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SHORT COMMUNICATION

Urine headspace analysis in medical diagnostics

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ABSTRACT

The analysis of the volatile organic compounds in the bodily fluids such as urine may provide useful information on the patients' general health. The potential application of urine headspace analysis could facilitate the diagnostics of various diseases such as metabolic disorders, urinary tract diseases and gastrointestinal conditions. Described in this paper are the currently available techniques of urine sample analysis as well as their different application in medical diagnostics.

Keywords**:** medicine, urine, VOC, biomarkers, medical diagnostics

1. INTRODUCTION

The urine headspace analysis has gained an increasing interest due to the possibility of its use in the assessment of patients' general health, as well as in the identification of various conditions. However, urine odour analysis may be one of the oldest methods of disease detection. Already in Ancient Greece, physicians knew that it can provide clues about the patient's condition and thus, facilitate the diagnostics process. The presence of various urinary tract infections may result in the change of the urine headspace composition. It has been

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established that dogs are able to distinguish between urine samples collected from cancer patients and healthy controls [1,2]. Moreover, in the case of conditions such as the fish-odour syndrome and the maple syrup urine disease, the change of the smell is so distinctive that is often used as a diagnostics tool [3]. Urine is an organic fluid composed of numerous constituents, the composition of which may vary based on numerous factors such as gender, age or diet [4–6]. Since urine headspace composition is affected by both exogenous and endogenous factors [7,8], urinary volatiles analysis may provide information on absorption of certain substances from the environment and the processes taking place inside the organism [9–11]. For this reason, numerous studies have been performed in order to evaluate whether the determination of the concentration of volatile organic compounds (VOCs) present in the urine headspace can be used in medical diagnostics.

2. SAMPLES ANALYSIS

2. 1. Gas Chromatography

There are several technologies currently used in the analysis of urine headspace composition, some of which are listed in Table 1. However, gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC-MS) are the gold standards, widely used in the diagnostics-related studies. They have been successfully applied in the investigation of diseases mechanisms, as well as in the qualitative and quantitative analysis of volatile organic compounds (VOCs) present in urine. Moreover, there are also ongoing studies on the possibility of using gas chromatography to diagnose diseases such as tuberculosis (TB) or diabetes (DM2) [9–11].

Tuberculosis is a potentially deadly, infectious disease caused by *Mycobacterium tuberculosis*. Due to the possibility of its asymptomatic development, it is not possible to diagnose TB solely on the basis of its symptoms. Currently, its diagnosis is made based on the tuberculin skin test, however, its reliability may vary since the use of certain drugs, impairment of the immune system or the course of other diseases can cause false-positive or negative results [12,13]. For these reasons, numerous efforts are underway in order to apply modern techniques such as gas chromatography as a non-invasive method of tuberculosis diagnosis. The results of the studies suggest that the use of urine analysis will make it possible to distinguish between patients with TB and healthy controls or patients suffering from respiratory diseases such as chronic obstructive pulmonary disorder [11].

Type 2 diabetes is a metabolic disorder in which blood sugar levels are elevated due to the insulin resistance. Currently, blood tests are used in order to diagnose DM2 and to determine blood glucose levels during treatment. However, since some of the changes in the concentration of various metabolites caused by diabetes are detectable not only in serum but also in urine, numerous studies have been conducted in order to evaluate whether it might be possible to diagnose DM2 based on the analysis of urine composition. In the study conducted using GC-MS, several volatile organic compounds present in the sample's liquid fraction have been identified as potential biomarkers of diabetes [9,14].

2. 2. Ion Mobility Spectrometry

Ion mobility spectrometry (IMS) is an analytical technique in which the separation and identification of ions are made based on the differences in their mobility. IMS devices are usually equipped with a drift tube (DTIMS) in which the ions move in a uniform electric field through the inert gas. The speed of drift depends on the intensity of the electric field as well as on the mobility of the ions and, therefore, it may be used to separate and identify the components of the sample. Moreover, alternative ion mobility techniques such as travelling wave (TWIMS) and field asymmetric wavelength IMS (FAIMS) may be used in diseases-related studies [15].

With the use of ion mobility spectrometry, it is possible to obtain the results of the analysis in a much shorter time than in the case of e.g. gas chromatography. In addition, portable IMS analysers may be used in the fast, noninvasive diagnosis of disorders such as dysbiosis or bowel diseases.

Gut dysbiosis is a condition in which the composition of the gastrointestinal microbiota is altered. The microbial imbalance may be caused by dietary changes, repeated exposure to antibiotics or conditions such as infections and inflammations. Since chronic dysbiosis can lead to the growth of harmful bacteria and in effect cause diseases, it is essential that the microbial imbalance is quickly diagnosed and treated. However, gut dysbiosis lacks specific symptoms and its diagnosis is rather difficult as it may be confused with numerous bowel diseases [16,17]. Because of that, it is investigated whether gut dysbiosis can be diagnosed based on the analysis of volatile organic compounds present in urine's headspace. Arasaradnam *et al.* found with the use of FAIMS, that the loss of bacterial diversity results in significant changes in the concentration of volatile organic compounds [18].

Inflammatory bowels diseases (IBDs) such as Crohn's disease and ulcerative colitis are a group of idiopathic inflammatory bowel disorders. They are one of the most common conditions related to the gastrointestinal tract, their prevalent symptoms are abdominal pain and altered bowel habits, however, they may be associated with other more or less specific symptoms. The gold standard in the diagnostics of IBDs is the biopsy of the pathological tissues, nevertheless, this technique is invasive and rather unsuitable for screening purposes.

Another approach is the analysis of faeces, namely determination of calprotectin in stool samples. It is routinely used in both diagnostics and monitoring of bowel disorders, however, it was suggested that the use of non-steroidal anti-inflammatory drugs such as aspirin cause the increase of the calprotectin levels which may result in the false-positive results [19]. Moreover, due to the taboo associated with the faeces sampling, it is possible that patients might be reluctant to participate in screening and diagnostic procedures [20,21]. Because of that, numerous studies have been performed in order to evaluate whether inflammatory bowel diseases can be diagnosed using the analysis of urine's headspace since the collection of urine has much higher acceptance rate among the patients [21]. The results of the recent studies suggest that the diagnosis of IBD may be possible using field asymmetric wavelength IMS [22,23]. The research indicates that it may be possible to distinguish not only between the samples collected from the healthy controls and from patients suffering from inflammatory bowel disease but also to differentiate between patients experiencing remission and those with an active disease [24,25].

2. 3. Selected ion flow tube mass spectrometry

Selected ion flow tube-mass spectrometry (SIFT-MS) is a technique in which the volatile compounds' ionisation is achieved through reactions with precursor ions such as H_3O^+, NO^+ or O_2^+. The sample is introduced along with the inert gas stream into the analyser, where the volatiles undergo ionization.

Then, the quantitative analysis of the resulting ions takes place in the quadrupole mass analyser (qMS). With the use of SIFT-MS device (Figure 1), direct and immediate analysis of numerous compounds may be performed simultaneously which may facilitate not only the diagnosis of diseases but also the continuous assessment of organ function. Because of its advantages, selected ion flow tube-mass spectrometry is used in various clinical studies e.g. in order to determine levels of acetone and ammonia in urine's headspace collected from women during ovulation [26] or to evaluate whether the increased concentration of acetonitrile in the headspace of urine may be related to recent smoking behaviour [27]. Moreover, the potential use of SIFT-MS in the diagnosis of immune thrombocytopenia (ITP) has been recently investigated.

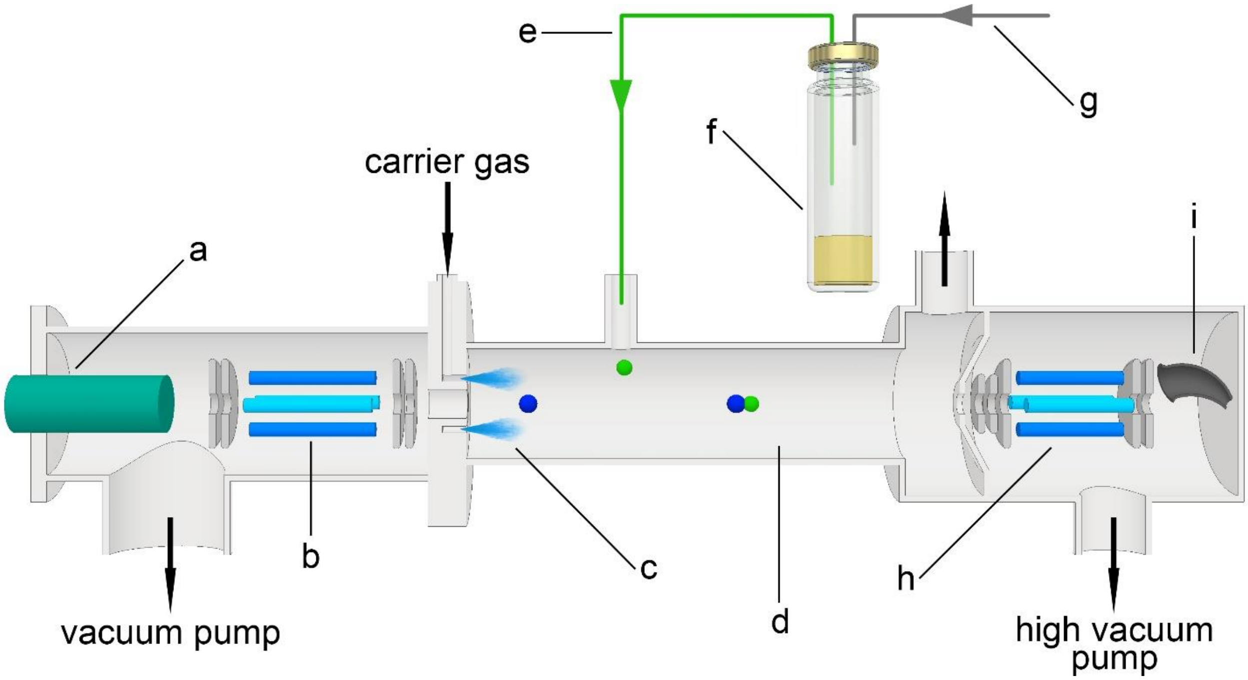

Figure 1. Schematic representation of SIFT-MS device: a) electrical discharge ion source, b) injection qMS, c) helium flow, d) flow tube, e) analytes, f) urine sample, g) carrier gas, h) qMS, i) channeltron.

ITP is an idiopathic, autoimmune disorder characterized by abnormally low levels thrombocytes and mucocutaneous haemorrhages. It is estimated that 5 in 100,000 children may suffer from this disease, though since thrombocytopenia may be asymptomatic or give only mild symptoms it is often undiagnosed. Moreover, its diagnosis is usually made by exclusion of other disorders as there is no targeted test for ITP [28].

However, recent studies have shown that it is possible to distinguish between the urine of healthy subjects and patients with immune thrombocytopenia using SIFT-MS with the accuracy of 88% [29]. Based on the obtained results it is possible to say that selected ion flow tube-mass spectrometry may find its application in the diagnostics of ITP.

2. 4. Electronic nose

An electronic nose (e-nose) is a device designed for rapid analysis of mixtures of volatile chemical compounds using statistical methods. Depending on its application, e-noses may employ a set of chemical sensors, ultrafast-gas chromatography or mass spectrometry. Data obtained through their use is compared with the data present in the database, which make it possible to distinguish between the headspace of samples collected from healthy controls and from those suffering from a given disease. So far, various studies have been conducted on the subject of the use of an electronic nose in order to diagnose urinary tract diseases. The possibility of using e-nose to diagnose prostate cancer has been investigated. Prostate cancer is one of the most common types of cancer among men. Initially, its development can be asymptomatic, and the symptoms appearing at the later stages of the disease are usually not characteristic which makes it difficult to make a diagnosis. Currently, it is diagnosed primarily based on a histopathological assessment of tumour sections. However, the results of the research suggest that the use of urine headspace analysis using e-nose will make it possible to distinguish between patients with prostate cancer and healthy patients or patients suffering from other urinary tract diseases [25,30,31].

Table 1. Advantages and disadvantages of the use of selected instrumental techniques used in the urine headspace analysis.

Technique	Advantages	Disadvantages
GC-MS	▪ Qualitative and quantitative analysis ▪ High sensitivity	▪ Sample preparation is needed ▪ Long time of an analysis ▪ Complexity of use
IMS	▪ Direct analysis ▪ Portability ▪ High sensitivity	▪ No complete profile analysis ▪ Complex data analysis
SIFT-MS	▪ Qualitative and quantitative analysis ▪ Real-time analysis ▪ Direct analysis	▪ Lack of portability ▪ Complexity of use ▪ High cost
E-nose	▪ Short time of an analysis ▪ Low cost ▪ Ease of use ▪ Portability	▪ No possibility of qualitative and quantitative analysis ▪ Only a holistic analysis

3. CONCLUSIONS

The possibility of the application of volatile organic compounds analysis in medical diagnostics has gained considerable attention, due to a variety of VOCs being reported as potential disease biomarkers. Since they are often present in the headspace of urine and other bodily fluids, their analysis may be performed with the use of various techniques that do not require time-consuming sample preparation. The patients' rate of acceptability for urine samples collection is much higher than in the case of faeces which may facilitate its application in screening and diagnostics procedures. Moreover, using urine's headspace analysis is non-invasive and, in contrast to blood collection, it does not cause discomfort to the patient being examined. Hence, a quick, non-invasive analysis of urine headspace may be routinely performed in a clinical environment.

References

[1] C.M. Willis et al., Olfactory detection of human bladder cancer by dogs: proof of principle study, *Bmj* 329 (7468) (2004) 712–717.

[2] J.-N. Cornu et al., Olfactory Detection of Prostate Cancer by Dogs Sniffing Urine: A Step Forward in Early Diagnosis, *Eur. Urol.* 59 (2) (2011) 197–201.

[3] D.G. Burke et al., Profiles of Urinary Volatiles from Metabolic Disorders Characterized by Unusual Odors, *Clin. Chem.* 2910 (10) (1983) 1834–1838.

[4] I. Demkowska, Ż. Polkowska, and J. Namieśnik, Non-invasive biological fluid matrices analysed to assess exposure to environmental tobacco smoke, *J. Expo. Sci. Environ. Epidemiol.* 21 (6) (2011) 656–661.

[5] Ż. Polkowska et al., Biological Fluids as a Source of Information on the Exposure of Man to Environmental Chemical Agents, *Crit. Rev. Anal. Chem.* 34 (2) (2004) 105–119.

[6] K. Kozłowska, Ż. Polkowska, and J. Namieśnik, Effect of treated swimming pool water on the levels of trihalomethanes in swimmer's urine, *Toxicol. Environ. Chem.* 88 (2) (2006) 259–272.

[7] Ż. Polkowska et al., Relationship between volatile organohalogen compounds in drinking water and human urine in Poland, *Chemosphere* 53 (8) (2003) 899–909.

[8] N. Jakubowska et al., Procedure of determination of volatile trihalomethanes in human urine with pervaporation and gas chromatography, *Int. J. Environ. Anal. Chem.* 87 (6) (2007) 449–457.

[9] K. Yuan et al., A GC-based metabonomics investigation of type 2 diabetes by organic acids metabolic profile, *J. Chromatogr. B* 850 (2007) 236–240.

[10] M. Phillips et al., Breath biomarkers of active pulmonary tuberculosis, *Tuberculosis* 90 (2) (2010) 145–151.

[11] K.M. Banday et al., Use of Urine Volatile Organic Compounds To Discriminate Tuberculosis Patients from Healthy Subjects, *Anal. Chem* 83 (2011) 5526–5534.

[12] R.E. Huebner, M.F. Schein, and J.B.J. Bass, The tuberculin skin test, *Clin. Infect. Dis.* 17 (6) (1993) 968–975.

[13] G.M. Lordi and L.B. Reichman, Tuberculin Skin Testing, *in* Schlossberg D. (Ed.), Clin. Top. Infect. Dis., 1st ed., *Springer*, New York, (1988).

[14] K.K. Pasikanti, P.C. Ho, and E.C.Y. Chan, Gas chromatography/mass spectrometry in metabolic profiling of biological fluids, *J. Chromatogr. B* 871 (2008) 202–211.

[15] S. Armenta, M. Alcala, and M. Blanco, A review of recent, unconventional applications of ion mobility spectrometry (IMS), *Anal. Chim. Acta* 703 (2011) 114–123.

[16] M. Zeng, N. Inohara, and G. Nuñez, Mechanisms of inflammation-driven bacterial dysbiosis in the gut, *Mucosal Immunol.* 10 (2017) 18–26.

[17] W.H. Moos et al., Microbiota and Neurological Disorders: A Gut Feeling, *Biores. Open Access* 5 (1) (2016) 137–145.

[18] R.P. Arasaradnam et al., Evaluation of gut bacterial populations using an electronic e-nose and field asymmetric ion mobility spectrometry: further insights into 'fermentonomics,' *J. Med. Eng. Technol.* 36 (7) (2012) 333–337.

[19] J.P. Gisbert and A.G. Mcnicholl, Clinical Review Questions and answers on the role of faecal calprotectin as a biological marker in inflammatory bowel disease, *Dig. Liver Dis.* 41 (2009) 56–66.

[20] S. Jewitt, Geographies of shit: spatial and temporal variations in attitudes towards human waste, *Prog. Hum. Geogr.* 35 (5) (2011) 608–626.

[21] E. Westenbrink et al., Development and application of a new electronic nose instrument for the detection of colorectal cancer, *Biosens. Bioelectron.* 67 (2015) 733–738.

[22] D.C. Baumgart and S.R. Carding, Inflammatory bowel disease: cause and immunobiology, *Lancet* 369 (9573) (2007) 1627–1640.

[23] S.B. Hanauer, Inflammatory bowel disease: epidemiology, pathogenesis, and therapeutic opportunities, *Inflamm. Bowel Dis.* 12 Suppl 1 (2006) S3-9.

[24] R.P. Arasaradnam et al., A Novel Tool for Noninvasive Diagnosis and Tracking of Patients with Inflammatory Bowel Disease, *Inflamm. Bowel Dis.* 19 (5) (2013) 999–1003.

[25] L. Capelli et al., Application and Uses of Electronic Noses for Clinical Diagnosis on Urine Samples: A Review, *Sensors* 16 (10) (2016) 1708.

[26] A.M. Diskin, P. Spaněl, and D. Smith, Increase of acetone and ammonia in urine headspace and breath during ovulation quantified using selected ion flow tube mass spectrometry, *Physiol. Meas. Physiol. Meas* 24 (24) (2003) 191–191.

[27] G.-M. Pinggera et al., Urinary acetonitrile concentrations correlate with recent smoking behaviour, *BJU Int.* 95 (3) (2005) 306–309.

[28] B. Zhou et al., Multi-dysfunctional pathophysiology in ITP, *Crit. Rev. Oncol. Hematol.* 54 (2) (2005) 107–16.

[29] C.A. Batty et al., Differences in microbial metabolites in urine headspace of subjects with Immune Thrombocytopenia (ITP) detected by volatile organic compound (VOC) analysis and metabolomics, *Clin. Chim. Acta* 461 (2016) 61–68.

[30] M. Bernabei et al., A preliminary study on the possibility to diagnose urinary tract cancers by an electronic nose, *Sensors Actuators B Chem.* 131 (1) (2008) 1–4.

[31] A. D 'Amico et al., A novel approach for prostate cancer diagnosis using a gas sensor array, *Procedia Eurosensors* XXVI, (2012): pp. 1113–1116.