Caspian J. Chem. 2(2013) 59-64

Caspian Journal of Chemistry

http://caschemistry.journals.umz.ac.ir

Application of Ion Mobility Spectrometry for Determination of Morphine in Human Urine

Ali Sheibani*, M. Reza Shishehbore, Shohrah Vafaie-Shahi

Department of Chemistry, Yazd Branch, Islamic Azad University, Yazd, Iran

* Corresponding author: E-mail address: alisheibani@ymail.com

Received 30 January 2013|Received in revised form 19 April 2013|Accepted 4 May 2013

Abstract: In this study, a rapid, simple and sensitive ion mobility spectrometry (IMS) method with corona discharge as ionization source was described for the morphine determination in human urine. Morphine was extracted and purified from urine samples using solid phase extraction procedure with C_{18} column. It can offer the clean extracts which no extra peaks were observed in IMS. Under operating experimental conditions (Temperature; injection: 220 and oven: 180 ^{o}C, Flow rate; carrier: 300 and drift: 600 mL min^{-1}, Voltage; corona: 2200 and drift: 6700 V), developed method showed good linearity in the ranges of 0.44 to 6.91 and 6.91 to 22 ng mL^{-1} with correlation coefficients (R^2) of 0.9979 and 0.9966, respectively. The limit of detection was 0.1 ng mL^{-1}, and precision as relative standard deviation was 11%. The capability of the proposed method was evaluated by the analysis of human urine as a real sample that satisfactory results were obtained.

Key words: Ion mobility spectrometry (IMS), Morphine, Human urine, Corona discharge

1. Introduction

The use of human urine as a diagnostic tool in therapeutic drug monitoring, detection of compound abused as illicit drug and pharmacokinetic studies has been attended [1-3]. Morphine (5α,6α-didehydro-4,5-epoxy-17-methymorphinan-3,6-diol: Figure 1) is used to reduce severe pain in patients and for the relief of moderate cancer-related pain [4]. However, morphine can be toxic in excess and when abused. Therefore, the determination of morphine is an important subject. Different methods have been developed for the determination of morphine including chromatographic, capillary electrophoresis, sequential injection analysis and kinetic methods [5-8]. Ion mobility spectrometry (IMS) is an analytical technique with the major advantages such as low detection limit and fast response which used to determining of a broad range of compounds [9-11].

In 2001 Margaret et al. determined morphine in human urine without sample preparation using high-field asymmetric waveform ion mobility spectrometry (FAIMS) with mass spectrometric detection method [12].

In 2006 Khayamian et al. reported the quantitative analysis of morphine and noscapine using IMS in standard solutions [13]. In this work, the application of IMS method was developed for the determination of morphine in human urine as a real sample. Corona discharge in positive mode was used for ionization of morphine. The analytical parameters of the proposed method are comparable with to those of the other methods.

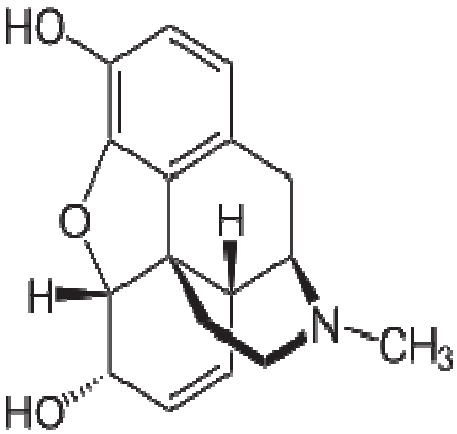

Figure 1. Chemical structure of morphine.

2. Experimental

2.1. Chemicals and materials

Morphine sulfate as ampoule (10 mg L^{-1}) was obtained from Temad Co. of IRAN. All solvents and materials were prepared by Merck (Darmstadt, Germany). Distilled water was used for the solution preparation. C_{18} columns (Supelco Inc., 100 mg) were purchased of Sigma-Aldrich (St. Louis, Mo, USA) for the extraction and clean up of morphine from urine samples. Human urine samples were obtained from healthy volunteers and stored at 4 ^{o}C until analysis.

2.2. Instrumentation

The ion mobility spectrometer used in this study was constructed at Isfahan University of Technology (Isfahan, IRAN). The instrument was equipped with a corona discharge ionization source. The operating conditions of IMS (voltage, flow rate, temperature and pulse width) for obtaining the ion mobility spectrum of morphine are given in Table 1.

2.3. Extraction procedure

The extraction and clean up of morphine from human urine samples were performed by solid phase extraction technique according to the Ref. [8].

20 mL of urine sample was diluted with 10 mL of water, and then 5 mL of it centrifuged at 14000 rpm for 5 min. 0.2 mL of supernatant was mixed with 0.2 mL of water and 2 mL of ammonium carbonate buffer (0.01 M, pH=9.3), passed through the C_{18} column that conditioned with 1 mL methanol, 1 mL water and 2 mL ammonium carbonate. The column was washed with 1 mL of water and 2 mL of ammonium carbonate. Finally, the analyte was eluted with 1 mL methanol in flow rate 1 mL min^{-1}. One microliter of it was injected into the IMS.

Table 1: The operating experimental conditions for determination of morphine.

Parameter	Setting
Drift voltage	6500 (V)
Corona voltage	2200 (V)
Flow of drift gas (N_2)	600 (mL min^{-1})
Flow of carrier gas (N_2)	300 (mL min^{-1})
Injection port temperature	220 ($^{\circ}$C)
IMS cell temperature	180 ($^{\circ}$C)
Pulse width	100 (µs)

3. Results and discussion

3.1. Ion mobility spectrum of the extracted morphine from human urine

Figure 2 shows the ion mobility spectra of drug free urine sample spiked with morphine before (lower) and after (upper) extraction based on the mentioned procedure. The spectra were obtained in the optimum conditions reported in Table 1.

According to this Figure, C_{18} column can provide the clean extracts which no extra peaks were observed in the region where the analyte peak appeared. The morphine spectrum showed two ion peaks that the shorter peak was disappeared rapidly. The higher peak at about 10.3 ms was stable that used for analysis. These peaks might be produced from addition or separation proton and H_2O to morphine molecules. This behavior has also been reported in Ref. [13]. Coupling of IMS to a mass spectrometer must be used for the characterization of the chemical formula of the product ions.

3.2. Optimization of temperature

The operating parameters of IMS such as voltage, flow rate, and pulse width were fixed at constant values (Table 1). Among, temperature is an important parameter that should be optimized.

The effect of the injection port temperature on the determination of morphine was studied in the range of 160 to 220 ^{o}C, shown in Figure 3.

According to this Figure, increasing temperature up to 220 ^{o}C caused an increase in the signal intensity, and at higher values it is constant. Therefore, 220 ^{o}C was selected as the optimum injection temperature. Under the optimum injection port temperature (220 ^{o}C), the IMS cell temperature (oven) was changed in the range of 140 to 200 ^{o}C (Figure 3). At 180 to 200 ^{o}C, the signal intensity is highest; therefore 180 ^{o}C was selected as the best temperature.

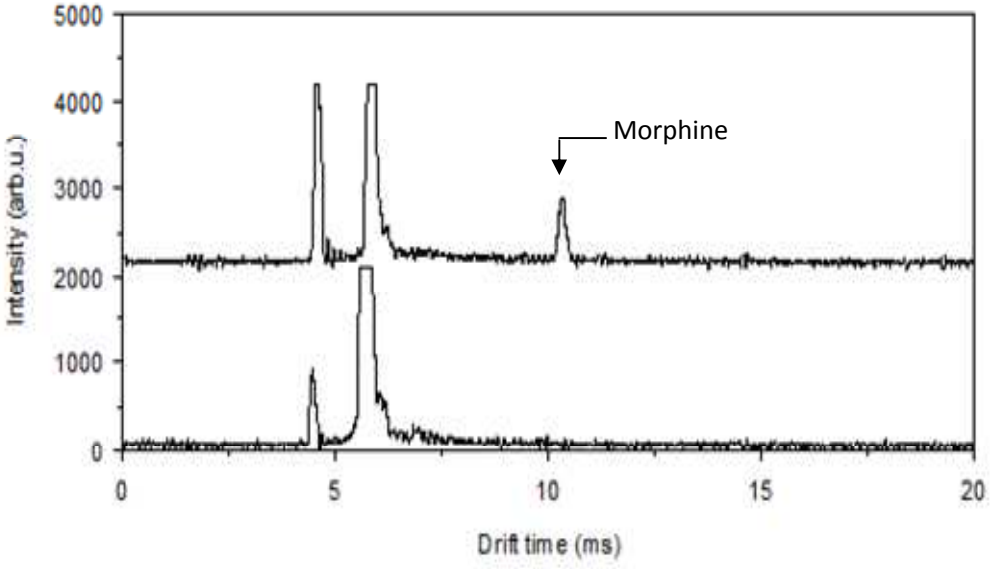

Figure 2. Ion mobility spectra of urine sample spiked with morphine after extraction using C18 (upper) and without it (lower).

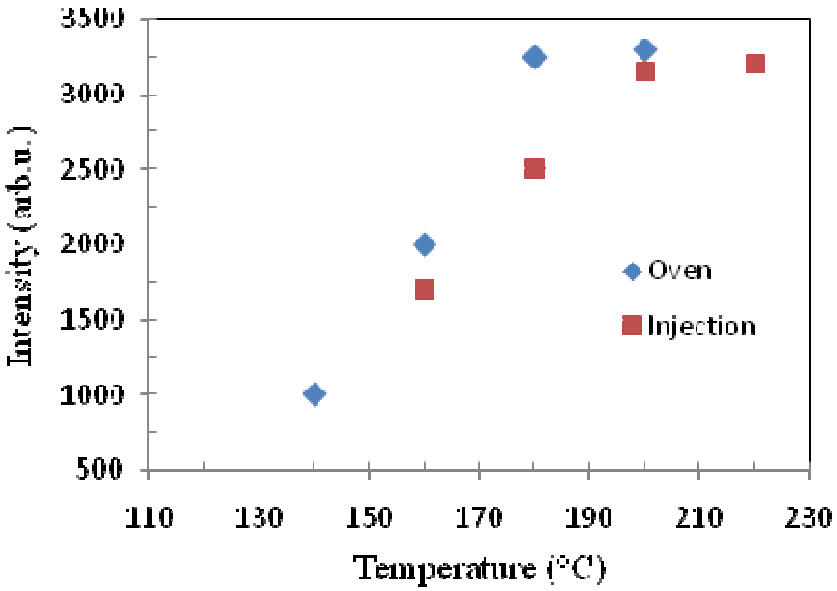

Figure 3. Effect of the injection and oven temperatures on the signal intensity.

3.3. Quantitative analysis

Under optimum conditions**,** the linearity (LDR), limit of detection (LOD: $3s_b/m$), and precision (RSD) were investigated and reported. For obtaining the calibration curve, standard samples were prepared by spiking the 1 mL blank human urine samples with known morphine concentrations. The proposed method was linear with two segments over the range 0.44 to 6.91 and 6.91 to 22 ng mL^{-1}. The regression equations were Y = 0.41 C + 0.14 and Y = 0.02 C + 0.26 with correlation coefficients (R^2) of 0.9979 and 0.9966, respectively, where Y is the instrument response (sum of the total peaks height) and C is morphine concentration (ng mL^{-1}). The wide linear range of this method covers the cut off concentration for morphine in human urine. The LOD based on the first part of calibration curve and RSD were 0.1 ng mL^{-1} and 11%, respectively. The analytical parameters of the proposed method are compared to those of other method for the determination of morphine, given in Table 2. With regard to this Table, the LDR (two linear ranges), LOD (the first part of calibration curve), and RSD of the proposed method are better or comparable with them. Furthermore, the simplicity, fast response and low cost are other advantages for the developed method.

Table 2: Comparison of the LDR, LOD and RSD% in different methods for determination of morphine.

Method	LDR (ng mL^{-1})	LOD (ng mL^{-1})	RSD%	Sample	Ref.
Kinetic	70-7980	30	0.6	Urine	8
Voltammetry	10-3100	3	1.1	Plasma	14
FAIMS-MS	6.5-600	0.6	3	Urine	12
HPLC-EC	1.2-60	0.001	8.7	Plasma	2
GC-MS	5-500	1	15.7	Saliva	15
IMS	0.44-22	0.1	11	Urine	This work

Table 3: Determination of morphine (ng mL^{-1}) in spiked human urine samples.

Sample	Added	Found*	Recovery (RSD%)
1	1.0	1.03 ± 0.11	103 (10.7)
2	15.0	14.39 ± 1.23	96 (8.5)

3.4. Application

In order to evaluate the capability of the developed method, it was applied for the determination of morphine in spiked human urine samples. Two samples (at two linear ranges) were prepared based on the mentioned procedure and analyzed (Table 3). According to the recovery results, the determination of morphine in human urine samples can be performed using the IMS method.

4. Conclusion

The IMS with positive corona discharge ionization has been developed for the determination of morphine in human urine. The developed method offers wide linear range and low detection limit for morphine that are comparable to those of other methods in the determination of morphine. . Moreover, the simplicity, low cost and rapid are other advantages for the developed method.

Acknowledgment

The authors are thankful to the Islamic Azad University, Yazd branch for the support of this work.

5. References

[1] Y. He, A. Vargas, Y.J. Kang, Anal. Chim. Acta, 589 (2007) 225.

[2] H. He, S.D. Shay, Y. Caraco, M. Wood, A.J.J. Wood, J. Chromatogr. B, 708 (1998) 185.

[3] J.F. Wilson, B.L. Smith, P.A. Toseland, I.D. Watson, J. Williams, A.H. Thomson, N.E. Capps, G. Sweeney, L.N. Sandle, Forensic Sci. Int., 119 (2001) 23.

[4] J. Stjernsward, Cancer Surv., 7 (1988) 195.

[5] M.E. Bosch, A.R. Sanchez, F.S. Rojas, C.B. Ojeda, J. Pharm. Biomed. Anal., 43 (2007) 799.

[6] Q.L. Zhang, J.J. Xu, X.Y. Li, H.Z. Lian, H.Y. Chen, J. Pharm. Biomed. Anal., 43 (2007) 237.

[7] A.M. Idris, A.O. Alnajjar, Talanta, 77 (2008) 522.

[8] A. Sheibani, M.R. Shishehbore, E. Mirparizi, Spectrochim. Acta A, 77 (2010) 535.

[9] T. Keller, A. Keller, E. Tutsch-Bauer, F. Monticelli, Forensic Sci. Int., 161 (2006) 130.

[10] W. Vautz, S. Sielemann, J.I. Baumbach, Anal. Chim. Acta, 513 (2004) 393.

[11] A. Sheibani, M. Tabrizchi, H.S. Ghaziaskar, Talanta, 75 (2008) 233.

[12] A.M. Margaret, E. Barbara, A.B. David, W.P. Randy, G. Roger, J. Anal. Toxicol., 25 (2001) 81.

[13] T. Khayamian, M. Tabrizchi, M.T. Jafari, Talanta, 69 (2006) 795.

[14] A. Niazi, A. Yazdanipour, Chinese Chem. Lett., 19 (2008) 465.

[15] K. Javidnia, R. Miri, D. Miri, Iran J. Med. Sci., 31 (2006) 213.