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Rapid and Accurate Stability-Indicating Assay for Nitroglycerin

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Abstract □ A rapid high-pressure liquid chromatographic method for determining the nitroglycerin concentration in liquid dosage forms and intravenous admixture solutions is presented. A coefficient of variation of less than 1.8% was achieved over the concentration range most commonly encountered (50–500 μg/ml). A variable wavelength detector (λ = 218 nm) and a micro-alkyl phenyl column were employed. The mobile phase was acetonitrile–tetrahydrofuran–water (26:10:64). Total analysis time was 12 min.

Keyphrases □ Nitroglycerin—assay of liquid dosage forms and intravenous solutions, stability, degradation products □ Stability—nitroglycerin in intravenous solutions □ High-pressure liquid chromatography—analysis, nitroglycerin liquid dosage forms and intravenous solutions □ Cardiac vasodilators—nitroglycerin, analysis, liquid dosage forms and intravenous solutions

Intravenous nitroglycerin (glyceryl trinitrate) is commonly used for patients with acute myocardial infarction. Several methods of preparing such solutions for human patients have been reported (1–3). Recent reports indicated that intravenous nitroglycerin solutions lose potency when prepared or stored in certain containers (4–6). This loss may be due to degradation or adsorption. Thus, a rapid and accurate assay of nitroglycerin in intravenous solutions is needed.

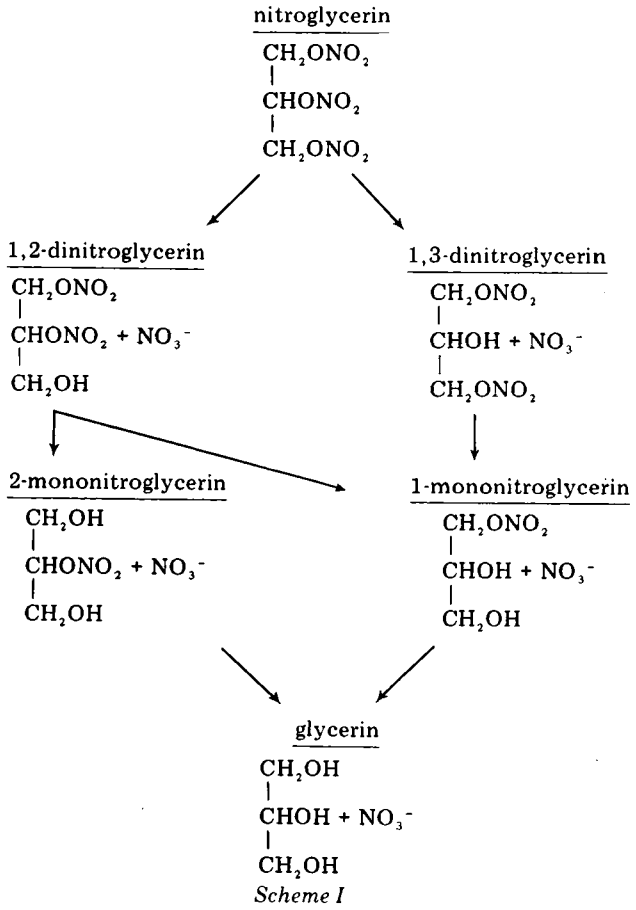
BACKGROUND

Nitroglycerin is the triester of nitric acid and glycerol. Breakdown occurs by a stepwise loss of nitrate groups (Scheme I).

Spectrophotometric methods require little instrumentation but are time consuming and complex and do not indicate stability (7–10) or differentiate nitroglycerin, mononitroglycerin, dinitroglycerin, and endogenous nitrates. TLC (11–13) is selective but not quantitative. Although polarographic methods (14, 15) offer both selectivity and sensitivity, they require sophisticated instrumentation not available in many laboratories.

A kinetic method of analysis (16) based on the transient appearance of a chromophoric intermediate during base hydrolysis offers speed and specificity but lacks sensitivity. GLC methods (17–21) give sensitivity for nitroglycerin in excess of that required. Some give the desired selectivity. However, all require a time-consuming extraction into an organic solvent.

Two normal phase high-performance liquid chromatographic methods were reported (22, 23). Both reports failed to discuss interference from excipients and breakdown products, and both methods require extraction into an organic solvent. Assay sensitivity is potentially a problem when applying these methods to intravenous nitroglycerin admixture solutions.



Scheme I

A new method is described here that allows the rapid direct measurement of nitroglycerin in intravenous admixture solutions, offers adequate sensitivity, and provides the potential for measuring breakdown products.

EXPERIMENTAL

Materials—Nitroglycerin[1] [10% in lactose or 1% (v/v) in ethanol], isosorbide dinitrate[2] (25% in lactose), and sublingual nitroglycerin tablets[3]

[1] ICI, Wilmington, Del.
[2] Napp Chemicals, Lodi, N.J.
[3] Eli Lilly and Co., Indianapolis, Ind.

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Table I—Accuracy and Precision Data

Nitroglycerin Concentration, μg/ml	Peak Height Ratios (Nitroglycerin/Isosorbide Dinitrate)				Mean	Adjusted Group Mean	Group *SD*
	Sample 1	Sample 2	Sample 3	Sample 4			
500	3.955	4.028	4.120	4.048	4.047	100.2	1.8
400	3.257	3.242	3.213	3.225	3.234	100.1	0.6
300	2.367	2.393	2.407	2.390	2.392	98.8	0.5
200	1.622	1.598	1.615	1.605	1.610	100.0	0.7
100	0.803	0.811	0.807	0.806	0.807	100.5	0.4
50	0.403	0.400	0.398	0.398	0.400	100.8	0.6
Overall	—	—	—	—	—	100.1	1.5

Table II—Admixture Measurement Data

Intravenous Solution	Theoretical Nitroglycerin Concentration, μg/ml	Measured Concentration, μg/ml				Mean, μg/ml	*SD*
		Bottle A	Bottle B	Bottle C	Bottle D		
Normal saline	50	47.5	51.3	48.5	55.5	50.6	3.4
Dextrose (5%)	50	49.5	50.0	52.0	48.4	50.0	1.5
Normal saline	100	104.1	100.8	101.8	99.3	101.5	2.0
Dextrose (5%)	100	100.5	100.7	98.7	99.4	99.8	0.9

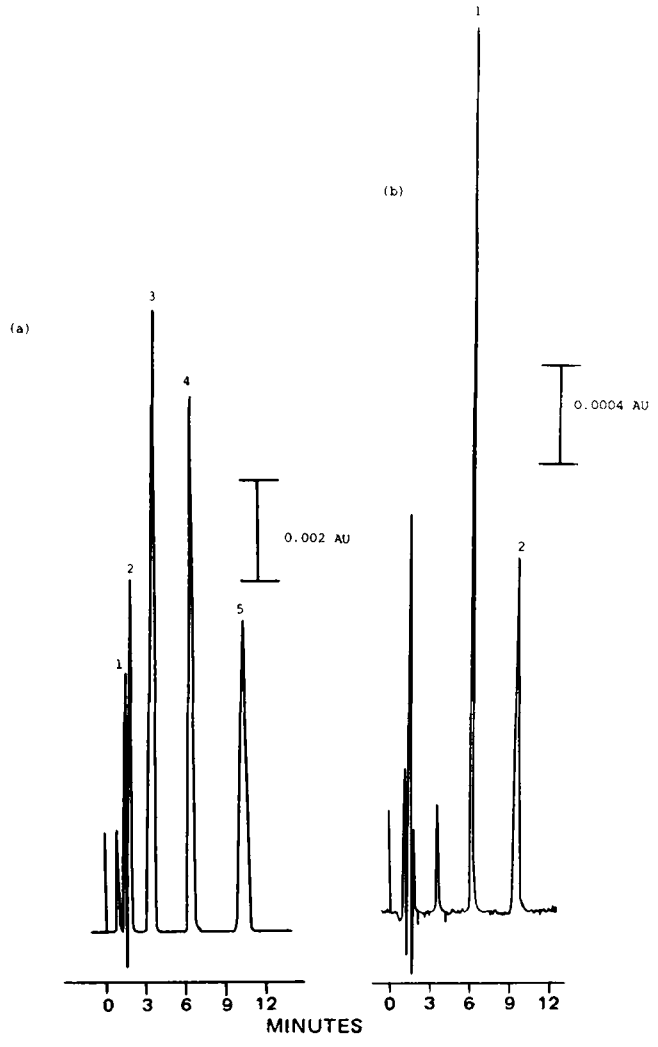

Figure 1—(a) *Chromatogram of a mixture of all the components of interest. Key: 1, glycerin; 2, mixture of the two mononitroglycerins; 3, mixture of the two dinitroglycerins; 4, isosorbide dinitrate; and 5, nitroglycerin. (b) Actual chromatogram of nitroglycerin (50 μg/ml) in 0.9% NaCl using a 40-μl injection. Key: 1, isosorbide dinitrate; and 2, nitroglycerin.*

USP were used as received. 1,2-Dinitroglycerin, 1,3-dinitroglycerin, 1-mononitroglycerin, and 2-mononitroglycerin were synthesized[4] using literature methods (24). Glass-distilled acetonitrile, methanol, and tetrahydrofuran were used for all procedures[5]. Five percent dextrose in water USP (D_5W) and sodium chloride injection USP (0.9% NaCl) in 250-ml glass bottles were used for admixture solutions[6]. Purified water USP was purified[7] further prior to use.

Instrumentation—The liquid chromatographic system consisted of a solvent pumping system[8], a septumless syringe-loading sample injector[9], a variable wavelength detector[10], and a 10-mv recorder[11]. A 30-cm × 3.9-mm column packed with alkyl phenyl bonded to silica gel[12] (10 μm), a detector wavelength of 218 nm, and a chart speed of 20 cm/hr were employed. Sample injections of 10–40 μl were used.

Mobile Phase—Approximately 1 liter of mobile phase was prepared fresh daily by thoroughly mixing 260 ml of acetonitrile, 100 ml of tetrahydrofuran, and 640 ml of water. The mobile phase was filtered through a 0.5-μm filter[13] prior to use and pumped at a constant rate of 2 ml/min, which yielded a pressure of less than 1600 psi.

Standard Curve—An accurately weighed sample of nitroglycerin (or isosorbide dinitrate), equivalent to 100 mg of active ingredient, was placed in a 100-ml volumetric flask and either dissolved in 10 ml of alcohol USP and brought to volume with water or dissolved in approximately 95 ml of water by vigorous agitation and then brought to volume with water. Lower concentrations were obtained by proper dilutions with water, 5% (v/v) ethanol, or appropriate intravenous solutions.

Standard concentrations of 500, 400, 300, 200, 100, and 50 μg/ml were prepared in quadruplicate. To 1.0 ml of each standard or sample was added 150 μl of isosorbide dinitrate (0.5 mg/ml), the internal standard; each was vortexed[14] for 5 sec and chromatographed. Peak heights were measured for nitroglycerin and isosorbide dinitrate. The peak height ratio (nitroglycerin/isosorbide dinitrate) was then plotted *versus* the nitroglycerin concentration to yield a calibration curve.

Admixtures—Admixture solutions were prepared to test the practicality of the method. Either 25 or 50 ml of intravenous solution was withdrawn from the intravenous bottle with a syringe[15], and then 25 or 50 ml of a 0.5-mg/ml nitroglycerin solution was added to the bottle, also with a syringe, to yield a theoretical concentration of 50 or 100 μg/ml. The solutions were thoroughly mixed and then sampled with a syringe.

[4] Midwest Research, Kansas City, Mo.
[5] Burdick & Jackson, Muskegon, Mich.
[6] McGaw Laboratories, Irvine, Calif.
[7] Milli-Q water purification system, Millipore Corp., Bedford, Mass.
[8] Series 2, Perkin-Elmer Corp., Norwalk, Conn.
[9] Model 7105, Rheodyne, Berkeley, Calif.
[10] LC-55A, Perkin-Elmer Corp., Norwalk, Conn.
[11] Model 023, Perkin-Elmer Corp., Norwalk, Conn.
[12] μBondapak phenyl, Waters Associates, Milford, Mass.
[13] Millipore Corp., Bedford, Mass.
[14] Votex Genie mixer, Scientific Products, McGaw Park, Ill.
[15] Becton, Dickinson and Co., Rutherford, N.J.

RESULTS AND DISCUSSION

The direct measurement of nitroglycerin in intravenous solutions in the presence of the anticipated breakdown products, glycerin, 1-mononitroglycerin, 2-mononitroglycerin, 1,2-dinitroglycerin, and 1,3-dinitroglycerin, is achieved for the first time. The potential to quantitate the anticipated breakdown products exists but has not yet been pursued, and no effort to resolve the dinitroglycerins or the mononitroglycerins was made. Other components of a potential intravenous formulation or admixture such as dextrose, sodium chloride, phosphate, and acetate ions or alcohol do not interfere with the quantitation of the nitroglycerin. A chromatogram illustrating the obtainable separations is seen in Fig. 1.

The described procedure, using 1 ml of sample or standard and 150 μl of internal standard, allows an accurate assay of nitroglycerin over the concentration range commonly encountered in nitroglycerin liquid dosage forms and intravenous admixture programs (50–500 μg/ml). Quantitation down to 5 μg/ml is possible with smaller quantities of internal standard, and the detection limit of 0.5 μg/ml is achievable with 100-μl injections. Still greater sensitivities are possible through the use of larger injection volumes or increased percentages of acetonitrile, which brings the peaks closer to the origin of the chromatogram, resulting in sharper peaks. However, this increase in sensitivity is counterbalanced by the decreased resolution of potential breakdown products.

Accuracy and Precision—Four replicate nitroglycerin samples (500, 400, 300, 200, 100, and 50 μg/ml) were chromatographed (Table I). A correlation of better than 0.999 was consistently obtained, as was a coefficient of variation of less than 1.8%.

Applicability—Hospitals presently employ three procedures to make standard nitroglycerin solutions, which are then used to prepare intravenous admixtures. Sublingual tablets are crushed and dissolved in sterile water for injection. Nitroglycerin in lactose is dissolved in a similar manner. Alcoholic nitroglycerin solutions are diluted to the required concentrations. Solutions prepared from each of these nitroglycerin materials were successfully analyzed by the described method, and no interferences were encountered in any instance.

A standard, 0.5 mg/ml, prepared from nitroglycerin in lactose was used to prepare a series of intravenous admixtures. This series was then analyzed to illustrate method practicality (Table II).

Unlike a methanol–water mobile phase, the acetonitrile–tetrahydrofuran–water mobile phase does not precipitate the lactose from the sample. Thus, the rapid pressure increases seen with methanol–water mixtures due to the clogging of column inlet filters are not encountered. The tetrahydrofuran is necessary only to resolve the glycerin and the mononitroglycerins. If this resolution is not required, the tetrahydrofuran portion of the mobile phase can be replaced with an equivalent amount of acetonitrile. The presence or absence of salts (*i.e.*, sodium nitrate and sodium phosphate, monobasic) or a variation of the mobile phase pH (pH 4–7.5) has no effect on resolution or retention times of components.

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