High-Performance Liquid Chromatographic Determination of Nitroglycerin in Sublingual, Sustained-Release, and Ointment Dosage Forms

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Abstract □ A rapid, precise, sensitive, and specific assay for nitroglycerin in sublingual, sustained-release, and ointment dosage forms using high-performance liquid chromatography is described. The nitroglycerin dosage forms were dissolved in methanol, filtered, and injected directly into the liquid chromatograph. A variable-wavelength UV detector operated at 220 nm and a C_{18} microporous silica column were employed. The mobile phase was methanol–water (40:60).

Keyphrases □ Nitroglycerin—high-performance liquid chromatographic determination in sublingual, sustained-release, and ointment dosage forms □ Dosage forms—ointment, sublingual, sustained-release, high-performance liquid chromatographic determination of nitroglycerin □ High-performance liquid chromatography—determination of nitroglycerin in sublingual, sustained-release, and ointment dosage forms

Several techniques for the determination of nitroglycerin in commercial dosage forms have been reported (1–5). Some limitations of these methods include: the time-consuming and nonspecific determination of the compendial assay (1), the poor sensitivity and lack of precision of the IR method (2, 3), possible decomposition in the GC method (4), and the inability to distinguish among the decomposition products using the polarographic method (5).

High-performance liquid chromatography (HPLC) as a determinative technique appears to offer the best approach (6, 7). As reported here, it is both rapid and stability indicating. A single procedure is applicable to more than one dosage form, and individual tablets can be analyzed at low dosage levels. The latter is necessary when analyzing nitroglycerin products because nitroglycerin migrates from tablet to tablet on exposure to air (8, 9). The use of HPLC eliminates interferences from other organic or inorganic nitrates and allows nitroglycerin to be distinguished from its mono- and dinitrate degradation products.

EXPERIMENTAL

Reagents—HPLC-grade methanol[1] and water[1] were used. All other chemicals and solvents were reagent grade and were used without further purification. A 10% nitroglycerin in lactose triturate[2] was used as a reference standard and was assayed by the USP phenoldisulfonic acid method (1). Authentic 1,2- and 1,3-dinitroglycerin and 1- and 2-mononitroglycerin[3] were used to determine the presence of any degradation products; pentaerythritol tetranitrate[4] and isosorbide dinitrate[5] were used as internal standards.

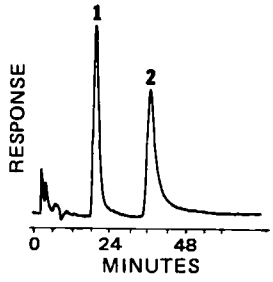

Figure 1—*Chromatogram of sublingual tablet sample. Key: (1) nitroglycerin; (2) pentaerythritol tetranitrate.*

Apparatus—The liquid chromatographic system consisted of a dual-head reciprocating piston positive-displacement pump[6], a septumless syringe-loaded loop injector with a 20-μl loop[7], a variable-wavelength UV detector[8] operated at 220 nm, a 10-mV recorder[9], and a 3.9 mm × 30-cm C_{18} microparticulate column[10] with a 4 cm × 4.6-mm guard column packed with pellicular octadecyl reverse-phase material[11]. The solvent system was methanol–water (40:60). The flow rate was 1 ml/min with a pressure of ~1600 psi.

Nitroglycerin Standard Solution—An accurately weighed quantity of nitroglycerin reference standard was dissolved in the internal standard solution (0.1 mg of pentaerythritol tetranitrate/ml of methanol) to obtain a concentration of ~0.075 mg/ml.

Extraction—*Sublingual Tablets*—Sufficient powdered tablet composite or individual powdered tablets (if individual tablet assays were required) were transferred to a 25-ml glass-stoppered Erlenmeyer flask and diluted with internal standard solution to give a nitroglycerin concentration of 0.075 mg/ml. The mixture was sonicated for 2 min then mechanically shaken for 30 min. The resulting solution was filtered through 0.7-μm filter paper[12] and injected into the chromatograph.

Ointment—Sufficient ointment was transferred to a 25-ml glass-stoppered Erlenmeyer flask and diluted with internal standard solution to give a nitroglycerin concentration of 0.075 mg/ml. The mixture was warmed in a 50° water bath and shaken intermittently until the ointment dispersed. The ointment solidified after being removed from the bath and shaken vigorously. The heating and shaking operation was then repeated. The solution was filtered through a 0.7-μm filter paper[12] and injected into the chromatograph.

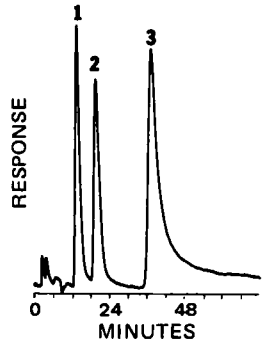

Figure 2—*Chromatogram of sustained-release tablet sample that contains phthalates. Key: (1) isosorbide dinitrate; (2) nitroglycerin; (3) phthalate excipient.*

[1] J. T. Baker Chemical Co., Phillipsburg, N.J.

[2] ICI Americas, Wilmington, Del.
[3] Arnar-Stone Labs, McGaw Park, Ill.
[4] Atlas Chemical Industries, Wilmington, Del.
[5] USP Reference Standard, United States Pharmacopeial Convention, Rockville, Md.
[6] Model 100A, Altex Scientific, Berkeley, Calif.
[7] Model 210 injector with 210-06 loop, Altex Scientific, Berkeley, Calif.
[8] Model 155-40, Altex Scientific, Berkeley, Calif.
[9] B-D-41, Kipp & Zonen, Delft, The Netherlands.
[10] μBondapak C_{18}, Waters Associates, Milford, Mass.
[11] μBondapak C_{18}/Corasil, Waters Associates, Milford, Mass.
[12] Gilman Instrument Co., Ann Arbor, Mich.

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Table I—Nitroglycerin Found in Sublingual, Sustained-Release, and Ointment Dosage Forms and in Synthetic Mixtures

Manufacturer	Tablet Concentration, mg	Found, %	Spike Added, mg	Spike Recovery, %	Synthetic Mixture Added, mg	Synthetic Mixture Recovery, %
			Sublingual Tablets[a]			
1	0.15	104.4	0.079	99.3	0.147	100.0
	0.30	108.7	0.193	100.7	0.302	100.3
	0.40	103.3	0.204	100.9	0.423	100.9
	0.60	108.7	0.335	99.7	0.615	100.9
2	0.15	98.0	0.081	99.3	0.146	99.3
	0.30	106.7	0.169	99.7	0.298	100.9
	0.40	102.3	0.192	101.0	0.421	100.9
	0.60	105.0	0.337	100.9	0.614	99.7
			Sustained-Release Tablets and Capsules[b]			
1	6.5	93.2	4.29	101.8	0.602	100.9
2	2.5	90.1	1.15	100.9	0.248	99.8
	6.5	94.8	3.10	101.8	0.500	100.5
	9.0	92.5	3.65	99.8	0.896	99.7
3	2.5	114.0	0.251	98.3	—[c]	—[c]
	6.5	106.5	0.643	99.4	—[c]	—[c]
4	2.6	91.0	0.243	97.2	—[c]	—[c]
	6.5	93.1	0.635	98.1	—[c]	—[c]
			2% Ointments[d]			
1		101.0	1.01	99.7	1.745	99.5
2		113.3	0.925	99.2	2.037	99.7
3		110.0	1.127	99.4	1.975	99.3

[a] Average recovery for all manufacturers, 100.2%; coefficient of variation, 0.753%; correlation coefficient, 0.9998. [b] Average recovery for all manufacturers, 99.4%; coefficient of variation, 1.83%; correlation coefficient, 0.9999. [c] Formulations not available for preparation of synthetic mixtures. [d] Average recovery for all manufacturers, 99.4%; coefficient of variation, 0.253%; correlation coefficient, 0.9997.

Sustained-Release Tablets or Capsules—This procedure required an alternate internal standard (isosorbide dinitrate) be used if phthalates were present as an excipient since phthalates have approximately the same retention time (R_t) as pentaerythritol tetranitrate under the conditions described. The presence of phthalates was detected by injecting a portion of sample dissolved in methanol into the chromatograph (a peak with the same R_t as that of pentaerythritol tetranitrate indicated the presence of phthalates). In such cases, isosorbide dinitrate (0.1 mg/ml of methanol) was used as the internal standard solution. Sufficient powdered sustained-release composite was transferred to a 25-ml glass-stoppered Erlenmeyer flask and diluted with the appropriate internal standard solution, giving a nitroglycerin concentration of 0.075 mg/ml. The mixture was sonicated for 2 min, then mechanically shaken for 30 min. The solution was filtered through 0.7-μm filter paper[12] and injected into the chromatograph.

Quantitation—The drug was quantitated by using the internal standard method, which compares peak height ratios. The internal standard for the sublingual tablets, ointments, and sustained-release dosage forms that did not contain phthalates was pentaerythritol tetranitrate; isosorbide dinitrate was used for the sustained-release dosage forms that contained phthalates.

RESULTS AND DISCUSSION

Figure 1 shows a typical chromatogram of 1.5 μg of nitroglycerin from a composite of sublingual tablets. Figure 2 shows a chromatogram of 1.5 μg of nitroglycerin from a composite of sustained-release tablets employing isosorbide dinitrate as the internal standard. Ointment and sustained-release capsules not containing phthalates gave chromatograms similar to those of the sublingual tablets because they contain no excipient interferences in the area of either nitroglycerin or pentaerythritol tetranitrate. The R_t with 40% methanol in water was ~12 min for isosorbide dinitrate, 18 min for nitroglycerin, and 32 min for pentaerythritol tetranitrate.

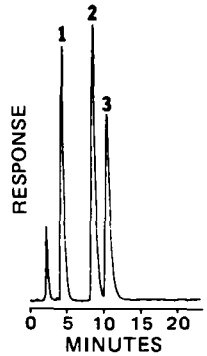

Figure 3—*Chromatogram of degradation products of nitroglycerin. Key: (1) 1- and 2-mononitroglycerin; (2) 1,3-dinitroglycerin; (3) 1,2-dinitroglycerin.*

During the development of this assay, a number of variations were tried. Methanol was chosen as the extracting solvent because of its ability to separate nitroglycerin and its mono- and dinitroglyceryl degradation products from the excipients in the various dosage forms. Methanol gave a cleaner solution than dimethyl sulfoxide; the latter dissolved the active ingredient as well as the excipients. Methanol and acetonitrile performed equally well as the mobile phase; methanol was chosen because it is less costly. Pentaerythritol tetranitrate was chosen as the internal standard because a number of samples contained excipients whose R_t value was the same or slightly different from that of isosorbide dinitrate, thereby causing peak distortion. One manufacturer's sustained-release tablets contained a phthalate with the same R_t as pentaerythritol tetranitrate, necessitating the use of isosorbide dinitrate as the internal standard.

The HPLC method described is capable of separating nitroglycerin from its mono- and dinitroglyceryl degradation products. Since these degradation products are more polar than nitroglycerin, they travel through the reverse-phase column faster and are eluted soon after the solvent front. To determine the degradation products, the methanol concentration of the mobile phase must be decreased, thereby increasing the R_t value of the other nitrate esters.

While detection at 254 nm may be possible when assaying nitroglycerin in a composite sample, it is not suitable for determining individual sublingual tablet uniformity because of the small quantity of active ingredient present in a single tablet. Detection at 220 nm gives a 60-fold enhancement in sensitivity with quantitative results comparable to those obtained at 254 nm, thus making individual tablet analysis possible.

The internal standard method of calculation was applied to the quantitative determination of nitroglycerin in the various dosage forms. The relative percentage of nitroglycerin found in the various dosage forms as well as the recovery data appear in Table I.

Synthetic mixtures (analogous to commercial formulations) were prepared by adding known amounts of the nitroglycerin triturate to the excipients according to the manufacturer's specifications. With these synthetic mixtures, the method demonstrated linearity from 0.05 to 1.5 mg/ml of original sample solution with a correlation coefficient >0.999 (10). Spiked samples of each dosage form were prepared using standard addition procedures. Weighed amounts of the triturate were added to previously assayed dosages containing the active ingredient. These preparations were then analyzed as described above (Table I).

This assay provides a rapid, sensitive, and specific method for the

determination of nitroglycerin in various dosage forms. The degradation products of nitroglycerin can be determined by a simple change of the mobile solvent system (methanol–water, 20:80) (Fig. 3). The assay is faster, more specific, and provides more stability-indicating information than the USP method.

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Hydrazine Levels in Formulations of Hydralazine, Isoniazid, and Phenelzine Over a 2-Year Period

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Abstract □ Hydrazine levels in formulations of hydralazine, isoniazid, and phenelzine have been measured over a 2-year period under ambient conditions and under temperature and humidity stress. Hydralazine tablets are stable under ambient conditions, but the hydrazine level in an injectable formulation increased from 4.5 to 10 μg/ml over a 23-month period. Isoniazid tablets are also stable, but hydrazine levels in an elixir and a pyridoxine combination product doubled to 44 μg/ml and 19 μg/tablet, respectively. Levels in phenelzine tablets appeared to remain constant at ~60 μg/tablet, with considerable tablet-to-tablet variation.

Keyphrases □ Hydrazine—levels in formulations of hydralazine, isoniazid, and phenelzine over a 2-year period □ Hydralazine—hydrazine levels in formulations over a 2-year period □ Isoniazid—hydrazine levels in formulations over a 2-year period □ Phenelzine—hydrazine levels in formulations over a 2-year period

Previous work demonstrated the presence of hydrazine in an isoniazid injectable product (1) and showed that isoniazid may hydrolyze to hydrazine (2, 3). Because hydrazine poses a risk of cancer in humans (4, 5), these observations prompted an assessment of other drugs derived from hydrazine that are available in Canada. These include carbidopa, hydralazine, isocarboxazid, and phenelzine, in addition to isoniazid. Hydrazine levels in isoniazid single-component tablet formulations were determined by TLC (2) and high-performance liquid chromatography (HPLC) (6) and by modifying a GLC procedure originally developed for the determination of phenelzine in urine (7). Methods for the determination of hydrazine in formulations of hydralazine (8), isoniazid elixir, isoniazid–pyridoxine combination tablets (8), and phenelzine (9) have also been developed. A 2-year normal and accelerated aging study of hydrazine formation in formulated products of hydralazine, isoniazid, and phenelzine has been completed and the results are reported in this paper.

Hydrazine is used in some syntheses of hydralazine (10), isoniazid (11), and phenelzine (12), and its presence in a formulated drug product may result from improper purification of the drug. All three drugs are known to degrade to hydrazine in solution (6, 9, 11), but there does not appear to be any published information on the formation of hydrazine in formulations of these drugs.

EXPERIMENTAL

Sample Preparation—Drug formulations were obtained directly from the manufacturer. All tablet samples were transferred to amber bottles for storage with at least five tablets in each bottle, and 1.0-ml aliquots of the elixir were transferred to culture tubes and securely capped. Phenelzine tablets from lot D were sealed in glass tubes (22 × 220 mm) by drawing out the top of the tube in a flame. The 100% relative humidity condition was achieved by sealing a 12-ml centrifuge tube containing 4 ml of water in with the tablets; tablets were not in direct contact with the water.

Storage Conditions—All formulations were aged under the temperature and humidity conditions given in Tables I, II, and III. Except as noted above, the humidity was controlled by placing silica gel (0% relative humidity) or an aqueous solution of sodium chloride (75 and 80% relative humidity) (13) in desiccators along with the product to be aged. The desiccators were placed in ovens[1] at the appropriate temperatures. Temperature variations were within 1.0° over the time of the experiments.

Procedure—All products were analyzed for hydrazine at the start of each study and at appropriate times thereafter. Assays were done in duplicate, usually on composites of five tablets, or composites of three ampules of an injectable or elixir or, for phenelzine, on single tablets. Hydrazine content (as the benzaldehyde derivative) in hydralazine (8), isoniazid–pyridoxine tablets, and isoniazid elixir (8) was determined by GLC using a 2% OV-101 column and a nitrogen–phosphorus detector. In phenelzine products and for some isoniazid analyses, hydrazine content (as the benzaldehyde derivative) was determined by HPLC (9) on a 5-μm silica gel column with a mobile phase of 6% chloroform in *n*-hexane and detection at 313 mm. In isoniazid single-component tablets after 2.5 and 6.8 months, hydrazine, as the acetone derivative, was determined by GLC on a 3% OV-225 column with a flame-ionization detector (7). The initial assessment of hydrazine in isoniazid tablets was made by TLC with

[1] Thelco Model 6 M and Freas Model 815, Precision Scientific.

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