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Automated solid-phase extraction and high-performance liquid chromatographic determination of nitrosamines using post-column photolysis and tris(2,2′-bipyridyl) ruthenium(III) chemiluminescence

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Abstract

A sensitive and selective post-column detection system for nitrosamines is described. The principle upon which the detector works is that UV irradiation of aqueous solutions of nitrosamines leads to cleavage of the N–NO bond. The amine generated is subsequent detected by chemiluminescence using tris(2,2′-bipyridyl) ruthenium(III), which is on-line generated by photo-oxidation of the ruthenium(II) complex in the presence of peroxydisulfate. Factors affecting the photochemical and chemiluminescent reactions were optimized to minimise their contribution to the total band-broadening. This detection system was tested for *N*-nitrosodimethylamine, *N*-nitroso-diethylamine, *N*-nitrosomorpholine, *N*-nitrosopiperidine and *N*-nitrosopyrrolidine, which were separated on an ODS column by isocratic reversed-phase chromatography with acetonitrile–water containing 5 mM acetate buffer at pH 4.0. A linear relationship between analyte concentration and peak area was obtained within the range 0.13–500 $\mu g\,l^{-1}$ with correlation coefficients greater than 0.9995 and detection limits of between 0.03 and 0.76 $\mu g\,l^{-1}$. Intra- and inter-day precision values of about 1.2% RSD ($n = 11$) and 2.5% RSD ($n = 10$), respectively, were obtained. The sensitivity may increase from 9 to 280 times with respect to UV detection, depending on the nitrosamine in question. An automated solid-phase extraction (SPE) system was used in conjunction with HPLC to determine nitrosamine residues in waters. Detection limits within the range 0.10–3.0 $ng\,l^{-1}$ were achieved for only 250 ml of sample.

Keywords: Nitrosamines; Automated solid-phase extraction; HPLC; Chemi-luminescence detection; Photohydrolysis; tris(2,2′-Bipyridyl)ruthenium(III) photo-generated; Water analysis

1. Introduction

N-Nitrosoamines (NAm) are known to be carcinogenic in a variety of animal species [1,2]. NAm require metabolic activation by cytochrome P450 for their carcinogenicity [3], yielding an alkylating agent that may react with DNA and protein [4]. The carcinogenicity of NAm, and the apparent ease of formation with which they are formed in air, water, soil and even in man, when the appropriate amine and nitrite precursors are present, makes the analytical determination of the compounds important. For a particular analytical technique to be useful for trace analysis of NAm in environmental and biological systems, it must be fast, sensitive, and specific, while, at the same time, providing a high degree of accuracy and precision.

High-performance liquid chromatography (HPLC) is a useful technique for trace quantities of *N*-nitroso compounds and specific post-column approaches based on the cleavage of the N–NO bond have been reported. This cleavage can be carried out by hydrolysis with aqueous mineral acids and heating [5] or UV irradiation [6,7] or by denitrosation with hydrobromic acid–acetic anhydride [8,9]. The amine or nitrite thus released can be detected. Nitrite has been monitored by spectrophotometry with Griess-type reagents [10,11], or by measuring the fluorescence of Ce^{3+} formed when this anion is oxidised with Ce^{4+} [5] or by chemiluminescence (CL) using the classical atmospheric nitrogen oxide detector [12].

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Detection of amines has been carried out by reaction with dansyl chloride to the fluorescent dansyl derivatives [8,13]. Increasing sensitivity was achieved using bis(2-nitrophenyl) oxalate and hydrogen peroxide as post-column CL reagents for the dansyl derivatives [9].

In recent years, CL as a detection technique for use with HPLC has become more attractive due to its high sensitivity, wide linear range and simple instrumentation [14]. The CL from tris(bipyridyl)ruthenium(III) [$Ru(bpy)_3^{3+}$] has emerged as a versatile detection method for the determination of a variety of compounds containing amine functionality [15,16]. Common to all these applications is the formation of $Ru(bpy)_3^{3+}$, which is then reduced by the analyte to emit light. The active reagent $Ru(bpy)_3^{3+}$ has been generated by chemical, photochemical and electrochemical oxidation and in situ electrochemiluminescence (ECL) [17]. Despite the large theoretical potential of ECL, only certain reactions have been found widespread applicability in analytical science, because significant limitations such as the presence of interfering species, the complex optimisation and the lack of reproducibility make ECL less attractive than conventional CL [18]. In previous works, it was found that the photochemically assisted oxidation of $Ru(bpy)_3^{2+}$ to $Ru(bpy)_3^{3+}$ in the presence of peroxydisulfate is very effective and reproducible and it has been successfully used in the CL determination of several analytes [19–22].

The aim of this work was to develop a simple, automatic and sensitive HPLC method for the determination of NAm, which can be easily applied to the analysis of real samples. The post-column detection system used two photoreactors, in one of which $Ru(bpy)_3^{3+}$ is generated by on-line photo-oxidation of $Ru(bpy)_3^{2+}$ with peroxydisulfate while in the other the NAm eluted from the column are first photodegradated to the corresponding amines, which react with $Ru(bpy)_3^{3+}$ to give strong CL.

Finally, a solid-phase extraction (SPE) system was used in conjunction with HPLC to determine NAm in natural waters. In the proposed approach, sample preparation, sample concentration, separation and detection were all automated.

2. Experimental

2.1. Reagents

All the reagents were of analytical grade. Ultrapure water from a Milli-Q plus system (Millipore Ibérica, Madrid, Spain) was used throughout. Acetonitrile, acetone and methanol were of HPLC grade (Romil, Loughborough, UK). *N*-Nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitroso-morpholine (NMOR), *N*-nitrosopiperidine (NPIP) and *N*-nitrosopyrrolidine (NPYR) were obtained from Sigma-Aldrich (Madrid, Spain). tris(2,2′-Bipyridyl)ruthenium(II) from Fluka (Steinheim, Switzerland) and potassium peroxydisulfate and Dowex 2X8-100 ion exchanger from Merck (Darmstadt, Germany).

Standard solutions of NAm were prepared by dissolving the pure compounds in water (10 μg ml^{-1}). Working NAm solutions were prepared by diluting the standard solutions. All solutions were filtered with a 0.45 μm membrane filter (Millipore Ibérica) and stored in the dark at 4 °C.

2.2. Apparatus

The instrumental setup used in this study (Fig. 1) consisted of an HPLC Beckman Coulter (Fullerton, CA, USA) instrument composed of a System Gold 125 NM solvent Module, a System Gold 186 diode array detector and a Rheodyne injection valve. An Aspec XLi (Gilson, Middleton, WI, USA) was used for the injection of the sample into an Ultrasphere column (Beckman) (5 μm C_{18}, 250 mm × 4.6 mm). The CL detector was a Camspec CL-2 (Cambridge, UK) luminometer equipped with a three-port flow cell (two inlets and one exit). The CL detector was connected to the HPLC equipment by means of an SS420x interface (Beckman).

The post-column detection system is depicted schematically in Fig. 1. Two Gilson Miniplus-3 peristaltic pumps were used for the addition of reagents. Except for the pump tubing (Tygon), PTFE tubing (0.5 mm I.D.) was used through the manifold. Two photoreactors, each consisting of PTFE tubing (0.5 mm I.D., length L_1 = 300 cm and L_2 = 100 cm) coiled around Spectronic (Westbury, NY, USA) rod-shape low pressure mercury lamps (50 mm × 5 mm diameter) were incorporated in the manifold. The lamps operated at 6 W and the main spectral line was at 254 nm. Both photoreactor-lamp assemblies were housed in fan-ventilated metal boxes covered with aluminium foil to increase the photon flux by reflection.

All extraction steps were performed automatically using the Aspec XLi coupled to a Gilson 306 pump so that large volumes of sample could be used. Strata X, surface modified styrene–divinylbenzene polymer (Phenomenex, CA, USA), was used as the solid phase in the extraction.

2.3. Basic procedure

The NAms were separated on an ODS column with an isocratic elution program at a flow rate of 1.2 ml min^{-1}. The mobile phase consisted of 5 mM acetate buffer at pH 4 and acetonitrile (95:5, v/v). The outlet of the chromatographic column was connected to the photohydrolytic reactor L_1 where the NAms were converted to the corresponding amines. The effluent from the photolysis coil was added to the 100 mM phosphate buffer at pH 7 through a T-connector and finally directed to one of the flow cell ports. $Ru(bpy)_3^{3+}$ was on-line generated by photochemically assisted oxidation of 2 mM $Ru(bpy)_3^{2+}$ with 1.5 mM peroxydisulfate along the photoreactor L_2 and pumped to the other inlet of the flow cell. Thus, the mixing occurred in the flow cell. Finally, the CL emitted light was measured without wavelength discrimination. The tubing between the two photoreactors and the flow cell was covered with black insulating tape to prevent a fiber optic effect from introducing stray light into the detector.

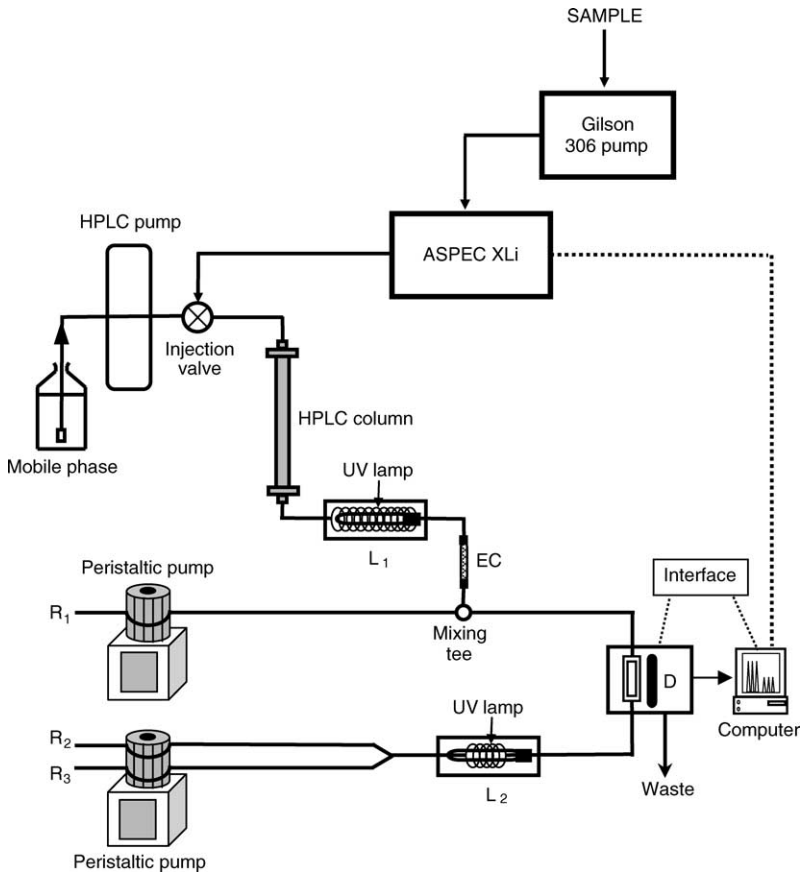


Fig. 1. Assembly used for automated SPE and HPLC determination of NAm in waters using on-line post-column CL detection. R_1: 0.1 M phosphate buffer of pH 7.0; R_2: 1.5×10^{-3} M peroxydisulfate and 0.03 M phosphate buffer of pH 5.7; R_3: 2×10^{-3} M $Ru(bpy)_3^{2+}$; L_1, L_2: photoreactors; EC: exchange minicolumn; D: CL detector.

2.4. Automated extraction and NAm concentrations

Strata X cartridges (200 mg) with a 3-ml reservoir were conditioned with 2 ml of acetone followed by 2 ml of ultrapure water separated by 0.5 ml of air, prior to application of the sample. The water samples (250 ml) were pumped through the cartridge at a flow rate of 5 ml min^{-1} using a Gilson 306 pump which was controlled by the software of the Aspec. The cartridge was washed with 1 ml ultrapure water and 1 ml air. The retained NAm were eluted at 1 ml min^{-1} with 2 ml of acetone followed by 3 ml air. The eluate was evaporated to near dryness at 45 °C by passing an air stream for 1 min. The residue was dissolved with 0.5 ml of ultrapure water. A 20 μl aliquot was injected by the Aspec into the chromatographic system.

The HPLC system and the SPE worked simultaneously, i.e., while sample 1 was being analysed, sample 2 was extracted in the Aspec.

2.5. Sample preparation procedure

The water samples were collected, filtered through a 0.45 μm membrane filter, acidified to pH 2 with hydrochloric acid, stored at 4 °C and analysed within two days of collection. Prior to the analysis, the samples were neutralised with sodium hydroxide. NAm were added to water samples by placing 10 ml of a NAm mixture solution (containing between 0.5 and 24 ng of each NAm) in a volumetric flask and making up to 1000 ml with the water sample.

3. Results and discussion

3.1. Study of the post-column detection system

To facilitate the study of the post-column reactions, no chromatographic column was used.

3.1.1. On-line photogeneration of $Ru(bpy)_3^{3+}$

The procedure for the generation of the Ru(III) complex was selected on the bases of our previous studies [21,22]. The optimization of the FI system outlined in Fig. 1 was carried out using dimethylamine or pyrrolidine as model compounds in order to avoid the influence of the variables affecting the photoconversion of NAm into its corresponding amine. The variables studied were pH, peroxydisulfate

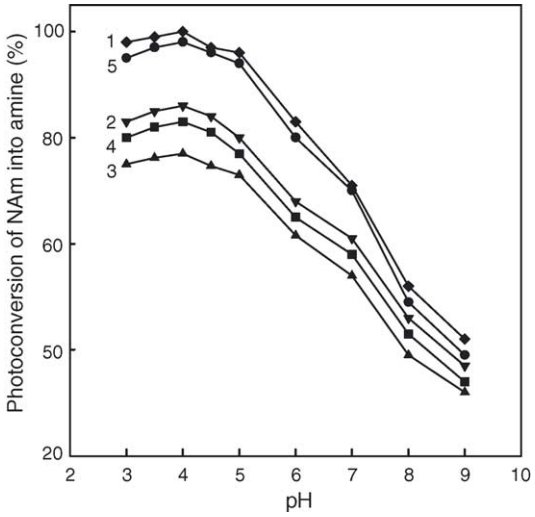


Fig. 2. Effect of pH on the photoconversion of NAm into amines. (1) NDMA; (2) NDEA; (3) NMOR; (4) NPIP; (5) NPYR. Conditions: NAm concentration, 5×10^{-6} M; Ru(III) complex concentration, 2×10^{-3} M.

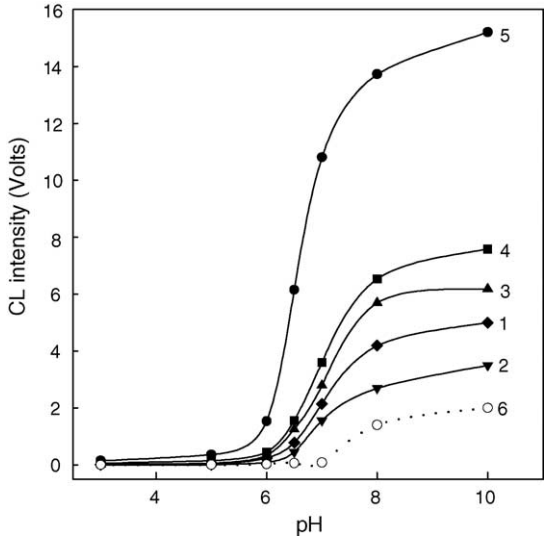
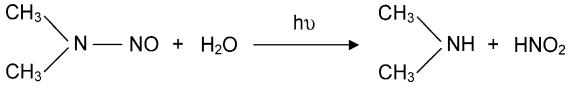


Fig. 3. Effect of pH on $Ru(bpy)_3^{3+}$/amines CL reaction. (1) DMA; (2) DEA; (3) MOR; (4) PIP; (5) PYR; (6) blank. Conditions: amine concentration, DMA: 75 ng ml^{-1}; DEA:100 ng ml^{-1}, MOR, PIP, PYR:25 ng ml^{-1}; Ru(III) complex concentration, 2×10^{-3} mol l^{-1}.

concentration, $Ru(bpy)_3^{2+}$ concentration, flow rate, length of photoreactor (L_2). The greatest CL signal was obtained using the following conditions: $Ru(bpy)_3^{2+}$ (2×10^{-3} M), peroxydisulfate (1.5×10^{-3} M $K_2S_2O_8$ buffered at pH 5.7), photoreactor L_2 (length 100 cm) and flow rate (1.2 ml min^{-1}).

3.1.2. Photolysis of NAm

Ultraviolet irradiation of aqueous NAm solutions led to a rapid decay of NAm and the formation of the corresponding amine and nitrite as the major products [6,7]. The reaction for NDMA is:



NO_3^- ions were generated along with NO_2^- ions, but at a low level. Kinetic studies had indicated a relatively strong pH dependence of the quantum yield for the direct photolysis of NAm [6]. Therefore, the influence of pH on the photoconversion of NDMA, NDEA, NMOR, NPIP and NPYR to the corresponding amine in the FI system was studied by injecting samples of each NAm (100 μl, 5×10^{-6} M) into a carrier of a buffer solution (0.01 M sodium dihydrogen phosphate) adjusted at different pH values between 3 and 9 with a flow rate of 1.2 ml min^{-1}. The efficiency of the photochemical reaction increased with increasing pH up to about 4 and then decreased at higher values (Fig. 2). A pH of 4.0 was considered appropriate for all NAm.

At a constant radiation intensity, the illumination time had a decisive effect on the photochemical reaction and hence on the sensitivity attained. The residence time of the analyte in the photoreactor L_1 can be selected by controlling the flow rate of the carrier stream and/or the length of the reactor. An irradiation time of 30 s was sufficient to achieve efficiency within the range 77–100% for the conversion of all NAm to amines. This time was obtained using a reactor length of 300 cm and a flow rate of 1.2 ml min^{-1} for the carrier stream.

It is worth noting that nitrite showed a strong inhibitory on the amine/$Ru(bpy)_3^{3+}$ CL reaction, a problem that was easily avoided by placing an anion exchanger minicolumn at the exit of photoreactor L_1 to retain the nitrite.

3.1.3. Optimisation of CL system

The efficiency of the light emission of the reaction between $Ru(bpy)_3^{3+}$ and the amines, DMA, DEA, MOR, PIP and PYR was greatly affected by the pH. Data obtained for the CL signal of each amine and background at various pH values are shown in Fig. 3. Although the background-corrected CL signals increased with increasing pH, values higher than 7.5 are not recommended because the backgrounds were very high as a result of the hydroxide ion reducing $Ru(bpy)_3^{3+}$ to $Ru(bpy)_3^{2+}$ with the emission of CL [23].

The total flow rate is a very important parameter in the CL reaction and should be regulated. When flow rates are too slow or too high, CL is not emitted in the flow cell and hence the emitter cannot be detected. Under the above selected conditions, CL increased with increasing flow rates; an overall flow rate of 6.4 ml min^{-1} was selected, because higher flow rates caused more noise, higher pressure in the connection and the excessive consumption of reagents.

3.2. Chromatographic conditions

In developing the procedure, both the appropriate HPLC conditions and the chemistry of the detection system were considered. Since the post-column reactions (photochemical and CL) are aqueous, the use of a reverse-phase LC column was essential. The Ultrasphere C_{18} column with

acetonitrile–water mixtures provided an excellent separation for the five NAm studied, but a problem was encountered in the CL flow system. Acetonitrile decreased the CL signal (10% and 20% acetonitrile in water reducing the CL by about 15% and 50%, respectively). We found 5% acetonitrile/95% 5 mM acetate buffer (pH 4.0) to be a suitable mobile phase because the resolution of the peaks was good, the photoconversion of NAm into amine took place under the recommended conditions and the CL signal was not affected. The flow rate of the mobile phase was set at 1.2 ml min^{-1} in order to attain the optimum flow rate of 2.4 ml min^{-1} at one of the inlets of the CL flow cell.

Fig. 4A and B show chromatograms of a mixture of NDMA, NDEA, NMOR, NPIP and NPYR using the post-column photochemical reaction—CL detection and photometric detection at 220 nm. From a comparison of the two chromatograms it may be concluded that the proposed detection system can indeed be employed for the sensitive detection of NAm. The sensitivity can increase from 9 to 280 times with respect to UV detection depending of the NAm.

3.3. Analytical figures of merit

In order to evaluate the quantitative performance of the proposed method, calibration graphs for each NAm were obtained by injecting standard solutions of the analytes in the concentration range 0.13–500 μg l^{-1}. Each point of the calibration graph is an average of at least three injections. The linearity between peak area and concentration was good for all the analytes. The regression equations and other characteristics parameters are shown in Table 1. The limits of detection were determined at a signal-to-noise ratio of 3. The reproducibility of the method was studied with 11 repeated injections of two sample solutions containing the analytes at two concentration levels, 5 and 80 μg l^{-1} for NDMA, NMOR, NPIP and NPYR and 10 and 100 μg l^{-1} for NDEA. The relative standard deviations (RSDs) were always less than 1.2%. The RSD values according to the retention time at the same day at each concentration level changed between 0.5% and 1.5%, indicating again good reproducibility. The inter-day precision of the method was studied by analysing two samples containing the five NAm at 5.0 and 20.0 μg l^{-1} concentration levels. On five consecutive days, each sample was injected 10 times every day. The RSDs for peak area were less than 2.5%.

Amines are tolerated because they are eluted as poorly resolved peaks before NAm. If any co-eluting amine is present

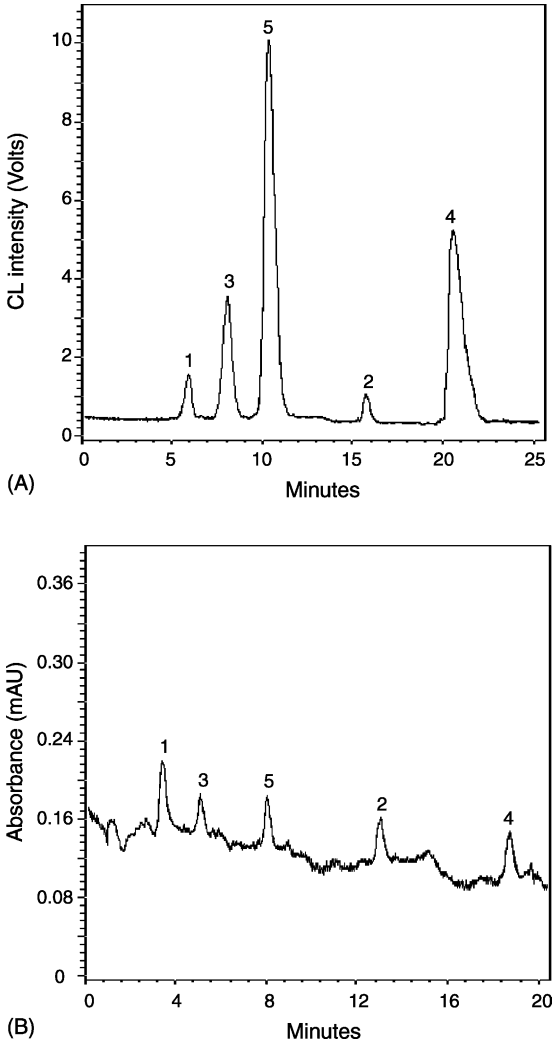


Fig. 4. Typical chromatograms of a standard solution of a mixture of NAm. (A) Photochemical–CL detection. (B) Photometric detection at 220 nm. Mobile phase: acetonitrile–5 mM acetate buffer pH 4.0 (5:95, v/v). NAm concentration, 15 ng ml^{-1}.

(at a concentration lower than 60 times of that of NAm) its effect can be obviated by comparing the measurements with those obtained by the same HPLC system when the mercury lamp of the photohydrolytic reactor (L_1) was turned off.

3.4. Analysis of real water samples

The levels of NAm in water consumption must be very low, EPA established an average clean up level of about 1 ng l^{-1}

Table 1
Calibration fits without preconcentration

Nitrosamine	Intercept (10^3 area units)	Slope (10^4 area units/μg l^{-1})	Correlation coefficient	Linear range (μg l^{-1})	DL (μg l^{-1})
NDMA	−6.8 ± 2.8	7.50 ± 0.03	0.9998	1.5–148	0.29
NDEA	−4.3 ± 0.7	3.50 ± 0.03	0.9997	4.2–510	0.76
NMOR	−33.5 ± 8.4	14.1 ± 0.4	0.9996	0.52–93	0.09
NPIP	28.9 ± 9.6	35.0 ± 0.6	0.9998	0.36–103	0.07
NPYR	11.2 ± 3.2	49.2 ± 0.4	0.9997	0.13–35	0.03

for individual NAm [24]; hence, analyte preconcentration prior to application of the proposed method is required in order to reach sensitivity levels below the legal limits. Several methodologies, such as liquid–liquid extraction, supercritical fluid extraction and SPE have been proposed [25–27]. We chose the SPE technique for its simplicity, rapidity, easy automation and economy.

Simple initial experiments to determine SPE cartridge suitability were conducted using 100 ml of 50 ng l^{-1} aqueous solutions of the five NAm and using methanol as an eluent. Initially, active carbon was selected because this solid phase has been used with NAm [28,29]. The carbon cartridges proved poor at simultaneously retaining all five NAm at acidic, neutral and basic pH values. Recoveries of between 12% and 34%, depending of each NAm, were obtained.

Polymer based cartridges were then investigated as they are much better able to simultaneously retain a wide range of polar and non-polar compounds, for which reason they are increasingly used in preference to other solid phases for multianalyte environmental and pesticide analysis [30,31]. As a surface modified styrene–divinylbenzene polymer (Strata X) gave the highest recoveries for the NAm, a cartridge containing 200 mg of this solid with a 3-ml reservoir was selected. For NAm desorption from the cartridge the solvents, acetone and methanol, were studied. Acetone was found to be the most suitable. The amount of solvent required for optimum elution was determined by eluting with different volumes of acetone and measuring the recoveries. The results demonstrated that 2 ml was the minimum volume of elution solvent that could be used since non-significant difference were achieved for higher volumes. The automated SPE procedure was checked for standard samples at 1–150 ng l^{-1} concentration levels. The recovery of the NAm remained constant when the water volume passing through the extraction cartridge was increased from 100 to 500 ml. The sample volume selected was 250 ml as a compromise between sensitivity and analysis time. Under these conditions, the determination of NAm at trace levels in water should be possible because of the enrichment factor of 500 in the preconcentration step. The recoveries of the five NAm were measured with spikes of 0.8, 20 and 100 ng l^{-1} in ultrapure water (Table 2). The differences in recovery observed between each NAm are explained by the differing polarities which affected their elution from the columns. Some of the loss of NDMA, the most volatile nitrosamine, occurs during the final concentration step, where the solvent is reduced to achieve an appropriate preconcentration.

In order to check the applicability of the proposed method to real matrices, water samples from different points of the province of Murcia were analysed. The environmental water samples were named as A1 and A2: tap water; A3 and A4: irrigation ditch water; and A5: Segura river. An aliquot (250 ml) of each sample was first analysed following the same procedure to provide ambient-level NAm concentrations; none of the samples gave peaks that interfered with the determination of these NAm (Fig. 5A). Samples were then fortified at two concentration levels for each NAm. The spiked concentrations were calculated employing the corresponding standard calibration graph for each NAm and its average recovery in ultrapure water. The samples were also analysed using the standard additions method in order to avoid any potential matrix effect. The results obtained by both procedures (Table 3) did not show significant differences. Fig. 5B shows

Table 2
NAm recoveries from ultrapure water samples

Nitrosamine	Fortification level (ng l^{-1})	Recovery[a] (%)
NDMA	10	39 ± 2
	50	40 ± 3
	100	40 ± 2
		40 ± 2[b]
NDEA	20	51 ± 1
	40	49 ± 3
	100	50 ± 2
		50 ± 2[b]
NMOR	3	56 ± 3
	10	54 ± 3
	100	55 ± 2
		55 ± 3[b]
NPIP	1	90 ± 1
	20	89 ± 2
	100	90 ± 2
		90 ± 2[b]
NPYR	0.8	59 ± 2
	5	61 ± 4
	100	60 ± 3
		60 ± 3[b]

[a] Mean of four determinations ± SD.
[b] Average.

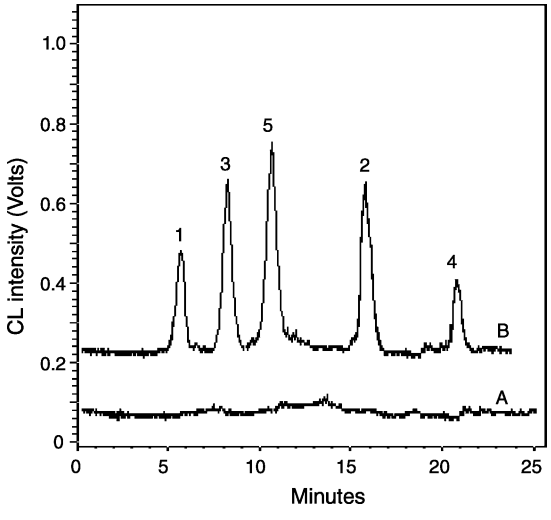


Fig. 5. Chromatograms of an irrigation ditch water sample: (A) ambient-level NAm concentration; (B) fortified with NDMA, NDEA, NMOR, NPIP and NPYR at 18.9, 46.9, 8.2, 1.1 and 2.7 ng l^{-1}, respectively.

Table 3
Determination of NAm in water samples

Sample	Spiked concentration ($ng\,l^{-1}$)					Found spiked concentration[a] ($ng\,l^{-1}$)				
	NDMA	NDEA	NMOR	NPIP	NPYR	NDMA	NDEA	NMOR	NPIP	NPYR
A1	9.0	20	2.4	1.0	0.70	8.9 ± 0.2^{b}, 9.0 ± 0.2^{c}	19.3 ± 0.2^{b}, 19.4 ± 0.2^{c}	2.4 ± 0.1^{b}, 2.3 ± 0.1^{c}	1.0 ± 0.1^{b}, 0.98 ± 0.1^{c}	0.68 ± 0.1^{b}, 0.69 ± 0.1^{c}
A2	7.5	17	3.0	0.95	0.50	7.4 ± 0.2^{b}, 7.4 ± 0.1^{c}	16.9 ± 0.2^{b}, 16.7 ± 0.2^{c}	3.0 ± 0.1^{b}, 3.1 ± 0.1^{c}	0.93 ± 0.1^{b}, 0.94 ± 0.1^{c}	0.48 ± 0.02^{b}, 0.51 ± 0.03^{c}
A3	8.0	19	2.0	0.90	0.60	8.0 ± 0.2^{b}, 7.9 ± 0.1^{c}	18.9 ± 0.3^{b}, 19.2 ± 0.2^{c}	1.9 ± 0.1^{b}, 2.0 ± 0.1^{c}	0.88 ± 0.03^{b}, 0.89 ± 0.02^{c}	0.61 ± 0.1^{b}, 0.59 ± 0.04^{c}
A4	9.5	18	2.6	1.5	0.80	9.5 ± 0.2^{b}, 9.3 ± 0.2^{c}	18.1 ± 0.2^{b}, 17.9 ± 0.2^{c}	2.5 ± 0.1^{b}, 2.4 ± 0.2^{c}	1.4 ± 0.1^{b}, 1.5 ± 0.1^{c}	0.79 ± 0.03^{b}, 0.80 ± 0.02^{c}
A5	7.7	16.9	2.2	1.1	1.0	7.6 ± 0.2^{b}, 7.7 ± 0.1^{c}	16.8 ± 0.2^{b}, 16.7 ± 0.2^{c}	2.1 ± 0.2^{b}, 2.2 ± 0.1^{c}	1.06 ± 0.03^{b}, 1.02 ± 0.04^{c}	1.04 ± 0.04^{b}, 1.02 ± 0.02^{c}

A1 and A2: tap water sample; A3 and A4: irrigation ditch water sample; A5: Segura river sample.
[a] Average of three determinations ± SD.
[b] Standard calibration graph method.
[c] Standard additions method.

the chromatogram of an irrigation ditch water spiked with the five NAm at concentration levels in the range 1.1–46.9 $ng\,l^{-1}$.

4. Conclusions

A fully automated SPE-HPLC-CL detection system has been developed for the determination of NAm. The CL detection is based on the cleavage of the N–NO bond of NAm to give the amine with subsequent CL detection with $Ru(bpy)_3^{3+}$, which was on-line generated by photo-oxidation of the Ru(II) complex. The main advantages of the proposed detection system are: (a) great stability and very low background of the baseline, (b) presence of very few peaks and (c) analyte derivatization is not required.

In the automated on-line preconcentration scheme proposed, problems with analyte losses or contamination are minimal because the extracts can be injected automatically into the chromatographic system. The linearity of the calibration graphs shows the suitability of the overall system, including adsorption, desorption, separation and detection. The limits of detection of NAm were set at sub $ng\,l^{-1}$ levels by sampling 250 ml of water. No significant differences were observed in the quantification of the analytes between the different sample types tested.

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