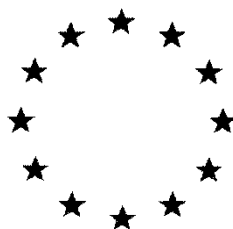


European Commission

**Second Programme for the Renewal of the Inclusion of Active Substances
in Annex I of Council Directive 91/414/EEC**



**Draft Renewal Assessment Report prepared according to the Commission
Regulation (EU) No 1141/2010**

AMITROLE

Volume 3 – Annex B.5

**Rapporteur Member State: France
Co-Rapporteur Member State: Hungary**

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B.5. ANALYTICAL METHODS

B.5.1. ANALYTICAL METHODS FOR THE DETERMINATION OF ACTIVE SUBSTANCE IN TECHNICAL ACTIVE SUBSTANCE AND IN FORMULATION

B.5.1.1. Analytical methods for the determination of active substance in the technical active substance

B.5.1.1.1. Analytical methods

The same analytical method as for the 1st inclusion of Amitrole is proposed.

Reference: Schmit, C. (1999) – Determination in technical active ingredient and formulated products by HPLC method
Method reference: C-ORGANIQUE-00383-E-TA, supersedes the method issued on 11 August, 1998

Principle of the method

The method derived from the CIPAC method 90/TC/(M)/- (Handbook J, page 4).
Amitrole content is determined by HPLC with UV detection ($\lambda = 215 \text{ nm}$) on silica diol phase using the external standard procedure.

Conclusion

The analytical method for the determination of Amitrole in technical material is a collaboratively tested standard CIPAC method. It can be regarded as validated and no additional validation data are necessary.
Nevertheless, the method has been validated in the study Adam, C. (2012), providing that there is no interference > 3%.

B.5.1.1.2. Applicability of existing CIPAC methods

See above.

B.5.1.2. Analytical methods for the determination of significant and/or relevant impurities and additives in the technical active substance

B.5.1.2.1. Analytical methods for the determination of significant and/or relevant impurities

Please refer to Volume 4, confidential part

B.5.1.2.2. Analytical methods for the determination of additives

Please refer to Volume 4, confidential part

B.5.1.3. Methods for the determination of the active substance in the plant protection product

B.5.1.3.1. Analytical methods

Reference: Amic, S. (2012) – Validation of an analytical method for Amitrole in a SL formulation CA 2735
Document No.: S11-04000

Principle of the method

The determination of Amitrole was based on method CIPAC 90/SL/M. It was performed by HPLC/UV ($\lambda = 215 \text{ nm}$).

GLP: yes

A full validation set was performed to demonstrate that the method is suitable for determination of Amitrole in a SL formulation CA2735.

Results

Specificity	Linearity	Accuracy	Repeatability
<3% of the total peak measured for Amitrole (chromatograms provided)	range 250-550 µg/mL n = 7 $y = 0.191674 x - 0.1045$ $r^2 = 0.9993$	mean recovery: 102.4%	RSD: 0.4298% n = 5

Conclusion

The analytical method for the determination of Amitrole in a SL formulation CA2735 is considered as validated in terms of specificity, linearity, accuracy and precision.

B.5.1.3.2. Applicability of existing CIPAC methods

The method used to analyze Amitrole in the formulation Weedazol TL derives from the CIPAC method 90/SL/(M).

B.5.1.4. Method for the determination of significant and/or relevant impurities in the plant protection product

There are no toxicologically, environmentally or ecotoxicologically relevant impurities present in the preparation CA2735. Therefore, analytical methods for the determination of impurities of concern are not required.

Nevertheless, an analytical method for the determination of the synergist ammonium thiocyanate in the preparation CA2735 has been submitted.

Reference: Adam, C. (2011) – Validation of analytical method for the ammonium thiocyanate determination in CA2735, Method reference: C-Organique-00585-TA, version 1
Document No.: 01316

Principle of the method

The determination of ammonium thiocyanate content in CA2735 was performed using a high liquid performance chromatography (HPLC) employing a conductivity detector and an external standard method.
(column: IonPac AS19: 250*4 mm (Dionex ref P/N 062886) coupled Guard column: IonPac AG19: 50*4 mm (Dionex ref P/N 062887))

GLP: yes

Results

Specificity

No significant difference between the retention time of ammonium thiocyanate in the reference item, in the technical ammonium thiocyanate and in the test item was observed.

There was no interference between ammonium thiocyanate and the co-formulants.

Linearity

In order to test the linearity of the method for detecting ammonium thiocyanate without and with formulants, a concentration range represented 40% to 140% of the working level of calibration was used.

Each linearity solution was injected once after a blank solvent injection.

Without co-formulants, the response was found to be linear over the range of concentrations 0.050 to 0.175 mg/mL. Typical calibration data were as follows, $y = 73.808x - 0.0842$ with $R^2 = 0.9999$ and $n = 6$

With co-formulants, the response was found to be linear over the range of concentrations 0.050 to 0.175 mg/mL. Typical calibration data were as follows, $y = 74.3446x - 0.1481$ with $R^2 = 0.9999$ and $n = 6$

Accuracy

The accuracy (analytical recovery) of the method was assessed by referring to the data obtained when testing the linearity with and without co-formulants.

The recoveries ranged from 98.76 to 100.37%.

Precision (repeatability)

Two calibration solutions and 6 test items solutions of 2.5 mg/mL were injected.

The method was shown to be repeatable with a mean ammonium thiocyanate content found at 19.644% with a relative standard deviation (RSD) of 0.61%. This experimental RSD was found to be lower than the Horwitz RSDr (1.71%).

Conclusion

The analytical method for the determination of ammonium thiocyanate in the preparation CA2735 is considered as validated in terms of specificity, linearity, accuracy and precision.

B.5.2. ANALYTICAL METHODS FOR THE DETERMINATION OF RESIDUES OF AMITROLE IN PLANT MATERIELS

The residue definition for monitoring in plant is: Amitrole

MRLs :

Grapes (table and vine): 0.01 mg/kg

Olives: 0.05 mg/kg

Citrus fruit: 0.01 mg/kg

Pome fruit: 0.01 mg/kg

Stone fruit: 0.01 mg/kg

Tree nut: 0.01 mg/kg

The intended uses correspond to commodities with high water content, high acid content and high oil content.

B.5.2.1. Analytical method for the determination of residues of Amitrole in plant materials

B.5.2.1.1. Analytical method for determination of residue in grapes, olives and apples

Reference: Gemrot, F. (2011a) - Validation of analytical method for Amitrole, triazolylalanine and aminotriazolylalanine in grapes

Document No.: S10-03263

Method: AGR/MOA/AMT-8

Sponsor: Nufarm SAS

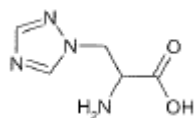
Test facility: Eurofins/ADME BIOANALYSES, Vergèze, France

Principle of the method

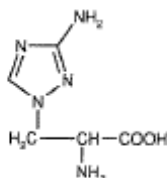
Residues were extracted by homogenisation with acetone/ultra pure water (75/25, v/v) mixture. After filtration an aliquot was taken and washed in the dichloromethane then in hexane. The aqueous phase was acidified then purified on cartridge MCX (500 mg /6 mL). After dry evaporation, the residue was dissolved in ultra pure water and inject in LC-MS/MS (ionisation mode: ESP^+ for Amitrole, ESI^+ for triazolylalanine and ESI^- for aminotriazolylalanine).

Structural formula of metabolites:

Triazolylalanine:



Aminotriazolylalanine:

Transitions used:

Amitrole:

m/z 85.1 → m/z 58.1 (quantification) and m/z 85.1 → m/z 57.1 (confirmation)

triazolylalanine:

m/z 157.1 → m/z 88.1 (quantification) and m/z 157.1 → m/z 69.8 (confirmation)

aminotriazolylalanine:

m/z 170.1 → m/z 83.1 (quantification) and m/z 170.1 → m/z 86.1 (confirmation)

GLP: yes**Results**Specificity

The reagent blank prepared according to the analytical method and the unfortified grapes specimens showed no significant interference (< 30% LOQ) at the retention time of Amitrole or its metabolites (triazolylalanine and aminotriazolylalanine).

Matrix effects of the instrument response presented was considered to be significant (>10% - suppression) for Amitrole in grapes. For this reason, matrix matched calibration standards were used when quantifying Amitrole in grapes. The matrix effect on the instrument response observed for triazolylalanine and aminotriazolylalanine in grapes was considered to be not significant (<10%). For this reason, non matrix-matched calibration standards could be used when quantifying triazolylalanine and aminotriazolylalanine.

Linearity

The linearity of the detector was checked. The calibration curve consisted of six to seven calibration standards covering the concentration range 0.00025 to 0.01 µg/mL for Amitrole and 0.00025 to 0.025 µg/mL for triazolylalanine and aminotriazolylalanine. This calibration curve was linear in this concentration range with a determination coefficient above 0.99.

Amitrole:

 $y = 629421x + 57$, $n = 6$, $R^2 = 0.9969$, mass transition m/z 85.1 → m/z 58.1 $y = 992157x + 62$, $n = 6$, $R^2 = 0.9999$, mass transition m/z 85.1 → m/z 57.1

triazolylalanine :

 $y = 476888x + 15$, $n = 7$, $R^2 = 0.9989$, mass transition m/z 157.1 → m/z 88.1 $y = 841095x - 38$, $n = 7$, $R^2 = 0.9974$, mass transition m/z 157.1 → m/z 69.8

aminotriazolylalanine :

 $y = 687449x + 52$, $n = 6$, $R^2 = 0.9999$, mass transition m/z 170.1 → m/z 83.1

$y = 437011x - 7.8$, $n = 7$, $R^2 = 0.9995$, mass transition m/z 170.1 $\rightarrow$ m/z 86.1

Recovery

Recovery data for Amitrole and its metabolites (triazolylalanine and aminotriazolylalanine) in grapes are given in the table below.

Matrix	Analyte	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Grapes	Amitrole	Primary mass transition m/z 85.1 $\rightarrow$ m/z 58.1			
		0.01	82	14	5
		0.1	97	7	5
		Confirmation mass transition m/z 85.1 $\rightarrow$ m/z 57.1			
		0.01	90	17	5
		0.1	98	9	5
	triazolylalanine	Primary mass transition m/z 157.1 $\rightarrow$ m/z 88.1			
		0.01	92	11	5
		0.1	103	3	5
		Confirmation mass transition m/z 157.1 $\rightarrow$ m/z 69.8			
		0.01	81	16	5
		0.1	101	5	5
	aminotriazolylalanine	Primary mass transition m/z 170.1 $\rightarrow$ m/z 83.1			
		0.01	97	5	5
		0.1	97	2	5
		Confirmation mass transition m/z 170.1 $\rightarrow$ m/z 86.1			
		0.01	76	14	5
		0.1	98	3	5

Mean recoveries are in the acceptable range.

Repeatability

Relative standard deviations (RSDs) are in the acceptable limits (see above).

Limit of quantification (LOQ)

The limit of quantification is determined to be 0.01 mg/kg for Amitrole and each metabolite in grapes.

Conclusion

The analytical method for the determination of Amitrole and its metabolites (triazolylalanine and aminotriazolylalanine) in commodities with high acid content (grapes) is considered as validated in terms of specificity, linearity, accuracy and precision. It is considered as highly specific according to guidance document SANCO/825/00 rev8.1.

Reference: Genrot, F. (2011b) - Validation of analytical method for Amitrole, triazolylalanine and aminotriazolylalanine in olives
Document No.: S10-03184
Method: AGR/MOA/AMT-2
Sponsor: Nufarm SAS
Test facility: Eurofins/ADME BIOANALYSES, Vergèze, France

Principle of the method

Residues were extracted by homogenisation with acetone/ultra pure water (75/25, v/v) mixture. After filtration an aliquot was taken and washed in the dichloromethane then in hexane. The aqueous phase was acidified then purified

on cartridge MCX (500 mg /6 mL). After dry evaporation, the residue was dissolved in ultra pure water and inject in LC-MS/MS (ionisation mode: ESP⁺ for Amitrole, ESI⁺ for triazolyllalanine and ESI⁻ for aminotriazolyllalanine).

Transitions used:

Amitrole:

m/z 85.1 → m/z 58.1 (quantification) and m/z 85.1 → m/z 43.1 (confirmation)

triazolyllalanine:

m/z 157.1 → m/z 88.1 (quantification) and m/z 157.1 → m/z 69.8 (confirmation)

aminotriazolyllalanine:

m/z 170.1 → m/z 83.1 (quantification) and m/z 170.1 → m/z 86.1 (confirmation)

GLP: yes

Results

Specificity

The reagent blank prepared according to the analytical method and the unfortified olive specimens showed no significant interference (< 30% LOQ) at the retention time of Amitrole or its metabolites (triazolyllalanine and aminotriazolyllalanine).

The matrix effect of the instrument response observed for Amitrole in olives was considered to be not significant (<10%). For this reason, non matrix-matched calibration standards could be used when quantifying Amitrole. Matrix effect on the instrument response presented was considered to be significant (>10% - enhancement) for triazolyllalanine and aminotriazolyllalanine in olive. For this reason, matrix matched calibration standards were used when quantifying triazolyllalanine and aminotriazolyllalanine in olive.

Linearity

The linearity of the detector was checked. The calibration curve consisted of seven calibration standards covering the concentration range 0.00025 to 0.025 µg/mL for Amitrole, triazolyllalanine and aminotriazolyllalanine. This calibration curve was linear in this concentration range with a determination coefficient above 0.99.

Amitrole:

$y = 2733134x + 289$, $n = 7$, $R^2 = 0.9971$, mass transition m/z 85.1 → m/z 58.1

$y = 5069913x + 542$, $n = 7$, $R^2 = 0.9962$, mass transition m/z 85.1 → m/z 43.1

triazolyllalanine :

$y = 1173662x + 16$, $n = 7$, $R^2 = 0.9984$, mass transition m/z 157.1 → m/z 88.1

$y = 2003051x + 195$, $n = 7$, $R^2 = 0.9988$, mass transition m/z 157.1 → m/z 69.8

aminotriazolyllalanine :

$y = 747675x + 35$, $n = 7$, $R^2 = 0.9988$, mass transition m/z 170.1 → m/z 83.1

$y = 303158x + 17$, $n = 7$, $R^2 = 0.9990$, mass transition m/z 170.1 → m/z 86.1

Recovery

Recovery data for Amitrole and its metabolites (triazolyllalanine and aminotriazolyllalanine) in olives are given in the table below.

Matrix	Analyte	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Olives	Amitrole	Primary mass transition m/z 85.1 → m/z 58.1			
		0.01	83	4	5
		0.1	99	7	5
		Confirmation mass transition m/z 85.1 → m/z 43.1			
		0.01	90	6	5
		0.1	102	7	5
	triazolylalanine	Primary mass transition m/z 157.1 → m/z 88.1			
		0.01	87	13	5
		0.1	92	3	5
		Confirmation mass transition m/z 157.1 → m/z 69.8			
		0.01	84	6	5
		0.1	94	3	5
	aminotriazolylalanine	Primary mass transition m/z 170.1 → m/z 83.1			
		0.01	90	18	5
		0.1	97	6	5
		Confirmation mass transition m/z 170.1 → m/z 86.1			
		0.01	93	14	5
		0.1	98	2	5

Mean recoveries are in the acceptable range.

Repeatability

Relative standard deviations (RSDs) are in the acceptable limits (see above).

Limit of quantification (LOQ)

The limit of quantification is determined to be 0.01 mg/kg for Amitrole and each metabolite in olives.

Conclusion

The analytical method for the determination of Amitrole and its metabolites (triazolylalanine and aminotriazolylalanine) in commodities with high oil content (olives) is considered as validated in terms of specificity, linearity, accuracy and precision. It is considered as highly specific according to guidance document SANCO/825/00 rev8.1.

Reference: Gemrot, F. (2012) - Validation of analytical method for Amitrole, triazolylalanine and aminotriazolylalanine in olives (Amendment)
Document No.: S10-03184
Sponsor: Nufarm SAS
Test facility: Eurofins/ADME BIOANALYSES, Vergèze, France

The reason for this study is to present the validation of a new analytical method for the confirmatory analyse of aminotriazolylalanine in olives by LC-MS/MS technique (same transition used for quantification and confirmation but with another chromatographic conditions).

Matrix	Analyte	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Olives	aminotriazolylalanine	Confirmation mass transition m/z 170.1 → m/z 83.1			
		0.01	74	4	5
		0.1	83	1	5

Reference: Amic, S. (2012) - Validation of analytical method for Amitrole, triazolyalanine and aminotriazolyalanine in apples
Document No.: S11-03999
Method: AGR/MOA/AMT-9
Sponsor: Nufarm SAS
Test facility: Eurofins/ADME BIOANALYSES, Vergèze, France

Principle of the method

Residues were extracted by homogenisation with acetone/ultra pure water (75/25, v/v) mixture. After filtration an aliquot was taken and washed in the dichloromethane then in hexane. The aqueous phase was acidified then purified on cartridge MCX (500 mg /6 mL). After dry evaporation, the residue was dissolved in ultra pure water and inject in LC-MS/MS (ionisation mode: ESP⁺ for Amitrole, ESI⁺ for triazolyalanine and ESI⁻ for aminotriazolyalanine).

Transitions used:

Amitrole:

m/z 85.1 → m/z 58.1 (quantification) and m/z 85.1 → m/z 57.1 (confirmation)

triazolyalanine:

m/z 157.1 → m/z 88.1 (quantification) and m/z 157.1 → m/z 69.8 (confirmation)

aminotriazolyalanine:

m/z 170.1 → m/z 83.1 (primary conditions) and m/z 170.1 → m/z 83.1 (confirmatory conditions)

GLP: yes

Results

Specificity

The reagent blank prepared according to the analytical method and the unfortified olive specimens showed no significant interference (< 30% LOQ) at the retention time of Amitrole or its metabolites (triazolyalanine and aminotriazolyalanine).

Matrix effect on the instrument response presented was considered to be significant (>20% - suppression) for Amitrole and triazolyalanine in apples. No matrix effect was observed for aminotriazolyalanine. Matrix matched calibration standards were used when quantifying Amitrole and its metabolites (triazolyalanine and aminotriazolyalanine) in apples.

Linearity

The linearity of the detector was checked. The calibration curve consisted of seven to eight calibration standards covering the concentration range 0.00025 to 0.05 µg/mL. This calibration curve was linear in this concentration range with a determination coefficient above 0.99.

Amitrole:

$y = 3619562x + 489$, $n = 7$, $R^2 = 0.9915$, mass transition m/z 85.1 → m/z 58.1

$y = 5729314x + 1156$, $n = 8$, $R^2 = 0.9908$, mass transition m/z 85.1 → m/z 57.1

triazolyalanine:

$y = 3390118x + 416$, $n = 8$, $R^2 = 0.9986$, mass transition m/z 157.1 → m/z 88.1

$y = 6210088x + 950$, $n = 8$, $R^2 = 0.9988$, mass transition m/z 157.1 → m/z 69.8

aminotriazolyalanine :

$y = 6230293x + 185$, $n = 8$, $R^2 = 0.9996$, mass transition m/z 170.1 → m/z 83.1 (primary conditions)

$y = 7088281x - 40$, $n = 8$, $R^2 = 1.0000$, mass transition m/z 170.1 → m/z 83.1 (confirmatory conditions)

Recovery

Recovery data for Amitrole and its metabolites (triazolylalanine and aminotriazolylalanine) in apples are given in the table below.

Matrix	Analyte	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Apples	Amitrole	Primary mass transition m/z 85.1 → m/z 58.1			
		0.01	108	6	5
		0.1	109	4	5
		Confirmation mass transition m/z 85.1 → m/z 57.1			
		0.01	108	7	5
		0.1	109	2	5
	triazolylalanine	Primary mass transition m/z 157.1 → m/z 88.1			
		0.01	96	3	5
		0.1	96	6	5
		Confirmation mass transition m/z 157.1 → m/z 69.8			
		0.01	96	3	5
		0.1	95	6	5
	aminotriazolylalanine	Primary mass transition m/z 170.1 → m/z 83.1 (primary conditions)			
		0.01	90	3	5
		0.1	91	2	5
		Confirmation mass transition m/z 170.1 → m/z 83.1 (confirmatory conditions)			
		0.01	92	2	5
		0.1	95	1	5

Mean recoveries are in the acceptable range.

Repeatability

Relative standard deviations (RSDs) are in the acceptable limits (see above).

Limit of quantification (LOQ)

The limit of quantification is determined to be 0.01 mg/kg for Amitrole and each metabolite in apples.

Conclusion

The analytical method for the determination of Amitrole and its metabolites (triazolylalanine and aminotriazolylalanine) in commodities with high water content (apples) is considered as validated in terms of specificity, linearity, accuracy and precision. It is considered as highly specific according to guidance document SANCO/825/00 rev8.1.

Reference: Ivanov, E. (2012) – Independent laboratory validation of analytical method for determination of Amitrole, Triazolylalanine and Aminotriazolylalanine residues in crops
Document No.: S12-00221
Sponsor: Nufarm SAS
Test facility: Eurofins Agroscience Services Chem GmbH, Hamburg, Germany

Principle of the method

Same as above (methods AGR/MOA/AMT-2 for olives, AGR/MOA/AMT-8 for grapes and AGR/MOA/AMT-9 for apples).

GLP: yes

Results

Specificity

Analysis of control specimens yielded no residues of Amitrole (>30% LOQ). Nevertheless, due to slight residues/interferences of aminotriazolylalanine in grape control specimens and of triazolylalanine in apples, the recoveries were corrected.

Significant matrix effects were found for Amitrole in apples, grapes (at 10*LOQ) and olives and for aminotriazolylalanine and triazolylalanine in grapes and apples by LC-MS/MS. For the validation of recoveries of aminotriazolylalanine and triazolylalanine in apples and grapes, as well as Amitrole in apples, olives and grapes (at 10*LOQ), matrix-matched calibration standards were therefore used for quantification and calibration.

Linearity

The linearity of the detector was checked. The calibration curve consisted of five to eight calibration standards covering the concentration range 0.15 to 25 ng/mL (in solvent) or 0.15 ng/mL to 10 ng/mL (in matrix). This calibration curve was linear in this concentration range with a determination coefficient above 0.99.

Olives:

Amitrole:

$y = 9097.6x + 1018.4$, $n = 6$, $R^2 = 0.9931$, mass transition m/z 85 $\rightarrow$ m/z 58

$y = 32577x + 4465.4$, $n = 6$, $R^2 = 0.9915$, mass transition m/z 85 $\rightarrow$ m/z 43

triazolylalanine :

$y = 30305x + 4313.2$, $n = 8$, $R^2 = 0.9992$, mass transition m/z 157 $\rightarrow$ m/z 88

$y = 65546x + 20559$, $n = 8$, $R^2 = 0.9971$, mass transition m/z 157 $\rightarrow$ m/z 70

aminotriazolylalanine :

$y = 19938x + 5640.1$, $n = 8$, $R^2 = 0.997$, mass transition m/z 170 $\rightarrow$ m/z 83

$y = 86375x + 7178.9$, $n = 8$, $R^2 = 0.9971$, mass transition m/z 170 $\rightarrow$ m/z 83

Grapes :

Amitrole:

$y = 151021x + 21702$, $n = 5$, $R^2 = 0.9965$, mass transition m/z 85 $\rightarrow$ m/z 58

$y = 239907x + 34042$, $n = 5$, $R^2 = 0.9969$, mass transition m/z 85 $\rightarrow$ m/z 57

$y = 84602x + 83052$, $n = 5$, $R^2 = 0.9975$, mass transition m/z 85 $\rightarrow$ m/z 58

$y = 132753x + 136910$, $n = 5$, $R^2 = 0.9971$, mass transition m/z 85 $\rightarrow$ m/z 57

triazolylalanine :

$y = 3737.3x + 496.63$, $n = 8$, $R^2 = 0.9998$, mass transition m/z 157 $\rightarrow$ m/z 88

$y = 8842.8x + 1449.3$, $n = 8$, $R^2 = 0.9995$, mass transition m/z 155 $\rightarrow$ m/z 68

aminotriazolylalanine :

$y = 9180.5x + 2213.3$, $n = 7$, $R^2 = 0.9992$, mass transition m/z 170 $\rightarrow$ m/z 83

$y = 9563.1x + 2000.2$, $n = 7$, $R^2 = 0.9997$, mass transition m/z 170 $\rightarrow$ m/z 83

$y = 35988x + 6256.7$, $n = 7$, $R^2 = 0.9994$, mass transition m/z 170 $\rightarrow$ m/z 83

Apples :

Amitrole:

$y = 66068x + 8873$, $n = 8$, $R^2 = 0.9932$, mass transition m/z 85 $\rightarrow$ m/z 58

$y = 104488x + 16261$, $n = 8$, $R^2 = 0.9927$, mass transition m/z 85 $\rightarrow$ m/z 57

$y = 104488x + 16261$, $n = 8$, $R^2 = 0.9927$, mass transition m/z 85 $\rightarrow$ m/z 57

triazolylalanine :

$y = 9981.8 x + 304.63$, $n = 8$, $R^2 = 0.9999$, mass transition m/z 157 $\rightarrow$ m/z 88

$y = 22127 x + 2301.3$, $n = 8$, $R^2 = 0.9998$, mass transition m/z 157 $\rightarrow$ m/z 70

$y = 6779.5 x + 1842.5$, $n = 6$, $R^2 = 0.9996$, mass transition m/z 157 $\rightarrow$ m/z 88

$y = 14898 x + 5206.5$, $n = 6$, $R^2 = 0.994$, mass transition m/z 157 $\rightarrow$ m/z 70

aminotriazolylalanine :

$y = 10774 x + 1647.9$, $n = 7$, $R^2 = 0.9982$, mass transition m/z 170 $\rightarrow$ m/z 83

$y = 49548 x + 2686.2$, $n = 7$, $R^2 = 0.999$, mass transition m/z 170 $\rightarrow$ m/z 83

$y = 56470 x - 951.39$, $n = 7$, $R^2 = 0.9979$, mass transition m/z 170 $\rightarrow$ m/z 83

Recovery

Recovery data for Amitrole and its metabolites (triazolylalanine and aminotriazolylalanine) in olives, grapes and apples are given in the table below.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

Matrix	Analyte	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Olives	Amitrole	mass transition m/z 85 → m/z 58 (quantitation)			
		0.01	98	9.1	5
		0.1	73	5.1	5
		mass transition m/z 85 → m/z 43 (confirmation)			
		0.01	101	9.3	5
		0.1	75	3.3	5
	triazolylalanine	mass transition m/z 157 → m/z 88 (quantitation)			
		0.01	77	1.5	5
		0.1	80	1.4	5
		mass transition m/z 157 → m/z 70 (confirmation)			
		0.01	83	2.8	5
		0.1	75	3.4	5
	aminotriazolylalanine	mass transition m/z 170 → m/z 83 (quantitation)			
		0.01	85	5.1	5
		0.1	73	2.9	5
		mass transition m/z 170 → m/z 83 (confirmation)			
		0.01	71	7.3	5
		0.1	81	6.8	5
Grapes	Amitrole	mass transition m/z 85 → m/z 58 (quantitation)			
		0.01	70	4.8	5
		0.1	84	6.1	5
		mass transition m/z 85 → m/z 57 (confirmation)			
		0.01	70	4.6	5
		0.1	83	5.8	5
	triazolylalanine	mass transition m/z 157 → m/z 88 (quantitation)			
		0.01	84	5.5	5
		0.1	74	6.6	5
		mass transition m/z 155 → m/z 68 (confirmation)			
		0.01	109	7.0	5
		0.1	91	13	5
	aminotriazolylalanine	mass transition m/z 170 → m/z 83 (quantitation) *			
		0.01	99	9.0	4
		0.1	70	6.3	5
		mass transition m/z 170 → m/z 83 (quantitation) **			
		0.01	142	6.3	4
		0.1	75	5.3	5
		mass transition m/z 170 → m/z 83 (confirmation)			
		0.01	101	6.9	5
		0.1	84	12	5
		mass transition m/z 170 → m/z 83 (confirmation)			
Apples	Amitrole	mass transition m/z 85 → m/z 58 (quantitation)			
		0.01	93	3.4	5
		0.1	77	3.0	5
		mass transition m/z 85 → m/z 57 (confirmation)			
		0.01	94	4.5	5
		0.1	77	2.5	5
	triazolylalanine	mass transition m/z 157 → m/z 88 (quantitation)			
		0.01	97	6.8	5
		0.1	78	3.3	5
		mass transition m/z 155 → m/z 70 (confirmation) *			
		0.01	70	4.7	5
		0.1	80	2.4	5
		mass transition m/z 155 → m/z 70 (confirmation) **			
		0.01	105	3.1	5

		0.1	84	2.3	5
	aminotriazolylalanine	mass transition m/z 170 → m/z 83 (quantitation)			
		0.01	101	2.4	5
		0.1	73	2.3	5
		mass transition m/z 170 → m/z 83 (confirmation)			
		0.01	89	9.0	5
		0.1	71	9.8	5

* corrected recoveries

** uncorrected recoveries

Repeatability

Relative standard deviations (RSDs) are in the acceptable limits (see above).

Limit of quantification (LOQ)

The limit of quantification is determined to be 0.01 mg/kg for Amitrole and its metabolites in each matrix.

Conclusion

The ILV for the determination of Amitrole and its metabolites (triazolylalanine and aminotriazolylalanine) in commodities with high oil content (olives), commodities with high acid content (grapes) and commodities with high water content (apples) is considered as validated in terms of specificity, linearity, accuracy and precision.

Additional methods have been submitted:

Reference: Todd, M. A. (2002) - Development and validation of analytical methods for the determination of residues of the herbicide Amitrole in grapes, juice, must, wine and deposit cards
Document No.: CPF/026
Sponsor: CFPI AGRO
Research laboratory: Huntingdon Life Sciences Ltd., Eye, England

Principle of the method

Grapes samples were extracted with acetone: water, followed by liquid/liquid washes with dichloromethane, then hexane and finally solid phase extraction clean-up using PRS cartridge columns. Following derivatisation (dry pyridine (1 mL) and BSTFA + TMCS (99:1 v:v) reagent (1 mL)), quantification was by gas chromatography using mass selective detection GC-MS (single ion monitored: m/z 285).

Must samples were cleaned-up by liquid/liquid washes with dichloromethane followed by solid phase extraction using PRS cartridge columns. Following derivatisation (dry pyridine (1 mL) and BSTFA + TMCS (99:1 v:v) reagent (1 mL)), quantification was by gas chromatography using mass selective detection GC-MS (single ion monitored: m/z 285).

Juice and wine samples were extracted by solid phase extraction using PRS cartridge columns. Following derivatisation (dry pyridine (1 mL) and BSTFA + TMCS (99:1 v:v) reagent (1 mL)), quantification was by gas chromatography using mass selective detection GC-MS (single ion monitored: m/z 285)

Deposition card (filter paper in Petri dishes) samples were extracted with an acetonitrile : water : triethylamine mixture. Quantification was by high performance liquid chromatography using ultraviolet detection HPLC/UV ($\lambda = 205 \text{ nm}$).

GLP: yes

ResultsSpecificity

Control (untreated) samples of grape, juice, must, wine and deposition cards were submitted to the developed methods. For all matrices the response in chromatograms corresponding to the retention time of Amitrole was less than the limit of detection and therefore reported as not detected.

Linearity

The response of the mass selective detector to Amitrole was found to be linear over the range of concentrations examined (0.0025 to 0.05 µg/mL).

Typical calibration data were as follows: $y = 1364000x - 538.7$, $r^2 = 0.9973$, $n = 7$.

The response of the ultraviolet detector to Amitrole was found to be linear over the range of concentrations examined (2.5 to 50 µg/mL).

Typical calibration data were as follows: $y = 62.61x - 13.65$, $r^2 = 0.9997$, $n = 7$.

Recovery

Recovery data for Amitrole in grapes, juice, must, wine and deposition cards are given in the table below.

Matrix	Analyte	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Grapes	Amitrole	0.01	83.0	6.4	5
		0.1	100.0	8.9	5
		0.5	96.1	5.7	5
Juice	Amitrole	0.01	88.6	3.5	5
		0.1	89.9	4.8	5
		0.5	85.1	5.9	5
Must	Amitrole	0.01	89.3	4.7	5
		0.1	80.9	8.0	5
		0.5	88.6	3.9	5
Wine	Amitrole	0.01	94.5	4.2	5
		0.1	96.4	2.3	5
		0.5	99.0	2.4	5
Deposition cards	Amitrole	0.1	88.5	2.3	3
		0.5	86.9	1.9	3
		3	91.8	0.7	3

Mean recoveries are in the acceptable range.

Remark: For deposition cards, number of sample at each fortification level is insufficient ($n = 3$ instead of $n = 5$). Nevertheless, as data on grapes (which is one of the intended uses) are acceptable, no further data required.

Repeatability

Relative standard deviations (RSDs) are in the acceptable limits (see above).

Limit of quantification (LOQ)

The limit of quantification is determined to be 0.01 mg/kg for Amitrole in grapes, juice, must and wine.

Conclusion

The analytical method for the determination of Amitrole in commodities with high acid content (grape, juice, must, wine, deposition cards) is considered as validated in terms of specificity, linearity, accuracy and precision. Nevertheless, it is not considered as highly specific according to guidance document SANCO/825/00 rev8.1. No data on the derivatisation efficiency has been provided.

Reference: Monnerie, P. (2007) – Analysis of Aminotriazole residues in wine – Performance assessment
Document No.: 60211BPL14

Sponsor: NUFARM SAS
Test facility: CGI laboratory, Ploemeur, France

The purpose of this study was to assess the performance of a method for analyzing Amitrole residues in wine, up to a limit of 10 µg/L.

The considered analytical method comprised 2 components:

- Method no. 1: analysis based on a liquid chromatography method coupled with fluorimetric detection following molecule derivation. This method should be used after wine dilution and purification by solid phase extraction over a polymeric cartridge. The laboratory had performed this method (excluding the purification part) for over 10 years on water and was accredited for this matrix.
- Method no. 2: analysis based on a liquid chromatography method coupled with triple quadrupole mass spectrometry (LC/MS/MS) after molecule derivation. This method should be used after wine purification by solid phase extraction over a polymeric cartridge.

The LC-MS/MS method is confirmed as being a good identification method, but that also presents acceptable quantification performance.

Only validation data for LC-MS/MS method are reported below:

Principle of the method

Following wine purification by solid-phase extraction (SPE), Amitrole is derived by fluorescamine, then the wine is analysed by liquid phase chromatography and mass detection (LC-MS/MS: ionisation mode: ESI⁺).

Transitions used:

Amitrole:
m/z 345 → m/z 317 and m/z 345 → m/z 327

GLP: yes

Results

Specificity

No residues > 30% of the LOQ are detected (non fortified red wine chromatogram provided).

Linearity

Analytical method performance was assessed by examination of the results obtained from five standards over a working range of 5 to 200 µg/L. Each standard point was injected 5 times, while the first point of the range and a water blank (derived) were injected 10 times. Processing of the results gave rise to the linear calibration function, thus validating the method's linearity as $R^2 = 0.9997$.

Amitrole:
 $y = 17.202 x - 22.886$, $n = 5$, $R^2 = 0.9997$

Recovery

Recovery data for Amitrole in wines are given in the table below.

Matrix	Analyte	Fortification level [µg/L]	Mean recovery [%]	RSD [%]	n
White wine	Amitrole	10	72.7	12.14	5
		100	68.8	5.35	5
Red wine	Amitrole	10	91.3	6.23	5
		100	79.5	5.00	5

Mean recoveries are in the acceptable range.

Repeatability

Relative standard deviations (RSDs) are in the acceptable limits (see above).

Limit of quantification (LOQ)

The limit of quantification is determined to be 10 µg/L for Amitrole in wine (white and red).

Conclusion

The analytical method for the determination of Amitrole in commodities with high acid content (red and white wines) is considered as validated in terms of specificity, linearity, accuracy and precision. It is not considered as highly specific according to guidance document SANCO/825/00 rev8.1. Nevertheless, as the ILV of this method is submitted below and validated on two mass transitions, no further data required. No data on the derivatisation efficiency has been provided.

Reference: Royer, A. (2008) – Independent laboratory validation of analytical method for the determination of Amitrole in red wine and white wine by HPLC mass spectrometry detection
Document No.: NUF/AMI/07001
Sponsor: NUFARM SAS
Test facility: ADME BIOANALYSES, Vergèze, France

Principle of the method

Wine is purified by a liquid/solid extraction using an Oasis HLB cartridge. After derivatisation with fluorescamine, the residue of Amitrole is determined by LC-MS/MS using two mass transitions (ionisation mode: ESP⁺).

Amitrole:

m/z 345.1 → m/z 317.3 (quantification) and m/z 345.1 → m/z 327.3 (confirmation)

GLP: yes

Results

Specificity

The reagent blank prepared according to analytical method for wine and the two unfortified red wine and the two unfortified white wine specimen samples, showed no significant interference (<30% LOQ) at the retention time of Amitrole.

Linearity

The linearity of the detector was checked. For LC-MS/MS, the calibration curve consisted of six calibration standards covering the concentration range 0.005 to 0.2 µg/mL. Sometimes, the standard calibration point at 0.2 µg/mL must be discarded. In this case, the fortification level at 100 µg/L must be diluted at half before derivatisation step. This calibration curve was linear in these concentration ranges with a determination coefficient above 0.99.

Amitrole:

y = 5147233 x + 4191.1, n = 6, R² = 0.9948, mass transition m/z 345.1 → m/z 317.3

$y = 3832475x + 3051$, $n = 6$, $R^2 = 0.9946$, mass transition m/z 345.1 $\rightarrow$ m/z 327.3

Recovery

Recovery data for Amitrole in wines are given in the table below.

Matrix	Analyte	Fortification level [µg/L]	Mean recovery [%]	RSD [%]	n
Red wine	Amitrole	Primary mass transition m/z 345.1 $\rightarrow$ m/z 317.3			
		10	92	6	5
		100	83	7	5
		Confirmation mass transition m/z 345.1 $\rightarrow$ m/z 327.3			
		10	93	5	5
		100	82	7	5
White wine	Amitrole	Primary mass transition m/z 345.1 $\rightarrow$ m/z 317.3			
		10	74	19	5
		100	84	16	5
		Confirmation mass transition m/z 345.1 $\rightarrow$ m/z 327.3			
		10	75	19	5
		100	85	15	5

Mean recoveries are in the acceptable range.

Repeatability

Relative standard deviations (RSDs) are in the acceptable limits (see above).

Limit of quantification (LOQ)

The limit of quantification is determined to be 10 µg/L for Amitrole in grapes (red and wine).

Conclusion

The ILV of the analytical method (study 60211BPL14) for the determination of Amitrole in commodities with high acid content (red and white grapes) is considered as validated in terms of specificity, linearity, accuracy and precision.

No data on the derivatisation efficiency has been provided.

Reference: Clemson, A. D. (1996) – Determination of Amitrole residues in vine leaves: validation of the analytical method and analysis of vine leaves treated with Weedazol TL
Document No.: 95/CPFOI7/1134
Sponsor: CFPI AGRO
Research laboratory: Huntingdon Life Sciences Ltd., Eye, England

Principle of the method

Amitrole was extracted from vine leaves (100 g) with aqueous acetone. Following filtration, the filtrate was extracted with dichloromethane. A tenth aliquot of the aqueous acetone phase was concentrated to approximately 10 mL (to remove the acetone) and then passed through activated Dowex-50W ion-exchange media. After elution with aqueous ammonia, the analyte was subjected to a column clean-up procedure on alumina. The amitrole was eluted from the alumina with water and the eluate evaporated to dryness. The residues were resuspended in mobile phase and determined by HPLC with electrochemical detection (HPLC/ECD).

GLP: yes

Results

Specificity

The control grapes sample showed no significant interference (< 30% LOQ) at the retention time of Amitrole.

Linearity

The linearity of the detector was checked. The calibration curve consisted of more than five calibration standards covering the concentration range 0.05 to 5.30 µg/mL. This calibration curve was linear in this concentration range with a determination coefficient above 0.99.

Amitrole:

$$y = 0.0177x - 2.6762, n > 5, R^2 = 0.9995$$

Recovery

Recovery data for Amitrole in vine leaves are given in the table below.

Matrix	Analyte	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Vine leaves	Amitrole	0.01	89.0	5.3	3
		0.025	86.4	12.3	3
		0.05	76.4	5.5	3
		0.1	90.7	18.5	3
		0.25	86.6	16.4	3
		0.5	80.7	16.8	3
		1.0	77.7	6.8	3

Mean recoveries are in the acceptable range.

Remark: At each fortification level, number of sample is insufficient (n = 3 instead of n = 5).

Repeatability

Relative standard deviations (RSDs) are in the acceptable limits (see above).

Limit of quantification (LOQ)

The limit of quantification cannot be determined.

Conclusion

The analytical method for the determination of Amitrole in commodities with high water content (vine leaves) is not considered as validated in terms of accuracy and is not considered as highly specific according to guidance document SANCO/825/00 rev8.1. Nevertheless, no further data required as other methods are considered as validated.

B.5.2.1.2. Applicability of the multi-residue method

The applicability of the DFG method S19 multi-residue method has not been demonstrated and is required. (December 2012: Nufarm stated that a study is ongoing and that the final report will be available by March 2013.)

B.5.2.2. Conclusion

An analytical method (Gemrot, F. 2011a, Gemrot, F. 2011b, Amic, S. 2012) and its ILV (Ivanov, E., 2012) using LC-MS/MS are considered as validated for the determination of Amitrole and its metabolites triazolyalanine and aminotriazolyalanine in plants (grapes, olives, apples) with **LOQ = 0.01 mg/kg** for each analyte.

B.5.3. ANALYTICAL METHODS (RESIDUES) FOR FOOD OF ANIMAL ORIGIN

Studies on nature or magnitude of residues in commodities of animal origin are not available, or required. Following the estimation of the dietary burden arising from the authorised uses of Amitrole, MRLs for livestock products are not required because livestock is not expected to be exposed to significant levels of Amitrole residues.

So, no analytical method is necessary.

B.5.4. ANALYTICAL METHODS FOR THE ANALYSIS OF RESIDUES OF AMITROLE IN SOIL, WATER AND AIR**B.5.4.1. Methods for the analysis of residues in soil**

The residue definition for monitoring in soil is: Amitrole

Reference: Amic, S. (2012) - Validation of analytical method for Amitrole in soil and water
Document No.: S11-04001

Principle of the method

Residues of Amitrole were extracted by homogenisation with a 75/25, v/v acetone/ultra water mixture. After filtration, an aliquot was acidified and purified over a MCX cartridge (500 mg / 6 mL). After evaporation, the dry extract was dissolved in ultra pure water and then analysed by LC-MS/MS with electropositive ion spray.

Amitrole:

m/z 85.1 → m/z 58.1 (quantification) and m/z 85.1 → m/z 57.1 (confirmation)

Characterization of soil control samples:

Source	Lufa Speyer	Lufa Speyer
Soil sample identification	2.1*	6S**
Organic matter (%)	0.62 ± 0.07	1.64 ± 0.12
pH (CaCl₂)	5.1 ± 0.4	7.1 ± 0.1
Cation exchange capacity (meq/100g)	3.8 ± 0.8	23.7 ± 7.0
Soil textural classification (USDA)	Sand	Clay
Sand content (% w/w) [2000-50 µm]	87.3 ± 1.2	22.2 ± 1.8
Silt content (% w/w) [50-2 µm]	10.1 ± 1.6	36.8 ± 2.0
Clay content (% w/w) [<2µm]	2.7 ± 1.1	41.0 ± 1.9

* Control matrix n°232 at Eurofins/ADME BIOANALYSES

** Control matrix n°234 at Eurofins/ADME BIOANALYSES

GLP: yes

Results

Only results concerning soil matrix are reported.

Specificity

The reagent blank prepared according to the analytical methods and the unfortified soil samples showed no significant interference (< 30% LOQ) at the retention times of Amitrole.

The matrix effect on the instrument response observed for Amitrole in soil (sandy and clay soil) was considered to not be significant (<20%). For this reason, non matrix-matched calibration standards could be used when quantifying Amitrole in soil.

Linearity

The linearity of the detector was checked. The calibration curve consisted of eight calibration standards covering the concentration range 0.00025 to 0.05 µg/mL. This calibration curve was quadratic in this concentration range with a determination coefficient above 0.99.

Amitrole:

$$y = -2.09 \cdot 10^7 x^2 + 4.08 \cdot 10^6 x + 219, n = 8, R^2 = 0.9996, \text{mass transition } m/z \ 85.1 \rightarrow m/z \ 58.1$$

$$y = -3.49 \cdot 10^7 x^2 + 6.55 \cdot 10^6 x + 243, n = 8, R^2 = 0.9990, \text{mass transition } m/z \ 85.1 \rightarrow m/z \ 57.1$$

Recovery

Recovery data for Amitrole in soil are given in the table below.

Matrix	Analyte	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Sandy soil	Amitrole	Primary mass transition m/z 85.1 → m/z 58.1			
		0.01	86	4	5
		0.1	90	3	5
		Confirmation mass transition m/z 85.1 → m/z 57.1			
		0.01	88	3	5
		0.1	89	3	5
Clay soil	Amitrole	Primary mass transition m/z 85.1 → m/z 58.1			
		0.01	93	7	5
		0.1	91	2	5
		Confirmation mass transition m/z 85.1 → m/z 57.1			
		0.01	92	8	5
		0.1	93	2	5

Mean recoveries are in the acceptable range.

Repeatability

Relative standard deviations (RSDs) are in the acceptable limits (see above).

Limit of quantification (LOQ)

The limit of quantification is determined to be 0.01 mg/kg for Amitrole in soil.

Conclusion

The analytical method for the determination of Amitrole in soil is considered as validated in terms of specificity, linearity, accuracy and precision. It is considered as highly specific according to guidance document SANCO/825/00 rev8.1.

$$LC_{50} \text{ earthworm} = 448 \text{ mg a.s./kg}$$

B.5.4.2. Methods for the analysis of residues in water

The residue definition for monitoring in water is: Amitrole

Reference: Amic, S. (2012) - Validation of analytical method for Amitrole in soil and water
Document No.: S11-04001

Principle of the method

The matrix was acidified and purified over a MCX cartridge (500 mg / 6 mL). After evaporation, the dry extract was dissolved in ultra pure water and then analysed by LC-LC-MS/MS with electropositive ion spray.

Amitrole:

m/z 85.1 → m/z 58.1 (quantification) and m/z 85.1 → m/z 57.1 (confirmation)

Characterization of water control samples:

Water type	Surface water	Drinking water
Source	“Le Tech” river	Tap water
pH*	7.2	7.3
Silt content (mg/L) (Method NF T90-029)	84	531
Dissolved Organic Carbon (DOC) (mg/L) (Method MS99999)	2.4	1.7
Total hardness as CaCO ₃ (mg/L) (Method NF EN ISO 9963-1 ^a)	65	307

a: accredited method (NF EN 17025)

* measured at Eurofins/ADME BIOANALYSES

GLP: yes

Results

Only results concerning water matrix are reported.

Specificity

The reagent blank prepared according to the analytical methods and the unfortified water samples showed no significant interference (< 30% LOQ) at the retention times of Amitrole.

The matrix effect on the instrument response observed for Amitrole in water (drinking and surface water) was considered to not be significant (<20%). For this reason, non matrix-matched calibration standards could be used when quantifying Amitrole in water.

Linearity

The linearity of the detector was checked. The calibration curve consisted of seven calibration standards covering the concentration range 0.25 to 12.5 µg/L. This calibration curve was linear in this concentration range with a determination coefficient above 0.99.

Amitrole:

$y = 3.53 \cdot 10^3 x + 345$, $n = 7$, $R^2 = 0.9967$, mass transition m/z 85.1 → m/z 58.1

$y = 5.38 \cdot 10^3 x + 610$, $n = 7$, $R^2 = 0.9970$, mass transition m/z 85.1 → m/z 57.1

Recovery

Recovery data for Amitrole in water are given in the table below.

Matrix	Analyte	Fortification level [µg/L]	Mean recovery [%]	RSD [%]	n
Drinking water	Amitrole	Primary mass transition m/z 85.1 → m/z 58.1			
		0.05	97	6	5
		0.5	94	2	5
		Confirmation mass transition m/z 85.1 → m/z 57.1			
		0.05	90	6	5
		0.5	92	2	5
Surface water	Amitrole	Primary mass transition m/z 85.1 → m/z 58.1			
		0.05	94	3	5
		0.5	92	2	5
		Confirmation mass transition m/z 85.1 → m/z 57.1			
		0.05	100	6	5
		0.5	92	1	5

Mean recoveries are in the acceptable range.

Repeatability

Relative standard deviations (RSDs) are in the acceptable limits (see above).

Limit of quantification (LOQ)

The limit of quantification is determined to be 0.05 µg/L for Amitrole in water (drinking and surface).

Conclusion

The analytical method for the determination of Amitrole in water (surface and drinking) is considered as validated in terms of specificity, linearity, accuracy and precision. It is considered as highly specific according to guidance document SANCO/825/00 rev8.1.

NOEC (daphnia) = 0.32 mg as/L and EC₅₀ (algae) = 2.1 mg as/L

Additional methods have been submitted:

Reference: Todd, M. A. (2004) – Development and validation of an analytical method for the determination of residues of the herbicide Amitrole in water
Document No.: CPF/021

Principle of the method

A sub-sample (10 mL) of the untreated water sample was measured into a polypropylene bottle (500 mL). The sample was fortified with Amitrole at this stage if required. Acetic acid (1 mL) and ultra pure water (300 mL) were added to the sample and mixed well. A reservoir (60 mL) was attached to a SCX cartridge. The cartridge was prepared by washing with methanol (5 mL) and water (5 mL), ensuring that it did not dry out. The sample was loaded onto the cartridge at a steady rate, allowing the eluate to drain to waste. The polypropylene bottle was rinsed with water (5 mL) and the rinsate passed through the cartridge to waste. The cartridge was washed with methanol (5 mL), allowing the eluate to go to waste. The cartridge was eluted with 1% ammonia in methanol (5 mL), collecting the eluate in a glass centrifuge tube (10 mL). The sample was evaporated to dryness at approximately 70°C under a steady stream of nitrogen, and reconstituted in an appropriate volume of water prior to quantitation by LC-MS (Ion monitoring: m/z 85.0).

Water characteristics:

Water type	Surface water
Source	local "Diss Mere"
pH	8.00
Dissolved oxygen	11.3 mg/L O ₂ at 14.8°C

Conductivity	570 μ S at 25°C
Alkalinity	175 mg/L CaCO ₃
Total hardness	170 mg/L CaCO ₃
Total Organic Carbon (TOC)	14.3 mg C/L
Dissolved Organic Carbon (DOC)	12.8 mg C/L

GLP: yes

Results

Specificity

Controls (untreated) were submitted to the methodology. There was no apparent response (i.e. > 20% of LOQ) in the chromatograms region corresponding to the Amitrole retention time.

Linearity

The chromatographic response to Amitrole was shown to be linear over the range of concentrations 0.5 - 100 ng/mL and gave the following typical equation: $y = 365.811 x + 342.172$. The coefficient of determination R^2 was 0.9995 with $n = 8$.

Recovery

Recovery data for Amitrole in water are given in the table below.

Matrix	Analyte	Fortification level [ng/L]	Mean recovery [%]	RSD [%]	n
Water (surface)	Amitrole	50	96.8	4.4	5
		100	104	2.3	5
		500	108	1.3	5

Mean recoveries are in the acceptable range.

Repeatability

Relative standard deviations (RSDs) are in the acceptable limits (see above).

Limit of quantification (LOQ)

The limit of quantification is determined to be 50 ng/L (= 0.05 μ g/L) for Amitrole in water (surface).

Conclusion

The analytical method for the determination of Amitrole in water (surface) is considered as validated in terms of specificity, linearity, accuracy and precision. It is not considered as highly specific according to guidance document SANCO/825/00 rev8.

Reference: Cohn, R. (2000) – Validation of an analytical method for the determination of the herbicide Amitrole in water
Document No.: 9CFP0102BPL1

Principle of the method

About 50 mL of sample was measured into a glass flask. The pH was adjusted with phosphoric acid (3.2 mol/L or 0.8 mol/L) at 4.20 ± 0.05 . Then an aliquot part of the sample was spiked with the test substance and 1 mL of this part was transferred in a 2-mL glass vial and stored at $4^\circ\text{C} \pm 2^\circ\text{C}$ in the dark for at least 30 minutes. Fifty μ L of

fluorescamine (5 g/l in acetonitrile) was gently added with a syringe. The glass vial was incubated at $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 1 hour and 100 μL were immediately injected in the chromatographic system (LC with fluorimetric detection).

Characteristics of water matrices:

Water type	Mineral water	
Source	VOLVIC	HEPAR
Calcium	9.9 mg/L	555 mg/l
Magnesium	6.1 mg/L	110 mg/L
Sodium	9.4 mg/L	14 mg/L
Potassium	5.7 mg/L	-
Chlorures	8.4 mg/L	-
Nitrates	6.3 mg/L	2.9 mg/L
Sulfates	6.9 mg/L	1479 mg/L
Silice	30.0 mg/L	-
Bicarbonates	65.3 mg/L	403 mg/L
Dry residus	109 mg/L	2580 mg/L
pH	7	7

GLP: yes

Results

Specificity

Controls (untreated) were submitted to the methodology. There was no apparent response (i.e. $> 30\%$ of LOQ) in the chromatograms region corresponding to the Amitrole retention time. The control samples showed no detection signal.

Linearity

The calibration curve over the range of 0.05 to 1.00 $\mu\text{g/L}$ gave the following typical equation: $y = -5589.1 + 398398x$. The coefficient of determination R^2 was 0.999 and $n = 5$.

Recovery

Recovery data for Amitrole in water are given in the table below.

Matrix	Analyte	Fortification level [$\mu\text{g/L}$]	Mean recovery [%]	RSD [%]	n
Soft water (VOLVIC)	Amitrole	0.05	106	9.5	5
		0.5	82	5.5	5
Hard water (HEPAR)	Amitrole	0.05	102	9.3	5
		0.5	79	3.7	5

Mean recoveries are in the acceptable range.

Repeatability

Relative standard deviations (RSDs) are in the acceptable limits (see above).

Limit of quantification (LOQ)

The limit of quantification is determined to be 0.05 $\mu\text{g/L}$ for Amitrole in water (mineral).

Conclusion

The analytical method for the determination of Amitrole in water (mineral) is considered as validated in terms of specificity, linearity, accuracy and precision. It is not considered as highly specific according to guidance document SANCO/825/00 rev8.1.

No data on the derivatisation efficiency has been provided.

B.5.4.3. Method for the analysis of residue in air

The residue definition for monitoring in air is: Amitrole

Reference: Todd, M. (2001) – Amitrole and ammonium thiocyanate: Validation of analytical methodology for the determination of residues on typical protective clothing and in associated samples generated from operator exposure studies

Document No.: CPF053/003333

Principle of the method

Air filter samples were extracted in ammonia: methanol (1:9 v/v), the extracts were evaporated to dryness and re-suspended in water prior to quantification of Amitrole by HPLC/UV ($\lambda = 205 \text{ nm}$).

GLP: yes

Only results for Amitrole and air filters are presented.

ResultsSpecificity

The unfortified specimens showed no significant interference ($> 20\% \text{ LOQ}$) in the region in the chromatograms corresponding to the retention time of Amitrole.

Linearity

The response of the detectors to Amitrole was shown to be linear over the range of concentrations 0.02 to 50 $\mu\text{g/mL}$.
Amitrole:

$$y = 71.532x + 1.240 \text{ with } R^2 = 1.000 \text{ and } n = 11$$

Recovery

Recovery data for Amitrole in air filters are given in the table below.

Matrix	Analyte	Fortification level [mg/filter]	Mean recovery [%]	RSD [%]	n
Air filters	Amitrole	0.01	87.6	2.4	7
		0.1	95.0	3.0	7
		1	98.7	0.6	7

Mean recoveries are in the acceptable range.

Repeatability

Relative standard deviations (RSDs) are in the acceptable limits (see above).

Limit of quantification (LOQ)

The limit of quantification is determined to be 0.01 mg/filter for Amitrole in air filters.

Conclusion

The analytical method for the determination of Amitrole in air filters is considered as validated in terms of specificity, linearity, accuracy and precision. It is not considered as highly specific according to guidance document SANCO/825/00 rev8.1. Nevertheless, sufficient methods are available in soil and water.

Some parameters requested in guidance document SANCO/825/00 rev 8.1 (extraction efficiency, retention capacity of the sorbent) are not reported. Furthermore, as part of the first field operator exposure study, it was demonstrated that the contamination through inhalation was negligible. In addition Amitrole is not volatile.

Nevertheless, as Amitrole is classified as harmful Xn, a fully validated method is required according to guidance document SANCO/825/00 rev 8.1. (December 2012: Nufarm stated that the validation is ongoing and that the final report will be provided by Q2 2013.)

$$AOEL_{\text{systemic}} = 0.001 \text{ mg/kg bw/d} \quad C = (0.001 * 0.1 * 60) / 20 = 0.0003 \text{ mg/m}^3 \text{ air}$$

B.5.5. ANALYTICAL METHODS (RESIDUES) FOR BODY FLUIDS AND TISSUES

Amitrole is not classified as toxic or very toxic, but is classified reprotoxic (cat. 3).

An analytical method has been submitted in urine:

Reference: Gemrot, F. (2011c) – Validation of an analytical method for the determination of Amitrole on typical protective clothing and in associated samples generated from the field operator exposure study ChR-11-8256 Document No.: S10-03673

Principle of the method

For urine, specimens were acidified then purified on cartridge MCX (500 mg / 6 mL). After evaporation, the residue was dissolved in ultra pure water. Finally, Amitrole residues present in the extracts were quantified by LC-MS/MS (ionization mode: ESI⁺).

Transitions used:

Amitrole:

m/z 85.1 → m/z 58.1 (quantification) and m/z 85.1 → m/z 57.1 (confirmation)

GLP: yes

Only results for urine are presented. For validation on other matrices see below B.5.6.

Results

Specificity

The unfortified specimens showed no significant interference (< 30% LOQ) at the retention time of Amitrole.

Linearity

The linearity of the detector was checked. The calibration curve consisted of seven calibration standards covering the concentration range 1.25 µg/mL to 125 µg/L. These calibration curves were linear in this concentration range, with a determination coefficient above 0.99.

$y = 1513x + 352$, $n = 7$, $R^2 = 0.9986$, mass transition m/z 85.1 → m/z 58.1

$y = 2354x + 999$, $n = 7$, $R^2 = 0.9984$, mass transition m/z 85.1 → m/z 57.1

Recovery

Recovery data for Amitrole in urine are given in the table below.

Matrix	Analyte	Fortification level [µg/L]	Mean recovery [%]	RSD [%]	n
Urine	Amitrole	Primary mass transition m/z 85.1 → m/z 58.1			
		5	88	6	7
		50	104	2	7
		2500	99	1	7
		Confirmation mass transition m/z 85.1 → m/z 57.1			
		5	89	11	7
		50	104	3	7
		2500	101	2	7

Mean recoveries are in the acceptable range.

Repeatability

Relative standard deviations (RSDs) are in the acceptable limits (see above).

Limit of quantification (LOQ)

The limit of quantification is determined to be 5 µg/L for Amitrole in urine.

Conclusion

The analytical method for the determination of Amitrole in body fluids (urine) is considered as validated in terms of specificity, linearity, accuracy and precision. It is considered as highly specific according to guidance document SANCO/825/00 rev8.1.

B.5.6. OTHER METHODS

An analytical method has been submitted (the same as in urine) for the determination of Amitrole in the specimens from the field operator exposure.

Reference: Gemrot, F. (2011c) – Validation of an analytical method for the determination of Amitrole on typical protective clothing and in associated samples generated from the field operator exposure study ChR-11-8256
Document No.: S10-03673

Principle of the method

For coverall, cotton undergarments, face/neck wipes and hand wash, specimens were shaken in ultra pure water for extraction. An aliquot was diluted in ultra pure water.

Finally, amitrole residues present in extracts were quantified by LC-MS/MS (ionization mode: ESI⁺).

Transitions used:

Amitrole:

m/z 85.1 → m/z 58.1 (quantification) and m/z 85.1 → m/z 57.1 (confirmation)

GLP: yes

Only results for coverall, cotton undergarments, face/neck wipes and hand wash are presented.

Results

Specificity

The unfortified specimens showed no significant interference (< 30% LOQ) at the retention time of Amitrole.

Linearity

The linearity of the detector was checked. The calibration curve consisted of 6-7 calibration standards covering the concentration range 0.00025 µg/mL to 0.05 µg/mL. These calibration curves were linear in this concentration range, with a determination coefficient above 0.99.

Recovery

Recovery data for Amitrole in coverall, cotton undergarments, face/neck wipes and hand wash are given in the table below.

Matrix	Analyte	Fortification level [µg/specimen]	Mean recovery [%]	RSD [%]	n
Coverall	Amitrole	Primary mass transition m/z 85.1 → m/z 58.1			
		10	83	3	7
		100	98	4	7
		5000	90	3	7
		Confirmation mass transition m/z 85.1 → m/z 57.1			
		10	86	7	7
		100	97	2	7
		5000	90	4	7
Cotton undergarment	Amitrole	Primary mass transition m/z 85.1 → m/z 58.1			
		1	89	5	7
		10	108	1	7
		500	104	2	7
		Confirmation mass transition m/z 85.1 → m/z 57.1			
		1	88	5	7
		10	116	1	7
		500	103	2	7
Face/neck wipes	Amitrole	Primary mass transition m/z 85.1 → m/z 58.1			
		0.1	93	10	7
		1	100	3	7
		50	98	3	7
		Confirmation mass transition m/z 85.1 → m/z 57.1			
		0.1	91	8	7
		1	100	2	7
		50	95	2	7
Hand wash	Amitrole	Primary mass transition m/z 85.1 → m/z 58.1			
		1	97	5	7
		10	109	4	7
		500	102	3	7
		Confirmation mass transition m/z 85.1 → m/z 57.1			
		1	97	8	7
		10	106	6	7
		500	102	1	7

Mean recoveries are in the acceptable range.

Repeatability

Relative standard deviations (RSDs) are in the acceptable limits (see above).

Limit of quantification (LOQ)

The limit of quantification is determined to be 10 µg/specimen in coverall, 1 µg/specimen in cotton undergarments and hand wash, 0.1 µg/specimen in face/neck wipes for Amitrole.

Conclusion

The analytical method for the determination of Amitrole in coverall, cotton undergarments, face/neck wipes and hand wash is considered as validated in terms of specificity, linearity, accuracy and precision.

The methods for the determination of Amitrole residues used in the residue trials and stability studies submitted in Volume 3 – B7 are presented below:

Reference: Amic, S. (2012) – Amitrole - Residue study on grapes in France in 2011
Eurofins report n° S11-01150
Sponsor: NUFARM SAS
Test facility: Eurofins/ADME BIOANALYSES, Vergèze, France

The method referenced as AGR/MOA/AMT-8 at Eurofins/ADME BIOANALYSES has been used for the analysis of Amitrole, triazolyalanine and aminotriazolyalanine in grapes. This method has been validated during the validation study S10-03263 (see above in monitoring part B.5.2.1.1.).

Validation data obtained during the analytical studies are summarised below and confirmed the validation of the method.

Matrix	Analyte	Specificity	Linearity		Recovery	
			Concentration range (µg/mL)	Correlation coefficient r^2 (n)	Fortification level (mg/kg) (n)	Mean Recovery (%)
Grape fruit	Amitrole	Interference <30% of LOQ	0.00025 – 0.025	0.9959 (7)	0.01 (1)	102
					0.1 (1)	106
	Triazolyalanine	Interference <30% of LOQ	0.00025 – 0.025	0.9996 (7)	0.01 (1)	97
					0.1 (1)	82
	Aminotriazolyalanine	Interference <30% of LOQ	0.00025 – 0.025	0.9994 (7)	0.01 (1)	109
					0.1 (1)	85

Conclusion

The analytical method for the determination of Amitrole and its metabolites triazolyalanine and aminotriazolyalanine is considered as validated.

Reference: Amic, S. (2011) – Amitrole - Residue study on olives in Spain in 2010
Eurofins report n° S10-03088
Sponsor: NUFARM SAS
Test facility: Eurofins/ADME BIOANALYSES, Vergèze, France

Reference: Amic, S. (2011) – Amitrole - Residue exploratory trial on processing olives in Spain in 2010
Eurofins ADME report n° S10-03541 N
Sponsor: NUFARM SAS
Test facility: Eurofins/ADME BIOANALYSES, Vergèze, France

The method referenced as AGR/MOA/AMT-2 at Eurofins/ADME BIOANALYSES has been used for the analysis of Amitrole, triazolyalanine and aminotriazolyalanine in olives. This method has been validated during the validation study S10-03184 (see above in monitoring part B.5.2.1.1.).

Validation data obtained during the analytical studies are summarised below and confirmed the validation of the method.

Study	Matrix	Analyte	Specificity	Linearity		Recovery	
				Concentration range (µg/mL)	Correlation coefficient r^2 (n)	Fortification level (mg/kg) (n)	Mean Recovery (%)
S10-03088	Table olive	Amitrole	Interference <30% of LOQ	0.00025 – 0.025	0.9977 (7)	0.01 (1)	86
						0.1 (1)	94
		Triazolyalanine	Interference <30% of LOQ	0.00025 – 0.025	0.9999 (7)	0.01 (1)	87
						0.1 (1)	94
		Aminotriazolyalanine	Interference <30% of LOQ	0.00025 – 0.025	0.9995 (7)	0.01 (1)	103
						0.1 (1)	91
	Processing olive	Amitrole	Interference <30% of LOQ	0.00025 – 0.025	0.9977 (7)	0.01 (1)	87
						0.1 (1)	89
		Triazolyalanine	Interference <30% of LOQ	0.00025 – 0.025	0.9999 (7)	0.01 (1)	90
						0.1 (1)	88
		Aminotriazolyalanine	Interference <30% of LOQ	0.00025 – 0.025	0.9995 (7)	0.01 (1)	101
						0.1 (1)	91
S10-03541 N	Processing olive	Amitrole	Interference <30% of LOQ	0.00025 – 0.025	0.9926 (6)	0.01 (1)	95
						1 (1)	103

Conclusion

The analytical method for the determination of Amitrole and its metabolites triazolyalanine and aminotriazolyalanine is considered as validated.

Reference: Biever, R. C. (1996) – Amitrole: The accumulation and degradation of Amitrole in apples
Springborn Lab report n° 95-08-6065
Sponsor: CFPI
Test facility: Springborn Laboratories, Inc., Wareham, USA

Principle of the method

Amitrole is extracted and concentrated from apples by ethanol partition; the apple-solvent mixture is shaken on a shaker table and filtered. A portion of the ethanolic extract is then partitioned onto cation exchange resin, the Amitrole residues are eluted from the column and the eluent is rotary evaporated to dryness. The Amitrole residues are dissolved in E-Pure water. An aliquot of this solution is analyzed by isocratic elution HPLC using electrochemical detection.

GLP: yes

Results

Specificity

The chromatograms (unfortified sample and fortified samples at each level) showed no significant interference (< 30% LOQ) at the retention time of Amitrole.

Linearity

The linearity of the detector was checked. The calibration curve consisted of 4 calibration standards in duplicate covering the concentration range 10 µg/L to 150 µg/L. This calibration curve was linear in this concentration range, with a determination coefficient of 0.9962.

Recovery

Recovery data for Amitrole in apples are given in the table below.

Matrix	Analyte	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Apples	Amitrole	0.01	89.5	-	2
		0.025	85.2	4.7	3
		0.05	82.7	8.2	3
		0.1	75.8	3.6	3

In addition, recovery data obtained during the analytical studies are summarised below.

Matrix	Analyte	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Apples	Amitrole	0.025	76.1	7.6	5
		0.1	77.9	5.1	6

Mean recoveries are in the acceptable range. By pooling results at 0.025 and 0.1 mg/kg, number of sample is acceptable.

Repeatability

Relative standard deviations (RSDs) are in the acceptable limits (see above).

Limit of quantification (LOQ)

The limit of quantification is determined to be 0.025 mg/kg (as the 1st fort. lev. is not considered as completely validated) in apples for Amitrole.

Conclusion

The method for the determination of Amitrole in apples was successfully validated in terms of specificity, linearity, accuracy and precision. Nevertheless, the method is not considered as highly specific, but can be used as pre-registration method.

Reference: Wilson, A. J. (1999) – Amitrole - Raw agricultural and processed commodity study on grapes in France and Spain
HLS report n° CPF024
Sponsor: CFPI AGRO
Research laboratory: Huntingdon Life Sciences Ltd., Eye, England

The analysis was conducted based on an analytical method previously developed by Huntingdon Life Sciences and documented in report no. 93/CPF017/0935. The method was further developed and validated in study number CPF/026 (see above in monitoring part B.5.2.1.1.) prior to any analyses in this study.

Validation data obtained during the analytical studies are summarised below.

Matrix	Analyte	Specificity	Linearity	Recovery
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			Concentration range (µg/mL)	Correlation coefficient r^2 (n)	Fortification level (mg/kg) (n)	Mean Recovery (%)
grapes, juice, must and wine	Amitrole	Interference <30% of LOQ	0.0025 – 0.05	0.9974 (7)	-	-
					-	-
deposition cards	Amitrole	Interference <30% of LOQ	2.5 - 50	0.9998 (7)	-	-
					-	-

Conclusion

No data concerning the recovery is provided. Nevertheless, as the analytical method has been validated in monitoring part B.5.2.1.1. in the same laboratory and on the same matrices, the analytical method for the determination of Amitrole and its metabolites triazolyalanine and aminotriazolyalanine is considered as validated.

Reference: Clemson, A. D. (1997) – Amitrole: determination of freezer storage stability of Amitrole residues in grapes
HLS report 95/CPF018/1407
Sponsor: CFPI AGRO
Research laboratory: Huntingdon Life Sciences Ltd., Eye, England

The analytical method used on this study for the quantitative analysis of Amitrole residues in grapes was previously validated at Huntingdon Life Sciences and the data reported in report No. 94/CPF017/0450. A novel GC-MS method has been developed and validated in report No. 96/CPF020/0540 for confirmation of the residue level found in the sample assayed after 726 days storage.

Principle of the method

Amitrole is extracted from grapes with aqueous acetone. Following filtration, the filtrate was extracted with dichloromethane. A tenth aliquot of the aqueous acetone phase was concentrated to approximately 10 mL (to remove the acetone) and then passed through activated Dowex-50W ion-exchange media. After washing with water, the Amitrole was eluted from the Dowex with aqueous ammonia. The eluate was dried by rotary evaporation under vacuum at about 60°C and the residue resuspended in water before loading onto an alumina column. After washing with a small volume of water, the Amitrole was eluted with a further volume of water. The eluate was evaporated to dryness under vacuum at about 60°C and the residue dissolved in mobile phase. The resuspended sample was transferred to vials for analysis by HPLC using electrochemical detection.

GLP: yes

Results

Specificity

The chromatograms (control grapes and control grapes fortified samples at each level) showed no significant interference (< 30% LOQ) at the retention time of Amitrole.

Linearity

HPLC analysis of standards to cover the range 50 – 5000 ng/mL Amitrole has been shown to produce a linear calibration with a coefficient of determination r^2 of greater than 0.99.

Recovery

Recovery data for Amitrole in grapes are given in the table below.

Matrix	Analyte	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Grapes	Amitrole	0.01	84.2	17.5	3
		0.025	96.0	11.3	3
		0.05	100.9	2	3
		0.1	86.6	12.3	3
		0.25	81.3	4.9	3
		0.5	100.1	10.1	3
		1	83.0	11.4	3

Mean recoveries are in the acceptable range.

Repeatability

Relative standard deviations (RSDs) are in the acceptable limits (see above).

Limit of quantification (LOQ)

The limit of quantification is determined to be 0.01 mg/kg in grapes for Amitrole.

Conclusion

The method for the determination of Amitrole in grapes was successfully validated in terms of specificity, linearity, accuracy and precision. Nevertheless, the method is not considered as highly specific, but can be used as pre-registration method.

Reference: Amic, S. (2011) – Determination of storage stability of Amitrole in grapes and olives under freezer storage conditions
Eurofins/ADME report n° S10-03264

The methods referenced as AGR/MOA/AMT-1 and AGR/MOA/AMT-2 were used for the analysis of Amitrole and its metabolites triazolyalanine and aminotriazolyalanine, respectively in grapes and olive matrices. These methods have been validated during the validation studies S10-03263 and S10-03184 (see above in monitoring part B.5.2.1.1.).

Validation data obtained during the analytical studies are summarised below.

Matrix	Analyte	Specificity	Linearity		Recovery	
			Concentration range (µg/mL)	Correlation coefficient r^2 (n)	Fortification level (mg/kg) (n)	Mean Recovery (%) at T0
Grapes	Amitrole	Interference <0.03%	0.00025 – 0.025	0.9959 (7)	0.1 (2)	101
	Triazolyalanine	Interference <0.03%	0.00025 – 0.025	0.9996 (7)	0.1 (2)	84
	Aminotriazolyalanine	Interference <0.03%	0.00025 – 0.025	0.9994 (7)	0.1 (2)	103
Olive	Amitrole	Interference <0.03%	0.00025 – 0.025	0.9944 (7)	0.1 (2)	100
	Triazolyalanine	Interference <0.03%	0.00025 – 0.025	0.9993 (7)	0.1 (2)	93
	Aminotriazolyalanine	Interference <0.03%	0.00025 – 0.025	0.9985 (7)	0.1 (2)	101

Conclusion

The analytical methods for the determination of Amitrole and its metabolites triazolyalanine and aminotriazolyalanine are considered as validated.

Reference: Biever, R. C. (2000) – Amitrole - Stability in apples stored under frozen conditions
Springborn lab (USA) report n° 12983.0896.6128.395

The method used in this stability study has been validated above (see reference: Biever, R. C. (1996)).

Conclusion

The analytical method for the determination of Amitrole is considered as validated with a LOQ of 0.025 mg/kg.

B.5.7. SUMMARY AND CONCLUSIONS

B.5.7.1. Analytical methods for determination of active substance and by-products in the active substance as manufactured, in the plant protection product and the assay of residue

Analytical method CIPAC method 90/TC/(M)- for the determination of Amitrole in technical material is considered as validated.

An HPLC/UV method based on CIPAC method 90/SL/M for the determination of Amitrole in the preparation CA2735 is considered as validated.

There are no toxicologically, environmentally or ecotoxicologically relevant impurities present in the preparation CA2735. Therefore, analytical methods for the determination of impurities of concern are not required. Nevertheless, an analytical method for the determination of the synergist ammonium thiocyanate in the preparation CA2735 has been submitted and considered as validated.

B.5.7.2. Analytical methods for determination of residues

Analytical methods for residue determination in food of plant origin

The residue definition for monitoring in plant is Amitrole.

An analytical method (Gemrot, F. 2011a and 2011b, Amic, S. 2012) and its ILV (Ivanov, E. 2012) using LC-MS/MS are considered as validated for the determination of Amitrole and its metabolites triazolylalanine and aminotriazolylalanine in plants (grapes, olives, apples) with **LOQ = 0.01 mg/kg** for each analyte.

The applicability of the DFG method S19 multi-residue method has not been demonstrated and is required. (December 2012: Nufarm stated that a study is ongoing and that the final report will be available by March 2013.)

Analytical methods for residue determination in soil, water and air

The residue definition for monitoring in soil is Amitrole.

An analytical method (Amic, S. 2012) using LC-LC-MS/MS is considered as validated for the determination of Amitrole in soil with **LOQ = 0.01 mg/kg**.

The residue definition for monitoring in water is Amitrole.

An analytical method (Amic, S. 2012) using LC-LC-MS/MS is considered as validated for the determination of Amitrole in drinking and surface water with **LOQ = 0.05 µg/L**.

The residue definition for monitoring in air is Amitrole.

An analytical method fully validated is required according to guidance document SANCO/825/00 rev 8.1. (December 2012: Nufarm stated that the validation is ongoing and that the final report will be provided by Q2 2013.)

Analytical methods for residue determination in body fluids

An analytical method (Gemrot, F., 2011c) using LC-MS/MS is considered as validated for the determination of Amitrole in body fluids (urine) with **LOQ = 5 µg/L**.

B.5.8. REFERENCES RELIED ON

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
IIIA 5.2.1/01	Amic, S	2012	Validation of an analytical method to analyze amitrole in CA 2735 Eurofins report n°S11-04000 GLP Not published	Y	NUFAR M
IIIA 5.2.5	Adam, C.	2011	Validation of analytical method used for ammonium thiocyanate content determination in CA2735. Nufarm report n° 01316 Not published	Y	NUFAR M
IIA 4.3/01	Todd M. A.	2002	Development and validation of analytical methods for the determination of residues of the herbicide amitrole in grapes, juice, must, wine and deposit cards HLS, UK Nufarm SAS Report N°: CPF/026 GLP: yes Unpublished	Y	NUFAR M
IIA 4.3/02	Gemrot F.	2011a	Validation of analytical method for amitrole, triazolyalanine and aminotriazolyalanine in grapes eurofins, France Nufarm SAS Report N°: S10-03263 GLP: yes Unpublished	Y	NUFAR M
IIA 4.3/07	Gemrot F.	2011b	Validation of analytical method for amitrole, triazolyalanine and aminotriazolyalanine in olives eurofins, France Nufarm SAS Report N°: S10-03184 GLP: yes Unpublished	Y	NUFAR M
IIA 4.3/07bis	Gemrot F.	2012	Validation of analytical method for amitrole, triazolyalanine and aminotriazolyalanine in olives-amendment- eurofins, France Nufarm SAS Report N°: S10-03184 GLP: yes Unpublished	Y	NUFAR M

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
IIA 4.3/08	Amic, S.	2012	Validation of analytical method for amitrole, triazolyalanine and aminotriazolyalanine in apples eurofins, France Nufarm SAS Report N°: S11-03999 GLP: yes Unpublished	Y	NUFAR M
IIA 4.3/03	Ivanov, E	2012	AMITROLE- Independent Laboratory Validation of Analytical Method for Determination of Amitrole, Triazolyalanine and Aminotriazolyalanine Residues in Crops Report N°: S12-00221 GLP: yes Not published	Y	NUFAR M
IIA 4.3/04	Monnerie P.	2007	Analysis of aminotriazole residues in wine - Performance assessment CGI laboratory, France Nufarm SAS Report N°: 60211BPL14 GLP: yes Unpublished	Y	NUFAR M
IIA 4.3/05	Royer A.	2008	Independent Laboratory Validation of analytical method for the Determination of Amitrole in red wine and white wine by HPLC mass spectrometry detection eurofins, France Nufarm SAS Report N°: NUF/AMI/07001 GLP: yes Unpublished	Y	NUFAR M
IIA 4.4/01	Amic, S.	2012	Validation of analytical method for determining amitrole in soils and waters eurofins, France Nufarm SAS Report N°: S11-04001 GLP: yes Unpublished	Y	NUFAR M
IIA 4.5/02	Todd M. A.	2004	Development and validation of an analytical method for the determination of residues of the herbicide amitrole in water HLS, UK Nufarm SAS Report N°: CPF/021 GLP: yes Unpublished	Y	NUFAR M
IIA 4.5/01	Colin R.	2000	Validation of an analytical method for the determination of the herbicide amitrole in water CGI laboratory, France Nufarm SAS Report N°: 9CFP01 02BPL 1 GLP: yes Unpublished	Y	NUFAR M

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IIA4.8/01	Gemrot, F.	2011c	Validation of an analytical method for the determination of amitrole on typical protective clothing and in associated samples generated (including urine) from operator exposure study ChR-11-8256. ADME Eurofins Nufarm SAS Report n° S10-03673 GLP: yes Unpublished	Y	NUFARM

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