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Quality control of pharmaceuticals containing clenbuterol by thermal lens spectrometry[1]

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Abstract

An ultrasensitive absorptiometric procedure for the determination of clenbuterol in pharmaceutical preparations was developed. Clenbuterol was diazotized with nitrite and coupled with 1-(naphthyl)ethylenediamine, and the absorbance of the azo dye formed was measured by both spectrophotometry and ultrasensitive thermal lens spectrometry (TLS). The TLS limit of detection was 1.5 ppb, 14-fold lower than with a Hewlett-Packard diode array spectrophotometer. Thus, the TLS procedure can be advantageously applied to quality control of clenbuterol at the individual dose level and in small samples. Repeatability as relative standard deviation was 1.5% (50 ppb, $n = 6$).

Keywords: Clenbuterol; Diazotization and coupling: *N*-(naphthyl)ethylenediamine; Thermal lens spectrometry

1. Introduction

Clenbuterol (CB) is applied in the treatment of pulmonary diseases and tocolysis, and is also frequently used as a β-agonist to illegally promote cattle growth [1,2]. Thus, many procedures to detect CB in the urine, eye and liver of meat-producing animals have been described. Screening of cattle urine and tissues is normally based on enzyme immunoassay [3,4], and confirmation is usually performed by gas chromatography–mass spectrometry [5–7]. Liquid chromatography with amperometric [8], spectrophotometric [9,10], or mass spectrometric detection [11] has also been described.

Spectrophotometric procedures are inadequate for detecting CB in cattle owing to the low sensitivity, but they can be useful in the quality control of pharmaceuticals. Owing to its high activity, CB is used at much lower doses than other bronchodilator epinephrine derivatives, e.g. 2–5 mg dose^{-1} for terbutaline and salbutamol but 0.02 mg dose^{-1} for CB. An excessive dose produces tachicardia, hypertension and cephalea, thus sensitive procedures for the quality control of the CB preparations are required.

The direct spectrophotometric determination of CB in the UV [12,13] lacks sensitivity. Both sensitivity and selectivity can be improved by coupling

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with *N,N*-dimethylaniline [9], and by post-column diazotization with nitrite and coupling with *N*-(1-naphthyl)ethylenediamine (NED) [10]. However, owing to the low doses used in the preparations, a large amount of sample (e.g. several tablets) is required. As shown in this work, the limit of detection of absorptiometric procedures can be further lowered by ultrasensitive thermal lens spectrometry (TLS) [14]. In water and other polar solvents the TLS limits of detection are five- to 50-fold lower than in conventional spectrophotometry, the dynamic range being about two decades [14,15].

To reach a very low limit of detection with TLS the following conditions should be met: (a) high molar absorptivity of the chromophore at the wavelength of the pump radiation, e.g. at 488–514.5 nm with an Ar^+ laser beam, which is most frequently used for pumping TLS experiments; (b) very large stability of the chromophore, i.e. irreversibility of the chromogenic reaction and resistance of the dye to hydrolysis; (c) low absorbance of the blank solution. As shown with other arylamines [16], the CB–NED azo dye meets the two former conditions, and the extracts of the preparations show some scattering but a negligible absorbance in the visible region. Scattering on suspended particles can give a high blank in spectrophotometry but TLS is blind to scattering [17]. This lead to a TLS limit of detection which was 14-fold lower than in spectrophotometry. Therefore, the procedure can be applied to quality control of pharmaceuticals at the dose level.

2. Experimental

2.1. Reagents

Analytical-grade clenbuterol hydrochloride (Sigma, Barcelona, Spain), sodium nitrite, sulphamic acid (Probus, Barcelona), *N*-(1-naphthyl)ethylenediamine dihydrochloride, sodium dodecyl sulphate (SDS) (Merck, Darmstadt, Germany) and HCl (Panreac, Barcelona) were used. Distilled and nanopure deionized water (Barnstead deionizer, Sybron, Boston, MA) was also used.

A 0.1 M HCl and 0.015 M SDS solution was prepared and stored for use as needed. A 100 ppm CB stock solution in the same HCl–SDS medium was prepared and stored at 4°C. For the spectrophotometric determination, the reagent concentrations were: 0.2 M sodium nitrite, 0.5 M sulphamic acid and 1×10^{-3} M NED. For the TLS determination the concentrations were: 0.01 M sodium nitrite, 0.025 M sulphamic acid and 1.25×10^{-5} M NED. The optimized procedures were applied to the Spiropent preparation (Europharma, Madrid, Spain) which contains 0.02 mg CB hydrochloride declared, lactose and other excipients per tablet.

2.2. Apparatus

The TLS setup with an Ar^+/He–Ne coaxial pump-probe configuration has been described elsewhere [18,19] (see scheme in Fig. 1). The 514.5 nm line of the Ar^+ laser with 100 mW pump power, modulated at 4 Hz, was used. A Hewlett-Packard 8452A diode array spectrophotometer and 1 cm optical path cells were also used.

2.3. Derivatization and measurement procedure

A CB standard solution or a sample aliquot is mixed with HCl–SDS solution to a volume of 15 ml in a 25 ml volumetric flask, 1 ml sodium nitrite

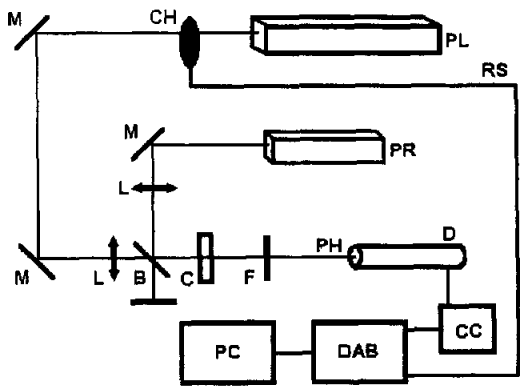

Fig. 1. Scheme of the dual-laser pump-probe coaxial thermal lens spectrometer: PL, pump laser head; CH, chopper; M, mirror; PR, probe laser head; RS, reference signal; CC, signal conditioning circuit; DAB, data acquisition board; PC, computer; L, lens; B, beamsplitter; C, cell; F, filter; PH, slit; D, photodiode detector.

solution is added and the mixture is allowed to react for 5 min; 1 ml sulphamic acid solution is added and after 10 min 2 ml of NED solution is also added. The volume is made up to 25 ml with HCl–SDS solution. Aliquots are taken with a syringe, filtered by pressing through a 0.22 μm nylon filter (MSI, Westboro, MA), and the absorbance at 506 nm or the TLS signal at 514.5 nm is measured.

2.4. Preparation of the sample and blank solutions

For the spectrophotometric procedure, four tablets of Spiropent were ground in a mortar, extracted three times with the HCl–SDS solution, the extracts were separated by centrifugation, the combined volume was made up to 25 ml with HCl–SDS solution, and the derivatization and measurement procedure above was applied. An internal blank was prepared by adding the sample after the addition of NED. At this stage of the procedure nitrite is not present so the CB is not derivatized. The absorbance of the internal blank was subtracted from that of the sample. Alternatively, background correction was performed by measuring the absorbance of the derivatized CB solutions at 450, 550 and 506 nm, and following the procedure given later in the text.

For the TLS procedure, a single tablet of Spiropent was ground and treated four times with the HCl–SDS solution. The combined centrifuged extracts were added to a 50 ml volumetric flask, 2 ml aliquots were taken, the derivatization procedure given above was applied and the TLS signal was measured. An internal blank prepared by adding the sample after the addition of NED was also used to correct the signal of the sample.

3. Results and discussion

3.1. Spectra and stability of the azo dye

In 0.1 M HCl, the underivatized CB solutions showed two absorption peaks at 246 and 300 nm, with $\varepsilon = 7400$ and 2900 mol^{-1} 1 cm^{-1} (five calibration points) respectively. The spectra of the CB azo dye at several pH values are shown in Fig. 2. The protonated species of the azo dye predominated in 0.1 M HCl, showing a maximum at 506 nm with $\varepsilon = 31\,400$ mol^{-1} 1 cm^{-1} (five calibration points, 2×10^{-6}–2×10^{-5} M range, $r^2 = 0.9999$), which is much higher than the values obtained with underivatized CB. Stability studies performed by measuring the absorbance of the azo dye solutions at 506 nm and at room temperature showed no significant changes within the 1–5 pH range for at least 2 h.

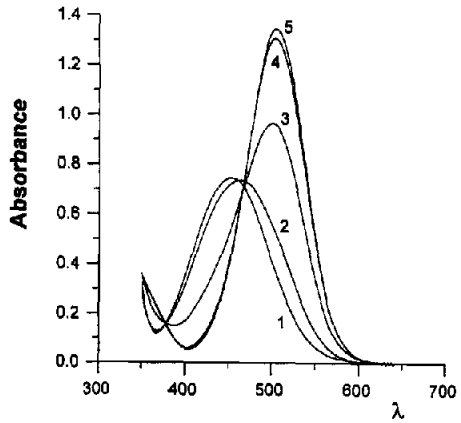

Fig. 2. Spectra of the CB–NED azo dye at several pH values. (1)–(5): pH = 4.9, 3.7, 2.6, 1.5 and 1.0 respectively.

3.2. Spectrophotometric determination

In 0.1 M HCl and at 506 nm, the background noise obtained with 10 blank solutions was $s = \pm 8 \times 10^{-4}$ absorbance units. Using the 3s criterion, a limit of detection of 6×10^{-8} M CB (equivalent to 21 ppb) results, the dynamic range being linear up to 6×10^{-5} M. With Spiropent, background correction was observed to be necessary, otherwise a +10% systematic error was produced. This error was adequately corrected by subtracting the absorbance of an internal blank which was prepared as explained above.

The spectrum of the internal blank showed a continuous decrease of the absorbance within the 450–550 nm range, thus suggesting that the apparent background absorbance was in fact scattering from suspended particles. This also suggested that the spectrum of the azo dye con-

tained the necessary information to correct the background. Thus, an alternative procedure for background correction was developed. As illustrated in Fig. 3, the absorbance of the azo dye solutions was measured at 450, 550 and 506 nm, and the background absorbance at 506 nm was calculated as

$$A_p = 0.44A_{450} + 0.56A_{550}$$

This value was subtracted from the absorbance at 506 nm. The difference in absorbances corresponded to a molar absorptivity of 20 400 mol^{-1} $l\ cm^{-1}$, which corresponded to a limit of detection of 1×10^{-7} M. Using this background correction procedure, three independent samples of Spiropent gave 0.0197 ± 0.0004 mg per tablet.

3.3. TLS determination

In TLS, the use of smaller reagent concentrations than in spectrophotometry usually leads to a reduction of the blank signal and background noise. How much the concentration of the derivatization reagents can be reduced depends on the stoichiometry and thermodynamic constant of the reactions involved. The CB concentration range encountered in TLS is much lower than in spectrophotometry, and so the concentrations of the reagents can be greatly reduced without reaching the limits imposed by the stoichiometry of the reactions. Further, quantitative formation of the azo dye at very low reagent concentrations was assured by the irreversibility of the diazotization and coupling reactions.

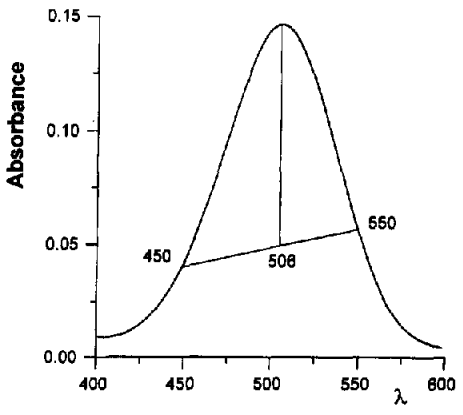

Fig. 3. Scheme for background correction: the weighted average of the absorbances at 450 and 550 nm was subtracted from the absorbance at 506 nm. This spectrum was obtained with a 4.46×10^{-6} M solution of derivatized CB.

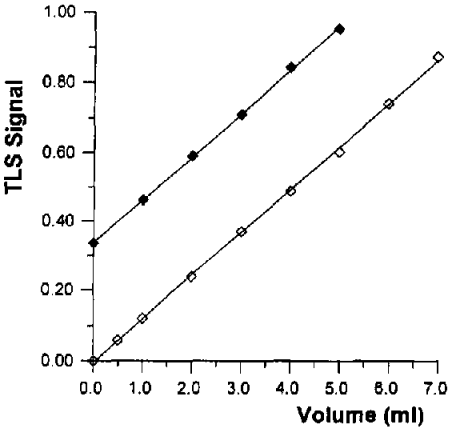

Fig. 4. TLS normal (◇) and standard addition (◆) calibration curves. On the x axis, 1 ml of the standard solution corresponds to 3.6×10^{-8} M CB.

For a CB concentration of 1×10^{-5} M, a reduction of the nitrite, sulphamic acid and NED concentrations to 1/5, 1/25 and 1/100 of the values used in spectrophotometry lead to no reductions in the TLS signal. Thus, the concentrations given in the recommended procedure were chosen (1/20 to 1/80 reduction of the reagent concentrations). The calibration curve was linear at least within the 1×10^{-8}–2.5×10^{-7} M range (seven points, $r^2 = 0.9997$). The limit of detection was 1.5 ppb ($n = 10$ independent blanks), 14-fold lower than with the HP diode array spectrophotometer. Repeatability, given as relative standard deviation, was 1.5% (50 ppb, $n = 6$).

To reveal possible matrix effects, the standard addition method was used. A series of 2 ml aliquots of the sample solution was taken and additions of CB were made. The CB concentration range was 3.5×10^{-8}–1.8×10^{-7} M. The slopes of the standard addition ($n = 6$, $r^2 = 0.9998$) and normal calibration curves were statistically the same (see also Fig. 4). The CB content found in single tablets of Spiropent was 0.0193 ± 0.0005 mg per tablet (mean of three different tablets) by direct calibration, and 0.0195 ± 0.0004 mg per tablet (mean of three

different tablets) by the standard addition method.

It has been shown that the TLS procedure is adequate for the evaluation of the CB content of pharmaceutical preparations below the single dose level and is useful in quality control, including study of the tablet-to-tablet dispersion.

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