

DEVELOPMENT OF A METHOD FOR THE DETERMINATION OF THIOSULTAP AND THIOCYCLAM RESIDUES IN RED PEPPER.

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INTRODUCTION

Thiosultap and thio cyclam are known as nereistoxin analogues; nereistoxin is a naturally occurring insecticide in the marine worm *L. heteropoda*. These compounds are extensively used as pesticides in rice, potatoes, fruit trees and vegetables. Despite thiosultap is **banned in the EU**, it is still authorized in other third countries like Brazil and Japan. There is little literature about experimental methodology for their analysis and GC/MS is not an appropriate technique because thiosultap degrades thermally to nereistoxin. This, along with the difficulties of its extraction from vegetable matrices, makes their analysis of great interest. In this communication, a new method for extraction and analysis of thiosultap and thio cyclam in red pepper has been validated.

Different extraction methods were tested to attain the best results in the recoveries. The final one combines acetonitrile extraction in an acidic medium, in order to enable the protonated form of these molecules, with ultrasonic extraction followed by a clean-up step with MgSO₄. The analyses were performed on a Linear Ion Trap Quadrupole LC-MS/MS in negative mode for thiosultap and in positive mode for thio cyclam and nereistoxin. Calibration curves covering three orders of magnitude were performed and they were linear over the concentration range studied (0.001 to 0.5 mg·kg⁻¹). Instrumental detection limits (LOD) were in the low µg·kg⁻¹ range. Good reproducibility was obtained at both studied concentration levels, with RSDs below 20 %. The average recoveries for pepper were 58% for thiosultap and 87% for thio cyclam.

EXTRACTION PROCEDURE

Weigh 15 g of blended Sample in a 50 ml Falcon tube

Add 2 ml HCl 2N and shake For 2 minutes; then add 13 ml of Acetonitrile with 1% Acetic Acid

Add 6 g MgSO₄ anhydrous and 1.5 g Sodium Acetate and shake vigorously for 1 minute

Sonicate for 4 minutes and centrifuge for 5 minutes at 3700 r.p.m.

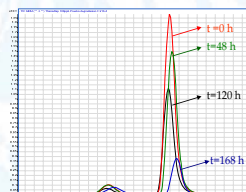
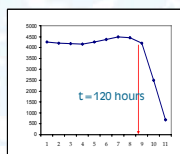
Take a 5 ml aliquot from supernatant and add 750mg MgSO₄ anhydrous

Shake for 20 seconds and centrifuge for 5 minutes at 3700 r.p.m.

Take the supernatant to a vial for analysis

DEGRADATION STUDIES

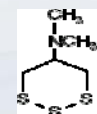
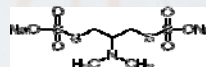
Stability studies of thiosultap in water were performed evaluating a 100 µg/kg solution of this compound in water. It was analyzed during seven days, after which a degradation of around 60% of thiosultap could be observed.



LC-MS/MS (QQQ) ANALYSIS

Structures:

Thiosultap



Thio cyclam



Chromatography:

• **Injection Volume:** 20 µl

• **Column:**

C₈ (ZORBAX Eclipse® XDB, Agilent Technologies) 150 mm x 4.6 mm (5-µm)

• **Mobile phase:**

Acetonitrile/H₂O (0.1% formic acid): 30 % ACN t=3m min, then to 100 % in 10 min, isocratic at 100% for 5 min Flow rate of 0.4 ml/min.

MS-MS conditions:

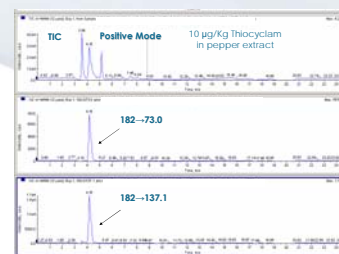
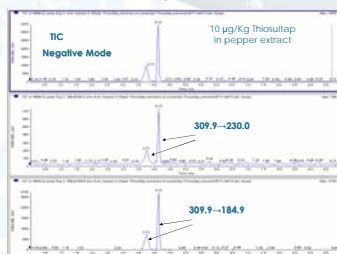
3200 Qtrap Applied Biosystems/MDS SCIEX

Ion Source: Turbo Spray

Ionization mode: positive and negative

Dwell time: 200 msec.

	Quantitation Transition	Confirmation Transition	Retention Time (min)
Thiosultap (negative mode)	309.9 → 184.9	309.9 → 230.0	3.8 and 4.1
Thio cyclam (positive mode)	182.0 → 73.0	182.0 → 137.1	4.16



METHOD VALIDATION

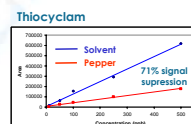
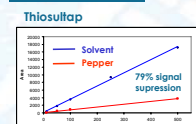
Recoveries

	Spiking Level			
	20 µg/kg		100 µg/kg	
	Recovery	RSD (%) n=5	Recovery	RSD (%) n=5
Thiosultap	58	17	40	6
Thio cyclam	87	3	93	3

Linearity

	Solvent		Pepper	
	Equation	R ²	Equation	R ²
Thiosultap	y = 34.6 x + 163.4	0.9976	y = 7.4 x + 96.6	0.9985
Thio cyclam	y = 1209.6 x + 7783.9	0.9949	y = 349.28 x + 6885.5	0.9955

Matrix effect



Limits of detection

- Thiosultap: 0.010 mg/Kg
- Thio cyclam: 0.001 mg/Kg