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A new approach to determine salicylic acid in human urine and blood plasma based on negative electrospray ion mobility spectrometry after selective separation using a molecular imprinted polymer

M.T. Jafari [a,*], Z. Badihi [a], E. Jazan [b]

[a] Department of Chemistry, Isfahan University of Technology, Isfahan 84156-83111, Iran
[b] Department of Chemistry, Islamic Azad University, Shahreza Branch, Shahreza, Isfahan 311-86145, Iran

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ABSTRACT

This paper deals with a method based on negative electrospray ionization ion mobility spectrometry (ESI-IMS) as a detection technique. The method was used to determine the salicylic acid in human urine and plasma after selective separation of salicylic acid (SA) via molecular imprinted polymer (MIP). The ion mobility spectrum of salicylic acid in negative mode and the reduced mobility value for its ion peak is reported in this paper for the first time. In order to combine the technique with negative ESI-IMS, suitable experimental conditions related to MIP (e.g., Soxhlet extraction) were selected. The method was exhaustively validated in terms of sensitivity, imprinting factor, enrichment factor, and sorption capacity. The linear dynamic range of 0.02–2.00 μg mL^{-1} and the relative standard deviation (RSD) below 6% were obtained for the analysis of SA through this method. The average recovery was calculated about 92% for the analyzed drug. Finally, human urine and plasma were analyzed and the feasibility of the proposed method was successfully verified by the efficient clean-up of the samples using MIP separation before the analysis by ESI-IMS.

1. Introduction

Salicylic acid (SA) or 2-hydroxybenzoic acid is a principal metabolite of aspirin in plasma which is widely used for the treatment of various skin ailments. Aspirin is easily hydrolyzed to SA and circulates in blood in the salicylate form [1,2]. This compound is used in pharmaceutical, topical preparations as a keratolytic for the treatment of acne, psoriasis and warts as well as an antimicrobial agent [3]. In equine sport, SA is prohibited at a concentration level higher than 750 μg mL^{-1} in urine or 6.5 μg mL^{-1} in plasma [4]. This drug is also carefully monitored in the urine and blood of animals to be slaughtered. Salicylic acid is also utilized as a preservative of food products, paste, glue, and also a whitening agent in cosmetic products [1,2]. In addition, SA as a colorless crystalline organic acid is widely used in synthesis of organic compounds. This compound can act as a plant hormone having important roles in plant growth, photosynthesis, and transpiration. Therefore, doping control laboratories have to establish a quantitative and qualitative method for the analysis of this drug in various biological matrixes. For the determination of salicylic acid in different samples, a number of analytical methodologies have been reported, including high performance liquid chromatography (HPLC) with various detection systems [2,5–7], capillary electrophoresis [8], liquid chromatography/quadruple-time of flight mass spectrometry (LC–QTOF–MS) [4,9], gas chromatography/mass spectrometry (GC–MS) [3,10,11] and a variety of electrochemical methods [12,13]. Usually, these standard methods have a good selectivity due to using a powerful separation techniques (e.g., GC, LC, …). However, most of these instruments are costly, laborious, and most importantly are blamed for the lack of portability. In addition, the analytical methods including GC separation or luminescence detection commonly require a tedious and time-consuming procedure for derivatization. Electrochemical methods for the analysis of SA also suffer from some shortcomings such as their results being ambiguous and corrupted by experimental artifacts [14].

Molecularly imprinted polymers (MIPs) are extensively cross-linked polymers containing specific recognition sites with a predetermined affinity for target molecules [15,16]. The procedure for synthesizing an MIP is based on the formation of a complex between a target (template) molecule and appropriate functional monomer/s that cross-link/s into matrix (macromolecular). Then, template is removed from the binding site and the imprinted polymer with a high affinity for the template molecule is obtained. This method is similar to that of enzymes and antibodies. However, MIPs are resistant not only to mechanical

* Corresponding author. Tel.: +98 311 391 2351; fax: +98 311 391 2350.
E-mail address: jafari@cc.iut.ac.ir (M.T. Jafari).

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stress, high pressure, and elevated temperature, but to acids, bases, organic solvents, and metal ions. In this study, salicylic acid that can form intramolecular hydrogen-bond between its two functional groups –COOH and –OH, was chosen as the template. Previously, SA has been separated by MIP and then quantified using UV detection or by electrochemical methods [17–19]. However, these detection systems have a relatively low sensitivity and selectivity compounded by long time response, and therefore may not be suitable for the analysis of some biological samples.

Ion mobility spectrometry (IMS) has been developed for detection and characterization of chemical compounds such as illicit drugs, explosives, pharmaceuticals, and environmental pollutants [20]. This technique can help distinguish compounds on the basis of differences in their reduced mass, charge, and collision cross-section under a weak electric field. This method is very sensitive (~ppb), simple, inexpensive, cost effective and very fast for detection of chemical compounds in various samples. Previously, we applied IMS in a positive operating mode as the detection system for MIP-based separation [21,22]. The results revealed that the MIP-IMS system has a good capability for the analysis of drugs (e.g., metronidazole and primidone) in biological samples. However, IMS in the negative operating mode has not been used as a detection system for MIP separation. Overall, our literature review brought forth a few papers reporting on the use of ESI-IMS in the negative mode.

Considering the discussions above, the objective of this work is to develop a simple and rapid technique for the separation and also determination of SA without using any additional derivatization methods. In this regard, the capability of the negative electrospray IMS after MIP separation for the analysis of the salicylic acid in human plasma and urine was investigated. The results validated the capability of the MIP-IMS for the analysis of SA in biological samples.

2. Experimental section

2.1. Chemicals

The standard drug of salicylic acid was purchased from Merck. Ethylene glycol dimethacrylate (EDMA), and 2, 2′-azobis (2-isobutyronitrile) (AIBN) were also purchased from Merck. 4-vinylpyridine (4-VPY) was prepared from Sigma Aldrich. The solvents, including dimethylformamide (DMF), acetone, HPLC-grade methanol, formic acid (FA) and acetic acid (ACA) were also purchased from Merck. Water was 18 MΩ deionized, prepared by Barnstead Ultrapure Water Systems.

2.2. Ion mobility spectrometry

The ion mobility spectrometer (IMS) used for this research was designed and constructed at Isfahan University of Technology and described previously [23]. In this work, electrospray ionization in the negative mode was applied as an ionization source. In brief, the main parts of the instrument include the following: the IMS cell, the electrospray needle, two high voltage power supplies, a pulse generator, an analog to digital converter and a computer. In this work, the IMS cell of the system was constructed from 16 aluminum rings 10 mm wide. These conducting rings were separated from each other by thin PTFE rings (1 mm in width). The IMS cell length was divided into three regions, the electrospray region (3 cm), the desolvation region (4 cm), and the drift region (11 cm in length). The drift and desolvation rings had the inner diameters of 4 and 2.1 cm, respectively. The aluminum rings were connected by a series of resistors to form the electric field gradient. The electrospray needle (P/N 7768-01, Hamilton, Reno, NV, U.S.A) was inserted into a Teflon tube to eliminate the corona discharge problem and fixed at one end of the cell. The Bradbury–Nielsen shutter grid was made of two series of parallel wires biased to a potential, creating an orthogonal field relative to the drift field, to block ion passage to the drift tube. The grid potential is removed for a short period of time by the pulse generator to admit an ion pulse to the drift region. Generally, this period of time was selected 200 μs. Preheated nitrogen was employed as the drift and the desolvation gases with flow rates of 500 and 900 mL min^{-1}, respectively. The default Faraday plate detector configuration consisted of a 21-mm-diameter stainless steel plate positioned ~1.0 mm behind the aperture grid. The high-speed A/D module (12-bit dynamic range) was used to measure the spectrometer output and to convert the analog signal to a digital one. All mobility data were collected by data acquisition software and each IMS spectrum was the average of 50 individual spectra. Table 1 summarizes the operating conditions under which the IMS spectra were taken.

2.3. Molecular imprinted polymer

Molecular imprinted polymer was synthesized using SA as a model template and 4-VPY as a complementary functional monomer. Zhang et al. [17] showed that 4-VPY has a more efficient imprinting effect for SA than acrylamide. This is due to electrostatic interaction between SA and 4-VPY monomers, resulting in the elimination or decreasing of the intra-molecular hydrogen bonding in SA. EDMA was also used as a cross-linker which was able to provide sufficient rigidity of the cross-linked polymer. This is necessary for formation and functioning of the template specific binding sites [24,25].

In this work, non-covalent method with SA was employed in the molecularly imprinted polymer synthesis. Thus, SA (1.0 mmol), functional monomer (4-VPY, 6.0 mmol), cross-linker (EDMA, 30.0 mmol) and initiating agent (AIBN, 0.3 mmol) were dissolved in acetonitrile (10 mL). The solution was transferred into a 20 mL glass tube and deoxygenated with N_2 stream for 15 min, sealed under nitrogen and then left to polymerize in an oil bath at 60 °C for 24 h. After polymerization, the glass tube was broken, and the polymer was mechanically grounded in a mortar. A steel sieve was employed to select particles with sizes ≤200 μm and a Soxhlet extraction system was also used to remove the analyte from the polymer. Afterward, the prepared MIP particles, kept at the room temperature overnight, were used through the SPE procedure. The preparation steps for MIP are shown in Fig. 1. As a reference, non-imprinted polymer (NIP) was also prepared in the same way but without the addition of the template (SA). To evaluate the imprinting effect and the non-specific absorptions, the selectivity of NIP and MIP was also compared.

Table 1
Typical operating conditions of IMS during the experimental runs.

Operating parameters	Setting
Needle voltage	−11.40 kV
Target electrode votage	−9.00 kV
Liquid flow rate	6 μL min^{-1}
Drift field	600 V cm^{-1}
Desolvation field	600 V cm^{-1}
Drift gas flow (N_2)	500 mL min^{-1}
Desolvation gas flow (N_2)	900 mL min^{-1}
Drift tube length	11 cm
Shutter grid pulse	0.2 ms
Number of IMS averages	50
Scan time	20 ms
Number of points per ion mobility spectrum	500

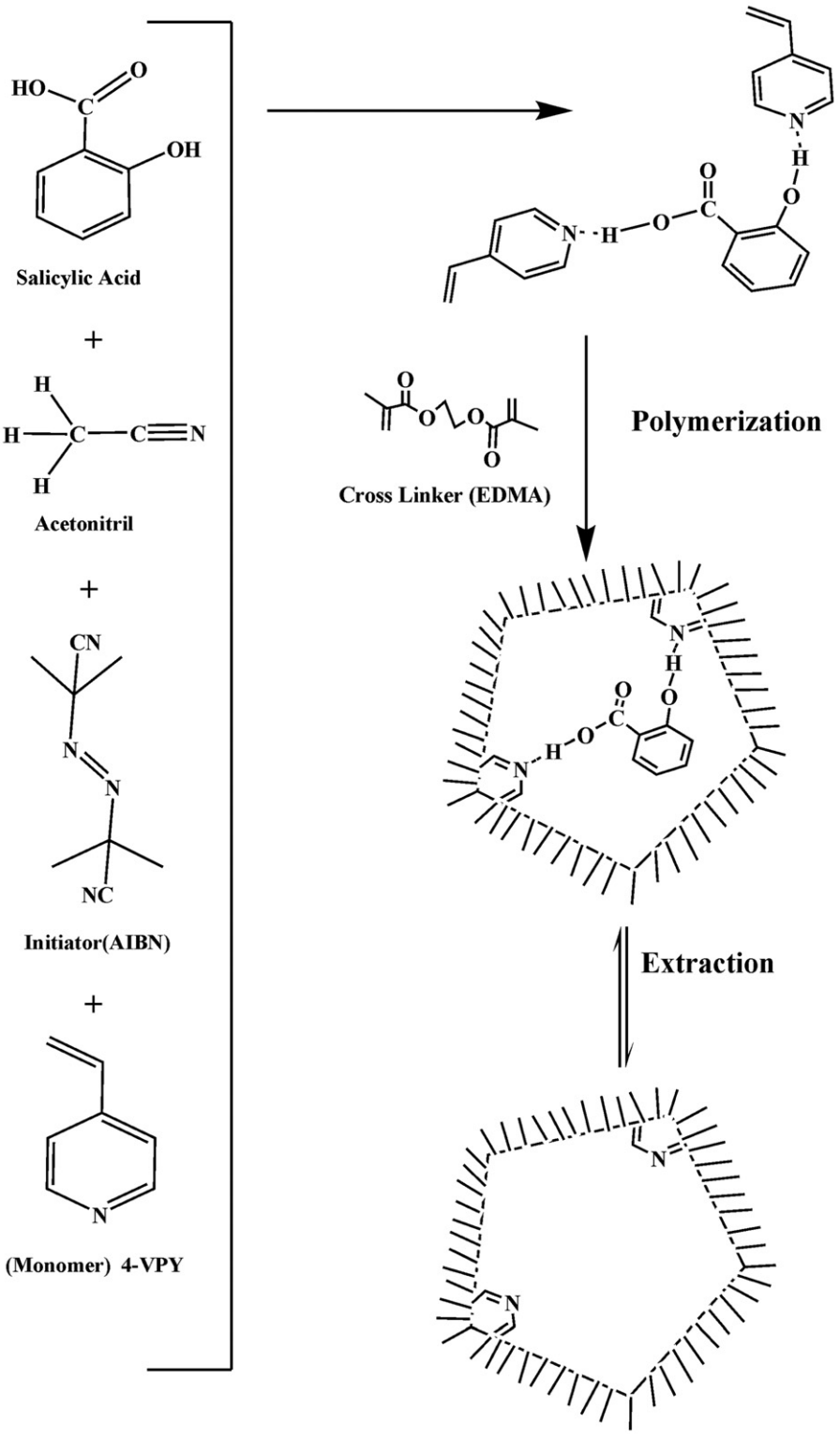

Fig. 1. Diagrammatic representation of hypothetical imprinted polymer formation using 4-VPY as the functional monomer and SA as the template.

2.4. Extraction of SA from the MIP

Removal of the SA print molecule from the MIP particles was accomplished through a Soxhlet extraction. For this purpose, 2.0 g SA-imprinted polymer was placed inside the extraction thimble. The extraction solvent (50 mL) was a mixture of methanol and formic acid (7:3). Heat was applied to the solvent flask, at a rate that caused filling and eventual emptying of the extraction chamber. The extraction was continued for 29 h to produce the anti-SA polymer particles. Quantification of the extracted SA was accomplished by using ESI-IMS. Soxhlet extraction was not performed on the non-imprinted polymer particles.

2.5. Preparation of columns

The polymer particles were dispensed into a stainless steel column measuring 5 cm in length and 0.4 cm in diameter until the column bed was fully packed. A 10 μm frit was used at the end of column fitting to ensure no loss of the MIP. Methanol (2 mL) was run through the column to ensure uniform particle packing. Afterward, imprinted polymer inside the column was eluted with 2.0 mL water. The column was capped and connected to a 10 mL gastight syringe (Hamilton, Reno, NV, and U.S.A) via a PTFE tube. Sample solution was delivered into the column by a programmable syringe pump (New Era Pump System Inc., and U.S.A). The preparation of a non-imprinted polymer column was achieved in a similar manner.

2.6. Real samples analysis

In this work, the capability of the method was investigated for the analysis of SA in human urine and plasma as the real samples. In this regard, amount of this compound in the urine and plasma of a healthy volunteer after percutaneous absorption was determined. Real samples were obtained 2 h after administration of drug (containing 40% SA) and moved to laboratory for the analysis, immediately. Before extraction, the urine sample was passed through a 0.45 μm syringe filter and then diluted 10 times with deionized water. Drug-free plasma sample was obtained from the volunteer before administration and stored at −20 °C. Prior to use, the plasma sample was allowed to thaw at room temperature. To clean up the plasma sample, 0.5 mL plasma was mixed with 4 mL mathanol and the sample was centrifuged for 10 min at 3800 rpm. A 1.0 mL aliquot of the methanol layer was placed into another glass vial; 2.0 mL methanol was added and centrifuged again at 3800 rpm for 10 min. One milliliter of the supernatant was diluted to 10 mL and 2.0 mL of the aliquot was injected onto the MIP column. After passing through the MIP, the column was washed by 2 mL of methanol: water, 0.5:9.5 with the flow rate of 0.04 mL min^{-1}. This solution washed out undesirable materials, which were chemically similar to the analyte or the analyte molecules adsorbed to non-specific binding point of MIP. Finally, the analyte was eluted with 2 mL pure methanol, and 20 μL of it was injected into ESI-IMS instrument. In this work, the standard addition method was used to cope with the matrix effect.

3. Results and discussion

3.1. Ion mobility spectrum

Comparison of the ion mobility spectrum of SA and the background spectrum of negative ESI-IMS is shown in Fig. 2. These spectra were recorded in the optimized conditions of IMS given in Table 1. In our previous work [26], several ions originating from electrospray solvent (methanol) were assigned as chloride, nitrite, formate, nitrate, and acetate in addition to some unknown ion peaks. As this figure displays, ion mobility spectrum of salicylic acid shows only one ion peak named SA. In this work, trinitrotoluene with the reduced mobility value of 1.52 $cm^2 V^{-1} s^{-1}$ [27] was used as the internal standard compound. By using it, the reduced mobility value of 1.75 $cm^2 V^{-1} s^{-1}$ was calculated for SA. Since there is not any report on the reduced mobility for the product ion originating from SA in the literature, the chemical formula of this ion could not be characterized. However, based on the results reported from electrospray mass spectrometer (ESI-MS) [4,28] it can be concluded that the most typical ion is characterized by the loss of proton. Therefore, the ion peak SA originating from the compound in this work might be due to the formation of $[M-H]^-$. In fact, the coupling of a MS to IMS is needed for a definite characterization of the product ion.

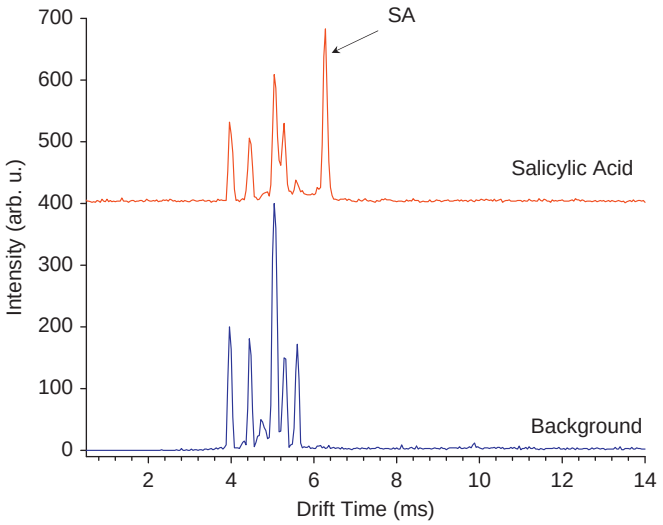

Fig. 2. The ion mobility spectrum of SA drug compared with that of the background obtained by ESI-IMS in negative mode.

3.2. Calibration curve and analytical parameters

For quantitative analysis, a series of standards in the range 0.01–10 μg mL^{-1} of SA were prepared in pure methanol and used to determine the analytical parameters of the instrument. When a standard solution of SA was sprayed, the SA peak appeared after a short time. After formation of the stable electrospray, the area of the ion peak was calculated and considered as response of ESI-IMS for each concentration level of the compound. Ten ion mobility spectra (each of them was averaged from 50 individual spectra) were acquired to obtain the averaged data points. The response of ESI-IMS was plotted against the concentration of this compound and calibration curve equation was developed by the least-squares method. As data show, good linearity ($r^2 < 0.99$) is observed for SA in the range 0.02–2.00 μg mL^{-1}. The linear dynamic range is about two orders of magnitude, which is typical for electrospray ionization source and for most IMS systems. Using the standard definition of $S/N=3$, the detection limit of 8 μg L^{-1} was determined for this compound. The relative standard deviation (RSD) was calculated 5.4%, for six replicates analysis of 0.5 μg mL^{-1} solution. The quantitative results obtained in this work are promising for development of this method as a powerful analytical tool for detection of SA in liquid samples.

3.3. Effect of solvent ratio and extraction time

In this work, target molecules inside the MIPs were removed by Soxhlet extraction using successive volumes of an appropriate solvent until the extraction monitoring by IMS showed undetectable levels of SA. Therefore, it is necessary to obtain the best solvent ratio for extraction of SA from the polymer. First, a mixture of methanol–acetic acid (1:1) was used as Soxhlet extraction solvent. However, no considerable decreasing in the signal was observed during extraction even after 160 h. Further investigations showed that when solution of acetic acid (ACA) in methanol was injected into ESI-IMS, the acetate ion signal of background was increased and another significant signal with the same drift time as that for SA was also appeared in spectrum

(data not shown). Therefore, when ACA was used for extraction of template, one of the ACA ions was completely overlapped with SA signal. Consequently, it was not possible to investigate the extraction efficiency when ACA solution was used for Soxhlet extraction. To overcome this problem, the Soxhlet extraction with various ratios of methanol:formic acid (9:1, 8:2, 7:3 and 1:1) at different times, from 2 to 16 h, was followed to obtain the optimum rebinding conditions. Fig. 3 shows that the best ratio of eluting solvent was 7:3, but because of bleeding SA from MIPs, the extraction time of 29 h was selected for the complete extraction of SA.

3.4. Effect of pH

The effect of pH of test solution on the performance of MIP was studied by varying the mobile phase pH in the range of 2–8 with the interval of 1 unit. The obtained results show that when the pH increased from 2 to 5, the adsorption of the analyte on the MIP-sorbent increased and then decreased while the pH reached 8. An explanation is that the pyridine group in binding site of MIP can be protonated at low pH, and the SA molecules can be deprotonated at high pH [17]. This is apparent from the results that the recovery was enhanced to 95% in pH 5. Therefore, the pH of the test solution was adjusted to 5 for all the experiments.

3.5. Effect of flow rate

Since the sample flow velocity dictated the time available for molecular diffusion, analyte selectivity of MIP was evaluated by varying the flow rate over a range of 0.01–0.12 mL min^{-1}. Standard solution of 10.0 mg L^{-1} SA was injected into the column, followed by adsorption of the bound analyte. The recoveries were calculated and plotted against the flow rate (Fig. 4). According to this figure, SA displayed a trend of the increased binding efficiency with decreasing flow rate, provided more time for the diffusion of SA molecules to the strong and selective binding sites inside the MIP particles. Based on the results, the most favorable diffusion to the selective binding of SA was achieved at the flow rate of 0.04 mL min^{-1}.

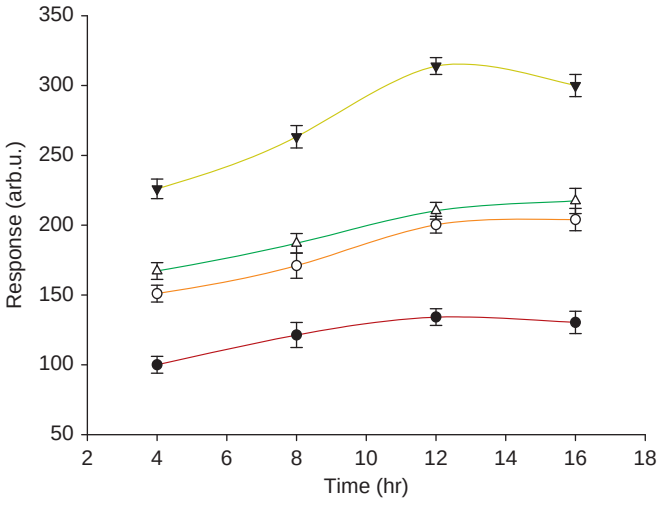

Fig. 3. The plots of the extracted SA using Soxhlet extraction versus the extraction time onto the different ratios of methanol: formic acid (●) 9:1, (○) 8:2, (▲) 7:3, and (△) 1:1.

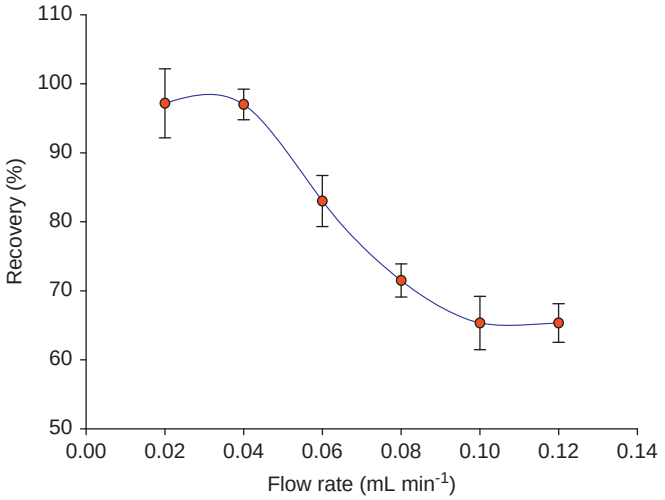

Fig. 4. Recoveries obtained for SA regarding to the amount of drug present in the solution after loading of 10.0 mg L^{-1} SA versus the flow rate of mobile phase.

3.6. Evaluation of MIP performance

In this work, some parameters such as enrichment factor (EF), imprinted factor (IF), and retention capacity (sorption capacity) were studied for evaluation of MIP performance. The enrichment factor was calculated through dividing the obtained concentration in the extraction phase (C_{org}) by the initial concentration in the spiked sample (C_{aq}), Eq. (1).

$$EF = \frac{C_{org}}{C_{aq}} \quad (1)$$

For this purpose, 100.0 mL aqueous solution (0.01 μg mL^{-1}) was passed through the column and the analyte was eluted by 2.0 mL methanol. After evaporation of solvent from 200.0 μL solution, remained material was resolved by 100.0 μL methanol. This solution was injected to the ESI-IMS for determination of the enrichment factor. In this work, the average enrichment factor of 97 was calculated for SA in aqueous solution. The imprinting factor was also calculated from the ratio of the quantity bound by the MIP to the quantity bound by the NIP as described in Eq. (2).

$$IF = \frac{k_d(MIP)}{k_d(NIP)} \quad (2)$$

where k_d is the distribution constant and determined by:

$$k_d = \frac{(C_i - C_f) \times V}{C_f \times m} \quad (3)$$

In Eq. (3), C_i and C_f are the analyte concentrations in the sample before and after extraction, respectively, V is the sample volume passing through the column, and m is the amount of polymer dispersed in column. The calculated IF in this work is 9.3, indicating the acceptable efficiency of bonding in the prepared MIP sorbent for SA.

Another critical step in any MIP-based protocol is the determination of retention capacity, as it allows the high extraction efficiency to be achieved. For determination of retention capacity, 0.055 g of imprinted polymer was added to 100 mL of the analyte standard solution (5.0 μg mL^{-1}), and the IMS signal of the supernatant was explored at different times. The results show that the signal was maximum at the initial time ($t=0$) and decayed rapidly until $t=50$ min. Then, the IMS signal was smoothly decreased with increasing of time and reached to a constant

value after about 280 min. The retention capacity was calculated to be 9.7 mg g^{-1} in this work.

Table 2
Recovery percentages of SA for spiked samples ($n=3$).

Sample	SA added (μg mL^{-1})	SA found (μg mL^{-1})	Recovery (%)
Urine	0.25	0.25 ± 0.04	100
	0.50	0.49 ± 0.09	98
	1.00	0.98 ± 0.01	98
Plasma	0.25	0.24 ± 0.02	96
	0.50	0.48 ± 0.02	96
	1.00	0.98 ± 0.09	98

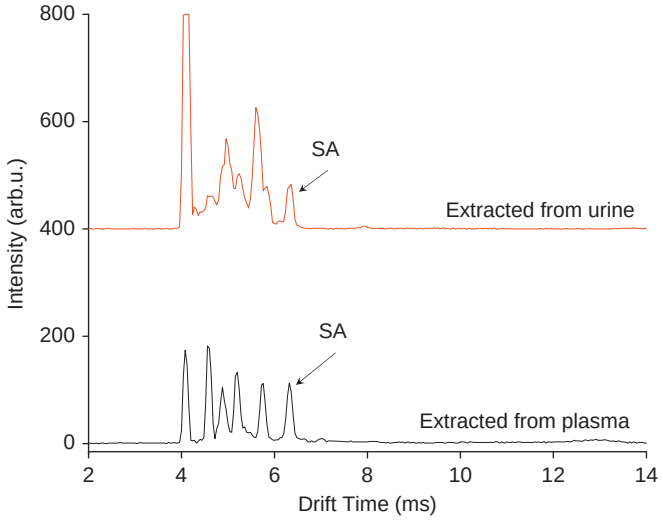

Fig. 5. The ion mobility spectra of SA extracted from urine and plasma obtained from a volunteer after administration of drug, 40% SA.

Table 3
Concentration of SA in diluted urine and plasma of volunteer after administration of drug, 40% SA, obtained using standard addition method ($n=3$).

Sample	SA added (μg mL^{-1})	SA found (μg mL^{-1})	Recovery (%)
Urine	–	0.022 ± 0.005	–
	0.1	0.107 ± 0.005	85
	0.2	0.191 ± 0.002	84
	0.4	0.403 ± 0.002	95
Plasma	–	0.023 ± 0.001	–
	0.1	0.111 ± 0.001	88
	0.2	0.196 ± 0.002	86
	0.4	0.399 ± 0.001	94

3.7. Real sample analysis

To demonstrate the capability of the MIP coupled to negative ESI-IMS system for the analysis of real samples, two categories of real samples including human plasma and human urine were analyzed. Thus, the drug of the samples was extracted according to the procedure described in Section 2.6. In this work, methanol was used for protein precipitation of plasma samples. However, during the extraction of plasma samples with MIP, a column blockage problem arose. To overcome this problem, two steps of protein precipitation were separately accomplished before the MIP separation (Section 2.6). For investigation of the method validation, the collected urine and plasma blanks were spiked by standard solutions of SA and then the analyte was extracted. The recovery values obtained from these experiments are shown in Table 2, revealing the capability of the ESI-IMS for analysis of SA in urine and plasma matrices. In this work, the ion mobility spectra originated from the urine and plasma blanks were also compared with the background of negative ESI-IMS alone (data not shown). According to this comparison, although the peak of chloride ion in the urine blank spectrum is greater than that of background; however, there is no ion peak with the same drift time as that for SA in both spectra of urine and plasma blanks.

For more investigation of the method capability, the urine and plasma obtained after administration by a volunteer were analyzed using standard addition technique. The ion mobility spectra of extracted SA from human urine and plasma samples are shown in Fig. 5. It is clearly observed in this figure that the major interferences of urine and plasma matrixes could be eliminated by MIP system. Ion mobility analysis of real samples was carried out by the previously mentioned method (Section 3.2), the results of which are shown in Table 3. Based on these results, the calculated recoveries for extraction of the analyte from the real samples were satisfactory, and consequently, the proposed combined system of MIP-IMS can be easily employed for the analysis of SA in biological fluid samples.

3.8. Comparison between the new method and other methods

Table 4 shows a comparison between some characteristics such as linear dynamic range, limit of detection, RSD and recovery values obtained in this work and those reported in different literatures involving pre-concentration and detection of SA in biological samples. In most cases, a separation method such as GC or HPLC has been combined with sample pretreatment methods for the analysis of SA. However, since these techniques suffer from several disadvantages such as the need for expensive solvents, long time for analysis, tedious derivatization process, they are not suitable for routine analysis of the samples. In addition, on the basis of the comparisons conducted in this table, it is obviously observed that when the method of MIP is combined

Table 4
Comparison of analytical parameters of the proposed method and other techniques for determination of SA in biological samples.

Analytical method	Sample type	Linear dynamic range (μg mL^{-1})	LOD (μg L^{-1})	RSD[a] (%)	Recovery (%)	Reference
HPLC-UV	Mouse skin	0.2–5.0	50	2.6 (4)	–	[29]
SPE-HPLC–MS	Human plasma	0.013–1.1	7	2.8–4.4(6)	76–81	[6]
Bienzymatic sensor	Cow urine and drugs	0.14–14	69	8 (6)	75–110	[30]
Spectrofluorimetry	Human serum	0.014–14	4.2	2.4 (5)	96–100	[14]
Sequential injection chromatography	Drug	3.1–3.0	1000	0.4–2.0 (8)	98–99	[31]
GC–MS	Human skin	–	50	5 (3)	93–103	[3]
SPME-Voltammetry	Mouse blood	0.069–28	690	1.7 (7)	99	[13]
LC–MS–MS	Horse urine	2.5–50	–	2.8 (6)	–	[4]
MIP-ESI-IMS	Human urine and plasma	0.02–2.0	8	5.4 (6)	84–100	Proposed method

[a] The replicates, n, are indicated in the parentheses.

with negative ESI-IMS, it allows us to have a sensitive and rapid analysis of SA drug with an acceptable recovery and repeatability.

4. Conclusions

This research expands and substantiates the use of the negative electrospray ionization ion mobility spectrometry (ESI-IMS) as a powerful detection technique after selective separation of SA by molecular imprinted polymers (MIP). The results obtained in this research showed that the negative ESI-IMS is effectively capable for providing highly selective and sensitive analysis of SA in biological samples, without using any additional derivatization methods. The analysis time in this method was much shorter than that of the methods such as LC, GC or CE, so, if the MIP sorbent is ready, the extraction and detection of a sample take about one hour. Furthermore, the reliability of this new method was equally acceptable. In addition to portability, IMS is much easier to use and is cost-effective compared to the other methods such as MS.

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