HPLC-MS-MS: A Powerful Tool for Sensitive Detection of Nitrophenols in Cloud Water Samples

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Introduction

Nitrophenols occur ubiquitously in the environmental; they are of biogenic as well as anthropogenic origin. Nitrophenols (NP) have been used in the past as herbicides such as *2,4-DNP* (2,4-dinitrophenol), *DNOC* (4,6-dinitro-o-cresol), and are still in use in the form of *Dinoterb* (2-tert-butyl-4,6-dinitro-phenol). Combustion processes in motor vehicles and atmospheric reactions of aromatic hydrocarbons such as benzene, toluene, and cresols, with NO_x and OH-radicals are other important sources. Due to their phytotoxicity it is important to determine their concentrations in the atmosphere, especially in liquid aerosols such as cloud water droplets.

Between July 1998 and March 1999 cloud water samples were collected at an ecosystem research site (800 m a.s.l.) in North eastern Bavaria, Germany. These samples were analysed for nitrophenols by GC-MS or HPLC-MS-MS techniques.

Sampling and Chemical Analysis

- the cloud water collectors were started automatically whenever the visibility was below 500 m
- winter: Caltech Heatable Rods Cloud water Collector (**CHRCC**)
- summer: Caltech Active Strand Cloud water Collector (**CASCC**)

GC-MS:
GC-MS in the electron impact mode was performed with a HP 5970 MSD with splittless injection (injector temperature: 250° C). Using a DB-5.625 fused silica capillary column (30 m x 0.25 mm, 0.25µm) for the direct analysis of nitrophenols, with a temperature gradient of 25°C/min from 50°C (1 min) to 140°C, then 8°C/min to 230 °C. For the separation of derivated nitrophenols, a RTX-200 column (30 m x 0.32 mm, 0.5 µm) with a temperature gradient of 10 °C/min from 80 °C (1 min) to 150 °C, then 20 °C/min up to 280 °C was used.

HPLC-MS-MS:
HPLC-MS-MS was performrmed with a PE Sciex API 3000 (TurboionSpray, negative ion mode) coupled to a PE 200 micro-pump and a PE Series 200 autosampler. 10 µL of standard solutions and cloud water samples were injected and separated by a Superspher 100 RP-18 endcapped (125 x 2 mm, Merck) with an isocratic gradient of 70 % AcCN and 30 % H_2O + 5 mM $NH_4CH_3CO_2$ + 0.1 % (v/v) CH_3CO_2H. For all analyses the following MS-MS transitions were monitored:
2-nitrophenol and 4-nitrophenol: 138.1 to 107.9; 2,4-dinitrophenol: 183.0 to 108.7; 4,6-dinitro-o-cresol: 196.9 to 136.9

Sample treatment

Cloud water samples were collected during each event and stored at -20 °C until analysis.

HPLC-MS-MS	GC-MS

- 1 mL of cloud water
- no sample preparation
- injection into HPLC-MS-MS

- 100 - 140 mL of cloud water
- acidify to pH 2
- addition of 20 mg NaCl
- addition of 100 µL of internal standard
- solid phase extraction (Chromabond HR-P, M&N) with 1,5 mL ethyl acetate
- addition of 20 µL triethylamine (pH 11)
- solvent evaporation

Derivatisation:
50 µL BSA and 50 µL eluate
1h at 80°C
Injection of 1 µL into GC-MS

Solvation 20 µL dichloromethane + 5 µL H_2SO_4
Injection of 1 µL of the organic phase into GC-MS
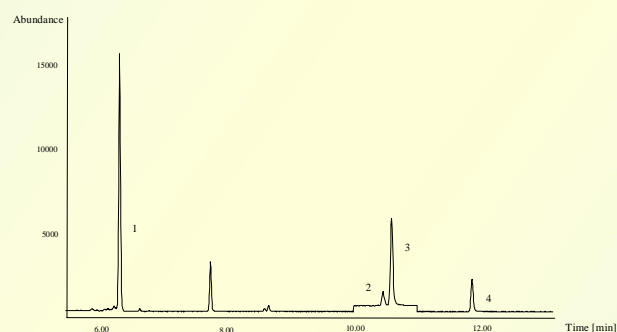
Results

Fig. 1: GC-MS separation of 2-NP (1), 2,4-DNP (2), 4-NP (3), and DNOC (4) without derivatisation (6 ng/µL of each compound).
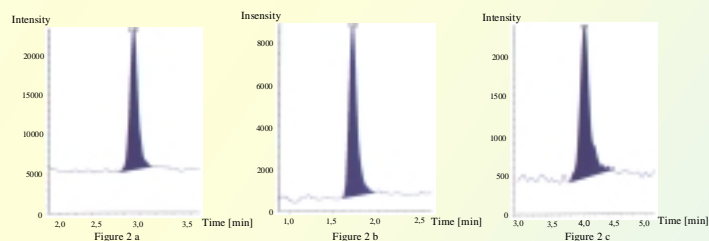

Fig. 2: 2/4-NP (a), 2,4-DNP (b), and DNOC (c) standard solutions analysed (1 pg/µL) by HPLC-MS-MS.

Detection limits: HPLC-MS-MS: < 0,1 pg/µL
GC-MS with derivatisation: < 100 pg/µL
GC-MS without derivatisation: 20 pg/µL - 400 pg/µL

Table 1: Concentrations (µg/L) of nitrophenols in cloud water (20 samples); analysed by HPLC-MS-MS.

	2/4-NP*	2,4-DNP	4,6-DNOC
min	< 0.1	< 0.1	< 0.1
max	73.3	26.8	8.0
median	12.6	2.4	0.8
mean	15.0	5.4	1.3

* Sum values of 2- and 4-nitrophenol, which were not separated from each other with HPLC

Table 2: Concentrations (µg/L) of nitrophenols in cloud water (5 samples); analysed by GC-MS.

	Phenol	2-NP	4-NP	2,4-DNP	4,6-DNOC
min	0.7	0.1	5	0.2	0.5
max	6.4	0.4	170	17	4.2
median	0.7	0.1	5	0.2	0.5
mean	2.1	0.2	58	7.88	2.6

Conclusions

using GC-MS:

- good separation of nitrophenols with or without derivatisation
- time consuming and complex sample preparation
- high limits of detection for di- and higher substituted nitrophenols

using HPLC-MS-MS:

- no sample preparation
- low limits of detection
- short analysing times