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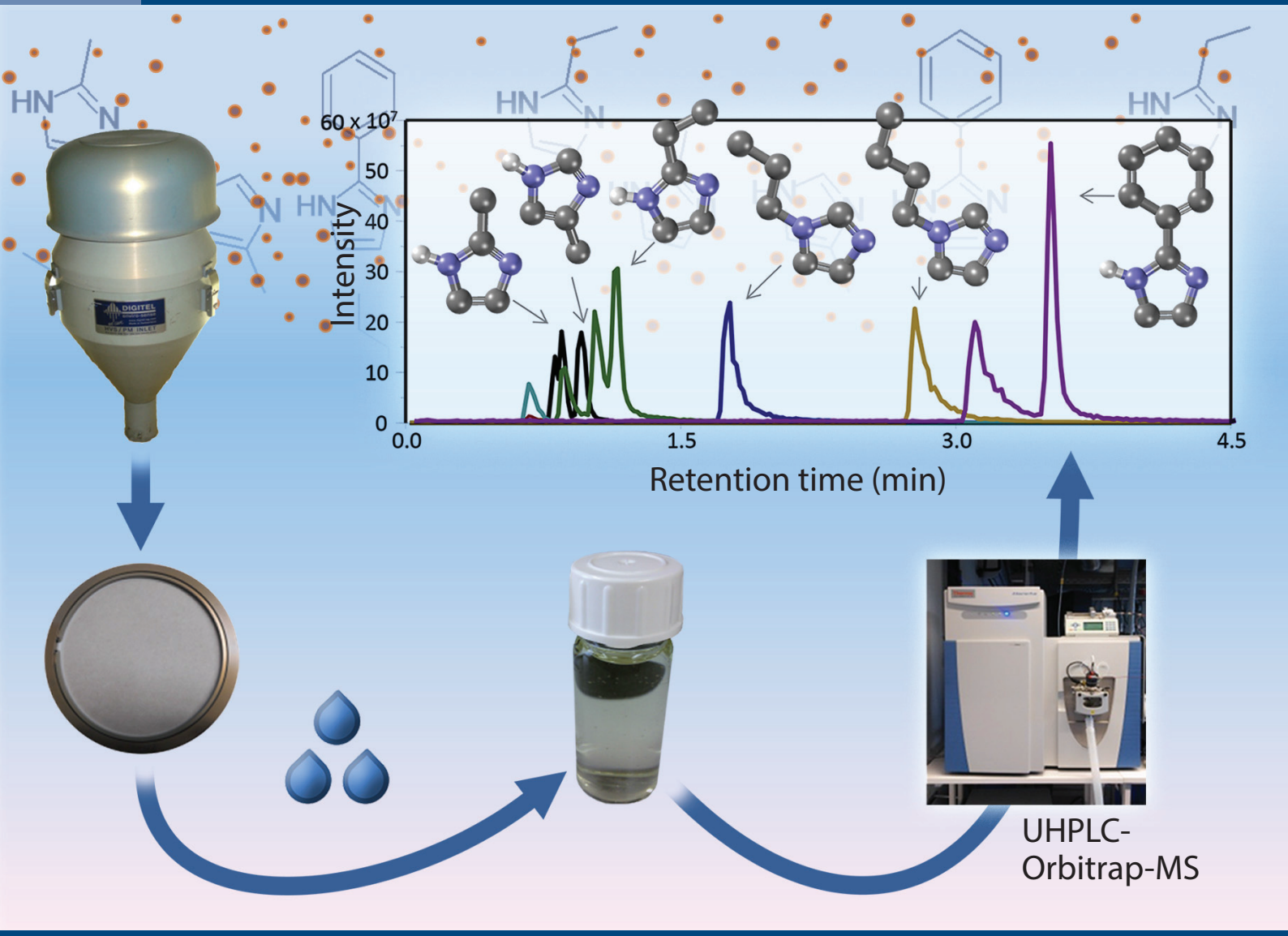

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RESEARCH ARTICLE

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Separation and quantification of imidazoles in atmospheric particles using LC–Orbitrap-MS

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A method using ultra-high performance liquid chromatography coupled to a high resolution Orbitrap mass spectrometer was developed to identify and quantify imidazoles in aqueous extracts of aerosol particles. The aqueous particle extract was used without further enrichment or sample clean-up. Five columns were tested for efficient separation of ten imidazoles and the Acquity HSS T3 column was chosen for further optimization. Low limits of detection (<25 nM) and good intraday and interday repeatability (<1.6 and <6%, respectively) were achieved. Investigation of matrix effects showed that external calibration is applicable when the loading of organic carbon in the sample is below 10 μg m^{-3}. The developed method was applied to ten real samples, and six out of the ten test imidazoles were successfully quantified, while six further imidazoles were qualitatively identified, among them 4-imidazolecarboxaldehyde and 4-methyl-5-imidazolecarboxaldehyde. Advantages of the method are the minimal sample preparation, the short run time for each sample, and the low detection limits. These allow for a fast and reliable quantification of imidazoles even in a large number of aqueous particle extract samples.

KEYWORDS
brown carbon, high resolution mass spectrometry, imidazoles, method development, organic aerosols

1 | INTRODUCTION

Imidazoles are constituents of organic aerosol particles and gained increasing attention in the past decade, since they might be part of atmospheric brown carbon [1], act as photosensitizers [2] or have negative health impacts [3]. Many laboratory studies confirmed the production of imidazoles during the reaction of dicarbonyl compounds with aldehydes in the presence of nitrogen containing compounds [4–6], suggesting mainly secondary formation pathways. Beside the laboratory studies, evidences of imidazoles in atmospheric particles were found by using MS [7–9]. Nevertheless, knowledge about the concentration levels of imidazoles in atmospheric particles is still scarce, although a deeper understanding of ambient concentrations is crucial to assess their overall atmospheric relevance. To the best of the authors' knowledge, to date, only one study from our laboratory reported the concentrations of five different imidazoles (4-methylimidazole,

Article Related Abbreviations: ACN, acetonitrile; 1,2DMI, 1,2-dimethylimidazole; 1,3DMI, 1,3-dimethylimidazolium; 1EI, 1-ethylimidazole; 1PhI, 1-phenylimidazole; 2EI, 2-ethylimidazole; FA, formic acid; 2PhI, 2-phenylimidazole; 4IC, 4-imidazolecarboxaldehyde; 4M5IC, 4-methyl-5-imidazolecarboxaldehyde; 4MI, 4(5)-methylimidazole; BI, 1-butylimidazole; MeOH, methanol; OC, organic carbon; PA, peak area; PI, 1-propylimidazole.

1-ethylimidazole, 2-ethylimidazole, 1-butylimidazole, and 2-imidazolecarboxaldehyde) in aerosol particles collected at an urban background station in Germany and two rural background stations in China [10]. Imidazoles were present at a concentration ranging from 0.2 to 14 ng m^{-3}, with 4-methylimidazole being most abundant. However, the origin of those imidazoles is still unclear. As mentioned above, one pathway might be a secondary formation involving glyoxal or methylglyoxal. However, a connection of imidazoles with highly polluted areas or biomass burning was also found [7,8, 10]. For a better understanding of possible sources and effects of imidazoles more field studies are necessary. To accomplish this task methods enabling a fast analysis with minimal sample preparation to determine imidazoles in aerosol particles are favourable. In the literature, besides one method to determine imidazoles in atmospheric particles [10], other methods exist to determine low molecular weight imidazoles in matrices like food and beverages [11–15]. Imidazoles of higher molecular weight (>250 Da) are often used as pharmaceutics and have recently been found in environmental waters [16,17]. These methods often use SPE as a sample pretreatment, which is cost intensive, time consuming, and prone to low recoveries [18]. Reducing the sample preparation steps as much as possible would allow for the analysis of a large variety of imidazoles and also enables a higher throughput of samples.

LC coupled to high resolution mass spectrometry proofed to be efficient in the characterization of organics extracted from aerosol particles [19]. The high resolving power and accurate mass detection of high resolution mass spectrometry instruments facilitates the identification of unknown compounds [20].

The aim of the present study is to develop and optimize a reproducible method with minimal sample preparation efforts that allows for fast screening of imidazole compounds in aqueous aerosol particle extracts. The method was optimized using a standard mixture containing ten commercially available imidazoles. These imidazoles were selected based on the findings of a previous study [10]. Furthermore, an attempt was made to identify further imidazoles in aerosol particles by comparing peaks observed in the field samples with commercially available imidazole compounds. For separation and identification an UHPLC coupled to an Orbitrap-MS system was used.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Optima LC–MS grade acetonitrile (ACN) and methanol (MeOH) were obtained from Fisher Scientific (Loughborough, UK). Ammonium hydroxide solution (5 M) was purchased from Fluka Analytical (St. Lois, US) and ammonium formate (≥99%) and formic acid (FA, puriss. ≥ 98%) from Sigma-Aldrich (St. Louis, US). All imidazole standards were obtained from Sigma-Aldrich (St. Louis, US) in high purity (Supporting Information Table S1), except 1-propylimidazole (PI), which was obtained from HPC Standards (Cunnersdorf, Germany). Stock solutions of the imidazoles were prepared by dissolving a desired amount of the standard in ultrapure water. Individual stock solutions were stored at −20°C. For the method optimization and first application to real samples a stock solution of 1 M standard mixture containing ten imidazoles was prepared in ultrapure water. The choice of model compounds was made based on findings from previous studies [10,21] and on availability of commercial standards. Those imidazole compounds were: PI, 2-methylimidazole (2MI), 4(5)-methylimidazole (4MI), 2-imidazolcarboxaldehyde, 1,3-dimethylimidazolium (1,3DMI), 1-ethylimidazole (1EI), 2-ethylimidazole (2EI), 1-butylimidazole (BI), 1-phenylimidazole (1PhI), and 2-phenylimidazole (2PhI). Another ten standard imidazole compounds were used to identify further imidazoles in aerosol particles: 4-imidazolecarboxaldehyde (4IC), 1,2-dimethylimidazole (1,2DMI), 4-phenylimidazole, 4-methyl-5-imidazolecarboxaldehyde (4M5IC), 4-imidazolecarboxylic acid, 2-methylimidazole-4-carboxaldehyde, 1-(2-methylimidazol-4-yl)ethanone, 1,2-dimethylimidazole-5-carboxaldehyde, 1,5-dimethylimidazole-4-carboxaldehyde, and 4,5-dimethylimidazole-2-carboxaldehyde.

2.2 | UHPLC-orbitrap-MS conditions

The system for separation and detection of imidazoles consisted of a Vanquish Horizon UHPLC System (including a binary pump, split sampler, column compartment and a diode array detector) and a Q Exactive Plus mass spectrometer with a heated electrospray ionisation source and an orbitrap analyser (all components from Thermo Scientific Waltham, USA).

Separation of the standard mixture was tested on the following five columns: Acquity UPLC HSS T3, Cortecs UPLC C18+, Cortecs UPLC T3 (all from Waters, Milford, USA), Scherzo SM-C18 (Imtakt, Portland, USA) and Accucore Vanquish C18+ UHPLC (Thermo Scientific, Waltham, USA). For further column specifications please refer to Supporting Information Table S2.

Various gradient systems were tested for the different columns. Ultrapure water with and without additives was used as aqueous eluent (eluent A). Both MeOH and ACN were tested as organic eluents (eluent B). Possible additives were 1% v formic acid, ammonium formate (25 and 50 mM) and ammonium hydroxide (0.5 and 5 mM). The initial vol-% B was varied from 0 to 5%. Furthermore, the gradient time until 50% of eluent B was reached at 7 min was varied and included isocratic segments of different lengths at the beginning of the analysis. After 50% B was reached, the mobile phase

composition was hold for another 2 min. For each run, 5 to 20 μL of the 10 μM mixture were injected with a dispense speed of 5 μL/s. The columns were held at 30°C for all runs and the samples stored in the auto sampler at 10°C. The flow rate varied between 0.2 and 0.5 mL/min. An overview over all tested gradient systems is given in Supporting Information Table S3. Between each run, the column was equilibrated. Equilibration times depended on the column (see Supporting Information Table S2).

After comparison of the five columns, the column with best separation capability was chosen for further optimization. For optimization, the following parameters were varied: initial amount of eluent B, isocratic conditions at the beginning of the gradient, the slope of the gradient, flow rate, and injection volume. An overview of the different conditions tested is given in Supporting Information Table S4.

All compounds were ionised with HESI operated in positive-ion mode by applying a voltage of 3 kV and analysed in full scan mode (scan range: 50–750 *m/z*). Sheath gas flow was set to 50 units, auxiliary gas to 10 units, capillary temperature to 280°C, and the probe heater to 250°C. While these conditions have proved to work reasonably well for small molecules, it is noted that for ultimate sensitivity, optimization of these settings might be beneficial. Raw data was acquired with ThermoXcalibur software bundle (4.0.27.19) and quantitative analysis was conducted using the QuanBrowser included in Xcalibur. Chromatograms were generated using FreeStyle 1.1 SP1 (Version 1.1.175.0) software (all software from Thermo Scientific, Waltham, USA).

2.3 | Aerosol particle sampling and sample extraction

In total ten aerosol particle samples (five summer and five winter) were extracted with water and analysed for the target imidazole compounds. Atmospheric particulate matter with an aerodynamic diameter up to 10 μm (PM_{10}) were collected between July 2014 and October 2015 at the research station Melpitz, which is located in a rural area approximately 50 km northeast of Leipzig, Germany [22]. PM_{10} samples were collected on quartz fibre filters (MK 360, Munktell, Sweden) with a Digitel DHA-80 filter sampler (Riemer, Hausen, Germany). In order to reduce organic background contamination, the filters were heated for 24 h at 105°C prior to sampling. Each sample was collected with a flow rate of 0.5 m^3/min over a period of 24 h and stored at −20°C until analysis. For the extraction, 16 filter punches (78.5 mm^2 each) were transferred into a 5 mL syringe (Omnifix, Braun, Melsungen, Germany) and covered with 2.5 mL ultrapure water. The syringes were shaken for 2 h at 420 rpm and the extract was filtered through a syringe filter (0.2 μm GHP Acrodisc, Pall, NY, USA). Both syringes and syringe filters were pre-cleaned with LC–MS grade MeOH. The extracts were stored at −20°C until analysis. Given the high sensitivity of the Orbitrap MS analyser, the samples could be injected into the LC–MS system without further clean-up or enrichment, which avoids further time consuming and error prone sample preparation steps.

2.4 | Method validation

The instrumental LOD and LOQ was determined by analysing a dilution series of the standard mixture in water. The LOD was set to be at S/N = 3 and the LOQ at S/N = 10. These experiments were performed in full-scan mode that was applied to real samples as well. It is noted that for ultimate sensitivity, the selected-ion-monitoring mode might be beneficial.

External calibration was carried out by analysing two dilution series of standard mixtures in water at 13 concentration levels ranging from 1 nM to 10 μM to determine the linearity.

Intraday repeatability was determined by nine injections of the standard mixture at the same day and interday repeatability by analysing three replicates of standard mixture at three different days. The repeatability is given as RSD of the corresponding peak area (PA).

Matrix effects were determined by spiking five aqueous particle extracts with 10 μM standard solution to yield a final concentration of standard compounds in the spiked sample of 1 μM. The aerosol particle samples are characterised by different loadings of organic carbon (OC), thus comprising different sample matrices. The recovery of the PA of imidazole compound in the extract compared to their PA in water was calculated according to Eq. (1).

$$Recovery\,(\%) = 100 * \frac{PA_{\text{imidazole}}\,(spiked\ extract) - PA_{\text{imidazole}}\,(extract)}{PA_{\text{imidazole}}\,(H2O)} \quad (1)$$

2.5 | Quantification of imidazole compounds

Quantification of imidazole compounds in aqueous particle extracts was carried out by external calibration with a dilution series of standard compounds in ultrapure water. Extracted ion chromatograms of $[M+H]^+$ protonated molecules were used for quantification (see Table 1 for individual *m/z* traces). Concentrations for the calibration curve ranged from 2.5 nM to 10 μM. All aqueous aerosol particle extracts were injected twice. A quadratic regression with weighting 1/x or $1/x^2$ was chosen, instead of linear due to an observed non-linear behaviour. More details are given in Section 3.2.

For comparison, quantification for five samples was done using standard addition. Each aqueous extract was spiked at six to seven concentration levels, with final concentrations of standard compounds up to 6 μM in solution. Concentration levels were chosen based on the results of the quantification with external calibration. For each compound and sample

TABLE 1 Figures of merit for the final UHPLC-Orbitrap MS method

m/z $[M+H]^+$ [a]	Compound (abbreviation)	Chemical formula	RT (min)	Repeatability: Intraday (RSD PA, *n* = 9, in %)	Repeatability: Interday (RSD PA, *n* = 12, in %)	LOD (S/N > 3) (nM)	LOQ (S/N > 10) (nM)	Calibration range (nM)	R^2 of quadratic calibration function	Weighting function
83.060	2-Methylimidazole (2MI)	$C_4H_6N_2$	0.82	1.2	1.9	2.5	5	5–2500	0.9992	1/x
83.060	4(5)-Methylimidazole (4MI)	$C_4H_6N_2$	0.92	1.0	2.0	1	5	5–2500	0.9990	1/x
97.040	2-Imidazolcarboxaldehyde (IC)[b]	$C_4H_4N_2O$	0.68	1.0	5.3	<1	<1	2.5–500	0.9897	1/x
97.076	1,3-Dimethylimidazolium (1,3DMI)	$C_5H_9N_2$	0.82	0.9	2.2	25	50	50–10 000	0.9912	$1/x^2$
97.076	1-Ethylimidazole(1EI)	$C_5H_8N_2$	0.95	1.5	2.4	10	25	25–500	0.9885	$1/x^2$
97.076	2-Ethylimidazole(2EI)	$C_5H_8N_2$	1.05	0.9	2.0	2.5	10	10–1000	0.9988	1/x
111.092	1-Propylimidazole (PI)	$C_6H_{10}N_2$	1.60	1.1	2.8	10	25	25–10 000	0.9987	1/x
125.107	1-Butylimidazole (BI)	$C_7H_{12}N_2$	2.70	0.9	2.0	5	25	25 -10 000	0.9993	1/x
145.076	1-Phenylimidazole (1PhI)	$C_9H_8N_2$	3.01	0.8	1.8	5	25	25–10 000	0.9992	1/x
145.076	2-Phenylimidazole (2PhI)	$C_9H_8N_2$	3.51	1.3	0.8	1	5	5–5000	0.9989	1/x

[a] 1,3-Dimethylimidazolium is already present as a cation.
[b] Results based on hydrated form of 2-imidazolecarboxaldehyde.

three out of the seven concentration levels were chosen in a way that they were close to the expected concentration in the sample and showed linearity between the signal intensity and concentration.

3 | RESULTS AND DISCUSSION

3.1 | Method optimization

3.1.1 | Selection of the UHPLC column

Several columns with different chemistries were tested for imidazoles separation as described in Section 2.2. The best observed gradient conditions are summarized in Table 2 and the resulting chromatograms are displayed in Figure 1. In the following paragraphs, the results for the individual columns are briefly discussed.

The Scherzo SM-C18 column provides a mixed mode chemistry which allows for anionic and cationic exchange as well as hydrophobic interaction. The observed retention for all analytes in the present study was high and the analytes were distributed evenly over the gradient. For both MeOH and ACN eluents, all isomers were separated when 25 mM NH_4HCO_3 was used as a modifier in both water and organic eluent. However, peak shapes were rather bad. Several imidazoles showed strong fronting (e.g. imidazolecarboxaldehyde) or tailing (e.g. 1-butylimidzole).

The Accucore Vanquish C18+ column carries positive charges on the stationary phase, which can improve peak shapes of basic compounds. In the present study, under acidic conditions, the separation was insufficient for the ethylimidazole isomers. Peak shapes improved significantly under basic conditions (pH 10, 5 mM NH_4OH as additive in both eluents) for both MeOH and ACN. The phenylimidazole and methylimidazole isomers were separated completely. However, only two instead of three signals were detected for the ethylimidazole isomers. Since the pH tested was outside the columns specifications, which would likely hamper a long-term application of the system, and the ethylimidazole isomers were not separated effectively, this column was not considered further.

The Cortecs C18+ column has a similar separation mechanism as the Accucore Vanquish C18+ column. Here, for MeOH and ACN gradients, peak shapes were not significantly improved. The isomers were not separated, peak broadening occurred and with the ACN gradient two peaks were observed for 1-propylimidazol.

Comparison of MeOH and ACN gradients for the Cortecs T3 column showed that peak shapes were improved for acidic ACN gradients, especially if the flow rate was increased. However, no sufficient separation of isomers could be achieved with the ACN gradient. In contrast, the MeOH

TABLE 2 Overview of best conditions for chromatographic separation obtained for each column

Column	Eluent	Eluent additive	Gradient profile	Flow [mL/min]	Injection volume [μL]	Comment
Scherzo SM-C18	H_2O/MeOH	25 mM NH_4HCO_3	min 0: 3%B, min 7: 50%B, min 13: 50% B	0.3	5	Poor peak shapes
Accucore Vanquish C18+ UHPLC	H_2O/ACN	5 mM NH_4OH	min 0: 3%B, min 4: 3%B, min 7: 50%B, min 9: 50% B	0.3	5	Only two signals for ethylimidazole isomers
Cortecs UPLC T3	H_2O/MeOH	0.1% FA	min 0: 0%B, min 7: 50%B, min 9: 50%B	0.3	10	Peak broadening
Cortecs C18+	H_2O/MeOH	0.1% FA	min 0: 1%B, min 7: 50%B, min 9: 50%B	0.2	10	Isomers not separated and peak broadening
Acquity UPLC HSS T3	H_2O/MeOH	0.1% FA	min 0: 3% B, min 2: 3% B, min 7: 50%B, min 9: 50% B	0.3	5	Satisfactory peak shape, peak intensity, and separation of isomers

gradient, although showing broader peaks, resulted in a good separation.

An acidic MeOH gradient resulted in a satisfactory peak shape, peak intensity and separation of isomers on the Acquity HSS T3 column. Separation improved for low organic content at the beginning of the gradient. Compared to the Cortecs T3 column with solid-core particles, the Acquity HSS T3 column with fully-porous particles showed better or similar peak forms, separation and intensities for most imidazoles. Therefore, the Acquity HSS T3 column with a MeOH gradient was chosen for further optimization.

3.1.2 | Optimization of chromatographic conditions

The following parameters were selected for further optimization of the chromatographic conditions, that is, retention time, peak shape and resolution of peaks, with the Acquity HSS T3 column: the composition of the mobile phase, the gradient profile including an initial isocratic step, injection volume and flow rate. An overview of the optimization experiments is given in Supporting Information Table S4.

The initial amount of eluent B varied between 0 and 5%. The influence of the composition of the mobile phase on the separation efficiency of the isomers is shown in Supporting Information Figure S1. While peak shape for 1,3DMI improved with increased eluent B at the beginning, the methylimidazole isomers were less sufficiently separated under such conditions. An initial amount of 3% for eluent B was found to be the best compromise between peak shape and separation.

Variation of the gradient profile including an initial isocratic step at the beginning of the analysis has little to no influence on early eluting imidazoles, that is within the first 2 min. With decreasing isocratic time the retention time of BI, PI, and both phenylimidazoles also decreased. The peak shape of the phenylimidazole isomers remained the same, whereas the peak shape for PI and BI improved significantly. As an example, the influence of the isocratic time step on the peak shape of BI is given in Supporting Information Figure S2. Based on these results, the linear gradient of the eluent was directly started after injection without any isocratic time.

The influence of the gradient slope was tested by varying the time until 50% eluent B was reached (1 to 9 min, see Supporting Information Figure S2). The influence of the gradient slope was found to be small and 7 min was chosen as the best result.

Variation of the injection volume (3 to 20 μL) showed an expected trend of increasing peak intensities with increasing injection volume (see Supporting Information Figure S3). Nevertheless, an injection volume of 15 μL or higher resulted in an additional peak at m/z = 83.060. Further experiments revealed that this peak is independent from the concentration of methylimidazoles. An additional peak also occurred at m/z = 97.076 at high concentrations, but disappeared at atmospheric relevant concentrations. These additional peaks might be a result of distorting peaks due to high column loading. To avoid this, an injection volume of 10 μL was chosen for further experiments.

The last parameter optimized was the flow rate. Flow rates were altered between 0.3 and 0.5 mL/min (see Supporting Information Figure S4). Although high flow rates were found to improve peak shapes, compounds also eluted earlier and closer to the dead volume where highest matrix effects are to be expected. The optimum was found to be a flow rate of 0.4 mL/min.

Using the optimized method, all ten target imidazoles eluted within the first 5 min. To achieve a faster screening of field samples the final method was shortened, that is, instead of 7 min the linear gradient increased for 5 min with the same slope (35.5% eluent B reached after 5 min). Afterwards, eluent B was increased to 100% within 1 min and held for 2 min. Extracted ion chromatograms of the target imidazoles using the final method is shown in Figure 2.

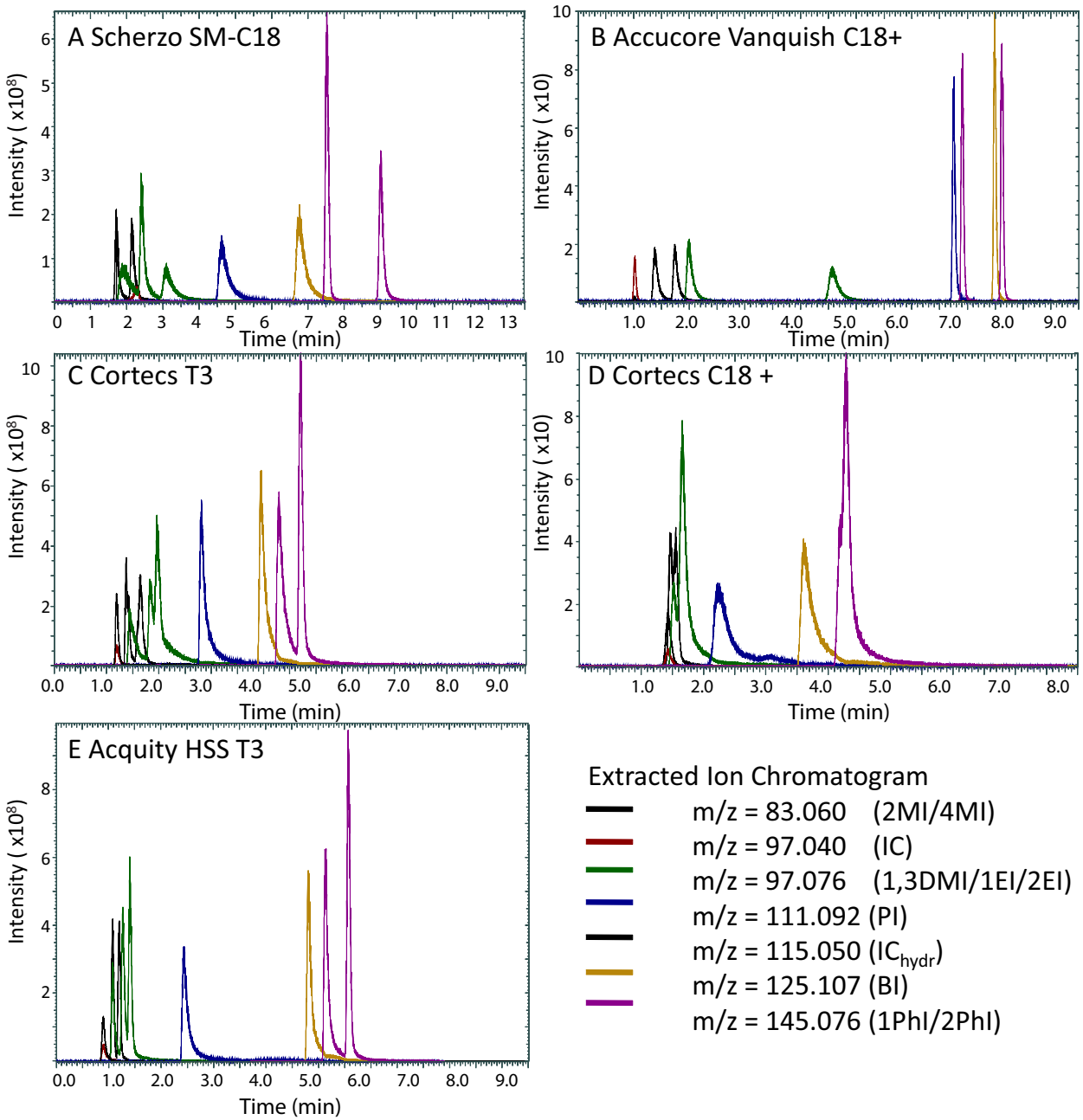

FIGURE 1 Extracted ion chromatograms of the standard imidazole mixture for each tested column. Shown are the best obtained results for each column as summarized in Table 2

3.2 | Method validation

The final method was validated by analysing standard mixtures in water. Figures of merit of the final method are summarized in Table 1.

It should be noted that imidazolecarboxaldehyde forms hydrated compounds and can be oxidized in solution over time, that is, hydrated imidazolecarboxaldehyde and imidazolecarboxylic acid are formed making an accurate quantification of imidazolecarboxaldehyde difficult. Imidazolecarboxaldehyde and its derivatives are further discussed in Section 3.3. The given values for LOD and LOQ for imidazolecarboxaldehyde are determined based on the initial concentration of imidazolecarboxaldehyde in the standard mixture.

The LOD were found to lie between 1 and 25 nM and the LOQ ranged from 1 to 50 nM. These LODs are generally in a similar range as the instrumental LODs of a previous published method, that used CE–TOF–MS to determine imidazoles in aerosol particles [10] (see Table 3). Compared to the previously published method the LODs of hydrated imidazolecarboxaldehyde and 4-methylimidazole are lower by a factor of 25 and 2.5, respectively. 2-Methylimidazole and 4-methylimidazole products are also often found in food

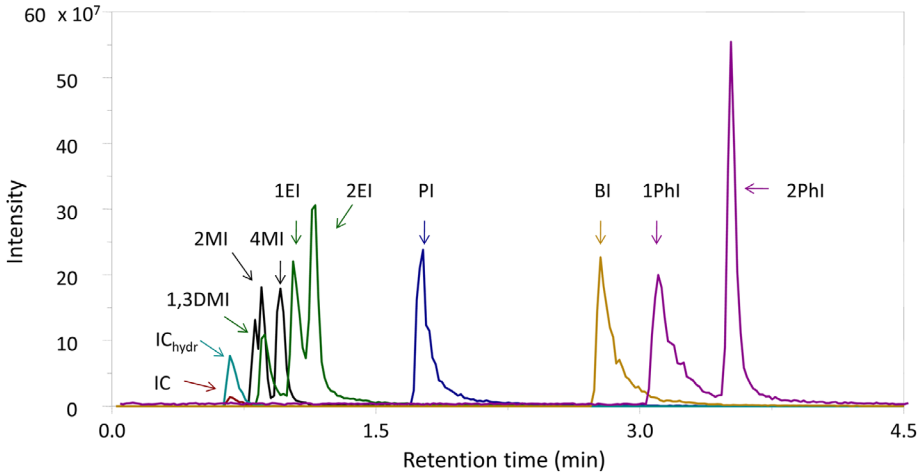

FIGURE 2 Extracted ion chromatograms of imidazole standard mixture with optimized parameters on the Acquity HSS T3 column: Eluent: H_2O/MeOH, Additive: 0.1% FA, gradient profile: min 0: 3% B, min 7: 50% B, flow 0.4 mL/min, injection volume 10 µL. See Table 1 for compound name abbreviations

TABLE 3 LOD determined in the present study in comparison with LODs determined in other studies. The literature overview includes aerosol particles and food and beverages as matrices. See Table 1 for compound name abbreviations

LOD (nM)								
Aqueous aerosol particle extract			**Food and beverages**					
2MI	2.5		60[b]		120 [b]	24[a]	10.4	
4MI	1	2.5 [a]	60[b]	1.2 [a]	60[b]	24[a]	9.9	4.9
1EI	10	2.5[a]						
2EI	2.5	2.5[a]						
IC_{hydr}	< 1	25 [a]						
BI	5	2.5 [a]						
Sample preparation	–	SPE	SPE	SPE	SPE	Dilution with eluent	Dilution with H_2O	SPE
Analysis method	UHPLC–MS	CE–TOF–MS	UHPLC–MS/MS	HPLC–MS	UHPLC–MS/MS	HPLC–MS/MS	UHPLC–MS/MS	HPLC–MS
Reference	This study	[10]	[11]	[12]	[23]	[24]	[30]	[31]

[a]LODs are instrumental LODs. Sample preparation is not included.
[b]LOQ.

or beverages [11,23,24] by using HPLC or UHPLC coupled with MS. A comparison of LODs determined in these studies is given in Table 3. In general, the method used in the present study performs better, that is, with lower LODs or LOQs, than the previously published methods.

A non-linear relationship of concentration to response was observed in the tested concentration range (see Supporting Information Figure S5 for example). Thus, a quadratic regression was chosen. To reduce the error especially for lower concentrations, a 1/x or $1/x^2$ weighting was applied. The applied weight function for each compound is given in Table 1.

Intraday repeatability was below 2% for all compounds and interday repeatability was below 3%, except for imidazolecarboxaldehyde (5.3%). 2-Imidazolecarboxaldehyde and its hydrated form are in equilibrium. As will be discussed in Section 3.3, 2-imidazolecarboxaldehyde oxidizes to form 2-imidazolecarboxylic acid over time. A shift in the equilibrium and the ongoing oxidization process leads to a lower interday repeatability.

3.3 | Imidazolecarboxaldehyde and its derivatives

In 2013, a laboratory study showed that 2-imidazolecarboxaldehyde is a potential photosensitiser, that is, it might trigger further reactions in the atmosphere [2]. This discovery led to an increasing interest in 2-imidazolecarboxaldehyde and a number of follow-up studies were published that seem to confirm its importance in the atmosphere [25–27] the knowledge about its ambient concentrations is still scarce. The method developed in the present study might therefore facilitate the quantification of imidazolecarboxaldehyde and

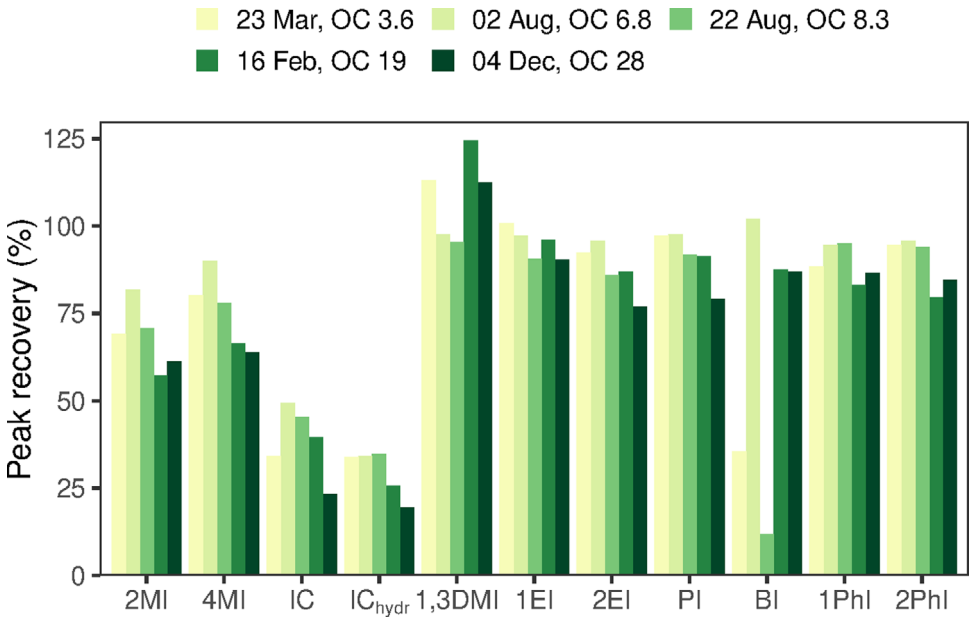

FIGURE 3 Matrix effects displayed as recovery of peak area in samples with different OC loading compared to the standard solution. Aqueous particle extracts were spiked with 1 μM of standard mixture. OC values are given in μg m^{-3}. See Table 1 for compound name abbreviations

its hydrated form to get new insights into the impact that these compounds might have in the atmosphere. A difficulty in the quantification is that 2-imidazolecarboxaldehyde is not stable in aqueous solutions [28]. The standard mixture used in the present study was prepared using a 2-imidazolecarboxaldehyde standard (purity 97%). Besides signals for imidazolecarboxaldehyde (*m/z* = 97.037), also signals for *m/z* = 113.035 and m/z = 115.050 were observed, where the signals at *m/z* = 97.037 and *m/z* = 115.050 co-eluted at 0.68 min. The signal at *m/z* = 113.035 appeared somewhat later at 0.85 min. The observed additional signals most likely represent hydrated 2-imidazolecarboxaldehyde (*m/z* = 115.050) and 2-imidazolecarboxylic acid (*m/z* = 113.035), respectively. Comparison with commercially available 2-imidazolecarboxylic acid confirmed the presence of this compound in the standard mixture. The signal intensity for hydrated 2-imidazolecarboxaldehyde is stronger than 2-imidazolecarboxaldehyde, whereas the signal intensity for 2-imidazolecarboxylic acid is comparatively weak.

Furthermore, the stability of 2-imidazolecarboxaldehyde was tested over time (see Supporting Information Figure S6). A comparison of the resulting PAs of the three derivatives shows a high variation between individual measurements. Moreover, one can see a trend of decreasing 2-imidazolecarboxaldehyde and increasing PA for 2-imidazolecarboxylic acid. For hydrated 2-imidazolecarboxaldehyde compared to non-hydrated 2-imidazolecarboxaldehyde there is no observable strong trend over time (Supporting Information Figure S6), which indicates an equilibrium between those two compounds in the standard solution. The increase in PA for 2-imidazolecarboxylic acid might be due to oxidation of small fractions of 2-imidazolecarboxaldehyde over time. This oxidation process might contribute to the observed high intraday repeatability for 2-imidazolecarboxaldehyde. This finding is of importance for analysing field samples. A long storage time of particle extracts might lead to a conversion from 2-imidazolecarboxaldehyde to 2-imidazolecarboxylic acid and therefore to biased concentrations. It is therefore recommended to freshly prepare standard solutions on a regular basis and to avoid long storage of aqueous filter extracts before analysis.

3.4 | Method application

3.4.1 | Matrix effects

It is known that the sample matrix might have significant influence on the peak intensity. Ion suppression or enhancement may occur due to co-eluting compounds [29]. To study possible matrix effects, five ambient particle samples with different OC loadings were spiked with the standard mixture. The peaks in the real sample were then compared to the peaks of the standard solution as described in Section 2.4. The results, presented in Figure 3, show that the sample matrix has only a small effect on the obtained PA for 1,3DMI, 1EI, 2EI, PI, 1PhI, and 2PhI, with average peak recoveries between 87 and 109%. Regarding 2MI and 4MI ion suppression can be observed, with an average recovery of the PA of 68 and 76%, respectively. The highest ion suppression was found for 2-imidazolecarboxaldehyde and hydrated 2-imidazolecarboxaldehyde with average peak area of 30

TABLE 4 Comparison of results for external calibration and standard addition. Values are given in ng m^{-3}. See Table 1 for compound name abbreviations

	23 Mar 2015		02 Aug 2015		22 Aug 2015		16 Feb 2015		04 Dec 2014	
	ExCal	SA	ExCal	SA	ExCal	SA	ExCal	SA	ExCal	SA
2MI	0.75	1.11	0.12	0.12	0.17	0.21	2.61	4.76	5.59	11.3
4MI	0.62	0.72	0.13	0.10	0.23	0.21	1.36	2.74	4.86	4.76
2EI	0.19	0.21	0.06	0.06	0.07	0.07	0.55	0.86	2.40	4.21
PI	0.05	0.08	<LOD	0.01[a]	<LOD	<LOD	0.06	0.08	0.14	0.12
BI	0.15	0.15	<LOD	<LOD	0.03[a]	0.04[a]	0.21	0.24	0.58	0.76
2PhI	0.04	0.05	<LOD	<LOD	0.01[a]	0.09[a]	0.03[a]	0.31[a]	0.10	0.11

[a]Values are below LOQ.
ExCal, external calibration; SA, standard addition.

and 38%, respectively. Thus, it appears that early eluting imidazoles are more affected by ion suppression. This might be caused by co-eluting very polar compounds that are more abundant in the beginning of the analysis. Moreover, a slight trend towards increasing ion suppression with increasing OC content was noticed for most compounds, except 1,3DMI and BI. From Figure 3 it is apparent that the PA recovery for BI is highly variable. The reason for this is still unclear and needs further investigation.

The RSD regarding PA recovery is below 15% all imidazoles, except for BI, 2-imidzolecarboxaldehyde and hydrated 2-imidzolecarboxaldehyde (RSD 60, 27 and 23%, respectively). The low variation of peak recovery with different samples could mean that the ion suppression is independent of the OC loading. The observed high peak recoveries and low variations across the samples suggest that an external calibration might be sufficient enough to quantify imidazoles in aqueous particle extracts. Therefore, imidazoles were quantified by, external calibration as described in Section 2.5. The results were compared to those of the standard addition (described in Section 2.5). Standard addition is used to compensate for the matrix effects in a sample. If the matrix effects on the quantification are low, the results of external calibration and standard addition method should be comparable. Out of the ten model imidazoles, seven could be identified and quantified. Concentration trends will be discussed further in Section 3.4.2. The comparison between external calibration and standard addition is shown in Table 4. Concentrations determined by standard addition and external calibration are comparable for PI and 2PhI, as could be expected from the high peak recoveries observed before. The observed concentrations are also comparable for BI, although the peak recovery experiment showed a high variability among samples with different loadings. An external calibration is also sufficient to quantify 2EI, although the differences between external calibration and standard addition seem to increase with higher OC loading of the sample. For the sample with the highest OC loading (OC 28 μg m^{-3}), the external calibration underestimates the concentration of 2EI by a factor of 1.5. An underestimation with higher OC loadings was also found for the two methylimidazoles, where the two quantification methods differed by a factor of 2.

Therefore, for an accurate quantification of the methylimidazoles and 2EI in samples with high OC loading, the standard addition method is favourable. Nevertheless, since standard addition is very labour intensive, for a faster screening of samples and determination of concentration trends quantification by external calibration can be an acceptable alternative for low to medium loaded samples. Thus, in the following section the results for quantification with external calibration are presented.

3.4.2 | Quantification of imidazoles in aerosol particles

The optimized method was applied to ten field samples collected at the rural background station Melpitz, Germany. The field samples included five summer and five winter samples. Determined concentration ranges and average concentration are presented in Figure 4. Concentrations for each individual sample are summarized in Supporting Information Table S5. It should be noted that for the identified compounds other isomers with the same *m/z* ratio might be present that elute within the same retention time.

Comparing the summer and winter samples, higher concentrations in winter are clearly observed for all imidazole compounds. Similar findings were reported by Teich et al. [10], where a connection between increased levels of imidazole compounds and polluted air masses was identified. Moreover, it was concluded that imidazole compounds may derive from biomass burning aerosols which corroborated findings from other studies that investigated biomass burning aerosols by MS [7,8]. In the present study, higher imidazole concentrations are accompanied by increased potassium concentrations in winter (data not shown), indicating a connection to biomass burning aerosol. However, the small number of samples analysed in the present study does not allow for a thorough investigation of emission sources, which

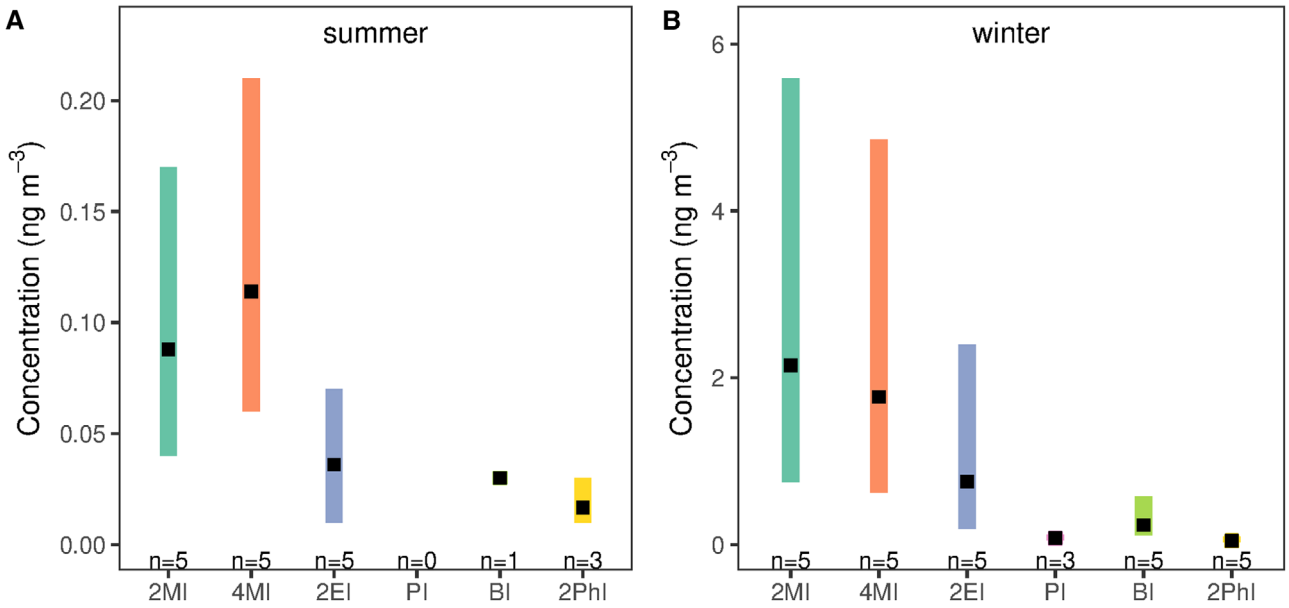

FIGURE 4 Ambient aerosol particle concentrations of imidazoles in (A) 5 summer and (B) 5 winter field samples collected at the rural background station Melpitz, Germany. The black square represents the mean value; the bar represents the concentration range. In the graph, all concentrations above the instrumental LOD are considered and the number of data points indicated with n. See Table 1 for compound name abbreviations

will therefore subject of a subsequent study with a larger data set.

In the present study, highest concentrations were observed for 2- and 4MI. This also corresponds to the findings from Teich et al. [10]. However, Teich et al. [10] were not able to separate the isomers 2- and 4MI and only reported the sum concentrations.

1EI, 2-imidazolecarboxaldehyde, and 1PhI could not be detected in the present study. Due to the limited number of aerosol particle samples, these imidazole compounds might simply be not present in the investigated samples. However, since a previous study were able to detect 1EI and 2-imidazolecarboxaldehyde in ambient aerosol particles [10], it is also possible that an enrichment step prior analysis is necessary to detect these compounds.

3.5 | Qualitative identification of further imidazoles

To identify further imidazoles in the samples, the aqueous extracts were spiked with a mixture of commercially available imidazole compounds (see Supporting Information Table S1).

Comparison of standard compounds with peaks in the sample (see Figure 5) revealed that six other imidazole compounds could be qualitatively identified, that is, 4IC, 1,2DMI, 4-imidazolecarboxylic acid, 4M5IC, 1-(2-methylimidazol-4-yl)ethanone, 4-phenylimidazole. Of those compounds 1,2DMI seems to be present in all samples. All identified compounds in the individual samples are summarized in Supporting Information Table S6.

Analysis of the individual aqueous standard solutions showed that, similar to 2-imidazolecarboxaldehyde, the hydrated and oxidized derivatives are present in solution for the other aldehyde compounds (compare Supporting Information Figure S7). This needs to be considered when attempts for quantification are made. As mentioned before, previous studies 2-imidazolecarboxaldehyde suggested as a photosensitiser, that is, it might trigger secondary reactions in the atmosphere, which could lead to aerosol particle growth [2, 25]. The identification of further imidazolecarboxaldehydes is interesting, since they might be involved in similar processes.

4 | CONCLUDING REMARKS

A new UHPLC-ESI-Orbitrap-MS method was developed to determine imidazole compounds in aqueous extracts of aerosol particles. Low LODs and a good repeatability were achieved and the method was successfully applied to real samples. The tested imidazole compounds eluted within the first 5 min. An external calibration proved to be sufficient for quantification of imidazole compounds in real samples with a low to medium loading of OC. Together with an injection of aqueous particle extract into the UHPLC system without further enrichment or clean-up steps, this method enables a fast screening of a large number of samples. The developed method was applied to ten real samples and six out of the ten test imidazoles were successfully quantified. Moreover, six other imidazoles were identified, among them 4IC and

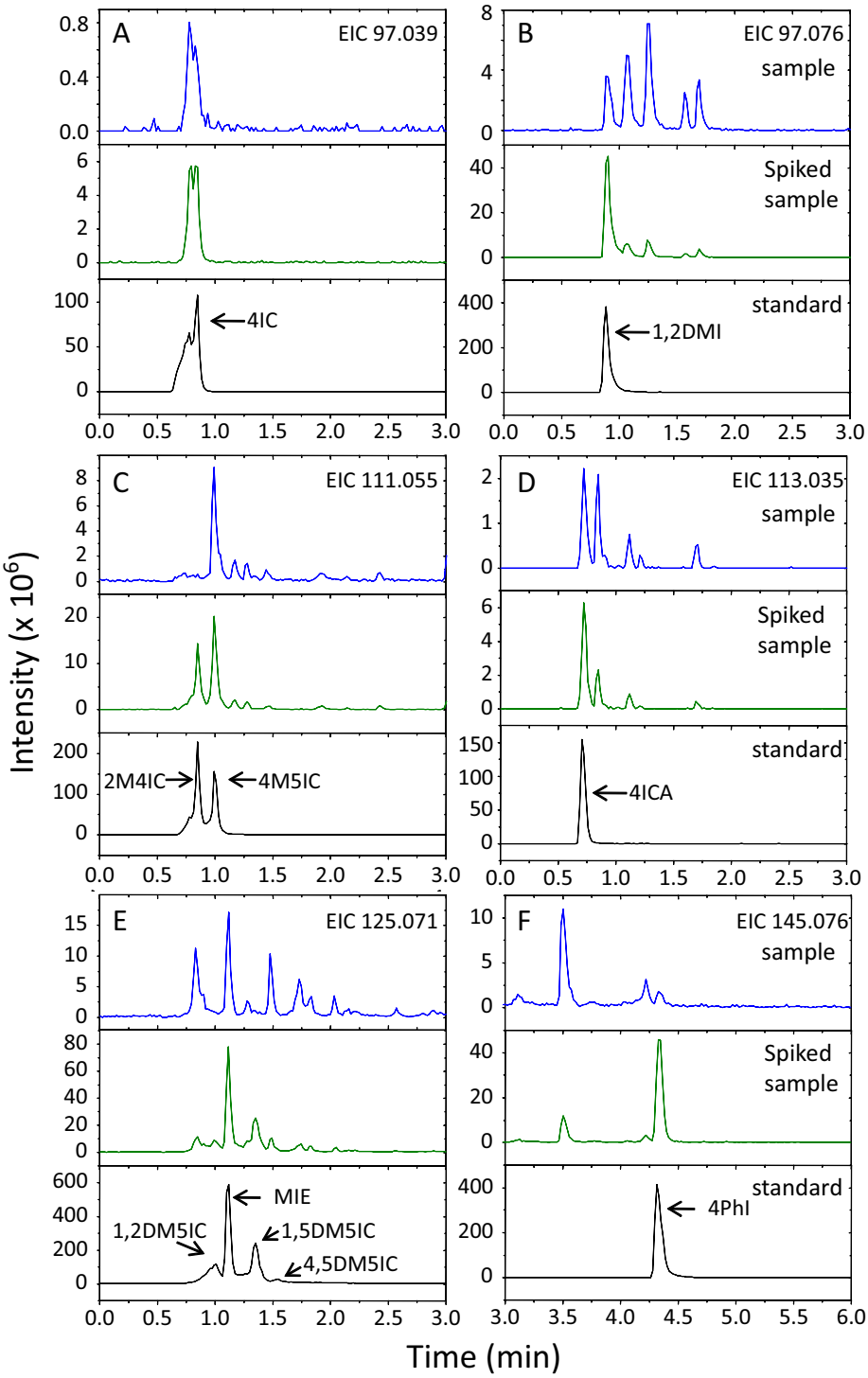

FIGURE 5 Example for peaks identified as imidazole compounds. The top panel in each graph shows the non-spiked sample, the middle one the spiked sample and the bottom the aqueous standard solution (1 μM). Extracted ion chromatograms were obtained from the following samples (A) 14 Aug 2015 (B) 02 Aug 2015 (C) 23 Mar 2015 (D) 14 Aug 2015 (E) 04 Dec 2014 (F) 04 Dec 2015. See Section 2.1 for abbreviations

4M5IC. Future studies may include field samples to investigate ambient concentrations and assess their possible impact or samples from chamber studies to investigate if these imidazoles are formed secondarily or are emitted primarily into the atmosphere.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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