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Short communication

# High-performance liquid chromatography using electrochemical detection for the determination of prazosin in biological samples

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**Abstract**

For the quantitation of prazosin a sensitive high-performance liquid chromatographic (HPLC) method was developed. This HPLC analysis method uses an electrochemical detection technique for the identification and quantitation of prazosin. In this assay the serum samples were deproteinized by using a simple acetonitrile precipitation technique that was followed by *n*-hexane extraction. Prazosin in the deproteinized serum sample was separated by an isocratic elution with an ODS Hypersil HPLC column (150 × 4.6 mm) using a mobile phase consisting of 0.05 *M* $Na_2HPO_4$–acetonitrile (60:40), pH 8.4. Prazosin that was eluted from the column was detected using a Coulochem II electrochemical detector. The precision of this assay method was assessed by performing inter- and intra-assay analyses by spiking prazosin free fetal bovine serum samples with 20 and 40 ng/ml concentrations of prazosin. In the intra-assay the recovery was 95.40 ± 4.82% and 97.80 ± 3.40%, respectively, for 20 and 40 ng/ml concentrations of prazosin that were used to spike the serum samples. This electrochemical detection HPLC assay method could be very useful in monitoring plasma levels of prazosin.

## 1. Introduction

Prazosin is an oral, quinazolin derivative that selectively blocks postsynaptic $\alpha_1$-adrenergic receptors in arterioles and veins leading to the dilation of systemic vascular and venous capacitance beds [1]. In recent years, prazosin is frequently used in young children and in infants for the treatment of various cardiovascular disorder conditions such as congestive heart failure and diminished cardiac function [2]. Most often the dosage of prazosin utilized for children is determined either by an extrapolation of the adult dosage or by monitoring therapeutic responses after drug administration. Hence, an effective prazosin therapy in young children warrants a precise quantitation of the drug in the plasma as a prerequisite for the establishment of pharmacokinetic parameters. So far, the pharmacokinetics analysis of prazosin in young children has been difficult to achieve since this drug is administered in low doses: in the range of 0.01–0.05 mg/kg body weight [2]. In addition, the currently available UV and fluorescence detection HPLC assay methods require 1.0 ml plasma samples which may be difficult to obtain

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from newborn babies. Therefore, estimation of prazosin concentration in smaller sample volumes requires a very sensitive HPLC method. In this paper we describe the use of an electrochemical detection HPLC system, which utilizes a relatively small volume of serum sample (100 μl) for prazosin analysis. This method also offers higher sensitivity and selectivity for prazosin.

## 2. Experimental

### 2.1. Reagents

Prazosin [1-(4-amino-6,7- dimethoxy-2-quinazolinyl)-4-(2-furonylcarbonyl)piperazine] was purchased from Sigma (St. Louis, MO, USA). HPLC grade acetonitrile was obtained from Mallinkrodt (St. Louis, MO, USA) and HPLC water was obtained from Baxter Scientific (Chicago, IL, USA). Other reagents used in the assay were of analytical grade and all aqueous solutions used in the assays were made using the HPLC grade water.

### 2.2. Prazosin standards

A stock solution of prazosin was made by suspending 5 mg prazosin in 5 ml of distilled water in a volumetric flask and keeping the flask in a water bath that was heated to 90°C for 2 to 3 min. The flask was vigorously agitated for 60 to 90 s until the compound completely dissolved and then the volume was made to 50 ml with HPLC water. This stock solution when stored at 4°C in the dark, was stable upto 1 month without any decrease in the original concentration. Whenever necessary, the stock solution was diluted further to make working standards.

### 2.3. Chromatography

In this method a HPLC system fitted with an ODS Hypersil column (150 × 4.6 mm), a Coulochem II electrochemical detector and an analytical cell (No. 5014) from ESA (Bedford, MA, USA) was used. The mobile phase consisted of 0.05 *M* $Na_2HPO_4$–acetonitrile (60:40), pH 8.4. The potential used for our analysis of prazosin was +500 mV on channel 1 in the detector, which was determined by a hydrodynamic voltammogram (HDV). The detector response was set to give a full-scale detection for 1 μA current output received from the analytical cell. During the analysis, the potential of channel 2 was kept at +0.00 mV and the guard cell had a potential of +300 mV. The HPLC was operated in a constant flow mode and the flow-rate was kept at 1.0 ml/min.

### 2.4. Hydrodynamic voltammogram

The hydrodynamic voltammogram was developed by injecting 20 μl of 250 ng/ml concentration of standard prazosin and measuring the current produced by prazosin at the electrodes for various applied potentials ranging from −900 mV to +900 mV. At the end of the analyses a plot of the area under the peak vs applied potential was generated and this represented the hydrodynamic voltammogram for prazosin.

### 2.5. Extraction procedure

Extraction of prazosin from serum samples was achieved by a slightly modified method of Lin et al. [3]. Fetal bovine serum (100 μl) samples spiked with prazosin was precipitated using 200 μl of HPLC grade acetonitrile and then the precipitate was removed by centrifuging the tubes at 9000 *g* for 5 min. The supernatant fraction was separated from the precipitate and mixed with 250 μl of water-saturated *n*-hexane. The water-saturated *n*-hexane was prepared by shaking *n*-hexane in a separating funnel with distilled water for 30 min and then separating the organic layer from the aqueous layer. The samples mixed with the water-saturated *n*-hexane were agitated vigorously on a vortex mixer for 120 s and then centrifuged at 10 000 rpm for 5 min. The *n*-hexane fraction remained as the top layer which was aspirated and discarded. This extraction step was repeated twice and then the acetonitrile fraction was evaporated to dryness under nitrogen. Dried fractions were redissolved

in 100 μl of mobile phase and then 50 μl of the extract was injected in the HPLC for prazosin quantitation. Since the extraction procedure showed 95 ± 5% recovery, internal standards were not incorporated into our analysis samples.

### 2.6. Recovery and reproducibility

The precision of the HPLC assay was established by performing intra- and inter-assay reproducibilities by utilizing fetal bovine serum samples that are spiked with known concentrations of prazosin. The intra-assay was performed by adding 20 and 40 ng/ml concentrations of prazosin to the fetal bovine serum samples and then quantitating prazosin by the above-described assay method after extraction. The inter-assay reproducibility was determined by consecutively adding a 20 ng/ml concentration of prazosin to a single sample of fetal bovine serum for five successive runs and quantitating the extracted prazosin levels after each addition.

## 3. Results and discussion

The mobile phase used in our HPLC analysis, 0.05 *M* $Na_2HPO_4$–acetonitrile (60:40) with pH 8.4, gives a good separation of prazosin. In this chromatographic system prazosin was eluted as an asymmetrical peak at 5.7 min retention time ($t_R$) following injection into the $C_{18}$ reversed-phase column. The mobile phase used in this system also offered a suitable environment for electrochemical activation and detection of prazosin. Since prazosin was undergoing both oxidation and reduction changes, depending on the voltage applied to the electrodes during detection, the HDV analysis was performed to

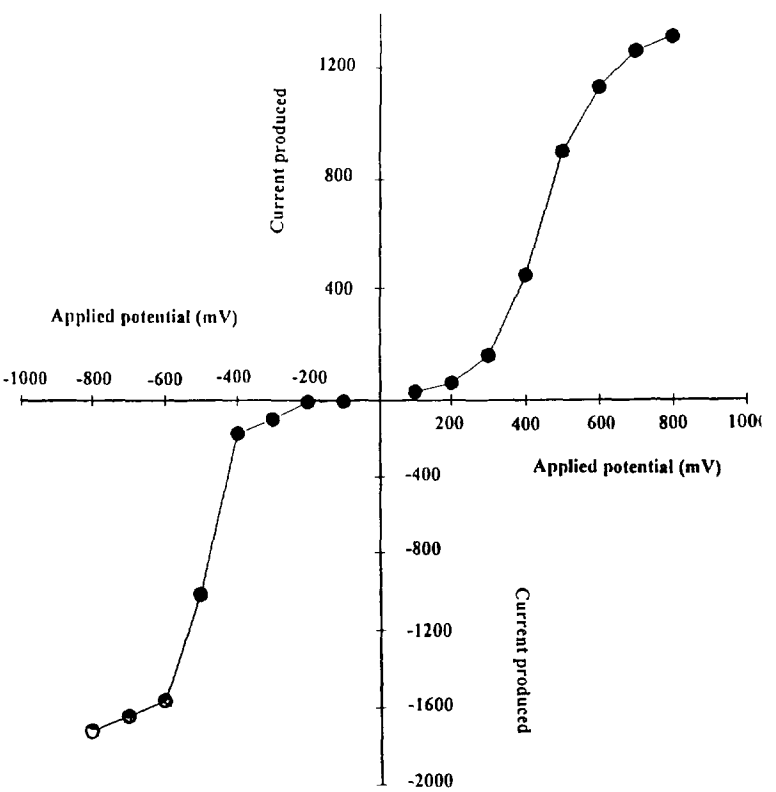


Fig. 1. The hydrodynamic voltammogram (HDV) of prazosin. The HDV was developed by plotting the amount of current (mA) produced by 5 ng of prazosin vs various oxidation and reduction potentials.

optimize the condition for an efficient detection of prazosin in our electrochemical detector. Fig. 1 illustrates the HDV corresponding to a range of oxidation and reduction potentials used for prazosin detection. From the HDV, a minimum potential required for the detection electrodes to produce maximum current response by oxidizing prazosin was determined. The ideal potential for oxidative detection of prazosin was found to be + 500 mV which was later used in the detector for all our sample analyses. The responses to various amounts of standard prazosin are shown in Fig. 2. This three-dimensional graph illustrates that the amount of current produced by prazosin (retention time 5.7 min) is increased with increasing amount of the drug injected into the HPLC. A calibration curve was prepared from the results of this experiment, by plotting the amount of prazosin vs the current produced as shown in Fig. 3. The calibration curve indicates a linear relationship between the amount of prazosin injected into the HPLC system and the amount of current produced by the detector. Also, the detector response is found to be linear from 5 ng/ml to 250 ng/ml of prazosin concentration (Fig. 3), reaching a saturation point beyond this limit. Under this condition the lower

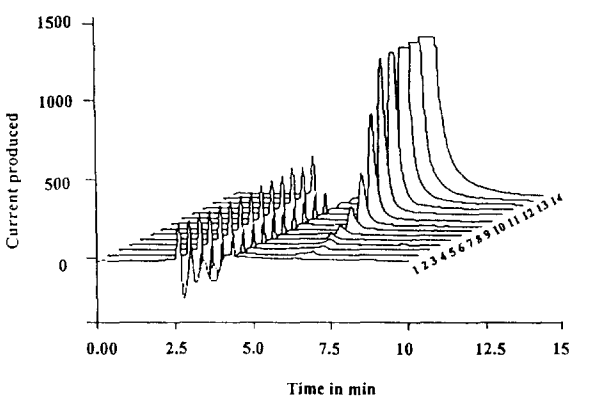


Fig. 2. Three-dimensional plot of chromatographic peaks obtained by injecting 20 $\mu$l of each concentration of prazosin into the HPLC. The concentrations of prazosin used for obtaining the chromatograms are as follows: 1 = 0.05 ng/ml; 2 = 0.5 ng/ml; 3 = 1.0 ng/ml; 4 = 2.5 ng/ml; 5 = 5.0 ng/ml; 6 = 10.0 ng/ml; 7 = 50 ng/ml; 8 = 100 ng/ml; 9 = 250 ng/ml; 10 = 500 ng/ml; 11 = 1000 ng/ml; 12 = 2000 ng/ml; 13 = 4000 ng/ml; 14 = 5000 ng/ml.

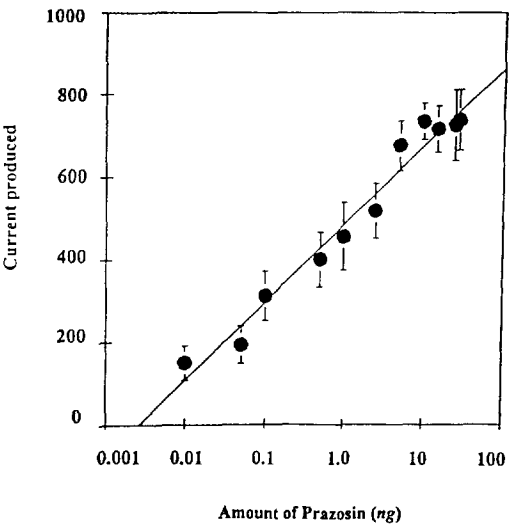


Fig. 3. Prazosin standard curve developed by plotting the amount of current (nA) produced by the electrochemical detector vs the amount of prazosin injected. The plot demonstrates the linearity of prazosin up to 250 ng/ml concentration. The signal-to-noise ratio employed for determining the lower detection limit was 3.

detection limit for prazosin was found to be 2.5 ng/ml and the peak height for this concentration was three times the noise level at 1 $\mu$A full-scale detection setting.

A representative chromatogram of deproteinized, blank, serum sample is shown in Fig. 4a. Acetonitrile precipitation of serum proteins followed by the extraction of interfering compounds by *n*-hexane offered a reliable method to clarify prazosin in the serum samples. Previously reported HPLC methods for measuring prazosin in biological samples involved either a complex extraction procedure, with series of extractions into various organic solvents [4] or solid extraction procedures using cartridge columns [5].

These methods are generally time-consuming due to multiple steps involved and often requires sample volumes in the range of 1.0 ml per analysis. Our method described in this article involves a simple protein precipitation method combined with a single *n*-hexane extraction step using 100 $\mu$l of serum samples. Our method also provided clean samples for HPLC analysis which separated prazosin as a symmetrical peak with-

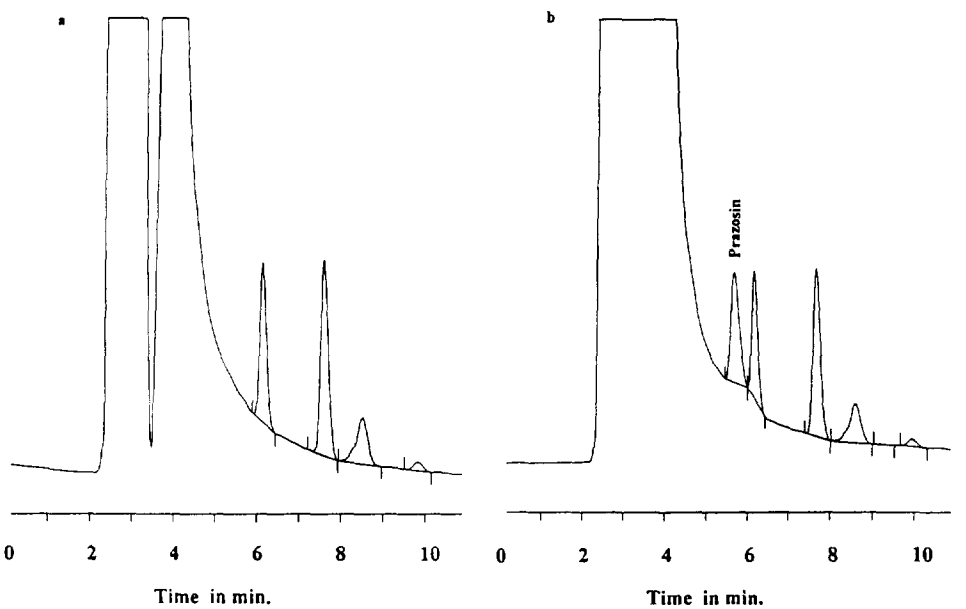


Fig. 4. Representative chromatograms of blank serum (a) and serum spiked with 20 ng/ml prazosin (b). The samples were deproteinized and separated using the $C_{18}$ reversed-phase column and detected using the Coulochem II electrochemical detector.

out any tailing. When fetal bovine serum samples were spiked with known quantities of prazosin and injected into the HPLC, prazosin was eluted as a separate peak (Fig. 4b) at 5.7 min. These samples did not show any other peaks that were eluted at the same retention time ($t_R$) as prazosin. The within-day precision and reproducibility of the assay method was assessed by performing inter- and intra-assay recoveries using prazosin-free fetal bovine serum samples spiked with known quantities of prazosin. In the intra-assay, when the serum samples were spiked with 20 ng/ml concentration of prazosin, the recovery was 95.40 ± 4.82% and this percentage of recovery was higher when the serum samples were spiked with 40 ng/ml concentration of prazosin (Table 1). The coefficient of variation in the intra-assay was 5.05% and 3.40%, respectively, for the concentrations of 20 ng/ml and 40 ng/ml (Table 1). The coefficient of variation in the inter-assay was 2.47% for 20 ng/ml concentration of prazosin, indicating an acceptable level of recovery and precision.

The assay described in this paper is a highly sensitive, which also maintained the accuracy and selectivity of previously described techniques. Our method is designed for relatively smaller-volume serum samples (100 μl). The

Table 1
Coefficient of variation for the prazosin assay ($n = 5$)

| Added prazosin (ng/ml) | Recovered (ng/ml) | Recovery (%) | C.V. (%) |
|---|---|---|---|
| *Intra-assay* | | | |
| 40.0 | 39.10 ± 1.36 | 97.80 ± 3.40 | 3.40 |
| 20.0 | 19.08 ± 0.96 | 95.40 ± 4.82 | 5.05 |
| *Inter-assay* | | | |
| 20.0 | | 93.46 ± 2.31 | 2.47 |

new assay method looks promising for the analysis of prazosin in microdialysis experiments also.

## Acknowledgements

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