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Journal of Chromatography B, 663 (1995) 309–313

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

High-performance liquid chromatographic determination of terguride in solid dosage forms and plasma

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First received 19 May 1994; revised manuscript received 20 September 1994

Abstract

A simple and rapid HPLC method was developed to determine terguride in terguride hydrogenmaleate, coated tablets and plasma. The assay was carried out on a glass column of SGX CN (150 × 3.3 mm I.D.) using methanol and phosphate buffer solution (pH 7.0) as the mobile phase, with detection at 227 nm. Terguride was quantified using promethazine as an internal standard. The tablet matrix was extracted into methanol. Plasma samples were deproteinated with acetonitrile and the supernatant was injected into the HPLC system. The method is linear, quantitative and reproducible.

1. Introduction

Terguride, 1-[(5*R*,8*S*,10*R*)-6-methyl-8-ergolinyl]-3,3-diethylurea, is a semi-synthetic derivative of ergot alkaloids. According to its structure (Fig. 1) it belongs to the ergolinylurea group, analogous to lisuride, its *trans*-dihydro derivative. Terguride is effective on dopamine receptors, inhibits adenohypophyseal secretion of prolactin and adjusts secretion of gonadotropin. Terguride is an anti-hyperprolactinaemic drug [1,2], used in the treatment of Parkinson's disease, and has potential use in the indication sphere of neuroleptics.

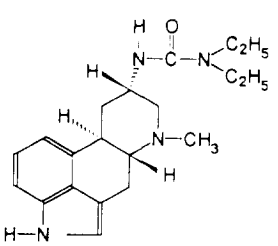

Fig. 1. Structure of terguride.

Terguride can be assayed by visible spectrophotometric methods after conversion into a coloured product by the van Urk reaction (with *p*-dimethylaminobenzaldehyde) [3]. It is also possible to use fluorescence of the indolyl group. Vachek and Svátek [4] assayed terguride spectrophotometrically at 409 nm (after reaction with tropaeolin OO) and fluorimetrically in terguride substance and in pharmaceutical dosage forms. A radioreceptor assay [5] has been used to determine terguride, tritiated in positions 9 and 10 ([^{3}H] terguride), in plasma.

The aim of this study was to develop a high-

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0378-4347/95/$09.50
SSDI 0378-4347(94)00438-2

performance liquid chromatographic (HPLC) method for the determination of terguride and its salt terguride hyrogenmaleate. No HPLC method has been reported previously. The applicability of the method was demonstrated in the assay of terguride in tablets and rat plasma samples.

2. Experimental

2.1. Reagents and chemicals

Terguride and terguride hydrogenmaleate were supplied by Galena (Opava-Komárov, Czech Republic) and Mysalfon coated tablet by Léčiva (Prague, Czech Republic). Methanol, acetonitrile, potassium dihydrogenphosphate and disodium phosphate were purchased from Lachema (Brno, Czech Republic). Promethazine chloride (internal standard) was obtained from Léčiva. All chemicals were of analytical-reagent grade, except methanol (HPLC grade), and water was doubly distilled. Mixed phosphate buffer solution (pH 7.0, 6.6 m*M*) was prepared by adding to 413 ml of 6.6 m*M* potassium dihydrogenphosphate solution a sufficient volume of 6.6 m*M* disodium phosphate solution to adjust the pH to 7.0 and mixing.

2.2. Chromatography

The HPLC system consisted of a Varian (Palo Alto, CA, USA) Model 8500 pump, an LCD 2040 variable-wavelength UV detector (Laboratory Instruments, Prague, Czech Republic) and an SP 4100 integrator (Spectra-Physics, Santa Clara, CA, USA). Analytical samples were introduced on to the column using an LCI 30 injection valve (Laboratory Instruments) with 3- and 10-μl loops. The analytical glass column (150 × 3.3 mm I.D.) contained Separon SGX CN (5 μm) (Tessek, Prague, Czech Republic). The mobile phase was methanol–phosphate buffer solution (pH 7.0). The flow-rate was set at 0.5 ml/min. The detector wavelength was adjusted to 227 nm.

2.3. Preparation of standard solutions

Stock standard solutions of terguride, terguride hydrogenmaleate and promethazine chloride (internal standard) were prepared in methanol by dilution of 1 mg/ml solutions. The stock standard solutions of terguride and terguride hydrogenmaleate were kept in the dark at room temperature.

Tablets

Calibration standards were prepared by placing aliquot volumes of stock standard solution and 500 μl a solution of the internal standard in a 10-ml volumetric flask, diluting to volume with methanol and mixing. Five concentrations of terguride (1, 5, 10, 20 and 30 μg/ml) were used to construct the calibration graph.

Plasma

Drug-spiked rat plasma standards were prepared by adding an appropriate volume of the stock standard solution of terguride and 40 μl of the stock standard solution of the internal standard to 0.25 ml of control rat plasma. Five concentrations of terguride (1, 8, 40, 100 and 160 μg/ml) were used to construct the calibration graph.

The concentration of terguride was calculated from the linear regression equation of the calibration graph constructed by plotting the peak-area ratio (y) versus the concentration of terguride (x).

2.4. Sample preparation

Tablets

Ten coated tablets were weighed and the average mass of a tablet was calculated. The tablets were powdered in a porcelain mortar. An accurately weighed amount of powder equivalent to 0.500 mg of terguride was extracted with 40 ml of methanol in a 100-ml glass-stoppered flask by shaking for 10 min. The extract was filtered into a 50 ml volumetric flask containing 0.5 ml of the internal standard solution and diluted to volume with methanol. This solution (3 μl) was injected into the HPLC column.

Plasma

A 0.25-ml volume of rat plasma sample was pipetted into a 10-ml glass-stoppered centrifuge test-tube, 40 μl of the internal standard solution were added and the sample was shaken for 5 min. Acetonitrile (0.5 ml) was then added and protein was precipitated by shaking for 5 min. Following centrifugation at 1930 *g* for 5 min, the supernatant was separated and 10 μl were injected into the HPLC column.

3. Results and discussion

HPLC determination of terguride was investigated. Using an SGX CN column, various mixtures of methanol–phosphate buffer solution were tested as the mobile phase. A pH of 7.0 of the phosphate buffer solution proved to be the optimum. For the terguride assay procedure a mobile phase of methanol–phosphate buffer (80:20, v/v) was found to permit adequate resolution of terguride and the internal standard (promethazine chloride) in reasonable time. The retention times were 3.1 and 7.0 min for terguride and the internal standard, respectively (Fig. 2A).

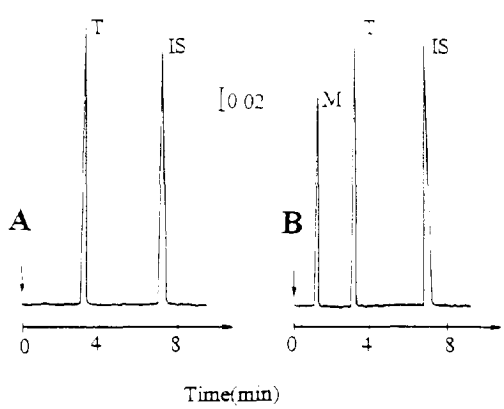

Fig. 2. Typical chromatograms for terguride (T) and the internal standard (promethazine chloride, I.S.). (A) Standard solution of terguride base (5 μg/ml) and I.S. (30 μg/ml); (B) standard solution of terguride hydrogenmaleate (7 μg/ml) and I.S. (30 μg/ml). M = maleate. Column, SGX CN (5 μm); mobile phase, methanol–0.0066 *M* phosphate buffer (pH 7) (80:20, v/v) at a flow-rate of 0.5 ml/min; detection wavelength, 227 nm; injection volume, 3 μl.

Quantification of terguride was based on least-squares linear regression analysis. Under the same chromatographic conditions terguride hydrogenmaleate was analysed (Fig. 2B). In the assay 1.341 mg of terguride hydrogenmaleate was equivalent to 1.000 mg of terguride base.

3.1. Analysis of tablets

This method was used for the analysis of coated tablets. No excipients interfered with the analyte drugs. The chromatogram of the extract from tablets is nearly coincident with that of terguride hydrogenmaleate in Fig. 2B. The calibration graph was linear over the concentration range studied; the regression equation was $y = 0.07235x - 0.07943$ ($r = 0.9998$).

Recovery studies over the range of 80–120% of the label claim were performed by adding known amounts of terguride to the amounts of the excipient mixtures equivalent to those used in the tablets. These samples were extracted as described above and terguride was determined from the peak-area ratios. The results of the recovery studies (see Table 1) show that quantitative recovery is obtained and the method is accurate and precise. The precision is given by the standard deviation (S.D.) of the recoveries and accuracy is represented by the 100% value of the recovery which is included between the confidence limits of the average value.

The analysis of Mysalfon Spofa tablets (strength 0.100 and 0.500 mg per tablet) was performed. The mean results were 99.5% and 99.4% terguride, respectively (average of five determinations, range 98.54–100.81% in both

Table 1
Recovery of terguride added to a mixture of excipients

Amount added (μg)	Recovery (mean ± S.D., $n = 5$) (%)
80.0	100.3 ± 1.5
100.0	99.7 ± 1.6
120.0	99.7 ± 1.7
400.0	99.6 ± 1.3
500.0	100.2 ± 1.0
600.0	100.2 ± 1.1

assays) and the coefficients of variation (C.V.) were always less than 1.7%.

3.2. Analysis of plasma

The determination of terguride in rat plasma was examined after its extraction into an organic solvent. The plasma sample was extracted into methylene chloride without pH adjustment and with addition of sodium hydroxide solution (5 mol/l), but the recovery was very low (about 60%). An attempt was made to adjust the plasma sample by precipitation of proteins. Methanol and acetonitrile were tested as protein precipitants. Acetonitrile produced higher recovery values and better protein precipitation than methanol. Using acetonitrile, the recovery of terguride in plasma ranged from 91.6% to 86.2% (Table 2). The supernatant was kept in the dark at room temperature.

Even though acetonitrile was used for the adjustment of plasma samples, it was nevertheless necessary to change the ratio of methanol and phosphate buffer solution in the mobile phase in order that the peak of the plasma residue did not interfere with the peak of terguride. Therefore, a mobile phase of methanol–phosphate buffer (70:30, v/v) was used for the determination of terguride in plasma, the other chromatographic conditions, including the internal standard (promethazine), remaining the same. Peaks corresponding to terguride and promethazine were eluted in 4.2 and 9.4 min, respectively. Fig. 3 shows typical chromatograms for blank and control plasma samples.

The calibration graph for plasma spiked with 1–160 μg/ml of terguride was linear; the regression equation was $y = 0.0947x - 0.0294$ ($r = 0.9997$). The within-day precision was calculated from the analysis of five plasma samples at four terguride concentrations. The day-to-day reproducibility was investigated during a 4-week period. Measured concentrations and C.V.s are given in Table 3; the C.V.s were all less than 5%. The limit detection for terguride in plasma was 60 ng/ml and the limit of quantification was 160 ng/ml.

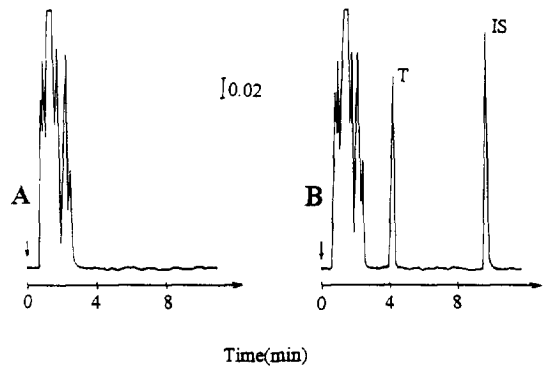

Fig. 3. Typical chromatogram for terguride (T) and the internal standard (I.S.) in rat plasma. (A) Bank plasma; (B) control sample spiked with standard solutions of terguride (8 μg/ml) and internal standard (40 μg/ml). Column, SGX CN (5 μm); mobile phase, methanol–0.0066 *M* phosphate buffer (pH 7) (70:30, v/v) at a flow-rate of 0.5 ml/min; detection wavelength, 227 nm; injection volume, 10 μl.

Table 2
Recovery of terguride from rat plasma

Concentration (μg/ml)	Recovery (mean ± S.D., $n = 5$) (%)
8.0	86.21 ± 2.1
80.0	90.73 ± 1.8
140.0	91.46 ± 2.3

Table 3
Assay precision and accuracy of determination of terguride in rat plasma

Concentration (μg/ml)		*n*	C.V. (%)
Actual	Found (mean ± S.D.)		
Within-day			
4.0	4.1 ± 0.2	5	3.9
20.0	20.9 ± 0.5	5	2.4
80.0	80.8 ± 2.2	5	2.7
140.0	142.3 ± 2.1	5	2.4
Day-to-day			
4.0	4.1 ± 0.2	13	4.2
20.0	21.1 ± 0.6	13	2.9
80.0	82.3 ± 2.4	13	2.9
140.0	142.8 ± 3.5	13	2.5

3.3. Separation from other ergot alkaloids

The method was applied to the separation of terguride from some other ergot alkaloids, as shown in Fig. 4. Simply changing the mobile phase composition to methanol–phosphate buffer (60:40, v/v) makes it possible to separate terguride from lisuride and ergotamine. The retention times were 7.2, 5.1 and 4.2 min, respectively.

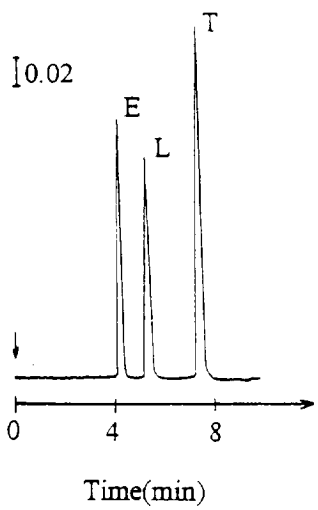

Fig. 4. Typical chromatogram for the separation of terguride (T) (5 μg/ml), lisuride (L) (3 μg/ml) and ergotamine (E) (5 μg/ml). Column, SGX CN (5 μm); mobile phase, methanol–0.0066 *M* phosphate buffer (pH 7) (60:40, v/v) at a flow-rate of 0.5 ml/min; detection wavelength, 227 nm; injection volume, 3 μl.

4. Conclusions

An HPLC method for the determination of terguride in pharmaceutical preparations and plasma samples was developed. The method was used to assay commercially available tablets, but it can also be applied to the assay of terguride in pharmaceutical injection formulations. It was possible to use the proposed HPLC method for the determination of terguride in plasma for the determination of actual plasma terguride levels or in the pharmacokinetic studies of terguride. The pharmacokinetic profile and metabolism of terguride will be considered in a future paper.

References

[1] A. Černý, J. Beneš, J. Vachek, M. Pešák, J. Stuchlík, M. Stuchlík, P. Sedmera, M. Flieger and J. Vokoun, *Collect. Czech. Chem. Commun.*, 52 (1987) 1331.
[2] L. Trejbalová, *Farmakoterap. Zprávy, Spofa*, 36 (1990) 55.
[3] B. Kakáč and J. Vejdělek, *Handbuch der Photometrischen Analyse Organischer Verbindungen*, Verlag Chemie, Weinheim, 1974, pp. 766–767.
[4] J. Vachek and E. Svátek, *Česk. Farm.*, 33 (1984) 10.
[5] R. Lapka, S. Šmolík and Z. Franc, *J. Pharmacol. Methods*, 11 (1984) 263.