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Determination of N-Nitrosamines by Gas Chromatography Coupled to Quadrupole–Time-of-Flight Mass Spectrometry in Water Samples

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Abstract: An analytical method based on high-resolution quadrupole–time-of-flight (QToF) mass spectrometry has been developed as an alternative to the classical method, using a low-resolution ion trap (IT) analyzer to reduce interferences in N-nitrosamines determination. Extraction of the targeted compounds was performed by solid-phase extraction (SPE) following the United States Environmental Protection Agency (USEPA) -521 method. First, both electron impact (EI) and positive chemical ionization (PCI) using methane as ionization gas were compared, along with IT and QToF detection. Then, parameters such as limits of detection (LOD) and quantification (LOQ), linearity, and repeatability were assessed. The results showed that the QToF mass analyzer combined with PCI was the best system for the determination of the N-nitrosamines, with instrumental LOD and LOQ in the ranges of 0.2–4 and 0.6–11 ng mL^{-1}, respectively, which translated into method LOD and LOQ in the ranges of 0.2–1.3 and 0.6–3.9 ng L^{-1}, respectively. The analysis of real samples showed the presence of 6 of the N-nitrosamines in influent, effluent, and tap water. N-nitrosodimethylamine (NDMA) was quantified in all the analyzed samples at concentrations between 1 and 27 ng L^{-1}. Moreover, four additional nitrosamines were found in tap and wastewater samples.

Keywords: gas chromatography–mass spectrometry (GC-MS); high-resolution mass spectrometry (HRMS); N-nitrosamines; water samples; positive chemical ionization (PCI)

1. Introduction

The occurrence of N-nitrosamines in surface water, wastewater, and finished drinking water is a relevant issue of environmental and public health significance because many N-nitrosamines are probable human carcinogens, mutagens, and teratogens [1]. Many industrial manufacturing processes, such as those for rubber, cosmetics, food, and detergent, are sources of these compounds. In addition, potential sources of nitrosamines include postcombustion CO_2 capture using amine-based scrubbing systems, tobacco smoke, and disinfection of drinking water. Nitrosamines have been found in surface and groundwater, in wastewater treatment plant influents and effluents, as well as in drinking water [2]. The occurrence of N-nitrosamines in drinking water or treated wastewater may be caused by the pollution of raw waters by nitrosamines or their formation during water treatment processes, such as chloramination or chlorination. They have been found in water as by-products of oxidized amines after chlorination treatment in wastewater treatment plants (WWTPs) [3]. In California, United States, N-nitrosodimethylamine (NDMA) has been found at concentrations over 100 ng L^{-1} in effluent waters

and below 10 ng L^{-1} in surface waters, both after chlorination treatments [4]. Nevertheless, the reaction of chloramines with amine precursors is likely the dominant mechanism responsible for NDMA formation in water [5–7]. Specific NDMA precursors in wastewater-impacted source waters may include tertiary amine-containing pharmaceuticals or other quaternary amine-containing constituents of personal care products [8]. Recent studies have demonstrated that NDMA formation by chlorination is a low-yield process, resulting in concentrations below 12% compared to the total chlorine loss after the treatment at a pH in the range of 6 to 8 [8].

Due to the high carcinogenic activity of some nitrosamines, the World Health Organization (WHO) has included NDMA in its guidelines for drinking water quality, with a guideline value of 100 ng L^{-1} [9]. Nitrosamines are on the chemical contaminants candidate list of the U.S. Environmental Protection Agency (EPA), however to date, major regulations are still at a regional scale. For instance, the government of California set a notification level of 10 ng L^{-1} and response level of 100 ng L^{-1} for NDMA, N-nitrosodiethylamine (NDEA), and N-nitrosodipropylamine (NDPA), on the basis of the 10^{-6} risk level estimates, which ranges from 1 to 15 ng L^{-1} for seven nitrosamines [10].

Research on nitrosamine occurrence in waters would not be possible without the development of sensitive methods and analytical procedures, particularly considering the low concentration levels that need to be determined. The methods currently used for the determination of nitrosamines are mostly based on enrichment of nitrosamines by solid-phase extraction (SPE) [11,12], solid-phase microextraction (SPME) [13], or dispersive SPE [14] and chromatographic analysis with mass spectrometry (MS) detection, either gas chromatography (GC) [12,15] or liquid chromatography (LC) [16]. The U.S. EPA-521 method [11] for the determination of seven nitrosamines in drinking water proposes an SPE using coconut charcoal as the sorbent, dichloromethane as the elution solvent, concentration of the extract to less than 1 mL, and GC coupled to tandem MS (GC-MS/MS) detection by chemical ionization source operating in positive (PCI) mode and using an ion trap mass analyzer (GC-PCI–MS/MS). Electron impact (EI) sources could also be used, however nitrosamine fragmentation in EI sources provides smaller ions that could be interfered with by background ions, misleading the identification [12]. Thus, a softer ionization source such as PCI is recommended in the literature [11–14,17–21], with which fragmentation would be lower. However, even without fragmentation, due to the low molecular weight of nitrosamines, background ions can interfere with their determination. Such ion interference could theoretically be solved by using high-resolution mass spectrometry (MS).

Thus, the aim of this work is to develop an alternative to low-resolution ion trap (IT) mass analyzers. The hypothesis to be tested is whether a high-resolution quadrupole–time-of-flight (QToF) instrument can lead to better performance due to its selectivity and overcome the abovementioned problems of low-resolution MS. Although a methodology based on magnetic sector high-resolution mass spectrometry was already published some years ago [22], we herein would like to demonstrate that GC-QToF instruments, often used for qualitative purpose [23–25], can compete with such older technologies also for quantitative applications. Therefore, a comparison between IT and QToF analyzers and both EI and PCI MS ionization sources was performed. Finally, real water samples, including wastewater and tap water, were analyzed to determine the considered compounds with GC-PCI-QToF after SPE following the U.S. EPA-521 method. The nitrosamines considered were those amenable by GC-MS, reported previously in drinking, surface, and wastewater [18,26,27] and included in the U.S. EPA-521 method, i.e., NDMA, N-nitrosomethylethylamine (NMEA), NDEA, NDPA, N-nitrosodibutylamine (NDBA), N-nitrosopiperidine (NPIP), N-nitrosopyrrolidine (NPYR), and N-nitrosomorpholine (NMOR).

2. Materials and Methods

2.1. Chemicals

NDMA, NMEA, NDEA, NDPA, NDBA, NPIP, and NPYR were purchased from Sigma-Aldrich (Steinheim, Germany) as a mixed (EPA-521 Mix) 2000 µg mL^{-1} solution in dichloromethane (DCM).

NMOR was individually purchased from Sigma-Aldrich as 5000 μg mL^{-1} solutions in methanol (MeOH). The properties and structures of these compounds are shown in Table S1 (Supporting Information). Surrogate standards (NDEA-d10 and NPYR-d8) were individually purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA) as 1000 μg mL^{-1} solutions in deuterated dichloromethane. Mixed stock solutions of 100 μg mL^{-1} were prepared in MeOH and diluted as necessary in MeOH for water fortification or in ethyl acetate (AcOEt) for injection in the GC-MS system. All standards solutions were kept at −20 °C. Coconut charcoal SPE cartridges (2 g) were supplied from Supelco (Sigma-Aldrich, Milwaukee, WI, USA). Ultrapure deionized water (18.2 MΩ cm^{-1}) was obtained from a Milli-Q Gradient A-10 system (Millipore, Bedford, MA, USA). MeOH (Reagent European Pharmacopoeia, gradient grade for liquid chromatography) and AcOEt (for gas chromatography MS) were supplied by Merck (Damstard, Germany). Dichloromethane (DCM) for pesticide residue analysis (>99.8%) was obtained from VWR Prolabo (Fontenay-sous-Bois, France).

2.2. Samples

Grab samples were obtained from the influent and effluent of an urban wastewater treatment plant (WWTP) in Galicia (Spain) equipped with a primary and secondary treatment. These samples were stored at 4 °C for 24–48 h until analysis. Tap water was obtained from the laboratory at three different times and analyzed immediately after collection. All samples were filtered before analysis through 0.45 μm nitrocellulose filters (Millipore, Milford, MA, USA) to remove particulate matter.

2.3. Sample Preparation

Extraction of nitrosamines from water samples was carried out according to the U.S. EPA-521 method [11]. Briefly, 500 mL of filtered water was taken and then 20 ng of NDEA-d10 (as surrogate standards for NDMA, NMEA, and NDEA) and 20 ng of NPYR-d8 (surrogate for the remaining nitrosamines) were added. Samples were percolated through the coconut charcoal (2 g) SPE cartridges at a flow rate of 10 mL min^{-1}. SPE cartridges were dried under nitrogen stream for 45 min. After drying, compounds were eluted by gravity using 15 mL of DCM, and the resultant extract was concentrated in a Syncore (Büchi Labortechnik AG, Meierseggstrasse, Switzerland) system (water bath at 50 °C, kept at 175 r.p.m. and 50,000 Pa pressure) down to approximately 0.3 mL. AcOEt was added to the extract and transferred to graduated glass tubes for further concentration to 100 μL by a gentle nitrogen stream, with the precaution of avoiding dryness of the extract in order to minimize the loss of nitrosamines by volatilization. Therefore, the solvent of the extract was changed to AcOEt thanks to the higher volatility of DCM. Finally, this extract was injected in the GC-MS system.

2.4. Determination Conditions

Determination of nitrosamines was carried out using two GC-MS instruments: an Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) with an Agilent 7693 automatic sampler combined with an Agilent 7200 QToF MS instrument; and a Varian (Walnut Creek, CA, USA) 450-GC coupled to an ion trap (Varian 240MS).

In both instruments, the volume injected was 2 μL in the splitless mode of operation, with an injector temperature established at 250 °C with a splitless time of 1 min. Three GC capillary columns were tested: HP-5MS (5% phenyl-methylpolysiloxane, 30 m × 0.25 mm × 0.25 μm film thickness, Agilent Technologies), a DB-WAXetr (extended temperature range high polarity polyethylene glycol, 30 m × 0.25 mm, 0.50 μm film thickness), and HP-35MS (35% phenyl-methylpolysiloxane, 30 m × 0.25 mm, 0.25 μm film thickness, Agilent Technologies). Under final conditions, the DB-WAXetr column was used for the sample analysis. The solvent delay was set at 6 min. The oven temperature program was as follows: 60 °C kept for 1 min; then ramped to 100 °C at 10 °C min^{-1} and kept for 1 min; and a final ramp to 245 °C at 15 °C min^{-1} and held for 2 min. Helium (99.9999%, Praxair, Spain) was used as carrier gas at a constant flow rate of 1 mL min^{-1}.

The GC-QToF-MS was operated in the 2 GHz extended dynamic range mode, which provides a full width half maximum (FWHM) resolution of ca. 4000 at *m*/*z* 75 and ca. 6000 at *m*/*z* 187. The MS spectra were acquired and stored in the profile mode. MS sources conditions were: PCI using methane (99.95%) as ionization gas (20% pressure) at 150 μA of filament emission current and EI working at 70 eV with the filament emission set at 30 μA. The transfer line, PCI source, EI source, and MS quadrupole temperatures were set at 245 °C, 300 °C, 230 °C, and 150 °C, respectively. Full-scan acquisition mode was set in the range of 40–650 *m*/*z* with an acquisition rate of 5 spectrum s^{-1}. For the high-resolution mass spectrometry (HRMS) calibration to be maintained during the analysis sequence, perfluorotributylamine (PFTBA) was infused in the MS every 4 injections, according to the manufacturer specifications.

In the GC-IT-MS instrument, an external ionization configuration of the MS source was used. The transfer line, MS source, manifold, and ion trap temperatures were 280 °C, 200 °C, 50 °C, and 150 °C, respectively. Full-scan mode of acquisition was set in the range of 40–230 *m*/*z*, with an acquisition rate of 2 spectrum s^{-1}, average scans of 3 μScans, and target total ion chromatogram (TIC) of 20,000 counts. For EI, the filament emission current was set at 30 μA, while in PCI, using methane as an ionization gas, it was set at 100 μA. The damping gas was set at 2.5 mL min^{-1}. MS/MS experiments were also tested with spectra acquired in the GC-(PCI)-IT-MS system at 3 spectra s^{-1}.

2.5. Method Validation

Analytes were quantified using NDEA-d10 as surrogate internal standards (IS) for NDMA, NMEA, and NDEA; and NPYR-d8 for the rest of the nitrosamines.

Linearity was assessed by a 9-point (1, 2, 5, 10, 20, 50, 100, 200, 500 ng mL^{-1}) calibration curve ranging from the instrumental quantification limits (IQL) to 500 ng mL^{-1} (IS concentration: 200 ng mL^{-1}). Instrumental detection limits (IDL) and IQL were estimated from the lowest calibration standards as the concentrations providing a signal to noise ratio (*S*/*N*) of 3 and 10, respectively. Intra-day and inter-day instrumental precision were assessed from the relative standard deviation (%RSD) of six injections of a standard of 50 ng mL^{-1} performed over 24 h (intra-day precision) or over three weeks (inter-day precision).

Validation of the SPE-GC-MS method was performed in wastewater samples spiked at two levels with 5 ng and 100 ng of all the analytes (10 and 200 ng L^{-1} referred to the sample, respectively) and 20 ng of IS before extraction. Additional aliquots were spiked only with IS and processed simultaneously in order to account for the background levels. Method detection and quantification limits (MDL and MQL) were estimated from the measured concentrations in spiked wastewater samples (n = 3), downscaling the levels for which the *S*/*N* values are 3 (MDL) and 10 (MQL). Accuracy—determined for the wastewater samples spiked with 5 ng and 100 ng of all the analytes—was expressed as the average recovery, calculated from calibrations performed with standards in AcOEt by the internal standard method from the nominal spiking value. Precision was expressed as the %RSD from the average concentration measured (n = 3).

3. Results and Discussion

3.1. Capillary Column Selection

Three different capillary columns were tested using the temperature program described in Section 2.4 to obtain the best chromatographic separation. In order from less to more polar, the columns were: HP-5MS, HP-35MS, and DB-WAXetr. A 10 μg mL^{-1} mix standard was injected in all three cases. The compounds were identified based on their EI spectra using the National Institute of Standards and Technology (NIST) library. As shown in Figure 1, by using the HP-5MS capillary column, all compounds eluted before 13 min, with a co-elution of NPYR, NMOR, and NDPA at 6.5 min. Jurado-Sánchez et al. obtained a separation at baseline for seven common nitrosamines using the same HP-5MS capillary column, but a longer oven program was used, with a total runtime of 22 min and

without the inclusion of NDPA [28]. With the HP-35MS capillary column, a better separation was observed, although NMOR and NPYR co-eluted at 8 min. The DB-WAXetr, which was the most polar and with higher film thickness column, was able to separate all nitrosamines at baseline with good peak shapes, without compromising the analysis time and with suitable retention of the most polar and volatile compounds, such as NDMA.

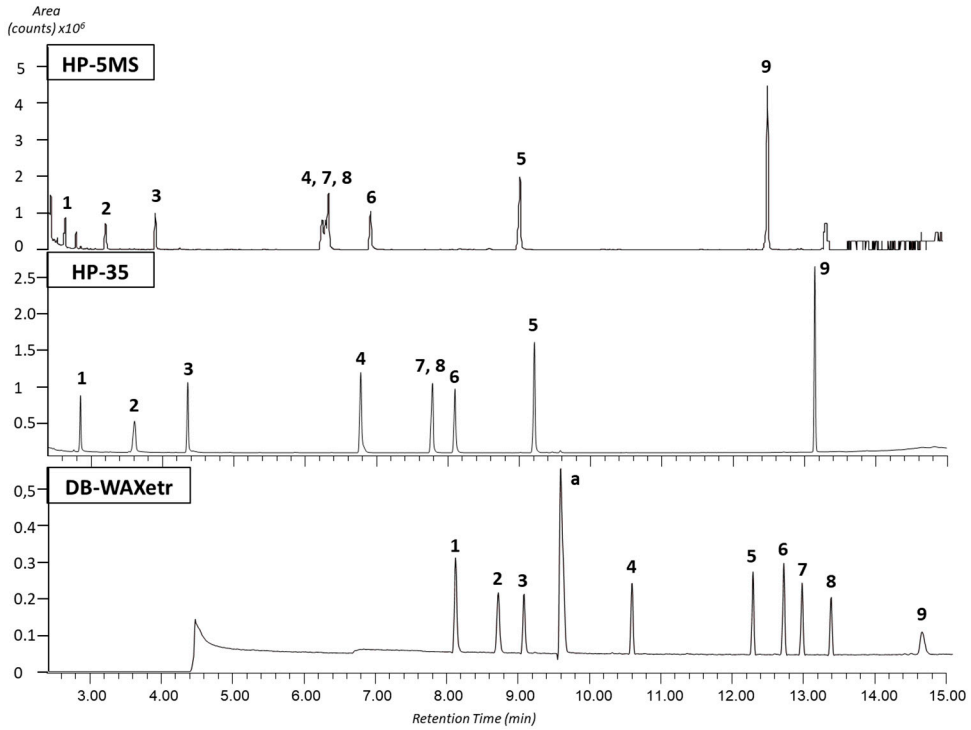

Figure 1. Total ion chromatograms obtained for the 3 tested capillary columns: (1) N-nitrosodimethylamine (NDMA), (2) N-nitrosomethylethylamine (NMEA), (3) N-nitrosodiethylamine (NDEA), (4) N-nitrosodipropylamine (NDPA), (5) N-nitrosodibutylamine (NDBA), (6) N-nitrosopiperidine (NPIP), (7) N-nitrosopyrrolidine (NPYR), (8) N-nitrosomorpholine (NMOR), (9) N-nitrosodiphenylamine (NDPhA), (a) column bleed.

3.2. Mass Spectrometry

A mix of eight nitrosamines (10 μg mL^{-1} each) was injected in both GC-MS systems with both ionization sources. Selected quantifier and qualifier ions are shown in Table 1. The MS/MS spectra were also recorded using a PCI source and IT analyzer as recommended the EPA methods [11], but using methane as ionization gas, as the option available in the lab. However, the LOD achieved were far higher (between 4 and 29 times higher) than in single-stage MS mode, so this option was not considered further. In fact, the U.S. EPA method 521 already reports on lower LOD using MS/MS vs. MS, but recommends its use due to the lower selectivity of the IT-MS being operated in single-stage MS.

A relevant issue in the GC-ToF-MS system with the PCI source was that a Gaussian profile for the NMEA peak was not observed when the spectra was acquired in centroid mode (Figure S1A), due to an isobaric interference between a background ion at *m*/*z* 89.0674 and NMEA with *m*/*z* 89.0709. In order to solve this problem, the acquisition mode was switched from centroid to profile mode. Thus, a better peak shape for NMEA was obtained, as shown in Figure S1B. Furthermore, the use of the profile mode of acquisition results in a realistic baseline that can be used to estimate LOD, since the centroid mode has a cut-off value that results in a flat baseline (there is actually no visible baseline).

Table 1. Retention time and quantification (first *m*/*z* value) and qualifier ions (subsequent *m*/*z* values) used in each system. Note: electron impact = EI; positive chemical ionization = PCI.

Compound	Retention Time (min)	*m*/*z* (Relative Abundance)			
		GC-IT-MS		GC-QToF-MS	
		EI	PCI	EI	PCI
NDMA	7.6	74 (100), 42 (229), 44 (85)	75 (100), 43 (10), 115 (3)	74.0475 (100), 44.0495 (24)	75.0553 (100), 115.0866 (2)
NMEA	8.3	88 (100), 42 (400), 71 (105)	89 (100), 61 (35), 117 (15)	88.0631 (100), 71.0604 (65)	89.0709 (100), 129.1022 (3)
NDEA	8.7	102 (100), 42 (280), 56 (90)	103 (100), 131 (28), 75 (5)	102.0788 (100), 85.0760 (32)	103.0866 (100), 143.1179 (5)
NDPA	10.2	113 (100), 70 (430), 41 (250)	131 (100), 159 (30), 89 (4)	113.1073 (100), 70.0655 (380)	131.1179 (100), 159.1492 (10)
NDBA	11.9	99 (100), 84 (240), 41 (180)	159 (100), 187 (28), 103 (2)	99.0920 (100), 84.0812 (240)	159.1492 (100), 187.1805 (15)
NPIP	12.3	114 (100), 42 (97), 84 (40)	115 (100), 143 (26), 84 (3)	114.0788 (100), 84.0808 (35)	115.0866 (100), 155.1179 (4)
NPYR	12.5	100 (100), 41 (92), 68 (37)	101 (100), 129 (19), 70 (5)	100.0631 (100), 68.0493 (16)	101.0709 (100), 141.1022 (4)
NMOR	12.9	86 (100), 56 (155), 116 (6)	117 (100), 145 (24), 86 (11)	86.0600 (100), 116.0580 (15)	117.0659 (100), 87.0679 (12)
NDEA-d10	8.7	112	113	112.1415	113.1494
NPYR-d8	12.5	108	109	108.1133	109.1212

3.3. Instrumental Performance

The performance for the two GC-MS systems (IT and QToF analyzers) operating with the two different ionization modes (EI and PCI) was evaluated using the following criteria: linearity, repeatability, IDLs, and IQLs. The results are presented in Table 2. The linearity was evaluated with standards at 7–9 different concentration levels (depending on the compound) in the range of LOQ-500 ng mL^{-1}, with the IS level remaining at 200 ng mL^{-1}. The peak area divided by the IS peak area was plotted versus concentrations, fitting a linear model with determination coefficients (R^2) higher than 0.991 in both instrument and ionization modes (Figure S2). Moreover, the Durbin–Watson statistic tests provided a *p*-value greater than 0.05 for all nitrosamines, indicating no significant correlation in the residuals at the 95% confidence level. The intra-day and inter-day precision were evaluated as IS corrected peak area by injections of a 50 ng mL^{-1} standard mixture, obtaining RSD values below 15% and 16% in all systems, respectively. As shown in the Table 2, GC-IT-MS with EI source provided the highest average intra-day RSD (7%), while the lowest average intra-day RSD was obtained with GC-QToF-MS with the PCI source (1.6%), with a maximum individual value of 2.2% for NMOR. IDLs and IQLs were estimated as the lowest concentration providing a signal-to-noise ratio of 3 and 10, respectively, by direct injection of the lower levels standards of the calibration curve, obtaining the lowest IDLs when the QToF system with PCI was used (0.2–4 ng mL^{-1}).

Table 2. Gas chromatography-mass spectrometry (GC-MS) performance for both instruments using EI and PCI sources. Note: IDL = instrumental detection limit; IQL = instrumental quantification limit.

	GC-IT-MS								**GC-QToF-MS**							
	EI				**PCI**				**EI**				**PCI**			
	R^2 [a]	%RSD [b]	IDL [c] (ng mL^{-1})	IQL [c] (ng mL^{-1})	R^2 [a]	%RSD [b]	IDL [c] (ng mL^{-1})	IQL [c] (ng mL^{-1})	R^2 [a]	%RSD [b]	IDL [c] (ng mL^{-1})	IQL [c] (ng mL^{-1})	R^2 [a]	%RSD [b]	IDL [c] (ng mL^{-1})	IQL [c] (ng mL^{-1})
NDMA	1.0000	4/6	3	10	0.9997	3/5	3	8	0.9997	2/10	1.3	4	0.9995	1.6/6	1.6	4.9
NMEA	0.9835	15/15	15	50	0.9984	8/11	15	50	0.9993	11/13	5.6	19	0.9996	1.8/10	4	11
NDEA	1.0000	5/4	3	8	0.9998	7/4	5	17	0.9991	1/2	2.0	5.9	0.9994	1.5/10	0.3	0.9
NDPA	1.0000	5/5	2	7	0.9986	4/2	3	8	0.9998	4/2	4.8	16	0.9998	1.7/10	0.2	0.6
NDBA	0.9997	5/11	1	9	0.9997	8/5	15	50	0.9992	5/8	8.8	29	0.9992	1.4/7	1	2
NPIP	0.9995	4/5	1	3	0.9991	6/3	1	3	0.9992	4/1	1.1	3.4	0.9993	1.7/12	1	1.2
NPYR	0.9998	7/5	1	4	0.9986	8/3	1	3	0.9986	5/10	1.3	4	0.9994	1.1/6	0.3	0.8
NMOR	0.9999	9/16	3	8	0.9992	3/3	2	6	0.9979	3/6	19	63	0.9995	2.2/8	1	2.8
average	0.9978	7/8	3.6	12.4	0.9991	6/5	5.6	18.1	0.9991	4/7	5.4	17.5	0.9995	1.6/9	1.2	3.0

Note: [a] R^2, linearity is expressed by the determination coefficient in the range of 1–500 ng mL^{-1}; [b] %RSD, intra-day or inter-day (over a three-week period) precision expressed as the RSD (%) (n = 6) in the 50 ng mL^{-1} level; [c] IDLs and IQLs were estimated at a signal-to-noise ratio of 3 and 10, respectively.

3.4. Performance with Real Samples after SPE

The different GC-MS systems were also compared in terms of overall method LOD (MDL) and LOQ (MQL) using 500 mL of effluent wastewater as real matrix after the SPE following the U.S. EPA 521 method (see Experimental). As presented in Table 3, GC-PCI-QToF-MS clearly provided the lowest average MDL (0.5 ng L^{-1}) and MQL (1.6 ng L^{-1}) values, being approximately 1 order of magnitude better than those obtained with the other 3 approaches.

Table 3. Accuracy of the method detection limit (MDL) and method quantification limit (MQL), expressed as recoveries (R%), intra-day precision (expressed as RSD), and mass accuracy (average error in mDa) obtained from a spiked (10 ng L^{-1} and 200 ng L^{-1}) wastewater effluent sample.

	MDLs (ng L^{-1})				MQLs (ng L^{-1})				R%		RSD		Mass Accuracy (mDa)	
									10 ng L^{-1}	200 ng L^{-1}	10 ng L^{-1}	200 ng L^{-1}	10 ng L^{-1}	200 ng L^{-1}
	GC-IT-MS		GC-QToF-MS		GC-IT-MS		GC-QToF-MS		GC-QToF-MS					
	EI	PCI	EI	PCI	EI	PCI	EI	PCI	PCI					
NDMA	7.2	1.3	1.7	0.4	21.6	3.9	5.1	1.2	119	62	3.2	5.2	0.20	0.15
NMEA	20	24	2.2	1.3	60	72	6.6	3.9	65	66	0.75	6.5	0.35	0.90
NDEA	2.0	1.8	1.2	0.2	6	5.4	3.6	0.6	119	89	2.1	5.2	0.20	0.10
NDPA	5.8	7.6	3.9	0.4	17.4	22.8	11.7	1.2	101	109	5.6	4.2	0.32	0.13
NDBA	12	6.7	5.4	0.6	36	20.1	16.2	1.8	107	113	5.1	4.6	0.35	0.35
NPIP	3.6	1.2	1.0	0.4	10.8	3.6	3	1.2	117	106	2.1	2.5	0.20	0.55
NPYR	4.8	1.2	3.9	0.4	14.4	3.6	11.7	1.2	118	105	4.5	6.7	0.65	0.45
NMOR	10	3.9	22	0.6	30	11.7	66	1.8	92	106	0.97	5.0	0.52	0.70
average	8.2	6.0	5.2	0.5	24.5	17.9	15.5	1.6	105	95	3.0	5.0	0.35	0.42

The estimated MDLs obtained by GC-PCI-QTOF were in the range of 0.2 ng L^{-1} (NDEA)–1.3 ng L^{-1} (NMEA). These values are comparable to those reported in the literature (Table S2). Cheng et al., using a 500 mL of sample, reported MDLs in the range of 0.3–1.8 ng L^{-1}, using SPE and a GC-PCI-MS/MS system with a slightly different protocol from the EPA-521 method [17]. The U.S. EPA-521 method [11] reported MDLs in the range of 0.3–0.7 ng L^{-1}, also considering a volume of 500 mL and exactly the same SPE protocol, but via large-volume injection of 20 µL of extract then GC-PCI-IT-MS/MS. The proposed GC-PCI-QTOF-MS method can provide MDLs at the same order of magnitude as the U.S. EPA method by injecting 10 times less extract, thus being a good alternative to that method, particularly considering that IT instruments are losing popularity and that using methanol or acetonitrile as the reaction gas is not feasible with all instruments. Furthermore, the method proposed here was validated with a more complex matrix than the EPA method (effluent wastewater vs. tap water), demonstrating its high selectivity.

Overall accuracy and precision for the method were assessed with the GC-PCI-QTOF system using effluent water that was spiked at 10 ng L^{-1} and 200 ng L^{-1}, so as to verify its performance before its application in real samples. A chromatogram of an effluent water sample spiked at 10 ng L^{-1} is shown in Figure 2. Results are shown in Table 3, in which all compounds show recoveries in the range of 90–120%, except for NDMA and NMEA, with relative standard deviations below 7% and mass accuracies exhibiting an error below 1 mDa.

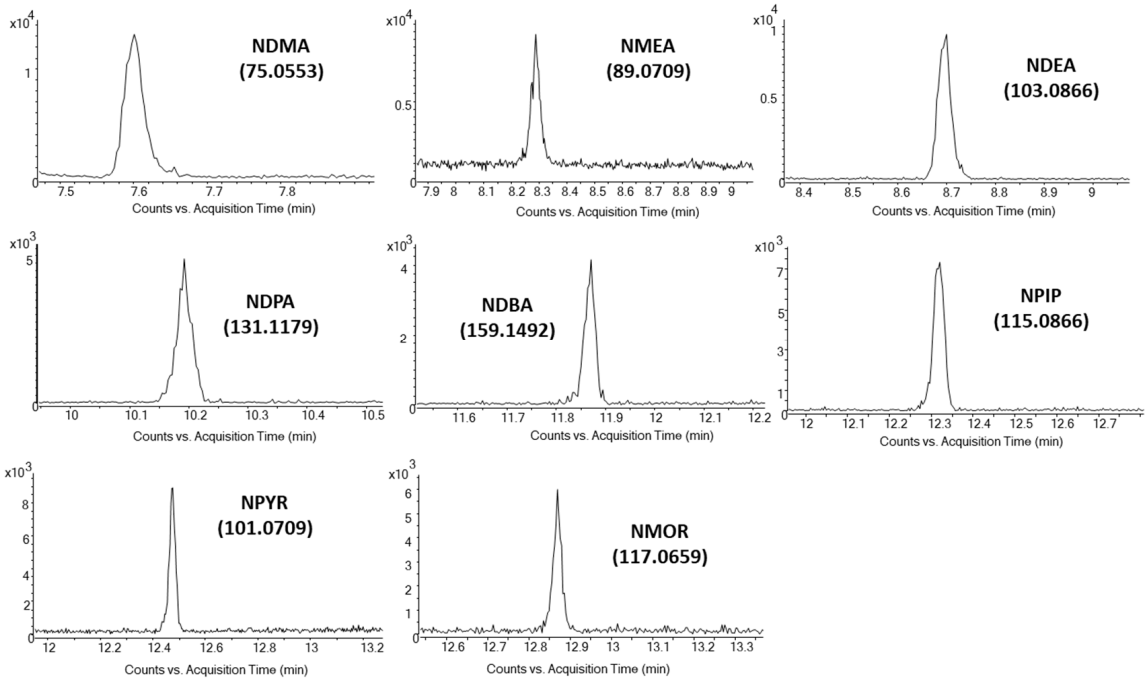

Figure 2. Extracted ion chromatograms (±50 ppm) for an effluent water sample spiked at 10 ng L^{-1}.

3.5. Analysis of Real Samples

Finally, the GC-PCI-QTOF method was used for the analysis of real samples after SPE. The samples considered were 2 wastewater effluent (n = 3 replicates) and 2 wastewater influent (n = 3) samples, both collected as grab samples on two different days; and 3 tap water (n = 3) samples collected at three different times on the same day. Samples were analyzed as explained in Sections 2.3 and 2.4, and the levels detected are presented in Table 4, with RSD values below 20%.

Table 4. Concentrations (ng L^{-1}) ± standard deviation found in real samples collected on two different days (1 and 2). Sampling times are indicated between parentheses for tap water samples collected in the same day. Note: LOD = limit of detection.

	NDMA	NMEA	NPYR	NDEA	NPIP	NMOR	NDPA	NDBA
Influent wastewater (1)	4.6 ± 0.5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Influent wastewater (2)	27 ± 2	<LOD	<LOD	15.1 ± 0.6	<LOD	<LOD	<LOD	<LOD
Effluent wastewater (1)	4.4 ± 0.1	<LOD	<LOD	2.8 ± 0.2	<LOD	2.7 ± 0.5	<LOD	3.1 ± 0.4
Effluent wastewater (2)	2.6 ± 0.3	<LOD	<LOD	3.35 ± 0.05	<LOD	3.0 ± 0.2	<LOD	8 ± 1
Tap water (8:45 h)	2.2 ± 0.2	<LOD	<LOD	2.8 ± 0.1	<LOD	1.72 ± 0.01	<LOD	7.1 ± 0.2
Tap water (12:30 h)	5 ± 1	<LOD	<LOD	4.5 ± 0.7	2.7 ± 0.3	2.4 ± 0.3	<LOD	9 ± 1
Tap water (15:45 h)	1.5 ± 0.2	<LOD	<LOD	<LOD	1.8 ± 0.1	1.8 ± 0.3	<LOD	4.3 ± 0.7

In drinking water, three nitrosamines (NDMA, NMOR, and NDBA) were found in all analyzed samples, while NPIP and NDEA were found in two of them, at concentrations ranging between 1.5 and 9 ng L^{-1}. These levels are in agreement with those found in the literature in tap and treated drinking water, which are usually less than 10 ng L^{-1} [26,29,30]. These levels would be below the 10 ng L^{-1} reporting level set by the authorities of California [10] and far below the 100 ng L^{-1} level considered as the guideline value by the WHO for NDMA in drinking water [9].

NDMA, NDEA, NMOR, and NDBA were the analyzed compounds found in the wastewater samples, with concentrations ranging from 2.6 to 27 ng L^{-1}. The levels found were lower than those reported in the literature by Llop et al. [18] (i.e., levels ranging from 11 ng L^{-1} (NDPA) to 139,718 ng L^{-1} (NMOR)), or the high (up to 5000 ng L^{-1}) and variable concentrations of NDMA reported in effluents of three WWTPs by Zhou et al. [31]. However, they are similar to those reported by Krauss et al. in

WWTPs in Switzerland, where in primary effluents, NDMA, NMOR, NDBA, NPIP, and NDEA were found at concentrations typically in the range of 5–25 ng L^{-1}, while in the secondary effluent NDMA concentrations were usually lower than 10 ng L^{-1} [27].

4. Conclusions

Results show that GC-PCI-QToF-MS can provide sufficiently low LODs for the determination of nitrosamines in water samples, with excellent selectivity as compared to existing methods. Thus, the GC-QToF can be a valuable alternative to the EPA method 521, as it does not require large-volume injection. The method was finally applied to real drinking and wastewater samples, where several nitrosamines were detected at the 1–27 ng L^{-1} level.

Supplementary Materials: The following are available online at http://www.mdpi.com/2297-8739/7/1/3/s1. Table S1. Structure and properties of the N-nitrosamines considered in this work. Table S2: Compilation of MDLs reported in the literature for the determination of nitrosamines in water. Figure S1. Isobaric interference for NMEA in PCI acquisition in centroid (A) and profile modes (B). Extracted ion chromatograms (EIC) with ±50 ppm. Figure S2: Calibration curves obtained by: (a) GC-EI—MS (IT), (b) GC-PCI—MS (IT), (c) GC-EI-MS(QTOF), and (d) GC-PCI-MS(QTOF).

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