

Use of Gas Chromatography-(Time-Of-Flight) Mass Spectrometry for Analysis of Metabolites from Solanum Species

T. Shepherd, G. Dobson, R. Marshall, S. R. Verrall, S. Conner, D. W. Griffiths, D. Stewart and H. V. Davies



Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, United Kingdom

A metabolite profiling technique using a high throughput GC-Time-Of-Flight (TOF) MS for data acquisition, followed by automated data analysis, has been developed for measuring the metabolite distribution within plants.

We are using this approach to study substantial equivalence and unintended effects of genetic modification in Solanum species. In addition, metabolite variation within Solanum germplasm collections is being measured with the objective of exploring phytochemical diversity and relating metabolite distribution to phenotypic characteristics.

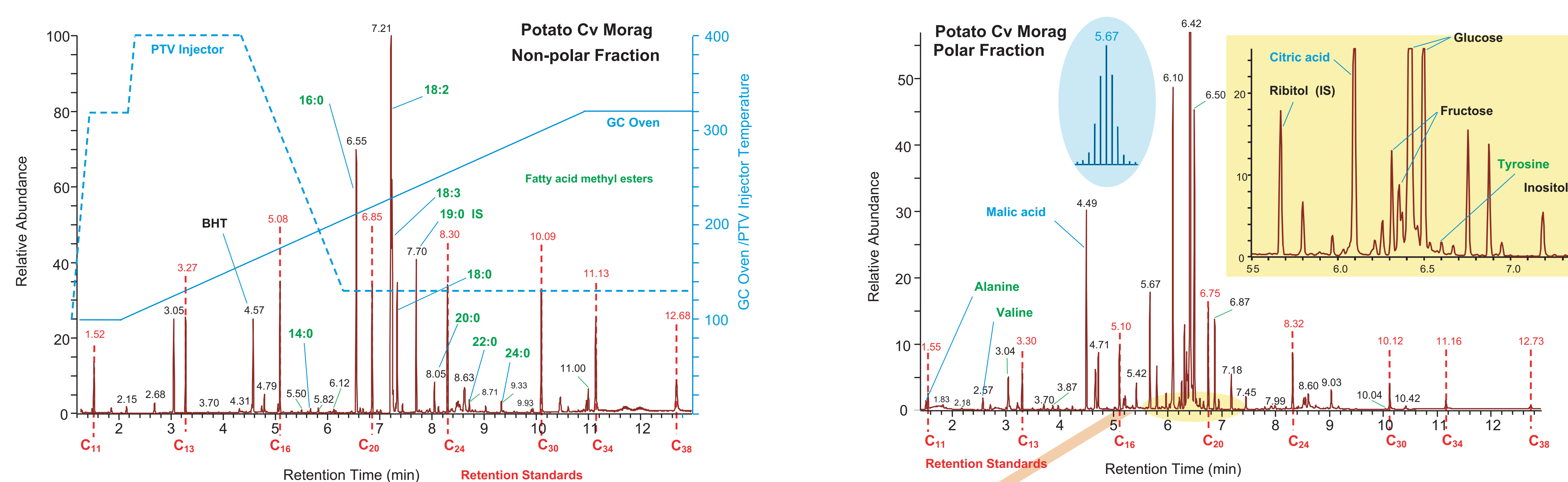
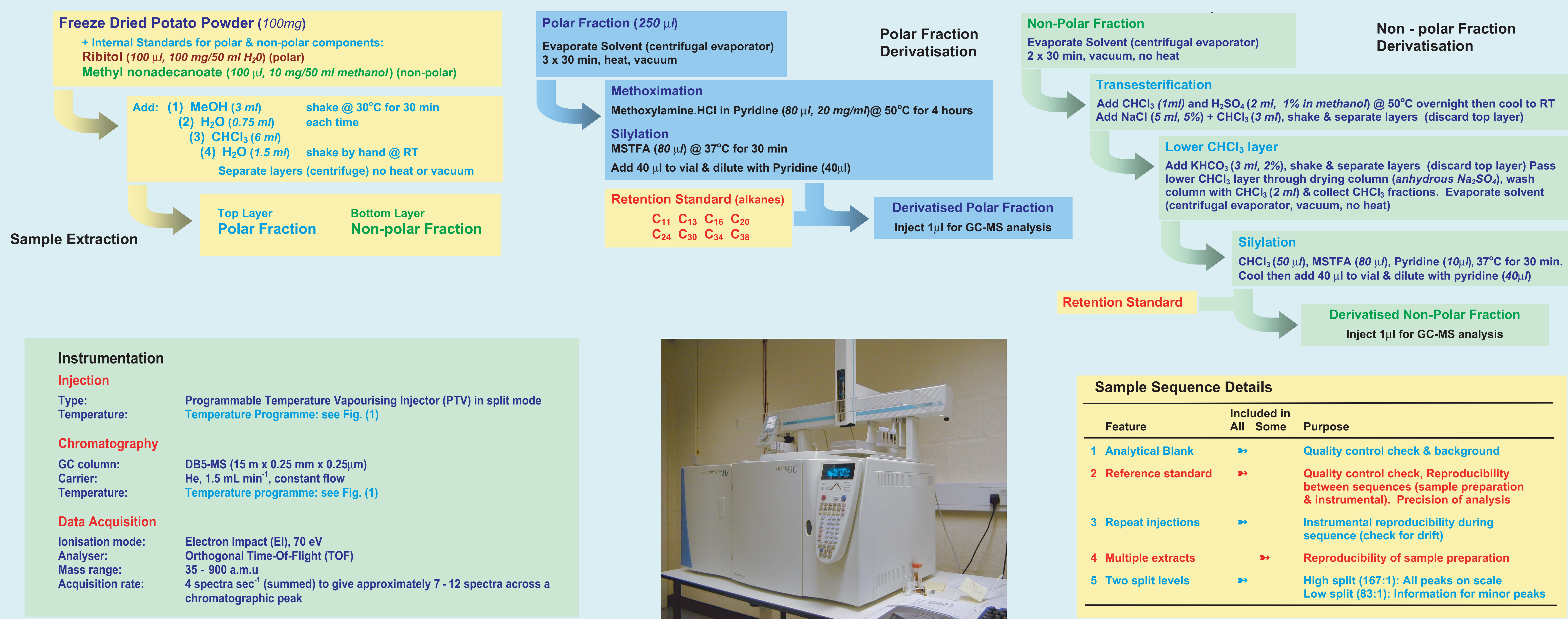


Figure 1. Total Ion Chromatograms (TIC) of polar and non-polar extracts of freeze dried potato tubers

- 1 AMDIS™ is used with representative samples to verify the presence of individual components, to deconvolute co-eluting peaks and to help identify ion(s) characteristic of each component
- 2 Having selected ion(s) suitable for detection of each component, a time window is defined for each component relative to an adjacent retention standard
- 3 Xcalibur™ is used to generate Selected Ion Chromatograms (SIC) for each component, including an appropriate internal standard (IS), within the appropriate time windows
- 4 Ratios are calculated for each component relative to the IS using the SIC areas and the values are downloaded into an Excel™ spreadsheet for further data analysis

Statistical treatment of data eg:
• Principle Components
• ANOVA

Data Analysis

Identification of compounds is based on:
• analysis of standards
• comparison with mass spectral libraries & databases
• literature data
• extrapolation from known compounds

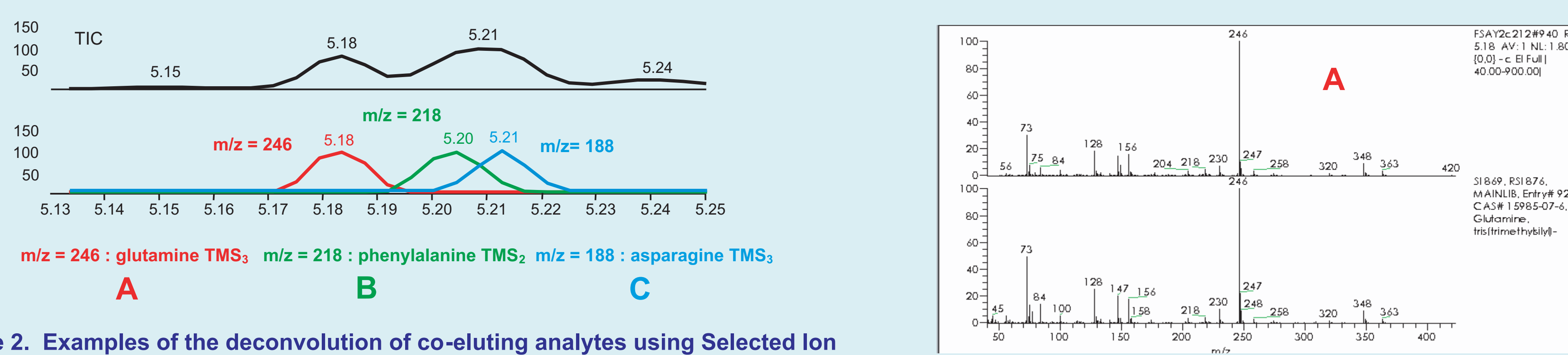
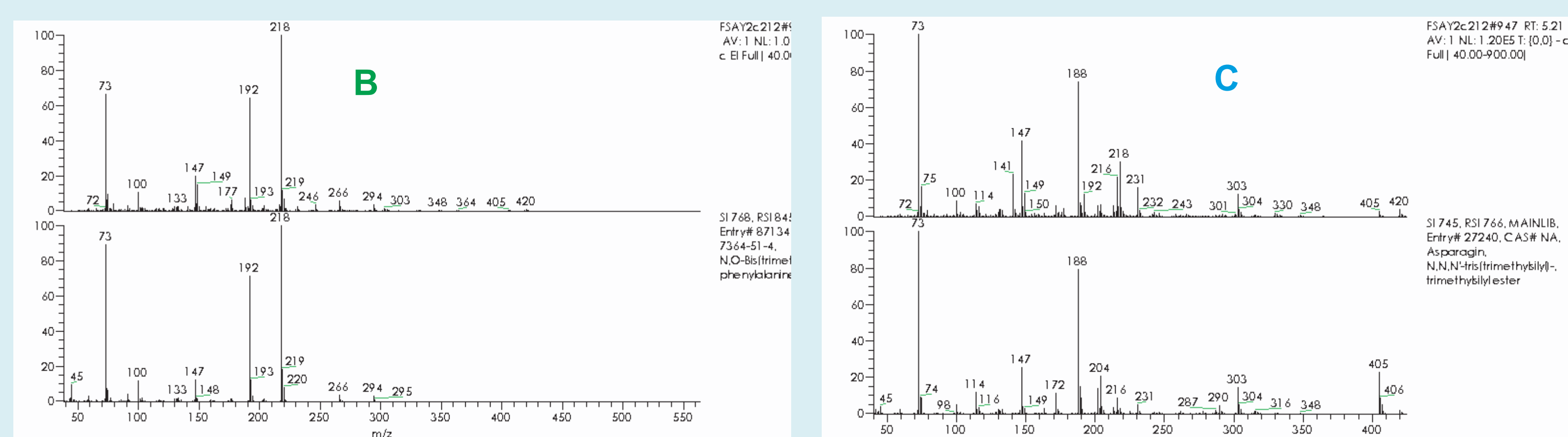


Figure 2. Examples of the deconvolution of co-eluting analytes using Selected Ion Chromatograms (SIC) for characteristic fragment ions.



Comparison of mass spectra of analytes with entries in the NIST™ library of mass spectra

Figure 3. Optimization of methoximation conditions.
Effect of A. Temperature (reaction time 45 min) and B. Reaction time (temperature of 50°C) on sugar methoximation. At low temperatures and short reaction times methoximation of monosaccharides is incomplete. At high temperatures and long reaction times methoximation is complete but sucrose degrades. Optimum conditions are 50°C for 4 hours

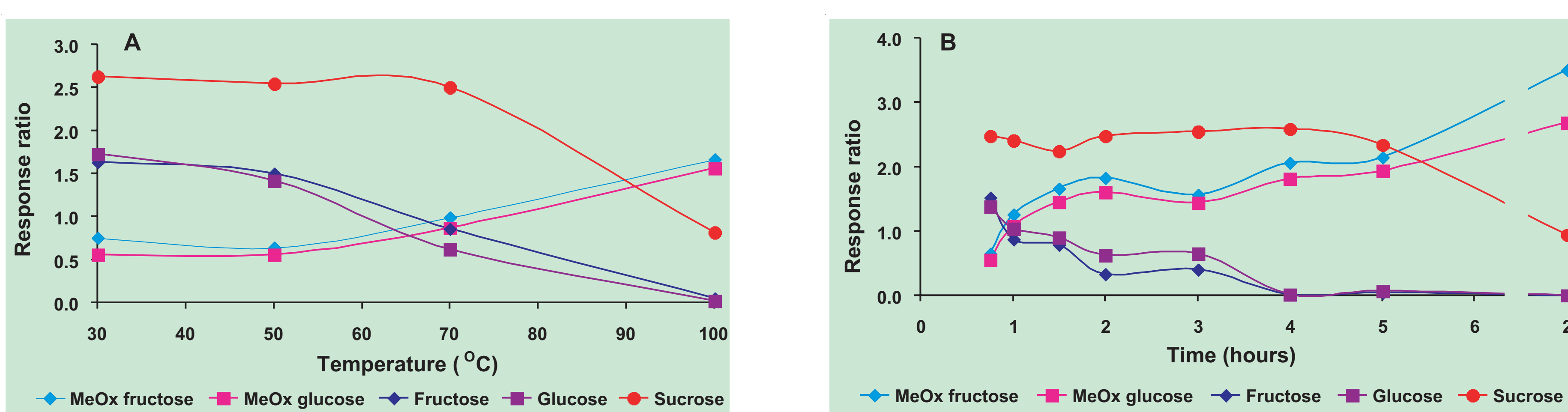
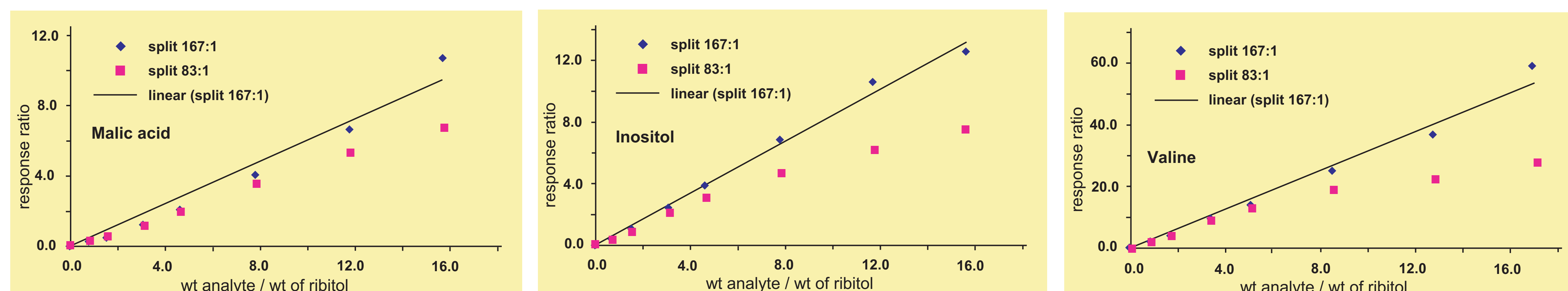


Figure 4. Linearity of analysis

At a split ratio of 167:1 variation in analyte levels gives a linear response relative to a fixed amount of the internal standard. At a split of 83:1 the response becomes non-linear at higher analyte concentrations. Data shown is for representative non polar analytes



Characteristics of profiling technique:

- Short analysis times & high sample throughput
- Reproducible analyses
- Linearity of response
- 300 - 600 metabolites detected at higher sample loadings

We acknowledge the support of the Food Standards Agency and the Scottish Executive Environment and Rural Affairs Department