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Non-porous silica for ultrafast reversed-phase high-performance liquid chromatographic separation of aldehyde and ketone 2,4-dinitrophenylhydrazones

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Abstract

Ultrafast high-performance liquid chromatographic separation of aldehyde and ketone 2,4-dinitrophenylhydrazones was carried out on reversed-phase 1.5 μm non-porous silica. Separation times of DNPH and a series of 12 aldehyde and ketone derivatives or potentially interfering compounds were less than 4 min compared to approximately 15 min using conventional columns. Formation of the formaldehyde derivative in solution was monitored using injection frequencies of 30 s for 15 min. The temperature dependence of the separation showed differences between the hydrazones of aliphatic aldehydes and those of unsaturated aldehydes. © 1997 Elsevier Science B.V.

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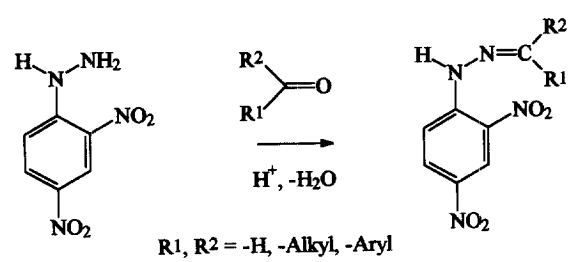
1. Introduction

Aldehydes and ketones are frequently used as intermediates and solvents in the production of organic chemicals and polymers. Further applications of aldehydes include disinfection in the food industries and medical sterilization. Lower aldehydes are formed in significant amounts as combustion by-products. Therefore, aldehyde and ketone determination has to be performed for a great variety of samples, including liquid and gas phases.

Among a large number of photometric, fluorimetric and chromatographic techniques for aldehyde and ketone monitoring, the 2,4-dinitrophenylhydrazine (DNPH) method has gained outstanding importance. The carbonyl compounds are derivatized with DNPH in acidic media, thus yielding the corresponding hydrazones [1,2] (see Scheme 1).

Separation of the reaction products is performed using high-performance liquid chromatography



R1, R2 = -H, -Alkyl, -Aryl

Scheme 1.

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(HPLC) with UV–Vis detection at approximately λ=360 nm, depending on the absorption maxima of the respective hydrazones [3,4].

Various sampling techniques are applied for gas phase aldehyde and ketone derivatization, including acidified solutions of DNPH in impingers [5–7], DNPH-coated solid sorbents in test tubes [8–10] and passive sampling devices [11,12].

The DNPH method allows the simultaneous determination of several different aldehydes in complex matrices. Typical times for chromatography vary from 10 min to more than 30 min. The separation of similar aldehyde hydrazones, e.g. those of propanal, acrolein and acetone is difficult, but can be achieved using specially adapted binary (water–acetonitrile) [13] or ternary (with addition of tetrahydrofuran or methanol) [14,15] gradients. With increasing alkyl chain length, the number of isomeric aldehydes increases, and the separation of ten different C_4 aldehyde and ketone hydrazones has not been achieved so far [16]. Ozone [17,18] and nitrogen dioxide [19] have been identified as chemical interferents when using the DNPH method, as their reaction with the reagent yields products which may coelute with the formaldehyde hydrazone. Whilst the main reaction product of DNPH and nitrogen dioxide has been identified as 2,4-dinitrophenylazide (DNPA) [19], the products of DNPH and ozone have not been characterized yet. DNPA and formaldehyde 2,4-DNPhydrazone may be separated by reversed-phase HPLC with only slightly modified elution conditions [19]. Dual-wavelength detection may be used as a tool for the identification of chemical interferences [16].

The DNPH method has been introduced as a standard procedure by several national standardization organisations and is currently under discussion as a standard method within the European Union. Several manufacturers are currently offering reversed-phase HPLC columns for aldehyde and ketone determination using the DNPH method. For further improvements, an increase of the chromatographic resolution should improve the separation of related compounds, e.g. of isomers and of chemical interferences from the hydrazones. A reduction of analysis time would enhance the effectiveness of the method, decrease the consumption of mobile phase and therefore reduce analysis costs.

HPLC columns based on non-porous silica stationary phases with particle sizes around 1.5 μm have been introduced recently. Procedures for the synthesis of the highly monodisperse particles have been described [20,21]. Their general properties and some applications are summarized by Hanson and Unger [22,23]. These phases permit a strong reduction of analysis time without loss of chromatographic resolution. Reversed-phase C_{18} columns based on 1.5 μm non-porous stationary phases have been mainly applied for the rapid analysis of proteins [24–27]. Within this work, the application of reversed-phase non-porous silica stationary phases for the separation of DNPH derivatives is introduced. On-line monitoring of the derivatization of formaldehyde with DNPH has been performed.

2. Experimental

2.1. Chemicals

All chemicals (aldehydes, ketones and DNPH) were purchased from Aldrich (Steinheim, Germany) in the highest quality available. Mineral acids and sodium azide were Merck (Darmstadt, Germany) analytical grade. Acetonitrile for HPLC mobile phases was Merck LiChroSolv gradient grade. Water for HPLC mobile phases was Milli Q from Millipore (Eschborn, Germany).

2.2. Synthesis

All hydrazones described here were prepared according to a modified procedure [16] based on the work of Behforouz et al. [28]. The DNPA standard was synthesized based on a procedure published by Bailey and Case [29].

2.3. Air sampling and sample preparation

Air sampling and sample preparation were performed according to the procedures described in Refs. [10,16].

2.4. HPLC instrumentation

2.4.1. System A

The high-pressure gradient system was based on two HPLC pumps (Model 2150 from LKB, Bromma, Sweden) and a HPLC controller Model 2152. For isocratic separations, a HPLC pump (Model 420 from Kontron Instruments, Zürich, Switzerland) was used. The injection unit consisted of an injector (Model 8125 from Rheodyne, Cotati, CA, USA) with a 1.5 μl loop. For detection, a HPLC-capillary detector 433 (Kontron) with a modified detector cell (1 μl) was used.

2.4.2. System B

The high-performance liquid chromatograph consisted of the following components: two pumps LC-10AS (Shimadzu, Duisburg, Germany), detector SPD-10AV (Shimadzu) with a cell volume of 8 μl, autosampler SIL-10A (Shimadzu), software Class LC 10 Version 1.4 (Shimadzu), and controller unit CBM-10A (Shimadzu). Injection volume was 5 μl.

The two reversed-phase columns based on non-porous silica were Micra NPS TAS 1.5 μm (C_{30} alkyl chain length, 33×4.6 mm; Micra Scientific, Northbrook, IL, USA) and Micra NPS ODS 1 1.5 μm (C_{18} alkyl chain length, 33×4.6 mm; Micra Scientific). The reversed-phase column based on porous silica was Merck LiChroSpher RP-18 5μm (C_{18} alkyl chain length, 250×3 mm; Merck)

2.5. HPLC analysis

Different isocratic and binary gradient eluents consisting of acetonitrile and water were selected for both Micra NPS columns and the Merck LiChroSpher column as listed below:

- Method 1: column: Micra NPS TAS, 1.5 μm, HPLC system: A

Gradient

Elution volume (ml)	0.0	3.0	3.75 (stop)
$c(CH_3CN)$ (%)	25	62	62

- Method 2: column: Micra NPS ODS1, 1.5 μm, HPLC system: A

Gradient

Elution volume (ml)	0.0	3.3	5.1	6.0 (stop)
$c(CH_3CN)$ (%)	20	35	91	91

- Method 3: column: Micra NPS TAS, 1.5 μm, HPLC system: A

  Isocratic, mobile phase: CH_3CN–H_2O (30:70, v/v).

- Method 4: column: LiChroSpher RP-18, 5 μm, HPLC system: B

Gradient

Elution volume (ml)	0.0	0.62	4.03	4.96	7.13	8.99	12.09 (stop)
$c(CH_3CN)$ (%)	49	49	65	80	80	49	49

Detection wavelength was λ=360 nm for all investigations. Gradient profiles displayed in the chromatograms are the effective profiles determined by using methanol with 500 ppm toluene instead of acetonitrile and methanol instead of water as mobile phases for the individual gradients. The increasing toluene concentration was used to monitor the gradient profile (λ=254 nm). It should be noted that these gradient profiles report the composition of the mobile phase at the head of the column. The resulting time delay between chromatogram and gradient profile is due to the dead volume of the respective column.

3. Results and discussion

The separation of the most significant aldehyde and ketone 2,4-DNPhydrazones was carried out using the two differently modified reversed-phase non-porous silica columns NPS TAS 1.5 μm and NPS ODS1 1.5 μm. All aldehydes and ketones described here are listed in the EPA-method TO-11. Fig. 1 shows the separation on NPS TAS 1.5 μm, whereas in Fig. 2 the respective chromatogram on NPS ODS1 1.5 μm is presented. For comparison, separation of these hydrazones under HPLC-conditions as described in Ref. [16] is shown in Fig. 3.

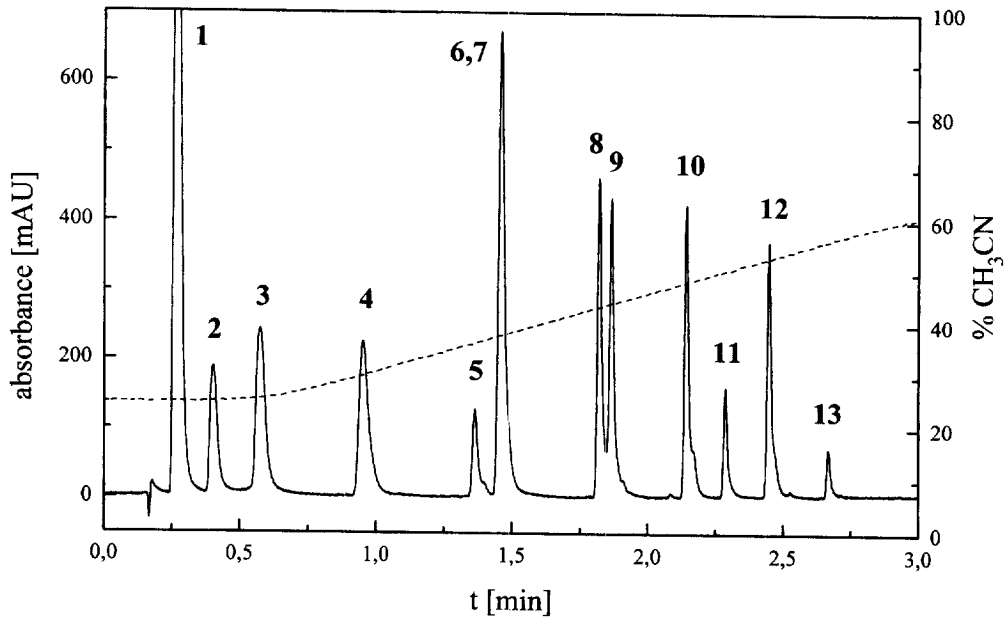

Fig. 1. Separation of a series of aldehyde and ketone hydrazones on NPS TAS. Chromatographic conditions: see method 1. Temperature: ambient. Flow-rate: 1.5 ml/min. Peaks: 1=DNPH, 2=DNPA, and the 2,4-DNPhydrazones of 3=formaldehyde, 4=acetaldehyde, 5=acetone, 6=acrolein, 7=propanal, 8=crotonaldehyde, 9=butanal, 10=pentanal, 11=benzaldehyde, 12=hexanal, 13=*p*-tolualdehyde.

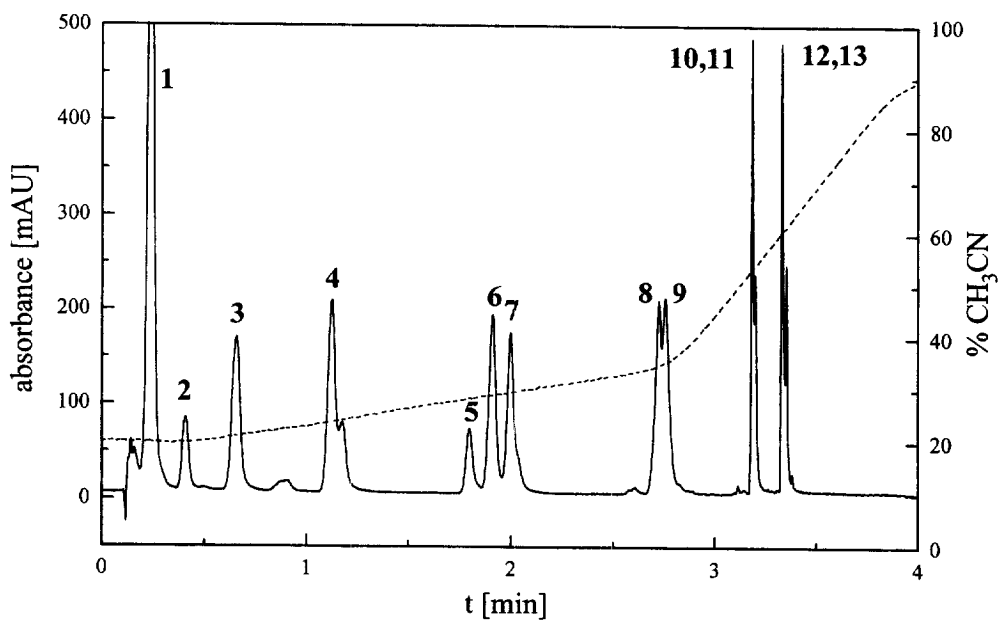

Fig. 2. Separation of a series of aldehyde and ketone hydrazones on NPS ODS1. Chromatographic conditions: see method 2. Temperature: ambient. Flow-rate: 1.5 ml/min. Characterization of the hydrazones: see Fig. 1.

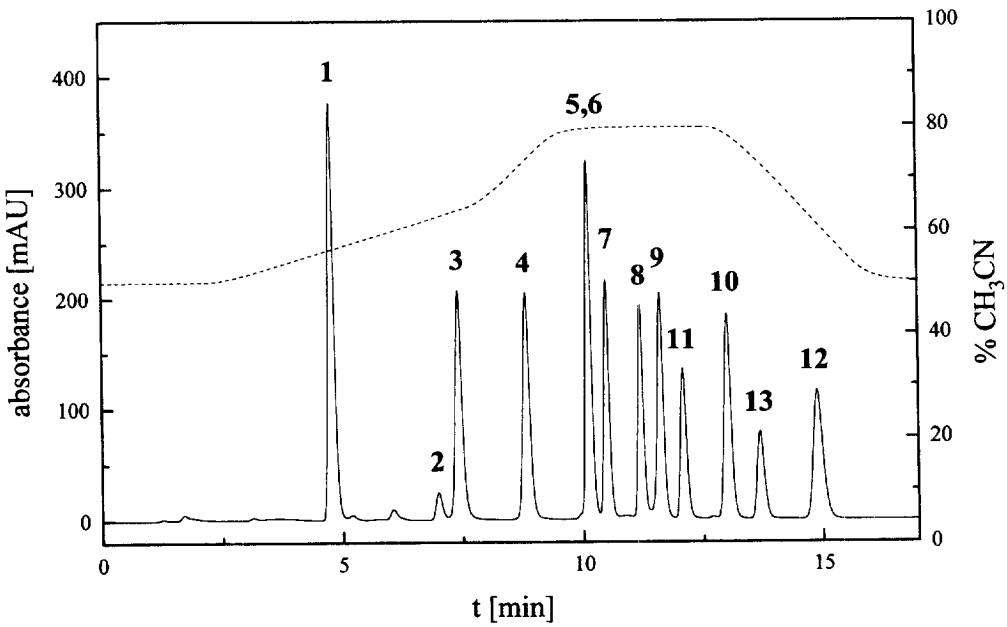

Fig. 3. Separation of a series of aldehyde and ketone hydrazones on Merck LiChroSpher RP-18. Chromatographic conditions: see method 4. Temperature: ambient. Flow-rate: 0.62 ml/min. Characterization of the hydrazones: see Fig. 1.

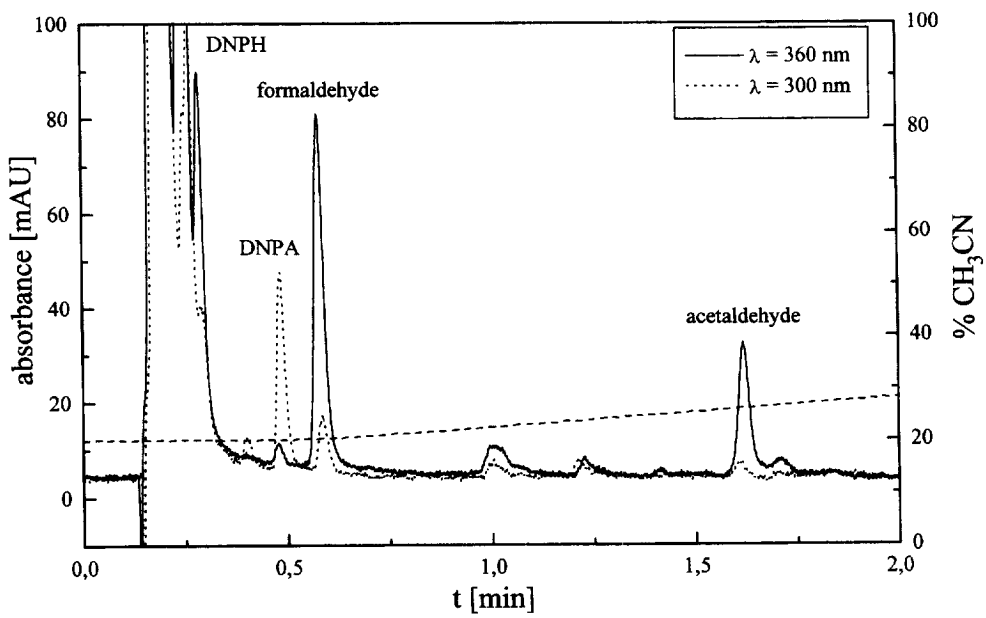

Fig. 4. Chromatogram of an automobile exhaust sample using the NPS ODS1 column. Chromatographic conditions: see method 2. Temperature: ambient. Flow-rate: 1.3 ml/min.

As expected, separation time could be reduced drastically from approximately 15 min using the classical reversed-phase C_{18} column to less than 4 min without loss of separation performance. Depending on the individual column and the elution conditions, the complete hydrazone series of aliphatic (C_1 to C_6 saturated, C_3 and C_4 unsaturated) and aromatic (benzaldehyde, *p*-tolualdehyde) aldehydes including acetone is analyzed within less than 4 min. Both columns are well suited for separation of the DNPhydrazones. DNPH is baseline separated from formaldehyde hydrazone. The NPS TAS column is favourable for the separation of the hydrazones of the long-chain aliphatic aldehydes, whereas the NPS ODS1 column shows better separation for the group of C_3 aldehyde hydrazones.

The DNPH method is particularly useful for industrial applications, including the determination of aldehydes in automobile or industrial exhaust. An automobile exhaust sample has been analyzed using the NPS ODS1 column and the elution conditions as stated above. The respective chromatogram is presented in Fig. 4.

Again, baseline separation of DNPA and the formaldehyde hydrazone is observed. Dual-wavelength detection at λ=360 nm and λ=300 nm confirms the excellent separation of these two compounds and the applicability of the column for automobile exhaust analysis.

The temperature dependence of the hydrazone separation in the temperature range of 20–30°C on NPS ODS1 is presented in Fig. 5.

Retention of all components in the chromatograms is reduced with increasing temperature. As an interesting effect, the retention times of the hydrazones of the unsaturated aldehydes acrolein and crotonaldehyde are decreasing more with increasing temperature than those of the saturated hydrazones with identical chain lengths. The C_4 aldehyde hydrazones coelute at 20°C, but are baseline separated at 28°C. Acrolein 2,4-DNPhydrazone coelutes with the propanal hydrazone at lower temperatures, but moves to shorter retention times to coelute partially with the acetone hydrazone at 30°C. At a temperature of 28°C, the optimum resolution of all C_3 hydrazones could be achieved.

Due to the long analysis times in chromatography of the DNPH derivatives, it is difficult to obtain

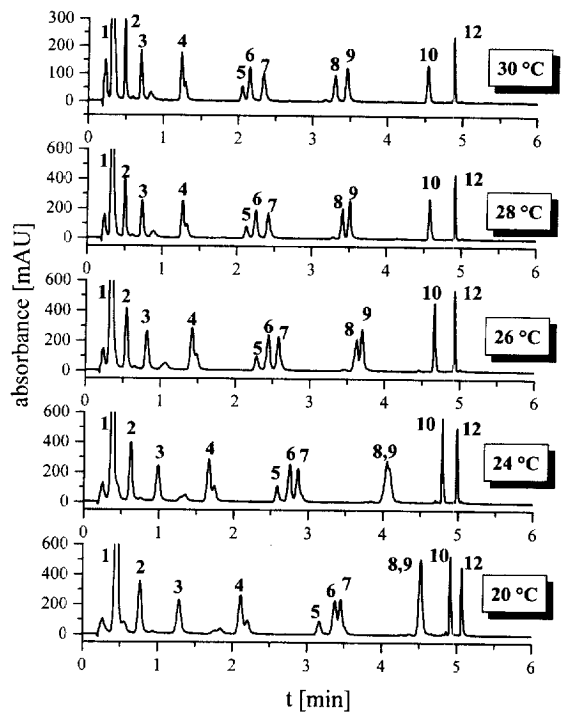

Fig. 5. Temperature dependence of the hydrazone separation on the NPS ODS1 column. Chromatographic conditions: see method 2. Flow-rate: 1.0 ml/min. Characterization of the hydrazones: see Fig. 1.

HPLC information about the reaction times of the aldehydes with DNPH in solution. In general, this information could be obtained using spectrophotometry, as acidified DNPH and the hydrazones exhibit significantly different absorption maxima. On the other hand, the simulation of realistic sampling conditions requires a large excess of reagent compared to the analyte. In this case, formation of a very small amount of product in the presence of this reagent excess cannot be monitored using UV–Vis spectroscopy. As the non-porous silica stationary phases allow an extremely rapid analysis of the DNPH derivatives, injection frequency for HPLC can be increased to two injections per minute. This allows a reliable monitoring of product formation, as can be seen in Fig. 6a. Baseline separation of the reagent and the product of the reaction can still be achieved under these separation conditions, as demonstrated in Fig. 6b.

In Fig. 7, peak height and peak area of the

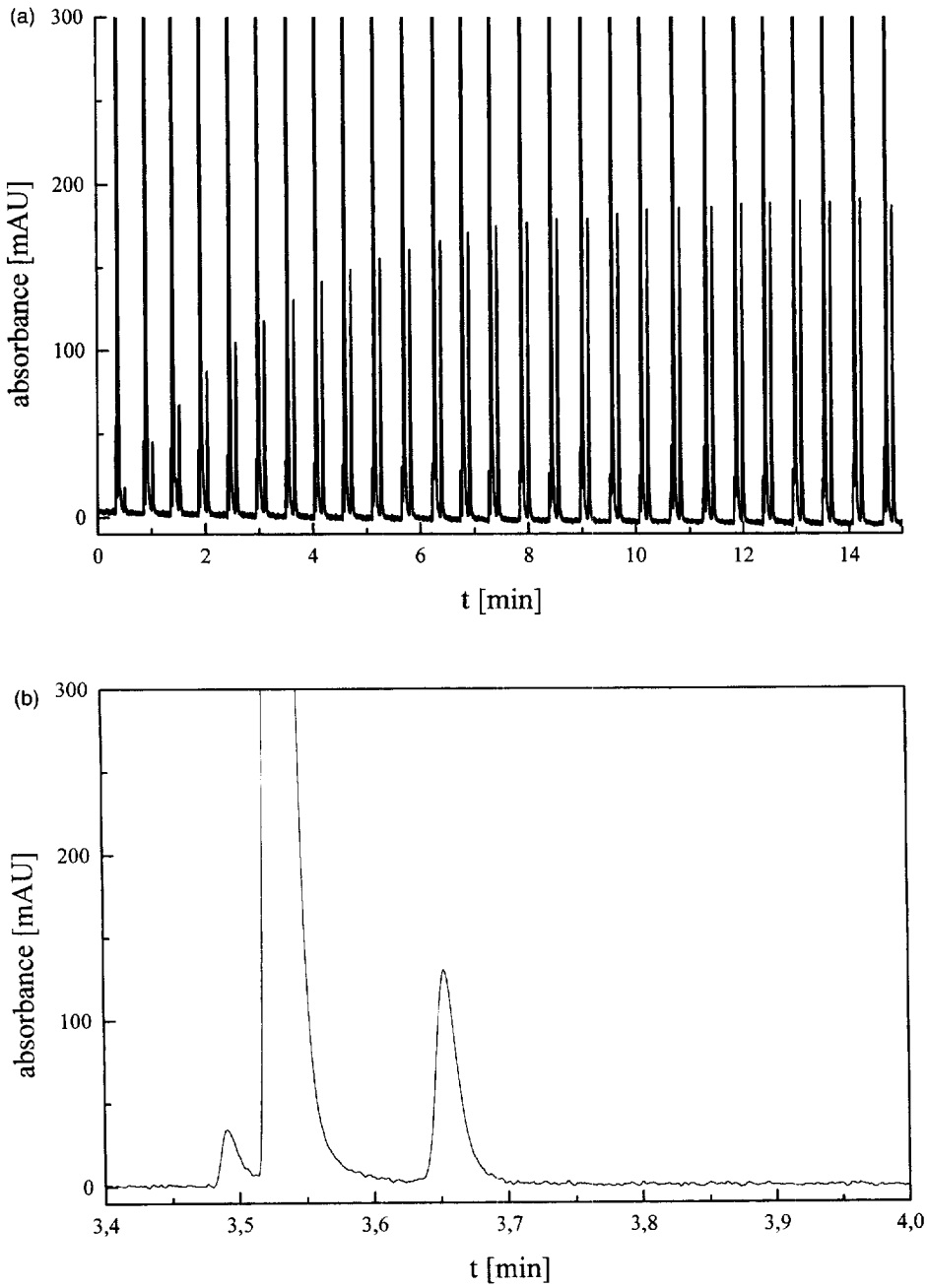

Fig. 6. (a) Formation of formaldehyde 2,4-DNPhydrazone monitored by multiple sample injection. Concentrations: $5 \cdot 10^{-4}$ *M* DNPH, $5 \cdot 10^{-5}$ *M* formaldehyde, 0.05 *M* H_2SO_4 in acetonitrile–water (30:70, v/v). Chromatographic conditions: see method 3. Temperature: ambient. (b) Section of Fig. 6a.

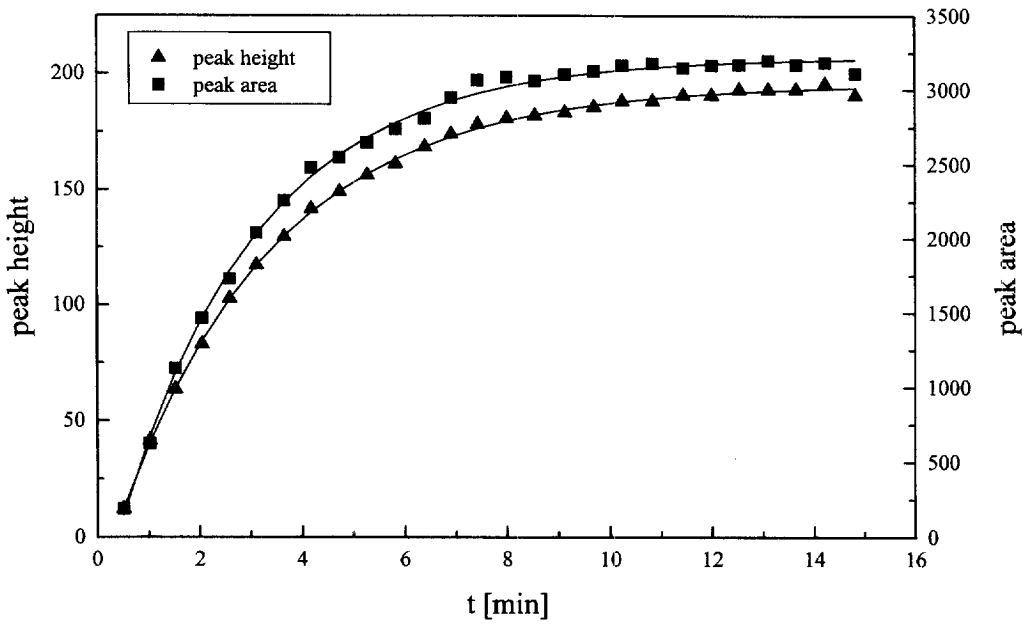

Fig. 7. Peak height and peak area of the formaldehyde hydrazone versus reaction time.

formaldehyde hydrazone peak from Fig. 6a are plotted versus the reaction time. It should be noted that the times listed for the peak areas and peak heights include a time delay of 10 s caused by the separation process.

From Figs. 6 and 7, it is obvious that separation of the hydrazones on reversed-phase non-porous silica columns is an excellent tool for the on-line monitoring of fast reactions in solution. Injection frequency is enhanced to two samples per minute using the separation conditions described here. It should be noted that injection frequency is limited more by the possible injection frequency for manual or automatic injection than by the separation conditions. As demonstrated in this example, ultrafast HPLC separation based on reversed-phase non-porous stationary phases may show new aspects to applications of liquid chromatography, including industrial process control.

4. Conclusions

Analysis times for the separation of aldehyde and ketone 2,4-dinitrophenylhydrazones can be shortened drastically without loss of chromatographic performance on reversed-phase non-porous silica stationary phases, thus enhancing the effectiveness of the process. The reagent, several of the corresponding hydrazones and some interfering compounds known to occur in gas phase real samples are baseline separated. The fast chromatographic separation permits high resolution on-line monitoring of the hydrazone formation.

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References

[1] C.F.H. Allen, J. Am. Chem. Soc. 52 (1930) 2955–2959.

[2] O.L. Brady, J. Chem. Soc., (1931) 756–759.
[3] Z. Rappoport and T. Sheradsky, J. Chem. Soc. B, (1968) 277–291.
[4] F. Bohlmann, Chem. Ber. 84 (1951) 490–504.
[5] D. Grosjean, K. Fung, Anal. Chem. 54 (1982) 1221–1224.
[6] F. Lipari, S.J. Swarin, J. Chromatogr. 247 (1982) 297–306.
[7] D. Grosjean, Environ. Sci. Technol. 16 (1982) 254–262.
[8] R.H. Beasley, C.E. Hoffmann, M.L. Rueppel, J.W. Worley, Anal. Chem. 52 (1980) 1110–1114.
[9] X. Zhou, K. Mopper, Environ. Sci. Technol. 24 (1990) 1482–1485.
[10] N. Binding, S. Thiewens, U. Witting, Staub-Reinhalt. Luft 46 (1986) 444–446.
[11] J.-O. Levin, K. Andersson, R. Lindahl, C.-A. Nilsson, Anal. Chem. 57 (1985) 1032–1035.
[12] J.-O. Levin, R. Lindahl, Analyst 119 (1994) 79–83.
[13] P.R. Houlgate, K.S. Dhingra, S.J. Nash, W.H. Evans, Analyst 114 (1989) 355–360.
[14] C.H. Risner, P. Martin, J. Chrom. Sci. 32 (1994) 76–82.
[15] M. Possanzini, V. Di Palo, Chromatographia 40 (1995) 134–138.
[16] W. Pötter, U. Karst, Anal. Chem. 68 (1996) 3354–3358.
[17] R.R. Arnts, S.B. Tejada, Environ. Sci. Technol. 23 (1989) 1428–1430.
[18] D.F. Smith, T.E. Kleindienst, E.E. Hudgens, J. Chromatogr. 483 (1989) 431–436.
[19] U. Karst, N. Binding, K. Cammann, U. Witting, Fres. J. Anal. Chem. 345 (1993) 48–52.
[20] W. Stöber, A. Fink, E. Bohn, J. Colloid Interface Sci. 26 (1968) 62–69.
[21] T.J. Barder and P.D. DuBois, US 4983369 (1989).
[22] M. Hanson, K.K. Unger, LC·GC 9 (1996) 650–656.
[23] M. Hanson, K.K. Unger, LC·GC 9 (1996) 741–746.
[24] R. Janzen, K.K. Unger, H. Giesche, J.N. Kinkel, M.T.W. Hearn, J. Chromatogr. 397 (1987) 91–97.
[25] R. Janzen, K.K. Unger, H. Giesche, J.N. Kinkel, M.T.W. Hearn, J. Chromatogr. 397 (1987) 81–89.
[26] G. Jilge, R. Janzen, H. Giesche, K.K. Unger, J.N. Kinkel, M.T.W. Hearn, J. Chromatogr. 397 (1987) 71–80.
[27] K.K. Unger, G. Jilge, R. Janzen, H. Giesche, J.N. Kinkel, Chromatographia 22 (1986) 379–380.
[28] M. Behforouz, J.L. Bolan, M.S. Flynt, J. Org. Chem. 50 (1985) 1186–1189.
[29] A.S. Bailey, J.R. Case, Tetrahedron 3 (1958) 113–131.