ADVANCED SOFTWARE FOR MULTI-ANALYTE SCREENING OF TOF-MS DATA USING LIBRARY SEARCHING AND ACCURATE MASS CONFIRMATION

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INTRODUCTION

Although library searching of mass spectra has been available for many years, its full potential has never before been realised as a tool for screening of both known and unexpected compounds. Recent developments in Time of Flight MS and associated software have enabled high mass accuracy full spectral data to be acquired and processed easily and quickly. The work outlined here demonstrates the use of new, advanced data processing software which automatically and effectively detects peaks from complex chromatographic data; searches the deconvoluted spectra against libraries created from either real or theoretical data and scores the results based on mass accuracy or proximity of the measured to theoretical isotope ratios.

METHODS

For fast, high efficiency separations LC data was acquired using an ultra-performance liquid chromatograph with 1.7mm column particle size. This was coupled to an electrospray ionisation LC-ToF mass spectrometer with enhanced ion optics to maximise spectral resolution and mass accuracy. For other analytes, a traditional gas chromatograph was coupled to a new GC-ToF mass spectrometer equipped with an electron impact ionisation source, again acquiring elevated resolution accurate mass data. Both mass spectrometers use novel ion optics to enhance their dynamic range and ensure that good quality data with accurate ion ratios were obtained. In order to fully investigate the applicability of the new software module, a wide range of compounds in matrices of varying complexity were analysed.

Extraction Methods

Details in Waters Application Notes 720001437EN, 720001607EN & 720001675EN.

LC-MS Methods

A Waters Acquity UPLC system coupled to a Waters LCT Premier ToF MS were employed. The column used was an Acquity UPLC BEH C_{18} 2.1x100 mm, 1.7 µm @ 40 °C. The gradient and mobile phases were dependent on the analysis. The mass spectrometer was operated in W-mode for elevated resolution (>10,000 FWHM) and real-time exact mass data was acquired by reference to an independently sampled reference (Leucine Enkephalin, $[M+H]^+$ = 556.2771Da, $[M-H]^-$ = 554.2615Da.

GC-MS Methods

An Agilent 6890N GC with 7683B autosampler was coupled to a Waters GCT Premier ToF MS. 1µL sample was injected by Cyro-cooled PTV in solvent vent mode onto a DB-5MS column (20 m x 0.18 mm i.d. x 0.18 mm). Exact mass data was acquired by reference to 2,4,6-tris(trifluoromethyl)-1,3,5-triazine, 284.9949Da at a rate of 10 spectra s^{-1}.

Software

MassLynx v4.1 with the ChromaLynx application manager.

RESULTS AND DISCUSSION

Several datasets were acquired encompassing various applications using a range of techniques. Investigations were first carried out in order to ascertain the ability of the software to detect analyte peaks from complex chromatograms. Figure 1 above shows a section of the LC chromatogram from a milk sample spiked with a selection of veterinary drugs. It is apparent that peaks from indigenous components of the matrix are considerably more intense than, and indeed mask, the peaks from the analytes of interest. However, by plotting extracted ion chromatograms, those components are easily found manually.

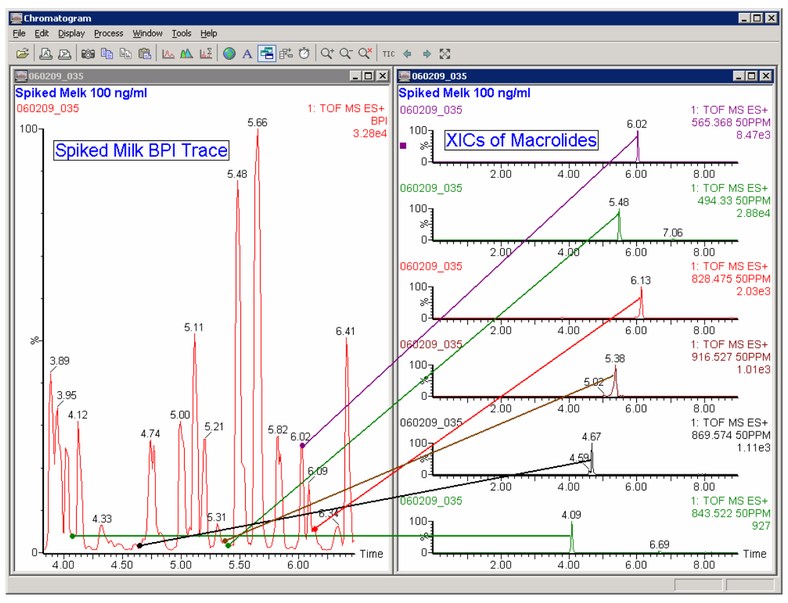

Figure 1. Raw chromatogram and extracted analytes from a spiked milk sample

When processing the data using ChromaLynx, the *n* most intense spectral peaks (8 in this case) from each scan are extracted. By applying automatic peak detection parameters, 166 distinct chromatographic peaks were found. The mass spectrum from each peak is then deconvoluted and compared to a library created from spectra of standards, and any 'hits' are displayed by match quality. Other screening parameters (retention time, polarity, cone voltage) can then be applied to create a list of candidates, ranked as positive, tentative or negative depending on the library match (either forward or reverse fit). Figure 2 shows the final, screened results for the milk sample, and proves that the peak detection and library screen have been successful.

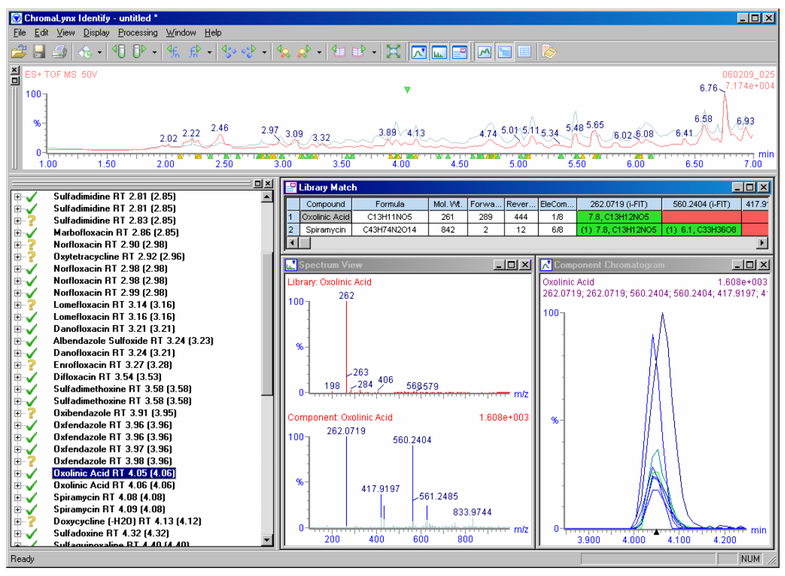

Figure 2. ChromaLynx screening results for milk sample spiked with 41 veterinary drugs

Table 1 below summarises the concentrations at which each of the 41 compounds are successfully identified from a typical screening experiment. Notably, for all regulated compounds, this concentration is at or below the Maximum Residue Limit (MRL)—the level above which produce must be withdrawn from sale.

		MRL/ µg/L	Screened Level	LoD/ µg/L
Benzimidazoles	Albendazole	100	100 (25)	0.10
	Albendazole Sulfoxide	100	25 (10)	0.20
	Fenbendazole	10	10	0.20
	Oxfendazole	10	10 (5)	0.10
	Oxfendazole Sulfone		100 (25)	0.10
	Mebendazole		100 (25)	0.20
	Levamisole		10 (5)	0.25
	Thiabendazole	100	10	0.20
	Flubendazole		10	0.05
	Oxibendazole		25 (5)	0.05
Macrolides	Spiramycin	200	25	0.30
	Tilmicosin	50	10 (5)	0.50
	Tylosin	50	25	1.00
	Josamycin		25	0.20
	Tiamulin		5 (1)	0.05
	Valnemulin		10	0.10
	Lincomycine	150	10	0.20
Tetra-cyclines	Doxycycline (-H2O)	100	(25)	0.40
	Tetracycline	100	(100)	1.00
	Oxytetracycline	100	(50)	0.50
	Chlorotetracycline	100	(50)	4.00

		MRL/ µg/L	Screened Level	LoD/ µg/L
Quinolones	Nalidixic Acid		(25)	0.10
	Oxolinic Acid		50 (10)	0.10
	Flumequine	50	100 (10)	0.10
	Norfloxacin		100 (50)	1.00
	Ciprofloxacin		*	1.00
	Lomefloxacin (IS)		50	0.50
	Enrofloxacin	100	10	
	Marbofloxacin	75	10	0.20
	Difloxacin		25 (5)	0.20
	Danofloxacin	30	50 (25)	0.20
	Cinchophen		100 (10)	0.10
	Sarafloxacin		50 (25)	0.20
Sulfonamides	Dapsone	100	(25)	0.10
	Sulfadiazine	100	(100)	0.25
	Sulfamethoxazole	100	*	0.15
	Sulfamethazine	100	*	0.20
	Sulfadimethoxine	100	100 (10)	0.15
	Sulfadimidine	100	25 (10)	0.25
	Sulfadoxine	100	25 (10)	0.05
	Sulfaquinoxaline		100 (25)	0.20

* = not in library

Table 1. Summary of milk screening results. Bracketed concentrations are where a tentative match has been made. No brackets for a positive match.

When GC-MS is the analytical method, extremely complex chromatograms are often observed. Figure 3 shows the software has successfully differentiated three co-eluting components from a mixture of pesticides. This method was applied to various food samples, including cucmber, grapefruit and sweet pepper.

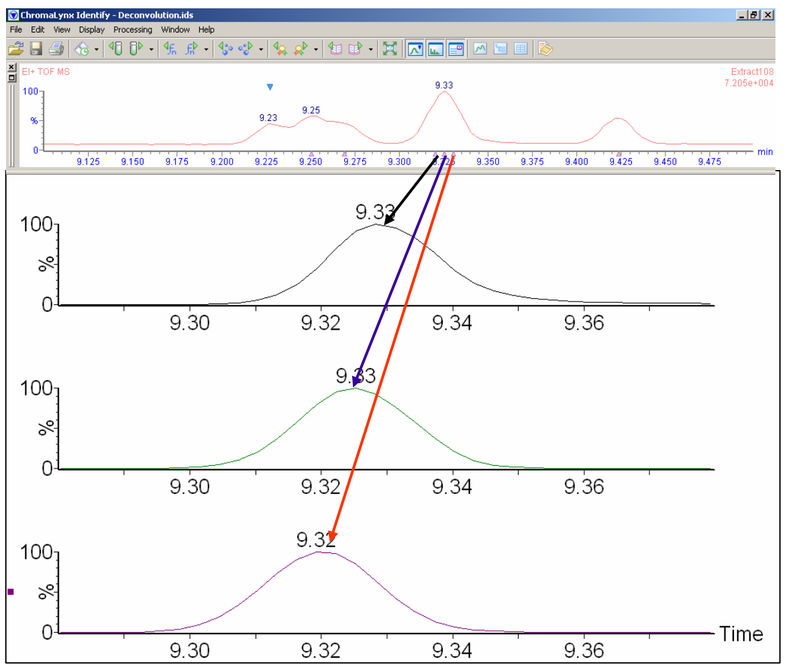

Figure 3. Automatic and manual peak detection of three co-eluting pesticides in a complex GC chromatogram

EI ionisation allows the searching of extensive libraries such as those supplied by NIST and Wiley, enabling the identification of over 200,000 compounds. Figure 4 below shows the results of such a search performed on a cucumber extract, containing over 500 peaks.

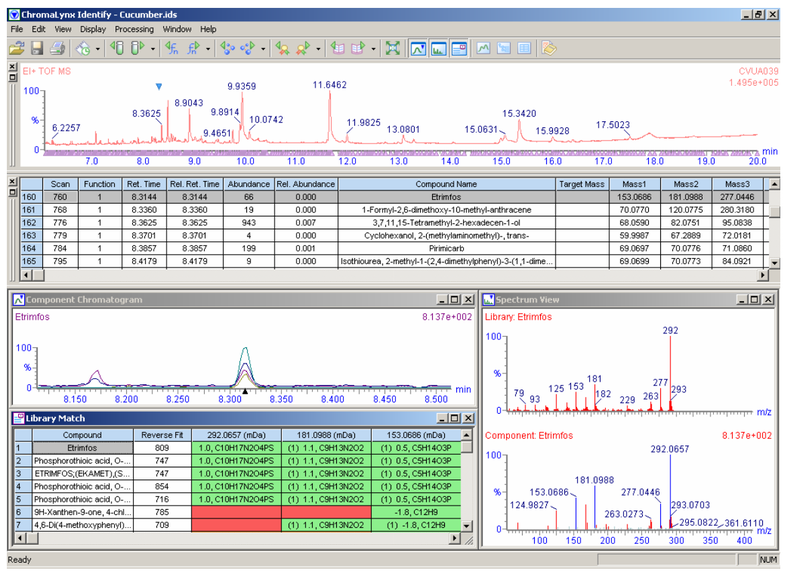

Figure 4. Results of a ChromaLynx search from cucumber using the NIST library

Where applicable libraries are not available, and standards are scarce or prohibitively expensive, it is advantageous to use a 'look-up table' to search for compounds of interest by their exact mass. ChromaLynx extends this idea by employing spectra created by an isotope modelling algorithm, which automatically contains the exact mass and the correct distribution of elemental isotopes. By using this spectrum, it becomes possible to get identification of screened compounds. Figure 5 shows the measured and theoretical spectra of Albendazole ($C_{12}H_{15}N_3O_2S$, $[M+H]^+$ = 266.0963Da) as well as the elemental composition performed on the acquired data. Here, possible formulæ are calculated within 5ppm of the measured mass and usually displayed by ΔM. The new software, however, contains a scoring algorithm called i-FIT, which compares the measured isotopic ratios to those of the suggested elemental composition. The closer the match, the lower the score, and in this example, the results are ranked thus. The top score (closest match) is the correct formula.

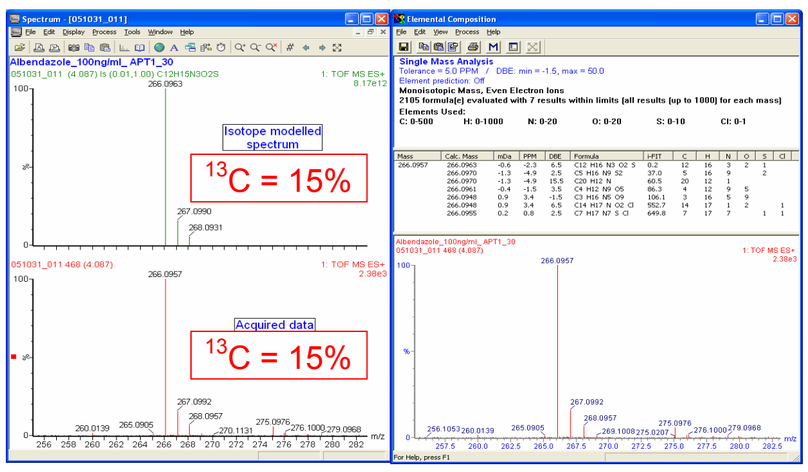

Figure 5. Acquired spectrum of Albendazole compared to theoretical isotope model plus elemental composition results ranked by i-FIT

ChromaLynx automatically uses the formula from the library as the defining parameter in an elemental composition search. Candidates are subject to this scoring system as shown in figure 6—the green shaded box indicates that the mass is correct to <5ppm, and the i-FIT score of 13.9 indicates a good agreement. The analyst obtains the nominal mass, retention time and can readily confirm these positive results by their exact mass and isotopic distribution.

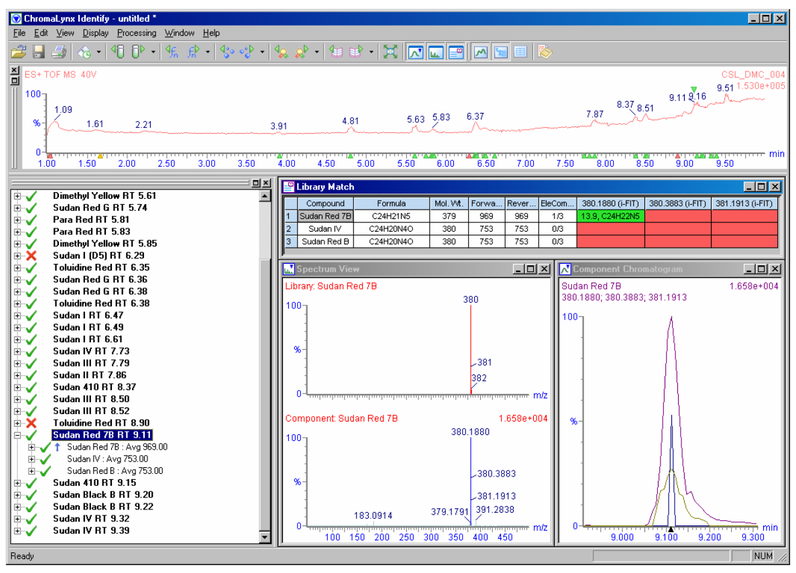

Figure 6. A mixture of illegal dyes screened using a theoretical library.

CONCLUSION

- The automatic peak detection software effectively detects chromatographic peaks in complex matrices, even when components co-elute
- The resulting de-convoluted spectra are suitable for library matching against reference spectra
- The methods presented are suitable for the screening of multiple residues at relevant concentration levels in a variety of matrices.
- Spectra with high mass accuracy and correct isotope ratios are valuable aids to the identification of contaminants, and may be appropriate for confirmatory analysis

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