Journal of Chromatography, 298 (1984) 399–406
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,819

TRACE ANALYSIS OF ALDEHYDES BY PRE-COLUMN FLUORIGENIC LABELING WITH 1,3-CYCLOHEXANEDIONE AND REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

WILLIAM L. STAHOVEC* and KENNETH MOPPER*,*
College of Marine Studies, University of Delaware, Lewes, DE 19958 (U.S.A.)
(Received April 10th, 1984)

SUMMARY

A reagent used for the analysis of total aldehyde concentrations, 1,3-cyclohexanedione, was adapted to the trace analysis of aldehydes using high-performance liquid chromatography. Experiments were performed to determine the optimal reagent composition, stability of the derivatives, reproducibility and reagent clean up. Many of the optimal reaction conditions with 1,3-cyclohexanedione were found to be very similar to those with an analogous reagent, dimedone. However, 1,3-cyclohexanedione has some advantages, namely that it is more soluble in water than dimedone, making reagent preparation easier, and the derivatization reaction occurs at a lower temperature. Removal of contaminants from the reagent is readily achieved with a method employing disposable cartridges of reversed-phase material. Applications to environmental samples are reported.

INTRODUCTION

We recently reported a method using 5,5-dimethyl-1,3-cyclohexanedione (dimedone) as a pre-column derivatization reagent for the fluorimetric analysis of low-molecular-weight aldehydes using high-performance liquid chromatography (HPLC)[1]. Here we report on the chromatographic applicability of a similar reagent, 1,3-cyclohexanedione (dihydroresorcinol) (CHD), which, like dimedone, has been previously used for the determination of total aldehydes[2]. Despite the similar structures of these compounds (Fig. 1), there are some pronounced differences in their properties that may influence which one is more suitable for a particular purpose.

EXPERIMENTAL

Apparatus

The HPLC system employed was an Eldex Chromat-A-Trol gradient control-

* Present address: Rosenstiel School of Marine and Atmospheric Science, University of Miami, FL 33149, U.S.A.

0021-9673/84/$03.00

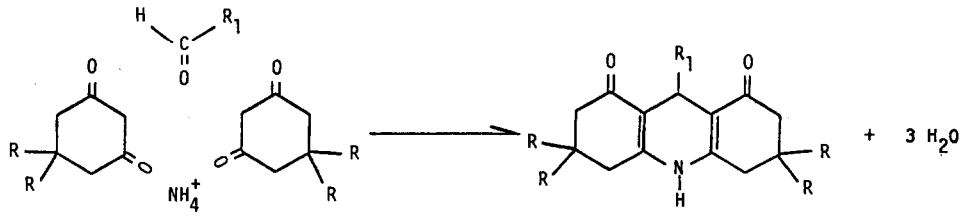

Fig. 1. Overall reaction of CHD and dimedone with aldehydes and ammonium. CHD, R = H; dimedone, R = CH_3.

ler (Eldex Labs., Menlo Park, CA, U.S.A.) with an Eldex Model AA pump, and a Valco Model CV-6UHpa-N60 sample injector (Valco, Houston, TX, U.S.A.) with a 20-μl sample loop. The analytical column used was an Adsorbosphere (Applied Science, State College, PA, U.S.A.) 3 μm, ODS (100 × 4.6 mm I.D.) column. Detection of the derivatives was accomplished with a Gilson 121 fluorimeter (Gilson Medical Electronics, Middletown, WI, U.S.A.) with a 305–395 nm excitation filter and a 450 ± 3.5 nm emission filter. Peak integrations were performed on a Hewlett-Packard (Avondale, PA, U.S.A.) 3390A integrator.

Chemicals

Chemicals used in the derivatization and solvents used in the mobile phases were of analytical-reagent or HPLC grade. CHD (>99% purity) was obtained from Fluka (Buchs, Switzerland). Deionized water was obtained from a Millipore (Milford, MA, U.S.A.) Q-water system. All mobile phases were filtered (0.45 μm) prior to use.

Aldehyde standards were of the purest grade available (Aldrich, Milwaukee, WI, U.S.A.; Sigma, St. Louis, MO, U.S.A.). The formaldehyde standard was prepared from a 40% (w/w) aqueous solution. Stock solutions (10 m*M*) were prepared in 50% (v/v) acetonitrile–water or pure acetonitrile, depending on the solubility of the carbonyl. From stock solutions, mixed standards (1 m*M* each) were prepared. Aliquots of these standards were further diluted with water to prepare the solutions used during the optimization experiments. Stock solutions and mixed standards were stored at 4°C.

Derivatization

The reagent was prepared by dissolving 25 g of ammonium acetate, 1.0 g of CHD and 8.0 ml of concentrated HCl in 75 ml of water and diluting to 100 ml with water. To remove contaminants (Fig. 2), the reagent was heated at 60°C for 1 h. The reagent was then cooled and passed sequentially through a 3-g C_{18} Bond-Elut cartridge (Analytichem International, Harbor City, CA, U.S.A.) fitted into a 1-g C_{18} Sep-Pak cartridge (Waters Assoc., Milford, MA, U.S.A.). A 1-ml volume of carbonyl-free reagent was added to 1-ml of sample and heated in a PTFE-sealed test-tube for 1 h at 60°C, then cooled in an ice-bath. An aliquot of this solution was injected directly on to the HPLC column.

HPLC conditions

During optimization of the derivatization procedure, HPLC separations were

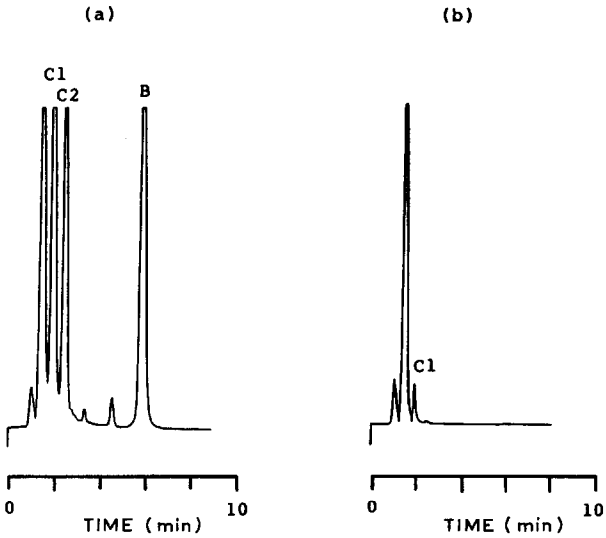

Fig. 2. Chromatograms of CHD reagent blank (a) prior to clean-up and (b) after clean-up with Bond-Elut-Sep-Pak combination. Peaks: C1 = formaldehyde; C2 = acetaldehyde; B = benzaldehyde.

performed isocratically at a flow-rate of 1.0 ml/min using acetonitrile–water (30:70, v/v). For environmental samples, gradient elutions using methanol (B) and water (A) were used. The gradient employed was 40% B to 85% B in 10 min; isocratic at 85% B for 6 min; 85% B to 100% B in 2 min; 100% B to 40% B in 3 min. A new sample could be injected every 35 min. All chromatographic runs were performed at room temperature. A typical chromatogram of a mixed standard is shown in Fig. 3.

RESULTS AND DISCUSSION

Effect of pH on reagent reactivity

As the pH of the reaction solution was found to have a dramatic effect on the dimedone reagent[1], a similar study was conducted for CHD. The composition of the reaction solution was kept constant at 1.6 *M* ammonium acetate, 45 m*M* CHD, 2.5 μM formaldehyde and acetaldehyde and 5.0 μM butanal. The pH of the reaction solution was adjusted with concentrated HCl. As can be seen in Fig. 4, the optimal pH for all aldehydes tested is about 5. CHD thus differs from dimedone[1], in which formaldehyde reacts best at about pH 7 while other aldehydes react best at pH 2–4. The pH of the reaction solution used in the normal derivatization method (see *Derivatization*) is 5.1.

Effect of ammonium acetate concentration

The effect of varying the ammonium acetate concentration on CHD reactivity was found to be almost identical with the effect on the dimedone reagent[1]. The optimal concentration for ammonium acetate in the reaction solution was found to be 12.5 g per 100 ml (1.6 *M*). Low reactivity at higher ammonium acetate concentrations may be due to the high viscosity of the reaction solution at the high salt concentrations.

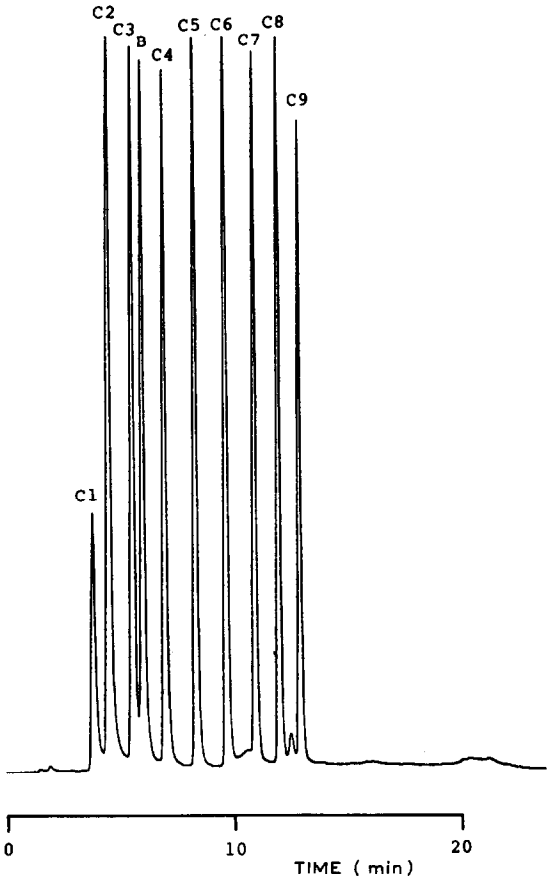

Fig. 3. Reversed-phase separation of selected aldehyde–CHD derivatives. Each peak represents 20 pmol. Peaks: C1 = formaldehyde; C2 = acetaldehyde; C3 = propanal; C4 = butanal; C5 = pentanal; C6 = hexanal; C7 = heptanal; C8 = octanal; C9 = nonanal; B = benzaldehyde.

Effect of CHD concentration

The concentration of CHD in the reaction solution was varied from 3.0 to 90.0 m*M*. The concentrations of the other constituents in the solution were held constant: ammonium acetate at 12.5 g per 100 ml (1.6 *M*), concentrated HCl at 4 ml per 100 ml formaldehyde and acetaldehyde at 2.5 μM and butanal at 5.0 μM. As with dimedone[1], formaldehyde reacted well even at low CHD concentrations. For other aldehydes, the reactivity increased with increasing CHD concentration, but leveled off above 30 m*M*. This concentration corresponds to a CHD to aldehyde molar ratio of about 4500. Therefore, for quantitative analysis at the micromole level and below, a large excess of reagent is required.

Reaction time at 60°C

A time series study was performed using the normal reaction conditions (see *Derivatization*). The results indicated that a reaction time of 1 h was sufficient for all

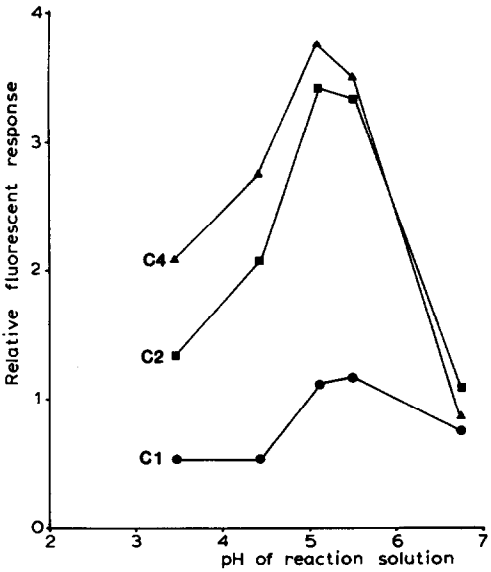

Fig. 4. Effect of pH on the reaction of CHD with aldehydes. Reaction conditions as in text. C1, Formaldehyde; C2, acetaldehyde; C4, butanal.

aldehydes studied. The fluorescent responses did not increase significantly at longer reaction times.

Fluorescent response

An equimolar standard solution of eleven aldehydes was derivatized by the normal procedure. Derivatives were analyzed by HPLC and their responses, as peak areas, were normalized to that of acetaldehyde (Table I). In general, the responses were fairly uniform. As with dimedone[1], acrolein and crotonaldehyde gave very poor relative responses. The unsaturated bonds in these molecules may interact with the fluorophore, lowering the quantum yield, or may hinder the derivatization reaction itself.

TABLE I

FLUORESCENT RESPONSES OF ALDEHYDE–CHD DERIVATIVES

Aldehyde	*Response**	*Aldehyde*	*Response**
Formaldehyde	0.5	Heptanal	0.6
Acetaldehyde	1.0	Octanal	0.6
Propanal	0.8	Benzaldehyde	0.8
Butanal	0.8	Acrolein	<0.1
Pentanal	0.7	Crotonaldehyde	<0.1
Hexanal	0.8		

* Response values are peak areas normalized to the response of acetaldehyde.

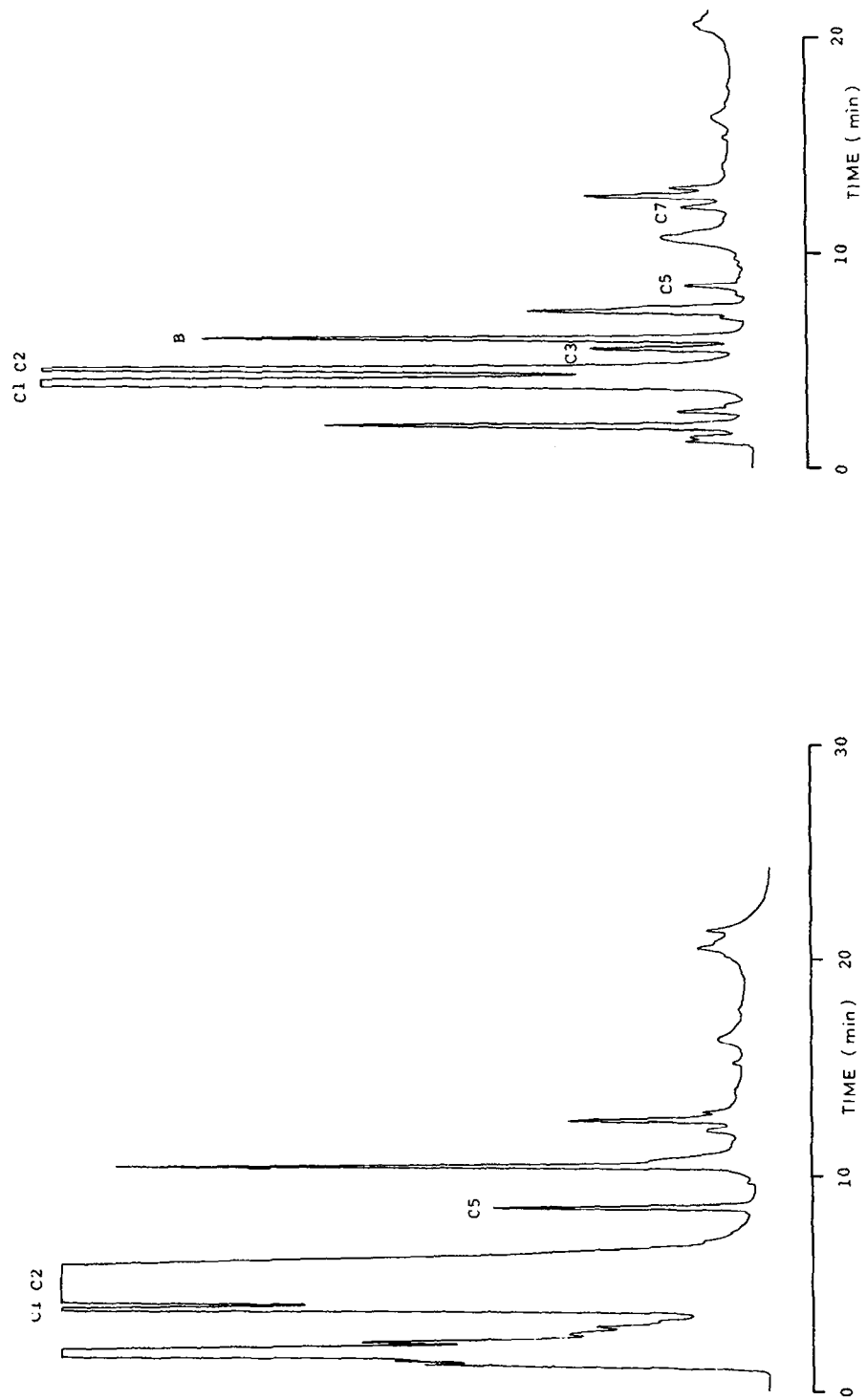

Fig. 5. Chromatogram from direct injection of 20 μl of a derivatized red wine sample. Peaks C1 = formaldehyde; C2 = acetaldehyde; C5 = pentanal.

Fig. 6. Chromatogram of CHD-derivatized aldehydes in automobile exhaust collected in methanol–water (20:80, v/v). Peaks: C1 = formaldehyde; C2 = acetaldehyde; C3 = propanal; B = benzaldehyde; C7 = heptanal.

Reproducibility and stability

A standard solution (2.5 μM formaldehyde, 2.5 μM acetaldehyde and 5.0 μM butanal) was derivatized using the normal procedure (see *Derivatization*). The same solution was analyzed at regular intervals for over 4 h. Between sample injections, the reacted solution was kept in an ice-bath in the dark. This procedure was performed in triplicate and the results, in terms of peak areas, are given in Table II. It can be seen that the reproducibility is excellent; however, slight degradation of the derivatives is evident after 4 h.

TABLE II

STABILITY AND REPRODUCIBILITY OF CHD DERIVATIVES

Results presented as average peak areas ± standard deviations (arbitrary units) for three samples.

Aldehyde	*Fresh*	*After 1 h*	*After 4 h*
Formaldehyde	4.48 ± 0.01	4.38 ± 0.03	4.31 ± 0.03
Acetaldehyde	7.83 ± 0.08	7.85 ± 0.08	7.69 ± 0.09
Butanal	11.08 ± 0.05	11.00 ± 0.04	10.87 ± 0.07

Environmental samples

An aliquot of a red wine was derivatized directly, and the resulting chromatogram is depicted in Fig. 5. All peaks are CHD derivatives. The largest peak was that of acetaldehyde, an oxidation product of ethanol. We also analyzed automobile exhaust, which was collected by passing exhaust through methanol–water (20:80, v/v) (Fig. 6). High concentrations of formaldehyde, acetaldehyde and benzaldehyde were also found using dimedone in our earlier study[1].

Additional comments

As expected, the CHD reagent was similar to the dimedone reagent. For example, the effect of ammonium acetate and CHD concentrations and fluorescent responses of the derivatives are almost identical for both CHD and dimedone.

However, there are some notable exceptions. One is that CHD is considerably more soluble than dimedone in water, which makes reagent preparation much easier and a more concentrated reagent possible.

In addition, removal of contaminants from the CHD reagent required a different procedure than for the dimedone reagent. The latter could easily be cleaned by organic solvent extraction or by passing the pre-reacted reagent through a single C_{18} Sep-Pak cartridge[1]. These methods were not effective for CHD. However, it was found that by passing the pre-reacted CHD reagent sequentially through a Bond-Elut cartridge fitted into a Sep-Pak cartridge removed all but the most hydrophilic contaminants. The reactivity of the reagent was unaffected by this clean-up procedure.

The reaction temperature is another major difference between the two reagents. CHD reacts very well at 60°C, far below the 100°C optimal reaction temperature for dimedone. This lower reaction temperature may be more desirable for thermolabile samples (*e.g.*, certain biological preparations).

Finally, CHD derivatives displayed different chromatographic behavior than dimedone derivatives. It was found that CHD derivatives were easier to resolve by HPLC. The increased selectivity may be due to the less bulky nature of the CHD derivatives (Fig. 1). Further, unlike dimedone, better selectivity was obtained with methanol than acetonitrile in the mobile phases. For example, although butanal is easily resolved from benzaldehyde using methanol (Fig. 3), these compounds could not be resolved using acetonitrile. The use of methanol in the mobile phase has other advantages. For example, methanol is considerably cheaper and not as toxic as acetonitrile. Given these advantages, we consider CHD to be superior to dimedone for the chromatographic analysis of aldehydes.

ACKNOWLEDGEMENT

This work was supported by a grant from the National Science Foundation (OCE-8207788).

REFERENCES

1 K. Mopper, W. L. Stahovec and L. Johnson, *J. Chromatogr.*, 256 (1983) 243.
2 E. Sawicki and C. R. Sawicki, *Aldehydes—Photometric Analysis*, Vol. 1, Academic Press, New York, 1975.