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Determination and degradation study of haloperidol by high performance liquid chromatography

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Abstract

A specific, high performance liquid chromatographic method was developed for the assay of haloperidol, together with an adequate separation of its degradation products. The method is based on the use of an octadecylsilane stationary phase column under isocratic conditions. The mobile phase consisted of 50 mM sodium phosphate monobasic pH 2.5–acetonitrile–THF–TEA (63:34:3:0.1, v/v/v/v) adjusted with *o*-phosphoric acid to a pH of 2.5. The degradation was performed in hydrochloric acid, sodium hydroxide and hydrogen peroxide. The main degradation products were identified. Application of the assay of haloperidol in tablet formulations is presented.

Keywords: Pharmaceuticals; Haloperidol; Liquid chromatography; Degradation products

1. Introduction

Haloperidol, 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4fluorophenyl)-1-butanone, a major tranquilizer, is used in the treatment of schizophrenia, mania and neurological disorders with hyperkinesia [1].

A number of methods have been reported for its quantitation in dosage forms. They include acidimetric titration in non aqueous medium [2], UV spectrophotometry [3,4], derivative spectrophotometry [5], fluorimetry [6], colorimetry [7–11], polarography [12], H-nuclear magnetic resonance [13], differential pulse adsorptive voltammetry [14], densitometry [15], GC [16], HPLC [17–24], CE [25] and MEKC [26].

However, there is no report of stress testing of haloperidol using acidic, basic and hydrogen peroxide medium to develop a HPLC method suitable to quantify this active ingredient in presence of its degradation products.

The present study was conducted in order to develop and validate a HPLC method allowing the quantitation of haloperidol in the presence of its likely degradation products. The considered compounds are reported in Fig. 1. In addition, identification of the obtained degradation products was investigated by comparison of their re-

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tention times and UV spectra with those of reference substances using a photodiode array detector.

2. Experimental

2.1. Samples

Haloperidol (HAL), 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-hydroxyphenyl)-1-butanone (**b**), *trans*-4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone, *N*-oxide monohydrate (**d**), *cis*-4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone *N*-oxide (**e**),), 4-[4-(4-chlorophenyl)-3,6-dihydro-1(2*H*)-pyridinyl]-1-(4-fluorophenyl)-1-butanone (**f**), were kindly provided by Janssen Pharmaceutica (Beerse, Belgium), that of both 4-(4-chlorophenyl)-4-hydroxy piperidine (**a**) and 4-fluorobenzoic acid (**c**), were purchased from Acros (NJ, USA). The pharmaceutical formulation used in this study was Haldol tablets (Laboratoires Janssen-Cilag, France).

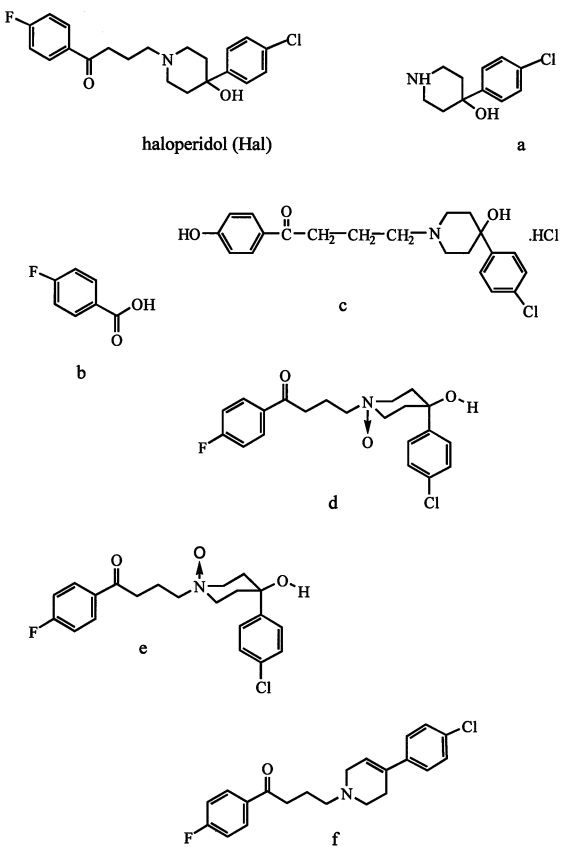

Fig. 1. Structure of haloperidol and its suggested degradation products.

2.2. Reagents

Acetonitrile, tetrahydrofuran (THF) and triethylamine (TEA) were of HPLC grade, from Fisher chemicals (UK). Hydrogen peroxide, hydrochloric acid, phosphoric acid, sodium hydroxide and sodium phosphate monobasic were purchased from Prolabo (France). Sodium nitrate was from Fluka (Switzerland). Water was bidistilled. All solid and liquid reagents were reagent grade.

2.3. Apparatus

A Shimadzu HPLC system (Kyoto, Japan) composed of an LC-10AT VP pump equipped with a 7725i Rheodyne (CA, USA) injector, an SPD-10 A VP variable UV–VIS detector and a C-R8A Chromatopac integrator was used. For the photodiode array study, an SPD-M10AV detector and LC-work station software, both from Shimadzu, were used. The pH values were measured with a Schott CG 825 pH meter.

2.4. Chromatographic conditions

The separation was performed on a 25 cm × 4.6 mm i.d. Spheri-5 RP-18 column (Applied Biosystem, Foster City, CA, USA). The flow rate was 1.0 ml min^{-1}. The injection volume was 20 μl. The detection wavelength was set at 248 nm. The mobile phase consisted of 50 mM phosphate buffer, pH 2.5–acetonitrile–THF–TEA (63:34:3:0.1, v/v/v/v).

To determine the influence of mobile phase pH on the separation of haloperidol and its suggested degradation products, five different mobile phases were prepared at pHs of 2.5, 3.5, 4.5, 5.5 and 6.5. For the calculation of the capacity factor, a solution of sodium nitrate (10 μg ml^{-1}) was used as a

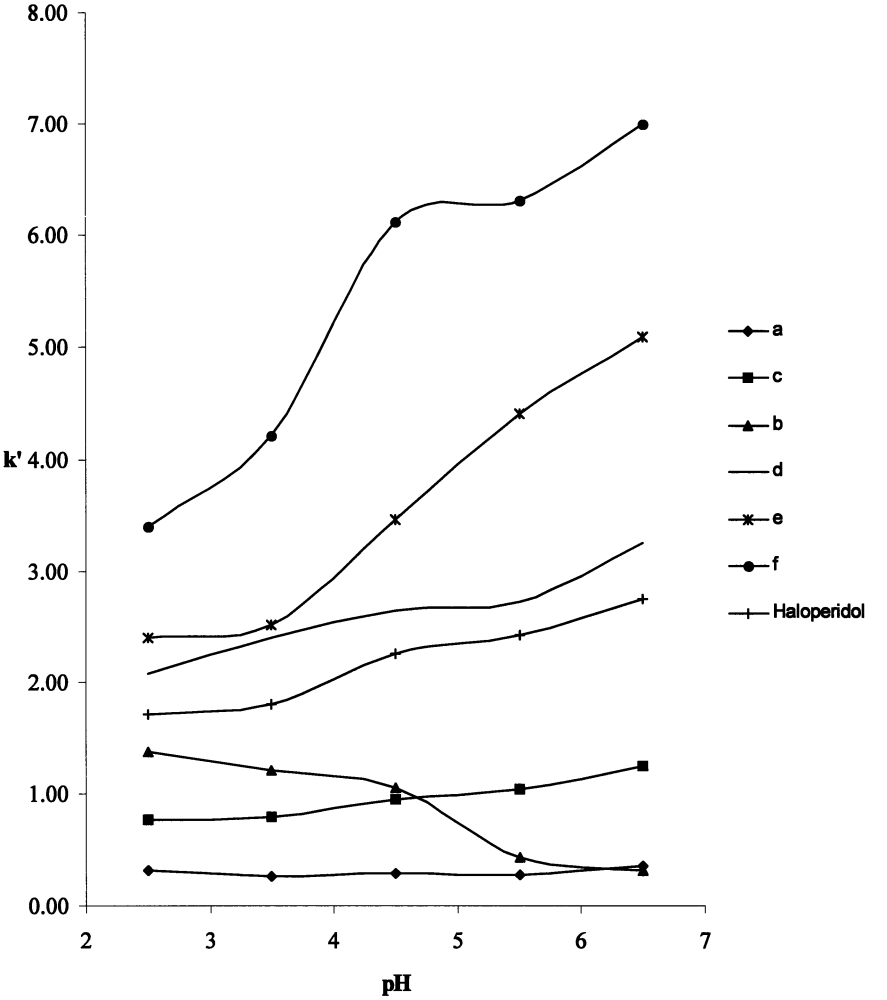

Fig. 2. Effect of mobile phase pH on capacity factors of haloperidol and its suggested degradation products (a–f).

non-retained substance in order to determine the void retention.

2.5. Preparation of samples solutions

Quantities between 2.4 and 3.3 mg of haloperidol and the examined products were dissolved separately in 10 ml of methanol and were labeled as stock solutions. For the determination of the retention time of the different compounds, reference solutions were separately prepared by diluting 1 ml of each stock solution to 10 ml with methanol. To optimize and evaluate the separation of all the analytes from each other, a mixture of the seven substances containing 1 ml from each stock solution was prepared in a 10 ml volumetric flask and was diluted to volume with methanol.

2.6. Calibration solutions and sample assay

In order to check the response linearity of the method, five calibration solutions over the range of the desired concentrations were prepared by appropriate dilutions of the calibration stock solution of haloperidol (1000 μg ml^{-1}). Methanol was used as solvent for all preparations.

For tablets, 20 units were weighed and powdered. Accurately weighed portions of the powder equivalent to the weight of one tablet were placed in a 25 ml volumetric flask. Each sample was sonicated for 5 min with 25 ml of methanol and filtered through 0.22 μm membrane filters. The filtrate was diluted with methanol in order to obtain a final concentration of 30 μg ml^{-1}. The obtained solutions were injected as described in the previous preparation.

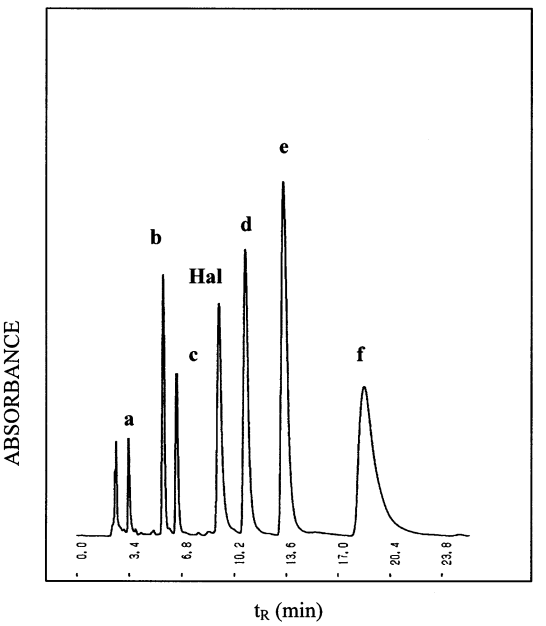

Fig. 3. HPLC chromatogram of haloperidol and its suggested degradation products (a–f).

Table 1
Statistical study of linearity of haloperidol

Range of concentration (μg ml^{-1})	10–50	
Slope	17765.4 (RSD% = 3)	
Intercept	−6506.8	
Correlation coefficient	0.9995	
		Theoretical values
Comparison of intercept with 0 (*t*-test)	0.54 (NS)	$t_{(0.05;\ 13)} = 2.16$
Homogeneity of variance (test of Cochran)	0.41 (NS)	$C_{(0.05;\ 5;.2)} = 0.68$
Existence of a significant slope (*F*-test)	1814.4 (HS)	$F_{(0.05;\ 1;\ 13)} = 4.67$
Validity of adjustment (*F*-test)	0.10 (NS)	$F_{(0.05;\ 3;\ 10)} = 0.68$

ns: not significant. HS: highly significant.

2.7. Validation parameters

Linearity, accuracy and precision were determined according to the statistical method of validation described previously [27]. The percentage recovery of the haloperidol was computed from the regression equation.

2.8. Haloperidol degradations

20 mg of haloperidol was mixed separately in 40 ml of 1N HCl, 1N NaOH and 30% H_2O_2. The mixtures obtained with either hydrochloric acid or sodium hydroxide were refluxed for 5 h, while the one obtained with hydrogen peroxide was heated at 70 °C for 5 h.

Each resulting solution was cooled at room temperature and filtered. For acidic and basic degradation solutions, an aliquot of 1 ml was neutralized and diluted with methanol to 4 ml while for the hydrogen peroxide one, an aliquot of 1 ml was diluted with methanol to 20 ml. All these solutions were analyzed using HPLC.

3. Results and discussion

3.1. Development of the optimum mobile phase

Peak tailing in reversed phase liquid chromatography of haloperidol was observed. Thus, the addition of triethylamine to the mobile phase was essential to improve the sharpness of the haloperidol peak. On the other hand, the effect of the eluent pH on the capacity factor of each of the examined compounds with a mobile phase of acetonitrile–50 mM phosphate buffer–TEA (40:60:0.1, v/v) was investigated. As can be seen in Fig. 2, an increase of pH led to the increase of the capacity factor of haloperidol and compounds **b**,

Table 2
Precision of the method

	Repeatability ($n = 6$ within 1 day)			Reproducibility ($n = 18$ within 3 days)		
Concentration of haloperidol (μg ml^{-1})	10	30	50	10	30	50
Found mean	10.2	30.2	50.2	10.1	29.7	50.9
±SD	0.2	0.2	0.8	0.1	0.1	0.3
RSD (%)	2.0	0.7	1.6	0.1	0.3	0.6

d, **e** and **f**, which behave as bases, while the capacity factor of 4-fluorobenzoic acid decreased with increasing pH. This result corresponds to the usual behavior of retention versus pH of bases and monoprotic acids [28,29]. However, it is worth noting that this change in the mobile phase pH did not lead to a complete separation. Nevertheless, it appears that at pH < 3, better separation was obtained without dramatic change of the retention times of the different compounds. This suggests the use of a pH of 2.5 to avoid a significant variation of the resolutions with a small change of pH. On the other hand, the addition of an optimum concentration of THF to the mobile phase with decreasing the one of acetonitrile yielded a sufficient separation. However, compound **a** was not retained. Thus, a decrease of acetonitrile concentration with higher proportion of buffer concentration was tried and a good result was obtained. The selected mobile phase for further method validation consisted of 50 mM phosphate buffer–acetonitrile–THF–TEA (63:34:3:0.1, v/v/v/v). The apparent pH was readjusted to 2.5 with phosphoric acid. The complete separation under the suggested conditions is depicted in Fig. 3, which shows a chromatogram of the solution of the seven compounds.

3.2. Linearity

Three five-point calibration curves, performed on three different days, were plotted as the peak area versus concentration. Table 1 lists the linearity regression results. The chromatographic analysis was linear in the working range for standard solutions with a correlation coefficient of 0.999. The mean slope had a low RSD (%) and the mean intercept was not significantly different from the theoretical value of zero.

3.3. Precision and accuracy

Repeatability was evaluated by injecting haloperidol standard solution at three different levels six times in the same day. Reproducibility was assessed by 18 determinations of a haloperidol standard solution, at three different concentrations for 3 consecutive days (six determinations per day for each concentration). The obtained RSD values for the intra-day and inter-day were less than 2% (Table 2), indicating a satisfactory result. The accuracy of the method was demonstrated by recovery experiments, using the standard addition technique. Three different levels of standard haloperidol were added to preanalyzed tablets. As shown in Table 3, satisfactory recoveries were obtained, and no significant differences were observed between the amount of haloperidol added and the amount found, which indicated the accuracy of the method.

Table 3
Results of accuracy/recovery

Amount added (μg ml^{-1})	Amount found (μg ml^{-1})	Recovery (%)	RSD%
9.98	10.17	101.7	2.1
19.96	19.75	98.9	1.7
29.94	30.06	100.4	2.1
39.55	39.25	99.2	1.0
49.06	49.28	100.4	0.4

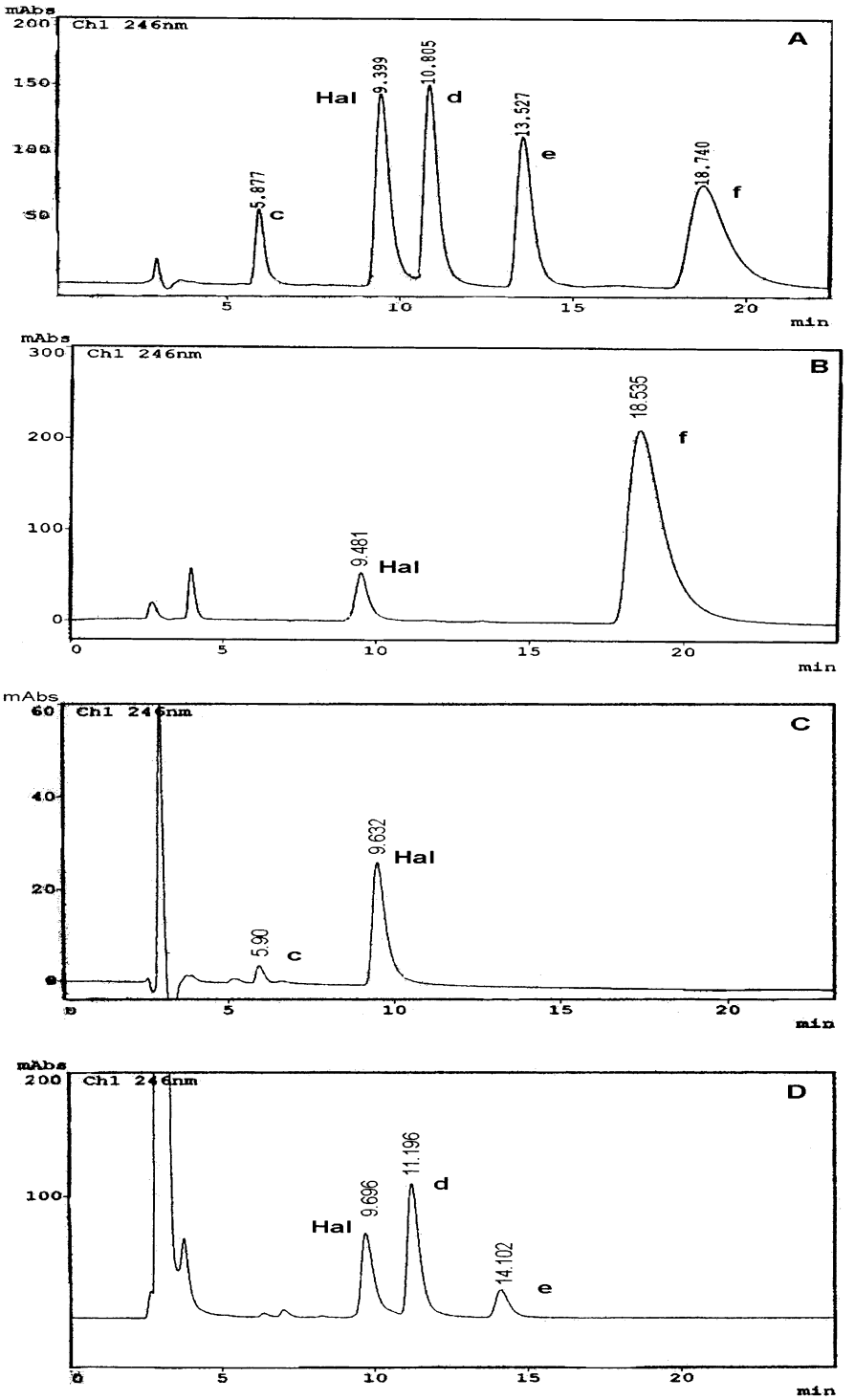

Fig. 4. HPLC chromatograms from haloperidol degradation study: (A) a synthetic mixture of haloperidol, compounds **c**, **d**, **e** and **f**; (B) acidic degradation; (C) basic degradation; (D) hydrogen peroxide degradation.

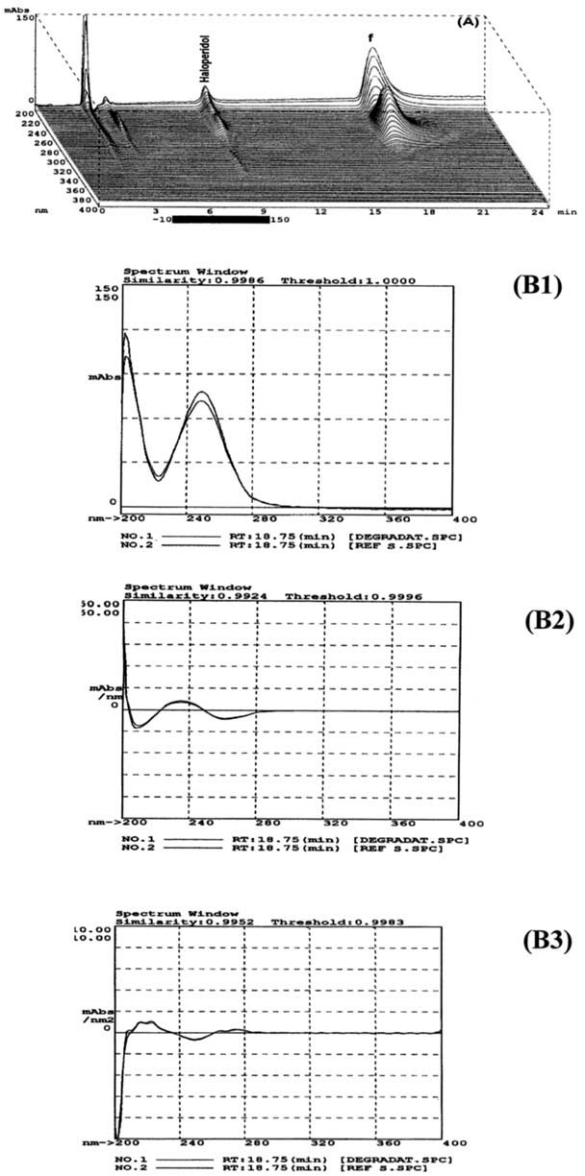

Fig. 5. Example of diode-array analysis from acidic degradation of haloperidol: (A) three dimensional HPLC chromatogram; (B1), (B2) and (B3) are the superimposed UV spectra of dehydrated haloperidol (compound **f**) obtained from degradation product (No. 1: $t_g = 18.75$ min) and reference substance (No. 2: $t_g = 18.75$ min), their first and second derivative spectra, respectively.

3.4. LOD and LOQ

The detection limit, defined as the lowest concentration of the analyte that can be clearly detected, is estimated as three times the signal to noise ratio [30]. It was found to be 15 ng ml^{-1}. The quantitation limit with a signal to noise of 10:1 was estimated to be 50 ng ml^{-1}.

3.5. Degradation studies

The resulting chromatogram for a standard mixture with those of haloperidol solutions obtained under stressed conditions is shown in Fig. 4. The degradation products are well resolved from haloperidol and did not interfere with its determination.

Degradation peaks were identified by their retention times, their diode-array spectra and their corresponding first and second derivative ones, which were identical to the reference substances available in our laboratory. On the other hand, the comparison of these diode array spectra with the haloperidol one taken during the upslope, apex and downslope did not reveal any Coeluting products. A representative diode-array spectrum of a sample preparation is shown in Fig. 5.

In the chromatogram obtained from the acidic medium, one degradation product appeared at $t_R = 18.7$ min and was identified as the compound **f**. This result indicates that dehydration of haloperidol occurred in acidic medium. This decomposition by dehydration in acidic medium was reported for other hydroxylated compounds [31,32].

The solution obtained from refluxed haloperidol in sodium hydroxide led to a chromatogram with also one major degradation product ($t_R = 5.9$

Table 4
Assay of haloperidol in commercial tablets

Brand	Label claim	Amount found (mg)	RSD (%)
Haldol tablets	1 mg/tablet	1.0	2.6
Haldol tablets	5 mg/tablet	5.0	1.7
Haldol tablets	5 mg/tablet	4.9	0.9

min), which corresponds to compound **b**. This result indicates that haloperidol, like droperidol [33], undergoes a nucleophilic substitution of the fluorine atom by the hydroxyl group. As is well known, this reaction is favored by the presence of ketone functionality in the *para* position [34].

For the degradation performed by hydrogen peroxide, two major products appeared at $t_R =$ 11.2 min and $t_R = 14.1$ min and were attributed to the *trans* (**d**) and the *cis* (**e**) isomers of haloperidol *N*-oxide, respectively. However, it is worth noting that the *trans* isomer is the major compound, which appears more stable. Similar results about the formation of *N*-oxide derivatives under such conditions were reported for other heterocyclic compounds containing nitrogen atom [32,33,35,36].

3.6. Assay of haloperidol

The proposed method was applied to the determination of haloperidol in tablets. The results reported in Table 4 were in good agreement with the label values. Moreover, the low values of RSD indicate that the method is precise and accurate.

4. Conclusion

An isocratic HPLC method with good precision has been developed for haloperidol. The method enables the separation of the active ingredient and its chemical degradation compounds obtained in different media. 4-[4-(4-Chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-hydroxyphenyl)-1-butanone, 4-[4-(4-chlorophenyl)-3,6-dihydro-1(2*H*)-pyridinyl]-1-(4-fluorophenyl)-1-butanone and both *trans* and *cis* isomers of haloperidol *N*-oxide were identified as degradation products.

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