The determination of Ziram residues in fruit by LC-MS/MS

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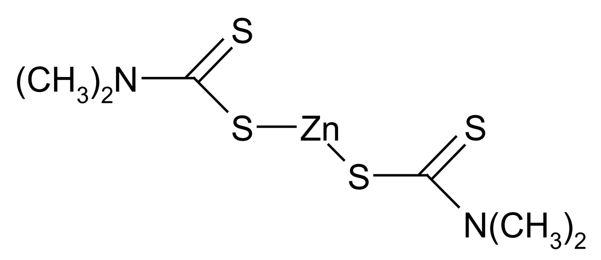
Introduction

- Ziram (zinc bis(dimethyldithiocarbamate)) is a widely used fungicide used in agriculture to control and prevent the spread of a variety of fungal infections
- Ziram is commonly used on stone fruit, apples, pears, grapes and almonds. In addition, Ziram is also used on soil, as well as a seed treatment
- It is also used in industry where it is an additive in adhesives and paints and as an accelerator in the manufacturing process of some rubber materials
- The analysis of dithiocarbamates is generally performed by the measurement of liberated CS_2, following decomposition in the presence of $SnCl_2$/HCl
- This technique does not distinguish between the other related compounds that also produce CS_2, in this way
- There was a requirement to develop and validate a modern analytical method that was specific to Ziram/dimethyl dithiocarbamate pesticides
- This method had to be suitable for routine use for the analysis of samples generated in crop residue trials

Challenges

- Ziram has limited solubility and stability in the majority of common extraction solvents
- Limited stability when in contact with acidic plant juices
- The decomposition analysis technique cannot distinguish between other similar analytes and naturally occurring materials that also produce CS_2
- A fast efficient LC-MS method was therefore required, that could complement the current CS_2 screening technique

Thiram

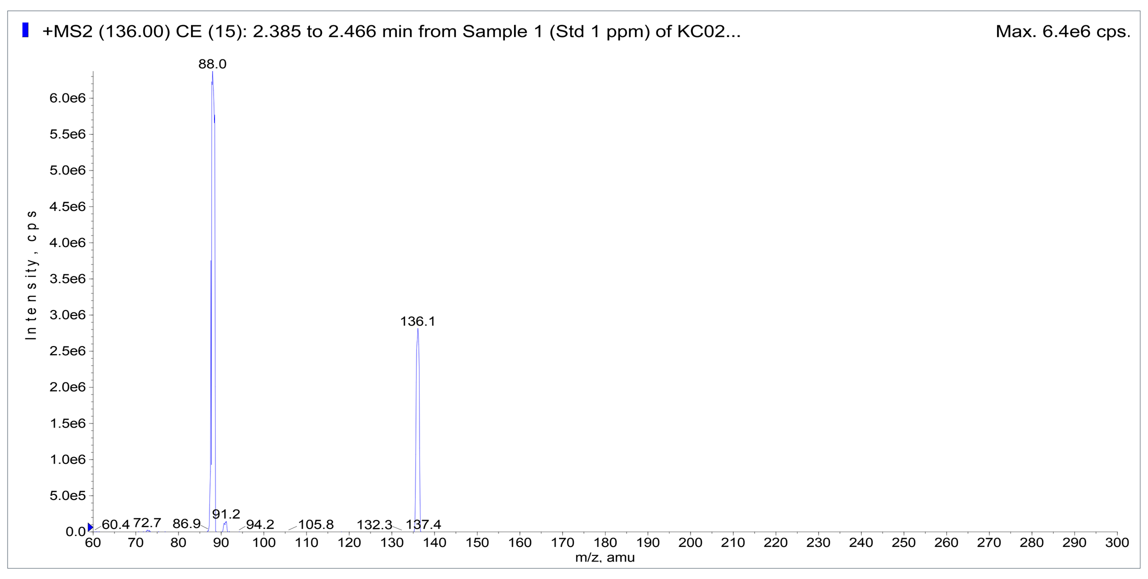


Molecular formula : $C_6H_{12}N_2S_4Zn$ Molar mass : 305.8 g/mol

Summary of extraction and clean-up procedure

- As dimethyl dithiocarbamate pesticides are usually analysed by the non selective CS_2 approach, an alternative LC-MS/MS method was desirable
- The use of LC-MS/MS with atmospheric pressure chemical ionisation (APCI) was investigated to enable sample analysis to very low levels with a high degree of confidence in the data produced
- Frozen crop samples were prepared for analysis by homogenisation with dry-ice
- The analytical method involved initial extraction into a mixture of EDTA, cysteine and iodomethane by mechanical shaking
- This approach had previously been developed and routinely used for EBDC analysis[1], and was subsequently modified for Ziram analysis
- The extract was centrifuged and the supernatant was allowed to stand to allow methylation of the dimethyl dithiocarbamate
- An aliquot was diluted with a methanol/water solution prior to quantification by LC-MS/MS
- No further sample clean-up stage is required as there were no observable matrix effects using this approach
- This method allows a range of fruit sample types to be analysed, using this quick, efficient, robust and reliable technique
- The analytical method was developed to utilise LC/MS-MS which significantly reduced the analysis time to approximately six minutes per sample injection
- Two MS/MS ion transitions can be simultaneously monitored to demonstrate a suitable confirmatory technique, if required
- In addition, the use of the alternative CS_2 technique can offer another method of confirmation if used alongside the Ziram specific LC-MS/MS method

The MS/MS scan of the protonated methylated derivative (*m/z* 136) showed fragmentation to produce daughter ions at *m/z* 88 and *m/z* 73



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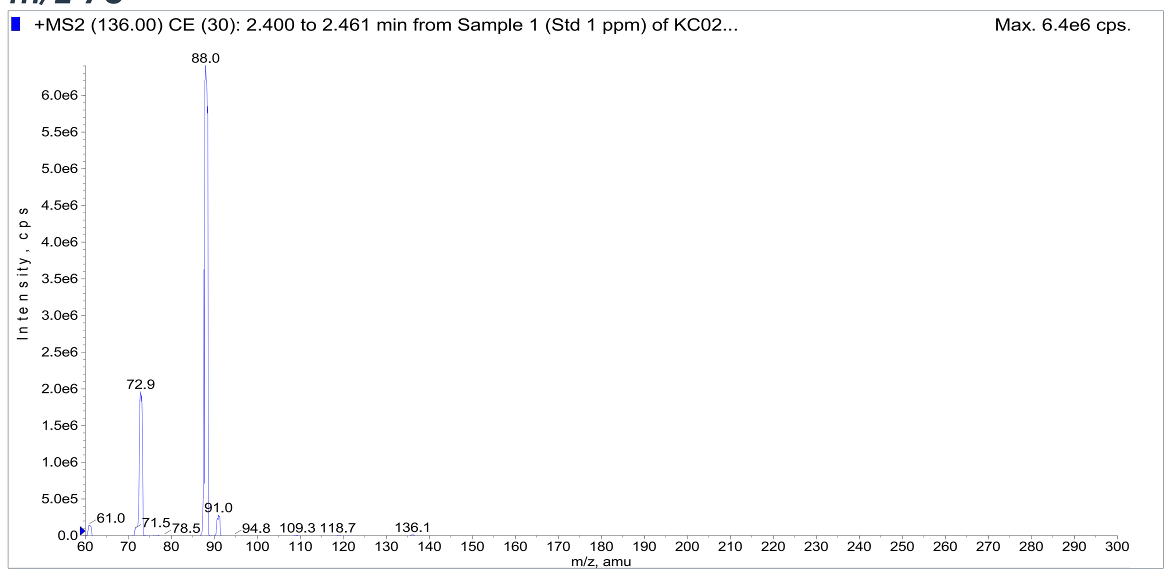
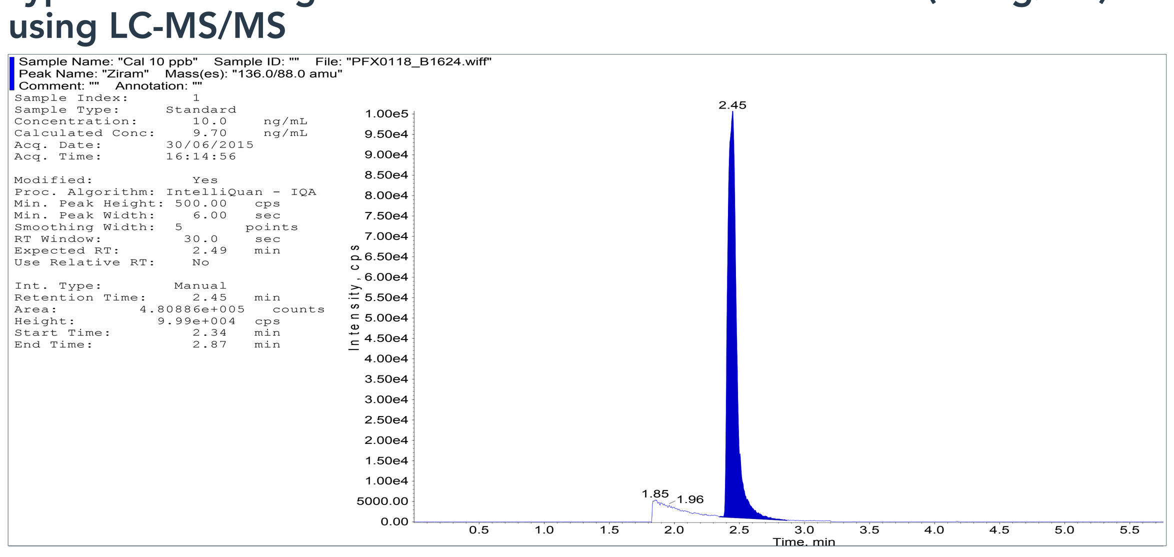
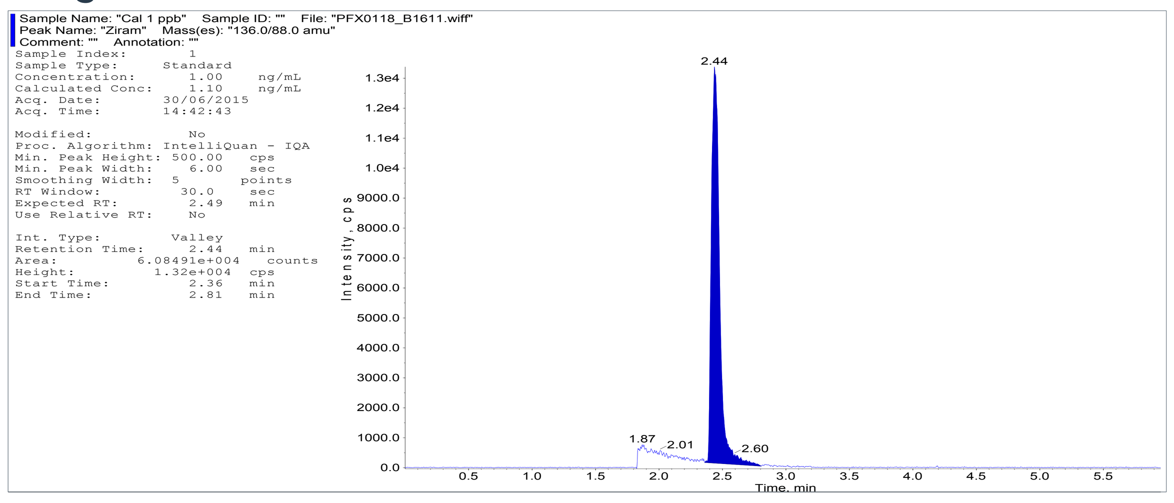


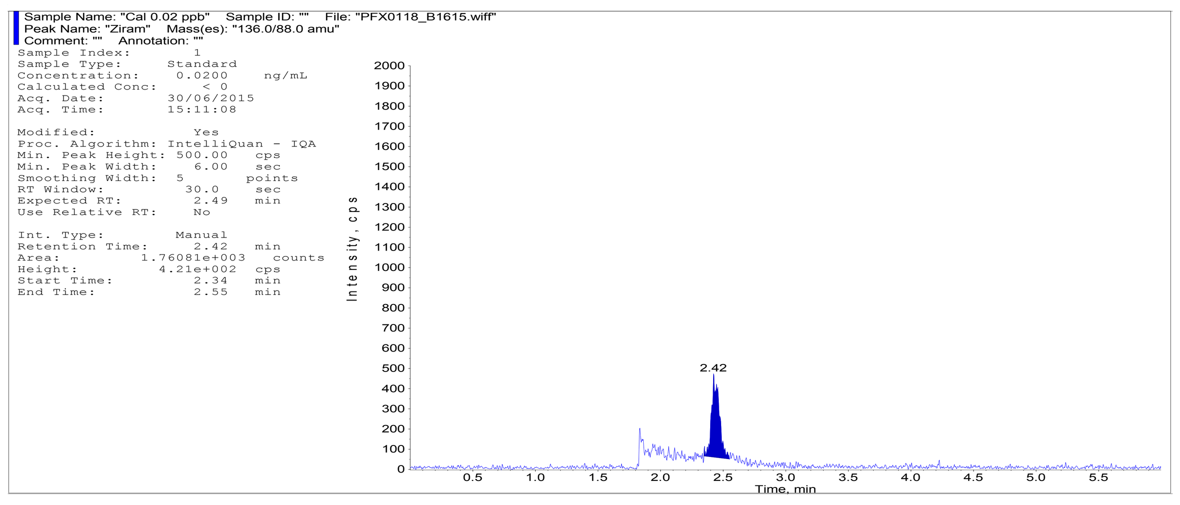
Typical chromatogram of a Ziram calibration standard (10 ng/mL) using LC-MS/MS



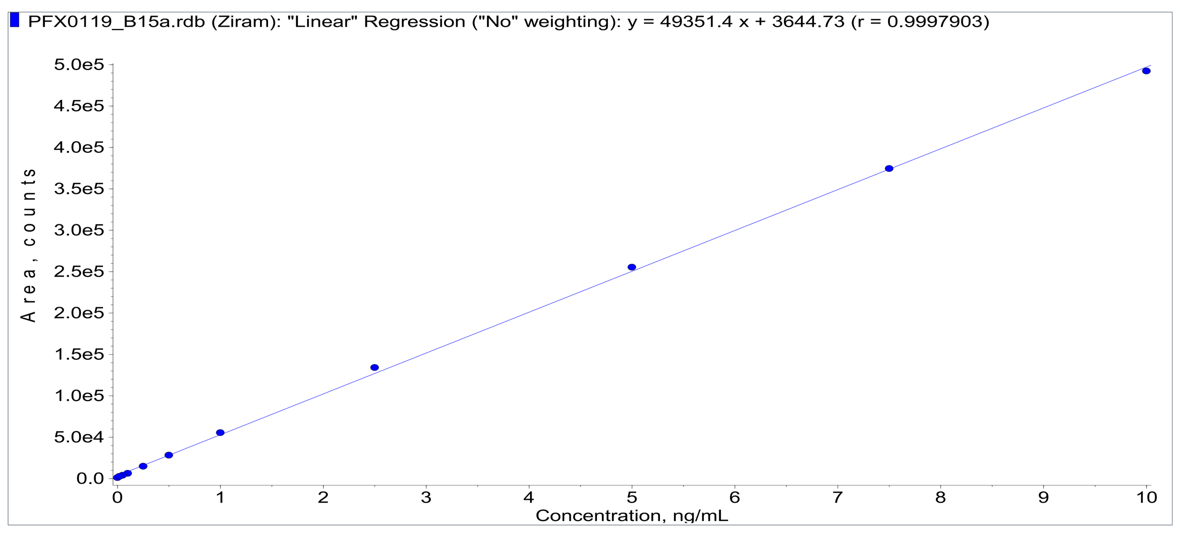
Typical chromatogram of a Ziram calibration standard (1 ng/mL) using LC-MS/MS



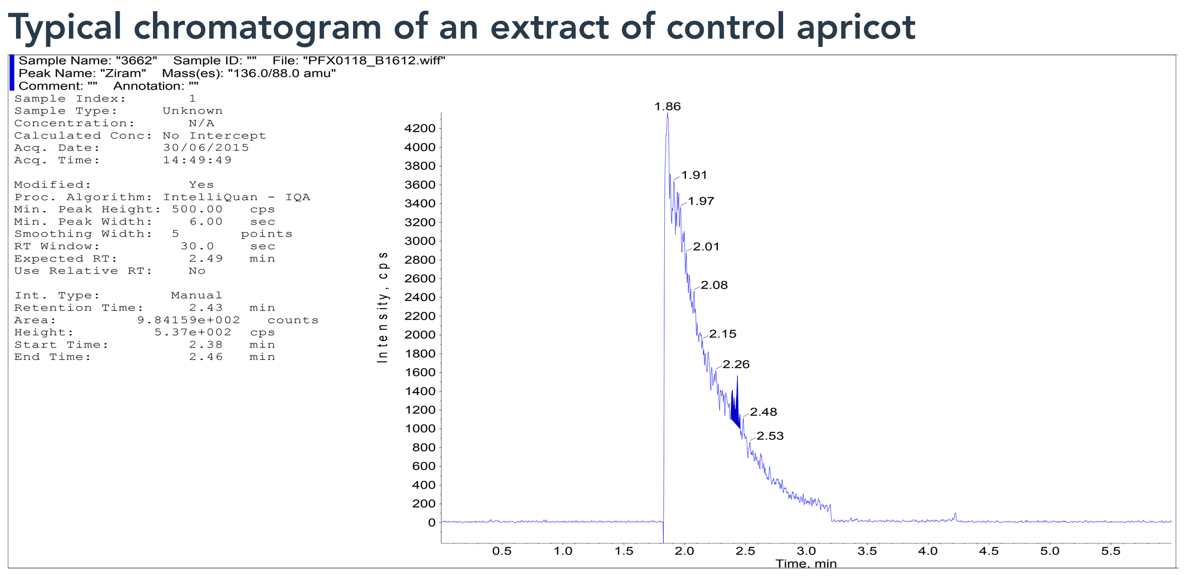
Typical chromatogram of a Ziram calibration standard (0.02 ng/mL) using LC-MS/MS



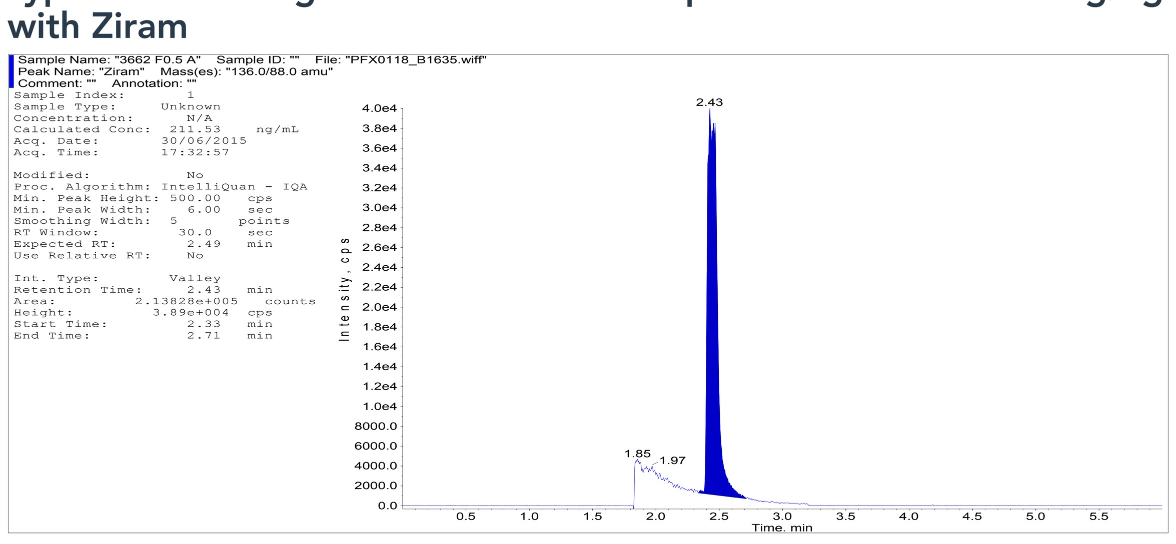
Standard calibration graph for Ziram (0.02 - 10 ng/mL) using LC-MS/MS



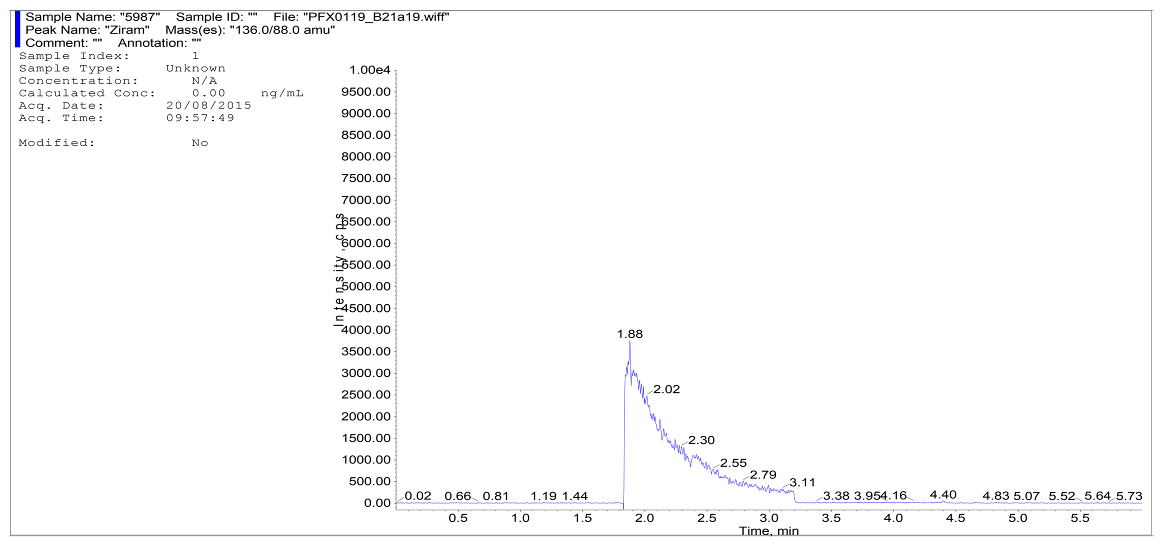
Typical chromatogram of an extract of control apricot



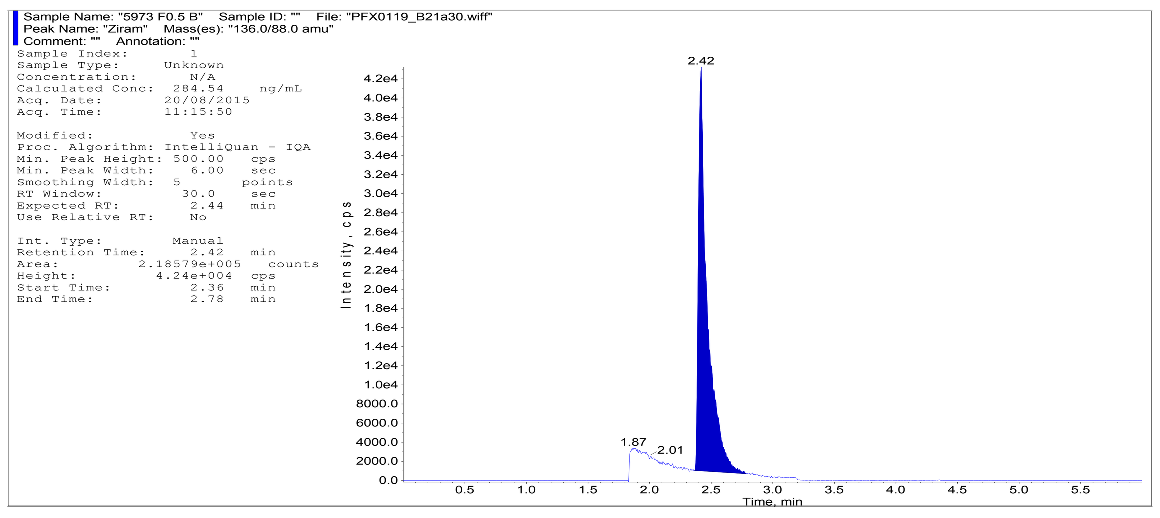
Typical chromatogram of an extract of apricot fortified at 0.5 mg/kg with Ziram



Typical chromatogram of an extract of control peach



Typical chromatogram of an extract of peach fortified at 0.5 mg/kg with Ziram



The validation data generated for Ziram in apricot is summarised as follows:

Fortification level (mg/kg)	Replicate	Concentration detected (mg/kg)	Recovery (%)	Mean (%)	CV (%)
Control	A	ND	-		
Control	B	ND	-		
0.01	A	0.0094	94		
0.01	B	0.0082	82		
0.01	C	0.0079	79	82	8.7
0.01	D	0.0081	81		
0.01	E	0.0075	75		
0.5	A	0.594	119		
0.5	B	0.507	101		
0.5	C	0.515	103	105	7.4
0.5	D	0.501	100		
0.5	E	0.517	103		
Overall Mean (%)					94
Overall CV (%)					15.0

The validation data generated for Ziram in peach is summarised as follows:

Fortification level (mg/kg)	Replicate	Concentration detected (mg/kg)	Recovery (%)	Mean (%)	CV (%)
Control	A	ND	-		
Control	B	ND	-		
0.01	A	0.0108	108		
0.01	B	0.0115	115	111	3.4
0.01	C	0.0109	109		
0.5	A	0.461	92		
0.5	B	0.479	96	93	2.8
0.5	C	0.457	91		
Overall Mean (%)					102
Overall CV (%)					9.9

Conclusions

- It is possible to analyse Ziram in fruit matrices using LC-MS/MS, following methylation
- This gives rise to short analysis times, enabling a large number of samples to be quantified in a single batch
- The use of LC-MS/MS means that extraction and clean-up procedures can be simplified due to the high instrument selectivity obtained
- This technique offers an improvement over the CS_2 approach, being more specific to the dimethyl dithiocarbamates and avoiding possible false positive results
- The analytical method has been validated on apricots and peaches at two concentration levels
- The method was demonstrated to be robust with recovery values falling within the range 75-119%
- The overall mean recoveries between 94-102 % and coefficient of variation values of ≤15% show that this method is suitable for routine sample analysis
- Additional confirmatory data can be acquired using a second MS/MS transition, as well as obtaining information by performing additional analyses using the CS_2 technique

References

1. Stephen Brewin, et al. (2006) *The Analysis of Ethylene Bisdithiocarbamate (EBDC) Fungicides in Various Commodities by the use of LC-MS/MS.* Sixth European Pesticide Residue Workshop, Corfu 21-25

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