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DETERMINATION OF IMPURITIES IN PROPRANOLOL HYDROCHLORIDE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON DYNAMICALLY MODIFIED SILICA*

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SUMMARY

A rapid high-performance liquid chromatographic method has been elaborated for the separation and determination of small amounts of impurities in propranolol hydrochloride. The separation was achieved on a column of bare silica (Zorbax SIL) with methanol–water–0.2 *M* phosphate buffer pH 8.0 (70:25:5) containing 2.5 m*M* of cetyltrimethylammonium (CTMA) bromide as the eluent. The concentrations of methanol and CTMA as well as the pH of the phosphate buffer were found greatly to affect the separation. The selectivity of the system towards a mixture of propranolol and three possible impurities was investigated using different brands of silica. Only minor variations were found relative to those of a chromatographic system based on chemically bonded ODS silicas from the same sources. The method is also suitable for identification purposes, being able to separate most β-blocking drugs of structures similar to that of propranolol.

INTRODUCTION

Propranolol (I) is a β-adrenergic blocking agent widely used in the medical treatment of hypertension and angina pectoris. The drug is often prescribed in rather high doses (up to 0.5–1 g per day) and for an extended time, and hence strict demands on its purity are set by modern pharmacopoeias. A test for related substances by thin-layer chromatography (TLC) is included, *e.g.*, in the *British Pharmacopoeia*[1] stipulating a limit for the contents of individual impurities of 0.2%. The structures of propranolol and of related substances which might possibly occur as impurities arising from its synthesis are shown in Table I. Compounds II–IV are the most probable impurities, as V and VI are rather unstable. The separation and detection of these impurities, at the low level required (0.2%), would be at the limit of usefulness of the TLC method. For this reason and because in the future the impurity level allowed may be decreased further, a high-performance liquid chromatographic (HPLC) method could prove valuable.

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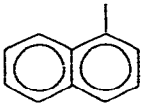
TABLE I

STRUCTURE OF PROPRANOLOL AND POSSIBLE IMPURITIES OF THE GENERAL FORMULA R–O–R_1

R =



Substances	R_1
I (Propranolol)	$-CH_2-CHOH-CH_2-NH-CH(CH_3)_2$
II	$-CH_2-CHOH-CH_2OH$
III	$-CH_2-CHOH-CH_2-N(CH(CH_3)_2)-CH_2-CHOH-CH_2-O-R$
IV	$-CH_2-CHOH-CH_2-O-R$
V	$-CH_2-CH(O)CH_2$
VI	$-CH_2-CHOH-CH_2Cl$

Several HPLC methods have been published for the assay of propranolol in plasma or urine (*e.g.*, refs. 2–5) and even for the simultaneous determination of the two stereoisomeric forms[6–8]. Furthermore, the problem of peak tailing on bonded phase column materials has been discussed[9,10]. None of these methods was designed for testing the purity of the drug and furthermore they were all based on the use of bonded phase materials. It has been demonstrated by several authors (*e.g.*, refs. 11–14) that the standardization of HPLC methods based on chemically bonded materials is problematic due to brand-to-brand variations in selectivity. Recently, however, it was shown that similar separations could be executed on columns of bare silica dynamically coated with long chain quaternary ammonium compounds[15,16]. Solid phase-induced variations in selectivity using this technique were of only minor importance[17].

The present investigation was performed with a view to elaborating an adequate HPLC method to separate and determine possible impurities in propranolol hydrochloride by use of the dynamic coating approach.

EXPERIMENTAL

Chemicals

Samples of propranolol hydrochloride and other β-blocking agents were of pharmacopoeial quality. Propranolol impurities were supplied by I.C.I. (Macclesfield, Great Britain). Dodecyltrimethylammonium (DTMA) bromide was obtained from Sigma (St. Louis, MO, U.S.A.). All other reagents were of analytical grade from E. Merck (Darmstadt, G.F.R.).

Chromatography

A liquid chromatograph consisting of an Altex Model 110 solvent metering pump, a Pye Unicam LC UV spectrophotometer detector operated at 254 or 292 nm and a Rheodyne Model 7120 injection valve equipped with a 50-μl loop was used. Chromatograms were recorded on a Kipp & Zonen Model BD-8 recorder and retention data were measured and processed by means of a Hewlett-Packard Model 3353 A laboratory data system.

All experiments were performed on columns of 120 or 250 × 4.6 mm (Knauer, Oberursl, G.F.R.) packed as described earlier[18] with silica or chemically bonded octadecylsilyl (ODS) silica (see Table II). For chromatography on dynamically coated columns the eluents were mixtures of methanol, water and potassium phosphate buffer of various pH values and with the addition of various amounts of cetyltrimethylammonium (CTMA) bromide. The columns were equilibrated by elution overnight. During chromatography the analytical column was guarded by a silica precolumn situated between the pump and the injection valve. Following each experiment the column was brought to its initial state by rinsing with methanol–0.05 *M* phosphoric acid (1:1) and finally with methanol. For bonded phase chromatography the eluent was methanol–0.01 *M* phosphoric acid (80:20) containing 5 m*M* sodium dodecanesulphonate and 2.5 m*M* DTMA bromide.

Determination of the amount of CTMA adsorbed onto the Zorbax SIL column was performed by the elution method previously described[19].

Test and standard solutions

For impurity testing, 0.2% solutions of the individual propranolol hydrochloride samples in the eluent were used. A 0.0004% solution of propranolol hydrochloride in the eluent was employed as the external standard. Fifty microlitres of each solution were injected.

For identification purposes, a solution in the eluent containing 0.05–0.5% of the individual β-blocking agents, depending upon their absorbance at 254 nm, was used. Twenty microlitres of the solution were injected.

RESULTS AND DISCUSSION

The HPLC method was elaborated using a test solution containing 0.2% of propranolol hydrochloride and *ca.* 0.0006% of each of three possible impurities (II–IV) which were available at that time (*cf.*, Table I). Several parameters will influence the retention and selectivity in a chromatographic system based on the dynamic coating approach[17,19]. The starting point in chosing the actual eluent was a previously used mixture, methanol–water–potassium phosphate buffer pH 7.5 (50:45:5) containing 2.5 m*M* CTMA. To optimize the system, the influence on separation of the methanol concentration, CTMA concentration and buffer pH was investigated.

The influence of the methanol concentration on retention of the four test substances is shown in Fig. 1. It appears that an increase in methanol concentration is required to obtain reasonably low retentions. For the remaining experiments 70% methanol was chosen.

Fig. 2 illustrates the influence of the CTMA concentration on retention. The four compounds are clearly affected to different extents, *i.e.*, impurities III and IV to a

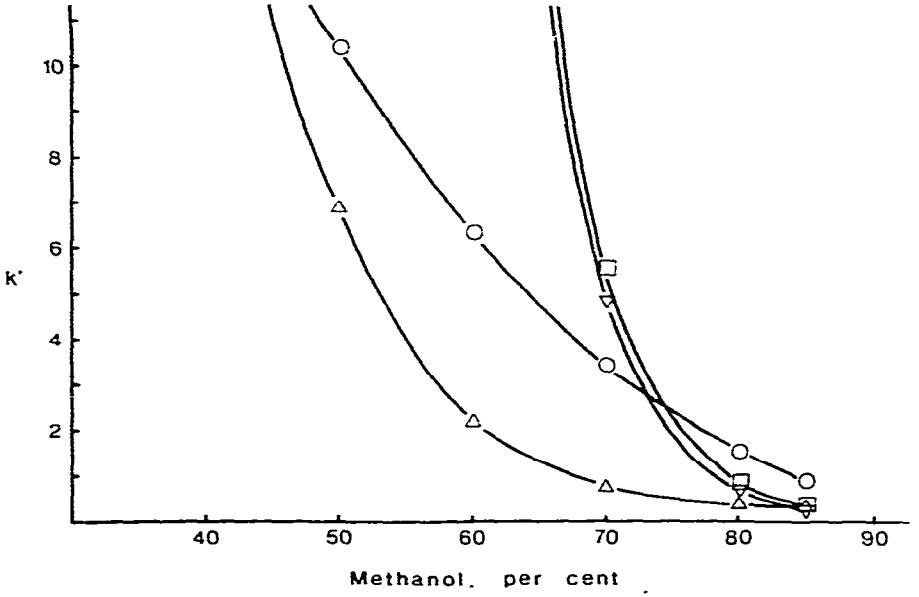


Fig. 1. Influence of methanol concentration on retention. △, II; ○, propranolol; ▽, III; □, IV. Column: LiChrosorb Si 60 (120 × 4.6 mm I.D.). Eluents: various methanol–water mixtures containing 5% of 0.2 *M* potassium phosphate buffer pH 7.5 and 2.5 m*M* CTMA.

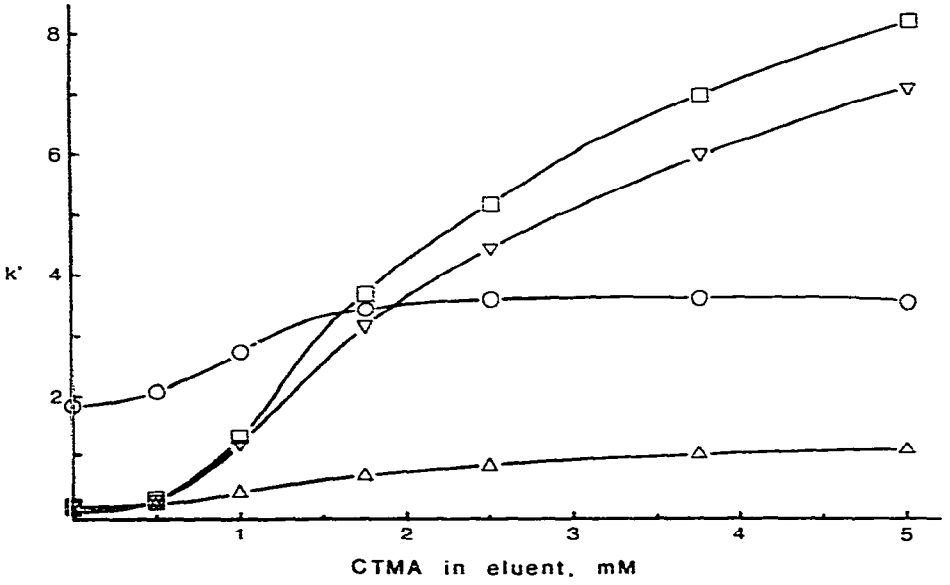


Fig. 2. Influence of CTMA concentration on retention. Eluents: methanol–water–0.2 *M* potassium phosphate buffer pH 7.5 (70:25:5) containing various amounts of CTMA. Symbols and other chromatographic conditions as in Fig. 1.

much higher degree than impurity II and propranolol. It can be deduced that the retention mechanism, at the pH of the buffer used (7.5), for all compounds is based mainly on reversed phase partition. The higher the CTMA concentration the larger will be the amounts adsorbed onto the silica surface and hence the influence on the molecules III and IV is expected to be more pronounced due to their larger hydrophobic moieties. However, for propranolol, a cation-exchange mechanism cannot be exluded since only this compound is retained on the column when no CTMA is present. If cation exchange were the dominant mechanism in the retention of the two bases (propranolol and impurity III) a decrease in retention would be expected with increasing CTMA concentration[17].

From Fig. 2 it might have been concluded that a rather low CTMA concentration would prove advantageous by placing all impurity peaks ahead of the main peak. The low concentrations, however, would also imply an unsuitably small k' for impurity II and also the necessity of long equilibration times. In conclusion it was decided to employ a CTMA concentration of 2.5 mM and attempt to achieve an optimal separation by adjustment of pH. This proved possible as Fig. 3 demonstrates. Increasing the pH will cause increased adsorption of CTMA[20] and hence increase the retention for compounds which are chromatographed according to reversed phase partition, as is seen for all four compounds up to pH $\approx$ 7. At higher pH values the retention of propranolol is found to decrease, a phenomenon which has been observed for several other compounds[20], the mechanisms underlying which are under study. From Fig. 3 it is seen that the efficiency of the separation is improved at high pH values, but in order to prevent extensive dissolution of silica, pH 8.0 was chosen as a compromise. The coverage of the silica surface with CTMA was determined by

Fig. 3. Influence of buffer pH on retention. Eluents: methanol–water–0.2 M potassium phosphate buffer (70:25:5) of various pH containing 2.5 mM CTMA. Symbols and other chromatographic conditions as in Fig. 1.

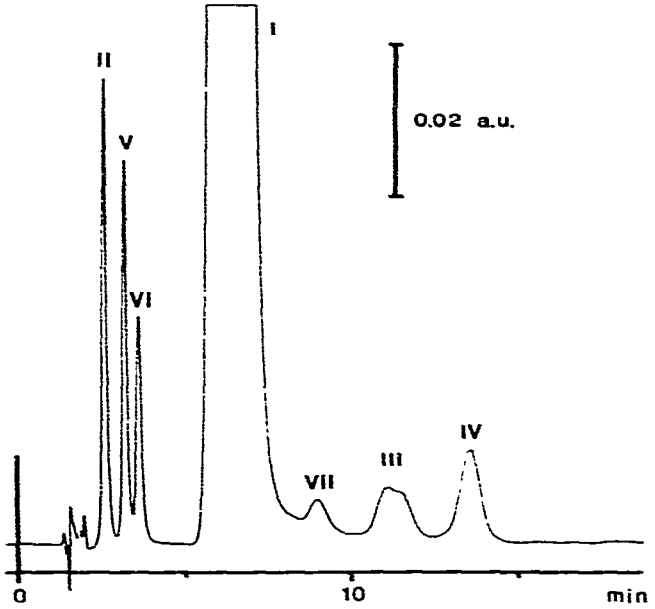


Fig. 4. Chromatogram of propranolol spiked with ≈ 0.3% of each of five possible impurities. Column: Zorbax SIL (120 × 4.6 mm I.D.). Eluent: methanol–water–0.2 *M* potassium phosphate buffer pH 8.0 (70:25:5) containing 2.5 m*M* CTMA. Detection wavelength: 292 nm. Flow-rate: 1 ml/min. Peak identification: as in Table I, except for VII, an unknown impurity.

eluting the column with acidified methanol; it was found that 0.26 mmol CTMA were adsorbed per g silica corresponding to ≈0.5 CTMA ions per nm^2. The performance of the final chromatographic system is shown in Fig. 4 and it appears from the chromatogram that the two impurities V and VI, which were also studied at this stage, are well separated from propranolol.

In order to compare the variations in selectivity of various brands of column materials to those of the corresponding chemically bonded ODS materials a separation method utilizing the latter was required. The method of Sokolowski and Wahlund[9] was used with a few modifications; in order to prevent tailing of the two bases (propranolol and impurity III), 2.5 m*M* DTMA was added to the eluent, and to achieve sufficient retention of the same compounds it was found necessary to include a sulphonate counterion. Using 80% methanol in aqueous 0.01 *M* phosphoric acid, the addition of 5 m*M* sodium dodecanesulphonate was found adequate. Fig. 5 shows a separation using the bonded phase chromatographic system. It appears that the peaks corresponding to impurities V and VI coincide with the propranolol peak.

The two separation methods have been tried out using supports from all suppliers which were able to deliver silica as well as chemically bonded ODS silica in bulk. The selectivities of the individual chromatographic systems towards a mixture of propranolol and impurities II–IV, as expressed by the separation factor of each impurity from propranolol, appear in Table II. It can be seen that the variations in selectivity of the systems based on bonded phase materials are considerably larger than those of the systems based on dynamically coated silica, resulting even in the

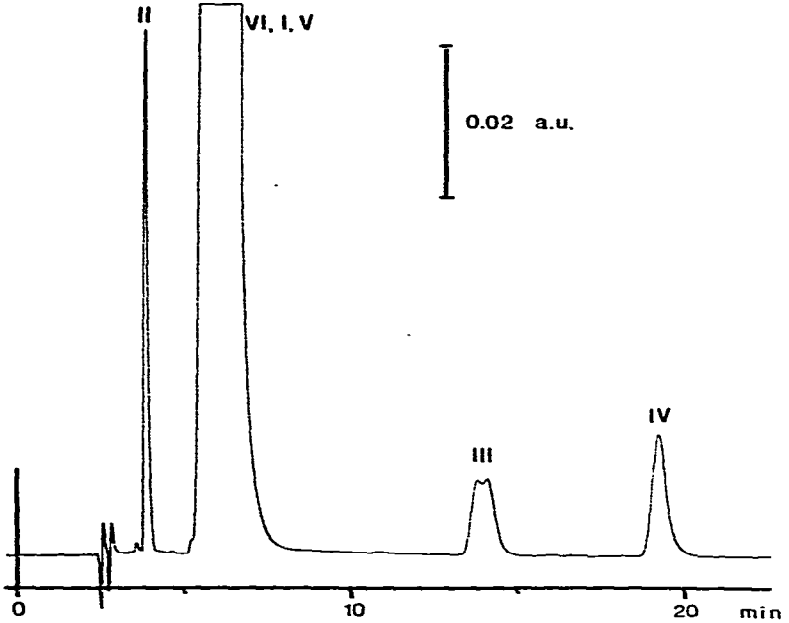


Fig. 5. Chromatogram of propranolol and possible impurities as in Fig. 4. Column: Zorbax ODS (250 × 4.6 mm I.D.). Eluent: methanol–0.01 *M* phosphoric acid (80:20) containing 5 m*M* sodium dodecanesulphonate and 2.5 m*M* DTMA. Peak identification and other chromatographic conditions as in Fig. 4.

TABLE II

SEPARATION FACTORS BETWEEN PROPRANOLOL AND THREE POSSIBLE IMPURITIES MEASURED ON EIGHT DIFFERENT SILICA COLUMNS AND EIGHT DIFFERENT ODS-SILICA COLUMNS

Column material	*Pore size (nm)*	*Separation factor*		
		II	*III*	*IV*
Silica				
LiChrosorb Si 60	6	0.26	1.86	2.22
Nucleosil 50-5	5	0.26	1.76	2.13
Zorbax SIL	7	0.24	1.76	2.08
Partisil 5	7–8	0.25	1.66	1.98
Spherisorb S 5W	8	0.28	1.74	2.01
LiChrosorb Si 100	12	0.31	1.39	1.60
Nucleosil 100-5	10	0.26	1.43	1.66
Hypersil	10	0.26	1.40	1.66
ODS silica				
LiChrosorb RP-18		0.40	2.43	3.81
Nucleosil 5 C18		0.61*	2.40	4.69
Hypersil ODS		0.38	2.62	3.34
Zorbax ODS		0.38	2.97	4.38
Partisil 10 ODS		1.19*	2.05	4.48
Partisil 10 ODS 2		0.61	3.21	6.53
Partisil 10 ODS 3		0.56	2.42	3.71
Spherisorb S5 ODS		0.60	1.84	2.68

* The peak coincides with the propranolol peak in the chromatogram.

inability to separate impurity II from propranolol on two columns of the former type. There seems to be a minor difference in the magnitude of the separation factors for silica materials of 5–8 nm, or 10–12 nm in pore size, respectively. As shown previously[17], the shape of the isotherms for adsorption of CTMA on silica do depend on the pore size, and hence a certain difference in selectivity can result. Thus, if a high degree of standardization of a reversed-phase HPLC method based on a dynamically coated phase is required, it may be necessary to prescribe, apart from a minimum column surface area, the use of a column material of a particular pore size.

Utilizing the dynamically coated chromatographic system described above a series of samples of propranolol hydrochloride have been analyzed. For quantitation external standardization was used. Detector response linearities for each of the five possible impurities were established up to a content of 1 % in propranolol hydrochloride. The precision of the method was investigated by analyzing eight individually prepared solutions of a sample of propranolol hydrochloride spiked with 0.25 % of each of the five possible impurities; relative standard deviations ranged between 3.8 and 7.3 %. The results of the analysis are shown in Table III. Only impurities which have been found in one or more of the samples are included. Compounds IV, V or VI were not detected, thus their content does not exceed *ca.* 0.005 %. It appears that impurity III is only detected in rather old samples, whilst the more recent ones tend to be of a higher degree of purity. In certain recent samples, however, an unknown impurity (VII) was also detected. In Fig. 6 are displayed chromatograms of samples A, D and G.

TABLE III

RESULTS OF THE ANALYSIS OF THREE OLD (A–C, MORE THAN 6 YEARS) AND FOUR RECENT (D–G, LESS THAN 6 YEARS) SAMPLES OF PROPRANOLOL HYDROCHLORIDE, AND OF THE BP AUTHENTIC SPECIMEN OF PROPRANOLOL HYDROCHLORIDE (H)

Sample	*Amount of impurity (%)*		
	II	*III*	*VII*
A	0.02	0.38	–
B	0.01	0.01	0.01
C	0.12	0.02	0.01
D	0.01	–	0.12
E	0.01	–	0.11
F	–	–	–
G	–	–	–
H	–	0.14	0.02

Fig. 7 depicts the separation of a mixture of all common β-blocking agents marketed, which are structurally related to propranolol. It appears that most components are separated from each other and all of them from propranolol; thus the method might also be valuable for identification purposes.

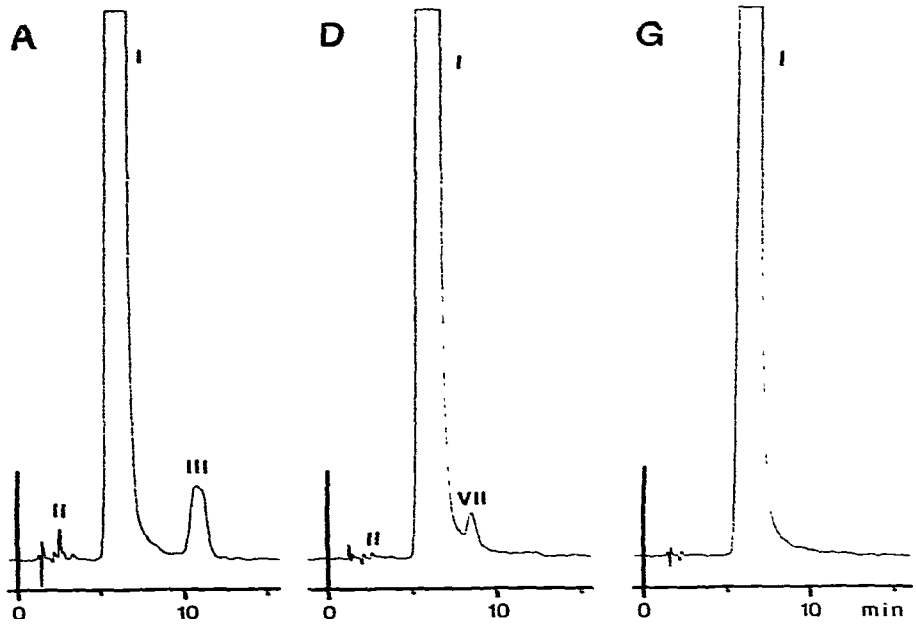


Fig. 6. Chromatograms of an old (A) and two recent (D, G) samples (*cf.*, Table III) of propranolol hydrochloride. Chromatographic conditions and peak identification as in Fig. 4.

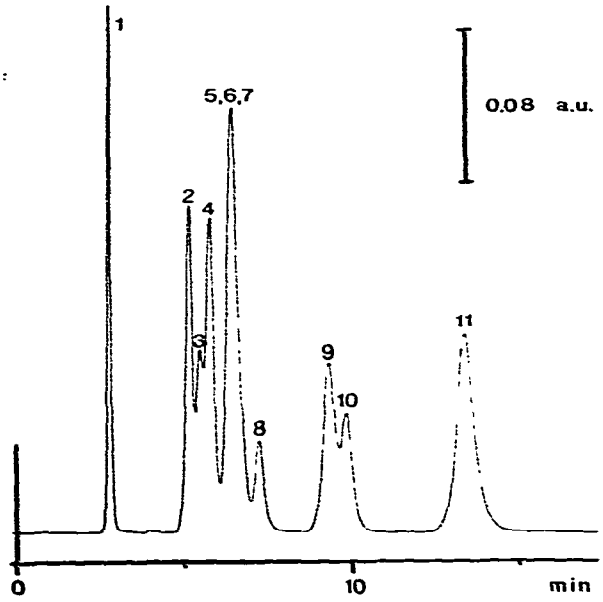


Fig. 7. Chromatogram of a mixture of β-blocking drugs. Columns: Zorbax SIL (250 × 4.6 mm I.D.). Detection wavelength: 254 nm. Other chromatographic conditions as in Fig. 4. Peaks: 1 = maleate; 2 = sotatol; 3 = atenolol; 4 = practolol; 5 = timolol; 6 = acebutolol; 7 = metoprolol; 8 = oxprenolol; 9 = pindolol; 10 = alprenolol; 11 = propranolol.

CONCLUSIONS

An HPLC method based on dynamically modified silica has been elaborated and shown to be suitable for the separation and determination of impurities in propranolol hydrochloride. It was possible to standardize the method to a degree where it is independent of the brand of silica used by specifying, besides the eluent composition, a minimum column surface area and possibly a particular pore size. Hence the method has been shown to be superior to an alternative HPLC method based on chemically bonded ODS silica since the latter exhibited large brand-to-brand variations in selectivity towards a test mixture containing propranolol and three possible impurities.

The method is also suitable for the separation of propranolol from all other β-blocking agents of similar structure.

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