Analysis of Metabolites from Solanum Species using Gas **Chromatography-(Time-Of-Flight) Mass Spectrometry and Automated Data Analysis**

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Metabolite profiling of plant species using high throughput GC-MS techniques, linked to automated data processing, is becoming a highly useful tool for the study of metabolite distribution within plant populations. We have developed a metabolite profiling technique based on the use of GC-Time-Of-Flight (TOF) MS for data acquisition, followed by automated data analysis using a combination of the AMDISTM and XcaliburTM software programmes.

The profiling technique is being used to measure metabolite variation within Solanum germplasm collections in an attempt to relate metabolite distribution to phenotypic characteristics and to look for phytochemical diversity.

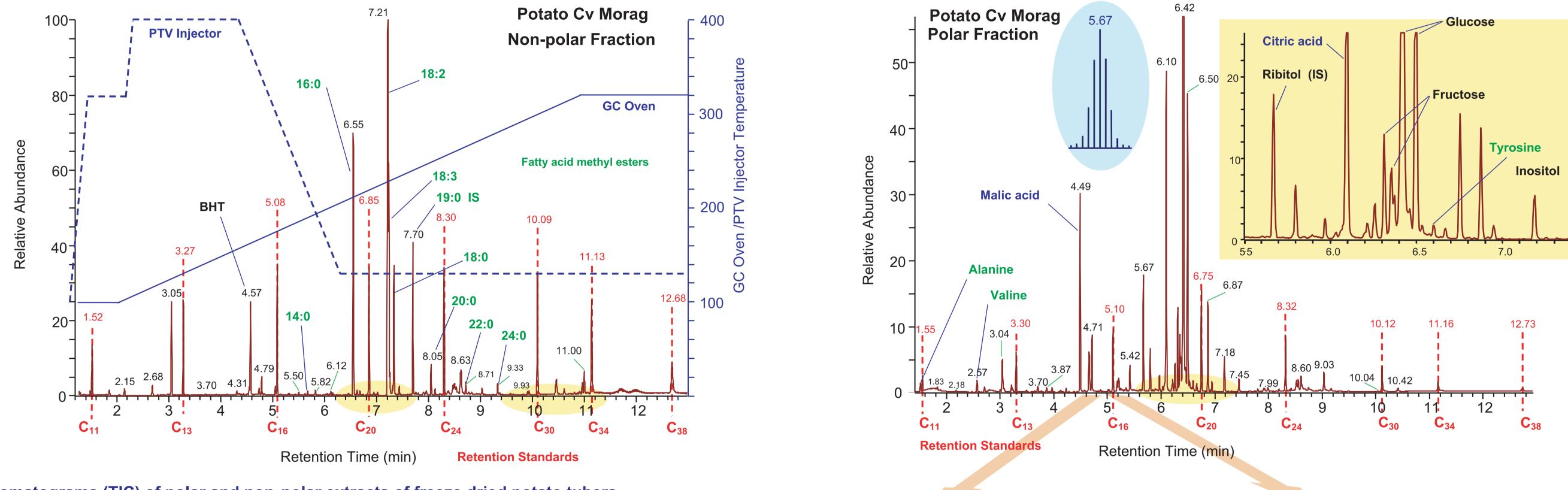
Freeze Dried Potato Powder (100mg) + Internal Standards for polar & non-polar components **Polar Fraction (250** μ*l*)

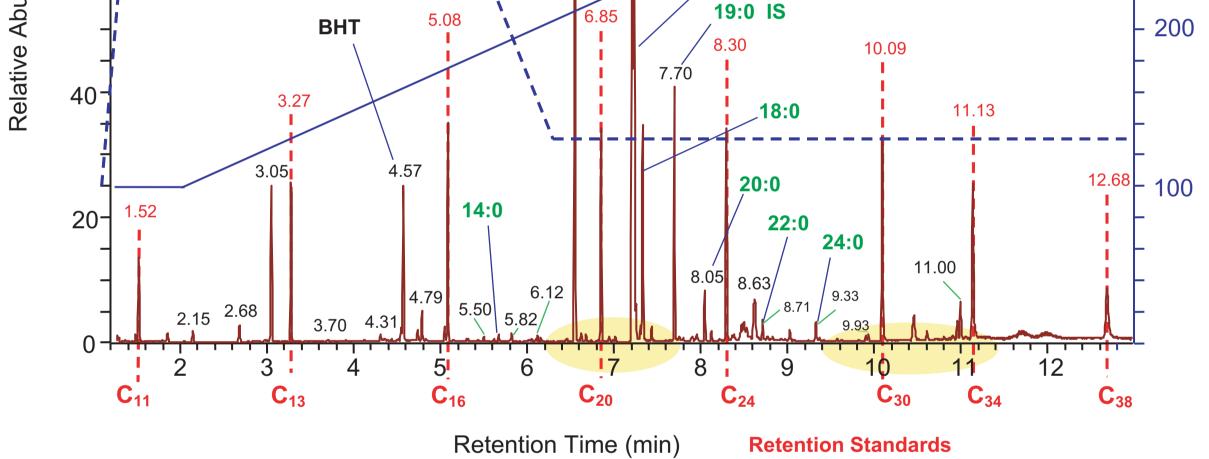
Polar Fraction

Non-Polar Fraction Evaporate Solvent (centrifugal evaporator)

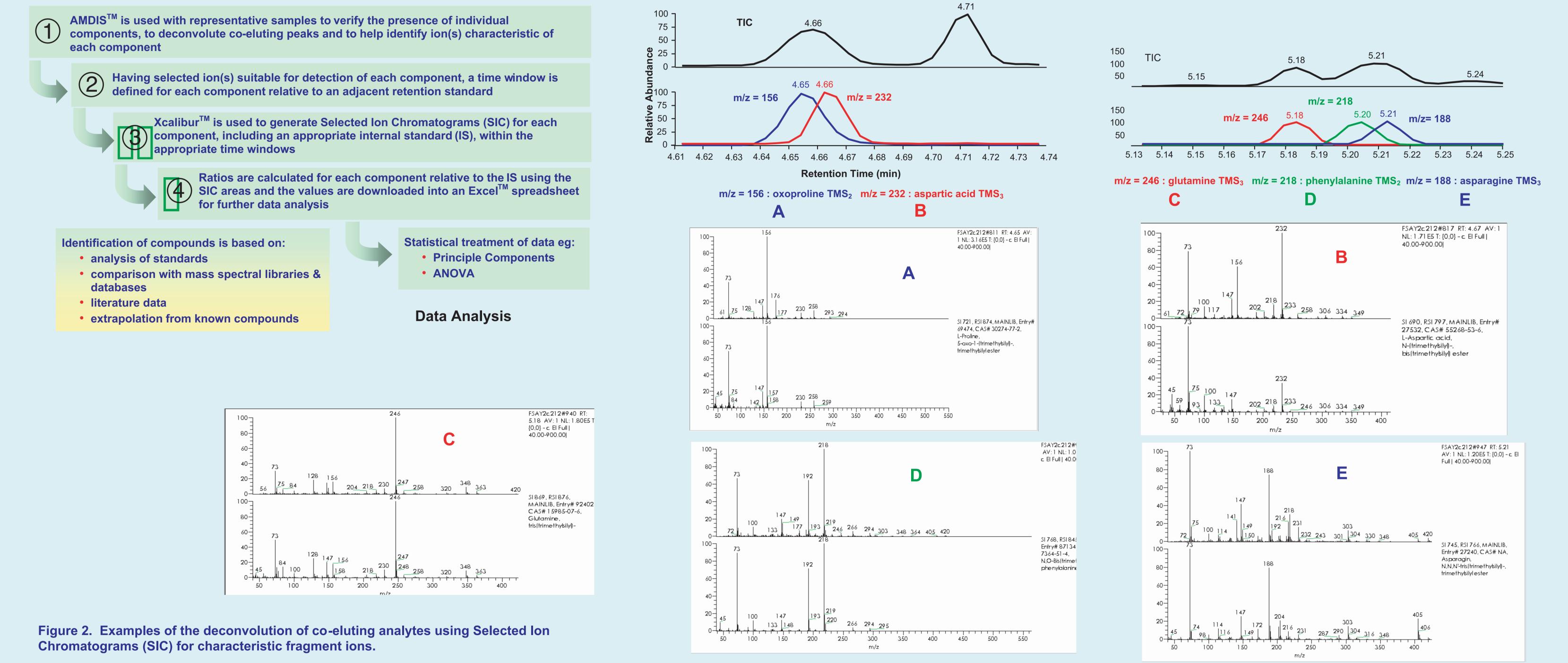
Non - polar Fraction Derivation

ds for polar & non-polar components: 0 <i>mg/50 ml H</i> ₂0) (polar) anoate (100 μ/, 10 mg/50 ml isohexane) (non-polar)	· · · · · · · · · · · · · · · · · · ·	Solvent (centrifugal evaporator) heat, vacuum	Derivatisation	-	Solvent (centrifugal evapo vacuum, no heat	rator)	Derivatisation
MeOH (3 <i>ml</i>) shake @ 30° C for 30 min 2) H ₂ O (0.75 <i>ml</i>) each time			<i>ml</i>)@ 100°C for 45 min				overnight then cool to RT hake & separate layers (discard top layer)
 (3) CHCI₃ (6 <i>ml</i>) (4) H₂O (1.5 <i>ml</i>) shake by hand @ RT Separate layers (centrifuge) no heat or vacuum 		MSTFA (80 μl) @ 37°C for 30 min			Add KHCO ₃ lower CHCl ₃	(3 <i>ml,</i> 2%), s layer throug	hake & separate layers (discard top layer) Pass gh drying column (<i>anhydrous Na₂SO₄</i>), wash
Top LayerBottom LayerPolar FractionNon-polar Fraction		Retention Standard (alkanes) C ₁₁ C ₁₃ C ₁₆ C ₂₀				evaporator,	
nple Extraction						Silylation CHCl ₃ (50 μ/), MSTFA (80 μ/), Pyridine (10μ/), 37°C for 30 min Cool then add 40 μl to vial & dilute with pyridine (40μ/)	
				Rete	ention Standard		Derivatised Non-Polar Fraction Inject 1μl for GC-MS analysis
					Semula Seguence F		
	de			-		Included in	Purpose
					1 Analytical Blank	>	Quality control check & background
He, 1.5 mL min ⁻¹ , constant flow		TEMPUSTOF MS			2 Reference standard	3>	Quality control check, Reproducibility between sequences (sample preparation & instrumental). Precision of analysis
					3 Repeat injections	3 +	Instrumental reproducibility during sequence (check for drift)
					4 Multiple extracts	3+	Reproducibility of sample preparation
4 spectra sec ⁻¹ (summed) to give approximately 7 - 12 spectra acro chromatographic peak	ss a				5 Two split levels		High split (167:1): All peaks on scale Low split (83:1): Information for minor peaks
	 (2) H₂O (0.75 m) each time (3) CHCl₃ (6 m) (4) H₂O (1.5 m) shake by hand @ RT Separate layers (centrifuge) no heat or vacuum Top Layer Bottom Layer Polar Fraction Non-polar Fraction Programmable Temperature Vapourising Injector (PTV) in split mod Temperature Programme: see Fig. (1) DB5-MS (15 m x 0.25 mm x 0.25µm) He, 1.5 mL min ⁻¹ , constant flow Temperature programme: see Fig. (1) Electron Impact (EI), 70 eV Orthogonal Time-Of-Flight (TOF) 35 - 900 a.m.u	anoate (100 µl, 10 mg/50 ml isohexane) (non-polar) MeOH (3 ml) shake @ 30°C for 30 min (2) H ₂ O (0.75 ml) each time (3) CHCl ₃ (6 ml) each time (4) H ₂ O (1.5 ml) shake by hand @ RT Separate layers (centrifuge) no heat or vacuum Top Layer Bottom Layer Polar Fraction Non-polar Fraction Programmable Temperature Vapourising Injector (PTV) in split mode Temperature Programme: see Fig. (1) DB5-MS (15 m x 0.25 mm x 0.25µm) He, 1.5 mL min ⁻¹ , constant flow Temperature programme: see Fig. (1) Electron Impact (El), 70 eV Orthogonal Time-Of-Flight (TOF) 35 - 900 a.m.u	anoate (100 μl, 10 mg/50 ml isohexane) (non-polar) MeOH (3 ml) shake @ 30°C for 30 min (2) H ₂ O (0.75 ml) each time (3) CHCl ₃ (6 ml) each time (4) H ₂ O (1.5 ml) shake by hand @ RT Separate layers (centrifuge) no heat or vacuum Methoximation Top Layer Bottom Layer Polar Fraction Non-polar Fraction Programmable Temperature Vapourising Injector (PTV) in split mode Temperature Programme: see Fig. (1) DB5-MS (15 m x 0.25 mm x 0.25 m) He, 1.5 mL min ⁻¹ , constant flow Temperature programme: see Fig. (1) Electron Impact (EI), 70 eV Orthogonal Time-0f-Flight (TOF) 35 - 900 am.u	Anoate (100 µl, 10 mg/50 ml isohexane) (non-polar) MeOH (3 m) shake @ 30°C for 30 min each time (3) CHCl ₃ (6 m) (4) H ₂ O (1.5 m) shake by hand @ RT Separate layers (centrifuge) no heat or vacuum Top Layer Bottom Layer Polar Fraction Non-polar Fraction Programmable Temperature Vapourising Injector (PTV) in split mode Temperature Programme: see Fig. (1) DB5-MS (15 m x 0.25 mm x 0.25µm) He, 1.5 mL min ¹ , constant flow Temperature programme: see Fig. (1) Electron Impact (EI), 70 eV Orthogonal Time-OF-Hight (TOF) S1- 900 a.m.u	anoate (100 µl, 10 mg/50 ml isohexane) (non-polar) MeOH (3 m) shake @ 30°C for 30 min 2) H ₂ O (0.75 m) each time (3) CHCL (6 m) (4) H ₂ O (1.5 m) (4) H ₂ O (1.5 m) shake by hand @ RT Separate layers (centrifuge) no heat or vacuum Methoxyamine.HCl in Pyridine (80 µl, 20 mg/ml)@ 100°C for 45 min Top Layer Bottom Layer Polar Fraction Non-polar Fraction Vertice (50 µl, 10 mg/50 ml isohexane) Criptice (20 µl) @ 37°C for 30 min Add 40 µl to vial & dilute with Pyridine (40µl) Derivatised Polar Fraction Top Layer Bottom Layer Polar Fraction Non-polar Fraction Non-polar Fraction Criptice (20 C C c c c c c C c C c C c C c C c C c	Ancate (100 µ, 10 mg/50 ml isobexare) (non-polar)	ancade (100 µl, 10 mg/50 ml isobexare) (non-polar) IMCOM (2 mn) scheka @ 30°C for 30 min cach time (30 µl, 20 mg/m/@ 100°C tor 45 min Silylation (3) CHCls (7m) chack by hand @ RT Separate layers (centrifuge) no heat or vacuum Methoximation Methoximation More Layer Polar Fraction Bottom Layer Polar Fraction Non-polar Fraction Non-polar Fraction Non-polar Fraction Programmable Temperature Vapourising Injector (PTV) in split mode Temperature Programme: see Fig. (1) DB5-MS (15 m x 0.25 mm x 0.25 m) He to vacuum Electron Impact (E), 70 eV Orthogonal Time-Of-Flight(TCOF) 35 - 900 ann 40 with 30 with value (5 min with solution with programme: see Fig. (1)





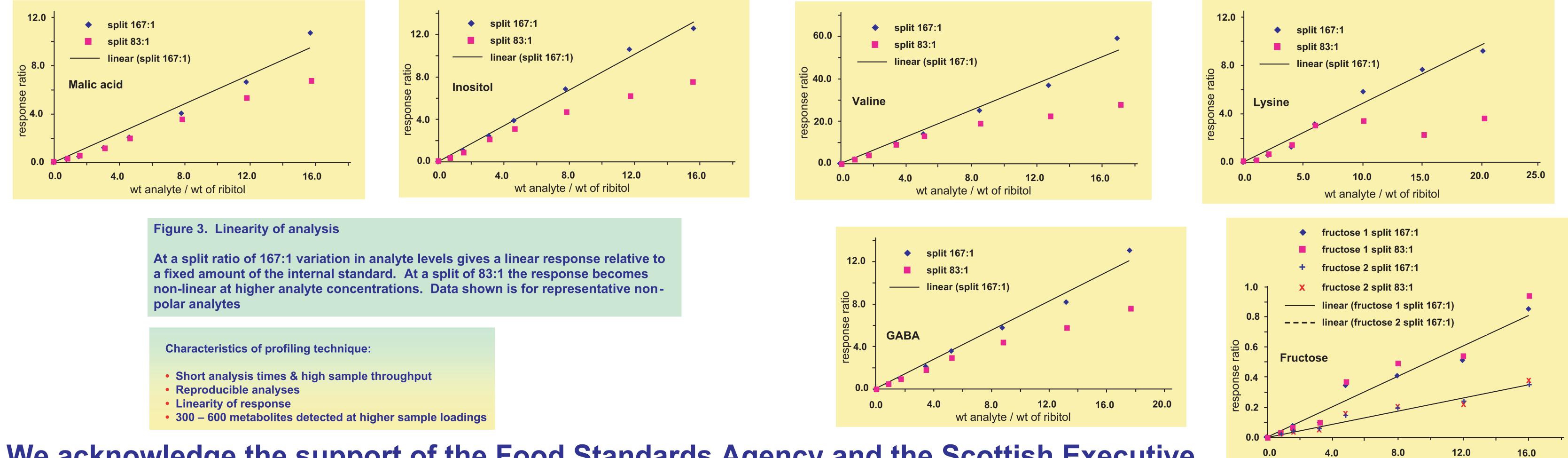




Comparison of mass spectra of analytes with entries in the NIST[™] library of mass spectra

0.0

wt analyte / wt of ribitol



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