High-Performance Liquid Chromatographic Determination of The Nitrate Esters Isosorbide Dinitrate, Pentaerythritol Tetranitrate, and Erythrityl Tetranitrate in Various Tablet Forms

CAROLYN S. OLSEN ˣ **and HENRY S. SCROGGINS**

Received April 11, 1983, from the *Food and Drug Administration, 4298 Elysian Fields Avenue, New Orleans, LA 70122.* Accepted for publication June 22, 1983.

Abstract □ A reliable, sensitive, and specific assay for isosorbide dinitrate, pentaerythritol tetranitrate, and erythrityl tetranitrate in sublingual, uncoated, sustained-release, and chewable dosage forms, using high-performance liquid chromatography, is described. The nitrate ester 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 reverse-phase C_{18} microporous silica column were employed. The mobile phase was methanol–water (40:60). The proposed method is quantitative and reproducible.

Keyphrases □ Isosorbide dinitrate—HPLC, tablet forms □ Pentaerythritol tetranitrate—HPLC, tablet forms □ Erythrityl tetranitrate—HPLC, tablet forms

Isosorbide dinitrate (I), pentaerythritol tetranitrate (II), and erythrityl tetranitrate (III) are members of a group of vasodilator drugs that are useful in the treatment of angina pectoris and ischemia of skeletal muscles. Since these nitrate esters have a slower onset of action and their duration is longer than nitroglycerin (IV), they are beneficial in the prevention of attacks, but not in the management of the acute attack (1).

Nitrate esters have been determined by polarographic (2–4), IR, spectrophotometric (5–8), and colorimetric (9–11) procedures. The basis for several procedures (12, 13) is nitration of phenoldisulfonic acid with the nitrate moiety of the ester and subsequent formation of a colored ion in a basic solution. These standard methods are time consuming and complex, and the colorimetric method is nonspecific for the active ingredient.

The objective of this investigation was to devise a simple, rapid, and specific procedure that would be applicable to the three nitrate esters cited above and to the majority of other nitrate esters. High-performance liquid chromatography (HPLC) as a determinative technique offered the best approach (14, 15). This straightforward procedure has already been successfully used to quantitatively determine IV (16, 17). This paper presents a method for the analysis of I, II, and III in various dosage forms by reverse-phase HPLC with IV as the internal standard. This method is simple, rapid, and reproducible.

EXPERIMENTAL SECTION

Reagents and Materials—HPLC-grade methanol[1] and water[1] were used. All other chemicals and solvents were reagent grade and were used without further purification. Ten percent of II[2], 10% of III[3], and 25% of I[4], all as lactose triturates, were used as reference standards; II and III were assayed by the USP phenoldisulfonic acid method (18). A 10% IV[5] triturate was used as an internal standard.

Instrumentation—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, and a 10-mV recorder[9]. A 30 cm × 3.9-mm i.d. 10-μm C_{18} microparticulate column[10] with a 4 cm × 4.6-mm guard column packed with pellicular octadecyl reverse-phase material[11] was used at ambient temperature. The mobile phase was methanol–water (40:60). The flow rate was 1 mL/min with a pressure of 1600 psi.

Solution Preparation—The internal standard, IV, was prepared as a 75-μg/mL solution in methanol and filtered through 0.7-μm filter paper[12]. To prepare a standard solution, 1 mg of I, 2 mg of II, or 3 mg of III was accurately weighed into a 50-mL glass-stoppered Erlenmeyer flask and diluted with 10.0 mL of internal standard solution. The desired solution was sonicated for 2 min, mechanically shaken for 30 min, and then filtered through 0.7-μm filter paper[12]. Samples of each drug were prepared by weighing and finely powdering 20 tablets. An accurately weighed portion of the powder, equivalent to 1 mg of I, 2 mg of II, or 3 mg of III, was transfered to a 50-mL glass-stoppered Erlenmeyer flask and diluted with 10.0 mL of internal standard solution,

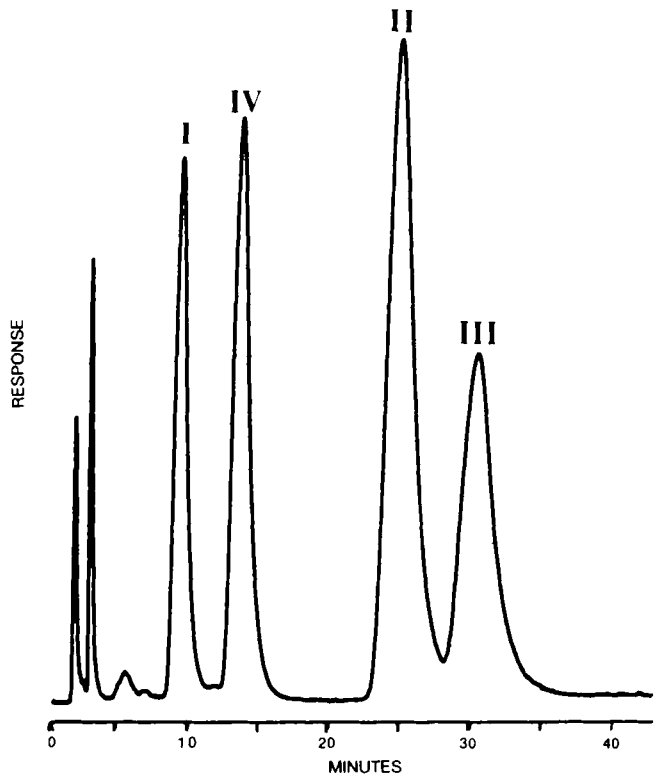

Figure 1—*Chromatogram of nitrate ester mixture. Key: (I) isosorbide dinitrate; (IV) nitroglycerin; (II) pentaerythritol tetranitrate; (III) erythrityl tetranitrate.*

[1] J. T. Baker Chemical Co., Phillipsburg, N.J.
[2] Atlas Chemical Industries, Wilmington, Del.
[3] Burroughs Wellcome, Research Triangle Park, N.C.
[4] USP Reference Standard, United States Pharmacopeial Convention, Rockville, Md.
[5] ICI Americas, Wilmington, Del.
[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] μ-Bondapak C_{18} (10 μm); Waters Associates, Milford, Mass.
[11] μ-Bondapak C_{18}/Corasil; Waters Associates, Milford, Mass.
[12] Gilman Instrument Co., Ann Arbor, Mich.

0022-3549/84/0900-1303$01.00/0

Table I—Assay Results for Tablet Forms

Dosage Form	Tablet Conc., mg	Found, mg	Found, %
	Isosorbide Dinitrate		
Sublingual	2.5	2.34	93.6
Sublingual	2.5	2.40	96.0
Sustained-release	40	40.57	101.4
Sustained-release	40	38.53	96.3
	Pentaerythritol Tetranitrate		
Uncoated	10	9.04	90.4
Uncoated	10	10.05	100.5
Uncoated	10	9.17	91.7
Sustained-release	80	84.00	105.0
	Erythrityl Tetranitrate		
Chewable	10	10.80	108.0
Sublingual	10	10.13	101.3
Sublingual	10	10.36	103.6

sonicated for 2 min, mechanically shaken for 30 min, and filtered through 0.7-μm filter paper[12].

Assay of Standard and Sample—Using the HPLC parameters described, three 20-μL portions of the standard and sample filtrates were chromatographed. The calculation of the peak area ratio, R, was determined from the area of I/the area of internal standard peak. The concentration of I (C) was then determined from:

$$C_{sample} = C_{standard} \times (R_{sample}/R_{standard})$$

The percent of I was determined from the percent of label = (sample concentration/theoretical sample concentration) × 100.

Precision and Accuracy Studies—Twenty tablets were weighed and triturated to a fine powder. From this powder, seven samples were weighed and treated as described above. Triplicate injections were made of each sample. The 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 (19).

RESULTS AND DISCUSSION

Figure 1 shows a typical chromatogram of a mixture of 2 μg of I, 4 μg of II, and 6 μg of III, using 1.5 μg of IV as the internal standard. The retention time with 40% methanol in water was ~10 min for I, 14 min for IV, 25 min for II, and 30 min for III.

During the development of this method, a number of variations were tried. Methanol was chosen as the extracting solvent because of its ability to solubilize the nitrate ester from its excipients in the various dosage forms. Methanol and acetonitrile performed equally well as the mobile phase; however, methanol was chosen because it is less costly. Compound IV was chosen as the internal standard because it does not interfere with the nitrate esters or any of the excipients present in the various dosage forms.

The various nitrate esters were separated by the HPLC method described. Detection with 220 nm gave twice the sensitivity with quantitative results compared with those obtained at 254 nm. Commercial preparations were analyzed by the proposed HPLC method. Results are given in Table I.

Quantitation was based on the nitrate ester-to-internal standard peak area ratio. Linearity was obtained between 0.2–40 μg of I, 0.4–50 μg of II, and 0.6–50 μg of III. The correlation coefficients were 0.9980, 0.9999, and 0.9999 for I, II, and III, respectively. The precision of the method showed an *RSD* of 1.56% ($n = 7$) (Table II). The overall percent recoveries (±*SD*) from the spiked sample mixtures were 100.8 (±1.4) for I, 101.1 (±0.5) for II, and 101.5 (±0.6) for III.

Table II—Precision of HPLC Assay of Isosorbide Dinitrate

Tablet	Absorbance[a]	Found, mg	Percent of Label Found
1	0.5500	2.43	97.2
2	0.5618	2.48	99.2
3	0.5694	2.51	100.4
4	0.5793	2.56	102.4
5	0.5657	2.50	100.4
6	0.5616	2.48	99.2
7	0.5656	2.50	100.0
Mean			99.8
SD (σ) =			1.56
RSD[b]			1.57%

[a] Factor = 4.4145. [b] *RSD* derived from 100 ($\sigma/\bar{x}$).

The HPLC assay provides a rapid, sensitive, and specific method for the determination of the nitrate esters in the various dosage forms. This method is faster and more specific than the present official methods.

REFERENCES

(1) P. Needleman and E. M. Johnson, Jr., in "The Pharmacological Basis of Therapeutics," L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N.Y., 1980, p. 819.

(2) "The United States Pharmacopeia," 20th rev., U.S. Pharmacopeial Convention, Rockville, Md., 1980, p. 434.

(3) G. C. Whitnack, J. N. Nielson, and E. St. Clair Gantz, *J. Am. Chem. Soc.*, **74**, 4997 (1952).

(4) G. C. Whitnack, M. M. Mayfield, and E. St. Clair Gantz, *Anal. Chem.*, **27**, 899 (1955).

(5) "Official Methods of Analysis of the Association of Official Analytical Chemists," 13th ed., Association of Official Analytical Chemists, Arlington, Va., 1980, p. 679.

(6) J. Carol. *J. Assoc. Offic. Agr. Chem.*, **43**, 259 (1960).

(7) J. Rosenstein, *J. Assoc. Offic. Agr. Chem.*, **47**, 469 (1964).

(8) H. L. Fung, P. Dalecki, E. Tse, and C. T. Rhodes, *J. Pharm. Sci.*, **62**, 696 (1973).

(9) "The United State Pharmacopeia," 20th rev., U.S. Pharmacopeial Convention, Rockville, Md., 1980, p. 288.

(10) "The United State Pharmacopeia," 20th rev., U.S. Pharmacopeial Convention, Rockville, Md., 1980, p. 597.

(11) F. K. Bell, *J. Pharm. Sci.*, **53**, 752 (1964).

(12) J. R. Hohmann and J. Levine, *J. Assoc. Offic. Agr. Chem.*, **47**, 471 (1964).

(13) E. Sarnoff, *J. Assoc. Offic. Agr. Chem.*, **39**, 630 (1956).

(14) W. G. Crouthamel and B. J. Dorsch, *J. Pharm. Sci.*, **68**, 237 (1979).

(15) D. M. Baaske, J. E. Carter, and A. H. Amann, *J. Pharm. Sci.*, **68**, 481 (1979).

(16) C. S. Olsen and H. S. Scroggins, *J. Pharm. Sci.*, **72**, 963 (1983).

(17) L. Gelber, *J. Pharm. Sci.*, **69**, 1084 (1980).

(18) "The United States Pharmacopeia," 20th rev., U.S. Pharmacopeial Convention, Rockville, Md., 1980, p. 552.

(19) C. Ko, F. Marzian, and C. Janicki, *J. Pharm. Sci.*, **69**, 1081 (1980).