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Development of a hydrophilic interaction liquid chromatography–mass spectrometry method for detection and quantification of urea thermal decomposition by-products in emission from diesel engine employing selective catalytic reduction technology

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ABSTRACT

The use of urea based selective catalytic reduction (SCR) technology for the reduction of NOx from the exhaust of diesel-powered vehicles has the potential to emit at least six thermal decomposition by-products, ammonia, and unreacted urea from the tailpipe. These compounds may include: biuret, dicyandiamine, cyanuric acid, ammelide, ammeline and melamine. In the present study, a simple, sensitive and reliable hydrophilic interaction liquid chromatography (HILIC)-electrospray ionization (ESI)/mass spectrometry (MS) method without complex sample pre-treatment was developed for identification and determination of urea decomposition by-products in diesel exhaust. Gradient separation was performed on a SeQuant® ZIC-HILIC column with a highly polar zwitterionic stationary phase, and using a mobile phase consisting of acetonitrile (eluent A) and 15 mM ammonium formate (pH 6; eluent B). Detection and quantification were performed using a quadrupole ESI/MS operated simultaneously in negative and positive mode. With 10 μL injection volume, LODs for all target analytes were in the range of 0.2–3 μg/L. The method showed a good inter-day precision of retention time (RSD < 0.5%) and peak area (RSD < 3%). Satisfactory extraction recoveries from spiked blanks ranged between 96 and 98%. Analyses of samples collected during transient chassis dynamometer tests of a bus engine equipped with a diesel particulate filter (DPF) and urea based SCR technology showed the presence of five target analytes with cyanuric acid and ammelide the most abundant compounds in the exhaust.

1. Introduction

In response to health concerns, regulators have promulgated more stringent emissions standards for particulate matter (PM) and NOx from diesel engines. PM emissions are controlled in part by diesel particulate filters (DPFs) that incorporate either a passive or active strategy for regenerating the filter substrates. Passive DPFs use a combination of exhaust gas temperature (heat) and a catalyst to initiate the regeneration process while active DPFs use an outside source to provide the heat for regeneration. Urea-selective catalytic reduction (SCR) and NOx adsorber catalyst are emerging as the current leading contenders for NOx control in diesel engines. Urea-SCR technology can substitute the use of NH_3, which poses problems related to its toxicity and has the advantage of being less sensitive to sulfur as compared the NOx absorber-type catalysts [1–5]. Nevertheless, it is important to understand the effects that engine and after-treatment technologies may have on the reduction or formation of the full spectrum of chemical species that are not currently identified.

Urea thermal decomposition reaction is a two-step process that includes the formation of ammonia, and isocyanic acid as an intermediate product [6–8]. Ammonia is then involved in several reactions, which ultimately lead to the denitrification of flue gas [1,4,8]. The isocyanic acid is very reactive and can initiate the formation of larger molecular weight compounds such as cyanuric acid, biuret, melamine, ammeline and ammelide [6,7,9–11]. Storey et al. [3] reported also the formation of dicyandiamide. However, the effect of urea-SCR technology in the chemical composition of the gaseous and PM diesel emissions is still ambiguous. In addition, the toxicity data base for these compounds, in general, is incomplete [12]. Thus, the quantification of these compounds is essential for identification of potential adverse environmental and

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health effects and to ensure that the emission inventories used in atmospheric modeling activities and in health risk assessments are accurate.

Various chromatographic approaches have already been developed and applied for the analysis of this class of chemicals in variety matrices. Previously, reversed-phase liquid chromatography (RPLC) with UV or diode array detection has been reported for the analysis of cyanuric acid in swimming pool waters and air [13], or melamine and its derivatives in natural waters and industrial products, and in air [14]. Recently, as an urgent response to toxicity incidents in 2007 and 2008 involving melamine [15–17] extensive efforts have been made for determining melamine and its analogs (ammeline, ammelide and cyanuric acid) in food/feed and animal tissues. In these incidents, the melamine was added deliberately to pet food and infant formula to boost their apparent protein content. Melamine alone is of low toxicity; however, combination with cyanuric acid leads to crystal formation and subsequent kidney toxicity [16]. Many novel analytical methods, based on gas chromatography–mass spectrometry (GC–MS) [18,19], capillary electrophoresis with UV [20,21], RPLC [22,23] and hydrophilic interaction liquid chromatography (HILIC) with UV and MS detection [24,25] have been developed. However, little is known about the determination of these highly polar nitrogenous compounds in emissions from diesel engines employing urea-SCR after-treatment technology. To our best knowledge, an anion-exchange chromatography with UV detection developed by Koebel and Elsner [9] is the only method reported to study the formation of thermal degradation by-products of the urea-SCR process.

The purpose of this work was to establish a reliable and sensitive method using LC-ESI/MS for identification and quantification of urea and its thermal decomposition by-products in emissions from diesel engine employing SCR technology. Recent advantages in LC–MS capabilities made this technique an attractive alternative to GC–MS because it offered the advantage of omitting a derivatization step. This was achieved by exploiting the advantages of RPLC and HILIC separations combined with ESI mass spectrometry giving molecular weight information. HILIC is a useful technique for the retention of polar analytes that offers a difference in selectivity compared to traditional RPLC [26,27]. Different LC columns, which included a reversed-phase Zorbax SB-Aq and HILIC columns (viz., Atlantis™ HILIC Silica, Cogent Diamond Hydride™ and SeQuant® ZIC-HILIC), were tested. The best performance was obtained using the SeQuant® ZIC-HILIC column with a highly polar zwitterionic stationary phase. The developed HILIC-ESI/MS method was validated and successfully applied to the analysis of real-world samples following a simple sample pre-treatment procedure using acetonitrile/formic acid extraction.

2. Experimental

2.1. Reagents and standards

Double deionized water (DDW; 18 MΩ; Barnstead, Dubuque, IA, USA) was used for the preparation of all solutions. HPLC grade acetonitrile (ACN), methanol and ammonium formate were purchased from Fisher Scientific (Ottawa, ON, Canada). Formic acid (98+%) was obtained from Acros Organics (Geel, Belgium). Cyanuric acid (CYA), melamine (MEL), dicyandiamide (DICY), biuret (BIU) and urea were purchased from Sigma–Aldrich (Toronto, ON, Canada). Ammeline (AML) was obtained from MP Biomedicals (Aurora, OH, USA). Isotopically labeled melamine ($^{13}C_3$, 99%; amino-$^{15}N_3$, 98%; chemical purity, ≥98%; Mw = 132.08) and cyanuric acid ($^{13}C_3$, 99%; $^{15}N_3$, >98%; chemical purity, 90%; Mw = 135.03) were supplied by Cambridge Isotope Laboratories (Andover, MA, USA).

Stock solutions of CYA, MEL, DICY, BIU, AML and urea (each 100 μg/mL) were prepared by dissolving appropriate amounts of these compounds in a mixture of ACN/water (1:1, v/v). Ammelide (AMD; Tokyo Chemical Industry Co., Tokyo, Japan) was prepared in 15% (v/v) aqueous formic acid solution. All stock solutions were sonicated for 20 min or until dissolved. To avoid formation of water-insoluble melamine–cyanurate complex [25], the mixed standards were prepared weekly by diluting the appropriate amount of stock solutions in a 2% (v/v) formic acid/ACN mixture. All diluted standards were prepared daily from the mixed stock standard solutions. Quality control standards were prepared from certified standard solutions purchased from Delta Scientific Laboratory Products Ltd. (Mississauga, ON, Canada). All standards were stored at 4 °C.

2.2. Instrumentation and methods

All analyses were performed on an Agilent 1100 series LC system (Agilent Technologies, Wilmington, DE, USA) consisting of a vacuum degasser, binary pump, thermostated column compartment, and autosampler. Detection was carried out utilizing a single quadrupole MS (MSD SL; Agilent Technologies) with a pneumatically assisted ESI source as the interface. The LC-ESI/MS system was controlled, and data were acquired and processed using the Agilent ChemStation software (Rev. B.02.01-SR2).

For chromatographic separation, four analytical columns with compatible guard columns were compared. The tested analytical columns included Zorbax SB-Aq column (150 mm × 2.1 mm i.d., 3.5 μm particle size; Agilent), Atlantis™ HILIC silica column (150 mm × 2.1 mm i.d., 3.0 μm particle size; Waters Corporation, Milford, MA, USA), Cogent Diamond Hydride™ column (150 mm × 2.1 mm i.d., 4.2 μm particle size; MicroSolve Technology, Eatontown, NJ, USA) and SeQuant® ZIC-HILIC column (150 mm × 2.1 mm i.d., 3.0 μm particle size; Canadian Life Science, Inc., Peterborough, ON, Canada). All analyses were performed at 25 ± 0.1 °C.

The mobile phases were prepared by diluting 1.0 M ammonium formate in DDW, and the pH was adjusted using formic acid. The organic modifier, methanol or ACN, was mixed on-line using the binary pump. Prior to use, all eluents were filtered through a 0.2 μm filter with applied vacuum. Mobile phase compositions and sample injection volumes were optimized for each column.

Analytes were detected with ESI in both positive and negative modes. The optimized operating parameters for both modes were as follows: desolvation gas flow (12 L/min), nebulizer pressure (35 p.s.i.; 241 kPa); desolvation temperature (350 °C). Nitrogen gas of 99.9% purity, generated from pressurized air by a nitrogen generator (Parker Hannifin Corporation, Tewksbury, MA, USA) was used as the nebulizer and the desolvation gas. Capillary voltage was set up at 2000 V and −3000 V for positive and negative modes, respectively. Fragmentation voltages were set up at 120 V and −90 V for positive and negative modes, respectively.

2.3. Quantification and validation

The identification and confirmation of the target compounds in real-world samples were performed by matching their retention times and mass spectra with those of the standards. In addition, the most intense fragment ions, such as *m*/*z* 44 for urea, *m*/*z* 68 for DICY, *m*/*z* 61 for BIU, *m*/*z* 85 for MEL, and *m*/*z* 86 AML, were monitored in the positive mode. Besides, fragment ions with *m*/*z* 42 for CYA and *m*/*z* 84 for AMD were checked in the negative mode. Although AMD was detected in both positive and negative modes, the signal-to-noise ratio in negative mode was much better and thus the AMD signal in the negative mode was used for quantification. Final quantification was performed in a selected ion monitoring (SIM) mode for the quasi-molecular (parent) ions of the

target analytes using external calibration. Calibration curves were generated using a linear regression analysis. The calibration and QC standards were analyzed repetitively between samples within each analytical sequence.

The limit of detection (LOD) and limit of quantification (LOQ) were defined as the concentration at which the parent ion has a signal-to-noise (S/N) ratio of 3 and 10, respectively. The accuracy was evaluated by applying the entire sample preparation procedure to filter blanks spiked with a mixture of analytes at known concentration 25 μg/L (MEL, AML and AMD) and 250 μg/L (urea, DICY, BIU, and CYA), and tested in triplicates. To evaluate the matrix effect, the sample filters were spiked with known amounts of isotopically labeled MEL (5 μg/L) and CYA (50 μg/L).

2.4. Samples and sample preparation

The particulate matter (PM), emitted from an urban transit bus equipped with a diesel particulate filter (DPF) and the urea-SCR system, were obtained from the Emission Research and Measurement Section of Environment Canada, as part of the Program for Energy Research and Development Project. The bus was operated on a chassis dynamometer over different driving modes to emulate city driving conditions including the Braunschweig Cycle (frequent stops), Manhattan Drive Cycle (SAE Man; frequent stops and very low speed), Urban Dynamometer Drive Schedule (SAE UDDS; higher speed operation) and Orange County Drive Cycle (SAE OCTA; reflects a wide variety of accelerations, decelerations and cruise operations). Exhaust samples were collected and diluted using a constant volume sampling system. The samples were collected on Teflon filters with 2 μm pore size, 47-mm i.d. and 46 μm thickness (Teflo membrane; PTFE with polymethylpentene supporting o-ring) at a constant flow-rate of 35 L/min for 29–36 min, dependant on the drive cycle, at 23 °C. These filters were used as received from the supplier (Pall Life Sciences, OEM Materials and Devices, Ann Arbor, MI, USA). In all experiments, ultra-low sulfur diesel (<15 ppm S; commercially available) was used. After weighing, all filters were stored in the freezer until analysis.

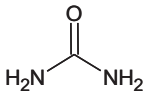
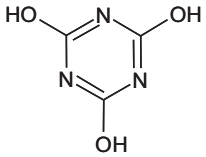
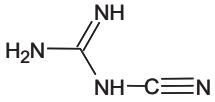
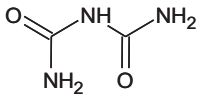
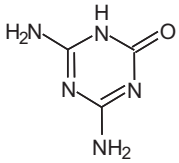
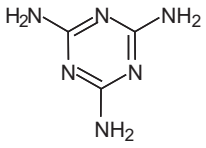
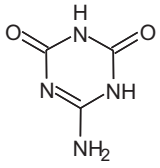
Prior to extraction, the polymethylpentene support ring of each filter was cut with a clean stainless steel cutter. All filters were then placed in glass extraction vials (1.5 mL; Agilent), and spiked with MEL (50 μL of 100 μg/L) and CYA (50 μL of 1000 μg/L) isotopically labeled surrogate standards to produce individual known concentrations of 5 μg/L and 50 μg/L, respectively, in the final extract. Before extraction, Teflon filters were wetted using 20 μL of isopropanol. Next, the samples were sonicated for 30 min with 1 mL of 2% (v/v) formic acid/ACN mixture. Then, the extract was filtered through a 0.2 μm porosity PTFE syringeless filter device with polypropylene housing (Mini-UniPrep™ Syringeless Filter; Whatman, Florham Park, NJ, USA). Blank samples were treated similarly. Finally, the filtered samples were transferred into LC vials (200 μL, Agilent) for LC-ESI/MS analysis. Analyses were performed within the established linear dynamic range. If required the extracts were diluted accordingly to bring their concentration levels within the linear dynamic range.

3. Results and discussion

3.1. Optimization of the chromatographic separation

For LC separation of the target analytes listed in Table 1, the performance of different analytical columns with a reversed-phase and HILIC mode stationary phases were evaluated. Initially, a RPLC with a Zorbax SB-Aq column was tested (Fig. 1a). The Zorbax SB-Aq column was chosen as it was specifically designed to retain highly polar compounds allowing the use of highly aqueous mobile phases

Table 1
Compounds analyzed, structure and peak identity.

Analyte	Peak ID	M_r	Molecular formula
Urea	1	60.06	
Cyanuric acid (CYA)	2	129.07	
Dicyandiamide (DICY)	3	84.08	
Biuret (BIU)	4	103.08	
Ammeline (AML)	5	127.10	
Melamine (MEL)	6	126.12	
Ammelide (AMD)	7	128.07	

[28]. However, except for MEL, the target compounds were not well retained on the RPLC column under the optimized conditions. No significant improvement in the analyte retention was obtained by increasing the percentage of aqueous mobile phase or by changing a pH. In addition, a high water percentage in the mobile phase leads to lower sensitivity of the MS detection.

Next, the suitability of three HILIC mode columns was investigated. HILIC coupled to MS is a valuable complementary approach to RPLC for the analysis of hydrophilic and polar compounds [26,29]. From a practical perspective, HILIC offers an attractive alternative to the normal phase LC mode [29]. The high amount of polar organic mobile phase (often ACN) used in HILIC is especially compatible with ESI/MS, resulting in high sensitivity. By far, HILIC/MS has been reported to be the most suitable method for determining melamine and its analogs (ammeline, ammelide and cynauric acid) in food and biological samples [16,25–27,30]. Fig. 1b shows a baseline separation of six studied analytes using Atlantis™ HILIC Silica column. A gradient elution with a mobile phase composed of ACN (solvent A) and 15 mM ammonium formate buffer (pH 6; solvent B) was used. Atlantis™ HILIC Silica is an underivatized silica-based column that provides enhanced retention of charged polar bases due to combination of hydrophilic and cation-exchange interaction [31,32]. In comparison to the reversed-phase

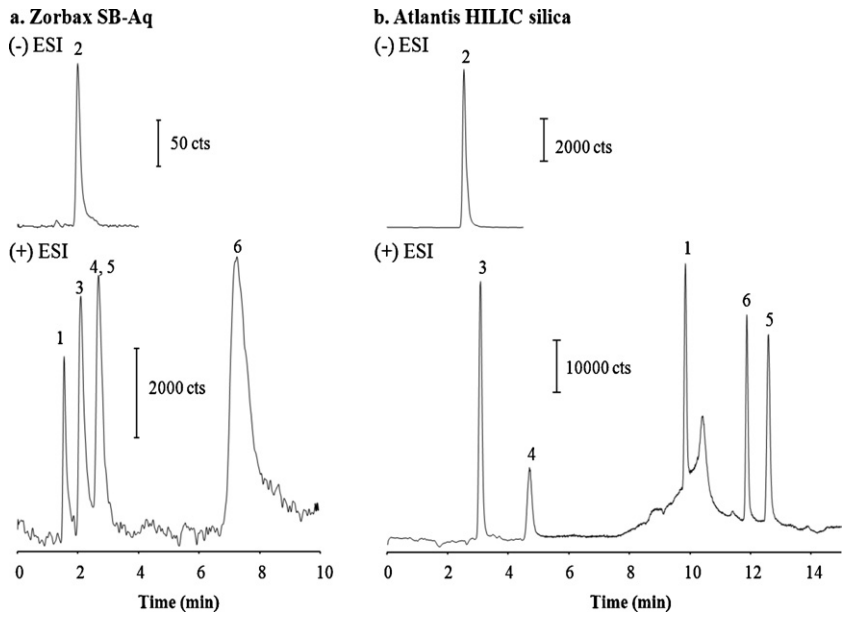


Fig. 1. Separation of a model solution of urea and five thermal decomposition by-products by LC-ESI/MS. LC conditions: (a) column: Zorbax SB-Aq (150 mm × 2.1 mm i.d., 3.5 μm); mobile phase: 5:95 (v/v) methanol–5 mM ammonium formate (pH 6); flow rate: 0.3 mL/min; injection volume: 5 μL; sample matrix: DDI water; sample concentration: 500 μg/L of each analyte; (b) column: Atlantis™ HILIC Silica (150 mm × 2.1 mm i.d., 3 μm); mobile phase: ACN (eluent A)–15 mM ammonium formate (pH 6; eluent B); elution profile: isocratic step at 1% eluent B for 1 min followed by linear gradient step at a slope of 2.78% eluent B per min for 15 min; flow rate: 0.3 mL/min; column temperature 25 °C; injection volume: 10 μL; sample matrix: 2% (v/v) formic acid/ACN; sample concentration: 500 μg/L (CYA, BIU and urea); 200 μg/L (DICY, AMD); 50 μg/L (AML, MEL). For MS conditions and peaks identification see Section 2.2 and Table 1, respectively.

Zorbax SB-Aq, a better retention and separation efficiency with a complementary selectivity was obtained. Due to the use of a higher percentage of organic solvent in the mobile phase, a considerable MS sensitivity increase of at least one order of magnitude was also achieved. However, a deterioration of the separation performance of the Atlantis™ HILIC Silica column within time was observed under the optimized conditions (pH 6). This is probably due to the fact that this unbounded silica-based stationary phase is more susceptible to particle dissolution at pH ≥ 6 [32]. Also, acidic compounds such as CYA exhibited weak retention, which is probably attributed to the repulsion of these negatively charged solutes from the ionized silanols. A better separation performance was obtained using a Cogent Diamond Hydride™ column. This stationary phase has a silicon hydride surface with low coverage of attached organic moiety (~2% carbon) [33], and allows operation without deterioration up to pH 7 [34]. As a result, this stationary phase demonstrated a better robustness at the operated pH. As shown in Fig. 2a, the Cogent Diamond Hydride™ column showed a similar selectivity to that achieved by Atlantis HILIC silica. However, both stationary phases failed to provide adequate retention for CYA.

The best separation of analytes of interest was obtained with a SeQuant® ZIC-HILIC column, which is a highly polar zwitterionic stationary phase specially designed for separating polar and ionized analytes under HILIC conditions [26,27,35]. Due to a high polarity of stationary phase, the sulfobetaine-type zwitterionic functional groups strongly adsorb water and form an immobilized water-rich layer on the stationary phase which promotes hydrophilic partitioning as a primary retention mechanism [36]. The combination of the hydrophilic partitioning and weak electrostatic interactions results in a unique selectivity and allows separating negatively and positively charged analytes simultaneously [37]. Using a mobile phase consisting of a binary gradient ACN (solvent A)–15 mM ammonium formate (pH 6; solvent B) permitted the separation of 7 target analytes in less than 9 min. The corresponding chromatogram under the optimized conditions is shown in Fig. 2b. In contrast to other tested columns, the zwitterrionic column showed greater retention for the negatively charged species (CYA and AMD). This behaviour can be attributed to some ion exchange contribution to the retention of ionized solutes. In addition, the SeQuant® ZIC-HILIC column showed good stability and low column bleeding, suggesting that the column is suitable for high-sensitivity HILIC-LC/MS analyses. Thus, this column was chosen for further experiments.

3.2. Optimization of the MS detection

Under ESI conditions, analytes namely urea, BIU, DICY, MEL and AML acquire positive charge $[M+H]^+$, whereas AMD and CYA are dominated by negative charges $[M-H]^-$. Thus, simultaneous detection mode with the MS polarity alternating between positive and negative modes was used to detect all analytes (acidic and basic) in a single run. In order to obtain the best detection sensitivity, the ESI parameters were optimized by monitoring the $[M+H]^+$ ions (m/z 61 for urea, m/z 85 for DICY, m/z 104 for BIU, m/z 127 for MEL, m/z 128 AML and m/z 129 for AMD), and $[M-H]^-$ ions (m/z 127 for AMD and m/z 128 for CYA) under the optimal separation conditions. The optimized operating parameters are listed in Section 2.2. In comparison to single runs, no critical loss in sensitivity (<20%) was observed by using simultaneous detection mode.

3.3. Analytical performance

Under optimum conditions, the performance and reliability of the HILIC/MS method with SeQuant® ZIC-HILIC column were assessed by determining its analytical figures of merit such as precision, linearity, LOD, LOQ and accuracy.

Intra-day (within day) and inter-day (between days) precision of retention time and peak area were evaluated at a concentration of 25 μg/L (MEL, AML and AMD) and of 250 μg/L (urea, DICY, BIU, and CYA) over a 4-day period. An excellent precision of retention times with the relative standard deviations (R.S.D.) less than 0.5%, and a good intra-day precision of the peak area (<3% R.S.D.) were obtained (Table 2). The between-days repeatability of the peak area was satisfactory with R.S.D. ranging between 3.0 and 10.2%

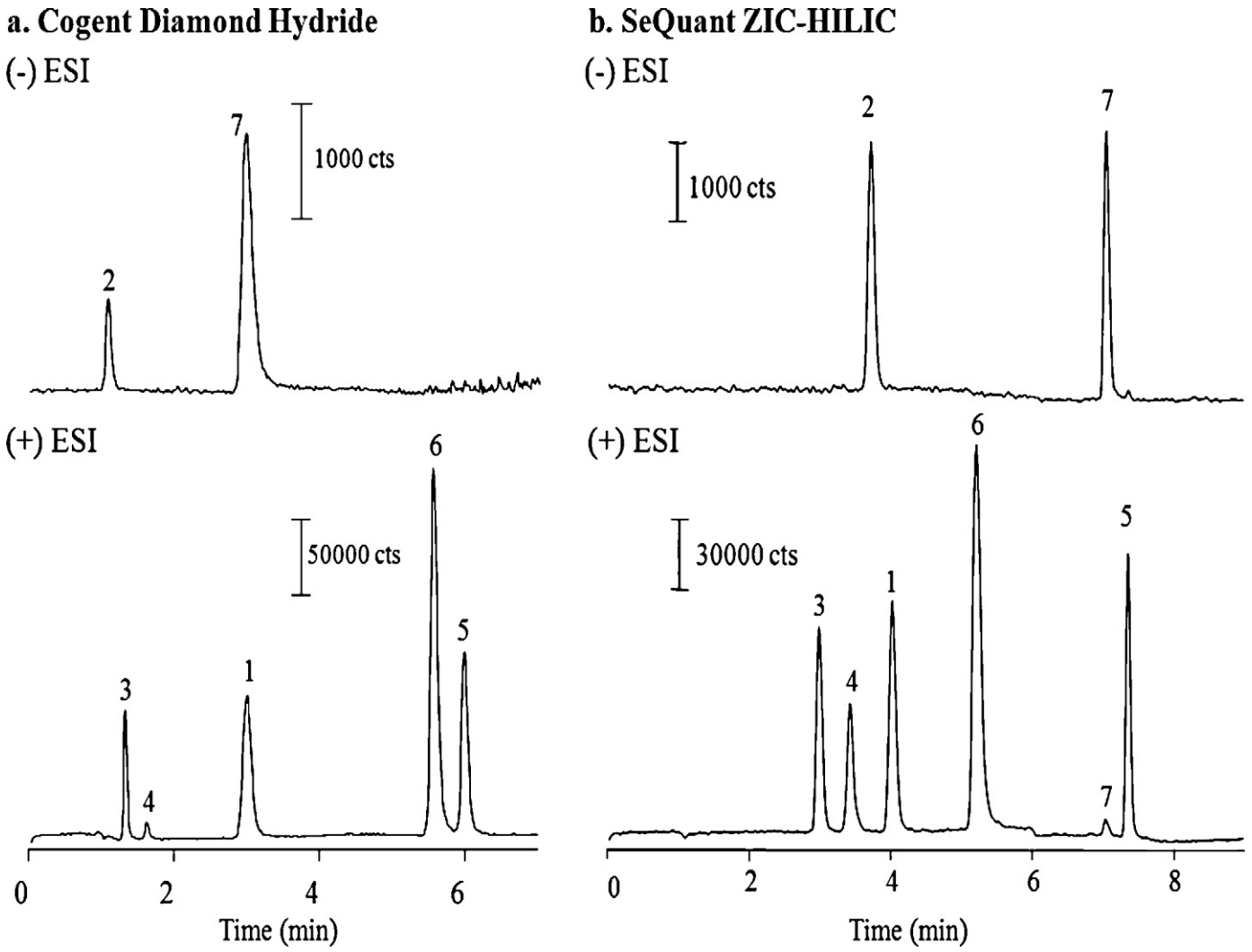


Fig. 2. Separation of a model solution of urea and six thermal decomposition by-products by LC-ESI/MS. LC conditions: (a) column: Cogent Diamond hydride™ (150 mm × 2.1 mm i.d., 4 μm); elution profile: isocratic step at 5% eluent B for 0.5 min followed by linear gradient step at a slope of 5.2% eluent B per min for 8 min; (b) column: SeQuant® ZIC-HILIC (150 mm × 2.1 mm i.d., 3 μm); elution profile: isocratic step at 5% eluent B for 0.5 min followed by linear gradient step at a slope of 4.59% eluent B per min for 9 min; flow rate: 0.5 mL/min, column temperature 25 °C; injection volume: 10 μL; sample matrix: 2% (v/v) formic acid/ACN; concentration: 500 μg/L (CYA, BIU and urea); 200 μg/L (DICY, AMD); 50 μg/L (AML, MEL). For MS conditions and peaks identification see Section 2.2 and Table 1, respectively.

(Table 2). Similar performance was obtained at a lower concentration of 5 μg/L (MEL, AML and AMD) and 25 μg/L (urea, DICY, BIU, and CYA; data not shown).

For estimation of the linear dynamic range, seven-point calibration curves injected in triplicate were constructed in the range listed in Table 2. Using a least-square regression analysis, the correlation coefficients (r) were greater than 0.998 (Table 2).

Limits of detection (LODs) were calculated from the injection (10 μL) of standard solutions at the concentration of a compound giving a signal-to-noise ratio of 3, and were in the range of 0.2 and

Table 2
Analytical characteristics of the HILIC-LC-ESI/Q-MS method.

Analyte	LOD[a] (μg/L)	LOQ[a] (μg/L)	Intra-day repeatability[b] R.S.D. (%)		Inter-day repeatability[c] R.S.D. (%)		Recovery[c] (%)	Correlation coefficient (r)	Dynamic range (μg/L)
			Retention time	Peak area	Retention time	Peak area			
CYA	1.0	3.0	0.22	2.4	0.48	5.2	96 ± 4	0.998	3.0–1000
DICY	0.5	1.7	0.25	2.8	0.33	3.2	98 ± 3	0.998	1.7–500
BIU	3.0	10	0.22	2.9	0.30	3.3	97 ± 2	0.998	10–1000
Urea	1.5	5.0	0.27	1.9	0.46	4.1	98 ± 4	0.999	5.0–1000
MEL	0.2	0.6	0.21	1.3	0.45	3.0	97 ± 3	0.999	0.6–50
AML	0.3	1.0	0.17	2.5	0.21	3.2	98 ± 2	0.998	1.0–100
AMD	0.4	1.5	0.13	3.0	0.2	10.2	95 ± 1	0.998	1.5–200

Experimental conditions: see Fig. 2b.
[a] Injection volume 10 μL.
[b] $N = 4$.
[c] $N = 10$.

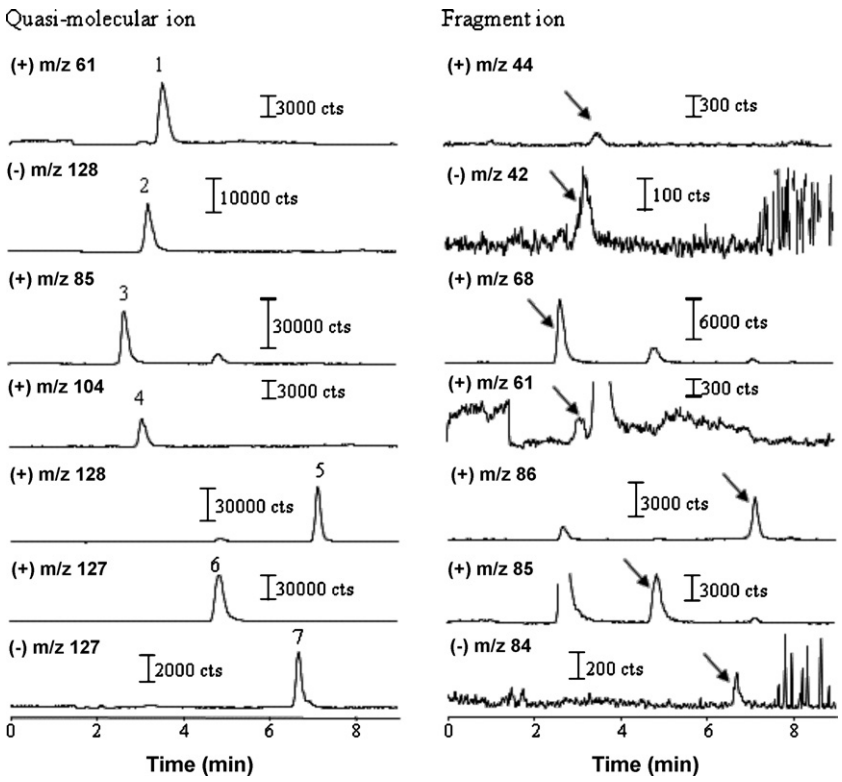


Fig. 3. Example of extracted ion chromatograms of the quasi-molecular (parent) ions and fragment ions for target analytes using SeQuant® ZIC-HILIC column. Sample concentration: 500 μg/L (CYA, BIU and urea); 200 μg/L (DICY, AMD); 50 μg/L (AML, MEL). For peaks identification and analytical conditions see Table 1 and Fig. 2b, respectively.

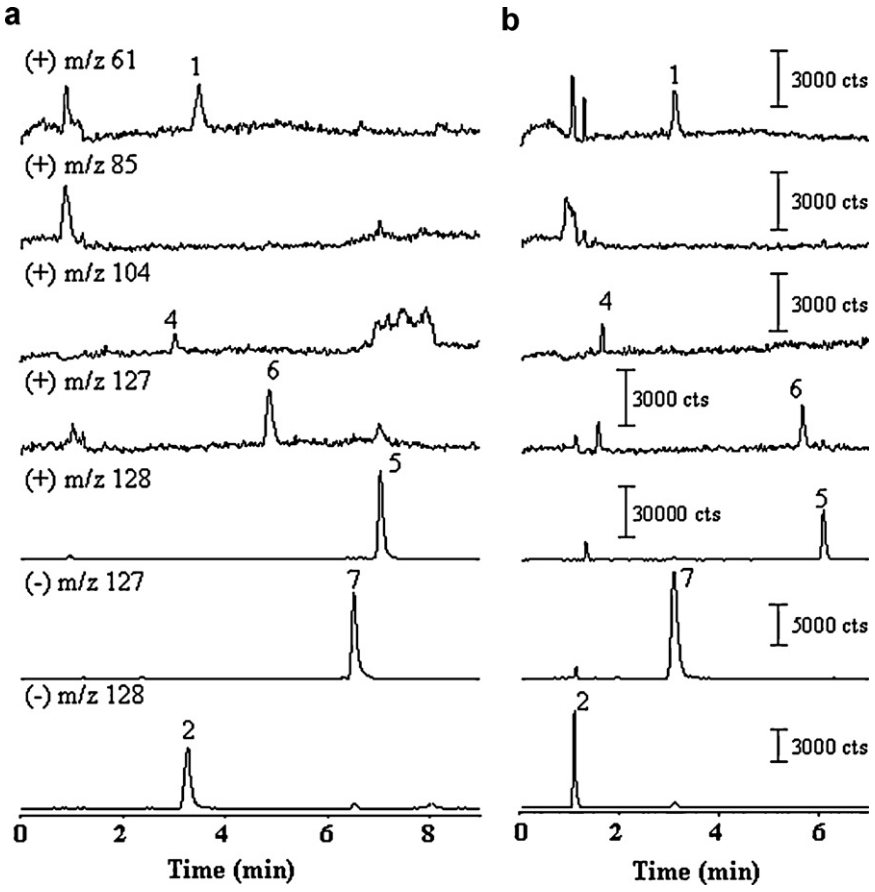


Fig. 4. Example of extracted ion chromatograms of a real sample from a DPF-urea SCR-equipped bus engine using (a) SeQuant® ZIC-HILIC column and (b) Cogent Diamond Hydride column. For peaks identification and analytical conditions see Table 1 and Fig. 2, respectively.

Table 3
Concentration of target compounds detected in diesel $PM_{2.5}$ emitted from urban transit bus equipped with a DPF and urea-SCR.

Samples/driving mode	PM mass (μg/filter)	Urea (μg/L)	DICY (μg/L)	BIU (μg/L)	MEL (μg/L)	AML (μg/L)	AMD (μg/L)	CYA (μg/L)
Blank		<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
T9381 (Braunschweig)	8.5	332 (39)	<LOD	62 (7.3)	7.1 (0.84)	80 (9.4)	959 (113)	365 (43)
T9372 (SAE Man)	8.5	91 (11)	<LOD	87 (10)	3.0 (0.36)	76 (9.0)	1094 (129)	563 (66)
T9411 (SAE OCTA)	13.5	110 (8.1)	<LOD	72 (5.3)	6.7 (0.50)	198 (15)	2099 (156)	502 (37)
T9416 (SAE UDDS)	30.0	22 (0.7)	<LOD	86 (2.9)	11 (0.37)	137 (4.6)	1314 (44)	320 (11)

Values in parenthesis are calculated in ng/μg of PM; Braunschweig Cycle (frequent stops), SAE Man: Manhattan Drive Cycle (frequent stops and very low speed); SAE OCTA: Orange County Drive Cycle (reflects a wide variety of accelerations, decelerations and cruise operations); SAE UDDS: Urban Dynamometer Drive Schedule (higher speed operation); Experimental conditions: see Fig. 2b.

3 μg/L (Table 2). The LOQs, based on a signal-to-noise ratio of 10, ranged from 0.6 to 10 μg/L (Table 2). However, in order to confirm identity of peaks using the fragment ions, at least 10 times higher concentrations than LODs are required (Fig. 3).

Extraction recovery and matrix effect were evaluated by analyzing spiked blank filters and samples. Using spiked blank filters, the extraction procedure accuracy ranged between 96 ± 4 and 98 ± 4% (Table 2). A straightforward liquid extraction method using acidified acetonitrile was employed. The mean recoveries of surrogates from the spiked samples were 101 ± 2% and 99 ± 2% for MEL and CYA, respectively. Thus, the extraction recoveries of the method were excellent, and the time of sample preparation was minimized. Overall, the above mentioned validation parameters indicate the accuracy, precision, linearity, and sensitivity of the analytical method and reliability of the extraction procedure.

3.4. Method application

The proposed analytical methodology was applied to different PM samples emitted from an urban transit bus equipped with the DPF and urea-SCR system, and tested over four driving cycles to emulate various city driving conditions. Fig. 4 shows typical extracted ion chromatograms for the studied samples. The quantitative data of four analyzed samples are reported in Table 3. Except for DICY, all target compounds were detected in the analyzed samples. CYA and AMD were the most abundant urea decomposition by-products. CYA, a product of trimerization of isocyanic acid, was reported to be formed in significant amounts at the lower catalyst temperature around 200 °C, and starts to decompose at the temperature above 275 °C. AMD is formed from BIU (~200 °C) and during the reaction of CYA with ammonia [6,7,10]. Other compounds, such as AML and MEL reported to be formed at the higher catalyst temperature of 225 °C and 300 °C, respectively [6,7], were present at a relatively low level. Blank filters showed no indication of contaminations that may interfere with the analysis since none of the target analytes were detected in blank extracts (Table 3).

All analytes were identified by comparing the retention times of chromatographic peaks of the matched quasi-molecular (parent) with those of the target analytes. To avoid positive findings of compounds in the real-world samples by single quadrupole MS, the most intense fragment ion of each analyte was also monitored for analyte identity confirmation. Using criteria that the intensity ratio of the fragments to the quasi-molecular ion intensity should be within 20% of the standard value [38], most of the target analytes were confirmed. The only exception was urea and BIU, as they were detected at low levels where their peak identity confirmations were not possible (Fig. 3).

Further, the identity of target analytes in the analyzed samples was confirmed by analyzing samples using a Cogent Diamond Hydride™ column (Fig. 4b). As discussed in Section 3.1 (Fig. 2a), the column provides a satisfactory separation with a complementary selectivity and a good retention time precision (R.S.D. < 0.5%). Continuing work in our laboratory is focused on employing triple quadrupole MS to confirm the analyte identity at trace level in a single run which is not always possible by LC–single quadrupole MS.

4. Conclusions

A new HILIC-ESI/MS method has been developed and validated for the identification and quantification of urea thermal decomposition compounds, namely cyanuric acid, biuret, dicyandiamide, ammeline, ammelide and melamine, in emissions from diesel engines employing urea-SCR after-treatment technology. Different RPLC and HILIC columns were tested. The best baseline separation of all analytes of interest was obtained using a highly polar zwitterionic stationary phase (SeQuant® ZIC-HILIC column) with a mobile phase composed of acetonitrile and ammonium formate (pH 6). In the SIM mode with ESI operated simultaneously in negative and positive mode, LODs were in the range of 0.2-3 μg/L (injection volume of 10 μL). The proposed method includes a simple extraction procedure, and offers a combination of high sensitivity, simplicity and relatively short time of analysis. The applicability was verified by the determination of urea-decomposition by-products and unreacted urea in PM emitted from a DPF-urea SCR-equipped diesel bus engine operated over four different driving modes.

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