Printed in the Republic of Korea
ANALYTICAL SCIENCE & TECHNOLOGY
Vol. 33 No. 4, 169-176, 2020
https://doi.org/10.5806/AST.2020.33.4.169

Alternative chromatographic method for the assay test of terbutaline and salbutamol using ionic liquid assisted aqueous mobile phase

Xuan-Lan Mai[1], Yusung Choi[1], Quoc-Ky Truong[2], Thi-Ngoc-Van Nguyen[3], Sang Beom Han[4], and Kyeong Ho Kim[1,★]

[1]*College of Pharmacy, Kangwon National University, Chuncheon 24341, Korea*
[2]*Faculty of Pharmacy, University of Medicine Pham Ngoc Thach, Vietnam*
[3]*Faculty of Pharmacy, Can Tho University of Medicine and Pharmacy, Vietnam*
[4]*College of Pharmacy, Chung-Ang University, Seoul 06974, Korea*

(Received May 7, 2020; Revised July 17, 2020; Accepted July 20, 2020)

Abstract: Separation of basic compounds using reverse phase chromatography on a silica-based stationary phase represents a major challenge, because of the interaction between the cationic sites of the basic compounds with the anionic silanols of the stationary phase. This study presents a simple, reliable, and organic solvent - free liquid chromatographic method for the determination of terbutaline and salbutamol, in which a room temperature ionic liquid (RTIL) is used as mobile phase additive. We investigated various mobile phase parameters affecting the retention of the two compounds, such as types and concentration of RTILs and, pH of the mobile phase were investigated. The developed method was validated according to International Conference on Harmonization (ICH) guidelines and successfully applied effectively to determine salbutamol sulfate in pharmaceutical preparations.

Key words: ionic liquid, salbutamol, terbutaline, reverse phase HPLC

1. Introduction

Liquid chromatography (LC)-based researches generate about more than 34 million L of organic solvent waste annually.[1] To reduce the negative impacts of the organic solvent waste from laboratories to the environment, green chemistry studies have become trending in analytical sciences. The green analytical chemistry approaches include the miniaturization of the sample preparation techniques and determination devices, the application of solvent-less extraction techniques and the introduction of less toxic solvents.[2-6] Room temperature ionic liquids (RTILs) have been attracted widespread interest as "green" mobile phase additives in LC because RTILs are easily recyclable and produce less pollution; their negligible vapor pressure help avoiding the loss of solvent to the atmosphere and reducing potential exposure risks to workers.[7,8]

Basic compounds (pKa > 9) are difficult to retain in reverse phase liquid chromatography (RPLC) on silica-based stationary phases, because they are ionized

★ Corresponding author
Phone : +82-(0)33-250-6918 Fax : +82-(0)33-259-5631
E-mail : kyeong@kangwon.ac.kr

in the typical pH range (2-8) of the mobile phases used in RPLC. In this case, ion-pairing reagents are usually employed.[9] However, these chemicals can result in irreversible damage to the column performance such as changing in selectivity or longer equilibration times while organic solvents still necessary to satisfy chromatographic behaviors.[10] On the other hand, the remaining silanol groups of the stationary phase could also be involved in another mechanism. The interaction between the cationic sites of the basic compounds and the anionic silanols of the stationary phase produces peak tailing and long retention times.[7] In contrast to ion-pairing reagents, the use of RTILs with short alkyl chains does not affect the column performance. RTILs are also demonstrated to effectively improve the resolution and suppress tailing of analyte peaks.[11,12] Recently, thiamine, urazamide, and melamine were successfully determined in pharmaceutical preparations, using RPLC with a RTIL-modified mobile phase. [13-15]

Terbutaline (5-[2-(tert-butylamino)-1-hydroxyethyl] benzene-1,3-diol) and salbutamol (4-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl)phenol) (*Fig.* 1), are among the most common agents prescribed for the treatment of numerous respiratory disorders.[16] For the assay tests of these compounds, common mobile phase components are methanol (or acetonitrile) with buffer solution and ion pair solution (solution of sodium heptanesulfonate or hexanesulfonate).[17,18]

In this paper, we developed an RP-HPLC method for the simultaneous determination of two basic compounds, terbutaline and salbutamol, using a mobile phase modified with RTILs. The method does not involve modification with organic solvents such as methanol or acetonitrile, and thus meets the requirements of green chemistry. The present method was also compared with conventional methods for the determination of the two compounds, described in the USP 39 and BP 2013 specifications.

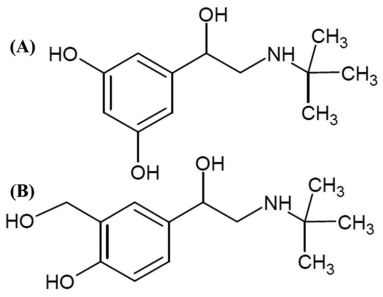

Fig. 1. Chemical structures of terbutaline (A) and salbutamol (B).

2. Experimental

2.1. Chemicals and reagents

Terbutaline sulfate and salbutamol sulfate were provided by Shinpoong Pharmaceutical Co., Ltd. (Ansan, Korea). The RTILs, i.e., 1-ethyl-3-methylimidazolium chloride ([EMIM][Cl]), 1-ethyl-3-methylimidazolium bromide ([EMIM][Br]), 1-ethyl-3-methylimidazolium hydrogen sulfate ([EMIM][HSO_4]), 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIM][BF_4]), 1-ethyl-3-methylimidazolium hexafluorophosphate ([EMIM][PF_6]), 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]), 1-hexyl-3-methylimidazolium hexafluorophosphate ([HMIM] [PF_6]), and 1-butylpyridinium hexafluorophosphate ([Bpy][PF_6]), were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetic acid, formic acid, and phosphoric acid (≥ 99.5 %) were purchased from Daejung (Siheung, Korea). Ammonium acetate (95.0-101.0 %) was purchased from Duksan (Ansan, Korea). Ammonium formate and potassium phosphate monobasic (≥ 95 %) were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). HPLC-grade methanol was obtained from Daejung (Siheung, Korea), whereas salbutamol sulfate tablets were purchased from Bukwang Pharmaceutical Co., Ltd. (Ansan, Korea). All other reagents were of analytical grade. Deionized water was prepared in our laboratory using an Aqua Max water purification system (Young Lin Instrument Co., Ltd., Anyang, Korea).

2.2. Instrumental conditions

The analytical chromatography experiments were performed on a 1100 HPLC system equipped with a G1379A degasser, a G1312 binary pump, a G1313 auto-sampler, a G1316 Colcom column oven, and a G1314AVWD detector (Agilent Technology, Santa

Clara, USA). An ultrasonic cleaner (60 Hz, Wiseclean, Seoul, Korea) was used for degassing the mobile phase. The pH of the buffer solution was determined with a SevenEasy pH meter (Mettler Toledo, Columbus, OH, USA).

Terbutaline and salbutamol were eluted on an Aegispak C8 column (150 × 4.6 mm, 5.0 μm, Seongnam, Korea) at 28 °C. The chromatographic separations were performed using a mobile phase consisting of 10 mmol/L potassium dihydrogen phosphate (pH 3.0, adjusted with phosphoric acid) and 40 mmol/L [EMIM][Br]. The flow rate and injection volume were 1.0 mL/min and 10 μL, respectively, whereas the detection wavelength was 276 nm.

The developed method was compared with the official analytical procedures described in USP 39 and BP 2013. Following the USP 39, terbutaline sulfate was determined by ion pair chromatography with a C18 column (150 × 4.6 mm, 5.0 μm). The mobile phase consisted of methanol and an ion pair solution of sodium 1-hexansulfonate and ammonium formate (23:77), previously adjusted to pH 3.0. The flow rate and injection volume were 1.0 mL/min and 20 μL, respectively, and the detection wavelength was 276 nm. Following the BP 2013, salbutamol was determined by ion pair chromatography with a C8 column (150 × 4.6 mm, 5.0 μm), with a mobile phase consisting of acetonitrile and an ion pair solution of sodium heptanesulfonate and potassium dihydrogen phosphate (22:78), previously adjusted to pH 3.65. The flow rate, injection volume, and detection wavelength were 1.0 mL/min, 20 μL, and 220 nm, respectively.

2.3. Sample Preparation

All stock solutions were prepared weekly. One hundred milligrams of terbutaline sulfate and salbutamol sulfate were dissolved in 20 mL of water to produce stock solutions with a concentration of 5000 μg mL^{-1} each. Working solutions were prepared daily by diluting appropriate aliquots of standard stock solutions with water.

2.4. Validation studies

The assay was validated for concentrations in the range of 50-2000 μg mL^{-1} for each analyte. The validation was conducted according to the International Conference on Harmonization (ICH, Harmonized Tripartite Guidelines 2005) guideline Q2 (R1), to assess the linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy, and robustness of the method.[19]

Linearity was constructed with six different concentrations: 50, 100, 200, 500, 1000, and 2000 μg mL^{-1}. Detection and quantification limits were based on signal-to-noise ratios of 3:1 and 10:1, respectively.

The intra-day precision was calculated from six replicate injections of three concentrations in the same day, whereas the inter-day precision and accuracy were estimated from three replicate injections of three concentrations on three consecutive days.

Recovery tests were performed by adding known amounts of standard solutions at low (80 % of the known amount), intermediate (same as the known amount), and high (120 % of the known amount) levels. The spiked samples were then extracted, processed, and quantified according to the methods discussed above. Three replicates were performed for each test.

2.5. Application of the method

Available commercial tablets containing 4.82 mg of salbutamol sulfate (equivalent to 4.0 mg of salbutamol) were used for testing the suitability of the proposed method. Twenty tablets were weighed and powdered; then, a quantity of powder containing the equivalent of 50 mg of salbutamol sulfate was transferred to a 50-mL volumetric flask, and 15 mL of ethanol was added to the flask. The mixture was shaken vigorously for 2 min, followed by addition of approximately 20 mL of water. The resulting mixture was then sonicated for 30 min to promote the dissolution of salbutamol sulfate. Water was added to the mark and 5 mL of the resulting solution was diluted to 10 mL with water, before filtering through a 0.45 μm membrane to obtain the sample ready for injection into the HPLC system.

3. Results and Discussion

3.1. Chromatographic procedures

Terbutaline and salbutamol are small-sized, organic, basic compounds (pKa values calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 are 9.11 ± 0.1 and 9.6 ± 0.1, respectively). Retention and peak shape issues are considered as common challenges in separation such analytes. Preliminary experiments showed that terbutaline and salbutamol were retained strongly on C8 and C18 stationary phases with acidic eluent (pH 3.0) without organic solvent: capacity factors (k') were more than 5.0 and 20.0, respectively. Band broadening and severe tailing (peak asymmetric factor As were more than 5.0 for both compounds) were observed in preliminary experiments (*Fig.* 2). Thus, in further experiments, various phase parameters affecting the retention of the two compounds were investigated.

3.1.1. Selection of HPLC columns

The two most commonly used stationary phases in reverse phase HPLC were tested in this study: an Aegispak C8 column (150 mm × 4.6 mm, 5 μm), an Aegispak C18 F column (150 mm × 4.6 mm, 5 μm) and Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 μm). The mobile phase included 30 mM [EMIM][Br] and was used equally. As C18 stationary phase has longer alkyl chains which provide greater hydrophobic interaction than C8 column, the retention of terbutaline and salbutamol were less favorable in C18 column: t_R more than 30 min while on C8 column, both compounds eluted less than 20 min. Therefore, C8 column was chosen for further experiments.

3.1.2. Effects of chaotrope type

Selecting a suitable RTIL is a major consideration on retention control of the analytes. Different RTILs were carefully studied: [EMIM][Cl], [EMIM][Br], [EMIM][HSO_4], [EMIM][BF_4], [EMIM][PF_6], [BMIM]

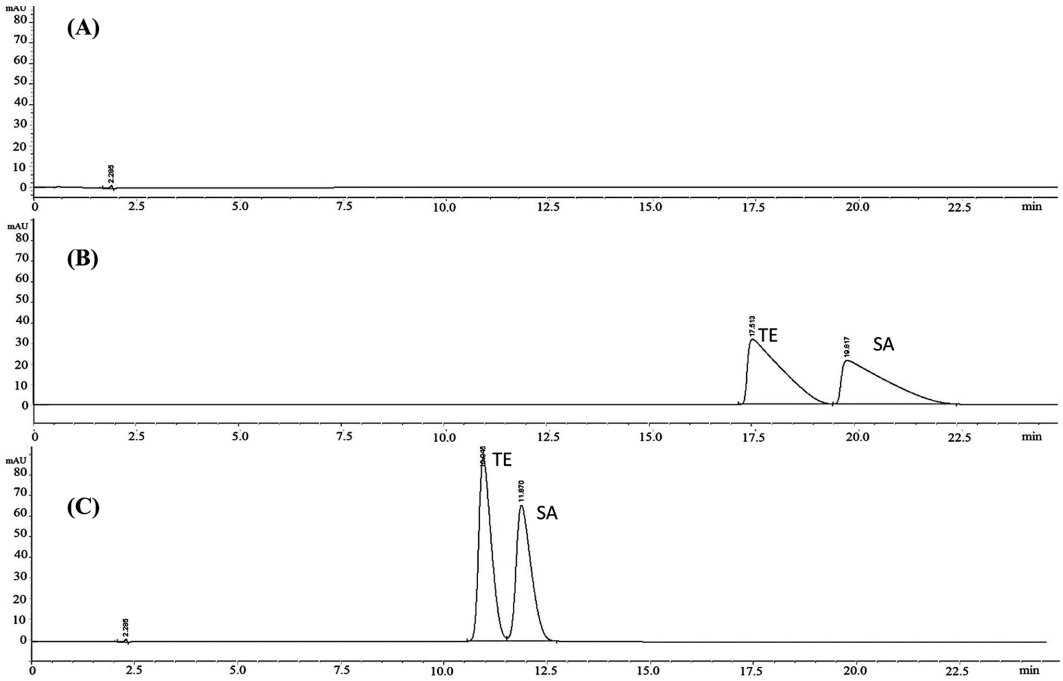

Fig. 2. Typical chromatograms of blank (A); standard mixtures eluted with mobile phase without the addition of RTIL (B) and with [EMIM][Br] (C). Condition: Aegispak C8 column (150 × 4.6 mm, 5.0 μm) 28 °C. Mobile phase consisted of 10 mmol/L potassium dihydrogen phosphate (pH 3.0 adjusted with phosphoric acid) and 40 mmol/L [EMIM][Br]. Flow rate was 1.0 mL/min, injection volume was 10 μL, and detection was at 276 nm. TE: Terbutaline; SA: Salbutamol.

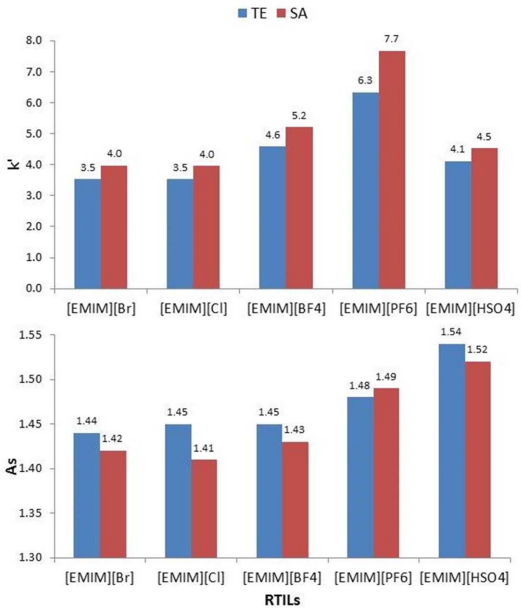

Fig. 3. Effects of different types of RTILs on capacity factor (k') and asymmetric factor (As).

$[PF_6]$, [HMIM]$[PF_6]$, and [Bpy]$[PF_6]$. However, when [BMIM]$[PF_6]$, [HMIM]$[PF_6]$, and [Bpy]$[PF_6]$ were used, a certain amount of organic solvent (methanol) had to be included in the mobile phase due to their low solubility. Because the main purpose of this study is to omit the use of organic solvents, these long-alkyl chain RTILs were not investigated any further. Varying chaotropic anion (HSO_4^-, Cl^-, Br^-, BF_4^- and PF_6^-), five [EMIM]-based RTILs were tested. *Fig.* 3 illustrates the effects of RTIL type on capacity factor and asymmetric factors of analytes. [EMIM][Br] resulted in the most suitable retention and peak shapes among the chaotropes investigated (Br^-, Cl^-, BF_4^-, PF_6^-, HSO_4^-), and was thus selected for the following tests.

3.1.3. Effects of RTIL concentration

The concentration of RTILs is able to affect the retention and separation of analytes. By varying the [EMIM][Br] concentration (0, 10, 20, 30, 40 and 50 mmol/L) in the mobile phase, the chromatographic performance of terbutaline and salbutamol were tested. As shown in *Fig.* 4 the addition of [EMIM][Br], even with a small concentration (10, 20 mmol/L) in mobile phase, significantly reduced the lengthy retention and peak tailing of the analytes in column. When the concentration of [EMIM][Br] increased from 20 to 50 mmol/L, retention times of the analytes slightly decreased, asymmetric factors were improved and number of theoretical plates were increased. The selectivity of the two compounds was less than 1.4 at a [EMIM][Br] concentration of 50 mmol/L, 40 mmol/L was chosen as the optimal concentration.

3.1.4. Effects of pH

Since the ionization stage of the analytes is greatly influenced by pH of aqueous mobile phase, the effect of buffer pH was also evaluated. The chromatographic behavior of terbutaline and salbutamol was examined at pH values using various pH values (2.5, 3.0, 3.5, 4.0, 4.5, 5.0). The pH adjustment was performed using a phosphate buffer at pH 2.5-3.5 and an ammonium acetate buffer at pH 4.0-5.0 by adding phosphoric acid and acetic acid, respectively. With an increase of the mobile phase pH, both retention time and resolution of analytes increased but there is no differences in peak symmetry. Considering between appropriate retention time and resolution of the analytes, the pH value of the mobile phase was adjusted to 3.0 in the following experiments.

3.2. Method Validation

3.2.1. Specificity & system suitability

The specificity was determined using the chromatographic results of the analytes. No interference was observed for the retention time of terbutaline and salbutamol when blank samples were analyzed (*Fig.* 2).

System suitability was tested by performing six replicate injections and determining retention times and peak area ratios, theoretical plate numbers (N), resolution (Rs), and symmetry factor (As) for the analytes of interest. The relative standard deviations (RSD) of these properties were used as indicators of system suitability. The retention time of terbutaline was about 10.53 min, that of salbutamol was 11.58 min. RSD of retention time of two compounds were less than 0.30 %. RSD of peak areas of two compounds were less than 0.20 %. The mean values of N for

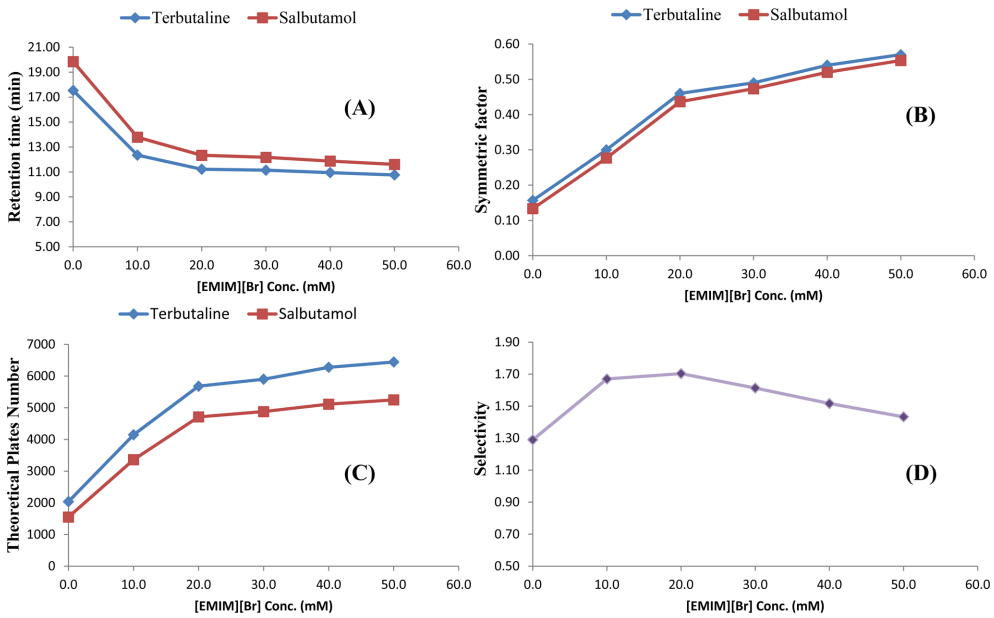

Fig. 4. Effects of [EMIM][Br] concentration on chromatographic behaviors of analytes.

Table 1. Results of linearity validation (n=6)

Parameter	Tebutaline	Salbutamol
Regression equation	y = 3.7271x + 46.370	y = 3.5460x – 42.936
Range (μg mL^{-1})	50 – 2000	50 – 2000
Coefficient of determination (r^2)	0.9997	0.9997
Number of data points	6	6
Slope ± SD	3.7271 ± 0.0074	3.5460 ± 0.0189
Intercept ± SD	46.37 ± 2.696	42.936 ± 2.417
LOD/LOQ (μg mL^{-1})	2/5	2/5

terbutaline, salbutamol were about 6000 and 5100 respectively. As value were 1.8 and 1.9, respectively.

3.2.2. Linearity

Calibration curves showed good linearity (correlation coefficient was 0.9997) in the concentration range 50-2000 μg mL^{-1}. The LOD and LOQ concentration were estimated to be 2.0 and 5.0 μg mL^{-1} for both analytes (*Table* 1).

3.2.3. Precision

The precision of method was evaluated using intra- and inter-day variations of three concentrations: 400, 500 and 600 μg mL^{-1}. The results of precision were reported in *Table* 2. Intra-day precision (RSD) ranged from 0.23 to 1.11 % with the accuracy from 97.53 to 100.58 %. Inter-day precision (RSD) ranged from 0.34 to 1.02% with the accuracy from 97.52 to 100.78 %.

Table 2. Results of precision validation of the method

Conc. (μg mL^{-1})	Precision (RSD %)			
	Intra-day (n=5)		Inter-day (n=11)	
	Terbutaline	Salbutamol	Terbutaline	Salbutamol
400	1.11	0.79	1.02	1.02
500	0.23	0.43	0.34	0.38
600	0.45	0.38	0.48	0.89

3.2.4. Recovery

The recovery (n = 3) was investigated by standard

Table 3. Results of recovery tests for commercial drugs (n=3)

Added conc. (μg mL^{-1})	Measured conc. (μg mL^{-1})	Recovery	
		Mean (%)	RSD (%)
400	404.43	101.11	1.302
500	503.08	100.62	0.672
600	603.44	100.57	0.058

addition method at three different levels (400, 500 and 600 μg mL^{-1}). The mean recovery was 100.57-101.11 % (*Table* 3). These results suggested that there was no interference from excipients in determining content of analytes in pharmaceutical preparations.

3.2.5. Robustness

Two factors (parameters) were selected from the analytical procedure to be examined in the robustness testing: Flow rate 1.0 ± 0.1 mL/min and pH 3.0 ± 0.2. In all the deliberate varied chromatographic conditions, all analyte peaks were adequately resolved and elution orders remained unchanged. Relative standard deviations of peak ratios were not more than 0.90 % indicated that these minor changes from the optimized conditions barely affected the peak area ratio of the studied analytes.

3.3. Application

To evaluate the applicability of the method, commercial salbutamol sulfate tablet sample was analyzed by the proposed method. The samples were prepared as mentioned in "Material and Method". The average content of salbutamol in the formulation was 97.4 %, RSD% was 0.62 %.

3.4. Comparison with current official analytical methods

The chromatographic method developed in this work was compared to the official methods described in the USP 39 and BP 2013 monographs. Both procedures used more than 20 % of organic solvent in mobile phase. Typical chromatograms are shown in *Fig.* 5. Compared with the present method, USP method is less selective for the determination of terbutaline and salbutamol as the two peaks were

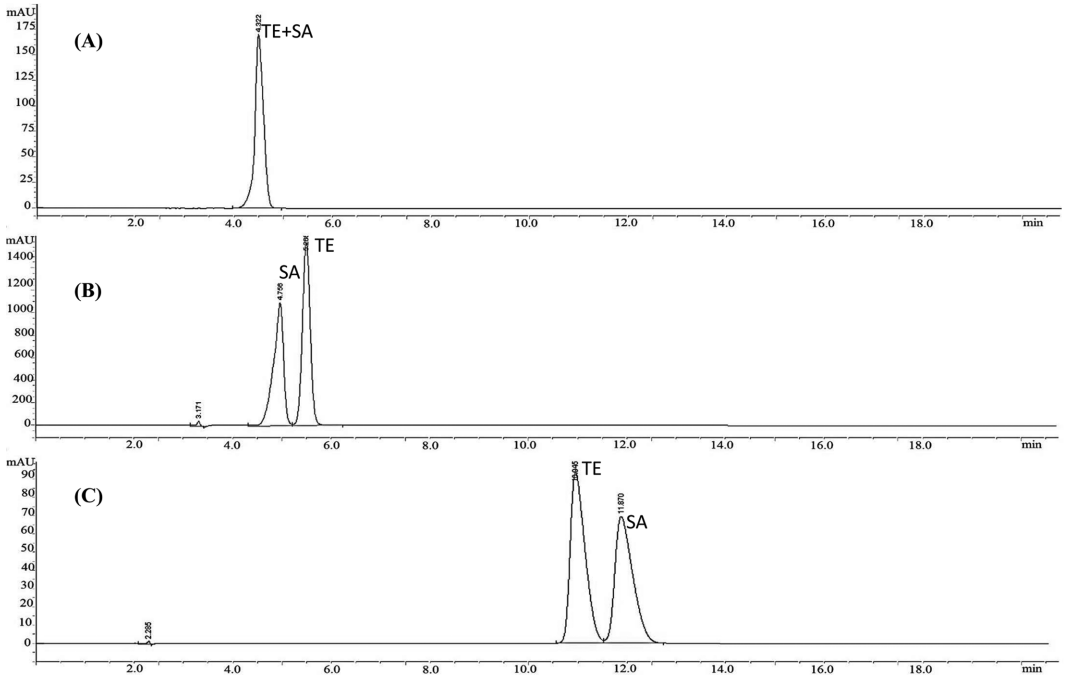

Fig. 5. Typical chromatograms obtained from USP 39 method (A); BP 2013 (B) and the proposed method (C). TE: Terbutaline; SA: Salbutamol.

identical, while a fronting peak was observed for salbutamol in the case of BP method.

4. Conclusions

The analysis of basic compounds, which account for over 70 % of pharmaceuticals, represents a long-standing challenge for RP separations.[20] In the present study, we described an effective RP-HPLC method using an aqueous mobile phase modified with RTILs for the simultaneous determination of two basic compounds, terbutaline and salbutamol. [EMIM][Br] was the most suitable mobile phase additive among the investigated RTILs. The influence of concentration of [EMIM][Br], mobile phase pH, and column temperature on the chromatographic behavior of the analytes was investigated in detail. The sensitivity, accuracy, and precision of the method were found to be satisfactory. The proposed method was successfully applied to quantify salbutamol sulfate in pharmaceutical preparations. In comparison with other official methods, this method was not only found to be less hazardous, as no organic solvents were used, but also turned out to be superior in terms of selectivity and peak shapes.

Acknowledgements

This research did not receive any specific grant from public, commercial, or non-profit funding agencies. The authors thank the Institute of New Drug Development Research and the Central Laboratory of Kangwon National University for the use of their analytical equipment.

References

1. Y. Gaber, U. Tornvall, M. A. Kumar, M. A. Amin and R. Hatti- Kaul, *Green Chem.,* **13**, 2021-2025 (2011).
2. A. Rios and M. Zougagh, *Trends Anal. Chem.,* **43**, 174-188 (2013).
3. F. T. G. van den Brink, W. Olthuis, A. van den Berg and M. Odijk, *Trends Anal. Chem.*, **70**, 40-49 (2015).
4. F. Pena-Pereira, I. Lavilla and C. Bendicho, *Trends Anal. Chem.*, **29**, 617-628 (2010).
5. H. M. Mohamed, *Trends Anal. Chem*., **66**, 176-192 (2015).
6. M. Tobiszewski, *Anal. Methods,* **8**, 2993-2999 (2016).
7. A. Berthod, M. J. Ruiz-Ángel and S. Carda-Broch, *J. Chromatogr. A,* **1184**, 6-18 (2008)
8. A. Romero, A. Santos, J. Tojo and A. Rodríguez, *J. Hazard. Mater.*, **151**, 268-273 (2008).
9. S. H. Hansen and S. Pedersen-Bjergaard, 'Bioanalysis of Pharmaceuticals: Sample Preparation, Separation Techniques, and Mass Spectrometry' Wiley, Chinchester, 2015.
10. J. W. Dolan, *LCGC Eur.*, **21**, 258-263 (2008).
11. L. He, W. Zhang, L. Zhao, X. Liu and S. Jiang, *J. Chromatogr. A*, **1007**, 39-45 (2003).
12. X. Xiaohua, Z. Liang, L. Xia and J. Shengxiang, *Anal. Chim. Acta.*, **519**, 207-211 (2004).
13. J. H. Suh, J. H. Kim, J. H. Jung, K. H. Kim, S. G. Lee, H. D. Cho, Y. Jung and S. B. Han, *Bull. Korean Chem. Soc.*, **34**,1745-1750 (2013).
14. N. Seo, Y. R. Lee, H. S. Park, Q. K. Truong, J. Y. Lee, H. K. Chung, Y. Choi, B. Kim, S. B. Han and K. H. Kim, *Arch. Pharm. Res.*, **40**(3), 364-372 (2017).
15. J. H. Suh, J. H. Jung, B. Kim, H. D. Cho, J. Kim, T. Eom, M. Park, Y. Wang and S. B. Han, *J. Chromatogr. A*, **1496**, 174-179 (2017).
16. D. P. Tashkin and L. M. Fabbri, *Respir. Res.*, **11**,149 (2010).
17. The United States Pharmacopeial Convention, 'The United States Pharmacopeia USP 39 NF 34', Rockville, 2016.
18. Medicines & Healthcare Products Regulatory Agency, 'British Pharmacopoeia', 7th Edition, London, 2013.
19. ICH, 'ICH Harmonised Tripartite Guideline Q2(R1): Validation of Analytical Procedures', ICH, Geneva, 2005.
20. D. V. McCalley, *J. Chromatogr. A*, **1217**, 858-80 (2010).

Authors' Positions

Xuan-Lan Mai : Researcher
Yusung Choi : Graduate student
Quoc-Ky Truong : Researcher
Thi-Ngoc-Van Nguyen : Associate Professor
Sang Beom Han : Professor
Kyeong Ho Kim : Professor