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ARTICLE

Trace-level quantification of *N*-nitrosopiperazine in treated wastewater using supported liquid extraction and hydrophilic interaction chromatography mass spectrometry

Anthony Lapointe, Stéphanie Gallant, Simon Comtois-Marotte, Alexandra Furtos, and Karen C. Waldron

Abstract: Regenerable amine-based solvents used for post-combustion CO_2 capture, primarily monoethanolamine and piperazine, are known to undergo degradation and secondary reactions over time forming, amongst other species, *N*-nitrosamines. These carcinogenic species can eventually make their way from treated wastewater into environmental waters. The United States Environmental Protection Agency (US EPA) recommends that the concentration of *N*-nitrosamines in surface water not exceed 1.24 μg/L. We have developed a straightforward method to quantify *N*-nitrosopiperazine in treated wastewater by hydrophilic interaction liquid chromatography – mass spectrometry (HILIC–MS) after sample preparation by supported liquid extraction (SLE). To achieve the best extraction recovery and method limits of quantification (MLOQ), standards were prepared in a high-salt synthetic matrix to mimic the treated wastewater effluent. To further improve the MLOQ, the drying steps after extraction were optimized. HILIC separation of the highly polar analytes was achieved using an ethylene-bridged hybrid amide stationary phase. Detection was achieved using a triple quadrupole mass spectrometer operated in positive electrospray ionisation and multiple reaction monitoring mode, providing a final MLOQ of 0.25 μg/L for *N*-nitrosopiperazine. Validation of the method was carried out to ensure good confidence in the data obtained for a treated wastewater sample from a post-combustion CO_2 capture facility. In addition, *N*-nitrosopiperazine was quantified with the developed SLE-HILIC–MS method in eight degraded carbon capture samples that had not yet undergone wastewater treatment.

Key words: *N*-nitrosamines, carbon capture and storage, amine scrubbing, solid-supported liquid-liquid extraction, LC–MS.

Résumé : On sait que les solvants régénérables à base d'amines employés pour la capture du CO_2 dans les fumées de combustion, principalement la monoéthanolamine et la pipérazine, subissent une dégradation et des réactions secondaires au fil du temps pour former, entre autres, des *N*-nitrosamines. Ces espèces cancérogènes peuvent éventuellement contaminer les eaux usées traitées et se retrouver dans l'environnement. La United States Environmental Protection Agency (US EPA) a établi la limite environnementale de concentration de *N*-nitrosamines dans les eaux de surface à 1,24 μg/L. Nous avons mis au point une méthode simple pour quantifier la *N*-nitrosopipérazine dans les eaux usées traitées. L'échantillon est d'abord traité par extraction liquide-liquide sur support solide (SLE), puis soumis à la chromatographie liquide d'interaction hydrophile couplée à la spectrométrie de masse (HILIC–MS). Afin d'obtenir les meilleurs taux d'extraction et les plus faibles limites de quantification de la méthode (LQM), nous avons préparé une série d'étalons dans une matrice synthétique à teneur élevée en sels imitant les effluents d'usines de traitement des eaux usées. Nous avons optimisé l'étape de séchage après l'extraction afin d'améliorer davantage la LQM. Nous avons effectué la séparation des analytes très polaires par HILIC à l'aide d'une phase stationnaire d'amide avec particules hybrides à ponts éthylène. La détection était assurée par un spectromètre de masse à triple quadripôle utilisé en mode de suivi de réactions multiples et dont la source d'ionisation par électronébulisation était réglée en mode positif. Nous avons obtenu une LQM optimisée de la *N*-nitrosopipérazine de 0,25 μg/L. Par la suite, nous avons procédé à la validation de la méthode pour assurer la validité des données obtenues avec des échantillons d'eaux usées traitées provenant d'une usine de capture du CO_2 dans les fumées de combustion. Enfin, à l'aide de la méthode SLE–HILIC–MS que nous avons mise au point, nous avons quantifié la *N*-nitrosopipérazine dans huit échantillons de capture de carbone dégradés qui n'avaient pas encore été soumis au traitement des eaux usées. [Traduit par la Rédaction]

Mots-clés : *N*-nitrosamines, capture et séquestration du carbone, lavage aux amines, extraction liquide-liquide sur support solide, LC–MS.

Introduction

One of the main challenges of this century is the reduction of greenhouse gas emissions, particularly CO_2 from the burning of fossil fuels. It is an unfortunate reality that fossil fuel powered electricity plants will be around for some time because of increasing demand worldwide for energy. In Canada, coal-fired power accounted for 9% of electricity generation and natural gas or oil-fired power accounted for 10% in 2017.[1] By the end of 2016, 30% the United States' electricity was generated by coal-fired plants and 34.5% by natural gas or oil.[2] During the slow but steady transition to clean-energy technologies, the implementation of carbon capture and storage (CCS) technologies at the source is essential to mitigate the devastating effects of CO_2 on global warming.[3,4] CCS includes several diverse technologies including post-combustion

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capture of CO_2 by amine scrubbing, which is based on chemical absorption using regenerable aqueous amine-based solvents.[5,6] The benchmark solvent is monoethanolamine (MEA) at 30 wt%, but many others are in use: diethanolamine (DEA), *N*-methylethanolamine, *N*-methyl diethanolamine (MDEA), piperazine, and blends of these solvents to name a few.[7–17]

Amine-based solvents can absorb more than 90% of the CO_2 generated in flue gas, which would otherwise be emitted into the atmosphere.[4,17] Heating the CO_2-rich solvent at temperatures generally higher than 100 °C strips off the CO_2 with high purity for storage and sequestration, and the regenerated aqueous amine is pumped back to the flue for subsequent rounds of absorption. However, the carbon capture scrubbing process has some shortcomings; the aqueous amine-based solvent undergoes irreversible degradation with repeated cycles of regeneration caused mainly by two mechanisms: oxidative and thermal degradation.[9,18–20] A few of the known degradation products observed with MEA, piperazine, and piperazine blends are listed in Supplementary Table S1.[13,19,21–23] The presence of degradation products can reduce the efficiency of the CO_2 capture process[9] leading to financial cost and the obvious environmental burden of unintended emissions. Of additional concern is the formation of toxic, carcinogenic *N*-nitrosamines.[19,28,31,32,50] Nitrogen oxides (NOx) are present in flue gases and NO_2 absorption by the amine solvent as nitrite leads to nitrosation of the solvent at the high temperatures used in the desorber, which is the predominant pathway to accumulation of nitrosamines.[24–26] Even in the absence of NO_2, the presence of NO during aqueous-amine scrubbing was shown to lead to formation of *N*-nitrosamines.[13,27] Of importance to human health and the environment is contamination of downwind airsheds and surface water by all degradation products volatilized from the CO_2 scrubbing process,[26,28,29] as well as contamination in treated wastewater from the CCS facility.

N-Nitrosamines were reported to be carcinogenic in the early 1970s,[30] and this has been reiterated recently by groups studying nitrosamine detection in water, frequently present as disinfection by-products of chloramination during water purification[31–39] and not just as a result of industrial processes,[40] which the current work pertains to. According to the United States Environmental Protection Agency's guidelines for human health ambient water quality,[41] the recommended upper limits of *N*-nitrosamines for consumption of water are below 1 ng/L and the limit for consumption of organisms is 1.24 μg/L, e.g., in surface waters. It is therefore essential to monitor the concentration of *N*-nitrosamines, and *N*-nitrosopiperazine, in treated wastewater from industrial facilities before any potential release into environmental water. In addition, monitoring nitrosamine levels before a wastewater's treatment can be used to evaluate the efficacy of treatment methods. Some of these methods include using biological processes that combine an aerobic step and an anaerobic step to reduce the levels of organic compounds[42] and (or) UV treatment combined with hydrogen peroxide,[43] to name a few. Jackson et al. showed that *N*-nitrosopiperazine will degrade slowly when exposed to UV light.[44] It should be noted that the presence of *N*-nitrosamines in tobacco products and foods is well documented.[45,46]

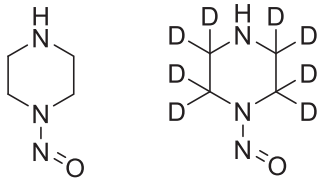
Treated wastewater is a relatively complex matrix with a high salt concentration that needs to be either extensively diluted or subjected to analyte extraction for compatibility with MS analysis, particularly to avoid ion suppression.[47] Extensive dilution is not always a viable option when very low detection or quantification limits are required, so various extraction and enrichment techniques are employed. Many chromatographic and mass spectrometric (MS) methods have been reported to quantify a variety of *N*-nitrosamines in wastewater. Usually, these involve determination of more commonly known *N*-nitrosamines such as *N*-nitrosodimethylamine or *N*-nitrosodiethylamine. Nine different *N*-nitrosamines were quantified in wastewater by Krauss and Hollender using solid-phase extraction (SPE) followed by liquid chromatography (LC) coupled to a hybrid MS instrument composed of an Orbital trap and a linear ion trap.[31] Their excellent limit of quantification (LOQ) was on the order of low ng/L (below parts per billion) level, which was achieved by using sophisticated MS instrumentation — which may not be readily available or affordable in many labs — and by a long extraction procedure requiring tandem SPE cartridges; reversed phase HLB (hydrophilic–lipophilic balance) sorbent to remove interfering matrix compounds followed by carbonaceous sorbent to extract the most polar nitrosamines. A sensitive method developed by Yoon et al. to quantify eight *N*-nitrosamines also used tandem SPE cartridges, namely an aminopropyl sorbent plus an activated charcoal sorbent, followed by gas chromatography (GC) coupled to a triple quadrupole MS.[36] GC–MS is a good technique for the analysis of low molecular weight nitrosamines and the authors achieved method limits of detection (LODs) as low as 0.1 ng/L for 200 mL water samples. Gerrity et al. used SPE–GC coupled to an ion trap MS to investigate the impact of ozonation treatment on *N*-nitrosamine concentrations in treated wastewater[48] and reported LOQs in the low ng/L range. A single extraction cartridge made in-house and composed of vinyl/divinylbenzene polymer was used by Qian et al. to achieve superb method LODs of 0.01 ng/L for *N*-nitrosamines in wastewater by SPE–HPLC–MS/MS using only 100 mL of sample.[37]

Despite the many ultra-sensitive determination methods reported for a range of *N*-nitrosamines, the more polar *N*-nitrosopiperazine is less volatile and thus harder to quantify at trace levels using traditional SPE, reversed phase LC–MS, or GC–MS based methods. For *N*-nitrosopiperazine, one of the few reported methods to quantify it in a matrix similar to wastewater gave a mediocre LOD of 575 μg/L by LC–UV.[49] An LC–MS method using a triple quadrupole analyzer and having an instrumental LOQ of 0.1 to 1 μg/L was used to monitor the environmental fate of *N*-nitrosopiperazine and other nitrosamines and nitramines, although the hydrolytic and photolytic stability studies were made in buffered aqueous solutions, not wastewater.[23,50] The quantification of nitrosamine degradation products in amine solvents used for CO_2 capture, including *N*-nitrosopiperazine, was briefly discussed in a recent review by Cuccia et al. where dilution was the only sample treatment mentioned.[19]

A method that is sensitive and reliable using accessible LC–MS instruments is needed for determination of more polar nitrosamines like *N*-nitrosopiperazine. In the present work, a method was developed using supported liquid extraction (SLE)[51,52] (also known as solid-supported liquid–liquid extraction[53,54]) for treated wastewater sample preparation followed by hydrophilic interaction liquid chromatography (HILIC) coupled to a triple quadrupole MS to target an LOQ of < 1 μg/L. SLE is a variation of classical liquid–liquid extraction, carried out in a cartridge. Aqueous sample is added to the inert, high surface to area diatomaceous earth particles, which become wetted by adsorption and absorption or dispersion to create an aqueous, immobilized liquid layer with no breakthrough of sample.[51] After 5–10 min, an immiscible organic solvent is passed slowly through the cartridge, ideally in two aliquots, to extract organo-soluble species, leaving unwanted salts trapped in the immobilized aqueous layer. There is no preconditioning of the SLE cartridge or "clean-up" step involved. This is opposite to SPE in which salts are washed out of the sorbent bed before analyte elution. In the current work, the solvent drying steps following SLE elution were optimized to improve accuracy and extraction recovery. A synthetic matrix-matched solution for preparing the calibration curves was used to mimic the high salt content of the treated wastewater effluent, which was provided from a CCS facility using post-combustion CO_2 capture technology with a proprietary regenerable amine.

The developed SLE–HILIC–MS method was validated for quantification of *N*-nitrosopiperazine (Fig. 1) and a second *N*-nitrosamine of confidential structure (referred to as Molecule X) in treated wastewater, as well as in samples of degraded CO_2 capture solvent.

Fig. 1. Structures of *N*-nitrosopiperazine and internal standard *N*-nitrosopiperazine-d_8.



Internal calibration was carried out using the deuterated internal standard *N*-nitrosopiperazine-d_8 (Fig. 1).

Experimental

Materials and methods

The standards *N*-nitrosopiperazine and Molecule X were obtained with a purity of 99% from the supplier (name withheld) of the CO_2 capture solvent. The internal standard *N*-nitrosopiperazine-d_8 was obtained from Toronto Research Chemicals (Toronto, ON). LCMS-grade acetonitrile (ACN), LCMS grade water, and dichloromethane (DCM) were obtained from Fisher Scientific (St. Laurent, QC). Ammonium formate and 98% formic acid were obtained from Sigma-Aldrich (Oakville, ON). Ethanol was obtained from Les Alcools de Commerce (Boucherville, QC). Stock solutions of *N*-nitrosopiperazine and Molecule X were made at 100 μg/L and of *N*-nitrosopiperazine-d_8 at 50 mg/L, all prepared in LCMS-grade water. Dilutions thereafter were made in synthetic matrix as described below. We note that all handling of *N*-nitrosamine compounds and organic solvents in this research project was done while wearing personal safety protection gear and in a chemical fume hood or well vented workspace.

Sample collection and synthetic matrix

The synthetic matrix used for calibration curves was prepared by the same supplier as the carbon capture solvent and standards. Although its exact composition is confidential, the synthetic matrix contained a mixture of amines, sodium sulfate, other salts, and water with a conductivity matched to the treated wastewater sample. Standards were prepared by dilution of stock solutions in the synthetic matrix and then extracted by SLE following the procedures below before measurement by HILIC–MS. A wastewater sample was obtained that had undergone treatment similar to that typically used at wastewater treatment plants such as UV ozonation combined with a biotreatment.[42,43] Because the concentration of *N*-nitrosamines is known to vary throughout a water treatment process,[31] three or four samples were collected during each stage of treatment, pooled, and then homogenized to provide a sample of averaged *N*-nitrosamines concentration. The treated wastewater sample was stored in darkness at room temperature and analyzed by SLE–HILIC–MS.

In addition to the wastewater sample, we received eight samples of degraded CO_2 capture solvent collected from different batches after many regeneration cycles. These eight samples had not yet been submitted to wastewater treatment, but quantification of *N*-nitrosopiperazine and Molecule X are important factors for optimization of the treatment procedures. Samples were diluted 10-fold in LCMS-grade water before extraction and analysis by SLE–HILIC–MS. Each sample was analysed in duplicate.

SLE with two drying steps

Calibration curves were prepared from the stock solutions diluted in synthetic matrix with concentrations varying from 0.01 to 0.50 μg/L in both *N*-nitrosopiperazine and Molecule X in the mixture, and a constant concentration of 10 μg/L internal standard, *N*-nitrosopiperazine-d_8. Aliquots of 50.0 mL standard mixture or 50.0 mL sample spiked with 10 μL internal standard (10 μg/L final concentration) were gravity loaded onto SLE cartridges (ChemElut, Agilent Technologies, Oakville, ON) and allowed to adsorb on the diatomaceous earth packing for 5 min. A first elution was performed with 50 mL DCM followed by a second elution with an additional 50 mL of DCM, both by gravity, as recommended by the manufacturer. It is well known that multiple extractions are more efficient than a single large-volume extraction. To achieve solvent exchange and enrichment, the pooled extracts (100 mL) were evaporated to dryness on a rotary evaporator. Two sequential additions of 3.5 mL acetonitrile into the round-bottom flask were used for solubilisation. The 7 mL sample was then transferred to a 15 mL Falcon tube, dried with a gentle stream of nitrogen, and reconstituted in 500 μL acetonitrile prior to injection of 2 μL in the HILIC–MS. This gave an overall enrichment factor of 100-fold, i.e., from 50 to 0.5 mL.

SLE with one drying step

The one drying step procedure included the same steps as above up to reconstitution in acetonitrile after rotary evaporation. Solubilisation was achieved by adding two sequential aliquots of 2.5 mL acetonitrile to the dried residue in the round-bottom flask with no additional drying or reconstitution steps. The final volume of 5.0 mL, providing an overall enrichment factor of 10-fold, was transferred to a sample vial and 5 μL injected for HILIC–MS analysis. The standard mixtures for the calibration curve were prepared at concentrations 10 times higher than above; from 0.1 to 5.0 μg/L in *N*-nitrosopiperazine and Molecule X with a constant concentration of 10 μg/L *N*-nitrosopiperazine-d_8. The one drying step SLE procedure was expected to give higher MLOD and MLOQ values because of the lower enrichment factor but better extraction recovery and reproducibility.

LC–MS procedure

Analyses were performed on a 6410 Triple Quad mass spectrometer coupled to an 1100 series HPLC system, both from Agilent Technologies. The separation was performed in HILIC mode on a Waters XBridge BEH Amide column (100 × 3.0 mm, 2.5 μm particle size) (Boucherville, QC) maintained at 30 °C. Analytes were eluted at 0.5 mL/min in gradient mode. The aqueous eluent (A) contained 10 mmol/L ammonium formate, 5% ethanol, and 0.05% formic acid and the organic eluent (B) was acetonitrile. The mobile phase gradient was as follows: 2 min at 5% A; increase to 40% A over 6 min; hold at 40% A for 2 min; decrease to 5% A over 2 min; hold at 5% A for 5 min for re-equilibration. The ionization mode was positive electrospray (+ESI). The solution used for calibration of the MS was Agilent's ESI tuning mix (part no. G2421A, Agilent Technologies) composed of a variety of phosphazene analogs. The triple quadrupole MS was used in multiple reaction monitoring mode. The transition monitored for *N*-nitrosopiperazine was *m*/*z* 116.1 → 85.1 and for *N*-nitrosopiperazine-d_8 was *m*/*z* 124.0 → 93.0. These two transitions correspond to the loss of one nitrogen and one oxygen of the nitroso group. The transition for Molecule X is confidential. The MS source was heated to 300 °C and the flow of the nitrogen collision gas was 11 L/min. The collision energy used for Molecule X was 20 V, and that used for *N*-nitrosopiperazine and *N*-nitrosopiperazine-d_8 was 12 V. These conditions were optimized to give the best results.

Method validation

Analysis of wastewater is highly dependent on the sample preparation method; thus, all validation studies were based on the full SLE–HILIC–MS method. The method limits of detection (MLOD) and quantification (MLOQ) were calculated, respectively, as 3 times and 10 times the standard deviation of duplicate peak height ratio measurements at the lowest concentration divided by the slope of the appropriate internal calibration curve, depending on the SLE protocol. MLOD and MLOQ were also evaluated using the sample blank consisting of synthetic matrix alone, extracted, and analysed by SLE–HILIC–MS. Noise on the blank was determined as the

Table 1. Results showing sensitivity and linearity of internal calibration curves, extraction recovery, method detection limits (MLOD), and method quantification limits (MLOQ) for the two SLE extraction procedures and analysis by HILIC–MS.

	Range or spike level	*N*-Nitrosopiperazine	Molecule X
Two drying step SLE protocol			
Calibration curves	0.01–0.50 μg/L	$y = 0.531x + 0.0283$ ($R^2 = 0.9918$)	$y = 0.7811x + 0.2091$ ($R^2 = 0.9924$)
Extraction recovery when standards prepared in water ($n = 3$)	Spiked at 0.50 μg/L	6%±5%	34%±3%
Extraction recovery ($n = 3$)	Spiked at 0.50 μg/L	22%±14%	57%±2%
MLOD (μg/L)[a]		0.10	0.02_7
MLOQ (μg/L)[a]		0.33	0.09_1
One drying step SLE protocol			
Calibration curves	0.10–5.0 μg/L	$y = 0.4533x + 0.0044$ ($R^2 = 0.9978$)	$y = 0.3937x + 0.1060$ ($R^2 = 0.9955$)
Extraction recovery ($n = 3$)	Spiked at 1.0 μg/L	50%±2%	47%±4%
MLOD (μg/L)[b]		0.07_5	0.14
MLOQ (μg/L)[b]		0.25	0.49

Note: Standards were prepared in synthetic matrix unless otherwise indicated. Uncertainties are reported as standard deviations. Internal calibration curves prepared using 10 μg/L *N*-nitrosopiperazine-d_8.

[a]Calculated using slopes of internal calibration curves (0.01–0.50 μg/L) and standard deviation of replicate ($n = 2$) measurements at 0.01 μg/L. Values calculated using the synthetic matrix blank and external calibration were 2 times lower for Molecule X and 10 times lower for *N*-nitrosopiperazine.

[b]Calculated using slopes of internal calibration curves (0.10–5.0 μg/L) and standard deviation of replicate ($n = 2$) measurements at 0.10 μg/L. Values calculated using the synthetic matrix blank and external calibration were the same or lower by less than a factor of 2.

difference between the maximum and minimum signal over a 60 s period, and slopes from the respective external calibration curves were used to get concentration units. Determination of the instrumental limits of detection and quantification for the two analytes was deemed immaterial because they are directly correlated to the quality and sensitivity of the MS system used.

To evaluate extraction recovery, six identical synthetic matrix solutions were used. Standards (analytes and deuterated internal standard) were spiked into the first three solutions at 0.5 μg/L or 1.0 μg/L for the two drying step or one drying step SLE protocols, respectively, before extraction and then into the remaining three solutions after extraction, just before HILIC–MS analysis. The average peak height ratio from the solutions spiked before extraction ($n = 3$) were divided by the average peak height ratio for the solutions spiked after extraction ($n = 3$) to calculate percent extraction recovery for the two analytes (Table 1).

The following validation parameters were evaluated for standards extracted using the one drying step SLE protocol and HILIC–MS analysis because our target MLQ was in fact 1 μg/L *N*-nitrosopiperazine: precision, accuracy, column efficiency (N), resolution, and peak tailing factor. Inter-day precision was determined as the percent RSD ($n = 5$) in peak height ratio for replicates prepared at 1 μg/L and 2 μg/L the same day. The procedure was repeated a second day to determine intra-day ($n = 2$) precision by comparing the averages of replicates from each day. The method's inter-day ($n = 5$) and intra-day ($n = 2$) accuracies were determined at two levels by comparing the average calculated concentration from five replicates with the true value spiked into synthetic matrix. For 10 replicate analyses at 1 μg/L, the average number of theoretical plates, N, for each analyte and the resolution of neighbouring peaks were calculated using peak widths at half height. The average peak tailing factor (TF) was calculated using eq. 1, where A and B represent the peak half-widths at 5% of the peak height. The first half (front) of the peak is represented by A, while the other half (tail) of the peak is represented by B.[55]

$$\text{TF} = \frac{A + B}{2A} \quad (1)$$

Results and discussion

There is abundant literature surrounding the trace-level analysis of several *N*-nitrosamines in water and wastewater formed as water disinfection by-products or from industrial use of amines. To achieve quantification at the ng/L level required for safe drinking water, extraction and enrichment prior to LC–MS and GC–MS has mostly been achieved by SPE using either reversed phase media with polar functionality or tandem sorbents to extract nonpolar and polar analytes. As mentioned in the Introduction, there are few reports on the sensitive determination specifically of *N*-nitrosopiperazine in wastewater. The SLE technique for extraction of polar analytes was chosen to help overcome the challenge of working with treated wastewater, a matrix containing a significant level of salts, as was the case for the effluent sample. The main advantage of SLE compared with liquid–liquid extraction is that it is less time consuming, and there is no emulsion formed because there is no shaking involved. The choice of dichloromethane as the SLE extraction solvent, which has been used to extract chemical warfare agents from water,[54] was based on preliminary tests (data not shown) with several solvents including ethyl acetate, reported to be effective in other applications.[52,53]

Separation of the two analytes was initially tested by reversed phase LC; however, *N*-nitrosopiperazine was not well retained, so HILIC mode was used. SLE extraction into organic solvents provides an additional advantage to HILIC-based separations because they are the weaker eluent in this LC mode. Ideally, determination of *N*-nitrosopiperazine in wastewater using the SLE–HILIC–MS method should be done by standard additions because of the matrix variability between samples. Unfortunately, this approach is time consuming and expensive given the higher number of SLE cartridges that would be needed for method development and application.

SLE with two drying steps

The first SLE–HILIC–MS method developed included two drying steps to achieve a pre-concentration factor of 100 because we thought an MLOQ of < 0.1 μg/L would be needed for *N*-nitrosopiperazine. Figures 2*a* and 2*b* show the internal calibration curves for *N*-nitrosopiperazine and Molecule X, respectively, prepared in synthetic matrix.

Linearity was good for both analytes using internal calibration with $R^2 > 0.99$, which surpassed that of external calibration where $R^2 = 0.94$ and 0.85 for *N*-nitrosopiperazine and Molecule X, respectively (data not shown). In our preliminary tests, very low extraction recoveries (6% for *N*-nitrosopiperazine and 34% for Molecule X; see Table 1) were obtained using LCMS-grade water as the matrix. Therefore, a synthetic matrix solution rich in salt species was prepared to mimic the composition of the wastewater, particularly with respect to conductivity. The SLE technique works simi-

Fig. 2. Internal calibration curves for (*a*) *N*-nitrosopiperazine ($y = 0.531x + 0.0283$, $R^2 = 0.9918$) and (*b*) Molecule X ($y = 0.7811x + 0.2091$, $R^2 = 0.9924$) prepared in synthetic matrix, extracted using the two drying step SLE sample preparation protocol, and analysed by HILIC–MS. Error bars represent the standard deviation of duplicate measurements for the full method. Instrumental conditions are given in the Experimental section.

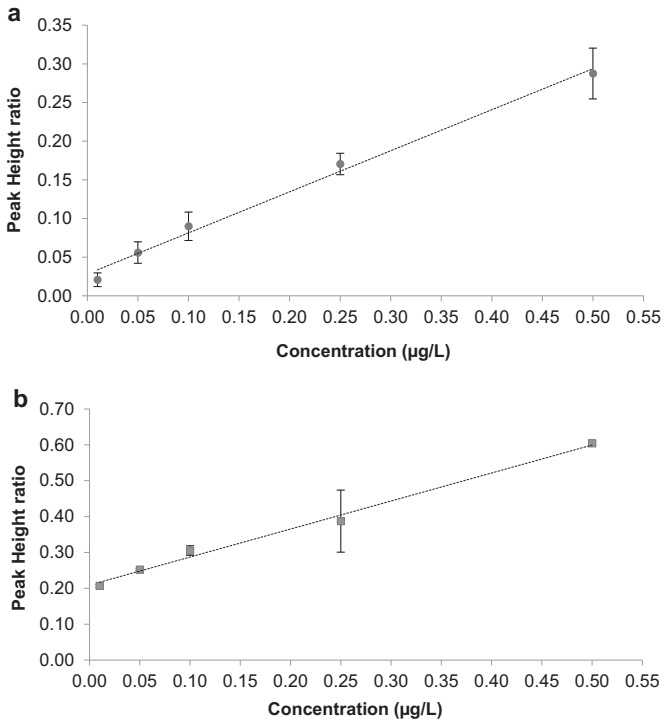
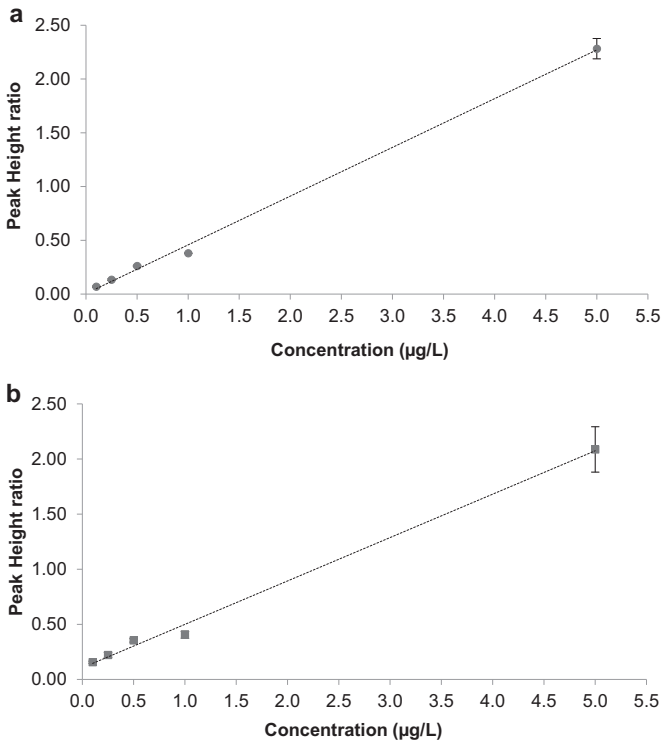


Fig. 3. Internal calibration curves for (*a*) *N*-nitrosopiperazine ($y = 0.4533x + 0.0044$, $R^2 = 0.9978$) and (*b*) Molecule X ($y = 0.3937x + 0.10$, $R^2 = 0.9955$) prepared in synthetic matrix, extracted with the one drying step SLE sample preparation protocol, and analysed by HILIC–MS. Error bars represent the standard deviation of duplicate measurements for the full method. Instrumental conditions are given in the Experimental section.



larly to classical liquid–liquid extraction where the partition of analytes into the organic phase can be enhanced by increasing the ionic strength of the aqueous phase. Extraction recovery improved to 22% for *N*-nitrosopiperazine and 57% for Molecule X at a spike level of 0.5 µg/L when these standards were spiked into the synthetic matrix (Table 1), although these were still lower recoveries than expected and not very reproducible, particular for *N*-nitrosopiperazine. The MLOQ obtained for the analytes using the two drying step SLE protocol were 330 and 91 ng/L for *N*-nitrosopiperazine and Molecule X, respectively. Although these were deemed acceptable, it was suspected that using two drying steps might lead to non-specific sample losses. The nitrogen drying step was therefore removed and reconstitution of the sample was made directly in 5 mL acetonitrile. This reduced the preconcentration factor to 10-fold, which we expected would increase the MLOQ for the presumed trade-off of better recovery.

SLE with one drying step

Simplifying the extraction protocol by removing the nitrogen-drying step had the advantage of reducing the duration of the SLE protocol by almost 50 min. Supplementary Table S2 shows a breakdown of the approximate time needed for both SLE protocols. Internal calibration curves initially prepared to cover the range 0.01 to 0.5 µg/L for standards in synthetic matrix extracted with the one drying step SLE–HILIC–MS method were linear for *N*-nitrosopiperazine but not as good for Molecule X ($R^2 = 0.9710$), as shown in Supplementary Fig. S1. Because the target MLOQ was actually 1 µg/L as requested by the supplier of the treated wastewater sample, the calibration range was modified to cover 0.10–5.0 µg/L using the one drying step SLE protocol. Figures 3*a* and 3*b* show the internal calibration curves for *N*-nitrosopiperazine and Molecule X, respectively, prepared in synthetic matrix.

As seen in Table 1, linearity for the 10-fold higher calibration range with the faster SLE protocol was fairly good for each analyte ($R^2 > 0.995$). The 10-fold lower enrichment factor actually led to better method detection and quantification limits for *N*-nitrosopiperazine, presumably because of improved extraction recovery (MLOD, 75 ng/L; MLOQ, 250 ng/L). More than a 2-fold increase in extraction recovery was obtained for *N*-nitrosopiperazine (from 22% to 50%) with better reproducibility, whereas recovery for Molecule X decreased to 47% (Table 1). Albero et al. achieved extraction recoveries ranging from 70% to 120% for determination of pharmaceuticals in biosolids, the material resulting from treated sewage sludge in wastewater treatment plants.[52] Their extraction process included liquid extraction from the solid sample followed by SLE clean-up.

There was some concern that evaporating the pooled DCM extracts to dryness is not recommended because it can lower recoveries.[38,56] We decided to investigate incomplete drying by adding a small amount of acetonitrile (2.0 mL) to the 100 mL pooled DCM extract before rotary evaporation. The extraction recoveries obtained for *N*-nitrosopiperazine and Molecule X, both spiked at 1 g/L, showed no significant differences (less than ±3%) between using incomplete drying and complete drying of the DCM eluate. Therefore, evaporating the extraction solvent to dryness before adding 5 mL acetonitrile was retained for the one drying step SLE protocol. An example of the SLE–HILIC–MS analysis is shown in Fig. 4.

The developed one drying step SLE–HILIC–MS method exceeded the objective of reaching a MLOQ of 1 µg/L, even though extraction recovery for this analyte was a modest 50%. Additional validation studies were made for analytical parameters assessed at 1 and 2 µg/L with the developed method, as summarized in Table 2. The repeatability of the method was evaluated as the percent RSD in

Fig. 4. MRM extracted ion chromatograms of *N*-nitrosopiperazine (1.0 μg/L), Molecule X (1.0 μg/L), and *N*-nitrosopiperazine-d_8 (10 μg/L) obtained with the one drying step SLE–HILIC–MS method. Details regarding extraction, chromatography, and mass spectrometry parameters are given in the Experimental section. [Colour online.]

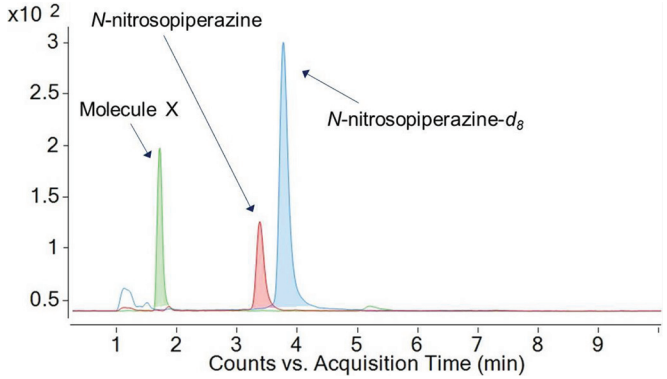


Table 2. Additional validation results for the one drying step SLE–HILIC–MS procedure with standards prepared in synthetic matrix.

	Concentration (μg/L)	*N*-Nitrosopiperazine	Molecule X
Intra-day precision[a] ($n = 5$)	1	3.0% RSD	4.0% RSD
	2	3.8% RSD	2.5% RSD
Inter-day precision[b] ($n = 2$)	1	2.7% RSD	5.0% RSD
	2	2.8% RSD	3.3% RSD
Intra-day accuracy ($n = 5$)	1	90%±3%	97%±3%
	2	88%±5%	95%±6%
Inter-day accuracy ($n = 2$)	1	93%±4%	99%±6%
	2	93%±2%	93%±5%
Theoretical plates[c] ($n = 10$)	1	2842±43	1260±56
Resolution[c] ($n = 10$)	1	1.48±0.04[d]	7.05±0.09[e]
TF[f] ($n = 10$)	1	1.12±0.06	0.96±0.05

Note: Uncertainties are reported as standard deviations unless otherwise indicated.

[a]Calculated for ratios of analyte peak height to internal standard peak height. Precision in absolute peak height ranged from 6.4% to 7.9% RSD for the three compounds in the mixture.

[b]Based on peak height ratios for five replicates per day over 2 days.

[c]Calculated using peak width at half-height. N = 3367 ± 263 plates for *N*-nitrosopiperazine-d_8.

[d]For *N*-nitrosopiperazine and *N*-nitrosopiperazine-d_8 peaks.

[e]For Molecule X and *N*-nitrosopiperazine peaks.

[f]Tailing factor (TF) = 1.07 ± 0.07 for *N*-nitrosopiperazine-d_8.

peak height ratio (analyte to internal standard). It ranged from 2.5% to 4.0% RSD intra-day and from 2.7% to 5.0% RSD inter-day. These values compare well to other studies using SLE where peak area precision varied from 2.9% to 10.8% RSD for polyphenols in wine and from 1.5% to 12% RSD for pharmaceuticals in biosolids.[52,53] Accuracy measurements were based on the difference between the concentration determined using the calibration curve and the exact (i.e., expected) value spiked into matrix. Both intra- and inter-day accuracies were better than 88% (Table 2). The peak efficiencies, tailing factors, and resolution were determined at 1 μg/L for all three compounds to verify the system suitability of the HILIC–MS analysis. The tailing factor was less than 1.2 for both analytes, which doesn't indicate the presence of tailing peaks according to Snyder et al.[55] Nonetheless, the number of theoretical plates, N, and resolution, R, were calculated using the equations for peak width at half-height.[55] The results presented in Table 2 were deemed acceptable for the developed method.

Analysis of treated wastewater from a carbon capture facility

A sample of degraded carbon capture solvent that had been submitted to wastewater treatment was divided into two aliquots and analysed using the one drying step SLE–HILIC–MS method. The concentration of *N*-nitrosopiperazine was determined to be 8.9 ± 0.3 μg/L (3.7% RSD) using extrapolation of the 0.1–5.0 μg/L calibration curve. This was justified by studies showing that the one drying step SLE–HILIC–MS method is linear past 10 μg/L (Supplementary Fig. S2). The other analyte, Molecule X, was not detected in the sample, implying that it was efficiently removed during the supplier's wastewater treatment process.

Analysis of degraded carbon capture solvent

Although the SLE–HILIC–MS method was developed for trace analysis of *N*-nitrosopiperazine in wastewater, it was also applied to a series of degraded aqueous amine samples from different carbon capture facilities (confidential) after many cycles of regeneration. Eight different samples of "used" carbon capture solvent that had not yet been subjected to wastewater treatment were each divided into two aliquots and analysed for *N*-nitrosopiperazine using the one drying step SLE–HILIC–MS method. It should be noted that the samples had high concentrations of other amine species that were quantified previously by a dilute and shoot LCMS method that had rendered *N*-nitrosopiperazine below the detection limit. The results shown in Table 3, ranging from 0.7 to 92 μg/L *N*-nitrosopiperazine, are the means of duplicate analyses. Even though one-half of the samples fell within the linear range of the SLE–HILIC–MS internal calibration curves, external calibration had to be used because the internal standard was not consistently detected. The high concentration of salts, as well as Molecule X, apparently had a negative impact on the extraction of the internal standard during SLE. As the external calibration curves were linear up to 50 μg/L (Supplementary Fig. S3), the results for samples A–F can be considered as confident, whereas those for samples G and H have less confidence because the concentrations were determined by extrapolation.

The reproducibility of sample B for *N*-nitrosopiperazine was evaluated using five aliquots of 50 mL prepared identically by the one dying step SLE–HILIC–MS method and external calibration. The concentration precision was 9% RSD. This value, which was slightly higher than the absolute peak height precision observed during method validation (see footnotes in Table 2), was not unexpected because the degraded carbon capture solvent is much more complex than the synthetic matrix. Combined with the fairly high percent RSD values seen in Table 3, these results suggest there is high variability in the salt content, which affects the efficiency of SLE extraction and thus analyte recovery. To verify this hypothesis, we measured the conductivity of our solutions; it was 14.8 mS/cm for the synthetic matrix used in method development versus 35.2–63.1 mS/cm for the eight carbon capture samples. This confirmed the presence of higher amounts of salts in the matrices studied in Table 3, although there was no direct correlation between conductivity and percent RSD in *N*-nitrosopiperazine concentration determinations. On the other hand, the two carbon capture solvent samples having the highest conductivity (>60 mS/cm) also had the highest concentrations of *N*-nitrosopiperazine, namely G and H. Although we did not rigorously test it, extraction recoveries are correlated to the presence of salts, as shown in Table 1 for the two drying step SLE protocol. The mean concentrations of *N*-nitrosopiperazine in the carbon capture solvents in Table 3 were, in fact, weakly correlated (R^2 = 0.697) to their conductivities. We also observed that the conductivity of at least one sample did not scale with dilution. Sample F, which had 0.7 ± 0.2 ppb *N*-nitrosopiperazine (Table 3) and a conductivity of 46.2 mS/cm when analysed, needed 10-fold dilution to reach a conductivity of 12.1 mS/cm. The obvious conundrum to the conductivity problem is that dilution of samples to match the conductivity of the calibration standards jeopardizes detection of analytes present at trace levels in their matrix. The use of the SLE extraction procedure should therefore start with assessment of

Table 3. Results for the determination of *N*-nitrosopiperazine in eight degraded carbon capture samples (A–H) obtained prior to wastewater treatment.

	A	B	C	D	E	F	G	H
Concentration (μg/L)[a]	4.5±1.0	2.3±0.4	7.3±1.5	3.4±0.1	5.1±0.3	0.7±0.2	53±8	92±10
RSD (%; n = 2)	22	16	20	3.5	5.5	23	14	11

[a]Mean and standard deviation (n = 2) obtained using external calibration curves (Supplementary Fig. S3).

sample conductivities then preparation of matrix-matched calibration standards to avoid excessive sample dilution.

Conclusion

A method was developed to quantify *N*-nitrosopiperazine at the ppb level by SLE extraction combined with LC–MS analysis and was applied to samples of treated wastewater and degraded carbon capture solvent. A second analyte related to the CCS solvent, Molecule X, was not detected in treated wastewater effluent indicating that the treatment procedure was efficient for its removal. The concentration of *N*-nitrosopiperazine ranged from 0.7 to 92 μg/L in samples of degraded carbon capture solvent before wastewater treatment compared with 8.9 μg/L in a separate wastewater sample provided after treatment. Unfortunately, the pre-wastewater treatment concentration of *N*-nitrosopiperazine was not known for this sample.

The SLE–HILIC–MS method is straightforward and allows sub-ppb quantification of *N*-nitrosamines with more affordable instrumentation than several other published LC–MS methods and offers an alternative to the several GC–MS methods published[31,36] that are not as good for determining nitrosamines of higher molar mass. The level of dissolved salts in environmental and industrial samples can vary immensely, which affects the performance of SLE. Adjustment of either the samples or the standards, or both, to achieve similar conductivities should improve extraction recovery and lead to better accuracy and precision for the SLE method. Although the current work focused specifically on *N*-nitrosopiperazine and the confidential analyte Molecule X, other *N*-nitrosamines of known carcinogenicity[30,35] could be added to the developed method for determinations in industrial and treated wastewaters at low ppb levels with good specificity by using MRM transitions in the mass spectrometric determination. Similarly, a wide range of analytes, from pesticides and herbicides to personal care products to pharmaceuticals could be determined in wastewater using the straightforward SLE method and the appropriate column for LC–MS analysis. Improvements in sensitivity apart from better MS instrumentation can be achieved by doubling the sample loaded using 100 mL ChemElut SLE cartridges and (or) through optimization of sample pH and mixed organic solvents for elution.

Supplementary data

Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjc-2020-0082.

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