

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

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Introduction

Most of the commercial GC/MS systems use ionization under vacuum conditions: electron ionization (EI) and chemical ionization (CI). Atmospheric pressure ionization sources (API), which are probably the key of the "overnight success" of MS-detectors in analytical sciences due to coupling with liquid chromatography, are rarely used with GC instruments. A recently introduced multipurpose AP source [1] created the opportunity to reconsider the importance of AP ionization for GC. Here, we present an analytical evaluation of GC/APCI-MS showing the benefits of soft atmospheric pressure chemical ionization for GC in combination with an orthogonal-acceleration ultra high-resolution TOF mass spectrometer (UHR-TOF-MS) maXis. First data of clinical cerebrospinal fluid (CSF) samples from CRPS patients are presented to demonstrate the huge potential of GC/APCI-UHR-TOF-MS in metabolic profiling.

GC/APCI UHR-TOF-MS

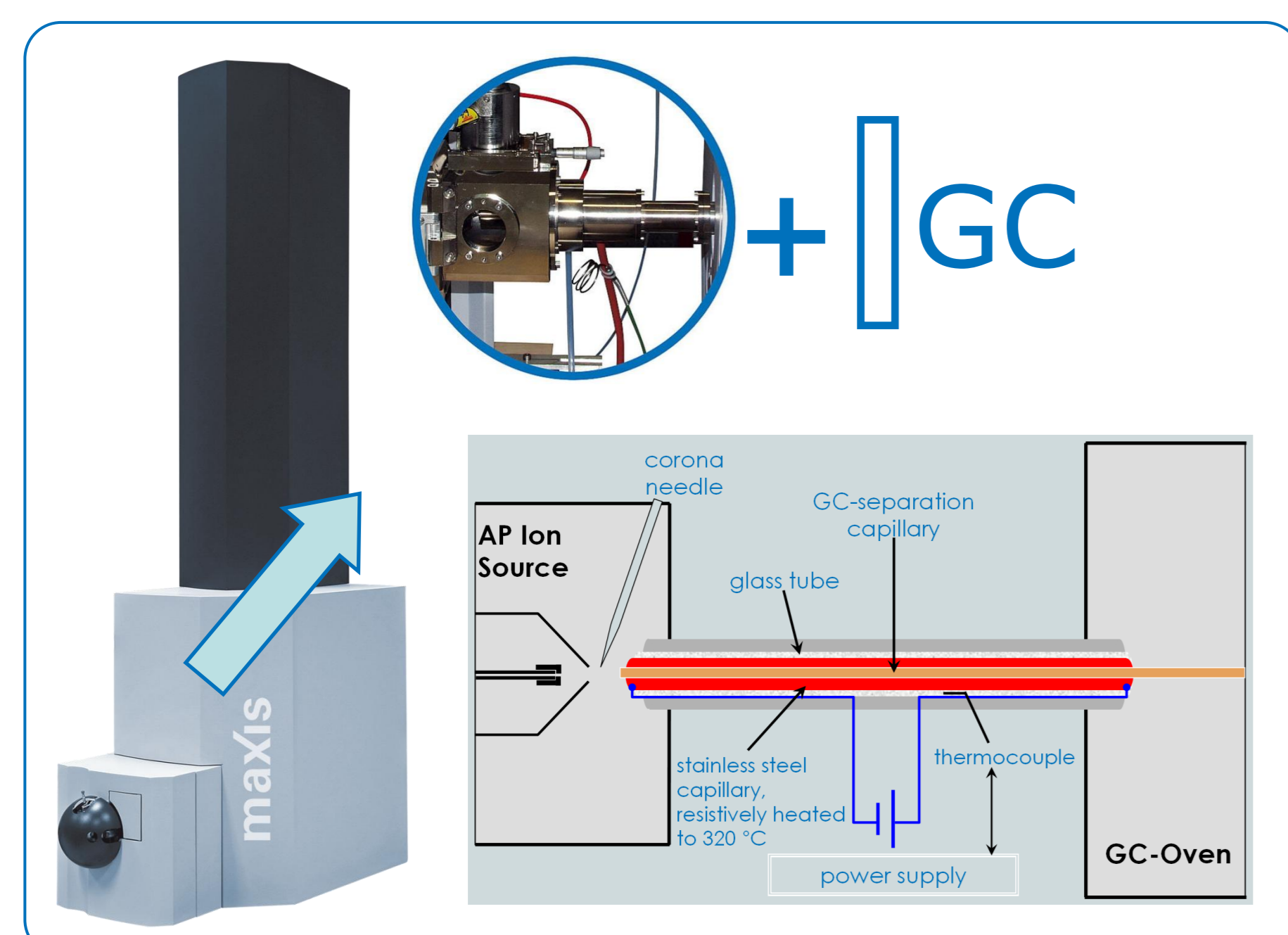


Fig. 1 GC/APCI UHR-TOF MS instrument: Transfer line enlarged & source schematics.

[1] Schiewek et al (2008) Development of a multipurpose ion source for LC-MS and GC-API MS, Analytical Bioanalytical Chemistry, v.392, p.87

Strategy for Analytical Method Development

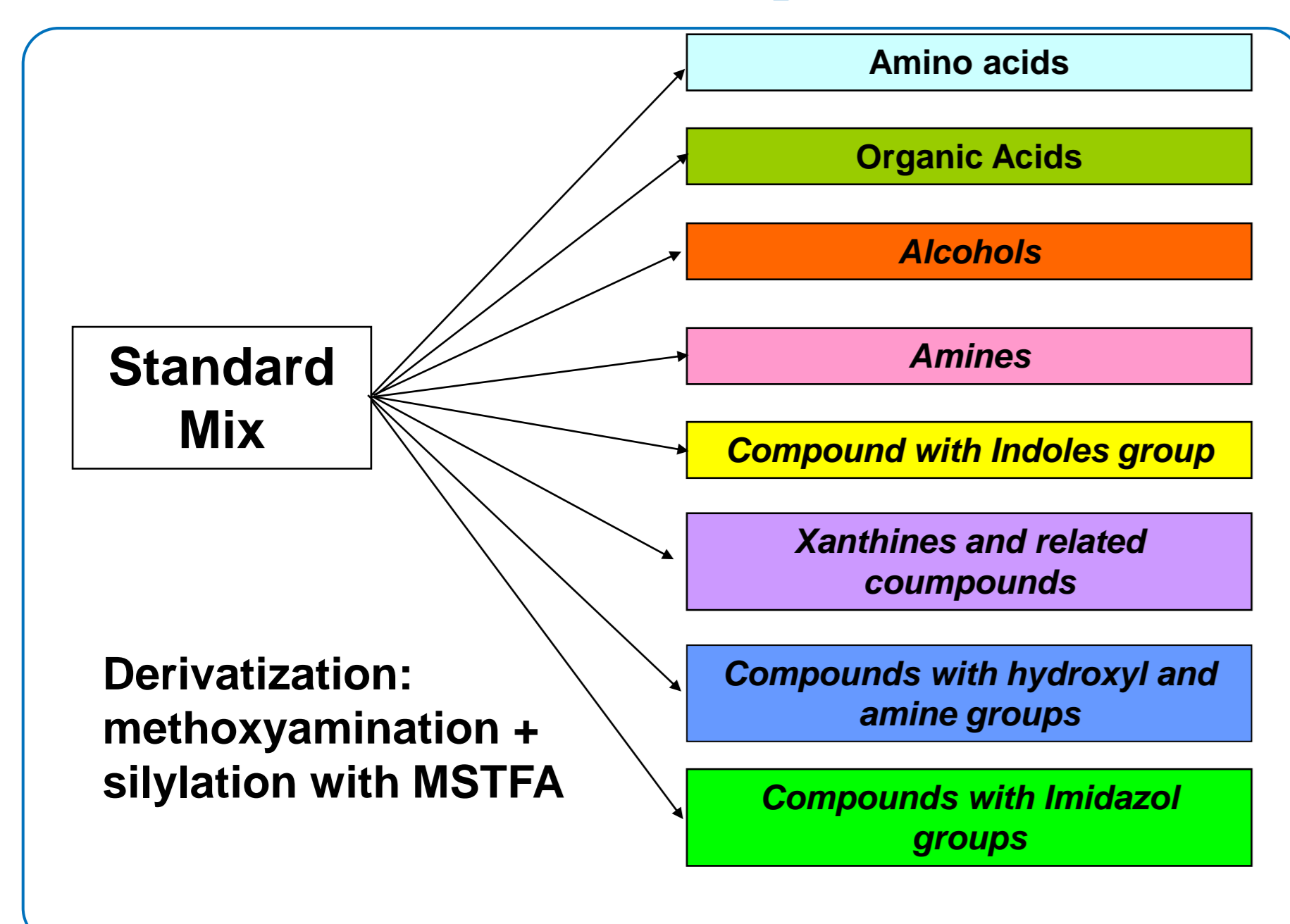


Fig. 2 Standard Mixture for analytical method development covering different compound classes and polarity.

The first step towards a GC method applicable to metabolic profiling is the composition of a standard mixture covering simultaneously both a variety of compound classes found in biological samples as well as different polarity ranges & molecule sizes. The compound classes covered in the standard mixture are listed in Fig. 2. A chromatogram of the standard mixture is presented in Fig. 3. Excellent repeatability was obtained, with relative standard deviations (RSDs) of peak areas between 0.7% and 2.1% in the intra-day study, and between 3.8% and 6.4% in the inter-day study. The analytical method optimization as well as analytical parameters are described in detail in [2].

[2] Carrasco-Pancorbo et al (2009) submitted for publication

Results Standard Mixture

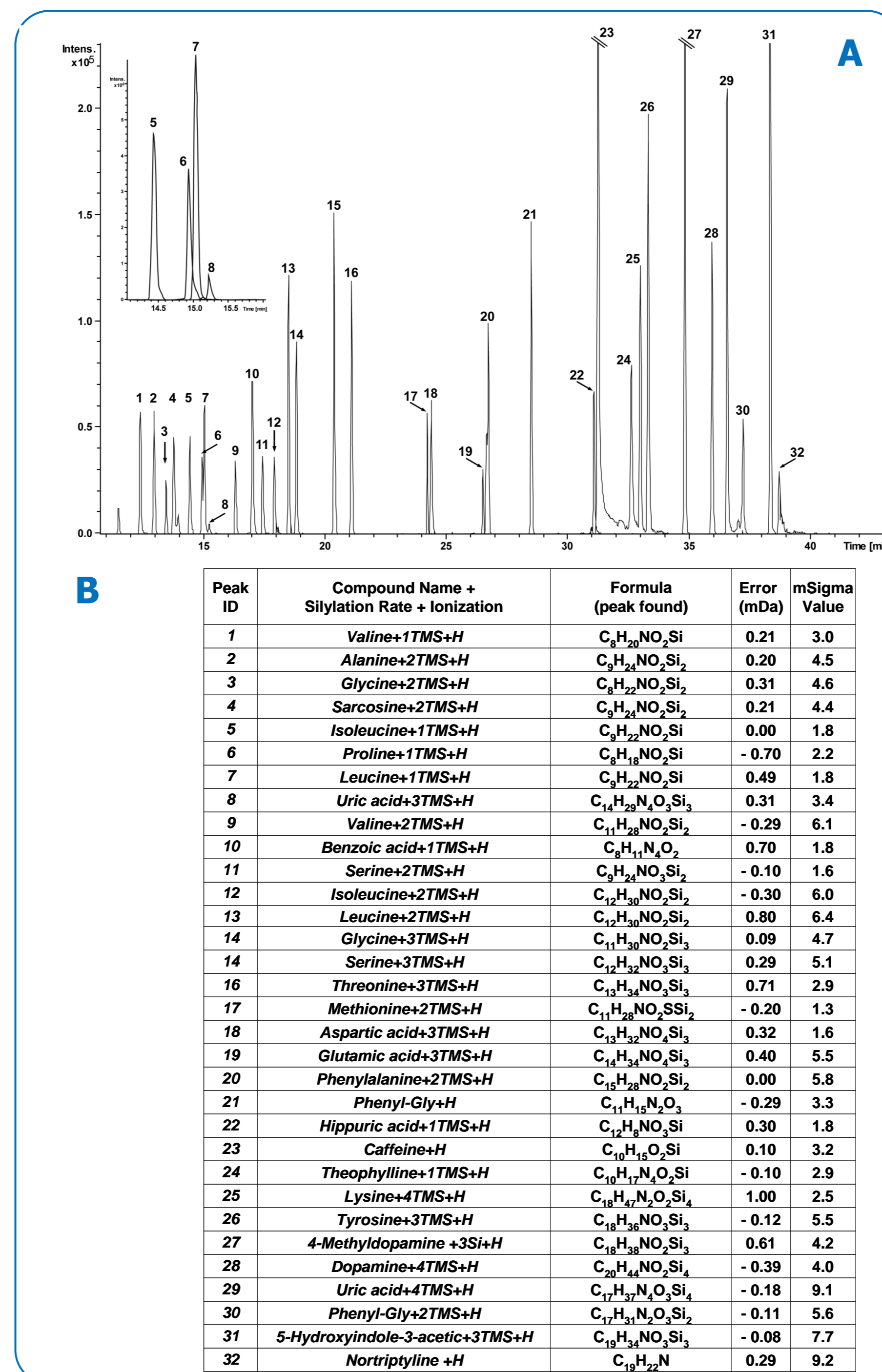


Fig. 3 Separation of the derivatized standard mixture (100 μM) (A) & assignment of trimethylsilyl (TMS)-derivatives (B).

The separation of the standard mixture is shown in Fig. 3, while Fig. 4 is giving more details on the different ionization behaviour of TMS derivatives at EI and APCI conditions. In both examples, similar key fragments (e.g. 144m/z for valine) derived from in-source CID can be observed for both ionization techniques. In the APCI spectra, the EI fragment of 73m/z is not observed. The big advantage of APCI ionization in combination with an accurate mass analyzer is the simple assignment of the molecular formulae to both the pseudomolecular ion [M+H]⁺ and the fragments as demonstrated in Fig. 4.

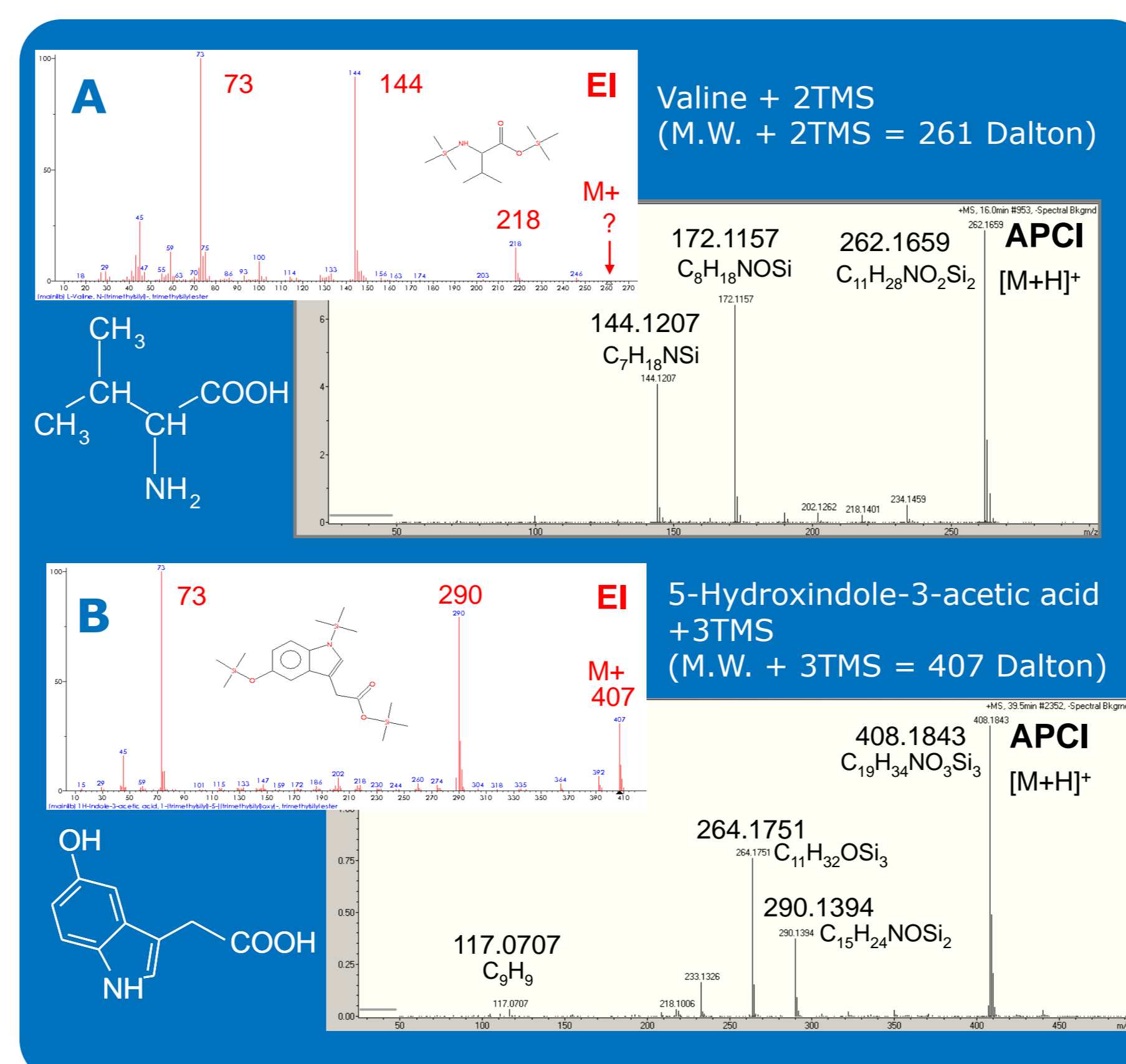


Fig. 4 Comparison of APCI and EI spectra of silylated A. valine and B. 5-hydroxyindole-3-acetic acid.

Material and Methods

Cerebrospinal Fluid (CSF) Sample Preparation

CSF extracts were prepared by adding cold methanol precipitation (total time 2 hours). Afterwards they were centrifuged and the supernatant was evaporated under N₂.

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 (1 μL) 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).

Mass spectrometry method

The maXis was operated in APCI positive mode at 2.0bar nebulizer pressure, 200°C dry gas temperature (N₂) and a dry gas flow rate of 4L/min. The scan range was set to 100-1000m/z, number of summations to 5000x.

CSF Analysis & Profiling

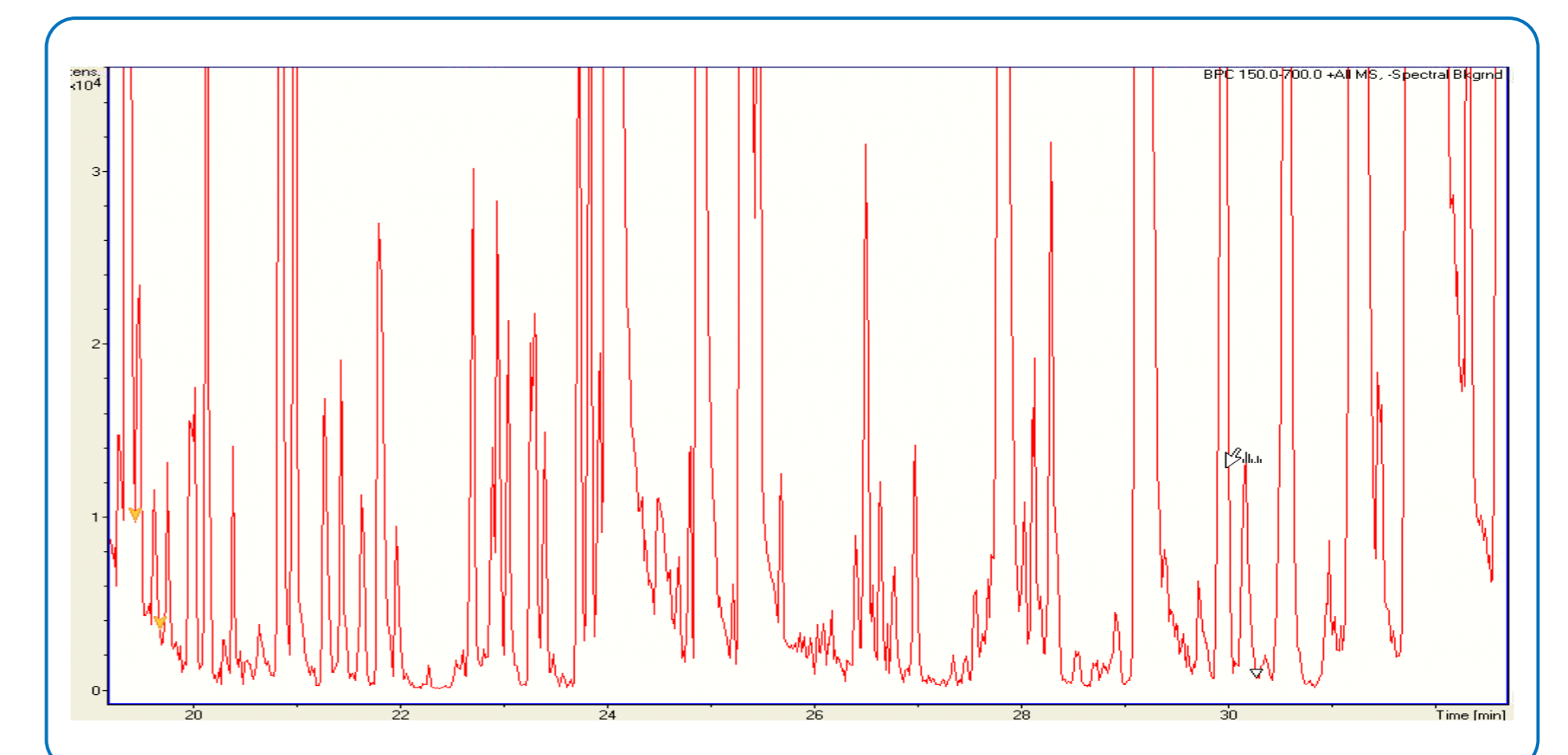


Fig. 5 Chromatogram of derivatized CSF sample.

After optimization of the method using the standard mixture, the analysis of CSF real samples from patients suffering from Complex Regional Pain Syndrome (CRPS) was performed (see Fig. 5.) CRPS is a chronic pain condition characterized by a variety of sensory and autonomic disturbances. CRPS usually develops after an injury. However, the pathogenesis remains unclear and consequently no diagnostic laboratory tests do exist nowadays. Metabolic profiling, as performed here, can provide insight into mechanisms of disease and novel prognostic or diagnostic markers.

In total, a set of 32 CSF samples was measured: 12 controls, 12 CRPS patients and one pooled CSF sample as quality control (QC, 8 technical replicates). The principle component analysis (PCA) of CSF samples from control and CRPS patients is presented in Fig. 6. The QC sample represents one identical pooled CSF sample. The observations are labeled by the order of measurement. Clearly, the variation is associated to the derivatization order in the batch.

Therefore, another effort to optimize derivatization conditions and waiting times prior to analysis needs to be made. This is necessary to improve reproducibility and robustness of the method to a level acceptable for statistical analysis.

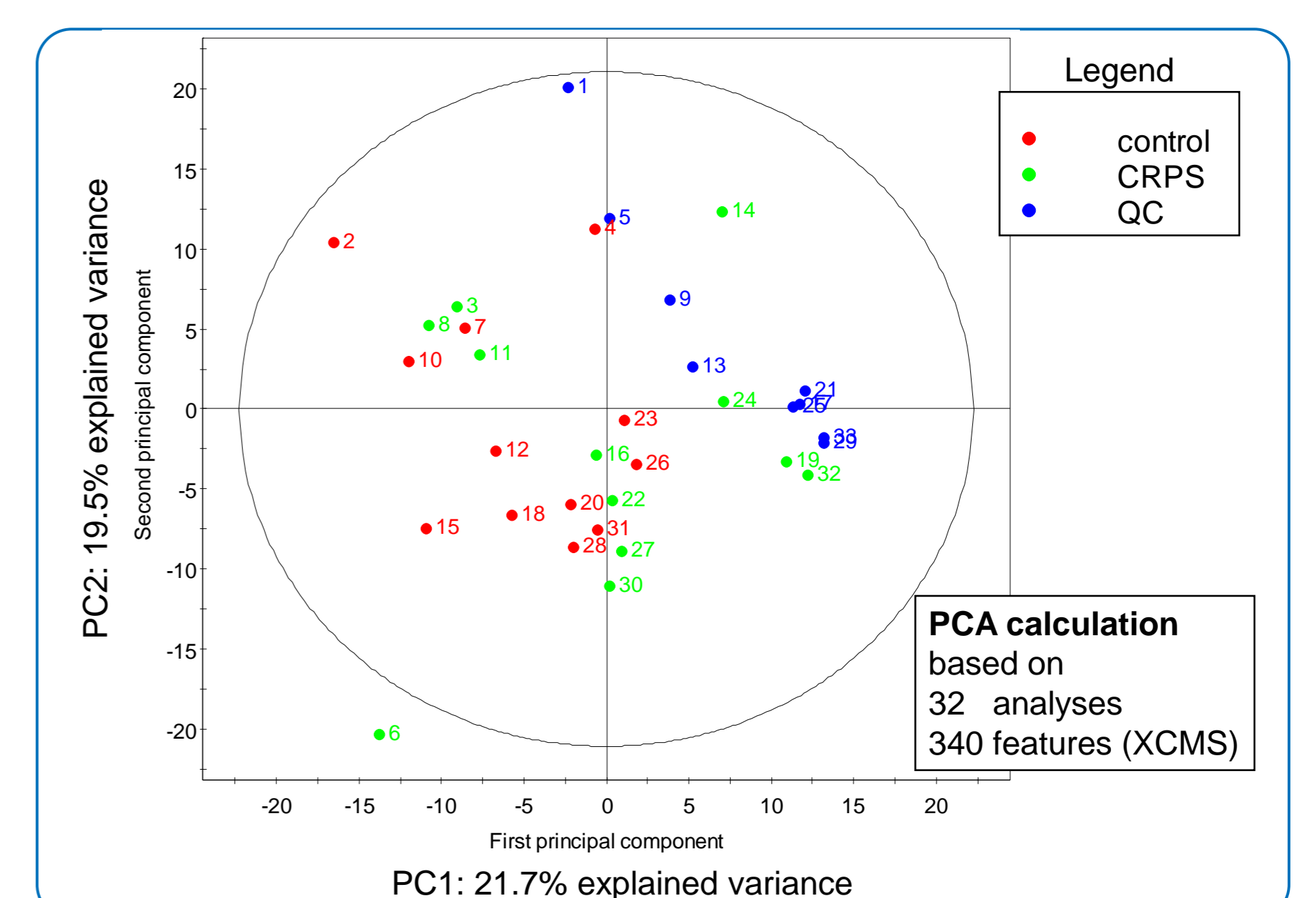


Fig. 6 PCA analysis of CSF samples from control & CRPS patients, and CSF quality control sample.

Conclusions

- The derivatization reaction is a crucial step in GC-MS analysis. It is mandatory to optimize its conditions in depth as it can be the major source of variation in complex matrices.
- During GC/APCI ionization of TMS derivatives the predominant ion observed is [M+H]⁺.
- The comparison of APCI and EI spectra reveals similarities and differences concerning presence/absence of the (pseudo) molecular ion and key fragments.
- The combination of GC/APCI with UHR-TOF MS directly enables the assignment of molecular formulae to the TMS derivatives and its fragments. This significantly facilitates spectra interpretation.

Future directions

- To continue the study of the analyses obtained for CSF samples to identify more compounds in the profile.
- To improve derivatization robustness by applying automated in-line derivatization.
- To create a GC/APCI-MS database for automatic compound assignment.