Validation of High-Performance Liquid Chromatographic Methods for Analysis of Sustained-Release Preparations Containing Nitroglycerin, Isosorbide Dinitrate, or Pentaerythritol Tetranitrate

LOREN GELBER[x] and ANDREW N. PAPAS

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Abstract □ The assay of sustained-release tablets or capsules containing nitroglycerin, isosorbide dinitrate, or pentaerythritol tetranitrate by high-performance liquid chromatography is described. Acetonitrile was found to be the sample preparation solvent with the most general applicability to these products. Anisole was used as an internal standard for nitroglycerin and pentaerythritol tetranitrate, while 4-chloroacetanilide was used for isosorbide dinitrate. The method, which uses a C_{18} bonded-phase column, a methanol–water mobile phase, and 214-nm detection, was shown to be accurate, linear, and reproducible.

Keyphrases □ High-performance liquid chromatography—analysis of sustained-release preparations containing nitroglycerin, isosorbide dinitrate, or pentaerythritol tetranitrate □ Sustained-release preparations—high-performance liquid chromatographic analysis of preparations containing nitroglycerin, isosorbide dinitrate, or pentaerythritol tetranitrate □ Nitroglycerin—high-performance liquid chromatographic analysis of sustained-release preparation □ Isosorbide dinitrate—high-performance liquid chromatographic analysis of sustained-release preparation □ Pentaerythritol tetranitrate—high-performance liquid chromatographic analysis of sustained-release preparation

Recently, the analysis of nitroglycerin ointment was reported by this laboratory (1). Nitroglycerin and the related drugs, isosorbide dinitrate and pentaerythritol tetranitrate, are also formulated as sustained-release capsules and tablets. Pharmaceutical glaze and/or various cellulosic polymers are used to delay release of the active ingredient. Sustained-release nitroglycerin tablet analysis by column partition chromatography (2), sustained-release isosorbide dinitrate by GLC (3), and sustained-release pentaerythritol tetranitrate analysis by color development with phenoldisulfonic acid (4) have been reported. However, high-performance liquid chromatography (HPLC) has advantages over all of these, since it is less time consuming than column chromatography, more specific than phenoldisulfonic acid, and does not subject the samples to heat, which may cause thermal decomposition (5).

The chromatography used for nitroglycerin ointment was based on a previously described method (6) and was adapted to the sustained-release preparations by varying the methanol content of the mobile phase to achieve the desired separation. A study was made of the ability of various solvents to extract the drug from the sustained-release matrix, since this is the most critical step in the analysis.

EXPERIMENTAL

Materials—The water[1] and acetonitrile[2] used were HPLC grade; methanol[3] was distilled in glass; dimethyl sulfoxide, dicalcium phosphate, calcium sulfate, stearic acid, and ferric oxide[4] were ACS reagent grade; and magnesium stearate[4] was laboratory grade. Intestinal fluid was simulated intestinal fluid T.S. (7) without enzymes. Ethyl cellulose, carnauba wax[4], povidone[5], talc[6], colloidal silica[7], pharmaceutical glaze[8], nonpareil seeds[9], starch[10], lactose, polyethylene glycol, guar gum[11], carboxypolymethylene, hydrogenated vegetable oil[12], hydroxymethylpropylcellulose[13], zein[14], microcrystalline cellulose[15], sodium starch glycolate[16], dried malt syrup[17], FD&C blue No. 1, FD&C yellow No. 5, and D&C yellow No. 10[18] were used as received without further purification.

Standard nitroglycerin was as described previously (1), standard isosorbide dinitrate triturate[19] assayed at 25.06% by the compendial method (8), and standard pentaerythritol tetranitrate triturate[20] assayed at 18.52% by the compendial method (9).

Instrumentation—Instrumentation was as reported previously (1). For Table I, HPLC column 1 was μBondapak C_{18}[21] and column 2 was Ultrasphere ODS[22]. In addition, for analysis of authentic mixtures an electronic integrator[23] was used.

Chromatographic Conditions—Typical chromatographic parameters [calculated as reported previously (1)] are summarized in Table I.

Procedure—Twenty tablets or the pellet contents of 20 capsules from the sample to be analyzed were accurately weighed and ground until just reduced to a fine powder. An appropriate weight of composite was transferred to a glass-stoppered Erlenmeyer flask or centrifuge tube and 10.0 or 20.0 ml of the solvent being tested, containing internal standard (except as noted) was added. Flasks or tubes were stoppered, sealed with paraffin film and vigorously shaken with a wrist-action mechanical shaker for 1 hr. The following concentrations were used: nitroglycerin, 0.1 mg/ml in intestinal fluid, 1 mg/ml in dimethyl sulfoxide, and 0.5 mg/ml in acetonitrile; isosorbide dinitrate, 0.25 mg/ml in water, 0.5 mg/ml in dimethyl sulfoxide, and 0.5 mg/ml in acetonitrile; pentaerythritol tetranitrate, 1 mg/ml in dimethyl sulfoxide, and 0.5 mg/ml in acetonitrile. For nitroglycerin and pentaerythritol tetranitrate, anisole, as reported previously (1), was used as an internal standard, except for the intestinal fluid trials, where no internal standard was used. For isosorbide dinitrate, 4-chloroacetanilide was used as an internal standard in all solvents. Internal standards were selected for suitable retention parameters; it was not considered desirable to use compounds of similar chemistry as internal standards due to the difficulties in handling polynitro compounds. The solutions were filtered[24] except for dimethyl sulfoxide solutions, which were centrifuged. The clarified solutions were injected onto the liquid chromatograph. All detection was at 214 nm.

[1] J. T. Baker, Phillipsburg, N.J.
[2] Fisher Scientific Co., Fairlawn, N.J.
[3] Burdick and Jackson Laboratories Inc., Muskegon, Mich.
[4] Pfaltz and Bauer Inc., Stamford, Conn.
[5] GAF Corp., New York, N.Y.
[6] Whittaker, Clark and Daniels Inc., Plainfield, N.J.
[7] Syloid 244, W. R. Grace and Co., Davison Chemical Division, Baltimore, Md. and Cab-o-sil, Cabot Corp., Cab-o-sil Division, Boston, Mass.
[8] William Zinser and Co. Inc., Somerset, N.J.
[9] SCM SnowCrest, Salem, Mass.
[10] Colorcon Inc., West Point, Pa.
[11] Sigma Chemical Co., St. Louis, Mo.
[12] Bolar Pharmaceutical Co., Copaigue, N.Y.
[13] Forest Labs, New York, N.Y.
[14] Eastman Kodak Co., Rochester, N.Y.
[15] Avicel pH 101, FMC Corp., Philadelphia, Pa.
[16] Explotab, Generichem Corp., Little Falls N.J.
[17] Stanofill, Standard Brands, New York, N.Y.
[18] Division of Colors, Bureau of Foods, Food and Drug Administration, Washington, D.C.
[19] Barr Laboratories, Northvale, N.C.
[20] Bolar Pharmaceutical Company, Copaigue, N.Y.
[21] Waters Associates, Milford, Mass.
[22] Altex Scientific Inc., Berkeley, Calif.
[23] Model 3390A, Hewlett-Packard, Avondale, Pa.
[24] Grade 1, Whatman Ltd., England, followed by AAWP or FHLP, Millipore Corp., Bedford, Mass.

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Table I—Typical Chromatographic Parameters

Compound	Sample Solvent[a]	Mobile Phase Percent Methanol	k'	Plate Count	Resolution
Nitroglycerin	Dimethyl sulfoxide	50	5.1	2240	1.95
Anisole	Dimethyl sulfoxide	50	6.0	2850	1.95
Nitroglycerin	Acetonitrile	60	2.6	1745	3.8
Anisole	Acetonitrile	60	3.6	2570	3.8
Isosorbide dinitrate	Dimethyl sulfoxide	40	4.9	1370	3.9
4-Chloroacetanilide	Dimethyl sulfoxide	40	7.7	1140	3.9
Isosorbide dinitrate	Acetonitrile	55	2.8	1270	2.3
4-Chloroacetanilide	Acetonitrile	55	3.7	1290	2.3
Pentaerythritol tetranitrate	Dimethyl sulfoxide	50	7.4	1770	3.9
Anisole	Dimethyl sulfoxide	50	5.2	840	3.9
Pentaerythritol tetranitrate	Acetonitrile	60	5.5	1725	1.8
Anisole	Acetonitrile	60	4.6	2130	1.8

[a] Dimethyl sulfoxide sample solvent runs on column 1, acetonitrile sample solvent runs on column 2.

Table II—Comparison of Sample Preparation Solvents for Nitroglycerin

Manufacturer	Dosage Form	Intestinal Fluid (pH 8)		Dimethyl Sulfoxide		Acetonitrile	
		A[a]	R[b]	A	R	A	R
1	6.5-mg capsule	n.d.[c]	n.d.	100.0	100.5	103.4	99.4
	2.5-mg capsule	83.8	n.d.	92.9	99.3	90.0	102.7
2	6.5-mg capsule	107.2	98.8	98.2	102.5	94.5	97.7
	2.5-mg capsule	92.6	110.4	n.d.	n.d.	94.4	101.2
3	6.5-mg tablet	n.d.	n.d.	86.8	119.4	116.9	102.4
4	2.6-mg tablet	n.d.	n.d.	84.0	94.7	79.8	99.3

[a] A is the percent of the declared assay. [b] R is the percent recovery from the spiked sample. [c] n.d. is not determined.

Table III—Comparison of Sample Preparation Solvents for Isosorbide Dinitrate

Manufacturer	Dosage Form	Water		Dimethyl Sulfoxide		Acetonitrile	
		A[a]	R[b]	A	R	A	R
3	40-mg tablet	105.0	104.8	n.d.[c]	n.d.	100.6	96.3
5	40-mg tablet	87.7	97.2	98.6	97.4	99.7	99.4
6	40-mg tablet	101.0	101.0	n.d.	n.d.	94.6	99.8
7	40-mg tablet	73.5	88.6	96.9	98.3	99.8	98.2

[a] A is the percent of the declared assay. [b] R is the percent recovery from spiked sample. [c] n.d. is not determined.

Spiked samples were prepared from one-half the appropriate composite weight and one-half the appropriate standard weight and treated as described. Authentic samples were prepared by combining individual weighings of the appropriate excipients and standards and analyzing them according to the method.

RESULTS AND DISCUSSION

The results of solvent comparison (reported as the average of two determinations) are presented in Tables II–IV. Solvents were selected to span a range of polarities consistent with the solubility properties of the analytes and compatability with reverse-phase HPLC. Preliminary trials with methanol and water–methanol gave poor recoveries for isosorbide dinitrate. Since a single sample solvent for all products was considered desirable, no further consideration was given to methanol. The data indicate that acetonitrile is the solvent with the most general applicability to these products. Aqueous solvents gave good extraction of the active ingredient in only about one-half of the cases in which they were tried. In addition, an interfering excipient peak was observed when 2.5-mg nitroglycerin capsules from manufacturer No. 2 were prepared with intestinal fluid. Since pentaerythritol tetranitrate is practically insoluble in water (8) aqueous extraction was not attempted for this drug. Due to their solubility in dimethyl sulfoxide, cellulose derivative excipients caused interference (a broad peak between dimethyl sulfoxide and nitroglycerin in samples from manufacturer No. 3) when dimethyl sulfoxide was the solvent. Acetonitrile has further advantages over dimethyl sulfoxide, since it can be filtered through nylon or perfluoroelastomer membranes and does not give a large solvent peak, allowing a more polar mobile phase to be used. The most serious disadvantage of dimethyl sulfoxide is that the solvent peak obscures the presence of any incompletely nitrated impurities, which are reported to elute before the fully nitrated product (6). These impurities can be detected if acetonitrile is the extracting solvent.

Since acetonitrile was the best solvent, the methods in which it was used were subjected to further validation. Authentic mixtures containing the excipients and drug expected in these products at the levels most likely to be used in commercial formulations were analyzed. In the case of manufacturers No. 1 and No. 2, where two potency levels were under consideration, authentic samples were prepared corresponding to the lower potency. Since the formulations of the two potencies are similar, the lower potency is more likely to reveal any possible interferences. For nitroglycerin the excipients used were nonpareil seeds, talc, colloidal silica, povidone, pharmaceutical glaze, magnesium stearate, dicalcium phosphate, ferric oxide, ethyl cellulose, hydroxymethylpropylcellulose, and D&C yellow No. 10. The excipients used for isosorbide dinitrate were starch, lactose, talc, pharmaceutical glaze, ethylcellulose carboxypolymethylene, magnesium stearate, stearic acid, colloidal silica, hydroxymethylpropylcellulose, hydrogenated vegetable oil, povidone, FD&C blue No. 1, and FD&C yellow No. 5. For pentaerythritol tetranitrate the excipients studied were carnauba wax, hydrogenated vegetable oil, zein,

Table IV—Comparison of Sample Preparation Solvents for Pentaerythritol Tetranitrate

Manufacturer	Dosage Form	Dimethyl Sulfoxide		Acetonitrile	
		A[a]	R[b]	A	R
6	80-mg tablet	100.2	100.8	104.1	102.1
7	80-mg capsule	101.3	101.6	107.2	101.5
8	80-mg tablet	104.6	100.0	107.0	101.8

[a] A is the percent of the declared assay. [b] R is the percent recovery from spiked sample. [c] n.d. is not determined.

Table V—Authentic Recoveries, Acetonitrile Sample Preparation Solvent

Formulation Type[a]	Recovery of Label Claim, %	
Nitroglycerin		
1	97.5	100.8
2	101.1	100.5
3	100.3	100.4
4	98.3	101.8
5	101.3	99.7
	average	100.1
	CV[b] 1.25%	
Isosorbide Dinitrate		
3	100.6	101.6
5	97.8	98.3
6	102.0	100.6
7	100.0	99.2
	average	100.0
	CV 1.50%	
Pentaerythritol Tetranitrate		
6	99.0	99.2
7	99.9	100.1
8	98.2	99.8
	average	99.4
	CV 0.72%	

[a] Formulation types correspond by number to manufacturer numbers in previous tables. [b] Coefficient of variation.

magnesium stearate, talc, dried malt syrup, microcrystalling cellulose, sodium starch glycolate, calcium sulfate, stearic acid, starch, colloidal silica, lactose, polyethylene glycol, guar gum, FD&C blue No. 1 and FD&C yellow No. 5. Placebo mixtures without active ingredient or internal standard gave no peaks past the solvent front. Excipient peaks would not interfere with the detection of incompletely nitrated impurities.

The results of assays of authentic mixtures containing active ingredient are given in Table V. The average recoveries of 99.4–100.1% with 0.72–1.50% *CV* indicate that the accuracy and reproducibility of the method are acceptable for the analysis of this type of product. The results of the spiked authentic samples demonstrate that 60-min shaking time is adequate to extract the drug. Since this is compatible with requirements of chromatograph start-up and equilibration, shorter times were not investigated.

Linearity was tested using authentic mixtures. For nitroglycerin linearity was demonstrated from 0.2 to 0.8 mg/ml, representing 40–160% of label declaration. A correlation coefficient of 0.9990 was obtained (10) from the analysis of 0.2-, 0.4-, 0.5-, 0.6-, and 0.8-mg/ml samples. For isosorbide dinitrate linearity was demonstrated from 0.27 to 0.8 mg/ml, representing 54–160% of label declaration. A correlation coefficient of 0.9998 was obtained (10) by measuring 0.27, 0.45, 0.55, 0.63, and 0.80 mg/ml. For pentaerythritol tetranitrate linearity was demonstrated from 0.5 to 1.5 mg/ml, representing 50–150% of label declaration. A correlation coefficient of 0.9999 was obtained (10) by measuring 0.5, 0.78, 1.0, 1.24, and 1.5 mg/ml.

It appears that reverse-phase HPLC, after sample preparation with acetonitrile, is a useful method for the analysis of nitroglycerin, isosorbide dinitrate, and pentaerythritol tetranitrate sustained-release tablets and capsules. Since nitroglycerin hydrolysis products were recently demonstrated to elute substantially prior to nitroglycerin in this system (11), the method is anticipated to be stability indicating for nitroglycerin.

REFERENCES

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

(2) A. M. Soeterboet and M. VanThiel, *Pharm. Weekbl.*, **110**, 169 (1975).

(3) D. G. Prue, R. N. Johnson, and B. T. Kho, *J. Assoc. Off. Anal. Chem.*, **60**, 1341 (1977).

(4) V. D. Gupta, *J. Pharm. Sci.*, **67**, 717 (1978).

(5) P. Vouros, B. A. Petersen, L. Colwell, and B. L. Karger, *Anal. Chem.*, **49**, 1039 (1977).

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

(7) "The United States Pharmacopeia," 19th rev., U.S. Pharmacopeial Convention, Rockville, Md., 1975, p. 765.

(8) *Idem.*, p. 276.

(9) "The National Formulary," 14th ed., U.S. Pharmacopeial Convention, Rockville, Md., 1975, p. 539.

(10) R. G. D. Steel and J. H. Torrie, "Principles and Procedures of Statistics," McGraw-Hill, New York, N.Y., 1960, p. 188.

(11) C. C. Wu, T. D. Sokoloski, A. M. Burkman, and L. S. Wu, *J. Chromatogr.*, **216**, 1981, p. 239.

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Titrimetric Determination of Ascorbic Acid with 2,6-Dichlorophenol Indophenol in Commercial Liquid Diets

DAVID EMLYN HUGHES

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Abstract □ The titrimetric determination of ascorbic acid in the presence of a variety of potentially physically and chemically interfering species in commercial liquid diets is presented. The titrant and indicator was a solution of 2,6-dichlorophenol indophenol. Iron(II), copper(II), cysteine, glutathione, sulfite, and tin(II) do not interfere.

Keyphrases □ Ascorbic acid—titrimetric determination with 2,6-dichlorophenol indophenol in commercial liquid diets □ 2,6-Dichlorophenol indophenol—titrimetric determination of ascorbic acid in commercial liquid diets □ Titrimetric determination—ascorbic acid determination with 2,6-dichlorophenol indophenol in commercial liquid diets

Ascorbic acid frequently appears in multicomponent media, except in simple synthetic preparations such as ascorbic acid injection (1). In commercial liquid diets, for example, it is accompanied by proteins, amino acids, saccharides, lipids, and minerals (2). Methods involving oxidation of ascorbic acid are complicated by oxidizable metal ions, notably iron(II) and tin(II) (3).

The titrimetric oxidation of the two enolic groups of

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