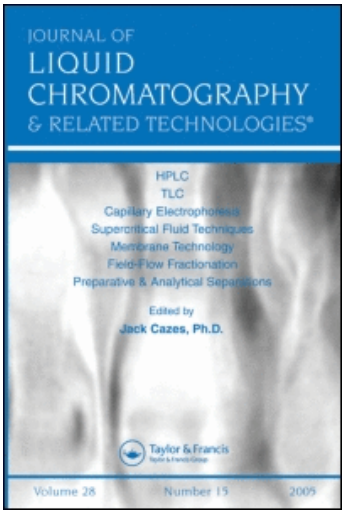

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Fast Determination of Haloperidol in Pharmaceutical Preparations Using HPLC with a Monolithic Silica Column

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Fast Determination of Haloperidol in Pharmaceutical Preparations Using HPLC with a Monolithic Silica Column

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Abstract: A rapid, sensitive, and reproducible HPLC method was developed and validated for the analysis of haloperidol in pharmaceutical preparations. The analysis was carried out on a monolithic silica column Chromolith Performance RP-18e (100 × 4.6 mm). The mobile phase consisted of sodium phosphate (100 mM, pH 3.0)-acetonitrile (70 : 30, v/v) at a flow rate from 1.0 mL/min with UV detection at 230 nm. The detection limit of haloperidol was 1 ng while the linearity range was 1 to 100 ng. The retention time and capacity factors were 4.26 min and 2.41, respectively.

Keywords: Haloperidol, HPLC, Monolithic column, Pharmaceutical preparations

INTRODUCTION

Due to the costly chemicals used in high performance liquid chromatography (HPLC) and importance of time, the speed of analysis is becoming increasingly important in many application areas of HPLC, such as pharmaceutical analysis and toxicology laboratories, in order to increase throughput and reduce costs. Some drugs and biomedically important compounds are bases and the analysis of these remains problematic due to poor peak shapes, which are

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often experienced in reversed phase chromatography.[1] In the past, attempts have been made to decrease analysis times by using short columns with smaller particles (smaller than the standard 5 μm). Of course, these columns offered good efficiency with higher flow rates but resulted in high plug and back pressure.[2] Therefore, researchers have been trying to overcome the problem of high pressure drop associated with the use of small particles by employing ultra high-pressure liquid chromatography (UHPLC),[3] capillary electro-chromatography (CEC),[4] and by open tube liquid chromatography.[5] Recently, columns made of a single piece of monolithic silica were introduced as the alternatives to particle-based columns. These columns possess a biporous structure consisting of larger macropores (2 μm) that permit high flow rates with low back pressure and smaller mesopores (13 nm), providing a high surface area for high efficiency.[6] Therefore, it is possible to perform analyses with high linear flow velocity but without significantly reduced separation efficiency. Some reviews[7,8] have been published claiming the fast and economic analysis by these columns for a variety of compounds.

Haloperidol (Figure 1) is a butyrophenone antipsychotic, a D_2, D_3, and D_4 dopamine receptor antagonist, and is used in the therapy of patients with acute and chronic schizophrenia. The determination of trace amount of drugs in biological samples and pharmaceutical preparations is a well-known problem. Drug testing is an integral part of pharmaceutical analysis and routine quality control monitoring of drug release characteristics. In view of this, attempts have been made to develop a fast, sensitive, and reproducible HPLC method for the analysis of haloperidol in pharmaceutical preparations and the results are presented herein.

EXPERIMENTAL

Chemicals and Reagents

Haloperidol was obtained from RBI-Sigma, USA, and the pharmaceutical preparations of haloperidol, i.e., Halodol (5 mg/mL) ampoules were purchased from Janssen Pharmaceutica, Beerse, Belgium. The standard solutions of different concentrations (1–100 μg/mL) of haloperidol were prepared in methanol. The dilutions of the halodol ampoules were also carried out by methanol. Purified water was prepared using a Millipore Milli-Q (Bedford, M.A.,

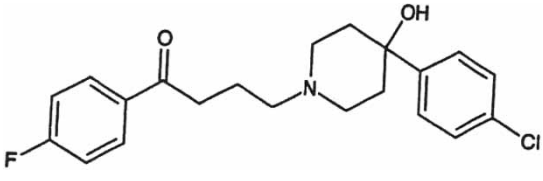

Figure 1. Chemical structure of haloperdol.

U.S.A.) water purification system. Acetonitrile and methanol, of HPLC grade, and *o*-phosphoric acid of A.R. grade were purchased from Fisher Scientific (Fairlawn, New Jersey, USA). Sodium dihydrogen orthophosphate ($NaH_2PO_4 \cdot 2H_2O$) of A.R. grade was obtained from BDH Limited, Poole, England. Phosphate buffers were prepared by the standard methods.

Instruments Used

The HPLC system consisting of Waters solvent delivery pump (model 510, Milford, Massachusetts, USA), Waters injector (model WISP 710B), Waters tunable absorbance detector (model 484), and Waters integrator (model 740) was used in this study. The monolithic silica column, Chromolith Performance RP-18e 100-4,6 (100×4.6 mm) was purchased from Merck, Darmstadt, Germany. The pH meter used was Orion Research (model 611), Orion Research Incorporation, USA. Millipore Milli-Q (Bedford, M.A., U.S.A.) was used for deionized water.

Chromatographic Conditions

An aliquot of 10 μL of standard and pharmaceutical preparations of haloperidol was injected on to a HPLC system separately and respectively. The mobile phase used in this study was phosphate buffer (100 mM, pH 3.0)-acetonitrile (70 : 30, v/v) with a flow rate of 1 mL/min. The mobile phase was filtered and degassed before use. The chart speed was kept constant at 2 cm/min. All the experiments were carried out at $23 \pm 1^{\circ}C$ with the UV detection at 230 nm. The chromatographic parameters such as retention time and capacity factor (k) were calculated. The identification of the separated haloperidol in pharmaceutical preparations was confirmed by running the chromatogram of the standard compound under the identical chromatographic conditions. The internal addition method was also applied for the confirmation of the studied molecule in ampoule formulations.

Preparation of Standard Stock Solution

Stock solutions of haloperidol were prepared by accurately weighing 50 mg of the drug and dissolving in methanol in 100 mL volumetric flasks. Serial dilutions (1–100 ng/mL) were carried out, using methanol to obtain the concentration ranges required.

Sample Preparation from Halodol Ampoule

To estimate the concentration of haloperidol in ampoule formulations, the liquid contents of the ampoule were taken out in a stoppered glass tube and

was used to prepare the dilute solutions of haloperidol with methanol. Dilution was also carried out by ethanol, buffers, and mobile phase, which have no effect on the chromatographic behavior of haloperidol analysis in the ampoule. There was no interference in the chromatographic studies by using the dilute solutions of haloperidol in the ampoule and, hence, no special sample preparation was required for the analysis of haloperidol from the ampoule. Haloperidol analysis was carried out in several ampoule preparations.

Quantitation and Linearity

Equal volume (10 μL) of the standard and the assay preparations that contain haloperidol in methanol were loaded onto the HPLC device and the chromatograms were recorded. Calibration standards of each concentration were analyzed in triplicate. Calibration curves of haloperidol were constructed using the observed peak area versus nominal concentrations of the analyte.

Specificity

The specificity of the method was investigated by observing any interference encountered from excipients present in the formulations. It was shown that haloperidol does not interfere with the proposed method.

Validation

The limit of detection (LOD) and the limit of quantitation (LOQ) were determined as 3 and 5 times the baseline noise, respectively, following the United States Pharmacopoeia.[9] The results of the statistical analysis of the experimental data such as the standard deviation, correlation coefficients, and confidence limit were calculated by Microsoft Excel software program. The good linearity of the calibration graphs and the negligible scatter of experimental points are clearly evident by the values of the correlation coefficient and standard deviation.[10] The robustness of the method is demonstrated by the versatility of the experimental factors that affect the peak area.

RESULTS AND DISCUSSION

Chromatography

Chromatographic parameters such as retention time and capacity (k) factors for the separated haloperidol are calculated and are given in Table 1. A

Table 1. Retention time and capacity (k) factor for haloperidol

Compound	t_R	k	SD	R^2	Confidence limit
Haloperidol standard	4.26	2.41	± 0.05	0.9999	99.6
Haloperidol ampoule	4.29	2.41	± 0.05	0.9999	99.6

Chromatographic conditions:
Column: Monolithic silica column (100 × 4.6 mm).
Mobile phase: Phosphate buffer (100 mM, pH 3.0)-acetonitrile (70 : 30, v/v).
Flow rate: 1 mL/min.
Detection: UV at 230 nm.
Experimental temp.: 23 ± 1°C.

typical chromatogram of the separated haloperidol (standard solution) is given in Figure 2. The higher values of retention time (4.26 for standard and 4.29 for pharmaceutical preparations) and k (2.41) (Table 1) indicated the good chromatographic separation of haloperidol in the standard and pharmaceutical preparations.

A chromatogram of the standard haloperidol was also recorded under the identical chromatographic conditions. Haloperidol in pharmaceutical preparations was identified by comparing its retention time with the retention time of this compound in standard solution. The confirmation of haloperidol in ampoule preparation was carried out by the internal addition method. Calibration curves was plotted for haloperidol and used to determine its concentrations in pharmaceutical preparations. To optimize the chromatographic conditions, various combinations of phosphate buffer-acetonitrile, water-acetonitrile were tested. Besides, buffers, with different concentrations and pHs, alone or with methanol were also used. As a result of extensive experimentation the optimized HPLC conditions were developed and reported herein (Table 2).

pH of the reported mobile phase was 3.5 and, at this pH haloperidol existed as ammonium cation and, hence, this molecule interacted with the silonol groups through some electrostatic forces. Besides the dispersion forces, hydrogen bonding, van der Waal forces, and steric effect are also playing some role in the separation phenomenon of the reported drug on monolithic silica column.

Analysis of Haloperidol in Pharmaceutical Formulations

The analysis of haloperidol in pharmaceutical preparations was carried out by the optimized and reported HPLC method and the values of chromatographic parameters are given in Table 1. Different dilutions of haloperidol concentrations from ampoule preparations were prepared by methanol. A typical chromatogram of the separated haloperidol in pharmaceutical preparations is shown in Figure 3, which clearly indicates the base line separation of this compound. The quantitative estimation of haloperidol was carried out by comparing the peak area of

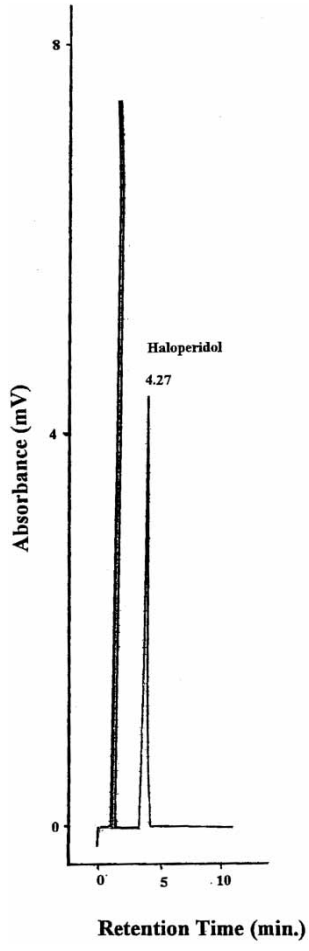

Figure 2. Chromatogram of haloperidol (standard solution) on monolithic silica column (100 × 4.6 mm) using phosphate buffer (100 mM, pH 3.0)-acetonitrile (70 : 30, v/v) as the mobile phase at 1 mL/min flow rate with UV detection at 230 nm.

haloperidol in pharmaceutical preparations with the peak area of standard compound. The percentage recoveries of haloperidol was 99.8% (Table 2). Attempts have been made to optimize the chromatographic parameters to get the maximum recoveries and the results are given in Table 2. It has been observed that, at higher concentrations and pHs of phosphate buffer and wavelengths of detection, poor recoveries have been experienced. It is interesting to note that lower amounts of acetonitrile (10 and 20%) resulted in poor detection of haloperidol, while at higher concentrations of acetonitrile the elution was very fast. There was no marked effect on the recoveries of haloperidol on varying its loading amount into an HPLC instrument, however, an aliquot of 10 μL of sample loading has given the maximum recovery.

Table 2. Effect of HPLC Experimental parameters on the analysis of haloperidol in pharmaceutical preparations

Parameter	Variation	Recovery (%)
Conc. of MP	25 mM	99.5
	50 mM	99.6
	100 mM	99.8
	200 mM	96.0
	500 mM	95.0
pH of MP	2	99.6
	3	99.8
	4	99.4
	6	99.2
	8	96.1
		96.0
Amount of acetonitrile (%) detection	10	Poor
	20	Late elutio
	30	99.8
	40	99.7
	50	Fast elutio
Wavelength (nm)	220	Interference
	230	99.8
	254	90.2
	280	80.5
Sample loading amount	5	99.5
	10	99.8
	15	99.7
	20	99.7

Experimental conditions: as in Table 1.

Validation of the Methods

Specificity

The specificity of the method was investigated by observing any interference from excipients present in the pharmaceutical preparations. It was shown that haloperidol does not interfere, thus the HPLC method presented in this study is selective for the studied drug. The specificity was also demonstrated by induced degradation of the haloperidol sample by treating it with 100 mM hydrochloric acid and 100 mM sodium hydroxide, separately and respectively, and storing the sample at room temperature for 24 h; the recovery of haloperidol was 99.8% in both the cases. The possible photo-degradation of haloperidol in methanolic solution and mobile phase (1 mg/mL) was also studied by

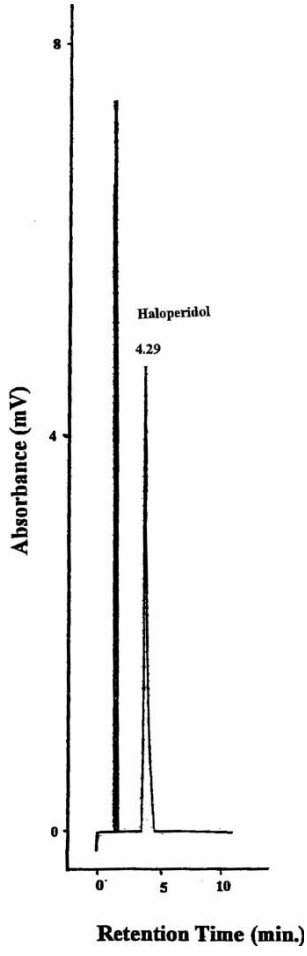

Figure 3. Chromatogram of haloperidol in pharmaceutical preparation on monolithic silica column (100 × 4.6 mm) using phosphate buffer (100 mM, pH 3.0)-acetonitrile (70 : 30, v/v) as the mobile phase at 1 mL/min flow rate with UV detection at 230 nm.

exposing various samples of haloperidol to direct sunlight and darkness for 7 days. No marked difference was observed in the concentration of haloperidol, which indicated the photo-stability of the drug.

Linearity

The linearity of calibration curves (peak area vs. concentration) for haloperidol in pure solution, as well as in dosage forms, were checked over the concentration ranges of about 1–100 ng/mL with correlation coefficient (R^2) of greater than 0.999, as determined by least squares analysis.

Limit of Detection (LOD), Limit of Quantitation (LOQ), and Accuracy

The limit of detection (LOD) and limits of quantitation (LOQ) were calculated for the calibration graphs of haloperidol as three and five times of the noise level for LOD and LOQ, respectively. The values for LOD and LOQ were 1 and 3 ng/mL respectively. The accuracy of the method was tested by analyzing different samples of haloperidol at various concentration levels in pure solutions. The results were expressed as percent recoveries of the particular components in the samples, which were in agreement with the reference method.[11]

Stability of Analytical Solutions

The stabilities of sample solutions of haloperidol were tested over one week's time. The freshly prepared and stored samples were analyzed by the optimized proposed HPLC method. The percent difference observed was in the range of 0.09–0.10 (Table 3), indicating the good stability of haloperidol for one week. The standard deviation of the stability analysis was ± 0.04, while the values of correlation coefficient were in the range of 0.9997 to 0.9998. The confidence limits were found to be 99.5 to 99.6%.

Robustness

The optimum HPLC conditions set for this method have been slightly modified for samples of haloperidol as a means to evaluate the method's ruggedness. The small changes made include the composition and pH of the mobile phase, flow rate, the detection wavelength, and sampling amount. It was observed that there was no remarkable variations in HPLC results, which indicated good reproducibility of the method. Considering the modification in the system suitability parameters and the specificity of the method, as

Table 3. The intra- and inter days data for haloperdol (stability of haloperidol within a week) in methanolic solution of 1 mg/mL

Days	Difference (%)	Standard deviation	Correlation coefficient	Confidence limit
1	0.09	± 0.04	0.9999	99.6
2	0.09	± 0.04	0.9999	99.6
3	0.10	± 0.04	0.9998	99. 6
4	0.10	± 0.04	0.9998	99.5
5	0.11	± 0.04	0.9998	99.5
6	0.11	± 0.04	0.9998	99.5
7	0.11	± 0.04	0.9997	99.5

$n = 3$.

Difference (%) = [quantity found in fresh solution – quantity found in stored solution] $\times$ 100/(quantity found in fresh solution).

well as carrying out the experiment at room temperature, we would conclude that the method conditions are robust.

The developed chromatographic method was validated by carrying out three sets (n = 3) of the chromatographic runs under the identical experimental conditions. It is interesting to note that the values of chromatographic parameters (Table 1) are almost equal when analyzing standard solutions and pharmaceutical preparations, which indicates the robustness of the HPLC method. The validation of the developed method was ascertained by regression analysis using Excel Microsoft program and the results for chromatographic studies are given in Table 1. A perusal of Table 1 indicates that the values of standard deviation (SD), correlation coefficient (CC), and confidence limit (CL) were ± 0.05, 0.9999 and 99.5, respectively.

The data of peak area versus drug concentration was treated by linear least square regression analysis. Linearity was calculated according to DIN 32645 with B.E.N. Version 2.0 (B.E.N., Herbold M., Schmitt G., Inst. Of Legal and Traffic Medicine) and the values were in the range of 1 to 100 ng. The values of correlation coefficients varied from 0.9998 to 0.9999, while the values of confidence limit were 99.6% for the drug studied. The values of the detection limits were also calculated and were found to be 1 ng in both standard and pharmaceutical preparations. These values of validation indicated the good reproducibility of the chromatographic methods.

Conclusion

The developed chromatographic method is simple, fast, and reproducible for the separation and identification of the haloperidol in pharmaceutical preparations. The method utilizes a reversed phase monolithic silica column technology with UV detection. This method has been validated with good validation values, which were in agreement with United States Pharmacopoeia.[10] Therefore, the reported method can be used successfully for the analysis of haloperidol in any pharmaceutical preparation.

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REFERENCES

1. Rogers, S.D.; Dorsey, J.G. Chromatographic silanol activity test procedures: the quest for a universal test. J. Chromatogr. A. **2000**, *872*, 57–65.

2. Synder, L.R.; Kirkland, J.J.; Glajch, J.L. *The column in practical HPLC method development*; John Wiley & Sons Inc: New York, 1997, 174–232.
3. MacNair, J.E.; Lewis, K.C.; Jorgenson, J.W. Ultra-high pressure reversed-phase liquid chromatography in packed capillary columns. Anal. Chem. **1997**, *69*, 983–989.
4. Dittmann, M.M.; Rozing, G.P. Capillary electrochromatography—A high efficiency microseparation technique. J. Chromatogr. A **1996**, *744*, 63–74.
5. Tock, P.P.H.; Duijsters, P.P.E; Kraak, J.C.; Poppe, H. Theoretical optimization of open tubular columns for liquid chromatography with respect to mass loadability. J. Chromatogr. **1990**, *479*, 200–208.
6. Cabrera, K.; Lubda, D.; Eggenweiler, H.M.; Minakuchi, H.; Nakanishi, K. A new monolithic-type HPLC column for fast separations. J. High. Resolut. Chromatogr. **2000**, *23*, 93–99.
7. Rogers, S.D.; Dorsey, J.G. Chromatographic silanol activity test procedures: The quest for a universal test. J. Chromatogr. A **2000**, *892*, 57–65.
8. Tanaka, N.; Kobayashi, H.; Ishizuka, N.; Minakuchi, H.; Nakanishi, K.; Hosoya, K.; Ikegami, K. Monolithic silica columns for high-efficiency chromatographic separations. J. Chromatogr. A **2002**, *965*, 35–49.
9. *The United State Pharmacopeia*, 24th Ed.; United States Pharmacopeial Convention: Rockville, MD, 2000; 2150–2151.
10. Miller, J.C.; Miller, J.N. *Statistics for Analytical Chemistry*; Wiley: New York, 1984.
11. Dinesh, N.D.; Vishukumar, B.K.; Nagaraja, P.; Made Gowda, N.M.; Rangappa, K.S. Stability indicating RP-LC determination of sildenafil citrate (Viagra) in pure form and in pharmaceutical samples. J. Pharm. Biomed. Anal. **2002**, *29*, 743–748.

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