Development of an LC/MS Method for the Analysis of Rodenticides

Application Note

Environmental

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Introduction

Rodents such as rats and mice must be controlled because they destroy food supplies and serve as vector hosts for human diseases such as hantavirus. Although individual animals or small groups can be removed by trapping, rodenticides are frequently used in rodent control. Most rodenticides are also toxic to humans and domesticated animals such as dogs.

Anticoagulant poisons work by interfering with the blood clotting mechanism. After repeated ingestion of relatively small doses, a lethal dose accumulates and causes death due to internal bleeding. Anticoagulant poisoning can also cause spontaneous bleeding from the nose, gums and the gastrointestinal and urinary tracts.

In this work, an LC/MS method was developed to monitor several coumarin rodenticides in complex matrices.

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Experimental

All experiments were done on an Agilent 1100 Series LC/MSD system that was comprised of a binary pump, vacuum degasser, autosampler, thermostatted column compartment with column-switching valve, diode-array detector, and an LC/MSD. The LC/MSD was used with either the electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) source. Complete system control and data evaluation was done on the Agilent ChemStation for LC/MS.

Reagent grade chemicals and HPLC grade solvents were used for preparing mobile phases and standards.

The compounds used for this study are all classified as coumarin rodenticides due to the common coumarin moiety in the structures (Figure 1). Method development included assessing ionization technique (ESI and APCI), ionization polarity and fragmentor voltage to determine the best conditions for the three analytes.

In order to test the performance of the method, spiked sausage extract and dog stomach extract were analyzed. The samples were spiked at either 0.5 or 5 ppm, then subjected to a general extraction and sample cleanup.[1] The spiked samples were extracted in acetone for four hours. The acetone was then evaporated to a volume of approximately 50 ml and extracted with 100 ml of methylene chloride. The acetone layer was dried with anhydrous sodium sulfate, filtered, and evaporated to dryness. After redissolving in 2 ml of acetone, the extract was applied to a column containing 15 g silica gel and 1 g charcoal. After elution with methylene chloride/benzene/acetone (10/2/2 v/v), the eluate was evaporated to dryness and redissolved in 5 ml of methanol. The sample extracts were then analyzed by ESI-LC/MS.

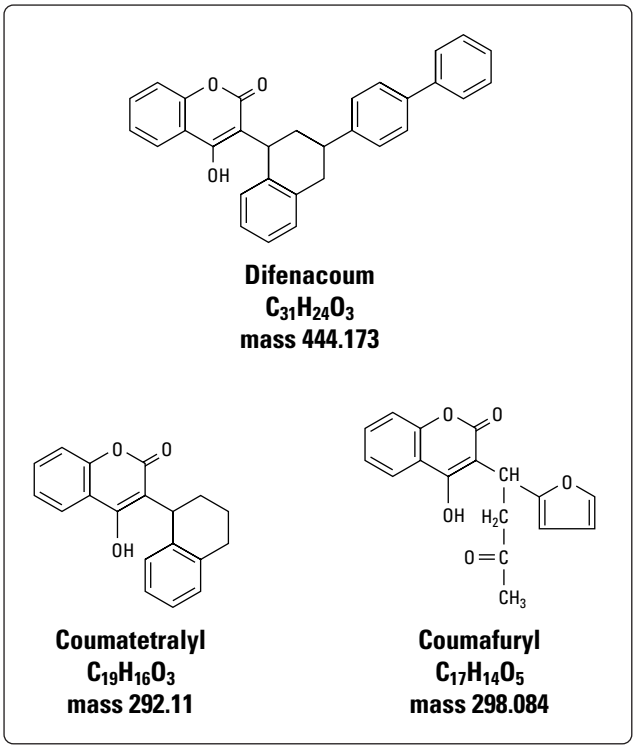

Figure 1. Coumarin rodenticides.

Results and Discussion

Both coumafuryl and coumatetralyl showed poor response in positive mode ESI compared to negative mode ESI (Figure 2). The positive mode response for these compounds was better in APCI than ESI, but the negative mode APCI response for coumatetralyl was weak compared to ESI. Based on these results, ESI negative mode was selected for the optimized method.

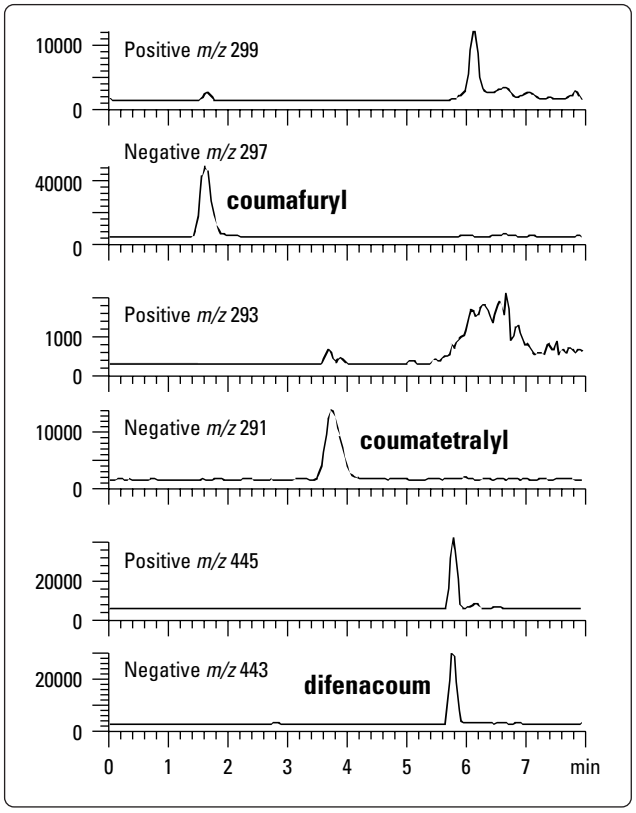

Figure 2. Comparison of positive and negative ionization modes for electrospray LC/MS.

ANALYSIS METHOD

Chromatographic Conditions

Column:	150 × 2.1 mm Zorbax® XDB-C18, 5 µm (p/n 993700-902)
Mobile phase:	A = 2 mM ammonium acetate in water B = methanol
Gradient:	start with 30% B at 2 min 50% B at 4 min 100% B
Flow rate:	0.4 ml/min from 0 to 2 min, then 0.5 ml/min
Column temp:	50°C
Injection vol:	2 µl
Diode-array detector:	signal: 280, 16 nm; reference: 550, 100 nm

ESI-MS Conditions

Source:	ESI
Drying gas flow:	10 l/min
Nebulizer:	40 psig
Drying gas temp:	350°C
Vcap:	2500 V (positive and negative)
Stepsize:	0.1
Peakwidth:	0.2 min
Time filter:	On
Scan:	*m/z* 150–500
SIM ions (negative mode):	296, 297, 298 Coumafuryl 290, 291, 292 Coumatetralyl 442, 443, 444 Difenacoum
Fragmentor:	variable 180 V (50–275) 100 V (280) 120 V (400)

Typically, positive ions will fragment more easily than negative ions, so the fragmentor voltage was optimized for both modes. For example, difenacoum showed extensive fragmentation at 160 V in positive ion mode but no fragmentation at the same voltage in negative ion mode. Optimized fragmentor voltages were used for each ion in the final method.

For samples extracted from a complex matrix, the deprotonated molecule as well as the ions 1 *m/z* above and below can be monitored to check for coeluting artifacts. In the final method, this strategy was employed so a set of three ions was used for each analyte. The ions for each analyte were time-programmed as a SIM ion group in order to maximize the dwell time and thus maintain maximum sensitivity. Figure 3 shows the results for the spiked sausage extract. For this analysis, data was collected in scan mode because the coumafuryl and difenacoum were present in sufficient concentration. The mass spectra from the spiked analytes show good quality at the 5 ppm level. The dog stomach extract was spiked at a lower level (0.5 ppm) so SIM analysis was done for this sample (Figure 4). Only the spiked analyte, coumafuryl, shows a significant signal.

This work demonstrates that anticoagulant rodenticides can be detected by APCI and ESI in both positive and negative ion modes. Negative mode ESI-LC/MS was found to be the best choice for the three target compounds. This LC/MS method was capable of detecting the rodenticides in complex matrices.

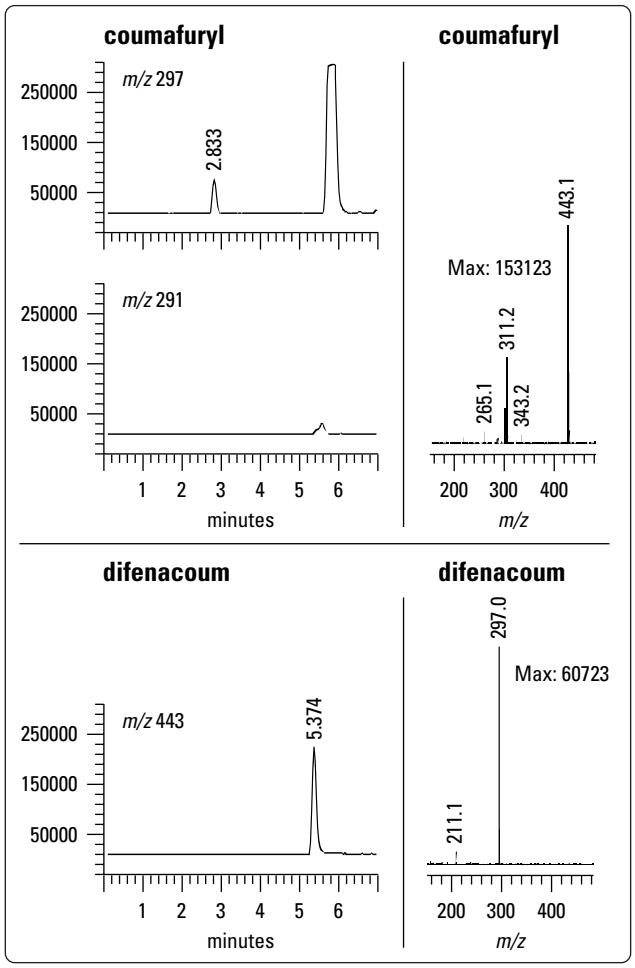

Figure 3. Extracted ion chromatograms (left) and mass spectra (right) from the analysis of sausage extract spiked with 5 ppm of coumafuryl and difenacoum. Data was collected in negative ion using scan mode.

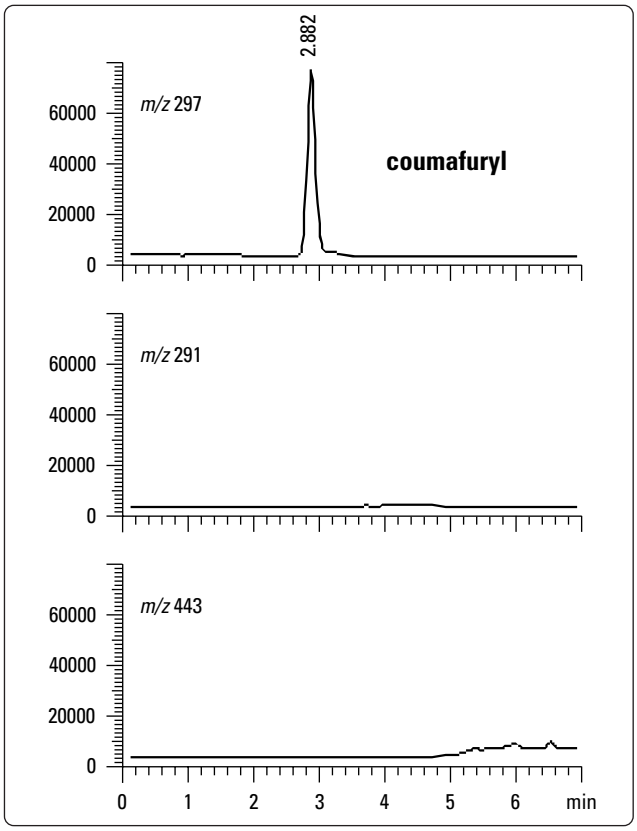

Figure 4. Extracted ion chromatograms from the analysis of dog stomach extract spiked with 0.5 ppm of coumafuryl. Data was collected in negative ion using SIM mode.

References

1. Faucannet, V., Pouliquen, H., and Pinault, L., *Journal of Analytical Toxicology*, **27**, 1997.

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