Journal of Chromatography, 193 (1980) 255–263

CHROM. 12,633

SEPARATION AND QUANTITATION OF BROMPERIDOL IN PHARMACEUTICAL PREPARATIONS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY

E. VAN DEN EECKHOUT, G. A. BENS and P. DE MOERLOOSE

Department of Pharmaceutical Chemistry and Drug Quality Control, State University of Ghent, Faculty of Pharmaceutical Sciences, De Pintelaan 135, B-9000 Ghent (Belgium)

(First received September 14th, 1979; revised manuscript received December 19th, 1979)

SUMMARY

A high-performance thin-layer chromatographic (HPTLC) method and a high-performance liquid chromatographic (HPLC) method were developed for the determination of bromperidol, a neuroleptic drug in the butyrophenone series. The HPTLC uses two different solvent systems: benzene–ethyl acetate–diethylamine (90:5:5), suitable for the quantitative determination of bromperidol, and the organic layer of ethanol–ammonia–dichloromethane (5.0:1.5:93.5), which separates eight of the nine butyrophenones studied. The HPLC uses a C_{18} reversed-phase column with acetonitrile–water–diethylamine (50:50:0.1) as eluent, which is suitable for the quantitative determination of bromperidol and is highly specific as it separates all nine butyrophenones studied.

INTRODUCTION

Bromperidol is a neuroleptic in the butyrophenone series (Fig. 1). Butyrophenones in pharmaceutical preparations have been determined spectrophotometrically by UV absorption[1–4] and by colorimetry[5,6], by a fluor determination[7,8] and spectrofluorimetrically[9,10], but these methods all lack specificity. A method for the determination of bromperidol in pharmaceutical preparations has not yet been published. We describe here specific and reproducible high-performance thin-layer chromatographic (HPTLC) and high-performance liquid chromatographic (HPLC) methods for the determination of bromperidol.

EXPERIMENTAL

Chemicals and materials

All of the butyrophenones studied, the degradation product 4-[4-(4-bromophenyl)-3,6-dihydro-1(2*H*)-pyridinyl]-1-(4-fluorophenyl)-1-butanone and the im-

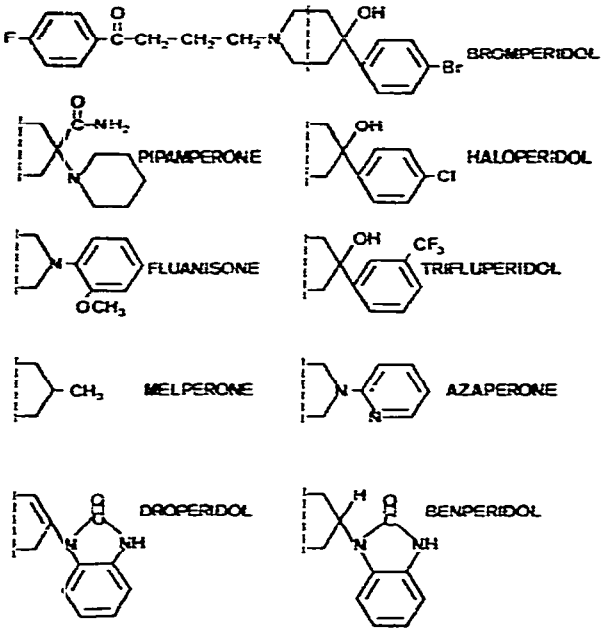


Fig. 1. Structures of the butyrophenones studied.

purities 4-(4-bromophenyl)-1,2,3,6-tetrahydropyridine and 4-(4-bromophenyl)-4-hydroxypiperidine were supplied by Janssen Pharmaceutica, Beerse, Belgium.

All solvents used were of reagent grade.

The column packing material was RSil C18 HL (5 μm) (RSL, Elze, Belgium) (HL represents high loading, *i.e.*, 18% of bonded organic material).

The columns (100 × 4.6 mm I.D.) were packed by a slurry technique (a slurry of silica C_{18} in glycerol–methanol, 25:75).

HPTLC silica gel 60 plates (10 × 10 cm) without a fluorescence indicator (E. Merck, Darmstadt, G.F.R.) were used.

Preparation of standards and samples of tablets, solutions and injections for HPTLC

A bromperidol stock solution containing 2.50 mg/ml of bromperidol in methanol and an azaperone (internal standard) stock solution containing 0.5 mg/ml of azaperone in methanol were prepared.

Standards containing 0.30, 0.35, 0.40 and 0.45 mg/ml of bromperidol and 0.20 mg/ml of azaperone in methanol were prepared.

Tablets. The mean weight of 20 tablets was determined. After grinding an amount of tablet powder corresponding to 18 mg of bromperidol was accurately weighed and transferred into a 50-ml volumetric flask to which 20.0 ml of azaperone stock solution were added. The solution was made up to 50.0 ml with methanol. After ultrasonic dispersion for 5 min, the suspension was filtered, the first 10 ml of filtrate being discarded.

Solutions. Solutions were diluted with methanol to obtain a final concentration of 0.40 mg/ml of bromperidol. Azaperone was added as the internal standard at a concentration of 0.20 mg/ml.

Injections. Injections were diluted with methanol to a final concentration of 0.40 mg/ml of bromperidol. Azaperone was added as the internal standard at a concentration of 0.20 mg/ml.

Preparation of standards and samples for HPLC

The standard and samples prepared for HPTLC were diluted 10-fold with acetonitrile–water (1:1).

Quantitative HPTLC

All HPTLC separations were carried out using 10 × 10 cm pre-coated HPTLC silica gel plates. In order to ensure a low background, ultraviolet-absorbing materials were removed with a pre-development wash with methanol. The plates were then dried for 10 min at 100 °C and used after cooling.

Samples were spotted by means of an application system (Nanomat; Camag, Muttenz, Switzerland) with 200-nl fixed-volume platinum–iridium capillary pipettes, resulting in spot diameters of less than 2 mm. Samples were spotted using the data pair technique. Unknowns and standard solutions were spotted in such a way as to give a pair for each concentration, with the wo spots being about a half plate-width apart[11]. After sample application the plate was held in a stream of nitrogen to ensure removal of methanol. After this drying step, the plates were allowed to stand in the ambient atmosphere for 5 min before chromatography.

The plates were developed with benzene–ethyl acetate–diethylamine (90:5:5) (solvent system I), using a solvent-saturated paper-lined HPTLC separating chamber of dimensions 6.5 × 12 × 14 cm (Desaga, Heidelberg, G.F.R.). The solvent front travelled 6 cm. After development, the plates were dried briefly under a stream of nitrogen and were then ready for scanning without further processing.

Quantitation of HPTLC spots

Quantitation of the developed HPTLC plates was accomplished utilizing a Zeiss KM-3 chromatogram spectrophotometer in the reflectance mode for UV absorption. A deuterium light source was used with the monochromator set at 254 nm. The slit was 3.5 × 0.1 mm, the scanning speed 2 cm/min and the paper speed 2 cm/min.

Peak heights were measured manually. The concentration of bromperidol in the unknown sample was calculated from calibration graphs constructed by plotting the peak-height ratios (drug to internal standard) *versus* amount of drug in the reference spots. Each point on the calibration graph was the mean of two determinations. All calibration graphs were linear in the concentration ranges used.

Qualitative separation of bromperidol from other butyrophenones by HPTLC

The qualitative separation of bromperidol from other butyrophenones was carried out on HPTLC plates with a solvent system consisting of the organic layer of ethanol–ammonia–dichloromethane (5.0:1.5:93.5) (solvent system II).

Quantitative HPLC

A Waters Model M-6000A chromatography pump (Waters Assoc., Milford, MA, U.S.A.) equipped with a variable-wavelength detector (Varichrom; Varian, Palo Alto, CA, U.S.A.) and a Varian Model 9176 were used. The temperature of the

column and the flow cell was maintained at 25 °C. Detection was effected at 247 nm. The eluent, consisting of acetonitrile–water–diethylamine (50:50:0.1), was pumped at a rate of 1.0 ml/min and at 25 °C.

Samples were injected through a six-way Valco valve, with a 10-mm³ fixed loop. The quantitative evaluation of the chromatograms was carried out by peak-height measurement. The concentrations in the unknown samples were calculated from calibration graphs constructed by plotting the peak-height ratios (drug to internal standard) *versus* amount of drug in each standard.

Qualitative HPLC

Qualitative separation of bromperidol from other butyrophenones was carried out using the conditions specified under *Quantitative HPLC*.

RESULTS AND DISCUSSION

HPTLC

The choice of the solvent system is of great importance in HPTLC, as the size of the spot will vary from one system to another and influence the sensitivity and the reproducibility of the analysis. We experienced this phenomenon with solvent systems other than that used here, *e.g.*, benzene–methanol–diethylamine (80:20:1) gave no reproducible results for quantitative purposes. Solvent system II, used for the qualitative separation of different butyrophenones, cannot be used for the quantitative analysis of solutions owing to interference from methyl *p*-hydroxybenzoate present in bromperidol solutions. The R_F values for the butyrophenones studied and for methyl and propyl *p*-hydroxybenzoate in solvent systems I and II are given in Table I.

In solvent system I there is no interference on either bromperidol or azaperone due to accompanying substances in the solutions. In solvent system II methyl *p*-hydroxybenzoate chromatographs in almost the same position as bromperidol and interferes in the quantitative determination of the latter.

TABLE I

R_F VALUES OF BUTYROPHENONES AND METHYL AND PROPYL *p*-HYDROXYBENZOATE STUDIED BY HPTLC IN SOLVENT SYSTEMS I AND II

Compound	R_F	
	Solvent I	*Solvent II*
Pipamperone	0.13	0.07
Droperidol	0.13	0.31
Benperidol	0.16	0.36
Haloperidol	0.48	0.44
Bromperidol	0.48	0.44
Trifluperidol	0.48	0.51
Melperone	0.77	0.55
Azaperone	0.65	0.69
Fluanisone	0.77	0.93
Methyl *p*-hydroxybenzoate	0.00	0.46
Propyl *p*-hydroxybenzoate	0.00	0.48

Table II gives the results for the analysis of tablets, solutions and injections expressed as a percentage of the theoretical values. The coefficient of variation, calculated for each formulation on data obtained for five different solutions analysed in duplicate, was 2–3%.

TABLE II

RESULTS OBTAINED BY HPTLC AND HPLC FOR THE QUANTITATIVE DETERMINATION OF BROMPERIDOL IN TABLETS, SOLUTIONS AND INJECTIONS

Mean (n = 10) expressed as a percentage of the theoretical value. C.V. = coefficient of variation expressed as a percentage of the mean.

Method	*Tablet (5 mg) ± C.V. (%)*	*Tablet (10 mg) ±C.V. (%)*	*Solution (2 mg) ±C.V. (%)*	*Solution (10 mg) ±C.V. (%)*	*Injection (5 mg) ±C.V. (%)*
HPTLC	97.19 ± 2.96	100.86 ± 2.59	107.62 ± 2.18	105.52 ± 2.42	104.11 ± 2.71
HPLC	98.40 ± 1.23	102.47 ± 1.89	106.00 ± 1.47	105.33 ± 0.64	106.73 ± 0.96

The separation of bromperidol from the following possible degradation product (1) and impurities (2 and 3) was checked in solvent system I: (1) 4-[4-(4-bromophenyl)-3,6-dihydro-1(2*H*)-pyridinyl]-1-(4-fluorophenyl)-1-butanone; (2) 4-(4-bromophenyl)-1,2,3,6-tetrahydropyridine; (3) 4-(4-bromophenyl)-4-hydroxy-piperidine.

Fig. 2 shows the HPTLC results for bromperidol, azaperone and the possible degradation product and impurities, and it can be seen that none of the latter interferes with bromperidol or azaperone.

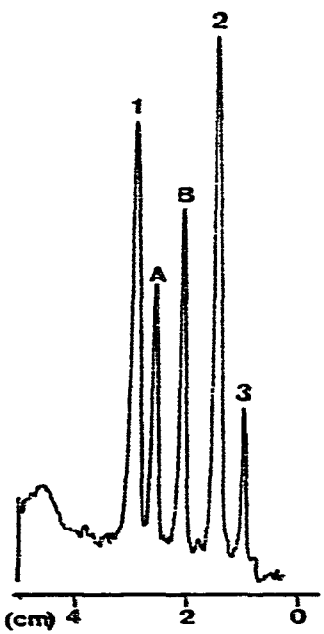
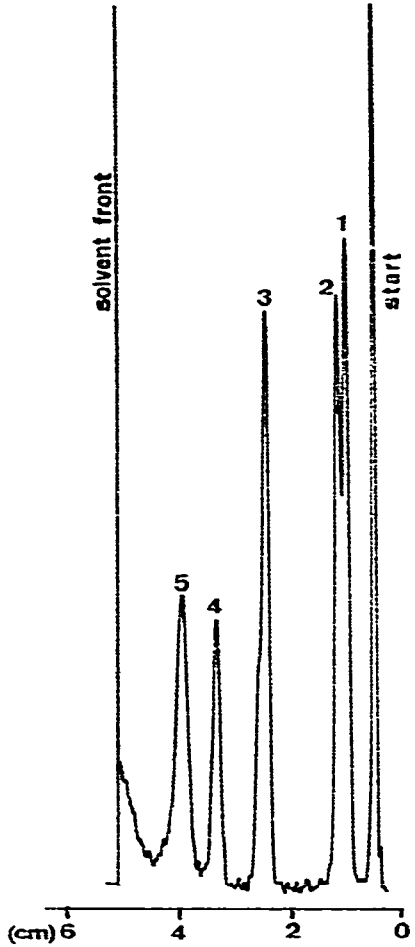


Fig. 2. HPTLC results for bromperidol (B), azaperone (A), degradation product (1) and impurities (2,3) in solvent system I (benzene–ethyl acetate–diethylamine, 90:5:5).

Separation of bromperidol from other butyrophenones by HPTLC. The separation of the following nine butyrophenones was investigated: pipamperone, droperidol, benperidol, haloperidol, bromperidol, trifluperidol, melperone, azaperone and fluanisone (Fig. 1).

In solvent system I, five of the nine butyrophenones were separated (Fig. 3), and in solvent system II eight of the nine butyrophenones were separated (Fig. 4). However, solvent system II cannot be used for quantitative work owing to interference from accompanying substances in the solutions. Bromperidol could not be separated from haloperidol. In 50 other solvent systems tried, the separation of bromperidol from haloperidol remained unsuccesful.



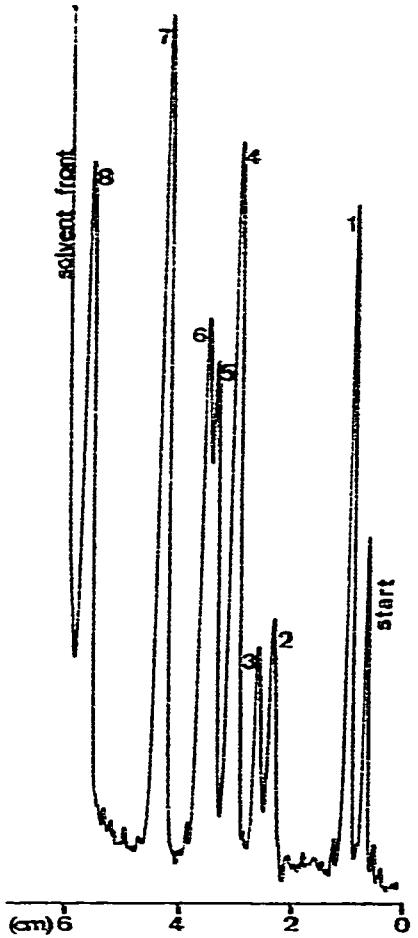


Fig. 3. HPTLC results for butyrophenones in solvent system I (benzene–ethyl acetate–diethylamine, 90:5:5). Peaks: 1 = pipamperone + droperidol; 2 = benperidol; 3 = bromperidol + trifluperidol + haloperidol; 4 = azaperone; 5 = melperone + fluanisone.

Fig. 4. HPTLC results for butyrophenones in solvent system II (organic layer of ethanol–ammonia–dichloromethane, 5.0:1.5:93.5). Peaks: 1 = pipamperone; 2 = droperidol; 3 = benperidol; 4 = haloperidol + bromperidol; 5 = trifluperidol; 6 = melperone; 7 = azaperone; 8 = fluanisone.

HPLC

Table II gives the results for the analysis of tablets, solutions and injections expressed as a percentage of the theoretical values. The coefficient of variation, calculated for each formulation on data obtained for five different solutions analysed in duplicate, was 1–2%. From Table II it can be concluded that the results of both techniques agree well.

Separation of bromperidol from other butyrophenones, impurities and a possible degradation product by HPLC. Fig. 5 shows the HPLC results for the nine butyrophenones studied. It can be seen that they are all well separated, even haloperidol and bromperidol, which were not separated by HPTLC. Table III gives the capacity factors (k') of the butyrophenones, impurities and degradation product on the reversed-phase column. Fig. 6 shows the HPLC results for bromperidol, azaperone and compounds (1), (2) and (3). It can be seen that with the HPLC system used, bromperidol and azaperone are separated from the possible degradation product and impurities.

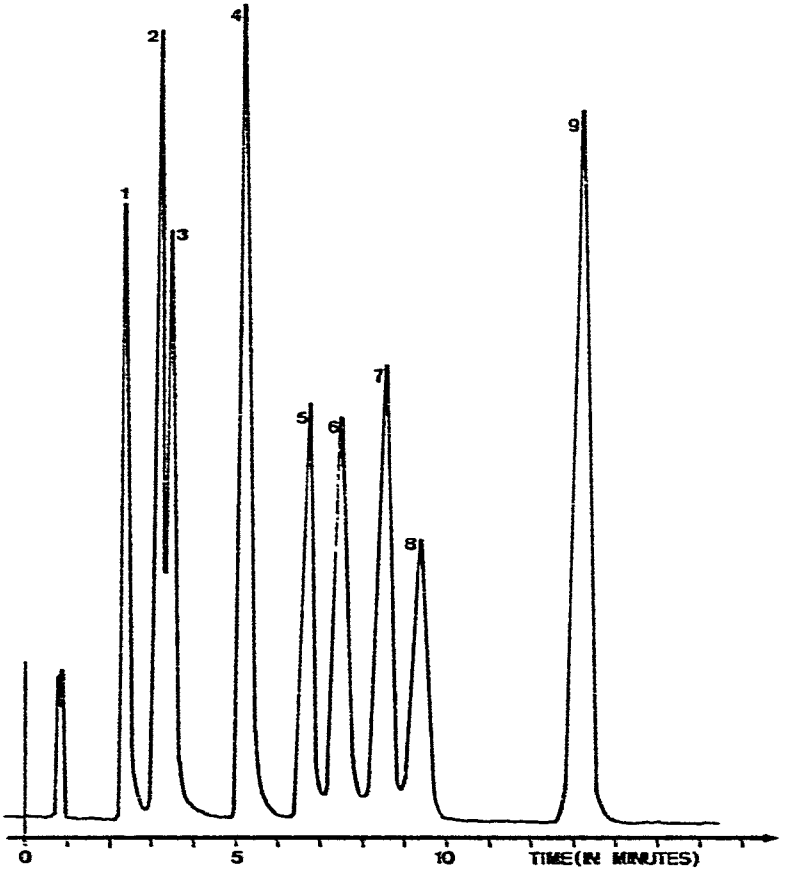


Fig. 5. HPLC results for butyrophenones. Peaks: 1 = pipamperone; 2 = droperidol; 3 = benperidol; 4 = azaperone; 5 = haloperidol; 6 = bromperidol; 7 = fluanisone; 8 = trifluperidol; 9 = melperone.

TABLE III

CAPACITY FACTORS $\left(k' = \frac{t_R - t_0}{t_0}\right)$ OF THE BUTYROPHENONES, IMPURITIES AND DEGRADATION PRODUCT TESTED

Compound	*k'*	*Compound*	*k'*
Pipamperone	1.94	Fluanisone	9.72
Droperidol	3.04	Trifluperidol	10.77
Benperidol	3.35	Melperone	15.56
Azaperone	5.56	Degradation product (1)	39.68
Haloperidol	7.47	Impurity (2)	22.73
Bromperidol	8.37	Impurity (3)	9.09

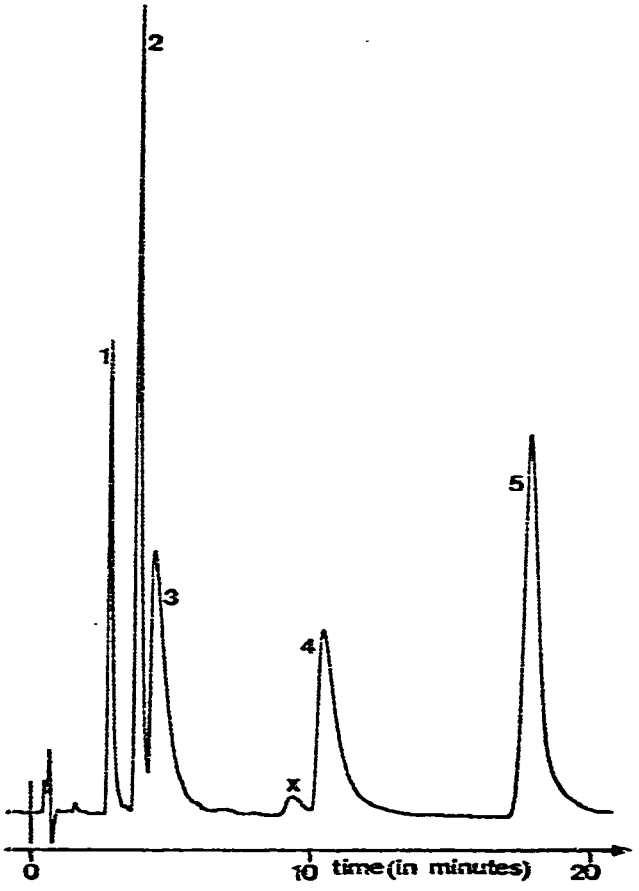


Fig. 6. HPLC results for bromperidol, azaperone, a degradation product and impurities. Peaks: 1 = azaperone; 2 = bromperidol; 3 = impurity (3); 4 = impurity (2); 5 = degradation product (1).

CONCLUSION

The HPLC and HPTLC methods described are very suitable for the determination of bromperidol in pharmaceutical preparations. The results for both techniques agree well. HPLC has the advantage over HPTLC of being more precise, with a coefficient of variation of 1–2% compared with 2–3%. HPLC is more specific than HPTLC as it separates all nine butyrophenones studied, even the two structurally very similar compounds haloperidol and bromperidol. The separation of bromperidol from a degradation product and impurities is achieved equally well by HPTLC and HPLC. In our HPTLC system, eight of the nine butyrophenones studied can be separated, but we did not succeed in separating haloperidol from bromperidol. HPTLC has the advantage over HPLC of being more rapid; in the same time period four times as many samples can be examined by HPTLC than by HPLC.

ACKNOWLEDGEMENTS

Janssen Pharmaceutica are gratefully acknowledged for the gift of butyrophenones, degradation products, impurities and pharmaceutical preparations.

REFERENCES

1 P. J. Demoen, *J. Pharm. Sci.*, 50 (1961) 350.
2 L. P. Chekova, F. M. Shemyakin and L. N. Molnar, *Farmatsiya (Moskow)*, 21 (1972) 41.
3 J. J. Thomas and L. Dryon, *J. Pharm. Belg.*, 22 (1967) 163.
4 E. Pawelczyk, Z. Plotkowiak and F. Miszuk, *Chem. Anal. (Warsaw)*, 16 (1971) 643.
5 T. Tyfczynska, *Diss. Pharm. Pharmacol.*, 20 (1968) 459.
6 A. Haemers and W. Van den Bossche, *J. Pharm. Pharmacol.*, 21 (1969) 531.
7 H. Soep, *J. Chromatogr.*, 6 (1961) 122.
8 W. Van den Bossche, A. Haemers and P. De Moerloose, *Pharm. Weekbl.*, 104 (1969) 445.
9 W. Baeyens, *Pharm. Weekbl.*, 112 (1977) 681.
10 W. Baeyens and P. De Moerloose, *Pharmazie*, 32 (1977) 764.
11 H. Bethke, W. Santi and R. W. Frei, *J. Chromatogr. Sci.*, 12 (1974) 392.