Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Journal of Chromatography A, 996 (2003) 95–102
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Isomerization of aldehyde-2,4-dinitrophenylhydrazone derivatives and validation of high-performance liquid chromatographic analysis

Shigehisa Uchiyama[a,*], Masanori Ando[a], Shohei Aoyagi[b]

[a]*Division of Environmental Chemistry, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo, 158-8501, Japan*
[b]*Faculty of Engineering, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan*

Received 18 December 2002; received in revised form 28 February 2003; accepted 25 March 2003

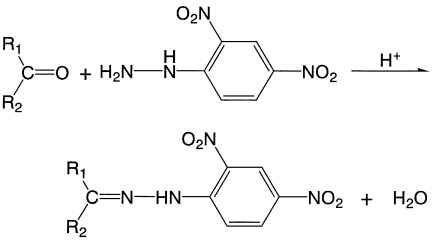
Abstract

The most widely used method for qualitative and quantitative analysis of carbonyl compounds is the 2,4-dinitrophenylhydrazine method through the formation of 2,4-dinitrophenylhydrazone derivatives. However, this method may cause an analytical error because 2,4-dinitrophenylhydrazones have both *E*- and *Z*-stereoisomers. Purified aldehyde-2,4-dinitrophenylhydrazone demonstrated only the *E*-isomer. However under UV irradiation and the addition of acid, both *E*- and *Z*-isomers were seen. The spectral patterns of *Z*-isomers were different from those of *E*-isomers and the absorption maximum wavelengths were shifted towards shorter wavelengths by 5–8 nm. An equilibrium *Z*/*E* isomer ratio was observed in 0.02–0.2% (v/v) phosphoric acid solutions. In the case of acetaldehyde- and propanal-2,4-dinitrophenylhydrazones, the equilibrium *Z*/*E* isomer ratios were 0.32 and 0.14, respectively. However, when irradiated with ultraviolet light at 364 nm, the isomer ratios were increased beyond this constant ratio and reached 0.55 and 0.33, respectively. Zero-order rates for decreases of aldehyde derivatives were observed under UV irradiation (364 nm), however the decreases of concentration were not observed in phosphoric acid solutions. The best method for the determination of aldehyde-2,4-dinitrophenylhydrazones by HPLC is to add phosphoric acid to both the sample and the standard solution, to form a 0.02–1% acid solution.

Keywords: Derivatization, LC; Validation; Aldehyde-2,4-dinitrophenylhydrazone

1. Introduction

The specific reaction of carbonyl compounds with 2,4-dinitrophenylhydrazine (DNPH) forming the corresponding 2,4-dinitrophenylhydrazones is one of the most important qualitative and quantitative methods in organic analysis. It was first published by Allen [1] and Brady [2]. The carbonyl compounds react with acidified DNPH in solid or liquid phase as follows:



*Corresponding author. Tel.: +81-3-3700-9298; fax: +81-3-3700-9298.
E-mail address: uchiyama@nihs.go.jp (S. Uchiyama).

0021-9673/03/$ – see front matter
doi:10.1016/S0021-9673(03)00542-9

The main advantage of the DNPH method is the ability to analyze various aldehydes and ketones simultaneously in a complex mixture. Sampling can be performed using acidic solutions of DNPH in impingers [3] or with acidic solid sorbents using a DNPH-coated cartridge. A number of cartridge devices containing solid sorbents coated with DNPH have recently been provided for sampling aldehydes in air. The solid sorbents include XAD-2 [4,5], silica gel [6,7], glass beads [8], octadecylsilane bonded silica gel [9], Florisil [10], and glass fiber filters [11]. Recently, DNPH-coated silica gel has been widely used for active [12] and diffusive [13] sampling methods. Due to the importance of the method, it has been introduced as a standard procedure by several national standardization bodies. Recent research has resulted in the identification of chemical interferences caused by the presence of ozone [14–16] or nitrogen dioxide [17] in air samples.

The formation of isomeric 2,4-dinitrophenylhydrazones from unsymmetrical carbonyl compounds in the liquid phase has since long been known [18–20]. Behforouz et al. [21] and Tayyari et al. [22] reported that a trace of acid catalyzed the *E–Z* isomerization which was detected via melting point anomalies. However, until recently, this new evidence seemed irrelevant with regard to possible analytical problems it would cause in the determination of airborne aldehydes and ketones. In particular, there is no information available with respect to the occurrence and the extent of isomerization when solid sorbent samplers are used.

Usually, 2,4-dinitrophenylhydrazone derivatives extracted from solid sorbent are separated by means of high-performance liquid chromatography (HPLC) and detected using UV spectrophotometry at 360 nm (depending on the absorption maximum of the hydrazones). In this study, the influence of UV irradiation and the addition of acid on isomerization were examined. As a result, the most appropriate method for the determination of aldehyde-2,4-dinitrophenylhydrazones by HPLC or gas chromatography (GC) is proposed.

2. Experimental

2.1. Apparatus

The HPLC system (Shimadzu, Kyoto, Japan) used included two LC-10ADvp pumps, an SIL-10ADvp autosampler, an SPD-10Avp ultraviolet absorbance detector adjusted to 360 nm and an SPD-M10Avp photo diode array detector. The analytical columns used were 250 mm×4.6 mm I.D. stainless steel tubes packed with Zorbax Bonus-RP, 5 μm particle size (Bonus-RP, Agilent, Palo Alto, CA, USA), Discovery RP-Amide C_{16}, 5 μm particle size (Amide C_{16}, Supelco, Bellefonte, PA, USA), and Discovery C_{18}, 5 μm particle size (C_{18}, Supelco). The eluents were acetonitrile and water. A gradient combination of acetonitrile–water was employed as follows:

Time (min)	0	5	25	40	60
Acetonitrile (%)	40	40	58	70	70

The flow-rate of the mobile phase was 1.5 ml/min; the column temperature was 40 °C and the injection volume was 20 μl. The TFL 40 UV transilluminator (UVP, San Gabriel, CA, USA) was set at 364 nm, 25 W×4 tubes were used for UV irradiation. The LC–MS equipment was a Finnigan AQA (ThermoQuest, San Jose, CA, USA).

2.2. Reagents

The water used in HPLC and sample preparation was deionized and further purified using a Milli-Q water system (Millipore, Bedford, MA, USA). Acetonitrile was HPLC grade (Wako, Osaka, Japan). 2,4-DNPH (containing approx. 50% water) and phosphoric acid (85%, w/v, solution in water) pure grade were obtained from Wako. The standard reagents of 2,4-dinitrophenylhydrazone derivatives of formaldehyde, acetaldehyde, propanal, butanal, pentanal, hexanal, heptanal, octanal, nonanal and decanal were commercial synthetic products (99%, Chem Service, West Chester, PA, USA). Solid-phase extraction tubes packed with 2,4-DNPH coated silica gel (DNPH-cartridge) were obtained from Supelco (LpDNPH S10) and contained 1 mg 2,4-DNPH.

2.3. E to Z isomerization by ultraviolet irradiation

Solutions of aldehyde-2,4-dinitrophenylhydrazone in acetonitrile (1000, 100, and 10 μmol/l) were prepared by dissolving 100, 10 and 1 μmol of acetaldehyde-2,4-dinitrophenylhydrazone or pro-

panal2,4-dinitrophenylhydrazone in 100 ml acetonitrile. A 20 mm optical path length cylindrical quartz cell with screw cap (cell volume 35 ml, GL Sciences, Tokyo, Japan) was filled with this mixture and then attached to the surface of the UV transilluminator. The solution was irradiated continuously at 364 nm and analyzed every 30 min by HPLC. Isomer ratios were calculated from their peak areas at 362 nm.

2.4. E to Z isomerization with phosphoric acid

A 5-ml volume of 100 μmol/l acetaldehyde-2,4-dinitrophenylhydrazone and propanal-2,4-dinitrophenylhydrazone solutions was prepared in 10-ml volumetric flasks. Aliquots (10–1000 μl) of the phosphoric acid/acetonitrile solutions (0.01–10%, v/v) were added and immediately diluted to 10 ml with acetonitrile. After 0.5, 9, 18 and 27 h, the solutions were examined by HPLC. Isomer ratios were calculated from their peak areas at 362 nm.

2.5. Air sample analysis

The measurement of aldehydes in air was carried out in accordance with an established active sampling method [12]. An indoor air sample (144 l) was drawn through the DNPH-cartridge using a pumping system (APN-085V-1; Iwaki, Tokyo, Japan) provided with a mass flow controller (Model SEC-400 MARK3; STEC, Kyoto, Japan) set to 100 ml/min. The DNPH-cartridges were eluted in reversed direction by gravity feed with 5 ml of acetonitrile. The eluate was analyzed by HPLC.

3. Results and discussion

3.1. Optimization of the HPLC method

Analytical conditions for *E*- and *Z*-isomers were examined for the acetaldehyde-2,4-dinitrophenylhydrazone derivative using three different HPLC columns (Fig. 1). Only one peak appeared on the C_{18} column. On the Bonus RP and Amide C_{16} columns there was one more significant component that eluted just before the main acetaldehyde-2,4-dinitrophenylhydrazone peak. LC–MS analysis of this peak suggested that the additional component was a

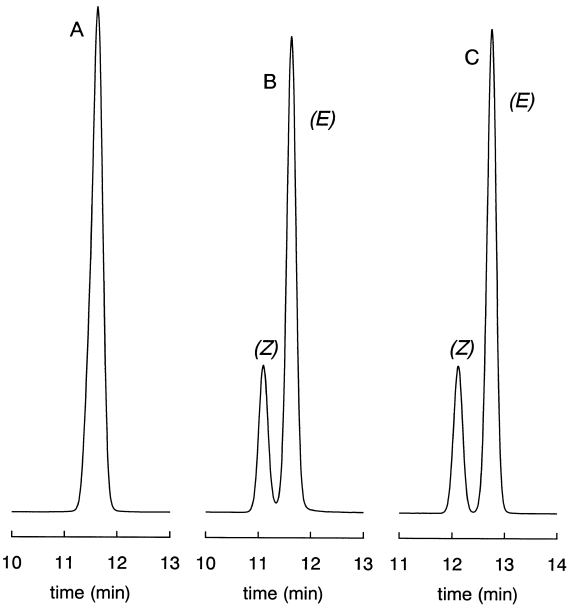
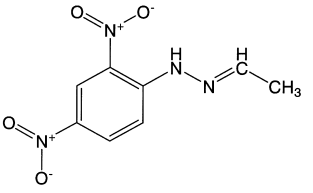
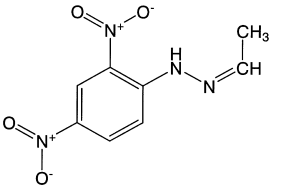


Fig. 1. Chromatographic profiles of acetaldehyde-2,4-dinitrophenylhydrazone on three columns (A: C_{18}, B: Amide C_{16}, C: Bonus RP).

stereoisomer of the main acetaldehyde-2,4-dinitrophenylhydrazone derivative because it gave a similar mass spectrum.



E-isomer



Z-isomer

The HPLC separation of the *E*- and *Z*-isomers of acetaldehyde-2,4-dinitrophenylhydrazone was the

most difficult case, which succeeded by using the Bonus RP and RP-Amide C_{16} columns. Those are alkylamide reversed-phase type columns based on high-purity silica. The presence of the alkylamide group gives these columns a different selectivity compared to the simple hydrophobic affinity of the C_{18} alkyl groups for the C_{18} columns [23]. Due to the presence of both alkyl and alkylamide groups on the RP-Amide C_{16} column, two retention mechanisms are available—a mechanism based on affinity with hydrophobic alkyl groups and a mechanism based on interaction between the polar groups of the 2,4-dinitrophenylhydrazone derivatives with the amide group. Therefore, amide columns such as Bonus-RP or RP-Amide C_{16} provide unique selectivity and applicability resulting in sufficient resolution between *E*- and *Z*-isomers of the 2,4-dinitrophenylhydrazone derivative. In the chromatograms produced using amide columns, the acetaldehyde-2,4-dinitrophenylhydrazone isomers are completely separated. The isomers of other C3–C10 straight-chain aldehyde-2,4-dinitrophenylhydrazones were completely separated using the three columns mentioned; besides it was difficult to obtain a chromatogram corresponding to the non-separate isomers.

3.2. UV–visible spectra of isomers

UV–visible spectra of *E*- and *Z*-isomers of acetaldehyde-2,4-dinitrophenylhydrazone derivatives are presented in Fig. 2. The *E*- and *Z*-isomers showed different spectral profiles and different absorption maxima (λ_{max}) of 365 and 360 nm, respectively. Spectral profiles of other aldehyde-2,4-dinitrophenylhydrazones were similar to that of acetaldehyde-2,4-dinitrophenylhydrazone, and in each case the absorption maximum for the *Z*-isomer was shifted towards shorter wavelength by 5–8 nm compared to the *E*-isomer (see Table 1).

The accurate isomer ratio should be obtained from the concentrations of *E*- and *Z*-isomers. However, it is impossible to determine the concentration of *Z*-isomer because there are no standard reagents of only *Z*-isomers. Therefore, the isomer ratio was defined as the peak area ratio, and calculated from the middle value (362 nm) of absorption maxima of *E*- and *Z*-isomers.

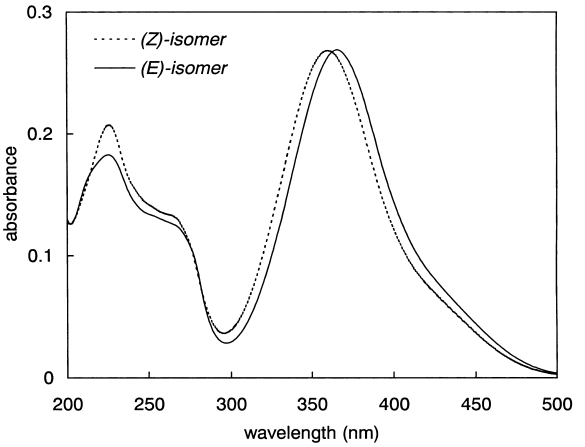


Fig. 2. UV spectrum of acetaldehyde-2,4-dinitrophenylhydrazone derivatives.

3.3. E to Z isomerization by ultraviolet irradiation

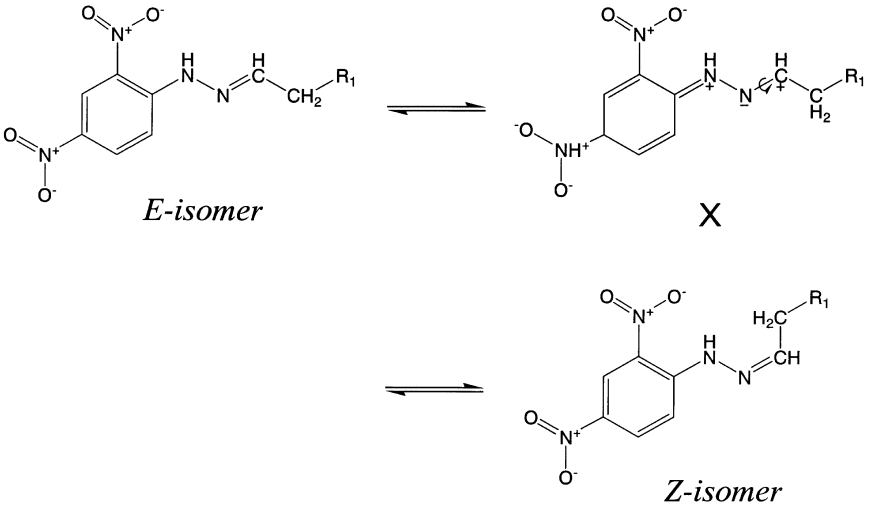
HPLC analysis of the purified aldehyde-2,4-dinitrophenylhydrazones revealed only the *E*-isomers. However under UV irradiation, both *E*- and *Z*-isomers were seen. Fig. 3 shows changes in the *Z*-to-*E* ratio for acetaldehyde-2,4-dinitrophenylhydrazone and propanal-2,4-dinitrophenylhydrazone following irradiation at 364 nm. In the case of acetaldehyde-2,4-dinitrophenylhydrazone, the *Z*- to *E*-isomer ratio at the initial concentration of 1000 μmol/l increased depending on the irradiation time and reached a value of 0.35 at 480 min. The isomerization rate for an initial concentration of 100 μmol/l was faster than that of 1000 μmol/l, and the *Z*- to *E*-isomer ratio reached a value of 0.55 presumably corre-

Table 1
The wavelengths of maximum absorption of *E*- and *Z*-isomers

Derivative	(*Z*)-isomer λ_{max} (nm)	(*E*)-isomer λ_{max} (nm)
(Formaldehyde-DNPH)	(356)	
Acetaldehyde-DNPH	360	365
Propanal-DNPH	358	366
Butanal-DNPH	358	365
Pentanal-DNPH	358	365
Hexanal-DNPH	358	365
Heptanal-DNPH	358	365
Octanal-DNPH	358	364
Nonanal-DNPH	358	364
Decanal-DNPH	358	364

sponding to a dynamic equilibrium. The isomerization rate for the initial concentration of 10 μmol/l was much faster than that of 100 μmol/l solution, and the *Z*- to *E*-isomer ratio reached a value of 0.51 at 60 min. After that the isomer ratio decreased gradually. Propionaldehyde-2,4-dinitrophenylhydrazone showed a very similar variation compared to the acetaldehyde derivative with a low ratio of 0.18 (1000 μmol/l) or 0.33 (100 μmol/l) at 480 min.

The strong UV band in the ultraviolet between 350 and 390 nm has been attributed to electronic transitions involving the excited resonance form of X [18]. 2,4-Dinitrophenylhydrazones most likely isomerize according to the following reaction:



The different isomer ratios observed for acetaldehyde- and propanal-2,4-dinitrophenylhydrazone in Fig. 3 can be related to the steric effect of the alkyl substituent on the sp2-carbon atom.

Zero-order decreases of the concentrations in aldehyde derivatives due to degradation were observed under UV irradiation (364 nm), i.e., the rate of decrease was not dependent on the initial concentration level. For the acetaldehyde-2,4-dinitrophenylhydrazone derivative, the rates of decrease at the initial concentration of 1000, 100, and 10 μmol/l were −0.020, −0.019, and −0.013 μmol/l/min, respectively. Propionaldehyde-2,4-dinitrophenylhydrazone derivative showed similar decrease rates of −0.017, −0.018, and −0.013 μmol/l/min, respectively. Analysis of formaldehyde-2,4-dinitrophenylhydrazone under UV irradiation (364 nm) also revealed a zero-order decrease of the concentration; the same decrease rate of −0.0513 μmol/l/min was observed at 1000 and 100 μmol/l. Fig. 4 shows the decreases of aldehyde-2,4-dinitrophenylhydrazone derivatives dependent on the UV irradiation at 364 nm. At an initial concentration of 10 μmol/l, the decrease of formaldehyde 2,4-dinitrophenylhydrazone was non-linear and the concentration dropped to 0.72 μmol/l after irradiation for 480 min.

3.4. E to Z isomerization with phosphoric acid

As mentioned before, *Z*-isomers were not detected in the case of purified aldehyde-2,4-dinitrophenylhydrazones, However, isomerization was observed not only under UV irradiation but also upon addition of trace quantities of phosphoric acid. Fig. 5 shows the variation of *Z*- to *E*-isomer ratios of acetaldehyde- and propanal-2,4-dinitrophenylhydrazone derivatives with various phosphoric acid concentrations. With a trace of phosphoric acid (0.0001%), little isomerization was observed whereas the isomer

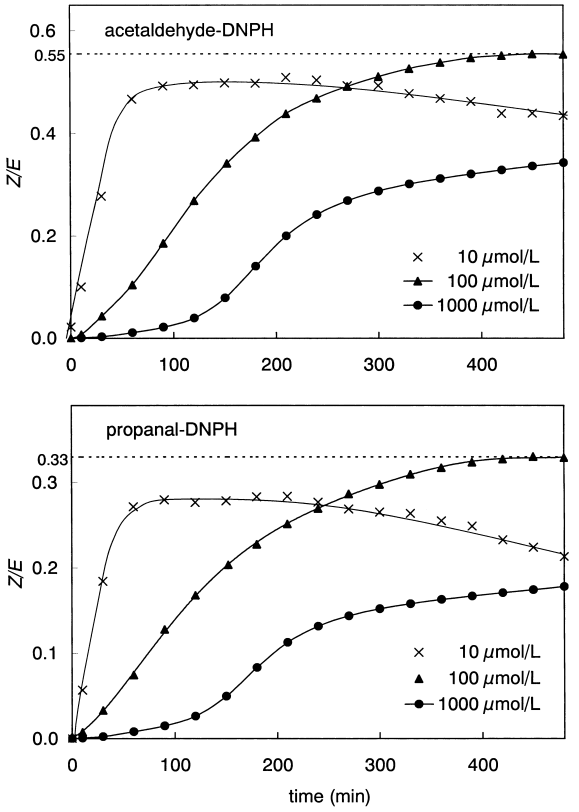


Fig. 3. The changes in the isomer ratios with UV ($\lambda = 364$ nm) irradiation time.

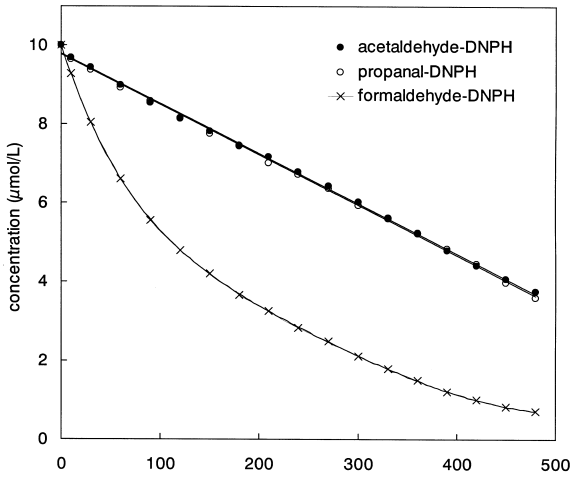
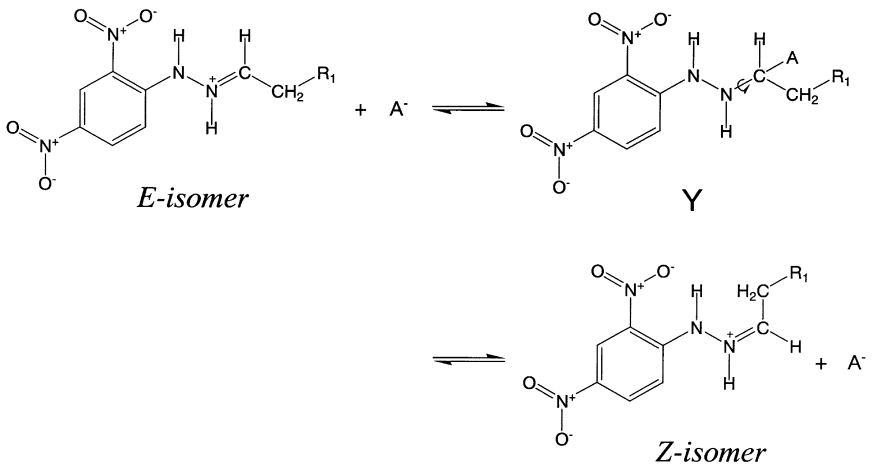


Fig. 4. The decreases of aldehyde-2,4-dinitrophenylhydrazone derivatives dependent on the UV irradiation at 364 nm.

ratio increased dramatically with 0.005% phosphoric acid; an equilibrium isomer ratio of approximately 0.32 was determined. The equilibrium isomer ratio (0.32) was reached at lower concentration of acid when the reaction time was longer. After reaction for 18 h, the same curve profile corresponding to the equilibrium isomer ratio was observed in a wide range of phosphoric acid concentrations from 0.001 to 0.01%. Propanal- and other aldehyde-2,4-dinitrophenylhydrazone derivatives showed a similar behavior.

In all reaction systems using phosphoric acid, no decrease of concentration due to degradation was detected.

In acidic solution, the protonated aldehyde-2,4-dinitrophenylhydrazones most likely isomerize according to the following reaction [24]:



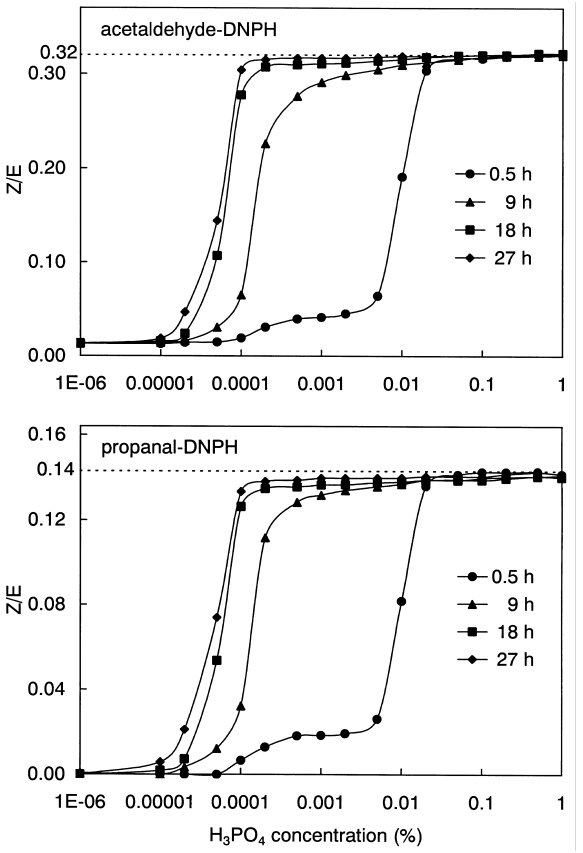


Fig. 5. The changes in the isomer ratios with phosphoric acid.

Presumably, the isomerization involves addition of nucleophile (phosphate ion or water) to the protonated *E*- or *Z*-isomers. From the 0.5 h curves shown in Fig. 5 it appears that the reaction is approximately half-way at a 0.01% phosphoric acid. The effect of the alkyl substituent on the rate of isomerization is marginal.

Fig. 6 shows the chromatograms of the standard solution of C1–C10 aldehyde-2,4-dinitrophenylhydrazone derivatives (50 μmol/l) in 0.1% (v/v) phosphoric acid and the acid-free solution, and a representative chromatogram for the measurement of indoor air. The air sample was obtained from residential indoor air at living room in Japan. The eluate from the air sample was an acidic solution because the DNPH-cartridge contained phosphoric acid as essential catalyst for reaction of 2,4-DNPH and carbonyl compounds. Therefore, both *E*- and *Z*-isomers were observed when the eluate from a

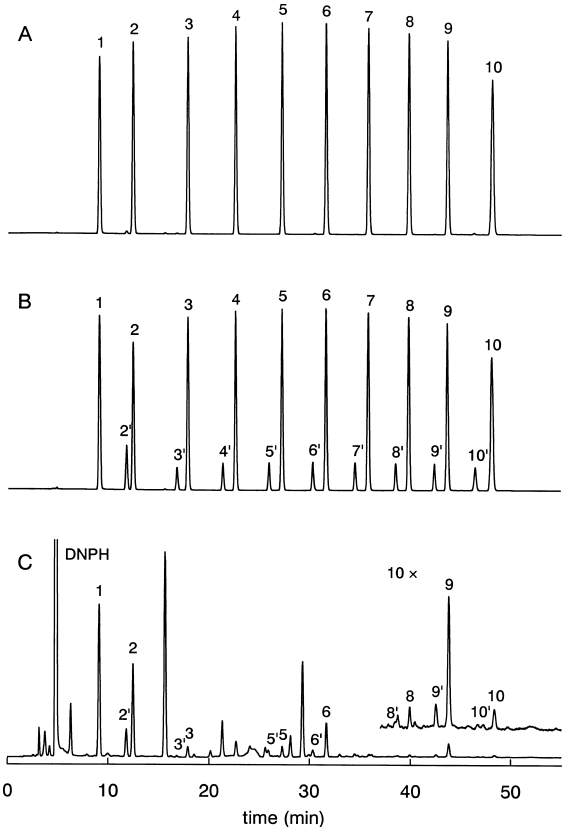


Fig. 6. Chromatograms of C1–C10 aldehyde-2,4-dinitrophenylhydrazone derivatives. (A) Standard solution. (B) Standard solution containing 0.1% of phosphoric acid. (C) Sample solution from indoor air. 1 = Formaldehyde-DNPH, 2 = acetaldehyde-DNPH, 3 = propanal-DNPH, 4 = butanal-DNPH, 5 = pentanal-DNPH, 6 = hexanal-DNPH, 7 = heptanal-DNPH, 8 = octanal-DNPH, 9 = nonanal-DNPH, 10 = decanal-DNPH.

Table 2
Z/*E* isomer ratio of various aldehydes 2,4-dinitrophenylhydrazone derivatives in standard and sample solution with 0.1% (v/v) phosphoric acid

Derivative	*Z*/*E*	
	Sample solution	Standard solution
Acetaldehyde-DNPH	0.32	0.32
Propanal-DNPH	0.14	0.14
Butanal-DNPH	Not detected	0.15
Pentanal-DNPH	0.29	0.15
Hexanal-DNPH	0.25	0.16
Heptanal-DNPH	Not detected	0.15
Octanal-DNPH	0.15	0.15
Nonanal-DNPH	0.15	0.15
Decanal-DNPH	0.15	0.16

DNPH-cartridge was analyzed by HPLC. While only one peak of *E*-isomer appears for the acid-free standard solution, double peaks of *Z*- and *E*-isomers are detected using the acid solution. The *Z*/*E* isomer ratios of all derivatives studied are shown in Table 2. These isomer ratios were invariant and independent of concentration. The isomer ratios of the standard solution were almost equal to the sample solution except for pentanal-2,4-dinitrophenylhydrazone and hexanal-2,4-dinitrophenylhydrazone. When comparing the sample solution with standard solution, many unknown peaks are observed in the sample solution. Therefore, small *Z*-isomer peaks of pentanal-2,4-dinitrophenylhydrazone and hexanal-2,4-dinitrophenylhydrazone may overlap with unknown peaks.

4. Conclusions

Purified C2–C10 straight-chain aldehyde-2,4-dinitrophenylhydrazone derivatives contains only the *E*-isomer. However, this *E*-isomer can partly convert into the *Z*-isomer upon UV irradiation or addition of acid solution. The UV spectral pattern of the *Z*-isomer was different from that of the *E*-isomer: the absorption maximum wavelengths were lower for the *Z*-isomer by 5 to 8 nm. The isomer ratio of acetaldehyde-2,4-dinitrophenylhydrazone in 0.02–1.0% (v/v) phosphoric acid–acetonitrile solution attained an equilibrium value of 0.32 within 30 min. However, when an acid free acetonitrile solution of acetaldehyde-2,4-dinitrophenylhydrazone was irradiated with ultraviolet light of 364 nm for 480 min, a dynamic equilibrium was established reaching a value of 0.55.

The quantitative analysis of carbonyl compounds in air or water using DNPH is usually conducted in the presence of an acid catalyst. Consequently the solution of the crude extract prepared for HPLC or GC analysis contains both *E*- and *Z*-isomers. Summing peak areas of both isomers is only possible when the isomer ratio is constant, i.e., when equilibrium is attained since the absorption maxima of the *E*- and *Z*-isomers occur at different wavelengths. In conclusion, the best method for the determination of aldehyde-2,4-dinitrophenylhydrazones by HPLC or GC is to add phosphoric acid to both the sample and the standard solution, forming a 0.02–1.0% acid solution.

Acknowledgements

This work was supported by a grant from the Health Science Research and from the Ministry of Health and Welfare of the Japanese Government.

References

[1] C.F.H. Allen, J. Am. Chem. Soc. 52 (1930) 2955.
[2] O.L. Brady, J. Chem. Soc. (1931) 756.
[3] D. Grosjean, Environ. Sci. Technol. 16 (1982) 254.
[4] G. Andersson, K. Andersson, C.A. Nilsson, J.O. Levin, Chemosphere 8 (1979) 823.
[5] K. Andersson, C. Hallgren, J.O. Levin, C.A. Nilsson, Chemosphere 10 (1981) 275.
[6] R.K. Beasley, C.E. Hoffmann, M.L. Rueppel, J.W. Worley, Anal. Chem. 52 (1980) 1110.
[7] J.P. Guenier, P. Simon, J. Delcourt, M.F. Didierjean, C. Lefevre, J. Muller, Chromatographia 18 (1984) 137.
[8] D. Grosjean, K. Fung, Anal. Chem. 54 (1982) 1221.
[9] K. Kuwata, M. Uebori, H. Yamasaki, Y. Kuge, Y. Kiso, Anal. Chem. 55 (1983) 2013.
[10] F. Lipari, S. Swarin, J. Environ. Sci. Technol. 19 (1985) 70.
[11] J.O. Levin, K. Andersson, R. Lindahl, C.A. Nilsson, Anal. Chem. 57 (1985) 1032.
[12] Method for the Determination of Formaldehyde in Ambient Air Using Adsorbent Cartridge Followed by High Performance Liquid Chromatography (HPLC), Compendium Method TO-11, US Environmental Protection Agency, Office of Research and Development, Research Triangle Park, NC, 1998.
[13] S. Uchiyama, S. Hasegawa, Atmos. Environ. 33 (1999) 1999.
[14] R.R. Arnts, S.B. Tejada, Environ. Sci. Technol. 23 (1989) 1428.
[15] D.F. Smith, T.E. Kleindienst, E.E. Hudgens, J. Chromatogr. 483 (1989) 431.
[16] D.R. Rodier, L. Nondek, J.W. Birks, Environ. Sci. Technol. 27 (1993) 2814.
[17] U. Karst, N. Binding, K. Cammann, U. Witting, Fresenius J. Anal. Chem. 345 (1993) 48.
[18] F. Ramirez, A.F. Kirby, J. Am. Chem. Soc. 76 (1954) 1037.
[19] V.P. Uralets, J.A. Rijks, P.A. Leclercq, J. Chromatogr. 194 (1980) 135.
[20] N. Binding, W. Müller, U. Witting, Fresenius J. Anal. Chem. 356 (1996) 315.
[21] M. Behforouz, J.L. Bolan, M.S. Flynt, J. Org. Chem. 50 (1985) 1186.
[22] S.F. Tayyari, J.L. Speakman, M.B. Arnold, W. Cai, M. Behforouz, J. Chem. Soc., Perkin Trans. 2 (1998) 2195.
[23] K. Kallury, P. Shieh, R. Paschal, N. Cooke, Supelco Rep. 17 (1998) 5.
[24] G.J. Karabatsos, F.M. Vane, R.A. Taller, N. Hsi, J. Am. Chem. Soc. 86 (1964) 3351.