Determination of acidic catecholamine metabolites in plasma and cerebrospinal fluid using gas chromatography-negative-ion mass spectrometry

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DETERMINATION OF ACIDIC CATECHOLAMINE METABOLITES IN PLASMA AND CEREBROSPINAL FLUID USING GAS CHROMATOGRAPHY—NEGATIVE-ION MASS SPECTROMETRY

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SUMMARY

A method for the assay of acidic catecholamine metabolites in biological fluids using capillary gas chromatography—electron-capture negative-ion mass spectrometry is described. The method combines acetylation of phenolic hydroxy groups in buffered aqueous solution followed by pentafluorobenzyl ester formation and acetylation of aliphatic hydroxy groups under anhydrous conditions. The resulting per-O-acetyl carboxypentafluorobenzyl esters provided excellent negative-ion mass spectra with intense and diagnostic anions. The sensitivity of the analysis using electron-capture negative-ion mass spectrometry exceeds that using electron-impact mass spectrometry by two to three orders of magnitude. Analysis of acidic catecholamine metabolites in human lumbar cerebrospinal fluid and plasma were performed with good precision ($\sigma_{rel} < 5\%$) at the low nanomoles per litre level.
INTRODUCTION

Catecholamines (CA) and their metabolites are of considerable interest in neurological research [1], in clinical diagnosis [2] and in stress-related problems. Catecholamines exert their role primarily as neurotransmitters. The concentrations of these amines and most of the metabolites are normally at the low nanomoles per litre level in plasma and cerebrospinal fluid (CSF) [3]. Various methods have been developed to measure these compounds. Methods employing gas chromatography–mass spectrometry (GC–MS) have proved to be most specific and sensitive. Faull and Barchas [4] have recently reviewed this topic. They summarized several derivatization methods for acidic CA metabolites two of which were suitable for GC–electron-capture negative-ion MS (EC-NIMS). The recent highly sensitive MS technique might be of particular value for the determination of CA and associated metabolites in plasma and CSF. Shimamura et al. [5] reported its application to the determination of trace amounts of labelled isomers of some CA metabolites in urine. EC-NIMS, however, is a less universal technique than the classical electron-impact (EI) and positive-ion chemical-ionization (PICI) MS methods. A limiting condition in using GC–EC-NIMS is the relatively small dynamic range. Owing to exhaustion of thermal electrons in the ion source, a non-linear response might be obtained if large amounts of compounds with electron affinity co-elute with the compound of interest. The potential utility of GC–EC-NIMS has become recognized in biomedical research, as revealed from recent publications [6–13].

In this paper we report a sensitive assay for acidic CA metabolites in biological fluids by GC–EC-NIMS. Basically, the method combines two convenient chemical treatments, providing suitable derivatives in high yields. First, the phenolic hydroxy groups were acetylated in aqueous solution [14–17] and subsequently extracted with an organic solvent. This method facilitates an efficient extraction and improves the selectivity because extractions were accomplished from a less acidic aqueous sample than that generally used [2]. Secondly, the acetyl derivatives were converted into their pentafluorobenzyl esters (PFB), providing an excellent electrophore for EC-NIMS analysis [7, 9]. Non-acetylated aliphatic hydroxy groups were subsequently acetylated under anhydrous conditions. The final products, the per-O-acetylcarboxy-PFB derivatives (AC-PFB) were stable, volatile and highly electronegative compounds, making reliable determinations of acidic CA metabolites at the nanomoles per litre level possible using GC–EC-NIMS.

EXPERIMENTAL

Gas chromatography–mass spectrometry

GC–MS experiments were performed using a Kratos MS-80 instrument (Kratos, Manchester, U.K.) with negative-ion detection capability. The instrument was operated at a resolution $R = 1000$ (10% valley definition). The moderating gas for EC-NIMS was ammonia. Ionization was initiated with 50–80 eV electrons with an emission current of 1.5 mA. The source temperature was 225°C unless indicated otherwise. The chromatographic
column was a CPSIL-19 chemical bonded fused-silica capillary, 20 m × 0.22 mm I.D. (Chrompack, Middelburg, The Netherlands). Helium was used as the carrier gas with a flow-rate of 0.55 ml min⁻¹. The oven temperature was increased from its initial temperature of 260°C 2 min after injection at a rate of 5°C min⁻¹ to 300°C. The injection temperature was 300°C and the GC–MS interface was maintained at 260°C. Samples were introduced using a solid injector system (glass falling needle, Chrompack [18]). The capillary column was inserted directly into the ion source as described previously [19].

**Materials and reagents**

Homovanillic acid (HVA), vanillylmandelic acid (VMA), dihydroxyphenylacetic acid (DOPAC) and dihydroxymandelic acid (DOMA) were obtained from Janssen Pharmaceutica (Beerse, Belgium) and vanillylactic acid sodium salt (VLA) from Sigma (St. Louis, MO, U.S.A.).

Acetic anhydride (AA), triethylamine (TEA), deuterium oxide (²H₂O) and deuterium chloride (²HCl) were purchased from Merck (Darmstadt, F.R.G.).

**Buffers.** The buffers used were phosphate buffer (1 M K₂HPO₄, the pH adjusted with concentrated phosphoric acid to 7.7) and phosphate–carbonate buffer (1 M K₂HPO₄ mixed with a saturated K₂CO₃ solution 1:1 by volume and the pH adjusted to 10.5 with concentrated phosphoric acid).

**Synthesis of deuterium-labelled internal standards**

Ring-deuterated 2,5,6-²H₃ isomers of HVA (HVA-d₃), DOPAC (DOPAC-d₃) and VLA (VLA-d₃) were synthesized according to the method of Muskiet et al. [20]. [2,5,6-²H₃] VMA (VMA-d₃) was obtained from Mass Analys (Bromma, Sweden). All deuterated isomers contained less than 1% of the parent compounds.

**Analytical system**

A scheme of the analytical system is presented in Fig. 1.

**Plasma pre-treatment**

For the separation of CA metabolites from interfering phenylalanine, plasma samples (1 ml) were diluted three times with 0.1 M HCl, saturated with NaCl, and 50 µl of a solution of deuterated isomers in 0.1% ²HCl in ²H₂O were added, followed by the addition of 0.1 ml of a 0.03 M solution of ascorbic acid as an antioxidant. This solution was extracted twice with 4 ml of ethyl acetate with vigorous mixing for 5 min. Phase separation was facilitated by centrifugation (5 min at 2400 g). Between the phases a gel layer was formed containing ca. 1 ml of ethyl acetate and water. After the first extraction 3 ml of ethyl acetate were available and after the second extraction 4 ml. The combined supernatants (7 ml) were back-extracted into 2 ml of phosphate buffer (pH 7.7) with vigorous mixing for 2 min. The organic layer was aspirated.

**Aqueous acetylation**

**Plasma back-extract.** To the aqueous solution obtained as described above, 30 µl of AA were added with continuous mixing for 1 min. Then 0.5 ml of
<table>
<thead>
<tr>
<th>STEP</th>
<th>ADDITION</th>
<th>PROCESS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma pre-treatment</strong></td>
<td>2 ml 0.1 M HCl saturated with NaCl</td>
<td><strong>Plasma</strong> (1 ml)</td>
</tr>
<tr>
<td></td>
<td>0.1 ml 0.03 M ascorbic acid</td>
<td>Extraction (2x)</td>
</tr>
<tr>
<td></td>
<td>0.05 ml internal standard</td>
<td>Combined supernatants</td>
</tr>
<tr>
<td></td>
<td>2 x 4 ml ethyl acetate</td>
<td>Back-extraction</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reaction (1 min)</td>
</tr>
<tr>
<td></td>
<td>2 ml 1 M phosphate (pH 7.7)</td>
<td></td>
</tr>
<tr>
<td><strong>Aqueous acetylation</strong></td>
<td>30 µl acetic anhydride</td>
<td></td>
</tr>
<tr>
<td>(step I)</td>
<td>0.5 ml carbonate-phosphate buffer (pH 10.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 µl acetic anhydride</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.8 ml 6 M HCl</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 x 4 ml ethyl acetate</td>
<td></td>
</tr>
<tr>
<td><strong>PFB esterification</strong></td>
<td>5 µl PFB-Br, 10 µl TEA</td>
<td></td>
</tr>
<tr>
<td>(step II)</td>
<td>50 µl acetonitrile</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 ml ethyl acetate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 ml 0.1 M HCl</td>
<td></td>
</tr>
<tr>
<td><strong>Anhydrous acetylation</strong></td>
<td>50 µl ethyl acetate</td>
<td></td>
</tr>
<tr>
<td>(step III)</td>
<td>50 µl acetic anhydride</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 µl triethylamine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 ml ethyl acetate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 ml 0.1 M HCl</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 µl hexane</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Scheme of the analytical procedure for GC–EC-NIMS analysis of acidic catecholamine metabolites. TEA = triethylamine; PFB = pentafluorobenzyl.
the phosphate—carbonate buffer and 30 μl of AA were added. This mixture was allowed to react at room temperature for 10 min.

**CSF, urine and standard solutions (authentic samples, no pre-treatment necessary).** Samples (0.5 ml of CSF, 0.1 ml of urine) were spiked with suitable amounts of deuterated isomers. Then 2 ml of 1 M phosphate buffer (pH 7.7) were added and subsequently processed as described for plasma back-extracts.

**Preparation of PFB esters and anhydrous acetylation**

The aqueous reaction medium was acidified to pH 3 with 0.8 ml of 6 M HCl. The derivatives formed were extracted twice with 4 ml of ethyl acetate. The combined organic layers were evaporated with a stream of nitrogen at 60°C. The residue was treated with 5 μl of PFB-Br and 10 μl of TEA in 50 μl of acetonitrile for 5 min at room temperature [12]. Then 3 ml of ethyl acetate were added and the solution washed with 1 ml of 0.1 M HCl. The organic layer was evaporated under nitrogen at 60°C and the residue treated with 100 μl of a mixture of AA and TEA in ethyl acetate (45:10:45, v/v) at 60°C for 30 min. The reaction product was dissolved in 3 ml of ethyl acetate and washed with 1 ml of 0.1 M HCl. The organic layer was evaporated and the residue stored until analysis. Before analysis, the residue was dissolved in 50 μl (plasma and CSF samples) or 1 ml (urine samples) of hexane. A 2-μl volume was placed on the falling needle and the solvent was evaporated.

**Recovery**

HVA-d₃ (9.88 μg), DOPAC-d₃ (1.68 μg) and VMA-d₃ (4.88 μg) were derivatized as described for standards and subsequently dissolved in 40 ml of ethyl acetate (reference solution). Of this solution, 50 μl were used for recovery estimates. The yields of various steps in the procedure (Fig. 1) were determined by processing 11.9 ng of HVA, 4.7 ng of DOPAC and 7.1 ng of VMA. The reference compounds were introduced into the procedure before each step to be investigated.

**RESULTS AND DISCUSSION**

In recent years, a wide variety of methods for GC—EIMS analysis of acidic CA metabolites in biological material have been described [4]. Only a limited number of these are suitable [21, 22] and have been applied to biological samples [13] using GC—EC-NIMS. In these methods the metabolites were derivatized to their N,O,S-perfluoroacylcarboxyperfluoroalkyl esters.

In this work we propose the omission of N,O,S-perfluoroacylation for negative-ion analysis of these metabolites because it does not significantly improve the sensitivity and it will increase the background signal. Using a selective detector such as the EC-NIMS system, a marked reduction in the background might be expected if the non-relevant compounds remain undetected. The acidic CA metabolites contain phenolic hydroxy groups and a carboxy group; both may be selected to react with an electrophoric reagent. Esterification with PFB-Br was preferred, based on recent experiments with favourable EC-NIMS characteristics of PFB esters [5, 7—9, 12]. However, it appeared that this reagent also reacted to some extent with phenolic
hydroxy groups [23], resulting in products of low volatility and difficult to identify. To prevent this side-reaction it was necessary to block this hydroxy group by acetylation prior to esterification. This problem was not reported by Shimamura et al. [5]. In their method, PFB esters of HVA and VMA were converted into their O-trimethylsilyl (TMS) ethers. The rate of the reaction between PFB-Br and phenolic OH will depend on the pH of the aprotic reaction medium. Their esterification conditions used (briefly, PFB-Br in acetone with carbonate as catalyst) may explain this contrast. We preferred a stronger catalyst (TEA) to ensure a quantitative yield [12].

Acetylation of the phenolic hydroxy group had to be carried out in aqueous solution [14] because mixed anhydrides of HVA and DOPAC were formed by anhydrous acetylation. In addition to its blocking function, aqueous acetylation prevented dihydroxyphenyl metabolites from oxidative degradation and improved their recovery during organic solvent extraction.

**Methodology**

The proposed method, as outlined in Fig. 1, consists of a three-step derivatization scheme. It appeared to be essential that in steps II and III the excesses of reagents and by-products formed were removed by washing. In addition, contrary to earlier reports [14, 15], we observed a 10–20% acetylation of aliphatic hydroxy groups of VMA, DOMA and VLA in step I, making anhydrous acetylation in the third step obvious. The presence of the carboxy-PFB group considerably reduced the reactivity of the aliphatic hydroxy group.

![Fig. 2. Selected-ion recordings of standards, HVA, DOPAC, VMA, VLA and DOMA. Offset time axis, 2 min 15 s.](Image)
towards acetic anhydride. Addition of a basic catalyst (TEA) markedly increased the rate of reaction so that at 60°C the acetylation was completed within 30 min.

Gas chromatography—mass spectrometry

The AC-PFB derivatives of acidic CA metabolites showed good chromatographic characteristics. However, analysis at the 1–10 pg on-column level required an extensively cleaned system. At this level the results might even be adversely affected by the presence of non-volatile residues due to insufficient clean-up in step II and III. The separation of the most prominent endogenous metabolites on the CPSIL-19 fused-silica capillary column is shown in Fig. 2.

<table>
<thead>
<tr>
<th>TABLE I</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAIN CHARACTERISTICS OF ELECTRON-IMPACT MASS SPECTRA OF ACETYL-PENTAFLUOROBENZYL DERIVATIVES OF ACIDIC CATECHOLAMINE METABOLITES</td>
</tr>
<tr>
<td>Compound</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>HVA</td>
</tr>
<tr>
<td>VMA</td>
</tr>
<tr>
<td>DOPAC</td>
</tr>
<tr>
<td>VLA</td>
</tr>
<tr>
<td>DOMA</td>
</tr>
</tbody>
</table>

The EI mass spectra are summarized in Table I. EIMS and PICIMS were used to confirm the identity of the derivatives formed. The EC-NI mass spectra (Fig. 3) consisted of ions arising from losses of CH₂C₆F₅ (ion a, Fig. 4) and COOCH₂C₆F₅ (ion b) from the ionized molecular species, respectively. In addition, the EC-NI mass spectrum of DOMA-AC-PFB showed an intense peak at −m/z 223, which corresponds to the expulsion of ketene from ion b. The abundance of a and b depended strongly on the molecular structure and the ion source temperature. High source temperatures favoured the formation of b at the expense of a (Fig. 5) for all compounds except VLA. To prevent condensation during analysis a high source temperature (≥ 225°C) was required. The observed increase in sensitivity for EC-NIMS versus EIMS was primarily due to an approximately two orders of magnitude higher ionization efficiency and secondly to lesser fragmentation. The gain in sensitivity obtained for the individual compounds is shown in Table II. The limits of detection of the indicated ions (Table II) using selected-ion monitoring in general exceeded 0.2 pg on-column.

Quantitation in biological samples

Isolation of acidic CA metabolites from plasma samples was necessary for reliable DOPAC analysis. Otherwise, plasma phenylalanine (Phe) will occasionally disturb the recording of DOPAC ion traces. For CSF and urine this pre-purification was not required because the ratio of the concentrations of DOPAC and Phe in these was much higher than that in plasma.
Various steps in the sample handling procedure were examined for the recovery of HVA, DOPAC and VMA in the nanogram range (6–10 ng). As shown in Table III, saturation of the sample with NaCl (salting-out) improved the recovery of VMA. In addition, the presence of ascorbic acid (antioxidant) during extraction and back-extraction was necessary for the recovery of DOPAC. Using both salting-out and ascorbic acid, the recoveries of all three compounds were more than 90%. The large amount of ascorbic acid added was not seen in the chromatogram.

The calculated partition constants of the AC derivatives of HVA, DOPAC and VMA for the system ethyl acetate–acidic medium (pH < 3) were 6.1,
Fig. 4. General structure of acetylpentafluorobenzyl derivatives of acidic catecholamine metabolites and their most prominent fragments (ions a and b) under ECNI.

<table>
<thead>
<tr>
<th>Compound</th>
<th>x</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>a m/z</th>
<th>b m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>HVA</td>
<td>0</td>
<td>COCH₃</td>
<td>CH₃</td>
<td>H</td>
<td>-</td>
<td>223</td>
<td>179</td>
</tr>
<tr>
<td>DOPAC</td>
<td>0</td>
<td>COCH₃</td>
<td>COCH₂</td>
<td>H</td>
<td>-</td>
<td>251</td>
<td>207</td>
</tr>
<tr>
<td>VMA</td>
<td>0</td>
<td>COCH₃</td>
<td>CH₃</td>
<td>O-COCH₃</td>
<td>-</td>
<td>281</td>
<td>237</td>
</tr>
<tr>
<td>DOMA</td>
<td>0</td>
<td>COCH₃</td>
<td>COCH₃</td>
<td>O-COCH₃</td>
<td>-</td>
<td>309</td>
<td>265</td>
</tr>
<tr>
<td>VLA</td>
<td>1</td>
<td>COCH₃</td>
<td>CH₃</td>
<td>H</td>
<td>O-COCH₃</td>
<td>295</td>
<td>251</td>
</tr>
</tbody>
</table>

Fig. 5. Influence of source temperature on fragmentation. a = [M - CH₂C₆F₅]⁻; b = [M - CO₂CH₂C₆F₅]⁻.

8.6 and 4.3, respectively. Two-fold extraction with a phase ratio of 4:3 (Vorg/Vaq) provided quantitative recoveries (> 99%) of the acetyl derivatives. Extractions following PFB treatment and anhydrous acetylation were also quantitative.
TABLE II

**COMPARISON OF THE SENSITIVITY OF ACETYL-PENTAFUROBENZYL DERIVATIVES OF ACIDIC CATECHOLAMINE METABOLITES ANALYSED BY EIMS AND EC-NIMS**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Analysed ion EIMS</th>
<th>Analysed ion EC-NIMS</th>
<th>Intensity ratio, EC-NIMS/EIMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>HVA</td>
<td>362</td>
<td>−223</td>
<td>420</td>
</tr>
<tr>
<td>DOPAC</td>
<td>348</td>
<td>−207</td>
<td>280</td>
</tr>
<tr>
<td>VMA</td>
<td>420</td>
<td>−237</td>
<td>715</td>
</tr>
<tr>
<td>VLA</td>
<td>374</td>
<td>−295</td>
<td>90</td>
</tr>
<tr>
<td>DOMA</td>
<td>406</td>
<td>−223</td>
<td>295</td>
</tr>
</tbody>
</table>

TABLE III

**RECOVERY ESTIMATES FOR SOME STEPS OF THE PROCEDURE**

<table>
<thead>
<tr>
<th>Step</th>
<th>Recovery (mean, n = 3) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HVA (11.9 ng)</td>
</tr>
<tr>
<td>Back-extraction without ascorbic acid</td>
<td>96</td>
</tr>
<tr>
<td>Extraction plus back-extraction:</td>
<td></td>
</tr>
<tr>
<td>Without NaCl and ascorbic acid</td>
<td>82</td>
</tr>
<tr>
<td>Standard procedure</td>
<td>97</td>
</tr>
<tr>
<td>Extraction of O-acetylts</td>
<td>99</td>
</tr>
<tr>
<td>Overall recovery</td>
<td>94</td>
</tr>
</tbody>
</table>

The precision of the method was determined by analysing five plasma samples individually. The relative standard deviations obtained were 1.8, 1.6 and 4.5% for HVA, DOPAC, and VMA, respectively.

**Control values**

*Plasma.* The method was applied to plasma samples drawn from non-fasting, healthy adults. The levels of HVA and DOPAC obtained were found to be $12.1 \pm 6.7$ and $3.0 \pm 1.3$ ng ml$^{-1}$, respectively (mean ± S.D., $n = 10$).

In another series of randomly collected plasma samples from healthy adults, which were stored at $-20^\circ$C for several weeks or months, the HVA and VMA levels were 11.97 and 7.26 ng ml$^{-1}$, respectively (pooled plasma, $n = 20$). The DOPAC level appeared to be very low (0.3 ng ml$^{-1}$), probably owing to oxidation during storage.

These results were in good agreement with normal values obtained with other techniques (Table IV).

*CSF.* HVA, DOPAC and VMA were determined in randomly collected CSF samples from hospitalized adults, not suffering from neurological or mental diseases. The concentrations obtained were HVA, $34.3 \pm 21$ ng ml$^{-1}$ and DOPAC $0.91 \pm 0.34$ ng ml$^{-1}$ (mean ± S.D., $n = 14$). The correlation
TABLE IV

COMPARISON BETWEEN THE LEVELS OF HVA, DOPAC AND VMA IN PLASMA OR SERUM OF NORMAL ADULTS, OBTAINED BY DIFFERENT WORKERS AND WITH DIFFERENT TECHNIQUES

<table>
<thead>
<tr>
<th>Reference</th>
<th>Method</th>
<th>Level (mean ± S.D.) (ng ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HVA</td>
<td>DOPAC</td>
</tr>
<tr>
<td>3</td>
<td>GC-EIMS</td>
<td>11.7 ± 1.9</td>
</tr>
<tr>
<td>24</td>
<td>GC-EIMS</td>
<td>11.3 ± 4.2</td>
</tr>
<tr>
<td>25</td>
<td>HPLC</td>
<td>9.8 ± 0.5</td>
</tr>
<tr>
<td>26</td>
<td>HPLC</td>
<td>12.0 ± 3.8</td>
</tr>
<tr>
<td>27</td>
<td>HPLC</td>
<td>12.9 ± 3.2</td>
</tr>
<tr>
<td>This work</td>
<td>GC-EC-NIMS</td>
<td>12.1 ± 6.7</td>
</tr>
</tbody>
</table>

between HVA (y) and DOPAC (x) was y = 52.2x − 10 with a correlation coefficient r = 0.84 (n = 12). In a pool of equal volumes of these samples (n = 14) the following values were obtained (means of duplicate data): HVA 34.2 ng ml⁻¹, DOPAC 0.99 ng ml⁻¹ and VMA 1.04 ng ml⁻¹. VMA measurement was included in the pooled CSF samples, although this metabolite is not relevant for studies on the dopamine metabolism in brain. The CSF levels obtained did not agree well with those given by Karoum [3] (HVA 51 ± 8 ng ml⁻¹, DOPAC 3 ± 1.6 ng ml⁻¹ and VMA 0.46 ± 0.18 ng ml⁻¹), whereas the HVA value in CSF was in accord with that reported by Vogt et al. [28] (HVA 30.9 ng ml⁻¹) and Bottiglieri et al. [29] (HVA 29.5 ng ml⁻¹).

CONCLUSION

The per-O-acetylcarboxy-PFB derivatives of acidic CA metabolites have been shown to be very suitable for both qualitative and quantitative GC-EC-NIMS analysis. The EC-NI mass spectra contained abundant and structurally diagnostic anions. The strongly electrophoric carboxy-PFB group provided a high ionization efficiency, which allowed profiling at the sub-nanomoles per litre level of several acidic catecholamine metabolites in biological materials.

REFERENCES