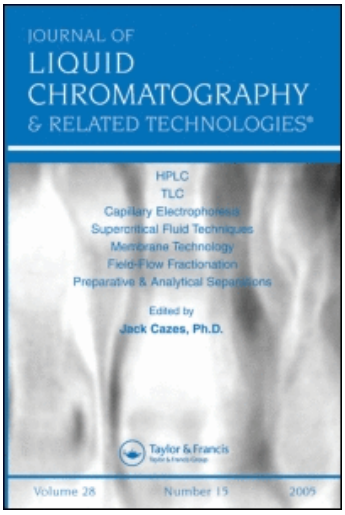

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NORMAL-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD WITH DANSYLATION FOR THE ASSAY OF PIPERAZINE CITRATE IN DOSAGE FORMS

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ABSTRACT

The assay of the anthelmintic agent piperazine citrate in dosage forms has been accomplished by normal-phase HPLC after converting the drug to a UV-absorbing dansyl (DNS) derivative. For this purpose, an aliquot of an aqueous extract or dilution from tablets, powders, effervescent granules or syrups was first mixed with a solution of 1-benzylpiperazine, the internal standard, and next with solutions of DNS-chloride and sodium carbonate. Sonication of the reaction mixture led to the formation of the DNS-derivatives, with bis-DNS-piperazine separating as a crystalline product. After extraction into chloroform, and dilution of the extract with mobile phase, the DNS derivatives were separated on a cyanopropyl column with hexane-isopropanol (85:15) as the mobile phase. At a flow rate of 1.5 mL/min and a detection wavelength of 335 nm, DNS-1-benzylpiperazine and bis-DNS piperazine eluted at 4.0 and 8.5 min, respectively. Detector responses were linearly related to on-column concentrations of piperazine citrate ranging from 8-50 μg. The recovery of analyte from synthetic formulations simulating the various dosage forms was in all cases > 99.0% (range 99.7-102.0%). Assay results by the proposed method agreed closely with those obtained by the gravimetric method of USP 23.

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INTRODUCTION

Piperazine, a cyclic ethylenediamine, is an anthelmintic agent used to treat human and animal infestations by roundworms and pinworms (1). Following oral administration, piperazine and its salts interact with susceptible intestinal worms to cause reversible muscle paralysis, presumably by blocking the response of muscles to acetylcholine (2,3). The paralyzed worm is eventually expelled in the stools by the normal peristaltic activity of the intestine (2).

Piperazine citrate is the salt recognized as official in the United States (4); however, the hexahydrate form as well as the adipate, tartrate and phosphate salts are also considered to be therapeutically important in foreign markets (5,6).

Piperazine and its salts have been analyzed by a variety of analytical methods that have included gravimetry (4,7), UV-Vis spectrophotometry (8-12), IR spectrophotometry (7,13), NMR spectroscopy (14), turbidimetry (15), fluorometry (16), polarography (17,18), titrimetry (4-6,19-21), and gas-chromatography (22,23). In the USP (4), the drug substance is assayed by a nonaqueous titration while a tedious gravimetric approach is required for the dosage forms. More recently, piperazine has been assayed in dosage forms by HPLC methods, either on a cationic ion-exchange column with refractometric detection (24), or by the reversed-phase (RP)mode with photometric detection in the visible range after derivatization with 4-chloro-7-nitro-benzofurazane (NBD)-chloride (25).

The purpose of this report is to describe a HPLC assay method for piperazine citrate which utilizes precolumn derivatization with DNS-chloride at room temperature, followed by normal-phase (NP)-HPLC with photometric detection in the UV range. This method was found suitable for the assay of the title drug in commercial tablets, powders, effervescent granules and syrups.

EXPERIMENTAL

Samples and Materials

The piperazine citrate was a USP reference standard (U.S. Pharmacopeial Convention, Inc.). The dansyl chloride (DNS-Cl), anhydrous sodium sulfate (Sigma Chemical Co.), and 1-benzylpiperazine (Aldrich Chemical Co.) were used as received. Tablets (275.75 mg), syrups (1.25 g/5 mL, 650 mg/5 mL, or 550 mg/5 mL), effervescent granules (0.2 g/5.71 g) and powders(1.65 g/envelope) were obtained from domestic and foreign commercial sources. The acetone, isopropanol and hexane were of HPLC grade (J.T. Baker); the anhydrous sodium carbonate was of analytical reagent grade (Fisher).

Reagents

a. DNS-Cl solution - Prepared by dissolving DNS-Cl in HPLC grade acetone and filtering. This solution, containing 5mg/mL, was stored in an amber glass bottle and in the refrigerator.

b. Basic solution - Prepared by dissolving 550 mg of anhydrous sodium carbonate in 300 mL of water, adding 300 mL of HPLC grade acetone, and mixing. This solution was stored in an amber glass bottle at room temperature.

c. Internal standard solution - Prepared by dissolving 1-benzylpiperazine in acetone-water (1+1) to a concentration of 4 mg/mL.

Sample Preparations

a. Piperazine citrate standard preparation - An accurately weighed quantity of piperazine citrate (about 200 mg) was transferred to a 100 mL volumetric flask, dissolved in about 50 mL of water, brought to volume with water, and mixed.

b. Tablet preparation - A group of 20 piperazine citrate tablets were weighed and reduced to a fine powder. A portion of powder, equivalent to about 200 mg of piperazine citrate, was transferred to a 100 mL volumetric flask, mixed with about 50 mL of water, and sonicated for about 15 min. After bringing to volume with water and mixing, the solution was filtered through a 0.45 μm membrane filter with the aid of pressure.

c. Syrups - A 5.0 mL volume of syrup was transferred to a 100 mL volumetric flask, diluted to volume with water, and mixed. An aliquot of the solution, containing about 200 mg of piperazine citrate (15.0, 30.0 or 40.0 mL aliquots for syrups containing 1.25 g/5 mL, 650 mg/5 mL, or 550 mg/5 mL, respectively), was transferred to a 100 mL volumetric flask, diluted to volume with water, and mixed.

d. Effervescent granules and powders - An accurately weighed quantity of granules or powder, equivalent to about 200 mg of piperazine citrate, was transferred to a 100 mL volumetric flask, and dissolved in about 50 mL of water (added slowly and with gentle swirling). After sonication for 10 min, the solution was brought to volume with water, mixed, and filtered through a 0.45 μm membrane filter with the aid of pressure.

e. Synthetic piperazine tablet preparation - To a 100 mL volumetric flask, an accurately weighed quantity of piperazine citrate (about 200 mg) and a starch-lactose (1+1) mixture (about 100 mg) was added. After the addition of 50 mL of water, the mixture was sonicated for 15 min, diluted to volume with water, and mixed. A portion of the solution was filtered through a 0.45 μm membrane filter with the aid of pressure.

f. Synthetic piperazine citrate syrup preparation - An accurately weighed quantity of piperazine citrate (about 100, 130 or 250 mg) was transferred to a 100 mL volumetric flask, mixed with 1 mL of simple syrup, diluted to volume with water, and mixed.

g. Synthetic effervescent granules preparation - An accurately quantity of piperazine citrate (about 200 mg), together with sodium bicarbonate (2.46 g), tartaric acid (1.94 g), and citric acid (0.63 g) were transferred to a 100 mL volumetric flask, dissolved in about 50 mL of water (added slowly with gentle swirling), and sonicated for about 10 min. The solution was diluted to volume with water, mixed, and filtered through a 0.45 μm membrane filter with the aid of pressure.

Dansylation Method

To a 125 mL glass-stoppered Erlenmeyer flask, 3.0 mL of sample preparation, 2.0 mL of internal standard solution, 10 mL of DNS-Cl, and 10 mL of basic solution, were added in succession and mixed with gentle swirling. The flask was loosely stoppered, placed in an ultrasonic bath, sonicated for 10 min, and allowed to stand in the dark for at least 30 min. The reaction mixture was diluted with water (20 mL), mixed with chloroform (20 mL), and vigorously shaken for about 1 min. After allowing the phases to separate, the chloroform layer was carefully removed by aspiration with a pipet, and passed through a layer of anhydrous sodium sulfate, held on a funnel fitted with a glass wool pledget, into an amber glass vial. A 2.0 mL volume of the filtrate was transferred to a 5 mL volumetric flask, diluted to volume with mobile phase, and a portion of the solution was immediately injected into the liquid chromatograph.

HPLC Method

a. Apparatus - Consisting of a Series 10 liquid chromatograph, LC 90 UV spectrophotometric detector (Perkin-Elmer Corporation), and ChromJet electronic integrator (Spectra-Physics). Samples were introduced through a Model 7125 injection valve fitted with a 20 μL sample loop (Rheodyne).

b. Chromatographic conditions - Separations were performed on a 25 cm x 4.6 mm i.d., CN5 SG cyanopropyl, 5 μm, column (Burdick & Jackson). Samples were eluted with a mobile phase composed of hexane-isopropanol (85:15). The flow rate was 1.5 mL/min, and the detection wavelength was 335 nm.

Calculations

The quantity of piperazine citrate in the sample preparation was calculated from one of the following equations:

$$\text{mg/tablet} = (R_{sp}/R_{st}) \times C \times (W_1/S) \times 100$$
$$\text{mg/g effervescent granules} = (R_{sp}/R_{st}) \times C \times (W_2/S) \times 100$$
$$\text{mg/envelope} = (R_{sp}/R_{st}) \times C \times W_3/S) \times 100$$
$$\text{mg/5 mL syrup} = (R_{sp}/R_{st}) \times C \times 100F$$

where R_{sp} and R_{st} = peak responses of sample/internal standard in the sample preparation and standard/internal standard in the standard preparation, respectively; C = the amount of piperazine citrate in the standard preparation, mg/mL; W_1 = the average tablet weight, mg; S = the amount of sample taken for the analysis, mg; W_2 = 5,710 mg; W_3 = the weight of the contents of one envelope, mg; and F = 100/15 for 1.25 g/5 mL syrup, 100/30 for 625 mg/5 mL syrup, and 100/40 for 550 mg/5mL syrup.

To convert the value in piperazine citrate to that in piperazine hexahydrate, multiply the value by 0.8968 (obtained from [mol. wt. piperazine hexahydrate/mol. wt. piperazine] x [3 mol. wt. piperazine/mol. wt. piperazine citrate]). The reverse conversion can be accomplished by dividing by the same factor.

RESULTS AND DISCUSSION

DNS-Cl is an electrophilic reagent that has been widely used for the precolumn derivatization of a number of drugs of therapeutic interest (26). The formation of the DNS-derivatives is usually carried out with an excess of DNS-Cl in an aprotic solvent and under alkaline conditions, by leaving the reaction mixture to stand for several hours at room temperature or for shorter periods at elevated (30-50°C) temperatures (27). In this manner, primary and secondary amines with little or no UV absorbing properties are converted to stable sulfonamides that are analyzable by HPLC with either photometric or fluorometric detection (26,27). In the present study, formation of bis-DNS-piperazine occurred over several hours upon standing, and in less than 5 minutes upon sonication of the reaction mixture. In both instances, the derivative separated as a light yellow crystalline product, m.p. 258-260°C, whose mass spectrum exhibited abundant peaks at m/e 84,155, 171, 318, and 552, and a molecular ion peak corresponding to a molecular weight of 552.14.

A sample preparation amenable to direct NP-HPLC analysis was obtained by extracting the aqueous reaction mixture with chloroform, drying the chloroform extract with anhydrous sodium sulfate and diluting the extract with the mobile phase. Alternatively one could use a RP-HPLC approach, but this course of action will complicate the analytical procedure because it will require to evaporate the chloroform extract to

Table 1

Retention times relative to DNS-piperazine of DNS-derivatives of N-containing heterocyclic compounds of potential utility as an internal standard

DNS-derivative	Column[a]			
	A[b]	B[b]	C[b]	D[c]
Piperazine	1.00	1.00	1.00	1.00
2-Methylpiperazine	0.95	0.94	0.89	0.93
1-Benzylpiperazine	0.50	0.36	0.31	0.48[d]
1-Phenylpiperazine	0.46	0.41	0.45	0.47[d]
1-(2-Aminoethyl)piperazine	2.41	2.05	-e	2.94
Piperidine	0.42	0.32	0.36	0.41[d]
4-Benzylpiperidine	0.41	0.36	0.40	0.41[d]
4-Hydroxypiperidine	0.95	0.54	0.74	1.79

[a]Column identification: A= CN5 SG cyanopropyl (Burdick & Jackson); B = Microsorb-MV CN (Rainin); C = μBondapak CN (Waters); D = Econosphere CN (Alltech).
[b]Solvent: hexane-isopropanol (85:15), 1.5 mL/min.
[c]Solvent: hexane-isopropanol (97.5:2.5), 1.2 mL/min.
[d]Only partially resolved from the solvent front.
[e]Derivative was retained on the column.

dryness, and to reconstitute the residue in a suitable solvent. In comparison to other procedures for the precolumn derivatization of piperazine citrate (25), the use of DNS-Cl offers advantages such as the possibility of conducting the reaction at ambient temperature, of omitting the addition of an amine to the mobile phase, and of monitoring the elution within the UV spectral range.

Several hydroxy- phenyl- and methyl-piperazine and piperidine compounds were evaluated for their suitability as an internal standard. Table 1 lists the retention times of the corresponding DNS-derivatives on various brands of cyano columns relative to that of piperazine. On the basis of its ready reaction with DNS-Cl to form a DNS-derivative that was quantitatively coextracted with that of piperazine, and chromatographically well resolved from the peak of bis-DNS-piperazine (R >2.0), 1-benzylpiperazine was judged as appropriate. However, at the completion of this study, it was verified that homopiperazine could serve as an attractive alternative to 1-benzylpiperazine by virtue of

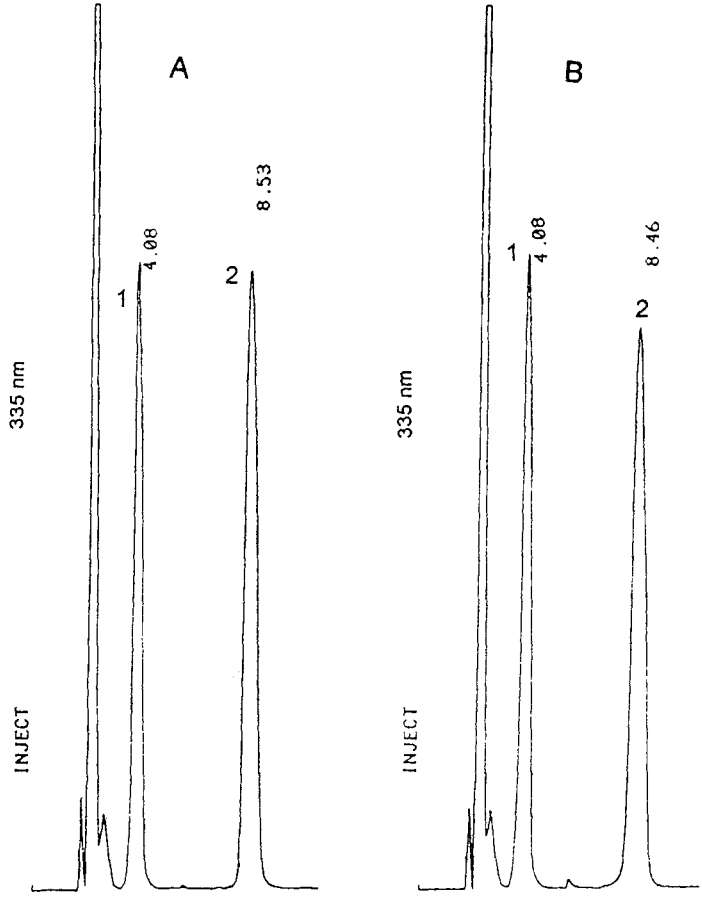

Figure 1. Typical high-performance liquid chromatograms of 1, DNS-1-benzylpiperazine and 2, bis-DNS-piperazine in (A) a standard preparation and (B) a commercial syrup. Flow rate was 1.5 mL/min.

its more convenient (ca. 6.5 min) retention time. In addition, it was verified that the elution behavior of certain DNS-derivatives varied from column to column, notably on the μBondapak cyano and Econosphere CN. For instance, the μBondapak CN column was the least retentive of all the columns tested, with some DNS-derivatives eluting with the solvent front even when the concentration of isopropanol in the mobile phase was less

Table 2
Results of recovery of piperazine citrate from spiked synthetic dosage forms by proposed HPLC method

Synthetic formulation	Piperazine citrate found, % of added			
	Run 1	Run 2	Mean	SD
Tablet, 200 mg	99.5	99.8	99.7	0.15
Syrup, 250 mg/mL	99.7	100.7	100.2	0.71
Syrup, 130 mg/mL	99.9	99.5	99.7	0.28
Syrup, 100 mg/mL	101.9	99.7	100.8	1.55
Granules, 0.2 g/5.71 g	102.7	102.9	102.8	0.14

than 5 parts per 100. In contrast, the Econosphere CN column retained certain DNS-derivatives (e.g. DNS- 1-(2-aminoethyl)piperazine) under the recommended experimental conditions. Typical separations of the DNS derivatives of the analyte and internal standard are presented in Figure 1.

A linear relationship between detector responses (ratio of peak heights or areas of bis-DNS-drug to DNS-internal standard) and on column concentrations of piperazine citrate was observed over the concentration range of 8-50 μg (line equation, $y = 0.86x - 0.026$; $r = 0.999$), with the curve passing through the origin. Based on these results, assays were routinely conducted using a sample preparation that contained about 2 mg/mL of analyte. The reproducibility of the method was assessed on the basis of peak areas (RSD = 1.92%) and peak heights (RSD = 0.58%) for six consecutive injections of a standard mixture of bis-DNS-piperazine and DNS-1-benzylpiperazine. The accuracy of the proposed method was assessed by spiking synthetic formulations, prepared to simulate tablets, syrups and effervescent granules, with known amounts of piperazine citrate and subjecting the samples to the assay procedure described for commercial dosage forms. As presented in Table 2, the mean recovery ($n = 2$) of piperazine citrate by the proposed HPLC method was in all cases better than 99.5% (range 99.7-102.0%) of added. No recovery study was conducted on a synthetic powder dosage form owing to the fact that the formulation ingredients were not disclosed by the manufacturer.

Table 3

Results of the assay of piperazine citrate in commercial dosage forms by proposed DNS-HPLC method and USP 23 gravimetric method

Lot No.	Piperazine citrate found, % of declared					
	HPLC			Gravimetry		
	Run 1	Run 2	Mean	Run 1	Run 2	Mean
	Tablets, 275.5 mg/tablet[a]					
1	100.1	101.3	100.7	99.2	98.0	98.6
	Effervescent granules, 0.2 g/5.71 g[b,c]					
1	103.0	103.7	103.4	104.2	104.9	104.5
2	103.7	103.8	103.8	104.8	104.7	104.8
	Powder, 1.65 g/envelope[d]					
1	99.9	101.3	100.6	99.5	98.8	98.7
	Syrup A, 1.25 g/5 mL[b]					
1	85.9	87.2	86.6	85.4	85.5	85.5
2	85.9	86.9	86.4	85.5	85.3	85.6
3	87.4	87.6	87.5	85.8	85.8	85.8
	Syrup B, 625 mg/5 mL[b]					
1	93.3	91.8	92.6	90.9	91.7	91.8
	Syrup C, 550 mg/5 mL[c]					
1	85.2	84.0	84.6	83.3	82.2	82.8
2	84.0	85.1	84.5	83.4	82.9	83.2
3	83.1	84.3	83.7	82.7	83.3	83.0

[a]Manufactured in U.S.A.
[b]Manufactured in the Philippines.
[c]Declared as piperazine hexahydrate.
[d]Manufactured in Peru.

Assay values for commercial dosage forms of piperazine citrate by the proposed HPLC method are summarized in Table 3. These values agreed closely with those obtained by the gravimetric method of USP 23, which is based on the precipitation of piperazine as the picrate salt. Intermethod assay differences based on the labeled amount were about 2.1% for tablets, 0.7-1.9% for syrups, 1.9% for powders, and 1.1% for effervescent granules. Except for two brands of syrup (syrups A and C), all of the samples conformed to the compendial requirements for labeled amounts. No interferences were noted from either excipients of tablets and effervescent granules or from ingredients of syrups.

In summary, the analysis of piperazine citrate in commercial solid and liquid dosage forms can be accomplished in a simple, specific and accurate manner by the NP-HPLC method presented here. This method will yield assay results that are comparable to those obtained by the slower and more cumbersome gravimetric method of USP 23.

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