CHROM. 4815

QUANTITATIVE ANALYSIS OF NITRO COMPOUNDS IN THE MICRO- TO PICOGRAM RANGE BY A COMBINATION OF THIN-LAYER AND VAPOR PHASE CHROMATOGRAPHY WITH THE NICKEL-63 ELECTRON CAPTURE DETECTOR

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(Received May 4th, 1970)

SUMMARY

A method has been developed for the quantitative analysis of nitro compounds in the micro- to picogram range by a combination of thin-layer and vapor phase chromatography employing the nickel-63 electron capture detector. The relative electron absorptivities of ten nitro compounds have been measured with 1,3,5-trinitrobenzene as a standard. Experimental variables affecting the nickel-63 electron capture detector are presented.

INTRODUCTION

Recently we reported a method for the quantitative analysis of polynitroaromatic compounds in complex mixtures by combination of thin-layer chromatography (TLC) and visible spectrometry[1]. The method is based on the formation of a colored "Meisenheimer" complex between the polynitroaromatic compound and ethylenediamine (EDA) in dimethylsulfoxide (DMSO) solution[2] after separation and extraction from thin-layer plates. This method has proved particularly useful in those cases where stable colored complexes were formed which possessed relatively high molar extinction coefficients (15 to 20 $\times$ 10^4) and whose solutions obeyed Beer's law. However, there are instances where the method fails. Cyclotrimethylenetrinitramine (RDX), for example, forms no colored complex with EDA in DMSO solution[2]. In addition, Beer's law was found not to hold for both the *meta*- and *para*-dinitrobenzenes[2], while variable results were obtained with picryl chloride in EDA–DMSO solutions[3]. In other cases the development of the colored complex was quite slow as with 1,3,5-trimethyl-2,4,6-trinitrobenzene[2]. Rationales for these results have been presented[2] in terms of chemical interactions involving both displacement of nitro groups and irreversible reactions between the polynitroaromatic compound and the nucleophilic base, ethylenediamine.

It appeared that quantitative analysis of nitro compounds in amounts usually

encountered in TLC separations (10^{-5} to 10^{-6} g) might be possible by vapor phase chromatography (VPC) with an extremely sensitive detector. In this regard, the nickel-63 electron capture detector has been most valuable in pesticide residual analysis where amounts as low as 10^{-12} g of halogen containing pesticide have been detected[4–7]. Furthermore, LOVELOCK AND LIPSKY[8] found, for example, that the electron affinity of nitrobenzene was about 35% that of hexachlorobenzene, C_6Cl_6, a compound which was detectable at the picogram (10^{-12} g) level. We wish now to report a method for the quantitative analysis of nitro compounds by a combination of TLC and VPC employing the nickel-63 electron capture detector.

EXPERIMENTAL

Preparation of Silica Gel HF-254 thin-layer plates

Thirty grams of fluorescent Silica Gel HF-254 (Brinkman Inst. Co.) was vigorously slurried for 2 min in 65 ml of distilled water in a 500 ml erlenmeyer flask to make 11 to 12 10 × 20 cm TLC plates with a Camag applicator. The plates were allowed to dry partially at room temperature for 3–4 h, then in a drying oven at 110° for 1.5 h. The dried plates were stored at room temperature in a closed container.

Developing solvents

Nitro compounds were developed with (a) benzene–hexane–pentane (50:40:10), and (b) benzene–hexane–pentane–acetone (50:40:10:10) depending on the separation desired (see Table I).

TABLE I

R_F VALUES OF VARIOUS NITRO COMPOUNDS ON SILICA GEL HF-254 PLATES VISUALIZED UNDER 2537 Å UV LIGHT

Compound	R_F *value*[a]	R_F *value*[b]
Cyclotrimethylenetrinitramine (RDX)	0.02	0.06
2,3,4-Trinitrotoluene	0.13	0.24
1,2-Dinitrobenzene	0.18	0.45
2,4,6-Trinitroanisole	0.20	0.74
1,3,5-Trinitrobenzene	0.21	0.76
2,4,6-Trinitrotoluene	0.28	0.83
1,3-Dimethyl-2,4,6-trinitrobenzene	0.34	1.00
2,4,6-Trinitro-1-chlorobenzene	0.34	0.84
1,3,5-Trimethyl-2,4,6-trinitrobenzene	0.51	1.00
1,3-Dichloro-2,4,6-trinitrobenzene	0.56	0.90
1,3,5-Trichloro-2,4,6-trinitrobenzene	0.84	0.95

[a] Developing solvent (a).
[b] Developing solvent (b).

Zone visualization on TLC plates

Developed zones containing the nitro compounds were visualized under a 2537 Å UV light source with a Chromato-Vue Cabinet (Ultra Violet Products Inc., San Gabriel, Calif., U.S.A.) and appeared as dark spots against a yellowish-green background. Limits of detection were found to be approximately 4×10^{-7} g/spot.

Vapor phase chromatography

An F & M Model 5754A Research Gas Chromatograph equipped with a Model 5763A electron capture (EC) Nickel-63 detector and pulser kit together with a Model 7128A Moseley Dual Channel Recorder was used for all the compounds studied.

Septa were cleaned by thoroughly washing with distilled water, acetone, and hexane (GC spectrophotometric quality solvent, Baker Chem. Co.) and finally heated at 250° overnight under a vacuum of approximately 1 mm Hg.

Column preparation

The following general procedure is recommended to provide an even coating of the liquid phase on the solid support and to avoid the formation of fines encountered in any mechanical grinding of the solid support and is illustrated for the preparation of a 2.53% Apiezon M liquid hydrocarbon phase on 60/80 mesh Diatoport-S. A total of 0.26 g of Apiezon M was dissolved in 50 ml of boiling benzene (special quality recommended for pesticide residual analyses, No. 1043, Mallinckrodt Chem. Works) and then added to a boiling mixture of 10.0 g of 60/80 mesh Diatoport-S (Hewlett Packard ST-120-1) and 50 ml benzene. The mixture was then boiled fairly vigorously in a fume hood until most of the benzene had been removed, and the damp cake was stirred occasionally with a clean plastic spatula until no vapors of benzene were detected. The dry, coated support was then vacuum loaded by means of a water aspirator into a clean 4 ft. × ¼ in. glass column, and conditioned at 200° with a flow of Ar–CH_4 (95:5) at 70 ml/min for 3 h. During the conditioning period the column was disconnected from the nickel-63 detector to avoid contamination.

The following columns were used for all the nitro compounds studied: 4 ft. × ¼ in. glass, packed with 1.18%, 2.04%, 2.92%, 3.51%, and 5.33% Apiezon M liquid hydrocarbon and 3.75% silicone grease DC-11 on 60/80 mesh Diatoport-S. Methylene chloride was used as solvent in the preparation of the silicone coated column, while benzene was used to prepare the Apiezon M columns.

Analytical procedure

Since the nickel-63 detector is extremely sensitive, it is also quite easily contaminated or overloaded. As little as 1×10^{-6} g of 1,3,5-trinitrobenzene was found to overload the detector and resulted in almost complete loss of sensitivity. The contaminant was completely removed only after baking out the detector at 300° with a flow of Ar–CH_4 (95:5, v/v) also at 300° for a period of 12 h. Therefore, it is necessary to use two sets of Hamilton syringes: (1) one for concentrated solutions and spotting TLC plates, and (2) another for VPC analyses. These syringes were kept thoroughly clean by washing with acetone followed by high purity benzene between injections.

Stock solutions containing known amounts of nitro compounds in concentrations of approximately 2×10^{-6} g/μl in benzene or benzene–acetone (90:10) were accurately spotted by means of a 10 μl (No. 701) or 50 μl (No. 705) Hamilton syringe onto Silica Gel HF-254 TLC plates. The volume spotted in each case was no more than 5 μl/spot and the total number of spottings for a 10 × 20 cm plate was no more than 8. The spotted plate was allowed to air dry for about 1 min, then developed by an ascending technique in a 16 × 21 × 26 cm glass, rectangular developing chamber fitted with a ground glass cover. After the solvent front had traveled a distance of between 10 and 14 cm, the plate was removed from the developing chamber and

allowed to dry in a vacuum hood for about 10 min, before visualization under 2537 Å UV light.

The zones appeared as dark spots against a yellow-green background under UV light and were marked and scraped off into a small beaker prior to extraction as previously described[1]. This procedure was modified with equally good results by scraping the zones directly into a 10 ml volumetric flask and extracting with benzene or benzene–acetone (90:10) without physically removing the silica gel support. The volume occupied by the silica gel is negligible and is compensated for by the addition of an internal standard. For zones with low R_F values, for example RDX, it was found best to add 1 ml of acetone to the silica gel in the volumetric flask, swirl for about 1 min, then dilute with benzene to make 10 ml of solution. To this solution was added 24 μl of a solution of 1,2-dinitrobenzene (1.804×10^{-7} g/μl) as internal standard (IS) to make a final concentration of 4.33×10^{-10} g/μl. Concentrations of nitro compounds in solution varied from 4 to 70×10^{-10} g/μl depending on the relative responses of the individual compound (see Table VIII). Injections of 1.0 to 2.8 μl of these solutions were made with 2.8 μl solvent "back flush". "Back flush" was accomplished by drawing 2.8 μl of solvent into a 10 μl syringe, followed by about 0.2 μl air, and finally by 1.0 to 2.8 μl of sample solution. Sample injection was followed by injection of a standard solution containing a known concentration of the nitro compound with the same concentration of internal standard as in the sample solution.

The grams, g_{tlc}, of nitro compound extracted from the TLC plate into 10 ml of solvent were found from the expression,

$$g_{tlc} = (nh_{tlc}/h_{std})(C_{std})(10^4) \quad (1)$$

where nh_{tlc}, h_{std}, and C_{std} are the normalized sample peak height, standard peak height, and standard concentration of nitro compound in 10 ml of solution expressed in g/μl.

The normalized sample peak height, nh_{tlc}, is readily found by,

$$nh_{tlc} = (h'_{IS}/h''_{IS})(h_{tlc}) \quad (2)$$

where h'_{IS} and h''_{IS} are the internal standard peak heights for the standard and sample (TLC extract) solutions, respectively.

The total number of grams of nitro compound, g_{total}, in volume, V, from which the TLC spottings were made, may be calculated from the expression,

$$g_{total} = g_{tlc}(V/\mu l_{tlc}) \quad (3)$$

where μl_{tlc} is the total volume of solution spotted on the TLC plate. In practice, it is not necessary to know the precise volume of the TLC extraction, as long as known aliquots of internal standard are injected into the TLC extract and standard solutions (see Table II). The concentrations of internal standard and standard nitro compound in 10 ml of solution must, however, be known exactly.

RESULTS AND DISCUSSION

Thin-layer and vapor phase chromatographic analysis of 1,3,5-trinitrobenzene (TNB)

The vapor phase chromatographic analysis of TNB after development and

TABLE II

ANALYSIS OF 1,3,5-TRINITROBENZENE (TNB) BY A COMBINATION OF THIN-LAYER AND VAPOR PHASE CHROMATOGRAPHY

Solution	*Peak heights*[a]		*Normalized peak height TNB (mm)*	*μg TNB*[b] *found*
	IS (mm)	*TNB (mm)*		
TLC extract	211[c]	163	164 (nh_{tlc})	17.4[d]
VPC standard[e]	213[f]	167	167 (nh_{std})	

[a] IS = internal standard, 1,2-dinitrobenzene.

[b] Calculated from: $(\mu l\ IS_{sample})/(\mu l\ IS_{standard}) \times (nh_{tlc})/(nh_{std}) \times C_{std} \times 10^{4}$.

[c] $\mu l\ IS_{sample} = 12$; that is, 12 μl of internal standard (1.804×10^{-7} g/μl) were injected into about 5 ml of benzene TLC extract.

[d] Compared to 17.7×10^{-6} g of TNB actually spotted on the plate.

[e] $C_{std} = 3.536 \times 10^{-9}$ g/μl.

[f] $\mu l\ IS_{standard} = 24$; that is 24 μl of internal standard were injected into 10.00 ml of TNB standard.

extraction from a TLC plate is illustrative of the general method of analysis (see Table II). A total of 17.68×10^{-6} g of TNB was applied in two spots on a fluorescent TLC plate. The plate was developed with benzene–hexane–pentane (50:40:10) and zones were located under 2537 Å UV light, scraped off and extracted with 4 to 5 ml of benzene without filtering. To this solution was added 12 μl of stock (1.804×10^{-7} g/μl) 1,2-dinitrobenzene as internal standard (IS). The concentration of standard TNB solution in 10 ml benzene was 3.536×10^{-9} g/μl to which had been added 24 μl of 1,2-dinitrobenzene internal standard. Comparison of the extract and standard solutions was made by injecting 1.8 μl of each solution together with 2.8 μl benzene solvent "back flush" into the chromatograph. A 2.53% Apiezon M on 60/80 mesh Diatoport-S column was used isothermally at 150° with a flow/purge rate of 143 ml/min with Ar–CH_4 (95:5, v/v), an injection temperature of 160°, a detector temperature of 275°, a pulse interval of 150 μsec, attenuation of 64, and a chart speed of 6.35 mm (0.25 in.)/min. The results of the analysis (see Table II) indicate 17.4×10^{-6} g of TNB present in the TLC extract as compared to 17.7×10^{-6} g actually spotted on the plate.

The results of analyses of several other nitro compounds after development on TLC plates may be found in Table III. The accuracy of the method is of the order of 2–3% under optimum conditions. Since ratios of the chromatographic peak heights are used to analyze the nitro compounds, serious errors may be introduced when the peak heights are small, or when the peak heights of internal standard, sample and standard are widely different. The maximum reading with the recorder chart paper used (precision chart paper, Hewlett-Packard No. 927–1010) is 10 in. or 254 mm. Since drift and noise from the combined sources of column bleed, detector, electrometer, and recorder introduce an uncertainty of about 0.1 in. or 2.5 mm for a particular reading, best analytical results will be obtained when

$$h_{IS} \cong h_{std} \cong h_{sample} \cong 127 \text{ to } 254 \text{ mm}.$$

Concentrations of standard and internal standard can be adjusted readily from standard concentrated solutions (10^{-5} to 10^{-6} g/μl) by diluting with 10 or 50 μl Hamil-

TABLE III

ANALYSES OF VARIOUS NITRO COMPOUNDS BY A COMBINATION OF THIN-LAYER AND VAPOR PHASE CHROMATOGRAPHY

Compounds	*μg applied to TLC plate*	*μg found*[a]
2,4,6-Trinitro-1-chlorobenzene	66.9; 66.9	67.4; 65.6
2,4,6-Trinitrotoluene	6.84; 6.84	6.62; 6.82
1,3,5-Trinitrobenzene	17.7	17.4
1,3-Dimethyl-2,4,6-trinitrobenzene	17.5	17.3
1,3,5,-Trimethyl-2,4,6-trinitrobenzene	13.3	13.0
2,4,6-Trinitrotoluene[b]	10.6	10.8
Cyclotrimethylenetrinitramine (RDX)[b]	49.7; 66.2	46.8; 66.6[c]

[a] By VPC analysis after development of TLC plate and extraction into 10 ml of solvent. Benzene was used as the extraction solvent in all cases except for RDX where benzene–acetone (90: 10) was used.

[b] A mixture of RDX and 2,4,6-trinitrotoluene in acetone solutions with concentrations of 3.31 and 2.65 × 10^{-6} g/μl, respectively.

[c] RDX and 2,4,6-trinitrotoluene were analyzed on separate TLC plates since the height response (gram basis) was only about 1/4 that of 2,4,6-trinitrotoluene (see Table VIII).

ton syringes into 10 ml volumetric flasks to make standard VPC solutions (10^{-8} to 10^{-10} g/μl) to meet the above criteria. Concentrations of sample in the TLC extract can be controlled by (a) size of the aliquot spotted on the TLC plate, and (b) the volume of the TLC extraction solvent.

The solvents of choice for TLC zone extraction are (a) benzene, or (b) benzene–acetone (90:10). Methanol and ethanol were found to be unsatisfactory since reaction between solvent and nitro compound was detected in some instances. For example, no peak for RDX was observed at all when a solution of RDX in methanol was injected at 180°. *n*-Hexane is a solvent ideally suited for work with the nickel-63 detector. However, it has the disadvantage of not being polar enough to extract quantitatively the relatively polar nitro compounds from silica gel layers.

TABLE IV

NICKEL-63 DETECTOR RESPONSE AND LINEAR RANGE FOR 1,3,5-TRINITROBENZENE

ng on column[a]	*Detector response (mm/ng)*[b]	
3.18	17.2	
6.36	17.6	
12.7	17.1	
15.9	17.5	
18.9	15.1	detector saturation
22.3	13.1	detector saturation
25.5	11.7	detector saturation

[a] 4 ft. × 1/4 in. glass; 2.53% Apiezon M on 60/80 mesh Diatoport-S. Conditions in order: flow rate, ml/min, Ar–CH_4 (95 : 5, v/v); column temp.; injection temp.; detector temp.; pulse interval; injection solvent: 250 ml/min; 150°; 155°; 275°; 150 μsec; benzene, respectively.

[b] Calculated for attenuation 64; retention time, 4.0 min.

TABLE V

VARIATION OF NICKEL-63 DETECTOR RESPONSE TO 1,3,5-TRINITROBENZENE WITH GAS FLOW THROUGH COLUMN

Flow rate (ml/min)[a]	*Retention time (min)*[b]	*Dectector response (mm/ng)*[c]
67	10.2	25.9
113	6.7	26.7
143	5.6	26.6
167	4.8	22.5
200	4.2	20.2
250	4.0	17.9

[a] 4 ft. × 1/4 in. glass column; 2.53% Apiezon M on 60/80 mesh Diatoport-S. Conditions in order: column temp.; injection temp.; detector temp.; pulse interval; injection solvent: 150°; 160°; 275°; 150 μsec; benzene, respectively.
[b] Measured from solvent-pressure peak.
[c] Attenuation 64.

Nickel-63 detector response

The upper limit for linear detector response was found to be about 1.6×10^{-8} g of TNB for injected samples operating with a pulse interval of 150 μsec; flow/purge rate of 250 ml/min with Ar–CH_4 (95:5, v/v); detector temperature of 275°; and column temperature of 150° (see Table IV). This upper limit or saturation point will vary somewhat depending on the particular operating conditions employed. For example, this limit would be expected to decrease on raising the column temperature to 160° since a greater concentration of the TNB would be in the detector for a shorter period than would be true with a column temperature of 150° (see Tables VI and VII). On the other hand, within certain limits, the detector response appears to be independent of carrier gas flow (see Table V). However, it is always best to check detector response for linearity by making several injections of a particular nitro compound at different concentrations containing identical internal standard concentrations and normalizing sample peak heights. A constant value of height response in mm/ng within ± 3% indicates linearity. A lower value for the height response in going to a more concen-

TABLE VI

VARIATION OF NICKEL-63 DETECTOR RESPONSE TO 1,3,5-TRINITROBENZENE WITH COLUMN TEMPERATURE

Column temperature (°C)[a]	*Retention time (min)*[b]	*Detector response (mm/ng)*[c]
150	9.7	12.5
160	4.4	18.2

[a] 4 ft. × 1/4 in. glass; 3.51% Apiezon M on 60/80 mesh Diatoport-S. Conditions in order: flow rate, ml/min; injection temp.; detector temp.; pulse interval; injection solvent: 168 ml/min; 160°; 275°; 150 μsec; benzene, respectively.
[b] Measured from solvent-pressure peak.
[c] Attenuation 64.

TABLE VII

VARIATION OF RETENTION TIME AND NICKEL-63 DETECTOR RESPONSE TO 1,3,5-TRINITROBENZENE WITH DIFFERENT COLUMNS

Percent liquid phase on column[a]	*Column temp. (°C)*	*Flow rate (ml/min)*	*Retention time (min)*[b]	*Detector response (mm/ng)*[c]
1.18% Apiezon M	170	200	1.3	67.8
3.75% Silicone Grease, DC-11	160	190	1.8	26.2
2.92% Apiezon M	160	250	3.7	27.6
2.53% Apiezon M	150	250	4.0	17.3
Mixture (3.75% Silicone Grease, DC-11 and 3.51% Apiezon M, 50: 50, w/w)	150	184	4.7	16.2
2.04% Apiezon M	150	168	5.8	17.9
5.33% Apiezon M	160	182	9.3	14.4
3.51% Apiezon M	150	168	9.7	12.5

[a] 4 ft. × 1/4 in. glass; solid support, 60/80 mesh Diatoport-S. Conditions in order: injection temp.; detector temp.; pulse interval; injection solvent: 165°; 275°; 150 μsec; benzene, respectively.

[b] Measured from solvent-pressure peak.

[c] Attenuation 64.

trated solution of the nitro compound indicates saturation of the detector (see Table IV). Erratic detector responses may indicate decomposition of the nitro compound either in the injection port or on the column or may indicate a defective septum.

Tables IV through VIII are useful for predicting detector height responses for

TABLE VIII

RELATIVE ELECTRON ABSORPTIVITY RESPONSES OF VARIOUS NITRO COMPOUNDS

Compound	*Retention time (min)*[a]	*Relative electron absorptivity*[b,d]	
		Gram basis	*Mole basis*
Cyclotrimethylenetrinitramine (RDX)	6.3	0.70	0.73
2,4,6-Trinitro-1-chlorobenzene	4.0	0.67	0.78
1,3,5-Trinitrobenzene	3.6	1.0	1.0
1,3-Dichloro-2,4,6-trinitrobenzene	4.4	0.95	1.3
2,4,6-Trinitroanisole	3.2	1.4	1.6
2,4,6-Trinitrotoluene	2.9	2.9	3.1
2,3,4-Trinitrotoluene	3.6	3.2	3.4
1,3,5-Trichloro-2,4,6-trinitrobenzene	3.1	3.2	4.8
1,3-Dimethyl-2,4,6-trinitrobenzene	6.4[c]	4.9	5.5
1,2-Dinitrobenzene	0.64	10	8.2
1,3,5-Trimethyl-2,4,6-trinitrobenzene	4.2	8.2	9.8

[a] Measured from solvent-pressure peak, 4 ft. × 1/4 in. glass column, packed with 2.53% Apiezon M on 60/80 mesh Diatoport-S. Conditions in order: flow rate, ml/min, Ar–CH_4 (95: 5, v/v); column temp.; injection temp.; detector temp.; pulse interval; injection solvent: 250 ml/min; 155°; 155°; 275°; 150 μsec; benzene, respectively.

[b] Relative height response: 1.0 = 21.2 mm/ng for attenuation 64.

[c] Same conditions as in (a), except: 4 ft. × 1/4 in. glass column packed with 2.92% Apiezon M on 60/80 mesh Diatoport-S with a flow rate of 130 ml/min.

[d] For conditions as in (c), 1.0 = 30.9 mm/ng for attenuation 64.

a number of nitro compounds relative to 1,3,5-trinitrobenzene under a variety of conditions.

Injection temperatures up to 200° were found to be acceptable for the quantitative analysis of nitro compounds; however, column temperatures greater than 170° should be avoided. Presumably interaction between the nitro compound and the liquid phase occurs readily at column temperatures near 180°. There is also the further possibility that decomposition of the nitro compound itself takes place at these elevated temperatures especially for those compounds with long retention times.

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation for several helpful discussions with D. J. GLOVER, C. W. DICKINSON, and C. BOYARS of this laboratory. The encouragement and interest of J. M. ROSEN was especially helpful throughout all phases of the work. Thanks are also due to B. O. WILKERSON, who carefully prepared all the thin-layer plates used in this study.

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