Journal of Chromatography, 345 (1985) 184—186
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2766

Note

Quantitation of gallamine (Flaxedil) in human plasma using high-performance liquid chromatography

McNAMA J. SHAO*, KEVIN D. FALLON, SAMIA N. KHALIL and EZZAT ABOULEISH

Department of Anesthesiology, University of Texas Health Science Center at Houston, 6431 Fannin Street, Houston, TX 77030 (U.S.A.)

(First received March 19th, 1985; revised manuscript received June 19th, 1985)

Gallamine, first synthesized in France in 1947, has been used as a non-depolarizing neuromuscular blocking agent in anesthetic practice since the early fifties. Several methods have been used to assay gallamine triethiodide in biological fluids in order to determine its pharmacokinetic and pharmacodynamic properties in man. Plasma concentrations have been determined by fluorimetry after formation of a complex with Rose Bengal [1—4]. Cohen [5], however, found non-uniform results by fluorescence due to high blanks resulting from excess uncomplexed dye, precipitates at solution interphases, and loss of dye-complexed fluorescence during the extraction.

This report describes a method for the quantitation of nanogram amounts of gallamine in human plasma using reversed-phase high-performance liquid chromatography (HPLC) with UV detection. Since gallamine contains three quaternary ammonium salts and is silica-ionic at neutral pH, we were able to separate the drug by using a bonded C_{18} column and an ion-pairing reagent [6].

EXPERIMENTAL

Chemicals

Gallamine, 99% purified, was purchased from ICN Pharmaceutics (Plainview, NY, U.S.A.). Acetonitrile and phosphoric acid (HPLC grade) and dibasic anhydrous sodium phosphate (analytical-reagent grade) were all purchased from Fisher Scientific (Houston, TX, U.S.A.). 1-Octane sulfonic acid, sodium salt (HPLC grade) was purchased from Aldrich (Milwaukee, WI, U.S.A.). The distilled water was purified through a Milli-Q purified water system (Millipore, Bedford, MA, U.S.A.).

0378-4347/85/$03.30

HPLC apparatus and mobile phase system

We used an HP 1090 HPLC system consisting of a diode-array detector, binary DR5 pump, HP 3392A integrator, autosampler, HP 9121D flexible-disc drive and HP 85 personal computer (Hewlett-Packard, Palo Alto, CA, U.S.A.). A μBondapak octadecylsilane column (particle size 10 μm, 30 cm × 3.9 mm I.D., Waters Assoc., Milford, MA, U.S.A.) was used in an isocratic mode at ambient temperature. The UV detector was set at 230 nm on 0.012 absorbance units full scale (a.u.f.s.). The mobile phase consisted of 2% acetonitrile in an aqueous mixture of 5 mmol/l octane sulfonic acid and 5 mmol/l dibasic sodium phosphate. The final pH was 6.0, adjusted by 1 mol/l phosphoric acid. The eluent was filtered through a 0.45-μm filter (Rainin, Woburn, MA, U.S.A.), and degased prior to use. The integrator was set at 0.2 cm/min with 8 mV equal to full scale. The flow-rate was set to 2 ml/min for 2—3 h in order to equilibrate the column. Then, the flow-rate was lowered to 0.2 ml/min, and about 0.5 h later, the HPLC system was ready to run analyses.

Sample processing

Acetonitrile (0.5 ml) was added to 0.5 ml of human plasma or standards in a polypropylene centrifuge tube (Oak-Ridge type, round bottom). The contents were vortexed for 2 min, and centrifuged at 15 000 *g* for 30 min (4°C) on a J-21 centrifuge (Beckman, Palo Alto, CA, U.S.A.). A 0.5-ml volume of the aqueous phase was pipetted into a 10 × 75 mm test-tube with 0.5 ml of mobile phase. The contents were mixed for 2 min. Of this mixture 5 μl were injected onto the HPLC system for each assay.

RESULTS

We found a detection limit of about 900 ng/ml in human plasma. Gallamine has a retention time of approximately 9.38 min. Fig. 1 shows chromatograms of a human plasma sample with gallamine and a human plasma blank. The concentration of gallamine standard and area count were linearly related over the

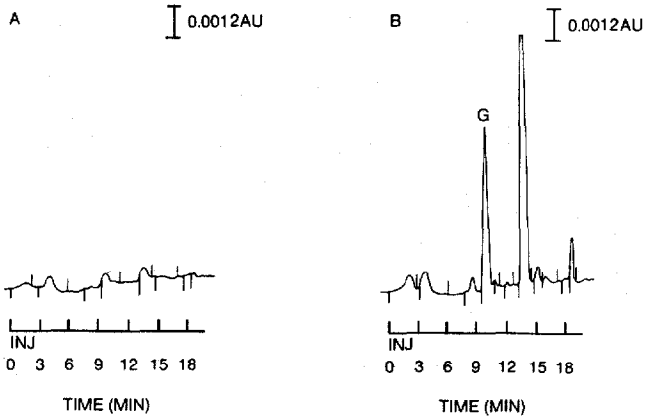

Fig. 1. Chromatograms of a human plasma blank (A) and a human plasma sample from a clinical study (B). The concentration of gallamine (G) is 13.8 μg/ml.

range 4—50 μg/ml with a correlation coefficient (r) of 0.9490 (n=5). The standards were obtained by dissolving gallamine in a plasma blank. To assess analytical recovery, we spiked human drug-free plasma with concentrations of 4, 10, 20, 25, and 50 μg/ml gallamine. Each level was extracted in triplicate, and analyzed according to the procedure. We found levels of 3.72, 10.68, 18.6, 23.1, and 49.42 μg/ml, respectively, with an overall recovery of 96.8±11.4%. We also determined the within-run precision for this method. The coefficient of variation (C.V.) was 8.0% (n=5) at 4 μg/ml gallamine.

DISCUSSION AND CONCLUSION

Gallamine is a potent neuromuscular blocking agent. Since the clinical doses of 1—2 mg/kg body weight are usually administered intravenously, methods of high sensitivity are required for study of the pharmacokinetic and pharmacodynamic behavior of the drug. Using the described conditions, we were able to resolve the gallamine peak from unknown background peaks in the plasma at a flow-rate of 0.2 ml/min. When we attempted to increase the flow-rate to 0.5—2 ml/min, we found that the resolution for gallamine was unsatisfactory. Although the fluorimetric determination has a detection limit of 0.02 μg/ml in human plasma, we found that the variation in the fluorescence intensity was great due to impurities in the Rose Bengal reagent, the complex sample preparation steps, and the high fluorescence background. Therefore, a reversed-phase ion-paired HPLC method was developed that is simple, rapid, precise, selective and sensitive, and permits the quantitation of gallamine in the plasma of patients undergoing therapy with this drug.

ACKNOWLEDGEMENT

We thank Dr. Alan H.B. Wu for his helpful consultations and review of the manuscript and Mrs. Terri Hudsonpillar for her assistance in the preparation of this manuscript.

REFERENCES

1 S. Agonston, G.A. Vermeer, U.W. Kersten and A.H.J. Scaf, Br. J. Anaesth., 50 (1978) 345.
2 M.I. Ramzan, E.J. Triggs and C.A. Shanks, Eur. J. Clin. Pharmacol., 17 (1980) 135.
3 M.I. Ramzan, E.J. Triggs and C.A. Shanks, Eur. J. Clin. Pharmacol., 17 (1980) 145.
4 M.I. Ramzan, C.A. Shanks and E.J. Triggs, Br. J. Clin. Pharmacol., 12 (1981) 141.
5 E.N. Cohen, J. Lab. Clin. Med., 61 (1963) 338.
6 B.A. Bidlingmeyer. J. Chromatogr. Sci., 18 (1980) 525.