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Short communication

Determination of oxamniquine in capsules by HPLC

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Abstract

A sensitive, accurate, reliable and easy method was developed for the quantification of oxamniquine in capsules using high-performance liquid chromatography (HPLC) with UV detection. This technique provided conditions for the separation of the active ingredient from the dosage form by extraction in methanol. Isocratic reversed phase chromatography was performed using methanol, water, and triethanolamine (60:40:0.099, v/v/w) (System C) or methanol, acetonitrile, water and formic acid (40:30:30:0.083, v/v/w) (System D) as mobile phase, a stainless steel column (125 × 4 mm i.d., 5 μm) filled with LiChrospher 100 RP-18 (Merck), column temperature of 28 ± 2 °C and detection at 260 nm. The calibration curves were linear over a wide concentration range (1.0–20.0 μg ml^{-1} of oxamniquine) to the Systems C and D with good correlation factor (0.9990 and 0.9982, respectively). The average content obtained were 100.1 ± 1.5% (System C) and 102.4 ± 0.8% (System D). The presence of lactose, starch, magnesium stearate and sodium laurylsulphate did not interfere in the results of the analysis. The above findings showed the proposed method to be both simple and added advantage of allowing for fast analysis.

Keywords: Oxamniquine; High-performance liquid chromatography (HPLC); Capsules

1. Introduction

Oxamniquine (Fig. 1) (1,2,3,4-tetrahydro-2-[[(1-methyl-ethyl)amino]methyl]-7-nitro-6-quinoline-methanol) is schistosomal agent that is indicated for the treatment of *Schistosoma mansoni* infection in South America and Africa. Moreover, it is well known fact that this drug is effective against mature and immature worms in some phases of the infestation [1].

Few methods have been reported to quantify this drug. A nonaqueous titrimetric method using perchloric and acetic acids was described [2]. A

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spectrophotometric methods to determine oxamniquine in raw material and dosage forms have been reported [3–5]. A sensitive method, which depends upon the polarographic activity of oxamniquine in a Britton-Robson buffer, whereby a well-defined cathodic wave, followed by a more negative ill-defined one, was produced over the whole pH range was described [6]. Oxamniquine and one structural analogue used as the internal standards were extracted from serum with ether [7]. After derivatization with *N*,*O*-bis(trimethylsilyl)acetamide, oxamniquine was determined as its trimethylsilyl ether derivative by GLC using an electron-capture detector. A sensitive and reliable high-performance liquid chromatography (HPLC) assay procedure to quantify oxamniquine in plasma or urine was developed [1]. However, an HPLC method to determine this drug in dosage forms has not been reported. Because of their selectivity, sensitivity and versatility, the development of HPLC methods has received considerable attention in the quality control of drugs [8]. The purpose of this study was to develop a simple, fast, and effective method to determine oxamniquine in capsules.

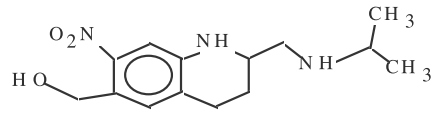

Fig. 1. The chemical structure of oxamniquine.

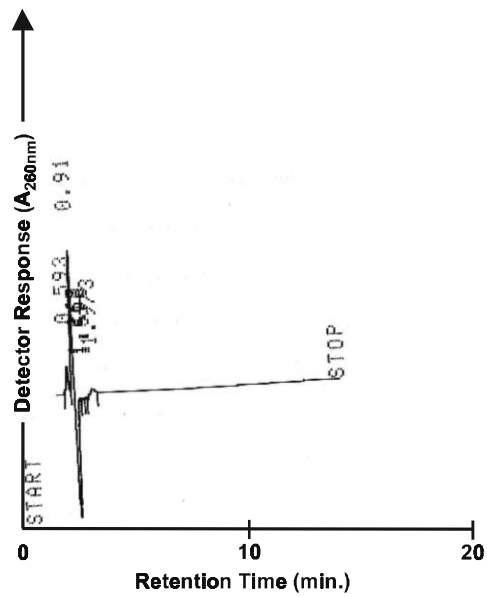

Fig. 2. HPLC chromatograms of placebo sample (System C).

2. Experimental

2.1. Samples

The oxamniquine used in this study (assigned purity 99.0%) was kindly provided by Pfizer Central Research (Sandwich, Kent, UK).

Oxamniquine capsules were obtained commercially. The capsules were claimed to contain 250 mg of drug and lactose, cornstarch, sodium laurylsulphate and magnesium stearate as excipients.

2.2. Reagents and solvents

Methanol (Mallinckrodt) HPLC grade, water filtered through a Milli-Q apparatus (Millipore), acetonitrile (Mallinckrodt) HPLC grade formic acid (Merck) and triethanolamine (Mallinckrodt) of an analytical grade were also used.

Table 1
Mobile phases tested in separation of oxamniquine

Mobile phase	Methanol	Water	Acetonitrile	Triethanolamine	Formic acid
A	–	30	70	–	–
B	60	40	–	–	–
C	60	40	–	0.099	–
D	40	30	30	–	0.083

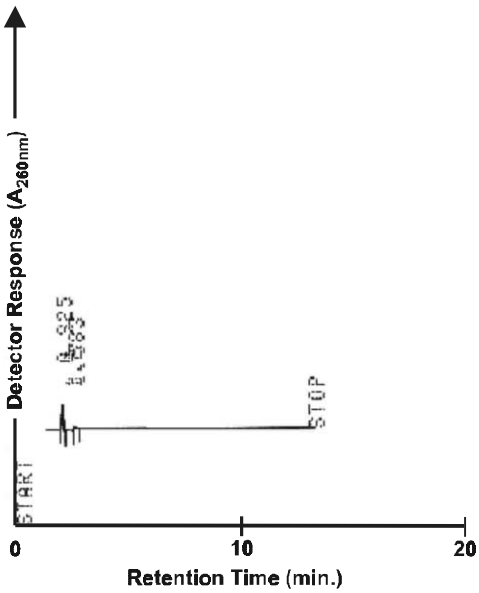

Fig. 3. HPLC chromatograms of placebo sample (System D).

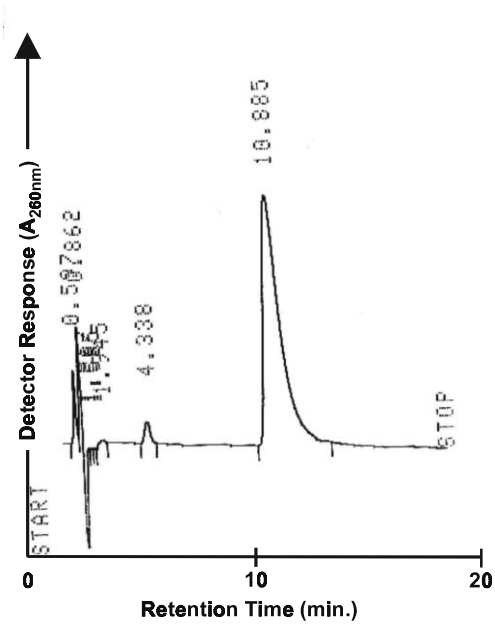

Fig. 4. HPLC chromatograms of commercial sample (System C).

2.3. Instrumentation and conditions

A Shimadzu System consisting of the following components was used: Solvent Delivery Module LC-9A, Ultraviolet–Visible Spectrophotometric Detector SPD-6AV Module, Column Oven CTO-6A Module and System Controller SCL-6B Module. Fixed loop injector (Rheodyne, 20 μl) was utilized to carry the sample onto the column.

Chromatographic separation was accomplished using a LiChrospher 100 RP-18 (Merck) stainless steel column (125 × 4 mm id., 5 μm particle size) and a LiChrospher 100 RP-18 (Merck), 4 × 4 mm, 5 μm guard column was used with methanol, water and triethanolamine (60:40:0.099, v/v/w) (System C) or methanol, acetonitrile, water and formic acid (40:30:30:0.083, v/v/w) (System D) was filtered through a 0.45 μm Millipore filter and degassed in helium gas before use. Isocratic separation was performed at a flow rate of 1.2 ml min^{-1}, with the column temperature was set at 28 ± 2 °C. The sensitivity was 0.005 A.U.F.S. and the chart speed was 0.3 cm min^{-1}.

2.4. Procedure

2.4.1. Oxamniquine standard

Stock solution of the oxamniquine standard in methanol (0.5 mg ml^{-1}) was freshly prepared by

Table 2
Analysis results for oxamniquine capsules (250 mg) by HPLC

Theoretical amount (mg)	Experimental amount [a](mg) ± CV%		Purity (%)	
	System C	System D	System C	System D
250	255.0 ± 1.7	256.8 ± 0.4	102.0	102.7
250	241.0 ± 1.2	256.8 ± 1.0	96.4	102.3
250	255.0 ± 1.7	255.8 ± 1.0	102.0	102.3

[a] Mean of three determinations.

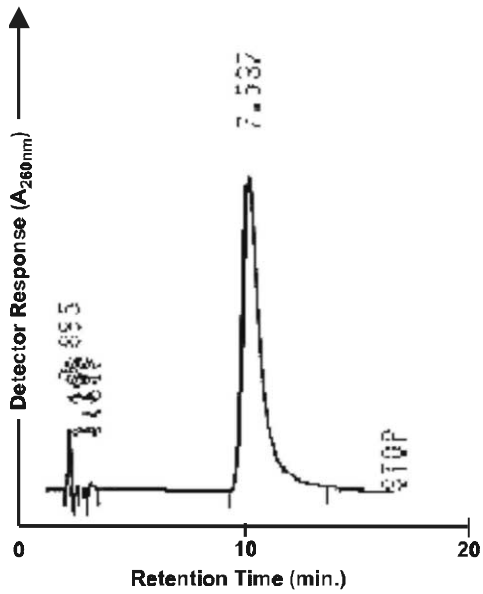

Fig. 5. HPLC chromatograms of commercial sample (System D).

accurately weighing 5 mg oxamniquine reference substance, transferring to a 10-ml volumetric flask, the addition of 5 ml methanol, followed by adding methanol to make up the volume. The working solutions were prepared by diluting the stock solution in methanol.

The calibration curves were linear throughout the concentration range under investigation, from 1.0 to 20.0 μg ml^{-1} (Systems C and D).

Quantification of oxamniquine was achieved by regression analysis of the peak areas against concentration. All determinations were conducted in triplicate.

2.4.2. Assay of oxamniquine in capsules

The contents of five capsules, each containing 250 mg of oxamniquine, were pulverized using a mortar and pestle. An aliquot of this material, equivalent to 50 mg of oxamniquine, was accurately weighed and transferred into a 100-ml volumetric flask. Methanol was added and the flask was shaken for 30 min. The volume was completed to 100 ml, after which the solution was filtered through paper and the first 5-ml discarded. An aliquot of 5 ml of the filtrate was diluted with methanol to obtain a 5 μg ml^{-1} solution. The solution was then filtered through a 0.22 μm Millipore filter and 20.0 μl were analyzed by HPLC employing Systems C and D.

2.4.3. Simulated sample

Simulated sample containing 250 mg of oxamniquine, 40 mg of lactose, 40 mg of starch, 0.125 mg of sodium laurylsulphate, and 3.3 mg of magnesium stearate were prepared and analyzed by the same HPLC procedure. The samples were homogenized (using a mortar and pestle) and the correspondent weight of one capsule was submitted to the analytical procedure.

2.4.4. Placebo sample

Placebo sample containing 40 mg of lactose, 40 mg of starch, 0.125 mg of sodium laurylsulphate, and 3.3 mg of magnesium stearate were prepared and analyzed by HPLC procedure. The samples were homogenized (using a mortar and pestle) and the correspondent weight of one capsule was submitted to the analytical procedure.

3. Results and discussion

Based on the extraction technique described in the literature [2–6] to determine of oxamniquine in capsules, various mobile phases were tested in order to establish the ideal conditions for analysis, using acetonitrile and water (System A); methanol and water (System B); methanol, water and triethanolamine (System C); methanol, acetonitrile, water and formic acid (System D) (Table 1).

An extraction method described in literature [4] for a pharmaceutical dosage form was used to establish experimental parameters for the analytical methodology applied to oxamniquine in commercial pharmaceutical preparations.

Separation of oxamniquine was achieved by using Systems C and D, the LiChrospher 100 RP-18 stationary phase and detection at 260 nm.

The correlation coefficients for the calibration plots of two systems were 0.9999 and 0.9989 (Systems C and D, respectively) for oxamniquine (1.0–20.0 μg ml^{-1}). Least square linear regression analysis was used to determined the slope, y-intercept and correlation coefficients as: $Y =$

$113162.0X - 1068.9$ (System C) and $Y = 124051.0X - 78808.0$ (System D), where Y and X are the peak area and concentration, respectively.

The statistical date were analyzed by t-test and precision values of the assays were 0.8137 and 0.5780% for Systems C and D, respectively.

The experimental conditions, as established for commercial pharmaceutical preparations of oxamniquine, was based under the principle of this method. Good separation was obtained, as it is shown by the 10.7 min (System C) and 7.6 min (System D) retention time.

In an attempt to detect interference, simulated and placebo samples were prepared and analyzed. Excipients used in these preparations were those most commonly used by the pharmaceutical industry. The presence of lactose, starch, magnesium stearate and sodium laurylsulphate did not interfere in the results of the analysis (Figs. 2 and 3).

Simulated sample, containing the same amount as these used in commercial samples of oxamniquine, was used to calculate recovery values and presented average value of $101.5 \pm 2.2\%$ (System C) and $100.6 \pm 0.8\%$ (System D), respectively. Results referred to the average of three assays and they are in good agreement with the values acceptable in the validation of an analytical procedure (80–120%) [9,10].

This preliminary study allowed the application of the method developed in commercial sample. Table 2 summarizes the results obtained in this study demonstrate the efficiency of the proposed methods and show mean recoveries of the oxamniquine in pharmaceutical preparations 100.1% (System C) and 102.4% (System D) with relative standard deviation values 1.5 and 0.8%, respectively. The calculated values correspond to the average of three analyses, which are in accordance to the values presented in USP XXIII [4]. Figs. 4 and 5 show the chromatograms and the results of the analyses.

4. Conclusion

The HPLC methods developed in this study proved to be simple, precise and effective, facilitating the determination of oxamniquine in capsules.

The presence of lactose, starch, magnesium stearate, and sodium laurylsulphate did not interfere in the results of the analysis.

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