Journal of Chromatography, 339 (1985) 321—330
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2517

IMPROVED GAS CHROMATOGRAPHIC—MASS SPECTROMETRIC ASSAY FOR HALOPERIDOL UTILIZING AMMONIA CHEMICAL IONIZATION AND SELECTED-ION MONITORING

PATRICIA A. SZCZEPANIK-VAN LEEUWEN

Clinical Pharmacokinetics Laboratory, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60680 (U.S.A.)

(First received September 13th, 1984; revised manuscript received November 28th, 1984)

SUMMARY

An improved method for the analysis of haloperidol in human serum, utilizing gas chromatography—ammonia chemical ionization mass spectrometry is described. A tetradeutero analogue of haloperidol is utilized as the internal standard, while a second drug, thioridazine, is added as a priming compound. The characteristic high sensitivity and selectivity of selected-ion monitoring combined with the added accuracy provided by incorporation of a labeled internal standard provide a reliable method for the quantitation of low levels of haloperidol.

INTRODUCTION

Haloperidol, a neuroleptic butyrophenone, is widely employed in the treatment of neuropsychiatric disorders. However, the relationship between daily dose and steady-state plasma concentration in patients receiving the drug is poor, indicating a need for monitoring the plasma levels if a relationship between drug plasma concentration and pharmacological effect can be found. Several groups of investigators have looked at the relationship between haloperidol plasma or serum concentration and therapeutic outcome in neuropsychiatric patients [1—5]. Studies reporting a positive relationship show large inter-patient variation in the optimal plasma level range [1—3]; other studies were unable to demonstrate a relationship at all [4, 5].

Huntington's disease is a chronic degenerative disease of the central nervous system. Primary treatment consists of the use of dopamine receptor site antagonists, primarily the phenothiazine derivatives and butyrophenones, to pharmacologically alter neurotransmitter inbalances [6]. The most widely

0378-4347/85/$03.30

employed drug of this group in the United States is haloperidol [7]. While patient response is highly variable, the reltionship between clinical response and plasma haloperidol concentration in these patients has not yet been examined.

There are many possible reasons for differences in the relationship between haloperidol plasma concentration and response; one which must be considered is the analytical methodology. Despite the number of gas chromatographic (GC) and GC—mass spectrometric (MS) procedures available [8—14], chemical analysis of plasma concentrations of haloperidol encountered in patients receiving dosages of less than 10 mg per day remains difficult. Butyrophenones, in general, are highly adsorptive compounds; the major problem affecting the accuracy of present GC and GC—MS assays for haloperidol is non-linear adsorption at the nanogram level [12].

This paper describes a highly selective and sensitive method for the analysis of haloperidol utilizing ammonia chemical ionization GC—MS and selected-ion monitoring (SIM). The accuracy of the assay is improved through the use of a deuterated analogue of haloperidol, which has been employed as both the internal standard and a carrier, and the addition a second drug, thioridazine, which added to the injection mixture acts as a chaser and priming compound to further reduce the adsorption of haloperidol in the GC step. The assay was used to determine the serum concentration of haloperidol in 23 patients with Huntington's disease receiving haloperidol therapy.

EXPERIMENTAL

Patient samples

Whole blood samples were drawn by glass syringe from Huntington's disease patients treated orally with haloperidol at several Chicago area medical centers. Samples were transferred to glass centrifuge tubes with PTFE-lined caps and allowed to clot for at least 30 min. They were then centrifuged, the serum was harvested and frozen at −20°C until analysis.

Standards and reagents

High-purity hexane and methanol were purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Isobutanol, reagent grade, was purchased from Eastman-Kodak (Rochester, NY, U.S.A.). Certified 1 *N* sodium hydroxide and 0.1 *N* sulfuric acid solutions were purchased from Fisher (Fair Lawn, NJ, U.S.A.). Thioridazine, 10-[2-(1-methyl-2-piperidyl)ethyl]-2-(methylthio)-phenothiazine, was obtained as Mellaril concentrate (30 mg/ml thioridazine hydrochloride in 3% alcohol—water) from Sandoz Pharmaceuticals (East Hanover, NJ, U.S.A.). Haloperidol, 4-[4-(4-chlorophenyl)-4-hydroxy-1 piperidinyl]-1-(4-fluorophenyl)-1-butanone (McN-JR-1625), and the 2,3,5,6-tetradeutero-4-fluorophenyl analogue (*d*4-haloperidol) were supplied by courtesy of McNeil Pharmaceuticals (Spring House, PA, U.S.A.).

Stock solutions

A stock solution A of 1.002 mg/ml haloperidol in methanol was prepared. Dilutions of the stock solution with methanol were made to give haloperidol solutions with the following concentrations: 100.2 μg/ml (B), 10.02 μg/ml

(C), 1.002 μg/ml (D), and 100.2 ng/ml (E). The internal standard solution was prepared by dissolving 40.02 mg of *d*4-haloperidol in 100 ml methanol to give a final concentration of 400.2 ng/ml. A 100-ml volume of the thioridazine spiked solution (2.0 μg/ml) was prepared from a 1:500 dilution of a stock solution containing 1.00 mg of thioridazine · HCl in 10.0 ml methanol. All solutions were stored at −20°C. No decomposition was observed over the course of the study.

Standard curve and quality control standard

Appropriate amounts of haloperidol from the prepared solutions were aliquoted into each of five flasks, the solvent was evaporated and freshly collected, drug-free human serum was added to give the following haloperidol concentrations: 2.00, 5.01, 10.02, 20.04 and 40.08 ng/ml. After equilibration for 30 min, the haloperidol dilutions and remaining blank serum were divided into 2.0-ml aliquots in 10-ml PTFE-lined screw-cap tubes and frozen at −20°C until needed for analysis. A quality control standard was prepared daily by adding 100 μl of haloperidol solution E (100.2 ng/ml) to a 2-ml blank serum aliquot. All standards were processed according to the method described below.

Extraction procedure

The procedure of Hornbeck et al. [14] was followed with minor modification. Serum samples were extracted from 2-ml aliquots in 10-ml PTFE-lined screw-cap glass tubes. Each sample was spiked with 50 μl of the *d*4-haloperidol internal standard solution (400.2 ng/ml) and allowed to equilibrate 30 min after vigorous wrist-action mixing. The samples were made alkaline with 400 μl of 0.5 *M* sodium hydroxide and extracted with 3 ml of 1.5% isobutanol in hexane by agitation on a mechanical shaker for 10 min, followed by centrifugation at 1400 *g* for 10 min. The supernatant was transferred to a second tube containing 1 ml of 0.05 *M* sulfuric acid and extracted into the aqueous phase as previously described. The organic phase was discarded and the aqueous layer was washed with 2 ml of the isobutanol—hexane extraction solvent. After re-alkalinization with 300 μl of 0.5 *M* sodium hydroxide, the aqueous phase was extracted as before. The organic phase was transferred to a 3-ml conical-bottom reaction vial containing 50 μl of the thioridazine solution (100 ng total). The extracts were evaporated to dryness under nitrogen in a 40°C water bath and frozen at −20°C until analysis (overnight). Prior to injection into the GC—MS system, the dry samples were dissolved in 10 μl methanol with vortexing.

Instrumentation

A Finnigan 4510 gas chromatograph—mass spectrometer coupled to an INCOS data system (Finnigan-MAT, Sunnyvale, CA, U.S.A.) was used for this study. The samples were introduced through a 0.61 m × 2 mm I.D. silanized glass column packed with 3% SP-2100 on 80—100 mesh Supelcoport (Supelco, Bellefonte, PA, U.S.A.). The column was initially conditioned using a helium flow-rate of 35 ml/min and the following temperature program: hold at 50°C for 1 h, heat from 50°C to 310°C at 5°C/min, hold at 310°C for 16 h (overnight). The column was then treated with approximately 40 μl of Silyl-8 (Pierce, Rockford, IL, U.S.A.) at 250°C and allowed to run for 30

min at that temperature before connecting to the mass spectrometer inlet. Daily priming of the column consisted of injection of 2 μl methanol twice, 1 μl thioridazine twice and finally 2 μl methanol twice.

The injector and column oven temperatures were 245°C and 235°C, respectively. Zero-grade helium at a flow-rate of 30 ml/min was used as the carrier gas and was admitted into the mass spectrometer through the direct transfer line. The gas chromatograph divert valve was open at injection to minimize solvent flow to the mass spectrometer and closed 45 sec after injection to admit the total sample for analysis. Samples were injected every 5—6 min.

Analysis was carried out in the chemical ionization (CI) mode with ammonia added through the make-up gas inlet to give an inlet pressure reading of 33.3 Pa. (The combined helium carrier and ammonia reagent gas inlet pressure reading was 130.6 Pa.) The GC—MS interface oven and transfer line were maintained at 245°C, the manifold heater at 90°C and the ionizer at 120°C. The ionization current was 0.2 mA with an electron energy of 70 eV; the pre-amp sensitivity setting was $1 \cdot 10^{-8}$ A.

The multiple-ion detection software provided by the instrument manufacturer was used to moniter the ions at *m/z* 376 and 380, corresponding to the *d*0- and *d*4-haloperidol. A dwell-time bias in favor of the unlabelled haloperidol was used to improve sensitivity of detection. The INCOS target compound analysis (TCA) and quantitation programs provided were modified to correct for ^{13}C-natural abundance contributions and incomplete labeling of the internal standard and for noise across the peak, and were used for computation of the data.

RESULTS

The need to accurately measure serum haloperidol concentrations below 10 ng/ml on reasonable size patient samples led to an examination of alternative CI reagent gases for the GC—MS analysis of this drug. Hornbeck et al. [14] have suggested that methane—ammonia gives a three-fold sensitivity over methane for haloperidol. In our laboratory, ammonia gave less fragmentation and thus a greater ion yield of protonated molecular ions (MH^+) than did methane or isobutane and was used as the reagent gas in this study. The ion yield of suitable ions from methane and isobutane negative chemical ionization (NCI) analysis was also compared with that from positive chemical ionization (PCI); methane NCI proved to be equally sensitive and isobutane NCI less sensitive (NCI production of suitable ions was approximately one fourth that of PCI) for this compound.

To further minimize losses in the extraction process and decrease adsorption on the GC column, a tetradeutero-labeled analogue of haloperidol was used as the internal standard. A stable isotope-labeled analogue is the ideal internal standard as its behavior is identical to the unlabeled haloperidol in the extraction process as well as the analytical process, eliminating the strict requirement for quantitative recovery in these steps. In this instance, the added bulk of the deutero haloperidol also acts as a carrier for the nanogram quantities of drug to be quantitated, thus aiding recovery.

The ammonia CI mass spectra of the unlabeled and *d*4-labeled haloperidol are shown in Fig. 1A and B. The protonated molecular ions are the major ions

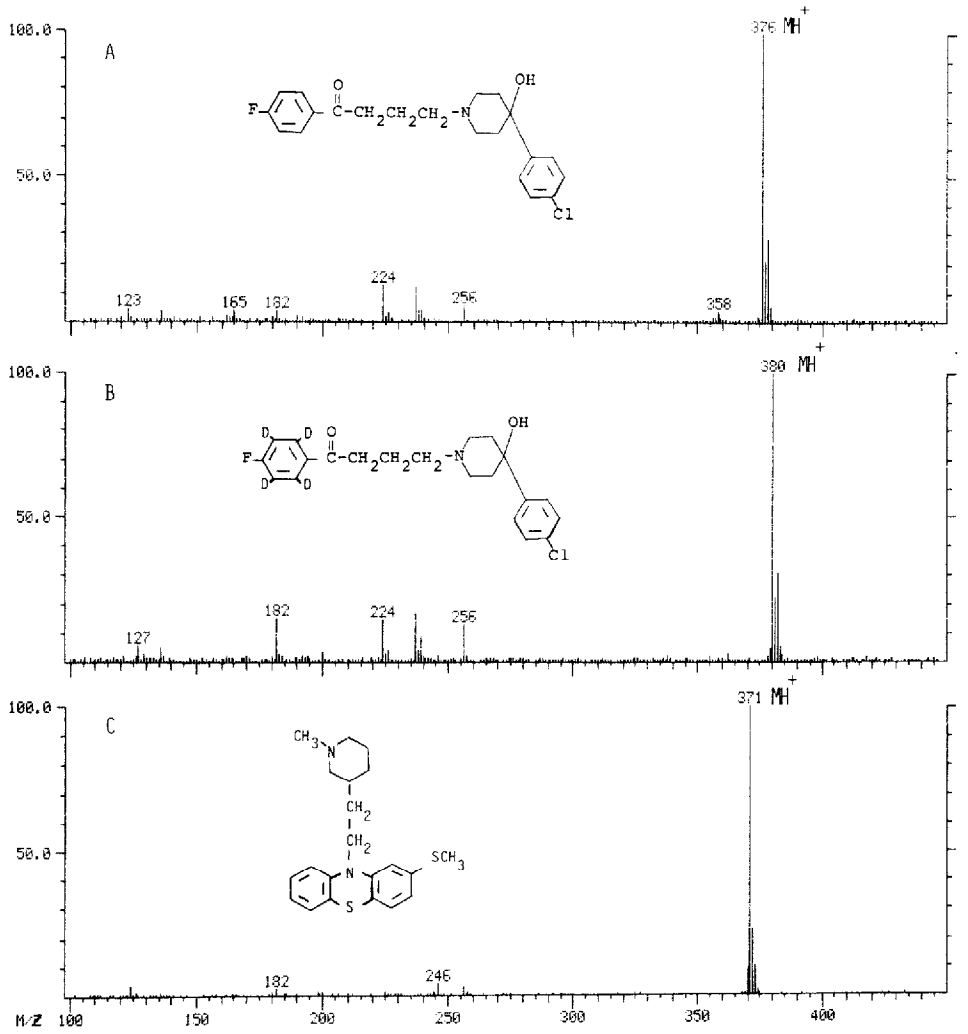

Fig. 1. Ammonia CI mass spectra of haloperidol (A), *d*4-haloperidol (B) and thioridazine (C). Samples were introduced through the gas chromatograph.

in the spectra, with the isotopic molecular ion cluster comprising greater than 70% of the total ion production. Minor ions at *m/z* 224 and *m/z* 237, common in the electron impact (EI) mass spectra, are also observed. MS analysis showed the *d*4-haloperidol to be greater than 98% labeled, minimizing any contribution from residual unlabeled drug in the quantitation.

Before evaporation to dryness, 100 ng of a second drug, thioridazine, was added to each of the extracted serum samples. The presence of thioridazine greatly improves the peak shape and recovery of haloperidol, possibly by displacing it on the GC column. The ammonia CI mass spectrum of thioridazine is shown in Fig. 1C; no interfering ions from the thioridazine can be expected in the MS analysis of *d*0- or *d*4-haloperidol. The compounds separate well on a 0.61-m column packed with 3% SP-2100, the haloperidol eluting with a retention time of approximately 2.0 min, followed by thioridazine at 3.3 min.

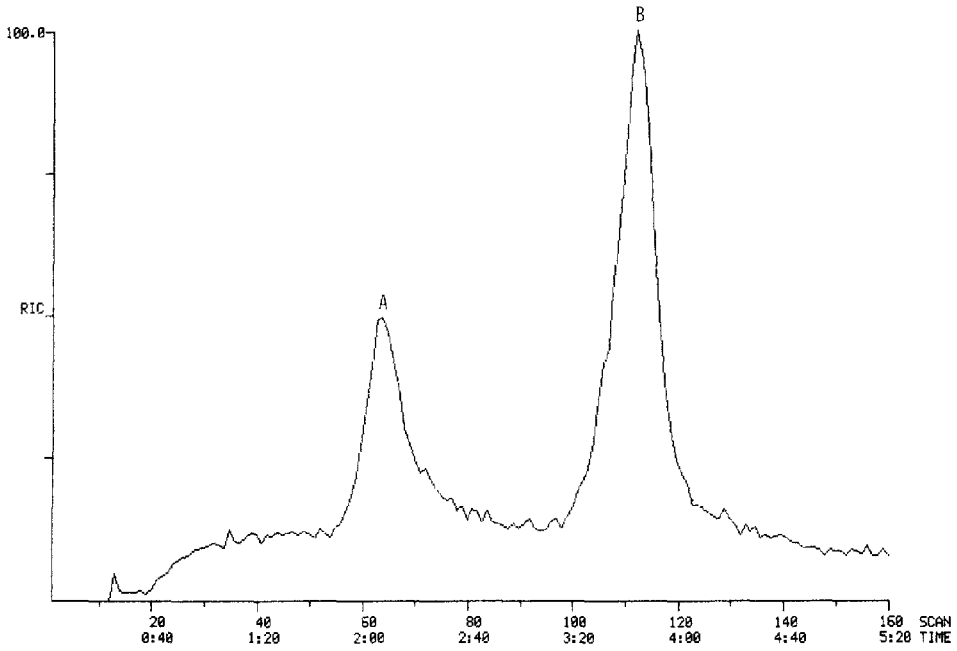

Fig. 2. Total-ion-current chromatogram of a mixture of haloperidol (A) and thioridazine (B) on a 0.61-m column packed with 3% SP-2100.

Fig. 2 shows the total-ion-current chromatogram of a mixture of haloperidol and thioridazine. The addition of thioridazine to the extracted serum samples eliminates memory effects due to the incomplete elution of the drug from the GC column, and permits standard and patients samples to be run in random order without affecting the quantitation. When haloperidol is used to prime the GC column, ghost peaks are commonly seen in subsequent injections. For this reason, thioridazine was also used for daily priming of the GC column; daily treatment with Silyl-8, which necessitates removal of the column in the instrument used, has been eliminated.

Mass chromatograms of the unlabeled and labeled haloperidol obtained from SIM-MS analysis of an actual serum sample spiked with 10 ng/ml of each compound are shown in Fig. 3. The GC conditions chosen give a minimum retention time for haloperidol while still allowing for accurate background calculation before and after the sample peak. The dwell times on the unlabeled drug and the labeled internal standard were 210 and 26 msec, respectively. The multiple-ion detector, set at *m/z* 376 and *m/z* 380 ± 0.250 a.m.u., exhibited a drift of less than ± 10 m.m.u. over the period of analysis (approximately 4 h).

Multiple injections of *d*0- and *d*4-labeled haloperidol were made on each day of analysis to determine contributions to the *m/z* 376 and *m/z* 380 ion abundances from incomplete labeling and from ^{13}C natural abundance ions. All subsequent samples were corrected for these contributions. A daily calibration curve consisting of five standard serum dilution samples was run with each set of patient samples. Internal standard (*d*4-haloperidol, 10 ng/ml) was added to each sample; the peak area ratio of *m/z* 376 to *m/z* 380 versus the concentration of unlabeled haloperidol (in ng/ml of serum) was examined by linear

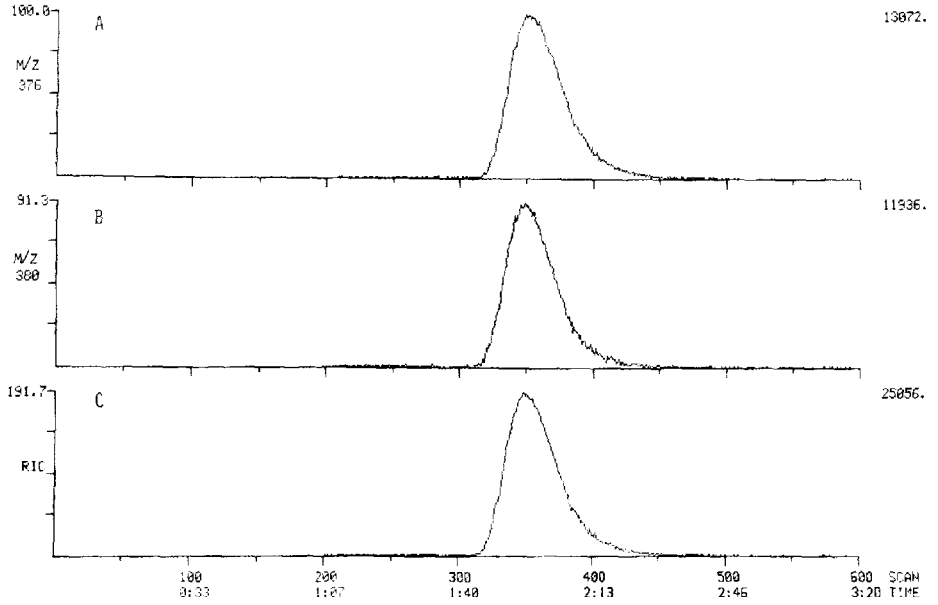

Fig. 3. Multiple-ion detection mass chromatograms of haloperidol (A) and *d*4-haloperidol (B) obtained from analysis of 2 ml of extracted serum spiked with 10 ng/ml of each compound. The displays are normalized to the maximum of each individual peak. The total-ion-current chromatogram is shown in C.

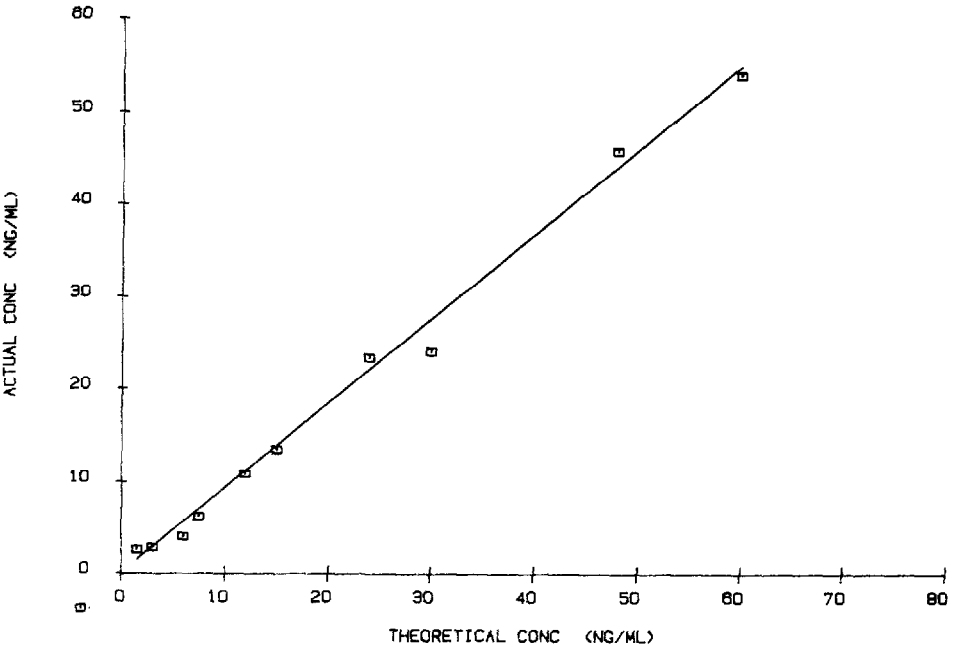

Fig. 4. Plot of the theoretical concentration of haloperidol in ten spiked serum samples versus the measured concentration. A 2-ml volume of extracted serum was used. Linear regression analysis gave the equation values $y = 0.913x - 0.125$; the correlation coefficient was 0.996.

regression analysis. A typical standard curve gave a slope and intercept of 0.110 and 0.074, respectively, and a correlation coefficient of 0.999. The mean linear regression correlation coefficient for seven runs was 0.995 ± 0.007. Four standard serum dilution curves covering the range 1.0—20.0 ng of haloperidol per ml of serum, run prior to analysis of patient samples, gave a linear curve with a mean correlation coefficient of 0.994 ± 0.009.

A plot of the actual concentration of haloperidol measured in ten spiked serum samples is shown in Fig. 4. Linear regression analysis gave a slope of 0.913 and an excellent correlation coefficient (0.996) for the concentration range 1.5—60.0 ng/ml haloperidol. The linear correlation did not hold at concentrations greater than 60.0 ng/ml, and no quantitation above this level was attempted.

The precision and accuracy of multiple measurements made on pooled serum samples spiked with known quantities of unlabeled haloperidol are given in Table I. These samples and the spiked serum curve samples described above were prepared by an outside source. The internal standard was added to each sample prior to extraction. The inter-assay precision for control serum samples spiked with 2—40 ng/ml haloperidol is shown in Table II. Quality control samples prepared fresh with each run by spiking 2 ml of serum with 100 μl of a 100.2 ng/ml standard solution of haloperidol gave a mean value of 4.42 ± 0.32 with a coefficient of variation (C.V.) of 7.3%. These values were not significant-

TABLE I

PRECISION AND ACCURACY OF DETERMINATIONS OF HALOPERIDOL IN HUMAN SERUM (n = 5)

Haloperidol concentration (ng/ml)			C.V.* (%)	Relative accuracy (%)
Theoretical	Observed	S.D.		
7.52	7.56	0.17	2.2	0.5
24.05	25.61	3.12	12.2	6.5
48.10	46.17	4.99	10.8	−4.0

*Coefficient of variation calculated from results for pooled drug-free human serum containing known quantities of unlabeled haloperidol.

TABLE II

INTER-ASSAY PRECISION OF CONTROL SAMPLES

Computations are based on seven runs made over a four-week period on spiked pooled serum.

Haloperidol concentration (ng/ml)			C.V. (%)
Theoretical	Observed	S.D.	
2.00	2.14	0.22	10.1
5.01	4.70	0.46	9.9
10.02	10.22	0.24	2.4
20.04	20.48	0.93	4.6
40.08	39.91	0.35	0.9

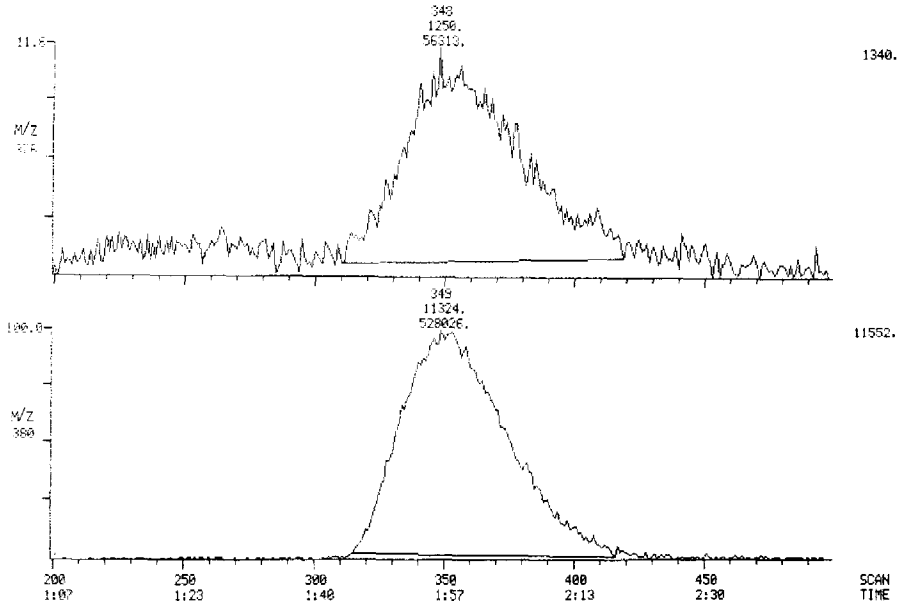

Fig. 5. Multiple-ion detection mass chromatograms showing the quantitation of haloperidol in 2 ml of extracted patient serum. The unlabeled haloperidol (m/z 376) and $d4$-haloperidol internal standard (m/z 380) peaks contain 280 pg/ml and 10 ng/ml, respectively. Data shown are uncorrected.

ly different from the 5.0 ng/ml frozen control samples above, indicating no deterioration of the haloperidol samples under the storage conditions used.

Serum samples from 22 patients receiving total daily doses of 1.0—40.0 mg (0.01—0.88 mg/kg per day) of haloperidol were analyzed using the method described. The measured serum concentration ranged from 0.3 to 28.7 ng/ml; Fig. 5 shows the quantitation of haloperidol in an actual patient sample using SIM-MS analysis. Dose adjustments were made when they were judged to be clinically necessary, and several patients were analyzed more than once. A minimum of one month was allowed between dosage adjustments to allow patients to reach a new steady state. One sample, from a patient receiving Mellaril only, gave a zero value, as did several pretreatment patient samples. The correlation between clinical response and serum haloperidol concentration in these patients will be reported elsewhere.

DISCUSSION

While the described assay gave satisfactory quantitation of haloperidol in patients receiving total doses of 1.0—40.0 mg per day, measurements in one patient whose daily intake ranged from 80.0 to 180.0 mg proved difficult due to the presence of an interfering peak. The retention time of this compound relative to haloperidol was 1.28; the EI mass spectrum of the compound was consistent with that of hydroxyhaloperidol. In agreement with the previous report by Forsman and Larsson [15], the reduced metabolite was estimated to be present at nearly the same concentration as haloperidol when high doses of the drug were administered. No evidence of the hydroxy metabolite was

observed in patients receiving 40 mg per day or less of haloperidol. Slight modification of the GC conditions and expansion of the standard curve should enable accurate measurement of haloperidol, as well as the hydroxy metabolite, in such patients.

Several investigators have used the chloro-substituted haloperidol (HAC) as their internal standard [10, 12, 13]. Because of the structural similarity of this compound to haloperidol and its greater retention time, one would suspect that the loss of haloperidol by adsorption on the GC column would be reduced with its use. However, HAC contains the same benzylic hydroxyl group probably responsible for the column-adsorption effect as does haloperidol, and reproducible recovery of the internal standard in assays where low nanogram amounts of HAC are used is not guaranteed.

In conclusion, the addition of carrier *d*4-haloperidol in combination with the use of ammonia as the CI reagent gas should theoretically improve the sensitivity of the GC—SIM-MS assay for haloperidol, possibly to the low picogram level. These modifications have not produced the higher sensitivity expected. However, the described method, which incorporates the use of a deuterated analogue of haloperidol as the internal standard, offers the only reliable solution to the adsorption problems encountered in the measurement of low levels of haloperidol. This addition, together with the excellent sensitivity and selectivity provided by ammonia CI in the SIM process and the convenience and improved peak shape gained using thioridazine, contributes to an improved assay for the measurement of haloperidol in patients receiving low doses of this drug.

REFERENCES

1 S.E. Ericksen, S.W. Hrut and S. Chang, Psychopharmacol. Bull., 14 (1978) 15.
2 R.C. Smith, J. Crayton, H. DeKirmenjian, D. Klass and J.M. Davis, Arch. Gen. Psychiatry, 36 (1979) 579.
3 J. Mendlewicz, P. Linkowski and A. Schoutens, in S. Dahl and E. Udin (Editors), Clinical Pharmacology in Psychiatry, Macmillan, London, 1981, p. 217.
4 K. Vaisanen, M. Viukari, R. Rimon and P. Raisanen, Acta Psychiatr. Scand., 63 (1981) 262.
5 V.A. Rama Rao, M. Bishop and A. Coppen, Brit. J. Psychiatry, 137 (1980) 518.
6 C.G. Goetz, W.J. Weiner and H.L. Klawans, in A. Barbeau (Editor), Disorders of Movement, Lippincott, Philadelphia, PA, 1981, p. 29.
7 S.P. Ringel, M. Guthrie and H.L. Klawans, Adv. Neurol., 1 (1973) 797.
8 I.A. Zingales, J. Chromatogr., 54 (1971) 15.
9 F. Marcucci, L. Airoldi, E. Mussini and S. Garattini, J. Chromatogr., 59 (1971) 174.
10 A. Forsman, E. Martensson, G. Nyberg and R. Ohman, Naunyn-Schmiedeberg's Arch. Pharmacol., 286 (1974) 113.
11 G. Bianchetti and P.L. Morselli, J. Chromatogr., 153 (1978) 203.
12 M. Franklin, Clin. Chem., 26 (1980) 1367.
13 M.A. Moulin, R. Camsonne, J.P. Davy, E. Poilpre, P. Morel, D. Debruyne, M.C. Bigot, M. Dedieu and M. Hardy, J. Chromatogr., 178 (1979) 324.
14 C.L. Hornbeck, J.C. Griffiths, R.J. Neborsky and M.A. Faulkner, Biomed. Mass Spectrom., 6 (1979) 427.
15 A. Forsman and M. Larsson, Curr. Ther. Res., 24 (1978) 567.