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

S Brewin, H Harper and A Blakely

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 CS₂, following decomposition in the

The MS/MS scan of the protonated methylated derivative (m/z 136) showed fragmentation to produce daughter ions at m/z 88 and m/z73



Typical chromatogram of an extract of control peach



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

- presence of SnCl₂/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 CS₂ screening technique



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

Summary of extraction and clean-up procedure

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

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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

Sample Name, "Cal 0.02 pp0" Sample ID Pi	Te. PPXD118_B1615.WIT	
Peak Name: "Ziram" Mass(es): "136.0/88.0 amu		
Samle Today:		
Sample Type: Standard	2000.	
Concentration: 0.0200 ng/mL		
Calculated Conc: < 0	1900	



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

Fortification level (mg/kg)	Replicate	Concentration detected (mg/kg)	Recovery (%)	Mean (%)	CV (%)
Control	A	ND	-		
Control	В	ND	-		
0.01	A	0.0094	94		
0.01	В	0.0082	82		
0.01	С	0.0079	79	82	8.7
0.01	D	0.0081	81		
0.01	E	0.0075	75		
0.5	А	0.594	119		
0.5	В	0.507	101		
0.5	С	0.515	103	105	7.4
0.5	D	0.501	100		
0.5	E	0.517	103		
					0.4

Overall Mean (%)

- + As dimethyl dithiocarbamate pesticides are usually analyzed by the non selective CS₂ approach, an alternative LC-MS/MS method was desirable
- + The use of LC-MS/MS with atmospheric pressure chemical ionization (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 homogenization 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 analysis¹, 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 analyzed, using this quick, efficient, robust and reliable technique
- + The analytical method was developed to utilize 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



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



Typical chromatogram of an extract of control apricot

Sample Name: "3652" Sample ID: " File:	"PFXD118_B1612.wiff"	
Comment ** Appointion: **	amur	
Sample Index: 1		
Sample Type: Unknown	75	1.04
Concentration: N/A	4300-	
Acq. Date: 30/06/2015	4000-	
Acq. Time: 14149169	3800-	1.91
Hodified: Yes	3600	
Proc. Algorithm: Intelliquen - IQ Min. Peak Height: 500.00 cps	A 3400-	1.97
Min. Peak Width: 6.00 sec	3200-	1
RT Window: 30.0 sec	3000	-2.01
Expected RT: 2.49 min	0. 2800	
ore relative with the	2600	2.00
Int. Type: Manual	> 2400-	A CONTRACTOR OF
Area: 9.84159#+002 coup	a 2200-	
Height: 5.37e+002 cps	5 2000-	-2.15
End Time: 2.46 min	<u>c</u> 1800-	2.76
	1600	a contraction of the second
	4400	n. (

Overall CV (%)

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

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

Conclusions

- + It is possible to analyze 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 CS₂ approach, being more specific to the dimethyl dithiocarbamates and avoiding possible false positive results

15.0

- to demonstrate a suitable confirmatory technique, if required
- + In addition, the use of the alternative CS₂ 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

+MS2 (136.00) CE (15): 2.38	35 to 2.466 min from Sample 1 (Std 1 ppm) o	d KC02	Max. 6.4e6 cps.
0.88			
6.0e6			
5.5e6			
5.De6			
4.5e6			
4.046			
3.5e6			
3.0e6	136.1		
2.5e6			
2.0e6	1		
1.546			
1.046			
5.045	104.6 133.2 197.4		
60 70 80 90 100	110 120 130 140 150 160 170 160 190	200 210 220 230 240 250	260 270 200 290 300



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

Use Relative RT: Int. Type: Retention Time: Ares: 2.1 Neight: 2.1 Start Time: End Time:	No Valley 2.43 3820+005 .890+005 2.71 2.71	min counts cps min min	inte nuity, 2p	2.4e4 2.2e4 1.8e4 1.6e4 1.2e4 1.2e4 1.0e4 8000.0 6000.0 2000.0		1.05 1.97				
Calculated Conc: Acq. Date: Acq. Time: Modified: Froc. Algorithm: Min. Peak Neight: Min. Peak Width: Smoothing Width: RT Window: Evented DT.	211.53 30/06/201 17:32:57 No IntelliQu 500.00 5 30.0	ng/mL s cps sec points sec		3.4e4 3.4e4 3.2e4 3.0e4 2.9e4						
Peak Name. "Zham" Comment. " Annota sample index: sample Type: Concentration:	Mass(cs). "I lon." I Unknown R/A	36.0/65.0 ams	r	4.064			2.42			

- + 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 $\leq 15\%$ 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 analyzes using the CS₂ 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

Envigo, Woolley Road, Huntingdon, Cambridgeshire, UK