assay was ~2%. Besides assaying for composite granulations, the HPLC assay also is applicable to content uniformity analysis.

In conclusion, the HPLC method is simple, precise, fast, specific, and accurate. A complete analysis takes ~10 min. All test compounds and known impurities can be detected. Since the analysis is conducted at ambient temperature, no thermal degradation of codeine is expected.

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# High-Performance Liquid Chromatographic Analysis of Nitroglycerin Ointment


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Received August 24, 1979, from the *Winchester Engineering and Analytical Center, Food and Drug Administration, Winchester, MA 01890.* Accepted for publication March 22, 1980.



**Abstract** □ The assay of ointments containing nitroglycerin is described. Sample preparation by direct dissolution in warm dimethyl sulfoxide appeared to be more accurate than extraction. Anisole was used as an internal standard. Chromatographic conditions included a $C_{18}$ microporous silica column and a methanol–water mobile phase (40:60). Detection at 215 nm was superior to detection at 254 nm.



**Keyphrases** □ Nitroglycerin—ointment, reversed-phase high-performance liquid chromatographic analysis □ Ointments—nitroglycerin, reversed-phase high-performance liquid chromatographic analysis □ High-performance liquid chromatography—analysis, nitroglycerin ointment


The use of topical nitroglycerin for patients with congestive heart failure was reported recently (1). Existing methods for the analysis of nitroglycerin (2–9) lack specificity (7), are too time consuming for convenient uniformity determinations, or require prolonged heating that could lead to decomposition of nitroglycerin, which occurs at 50–60° (10).

Several high-performance liquid chromatographic (HPLC) assays for nitroglycerin dosage forms were reported recently (11, 12). These methods can separate nitroglycerin from its mono- and dinitrate degradation products. Since HPLC has none of the disadvantages of the other methods, its applicability to the analysis of nitroglycerin ointment was studied. A sample preparation method was developed so that the method of Crouthamel and Dorsch (11) could be applied to ointment samples. It was chosen for initial investigation because it uses an octadecylsilane column, which is widely available in HPLC laboratories, and a simple, less expensive, water–methanol mobile phase. The method of Baske *et al.* (12) uses a less retentive phenyl column and a more expensive and complex water–acetonitrile–tetrahydrofuran mobile phase. Contrary to a previous observation (12), no problems of clogging of inlet filters were encountered.

## EXPERIMENTAL

**Materials**—The water used was HPLC grade[1]. The methanol was distilled in glass[2]. Dimethyl sulfoxide (I), butylated hydroxyanisole[3], isooctane, sodium acetate, acetic acid, potassium nitrate, ammonium hydroxide, lanolin, and petrolatum[4] were ACS reagent grade.

A 10% nitroglycerin on lactose triturate[5] was used as a standard and was calibrated by the USP phenoldisulfonic acid method for the assay of nitroglycerin (2). It assayed at 9.62%. The standard solutions were 1 mg/ml in methanol for Method 1, 2 mg/ml in I for Method 2, and 2 mg/ml in the internal standard solution for Method 2a.

**Instrumentation**—Two liquid chromatographic systems were used. The first system consisted of a dual-head reciprocating piston positive-displacement pump[6], a septumless syringe-loaded loop injector with a 10-μl loop[7], a variable-wavelength detector operated at 254 or 216 nm[8], a 10-mv recorder[9], and a 15-cm × 4-mm column of a slurry of chemically bonded octadecyl reversed-phase material[10] packed in aqueous sodium acetate and methanol according to the manufacturer's directions. The second system contained a dual-head reciprocating piston pump[11], an automatic injector[12], a variable-wavelength detector operated at 215 nm[13], a flat-bed recorder[14], and a 15-cm × 4.6-mm column of chemically

[1] J. T. Baker Chemical Co., Phillipsburg, N.J.
[2] Burdick & Jackson Laboratories, Muskegon, Mich.
[3] Sigma Chemical Co., St. Louis, Mo.
[4] Fisher Scientific Co., Fair Lawn, N.J.
[5] Marion Laboratories, Kansas City, Mo.
[6] Model 100A with preparative scale head, Altex Scientific Inc., Berkeley, Calif.
[7] Model 70-10 with model 70-11 loop filler port, Rheodyne Inc., Berkeley, Calif.
[8] Model 837 spectrophotometer, DuPont Instruments, Wilmington, Del.
[9] Servariter II, Texas Instruments, Houston, Tex.
[10] ODS-Hypersil, Hyperspheres (5 μm), Shandon Southern Products Ltd., Cheshire, England.
[11] Model 6000A, Waters Associates, Milford, Mass.
[12] WI-SP, Waters Associates.
[13] Model LC-75, Perkin-Elmer, Norwalk, Conn.
[14] Model 595, Linear Instruments Corp., Irvine, Calif.




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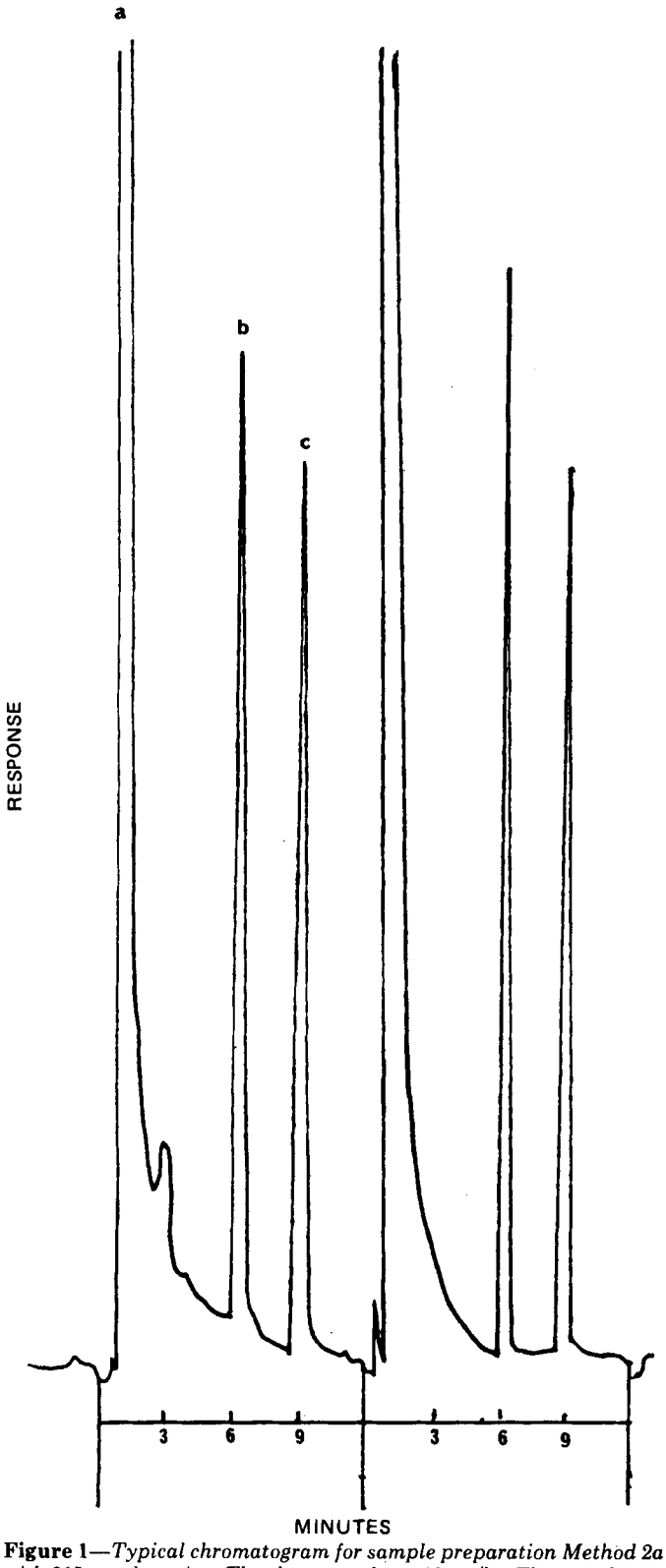


**Figure 1**—*Typical chromatogram for sample preparation Method 2a with 215-nm detection. The chart speed was 20 cm/hr. The sample was followed by the standard. Key: a, I; b, nitroglycerin; and c, anisole.*

bonded octadecyl reversed-phase material[15] commercially packed and used with a 4-cm × 4.6-mm precolumn dry packed with pellicular octadecyl reversed-phase material[16].

**Chromatographic Conditions**—The mobile phase was methanol–water (40:60) and was suction filtered through a 0.9-μm filter[17]. This mobile phase was chosen since it was the strongest mobile phase that gave adequate resolution of nitroglycerin from I. Anisole was the internal standard since it was well resolved from the peak of interest and from I or excipient peaks and is very stable and readily available. Diazepam, suggested as an internal standard by Crouthamel and Dorsch (11), was not resolved from nitroglycerin in System 1. The flow rate was 0.8 ml/min with a pressure of 800 psi for System 1 and 2 ml/min with 4400 psi for System 2.

Typical chromatographic parameters for System 1 were $k' = 5.4$ for nitroglycerin and 7.1 for anisole, $N = 2000$ for nitroglycerin and 2500 for anisole, and $R = 3.1$. For System 2, $k' = 8.6$ for nitroglycerin and 12.4 for anisole, $N = 3430$ for nitroglycerin and 5230 for anisole, and $R = 5.95$. The $N$ and $R$ values were evaluated using half-width formulas (13). Tailing (evaluated by dropping a vertical line from the peak maximum, drawing a horizontal line at the 10% peak height, and dividing the distance from the vertical line to the peak back by the distance from the vertical line to the peak front) was 3.4 for nitroglycerin and 3.3 for anisole in System 1 and 1.9 for nitroglycerin and 1.0 for anisole in System 2 (Fig. 1).

**Procedure**—*Method 1*—About 1 g of ointment (equivalent to 20 mg of nitroglycerin) and 20 ml of isooctane were shaken mechanically for 40 min. After the contents of the flask were transferred to a separator, the flask was washed with water to dissolve the remaining lactose; each water wash was used to extract gently the isooctane. The pooled water was shaken with isooctane. The original flask was washed twice with methanol. Both methanol portions were combined, used to extract the isooctane, and filtered[18]. This procedure was repeated twice, and the extract was taken to 25.0 ml with methanol.

*Method 2*—About 1 g of ointment was weighed accurately into a beaker, 10.0 ml of I was added, and the solution was heated on a steam bath for 5 min with occasional gentle agitation. After cooling, the coagulated solids were removed with a capillary pipet or left in the beaker when the solution was decanted into a glass-stoppered erlenmeyer flask. An internal standard solution was prepared by combining 0.1 ml of anisole with 10 ml of methanol and diluting 3 ml of the resulting solution to 75 ml with I. An aliquot of 0.50 ml of the internal standard solution was added to 2.0 ml of the sample or standard to produce solutions for injection.

*Method 2a*—About 1 g of ointment was weighed accurately into a 50-ml glass-stoppered centrifuge tube, 20.0 ml of the internal standard solution (0.0003 ml of anisole/ml in I) was added, and the solution was heated for 5 min on a steam bath with occasional vigorous shaking. The solution then was shaken mechanically for 30 min, which resulted in a ball of coagulated solid floating on top of the liquid, so that the clear liquid could be removed easily with a capillary pipet for injection into the liquid chromatograph.

System 1 was used to evaluate Methods 1 and 2; System 2 was used to evaluate Method 2a.

**Table I—Comparison of Sample Preparation Methods with 254-nm Detection [a]**

| Method | Sample Assay, % label claim | Spike Recovery, % added standard |
|---|---|---|
| 1 | 86.4 | 86.3 |
| | 84.7 | 92.7 |
| 2 | 94.9 | 100.1 |
| | 90.3 | 105.4 |

[a] Manufacturer 1.

## RESULTS AND DISCUSSION

Table I shows that Method 2, which makes a lipophilic sample suitable for injection into a reversed-phase HPLC system without extraction, is more accurate than Method 1. This fact is indicated most clearly by results of spiked samples, consisting of ~0.5–0.6 g of ointment and 100 mg of standard triturate (10 mg of nitroglycerin), taken through the method. Table II demonstrates that Method 2a gives improved extraction of nitroglycerin from the ointment and more precision due to the presence of the internal standard from the beginning of sample preparation and the increased agitation.

While detection at 254 nm is possible at the concentrations used here, the signal-to-noise ratio is low due to the fact that the UV spectrum of nitroglycerin exhibits a minimum at this wavelength. As shown in Table

[15] Ultrasphere ODS (5 μm), Altex Scientific Inc., Berkeley, Calif.
[16] μBondapak $C_{18}$ Corasil, Waters Associates.
[17] AAWP, Millipore Corp., Bedford, Mass.
[18] Grade 541, Whatman Ltd., England.

**Table II—Sample Preparation Method 2a with 215-nm Detection**

| Manufacturer | Assay, % | Recovery[a], % |
|---|---|---|
| 1 | 106.5 | 100.6 |
| | 106.6 | 102.7 |
| Average | 106.6 | 101.6 |
| 2 | 103.2 | 101.8 |
| | 100.4 | 101.9 |
| Average | 101.8 | 101.8 |
| 3 | 104.7 | 97.3 |
| | 104.0 | 100.2 |
| Average | 104.4 | 98.7 |

[a] Average recovery for all manufacturers = 100.7%.

**Table III—Comparison of Detection Wavelengths for Sample Preparation Method 2**

| | 254 nm | | 216 nm | |
|---|---|---|---|---|
| Manufacturer | Assay, % | Recovery, % | Assay, % | Recovery, % |
| 1 | 94.9 | 100.1 | 96.8 | 103.5 |
| | 91.0 | 105.4 | 92.6 | 98.8 |
| Average | 93.0 | 102.8 | 94.7 | 101.2 |
| 3 | 96.8 | 104.4 | 94.4 | 97.8 |
| | 98.1 | 104.8 | 93.1 | 101.0 |
| Average | 97.4 | 104.6 | 93.8 | 99.4 |

**Table IV—Authentic Recoveries for Sample Preparation Method 2a with 215-nm Detection**

| Manufacturer | Recovery of Label Claim[a], % |
|---|---|
| 1 | 99.2 |
| | 98.0 |
| 2 | 101.1 |
| | 99.3 |
| 3 | 99.3 |
| | 97.9 |

[a] Average = 99.1%; coefficient of variation = 1.14%.

III, detection at 216 nm gives comparable quantitative results (data were obtained with the same solutions). At 254 nm, peak heights were ~0.01 absorbance unit; at 216 nm, they were ~0.82 absorbance unit. This difference represents approximately an 80-fold enhancement in detection.

Thus, Method 2a and detection at 215 nm were best and were subjected to further validation. The three products tested were all currently marketed, nitroglycerin ointments. Three substances that represented expected excipients, lanolin, butylated hydroxyanisole, and petrolatum, were analyzed individually by this method at levels likely to be used in commercial formulations. Petrolatum gave no peaks, and lanolin gave only a peak before I. Butylated hydroxyanisole gave a peak with a $k'$ value six times that of nitroglycerin and a height 1.4% of that of nitroglycerin (at 254 nm, the butylated hydroxyanisole peak was 4.5% of that of nitroglycerin). This finding indicates that none of the excipients interferes with the method.

By combining individual weighings of the appropriate excipients, authentic mixtures were prepared and assayed (Table IV). The average recovery of 99.1% with a coefficient of variation of 1.14% indicates that the accuracy and reproducibility of the method are acceptable for the analysis of this type of product. With authentic mixtures, the method demonstrated linearity from 0.25 to 1.50 mg/ml, representing 25–150% of the label declaration of 2% nitroglycerin [correlation coefficient >0.9997 (14); levels measured were 0.25, 0.50, 0.75, 1.00, 1.25, and 1.50 mg/ml]. These results indicate that sample preparation Method 2a with detection at 215 nm is appropriate for the analysis of nitroglycerin ointments.

Preliminary testing indicates that the system can be used for the analysis of isosorbide dinitrate, pentaerythritol tetranitrate, and erythritol trinitrate, which are well resolved from one another and from nitroglycerin. With a 50:50 methanol–water mobile phase, $k'$ values of 6.7, 17, and 18.9 were obtained for these three glycosides, respectively; the $k'$ value of nitroglycerin was 9.5. The analysis of these cardiac glycosides in sustained-release preparations will be reported later.

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