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Short communication

Detection of benzthiazide by high-performance liquid chromatography–thermospray mass spectrometry

Yunje Kim[a], Songja Park[a], Jongsei Park[a], Won Lee[b,*]

[a]*Doping Control Centre, Korea Institute of Science and Technology, P.O. Box 131, Seoul 130-650, South Korea*
[b]*Research Institute for Basic Sciences and Department of Chemistry, Kyung Hee University, Seoul 130-701, South Korea*

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Abstract

Benzthiazide, a banned drug in the Olympic Games, is difficult to confirm by GC–MS after methylation of urine extracts. Organic acids are added to the LC effluent to detect this drug by LC–thermospray (TSP) MS. An organic acid with a small pK_a value is useful for detecting benzthiazide in the positive-ion mode. In the negative-ion mode, the highest sensitivity for benzthiazide was obtained by using $HOCH_2COOH$. When LC–TSP-MS is used to detect benzthiazide, FCH_2COOH and $ClCH_2COOH$ are useful organic acids in the positive-ion mode and $HOCH_2COOH$, CH_3CH_2COOH and FCH_2COOH in the negative-ion mode. FCH_2COOH is useful in both the positive- and negative-ion modes. $ClCH_2COOH$ is a useful organic acid for detecting the molecular ion of benzthiazide.

1. Introduction

Benzthiazide (Fig. 1), a diuretic, has been included in the list of banned drugs since the 1988 Winter and Summer Olympic Games. This drug is misused to reduce the body mass and to increase the urine flow, leading to a decrease in the concentration of other doping agents [1]. This drug is currently subjected to a screening method using high-performance liquid chromatography with UV detection [2]. However, confirmation of positive cases could not be achieved by gas chromatography–mass spectrometry (GC–MS) after methylation of the urine extracts, as used to detect diuretics [3]. In this work, we tried to detect benzthiazide by liquid chromatography–mass spectrometry (LC–MS) using a commercial thermospray (TSP) interface.

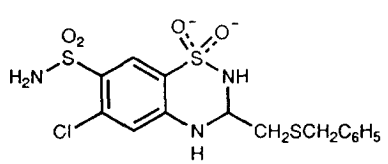

Fig. 1. Structure of benzthiazide.

When CH_3COONH_4 in the LC effluent (volatile buffer as an ionizing additive) was used to detect benzthiazide in the positive-ion mode of LC–TSP-MS, the drug could not be detected. We therefore tried to detect benzthiazide by using an LC effluent containing a different organic acid (FCH_2COOH, $ClCH_2COOH$, CH_3CH_2COOH, $HOCH_2COOH$ and SCH_2COOH), and the sensitivities of the drug to each of these acids in the LC effluent were compared.

* Corresponding author.

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2. Experimental

2.1. Chemicals

Water obtained with a Milli-Q water purification system (Millipore) was used throughout. Acids used in the mobile phase (Table 1) and methanol were of analytical-reagent grade from Merck (Darmstadt, Germany). Benzthiazide was obtained from Sigma (St. Louis, MO, USA).

2.2. Instrumentation

The HPLC system was Model HP 1090A (Hewlett-Packard, Palo Alto, CA, USA) equipped with an autoinjector for use in LC–MS. A Hewlett-Packard HP 5988A LC–TSP-MS system with an HP 9000-300 quadrupole mass spectrometer was used and a Model 7946 instrument was employed for data acquisition and processing.

2.3. Liquid chromatographic conditions

Solvent A consisted of 0.2 *M* of each carboxylic acid (HCOOH, CH_3CH_2COOH, $HOCH_2COOH$, $HSCH_2COOH$, $ClCH_2COOH$ or FCH_2COOH) and 0.2 *M* aqueous ammonia (1:1, v/v) and solvent B was methanol. The solvents were degassed and thoroughly purged with helium. The mobile phase composition was A–B (75:25), the flow-rate was 0.8 ml/min and the amount of benzthiazide injected was 2 μg.

2.4. Thermospray and mass spectrometric conditions

The TSP interface foreline pump maintained a pressure of 13.3–26.6 Pa in the cold trap, and two spectrometer diffusion pumps maintained a pressure of $2.66 \cdot 10^{-5}$–$5.32 \cdot 10^{-5}$ Pa in the manifold. The TSP temperatures were as follows: stem, 108–115°C (the stem temperature was determined by probe survey and was a temperature of 95% vaporization of mobile phase); tip, 180–205°C; and ion source, 276°C. In all the experiments the filament-on mode (ionization by an electron beam) was used. In this mode of operation, conventional negative-ion chemical ionization (NICI) can be carried out using the vaporized mobile phase as the CI reagent gas [4].

3. Results and discussion

In the negative-ion mode of the TSP buffer ionization process, it is assumed that the molecule studied by LC–MS contains hydrogen and is represented by HM; the anion of the additive, R^-, is the conjugate base of the organic acid in the LC effluent. When HM and R^- collide, there are two competing processes that can lead to products:

$$HM + R^- \rightleftharpoons HMR^- \quad (1)$$

$$HM + R^- \rightleftharpoons M^- + HR \quad (2)$$

Because Eq. 1 involves the formation of a hydrogen bond for almost all polar molecules,

Table 1
Physico-chemical properties of organic acids used in the mobile phase

Ionization solution	Molecular mass	Ion detection range (m/z)		pK_a	Boiling point (°C)
		Positive	Negative		
CH_3CH_2COOH	74.08	170–570	200–600	4.87	141
$HOCH_2COOH$	76.05	175–575	230–630	3.83	Decomposes
$HSCH_2COOH$	92.12	205–605	330–730	3.68	120
FCH_2COOH	78.04	170–570	240–640	2.95	168
$ClCH_2COOH$	94.05	235–635	270–670	2.92	189

this reaction is always favourable thermochemically [5]. The gas-phase acidity of HM may be compared with that of another acid, HR, whose conjugate base, R^-, competes with M^- for the proton in Eq. 2. The smaller the gas-phase acidity, the stronger is HM as an acid and the weaker is M^- as a base. Therefore, if the ionization process in TSP is in the gas phase, then the additive R^- will form HR from HM by proton abstraction when HR has a higher gas-phase acidity. In Fig. 2, all the spectra have an M^- ion, m/z 430, but do not have an HMR^- adduct ion. It is suggested that the gas-phase acidity of benzthiazide is lower than that of the organic acid. As a result, the proton affinity of benzthiazide must be weaker than that of the organic acid because this compound acts as a proton donor [6]. Therefore, if an organic acid with a higher pK_a value is used to detect benzthiazide, this drug is hardly detected by LC–TSP-MS. Fig. 3 shows that benzthiazide can be detected in the positive-ion mode by using

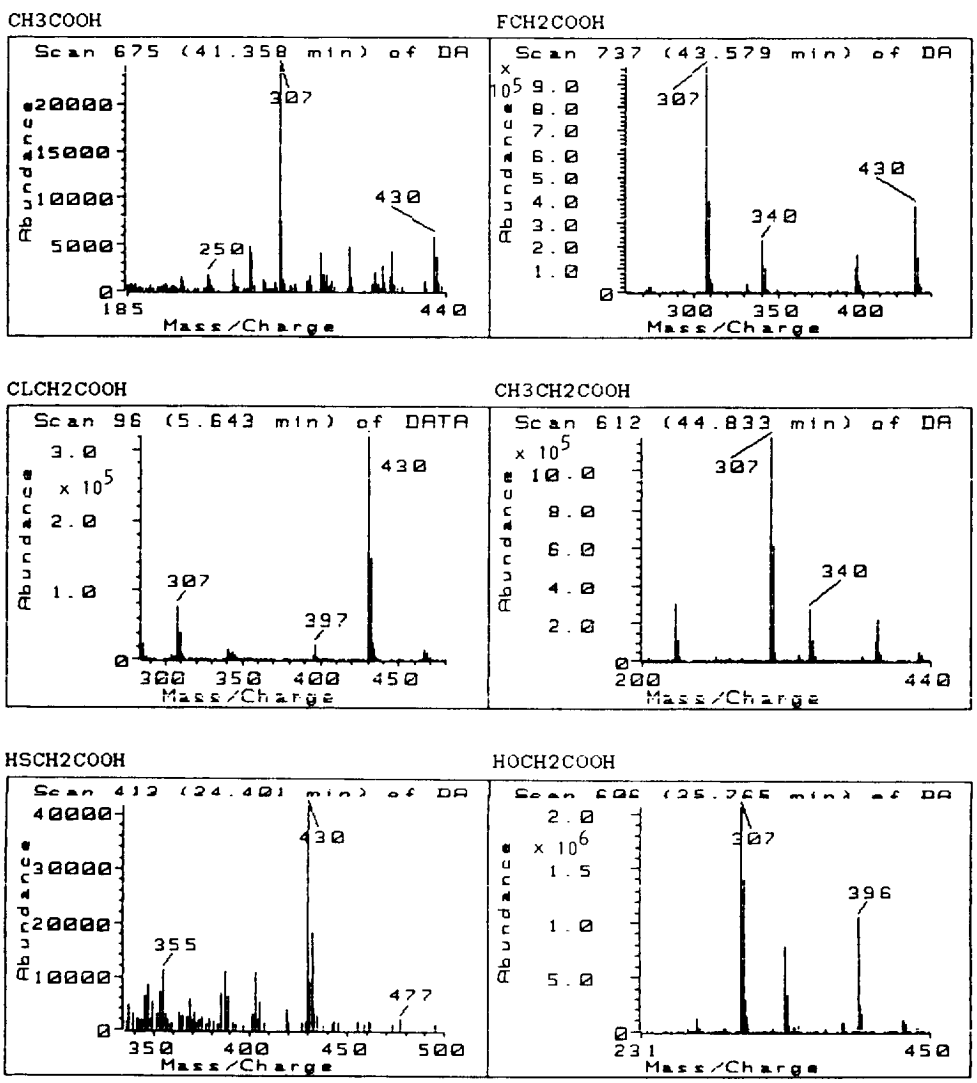

Fig. 2. Spectra of benzthiazide obtained with the use of different organic acids in LC–TSP-MS in the negative-ion mode.

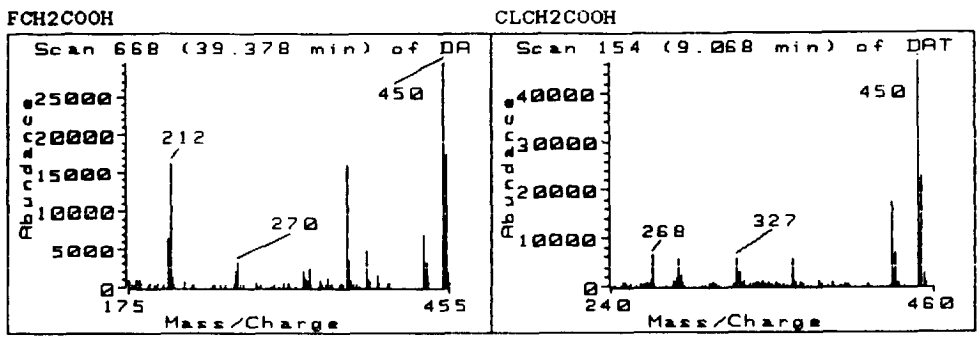

Fig. 3. Spectra of benzthiazide obtained with two organic acids in LC–TSP-MS in the positive-ion mode.

FCH_2COOH and $ClCH_2COOH$, which have smaller pK_a values than the other organic acids (see Table 1). We can predict that this drug will generate more $[MNH_4]^+$ adduct ion than $[MH]^+$ ion owing to smaller pK_a value and the proton affinities of FCH_2COOH and $ClCH_2COOH$, and the results show that the $[MNH_4]^+$ ion is the base peak.

When GC–MS was used to detect benzthiazide, it could not be detected by applying methylation to decrease the polarity. LC–MS in the positive-ion mode also could not detect this drug using CH_3COONH_4, which is generally used in LC–TSP-MS for the buffer ionization process. However, Table 2 shows that, in spite of using the positive-ion mode, the peak area of benzthiazide is $6.0 \cdot 10^4$ (FCH_2COOH) and $8.6 \cdot 10^4$ ($ClCH_2COOH$). In addition, in spite of using the positive-ion mode, the sensitivity of benzthiazide using these organic acids is higher than that in the negative-ion mode using CH_3COOH or $HSCH_2COOH$. Benzthiazide in the negative-ion mode shows the highest sensitivity when $HOCH_2COOH$ is used ($418.9 \cdot 10^4$). CH_3CH_2COOH ($201.7 \cdot 10^4$) and FCH_2COOH ($173.1 \cdot 10^4$) also give relatively high sensitivities. These three organic acids in the negative-ion mode give larger peak areas of benzthiazide than do the other acids. Therefore, if it is required to detect benzthiazide by LC–TSP-MS, FCH_2COOH or $ClCH_2COOH$ with a low pK_a value is useful in the positive-ion mode and $HOCH_2COOH$, CH_3CH_2COOH or FCH_2COOH is useful in the negative-ion mode. FCH_2COOH is a useful organic acid in both the positive- and negative-ion modes. The peak intensity of benzthiazide when using $ClCH_2COOH$ is lower than that with the other organic acids ($HOCH_2COOH$, CH_3CH_2COOH or FCH_2COOH), and the $[M]^-$ ion of benzthiazide is the base peak. This result is different from that with the fragment ion at m/z 307 as the base peak when the other organic acids are used. We assume that this is due to the boiling point of $ClCH_2COOH$ (189°C) being higher than those of the other acids (see Table 1); an organic acid with a high boiling point may remove fragment ions by pyrolysis because it is able to accommodate the input heat [7]. Hence $ClCH_2COOH$ is a useful acid for detecting the molecular ion of benzthiazide. When using FCH_2COOH and $ClCH_2COOH$, the sensitivity for benzthiazide was predicted to be higher than that with the other organic acids as in negative-ion chemical ionization (NICI) in GC–MS

Table 2
Comparison of sensitivity for benzthiazide with different organic acids in the positive- and negative-ion modes

Organic acid	Peak area ($\times 10^4$)	
	Positive-ion mode	Negative-ion mode
CH_3COOH	N.D.[a]	4.3
FCH_2COOH	6.0	173.1
$ClCH_2COOH$	8.6	43.9
$HOCH_2COOH$	N.D.	418.9
CH_3CH_2COOH	N.D.	201.7
$HSCH_2COOH$	N.D.	7.5

[a] N.D. = not detected.

because an element (F and CI, respectively) with high electronegativity is substituted. However, benzthiazide showed lower sensitivity when these organic acids were used than with the use of CH_3CH_2COOH and $HOCH_2COOH$ because it did not generate an $[HMR]^-$ adduct ion.

4. Conclusions

Because the gas-phase acidity of benzthiazide is high, an organic acid with a small pK_a value is useful for detecting this drug by LC–TSP-MS in the positive-ion mode. The base peak of benzthiazide is the $[MNH_4]^+$ adduct ion. When LC–TSP-MS in the negative-ion mode is used to detect benzthiazide, the highest sensitivity is obtained using $HOCH_2COOH$. In the positive-ion mode FCH_2COOH and $ClCH_2COOH$ are useful organic acids, and in the negative-ion mode $HOCH_2COOH$, CH_3CH_2COOH and FCH_2COOH are useful. FCH_2COOH is useful in both the positive- and negative-ion modes. If $ClCH_2COOH$, with a high boiling point, is used to detect benzthiazide in LC–TSP-MS, this acid is useful to detect the molecular ion of benzthiazide because the base peak of this drug is the $[M]^-$ ion.

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