Analytical Diagnosis of Chloralose Poisoning in Scottish Wildlife Crime Investigation

Scottish Agricultural Science Agency

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Introduction:

Chloralose (fig.2) is approved for use as a pesticide only for the control of mice indoors, and under specific licensing arrangements for the control of specific birds for public health reasons. However, this substance has been the subject of extensive abuse in Scotland to illegally poison non-target animals, particularly birds of prey[1,2]. Analytical supported diagnosis of poisoning is an important element of successful crime investigation. Historically GC techniques have been applied for this purpose, but these are both lengthy and labour intensive. Here we report studies on the application of LCMS based procedures for the quantitative analysis of chloralose in animal tissues.

Figure1. Common Buzzard (Buteo buteo), a frequent casualty of the illegal use of pesticides.

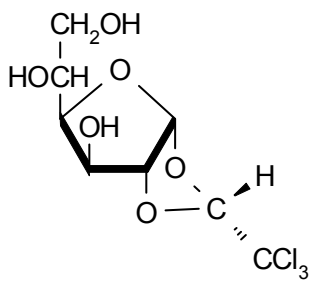
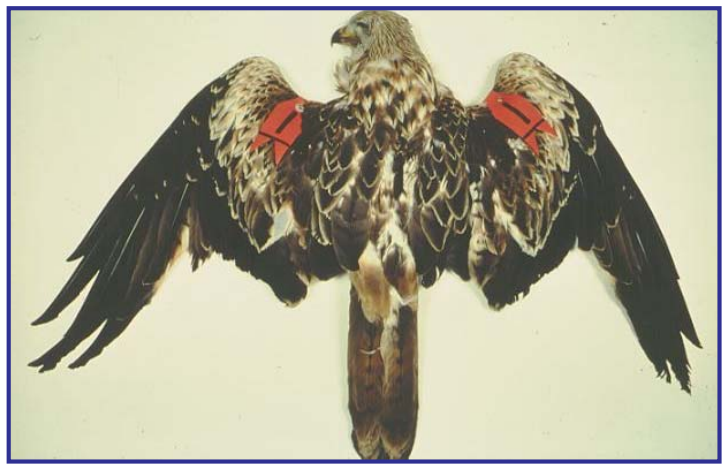


Figure 2. Chloralose [(R)-1,2-O-(2,2,2-trichloroethylidene)-α-D-glucofuranose]. $C_8H_{11}Cl_3O_6$ Rel. Mol. Wt=308. The technically pure material used in pesticide products occurs as a mixture of two isomers α and β, 85% and 15% respectively.

Figure 3. Red Kite (Milvus milvus), a poisoning victim resulting from the deliberate abuse of chloralose.



Experimental:

HPLC Method (Agilent 1100)

Column:	Hypersil C18 BDS 3µm (100 x 4.6 mm I.D.)
Mobile Phase:	Methanol / 10mM aqueous ammonium acetate pH 4.5 (55/45, v/v)
Flow-rate:	0.5 ml/min
Temperature:	35°C
Injection Volume:	20µl

Mass Spectrometry Method (Micromass Quattro Ultima)

Acquisition:	Electrospray negative ionisation Multiple reaction monitoring (MRM)
Collision gas:	Argon
Data system:	Mass Lynx 3.4

Analytical Procedure

Extraction:	Liver tissue - Maceration in methanol Digestive tract material - Tumbling in methanol
Clean-up	Not required, crude extract diluted with mobile phase and filtered
Detection	LCMSMS (transition of parent ions m/z 307 + m/z 309 → product-ion m/z 161)
Confirmation:	On-line LCMSMS (transition of parent ions m/z 307 + m/z 309 → product-ion m/z 189)

GC-based techniques usually involved solid phase extraction clean-up steps, derivatisation to the trimethylsilyl adduct, and possibly off-line GCMS confirmation.

Results and Discussion:

Basic MS parameters for chloralose were established experimentally following direct infusion of a solution in methanol into the mass spectrometer. The mass spectral data obtained using negative ionisation electrospray contained an intense molecular anion group (fig.4a). Two of these ions (m/z 307 & m/z 309) produced similar product-ion mass spectra when subjected to collision induced dissociation (fig.4b). Chloralose proved amenable to reverse-phase HPLC using simple binary mixtures of either acetonitrile or methanol with water, however the native chromophore offered poor sensitivity for spectroscopic detection. Specific HPLC isocratic elution conditions for an assay, that resolved the isomeric forms of chloralose, were developed using mass spectrometric detection. Optimised LCMS and LCMSMS methods were tested using solvent standards and fortified matrix extracts. LCMSMS offered superior sensitivity and selectivity, and was adopted for subsequent experimental work. A further refinement was to introduce post-column flow splitting into the electrospray ion-source, which reduced maintenance requirements without compromising sensitivity. Tissues extracted with methanol produced better defined peak shapes (fig.5) than those extracted with acetonitrile, although both solvents provided satisfactory extraction. The calibration obtained from standards in pure solvent (fig.6) was linear over the range 0.02 to 1.3 µg/ml. Tests on standards made up in matrix extract solutions demonstrated that calibration was essentially free from adverse effects from matrix components (fig.6). Recovery experiments on fortified tissues were performed using calibration against standards prepared in pseudo-matrix extracts from chicken liver or muscle tissue. Crude extracts were simply diluted in the HPLC mobile phase and filtered (0.45µm) prior to analysis. Recovery of chloralose from fortified tissues was acceptable for the intended purpose of the procedure (Table 1), and matched that achieved by GC-based techniques. The routine limit of detection was determined experimentally to be approximately 0.007 µg/ml, equivalent to 0.3 mg/kg in liver tissue (fig.7). This is well below the usual residue range (5-130 mg/kg) in liver tissue associated with fatal poisoning. Evidence for the confirmation of residues was provided by the simultaneous acquisition of data from an alternative structurally diagnostic precursor ion → product-ion transition i.e. m/z 307 + m/z 309 → m/z 189. No problems have been experienced in applying the procedure on a routine basis. Case work examples of the detection of chloralose residues in avian tissues are shown in Figures 8 & 9.

Table I Recovery of chloralose from fortified liver and muscle tissues

	Fortification level mg/kg	Percentage recovery	Percentage RSD (n)
Liver	5	90.7	5.6 (6)
Liver	29.3	80.2	9.4 (9)
Muscle	29.3	93	9.7 (5)
Muscle	236.6	90	6.3 (7)

Figure 4. (a) Molecular anion isotope group characteristic of chloralose, yielded in negative ionisation electrospray, (b) Product-ion mass spectrum produced following collision induced dissociation of m/z 307 precursor ion.

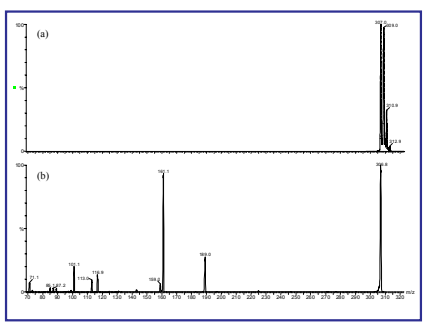


Figure 5. Reconstructed ion chromatograms illustrating effect of solvent (precursor ions m/z 307+ m/z 309 → product-ion m/z 161), for chloralose standards (1.5 ug/ml). Matrix concentrations equivalent to 0.025g/ml.

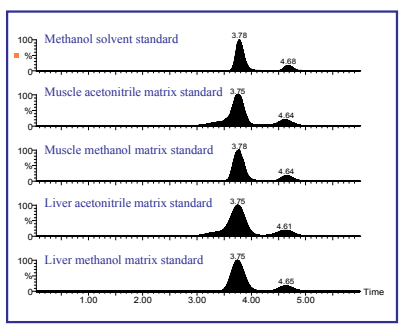


Figure 6. Calibration curves obtained for chloralose assay.
Methanol solvent standard,
Liver methanol matrix,
Liver acetonitrile matrix,
Muscle methanol matrix,
Muscle acetonitrile matrix.
Other details as in figure 5.

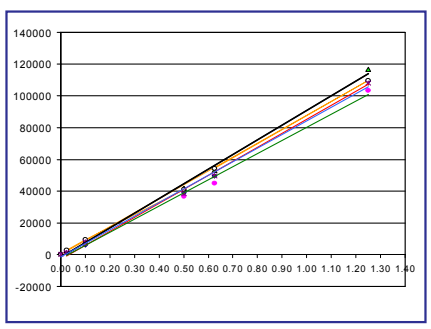


Figure 7. Determination of practical limit of detection for assay. Response from a chloralose standard at 0.00734 ug/ml prepared in muscle matrix extract (residue equivalent to 0.3 mg/kg in liver tissue).

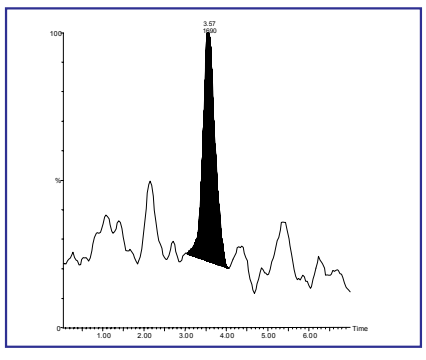


Figure 8. Detection and confirmation of a chloralose residue (1203 mg/kg) in a sample of material recovered from the gullet of a buzzard.

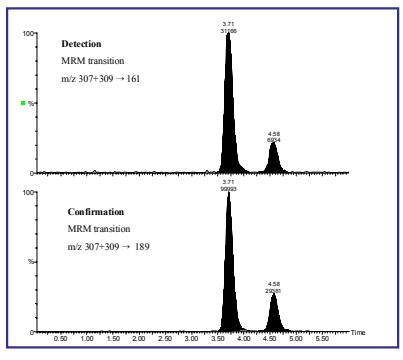
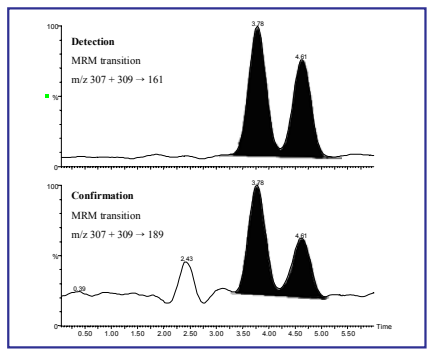


Figure 9. Detection and confirmation of a chloralose residue (5.2 mg/kg) in liver tissue from a buzzard.



Conclusions:

- A LCMSMS method has been successfully deployed to provide a specific screening tool for chloralose in wildlife incident investigation
- Appropriate confirmation data can be generated within the procedure
- Simple dilutions of crude extracts can be analysed directly
- There are no significant matrix effects on detection or calibration
- The limit of determination available meets the target specification
- Pseudo-matrix tissues can be used, sparing valuable material from relatively rare species
- Turnaround time is significantly reduced, and staff inputs decreased by ~ 60% compared to GC-based techniques

References:

1. Hamilton, GA, Ruthven, AD, Findlay, E, Hunter, K, & Lindsay, DA. Biological Conservation **21**, 315-326 (1981)
2. Hunter, K, Sharp, EA, & Melton, LM. *Pesticide Poisoning of Animals 2000: A Report of Investigations in Scotland*. SASA Report, 2001