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Short communication

Flow injection analysis of doxazosin mesylate using UV-detection

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Abstract

A flow injection analysis (FIA) of doxazosin mesylate (DOX) using UV detection is described in this study. The best solvent system was found to be consisting of 0.1 mol l^{-1} acetate buffer at pH 4 having 10%MeOH. A flow rate of 1 ml min^{-1} was pumped and active material was detected at 365 nm. The calibration equation was linear in the range of 1.3×10^{-5} to 6.4×10^{-5} mol l^{-1}. Limit of detection and limit of quantitation were calculated to be 1.6×10^{-6} and 4×10^{-6} mol l^{-1} with a RSD 1.27 and 1.16% ($n = 8$), respectively. The proposed method was applied to the determination of DOX in the pharmaceutical preparations. The results were compared with those obtained from UV-Spectrophotometry. The results showed that there is a good agreement between FIA method and UV-Spectrophotometry. The validation studies were realised by the related applications and the results were evaluated statistically. According to the results, insignificant difference was observed between the methods. © 2001 Published by Elsevier Science B.V.

Keywords: Determination of doxazosin mesylate; Flow injection analysis; Pharmaceutical application

1. Introduction

Doxazosin mesylate (DOX), [1(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(1,4-benzodioxan-2-yl-carbonyl) piperazine monomethansulphonate] is a postsynaptic α-1 adrenoreceptor antagonist (Fig. 1). DOX as a potent antihypertensive agent is effective when administered either orally or intravenously. It is intravenously. It is slowly eliminated in humans and its long half-life provides the basis for once-daily dosing [1,2]. Several HPLC methods have been employed for the determination of DOX. These studies cover the determination of active material in body fluids depending on the pharmacological evaluations [3–7]. In addition, a differential pulse [8], super imposed constant and super imposed increasing pulse polarographic method [9] an adsorptive stripping voltammetry [10,11], a

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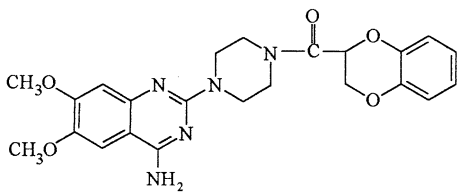

Fig. 1. The chemical structure of DOX.

cathodic stripping voltammetry [12] and a square-wave voltammetry [13] have been reported.

Flow injection analysis (FIA) is a new methodology characterized by its versatility, ease of automation, high sampling frequency and minimum sample treatment prior to injection into the system. The FIA techniques have found wide applications recently mainly due to reduction of the analysis time and reagents consumption compared to conventional manual procedures [14]. On the other hand, their high sensitivity makes them suitable for the determination of low concentrations of pharmaceuticals in biological fluids when used as detectors in HPLC. They can also optimize the detection of analyte independently from the way process occurring in the chromatographic column [15]. The aim of this study is the direct determination of DOX by FIA method and its application to pharmaceutical preparations.

2. Experimental

2.1. Apparatus and chemicals

The HPLC apparatus used a Model LC 6A pump equipped with a 20 μl manual loop injector, a Model SPD-A1O UV variable wavelength detector and a Model C-R7A integrator (all Shimadw, Japan). Spectrophotometric studies were done using a Model UV-2401 PC (Shimadzu, Japan). Standard DOX (99.8%) and Cardura® tablets containing 4 mg active material were kindly supplied from Pfizer Ilaçlan A.S. (Istanbul, Turkey). Standard DOX was used without further purification. Other chemicals were of analytical grade from Merck (Germany).

2.2. Solutions

A stock solution of DOX (1.3×10^{-3} mol l^{-1}) was prepared using MeoH%10. The dilutions were made in the range of 1.3×10^{-5}–6.4×10^{-5} mol l^{-1} with the same solvent. As a mobile phase an aqueous solutions of MeOH (10%, v/v) was used. The buffer solutions were prepared using 1 mol l^{-1} sodium acetate (pH 1–6) and 1 mol l^{-1} dipotassium hyrogen phosphate (pH 7–11) and their pH values were adjusted using 2 mol l^{-1} HCl or 2 mol l^{-1} KOH.

2.3. Application to the tablets

Twenty Cardura® tablets were weighed and finely powdered in a mortar. The average weight of a tablet was calculated. A sample equivalent to one tablet was weighed and transferred to a 100 ml calibrated flask, 1 ml acetate buffer (pH 4) and 50 ml MeOH%10 were added, magnetically stirred for 20 min and made up to volume with the same solvent. A sufficient amount of the solution was pipetted in a tube and it was centrifuged for 10 min. The supernatant was diluted to the predetermined values and injected into sample loop by means of a syringe.

3. Results and discussion

To investigate the percentage of MeOH, it was increased from 10 to 50% (v/v). It was found that the optimum concentration of MeOH, in view of peak morphology, was 10% (v/v). To determine the optimum flow-rate, it was changed from 0.3 to 3.5 ml min^{-1} and the best flow-rate was found to be 1 ml min^{-1}. The final concentration of buffer in the test solution was 0.1 mol l^{-1}. The peak areas versus pH is illustrated in Fig. 2. As seen in Fig. 2, morphologically good peaks were obtained around pH 4. The peak areas were significantly different above pH 6. However, these differences are minimum at pH values between 2 and 5. The ranges of these pH values are close to the p*K*a value of 4.8 for DOX [16]. Therefore, the buffer of pH 4 was chosen as working pH. The signals of the DOX at concentration ranging from $1.3 \times$

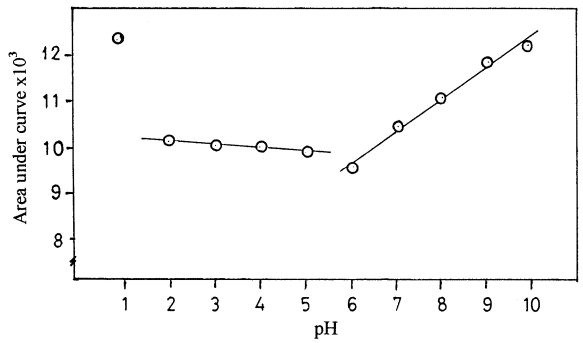

Fig. 2. Variation in the AUC values of DOX (6.4×10^{-5} mol l^{-1}) in relation to pH.

Table 1
Linearity and accuracy of FIA method for DOX

Parameters	Intra-day precision ($k = 1$; $n = 8$)	Inter-day precision ($k = 4$; $n = 32$)
Slope ± SD	$1.92 \times 10^{10} \pm 2599$	$2.17 \times 10^{10} \pm 2625$
Intercept	28368	26734
Correlation coefficient (r)	0.9996	0.9994
Slope ± CL ($P = 0.005$)	$1.92 \times 10^{10} \pm 2442$	$2.17 \times 10^{10} \pm 2716$

SD, standard deviation; CL, confidence limit; k, number of the set; n, number of the sample.

10^{-5} to 6.4×10^{-5} mol l^{-1} were obtained under the conditions described above and they are demonstrated in Fig. 3.

Although the prepared solutions give the same signals during a week time, it is not always possible to obtain the true stability of the molecule. For this aim, HPLC and TLC method are recommended.

The relationship between area under curve (AUC) and DOX concentration was found to be $AUC = 2 \times 10^{10} C$ (mol l^{-1}) + 27558.4; $r = 0.9998$. The detection limit (LOD, S/N = 3) and limit of calculation (LOQ, S/N = 10) were calculated to be 1.6×10^{-6} mol l^{-1} with RSD = 1.27% and 4×10^{-6} mol l^{-1} with RSD = 1.16% ($n = 8$), respectively.

Linearity and accuracy in the concentration range of 1.3×10^{-5}–6.4×10^{-5} mol l^{-1} were examined employing intra-day and inter-day (for 8 days) studies for the determination of DOX. Very accurate results were obtained for intra-day and inter-day experiments with a good correlation. The results were evaluated statistically and these are demonstrated in Table 1. These results indicate that the FIA method could be used for the analysis of DOX.

3.1. Application to the pharmaceutical dosage forms

The proposed technique was applied to the pharmaceutical dosage forms containing 4 mg DOX. The absorbances were monitored at 365 nm. The AUC values were used for calibration. UV-Spectrophotometry was chosen as a comparison method. The absorbances of the same solutions were measured at 365 nm using quartz cells. The relationship between absorbance

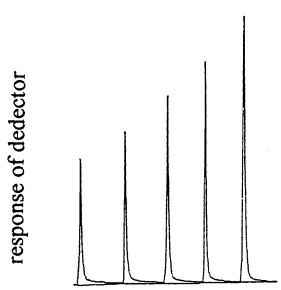

Fig. 3. The signals in the 1.3×10^{-5}–6.4×10^{-5} mol l^{-1} concentration range of DOX.

Table 2
The assay results of DOX in tablets[a]

	FIA	UV-Spectrophotometry
Mean	3.8	3.9
n	8	8
RSD%	1.2	0.8
CL	±0.15	±0.12
F-test of insignificant	3.68	$F_{0.05} = 4.12$ (table)
t-test of insignificant	1.75	$t_{0.05} = 2.18$ (table)

[a] Each tablet contains 4 mg of DOX.

(A) and concentration of DOX (C) was found to be $A = 10226.4C$ (mol l^{-1}) + 0.004; $r = 0.9999$.

The method validity was examined by applying it to the tablets. The ingredients in the tablets do not interfere in the experiments. All results of the assays were evaluated statistically and presented in Table 2.

High reproducibility was observed and insignificant differences between FIA and UV-Spectrophotometry at the 95% probability level. As a conclusion, the method proposed in this study is simple, accurate, precise and rapid. Therefore, it can be suggested for the routine analysis of DOX.

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