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Note

Sensitive analysis of butyrophenone neuroleptics by high-performance liquid chromatography with ultraviolet detection at 254 nm

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Haloperidol, spiroperidol and trifluperidol are used clinically as neuroleptics as well as pharmacological tools in the laboratory. Several methods are already available for the detection and assay of a variety of neuroleptics in plasma. Gas—liquid chromatography requires extensive sample purification but has the advantages of selectivity and accuracy when the internal standard ratio method is used [1, 2]. Radio-ligand displacement assays have high sensitivity and the widest application to different chemical classes but such methods require access to appropriate animal tissues as a source of specific-binding protein [3—5] or a specific antibody [6] and make use of radio-labelled materials. These techniques also lack selectivity, being unable, for example, to differentiate between the neuroleptic parent compound and a pharmacologically active metabolite with the result that drug levels can be overestimated [6]. In addition it is not easy to correct for the efficiency of recovery in a particular sample.

High-performance liquid chromatographic (HPLC) methods have been described for the analysis of haloperidol. Korpi et al. [7] applied isocratic reversed-phase HPLC and electrochemical detection on a glassy carbon electrode at +0.9 V to the assay of haloperidol and a metabolite. Electrochemical detectors are not, however, in wide use and routine high-sensitivity operation at high electrode voltages can be difficult. The strong ultraviolet (UV) absorption of butyrophenones (e.g. haloperidol, λ_{max} 247 nm; E =

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12 000) offers the possibility of UV detection to assay these compounds to the low nanogram range [8, 9]. In this work variable-wavelength detectors were used which are inherently more noisy than fixed-wavelength detectors. None of the HPLC methods describes the analysis of haloperidol or other butyrophenones from tissue samples. This paper describes a method for haloperidol analysis in brain samples with similar sensitivity to the other HPLC methods. UV detection at 254 nm is used since this is most often found in the detector on liquid chromatographs. Furthermore, other butyrophenones (spiroperidol, trifluperidol) can be measured in the same way and the application of trace enrichment to reduce the detection limits and simplify sample preparation is described.

EXPERIMENTAL

Apparatus

The liquid chromatograph consisted of a Spectra-Physics SP-8000 unit fitted with an SP-8310 UV detector (254 nm) or Micromeritics 760 variable-wavelength detector, an SP4000 plotter/integrator and a Waters U6K injector with a 2-ml loop. Columns were thermostatted at 30°C unless otherwise stated. A Waters μBondapak C_{18} (300 × 3.9 mm, 10 μm particle size) and a μBondapak Phenyl (300 × 3.9 mm, 10 μm particle size) columns were used and the mobile phases were 0.1% (v/v) trifluoroacetic acid (TFA) with 30% or 40% (w/v) acetonitrile (Burdick & Jackson Labs.).

A Varian 2200 UV—VIS spectrophotometer was used to obtain UV absorption spectra of 10 μg/ml solutions of butyrophenones in 0.05% (v/v) lactic acid. The spectrum of the dilute lactic acid solvent was subtracted from the drug spectra.

Drug administration

Butyrophenones (kindly supplied by Dr. Pierre Laduron, Janssen Pharmaceutica, Belgium) were dissolved in lactic acid, then diluted with distilled water to a final concentration of 5 mg/ml in 0.05% (v/v) lactic acid and stored at 4°C. Under these conditions, the solutions of butyrophenones are stable for more than one year [10]. Further dilutions from these stock solutions were made as necessary.

Male Long Evans Hooded (LEH) rats (200—300 g) received intraperitoneal (i.p.) injections of haloperidol dissolved in dilute lactic acid—methylparaben solution (2 ml/kg). At the appropriate time the animals were killed by decapitation, the brains dissected out and immediately frozen in isopentane on dry ice. Frozen brains were stored at −70°C until required for assay.

Sample extraction

Brains or parts thereof were homogenised in 8 vols. of cold aqueous acid (see Results for details) containing trifluperidol (50 or 500 ng) as internal standard. Samples were placed on ice for 10 min and then centrifuged at 5000 *g* for 30 min at 4°C. Aliquots of the supernatant (usually 1 ml) were made alkaline by the addition of 200 μl of 2 *M* sodium hydroxide per ml and then extracted with 2 vols. of heptane—isoamyl alcohol (97:3) by vigorous vortexing

[1]. The layers were separated by centrifugation and the organic phase transferred to a centrifuge tube containing 200 μl of 5 m*M* sulphuric acid for each 3 ml of organic phase. The butyrophenones were back-extracted into the aqueous phase and the organic layer was aspirated after centrifugation. Portions of the extract, usually 50 μl, were injected onto the liquid chromatograph. The internal standard peak height ratio method was used to calculate haloperidol content which was expressed relative to the wet weight of the tissue.

RESULTS

The UV absorption spectra of the three butyrophenones, haloperidol, spiroperidol and trifluperidol are given in Fig. 1. It can be seen that all three of these compounds absorb strongly in the region of 245—250 nm. The molar absorptivity at 254 nm was calculated to be 11 850, 16 010 and 9980 for the three drugs, respectively. All three show strong absorption below 210 nm and the spectrum of haloperidol also shows a shoulder centered around 220 nm.

The UV spectra suggest that these butyrophenones could be detected with high sensitivity with the 254-nm mercury line lamps most often found in HPLC—UV detectors. This prediction is confirmed by the chromatograms in

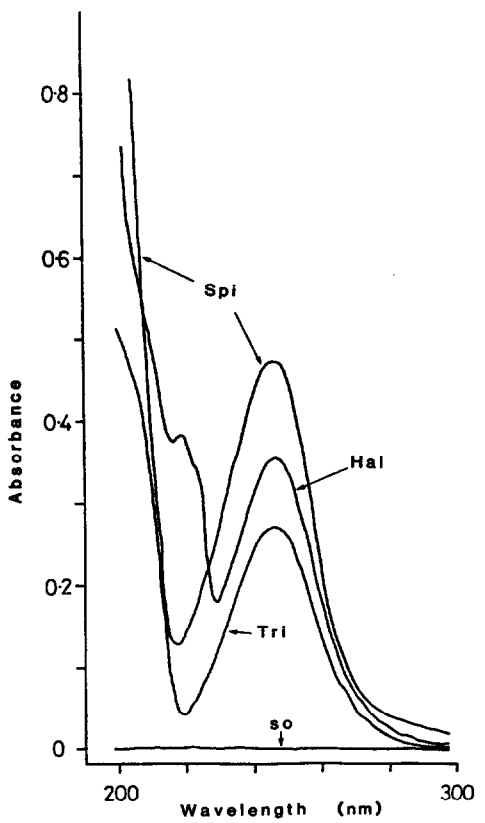

Fig. 1. UV absorption spectra of haloperidol (Hal), spiroperidol (Spi), and trifluperidol (Tri) and the solvent (so, 0.05% lactic acid). The concentration of butyrophenones was 10 μg/ml. The absorption spectrum of the solvent has been subtracted from all spectra.

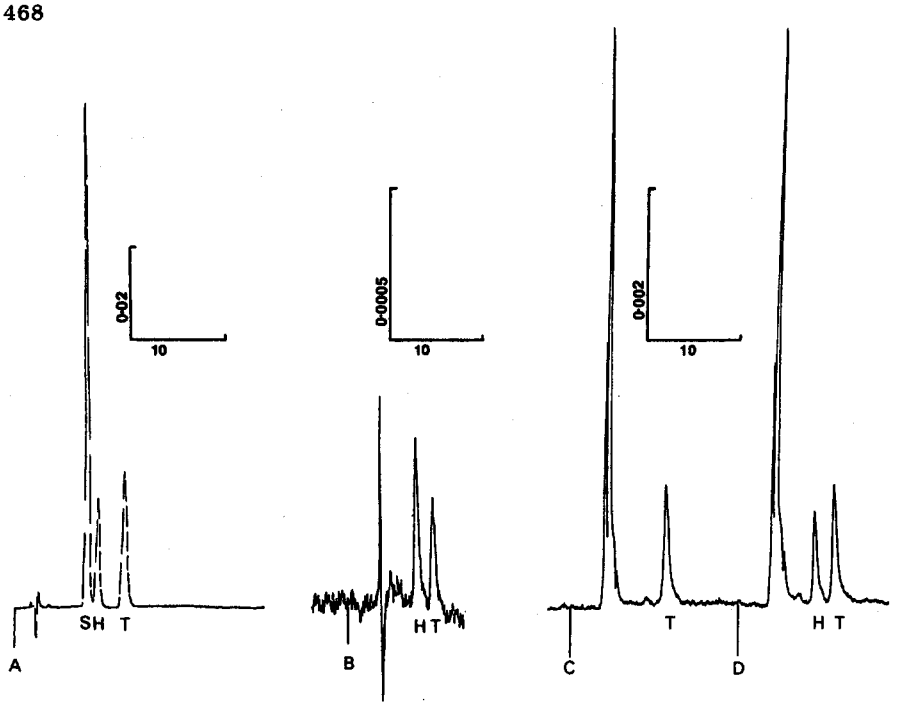

Fig. 2. Chromatograms of butyrophenones with detection at 254 nm. (A) Isocratic elution on a μBondapak C_{18} (300 × 3.9 mm) of 1.2 μg spiroperidol (S); 0.4 μg haloperidol (H); and 0.8 μg trifluperidol (T). Mobile phase, 0.1% TFA—30% acetonitrile; flow-rate, 1.2 ml/min; temperature, 23°C. (B) Chromatogram of 10 ng each of haloperidol (H) and trifluperidol (T). Conditions as in A except flow-rate (0.9 ml/min) and temperature (35°C). (C) Chromatogram of brain extract from vehicle-treated rat. Conditions as in B. T = trifluperidol, internal standard. (D) Chromatogram of brain extract from rat treated with 3 mg/kg haloperidol i.p. 2 h before sacrifice. H = haloperidol; T = trifluperidol, internal standard. Injection volume, 50 μl; Horizontal bar, 10 min; vertical bar absorbance (254 nm).

Fig. 2 where it can also be seen that the three drugs can be separated by isocratic elution on a μBondapak C_{18} column (Fig. 2A) and as little as 10 ng of each detected in a single injection (Fig. 2B). The sensitivity, calculated as twice baseline noise, was 1.5 ng for spiroperidol, 2 ng for haloperidol and 3 ng for trifluperidol. The response of the detector was linear from 10 ng to 10 μg.

The retention times of the three compounds were similar when the mobile phase contained either 0.1% TFA or 50 m*M* sodium acetate, pH 5 and with the same proportion of acetonitrile as organic modifier on the μBondapak C_{18} column. Chromatography on a μBondapak Phenyl column required a higher proportion of acetonitrile in the mobile phase for equivalent retention times but then there was no baseline resolution between spiroperidol and haloperidol. Better resolution on the phenyl-bonded phase was possible by reducing the proportion of acetonitrile to increase retention times or using gradient elution from 30% to 50% acetonitrile over 30 min. Similar results were obtained when methanol was used instead of acetonitrile as the organic modifier, except that the column temperature had to be increased or a higher proportion in the mobile phase was required.

Attempts were made to increase the sensitivity of detection by using low UV wavelengths. The peak height response to each compound was as predicted

by their UV absorption spectra. There was a maximum response at 247 nm; at 210 nm and below, peak height increased with decreasing wavelength but baseline noise also increased. For example, at 206 nm the peak response had doubled over that at 254 nm while baseline noise had increased four-fold. In anticipation of using the 214-nm zinc line, this wavelength was also evaluated. As would be predicted from Fig. 1, the response to haloperidol increased relative to 254 nm while the others decreased.

Extraction of butyrophenones from tissues was first attempted by precipitation of proteins with either 5% trichloroacetic acid (TCA) or 0.1 *M* perchloric acid (PCA), followed by organic extraction as described under Experimental. With either of these precipitants, however, there was poor recovery (< 10%) when drugs were added to brain tissues before homogenisation (Table I). Recovery of haloperidol or trifluperidol from TCA or PCA solutions was considerably higher than from brain homogenates. Protein precipitation, either by boiling the brain homogenate or with zinc sulphate—sodium hydroxide, also gave poor (< 10%) recoveries of butyrophenones. If the brains were homogenised in dilute hydrochloric acid, drug recovery was about 50% (Table I), so that this method was adopted for further investigation. The data in Table I also show that the recovery of haloperidol and trifluperidol closely follow each other so that one drug can be used as the internal standard to correct for the extraction efficiency of the other. Spiroperidol is also extracted in parallel with haloperidol and trifluperidol. The recovery from brain homogenate of added haloperidol (40—200 ng) was 44 ± 2% and for the amount of haloperidol calculated to be present in replicate estimations of the same brain homogenate, the coefficient of variation was 6.7% (n = 5).

Representative chromatograms of brain extracts from rats that had been treated with vehicle or haloperidol (3 mg/kg, i.p.) 2 h before sacrifice are

TABLE I

RECOVERY OF HALOPERIDOL AND TRIFLUPERIDOL FROM BRAIN HOMOGENATES BY SEVERAL ORGANIC EXTRACTION PROCEDURES

Rat brain was homogenized in 4 vols. of distilled water and haloperidol and trifluperidol were added to 500 ng/ml. An equal volume of double-concentrated acidic reagent was added and then extracted as described in the text. For "reagent alone", distilled water was used instead of the brain homogenate.

Reagent	Molarity	Percentage recovery (mean ± S.D.)			
		From acid alone		From brain homogenate	
		Haloperidol	Trifluperidol	Haloperidol	Trifluperidol
Trichloroacetic acid	0.3	67 ± 2	68 ± 4	10 ± 2	13 ± 3
Perchloric acid	0.1	63 ± 1	67 ± 3	25 ± 4	26 ± 2
Hydrochloric acid	0.01	73 ± 4	69 ± 5	45 ± 1	44 ± 1
Hydrochloric acid	0.05	—	—	50 ± 3	50 ± 4
Hydrochloric acid	0.1	—	—	49 ± 6	48 ± 3

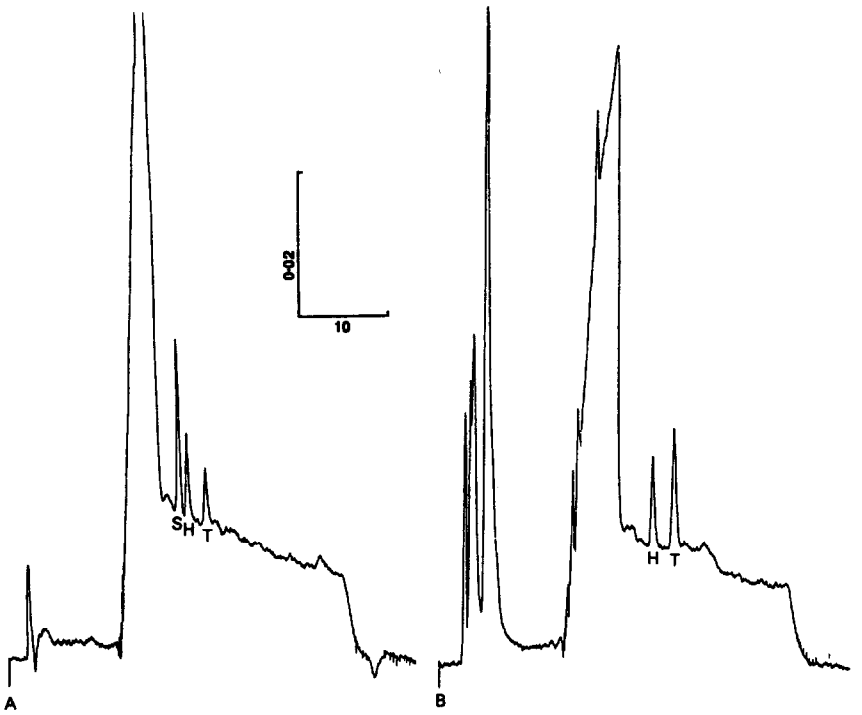

Fig. 3. (A) Step-gradient chromatogram of 25 ng each of spiroperidol (S), haloperidol (H) and trifluperidol (T). See text for details. (B) Step-gradient chromatogram of brain extract from a rat treated with 0.25 mg/kg haloperidol (H) i.p. T = trifluperidol, internal standard. Horizontal bar, 10 min; vertical bar, absorbance 254 nm.

shown in Fig. 2C and D, respectively. The trifluperidol internal standard peak was present in both chromatograms while haloperidol was present only in the extract from the drug-treated animal. The mean (± S.E.M.) haloperidol content of four different brains from animals receiving the same treatment was 2.28 ± 0.25 μg/g of tissue. These results were obtained with extracts that represented a small proportion of the total brain tissue. By the use of trace enrichment and step-gradient chromatography it was possible to easily quantify brain haloperidol 2 h after a dose of 0.25 mg/kg. In Fig. 3A, the column was equilibrated with 0.1% TFA and an injection containing 25 ng of spiroperidol, haloperidol and trifluperidol in 1.5 ml of 5 m*M* sulphuric acid was made onto the column. After 5 min the mobile phase was switched to 40% acetonitrile in 0.1% TFA and chromatography continued for a further 25 min. All three butyrophenones were resolved by this procedure and readily detected at 254 nm. For Fig. 3B, half of the brain was homogenized in 8 ml of 10 m*M* hydrochloric acid and then extracted as described above after the addition of 250 ng of trifluperidol as internal standard. The final sulphuric acid extract had a volume of 1.5 ml. A portion of this (750 μl) was diluted to 1.5 ml and chromatographed as for the standard in Fig. 3A. Fig. 3B then, represents the haloperidol content of only one quarter of the brain, calculated to be 0.19 μg/g of tissue for this sample and 0.18 μg/g of tissue for a second animal treated in

the same way. The extraction efficiency for the internal standard was 44%. The detection limit for a 1-g tissue sample would be 10 ng for spiroperidol and 25 ng for haloperidol if the whole extract (1.5 ml) was injected.

DISCUSSION

The work reported in this paper has demonstrated that butyrophenone neuroleptics can be quantified with high sensitivity by UV detection at 254 nm after reversed-phase HPLC. The sensitivity of the method would only be marginally improved by detection at 247 nm or lower (200—210 nm) wavelengths, and would then be accompanied by a reduction in the signal-to-noise ratio (i.e. increased noise relative to that of the high energy mercury line emission at 254 nm). Detection at 254 nm also allows the use of methanol in the mobile phase (when an octyl or hexyl reversed-phase column would be preferred) avoiding the higher cost and toxicity of acetonitrile, and reduces the possibility of interference from tissue contaminants and other drugs [7]. The use of the 214-nm line of a zinc lamp may be applicable to haloperidol analysis and also aid in its chemical characterisation by the 254 nm/214 nm response ratio.

The primary intention for this work was to devise a method for quantifying tissue butyrophenone content. It was initially hoped to extract tissues by using efficient protein precipitants and then inject the protein-free extract directly onto the liquid chromatograph, but recoveries were low. The poorer recovery in the presence of brain tissue may also be the result of the partitioning of these extremely lipophilic compounds into the precipitated membranes. Recovery from brain was increased by the use of dilute hydrochloric acid, but the resultant extract was too impure for direct injection routinely so that further purification became necessary.

The solvent extraction reported by Forsman et al. [1] for gas chromatographic analysis gave adequate recovery and the chromatograms were not complicated by any tissue-derived peaks (Fig. 2C and D). Haloperidol levels in rat brain areas after doses of 1—3 mg/kg were readily quantified with this method. The trace enrichment method reduces the detection limit for tissue haloperidol. From the data in Fig. 3B it can be shown that small brain areas (e.g. one twentieth of a rat brain, approximately 75 mg tissue) could be successfully analysed by increasing the proportion of the extract to be injected, or butyrophenone content could be measured after even lower doses (e.g. 0.05 mg/kg) had been administered. Since the sensitivity of this method is very similar to the gas chromatographic method [1, 2], it follows that plasma samples can also be analysed for butyrophenones by reversed-phase HPLC with UV detection [3, 4]. HPLC methods have the advantage of being less dependent upon extremely low injection volumes so that some of the volume-reducing extraction steps and solvent evaporations can be omitted when isocratic chromatography is employed and hence reduce extraction losses. The step-gradient modification is even less dependent on the use of small injection volumes.

In conclusion, it has been shown that haloperidol can be measured in tissue samples by a combination of reversed phase chromatography and highly

sensitive UV detection. This method can be extended to spiroperidol, trifluperidol and probably other butyrophenones with minor modifications. It is suggested that this method could be applied to the analysis of butyrophenones in plasma and that the sensitivity could be further increased by using larger plasma samples and trace enrichment.

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REFERENCES

1 A. Forsman, E. Martensson, G. Nyberg and R. Ohman, Naumyn-Schmiedeberg's Arch. Pharmacol., 286 (1974) 1367.
2 G. Bianchetti and P.L. Morselli, J. Chromatogr., 153 (1978) 203.
3 I. Creese and S.H. Snyder, Nature (London), 270 (1977) 180.
4 R.C. Smith, G. Vroulis, C.H. Misra, J. Schooler, C. DeJohn, P. Korivi, D.E. Leelavathi and D. Arzu, Commun. Psychopharmacol., 4 (1980) 451.
5 B.M. Cohen, M. Herschel and E. Miller, Neuropharmacology, 19 (1980) 663.
6 R.E. Poland and R.T. Rabin, Life Sci., 29 (1981) 1837.
7 E.R. Korpi, B.H. Phelps, H. Granger, W.-H. Chang, M. Linnoila, J.L. Meek and R.J. Wyatt, Clin. Chem., 29 (1983) 624.
8 K. Miyazaki, T. Arita, I. Oka, T. Koyama and I. Yamashita, J. Chromatogr., 223 (1981) 449.
9 P.I. Jatlow, R. Miller and M. Swigar, J. Chromatogr., 227 (1982) 233.
10 P.J.A.W. Demoen, J. Pharm. Sci., 50 (1961) 350.