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ANALYSIS OF LIGNAN PHYTOESTROGENS BY NEGATIVE ION LC-APCI-MRM-MS

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OVERVIEW

- Analysis of lignan phytoestrogens without the need for derivatisation has been effected.
- Quantitation of these compounds in non-hydrolysed and hydrolysed extracts for a variety of samples has been carried out.
- Linearity of response has been shown over 4 orders of magnitude with LLOQ (S/N=10) of 5pg on column.

INTRODUCTION

Lignan phytoestrogens are biologically active compounds derived from plant food such as whole grains, legumes and vegetables. It is thought that the consumption of foods containing high levels of lignan precursors may decrease the risk of hormone dependent cancers. These compounds may also exert their chemoprotective effects in a variety of ways such as antioxidant activity and inhibition of tumor growth [1-2]. Previous methods for the analysis of these species utilises GC-MS with extensive clean-up and derivatisation [3-8]. We now present a rapid, sensitive and selective method for the analysis of these compounds in biological fluids without the need for derivatisation.

EXPERIMENTAL

LC-APCI-MRM-MS analyses were carried out on a Quattro-Ultima (Micromass, Manchester, UK) in conjunction with a Waters 2690 (Waters Corporation, Milford, MA) liquid chromatography system in negative ion mode. Separations were performed on a 75 x 4.6mm Max-RP C12 (5µm) column (Phenomenex, Macclesfield, UK) at a flow rate of 0.7ml/min at 25°C. Mobile phases A and B, water and acetonitrile respectively, were applied in a linear gradient from 35%B to 70%B. The gradient was applied after 2 minutes under initial conditions. Optimum MRM conditions were found by infusion of a 10pg/µl solution of standards teed into a flow of 700µl/min of 50% B.

Standards of the phytoestrogen lignans were dissolved in 35% acetonitrile and serial dilutions of these were made to create the calibration lines. Food and urine samples were prepared according to in-house protocols and supplied at various stages of the extraction by Dr. P Grace.
The new APcI probe uses a higher power heater design that provides higher efficiency droplet evaporation with the nebulisation processes and droplet residence times being optimised by the use of the nebulisation support gas.

RESULTS

MS/MS spectra obtained via infusion for the 4 phytoestrogens enterolactone, enterodiol, matairesinol and secoisolariciresinol are shown in **Figure 1a - 1d**. Also shown are the transitions used in subsequent MRM experiments. An example chromatogram resulting from a 10µl injection of a 10pg/µl standard is shown in **Figure 2**. Chromatographic conditions were chosen due to the expected presence of isobaric and structurally related species in extracted samples. Interference seen in the chosen MRM channels from these species therefore requires complete chromatographic separation [9].

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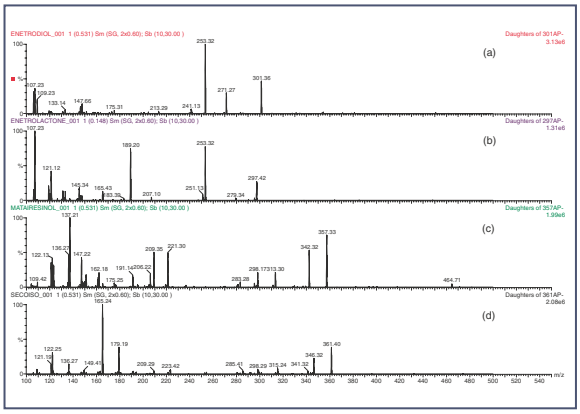

Figure 1. MS/MS spectra obtained by infusion of phytoestrogen standards (cone voltage =75(V)); a) enterodiol Collision Energy=29eV; b) enterolactone CE=25; c) matairesinol CE=22; d) secoisolariciresinol CE=22

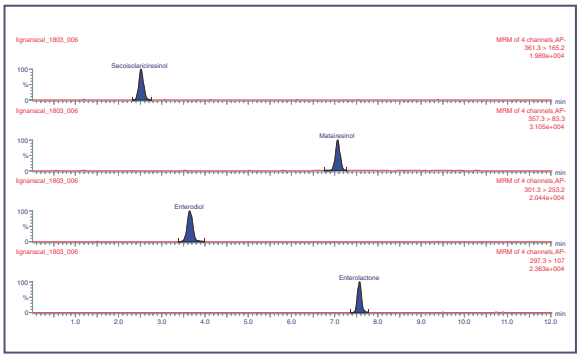

Figure 2. 100pg lignan phytoestrogens on column; source temp (C)= 140, heater temp (C)=600, corona (µA)=25, desolvation gas (l/hr)=250

Linearity of response for all 4 analytes was observed over the range 1-50000pg with a minimum coefficient of determination = 0.9994 when a 1/X weighting was applied (**Figure 3**).

Figures 4, 5 and 6 show chromatograms resulting from injection of non-hydrolysed urine, hydrolysed urine and aparagus extracts respectively, demonstrating the capacity of this method for quantitative analysis of lignan phytoestrogens in these matrices.

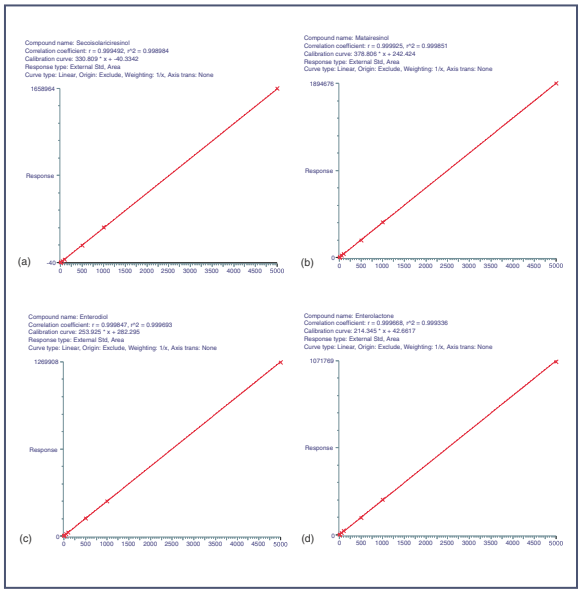

Figure 3. Calibration lines for a) secoisolariciresinol; b) matairesinol; c) enterodiol; and d) enterolactone

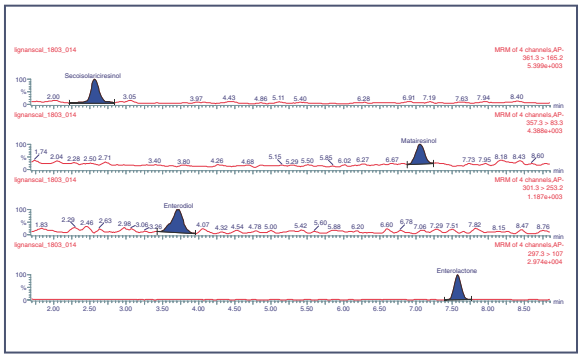

Figure 4. 10µl injection of a non-hydrolysed urine extract

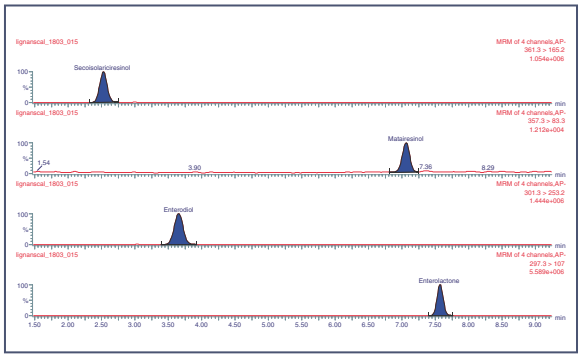

Figure 5. 10µl injection of a hydrolysed urine extract

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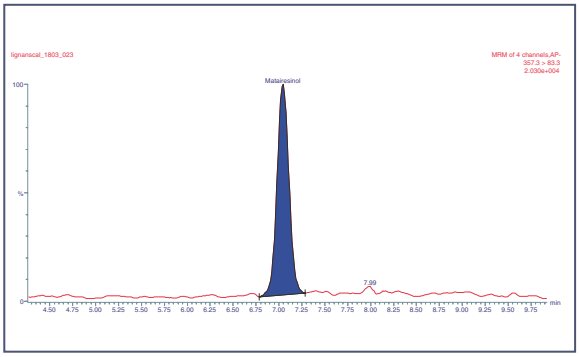


Figure 6. 10µl injection of an asparagus extract (matairesinol trace only)

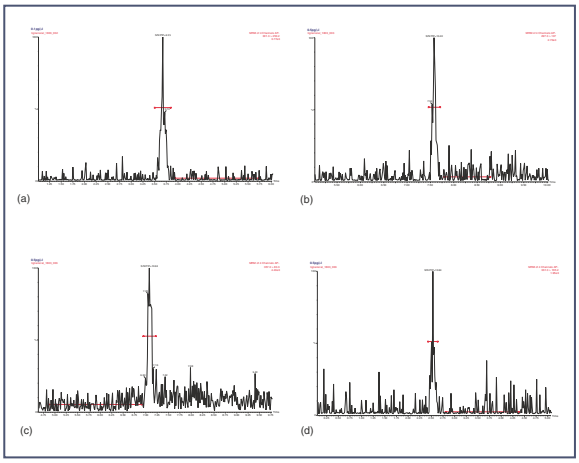
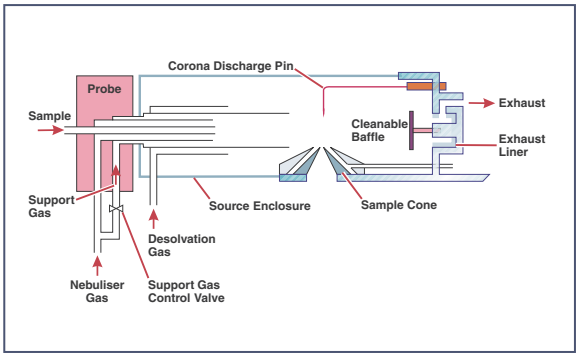


Figure 7. Raw data showing the S/N ratios for phytoestrogens at LLOQ (5 pg on column); a)enterodiol (1pg), b)enterolactone, c) matairesinol, d) secoisolariciresinol



CONCLUSION

The Quattro-Ultima, incorporating the new APcI probe, has been shown to be capable of quantitatively identifying 4 lignan phytoestrogens from biological matrices. Linearity of response over 4 orders of magnitude has been demonstrated with limit of detection <5pg on column for all analytes examined.

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