An automated GC-MS method based on ion-pairing micro-extraction with in-liner derivatisation for the analysis of phenolic acids in plasma

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Adding Vitality to life

1. Introduction

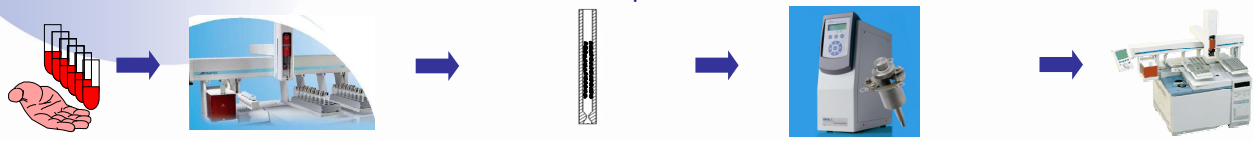
In recent years, phenolic compounds have been associated with various health benefits, resulting in an increased interest in their presence in plasma. For their analysis, however, several analytical challenges have to be overcome:

1. Typical concentration levels are sub-ppb
2. The presence of abundant, chemically-comparable compounds
3. Phenolic compounds represent a wide polarity range
4. Availability of only very small sample volumes

Here we present the development of a new automated method for the analysis of phenolic acids in plasma based on ion-pairing 'micro-extraction on a packed sorbent (MEPS)' coupled on-line to a GC-MS system. Derivatisation is performed *in-situ* using the ion-pairing reagent also for derivatisation.

2. Method development

Schematic of the automated extraction/derivatisation procedure



Plasma is manually placed in autosampler vials

MEPS is performed in the GC autosampler

The MEPS effluent is directly injected into the sintered-bed liner of the PTV system

Thermochemolysis is performed in the PTV injector with direct transfer of the derivatised sample into the GC system

The sample is analysed by GC-MS

Optimisation of the extraction method

A C18 MEPS bin was used, and for simplification of the large-volume injection a maximum of 40 µL elution volume was considered. Initially, the obtained recoveries were rather low. Two problems were observed: MEPS breakthrough and inefficient elution. To overcome these problems, an ion-pairing reagent was added to the sample (Fig. 1) and multiple elution steps were performed (Fig 2). Also, additional improvements were achieved by adding a drying step after extraction and a step for mixing the analytes prior elution and injection.

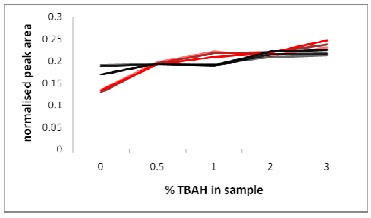


Figure 1: Recoveries of six phenolic acids with increased percentage TBAH in the sample

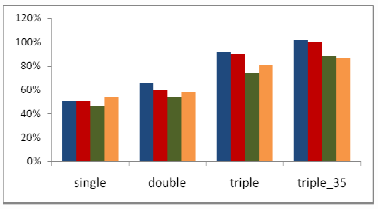


Figure 2. The effect of multiple injection steps on the recovery of four phenolic acids

Optimisation of in-liner derivatisation

Automated derivatisation was achieved by performing thermochemolysis in the liner of the PTV-injector (Fig. 3). Tetra-butyl ammonium hydroxide (TBAH) was used both as the ion-pair and the derivatisation reagent, resulting in butylation of the acids and alcohols-groups. The incubation time and temperature as well as the final injection temperature had the highest impact on the recoveries of the phenolic acids.

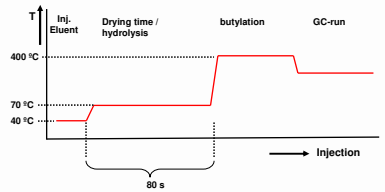


Fig. 3: Automated injection procedure after MEPS for in-liner butylation of the phenolic acids by means of thermochemolysis

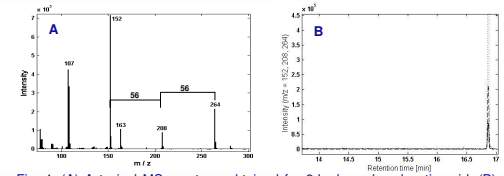
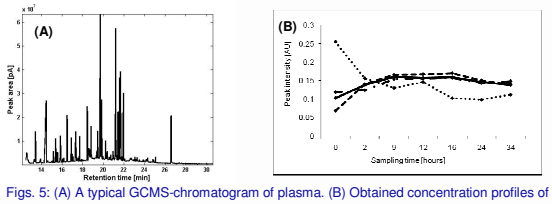


Fig. 4: (A) A typical MS-spectrum obtained for 3-hydroxyphenylacetic acid. (B) GC chromatograms of the characteristic ions of the phenolic acid

Detected peaks can be identified through the incorporation of butyl-groups to all alcohol- and acid functions. Method optimisation should also ensure that only fully-derivatised compounds are obtained.

3. Application to plasma

The optimised method was tested on human plasma taken at several time points following a tea intervention. Fig. 5a shows a typical GC-MS chromatogram obtained. When using extracted ion chromatograms, concentration profiles of phenolic acids can be determined (Fig. 5b).



Figs. 5: (A) A typical GCMS-chromatogram of plasma. (B) Obtained concentration profiles of four phenolic acids

4. Conclusion

A fully-automated extraction/derivatisation method for the analysis of phenolic acids in plasma has been developed. MEPS in combination with THM-GC-MS allows the quantitative analysis of phenolics in plasma even at the presence of abundant similar compounds such as fatty acids. Method performance characteristics are:

- Dynamic range: 10 to 5000 ng/mL
- Limit of detection: < 10 ng/mL (lowest standard); some higher (100 ng/mL)
- Linearity: $R\_sq > 0.99$
- Repeatability of whole method (sample prep, extraction, derivatisation, analysis): ~10 %
- Carry-over: < 1 % for most compounds (but some around 30 %!)
- Day-to-day repeatability: ~20 %

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