Journal of Chromatography B, 879 (2011) 296–298

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Short communication

Determination of hippuric acid in human urine by ion chromatography with conductivity detection

Fuyong Zhao [a,b], Zonghua Wang [b], Hui Wang [a], Mingyu Ding [a,*]

[a] Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology (Ministry of Education), Department of Chemistry, Tsinghua University, Beijing 100084, PR China
[b] Laboratory of Fiber Materials and Modern Textile, the Growing Base for State Key Laboratory, Qingdao University, Shandong 266071, PR China

ARTICLE INFO

Article history:
Received 4 November 2010
Accepted 20 December 2010
Available online 28 December 2010

Keywords:
Ion chromatography
Hippuric acid
Urine

ABSTRACT

A simple, rapid, precise and eco-friendly ion chromatography (IC) method for the determination of hippuric acid (HA) in human urine was proposed in this paper. The separation was carried out an anion exchange column with 2.0 mmol L^{-1} Na_2CO_3 + 2.0 mmol L^{-1} $NaHCO_3$ as mobile phase at the flow-rate 0.7 mL min^{-1}. A suppressed conductivity detector was used and the detection limit was 1.0 μg L^{-1} (S/N = 3) for hippuric acid. The analysis time for one run was 30 min under the optimized IC condition. The recovery of hippuric acid was 93.2–98.0% while the relative standard deviation (RSD) was 1.4–2.3% by seven measurements.

1. Introduction

Toluene is a common organic solvent widely used in chemical industry. It has been reported that exposure to high concentrations of volatile organic compounds (VOCs) such as toluene will lead to a series of diseases such as acute and chronic respiratory effects, functional alterations of the central nervous system, mucous and dermal irritations, and chromosome aberrations [1–5]. Hippuric acid (HA) is one kind of metabolite of toluene in human body. Therefore, HA is a physiological component of human urine if toluene was inhaled. Also, the content of HA in human urine actually is mainly confirmed as a diagnostic marker of exposure to toluene [6–8]. In order to diagnose patients who are suffering from a series of diseases caused by elevated HA levels, the determination of HA in human urine is necessary.

The most common method used for the separation and determination of HA in urine is chromatography such as GC or GC–MS [9–11], HPLC or HPLC–MS [12,13] and CE [14]. Usually GC and GC–MS methods need a pre-column derivatization, and the derivatization operation is rather complicated and time consuming. Furthermore GC and GC–MS methods have a narrow linear range. HPLC and HPLC–MS methods often need to use organic solvents as mobile phase such as acetonitrile, methanol which are not eco-friendly and harmful to experimenter. CE method has a rather poor repeatability though it needs only a small amount of organic solvents as mobile phase.

A simple, precise and eco-friendly anion exchange chromatography (AEC) method for the determination of HA in human urine was proposed in this paper. The AEC method used only inorganic mobile phase which is eco-friendly. Also this AEC method had a high detection sensitivity which can compare beauty with HPLC–MS. Therefore this AEC method is superior to GC, GC–MS, HPLC, HPLC–MS and CE methods in a synthetical consideration of simplicity, running cost, repeatability and environment pollution. This AEC method is suitable for routine clinical analysis of HA.

2. Experimental

2.1. Apparatus

The ion chromatograph employed in this work was a Metrohm 850 Professional IC (Metrohm, Herisau, Swizerland). A Metrosep A5 150 anion exchange column (150 mm × 4.0 mm i.d., 5 μm particle size) was used for the separation of HA, and a suppressed conductivity detector (Metrohm, Herisau, Swizerland) with a 20 μL flow cell was used. An RP-18 guard column was fitted up-stream of the analytical column. A centrifugal machine was used to separate undissolved substances in urine samples.

2.2. Chemicals and reagents

Ultrapure water (18.2 MΩ) was produced by a Millipore water purification. All chemicals and solvents were analytical pure grade. Standard stock solution (1000 mg L^{-1}) of HA (Wako, Osaka, Japan) was prepared by dissolving HA in water adding 5 mL 1 mol L^{-1} NaOH. The solution was stored in colourless glass bottle and kept

* Corresponding author. Tel.: +86 10 62797087; fax: +86 10 62782485.
E-mail address: dingmy@mail.tsinghua.edu.cn (M. Ding).

1570-0232/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2010.12.015

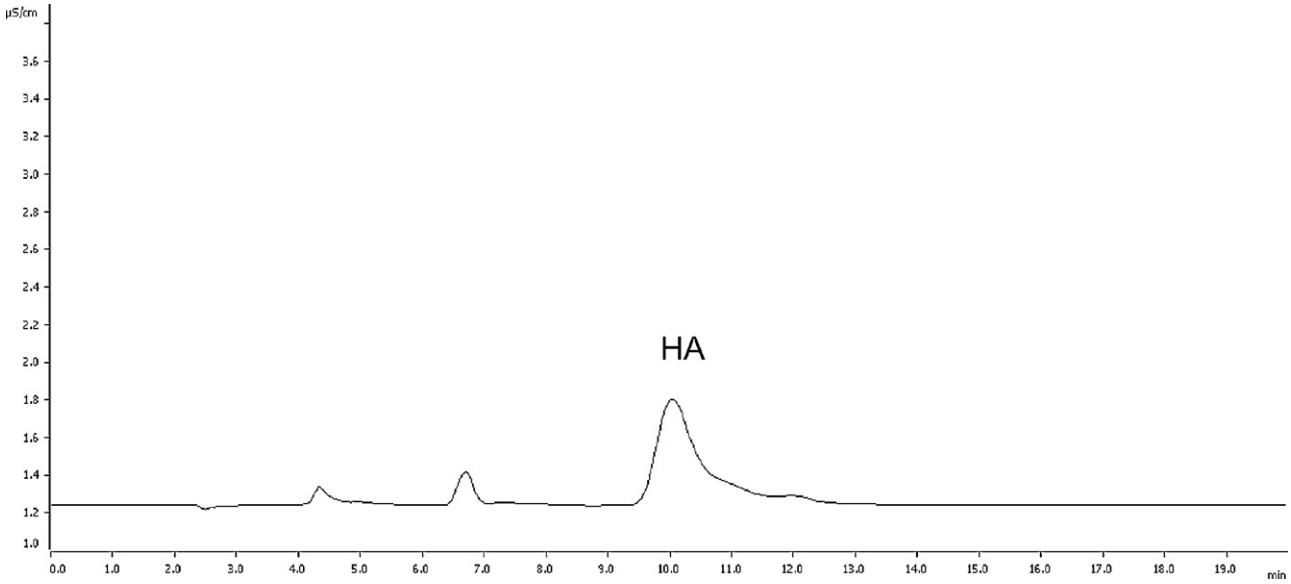

Fig. 1. Chromatogram of a standard solution of HA (10 mg L^{-1}).

at 4 °C. Fresh working solutions were prepared by an appropriate dilution of the stock solution before use.

2.3. Sample preparation

The urine samples were obtained from three healthy students in our lab (two males and one female). None of them suffered from systemic disease that could affect the urine. The samples were centrifuged for 10 min (4000 r/min). The supernatant was filtrated by a 0.22 μm infiltration head (MCM, Agela, Tianjin, China) and diluted by ultrapure water at 1:10 (v/v). Then take 2 mL diluted sample to pass through an actived C18 SPE column (Agela, Tianjin, China) in order to wipe off organic interfering components such as proteins, and 2 mL mobile phase (2 mmol L^{-1} Na_2CO_3 + 2 mmol L^{-1} $NaHCO_3$) was passed through the SPE column to elute the weakly retained compounds including a part of analyte (HA). The total collected solution (2 mL diluted sample + 2 mL mobile phase) was used for IC analysis.

2.4. Chromatographic conditions

An isocratic elution was adopted with 2 mmol L^{-1} Na_2CO_3 + 2 mmol L^{-1} $NaHCO_3$ as mobile phase at a flow-rate of 0.7 mL min^{-1}. A suppressed conductivity detector was employed. The total run time was 30 min. The column oven was maintained at 30 °C. Samples were injected using a 20 μL loop injector.

3. Results and discussion

3.1. IC performances and optimization

There is a carboxyl group in weak acidic HA molecule. HA could be existed mainly as HA anions in basic solution. The HA anions are retained on anion exchange column by ion exchange mechanism. The separation of HA from sample matrix was carried out on a Metrosep A5 150 anion exchange column with an isocratic elution. In order to establish an eco-friendly analytical method, the

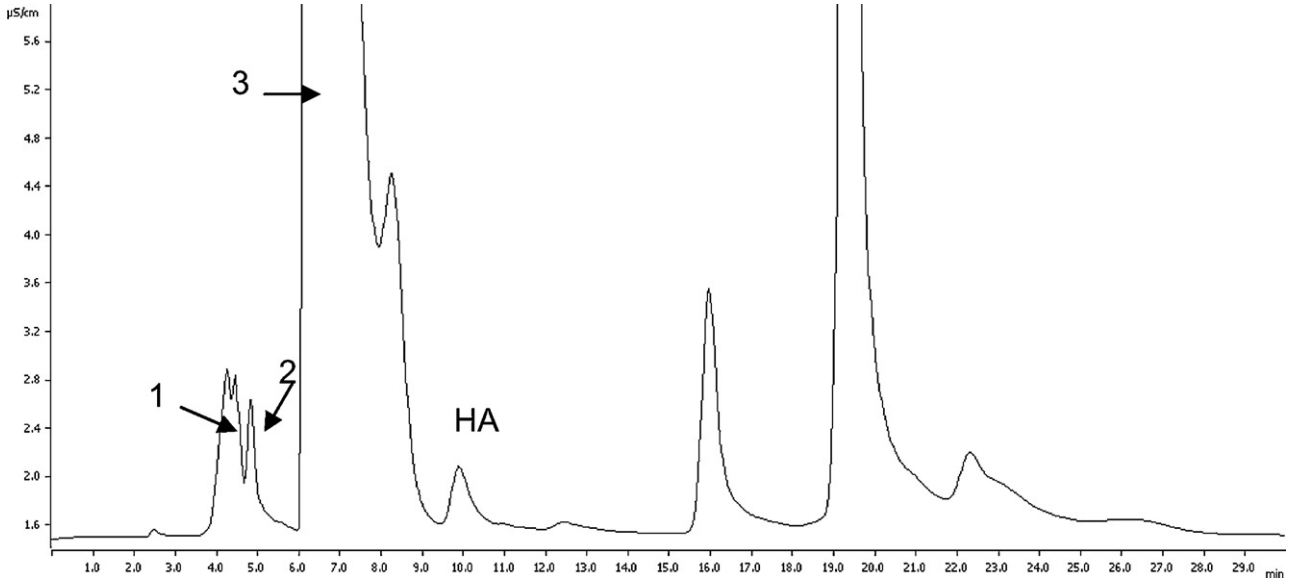

Fig. 2. Chromatogram of urine sample spiked HA, 1 – lactic acid; 2 – formic acid; 3 – chloridion.

Table 1
Reproducibility of the analytical method for HA ($n = 7$).

	1.0 mg L^{-1}		100 mg L^{-1}	
	Retention time (min)	Peak area (μS cm^{-1})	Retention time (min)	Peak area (μS cm^{-1})
Average	9.80	0.04	9.75	4.64
RSD%	0.08	0.71	0.08	0.05

Table 2
Recovery of different concentration of HA.

	Concentration added (mg L^{-1})	Concentration founded (mg L^{-1})	Recovery (%)	RSD (%)
HA	10	9.32	93.2	2.3
	20	19.3	96.5	1.9
	200	196	98.0	1.4

mobile phase should use no or low level of organic solvents. Often acetone or dipicolinic acid was used in the mobile phase for the separation of HA. In this work, the mixture of Na_2CO_3 and $NaHCO_3$ solution was selected as mobile phase to propose a completely eco-friendly method. A series of mobile phases with different ratios of Na_2CO_3 and $NaHCO_3$ were attempted keeping the total concentration of Na_2CO_3 and $NaHCO_3$ at 4 mmol L^{-1}. The results showed that the HA peak and other unidentified peaks were perfectly separated within 30 min when the mobile phase was 2 mmol L^{-1} Na_2CO_3 + 2 mmol L^{-1} $NaHCO_3$ with flow-rate 0.7 mL min^{-1}. The chromatogram of a 10 mg L^{-1} standard HA solution under the selected chromatographic conditions was shown in Fig. 1.

The coexisting organic compounds such as proteins in urine sample could be adsorbed onto the separation column to play down column performance and perhaps interfere the analysis of HA. A C18 SPE column was used to remove the coexisting components. Fig. 2 shows the chromatogram of a urine sample spiked with HA. As shown in Fig. 2, the HA peak (t_R = 9.80 min) and other peaks causing from anionic compounds were perfectly separated within 30 min. Since no interfered peaks appeared in the next determination, we could confirm that all the components were washed from the column in 30 min.

3.2. Calibration and reproducibility

The calibration graph was linear from 1.0 to 100 mg L^{-1} with a correlation coefficients of 0.9998 for HA. The linear equation is $y = 0.0466x - 0.0073$, here y is peak area (μS cm^{-1}) and x is concentration (mg L^{-1}) of HA. The detection limit of HA was 1.0 μg L^{-1} (S/N = 3). Two different concentrations (1.0 and 100 mg L^{-1}) of HA were injected seven times for testing the reproducibility of the offered method. As shown in Table 1, the relative standard deviations (RSD) of both retention time and peak area are very low. It indicates that the proposed method is stable and reliable.

3.3. Analysis of real samples and recovery

In order to assess the applicability of the proposed method in the determination of hippuric acid in real samples, three urine samples offered by three volunteers (two males and one female) were determined. HA was not detected in the three samples. At the same time, recovery test was made by adding HA to the real urine sample with different concentrations. Fig. 2 is the chromatogram of a real urine sample spiked HA. As shown in Fig. 2, the peak of HA was well separated from other peaks which from other anionic coexisting compounds such as lactic acid, formic acid and chloridion. Good recovery and RSD were obtained as shown in Table 2.

4. Conclusions

A simple and eco-friendly ion chromatographic method for the determination of HA in human urine was proposed in this work. Comparing with other chromatographic methods such as GC and HPLC, the proposed IC method used eco-friendly mobile phase (not containing organic solvent), and avoided complicated sample pretreatment. Furthermore the results shown that the proposed method has the advantages of easy operation, high sensitivity and accuracy. The method is suitable for routine clinical analyses of HA in human urine.

References

[1] M. Ogata, K. Tomokuni, Y. Takatsuka, Br. J. Ind. Med. 20 (1969) 330.
[2] M.D. King, R.E. Day, J.S. Oliver, M. Lush, J.M. Watson, Br. Med. J. Clin. Res. 283 (1981) 663.
[3] D. Otto, H. Hudnell, D. House, L. Mølhave, W. Counts, Arch. Environ. Health 47 (1992) 23.
[4] Y. Uchida, H. Nakatsuka, H. Ukai, T. Watanabe, Y.T. Liu, M.Y. Huang, Y.L. Wang, F.Z. Zhu, H. Yin, M. Ikeda, Int. Arch. Occup. Environ. Health 64 (1993) 597.
[5] Z. Chen, S.J. Liu, S.X. Cai, Y.M. Yao, H. Yin, H. Ukai, Y. Uchida, H. Nakatsuka, T. Watanabe, M. Ikeda, Occup. Environ. Med. 51 (1994) 47.
[6] S. Waidyanatha, N. Rothman, G. Li, M.T. Smith, S. Yin, S.M. Rappaport, Anal. Biochem. 327 (2004) 184.
[7] A.C. Schoots, J.B. Dijkstra, S.M. Ringoir, R. Vanholder, C.A. Cramers, Clin. Chem. 34 (1988) 1022.
[8] C.M. Jone, A.H. Wu, Clin. Chem. 34 (1988) 2596.
[9] P. Kongtip, J. Vararussami, V. Pruktharathikul, J. Chromatogr. B 751 (2001) 199.
[10] Y. Ohashi, T. Mamiya, K. Mitani, B.L. Wang, T. Takigawa, S. Kira, H. Kataoka, Anal. Chim. Acta 566 (2006) 167.
[11] D. de Carvalho, V.L. Lauchote, P.S. Bonato, C.R.H. Queiroz, A.C. Santos, S.A. Dreossi, Int. Arch. Occup. Environ. Health 63 (1991) 33.
[12] A.S. Mehanna, M. Dowling, J. Pharm. Biomed. Anal. 19 (1999) 967.
[13] N. Penner, R. Ramanathan, J. Zgoda-Pols, S. Chowdhury, J. Pharm. Biomed. Anal. 52 (2010) 534.
[14] C. Zuppi, D.V. Rossetti, A. Vitali, F. Vincenzoni, B. Giardina, M. Castagnola, I. Messana, J. Chromatogr. B 793 (2003) 223.