Introduction

Atmospheric Pressure Chemical Ionisation (APCI) is a well-established ionisation technique for liquid samples. Although LC/MS is mostly carried out using the electrospray interface, APCI and the relatively new Atmospheric Pressure Photoionisation are viable alternatives.

ESI and APCI are complementary in lipid analysis. Whereas polar lipids such as phospholipids or many sphingolipids are preferably ionised by electrospray, APCI comes into play for less polar compounds.

Materials and Methods

The measurements described here were performed using a Finnigan LCQ classic ion trap mass spectrometer (ThermoElectron, San Jose, CA, USA) with APCI interface.

HPLC was carried out using a Spectra System P4000 pump with autosampler AS 3000 (ThermoElectron).

Separation was achieved on reversed phase RP18 columns (4x 125 mm, Macherey-Nagel, Düren) in case of cholesterol and its oxidation products, and on normal phase LiChrospher columns, respectively (4 x 125 mm, Merck, Darmstadt), in case of stratum corneum ceramides.

Analysis of cholesterol oxidation products:

• Matrix: solvent extracts from food samples
• Poor ionisation in ESI, therefore APCI as an alternative
• Separation and quantification of cholesterol and its oxidation products in the same run
• Fewer artefacts than with GC/MS
• MS/MS of limited value because of isobaric positional isomers

Analysis of Stratum corneum ceramides:

• Matrix: solvent extracts from the horny layer in-vitro and ex-vitro
• Preseparation of ceramides on normal phase material: separation according to number and position of hydroxy groups, rel. independent from chain length
• Analysis of ceramide classes by means of MS to investigate molecular composition incl. chain length
• Possibilities: TLC-RPLC-ESI-MS or (SPE)-NPLC-APCI-MS
• MS/MS for structure identification

Results and Discussion

We have investigated the oxidation of cholesterol during food processing. A new reversed phase LC/APCI-MS method was developed for 25-hydroxycholesterol, cholestan-3β-5α-6β-triol, 7β-hydroxycholesterol, 7-ketol, 5,6α-epoxycholesterol, and cholesterol.

In another study the ceramides of human stratum corneum were analysed by means of normal phase LC/APCI-MS. In this case, the use of APCI instead of ESI was mainly motivated by the compatibility to normal phase solvents with low polarity. Furthermore, the most lipophilic ceramide species such as ceramide [EOS] show a considerably increased response with APCI. In conjunction with an ion trap mass analyzer, structural elucidation was possible using MS/MS.

Conclusion

In general, APCI is a versatile, reliable ionisation method in lipid analysis. Care should be taken to prevent artefact formation due to the loss of water or carbon dioxide, if applicable. Whereas older APCI interfaces show optimal performance at high flow rates around 1 ml/min, new interfaces can operate with lower flow rates as obtained using LC columns with 1 or 2 mm ID.

References