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Determination of analytical error function for β-blockers as a possible weighting method for the estimation of the regression parameters

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Abstract

Three analytical methods have been developed and validated for the quantification of β-blockers (celiprolol, bisoprolol and oxprenolol) using high performance liquid chromatography (HPLC) with UV detection. The methods were determined to be linear, precise and accurate (RSDs were lower than 5%), which allowed the quantitation of β-blockers assayed at concentrations in the range 25–0.78 μg ml^{-1}. After validation of reversed-phase HPLC methods, their analytical error functions were established by a rapid, simple and economical procedure. The discrimination of the best function for each active principle was performed by an appropriate polynomial statistical analysis, yielding SD (μg ml^{-1}) $= 0.0295 + 0.0124C - 3.88 \times 10^{-4}C^2$ for celiprolol, $0.0199 + 0.011C - 1.27 \times 10^{-5}C^3$ for bisoprolol; and $0.0183 + 0.0089C - 9.68 \times 10^{-6}C^3$ for oxprenolol. These analytical error functions are an alternative to the weighting methods used in parameter estimation of β-blockers.

Keywords: β-Blockers; Reversed-phase HPLC; Analytical error function; Weighting method; Variance model; Non-linear regression

1. Introduction

To fit a model to experimental data obtained by different analytical methods and techniques and to estimate the parameters inherent to the model that best defines the process studied is usual practice in pharmaceutical studies.

Different methods of parametric estimation such as non-linear regression usually require the weighting of primary data in order to obtain good estimators of the regression parameters [1–3]. Since issues related to the choice of weighting methods ($1/C$, $1/C^2$, 1/variance) are not entirely solved and there is no universal solution for all cases [4–8], an analytical error function can be used as an alternative valid weighting method in the regression problem [9,10]. Nevertheless, prediction of the analytical error function is not possible a priori, since error is associated with the characteristics of the active principle, analytical

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method and technique used, so this function must be determined [11].

β-Blockers are widely used in cardiovascular disorders and their efficacy is well established [12,13]. Many reports of chromatographic methods reflect the predominance of high performance liquid chromatography (HPLC) as the most favoured technique of pharmaceutical analysis [14]. In this work three β-blockers have been assayed: celiprolol, bisoprolol and oxprenolol.

Therefore, the objectives of the present study were twofold. First, to develop and validate three analytical methods for the quantification of all three β-blockers assayed by HPLC using a suitable chromatographic column and mobile phase. Second, after the validation of the reversed-phase HPLC methods, to determine their analytical error functions in order to provide a suitable data-weighting method throughout their working range.

2. Material and methods

2.1. Reagents and materials

Celiprolol, bisoprolol and oxprenolol were provided by Rhone-Poulenc Pharma (Madrid, Spain), Merck-Igoda (Barcelona, Spain) and Ciba-Geigy (Barcelona, Spain) respectively. Their chemical structures are represented in Fig. 1.

2.2. Apparatus

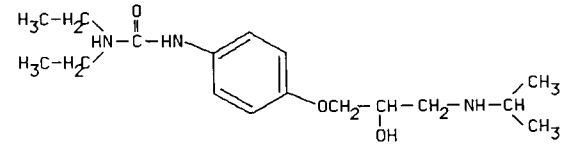
The HPLC system consisted of a Kontron (Model 420) instrument (Kontron Instruments, Barcelona, Spain) equipped with an automatic sampling system with a variable volume injector (Model 465), two pumps (Model 420), a mixer (Model 491), a capillary UV–visible detector with variable wavelength (Model 433) and a computerized integration system data output (Model MT-450). Liquid chromatographic analyses were performed on a C_{18} column (12.5 cm × 4 mm i.d.) packed with 5 μm Nucleosil (Teknokroma, Barcelona, Spain) operating at room temperature.

2.3. Chromatography

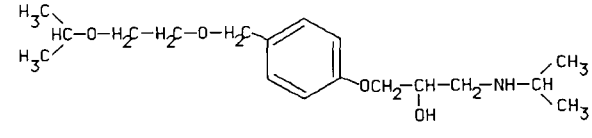
The mobile phase for all active principles was composed of acetonitrile (solvent A) and phosphate buffer (solvent B), with 0.2% (w/v) of triethylamine and with the pH adjusted to 3 with orthophosphoric acid 85% (0.067 M 30:70, v/v pH*3). The flow rate was 0.8 ml min^{-1}. The injection volume was 40 μl for both bisoprolol and oxprenolol and 20 μl for celiprolol. The UV detection was accomplished at 225, 224 and 232 nm for bisoprolol, oxprenolol and celiprolol respectively, at 0.05 AUFS and 0.5 s response time.

Standard solutions of each active principle were obtained by suitable dilution from stock solutions prepared at 0.25 mg ml^{-1} in phosphate buffer (pH 7.4, 0.067 M). The concentration range of the calibration curves was 25–0.78 μg ml^{-1}. The limits of quantitation were also determined.

Celiprolol



Bisoprolol



Oxprenolol

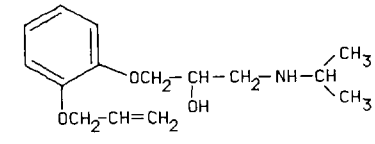
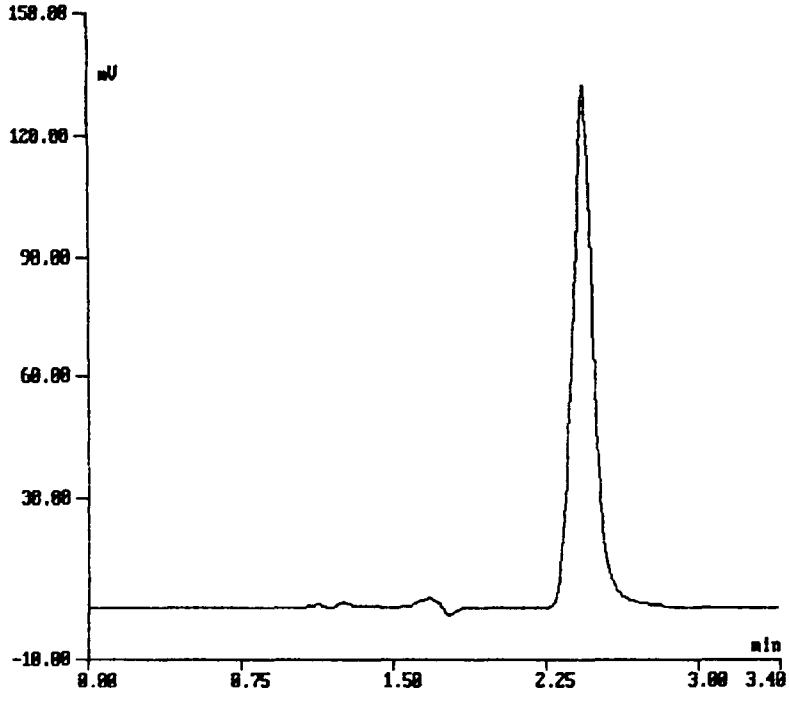
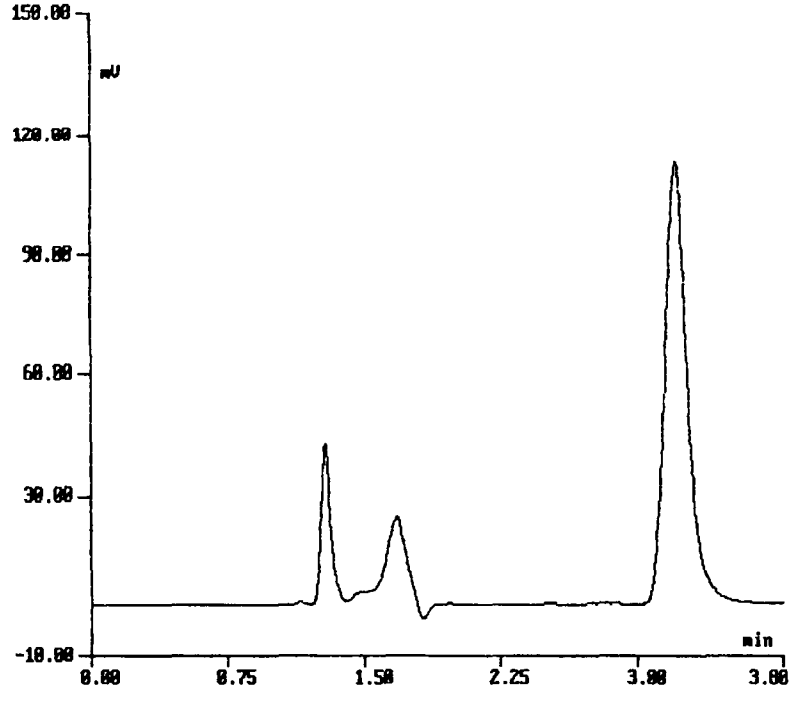


Fig. 1. Structure of β-blockers assayed.

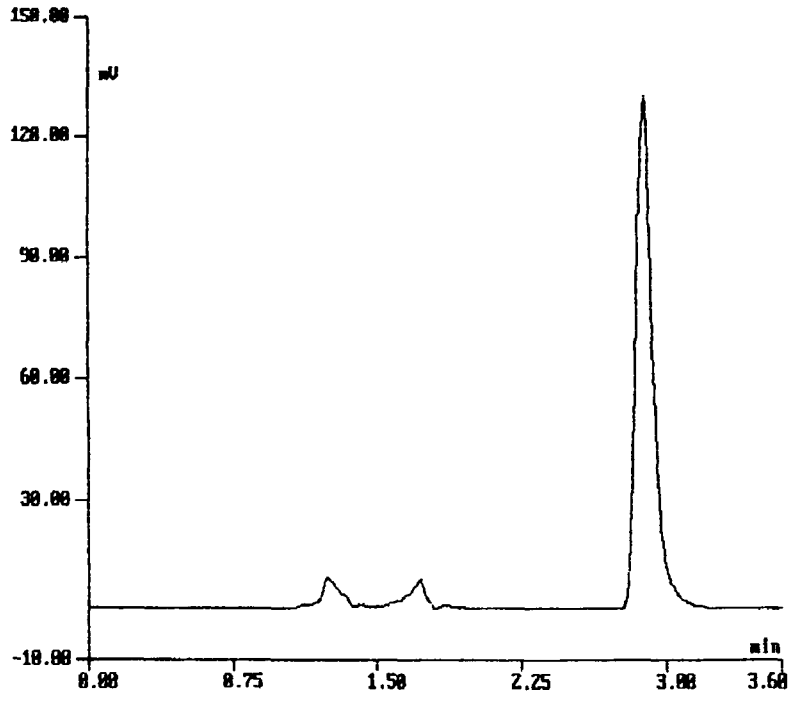


(a)



(b)

Fig. 2(a) and (b).



(c)

Fig. 2. Representative chromatograms of celiprolol (a), bisoprolol (b) and oxprenolol (c) at 12.5 μg ml^{-1}.

2.4. Validation

Evaluation of the reversed-phase HPLC methods was based on proportionality (linearity assay), precision (repeatability and reproducibility assays) and accuracy [15–17].

2.4.1. Linearity

Consisted in the determination of the same concentration range as the calibration curve, covering six concentrations: 25, 12.5, 6.25, 3.125, 1.56 and 0.78 μg ml^{-1}. Each concentration was analysed in triplicate.

2.4.2. Precision and accuracy

Three concentrations within the linearity range (low, medium and high) were selected: 25, 6.25 and 1.56 μg ml^{-1}. Five standard solutions of each concentration were prepared and analysed in triplicate (repeatability assay). This assay was repeated for 5 days (reproducibility assay).

2.5. Analytical error

The procedure used to obtain the error function of each analytical method previously validated was as follows: six calibration curves were obtained on the same day and repeated on five subsequent days. Each day, the mean and standard deviation of each standard concentration were obtained from the calibration curve. After that, those standard deviations (as dependent variable) and their theoretical concentration values (as independent variable) were regressed using polynomial analysis in order to establish the best function that would relate both variables, whose general equation is:

$$SD = A_0 + A_1 \cdot C + A_2 \cdot C^2 + A_3 \cdot C^3$$

where SD corresponds to the standard deviation associated with the measurement of each concentration value and C corresponds to theoretical concentration values.

Table 1
Chromatographic conditions of β-blockers assayed

Drug	Mobile phase (% acetonitrile)	Injection volume (μl)	Wavelength (nm)	Response time (min)	Limit of quantitation (μg ml^{-1})
Celiprolol	30	20	232	2.3	0.195
Bisoprolol	30	40	225	3.2	0.098
Oxprenolol	30	40	224	2.9	0.098

Table 2
Results obtained from validation assays

Drug	Theoretical concentration (μg ml^{-1})	Intra-day RSD (%)	Inter-day (%)	Linear regression curve	r
Celiprolol	25.00	1.07	0.01	$y = 0.644x - 0.228$	0.9999
	6.25	2.48	0.43		
	1.56	2.42	1.47		
Bisoprolol	25.00	1.05	0.02	$y = 0.642x - 0.008$	0.9999
	6.25	1.93	0.40		
	1.56	1.29	1.18		
Oxprenolol	25.00	1.35	0.01	$y = 0.548x - 0.176$	0.9999
	6.25	1.04	0.30		
	1.56	0.74	0.99		

3. Results and discussion

3.1. Chromatograms

Fig. 2a–c shows representative chromatograms at 12.5 μg ml^{-1}. Chromatographic conditions of the active principles are shown in Table 1. They were resolved and quantified satisfactorily by these reversed-phase HPLC methods and their retention times were 2.3, 3.2 and 2.9 min for celiprolol, bisoprolol and oxprenolol respectively.

A minimum signal-to-noise ratio of 5:1 was obtained with the lowest concentrations, allowing a quanititation limit of 0.195 μg ml^{-1} for celiprolol and 0.098 μg ml^{-1} for bisoprolol and oxprenolol. Thus, the limit of quantitation used (0.78 μg ml^{-1}) is higher than the absolute limit of the assays. As can be seen the injection volume is double for bisoprolol and oxprenolol relative to celiprolol.

3.2. Validation

The results obtained in the validation assay procedure are summarised in Table 2. In linearity assay, the response factors expressed by the percentage of the RSD were 4.3, 2.49 and 4.1% for celiprolol, bisoprolol and oxprenolol respectively. The regression equations obtained by unweighted least-squares linear regression are represented by $y = ax + b$ where y is peak area and x is concentration. Good linearity between the peak area and concentration was observed for all active principles with correlation coefficients of 0.9999. Maximum RSD values in the repeatability and reproducibility assays were respectively 2.48 and 1.47% for celiprolol, 1.93 and 1.18% for bisoprolol and 1.35 and 0.99% for oxprenolol. Accuracy expressed as the percentage of the mean recovery was confirmed after application of Student's t-test. No significant differences ($P > 0.05$) appeared between the mean recovery and 100% in any of the active principles.

3.3. Analytical error procedure

Coefficient values of the general equation obtained by polynomial regression fitting are shown in Tables 3–5 for celiprolol, bisoprolol and oxprenolol respectively. In order to discriminate the function that best fits the experimental data, the corresponding statistical study was performed. The results obtained are shown in Tables 6–8 for celiprolol, bisoprolol and oxprenolol respectively. From these Tables the most probable function for each active principle can be selected. The selection was made by the Stepwise procedure [18] which permitted us to discriminate the best fitting when the differences among functions in the coefficient of correlation, F, standard error of estimate and level of probability values did not differ significantly. Taking into account these results, the analytical error functions chosen are the following (Fig. 3a–c): SD (μg ml^{-1}) $= 0.0295 + 0.0124C - 3.88 \times 10^{-4}C^2$ for celiprolol; $0.0199 + 0.011C - 1.27 \times 10^{-5}C^3$ for bisoprolol; and $0.0183 + 0.0089C - 9.68 \times 10^{-6}C^3$ for oxprenolol.

Table 3
Coefficient values of the equations from polynomial regression fitting for celiprolol

Variable regression	A_0	A_1	A_2	A_3
C	0.0573	0.0024		
C^2	0.0680		6.49×10^{-5}	
C^3	0.0710			2.05×10^{-6}
C, C^2	0.0295	0.0124	-3.88×10^{-4}	
C, C^3	0.0353	0.0087		-1.01×10^{-5}
C^2, C^3	0.0509		8.80×10^{-4}	-3.23×10^{-5}
C, C^2, C^3	0.0182	0.0202	-0.0013	2.45×10^{-5}

Table 4
Coefficient values of the equations from polynomial regression fitting for bisoprolol

Variable regression	A_0	A_1	A_2	A_3
C	0.0477	0.0029		
C^2	0.0607		8.01×10^{-5}	
C^3	0.0645			2.46×10^{-6}
C,C^2	0.0144	0.0148	-4.64×10^{-4}	
C,C^3	0.0199	0.011		-1.27×10^{-5}
C^2, C^3	0.0371		0.0012	-4.45×10^{-5}
C, C^2, C^3	0.0266	0.0064	5.11×10^{-4}	-2.64×10^{-5}

Table 5
Coefficient values of the equations from polynomial regression fitting for oxprenolol

Variable regression	A_0	A_1	A_2	A_3
C	0.0394	0.0028		
C^2	0.0510		8.14×10^{-5}	
C^3	0.0545			2.64×10^{-6}
C, C^2	0.0142	0.0118	-3.51×10^{-4}	
C, C^3	0.0183	0.0089		-9.68×10^{-6}
C^2, C^3	0.0321		9.82×10^{-4}	-3.57×10^{-5}
C, C^2, C^3	0.0250	0.0044	5.08×10^{-4}	-2.33×10^{-5}

Table 6
Polynomial statistical analysis of the analytical error function for celiprolol

Variable regression	r^2	SE	F	P
C	0.2487	0.0364	11.256	1.96×10^{-3}
C^2	0.1269	0.0393	4.942	0.0330
C^3	0.0820	0.0403	3.036	0.0905
C, C^2	0.4902	0.0296	17.830	5.62×10^{-6}
C, C^3	0.4709	0.0301	16.574	1.04×10^{-5}
C^2, C^3	0.3953	0.0322	12.440	9.42×10^{-5}
C, C^2, C^3	0.4940	0.0295	12.390	1.53×10^{-5}

As can be seen, the analytical errors do not fit any pattern foreseen a priori, but, rather, they can be described by several different functions: linear and non-linear functions. For example, the errors corresponding to celiprolol, bisoprolol and oxprenolol are described by non-linear functions. This variety of functions applicable to the description of errors is found in spite of the fact that the chemistry of all three active principles is based on the aryloxypropanolamine structure (Fig. 1). In addition, they were quantified with the same analytical technique (reversed-phase HPLC). Moreover, considering celiprolol and bisoprolol together or celiprolol and oxprenolol together, their analytical methods used the same mobile phase but different injection volumes and wavelengths of UV detection (Table 1). Finally, the analytical methods used for bisoprolol and oxprenolol were very similar since both of them

have equal mobile phase and injection volume and their wavelengths are practically the same and, only in this case, both of them have the same pattern of non-linear function. From the above, it appears clear that it is necessary to determine the analytical error function of each active principle individually.

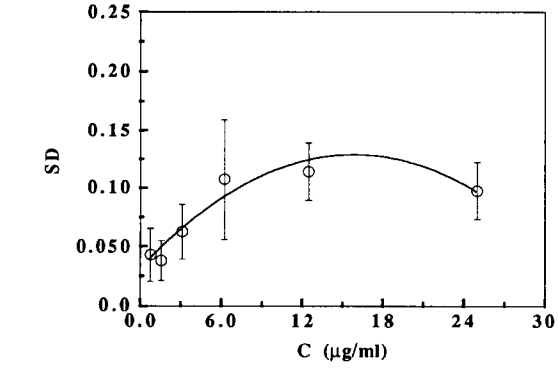
One of the main applications of the analytical error function as a possible weighting method is in the pharmacokinetic data parametric estimation [19–21]. In this case, the sources of the total error known can come from an inappropiate design of the study with incorrect sampling times, which can be prevented before starting experiments, also, from incorrect specifications of the kinetic model used, which may be reduced by performing a statistical discrimination of alternative models [22], and finally from the quantification of active principle concentrations studied [23], which can be minimized with the analytical error function determined in the way described in the present work.

Table 7
Polynomial statistical analysis of the analytical error function for bisoprolol

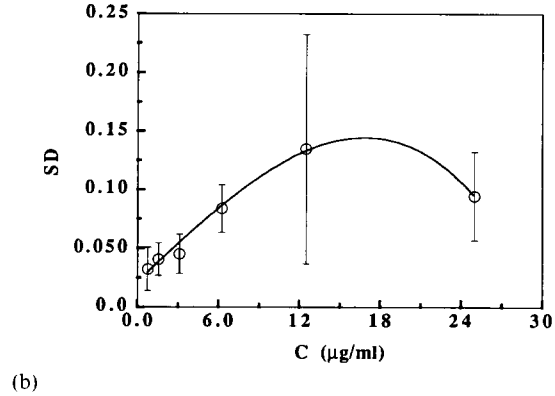
Variable regression	r^2	SE	F	P
C	0.2068	0.0502	8.8630	5.33×10^{-3}
C^2	0.1072	0.0533	4.0820	0.0513
C^3	0.0654	0.0545	2.3800	0.1321
C, C^2	0.3870	0.0435	12.0480	1.18×10^{-4}
C, C^3	0.3959	0.0432	12.4710	9.25×10^{-5}
C^2, C^3	0.3928	0.0433	12.3220	1.01×10^{-4}
C, C^2, C^3	0.3805	0.0437	8.1660	3.54×10^{-4}

Table 8
Polynomial statistical analysis of the analytical error function for exprenolol

Variable regression	r^2	SE	F	P
C	0.4127	0.0292	19.674	1.30×10^{-4}
C^2	0.2449	0.0331	9.083	5.43×10^{-3}
C^3	0.1671	0.0348	5.617	0.0249
C, C^2	0.6630	0.0217	29.526	1.60×10^{-7}
C, C^3	0.6770	0.0213	31.393	9.02×10^{-8}
C^2, C^3	0.6776	0.0213	31.481	8.78×10^{-8}
C, C^2, C^3	0.6723	0.0214	20.828	4.34×10^{-7}



(a)



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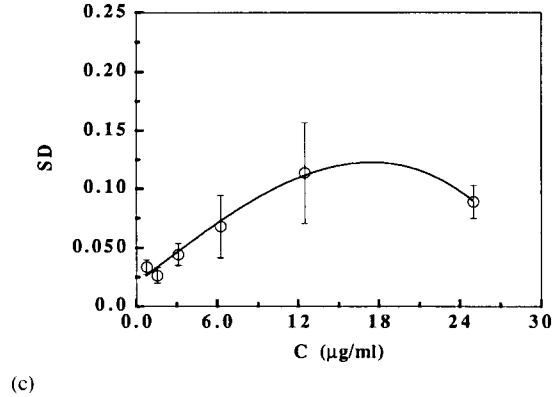

(c)

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

In our study of the analytical error function, the posible effect of the active principle extraction process from a biological matrix in which it is

included was not taken into account, and this effect should be considered in a possible pharmacokinetic data parametric estimation.

4. Conclusions

Results proved that these analytical methods have acceptable precision, accuracy and linearity between the peak area and concentration. In none of these methods did any of the RSDs surpass the maxima permitted of 5, 3 and 5% for linearity, repeatability and reproducibility assays respectively. Moreover, these methods allow the quantification of a large number of samples daily, since a single mobile phase and a type of reversed-phase column are used for the determination of all three active principles.

The error function for each validated analytical method has been determined by a rapid, simple and economical procedure. The analytical error function established for each active principle allows one first to determine the variance associated with a concentration value within the working calibration curve range and second to use it as a heteroscedastic weighting method (1/variance) for the parameter estimation.

Independent of other errors such as incorrect model specifications, inappropiate experimental designs and the uncertainty (Stochastic control), the use of this weighting method may lead to a better quantification of the active principles since it explains, at least, a part of the total error produced in parameter estimation.

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