DETERMINATION OF ALDEHYDES AND KETONES IN THE GAS PHASE

by

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ABSTRACT

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A recently developed method for the quantitative detection of carbonyl compounds in ambient air was investigated, specifically in order to further standardize and improve the process. The method involves drawing a gas phase sample of carbonyl compounds through impingers containing a 2,4-Dinitrophenylhydrazine solution in acetonitrile solvent. The resulting hydrazone solution is then injected directly into the hplc for separation on a reversed phase column and quantitative determination by uv absorption at 254 nm. and 360 nm. simultaneously. The critical feature of the method (based upon a standard analytical derivatization reaction) is the use of the acetonitrile solvent. This allows for direct injection of the sampling solution into the hplc with no workup (i.e. hydrazone extraction) necessary. This modification accounts for improved detection efficiency over previous 2,4-DNPH methods.

My deepest thanks go to Professor L.A. Hull for his insight into and guidance through the trials and accomplishments of doing research. Additionally, to my fellow chemistry graduates of 1983 who listened to and understood the difficulties because they shared them. Their presence and support made the difference.

Clyde A. Kelly

Clyde A. Kelly

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

INTRODUCTION

Aldehydes and ketones form a major class of air pollutants produced by many industrial processes, as a result of partial combustion of hydrocarbons, and as products of aliphatic and aromatic hydrocarbon photooxidation (primarily as a result of ozone-olefin reactions (2)). Formaldehyde and many other aldehydes have long been recognized as irritants of the skin, eyes and nasopharyngeal membranes (3). Recently the Federal Consumer Product Safety Commission banned urea-formaldehyde foam housing insulation in response to 2,200 complaints of flu-like symptoms, respiratory illnesses and other health problems associated with formaldehyde fumes from the insulation. Additionally, scientific studies, such as the one conducted by the Chemical Industry Institute of Toxicology finding that rats exposed to 15 ppm. formaldehyde over a two year period developed cancerous nasal tumors, potentially mark formaldehyde as a carcinogen (4). Quantitative determination of aldehydes and ketones in the gas phase has therefore become an important goal of the environmental and analytical chemist.

Several techniques for the analysis of carbonyl compounds in the gas phase have been proposed and investigated. These include the 3-methyl-2-benzothiazolone (MBTH) method, the chromotropic acid method of formaldehyde determination, long path fourier transform infra red spectroscopy (lpir), 2-diphenylacetyl-1,3-indandione-1-hydrazone (DIAH) with fluorescence detection and the 2,4-dinitrophenylhydrazine (DNPH) method with uv detection. The MBTH method is a nonselective colorimetric method for low molecular weight aliphatic aldehydes and is therefore insensitive to higher molecular weight and unsaturated aldehydes (5). The chromotropic acid technique is specific for formaldehyde only. The DIAH method is a new and viable method and its further investigation (particularly the application of fluorescence detection) is a planned continuation of this project.

Reaction of carbonyl compounds with 2,4-DNPH is a standard test (both sensitive and dependable) for the presence of aldehydes and ketones. These same characteristics make this reaction a powerful analytical tool when applied to carbonyl compounds in the gas phase. The reaction proceeds according to the following equation.

$${>}C{=}O + H_2N{-}NH{-}DNP \rightarrow {>}C{=}N{-}NH{-}DNP + H_2O \qquad \text{equation \#1}$$

Previous to the improvements pursued in this and related work, the practiced DNPH method involved derivation in an acidic aqueous solution followed by filtration, extraction, evaporation to dryness and dilution in a solvent suitable for gas chromatographic (gc)(6) or high performance liquid chromatographic (hplc)(7,8) analysis. Thus a combination of water interferences (as a possible result of Le'Chatelier's principle, see equation #1 above), extensive sample preparation and small sample handling lead to recoveries of 60 to 85% (5).

This work proposes to improve upon the method by using acetonitrile (ACN) as the solvent for sample collection and derivation. This has the advantages of being an hplc compatible solvent and therefore requiring no post-sampling workup, as well as eliminating water interference. The resultant ease of sampling plus the relatively short analysis time (less than ten minutes for those carbonyl compounds studied) suggest that this method would be particularly attractive in situations where a large number of samples must be analyzed (9).

The method investigated was developed and reported by

R. Kuntz, W. Lonneman, G. Namie, and L.A. Hull (9) working at the U.S. Environmental Protection Agency. This work is developmental to theirs and dealt primarily with method standardizarion, definition of some parameters and preliminary implimentation. Several hydrazone "standards" of common carbonyl compounds were prepared in order to calibrate hplc responses. The optimization of analytical separations by choice of column and use of solvent programming was investigated. The useful shelf life of the DNPH (i.e. the rate of contamination) was identified. This parameter will prove to be particularly important in field studies in which samples must be shipped to a laboratory for analysis (10).

EXPERIMENTAL

The work with 2,4-DNPH was carried out in methanol and/or acetonitrile (both spectral grade). One or the other of these was used in the cleaning and rinsing of all equipment which came in contact with the hydrazine and its derivatives. Their use in other processes is as described below.

A. Recrystallization of 2,4-DNPH

1. Method

6 gm. of 2,4-DNPH were placed in 100. ml of ACN. The mixture was then heated to the boiling point with stirring to dissolve the DNPH. The resultant solution was hot suction filtered to remove any solid residue and transferred immediately to a beaker. Crystal formation was effected by allowing the solution to cool to room temperature and then on ice. The crystals were recovered by suction filtration with a minimum of atmospheric exposure to prevent the introduction of contaminants (11).

2. Evaluation of purity

A melting point was taken and compared to literature values, see Table #1. For a quantitative evaluation of purity, a portion of the DNPH sampling solution was prepared according to the method described in section C below. Analysis of this solution by hplc according to the directions of section D.3. below will allow for quantitative determination of the DNPH purity.

3. Recrystallize again if necessary (see discussion section for criteria of purity).

B. Preparation of 2,4-dinitrophenylhydrazone Standards

In order to generate high purity hydrazone samples of several carbonyl compounds the following derivation, extraction, recrystallization scheme was followed. Table #1 contains necessary physical data for use of these chemicals.

1. Preparation of the 2,4-DNPH reagent

4.0 gm. of recrystallized DNPH was dissolved in 20. ml of conc. sulfuric acid. 30 ml of water was slowly added to this solution with stirring (being careful not to splatter). This solution

TABLE 1

Physical constants of some chemicals used (17)

Substance	Density (gm/ml)	Molecular weight	Lit. mp(C)
Formaldehyde	0.815	30.03	
Acetaldehyde	0.7834	44.05	
Acetone	0.7908	58.05	
Propanal	0.8058	58.08	
Benzaldehyde	1.0415	106.13	
2-Butanone	0.805	72.10	
2,4-DNPH		198.14	194
DIAH		354.41	
Acetonitrile	0.7828	41.05	
2,4-DNPH deriv of:			
Formaldehyde		210.15	167
Acetaldehyde		224.17	157
Acetone		238.17	128
Propanal		238.20	150
2-Butanone			115
Benzaldehyde		286.25	237
Cyclohexanone			160

* * * * *

was then diluted with 100. ml of non-denatured ethanol. The reagent solution was filtered if a solid had precipitated (12)

2. The carbonyl compound (liq) was then added to about 40 ml of the DNPH solution with stirring until precipitation of the hydrazone ceased (at this point the solution was a thick mass of hydrazone crystals).

3. The mass was then suction filtered.

4. The purity was again checked by m.p. and/or hplc. For the hplc purity check, a portion of the hydrazone was dissolved in spec. grade ACN. The resultant area of the hydrazone peak divided by the area of all species eluted was considered to be the percent purity. More strictly the relative concentrations derived from the hplc response calibrations (Table #2) should be used. Recrystallization is generally recommended.

5. Recrystallization:

The hydrazone was dissolved in a minimum amount of diglyme (bis-(2-methoxyethyl) ether) with heating and stirring (about 0.3 gm hydrazone per 10 ml diglyme). The recrystallization could alternately be done in methanol. The solubility in methanol is lower than in diglyme. After complete solution, water was added dropwise to

TABLE 2 HPLC Calibration Standards for 2,4-Dinitrophenylhydrazine Derivatives (18)

Compound	M.P.(°C, exp)	M.P.(°C,Lit)	% Purity	Calibration Range [a]	Wave length (nm)	Response Factor [b] (X10^7)	Correlation Coefficient [c]
Formaldehyde	166-7	167	99.5	4.04ppm-5.74ppb	254 360	3.39 1.62	.9999+ .9999+
Acetaldehyde	157-8	157	99.7	2.10ppm-5.99ppb	254 360	2.95 1.41	.9998+ .9997+
Acetone	124-5	128	99.8	2.02ppm-5.74ppb	254 360	3.30 1.57	.9999+ .9999+
Propanal	149-51	150	99.8	2.06ppm[d]	254 360	3.24 1.51	d d
2-Butanone	113-4	115	99.6	2.02ppm-5.74ppb	254 360	3.54 1.68	.9999+ .9999+
Benzaldehyde	238-39	237	99.6	2.02ppm-5.74ppb	254 360	2.74 1.51	.9999+ .9999+
Cyclohexanone	159-60	160	99.4	1.96ppm-5.57ppb	254 360	3.04 1.58	.9999+ .9999+

a. Gas phase concentration equivalent if a 30.0ℓ sample of air is passed through 4.00ml of solution.

b. Expressed as Molarity/mm^2 for 0.08 Abs, 10mm/min, determined from slope of conc-response plot.

c. Pearson's r value for least squares analysis of concentration-response plot.

d. Based on one concentration.

cloudy. This assured that the hydrazone solution was saturated. The saturated solution was then hot suction filtered and transferred immediately to a beaker. After allowing to cool to room temperature and then on ice, cold water was added dropwise to cessation of crystal formation. The liquid was then suction filtered off and the crystals transferred to a vial and dryed in a vacuum oven. The purity was checked by mp and hplc (see #4 above)

C. Preparation of DNPH sampling solution

This solution is used for the sampling of all carbonyl compounds (liquid and gas phase). The quantity of DNPH should always exceed the quantity of carbonyl compounds.

In a 1.0 l volumetric flask was dissolved 0.50 gm of purified DNPH in 1.0 l of hplc grade ACN. Just prior to use, conc. sulfuric acid was added (0.2 ml per one liter of DNPH solution). (11)

D. The purity of all carbonyl compounds was checked by nmr prior to their use. If impurities were detected a portion of the carbonyl compound was distilled and rechecked for purity before use.

E. Analytical Instrumentation

1. A Varian-Aerograph Cary 118 uv/vis absorption spectrophotometer was used to identify absorbance maxima of hydrazones. A sample spectrum for the acetone hydrazone can be found in Appendix #1.

2. A Hitachi Perkin-Elmer High Resolution nmr spectrometer model number R-24A was used for the evaluation of purity of liquid carbonyl compounds (as obtained from the shelf and/or after distillation.)

3. Analysis of hydrazones was done on a Varian-Aerograph hplc with builtin 254 nm. uv detector and a Tracor 970A variable wavelength detector set with uv lamp at 360 nm. Data was recorded on a Linear dual pen recorder.

a. Column- Alltech C18 reversed phase 25 cm x 4.6 mm 10 micron column

b. Conditions of analysis:

Isocratic 65% ACN/35% water

Flow rate 1.5 ml/min

Abs(254)=constant (for qualitative analysis)

Abs(360)=variable (for quantitative analysis)

c. Care must be taken to check the specifications found in the manufacturer's literature on prefilters used for the hplc. Many "organic solvent" filters are not acceptable for use with acetonitrile. In our lab glass fiber filter paper (with an effective retention of 1.5 microns) was cut to size for sample prefiltering.

d. In analyzing and comparing data all hplc responses were corrected to a standard of abs=08 and chart speed=1 cm/min.

example: For a given analysis on hplc at 360 nm., the abs was set at .04 to provide sufficient sensitivity, the chart speed (c.s.) was set at 4 cm/min to yield an easily measureable peak area. To standardize this data (i.e. the area obtained) divide by 2 (abs .04->abs .08) and divide by 4 (c.s.=4 -> c.s.=1 cm/min).

e. Most of the analyses done were monitored and quantified at 360 nm. This was due to the greater ease in adjusting the absorbance (abs) of the variable wavelength detector in the middle of a run. The adjustment of the absorbance yields peaks of maximum height for varying concentrations of eluent. Additionally, detection at 360 nm. proved to be more sensitive than at 254 nm. (see hplc calibration standards, Table #2)

f. A reference solution containing equimolar amounts of several standard hydrazones was prepared-see Appendix #2. These carbonyl compounds were chosen because they account for some of the more commonly occurring gas phase carbonyl compounds as well as providing a good range of retention times for the characterization of eluents. Table #3 lists the components of the reference solution along with their retention times.

The reversed phase column separates according to side chain size with smaller side chains eluting first. For a given carbon skeleton (e.g. C3) the ketone (acetone) elutes before the aldehyde (propanal) probably because the side chain of propanal is two carbons while those of acetone are one carbon in length. Similarly, straight chain carbonyl compounds would be expected to have longer retention times than branched chain carbonyl compounds for a given carbon number.

Column separation parameters were evaluated using this reference solution in an attempt to obtain baseline resolution as well as short analysis time.

F. Bag sampling

TABLE 3

Standard Hydrazones and Their Retention Times

Component	Retention Time (min. ± 0.10)
2,4-DNPH	2.65
2,4-DNPH deriv. of:	
Formaldehyde	3.85
Acetaldehyde	4.80
Acetone	6.10
Propanal	6.55
Methyl Ethyl Ketone	
-syn	8.10
-anti	8.85 ± 0.2
Benzaldehyde	9.95 ± 0.2
Cyclohexanone	12.40 ± 0.2

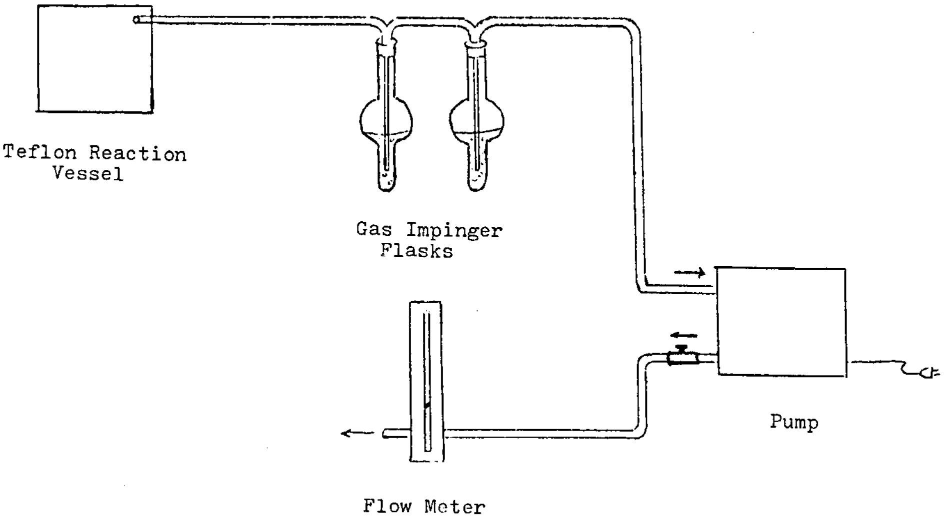
* * * * *

Standardized sampling of carbonyl compounds in the gas phase was done by drawing known volumes of a carefully measured carbonyl compound (or mixture of carbonyl compounds) in the gas phase from Tedlar bags through two impingers in series, each containing 10.0 ml of DNPH. The gas flow rate was set with the needle valve of a calibrated flow meter to between 0.1 and 1.0 l/min depending upon the total sample size. A schematic of the equipment set-up for bag sampling can be found in Figure #1. All gas phase sampling was done through two impingers in series in order that the efficiency of collection of the first impinger could be checked. A blank was prepared for a given day of sampling by storing an aliquot of the DNPH sampling solution (with acid catalyst added on the day of sampling) in a teflon capped vial. This choice of blank was made after ascertaining that the baseline, consisting of the DNPH reagent peak plus major hydrazone contaminants, was largely a function of storage time of the DNPH solution and negligible change resulted from drawing "clean" air through the sampling solution. The method of calibration of the flow meter as well as the method of preparation of Tedlar sampling bags can be found in reference #13.

1. Sampling procedure from Tedlar bags

Figure 1:

Bag Sampling Apparatus



Concentrated sulfuric acid was added to an aliquot of DNPH sampling solution which was to be used for the day's sampling according to the ratio 0.2 ml/1.0 l DNPH solution. 10.0 ml of the resultant solution was then added to each impinger used. Without the bubblers connected, the flow rate was adjusted according to a flow meter calibration curve so that the total sampling time would be between two and twenty minutes (sampling was done in the low ppm and high ppb range with volumes of between two and twelve liters of gas sampled). Connections were then made between the two impingers, between the pump and the second impinger and finally between the Tedlar bag and the first impinger. The pump was started along with a stopwatch. The flow meter was then rechecked for final flow rate adjustments. After sampling was complete and the pump was turned off the impingers were disconnected from the Tedlar bag, from each other and from the pump. An aliquot from each impinger was then stored in a teflon capped vial for future analysis by hplc. Due to evaporation of the ACN solvent, the solution in the impinger should be diluted back up to 10.0 ml by addition of ACN after sampling and before storage. This step will prove to be particularly important in quantitative analyses involving more than two liters of sampled air. Finally, the impingers were rinsed with ACN before reuse.

2. Preparation of carbonyls of known concentration in the gas phase

Using a tank of hydrocarbon free grade compressed air and a calibrated flow meter, a Tedlar bag was filled with a known quantity of air.

In a teflon capped vial 3.900 ml of ACN was weighed out on an analytical balance (using the density value found in Table #1). Using a calibrated 10.0 microliter syringe, weighed aliquots of various carbonyl compounds were then added to the ACN. From these weight measurements, actual liquid volumes could be calculated to the nearest tenth of a microliter (using densities). If necessary, further serial dilutions of this carbonyl solution could be made. Using a 10.0 microliter syringe an aliquot of this diluted solution of carbonyl compounds was then injected into a known volume of air in a Tedlar bag. The actual volume of carbonyl solution added was determined by using an analytical balance and taking weight measurements of the ACN solution before and after injection. The solution was assumed to have the density of ACN. The ACN/carbonyl solution was stored under refrigeration after use. After allowing sufficient time for the system to equilibrate (the length of time determined by the total disappearance of the liquid phase droplets from the inside of the Tedlar bag (for eight microliters in twenty liters of air approximately 15 minutes was allowed) sampling was done as described in section #1 above. After sampling, serial dilutions were made by adding known quantities of hydrocarbon free air to the Tedlar bag containing the gas phase carbonyl compounds. For the determination of sampling efficiency, it was necessary to compare the experimentally determined carbonyl concentrations to the expected concentrations (i.e. the maximum carbonyl recovery expected). The method is as follows.

Calculations of Ideal Recovery from Bag Sampling

An approximately equimolar solution of three carbonyls acetaldehyde, acetone and benzaldehyde was prepared in ACN (spec. grade) using a 10.0 microliter syringe and an analytical balance as described above. This solution was labeled "A". A known volume of this solution was then injected into a Tedlar bag and sampled through DNPH in impingers as described in section #2 above. This process yields an experimental ppm detected for each carbonyl compound (for an explanation of the conversion of peak areas to ppm see the Discussion section).

A weighed aliquot of solution A was then diluted in ACN and this solution was labeled "B".

Example:

(Vial)	wt.	28.6895 gm
(Vial + ACN)	wt.	31.0745 gm

wt. ACN = 2.3850 gm = 3.0468 ml*

(Vial + ACN + aliquot of sol'n A) wt.	31.1435 gm

wt. A added = 0.0690 gm = 0.0882 ml**

net vol. sol'n B = 3.135 ml

* Obtained by using the density of ACN (see Table #1)
** Obtained by using the density of ACN, since the contribution of carbonyls to density is essentially negligible.

Therefore the ratio (vol. sol'n A/net vol. sol'n B) was known. In a similar manner a known volume of solution B was injected into a known volume of the DNPH sampling solution yielding the ratio (vol. sol'n B/net vol. DNPH). This was then converted to the ratio (vol. sol'n A/net vol. DNPH) as follows;

$$\frac{\text{vol. sol'n B}}{\text{net vol. DNPH}} \times \frac{\text{vol. sol'n A}}{\text{net vol. sol'n B}} = \frac{\text{ml sol'n A}}{\text{1.0 l DNPH}} \quad (1)$$

A portion of the resultant DNPH solution was then injected into the hplc. From the peak area obtained for each component, the concentration of each carbonyl compound in the DNPH solution was calculated using the hplc calibration factors (Table #2). The carbonyl compound, acetone, will be used as an example.

Result of hplc plus calculations: $\frac{\text{moles acetone}}{\text{1.0 l DNPH}}$ (2)

Expression (2) was then divided by expression (1) to yield

$$\frac{\text{mole acetone}}{\text{ml sol'n A}}$$

Therefore,

$$\frac{\left(\frac{\text{mole acetone}}{\text{ml sol'n A}}\right) \times \text{ml sol'n A injected*}}{(\text{no. 1 air})\left(\frac{\text{4.117 E-2 mole gas **}}{\text{1.0 liter air}}\right)} \times (1\ \text{E6}) = \text{PPM}$$

* from experimental procedure e.g. $\left(\frac{0.008 \text{ ml sol'n A}}{20.0 \text{ l air}}\right)$

** assuming ideal gas behavior and T=298 K

RESULTS AND DISCUSSION

Column Evaluation

Accurate determination of individual aldehyde and ketone concentrations is dependent upon the extent to which the analytical conditions allow for the quantitative separation of the components of interest. In order to measure the effectiveness of analysis of a given column and separation conditions from a sample mixture, four parameters were evaluated:

Efficiency (N)=number of theoretical plates
Selectivity (α)
Capacity (k')=partition ratio
Resolution (R)

The method of determination of each of these parameters can be found in Appendix #3.

The efficiency of a liquid chromatographic column is greatest for compounds with k' values between 2 and 6. However, acceptable values are between 1 and 15. k' essentially represents the time constraint of an analysis. That is, small k' values indicate that components elute close to the unretained solvent while large k′ values improve separation but lead to long analysis times (14).

Column selectivity, α, is a measure of the relative separation of two peaks. Values greater than 1.05 are generally accepted to provide 98% separation of peaks (15).

The resolution may be improved by improving the efficiency, N. An efficient column keeps the bands from spreading and/or gives very narrow peaks. A well packed column of particle size 10 microns should be between 3500 and 10,000 theoretical plates (16,p.101).

Generally a resolution value, R, of 1.0 or greater is required for good quantitative or qualitative work. Figure #2 shows peak shapes and corresponding R values for various partly resolved pairs of bands (16).

These parameters were used to compare two different hplc columns and then to determine the effect of solvent programming on the better of the two columns. The results of these determinations are compiled in Table #4. From this table it is clear that conditions "c" (solvent programming) provided the best separations as indicated by the higher N, R, and k′ values. However, there are disadvantages to solvent programming which make its use impractical for present separations. While actual analysis time is comparable between conditions c and b (13.0 minutes for elution of the cyclohexanone hydrazone

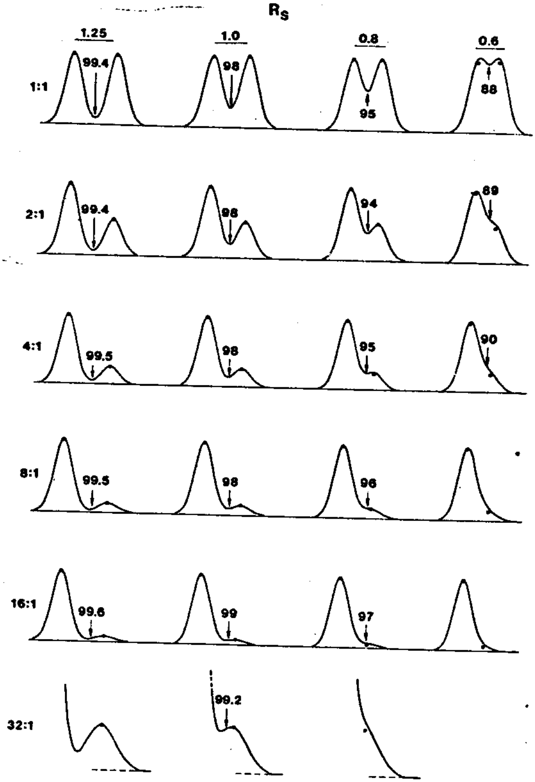


Figure 2. Standard curves for resolution (16)

TABLE 4

Column Parameters

2,4-DNPH deriv. of:	N			α			k'			R		
	a	b	c	a	b	c	a	b	c	a	b	c
2,4-DNPH	1869	2873	3100				1.03	0.81	1.24			
				1.41	1.99	2.15				1.84	5.43	7.07
Formaldehyde	2241	4397	5615				1.44	1.62	2.67			
				1.17	1.38	1.34				1.05	3.43	4.06
Acetaldehyde	2284	4530	7015				1.69	2.23	3.59			
				1.20	1.39	1.29				1.26	4.03	4.60
Acetone	2454	5341	10607				2.03	3.11	4.65			
				f	1.02d	f				0.0	1.10d	f
Propanal	f	5454d	f				f	4.4d	f			
				f	f	f				f	f	f
MEK- syn	2228	5692	14884				2.56	4.53	6.01			
				1.0	1.08	1.05				0.0	1.13	1.35
- anti		5574	16273					4.90	6.33			
				1.14	1.16	1.09				1.09	2.22	2.38
Benzaldehyde	2145	5461	18943				2.92	5.68	6.90			
				1.09	1.28	1.13				0.71	3.87	3.62
Cyclohexanone	1963	5366	21319				3.17	7.27	7.81			

a- Varian Micropak MCH-10 C18 reversed phase column; Isocratic 65% ACN

b- Alltech MCH-10 C18 reversed phase column; Isocratic 65% ACN

c- Alltech MCH-10 C18 reversed phase column; Solvent programming t=0 - 10 min 55% - 75% ACN

d- Data taken in a separate analysis

f- Data not available

by solvent programming versus 12.5 minutes for isocratic) the programming requires that the column be brought back to initial conditions for the subsequent analysis. Total analysis time increases by three to five minutes per sample. Therefore, the use of column b run isocratically at 65% ACN gives separations which are most nearly within the ranges desired (as described above), yielding the necessary compromise between short analysis time and quantitative separation.

Experimentally, the improved separation obtained with column b allowed for the resolution of several pairs of components which were of identical molecular weight but different structures-refer to Appendix #2. Most notably, the separation of the acetone hydrazone from the propanal hydrazone (both C3 carbonyls) was effected with quantitative separation. Additionally two peaks were resolved from what had, on previous columns, been one 2-butanone hydrazone (methyl ethyl ketone) peak.

It is proposed that the 2-butanone hydrazone peaks result from the two stereoisomeric structures (below) of the hydrazone.

From steric considerations, it is predicted that the first conformation would be the more abundant as a result of decreased steric hindrance with the DNP unit. Analytically, the first conformation might be expected to elute last on a reversed phase column since the side chain which is more available for binding with the stationary phase is a C2 rather than a C1 as in the latter conformation. The reversed phase column separates according to side chain size with smaller side chains eluting first. The experimental results support these assumptions since the later of the two peaks is also the larger of the two.

Separation of Early Retention Time Components

Several unidentified peaks recurred throughout the analytical work done. Two peaks were consistently detected at 254 nm. after the DNPH reagent peak and before the formaldehyde hydrazone retention time. The detection at 254 nm and not at 360 nm suggests an aromatic compound without the extended nitro-conjugated ring functionality. The first of these two appeared as a shoulder to the DNPH peak and its area could not be measured with accuracy. By using solvent programming baseline separation was achieved among these early retention time components. (Refer to Appendices #4 and #5 for a comparison of the spectra obtained isocratically and with solvent programming respectively). This method of early retention time separation may prove valuable in later analyses.

Rate of Contamination of DNPH Sampling Solution

Samples of the DNPH sampling reagent stored with acid catalyst showed less than 0.5% contamination (based upon relative peak areas of DNPH to all eluents obtained from hplc) after 16 days of storage. This fact together with Figure #3, which shows the build-up of contamination of the DNPH reagent in several different storage containers versus time, suggests that the useful shelf life of the DNPH sampling solution is on the order of three to four weeks (presumably depending upon the local ambient concentrations of carbonyl compounds). After approximately three weeks of storage, a steady rate of contamination made the DNPH reagent unsuitable for quantitative analyses. From Figure #3 it is clear that storage of the solution with acid catalyst in the teflon capped vial is less prone to contamination than storage in any other container tested. This form of storage would presumably apply to aliquots of the DNPH reagent which had already been used for gas phase sampling and therefore already contained acid catalyst.

In all of the samples stored with acid the major contaminant after two weeks was an unknown component which eluted before the DNPH reagent. This component typically accounted for approximately 50% of the impurities. The other major contaminant was an unknown

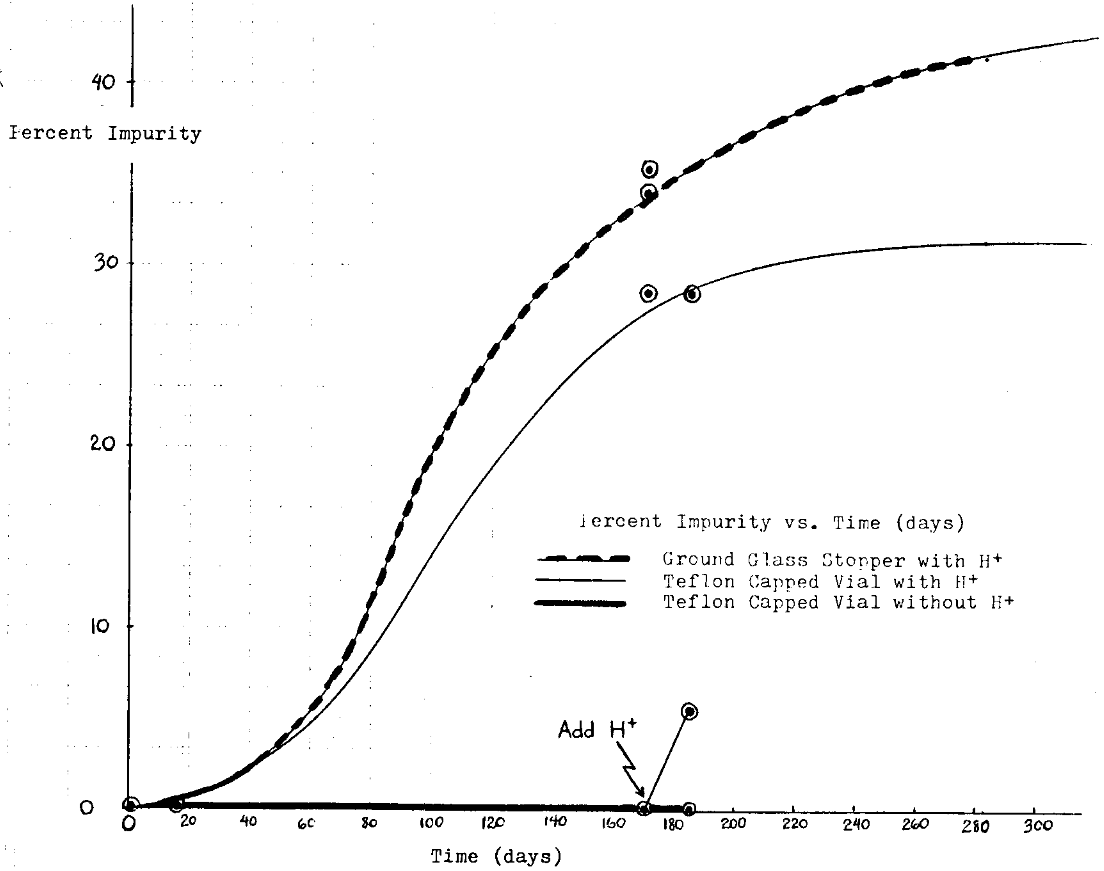


Figure 3. Buildup of Contaminants in 2,4-DNPH Reagent Solution

component which eluted just after the acetone hydrazone. This compound similarly accounted for approximately 50% of the total contamination. Contamination by any of the "standard" hydrazones was negligible. Refer to Table #5 for a list of contaminant concentrations in six month old DNPH reagent samples.

Storage of the DNPH under a plastic cap resulted in a contamination that, upon analysis by hplc, gave a constant elution of components which totally obscured the baseline from the DNPH reagent peak to beyond fifteen minutes (for reference, the cyclohexanone hydrazone elutes at about 13 minutes).

Practical Solution for DNPH Storage

The DNPH reagent solution was effectively stored without acid catalyst in a volumetric flask for two months at which time bag sampling analyses were done which proved consistent with analyses using fresh DNPH reagent. The use of the ground glass stoppered container rather than 4 ml. teflon capped vials was necessary for the storage of the bulk reagent solution.

In all samples stored without acid the major contaminant upon addition of the acid catalyst was the acetone hydrazone. This typically accounted for approximately 75% of all contamination. The formaldehyde, acetaldehyde, and benzaldehyde hydrazones appeared sporadically in trace quantities. Two unknown

TABLE 5

Contamination of 2,4-DNPH solutions after 171 days of storage

Container	r_N*(min)	Source (2,4-DNPH deriv. of)	% Impurity
Plastic Capped	3.2	Unknown	15.9
	6.7	Unknown	17.1
	7.9	Unknown	1.1
			net 34.1%
Ground glass stopper	3.2	Unknown	19.7
	6.7	Unknown	15.6
			net 35.3%
Teflon Capped vial, stored with H^+	3.2	Unknown	16.0
	6.2	Acetone	trace
	6.9	Unknown	12.5
			net 28.5%
Teflon Capped vial, stored with no H^+	2.9	Unknown	trace
	6.2	Acetone	trace
	8.4	Benzaldehyde	trace
			net trace
Teflon Capped vial, stored with no H^+. H^+ added on day of analysis	3.1	Unknown	0.06
	6.3	Acetone	0.09
	8.3	Benzaldehyde	trace
			net 0.15%

* * * * *

* r_N = Retention time

compounds consistently eluted as shoulder peaks to the DNPH reagent peak. These accounted for approximately 25% of all contamination (see Table #5).

Due to the presence of contaminants in the DNPH sampling solution it was standard procedure to prepare a blank at the time of sampling. Thus background levels of contamination as a result of storage could be evaluated for a sample of DNPH reagent and subtracted from subsequent analyses. This method of correcting for contaminations in the DNPH was applied to all bag sampling done.

In bag samplings, acid catalyst was added to stored DNPH just prior to use. A blank was then set aside for each day's sampling. An interesting phenomena arose from this practice which may warrant some further investigation. In analyzing the solution collected in the second impinger, it was consistently found that the level of contamination was lower than in the blank. If the level of contamination does decrease upon sampling, this would lend a potentially large source of error to the method of subtracting out background contaminations. In several analyses, the amount of contaminants was equal to the amount of the carbonyl compound analyzed for (this was particularly true for the acetone hydrazone). In such a case, the data collected is of questionable validity and should be verified by duplication of the experiment using fresh DNPH reagent solution.

Alternately, it may be possible to unequivocably establish the level of background contaminations.

Use of hplc Response Factors

By making serial dilutions of the reference solution, made from weighed samples of the isolated hydrazones, it was possible to construct a peak area versus concentration graph such as Appendix #6 for each hydrazone of interest at 254 nm as well as at 360 nm. The slope of these graphs are the response factors of the uv detector at a given wavelength for a given hydrazone (between the given concentration ranges). This data is compiled in Table #2. These response factors allow for the quantitative determination of the hydrazones of interest from the peak areas obtained during analysis by standardizing the peak areas to abs = .08 , chart speed = 1 cm/min and then multiplying by the response factor for a given wavelength of detection.

Sources of Error in Bag Sampling

A summary of the steps involved in bag sampling and the error which they propagated are compiled in Table #6. The resultant percent errors have a cumulative effect. Therefore, a minimum method uncertainty could be set at 20%. For experiments in which both an experimental result and an expected result were calculated, both numbers would carry an uncertainty.

TABLE 6

Sources of Error in Bag Sampling

Procedure	Error Propagated	Notes
1. Preparation of carbonyl solution	0	By using weight measurements, the error was negligible
2. Filling and diluting Tedlar bags	3-5%	Errors in calibration of flow meter
3. Injection of a sample into Tedlar bags	5%	Small sample size result in relatively large uncertainties
4. Gas phase sampling	3-5%	See #2 above
5. Analysis of data	10%	From measurement of peak heights and half widths

* * * * *

Gas Phase Sampling Results

Using the hplc response factors to convert peak areas into hydrazone concentrations (which equals carbonyl concentrations assuming 100% reaction of DNPH with each carbonyl compound) and subtracting any reagent hydrazone contaminations as determined from the blank yields the concentration (in moles/liter of DNPH solution) of a carbonyl compound trapped during sampling. This was multiplied by the volume of DNPH solution used in sampling (0.01 l) to find the number of moles of a given carbonyl compound actually trapped. This number was divided by the number of moles of air sampled, to yield the fraction, ppm or ppb as follows.

Determination of ppm Carbonyl from Peak Areas

For a given carbonyl compound:

(Peak area experimental) - (Peak area blank) = (Peak area corrected)

$$(\text{Peak area corrected}) \times \text{Response factor} = \left(\frac{\text{mole carbonyl}}{\text{l. l DNPH soln}}\right)$$

x (0.01 l DNPH solution used = moles of carbonyl

Assuming ideal gas behavior for air:

$$PV = nRT$$

$$\frac{n}{\text{l. l air}} = \frac{(1\ \text{atm})}{(0.08206\ \text{atm.l./Kmole})(298\ \text{K})} =$$

$$4.117\ \text{E-2 mole gas/l. l air}$$

Therefore,

$$\left(\frac{\text{moles carbonyl}}{(\text{number of liters air sampled})(4.117\ \text{E-2})}\right) \times 1\ \text{E6} = \text{PPM}$$

Gas Phase Sampling Continued

The initial studies were directed towards determining the effective concentration range of carbonyl compounds over which the DNPH method gave accurate analyses. Using the methods described, serial dilution were made to generate gas phase samples of acetaldehyde, acetone and benzaldehyde in the range of 125 ppb - 2 ppm. This preliminary work was intended to establish the direction that more quantitative investigation should pursue. The following results and conclusion therefore address themselves to this purpose.

In the two runs made, recovery of the acetaldehyde and acetone hydrazones was on the order of 30%-50%. The recovery of the benzaldehyde hydrazone varied widely from about 94% to 0%.

In the sampling done at 2 ppm, the benzaldehyde hydrazone was consistently not recovered. The benzaldehyde hydrazone was detected at all lower concentrations except in one experiment at 1 ppm and the other experiment at 250 ppb in which none of the benzaldehyde hydrazone was detected. In the runs in which any of the hydrazone compounds were detected there was no pattern to their level of recovery (i.e. they showed marked scattering). The benzaldehyde hydrazone seemed particularly sensitive to this scattering. This would tend to suggest that there were recovery problems in the present system which were specific to the benzaldehyde hydrazone, or which affected the benzaldehyde hydrazone to the greatest degree.

It was proposed that, in addition to errors propagated by the unfamiliarity with the techniques, the results may have been subject to some amount of adhesion of the carbonyl compounds to the walls of the Tedlar bags. To test this proposal a final experiment was run in which incremental 1 ppm additions of the ACN solution of carbonyl compounds were made to a Tedlar bag to provide a concentration range of approximately 1 to 3 ppm. Assuming the existence of the adhesion phenomena, a "wall saturation" level should be reached above which point incremental injections should yield incremental recoveries of the carbonyl compounds. The results of this experiment can be found in Table #7. Consistent recoveries were recorded of the acetaldehyde and acetone hydrazones at approximately 100% (within experimental error) and of the benzaldehyde hydrazone at 0%. This suggests that the wall saturation level had been reached for the acetaldehyde and acetone hydrazones but that the benzaldehyde hydrazone was still not at saturation level after the final addition. This is inconsistent with the data of previous experiments in which the benzaldehyde

TABLE 7

Collection Efficiency in Bag Sampling

Hydrazone Component	PPM^+	$\frac{PPMc}{PPMe}$	PPM^+	$\frac{PPMc}{PPMe}$	PPM^+	$\frac{PPMc}{PPMe}$	Avg % Recovery
Acetaldehyde	0.60	$\frac{0.63}{0.60}$	0.63	$\frac{1.23}{1.23}$	0.62	$\frac{2.04}{1.85}$	110%
Acetone	0.68	$\frac{0.62}{0.68}$	0.70	$\frac{0.90}{1.38}$	0.70	$\frac{2.00}{2.08}$	96%
Benzaldehyde	0.29	$\frac{0.0}{0.29}$	0.30	$\frac{0.0}{0.59}$	0.30	$\frac{0.0}{0.89}$	0%

* * * * *

PPM^+ represents the incremental addition of the carbonyl compound to the bag sample

PPMc represents the ppm collected as determined by analysis by hplc

PPMe represents the ppm expected assuming 100% recovery was consistently recovered at concentrations below 2 ppm.

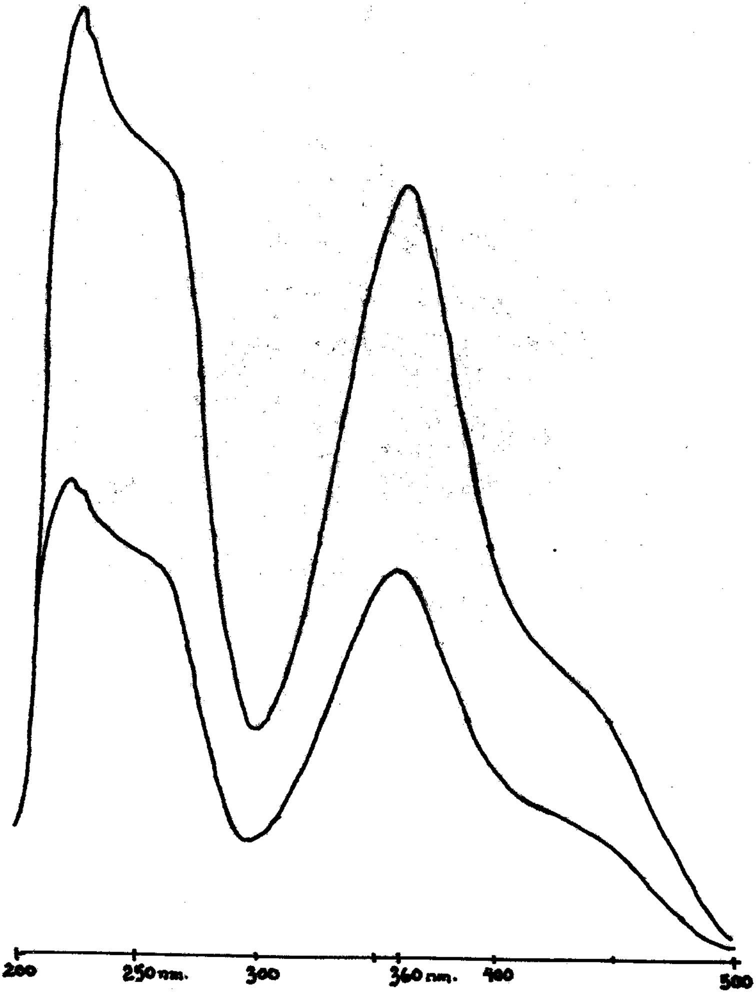
It would seem that at the outset of bag sampling the limiting factor is the proficiency of the experimenter (as is true of most processes). Assuming the absence of peculiarities in the method to benzaldehyde, the flaw in technique which yielded early inconsistencies had yet to be eliminated in this last experiment.

FUTURE WORK

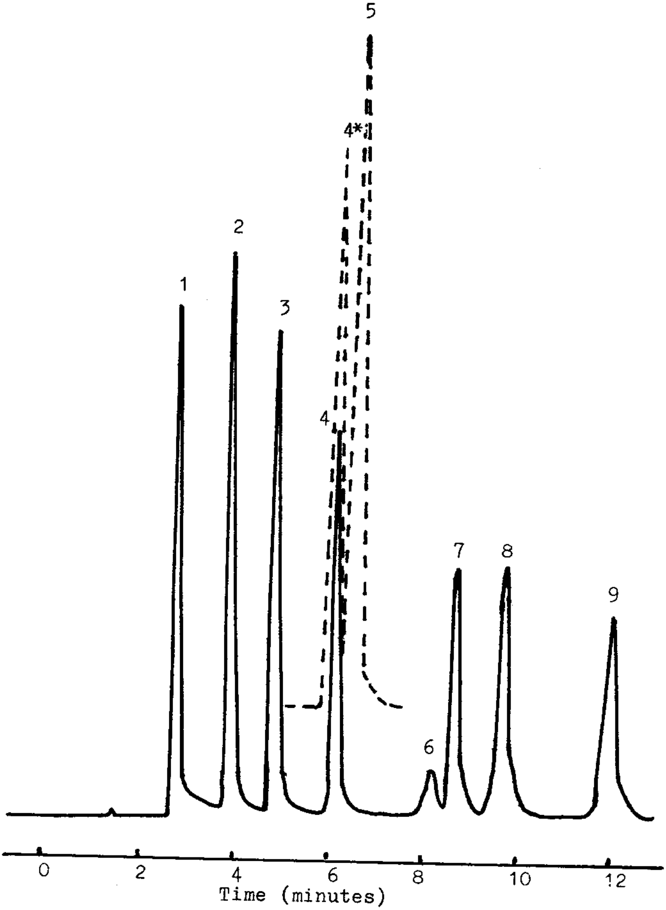
The method evaluation studies have yet to be completed. These include studies of the derivatized solution stability, the concentration range which can quantitatively be sampled, the possible interference of the DNPH-ACN by standard air contaminants, and the effect of water interference in the form of local humidity.

Acetonitrile has proven to be a valuable solvent because it is water solubility (which makes the sampling solution hplc compatibile and therefore requires no sample workup), it is polar, (which dissolves the reactants and products well), and it does not contain O-H groups (which would, like a water solvent, interfere with the derivatization reaction). However ACN has the disadvantage of being highly volatile. As a result there is a volume loss during sampling which must be accounted for by post sampling dilution of the sampling solution. The introduction of ACN into the environment of the worker in the form of exhaust gases from the sampling may prove to be objectionable, since ACN vapors are harmful and exposure is regulated. In addition, there is the possibility of a side reaction with the derivatizing reagents in which ACN is subject to nucleophilic attack under the conditions of acid catalysis. For these reasons, alternative solvents should be investigated which possess the desirable characteristics of ACN but which are less volatile. Bis-(2-methoxyethyl) ether (diglyme), with a boiling point of 162 C, is a proposed alternate solvent and its use should be investigated. Diglyme has been used for derivatization of aldehydes and ketones in the liquid phase (14).

It is proposed to extend the present studies to investigate the use of DIAH (2-diphenylacetyl-1,3-indandione-1-hydrazone) with hplc separation and fluorescence detection. Assuming comparable reactivity, this would presumably lower the detection limits of the method since fluorescence is generally more sensitive than absorption spectroscopy.



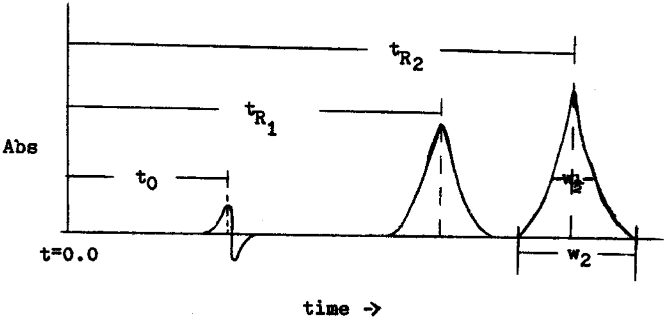
Appendix 1. UV spectrum of the Acetone 2,4-Dinitrophenylhydrazone



Appendix 2. HPLC of the reference solution of carbonyl compounds. Peak 1, DNPH reagent; peak 2, formaldehyde derivative; peak 3, acetaldehyde derivative; peak 4, acetone derivative; peak 5, propanal derivative (taken along with peak 4* in a separate chromatogram); peak 6, methyl ethyl ketone (-syn?); peak 7, methyl ethyl ketone (-anti?); peak 8, benzaldehyde derivative peak 9, cyclohexanone derivative

APPENDIX 3

Evaluation of Column Parameters

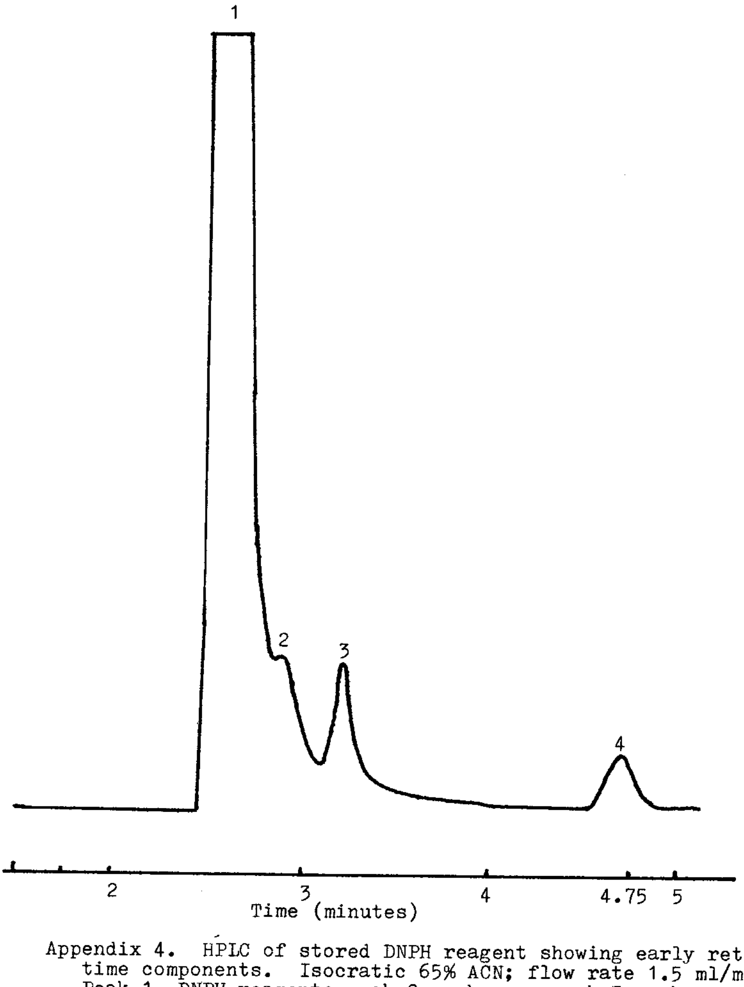


Efficiency = Theoretical plates = N = $5.5 \left(\frac{t_R}{w_{\frac{1}{2}}} \right)^2$

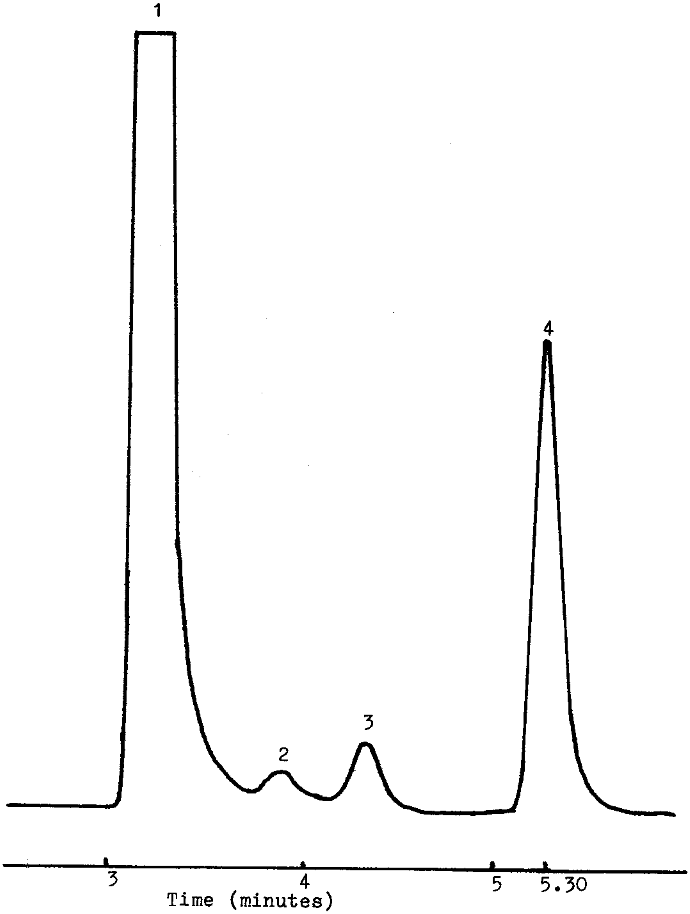
Capacity = Partition Ratio = k' = $\frac{t_R - t_0}{t_0}$

Resolution = R = $\left(\frac{t_{R_2} - t_{R_1}}{\left(\frac{w_2 - w_1}{2} \right)} \right)$

Selectivity = α = $\left(\frac{t_{R_2} - t_0}{t_{R_1} - t_0} \right)$



Appendix 4. HPLC of stored DNPH reagent showing early retention time components. Isocratic 65% ACN; flow rate 1.5 ml/min Peak 1, DNPH reagent; peak 2, unknown; peak 3, unknown; peak 4, formaldehyde derivative



Appendix 5. HPLC of stored DNPH reagent showing early retention time components. Solvent programming 55%-75% ACN in 10 minutes. Peak 1, DNPH reagent; peak 2, unknown; peak 3, unknown; peak 4, formaldehyde derivative

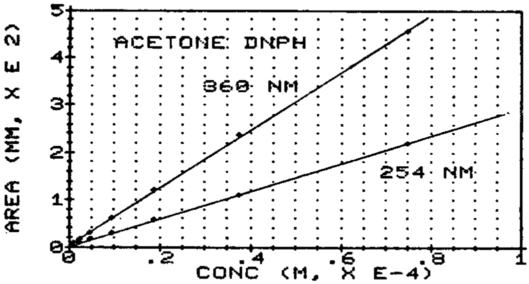
```
FOR THE DATA IN ACETONE360.DATA.

MOLARITY (XE-4) AREA (MM^2, .08 ABS)
--------------- ------------------

4.12            2630
.749000001      455
.375            239
.187            122
.0936           63.1
.0468           30.2
.0234           16.7
.0117           9.22


         Y = M * X + B

SLOPE = 6378428.42 MM^2/MOL/LITER
RESPONSE= 1.56778431E-07 MOL/LITER*MM^2
INTERCEPT = -1.35573681 MM^2
PEARSON'S R= .999952574
```



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FOR THE DATA IN ACETONE254.DATA.

MOLARITY (XE-4) AREA (MM^2, .08 ABS)
--------------- ------------------

4.12            1250
.749000001      221
.375            112
.187            56.9
.0936           29.1
.0468           14.6
.0234           7.43000001
.0117           3.52


         Y = M * X + B

SLOPE = 3033473.64 MM^2/MOL/LITER
RESPONSE= 3.29655082E-07 MOL/LITER*MM^2
INTERCEPT = -.770874609 MM^2
PEARSON'S R= .999985147
```

AREA (MM, X E 2)

Appendix 6. Data for determination of HPLC response factor for the acetone 2,4-Dinitrophenylhydrazone

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