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Determination of celiprolol and oxprenolol in human plasma by high-performance liquid chromatography and the analytical error function

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Abstract

Two reversed-phase HPLC methods with UV detection to quantify celiprolol and oxprenolol in human plasma are described. The analytical methods for the determination of both drugs used the same reversed-phase HPLC column, mobile phase and extraction procedure. Linearity was obtained in the ranges 15.63–1000 and 25–800 ng/ml for celiprolol and oxprenolol, respectively. Intra-day and inter-day variation was lower than 14%. After validation of the methods, analytical error functions were established as S.D. (ng/ml)=3.096+0.041C for celiprolol and S.D. (ng/ml)=8.906+8.075·$10^{-8}C^3$ for oxprenolol.

Keywords: Celiprolol; Oxprenolol

1. Introduction

Celiprolol and oxprenolol are two safe and well-tolerated β-blockers, with some differences in their pharmacokinetic and pharmacological properties. They are effective and widely used to treat cardiovascular disorders such as essential hypertension, cardiac arrhythmia and angina pectoris [1,2].

Several non-enantiospecific and enantiospecific liquid chromatographic methods for the determination of celiprolol and oxprenolol in human plasma have been published [3–10]. None has reported the error function associated with the analytical method. The problem of choosing weights in non-linear regression analysis of pharmacokinetic data is well known [11,12], especially when there are few experimental points as in therapeutic drug monitoring [13]. Thus, it would be useful to have a practical way of obtaining the estimated standard deviation with which a single determination of a serum drug concentration is measured [14,15].

In a previous work, a study of three analytical method error functions for the quantification of celiprolol, bisoprolol and oxprenolol in a simple matrix (phosphate buffer, pH 7.4) was carried out [14]. Different non-linear functions applicable to the description of errors were found, which confirmed the need to determine the analytical error function of each drug with its corresponding analytical method of quantification individually.

Thus, the objectives of the present study were twofold. First, to develop and validate two analytical

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methods for the quantification of two β-blockers in human plasma. Second, after the validation of both the HPLC methods, to determine their analytical error functions in order to provide a suitable data-weighting method covering their working range.

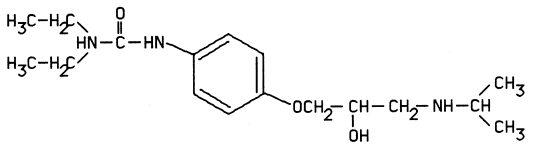
2. Experimental

2.1. Reagents and materials

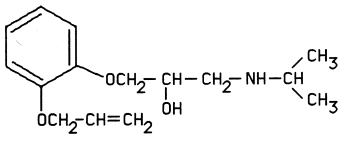
Celiprolol and oxprenolol were provided by Rhone-Poulenc Rorer (Alcorcón, Madrid, Spain) and Novartis Farmacéutica (Barcelona, Spain), respectively. Their chemical structures are represented in Fig. 1. Acetonitrile was HPLC grade and was purchased from Scharlau (Barcelona, Spain). Triethylamine, 85% phosphoric acid, 97% sodium hydroxide and dichloromethane were analytical grade and were purchased from Fluka (Buchs, Switzerland), Probus (Badalona, Barcelona, Spain), and Panreac (Montcada i Reixac, Barcelona, Spain) respectively.

2.2. Equipment

The HPLC system consisted of a 422 Kontron (Kontron Instruments, Barcelona, Spain) equipped with two 422 pumps, a rheodyne 7161 injector with a 100-μl sample loop, a 491 mixer, a 432 capillary UV–visible detector with variable-wavelength and an INT-450 computerized integration system data output.



Celiprolol



Oxprenolol

Fig. 1. Chemical structure of β-blockers assayed.

2.3. Sample preparation

For each sample, 100 μl of 1 *M* sodium hydroxide and 6 ml of dichloromethane were added to 1 ml of human plasma. The tube was capped and the contents were mixed for 10 min on a rotary mixer and centrifuged for 5 min at 2000 *g*. The upper aqueous layer was discarded and 5 ml of the organic layer was transferred to a conical glass tube and evaporated to dryness at 50±1°C. The dry residue was redissolved in 150 μl of mobile phase and 100 μl were injected into the HPLC system using a 100-μl Hamilton syringe.

2.4. Chromatography

Liquid chromatographic analyses were performed on a column packed with 5 μm Nucleosil RP-18 (125×4 mm I.D.) (Teknokroma, Barcelona, Spain) operating at room temperature.

The mobile phase for both active principles was an isocratic mixture of acetonitrile–HPLC water with 1.2% (w/v) of triethylamine and the pH adjusted to 3 with 85% orthophosphoric acid (16:84, 20:80, v/v, pH 3). The flow-rate was 1 ml/min. The injection volume was 100 μl and the UV detection was accomplished at 232 and 220 nm for celiprolol and oxprenolol, respectively (0.05 a.u.f.s. and 0.5 s response time).

Standard solutions of β-blockers dissolved in drug-free human plasma were obtained by suitable dilution from stock solutions prepared at 300 μg/ml. The concentration ranges for the calibration curves in human plasma were 15.63–1000 and 25–800 ng/ml for celiprolol and oxprenolol, respectively. The limits of quantitation were also determined.

2.5. Validation

Evaluation of the reversed-phase HPLC methods was based on proportionality (linearity assay), preci-

sion (repeatability and reproducibility assays) and accuracy [16–18].

2.5.1. Linearity

Linearity was determined using the same concentration range as the calibration curve, covering seven concentration levels: 1000, 500, 250, 125, 62.5, 31.25 and 15.63 ng/ml for celiprolol and six concentration levels: 800, 400, 200, 100, 50 and 25 ng/ml for oxprenolol. Each concentration was analysed in triplicate.

2.5.2. Precision and accuracy

Three concentrations within the linearity range (low, medium and high) were selected: 500, 125 and 31.25 ng/ml and 400, 100 and 25 ng/ml for celiprolol and oxprenolol, respectively. Five standard solutions of each concentration were spiked to drug-free human plasma and analysed (repeatability assay). The assay was repeated on 5 days (reproducibility assay).

2.5.3. Recovery

The absolute extraction recovery of both β-blockers from human plasma was assessed by comparing the peak areas obtained from the standard stock solutions of the compounds with those of drug-free plasma spiked with the drug.

2.6. Analytical error function

The study of the analytical error function was carried out using the same ranges of the calibration curves of celiprolol and oxprenolol. The concentration levels were 1000, 500, 250, 125, 62.5, 31.25 and 15.63 ng/ml for celiprolol and 800, 400, 200, 100, 50 and 25 ng/ml for oxprenolol. The procedure used to obtain the error function of each validated analytical method was the same as previously described [14,15]. The best functionalization between the standard deviation (S.D.) obtained for each concentration level of the calibration curve, and the theoretical values (C), were calculated using multiple regression, applying the stepwise forward selection method.

3. Results and discussion

3.1. Chromatogram

Fig. 2a–d shows representative chromatograms (a) blank plasma and (b) blank plasma spiked with 125 ng/ml celiprolol and (c) blank plasma and (d) blank plasma spiked with 100 ng/ml oxprenolol. Chromatographic conditions of β-blockers studied are shown in Table 1. Resolution and quantification were satisfactory using these reversed-phase HPLC methods and the retention times were 9.5 and 7.0 min for celiprolol and oxprenolol, respectively.

A minimum signal-to-noise ratio of 5:1 was obtained with the lowest concentrations, allowing a quantitation limit of 7.81 and 12.50 ng/ml for celiprolol and oxprenolol, respectively. Thus, the limits of quantitation used (15.63 and 25 ng/ml) were higher than the absolute limits of the assays. The injection volume was the same for both drugs.

3.2. Recovery

The analytical recovery in the plasma sample averaged 90% for celiprolol and 85% for oxprenolol over the entire concentration ranges.

3.3. Validation

In the linearity assay, the response factors expressed by the coefficient of variation (C.V.) were 11.61 and 12.62% for celiprolol and oxprenolol, respectively. The regression equations obtained by unweighted least-squares linear regression were $y=0.1901+0.4267x$, $r^2=0.9983$, and $y=0.9855+0.1454x$, $r^2=0.9982$, where y is peak area and x is concentration. A good linear relationship between the peak area and concentration was observed. The results obtained in repeatability and reproducibility assays are summarised in Table 2. Maximum C.V values were 5.30 and 4.99% in the repeatability assay and 4.85 and 13.80% in the reproducibility assay for celiprolol and oxprenolol, respectively. Accuracy, expressed as the percentage of the mean recovery, was confirmed after application of the Student's t-test. No significant differences ($P>0.05$) were found between the mean recovery and 100% recovery for either drug.

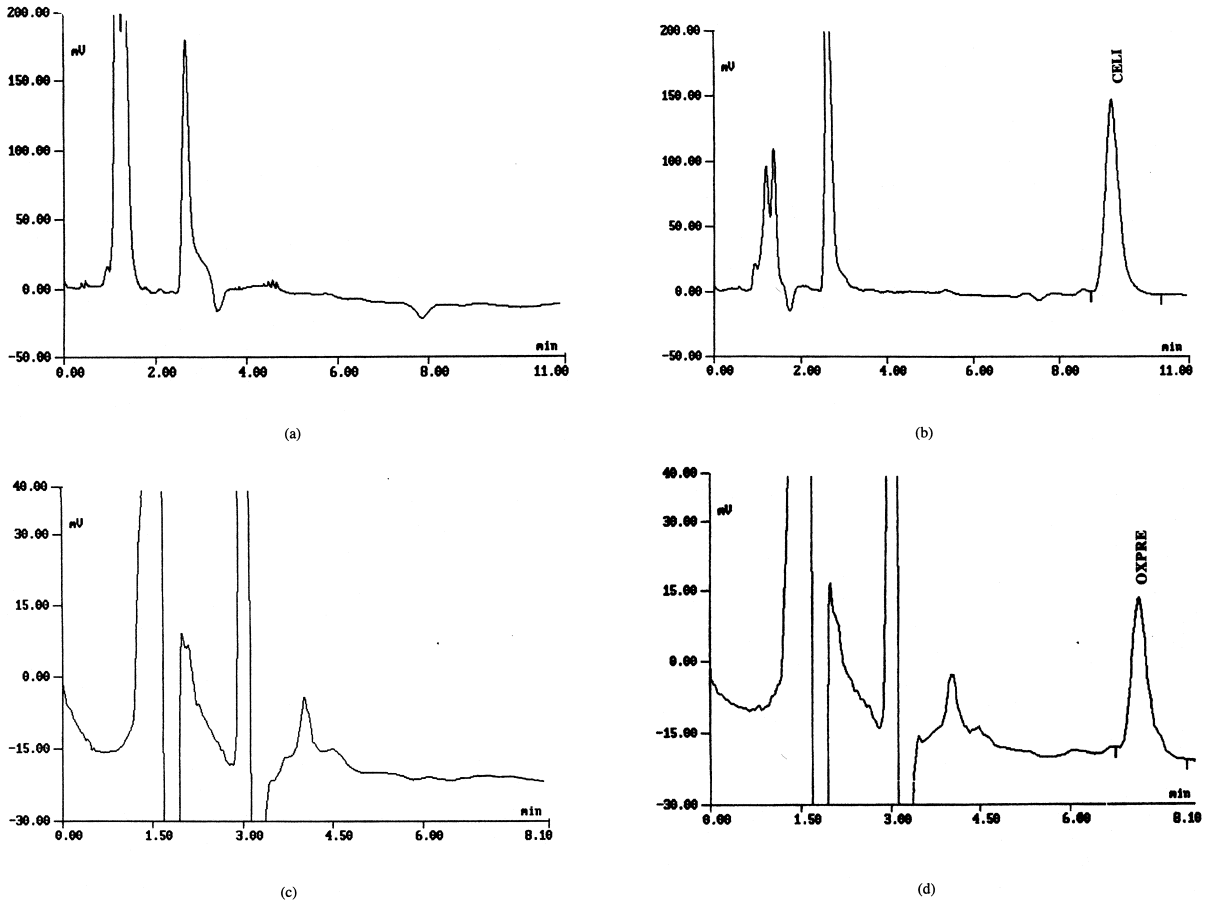


Fig. 2. Representative chromatograms of (a) blank plasma of celiprolol; (b) blank plasma spiked with 125 ng/ml of celiprolol; (c) blank plasma of oxprenolol; (d) blank plasma spiked with 100 ng/ml of oxprenolol.

3.4. Analytical error procedure

The best analytical error functions obtained with the stepwise forward selection method were the following: S.D. (ng/ml)=3.096+0.041C for celiprolol and S.D. (ng/ml)=8.906+8.075·$10^{-8}C^3$ for oxprenolol. Fig. 3a and b show the fit of these functions to the mean values of S.D. obtained for each theoretical concentration (error bars represent the S.D. of the mean values of the 5 analysis days). So, the error corresponding to celiprolol and oxprenolol is described by a linear and a non-linear function, respectively, in spite of the fact that the same reversed-phase HPLC column, mobile phase

Table 1
Chromatographic conditions of drugs studied

β-Blocker	Mobile phase (% acetonitrile)	Injection volume (μl)	Wavelength UV (nm)	Response time (min)	Limit of quantitation (ng/ml)
Celiprolol	16	100	232	9.5	7.81
Oxprenolol	20	100	220	7.0	12.50

Table 2
Intra-day (1 representative day) and inter-day precision for celiprolol and oxprenolol in plasma

β-Blocker	Concentration added (ng/ml)	Intra-day (n=5)		Inter-day (n=5)	
		Concentration found (ng/ml)	C.V. (%)	Concentration found (ng/ml)	C.V. (%)
Celiprolol	500.00	499.49	4.02	499.09	0.21
	125.00	124.03	1.27	122.74	2.04
	31.25	32.73	5.30	34.41	4.85
Oxprenolol	400.00	400.39	1.17	399.13	0.26
	100.00	97.19	6.28	101.16	4.15
	25.00	27.41	4.99	24.86	13.80

C.V.=coefficient of variation.

and extraction procedure was used. Nevertheless, some of the chromatographic conditions (Table 1) and calibration curve ranges are different from both drugs. At this point, we would like to underline the importance of the correct selection of the calibration curve range according to the concentrations of the analytical plasma samples when analytical error function is used as a possible weighting method [19].

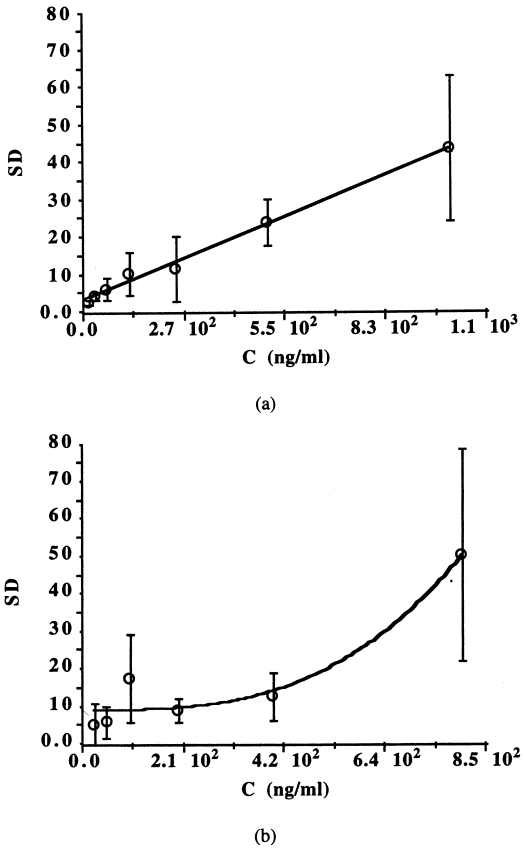


Fig. 3. Mean values and standard deviations obtained in the study of the analytical error function vs. theoretical concentrations from the calibration curves for (a) celiprolol and (b) oxprenolol.

We have previously determined nonlinear analytical error function for the same active principles [14]. Although the same analytical technique (reversed-phase HPLC with UV detection), in comparison, was employed, there are some differences to consider. On the one hand, chromatographic conditions and the effect of the extraction method taken into account in the present study, since plasma concentrations are measured. On the other hand, concentration ranges were totally different in both studies, not being possible to extrapolate the analytical error function out of the calibration curve range where it is used as a weighting method. One more time, these results show that analytical errors do not fit any pattern foreseen a priori, but, rather, the analytical error function of each drug has to be determined individually.

From these error functions the variance associated with a concentration value within the working calibration curve range can be calculated and its reciprocal ($1/V$) used as an alternative weighting method in pharmacokinetic parameter estimation [19–22].

4. Conclusions

The data demonstrated that these analytical methods have acceptable linearity, precision and accuracy

between the peak area and concentration. The maximum C.V. values were 12.62, 6.28 and 13.80% for the linearity, repeatability and reproducibility assays respectively. Moreover, these methods can be used for analysis of a large number of human plasma samples each day, since the same type of reversed-phase column, drug extraction process and mobile phase are used in the determination of both drugs.

The analytical error function established for each β-blocker could be used as a possible weighting method, which would contribute significantly to reduce overall variability in pharmacokinetic studies of these β-blockers.

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