

## Spectrofluorimetric determination of tacrine in pharmaceuticals and spiked human serum

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**A spectrofluorimetric method to determine tacrine is proposed and applied to the determination of tacrine in human serum and pharmaceuticals. The fluorimetric method allows the determination of 1–70 ng ml<sup>-1</sup> of tacrine in aqueous solutions containing acetic acid–sodium acetate buffer (pH 5.6) with  $\lambda_{\text{exc}} = 242$  nm and  $\lambda_{\text{em}} = 362$  nm.**

**Keywords:** Tacrine determination; fluorescence; serum; pharmaceuticals

Tacrine hydrochloride (THA, 9-amino-1,2,3,4-tetrahydroacridine monohydrochloride) (Fig. 1) is a centrally acting reversible cholinesterase inhibitor, which has been used in the management of anti-cholinergic overdoses, for intractable pain of terminal carcinoma, as a decurarizing agent, and more recently in the treatments of tardive dyskinesia and for patients with Alzheimer's disease, being the first drug approved by the US Food and Drug Administration for this disease; in this case, data indicate the existence of a therapeutic window with effective blood levels of THA between 5 and 80 ng ml<sup>-1</sup>.

Several reports describe high-performance liquid chromatography methods for the analysis of plasma concentrations of THA alone using either ultraviolet<sup>1–3</sup> or fluorescence detection.<sup>4–7</sup> The methods with UV detection show detection limits well above that desired for a clinically useful assay method to monitor THA levels in blood samples of human subjects.

This work constitutes the first stage of a research schedule focused on the proposal of new analytical procedures for the determination of tacrine as alternatives to the HPLC methods. This article describes its spectrofluorimetric determination in serum and also has been applied to its determination in pharmaceuticals.

The spectrofluorimetric features of THA tested in our experiments are as follows: the excitation wavelength exhibits a maximum at 242 nm; at this value, THA solutions show emission spectra whose maxima occur at 362 nm within a pH range from 1 to 7. The fluorescence intensity measured at 362 nm with excitation at 242 nm remains unaffected by total acetate buffer concentrations lower than 0.03 M and total ionic strength up to 1 M.

### Experimental

#### Reagents

Tacrine hydrochloride (THA) was kindly provided by Warner-Lambert/Parke-Davis (Ann Arbor, MI, USA). Concentrated acetic acid, sodium acetate, sodium hydroxide, isopropanol and chloroform, were of analytical-reagent grade and purchased

from Merck (Darmstadt, Germany). High-purity water was obtained from a Millipore (Milford, MA, USA) Milli-Q Plus system. Stock standard THA solution of 100 µg ml<sup>-1</sup> and working standard solution of 1 µg ml<sup>-1</sup> were prepared by dissolving the THA in high-purity water; both solutions were stable for several months at room temperature. Serum samples were obtained from clinical patients. To adjust the pH of the solutions, an acetic acid–sodium acetate 0.1 M buffer of pH 5.6 was used.

#### Apparatus

Fluorescence intensity was measured on a Perkin-Elmer (Norwalk, CT, USA) LS-5 luminiscence spectrometer equipped with a xenon-lamp and a Acer Model 1030 computer working with the FLUORPACK software from Sciware (Mallorca, Spain). All the measurements took place in a standard 10 mm pathlength quartz cell, thermostated at 25.0 ± 0.5 °C, with 5 nm bandwidths for the emission and excitation monochromators.

A Philips (Eindhoven, Netherlands) Model PU-8720 UV/VIS spectrophotometer was used for the absorbance measurements.

The pH was measured on a Crison (Barcelona, Spain) microPH 2002 pH-meter.

Centrifugation of serum samples was carried out with a Sigma (Osterode, Germany) Laborzentrifugen 4-10.

For agitation in the serum extraction procedure, a Selecta (Barcelona, Spain) Vibromatic 384 was used.

#### Samples

The proposed procedure for the determination of THA was applied to one Spanish commercialized pharmaceutical formulation (Cognex capsules). Human sera were kindly provided from hospitals in the city.

#### Capsule treatment

The total content of the capsule is weighed, dissolved in water and diluted to 1 l with water. Filter the solution through a 0.45 µm filter and take suitable aliquots for the determination of THA.

#### Sera preparation

Serum (0.5 ml) spiked with a maximum of 0.25 ml of THA solution (of suitable concentration) is poured into a 15 ml centrifuge tube with a thread lock and then, 0.5 ml of 1 M sodium hydroxide solution, 0.6 ml of isopropanol and 6 ml of chloroform are added. The tube is vigorously shaken in a mechanical shaker for 15 min and then centrifuged (2500g) for 5 min. The organic layer is transferred to a reservoir for collecting subsequent organic phases. The aqueous phase is treated with 4 ml of chloroform, agitated for 10 min by the mechanical shaker and centrifuged for 5 min. The organic phase is transferred again to the reservoir and the treatment of the remaining aqueous phase is repeated again. The final organic extract is also transferred to the reservoir and all the combined organic extracts are then evaporated to dryness under a nitrogen stream. The tube is removed immediately after drying and the

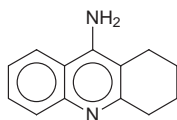


Fig. 1 Formula of tacrine (THA).

solution reconstituted with 5 ml of water. For the determination procedure prepare a blank under the same conditions but without THA spiking.

### Spectrofluorimetric determination of THA

Into 10 ml calibrated flasks place suitable aliquots of working solution of THA containing 10–70 ng of THA; add 2 ml of 0.1 M acetic acid–sodium acetate buffer (pH 5.6) and dilute to the mark with water. Thermostat at  $25 \pm 0.1^\circ\text{C}$  and measure the fluorescence at 362 nm using an excitation wavelength of 242 nm against a blank solution. Determine the concentration of THA in the sample from a calibration graph prepared under identical conditions. The prepared solutions remain stable for at least 24 h.

## Results and discussion

### Figures of merit and validation of the spectrofluorimetric determination of tacrine

#### Selectivity: effect of interfering substances

A study of some potential interferents in the spectrofluorimetric determination of THA was performed by selecting them as the excipients often used in tablet formulations.

Samples containing a fixed amount of THA ( $50\text{ ng ml}^{-1}$ ) and variable concentrations of excipients were measured. Lactose, sucrose, glucose and fructose do not cause interference at weight ratios of excipient/THA  $\leq 10\,000$ .

#### Linearity of the response

A series of standard solutions (four replicates) of THA were prepared following the procedure described in the Experimental section. The calibration graph fluorescence intensity ( $Y$ ) vs. THA concentration ( $X$ ) was found to be linear over the range  $1.0\text{--}70.0\text{ ng ml}^{-1}$  of THA:

$$Y = (0.5 \pm 0.8) + (6.36 \pm 0.03) X \quad (n = 10, r = 0.9997) \quad (1)$$

The application of Student's  $t$ -test shows that the intercept is insignificant and accordingly, the straight line passes through the origin.

#### Accuracy study: recovery assays from spiked sera

Serum samples were spiked with THA to obtain concentrations of 40 and  $80\text{ ng ml}^{-1}$  and treated according to the spectrofluorimetric procedure described above. Poor recoveries were found ranging from 74 to 77%, values very similar to those obtained by other authors.<sup>5,7</sup> In order to avoid matrix effects affecting the results obtained by applying external calibration, the standard additions method was used.<sup>8</sup> The results obtained for the additions of 40 and  $80\text{ ng ml}^{-1}$  (five replicates) were  $40.3 \pm 1.5$  and  $78.1 \pm 2.5\text{ ng ml}^{-1}$ , respectively. This leads to recoveries of  $1.01 \pm 0.04$  and  $0.98 \pm 0.03$ . The application of Student's  $t$ -test indicates that the method is accurate (null hypothesis accepted).<sup>9</sup>

#### Precision

Eleven replicates carried out on different days within a month of a target solution of  $40\text{ ng ml}^{-1}$  were made by using the proposed procedure. The result was  $39.9 \pm 0.2$ , which leads to an RSD of 0.5%.

#### Detection and quantitation limit

According to the Analytical Methods Committee,<sup>10</sup> the detection limit (LOD) is the concentration of THA corresponding to a signal equal to the blank mean ( $Y_B$ ) plus three times the standard deviation of the blank ( $s_B$ ). Eleven blank measurements gave as an average a signal blank of  $Y_B = 0.3$  and a standard deviation of  $s_B = 0.4$ . Thus, the analytical signal corresponding to LOD is  $Y_B + 3s_B$ . This value was calculated with the equation of the calibration line, giving  $0.24\text{ ng ml}^{-1}$ .

**Table 1** Results from the analysis of capsules of Cognex by the proposed method and for direct spectrophotometric determination at 239.8 nm

Sample	Proposed method*	Spectrophotometric method*
Cognex 10	$4.43 \pm 0.06$	$4.38 \pm 0.03$
Cognex 20	$8.66 \pm 0.06$	$8.79 \pm 0.11$
Cognex 30	$13.32 \pm 0.24$	$13.68 \pm 0.07$
Cognex 40	$17.56 \pm 0.14$	$17.55 \pm 0.12$

\* Percentage (w/w) of THA. Average for three determinations  $\pm$  standard deviation.

From the calibration straight line it is also possible to estimate the quantitation limit as the THA concentration corresponding to the ratio between three times the standard deviation of the intercept and the slope of the calibration line.<sup>9</sup> This leads to a quantitation limit of  $0.38\text{ ng ml}^{-1}$ .

### Analysis of pharmaceutical samples

Capsules of Cognex (Parke-Davis) with THA label contents of 10, 20, 30 and 40 mg, and total weights around 250 mg were prepared according to the procedure previously described in the experimental section and analysed with the proposed method using external calibration. The results were compared with those obtained from an independent method (direct spectrophotometric determination at 239.8 nm) in Table 1. As can be observed there is good agreement between both methods. This is also statistically proved according the paired  $t$ -test.<sup>9</sup>

## Conclusions

The results obtained show that the proposed method may be a useful procedure to determine tacrine in spiked human serum at the levels obtained after the administration of normal clinical doses and it would be a method of choice for tacrine monitoring in patients. The method has also been applied to the determination of the active constituent in commercial pharmaceuticals.

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