

# GC/APCI-TOF MS: a new valuable tool for analysis of biofluids in metabolomics studies

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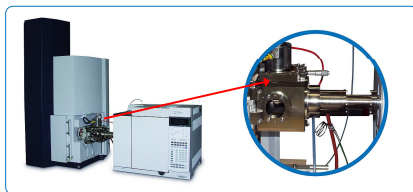
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## Introduction

Gas chromatography-mass spectrometry (GC-MS) is used widely for metabolomics studies in medicine, animal and plant sciences. Indeed the combination of GC with MS, providing the possibility to reveal a chemical identity of studied compounds on basis of retention time and mass information, is an extremely powerful tool. So far, GC-MS has mainly been used with electron ionization (EI) and chemical ionization (CI), which have as main advantage a possibility to use exhaustive commercial and open source libraries to identify compounds of interest. However, both EI and CI are rather harsh the fragmentation techniques, and sometimes it is problematic to identify the molecular ion (the precursor) and to come to a correct conclusion about the identity of a compound. A softer ionization technique, like Atmospheric Pressure Chemical Ionization (APCI) could be a possible alternative to "classical methods". Moreover, GC-APCI combined with modern TOF analyzer, which provides excellent mass accuracy and resolving power has a potential to open a new horizons in GC-based metabolic profiling.

The aim of the study is to explore analytical limits of APCI-GC and its applicability for human body fluids analysis - cerebrospinal fluid (CSF). To carry out the optimization, calculation of the analytical parameters and validation of a method a standard mix consisting of 35 compounds was designed. Compounds were selected with the aim to cover a wide range of polarity and molecular weight typical for biological samples.

Fig. 1 GC/APCI-TOF MS instrument used in this study. (Transfer line enlarged)



## Method

### Standard mixture

Individual stock solutions of the 35 compounds (see Table 1 and results) were prepared in methanol at a concentration of 1 mM.

### CSF

CSF extracts were prepared by cold methanol precipitation (total time 2 h) and subsequent centrifugation. The supernatant was evaporated under N<sub>2</sub>.

### Derivatization reaction

Derivatization reaction was based on a two step procedure: methoxyamination (60 min at 40°C) and silylation with MSTFA+1%TMCS (30 min at 40°C).

### Chromatographic method

The samples (1µL) were injected in a HP-5-MS column (30 m, 0.25 mm ID, 0.25 µm film) and analyzed by a temperature gradient of 5°C/min over 57 min (oven initial T= 70°C kept over 5 min).

### MS method

The transfer line temperature to the MS was 280°C. The APCI source was operated in positive mode, temperature and flow rate of the dry gas (nitrogen) were 250°C and 5.00 l/min, respectively, APCI vaporizer temperature was 450 °C; the pressure of the nebulizer gas (nitrogen) was set to 2 Bar, and the voltage of the corona discharge needle was +2000 nA. Capillary voltage was set at -1000V and the end-plate offset at -1000 V.

MS analysis was performed using a MicroTOF orthogonal-accelerated TOF mass spectrometer (oaTOF-MS, Bruker Daltonik, Bremen, Germany). Spectra were acquired in a mass range from 50–1000 m/z, at repetition rate of 0.7Hz. Mass calibration was done based on cyclic polysiloxane background ions typical for GC-MS.

## Results

The derivatization as well as the separation was optimized using the standard mixture. Figure 2A shows the MS spectrum of monosilylated theophylline, in Fig 2B the separation of the 25 compounds (100µM) that were detected successfully from 35 standards is displayed. The missing compounds (arginine, cysteine, histidine, creatinine, 4-nitrobenzoic acid, glutathione, homovanillyl alcohol, folic acid, thyroxine, Leucine-Enkephaline) are not easily amenable to GC analysis due to thermal instability or require different derivatization techniques. Table 1 summarizes all detected compounds including different degrees of silylations, the mass accuracy as well as mSigma values. The mSigma value is representing a measure for the goodness of fit of the measured to the theoretical isotopic pattern. Mass accuracies are in average well below 1ppm, mSigma values at 4.3.

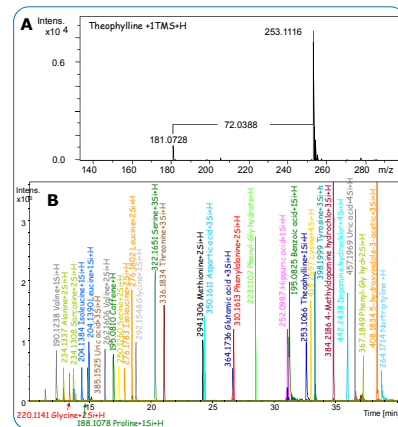


Fig. 2 A: MS spectrum of theophylline; B: Extracted ion chromatograms of the compounds in the standard mixture.

### Determination of analytical parameters

We carried out a study to check the repeatability and reproducibility of the proposed method. Calibration curves for the compounds under study were established and the detection (LOD) and quantification limits (LOQ) were calculated. The LOD values in the nanomolar range are also presented in Table 1.

### CSF Analysis

We applied the developed method to the analysis of human CSF samples. A complex chromatographic pattern with 300 or more peaks was obtained (Fig. 3). Some of the peaks present in the profile have been already identified with standards. The identification process is still ongoing. In order to facilitate & speed up identification, the generation of an MS database containing the GC-APCI-MS spectra in parallel to the well established GC-EI-MS databases would be beneficial for the future.

Compound	Formula	RT	m/z exp.	m/z calc.	Mean Error	mSigma	LOD (nM)
Valine +2Silyl-H	C <sub>12</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub> S <sub>2</sub>	12.4	190.1258	190.1258	1.1	3.4	29
Alanine +2Silyl-H	C <sub>10</sub> H <sub>18</sub> N <sub>2</sub> O <sub>5</sub> S <sub>2</sub>	13.0	214.1340	214.1340	0.9	5.1	49
Glycine +2Silyl-H	C <sub>8</sub> H <sub>14</sub> N <sub>2</sub> O <sub>5</sub> S <sub>2</sub>	13.5	200.1181	200.1181	1.4	4.6	-
Sarcosine +2Silyl-H	C <sub>10</sub> H <sub>18</sub> N <sub>2</sub> O <sub>5</sub> S <sub>2</sub>	13.8	224.1372	224.1340	0.0	5.1	-
Isoalloxine +2Silyl-H	C <sub>14</sub> H <sub>16</sub> N <sub>4</sub> O <sub>5</sub> S <sub>2</sub>	14.4	264.1414	264.1414	0.0	1.8	25
Proline +1Silyl-H	C <sub>8</sub> H <sub>12</sub> N <sub>2</sub> O <sub>5</sub> S	14.9	188.1108	188.1101	-3.7	2.2	73
Leucine +2Silyl-H	C <sub>14</sub> H <sub>24</sub> N <sub>2</sub> O <sub>5</sub> S <sub>2</sub>	15.3	240.1400	240.1414	2.4	1.8	25
Orn. Acid +3Silyl-H	C <sub>12</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub> S <sub>3</sub>	15.2	305.1545	305.1544	0.0	3.4	-
Valine +2Silyl-H	C <sub>12</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub> S <sub>2</sub>	16.3	262.1656	262.1653	-1.1	6.1	-
Caffeine +H	C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub>	17.0	195.0870	195.0877	3.6	1.8	39
Serine +2Silyl-H	C <sub>10</sub> H <sub>18</sub> N <sub>2</sub> O <sub>5</sub> S <sub>2</sub>	17.4	205.2200	205.2200	-0.4	3.6	-
Isoalloxine +2Silyl-H	C <sub>14</sub> H <sub>16</sub> N <sub>4</sub> O <sub>5</sub> S <sub>2</sub>	17.9	276.1813	276.1810	-1.1	8.9	-
Leucine +2Silyl-H	C <sub>14</sub> H <sub>24</sub> N <sub>2</sub> O <sub>5</sub> S <sub>2</sub>	18.5	276.1803	276.1810	2.9	6.4	-
Leucine +3Silyl-H	C <sub>16</sub> H <sub>26</sub> N <sub>2</sub> O <sub>5</sub> S <sub>3</sub>	18.8	292.1578	292.1579	0.3	4.7	25
Serine +3Silyl-H	C <sub>12</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub> S <sub>3</sub>	20.4	322.1683	322.1684	0.9	5.1	36
Threonine +3Silyl-H	C <sub>12</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub> S <sub>3</sub>	21.1	336.1834	336.1841	2.1	3.9	35
Methionine +2Silyl-H	C <sub>14</sub> H <sub>22</sub> N <sub>2</sub> O <sub>5</sub> S <sub>2</sub>	24.2	294.1376	294.1374	-0.7	1.3	45
Aspartic Acid +2Silyl-H	C <sub>12</sub> H <sub>18</sub> N <sub>2</sub> O <sub>5</sub> S <sub>2</sub>	24.3	300.1361	300.1361	0.9	1.9	38
Glutamic Acid +2Silyl-H	C <sub>13</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub> S <sub>2</sub>	24.7	314.1512	314.1510	-1.1	5.0	48
Phenylalanine +2Silyl-H	C <sub>16</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub> S <sub>2</sub>	26.7	310.1653	310.1653	0.0	5.8	33
Phenyl-Glycine +H	C <sub>14</sub> H <sub>16</sub> N <sub>2</sub> O <sub>5</sub>	28.5	223.1080	223.1077	-1.3	3.3	19
Proline +3Silyl-H	C <sub>10</sub> H <sub>18</sub> N <sub>2</sub> O <sub>5</sub> S <sub>3</sub>	31.3	252.1047	252.1050	1.2	3.8	17
Biotinyl Acid +2Silyl-H	C <sub>17</sub> H <sub>24</sub> N <sub>2</sub> O <sub>5</sub> S <sub>2</sub>	31.3	195.0833	195.0836	0.5	3.2	12
Theophylline +2Silyl-H	C <sub>10</sub> H <sub>14</sub> N <sub>4</sub> O <sub>5</sub> S <sub>2</sub>	32.6	253.1116	253.1115	-0.4	1.9	15
Lysine +4Silyl-H	C <sub>18</sub> H <sub>28</sub> N <sub>4</sub> O <sub>5</sub> S <sub>4</sub>	33.0	435.2009	435.2000	-2.3	2.5	22
Tyrosine +3Silyl-H	C <sub>18</sub> H <sub>22</sub> N <sub>2</sub> O <sub>5</sub> S <sub>3</sub>	33.3	368.1979	368.1979	-0.2	5.5	19
4-Methylpyridine +3Silyl-H	C <sub>14</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub> S <sub>3</sub>	34.8	384.2159	384.2205	1.6	4.2	18
Dopamine +4Silyl-H	C <sub>18</sub> H <sub>26</sub> N <sub>2</sub> O <sub>5</sub> S <sub>4</sub>	35.9	442.2448	442.2444	-0.9	4.0	19
Orn. Acid +4Silyl-H	C <sub>16</sub> H <sub>24</sub> N <sub>2</sub> O <sub>5</sub> S <sub>4</sub>	36.3	407.1992	407.1993	-0.4	9.1	24
Phenyl-Glutamine +2Silyl-H	C <sub>18</sub> H <sub>22</sub> N <sub>2</sub> O <sub>5</sub> S <sub>2</sub>	37.2	342.1860	342.1868	0.3	5.2	17
Hydroxyindol-3-acetic Acid +2Silyl-H	C <sub>18</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub> S <sub>2</sub>	38.3	408.1843	408.1841	-0.2	7.7	17
Homocysteine +H	C <sub>9</sub> H <sub>14</sub> N <sub>2</sub> O <sub>5</sub>	38.7	264.1744	264.1747	1.1	9.2	66

Tab. 1 Summary of mass accuracies, mSigma values and detection limits (LOD) for the standard mixture.

## ASMS 2009, WPD-118

## CSF analysis

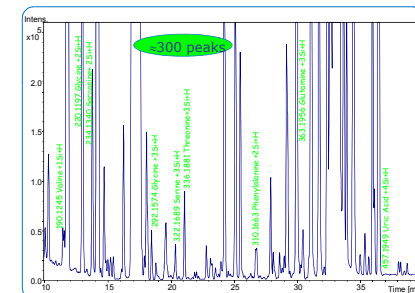


Fig. 3 Basepeak chromatogram of derivatized CSF sample.

## Conclusions

- Derivatization reaction is a crucial step and it is necessary to optimize its conditions in depth.
- In the positive mode, mostly [M+H]<sup>+</sup> ions are observed.
- 25 compounds in the standard mixture were successfully detected.
- Mass accuracies were excellent for the standards with errors of 1ppm and mSigma values at 4.3 in average.
- The LOD values for the new GC-APCI-TOF-MS method were in the nanomolar range.
- The GC-APCI-TOF MS method developed is an alternative for the analysis of metabolites in biological fluids.