Journal of Chromatography, 216 (1981) 239–249
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,129

SEPARATION, IDENTIFICATION AND QUANTITATION OF NITROGLYCERIN AND ITS METABOLIC OR HYDROLYSIS PRODUCTS

CHIEN CHIN WU, THEODORE D. SOKOLOSKI*, ALLAN M. BURKMAN and LEI SHU WU
College of Pharmacy, Ohio State University, Columbus, OH 43210 (U.S.A.)
(First received May 12th, 1981; revised manuscript received June 16th, 1981)

SUMMARY

The characteristics of high-performance liquid chromatographic and gas chromatographic–electron capture assay systems are described for the separation of nitroglycerin from its metabolic and hydrolysis products: 1,2-dinitroglycerin, 1,3-dinitroglycerin, 1-mononitroglycerin and 2-mononitroglycerin. The methods quantitate the amount of parent compound and its major metabolites and were used to measure the rate of acid hydrolysis of nitroglycerin and its dinitro compounds. Assignment of the several peaks in the chromatograms is unequivocal as shown through the use of synthesized metabolites and hydrolysis studies.

INTRODUCTION

Nitroglycerin is one of the oldest drugs in use and still the drug of choice in the treatment of angina pectoris[1]. It is also the major component in the manufacture of dynamite. Its analysis and that of its denitration products is therefore of current interest. *In vivo*, nitroglycerin is metabolized primarily in the liver by a partial denitration process catalyzed by glutathione-S-transferase to form 1,3-dinitroglycerin and 1,2-dinitroglycerin[2]. Denitration of dinitroglycerin proceeds at only 2–5% of the rate found for nitroglycerin and mononitroglycerin is practically uneffected by liver enzyme[3]. *In vitro*, breakdown of nitroglycerin occurs by a stepwise loss of nitroxyl groups upon acid[4–7] or alkaline[4,5,8] hydrolysis. Both *in vitro* and *in vivo* degradation thus follow the same scheme of successive removal of nitroxyl groups.

Separation, identification, and convenient quantitation methods for mixtures of nitroglycerin and its denitration products have not been optimized. Identification of nitroglycerin and its principle metabolites by extraction, derivitization, infra red (IR) and mass spectral analysis has been problematical[4,6,8–11].

A combination of radiochemical and thin-layer chromatographic (TLC) techniques was widely used in the identification and quantitation of nitroglycerin and its metabolites[3,5,7]. The techniques required tedius radioisotope manipulation and only partial resolution was obtained. Several high-performance liquid chromatographic (HPLC) methods have been reported for the analysis of nitroglycerin and its decomposition products[12–16]. In the use of reversed-phase columns, either no attempt was

0021-9673/81/0000–0000/$02.50

made[14,15] or insufficient information was made available regarding the resolution of isomeric dinitroglycerins and mononitroglycerins. Spanggord and Keck[16] reported a method capable of resolving nitroglycerin and its four degradation products on one chromatogram with a normal-phase column and a gradient system. The main disadvantages in this method are the cost of the thermal energy analyzer detector used and its inability to meet the sensitivity needed for biological systems (less than 2 ng/ml; ref. 17).

Gas chromatography (GC) has received the greatest attention in the analysis of organic nitrates because of its assay speed and sensitivity. Although nitroglycerin is thermally unstable, quantitative data are attainable with careful experimental design and attention. The first complete GC resolution of nitroglycerin and its two isomeric dinitroglycerins was reported by Trowell[18]. The method required derivatization of dinitroglycerin[5] and was not very sensitive in its detection of nitroglycerin. A simultaneous two-column analysis for nitroglycerin and the two dinitroglycerins was proposed by Rosseel and Bogaert[19]. With a 3.5% QF-1 column nitroglycerin was separated but 1,2- and 1,3-dinitroglycerin remained unresolved and with a 3% XE-60 column nitroglycerin and 1,3-dinitroglycerin had the same retention time. In their pharmacological studies, Armstrong *et al.*[20] reported that nitroglycerin and a mixture of dinitroglycerins could be quantitated by separating these compounds using differential solvent extractions and analyzing the extracts using an OV-101 column. The method suffered from multiple solvent extractions needed and the non-selective column used.

In this study, the characteristics of HPLC and gas–liquid chromatographic separation systems capable of resolving nitroglycerin and its individual isomeric degradation products are described. These chromatographic methods were applied to a study of the kinetics of degradation of nitroglycerin and 1,2-dinitroglycerin and 1,3-dinitroglycerin under acid conditions. The results obtained show that the techniques developed provide a convenient and reliable method to separate, quantitate and identify nitroglycerin, 1,2- and 1,3-dinitroglycerin and 1- and 2-mononitroglycerin. Another paper considers optimization of the gas chromatographic method for quantitation of nitroglycerin and its metabolites in plasma[21].

EXPERIMENTAL

All reagents used were analytical grade unless otherwise indicated. Water was HPLC grade from Mallinckrodt (Paris, KY, U.S.A.). Methanol from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.) was distilled from glass. Pentane and ethyl acetate (for pesticide residue analysis) were purchased from J. T. Baker (Phillipsburg, NY, U.S.A.). *o*-Iodobenzyl alcohol was obtained from Aldrich (Milwaukee, WI, U.S.A.) and 1,2-dibromopropane-3-ol, 1,3-dibromopropane-2-ol and 1-chloropropane-2,3-diol were obtained from Eastman Kodak (Rochester, NY, U.S.A.). These compounds were used to synthesize 1,2-dinitroglycerin, 1,3-dinitroglycerin and 1-mononitroglycerin respectively according to the method of Dunstan *et al.*[4]. 2-Mononitroglycerin was obtained as an HPLC eluate of a nitroglycerin acid hydrolysis mixture. The synthesized organic nitrates were isolated and purified using a silica column with a mixture of ethylacetate and benzene as eluent. A 99% purity was found as determined by a TLC method described by Crew and DiCarlo[5] and by an

HPLC system developed in our laboratories that is described below. Identity was determined by nitrate[22] and IR analysis[9] or by comparing R_F values[5] and GC retention times[19] with literature values. Nitroglycerin as a 10% adsorbate on lactose was obtained from ICI America (Wilmington, DE, U.S.A.). Very pure nitroglycerin was prepared by extracting an aqueous solution of the drug with pentane, separating the supernatant and removing the pentane using nitrogen, redissolving the nitroglycerin in water and then repeating the pentane extraction procedure two more times. The purity of the nitroglycerin thus obtained in aqueous solution (99.9%) was determined by HPLC and TLC. Nitroglycerin content was standardized using a colorimetric assay previously reported[22].

The liquid chromatography system consisted of a Beckman Model 110 A solvent metering pump (Beckman Instruments, Fullerton, CA, U.S.A.), a Rheodyne Model 7125 septumless syringe loaded loop injector with a 20-μl loop (Rheodyne, Berkeley, CA, U.S.A.), an MPLC RP-18 guard column (Brownlee Labs., Santa Clara, CA, U.S.A.), a 250 × 4.6 mm LiChrosorb RP-18 10 μm particle column (Unimetrics, Anaheim, CA, U.S.A.), a Perkin-Elmer LC-75 spectrophotometric detector (at 205 nm) (Perkin-Elmer, Norwalk, CT, U.S.A.) and a Shimadzu Chromatopac C-RIA recording data processor (Shimadzu Scientific Instruments, Columbia, MD, U.S.A.). Mixtures of methanol and water were used as mobile phases. Capacity factors (k'), resolution factors (R_s), and selectivity (α) were calculated using standard formulae[23]. The variation in the retention times was ± 1% when the studies are run on the same day and about ± 4% in the day to day variation and hence negligible.

A Varian 3700 series dual column gas chromatograph (Varian, Palo Alto, CA, U.S.A.) equipped with a 8 mCi ^{63}Ni electron-capture detector coupled with a Shimadzu Chromatopac C-RIA recording data processor was used in this study. Carbowax 20M-TPA (3%) (Applied Science Labs, State College, PA, U.S.A.) was used as a stationery phase on 60–80 mesh Supelcoport (Supelco, Beffefonte, PA, U.S.A.). The coated material was packed into a 42 cm × 2 mm silanized glass column. The temperatures of the injection port, column and detector were 150, 137 and 200°C, respectively. The carrier gas was 5% methane in argon used at a flow-rate of 20 ml/min. The parameters used to evaluate the GC column were calculated using the same factors (k', R_s, α) described for HPLC. The variation in GC retention times was ±1.5% when studies were run on the same day and about a 5% variation was seen from day to day.

In studies of the acid hydrolysis of nitroglycerin and 1,2- and 1,3-dinitroglycerin the following procedures were used. Nitroglycerin solutions (two at each concentration) were prepared at 0.8, 0.4 and 0.2 mg/ml by dissolving pure material in 4 *N* HCl and then stored at 37°C in a water bath. At various periods of time, 0.5 ml of the solution was removed, cooled to 0°C and neutralized with 4 *N* NH_4OH. A 20-μl volume of the neutralized solution was directly injected onto the HPLC system. Another 0.5 ml of neutralized solution was extracted with ethyl acetate and diluted as needed for chromatographic analysis. A 5-μl volume of this diluted solution was injected onto the gas chromatograph for the analysis of nitroglycerin and its degradation products. The chromatograms thus obtained were compared with those obtained for a known mixture of purified nitroglycerin and synthetic dinitroglycerins and mononitroglycerins. The extent of degradation was calculated from the chromatograms using an external standard method in HPLC and an internal standard meth-

od in GC where peak areas or ratios were compared against the cabibration curve. A blank (without drug) and a control (without acid) were carried out using the same procedure. No interfering peaks appeared in either the HPLC or GC chromatograms when analyzing the blank samples and no detectable decomposition occurred in the control samples. The same procedure was repeated in a kinetic analysis of the acid degradation of 1,2-dinitroglycerin and 1,3-dinitroglycerin. The only difference was in the starting materials.

In addition to functional group determination by chemical and IR analysis, the criteria for identification used in this study for the organic nitrates was essentially a comparison of chromatographic retention behavior of synthetic compounds with that of degradation products obtained from acid hydrolysis of pure materials.

RESULTS AND DISCUSSION

High-performance liquid chromatography

The dependence of the chromatographic resolution of a mixture of the organic nitrates with methanol concentration is given in Fig. 1. Using 60% methanol in water (Fig. 1A), the retention times for nitroglycerin, a mixture of dinitroglycerins and a mixture of mononitroglycerins are 8.9, 5.0 and 3.5 min, respectively. This concentration of methanol thus would be useful for in-process nitroglycerin assay in the pharmaceutical industry. When 40% methanol in water was used (Fig. 1B), nitroglycerin, and 1,2- and 1,3-dinitroglycerin were separated with retention times of 26.5, 8.4 and 7.1 min, respectively, but the mononitroglycerins were not separated (retention time 3.8 min). Methanol (40%) would be most convenient to follow the formation of the dinitro compounds. With pure water as a mobile phase (Fig. 1D) a complete separa-

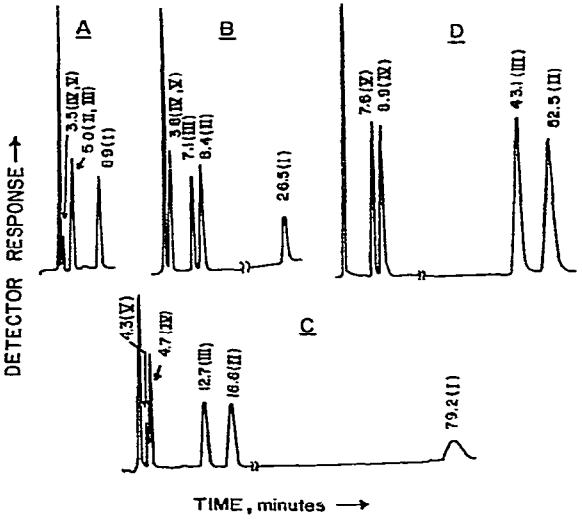

Fig. 1. The effect of methanol–water mixtures on the HPLC resolution of a mixture of nitroglycerin (I), 1,2-dinitroglycerin (II), 1,3-dinitroglycerin (III), 1-mononitroglycerin (IV) and 2-mononitroglycerin (V). Numbers above the peaks are retention times in minutes. The various percentages of methanol used were: A, 60%; B, 40%; C, 20%; and D, 0%.

TABLE I

RESOLUTION AND SELECTIVITY FACTORS FOR SEVERAL ORGANIC NITRATES IN A SERIES OF WATER–METHANOL MIXTURES IN THE HPLC SYSTEM

*Factor**	*Methanol (%)*			
	60	*40*	*20*	*0*
R_{s1}	0	0	0.7	1.41
α_1	1	1	1.33	1.31
R_{s2}	0	1.39	2.28	2.32
α_2	1	1.32	1.41	1.23

* R_{s1} and R_{s2} are the resolution factors for the two mononitroglycerins and the two dinitroglycerins respectively and α_1 and α_2 are their corresponding selectivity factors.

tion of the two dinitroglycerins and two mononitroglycerins was achieved with retention times of 52.5 and 43.1 min for 1,2- and 1,3-dinitroglycerin and 8.9 and 7.6 min for 1- and 2-mononitroglycerin. When capacity factors (k') were plotted as a function of methanol concentration, the normal linear relation between log k' and the methanol concentration is found for nitroglycerin and each of the four metabolites. Resolution and selectivity factors are given in Table I. Only the values for 1,2- and 1,3-dinitroglycerin and for 1- and 2-mononitroglycerin are listed because these values are probably of greater use to others in selecting a desired separation system. Gradient elution, unavailable to us in this study, would undoubtably separate all organic nitrates in a mixture.

The response of the UV detector at 205 nm to all organic nitrates was linear from 5 to 300 μg/ml. With 40% methanol, nitrophenol can be used as internal standard, retention time 10.7 min. Because of the consistency of detector response and the simplicity of sample preparation, accurate quantitation can be obtained without the use of an internal standard. An external standard method was adopted in the hydrolysis studies that are described below. The assay precision for nitroglycerin, 1,2-dinitroglycerin and 1,3-dinitroglycerin was within $\pm$ 5% when five samples of each of the organic nitrates were assayed at concentrations of 50, 100 and 200 μg/ml.

Gas chromatography

The gas chromatogram given in Fig. 2A shows the separation of a mixture of nitroglycerin, 1,2-dinitroglycerin, 1,3-dinitroglycerin and *o*-iodobenzyl alcohol (internal standard). With the 3% Carbowax 20M-TPA glass column, good resolution was obtained with retention times 3.2, 4.1, 7.7 and 9.6 min for nitroglycerin, internal standard, 1,2-dinitroglycerin and 1,3-dinitroglycerin, respectively. Dinitroglycerins were studied since they are the primary metabolites of nitroglycerin. Resolution (R_s) and selectivity (α) factors are given in Table II.

The ratio of peak areas of nitroglycerin to dinitroglycerin at the same concentration was found to be 2:1. Detector response to organic nitrates was linear from 7.5 to 500 ng/ml. Using an internal standard (*o*-iodobenzyl alcohol) method, the assay precision was $\pm$ 6% at 50, 100 and 200 ng/ml. This system provides a separation of nitroglycerin and underivatized 1,2- and 1,3-dinitroglycerin which has not previously been reported in a single chromatogram. Electron-capture detection gives enough

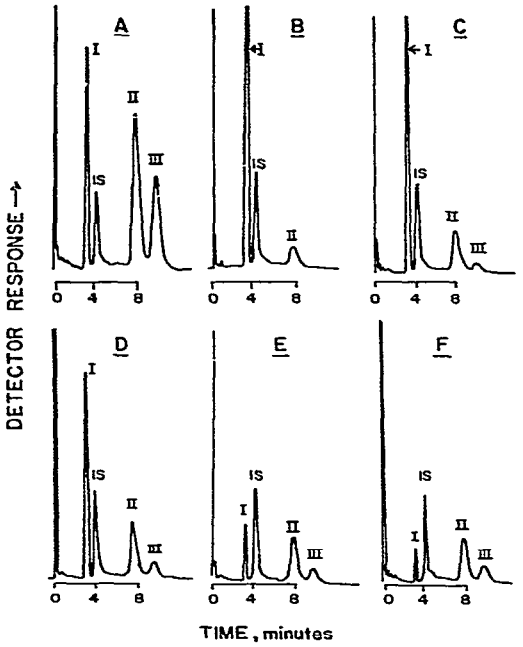

Fig. 2. GC separations of nitroglycerin (I), 1,2-dinitroglycerin (II), 1,3-dinitroglycerin (III) and *o*-iodobenzyl alcohol (internal standard, IS). Their retention times (min) were: I, 3.2; IS, 4.1; II, 7.7; III, 9.6. A is a known mixture of the four components. B through F are chromatographed samples obtained from the hydrolysis of nitroglycerin (initially at 400 μg/ml) in 4 *N* HCl at 37°C at various times in hours: B, 0; C, 10; D, 24; E, 48; and F, 60.

sensitivity to meet the picogram detection requirement of biological studies. Such an application is presented in another paper[21].

Thermal decomposition on a GC column is a recognized problem in the analysis of organic nitrates[24,25]. Some decomposition (≈ 10%) occurred on almost all columns tested in our investigations, but thermal decomposition does not change the results obtained in the analysis of degradation products in this study. Furthermore, the short columns adopted minimize this effect.

TABLE II

RESOLUTION (R_s) AND SELECTIVITY FACTORS (α) FOR SEVERAL ORGANIC NITRATES IN THE GC SYSTEM

NG = nitroglycerin; IS = internal standard; 1,2-DNG = 1,2-dinitroglycerin; 1,3-DNG = 1,3-dinitroglycerin.

Factor	*Nitrate system*			
	NG/IS	*NG/1,2-DNG*	*NG/1,3-DNG*	*1,2-DNG/1,3-DNG*
R_s	1.41	5.07	7.5	1.25
α	1.30	2.95	3.05	1.25

Acid hydrolysis

The study of nitroglycerin and dinitroglycerin hydrolysis served several purposes: it provided an application and a test of the HPLC and GC systems; it provided information to substantiate the identity of hydrolysis products; and, it gave information with regard to the relative stability of organic nitrates to acid attack. In order to get accurate retention times for comparison, isocratic HPLC systems were used.

Fig. 3A for nitroglycerin at 400 μg/ml at time zero shows that the nitroglycerin used was pure. Fig. 3B–F shows the disappearance of nitroglycerin and appearance of dinitroglycerins and mononitroglycerins at different reaction times. Rapid disappearance of nitroglycerin (peak at 8.9 min) was accompanied by rapid appearance of dinitroglycerins (peak at 5.0 min). The latter reached a maximum at 24 h and then declined at a slower rate. Mononitroglycerins (peak at 3.5 min) continuously increased at a slow rate over the 60-h study. Methanol (60 %) in water was used in this study. The degradation products were further analyzed with 40 % methanol or pure water as the mobile phase which resolves the dinitroglycerins and the mononitroglycerins. The chromatogram in Fig. 4A illustrates the resolution of dinitroglycerins in the 24-h sample (corresponds to the sample of Fig. 3C). The previous unresolved dinitroglycerins were clearly separated with 1,2-dinitroglycerin and 1,3-dinitroglycerin appearing at a ratio of 2:1. Similarly, the chromatogram of a 48-h sample shown in Fig. 4B

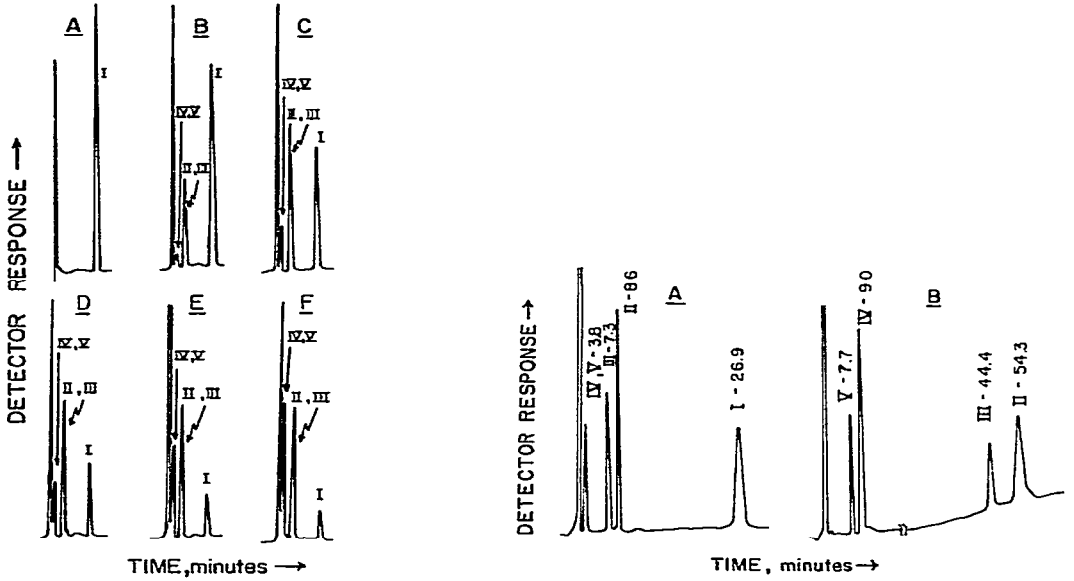

Fig. 3. HPLC chromatograms for samples of nitroglycerin (400 μg/ml initially) in 4 *N* HCl at 37°C at different reaction times. The mobile phase was 60 % methanol in water. In the chromatograms: I = nitroglycerin; II = 1,2-dinitroglycerin, III = 1,3-dinitroglycerin; IV = 1-mononitroglycerin; and V = 2-mononitroglycerin. The several samples were run after a specified length of time in hours: A = 0; B = 10; C = 24; D = 33.5; E = 48; and F = 60. The retention time for nitroglycerin (I) is about 8.9 min, for the dinitro mixture 5.0 min and for the mononitro mixture 3.5 min.

Fig. 4. HPLC chromatograms for samples of nitroglycerin (400 μg/ml initially) in 4 *N* HCl at 37°C at reaction times of 24 h (A) and 48 h (B). The mobile phase was 40 % methanol (A) or pure water (B). In the chromatograms: I = nitroglycerin; II = 1,2-dinitroglycerin; III = 1,3-dinitroglycerin; IV = 1-mononitroglycerin; V = 2-mononitroglycerin. The numbers adjacent to the peaks are retention times.

illustrates the resolution of the two isomeric dinitroglycerins and the two isomeric mononitroglycerins using pure water as the mobile phase. 1-Mononitroglycerin and 2-mononitroglycerin were found at a ratio of about 2:1.

The acid degradation of nitroglycerin was further studied using the GC system. GC chromatograms shown in Fig. 2B–F correspond to the HPLC chromatograms shown in Fig. 3A–C and E–F. Fig. 2B indicates that some thermal decomposition occurred when pure nitroglycerin was injected into the gas chromatograph. The only thermal decomposition product observed had a retention time similar to that of 1,2-dinitroglycerin. Nitroglycerin (peak at 3.2 min) disappeared rapidly with the same rate constant as that observed in the HPLC analysis. The ratio of 1,2-dinitroglycerin to 1,3-dinitroglycerin (Fig. 2C–E) is slightly higher than that observed in the HPLC analysis. A possible explanation for this difference may be due to thermal decomposition of nitroglycerin where the degradation product has a retention time equal to that of 1,2-dinitroglycerin.

The linear relationship found between the logarithm of nitroglycerin remaining and time at each of the three initial concentrations used (Fig. 5) shows that the reaction of nitroglycerin in 4 *N* HCl and 37°C is pseudo-first order. The average rate constant obtained from the slopes was $4.27 \cdot 10^{-2}$ h^{-1} (half life 16.1 h). This result is very close to that obtained by other workers[7] using a TLC method, half-life 16.33 h at an initial nitroglycerin concentration of 1 mg/ml.

The semilogarthmic plot for the degradation of 1,2-dinitroglycerin and 1,3-dinitroglycerin in 4 *N* HCl at 37°C is shown in Fig. 6. As in the hydrolysis of nitroglycerin, the degradation of the dinitro compounds is pseudo-first order but with a slower rate constant. The average rate constants obtained from the slopes of the lines in Fig. 6 were $2.15 \cdot 10^{-2}$ h^{-1} for 1,2-dinitroglycerin and $1.91 \cdot 10^{-2}$ h^{-1} for 1,3-dinitroglycerin (half-lives 32.1 and 36.2 h, respectively).

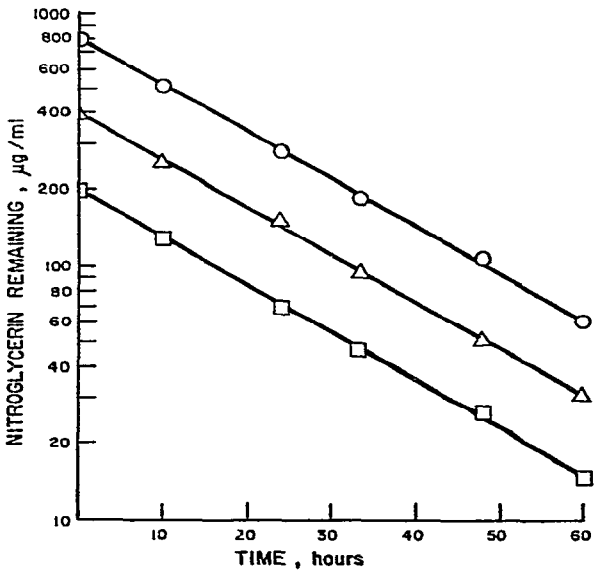

Fig. 5. Relationship between the logarithm of nitroglycerin remaining (µg/ml) and time for the hydrolysis of the drug in 4 *N* HCl at 37°C at different initial concentrations: ○, 800 µg/ml; △, 400 µg/ml; □, 200 µg/ml.

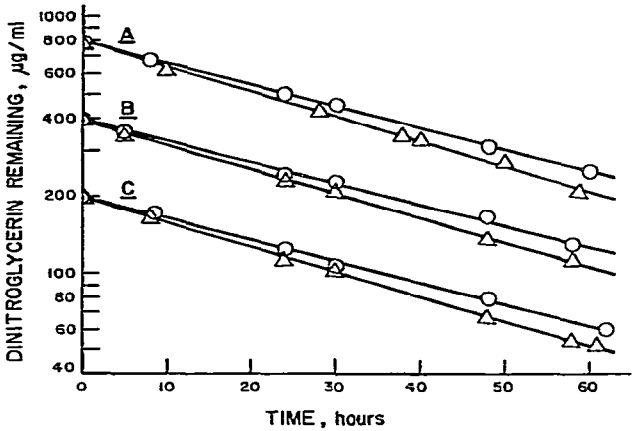

Fig. 6. Relationship between the logarithm of 1,2-dinitroglycerin (△) and 1,3-dinitroglycerin (○) remaining (μg/ml) and time for their hydrolysis in 4 *N* HCl 37°C. Three initial concentrations were used: A, 800 μg/ml; B, 400 μg/ml; C, 200 μg/ml.

It has been reported that organic nitrate hydrolysis occurs more readily in alkaline than acid solution and only very slowly in a neutral medium[4,5,26,27]. Despite extensive study, the reaction mechanism remains unclear. Organic nitrates can undergo hydrolysis by several potential pathways[28,29] (depending on the acids used) to yield the expected alcohol as well as other by-products. In previous TLC studies[5,7], hydrochloric acid proved to be superior to other acids in the avoidance of side reactions such as oxidization or esterification. During acid hydrolysis, the formation of the corresponding alcohol from an organic nitrate may start with the formation of an oxonium ion on the alkoxyl atom[30]. Then a reaction between the oxonium ion and water leads to a complex which decomposes to yield a nitric acid molecule and a new oxonium ion. Further deprotonation gives the final alcohol[7]. The absence of interfering peaks in the analysis of degradation products of organic nitrates in either HPLC or GC chromatograms in this study suggests that organic nitrates are hy-

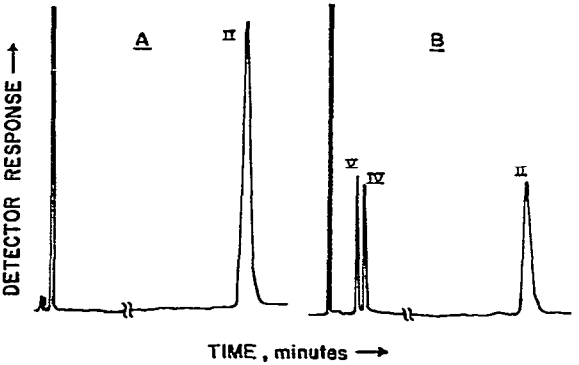

Fig. 7. HPLC chromatograms for samples of 1,2-dinitroglycerin hydrolyzed in 4 *N* HCl at 37°C (initial concentration 400 μg/ml) at time zero (A) and 36 h (B). The peaks are: II, 1,2-dinitroglycerin; IV, 1-mononitroglycerin; V, 2-mononitroglycerin. The chromatograms were developed using pure water as the mobile phase with retention times (min) of 52.5 (II), 8.9 (IV) and 7.6 (V).

drolyzed in HCl by a stepwise loss of nitroxyl groups to form the corresponding alcohols.

Results typified by those in Fig. 7 show that hydrolysis of 1,2-dinitroglycerin yields equal amounts of the two isomeric mononitroglycerins. This suggests that there is no difference in the rate of attack on a primary or secondary nitroxyl group. If the rate of loss of a nitroxyl group is independent of its position (primary or secondary), the ratio of degradation products will depend on the ratio of the available number of primary and secondary nitroxyl groups on the substrate. This could explain why acid hydrolysis of nitroglycerin (Fig. 4A) yields 1,2- and 1,3-dinitroglycerin at a ratio of 2:1 (two primary and one secondary nitroxyl groups on nitroglycerin). Since the rate constants of 1,2- and 1,3-dinitroglycerin were similar (Fig. 6), degradation products of nitroglycerin should yield 1-mononitroglycerin and 2-mononitroglycerin at a ratio of about 2:1. This observation is demonstrated in Fig. 4B. The ratio of the rate constant of nitroglycerin to that of the dinitroglycerins (about 2:1) in acid hydrolysis can not be explained solely on probability difference (3:2). The rate constant differences may be explained by strain differences due to nitroxyl interactions. There should be a greater strain in the trinitro compound leading to a greater reactivity. This might also explain why 1,2-dinitroglycerin has a slightly higher degradation rate than 1,3-dinitroglycerin.

An analysis of the dinitroglycerin acid degradation products offers information concerning identity of the dinitroglycerins and the mononitroglycerins as well. The chromatograms of Fig. 7A and 8A show that the initial dinitroglycerin samples are quite pure. The hydrolysis of 1,2-dinitroglycerin yields equal amounts of the isomeric mononitroglycerins (Fig. 7B) and the hydrolysis of 1,3-dinitroglycerin produces only 1-mononitroglycerin (Fig. 8B). It is evident that these degradation patterns can distinguish 1,2-dinitroglycerin from 1,3-dinitroglycerin and 1-mononitroglycerin from 2-mononitroglycerin.

The retention times for these degradation products correspond to those obtained for the synthetic materials and thus support the assignment of the chromatographic peaks. Further confirmation was made by collecting the eluent under each peak of the chromatogram in the acid hydrolysis mixtures, mixing them with the corresponding synthetic compound and then co-chromatographing in HPLC or GC.

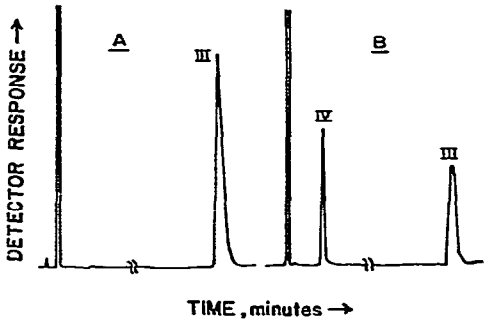

Fig. 8. HPLC chromatograms for samples of 1,3-dinitroglycerin hydrolyzed in 4 *N* HCl at 37°C (initial concentration 400 μg/ml): A, time zero; B, 36 h. The peaks with their retention times in minutes are: III, 1,3-dinitroglycerin, 43.1 and IV, 1-mononitroglycerin, 8.9. The chromatograms were developed using water as the mobile phase.

A single symmetrical peak with no shoulder was obtained in each case which provides further support for the proper assignment of peaks. In these assignments, it must be appreciated that unequivocal identification of an inknown compound by chromatographic methods alone is tenuous. In this study, high-resolution and reproducible HPLC and GC separation systems were developed and a simultaneous analysis of degradation products was made using a non-polar HPLC column with a universal detector (UV at 205 nm) and a polar GC column with a specific detector (electron capture). This combination should decrease error due to two or more components eluting under one peak or in basing the identify of an unknown compound by mistakenly comparing it with a chemically different compound having an identical retention time.

ACKNOWLEDGEMENT

This work was supported by NIH Grant No. HL-23303 of the Public Health Service, U.S.A.

REFERENCES

1 C. DeGraff, in W. Modell (Editor), *Drugs of Choice —1978–1979*. The C. V. Mosby Company, Saint Louis, MO, 1979, p. 397.
2 P. Needleman, D. J. Blehm, A. B. Harkey, E. M. Johnson, Jr. and S. Lang, *J. Pharmacol. Exp. Ther.*, 179 (1971) 347.
3 P. Needleman and F. E. Hunter, Jr., *Mol. Pharmacol.*, 1 (1965) 77.
4 J. Dunstan, J. V. Griffiths and S. A. Harvey, *J. Chem. Soc.*, (1965) 1319.
5 M. C. Farmer, *J. Chem. Soc.*, 117 (1920) 806.
7 M. T. Rosseel, M. G. Bogaert and D. De Keukeleire, *Bull. Soc. Chem. Berg.*, 83 (1974) 211.
8 W. Will, *Ber.*, 41 (1908) 1107.
9 T. Urbanski and M. Witanowski, *Trans. Faraday Soc.*, 59 (1963) 1046.
10 R. T. M. Fraser and N. C. Paul, *J. Chem. Soc. B.*, (1968) 659.
11 H. Takagi, N. Washida, H. Akimoto and M. Okunda, *Anal. Chem.*, 53 (1981) 175.
12 C. D. Chandler, G. R. Gibson and W. T. Bolleter, *J. Chromatogr.*, 100 (1974) 185.
13 W. G. Crouthamel and B. Dorsch, *J. Pharm. Sci.*, 68 (1979) 237.
14 D. M. Baaske, J. E. Carter and A. H. Amann, *J. Pharm. Sci.*, 68 (1979) 481.
15 L. Gelber, *J. Pharm. Sci.*, 69 (1980) 1084.
16 R. J. Spanggord and R. G. Keck, *J. Pharm Sci.*, 69 (1980) 444.
17 P. W. Armstrong, J. A. Armstrong and G. S. Marks, *Circulation*, 59 (1979) 585.
18 J. M. Trowell, *Anal. Chem.*, 42 (1970) 1440.
19 M.-T. Rosseel and M. G. Bogaert, *J. Chromatogr.*, 64 (1972) 364.
20 J. A. Armstrong, G. S. Marks and P. W. Armstrong, *Mol. Pharmacol.*, 18 (1980) 112.
21 C. C. Wu, T. D. Sokoloski, A. M. Burkman, M. F. Blanford and L. S. Wu, *J. Chromatogr.*, submitted for publication.
22 T. W. Dean and D. C. Baun, *Amer. J. Hosp. Pharm.*, 32 (1975) 1036.
23 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, John Wiley, New York, 1974.
24 E. Camera and D. Pravisani, *Anal. Chem.*, 36 (1964) 2108.
25 J. M. Trowell and M. C. Philpot, *Anal. Chem.*, 41 (1969) 166.
26 S. C. J. Olivier and G. Berger, *Rec. Trav. Chim. Pays-Bas*, 41 (1922) 637.
27 J. W. Baker and A. J. Neale, *J. Chem. Soc.*, (1955) 608.
28 J. W. Baker and D. M. Easty, *J. Chem. Soc.*, (1952) 1193.
29 R. Boschan, R. T. Merrow and R. W. Van Dolah, *Chem. Rev.*, 55 (1955) 485.
30 M. J. Dewar, M. Shanshal and S. D. Worley, *J. Amer. Chem. Soc.*, 91 (1969) 3590.