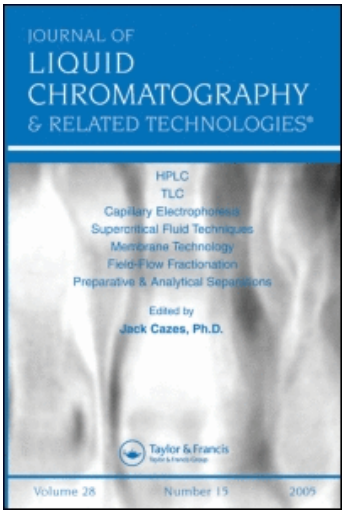

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High-Performance Liquid Chromatographic Assay for the Determination of Haloperidol in Plasma

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY FOR THE DETERMINATION OF HALOPERIDOL IN PLASMA

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ABSTRACT

A sensitive, reproducible and accurate high performance liquid chromatographic (HPLC) method for the quantitative determination of haloperidol in plasma has been developed and validated. Sample preparation involves extraction of haloperidol and diazepam (internal standard) from 0.5 mL plasma. The separation was carried out in a stainless steel, resolve C_{18} column with a mobile phase composed of a mixture of 55% methanol and 45% HPLC water containing 0.2 M ammonium acetate and adjusted to an apparent pH 7.2. The mobile phase was pumped at a flow rate of 1.5 mL/min. The column oven temperature was adjusted at 38°C and the effluent was monitored at 249 nm. The retention times for the internal standard and haloperidol were found to be 5.1 and 6.3 minutes, respectively. Peak-height ratios of the drug to the internal standard were used for the quantification of haloperidol in the plasma samples. The average (±SD) absolute and relative recovery of haloperidol were

97±3.6% and 100 6±1.52%, respectively. The intraday coefficients of variation (CVs) ranged from 1.74 to 4.68%, while the interday CVs varied from 2.31 to 5.23%. The detection limit for haloperidol in plasma was found to be 5 ng/mL.

INTRODUCTION

Haloperidol is a potent neuroleptic drug of the butyrophenone series, widely prescribed for the treatment of acute and chronic psychotic syndromes, abnormal movements and confused states in elderly and hospitalized patients[1]. The drug is readily absorbed from the gastrointestinal tract. A serum half-life ranging from 10 to 19 hours and an oral bioavailability of about 60% were reported.[2] This low bioavailability is attributed to the first-pass effect. Large intersubject variation in the pharmacokinetics of haloperidol in both healthy volunteers and psychiatric patients was reported.[1-3] Because of the overall low plasma levels following single dose of haloperidol and due to the need for close plasma drug monitoring, specially in psychiatric patients, a highly sensitive, simplified, and accurate assay for the drug is essential.

Several analytical methods have been reported for the assay of haloperidol in biological fluids. These include: gas chromatography (GC),[4,5] gas chromatography combined with mass spectroscopy (GCMS),[6] radioreceptor assay,[7,8] radioimmunoassay (RIA),[9,10] and high performance liquid chromatographic assay.[11-15] The GC and GCMS methods required highly sophisticated equipment and are not amenable to rapid and routine clinical assay. The radioreceptor assay is not specific. The RIA methods are sensitive, but need expensive materials and impractical for routine analysis. The HPLC methods proved to be sensitive and convenient techniques for the determination of haloperidol in biological fluids. Some of the previous methods needed a relatively large plasma volume (2 mL) which may not be always available.[15] In others an electrochemical detector was necessary for conducting the assay.[14]

In this report, a simple, sensitive and reproducible HPLC assay for the determination of haloperidol in plasma is described.

MATERIALS AND METHODS

Materials

Haloperidol and the internal standard diazepam (Sigma Chem. Co., St. Louis, MO, U.S.A.), glacial acetic acid, ammonium acetate and hydrochloric acid (BDH Chemicals Ltd., Poole, U.K.), sodium hydroxide (E. Merck AG, Darmstadt, Germany) were used without further purification. Methanol, chloroform and ether (BDH Chemicals Ltd., Poole, U.K.) were HPLC grade.

Instruments

The following instruments were utilized: A Model LC-10 AD solvent delivery pump, a Model SPD-10 AV UV-Vis detector, a Model CTO-10 A column oven, and a Model C-R4A Chromatopac computing integrator (Shimadzu Corporation, Koyato, Japan), a Model 7010 Rheodyne injector (Rheodyne Inc., Catati, CA, U.S.A.), stainless steel column (Resolve C_{18}, 150 mm length x 3.9 mm i.d., 5 μm particles, Waters Associates, Milford, MA, U.S.A.), and a Model CFC-301 Gallenkamp centrifuge (Gallenkamp, Loughborough, England).

Standard Stock Solutions

An accurately weighed sample of 10 mg haloperidol and 10 mg diazepam were dissolved in methanol in two separate 100 mL volumetric flasks to give standard stock solution of 100 μg/mL.

Chromatographic Conditions

The mobile phase was a mixture of 55% methanol and 45% HPLC water containing 0.2M ammonium acetate and adjusted to an apparent pH 7.1-7.3 with acetic acid. It was degassed daily by passing it through 0.45 μm membrane filter (Millipore, Bedford, MA, U.S.A.).

The mobile phase was pumped at a flow rate of 1.5 mL/min, which produced back-up pressure of about 210 kg/cm^2. The column oven temperature

was adjusted at 38°C. The effluent was monitored at 249 nm and attenuation at 0.0005 AUFS. The chart speed was 2.5 mm/min.

Analytical Procedure

To a screw-capped (silicone coated) 15 mL glass test tube, 0.5 mL plasma and 25 µl of the internal standard (1 µg/mL) were added. The mixture was shaken on a vortex mixer for 30 sec. Five millilitres ether and 3 mL of 0.1N HCI were added for extraction and the mixture was shaken on a vortex mixer for one min and centrifuged for 3 min. at 3000 rpm. The aqueous phase containing the drug was transferred to 15 mL glass stoppered centrifuge tube containing 7 mL chloroform and 0.5 mL 1N NaOH. The mixture was shaken gently for 2 min and the chloroform layer was transferred to another 10 mL glass centrifuge tube and evaporated to dryness under vacuum at 45°C. The residue was reconstituted with 0.5 mL mobile phase, vortexed for 1 min and an aliquot of 20 µl was directly injected into the loop injector.

Application

The in vivo percutaneous absorption of haloperidol from experimental methylcellulose gel formulation developed in our laboratory were studied in rabbits. Ten white New Zealand male rabbits weighing 4.2-4.5 kg were utilized. The hairs were removed from the back of the rabbits with an animal clipper. The gel formulation containing a dose of 10.0 mg haloperidol was spread uniformLy to the back of each rabbit over an area equal to 3.14 cm^2. Blood samples (1.5 mL) were collected from the marginal vein of the left ear via a cannula into heparinized tubes before drug administration and at 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0 and 24.0 hrs post-dosing. The plasma was harvested and stored at -20°C pending analysis.

RESULTS AND DISCUSSION

The solvent system used provided good separation of the drug and its internal standard with no interference from plasma substances. Figure 1 represents a typical chromatogram of: blank plasma (A), plasma containing the drug (B), and plasma containing haloperidol and the internal standard (C). Using the assay method the retention times for the internal standard and

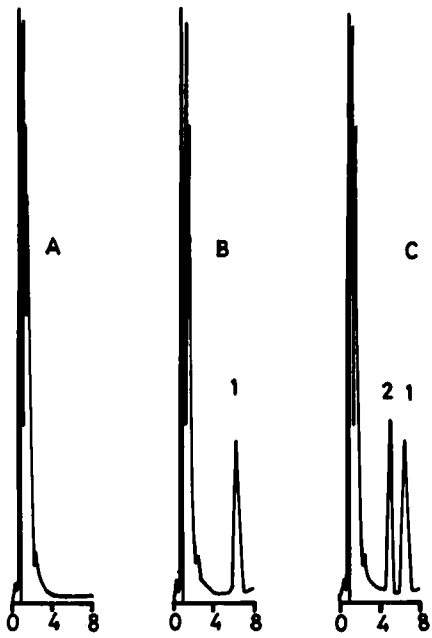

Figure 1. Chromatograms from (a) blank human plasma, (b) plasma spiked with haloperidol and (c) human plasma spiked with haloperidol and the internal standard.

1 = Haloperidol
2 = Diazepam (Internal Standard)

haloperidol were 5.1 and 6.3 minutes, respectively. The presence of ammonium acetate (0.2 M) in the mobile phase and heating the column to 38°C improved separation and yielded sharp peaks.

Quantification

Peak-height ratios of the drug to the internal standard were used in preparing four different standard curves in plasma and mobile phase by spiking 0.5 mL drug-free plasma samples and 0.5 mL mobile phase samples with the drug standard to produce a final concentration of 10, 20, 40, 60 and 100 ng/mL haloperidol. The standard plots were constructed over a period of three weeks. Least squares linear regression analysis of the mean standard calibration lines

for mobile phase and plasma samples resulted in the following equations:

Y= 0.0097 X -0.0077, r = 0.999 (Plasma)
and Y = 0.0102 X -0.0048, r = 0.999 (Mobile phase)

Analysis of variance of the slope, intercept and correlation coefficient of the four standard curves from plasma indicated non-significant difference ($F=5.11$, $p>0.05$). These results confirm the linearity of the standard curves and the excellent reproducibility of the assay method.

Recovery

The absolute recovery of haloperidol and the internal standard (diazepam) were determined by comparing the peak-height of the drug obtained from spiked plasma with the peak-heights obtained by the direct injection of pure aqueous drug standard at three different concentrations (30, 50 and 80 ng/mL). The relative recovery of the drug was calculated by comparing the concentrations obtained from the drug-supplemented plasma to the actual added concentrations. The results of the recovery studies are shown in Table 1. The average absolute and relative recovery of haloperidol were found to be 97±3.6% and 100.6±1.52%, respectively.

Table 1

Absolute and Relative Recovery of Haloperidol from Human Plasma*

Conc. (ng/mL)	Mean Peak Heights (cm)		Absolute Recovery %	Relative Recovery % Mean±SD
	Aqueous	Plasma		
30	0.86±0.05	0.80±0.2	93.02	100.94±5.25
50	1.30±0.08	1.28±0.04	98.46	98.73±2.95
80	1.89±0.11	1.90±0.14	100.53	106.3±2.4
Diazepam (I.S.)				
50	1.82±0.08	1.77±0.07	97.25	

*Twelve replicate analyses of each concentration.

Precision

The intraday precision was evaluated by replicate analysis of plasma samples containing haloperidol at three different concentrations (30, 50 and 80 ng/mL). The intraday precision showed a coefficient of variation (CV) of 1.74 to 4.68% (Table 2). The interday precision was similarly evaluated over 3-weeks period. The interday CVs ranged from 2.31 to 5.23% (Table 2).

Table 2

Intraday and Interday Precision of Haloperidol in Human Plasma

Intraday*			Interday**		
Added Conc. (ng/mL)	Measured Conc. (ng/mL)	Bias***	Added Conc. (ng/mL)	Measured Conc. (ng/mL)	Bias***
30			30		
Mean	30.7	2..3	Mean	30.3	1.0
S.D.	1.2		S.D.	1.59	
CV%	3.93		CV%	5.23	
50			50		
Mean	48.74	-2.52	Mean	49.36	-1.28
S.D.	2.28		S.D.	1.48	
CV%	4.68		CV%	3.0	
80			80		
Mean	83.18	3.97	Mean	85.1	6.38
S.D.	1.45		S.D.	1.96	
CV%	1.74		CV%	2.31	

*Mean values represent five different plasma samples for each concentration.
**Interday precision was determined from 12 different runs over 3-weeks period at the three concentrations.
***Bias=100 X (measured concentration-added concentraton) / added concentration.

Sensitivity

The minimum detectable amount which is defined as the amount in nanograms that give peak height equals to twice the background noise was found to be 5 ng of haloperidol per 1 mL of plasma samples.

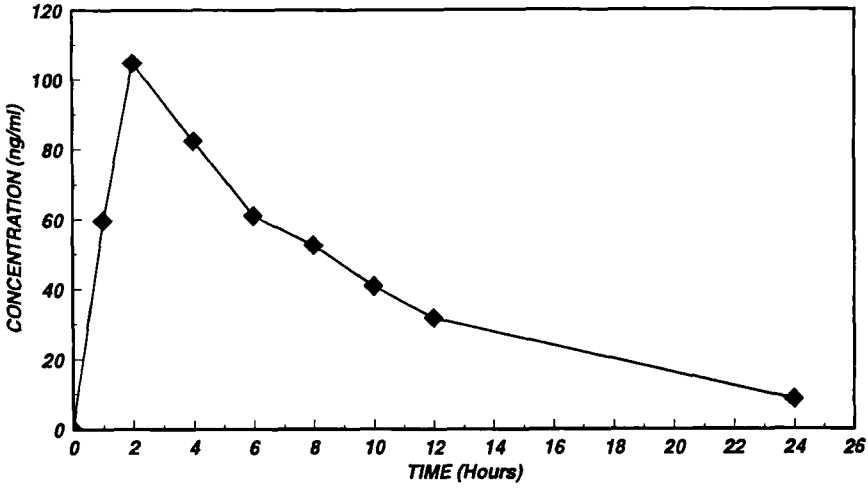

Figure 2. Mean plasma concentration-time profile of haloperidol after transdermal administration of 10 mg dose to ten rabbits.

Specificity

The specificity of the method was evaluated by analysing drug-free blank plasma from several healthy male volunteers. No interference from endogenous plasma constituent was observed at the retention times of haloperidol and the internal standard.

Application

Figure 2 shows the mean plasma concentration-time profile of haloperidol after transdermal administration of haloperidol (10 mg) from the developed gel formulation to each rabbit. The mean pharmacokinetic parameters calculated from the individual rabbit data (AUC, C_{max}, T_{max}, K_{el}, $t_{1/4}$ and MRT) are presented in Table 3.

Table 3

Mean (±SD) Pharmacokinetic Parameters of Haloperidol after Transdermal Administration of 10 mg Dose to 10 Rabbits

Parameters	Value
AUC (ng.hr/mL)	1027.14±254.84
C_{max} (ng/mL)	108.60± 36.20
T_{max} (hr)	2.20±0.42
K_{el} (hr^{-1})	0.121±0.013
$t_{1/2}$ (hr)	5.77±0.64
MRT (hr)	8.94±1.59

AUC = Area under the plasma concentration-time curve.
C_{max} = Peak plasma concentration. T_{max} = Peak time.
K_{el} = Elimination rate constant.
$t_{1/2}$ = Elimination half-life.
MRT= Mean residence time.

CONCLUSION

The HPLC method developed in this study has the sensitivity, accuracy and reproducibility which makes it valuable in many applications, specifically in pharmacokinetic studies and blood level monitoring of haloperidol.

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REFERENCES

1. F. Holley, J. Mgliozzi, D. Stanski, L. Lombrozo, L. Hollister, Clin. Pharmacol. Ther., **33**, 477-484 (1983).

2. A. Forsman, R. Ohman, Curr. Ther. Res., **20**, 319 (1976).

3. P.L. Morselli, G. Bianchetti, and M. Dugas, Ther. Drug. Monit., **4**, 51-58 (1982).

4. M. Franklin, Clin. Chem., **26**, 1367-1368 (1980).

5. G. Bianchetti, P. Morselli, J. Chromatogr., **153**, 203-209 (1978).

6. B. Pape, J. Anal. Toxicol., **5**, 113-117 (1981).

7. L. Tune, I. Crease, J. Depaulo, P. Slavney, S. Synder, J. Nerv. Ment. Dis., **3**, 60-63 (1981).

8. A. Kurland, A. Nagaraju, T. Hanlon, E. Wilinson, J. Clin. Pharmacol., **21**, 42-47 (1981).

9. Y. Terauchi, S. Ishikawa, S. Oida, M. Nakao, A. Kagemoto, T. Oida, Y. Utsui, Y. Sekine, J. Pharm. Sci., **79**, 432-436 (1990).

10. R. Poland, R. Rubin, Life Sci., **29**, 1837-1845 (1981).

11. D. Wilhelm, A. Kemper, J. Chromatogr., **525**, 218-224 (1990).

12. K. Midha, J. Cooper, E. Hawes, J. Hubbard, E. Korchinski, G. McKay, Ther. Drug Monit., **10**, 177-183 (1988).

13. R. Miller, C. Devane, J. Chromatogr., **374**, 405-408 (1986).

14. K. Yokogawa, E. Nakashima, F. Ichimura, T. Yamana, Chem. Pharm. Bull., **33**, 4581-4586 (1985).

15. P. Jatlow, R. Miller, M. Swigar, J. Chromatogr., **227**, 233-238 (1982).

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