

Metamitron, Desaminometamitron Final Report

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**Final Report** 

# Validation of an Analytical Method for the Determination of Metamitron and Desaminometamitron in Drinking Water and Surface Water

**Data Requirement** 

EC Guidance document on residue analytical methods, SANCO/825/00 rev. 7 (17/03/2004)

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# 3 Study Objective

The aim of the current study was the validation of an analytical method (postregistration monitoring) for the determination of metamitron and its metabolite desaminometamitron in drinking water and surface water to meet the requirements of guideline SANCO/825/00 rev. 7 (17/03/2004). The analytical method should also be suitable as a confirmatory method.

# 4 Materials and Methods

Common name:	Metamitron	
Structure:	$ \begin{array}{c} \swarrow & N - N \\ \swarrow & \swarrow & N \\ & \swarrow & N \\ & & N \\ & O & NH_2 \end{array} $	
CAS No .:	41394-05-2	
Supplier:	Labor Dr. Ehrenstorfer, Augsburg, Germany	- 80
GAB code:	20041128	
Lot number:	30821	
Purity:	99.4 %	
Certificate of analysis:	05 September 2003	
Expire date:	01 September 2009	
Storage:	Refrigerated (nominally 4 °C)	3

#### 4.1 Test Items

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Common name:	Desaminometamitron		
Structure:	$\bigvee_{O}^{N-N} \xrightarrow{H}_{CH_3}^{N-N}$		
CAS No .:	N/A		
Supplier:	Labor Dr. Ehrenstorfer, Augsburg, Germany		
GAB code:	20041318		
Lot number:	30603		
Purity:	99.0 %		
Certificate of analysis:	05 Jun 2003		
Expire date:	05 Jun 2007		
Storage:	Refrigerated (nominally 4 °C)		

Stock solutions of the test items (effective concentrations 1000  $\mu$ g/mL) were prepared in methanol. Further dilutions containing mixtures of the two test item (10  $\mu$ g/mL, 1  $\mu$ g/mL) were prepared in methanol/water (50/50, v/v). Calibration standards for HPLC analysis (0.005 ng/mL to 1 ng/mL, see Figure 1 to Figure 4 in the appendix) were prepared in HPLC grade water.

All standard solutions were stored refrigerated (4 °C nominally) in the dark.

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# 4.2 Sample Material

Chemical and physico-chemical parameters were determined at CIP GmbH, Pforzheim (non-GLP).

# 4.2.1 Drinking Water

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Origin:	D-76316 Malsch, Germany
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Parameter	Value	Unit	Method of Determination
pH (at 20°C)	7.6		DIN 38404-C 5
Specific electric conductivity (at 20°C)	558	µS/cm	DIN EN 27888
Total hardness	2.9	mmol/L	Complexometric titration
Spectral absorption coefficient (at 254 nm)	0.3	m-1	DIN 38404-C3
Dissolved organic carbon	0.77	mg/L	Infrared detection

### 4.2.2 Surface Water

Origin: Appearance: River "Enz", D-75173 Pforzheim/Germany Slightly brown colour, odorless

Parameter	Value	Unit	Method of Determination
pH (at 20°C)	7.6	1.44	DIN 38404-C 5
Specific electric conductivity (at 20°C)	388	µS/cm	DIN EN 27888
Total hardness	2.2	mmol/L	Complexometric titration
Spectral absorption coefficient (at 254 nm)	3.5	m-1	DIN 38404-C3
Total organic carbon	105	mg C/L	DIN EN 1484 (H3)
Silt content	56	mg/L	Filtration



# 4.3 Procedure for Determination of the Test Items

#### 4.3.1 Principle of the Method

Metamitron and desaminometamitron were determined by direct injection of an aliquot of the water sample in the HPLC/MS-MS system.

#### 4.3.2 Equipment

Volumetric pipettes HPLC with MS/MS detector (details see below) Glass vials for HPLC measurement

#### 4.3.3 Reagents

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Acetonitrile, HPLC garde (Sigma-Aldrich No. 34851) Methanol, LCMS grade (Riedel-de Haën No. 34966) Water, HPLC grade (Merck No. 1.15333)

Acetic acid, p.a. (Merck No. 1.00063)

#### 4.3.4 Sample Preparation

From the homogeneous water sample an aliquot is filled into a 1.5 ml glass vial for HPLC/MS-MS analysis. In case of fortification (recovery) experiments a 10 mL aliquot of the homogeneous water sample is taken and fortified with 10  $\mu$ L of a solution containing 0.05  $\mu$ g/mL respectively 0.5  $\mu$ g/mL of each of the substances under investigation. From this fortified sample an aliquot is filled into a 1.5 ml glass vial for HPLC/MS-MS analysis.

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# 4.3.5 Analysis by HPLC-MS/MS

HPLC system:	MS Surveyor pump with autosampler
Column:	Phenomenex Synergi Fusion-RP 80A, 50 mm x 2.1 mm i.d., 4 µm mean particle size (No. 00B-4424-B0)
Injection volume:	50 μL

Flow rate: 0.5 mL/min

Temperature: 40 °C

Mobile phase:

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B: Acetonitrile

A: Water

C: 1 % acetic acid in water

	Time (min)	% A	% B	% C	Gradient	
	0.00	90	5	5	-	
	0.50	90	5	5	-	
	5.00	35	60	5	linear	
	5.01	90	5	5		
	7.00	90	5	5	-	
Retention times:	Desamin Metamiti	ometami on	tron 2. 2.	6 min 7 min		
Detector:	ThermoFinnigan TSQ Quantum triple quadrupole syst					
	Ionization mode:			ESI		
	Source polarity:			oositive		
	Spray voltage:			4200 V		
	Capillary temperature:			280 °C		
	Capillary offset:			35 V		
	Sheath gas flow:			35 units		
	Auxiliary gas flow:			0 units		
	Collision gas:			Nitrogen 3.0 mTorr		
	Scan width:			0.1 amu		
	Ouadrupole 1 width:			0.7 amu		
	Quadrup	Quadrupole 3 width:			0.7 amu	
	Flow to detector:			2.0 – 4.0 r	nin	
	Split befo	ore detect	or: a	pprox. 1:	5	
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Compound	Parent m/z	Dwell time (sec)	Collision energy (V)	Fragment ions (m/z)	lon type
Metamitron	203.0	0.1	31	104.1	qualifier
	203.0	0.1	21	175.0	<u>quantifier</u>
Desamino-	188.1	0.1	28	104.1	qualifier
metamitron	188.1	0.1	27	160.0	<u>quantifier</u>

Source and detection parameters for MS/MS experiments:

Within the sequence, a calibration curve was constructed by injecting standard solutions within the range from 1 ng/mL to 0.005 ng/mL (9 calibration points, see Figure 1 to Figure 4). Injections of sample extracts were interspersed with injections of standards (0.5 ng/mL and 0.05 ng/mL) after 2-4 samples to verify the detector response and to adjust the calculated concentration of samples to the mean of the two bracketed standards.

The quantification was performed by the peak area of quantifier ions using external standards in HPLC grade water.



### 4.3.6 Calculation of Residues

The determined concentration correlates to the concentration in the water sample.

Recoveries were calculated by the following equation:

$$\operatorname{Rec} = \frac{R_{\text{found}}}{R_{\text{fortified}}} \cdot 100 \%$$

Rec	recovery (%)
<b>R</b> <sub>found</sub>	residue determined (mg/kg)
<b>R</b> <sub>fortified</sub>	fortification level (mg/kg)

# 5 Deviations from the Study Plan

No deviations from the study plan dated 16 March 2007 occurred.

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# 6.2 Repeatability (Precision)

The relative standard deviations (RSD) at the quantifier ions for each fortification level ranged from 2 % to 8 % for metamitron and 2 % to 9 % for desaminometamitron.

These values are within the guideline requirements ( $\leq 20$  % RSD).

#### 6.3 Blanks

Two untreated control samples were analysed from each water type. Residues of the test items were not detectable in these samples (i.e. < 30 % of LOQ or  $< 0.015 \mu g/L$ , see also chromatograms in the appendix).

#### 6.4 Limit of Quantification and Limit of Detection

The limit of quantification is defined as the lowest fortification level with mean recoveries ranging between 70 % and 110 % at a relative standard deviation not exceeding 20 % and blanks not exceeding 30 %. These conditions are fulfilled for both test items at the 0.05  $\mu$ g/L fortification level.

#### 6.5 Linearity

For analysis of the test items the detector response was linear within the range from 0.005 ng/mL ( $\mu$ g/L) to 1 ng/mL ( $\mu$ g/L) (0.25 pg to 50 pg injected)

#### 6.6 Specificity

The method is highly specific for metamitron and desaminometamitron (mass transitions from the positively charged molecule ions to two typical fragments in MS/MS mode).

The retention times of the test items in solvent matched the retention times in extracts from fortified samples. No peak interferences occurred at the retention time of metamitron and desaminometamitron at the quantifier ions. At the qualifier ions, an interfering peak next to the retention time of desaminometamitron was observed with about the same order of magnitude as the concentration at LOQ. However this had no impact on validity of study, because identification of the substance and also peak integration was not affected.



#### 6.7 Matrix Effects

Since the samples were injected unchanged and quantified against standards in HPLC grade water, no matrix effect had to be determined.

#### 6.8 Storage Stability of the Test Items in Final Sample Extracts

The storage stability of the test items in the final extract had not to be tested, since all samples were analysed within 24 h after extraction.

#### 6.9 Storage Stability of the Test Items in Stock Solution

Standard solutions of the test items (1  $\mu$ g/mL each as intermediate dilution) in methanol/water (50/50, v/v) were stored refrigerated in the dark and used within 1 day after preparation. The storage stability in this solvent mixture was tested by comparison of the measured concentration of an aged standard solution (1  $\mu$ g/mL, storage for 11 days at 4-7 °C, and diluted to 0.5 ng/mL in HPLC grade water) versus a solution from a freshly prepared stock solution (final concentration 0.5 ng/mL in HPLC grade water).

The concentrations of metamitron and desaminometamitron in the aged solution were 100 % and 101 % of the fresh standard solution, indicating the stability of both test items under the given conditions.

# 7 Discussion and Conclusions

A residue method for determination of metamitron and its metabolite desaminometamitron in drinking water and surface water has been successfully validated.

- Validation of the method demonstrated that it was highly specific to the test items with a LOQ of 0.05 μg/L for each compound.
- The results of this study indicate that the recovery (accuracy) and the precision were within acceptable limits of 70 % 110 % for mean recovery and < 20 % RSD at each fortification level.
- Interferences from the control matrices at the quantifier ions used were not detectable. A minor interference at the qualifier ion next to the desamino-metamitron peak was found to be negligible.
- The requirements were met by the data obtained for the quantifier and qualifier mass transitions of both analytes.

The analytical method fulfils all requirements of guideline SANCO/825/00 rev. 7 and is also suitable as a confirmatory method.