The quantitative and qualitative multi-residue determination of over 150 multi-class chemical contaminants in vertebrate animal tissues using QuEChERS extraction, SPE clean-up and LCMSMS.

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Introduction

The Wildlife Incident Investigation Scheme (WIIS) operated on behalf of the Scottish Government by the Chemistry Branch of Science and Advice for Scottish Agriculture (SASA) investigates incidents of suspected poisoning and chemical contamination of wildlife, beneficial insects, companion animals and livestock.

A Quick Easy Cheap Effective Rugged Safe (QuEChERS) approach has been developed that allows the multi-class chemical contaminants of interest to be analysed in a single extract. The procedure is straightforward and has eliminated the traditional need to use three separate multi-residue extraction and LCMSMS acquisition methods. A highly sensitive LCMSMS Shimadzu 8050 instrument is capable of simultaneous acquisition of hundreds of multiple reaction monitoring (MRM) transitions incorporating fast polarity switching. Reliable analytical data are generated collecting sufficient number of data points across each chromatographic peak.

The method has been successfully validated in a variety of matrices: chicken liver, lamb kidney and chicken muscle tissue where muscle tissue is used as a pseudo matrix for digestive tract material. All matrices have been purchased locally and are free from residues.

Experimental

Initial Extraction (QuEChERS)

1g of chopped liver or kidney tissue or cryomilled chicken muscle was placed in a 50ml centrifuge tube with 5ml of acetonitrile and a ceramic homogeniser and vortexed for 1 min. The extraction salts (4g Na2SO4, 1g NaCl) were added to each tube. The tubes were capped tightly and vigorously shaken by hand for 1 minute then centrifuged at 4000rpm for 5 minutes. (The volume of the extraction solvent was adjusted to account for the volume of the spike solution to give 5ml in total)

d-SPE Cleanup

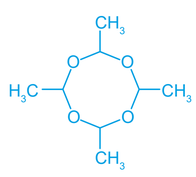
A 3ml aliquot of the supernatant was pipetted into a 15ml disperdive SPE tube, which contained 50mg of PSA, 150mg of C18EC and 900mg of anhydrous Na2SO4. The tube was tightly capped and vortexed for 1min and then centrifuged at 3000 x g for 5 minutes. The upper layer of the sample extract was then filtered (0.45µm PTFE) into a vial ready for LCMSMS. Calibration standards were prepared in appropriate liver, kidney or chicken muscle matrix.

Set-up for the Shimadzu LCMS 8050 and Shimadzu Nexera UHPLC

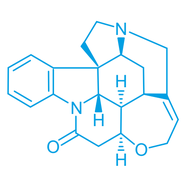
- Run time: 17 min
- Flow rate: 0.4 mL/min
- Eluent A: Methanol/H2O 5/95 v/v + 5mM ammonium acetate
- Eluent B: Methanol + 5mM ammonium acetate
- Gradient elution
- Column: Phenomenex Kinetex 2.6 µm, C18, 50 x 4.6 mm with Phenomenex Security Guard cartridge
- Injection volume: 5 µl
- Minimum of 2 MRMs acquired per compound
- Polarity switching

Results

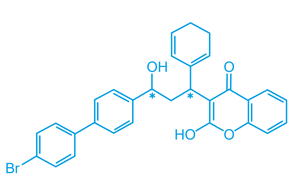
The procedure was validated following a series of recovery experiments whereby a minimum of 5 replicate spike samples were analysed to generate within laboratory recovery mean values and measurement repeatability (coefficient of variation CV%, Table 1), measurement uncertainty (Table 2) and to set a limit of determination (LOD = 0.003 mg/kg).



Metaldehyde



Strychnine



Bromadiolone

Table 1: Recovery data for spiked liver tissue

			Fortification Level 0.01 mg/kg					Fortification Level 0.05 mg/kg				
	Mass	Formula	Min	Max	n	Mean	%CV	Min	Max	n	Mean	%CV
Metaldehyde	176.21	C8H16O4	65	96	6	84	13	67	81	6	74	7
Strychnine	334.41	C21H22N2O2	80	86	6	82	3	76	87	6	80	5
Bromadiolone	527.41	C30H23BrO4	91	102	6	96	4	79	82	6	80	1
Warfarin	308.33	C19H16O4	87	94	6	91	3	102	105	6	104	1
Closantel	663.08	C22H14Cl2I2N2O2	83	111	6	98	9	91	102	6	93	5
Eprinomectin	914.14	C50H75NO14	64	98	6	79	17	59	80	6	70	10

Table 2: Expanded Uncertainty data

Commodity	Expanded Uncertainty %
Chicken Muscle	15
Chicken Liver	15
Lamb Kidney	13

Equivalence tests for liver samples

Liver tissues from two previously healthy dogs that died suddenly were extracted using the QuEChERS method and the results were compared with values obtained using two separate multi-residue methods.

Table 3: Liver tissue residues from Dog 1

Compound	Dog 1		
	QuEChERS (mg/kg)	Method 1 (mg/kg)	Method 2 (mg/kg)
Metaldehyde	45	41	
Bromadiolone	0.067		0.035

Figure 1

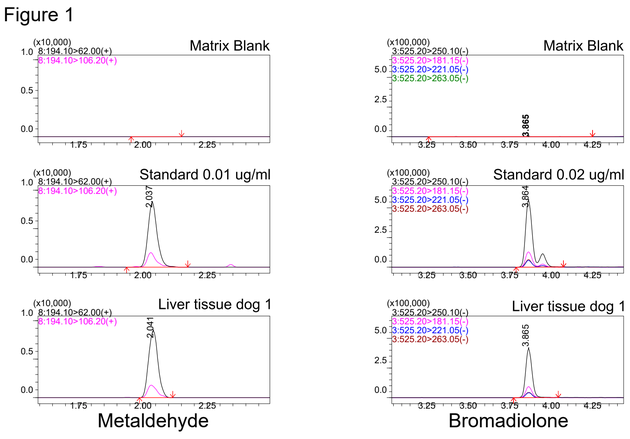


Figure 1 shows liver tissue extract from Dog 1, poisoned with Metaldehyde and with background residue of Bromadiolone.

Table 4: Liver tissue residues from Dog 2

Compound	Dog 2		
	QuEChERS (mg/kg)	Method 1 (mg/kg)	Method 2 (mg/kg)
Strychnine	2.08	1.99	
Bromadiolone	0.005		not tested

Figure 2

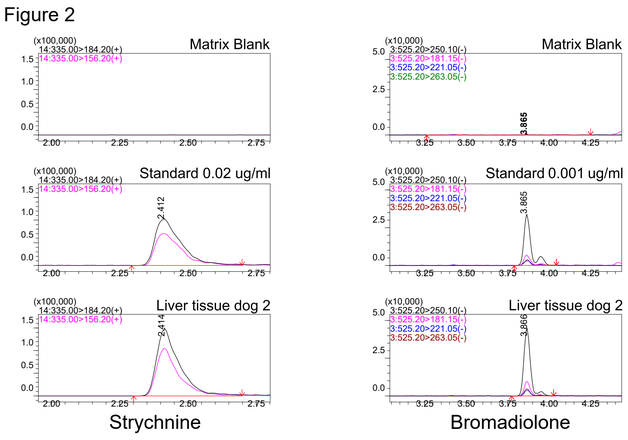


Figure 2 shows liver tissue extract from Dog 2, poisoned with Strychnine and with background residue of Bromadiolone.

Conclusions

This newly introduced QuEChERS extraction protocol has:

- Reduced the time spent on sample preparation from 4 to 1 day.
- Decreased the amount of solvent required to carry out the analysis from 300ml per sample to only 5ml.
- Increased the range of analytes from 118 to 158 due to the addition of a selection of veterinary medicines.
- Reduced the optimal amount of sample required for testing from 15g to 1g.

Much faster analytical determinations for a wider range of contaminants on every sample tested provide in depth information on contaminants non-target species are exposed to. The shorter turnaround times will be of great benefit to the agencies carrying out the investigative processes wherever poisoning is suspected.

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