A Case of Pesticide Poisoning: The Use of a Broad-Scope Tof Screening Approach in Wildlife Protection

INTRODUCTION

As fragile ecosystems struggle to survive the impact of human domination of the environment, wildlife protection becomes increasingly important. While it is always preferable to safeguard living specimens in their native habitats, sadly, it is sometimes necessary to deal with the consequences of human interaction with vulnerable animals. Here we describe the use of a ToF screening approach in an incident of pesticide poisoning of a protected bird of prey.

> ***Up until the mid-19th century, red kites were persecuted extensively as vermin in the UK. The species was brought back from the brink of extinction by an on-going conservation effort.***

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The red kite (family: accipitridae, latin name: milvus milvus), shown in *Figure 1*, is a bird of prey that belongs to the same family as hawks, vultures, and eagles. This species has approximately 18,000 to 24,000 current breeding pairs in Europe, with around two thirds of this population found in Germany, and further significant populations in France and Spain. Up until the mid-19th century, red kites were persecuted extensively as vermin in the UK. The species was brought back from the brink of extinction by an on-going conservation effort. There are now just over 1,000 breeding pairs in the UK, mainly located in central Wales, along the spine of central England and at various sites in Scotland [1,2].

Figure 1. A red kite (milvusmilvus) in flight.

The red kite is primarily a scavenger that feeds on worms, small mammals, and carrion. Its feeding habits make it particularly susceptible to pesticide poisoning, either accidental – when it feeds on creatures that have previously been killed by pesticides; or intentional – when people spike pesticides into carrion, either to kill animals such as foxes and crows, or to target the birds themselves.

In the UK, the red kite is protected under the Wildlife and Countryside Act of 1981, and, under Schedule 1, Part I, of this act, they are 'protected by special penalties' [3]. The birds are afforded additional, wider protection in Scotland, as a result of the Nature Conservation (Scotland) Act of 2004 [4]. If red kite carcasses are discovered by police or wildlife protection officers, and pesticide poisoning is suspected, they are often brought to SASA (Science and Advice for Scottish Agriculture – a division of the Scottish government). Here, samples are analysed to identify the cause of death and, if necessary, the particular type or types of pesticide used.

This application note describes the use of Waters® ACQUITY UPLC® coupled with Xevo™ G2 QTof, along with POSI±IVE™ Software and the MassFragment™ tool, to screen samples from the gullet of a red kite carcass suspected of poisoning by pesticides, and to identify which pesticides were used. We were able to demonstrate the unequivocal detection and identification of the pesticide poisons ingested by the red kite.

EXPERIMENTAL

Sample preparation

- The gullet contents were removed from the red kite carcass and 2.0g were extracted into 5mL of ethyl acetate.
- A 1mL aliquot was then solvent exchanged into methanol and made up to 400mL, i.e. 0.4g of gullet content extract in 400mL.
- This sample was passed through a 0.2µm syringe filter with no further cleanup prior to analysis.

LC conditions

LC system:	ACQUITY UPLC
Runtime:	5.00 min
Column:	ACQUITY BEH C_{18} 1.7mm, 2.1 x 50mm
Column temp:	45°C
Mobile phase A:	10mL of 1 M aqueous ammonium acetate solution and 990mL water
Mobile phase B:	10mL of 1 M aqueous ammonium acetate solution and 990mL methanol
Flow rate:	0.6mL/min
Injection volume:	3.0mL

Mobile phase gradient is detailed in *Table 1*.

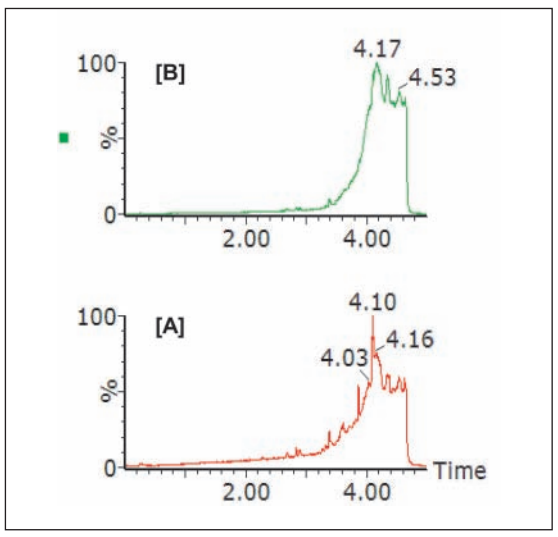


Figure 2. [A] Low energy MS^E TIC from broad-scope screening of red kite gullet contents; [B] High energy MS^E TIC from broad-scope screening of red kite gullet contents.

Table 1. ACQUITY UPLC mobile phase gradient.

	Time (min)	Flow rate (mL/min)	%A	%B	Curve
1	Initial	0.60	98	2	0
2	0.10	0.60	98	2	6
3	3.75	0.60	1	99	6
4	4.25	0.60	1	99	6
5	4.26	0.60	98	2	11
6	5.00	0.60	98	2	6

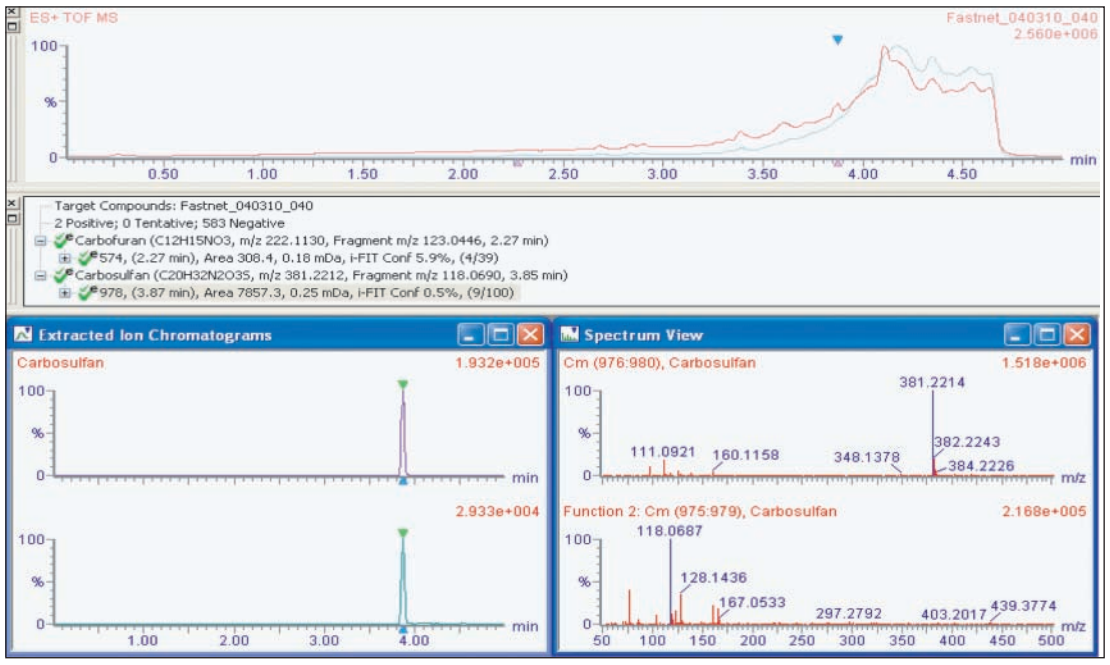
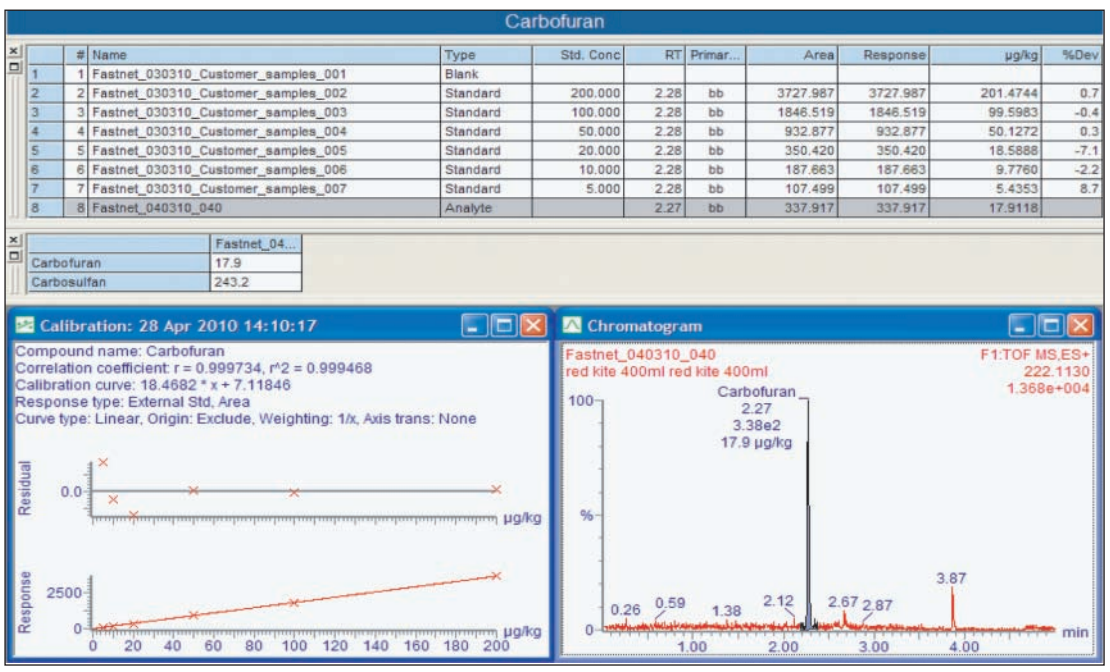


Figure 3. ChromaLynx XS Identify results browser showing MS^E precursor and fragment ion data identifying carbofuran and carbosulfan as the potential pesticide poisons.



#	Name	Type	Std. Conc	RT	Primar...	Area	Response	µg/kg	%Dev
1	Fastnet_030310_Customer_samples_001	Blank							
2	Fastnet_030310_Customer_samples_002	Standard	200.000	2.28	bb	3727.987	3727.987	201.4744	0.7
3	Fastnet_030310_Customer_samples_003	Standard	100.000	2.28	bb	1846.519	1846.519	99.5983	-0.4
4	Fastnet_030310_Customer_samples_004	Standard	50.000	2.28	bb	932.877	932.877	50.1272	0.3
5	Fastnet_030310_Customer_samples_005	Standard	20.000	2.28	bb	350.420	350.420	18.5888	-7.1
6	Fastnet_030310_Customer_samples_006	Standard	10.000	2.28	bb	187.663	187.663	9.7760	-2.2
7	Fastnet_030310_Customer_samples_007	Standard	5.000	2.28	bb	107.499	107.499	5.4353	8.7
8	Fastnet_040310_040	Analyte		2.27	bb	337.917	337.917	17.9118	

	Fastnet_04...
Carbofuran	17.9
Carbosulfan	243.2

Figure 4. TargetLynx Quantify results browser showing carbofuran and carbosulfan quantified using pesticide solvent standard calibration curves.

Butylate	C11H23NOS	3.43	
Carbaryl	C12H10NO2NH4	2.13	
Carbendazim	C9H9N3O2	1.82	
Carbofuran	C12H15NO3	2.27	
Carbofuran-3-hydroxy	C12H14NO4	1.72	
Carbofuran-3-keto	C12H13NO4	1.98	
Carbofuran-3-hydroxy-7-phenol	C10H12O3		CarboFuran
Carbofuran-3-keto-7-phenol	C10H10O3		CarboFuran
Carbofuran-7-phenol	C10H12O2		CarboFuran
Carbosulfan	C20H32N2O3S	3.85	
Carboxin	C12H13NO2S	2.33	
Chlorbromuron	C9H10BrClN2O2	2.83	
Chlorfenvinphos	C12H14Cl3O4P	3.23	

Figure 5. A section of the targeted pesticide database with the key compounds highlighted.

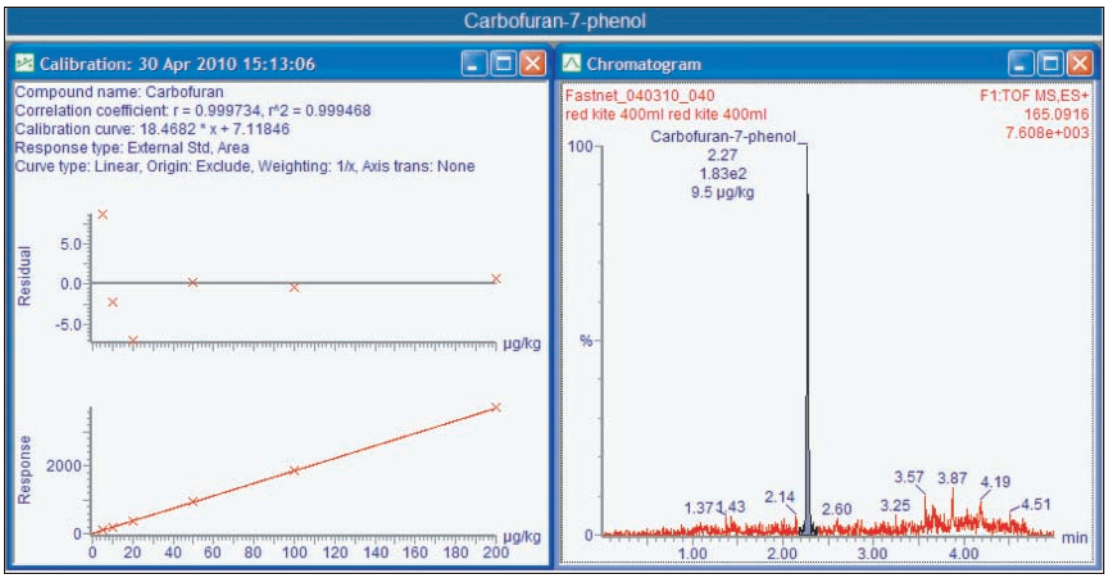


Figure 6. The metabolite 7-phenolcarbofuran was also identified and quantified using the calibration curve for carbofuran.

MS conditions

MS system: Xevo G2 QTof
Ionisation mode: ESI positive
Analyser: Resolution mode
Scan time: 0.1 s
Capillary voltage: 1.0 kV
Sampling cone: 30.0
Source temp: 120°C
Desolvation temp: 550°C
Desolvation gas: 1000 L/hr
Cone gas: 50 L/hr
Mass range: 50 to 1000 m/z

MS^E conditions

Low energy: 6.0
High energy ramp: 25.0 – 35.0

LockSpray conditions

Compound: Leucine enkephalin
Masses: m/z 556.2771 and m/z 278.1141
Flow rate: 20mL/min
Capillary voltage: 3.0kV
Collision energy: 21.0

RESULTS AND DISCUSSION

The generic screening method given above was used to screen extracted gullet contents of the red kite for pesticide residues. The low energy MS^E precursor ion total ion chromatogram (TIC) and the high energy MS^E fragment ion TIC acquired from this screening analysis are shown in *Figure 2*.

Matrix-matched standards were not available for the red kite sample; however, the sample data were processed using POSI±IVE Software with pesticide solvent standards in order to provide identification and help assess the magnitude of the compounds that poisoned the red kite.

Figure 3 shows the ChromaLynx™ XS Identify results browser from POSI±IVE data processing. Here, 585 compounds were targeted from which two were automatically found and identified as carbofuran and carbosulfan. Data for both the MSE low energy precursor ions and the high energy fragment ions are displayed. The accuracy of the exact mass ions, as shown for carbosulfan (precursor ion: m/z 381.2212, fragment ion: m/z 118.0690) with DM values of +0.2 mDa and -0.3 mDa respectively, provides added confidence that the results are correct.

Figure 4 shows the TargetLynx™ Quantify Browser from POSI±IVE data processing. Here pesticide solvent standards have been used to quantify the identified pesticide poisons.

Once the toxic compounds had potentially been identified as carbofuran and carbosulfan, additional metabolites of these two pesticides, previously identified in work by Soler, et al.[5], were included in the targeted screening database, and the data were re-processed using POSI±IVE Software.
Figure 5 shows a section of the targeted compound database used, with the added metabolites and parent compounds highlighted.

Standards were not available for some of the metabolites of interest; however, POSI±IVE provides the opportunity to identify a similar compound on the list for use as a standard from which to quantify. In *Figure 5*, 3-hydroxy-7-phenolcarbofuran, 3-keto-7-phenolcarbofuran, and 7-phenolcarbofuran, if present in the sample, would each be quantified using the calibration curve for carbofuran.

After re-processing, the metabolite 7-phenolcarbofuran was also identified and quantified using the solvent calibration curve for carbofuran, as shown in *Figure 6*.

Further compound confirmation was carried out using the MassFragment tool. Structures were assigned to the MS^E fragment ion spectra acquired from the relevant extracted ion chromatograms (XIC), based on accurate and precise exact mass data.

Figure 7 shows MassFragment-assigned structures for the fragment ions seen at 2.27 min, and *Figure 8* shows similar information for the fragment ions acquired at 3.87 min.

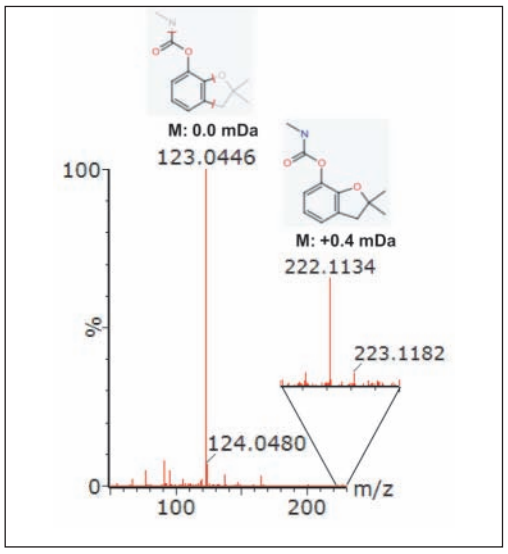


Figure 7. MS^E high energy fragment ion spectrum showing mass accuracy for carbofuran precursor ion and primary fragment ion at 2.37 min, with structural assignments from MassFragment.

CONCLUSIONS

- Broad-scope pesticide screening of the extracted red kite gullet samples enabled the detection and identification of the pesticides that poisoned the bird of prey. Carbosulfan and carbofuran were identified, with a high degree of confidence, as the pesticides used in this poisoning case.
- The MS^E functionality of Xevo G2 QTof enables the acquisition of both low energy (precursor ion) and high energy (fragment ion) data in one rapid screening run.
- The highly reproducible and precise exact mass data affords increased confidence in the accuracy of the results.
- POSI±IVE Software, along with the MassFragment tool, provide a powerful data processing approach for pesticide screening and unequivocal compound identification.

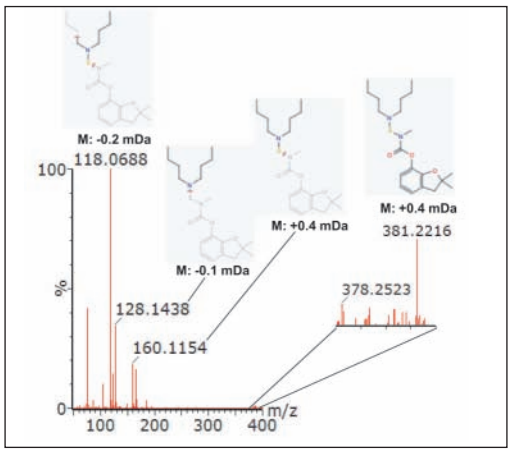


Figure 8. MS^E high-energy fragment ion spectrum showing mass accuracy for carbosulfan precursor ion and primary fragment ions at 3.87 min, with structural assignments from MassFragment.

ACKNOWLEDGEMENTS

With thanks to SASA for providing extracted samples and pesticide standards.

REFERENCES

[1] Website: http://www.redkites.co.uk/

[2] Website: http://www.rspb.org.uk/wildlife/birdguide/name/r/redkite/index.aspx

[3] Website: http://www.jncc.gov.uk/page-3614

[4] Website: http://www.opsi.gov.uk/legislation/scotland/acts2004/asp_20040006_en_1

[5] Soler C, Hamilton B, Furey A, James KJ, Manes J, and Pico Y. Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry Analysis of Carbosulfan, Carbofuran, 3-Hydroxycarbofuran and Other Metabolites in Food. Analytical Chemistry, 2007; 79: 1492-1501.

South African Doping Control for 2010 World Cup

Agilent Technologies, Inc instruments were selected by the South African Doping Control Laboratory as the primary provider of gas-phase testing instruments for the 2010 World Cup. The South African Doping Control Laboratory is certified by the World Anti-Doping Agency and will be responsible for all doping testing during the competition.

The South African Doping Control Laboratory is equipped with state-of-the-art Agilent gas chromatography (GC) and mass spectrometry (MS) instruments to confirm the chemical identity of suspected banned substances found in testing samples. Agilent provided five GC/MSD systems and an Agilent 7000 Series Triple Quadrupole GC/MS system. Agilent technicians provided technical support throughout the event.

"The use of the 7000 Series Triple Quadrupole GC/MS system from Agilent has raised the standard of doping testing to a higher level," said Dr Pieter J. van der Merwe, Director, South African Doping Control Laboratory. "This instrument is significantly increasing the sensitivity and specificity of detection."

"Agilent is proud of our leadership role in anti-doping testing instruments, which dates back to the 1970s," said Mike McMullen, President of Agilent's Chemical Analysis Group. "We are honoured that the South African Doping Control Laboratory selected Agilent to help ensure a level playing field and fair competition at the 2010 World Cup."

MASS SPECTROMETRY Circle no. 510

Automated High Throughput LC-MS Solution for Water and Beverage

Thermo Fisher Scientific, Inc announced the launch of the Thermo Scientific EQuan MAX, an automated high throughput Liquid Chromatography-Mass Spectrometry (LC-MS) solution for the analysis of contaminants in water and beverages. This innovative system with high resolution and accurate mass (HRAM) boasts new screening and quantitative capabilities that provide enhanced system flexibility and productivity.

EQuan MAX™ combines with the power of the Exactive™ LC-MS system. The Exactive is a high-resolution benchtop LC-MS system, powered by Oribtrap™ technology, designed for high throughput screening of target and non-target compounds. In addition, the EQuan MAX can also be used with the full range of Thermo Scientific TSQ series triple stage quadrupole mass spectrometers.

EQuan MAX is a comprehensive solution for the analysis of pesticides, pharmaceuticals, personal care products, endocrine disruptors and perfluorinated compounds in environmental water, drinking water and beverages. With EQuan MAX, samples are directly injected for LC/MS analysis, eliminating the need for off-line sample preconcentration. Its unique online sample preparation technique reduces analysis time from days to minutes, designed to increase lab productivity.

Its large injection volume (1-20ml) improves detection limits over conventional LC-MS analysis. In addition to large injection volumes, EQuan MAX now supports injection volumes as low as 1µL. Switching between injection volumes can be automated for overnight operation and increased productivity, without the need to manually change plumbing configurations.

The new EQuan MAX autosampler and plumbing provide out-of-the-box compatibility with the Thermo Scientific Accela 600 high-pressure liquid chromatography (HPLC) and Thermo Scientific Accela 1250 ultra-high pressure liquid chromatography (U-HPLC) systems. The new autosampler is also more compact, reducing total system footprint from approximately 5 to 2.5ft^2, saving valuable lab space.

MASS SPECTROMETRY Circle no. 511

ICP-MS Systems Help Ensure Safety of Chinese Herbal Medicines

PerkinElmer, Inc announced that the Company's Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) technology can help insure that traditional Chinese herbal medicines comply with safety guidelines.

Amid concerns that traditional herbal ingredients may contain potentially toxic contaminants, PerkinElmer's broad portfolio of high performance analytical solutions are well suited for the safety and quality testing of traditional Chinese herbal medicines. PerkinElmer's ICP-MS systems, such as the ELAN 9000, offer exceptional analytical sensitivity and accuracy and feature superior detection capacity.

Traditional Chinese herbal medicines are generally perceived by consumers as being natural and having few side-effects. Due to the complexity of the medicinal plants, their inherent biological variation and environmental influences, it is essential to evaluate the safety, efficacy and quality of these herbal ingredients to ensure consumer safety.

Awareness of the potential for contamination has led to the increased demand for quantitative evaluation of inorganic impurities. The Chinese Pharmacopoeia 2010 (CP 2010) guidelines, due to take effect later this year, will set legal limitations on the levels of certain critical elements, including lead and arsenic, in medicinal products intended for frequent use by infants and the elderly. PerkinElmer's range of ELAN ICP-MS instruments, as well as the new NexION 300 ICP-MS offering, which both combine superior detection limits with ease of use and high sample throughput, can help to assess traditional Chinese herbal ingredients for CP 2010 compliance.

Designed for the quantification of elemental isotopic concentrations and ratios, PerkinElmer's ELAN ICP-MS range offers multi-element capacity, allowing the detection of multiple analytes per sample to enable quicker analysis. Operating at or below the parts-per-trillion detection range, ELAN ICP-MS systems can provide the levels of sensitivity and reproducibility necessary for the accurate determination of the inorganic profile of Chinese herbal medicines.

MASS SPECTROMETRY Circle no. 512