Journal of Chromatography, 136 (1977) 271–277

CHROM. 9973

SEPARATION AND QUANTITATIVE DETERMINATION OF TRACES OF CARBONYL COMPOUNDS AS THEIR 2,4-DINITROPHENYLHYDRAZONES BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY

SAMI SELIM

FMC Corporation, Agricultural Chemical Division 100 Niagara Street, Middleport, N.Y. 14105 (U.S.A.)

(Received December 21st, 1976)

SUMMARY

A method for the quantitative conversion of traces of aldehydes and ketones to their 2,4-dinitrophenylhydrazones at room temperature is presented. The 2,4-dinitrophenylhydrazones of a number of aliphatic and aromatic carbonyl compounds have been prepared. The compounds were separated on a reversed-phase μBondapak C_{18} column. The method is valuable in the quantitative determination of traces of low-molecular-weight aldehydes and ketones. Derivatives of identical molecular weight can be easily separated by high-pressure liquid chromatography but not by gas chromatography under the conditions tried.

INTRODUCTION

High-pressure liquid chromatography (HPLC) is becoming a powerful technique for the analysis of trace components. Liquid chromatography (LC) has many of the advantages of gas chromatography (GC) such as speed and accurate quantitative analysis, and some added advantages such as capability to analyze high-molecular-weight or thermally unstable compounds. However the applicability of LC to trace analysis has been limited because of the few types of detectors commercially available. Introduction of the variable-wavelength detector and the development of totally porous small-particle packings are allowing separations at least as good as those obtained by GC, with the needed sensitivity.

Nanogram quantities of certain groups of compounds such as aliphatic alcohols, aldehydes, ketones, acids, etc., cannot be selectively detected by GC or LC. One approach to overcoming the detection problem is to tag the compound by adding a group that renders it detectable. This approach has been used extensively in GC and is gaining in importance in HPLC.

Derivatives that absorb strongly in UV light have been prepared for HPLC. Hydroxy steroids have been benzoylated[1], the 3,5-dinitrobenzoates of glycols have been formed[2], and hexachlorophene has been detected via its *p*-methoxybenzoate

derivatives[3]. The HPLC response of fatty acids has been improved by the formation of benzyl[4], *p*-nitrobenzyl[5], 2-naphthacyl[6], and phenacyl[7,8] esters.

Carbonyl compounds have received much attention recently because of an increasing realization of their importance when present in trace amounts in air pollution, vegetable flavoring, cigarette smoke, and the aroma of commercial beverages. The method most commonly used to determine traces of these compounds is to form the 2,4-dinitrophenylhydrazone and determine that derivative by GC or HPLC. The detectability of 17-keto steroids has been enhanced by the formation of the 2,4-dinitrophenylhydrazone[9] such that as little as 10 ng of steroids was detected[10]. The 2,4-dinitrophenylhydrazones of a number of aliphatic and aromatic carbonyl compounds have been synthesized and successful separation of C_1–C_5 2,4-dinitrophenylhydrazones was obtained on pellicular LC columns[2,11]. It was reported[2] that the retention times of the 2,4-dinitrophenylhydrazones of carbonyl compounds with six or more carbons tend to merge because the solubilities in the hydrocarbon mobile phase are nearly the same. Adsorption chromatography was used in all the reported HPLC separations.

The quantitative conversion of aldehydes and ketones to their corresponding 2,4-dinitrophenylhydrazones on a microscale has not been investigated.

In this article, the quantitative conversion of aldehydes and ketones to their corresponding 2,4-dinitrophenylhydrazones on a microscale at room temperature is reported. The separation of same-molecular-weight aldehydes and ketones could not be accomplished by GC but was achieved by HPLC. The separation of the 2,4-dinitrophenylhydrazones of aldehydes and ketones has been extended to higher molecular weights, aldehydes and ketones (above 6 carbon) by using reversed-phase HPLC.

EXPERIMENTAL

Preparation of 2,4-dinitrophenylhydrazine reagent

The 2,4-dinitrophenylhydrazine reagent was prepared by adding 0.25 g of 2,4-dinitrophenylhydrazine to 100 ml of 6 *N* hydrochloric acid.

Preparation of 2,4-dinitrophenylhydrazones

The 2,4-dinitrophenylhydrazones were prepared by standard procedures[12] and purified by recrystallization from ethanol.

Conversion of propionaldehyde to its 2,4-dinitrophenylhydrazone on a microscale

To each of six Erlenmeyer flasks, 25 ml of water, 0.2 ml of the 2,4-dinitrophenylhydrazine reagent, 20 μg of propionaldehyde and 10 ml of isooctane were added. The mixtures were stirred on a magnetic stirrer. At intervals, the two-phase mixture was transferred to a separatory funnel and the two phases separated. The aqueous phase was extracted with 10 ml of isooctane, and the two isooctane fractions were combined and extracted twice with 10 ml of acetonitrile. The acetonitrile extract was concentrated and injected onto the liquid chromatograph (system II, see below).

High-pressure liquid chromatography

A Waters Assoc. ALC 202 liquid chromatograph equipped with a U6K

injector and a Schoeffel spectrophotometer was used in this study. Two HPLC systems were studied.

System I. Adsorption chromatography on a prepacked 30-cm Microporasil column (Waters Assoc.) using ethyl acetate–hexane (1:49) as the mobile phase.

System II. Reversed-phase chromatography on a 30-cm prepacked μBondapak C_{18} (Waters Assoc.) column using different ratios of acetonitrile–water as the mobile phase.

Gas chromatography

A Hewlett-Packard 5700A gas chromatograph equipped with a Ni 63 electron capture detector was used. This instrument features on-column injection and direct connection of the column to the detector. Glass columns (4 ft. × 6 mm O.D. × 2 mm I.D.) packed with various liquid phases on Chromosorb W AW DMCS (80–100 mesh) were used. The flow-rate of the argon–methane (95:5) gas was 65 ml/min.

RESULTS AND DISCUSSION

The reaction between propionaldehyde and 2,4-dinitrophenylhydrazine was studied on a microscale at room temperature. Propionaldehyde was added to an excess of the 2,4-dinitrophenylhydrazine reagent. The solution was extracted at intervals and injected onto the HPLC. After 30 min, 67% of propionaldehyde was converted to the corresponding 2,4-dinitrophenylhydrazone. Allowing the reaction to proceed for 15 h did not substantially improve the yield (Table I). These results indicate that an equilibrium was reached in the aqueous phase when 70.6% of the derivative had been formed:

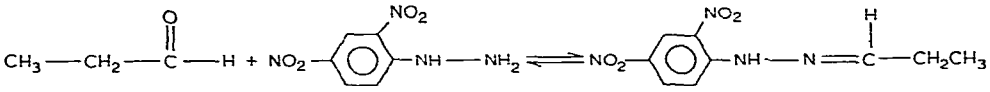

TABLE I

CONVERSION OF PROPIONALDEHYDE TO ITS 2,4-DINITROPHENYLHYDRAZONE AT ROOM TEMPERATURE IN A ONE-PHASE REACTION MEDIUM

Time (h)	*Added (μg)*	*Found (μg)*	*Recovery (%)*
½	20	13.4	67
1	20	12.4	62
2	20	13.5	67.7
4	20	12.6	63.3
6	20	12.4	62.2
15	20	14.1	70.6

When the same reaction is carried out with gram quantities, the 2,4-dinitrophenylhydrazone of propionaldehyde precipitates immediately due to its low solubility in the aqueous phase. The removal of the derivative from the aqueous phase by precipitation shifts the equilibrium toward the formation of more derivative and the reaction is almost quantitative. The water-insolubility of the 2,4-dinitrophenylhydra-

TABLE II

CONVERSION OF PROPIONALDEHYDE TO ITS 2,4-DINITROPHENYLHYDRAZONE AT ROOM TEMPERATURE IN A TWO-PHASE REACTION MEDIUM

Time (min)	*Added (μg)*	*Found (μg)*	*Recovery (%)*
5	20	10.7	53.5
10	20	15.1	75.3
15	20	17.6	88.1
20	20	19.8	99
25	20	20	100.4
30	20	20	100.7

zone of propionaldehyde indicates that the reaction could be made quantitative at the microscale by using an aqueous–organic two-phase reaction medium. Propionaldehyde and the 2,4-dinitrophenylhydrazine reagent are water-soluble. Propionaldehyde reacted with the 2,4-dinitrophenylhydrazine reagent in the aqueous phase. Partitioning of the derivative into the organic phase shifted the equilibrium of the reaction to the right and allowed the reaction to go to completion. Propionaldehyde was quantitatively converted to its 2,4-dinitrophenylhydrazone in 20 min (Table II, Fig. 1).

The HPLC separation of the 2,4-dinitrophenylhydrazones of carbonyl compounds offers many advantages over the GC separation. The reported GC methods capable of separating different aldehyde derivatives and ketone derivatives are not capable of separating aldehyde derivatives from ketone derivatives of the same molecular weight (Fig. 2). GC columns having liquid phases ranging in polarity from OV-1 to OV-25 were tried in this laboratory to separate the 2,4-dinitrophenylhydrazones of acetone and propionaldehyde without success. Base-line separation of the 2,4-di-

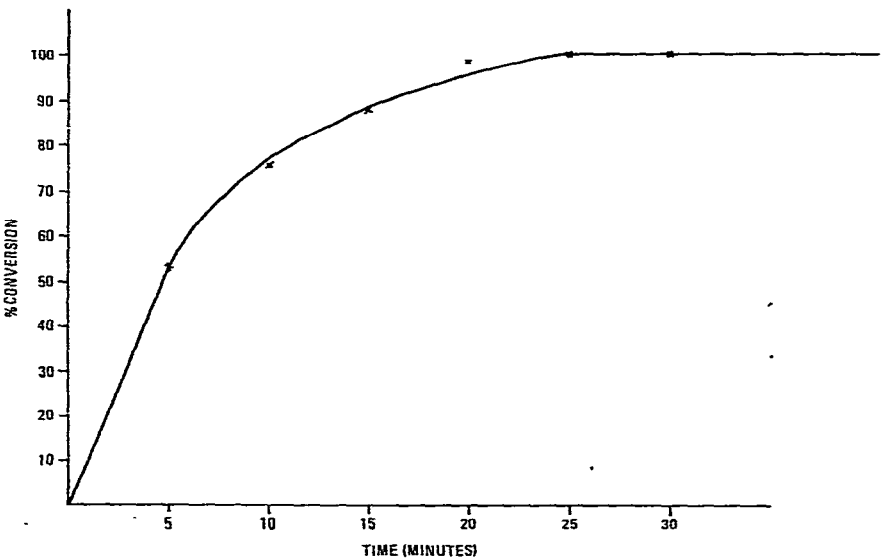

Fig. 1. Rate of conversion of propionaldehyde to propionaldehyde 2,4-dinitrophenylhydrazone using a two-phase reaction medium.

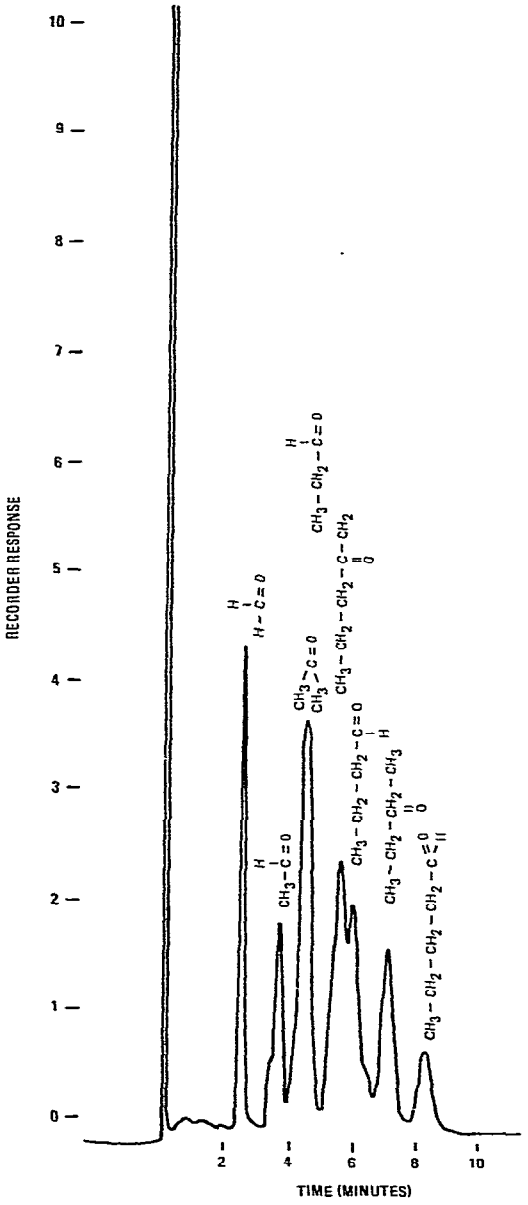

Fig. 2. Gas chromatogram of a mixture of 2,4-dinitrophenylhydrazones of carbonyl compounds on a 2% OV-17 column.

nitrophenylhydrazone of propionaldehyde from the 2,4-dinitrophenylhydrazone of acetone was achieved on reversed-phase HPLC using a acetonitrile–water (3:2) mobile phase (Fig. 3). Aldehydes and ketones with four or five carbons were also separated (Fig. 3). As expected, higher-molecular-weight aldehyde and ketone 2,4-dinitrophenylhydrazones were retained longer on the reversed-phase column. The 2,4-dinitrophenylhydrazone of the aromatic aldehydes α-tolualdehyde and salicyl-

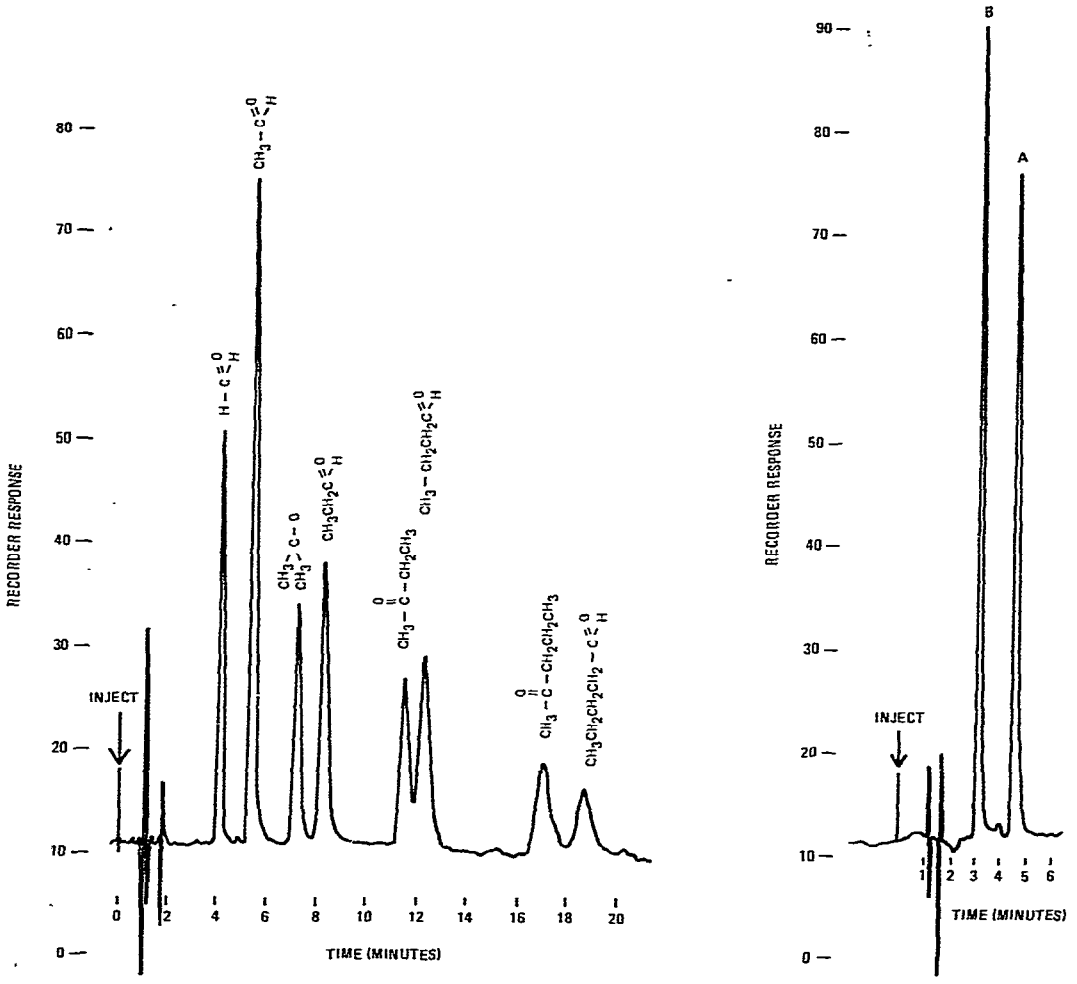

Fig. 3. LC of a mixture of 2,4-dinitrophenylhydrazones of aldehyde and ketone. Mobile phase acetonitrile–water (3:2). Flow-rate, 3 ml/min; pressure, 1000 p.s.i.; column temperature ambient; UV detection at 336 nm, 0.04 a.u.f.s.

Fig. 4. LC of a mixture of the 2,4-dinitrophenylhydrazone of (A) tolualdehyde and (B) salicylaldehyde. Conditons are the same as in Fig. 3, except a acetonitrile–water (13:7) mobile phase was used.

aldehyde were easily separated on the same column using a acetonitrile–water (13:7) mobile phase (Fig. 4). 2,4-Dinitrophenylhydrazones of aldehydes with eight, nine or ten carbons were separated on the same column using acetonitrile–water (3:1). 2,4-Dinitrophenylhydrazones of even higher aldehydes and ketones should not be difficult to separate but would probably require a higher concentration of acetonitrile. Shorter columns could also be used effectively.

Reversed-phase LC offers some advantages over adsorption chromatography for the separation of non-polar derivatives. When adsorption chromatography is used, the retention of a compound on the column depends on the interaction of the compound with the packing and on the solubility of the compound in the mobile

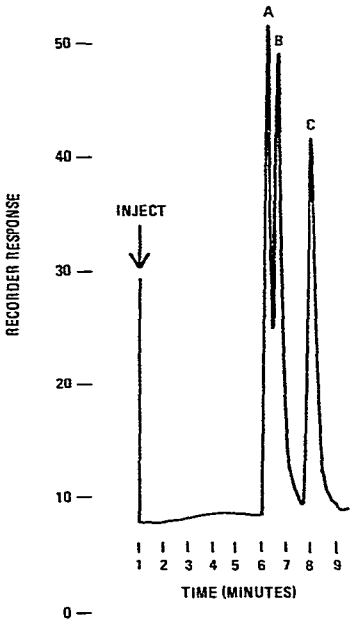

Fig. 5. LC of a mixture of the 2,4-dinitrophenylhydrazone of (A) 4-heptanone, (B) 3-heptanone and (C) 2-heptanone on a 1 ft. × ¼ in. microporasil column; mobile phase, ethyl acetate–hexane (2:98). Flow-rate, 3 ml/min; pressure, 1000 p.s.i.; column temperature, ambient; UV detection at 336 nm, 0.04 a.u.f.s.

phase. It has been found[2] that compounds containing six or more carbon atoms could not be separated on a Corasil II column using ethyl acetate–hexane (3:97) as mobile phase. The solubilities of these compounds in the mobile phase were nearly the same. Decreasing the ethyl acetate concentration to 2% and using a Microporasil column allowed separation of the 2,4-dinitrophenylhydrazones of three heptanones (Fig. 5). However, the 2,4-dinitrophenylhydrazones of aldehydes with eight or nine carbons could not be separated.

REFERENCES

1 F. A. Fitzpatrick and S. Siggia, *Anal. Chem.*, 45 (1973) 2310.
2 M. A. Carey and A. F. Persinger, *J. Chromatogr. Sci.*, 10 (1972) 537.
3 P. J. Porcaro and P. Shubiak, *Anal. Chem.*, 44 (1972) 1865.
4 I. R. Plitzer, G. W. Griffin, B. J. Dowty and J. L. Laseter, *Anal. Lett.*, 6 (1973) 539.
5 W. Morozowich, *APLA Acad. Pharm. Sci. Abstr.*, 69 (1972).
6 M. J. Cooper and M. W. Anders, *Anal. Chem.*, 46 (1074) 1849.
7 H. D. Durst, M. Milano, E. J. Kikta, Jr., S. A. Connelly and E. Grushka, *Anal. Chem.*, 47 (1975) 1797.
8 R. F. Borch, *Anal. Chem.*, 47 (1975) 2437.
9 F. A. Fitzpatrick, S. Siggia and J. Dingman, Sr., *Anal. Chem.*, 44 (1972) 2211.
10 R. A. Henry, J. A. Schmit and J. F. Diekman, *J. Chromatogr. Sci.*, 9 (1971) 513.
11 L. S. Papa and L. P. Turner, *J. Chromatogr. Sci.*, 10 (1972) 744.
12 R. L. Shriner, R. C. Fuson and D. Y. Curtin, *The Systematic Identification of Organic Compounds*, Wiley, New York, 5th ed., 1965.