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## Preliminary study on application of urine amino acids profiling for monitoring of renal tubular injury using GLC-MS\*

### Zastosowanie profilowania aminokwasów metodą GLC-MS w moczu w diagnozowaniu uszkodzenia kanalików nerkowych

#### Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
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#### Summary

##### Background:

The early diagnosis of the nephrotoxic effect of xenobiotics and drugs is still an unsolved problem. Recent studies suggest a correlation between the nephrotoxic activity of xenobiotics and increased concentration of amino acids in urine. The presented study was focused on the application of GLC-MS method for amino acids profiling in human urine as a noninvasive method for monitoring of kidney condition and tubular injury level.

##### Material and Methods:

The analytic method is based on the conversion of the amino acids present in the sample to tert-butyldimethylsilyl (TBDMS) derivatives and their analysis by gas-liquid chromatography-mass spectrometry (GLC-MS). The procedure of urine sample preparation for chromatographic analysis was optimized.

##### Results:

The presence of 12 amino acids in most of the tested healthy human urine samples was detected. The significant differences in the levels of particular amino acids between patients with tubular injury and healthy controls were found, especially for lysine, valine, serine, alanine and leucine (on average 30.0, 7.5, 3.6, 2.9 and 0.5 fold respectively).

##### Conclusions:

We found that this approach based on GLC-MS detection can be used in nephrotoxicity studies for urine amino acids monitoring in exposure to xenobiotics and drugs.

##### Key words:

Urinary amino acids • GLC-MS • MTBSTFA silylation • nephrotoxicity biomarkers • tubular injury

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## INTRODUCTION

The kidney, as part of an excretory system, is highly susceptible to toxic damage [22]. The human kidney plays a major role in homeostasis of the body's total amino acid pool [35]. This is achieved through the synthesis, degradation, filtration, reabsorption and urinary excretion of amino acids and peptides. Because free amino acids present in the blood plasma are filtered at the level of the kidney glomeruli in substantial amounts (more than 50 g per day in humans), their reabsorption by the kidney proximal tubules plays a crucial role in organism homeostasis, since it prevents amino acid loss with the urine [32,34].

The development of metabolomic analysis increased the interest of amino acids profile investigation, especially that the urine is not an invasive source. Gas chromatography (GC) connected with mass spectrometry (MS) is widely used for amino acid analysis because of its high resolution, good sensitivity and relatively low cost of analysis [1,3,12,23,32]. A variety of new efficient and precise methods for the determination of amino acid content in physiological fluids, based on mass spectrometry connected with liquid chromatography have been developed. Nevertheless gas chromatography still proves its applicability.

There are still no standard markers that would allow for a quick and reliable diagnosis of tubular injury at an early stage. Taking the renal reserves, as well as kidney sensitivity limits, into consideration, it is often impossible to diagnose minor dysfunction using standard biomarkers. It is clear, however, that early histomorphological changes can be detected before the levels of physiological markers (urea and creatinine) increase in the urine of animals with kidney injury [20]. There is growing interest in the determination of amino acid level in urine as a noninvasive nephrotoxicity diagnostic method.

Metabolomics has significantly developed over the past ten years because of a growing interest among scientists as well as more advanced instruments making analysis more precise and sensitive [33]. Metabolome analysis has currently been performed using two main technologies: nuclear magnetic resonance (NMR) and mass spectrometry (MS). Both of them can be effectively

applied to analyze metabolite profiles in physiological fluids [10,21]. The core advantage of MS instruments is their high sensitivity which allows using them in combination with separation techniques as liquid and gas chromatography (LC and GC, respectively). GC/MS has been broadly applied to analyze metabolite due to reproducible chromatography and high chromatographic resolution [7].

A very important section of metabolite profiling are the amino acids. The development of metabolomic studies brought new information about amino acid profiles in urine. Initially amino acid analysis has been performed by either cation-exchange chromatography followed by post-column derivatization with ninhydrin or reversed-phase liquid chromatography coupled to UV absorbance [17,24]. Recently a great progress in the development of methods for free amino acid analysis in physiological fluids has been observed [13]. The methods described so far are isotope dilution reversed phase liquid chromatography-tandem mass spectrometry (LC-MS/MS) of underivatized [24] and propyl chloroformate derivatized amino acids [8] gas chromatography-mass spectrometry (GC-MS) of pentafluorobenzylated amino acids [6], enhanced <sup>13</sup>C NMR spectroscopy [31], capillary electrophoresis time-of-flight mass spectrometry [32] and iTRAQ® derivatized amino acids [12].

The study conducted on animals by Boudonck and colleagues as well as by Portilla showed an increase of amino acid level in urine under the influence of drugs with nephrotoxic activity (cisplatin, gentamicin, tobramycin) [1,26]. During kidney injury the observed level of amino acids in urine was increased, while it decreased in kidney tissue. Furthermore, higher secretion of amino acids and dipeptides could be observed after only one day of cisplatin administration, while histopathological tubular injury could not yet be detected [1]. As was shown in a study on mice, the harmful effect of cisplatin could be observed within 48 hours. After that time the urine levels of glucose and amino acids such as alanine, valine, leucine and methionine as well as the presence of tricarboxylic acid cycle metabolites (lactate, pyruvate) increased [26]. The mentioned studies point out a direct correlation between the nephrotoxic activity of xenobiotics and the increase of amino acid concentration in urine, which can be helpful for the diagnosis

of pathologic kidney dysfunctions as well as for drug/xenobiotic nephrotoxicity research.

There is also an array of well-known kidney dysfunctions which cause problems in the amino acid reabsorption, such as Hartnup disorder, characterised by malfunctioning of the transporter B<sup>0</sup>AT1 (SLC6A19), which leads to excretion of neutral amino acids (tryptophan, alanine, asparagine, glutamine, histidine, isoleucine, leucine, phenylalanine, serine, threonine, tyrosine and valine) in urine [30]. Cystinuria is a metabolic dysfunction provoked by kidney insufficiency in the reabsorption of cystine, lysine, arginine and ornithine, which results in excessive excretion of these amino acids in urine [4]. The Fanconi syndrome (FS) is a disease caused by proximal tubule failure characterized by a disorder of reabsorption of amino acids, glucose, phosphates, urea acid, citrates, low molecular weight proteins as well as magnesium, potassium and calcium ions, HCO<sub>3</sub><sup>-</sup> and water [8,21]. In all dysfunctions connected with amino acid reabsorption impairment, an amino acid profiling method in urine could be helpful in diagnosis and for screening purposes.

The main goal of the presented work was to develop a sensitive analytical procedure for the determination of amino acid profile in human urine. Based on the results obtained in the animal model [1] we expect that such amino acid profiling could be applied in the early diagnosis of kidney disease. The analysis was performed using gas-liquid chromatography coupled with mass spectrometry (GLC-MS). Three methods and a variety of conditions for amino acids derivatization were tested; the final method is based on the efficient conversion of the analytes to tert-butyldimethylsilyl derivatives and their analysis by GLC-MS. Also the procedure for preparing urine samples for chromatographic analysis was optimized. We investigated the implementation of GLC-MS method and MTBSTFA silylation procedure of amino acids profile in healthy human urine. In addition, we examined developed method on heroin addicted patients with co-existing HIV/HCV infection. The urine from 7 patient, a part of 83 group described before [9] with proven renal tubular injury was used as a model to check if the method is useful to find the differences in aminoacids profile between healthy and pathological urine.

## MATERIALS AND METHODS

### Materials

Standards of 20 amino acids (alanine, glycine, valine, leucine, isoleucine, proline, hydroxyproline, methionine, serine, threonine, phenylalanine, asparagine, cysteine, glutamine, glutamic acid, ornithine, aspartic acid, lysine, histidine and tyrosine) were used for optimization of the derivatization method (Fluka, Merck). The internal standard a-aminoisobutyric acid, and derivatization agents BSTFA (N,O-bis(trimethylsilyl) trifluoroacetamide), MTBSTFA (N-(tert-butyldimethylsilyl)-

N-methyltrifluoroacetamide, >97%) and urease from *Canavalia ensiformis* (Jack bean) (50 units per sample) were purchased from Sigma-Aldrich. HFBA (heptafluorobutyric acid), methanol, acetone, acetonitrile, chloroform, ethyl acetate and acetyl chloride were purchased from Merck. All solvents were of purity suitable for GC trace analysis. Derivatization reactions were performed in a sampling glass vial with aluminum-lined screw caps.

The first morning urine from 7 women 25 to 43 years old, addicted to heroin with coexisting HIV and HCV infection with tubular injury was collected. The tubular injury markers were determined before the GLC-MS analysis of urine.

As the control, the first morning urine was collected from 18 healthy adult donors (9 women and 9 men) 24 to 25 years old.

### Sample preparation for urinary amino acids measurement in urine

The urine samples were centrifuged for 10 minutes at 3000 rpm and then stored at -80°C until further analysis. Subsequently the creatinine concentration was determined by Jaffe's reaction.

Marker proteins: beta 2 microglobuline (β<sub>2</sub>M), interleukin IL-18 (IL-18), neutrophil gelatinase-associated lipocalin (NGAL), alpha and pi glutathione S-transferase (α/π GST) urine concentrations were determined by ELISA (Argutus Medical, BioPorto Diagnostic, Immunodiagnostik, MBL).

## Methods

Urine samples were thawed and extracted as follows: the sample was filtered through a 0.2 mm filter and 300 ml of urine was then transferred to a reaction vial. The internal standard (a-aminoisobutyric acid, 125 nM) and urease from *Canavalia ensiformis* (Jack bean, Sigma-Aldrich) (50 units per sample) were added to the sample and incubated at 37°C for 30 minutes.

After incubation proteins were precipitated from urine for 20 minutes at -20°C using a methanol/acetone 7:3 (v/v) mixture. After centrifugation the supernatant was transferred into a glass reaction vial and evaporated under a stream of nitrogen at 40°C. The samples were then frozen and stored at -80°C for chromatography analysis. The derivatization reaction was carried out with MTBSTFA as in the case of standards.

### Optimization of derivatization procedure

The standard mixture of 20 amino acids (solution containing 2.5 nmol of each amino acid), with the addition of 2.5 nmol of a-aminoisobutyric acid as an internal standard, was dispensed in a glass vial, evaporated at 40°C using a stream of dry nitrogen and then stored at -80°C until further analysis.

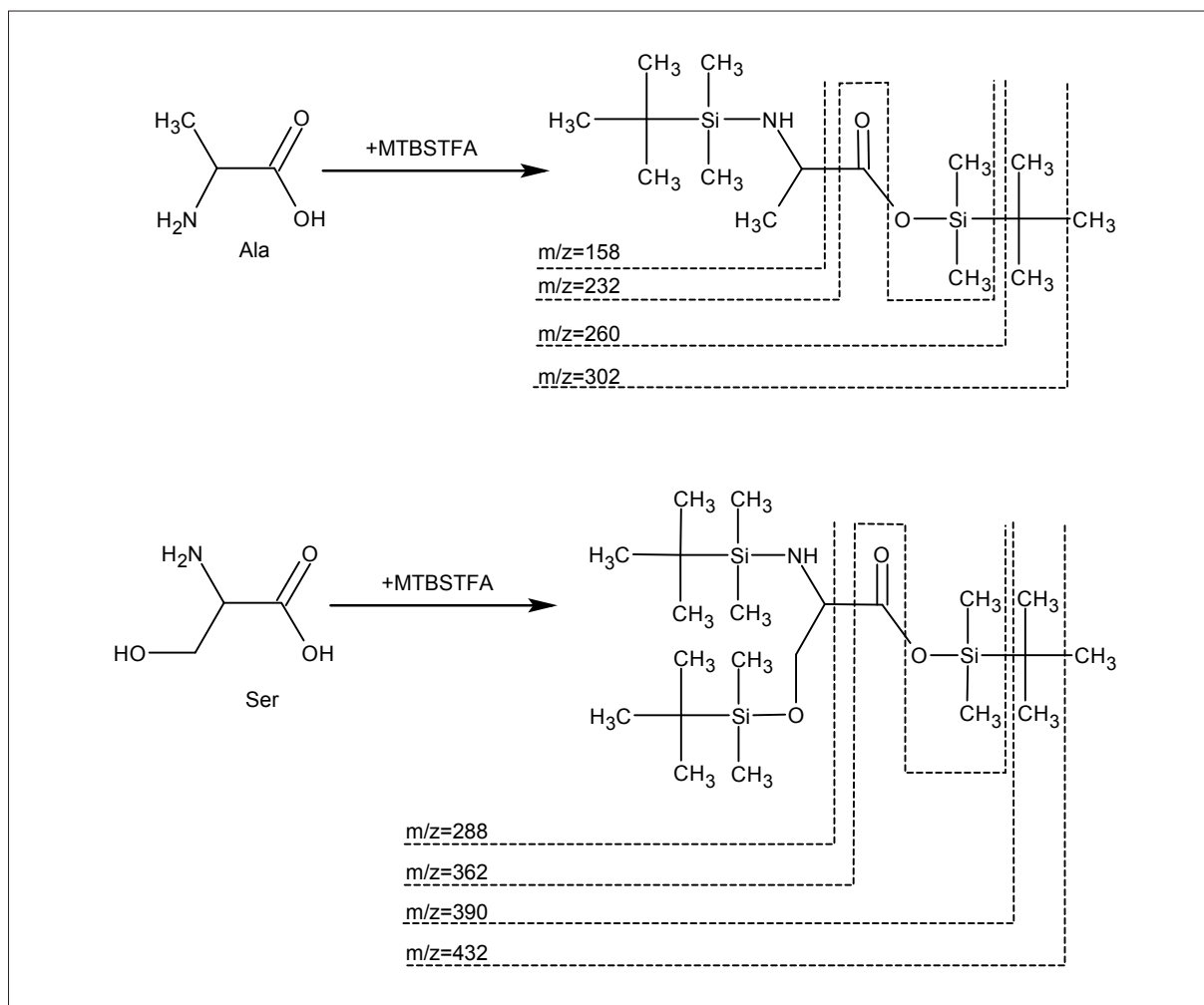


Fig. 1. Fragmentation pattern of the double and tripple TBDMS-derivatives

Three different derivatization methods were tested for optimization: esterification with n-butanol, silylation with BSTFA and silylation with MTBSTFA. Only the silylation with MTBSTFA gave proper derivatization efficiency and GC resolution for amino acids studied.

#### Silylation with MTBSTFA

Equal amounts of MTBSTFA and acetonitrile were added to dried extract and the vial was heated for 4 hours at 100°C. Exemplary derivatizations are shown in Figure 1.

#### GLC-MS analysis

GLC-MS analysis of the derivatized amino acids was performed using the Thermo Scientific Focus GC ITQ 700 equipped with a Restek Rxi-5MS column. The injector temperature was 250°C whereas the oven temperature was kept isothermal at 120°C for 4 min, then programmed at 12° min<sup>-1</sup> from 120°C to 180°C, which was held isothermal for 5 min. Helium was used as a carrier gas at a flow rate of 10 ml min<sup>-1</sup>, and 1 µl of the sample was injected. Respec-

tive amino acid derivative was identified by its retention time and mass spectrum and its amount in the sample was calculated by comparing the peak area with that obtained during analysis of amino acid standard mixture.

All procedures were in accordance with the ethical standards of the bioethics committee on human experimentation, permission number KB 157/2010.

## RESULTS

#### Derivatization conditions

Among the three tested methods the procedure with MTBSTFA provided the best results. Heating the samples at 100°C in acetonitrile and MTBSTFA for 4 hours was found to be the optimum overall derivatization procedure. Derivatives of all 20 tested amino acids were obtained (Fig. 2). Increase of the reaction time had no effect on efficiency of derivatization of any amino acid. That method has been chosen for further analysis of amino acids in urine.

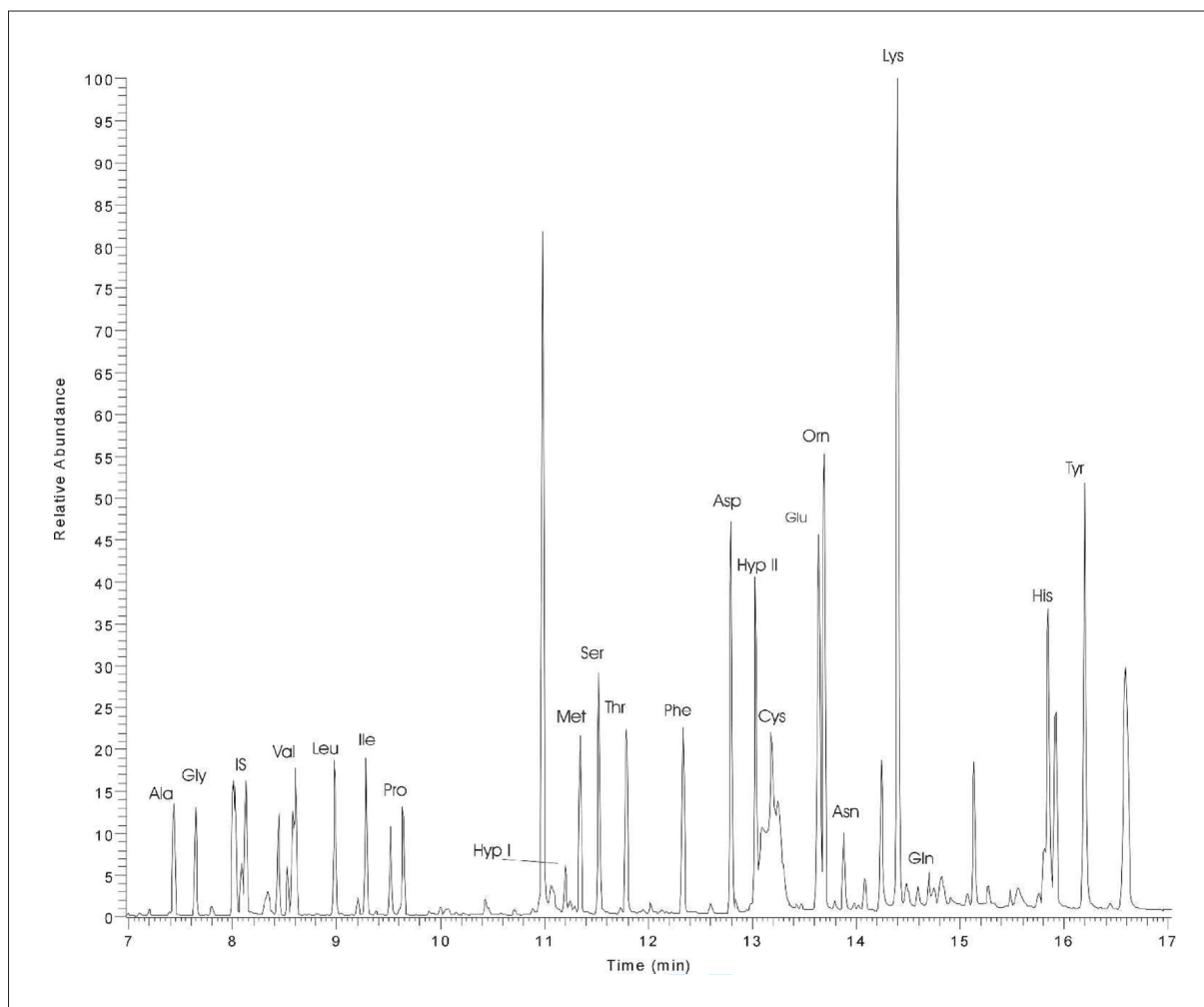


Fig. 2. GC-MS chromatogram for the analysis of an amino acid standard mixture after derivatization with MTBSTFA

### Analysis of amino acid standards

The described conditions of analysis (method of derivatization and GLC-MS parameters) gave a chromatogram, showing the tert-butyldimethylsilyl derivatives of all analysed amino acids (Fig. 2). Identification of individual amino acid derivatives based on their retention times and mass spectra. Each active hydrogen in an amino acid molecule is replaced by a TBDMS residue, which proportionally increases the molecular weight of the derivative by 114 Da per replaced hydrogen. Such derivatives are subjected to characteristic fragmentation, with the elimination from the derivative molecule following moieties:  $-CH_3$  (M-15),  $-C_4H_9$  (M-57),  $C_4H_9$  and CO (M-85) or TBDMS-COO (M-159) [11] (Fig.1) [11], although for derivatives of higher molecular mass those ions are unstable, undergoing further fragmentation. Basic mass spectra of all analysed amino acid derivatives are shown in Fig.3.

Tertbutyldimethylsilyl derivatives were eluted from the chromatography column between 7.44 and 16.20 minutes, in the following order: Ala (7.44), Gly (7.64), IS (8.13), Val

(8.60), Leu (8.98), Ile (9.28), Pro (9.64), HypI (11.20), Met (11.35), Ser (11.52), Thr (11.78), Phe (12.33), Asp (12.80), HypII (13.03), Cys (13.18), Glu (13.64), Orn (13.69), Asn (13.88), Lys (14.40), Gln (14.64), His (15.85), Tyr (16.20) (Fig. 2). During the silylation 2 derivatives of hydroxyproline (HypI, HypII) are formed, with two or three active hydrogens replaced with silyl groups; therefore on the chromatogram Hyp is represented by two separate peaks (Fig. 2). A summary of the major ions and molecular mass of derivatives from each amino acid standard is presented in the Table 1.

### Analysis of amino acids in urine samples

An analysis of the amino acid levels in physiological urine was performed. During the analysis a vast peak of urea derivative was dominating in the chromatogram. To remove urea from the sample, an enzymatic hydrolysis with urease was implemented. The procedure improved the analysis and the urea derivative was not detected in the urine samples. We have also confirmed that the urease did not decay to free amino acids in the hydrolysis, and it

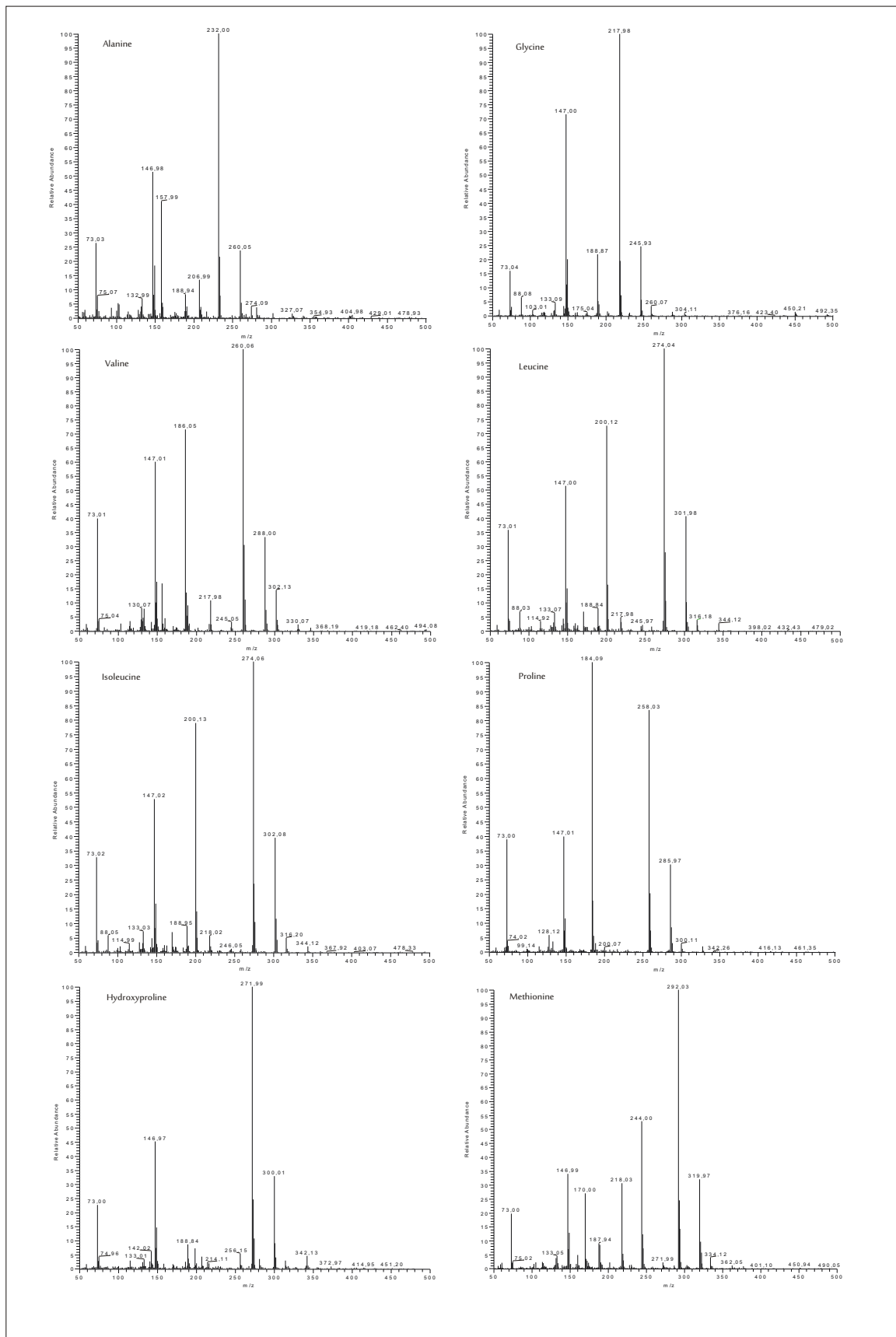


Fig. 3. Mass spectra of TBDMS-derivative of investigated amino acids

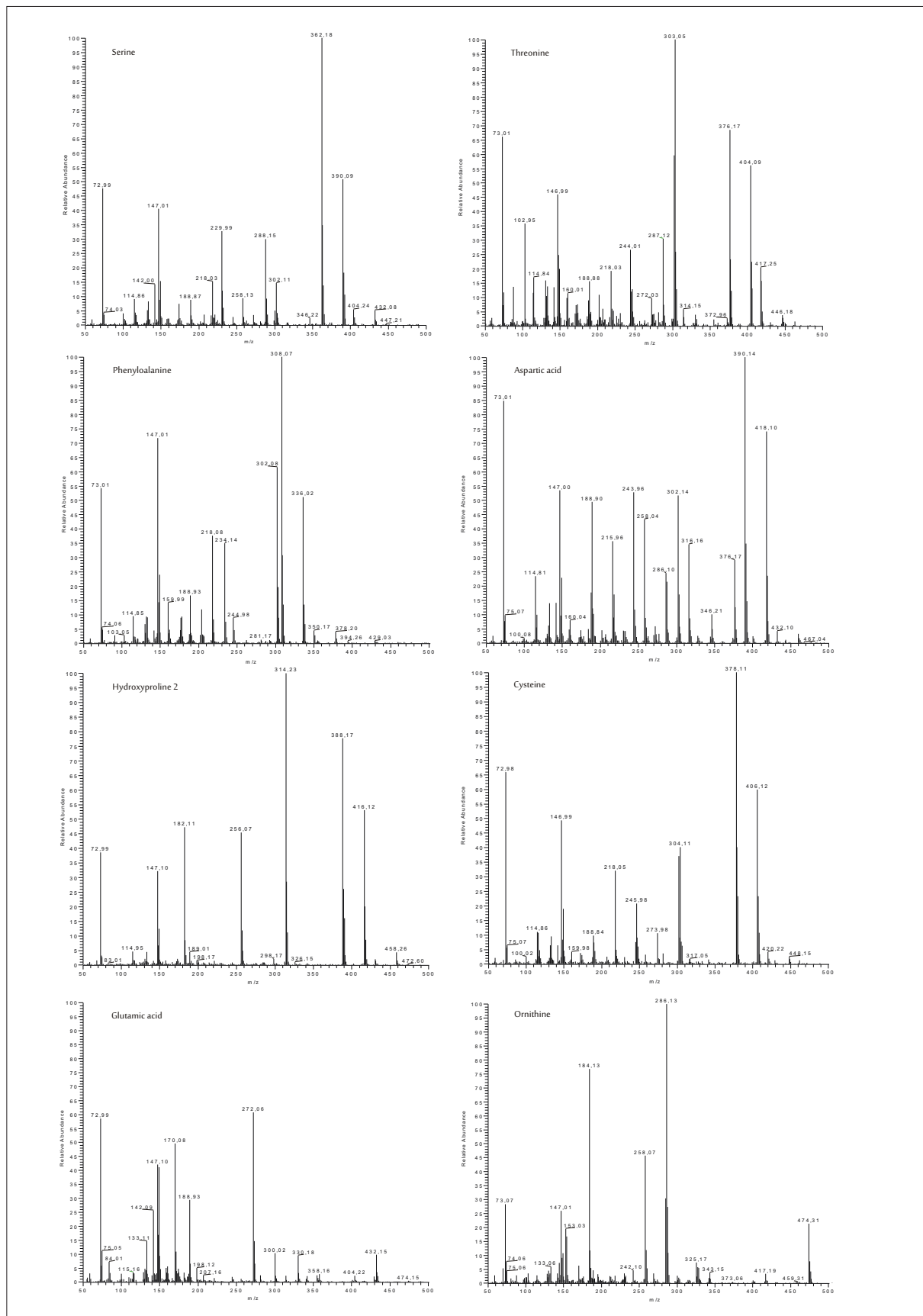


Fig. 3. Mass spectra of TBDMS-derivative of investigated amino acids

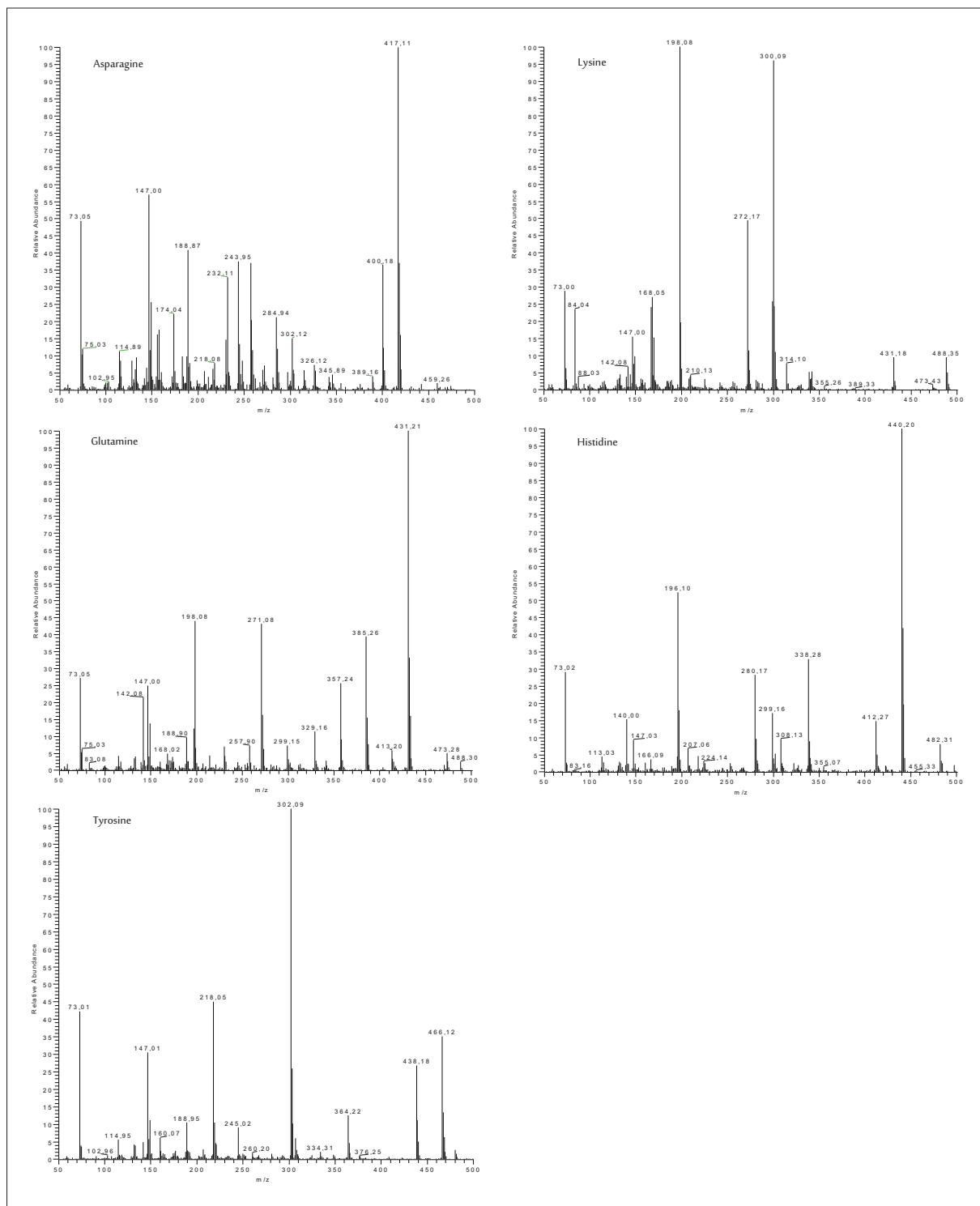


Fig. 3. Mass spectra of TBDMS-derivative of investigated amino acids



**Table 1.** Mass fragment ions of tert-butyldimethylsilyl derivatives of all tested amino acids generated by GLC–MS analysis

Amino acid	Major fragment ions (m/z)	Molecular mass of derivatives	
		+ 2 x TBDMS	+ 3 x TBDMS
Alanine	158, 189, 207, 232, 260	317	
Glycine	189, 218, 246, 260, 288	303	
Internal standard	172, 173, 174, 246, 274	331	
Valine	186, 218, 260, 288, 302	345	
Leucine	189, 200, 274, 302, 303	359	
Isoleucine	189, 200, 218, 274, 302	359	
Proline	184, 185, 258, 286, 300	343	
Hydroxyproline (2 silyl)	189, 198, 256, 272, 300, 342	340	
Methionine	170, 218, 244, 292, 320	377	
Serine	218, 230, 288, 362, 390		447
Threonine	218, 244, 303, 376, 404		442
Phenylalanine	218, 234, 302, 308, 336	393	
Aspartic acid	189, 190, 244, 302, 390		475
Hydroxyproline (3 silyl)	182, 256, 314, 388, 416		454
Cysteine	246, 302, 304, 378, 406		517
Glutamic acid	170, 272, 300, 330, 432		489
Ornithine	184, 185, 258, 286, 474		455
Asparagine	189, 232, 257, 400, 417		474
Lysine	168, 170, 198, 272, 300	410	
Glutamine	198, 271, 357, 385, 431		488
Histidine	196, 280, 299, 338, 440		497
Tyrosine	218, 302, 364, 438, 466		523

therefore did not affect the concentration of amino acids in the urine sample (data not shown).

In the analysis of the urine samples, retention times of derivatives and mass spectra of the detected amino acids were identical to standards.

Generally the presence of 12 amino acids (alanine, glycine, valine, leucine, isoleucine, hydroxyproline, serine, threonine, cysteine, lysine, histidine, tyrosine) was found in tested urine samples (Tab. 2).

#### Analysis of amino acid profiles in samples from patients with tubular injury and healthy controls

The amino acid profiles obtained after analysis of samples from healthy individuals were compared to the profiles of patients with tubular injury (patients addicted to heroin with coexisting HIV and HCV infection). The tubular injury of patients was confirmed by biochemical analysis. The increased level of alpha-GST-isoenzyme, 3.2 ug/l, control 1.1 ug/l, may suggest proximal tubule injury. The level of another marker of renal proximal tubular dysfunction:  $\beta$ 2M, was 0.6 mg/l (0.2 mg/ml in

control). Individual data for biochemical parameters are summarized in Tab.3. In the tested group of 7 women, significantly higher levels of most observed amino acids was obtained (Tab.3, Fig 4). Comparing to control group of healthy donors, the most significant increase was detected for lysine, valine, serine, alanine and leucine, (on average about 30.0, 7.5, 3.6, 2.9 and 0.5 times respectively). A very high level of phenylalanine and the presence of asparagin acid, leucine, proline and cysteine was observed among women with tubular injury (Tab.3, Fig 4).

#### DISCUSSION

Kaspar and colleagues compared the precision and accuracy of gas chromatography/mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) of propyl chloroformate and iTRAQ® derivatized amino acids, respectively, to conventional amino acid analysis method [12]. Their results obtained for amino acids indicate that the GC-MS analysis is the most effective method available, as it exhibits higher reproducibility and allows for automated sample pretreatment.

**Table 2.** Concentration range and average values (with standard deviation) of the amino acids identified in human urine

Amino acid	Healthy Women		Women with renal failure		Healthy Men	
	Concentration range (ug/mg creatinine)	Mean value (ug/mg creatinine)	Concentration range (ug/mg creatinine)	Mean value (ug/mg creatinine)	Concentration range (ug/mg creatinine)	Mean value (ug/mg creatinine)
Alanine	0.2-0.7	0.45 ± 0.1	0.8 – 1.8	1.3 ± 0.4	1.7-10.5	4.93 ± 1.3
Glycine	0.3-10.8	2.88 ± 1.6	1.1 – 6.6	3.2 ± 1.8	5.7-13.0	9.52 ± 1.3
Valine	0-0.1	0.04 ± 0.02	0.0 – 1.1	0.3 ± 0.4	0-1.5	0.54 ± 0.3
Leucine	0	0	0.3-1.4	0.5 ± 0.4	0.5-1.4	0.93 ± 0.2
Isoleucine	0-0.1	0.02 ± 0.02	0.0 – 0.6	0.1 ± 0.2	0	0
Proline	0	0	0.0- 0.8	0.1 ± 0.3	0	0
Hydroxyproline (2 silyl)	0.3-0.8	0.46 ± 0.09	0.0 – 1.5	0.2 ± 0.6	0.4-7.1	4.16 ± 1.1
Serine	0.3-3.8	1.83 ± 0.5	3.1 – 11.8	6.6 ± 3.2	6.7-31.0	20.02 ± 4.4
Threonine	0.1-1.0	0.41 ± 0.2	0.9 – 1.3	1.1 ± 0.2	2.1-9.7	4.31 ± 1.4
Phenylalanine	0	0	0.0- 20.2	6.7 ± 8.7	0	0
Aspartic acid	0	0	0.0- 1.8	0.0 ± 0.1	0	0
Cysteine	0	0	0.0 – 1.3	0.4 ± 0.5	0-3.5	1.7 ± 0.8
Lysine	0-0.2	0.04 ± 0.03	0.0 – 2.7	1.2 ± 1.0	0 – 1.9	0.55 ± 0.4
Histidine	0.4-5.0	2.15 ± 0.7	0.0 – 2.8	1.8 ± 1.1	10.6 – 56.8	22.13 ± 7.5
Tyrosine	0.2-0.7	0.37 ± 0.1	1.1-1.4	0.4 ± 0.6	1.7-13.8	4.52 ± 1.8

Due to the presence of both the amino and carboxyl groups and additional functional groups (alcohol hydroxyl, phenol hydroxyl, second carboxyl, carboxamide, second amino, guanidine, indole, imidazole, disulfide, thiol) the amino acids must be derivatized to achieve sufficient volatility for GC analysis [14].

Three methods of derivatization have been tested: n-butanol esterification, followed by acylation with heptafluorobutyric anhydride, silylation with BSTFA (results not shown) or silylation with MTBSTFA. Esterification, requiring a butanolysis step in acidic conditions, results in partial hydrolysis of amide groups; therefore it was not suitable for analysis of glutamine and asparagines. Bistrimethylsilyl derivatives after BSTFA treatment are less stable and more prone to moisture degradation than tert-butyl dimethylsilyl derivatives after reaction with MTBSTFA; therefore the latter method was used for the analysis of amino acids in urine in the present work.

The derivatization method used and established GLC-MS methodology enabled identification of all tested amino acids. A significant problem during the analysis was the presence of urea in the sample. Urea after reaction with MTBSTFA gave a derivative interfering with several amino acids during detection in GLC-MS analysis (data not shown). The sample treatment with urease, before the derivatization step, decreased the level of urea derivative to a minimum. It was found that addition of urease did not influence the levels of free amino acids in the sample.

Only 12 amino acids out of 20 tested standards were identified in the analysed urine samples; the other 8 were absent or below the detectable level. It may be a result of physiological decomposition or reabsorption, as on average 95% of amino acids are reabsorbed by the proximal tubules [2,24].

The study by Lamont and colleagues on humans showed that there are differences in amino acid metabolism according to gender [16]. Earlier, Proenza and colleagues proved that differences in amino acid concentrations in blood exist not only according to gender but also to age [27].

The comparison of two independent control groups (women and men) was intended as a test of the method applicability, as differences of urine amino acid levels according to gender are well described in the literature. Our study has shown significant gender differences in the concentration of amino acids in urine, what confirmed the method suitability for urine amino acid profiling. As the healthy studied group was age homogeneous (all patients were aged 24 or 25) we have no data concerning age dependency [19].

Our preliminary results, obtained using the presented method, concerning the level of amino acids in urine of women (study group of 7 drug-addicted women with coexisting HIV and HCV infection) showed that the amino acid concentration (alanine, leucine, serine, lysine, valine) was significantly higher than in the

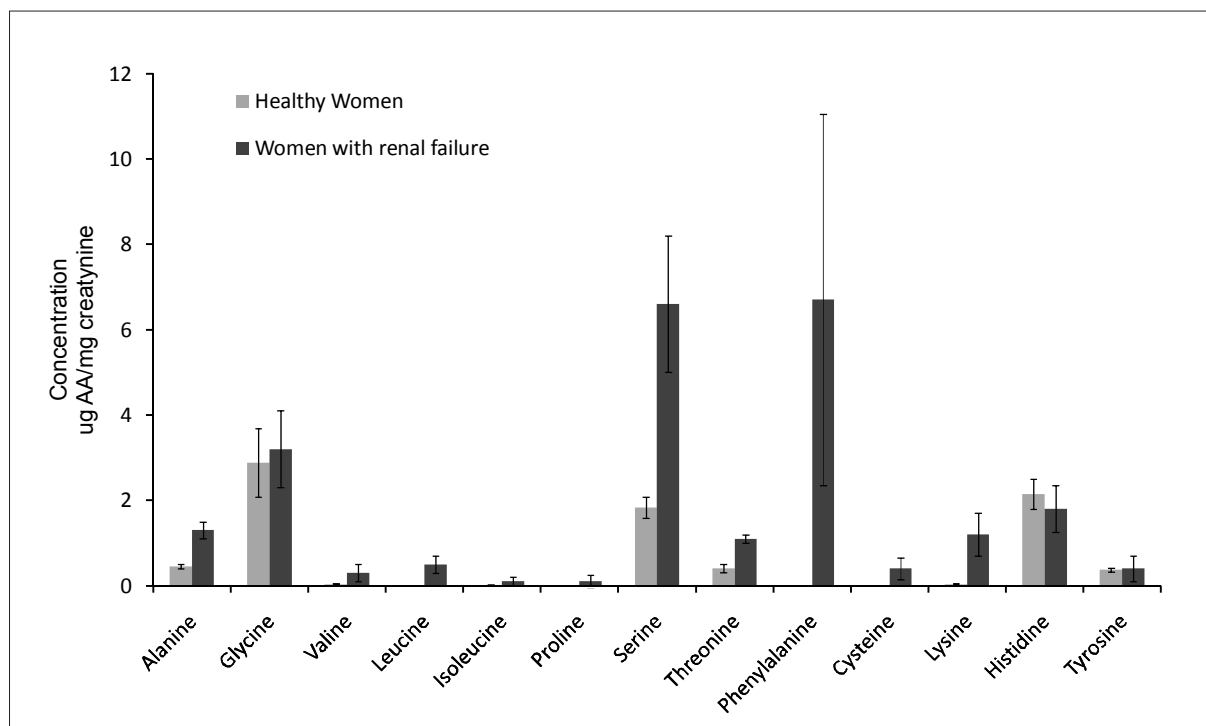


Fig. 4. Summary of concentration (with standard deviation) of the amino acids identified in human urine

Table 3. Biochemical parameters of patients with renal failure

Sample	Age	period of drug addiction [years]	IL-18 [pg/ml]	NGAL [ng/ml]	GST- $\alpha$ [ $\mu$ g/l]	GST- $\pi$ [ $\mu$ g/l]	$\beta$ 2- mikro [mg/l]
1	43	25	n.d.	n.d.	n.d.	n.d.	n.d.
2	25	8	11.2	0.4	2.4	4.4	0.5
3	40	27	26	0.2	2.4	2.9	0.4
4	30	11	28.6	0.3	0.8	2.6	0.3
5	31	5	44.4	0.3	3.6	2.3	1
6	25	10	67.4	1.1	8.8	6.8	1.1
7	36	20	11.2	0.1	1.2	2.6	0.3
	average:		31.5	0.4	3.2	3.6	0.6
	healthy:		8.87	0.27	1.08	3.25	0.2

control group. A high level of phenylalanine and the presence of asparagin acid, leucine, proline and cysteine was also detected in the study group. Increased urinary  $\beta$ 2M value can show a significant disorder of proximal tubular reabsorption or glomerular filtration rate decline. Recent studies have suggested that measurement of the urinary excretion of glutathione S-transferases (GST) *alpha* and *pi* might allow differentiation between proximal and distal tubular cell injury [29]. The increased level alpha-GST-isoenzyme located in the proximal renal tubules may suggest, that drug addicts, are particularly vulnerable to proximal tubule injury, in contrast to the level of pi-GST which did not differ from healthy subjects (Table 3). We assume that

the observed changes in the level of amino acids may be used as a new biomarker of renal tubules condition, as it has been presented by a group Boudonck [1] on animals. The presented method of monitoring renal tubules, can be used in therapies based on the highly nephrotoxic drugs.

The higher level of selected amino acids may be related to kidney dysfunction caused by drug abuse, coexisting HIV and HCV infection and sustained use of antiretroviral drugs. This preliminary study confirmed that the increased excretion of amino acids in urine can occur under the influence of pathogenic factors or nephrotoxic activity of xenobiotics.

Presented application of amino acid profile in urine, for monitoring of nephrological status of the patient, can be further developed into a tandem spectrometry method, utilizing multi-reaction monitoring (MRM), what significantly improves specificity and sensitivity of the method especially in the analysis of complex matrices. Application of tandem spectrometry will additionally extend the capabilities of the amino acid profiling for nephrotoxicity monitoring. The efficiency of such a methodology has been presented for methylformate derivatization of several amino acids and metabolites [15].

The analysis by gas chromatography-mass spectrometry provides new opportunities for detection of amino acids as a diagnostic tool and gives more useful information than the study of individual markers of tubular injury. Complex analysis of human urine allows for the creation of an amino acid profile characteristic for the injury of specific nephron parts. Such a noninvasive method can be developed in xenobiotic nephrotoxicity studies. The

presented results are preliminary, the applicability of GLC-MS amino acid profiling using TBDMS-derivatives for monitoring of renal tubular injury requires further, larger scale studies.

## CONCLUSIONS

Developed method (GLC-MS analysis of TBDMS-derivatives) enables the quantification of amino and carboxylic group containing metabolites in human urine. We found that this approach based on GLC-MS detection can be used in nephrotoxicity studies for urine amino acids monitoring in exposure to xenobiotics and drugs.

## Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article. Research funding played no role in the study design; in the collection, analysis, and interpretation of data or in the decision to submit the report for publication.

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