Journal of Chromatography, 494 (1989) 397–402
Biomedical Applications
Elsevier Science Publishers B V , Amsterdam — Printed in The Netherlands

CHROMBIO 4872

Note

Determination of tacrine and its 1-hydroxy metabolite in plasma using column liquid chromatography with ultraviolet detection

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(First received April 5th, 1989, revised manuscript received May 22nd, 1989)

Tacrine or tetrahydroaminoacridine (THA, Fig 1) is a potent acetylcholinesterase inhibitor, which has recently been employed in the therapy of Alzheimer's disease [1] To be able to study the kinetics of this drug a method was designed which has sufficient sensitivity to monitor plasma levels of THA and its 1-hydroxy metabolite (THA-1-ol, Fig 1) down to a few nanograms per millilitre of plasma A previous method based on high-performance liquid chromatography (HPLC) [2] had a limit of determination of 50 ng/ml, which, according to preliminary clinical investigation, is out of range Another HPLC method had similar drawbacks [3] Recently an HPLC method was published utilizing fluorescence detection, which had a sensitivity of the same order as the method to be presented here [4] However, that report did not describe the measurement of the metabolite of THA, and neither did the two other methods referred to The present method was also based on HPLC, and designed with simplicity and optimal sensitivity in mind

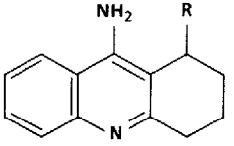

Fig 1 Chemical structures of tacrine (R = H, 9-amino-1,2,3,4,-tetrahydroacridine) and its 1-hydroxy metabolite (R = OH, 9-amino-1,2,3,4-tetrahydroacridine-1-ol)

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0378-4347/89/$03 50

EXPERIMENTAL

Standards and chemicals

Tacrine and its 1-hydroxy metabolite were gifts from Nobel Kemi (Sollentuna, Sweden) Organic solvents and other chemicals used were of standard analytical and HPLC quality (Merck, Darmstadt, F R G)

Column liquid chromatography

The column liquid chromatographic system consisted of an M 45 pump, a Rheodyne injector (Millipore Waters, Milford, MA, U S A) and a 655A variable-wavelength UV detector (Hitachi, Tokyo, Japan) operated at 240 nm The column (150×4 6 mm I D , stainless steel) was slurry-packed with Nucleosil C_{18} (5-μm particles, Macherey-Nagel, Duren, F R G) and eluted with acetonitrile–0.02 *M* phosphate buffer, pH 2 7 (20 80, v/w) at a flow-rate of 1 1 ml/min When the metabolite, THA-1-ol, was analysed the eluent had an acetonitrile content of 15%

Plasma assay

In a 10-ml screw-capped tube, a 1-ml plasma sample was added to 0 5 ml of 0 5 *M* NaOH solution and 5 ml of dichloromethane The mixture was shaken gently on a shake board for 15 min and then centrifuged for 5 min (500 *g*) After removal of the aqueous phase, 4 ml of the organic phase were transferred to a conical-bottomed tube, and the solvent was evaporated by ventilating with nitrogen gas (heating block, ca 40°C) The residue was dissolved in 150 μl of the mobile phase, and normally 50 μl was injected into the chromatograph For the lowest levels measurable this amount was doubled

RESULTS AND DISCUSSION

Extraction and recovery

The recovery was calculated by comparing the chromatographic peaks obtained from spiked plasma samples processed through the method with those resulting from injected standards of THA and THA-1-ol dissolved in the eluent Extraction of THA and THA-1-ol from plasma samples with dichloromethane as described gave an absolute recovery of 93 ± 2% for both substances To prevent the formation of emulsions during the extraction of plasma samples under alkaline conditions with the chlorinated solvent, these extractions were carried out on a shake board under gentle shaking The temperature of the heating block used for the evaporation did not seem to be critical, and was held at around the boiling point of the solvent

Column liquid chromatography

Fig 2 shows chromatograms of plasma samples, containing THA and THA-1-ol, obtained after processing them through the method described above Fig 3 shows chromatograms of plasma analysed with an eluent containing 15% acetonitrile. If THA-1-ol is to be quantified simultaneously with THA, this eluent with a slightly lower content of acetonitrile can be used. The detectability of THA will, however, be somewhat less owing to the broadening of the THA peak (retention time 14 min compared with 6 min) The eluent with higher acetonitrile content cannot be used for determining THA-1-ol because it frequently interferes with plasma background peaks (as well as peaks representing minor metabolites of THA) of low retention Plasma samples from a few patients have been observed to generate late eluting peaks, which might cause disturbances on repeated injections The use of an internal standard was

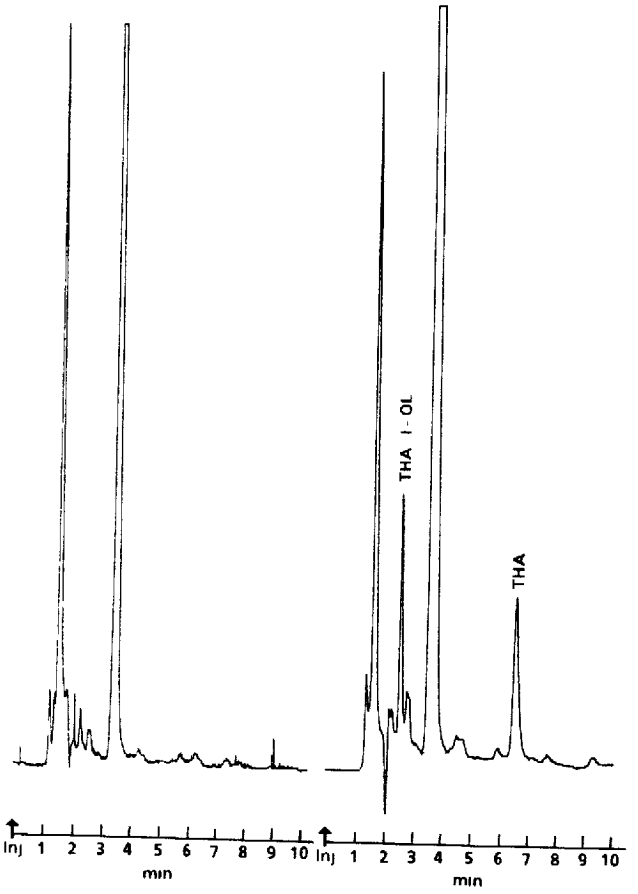

Fig 2 (Left) Chromatogram of a blank plasma sample (Right) Chromatogram of a plasma sample containing 10 ng/ml THA

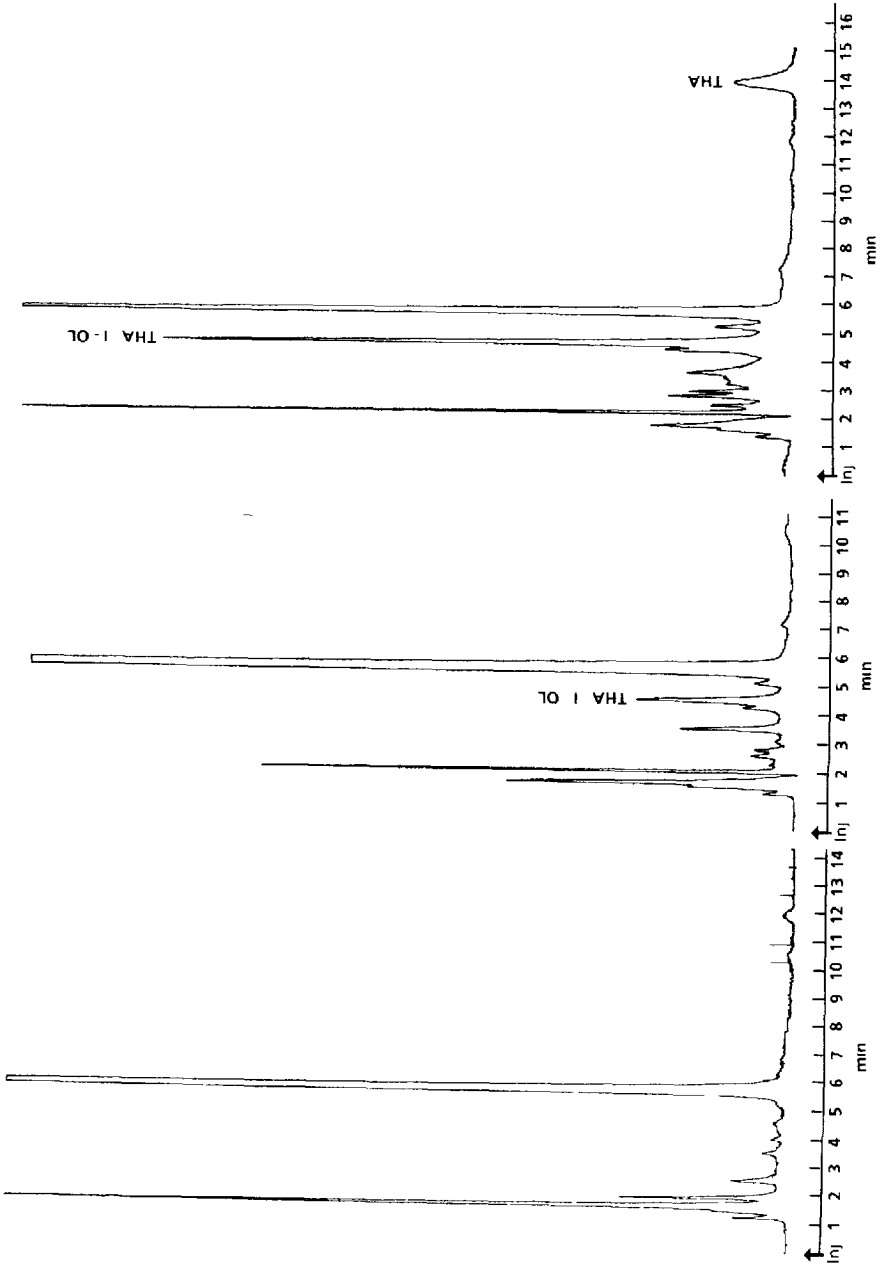

Fig 3 (Left) Chromatogram of a blank plasma sample obtained when eluting the column with a mobile phase containing 15% acetonitrile (Centre) Chromatogram of a plasma sample spiked with 30 ng/ml THA-1-ol (Right) A sample from a patient containing 10 ng/ml THA and 130 ng/ml THA-1-ol

not considered advantageous, since the extraction procedure applied consisted of only one extraction step and an exact volume of the organic solvent extract was simple to transfer in a reproducible way

Standard curve and precision

Calibration graphs were constructed by spiking samples of plasma with different concentrations of THA and THA-1-ol and analysing them according to the method The resulting peak heights were plotted versus the concentrations of THA The curves were linear and passed through the origin (correlation coefficients were 0 9999 and 0 9993 for THA and THA-1-ol, respectively). The coefficient of variation (C V) was determined to 2.7% for the 5 ng/ml level of THA ($n=10$, $4\ 98 \pm 0\ 07$) using an eluent containing 20% acetonitrile The C V at 5 ng/ml for THA-1-ol was 4% ($n=10$, $5\ 04 \pm 0\ 10$) The detection limit, at a signal-to-noise ratio of 2, was ca 0 3 ng/ml for both compounds

Application

Fig 4 shows plasma concentration ~ time curves for THA and THA-1-ol resulting after oral administration of a morning dose of 25 mg of THA to a patient, who was on a dose regimen of 75 mg of THA daily and in steady state. A study of patients suffering from Alzheimer's disease, in which the present method was used for determining THA, has been published [5]

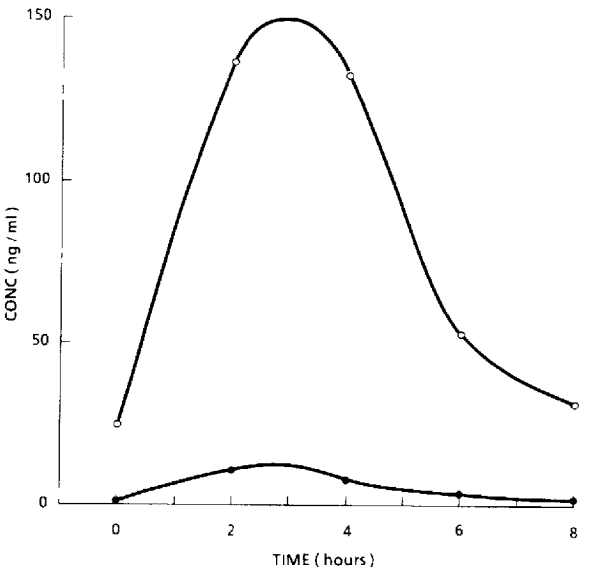

Fig 4 Plasma concentration-time curves of THA (●) and THA-1-ol (○) obtained from a patient given a final morning dose of 25 mg of THA after being on a daily dose regimen of 75 mg THA for 6 weeks

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