High-Performance Liquid Chromatographic Assay for Partially Nitrated Glycerins in Nitroglycerin

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Abstract □ A high-performance liquid chromatographic method for determining the concentration of mononitroglycerins and dinitroglycerins in the commonly used pharmaceutical raw material nitroglycerin (10% w/w) on lactose USP is presented. A coefficient of variation of <5% for the partially nitrated glycerins was achieved over the range examined (0–4.0 μg/mg). Thirteen lots of raw material were examined and found to contain a total of <0.13% w/w partially nitrated glycerins. A variable wavelength detector (λ = 218 nm) and a microphenyl column were employed. The mobile phase was acetonitrile–water (36:64) pumped at 2 ml/min. The internal standard was isosorbide dinitrate. Total analysis time was 12 min.

Keyphrases □ High-performance liquid chromatography—assay for partially nitrated glycerins in nitroglycerin □ Nitroglycerin—high-performance liquid chromatographic assay for partially nitrated glycerins □ Glycerin—partially nitrated, high-performance liquid chromatographic assay for nitroglycerin

Nitroglycerin has been used orally and topically for many years for the treatment of angina pectoris. Recently, research has led to several new indications for nitroglycerin, all of which take advantage of the benefits of intravenous administration (1–3). The increase in the use of intravenous administration of nitroglycerin has prompted the FDA to seek additional data on the stability and purity of the raw materials used in the manufacture of drugs. To supply this data, a rapid and accurate assay for the partially nitrated glycerins (1,2-dinitroglycerin, 1,3-dinitroglycerin, 1-mononitroglycerin, and 2-mononitroglycerin) in nitroglycerin is needed.

BACKGROUND

Nitroglycerin is the triester of nitric acid and glycerol and is synthesized from glycerol and nitric acid using sulfuric acid as a catalyst. The addition of the nitro groups presumably proceeds in a stepwise manner. Metabolism and the nonexplosive chemical degradation occur by an opposite process—a stepwise loss of nitro groups. Partial completion of any of these processes would result in the contamination of nitroglycerin with partially nitrated glycerins (Scheme I).

Another potential source of partially nitrated glycerins in nitroglycerin is contamination. Most of the nitroglycerin made is intended for use as explosives. Pharmaceutical nitroglycerin is a small proportion of the production. Several explosive mixtures contain significant quantities of partially nitrated glycerins by design, and since they are made by the same manufacturer as the nitroglycerin intended for pharmaceutical use, the potential for cross contamination exists.

There are two widely used colorimetric methods for quantitating nitroglycerin which can be made stability indicating (4, 5). One is the basis for compendial monographs (4). However, the colorimetric methods and a published kinetic assay (6) do not allow the direct quantitation of partially nitrated glycerins.

Gas chromatographic (GC) and high-performance liquid chromatographic (HPLC) methods offer the potential to quantitate the partially nitrated glycerins in nitroglycerin. However, all GC methods require a tedious extraction into an organic phase (7–13), and only two have been employed for dosage-form types of solutions (7, 13), while the rest were designed for blood level assays. The HPLC methods are faster since they do not employ an extraction step (14–17). Of all the chromatographic methods only four demonstrate the potential quantitation of partially nitrated glycerins (12–15) and none specifically do so.

A sensitive HPLC method is described here that specifically allows the rapid direct quantitation of partially nitrated glycerins in nitroglycerin raw materials.

EXPERIMENTAL

Materials—Nitroglycerin[1] (10% w/w on lactose, USP) and isosorbide dinitrate[2] (25% w/w on lactose) were used as received. 1,2-Dinitroglycerin (10% w/w on lactose), 1,3-dinitroglycerin (10% w/w on lactose), 1-mononitroglycerin, and 2-mononitroglycerin were synthesized using literature methods (18). All standards were calibrated by the USP phenoldisulfonic acid assay for nitroglycerin (19). Glass-distilled acetonitrile and methanol were used for all procedures[3]. Purified water was further purified[4] prior to use.

Instrumentation—The liquid chromatographic system consisted of a solvent pumping system[5], an automatic fixed-loop sample injector[6], a variable wavelength detector[7], and a 10-mv recorder[8]. A 30 cm × 3.9-mm column packed with alkylphenyl-bonded silica gel[9] (10 μm), a detector wavelength of 218 nm (AUFS = 0.05), and a chart speed of 20 cm/hr were employed. Sample injections of 100 μl were used.

Mobile Phase—Approximately 1 liter of mobile phase was prepared fresh daily by thoroughly mixing 360 ml of acetonitrile and 640 ml of water. The mobile phase was always filtered through a 0.5-μm filter[4] prior to use. The mobile phase was pumped at a constant rate of 2 ml/min which yielded a pressure of <2000 psi.

Standard Curve—Stock standard solutions were prepared by transferring an accurately weighed sample of partially nitrated glycerin equivalent to 25 mg of active ingredient to a 50-ml volumetric flask. They were then dissolved in 5 ml of alcohol (USP) and brought to volume with water.

Working standard concentrations of ~20, 10, 5, and 2.5 μg/ml in both a mononitroglycerin and a dinitroglycerin were prepared from the stock standards by diluting in water 2-mononitroglycerin (or 1-mononitroglycerin) stock standard solution and 1,2-dinitroglycerin (or 1,3-dinitroglycerin) stock standard solution.

An accurately weighed sample of isosorbide dinitrate (25% w/w on lactose) of 500 mg was placed into a 250-ml volumetric flask, dissolved in 25 ml of alcohol and swirled for 5 min. It was brought to volume with water and mixed well. Ten milliliters of this solution was diluted to 100 ml to yield a final concentration of 50 μg/ml.

An accurately weighed sample of nitroglycerin (10% w/w on lactose), equivalent to 50 mg of active ingredient, was placed in a 100-ml volumetric flask, dissolved in 10 ml of alcohol, and brought to volume with water.

To 1.0 ml of each standard or sample was added 500 μl of isosorbide dinitrate (50 μg/ml) internal standard. Each solution was vortexed[10] for 5 sec and chromatographed. Peak heights for the partially nitrated glycerins and isosorbide dinitrate were measured manually. The peak areas were measured by a laboratory data system[11]. The peak area ratios

[1] ICI Americas, Wilmington, Del.
[2] Napp Chemicals, Inc., Lodi, N.J.
[3] Burdick and Jackson, Muskegon, Mich., or J. T. Baker, Phillipsburg, N.J.
[4] Millipore Corp., Bedford, Mass.
[5] System 2/2, Perkin-Elmer Corp., Norwalk, Conn.
[6] Model 420 Perkin-Elmer Corp., Norwalk, Conn.
[7] LC-55B, Perkin-Elmer Corp., Norwalk, Conn.
[8] Model 023, Perkin-Elmer Corp., Norwalk, Conn.
[9] μBondapak Phenyl, Waters Associates, Milford, Mass.
[10] Vortex Genie Mixer, American Scientific Products, McGaw Park, Ill.
[11] Model 3354, Hewlett-Packard Corp., Avondale, Pa.

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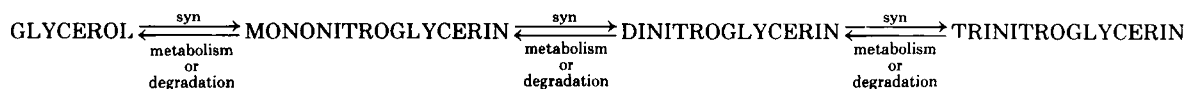

Scheme I

were then plotted *versus* concentration to yield a calibration curve. Identical results were obtained using peak height ratios.

RESULTS AND DISCUSSION

The direct quantitation of the partially nitrated glycerins in nitroglycerin (10% w/w on lactose) is reported for the first time. It is based on a modification of an HPLC procedure for nitroglycerin which has been used successfully for over 3 years on almost 10,000 samples (14). Alcohol has been used to speed the dissolution of the various nitrates but is not required.

This procedure does not resolve the dinitroglycerins from one another, nor does it resolve the mononitroglycerins from one another. For the purpose of quantitating partially nitrated glycerins in nitroglycerin raw material the resolution of the isomers is unnecessary. It was demonstrated that not only do the pairs of isomers coelute, but they yield superimposable calibration lines. Thus, it is simpler and more practical to quantitate the isomers employing only one mononitroglycerin and one dinitroglycerin standard. Chromatograms illustrating the obtainable separations are shown in Fig. 1.

The described procedure, using 1 ml of sample or standard and 0.5 ml of internal standard, allows an accurate assay of partially nitrated glycerins over the concentration range commonly encountered in nitroglycerin raw materials (0.0–4.0 μg/mg). It is possible to detect the partially nitrated glycerins down to a level of 0.02 μg/mg by using the maximum detector sensitivity of AUFS 0.002. Even greater sensitivities are theoretically achievable using greater sample sizes or an extraction, but were not required for this study.

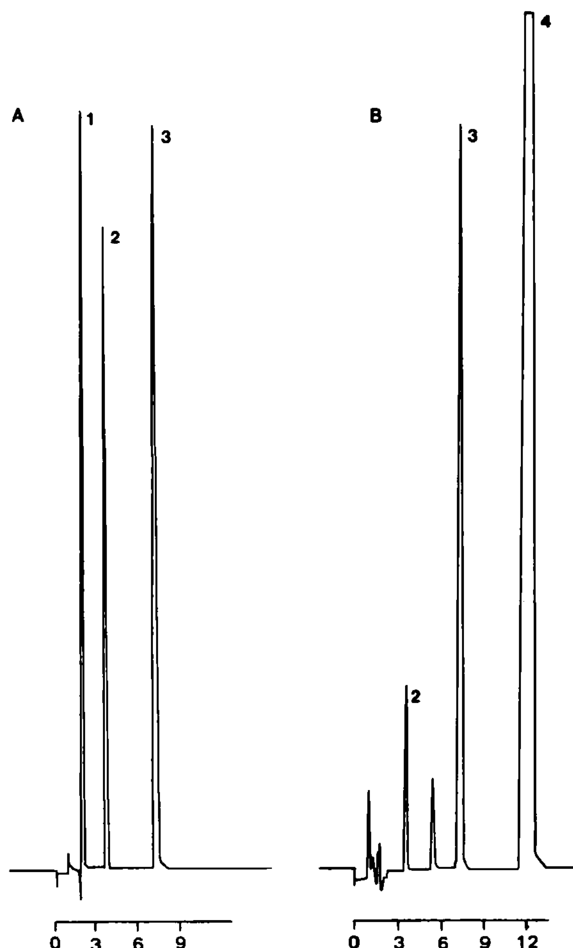

Figure 1—*A—Typical chromatogram of a partially nitrated glycerin standard. Combined partially nitrated glycerin concentration equal to ~0.6% w/w in raw material. Key: (1) mononitroglycerins peak of 14.15 μg/ml; (2) dinitroglycerins peak of 16.40 μg/ml; (3) isosorbide dinitrate. B—Typical chromatogram of a nitroglycerin on lactose raw material sample. No mononitroglycerins detected. Key: (2) dinitroglycerins peak of ~0.1% w/w in raw material; (3) isosorbide dinitrate; (4) trinitroglycerin.*

Table I—Levels of Partially Nitrated Glycerins [a]

Lot	Mononitroglycerins, % w/w in Sample[b]	Dinitroglycerins, % w/w in Sample
A		0.12
B	0	0.10
C	0	0.10
D	0	0.11
E	0	0.11
F	0	0.08
G	0	0.09
H	0	0.09
I	0	0.08
J	0	0.08
K	0	0.07
L	0	0.09
M	0	0.09

[a] Thirteen lots of nitroglycerin (10% w/w on lactose), raw material in percent.
[b] 0 = < 0.001%.

Accuracy and Precision—Four replicate, partially nitrated glycerin standards (20, 10, 5, and 2.5 μg/ml) were chromatographed. A correlation coefficient of >0.99 was consistently obtained for both partially nitrated glycerins, as was a coefficient of variation of <3% for the dinitroglycerins and 5% for the mononitroglycerins. Finally, accuracy for the mono- and dinitroglycerins of >95 and 98%, respectively, were found.

Applicability—The method has been applied to nitroglycerin in alcohol, nitroglycerin in propylene glycol (USP), and nitroglycerin (10% w/w on lactose). The practicality of the method was demonstrated by the analysis of 13 lots of nitroglycerin triturate (Table I). In all instances the level of mononitroglycerins was zero. Under the conditions of the experiment this was equivalent to <0.05 μg/ml in the assay solution or 0.001% w/w in the raw material. The dinitroglycerins were present at levels of <0.15% (w/w) in all samples, even after storage at room temperature for 4 years.

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Simultaneous Determination of Acetaminophen, Guaifenesin, Pseudoephedrine, Pholcodine, and Paraben Preservatives in Cough Mixture by High-Performance Liquid Chromatography

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Received August 3, 1981, from the *Quality Control Department, Fisons Pty. Ltd., Sydney, Australia.* Accepted for publication March 25, 1982.

Abstract □ The separation and simultaneous determination, by high-performance liquid chromatography, of acetaminophen (I), guaifenesin (II), pseudoephedrine hydrochloride (III), and pholcodine (IV), together with a series of parabens (methyl to butyl, V–VIII) in a cough mixture, has been demonstrated using a chemically bonded octadecylsilane stationary phase with a mobile phase of methanol–water–acetic acid (45:55:2) containing the ion-pairing agent octanesulfonic acid. Retention volumes for the active ingredients were 3.8 ml, 5.4 ml, 9.4 ml, and 15.6 ml for compounds I–IV, respectively. Corrected retention volumes for the parabens [5.4 ml for methyl (V), 9.6 ml for ethyl (VI), 18.5 ml for propyl (VII), and 37.9 ml for butyl (VIII)] showed an exponential relationship with chain length of the esterifying alcohols. Excipients did not interfere with the estimation of any of the compounds, hence pretreatment of the sample was unnecessary. Average recoveries of the active ingredients and of the parabens from laboratory prepared samples were essentially 100% of theoretical with standard deviations of 1.7, 0.3, 1.5, 0.3, 0.3, 3.3, 0.7, and 2.7% for I–VIII, respectively.

Keyphrases □ Acetaminophen—simultaneous determination of guaifenesin, pseudoephedrine, pholcodine, and paraben preservatives in cough mixture by high-performance liquid chromatography □ Pseudoephedrine—simultaneous determination of acetaminophen, guaifenesin, pholcodine, and paraben in preservatives in cough mixture by high-performance liquid chromatography □ Paraben—simultaneous determination of acetaminophen, guaifenesin, pseudoephedrine, and pholcodine in preservatives in cough mixture by high-performance liquid chromatography □ High-performance liquid chromatography—simultaneous determination of acetaminophen, guaifenesin, pseudoephedrine, pholcodine, and paraben preservatives in cough mixture

High-performance liquid chromatography (HPLC) has become a powerful tool for the analysis of pharmaceutical products. Mixtures used for the treatment of coughs and colds may be complexes containing several active ingredients including a decongestant, an antihistamine, frequently an analgesic, preservatives, dyes, and flavors. The active materials cover a range of structures with widely varying polarities and include both acidic and basic compounds.

A number of conventional methods have been applied to the present series. Pholcodine has been estimated by UV spectrophotometry following separation by TLC and colorimetry (1, 2). Pseudoephedrine and acetaminophen have been determined spectrophotometrically (3, 4) and by GLC (5, 6). Guaifenesin has been determined in pharmaceutical preparations by GLC (7, 8). The parabens may be assayed by GLC (9) or by UV spectroscopy following sample clean-up by column chromatography (10).

Spectrophotometric, GLC, or methods requiring TLC separation when applied to samples such as cough mixtures can be lengthy and/or subject to interferences by the matrix of the sample, and they are generally not suitable for simultaneous assay. The simultaneous assay of the drugs and preservatives described in this report cannot be achieved by any of the techniques mentioned here.

The application of HPLC procedures to various combinations of drugs and parabens has been reported (11–19), and some attention has been given to the effect of carbon chain length of the alkylsulfonic acid ion-pairing agents on retention times of several drugs (20, 21). The effect of chain length on resolution of mixtures of materials with varying polarity was tested, and a procedure was developed by which eight components, four active materials and four preservatives, may be determined with one injection.

EXPERIMENTAL

Materials—All active ingredients and paraben preservatives were of BP quality except guaifenesin which conformed to BPC standard. All were used without further purification.

Mobile Phase—The mobile phase, methanol[1]–water–glacial acetic acid (45:55:2) containing 0.005 *M* octanesulfonic acid[2], was filtered through a 0.45-μm filter[3]. The flow rate was 2.5 ml/min.

Instrumentation—The liquid chromatograph consisted of a constant flow pump[4], a low-pressure injector[5], a dual channel absorbance detector[6] set at 254 and 280 nm, and a 30-cm × 4-mm i.d. octadecylsilane column[7]. Outputs from the 280-[8] and 254-nm[9] channels were quantitated. Separate monitoring at 254 nm was required for pseudoephedrine which is not detected on the 280-nm channel.

Standard Solutions—The stock solution of analytes in methanol contained 25.00, 5.00, 5.00, 0.75, 0.61, 0.20, 0.33, and 0.20 mg/ml of I–VIII, respectively. Aliquots (2, 3, 4, 5, 6, and 7 ml) of this solution were diluted to 100 ml with water and filtered through a 0.45-μm filter[10]. A calibration

[1] Methanol BDH, redistilled in glass.
[2] PIC B8, Waters Associates, Milford, Mass.
[3] Millipore Type FH organic.
[4] Model 6000A, Waters Associates, Milford, Mass.
[5] Model U6K, Waters Associates, Milford, Mass.
[6] Model 440, Waters Associates, Milford, Mass.
[7] μ-Bondapak C-18, Waters Associates, Milford, Mass.
[8] Data Module, Waters Associates, Milford, Mass.
[9] Varian CDS III integrator.
[10] Millipore Type HA aqueous.

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