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Determination of carbonyl compounds in waters using triazine-based hydrazine reagents and liquid chromatography

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Triazine-based hydrazine reagents have been developed and applied for the determination of aldehydes in waters. *N*-Methyl-4-*N*′,*N*′-dimethylamino-6-(4′-methoxy-1′-naphthyl)-1,3,5-triazine-2-hydrazine (MDMNTH) and 4-*N*,*N*-dimethylamino-6-(4′-methoxy-1′-naphthyl)-1,3,5-triazine-2-hydrazine (DMNTH) react with these carbonyl compounds with very high yields under the formation of the respective hydrazones, which are separated by reversed-phase liquid chromatography with binary gradients of acetonitrile and water. Organic modifiers are added to obtain improved peak shapes. UV/vis as well as fluorescence detection has been applied successfully to quantify the hydrazones. The derivatization of aqueous solutions of the aldehydes has been optimized. Limits of detection are in the range between 0.1 μM and 1.0 μM depending on the analyte and the reagent used for derivatization. Although calibration *via* external standards is possible for higher analyte concentrations, best results are obtained by calibration *via* the derivatization reaction. Compared to the US EPA method 8315A, the newly developed method is characterized by very easy sample preparation, lower limits of detection without enrichment and excellent reproducibility with an average RSD of 1.5% with DMNTH as reagent and 3.5% with MDMNTH as reagent for a formaldehyde concentration of 1.0×10^{-5} mol L^{-1}. Aldehyde-spiked mineral water samples were analyzed successfully with the new method.

Introduction

Carbonyl compounds, especially short-chain aldehydes, are important organic pollutants in gaseous as well as in liquid samples. As aldehydes are somewhat unstable compounds, most methods for their determination are based on chromatographic techniques with pre-column derivatization of the analytes. Typically, the reagent and its derivatives are separated by reversed-phase liquid chromatography. Hydrazine reagents are a very popular group of derivatizing agents for carbonyl compounds, with 2,4-dinitrophenylhydrazine (DNPH) being most widely used.[1–8] Detection is performed using UV/vis spectroscopy in most cases, but mass spectrometry with atmospheric pressure chemical ionization has been suggested as a tool to improve selectivity.[9–11]

A quantitative reaction of the carbonyls with the reagent is desirable, as it allows external calibration and avoids a more laborious calibration *via* the derivatization reaction with calibration solutions of known content. In gas phase analysis, suitable sampling techniques which provide excellent contact between analyte and reagent have been developed. Solutions of the reagent in impingers as well as reagent coated on solid sorbents in test tubes have proved to be reliable and to provide quantitative data with external calibration. However, for a reaction between reagent and analyte in the liquid phase, a dependency of the reaction yield on the concentration is observed. Even in the presence of a large excess of reagent, only very low yields are observed at low concentrations, and quantitative reaction occurs only at very high concentrations of the analyte. The EPA method 8315A[12] is therefore based on derivatization of carbonyls in liquid samples with DNPH and calibration *via* the reaction. A major goal of this study is to provide a method which is characterized by improved reaction yields, which should allow an external calibration at least for the most reactive aldehydes.

Recently, we have presented a new group of hydrazine reagents based on a triazine backbone.[13] This may be modified by three functional groups, typically a reactive moiety (the hydrazine function), a detectable moiety (a chromophor or a fluorophor) and a third group, for example a dimethylamino group, to improve detectability by mass spectrometry.[14] Linkage of the functional groups to the molecule backbone is performed by nucleophilic substitutions or Friedel–Crafts alkylations. 4-*N*,*N*-Dimethylamino-6-(4′-methoxy-1′-naphthyl)-1,3,5-triazine-2-hydrazine (DMNTH) was synthesized and applied for the first time in the determination of carbonyls. Initial investigations on these reagents proved their very good reactivity, especially with regard to aliphatic short chain aldehydes.[13]

Nitrogen dioxide is known to interfere with the determination of carbonyls using DNPH due to potential co-elution of the reaction product, 2,4-dinitrophenylazide (DNPA), with the formaldehyde hydrazone.[15] The co-elution may easily be identified by dual wavelength detection in HPLC,[16] and it can be avoided when using appropriate separation conditions. However, the formation of DNPA can also be used to quantify nitrogen dioxide in air samples[17] or nitrite in aqueous samples.[18] The reaction of the newly developed *N*-alkylated hydrazine reagents with nitrite leads to the formation of the respective stable *N*-alkylated amines,[19,20] some of which even allow the fluorescence spectroscopic determination of nitrite in waters.[21] Therefore, *N*-alkylated hydrazine reagents are attractive alternatives to the non-alkylated reagents.

In this work, we are focusing on the determination of carbonyls in waters, in the presence of potential interference from nitrite. A new alkylated derivatizing agent is presented and compared with DMNTH regarding the quantification of aldehydes in aqueous samples.

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Experimental

Chemicals

All chemicals were purchased from Aldrich Chemie (Steinheim, Germany) in analytical grade specification. Acids were Merck (Darmstadt, Germany) analytical grade. Triethylamine was from Fluka (Neu-Ulm, Germany). Acetonitrile for HPLC was Merck gradient grade. The mineral water was Volvic naturelle with the following specified composition: calcium, 9.9 mg L^{-1}; magnesium, 6.1 mg L^{-1}; sodium, 9.4 mg L^{-1}; potassium, 5.7 mg L^{-1}; chloride, 8.4 mg L^{-1}; silica, 30.0 mg L^{-1}; sulfate, 6.9 mg L^{-1}; hydrogencarbonate, 65.3 mg L^{-1}.

Synthesis

***N*-Methyl-4-*N*′,*N*′-dimethylamino-6-(4′-methoxy-1′-naphthyl)-1,3,5-triazine-2-hydrazine (MDMNTH).** MDMNTH was prepared from 2-chloro-4-dimethylamino-6-(4′-methoxy-1′-naphthyl)-1,3,5-triazine (CDMNT), the synthesis of which is described in ref. 13. While cooling with ice, 4.25 mL of methylhydrazine (80 mmol) were added to a suspension of 5 g of CDMNT (16 mmol) in 85 mL of acetonitrile. After refluxing for 1 h, the mixture was cooled to room temperature and 170 mL of de-ionized water were added. The precipitate was filtered off and washed with de-ionized water. The yield was 60%.

Aldehyde–MDMNT hydrazones. MDMNTH, 100 mg (3.1×10^{-4} mol), was dissolved in 15 mL of acetonitrile and 5 mL of deionized water. A 50% molar excess of the aldehyde (4.7×10^{-4} mol) was added. The mixture was stirred for 4 h at room temperature, then 20 mL of de-ionized water were added. The MDMNT hydrazones precipitated as white crystalline material and were washed with de-ionized water. As the degree of purity was very high, according to HPLC investigations (for conditions, see below), recrystallization of the DMNT hydrazones was not necessary. Yields ranged from 60% to 95%.

***N*-Methyl-4-*N*′,*N*′-dimethylamino-6-(4′-methoxy-1′-naphthyl)-1,3,5-triazine-2-amine (MDMNTA).** MDMNTH, 200 mg (6.2×10^{-4} mol) was dissolved in 40 mL of acetonitrile and 4 mL of concentrated hydrochloric acid. A 20% molar excess of sodium nitrite (32 mg, 4.7×10^{-4} mol in 5 mL of water) was added. MDMNTA precipitated as pale yellow crystalline material; it was filtered off and washed with distilled water.

DMNTH and aldehyde DMNT hydrazones. The substances were prepared according to the procedure described in ref. 13.

All products were fully characterized by means of ^{1}H NMR, IR, UV, MS and elemental analysis.

Photometer

The HP 8453 diode array spectrophotometer (Hewlett-Packard, Waldbronn, Germany) with software HP Chem Station 845 (biochemical UV/vis-system) was used.

Spectrofluorimeter

The RF-5301 PC spectrofluorimeter (Shimadzu, Duisburg, Germany) with software version 1.10 was used.

UV/visible absorption and fluorimetric measurements

The UV/vis and fluorimetric measurements of DMNTH and the corresponding hydrazones are described in ref. 13. The UV/visible measurements of MDMNTH and the corresponding hydrazones were performed in acetonitrile as solvent as well as in a binary mixture consisting of acetonitrile (50%) and water containing the additives triethylamine and trifluoroacetic acid (according to the HPLC mobile phase) (50%). The concentration of all solutions was within the range from 2.8×10^{-5} to 6.5×10^{-5} mol L^{-1}. The UV/vis spectra were recorded in the range from 200 to 400 nm, while the fluorimetric spectra were recorded from 200 to 600 nm. The spectroscopic data are listed in Table 1.

HPLC instrumentation and analysis

A high-performance liquid chromatograph consisting of the following components was used: two LC-10AS pumps (Shimadzu, Duisburg, Germany), an SPD-M10Avp diode array detector (Shimadzu), an RF-10Axl fluorescence detector (Shimadzu), an SIL-10A autosampler (Shimadzu), Class LC-10 Version 1.6 software (Shimadzu) and a CBM-10A controller unit (Shimadzu). The injection volumes were 5 and 50 μL, as stated below. The column material for all separations was Supelco Discovery RP-18 (Supelco, Deisenhofen, Germany): particle size, 5μm; pore size, 200 Å; column dimensions, 150 mm × 4.6 mm; and guard column, 20 mm × 4 mm.

For the separation of DMNTH and the corresponding hydrazones, binary gradients consisting of acetonitrile and a mixture of water–triethylamine–acetic acid (preparation: 500 mL of water with 2415 μL of triethylamine and 975 μL of acetic acid, pH ≈ 7.5) with the following profile were selected (see Table 2).

For the separation of MDMNTH and the corresponding hydrazones, binary gradients consisting of acetonitrile and a

Table 2 Acetonitrile solvent gradient for gradient A

Time/min	0	0.1	8.5	17.0	17.5	19.5	21.5 (stop)
c CH_3CN (%)	38	38	46	90	90	38	38
Flow/mL min^{-1}	1.5						

Table 1 UV/vis spectroscopic and fluorimetric data for a series of MDMNT hydrazones

MDMNT hydrazone of	$\lambda_{max.}$ UV in HPLC eluent	$\varepsilon_{(315\ nm)}/10^3$ L mol^{-1} cm^{-1} in HPLC eluent	$\lambda_{max.}$ (excitation) in acetonitrile	$\lambda_{max.}$ (emission) in acetonitrile	$\lambda_{max.}$ (excitation) in HPLC eluent	$\lambda_{max.}$ (emission) in HPLC eluent
Formaldehyde	339	10.8	340	392	358	433
Acetaldehyde	339	10.0	347	399	358	432
Propanal	339	9.4	346	389	360	432
Butanal	339	9.7	353	399	358	432
Pentanal	339	9.5	349	400	358	432
Benzaldehyde	315	37.0	349	463	361	434
p-Tolualdehyde	315	62.1	343	477	355	432

mixture of water–triethylamine–trifluoroacetic acid (preparation: 500 mL of water with 500 μL of triethylamine and 375 μL of trifluoroacetic acid, pH ≈ 4.0) with the following profile were selected (see Table 3).

When using UV/vis detection, the detection wavelength for DMNTH, MDMNTH and derivatives was 315 nm. In the case of fluorescence detection the excitation wavelength was 332 nm and the emission wavelength was 395 nm for DMNTH and the derivatives, while the excitation wavelength was 358 nm and the emission wavelength 432 nm for MDMNTH and the derivatives.

Calibration standards for external solutions

Individual stock standard solutions for each of the analyte hydrazones were prepared by dissolving accurately weighed amounts in acetonitrile. Using the individual stock solutions of each target analyte derivative, a 5.0×10^{-5} M secondary standard solution was made by diluting these solutions with acetonitrile. Two calibration curves for two different concentration ranges were recorded after diluting the secondary standard with acetonitrile: (a) 1.0×10^{-7} mol L^{-1} to 1.0×10^{-6} mol L^{-1} (50 μL of the standard solution were injected into the LC system); and (b) 1.0×10^{-6} mol L^{-1} to 5.0×10^{-5} mol L^{-1} (5 μL of the standard solution were injected into the LC system).

Preparation of the reagent solutions for internal calibration and real sample analysis

The reagent solutions were prepared by adding 6.3 mL of 0.1 M hydrochloric acid to 25 mL of a 3.2×10^{-3} mol L^{-1} DMNTH or MDMNTH solution in acetonitrile.

Preparation of analyte solutions

A 2.0×10^{-3} M stock solution of the target analytes was prepared by diluting an appropriate amount of the corresponding analyte in acetonitrile. Secondary standards were prepared in a concentration range of 1.0×10^{-7} mol L^{-1} to 1.0×10^{-4} mol L^{-1}. Therefore, the appropriate amounts of the stock solution were made up to 10 mL with de-ionized water.

Spiking of mineral water with analyte solutions

The spiked mineral waters were prepared with analyte concentrations of 1.0×10^{-5} mol L^{-1} and 1.0×10^{-6} mol L^{-1} using mineral water for dilution of the secondary analyte standards.

Preparation of analyte solutions in the matrix of mineral water and nitrite

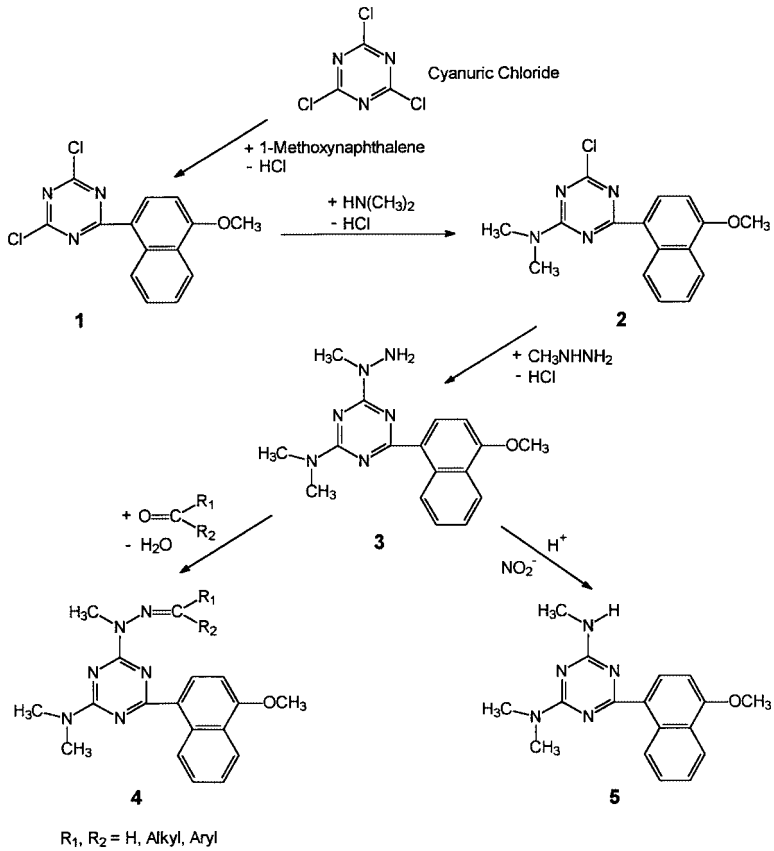
Spiked samples were prepared in a concentration of 1.0×10^{-5} mol L^{-1} by adding 1.0 mL of an 8.7×10^{-5} mol L^{-1} nitrite solution to 10 mL of the mineral water.

Derivatization procedure

A 500 μL volume of the analyte solution was added to 500 μL of the reagent solution in an autosampler vial. After 30 min reaction time at room temperature, the reaction mixture was injected into the LC system.

Table 3 Acetonitrile solvent gradient for gradient B

Time/min	0	7.5	16.0	19.0	19.5	21.5	23.5 (stop)
c CH_3CN (%)	34	34	55	100	100	34	34
Flow/mL min^{-1}	1.5						



Scheme 1 Scheme for the synthesis of the triazine-based hydrazine reagent MDMNTH, including the derivatization of carbonyls and the oxidation of the reagent in the presence of nitrite in acidic media. 1, 2,4-Dichloro-6-(4′-methoxy-1′-naphthyl)-1,3,5-triazine (DMNT); 2, 4-chloro-6-(4′-methoxy-1′-naphthyl)-1,3,5-triazine (CDMNT); 3, MDMNTH; 4, MDMNT hydrazone; 5, MDMNTA.

Results and discussion

N-Methyl-4-*N*′,*N*′-dimethylamino-6-(4′-methoxy-1′-naphthyl)-1,3,5-triazine-2-hydrazine (MDMNTH) was synthesized as a new derivatizing agent in a similar way as was previously described for DMNTH.[13] The respective reactions are depicted in Scheme 1. A Friedel–Crafts alkylation was used to couple 1-methoxynaphthalene to cyanuric chloride. Following a nucleophilic substitution of another chlorine atom at the triazine ring by dimethylamine, the target molecule MDMNTH was finally obtained by a substitution of the last chlorine atom at the triazine ring with methyl hydrazine. As is also presented in Scheme 1, MDMNTH reacts with aldehydes and ketones to form the respective hydrazones. A series of 7 hydrazones was prepared as calibration standards as described in the Experimental Section. The UV/vis spectroscopic and fluorescence spectroscopic data of the derivatives are summarized in Table 1. The benzaldehyde derivative of DMNTH was prepared in a similar way.

Preliminary investigations had confirmed that the reaction product of MDMNTH and nitrite in acidic media is *N*-methyl-4-*N*′,*N*′-dimethylamino-6-(4′-methoxy-1′-naphthyl)-1,3,5-triazine-2-amine (MDMNTA), as presented in Scheme 1. For further investigations, a larger amount of this substance was prepared, as described in the Experimental section, and fully characterized as the reagent and its carbonyl derivatives.

The HPLC separation of the DMNT hydrazones was optimized in a previous paper.[13] A Supelco Discovery RP-18 column was found to be very suitable as the stationary phase. A binary gradient consisting of acetonitrile and water with additives of triethylamine and acetic acid in the aqueous phase (pH 7.5) was selected, as an excellent peak shape was obtained under these conditions. For the MDMNT hydrazones, however, the use of these conditions resulted in severe peak tailing. Therefore, a more acidic eluent consisting of acetonitrile and water with triethylamine and trifluoroacetic acid as additives in the aqueous phase (pH 4) was selected.

The chromatogram of a mixture of the DMNTH reagent and several hydrazone standards using both UV/vis and fluorescence detection is presented in Fig. 1. A baseline separation of the analytes is easily achieved. All DMNT hydrazones of asymmetric carbonyls are characterized by two peaks, which can be associated with the two hydrazone isomers formed in the reaction. Owing to the fact that peak N disappears when an aldehyde is added, it is assumed that it is associated with the reagent, for example to an adduct of the reagent with solvent molecules or a dimer of the reagent. It is obvious that the formaldehyde derivative is characterized by a very high peak in the fluorescence chromatogram compared with the UV/vis chromatogram. On the other hand, the fluorescence intensity of the derivatives of aromatic hydrazones is relatively low.

The respective chromatograms of MDMNTH and its derivatives are presented in Fig. 2. While the separation of the reagent excess and the formaldehyde derivative is difficult, all other peaks are easily separated again. As with DMNTH, the relative fluorescence intensity (compared with the UV/vis peaks) of the formaldehyde derivative is high. However, the significant difference compared with DMNTH is that only one isomer is observed for the hydrazones of asymmetric carbonyls.

As nitrite can occur in significant concentrations in aqueous samples, its reaction with the triazine-based reagents was investigated. In Fig. 3, a chromatogram of DMNTH after reaction with nitrite is shown. The concentration of DMNTH was 1.3×10^{-3} mol L^{-1} in a solution that contained 8.7×10^{-6} mol L^{-1} (0.4 mg L^{-1}) of nitrite. One major reaction product is observed with both UV/vis and fluorescence detection, while some smaller peaks also occur.

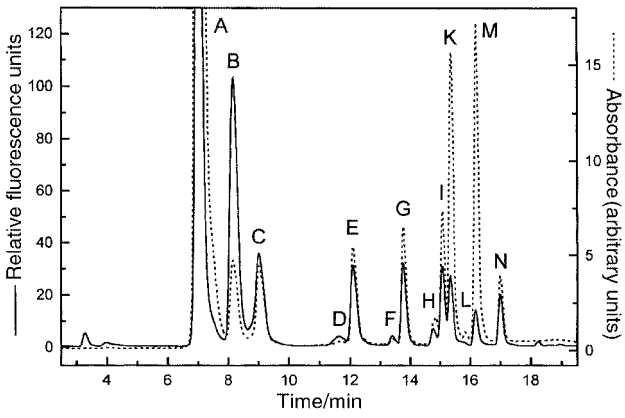


Fig. 1 Chromatogram of DMNTH and selected hydrazones with UV/vis detection (313 nm) and fluorescence detection (exc. 332 nm, em. 395 nm). The concentration of DMNTH in acetonitrile was 1.3×10^{-3} mol L^{-1}, the concentration of the DMNT hydrazones in acetonitrile was 3.0×10^{-5} mol L^{-1}. A, DMNTH; B, formaldehyde DMNT hydrazone; C, acetaldehyde DMNT hydrazone; D and E, isomers of propanal DMNT hydrazone; F and G, isomers of butanal DMNT hydrazone; H and I, isomers of pentanal DMNT hydrazone; I and K, isomers of p-tolualdehyde DMNT hydrazone; L and M, isomers of benzaldehyde DMNT hydrazone; N, peak which may be associated with the reagent.

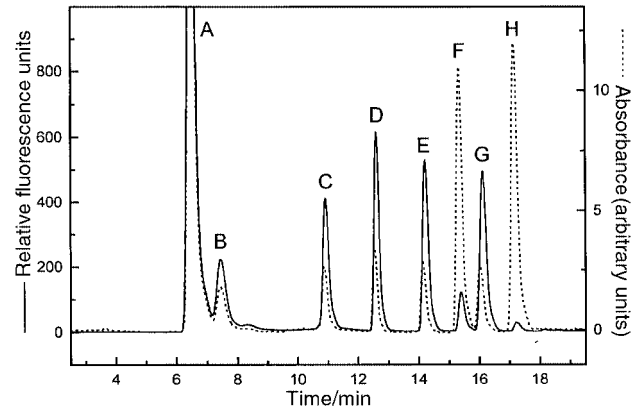


Fig. 2 Chromatogram of MDMNTH and selected hydrazones with UV/vis detection (315 nm) and fluorescence detection (exc. 355 nm, em. 432 nm). The concentration of MDMNTH in acetonitrile was 1.3×10^{-3} mol L^{-1}, the concentration of the DMNT hydrazones in acetonitrile was 1.5×10^{-5} mol L^{-1}. A, MDMNTH; B, formaldehyde MDMNT hydrazone; C, acetaldehyde MDMNT hydrazone; D, propanal MDMNT hydrazone; E, butanal MDMNT hydrazone; F, pentanal MDMNT hydrazone; G, *p*-tolualdehyde MDMNT hydrazone; H, benzaldehyde MDMNT hydrazone.

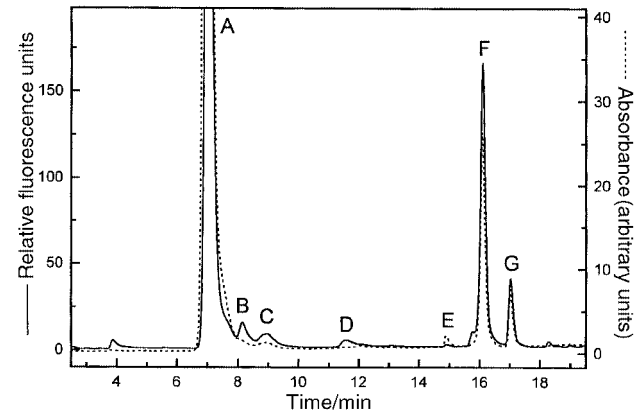


Fig. 3 Chromatogram of the products derived from the reaction between DMNTH and nitrite with UV/vis detection (315 nm) and fluorescence detection (exc. 332 nm, em. 395 nm). The concentration of DMNTH was 1.3×10^{-3} mol L^{-1}. The solution contained 8.7×10^{-6} mol L^{-1} (0.4 mg L^{-1}) nitrite. A, DMNTH; B,C,D,E, peaks of reaction side products; F, main reaction product; G, peak which may be associated with the reagent.

The analogous experiment for MDMNTH is documented in Fig. 4. Only one reaction product is observed, which was identified as MDMNTA (see Experimental section). No significant other peaks are visible in both the UV/vis and the fluorescence chromatogram.

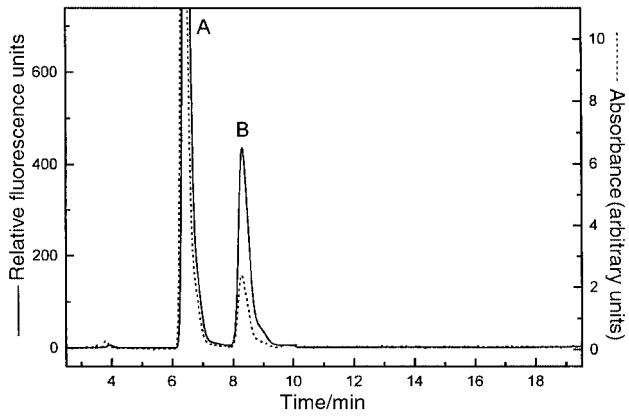


Fig. 4 Chromatogram of the products derived from the reaction between MDMNTH and nitrite with UV/vis detection (315 nm) and fluorescence detection (exc. 355 nm, em. 432 nm). The concentration of MDMNTH was 1.3×10^{-3} mol L^{-1}. The solution contained 8.7×10^{-6} mol L^{-1} (0.4 mg L^{-1}) nitrite. A, MDMNTH; B, MDMNTA.

A large number of experiments were carried out to investigate the potential of both reagents for the quantification of carbonyl trace concentrations in waters. For the concentration range between 0.1 mM and 0.1 μM, five carbonyls were diluted in 13 steps, and a recovery experiment was carried out in triplicate for any concentration. It should be noted that the injection volume for samples and standards was 5 μL for the concentration range between 2.5 μM and 0.1 mM, while it was 50 μL for the concentration range from 0.1 to 1 μM. Quantification was performed using UV/vis and fluorescence detection. External calibration with hydrazone standard solutions and calibration *via* the reaction with the hydrazine reagents were carried out. In Tables 4 and 5, the recovery rates obtained are summarized for DMNTH and MDMNTH, respectively. The relative standard deviation (RSD) for each measurement is based on triple measurements. Limits of quantification are the lowest values in each column provided in Tables 4 and 5.

It is obvious that the recovery rates for DMNTH (Table 4) are relatively constant for the individual analytes within the complete concentration range, with the RSDs increasing to lower concentrations. For external calibration, the recovery

Table 4 Recovery rates obtained for selected carbonyls in aqueous samples using DMNTH as reagent

	Recovery rate ± RSD (%), DMNTH as reagent																	
	Formaldehyde				Acetaldehyde				Butanal				Benzaldehyde				*p*-Tolualdehyde			
	Ext. cal.		Cal. v. react.		Ext. cal.		Cal. v. react.		Ext. cal.		Cal. v. react.		Ext. cal.		Cal. v. react.		Ext. cal.		Cal. v. react.	
Conc. of aldehyde mol l^{-1}	Fluor.	UV/vis	Fluor.	UV/vis	Fluor.	UV/vis	Fluor.	UV/vis	Fluor.	UV/vis	Fluor.	UV/vis	Fluor.	UV/vis	Fluor.	UV/vis	Fluor.	UV/vis	Fluor.	UV/vis
1.0×10^{-4}	91±2	91±1	96±2	100±1	87±2	78±1	101±2	98±1	89±1	84±1	98±1	101±2	106±1	107±1	103±1	102±1	92±1	98±1	102±2	98±1
7.5×10^{-5}	92±1	90±1	97±1	100±1	89±1	78±1	102±1	98±1	89±1	85±1	98±1	102±1	104±1	105±1	101±1	100±1	92±1	98±1	102±1	98±1
5.0×10^{-5}	91±1	90±1	97±1	100±2	88±1	77±1	101±2	97±2	90±2	87±1	99±2	103±1	107±4	106±1	103±4	101±1	89±1	97±1	99±1	97±1
2.5×10^{-5}	91±1	90±2	97±2	99±2	87±1	77±1	100±1	97±1	87±3	84±1	95±4	100±1	101±1	103±1	98±1	99±1	88±1	96±2	98±1	96±2
1.0×10^{-5}	91±1	90±2	97±1	99±2	86±1	79±1	99±1	99±1	88±4	82±1	97±4	98±1	101±2	103±2	98±2	98±2	91±8	101±2	101±9	101±2
7.5×10^{-6}	91±1	90±1	97±1	99±2	86±1	81±1	100±1	100±1	86±4	85±1	95±5	101±2	101±2	104±1	98±2	100±1	87±9	99±2	96±10	99±2
5.0×10^{-6}	92±2	89±2	98±2	99±3	85±2	82±4	98±2	98±2	87±4	88±1	95±4	105±1	103±1	105±4	99±1	100±4	84±10	103±1	93±11	103±1
2.5×10^{-6}	94±4	91±3	100±4	101±4	87±1	82±2	100±1	100±1	89±2	84±3	98±2	100±3	101±2	108±2	98±2	103±2	90±6	106±1	100±7	106±1
1.0×10^{-6}	103±7	91±3	102±4	102±4	88±4	81±6	101±5	101±5	92±4	83±2	101±4	98±2	99±2	103±7	96±2	99±6	90±3	101±2	100±3	100±2
7.5×10^{-7}	104±6	97±6	110±6	107±7	87±5	81±1	101±6	101±6	91±5	82±1	101±5	98±1	100±1	102±4	97±1	98±4	91±2	103±4	101±2	103±4
5.0×10^{-7}	92±3	86±4	98±3	95±4	82±11	81±4	95±15	95±13	101±5	84±1	111±5	100±1	105±3	101±5	101±3	97±5	97±2	101±6	108±3	101±6
2.5×10^{-7}	92±3	83±2	97±3	92±2		76±11		105±15	103±6	79±1	113±6	94±1	110±2	108±9	107±2	104±8		99±9		99±9
1.0×10^{-7}	100±8		106±9																	

Table 5 Recovery rates obtained for selected carbonyls in aqueous samples using MDMNTH as reagent

	Recovery rate ± RSD (%), MDMNTH as reagent																	
	Formaldehyde				Acetaldehyde				Butanal				Benzaldehyde				*p*-Tolualdehyde			
	Ext. cal.		Cal. v. react.		Ext. cal.		Cal. v. react.		Ext. cal.		Cal. v. react.		Ext. cal.		Cal. v. react.		Ext. cal.		Cal. v. react.	
Conc. of aldehyde mol l^{-1}	Fluor.	UV/vis	Fluor.	UV/vis	Fluor.	UV/vis	Fluor.	UV/vis	Fluor.	UV/vis	Fluor.	UV/vis	Fluor.	UV/vis	Fluor.	UV/vis	Fluor.	UV/vis	Fluor.	UV/vis
1.0×10^{-4}		94±1		97±1		77±2		90±2		78±1		92±1		89±3		96±4	92±3	93±2	94±3	95±2
7.5×10^{-5}		95±2		97±1		82±1		96±2		85±1		99±1	89±4	92±4	89±2	98±3	96±4	97±3	96±2	98±2
5.0×10^{-5}		94±1		97±1		88±5		104±6		87±1		102±1	90±1	93±2	91±1	100±2	97±2	98±1	99±2	100±1
2.5×10^{-5}	88±4	90±3	94±4	93±3	75±4	87±6	91±4	102±7		86±2		101±2	88±1	90±1	89±1	98±1	95±1	95±1	96±1	97±1
1.0×10^{-5}	94±3	98±4	101±3	102±4	73±4	84±8	89±5	99±10	83±5	82±2	93±5	96±2	87±3	90±1	88±3	97±1	93±2	95±1	95±2	97±1
7.5×10^{-6}	96±7	103±6	103±7	107±7	76±5	89±8	93±6	104±10	87±5	86±1	97±5	101±1	92±4	93±1	93±4	100±2	98±3	98±1	99±3	100±1
5.0×10^{-6}	96±6	102±12	103±6	105±12	77±5	88±1	94±6	104±2	87±5	87±2	97±5	102±2	91±3	92±3	92±3	100±3	98±2	98±3	100±2	100±3
2.5×10^{-6}					74±5	86±9	90±7	101±10	83±3	87±3	94±4	102±4	88±2	93±1	89±2	101±1	96±2	98±1	97±2	100±1
1.0×10^{-6}					76±8		93±10		89±4	89±4	100±4	104±5	97±2	102±4	98±2	110±4	106±5	108±5	108±5	110±4
7.5×10^{-7}					96±19		117±23		101±5		113±6		114±3		115±3		102±17		104±17	
5.0×10^{-7}					93±19		113±24		97±5		109±5		113±2		114±2		109±4		110±4	
2.5×10^{-7}					75±26		92±32		91±4		102±5		111±2		112±2					
1.0×10^{-7}									73±3		82±3		113±3		114±3					

Table 6 Recovery rates obtained for selected carbonyls in spiked water samples

Concn. of aldehyde and nitrite/ mol l^{-1}	Spiked mineral water, recovery rate ± RSD (%), DMNTH as reagent																	
	Formaldehyde				Acetaldehyde				Butanal				Benzaldehyde				*p*-Tolualdehyde			
	Ext. cal.		Cal. v. react.		Ext. cal.		Cal. v. react.		Ext. cal.		Cal. v. react.		Ext. cal.		Cal. v. react.		Ext. cal.		Cal. v. react.	
	Fluor.	UV/ vis	Fluor.	UV/ vis	Fluor.	UV/ vis	Fluor.	UV/ vis	Fluor.	UV/ vis	Fluor.	UV/ vis	Fluor.	UV/ vis	Fluor.	UV/ vis	Fluor.	UV/ vis	Fluor.	UV/ vis
1.0×10^{-5}	91 ± 3	90 ± 2	96 ± 2	100 ± 2	88 ± 5	77 ± 2	99 ± 2	96 ± 2	92 ± 2	88 ± 1	101 ± 2	105 ± 1	106 ± 2	104 ± 1	103 ± 2	99 ± 1	91 ± 4	103 ± 4	99 ± 3	100 ± 2
1.0×10^{-5} nitrite: 8.7×10^{-6}	88 ± 2	88 ± 1	93 ± 2	98 ± 1	85 ± 7	75 ± 1	98 ± 8	85 ± 1	91 ± 9	87 ± 1	101 ± 10	103 ± 1	102 ± 2	101 ± 1	99 ± 1	97 ± 1	[a]	[a]	[a]	[a]
1.0×10^{-6}	99 ± 2	94 ± 1	105 ± 2	104 ± 1	93 ± 12	74 ± 11	108 ± 14	93 ± 14	92 ± 8	83 ± 1	101 ± 8	99 ± 1	104 ± 2	97 ± 1	101 ± 2	93 ± 4	86 ± 4	103 ± 3	103 ± 4	103 ± 3

Concn. of aldehyde and nitrite/ mol l^{-1}	Spiked mineral water, recovery rate ± RSD (%), MDMNTH as reagent																			
	Formaldehyde				Acetaldehyde				Butanal				Benzaldehyde				*p*-Tolualdehyde			
	Ext. cal.		Cal. v. react.		Ext. cal.		Cal. v. react.		Ext. cal.		Cal. v. react.		Ext. cal.		Cal. v. react.		Ext. cal.		Cal. v. react.	
	Fluor.	UV/ vis	Fluor.	UV/ vis	Fluor.	UV/ vis	Fluor.	UV/ vis	Fluor.	UV/ vis	Fluor.	UV/ vis	Fluor.	UV/ vis	Fluor.	UV/ vis	Fluor.	UV/ vis	Fluor.	UV/ vis
1.0×10^{-5}	89 ± 5	97 ± 4	95 ± 5	101 ± 4	74 ± 4	80 ± 1	90 ± 5	94 ± 1	84 ± 2	94 ± 1	94 ± 2	99 ± 2	88 ± 2	91 ± 1	88 ± 1	98 ± 1	94 ± 2	94 ± 1	95 ± 1	96 ± 1
1.0×10^{-5} nitrite: 8.7×10^{-6}	93 ± 6	102 ± 4	99 ± 6	105 ± 3	75 ± 4	83 ± 1	91 ± 5	97 ± 1	84 ± 3	86 ± 2	94 ± 3	101 ± 2	88 ± 1	93 ± 1	89 ± 1	100 ± 1	97 ± 1	97 ± 1	98 ± 1	99 ± 1
1.0×10^{-6}									101 ± 1		113 ± 1		113 ± 1		114 ± 1		113 ± 1		115 ± 1	

[a] *p*-Tolualdehyde cannot be quantified using the DMNTH method due to co-elution of its hydrazone and the major reaction product of DMNTH and nitrite (see text).

rates range from 77 to 100%, thus enabling a first estimation of the aldehyde concentration in water samples based on external calibration. In the case of calibration *via* the reaction, excellent recoveries between 92% and 113% are observed throughout the complete data matrix. In some cases, the limit of quantification is 0.5 μM. In the case of MDMNTH (Table 5), however, some problems are observed. On the one hand, the signal for the formaldehyde hydrazone cannot be discriminated from the reagent peak at low concentrations, on the other hand, the sensitivity range of the fluorescence detector does not allow the determination of very high concentrations of most of the analytes using the same conditions as the lowest concentrations. The latter problem could easily be solved in the case of high aldehyde concentrations by adjusting the detector gain appropriately. For UV/vis detection of the MDMNTH derivatives, the molar absorptivity is reduced in the acidic buffer system, thus resulting in higher limits of detection. Regarding the recovery rates, similar observations are made as in the case of DMNTH.

A mineral water was spiked with two concentrations of the carbonyls (1 and 10 μM) and another 10 μM solution additionally with 8.7 μM nitrite as potential interferent. Recovery rates for both reagents were determined again using both calibration approaches and both detectors. The data are presented in Table 6. The relative standard deviations are based on triple measurements. Again, the recovery rates based on calibration *via* the reaction are excellent, while those based on external calibration are still acceptable. For DMNTH, no significant influence of the nitrite on the recovery of the carbonyls is observed, except for *p*-tolualdehyde. In this case, the analyte cannot be quantified due to co-elution of the major reaction product of DMNTH with nitrite. For MDMNTH, no significant influence of the nitrite on the recovery of all carbonyls is observed.

Therefore, the new method can be considered as a promising approach for the quantification of carbonyls in water samples. A calibration *via* the reaction is preferable compared to external calibration. The method requires no sample preparation except the derivatization step. The applicable concentration range extends to three decades and covers the complete range of analytical relevance for most applications.

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