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Determination of chloralose residues in animal tissues by liquid chromatography-electrospray ionisation tandem mass spectrometry

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Abstract

A relatively rapid and specific method for the determination of chloralose in animal tissues by LCMSMS was developed. Isocratic reverse phase HPLC was used to introduce samples for electrospray negative ionisation tandem mass spectrometry. Methanol extracts were diluted to approximate the mobile phase composition, then filtered prior to analysis. Residues were identified by monitoring the multiple reaction monitoring (MRM) transitions of precursor ions mass:charge (*m*/*z*) 309 and 307 to a common *m*/*z* 161 product ion. Qualitative and quantitative confirmation data were acquired simultaneously by monitoring alternative MRM transitions. Calibration was linear over a working range of 0.025–1.3 μg/ml, and the limit of quantitation (LOQ) was 0.28 mg/kg for liver. The mean recovery was 88.5% from chicken muscle tissue fortified at 198–237 mg/kg, and ranged from 81.3 to 94.3% from liver tissue fortified at 1–52 mg/kg. The method is compared to a gas chromatography (GC) procedure previously employed.

Keyword: Chloralose

1. Introduction

The alpha-isomer of the glucose derivative chloralose (Fig. 1) induces a narcotic effect in animals that ingest the substance. It also retards metabolic activity, which can in turn cause a lowering of body temperature to a fatal level [1]. Chloralose has been used as a rodenticide in a number of countries, usually with the active ingredient being a technical preparation of the α-isomer, but such preparations usually contain ∼15% as the β-isomer. Alpha-chloralose is approved for use as a rodenticide in the United Kingdom, for the control of mice in indoor situations [2]. The substance is also approved for use in specific bird control operations, for public hygiene reasons, under licensing arrangements with the Rural Affairs Departments of central government. In both cases access to high concentration preparations, for the production of baits, is restricted to professional pest control operators. Ready-prepared bait material, containing 4% (w/w) chloralose for mouse control, has been the only formulation available to members of the general public [3].

Despite such legal controls on pesticide formulations, the substance has been the subject of abuse in the illegal poisoning of non-target animals in Scotland over many years [4,5], the most frequent victims of poisoning being birds of prey, birds of various corvid species, and companion animals such as cats and dogs. Illegal baits are most frequently prepared either using the carcasses of rabbits, hares, game birds or pigeons to target predatory or scavenging animals; or using eggs, grain or bread to target other animals. Chloralose is rapidly absorbed from the gastrointestinal tract and is mainly excreted in urine, partly as the parent compound and partly as glucuronides [6,7]. Investigation of a fatal human poisoning demonstrated the presence of chloralose residues in a wide range of tissues and body fluids [8]. In most of these sample materials the major part of the total chloralose residue was present as free chloralose. Residues of free chloralose have also been detected in the muscle, liver, kidney and brain tissues of poisoned birds from a laboratory study [9], with concentrations in liver ranging from 31 to 40 mg/kg. Examination of vertebrate animals undertaken over a number of years at SASA, indicates that a much wider range of liver concentrations is likely to be observed in field poisonings. In the majority of confirmed poisoning cases ($n = 233$), liver residues were in the range of 10–130 mg/kg

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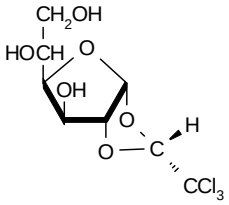

Fig. 1. Chemical structure of α-chloralose [(*R*)-1,2-*O*-(2,2,2-trichloroethylidene)-α-D-glucofuranose], MW: 309.5.

(residues in digestive tract material from the same animals were in the range of 100–10,000 mg/kg). Positive identification of the toxicant and reliable estimation of residue levels are the prime requirements in diagnosing poisoning, rather than highly precise quantification of residues.

Early analytical methods for the determination of chloralose usually involved indirect determination or colorimetry [10–14]. Subsequently, analyses by gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS) following derivatisation of chloralose or the indirect determination as chloral [6,7,15–17] were reported. A selective GC procedure, utilising electron capture detection of the trimethylsilyl (TMS)-derivative for the determinative stage, was applied for residues in animal tissues [9,18]. A modification of this latter procedure has been employed at the SASA laboratory for some time. Although the resulting method was capable of providing the necessary sensitivity and selectivity, it was relatively labour intensive and time consuming and ideally had to be supported with off-line GC-MS confirmation. Here we report the development of a method for the determination of chloralose based on the use of liquid chromatography with tandem mass spectrometric detection (LCMSMS), and compare it with the GC procedure previously employed.

2. Experimental

2.1. Materials and apparatus

Ethyl acetate (Super Purity Grade, Romil Chemicals Ltd.) was obtained from Anderson, Gibb & Wilson, Edinburgh, UK. Other solvents were supplied by Rathburn Ltd., Walkerburn, UK. Hexamethyldisilazane (HMDS), trimethylchlorosilane (TMCS), and chloralose were purchased from Sigma Ltd., Poole, UK. Extrelut cartridges and all other chemicals, which were of AnalaR Grade where available, were supplied by Merck Ltd., Loughborough, UK. Pyridine was stored over potassium hydroxide pellets. Gelman HPLC sample filters (PTFE, 0.45 μm) were obtained from Fisher Scientific Ltd., Loughborough, UK.

Tissue samples were homogenised with an Ultra-Turrax 18N tissue disperser. The GC system consisted of a Varian 3400 GC fitted with an electron capture detector, a 1093 SPI injector, and an 8200 autosampler. Xchrom software was used for data processing. GC-MS confirmations were carried out using a Finnigan GCQ Tandem ion trap system, equipped with a CTC A200S autosampler, and the resulting data processed using Xcalibur software (version 1.2). The LCMS system comprised a Micromass Quattro Ultima tandem mass spectrometer coupled to an Agilent Technologies 1100 series liquid chromatograph. The HPLC system included a quaternary pump module, an auto-injector/autosampler module, a column heater module, and a vacuum degassing module. A computer workstation, employing MassLynx software (version 3.4), was used for instrument control and data processing for the overall LCMS system.

2.2. LCMS procedure

2.2.1. Extraction

Tissue material was chopped and a portion (5 g) weighed into a large tube. Anhydrous sodium sulphate (12.5 g) was added and the sample homogenised in methanol (∼35 ml). The resulting extract was filtered, through Whatman No. 1 Filter Paper, into a volumetric flask (100 ml). The residual material was re-homogenised in methanol, and the extract filtered into the volumetric flask with washings from the homogeniser. The extract was then made up to volume with methanol.

Digestive tract material was weighed (10 g) into a conical flask, and methanol (100 ml) added. The stoppered flask was placed in an ultrasonic bath for approximately 2 min, removed and tumbled for 1 h. The resulting extract was filtered through Whatman No. 1 Filter Paper into a volumetric flask (200 ml) with washings. The extract was made up to volume with methanol.

2.2.2. LCMSMS analysis

Separations were carried out on a Hypersil C_{18} BDS 3 μm column (100 mm × 4.6 mm i.d.) using a mobile phase consisting of methanol per 10 mM aqueous ammonium acetate solution pH 4.5 (55/45, v/v) at a flow rate of 0.5 ml/min. The column was maintained at a temperature of 35 °C, and the injection volume was set to 20 μl. A proportion (15–20 μl/min) of the column effluent was diverted to the mass spectrometer using a flow splitter. The mass spectrometer was operated in electrospray negative ionisation mode (cone voltage: 35 V). Multiple reaction monitoring (MRM) data acquisition for the transitions mass:charge (*m/z*) 307 + 309 → 161, and 307 + 309 → 189, was achieved using argon as the collision gas, a collision energy of 13 eV, and a dwell time of 0.50 s. Nitrogen (set at approximately 450 l/h) was used for the nebulising and desolvation gases, and the desolvation temperature was set at 350 °C.

An intermediate standard of chloralose was prepared by diluting a methanol stock solution with HPLC mobile phase. This solution was then used to prepare matrix-matched standards in the range of 0.025–1.3 μg/ml chloralose. A matrix solution prepared from chicken muscle tissue, was added such that the final concentration of matrix was equivalent to 0.025 g/ml. Sample extracts were quantitatively diluted

in HPLC mobile phase at 40 ml/g of tissue for liver or kidney tissue, and 200 ml/g for digestive tract materials. Bracketed sequences of standards and samples were run on the LCMSMS system, and residue values interpolated from the relevant calibration curve.

2.3. GC procedure

2.3.1. Sample preparation

Liver tissue and digestive tract material were extracted essentially as described for the LCMSMS method, except that acetonitrile was used instead of methanol. Crude extracts were concentrated by rotary evaporation and made up to volume in methanol/water (approximately 1:9 v/v). Clean-up was achieved by applying an aliquot to an Extrelut SPE cartridge and after equilibration, eluting with ethyl acetate. The cleaned up extracts and working standards solutions were evaporated to dryness and the residual material derivatised with HMDS and TMCS in pyridine to form the TMS-adducts. Final solutions were made up in hexane.

2.3.2. GC analysis and GC-MS confirmation

Separations were carried out on a DB-608 capillary column (30 m × 0.32 mm i.d., 0.5 μm film thickness). Injection (1 μl) was in splitless mode, with an injector programme 50 °C for 1 min, up to 200 °C at 140 °C/min. The column oven programme was 50 °C for 2 min, up to 100 °C at 25 °C/min, up to 240 °C at 10 °C/min (held for 11.7 min). Nitrogen carrier gas was supplied at a flow rate of 5 ml/min. The electron capture detector was operated at 300 °C with a nitrogen make up gas at 30 ml/min. Bracketed sequences of standards and samples were run on the GC system and residue values interpolated from the relevant calibration curve.

Residues were confirmed by re-running appropriate extracts on a bench-top GC-MS system and acquiring full scan EI data. Separations were carried out using a DB-5 column (30 m × 0.025 mm i.d., 0.25 μm film thickness) fitted with a 1 m retention gap (deactivated fused silica tubing). Sample injection was made in splitless mode at 250 °C. The column oven programme was 40 °C for 2 min, up to 200 °C at 25 °C/min, then up to 250 °C at 10 °C/min. Helium carrier gas was supplied at a constant linear velocity of 40 cm/min. The transfer line was maintained at 275 °C, and the ion source operated at 180 °C.

3. Results and discussion

HPLC has been evaluated within the SASA laboratory as a tool to estimate the purity of batches of technically pure chloralose and other powders seized by officials investigating illegal poisoning incidents. Simple isocratic conditions in reverse phase chromatography, using a C_{18} column and a mobile phase of acetonitrile/water, permit the resolution of the isomeric forms of chloralose. The compound has an extremely poor chromophore for UV-spectroscopic detection, the optimum detection wavelength being 192 nm. Hence, although conventional HPLC is suitable for formulation analysis, it is never likely to form the basis of a sensitive and selective residue method. The use of mass detection in conjunction with HPLC has provided highly specific methods in a number of areas of analysis, and was considered to offer significant efficiency gains in relation to the determination of chloralose.

MS detection parameters were established and optimised by the direct infusion of a solution of chloralose in methanol into the mass spectrometer. The mass spectrum yielded using negative electrospray ionisation contained an intense $[M - H]^-$ anion isotope group (Fig. 2a). Two of these ions, at *m/z* 307 and 309, produced similar product-ion mass spectra when subjected to collision-induced dissociation using argon as the collision gas (Fig. 2b), the most abundant product ions being *m/z* 161 and 189. These collision-induced transitions were interpreted as corresponding to the loss of $C_2H^{35}Cl_3O$ and $CH^{35}Cl_3$ neutral moieties from the molecular *m/z* 307 anion, and to the loss of $C_2H^{35}Cl_2{}^{37}ClO$ and $CH^{35}Cl_2{}^{37}Cl$ from the molecular isotope anion *m/z* 309.

Candidate HPLC conditions for a chloralose assay were tested using LCMS monitoring. Elution from a 3 μm C_{18} reverse phase column was readily controlled by varying the organic modifier concentration in a simple binary mobile phase consisting of methanol per 10 mM aqueous ammonium acetate solution (pH 4.5). The optimum conditions permitting the resolution of the isomeric forms of chloralose were established (although this was not necessarily a pre-requisite for the application in question). Using these conditions, the linearity of responses in both LCMS and LCMSMS modes were tested using solvent standards of varying concentration and with matrix-matched standards, using blank liver and muscle tissue extracts prepared from chicken. Crude extracts were diluted in mobile phase solution and subject only to filtration through HPLC sample filters. These experiments demonstrated the superior selectivity and relative sensitivity available in the LCMSMS mode, which was then adopted as the basis for further development.

Chloralose is readily soluble in diethyl ether, alcohols, and acetic acid [1]. Diethyl ether and diethyl ether/chloroform mixtures have been used to extract residues from animal tissues or physiological fluids [6–9], however, they have the disadvantage of co-extracting a significant proportion of lipid material. In-house development of the GC-ECD method had demonstrated that acetonitrile was a suitable extractant. Given that one of the aims of investigating mass detection methodologies was to develop a simple procedure, both acetonitrile and methanol were tested as potential extractants. Ideally these solvents would extract less lipid material than other solvents, perhaps eliminating the requirement for a sample clean-up stage, and facilitate minimal onward sample manipulation prior to final determination. Extraction of chloralose from fortified liver tissue samples and muscle tissue samples was tested, using LCMSMS monitoring of the

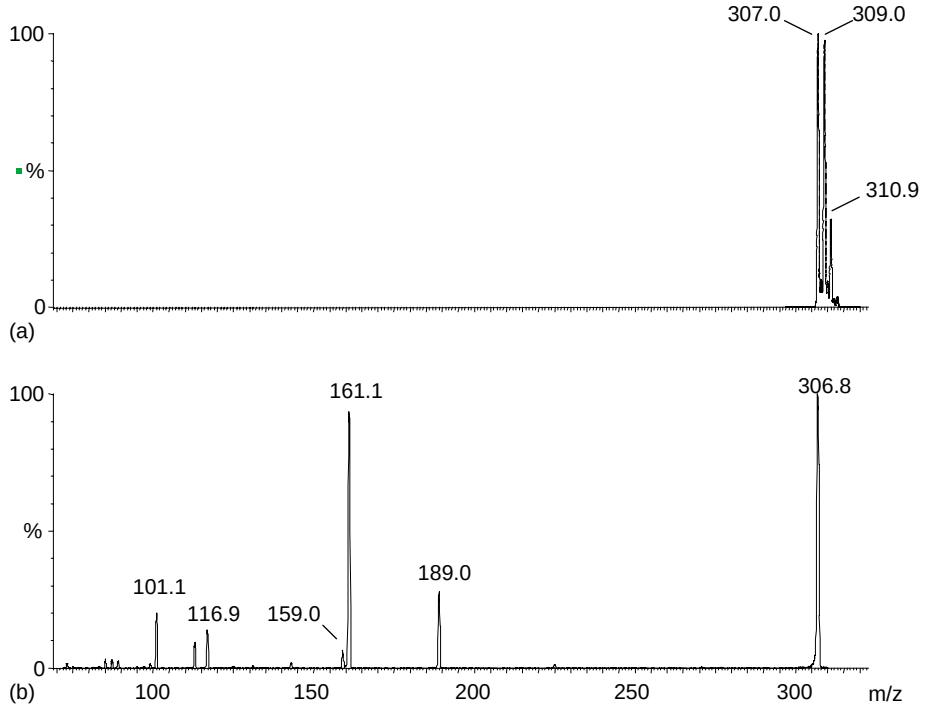

Fig. 2. Mass spectra generated from infusion of a chloralose solution in methanol: (a) molecular anion isotope group yielded in negative ionisation electrospray mode, (b) product ion spectrum produced following collision induced dissociation of *m*/*z* 307 precursor ion.

transition of the precursor ion *m*/*z* 307 to the product ion *m*/*z* 161. There was little difference in the performance of either solvent used to extract chloralose. Scrutiny of the relevant reconstructed ion chromatograms indicated that methanol extracts yielded peak shapes that were better defined than those obtained with acetonitrile extracts, where broad leading fronts of the same width as the main α-chloralose peak were exhibited. The superior peak definition and marginally better recovery obtained led to the selection of methanol as the extractant. Preliminary studies were conducted to evaluate the effect of various matrix solutions on the linearity and effective range of the calibration curve for chloralose. At the final working dilutions of the matrices tested, there was a limited enhancement (5–10%) of the LCMSMS response observed for chloralose in methanol/matrix extracts compared with solvent standards.

Detection parameters for the method were optimised to use the sum of the responses for both the *m*/*z* 307 and 309 precursor ion transitions to the product ion *m*/*z* 161 for screening purposes. Transitions from both precursor ions to the *m*/*z* 189 product ion were monitored to provide confirmatory data. Post-column flow splitting (reducing the effective flow-rate to the mass spectrometer to 15–20 μl/min) was introduced, to minimise maintenance requirements for the ion-source. This was accomplished with no compromise to the sensitivity of the assay. Chicken muscle tissue was chosen as a universal pseudo-matrix material for matrix-matched calibration standards because of the limited differences in ion enhancement effects between tissue types (<5%), and because of its ready availability. The responses of matrix-matched calibration standards containing 0.025–4.2 μg/ml chloralose were measured. A linear calibration line was obtained over the range 0.025–1.3 μg/ml, thereafter the response per unit concentration declined. Linearity over the lower concentration range was tested for lack of fit by plotting the residual of response from the linear regression [19]. The residuals for duplicate measurements gave a random distribution close to zero. Residuals obtained using higher concentrations showed a systematic and significant deviation from zero. Evaluation of these data suggested that divergence from linearity was likely at concentrations above 1.5 μg ml. Accordingly the maximum concentration level used for calibration purposes was conservatively set at 1.3 μg/ml.

Recoveries of chloralose from fortified chicken liver and muscle tissues were acceptable. The mean recovery ranged from 81.3 to 94.3% for liver tissue, and from 87.2 to 88.5% for muscle tissue. These values compared favourably with those achieved using the in-house GC-ECD method (Table 1). The lowest effective concentration that could be reliably measured was assessed by testing decreasing chloralose concentration levels in fortified liver tissues ($n = 6$). The limit of quantitation was defined as the lowest concentration at which a minimum mean recovery of 65%, repeatability of ≤20%, and a signal to noise ratio ≥3 could be achieved. The LOQ was experimentally determined as 0.28 mg/kg for liver tissue, where a mean recovery of 68.4%, repeatability of 13.2%, and an average signal to noise ratio of 3.9 were achieved. At this level only the major alpha-chloralose component from the test

Table 1
Recoveries of chloralose from fortified tissues

Tissues	Fortification (mg/kg)	Percentage recovery		CV (%)	*n*
		Mean	Range		
Gas chromatography					
Muscle	200	81.3	72–110	11.6	23
Liver	25	80.2	73–91	6.4	16
Liver	5	75.9	70–88	8.0	9
LCMSMS					
Muscle	198–237	88.5	77–98	7.5	22
Muscle	29.3	87.2	73–102	10.7	10
Liver	52.4	84.1	80–90	4.4	6
Liver	29.3	81.3	72–93	7.1	16
Liver	22.0	85.9	82–89	3.1	6
Liver	5.0	92.4	85–107	7.0	13
Liver	1.0	94.3	88–102	5.9	6

At each level, samples were fortified with chloralose at least 10 min prior to extraction. Samples for residue analysis were prepared as described in the text.

Table 2
Residues (mg/kg) of chloralose, detected by LCMSMS, in the tissues of poisoned animals

Species	Digestive tract material		Liver tissue
Cat	Stomach content material	2,850	81
Red kite	Gullet content material	5,650	113
Buzzard	Gullet content material	1,420	5.2
Buzzard	Gullet content material	4,020	Not available
Buzzard	Gullet content material	101	1.1
Buzzard	Gullet content material	2,090	182
Buzzard	Gullet content material	2,380	35
Sparrowhawk	Stomach content material	1,405	124
Buzzard	Gullet content material	730	44
Rook	Gizzard content material	2,040	174
Rook	Gizzard content material	206	20
Rook	Gizzard content material	2,500	104
Crow	Not available	–	30
Buzzard	Gullet content material	4,760	49
White-tailed eagle	Gullet content material	482	23
Red kite	Gullet content material	2,500	87
Red kite	Gullet content material	1,220	58
Red kite	Gullet content material	630	500
Buzzard	Gullet content material	7,360	107
Buzzard	Gullet content material	11,600	155

Quoted as sum of α- and β-isomers.

substance used could be determined. The LOQ was well below the initial minimum target value for the assay and compared favourable with that achieved for human urine and plasma using LCMS [8]. Sample dilutions used routinely in the assay were set to maximise the chance of first time compliance within the calibration range. Lower limits of quantitation may well be achievable by reducing the effective dilution in sample preparation. Intra-day and inter-day precisions were estimated from analyses of sets of liver tissue samples ($n = 6$), each fortified at one of four concentration levels, conducted in a single day (intra-) and over several days (inter-). The intra-batch precision varied from 2% at higher concentrations (20–50 mg/kg), to 5.1% at lower concentrations (1–5 mg/kg). The inter-batch precision ranged similarly according to concentration from 4.5 to 8.1%.

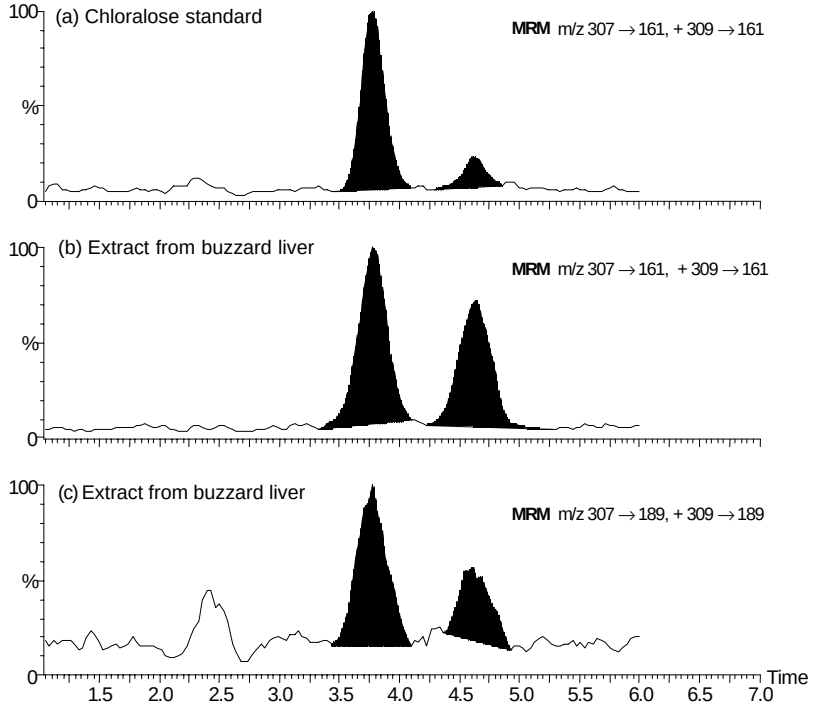

Fig. 3. Detection and confirmation of a chloralose residue in the liver tissue of a common buzzard (*Buteo buteo*): (a) chloralose standard, (b) detection of residue (=5.2 mg/kg), (c) confirmation.

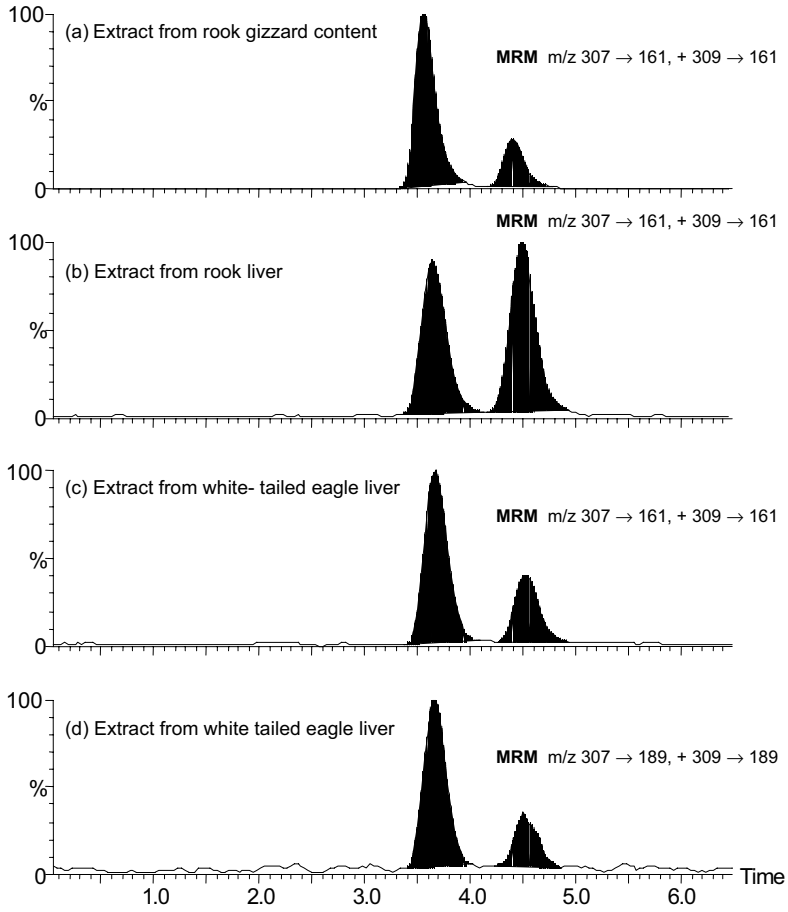

Fig. 4. Detection of chloralose in tissues from a rook (*Corvus frugilegus*) and a white-tailed eagle (*Haliacetus albicilla*): (a) gizzard content of rook, residue = 206 mg/kg; (b) liver tissue from rook, residue = 19.7 mg/kg; (c) liver tissue from white-tailed eagle, residue = 22.6 mg/kg; (d) confirmation of residue in liver tissue from white-tailed eagle.

Practical deployment of the method has demonstrated the powerful discrimination that LC-MSMS offers for screening animal tissues for chloralose in poisoning investigations. To date, residues in the range of 1–11,600 mg/kg have been detected in tissues from a variety of birds of prey and mammalian species (Table 2). In all cases it has been possible to provide additional evidence to confirm the identity and magnitude of the residue using the alternative MSMS transitions. There has been no indication of interference from any matrix related components. The application of the assay to material from poisoning incidents is shown in Figs. 3 and 4. The former illustrates detection and confirmation at the lower end of the anticipated range of interest, and the latter demonstrates the changes in isomeric distribution sometimes associated with some residues detected in liver tissue or other materials. It had been possible to apply the GC-ECD method to occasional blood or faecal samples from birds that might have suffered sub-lethal exposure to chloralose. Although no such samples types have been submitted for testing during initial deployment of the LC-MSMS method, there is no reason to suspect that they would not be amenable to the method.

Direct analyst inputs for a standard batch of samples was estimated to be approximately 4 h, with completion being achievable within a single working day. This represented a considerable efficiency saving over the in-house GC-ECD method where comparable direct analyst inputs were estimated to be approximately 12 h, with completion likely to be achieved on the third working day.

4. Conclusions

LC-MSMS can provide a simple and relatively quick analysis of the toxicant chloralose in animal tissues. Sample preparation can be limited to solvent extraction, dilution in HPLC mobile phase, and sample filtration. The selectivity of the determination eliminates the need for any sample clean-up, and the sensitivity achievable is more than satisfactory for the diagnosis of poisoning (or to confirm exposure) in animals. Additional confirmatory data can be acquired simultaneously. There is scope for lowering the limit of determination, should this prove necessary, by adjustment of the sample dilution employed. Significant ef-

ficiency gains (∼65%) are made over the previous methodology employed for this purpose, by eliminating the need for clean-up, derivatisation, off-line confirmation, as well as time consuming concentration steps involving solvent evaporation.

References

[1] C.D.S. Tomlin (Ed.), The Pesticide Manual, British Crop Protection Council, 1997.
[2] Pesticides 2001, Your Guide to Approved Pesticides, PSD/HSE, The Stationary Office, 2001.
[3] Pesticides 2000, Your Guide to Approved Pesticides, PSD/HSE, The Stationary Office, 2000.
[4] G.A. Hamilton, A.D. Ruthven, E. Findlay, K. Hunter, D.A. Lindsay, Biol. Conserv. 21 (1984) 315.
[5] K. Hunter, E.A. Sharp, L.M. Melton, Pesticide Poisoning of Animals 2000: a Report of Investigations in Scotland, SASA Report, Edinburgh, UK, 2001.
[6] P. Daenens, N. Bruneel, M. Van Boven, in: A. Kovatis (Ed.), Toxicological Aspects. Proceedings of the Ninth International Congress of the European Association of Poison Control Centres, Salonika, Greece, 1980.
[7] S. Savin, B. Cartigny, N. Azaroual, L. Humbert, M. Imbenotte, D. Tsouria, G. Vermeersch, M. Lhermitte, J. Anal. Toxicol. 27 (2003) 156.
[8] A. Tracqui, S. Doray, B. Ludes, Toxicorama 11 (1999) 125.
[9] E.M. Odam, H.P. Wardall, S. Bailey, E. Findlay, Analyst 109 (1984) 1335.
[10] A. Lespagnol, J. Paris, R. Merville, Bull. Soc. Chim. Biol. Fr. 24 (1942) 117.
[11] H. Griffon, Ann. Pharm. Fr. 6 (1948) 165.
[12] M. Fleury, J. Jolly-Colin, Ann. Pharm. Fr. 8 (1950) 15.
[13] L. Hartman, Analyst 81 (1956) 67.
[14] S. Bailey, Analyst 97 (1972) 676.
[15] N.C. Jain, H.L. Kaplan, R.B. Forney, F.W. Hughes, J. Forensic Sci. 12 (1967) 497.
[16] C. C Sweeley, R. Bentley, M. Kakita, W.W.J. Wells, J. Am. Chem. Soc. 85 (1963) 2497.
[17] P. Kintz, S. Doray, V. Cirimele, B. Ludes, Forensic Sci. Int. 104 (1999) 59.
[18] P. Brown, A. Charlton, M. Cuthbert, L. Barnett, L. Ross, M. Green, L. Gillies, K. Shaw, M. Fletcher, J. Chromatogr. B 754 (1996) 463.
[19] Analytical Methods Committee, The Royal Society of Chemistry, R. Soc. Chem. Anal. 119 (1994) 2363.