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Determination of atenolol by the micelle-stabilized room-temperature phosphorescence methodology

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ABSTRACT: A micellar-stabilized room-temperature phosphorescence (MS–RTP) method for the determination of atenolol has been developed in micellar solutions of sodium dodecylsulphate (SDS) in the presence of thallium(I) as a heavy atom and sodium sulphite as an oxygen scavenger. The effects of thallium(I) nitrate, SDS and sodium sulphite concentrations on atenolol MS–RTP intensity were studied. Optimized conditions to obtain maximum sensitivity were 0.015 mol/L thallium(I) nitrate, 0.1 mol/L SDS and 0.0075 mol/L sodium sulphite. The maximum phosphorescence signal was completely developed in 10 min and the intensity was measured at $\lambda_{ex} = 272$ nm and $\lambda_{em} = 412$ nm. The linear range of application obtained was 2.01–16.00 μg/mL. The detection limit estimated from the least-squares regression analysis was 0.86 μg/mL and the relative standard deviation of 10 replicates was 1.7%. The proposed method was applied to the determination of atenolol in a pharmaceutical formulation. The quantitation was carried out by means of standard calibration, standard-additions calibration and Youden calibration. These three experiments were necessary to evaluate the presence of constant and proportional errors due to the matrix. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: atenolol; phosphorimetry; room-temperature phosphorescence; micelle; matrix effect

INTRODUCTION

Atenolol, designed chemically as 4-(2-hydroxy-3-isopropylaminopropoxy) phenylacetamide (Fig. 1), is a β-adrenaceptor antagonist commonly known as a β-blocker (1). β-Blocking drugs affect the heart and circulatory system (arteries and veins). Atenolol is used to lower blood pressure and heart rate, to reduce chest pain (angina pectoris) and to reduce the risk of recurrent heart attacks.

Different analytical methods have been developed for the quantitative determination of this drug in dosage forms. Most of them make use of chromatographic techniques (2–15); others, in a lesser quantity, use spectrophotometry (16–22), fluorometry (23, 24) and capillary electrophoresis (25–28) and some electrochemical sensors have also been developed (29–32). The *United States Pharmacopeia*, USP 28 (33), describes a reversed-phase HPLC method based on UV detection for its determination. In the analytical literature reviewed for atenolol, no references were found describing any phosphorimetric technique for the quantitation of this β-blocker.

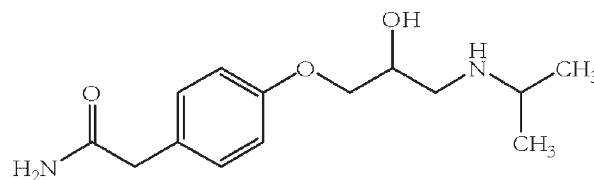

Figure 1. Chemical structure of atenolol.

The phosphorescence of different organic compounds has been normally observed at cryogenic temperatures (77K) and at room temperature using adequate solid supports (solid-phase phosphorescence, SPP) (34–39), and in some instances analytes could be made to phosphoresce in solution at room temperature when the non-radiative pathways were minimized (room-temperature phosphorescence in the liquid state, RTPL) (38, 40, 41). The addition of a heavy atom perturber is necessary to achieve room-temperature phosphorescence. The heavy atom produces an effective S_1–T intersystem crossing with a subsequent enhancement of the phosphorescence emission.

Micellar-stabilized room-temperature phosphorescence (MS–RTP) is an interesting manifestation of the external heavy-atom effect (42). Basically, MS–RTP achieves its effect by utilizing the amphiphilic character of micellized surfactant ions to compartmentalize the solubilized luminophore molecules in a submicroscopic

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region surrounded by a high concentration of heavy-atom counter-ions that favours the intersystem crossing in the luminophore. As in traditional phosphorimetry, the problem associated with oxygen quenching also remains. Deoxygenation by sulphite has become a successful method (43).

In this paper, a MS–RTP method for the determination of atenolol is described. The method is based on the RTP of atenolol in aqueous sodium dodecylsulphate (SDS) using thallium(I) as an external heavy atom. The procedure is very simple and its applicability has been demonstrated in a pharmaceutical formulation.

The sample has analytical problems, due to the complex and non-reproducible matrix. Sample components different from the analyte, called the matrix, may be the cause of a disturbance that affects either the measurement system or the generated analytical signal. The detection and the correction of constant and proportional systematic errors have been extensively studied (44–46). These studies require the establishment of a standard-added curve (by application of the standard additions method) to avoid proportional errors and the measurement of the true sample 'blank' from a Youden curve to evaluate the constant errors.

MATERIALS AND METHODS

Apparatus

All recordings of uncorrected phosphorescence spectra and phosphorimetric measurements were carried out on a Perkin-Elmer LS-50B luminescence spectrometer equipped with a pulsed xenon lamp (10 μs half-width, 60 Hz), an R 928 photomultiplier tube and a computer working with FL Winlab software. All the measurements took place in a standard 10 mm path-length quartz cell with band path of 5 and 20 nm for the excitation and emission monochromators, respectively. A gate time of 3 ms and a delay time of 0.02 ms were used throughout.

Reagents

Thallium(I) nitrate (Alfa Inorganics Ventron, Beverly, MA, USA) was recrystallized three times from water and dried under vacuum over silica gel. As thallium(I) salts are very toxic, the analyst must take precautions.

Sodium dodecyl sulphate (SDS) 99% was supplied by Sigma (St. Louis, MO, USA) and sodium sulphite by Merck (Darmstadt, Germany). These chemicals were used as received. Atenolol was supplied by IPCA Laboratories Ltd. (India); the content was determined by the USP 28 method and was found to be 99.6%. A dosage form containing atenolol was purchased from a local market. All solutions were prepared with double-distilled water. A working standard solution of 120 μg/mL atenolol (60.0 mg dissolved in 500 mL water) was freshly prepared. A stock standard solution of 0.15 mol/L thallium(I) nitrate was used. Stock standard solutions of 0.5 mol/L SDS and 0.075 mol/L sodium sulphite were prepared daily.

General procedure

A suitable aliquot of the atenolol standard solution was transferred to a 10.0 mL volumetric flask. Then 2 mL 0.5 mol/L SDS, 1 mL 0.15 mol/L thallium(I) nitrate and 1 mL 0.075 mol/L sodium sulphite were successively added and made up to volume with water. Final concentrations obtained were 0.1 mol/L SDS, 0.015 mol/L thallium(I) nitrate and 0.0075 mol/L sodium sulphite. After a thorough mixing, the flask was kept in a water-bath at 20.0 ± 0.5°C for 10 min. A portion of this solution was transferred to the quartz cell, the spectrum was recorded and the relative phosphorescence intensity was measured at wavelength of 412 nm with an excitation wavelength of 272 nm. A reagent blank lacking atenolol was prepared and measured following the same procedure.

Procedure for tablets

Ten tablets were weighed in order to find the average mass of each tablet. Then the contents were powdered and homogenized; 100 mL water were added to a quantity equivalent to 100 mg atenolol. The solution was sonicated for 20 min and filtered through a 0.22 μm nylon membrane. The filtrate was placed into a 500.0 mL volumetric flask and diluted with water. Suitable aliquots of this solution were taken throughout the analysis.

RESULTS AND DISCUSSION

Spectral characteristics

Figure 2 shows the MS–RTP excitation and emission spectra of atenolol in SDS micellar media using thallium(I) as heavy atom perturber and sodium sulphite as chemical deoxygenator. The MS–RTP spectrum of a blank solution prepared in the absence of atenolol was obtained under the same conditions. The excitation spectrum of atenolol corresponds to the transition from the ground singlet state to the first singlet excited state (1). In preliminary studies the emission spectrum of atenolol was recorded with an excitation wavelength of 224 nm that corresponds to the transition from the ground singlet state to the second singlet excited state. Although this transition is more intense than that finally selected, it is not adequate because of the great signal arising from the blank.

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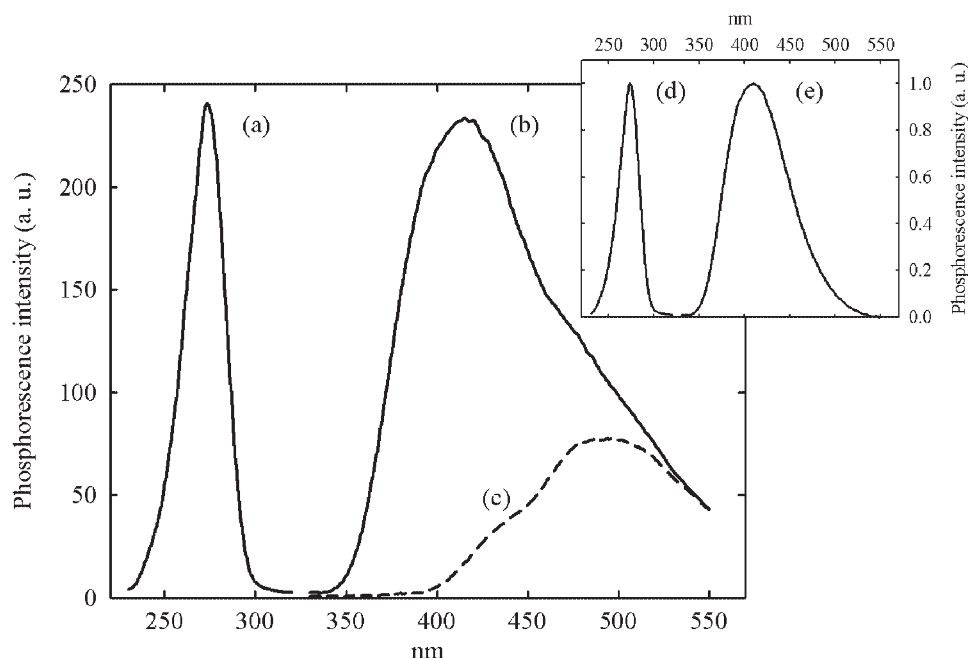

Figure 2. MS–RTP spectra. (a) Excitation spectrum of atenolol (λ_{em} = 412 nm); (b, c) emission spectra (λ_{ex} = 272 nm) of atenolol and blank solution, respectively; (d) normalized excitation and (e) difference emission spectra of atenolol. Conditions: atenolol, 22.40 µg/mL (8.4×10^{-5} mol/L); Na_2SO_3, 0.0052 mol/L; SDS, 0.1 mol/L; thallium(I), 0.016 mol/L.

Optimization of experimental variables

Some experimental variables were investigated to establish the optimum conditions for the analysis. The variables studied were thallium(I) nitrate, SDS and sodium sulphite concentrations.

As thallium(I) produces an effective spin orbital coupling that favours the intersystem crossing between singlet and triplet states producing the phosphorescence emission, it is required to know the optimal concentration to be used. The effect of thallium(I) concentration on the MS–RTP signal was examined in the range 0.000–0.030 mol/L. The results are shown in Fig. 3. The emission increased with increasing thallium(I) concentration up to 0.015 mol/L and decreased above this concentration. Therefore, 0.015 mol/L thallium(I) was selected for the present study.

The effect of SDS concentration was investigated by preparing samples with SDS concentrations in the range 0.00–0.25 mol/L. As Fig. 4 shows, the phosphorescence intensity of atenolol increased as SDS concentration increased and reached the maximum value at 0.10 mol/L, decreasing slowly above this concentration. This is about 12 times the SDS critical micellar concentration, 8.1×10^{-3} mol/L (47). 0.10 mol/L SDS was selected for the subsequent work.

The behaviour of sodium sulphite as deoxygenation scavenger selected is illustrated in Fig. 5. As the concentration of sodium sulphite increased, a steep increment was observed until the concentration reached

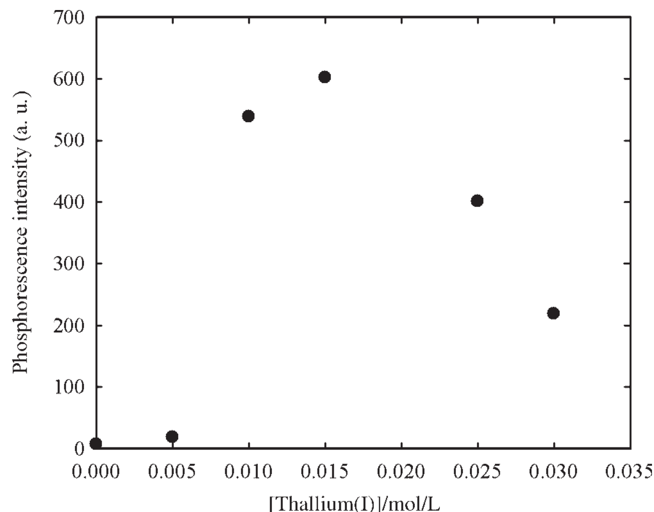

Figure 3. Effect of thallium(I) nitrate concentration on the MS–RTP signal intensity. Conditions: atenolol, 25.49 µg/mL (9.6×10^{-5} mol/L); Na_2SO_3, 0.0050 mol/L; SDS, 0.1 mol/L.

0.0075 mol/L. This concentration was chosen as the optimal value. The decrease in the signal for higher concentrations of the scavenger was produced by the displacement of thallium(I) from the micelle because of the high concentration of sodium in the solution (43). The system under study did not require buffering the solutions because MS–RTP intensities were higher when the pH was about 5.0–6.5.

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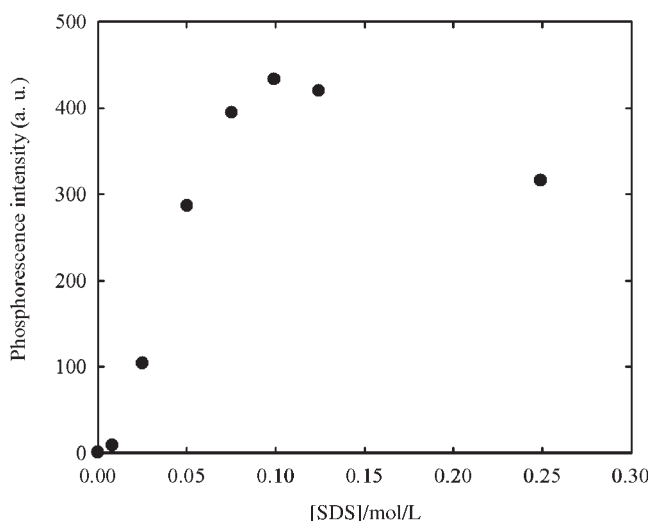

Figure 4. Variation of phosphorescence intensity of atenolol with SDS concentration. Conditions: atenolol, 25.49 µg/mL (9.6×10^{-5} mol/L); Na_2SO_3, 0.0075 mol/L; thallium(I), 0.015 mol/L.

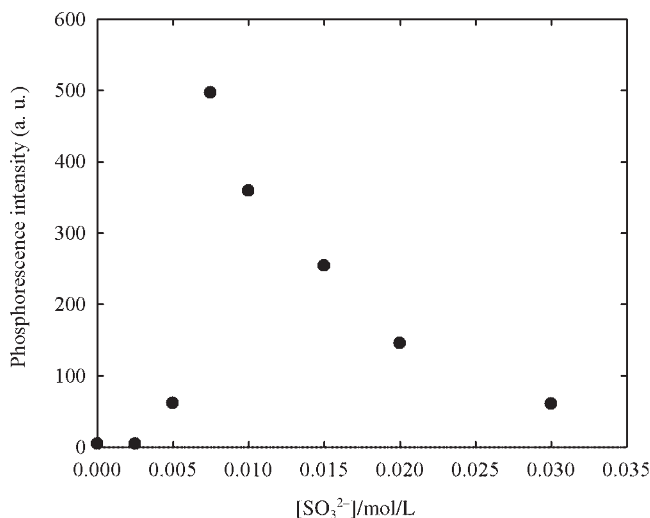

Figure 5. Behaviour of phosphorescence intensity of atenolol with respect to sodium sulphite concentration. Conditions: atenolol, 25.49 µg/mL (9.6×10^{-5} mol/L); thallium(I), 0.015 mol/L; SDS, 0.1 mol/L.

Under the optimal operating conditions outlined above, the maximum phosphorescence signal appeared after 10 min and remained stable for at least 60 min.

Interfering effects

In order to assess the possibility of analytical application of the method, the effects of some common excipients present in the studied formulation were investigated. The specificity of this determination was evaluated by adding some compounds used in atenolol processing (magnesium stearate, dioctyl sodium sulphosuccinate and corn starch) and tested to see whether the added

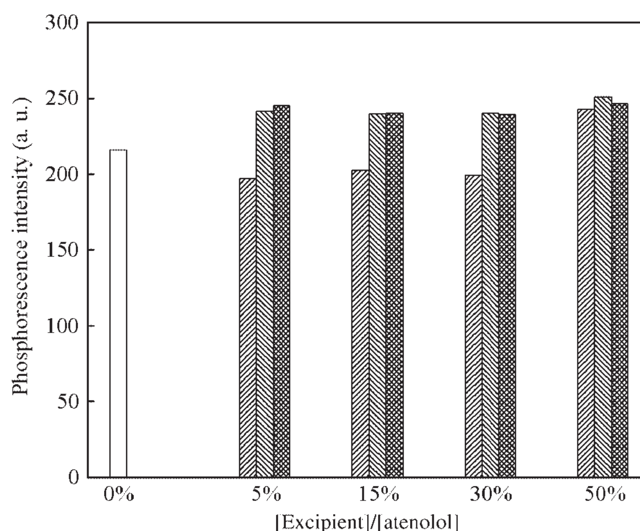

Figure 6. Influence of some commercial excipients on the phosphorescence intensity of atenolol (□), magnesium stearate (▨), dioctyl sodium sulphosuccinate (▧) and corn starch (▩). Conditions: atenolol, 8.0 µg/mL (3×10^{-5} mol/L); [excipient]/[atenolol], w/w.

excipients affected the MS–RTP signal of atenolol. Figure 6 shows the results obtained. The signal suffered the same increment (ca. 16%) when dioctyl sodium sulphosuccinate and corn starch were tested in different proportions with respect to atenolol. A slight decrease (ca. 6%) was observed for low levels of magnesium stearate and a subsequent increment (ca. 16%) for the highest level studied.

Analytical characteristics

The introduction of a novel analytical method must be supported by consistent information about its quantitative potentialities; this is critical for whoever considers its utilization for a specific application (48). The analytical figures of merit evaluated for the proposed method were the detection and quantitation limits, the linear range and the repeatability. All this information were deduced from the results of a calibration graph with several replicates at each analyte level.

The optimized procedure was applied to adequate aliquots of the 120 µg/mL atenolol standard solution. The final concentrations of the standards (C_i) subjected to the analysis were in the range 0.53–26.63 µg/mL. The response standard deviations at each analyte level (s_i) were estimated from the replicate values of the phosphorescence intensity (P_i); it was verified graphically that s_i shows uniformity and the homocedasticity of the data was confirmed by means of the Cochran's test (49). The fit of P_i to C_i was therefore carried out by means of least-squares regression analysis.

The linear range of the calibration plot was estimated according to the *F*-test for the lack of fit (49), at a 0.05

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Table 1. Results of the least-squares regression of phosphorescence intensity (P_i/a.u.) against atenolol concentration (C_i/µg/mL), upper limit of the linear range, detection limit and quantitation limit

						LD[f]		
n[a]	Intercept ± s[b]	Slope ± s[b]	$s^{c}_{P/C}$	r[d]	UL[e]	LSR[g]	CA[h]	LQ[i]
50	12.612 ± 1.237	24.495 ± 0.235	6.182	0.9977	16.00	0.86	0.76	2.01

[a]Number of calibration data points.
[b]Standard deviation.
[c]Residual standard deviation.
[d]Correlation coefficient.
[e]Upper limit of the linear range.
[f]Detection limit, calculated from the:
[g]Least squares regression analysis, calculated from the:
[h]Classic approach.
[i]Quantitation limit.

significance level. The results of the regression analysis through points below the upper limit of the linear range are gathered in Table 1.

The detection limit was estimated from the prediction bands calculated in the least-squares regression analysis, following calculation procedures given by Zorn and co-workers (50). Its value is compared in Table 1 with that obtained by the classical approach, in which the limit of detection is defined as the analyte concentration that gives a signal equal to the blank signal plus three standard deviations of the blank (51). No significant difference between both values could be detected by means of a t-test, at a 0.05 significance level. The quantitation limit was calculated as the analyte level at which the relative standard deviation (RSD = s_i/P_i) is 0.10 (48).

In order to study the precision of the method, 10 aliquots of the same standard, containing 5.11 µg/mL atenolol, were separately treated according to the optimized procedure and measured on the same day; a RSD of 1.7% was obtained.

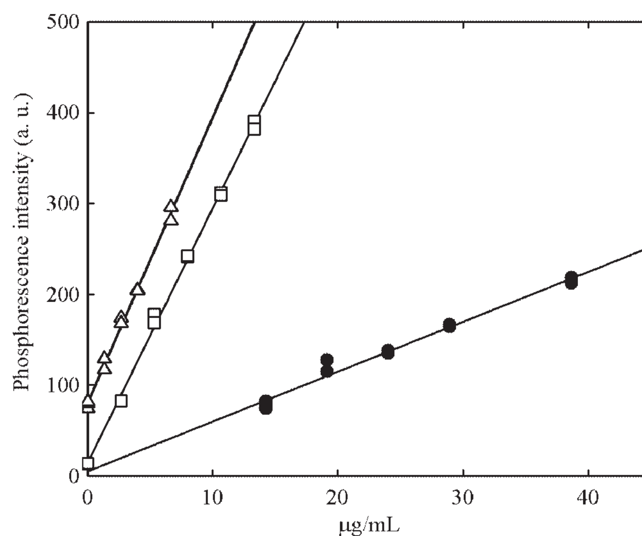

Figure 7. Calibration graphs: (□) standard; (△) standard additions and (●) Youden plot (µg/mL refers to the amounts of atenolol standard, atenolol added and sample per mL solution, respectively).

Analytical application

To investigate the applicability of the proposed method to real samples, we analysed atenolol in tablets. As Fig. 6 shows, the MS–RTP signal of atenolol was affected by some compounds used in tablet processing. Therefore, calculation methods that enable correction for matrix effects, such as those proposed by Cardone (44) and Castells *et al.* (45), must be used for the quantitative evaluation of atenolol in pharmaceutical formulations. Three calibration experiments, standard calibration, standard-additions calibration and Youden calibration, are required to obtain the data set necessary to carry out the corresponding statistical protocol (Fig. 7). The calibration samples were analyzed according to the optimized analytical procedure.

The first calibration experiment was to obtain the standard calibration plot. Adequate aliquots of the atenolol standard solution were transferred to a series of 10.0 mL calibrated flasks in order to attain a final concentration range of 0.00–13.32 µg/mL. Table 2 shows the statistical parameters obtained after applying least-squares linear regression analysis to this dataset.

The extrapolation to zero amount of response measured in the presence of finite amounts of analyte is an estimate of the *standard blank* or *reactive blank*. In our study, the intercept on the ordinate was significantly different from zero, indicating that a constant error, independent of the analyte amount, contributed to the analytical signal.

Contents of atenolol in the formulation were roughly estimated by means of the corresponding standard calibration plot. Then, the standard additions calibration was applied. Equal volumes of the sample solution were spiked with the atenolol standard solution at 0%, 50%, 100%, 150% and 250% of the expected level. Linear least-squares regression of analytical signal vs. amount of atenolol added was established (Table 2). The slopes of the standard calibration line and the standard

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Table 2. Results of the least-squares regression analysis of the three calibration graphs

Type of calibration	n^a	Intercept ± s^b	Slope ± s^b	$s_{P/C}^c$	r^d
Standard	12	14.730 ± 3.256	28.041 ± 0.404	6.363	0.9990
Standard additions	12	81.186 ± 2.462	31.400 ± 0.725	5.926	0.9973
Youden	12	5.491 ± 5.590	5.488 ± 0.226	6.761	0.9916

[a]Number of calibration data points.
[b]Standard deviation.
[c]Residual standard deviation.
[d]Correlation coefficient.

additions line were compared by means of a *t*-test. The pharmaceutical formulation showed significant differences with the standards, indicating the presence of an analyte/matrix interactional effect.

The Youden calibration plot was constructed with increasing amounts of sample. Therefore, five different aliquots of the ground tablets solution were treated according to the optimized procedure. The Youden plot is a representation of signal response against amount of sample. The intercept on the ordinate of the Youden plot is known as the *total Youden blank* and estimates constant errors, such as those associated with the reactive blank and those arising from the presence of the matrix. The difference between this intercept and that of the standard plot is called the *Youden blank* and indicates a bias exclusively due to sample matrix effect. As both plots were obtained from different independent variables, it was not possible to compare their corresponding intercepts by means of a statistical test. In our study, the existence of the above-mentioned difference was assumed because the value of the Youden calibration intercept was not included within the confidence interval value of the standard calibration intercept (46).

The content of atenolol was calculated from the ratio of the difference between the ordinate intercepts of the standard additions and the Youden plots to the slope of the standard additions plot. The assay result was 22.6 ± 0.4 mg atenolol/tablet. This value agreed with the nominal content (25 mg/tablet) and with that obtained by the official method of the USP 28 (21.3 mg/tablet) (33).

CONCLUSION

A new and highly sensitive method has been developed for the determination of atenolol. The developed method is suitable for the routine assay and quality control of pharmaceuticals. Furthermore, this method is simpler and less time consuming than the proposed HPLC official method.

Matrix effects are overcome by means of a careful quantitative evaluation. A Youden calibration and a standard additions calibration, together with the standard calibration, are necessary to take into account the constant and proportional errors associated with the presence of matrix.

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