DETERMINATION OF NITROAROMATIC PHOTODECOMPOSITION PRODUCTS OF TECNAZENE USING LIQUID CHROMATOGRAPHY WITH AMPEROMETRIC DETECTION

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Isocratic liquid chromatographic methods with mobile phases consisting of 20 mmol l^{-1} sulfuric acid and 60% acetonitrile (for 9 quickly eluted nitroaromatic compounds) or 75% acetonitrile (for 2 strongly retained nitrobenzenes) at a flow rate of 1.2 ml min^{-1} using a DC amperometric detection with a glassy carbon working electrode at –0.5 V *vs* Ag/AgCl reference electrode have been developed for the determination of 11 nitroaromatic compounds. The overall repeatability of the retention times was better than 0.5% and the repeatability of the peak areas ranged from 1.2 to 8.3% (n = 7) based on the manual injection of 300 µl of standard solution. Limits of detection (LODs for 3.S/N) of 25–250 nmol l^{-1} were obtained. The developed methods have been applied to the determination of nitroaromatic decomposition products in the UV-irradiated samples of a tecnazene (1,2,4,5-tetrachloro-3-nitrobenzene) solution. Due to the high selectivity of the DC amperometric detection in reductive mode, no interferences occurred in the analyses of UV-irradiated tecnazene solutions although high concentrations of other compounds (*e.g.*, phenols, chlorophenols, inorganic anions, and carboxylic acids) were present.

Keywords: Nitroaromatic compounds; Liquid chromatography; DC amperometric detection; Photodecomposition products; Tecnazene; Nitrobenzene; Fungicides.

Nitroaromatic compounds (NACs) are widely used by agricultural industry as pesticides, by industry as chemical raw materials and dyes and by the military as explosives[1–3]. The extensive use of NACs has resulted in the accidental and also intentional introduction in the environment both due to their usage and as a result of improper disposal treatment. The fact that NACs are considered to be toxic compounds[4,5] has led to extensive development of analytical methods using alternative detection systems for the determination of NACs [6–8].

Tecnazene (1,2,4,5-tetrachloro-3-nitrobenzene) is a fungicide used on potatoes for the purpose of sprout suppression and dry rot control. Despite the fact that it has been used commercially since 1947, the behaviour of tecnazene in the environment is not well understood. Tecnazene and its metabolites have been identified in sediments and fish downstream of potato washing and processing plants[9].

In our previous papers dealing with the photodecomposition of tecnazene[10,11], the major decomposition products were determined to be simple inorganic (chloride, nitrite, nitrate, carbonate) and organic (carboxylic acids) anions[10], and phenols and chlorophenols[11]. Next to those compounds also NACs were expected to be photodecomposition products, thus the monitoring of NACs is of a great importance.

The determination of NACs is usually carried out using high performance liquid chromatographic systems with conventional detection systems[12,13]. Using the HPLC-UV systems in complex mixtures can result in an improper determination of some NACs that could be co-eluted with other UV-absorbing compounds. Therefore, a sensitive and highly selective detection method for the determination of NACs has to be developed. Based on the above-mentioned requirements, amperometric detection seemed to be a good choice due to its good sensitivity and oxidation/reduction properties where only compounds that can be oxidised (phenols, amines) or reduced (nitro compounds) can be determined at a time.

In the 80's, Krull *et al.*[14] used HPLC with photolysis-amperometric detection (AMD) for the trace determination of explosives and Bratin *et al.*[15] used liquid chromatography (LC) with reductive EC detection for the determination of explosive mixtures. Murayama and Dasgupta[6] suggested an EC detection of nitro-substituted polycyclic aromatic hydrocarbons (NPAHs) as a pre-detection step for the subsequent fluorescence detection of these compounds. MacCrehan *et al.*[7] used LC with EC detection in amperometric reductive mode for the determination of some NPAHs.

In our work the ultimate goal was to develop a sensitive detection method for the determination of NACs in samples containing also other compounds that can be very often present in real samples (phenols, chlorophenols, amines, *etc.*) and that usually interfere with the determination of NACs when using conventional detection methods. The developed analytical methods should be used to determine typical nitroaromatic decomposition products in the UV-irradiated samples of tecnazene solution.

EXPERIMENTAL

Chemicals and Apparatus

All chemicals were of analytical grade purity and were supplied by Sigma–Aldrich, Lancaster and Fluka. Mobile phases for the LC determination of NACs were prepared using acetonitrile of HPLC grade (Fischer Scientific, U.K.), sulfuric acid (95–97% for analysis) or glacial acetic acid (both Riedel de Haen), and high-purity water (Purite Select, Purite Ltd., U.K.).

Stock solutions of NACs (nitrobenzene, Fluka, ≥99%; 2-nitrophenol, Lancaster, ≥98%; 4-chloro-2-nitrophenol, Lancaster, ≥98%; 1-chloro-2-nitrobenzene, Lancaster, ≥99%; 1-chloro-3-nitrobenzene, Lancaster, ≥98%; 2,4-dichloro-6-nitrophenol, Fluka, ≥98%; 1,2-dichloro-3-nitrobenzene, Lancaster, ≥97%; 1,4-dichloro-2-nitrobenzene, Lancaster, ≥98%; 1,3-dichloro-5-nitrobenzene, Lancaster, ≥99%; 1,2,4-trichloro-5-nitrobenzene, Sigma–Aldrich, recrystallised) were prepared by dissolving the appropriate chemical in 50% v/v acetonitrile/water mixture. In the case of less soluble chemicals, the stock solutions were prepared by dissolving the appropriate chemical in pure acetonitrile. Standard solutions were freshly prepared every day in 10% v/v acetonitrile/water mixture using the stock solutions. Stock solutions were stored in a cold room at 4 °C in order to minimize instability of NACs.

For the preparation of samples containing decomposition products, 1 l of 50 μmol l^{-1} solution of tecnazene (1,2,4,5-tetrachloro-3-nitrobenzene) in 10% v/v acetonitrile/water mixture was irradiated by UV light. Tecnazene (13 mg, Sigma–Aldrich, recrystallised from acetonitrile) was dissolved in 100 ml of acetonitrile and after all tecnazene was dissolved, 900 ml of high-purity water was added. The acetonitrile/water solution was used to increase the initial concentration of tecnazene and thereby concentration of possible decomposition products in irradiated samples.

LC determination of NACs was carried out using a Dionex DX-500 chromatographic system equipped with an ED40 amperometric detector in DC amperometric mode with a glassy carbon working electrode and a silver reference electrode, GP40 gradient pump, AS40 autosampler and NS1 (crosslinked ethylvinylbenzene/divinylbenzene polymer, 10 μm, substrate crosslinking 55%, reversed phase) analytical column (250 × 4 mm) with NG1 (50 × 4 mm) guard column (all parts Dionex Corp., U.S.A.). The mobile phases (20 mmol l^{-1} sulfuric acid in 60 and 75% acetonitrile for methods *1* and *2*, respectively) were prepared and kept under the helium atmosphere. All experiments were done at constant temperature 25 °C. For control analyses of standard solutions, a UV detector (Perkin–Elmer, LC-90, U.S.A.) was connected to the LC system through the UI-20 Universal Interface (Dionex Corp., U.S.A.).

Data Evaluation and Calibration

Data evaluation and peak identification were carried out using PeakNet 4.30 software (Dionex Corp., U.S.A.). Calibration standards of NACs at different concentrations were used for the quantification of the decomposition products in UV-irradiated tecnazene solution. Statistical evaluation was carried out using the Excel 97 spreadsheet software (Microsoft, U.S.A.).

UV Apparatus

For the preparation of samples containing decomposition products, a 1 l reaction vessel was wrapped in aluminium foil, filled with 1 l of 50 μmol l^{-1} tecnazene solution in 10% v/v

acetonitrile/water mixture and the content was stirred with a magnetic stirrer (IKAMAG, Janke & Kunkel Labortechnik, Germany). UV irradiation of the solution was carried out using an Engelhard Hanovia (U.K.) medium pressure mercury vapour photochemical reactor lamp (0.6 kW) jacketed with a water-cooled quartz sleeve (see ref.[10] for details).

RESULTS AND DISCUSSION

Liquid Chromatographic Methods

The mobile phase for the liquid chromatographic-amperometric determination (LC-AMD) of NACs has to be chosen very carefully because the nitro group of the benzene ring can be easily electrochemically reduced and therefore detected by the AMD detector only under acidic conditions. Therefore, acetic acid and sulfuric acid were tested in the mobile phase consisting of various concentrations of acetonitrile. The simultaneous UV detection at 215 nm was performed to control the response of NACs in the UV region, where all NACs in the standard solution were detected.

In the case of acetic acid (0.1 up to 1% v/v) that is commonly used in the HPLC determinations using the UV detection[16–18], no response was observed for any of the NACs in the standard solution although different values of potential for the AMD detector were applied. Thus the mobile phase containing sulfuric acid was chosen for further LC-AMD experiments and analyses.

In the case of the AMD detector potential applied to the working electrode, the maximum negative potential cannot exceed the value of –0.8 V [19]. The potential values below –0.35 V showed a significant decrease in the reduction ratio of NACs and although the noise level has decreased at the same time the method sensitivity was sacrificed. On the contrary, increasing the absolute value of negative potential up to –0.55 V has led to an increased signal-to-noise ratio. Higher values of negative potential led only to an increased noise level while the signal intensity of NACs remained nearly unchanged. For these reasons the optimal potential applied to the working electrode should be close to the value of –0.55 V.

Three different concentrations of sulfuric acid and four different potential values were tested (5, 10 and 20 mmol l^{-1} sulfuric acid at –0.4, –0.45, –0.5 and –0.55 V). Finally, the best signal-to-noise ratio for most of the nitroaromatic compounds was achieved with the mobile phase containing 20 mmol l^{-1} sulfuric acid and the potential value of –0.5 V. The dependence of amperometric response on potential value at three different concentrations of sulfuric acid for 4-chloro-2-nitrophenol is depicted in Fig. 1.

The dependence curves for other NACs showed similar relationships. The only exception was nitrobenzene, for which the amperometric response was very low for potential values ranging from –0.35 to – 0.45 V. The detector response has increased significantly in the range from –0.45 to –0.55 V. This fact can be explained by the value of potential when the full reduction of nitrobenzene occurs, which is quoted as –0.6 V [20]. As mentioned before, the noise level increased rapidly when using a potential more negative than –0.55 V and therefore the optimal value of potential for the LC-AMD determination of NACs was established as –0.5 V though in the case of nitrobenzene, the sensitivity was slightly sacrificed.

Two parameters affecting the analysis time were studied, the concentration of acetonitrile in the mobile phase and the flow rate at which the analysis was conducted. In the initial experiments, a gradient method for the separation of all NACs was tested but the increasing concentration of acetonitrile led to a strongly drifting baseline and therefore no quantitative determination of NACs was possible. For that reason, NACs were divided into two groups and determined separately using two isocratic methods, where the baseline drift was minimised.

Using 20 mmol l^{-1} sulfuric acid in 60% v/v acetonitrile, separation of nine quickly eluted nitroaromatic compounds can be achieved in less than 25 min. Full separation of the remaining NACs with higher affinity to the column packing (namely 1,2,4-trichloro-5-nitrobenzene and tecnazene plus the most strongly retained compound from the previous run – 1,3-dichloro-

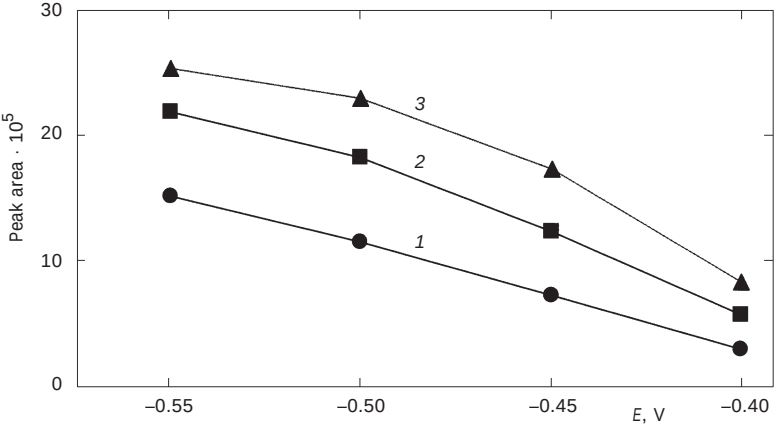

FIG. 1
Dependence of amperometric response on potential value at three different concentrations of sulfuric acid for 4-chloro-2-nitrophenol (in mmol l^{-1}): *1* 5, *2* 10, *3* 20

5-nitrobenzene) can be achieved in additional 16 min using 20 mmol l^{-1} sulfuric acid in 75% v/v acetonitrile (see Figs 2a and 2b).

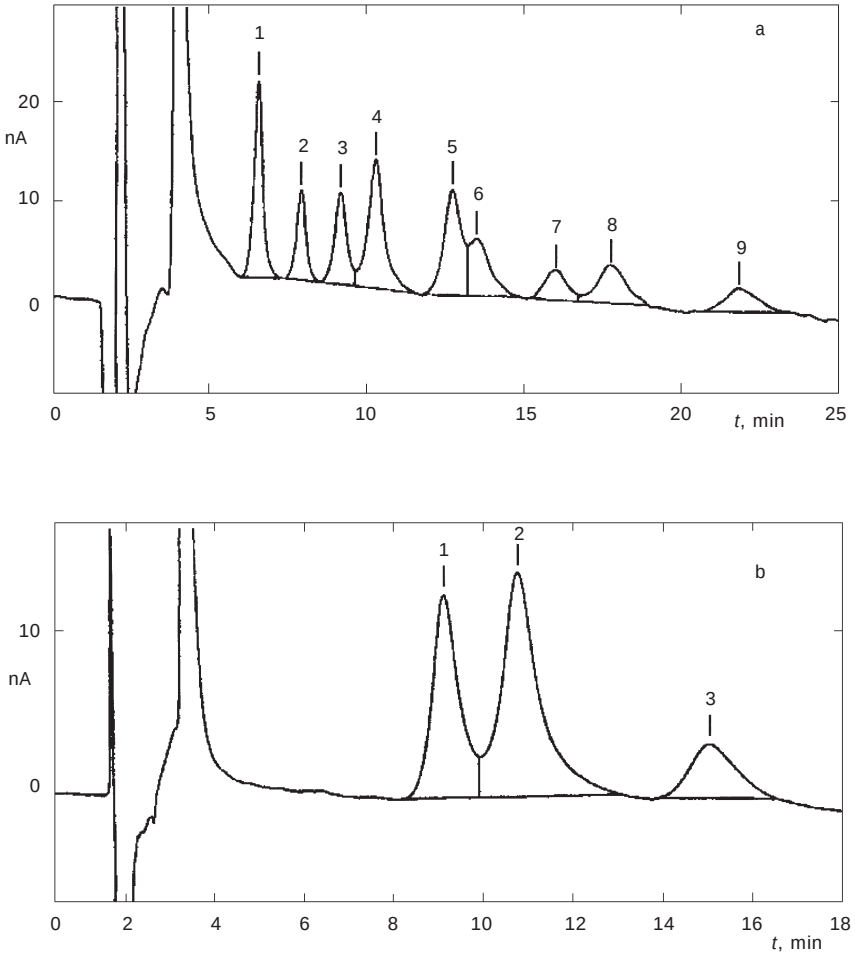

FIG. 2

Separation of nine nitroaromatic compounds (a) and two more retentive ones (b) at calibration level 5 c_{min} (see Table I). LC conditions see Table I. a Method *1*, peak description: 1 2-nitrophenol, 2 nitrobenzene, 3 4-chloro-2-nitrophenol, 4 1-chloro-2-nitrobenzene, 5 1-chloro-3-nitrobenzene, 6 2,4-dichloro-6-nitrophenol, 7 1,2-dichloro-3-nitrobenzene, 8 1,4-dichloro-2-nitrobenzene, 9 1,3-dichloro-5-nitrobenzene (the last peak from method *1* is used in method *2* to show that no coelution of compounds 1 and 2 takes place under the conditions of method *2*). b Method *2*, peak description: 1 1,3-dichloro-5-nitrobenzene, 2 1,2,4-trichloro-5-nitrobenzene, 3 tecnazene

The flow rate for both methods was chosen as a compromise between the analysis time and the backpressure in the column system and was set at 1.2 ml min^{-1} for both methods. At this flow rate the backpressure was below the limit value and the analysis time was reduced to minimum while the resolution for single NACs was not sacrificed.

The calibration curves were strictly linear in the whole calibration ranges and correlation coefficients r^2 ranged from 0.995 (nitrobenzene) to 0.999 (4-chloro-2-nitrophenol) for method *1* and from 0.998 (1,2,4-trichloro-5-nitrobenzene) to 0.999 (tecnazene) for method *2*. RSD (n = 7, calibration level 5 c_{min}, see Table I) values were in the range from 0.3 to 0.5% and from 1.2 to 8.3% for the retention times and peak areas, respectively, for method *1* and from 0.2 to 0.4% and from 2.4 to 7.1% for the retention times and peak areas, respectively, for method *2*. Concentration levels, RSD values and detection limits (based on 3 S/N criteria) calculated for the sample volume 300 µl are summarised in Table I.

TABLE I
Concentration ranges, LODs at 3 S/N level, RSD values at the calibration level 5 c_{min} (n = 7) of the calibration curves (n = 7) for 11 nitroaromatic compounds (correlation factor r^2 = 0.995–0.999, sample volume is 300 µl)

Nitroaromatic compounds	$c_{min} - c_{max}$ µmol l^{-1}	RSD, %		LOD nmol l^{-1}
		R.T.	P.A.	3 S/N
2-Nitrophenol[a]	2–20	0.4	4.0	50
Nitrobenzene[a]	4–40	0.4	6.8	250
4-Chloro-2-nitrophenol[a]	0.15–1.5	0.4	6.3	25
1-Chloro-2-nitrobenzene[a]	1–10	0.4	5.8	250
1-Chloro-3-nitrobenzene[a]	5–50	0.5	1.3	50
2,4-Dichloro-6-nitrophenol[a]	0.1–1	0.3	6.3	25
1,2-Dichloro3-nitrobenzene[a]	0.5–5	0.5	8.3	100
1,4-Dichloro-5-nitrobenzene[a]	0.4–4	0.5	2.0	100
1,3-Dichloro-5-nitrobenzene[a–c]	0.2–2	0.5	1.2	50
1,2,4-Trichloro-5-nitrobenzene[b]	0.5–5	0.2	2.4	100
Tecnazene[b]	2–20	0.4	7.1	250

[a] Method *1*: 20 mmol l^{-1} sulfuric acid in 60% acetonitrile at 1.2 ml min^{-1} and –0.5 V.
[b] Method *2*: 20 mmol l^{-1} sulfuric acid in 75% acetonitrile at 1.2 ml min^{-1} and –0.5 V.
[c] Calculated for the determination using method *1*.

When performing the real sample analyses, interfering compounds that are present in the real samples can contaminate electrodes of the AMD detector and therefore stability and reproducibility of the AMD detection system has to be checked periodically. During the analyses of UV-irradiated tecnazene solutions, where also high concentrations of phenols and chlorophenols[11] were present in addition to simple inorganic and organic anions[10], no decrease in the AMD detection system sensitivity was observed and also the reproducibility (over the period of three months) remained unchanged. Therefore no cleanup and subsequent equilibration and recalibration of the AMD detection system were needed during the whole experiment.

Determination of Photodecomposition Products

Both methods were used for the determination of NACs in the UV-irradiated solutions of tecnazene (9 samples were collected and analysed, details on irradiation time for all 9 samples are given in Table II). The typical chromatograms of tecnazene solution irradiated for 90 min, where the concentrations of interfering solutes were approximately 10-fold higher than those of nitroaromatic compounds, are shown in Figs 3a and 3b. The only problem that occurred during the qualitative analysis of real samples was the lack of commercially available standards of NACs and therefore an unidentified peak occurs in the chromatogram.

TABLE II
Concentration of decomposition products of tecnazene and of tecnazene (in μmol l^{-1}) at different irradiation times (calculated from three consecutive injections)

Compound	*t*, min								
	0	5	10	20	30	45	60	90	120
4-Chloro-2-nitrophenol	0	0	0	0	0.16	0.39	0.49	0.51	0.30
2,4-Dichloro-6-nitrophenol	0	0	0.29	0.43	0.47	0.63	0.68	0.62	0.40
1,3-Dichloro-5-nitrobenzene	0	0	0.10	0.13	0.25	0.36	0.58	0.38	0.27
Tecnazene	50.00	39.36	31.81	18.98	12.63	9.46	6.65	3.04	0.62

For the same reason, 1,2,4-trichloro-5-nitrobenzene had to be used as a standard instead of 1,2,5-trichloro-3-nitrobenzene or 1,2,4-trichloro-3-nitrobenzene that are more likely the decomposition products of UV irradiation of tecnazene. As the result of the LC-AMD determination, five NACs were detected of which three were identified and quantified as 4-chloro-2-nitrophenol, 2,4-dichloro-6-nitrophenol and 1,3-dichloro-5-nitrobenzene.

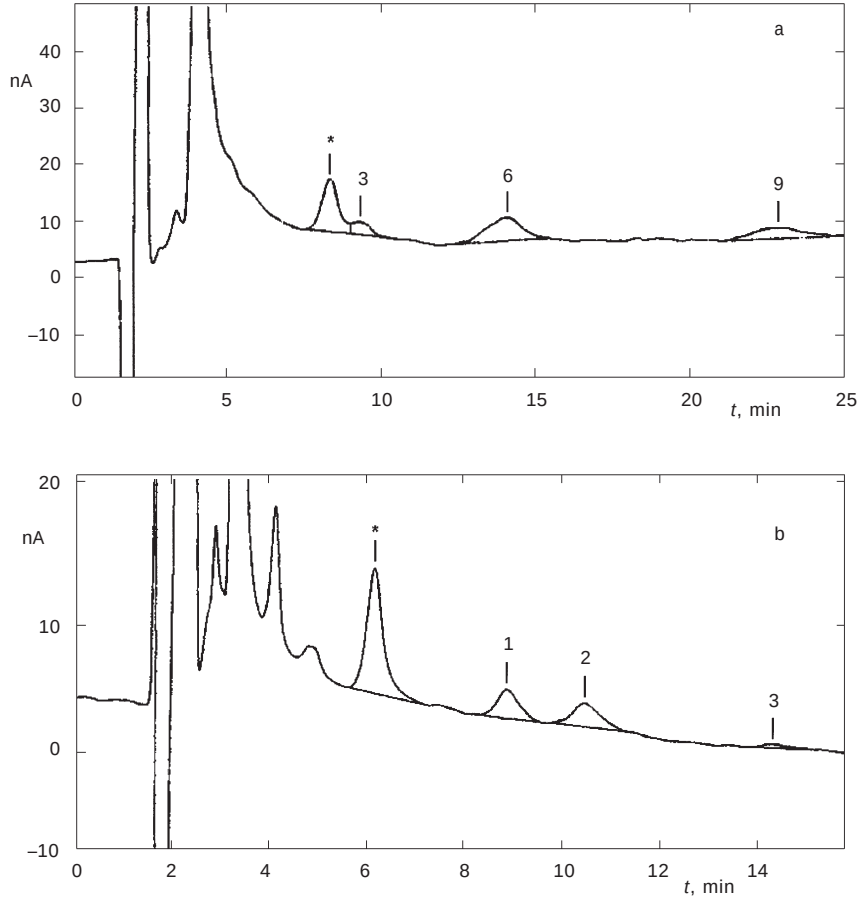

FIG. 3

An LC analysis of tecnazene solution UV-irradiated for 90 min (for LC conditions see Table I). a Method *1*, peak description: * unknown, 3 4-chloro-2-nitrophenol, 6 2,4-dichloro-6-nitrophenol, 9 1,3-dichloro-5-nitrobenzene. b Method *2*, peak description: * 2,4-dichloro-6-nitrophenol (as identified in Fig. 3a using method *1*), 1 1,3-dichloro-5-nitrobenzene, 2 trichloronitrobenzene (the confirmation of positional isomers was not possible since only one isomer was commercially available), 3 tecnazene

The quantitative results for these three compounds at different irradiation times are summarised in Table II. The fourth compound with the same retention time as 1,2,4-trichloro-5-nitrobenzene is probably one of the positional isomers of trichloronitrobenzene that are more likely the decomposition products. These trichloronitrobenzenes are not commercially available and therefore the comparison of retention times of standard and the real sample peak was not possible. Spiking the UV-irradiated tecnazene solution with the standard of 1,2,4-trichloro-5-nitrobenzene showed that the peak in UV-irradiated tecnazene solution and standard solution peak are co-eluted having the same retention time. According to the chromatographic behaviour of trichloro isomers of aromatic phenols on the NS1 chromatographic column, where all trichlorophenols are eluted with nearly the same retention time, similar behaviour of the trichloronitrobenzenes could be expected. Therefore it can be proposed that the peak in the irradiated tecnazene solution corresponds to one (or mixture) of 1,2,4-trichloro-3-nitrobenzene or 1,2,5-trichloro-3-nitrobenzene. This peak was not quantified.

The fifth peak in real samples did not match with retention times of any of the NACs in the standard solution and, since all commercially available NACs were tested for its retention times, the identification of this peak was not possible.

According to the results obtained in the experiments, it can be suggested that the chlorinated nitrobenzenes and nitrophenols (4-chloro-2-nitrophenol, 2,4-dichloro-6-nitrophenol and 1,3-dichloro-5-nitrobenzene, and one (or mixture) of 1,2,4-trichloro-3-nitrobenzene or 1,2,5-trichloro-3-nitrobenzene) are significant intermediates. The formation of nitrite and nitrate anions[10] could indicate the replacement of the ring nitro group by hydroxy group or just releasing the nitro group and subsequent replacement by a proton while the early release of chloride[10] is an evidence of a dechlorination pathway of tecnazene decomposition. The symmetrical halving of the benzene ring into C_3 molecules and symmetrical formation of the C_2 molecules is more likely while the non-symmetrical opening of the benzene ring to form C_4 and/or C_1 molecules is less probable in the second step[10,11].

CONCLUSIONS

The developed LC-AMD system has proved to be an effective analytical tool for the determination of nitroaromatic compounds in samples with complex matrices due to its high reduction/oxidation selectivity. No interfer-

ence of phenols and/or chlorophenols in the irradiated tecnazene solutions has occurred, although the concentrations of these compounds were approximately 10-fold higher than those of NACs. This approach can be very advantageous in analyses of samples that contain not only the NACs but also high concentrations of other substances (*e.g.* phenols, chlorophenols or amines).

The developed LC-AMD methods have been applied to the determination of NACs in UV-irradiated tecnazene solution. The results showed that NACs are the photodecomposition products next to the anions and to the phenols and chlorophenols. Application to the wastewater from the potato washing and processing plants and river water was unsuccessful since the worse LODs. In large quantities of water, microbial and photochemical degradation processes reduce the concentration of tecnazene and its degradation products down the LODs.

The main disadvantage of the developed analytical methods lies in the long analysis time that causes higher analysis costs and bigger amount of the used mobile phase.

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