Journal of Chromatography, 422 (1987) 353–355
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3862

Letter to the Editor

High-performance liquid chromatographic determination of pyridostigmine in plasma

Sir,

Pyridostigmine, which is used in the treatment of patients with myasthenia gravis, has been determined by spectrophotometric [1] and gas or liquid chromatographic analysis [2–7]. High-performance liquid chromatography (HPLC) is a more sensitive method, and Ellin et al. [2] published a method for measuring pyridostigmine in plasma and urine using Sep-Pak C_{18} cartridges on porous silica (Waters Assoc., Milford, MA, U.S.A.).

We have assayed plasma levels of pyridostigmine using a Bond Elut CBA cation-exchange column (Analytichem International, Harbor City, CA, U.S.A.), and achieved a limit of detection of 10 ng/ml for pyridostigmine in plasma.

EXPERIMENTAL

Pyridostigmine bromide (3-dimethylcarbamoyloxy-1-methylpyridinium bromide) was obtained from Roche (Tokyo, Japan). The internal standard, pralidoxime iodide (2-pyridine aldoxime methyl iodide), was obtained from Sumitomo (Osaka, Japan). A 1.0-ml aliquot of serum or plasma were transferred to a 10-ml centrifuge tube, and 0.5 ml of 10% trichloroacetic acid was added. The contents of the tube were vortex-mixed for 10 s and centrifuged at 1500 *g* for 10 min. The supernatant was transferred to another centrifuge tube and 250 μl of 0.6 *M* sodium hydroxide were added. The contents were vortex-mixed for 10 s. A 1.0-ml aliquot of the aqueous layer was placed in a Bond Elut 1-ml carboxylic acid (CBA) column, which was hung in a centrifuge tube, and centrifuged at 90 *g* for 5 min. The Bond Elut column was washed with 1.0 ml of water, 1.0 ml of 0.05 *M* acetic acid and 1.0 ml of methanol (R.G.). Finally, 1.0 ml of 1 *M* hydrochloric acid in methanol solution was added for sample elution, and the column was hung in another centrifuge tube and centrifuged at 90 *g* for 5 min. The tube was taken to dryness over nitrogen gas in a water-bath set at 40°C. The dry residue was dissolved in 100 μl of internal standard solution (1 mg of pralidoxime iodide in 100 ml of 50% acetonitrile), and 80 μl of this sample were injected into the liquid chromatograph.

The liquid chromatographic system consisted of a Waters Assoc. Model 6000A

0378-4347/87/$03.50

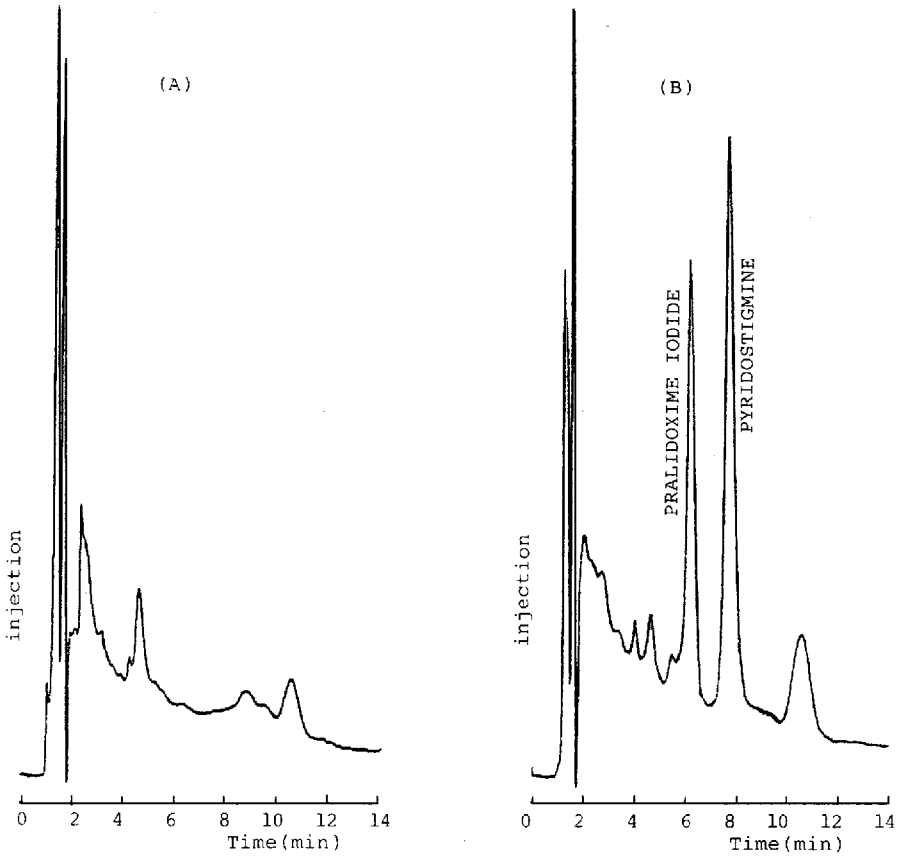

Fig. 1. Liquid chromatograms of (A) human plasma blank passed through a Bond Elut cartridge and (B) pyridostigmine isolated from human plasma by a Bond Elut cartridge. Pralidoxime iodide is the internal standard.

and Model M45 high-pressure pump, a U6K loop injector, a Model 480 variable-wavelength UV detector, a Model 680 automated gradient controller and a data module M730.

The mobile phase consisted of 0.1% triethylamine (R.G.) (pH 3.2 by acetic acid) in water and acetonitrile (R.G.) (50:50, v/v). The mobile phase flow-rate was 1.0 ml/min. The UV detector was set at 270 nm and was operated at 0.005 a.u.f.s. A prepacked 100×5 mm I.D. Radial-Pak CN column, particle size 10 μm (Waters Assoc.), was used at ambient temperature.

RESULTS

The chromatograms show that the separation of pyridostigmine and pralidoxime iodide from endogenous substance was good (Fig. 1). The retention time of pyridostigmine was 7.9 min and of pralidoxime iodide 6.2 min. The ratio of the peak height of pyridostigmine to that of the internal standard plotted against

drug concentration was linear between 10 and 200 ng/ml, with a correlation coefficient of 0.998.

The recoveries of pyridostigmine from human plasma to which 50, 100 and 200 ng/ml had been added were 83.8 ± 8.4, 86.5 ± 6.2 and $95.0 \pm 6.4\%$ ($n = 5$), respectively. A limit of detection of 2 ng/ml (signal-to-noise ratio > 2) was achievable for all analyses.

In conclusion, the proposed HPLC assay for pyridostigmine in plasma is more sensitive than other reported HPLC assays [2, 6].

Hospital Pharmacy,
Saga Medical School,
Nabeshima-Sambonsugi,
Nabeshima-machi,
Saga 840-01
(Japan)

H. MATSUNAGA
T. SUEHIRO
T. SAITA
Y. NAKANO
M. MORI
K. TAKATA*

Department of Internal Medicine,
Saga Medical School,
Nabeshima-Sambonsugi,
Nabeshima-machi,
Saga 840-01 (Japan)

K. ODA

1 H. Coper, G. Dehyle and K. Dross, Z. Klin. Chem. Klin. Biochem , 12 (1974) 273.
2 R.I. Ellin, P. Zvirblis and M.R. Wilson, J. Chromatogr., 228 (1982) 235.
3 K. Chan, N.E. Williams, J.D. Baty and T.N. Calvey, J. Chromatogr., 120 (1976) 349.
4 S.L. Cohan, J.L. Pohlmann, J. Mikszewski and D.S. O'Doherty, Neurology, 26 (1976) 536.
5 T.N. Calvey and K. Chan, Clin. Pharmacol. Ther., 21 (1977) 187.
6 M.G.M. De Ruyter, R. Cronnelly and N. Castagnoli, Jr. J. Chromatogr., 183 (1980) 193.
7 M.C. White, P. De Silva and C.W.H. Harvard, Neurology, 31 (1981) 145.

(First received April 14th, 1987; revised manuscript received July 7th, 1987)