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## Note

# Measurement of haloperidol in human plasma using reversed-phase high-performance liquid chromatography

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Dosage and plasma concentrations of the potent antipsychotic drug haloperidol vary over a range of almost two orders of magnitude with its present pattern of usage. Concentrations of 2—5 ng/ml seen after small dosages, push most analytical techniques to the limit of their sensitivity. Despite this, the relatively simple metabolism of haloperidol [1] makes it particularly attractive for studies relating plasma concentrations to various clinical and biochemical measures.

Gas chromatography using electron-capture [2] and nitrogen detectors [3, 4] as well as mass fragmentography [5, 6] have been applied to the measurement of haloperidol in plasma. Radioimmunoassay procedures have been reported [7, 8] and recently, Creese and Snyder [9] have published a radioreceptor binding assay for determing total plasma concentrations of active antipsychotic compounds as a class. We describe a high-performance liquid chromatography (HPLC) procedure for the quantitation of haloperidol concentrations in plasma*.

## MATERIALS AND METHODS

### *Apparatus*

We used a Series 2 high-pressure liquid chromatograph equipped with a Model 65T variable-wavelength detector (Perkin-Elmer, Norwalk, CT, U.S.A.),

* After submission of this manuscript, an HPLC procedure for haloperidol was published by Miyazaki et al. [10]. The above referenced procedure required an evaporation step, and as described with 250 nm detection, had a lower sensitivity limit of 5 ng/ml.

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a Model 7120 injector with a 100-μl loop (Rheodyne, Berkeley, CA, U.S.A.) and a 250-mm reversed-phase column (Bio-sil ODS-10 (10 μm), Bio-Rad Labs., Rockville Center, NY, U.S.A.). As alternatives, LiChrosorb RP-8 (10 μm), and Spherisorb C6 (5 μm) (Applied Science, State College, PA, U.S.A.) columns were also evaluated. Chromatograms were recorded on a Linear Instruments (Irvine, CA, U.S.A.) strip chart recorder set to 0.01 a.u.f.s.

*Reagents and standards*

Haloperidol and chlorohaloperidol were gifts of McNeil Pharmaceutical (Springhouse, PA, U.S.A.). Desipramine was a gift of USV Pharmaceutical Corp. (Scarsdale, NY, U.S.A.), Hexane, acetonitrile, and methanol, all distilled in glass, were purchased from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). Isoamyl alcohol, reagent grade, was redistilled in glass prior to use. All other reagents were obtained from usual commercial sources.

Stock standard solutions of haloperidol, chlorohaloperidol and desipramine (1 mg/ml calculated as free base) were prepared in acidified methanol (0.1 *M* hydrochloric acid in methanol) and stored at about —15°C.

Plasma standards over a concentration range of 2—30 ng/ml were prepared by appropriately supplementing drug-free plasma with 1:100 dilution of the stock standard in 0.01 *M* hydrochloric acid. Although we prepared the plasma standards freshly on each day of analysis, an aliquoted and frozen plasma control was stable at —15°C for at least six months.

A working internal standard(s) solution was prepared by adding 5 μl of desipramine and 10 μl of chlorohaloperidol stock solutions to 10 ml of 0.1 *M* hydrochloric acid.

Serum or plasma was stored frozen at —15°C until the time of analysis.

*Procedure*

Two ml of plasma or serum (samples, plasma standards, or controls) and exactly 100 μl of working internal standard solution were added in succession to PTFE-lined screw-top glass test tubes, mixed, and allowed to stand for 5 min. Following addition of 0.2 ml of 2 *M* sodium hydroxide, the sample was extracted for 20 min on a rotator with 10 ml of hexane containing 2% isoamyl alcohol. After centrifugation for 5 min, the organic (upper) phase was transferred to 12-ml PTFE-lined screw-capped conical centrifuge tubes. This can be accomplished with a pasteur pipette or, more efficiently, the tube can be immersed briefly in an acetone—dry ice bath, and the unfrozen supernatant organic phase decanted.

About 150 μl of 0.1 *M* hydrochloric acid were added and after vigorous mixing using a vortex type mixer, and a 5-min centrifugation, most of the organic (upper) layer was aspirated and discarded. Approximately 100 μl of the aqueous (hydrochloric acid) phase were submitted to chromatography.

*Chromatography conditions*

The mobile phase consisted of 40% (v/v) acetonitrile in phosphate buffer, pH 3.8 (6.5 g $KH_2PO_4$ in 1000 ml water adjusted to pH 3.8—4.0 with orthophosphoric acid). The optimal proportion of acetonitrile varied with the age and condition of the column. The column oven was maintained at 50°C. The

overall performance of the system was verified periodically by injecting about 20 ng each of a mixture of haloperidol, chlorohaloperidol and desipramine.

## RESULTS AND DISCUSSION

Fig. 1 illustrates chromatograms resulting from the analysis of serum from two patients who had been treated with haloperidol. Fig. 2 shows serial steady state concentrations in a patient maintained on a stable dose of haloperidol. Table I illustrates steady state levels in ten patients receiving various doses of haloperidol. Steady state concentrations are known to vary considerably between individuals on the same dosage. [11].

Peak height ratios of haloperidol to each of the internal standards showed a linear relationship to serum haloperidol concentration over a range of at least 2—60 ng/ml. While concentrations as low as 1 ng/ml could be detected, 2 ng/ml

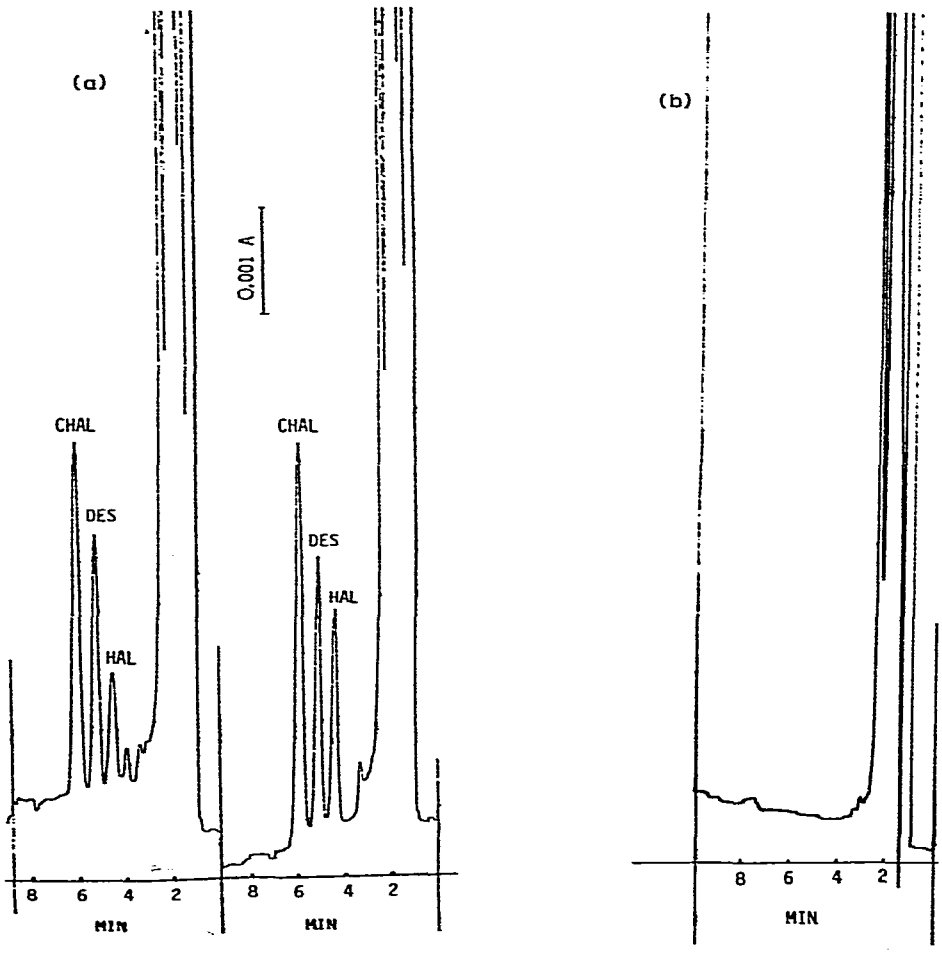


Fig. 1. (a) Chromatogram of extracts from patient sera containing 9.4 ng/ml (left) and 17.1 ng/ml (right) of haloperidol, respectively. Peaks: HAL = haloperidol; DES = desipramine; CHAL = chlorohaloperidol. (b) Chromatogram of extract from a blank (haloperidol-free) serum.

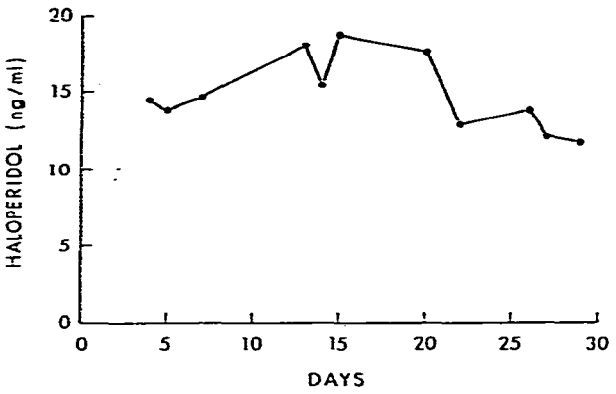


Fig. 2. Steady state serum haloperidol concentrations in a patient receiving 17 mg of drug per day. Values are a.m. trough concentrations, approximately 10 h after last dose.

TABLE I

ILLUSTRATIVE STEADY STATE TROUGH HALOPERIDOL CONCENTRATIONS

Values are trough concentrations collected a.m. before morning dose, approximately 10 h after last dose.

| Dose (mg/day) | Haloperidol concentration (mg/ml) |
|---|---|
| 10 | 3.8 |
| 10 | 7.8 |
| 12 | 7.7 |
| 12 | 5.9 |
| 15 | 17.5 |
| 17 | 17.2 |
| 20 | 6.0 |
| 40 | 32.1 |
| 40 | 32.2 |
| 80 | 105 |

is probably the lower limit for useful quantitation. For measurement of concentrations below 5 ng/ml, a UV detector capable of maintaining a reasonably stable and smooth baseline at 0.01 a.u.f.s., at 195 nm, is needed. Most modern variable-wavelength detectors can achieve this.

The within-day and between-day coefficients of variation were 5.0 and 7.3%, respectively, at a concentration of 5 ng/ml. Multiple drug-free sera were tested and found to be free of interfering peaks. Absolute uncorrected recovery achieved with the described double extraction procedure was 78%, but was compensated by carrying serum or plasma standards through the extraction procedure.

When measuring very low concentrations of drugs, trace contamination (endogenous or exogenous) can significantly distort the data. We have therefore found it useful to use two internal standards in that consistency in their peak height ratio provides assurance that otherwise undetectable interfering peaks

are not co-eluting with either internal standard. In the absence of interferences, either desipramine or chlorohaloperidol can be used to calculate haloperidol concentrations. Basic drugs tested that did not interfere included caffeine, nicotine, cogentin, codeine, meperidine, normeperidine, propoxyphene, chlordiazepoxide, norchlordiazepoxide, flurazepam, desalkylflurazepam, and various antihistamines. Nortriptyline eluted close to chlorohaloperidol, necessitating that desipramine be used as the internal standard. Concurrent administration of imipramine or desipramine of course necessitates use of chlorohaloperidol for quantitation. While chloropromazine itself did not interfere, the easily recognized cluster of chloropromazine metabolites may obscure the peaks of interest. Concurrent use of two neuroleptics is uncommon in clinical practice, and certainly not likely in clinical research studies.

We found that the ODS column provided the best overall performance in routine use. There are, however, selected instances of polypharmacy when the alternative columns are helpful. Using the ODS column, nordiazepam and diazepam were not adequately resolved from haloperidol and chlorohaloperidol, respectively. The presence of these interferences can be detected by distortion of the ratio between the two internal standards. Should it be necessary to measure haloperidol in patients receiving diazepam, this benzodiazepine and its major metabolite were well separated from haloperidol and chlorohaloperidol on a LiChrosorb RP-8 column. Similarly, diphenhydramine (Benadryl) co-eluted with haloperidol. Although alternative medications to diphenhydramine are readily available, it was separated from haloperidol (using 33% acetonitrile) on the Spherisorb C6 column, although retention times were much longer. Desipramine did not adequately separate from haloperidol on the C6 column or from nordiazepam on the RP-8 column. Therefore, with either alternative column only one internal standard (chlorohaloperidol) is suitable.

Various options are now available for measurement of haloperidol in plasma. Some radioimmunoassays have been reported to yield higher concentrations than gas—liquid chromatography [12] suggesting that the former may cross-react with one or more circulating metabolites. On the other hand, the receptor binding assay has been reported to produce higher concentrations than a radioimmunoassay [13]. The receptor binding assay [8] may be especially attractive for correlating total active drug concentration with various measures of pharmacological activity. On the other hand, a specific physical measurement (e.g., chromatography) is more desirable for any studies which include pharmacokinetic considerations.

Evaluating gas—liquid chromatography using a variety of columns we were troubled by apparent adsorptive losses, and non-linear recovery. We had much greater success with reversed-phase HPLC which was also more efficient in that sample preparation was simpler and no evaporation step was required.

Sufficient data are not yet available to support a major clinical role for the therapeutic monitoring of antipsychotic drugs. However, there are preliminary data to suggest that haloperidol may be a good candidate in this instance [13—16]. Certainly, its apparent lack of major active metabolites suggests that measurement of the parent compound may be useful in clinical correlation studies. The HPLC procedure described is sufficiently sensitive to cover the range of concentrations anticipated after clinical use.

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