

## Note

### Determination of Yohimbine hydrochloride in horse serum using high-performance liquid chromatography

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Yohimbine hydrochloride (YOH), primarily an  $\alpha_2$ -adrenergic antagonist, has been used experimentally as an antagonist for anesthesia and sedation in animals. Used alone or in combination with 4-aminopyridine, YOH antagonizes xylazine sedation in dogs<sup>1</sup> and cattle<sup>2</sup>, ketamine anesthesia in cats<sup>3-5</sup>, xylazine plus large dose atropine immobility in dogs<sup>6</sup> and xylazine plus ketamine sedation in horses<sup>7</sup>. Yohimbine plus 4-aminopyridine and naloxone have been used to antagonize pentobarbital anesthesia in dogs following pretreatment with xylazine, acepromazine, diazepam, morphine and fentanyl plus droperidol<sup>8-10</sup>. In these studies, YOH showed a prolonged duration of action because relapses to unconsciousness were not observed after YOH-induced arousal. However, the biological half life of YOH has not been determined.

To study the pharmacokinetics of any drug, the elimination and distribution rates are calculated from serum or plasma following a single intravenous (i.v.) bolus injection. Precise determinations of drug concentrations at various times are necessary. Existing methods of analysis of YOH were reviewed and found to be inadequate due to either poor extraction recovery<sup>11</sup>, complexity of procedure<sup>11,12</sup>, or high quantitation limit<sup>12</sup>. The extraction method<sup>13</sup> was not quantitative and did not result in sufficient sample cleanup for high-performance liquid chromatographic (HPLC) analysis using a UV detector. Therefore, modification of the analytical method was required. Extraction procedures and quantitation using HPLC were developed to circumvent these problems.

#### EXPERIMENTAL

##### *Samples and materials*

YOH was purchased from Sigma (St. Louis, MO, U.S.A.). A stock solution of YOH (10 mg/l) in mobile phase was prepared and stored at room temperature. A small quantity of this solution was prepared daily. Analytical grade solvents (J. T. Baker, Philipsburg, NJ, U.S.A.) were used without further purification. Sep-Pak C<sub>18</sub> cartridges were purchased from Waters Assoc. (Milford, MA, U.S.A.).

Blood samples were obtained from several horses by i.v. collection from the jugular vein. Samples were allowed to clot, centrifuged at 1000 g for 10 min, and the serum was separated and stored at -20°C until the analysis was performed.

### Sample extraction

Serum (2 ml) was added to a test tube containing 5 ml of distilled water and vortexed for 5 s. Using a 10-ml syringe the extract was gently (about 2 drops/s) passed through a Sep-Pak C<sub>18</sub> cartridge which had been prewet with 2 ml of methanol followed by 10 ml of distilled water. The cartridge was then rinsed with 3 ml of distilled water. The residue remaining in the Sep-Pak was eluted with 6 ml of ethyl acetate. The organic layer was carefully separated and dried under a stream of nitrogen at 37°C. The residue was dissolved in 0.2 ml of the mobile phase and analyzed by HPLC.

### Chromatographic conditions

The chromatography was carried out using a Waters Model 6000A solvent delivery system equipped with Waters Model 440 absorbance detector utilized at 280 nm. A Waters  $\mu$ Bondapak C<sub>18</sub> (30 cm  $\times$  3.9 mm I.D.) column was operated at room temperature. Chromatograms were recorded on a Fisher Recordall, Series 5000 strip chart recorder.

The mobile phase consisted of a thoroughly degassed mixture of acetonitrile- $1.2 \cdot 10^{-3}$  M aqueous hydrochloric acid-methanol (60:10:30). The retention time of YOH is very sensitive to the acid content. If interferences are encountered, the relative elution of YOH can be shifted to a suitable location of the chromatogram by altering the pH of mobile phase. A constant flow-rate of 1 ml/min was maintained.

### Analyses

The chromatographic column was preconditioned by running the mobile phase through the column with a flow-rate of 0.5 ml/min for several days. Several injections of the standard solution of YOH were made prior to sample analysis. A calibration curve was obtained by injecting 40  $\mu$ l of various YOH standards (1000, 500, 200, 100, 50 and 10 ng/ml) in mobile phase and measuring the peak height. In order to monitor the retention time and the recorder response, injection of 20  $\mu$ l of standard solution (1000 ng/ml of YOH in mobile phase) was repeated after every fifth sample injection.

TABLE I  
RECOVERY OF YOH FROM FORTIFIED CONTROL HORSE PLASMA

Spiking level (ng/ml)	Recovery* (%)
500	98.9 $\pm$ 1.1
50	104.0 $\pm$ 3.1
20	97.4 $\pm$ 1.7
10	95.6 $\pm$ 2.3
5	104.0 $\pm$ 3.0
1	105.1 $\pm$ 4.5

\* Mean  $\pm$  S.E. ( $n = 3$ ).

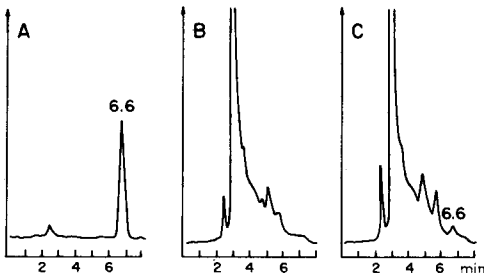


Fig. 1. Chromatograms of standard (A), control sample (B) and sample fortified with 1 ng/ml of YOYH (C). Retention time for the YOYH peak was 6.6 min.

## RESULTS AND DISCUSSION

Recovery of YOYH is shown in Table I and a detection limit of 1 ng/ml is shown in Fig. 1 (a concentration with the peak height of 2% of the full scale was considered the limit of detectability). Detector response was found to be linear in a working range of 1–1000 ng/ml. This method resulted in better extraction recovery (95–105%) and cleaner extracts than previously reported procedures.

This technique allows for the analysis of small amounts of sample. The simplicity, brevity and small sample size requirements of the method make it most convenient for pharmacokinetic studies. In contrast to the existing procedures, this method can be performed using a conventional UV detector. In addition to the above features, this technique leads to an extremely clean extract which enhances the life of the chromatographic column and consequently produces a cleaner chromatogram and less expensive analysis. This process has also successfully applied to cattle and dog plasma.

## REFERENCES

- 1 R. C. Hatch, N. H. Booth and J. C. Clark, *Am. J. Vet. Res.*, 43 (1982) 1009–1014.
- 2 J. V. Kitzman, N. H. Booth and R. C. Hatch, *Am. J. Vet. Res.*, 43 (1982) 2165–2169.
- 3 R. C. Hatch and T. Ruch, *Am. J. Vet. Res.*, 35 (1974) 35–39.
- 4 W. H. Hsu, *Calif. Vet.*, 7 (1983) 19–21.
- 5 R. C. Hatch, N. H. Booth and J. V. Kitzman, *Am. J. Vet. Res.*, 44 (1983) 417–423.
- 6 B. M. Wallner, R. C. Hatch and N. H. Booth, *Am. J. Vet. Res.*, 43 (1982) 2259–2265.
- 7 J. V. Kitzman, R. C. Wilson and R. C. Hatch, *Am. J. Vet. Res.*, 45 (1984) 875–879.
- 8 R. C. Hatch, J. D. Clark and N. H. Booth, *Am. J. Vet. Res.*, 44 (1983) 2312–2319.
- 9 R. C. Hatch, J. D. Clark and N. H. Booth, *Am. J. Vet. Res.*, 34 (1973) 1321–1331.
- 10 M. F. Cronin, N. H. Booth and R. C. Hatch, *Am. J. Vet. Res.*, 44 (1983) 2037–2042.
- 11 M. R. Goldberg, L. Speier and D. Robertson, *J. Liq. Chromatogr.*, 7 (1984) 1003–1012.
- 12 B. Diquet, L. Doare and G. Gaudel, *J. Chromatogr.*, 311 (1984) 449–455.
- 13 J. A. Owen, S. L. Nakatsu, M. Condra, D. H. Surridge, J. Fenemore and A. Morales, *J. Chromatogr.*, 342 (1985) 333–340.