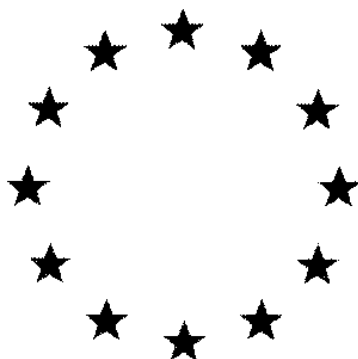


# European Commission



**Draft (Renewal) Assessment Report prepared  
according to the Commission Regulation (EC) No  
1107/2009**

**Daminozide (ISO); 4-(2,2-  
dimethylhydrazino)-4-oxobutanoic  
acid; *N*-dimethylaminosuccinamic  
acid**

**Volume 3 - B.5 (PPP) - Dazide Enhance**

Rapporteur Member State: Czech Republic  
Co-Rapporteur Member State: Hungary

**Version history page**

<b>Date</b>	<b>Version</b>	<b>Reason for revision</b>
April, 2018	Version 1	First draft
October, 2018	Version 2	Notifier's and co-RMS comments
June, 2019	Version 3	Update following the ECHA accordance check

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**B.5 METHODS OF ANALYSIS**

Details of the literature search undertaken are available. No relevant scientifically peer-reviewed open literature reference has been identified for plant protection product Dazide Enhance.

**B.5.1 Methods used for generation of pre-authorisation data****B.5.1.1 Analysis of the Plant Protection Product*****(a) Methods for the determination of the active substance and/or variant in the plant protection product***

The following study was submitted by the Notifier to cover the analysis of the active substance in the plant protection product Dazide Enhance.

<b>Reference:</b>	Bates, G.J.D. (2012): Validation of Analytical Method M741 'High Performance Liquid Chromatographic Determination of Daminozide in Technical Material and Formulations' for the FAL 2400 Formulation
Report No.:	J19126
Guideline:	SANCO/3030/99 rev. 4 (11/07/00)
GLP:	Yes
Published:	No
<b>Previous evaluation:</b>	Submitted for the purpose of renewal

**Principle of the method M741 (Daminozide in formulation FAL 2400)**

Samples (0.18-0.29 g) are weighed into 150 mL flasks and diethyl phthalate internal standard (100 mL) is added. The samples are sonicated for 10 minutes, filtered if necessary, and analysed by high performance liquid chromatography with UV detection at 220 nm, using an ACE C18 column and isocratic elution with a mobile phase water/acetonitrile/methanol. Quantification is performed using daminozide reference standard solutions containing diethyl phthalate as an internal standard.

Column	Ace 5µm C18 (250 mm x 4.6 mm)
Column temperature	30°C
Detection	UV 220 nm
Injection volume	2 µL
Flow rate	1 ml/min
Mobile phase	40/40/20 water/acetonitrile/methanol

**Specificity**

A small interfering peak was observed at the retention time of daminozide in the blank formulation. However, this peak contributes only 0.3% to the active substance peak, significantly below the allowable 3%, and is not considered significant. The specificity of the method has therefore been demonstrated.

Analyte identity was confirmed by retention time match with an analytical standard and by comparison of UV spectra.

**Linearity**

The linearity of detector response was demonstrated using six standard solutions across the nominal concentration range of 0.02 to 0.06 g daminozide, with a coefficient of determination ( $R^2$ ) of 0.9994 (regression line equation  $y = 9.508x + 0.008$ ).

**Precision (Repeatability)**

Repeatability data was generated from six sample solutions. The relative standard deviation (RSD) obtained was within the guideline requirements and is presented in table below.

Analyte	Number of Samples (n)	Mean Content (%)	RSD (%)	Acceptable RSD (%)
Daminozide	6	86.8	0.527	1.369

**Accuracy (Recovery)**

Recovery data was generated from six samples of blank formulation fortified with a known amount of daminozide. The mean percentage recovery obtained was within the guideline requirements and is presented in table below.

Analyte	Fortification Range (% of Nominal)	Number of Samples (n)	Mean Recovery (%)	RSD (%)	Acceptable Recovery (%)
Daminozide	75 - 125	6	100.3	0.348	98 - 102

**RMS comments and conclusion**

The method was validated on linearity, precision, accuracy and specificity in accordance with SANCO/3030/99 rev. 4. Thus, the method M741 is valid and suitable for determination of Daminozide in Dazide Enhance. Representative chromatograms attached.

***(b) Methods for determination of relevant impurities identified in the technical material or which may be formed during manufacture of the plant protection product or from degradation of the plant protection product during storage***

The following study was submitted by the Notifier to cover the analysis of the relevant impurities Unsymmetrical Dimethylhydrazine (UDMH) and N-Nitrosodimethylamine (NDMA) in the plant protection product Dazide Enhance.

<b>Reference:</b>	Knowles, R.J. (2010): Validation of GC Laboratories Ltd Method M487 'Determination of Unsymmetrical Dimethylhydrazine (UDMH) and N-Nitrosodimethylamine (NDMA) in Daminozide by Gas Chromatography' for the Dazide Enhance Formulation
Report No.:	J17929
Guideline:	Not stated
GLP:	Yes
Published:	No
<b>Previous evaluation:</b>	Submitted for the purpose of renewal

**Principle of the method M487 (UDMH and NDMA in formulation Dazide Enhance - former recipe)**

Samples (1 g daminozide) are transferred to a vial and p-xylene (1 mL) is added. The vial is shaken and allowed to stand for 10 minutes. The sample is syringe filtered (0.45 µm) and analysed by gas chromatography with nitrogen-phosphorous detection (GC-NPD) using Carbowax column. Quantification is performed using external UDMH and NDMA reference standard solutions.

Column	1.5 m x 4.0 mm i.d. glass packed with 6.6% Carbowax 20M on 80/120 Carbopack B
Column temperature	140°C
Injection temperature	200°C
Detector temperature	250°C
Detection	NPD
Injection volume	15 µL
Carrier gas flow-rate	Approx. 20 ml/min (15psi)

**Specificity**

Analyte identity was confirmed by retention time match with analytical standards. No interferences were observed at the retention times of interest in any control samples, demonstrating specificity of the method.

**Linearity**

The linearity of detector response was demonstrated using five standard solutions across the nominal concentration range of 0.1173 - 0.4692 µg/mL for UDMH and five standard solutions across the nominal concentration range of 0.1294 - 0.5176 µg/mL for NDMA. The results are presented in table below.

Analyte	Concentration Range (µg/mL)	Coefficient of determination (R <sup>2</sup> )	Regression line equation
UDMH	0.1173 - 0.4692	0.989	$y = 20.378x - 1.857$
NDMA	0.1294 - 0.5176	0.982	$y = 58.302x - 0.707$

**Precision (Repeatability)**

Repeatability data was generated from five determinations of a sample fortified with known amounts of UDMH and NDMA. The relative standard deviations (RSD) obtained were within the guideline requirements and are presented in table below.

Analyte	Number of Samples (n)	Mean Content (mg/kg)	RSD (%)	Acceptable RSD (%)
UDMH	5	0.565	3.51	11.5
NDMA	5	0.620	3.71	11.5

**Accuracy (Recovery)**

Recovery data was generated by the standard addition technique. Samples of formulation were fortified with known amounts of UDMH and NDMA at three concentrations. The mean percentage recoveries obtained were within the guideline requirements and are presented in table below.

Analyte	Fortification Range (mg/kg)	Number of Samples (n)	Mean Recovery (%)	Acceptable Recovery (%)
UDMH	0.1173 - 0.5865	3	104.6	75 - 125
NDMA	0.1294 - 0.6470	3	111.8	75 - 125

#### Limit of quantification (LOQ)

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been demonstrated to be 0.1173 mg/kg for UDMH and 0.1294 mg/kg for NDMA.

#### Confirmation

Confirmation by GC/MS is not possible due to the very low levels of UDMH and NDMA present. The formulation contains quantities of UDMH and NDMA undetectable by the method with the consequence that confirmation can only be carried out by comparison retention times from recovery samples and standards. In both case the retention times were essentially the same.

#### RMS comments and conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANCO/3030/99 rev. 4. Confirmatory analysis by GC/MS is not possible. Nevertheless the method M487 is suitable for determination of UDMH and NDMA in Dazide Enhance. Representative chromatograms attached.

#### *(c) Methods for the determination of relevant co-formulants or components of co-formulants, where required by the national competent authorities*

With respect to toxicological, eco-toxicological or environmental aspects the product Dazide Enhance does not contain any relevant formulants. Therefore, a special analytical method and validation is not needed.

#### CIPAC methods

A CIPAC method for the determination of Daminozide in SG formulations is not available.

#### **B.5.1.2 Methods for the Determination of Residues**

All methods for the determination of daminozide residues are included in Volume 3 CA\_B-5.

#### *(a) Methods In soil, water, sediment, air and any additional matrices used in support of environmental fate studies*

No new environmental fate studies on Dazide Enhance for which analytical methods are required are being submitted in support of the renewal of approval of daminozide.

#### *(b) Methods in soil, water and any additional matrices used in support of efficacy studies*

No new efficacy studies on Dazide Enhance for which analytical methods are required are being submitted in support of the renewal of approval of daminozide.

***(c) Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicology studies***

No new toxicology studies on Dazide Enhance for which analytical methods are required are being submitted in support of the renewal of approval of daminozide.

***(d) Methods in body fluids, air, and any additional matrices used in support of operator, worker, resident and bystander exposure studies***

No new operator, worker, resident or bystander exposure studies on Dazide Enhance for which analytical methods are required are being submitted in support of the renewal of approval of daminozide.

***(e) Methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies***

No new residues studies on Dazide Enhance for which analytical methods are required are being submitted in support of the renewal of approval of daminozide.

***(f) Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies***

No new ecotoxicology studies on Dazide Enhance for which analytical methods are required are being submitted in support of the renewal of approval of daminozide.

***(g) Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests***

<b>Reference:</b>	Knowles, R.J. (2007): Validation of Analytical Method M485 'High Performance Liquid Chromatographic Determination of Daminozide in Technical Material and Formulations' for the Daminozide WSG Formulation
Report No.:	J16147
Guideline:	SANCO/3030/99 rev. 4 (11/07/00)
GLP:	Yes
Published:	No
<b>Previous evaluation:</b>	Submitted for the purpose of renewal

**Principle of the method M485 (Daminozide in formulation Dazide Enhance - former recipe)**

Samples (containing between 0.35 and 0.5 g daminozide) are weighed into 100 mL volumetric flasks and pyridoxine hydrochloride internal standard (100 mL) is added. The flasks are sonicated for 10 minutes and aliquots are analysed by HPLC-UV at 220 nm, using a Phenomenex Luna C18 column and isocratic elution with a mobile phase of 98/2, v/v, water (+ perchloric acid)/acetonitrile. Quantification is performed using daminozide reference standard solutions containing pyridoxine hydrochloride as an internal standard.

Column	Phenomenex Luna 5 µm C18 (250 mm x 4.6 mm)
Column temperature	30 °C



Detection	UV 220 nm
Injection volume	10 µL by loop.
Flow rate	1 ml/min
Mobile phase	98/2 water plus 0.2% of 60% perchloric acid/acetonitrile

**Specificity**

No interferences were observed at the retention time of interest in any control samples, demonstrating specificity of the method. Analyte identity was confirmed by retention time match with an analytical standard and by comparison of UV spectra.

**Linearity**

The linearity of detector response was demonstrated using five standard solutions across the nominal concentration range of 0.04 - 0.125 g daminozide, with a coefficient of determination ( $R^2$ ) of 0.9978 (regression line equation  $y = 6.571x - 0.006$ ).

**Precision (Repeatability)**

Repeatability data was generated from five sample solutions. The relative standard deviation (RSD) obtained was within the guideline requirements and is presented in table below.

Analyte	Number of Samples (n)	Mean Content (%)	RSD (%)	Acceptable RSD (%)
Daminozide	5	83.3	0.477	1.377

**Accuracy (Recovery)**

Recovery data was generated from five samples of blank formulation fortified with a known amount of daminozide. The mean percentage recovery obtained was within the guideline requirements and is presented in table below.

Analyte	Fortification Range (% of Nominal)	Number of Samples (n)	Mean Recovery (%)	RSD (%)	Acceptable Recovery (%)
Daminozide	75 - 125	5	99.4	1.252	98 - 102

**RMS comments and conclusion**

The analytical procedure has been successfully validated in terms of specificity, linearity, precision and accuracy in accordance with the requirements of SANCO/3030/99 rev. 4. Thus, the method M485 is valid and suitable for determination of Daminozide in Dazide Enhance. Representative chromatograms attached.

Analytical method HPLC-UV (M485) and its validation used in study “Comb, A.L. (2003, FNA104/033834, Two Year Storage Stability)” is identical as AM (M485) used in study “Knowles, R.J. (2007, J16147)” above mentioned.

**B.5.2 Methods for Post-Authorisation Control and Monitoring Purposes**

All enforcement methods for the determination of daminozide residues are included in Volume 3 CA\_B-5.

**B.5.3 References relied on**

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Data protection claimed Y/N</b>	<b>Justification if data protection is claimed</b>	<b>Owner</b>
B.5.1.1/01	Bates, G.J.D.	2012	Validation of Analytical Method M741 'High Performance Liquid Chromatographic Determination of Daminozide in Technical Material and Formulations' for the FAL 2400 Formulation Amended Final Report GC Laboratories Ltd., UK. Report No. J19126 GLP Unpublished	N	Y	New data for AIR3 renewal	Fine Agrochemicals Limited
B.5.1.1/02	Knowles, R. J.	2010	Validation of GC Laboratories Ltd Method M487 'Determination of Unsymmetrical Dimethylhydrazine (UDMH) and N-Nitrosodimethylamine (NDMA) in Daminozide by Gas Chromatography' for the Dazide Enhance Formulation GC Laboratories Ltd., UK. Report No. J17929 GLP Unpublished	N	Y	New data for AIR3 renewal	Fine Agrochemicals Limited
B.5.1.2/01	Knowles, R. J.	2007	Validation of Analytical Method M485 'High Performance Liquid Chromatographic Determination of Daminozide in Technical Material and Formulations' for the Daminozide WSG Formulation GC Laboratories Ltd., UK. Report No. J16147 GLP Unpublished	N	Y	New data for AIR3 renewal	Fine Agrochemicals Limited
B.5.1.2/02	Comb, A.L.	2003	Dazide 85 WG Two Year Storage Stability Huntingdon Life Sciences Ltd, UK. Report No. FNA/104/033834 GLP Unpublished	N	Y	New data for AIR3 renewal	Fine Agrochemicals Limited