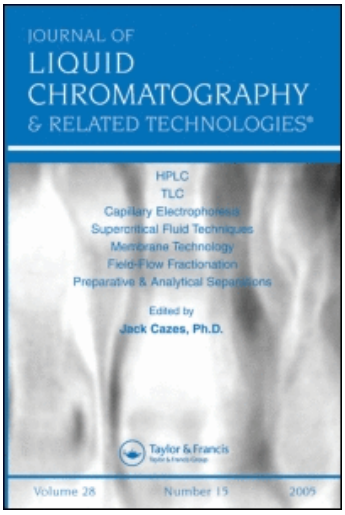

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Tetrahydroaminoacridine (THA) Analysis by HPLC

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TETRAHYDROAMINOACRIDINE (THA) ANALYSIS BY HPLC

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ABSTRACT

The potent acetylcholinesterase and butyrylcholinesterase inhibitor tetrahydroaminoacridine (THA) has been used to reverse the signs and symptoms of the acute anticholinergic syndrome in man and other animals. In order to study the pharmacokinetics of THA and related drugs we have developed an HPLC analytical method based on UV absorbance at 240 nm in TEAP buffer which permits quantitative determination in the ng/ml range in biological samples.

Tetrahydroaminoacridine (THA) is a potent acetylcholinesterase and plasma butyrylcholinesterase inhibitor[1]. THA has been used clinically to reverse the signs and symptoms of the central anticholinergic syndrome[2,3] often associated with overdosage of

tricyclic antidepressants and with overdosage of the illicit drug phencyclidine (PCP)[4]. We describe a rapid, non-destructive and sensitive HPLC analytical method which permits quantitative determination of THA in biological samples in the nanogram/ml range.

This method was developed in conjunction with our clinical research in the pharmacokinetics of THA in humans. THA is currently available to physicians as an investigational drug by application through the FDA.

MATERIALS AND METHOD

Reagents

TEAP buffer was prepared by titrating 0.10 N phosphoric acid (A.R. Mallinkrodt) to a pH = 2.75 with triethylamine (Sigma). The buffer solution was filtered through 0.008" Schieicher and Schuell filter paper and degassed by sonifaction for 3 minutes.

Plasma Samples

Two ml aliquots of human plasma spiked with known amounts of THA were adjusted to a pH of 9.0 with 400 ul of Tris buffer in a 5 ml conical glass-stoppered centrifuge tube. 500 ul of chloroform was added and the test tube was vigorously mixed for 1 min on a Vortex Mixer and then centrifuged at 2000g for 5 min. The 500 ul organic phase was isolated by removing the lower aqueous phase by careful aspiration with a micropipet. 100 ul of 0.1 M TEAP buffer (pH = 1.00) was added to the

500 ul organic phase, the test tube was vigorously mixed (Vortex Mixer) for 1 min, and then centrifuged at 2000g for 1 min. The 100 ul aqueous phase was removed by micropipetting and transferred to a 4 x 50 mm stoppered tube, 10 ul 0.5 m TEAP pH 4.0 was added; and the tube was mixed (Vortex Mixer, 10 sec). 20 ul of this aqueous THA extract was injected into the HPLC.

HPLC

We used an Altex HPLC System (Altex Instruments, Berkeley, CA, USA) made up of a Model 400 Solvent Programmer, two Model 100A Pumps, Injector Valve #905-42 (Rheodyne), Model 15 Variable Wavelength Detector with a 20 microliter flowcell (Hitachi), Model 385 Chart Recorder (Linear) and a Chromatography Data System, CDS 111 (Varian).

All experiments were run at room temperature. The mobile phase consisted of 0.10 M TEAP pH 2.75 and acetonitrile (75/25). The flow rate was 1.5 ml/min with a corresponding pressure of 1500 psi. The wavelength used to monitor eluted compounds was 240 nm, the absorption maximum for THA. (Fig. 1)

Columns and Column Packing

The column was a Bio-Rad Reversed Phase C_{18} 4 x 250 mm, Whatman Partisil - 10 ODS-2 packing material. A pre-column packed with Perisorb RP-18 (Merck) 30-40 u particle size was used in all work.

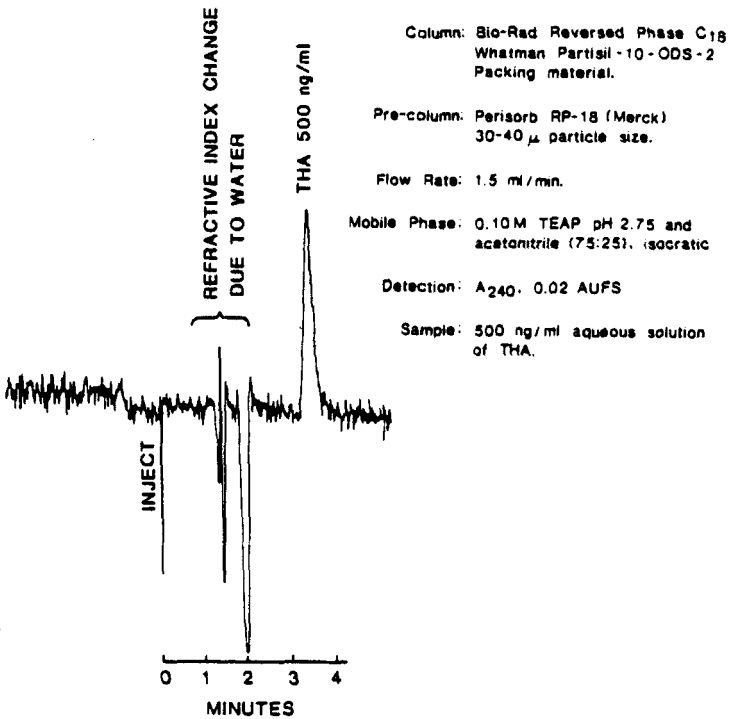

FIGURE 1

ISOCRATIC ANALYSIS OF TETRAHYDROAMINOACRIDINE (THA)

Results and Discussion

Calibration curves were linear and quantitative measurements were made to a lowest concentration of 50 ng/ml (detection limit 10 ng/ml) for extractions from aqueous standard solutions and from human plasma (or serum) samples containing added THA. The assay was linear and reproducible from 50 ng/ml THA added to blank human serum to 10,000 ng/ml. (Fig. 2) Ten separate extractions of one such spiked serum gave mean values of

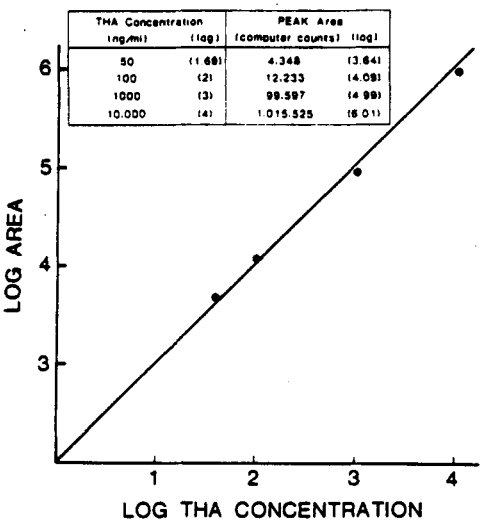

FIGURE 2
THA CALIBRATION CURVE

104.5 ng/ml ± 2.04 (S.E.M.). Ten separate injections of each of 4 spiked blank sera (50 ng/ml, 100 ng/ml, 1000 ng/ml and 10,000 ng/ml) gave mean calculated values of 48.1 ± 3.1, 103.6 ± 3.5, 995.9 ± 32.8, and 10,155 ng/ml ± 272.4 (S.E.M). This HPLC method allows determination, with minimal sample preparation, of THA blood (and urine, saliva, and/or CSF) levels in patients treated with THA.

Development of this method is useful in clinical investigations of the pharmacokinetics of THA and similar cholinergic anti-coma agents.

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