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Review

Analytical Procedures Used in Examining Human Urine Samples

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Abstract

The source of information about the state of our environment can be both the abiotic part of the environment (water, soil, air) and samples of the biotic part, including tissues and body fluids of humans, who are continuously exposed to a wide spectrum of xenobiotic chemicals.

The investigation of human body fluids (mainly blood and urine) can be a useful and interesting way to obtain information about the state of the environment.

However, in order to examine the composition of physiological fluids such as urine or blood, collected samples have to be pretreated prior to final determination, because they have a very complex matrix, precluding direct determination of analytes by any of the available analytical methods.

This paper is a review of the literature regarding:

- analyte isolation and/or enrichment techniques from samples of human urine prior to their final determination;
- final determination methods for a wide variety of analytes (both organic and inorganic) in urine samples.

The review includes both the primary form of pollutants that entered the human body as a result of environmental or workplace exposure and the products of their metabolism in the organism.

The determination of metabolites (biomarkers) in urine is often used as a quantitative indicator of exposure to a given substance (so-called biomonitoring).

Keywords: human urine, trace components, metals, volatile organic compounds, isolation, enrichment, biomarkers, biomonitoring

Introduction

The source of information about the state of our environment can be both the abiotic part of the environment (water, soil, air) as well as samples of the biotic part, including tissues and body fluids of humans, who are continuously exposed to a wide spectrum of xenobiotic chemicals. We can distinguish three fundamental kinds of exposure through which environmental pollutants can enter the human body:

Kinds of exposure:

Endemic exposure

- associated with human contact with drinking water, indoor air, outdoor air, and food ingestion

Workplace exposure

- associated with performing specific operations and activities at the workplace

Catastrophic exposure

- associated with exposure to pollutants resulting from unexpected events (ecological disasters)

Figure 1 illustrates the pathways through which chemicals may reach human organisms. The magnitude

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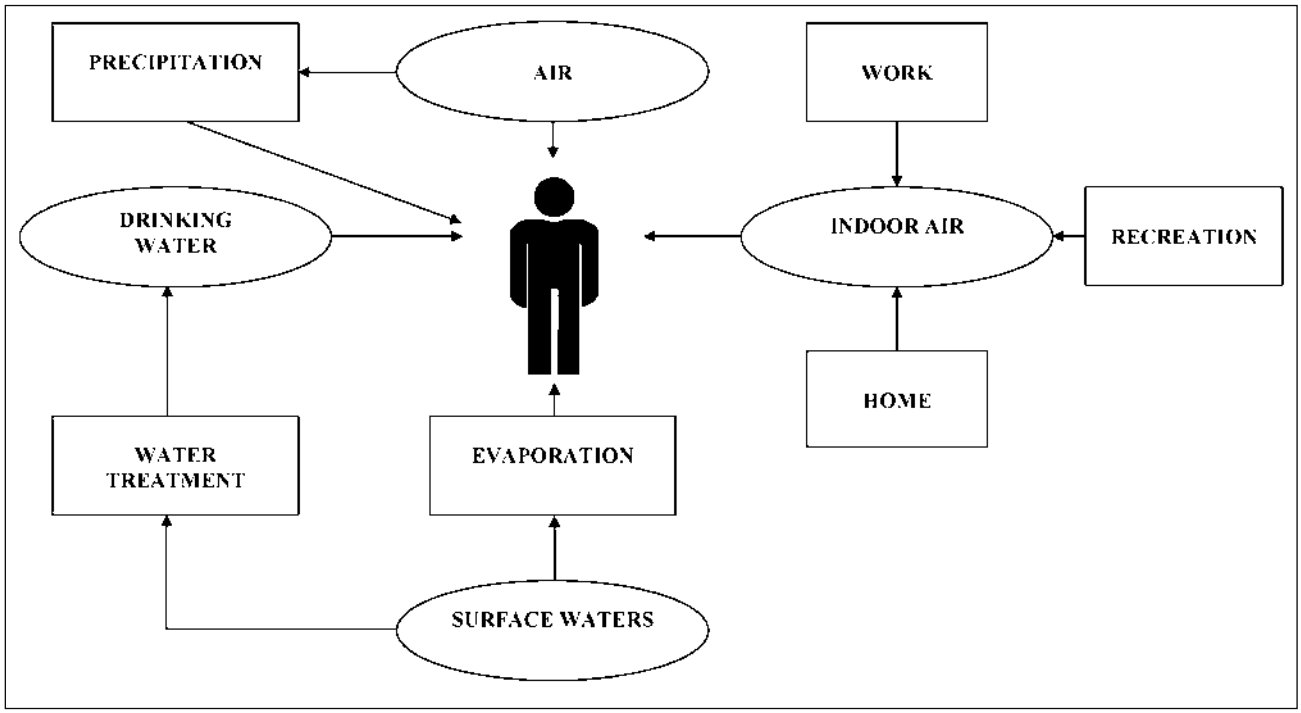

Fig. 1. The schematic diagram of pathways through which chemicals may reach human organisms.

of the individual inputs depends on a number of factors, including urban development, traffic volume, industrial development, occupation (industry, laboratories, offices, stores) and everyday activities including showering, washing and meal preparation, as well as recreation activities, e.g. attending swimming pools.

Xenobiotic chemicals can enter the human body via three paths: through the skin, through the respiratory system and through the digestive system. Depending on their physicochemical properties, these chemicals take different paths in the body. A fraction of them is cumulated in the tissues (bone, adipose, and parenchymatous) and the organs (brain, liver, kidneys), while the rest undergoes metabolic processes, and is excreted in a different form with the expelled air, bile, urine, saliva, or sperm. Renal excretion plays the greatest role in higher organisms.

The investigation of samples taken from humans presents a great analytical challenge. In order to determine metals and, most of all, a variety of organic compounds in such samples, they must be subjected to tedious and time-consuming sample preparation procedures. Samples of physiological fluids (including urine) have very complex matrices and in the majority of cases, direct determination of analytes by means of commonly used methods and techniques is impossible.

Recently, the literature available contains more and more information on:

- new analytical procedures for the investigation of physiological fluids, mainly human urine and blood;
- concentration levels of a wide variety of analytes in urine and blood samples both in the unchanged form and their metabolites;
- evaluation of the effect of environmental and occupational exposure on the concentration level of various types of chemical compounds in urine.

Environmental pollutants entering the human body are accumulated in various parts of the body or excreted (in the original form or as metabolites).

Irrespective of the way a chemical compound enters the body (except for intravenous injection), after penetrating the initial cellular barrier (such as the skin or mucous membrane) the compound first reaches the intercellular fluid. From the intercellular fluid the compound penetrates capillary blood vessels and thus enters the circulatory system, which distributes it throughout the entire body. A majority of toxic compounds do not cause damage at the site where they enter the body. The absorption process is the beginning of the path consisting of:

- absorption;
- distribution;
- biotransformation;
- accumulation;
- elimination of harmful substances.

In order to provoke symptoms of poisoning, a chemical or its metabolite must penetrate a target organ, which is susceptible to its action. At the same time, the concentration of the toxin must be sufficiently high and appear there at a definite time. The target organ is the point of anatomical preference for the appearance of symptoms of poisoning by a given substance. Figure 2 shows a schematic diagram of spreading of a poison, following its absorption by the organism.

Mechanisms of Absorption of Substances by the Body

Molecules of chemical compounds can penetrate the epithelial cells, forming external and internal protective barriers of the body as a result of:

a) passive transport through the cell membranes (i.e. passive diffusion);
b) active transport (involving metabolic energy);
c) permeation through pores (convective absorption) or intercellular channels of the epithelium;
d) specialized transport (phagocytosis, pinocytosis, endocytosis).

The majority of toxins penetrate the cell membranes by way of diffusion. The diffusion rate depends on such physicochemical properties of a toxin as [1]:

- solubility in water (polar compounds);
- solubility in lipids (nonpolar compounds) ;
- ability to bind to proteins.

Solubility in lipids is the most important property of a toxin used to assess its permeability through the cell membranes. It depends on polarity of a chemical compound.

The major paths of penetration of the human body by xenobiotics include (see Figure 2):

- the skin – is the barrier most difficult to penetrate by water and xenobiotics;
- the respiratory system – can absorb toxic compounds occurring in the form of gases, vapors, mists, and smokes. A large surface area of the respiratory tract and a minimal thickness of the "air – blood" barrier make absorption of gases and vapors through the lungs very quick and efficient.
- the digestive system – the digestive tract is one of the most important ways of absorption of exogenous substances [2]. In this way most drugs and chemical pollutants of water and food enter the body. The absorption of toxic compounds can take place over the entire length of the digestive tract: in the mouth, the esophagus, the stomach, and the intestines. Most toxic compounds are only slightly absorbed in the mouth and the esophagus primarily because they are rapidly transported to the stomach [3]. In the mouth are absorbed such compounds as nicotine, cocaine, cyanides and alcohol (which are not processed by gastric juice and metabolizing enzymes).

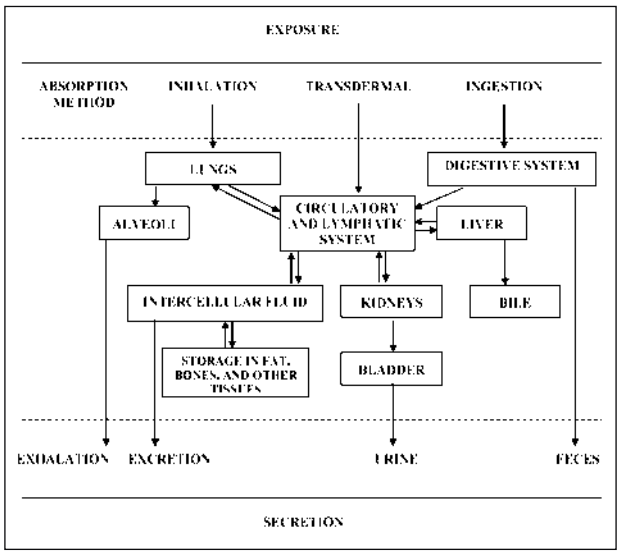

Fig. 2. The schematic diagram of transport of poison in organism following its absorption [1].

Transport, Biotransformation and Excretion of Xenobiotics

In order to reach the receptor in a target cell, an absorbed xenobiotic must be transported by the blood. The lymph participates in the distribution of some strongly lipophilic substances administered orally [4]. After the initial distribution phase (absorption), the rate at which a chemical compound penetrates the cell membranes and the availability of binding sites are the dominating factors affecting the final distribution of the compound in the body [2]. The time elapsed before the symptoms of poisoning of the body appear depends on how quickly the toxic concentration of a compound in the cytoplasm of cells of a given organ has been reached. This rate depends, among others, on physicochemical properties, such as solubility in lipids and degree of ionization [4,5]. Following absorption, in a few minutes the largest amount of a substance reaches the heart, the liver, the kidneys, the brain, and other blood-rich organs, where the majority of toxins are accumulated. A fraction of xenobiotic is retained in the organs for a long time (from a week to several years), sometimes initiating tumor growth, or undergoes biotransformation. The latter process usually results in deactivation of the toxin and excretion of its metabolites with urine.

Biotransformation and elimination of poisons takes place primarily in the liver and the kidneys. Toxins absorbed in the digestive tract are transported in blood from the stomach and the intestines to the liver. The liver is the site where the absorbed toxins undergo rapid transformation involving enzymes, which are especially abundant there. Some substances become soluble in water and are transported with blood to the kidneys. Once there, they are filtered, removed from blood as polar toxins and metabolites, and then excreted with urine. For example, benzene is metabolized in the liver to phenolic compounds, which are excreted in urine along with other metabolites. The determination of specific metabolites in urine is often used as a quantitative indicator of exposure to a specific substance. This process is called "biomonitoring", and the metabolite itself is called a "biomarker" or "bioindicator". Biomarkers are indicators of changes, which can take place in biological systems as a result of interactions with hazardous compounds of diverse nature and origin. These substances fall into two categories [6]:

- selective biomarkers (this kind is used in practice);
- nonselective biomarkers.

Biotransformation does not have to lead to a decreased toxicity of a substance. Some metabolites are significantly more toxic than the original toxin. For example, benzene undergoes metabolic transformation to phenol, which poisons bone marrow. Hence, phenol is a biomarker for exposure to benzene. Table 1 lists examples of chemical compounds and biomarkers detected in urine.

The fact that for the majority of biomarkers there are no data concerning the allowed concentrations in urine is a serious problem. Consequently, using biomarkers we can establish exposure to xenobiotics but without the knowledge of the risk caused by an ecotoxin [31].

Storage of chemical compounds or their metabolites in the body is an important stage of the path of xenobiotics in the system. In general, it can be stated that a successively fed chemical compound will be stored in the body if the processes of its elimination or biotransformation will be slower than the feeding rate. The best example illustrating this phenomenon is accumulation of ethanol in blood following extended periods of its consumption.

Some chemical compounds are stored in specific tissues. Such storage effectively removes poisons from the circulatory system and thus reduces their toxic effects. In some cases chemical compounds that are already stored can be displaced by other compounds having a greater affinity for the same receptors.

In addition to deactivation of a xenobiotic through its storage, living organisms try to protect themselves by initiating other, reserve abilities of functioning of some organs. Organs such as the lungs, the liver, or the kidneys can withstand a number of damage without visual symptoms. In such cases, changes in these organs can be observed only by histological examination [32].

A fraction of toxins absorbed by the human body is accumulated in various tissues and organs. The rest is excreted unchanged or as polar metabolites. The body has a tremendous ability to eliminate a wide variety of chemicals absorbed with the diet or as a result of exposure to the environment [2,4,5,33]. The majority of poisons, particularly polar substances, leave the body with exhaled air and urine.

Smaller amounts of chemicals are removed with saliva, bile, sweat, milk, and feces (see Figure 2).

In contrast, the elimination of nonpolar and nonvolatile compounds is difficult. They undergo biotransformation to metabolites which are more polar and more soluble in water, and are excreted mainly with urine. Considering the fact that a large number of compounds (in unchanged form or as polar metabolites) is excreted with urine, examination of samples of urine can be a source of valuable information about both the human environment and the transformation processes of pollutants absorbed by the body.

Table 1. Examples of biomarkers present in urine, which are used for assessment of human exposure to various xenobiotics.

Hazardous substance	Pollution source	Biomarker of exposure (metabolite) detected in urine
Inorganic compounds [7,8,9,10,11,12,13,14,15,16,17,18]		
chromium	air	chromium
nickel	drinking water	nickel
arsenic	air	arsenic
mercury	food	mercury
bismuth	drugs	bismuth
Organic compounds [19,20,21,22,23,24,25]		
benzene	air	phenol, catechol, hydroquinone, muconic acid
Polycyclic aromatic hydrocarbons	air	1-hydroxy-benzo(a)pyrene
toluene	air	o-cresol, hippuric acid, S,p-toluylmercapturic acid
Halogenated hydrocarbons [26,27,28,29,30]		
dichloromethane	air	Formaldehyde, carbon monoxide binding to Hb (COHb)
chloroform	air, drinking water, pool water	chloroform
1,1,1-trichloroethane	air, drinking water	trichloroethanol or its glucuronate
trichloroethene	air, drinking water	trichloroacetic acid, trichloroethanol or its glucuronate
tetrachloroethene	air, drinking water	trichloroacetic acid

Urine as a Source of Information About Condition of the Body

In general, the following human body fluids can be of analytical interest:

- cerebrospinal fluid,
- milk,
- sweat,
- tears,
- saliva,
- sperm,
- amniotic fluid,
- bile,
- lymph,
- blood,
- urine.

A number of sample preparation techniques for these fluids as well as analytical techniques for the determination of a variety of components at trace and ultratrace levels have been developed.

Thus far, blood has not been used as extensively as urine in the investigations of biological materials. This results from the fact that the collection of blood samples is an invasive process and the volume of samples collected does not exceed 20 mL. In some countries it is difficult to obtain permission to collect blood samples for examination.

Due to its properties and the method of sample collection, urine is used most often for the assessment of environmental and occupational exposure.

Urine is a fluid produced in the kidneys in the amount of about 1500 mL a day. In addition to water (95%), it contains harmful and unnecessary products of metabolism (mainly urea). Urine is a biological material whose analysis can be a source of valuable information about the functioning of the human body and about the effects of environmental pollutants thereon.

The collection and analysis of urine samples carries no risk and the sample volume obtained can be relatively large (up to 2 L/day). In clinical analyses, basic urinalysis includes specific gravity, color, transparency, pH, protein, glucose, ketones and bile pigments. A more thorough examination includes urinary sediment analysis, i.e. the number of epithelial cells, white and red blood cells, casts and mineral components [34].

Recently, in addition to typical morphological analysis of urine samples, the determination of a variety of chemical species has been attempted. Urine can contain certain chemical compounds, xenobiotics or their metabolites (biomarkers) which normally should not be there. Significant progress in medicine observed in recent years has made it possible to determine chemical compounds or their metabolites with high precision and sensitivity.

Review of Sample Preparation Techniques and Methods of Determination of Xenobiotics in Human Urine

Recent years have brought an increase in the number of papers dealing with the use of urine in the investigations allowing the assessment of human exposure to inorganic and organic pollutants present in the life and workplace environments. There are many factors affecting the content of these compounds in the human body. They can be classified in the following way [35].

Factors influencing levels of xenobiotics in the human body:

Factors associated with the workplace (occupational exposure)

- kind of work, performed operations (e.g. spraying, stripping),
- use of technical and individual protective devices (workers can carry chemical dusts (mainly metals) on their clothes and shoes, which leads to greater exposure in their homes,
- occupational hygiene and safety (safety goggles, masks, etc.)

Life style and standard (endemic exposure)

- eating habits
- quality of drinking water,
- smoking,
- alcohol drinking,
- personal hygiene,
- hobbies, chores,
- place of residence.

Other factors:

- age (small children can absorb xenobiotics to very high levels),
- sex,
- pregnancy (accumulation of pollutants in the placenta),
- genetic factors, illnesses.

Information on absorption and excretion of various types of pollutants as a result of both occupational and endemic exposure is compiled in Table 2.

Methods of Isolation of Metals from Human Urine Samples

The determination of metals and other elements in biological material is important not only for the assessment of environmental and occupational exposure and diagnostics of acute poisonings, but also in the diagnostics of diseases resulting from the deficiency of essential metals [66].

The detection of trace amounts of metals almost always requires oxidative decomposition of organic matter – in other words, mineralization [67]. The process is accompanied by the conversion of metallic xenobiotics to their ionic form. There are two methods of mineralization: dry and wet. The selection of one of these methods depends on an element being determined and the final determination technique.

Among the most commonly used methods of preparation of urine samples for the determination of metals are: wet digestion, microwave-assisted digestion, ultrasound-assisted digestion in oxidizing mixtures, as well as the simplest method, which is sample dilution (Table 3).

Dry decomposition is not used for urine samples, because it is tedious and unsuitable for liquid samples.

Techniques of Final Determination of Metals

A variety of analytical methods is used for the determination of metals. The more important methods include:

- Atomic absorption spectroscopy, both flame and flameless;
- Atomic emission spectroscopy;
- Inductively coupled plasma-mass spectrometry;
- Inductively coupled plasma-atomic emission spectrometry;
- Anodic stripping voltammetry;
- Neutron activation analysis.

The selection of one of the above methods depends on the kind of sample (matrix interference), on the amount of analyte in the sample, and on the accuracy desired.

The method used most often for the determination of metals in urine is atomic absorption spectroscopy (AAS). The determination of metals in a sample requires suitable pretreatment (matrix digestion techniques of organic samples were described above). Depending on sample complexity, one or more of the above digestion techniques can be used. A direct determination can also be carried out for example in diluted serum samples, in blood cells hemolysates, and in urine samples. AAS is a specific method; nonetheless, the presence in a sample of a number of chemicals can affect the atomization process and the results of determination. Matrix interference is a serious problem

Table 2. Literature data on intake and excretion by the human body of environmental pollutants associated with both occupational and endemic exposure.

Compound absorbed by the body	Compound determined in urine (metabolite)	Kind of exposure		References
		Occupational exposure (workplace atmosphere)	Endemic exposure lifestyle (water, food)	
		Subjects	Workplace/lifestyle	
Inorganic xenobiotics				
ARSENIC $_{inorganic}$	As_{total}	Workers in artistic glass plant	Healthy, nonsmoking subjects, 25-40 years old	[7]
	As^{3+} As^{5+} - metabolites methyl derivatives of arsenic(V) acid	People working with chromated copper arsenate used as wood preservative	Laboratory workers (control group)	[8]
BISMUTH	Unchanged form	-	Patients (suffering from stomach ulcers) using medicines containing bismuth	[10]
CHROMIUM	Unchanged form	Welders in aircraft plant (solders containing Cr)	Office workers (control group)	[12]
		Plasma cutters of stainless steel	-	[13,15]
CADMIUM	Unchanged form	Workers exposed to silver solder containing Cd		[15]
NICKEL	Unchanged form	Pregnant women (foundry workers in Russia) and their newborns	Pregnant women and their newborns in Norway	[16]
PLATINUM	Unchanged form	Workers with different occupations	-	[36]
MERCURY $_{total\ content}$	$Hg^0 + Hg^{2+}$	-	Randomly selected donors (having amalgam tooth fillings, eating large amounts of seafood)	[17]
		Gold shop workers (gold extraction and recovery – increased amount of mercury vapor in air)	Randomly selected donors	[18]
		-	Pregnant and lactating women and their newborns in Stockholm, Sweden	[37]

Table 2 continues on next page...

ZINC CADMIUM LEAD	Unchanged form	Zinc refinery workers simultaneously exposed to cadmium, zinc and lead	Control group	[38]
Organic xenobiotics				
METHANOL	Unchanged form	-	Healthy subjects from south-eastern Brazil (São Paulo)	[39]
METHYL FORMATE	Methanol Formic acid	Two groups of founders Group of volunteers (subjected to one-time exposure to methyl formate)	Control group	[40]
METHYL- tert-BUTYL ETHER (MTBE)	*tert*-Butyl alcohol	-	One-time study Single exposure through inhalation to MTBE (1ppm) for 10 min	[41]
TRIHALO-METHANES: Chloroform Dichloromethane Chlorodibromomethane Bromodichloromethane Bromoform Carbon tetrachloride Trichloroethene Tetrachloroethene	Unchanged form	Workers in analytical laboratory (using organic solvents)	Control group	[42-45]
		-	People using indoor swimming pools (effect of water chlorination)	[29,30]
DICHLOROMETHANE	Unchanged form	Workers in a printing factory	Control group	[26]
1,1,1-TRICHLOETHANE	Trichloroethanol	-	30 people living in urban Tokyo area	[27]
TRICHLOROETHENE TETRACHLOROETHENE	Trichloroacetic acid	Workers in a glass printing shop	-	[27,28]
BENZENE	S-methylmercapt-uric acid *trans*, *trans*-muconic acid Hydroquinone Catechol Phenol	Workers in glue and shoe factories in Tianjin, China	Control group	[19]
		Workers exposed to benzene and other aromatic solvents	Control group	[22]
	S-phenylmercapturic acid *trans*, *trans*-muconic acid	Children from several industrial areas of Korea exposed to benzene	Children from a mountain village– control group	[46]
TOLUENE	Unchanged form	Workers from rotogravure printing industry	Control group	[25]
	Mercapturic acids *o*-cresol Hippuric acid	Workers in a glass and ceramic fiber plant	Control group (laboratory workers)	[47]
STYRENE	Unchanged form	Workers in a plant manufacturing fiberglass boats, reinforced with resin	-	[48]
	Mandelic acid Mercapturic acids	Workers in resin-reinforced fiberglass plants exposed to a different extent to (protective equipment)	Control group	[49,50]

Table 2 continues on next page...

XYLENE	Mercapturic acids (*ortho*-, *meta*-, *para*-)	Workers in a paint factory exposed to a different extent to xylene	-	[51]
PYRENE	1-hydroxypyrene	Coking plant workers exposed to a different extent to PAH, Holland	Control group	[52]
		Coking plant workers exposed to a different extent to PAH, Poland	Control group	[53,54]
		Subjects exposed to a different extent to PAH, Budapest	Control group (from different countries)	[55,56]
		Workers in an engine repair workshop exposed to PAH (not using safety goggles)	Control group	[57]
		-	Smokers	[58]
		Workers in an artificial shooting target factory exposed to a different extent to PAH	Control group	[59]
		-	Children (playing on the floor of apartments, in which PAH-containing glue was used) Frankfurt am Main, Germany Control group (children living in safe dwellings)	[60]
		-	Policemen exposed to a different extent to PAH (traffic police and office workers)	[61]
17 DIURETICS	-	-	Hospital patients with chronic fluid/electrolyte disorders	[62]
ACROLEIN	Unchanged form	-	Three patients with cancers, tumors (treated with cyclophosphamides)	[63]
AROMATIC AMINES NITROARENES	-	-	Nonsmokers Smokers, Passive smokers, Germany	[64]
PVC MONOMER	Thiodiglycolic acid	Vinyl chloride monomer-exposed polyvinyl chloride workers	-	[65]

in flameless AAS. In order to eliminate this problem, the following operations can be used, depending upon the measuring technique, analyte, or type of matrix:

- background absorption correction,
- suitable calibration methods,
- separation of analyte from matrix by way of extraction, ion exchange, or hydride generation.

In atomic absorption spectroscopy there are four basic methods of atomization of analytes present in a given sample. Information about the various atomization methods is compiled in Table 4.

Each of these methods provides different possibilities, and the selection of one of them depends on the metal being determined, its concentration, type of sample, and sample size.

Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) has been frequently used for the final determination of metals in urine samples. This method involves the use of plasma to convert elements in a sample into their ionic form. The concentration of ions is measured directly in contrast with AAS, where the measurement is indirect,

Table 3. Compilation of sample preparation techniques in the determination of metals in urine [67].

Sample preparation technique	Description
Wet digestion	The most important role in analytical procedures is played by wet digestion. Currently, wet digestion involves heating the investigated material (both liquid and solid) with concentrated oxidizing agents, such as nitric acid, sulfuric acid, perchloric acid, and hydrogen peroxide. Their selection depends on properties of the element being determined (e.g. volatility). The sample matrix is decomposed by oxygen released from the oxidants at an elevated temperature. The acids or hydrogen peroxide are added gradually during the decomposition process of organic compounds until white fumes of the acid appear. The process is carried out in glass digesters of Kjeldahl or Bethge type. The digestion can be performed either in an open or a closed system. Using a closed digestion system, analyte losses can be avoided, secondary contamination prevented, and the digestion can be carried out at elevated temperature and pressure, thus reducing the duration of the process [13]. Increasingly, wet digestion techniques are assisted by microwaves, UV radiation or ultrasounds.
Ultrasonic digestion	Decomposition of the organic matrix of a sample can be accomplished using ultrasound as an energy source. A sample in a reaction vessel containing acids and possible oxidants is placed in an ultrasonic bath. It is a rapid technique, requiring smaller amounts of reagents, and less expensive than the microwave digestion described below. Ultrasonic digestion falls into the category of wet digestion methods in open systems.
Microwave digestion (in an open or closed system)	Wet digestion methods employing thermal conductivity, described above, are time-consuming (several to a dozen or so hours). They can be considerably accelerated by using microwave radiation as a source of energy for decomposition of samples with both organic and inorganic matrices. Microwave-aided sample digestion differs from classical digestion techniques in that the microwave energy (of a frequency 2450 MHz) is transferred directly to the sample without mediation of the containers in which the process is carried out. This results in a visible reduction of time of digestion. The technique was first introduced into analytical practice in 1975. The drawback of microwave digestion is incomplete sample decomposition in some cases.
UV digestion	UV digestion involves irradiation of a sample (often with H_2O_2 or $K_2S_2O_8$ added) in quartz reaction vessels using a low or high pressure UV lamp (λ=254 nm). This technique is frequently used to digest body fluids, and urine and blood serum in particular. UV digestion constitutes a very clean method of sample treatment (samples are not contaminated, the technique is environmentally friendly).
Sample dilution	Some methods of determination of metals call for simplified sample preparation. For example, if atomic absorption spectroscopy is used as a final determination method, in some cases time-consuming sample digestion can be avoided by a direct sample introduction into flame (flame technique) or a graphite cuvette (flameless technique). This simplified sample treatment can be used when determining in urine samples such metals as Pb, Zn, Tl, or Cr by flame AAS. In cases of determination in blood or urine samples such elements as Pb, Cd, Ni, or Fe by electrothermal AAS, the samples need only be diluted with ammonium phosphate.

through absorption of radiation. In the absence of spectral interference (which can occur as a result of overlap of a monoatomic ion peak with that of a polyatomic ion peak in the ICP-MS spectrum) and matrix effects, the method has a high accuracy, comparable to AAS. The method is particularly useful for the determination of heavy metals [68].

Table 5 compiles the literature data on the determination of inorganic xenobiotics and their metabolites in samples of human urine.

Methods of Isolation of Volatile and Semivolatile Organic Compounds from Body Fluids

The literature available contains numerous papers on a variety of diverse organic compounds present in different parts of the environment, and hence in the human body. These compounds are often toxic, although not all of them are included in the standards of environmental quality. The analytical results should initiate a revision of the existing standards.

Samples of urine or blood require special preparation prior to analysis proper. As mentioned previously, this is due to a complex matrix, low concentration levels of analytes and incompatibility of sample matrices with the chromatographic technique used. Consequently, the analytes present in urine and blood samples are subjected to isolation and/or enrichment. This process should preferably take place in one step. A compilation of most common analyte isolation and/or enrichment techniques is given in Table 6.

After completion of the isolation/enrichment operation, the analytes trapped on a sorbent bed have to

Table 4. Methods of analyte atomization in AAS [67].

Atomization technique	Description/method of sample introduction	Advantages/drawbacks	Application in analysis of biological materials	Limit of determination
Flame	- Sample is introduced into flame of a burner (acetylene/ air).	• Simplicity and speed of determination. • High precision of determinations. • Small matrix effects – easy to eliminate. • Small sample volume (1-2mL).	√ Determination of some metals naturally occurring in the body as well as being environmental pollutants that cause poisoning (Zn, Cu, and Mg in urine).	**Tenths of µg/mL**
Flameless with electrothermal atomization	- Sample is introduced into a graphite cuvette.	• Considerable lowering of detection limit (atomization takes place in a small, limited space). • Technique difficult and more time-consuming than flame. • Poorer precision of determinations. • Substantial matrix effect – difficult to eliminate. • Small sample volume (10-50µL)	√ Determination of some metals naturally occurring in the body (Cd, Pd in urine).	**ng/mL**
Cold vapor	- The technique makesuse of mercury being monoatomic vapor at ambient temperature. - $SnCl_2$ or $NaBH_4$ is added to the investigated sample (reduction of mercury compounds to elemental mercury). - Mercury is purged with a stream of air to a quartz flow cell in which absorption of radiation takes place.	• Elimination of matrix effect – separation of Hg from matrix. • Small sample volume (10-50µL).	√ Determination of mercury	**Several ng** in analyzed sample
Hydride generation	- The technique is based on formation of volatile hydrides by some elements (As, Bi, Sb, and Tl). - A reducing agent, $NaBH_4$, is added to the sample solution being examined. - The hydrides generated are transferred with a stream of inert gas to a quartz cell heated by flame or electrothermally, where they decompose and the elements being determined absorb radiation.	• Elimination of matrix effect - separation of matrix from analytes.	√ Determination of some metals naturally occurring in the body as well as being environmental pollutants and causing poisoning (As, Bi, Sb, and in urine and blood).	**Several ng** in analyzed sample

be released and the extract obtained reduced in volume (concentrated).

The sample preparation step must take into account the following factors:

- matrix of a urine sample,
- method of sample introduction onto a chromatographic column,
- chromatographic conditions,
- characteristics of detectors used (e.g. detection limit).

In many cases, the selection of an analytical method is limited by the sample size. However, in case of urine analysis this problem does not exist.

In the analysis of volatile and semivolatile organic compounds, a single-step isolation and/or enrichment process is preferred. This requirement is met by the techniques enabling simultaneous isolation of analytes from the matrix and their enrichment. The following techniques have been found to be most frequently used in the analysis of volatile and semivolatile organic compounds [67]:

- gaseous phase extraction;
- liquid phase extraction;
- solid phase extraction.

None of the above extraction techniques is universally applicable. The selection of an extraction technique limits the range and number of compounds that can be determined.

Gaseous Phase Extraction Techniques

These techniques fall into two categories:

Headspace analysis

- Static techniques
  - Headspace (HS) analysis

Table 5. Literature data on the determination of inorganic xenobiotics and their metabolites in samples of human urine.

Analyte	Sample storage and/or pretreatment	Isolation and/or final determination method	Limit of determination	References
[1]ARSENIC$_{inorganic}$ As$_{total}$ As^{3+} As^{5+} - its metabolites: methyl derivatives of arsenic acid	Urine samples were stored at -20°C; no pretreatment prior to final determination	ICP-MS HPLC/MS	-	[7]
[2]ARSENIC$_{inorganic}$ - its metabolites: methyl derivatives of arsenic acid	Urine samples were transferred into reaction tubes containing 4.0mL of 6M HCl, followed by addition of 1 tablet of antifoaming agent 20 mL of water	Hydride cold-trapping AAS	1.3 ng 1.3 ng 3 ng	[8]
[3]ARSENIC$_{inorganic}$ As^{3+} As^{5+} - its metabolites: methyl derivatives of arsenic acid	Urine samples were diluted with the mobile phase used for ion chromatography	Chromatographic separation of analytes (anion exchange) directly combined with the hydride generation AAS	1 µg/L 10 µg/L 2 µg/L 2 µg/L	[9]
BISMUTH	Wet digestion	Hydride generation AAS with flow injection analysis	320 pg/mL	[10]
	Wet digestion	Enrichment by ion exchange combined with Electrothermal Atomic Absorption Spectrometry	27 ng/L	[11]
CHROMIUM	Possible dilution of a urine sample prior to final determination	AAS with the Zeeman background correction	0.6 µg/L	[12]
	-	AAS with the Zeeman background correction	-	[13]
	-	AAS with the Zeeman background correction	-	[14]
CADMIUM	-	Electrothermal Atomic Absorption Spectrometry	-	[15]
NICKEL	Storage at -20°C, then at -70°C; thawing prior to analysis at 95°C for 1h, no further pretreatment	Electrothermal Atomic Absorption Spectrometry	10 nmol/L	[16]
	Wet digestion	Enrichment by ion exchange combined with Electrothermal Atomic Absorption Spectrometry	9 ng/L	[69]
PLATINUM	UV digestion	Adsorptive Voltammetry Technique	1 ng/L	[36]
MERCURY$_{vapor}$	Acid digestion of urine samples ($Hg^{2+} \rightarrow Hg^0$)	Cold -Vapor Atomic Absorption Spectrophotometry	0.5 ng/g	[17]
MERCURY$_{total\ content}$	Storage at -20°C, wet digestion	Cold Vapor Atomic Absorption Spectrophotometry	-	[18]
	Storage at -20°C, EDTA, Triton X-100 and ammonia solutions were added to urine samples	ICP-MS	-	[37]
ZINC CADMIUM LEAD	Storage at -20°C, Microwave-assisted wet digestion	AAS Flame Flameless	-	[38]
Sc, V, Mn, Fe, Co, Ni, Cu, Zn	Urine samples were diluted with water in a 1:9 ratio	ICP-MS	-	[70]

Table 6. Compilation of isolation and enrichment techniques used in the determination of various groups of organic compounds in samples of body fluids [71,72,73,74,75,76].

Isolation and/or enrichment technique	Analytes
Headspace *(HS, TLHS)*	Volatile and semivolatile nonpolar compounds
Purge and trap *(PT)*	Volatile and semivolatile nonpolar compounds
Distillation techniques	Volatile polar organic compounds
Liquid-liquid extraction *(LLE)*	Semivolatile and nonvolatile compounds
Solid phase extraction *(SPE, SPME)*	Semivolatile and nonvolatile compounds

- Dynamic techniques
  - Purge and Trap (PT) technique
  - Thin Layer Headspace (TLHS) technique

Distillation

Each of these techniques has its disadvantages and its uses are strictly limited.

Headspace Analysis Techniques

Headspace analysis techniques are based on partition of analytes between the liquid and gaseous phases. The analyte concentration in a condensed phase is determined by analyzing the headspace – the gaseous phase in contact with the analyzed sample. The most effective release from the liquid phase takes place for volatile and semivolatile nonpolar or weakly polar organic compounds [74,75].

Static headspace analysis is a technique wherein both contacting phases – aqueous (sample) and gaseous (receiving matrix) are stationary. The analysis is carried out in two steps: the investigated sample is placed in a closed container at a constant temperature, and the headspace is taken for analysis only after the thermodynamic equilibrium between the two phases has been established. Headspace sampling can be manual or automatic. The main advantages of headspace technique include simplicity, short time of analysis, high sensitivity, good precision, possibility of automation, and elimination of solvents from the analytical procedure. The fact that a fraction of the analyte is in the headspace can be considered a disadvantage [73,74,75,76].

In TLHS, a stream of gas is continuously passed through a sample or over a sample, and the analytes carried with it are retained in a sorbent bed or water. The technique can be combined with direct aqueous injection (DAI) of a sample onto a GC column of a chromatograph equipped with an electron capture detector (ECD). An example of this technique is TLHS-DAI-GC-ECD, developed in the Department of Analytical Chemistry of Chemical Faculty of Technical University of Gdańsk [42,43,77,78]. A schematic diagram of the apparatus for TLHS developed by the research group of professor Kozłowski is shown in Figure 3.

Main advantages of this technique include:
- elimination from analytical procedures of reagents and organic solvents, which would ultimately be discharged to the environment as wastes,;
- use of a stream of pure water (generated as a result of water vapor condensation) as medium trapping analytes,
- minimization of secondary contamination of samples through the solvents and sorbents used,
- possibility of direct analysis of samples of the receiving matrix without the removal of excess solvent (receiving matrix) due to a very small volume of the condensate [79,80].

Purge and Trap Technique (PT)

Purge and Trap is a dynamic technique, in which a stream of purge gas after passing through a liquid sample is directed to a sorbent trap. The technique is widely used for the determination of volatile and semivolatile organic compounds in a variety of aqueous matrices. The advantages of purge and trap include:
- high recovery of analytes
- low background of available systems ensure:
- low detection limit,
- short time of analysis,
- good precision of determinations,
- elimination of solvents from analytical procedure.

The headspace techniques described above are combined with gas chromatography with detection selective for a given analyte (FID, ECD, MS).

Distillation Techniques

Distillation techniques are used for the isolation of volatile and more polar organic compounds from liquid matrices. They enable the determination of analytes in samples with a high content of inorganic compounds and/or high-molecular-weight organic compounds, which would otherwise require complicated cleanup procedure prior to chromatographic analysis [81]. The basis of separation of a mixture into components is uneven distribution of individual components between the liquid phase and the gaseous phase. More volatile components are enriched

in the gaseous phase, which after condensation becomes the concentrate of these components – the distillate. The effectiveness of separation depends on the properties of mixture components, the apparatus used, and the distillation method. Due to the fact that distillation techniques permit effective separations of polar volatile compounds from nonvolatile ones, they are well suited for preparing samples for the analysis by Direct Aqueous Injection – Gas Chromatography - DAI-GC.

Liquid Phase Extraction

In liquid-liquid extraction (LLE), isolation is based on distribution of analytes between an organic solvent and an aqueous phase. The technique makes use of solvents immiscible with water, better dissolving analytes than water does, and volatile so that they can later be evaporated. LLE is used for the isolation of semivolatile and nonvolatile analytes.

Despite several important disadvantages, LLE is still widely used. Liquid-liquid extraction has a high detection limit, and handling of large sample volumes and automation are difficult. However, the major drawback of LLE is the need for use of large volumes of organic solvents [73,74,76,81].

Solid Phase Extraction

Solid Phase Extraction – SPE involves transfer of analytes from a liquid sample to a solid sorbent, followed by their release using either extraction with a solvent of high elution strength or, less often, thermal desorption. A large selection of solid sorbents available ensures high selectivity of extraction and an optimum degree of enrichment of pollutants being determined. This reduces the level of background and, consequently, the detection limit of analytical procedures. Typical sorbents used to trap analytes include:

- groups of porous organic polymers, including styrene-divinylbenzene copolymers,
- carbon sorbents,
- silica gels with chemically bonded phases containing various functional groups.

Isolation and enrichment by means of SPE can be carried out using extraction columns; the analyzed sample is passed (by gravity or under pressure) through a glass tube packed with a suitable sorbent.

The most important advantages of SPE include:

- ability to isolate/preconcentrate both volatile and nonvolatile analytes,
- applicability to extractions of both organic and inorganic components,
- possibility of storage of analytes trapped onto a sorbent bed,
- reduction in solvent volume used compared to LLE,
- large selection of solid sorbents,
- ease of automation,
- wide applicability.

A special variant of solid phase extraction is so-called Solid Phase Microextraction (SPME). In this case, the sorption medium is coated in the form of a thin layer on a fused silica fiber. SPME eliminates the need for any solvent, ensures rapid transport of analytes from samples to the sorbent, and simplifies introduction of the analytes into a chromatographic column [74,76,82].

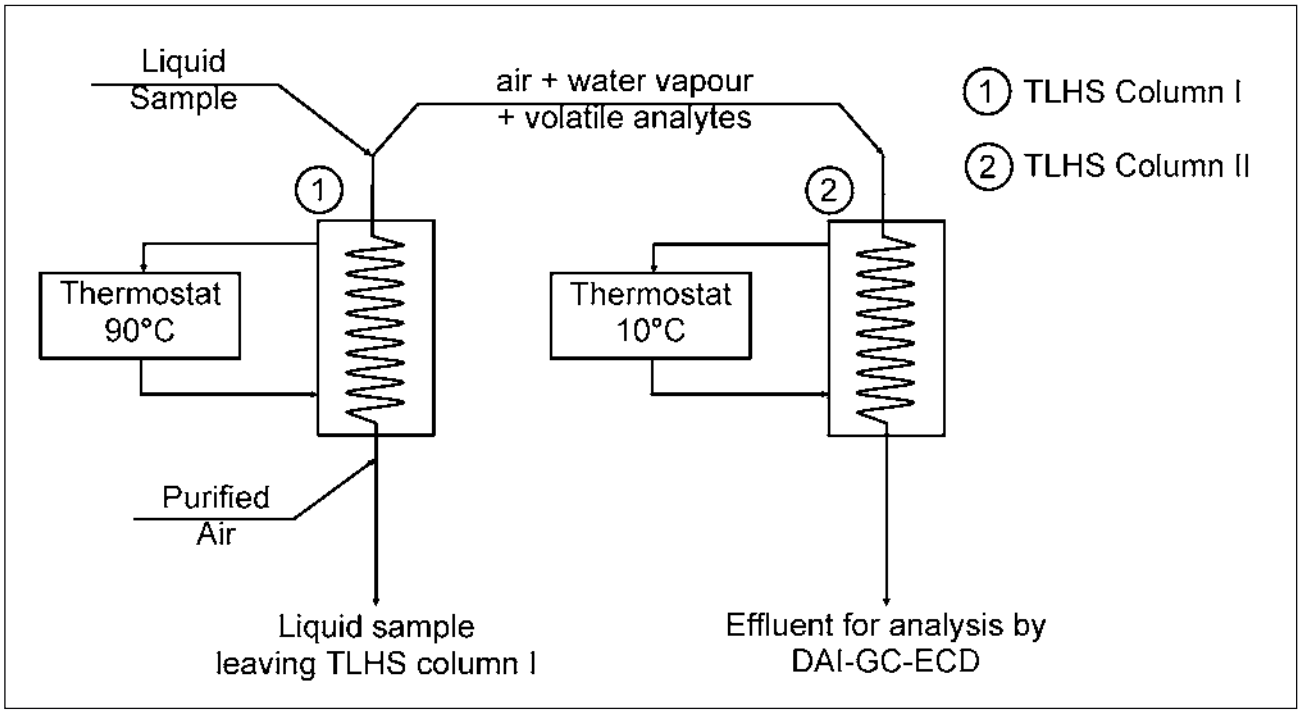

Figure 3. The scheme of the apparatus for TLHS with autogenous generation of liquid sorbent stream [45].

Methods of Final Determination of Organic Compounds Present in Urine Samples

The analysis of obtained extracts includes identification and determination of individual components and is feasible only by using chromatographic techniques. Among various chromatographic techniques, gas chromatography with selective detection (MS, ECD, FID, etc.) is most often used for the analysis of organic components of biological fluids. High performance liquid chromatography (HPLC) with fluorimetric detection has found less use for these assays. Information regarding the applicability of the above techniques for the determination of organic xenobiotics in urine samples is compiled in Table 7.

Conclusions

The number of papers on the determination of inorganic and organic compounds in urine and blood has been steadily growing since 1998. This fact seems to confirm the need for examination of biological fluids (mainly urine and blood), as such investigations can provide vital information on health hazards associated with exposure to both environmental pollutants, as well as occupational and lifestyle pollution.

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Table 7. Literature data on the determination of organic xenobiotics and their metabolites in human urine samples.

Analyte	Sample storage and/or pretreatment	Isolation and/or final determination method	Limit of determination	References
Alcohols				
Methanol	Manual headspace analysis (HS)	GC/FID	0.84 mg/L	[39]
Methanol Formic acid (indicators of occupational exposure to methyl formate)	Headspace analysis (HS)	GC/FID	-	[40]
Methyl- *tert*-butyl ether (MTBE) - its metabolite: tert-Butyl alcohol	Addition of antifoaming agent High-temperature (90°C) purge and trap (PT)	GC/MS	0.1 µg/L	[41]
Volatile organochlorine compounds/Halocarbons				
Trihalomethanes: Chloroform Dichloromethane Chlorodibromomethane Bromodichloromethane Bromoform Carbon tetrachloride Trichloroethene Tetrachloroethene	Thin-layer headspace (TLHS)	DAI/GC/ECD	0.001 µg/L	[42-44]
	Thin-layer headspace (TLHS)	DAI/GC/ECD	0.001 µg/L	[45]
Trichloroethanol Trichloroacetic acid (biomarkers of exposure to 1,1,1-trichloethane, trichloroethene, tetrachloroethene)	Hydrolysis of TCOH to its glucuronate Liquid-liquid extraction (LLE) -acidification (to pH 1.5), -cleanup on Sep-Pak C18 -addition of KOH and pyridine -addition of p-toluidylformic acid	GC/ECD Spectrophotometric determination (λ=500nm)	-	[22]
Trichloroethene - its metabolite: Trichloroacetic acid (biomarkers of exposure to trichloroethene)	Headspace analysis (HS)	GC/MS	-	[25]
Dichloromethane	Headspace analysis (HS)	GC/FID		[26]
Chloroform	Headspace analysis (HS)	GC/MS	-	[27]
Trihalomethanes THMs	Headspace analysis (HS)	GC/MS	0.1 µg/L	[28]

Table 7 continues on next page...

Polycyclic Aromatic Hydrocarbons (PAH)				
Benzene (and its metabolites)				
S-methylmercapturic acid* *trans*, *trans*-muconic acid Hydroquinone** Catechol Phenol*** (biomarkers of exposure to benzene)	Storage at -20°C until analysis	LC-ES-MS/MS* (Liquid chromatography-electrospray tandem mass spectrometry) LC-MS/MS** GS/MS***	-	[17]
	-urine sample was diluted (1:1) with distilled water -liquid-liquid extraction -derivatization	GS/MS	-	[18]
S-phenylmercapturic acid *trans*, *trans*-muconic acid	Storage at -20°C until analysis Acidification, liquid-liquid extraction (LLE) Solid phase extraction (SPE) Ion exchange	HPLC/UV	0.5 µg/L 4.8 µg/L	[46]
Toluene (and its metabolites)				
Toluene	Storage at -20°C until analysis Solid phase microextraction (SPME)	GS/MS	-	[19]
Sp-toluylmercapturic acid *o*-cresol Hippuric acid (biomarkers of exposure to toluene)	Liquid-liquid extraction (LLE)	GS/MS	-	[47]
STYRENE (and its metabolites)				
Styrene	Purge and trap (PT)	GC/FID	0.4 µg/L	[48]
Mandelic acid (biomarker of exposure to styrene)	Storage at 4°C in the refrigerator until analysis	HPLC	-	[49]
Mandelic acid* Mercapturic acids (biomarker of exposure to styrene)	Liquid-liquid extraction (LLE)	HPLC/UV GC/MS	10 µmol/L 1.5 µmol/L	[50]
Xylenes (and their metabolites)				
Hippuric acids (*ortho*-, *meta*-, *para*-) (biomarker of exposure to xylenes)	Storage at -18°C until analysis Acidification, liquid-liquid extraction (LLE)	HPLC/UV	15 mg/L	[51]
Pyrene (and its metabolite)				
1-hydroxypyrene (biomarker of exposure to PAH)	Storage at -20°C until analysis	HPLC with fluorimetric detection	-	[52]
	Enzymatic hydrolysis Solid phase extraction (SPE)	HPLC with fluorimetric detection	-	[53,54]
	Enzymatic hydrolysis Solid phase extraction (SPE)	HPLC with fluorimetric detection	-	[55,56]
	Enzymatic hydrolysis Solid phase extraction (SPE)	HPLC With fluorimetric-spectrophotometric detection	-	[57]
	Storage at -20°C until analysis Enzymatic hydrolysis Solid phase extraction (SPE)	HPLC with fluorimetric detection	0.3 µg/L	[58]
	Enzymatic hydrolysis Solid phase extraction (SPE)	HPLC with fluorimetric detection	-	[59]
	-	HPLC/FID	5 ng/L	[60]

Table 7 continues on next page...

Total Polycyclic Aromatic Hydrocarbons (PAH)	Storage at -70°C until analysis Enzymatic hydrolysis	HPLC with fluorimetric detection	0.05 µg/L	[83]
	Enzymatic hydrolysis Solid phase extraction (SPE)	HPLC with fluorimetric detection	-	[61] [84]
	Stabilization of urine samples by addition of methanol solution of p-toluidine; Storage at -20°C until analysis Solid phase extraction (SPE)	GC/MS	-	[85]
Methylhippuric acid Other PAHs (*o*-cresol, 1-naphthol, 2-naphthol) (biomarkers of exposure to PAH)	Thin-layer chromatography Storage at -20°C until analysis Hydrolysis Liquid-liquid extraction (LLE) Solid phase extraction (SPE)	Spectrophotometric determination GC/MS	-	[53]
Monohydroxy derivatives of PAH (biomarkers of exposure to PAH)	Enzymatic hydrolysis Solid phase extraction (SPE)	GC/MS	2.0-43.5 ng/L	[86]
Miscellaneous compounds				
17 Diuretics	Liquid-liquid extraction (LLE)	HPLC with fluorimetric detection	-	[62]
Acrolein	Headspace analysis (HS)	GC/MS	56-280 ng/L	[63]
Aromatic amines* Nitroarenes	Storage at -20°C until analysis Enzymatic hydrolysis Solid phase extraction (SPE) Acylation of aromatic amines*	GC/MS	0.001 ng	[64]
	Stabilization of urine samples by addition of methanol solution of p-toluidine; Storage at -20°C until analysis Solid phase extraction (SPE)	GC/MS	-	[85]
Thiodiglycolic acid	Storage at -20°C until analysis Acid hydrolysis	GC/MS	1 mg/L	[65]

AAS – atomic absorption spectroscopy, GC/MS – gas chromatography/mass spectrometry, HPLC – high performance liquid chromatography, GC/ECD – gas chromatography with electron capture detection, GC/FID – gas chromatography with flame ionization detection, HPLC/UV– high performance liquid chromatography with UV detection.

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