

Determination of Ultratrace Amitrol in Water Samples by *in situ* Derivatization-Solid Phase Extraction-Liquid Chromatography-Mass Selective Detector

Application

Environmental

Authors

Gerd Vanhoenacker, Tom Sandra, Frank David, Pat Sandra
Research Institute for Chromatography
Kennedypark 20, B-8500 Kortrijk, Belgium

Agilent contact:
Chin-Kai Meng
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808-1610
USA

Abstract

A sensitive method for the analysis of amitrol in water samples was developed using *in situ* derivatization with hexylchloroformate, solid phase extraction, and liquid chromatography/mass selective detector operated in the positive atmospheric pressure chemical ionization mode with selective ion monitoring. The limit of detection is approximately 0.02 ppb (0.02 µg/L) which is well below the EC advisory level of 0.1 µg/L.

Introduction

Amitrol is used as both an herbicide (weed killer) and as a photographic chemical. Although no acute, short-term, health effects are known at this time, it may be a carcinogen in humans since it causes thyroid and liver cancer in animals. It is,

therefore, on the Hazardous Substance List and cited by the EPA (Environmental Protection Agency) and NIOSH (National Institute for Occupational Safety and Health).

Amitrol was analyzed without any sample preparation at the ppb level (µg/L) in water samples by liquid chromatography/mass selective detector (LC/MSD) operated in positive electrospray ionization (ESI), selective ion monitoring (SIM) mode [1]. The method used an ion-pairing agent and large volume injection (250 µL).

For sub-ppb analysis of amitrol in water samples, it is necessary to resolve two issues. First, the low molecular weight (MW 84) gives an unspecific ion in mass spectrometry (MS) and second, the highly water-soluble compound is difficult to recover satisfactorily out of an aqueous matrix. Both issues can be solved by *in situ* derivatization with hexylchloroformate (Figure 1). The derivative has a specific mass at m/z 213 (M+H)⁺ and hydrophobicity (hexyl) is introduced in amitrol to allow solid phase extraction (SPE) enrichment on octadecylsilica (ODS) packing. A labeled standard is not commercially available and a solute with similar chemical characteristics namely 3-amino-5-methylthio-1H-1,2,4-triazole was selected as internal standard (IS). The standard is added to the water sample before derivatization and the (M+H)⁺ ion of the IS derivative is m/z 259.



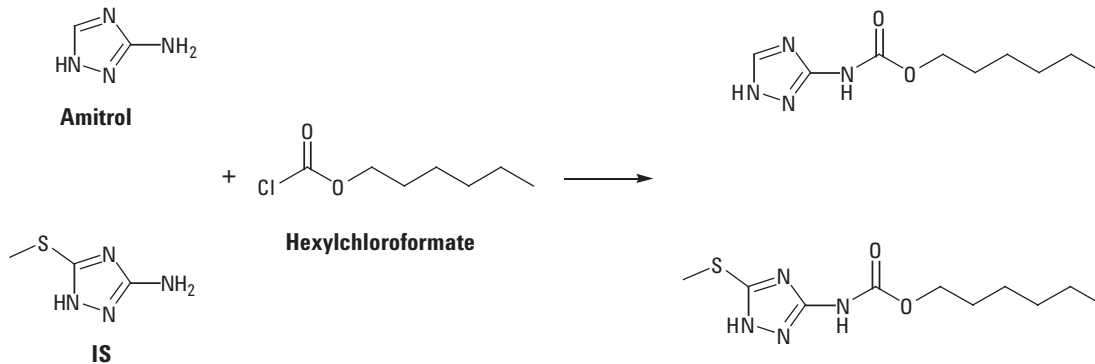


Figure 1. Derivatization of amitrol and IS with hexylchloroformate.

Experimental

Analyses were performed on an Agilent Technologies 1100 Series HPLC equipped with a binary pump, vacuum degasser, thermostated column compartment, and autosampler. An Agilent Technologies 1100 Series quadrupole MSD (SL version) was used for detection.

HPLC Conditions

Column:	Zorbax SB-C18, 3.5 μ m particle size 150 mm L \times 3-mm id (Agilent p/n 863954-302)		
Mobile phases:	Solvent A: 10-mM ammonium acetate in H ₂ O Solvent B: MeOH		
Gradient :	Time (min)	Solvent A	Solvent B
	0	35	65
	10	35	65
	15	0	100
	20	35	65
Analysis time:	20 min		
Post analysis time:	6 min		
Flow:	0.4 mL/min		
Column temperature:	30 $^{\circ}$ C		
Injection volume:	100 μ L		

MSD Conditions

Ionization mode:	APCI, positive polarity	
Selective Ion Monitoring (SIM) Parameters:	4 min Ion: 213 (Amitrol)	Gain: 10
	8 min Ion: 259 (IS)	Gain: 1
Fragmentor:	100 V	
SIM Resolution:	Low	
Vaporizer:	325 $^{\circ}$ C	
Drying gas (N ₂):	5.0 L/min	
Gas temperature:	350 $^{\circ}$ C	
Nebulizer pressure:	60 psig	
V _{cap} :	4000 V	
Corona:	4.0 μ A	

Sample Preparation

Chemicals and consumables

Methanol (MeOH, for LC)
Water (for LC)
Ammonium acetate (high purity)
Ethanol (EtOH, high purity)
Pyridine (high purity)
Acetonitrile (ACN, high purity)
Amitrol (Dr. Ehrenstorfer GmbH, Augsburg, Germany, N $^{\circ}$ C10240000)
3-Amino-5-methylthio-1H-1,2,4-triazole (IS) (Aldrich N $^{\circ}$ 19,068-3)
Hexylchloroformate (for derivatization, Aldrich N $^{\circ}$ 25,277-8)
SPE cartridge: AccuBOND II ODS-C18, 500 mg/3 mL (Agilent, p/n 188-1350)
Nylon membrane filter: 3 mm, 0.2 μ m pore size (Agilent, p/n 5061-3368)

Derivatization with Hexylchloroformate

1. To a raw, unfiltered sample volume of 50 mL, add 25 ng (250 μ L of a 100-ppb stock solution in MeOH) of the IS.
2. Add 2.5 mL of the reagent mixture H₂O/EtOH/Pyridine (60/32/8 v/v).
3. Add 200 μ L hexylchloroformate solution (100 μ L in 10 mL ACN).
4. Vortex the mixture for 30 seconds and load on a SPE cartridge.

SPE (Using the Agilent SPE Station, P/N 5185-5754)

Conditioning: 2 \times 3 mL ACN/MeOH 50/50 v/v

1 \times 3 mL MeOH

2 \times 3 mL H₂O

Loading: 50 mL derivatized sample

Washing: 2 \times 3 mL H₂O

Dry the cartridge under vacuum

Elution: 3 \times 1 mL ACN/MeOH 50/50 v/v

Evaporate to near dryness and redissolve in 200 μ L H₂O

Vortex for 10 seconds and filter through a membrane filter, pore size 0.2 μ m.

Results

The full-scan spectra for derivatized amitrol and IS are shown in Figure 2. The derivative of amitrol has a specific mass at m/z 213 ($M+H$)⁺ and the IS at m/z 259 ($M+H$)⁺. In both cases, the loss of the derivatization group gives ions at m/z 128 lower ($-C_7H_{12}O_2$) that can be used for confirmation.

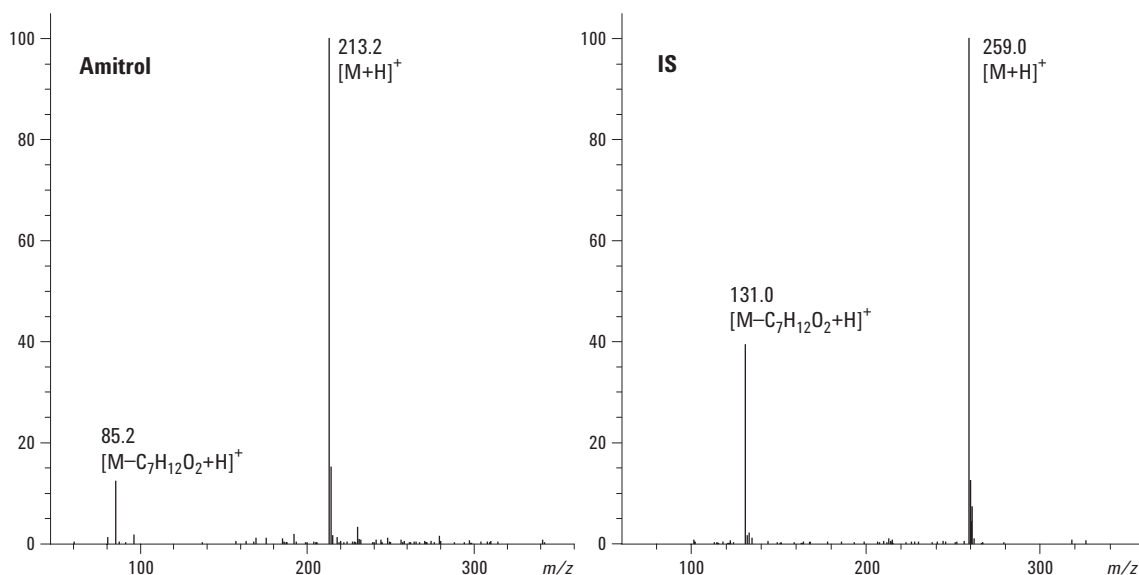


Figure 2. Mass spectra of derivatized amitrol and IS in scan mode.

Figure 3 shows the SIM analysis, monitoring the ions m/z 213 and 259, of a polluted surface water (amitrol at 0.05 ppb), a clean surface water, and the same surface water spiked at 0.1 ppb (IS at 0.5 ppb in all three samples).

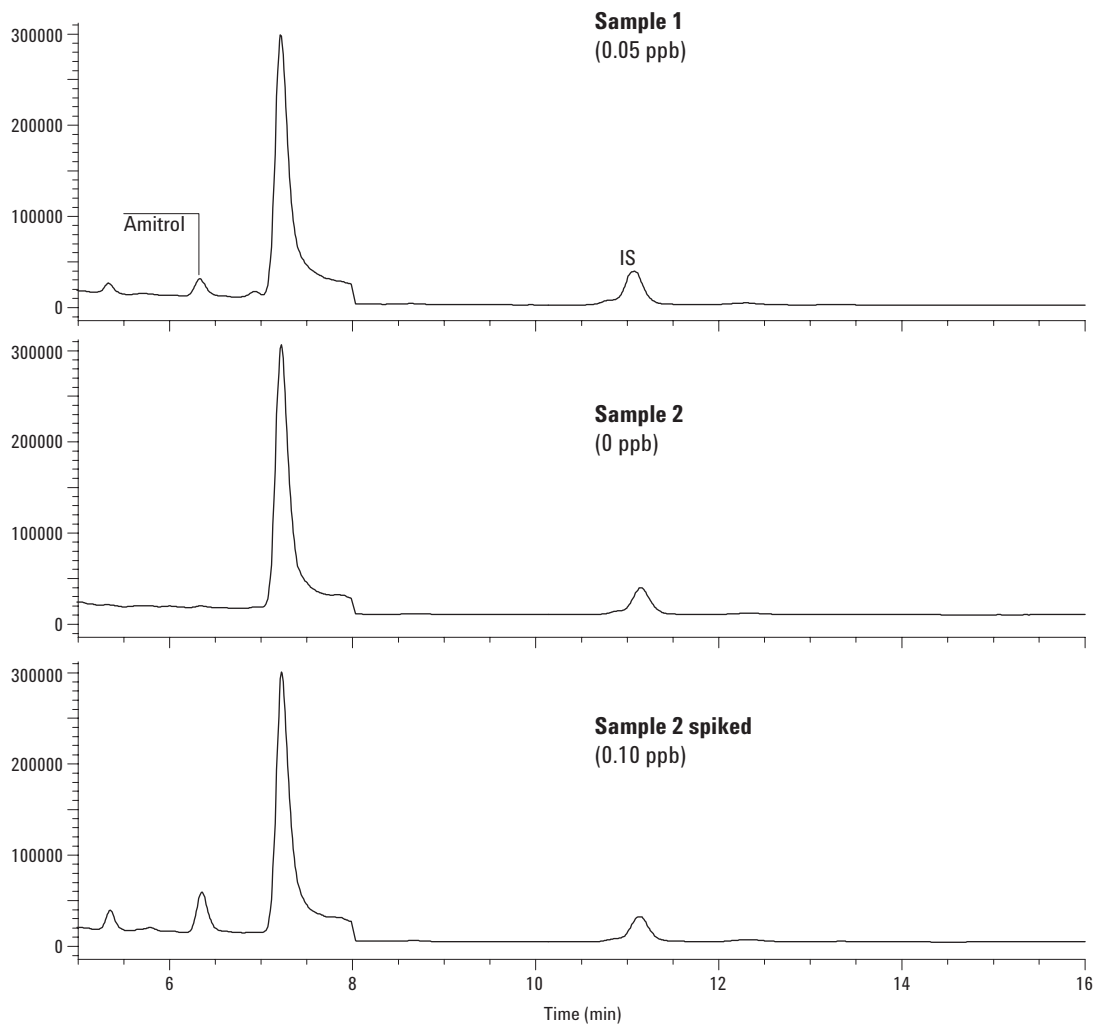
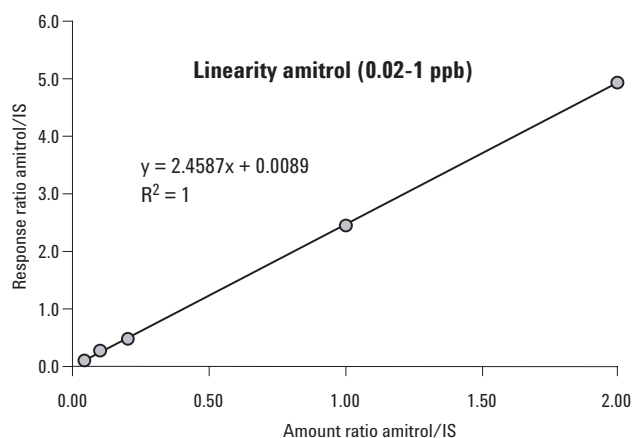


Figure 3. SIM analysis of real water samples and a spiked sample.

The linearity of the method was evaluated with standard solutions containing amitrol (0.02-1 ppb) and the IS (0.5 ppb). The solutes (IS stock solutions of 100 ppb) were added to blank water samples and the derivatization and extraction procedures were applied. In the range studied, a linear graph was obtained. The results are summarized in Figure 4.



Concentration amitrol (ppb)	Concentration IS (ppb)	Area amitrol/ Area IS
0.02	0.50	0.12
0.05	0.50	0.26
0.10	0.50	0.49
0.50	0.50	2.45
1.00	0.50	4.93

Figure 4. Calibration data for amitrol (0.02–1 ppb).

The repeatability of the analysis was verified by quantifying six standard solutions (50 mL each) at 0.05 ppb amitrol and 0.5 ppb IS. The complete derivatization and extraction procedure gave a RSD of 6.5%.

Conclusion

The described method provides ample sensitivity to quantify amitrol in water below the EC norm level of 0.1 ppb. In situ derivatization followed by SPE sample concentration was easily performed and reproducible with an IS.

Reference

1. N. Cullum and P. Stephens, "Rapid screening Method for the Analysis of Paraquat and Diquat by LC/MSD Using Selective Ion Monitoring and Large Volume Injection," Agilent Technologies, publication 5988-7720EN www.agilent.com/chem

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.

Agilent shall not be liable for errors contained herein or for incidental or consequential damages in connection with the furnishing, performance, or use of this material.

Information, descriptions, and specifications in this publication are subject to change without notice.

© Agilent Technologies, Inc. 2003

Printed in the USA
October 13, 2003
5989-0184EN

