Determination of Apraclonidine in Microdialysates from Eye Vitreous Humor by Microbore LCEC

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Purpose

Apraclonidine (F1) is a drug used for the treatment of post-surgical elevated intraocular pressure. Determination of apraclonidine in microdialysates from pig-eye vitreous humor was studied. The analyte was spiked in this matrix at 20 - 150 ng/mL, respectively, prior to microdialysis. Aliquots of samples were injected into a microbore liquid chromatograph, including a SepStik column and electrochemical detector.

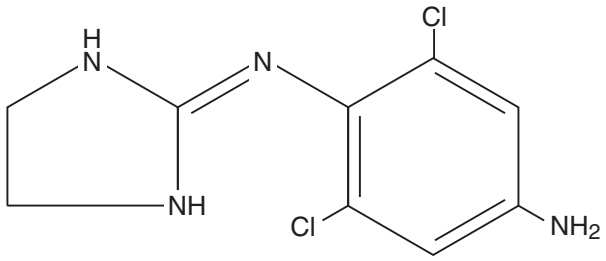

Figure 1. *Chemical structure of apraclonidine.*

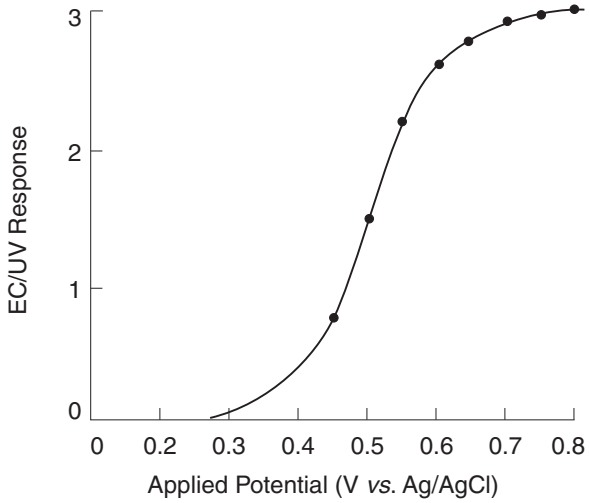

Figure 2*. Hydrodynamic voltammogram of apraclonidine.*

Conditions

System: Microbore capable Liquid Chromatograph
Detector: BASi Electrochemical Detector.
Electrochemical Detector Electrode: Glassy carbon, 3 mm (PN MF-1000)
Potential: +800 mV *vs*. Ag/AgCl
Columns: UniJet SepStik SCD, 5 µm, 150 x 1 mm (PN MF- 8914)
Mobile Phase: 10% acetonitrile in 20 mM sodium acetate (pH 6.5) containing 2 mM 1-decanesul-fonic acid. Flow rate, 200 µL/min
Detection Limit: 50 pg apraclonidine on column. Injection volume, 5 µL

Notes

A hydrodynamic voltammogram of apraclonidine was generated, F2, by injecting a constant amount of standard sample at a varying applied potential. An applied potential of +800 mV vs. Ag/AgCl was used for the remainder of this study. Typical chromatograms of microdialysates from apraclonidine in Ringer's solution (A), blank pig-eye vitreous humor (B), and apraclonidine spiked in vitreous humor (C) are presented in F3. The microdialysis recovery test results of apraclonidine from Ringer's solution using a polycarbonate membrane probe (membrane length, 4 mm) are presented in Table 1. The effects of flow rate of the perfusion solution on recovery are presented in Table 2. Calibration curves of apraclonidine at ranges from 0.05 to 2.00 ng (insert) and 20 to 200 ng injected are presented in F4.

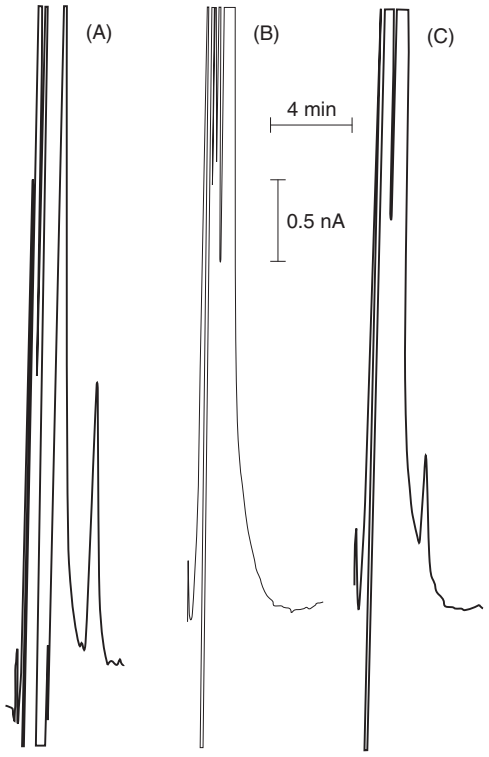

Figure 3*. Chromatograms of microdialysate from apraclonidine in Ringer's solution (A), blank pig-eye vitreous humor (B) and apraclonidine spiked in vitreous humor ©.*

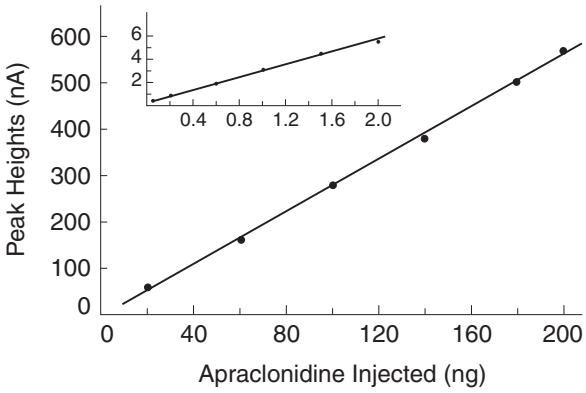

***Figure 4**. Calibration curves of apraclonidine.*

Sample Concentration (pg/µL)	Relative Recovery (%)*
20	22.73
60	18.70
100	21.23
150	22.65

***Table 1.** Recovery of apraclonidine from Ringer's solution by in vitro microdialysis. *The flow rate of the perfusion solution was 2 µL/min.*

Flow Rate (µL/min)	Relative Recovery (%)
0.5	65.7
1.0	41.9
1.5	37.1
2.0	22.4
2.5	21.3
3.0	17.0
3.5	16.2
4.0	11.2
4.5	10.2
5.0	8.8

***Table 2.** Effect of varying perfusion flow rates on recovery of apraclonidine from Ringer's solution by in vitro microdialysis.*

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