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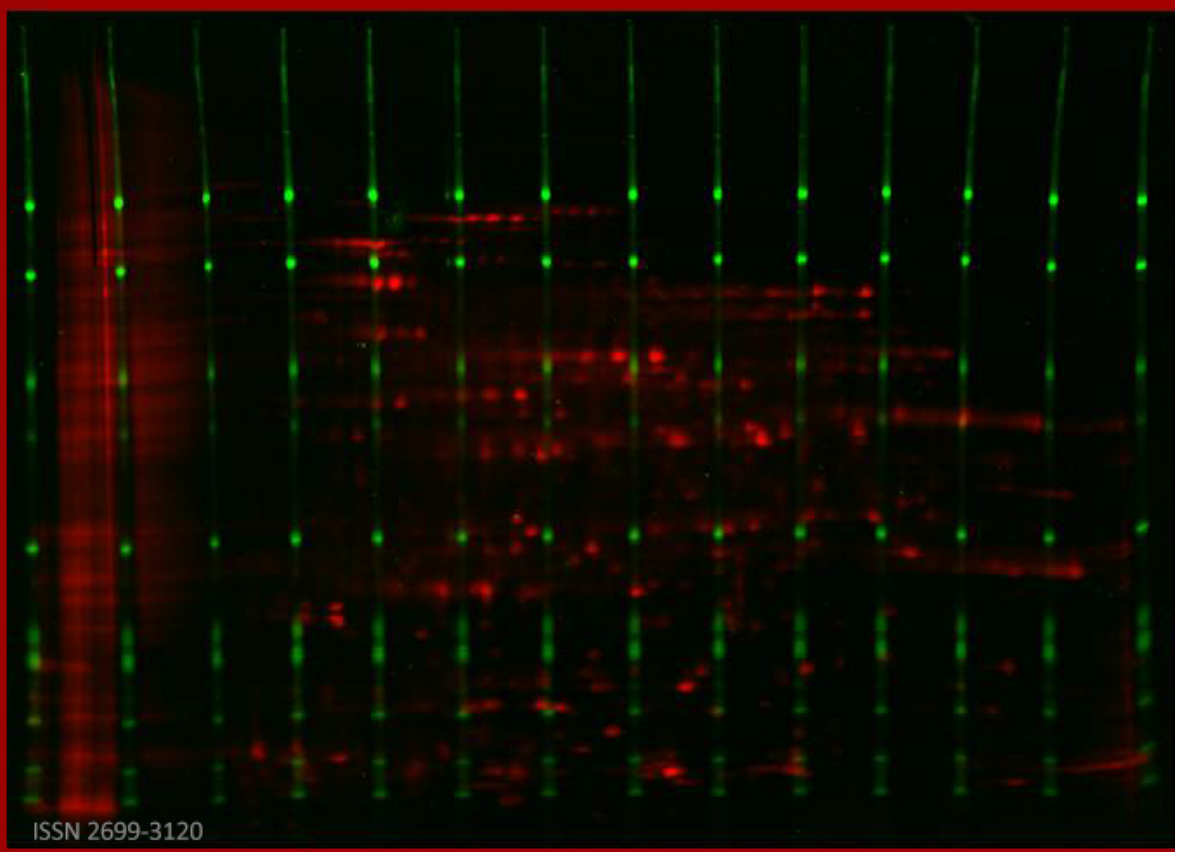

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Cover image
Mercator gel (run by D. Ackermann at CUP) representing the award-winning CoFGE technology for standardized gel electrophoresis

Protocol

Target analysis of underivatized biogenic amines with reversed-phase chromatography and quadrupole time-of-flight mass spectrometry

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Keywords: Polyamines, Q-TOF, spermine, spermidine

Abstract

Low-molecular-weight polyamines such as spermine and spermidine are ubiquitous in all forms of life and are involved in many cellular functions such as cell growth. Pathological altered polyamine concentration are associated with a variety of diseases. We thus aimed for their analysis from serum using reversed-phase liquid chromatography coupled to detection with quadrupole time-of-flight mass spectrometry. We tested 15 polyamines for their response, developed a multiplex target method, and refined it for five active substances including spermine and spermidine, which induced expression of the autophagy protein Atg8/LC3-II in a neuronal cell line.

Introduction

Spermine and spermidine are the most prominent polyamines in organisms. Low-molecular-weight polyamines have more than two amino groups and are aliphatic amino acid-derived polycations at physiological pH. They are synthetized in a multi-enzyme process from urea cycle-

derived ornithine and are involved in many fundamental cellular functions like cell growth and survival [1]. Pathologically altered polyamine concentrations are associated with diseases such as gastric cancer [2] and Parkinson disease [3]; inhibition of polyamine synthesis has been successfully tested as an effective cancer chemoprevention option in preclinical studies [1].
We were interested in the detection of biogenic amines in serum and in particular of those, which induced autophagy. To that end, we investigated 15 synthetic polyamines for their response in reversed-phase liquid chromatography (RP-LC) coupled to quadrupole time-of-flight mass spectrometry (Q-TOF-MS). A target multiplex method was developed for the simultaneous specific measurement of the five active substances including spermine and spermidine, which induced expression of the autophagy protein Atg8/LC3-II in a neuronal cell line [3].

Experimental

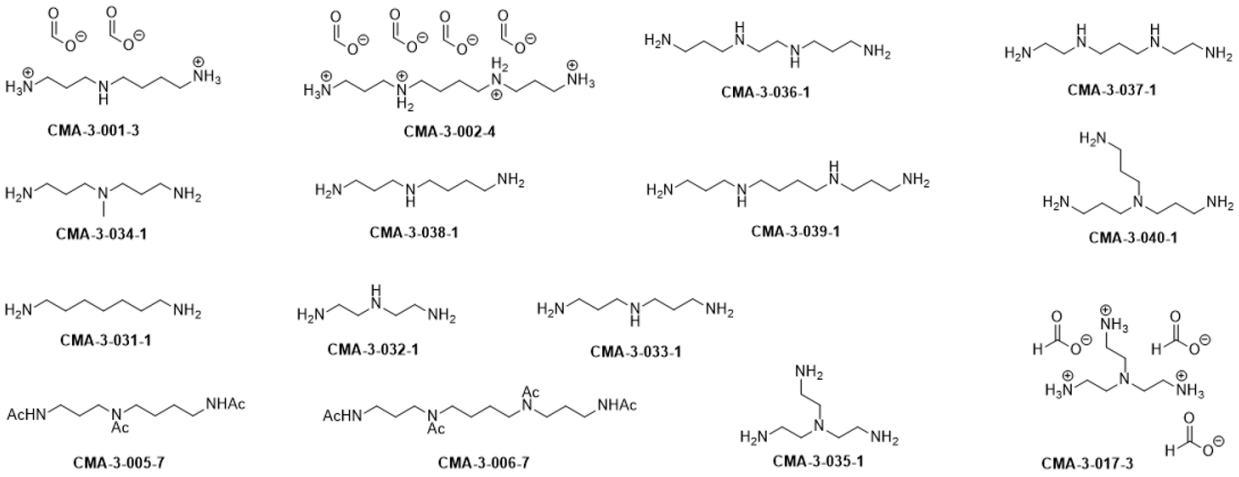
Polyamines (Table 1) were synthetized in the group of R. Gilmour, dissolved in water at 1 pmol/µl and separated with RP-LC on Q-TOF Premier coupled to nanoAcquity (Waters Corp.; ZORBAX ECLIPSE XCD-C18 column (0.3 x 150 mm, 3.5 µm; Agilent) with trap column (ZORBAX 300SB-C18, 5 µm, 5 x 0.3 mm, Agilent). The solvent system was: A: 0.1% aqueous formic acid (FA) and B: 0.1% FA in acetonitrile using 0.005% heptafluorobutyric adic as a modifier as suggested before [3, 4]. The flow rate was 8 µl/min for 34 min runs (3-11 min 5-40% B, 11-13 min 40% B, 13-18 min 100% B). MS source parameters were: capillary voltage 3.2 kV, desolvation temperature 320°C, cone gas 100 l/h, desolvation gas 500 l/h. The quadrupole was set to *m/z* 100 – 200 - 300 with 25% dwell and ramp time, respectively. Spectra were collected at a scan rate of 0.1 s and 0.01 s interscan delay in positive ion mode. A collision energy ramp from 6 to 14 eV was used for collision-induced dissociation (CID). Polyamines were measured at 5 or 10 (32-1, 33-1, 34-1, 38-1) pmol on-column using direct injection.

Results

The aim of this work was a method with which polyamines could be detected in serum following protein depletion by trichloroacetic acid precipitation. Using an 8 min-gradient from 5 to 40% organic solvent followed by 2 min of isocratic separation and a 5 min-gradient to 100% B, separation of the polyamines with different structure was achieved in the range from 13 to 18 min as is shown for the five most active compounds in Fig. 1. Three substances were supplied as salts (1-3, 2-4, 17-3) and appeared at the same *m/z* value as the corresponding uncharged polyamines as did isomeric structures (Table 1). Target-MS/MS was thus only feasible for LC-separated compounds as demonstrated in Fig. 1B/C for spermidine and its isomer, N^1-(3-aminopropyl, methyl) propane-1,4-diamine. Examples for CID fragmentation patterns of acetylated and regular polyamines are shown in Fig.s 3 and 4. These fragmentation profiles serve as markers for specific compound identification from complex matrices. A pseudo-MRM method was set up for the five compounds, which showed the best response in autophagy experiments (5-7, 32-1, 34-1, 38-1 (spermidine) and 39-1 (spermine)). It contained an overview scan to *m/z* 300 and four target MS/MS scans (*m/z* 104.11, 146.16, 203.22, 272.19).

Table 1: Synthetic polyamine standard substances and their major protonated ion in electrospray MS. Some of them appear at the same parent ion in MS as a result of common features in their basic structure. The compounds with most activity in the autophagy assay were 5-7, 32-1, 34-1, 38-1 (spermidine) and 39-1 (spermine).

#	Formula	m_{mono}	$[M+H]^+$
1-3	C9H23N3O4	237,1689	146,1579
34-1	C7H19N3	145,1579	146,1579
38-1	C7H19N3	145,1579	146,1579
35-1	C6H18N4	146,1531	147,1531
17-3	C9H24N4O6	284,1696	147,1531
2-4	C14H34N4O8	386,2377	203,2157
39-1	C10H26N4	202,2157	203,2157
5-7	C13H25N3O3	271,1896	272,1896
6-7	C18H34N4O4	370,2580	371,2580
31-1	C7H18N2	130,1470	131,1470
32-1	C4H13N3	103,1109	104,1109
33-1	C6H17N3	131,1422	132,1422
36-1	C8H22N4	174,1844	175,1844
37-1	C7H20N4	160,1688	161,1688
40-1	C9H24N4	188,2001	189,2001



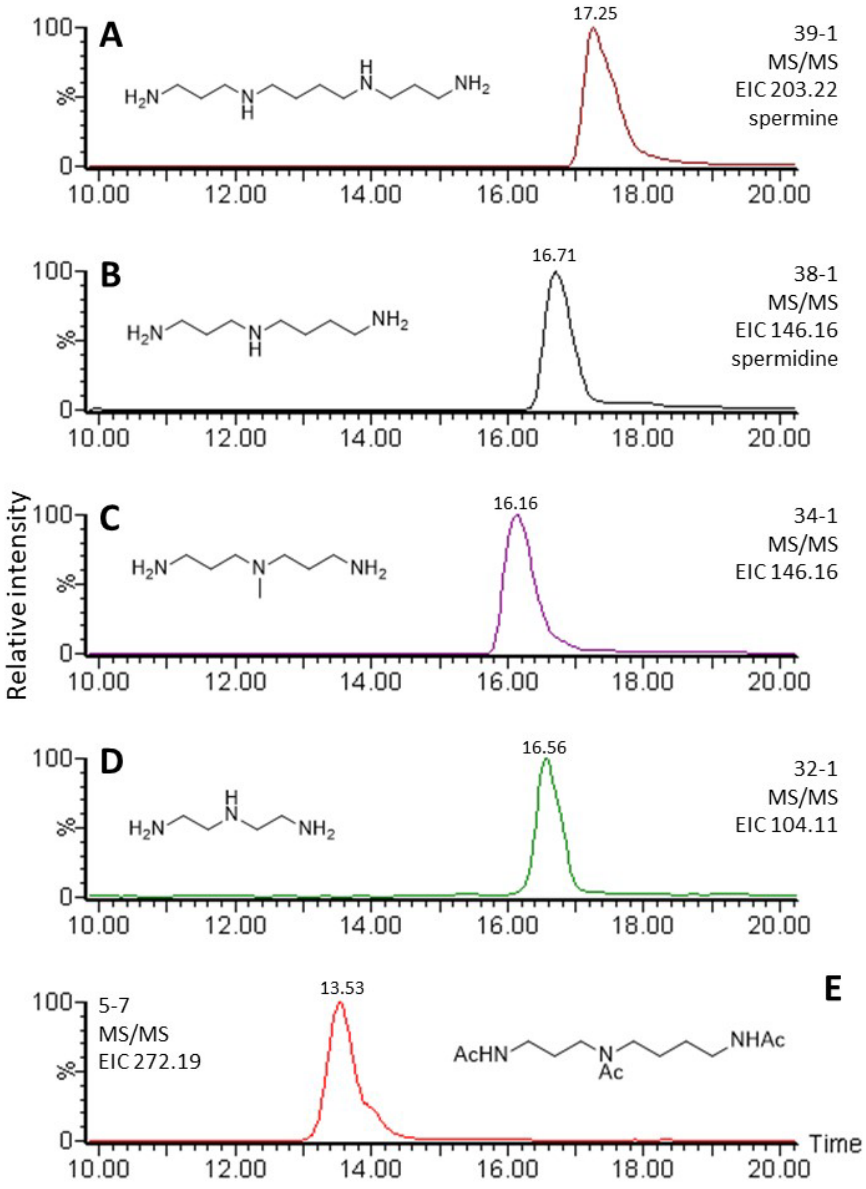


Figure 1: Extracted ion chromatograms (EIC) for the five most active polyamines (Table 1).

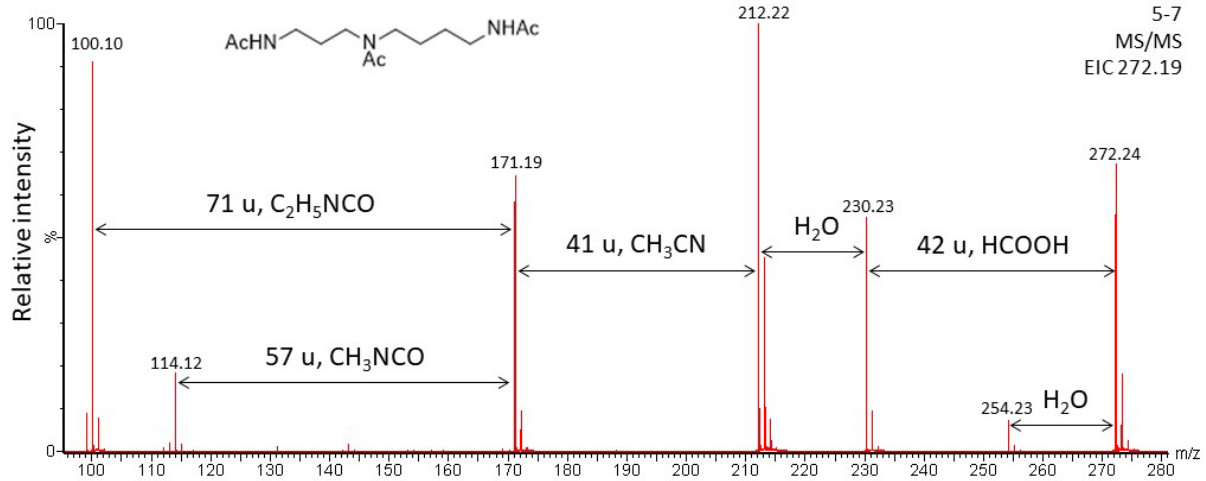


Figure 2: Fragmentation spectrum for compound 5-7, triactetylspermidine (Table 1), demonstrating the typical neutral losses.

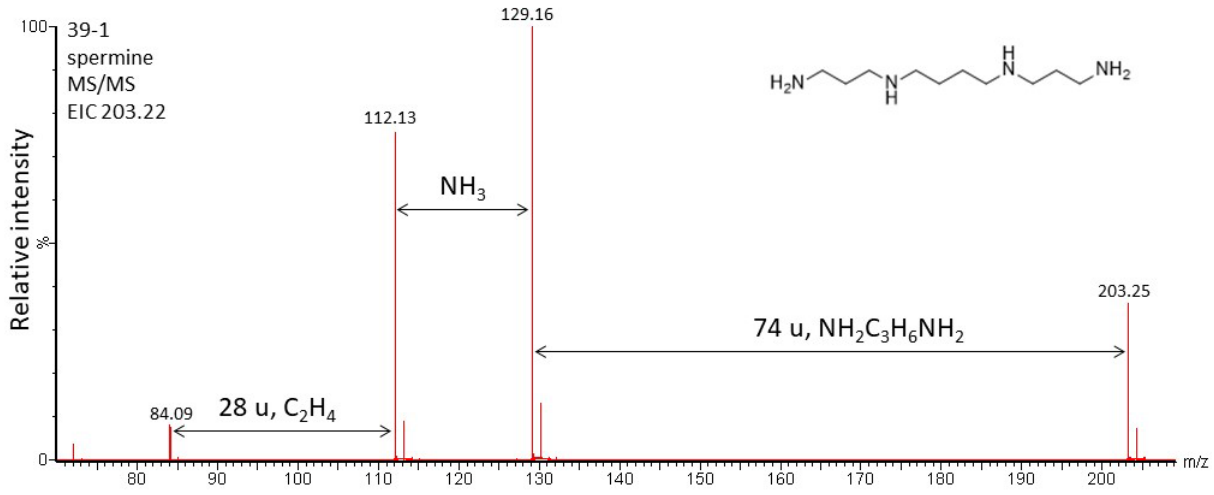


Figure 3: Fragmentation spectrum for compound 39-1, spermine (Table 1), illustrating the typical neutral losses.

Conclusion

An LC-MS based method was developed for the detection of 15 polyamines, which was ultimately refined for the measurement of the five most active compounds in an autophagy assay. Polyamine serum concentrations are expected at 4 to 40 µM so that our method with its high fmol to low pmol sensitivity is applicable unless poor recovery from serum hampers analysis. The direct measurement from protein-depleted serum as suggested by some authors [3] may not be the best way of sample preparation with respect to LC-column protection and pressure stability. Therefore, more specific isolation methods are still employed using, e.g., multiple solid-phase extraction steps [4] or derivatisation such as carbamoylation [5] and dansylation [6]. The best method for our purposes is still being investigated.

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