ORIGINAL PAPER

DOI: https://doi.org/10.20883/jms.2019.335

LC-MS/MS based targeted metabolomics method for analysis of serum and cerebrospinal fluid

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ABSTRACT

Introduction. Recent instrumentation and software advancement enabled to develop new, high-throughput targeted metabolomics methods for in-depth exploration of metabolome in a quantitative manner.
Material and Methods. The presented targeted metabolomics approach allows to analyze both of serum and CSF in the same way, with identical sample preparation procedures. The analyses were carried out using high-performance liquid chromatography system coupled to triple quadrupole tandem mass spectrometer with electrospray ion source (LC-ESI-QqQ-MS/MS).
Results. The applied targeted metabolomics approach enabled to determine a wide panel of metabolites from different chemical classes of compounds including: acylcarnitines, amino acids and biogenic amines, glycerophospholipids, sphingolipids and sum of hexoses. Finally, 148 metabolites in serum and 57 in cerebrospinal fluid were determined.
Conclusions. Here we presented the results of successful implementation of the method of analysis of low-molecular weight compounds in human serum and CSF using targeted metabolomics. The evaluation of selected groups of metabolites resulted in obtaining the mean concentrations of panel of metabolites in serum and CSF, which gives a valuable information about the metabolome of these matrices.

Keywords: liquid chromatography, tandem mass spectrometry, flow injection analysis.

Introduction

Metabolome, as a sum of small molecules which reflects, both of genetic and environmental factors affecting human's health at a given time, seems to be a perfect tool for describing physiological or pathological processes and in the result identifying biomarkers or introducing personalized treatment approach. To reach such important goals, a reliable and reproducible analytical methods are needed [1]. Until today, many different techniques were employed to investigate human metabolome such as separation techniques (gas chromatography (GC), liquid chromatography (LC)) coupled to mass spectrometry (MS) or nuclear magnetic resonance (NMR) spectroscopy [2]. None of these techniques seems to be perfect for reliable analysis of the whole metabolome yet. Thus, researchers' efforts are focused on developing tools which might enable identifying and quantifying as many

metabolites as possible. Until recently, untargeted metabolomics approach was prevailing in discovery-based investigations, but today due to instrumentation and software development we can apply high-throughput targeted metabolomics methods [3] to explore metabolome in a quantitative manner [4]. That approach allows the researchers to skip time consuming step of identification of relevant metabolites — characteristic for untargeted approach, and to focus on data interpretation taking into account clinical needs (e.g. data comparability to reference ranges). The aim of the study was the application of targeted metabolomics method allowing to analyze wide spectrum of metabolites from five different chemical classes of compounds in serum and cerebrospinal fluid (CSF) from human subjects, in the same way.

Material and Methods

Quantitative metabolomics of CSF and serum covers wide spectrum of analytes form different groups of compounds, including: 40 acylcarnitines, 21 biogenic amines, 21 amino acids, 90 glycerophospholipids (14 lysophosphatidylcholines and 76 phosphatidylcholines), 15 sphingolipids and sum of hexoses. It yields the total of 188 metabolites quantified simultaneously (**Figure 1**). The assays were performed using AbsoluteIDQ p180 kit (Biocrates Life Sciences AG, Innsbruck, Austria) according to standard operation procedures. The methodology is based on 2-layers, 96-deepwell plate supplied by manufacturer, with selected internal standards partially integrated with filter layer of the plate.

10 serum and 10 CSF samples were analyzed. All samples were collected from patients who has no diagnosed nervous system disorders. The research was performed in accordance with the Declaration of Helsinki. All study subjects gave written informed consent prior the sample collection. (Bioethics Committee of Poznan University of Medical Sciences — Decision no. 821/16 and 206/17). First step of analytical workflow was to add 10 µL of internal standards mixture containing rest of internal standards on the filter layer. Then phosphate buffered saline, seven calibration standards of different concentration and quality

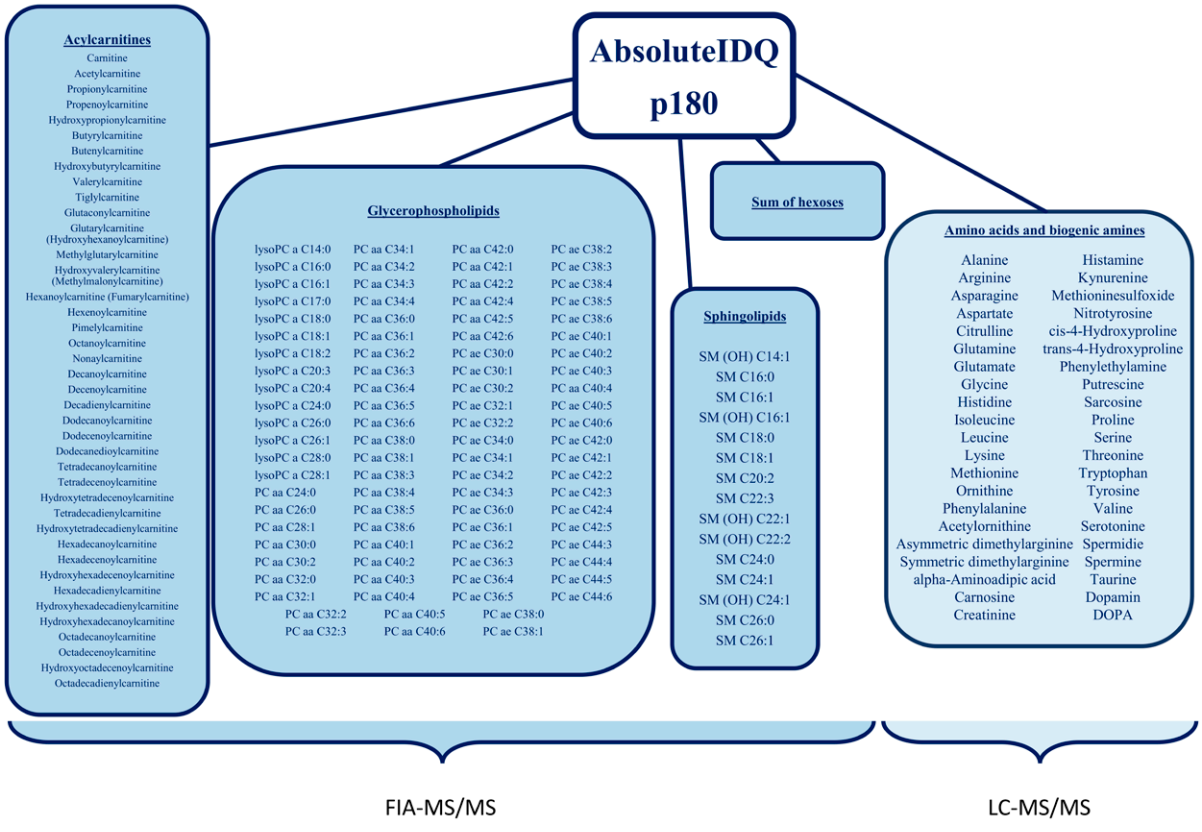

Figure 1. List of all metabolites analyzed using Absolute IDQ p180 methodology in FIA-MS/MS mode and LC-MS/MS mode

controls samples (QC) were put in the respective wells. 3 concentration levels (low, medium, high) of QC samples were analyzed. Biological samples were thawed in room temperature and pipetted on the appropriate spots in amount of 10 µL and 30 µL of serum and CSF, respectively. The plate was then dried under nitrogen flow for 30 minutes. During that time the derivatization agent was prepared by vortexing rigorously 300 µL of phenylisothiocyanate (PITC) with 5700 µL of mixture of pyridine and ethanol in water (1:1:1). After 30-minutes of drying, 50 µL of derivatization solution was put in each well. Covered plate was left for 20-minutes incubation at room-temperature. Next the second round of drying under nitrogen flow was performed for 60 minutes. Then 300 µL of extraction solvent containg 5 mM of ammonium acetate in methanol was pipetted to each well, after which covered plate was shaken at room temperature for 30 minutes using orbital plate shaker at 450rpm. Subsequently sample extracts were passed through the filter layer to the lower capture plate via nitrogen pressure. Finally sample extract was split into two analogous 96-well plates and diluted with 150 µL of water and 400 µL of FIA mobile phase for LC-MS/MS analysis and FIA-MS/MS analysis, respectively. Each plate was sealed with silicone mat to reduce evaporation and placed in autosampler.

The LC run as well as FIA run were carried out using high-performance liquid chromatography system 1260 Infinity (Agilent Technologies, Santa Clara, CA, USA) coupled to triple quadrupole tandem mass spectrometer 4000 QTRAP (Sciex, Framingham, MA, USA). Quantification of amino acids and biogenic amines was preceded by chromatographic separation (LC-MS/MS part) using 0.2% solution of formic acid (phase A) and 0.2% formic acid in acetonitrile (phase B) at the flow rate 0.5 mL/min. Gradient elution was as follows: 0–0.5 min, 100% phase A; 0.5–5.5 min, linear to 5% phase A; 5.5–6.5 min holding at 5% phase A; followed by returning to 100% phase A (6.5–7.0 min); and finally 7.0–9.5 min, 100% phase A. The chromatography was carried out on an ZORBAX Eclipse XDB-C18 (3.0 x 100 mm, 3.5 µm) column (Agilent Technologies, Santa Clara, CA, USA), with a pre-column (C18, 4.0 x 3.0 mm) SecurityGuard (Phenomenex, Torrance, CA, USA). In turn, remaining metabolites were determined by injecting sample into mobile phase, at isocratic flow, directly to mass spectrometer (flow injection analysis, FIA-MS/MS). These compounds were analyzed by mass spectrometer only, bypassing the chromatographic column, according to different m/z ratios.

Data acquisition and quantification were performed under control of Analyst 1.5.2 software (Sciex, Framingham, MA, USA). Sample management and data processing were carried out using MetIDQ version Boron software (Biocrates Life Sciences AG, Innsbruck, Austria). In the next step the raw data (obtained as chromatographic peaks) for QC samples and calibrators were manually reviewed and peak integrations were checked in order to validate the performance of both acquisition and quantitation methods of in-house MS-system. To confirm the reliability of the results, the QC samples evaluation using software build-in tool (MetVAL module, Biocrates Life Sciences AG, Innsbruck, Austria) was performed. Afterwards the concentrations of metaboliteswere calculated (µM) using MetSTAT module (Biocrates Life Sciences AG, Innsbruck, Austria). The metabolites with 50% or more of missing data (concentration below limit of detection) were excluded. Then, the remaining missing data was replaced with half of minimal determined concentration for appropriate metabolite.

Results

Two different matrices: serum and cerebrospinal fluid from human subjects were analyzed using targeted metabolomics approach, according to manufacturer's operation procedures. Quality assessment of the data was passed by QC samples analyzed in LC-MS/MS analysis, as well as QC samples analyzed in FIA-MS/MS analysis (all tested metabolites concentration levels were within reference ranges), indicating satisfactory accuracy and the performance of the method. In subjects' sera 148 metabolites were determined, including: 14 acylcarnitines, 21 amino acids, 13 biogenic amines, 84 glycerophospholipids, 15 sphingolipids and sum of hexoses. Conversely, in CSF 57 metabolites were determined, and they were as follow: 1 acylcarnitine, 18 amino acids, 5 biogenic amines, 23 glycerophospholipids, 9 sphingolipids and sum of hexoses (**Table 1**). It corresponds to 78.72% (serum) and 30.32% (CSF)

Table 1. The number of metabolites determined in serum and cerebrospinal fluid

Groups of metabolites	Metabolites included in the panel (No)	Biological matrix			
		Serum		Cerebrospinal fluid	
		Determined compounds (No)	% of determined metabolites	Determined compounds (No)	% of determined metabolites
Acylcarnitines	40	14	35,00	1	2,50
Aminoacids	21	21	100,00	18	85,71
Biogenic amines	21	13	61,90	5	23,81
Glycerophospholipids	90	84	93,33	23	25,56
Sphingolipids	15	15	100,00	9	60,00
Sum of hexoses	1	1	100,00	1	100,00
Total	188	148	78,72	57	30,32

of the total of 188 metabolites possible to quantitate with applied methodology. The full list of determined metabolites is given in **Table 2**. All possible amino acids and sphingolipids as well as sum of hexoses, were determined in human serum. In the remaining groups of compounds some metabolites were not observed in serum or they did not exceed limit of detection. Nevertheless, abovementioned targeted metabolomics approach enables determining 35.0% acylcarnitines, 61.90% biogenic amines and 93.33% glycerophospholipids amongst wider spectrum of compounds. In case of CSF the applied approach enables determining: sum of hexoses, 85.71%

Table 2. Metabolite concentrations determined in the biological matrices — serum and cerebrospinal fluid (means with standard deviation (SD); minimum and maximum; μM)

Class of compounds	Metabolites	Serum				Cerebrospinal fluid			
		Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Acylcarnitines	C0	39.860	13.527	15.000	63.900	–	–	–	–
	C2	5.451	2.916	1.390	12.600	0.457	0.208	0.108	0.754
	C3	0.313	0.100	0.167	0.454	–	–	–	–
	C4	0.159	0.049	0.095	0.242	–	–	–	–
	C5	0.103	0.030	0.048	0.150	–	–	–	–
	C10	0.215	0.117	0.084	0.402	–	–	–	–
	C12:1	0.124	0.047	0.049	0.186	–	–	–	–
	C14	0.032	0.016	0.013	0.059	–	–	–	–
	C14:1	0.071	0.039	0.022	0.139	–	–	–	–
	C14:2	0.022	0.012	0.009	0.041	–	–	–	–
	C16	0.112	0.037	0.043	0.159	–	–	–	–
	C18	0.048	0.013	0.028	0.067	–	–	–	–
	C18:1	0.120	0.051	0.039	0.206	–	–	–	–
	C18:2	0.037	0.012	0.012	0.050	–	–	–	–
Amino acids	Ala	396.200	70.345	311.000	516.000	30.040	9.076	21.700	49.000
	Arg	123.280	14.584	97.800	141.000	22.800	2.827	18.800	26.500
	Asn	54.230	8.545	38.700	68.800	6.590	1.146	4.500	8.200
	Asp	28.730	7.001	18.600	38.700	–	–	–	–
	Cit	28.650	6.521	19.900	40.900	2.041	0.561	1.460	3.230
	Gln	676.600	94.663	448.000	789.000	521.700	92.959	437.000	733.000
	Glu	64.510	29.944	31.500	118.000	–	–	–	–
	Gly	340.900	113.998	213.000	625.000	6.047	2.236	2.610	8.970
	His	90.320	20.711	65.200	143.000	15.220	3.409	11.100	20.900
	Ile	73.400	10.456	60.800	89.100	6.633	2.730	3.900	12.400
	Leu	141.700	21.375	118.000	184.000	15.300	5.711	9.270	26.200
	Lys	213.200	25.879	162.000	252.000	34.920	6.411	27.900	46.000
	Met	22.250	2.576	15.800	25.400	3.795	1.290	2.310	6.230

Table 2. Contd.

Class of compounds	Metabolites	Serum				Cerebrospinal fluid			
		Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Amino acids	Orn	86.430	20.572	57.100	123.000	6.097	1.935	3.970	10.200
	Phe	73.600	7.959	64.200	83.700	10.600	4.005	6.300	19.400
	Pro	212.200	66.758	115.000	314.000	–	–	–	–
	Ser	144.160	26.215	94.600	183.000	28.260	4.085	23.300	36.000
	Thr	122.110	20.364	94.000	153.000	30.600	6.439	21.300	41.300
	Trp	62.260	9.273	49.000	76.800	1.890	0.633	1.160	3.310
	Tyr	61.880	12.507	47.800	89.500	8.932	3.087	5.470	14.400
	Val	207.800	31.815	170.000	254.000	17.930	6.477	11.200	31.500
Biogenic amines	Ac–Orn	0.419	0.385	0.107	1.410	–	–	–	–
	ADMA	0.439	0.086	0.287	0.572	–	–	–	–
	Creatinine	85.120	12.080	69.300	105.000	68.790	13.983	44.300	90.700
	Kynurenine	2.482	0.539	1.810	3.510	–	–	–	–
	Met–SO	0.651	0.261	0.390	1.190	–	–	–	–
	Putrescine	0.106	0.034	0.025	0.143	0.132	0.028	0.090	0.177
	SDMA	0.355	0.224	0.173	0.757	–	–	–	–
	Serotonin	0.662	0.349	0.052	1.170	–	–	–	–
	Spermidine	0.144	0.041	0.086	0.220	–	–	–	–
	Spermine	0.148	0.004	0.141	0.156	–	–	–	–
	t4–OH–Pro	7.959	1.412	6.070	10.300	0.422	0.129	0.266	0.657
	Taurine	128.800	44.465	74.800	199.000	6.260	0.877	4.900	7.430
	total DMA	0.684	0.128	0.499	0.899	0.151	0.070	0.052	0.279
Glycerophospholipids	lysoPC a C16:0	92.670	17.678	68.300	120.000	–	–	–	–
	lysoPC a C16:1	2.586	1.165	1.460	4.760	–	–	–	–
	lysoPC a C17:0	4.299	3.338	1.380	8.670	–	–	–	–
	lysoPC a C18:0	28.300	7.132	20.300	42.600	–	–	–	–
	lysoPC a C18:1	18.160	4.167	12.100	25.000	–	–	–	–
	lysoPC a C18:2	19.790	4.945	12.700	27.100	–	–	–	–
	lysoPC a C20:3	2.165	0.756	1.360	3.630	–	–	–	–
	lysoPC a C20:4	7.167	1.691	5.040	9.610	–	–	–	–
	lysoPC a C26:0	0.312	0.139	0.136	0.611	–	–	–	–
	lysoPC a C26:1	0.186	0.080	0.053	0.309	–	–	–	–
	lysoPC a C28:0	0.346	0.168	0.172	0.716	–	–	–	–
	lysoPC a C28:1	0.474	0.180	0.246	0.845	–	–	–	–
	PC aa C24:0	0.087	0.067	0.039	0.243	–	–	–	–
	PC aa C28:1	2.653	0.899	1.370	4.250	–	–	–	–
	PC aa C30:0	7.266	4.216	3.080	14.200	–	–	–	–
	PC aa C30:2	0.741	0.353	0.402	1.450	0.002	0.001	0.001	0.004
	PC aa C32:0	14.809	3.563	7.690	18.900	0.310	0.119	0.176	0.589
	PC aa C32:1	15.264	7.597	7.170	32.500	0.104	0.030	0.060	0.162
	PC aa C32:2	2.374	1.150	0.681	4.320	0.005	0.003	0.002	0.009
	PC aa C32:3	0.544	0.229	0.281	1.010	–	–	–	–
	PC aa C34:1	209.900	41.065	136.000	280.000	1.658	0.615	0.792	3.020
	PC aa C34:2	303.200	68.618	143.000	397.000	0.120	0.125	0.031	0.367
	PC aa C34:3	12.426	4.076	5.540	17.700	–	–	–	–
	PC aa C34:4	1.624	0.673	0.722	2.890	–	–	–	–
	PC aa C36:0	3.880	1.733	1.950	6.830	–	–	–	–
	PC aa C36:1	50.970	14.887	31.600	73.600	0.225	0.074	0.119	0.374
	PC aa C36:2	182.910	53.762	85.100	287.000	0.202	0.069	0.107	0.318
	PC aa C36:3	120.220	26.770	71.500	156.000	0.089	0.047	0.048	0.208
	PC aa C36:4	187.600	35.991	112.000	233.000	0.210	0.078	0.110	0.366
	PC aa C36:5	23.210	7.030	12.500	32.200	–	–	–	–
	PC aa C36:6	0.965	0.293	0.415	1.340	–	–	–	–
	PC aa C38:0	3.332	0.623	2.310	4.120	–	–	–	–
	PC aa C38:1	1.189	0.508	0.201	2.150	0.006	0.004	0.003	0.013

Class of compounds	Metabolites	Serum				Cerebrospinal fluid			
		Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Glycerophospholipids	PC aa C38:3	50.940	12.632	35.000	69.000	0.075	0.033	0.040	0.148
	PC aa C38:4	107.970	24.259	69.900	143.000	0.211	0.068	0.119	0.326
	PC aa C38:5	52.010	11.978	34.400	76.100	0.058	0.023	0.037	0.103
	PC aa C38:6	90.610	15.929	71.200	114.000	0.078	0.029	0.041	0.117
	PC aa C40:2	0.302	0.068	0.166	0.382	–	–	–	–
	PC aa C40:3	0.619	0.196	0.405	0.923	–	–	–	–
	PC aa C40:4	3.560	1.032	2.250	5.020	0.017	0.006	0.010	0.024
	PC aa C40:5	9.881	2.927	6.590	15.400	–	–	–	–
	PC aa C40:6	30.280	4.597	23.400	37.000	–	–	–	–
	PC aa C42:0	0.597	0.142	0.401	0.864	–	–	–	–
	PC aa C42:1	0.291	0.062	0.207	0.401	–	–	–	–
	PC aa C42:2	0.241	0.052	0.151	0.326	–	–	–	–
	PC aa C42:4	0.197	0.045	0.136	0.269	–	–	–	–
	PC aa C42:5	0.352	0.100	0.217	0.566	–	–	–	–
	PC aa C42:6	0.461	0.169	0.160	0.738	–	–	–	–
	PC ae C30:0	0.459	0.165	0.282	0.744	–	–	–	–
	PC ae C30:1	0.380	0.203	0.182	0.829	–	–	–	–
	PC ae C32:1	2.458	0.564	1.390	3.590	0.020	0.007	0.011	0.031
	PC ae C32:2	0.810	0.401	0.459	1.520	–	–	–	–
	PC ae C34:0	1.806	0.552	1.120	2.610	–	–	–	–
	PC ae C34:1	9.610	2.304	5.310	13.400	0.072	0.025	0.033	0.115
	PC ae C34:2	9.781	3.198	3.750	15.700	0.053	0.020	0.023	0.084
	PC ae C34:3	7.035	2.620	2.480	11.400	–	–	–	–
	PC ae C36:0	1.024	0.375	0.677	1.760	–	–	–	–
	PC ae C36:1	7.992	1.698	5.590	10.700	–	–	–	–
	PC ae C36:2	11.576	3.178	6.340	18.000	–	–	–	–
	PC ae C36:3	7.687	2.199	3.700	10.200	0.012	0.008	0.002	0.023
	PC ae C36:4	18.869	5.013	8.390	26.300	–	–	–	–
	PC ae C36:5	18.060	8.069	10.600	32.000	0.057	0.050	0.014	0.151
	PC ae C38:0	1.993	0.486	1.350	2.860	–	–	–	–
	PC ae C38:1	0.967	0.449	0.437	1.830	–	–	–	–
	PC ae C38:2	1.616	0.684	0.908	2.730	–	–	–	–
	PC ae C38:3	4.488	1.181	2.970	6.790	–	–	–	–
	PC ae C38:4	14.376	3.235	9.760	20.400	–	–	–	–
	PC ae C38:5	17.869	4.074	8.790	23.400	0.026	0.017	0.010	0.060
	PC ae C38:6	8.255	1.793	4.290	10.600	0.010	0.006	0.004	0.022
	PC ae C40:1	1.307	0.300	0.781	1.690	–	–	–	–
	PC ae C40:2	1.975	0.503	1.390	2.950	–	–	–	–
	PC ae C40:3	1.117	0.292	0.754	1.750	–	–	–	–
	PC ae C40:4	2.308	0.560	1.510	3.550	–	–	–	–
	PC ae C40:5	3.396	0.680	2.170	4.740	–	–	–	–
	PC ae C40:6	5.177	0.882	4.100	6.240	–	–	–	–
	PC ae C42:1	0.308	0.080	0.201	0.440	–	–	–	–
	PC ae C42:2	0.552	0.149	0.302	0.797	–	–	–	–
	PC ae C42:3	0.737	0.146	0.492	0.963	–	–	–	–
	PC ae C42:4	0.684	0.269	0.182	1.070	–	–	–	–
	PC ae C42:5	1.700	0.661	0.955	2.410	–	–	–	–
	PC ae C44:3	0.127	0.033	0.084	0.198	–	–	–	–
	PC ae C44:4	0.426	0.147	0.289	0.798	–	–	–	–
	PC ae C44:5	1.913	0.661	1.150	3.580	–	–	–	–
	PC ae C44:6	1.270	0.427	0.681	2.130	–	–	–	–

Table 2. Contd.

Class of compounds	Metabolites	Serum				Cerebrospinal fluid			
		Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Sphingolipids	SM (OH) C14:1	4.420	1.879	1.980	7.540	–	–	–	–
	SM (OH) C16:1	3.023	1.016	1.940	4.840	0.007	0.007	0.001	0.021
	SM (OH) C22:1	11.779	3.000	7.100	17.300	0.019	0.006	0.010	0.030
	SM (OH) C22:2	11.338	3.677	7.020	17.900	0.024	0.006	0.016	0.033
	SM (OH) C24:1	1.142	0.332	0.702	1.750	–	–	–	–
	SM C16:0	99.890	22.959	65.900	139.000	0.319	0.140	0.147	0.552
	SM C16:1	13.075	4.911	7.560	23.700	0.034	0.016	0.010	0.062
	SM C18:0	22.480	4.706	19.100	33.100	0.338	0.121	0.185	0.544
	SM C18:1	11.338	3.519	8.040	19.200	0.080	0.028	0.036	0.115
	SM C20:2	0.677	0.493	0.257	1.540	–	–	–	–
	SM C22:3	4.709	5.538	0.443	14.100	–	–	–	–
	SM C24:0	20.330	6.065	12.800	30.200	0.049	0.028	0.028	0.116
	SM C24:1	58.660	13.230	39.600	80.200	0.160	0.072	0.073	0.304
	SM C26:0	0.153	0.059	0.091	0.278	–	–	–	–
	SM C26:1	0.463	0.127	0.331	0.705	–	–	–	–
Sum of hexoses	H1	5972.700	822.089	4480.000	6899.000	3756.500	552.259	2843.000	4497.000

amino acids, 60.00% sphingolipids, 25.56% glycerophospholipids, 23.81% biogenic amines and only 1 acylcarnitine, which corresponds to 2.50% of all acylcarnitines included in panel.

It is easily seen that the quantification of 148 metabolites in serum and 57 in CSF has result in changes of contribution of particular groups of metabolites determined in both fluids compared to their contribution in the whole set of 188 metabolites provided by the applied methodology. **Figure 2** presents the percentage of each of group metabolites in the spectrum of: 188 compounds evaluated in applied methodology (**Figure 2A**); 148 metabolites determined in serum (**Figure 2B**); 57 metabolites determined in CSF (**Figure 2C**). The change in numbers of determined acylcarnitines is the highest. Among 40 acylcarnitines possible to quantitate 14 was determined in serum and only 1 in CSF, which corresponds to 9% of metabolites of this group of compounds quantified in serum and 2% of metabolites quantified in CSF.

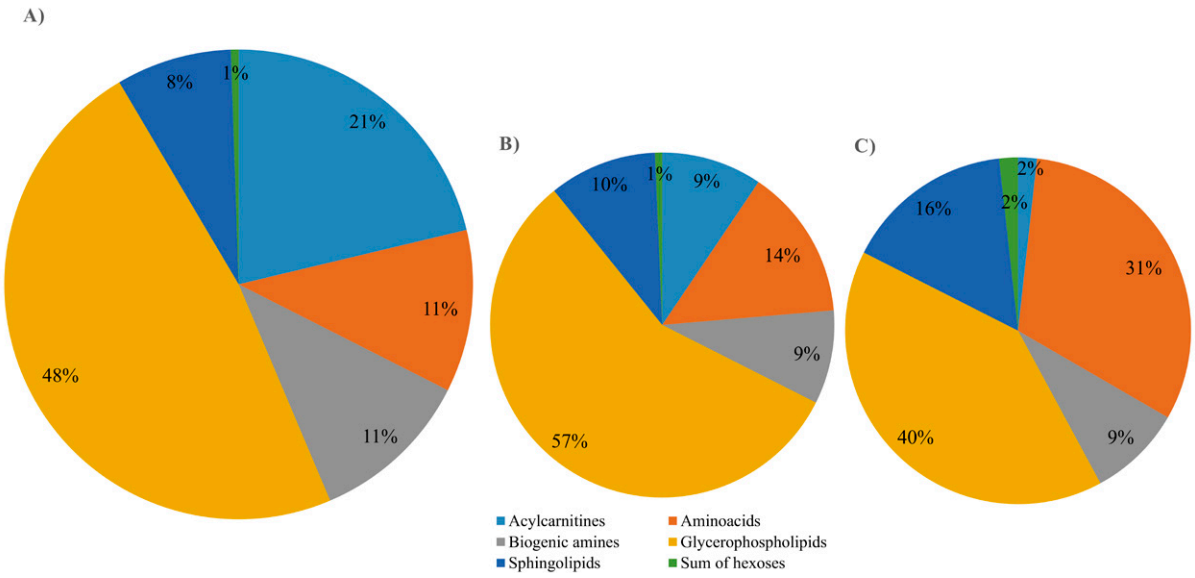

Figure 2. The percentage of each group metabolites in the spectrum of: 188 compounds evaluated in applied methodology (**A**); 148 metabolites determined in serum (**B**); 57 metabolites determined in CSF (**C**)

Discussion

As Aretz and Meierhofer [4] concluded, there is no perfect tool, nor methodology, allowing to reliably measure the entire metabolome at once. Nowadays we distinguish two complementary approaches in metabolomics: targeted metabolomics focused on selected metabolite or groups of metabolites [5, 6] and untargeted metabolomics focused on global profiling of the metabolome [7], very often without quantitative data [8]. Both of them have their own advantages and pitfalls [4].

Here we presented the results of successful implementation of method of the determination of low-molecular weight compounds in human serum and CSF. The assays were performed by using hyphenated mass spectrometric techniques: LC-MS/MS and FIA-MS/MS. This targeted strategy has emerged as a satisfactory compromise between wide spectrum of analyzed compounds (characteristic for untargeted approach) and highly selective and sensitive measurement of selected compounds (targeted approach; MRM mode). What is worth to emphasize, despite different composition of CSF and serum, presented method allows to analyze both matrices in the same way, with identical sample preparation procedures. During the whole analysis the only noteworthy difference between these matrices is an amount of sample pipetted onto the 96-well plate. It gives the invaluable tool for simultaneous analysis CSF and serum that might be crucial for central nervous system (CNS) disorders research. The better understanding of correlation between CSF and serum metabolome as well as the blood-brain barrier permeability for metabolites in different medical conditions could contribute to reducing the number of invasive diagnostic procedures such as lumbar puncture in the future [9]. The limitation of the study is limited number of analyzed samples resulting from invasiveness of CSF collection. That is why we recommend to analyze these data with respect to other metabolomics cohort studies.

Presented results from implementation of the method of evaluation of selected groups of metabolites resulted in obtaining the mean concentrations of panel of metabolites in serum and CSF, which gives a valuable information about metabolome of these matrices for other researchers focused directly on investigations of e.g. central nervous system disorders. This is crucial due to the fact of lacking wide-spectrum targeted metabolomics data of both serum and CSF from human subjects. And secondly, presented results are highly translatable and can be easily compared through the application of widely used methodology with confirmed interlaboratory reproducibility [10] and validation according to European Medicine Agency Guideline [11]. To conclude, we successfully implemented targeted metabolomics method allowing to analyze both of serum and CSF in the same way, and we applied it to analyze serum and CSF from human subjects. Evaluation of 148 metabolites in serum and 57 metabolites in CSF gives a tool for searching for disturbances in metabolism and enables determination of the broad variety of compound classes and confirms usefulness of described methodology for further studies on both serum and CSF metabolome.

Acknowledgements

Conflict of interest statement

The authors declare no conflict of interest.

Funding sources

There are no sources of funding to declare.

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Acceptance for editing: 2019-02-08
Acceptance for publication: 2019-03-09

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