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Journal of Chromatography A, 869 (2000) 251–259

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

2,4-Dinitro-3,5,6-trideuterophenylhydrazones for the quantitation of aldehydes and ketones in air samples by liquid chromatography–mass spectrometry

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Abstract

The quantification of carbonyl compounds in air samples using an internal calibration approach with stable isotope-labelled standards and HPLC–atmospheric pressure chemical ionization MS analysis is presented. 2,4-Dinitro-3,5,6-trideuterophenylhydrazine and various of its hydrazones have been synthesized and characterized for the first time. The respective stable isotope-labelled hydrazones of a series of aldehydes and ketones are applied as internal standards for the determination of the carbonyls in car exhaust samples. Various aldehydes are identified and quantified by MS detection. The results exhibit good agreement to quantification data obtained with UV detection.

Keywords: Air monitoring; Environmental analysis; Dinitrophenylhydrazones; Aldehydes; Ketones; Dinitrophenylhydrazines

1. Introduction

The 2,4-dinitrophenylhydrazine (DNPH) method has been established as one of the most powerful tools for the determination of aldehydes and ketones both in liquid and gaseous samples. The carbonyl compounds are derivatized with DNPH in acidic media under formation of the corresponding hydrazones. These are in most cases separated by means of reversed-phase liquid chromatography and detected spectroscopically by UV–Vis at wavelengths between 349 nm and 380 nm [1–4]. In recent years, chemical interferences have been observed in the presence of strong oxidants during sampling [5–7]. Especially in complex matrices, UV–Vis detection lacks selectivity to identify the interferents and to quantify the carbonyl compounds with the required selectivity and accuracy. The demand of higher selectivity is met by mass spectrometric (MS) detection for the identification of carbonyl compounds and unknown sample constituents. By the invention of ionization techniques at atmospheric pressure, coupling of liquid chromatography and mass spectrometry has made significant progress in recent years [8]. In the past, the ionization of DNPH derivatives had been performed using a moving belt interface [9] or a particle beam interface [10]. A procedure for the identification of carbonyls based on HPLC with atmospheric pressure chemical ionization mass spectrometry (APCI-MS) in the negative mode has first been published in 1998 by Kölliker et al. [11]. The method is characterized by very low detection limits in the nanomolar range and by the possibility to identify single carbonyls from a com-

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0021-9673/00/$ – see front matter
PII: S0021-9673(99)01240-6

plex mixture due to tandem MS in the used ion trap instrument. Similar results were obtained in a very recent publication by Grosjean et al., who identified a series of DNP-hydrazones by HPLC using diode array detection (DAD) and HPLC–APCI-MS with a single quadrupol instrument [12]. A method for the quantification of carbonyls based on HPLC–APCI-MS with calibration using isotope-labelled internal standards is described in [13]. Formaldehyde and acetaldehyde were quantified in automobile exhaust by means of [$^2H_2$]-formaldehyde DNP-hydrazone and [$^2H_4$]-acetaldehyde DNP-hydrazone as internal standards. The results are in good agreement to the ones of UV detection as standard detection technique. However, this approach is limited by the availability of labelled carbonyl compounds for the synthesis of the internal standards. A stable isotope-labelled derivatizing agent could solve this problem, as any desired standard would then be easily accessible.

We are presenting the synthesis and application of stable isotope-labelled DNPH and its hydrazones to enhance the availability of internal standards and the quantification of carbonyls by use of these standards in car exhaust samples.

2. Experimental section

2.1. Chemicals

All chemicals were purchased from Aldrich (Steinheim, Germany) in the highest quality available. As solvents for LC, acetonitrile, methanol and tetrahydrofuran (all in gradient grade quality from Merck, Darmstadt, Germany) were used. Air sampling was performed with coated DNPH cartridges (LpDNPH S10) from Supelco (Deisenhofen, Germany).

2.2. HPLC–MS instrumentation

The HPLC–MS system from Shimadzu (Duisburg, Germany) consisted of the following components: controller unit SCL-10Avp, degasser DGU-14A, two pumps LC-10ADvp, mixing chamber Model SUS (0.5 mL), autosampler SIL-10A, UV–Vis detector SPD-10AV, single quadrupol mass spectrometer LCMS QP8000 with atmospheric pressure ionization and software Class 8000 Version 1.11.

2.3. HPLC instrumentation

The HPLC system from Shimadzu consisted of the following components: controller unit CBM-10A, degasser GT-104, two pumps LC-10AS, mixing chamber Model SUS (0.5 mL), autosampler SIL-10A, photo diode array detector SPD-M10Avp.

2.4. HPLC conditions

All separations with the HPLC–MS system were performed using a Supelco Discovery C_{18} column of the following dimensions: particle size, 5 μm; pore size, 100 Å; length, 150 mm; I.D., 2.1 mm. An acetonitrile–water binary gradient at a flow-rate of 0.4 mL/min with the following profile was used:

time (min)	0	1	9	10	13	14	18	18.5
c (CH_3CN) (%)	49	49	78.1	100	100	49	49	Stop

The injection volume was 5 μL. The detection wavelengths were 300 nm and 360 nm.

The separations with HPLC and diode array detector were performed using a Supelco Discovery C_{18} column (Supelco, Deisenhofen, Germany) of the following dimensions: particle size, 5 μm; pore size, 100 Å; length, 150 mm; I.D., 4.6 mm. The same acetonitrile–water binary gradient as mentioned above was used at a flow-rate of 1.4 mL/min. The injection volume was 10 μL. Detection was carried out in a wavelength range from 190 to 500 nm.

2.5. MS conditions

All MS measurements were performed using APCI in the negative mode under the following conditions: nebulizer gas flow (N_2), 2 L/min; probe voltage, −3 kV; temperature of the APCI probe, 300°C; curved desolvation line (CDL) voltage, −20 V; CDL temperature, 250°C; deflector voltages, −35 V; detector gain, 1.5 kV. For SCAN mode measurements, a mass range from 100 to 400 m/z was selected, the integration time was 1.5 s. For selected ion monitoring (SIM) measurements, the integration

time was 1.5 s. All variations or further specifications to these parameters are mentioned at the respective analytical procedures.

2.6. Synthesis and characterization of 2,4-dinitro-3,5,6-trideuterophenylhydrazones

2,4-Dinitro-3,5,6-trideuterophenylhydrazine (d_3DNPH) was synthesized in two steps (see reaction scheme). The nitration is carried out by using [$^2H_5$]-chlorobenzene as educt and a mixture of $^2H_2SO_4$ and KNO_3 as nitration reagent [14]. In this procedure, 38 mL concentrated $^2H_2SO_4$ are mixed with 46 g KNO_3. 10 g [$^2H_5$]-chlorobenzene (85.4 mmol) are added dropwise within 30 min. The temperature should not exceed 80°C. The mixture is kept for 3 h at 80°C. After cooling, the mixture is poured under stirring onto 300 g of crushed ice. The product is extracted with toluene, washed with water and subsequently dried with $CaCl_2$. The toluene is removed in vacuo. The residue is dissolved in warm methanol and the product is allowed to crystallize in the refrigerator. Recrystallization from methanol is necessary in case that the mononitrochlorobenzene is present as well. This may be controlled by reversed-phase HPLC under the conditions stated above for analytical purposes.

The d_3DNPH was obtained by nucleophilic substitution of chloro-2,4-dinitro-3,5,6-trideuterobenzene with hydrazine [15]. 4.26 g Chloro-2,4-dinitro-3,5,6-trideuterobenzene (20.8 mmol) are dissolved in 20 mL ethanol. This solution is added dropwise to a mixture of 1.108 mL (22.85 mmol) hydrazine hydrate in 6 mL ethanol and 2.24 g (22.85 mmol) potassium acetate in 10 mL water. The reaction mixture is refluxed for 1 h and then cooled. The red-orange product is filtered and washed with hot ethanol (60°C) and hot water. DNPH is preferably stored in the presence of 10–20% water due to its potentially explosive properties as dry material.

All hydrazones were prepared according to the procedure of Behforouz et al. [16]. The products were recrystallized from ethanol. As both d_3DNPH and its hydrazones have not been described in literature previously, the spectroscopic data are listed in detail in the following.

2,4-Dinitro-3,5,6-trideuterophenylhydrazine. MS (EI, 70 eV), *m*/*z*: 201 (100%, $M^{+\cdot}$), 183 (14%), 155 (3%), 125 (36%), 95 (19%), 82 (64%); ^{1}H-NMR (C^2HCl_3, TMS), δ (ppm): 4.31 (s 2H, HN-N$\underline{H}_2$), 9.44 (s 1H, $\underline{H}$N-NH_2); IR (KBr): 3326 (s), 3293 (s), 2299 (s), 1524 (s), 1331 (s), 943 (s), 833 (s) cm^{-1}.

Formaldehyde d_3DNP-hydrazone. MS (EI, 70 eV), *m*/*z*: 213 (100%, $M^{+\cdot}$), 183 (23%), 155 (6%), 125 (13%), 82 (67%), 66 (46%), 54 (38%); ^{1}H-NMR (C^2HCl_3, TMS), δ (ppm): 6.73 (d 1H, N=CH_2), 7.16 (d 1H, N=CH_2), 11.13 (s 1H, $\underline{H}$-NN); IR (KBr): 3309 (s), 2921 (w), 1597 (s), 1522 (s), 1332 (s), 943 (m), 835 (m) cm^{-1}; UV (CH_3CN): λ_{max}= 349 nm; $\epsilon(\lambda_{max})$=20 500 L mol^{-1} cm^{-1}; EA: C_{calc}=39.47%, C_{found}=39.39%, N_{calc}=26.29% N_{found}=26.53%; m.p.: 178°C.

Acetaldehyde d_3DNP-hydrazone. MS (EI, 70 eV), *m*/*z*: 227 (100%, $M^{+\cdot}$), 183 (18%), 155 (13%), 125 (14%), 95 (11%), 82 (59%), 66 (22%); ^{1}H-NMR (C^2HCl_3, TMS), δ (ppm): 2.13 (d 3H, CHC$\underline{H}_3$), 7.70 (q 1H, C$\underline{H}$CH_3), 11.04 (s 1H, $\underline{H}$-NN); IR (KBr): 3289 (s), 2918 (w), 1599 (s), 1536 (m), 1331(s), 940 (m), 835 (m) cm^{-1}; UV (CH_3CN): λ_{max}=359 nm; $\epsilon(\lambda_{max})$=19 100 L mol^{-1} cm^{-1}; EA: C_{calc}=42.33%, C_{found}=42.23%, N_{calc}=24.67%, N_{found}=24.87%; m.p.: 176°C.

Propanal d_3DNP-hydrazone. MS (EI, 70 eV), *m*/*z*: 241 (100%, $M^{+\cdot}$), 183 (13%), 155 (21%), 125 (20%), 82 (60%), 66 (22%); ^{1}H-NMR (C^2HCl_3, TMS), δ (ppm): 1.23 (t 3H, -CH_2-C$\underline{H}_3$), 2.45 (m 2H, -C$\underline{H}_2$-CH_3), 7.57 (t 1H, N=C$\underline{H}$-C_2H_5), 11.02 (s 1H, $\underline{H}$-NN); IR (KBr): 3294 (s), 2984 (w), 2904 (w), 1598 (s), 1536 (m), 1327 (s), 926 (w), 830 (w) cm^{-1}; UV (CH_3CN): λ_{max}=360 nm; $\epsilon(\lambda_{max})$=20 100 L mol^{-1} cm^{-1}; EA: C_{calc}=44.85%, C_{found}=44.89%, N_{calc}=23.24%, N_{found}=23.53%; m.p.: 162°C.

Butanal d_3DNP-hydrazone. MS (EI, 70 eV), *m*/*z*: 255 (100%, $M^{+\cdot}$), 209 (5%), 183 (14%), 155 (25%), 125 (19%), 82 (36%), 66 (15%), 55 (62%); ^{1}H-NMR (C^2HCl_3, TMS), δ (ppm): 1.03 (t 3H, -CH_2-C$\underline{H}_3$), 1.66 (m 2H, CH_2-C$\underline{H}_2$-CH_3), 2.42 (m 2H, N=CH-C$\underline{H}_2$-CH_2), 7.55 (t 1H, N=C$\underline{H}$-CH_2), 11.02 (s 1H, $\underline{H}$-NN); IR (KBr): 3299 (s), 2953 (m), 2870 (m), 1599 (s), 1535 (m), 1332 (s), 943 (m), 833 (m) cm^{-1}; UV (CH_3CN): λ_{max}=361 nm; $\epsilon(\lambda_{max})$= 19 400 L $mol^{-1}cm^{-1}$; EA: C_{calc}=47.10%, C_{found}= 47.04%, N_{calc}=21.96% N_{found}=22.32%; m.p.: 129°C.

Pentanal d_3DNP-hydrazone. MS (EI, 70 eV), *m*/*z*: 269 (100%, $M^{+\cdot}$), 227 (22%), 209 (72%), 183 (9%), 155 (16%), 152 (28%), 125 (24%), 106 (27%), 82 (33%), 55 (48%); ^{1}H-NMR (C^2HCl_3, TMS), δ

(ppm): 0.97 (t 3H, -CH_2-C$\underline{H}_3$), 1.35 (m 2H, CH_2-C$\underline{H}_2$-CH_3), 1.59 (m 2H, CH_2-C$\underline{H}_2$-CH_2), 2.44 (m 2H, N=CH-C$\underline{H}_2$-CH_2), 7.55 (t 1H, N=C$\underline{H}$-CH_2), 11.00 (s 1H, $\underline{H}$-NN); IR (KBr): 3296 (s), 2948 (m), 2929 (m), 2868 (m), 1600 (s), 1535 (m), 1330 (s), 943 (w), 833 (w) cm^{-1}; UV (CH_3CN): λ_{max}=361 nm; $\epsilon(\lambda_{max})$=21 600 L mol^{-1} cm^{-1}; EA: C_{calc}=49.12%, C_{found}=48.93%, N_{calc}=20.82%, N_{found}=21.14%. m.p.: 118°C.

Hexanal d_3DNP-hydrazone. MS (EI, 70 eV), *m*/*z*: 283 (25%, $M^{+\cdot}$), 227 (10%), 209 (29%), 152 (11%), 106 (10%), 83 (100%); ^{1}H-NMR (C^2HCl_3, TMS), δ (ppm): 0.94 (t 3H, -CH_2-C$\underline{H}_3$), 1.37, 1.61 (m 6H, CH_2-$C_3\underline{H}_6$-CH_3), 2.42 (q 2H, N=CH-C$\underline{H}_2$-CH_2), 7.53 (t 1H, N=C$\underline{H}$-CH_2), 11.00 (s 1H, $\underline{H}$-NN); IR (KBr): 3297 (s), 2929 (s), 2869 (m), 1600 (s), 1535 (m), 1329 (s), 943 (w), 833 (w) cm^{-1}; UV (CH_3CN): λ_{max}=361 nm; $\epsilon(\lambda_{max})$=20 700 L $mol^{-1}cm^{-1}$; EA: C_{calc}=50.88%, C_{found}=50.53%, N_{calc}=19.78%, N_{found}=19.88%; m.p.: 116°C.

Acetone d_3DNP-hydrazone. MS (EI, 70 eV), *m*/*z*: 241 (100%, $M^{+\cdot}$), 184 (20%), 155 (7%), 125 (10%), 82 (13%), 81 (16%), 66 (11%); ^{1}H-NMR (C^2HCl_3, TMS), δ (ppm): 2.12 (d 6H, N=C(C$\underline{H}_3$)$_2$), 11.00 (s 1H, $\underline{H}$-NN); IR (KBr): 3312 (s), 2955 (w), 1596 (s), 1536 (m), 1330 (s), 935 (w), 841 (w) cm^{-1}; UV (CH_3CN): λ_{max}=363 nm; $\epsilon(\lambda_{max})$=20 000 L $mol^{-1}cm^{-1}$; EA: C_{calc}=44.85%, C_{found}=44.81%, N_{calc}=23.24%, N_{found}=23.57%; m.p.: 138°C.

2-Butanone d_3DNP-hydrazone. MS (EI, 70 eV), *m*/*z*: 255 (63%, $M^{+\cdot}$), 184 (22%), 155 (33%), 125 (22%), 82 (54%), 55 (100%); ^{1}H-NMR (C^2HCl_3, TMS), δ (ppm): 1.20 (t 3H, CH_2-C$\underline{H}_3$), 2.10 (d 6H, N=CC$\underline{H}_3$), 2.45 (q 2H, C$\underline{H}_2$-CH_3), 11.04 (s 1H, $\underline{H}$-NN); IR (KBr): 3320 (s), 2976 (s), 2307 (m), 1611 (s), 1528 (s), 1333 (s), 939 (s); 838 (m); UV (CH_3CN): λ_{max}=366 nm; $\epsilon(\lambda_{max})$=19 400 L mol^{-1} cm^{-1}; EA: C_{calc}=47.06%, C_{found}=47.26%, N_{calc}=21.96%, N_{found}=21.68%; m.p.: 124°C.

Cyclohexanone d_3DNP-hydrazone. MS (EI, 70 eV), *m*/*z*: 281 (100%, $M^{+\cdot}$), 246 (3%), 168 (12%), 155 (12%), 125 (8%), 99 (59%), 96 (18%), 81 (68%), 55 (73%); ^{1}H-NMR (C^2HCl_3, TMS), δ (ppm): 1.77 (m 6H, CH_2-$C_3\underline{H}_6$-CH_2), 2.40 (d 4H, N=C(C$\underline{H}_2$)$_2$), 11.18 (s 1H, $\underline{H}$-NN); IR (KBr): 3308 (s), 2944 (m), 2870 (w), 2856 (w), 1598 (s), 1528 (m), 1330 (s), 939 (m), 838 (w) cm^{-1}; UV (CH_3CN): λ_{max}=369 nm; $\epsilon(\lambda_{max})$=21 100 L mol^{-1} cm^{-1}; EA: C_{calc}=51.29%, C_{found}=51.24%, N_{calc}=19.93%, N_{found}=20.29%, m.p.: 175°C.

Benzaldehyde d_3DNP-hydrazone. MS (EI, 70 eV), *m*/*z*: 289 (100%, $M^{+\cdot}$), 242 (3%), 196 (6%), 155 (7%), 125 (5%), 107 (53%), 82 (22%), 77 (42%); ^{1}H-NMR (C^2HCl_3, TMS), δ (ppm): 7.48 (m 3H, aryl-H), 7.78 (m 2H, aryl-H), 8.14 (s 1H, N=C$\underline{H}$Ph), 11.33 (s 1H, $\underline{H}$-NN); IR (KBr): 3286 (s), 1577 (s), 1529 (m), 1330 (s), 944 (m), 834 (m) cm^{-1}; UV (CH_3CN): λ_{max}=379 nm; $\epsilon(\lambda_{max})$=30 500 L mol^{-1} cm^{-1}; EA: C_{calc}=54.03%, C_{found}=53.86%, N_{calc}=19.38%, N_{found}=19.69%; m.p.: 254°C.

p-Tolylaldehyde d_3DNP-hydrazone. MS (EI, 70 eV), *m*/*z*: 303 (100%, $M^{+\cdot}$), 183 (7%), 155 (5%), 125 (3%), 121 (53%), 91 (30%), 82 (14%); ^{1}H-NMR (C^2HCl_3, TMS), δ (ppm): 2.43 (s 3H, Ph-CH_3), 7.29 (s 2H, aryl-H), 7.67 (d 2H, aryl-H), 8.10 (s 1H, N=C$\underline{H}$Ph), 11.30 (s 1H, $\underline{H}$-NN); IR (KBr): 3285 (s), 2919 (w), 1607 (s), 1595 (s), 1576 (s), 1531 (m), 1334 (s), 945 (m), 834 (m) cm^{-1}; UV (CH_3CN): λ_{max}=384 nm; $\epsilon(\lambda_{max})$=28 300 L mol^{-1} cm^{-1}; EA: C_{calc}=55.50%, C_{found}=55.11%, N_{calc}=18.48%, N_{found}=18.59%; m.p.: 254°C.

Acrolein d_3DNP-hydrazone. MS (EI, 70 eV), *m*/*z*: 239 (100%, $M^{+\cdot}$), 192 (32%), 162 (16%), 146 (17%), 145 (18%), 119 (16%), 118 (14%), 92 (11%), 82 (18%), 66 (22%); ^{1}H-NMR (C^2HCl_3, TMS), δ (ppm): 5.73 (d 2H, -CH=C$\underline{H}_2$), 6.63 (m 1H, -C$\underline{H}$=CH_2), 7.80 (d 1H, N=C$\underline{H}$-CH), 11.14 (s 1H, $\underline{H}$-NN); IR (KBr): 3275 (s), 1659 (w), 1594 (s), 1536 (m), 1319 (m), 941 (m), 832 (m) cm^{-1}; UV (CH_3CN): λ_{max}=370 nm; $\epsilon(\lambda_{max})$=26 700 L mol^{-1} cm^{-1}; EA: C_{calc}=45.23%, C_{found}=45.19%, N_{calc}=23.43% N_{found}=23.67%; m.p.: 164°C.

Methacrolein d_3DNP-hydrazone. MS (EI, 70 eV), *m*/*z*: 253 (100%, $M^{+\cdot}$), 236 (13%), 206 (45%), 205 (26%), 191 (13%), 176 (14%), 159 (25%), 133 (11%), 94 (10%), 93 (11%), 66 (24%); ^{1}H-NMR (C^2HCl_3, TMS), δ (ppm): 2.06 (s 3H, -CH_3), 5.53 (d 2H, -CH=C$\underline{H}_2$), 7.83 (s 1H, N=C$\underline{H}$-CH), 11.16 (s 1H, $\underline{H}$-NN); IR (KBr): 3279 (s), 2929 (w), 1595 (s), 1536 (m), 1333 (s), 938 (m), 832 (m) cm^{-1}; UV (CH_3CN): λ_{max}=371 nm; $\epsilon(\lambda_{max})$=24 100 L mol^{-1} cm^{-1}; EA: C_{calc}=47.47%, C_{found}=47.38%, N_{calc}=22.13%, N_{found}=22.18%; m.p.: 200°C (dec.).

Crotonaldehyde d_3DNP-hydrazone. MS (EI, 70 eV), *m*/*z*: 253 (100%, $M^{+\cdot}$), 238 (31%), 236 (32%),

218 (7%), 206 (44%), 205 (34%), 192 (11%), 160 (14%), 159 (15%), 146 (5%), 133 (11%); ^{1}H-NMR ($C^{2}HCl_3$, TMS), δ (ppm): 1.95 (s 3H, -CH_3), 6.30 (m 2H, -C<u>H</u>=C<u>H</u>-), 7.74 (s 1H, N=C<u>H</u>-CH), 11.07 (s 1H, <u>H</u>-NN); IR (KBr): 3284 (s), 2927 (w), 1647 (w), 1595 (s), 1533 (m), 1330 (s), 938 (m), 833 (m) cm^{-1}; UV (CH_3CN): λ_{max}=376 nm; $\epsilon(\lambda_{max})$= 27 700 L mol^{-1} cm^{-1}; EA: C_{calc}=47.47%, C_{found}= 47.34%, N_{calc}=22.13%, N_{found}=22.16%; m.p.: 207°C (dec.).

2.7. Recovery rates using internal standards

Solutions of the deuterated and non-deuterated standards were mixed in different ratios (50:1, 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:10, 1:50) and analyzed by HPLC–MS using the following time programmes for the SIM traces for three hydrazone mixtures (Table 1). The hydrazones were analyzed in three mixtures to ensure baseline separation of all compounds and sufficient time windows for the program. This is necessary to evaluate the suitability of the calibration approach.

Every analysis was carried out three times. The peak areas of the SIM traces were integrated separately. The recovery rate of the non-deuterated compound was calculated based on the peak area of the deuterated compound [17].

Air sampling and sample preparation. The sampling volume of different car exhaust samples was 1.5 L at a flow-rate of 0.5 L/min using a personal sampling pump (Buck, Orlando, FL, USA). The car was stationary and under idling conditions, the engine had been running for 5 min. Every cartridge was equipped with a backup cartridge to control that the exhaust is only sampled on the first cartridge. The cartridges were eluted the same day with 10 mL of acetonitrile and left to rest another 20 min.

Analysis of the car exhaust samples. Each sample was analyzed in three different ways: (a) SCAN mode, (b) SIM mode with addition of deuterated standards, (c) UV detection (diode array 190–500 nm). Sample preparation for (b): 900 μL of each sample are mixed with 100 μL of a deuterated standard solution in two different concentrations (10^{-4}, 10^{-5} mol/L). This mixture is then injected into the HPLC–MS system and analyzed using the same time program as for the recovery rates. UV quantification is performed at 360 nm using the chromatographic conditions described for the HPLC–diode array detection system.

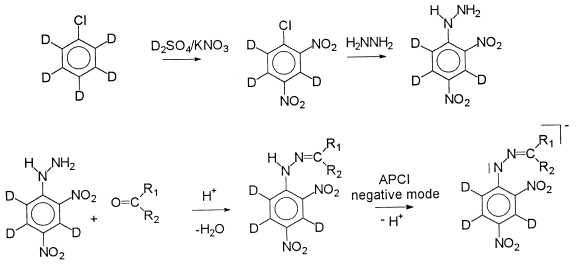
3. Results and discussion

The synthesis of 2,4-dinitro-3,5,6-trideuterophenylhydrazine (d_3DNPH) and its hydrazones and their application as internal standards for the quantification of aldehydes and ketones via HPLC–MS is the major aim of this work. Internal standards for the determination of carbonyls can be obtained by use of the labelled aldehydes as described in [13]. The use of labelled carbonyls is limited due to two reasons: Especially in case of the higher aldehydes or ketones, they are either not commercially available

Table 1
Time programmes for the SIM traces for three hydrazone mixtures

Mixture 1 containing the hydrazones of formaldehyde, acetaldehyde. acetone, propanal, methacrolein, cyclohexanone, *p*-tolylaldehyde, hexanal.					
t [min]	2.5–4.0	4.0–5.1	5.1–6.9	6.9–8.2	8.2–12.0
m/*z*	209, 212	223, 226	237, 240	249, 252	277, 279, 280, 282, 299, 302
Mixture 2 containing the hydrazones of acrolein, crotonaldehyde, butanal, benzaldehyde.					
t [min]	5.0–6.4	6.4–9.5			
m/*z*	235, 238	249, 251, 252, 254, 285, 288			
Mixture 3 containing the hydrazones of 2-butanone and pentanal.					
t [min]	6.4–8.2	8.2–10.5			
m/*z*	251, 254	265, 268			

or extremely expensive. The other reason is that exchange reactions are possible, if the deuterated carbonyls are used. We have therefore synthesized d_3DNPH according to the following equation. The two-step synthesis of the reagent consists of the nitration of [$^2H_5$]-chlorobenzene and the subsequent nucleophilic substitution of the chlorine atom by hydrazine.



This new trideuterated DNPH allows the synthesis of any desired internal hydrazone standard according to the standard procedure of Behforouz [16]. Exchange reactions of these hydrazones are very unlikely as the acidity of the aromatic hydrogen or deuterium atoms, respectively, is extremely low with a pK_a value of 43 [18].

APCI in the negative mode has proved the most versatile ionization method for the class of DNP-hydrazones [11–13]. The pseudomolecular anions of the hydrazones are obtained by abstraction of a proton from the hydrazone function. Fig. 1 shows the APCI-MS spectrum of acetone d_3DNP-hydrazone. The $[M\text{-}H]^-$ peak with m/z 240 is base peak of this spectrum. In this work, low accelerating voltages were applied to ensure that the $[M\text{-}H]^-$ ion is base peak of the spectra. For quantification purposes, the SIM trace of the respective $[M\text{-}H]^-$ ion is recorded.

Quantification was then performed using an internal calibration approach. The stable isotope-labelled standard differs by three mass units from the analyte in case of the d_3DNP-hydrazones. Both compounds exhibit similar chromatographic properties, although an isotope effect results in slightly shorter retention times for the deuterated compounds compared to the non-deuterated compounds. Therefore, standards and analytes are ionized under almost identical MS conditions. With known concentration of the isotope-labelled standard, the concentration of the analyte can be calculated on base of the ratio of the SIM trace peak areas. The recovery rates for various aldehydes determined with this technique are listed in Table 2. They can be found in a range from 88%

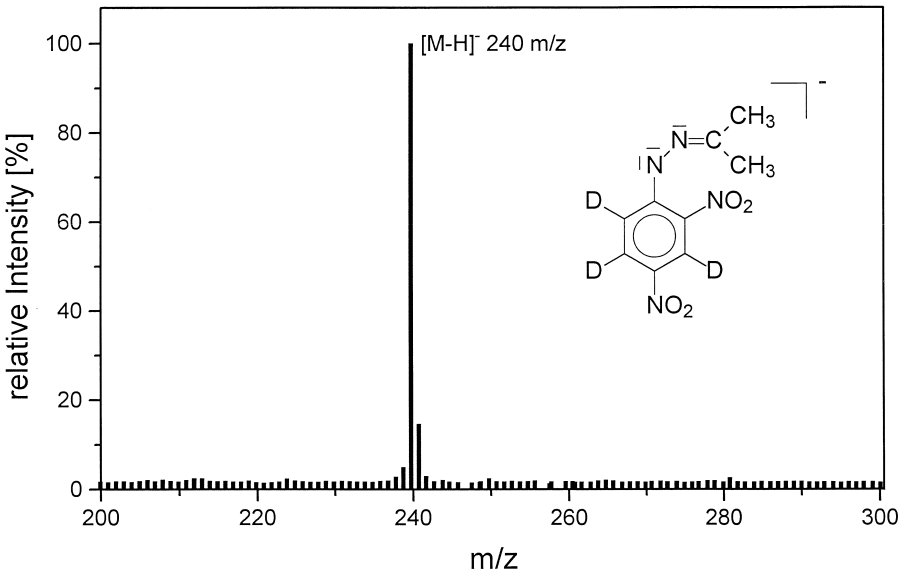


Fig. 1. APCI-MS spectrum of acetone d_3DNP-hydrazone.

Table 2
Recovery rates of various carbonyl compounds using internal standards

	Recovery (%)
Formaldehyde	88±10
Acetaldehyde	101±5
Acetone	95±6
Propanal	97±5
Acrolein	98±5
Methacrolein	103±6
Crotonaldehyde	100±8
Butanal	97±7
2-Butanone	100±5
Pentanal	91±4
Hexanal	101±6
Benzaldehyde	92±10
p-Tolylaldehyde	95±7

to 103% with standard deviations ($n=9$) from 4% to 10%. The recovery rate of formaldehyde is comparably low because the detection of the formaldehyde d_3DNP-hydrazone SIM trace with $m/z=212$ is disturbed by an unknown compound from the eluent exhibting the same mass-to-charge ratio. Especially for lower concentrations, it may be advantageous to use [$^2H_2$]-formaldehyde DNP-hydrazone [13] as internal standard in order to avoid this interference. However, the method delivers excellent recoveries for all other carbonyls investigated.

Limits of detection (LODs) are 0.1 μmol/L, limits of quantification 0.5 μmol/L. Best results are obtained when the ratio of concentration between deuterated and non-deuterated compounds is between 1:10 and 10:1. These results are in good agreement to data obtained with APCI-MS detection and external calibration with DNP-hydrazone standards [13]. The LODs of UV–Vis detection for the DNP-hydrazones cover a similar concentration range of 0.05 μmol/L to 0.1 μmol/L. However, selectivity of MS detection is by far superior compared to UV–Vis detection.

A series of car exhaust samples was analyzed regarding their content of carbonyl compounds. The UV ($\lambda=300$ nm and $\lambda=360$ nm) and MS chromatograms of the SIM traces at m/z 197 ([M-H]$^-$ of DNPH), m/z 209 ([M-H]$^-$ of formaldehyde DNP-hydrazone), m/z 223 ([M-H]$^-$ of acetaldehyde DNP-hydrazone), m/z 235 ([M-H]$^-$ of acrolein DNP-hydrazone), m/z 237 ([M-H]$^-$ of acetone DNP-hydrazone and propanal DNP-hydrazone), m/z 285 ([M-H]$^-$ of benzaldehyde DNP-hydrazone), m/z 299 ([M-H]$^-$ of tolylaldehyde DNP-hydrazone) of a regular fuelled car exhaust real sample (car D) are presented in Fig. 2. Significant amounts of DNPH and the aldehyde hydrazones are detected.

The quantification of selected aldehydes using UV detection is compared to MS detection based on internal calibration with d_3DNP-hydrazones. The respective data are provided in Table 3. Four samples were taken and analyzed as described in the experimental section. UV quantification was carried out at a detection wavelength of 360 nm. MS quantification was done by use of the internal standards. A closer look at the data of the individual aldehydes yields the following results: The formaldehyde concentrations determined by MS are always lower than the ones of UV detection. This result correlates well with the low recovery rate of 88%. The acetaldehyde contents correlate well for both detection techniques with differences between 1% and 6%. In the samples A and B, the presence of benzaldehyde was identified by MS detection, but the concentrations were below the limit of quantification (LOQ). The benzaldehyde concentrations of sample C and D show very good agreement.

The quantification in case of coeluting compounds with molecular masses differing by two mass units, e.g., acetone and acrolein, needs to be performed carefully. The reason is that the ^{13}C satellite of the acetone DNP-hydrazone exhibits the same m/z ratio like the internal standard acrolein d_3DNP-hydrazone. If the derivatives coelute, a quantification of acetone and acrolein in varying mixtures will therefore not be possible. The problem may be solved by a complete separation of these compounds. This may be achieved with a ternary gradient system consisting of methanol, tetrahydrofuran and water.

The analysis of car exhaust samples by HPLC–APCI-MS and the comparison to HPLC–UV demonstrates the superior selectivtity of MS detection. Internal standardization has proved a valuable tool for quantification of various aldehydes by HPLC–APCI-MS.

The synthesis of d_3DNPH and its hydrazones has therefore overcome the limited availability of stable isotope-labelled standards for the quantification of carbonyl compounds by means of HPLC–APCI-MS.

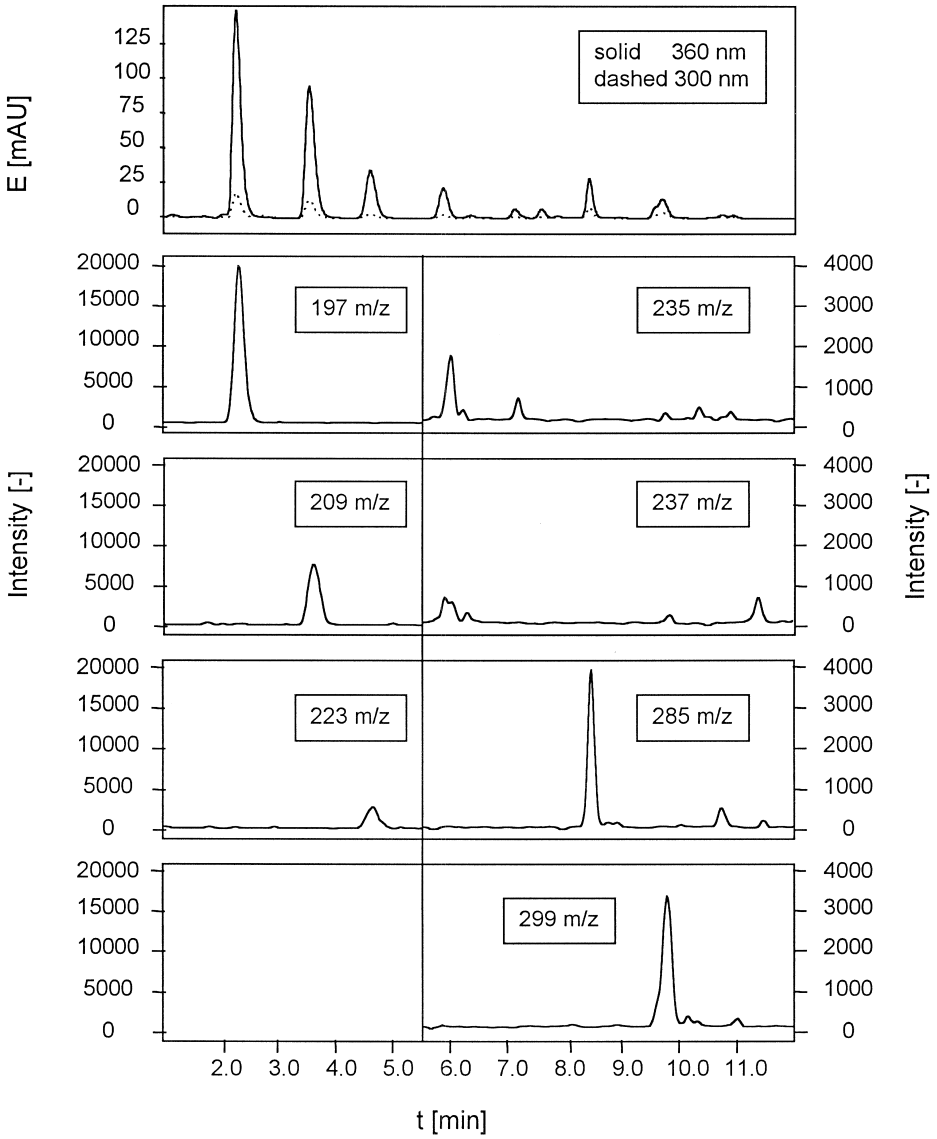


Fig. 2. UV and MS chromatograms (displayed as SIM traces) of a car exhaust real sample (car D).

Table 3
Concentrations of carbonyl compounds in car exhaust real samples in ppm using UV detection at 360 nm and MS detection with addition of internal standards

c [ppm]	Formaldehyde		Acetaldehyde		Propanal		Benzaldehyde	
	MS	UV	MS	UV	MS	UV	MS	UV
Car A	8.6	9.4	2.8	2.7	0.41	0.43	<LOQ	<LOQ
Car B	27.1	30.6	17.3	18.3	2.8	3.1	<LOQ	<LOQ
Car C	10.7	11.1	5.0	4.9	0.42	0.47	1.0	1.0
Car D	12.7	13.3	5.1	4.8	0.28	0.32	2.5	2.5

Acknowledgements

Financial support by the European Union ('Aldehydes' project, SMT4-CT97-2190) is gratefully acknowledged. We thank Supelco for providing the DNPH coated cartridges and the chromatographic columns. G.Z. thanks the Stiftung der Deutschen Wirtschaft (Berlin, Germany) for a scholarship.

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