## ORIGINAL PAPER

# Application of ion mobility spectrometry for the detection of human urine

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Abstract The aim of the present study was to evaluate the suitability of ion mobility spectrometry (IMS) for the detection of human urine as an indication of human presence during urban search and rescue operations in collapsed buildings. To this end, IMS with a radioactive ionization source and a multicapillary column was used to detect volatile organic compounds (VOCs) emitted from human urine. A study involving a group of 30 healthy volunteers resulted in the selection of seven volatile species, namely acetone, propanal, 3-methyl-2-butanone, 2-methylpropanal, 4-heptanone, 2-heptanone and octanal,

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Department of Anesthesiology and General Intensive Care, Innsbruck Medical University, Anichstrasse 35, 6020 Innsbruck, Austria which were detected in all samples. Additionally, a preliminary study on the permeation of urine volatiles through the materials surrounding the voids of collapsed buildings was performed. In this study, quartz sand was used as a representative imitating material. Four compounds, namely 3-methyl-2-butanone, octanal, acetone and 2-heptanone, were found to permeate through the sand layers during all experiments. Moreover, their permeation times were the shortest. Although IMS can be considered as a potential technique suitable for the detection, localization and monitoring of VOCs evolved from human urine, further investigation is necessary prior to selecting field chemical methods for the early location of trapped victims.

**Keywords** Ion mobility spectrometry · Volatile organic compounds · Urine markers · Location of trapped victims · Urban search and rescue operations

## Introduction

The early location of victims trapped in collapsed buildings is of particular importance for rescue teams after natural or man-made disasters. Currently, the use of canines is the method of choice in urban search and rescue operations [1]. Canines exhibit excellent scenting skills and are able to search relatively large areas in a short period of time. However, search dogs have a number of limitations: the information they provide must be correctly interpreted, their training is time-consuming and expensive and their working time is relatively short. Moreover, at the disaster scene canines can become temperamental, stressed and even frustrated when scenting dead victims [2]. Therefore, a demand exists for novel technical search tools which could complement or even replace their work.



In this context, knowledge of the human scent profile and its interaction with the disaster environment is crucial. Volatile markers of trapped victims can be released from different biological fluids (urine, blood, sweat) or tissues (skin or lungs). Their emission can be influenced by the medical status of the victim prior to and after collapse and the time for which they have been trapped. The resulting human scent may be modified by numerous interactions with the environment. In addition, the concentrations of species specific for human scent are extremely low, thus making their identification a challenging task.

Urine can be an important source of volatile markers of trapped persons. However, relatively few studies have investigated the profile of volatile organic compounds (VOCs) released spontaneously by non-modified human urine [3–5]. Volatile species emitted by human urine belong to numerous classes, such as hydrocarbons, aldehydes, ketones, furans, pyrroles, terpenes, sulfur-containing compounds and heterocyclic compounds. In a recent study, a set of 33 volatile markers that are omnipresent in human urine released spontaneously at human body temperature were detected and identified using solid-phase microextraction (SPME) and gas chromatography coupled with mass spectrometry (GC-MS) [3]. This set of species can be considered as an initial library to be used for detection of human urine.

For the detection, localization and monitoring of these VOCs in the field, portable, highly sensitive and selective instruments providing a relatively short response time need to be used. Ion mobility spectrometry (IMS) seems to meet these requirements. Currently, the IMS technique is used in biological analyses [6, 7], medical diagnostics [8–12] and food quality measurements [13, 14]. The IMS devices can be quite small and held in the hand. Portable IMS instruments are commonly used for the detection of chemical warfare agents and illegal drugs [15–17].

IMS consists of an ionization chamber, an ion-molecule injection shutter, an ion drift tube and an ion collector (Faraday plate) [8]. Different methods are used to ionize gas; usually radioactive sources such as <sup>63</sup>Ni are employed [8, 10]. An alternative way is the UV light [18, 19]. A carrier gas, usually air or nitrogen, introduces analyte molecules into the ion source where they undergo ionization. Afterwards, the ions are injected by opening an ion shutter into the drift region and separated according to ion mobility differences [8].

IMS is often coupled with standard gas chromatographic columns [18] or multicapillary columns (MCC) [8] for enhancing the separation of analysis. MCC characterize comparatively high flow rate and high sample capacity in comparison to single narrow columns [8].

The main goals of this study were firstly the evaluation of the suitability of the IMS technique for the detection of human urine emitted VOCs with or without permeation through quartz sand imitating trapment materials and secondly the selection of the most promising volatile urine markers which could be detected with this technique.

# **Experimental**

## Instrumentation

An ion mobility spectrometer (ISAS-Institute for Analytical Sciences, Dortmund, Germany) with a radioactive ionization source (<sup>63</sup>Ni) was applied for urine analysis. The principal parameters for the IMS are summarized in Table 1.

The device was equipped with a 20-cm-long polar multicapillary column (MCC; OV-5, Sibertech, Novosibirsk, Russia). The MCC consists of approximately 1,200 capillaries, with an inner diameter of 40  $\mu m$  and a film thickness of 0.2  $\mu m$  coupled to the  $^{63}\text{Ni-IMS}$  system. The flow rate of the carrier gas was 100 mL/min. Separation of analytes was performed isothermally at 30 °C. Gas samples were injected using a 10-mL sample loop installed on the six-way valve.

Each final ion mobility spectrum was an average of five measurements recorded for 100 ms with a frequency of 25,000 samples per second. Such a setting provided two ion mobility spectra per second of analysis. Further details on the IMS system applied are given elsewhere [20–22].

## Chemicals and standards

Identification parameters (drift times and retention times) were obtained on the basis of calibration mixtures prepared from pure compounds. An effort was made to prepare a separate standard for each compound. Primary standards

**Table 1** Parameters for multicapillary column (MCC) ion mobility spectrometry (IMS)

Parameter	MCC- <sup>63</sup> Ni-IMS				
Ionization source	<sup>63</sup> Ni (550 MBq)				
Length of the drift tube	120 mm				
Electrical field strength	333 V/cm				
Drift voltage	4 kV				
Shutter opening time	300 μs				
Drift gas	N <sub>2</sub> (99.9999%)				
Drift gas flow	100 mL/min				
Temperature (IMS)	23 °C (ambient)				
Pressure	950 hPa (ambient)				
Stationary phase of the MCC	OV-5 (polar)				
Temperature (MCC)	30 °C				



were prepared in a 1-L glass gas bulb (Supelco, Canada). Prior to use, the bulb was thoroughly cleaned with methanol and dried at 70 °C for at least 12 h. Afterwards, it was purged with pure nitrogen (99.9999%) for 20 min. Then, the bulb was evacuated using a vacuum pump and approximately 1 µL of pure compound was injected through a rubber septum. After the evaporation of the compound, the bulb was balanced with nitrogen. The final standard was prepared by transferring an appropriate volume of primary standard into a 3-L Tedlar bag (SKC, USA) filled with 1 L of pure nitrogen. The final concentrations of the analytes under study in secondary standards ranged from 20 to 80 ppb. A number of standards were purchased from Sigma-Aldrich (Vienna, Austria); 2heptanone (98%), 2-methyl-1-propanal (99%), 3-hexanone (98%), 3-methyl-2-pentanone (99%), dimethyl disulfide (99%), ethyl acetate (99.9%), furan (99%), hexanal (98%), methyl acetate (99.5%), pentanal (97%) and toluene (99.8%), 2-Butanone (99.5%), 2-methylbutanal (99%), 2pentanone (99%), 3-methyl-2-butanone (98.5%), 4-methyl-2-pentanone (99.7%), dimethyl sulfide (99%), dimethyl sulfone (98%) and isoprene (99.5%) were obtained from Fluka (Sigma-Aldrich, Vienna, Austria). Moreover, 2methyl-2-butenal (96%), 3-penten-2-one (better than 70%) and dimethyl trisulfide (98%) were provided by SAFC (Vienna, Austria), 3-methylfuran (98%), 4-heptanone (98%) and propanal (97%) were provided by Acros Organics (Fisher Scientific, Vienna, Austria), acetone (99.5%) by was provided by Merck (Merck, Vienna, Austria) and 1,2,3-trimethylbenzene (98%) was purchased from ChemSampCo (Vienna, Austria).

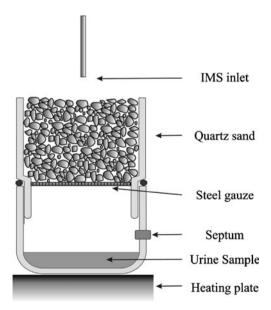


Fig. 1 The filling chamber. IMS ion mobility spectrometer

Table 2 Demographic data of volunteers

	Male	Female
Number of volunteers	20	10
Mean age (range) (years)	34 (22–53)	27 (23–29)
Number of smokers	2	2
Urine type		
Morning urine	12	9
Spontaneous urine	7	2

# Urine sampling

The urine collection was approved by the Ethics Commission of Innsbruck Medical University. Volunteers' urine was collected in three 10-mL plastic urine monovette vessels (Sarstedt, Germany), immediately after the volunteers had urinated. Prior to their use, urine monovettes were rinsed for 4 h with purified air at 50 °C to reduce possible contaminant emission during transport and storage. An effort was made to limit the urine storage in the urine monovettes to 3 h. Two types of urine were sampled: morning urine, which is expected to exhibit higher VOCs levels as it is more concentrated (particularly when the subject is dehydrated), and spontaneous urine.

## Detection of VOCs in the headspace of human urine

To investigate the possible interaction (e.g. overlapping, signal suppressing) between evolved urine VOCs, a series of measurements of the urine headspace was performed. They were accomplished using 1-L glass flasks. The flasks were thoroughly cleaned prior to the experiment and flushed with pure nitrogen to remove contaminants. Afterwards, 2 mL of urine sample was transferred from the monovette into the flask. The temperature of the glass flask was maintained at 36–37 °C to investigate the emission of VOCs at human body temperature. After 5 min, 10 mL of the headspace gas was introduced into the sample loop and analysed by MCC/IMS. Each measurement was repeated three times.

# Detection of VOCs permeating through debris material

In the ruins of collapsed buildings, volatiles are carried out by air currents and spread as plumes throughout the rubble; these plumes are either constant or transient. Consequently, interactions of VOCs with the debris materials (e.g. dust, wood, plastic, glass) are expected to significantly modify the urine scent. In this context, the permeative properties of urine markers have a crucial influence on the success of the search and rescue operation. A preliminary study on this aspect was done with a filling chamber as presented in



Table 3 Identification parameters of ubiquitous volatile organic compounds (VOCs) in human urine with their proton affinities [23]

Name	CAS no.	Proton affinity (kJ/mol)	Peak	Retention time (s)	Reduced ion mobility (cm <sup>2</sup> /Vs)	
1,2,3-Trimethylbenzene	526-73-8	no data	I	41.4	1.77	
2-Butanone	78-93-3	827.3	I	5.5	1.87	
			II	5.6	1.61	
2-Heptanone	110-43-0	No data	I	19.1	1.57	
			II	19.1	1.47	
			III	19.1	1.23	
2-Methyl-1-propanal	78-84-2	797.3	I	6	1.74	
			II	6	1.66	
2-Methyl-2-butenal	1115-11-3	843.7	I	8	1.81	
•			II	8	1.48	
2-Methylbutanal	96-17-3	No data	I	7	1.66	
2-Pentanone	107-87-9	832.7	I	8	1.77	
			II	8	1.58	
			III	11.3	1.46	
3-Hexanone	589-38-8	843.2	I	9	1.71	
			II	8	1.44	
			III	9	1.38	
3-Methyl-2-butanone	563-80-4	836.3	I	5	1.8	
			II	5	1.65	
			III	5.5	1.48	
3-Methyl-2-pentanone	565-61-7	No data	I	8.3	1.69	
			II	8.3	1.35	
3-Methylfuran	930-27-8	854	I	9	1.87	
	<i>350 27 0</i>		II	8.3	1.79	
			III	8.3	1.57	
			IV	8.3	1.45	
3-Penten-2-one	625-33-2	864.7	I	8	1.71	
			II	8	1.44	
			III	8.9	1.38	
4-Heptanone	123-19-3	845	I	15.6	1.62	
Тершпоно	120 17 0	0.0	II	18	1.48	
			III	14.5	1.27	
4-Methyl-2-pentanone	108-10-1	No data	I	8.9	1.7	
1 Methyl 2 pentanone	100 10 1	110 data	II	6.7	1.55	
			III	7.8	1.36	
Acetone	67-64-1	812	I	5.6	1.78	
Allyisothiocyanate	57-06-7	No data	Not measu		11,70	
Dimethyl disulfide	624-92-0	815.3	In RIP area			
Dimethyl sulfide	75-18-3	830.9	In RIP area			
Dimethyl sulfone	67-71-0	No data	In RIP area			
Dimethyl trisulfide	3658-80-8	No data	In RIP area			
Ethyl acetate	141-78-6	835.7	I I	6.6	1.49	
Furan	110-00-9	812	Not detecte		1.17	
Hexanal	66-25-1	No data	I	8.5	1.56	
Tionului	00-25-1	110 data	II	10	1.29	
Isoprene	78-79-5	826.4	I	44.2	1.65	
Tooptelle	10-17 <del>-</del> 3	020.7	II	44.2	1.53	
Isothiocyanocyclohexane	1122-82-3	No data	Not measu		1.JJ	
тьонносуаносуснонехане	1122-02-3	ino data	inoi illeasu	icu		



Table 3 (continued)

Name CAS no. Proton affinity (kJ/mol)		Peak	Retention time (s)	Reduced ion mobility (cm <sup>2</sup> /Vs)	
Methanethiol	74-93-1	773.4	Not detect	ed	_
Methyl acetate	79-20-9	821.6	I	6.1	1.68
N-Methylpyrrole	96-54-8	No data	Not measu	ıred	
Octanal	124-13-0	No data	I	43	1.4
			II	43	1.35
			III	43	1.11
Pentanal	110-62-3	796.6	I	6.7	1.64
			II	6.7	1.41
Propanal	123-38-6	786	I	1.1	1.75
•			II	1.1	1.71
Pyrrole	109-97-7	875.4	I	45.5	1.21
Toluene	108-88-3	784	Not detect	ed	

RIP reactant ion peak

Fig. 1. The chamber consists of a stainless steel cylinder with an open upper end having an internal diameter of 102 mm and a height of 81 mm. Steel gauze (mesh size 1 mm) divides the chamber into two parts (each 40 mm high). The top part was loosely packed with mimicking

debris quartz sand (Euroquarz, Germany) having a bulk density of 1.6 g/cm<sup>3</sup> and a particle of density 2.65 g/cm<sup>3</sup>. Three sizes of grain were tested: 0.5–1 mm, 1–2 mm and 4–8 mm. In the wall of the bottom part, an additional rubber septum (Supelco, Canada) was installed. The

**Table 4** VOCs identified in the headspace of human urine

Compound	Peak	Male		Female			
	number	Morning urine (12 samples)	Spontaneous urine (7 samples)	Morning urine (9 samples)	Spontaneous urine (2 samples)		
Acetone	I	12	7	9	2		
Propanal	I	12	7	9	2		
3-Methyl-2-butanone	I	12	7	9	2		
2-Methylpropanal	I	12	7	9	2		
Pentanal	I	10	2	2	1		
	II	8	3	3	2		
2-Pentanone	I	2	3	4	1		
	II	10	6	7	2		
2-Butanone	I	5	2	2	1		
3-Penten-2-one	I	9	5	5	1		
	II	11	6	7	2		
Hexanal	I	10	7	8	2		
4-Heptanone	I	12	7	9	2		
_	II	12	7	9	2		
	III	12	7	9	2		
2-Heptanone	I	12	7	9	2		
1,2,3- Trimethylbenzene	I	6	5	8	2		
Isoprene	I	8	2	3	0		
	II	5	5	5	1		
Octanal	I	12	7	9	2		



bottom of the filling chamber was kept at 30–31 °C. At the onset of the experiment, 1 mL of urine sample was injected through the septum. Permeation of VOCs through the quartz sand was studied by the MCC/IMS analyses of the headspace samples taken approximately 1 cm above the sand layer in the direction of the central axis of the chamber. The time instants for drawing the samples were defined as follows: one sample was taken before the urine injection; the next ones were drawn every 10 min for 180 min. For each size of grain, five experiments with urine of different volunteers were performed.

## Human subjects

A cohort of 30 healthy normal volunteers (20 males, 10 females) was recruited. All subjects gave informed consent to participate and completed a questionnaire describing their food and drug intake, health status, smoking status, etc. None of volunteers had urological diseases. Demographic data for the volunteers are presented in Table 2.

## Results and discussion

## Identification parameters

In a recent study, a set of 33 potential volatile urine markers released from unmodified human urine at human body temperature using SPME-GC-MS was identified [3]. The markers within this group were omnipresent in human urine

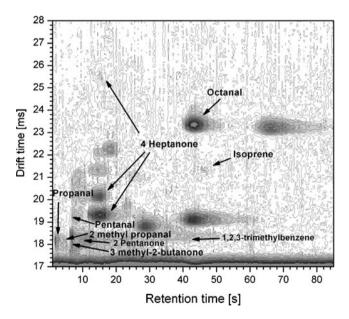


Fig. 2 An exemplary two-dimensional (contour) ion mobility spectrometry chromatogram of a human urine sample



 Table 5
 Filling chamber experimental conditions

Grain size (mm)	No.	Quartz mass (g)	Relative humidity (%)	Temperature (°C)
0.5–1	1	500.0	28.9	23.9
	2	500.0	23.4	23.9
	3	500.6	26.3	24.0
	4	500.3	22.0	24.2
	5	500.0	25.3	24.2
1-2	1	498.3	28.9	22.9
	2	503.6	22.3	23.4
	3	505.3	25.1	22.8
	4	500.0	23.8	23.0
	5	500.0	24.4	23.4
4–8	1	503.9	20.8	22.6
	2	503.0	23.5	22. 6
	3	501.3	26.9	22.8
	4	500.2	25.1	23.3
	5	500.0	21.7	23.8

with incidence higher than 80%. Consequently, the present study focused on the identification of VOCs from this group. The identification parameters—retention times and reduced ion mobilities—are presented in Table 3. The identification parameters for allyisothiocyanate, isothiocyanocyclohexane and N-methylpyrrole were not estimated owing to the lack of pure substances. Peaks for sulfur-containing compounds, namely dimethyl trisulfide, dimethyl sulfone, dimethyl sulfide and dimethyl disulfide, overlapped with the reactant ion peak. Consequently, these compounds could not be measured with the MCC/IMS system. However, under favourable conditions signals for these compounds could be extracted from the reactant ion peak using sophisticated algorithms. Furan, toluene and methanethiol were not detected. Finally, the original set of 33 potential urine markers was constrained to 23 compounds detectable with the IMS instrument. This set of VOCs served as a basic marker library for further investigations.

#### Detection of VOCs in the headspace of human urine

The compounds identified in the headspace of urine samples are presented in Table 4 together with their incidence. The compound was considered as detected if it was found at least two times out of three in the volunteer's urine. Urine of 30 volunteers was analysed: 21 morning urine samples and nine spontaneous urine samples. An exemplary two-dimensional IMS chromatogram of human's urine is shown in Fig. 2. Fourteen compounds were identified in the urine samples. Seven of them, namely

Table 6 Compounds detected above the quartz sand layer together with their permeation times in minutes

Compound	Grain	size (n	nm)												
	0.5–1					1–2				4-8					
	1	2	3	4	5	1	2	3	4	5	1	2	3	4 <sup>a</sup>	5 <sup>a</sup>
Acetone	40	40	40	40	40	30	30	30	30	30	20	20	20	70	-
Propanal	40	-	-	40	-	30	30	-	-	-	30	40	-	-	-
3-Methyl-2-butanone	40	40	40	40	40	30	30	30	30	30	20	20	20	40	50
2-Methylpropanal	40	50	40	40	40	30	30	50	30	-	40	-	-	70	110
Hexanal	-	-	-	-	40	-	-	30	30	-	30	-	-	-	-
4-Heptanone	-	-	130	110	-	70	70	70	60	60	60	60	-	-	-
2-Heptanone	130	90	80	90	40	80	90	80	70	70	50	50	80	-	110
1,2,3-Trimethylbenzene	-	-	-	-	170	50	50	40	40	40	30	-	50	160	-
Isoprene	150	-	-	100	90	-	110	100	90	100	70	70	10	170	-
	150	-	-	100	50	60	90	100	60	60	70	70	10	150	-
Octanal	40	50	40	50	40	30	30	30	30	30	20	20	20	70	50
Pyrrole	-	-	-	-	-	90	90	120	120	100	70	70	20	-	-

<sup>&</sup>lt;sup>a</sup> Spontaneous urine

acetone, propanal, 3-methyl-2-butanone, 2-methylpropanal, 4-heptanone, 2-heptanone and octanal, were found in all samples. A further two, hexanal and 2-pentanone, were detected in at least 80% of samples. No significant

Pyrrole
Octanal Isoprene
Acetone

Acetone

Acetone

Acetone

18

Acetone

Acetone

18

Acetone

**Fig. 3** Three-dimensional view of an ion mobility spectrometry spectrum showing volatile compounds of human urine sample which had passed through the layer of quartz sand (1–2 mm)

differences between the morning urine and the spontaneous urine were observed.

Identification of compounds which pass through rubble

In this experiment, the quartz sand was exposed to VOCs released by unmodified human urine. The mass of quartz, the air temperature and the humidity during the experiments are summarized in Table 5. The VOCs detected in the chamber headspace are listed in Table 6 together with their permeation times. The experimental conditions were comparable. Relative humidity ranged from 20.8 to 28.9% (median 24.4%), whereas ambient temperature was between 22.6 and 24.2 °C (median 23.4 °C). Eleven compounds were identified in the air above the quartz layer during the 3-hlong experiments. Ketones were represented by acetone, 3methyl-2-butanone, 2-heptanone and 4-heptanone. Another well-represented class was aldehydes, also with four representatives: propanal, 2-methylpropanal, hexanal and octanal. Within the remaining species there was one aliphatic hydrocarbon (isoprene), one aromatic hydrocarbon (1,2,3trimethylbenzene) and one heterocyclic compound (pyrrole). Interestingly, pyrrole, which was not detected in the headspace of urine samples, was found to permeate through the quartz layer. This observation could be explained by a suppression of the pyrrole signal in the IMS owing to the presence of other VOCs with significantly higher proton affinities and a similar retention time. Two compounds, namely 3-methyl-2-butanone and octanal, were detected during all experiments, and a further two, acetone and 2heptanone, were detected in all samples but one. The shortest



permeation times were noted for acetone, propanal, 3-methyl-2-butanone and octanal. 4-Heptanon, which is among the most abundant VOCs present in human urine [4, 5], was detected only in nine experiments. In general, the permeation times increased with the decrease of the quartz grain size. However, it must be remembered that the urine samples used during the experiments originated from different volunteers and the initial VOC concentrations were different. An exemplary three-dimensional IMS chromatogram of a human urine sample taken over the quartz sand is presented in Fig. 3.

## Conclusion

The present study aimed at evaluating the suitability of IMS for the detection of human urine during rescue operations after earthquakes or other disasters. For this purpose, IMS with a β-radiation source (<sup>63</sup>Ni) and a MCC was applied. Twenty-three VOCs which are ubiquitous in human urine (according to previous SPME-GC-MS studies [3]) were detected with this technique. Fourteen compounds from this group were found in the headspace of human urine; however, only seven were omnipresent. Additionally, the IMS technique was used to study the permeation of urine vapour through the mimicking rubble quartz sand. Eleven compounds were found to permeate through the quartz layer. Within this group, four compounds, namely acetone, 3methyl-2-butanone, 2-heptanone and octanal, are of particular interest as they were commonly detected above the quartz layer exposed to the urine vapours and additionally exhibited the shortest permeation times. These compounds can be regarded as very promising markers of human urine.

Within this context, IMS can be considered as a potential technique suitable for the detection, localization and monitoring of VOCs evolved from human urine for the location of victims trapped in collapsed buildings. The main advantages of this technique are its good sensitivity (up to parts per trillion level), the short time of analysis and the potential for miniaturization. However, the trapped environment in collapsed buildings and especially in major disasters is extremely complicated and sometimes dominated by dust and smoke. Consequently, further investigation is necessary prior to selecting field chemical methods for the early location of trapped victims.

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