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Note

Rapid, sensitive high-performance liquid chromatographic method for the analysis of buflomedil hydrochloride and its potential by-products

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Buflomedil hydrochloride [2′,4′,6′-trimethoxy-4-(1-pyrrolidinyl)butyrophenone] is a peripheral vasodilatator[1–4] currently widely employed in the treatment of peripheral vascular diseases. The synthesis of this compound is relatively simple, but impurities may occur (Fig. 1).

We have developed a rapid, sensitive high-performance liquid chromatographic (HPLC) method for the simultaneous separation and determination of buflomedil hydrochloride and related by-products using a reversed-phase Supelcosil LC-8 3-μm column and 0.005 *M* KH_2PO_4–acetonitrile as a mobile phase. Two HPLC methods[5,6] for the determination of buflomedil hydrochloride have been reported but, in both cases, sodium lauryl sulphate was used as an ion-pair reagent and moreover the separation from related impurities was not taken into account.

EXPERIMENTAL

Apparatus

Buflomedil hydrochloride and its potential by-products were separated on an Hewlett-Packard HPLC system consisting of a Model 1084 B liquid chromatograph equipped with a variable-wavelength detector set at 230 nm and a Model 79850 B LC terminal which served as a gradient controller and data station. Samples of 20 μl, prepared by dissolving the products in the mobile phase, were injected via a Model 79841 variable volume injector and separated on Supelcosil LC-8 3 μm, 3.3 cm × 4.6 mm I.D. The mobile phase, acetonitrile–0.005 *M* KH_2PO_4 pH 3, was filtered and deaerated before use and delivered according to the gradients indicated in Fig. 2. During analysis, both the solvents and column compartment were maintained at 40°C.

Chemicals and reagents

All the solvents used were HPLC-grade (Merck, Darmstadt, F.R.G.) except water. Distilled water was deionized and filtered through a Milli-Q water purification system. Monobasic potassium phosphate, 1,3,5-trimethoxybenzene as well as the other reagents were obtained from Janssen Chimica (Beerse, Belgium).

Buflomedil hydrochloride was synthesized according to the method described[7], while the by-products were separated by liquid chromatography on a silica-gel column using ethyl acetate–hexane (20:80, v/v) as the mobile phase.

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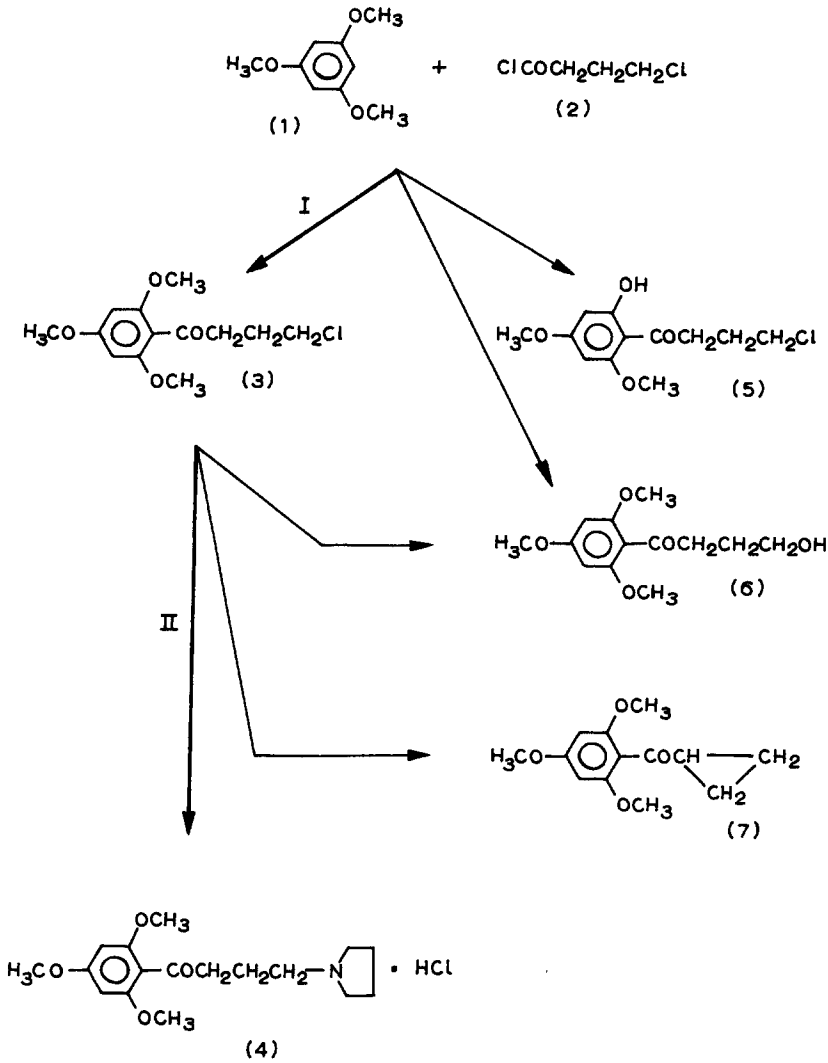

Fig. 1. Synthetic pathway for buflomedil hydrochloride, **1** = 1,3,5-trimethoxybenzene; **2** = 4-chlorobutyryl chloride; **3** = 2′,4′,6′-trimethoxy-4-chlorobutyrophenone; **4** = buflomedil hydrochloride; **5** = 2′-hydroxy-4′,6′-dimethoxy-4-chlorobutyrophenone; **6** = 2′,4′,6′-trimethoxy-4-hydroxylbutyrophenone; **7** = cyclopropyl(2,4,6-trimethoxyphenyl)methanone.

The structures were assigned by elemental analysis and ^{1}H and ^{13}C NMR spectroscopy.

Sensitivity, linearity and precision

The external standard technique was used to check the sensitivity, the linearity and the precision of the assay. The proportionality of the peak height to the amount of buflomedil hydrochloride and of all other products was measured in the range of 0–200

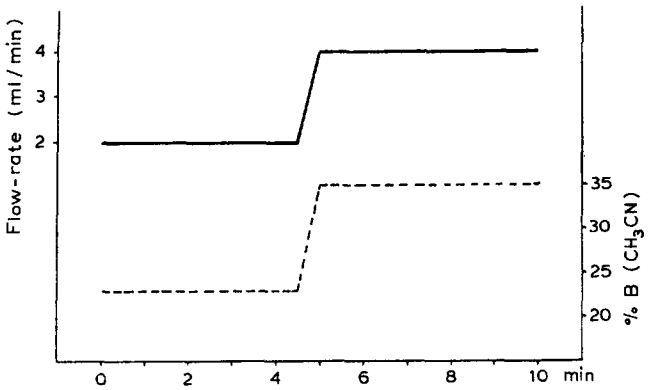

Fig. 2. Flow (———) and mobile phase (– – –) gradients.

μg/ml. The calibration graphs were linear in this range; the correlation coefficients and the detection limits for buflomedil hydrochloride and its by-products are reported in Table I.

The reproducibility of the chromatographic procedure was indicated by

TABLE I
LINEARITY AND SENSITIVITY OF THE ANALYTICAL PROCEDURE

Product[a]	*Slope*	*Correlation coefficient*	*Detection limit (μg on column)*
A	1.025	0.996	0.35
B	0.985	0.995	0.10
C	0.982	0.997	0.40
D	1.004	0.996	0.40
E	1.032	0.995	0.35
F	1.040	0.995	0.12

[a] A = 2',4',6'-Trimethoxy-4-hydroxybutyrophenone; B = buflomedil hydrochloride; C = 1,3,5-trimethoxybenzene; D = cyclopropyl(2,4,6-trimethoxyphenyl)methanone; E = 2',4',6'-trimethoxy-4-chlorobutyrophenone; F = 2'-hydroxy-4',6'-dimethoxy-4-chlorobutyrophenone.

TABLE II
PRECISION OF THE ANALYTICAL PROCEDURE
Products as in Table I. $n = 10$. Concentration of each product was 20 μg/ml.

Product	*Rel. standard deviation (%)*
A	± 0.35
B	± 0.32
C	± 0.40
D	± 0.25
E	± 0.37
F	± 0.25

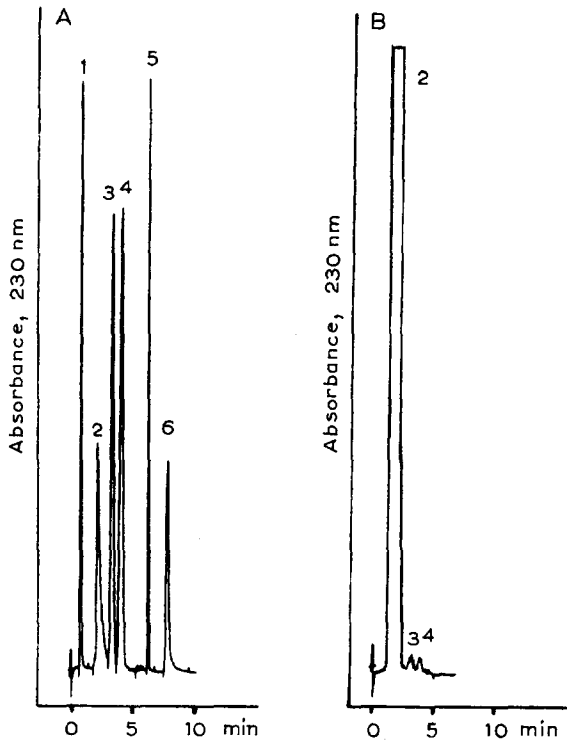

Fig. 3. Chromatograms of an artificial mixture (20 μg/ml) of each compound (A) and of 100 μg/ml of buflomedil, 0.5 μg/ml of impurities **1** and **7** (B). Peaks: 1 = 2′,4′,6′-trimethoxy-4-hydroxybutyrophenone; 2 = buflomedil hydrochloride; 3 = 1,3,5-trimethoxybenzene; 4 = cyclopropyl(2,4,6-trimethoxyphenyl)-methanone; 5 = 2′,4′,6′-trimethoxy-4-chlorobutyrophenone; 6 = 2′-hydroxy-4′,6′-dimethoxy-4-chloro-butyrophenone.

replicate injection of the same standard solutions; the relative standard deviations are reported in Table II.

RESULTS

The chromatograms shown in Fig. 3 are typical of an artificial mixture of buflomedil hydrochloride and related impurities. In chromatogram A the concentration of each product was 20 μg/ml and the attenuation was 0.0256 a.u.f.s., while in chromatogram B the concentration of buflomedil hydrochloride was 100 μg/ml while those of the impurities **1** and **7**, which are the most important were 0.5 μg/ml and the attenuation was 0.0064 a.u.f.s.

DISCUSSION

We have developed an high speed (less than 10 min) and highly sensitive HPLC method that allows the efficient and simultaneous separation and quantitation of buflomedil and synthesis-derived impurities.

According to the synthetic pathway (Fig. 1), in the first step, if the temperature is not well controlled, the presence of the Lewis acid can produce demethylation of an *o*-methoxy group with consequent formation of the impurity **5**.

In the second step there are three possible mechanisms of reaction: substitution of chlorine by pyrrolidine yields the expected product **4**; substitution of chlorine by hydroxyl, which may occur in basic media (second step) or in acidic media (first step in the presence of moisture); dehydrohalogenation with cyclization to yield impurity **7**. The impurity **7** is the most important because in raw buflomedil it is present in considerable amount and even after purification by crystallization it is possible to detect traces of this product. Some commercial samples of buflomedil, analyzed according to our method, do not show the presence of impurity **7**, nevertheless we think that it is very important to monitor this by-product.

In our method the use of KH_2PO_4 instead of sodium lauryl sulphate, and the low pH of the mobile phase, leads to a longer column life.

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