Original Article:

A method of GC-MS analysis of serum metabolites

Masoud Soheili[1], Afsaneh Arefi Oskouie[2], Mostafa Rezaei Tavirani[3], Ghazaleh Aliakbarzadeh[4], Mahmoud Salami[5,*]

[1]Student Research Committee, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Department of Basic Sciences, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Proteomics Research Center, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4]Department of Chemistry, Faculty of Science, University of Tehran, Tehran, Iran
[5]Physiology Research Center, Kashan University of Medical Sciences, Kashan, Iran

*Corresponding author: email address: salami-m@kaums.ac.ir (M.Salammi)

ABSTRACT

Introduction: Metabolites are the intermediate and natural low molecular weight products of metabolic reactions which naturally occur within cells. Metabolomics is a post-genomics study that analyzes metabolic profile in all biological samples. Gas chromatography-mass spectrometry (GC-MS) is a powerful technique that detects volatile components. This study evaluates the metabolite profile of serum with a simple method for derivatization. Methods: In this study, the metabolic profile of serum sample of 16 rats was analyzed using GC-MS after protein precipitation, using acetonitrile, and derivatize with a method of less in number and volume of materials. Acquisition chromatograms were pre-processed and analyzed using National Institute of Standards and Technology (NIST) library.Results: 35 metabolites with probability of >60% perfect match were detected according to spectrum fragments analysis by NIST library.
Conclusion: This study represents the metabolome profile of serum instead of the change of metabolites that can be evaluated with a simple derivatization method.

Keywords: metabolomics; rat; serum; GC-MS

INTRODUCTION

Metabolites are low-molecular-weight (<1 kDa) components in the biological sample such as urine and serum [1]. The metabolites are small molecules which interfere in the biochemical pathway essential for growth, development and reproduction mechanisms [2]. The flux of information from gene activation to translation affects the level and fluctuation of metabolites. The concentration of metabolites at a given time can represent the state of the organism. Metabolomics or metabolome profiling is the quantitative and qualitative study of the metabolites that provides a considerable capacity for diagnostics [3]. This approach is an exclusive top-down approach for studying complex systems that evaluate the end result. [4]. Biomarker discovery in research and clinical conditions is an advantage of metabolomics that provide diagnostic and prognostic information [5]. The chromatographic techniques in combinations with mass spectrometry facilitated the analysis of metabolome [6]. Gas chromatography-mass spectrometry (GC-MS) is a powerful technique that extensively applies in metabolomics [7]. The volatile components are detectable in GC-MS analysis. Limitation of GC-MS in metabolomics is the study of nonvolatile components [8]. A derivatization reaction is necessary to generate them suitably for separation on GC column [9]. While the previous studies focused on either various materials or high volume of each material for derivatization [10-12] in the present study, this study attempted to decrease both the quantity of materials and the volume of each for derivatization. Further, total serum metabolome was assessed instead of measuring the change of metabolites by others.

MATERIALS AND METHODS

Reagents

Pyridine, methoxylamine hydrochloride, N-methyl-N-(trimethylsilyl)- trifluoracetamide (MSTFA) and acetonitrile (HPLC grade) were purchased from Sigma-Aldrich (Poole, UK).

Sample collection and preparation

In this experimental study, a total of 16 male Wistar rats serum sample were obtained from Physiology Research Center, Kashan University of Medical Sciences. Blood samples were collected from animals into tubes. After 10 min, the clot was separated and the solvent was centrifuged at 3000g for 10 min. Then the supernatant (300mL) was protein precipitated (room temperature for 15 min) using acetonitrile (1:3 v/v) followed by centrifugation (3000 rpm, 10 min) at room temperature. All the acetonitrile solutions were clear after centrifugation indicating complete solubilization. An aliquot (400 mL) of the supernatant was evaporated to dryness under vacuum. All procedures were in accordance with the Guidelines of Ethical Committee, Deputy of Research, Kashan University of Medical Sciences, Kashan, Iran.

Sample derivatization

Samples had to be derivatised before injection using methoxylamine hydrochloride (20 µL; 40mg/mL in pyridine) at 28^{o}C for 90 min, followed by MSTFA and TMCS (80µL; 70^{o}C; 90min).

Gas chromatography/mass spectrometry (GC/MS) analysis

The sample was analyzed using an Agilent GC-MS system with Chemstation software. A sample of 1 µL was injected with a split ratio of 2 into the GC and then separated with a fused silica HP-5MS capillary column (30 m, 0.25 mm inside diameter, 0.25 µm thickness of the inner liquid in the column). In the chromatographic part, the injector temperature was 270 °C and the helium with a purity of 99.999 was used as carrier gas with a flow rate of 1.5 mL/min. The column temperature condition was as follows: It was initially kept at 40 °C for 3 min, ramped to 280 °C at 5 °C/min, and then held for 10 min. The inter-phase temperature and ion source temperature at mass spectrum were 180 and 150 °C, respectively. The metabolomic analysis was performed in Chemistry and Chemical Engineering Research Center and Analytical Chemistry Research Center, Kashan University.

DATA ANALYSIS

The total ion chromatograms (TIC) of all samples were extracted and subsequently imported into MATLAB environment for further data analysis. In order to remove different chromatographic artifacts, the obtained chromatograms were subjected to various pre-processing steps including baseline correction performing by asymmetric least squares (AsLS) algorithm and peak alignment by Correlation Optimizing warping (COW). The pre-processed chromatograms were then mean-centered and paretoscaled. Finally, each compound was compared with National Institute of Standards and Technology (NIST) as the standard mass spectra library for identification of them. For each peak, the software generated a list of similarities; the similarity indexes more than 60% were assigned compound names.

RESULTS

The spectrum of rat serum metabolites is depicted in figure 1. We detected about 35 metabolites in serum sample. All of the metabolites detected by GC-MS were identified using mass spectral libraries. The commercially available library, NIST, was used for identification of detected metabolites. According to spectrum fragments and its retention times, the derived metabolites are matched to the library and all fragments scored with a match probability percent. The probability of >60% was regarded as a perfect match (table 1). There are 35 metabolites detected through this study with probability of > 60%. Because of derivatization, all metabolites with trimethylsilyl are reported as suffix or pref

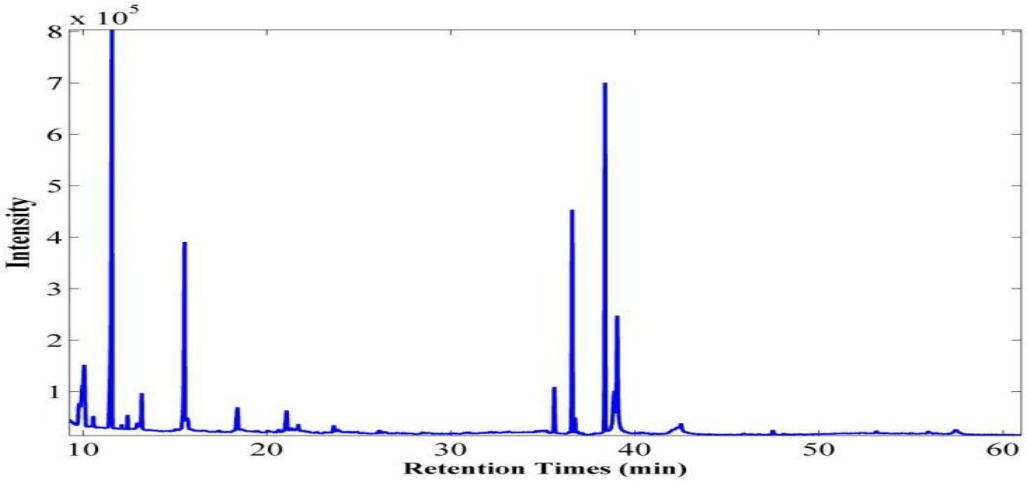

Figure1. The chromatogram of rat serum using GC-MS

Table 1. The metabolites of rat serum detected by GC-MS and using NIST library

RT (min)	Elemental composition	Prob.	MW	Description from best library match
7.971	C8H24O2Si3	89	236	Trisiloxane, octamethyl
8.106	C7H21NSi2	96	175	Silanamine, N,1,1,1-tetramethyl-N-(trimethylsilyl)-
9.775	C7H21NOSi2	95	191	Silanamine, N-methoxy-1,1,1-trimethyl-N-(trimethylsilyl)-
10.83	C7H18N2Si2	89	186	Silanamine, N,N'-methanetetraylbis[1,1,1-trimethyl-
11.558	C8H22O2Si2	73	206	1,2-Bis(trimethylsiloxy)ethane
13.7	C7H15NO3Si	70	189	Propanoic acid, 2-(methoxyimino)-, trimethylsilyl ester
14.49	C8H20O3Si2	67	220	Acetic acid, [(trimethylsilyl)oxy]-, trimethylsilyl ester
14.917	C8H19NO2Si	87	189	l-Valine, trimethylsilyl ester
16.143	C10H30O5Si5	88	370	Cyclopentasiloxane, decamethyl-
16.977	C10H24O3Si2	78	248	Butanoic acid, 3-[(trimethylsilyl)oxy]-, trimethylsilyl ester
17.045	C12H36O4Si5	80	384	Pentasiloxane, dodecamethyl-
19.671	C7H20N2OSi2	90	204	Urea, N,N'-bis(trimethylsilyl)-
20.172	C12H32O3Si3	76	308	Trimethylsilyl ether of glycerol
20.352	C9H27O4PSi3	91	314	Silanol, trimethyl-, phosphate (3:1)
20.884	C12H36O6Si6	97	444	Cyclohexasiloxane, dodecamethyl-
21.023	C11H29NO2Si3	83	291	Glycine, N,N-bis(trimethylsilyl)-, trimethylsilyl ester
21.718	C12H30O4Si3	71	322	Propanoic acid, 2,3-bis[(trimethylsilyl)oxy]-, trimethylsilyl
22.645	C12H26O2Si	60	230	Nonanoic acid, trimethylsilyl ester
25.103	C13H28O2Si	60	244	Decanoic acid, trimethylsilyl ester
25.165	C14H42O7Si7	94	518	Cycloheptasiloxane, tetradecamethyl-
26.679	C11H23NO3Si2	78	273	L-Proline, 5-oxo-1-(trimethylsilyl)-, trimethylsilyl ester
28.999	C16H48O8Si8	74	592	Cyclooctasiloxane, hexadecamethyl-
29.078	C15H39NO3Si3	68	365	Triethylamine, 2,2',2"-tris(trimethylsiloxy)-
30.574	C14H22O4Si2	94	310	1,2-Benzenedicarboxylic acid, bis(trimethylsilyl) ester
32.85	C15H32O4Si2	60	332	Azelaic acid, bis(trimethylsilyl) ester
33.924	C17H36O2Si	71	300	Tetradecanoic acid, trimethylsilyl ester
37.785	C19H40O2Si	88	328	Hexadecanoic acid, trimethylsilyl ester
38.301	C24H60O6Si6	75	612	Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-, scyllo-
38.398	C17H27NO2Si2	97	333	1H-Indole-3-propanoic acid, 1-(trimethylsilyl)-, trimethylsilyl
40.727	C21H40O2Si	69	352	9,12-Octadecadienoic acid (Z,Z)-, trimethylsilyl ester
41.271	C21H44O2Si	88	356	Octadecanoic acid, trimethylsilyl ester
43.789	C23H50O4Si2	90	446	Myristic acid, 2,3-bis(trimethylsiloxy)propyl ester

46.267	C25H54O4Si2	76	474	2-Monopalmitin trimethylsilyl ether
46.794	C25H54O4Si2	95	474	Hexadecanoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester
55.53	C30H54OSi	71	458	Cholesterol trimethylsilyl ether

DISCUSSION

A metabolite is any intermediate and small molecules substance produced during metabolism. Metabolite may also refer to the product resulting from drug metabolization by the body [13, 14]. They have various functions in the body including fuel, structure, signaling, stimulation and inhibition of enzymes. Also, it is directly involved in normal growth, development, and reproduction [15, 16].

This study aimed at evaluating the metabolite profile of serum, using GC-MS technique with a modified method with less amount and volume materials for derivatization. It revealed that there are 35 metabolites in serum with probability of up to 60%, known as a perfect match.

Metabolomics is a procedure which evaluates the metabolites in various conditions and determines system response to perturbations [17]. This approach profiles metabolites in different physiological states and can be used to monitor the changes of metabolites in biofluids [18]. Gas chromatography coupled with mass spectrometry (GC-MS) is a common analytical tool in metabolomics which provides high separation efficiency for biological mixtures [19]. Many investigations used various or high amounts of materials for sample derivatization. Ruoxu Liu and his co-workers used ribitol, methanol, chloroform, deionized water, N2 gas, metoxyamine hydrochloride, MSTFA and TMCS for derivatization [10]. While previous studies reported that 200µL of MSTFA is required for derivatization, the present study proved that 80 µL of MSTFA could be sufficient enough for the process [12]. However, the present study suggests a method with decreased quantity and volume of materials for derivatization. Moreover, total metabolome of serum instead the change of metabolites is presented which is measured in some other studies [20, 21]. This study, similar to some other studies detected 35 metabolites were which almost near to findings of others; however, it would be a limitation of this study where there are lots of metabolites that are ignored in our measurements.

CONCLUSION

This study represents the metabolome profile of serum instead the change of metabolites that can be evaluate with a simple derivatization method.

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"The authors declare no conflict of interest"

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