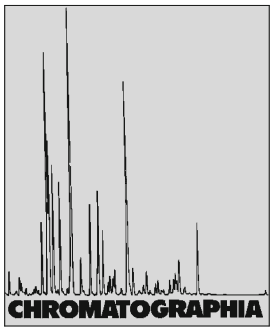
LC Simultaneous Determination of Thioctic Acid, Benfotiamine and Cyanocobalamin in Thiotacid Compound Capsules



2010, *72*, 327–330

Hesham Salem✉

Analytical Chemistry Department, Faculty of Pharmacy, October University for Modern Science and Arts (MSA University), October 6, Egypt; E-Mail: h_salem_eg@yahoo.com

Received: 10 January 2010 / Revised: 18 April 2010 / Accepted: 1 May 2010
Online publication: 3 June 2010

Abstract

A simple, selective, sensitive, precise, simultaneous liquid chromatographic analysis of capsules containing thioctic acid, benfotiamine and cyanocobalamin was described. Good chromatographic separation was achieved using a Zorbax C18 (4.6 cm × 250 mm, 5 μm) and a mobile phase consisting of acetonitrile–phosphate buffer pH 3.5 (15:85, *v/v*) at a flow rate of 0.9 mL min^{-1}. The ultraviolet detector was set a wavelength of 280 nm. Thioctic acid, benfotiamine and cyanocobalamin were eluted at 2.869, 3.752 and 13.689 min, respectively. The linear ranges for thioctic acid, benfotiamine and cyanocobalamin were 30–180, 4–24 and 0.025–0.150 μg mL^{-1}, respectively. The recoveries of thioctic acid, benfotiamine and cyanocobalamine in pharmaceutical preparation were all greater than 98% and their relative standard deviations were less than 2.0%. The limits of detection were 2.57, 0.19 and 0.003 μg mL^{-1} for thioctic acid, benfotiamine and cyanocobalamin, respectively.

Keywords

Column liquid chromatography
Polyneuropathy medicaments

Introduction

Thioctic acid, benfotiamine and cyanocobalamin are formulated together in capsules known as Thiotacid Compound capsules for diabetic polyneuropathy, diabetic retinopathy, neuritis, polyneuritis and neurodegenerative diseases.

Thioctic acid, 1,2-dithiolane-3-pentanoic acid, is used in the treatment of alcoholic liver disease, mushroom poisoning, heavy metal poisoning and glaucoma [1, 2]. Currently various methods, such as spectrophotometry [3–5], spectrofluorometry [6], gas chromatography-mass spectroscopy [7], liquid chromatography [8–11], electrochemistry [12, 13] and capillary electrophoresis [14] were reported for the determination of thioctic acid.

Benfotiamine, *N*-((4-amino-2-methyl-5-pyrimidinyl)methyl)-*N*-(4-hydroxy-2-mercapto-1-methyl-1-butenyl)formamide-*S*-benzoate dihydrogen phosphate. Benfotiamine's superior ability to penetrate cell membranes increases its bioavailability over conventional thiamin supplements. This allows for the use of benfotiamine to elicit all of the benefits of vitamin B1, such as the metabolism of carbohydrates, protein and fat, in a highly efficient manner [15].The literature survey reveals only pharmacological methods for the utility of benfotiamine [16, 17].

Cyanocobalamin, cobinamide cyanide phosphate 3-ester with 5, 6-dimethyl-1-α-D-ribofuranosylbenzimideazole inner salt, is essential to growth, cell reproduction, hematopoiesis, and nucleoprotein and myelin synthesis [1]. The reported methods for the determination of cyanocobalamin include spectrophotometry [18–20], spectrofluorometry [21, 22], voltametry [23] and liquid chromatography [24–32].

The article describes the development and validation of a simultaneous method for the LC-determination of thioctic acid, benfotiamine and cyanocobalamin in capsules.

Full Short Communication
DOI: 10.1365/s10337-010-1635-5
0009-5893/10/08

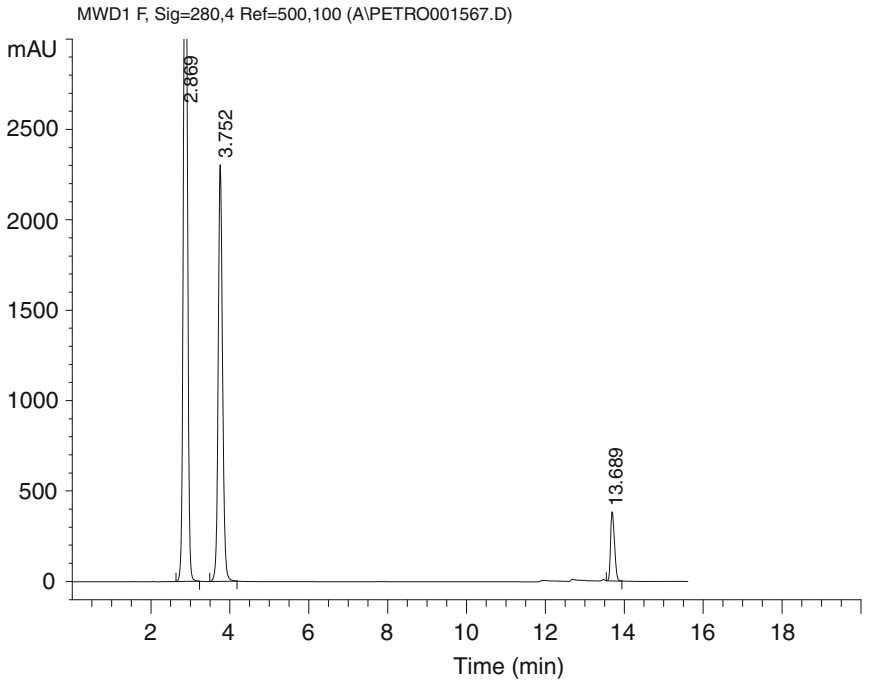


Fig. 1. Separation of thioctic acid, benfotiamine and cyanocobalamin at 2.869, 3.752 and 13.689 min, respectively, in Thiotacid Compound capsules

Experimental

Equipments

Agilent 1200 series, vacuum degasser, thermos tatted column compartment G1316A/G1316B, diode array and multiple wavelength detector SL, quaternary pump (Germany).

Chemicals

Thioctic acid, benfotiamine and cyanocobalamine were purchased from Eva Pharma for Pharmaceuticals & Medical Appliances, Cairo, Egypt. LC grade acetonitrile was purchased from Merck (Germany).

Pharmaceutical Preparation

Thiotacid Compound capsules; B.N. 24558 (Eva Pharma for Pharmaceuticals & Medical Appliances, Egypt), labeled to contain 300 mg thioctic acid, 40 mg benfotiamine and 250 mcg cyanocobalamin per tablet.

LC Procedure

Chromatographic Conditions

The analytical column was a Zorbax C18 (4.6 cm × 250 mm, 5 μm) and a mobile phase consisting of acetonitrile and phosphate buffer pH 3.5 (15:85, *v*/*v*) at a flow rate of 0.9 mL min^{-1} and at room temperature. The ultraviolet detector was set at a wavelength of 280 nm. Solutions and mobile phase were freshly prepared at the time of use.

Standard Solution Preparation

Stock solutions of thioctic acid, benfotiamine and cyanocobalamin were prepared daily by dissolving the appropriate amount of drug standards in the mobile phase to yield a final concentration of 3.0, 0.4 and 0.0025 mg mL^{-1}, respectively. Separate stock solutions were prepared for the calibration standards and quality control samples. Further, solutions were obtained by serial dilutions of stock solutions with mobile phase.

Preparation of Pharmaceutical Dosage Sample

The contents of 20 capsules were individually weighed, mixed and finely powdered in a mortar. Portions of the powder equivalent to 300 mg of thioctic acid, 40 mg of benfotiamine and 0.025 mg of cyanocobalamin were accurately weighed and diluted with mobile phase to get the final concentration of 30, 4 and 0.025 μg mL^{-1}, respectively.

Results and Discussion

Chromatograms of Samples

The aim of this research was to develop a new, simple, accurate, reproducible, sensitive LC method for the simultaneous determination of thioctic acid, benfotiamine and cyanocobalamin in pharmaceutical dosage form. A satisfactory separation of each drug from pharmaceutical excipients was obtained. To optimize the appropriate LC conditions for separation of the examined drugs, various reversed-phase columns, isocratic and gradient mobile phase systems were tried. The optimum wavelength for detection was 280 nm at which much better detector responses for the three drugs were obtained. The mobile phase was found to be suitable to improve the sharpness and thinness of thioctic acid, benfotiamine and cyanocobalamin peaks. The retention times for the investigated drugs were found to be 2.869 min (thioctic acid), 3.752 (cyanocobalamin) and 13.689 min (benfotiamine). No pharmaceutical excipients eluted at retention times of the peaks of interest (Fig. 1).

Calibration and Linearity

Calibration curves were constructed in the ranges of 30–180, 4–24 and 0.025–0.15 μg mL^{-1} for thioctic acid, benfotiamine and cyanocobalamin, respectively. The slope, intercept and regression coefficient for each compound were estimated.

Table 1. Statistical analysis of the results of authentic thioctic acid, benfotiamine and cyanocobalamin compared with official methods

	Thioctic acid		Benfotiamine		Cyanocobalamin	
	Official method [11]	Proposed method	A_{max} method	Proposed method	Official method [11]	Proposed method
X	100.06	100.07	100.14	100.0	99.52	100.15
N	5	6	5	6	5	6
±SD	0.74	0.63	0.35	0.40	1.76	1.72
RSD%	0.74	0.63	0.35	0.40	1.76	1.72
t		0.016		0.610		0.598
F		1.361		1.301		1.05

Table 2. Determination of thioctic acid, benfotiamine and cyanocobalamin in Thiotacid® Compound capsules

Thioctic acid			Benfotiamine			Cyanocobalamin		
Taken (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)	Taken (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)	Taken (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)
30	30.1	100.4	4	4.09	102.2	0.025	0.0252	100.9
60	59.9	99.2	8	8.04	100.5	0.050	0.0489	97.8
90	89.0	98.9	12	12.13	101.1	0.075	0.0759	101.3
120	120.3	100.3	16	15.81	98.8	0.100	0.0986	98.7
150	151.4	100.9	20	20.06	100.3	0.125	0.1250	100.0
180	179.1	99.6	24	23.98	99.9	0.150	0.1499	99.9
X		99.98			100.47			99.77
±SD		0.77			1.14			1.32
RSD%		0.77			1.14			1.32

Table 3. Reproducibility and precision

Injected amount (μg)	Intra-day (n = 4–5)			Inter-day (n = 5)		
	Observed amount, (μg) ± SD	RSD (%)	Accuracy (%)	Observed amount, (μg) ± SD	RSD (%)	Accuracy (%)
Thioctic acid						
30	30.33 ± 0.19	0.98	101.1	30.05	0.87	100.17
180	179.83 ± 0.32	1.28	99.91	179.91	1.17	99.95
Benfotiamine						
4	3.99 ± 0.38	0.84	99.75	4.02 ± 0.28	1.21	100.5
24	23.89 ± 0.89	1.12	99.54	24.21 ± 0.29	0.93	100.88
Cyanocobalamine						
0.025	0.0251 ± 1.19	1.29	100.4	0.0248 ± 0.73	1.82	99.20
0.15	0.152 ± 0.58	1.33	101.33	0.147 ± 1.12	1.32	98.00

Accuracy

Absolute recoveries of six different authentic concentrations of thioctic acid, benfotiamine and cyanocobalamin (Table 1) and the studied drugs in capsules (Table 2) were determined by assaying the samples as described above. Mean recoveries, standard deviations and the relative standard deviations were calculated by the standard method (Tables 1 and 2).

Precision

Intra-day precisions were assessed injecting standard solution four to five times during a day (this solution was extracted via the same procedure as the capsules) of each analyte at two different concentrations (low and high concentration). The resultant standard deviations were less than 2% for all (Table 3). Inter-day precision experiments were done after treatment of the standard solution in the same method of the capsule extraction, and then analyzed every day over 5 days (Table 3). All RSD% were lower than 2%.

Method Validated

The method was validated with regard to specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy and robustness.

Peak areas of thioctic acid, benfotiamine and cyanocobalamin of calibration standards were proportional to the concentration in serum and dosage forms over the ranges tested 30–180, 4–24 and 0.025–0.15 μg mL^{-1}, respectively. Each concentration was tested in triplicate. The slope values for thioctic, benfotiamine and cyanocobalamin were calculated as 4.937, 8.256 and 4505.0, respectively, with intercept values of 42.627, −3.440 and −7.173, respectively. The standard deviations of slope were calculated as 0.03297, 0.03110 and 48.544, respectively, and similarly standard deviations of intercept were calculated as 3.852, 0.4845 and 4.726, respectively, for thioctic acid, benfotiamine and cyanocobalamin. The calibration curves were fitted by linear least-square regression and showed correlation coefficients of not less than 0.9998.

The LODs and LOQs of thioctic acid, benfotiamine and cyanocobalamin were calculated on the peak area using the following equations: $LOD = 3.3 \times \sigma/S$, $LOQ = 10 \times \sigma/S$, where σ, is the standard deviation of the intercept of regression line of the drugs and S is the slope of the corresponding calibration curve [33]. The LODs of thioctic acid, benfotiamine and cyanocobalamin were found to be 2.57, 0.19 and 0.003 μg mL^{-1} and the LOQs were 7.80, 0.59 and 0.010 μg mL^{-1} for thioctic acid, benfotiamine and cyanocobalamin, respectively. Determination of authentic samples of thioctic acid, benfotiamine and cyanocobalamin and statistical analysis of the results obtained for the proposed method (Table 1); show that all the suggested measurements were equally precise and accurate to the official methods [11] for thioctic acid and cyanocobalamin and to A_{max} method for benfotiamine (Table 1).

Application to Pharmaceutical Dosage Form

The proposed method was successfully applied for the simultaneous determination of thioctic acid, benfotiamine and cyanocobalamin in Thiotacid Compound capsules without interference of the excipients present and without prior separation (Table 2). The utility of the method was also verified by applying the standard addition technique.

Conclusions

The chromatographic method described is adequate for quantitation of thioctic acid, benfotiamine and cyanocobalamin in pharmaceutical dosage forms at different concentration levels. It is very simple, accurate and effective and provided no interference peaks for endogenous components and pharmaceutical excipients. Acceptable values of precision and accuracy have been obtained for all levels by this method regarding the guidelines for assay validation. The separation of these drugs takes 13.68 min in one chromatogram, so a large number of samples can be analyzed in a short period of time. The method uses simple mobile phase and is very beneficial for column life. In summary, the method can be successfully applied to samples of pharmaceutical dosage form.

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