Journal of Chromatography, 416 (1987) 203–206
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3543

Note

Quantitation of merbarone in human plasma by high-performance liquid chromatography

Y.-W. STEVENS, T.D. WALSH and M.P. FANUCCHI*

Clinical Pharmacology Laboratory, Developmental Chemotherapy Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, Cornell University Medical School, New York, NY 10021 (U.S.A.)

(First received September 11th, 1986; revised manuscript received December 5th, 1986)

Merbarone, 5-(N-phenylcarboxamido)-2-thiobarbituric acid, has been shown to have potent antitumor activity in the mouse L1210 leukemia model system [1]. Encouraging results were also obtained against murine P388 leukemia, B16 melanoma and M5076 sarcoma [1,2]. Based on these findings, Phase I clinical trials were initiated with this drug. In order to study the pharmacokinetics of merbarone in these patients, we have modified the previously published method of Liao et al. [3]. Our modifications consisted of: (1) inclusion of an internal standard, thiopental sodium, to monitor drug loss during sample preparation; (2) development of a convex gradient for rapid elution of both merbarone and thiopental sodium; (3) addition of triethylamine to the mobile phase to obtain better peak definition; and (4) ultraviolet detection at 300 nm for simultaneously measuring merbarone and thiopental sodium with adequate sensitivity.

EXPERIMENTAL

Pure merbarone was supplied by the Drug Synthesis Branch, National Cancer Institute (Bethesda, MD, U.S.A.). The internal standard, thiopental sodium, of pharmaceutical purity, was obtained from Abbott Labs. (Chicago, IL, U.S.A.). The reagents used were: dimethyl sulfoxide, derivatization grade (Regis, Morton Grove, IL, U.S.A.); HPLC-grade methanol, ammonium acetate and Baker-analyzed magnesium chloride 6-hydrate, all supplied by J.T. Baker (Phillipsburg, NJ, U.S.A.); HPLC-grade triethylamine (Pierce, Rockford, IL, U.S.A.); and Milli-Q Type I reagent-grade water (Millipore, Bedford, MA, U.S.A.).

0378-4347/87/$03.50

Stock solutions containing 50, 100, 200, 300, 400 and 500 μg/ml merbarone were prepared in dimethyl sulfoxide and kept at −80°C until use. The plasma standard curve consisted of 0.5, 1, 2, 3, 4, 5, 10 and 20 μg/ml merbarone. These standard solutions were prepared by adding appropriate volumes of the stock solutions of merbarone to 1 ml of pooled plasma, stored at 4°C and used within two weeks. Recovery standards were prepared fresh for each run using the initial mobile phase of the gradient (40% methanol in 300 m*M* ammonium acetate–60 m*M* magnesium chloride–7.2 m*M* triethylamine). Our method of sample preparation and analysis was a modification of the procedure of Liao et al. [3] and Malspeis [4]. Briefly, 50 μl of plasma standard or 50 μl of patient plasma were mixed with 250 μl of methanol–dimethyl sulfoxide (85:15, v/v) containing 460 ng of thiopental sodium, the internal standard, by vortexing. The mixture was centrifuged at 15 600 *g* for 5 min. A 200-μl volume of the supernatant was diluted with 300 μl of Milli-Q water and then analyzed by high-performance liquid chromatography (HPLC) with ultraviolet detection. Injection volumes of 50 or 100 μl were used.

A C_{18} Nova-Pak reversed-phase column (15 cm×3.9 mm, 4 μm particle size, Waters Assoc. Milford, MA, U.S.A.) attached to a Bio-Rad precolumn (40 mm×4.6 mm ODS-10, Bio-Rad Labs., Richmond, CA, U.S.A.) was used. Analyses were performed at ambient temperature using a Waters liquid chromatograph consisting of two M6000A solvent delivery systems, a 710B WISP automatic sampler, a 730 data module and a 720 system controller. The ultraviolet spectra of merbarone and thiopental sodium were obtained using a Model 576 scanning spectrophotometer (Perkin-Elmer, Norwalk, CT, U.S.A.). The maximal wavelengths of merbarone and thiopental sodium were 305 and 291 nm, respectively, and were similar to that of thiobarbital [5]. We detected merbarone and thiopental sodium at 300 nm using a Lambda-Max Model 481 LC spectrophotometer (Waters Assoc.) with a sensitivity of 0.01 absorbance units full scale. The convex gradient employed (curve No. 5, manual for Model 720 system controller, Waters Assoc.) consisted of 40% methanol (in 300 m*M* ammonium acetate–60 m*M* magnesium chloride–7.2 m*M* triethylamine) to 80% methanol (in 100 m*M* ammonium acetate–60 m*M* magnesium chloride–7.2 m*M* triethylamine) over 15 min with a flow-rate of 1 ml/min. The retention times of merbarone and thiopental sodium were 4.36 and 10.53 min, respectively.

RESULTS AND DISCUSSION

Typical chromatograms of a plasma blank, a recovery standard, a plasma standard and a plasma sample obtained from a patient after intravenous administration of merbarone are shown in Fig. 1. Quantitation was based on linear least-squares regression analyses of the peak-area ratios (merbarone to internal standard) versus concentrations of merbarone in the plasma standard curve (0.5 – 20 μg/ml, $r=0.999$). The limit of detection of our method was 0.5 μg/ml merbarone with a signal-to-noise ratio at this level of 15:1. The mean recovery of merbarone from plasma at 0.5 μg/ml ($n=10$) and 20 μg/ml ($n=10$) was 94 and 97% with a coefficient of variation of 2.7 and 4.9%, respectively. The internal standard

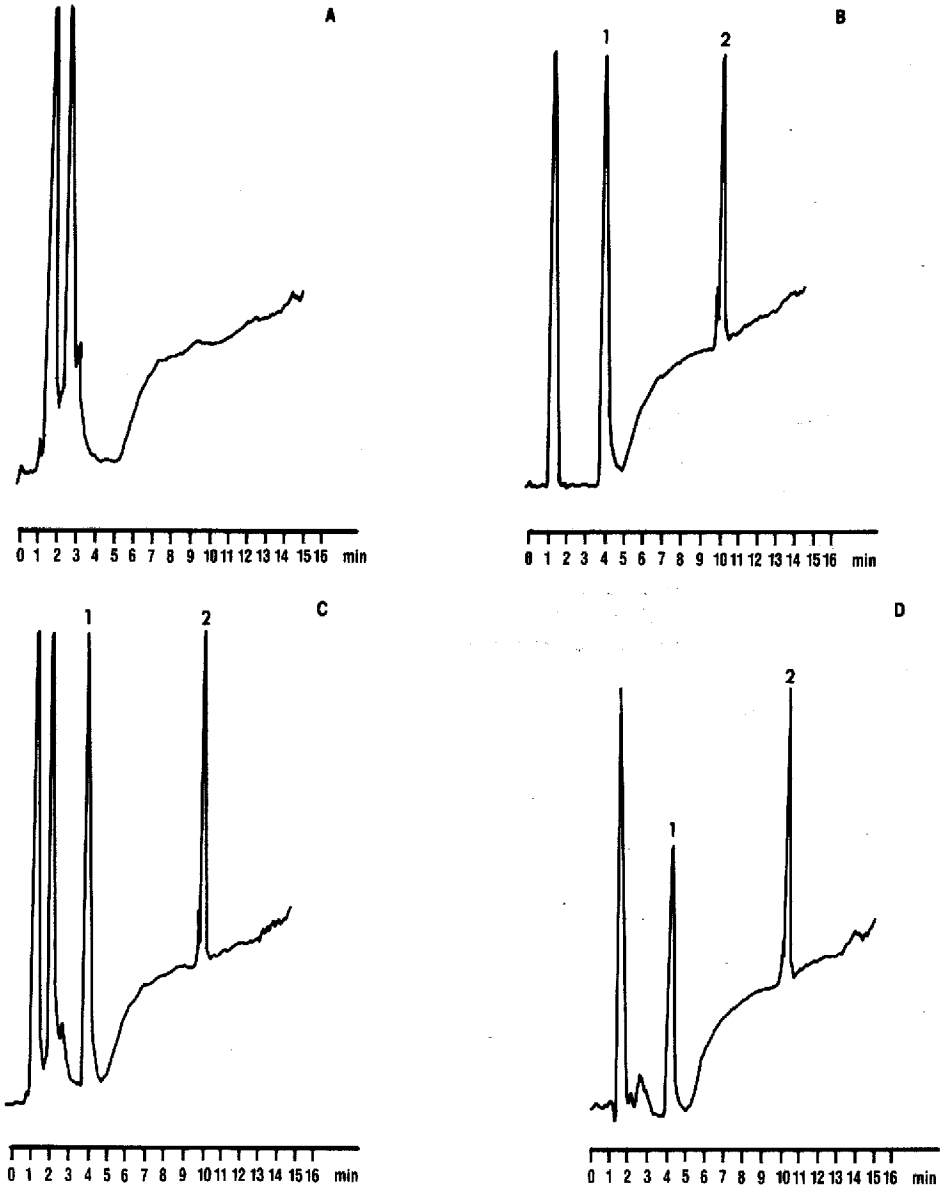

Fig. 1. Chromatogram of an injection of (A) a plasma blank, (B) a recovery standard of merbarone at 5 μg/ml and internal standard, (C) a plasma standard of merbarone at 5 μg/ml and internal standard, and (D) a plasma sample from a patient treated with merbarone. Peaks: 1 = merbarone; 2 = thiopental sodium.

was recovered quantitatively following protein precipitation. The coefficient of variation of injection for an extracted plasma standard containing 0.5 μg/ml merbarone was 6.7% ($n=10$) within runs and 7.4% ($n=10$) between runs on two consecutive days. The coefficient of variation of ten injections of a recovery standard containing 4 μg/ml merbarone was 1.1%. The accuracy of the method was

checked by including a quality-control sample with a calculated value of 5.0 μg/ml merbarone in plasma. The coefficient of variation of such samples was 4.5% with a mean ($\pm$S.D.) concentration of 4.80 $\pm$ 0.21 μg/ml merbarone.

In summary, we have developed a simple and reliable method for quantitating merbarone in plasma using an internal standard to monitor drug loss during sample preparation. In contrast to the gradient elution reported here, with the isocratic method of Liao et al. [3], merbarone was eluted with a longer retention time (12 min), and the internal standard, thiopental sodium, was retained on the column. We therefore modified the HPLC conditions to substantially reduce the retention time of merbarone and elute the internal standard while maintaining good separation between them. We have been using our method satisfactorily for several months to study the pharmacokinetics of merbarone in the plasma of cancer patients treated with this drug.

ACKNOWLEDGEMENTS

We wish to thank Dr. L. Malspeis for his suggestions. We are also grateful to Dr. C. Young for critical review of the manuscript, to Mr. G. Lokos for technical advice and to Ms. Shelley Sunshine and Ms. Adella Welch for preparation of the manuscript. Supported by NIH Grants CA 05826 and CA 08748, NIH Contract NO1 CM 57732 and a fellowship from the JM Foundation (T.D.W.).

REFERENCES

1 A.D. Brewer, J.A. Minatelli, J. Plowman, K.D. Paull and V.L. Narayanan, Biochem. Pharmacol., 34 (1985) 2047.
2 J. Plowman, K.D. Paull, V.L. Narayanan, A.D. Brewer and J.A. Minatelli, Proc. Am. Assoc. Cancer Res., 26 (1985) 251.
3 J.T. Liao, W.T. Collins, Jr., R.A. Hiles and R.V. Rutschilling, Appendix A, Addendum to Report No. SIBR-84-03, Phase II Report of Preclinical Continuous Intravenous Infusion Toxicity Study of Merbarone (NSC-336628) in Beagle Dogs, Toxicology Branch, National Cancer Institute, Bethesda, MD, 1985, internal publication.
4 L. Malspeis, personal communication.
5 M.T. Bush, P. Mazel and J. Chambers, J. Pharmacol. Exp. Ther., 134 (1961) 110.