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LC determination and degradation study of droperidol

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Abstract

A specific, high performance liquid chromatographic method for the determination of droperidol in the presence of its degradation products is described. The method is based on the use of an amide functionalized bonded phase column (LC-ABZ$^+$ Plus) and a simple mobile phase of methanol–sodium phosphate monobasic (0.05 M, pH 4.5) (40:60, v/v). It enables the resolution of eight compounds from the parent drug and from each other. The degradation was carried out in hydrochloric acid, sodium hydroxide and hydrogen peroxide. The main degradation products were identified. Application of the assay for a commercial preparation of droperidol for injection is presented. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Pharmaceuticals; Droperidol; Liquid chromatography; Degradation products

1. Introduction

Droperidol, 1-[1-[4(4-fluorophenyl)-4-oxobutyl]-1,2,3,6-tetrahydro-pyridin-4-yl]-2,3-dihydro-2H-benzimidazol-2-one, is an antipsychotic agent of the butyrophenone group which is widely used in psychiatry and anesthesiology.

Droperidol is thermosensitive [1]. Both refluxed bulk drug in hydrochloric acid [2] and heated injection solution [3] undergo hydrolysis leading to the formation of two degradation products: 1-[4-(4-fluorophenyl)-4-oxobutyl]-4-piperidinone and 1,3-dihydro-2H-benzimidazol-2-one.

Various methods have been described for the determination of droperidol. They employ UV spectrophotometry [2], derivative spectrophotometry [4], colorimetry [5], fluorimetry [6,7], voltammetry [8–10], gas chromatography [11–13] and high performance liquid chromatography (LC) [3,14–18]. Although two of them [3,15] allow for the separation of droperidol from the two aforementioned degradation compounds, these methods do not consider quantitation of droperidol where more degradation products could be present.

The first aim of this study was to develop a suitable HPLC method to quantify droperidol and to separate it from its likely degradation compounds. The considered compounds are reported in Fig. 1.

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The second aim of this investigation was to study the degradation of droperidol under various conditions, using a photodiode array detector. The main degradation products were identified by comparing their retention times and UV spectra with those of reference materials.

2. Experimental

2.1. Samples

Droperidol, 1,3-dihydro-1-(1,2,3,6-tetrahydro-4-pyridinyl)-2H-benzimidazol-2-one (**a**), 1-[4-(4-fluorophenyl)-4-oxobutyl]-4-piperidinone (**b**), 1,3-dihydro-2H-benzimidazol-2-one (**c**), 5-(4-fluorophenyl)-3,4-dihydro-2H-pyrrole ethanedioate (**d**),1-[1-[4(4-hydroxyphenyl)-4-oxobutyl]-1,2,3,6-tetrahydro-pyridinyl]-1,3-dihydro-2H-benzimidazol-2-one (**e**), 1,3dihydro-1-(methylene-2-propenyl)-2H-benzimidazol-2-one (**g**), 1-[1-[4(4-fluorophenyl)-4-oxobutyl]-1,2,3,6-tetrahydro-4-pyridinyl]-1,3-dihydro-2H-benzimidazol-2-one, N-oxide (**h**) were kindly provided by Janssen Pharmaceutica (Beerse, Belgium)), 4-fluorobenzoic acid (**f**) was purchased from Acros (NJ). The pharmaceutical formulation used in this study was Droleptan injectable (Laboratoires Janssen-Cilag, France).

2.2. Reagents

HPLC grade methanol was from Fisher chemicals (UK). Hydrogen peroxide, hydrochloric acid, phosphoric acid, sodium hydroxide and sodium phosphate monobasic were purchased from Prolabo (France). 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) 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 LC-ABZ$^+$ Plus column (Supelco, Bellefonte, PA). 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 methanol–sodium phosphate monobasic 0.05 M (40:60, v/v).

To determine the effect of buffer pH on the separation of droperidol and its suggested degradation products, five different mobile phases were prepared at pHs of 3.5, 4, 4.5, 5 and 5.5.

2.5. Preparation of sample solutions

Quantities between 4.8 and 7.9 mg of droperidol 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 nine 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 droperidol (1000 μg ml^{-1}). Methanol was used as solvent for all preparations.

The droperidol injection solutions of droperidol was diluted with methanol in order to obtain a final concentration of 25 μg ml^{-1}.

2.7. Validation parameters

Linearity, accuracy and precision were determined according to the statistical method of vali-

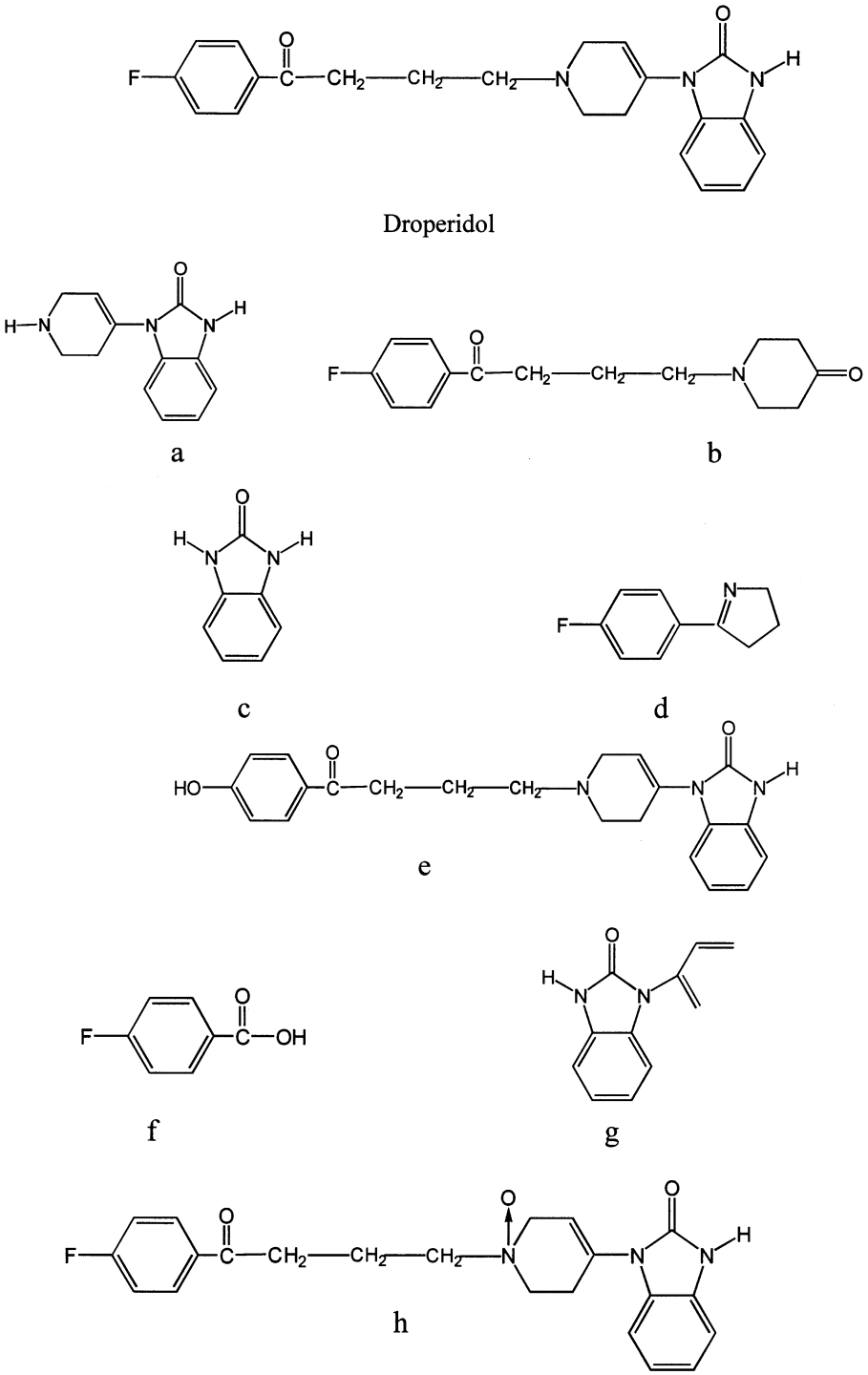

Fig. 1. Chemical structures of droperidol and its suggested degradation products.

dation described previously [19,20]. The percent recovery of the droperidol was computed from the regression equation.

2.8. Droperidol degradations

Twenty milligrams of droperidol was mixed separately in 40 ml of 1N HCl, 1N NaOH and 20 V 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 80 °C for 2 h.

Each resulting solution was cooled at room temperature and filtered. An aliquot of 1 ml was neutralized when it was necessary and diluted with methanol to 20 ml. All these solutions were analyzed using HPLC.

3. Results and discussion

3.1. Separation studies

A preliminary experiment performed with a mobile phase consisting of 50 mM phosphate buffer(pH 3)–methanol (50:50, v/v) yielded a result where some analytes were not retained. Thus, an eluent with a smaller proportion of organic solvent was tried and further used for the continuation of the study.

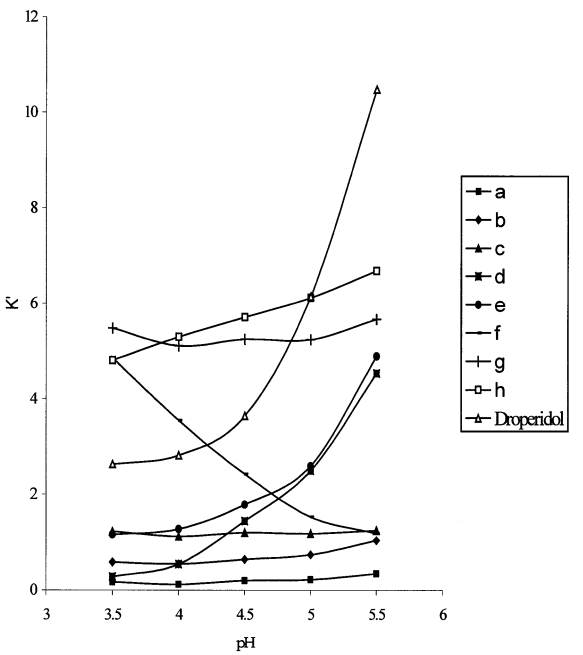

Fig. 2. Effect of buffer pH on capacity factor of droperidol and its suggested degradation products (**a**–**h**).

Fig. 2 shows the influence of pH on the capacity factor of each of the examined compounds with mobile phase of 50 mM phosphate buffer–methanol (60:40, v/v). This graphic representation shows that an increase of pH led, as expected, to the decrease of the retention time of 4-fluorobenzoic acid with a sigmoidal curve corresponding to the usual behavior of retention vs. pH of monoprotic acids [21], while, an increase of the retention time was obtained for droperidol and compounds **d**, **e** and **h**, which behave as bases [22,23]. However, the retention of the remaining analytes was slightly affected by changes in the mobile phase pH; this phenomenon has been observed for other nitrogen-containing compounds and might be due to their ionization state (uncharged or partially ionized) [24]. A pH of 4.5 appeared to be optimal for an adequate resolution with an acceptable analysis time and was selected for further method validation. The complete separation under the suggested conditions is depicted in Fig. 3, which shows a chromatogram of the solution of the nine compounds.

3.2. Linearity

Three 5-point calibration curves, performed on three different days, were plotted as the peak area vs. concentration. The linear regression results (Table 1) showed that the method was linear, with a correlation coefficient greater than 0.999. The mean slope had a low RSD (2.36%) and the mean intercept was not significantly different from the theoretical value of zero.

3.3. Precision and accuracy

Repeatability was assessed by injecting droperidol standard solution at three different levels six times in the same day. Reproducibility was evaluated by eighteen determinations of a droperidol

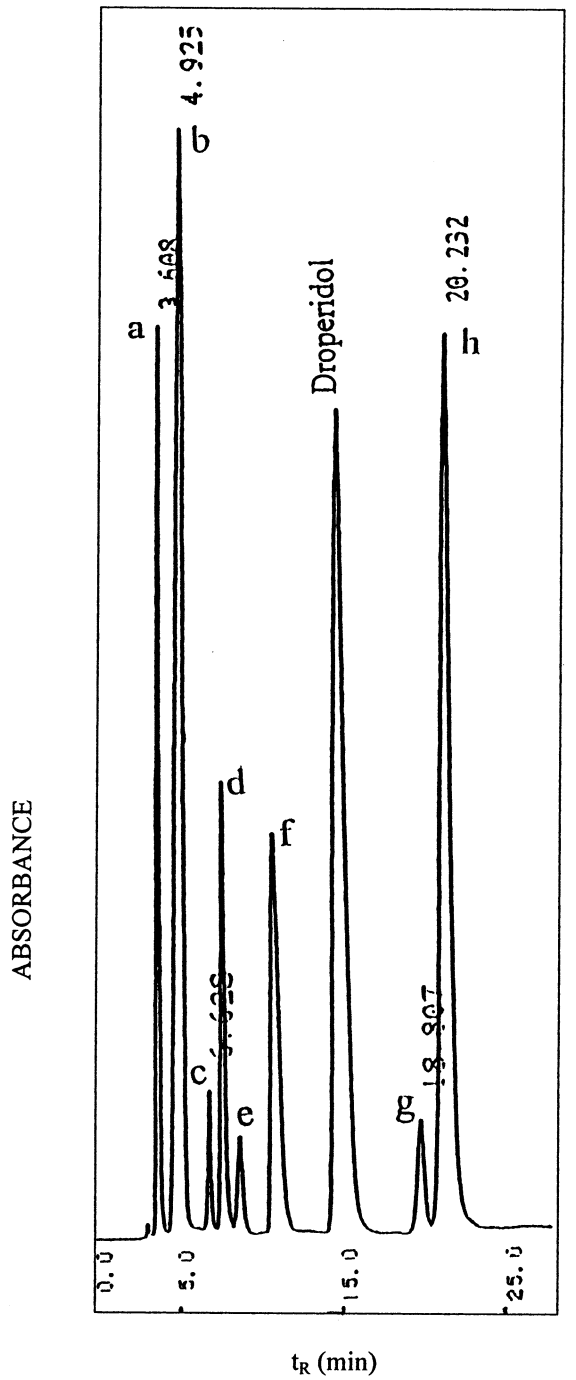

Fig. 3. HPLC chromatogram of droperidol and its suggested degradation products (**a**–**h**).

standard solution, at three different concentrations for three 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 spiking samples of droperidol solutions with known amounts of the active ingredient. Satisfactory recoveries (Table 3) were obtained, and no significant differences were observed between the amount of droperidol added and the amount found, which indicated the accuracy of the method.

3.4. LOD and LOQ

The detection limit, based on a signal to noise ratio of 3 and 20 μl injection, was found to be 0.1 μg ml^{-1}. The quantitation limit with a signal to noise of 10:1 and 20 μl injection was found to be 0.3 μg ml^{-1}.

Table 1
Statistical study of linearity of droperidol

Range of concentration (μg ml^{-1})	10–40	
Slope	22173 (RSD (%) = 2.36)	
Intercept	−9888.4	
Correlation coefficient	0.9994	
		Theoretical values
Comparison of intercept with 0 (*t* test)	0.619 (ns)	$t_{(0.05;\ 13)} = 2.16$
Homogeneity of variance (test of Cochran)	0.498 (ns)	$C_{(0.05;\ 5;\ 0.2)} = 0.68$
Existence of a significant slope (test of fisher)	27.792 (HS)	$F_{(0.05;\ 1;\ 13)} = 4.67$
Validity of adjustment (test of fisher)	0.0004 (ns)	$F_{(0.05;\ 3;\ 10)} = 0.68$

ns: not significant, HS: highly significant.

Table 2
Precision of the method

	Repeatability $n = 6$ within 1 day			Reproducibility $n = 18$ within 3 days		
Concentration of droperidol (μg ml^{-1})	10	25	40	10	25	40
Found mean	10.07	25.06	40.03	9.98	25.08	39.02
RSD (%)	0.79	0.44	0.82	1.00	0.40	0.60

Table 3
Accuracy/recovery of droperidol in synthetic preparations

Amount added (μg ml^{-1})	Amount found (μg ml^{-1})	Recovery (%)	RSD (%)
10	10.07	100.7	1.00
20	19.95	99.8	0.86
25	25.24	100.9	0.54
30	30.27	100.9	0.72
40	40.09	100.2	0.65

3.5. Degradation studies

The resulting chromatogram for a standard mixture with those of droperidol solutions obtained under stressed conditions are shown in Fig. 4. The degradation products are well resolved from droperidol and did not interfere with its determination. In addition, it's worth noting that change in ± 0.1 pH units did not significantly affect the resolution factors (Table 4). However, higher pH increased analysis time.

Degradation peaks were identified by their retention time, 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 droperidol 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, two degradation products appeared at $t_R = 5.3$ and 6.7 min and were attributed to the compounds **b** and **c**, respectively. This result indicates that droperidol behaves as an enamine, which is known for its sensitivity to electophilic attack and subsequent hydrolysis in acidic medium [25]. The same finding was obtained in a previous acidic degradation study of droperidol [2].

The solution obtained from refluxed droperidol in sodium hydroxide led to a chromatogram with only one major degradation product ($t_R = 8.8$ min), which corresponds to compound **e**. This result indicates that droperidol undergoes a nucleophilic substitution of the fluorine atom by the hydroxyl group. As well known, this reaction is favored by the presence of ketone functionality in the para position [25].

The degradation performed by hydrogen peroxide generated one major product ($t_R = 20.8$ min), which was identified as the droperidol N-oxide (**h**). Similar results about the formation of N-oxide derivatives under such conditions were reported for other heterocyclic compounds containing nitrogen atoms [26–28].

3.6. Assay of droperidol for injection

The proposed method was compared to the HPLC method of the USP [29] for the determination of droperidol in injection solutions. The results reported in Table 5 were in good agreement

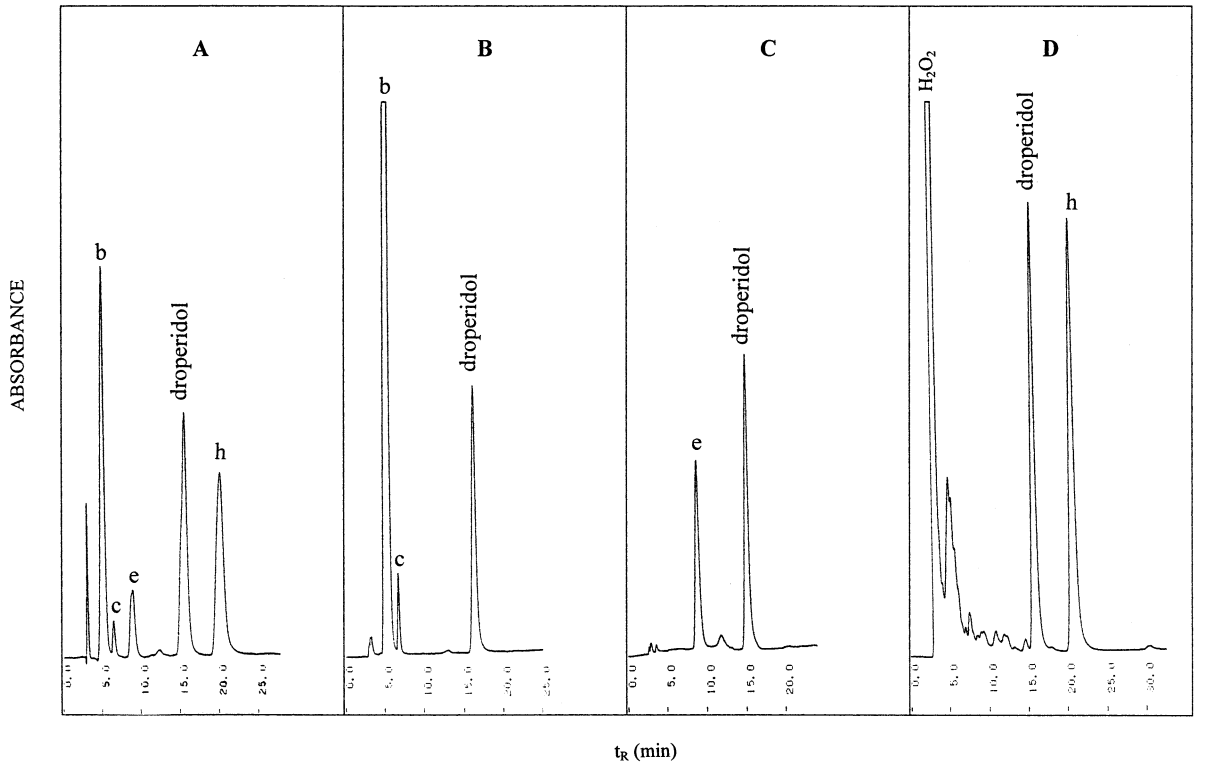

Fig. 4. HPLC chromatograms from droperidol degradation study: (A) synthetic mixture of droperidol, compounds **b**, **c**, **e**, and **h**; (B) acidic degradation; (C) basic degradation; (D) hydrogen peroxide degradation.

with the label values. Moreover, comparison between the two methods based on the *t* test shows no significant difference.

4. Conclusion

A simple and accurate HPLC method with good precision has been developed for droperidol; the method is capable of following its thermal degradation in different media. 1-[4-(4-fluorophenyl)-4-oxobutyl]-4-piperidinone,1,3-dihydro-2H-benzimidazol-2-one, 1-[1-[4(4-hydroxyphenyl)-4-oxobutyl]-1,2,3,6-tetrahydro-pyridinyl]-1,3-dihydro-2H-benzimidazol-2-one and droperidol N-oxide were identified as degradation products. The method could be used as a stability indicating HPLC assay for droperidol raw material and injection solution.

Table 4
Effect of buffer pH on the separation of droperidol and its identified degradation products

pH	Resolution			
	(**b**, **c**)	(**c**, **e**)	(**e**, droperidol)	(droperidol, **h**)
4.4	1.4	2.5	4.8	3.1
4.5	1.5	2.8	4.7	3.2
4.6	1.4	2.9	4.9	2.8

Table 5
Determination of droperidol in commercial formulations

Formulation	Labeled amount (mg ml^{-1})	The proposed method			USP method			Statistical study (Student test)	
		Found (mg ml^{-1})	Recovery (%)	RSD (%)	Found (mg ml^{-1})	Recovery (%)	RSD (%)	Theoretical value	Calculated values
Droleptan injection solution 1	5	4.98	99.6	1.4	5.01	100.2	1.36		0.52 (ns)
Droleptan injection solution 2	5	5.07	101.4	0.36	5.04	100.8	0.72	t (0.05, 4) = 2.776	1.71 (ns)
Droleptan injection solution 1	2.5	5.1	102.1	0.26	4.99	99.8	0.26		0.13 (ns)
Droleptan injection solution 2	2.5	4.99	99.97	1.63	4.96	99.2	0.19		2.18 (ns)

Tabulated data is the mean of three determinations, ns: not significant.

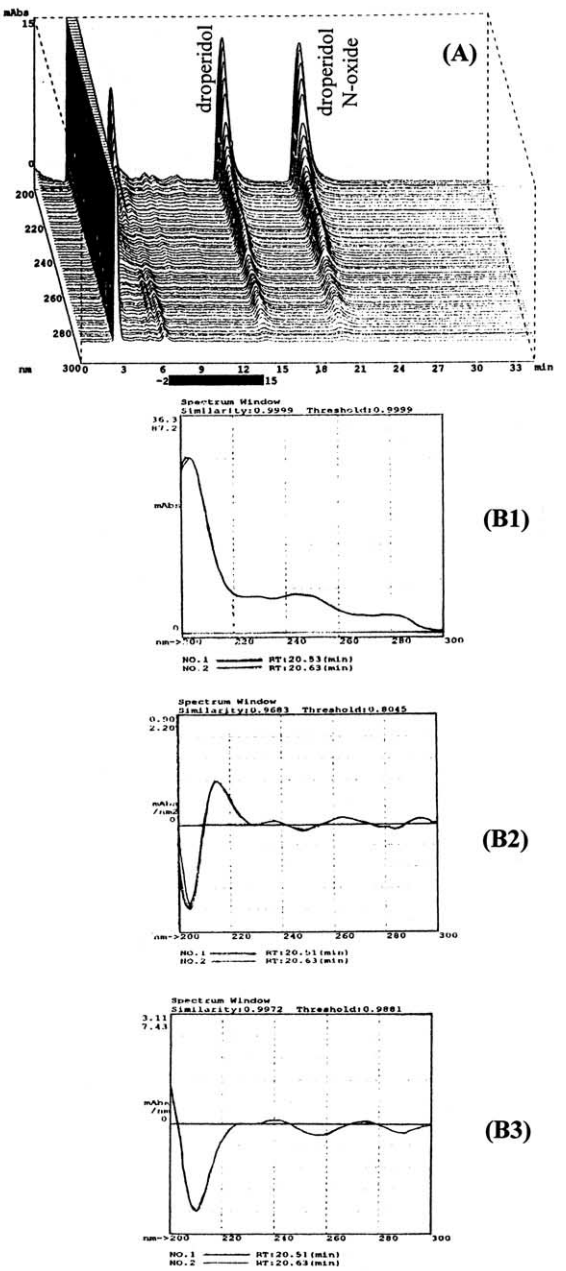

Fig. 5. Example of diode-array analysis form hydrogen peroxide degradation of droperidol: (A) three-dimensional HPLC chromatogram; (B1), (B2) and (B3) are the superimposed UV spectra of droperidol N-oxide obtained from degradation product ($t_R = 20.5$ min) and reference substance ($t_R = 20.6$ min), their first and second derivative spectra, respectively.

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References

[1] C.A. Janicki, R.K. Gilpin, in: K. Florey (Ed.), Analytical Profiles of Drug Substances, vol. 7, Academic Press, New York, 1978, pp. 171–192.
[2] C.A. Janicki, R.J. Brenner, B.E. Schwartz, J. Pharm. Sci. 57 (1968) 451–455.
[3] M. Dolezalova, J. Chromatogr. 286 (1984) 323–330.
[4] H. Trabelsi, F. Raouafi, M. Limam, K. Bouzouita, J. Pharm. Biomed. Anal. (2001), accepted for publication.
[5] A. Haemers, Van den Bossche, J. Pharm. Pharmacol. 21 (1969) 531–532.
[6] W. Baeyens, Pharm. Weekbl. 112 (1977) 681.
[7] W. Baeyens, Analyst 102 (1977) 525–530.
[8] S. Pap, J.-M. Kauffmann, J.-C. Vire, G.J. Patriarche, M.P. Prete, J. Pharm. Belg. 39 (1984) 335–340.
[9] H. Hopkala, L. Przyborowski, Pharmazie 44 (1989) 65.
[10] I. Biryol, S.A. Ozkan, J. Pharm. Biomed. Anal. 15 (1997) 1695–1701.
[11] J. Rosenfeld, M. Kawai, J.R.A. Rigg, J.K. Khandelwal, J. Chromatogr. 129 (1976) 387–392.
[12] D. Wilhelm, A. Kemper, J. Chromatogr. 525 (1990) 218–224.
[13] M.P. Quaglio, A.M. Bellini, F. Lambardi, Boll. Chim. Farm. 121 (1982) 276–284.
[14] R. Kruger, I. Mengel, H.J. Kuss, J. Chromatogr. 311 (1984) 109–116.
[15] D. Wilhelm, A. Kemper, J. Chromatogr. 525 (1990) 218–224.
[16] Seng To Tan, Peter J. Boniface, J. Chromatogr. 532 (1990) 181–186.
[17] J. Guichard, G. Pantex, J. Dubost, P. Baltassat, C. Roche, J. Chromatogr. 612 (1993) 269–275.
[18] K. Kumar, J.A. Ballantyne, A.B. Baker, J. Pharm. Biomed. Anal. 14 (1996) 1529–1533.
[19] J. Caporal-Gauthier, J.M. Nivet, P. Algranti, M. Guilloteau, M. Lallieu, J.J. N'guyen-Huu, R. Russotto, S.T.P. Pharma pratiques 2 (4) (1992) 205–239.
[20] J.M. Green, Anal. Chem. 68 (1996) 305A–309A.
[21] C. Horvath, W. Melander, I. Molnar, Anal. Chem. 49 (1977) 142.
[22] R. Rosset, M. Caude, A. Jardy, Chromatographies en Phases Liquides et Supercritique, Troisième édition, Masson, Paris, 1991, pp. 356–359.
[23] A. Pappa-Louisi, F. Zougrou, Chromatographia 44 (7) (1997) 348–354.
[24] J. Barbosa, R. Bergés, V. Sanz-Nebot, Chromatographia 51 (7) (2000) 417–427.

[25] J. Mathieu, R. Panico, Mécanismes Réactionnels en Chimie Organique, Deuxième édition, Hermann, Paris, 1980, pp. 451 and 352–353.
[26] K.A. Connors, G.L. Amidon, L. Kennon, Chemical Stability of Pharmaceuticals, Wiley, New York, 1979, pp. 267–268.
[27] B. Proksa, J. Pharm. Biomed. Anal. 20 (1999) 179–183.
[28] Y. Wu, X. Chen, L. Geir, O. Almarsson, D. Ostovic, A.E. Loper, J. Pharm. Biomed. Anal. 20 (1999) 471–485.
[29] The United States Pharmacopoeia (USP) 24-NF 19 Beta, US Pharmacopoeial convention, Rockville, MD, 614 (2000).