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Original Article

Application of ion mobility spectrometry for the determination of tramadol in biological samples

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ABSTRACT

In this study, a simple and rapid ion mobility spectrometry (IMS) method has been described for the determination of tramadol. The operating instrumental parameters that could influence IMS were investigated and optimized (temperature; injection: 220 and IMS cell: 190°C, flow rate; carrier: 300 and drift: 600 mL/minute, voltage; corona: 2300 and drift: 7000 V, pulse width: 100 μs). Under optimum conditions, the calibration curves were linear within two orders of magnitude with $R^2 \geq 0.998$ for the determination of tramadol in human plasma, saliva, serum, and urine samples. The limits of detection and the limits of quantitation were between 0.1 and 0.3 and 0.3 and 1 ng/mL, respectively. The relative standard deviations were between 7.5 and 8.8%. The recovery results (90–103.9%) indicate that the proposed method can be applied for tramadol analysis in different biological samples.

1. Introduction

Drug abuse is a patterned use of a substance (drug) in which the user consumes the substance in amounts or with methods which are harmful to themselves or others. This issue has been addressed in many countries [1,2]. Tramadol 2-[(dimethylamino) methyl]-1-(3-methoxyphenyl) cyclohexanol (Fig. 1) is a centrally acting analgesic drug used for treating moderate to chronic pain. This drug, like morphine and other narcotics, may also be abused [3,4]. Hence, the determination of tramadol in human biological samples is important because it can provide information about long-term abuse and is also helpful for forensic purposes. Various methods have been reported for tramadol determination such as high performance liquid chromatography (HPLC) [5,6], gas chromatography (GC) [7], capillary electrophoresis [8], spectrophotometry [9], and electrochemistry [10,11]. However, it is necessary to develop a rapid, simple, and sensitive method for the determination of tramadol in biological samples.

Ion mobility spectrometry (IMS) has been used for over 40 years since 1970. It is an analytical method for determination and characterization of trace substances present in different samples. In forensic sciences, IMS is used as a detection tool to prove the presence of latent traces of illicit drugs or explosives on surfaces of suspicious or confiscated material of evidence. The IMS instrumentation is comprised of four major components including: (A) ionization source; (B) ion gate; (C) drift region; and (D) detector. Neutral molecules are introduced into IMS and ionized with different procedures. Then, a bundle of ions, known as a swarm, is introduced into a voltage gradient or electric field (E, V/cm). The ion swarm attains an average velocity through the electric field, called the drift velocity (V_d, cm/second), at ambient pressure in a gas. The

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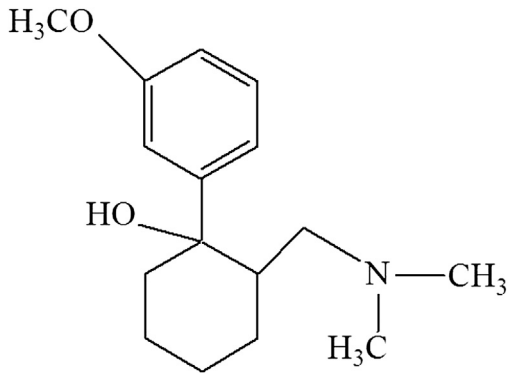

Fig. 1 – Chemical structure of tramadol.

average velocity (V_d) of ions can be calculated by $V_d = K.E$, where K is the mobility constant. In drift region, ions are separated based on their mobility. As a result, diverse ions reach the detector at different drift times, which are characteristic of the ions considered. A mobility spectrum is a plot of current or voltage signal versus drift time. The number of ions reaching the detector is a measure of the analyte concentration. Portability, speed, sensitivity, and low cost are the main advantages of IMS [12–14].

In this work, the IMS method was developed for the determination of tramadol. Corona discharge in positive mode is used for the ionization of the vaporized analyte molecules. The operating instrumental conditions of IMS such as temperature, voltage, and gas flow rate were investigated and optimized. The analytical parameters of the proposed method are comparable to those of other methods used for tramadol determination. The biological applications of the IMS method were evaluated by tramadol analysis in human plasma, saliva, serum, and urine samples.

2. Instrumentation and methods

2.1. Apparatus

The ion mobility spectrometer with a corona discharge ionization source was constructed at Isfahan University of Technology (IUT, Isfahan, Iran). Fig. 2 shows a schematic diagram of the spectrometer [15,16]. The main parts of instrument are: the IMS cell, the needle for producing the corona, two power supplies, a pulse generator, an analog to digital converter, and a computer. The IMS cell, including the ionization and drift regions, was inserted in an oven. The ionization region and drift tube consisted of 16 aluminum rings which were isolated from each other by thin Teflon insulators. The rings were connected using a series of resistors to make a potential gradient. The instrument was also equipped with a needle-to-plane corona discharge ionization source in positive and negative modes. A shutter grid was applied to produce an ion pulse to the drift region by the pulse generator. To display the ion mobility spectrum, an analog to digital converter (PicoScope, UK) was used. The total peak area of product ions over the acquisition time (from time of appearance until the disappearance of analyte peak; average time: 60 seconds and the maximal amplitude: 1 mv) was obtained using a laboratory made integrator program. The total peak area was considered as the IMS signal. The obtained peak information was further processed and analyzed with Microsoft Excel. The results are the averages of three experiments in each concentration.

2.2. Chemical and reagents

Tramadol hydrochloride (purity >99%) was obtained from Arya Co. (Tehran, Iran). A stock standard solution of tramadol hydrochloride (100 μg/mL) was prepared in distilled water. Blank human plasma, saliva, serum, and urine samples were obtained from healthy volunteers ($n = 3$, age = 20–25 years, male and female, nonsmokers; taken from the Shahvali Hospital of IAU-Yazd, Iran). C_{18} cartridge columns (Supelco Inc., 100 mg) were supplied from Sigma-Aldrich (St. Louis, Mo, USA). Ammonium bicarbonate, ethyl acetate, methanol, and sodium hydroxide were purchased from Merck (Darmstadt, Germany).

2.3. The extraction procedures of tramadol from different biological samples

The known amounts of tramadol were spiked to blank tissue of different biological samples (plasma, saliva, serum, and urine) and then, a liquid–liquid extraction or solid-phase extraction procedure (described below) was used for sample preparation [17,18].

Human plasma (1.0 mL) was placed into a glass tube containing 0.1 mL of NaOH (2.0 M) and mixed for 10 seconds, and

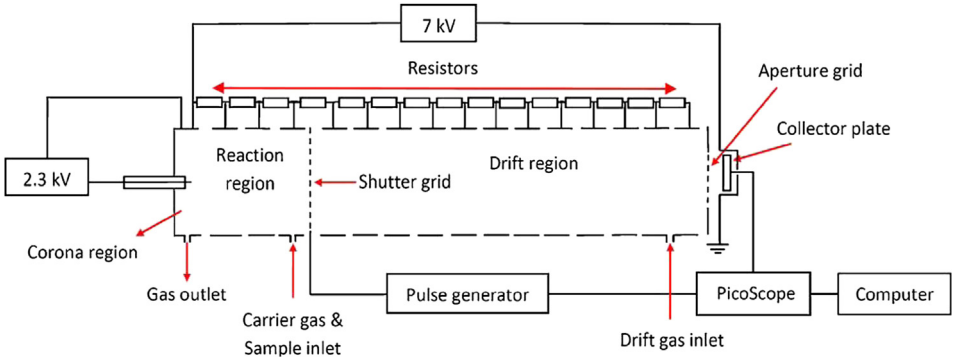

Fig. 2 – Schematic diagram of an ion mobility spectrometer (IMS). Reprinted with kind permission from Isfahan University of Technology (IUT).

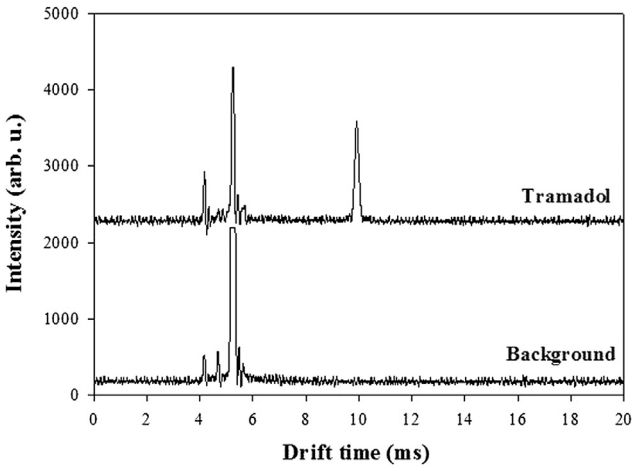

Fig. 3 – Ion mobility spectra of tramadol and background.

Table 1 – The operating instrumental conditions of ion mobility spectrometry (IMS).

Parameter	Setting
Corona voltage (kV)	2.3
Drift voltage (kV)	7.0
Flow rate of drift gas (N2, mL/min)	600
Flow rate of carrier gas (N2, mL/min)	300
Injection port temperature (°C)	220
IMS cell temperature (°C)	190
Pulse width (μs)	100

then 1.25 mL of ethyl acetate added. After vertical agitation (10 minutes), it was centrifuged at 10,000 rpm for 5 minutes and then 1.0 μL of the supernatant solution was injected into IMS. To apply the extraction of saliva, samples were first diluted twice with distilled water and then extracted (similar to plasma) and injected into IMS.

For sample preparation of human serum, 1.0 mL was placed into a glass tube and then 3.0 mL of methanol was added, mixed for 20 seconds, and centrifuged at 10,000 rpm for 10 minutes. A volume of 1.0 mL of the methanol layer was transferred to a clean glass tube and 2.0 mL of methanol was added and centrifuged again at 10,000 rpm for 5 minutes. Then, 1.0 mL of the supernatant solution was loaded on a C_{18} cartridge column that had been pretreated with 1.0 mL of methanol, 1.0 mL of water, and 2.0 mL of ammonium carbonate buffer (0.01 M, pH = 9.3). After loading, the C_{18} cartridge was washed with 1.0 mL of distilled water and 2.0 mL of ammonium carbonate buffer (0.01 M, pH = 9.3) and then tramadol eluted with 1.0 mL of methanol. Finally, 1.0 μL of this solution was introduced into the injection port of the IMS.

For urine samples, 5 mL of it was first diluted with 5 mL of distilled water and then centrifuged at 14,000 rpm for 5 minutes. A volume of 1.5 mL of solution was transferred to a glass tube and 2.0 mL of ammonium carbonate buffer (0.01 M, pH = 9.3) was added and centrifuged again at 14,000 rpm for 10 minutes; it was then applied to the C_{18} cartridge column (similar to the serum sample). Finally, 1.0 μL of the methanol solution was injected into the IMS.

2.4. *Method validation*

Method validation was performed based on the international conference on harmonisation (ICH)-guideline [19]. The calibration curves (linear dynamic ranges, LDRs) for tramadol in different biological samples were obtained by least squares linear regression. These were constructed using the several spiking levels within 0.5–45 ng/mL of tramadol in the blank tissue (drug free) from plasma, saliva, serum, and urine samples. Precision of the developed method was determined by repeatability and expressed as the relative standard deviation (RSD%). The formula of RSD% is: (standard deviation/average concentration) × 100. The accuracy of the method was reported as recovery by comparing the concentrations at three levels calculated from the calibration curve to their spiked concentrations. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using $3s_b/m$ and $10s_b/m$, respectively, where s_b is the standard deviation of the signal intensity for the blank sample and m is the slope of the calibration curve.

3. Results and discussion

Chromatographic techniques, especially HPLC, were considered as the first choice for tramadol analysis. However, these

Table 2 – Comparison of analytical parameters of the proposed method with other methods in the determination of tramadol at different samples.

Method	Sample	LDR (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)	RSD (%)	Recovery (%)	Ref.
HPLC-UV	Plasma	12.5–800	9.0	–	1.2	88.5	[20]
HPLC-FL	Saliva	100–6000	–	2.5	3.3	97.3	[17]
GC-MS	Oral fluid	10–100	–	10	2.4	87.7	[13]
GC-MS	Hair	0.5–5.0	0.5	–	6.7	90.7	[21]
HPLC-FL	Urine	$(2–100) \times 10^3$	–	2.5×10^3	10.6	97.1	[17]
HPLC-MS/MS	Urine (dog)	1–1000	5.0	10.0	2.7	82.0	[22]
IMS	Plasma	0.5–37.5	0.1	0.4	8.1	98.7–100.7	This work
	Saliva	0.8–35.0	0.2	0.6	7.8	97.4–100.8	
	Serum	0.5–40.0	0.3	1.0	8.8	90.0–99.4	
	Urine	0.5–45.0	0.1	0.3	7.5	99.3–103.9	

FL = fluorescence; GC = gas chromatography; HPLC = high performance liquid chromatography; IMS = ion mobility spectrometry; LDR = linear dynamic range; LOD = limit of detection; LOQ = limit of quantitation; MS = mass spectrometry; RSD = relative standard deviation.

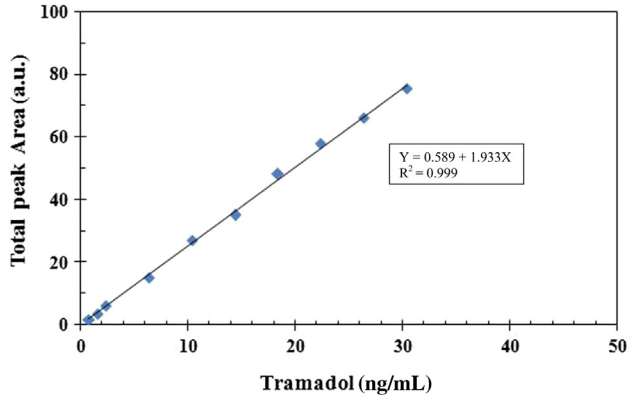

Fig. 4 – Calibration curve for tramadol extracted from spiked plasma samples.

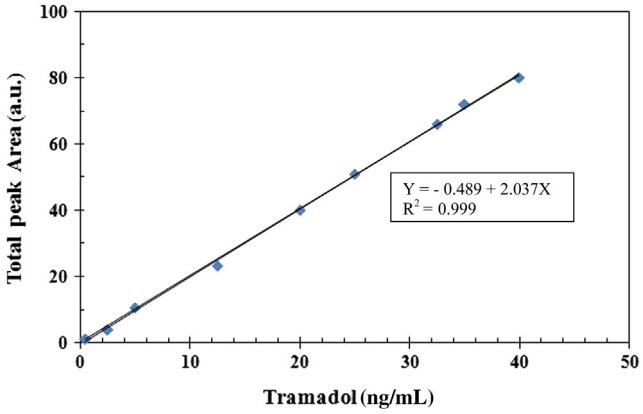

Fig. 6 – Calibration curve for tramadol extracted from spiked serum samples.

methods usually involve a long analysis time, expensive equipment and are solvent-consuming. In order to fulfill the growing needs for determining and control of tramadol, a simple and rapid IMS method was developed with the potential for use for the analysis of the drug in different biological samples.

3.1. *Optimization of the operating instrumental conditions of IMS*

The ion mobility spectra of tramadol and background are shown in Fig. 3. The analyte spectrum shows only one peak at about 10 milliseconds. In order to obtain the best sensitivity, the effective instrumental parameters including corona and drift voltages, injector port and cell temperatures, carrier and drift gas flow rates, and pulse width were investigated and optimized; the results are presented in Table 1. Among these, temperature (injection and oven) is an important parameter. The effect of the injection temperature on the signal intensity was studied in the range 180–230°C with the IMS cell temperature (oven) 180°C. The signal intensity increases until 220°C and remains constant at higher temperatures. Therefore, 220°C was selected for future study. Similarly, the effect of the IMS cell temperature on signal intensity was studied in the range 150–200°C, with the injection port temperature 220°C. The results show that the signal intensity improves with increase in the IMS cell temperature up to 190°C and then it does not change. Therefore, 190°C was selected as the optimum value of the IMS cell temperature.

3.2. *Analytical parameters*

The analytical parameters of the IMS method for the determination of tramadol are given in Table 2 [13, 17, 20–22]. Under the optimized conditions (Table 1), calibration curves (LDRs) were found to be linear within 0.5–45 ng/mL, with the correlation coefficients close to one; $R^2 \geq 0.998$ (Figs. 4–7). The concentrations were at the concentration level for the determination of tramadol in the tested real samples. At higher concentrations, saturation of the signal generated of the analyte occurred and the linearity of the calibration curves was destroyed; this status must be avoided. The ion mobility spectra of the extracted tramadol of different biological samples show that the position and shape analyte peaks are similar to Fig. 3. This means that the extraction procedures could provide clean extracts in which no extra peaks were observed in the region where the analyte appeared. The difference in the region of reactant ions can be concerned to the

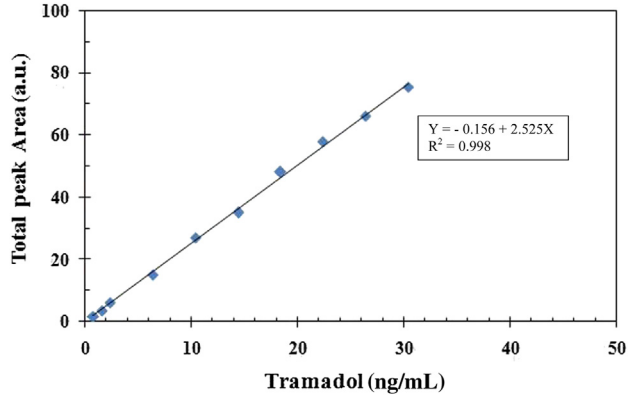

Fig. 5 – Calibration curve for tramadol extracted from spiked saliva samples.

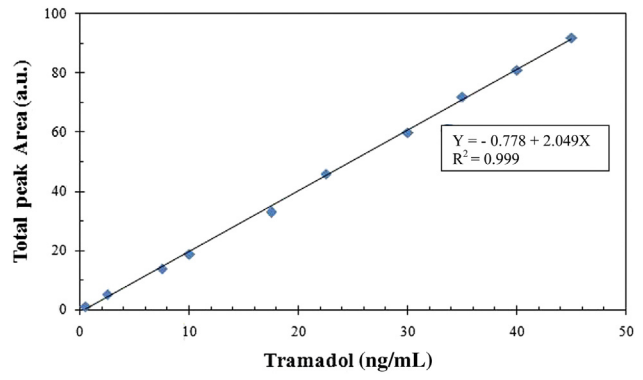

Fig. 7 – Calibration curve for tramadol extracted from spiked urine samples.

matrices from tested samples. The RSDs following eight measurements were found to be lower than 9%. LOD and LOQ values were 0.1–0.3 ng/mL and 0.3–1 ng/mL, respectively.

Analytical parameters of the proposed IMS are comparable with other methods reported in previous studies (Table 2). According to the results, the LODs and LOQs of the proposed method are better than those of other methods. This shows that IMS can determine trace levels of tramadol in biological samples. The LDRs and recovery data also are comparable with those reported in literature. Furthermore, the proposed method is simple, rapid, and does not need expensive equipment and hazardous solvents.

3.3. *Biological applications of proposed method*

Plasma, saliva, serum, and urine samples were analyzed to demonstrate the capability of the proposed method in determining tramadol in real samples. In this regard, spiked biological samples were prepared at three concentrations (2, 15, and 30 ng/mL) by addition of the standard solution of tramadol to blank samples. Then, tramadol was extracted according to the procedures described in the experimental section and injected into the IMS. The recoveries obtained (Table 2) demonstrate the capability of the developed method in the quantitative detection of tramadol in biological samples.

In humans, tramadol is metabolized to its metabolites. With regard to the chemical structure of these compounds, we expect that the product ions of them could be also created in the IMS. By contrast, real samples could include some other drugs, which may result in creating peaks which interfere with that of tramadol. Therefore, a preseparation technique may be required for quantitative analysis of real samples before the analyte is introduced into IMS.

Conflicts of interest

All authors declare no conflicts of interest.

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