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Application Note 01510

LC/MS/MS Analysis of Two Rodenticides on the Varian 1200L Triple Quadrupole LC/MS/MS

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Introduction

Currently, there are numerous types of domestic rodenticides in use. These poisons include many highly effective compounds, such as fluoroacetamide, sodium fluoroacetate and phosazetim; they also contribute to an increasing number of incidents of accidental swallowing, poisoning, suicide or second-hand poisoning in humans. Indadione type and coumarin type rodenticides (e.g. brodifacoum, bromadiolone, etc.) are also highly poisonous, as they interfere with the synthesis of prothrombin in the liver. Although particular rodenticides are promoted due to their relative safety, accidental swallowing and poisoning in humans does still occur. For this reason, it is imperative to develop highly effective and accurate methods to detect these compounds for diagnosis in biological samples in case poisoning occurs.

This method uses the Varian1200L Triple Quadrupole LC/MS/MS to detect and quantitate the sulfuric rodenticide ANTU (N-(1-naphthyl)thiourea) and the indandione rodenticide (diphacinone) down to a concentration of 1 ppb.

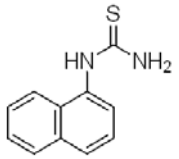

Figure 1. Molecular structure of the rodenticide ANTU (N-(1-naphthyl)thiourea).

Currently, the most common analytical methods used for rodenticide detection include HPLC and GC. These methods are simple in application but have limited evidential persuasive power. The LC/MS/MS method outlined herein is sensitive, qualitative and quantitative, making it a powerful tool for solving cases in the field of judicial public safety.

Instrumentation

- Varian 1200L Triple Quadrupole LC/MS/MS with ESI source
- Varian Prostar™ 210 Binary Solvent Delivery Modules
- Varian Prostar™ 410 AutoSampler

Materials & Reagents

10 µg/mL liquid standards for ANTU (N-(naphthyl)thiourea) and diphacinone were purchased from Sigma and Chem Service, respectively. All solvents used were chromatographically pure and formic acid was analytically pure.

Sample Preparation

Standard solutions of ANTU and diphacinone were used to prepare a standard calibration set of 1-125 ppb for ANTU, and 0.2-50 ppb for diphacinone.

HPLC Conditions

Column: Inertsil ODS-3, 5 µm, 50 x 2.0 mm ID (Recommended Varian Pursuit™ C18 Part No. A3000050X020)

Solvent A: Water with 0.1% formic acid

Solvent B: Methanol

LC Program:

Time (min:sec)	%A	%B	Flow (µL/min)
00:00	25	75	250
05:00	25	75	250

MS Parameters

Ionization Mode: ESI Negative

API Drying Gas: 20 psi at 300 °C

API Nebulizing Gas: 52 psi

Table 1. MS Transitions.

Analyte	Transition	Collision Voltage
ANTU	201 > 142	18 V
	201 > 167	10.5 V
Diphacinone	339 > 183	36 V
	339 > 67	12 V

Results & Discussion

In order to optimize the MS conditions for both of the standards, each was infused. The "MS/MS Breakdown" feature of the MS Workstation software automatically found the most prominent breakdown product ions with the corresponding collision energy. Figure 2 shows the breakdown curve for ANTU. On this graph, the most abundant product ion is shown as *m/z* 167, so this was the ion used for quantitation. For diphacinone, the most abundant product ion used for quantitation was *m/z* 183.

Figure 3 shows the product ion spectrum for ANTU, resulting from the breakdown of precursor ion *m/z* 201. While *m/z* 167 is the most abundant product ion, other product ions are observed at *m/z* 142 and 58. Figure 4 shows the chromatographic separation of diphacinone and ANTU.

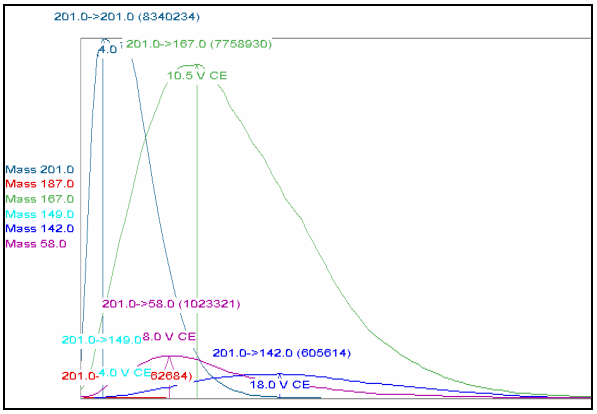

Figure 2. MS/MS breakdown curve for ANTU.

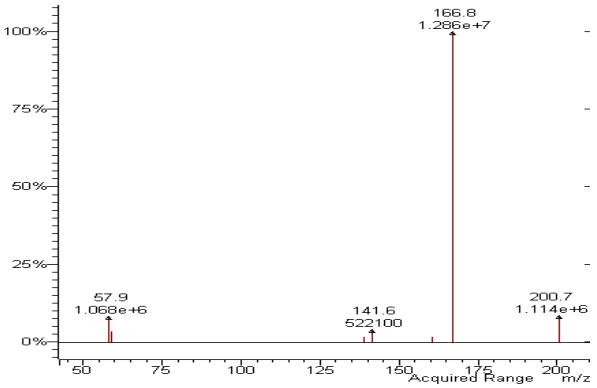

Figure 3. The MS/MS product ion spectrum for ANTU. The parent ion is *m/z* 201, and the most abundant product ion is *m/z* 167.

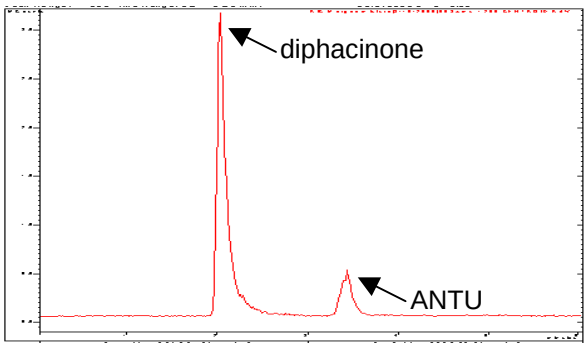

Figure 4. Total ion chromatogram (TIC) of diphacinone and ANTU.

The sensitivity of the Varian 1200L is demonstrated by the chromatogram of diphacinone at 0.2 ppb, shown in Figure 5. Although this is a very low concentration of sample, the chromatographic peak still has a signal-to-noise ratio of 83.

Calibration curves were found to be linear from 0.2 to 50 ppb for diphacinone, and 1 to 125 ppb for ANTU (Figures 6 and 7, respectively). The r^2 value for diphacinone is 0.999 and the r^2 value for the ANTU calibration curve is 0.998.

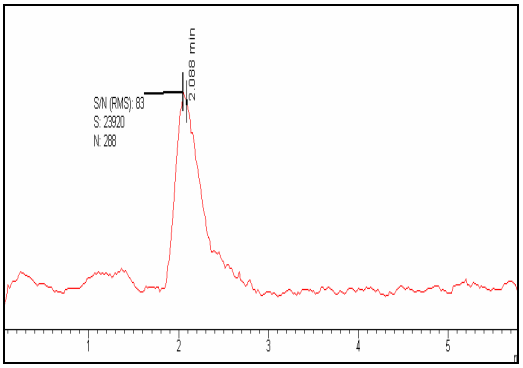

Figure 5. Chromatogram of diphacinone at 0.2 ppb. The S/N ratio is 83.

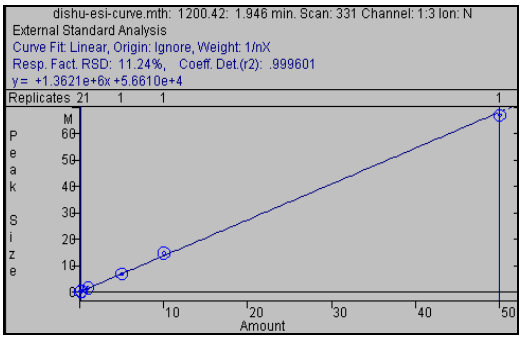

Figure 6. Calibration curve for diphacinone from 0.2 to 50 ppb. The r^2 value is 0.999.

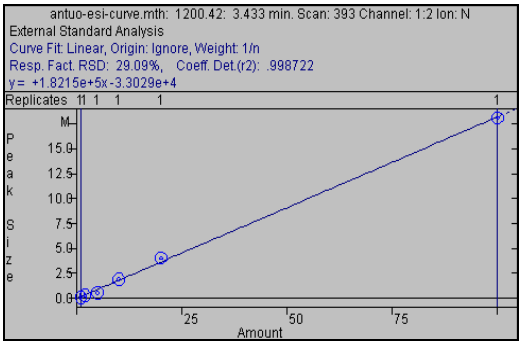

Figure 7. Calibration curve for ANTU from 1 to 125 ppb. The r^2 value is 0.998.

Conclusion

This LC/MS/MS method detects and quantitates two common rodenticides down to low ppb levels. For quantitation, this method is both sensitive and accurate, providing the confidence of MS/MS identification. In addition, the Varian 1200L is capable of qualitative analysis of unknowns and automated optimization for known and unknown compounds.

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