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Note

High-performance liquid chromatography of droperidol in whole blood

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Droperidol, 1-{1-[4-(*p*-fluorophenyl)-4-oxobutyl]-1,2,3,6-tetrahydro-4-pyridyl}-2-benzimidazolinone, belongs to the butyrophenone class of drugs used in psychiatry and anaesthesiology. Various methods of determining droperidol are available: colorimetry [1], spectrophotometry [2], fluorimetry [3,4], gas chromatography [5,6] and thin-layer chromatography [7,8]. Two high-performance liquid chromatographic (HPLC) methods, to our knowledge, have been reported [9,10]. One of these methods was used for the separation of nine butyrophenones, including droperidol [10], whilst the other was used for the determination of droperidol in pharmaceutical preparations [9]. HPLC has not yet been used for the determination of droperidol in whole blood. We describe here a selective and reproducible HPLC method for the determination of droperidol in blood. It is also applicable to the analysis of trifluperidol and haloperidol.

EXPERMINENTAL

Reagents and standards

Droperidol and its metabolites, trifluperidol hydrochloride and haloperidol, were all gifts of Janssen Pharmaceutica (Beerse, Belgium). Diltiazem hydrochloride was a gift from Goedecke (Berlin, F.R.G.). Acetonitrile, HPLC grade, was purchased from May and Baker (Dagenham, U.K.). *n*-Butyl chloride (synthesis grade, Merck) was distilled before use. All other chemicals were analytical reagent grade obtained from usual commercial sources.

Stock standard solutions of droperidol, trifluperidol, haloperidol and diltiazem (1 mg/ml calculated as free base) were prepared in ethanol and stored at −15°C.

Phosphate buffer was prepared by dissolution of 1.15 g ammonium dihydrogenphosphate in 1 l of water. Triethylamine (600 μl) was added and the pH adjusted to 3.75 with 1% orthophosphoric acid. This buffer was used in the

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preparation of the HPLC mobile phase and was prepared fresh each day

Blood droperidol standards over a concentration range of 10–50 ng/ml were prepared by supplementing blank transfusion blood with appropriate volumes of 2 μg/ml droperidol solution prepared by diluting stock standard solution with 0.09 *M* phosphoric acid.

Apparatus

The HPLC system consisted of an HP1050 quaternary pump, an HP1050 multiple-wavelength detector, and HP1050 autosampler with a high-pressure sampling valve (1–100 μl) supplied by Hewlett Packard (Waldbronn, F.R.G.) and a 220 mm × 4.6 mm I.D. C_{18} bonded 5-μm silica reversed-phase column (Brownlee Spheri 5) operated at room temperature. The retention times (in minutes) and peak height were measured using a Hewlett Packard HP3396A reporting integrator. The flow-rate was 1.2 ml/min and the UV detector was set at 248 nm.

The mobile phase consisted of 40% (v/v) phosphate buffer in acetonitrile. The overall performance of the system was verified periodically by injection of droperidol, haloperidol, trifluperidol and diltiazem standards.

Quantitation was effected using authentic spikes in blank transfusion blood and diltiazem as internal standard in the preparation of a calibration curve. Unknown concentrations were calculated using linear regression.

Procedure

A 2-ml volume of blood (samples, blood standards or blanks) and 60 ng of diltiazem (10 μl of 6 μg/ml solution) were added in succession to PTFE-lined screw-top glass test tubes, mixed and allowed to stand for 5 min. Concentrated ammonia (0.4 ml) was added followed by 3 ml of butyl chloride. The resultant mixture was vortex-mixed for 2 min. After centrifugation for 10 min at 2140 *g*, the upper organic layer was transferred to a clean tube. The extraction was repeated with a further 3 ml of *n*-butyl chloride. The combined organics were concentrated to about 2 ml under nitrogen at 40°C and 100 μl of 0.09 *M* phosphoric acid were added. The resultant solution was vortex-mixed for 1 min, centrifuged for 10 min and the upper organic phase aspirated and discarded. The tube was heated briefly at 40°C under nitrogen to remove residual butyl chloride and 30-μl portions of the remainder were subjected to chromatography.

RESULTS AND DISCUSSION

Preliminary work performed on gas chromatography with nitrogen–phosphorus detection failed to achieve sufficient sensitivity for droperidol at therapeutic levels. An alternative HPLC method was thus investigated.

HPLC mobile phase

The object in optimising the chromatography conditions was to achieve the elution of droperidol in a reasonable time with good peak shape using an isocratic system. The addition of a small amount of triethylamine to the mobile phase improved the chromatography markedly and resulted in both rapid elution and a symmetrical peak shape for droperidol.

The effect of pH, over the range 3.75–7.00, on the chromatography was studied. There is little difference between pH 3.75 and 7 in terms of retention time and peak shape, the capacity factors (k') being 1.87 and pH 3.75 and 2.04 at pH 6.8. A working pH of 3.75 was chosen because it gave a shorter retention time without loss of selectivity.

Method evaluation

Fig. 1 shows the chromatograms obtained from a blood blank, blood blanks spiked at 4 and 10 ng/ml droperidol and a patient's blood. The separation of droperidol and the internal standard, diltiazem, from endogenous substances and available metabolites of droperidol was good.

For the determination of droperidol, the method of internal standardization was used. A graph of the peak height of droperidol relative to that of diltiazem against drug concentration was linear over a range of at least 10–50 ng/ml with a correlation coefficient of 0.99. The precision and accuracy of the method are presented in Tables I and II.

The mean absolute recovery achieved with the described extraction procedure

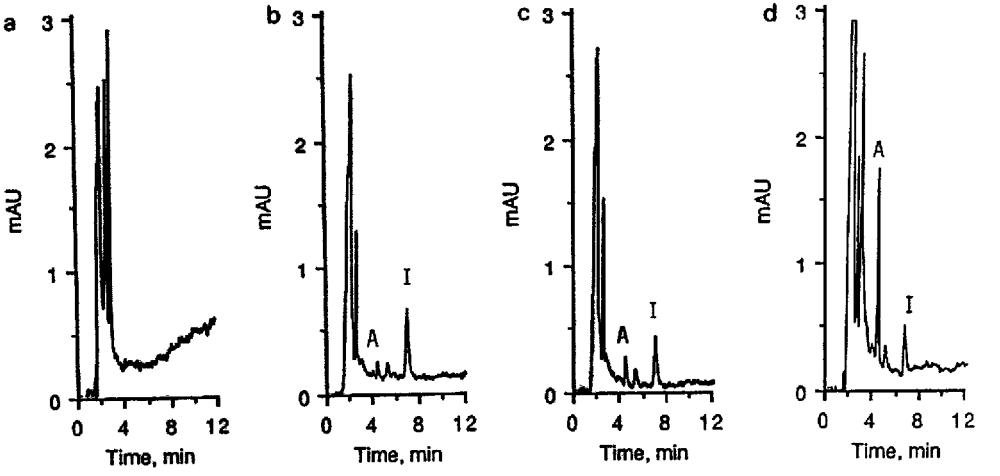

Fig. 1. High-performance liquid chromatography of (a) blank blood, (b) 4 ng/ml droperidol in blood, (c) 10 ng/ml droperidol in blood and (d) patient's blood. Peaks: A = droperidol, I = internal standard (diltiazem).

TABLE I

PRECISION AND EXTRACTION RECOVERY

Concentration added (ng/ml)	Mean concentration found (ng/ml)	Recovery (%)	Coefficient of variation (%)	*n*
Within-day				
4.40	3.32	75.5	7	6
8.80	5.55	63.1	3	6
50.00	43 07	86.1	2	4
Day-to-day				
4 40	2.87	65.2	7	6
8.00	5 79	65.8	7	6

TABLE II

ACCURACY OF THE METHOD

Concentration added (ng/ml)	Concentration determined (ng/ml)	Coefficient of variation (%)	*n*
4.40	4.10	10	6
44.0	42.0	5	5

was 76%, for both droperidol and the internal standard, but was compensated by carrying blood standards through the extraction procedure.

The sensitivity of a method is often expressed as the limit of detection (LOD). The LOD for spectrometric analysis is defined as the standard deviation of the baseline noise [11]. The measurement of the baseline noise is not ambiguous and is useful only if there is no interference from the matrix. If, for example, blood samples are analysed, there are normally peaks present from endogenous compounds, the number and amounts varying between samples. This results in poor repeatability at amounts near the LOD.

A more useful way to describe the sensitivity could be to calculate the instrumental limit of detection (ILOD) and the limit of quantitation (LOQ) [11]. The ILOD is the amount of an analyte giving a peak height three times the standard deviation of the baseline noise without any matrix interference, whereas the LOQ is the concentration of an analyte in the matrix that could be determined with a reasonable precision under the given analytical procedure. In our case the ILOD for droperidol was found to be 1.2 ng. The LOQ was *ca.* 4.0 ng/ml for a 2-ml blood sample.

Droperidol blood levels

The blood levels found post-mortem in a 29-year-old schizophrenic male who died of cardiac arrest following the administration of a therapeutic dose of droperidol (5 mg intramuscular) are shown in Table III.

To our knowledge no therapeutic whole blood levels have been reported. The results obtained are in the range of therapeutic droperidol plasma levels reported by Cressman *et al.* [12]. The variation in blood levels may be due to intersite variation or post-mortem redistribution [13].

Application

In order to examine the application of this method to analogues of droperidol, such as haloperidol and trifluperidol, the sensitivities, retention times and extraction recoveries for these two drugs were determined by the above method and the results are give in Table IV. Fig. 2 shows a chromatogram of a blank blood sample containing droperidol, haloperidol and trifluperidol.

As shown in Fig. 2, the peaks of the analogues were not subject to interference by endogenous substances from blank transfusion blood. Diltiazem is, however, not suitable as an internal standard for haloperidol quantitation. It therefore seems that the method is applicable to the determination of haloperidol and trifluperidol in blood but with slightly reduced sensitivity.

TABLE III

POST-MORTEM BLOOD LEVELS OF DROPERIDOL FOUND IN A MALE WHO DIED FOLLOWING THE ADMINISTRATION OF A THERAPEUTIC DOSE

Blood sample (site)	Droperidol level (ng/ml)
Heart	11
Unknown	46
Unknown	40

TABLE IV

RETENTION TIMES, EXTRACTION RECOVERIES AND RELATIVE PEAK-HEIGHT RATIOS OF DROPERIDOL, HALOPERIDOL AND TRIFLUPERIDOL

Compound	Retention time (min)	Recovery (%)	Relative peak-height ratio[a]
Droperidol	4.4	76	1.00
Haloperidol	7.4	88	0.85
Trifluperidol	8.7	84	0.69
Diltiazem	7.1	76	–

[a]Ratio of peak height of the compound to that of droperidol at equivalent concentration.

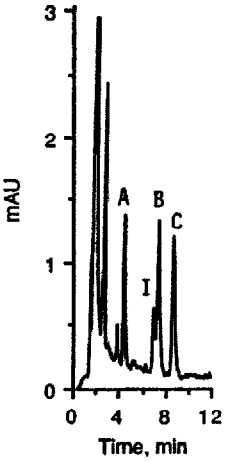

Fig 2. HPLC of droperidol (A), haloperidol (B), trifluperidol (C) and internal standard (I) All drugs were spiked at 50 ng/ml except diltiazem (30 ng/ml).

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