The determination of Thiram residues in fruit by UPLC-MS/MS

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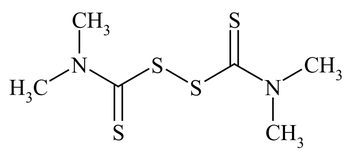
Introduction

- Thiram is a non-systemic dimethyl dithiocarbamate fungicide, mainly used in the field as well as to protect harvested crops during transport and storage
- Considered to be a general use pesticide, licensed for use on a range of crops including fruit, vegetables and ornamentals to prevent fungal diseases
- Also used as an animal repellent to prevent damage caused by rabbits, rodents and deer
- 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 Thiram
- This method had to be suitable for routine use for the analysis of samples generated in crop residue trials

Challenges

- Thiram can undergo decomposition when exposed to acidic plant juices
- It is also suggested that Thiram stability is adversely affected by the presence of copper ions[1]
- A fast efficient LC-MS method was required that could complement the current CS_2 screening technique

Thiram

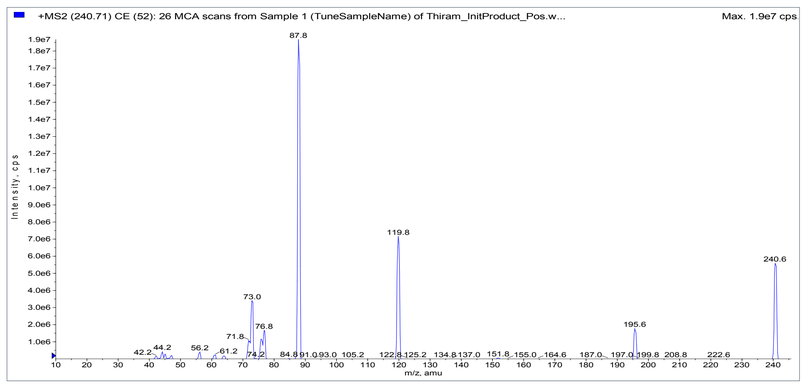


Molecular formula : $C_6H_{12}N_2S_4$ Molar mass : 240.44 g/mol

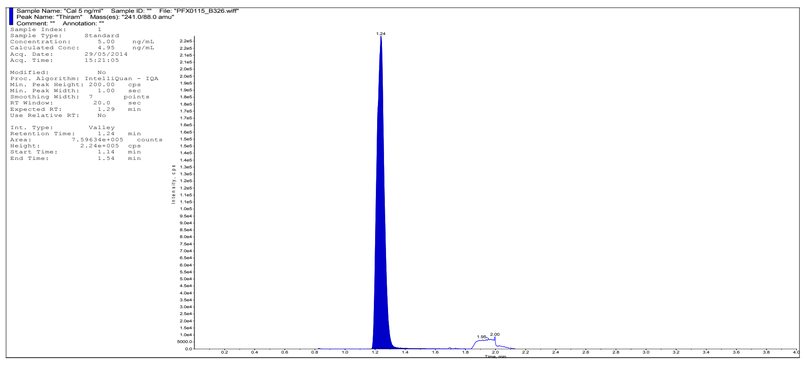
Summary of extraction and clean-up procedure

- Frozen crop samples were prepared for analysis by homogenisation with dry-ice
- Direct Thiram analysis in fruit (strawberry and apple) involved extraction by shaking with cold acetonitrile in the presence of anhydrous sodium sulfate
- An aliquot was diluted with an aqueous ETDA solution prior to quantification by UPLC-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 UPLC/MS-MS which significantly reduced the analysis time to approximately four minutes per sample injection
- Two MS/MS ion transitions can be simultaneously monitored to demonstrate a suitable confirmatory technique
- In addition, the use of the alternative CS_2 technique can offer another complimentary technique if used alongside the Thiram specific LC-MS/MS method

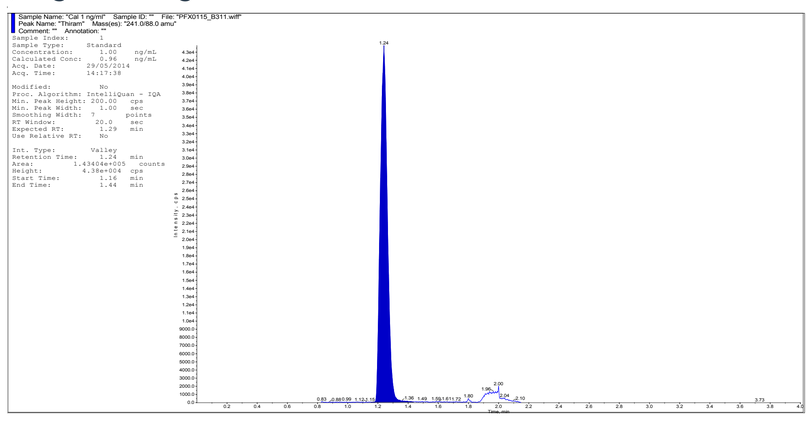
The MS/MS scan of *m/z* 241 showed fragmentation of the molecular ion to produce daughter ions at *m/z* 120 and *m/z* 88



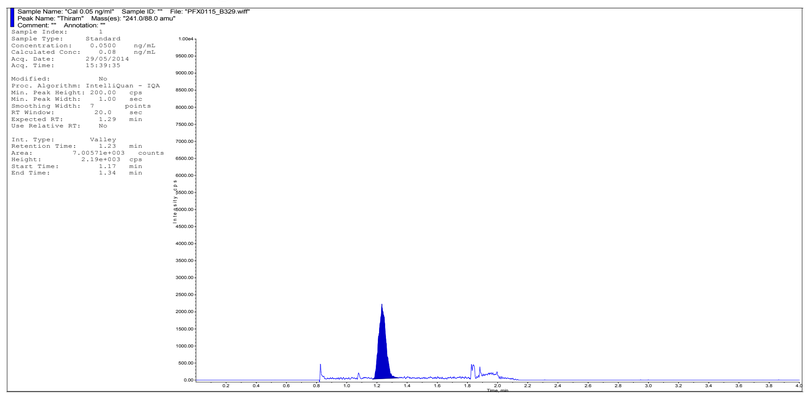
Typical chromatogram of a Thiram calibration standard (5 ng/mL) using UPLC-MS/MS



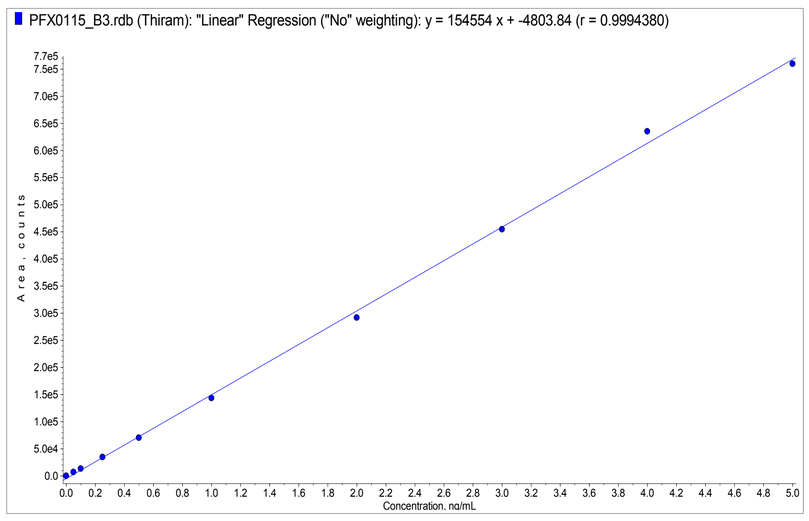
Typical chromatogram of a Thiram calibration standard (1 ng/mL) using UPLC-MS/MS



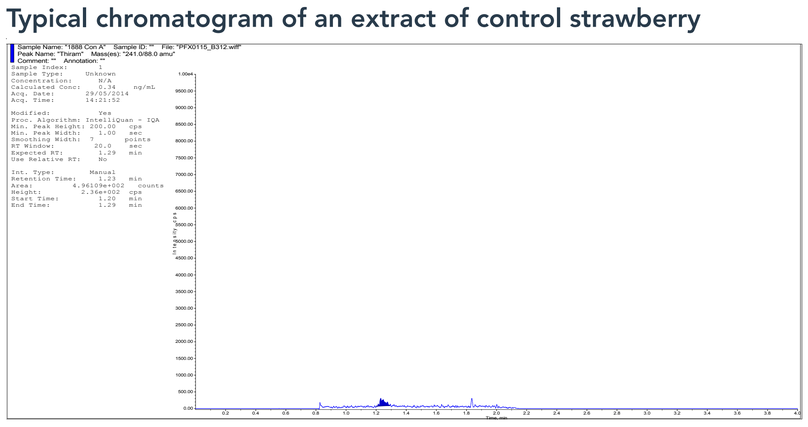
Typical chromatogram of a Thiram calibration standard (0.05 ng/mL) using UPLC-MS/MS



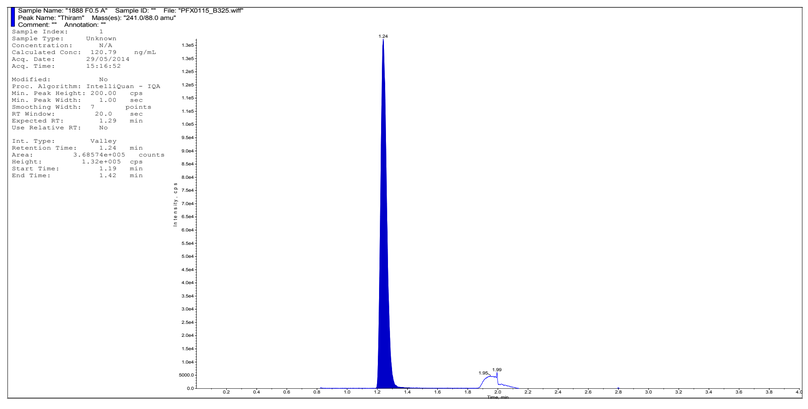
Standard calibration graph for Thiram (0.05-5 ng/ml) using UPLC-MS/MS



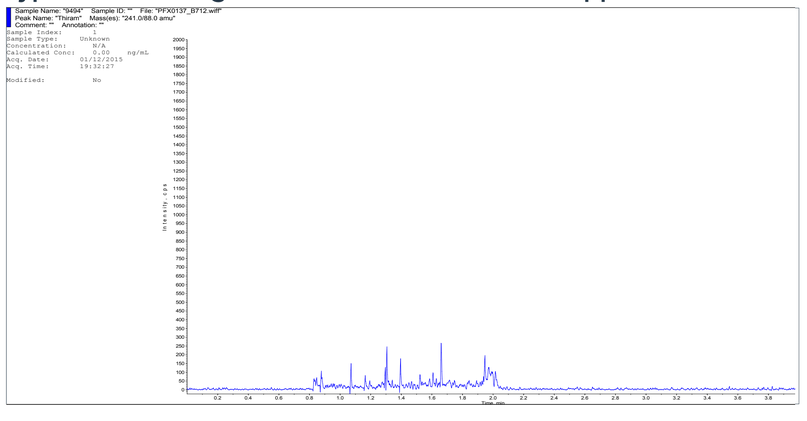
Typical chromatogram of an extract of control strawberry



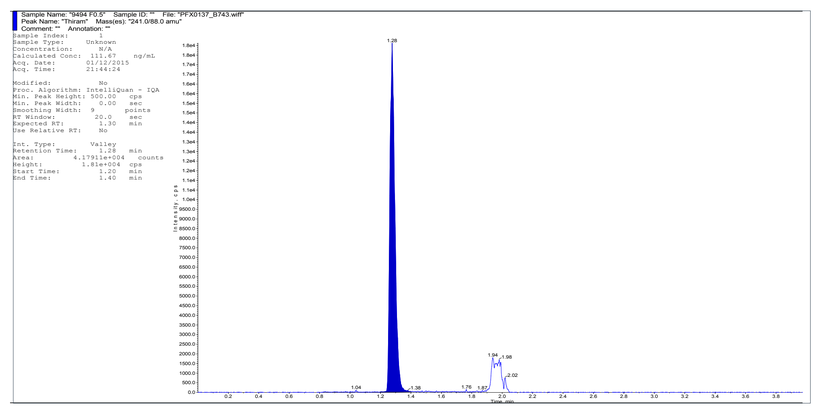
Typical chromatogram of an extract of strawberry fortified at 0.5 mg/kg with Thiram



Typical chromatogram of an extract of control apple



Typical chromatogram of an extract of apple fortified at 0.5 mg/kg with Thiram



The validation data generated for Thiram in strawberry 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	0.0073	73		
0.01	B	0.0085	85		
0.01	C	0.0085	85	83	7.0
0.01	D	0.0088	88		
0.01	E	0.0085	85		
0.5	A	0.483	97		
0.5	B	0.495	99		
0.5	C	0.410	82	94	7.3
0.5	D	0.479	96		
0.5	E	0.480	96		
Overall Mean (%)					89
Overall CV (%)					9.3

The validation data generated for Thiram in apple 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	0.0101	101		
0.01	B	0.0097	97		
0.01	C	0.0080	80	94	17.7
0.01	D	0.0076	76		
0.01	E	0.0117	117		
0.5	A	0.440	88		
0.5	B	0.527	105		
0.5	C	0.502	100	100	7.2
0.5	D	0.531	106		
0.5	E	0.508	102		
Overall Mean (%)					97
Overall CV (%)					12.9

Conclusions

- It is possible to analyse Thiram in fruit matrices using UPLC-MS/MS
- This gives rise to short analysis times, enabling a large number of samples to be quantified in a single batch
- The use of UPLC-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 specific to Thiram and avoiding possible false positive results
- The analytical method has been validated on strawberries and apples at two concentration levels with five replicates per level
- The method was demonstrated to be robust with recovery values falling within the range 73-117%
- The overall mean recoveries between 89-97 % and coefficient of variation values of <13% 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 further information by performing additional analyses using the CS_2 technique

References

1. Filipe O.M. et al, (2008). J Agric Food Chem., 56 (16) p.7347

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