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Note

Determination of duoperone in plasma by high-performance liquid chromatography

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(4-Fluorophenyl)[1-[3-[2-(trifluoromethyl)-10H-phenothiazin-10-yl]propyl]-4-piperidinyl]methanone (*E*)-2-butenedioate (1:1) (duoperone, Fig. 1) is a unique member of the phenothiazine class of compounds having a side-chain identical to that found in the tranquilizer lenperone [1]. Duoperone is a neuroleptic agent with an unusually long-acting duration of action, equalling or exceeding that of penfluridol. It is also effective as an antiemetic agent [2] in animal models.

In order to support the preclinical pharmacokinetic studies and the phase I tolerance studies in human, a selective high-performance liquid chromatographic (HPLC) method for duoperone in plasma was developed. The method was successfully applied to animal studies which will be described herein.

EXPERIMENTAL

Reagents

Acetonitrile, monobasic sodium phosphate and phosphoric acid were all purchased from Baker (Phillipsburg, NJ, U.S.A.). Hexane and acetone (analytical-reagent grade) were purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Duoperone and (4-fluorophenyl)[1-[4-[2-(trifluoromethyl)-10H-phenothiazin-10-yl]butyl]-4-piperidinyl]methanone (*E*)-2-butenedioate (1:1) (internal standard) (Fig. 1) 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 510; Waters Assoc., Milford, MA, U.S.A.), an autosampler (Waters, WISP Model

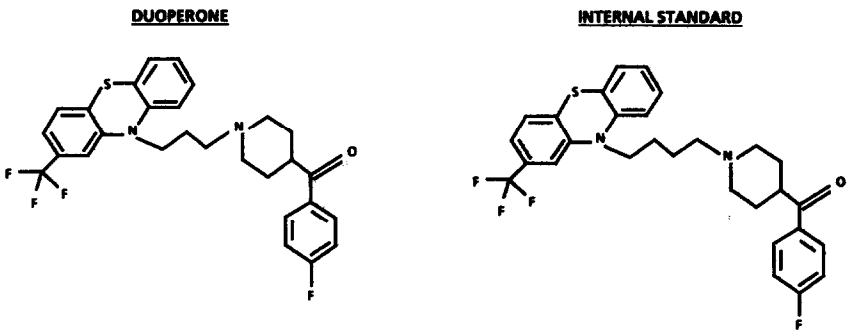

Fig. 1. Chemical structures of duoperone and the internal standard.

710B), a spectrofluorometric detector (Schoeffel Model 970, Schoeffel, Westwood, NJ, U.S.A.) and a column thermostat from Bioanalytical Sciences (West Lafayette, IN, U.S.A.).

The chromatographic separation was achieved on a 10-μm, 30 cm $\times$ 3.9 mm I.D. reversed phase μBondapak C_{18} column (Waters) and the column was kept at 40°C. The mobile phase used with this column was acetonitrile–0.04 *M* monobasic sodium phosphate–phosphoric acid (55:44.7:0.3) and it was delivered at a flow-rate of 1.5 ml/min. The compounds eluted were detected by a spectrofluorometric detector with excitation wavelength set at 258 nm and the emission wavelength set by a KV 470 filter. The output signal generated by the spectrofluorometer was processed by a computer-automated laboratory system (Computer Inquiry Systems, Walwick, NJ, U.S.A.) and Hewlett-Packard Model 1000 computer (Palo Alto, CA, U.S.A.). The chromatographic tracings were recorded on a 10-mV chart recorded (Hewlett-Packard).

Standard solutions

Stock solutions, corresponding to 100 μg/ml in methanol, were prepared for duoperone and the internal standard. Working solutions of duoperone at concentrations of 0.25, 0.5 and 1.0 μg/ml were prepared in the mobile phase. Different volumes of these working solutions were added to drug-free plasma to obtain standards ranging from 1 to 20 ng/ml. The stock internal standard solution was diluted with mobile phase to obtain the 100 ng/ml working solution.

Extraction procedure

A 1-ml volume of the standard or unknown plasma sample was transferred to a 125 $\times$ 16 mm culture tube containing 0.1 ml of the 100 ng/ml internal standard solution. A 5-ml volume of a hexane–acetone (1:1) solution was added to each tube. The solutions were mixed vigorously on a vortex mixer for 30 s centrifuged at 550 *g* for 5 min. The organic phase was transferred to a clean 15-ml centrifuge tube and evaporated to dryness with a stream of nitrogen at 50°C. Hexane (2 ml) and mobile phase (0.15 ml) were added to each tube. The solution was mixed thoroughly for 5 s and the two phases were separated by centrifugation as described before. The hexane (top) layer was aspirated off and discarded. The mobile phase

(bottom) layer was evaporated at 50°C under nitrogen. The dried residue was reconstituted in 0.2 ml of the mobile phase and 0.15 ml of it were injected into the HPLC system.

Precision, reproducibility and accuracy studies

To test the precision and reproducibility of this method, six standard curves for duoperone were run on consecutive working days. The range of the curves was 0–20 ng/ml. The coefficient of variation of the peak-height ratio was determined at each concentration level of the standard curve. The slope, intercept and the correlation coefficient of the daily standard curves were also calculated.

The accuracy of the method was determined by assaying 36 randomized samples prepared with various concentrations of duoperone.

Pilot study in dogs

A pilot study was conducted in dogs to evaluate the analytical method. After an overnight fast, one male and one female beagle dog were dosed with 2 mg/kg duoperone dissolved in 75% polyethylene glycol 300 in water. Blood samples were drawn over 48 h after oral administration. The plasma samples were assayed for duoperone.

RESULTS AND DISCUSSION

Duoperone is highly bound to plasma proteins. It cannot be quantitatively extracted from plasma even with polar solvent such as ethyl acetate. A mixture of acetone–hexane (1:1) is required to denature the proteins and extract the drug from the precipitate. However, when the extraction solvent is evaporated to dryness, a copious yellow residue was left behind which is insoluble in the mobile phase. An extra step of re-dissolving the lipoprotein residue in a small volume of hexane and back-extracting the drug and internal standard into the mobile phase is needed.

Although the sample preparation procedure is rather cumbersome, the extraction efficiency of duoperone is still over 80%. Fig. 2 shows typical chromatograms of drug-free plasma spiked with the internal standard and duoperone. The chromatogram of drug-free plasma shows no interference peaks at the retention time of duoperone.

The results of the six consecutive standard curves for duoperone were linear between 1 and 20 ng/ml with a slope of 0.121 ± 0.014 and a correlation coefficient of 0.997 ± 0.003. The intercept values of the regression lines were always negligible. The coefficient of variation of the average peak-height ratio in the six standard curves was generally less than 12%.

Table I shows the recovery values for the 36 spiked samples. The percentage duoperone found was generally within 5% of the theoretical concentration added.

Plasma samples obtained from dogs after oral administration of duoperone were analyzed by the method described above. Fig. 3 shows the chromatograms of the plasma samples obtained at 0 and 2 h after dosing. A few samples from the early time intervals would need dilution in order to bring the assay concentrations

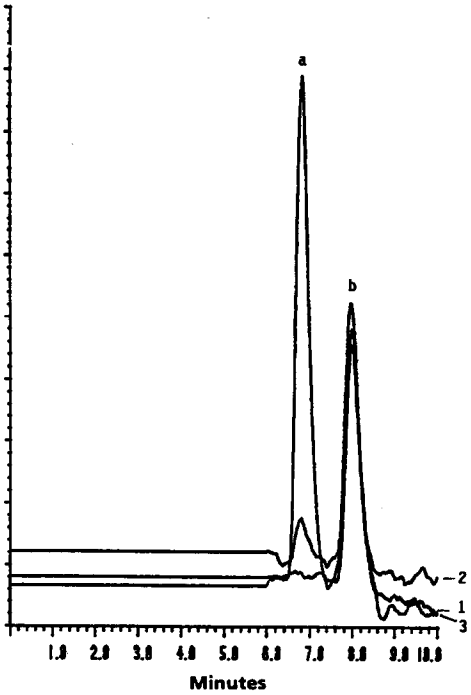

Fig. 2. Multiple plot of three chromatograms obtained from extracts of (1) drug-free plasma spiked with 0 ng/ml duoperone (a) and 10 ng internal standard (b), (2) drug-free plasma spiked with 2 ng/ml duoperone (a) and 10 ng internal standard (b) and (3) drug-free plasma spiked with 20 ng/ml duoperone (a) and 10 ng internal standard (b).

within the standard curve. Otherwise, the concentrations of most of the samples in other time intervals were within the linearity range of the method. No other drug-related peaks were observed in the chromatograms.

Fig. 4 shows the drug concentration–time profile for duoperone in a male beagle dog.

TABLE I

DETERMINATION OF UNKNOWN AMOUNTS OF DUOPERONE ADDED TO PLASMA

Concentration added (ng/ml)	*n*	Concentration found (mean ± S.D.) (ng/ml)	Coefficient of variation (%)	Found (%)
0	4	BQL*	–	–
1.17	6	1.2 ± 0.28	23.3	102.6
2.34	6	2.4 ± 0.25	10.4	102.6
3.90	4	4.0 ± 0.30	7.5	102.6
7.80	6	8.2 ± 0.89	10.9	105.1
12.68	4	12.7 ± 0.98	7.7	100.2
19.50	6	19.9 ± 2.36	11.9	102.1

*BQL = below quantifiable limit, i.e. concentration < 1 ng/ml.

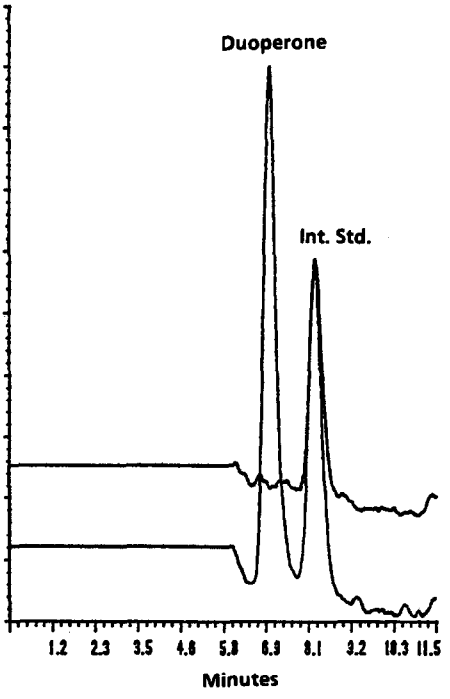

Fig. 3. Chromatograms of extracts of plasma obtained at 0 and 2 h after a dog was given 2 mg/kg duoperone orally.

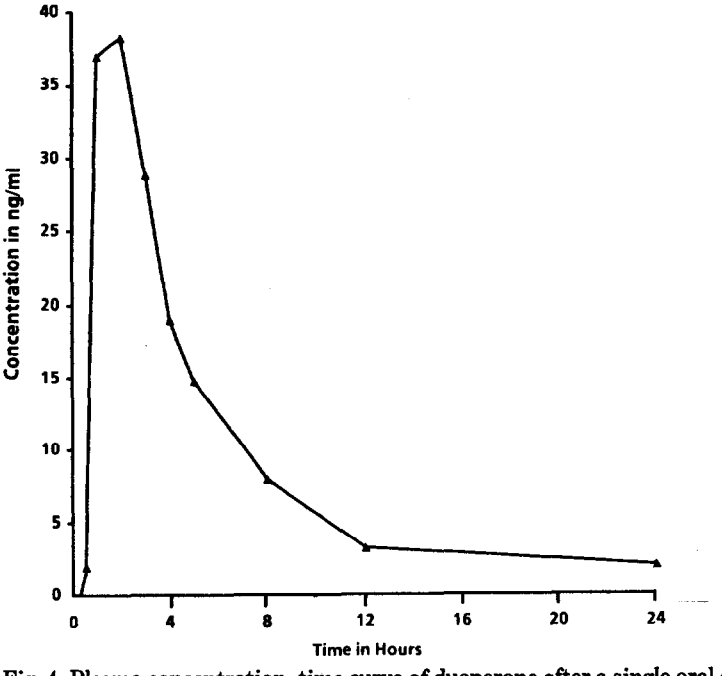

Fig. 4. Plasma concentration–time curve of duoperone after a single oral dose of 2 mg/kg.

CONCLUSION

A sensitive and selective HPLC method was developed for the determination of duoperone in plasma. The concentration–response curve was linear from 1 to at least 20 ng/ml when 1 ml of plasma was used for the assay. This method shows good precision and accuracy. It was utilized successfully in determining low nanogram levels of duoperone in plasma.

REFERENCES

1 R.F. Boswell, Jr., W.J. Welstead, Jr., R.L. Duncan, Jr., D.N. Johnson and W.H. Funderburk, J. Med. Chem., 21 (1978) 136–139.
2 D.N. Johnson, B.G. Turley, M.R. Jones, C.B. Coffin, C.A. Leonard and W.H. Funderburk, Prog. Neuro-Psycopharmacol., 3 (1979) 513–520.