Study Number 02-5700

Report Title S-metolachlor

Independent Laboratory Validation of an Analytical Method for the Determination of CGA-77102 and Degradates CGA-51202, CGA-354743, and CGA-37735 in Soil and Thatch, and CGA-77102 in Grass by High Performance Liquid Chromatography with Mass Spectrometric Detection. Novartis Report Number 127-99.

Author P G Evans

Summary

The analytical procedure documented in Novartis Report Number 127-99 (Appendix 14) was independently validated by laboratory personnel totally unfamiliar with the method and using equipment (supplies and instrumentation) not previously used by the method developer.

Control samples of soil, thatch and grass were fortified at two levels in quintuplet, and analysed using the supplied analytical procedure at the following rates:

- Soil and Thatch : S-metolachlor (also known as CGA-77102) and degradates CGA-51202, CGA-354743, and CGA-37735 , at 10 ppb (0.01 mg kg^{-1}) and 100 ppb (0.1 mg kg^{-1})

- Grass : CGA-77102 only, at 1 ppm (1 mg kg^{-1}) and 10 ppm (10 mg kg^{-1})

The recovery of CGA-77102 and degradates CGA-51202, CGA-354743, and CGA-37735 was determined for each sample and the results used to determine the validity of the method.

Validation was successful on the first attempt for grass and soil analysis. Similarly, analysis of CGA-77102, CGA-51202 and CGA-354743 in thatch was successful on the first attempt. However, two attempts at the validation were required for the analysis of CGA-37735 in thatch. On the initial attempt at the validation, low recovery levels were obtained for samples that were

fortified at the LOQ. Samples that were fortified at ten times the LOQ gave successful recoveries. A second attempt at the validation was made, which produced very similar recoveries. After consultation with the sponsor, it was agreed that the cause was likely to be matrix effects (suppression), and a modification to the method was required. The samples that were fortified with CGA-37735 at the LOQ were diluted to a similar dilution level as those fortified at ten times the LOQ. Reanalysis of these diluted samples gave acceptable recovery data.

The control samples analysed as part of this study showed amounts of CGA-77102, CGA-51202, CGA-354743, and CGA-37735 less than 30% of the LOQ.

1 Introduction

The aim of this study was to achieve an independent laboratory validation of the analytical method described in Novartis Report Number 127-99 (Appendix 14) for the determination of CGA-77102 and degradates CGA-51202, CGA-354743, and CGA-37735 in soil and thatch, and CGA-77102 in grass. Specifically to:

a) Prove that procedural recoveries fortified with CGA-77102 and degradates CGA-51202, CGA-354743, and CGA-37735 in soil and thatch, and CGA-77102 in grass, can be taken through the analytical method, and a mean recovery of 70% - 120% of the fortified amount can be achieved with an overall relative standard deviation (RSD) of ≤ 20%.

b) Demonstrate that the relationship between sample concentration and detector response is linear over the working range of the method.

The validation was carried out by personnel totally unfamiliar with the method and using equipment (supplies and instrumentation) not previously used by the method developer, thus meeting the requirements as permitted in OPPTS 850.7100 and EPA guidelines PR Notice 96-1.

2 Materials

2.1 Test and Reference Substance

The following analytical standards were utilised in this study.

Figure 1

Compound : S-metolachlor also known as CGA-77102

CAS Name : Acetamide, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)-, (S)-

CAS Number : 87392-12-9

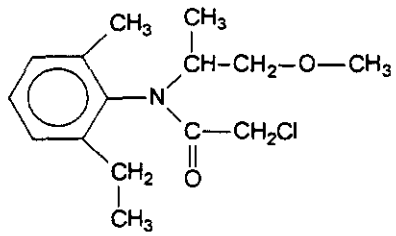


Figure 2

Compound : CGA-354743

CAS Name : Ethanesulfonic acid, 2-[(2-ethyl-6-methylphenyl)(2-methoxy-1-methylethyl)amino]-2-oxo-, sodium salt

CAS Number : Not Assigned

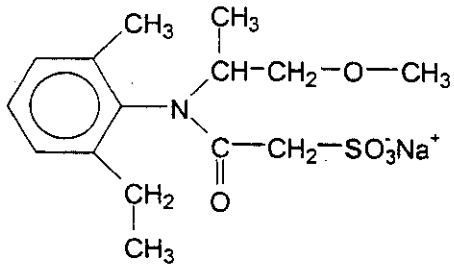


Figure 3

Compound : CGA-51202

CAS Name : Acetic acid, [(2-ethyl-6-methylphenyl)(2-methoxy-1-methylethyl)amino]oxo-

CAS Number : 152019-73-3

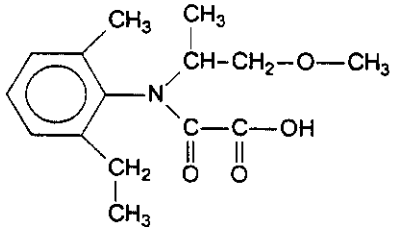
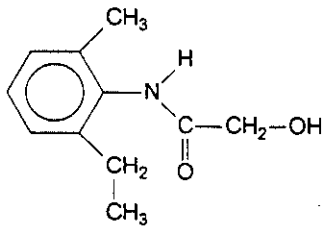


Figure 4

Compound : CGA-37735

CAS Name : Acetamide, N-(2-ethyl-6-methylphenyl)-2-hydroxy-

CAS Number : 97055-05-5



CGA-077102, analytical standard reference R228405/a (purity 99.6% w/w), expiry date February 2004.
CGA-354743, analytical standard reference 1634/met02/a (purity 99.0% w/w), expiry date March 2004.
CGA-051202, analytical standard reference 1634/met01/a (purity 100.0% w/w), expiry date June 2005.
CGA-037735, analytical standard reference 1634/met03/a (purity 95.0% w/w), expiry date May 2003.

The analytical standards were obtained from Syngenta Crop Protection, Analytical Development and Product Chemistry GS2131, Münchwilen AG, Breitenloh 5, CH-4333 Münchwilen. These standards are stored in the Environmental Sciences standard store at <-18°C and were used within their expiry date.

2.2 Test System

The matrices utilised in this study are detailed in Table 4.

Table 4. : Control Samples

Matrix Type	Sample Number	Trial Reference	Source
Soil	1	SMET-SOIL	Study Number 51-99 Research Options Inc. Montezuma, Georgia, USA
Thatch	1	SMET-THATCH	
Grass	1	SMET-GRASS	

3 Methods

3.1 Preparation and Stability of Analytical Standard Solutions

Stock standard solutions CGA-77102 and degradates CGA-51202, CGA-354743, and CGA-37735 were independently prepared in acetonitrile, according to the procedures described in the analytical method (Appendix 14). These were subsequently used to prepare working standard solutions in acetonitrile or mixed working standard solutions in acetonitrile:water (20:80%, v/v).

CGA-77102 and degradates CGA-51202, CGA-354743, and CGA-37735 in acetonitrile are assumed to be stable when stored in amber bottles at ≤ -18°C for up to 4 months after preparation.

CGA-77102 and degradates CGA-51202, CGA-354743, and CGA-37735 in acetonitrile:water (20:80%, v/v) are assumed to be stable when stored in amber bottles at < 8°C or ≤ -18°C for up to 4 months after preparation.

3.2 Fortification Levels

Recovery of the analyte through the analytical procedure was assessed by fortifying aliquots of soil or thatch with CGA-77102, CGA-51202, CGA-354743, and CGA-37735, or grass with CGA-77102. Five replicate recovery levels were carried out at the LOQ (0.01 mg kg^{-1} for soil and thatch, 1.0 mg kg^{-1} for grass) and five replicate recoveries were carried out at ten times the LOQ (0.1 mg kg^{-1} for soil and thatch, 10.0 mg kg^{-1} for grass). Fortification levels are summarised in Tables 5 and 6.

Table 5. : Fortification Levels – Soil and Thatch

Matrix	Fortification Level* (mg kg^{-1})	Number of Replicates
Soil or Thatch	Control	2
	0.01	5
	0.1	5

*Fortified with CGA-77102, CGA-51202, CGA-354743, and CGA-37735.

Table 6. : Fortification Levels – Grass

Matrix	CGA77102 only Fortification Level (mg kg^{-1})	Number of Replicates
Grass	Control	2
	1.0	5
	10	5

3.3 Analytical Procedures

3.3.1 Sample Analysis

Samples were analysed according to procedures described in detail in Novartis Report Number 127-99 (Appendix 14). The method was followed as written, with the following modifications:

- The instrument used for analysis was a PE Sciex API 3000 LC-MS-MS (see Appendix 1 for full details of analytical instrumentation).
- Instrument conditions – The chromatographic conditions listed in Table 3 of the method were modified slightly. The switch of polarity from negative to positive mode was reduced to 7 minutes from 10 minutes, due to the retention times of the analytes being shorter than expected.
- Re-analysis of CGA-37735 in thatch – the samples were required to be diluted (4xdilution), to eradicate the effect of matrix suppression, this was possible due to the increased sensitivity of this instrumentation used by the independent laboratory.

The percentage recovery obtained for each sample was calculated and these results were used to assess the relative standard deviation of the analytical method.

3.3.2 Detector Linearity

Standard solutions containing CGA-77102, CGA-51202, CGA-354743, and CGA-37735 at concentration levels from 0.005 to 0.1 $\mu g\ mL^{-1}$ in triplicate (equivalent to 0.1 to 2.0 ng of analyte injected on to the column) were analysed by LC-MS-MS, using the conditions specified in the analytical method. The mean detector response was plotted against standard concentration.

6 Recommendations

The following comments on the method indicate where minor improvements or clarification may be useful.

- In section II, part H, Time Required - using the equipment available in our laboratory, we found that the time required for analysis to be longer than that stated. It may be useful to indicate in the method that the time required for analysis may be greater than one day but no more than two, or otherwise quote 6 - 12 samples can be completed in one day, dependent on the facilities available.

- Section II part D, Analytical Procedure -

  - To increase the efficiency of the process it may be useful to load the samples onto the SPE columns using sample reservoirs.
  - At parts 2.12 - 2.16 and 3.9 - 3.12, the flow rate for loading the sample onto the columns is stated, it would also be helpful to state the flow rates for elution, clean-up etc.
  - At parts 2.16 and 3.12 – it may be beneficial to advise that if the glass vessels stated are not available, suitable alternative vessels may be used.

- In section 2 part I – state that matrix/suppression effects are possible. Dilution of samples may be necessary to overcome this, as long as the sensitivity of the analytical instrumentation allows.

- To suggest the use of bumping granules when using rotary evaporators.

7 Personnel and Testing Facilities

The study was carried out by P G Evans (Study Director) and P Edwards, Environmental Fate and Exposure Section, HAES, Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, UK (Telephone +44 (0) 1344 424701).

8 Study Dates

The study was carried out between 8th April 2002 and 2nd May 2002.

Instrument Description

Pump	:	Agilent 1100 series quaternary pump model number G1311A
Degasser	:	Agilent 1100 series model number G1322A
Column Oven	:	Agilent 1100 series model number G1316A fitted with column switching valve
Detector	:	Applied Biosystems API 3000 triple quadrupole mass spectrometer
Autosampler	:	CTC HTS PAL

Mass Spectrometer Conditions – CGA-77102

Interface	:	TurboIonSpray
Polarity	:	Positive
Nebuliser gas (NEB)	:	Nitrogen set at 12 (arbitrary units)
Curtain gas (CUR)	:	Nitrogen set at 10 (arbitrary units)
Temperature (TEM)	:	350°C
Ionspray voltage	:	5200 V
Collision gas setting (CAD)	:	Nitrogen set at 3 (arbitrary units)
Scan type	:	MRM
Q1 mass	:	284.02
Q3 mass	:	252.07
Dwell time	:	200 ms
Resolution Q1	:	Unit
Resolution Q2	:	Unit
Declustering potential (DP)	:	36 V
Focusing potential (FP)	:	230 V
Entrance potential (EP)	:	10 V
Collision energy (CE)	:	21 V
Collision cell exit potential (CXP)	:	10 V
Electron multiplier setting (CEM)	:	2600 V

Mass Spectrometer Conditions – CGA-51202

Interface	:	TurboIonSpray
Polarity	:	Negative
Nebuliser gas (NEB)	:	Nitrogen set at 12 (arbitrary units)
Curtain gas (CUR)	:	Nitrogen set at 10 (arbitrary units)
Temperature (TEM)	:	420°C
Ionspray voltage	:	-4500 V
Collision gas setting (CAD)	:	Nitrogen set at 3 (arbitrary units)
Scan type	:	MRM
Q1 mass	:	278.15
Q3 mass	:	206.20
Dwell time	:	200 ms
Resolution Q1	:	Unit
Resolution Q2	:	Unit
Declustering potential (DP)	:	-21 V
Focusing potential (FP)	:	-210 V
Entrance potential (EP)	:	-10 V
Collision energy (CE)	:	-16V
Collision cell exit potential (CXP)	:	-9 V
Electron multiplier setting (CEM)	:	2600 V

Mass Spectrometer Conditions – CGA-354743

Interface	:	TurboIonSpray
Polarity	:	Negative
Nebuliser gas (NEB)	:	Nitrogen set at 12 (arbitrary units)
Curtain gas (CUR)	:	Nitrogen set at 10 (arbitrary units)
Temperature (TEM)	:	420°C
Ionspray voltage	:	-4500 V
Collision gas setting (CAD)	:	Nitrogen set at 3 (arbitrary units)
Scan type	:	MRM
Q1 mass	:	328.18
Q3 mass	:	121.03
Dwell time	:	200 ms
Resolution Q1	:	Unit
Resolution Q2	:	Unit
Declustering potential (DP)	:	-66 V
Focusing potential (FP)	:	-340 V
Entrance potential (EP)	:	-10 V
Collision energy (CE)	:	-32 V
Collision cell exit potential (CXP)	:	-7 V
Electron multiplier setting (CEM)	:	2600 V

Mass Spectrometer Conditions – CGA-37735

Interface	:	TurboIonSpray
Polarity	:	Negative
Nebuliser gas (NEB)	:	Nitrogen set at 12 (arbitrary units)
Curtain gas (CUR)	:	Nitrogen set at 10 (arbitrary units)
Temperature (TEM)	:	420°C
Ionspray voltage	:	-4500 V
Collision gas setting (CAD)	:	Nitrogen set at 3 (arbitrary units)
Scan type	:	MRM
Q1 mass	:	192.01
Q3 mass	:	134.24
Dwell time	:	200 ms
Resolution Q1	:	Unit
Resolution Q2	:	Unit
Declustering potential (DP)	:	-46 V
Focusing potential (FP)	:	-320 V
Entrance potential (EP)	:	-10 V
Collision energy (CE)	:	-22V
Collision cell exit potential (CXP)	:	-9 V
Electron multiplier setting (CEM)	:	2600 V