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SEPARATION AND DETERMINATION OF DROPERIDOL, METHYL- AND PROPYLPARABEN AND THEIR DEGRADATION PRODUCTS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A stability-indicating isocratic reversed-phase high-performance liquid chromatographic method for the analysis of droperidol injection solutions has been developed. The separation was achieved on C_8 and C_{18} bonded silica with methanol–0.02 *M* aqueous phosphate buffer (pH 6.8) (65:35). The pH of the mobile phase significantly affects the separation. Four of six detected compounds were simultaneously determined. The calibration graphs were linear and the precision of the method was satisfactory.

INTRODUCTION

Droperidol, 1{1-[4-(*p*-fluorophenyl)-4-oxobutyl]-1,2,3,6-tetrahydro-4-pyridyl}-2-benzimidazolinone (Fig. 1), belongs to the butyrophenone class of drugs used in psychiatry and anaesthesiology. Various methods of determining droperidol are available: colorimetry[1], spectrophotometry[2], fluorimetry[3,4], gas chromatography[5–7] and thin-layer chromatography[7–9]. However, only one of them[2] deals with the determination of droperidol in the presence of its hydrolytic products and parabens, used as preservatives. The lack of specificity of the described spectrophotometric determination of droperidol is in this instance compensated for by three preceding extractions, which remove interfering hydrolytic products and parabens. Thus this method, which is also applied in the US Pharmacopeia[10], gives no information about the content of the degradation products.

The objective of this work was to develop a stability-indicating method suitable for studying the effect of temperature on the stability of the commercially available injection solutions containing droperidol (I) and the preservatives methylparaben (II) and propylparaben (III).

The degradation products considered were 2-benzimidazolinone (IV) and 4'-fluoro-4-(4-oxopiperidino)butyrophenone (V) (Fig. 1), reported[2] to be hydrolytic products of droperidol and *p*-hydroxybenzoic acid (VI), a hydrolytic product of methyl- and propylparaben.

High-performance liquid chromatography (HPLC) in the reversed-phase mode

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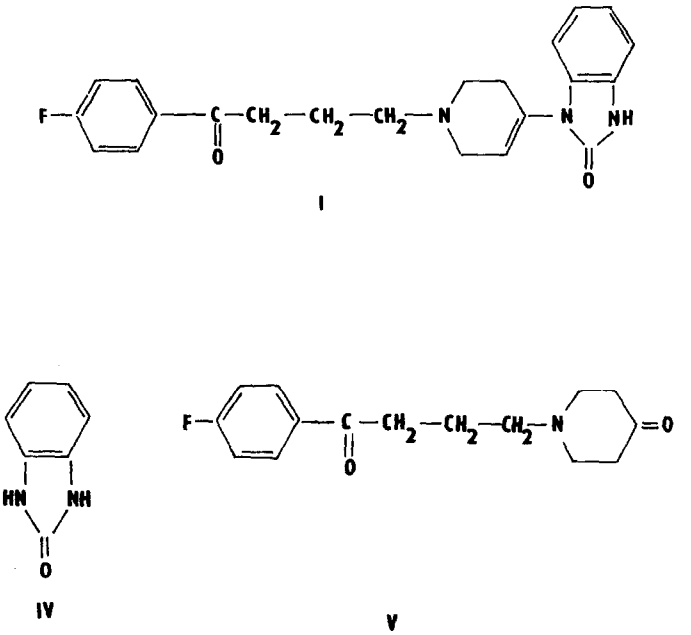

Fig. 1. Structural formulae of droperidol (I) and its two hydrolytic products (IV and V).

with UV spectrophotometric detection was chosen for the analysis of droperidol injection solutions. Liquid chromatography has not yet been used for the determination of droperidol in either pharmaceutical preparations or biological material. Only a reversed-phase liquid chromatographic procedure[11] has been reported for the separation of nine butyrophenones, including droperidol.

EXPERIMENTAL

Reagents and chemicals

Droperidol was a product of Gedeon Richter (Budapest, Hungary) and the hydrolytic products IV and V were prepared by complete hydrolysis of droperidol in 1 *M* hydrochloric acid[2]. Methylparaben and propylparaben were obtained from Léčiva Praha (Prague, Czechoslovakia). Methanol (Lachema, Brno, Czechoslovakia), acetonitrile (VEB Laborchemie, G.D.R.) and distilled water used in the mobile phase were distilled before use. All other chemicals were of analytical-reagent grade from Lachema.

For standard solutions, dried droperidol and recrystallized IV were dissolved in distilled water containing tartaric acid at a concentration of 1.5 mg/ml. Methyl- and propylparaben were first dissolved in a small amount of methanol and then distilled water containing tartaric acid was added to the required volume. The concentrations of droperidol, methyl- and propylparaben and IV in the standard solutions were in ranges 0.38–1.31, 0.08–0.49, 0.02–0.10 and 0.002–0.02 mg/ml, respectively.

Droperidol injection solutions, containing 25 mg of droperidol, 5 mg of methylparaben, 1 mg of propylparaben and 15 mg of tartaric acid in 10 ml, were diluted 1:1 prior to the chromatographic analysis.

Chromatography

All measurements were performed with HPLC apparatus consisting of a Varian Model 8500 liquid chromatograph, a Valco Model UHP-7K injector equipped with a 5-μl loop, a Varichrom UV50 variable-wavelength UV detector or a Variscan 635 UV detector and a Spectra-Physics Model SP 4100 computing integrator.

Three analytical HPLC columns, 250 × 4.6 mm I.D. Ultrasphere ODS (5 μm), 250 × 4.6 mm I.D. Ultrasphere Octyl (5 μm) and 250 × 2 mm I.D. MicroPak-CN (10 μm), were used.

The mobile phase used for quantitative determination was methanol–0.02 *M* phosphate buffer (pH 6.8) (65:35). The flow-rate was kept at 1.5 ml/min and the UV detector was operated at a wavelength of 230 nm and 0.5 or 2.0 a.u.f.s.

The capacity factors were calculated in the usual way from the retention times of the compound of interest and of an unretained compound (potassium nitrate).

RESULTS AND DISCUSSION

Chromatographic separation of droperidol, parabens and the hydrolytic products

The main problem in optimizing the chromatographic conditions was to achieve the elution of droperidol within a reasonable time and simultaneously a good separation of the remaining, much less retained, compounds, if possible with an isocratic system, which is more reliable.

Droperidol is very strongly retained on a non-polar or a moderately polar stationary phase employed in the reversed-phase mode. When a mobile phase composed of an organic modifier (methanol or acetonitrile) and water is used with any of the above-mentioned stationary phase, no peak of droperidol is observed.

The addition of a small amount of diethylamine or triethylamine to the mobile phase or the use of a mixture of methanol or acetonitrile and aqueous phosphate buffer improves the unsatisfactory behaviour of droperidol and symmetrical peaks are obtained on all the columns tested. The latter type of the mobile phase was found to be more suitable for our purpose.

Some experimental dependences measured during the investigations of the effect of the mobile phase composition and stationary phase on the retention of compounds I–IV are shown in Fig. 2. It can be seen that the control of the pH of the aqueous part of the mobile phase in the range 2.8–7.5 is essential for the optimization of the separation. A slight change in the pH of the mobile phase can affect the resolution, the order of elution and the time necessary for the whole chromatographic analysis.

Very similar results were obtained with both of the non-polar stationary phases C_8 (Ultrasphere Octyl) and C_{18} (Ultrasphere ODS). However, the CN bonded stationary phase (MicroPak-CN) proved to be less suitable for the separation as compounds II and IV were coeluted.

The use of acetonitrile instead of methanol produced no great differences in selectivity.

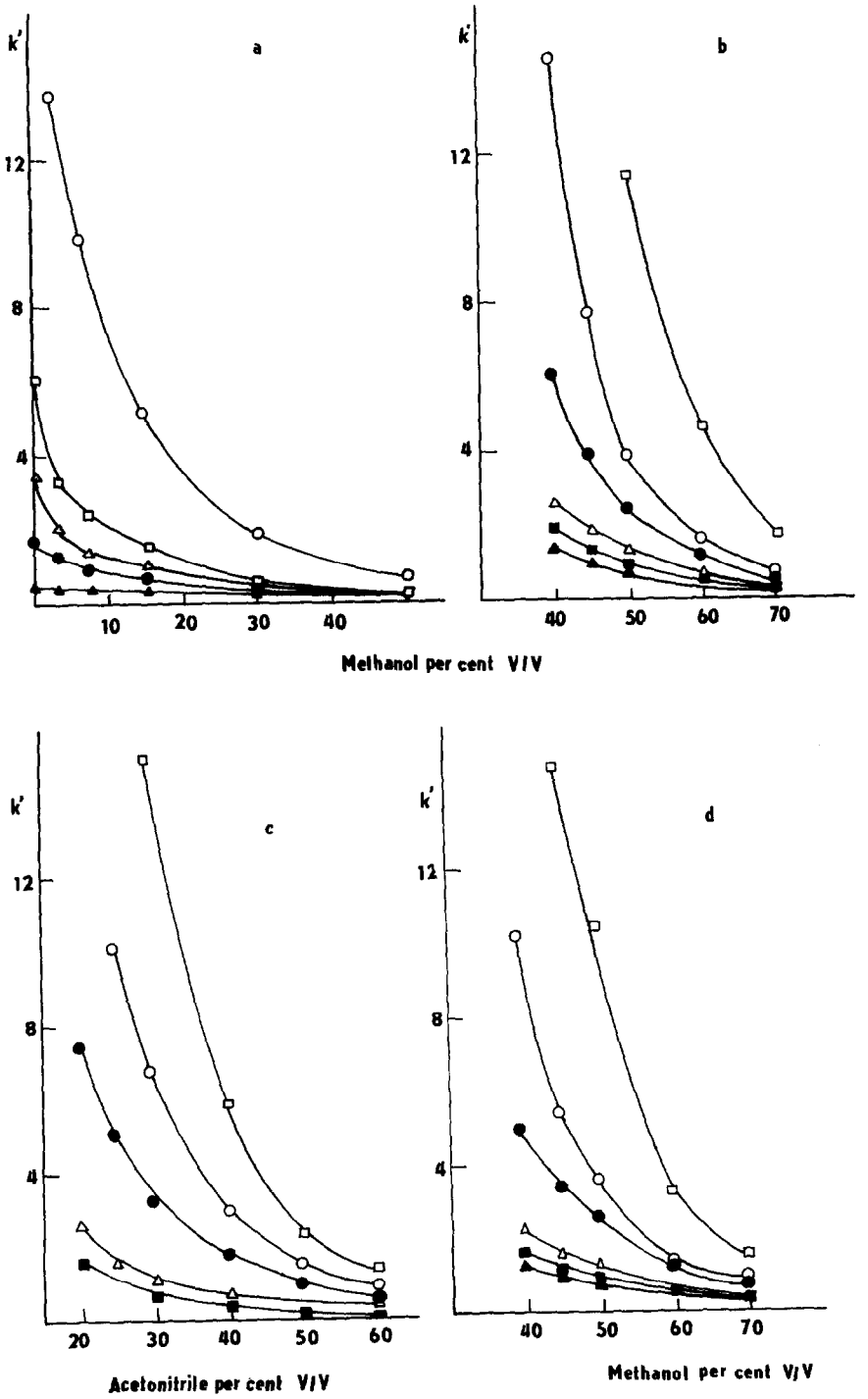
k'
a
12
8
4
10
20
30
40
b
40
50
60
70
Methanol per cent V/V
c
20
30
40
50
60
Acetonitrile per cent V/V
d
Methanol per cent V/V

Fig. 2.

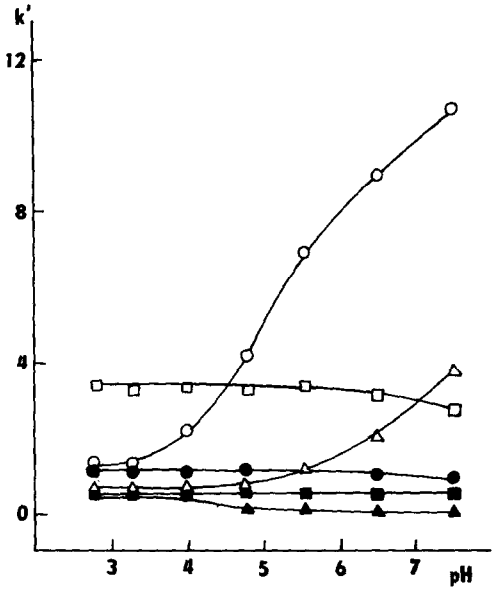

Fig. 2. Dependence of capacity factors, k', of I (○), II (●), III (□), IV (■), V (△) and VI (▲) on the content of an organic modifier and on the pH of the aqueous part of the mobile phase for various stationary phases. (a) MicroPak-CN and methanol–0.02 *M* phosphate buffer (pH 3.3); (b) Ultrasphere ODS and methanol–0.02 *M* phosphate buffer (pH 3.3); (c) Ultrasphere ODS and acetonitrile–0.02 *M* phosphate buffer (pH 3.3); (d) Ultrasphere Octyl and methanol–0.02 *M* phosphate buffer (pH 3.3); (e) Ultrasphere Octyl and methanol–0.02 *M* phosphate buffer (6:4).

The effect of pH was studied and the optimum composition of the mobile phase was found to be methanol–0.02 *M* phosphate buffer (pH 6.8) (65:35).

Table I reports the capacity factors (k') and separation factors (α) of the separated compounds for both Ultrasphere ODS and Octyl columns. Complete separation of all the compounds is achieved in both instances but the capacity factor of droperidol is lower when C_8 bonded silica is used and thus the whole analysis is faster. The degradation product of paraben (VI) is unretained on both columns.

TABLE I

CAPACITY FACTORS (k') AND SEPARATION FACTORS (α) OF THE SEPARATED COMPOUNDS FOR TWO NON-POLAR STATIONARY PHASES

Mobile phase: methanol–0.02 *M* phosphate buffer (pH 6.8) (65:35).

Compound	*Ultrasphere ODS*		*Ultrasphere Octyl*	
	k'	α	k'	α
VI	0		0	
IV	0.6		0.5	
II	0.9	1.6	0.8	1.5
V	2.2	2.3	1.7	2.0
III	2.7	1.2	2.1	1.2
I	8.4	3.1	5.3	2.5

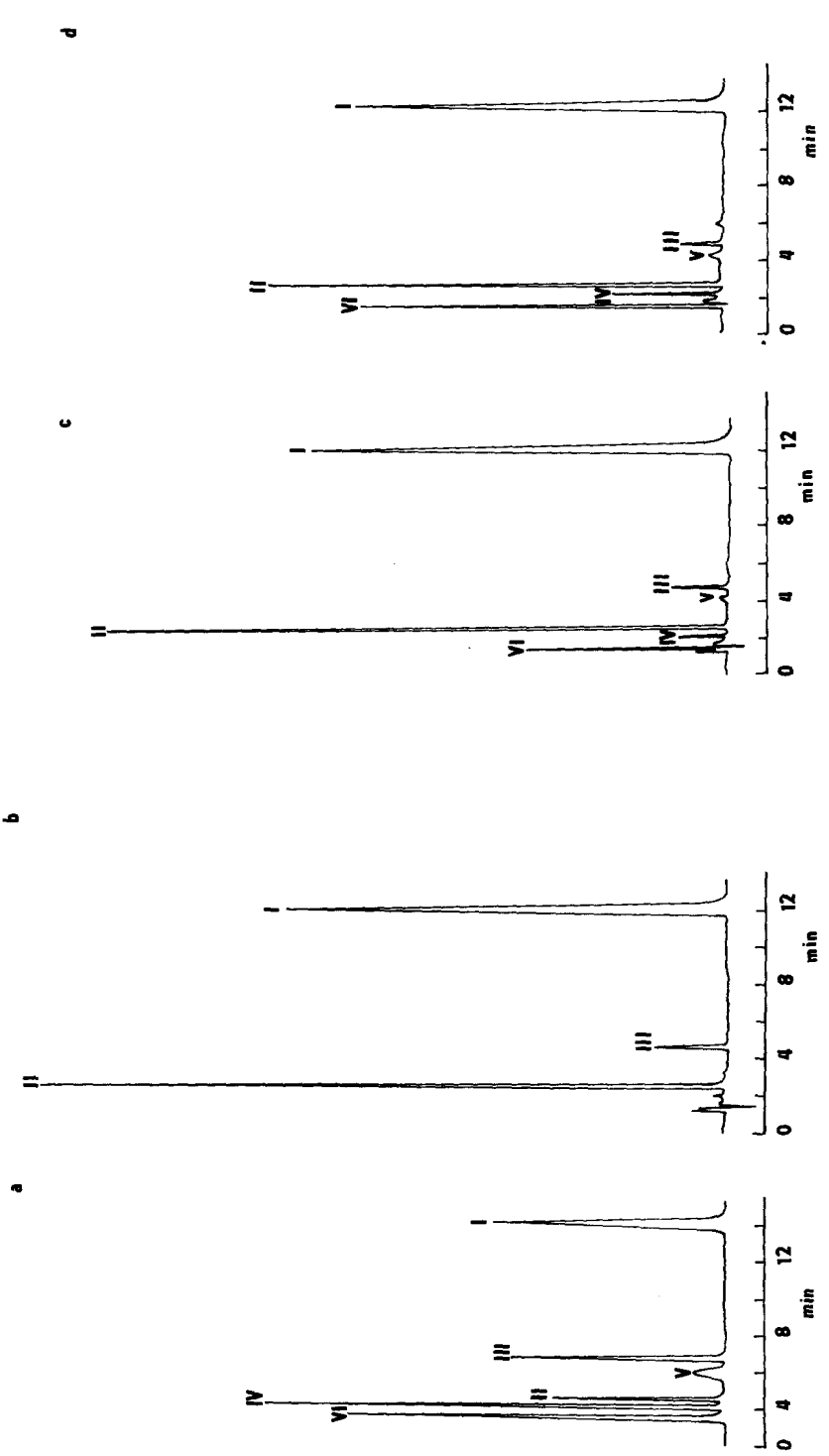

Fig. 3. Chromatograms of (a) a synthetic mixture of compounds I–VI, the droperidol injection solutions (declared contents of I, II and III in 10 ml are 25, 5 and 1 mg, respectively); (b) stored under normal conditions for 20 months; (c) kept at 60°C for 4 months; (d) kept at 70°C for 4 months. Column: Ultrasphere ODS. Mobile phase: methanol–0.02 *M* phosphate buffer (pH 6.8) (65:35). Detector: 230 nm, 0.5 a.u.f.s., peaks of droperidol registered at 2.0 a.u.f.s.

The complete separation under the suggested conditions is depicted in Fig. 3a, which shows a chromatogram of a synthetic mixture of all six compounds obtained with C_{18} bonded silica.

Quantitative evaluation

The stability-indicating capability of the suggested HPLC method is shown in Fig. 3b–d, where chromatograms of various droperidol injection solutions are presented. All six compounds were detected in the droperidol injection solutions heated to 60 or 70°C, but two of them (the degradation products V and VI) were not quantitated.

For the quantitative determination the method of external standardization was used.

The linearity of the method was tested by analysing standard solutions containing each compound at six concentration levels in the range listed in Table II. The lower value of the concentration range for the degradation product IV also corresponds to the limit of quantitation. All measured dependences of peak areas on concentration were linear, as the values of the correlation coefficient (r) given in Table II show.

TABLE II

LINEARITY AND PRECISION OF THE HPLC METHOD

Compound	*Amount injected (μg)*	*C.V. (%)*		*Concentration range (mg/ml)*	*Correlation coefficient r*
		n=5, within 1 day	*n=10, within 2 days*		
I	5.7	0.7	1.6	0.38 –1.31	0.9996
II	1.0	1.2	1.9	0.08 –0.49	0.9998
III	0.2	1.7	2.3	0.02 –0.10	0.9996
IV	0.2	1.0	1.2	0.002–0.02	0.9996

The precision of the method was evaluated from results of analyses of ten samples individually prepared by diluting (1:1) the droperidol injection solution kept at 70°C for 4 months. The coefficients of variation (C.V.) given in Table II show a satisfactory precision of the method.

The isocratic HPLC method suggested for the separation and determination of droperidol, parabens and hydrolytic products was used as a stability-indicating test. Minimal sample preparation is needed. The method is simple and precise, requires less time and gives more data than the pharmacopeial method.

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