

Wildlife Protection: Pesticide Poisoning Identified Using a UPLC-ToF

Screening Approach

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INTRODUCTION

In the food safety and environmental monitoring arenas the use of ToF screening approaches has steadily increased. Screening of food and environmental matrices for pesticide contamination is especially important because the use of pesticides has escalated, in an effort to meet global food demands. Pesticides, which were applied to crops, can potentially leech into soil and surface waters and may cause harm to plants and wildlife.



Figure 1. Red Kite (*milvus milvus*) in flight

Birds of prey, such as Red Kite (*milvus milvus*) shown in Figure 1, are particularly susceptible to poisoning by pesticides because they frequently feed on the insects, grubs, rodents and small mammals that are killed by pesticide compounds. These birds also fall victim to intentional poisoning, when people spike pesticides into carrion, either to kill animals such as foxes and crows, or to target the birds themselves.^{1, 2}

If red kite carcasses are discovered, for example by members of the public, RSPB staff or wildlife crime officers, and pesticide poisoning is suspected, they are often brought to SASA (Science and Advice for Scottish Agriculture—a division of the Scottish government). Here, samples are analysed in order to determine if pesticide residues are present in or on the test specimen and therefore implicated in the cause of death of the animal.

In this study we describe the use of Waters ACQUITY UPLC coupled with the newly released Xevo G2 QToF (Figure 2) to screen samples, supplied by SASA, from the gullet of a red kite carcass suspected of poisoning by pesticides and identify which pesticides were used. Data were processed using POSI±IVE software and screened using a targeted list of approximately 600 common pesticides. Further confirmation of the identity of the poisons was achieved using MassFragment software.



Figure 2. ACQUITY UPLC system coupled to Xevo G2 QToF

METHODS

Sample preparation

- 2 g Red Kite gullet contents extracted into 5 mL ethyl acetate
- 1 mL aliquot of ethyl acetate extract solvent exchanged into methanol and diluted to 400 µL
- Sample passed through 0.2 µm syringe filter with no further cleanup prior to injection

UPLC Conditions

- Column: ACQUITY BEH C₁₈, 1.7 µm, 2.1 x 50 mm @ 45 °C
- Flow rate: 0.6 mL/min
- Injection volume: 3.0 µL
- Mobile phase A: 10 mL of 1 M aqueous ammonium acetate solution and 990 mL water
- Mobile phase B: 10 mL of 1 M aqueous ammonium acetate solution and 990 mL methanol
- Generic UPLC screening gradient given in Table 1

Xevo G2 QToF Conditions

- Ionisation mode: ESI+
- Analyser: Resolution mode, >20K resolution
- Scan time: 0.1 s
- Capillary voltage: 1.0 kV
- Source temperature: 120 °C
- Desolvation temperature: 550 °C
- Mass range m/z 50— m/z 1000
- MS^E low energy: 6.0
- MS^E high energy: 25.0—35.0
- LockSpray compound: Leucine enkephaline
- LockSpray masses: m/z 556.2771 and m/z 278.1141

Data Acquisition and Processing

- Data were acquired using MassLynx 4.1
- Data were processed using POSI±IVE software, along with the MassFragment tool

Time (min)	Flow rate (mL/min)	%A	%B	Curve
Initial	0.60	98	2	0
0.10	0.60	98	2	6
3.75	0.60	1	99	6
4.25	0.60	1	99	6
4.26	0.60	98	2	11
5.00	0.60	98	2	6

Table 1. Generic UPLC screening gradient

RESULTS & DISCUSSION

The generic screening method, given in Table 1 above, was used to screen extracted gullet contents of the red kite for pesticide residues. The low energy MS^E precursor ion total ion chromatogram (TIC) and the high energy MS^E fragment ion TIC acquired from this screening analysis are shown in Figure 3.

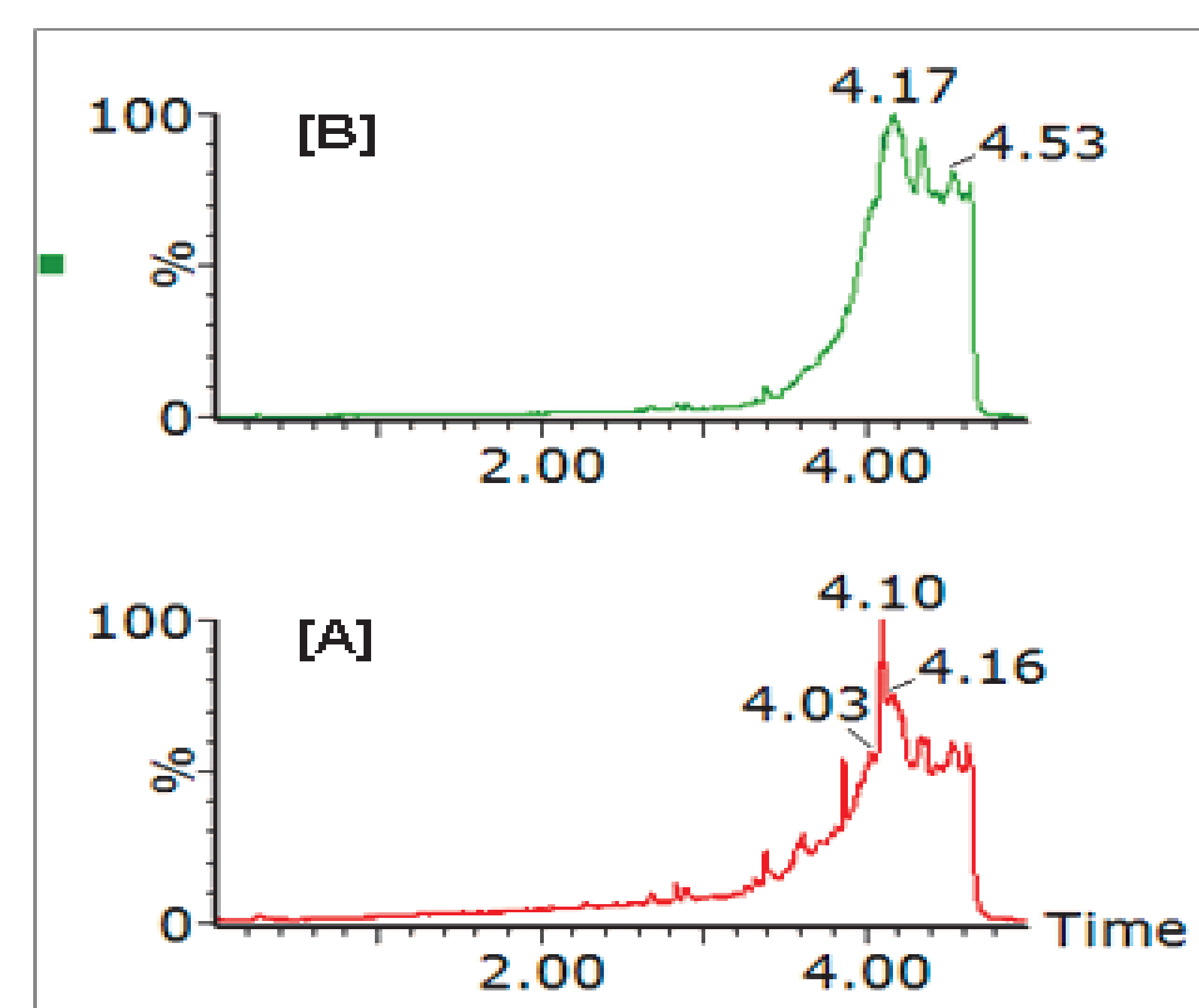


Figure 3. [A] Low energy MS^E TIC from broad-scope screening of Red Kite gullet contents [B] High energy MS^E TIC from broad-scope screening of Red Kite gullet contents

Figure 4 shows the ChromaLynx XS Identify results browser from POSI±IVE data processing. Here 585 compounds were targeted and automatically reduced to just two found and identified as carbofuran and carbosulfan. Data for both the MS^E low energy precursor ions and the high energy fragment ions are displayed. The accuracy of the exact mass ions, as shown for carbosulfan (precursor ion: m/z 381.2212, fragment ion: m/z 118.0690) with ΔM values of +0.2 mDa and -0.3 mDa respectively, gives added confidence that the results are correct. Carbofuran can be metabolised to carbosulfan, so both compounds could have been used independently or the carbosulfan found could be a metabolite of carbofuran.

Figure 5 shows the TargetLynx Quantify browser from POSI±IVE data processing. Here pesticide solvent standards have been used to semi-quantify the identified pesticide poisons. The browser window shows the relative amount of pesticide found, based on the responses from the calibration curve. This allows us to back calculate the approximate amounts of carbosulfan and carbofuran in the sample, taking into account the mass of sample taken, and the dilution factor. Using this method we identified carbosulfan at 145.9 mg/kg and carbofuran at 10.7 mg/kg.

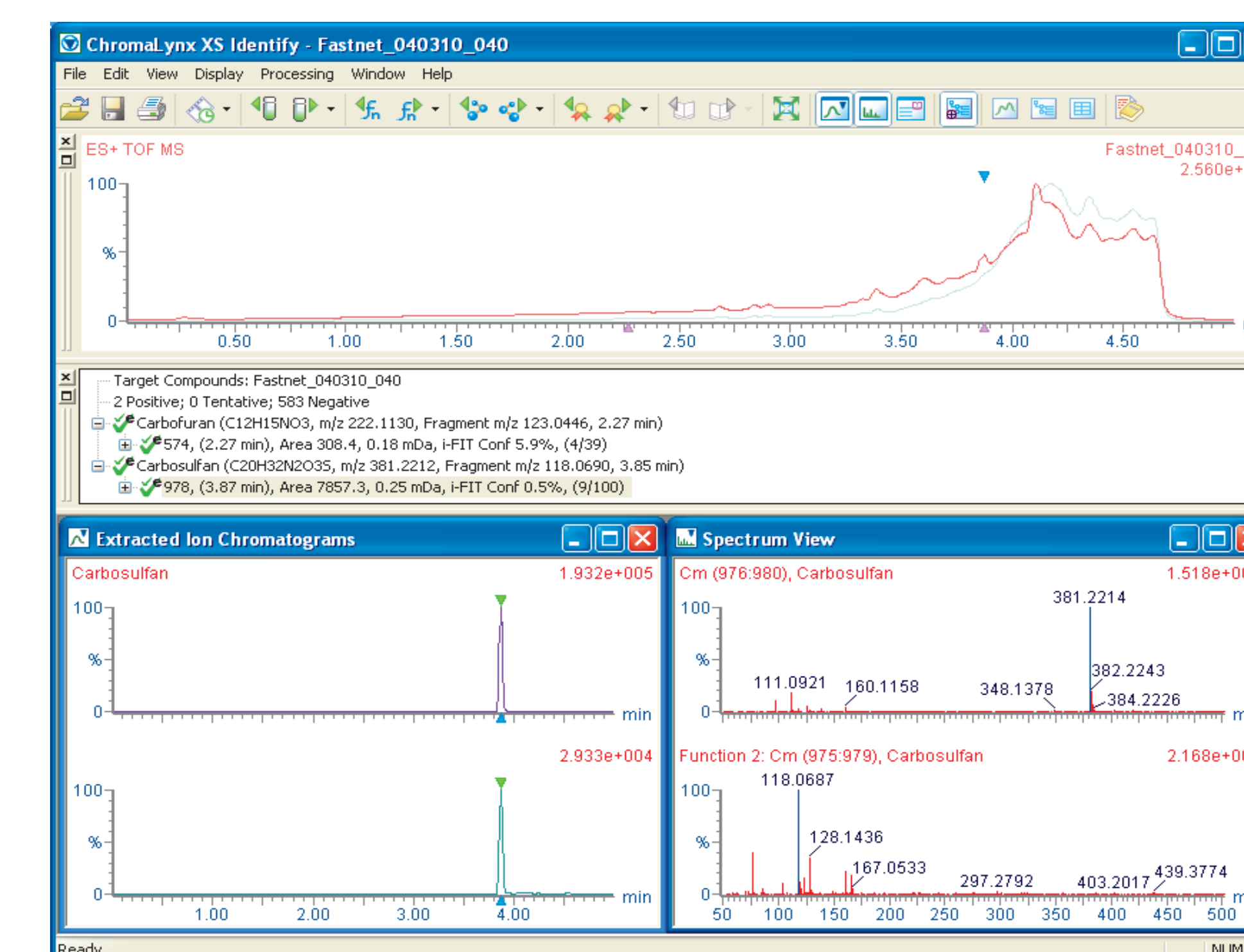


Figure 4. ChromaLynx XS results browser showing carbofuran and carbosulfan as the pesticide poisons

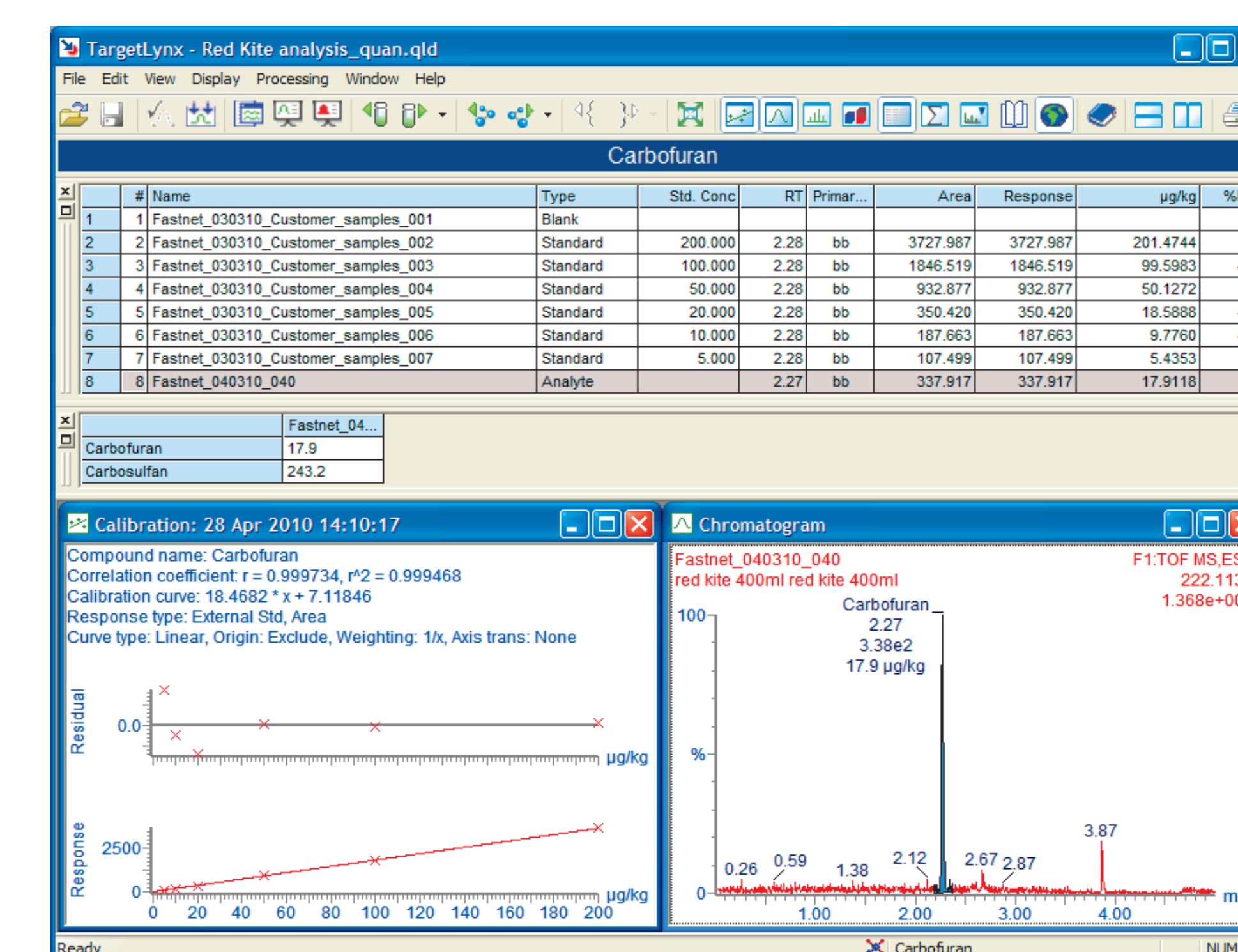


Figure 5. TargetLynx Quantify browser showing carbofuran and carbosulfan quantified using pesticide solvent standards

Further compound confirmation was carried out using the MassFragment tool. Structures were assigned to the MS^E fragment ion spectra acquired from the relevant extracted ion chromatograms, based on accurate and precise exact mass data.

Figure 6 shows MassFragment assigned structures for the fragment ions seen at 3.87 minutes, the retention time for carbosulfan. The precursor ion continues to be seen at low intensity in the high energy fragment ion spectrum, with very good exact mass. The fragment ions assigned by the MassFragment software also have excellent exact mass values, even for these very low masses. This gives further validation that the compound identification is correct.

CONCLUSIONS

Broad-scope pesticide screening of the extracted red kite gullet samples enabled the detection and identification of the pesticides used to poison the bird of prey. Carbofuran and carbosulfan were identified, with a high degree of confidence

MS^E functionality of Xevo G2 QToF enables the acquisition of both low energy (precursor ion) and high energy (fragment ion) data in one rapid screening run

The highly reproducible and precise exact mass data affords increased confidence in the accuracy of the results

POSI±IVE software, along with the MassFragment tool, provides a powerful data processing approach for pesticide screening and unequivocal compound identification

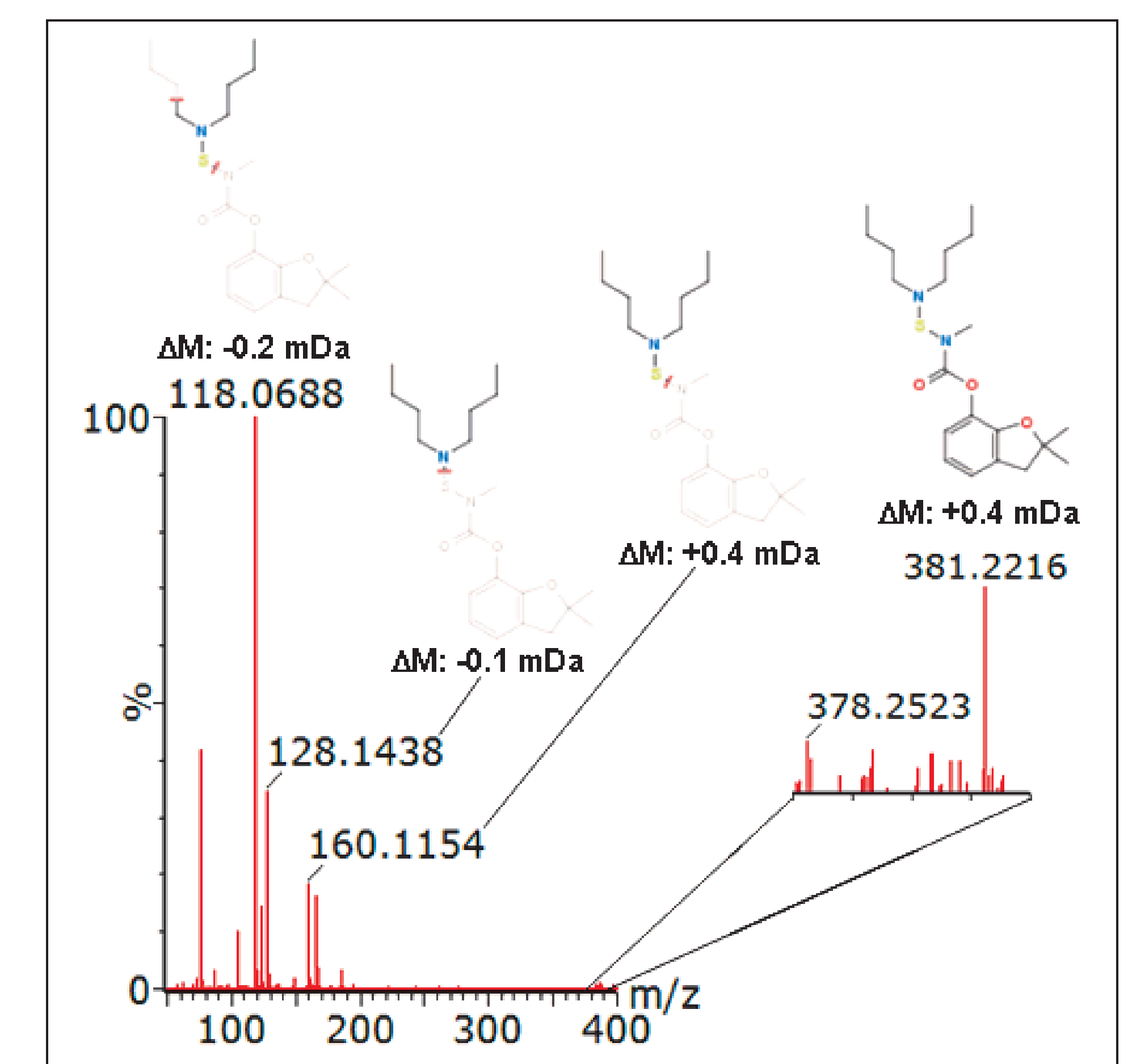


Figure 6. MS^E high energy fragment ion spectrum, with structural assignments from MassFragment

Acknowledgements

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References

1. Website: <http://www.redkites.co.uk/>
2. Website: <http://www.rspb.org.uk/wildlife/birdguide/name/r/redkite/index.aspx>