# Specific High-Performance Liquid Chromatographic Assay for Nitroglycerin in Dosage Forms

**WILLIAM G. CROUTHAMEL** *[x] and **BARBARA DORSCH**

Received March 1, 1978, from the *Department of Pharmacy, School of Pharmacy, University of Maryland at Baltimore, Baltimore, MD 21201.* Accepted for publication June 28, 1978. *Present address: Department of Biochemistry and Drug Metabolism, Hoffmann-La Roche, Nutley, NJ 07110.

**Abstract** □ A specific assay for nitroglycerin in dosage forms using high-performance liquid chromatography was developed. Sublingual nitroglycerin tablets are dissolved in 25 ml of water and injected directly into the chromatograph. Chromatographic conditions are: mobile phase, 60% methanol in water; flow rate, 2 ml/min; column, microparticulate reversed phase; and detection, 200 nm. The glyceryl mononitrate and dinitrate degradation products of nitroglycerin are separated from nitroglycerin and can be identified by altering the mobile phase composition of methanol.

**Keyphrases** □ Nitroglycerin—high-performance liquid chromatographic analysis in various dosage forms □ High-performance liquid chromatography—analysis, nitroglycerin in various dosage forms □ Vasodilators, coronary—nitroglycerin, high-performance liquid chromatographic analysis in various dosage forms

Nitroglycerin (glyceryl trinitrate) is one of the oldest drugs presently in use. It was synthesized in 1846 and was reported to produce a "migraine" when ingested (1). Initial clinical pharmacology research was begun in 1849 when Hering (2) tested nitroglycerin on several medical students.

## BACKGROUND

Virtually every analytical method available has been used in an attempt to quantitate nitroglycerin. One of the earliest reported analytical methods dates from 1901 when nitroglycerin was identified by exploding it with a hammer on an anvil following its extraction from the stomach of a cadaver (3). While relatively specific for nitroglycerin, quantitation with this assay was probably a problem. Other methods utilized include colorimetry (4), polarography (5), kinetic methods (6, 7), and, more recently, TLC (8) and GLC (9).

Many early methods lacked specificity and sensitivity, while the later methods are generally more complex and time consuming. The official USP content uniformity assay (10) of Bell (4) is based on alkaline hydrolysis of the nitrate ester to nitrate ion, followed by coupling with *N*-(1-naphthyl)ethylenediamine dihydrochloride to produce a purple compound with absorbance at 545 nm.

The automated version of this assay(11) has a sensitivity of approximately 5 μg/ml. While this assay may be specific for nitroglycerin and, therefore, stability indicating[1], supporting documentation has not been reported. Recent GLC methods are specific for nitroglycerin (9, 12, 13) but require extraction into organic solvents, evaporation, and detection by flame ionization or electron capture. Therefore, they are not applicable if rapid in-process quality control data are required. Analytical methods used to quantitate nitroglycerin were reviewed recently (14).

Significant changes in the potency of nitroglycerin tablets can occur with time, depending on tablet formulation and storage conditions (15). With the advent of the extemporaneous preparation of nitroglycerin injection in many medical centers, a rapid, specific, sensitive assay for nitroglycerin in dosage forms is needed.

## EXPERIMENTAL

**Equipment and Supplies**—All reagents were analytical grade unless indicated otherwise. Methanol[2] was glass distilled. A high-performance liquid chromatograph[3] with a variable wavelength UV detector[4] was used. A $C_{18}$ microparticulate[5] column was used in the separation.

Standard solutions of nitroglycerin were made from nitroglycerin spirit standardized against potassium nitrite using the phenoldisulfonic acid procedure (16). Samples of glyceryl mononitrate and glyceryl dinitrate were used as received. The two test formulations[6] were 400-μg sublingual tablets, Products A[7] and B[8].

**Automated Assay**—The procedure described in the USP (10) was modified. The concentration of *N*-(1-naphthyl)ethylenediamine dihydrochloride was reduced to 0.25%, the number of samples run per hour was reduced to 20 to allow better separation between samples, and the ratio of sample to wash was 1:4. Three standards were run at the beginning of each series, and one standard was run after every 10 samples.

**High-Performance Liquid Chromatographic (HPLC) Assay**—Individual tablets for analysis were placed in 25 ml of distilled water and shaken to ensure complete solution of the nitroglycerin. A 10-μl aliquot of the aqueous solution was injected directly into the chromatograph under the following conditions: mobile phase, 60% methanol in distilled water; flow rate, 2 ml/min; column, $C_{18}$ microparticulate[5]; and detection, 200 nm UV. Under these conditions, nitroglycerin had a retention time of 4 min.

Nitroglycerin injection, 50 μg/ml, prepared in a local hospital pharmacy, was assayed as described after appropriate dilution. No interfering peaks were found in the chromatograms of any sample. When identifying glyceryl mononitrate and dinitrate, the mobile phase methanol concentration was decreased to 40% but the procedure was otherwise unchanged.

Because of variability between manufacturers and even between columns for the same manufacturer, different $C_{18}$ microparticulate columns should be evaluated before the one best suited to the HPLC system is selected. Good results were obtained without an internal standard, and one was not routinely used. However, diazepam in a concentration of 20 μg/ml can be utilized as an internal standard; under the conditions described, it has a retention time of 8 min.

## RESULTS

Figure 1 shows a typical chromatogram of nitroglycerin from a 400-μg sublingual tablet dissolved in 25 ml of water. The retention time with 60% methanol in water was about 4 min. No interference was found from the constituents of the tablet. The standard curve for nitroglycerin was linear from 3 to 22 μg/ml, passed through the origin, and had a lower limit of detection of approximately 30 ng injected in the column (equivalent to a 3-μg/ml solution).

To determine the precision of this method as compared to the official USP content uniformity assay, a standard solution of nitroglycerin containing 18.3 μg/ml was assayed 10 consecutive times by both methods. The estimated concentration of the solution was 18.3 ± 0.48 μg/ml (mean ± *SD*) by HPLC and 18.3 ± 0.34 μg/ml by the USP method. The relative standard deviation of approximately 2.5% for HPLC is reasonable considering the low dose of the drug; under these conditions, about 183 ng was injected onto the column.

To compare the HPLC assay with the USP content uniformity assay under more realistic assay conditions, 10 tablets were individually dissolved in 25 ml of water as outlined under *Experimental.* Both Products A and B were tested since they contained different excipients (15) to

[1] S. A. Fusari, Parke-Davis and Co., Detroit, Mich., personal communication.
[2] Burdick & Jackson, Muskegon, Mich.
[3] Model 711.
[4] Spectromonitor II, Laboratory Data Control, Riviera Beach, Fla.
[5] μBondapak $C_{18}$, Waters Associates, Milford, Mass., or Datasorb ODS, Laboratory Data Control, Riviera Beach, Fla.
[6] Purchased in unopened bottles at a local pharmacy.
[7] Lot 0FT97A, Eli Lilly and Co., Indianapolis, Ind.
[8] Lot T L176, Parke-Davis and Co., Detroit, Mich.

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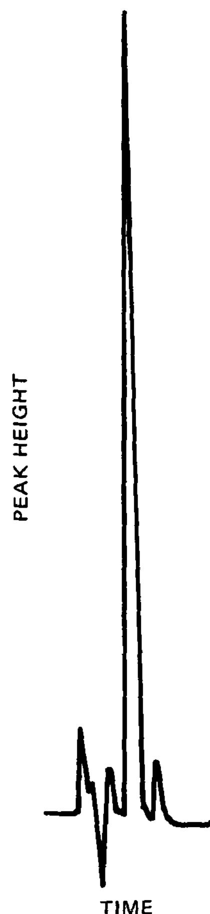


**Figure 1**—*Typical chromatogram for nitroglycerin from a 400-μg sublingual tablet.*

stabilize the nitroglycerin against volatilization. The results (mean ± *SD*) for Product A were 421.7 ± 25.6 and 420.1 ± 30.8 μg by the HPLC and USP methods, respectively. For Product B, the results were 398.0 ± 22.6 and 412.5 ± 24.5 μg, respectively.

No statistically significant differences were found between the two analytical methods with either product, although the mean concentration obtained with the USP assay was slightly higher for Product B. This same result was found when the experiment was repeated and may be due to a slight interaction with an excipient (such as the polyethylene glycol 400 stabilizer) in the product. No difference in weight variation or content uniformity was found between Products A and B.

The described method is capable of separating nitroglycerin from the glyceryl 1- and 2-mononitrate and glyceryl 1,2- and 1,3-dinitrate degradation products. Since the glyceryl mononitrates and dinitrates are more polar than nitroglycerin, they travel through the reversed-phase column faster than nitroglycerin and are eluted soon after the solvent front, making identification of the glyceryl mononitrates and dinitrates difficult. To separate these degradation products from nitroglycerin, the methanol concentration of the mobile phase must be decreased, thereby increasing the retention time of all nitrate esters. With a mobile phase of 40% methanol in water, the glyceryl mononitrate esters have retention times of approximately 2.00 and 2.75 min, the glyceryl dinitrates have retention times of approximately 3.5 and 3.75 min, and nitroglycerin has a retention time of approximately 11 min.

Although the tablets contained only small amounts of the glyceryl mononitrates and dinitrates, nitroglycerin solutions that were purposely degraded had much larger quantities of these degradation products.

## DISCUSSION

During the development of this assay, a number of variations were tried. Methanol and acetonitrile performed equally well as the mobile phase; methanol was chosen because it is less expensive, even though it must be used in higher concentration than acetonitrile. Because of the lower UV cutoff, acetonitrile may be a better mobile phase with a single-beam detector.

The effect of changes in mobile phase pH were studied to determine if pH changes could alter the nitroglycerin retention time. As might be expected for a neutral molecule, chromatographic characteristics were independent of mobile phase pH. As columns age, the retention times of these compounds tend to become shorter, so the methanol concentration may need to be modified when columns are changed or with time of use. Several variable wavelength UV detectors were evaluated, and the one used in this study provided the most stable baseline and the greatest absorbance. Since many compounds absorb UV light at 200 nm, relatively pure organic solvents should be used to minimize extraneous peaks in the chromatogram.

The problem of content uniformity of nitroglycerin tablets is perhaps best indicated by the large limits (75–135%) of potency established by the USP (10). The addition of polyethylene glycol 400 (Product B) or povidone (Product A) as a stabilizer in nitroglycerin tablets has reduced the nitroglycerin volatility problem, so the tablets presently available are usually well within the USP limits. Nevertheless, variations in nitroglycerin potency can still occur even with the stabilized formulations (15).

With the emergence of new nitroglycerin dosage forms with unknown stabilities, it will become increasingly important to monitor product performance. For example, nitroglycerin intravenous injection is presently extemporaneously compounded in many major medical centers due to its lack of commercial availability. No information is presently available on its stability under various storage conditions or in different intravenous solutions. Similarly, little information is available on the ointment dosage forms of nitroglycerin.

Although this HPLC assay is approximately equal to the automated USP content uniformity assay in precision, it has several features that may make it more desirable under certain circumstances. Since the assay specifically detects intact nitroglycerin and utilizes no chemical reactions, there is no likelihood of alterations in the assay due to interference with the reaction or its reagents. Since the assay physically separates nitroglycerin from its primary degradation products, no interference from these compounds should occur. In addition, the glyceryl mononitrate and dinitrate degradation products can be identified with this assay. Thus, much more stability information can be obtained than with the USP assay. Since the USP assay is subject to variations in color development with time, it is difficult to use when it is not automated.

In summary, this assay provides a rapid, precise, sensitive, and specific method for the analysis of nitroglycerin dosage forms, and it can be modified to provide information on the nitroglycerin degradation products as well. In stability studies, this assay provides more information than the conventional USP content uniformity method and is likely to be the analytical method of choice.

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