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Stability indicating LC method for the determination of pipamperone

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Abstract

A high performance liquid chromatographic method for the determination of pipamperone in the presence of one related impurity and its degradation products is described. The method is based on the use of an amide functionalized bonded phase column (LC-ABZ$^+$ Plus) and a mobile phase of acetonitrile-tetrahydrofuran-sodium phosphate monobasic (0.05 M, pH 6.5) (16:11:73, v/v/v). All peaks are eluted in <8 min. The method was demonstrated to be precise, accurate and specific. Degradation study showed that the drug is stable in acidic medium while it degrades under basic and oxidative conditions. The results indicated that the proposed method could be used in a stability assay.

Keywords: Pharmaceuticals; Pipamperone; Liquid chromatography; Degradation products

1. Introduction

Pipamperone, [1′-[4-(4-fluorophenyl)-4-oxobutyl]-[1,4′-bipiperidin]-4′carboxamide, is a sedative neuroleptic from the butyrophenone class. Its main therapeutic applications are for geriatric patients with psychometric aggressiveness, agitation or senile dementia [1].

A literature survey revealed few analytical methods for determination of pipamperone. Furthermore, its estimation in biological samples has been described by gas chromatography [2,3] and high performance liquid chromatography [4–7]. Differential-pulse polarography [8] and reversed phase liquid chromatographic [9] methods are reported for its determination in pharmaceuticals, but so far no data on specificity of the methods with respect to impurities is available.

The purpose of this work was to develop a procedure for the quantification of pipamperone and its separation, mainly, from its related substances. In addition a forced degradation studies of pipamperone were performed to define its degradation process and to provide an indication of the stability-indicating and specificity of the method. The considered impurities are reported in Fig. 1.

2. Experimental

2.1. Samples

Pipamperone, [1′-[4-(4-fluorophenyl)-4-oxobutyl]-[1,4′-bipiperidin]-4′-carboxamide dihydrochloride (**a**), 1′-[4-(4-fluorophenyl) - 4-oxobutyl]-[1,4′-bipiperidin]-4′-carboxylic acid (**b**), 1′-[4-(4-hydroxyphenyl)-4-oxobutyl]-[1,4′-bipiperidin]-4′-carboxamide (**c**), *cis*-1′-[1′-[4-(4-fluorophenyl)-4-oxobutyl]-[1,4′-bipiperidin]-4′-carboxamide, 1′-oxide (**d**) and 1′-[4-(2-fluorophenyl)-4-oxobutyl]-[1,4′-bipiperidin]-4′-carboxamide dihydrochloride (**f**) were kindly provided by Janssen Pharmaceutica (Beerse, Belgium). 4-fluorobenzoic acid (**e**) was purchased from Acros (NJ, USA). The pharmaceutical formulation used in this study was Dipiperon tablets (Laboratoires Janssen-Cilag, France).

2.2. Reagents

Methanol, acetonitrile and tetrahydrofuran (THF) were of HPLC grade, from Fisher chemicals (UK). Hydrogen perox-

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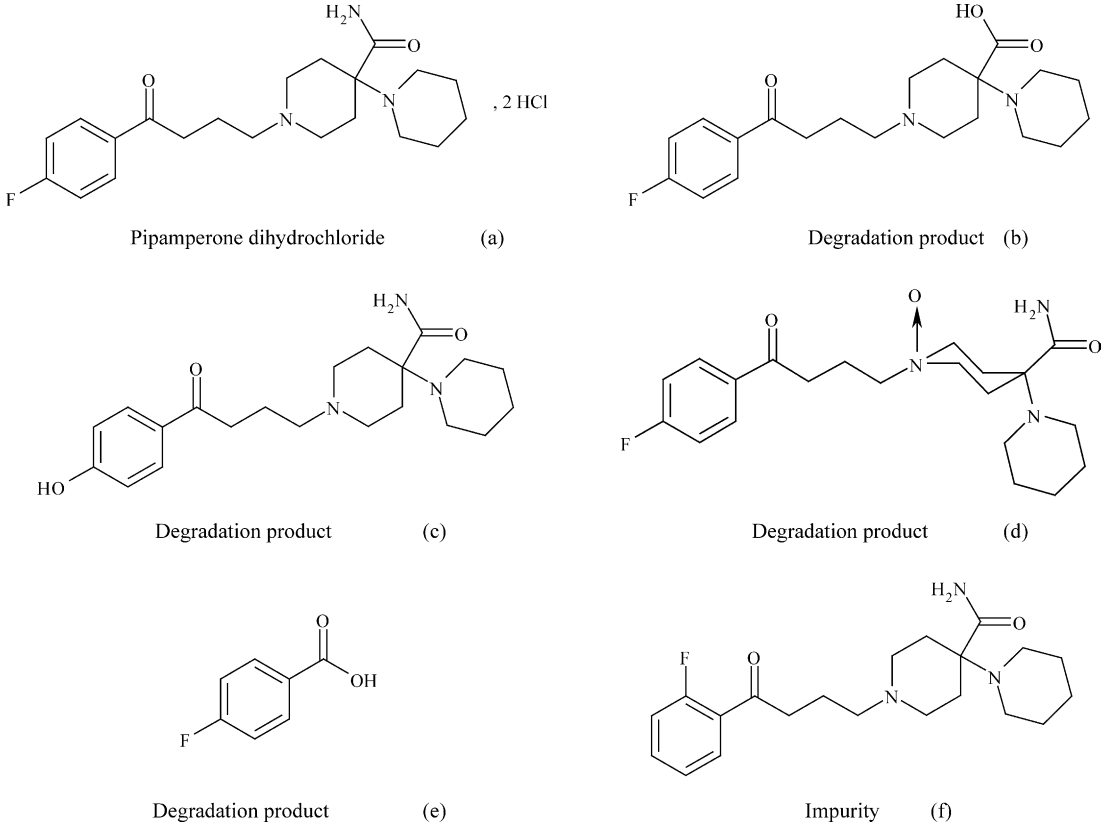

Fig. 1. Chemical structures of pipamperone dihydrochloride (a), its suggested degradation products (b–e) and impurity (f).

ide, 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 LC 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. LC-ABZ^{+} Plus column (Supelco, Bellefonte, PA, USA). The flow rate was 1.0 ml min^{-1}. The injection volume was 20 μl. The detection wavelength was set at 246 nm. The mobile phase consisted of acetonitrile-THF-sodium phosphate monobasic (0.05 M, pH 6.5) (16:11:73, v/v/v).

To determine the effect of buffer pH on the separation of pipamperone and its suggested degradation products, six different mobile phases were prepared at pHs of 4.5, 5, 5.5, 6, 6.5 and 7. For the estimation of the capacity factor, a solution of sodium nitrate (10 μg ml^{-1}) was used as a non retained substance in order to determine the void retention.

2.5. Preparation of sample solutions

Quantities between 7 and 10.9 mg of pipamperone dihydrochloride 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 six 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 pipamperone dihydrochloride (1000 μg ml^{-1}). Methanol was used as solvent for all preparations.

For tablets, 20 units were weighed and finely powdered. An accurately weighed amount of the powder equivalent to

40 mg of pipamperone dihydrochloride was transferred into a 100 ml volumetric flask and sonicated for 5 min with 100 ml of methanol. The resulting suspension was filtered through 0.22 μm membrane filter. A suitable aliquot of this filtrate was diluted with methanol in order to obtain a final concentration of 20 μg ml^{-1}. A 20 μl of the obtained solution was chromatographed. The determination of sample solution was carried out by using the calibration curve. The injection sequence included a blank solution (methanol), the five calibration standard solutions, a solution of excipients and finally the sample solution.

2.7. Validation parameters

Linearity, accuracy and precision were determined according to the statistical method of validation described previously [10,11]. The percentage recovery of the pipamperone was computed from the regression equation.

2.8. Pipamperone degradations

Forty milligrams of pipamperone dihydrochloride was mixed separately in 40 ml of 1N HCl, 1N NaOH and 20V 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 LC.

3. Results and discussion

3.1. Separation studies

To optimize the separation of the different compounds under isocratic conditions, the effect of both buffer pH and organic modifier were investigated. Fig. 2 shows the influence of pH on the capacity factor of each of the examined compounds with mobile phase of acetonitrile-50 mM phosphate buffer (30:70, 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 [12], while, an increase of the retention was obtained for all the remaining analytes, which behave as bases [13,14]. However, this change in the mobile phase pH did not lead to a complete separation. Nevertheless, it appears that better result was obtained at pH 6.5. On the other hand, a second mobile phase consisting on acetonitrile-50 mM phosphate buffer (27:73, v/v) led to an increase of the analysis time without improvement of separation. Therefore, the addition of an optimum concentration of tetrahydrofuran to this last mobile phase with decreasing the one of acetonitrile was tried [15] and a good result was obtained. The selected eluent yielding appropriate peak separation was acetonitrile-tetrahydrofuran-sodium phosphate monobasic (0.05 M, pH 6.5) (16:11:73, v/v/v). The chromatogram of the solution of the six compounds obtained under the suggested conditions is depicted in Fig. 3.

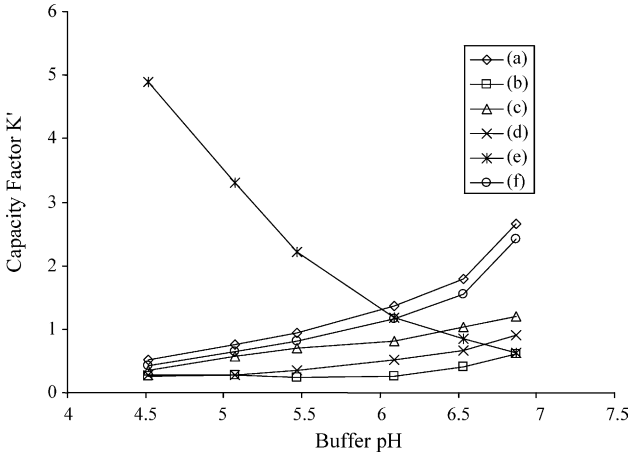

Fig. 2. Effect of buffer pH on capacity factor of pipamperone and its impurities.

3.2. Linearity

Three 5-point calibration curves, performed on three different days, were plotted as the peak area versus concentration. The results of regression analysis parameters summarized in Table 1 showed that the method was lin-

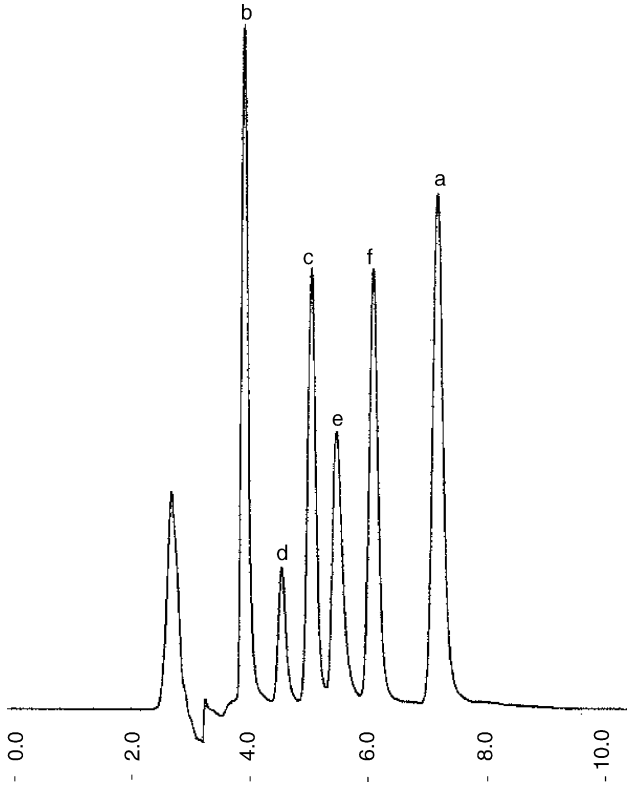

Fig. 3. LC chromatogram of pipamperone and its suggested impurities (a–f).

Table 1
Statistical study of linearity of pipamperone

Range of concentration ($\mu g\,ml^{-1}$)	10–30		
Slope	16208	(R.S.D. < 2%)	
Intercept	−5773		
Correlation coefficient	0.9994		
		Theoretical values	Conclusion
Comparison of intercept with 0 (*t*-test)	2.03	$t_{(0.05;\,13)} = 2.16$	NS
Homogeneity of variance (test of Cochran)	0.482	$C_{(0.05;\,5;\,2)} = 0.68$	NS
Existence of a significant slope (test of fisher)	16463	$F_{(0.05;\,1;\,13)} = 4.67$	HS
Validity of adjustment (test of Fisher)	0.522	$F_{(0.05;\,3;\,10)} = 3.7$	NS

NS: not significant. HS: highly significant.

Table 2
Precision of the method

	Repeatability ($n = 6$ within 1 day)						Reproducibility ($n = 18$ within 3 days)		
	Standard solutions			Sample solutions			Standard solutions		
Concentration of pipamperone ($\mu g\,ml^{-1}$)	10	20	30	10	20	30	10	20	30
Found mean	10.02	19.95	30.16	10.08	20.25	30.43	10.54	20.01	29.55
R.S.D. (%)	0.68	0.96	0.86	1.54	0.48	0.88	1.7	1.33	0.61

Table 3
Accuracy/recovery of pipamperone in synthetic preparations

Amount added ($\mu g\,ml^{-1}$)	Amount found ($\mu g\,ml^{-1}$)	Recovery (%)	R.S.D. (%) ($n = 3$)
10	9.95	99.5	1.00
15	14.86	99.1	0.60
20	20.01	100.0	0.11
25	24.72	98.9	1.00
30	30.21	100.7	0.11

ear, with a correlation coefficient greater than 0.999. The mean slope had a low R.S.D. (<2%) and the mean intercept was not significantly different from the theoretical value of zero.

3.3. Precision and accuracy

Repeatability was assessed by injecting a standard solution of pipamperone and one of the product sample at three

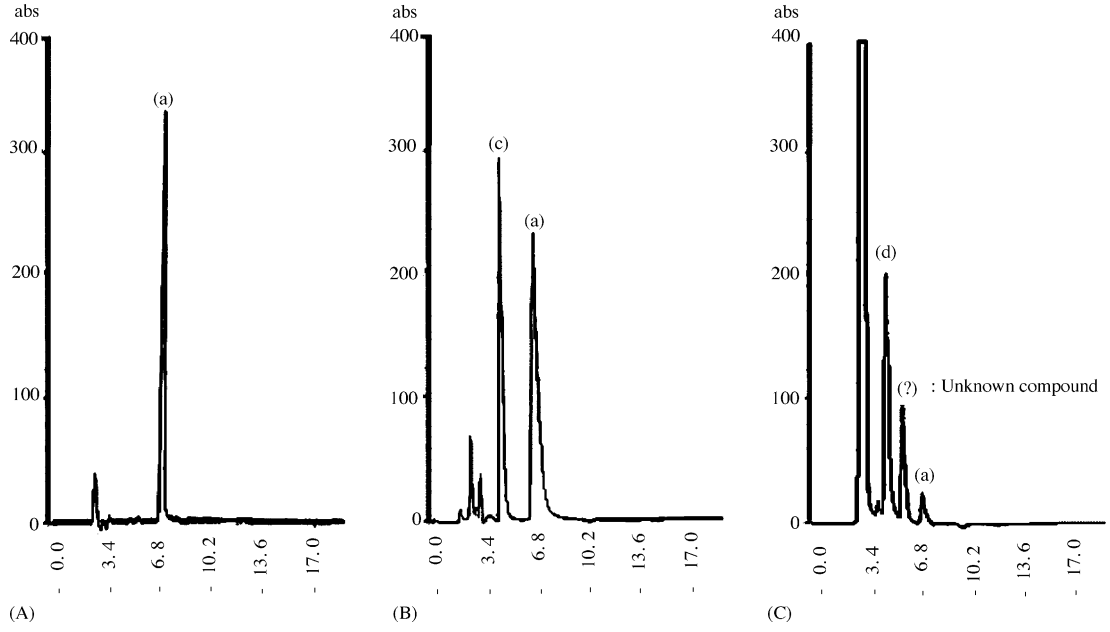

Fig. 4. LC chromatograms from pipamperone degradation study. (A) acidic degradation; (B) basic degradation; (C) hydrogen peroxide degradation.

different levels six times in the same day. Between-days precision was evaluated by 18 determinations of pipamperone standard solution, at three different concentrations for three consecutive days (six determinations per day for each concentration). The obtained R.S.D. 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. Different levels of standard pipamperone were added to pre-analyzed tablets. The determination was carried out using three replicates at each level. Satisfactory recoveries (Table 3) were obtained, and no significant differences were observed between the amount of pipamperone 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 3:1 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}.

3.5. Degradation studies

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

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 pipamperone 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.

The chromatogram obtained after acidic degradation shows only one peak, corresponding to pipamperone. This result indicates a good stability of this compound in acidic medium.

The solution obtained from refluxed pipamperone in sodium hydroxide led to a chromatogram with only one major degradation product (t_R = 5.1 min), which corresponds to compound **c**. This result indicates that pipamperone, like droperidol [16] and haloperidol [17], 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 [18].

The degradation performed by hydrogen peroxide generated two major products. One of them (t_R = 4.7 min) was identified as the *cis* pipamperone N-oxide (**d**). The second peak (t_R = 5.2 min) could be attributed to the *trans* isomer of pipamperone N-oxide. Similar results about the formation of N-oxide isomers under such conditions were reported for

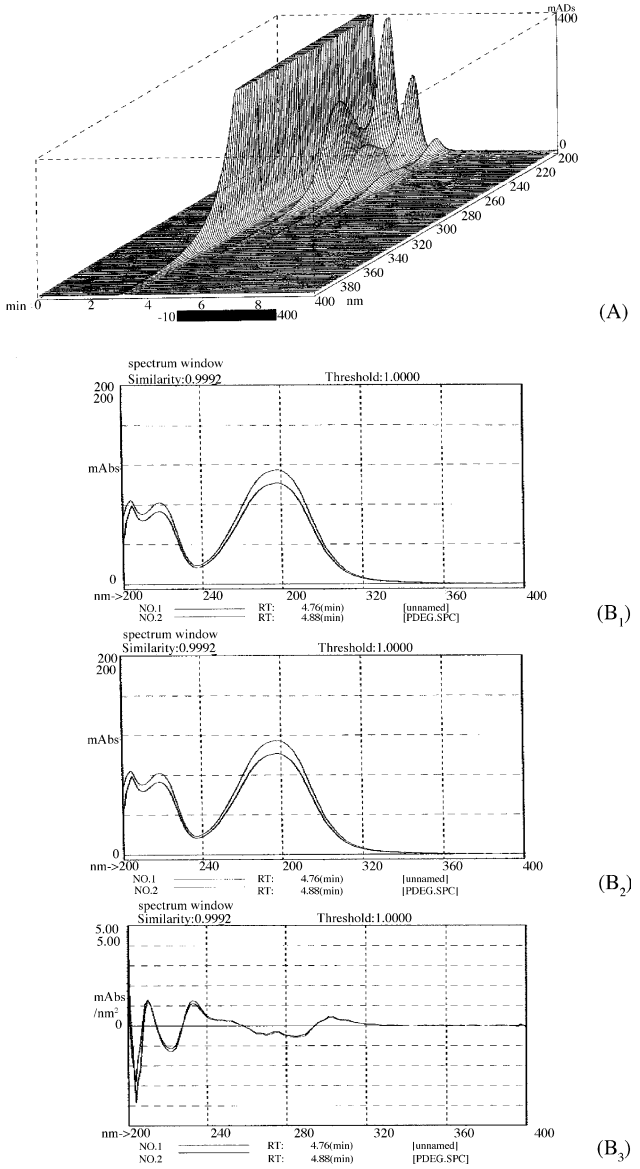

Fig. 5. Example of diode-array analysis from hydrogen peroxide degradation of pipamperone: (A) three-dimensional LC chromatogram; (B_1), (B_2) and (B_3) are the superimposed UV spectra of *cis*-pipamperone N-oxide obtained from degradation product (t_R = 4.7 min) and reference substance (t_R = 4.8 min), their first and second derivative spectra, respectively.

haloperidol [17] and loperamide [19]. Other N-oxide derivatives were also obtained by peroxide degradation of other heterocyclic compounds containing nitrogen atom [20–23].

3.6. Assay of pipamperone

The proposed method was applied to the determination of pipamperone in tablets formulation (Dipiperon 40 mg of pipamperone base). The mean average (three replicates) was found to be 39.5 mg corresponding to a mean recovery of 99.5% with an R.S.D. of 1.4%. This result was in good agreement with the label value. On the other hand, it should be pointed out that the chromatogram of the solution of excip-

ients is absolutely free of any peak indicating thus that no interference from the excipients is encountered.

4. Conclusion

A simple and rapid stability-indicating LC method has been developed for the determination of pipamperone in the presence of its impurity and degradation products. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The method is reliable and convenient for routine control and stability assays of pipamperone in both raw material and tablets.

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