co-elution peak in the blank excipient solution, and the impurity peaks were well separated from the principal component peaks.

3.2.2. Linearity and Range

Linearity and Range of the method were studied by injecting six different concentration levels of Cloprenaline Hydrochloride, Decloxizine Hydrochloride, and Bromhexine Hydrochloride in the range of 1.77-88.62 μg·mL^{-1}, 8.51 - 425.40μg·mL^{-1} and 3.35 - 167.28 μg·mL^{-1}, respectively. Regression curves were constructed from the relationship between peak area and analyte concentration. The regression curves were constructed from the peak areas versus the concentrations of the analyte. The measurement coefficients were all greater than 0.999, indicating satisfactory linearity of the method.

3.2.3. Accuracy

The recovery rate was used to evaluate the accuracy of the method. This was achieved by adding three principal

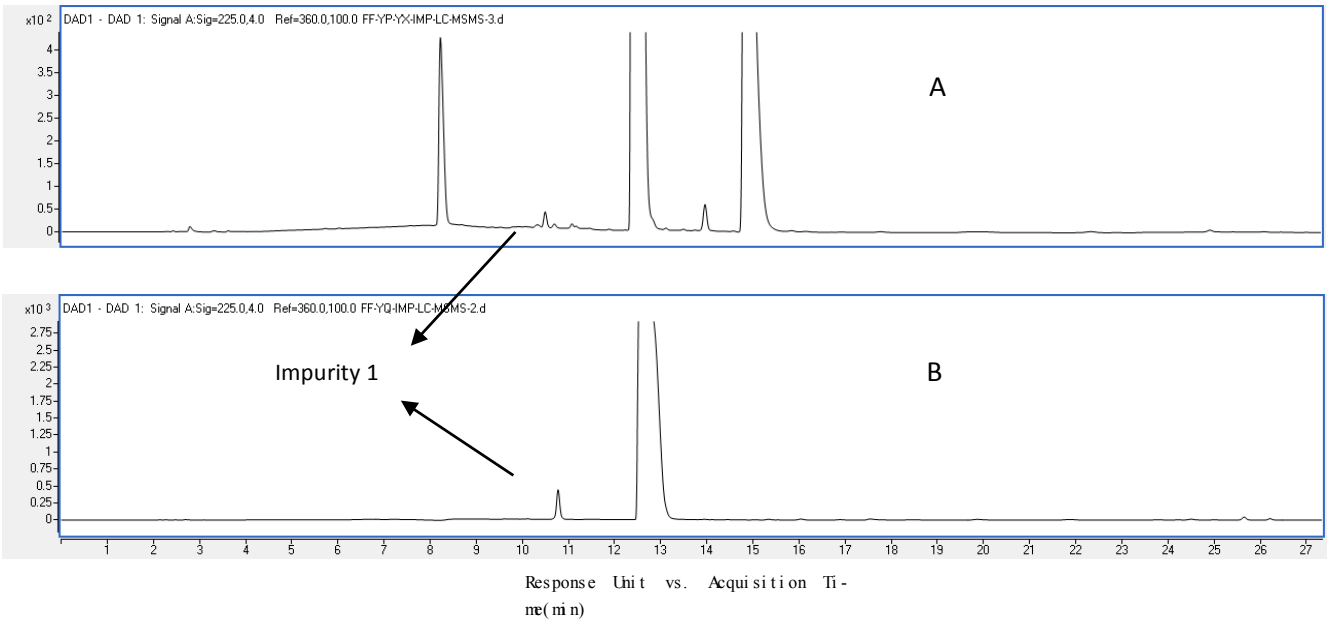


Fig. (3). The chromatogram of Compound Clorprenaline and Bromhexine Capsules and Decloxizine Hydrochloride in the first dimension. (**A**) Compound Clorprenaline and Bromhexine Capsules; (**B**) Decloxizine Hydrochloride.

component references at different concentration levels to the blank excipients. The recoveries of Cloprenaline Hydrochloride, Decloxizine Hydrochloride, and Bromhexine Hydrochloride were calculated, and they were in the range of 95.0 to 102.3%, all within the specified range of 80.0-120.0%.

3.2.4. LOD and LOQ

To evaluate the sensitivity of the developed method, the LOD and LOQ of Cloprenaline Hydrochloride, Decloxizine Hydrochloride and Bromhexine Hydrochloride were calculated with the signal-to-noise ratio (S/N) of 3:1 and 10:1, respectively. The LOQ solutions and LOD solutions were injected consecutively 6 times. The obtained LODs of Cloprenaline Hydrochloride, Decloxizine Hydrochloride, and Bromhexine Hydrochloride were 0.27 $\mu g \cdot mL^{-1}$, 0.20 $\mu g \cdot mL^{-1}$and 0.21 $\mu g \cdot mL^{-1}$, respectively. The LOQs were 0.82 $\mu g \cdot mL^{-1}$, 0.60 $\mu g \cdot mL^{-1}$ and 0.63 $\mu g \cdot mL^{-1}$, respectively. This limit is sensitive enough for the determination of impurities in Compound Clorprenaline and Bromhexine Capsules.

3.2.5. Robustness

Robustness was assessed by systematic variations in flow rate (0.9-1.1 $mL \cdot min^{-1}$) and column temperature (30-40 ℃). Compared with the conventional conditions, the impurity content difference was less than 0.05%. Therefore, the method can be considered robust to these small changes.

3.2.6. Sample Analysis

Using the established method, the contents of impurity 1 and impurity 2 were determined with reference of decloxizine hydrochloride and bromhexine hydrochloride, respectively. The content of impurity 1 and impurity 2 were 0.68% and 0.56% respectively. The developed HPLC-CAD methods were useful and effective for the quality control of Compound Clorprenaline and Bromhexine Capsules.

3.3. Identification of the Unknow Impurities by 2D LC-Q-TOF MS

3.3.1. Preliminary Analysis of Impurity Sources

Two large impurity peaks can be seen in the chromatogram of the sample solution under the recommended HPLC method. In order to identify the unknown impurities, all principal components were examined simultaneously by 2D LC-Q-TOF MS in electrospray ionization mode. Fig. (**3**) shows the chromatogram of the sample solution and Decloxizine Hydrochloride in the first dimension. Fig. (**4**) shows the chromatogram of impurity 1 in the sample solution and Decloxizine Hydrochloride in the second dimension. Fig. (**5**) shows the TIC scan of impurity 1 in the sample solution and Decloxizine Hydrochloride. It can be confirmed that impurity 1 came from Decloxizine Hydrochloride because, in LC chromatogram, the impurity 1 stayed for the same retention time, also mass data ion on $[M+H]^+$ of the impurity 1 in this drug and Decloxizine Hydrochloride were almost same.

Fig. (**6**) shows the chromatogram of the sample solution and Bromhexine hydrochloride in the first dimension. Fig. (**7**) shows the chromatogram of the sample solution and Bromhexine hydrochloride in the second dimension. Fig. (**8**) shows the TIC scan of impurity in sample solution and Bromhexine hydrochloride. It can be confirmed that impurity 2 in the sample solution came from Bromhexine hydrochloride because in LC chromatogram, the impurity 2 stayed for the same retention time, also mass data ion on $[M+H]^+$ of the impurity 2 in this drug and Bromhexine hydrochloride were almost same.

3.4. Structure Elucidation

Tables **2-4** shows ESI-MS^n mass data of Compound Clorprenaline and Bromhexine Capsules and their impurities in a sample solution in positive ion mode.

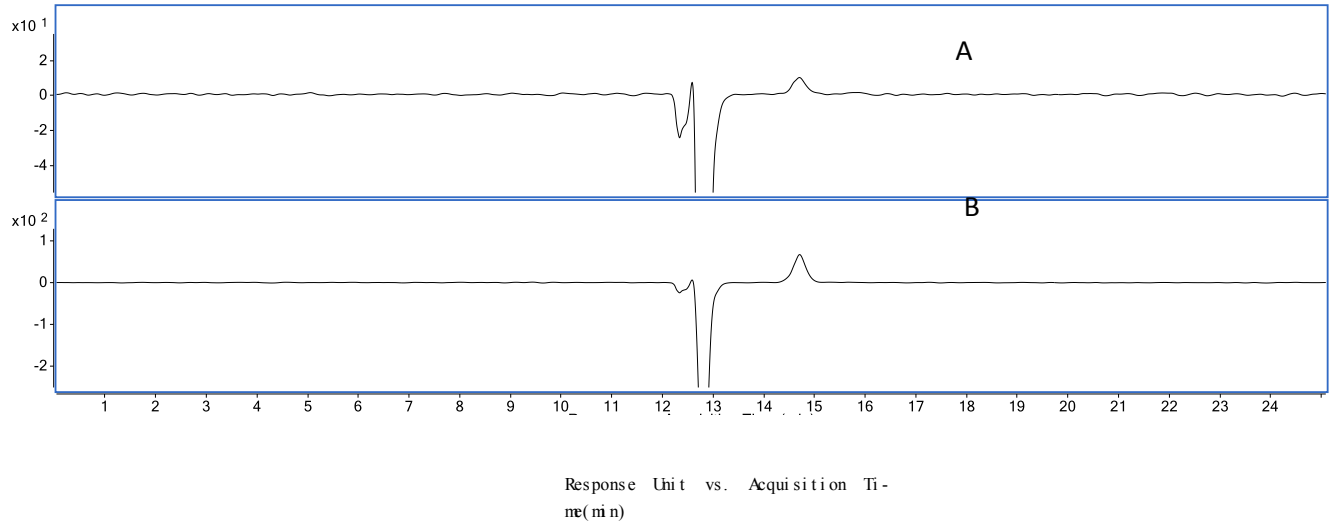


Fig. (4). The chromatogram of Compound Clorprenaline and Bromhexine Capsules and Decloxizine Hydrochloride in the second dimension. (**A**) Compound Clorprenaline and Bromhexine Capsules; (**B**) Decloxizine Hydrochloride.

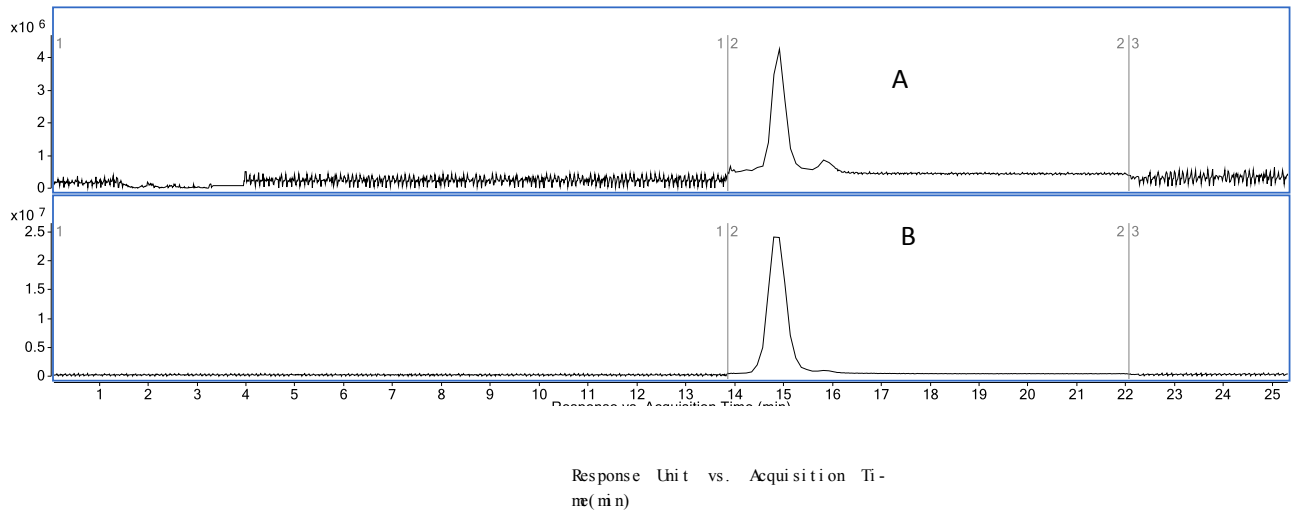


Fig. (5). The TIC scan of Compound Clorprenaline and Bromhexine Capsules and Decloxizine Hydrochloride. (**A**) Compound Clorprenaline and Bromhexine Capsules; (**B**) Decloxizine Hydrochloride.

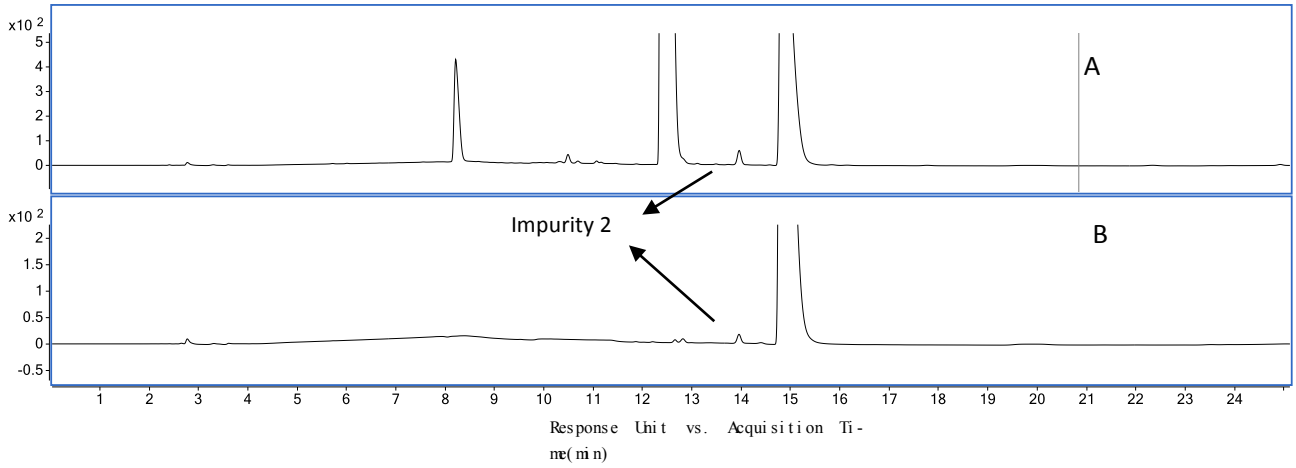


Fig. (6). The first-dimensional liquid chromatogram. (**A**) Sample solution; (**B**) Bromhexine Hydrochloride solution.

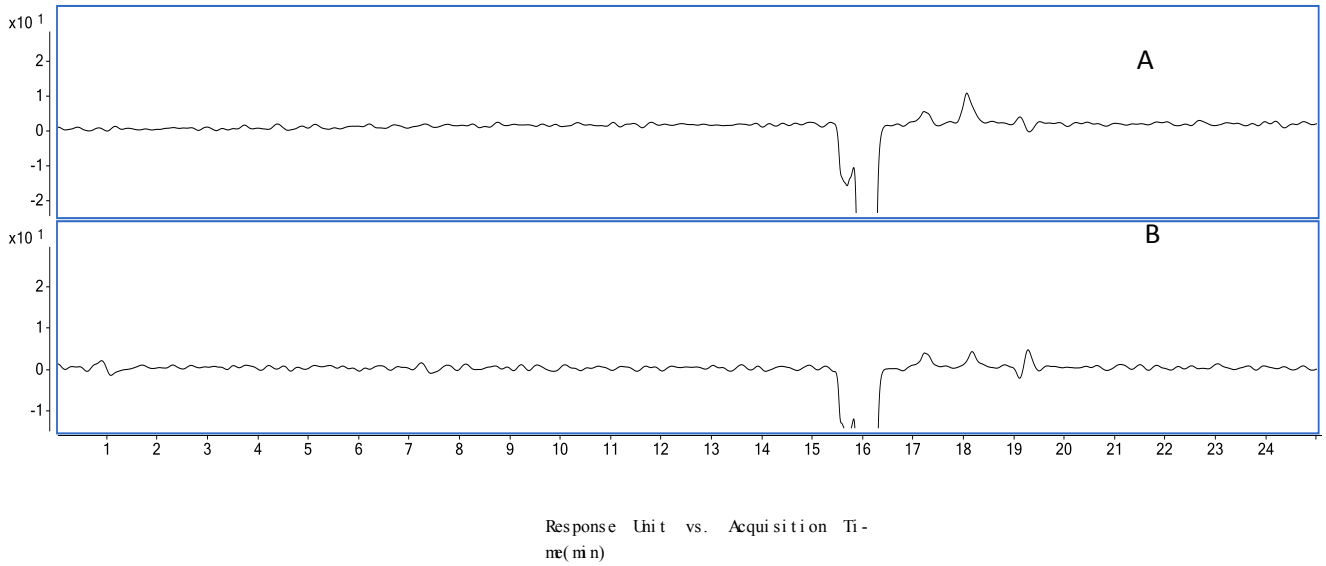


Fig. (7). The second-dimensional liquid chromatogram. (**A**) Sample solution; (**B**) Bromhexine Hydrochloride solution.

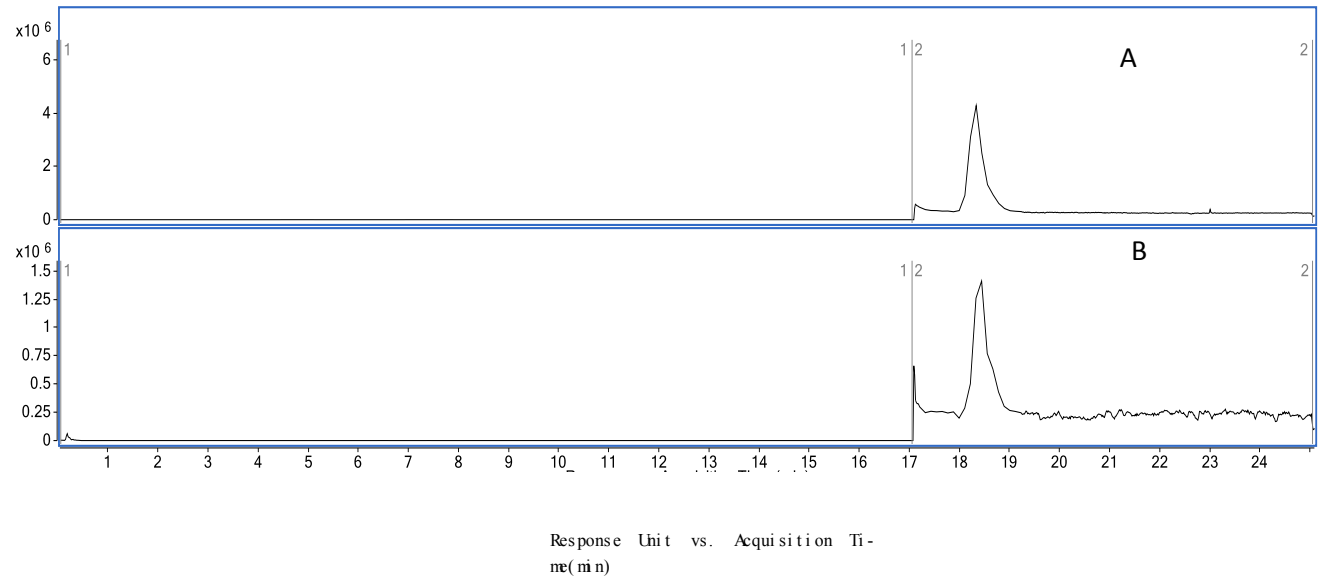


Fig. (8). TIC scan of degraded impurity. (**A**) In sample solution; (**B**) In Bromhexine Hydrochloride solution.

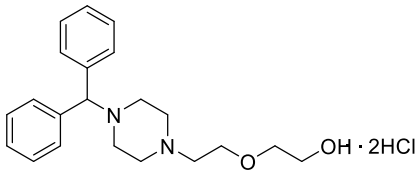
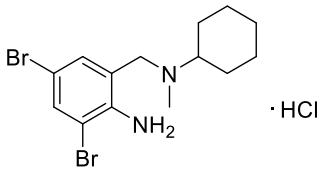
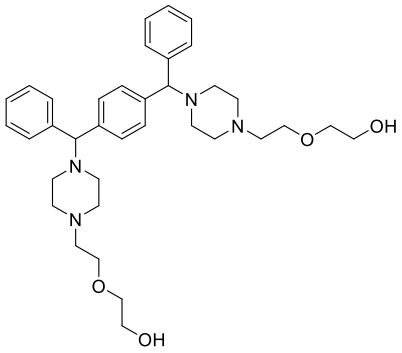
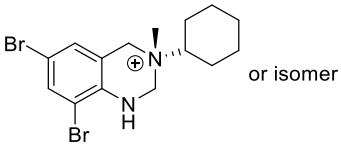
Table 2. ESI-MS exact and theoretical mass data for $[M+H]^+$ ions in positive ion modes.

Imp.	Formula	Observed $[M+H]^+$ (*m/z*)	Theoretical $[M+H]^+$ (*m/z*)	Deviation (ppm)
Decloxizine Hydrochloride	$C_{21}H_{28}N_2O_2$	341.2228	341.2224	-1.17
Bromhexine Hydrochloride	$C_8H_{10}N_4O_4S$	377.0049	377.0046	-0.80
Impurity 1	$C_{36}H_{50}N_4O_4$	603.3906	603.3905	0.17
Impurity 2	$C_8H_{10}N_4O_4S$	389.0032	389.0046	3.60

Table 3. ESI-MS and MS/MS exact mass data on major product ions in positive ion mode.

Imp.	$[M+H]^+$ (*m/z*)	MS/MS product ions (*m/z*)
Decloxizine Hydrochloride	341.2228	167.0853
Bromhexine Hydrochloride	377.0049	114.1280, 261.8860, 263.8836, 265.8818, 83.0852
Impurity 1	302.1987$[M+2H]^{2+}$, 603.3906$[M+H]^+$	429.2536, 296.1424, 173.1273
Impurity 2	387.0068, 389.0056, 391.0030	345.9598, 306.9246, 277.8988, 126.1272, 112.1125, 83.0849

Table 4. Structure of Decloxizine Hydrochloride and Bromhexine hydrochloride and proposed structures and sources of two impurities.

Imp.	Retention Time (min)	Impurity Source	Proposed Chemical Structure
Decloxizine Hydrochloride	12.399	Component	
Bromhexine hydrochloride	14.826	Component	
Impurity 1	10.493	From Decloxizine Hydrochloride	
Impurity 2	13.959	Degraded from Bromhexine hydrochloride	

3.5. Decloxizine Hydrochloride

Fig. (**9**) shows MS and MS/MS spectrum of Decloxizine Hydrochloride. The Decloxizine Hydrochloride $[M+H]^+$ precursor ion at m/z 341.2228 yielded the product ion at m/z 167.0853 by the breakdown of the C-N bond, which lost fragmentation at m/z 174 Da.

3.5.1. Impurity 1

The TOF high-resolution data confirmed that the elemental composition of impurity 1 was C36H50N4O4. The ESI-MS/MS spectrum of protonated $[M+H]^+$ ion of the impurity 1 displayed the product ions at m/z 429 by loss of fragmentation at m/z 174 Da (Fig. **10**), which displayed a similar fragmentation pattern as Decloxizine Hydrochloride. It can indicate that impurity 1 contained the same fragmentation structure as Decloxizine Hydrochloride. Besides, Fig. (**10**) shows that the MS spectrum of the impurity 1 displayed $[M+H]^+$ ion and [M+2H]2+ ion. However, the MS spectrum of Decloxizine Hydrochloride only displayed $[M+H]^+$ ion, which indicated that impurity 1 has two fragmentation structures as Decloxizine Hydrochloride.

Figs. (**11** and **12**) reveal the proposed pathways in the positive ionization of Dechlorohydroxyzine Hydrochloride and impurity 1 in detail.

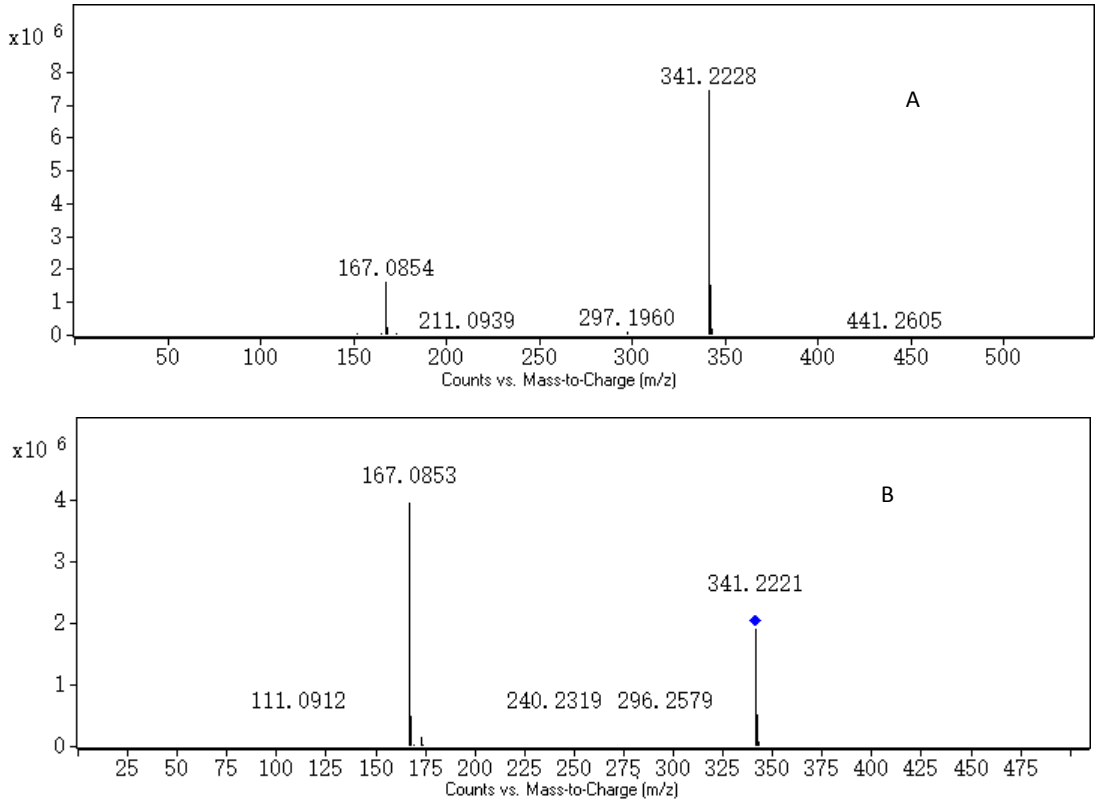


Fig. (9). MS and MS/MS of Decloxizine Hydrochloride. (**A**) MS spectrum; (**B**) MS/MS spectrum.

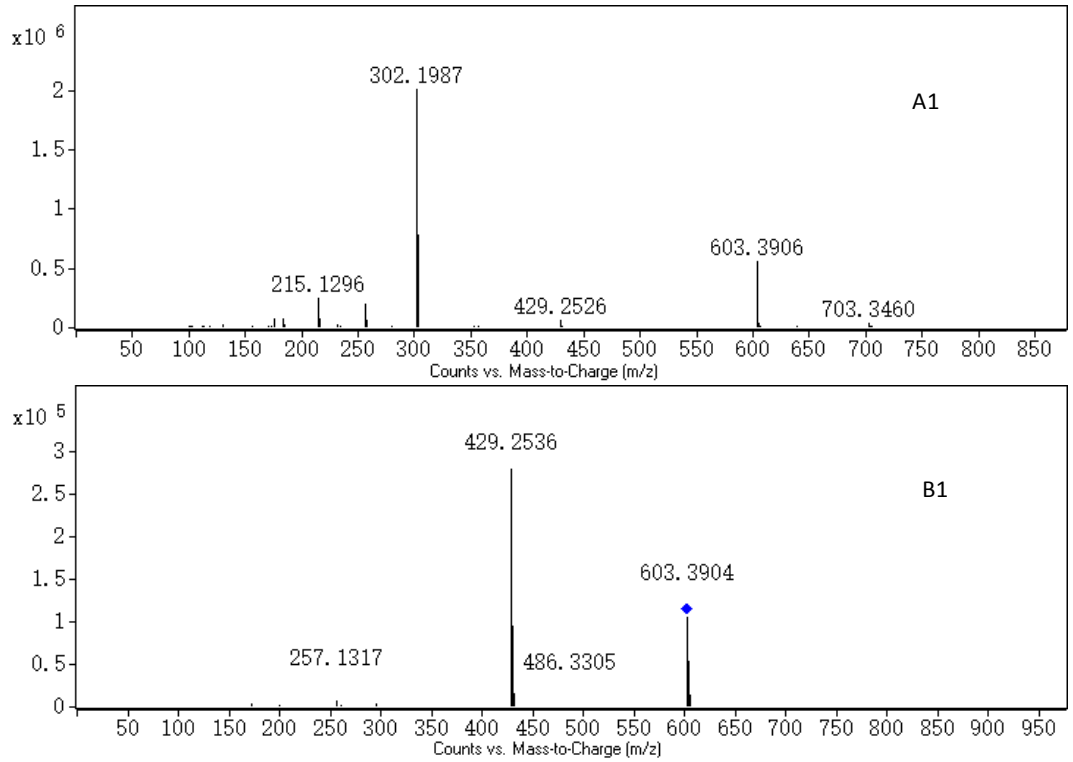


Fig. (10). Contd…

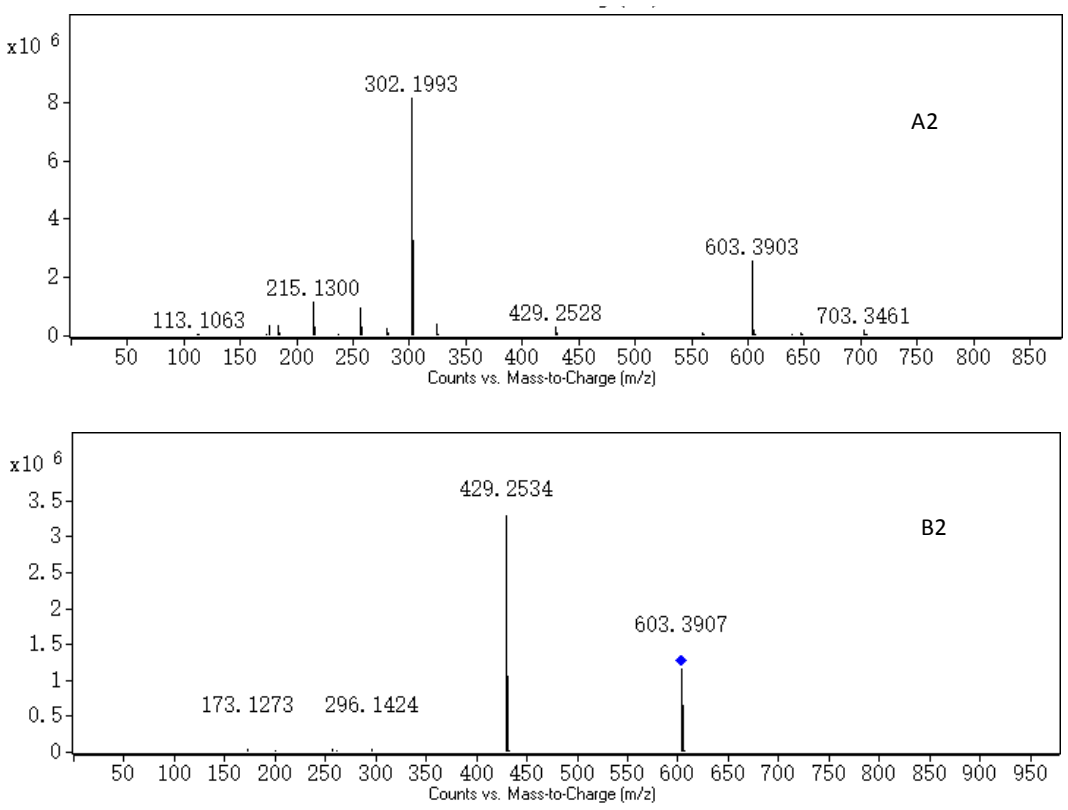


Fig. (10). The MS and MS/MS spectra of impurity 1. (**A1**) The MS spectrum of impurity 1 in sample solution; (**B1**) The MS/MS spectrum of impurity 1 in sample solution; (**A2**) The MS spectrum of impurity in Decloxizine Hydrochloride solution; (**B2**) The MS/MS spectrum of impurity in Decloxizine Hydrochloride solution

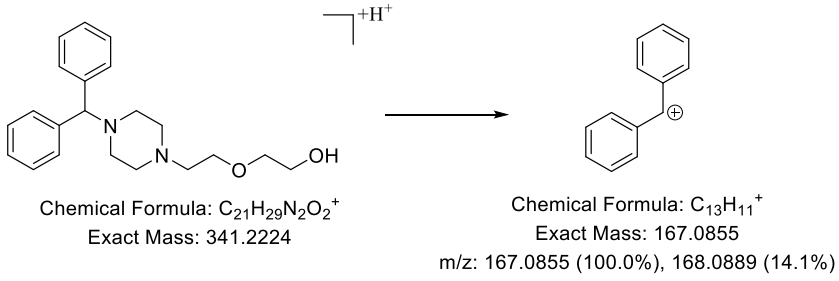


Fig. (11). The proposed fragmentation pathway of Decloxizine Hydrochloride.

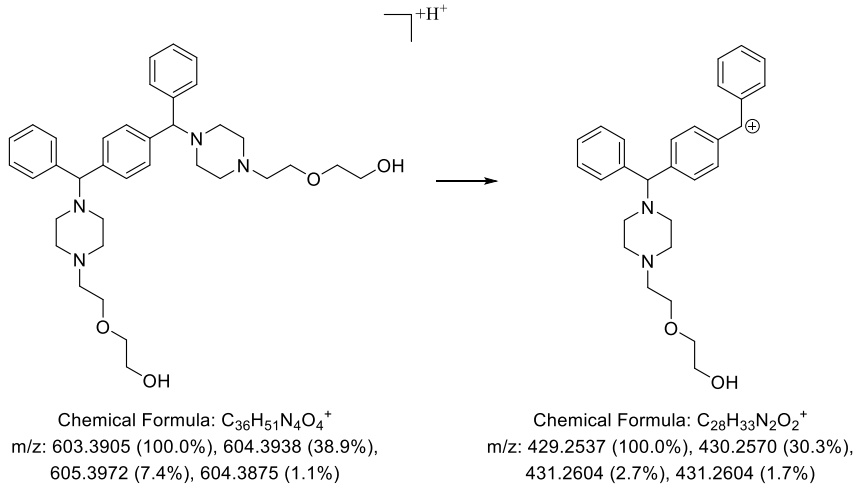


Fig. (12). The possilble fragmentation pathway of the impurity 1.

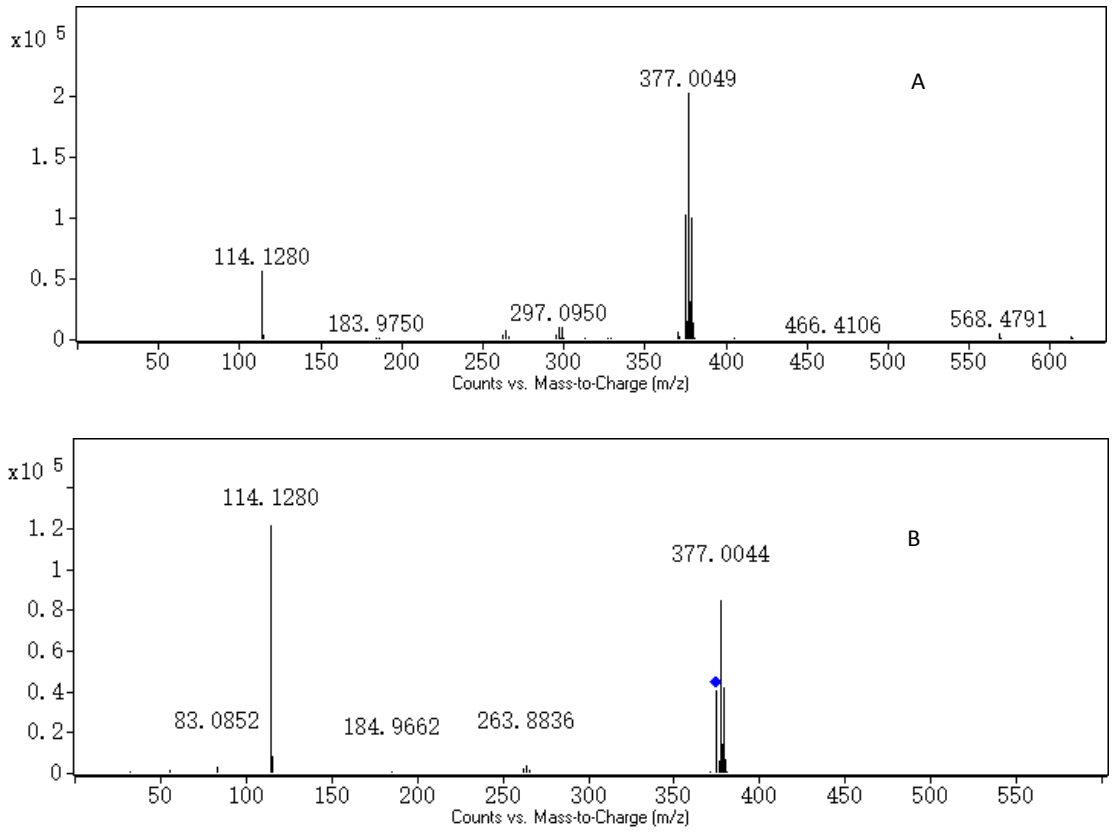
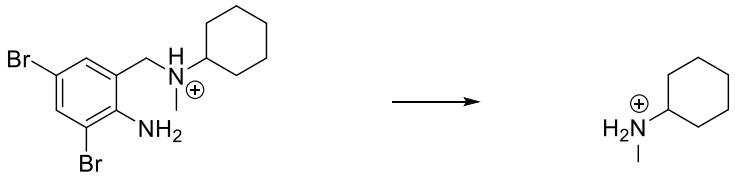


Fig. (13). The MS and MS/MS spectra of Bromhexine Hydrochloride (**A**) The MS spectrum; (**B**) The MS/MS spectrum.



Chemical Formula: $C_{14}H_{21}Br_2N_2^+$
m/z: 377.0046 (100.0%), 375.0066 (51.4%), 379.0025 (48.6%)

Chemical Formula: $C_7H_{16}N^+$
Exact Mass: 114.1277

Fig. (14). Possible fragment structure of Bromhexine Hydrochloride in positive ion mode.

3.6. Bromhexine Hydrochloride

Fig. (**13**) shows MS and MS/MS spectra of Bromhexine hydrochloride. Since two bromine atoms exist in the structure of bromhexine, the soft ionization process of Bromhexine hydrochloride in the ESI source produced the protonated ions $[M+H]^+$, $[M+H+2]^+$, and $[M+H+4]^+$, at the abundance ratio of 1:2:1, at m/z 375, 377, and 379, respectively. The $[M+H]^+$ precursor ion at m/z 377.0049 yielded the product ion at m/z 114.1280 by breakdown of C-N bond, along with mass at m/z 83 with low abundance. Fig. (**14**) illustrates the proposed chemical structures during fragmentation.

3.6.1. Impurity 2

The MS spectrum (Fig. **15**) showed that impurity 2 was produced as protonated ions $[M+H]^+$, $[M+H+2]^+$, and $[M+H+4]^+$, at the abundance ratio of 1:2:1, at m/z 387, 389, and 391, respectively. It behaved similarly to Bromhexine hydrochloride, so it can be inferred that impurity 2 has two bromine atoms. The TOF high-resolution data showed that the elemental composition of impurity 2 was $C_{15}H_{20}Br_2N_2$. The number of carbons in the molecular formula is one more than Bromhexine hydrochloride, and the elemental composition is $C_{14}H_{20}Br_2N_2$. The MS/MS spectrum of the precursor ion m/z 389 gave a fragment at m/z 83 with low abundance, m/z 126, m/z 112, the precursor ion m/z 389 also fragmented to a bunch of ions, at the abundance ratio of 1:2:1, at m/z 775, 277, and 279. And because the mass of $[M+H]^+$ ion m/z 389 was 12 Da more than $[M+H]^+$ ion m/z 377, similarly, the fragment ion m/z 126 was 12 Da more than m/z 114, also the impurity 2 originated from Bromhexine hydrochloride. Therefore, impurity 2 may involve a ring structure so that it can fragment to more fragmentation than Bromhexine hydrochloride. Fig. (**16**) illustrates the proposed chemical structures during fragmentation. Impurity 2 has the same structure as impurity E in EP 10.0 bromhexine hydrochloride quality standard.

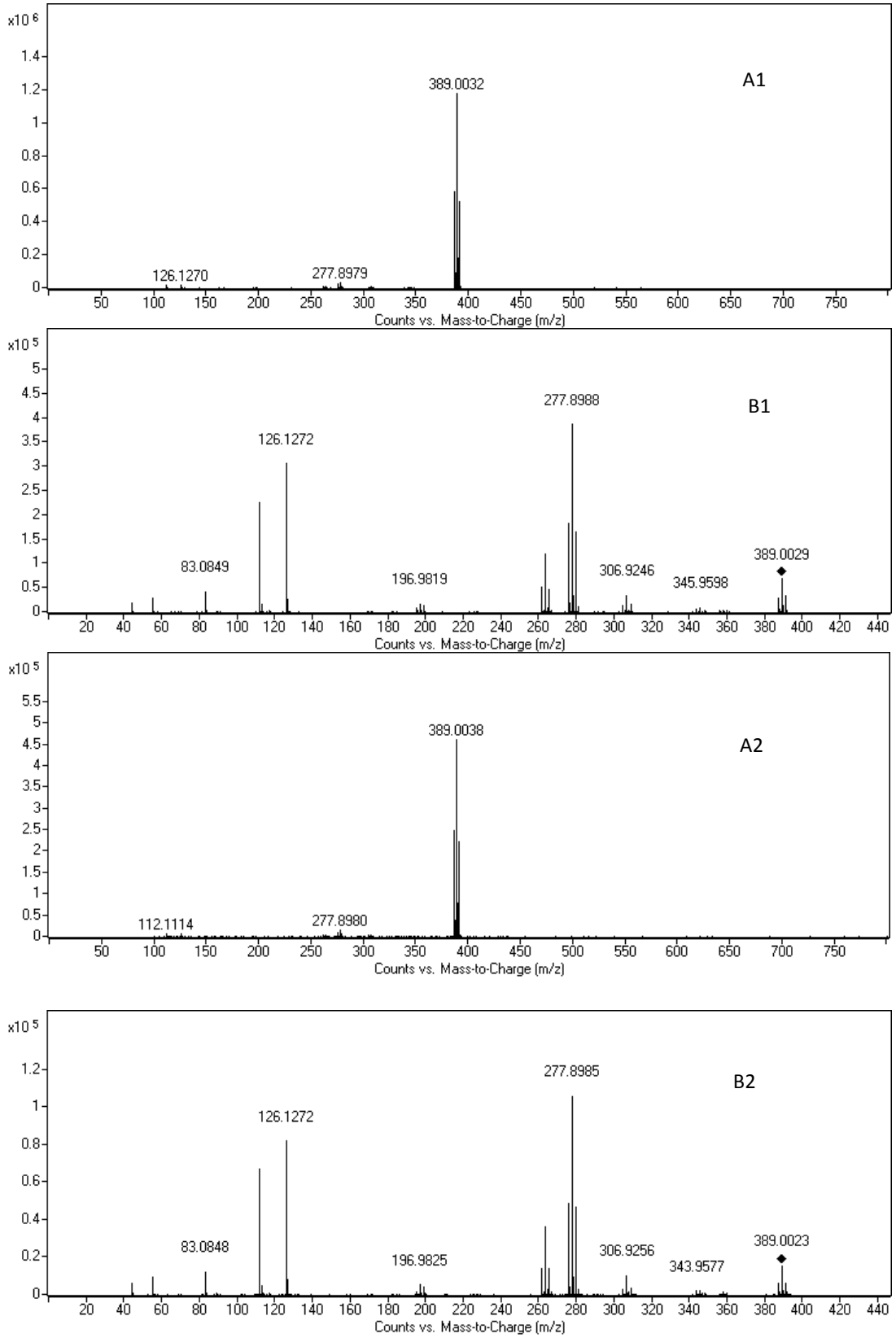


Fig. (15). The MS and MS/MS spectra of impurity 2. (**A1**) The MS spectrum of impurity 2 in sample solution; (**B1**) The MS/MS spectrum of impurity 2 in sample solution; (**A2**) The MS spectrum of impurity in Bromhexine Hydrochloride solution; (**B2**) The MS/MS spectrum of impurity in Bromhexine Hydrochloride solution.

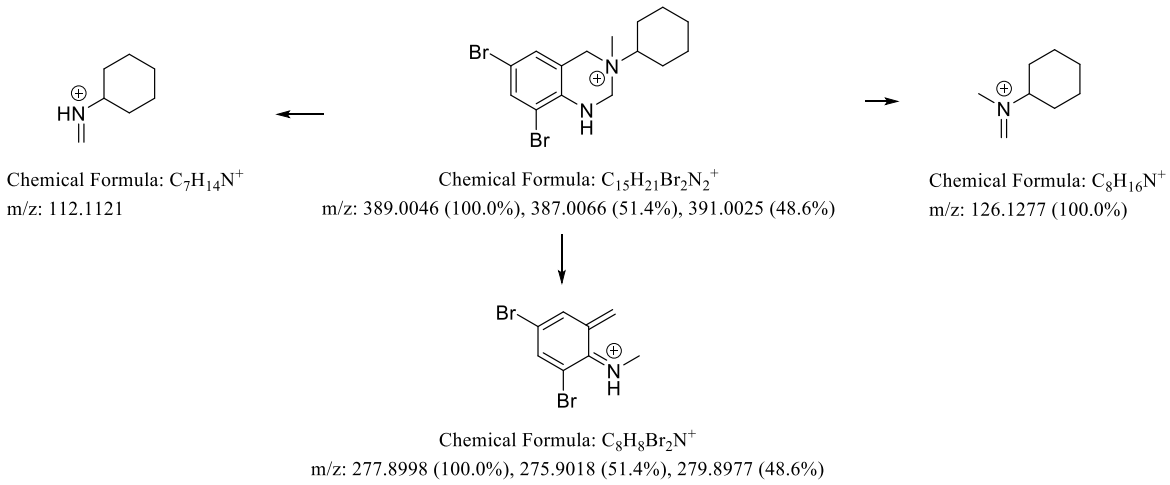


Fig. (16). Possible fragment structures of impurity 2 from Bromhexine Hydrochloride in positive ion mode.

CONCLUSION

For the quality control of multi-drug compound preparation, impurity is the focus and difficulty of research. There are many impurity types and sources. In addition to the impurities in each principal component, the chemical drug compound preparation may also contain degradation products produced by each principal component during storage or by interactions between principal components or between principal components and excipients. Due to the large number of impurities and the diversity of sources, the separation, qualitative and quantitative study of impurities are more difficult.

In this study, a novel HPLC method for the quantitative determination of impurities in Compound Clorprenaline and Bromhexine Capsules was established. The results of the study showed that the established method was stability-indicating and reliable. Moreover, unknown impurities in Compound Clorprenaline and Bromhexine Capsules were separated and characterized using 2D-LC-Q-TOF-MS in positive ESI mode. The structures of unknown impurities were characterized based on the MS/MS data. These results provide a sufficient basis for our subsequent study on the safety of Compound Clorprenaline and Bromhexine Capsules and also provide ideas for the impurity research of other compound preparations.

LIST OF ABBREVIATIONS

LoD = Limit of Detection

LoQ = Limit of Quantitation

DAD = Diode Array Detector

MSD = Mass Spectrometry Detector

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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