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Note

Improved high-performance liquid chromatographic method for the quantitation of *cis*-thiothixene in plasma samples using *trans*-thiothixene as internal standard

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In an earlier paper we reported a high-performance liquid chromatographic (HPLC) method for the separation of *cis* and *trans* forms of thiothixene [1]. We quantitated *cis*-thiothixene in plasma samples using mesoridazine as an internal standard. Since mesoridazine is also a commonly used antipsychotic drug and a metabolite of thioridazine we switched to another internal standard, thioproperazine, with equal success [2]. As reported earlier, setting the ultraviolet (UV) detector at 229 nm gave higher sensitivity [2]. Analysis of over 200 plasma samples using either of the above internal standards did not show any detectable amount of *trans*-thiothixene in any of the patient plasma samples. Thus we established that there is no biotransformation of the active drug, *cis*-thiothixene, into the inactive *trans*-thiothixene in humans. It therefore occurred to us that the non-biological non-drug, *trans*-thiothixene, would be the ideal standard for the quantitation of *cis*-thiothixene in human plasma samples. We now report here a simple HPLC method for the quantitation of *cis*-thiothixene with the *trans* form as an internal stnadard.

MATERIALS AND METHODS

Isoamylalcohol was Fisher-certified grade and all other solvents were HPLC grade from Burdick & Jackson Labs. or J.T. Baker. *cis*-Thiothixene and *trans*-thiothixene maleate standards were obtained from Pfizer Research Labs. through the kind courtesy of Dr. D.C. Hobbs.

Stock standard solution

Standard stock solutions of *cis*- and *trans*-thiothixene were separately

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prepared by accurately weighing 3.0 mg of *cis*-thiothixene and 3.45 mg of *trans*-thiothixene maleate and dissolving in 3 ml of methanol. Working standards were prepared from the stock solution to contain 10 μg/ml by dilution with water. Drug-free plasma was then spiked with *cis*-thiothixene to concentrations of 0, 2, 4, 6, 8, 10 and 20 ng/ml to prepare plasma calibration standards. All standards were stored at −20° C protected from light.

Extraction of plasma samples

Plasma (1 ml) and 10 μl of internal standard, *trans*-thiothixene (100 ng), were mixed with 1.0 ml of 2 *M* sodium carbonate (pH 9.8) and 5 ml of hexane-isoamyl alcohol (98.5:1.5). The mixture was vortexed twice for 15 sec each time, then centrifuged for 15 min at 700 *g*. The upper organic layer was transferred into a 5-ml centrifuge tube and evaporated under nitrogen. Finally, 0.5 ml of the solvent was used to rinse the sides of the tube and the solution evaporated to dryness under nitrogen. The residue was dissolved in 50 μl of mobile phase by vortexing for 15 sec, centrifuged for 5 min and 20 μl were injected into the HPLC system. From the peak height ratio of *cis*-thiothixene and the internal standard *trans*-thiothixene the plasma concentration is determined. A calibration curve from extracted plasma standards containing 0, 2, 4, 6, 8, 10 and 20.0 ng of *cis*-thiothixene and 100 ng internal standard was obtained.

HPLC conditions

A Bio-Rad 1310 HPLC pump and 1306 variable-wavelength UV detector, a Rheodyne 7125 sample injector with a 200-μl sample loop, and a Spherisorb 5-μm cyanopropyl HPLC column, 150 × 4.6 mm (Custom LC, U.S.A.), were used. The detector was set to 229 nm, 0.0025 a.u.f.s. sensitivity, as suggested earlier [1].

The mobile phase was 0.01 *M* potassium dihydrogen phosphate (pH 7.0)—acetonitrile—methanol (400:480:120), at a flow-rate of 2.0 ml/min.

RESULTS AND DISCUSSION

Relative retention data for *cis*- and *trans*-thiothixene and some commonly used antipsychotic drugs on our system are given in Table I. Thioproperazine has a relative retention time of 1.11, is distinctly separated as a peak and is distinguishable from *cis*- and *trans*-thiothixene. The percent recovery of *cis*- and *trans*-thiothixene added to plasma samples was the same and the mean percentage recovery of the internal standard (*trans*-thiothixene) was over 90% and ranged between 80% and 100%.

We have not found any interfering drugs or metabolites. Chromatograms of standards in drug-free plasma and patient samples are shown in Fig. 1. The plasma blank with ten different drug-free samples did not show any interference from endogenous compounds. The *trans*-thiothixene used as internal standard is almost free from the *cis* isomer. (Fig. 1, 0 ng). The patient samples (P_1 and P_2) showed 1.0 and 7.3 ng/ml, respectively. Calibration curves were run with every batch of plasma standards and had correlation coefficients (six points) of 0.994 ± 0.002 S.D. (n = 19). For routine analysis, we included in

TABLE I

RELATIVE RETENTION TIMES (RTT) OF PSYCHOTROPIC DRUGS AND METABOLITES TO *cis*-THIOTHIXENE

Compound	RTT	Compound	RTT
cis-Thiothixene	1.00	Trimipramine	1.53
trans-Thiothixene	1.24	Doxepin	2.00
Desmethylthiothixene	2.08	Amitriptylene	2.12
		Imipramine	2.18
Loxapine	0.44	Desmethyldoxepin	2.59
Clozapine	0.47	Notriptylene	2.65
Fluphenazine	0.50	Desipramine	2.88
Haloperidol	0.88	Maprotylene	2.79
Chlorohaloperidol	0.88	Protriptylene	2.94
Thioproperazine	1.11		
Chlorpromazine	1.89	Diazepam	0.26
Mesoridazine	3.00	Desmethyldiazepam	0.26
Thioridazine	3.06	Flurazepam	0.56
		Chlordiazepoxide	0.27
Trazodone	0.32		
Amoxapine	0.80	Benztropine	4.47

TABLE II

DATA ON STANDARD CALIBRATION AND PLASMA CONTROLS RUN ON DIFFERENT DAYS

No.	*r* Value	Low control (ng/ml)	High control (ng/ml)
1	0.9959	1.3	8.75
2	0.9946	0.7	7.4
3	0.9956	1.5	6.5
4	0.9930	0.25	7.5
5	0.9960	2.05	7.2
6	0.9960	1.1	7.3
7	0.9963	0.07	6.95
8	0.9907	1.08	6.8
9	0.9947	0.25	6.5
10	0.9950	0.14	7.4
11	0.9845	1.0	8.5
12	0.9909	1.4	8.2
13	0.9935	2.2	7.4
14	0.9936	2.9	7.6
15	0.9807	1.5	7.4
16	0.9969	1.9	8.9
17	0.9995	1.5	8.6
18	0.9920	2.6	8.6
19	0.9980	1.9	8.1
Mean ± S.D.	0.9937 ± 0.002	1.32 ± 0.8	7.6 ± 0.72

Equation $Y = 0.0092X \pm 0.024$

every batch of patient plasma samples a low and high plasma control as a quality control measure. The results of analysis on different days are shown in Table II. Similarly with two other batches of plasma controls, the assayed batches were 10.0 ± 0.63 and 36.8 ± 3.5 ng/ml (n = 10). To assess the validity of the assay method in a wide range of plasma levels that one may have to deal with in clinical samples we carried out the assay of spiked human plasma samples which were prepared for us at Pfizer Central Research Lab. (Groton,

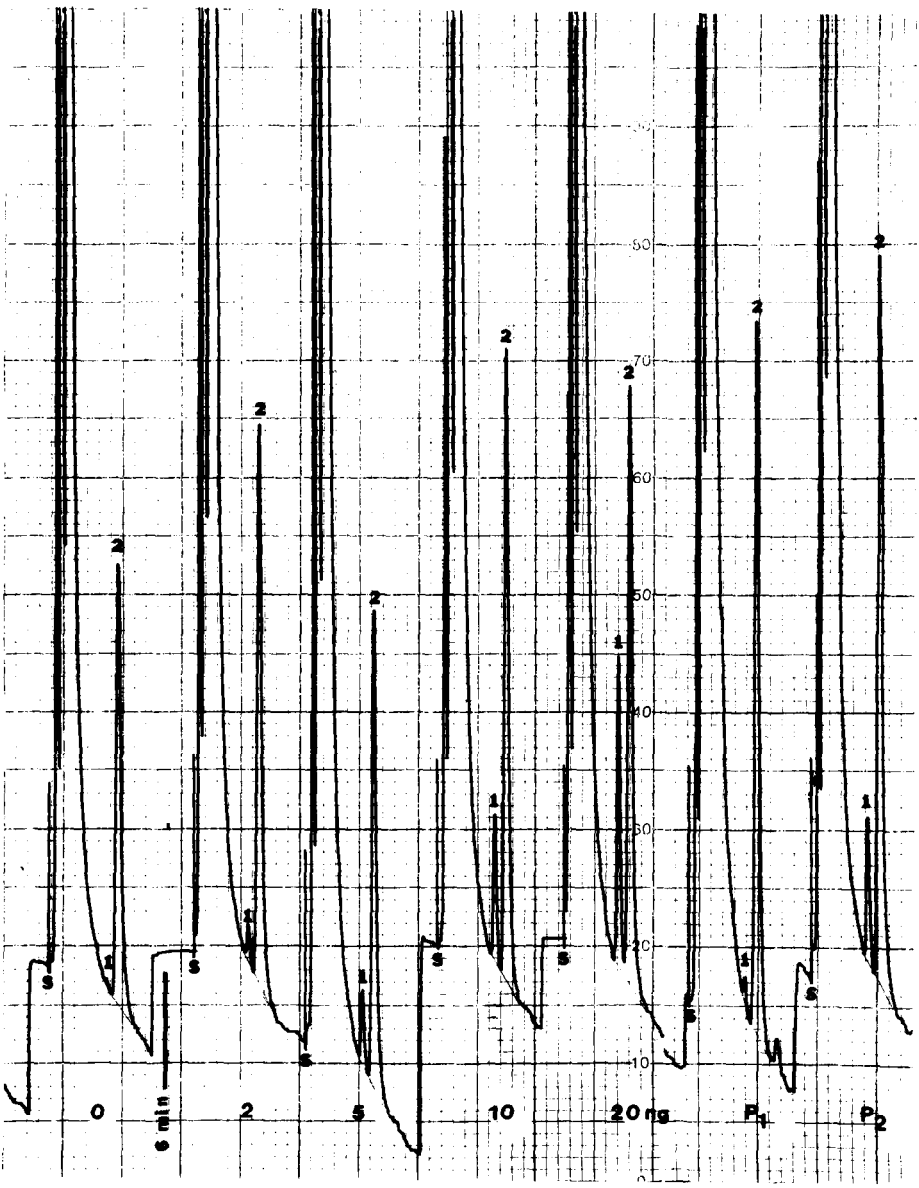

Fig. 1. Chromatograms of standards for calibration and patient plasma samples (P_1 and P_2). S = start: Chart speed 25 cm/h. Peaks: 1 = *cis*-thiothixene, 2 = *trans*-thiothixene (internal standard).

CT, U.S.A.). The samples were coded, frozen and sent to our laboratory for analysis. After the assay results were reported the code was broken and we were informed about the spiked levels and the assayed levels. These results are shown in Table III. These results indicate that reproducibility is good at all concentrations and accuracy is excellent at concentrations $\geqslant$ 12 ng/ml. The accuracy at the low concentrations is also adequate for clinical samples. Further we have not found any false positive in several plasma blanks analyzed. The minimum detectable level was 0.5 ng/ml *cis*-thiothixene. Plasma samples were from different clinical studies of patients receiving 5 to 70 mg thiothixene and from patients at MCV Hospital. Plasma levels ranged from undetectable to 40 ng/ml. This method has been in continuous and successful use in our laboratory for more than a year with adequate quality control procedures.

TABLE III

ASSAY OF THIOTHIXENE IN SPIKED HUMAN PLASMA

Spiked (ng/ml)	Found (ng/ml)	Ratio	Mean (S.D.)
0	0		
0	0		0 (0)
0	0		
0	0		
4	2.8	0.7	
4	2.6	0.65	0.67 (0.06)
4	2.9	0.73	
4	2.4	0.60	
8	7.5	0.94	
8	6.4	0.80	0.85 (0.07)
8	7.0	0.88	
8	6.3	0.79	
12	13.2	1.10	
12	12.9	1.07	1.08 (0.03)
12	12.5	1.04	
12	13.2	1.10	
16	16.4	1.03	
16	14.9	0.93	0.99 (0.06)
16	15.1	0.94	
16	17.0	1.06	
18	17.7	0.98	
18	18.3	1.02	0.95 (0.06)
18	16.3	0.91	
18	16.2	0.90	
35	34.5	0.99	
35	36	1.03	1.0 (0.02)
35	34.9	1.0	
35	34.5	0.99	

The *trans* form of thiothixene is added in excess to compensate for its lower UV absorbance and also to serve as a carrier for better extraction of the drug. We do not add *cis*-thiothixene to each sample, as in our earlier report [1], but upon repeated freeze—thaw cycles of the internal standard solution, a portion isomerises, so a blank plasma sample with added internal standard was included in each run. The isomerisation does not occur rapidly enough to affect results within a run. When we notice more than 0.5 ng of *cis*-thiothixene in our internal standard, we make a fresh internal standard solution.

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