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Note

Determination of lenperone in dog plasma by high-performance liquid chromatography

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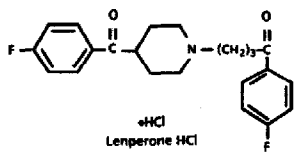
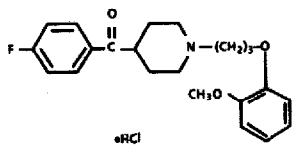
4′-Fluoro-4-[4-(*p*-fluorobenzoyl)piperidino]butyrophenone hydrochloride, lenperone, is a centrally acting drug related to the butyrophenone tranquilizers (Fig. 1). This compound has been synthesized [1], investigated pharmacologically [2] and found to be very effective as a major tranquilizer [3]. It has

LENPERONE



4′-fluoro-4-[4-(p-fluorobenzoyl)piperidino]butyrophenone hydrochloride

INTERNAL STANDARD



4-(p-fluorobenzoyl)-1-[3-(0-methoxyphenoxy)propyl]piperidine hydrochloride

Fig. 1. Chemical structures of lenperone and the internal standard.

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also been used in clinical trials for the treatment of anxiety and acute and chronic schizophrenia [4—9]. Lenperone is currently used either as an injectable or oral tranquilizer in dogs, cats, and swine [10].

In order to support the development of a new formulation, a selective high-performance liquid chromatographic (HPLC) method for detection of lenperone in dog plasma was developed. The method of assay and its application to analysis of samples obtained from a bioequivalence study in twenty dogs is described herein.

EXPERIMENTAL

Reagents

Acetonitrile, monobasic sodium phosphate, phosphoric acid, ammonium hydroxide and sulfuric acid (analytical-reagent grade) were all purchased from Baker (Phillipsburg, NJ, U.S.A.). Hexane and methanol (analytical-reagent grade) were purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Lenperone and 4-(*p*-fluorobenzoyl)-1-[3-(O-methoxyphenoxy)propyl]piperidine hydrochloride (internal standard) were synthesized by the A.H. Robins Company (Richmond, VA, U.S.A.).

High-performance liquid chromatography

The HPLC system consisted of a solvent delivery system (Waters Model M6000A; Waters Assoc., Milford, MA, U.S.A.), an autosampler (Waters, WISP Model 710B), and a fixed-wavelength detector (Waters Model 440).

The chromatographic separation was achieved on a 10-μm, 30 cm × 3.9 mm I.D., C_{18}, reversed-phase Bondapak column (Waters). The mobile phase used with this column was acetonitrile—0.05 *M* phosphate buffer (35:65). The mobile phase was adjusted to pH 3.5 and was delivered at a flow-rate of 1.5 ml/min at ambient temperature. The compounds eluted were detected by means of their ultraviolet absorbance at 254 nm. The output signal generated by the spectrophotometric detector was processed by a labortory system (Computer Inquiry Systems, Walwick, NJ, U.S.A.) automated by computer (Hewlett-Packard Model 1000; Hewlett-Packard, Palo Alto, CA, U.S.A.). The chromatographic tracings were recorded on a 10-mV chart recorder (Hewlett-Packard).

Standard solutions

Stock solutions, corresponding to 200 μg/ml in methanol, were prepared for lenperone and the internal standard. Standard solutions for lenperone were prepared at concentrations ranging from 5 to 160 ng/ml by spiking drug-free dog plasma with the appropriate concentrations of the drug. The stock solution of the internal standard was diluted in methanol to 200 ng/ml before being used to spike plasma samples.

Extraction procedure

Fig. 2 is a flow diagram of the procedure used for extracting lenperone from dog plasma. Samples (1 ml) of plasma that contained either standard or unknown concentrations of lenperone were transferred into culture tubes (125×16 mm). Diluted internal standard (1 ml), 0.5 ml of 5% ammonium

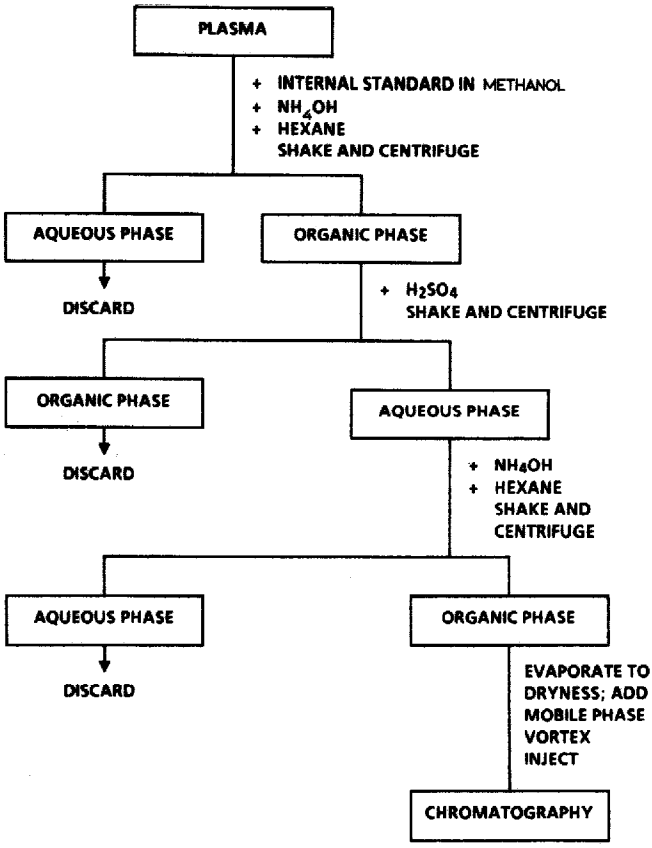

Fig. 2. Flow diagram of the procedure for the extraction of lenperone from dog plasma.

hydroxide and 8 ml of hexane were then added to each tube. The solutions were shaken for 10 min in a reciprocating shaker and centrifuged at 550 *g* for 5 min. The organic phase of the solution was then transferred to another culture tube (125X16 mm) that contained 1 ml of 0.05 *M* sulfuric acid. This solution was shaken for 10 min and centrifuged at 550 *g* for 5 min. The organic phase was then discarded. The remaining aqueous phase was made alkaline by the addition of 0.5 ml of 5% ammonium hydroxide. The drug and the internal standard were then back-extracted into 8 ml of hexane by shaking the solution for 10 min and centrifuging it at 550 *g* for 5 min. The organic phase was evaporated to dryness with a stream of nitrogen at 70°C, and the residue was reconstituted in 0.2 ml of mobile phase. A 100-μl volume of the solution was then injected into the HPLC system.

Precision, reproducibility and accuracy studies

To test the precision and reproducibility of this method, six individual standard curves for lenperone were run on consecutive days. The range of the curves was 0—160 ng/ml. The coefficient of variation was determined for the peak height of each curve at each concentration level; the slope, intercept and correlation coefficients of the daily standard curves were also calculated. The accuracy of the method was determined by assaying thirty randomized samples

spiked with various concentrations of lenperone. The concentrations of lenperone in these samples were unknown to the analyst at the time of analysis.

RESULTS AND DISCUSSION

Typical chromatograms for drug-free dog plasma, drug-free dog plasma spiked with internal standard and drug-free dog plasma spiked with 5 ng/ml lenperone and 200 ng/ml internal standard are shown in Fig. 3. The chromatograms for drug-free dog plasma showed no interference peaks at the retention times of lenperone and the internal standard.

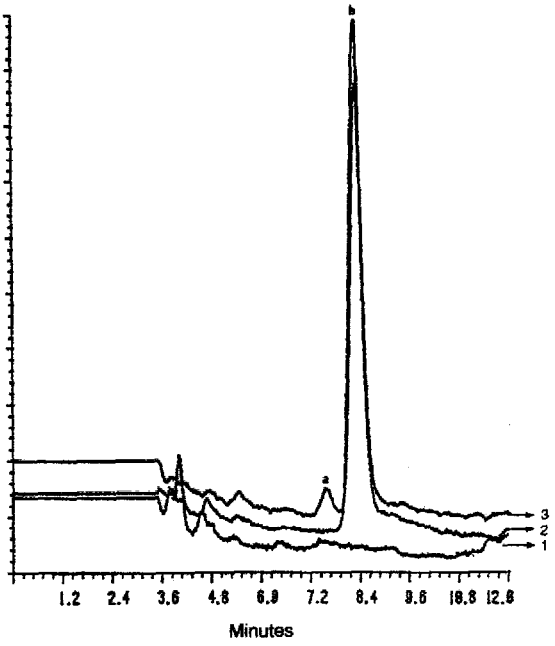

Fig. 3. Multiple plot of three chromatograms obtained from extracts of: (1) drug-free dog plasma; (2) drug-free dog plasma spiked with 200 ng/ml internal standard; (3) drug-free dog plasma spiked with 5 ng/ml lenperone (a) and 200 ng/ml internal standard (b).

TABLE I

DETERMINATION OF UNKNOWN AMOUNTS OF LENPERONE ADDED TO DOG PLASMA

Concentration added (ng/ml)	n	Concentration found (mean ± S.D.) (ng/ml)	Coefficient of variation (%)	Found (%)
0	4	BQL*	–	–
5	6	5.9 ± 0.7	11.2	117.6
10	4	9.6 ± 0.8	7.8	96.4
25	4	25.1 ± 0.7	2.8	100.4
75	4	81.2 ± 3.9	4.9	108.3
100	4	103.1 ± 3.0	2.9	103.1
150	4	163.0 ± 4.6	2.8	108.7

*BQL = Below quantifiable limit, i.e. mean concentration is less than 5 ng/ml.

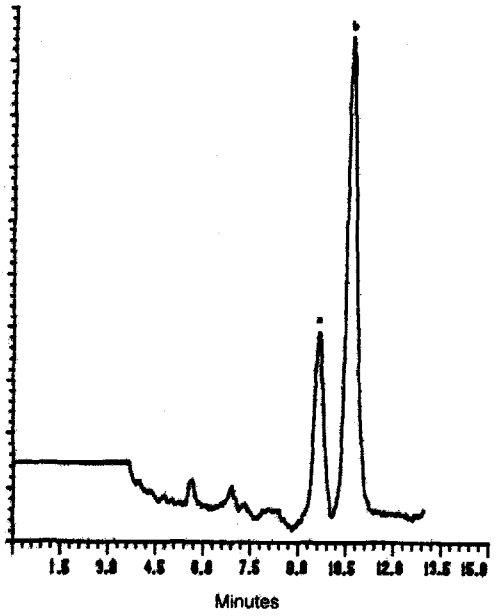

Fig. 4. Chromatogram of extracts from dog plasma 2 h after an oral dose of 10 mg of lenperone (tablet). Peaks: a = lenperone (38.4 ng/ml); b = internal standard (200 ng/ml).

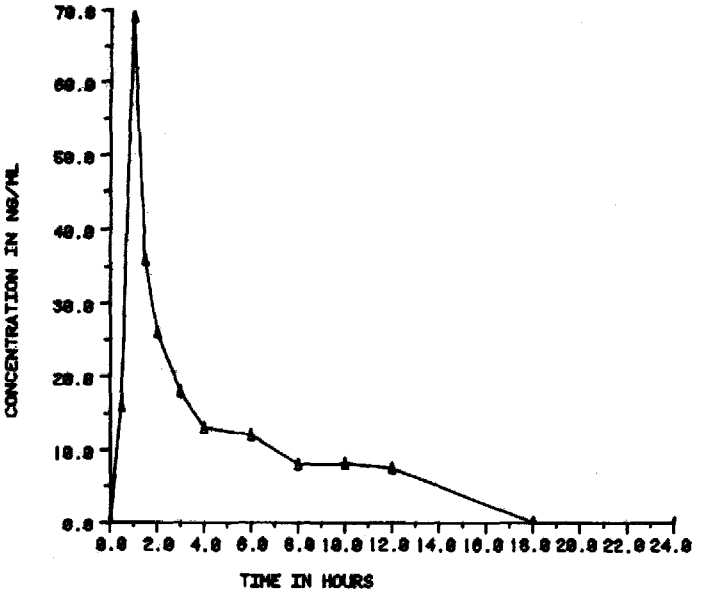

Fig. 5. Plasma concentration—time curve of lenperone after a single oral dose of 10 mg (two 5-mg tablets).

The results of the six consecutive standard curves for lenperone were linear between 0 and 160 ng/ml with a slope of 0.011 ± 0.0002. Good reproducibility was reflected by the correlation coefficient: r=0.9993 ± 0.0002. The intercept values of the regression lines were always negligible. The peak height of the curves for lenperone showed a coefficient of variation that was below 10% in the concentration range 5—160 ng/ml.

Table I shows the recovery values for the thirty spiked samples. The percentage lenperone found was generally within ±10% of the theoretical concentration added. When testing the accuracy of the method at the 5 ng/ml level, the lower limit of quantitation, the percentage found was 18% higher than the theoretical concentration added. The recovery of lenperone by the triple extraction steps used in this method was 85%.

This method was employed in the analysis of plasma samples obtained from a bioequivalence study in beagle dogs. Fig. 4 shows a chromatogram for extracts of a plasma sample obtained from a dog 2 h after the administration of a single, 10-mg, oral dose of lenperone. Fig. 5 shows a typical drug concentration–time profile for lenperone in one of the male dogs used in the study.

CONCLUSIONS

A sensitive and selective HPLC method was developed for the determination of lenperone in dog plasma. The concentration–response curve was linear from 5 to 160 ng/ml, when 1 ml of plasma was assayed. The method has a high degree of precision and accuracy. It was utilized successfully in the analysis of plasma samples obtained from a bioequivalency study of a new veterinary dosage form.

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