# A complementary multi-residue LC-MSMS method for faster laboratory investigations into suspected poisoning of vertebrate wildlife & domestic animals.

SASA
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## Introduction

The Wildlife Incident Investigation Scheme (WIIS) operated in Scotland by Science and Advice for Scottish Agriculture (SASA) Pesticides & Wildlife Branch investigates incidents of suspected poisoning and contamination of vertebrate wildlife, beneficial insects, companion animals and livestock where there is evidence to suggest that pesticides or biocides may be involved.

A multi-residue method has been developed that incorporates carbosulfan, chloralose, diclofenac, metaldehyde, nitroxynil and strychnine eliminating the traditional need to use individual methods for these pesticides and chemicals associated with poisoning incidents. The procedure is straightforward with no requirement for solvent evaporation or sample clean-up.

The method has been successfully applied in the analysis of a wide range of biological samples including liver, kidney, digestive tract material, suspected bait materials and suspicious chemicals. Consequently, time spent on sample preparation has been dramatically reduced allowing much faster analytical determinations and notification to investigating agencies.

## Experimental

### Sample Extraction

Chopped liver, kidney tissue, digestive tract or bait material (up to 5 g) was dried with anhydrous sodium sulphate then tumbled for one hour with 75 ml of methanol. The extract was filtered and transferred into a 100ml volumetric flask; internal standard (carbendazim) added and made up to volume. An aliquot was filtered through a 0.45 µm PTFE syringe filter into an autosampler vial prior to analysis.

Organic chicken muscle was used as a pseudo-matrix to prepare matrix-matched reference standards for LC-MSMS analysis.

### LC-MS Analysis

Separation was performed using an Agilent 1100 series HPLC system with methanol/water (5mM ammonium acetate) gradient elution on a Hypersil Gold, 3µ column with 15 minute cycle time, flow rate 0.5 ml/min and injection volume 20µl.

Electrospray ionisation was achieved using a Waters (Micromass) Quattro Ultima Triple Quadrupole Mass Spectrometer. This was operated in polarity switching mode and the target compounds were monitored during chromatographic separation using multiple reaction monitoring (MRM) combined with time-scheduled data acquisition sequences. Alternative MRM transitions were incorporated for simultaneous confirmation of positive residues.

## Results

With the exception of carbosulfan, mean recovery values all exceeded 70 % and %CV values were all < 20 % in both matrices. Carbosulfan is known to degrade hence poorer recoveries were obtained for chicken liver and at the lowest spiking level for chicken muscle. However the method is still considered acceptable for screening purposes. Further studies to include metabolites are planned.

### Recovery Data for Spiked Muscle Tissue

| | Fortification Level 5 mg/kg | | | | | Fortification Level 1 mg/kg | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Min | Max | n | Mean | %CV | Min | Max | n | Mean | %CV |
| Carbosulfan | 67 | 93 | 6 | 84 | 11 | 71 | 88 | 6 | 82 | 9 |
| Chloralose | 73 | 84 | 6 | 80 | 6 | 83 | 105 | 6 | 93 | 8 |
| Diclofenac | 86 | 100 | 6 | 92 | 6 | 82 | 89 | 6 | 85 | 3 |
| Metaldehyde | 70 | 95 | 6 | 82 | 10 | 65 | 107 | 6 | 92 | 16 |
| Nitroxynil | 76 | 98 | 6 | 84 | 9 | 80 | 84 | 6 | 82 | 2 |
| Strychnine | 73 | 91 | 6 | 81 | 9 | 76 | 83 | 6 | 80 | 3 |

| | Fortification Level 0.2 mg/kg | | | | |
|---|---|---|---|---|---|
| | Min | Max | n | Mean | %CV |
| Carbosulfan | 9 | 48 | 6 | 27 | 49 |
| Chloralose | 79 | 97 | 6 | 88 | 10 |
| Diclofenac | 63 | 90 | 6 | 78 | 15 |
| Metaldehyde | 87 | 113 | 6 | 99 | 9 |
| Nitroxynil | 80 | 94 | 6 | 88 | 7 |
| Strychnine | 66 | 103 | 6 | 80 | 16 |

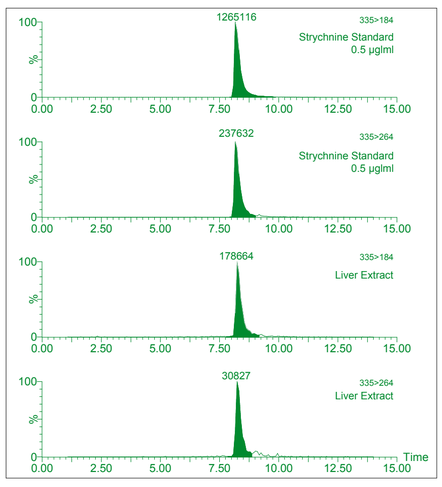
### Recovery Data for Spiked Liver Tissue

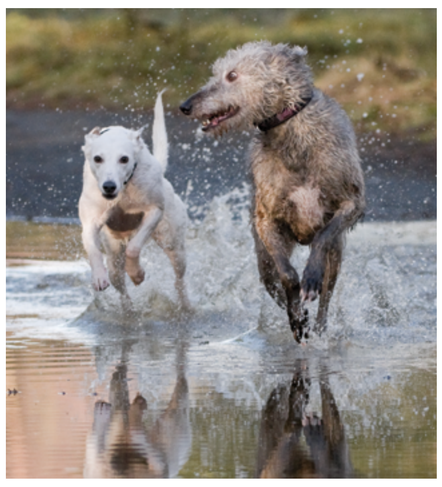
| | Fortification Level 5 mg/kg | | | | | Fortification Level 1 mg/kg | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Min | Max | n | Mean | %CV | Min | Max | n | Mean | %CV |
| Carbosulfan | 37 | 50 | 6 | 42 | 12 | 38 | 53 | 6 | 48 | 13 |
| Chloralose | 70 | 91 | 6 | 81 | 10 | 103 | 120 | 6 | 111 | 6 |
| Diclofenac | 85 | 108 | 6 | 99 | 8 | 92 | 108 | 6 | 100 | 6 |
| Metaldehyde | 93 | 117 | 6 | 105 | 10 | 73 | 126 | 6 | 99 | 18 |
| Nitroxynil | 111 | 125 | 6 | 116 | 7 | 80 | 106 | 6 | 96 | 10 |
| Strychnine | 80 | 90 | 6 | 85 | 4 | 78 | 92 | 6 | 84 | 6 |

| | Fortification Level 0.2 mg/kg | | | | |
|---|---|---|---|---|---|
| | Min | Max | n | Mean | %CV |
| Carbosulfan | 28 | 48 | 6 | 34 | 22 |
| Chloralose | 67 | 95 | 6 | 78 | 13 |
| Diclofenac | 65 | 87 | 6 | 80 | 10 |
| Metaldehyde | 94 | 141 | 6 | 112 | 15 |
| Nitroxynil | 78 | 107 | 6 | 99 | 11 |
| Strychnine | 77 | 110 | 6 | 85 | 14 |

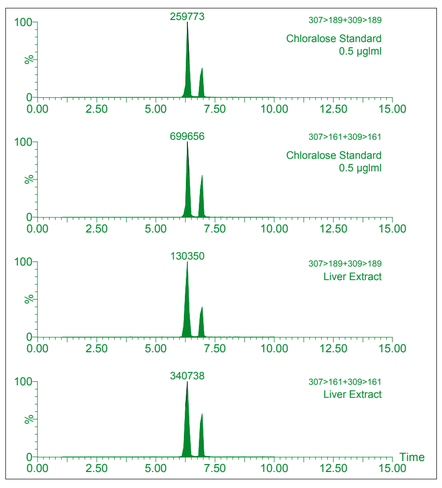
### Example of Positive Samples

*Liver tissue extract from a dog poisoned with strychnine in 2012. Residue level 1 mg/kg. (Positive ion mode)*



*Liver tissue extract from a buzzard poisoned with chloralose in 2011. Residue level 68 mg/kg. (Negative ion mode)*



## Conclusions

The method has been successfully validated for carbosulfan, chloralose, diclofenac, metaldehyde, nitroxynil and strychnine in animal tissues. The method has recently been assessed against ISO/IEC17025:2005 by United Kingdom Accreditation Service (UKAS) and added to our schedule of accreditation.

Several cases of poisoning have been successfully confirmed and early evidence provided to investigating officers.

*Red Kite (Milvus milvus)*

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