X-RAY SPECTROMETRY
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X-RAY Spectrometry

Sensitive determination of terazosin by x-ray fluorescence spectrometry based on the formation of ion-pair associates with zinc thiocyanate

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A new, simple and precise indirect method for the determination of terazosin through x-ray fluorescence (XRF) is proposed. The method is based on the precipitation of an ion-associate complex formed between terazosin and $[Zn(SCN)_4]^{2-}$ and the formation of a thin film on a membrane filter. The optimum conditions for ion-associate complex formation, such as pH, ionic strength, shaking time and stoichiometry of the complex, were studied. The complex was retained on the membrane filter, and the Zn Kα line was measured by XRF. The method presents the advantages of the separation of the drug from the matrix (excipients), the preconcentration of Zn, producing enhancement of XRF intensity though increased sensitivity, and the elimination of the matrix effects due to the thin film obtained. The lowest detection limit was 0.732 µg ml^{-1}. The validation studies were realized by the related applications and the results were evaluated statistically. The method was successfully applied to terazosin determination in tablets or in bulk. Copyright © 2007 John Wiley & Sons, Ltd.

INTRODUCTION

Terazosin hydrochloride dihydrate (THD): 2-[4-(2-tetrahydrofuranylcarbonyl]-1-piperazinyl-6,7-dimethoxy-4-quinazolinamine monohydro-chloride dihydrate (I) (Fig. 1) is a highly selective, potent 1-adrenoreceptor antagonist. It is an effective drug against hypertension[1] and benign prostatic hyperplasia.[2] Terazosin is presented for oral use in tablets containing 2, 5 and 10 mg as terazosin hydrochloride, for which the quality control of the final product and stability studies require high-sensitivity analytical methods.

The methods reported for the analysis of terazosin or similar drugs include different analytical methods and different sample forms such as in bulk, pharmaceuticals or biological fluids before intravenous and oral dosage: spectrophotometry,[3–5] spectrofluorimetry,[6,7] high-performance liquid chromatography (HPLC) with UV detection,[8,9] HPLC with fluorescence detection,[10–16] normal phase HPLC–electrospray mass spectroscopy,[17] stripping voltammetry.[18] Neither an official method nor an x-ray spectrometric determination through the ion-pair formation procedure has been reported for the assay of terazosin.[19–22] The formation of ion-pair complexes between pharmaceutical drugs and metal complexes has been used for the indirect determination of the drugs through atomic absorption spectrometry.[23,24] The main problem of this method is in the accumulation of errors when trying to dissolve the filter containing the sample to obtain the measuring solution.

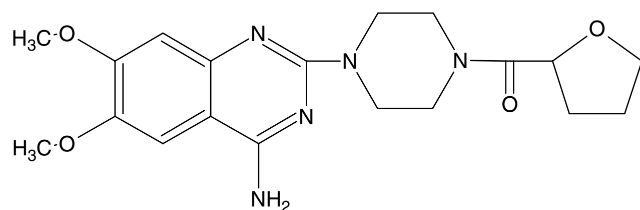

Figure 1. Terazosin formula.

Prior to assay of the drug using the reported methods, tedious sample pretreatment and long extraction procedures are necessary, which include back or direct extraction into an organic layer. Therefore, pharmaceutical quality control of the drug, both of the final products or of bulk, requires sensitive and simple analytical methods for the determination of terazosin without time-consuming pretreatment steps.

The method proposed here consists in the precipitation of the active drug (terazosin) in the form of an ion-associated complex formed with zinc thiocyanate. The solutions containing the insoluble ion-pair complexes are filtrated and retained on a membrane filter. The filter containing the Zn complex is presented as a thin film to the x-ray spectrometer and the Zn Kα line is measured.

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EXPERIMENTAL

Reagents and materials

The standard solution terazosin of 1×10^{-2} mol l^{-1} was prepared by dissolving 423.8 mg of the pure drug in water and diluting it to 100 ml with ultrapure water. Ultrapure water and analytical grade reagents were used to prepare all the solutions. Membrane filters of cellulose acetate and Teflon were from Millipore with 0.45 µm pore size. The terazosin tablets were from Rotiaz (Richmond Lab., 5 mg terazosin/tablet).

The standard solution of $[(SCN)_4]$ Zn 10^{-1} mol l^{-1} was prepared by weighing 1.79 g of $ZnSO_4 \cdot H_2O$ and 0.971 g of KSCN and dissolving in ultrapure water with concentrated HNO_3 and making up to 100 ml.

Apparatus

A Philips PW1400 x-ray fluorescence spectrometer (Almelo, The Netherlands) was used for the Zn Kα line measurement. The measurements parameters were; $2\theta = 63.57°$ for Zn Kα; Rh tube, 50 kV, 50 mA, LiF (200) crystal, 75–25% window width, 50 s counting time for peak and background. A gas proportional–scintillation counter in tandem arrangement was used for the analysis. The background was measured at Zn Kα $(2\theta + 1)°$, for 50 s. The pH of the solutions was measured by an Orion 701-A pH meter with a Ag/AgCl electrode.

Studies of the ion-pair formation and filtration conditions

Effect of pH

In order to establish the optimum pH for the ion-pair formation, sample solutions containing 6×10^{-4} mol l^{-1} terazosin and 1.4×10^{-2} mol l^{-1} $[(SCN)_4Zn]^{2-}$ were adjusted to different pH values with diluted solutions of NaOH and HCl, shaken during 35 min and filtered through a membrane filter. The Zn Kα line was measured on each filter membrane.

Influence of ionic strength

In order to establish the optimum ionic strength for the ion-pair formation, different quantities of NaCl were added to the sample solutions containing 6×10^{-4} mol l^{-1} terazosin and 1.4×10^{-2} mol l^{-1} $[(SCN)_4Zn]^{2-}$. The solutions were shaken for 35 min and filtered through a membrane filter. The Zn Kα line was measured on each filter membrane.

Effect of aging time

After adding the reagents to form the ion-pair complex and conditioning the pH and ionic strength, solutions were well shaken and left to stand for different periods of time and then filtered though a membrane filter. The quantity of ion-pair complex retained on the membrane was evaluated by measuring the Zn Kα line by XRF.

Determination of ion-pair complex stoichiometry

Solutions containing different terazosin/$[(SCN)_4Zn]^{2-}$ ratios were adjusted to pH 3.5 with hydrochloric acid and an ionic strength of 0.05 mol l^{-1} with sodium chloride. The solutions were filtered and Zn measured by XRF.

Analytical determination of the drug in solutions

Aliquots (0.5–2 ml) of 0.01 mol l^{-1} of terazosin solutions were quantitatively transferred into 25 ml flasks. To each flask, 3.5 ml of a standard solution of 10^{-2} mol l^{-1} zinc thiocyanate was added and the flask was filled to the mark with solutions of pH 3.5 and ionic strength 0.05 mol l^{-1}. The solutions were well shaken, left to stand for 35 min and then filtered through Millipore membrane filters. The metal ion concentration on each filter was determined using XRF.

Method validation

In order to demonstrate the validity of this method, ten tablets containing 5 mg terazosin each were weighed and finely powdered. The powder was divided into ten portions. The proposed method was applied to six portions and the average quantity of terazosin obtained was taken as the base value. Then, increasing quantities of terazosin were added to the other four aliquots of the sample and terazosin was determined applying the same method (Table 2).

The calibration data were analysed by the linear regression least-square fit method. The calibration graph equation was: $I = -89.34 + 176.20\,C$ with $R = 0.998$.

Assay for pharmaceutical preparations

Ten tablets containing 5 mg terazosin each were weighed and finely powdered. A portion of the powder, equivalent to 5 mg of terazosin, was treated with 5 ml of dichloroethane, shaken for 15 min to extract the poly(vinylpyrrolidone) (PVP), centrifuged and filtrated through Teflon membranes. The residue on the filter was treated with 5 ml ultrapure water at pH 3.5, shaken for 30 min, centrifuged and filtrated.

The filtrate was transferred to a 25 ml flask. Five milliliters of pH 3.5 water was added and shaken till dissolution. Then the zinc thiocyanate and sodium chloride solutions were added as described above. The sample was filled to the mark with 3.5 pH water, shaken and left to stand for 35 min and

Table 1. Optimum conditions and characteristics of the terazosin/$[Zn(SCN)_4]^{2-}$ system

Ion associate	Terazosin–zinc thiocyanate
pH	3.5
Ionic strength (mol l^{-1})	0.05
Time, t (min)	35
Stoichiometry	$(C_{19}H_{25}N_5O_4)_2$ $[Zn(SCN)_4]$
Linearity interval (mg ml^{-1}):	0.732–843

Table 2. Validation of the method for the determination of terazosin in a commercial pharmaceutical formula containing 5 mg terazosin

Sample	Base value ($n = 6$) (mg)	Quantity of terazosin added (mg)	Quantity found (mg)
1	4.83 ± 0.04	2	6.97 ± 0.045
2	4.83 ± 0.04	3	8.12 ± 0.042
3	4.83 ± 0.04	4	8.99 ± 0.041
4	4.83 ± 0.04	5	9.88 ± 0.037

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then filtered through a Millipore membrane. The metal ion concentration on the filter was determined using XRF.

RESULTS AND DISCUSSION

The principle of determination and the preconcentration method presented here are based on the conversion of the analyte (terazosin) into a collectible form by a filter and a detectable species by x-ray spectrometry. The requirement is to form an insoluble species that contains a metal. This was attained by forming an ion-pair complex between the drug and an ionic metal complex. In this way, the analyte retained on the filter formed a thin film and the metal could be measured by x-ray spectrometry.

The optimum conditions for ion-pair complex formation were necessary to be established. The effect of pH on ion-pair formation was studied by measuring the Zn Kα line at different pH values. The results are shown in Fig. 2. It can be seen that the highest Zn signal is obtained at approximately pH 3.5. The acid medium produces the protonation of amine groups in the drug, giving positive charges that allow the ion-pair association with the complex $[Zn(SCN)_4]^{2-}$ negatively charged. The ion-pair $(C_{19}H_{25}N_5O_4)_2[Zn(SCN)_4]$ formation curve as function of pH presents a typical behavior: (1) an ascending zone, in which the enhancement produced in Zn Kα line intensity by the formed insoluble ion-pair complex is a function of the availability of the drug as the pH increases; (2) a very small plateau in which all the Zn has been complexed; (3) a descending zone, in which the Zn hydroxide and deprotonation of drug amino groups begin to compete in an effective way with the ion-pair formation.

The variation of ionic strength produces a variable yield in the precipitate formation, so it was necessary to fix the best condition. Figure 3 shows that at high ionic strength values (more than 0.1 mol l^{-1}) the precipitate yielded is remarkably diminished, so the Zn Kα decreases in intensity. We establish an ionic strength of 0.05 mol l^{-1} as the optimum condition for the precipitation. At this value the precipitate obtained is coarse and easily filterable.

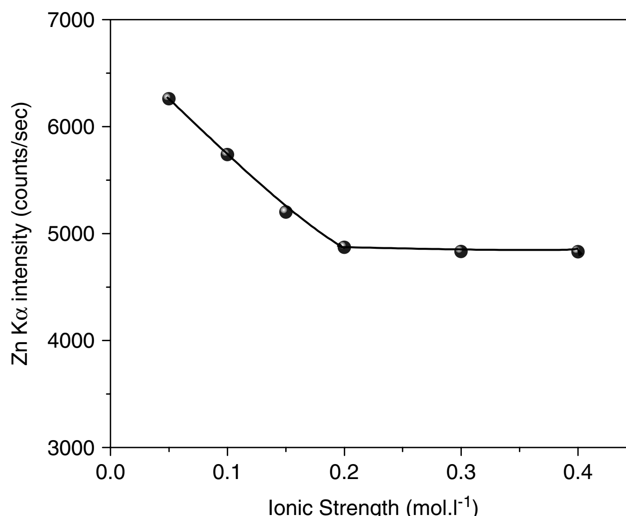

Figure 3. Ion-pair formation $(C_{19}H_{25}N_5O_4)_2Zn(SCN)_4$ as a function of ionic strength.

The aging time after adding and shaking the ion-pair-associated components plays an important role since as rest time increases, the precipitate volume is high with major particulates, improving the filtration step. Up to 35 min, the precipitates obtained are easily filterable and the quantity is adequate for x-ray measurements. Longer periods of time produce a large quantity of the precipitate but the x-ray intensity is not significantly enhanced, because of the film exceeding the critical thickness. In addition, the filtration step is very difficult and time consuming (Fig. 4).

The stoichiometric relation between the investigated drug and $[Zn(SCN)_4]^{2-}$ was performed to give an insight into the stoichiometric composition of the ion associate formed in solution and then precipitated. The method applied was that of the continuous molar ratio adapted to this method. The characteristic curve-break was observed at a cation/anion (terazosin/$[Zn(SCN)_4]^{2-}$) mol ratio of about 2, confirming the formation of an ion-associate complex (2 : 1) (Fig. 5).

The optimum conditions for the developed method are shown in Table 1.

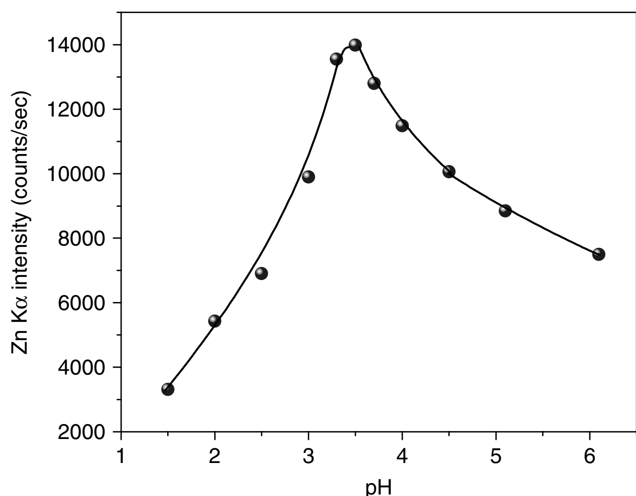

Figure 2. Ion-pair formation $(C_{19}H_{25}N_5O_4)_2Zn(SCN)_4$ as a function of pH.

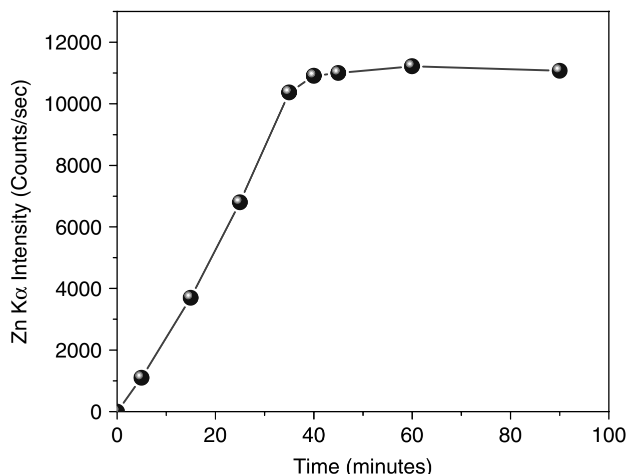

Figure 4. Effect of aging time on ion-pair terazosin–$Zn(SCN)_4^{2-}$ formation.

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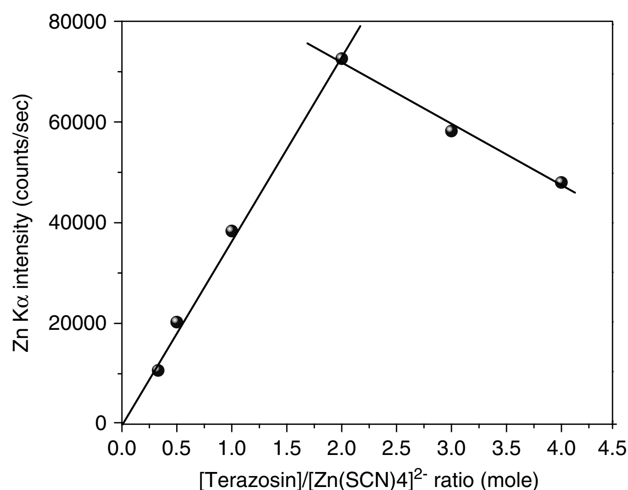

Figure 5. Determination of ion-pair complex terazosin–$[Zn(SCN)_4]^{2-}$ stoichiometry.

Table 3. Analysis of terazosin tablets (Rotiaz from Richmond Lab) ($n = 6$)

Sample ($n = 6$)	Terazosin content (mg)	Quantity found (mg)	E%
1	5	4.83 ± 0.040	2.6
2	10	10.24 ± 0.038	2.7

In this work, the ion-pair complex is directly measured on the membrane filter by x-ray spectrometry. The high sensitivity obtained is due to the fact of presenting a thin film to the x-ray spectrometer prepared through a preconcentration step. The preconcentration–separation method used increases the peak-to-background ratio due to the elimination of the matrix producing a very low background during measurements and the enhancement of the metal signal due to preconcentration of the measured metal (Zn) on the filter. The thin film eliminates the matrix interferences, since the absorption-enhancement effects are negligible because neither primary nor analyte x-rays lines are significantly absorbed in such a thin layer.

The developed method was applied to the determination of terazosin in commercial pharmaceutical samples containing 5 and 10 mg of terazosin. During sample preparation, it was necessary to add a separation step to eliminate some tablet excipients that avoid complex formation. PVP, because of its characteristics, constitutes an interference that competes with the terazosin in the ion-pair formation. So PVP must be separated during the sample treatment. Information on PVP solubility is available in the literature,[25–27] but not for terazosin. So, some solvents were found to dissolve PVP or the drug while the other remained insoluble. With this purpose, methanol, ketone, ethyl acetate and dichloroethane were evaluated. Terazosin showed the same behavior as PVP with the first three solvents. While dichloroethane dissolved PVP, the drug remained insoluble. So this solvent was used to separate this excipient from the drug. A Teflon membrane filter ought to be used in this step of analysis to avoid the dissolution of the membrane when filtering the insoluble terazosin. The other components of the matrix present in the tablet do not interfere with terazosin determination because they do not react with $[Zn(SCN)_4]^{2-}$. The results obtained are shown in Table 3.

The detection limit in the lowest concentration (C_{DL}) was 0.732 μg/ml, calculated as $C_{DL} = 3/m(I_B/t)^{1/2}$, where m is the slope, I_B is the background intensity at Zn Kα 2θ and t is the measuring time. The linear dynamic range was 0.732×10^{-3} to 1000 mg/ml.

CONCLUSIONS

The developed method provided a simple, accurate and reliable method for XRF determination of terazosin. The wide applicability of the newly developed method for routine quality control was well established by the assays of the drug in raw materials as well as in pharmaceutical preparations and shows a potential utility for other pharmaceutical drugs.

The method helps the analyst to overcome two drawbacks simultaneously: (1) the improved sensitivity through the preconcentration of the Zn; and (2) the elimination of the matrix effects by preparing a thin film and separating the analyte from the matrix.

The reagents used in the proposed method are inexpensive and readily available, and the procedures do not involve any critical reaction conditions or tedious sample preparation.

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