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ANALYSIS OF HALOPERIDOL TABLETS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY —AN INTER-LABORATORY STUDY

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SUMMARY

A high-performance liquid-chromatographic (HPLC) method for the determination of haloperidol in tablets was developed and evaluated by an inter-laboratory study. The spectrophotometric method of the British Pharmacopoeia 1973 was evaluated concurrently, and the accuracy and precision of the methods were compared. Two samples of a commercially available haloperidol tablet formulation were analysed by thirteen laboratories with satisfactory results for column performance and precision of assay. The total error standard deviations, S_D, for the HPLC method and the spectrophotometric method were 3.92 and 2.58%, respectively. The HPLC method is considered suitable for official testing purposes.

INTRODUCTION

In recent years, analysis of pharmaceuticals in dosage forms by chromatographic methods has become widespread. A number of high-performance liquid chromatographic (HPLC) procedures have been adopted as Pharmacopoeial referee methods and are used for the official testing of commercially available therapeutic goods. While HPLC methods have obvious attractions over many older pharmacopoeial procedures in terms of speed and selectivity, relatively little information has been made available on the precision and accuracy of chromatographic methods of pharmaceutical analysis under conditions of inter-laboratory usage. This paper describes an HPLC method for the major tranquiliser haloperidol {4-[4-(4-chlorophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone} in tablets and an inter-laboratory study of its precision and accuracy as compared with a pharmacopoeial procedure based on spectrophotometry.

The previous Australian official method for the determination of haloperidol in tablets was that in the 1973 edition of the *British Pharmacopoeia* (B.P.)[1]. This method involves direct extraction from the crushed tablet material, followed by measurement of the absorbance of the resulting solution at 245 nm. A difficulty arose in the use of this method for coloured haloperidol tablets because of interference from the dyestuffs. The experience of this laboratory was that, when the method was applied to coloured tablets, the results for haloperidol were up to 22% higher than the true

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contents. It therefore seemed appropriate to use a chromatographic method as an alternative, and an HPLC separation proposed by an Australian manufacturer[2] was considered for further development.

EXPERIMENTAL

Development of HPLC method

In the manufacturer's method, separation of haloperidol and the colouring material was achieved on a Waters μBondapak C_{18} column, using methanol–water–glacial acetic acid (80:20:1) as the mobile phase. Further work with this system showed that the relationship between detector response (peak-height ratio) and concentration was non-linear. Linearity and peak shape improved when the amount of haloperidol injected was decreased, but to obtain an acceptable response–concentration relationship it was necessary to add potassium chloride to the mobile phase. A mobile phase consisting of methanol–0.01 *M* potassium chloride–glacial acetic acid (60:40:2) was found to be suitable.

Fig. 1 shows a chromatogram obtained using this mobile phase at a flow-rate of 1.5 ml/min, with a UV detector operating at 254 nm. The dyestuff was completely retained by the column and was therefore resolved from haloperidol and from 2-naphthol, which was selected as an internal standard.

Tablets were prepared for analysis by grinding them to an even, fine powder. An accurately weighed portion of sample powder, equivalent to 2.5 mg of haloperidol, was then vigorously shaken for 5 min in 50 ml of the mobile phase which contained 0.05 mg/ml of 2-naphthol. The resulting solution was filtered prior to injection.

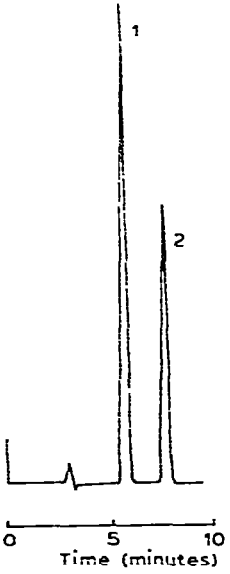

Fig. 1. Typical chromatogram from analysis of haloperidol tablets. Peaks: 1 = 2-naphthol; 2 = haloperidol.

Inter-laboratory trial of the method

In order to assess the suitability of the method for official testing of haloperidol tablets, an inter-laboratory trial was conducted, using thirteen participants. Each laboratory analysed two samples, consisting of tablets from separate batches of a commercially available 1.5-mg haloperidol formulation, by both the HPLC method and the spectrophotometric procedure of the B.P. 1973. In order to permit a valid comparison of the two methods, a sample formulation was selected which did not contain a dyestuff, so that interference in the B.P. assay was avoided.

The trial protocol specified use of a column packed with octadecylsilane-coated silica particles of mean diameter not more than 10 μm. Column dimensions of 25 cm × 2 mm I.D. and a nominal flow-rate of 1.0 ml/min were suggested.

Each laboratory received portions of the same haloperidol reference substance, which was checked for purity by HPLC before dispatch. Preliminary samples were not sent to the participating laboratories but criteria for resolution and reproducibility were to be met before participants proceeded to the analysis of the samples. Laboratories were asked to contact the National Biological Standards Laboratory if any difficulties were encountered or if any modifications to the method were desired.

A solution consisting of 0.1 mg/ml of haloperidol and 0.05 mg/ml of 2-naphthol in water–glacial acetic acid was used as a calibration solution. Prior to analysis of samples, six replicate injections of this solution were made and participating laboratories were asked to achieve a mean resolution factor of 3.0 with a coefficient of variation of less than 2.0%. The coefficient of variation of the peak-height ratios from the six chromatograms was also required to be less than 2.0%. A minimum height of 60% of full scale deflection was required for each peak. Some laboratories could not meet the requirement of not less than 3.0 for the resolution factor, R. However, it was considered that in view of current pharmacopoeial practice an R value greater than 2.0 was acceptable and the laboratories concerned were requested to proceed with analysis of the trial samples.

Laboratories analysed each sample once, using a mixture of powder from

TABLE I

DETAILS OF COLUMN PERFORMANCE

Laboratory No.	*Resolution factor (R)*	*Coeff. of variation of peak-height ratios*
1	3.77	1.71
2	4.28	2.63
3	3.59	0.35
4	4.85	1.26
5	2.09	0.26
6	2.76	0.33
7	2.65	0.40
8	2.47	0.71
9	2.71	0.02
10	3.26	0.01
11	3.00	0.35
12	3.39	0.82
13	6.50	2.4

TABLE II

ASSAY RESULTS

Laboratory No.	Percent recovery by			
	HPLC method		B.P. method	
	Sample A	Sample B	Sample A	Sample B
1	95.6	94.5	99.7	97.8
2	101.4	101.5	98.1	99.2
3	100.3	100.4	100.6	100.1
4	106.8	104.1	98.6	98.5
5	100.9	99.2	100.7	97.6
6	98.4	100.6	101.6	101.3
7	98.4	97.3	96.9	96.5
8	96.0	93.2	102.6	102.1
9	96.4	96.7	101.0	100.1
10	100.6	98.0	102.0	102.7
11	100.6	98.7	97.1	96.0
12	102.2	100.5	103.1	100.5
13	102.2	102.1	100.0	101.1
Mean	100.0	99.1	100.1	99.5
Standard deviation	3.2	3.2	2.0	2.1

twenty tablets in each case, and including 2-naphthol as an internal standard in the extracting solution of methanol–water–glacial acetic acid (80:20:2, v/v). Quantitation was achieved by comparison of the peak-height ratio with the mean peak-height ratio of the calibration solution.

RESULTS

Measurement of column performance

The values reported by the thirteen laboratories for the resolution factor (R) and coefficient of variation of peak-height ratio are shown in Table I. Laboratory 13 used a brand of column different from that used by all other laboratories and obtained a large value for R in addition to a reversal of elution order. This operator found that 2-naphthol was not practical as an internal standard and used an external-standard procedure.

Evaluation of the methods

Data from the analysis of the samples by the HPLC method are shown in Table II and Fig. 2. The mean results for content of haloperidol obtained for samples A and B were 100.0% and 99.2%, respectively. Laboratory 1 was the only participant to report the presence of decomposition products, which may have been partly responsible for the low assay values obtained by this operator. No attempt was made to compensate for the presence of these decomposition products in the computation of the haloperidol content.

Using the terminology of Youden and Steiner[3] the total error, precision (repeatability) error and bias (reproducibility) error of a method can be measured by the standard deviations S_D, S_R and S_B obtained from the expressions:

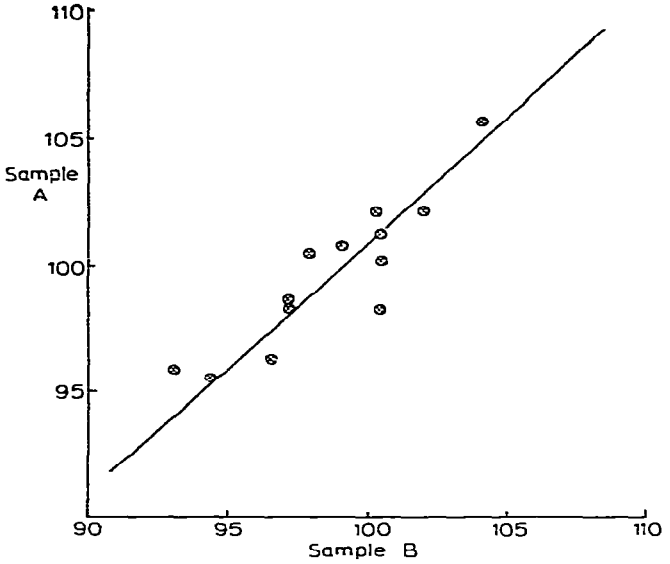

Fig. 2. Two-sample chart for the HPLC procedure.

$$S_D = \sqrt{\Sigma(T_i - T)^2/2(n-1)}$$
$$S_R = \sqrt{\Sigma(D_i - D)^2/2(n-1)}$$
$$S_B = \sqrt{(S_D^2 - S_R^2)/2}$$

Where T_i refers to the sum and D_i to the difference of the results for content of each sample for n estimates ($n = 13$ in the trial reported here).

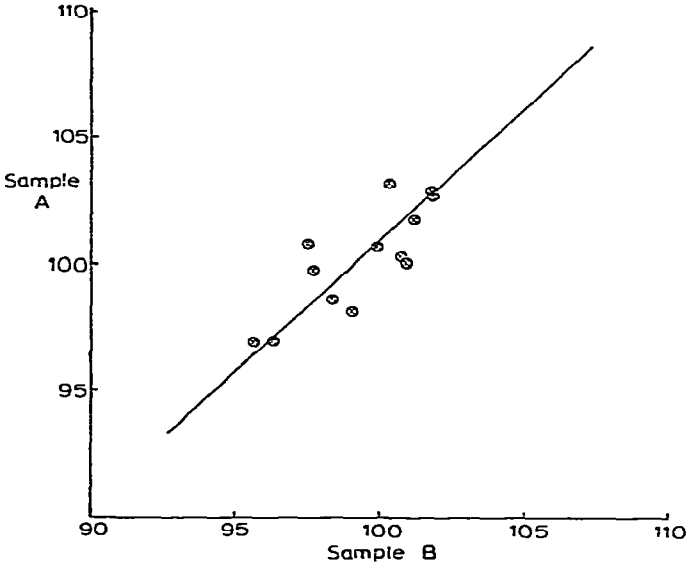

Fig. 3. Two-sample chart for the method of the B.P. 1973.

TABLE III

COMPARISON OF THE HPLC METHOD WITH THE B.P. METHOD. RELATIVE BIAS

Laboratory No.	*HPLC method* $A - B$	*B.P. method* $A + B$	D = *HPLC* − *BP*
1	190.1	197.5	−7.4
2	202.9	197.4	5.5
3	200.7	200.7	–
4	210.9	197.1	13.8
5	200.1	198.3	1.8
6	199.0	202.9	−3.9
7	195.7	193.4	2.3
8	189.2	204.7	−15.5
9	193.1	201.1	−8.0
10	198.6	204.7	−6.1
11	199.3	193.1	6.2
12	202.6	203.6	−1.0
13	204.3	201.1	3.2
Mean	199.0	199.7	−0.7
Standard deviation	5.9	3.9	7.5

For the HPLC method, the total error standard deviation S_D, is 4.20, the precision standard deviation, S_R, is 1.03 and the bias standard deviation, S_B, is 2.88. If the results from Laboratory 1 are rejected, because of the partial sample decomposition reported by that participant, the respective values for S_D, S_R and S_B are 3.92, 1.08 and 2.66.

The data obtained using the spectrophotometric method of the B.P. 1973 are shown in Table II and Fig. 3. The mean values obtained for samples A and B were 100.1% and 99.5%, respectively. The standard deviations S_D, S_R and S_B were found to be 2.76, 0.91 and 1.84.

Comparison of the methods

A comparison of the results obtained from the two procedures is shown in Table III. From the "difference" column it is apparent that the two test methods gave similar results, and use of a paired t test showed that no significant difference exists between the two methods with regard to the estimates of the mean contents of the two samples ($t_{12} = 0.30$). On the basis of the F test, at the 95% confidence level, there is no significant difference between the methods with regard to precision standard deviation (S_R), while bias standard deviation is significantly greater for the HPLC procedure. On the basis of these results, it is considered that the HPLC procedure is adequate for official testing of haloperidol tablets, with suitably low imprecision and bias, and with obvious selectivity advantages over the direct spectrophotometric method.

DISCUSSION

The most important point raised by participating laboratories concerned the difficulty in obtaining a suitable value for the resolution factor, R, and a range of

mobile phase compositions was used to achieve the required resolution. The composition of the mobile phase used by participants (methanol–electrolyte–acetic acid) ranged from 50:50:1 to 70:30:1. When the method is included in a standard, a range of solvent proportions will be specified with minimum column performance criteria. It is accepted that laboratories must be free to adjust mobile phase composition to achieve satisfactory resolution but it would seem necessary to set limits to this adjustment to avoid effectively different methods being used in a referee situation. A related problem is the task of appropriately specifying the type of column to be used in an official method. Possible approaches are to refer to commonly available commercial brands or to describe the column packing more closely, to take account of different methods of manufacture. This task is becoming increasingly difficult as the number of reversed-phase packings is rapidly proliferating. Majors[4] has listed over 30 octadecylsilane-bonded packings, all of which differ in percentage of phase loading, pore size and proportion of residual silanol groups.

Some laboratories neglected to use the electrolyte in the mobile phase as they considered that the peak shape obtained with methanol–water–acetic acid was symmetrical. Non-compliance with the trial protocol is always a potential problem with inter-laboratory trials, and also occurred in a previous study of an HPLC method conducted by this laboratory[5]. In the work described here, satisfactory results were obtained, but presumably over-all error in the HPLC method would have been less had all laboratories followed instructions more closely. A few participants were concerned that potassium chloride in the mobile phase could induce corrosion in the stainless steel of pumps and columns. This potential problem can be overcome by passifying the pump after use with 20–50% nitric acid solution[6]. It was found at this laboratory, after the trial, that sodium sulphate solutions, which do not produce significant corrosion of stainless steel, are as effective as potassium chloride solutions in ensuring linearity of response, and the method will be modified accordingly when used for official testing.

One laboratory commented that the peak-height ratios from the calibration solution varied less than the ratios of the electronically integrated areas, the coefficients of variation being 2.5% for the area ratios and 0.26% for the peak-height ratios. Scott and Reese[7] have pointed to the greater reliability of peak-height compared with peak-area measurement, and adoption of peak heights in a referee method also enables laboratories which do not have suitable integrators to carry out the official procedure.

The results of the inter-laboratory trial have shown that the HPLC method for haloperidol tablets compares favourably with the direct spectrophotometric procedure of the B.P. 1973 with regard to precision, but has greater systematic error. It may be possible to reduce the systematic error of the HPLC method by more closely specifying the procedure with regard to assurance of linear response of detector output and accurate temperature control of the column. Conditions of storage of the mobile phase might also be specified to ensure that evaporation of the volatile components does not occur. The HPLC method is, however, considered to be acceptable for the testing of haloperidol tablets, and is preferred to the spectrophotometric procedure for single-tablet analysis of low-dose (0.5 mg) formulations and for the assay of higher dose formulations containing dyestuffs.

During the course of the trial described in this paper, the method of the British

Pharmacopoeia for haloperidol tablets was modified to overcome the interference problems referred to above. The relevant monograph of the B.P. 1980 (see ref. 8) includes a spectrophotometric assay in which the powdered haloperidol tablets are successively triturated with portions of diethyl ether, which are then combined, and the drug substance is partitioned into dilute sulphuric acid. This relatively slow procedure successfully overcomes any interference from colouring materials, but in our hands gave low recoveries of drug substance and had lower precision than either the HPLC procedure or the method of the B.P. 1973. Analysis of sample B from the trial by this laboratory using the method of the B.P. 1980 gave a mean content of 94.8 % with a standard deviation of 1.7. ($n = 5$) This compares with the inter-laboratory results for the B.P. 1973 method of a mean content of 99.5 % with a precision standard deviation of 0.9, while the corresponding data for the HPLC method are 99.1 % and 1.08. The HPLC method is considered to be a realistic alternative to pharmacopoeial methods in terms of speed, bias, precision and selectivity, and to be suitable for official testing purposes.

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