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Analysis of nine nitrosamines relevant to occupational safety by ion mobility spectroscopy and preliminary gas chromatographic separation

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ABSTRACT

Nitrosamines have been identified as a probable human carcinogen and thus are of high concern in many manufacturing industries and various matrices (for example pharmaceutical, cosmetic and food products, workplace air or potable- and wastewater). This study aims to analyse nine nitrosamines relevant in the field of occupational safety using a gas chromatography-drift tube ion mobility spectrometry (GC-DT-IMS) system. To do this, single nitrosamine standards as well as a standard mix, each at 0.1 g/L, were introduced via liquid injection. A GC-DT-IMS method capable of separating the nitrosamine signals according to retention time (first dimension) and drift time (second dimension) in 10 min was developed. The system shows excellent selectivity as each nitrosamine gives two signals pertaining to monomer and dimer in the second dimension. For the first time, reduced ion mobility values for nitrosamines were determined, ranging from 1.18 to 2.03 $cm^2s^{-1}V^{-1}$. The high selectivity of the GC-DT-IMS method could provide a definite advantage for monitoring nitrosamines in different manufacturing industries and consumer products.

1. Introduction

N-Nitrosamines belong to a class of organic compounds that are characterized by the shared general structure R_2-NNO. A small subset of nitrosamine congeners, namely acyclic dialkylnitrosamines, are a potential potent human carcinogen, causing cancers of the colon, oesophagus and stomach and are highly toxic even at trace levels (nanogram per kilogram or nanogram per litre range). They have also been identified as critical environmental pollutants and health hazards due to their volatility and near-ubiquitous occurrence in many different matrices. Therefore, the need to monitor and investigate their presence across a range of industries is ever present within the framework of occupational and consumer safety [1,2].

Pathways of human exposure to the dangerous N-nitrosamines include inhalation, dermal contact as well as the consumption of contaminated food or pharmaceutical products. Their research has spurred the improvement of protection measures of workers and consumers against the exposure to nitrosamines [3], as well as the drive to develop more sensitive, accurate, robust and fast analysis methods to determine nitrosamine concentrations in various sample matrices. In particular, methods that do not solely focus on the lead substance N-nitrosodimethylamine (NDMA) are of high importance, as other nitrosamines like N-Nitrosodiethylamine (NDEA) pose an immediate health risk and need to be reliably analyzed as well [2]. This paper aims to lay the foundation for further research regarding nitrosamine analysis within the framework of occupational safety.

Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; DGUV, German Social Accident Insurance; DT-IMS, drift-tube ion mobility spectrometry; ECHA, European Chemicals Agency; FAIMS, field asymmetric ion mobility spectrometry; GC, gas chromatography; K0, reduced ion mobility; LOD, limit of detection; LOQ, limit of quantification; NDBA, N-Nitrosodi-n-butylamine; NDEA, N-Nitrosodiethylamine; ND-iso-PA, N-Nitrosodi-iso-propylamine; NDMA, N-Nitrosodimethylamine; ND-n-PA, N-Nitrosodi-n-propylamine; NIOSH, National Institute for Occupational Safety and Health; NMEA, N-Nitrosomethylethylamine; NMOR, N-Nitrosomorpholine; NO, nitrogen oxide; NPIP, N-Nitrosopiperidine; NPYR, N-Nitrosopyrrolidine; OSHA, Occupational Safety and Health Administration; PEROSH, Partnership for European Research in Occupational Safety and Health; TEA, thermal energy analyzer; TRGS, Technical Rules for Hazardous Substances (Technische Regel für Gefahrstoffe); UPLC-MS/MS, ultra-high performance liquid chromatography tandem mass spectrometry; UV, ultraviolet.

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In Germany, occupational exposure limit values, as well as protection and prevention measures to limit the worker's exposure to nitrosamines via inhalation or dermal contact, are defined in the German Federal Institute for Occupational Safety and Health document "Technical Rules for Hazardous Substances (Technische Regel für Gefahrstoffe, TRGS) 552″. Here, the tolerance and maximum acceptable concentrations for inhalative exposure of the lead substance NDMA are set at 0.75 and 0.075 μg/m^3, respectively. For want of toxicological data, these values have been transferred to other carcinogenic nitrosamines. At the same time, to limit the worker's exposure to a range of different, simultaneously occurring nitrosamines, the sum of all nitrosamines in workplace air must not exceed 0.75 μg/m^3. To meet these criteria, protection- and prevention measures, like wearing appropriate gloves, coats and goggles and exchanging precursor substances for benign alternatives, are to be set in place by employers [4].

In the US, OSHA (US Occupational Safety and Health Administration) has defined 9 nitrosamine compounds relevant to occupational safety in the rubber production industries: N-Nitroso-di-n-butylamine (NDBA), N-Nitroso-di-ethanolamine (NDELA), NDEA, NDMA, N-Nitroso-di-isopropylamine (ND-iso-PA), N-Nitroso-di-n-propylamine (ND-n-PA), N-Nitroso-morpholine (NMOR), N-Nitroso-piperidine (NPIP) and N-Nitroso-pyrrolidine (NPYR). For these substances, OSHA, the National Institute for Occupational Safety and Health (NIOSH), and the American Conference of Governmental Industrial Hygienists (ACGIH) propose no permissible exposure limits, however, label them a serious concern at any exposure level [5,6]. Ontario and Québec in Canada, as well as member states of the European Union, have not, as of now, set occupational exposure limits for nitrosamines, as reported by the European Chemicals Agency (ECHA) in June 2022 [7].

To date, several analytical methods are already implemented for nitrosamine analysis in different matrices. For the analysis of seven N-nitrosamines in workplace air, the German Social Accident Insurance (DGUV) has developed a method based on a coupling of a gas chromatography (GC) and a thermal energy analyzer (TEA) — a chemiluminescence detector specific for nitrogen oxides. This method has also been taken up by the Partnership for European Research in Occupational Safety and Health (PEROSH) and belongs to the repertoire of the OSHA analysis laboratories in the US. A defined volume of air is aspirated through a ThermoSorb-N cartridge with the help of a sampling pump to adsorb the volatile N-Nitrosamines that occur in gaseous form in the air at the workplace. In the TEA detector, nitrous oxide radicals are formed from the nitrosamines, which react with ozone and then emit specific electromagnetic radiation. Since the TEA detector is not able to provide substance identification, a second comparative measurement has to be performed, where the sample is irradiated with ultraviolet (UV) light over two to three hours. Due to their UV-lability, nitrosamine signals will then appear significantly less intensive [5,6,8–10].

The GC-TEA method, which has established itself as the state of the art for nitrosamine analysis in workplace air in Germany, offers only one dimension in terms of selectivity; the differing retention times of the gas chromatographic separation. The TEA detector only provides the recording of a current whenever a nitrogen oxide (NO) compound emits electromagnetic radiation after the reaction with ozone. That leaves the TEA detector itself cross sensitive to other NO-containing compounds, like nitramines, nitroaromatics, nitrate esters and nitroalkanes [8,9].

Other analytical methods have been implemented to detect nitrosamines in workplace air, for example LC-MS/MS (liquid chromatography coupled to tandem mass spectrometry). An application using adsorption cartridges for the enrichment of N-nitrosamines from air samples, followed by analysis by ultra-high performance (UP)-LC-MS/MS was investigated by Kim et al. Again, air samples were collected on ThermoSorb-N cartridges, eluted with dichloromethane and methanol, concentrated and then analyzed in approximately 10 min using an UPLC-MS/MS method [11]. While highly specific and sensitive, MS-based systems require a great deal of expert knowledge to operate, are costly and difficult to standardise.

Like mass spectrometric detection methods, chemiluminescence detection also requires sustaining a vacuum, which generates acquisition costs through additional vacuum pumps and a corresponding power consumption. In the case of the TEA, the latter is exacerbated by the step of pyrolysis at up to 850 °C. Moreover, it requires an additional oxygen supply for ozone generation. This makes the TEA detector, while a robust system, economically unfavorable and excludes the potential of developing a mobile on-site analysis system.

Ion mobility spectrometry (IMS) is a technique similar to a mass spectrometer, in that the analytes are first ionized and then selected or separated in an analyzer before they are detected. A distinction is made here between the Drift-Tube IMS analyzer (DT-IMS) and the field-asymmetric IMS (FAIMS), which can be compared with the mode of operation of a quadrupole MS. Thus, based on ion mobility, there is ion *separation* in the DT-IMS and ion *selection* in the FAIMS. In DT-IMS the difference in ion mobility is expressed by the time needed to cross the drift tube against a counter current of inert drift gas such as nitrogen, known as the drift time. The drift time depends on the mobility of an ion, which is determined by characteristic factors such as its size and mass. Thus, the drift time is a substance specific property that allows the generation of a database for later identification and offers a second dimension of selectivity next to the retention time on the GC column. Commercially available IMS instruments are generally economic in acquisition and maintenance, operate at atmospheric pressure and can be designed to be mobile systems for an on-site analytical approach [12,13].

To the best of the authors knowledge, no previous research has been carried out on the detection of nitrosamines using a GC coupled with an IMS detector, where the IMS is not only used for the separation, but also for the characterization of different nitrosamines [14]. In view of the shortcomings mentioned above, the goal of this work is to investigate the possibility of using a drift-tube ion mobility spectrometer to separate and detect nine different nitrosamines that are relevant to occupational safety.

2. Materials and methods

2.1. Reagents and solutions

Individual analytical standards of nine different nitrosamines (N-Nitrosodimethylamine (NDMA), N-Nitrosomethylethylamine (NMEA), N-Nitrosodiethylamine (NDEA), N-Nitrosodi-n-propylamine (ND-n-PA), N-Nitrosodi-iso-propylamine (ND-iso-PA), N-Nitrosomorpholine (NMOR), N-Nitrosopyrrolidine (NPYR), N-Nitrosopiperidine (NPIP), N-Nitrosodibutylamine (NDBA)) with a level of 0.1 g/L in methanol were purchased from Neochema GmbH (Bodenheim, Germany). A standard containing a mixture of the nine nitrosamines (each at 0.1 g/L in methanol) was also purchased from Neochema (Bodenheim, Germany). The purity was > 97% for all standards.

Standards were stored in a 1.5 mL amber glass vials until analysis. One μL of each respective standard was injected by the autosampler without further sample preparation. Single nitrosamine standards were used to confirm retention and drift times. Furthermore, the standard mix was employed to exclude any interactions between analytes during simultaneous ionization and detection with the ion mobility spectrometer. A molecular sieve (3 Å) was purchased from Carl Roth Gmbh & Co. Kg (Karlsruhe, Germany).

2.2. Apparatus

The drift tube ion mobility spectrometer based on tritium ionization was purchased from Gesellschaft für analytische Sensorsysteme (G.A.S., Dortmund, Germany) and coupled to a Shimadzu Nexis GC-2030 benchtop gas chromatograph (Duisburg, Germany). The GC system was equipped with a Chronect Robotic RTC PAL autosampler from CTC Analytics AG (Zwingen, Switzerland). The IMS voltages used for the

analysis of nitrosamines were set at 5 kV drift voltage, 50 mV blocking voltage and 2500 mV injection voltage. The drift tube was operated at 75 °C and purged/flushed with a nitrogen 5.0 flow of 150 mL/min after passing through a molecular sieve. The injection pulse width and the repetition rate were set to 150 µs and 21 ms, respectively. Detection was carried out in the positive mode. Signal averaging was set to 12.

Chromatographic separation was carried out on a Restek® Rtx-200 trifluorpropyl-methylpolysiloxane (100%) column (30 m x 0.25 mm x 0.25 µm). The column oven was heated according to programme 1 as shown in Table 1. The column flow of the carrier gas (helium 5.0) was set to 3 mL/min. Two additional GC temperature programmes at the same carrier gas flow were developed to assess the effect of different start- and end temperatures as well as different heating rates on the chromatographic separation of the nine nitrosamines (Table 1).

Nitrosamine standards were injected into the injection port at 250 °C with a split ratio of 1:200, which corresponds to an absolute mass of 0.5 ng of analyte on the chromatographic column.

Data evaluation was performed using the G.A.S. VOCal analysis software version 0.1.3.

3. Results and discussion

This work focuses on the analysis of N-nitrosamines via an analysis method based on gas chromatography coupled to a drift tube ion mobility spectrometer after a liquid injection of nitrosamine standards in methanol. The method is intended to form the basis to different sample preparation techniques in order to determine N-nitrosamine concentrations in different matrices relevant to occupational and consumer safety.

To evaluate the results, signal markers on the first and second dimension of the ion mobility spectrum (drift time and retention time) were taken into account. An overload of the detector due to the use of a liquid injection method was not observed.

3.1. Ionisation of nitrosamines

In ion mobility spectra, the analyte signals are located according to their retention time in the first dimension (y-axis) and their drift time in the second dimension (x-axis), while their intensity is indicated by color (heat chart). Previous analyses on an GC-IMS system equipped with a field asymmetric IMS (FAIMS) have shown experimentally that nitrosamine signals do not occur when operating in the negative ion mode (results not shown). Measurements were thus carried out in the positive IMS mode. The positive reactant ions are produced from nitrogen and water by the tritium ion source and appear as a continuous signal at a drift time of approximately 7.35 ms. In addition, each nitrosamine produces two signals in the IMS spectrum, pertaining to monomer and dimer that are formed during ionization (Fig. 1). The nitrosamine monomers and dimers have different velocities in the drift chamber according to their charge, mass and the collision cross-section, that is, their mobility [15], causing the differing drift times.

Two additional signals could be identified at the retention times 333.7 s (NMOR) and 361.3 s (NPIP), which may point to the formation of trimers or inter-analyte proton bound dimers.

Depending on temperature and humidity of the IMS system, reactant ions take the form of hydrated protons clustered with differing numbers of water molecules (n) and function as the proton/charge donors to ionise the analyte molecules [16]. This is supported by the nitrosamines' substantial dipole moments, which facilitate a rapid proton transfer, and which give reason to assume high proton affinities of the nitrosamines, although few have been published in the literature [17].

The interaction of protonated water clusters with the nitrosamines could follow different mechanisms. As one pathway, the proton can be transferred to the nitrosamine molecule while the remaining water moieties are released. Nitrosamines show a zwitterionic structure, being most basic at oxygen. While a protonated nitrosamine should exist in both N- and O-protonated forms after reacting with the water clusters, the O-protonated (Fig. 2a) species is the only stable form detected to date. It is more stable than the N-protonated (Fig. 2b) nitrosamine by 16 kcal/mol [18]. The O- and N-protonated isomers of a dialkylnitrosamine are shown in Fig. 2.

As a parallel pathway, the reactant ion water cluster may be remodeled during ionization, through a substitution of water molecules with the specific nitrosamine [19]

$$NNO + H^+(H_2O)_n \rightleftharpoons NNOH^+(H_2O)_{n-x} + xH_2O \quad (1)$$

where NNO is the molecule of a nitrosamine compound, $H^+(H_2O)_n$ is the reactant ion and $NNOH^+(H_2O)_{n-x}$ is the product ion of the nitrosamine compound.

Ions with different levels of hydration, i.e., with different n's, exist in equilibrium with each other and acquire and lose water molecules as they move along the drift tube [20]. Depending on the concentration of nitrosamines and on the IMS system (temperature and moisture level), a second nitrosamine analyte molecule can substitute another water moiety from the reactant ion cluster to form the corresponding proton bound dimer. [20,21]

$$NNOH^+(H_2O)_n + NNO \rightleftharpoons (NNO)_2H^+(H_2O)_{n-1} + H_2O \quad (2)$$

where $(NNO)_2H^+$ is the protonated dimer molecule. Again, as with the monomers, the dimer signal obtained in the IMS spectrum is composed of proton bound dimers clustered with differing numbers of water molecules (n), which exchange among each other within the confines of a set equilibrium [20].

3.2. Selectivity of the GC-DT-IMS method for nitrosamines

Prior to the separation of analytes according to their respective drift times via the IMS drift tube, all nine nitrosamines were separated on a gas chromatographic column. Their retention time increases with increasing molecular mass and boiling point, resulting in the following elution order: NDMA, NMEA, NDEA, ND-isoPA, ND-n-PA, NMOR, NPYR, NPIP and lastly NDBA. Retention times are shown on the y-axis of the resulting IMS spectrum, as shown in Fig. 1. For all three GC temperature programmes, the analytes are baseline separated on the Restek® Rtx-200 column. This confirms earlier work in which we compared the separation of the nine nitrosamines on three different columns of different packing composition and polarities, where the medium polar trifluorpropyl-methylpolysiloxane column was the only one to achieve the disconnected elution of the substances NMOR, ND-n-PA and NPYR (results not shown). Table 2 provides an overview of the retention times using the Rtx-200 column. With regard to the GC retention time, no analyte shows a greater relative deviation from the mean value than 0.6%, with the exception of the NMEA monomer and dimer (relative standard deviation 2.2%). The interval between signals on the y-axis of the IMS spectrum is no smaller than 6.4 s (NPYR and NPIP), which also demonstrates the adequate separation performance of the column installed in the GC-DT-IMS system. The measured values used for this assessment were from the fastest of the three GC temperature programmes tested (temperature programme 1 in Table 1). Thus, all nitrosamines can be adequately separated from each other according to their retention time with a total GC-IMS running time of approx. 600 s

Table 1
Overview of the two additional GC temperature programmes.

Parameter	Programme 1	Programme 2	Programme 3
Start temperature	80 °C (hold 1 min)	60 °C (hold 1 min)	60 °C (hold 2 min)
Ramp	16 °C/min	2 °C/min	7 °C/min
End temperature	210 °C (hold 1 min)	130 °C (hold 1 min)	180 °C (hold 5 min)

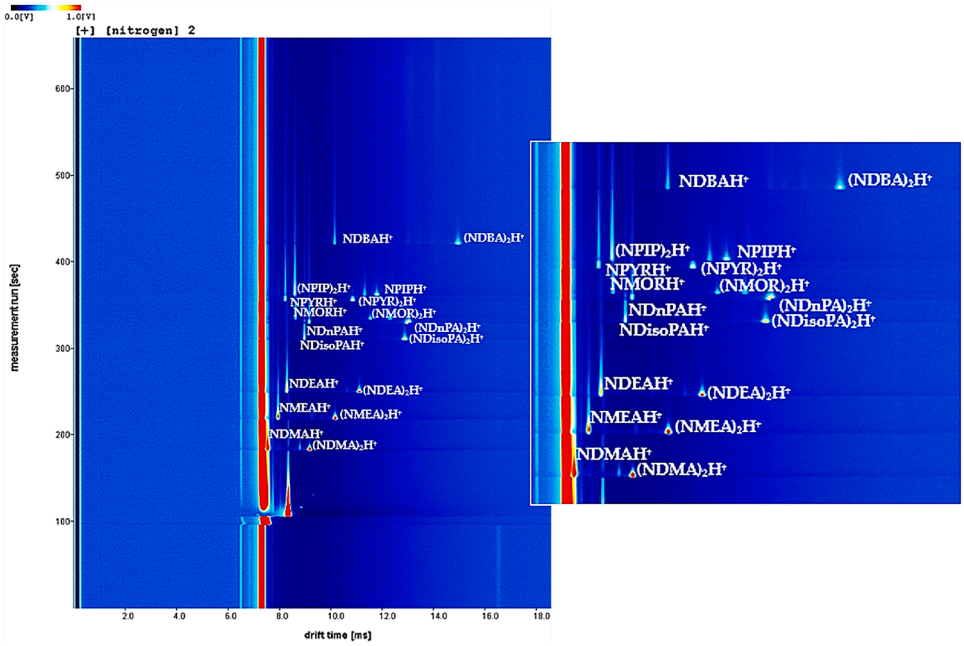

Fig. 1. IMS spectrum (heat chart) of a nine nitrosamine mix standard (0.1 g/L each), analyzed on a GC-DT-IMS system equipped with a Restek® Rxi-200 column (30 m x 0.25 mm x 0.25 μm) according to programme 1 in Table 1. The excerpt on the right is a magnification of the complete spectrum on the left.

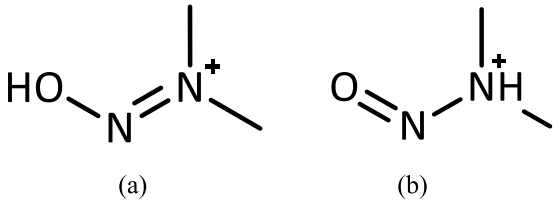

Fig. 2. Proposed structure for the (a) O- and (b) N-protonated nitrosamine after ionization by water cluster.

(10 min).

As the formation of both monomers and dimers gives signals at clearly distinct drift times for the same GC retention time, DT-IMS offers excellent selectivity in the second dimension, where all nine nitrosamines can be differentiated acutely. Fig. 1 shows the DT-IMS spectrum of a mixture of nine nitrosamines, where the monomer and dimer signals are marked. Individual drift times are listed in Table 2. The result shows a standard deviation of below 0.1% for all analytes except for the NMEA monomer, which deviated by 0.17% from the mean value. The drift tube IMS analyses were thus deemed to have high repeatability.

The drift times show sufficiently different values both for an individual nitrosamine and between the nitrosamines themselves which results in the advantage to use the mobility as a selective identification feature.

The separation of nitrosamine signals with respect to the combination of the two dimensions drift time and retention time can be seen in Fig. 3. Each nitrosamine is characterized by three markers: one each for the drift times of the analyte's monomer and dimer, and one for the retention time at which the monomer and dimer signals appear in the spectrum. This allows the creation of a matrix, in which the analytes position represents its difference with regard to the other analytes. Here, each data point represents a nitrosamine with all its selectivity traits regarding the IMS spectrum and no data points overlapping in either dimension.

Although the GC-IMS method primarily facilitates targeted analysis, due to the great selectivity and with the help of an IMS substance data base, it may be possible to use the technique for screening purposes.

Table 2
Retention times, absolute drift times and calculated reduced ion mobilities of nine nitrosamines (monomers and dimers). Data are mean values based on triplicate measurements on a GC-DT-IMS analysis system. Deviations are given as absolute values.

Analyte	Retention time t_R (s)	Drift time absolute t_D (ms)	Reduced ion mobility K_0 ($cm^2s^{-1}V^{-1}$)
NDMA_Dimer	182.4 ± 1.1	9.19 ± 4.50×10^{-3}	1.67 ± 8.20×10^{-4}
NDMA_Monomer	182.4 ± 1.1	7.57 ± 4.91×10^{-3}	2.03 ± 13.2 × 10^{-4}
NMEA_Dimer	215.0 ± 4.7	10.2 ± 9.47×10^{-3}	1.51 ± 14.0 × 10^{-4}
NMEA_Monomer	215.1 ± 4.6	7.98 ± 13.8 × 10^{-3}	1.93 ± 33.4 × 10^{-4}
NDEA_Dimer	248.7 ± 0.2	11.1 ± 8.59×10^{-3}	1.38 ± 10.7 × 10^{-4}
NDEA_Monomer	249.0 ± 0.2	8.30 ± 6.73×10^{-3}	1.85 ± 15.0 × 10^{-4}
NdnPA_Dimer	328.8 ± 0.0	13.0 ± 2.58×10^{-3}	1.18 ± 2.34×10^{-4}
NdnPA_Monomer	329.1 ± 0.0	9.18 ± 2.97×10^{-3}	1.68 ± 5.42×10^{-4}
NdisoPA_Dimer	310.0 ± 0.0	12.9 ± 5.90×10^{-3}	1.19 ± 5.47×10^{-4}
NdisoPA_Monomer	310.1 ± 0.2	8.98 ± 4.25×10^{-3}	1.71 ± 8.11×10^{-4}
NMOR_Dimer	333.7 ± 0.0	11.5 ± 4.73×10^{-3}	1.33 ± 5.46×10^{-4}
NMOR_Monomer	333.8 ± 0.2	8.63 ± 1.91×10^{-3}	1.78 ± 3.95×10^{-4}
NPYR_Dimer	354.8 ± 0.2	10.8 ± 3.26×10^{-3}	1.42 ± 4.26×10^{-4}
NPYR_Monomer	355.0 ± 0.0	8.22 ± 2.48×10^{-3}	1.87 ± 5.65×10^{-4}
NPIP_Dimer	361.3 ± 0.0	11.8 ± 7.33×10^{-3}	1.31 ± 8.13×10^{-4}
NPIP_Monomer	361.3 ± 0.0	8.59 ± 4.40×10^{-3}	1.79 ± 9.17×10^{-4}
NDBA_Dimer	420.9 ± 0.2	14.9 ± 4.39×10^{-3}	1.03 ± 3.03×10^{-4}
NDBA_Monomer	421.3 ± 0.0	10.1 ± 5.46×10^{-3}	1.52 ± 8.16×10^{-4}

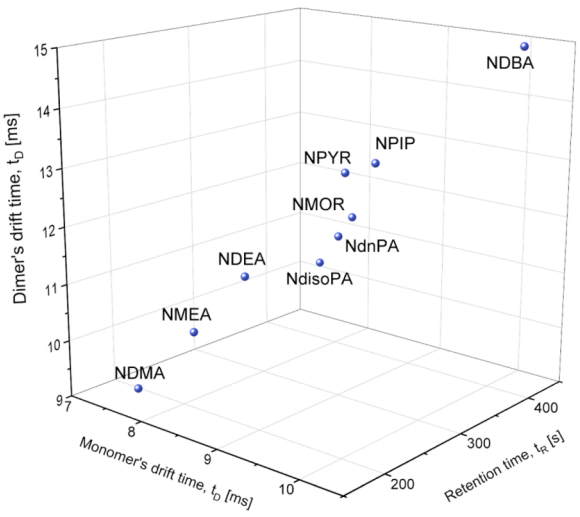

Fig. 3. Visual representation of the position of nitrosamine signals within the scope/frame of retention time, monomer and dimer drift time.

3.3. Determination of reduced ion mobilities

Additionally, reduced ion mobilities (K_0) were calculated according to Eq. (3). The reduced ion mobility value is independent of the used IMS system as the value is normalized to the length of the drift tube, the electric field, pressure and temperature [22]. Hence, it enables a better comparison of analyte signals between analysis systems. [23]

$$K_0 = \frac{d * T_0 * P}{E * T * P_0 * t_D} \quad (3)$$

With

K_0=reduced ion mobility [$cm^2*V^{-1}*s^{-1}$]
d = length of drift tube = 9.8 cm
T_0, P_0 = temperature and pressure under standard conditions =273.15 K and 1013.25 mbar)
P = atmospheric pressure at the time of measurement = 1013.25 mbar
E = electric field (5 kV)
t_D = drift time
T = drift tube temperature = 348.15 K

The reduced ion mobility was calculated for both the monomer and dimer signals of nine nitrosamines, using the absolute drift time values. Measurements and calculations were done in triplicate and give a relative standard deviation of K_0 below 0.1% for all analytes, except for NMEA monomer, which deviated by 0.17% from the mean value (Table 2). The reduced ion mobility, as well as the drift time, represents a selectivity marker as it quantifies the difference/distance of analyte signals in the IMS spectrum, albeit as a system independent value.

3.4. Quantification considerations

While the generation of two ion species (monomer and dimer) with specific drift time values per analyte results in excellent selectivity in the second dimension, it also leads to complications regarding the approach to quantification of the nitrosamines using the GC-DT-IMS system. The ratio of monomers to dimers that formed during ionization is dependent on the concentration of analytes being determined and on the supply of reactant ions, since the ionization process is a competitive one. Therefore, it is essential to consider both species when attempting a quantitative evaluation, especially when performing an external calibration.

Usually, the integration of signals over the retention time is used in order to employ peak areas for the evaluation. This approach requires the software to allow the complete deduction of the reactant ion signal to obtain an acceptable signal to noise ratio. The "VOCal" data analysis software, G.A.S., Dortmund, Germany, used for any quantitative data evaluation, does not provide a difference integration feature, but permits the definition of specific areas in the spectrum, for which either the signal intensities or signal volumes can be calculated. In this work, the peak volumes of monomers and dimers were summed up to represent a nitrosamine signal in its entirety.

Employing the signal volume instead of the intensities is bound to provide the highest sensitivities and the greatest linear range.

3.5. Limits of detection and quantification

To determine limits of detection and quantification (LOD and LOQ, respectively) of the instrument for the nine nitrosamines in a methanol eluent, an approach following the German DIN EN ISO 22065 and DIN 32645 was chosen. Ten repeat measurements of methanol spiked with nitrosamines at the estimated limit of quantification range were analyzed and the standard deviation was determined. The plotting of the signal volumes with each measurement is shown in Fig. 4 (left).

Independently recorded calibration measurements of five levels between 5 and 50 µg/mL provided the slope of the calibration curve for each nitrosamine. An exemplary calibration curve can be seen in Fig. 4 (right).

LOD and LOQ calculations were performed using the following equations:

$$x_{LOD} = \frac{s_y}{m} * t * \sqrt{\frac{1}{\tilde{N}} + \frac{1}{N}} \quad (4)$$

$$x_{LOQ} \approx 3 * x_{LOD} \quad (5)$$

With

x_{LOD} / x_{LOQ} = Limit of detection / limit of quantification (µg/mL)
s_y = standard deviation of the repeat measurements (a.u.)
m = slope of the calibration curve (a.u./µg/mL)
t = t-value for a one-tailed test at a confidence level of 95%
N = number of levels
$\tilde{N}$ = number of repeat measurements

Eq. (4) illustrates, that the limits of detection and quantification is positively correlated with the precision of the method. Since the measurements resulted in relative standard deviations between 1.25 and 3.68% for all nitrosamines, the precision of the method was considered to be sufficient. Table 3 shows the calculated LODs and LOQs for all nine nitrosamines.

The limits of detection and quantification of the GC-DT-IMS instrument for the analyzed nitrosamine range from 0.24–1.94 µg/mL and from 0.73–5.81 µg/mL, respectively, which corresponds to absolute masses of 1.22–9.67 pg and 3.66–29.0 pg on the column.

4. Conclusion

The main objective of this work was to develop a GC-DT-IMS method to selectively analyse nine different nitrosamines in a short amount of time. The current method for the analysis of nitrosamines in workplace air in Germany and the US are based on a coupling between GC and a thermal energy analyzer. However, the TEA detector does not allow for the generation of signals in the second dimension in addition to the gas chromatographic separation. This limits the GC-TEA in selectively identifying the nitrosamine analytes. Furthermore, employing a TEA for nitrosamine analysis is prone to cross-sensitivity, since the detector is specific for all nitroso compounds. Thus, as its main advantage, the system provides high selectivity for the analytes compared to the current

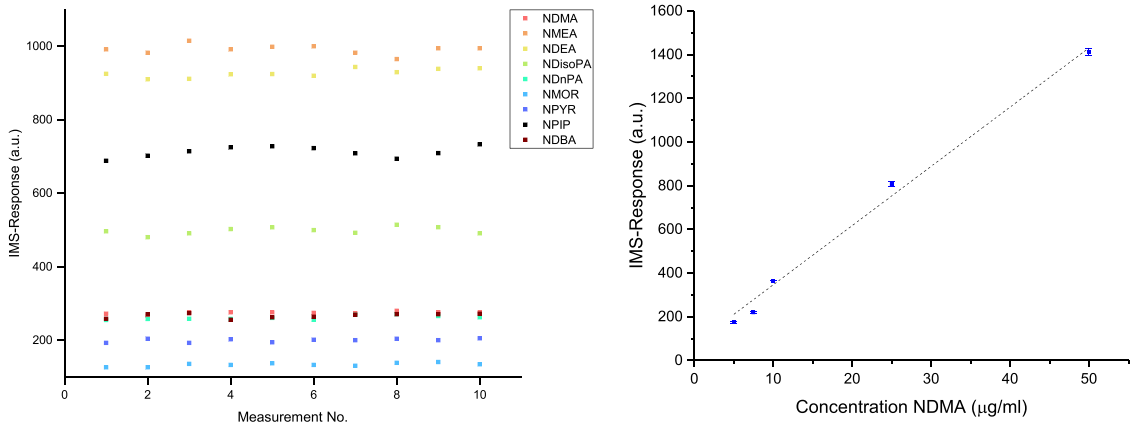

Fig. 4. (left) IMS-response (summed up monomer and dimer signal volumes for each nitrosamine) for ten repeat measurements. (right) Exemplary calibration curve (NDMA). Two repeat measurements were recorded for each level. The regression coefficient R^2 is 0.9925 and the regression equation is $y = 27.678x + 55.866$. Analysis conditions for both measurements are listed in Section 2.2 Apparatus.

Table 3
Calculated limits of detection and quantification for all nine nitrosamines, following the German DIN EN ISO 22065 and DIN 32645.

	LOD (µg/mL)	LOQ (µg/mL)
NDMA	0,24	0,73
NMEA	0,39	1,16
NDEA	0,38	1,13
ND-isoPA	0,86	2,59
ND-n-PA	1,24	3,72
NMOR	1,94	5,81
NPYR	0,83	2,50
NPIP	0,49	1,47
NDBA	0,82	2,46

state of the art. This work shows the possibility of GC-DT-IMS as a promising technique for nitrosamines analysis.

The simultaneous, baseline separated analysis of nine different nitrosamines is possible in approximately 10 min using a medium polar trifluorpropyl-methylpolysiloxane column after a liquid injection of 1 µL of standard in methanol. Furthermore, an overload of the detector as a result of the liquid injection was not observed, which leads to the conclusion that the drift tube IMS detector is well suited to be utilized for nitrosamine analysis in combination with standard liquid injection (albeit with a high split ratio) and a preliminary gas chromatographic separation.

To assess the sensitivity of the system toward nitrosamines, instrument detection and quantification limits were determined following the method described in the German DIN EN ISO 22065 and DIN 32645. The calculation showed a high precision of the method and LODs and LOQs in the 0.24 - 1.94 µg/mL and 0.73 - 5.81 µg/mL range, respectively, were achieved, depending on the nitrosamine of interest.

Further research on the sensitivity of the GC-DT-IMS system for nitrosamine analysis in real samples relevant to occupational and consumer safety, as well as the development of different sample preparation methods based on the findings of this work are currently in progress.

Author contributions

J.H. and Y.S. carried out the experiment and evaluated the data together with J.B. J.B. provided the visualization of data and molecule structures. J.H. wrote the manuscript together with J.B. and with input from S.S., T.M. and Y.S. S.S. and T.M. consulted on the formal aspects of the manuscript. M.W. supervised the work. U.T. reviewed and edited the manuscript. All authors have contributed substantially to the work reported, read, and agreed to the published version of the manuscript. All authors provided critical feedback and helped to shape the final version of the manuscript.

Availability of data

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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CRediT authorship contribution statement

Jana Hinz: Conceptualization, Investigation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Tessema F. Mekonnen:** Writing – review & editing. **Jonas Bergrath:** Writing – review & editing, Visualization. **Savanna Sewell:** Writing – review & editing. **Yannic Schneck:** Formal analysis, Writing – review & editing. **Michaela Wirtz:** Conceptualization, Supervision, Funding acquisition. **Ursula Telgheder:** Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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