Analytical Diagnosis of Chloralose Poisoning in Scottish Wildlife Crime Investigation


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. 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.

Experimental:

HPLC Method (Agilent 1100)
- Column: HyperC18 BDS 3 µm (100 x 4.6 mm I.D.)
- Mobile Phase: Methanol / 10 mM 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
- Collision gas: Argon
- Data system: Mass Lynx 3.4

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 (m/z 307). Two of these ions (m/z 307 & m/z 309) produced similar product-ion m/z 161 and 189. The technically pure material used in pesticide products occurs as a mixture of two isomers at 90.7% and 5.6% respectively.

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 m/z 161 and 189. The technically pure material used in pesticide products occurs as a mixture of two isomers at 90.7% and 5.6% respectively.

In contrast to GC, HPLC methods are not typically employed for the screening of illegal or medically controlled drugs. They are also usually restricted to the analysis of urine samples.

In conclusion, with the exception of GC, which is considered to lack sensitivity for the detection of chloralose residues in avian tissues, the LCMSMS method described above is capable of achieving 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.

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: