High-Pressure Liquid Chromatographic Determination of Thiothixene in Pharmaceutical Formulations

C. K. WONG, D. M. COHEN, and K. P. MUNNELLY ˣ

Abstract □ A method was developed for the quantitative determination of thiothixene in pharmaceutical formulations by high-pressure liquid chromatography after dissolution of the sample in methanol.

Keyphrases □ Thiothixene—high-pressure liquid chromatographic analysis, pharmaceutical formulations □ High-pressure liquid chromatography—analysis, thiothixene, pharmaceutical formulations □ Tranquilizers—thiothixene, high-pressure liquid chromatographic analysis, pharmaceutical formulations

Thiothixene, a thioxanthene derivative, is the *cis*-isomer of *N,N*-dimethyl-9-[3-(4-methyl-1-piperazinyl)propylidene]thioxanthene-2-sulfonamide. The thioxanthenes differ from the phenothiazines by replacement of the nitrogen in the central ring with a carbon-linked side chain fixed in space in a rigid structural configuration and an *N,N*-dimethylsulfonamide functional group bonded to the thioxanthene nucleus. Thiothixene is supplied as a capsule, injection, and solution and is effective in the management of manifestations of psychotic disorders (1).

Some methods for the quantitative determination of thiothixene in pharmaceutical formulations have appeared in compendial monographs. Three basic methods exist: a spectrophotometric assay, a paper chromatographic assay, and a colorimetric assay involving the formation of a methyl orange complex (2). However, these methods do not have the rapidity, simplicity, sensitivity, and selectivity found in high-pressure liquid chromatographic (HPLC) methodology. The subject of this report is the development of a simple, direct, and extremely rapid HPLC procedure for the quantitation of thiothixene in several pharmaceutical formulations.

EXPERIMENTAL

Equipment—A liquid chromatograph[1], operated at ambient temperatures and equipped with a UV detector for monitoring the column effluent at 254 nm, was used. The column was 1-m × 2.1-mm (i.d.) stainless steel tubing, dry packed with Corasil II[2]. An electronic integrator[3] was used to obtain peak areas.

Reagents—NF thiothixene reference standard, dried *in vacuo* at 100° for 3 hr, was used. *N*-(1-Naphthyl)ethylenediamine dihydrochloride[4] was the internal standard. Analytical reagent grade methanol[5], 95% ethanolamine[6], and distilled water were used to prepare the mobile phase.

Preparation of Mobile Phase—Add 0.37 ml of ethanolamine and 400 ml of water to 2.8 liters of methanol and shake vigorously. Degas prior to use.

Preparation of Standard Solution—Dissolve approximately 350 mg of *N*-(1-naphthyl)ethylenediamine dihydrochloride in 100 ml of methanol. Accurately weigh approximately 25 mg of thiothixene standard, transfer quantitatively to a 25-ml volumetric flask, add 5 ml of the *N*-(1-naphthyl)ethylenediamine dihydrochloride solution, and dissolve in and dilute to volume with methanol.

Preparation of Sample Solution—For thiothixene capsules, transfer as completely as possible the contents of not less than 30 capsules to a tared beaker and weigh. Mix and transfer an accurately weighed portion of the powder, equivalent to 25 mg of thiothixene, to a glass-stoppered centrifuge tube. Add 5 ml of the *N*-(1-naphthyl)ethylenediamine dihydrochloride solution and 20 ml of methanol, shake for 2 min, centrifuge, and use the supernate for analysis. For the thiothixene injection or solution, accurately pipet an aliquot of sample equivalent to 25 mg of thiothixene into a 25-ml volumetric flask. Add 5 ml of the *N*-(1-naphthyl)ethylenediamine dihydrochloride solution and dissolve in and dilute to volume with methanol.

Chromatography—Condition the column for 24 hr with the mobile phase at a flow rate of 0.5 ml/min. This procedure is necessary for newly packed columns; conditioning is not required for previously used columns. Inject 4 μl of the standard solution and adjust either the pressure or flow rate so that the *N*-(1-naphthyl)ethylenediamine dihydrochloride exhibits a retention time of approximately 4–5 min. Repeat if necessary.

Inject 4 μl of the standard solution followed by two 4-μl injections of the sample solution and one 4-μl injection of the standard solution, allowing sufficient time between injections for development of the chromatograms and return to baseline. The peak areas obtained are used for the calculations.

Calculations—Calculate the quantity of thiothixene in milligrams

Table I—Percent Recovery of Thiothixene by HPLC from Spiked Placeboes

Product	Day	Weight	Injection Number 1	2	3
Capsule[a]	1	1	100.6	99.2	99.1
		2	96.9	98.6	97.7
		3	100.1	97.1	99.0
	2	1	98.8	96.8	95.8
		2	101.5	98.0	98.8
		3	98.1	99.5	98.7
	3	1	97.1	94.7	97.9
		2	96.9	94.5	96.7
		3	96.8	95.5	96.3
Injection[b]	1	1	97.5	98.5	98.2
		2	104.0	100.7	102.7
		3	104.0	101.2	105.1
	2	1	100.0	100.9	101.8
		2	100.8	102.6	102.3
		3	101.3	103.7	102.6
	3	1	99.7	101.2	103.8
		2	103.7	99.2	98.1
		3	101.4	101.0	102.6
Solution[c]	1	1	100.2	98.9	96.9
		2	100.2	97.3	100.1
		3	98.4	99.1	99.3
	2	1	100.1	98.0	101.3
		2	96.6	99.7	98.6
		3	97.0	98.3	99.1
	3	1	100.7	98.6	101.8
		2	101.1	101.8	98.2
		3	98.7	98.4	98.0

[a] The average percent recovery was 97.8 with 95% confidence limits of 97.1–98.5 [b] The average percent recovery was 101.4 with 95% confidence limits of 100.6–102.2. [c] The average percent recovery was 99.1 with 95% confidence limits of 98.5–99.7.

[1] DuPont 820.
[2] Waters Associates.
[3] Hewlett-Packard 3370A.
[4] Fisher Scientific Co.
[5] Mallinckrodt.
[6] Aldrich Chemical Co.

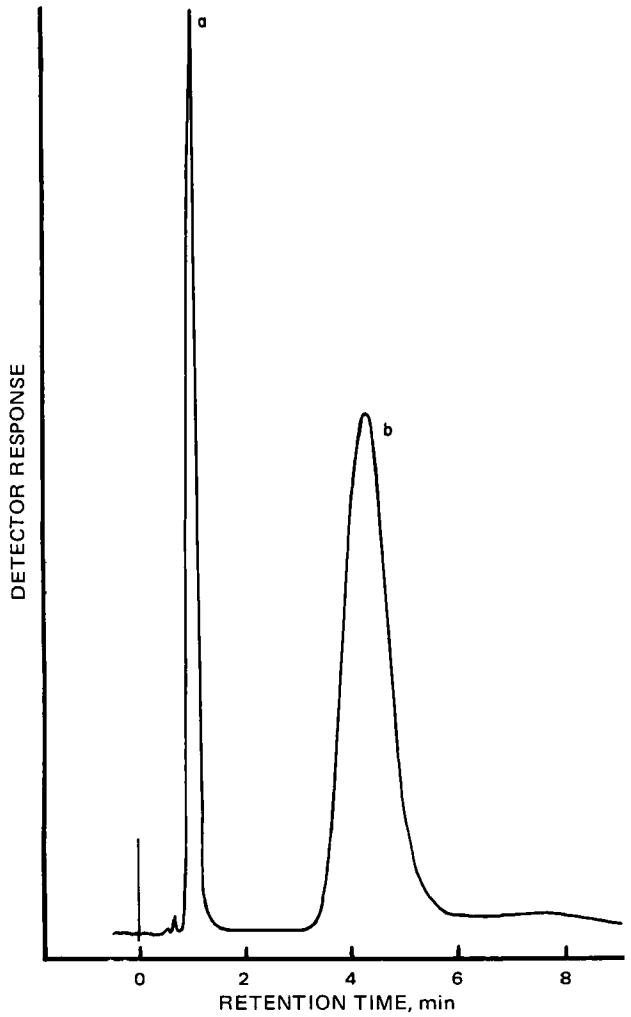

Figure 1—*Representative chromatogram. Key: a, thiothixene; and b, N-(1-naphthyl)ethylenediamine.*

per capsule or milliliter according to:

$$\text{mg/capsule} = \frac{R_{spl} \times W_{std}\ (\text{mg}) \times \text{average capsule weight (mg)}}{R_{std} \times W_{spl}\ (\text{mg})} \qquad \text{(Eq. 1)}$$

$$\text{mg/ml} = \frac{R_{spl} \times W_{std}\ (\text{mg})}{R_{std} \times W_{spl}\ (\text{ml})} \qquad \text{(Eq. 2)}$$

where R_{spl} is the ratio of the thiothixene peak area to the *N*-(1-naphthyl)ethylenediamine dihydrochloride peak area in a sample solution injection, R_{std} is the average ratio of the thiothixene peak area to the *N*-(1-naphthyl)ethylenediamine dihydrochloride peak area in the standard solution injections before and after the sample, W_{std} is the weight of the standard, and W_{spl} is the weight or volume of the sample.

RESULTS AND DISCUSSION

Initial studies were conducted utilizing methanol as the mobile phase, and acceptable peak symmetry and retention time were observed for thiothixene. *N*-(1-Naphthyl)ethylenediamine dihydrochloride appeared satisfactory as the internal standard, showing the greatest separation from thiothixene. However, its longer retention time (~30 min) resulted in a significant increase in the cycle time of an analysis. Further studies were initiated to modify the methanol mobile phase so as to decrease the retention time of the *N*-(1-naphthyl)ethylenediamine dihydrochloride without affecting the peak symmetry and retention of the thiothixene.

Modifiers for improving separation and retention in nonpolar mobile phases have been used for some time in liquid–solid chromatography (3). However, the use of modifiers in polar mobile phases, especially those containing large percentages of water, is unique in its application to HPLC. The addition of small amounts of ethanolamine to the methanol significantly decreased the retention of the *N*-(1-naphthyl)ethylenediamine dihydrochloride while only a minute decrease was observed for thiothixene. This decrease appears due to the conversion of the internal standard from the dihydrochloride to the free base. Excellent separation of the two components was maintained and the cycle time for an analysis decreased substantially when a mobile phase of methanol–ethanolamine (7560:1) was employed.

Although the resulting separation was considered optimum, the shape of the *N*-(1-naphthyl)ethylenediamine dihydrochloride peak required further improvement since the lack of a defined beginning and end presents considerable problems for quantitation by electronic integration. By the addition of water, the characteristic tailing for adsorption chromatography was eliminated (4). The mobile phase

Table II—Estimates of Precision for Determination of Thiothixene by HPLC in Pharmaceutical Formulations

Weights per Day	Injections per Weight	Estimates of Precision[a], % Capsule	Injection	Solution
1	1	±3.8	±4.3	±2.9
1	2	±3.3	±3.7	±2.1
1	3	±3.1	±3.5	±1.8
2	2	±2.9	±2.6	±1.5
2	3	±2.8	±2.4	±1.2
Injection to injection within a weight		±2.7	±3.2	±2.9

[a] Ninety-five percent of the individual results or averages of two, three, four, or six results will not vary from each other by more than the percentages quoted. These estimates include variability due to days, weights, and injections. The estimates of precision for injection to injection within a weight exclude variability due to days and weights.

Table III—Comparison of HPLC and Compendial Paper Chromatographic Methods for Determination of Thiothixene in Pharmaceutical Formulations

Product	Day	Sample	HPLC	Paper Chromatography
Capsule[a]	1	1	5.31	4.94
		2	5.13	4.99
		3	5.37	—
	2	4	5.26	5.18
		5	5.35	4.88
		6	5.16	—
	3	7	4.95	5.15
		8	5.08	5.05
		9	5.14	—
		Average	5.19	5.03
Injection[b]	1	1	2.01	2.01
		2	2.01	2.02
		3	1.99	—
	2	4	2.04	1.97
		5	2.03	2.04
		6	2.05	—
	3	7	2.02	2.01
		8	2.02	1.99
		9	2.00	—
		Average	2.02	2.01
Solution[c]	1	1	4.97	5.07
		2	5.05	4.92
		3	5.02	—
	2	4	5.00	5.06
		5	5.00	5.06
		6	5.02	—
	3	7	5.15	5.09
		8	5.13	5.09
		9	5.13	—
		Average	5.05	5.05

[a] Results are given in milligrams per capsule. [b] Results are given in milligrams per milliliter. [c] Results are given in milligrams per 5 ml.

reported in this study improved peak shape, with the resulting chromatogram resembling the characteristics of partition chromatography; all peaks were well defined and symmetrical (Fig. 1).

The accuracy and precision of the HPLC method were determined by the following experiments. Three weights of a placebo of each pharmaceutical formulation to which known quantities of thiothixene hydrochloride had been added were assayed per day for 3 consecutive days. The average percent recoveries of thiothixene were 97.8, 101.4, and 99.1 for the capsule, injection, and solution, respectively (Table I). The estimates of precision (Table II) were obtained using the analysis of variance statistical technique. Ninety-five percent of the individual results will not vary from each other (*i.e.*, from the mean) by more than ±3.8, ±4.3, and ±2.9% for the capsule, injection, and solution, respectively. The standard errors for the average of two injections per sample were ±1.6, ±1.8, and ±1.1% for the capsule, injection, and solution, respectively.

The proposed HPLC method was compared to the compendial paper chromatographic assays (2), and the results obtained (Table III) were in agreement. All samples were within the requirements of NF XIV for thiothixene formulations by the HPLC methodology (2). Because of the speed, accuracy, and precision of the proposed procedure, it represents a viable alternative to present compendial methodology.

REFERENCES

(1) "Physicians' Desk Reference," 29th ed., Medical Economics, Inc., Oradell, N.J., 1975, p. 1272.

(2) "The National Formulary," 14th ed., Mack Publishing Co., Easton, Pa., 1975, and 1st supplement, official July 1, 1975.

(3) "Modern Practice of Liquid Chromatography," J. J. Kirkland, Ed., Wiley–Interscience, New York, N.Y., 1971, p. 223.

(4) "Chromatography," E. Heftmann, Ed., Reinhold, New York, N.Y., 1964, p. 63.

ACKNOWLEDGMENTS AND ADDRESSES

Received July 21, 1975, from *Quality Control, Pfizer Inc., Brooklyn, NY 11206*

Accepted for publication September 26, 1975.

[x] To whom inquiries should be directed.

Structural Features and Protective Activity of Dexamethasone and Pregnenolone-16α-carbonitrile

A. TERZIS [*], P. KOUROUNAKIS [x], and T. THEOPHANIDES

Abstract □ Pretreatment with dexamethasone acetate or pregnenolone-16α-carbonitrile markedly diminished the pharmacological effect of zoxazolamine in rats. This prophylactic action was associated with significantly decreased plasma drug levels, which, in turn, were correlated with enhanced hepatic drug biotransformation, induced by the steroids. Dexamethasone proved to be more active than pregnenolone-16α-carbonitrile in this respect. The A-ring conformation as well as the distances of O-3–O-11, O-11–O-17, and the O-3–mean plane C-5–C-17 may be key factors in glucocorticoid activity, and the longer the distances, the greater the potency. These characteristics have no bearing on catatoxic activity for which the 16α-substituent appears to be a structural prerequisite.

Keyphrases □ Dexamethasone acetate—structural and conformational features, effect of pretreatment on pharmacological action, plasma concentrations, and liver homogenate metabolism of zoxazolamine, rats □ Pregnenolone-16α-carbonitrile—structural and conformational features, effect of pretreatment on pharmacological action, plasma concentrations, and liver homogenate metabolism of zoxazolamine, rats □ Structure–activity relationships—dexamethasone acetate and pregnenolone-16α-carbonitrile, structural features, prophylactic activity against zoxazolamine, rats □ Glucocorticoids—dexamethasone acetate and pregnenolone-16α-carbonitrile, structural features, prophylactic activity against zoxazolamine, rats □ Zoxazolamine—effect of pretreatment with dexamethasone acetate and pregnenolone-16α-carbonitrile on pharmacological action, plasma concentrations, and liver homogenate metabolism, rats

Numerous compounds induce liver microsomal enzymes and can be divided into three main classes: barbiturates, polycyclic aromatic hydrocarbons, and steroids (1–3). Pregnenolone-16α-carbonitrile (I), a synthetic "catatoxic" steroid devoid of any other known hormonal or pharmacological activity, and dexamethasone acetate, a synthetic glucocorticoid, are potent microsomal enzyme inducers (3). Pretreatment with I or dexamethasone protects experimental animals against numerous toxic agents, mainly *via* the induction of drug-metabolizing enzymes in liver microsomes (catatoxic mechanism). However, the protective effect of some steroids is attributed to their glucocorticoid activity (3, 4).

Because the mechanism of microsomal enzyme induction at the molecular level is still not known and little accurate structural information is available (5), an investigation was conducted on the structure–activity relationships of these and other similarly acting steroids. In this study, the prophylactic activity of I and dexamethasone against zoxazolamine was correlated with plasma drug concentrations and *in vitro* metabolism by the 9000×*g* liver homogenate fraction. Thus, the protective mechanism was demonstrated and the relative potency of these enzyme inducers was established.

In addition, the steroid structure was determined in an effort to elucidate the common structural and conformational prerequisites for their inductive properties. This information should prove useful in the continuing investigations of the structure–activity relationships of similarly acting steroids. Since information on the structure (6–8) and activity (9, 10) of fludrocortisone, cortisone, and 6α-methylprednisolone was available, it is also discussed.