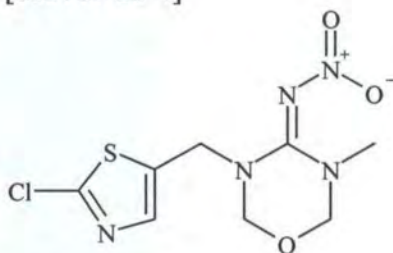


1. Introduction and Summary

1.1 Scope

The analytical procedure described is suitable for the determination of residues of thiamethoxam (Figure 1) in samples from the thiamethoxam dust deposition trials (glycerol/water dust traps, quartz sand Petri-dish traps, air filters and gauze netting samples) using an external standardisation procedure. The limit of quantification has been set at 1 ng per dust trap for the dust trap solutions (equivalent to 0.008 ng/mL for the glycerol/water (30/70, v/v)) 1 ng per quartz sand dust trap, 1 ng per gauze netting sample and 2.5 µg per air filter.

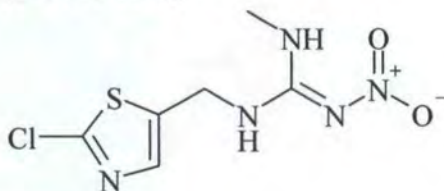
Common name: **Thiamethoxam**
Company code: CGA293343
Chemical name (IUPAC): 3-(2-chloro-thiazol-5-ylmethyl)-5-methyl-[1,3,5]oxadiazinan-4-ylidene-N-nitro-amine
CAS Registry No.: [153719-23-4]



Structural formula:
Molecular formula: $C_8H_{10}ClN_5O_3S$
Molecular mass: 291.7 g/mol

Batch: AMS 780/4
Purity: 99.7 %
Storage conditions: $20^{\circ}C \pm 4^{\circ}C$ (at Eurofins|ADME Bioanalyses)
Date of expiry: 30 June 2012

Common name: **CGA322704**
Chemical name (IUPAC): N-(2-chloro-thiazol-5-yl-methyl)-N'-methyl-N''-nitro-guanidine
CAS-Registry-No.: [131748-59-9]



Structural formula:
Molecular formula: $C_6H_8ClN_5O_2S$
Molecular mass: 249.7 g/mol

Batch:	BPS 1143/1
Purity:	98 %
Storage conditions	between 0 and 9°C (at Eurofins ADME Bioanalyses)
Date of expiry:	31 July 2010

1.2 Method Summary

1.2.1 Aqueous Dust Trap Solutions

A glycerol/chromasolv water dust trap solution (37.5/87.5, v/v) is shaken on a flatbed shaker for 30 minutes to solubilise any thiamethoxam residues adsorbed to particulate matter. An aliquot of the sample is diluted with ultra pure water and subjected to a Waters Oasis™ HLB solid phase extraction (SPE) procedure prior final determination by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The limit of quantification of the method is 1 ng per dust trap, equivalent to a residue of 0.008 ng/mL in the glycerol trapping solution.

1.2.2 Quartz Sand Dust Traps

Sand/glycerol/water samples consisted of 50 g of quartz sand that were moistened with 17 mL of glycerol/water (1/1, v/v) each. Water was added to the specimen material of sand/glycerol/water and the sample is shaken on a flatbed shaker at room temperature. After filtration the final extraction volume was made up to 200 mL using water. An aliquot of the sample is subjected to a Waters Oasis™ HLB solid phase extraction (SPE) procedure prior final determination by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The limit of quantification of the method is 1 ng per quartz sand dust trap.

1.2.3 Air Filters

An air filter sheet is shaken with methanol/ ultra pure water solution (50:50, v/v) to desorb thiamethoxam residues from the filter. An aliquot of the methanol/ultra pure water solutions is diluted with ultra pure water prior final determination by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The limit of quantification of the method is 2.5 µg thiamethoxam per air filter.

1.2.4 Gauze netting

50 x 50 cm gauze squares were moistened with approximately 15 mL of glycerol/water (1/1, v/v). A conditioned gauze square was extracted with methanol/water (1/1, v/v) by shaking on a flatbed shaker at room temperature. After filtration the final extraction volume was made up to 200 mL using water. The methanol amount of an aliquot was evaporated in a stream of nitrogen

and the initial volume of the aliquot is restored by adding water. An aliquot of the sample is subjected to a Waters Oasis™ HLB solid phase extraction (SPE) procedure prior final determination by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The limit of quantification of the method is 1 ng per gauze square sample.

2. Materials

The recommended equipment and reagents are described in Appendices 1 and 2. Equipment with equivalent performance specifications and reagents of comparable purity can be substituted provided that they can be shown to be suitable.

2.1 Apparatus

See Appendix 1 for a list of apparatus used during this method.

2.2 Reagents

All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used. See Appendix 2 for a list of reagents used in this method.

2.3 Preparation of Analytical Standards

It is recommended that the following precautions should be taken when weighing the analytical materials.

1. Ensure good ventilation.
2. Wear gloves and laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area immediately.

Weigh out accurately, using a five-figure balance, sufficient thiamethoxam and CGA322704 analytical standards to allow dilution in ethanol to give 200 µg/mL stock solutions in a volumetric flask. These standards should then be diluted by serial dilution to 10 µg/mL, 0.1 µg/mL and 0.01 µg/mL in ultra pure water to prepare mixed solutions. These standards should be used for the fortification of samples prior to extraction and for use as calibration standards for LC-MS/MS analysis.

When not in use, always store the standard solutions in a refrigerator at 4 °C (between 0 and 9°C) to prevent decomposition and/or concentration of the standard. It is recommended that analytical standards should be replaced with freshly prepared standards after six months.

2.4 Safety Precautions and Hazards

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in any doubt, consult the appropriate safety manual (e.g. Syngenta Laboratory Safety Manual), which contains recommendations and procedures for handling chemicals or a monograph such as 'Hazards in the Chemical Laboratory', Edited by S G Luxon, The Chemical Society, London (Reference 1).

Solvent Hazards

	Acetonitrile	Ethanol	Methanol	Acetic Acid
Harmful Vapour	✓	✓	✓	✓
Highly Flammable	✓	✓	✓	×
Harmful by Skin Absorption	✓	✓	✓	✓
Syngenta Hazard Category	SHC-C	SHC-B	SHC-C	SHC-C
OES Short Term (mg m ⁻³)	105	N/A	310	37
OES Long Term (mg m ⁻³)	70	1900	260	25

In all cases avoid breathing vapour. Avoid contact with eyes and skin.

Thiamethoxam has been designated as Syngenta toxicity classification SHC-B. The toxicity classification scale rates highly toxic chemicals as SHC-E and non-toxic chemicals as SHC-A.

2.5 Time Required for Analysis

The methodology is normally performed with a batch of 15 samples. One person can complete the analysis of up to 15 samples in 1 day (8 working hour period).

2.6 Work Stoppages

The analytical procedure can be stopped at various points for overnight and weekend breaks except where specified in the analytical procedure. Acceptable external standard recoveries will validate the work stoppages. Samples should be stored in sealed vessels at a temperature between 0 and 9°C.

2.7 Modifications and Potential Problems

- For preparation of aqueous HPLC mobile phases it has been found beneficial to use bottled HPLC grade water. This gives a reduced MS/MS background signal when compared to water from a laboratory water purification system.
- The method has been tested in these laboratories using an Applied Biosystems API 4000 triple quadrupole mass spectrometer and the sample dilutions are based on the

achievable detection limits using this instrument. Other suitable triple quadrupole mass spectrometers may be used for the analysis, however, it may be necessary to use different sample dilutions prior to analysis.

3. Analytical Procedure

3.1 Aqueous Dust Trap Solutions

3.1.1 Extraction

- a) The volume or weight of each field sample received must be recorded in the raw data and used to adjust the final analytical result. For example, the tare weight of sample containers used in the field phase could be assessed by individually weighing ten empty items, and then determining the mean container weight. By weighing field samples and subtracting the container weight, the field sample weight may be determined. Allowing for density, the weight of 125 ml glycerol/chromasolv water dust trapping solution [37.5/87.5 v/v] is 134g.
- b) Place the polypropylene centrifuge bottle containing the glycerol/chromasolv water dust trapping solution [37.5/87.5 v/v, ~125 mL] on a flatbed shaker and shake at a speed which visibly agitates the sample for 30 minutes. At least one untreated control solution of glycerol/water solution [37.5/87.5 v/v,] and two control solutions fortified with known amounts of thiamethoxam and CGA322704 in water should be analysed alongside each batch of samples to demonstrate acceptable performance of the method and allow recovery corrections to be made if desired.

3.2 Quartz Sand Trap

3.2.1 Extraction

50 g of quartz sand moistened with approximately 17 mL glycerol/water (1/1, v/v) dust trap samples were transferred to 250 mL plastic bottle, with 100 mL water rinsings. The sample was shaken on a flatbed shaker for about 30 min at ambient temperature. The sample was filtered through a filter paper into a 200 mL graduated flask. The plastic bottle was washed with water and the washing solution was passed through the filter to make up the final volume of 200 mL

3.3 Gauze Netting

3.3.1 Extraction

The moistened gauze was transferred to a 250 mL plastic bottle and 100 mL of methanol/water (1/1, v/v) was added. The bottle was shaken for 60 minutes on a flatbed shaker at ambient temperature. The solution was transferred into a 200 mL volumetric flask and the gauze square was shaken for five minutes in a further 100 mL of water. The aqueous solution was transferred to the volumetric flask and the volume was made up to 200 mL with water. A 25 mL aliquot was transferred into a volumetric flask and the methanol amount (nominal 6.25 mL) was evaporated under a stream of nitrogen in a water bath (45 °C). Finally the volume was made up to 25 mL with water.

3.3.2 Solid Phase Extraction

- a) Transfer an aliquot of the sample to be analysed from step 3.1.1 (12.5 mL) into a polypropylene centrifuge tube (25 mL size) and add ultra pure water (12.5 mL). Alternatively transfer an aliquot (25 mL) of the sample to be analysed from step 3.2.1 or step 3.3.1.
- b) Place a Waters Oasis™ HLB solid phase extraction cartridge (60 mg, 3 mL size) on a suitable vacuum manifold. Using a column connection adapter, attach a reservoir (70 mL size) to the top of the column. Add methanol (2 mL) and draw through under vacuum to the level of the top frit at a rate of approximately 2 mL min⁻¹, discarding the eluate. Add ultra pure water (2 mL) to the top of the cartridge and draw through under vacuum to the level of the top frit at the same rate, again discarding the eluate.
- c) Transfer the 25 ml sample aliquot onto the cartridge and allow to percolate through under gravity or low vacuum. Discard the eluate. Add ultra pure water (2.5 mL) to the reservoir and draw through the cartridge under vacuum, discarding the eluate.
- d) Remove the reservoir and adapter and dry the cartridge under high vacuum for 15 minutes.
- e) Place a collection tube (10 mL) in the manifold rack. Add acetonitrile (2 mL) to the top of the cartridge and draw through under gravity or low vacuum at a rate of approximately 2 mL min⁻¹, and dry the cartridge under high vacuum for a few seconds to collect the eluate in the tube.
- f) Evaporate the sample to dryness under a stream of clean, dry nitrogen in a heating block with the temperature set to 40 °C. Re-dissolve the sample in ultra pure water/methanol solution [90/10, v/v, 0.5 mL] with ultrasonication. Transfer the sample to an autosampler vial ready for final determination by LC-MS/MS.

3.4 Air Filters

Due to the size of the air filter, it is not possible to desorb a complete filter in one vessel. The filter therefore needs to be cut into smaller pieces so that desorption of thiamethoxam and CGA322704 residues can take place. After desorption, aliquots of the solutions used to carry out desorption are combined and analysed.

- a) For field specimens, carefully cut the air filter into four equal width pieces, ensuring that any dust that falls out of the filter was collected. Transfer the four pieces to wide mouth glass storage bottles (3 L size). For the validation, one sheet of filter is placed into a 3 L flask.
- b) At least one untreated control sample and two control samples fortified with known amounts of thiamethoxam and CGA322704 have been analysed alongside each batch of samples to demonstrate acceptable performance of the method and allow recovery corrections to be made if desired. Control and recovery samples have been generated

by cutting a strip of a clean air filter (fortifying if required), placing in a 3 L storage bottle and analysing alongside the treated samples.

- c) Add any dust collected during cutting of the filter to one of the storage bottles.
- d) Add ultra pure water/methanol solution [50/50 v/v, 2.5 L] to each of the bottles, cap the bottles and shake on a flatbed shaker at 250 cycles per minute for 30 minutes.
- e) Transfer aliquots (50 μL) from each of the four bottles to a single autosampler vial and add ultra pure water to the vial so that the final sample volume is 1 mL. The sample is ready for final determination by LC-MS/MS.

3.5 Preparation of LC-MS/MS Calibration Standards

Calibration standards for LC-MS/MS analysis have been prepared in ultra pure water/methanol solution [90/10 v/v, 1 mL]. For example, to prepare a mixed 0.0002 $\mu\text{g mL}^{-1}$ thiamethoxam and CGA322704 standard, transfer 20 μL of a 0.01 $\mu\text{g mL}^{-1}$ thiamethoxam standard to a volumetric flask (10 mL), add ultra pure water/methanol solution [9:1 v/v] and adjust to 10 mL.

4. Final Determination by LC-MS/MS

The following instruments and conditions have been found to be suitable for this analysis in this laboratory. Other instruments and conditions may be used, however optimisation may be required to achieve the desired separation and sensitivity. The operating manuals for the instruments should always be consulted to ensure safe and optimum use.

4.1 Instrument Conditions

Pump (two required)	:	Shimadzu LC-20 AD
Column Oven	:	Shimadzu CTO-20 AC
Detector	:	Applied Biosystems API4000 triple quadrupole mass spectrometer with Analyst™ software version 1.4.2

4.2 Chromatography Conditions

Column	:	Develosil RP aqueous, 3 μm , 150 mm x 3 mm
Mobile phase	:	Solvent A: acetonitrile:0.1% (v/v) acetic acid Solvent B: ultra pure water:0.1% (v/v) acetic acid

Gradient

Time (min)	% A	% B
0.0	5	95
1.0	5	95
4.0	100	0

6.0	100	0
7.0	5	95
10.0	5	95

Flow rate : 0.6 mL min⁻¹
Injection volume : 100 µL
Stop Time : 10 minutes
Injection protocol : Analyse calibration standard after 3 to 4 sample injections
Column oven temperature : 40°C

4.3 API4000 Mass Spectrometer Conditions

Interface : ElectroSpray
Polarity : Positive
Curtain gas (CUR) : Nitrogen set at 25 (arbitrary units)
Temperature (TEM) : 600°C
Ionspray voltage : 5500 V
Collision gas setting (CAD) : Nitrogen set at 4 (arbitrary units)
Gas 1 (GS1) : Air set at 50 (arbitrary units)
Gas 2 (GS2) : Air set at 60 (arbitrary units)
Scan type : MRM

MRM Conditions used for aqueous petri dish and filter samples

		Thiamethoxam (quantification)	Thiamethoxam (confirmatory)	CGA322704 (quantification)	CGA322704 (confirmatory)
Q1 m/z	:	292.2	292.2	250.2	250.2
Q3 m/z	:	211.2	181.1	169.2	132.1
Dwell time (ms)	:	100	100	100	100
Resolution Q1	:	Unit	Unit	Unit	Unit
Resolution Q3	:	Unit	Unit	Unit	Unit
Declustering potential (DP)	:	60 V	60 V	45 V	45 V
Entrance potential (EP)	:	10 V	10 V	10 V	10 V
Collision energy (CE)	:	17 V	30 V	18 V	22 V

Collision cell exit potential
(CXP)

:

15 V

18 V

11 V

8 V

MRM Conditions used for aqueous petri dish and filter samples

Analyte Monitored	Transitions Monitored	Declustering Potential (DP) [V]	Collision Energy (CE) [eV]	Cell Exit Potential (CXP) [V]	Dwell Time [ms]
Thiamethoxam	292/211 ^A	96	19	10	50
	292/181 ^A	96	33	10	100
	292/132 ^B	96	35	10	250
	292/108 ^B	96	57	10	250
CGA322704	250/169	51	21	20	150
	250/132	51	27	20	150

^A Ion mass transitions were used for gauze square extracts; ^B Ion mass transitions had to be used for sand/glycerol/water sample extracts due to significant matrix interferences for 292/211 and 292/181 at the retention time of thiamethoxam

Representative chromatograms for all matrices are shown in Appendix 4.

Protonated molecular ions generated in the ion source ($m/z = 292.2$ for thiamethoxam and $m/z = 250.2$ for CGA322704) were selected and subjected to further fragmentation by collisional activation. The most abundant ion ($m/z = 211.2$ for thiamethoxam and $m/z = 169.2$ for CGA322704) in the resulting daughter spectra are then monitored and used for quantitative analysis. Other abundant ions ($m/z = 181.1$ for thiamethoxam and $m/z = 132.1$ for CGA322704) were selected for confirmatory analysis. Typical chromatograms are shown in Appendix 4. Initial and final product scans showing the fragmentation and daughter ions for thiamethoxam and CGA322704 are presented in Appendix 6.

5. Calculation of Results

Residues may be calculated using an external standardisation procedure

Thiamethoxam and CGA322704 residues have been calculated in ng/dish or µg/filter for each sample as follows.

- a) Prepare standard solutions over a concentration range appropriate to the expected residues in the samples (for example, 50% LOQ to 20 x LOQ). An appropriate number of different concentrations within this range should be prepared (at least four).
- b) Make an injection of each sample solution and measure the areas of the peaks corresponding to thiamethoxam and CGA322704. Calibration standard solutions should be interspersed throughout the analysis, after a maximum of four injections of sample solutions.
- c) Generate calibration curve parameters using an appropriate regression package.

- d) The following equation can be rearranged and used to calculate residues as follows:

$$y = mx + c$$

Where y is the instrument response value, x is the standard concentration, m is the gradient of the line of best fit ("X-variable 1" in MS Excel) and c is the intercept value. An example of this equation generated using the experimental values of m and c should be included in the raw data, as should the "R-Squared" value for the regression.

Re-arrangement for x gives

$$x = \frac{y - c}{m}$$

- e) In dust traps, calculate the thiamethoxam and CGA322704 residues in the sample, expressed as ng/trap as follows:

$$\text{Residue (ng/trap)} = \frac{V_1 \times V_f}{V_2} \times n \times \text{Standard Conc } (\mu\text{g / mL})$$

Standard Conc. = Concentration of calibration standard ($\mu\text{g/mL}$)

Sample Conc. = Final sample concentration (mL mL^{-1})

V_1 = Total volume (125 mL)

V_2 = Aliquot volume (12.5 mL)

V_f = Final volume (0.5 mL)

n = Dilution of final extract (when applicable)

- f) In air filters, calculate the thiamethoxam and CGA322704 residues in the sample, expressed as $\mu\text{g}/\text{filter}$ as follows:

$$\text{Residue } (\mu\text{g}/\text{filter}) = \frac{V_1 \times V_f}{V_2} \times n \times \text{Standard Conc } (\mu\text{g / mL})$$

Standard Conc. = Concentration of calibration standard ($\mu\text{g/mL}$)

Sample Conc. = Final sample concentration (mL mL^{-1})

V_1 = Total volume (2.5 L)

V_2 = Aliquot volume (0.05 mL)

V_f = Final volume (1 mL)

n = Dilution of final extract (when applicable)

6. Control and Recovery Experiments

Fortification levels for procedural recoveries have been appropriate to the residue levels expected. A minimum of one control and two external recovery experiments have been run alongside each set of samples analysed (that is untreated samples accurately fortified with a known amount of thiamethoxam and CGA322704 prior to extraction).

Control and external recovery experiments have been completed as section 3 for each set of samples analysed. Provided the recovery values were acceptable they have been used to correct any thiamethoxam and CGA322704 residues found.

Recovery data is generally considered acceptable when the mean values are between 70% and 110% with a relative standard deviation (RSD) of <20%.

7. Specificity

If unexpected interference is observed at final determination, it is recommended that a reagent blank is taken through the analytical procedure to trace the source of the problem.

It is recommended that reagent blank samples be included in a sample set if contamination is suspected

7.1 Matrix

LC-MS/MS is a highly specific detection technique. Interference arising from the matrices tested has not been observed.

7.2 Reagent and Solvent Interference

Using high purity solvents and reagents no reagent interference has been found.

7.3 Labware Interference

The method mainly uses disposable labware. No interference from labware has been found.

This method uses disposable labware. All reusable glassware have been detergent washed and rinsed with HPLC grade methanol, acetone or acetonitrile prior to use.

8. Method Validation

8.1 Recovery Data and Repeatability

Validation data has been generated using the procedures described in Section 3. A summary of the method validation data is shown in Appendix 3.

Method validation has been carried out on the analytical procedures described in Section 3 and is reported in the Eurofins|ADME Bioanalyses Report S09-02420 (Reference 2). Further method validation has been carried out on the analytical procedures described in Section 3 and is reported in the Eurofins Dr. Specht GLP GmbH Report TK0022198-REG (Reference 3).

Full details are presented in Appendix 3.

8.2 Limit of Quantification and Limit of Detection

8.2.1 Limit of Quantification (LOQ)

The limit of quantification of the method is defined as the lowest analyte concentration in a sample at which the methodology has been validated and a mean recovery of 70-110% with a relative standard deviation of $\leq 20\%$ has been obtained. Generally, for accurate quantification, the response for an analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time.

The limit of quantification has been set at 1 ng per dust trap for the dust trap solutions (equivalent to 0.008 ng/mL for the glycerol/water (30/70, v/v)) 1 ng per quartz sand dust trap, 1 ng per gauze netting sample and 2.5 μg per air filter.

8.2.2 Limit of Detection (LOD)

The limit of detection of the method is defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. An estimate of the LOD can be taken as four times background noise. Note that the LOD may vary between runs and from instrument to instrument.

The limit of detection of this method was estimated and is presented in Appendix 3.

8.2.3 Matrix Effects

Suppression of the instrument response for thiamethoxam and CGA322704 has been observed in the glycerol/water dust trap tested using the above procedure in this laboratory.

No significant suppression or enhancement of the instrument response for thiamethoxam and CGA322704, has been observed in the air filter tested using the above procedure in this laboratory.

Significant matrix effects were found for sample extracts of sand/glycerol/water and gauze for both analytes. Therefore matrix-matched standard solutions were used for quantification. In case of samples that were diluted with solvent by at least factor 10, solvent standard solutions were used for quantification.

APPENDIX 1 APPARATUS

UK Suppliers

General laboratory glassware, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

Plastic centrifuge bottles, 50 mL size, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

Mechanical shaker for extraction of samples, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

Disposable test tubes (10 mL capacity) available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK. Part number CEK-151-010W.

Glass wide neck storage bottles, 3 litre size, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK. Part number CEK-151-010W.

Solid phase extraction vacuum manifold, available from Varian Limited, 6 Mead Road, Oxford Industrial Park, Yarnton, Oxford OX5 1QU, UK.

SPE column reservoirs and column connection adapters, available from Varian Limited, 6 Mead Road, Oxford Industrial Park, Yarnton, Oxford OX5 1QU, UK.

Oasis™ HLB solid phase extraction columns, 3 mL 60 mg size, available from Waters Ltd., 730 – 740 Centennial Court, Centennial Park, Elstree, Hertfordshire, WD6 3SZ, UK.

Techne Dri-block 3D sample concentrator, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

Ultrasonic bath e.g. Ultrawave U300/D, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

Crimp cap auto sampler vials and caps available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

API4000 HPLC-MS-MS system equipped with a TurboIonSpray source, available from Applied Biosystems, Lingley House, 120 Birchwood Boulevard, Warrington, Cheshire WA3 7QH, UK.

Shimadzu LC20AD HPLC system equipped with autosampler, quaternary pump, vacuum degasser and column compartment with column switching valve, available from Shimadzu UK Limited, Mill Court, Featherstone Road, Wolverton Mill South, Milton Keynes MK12 5RD

HPLC column, Develosil RP aqueous, 3 µm, 150 mm x 3 mm., available from Hichrom Ltd., 1 The Markham centre, Station road, Theale, Reading, RG47 4PE Berkshire, UK or www.hichrom.co.uk

Peak Scientific NM20ZA gas station, available from available from Peak Scientific Instruments Ltd, Fountain Crescent, Inchinnan Business Park, Inchinnan, Renfrew, PA4 9RE, UK.

APPENDIX 1 APPARATUS (continued)

US Suppliers

General laboratory glassware, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Plastic centrifuge bottles, 50 mL size, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Mechanical shaker for extraction of samples, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Disposable test tubes, 10 mL capacity, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Glass wide neck storage bottles, 3 litre size, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Solid phase extraction vacuum manifold, available from Varian Inc. 24021 Frampton Avenue, Harbor City, CA 90710, USA.

Oasis™ HLB solid phase extraction columns, 3 mL 60 mg size, available from Waters Corporation, 34 Maple Street, Milford, Massachusetts, 01757 - 3696, USA.

Techne Dri-block 3D sample concentrator, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Ultrasonic bath available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Crimp cap auto sampler vials and caps available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

API4000 HPLC-MS-MS system equipped with a TurboIonSpray source, available from Applied Biosystems, 850 Lincoln Center, Foster City, CA 94404-1128, USA.

Shimadzu LC20AD HPLC system equipped with autosampler, quaternary pump, vacuum degasser and column compartment with column switching valve, available from Shimadzu Scientific Instruments, 7102 Riverwood Drive, Columbia, MD 21046, U.S.A.

HPLC column, Develosil RP aqueous, 3 µm, 150 mm x 3 mm i.d, available from www.Hichrom.co.uk

Peak Scientific NM20ZA gas station, available from Peak Scientific Instruments, 1300 West Belmont Ave, Chicago, IL 60657, USA

APPENDIX 2 REAGENTS

All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used.

UK Suppliers

Methanol, ethanol, acetonitrile and water, high purity grade, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

Acetic Acid (98%), from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

Ultra pure water from a laboratory water purification system eg Elga Maxima available from Elga Ltd., High Street, Lane End, High Wycombe, Buckinghamshire HP14 3JH, UK.

Thiamethoxam analytical standard, available from Syngenta Crop Protection, Jealott's Hill Research International Research Centre, Bracknell, Berkshire RG42 6EY, UK.

US Suppliers

Methanol, ethanol, acetonitrile and water available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Acetic acid, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Ultra-pure water from a laboratory water purification system available from Waters Corporation, Milford, MA, USA.

Thiamethoxam analytical standard, available from Syngenta Crop Protection, Inc., P. O. Box 18300, Greensboro, NC 27419-8300.

APPENDIX 6: API4000 MS/MS TUNING PROCEDURE

Calibration of Instrument

The instrument must be mass calibrated on a regular basis using polypropylene glycol (PPG) solutions according to the manufactures instructions. Calibrate both mass resolving quadrupoles (Q1 and Q3).

Tuning of API4000 MS/MS Instrument for Thiamethoxam

Infuse a standard solution of thiamethoxam and CGA322704 (0.1 to 1.0 $\mu\text{g mL}^{-1}$) in ultra pure water directly into the mass spectrometer interface at a rate at of 10 – 20 $\mu\text{L min}^{-1}$. Roughly adjust the interface parameters (sprayer position, spray, heater and auxiliary gas flows, in addition to spray, and orifice voltage) for a sufficiently high parent ion signal at $m/z = 292.2$ for thiamethoxam and $m/z 250.2$ for CGA322704.

Using the Analyst software quantitative optimisation programme, tune the instrument for thiamethoxam and CGA322704, ensuring that the correct ions are selected (initial Q1 $m/z = 292$ and 250, product ions $m/z = 211$ and 169 for quantification or $m/z = 181$ and 132 for confirmatory) for the primary transition. If desired, manual tuning of the ion optics and collision energy can be carried out to ensure maximum sensitivity.

Connect the LC-pump via the autosampler directly to the MS/MS instrument. Perform repetitive flow injection of thiamethoxam standards using a mobile phase of 80:20 (v/v) ultra pure water:methanol at the required flow rate and at the intended split ratio. Tune the interface parameters (sprayer position, spray and heater gas flows, spray and orifice voltage) and the collision gas flow for maximum sensitivity.