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Short communication

Determination of toxic nitrophenols in the atmosphere by high-performance liquid chromatography

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Abstract

Seven HPLC columns were used for the optimization of the isocratic HPLC measurement of phenol, nitro- and dinitrophenols. A column constituted from 5 μm particles (100 Å) of silica-based C_{18} material was used for the analysis. Good separation of the analytes and their quantification in samples from the nitration of phenol in liquid and in gas phase in the laboratory was obtained. This approach allowed also to determine phenol in air samples.

Keywords: Air analysis; Environmental analysis; Nitrophenols; Phenols

1. Introduction

Organic compounds in rainwater derive from direct emissions from biogenic and anthropogenic sources and from photochemically induced reactions either in the gas or in the aqueous tropospheric phase. Among these, phenols (phenol, cresols, nitrophenols, nitrocresols) are totally anthropogenic and result from traffic. Their concentration in rain are in the range 1–12.8 μg l^{-1} (urban) and 0.12–0.48 μg l^{-1} (rural) [1] in the USA and have been found in the μg l^{-1}-range in the Rhein–Ruhr area in western Germany [2,3].

Concerning the analytical methodology adopted, speciation has been achieved by solid-phase extraction using both modified silica gel (C_{18}) and XAD resin-adsorbents and gas chromatography (GC) using capillary columns with specially deactivated weakly polar phases [4].

A simple analytical methodology for the speciation of phenols and nitrophenols is needed in order to study their origin and distribution. In fact, small differences in chemical structure determine the preferential toxicity in plants, fungi or animals and the specific phytotoxicity during seed germination or on the photosynthetic processes in plants [5–7].

This paper reports a procedure for the complete separation of phenol, nitrophenols and dinitrophenols using isocratic RP-HPLC and diode array detection to be used for environmental analysis in the atmosphere.

2. Experimental

2.1. Chemicals

The mobile phase used in isocratic runs was A–B

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(55:45). A was 0.005 *M* KH_2PO_4 (pH 4.5 with H_3PO_4)–CH_3CN (90:10). B was 0.005 *M* KH_2PO_4 (pH 4.5 with H_3PO_4)–H_3CN (25:75).

Solution A and B were filtered through 0.45 μm filters (Millipore, Bedford, MA, USA) and degassed with helium during analysis. All reference compounds were purchased from Fluka (Buchs, Switzerland). Acetic acid and methylene chloride were reagent grade products (Fluka) methanol and acetonitrile were HPLC grade product (J.T. Baker, Phillipsburg, NJ, USA). A silica gel solid-phase (SiOH) cartridge (4 cm×0.6 cm I.D., PTFE tubing, packed with 500 mg, size 40 μm) and a styrene–divinylbenzene (XAD-2) cartridge, same dimensions, were obtained from Superchrom (Milan, Italy).

2.2. The analyses

The instrument was a HPLC pump 600 E (Waters, Milford, MA, USA) equipped with a Reodyne 7125 injector with a 100 μl loop. The detector was a photodiode array detector 1040 (Hewlett-Packard, Palo Alto, CA, USA). The analytical column (25 cm×4.4 mm I.D.) used was a stainless-steel column packed with 5 μm particles (100 Å) of silica-based C_{18} material, Kromasil (Superchrom). The flow was 1 ml min^{-1}.

2.3. Sampling and sample preparation

The SiOH cartridge was rinsed with 10 ml methylene chloride, dried with helium and stored at 4°C prior use.

The air sample collection apparatus used was a SAM 1 (CEE/JRC, Ispra, Italy) precalibrated at 1 l min^{-1}. The SiOH cartridges after pretreatment or the XAD-2 cartridge were connected to the system with PTFE tubing. After sampling, 4 h, they were eluted with 1 ml of 1% acetic acid (SiOH) or 2 ml of methanol (XAD-2) under vacuum. A 100 μl volume of the sample was directly injected into the column.

3. Results and discussion

A preliminary study was devoted to the selection of the proper column to obtain good separation of the whole family of nitro- and dinitrophenols. Seven columns were tested for this purpose as shown in Table 1.

A silica-based C_{18} material, Kromasil (Superchrom) packed with 5 μm particles (100 Å) gave the best performance and was used for the analysis, probably the packing pressure used for the column increased the mass of packing material inside each column and increased the efficiency of the column [8]. Fig. 1 shows the results obtained in the HPLC analysis. Table 2 lists the result of the linearity of the column with a standard solution. The total analysis time was 18 min with a separation of all the components.

Linearity and detection limit (signal-to-noise

Table 1
Columns tested for the separation

Column	Dimension	Description	Particle size (μm)	Pore diameter (Å)	pH stability	% C
Kromasil C_{18} Superchrom	250×4.6 mm	Silica spheric, metal free (Na, Al, Fe <10 ppm)	5	100	2.0–7.5	16
Kromasil C_{18}	250×4.6 mm	Silica spheric, metal free (Na, Al, Fe <10 ppm)	5	100	2.0–7.5	19
Nucleosil C_{18}	250×4 mm	Silica spheric, totally porous	5	120	1.0–9.0	11
Nucleosil C_{18}	250×4 mm	Silica spheric, totally porous	5	100	1.0–9.0	24
LiChrospher	250×4 mm	Silica irregular, totally porous	5	100	–	–
Supelcosil Abzplus	250×4.6 mm	Silica spheric, bonded phase alkylamide	5	100	2.0–7.5	12
Polystyrene–divinylbenzene	250×4.6 mm	Polymeric divinylbenzene–polystyrene	5	100	1.0–13.0	

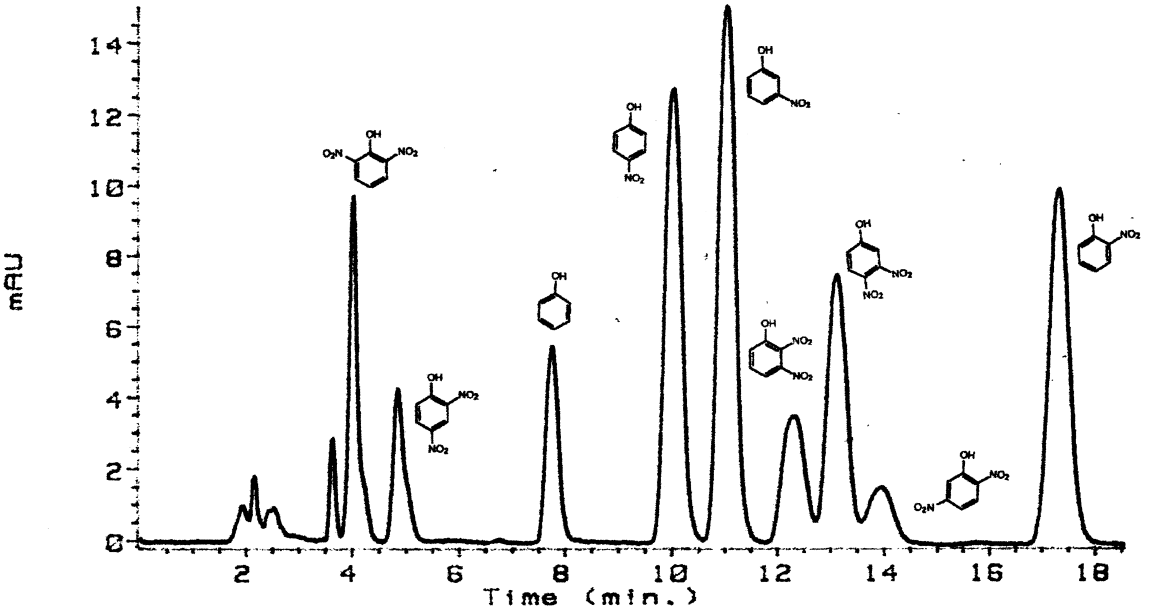

Fig. 1. An isocratic run with a mixture of reference compounds.

ratio=3) were those reported in Table 2 for all samples using detection at 230 nm. An increase in sensitivity could be obtained using the wavelengths of maximum absorption of the analytes.

The isocratic HPLC procedure was then used to analyze mixtures of phenols and nitrophenols both in laboratory and in field studies since the relative abundance of the isomeric nitrophenols may be significant for the identification of the sources. The samples of the laboratory studies were diluted approximately to 0.5 ng μl^{-1} because the concentration of phenol was high (Table 3) and were injected into the column. Table 3 shows the results obtained from samples deriving from the liquid or the gas phase nitration of phenol in the laboratory. Interestingly, the gas phase nitration gave essentially the *ortho*-nitration, whereas the liquid phase resulted in a ratio *ortho*-nitrophenol/*para*-nitrophenol=0.5–0.8. This finding was important since only 2-nitrophenol has been detected in urban rain in Los Angeles [1], thus suggesting that the nitration of phenol occurs in the tropospheric gaseous phase.

Hence, a methodology for field studies was available. However, the low concentration of phenolic

Table 2
Linearity and detection limit of the RP-HPLC column

Sample	r^2	Range of the sample injected (ng)	Detection limit (ng injected)
2,6-Dinitrophenol	0.998	8.4–83.3	6.5
2,4-Dinitrophenol	0.991	8.0–62.9	6.0
p-Benzoquinone	0.991	5.0–140.0	5.0
2,4,6-Trinitrophenol	0.994	5.0–100.0	5.0
Phenol	0.998	6.1–103.7	4.0
4-Nitrophenol	0.997	11.2–150.3	6.2
3-Nitrophenol	0.999	8.2–69.7	6.0
2,3-Dinitrophenol	0.999	11.4–96.9	6.4
3,4-Dinitrophenol	0.999	10.0–83.3	5.0
2,5-Dinitrophenol	0.997	11.4–96.9	6.0
2-Nitrophenol	0.996	8.2–140.1	5.4

Table 3
Results of the gas and the liquid phase nitration of phenol

Reactant	Phenol (mol)	2,6-Dinitro-phenol (mol %)	2,4-Dinitro-phenol (mol %)	*p*-Benzo-quinone (mol %)	2,4,6-Trinitro-phenol (mol %)	Phenol (mol %)	4-Nitro-phenol (mol %)	2-Nitro-phenol (mol %)
Nitric acid (65%) in water [9]	$2.69\cdot10^{-2}$	0.19	0.83	6.07	0	0	59.21	33.7
Nitric acid (65%) in chloroform	$2.51\cdot10^{-4}$	8.13	13.15	15.06	0	0	41.98	21.68
Photolysis of cerium(IV) ammonium nitrate [10]	$2.25\cdot10^{-2}$	0	0	5.32	0	0	56.97	37.71
Dinitrogen pentoxide (run 1) [11]	$8.93\cdot10^{-4}$	2.38	6.32	0	0.55	0	2.02	88.73
Dinitrogen pentoxide (run 2)	$8.93\cdot10^{-4}$	0	4.36	7.97	1.18	11.4	0.48	74.61

material in real samples both outdoor and indoor required trapping and the trapping efficiency of the traps had to be checked. A SiOH and a XAD-2 cartridge were used. Table 4 shows the recovery of all the analytes. Recovery was determined from samples having the phenolic compounds applied to the collection devices prior to pure air collection. The performance of the silica gel solid phase was better than that of the XAD-2 trap. The calibration of SiOH was linear, for all the analytes, r^2 were 0.991–0.999, in the range of 25–250 ng injected.

No interference between nitrophenols and methylphenols was possible because 2- and 4-methylphenols eluted between 11.5 and 12.5 min. Moreover, in real atmosphere 3-nitrophenol was absent. No presence of compounds eluted after 20 min was noticed in real air samples.

This method was used to determine the concentration of phenol in the atmosphere in the Bicocca University Campus located in the northern part of Milan. This region is frequently affected by heavy air pollution episodes both for high emissions and for stagnant meteorological conditions. Fig. 2 shows the HPLC analysis of the sample collected on 18 June 1998 at the Bicocca University campus. The presence of phenol (0.4 $\mu g\ m^{-3}$) and 4-nitrophenols (0.3 $\mu g\ m^{-3}$) in the sample were demonstrated both by the UV spectrum of the peaks at 7.23 min and 10.11 min, respectively, and their mass spectrum. The structures of further peaks in the sample will be determined by an HPLC–MS experiment and 2-nitrophenol and 2,5-dinitrophenol were absent.

Table 4
Recovery of the analytes from the traps

Analyte	r^2	Recovery (%)	
		SiOH	XAD-2
2,6-Dinitrophenol	0.996	88.80±2.40	–
2,4-Dinitrophenol	0.996	95.80±6.30	108.0
Phenol	0.998	108.1±9.40	81.50
4-Nitrophenol	0.997	103.9±2.00	69.60
3-Nitrophenol	0.998	109.6±3.80	69.40
2,3-Dinitrophenol	0.999	99.90±2.10	73.40
3,4-Dinitrophenol	0.992	72.10±7.20	76.20
2,5-Dinitrophenol	0.994	110.5±2.00	76.20
2-Nitrophenol	0.974	75.80±7.40	74.10

Recently several papers report the separation and the determination of nitro-, methyl-, and chlorophenols [12–14] from sea, waste and river water, using liquid chromatography-mass spectrometry or UV detection after post-column reaction. Indoor air is at present been studied [15] for the determination of phenol, cresols, hydroquinone and catechol by HPLC. Quite recently GC–MS [16,17] has been used for the detection of phenol and nitrophenols in cloud and air.

In conclusion, this simple isocratic HPLC approach allows to speciate phenol and nitrophenols both in laboratory samples and in the field, every 4 h and will be of great utility in environmental monitoring. Work is in progress in order to identify all analytes found in field samples.

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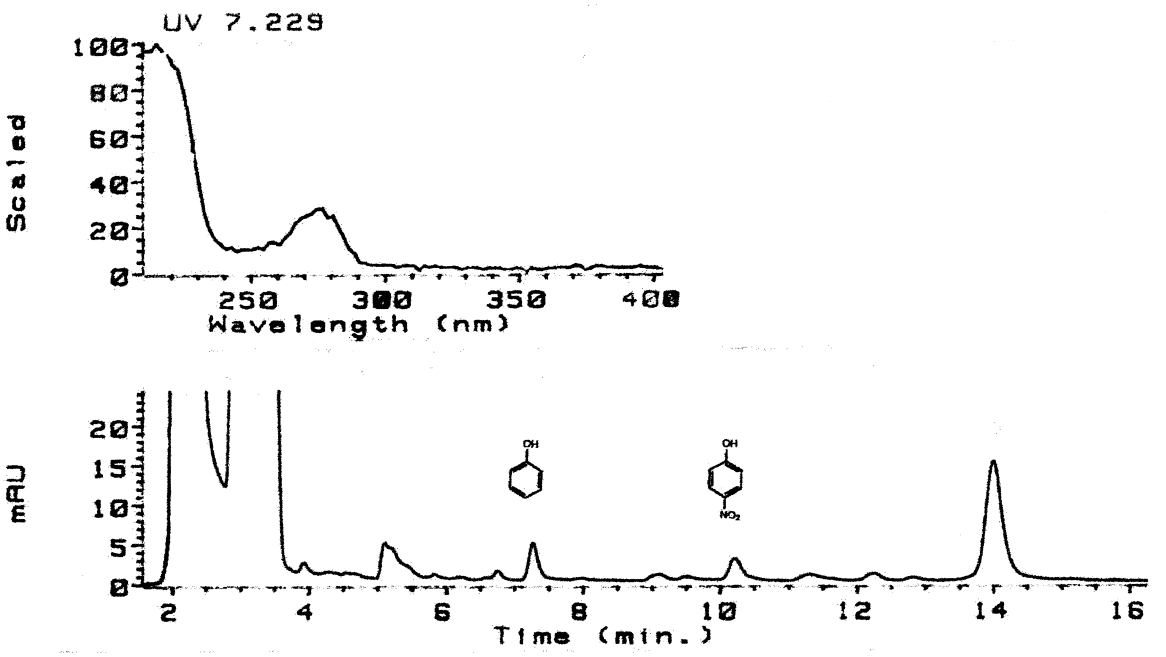

Fig. 2. HPLC analysis of a sample collected on 18 June 1998 at the Bicocca University Campus.

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