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Spectrofluorimetric determination of velnacrine in human serum and urine

I. Aparicio, M. Callejón *, M.A. Bello, J.C. Jiménez, A. Guiraúm

Department of Analytical Chemistry, Faculty of Chemistry, 41012 Seville, Spain

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Abstract

A simple, sensitive and rapid spectrofluorimetric method to determine velnacrine, a cholinesterase inhibitor, has been developed and validated. The influence of pH, temperature, ionic strength, presence of excipients, metal ions and surfactants on the fluorescence intensity has been studied. The proposed method allows the determination of 5–100 ng ml^{-1} of velnacrine in aqueous solution containing sodium acetate buffer (pH 5.6; 0.04 M) with $\lambda_{excitation}$ 242 nm and $\lambda_{emission}$ 359 nm. The detection and quantitation limits were 1.7 and 4.5 ng ml^{-1} respectively. The method was successfully applied to the determination of velnacrine in human serum and urine.

Keywords: Velnacrine; Serum; Urine; Spectrofluorimetry

1. Introduction

In 1988 Shutske et al. [1] synthesized velnacrine (9-amino-1,2,3,4-tetrahydroacridine-1-ol) (Fig. 1), which produces improvement in patients with Alzheimer's disease. Alzheimer's disease has been recognized as one of the most disabling conditions affecting the aged people and it is the major cause of dementia among elderly people. Velnacrine is a centrally acting cholinesterase inhibitor that restores the cholinergic neurotransmission deficit observed in patients with Alzheimer's disease [2–6]. Velnacrine also inhibits hepatic oxidative enzymes reducing the clearance rate of drugs that are metabolized through the hepatic oxidase system [7].

Several reports describe the determination of velnacrine in serum samples by high performance liquid chromatography (HPLC) using ultraviolet [8–10] or fluorimetric detection [11–13] and by capillary zone electrophoresis [14], in some of them the determination of velnacrine in urine samples is also described [9,11,14]. Also a differential pulse voltammetric method has been reported for the determination of velnacrine in urine [15]. Some of the above-mentioned methods are either not sufficiently sensitive for the determination of velnacrine in serum [15] or present low recovery [10–12] and only three methods have been reported for the determination of velnacrine in serum and urine.

Although fluorimetric methods are sensitive and simple, no fluorimetric method for the determination of velnacrine has been reported. In this paper, we present a spectrofluorimetric method for the determination of velnacrine which has been applied to the analysis of velnacrine in serum and urine samples.

2. Experimental

2.1. Chemical and reagents

Velnacrine was kindly provided by Warner–Lambert/Parke–Davis (Ann Arbor, MI). Acetic acid, sodium hydroxide and chloroform were of analytical reagent grade and purchased from Merck (Darmstadt, Germany). Surfactants were purchased from Fluka (Buchs, Germany). Deionized water was obtained from a Millipore (Milford, MA) Milli-Q Plus water purification system.

* Corresponding author.
E-mail address: mochon@us.es (M. Callejón).

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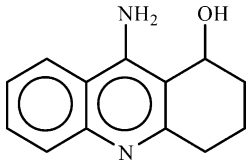

Fig. 1. Chemical structure of velnacrine.

Velnacrine stock standard solution of 100 μg ml^{-1} was prepared by dissolving velnacrine in deionized water. This solution is stable for several months at room temperature. Working standard solutions of velnacrine were prepared from stock standard solution by diluting the appropriate aliquot in deionized water.

Sodium acetate buffer solution (pH 5.6; 0.1 M) was prepared by adding the appropriate volume of the concentrated acetic acid to water and adjusting the pH with sodium hydroxide.

2.2. Instrumentation

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

Chromatography was performed on a Merck (Hitachi, Tokyo, Japan) with a 20 μl injector loop, a L-7100 pump and a L-7485 fluorescence detector. The system was controlled through an interface module and a personal computer. Separations were achieved using a LiChrospher® 100 RP-18, 5 μm, (250 × 4 mm I.D.) column with a LiChrospher® 100 guard column.

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

In the extraction procedure, samples were shaken on a Selecta shaker (Vibromatic 384, Barcelona, Spain) and centrifuged in a Sigma centrifuge (Laborzentrifugen 4–10, Osterode, Germany).

2.3. Spectrofluorimetric measurements

Suitable aliquots of velnacrine working solution, containing 50–1000 ng of velnacrine, and 2 ml of acetate buffer (pH 5.6; 0.1 M) were added to 10 ml volumetric flasks. Solutions were diluted to the mark with deionized water, thermostated at 25.0 ± 0.1 °C and fluorescence intensity (FI) was measured at 359 nm (emission wavelength, $\lambda_{emission}$) using an excitation wavelength ($\lambda_{excitation}$) of 242 nm.

2.4. Comparative HPLC method

Data obtained from the analysis of serum and urine samples were compared with a previously described HPLC method [11]. The mobile phase consisted of acetate buffer (0.2M, pH 4) and acetonitrile (87:13 v/v).

2.5. Sample preparation

2.5.1. Serum

Human serum (0.5 ml) spiked with a suitable amount of velnacrine solution was poured into 10 ml centrifuge tubes with screwcaps. 0.25 ml of 0.5 M sodium hydroxide solution and 8 ml of chloroform were added. The tubes were vigorously shaken for 10 min and centrifuged (2500 × g, 5 min). The organic phase was transferred to a 25 ml reservoir and the aqueous phase was treated with 8 ml of chloroform, shaken for 10 min and centrifuged (2500 × g, 5 min). The organic phase was transferred to the reservoir and evaporated to dryness under a nitrogen stream. The residue was reconstituted with deionized water, transferred to a 10 ml volumetric flask, 2 ml of acetate buffer solution were added and the solution was diluted to the mark with deionized water.

2.5.2. Urine

Human urine (2 ml) spiked with a suitable amount of velnacrine solution was poured into 50 ml separator funnels. 0.4 ml of 0.5 M sodium hydroxide solution and 8 ml of chloroform were added and the funnels were vigorously shaken for 10 min. After separation of the two phases by centrifugation (2500 × g, 5 min), the organic phase was transferred to a 25-ml reservoir. The aqueous phase was treated with 4 ml of chloroform and shaken for 10 min. The organic phase was transferred to the reservoir and evaporated to dryness under a nitrogen stream. The residue was reconstituted with deionized water and transferred to a 25-ml volumetric flask. Acetate buffer solution (5 ml) was added and the solution was diluted to the mark with deionized water.

3. Results and discussion

The excitation and emission spectra of velnacrine are shown in Figs. 2 and 3, respectively. The influence of pH, temperature, solution ionic strength and presence of metal ions, surfactants and excipients often used in tablet formulations, was studied.

Considerable decrease of velnacrine fluorescence intensity can be observed after pH 8 (Fig. 4). A pH value of 5.6 was selected because a good sensitivity is achieved and can be easily fixed with a sodium acetate buffer.

An important linear decrease of fluorescence intensity with temperature was observed (FI = (90.4 ± 0.5) + (−

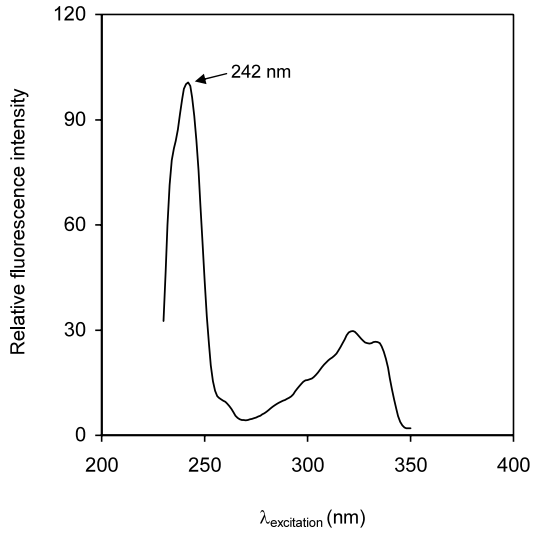

Fig. 2. Fluorescence excitation spectrum, at $\lambda_{emission}$ 359 nm, of 2 μg ml^{-1} velnacrine aqueous solution.

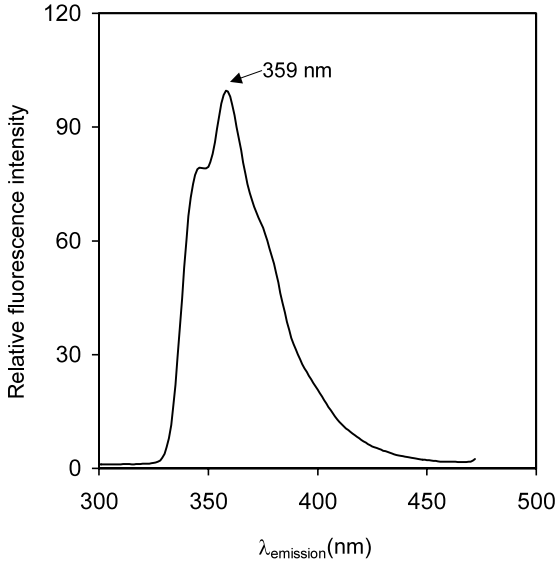

Fig. 3. Fluorescence emission spectrum, at $\lambda_{excitation}$ 242 nm, of 2 μg ml^{-1} velnacrine aqueous solution.

$1.63 \pm 0.02)T$; $r = 0.9990$), so measurements had to take place in a thermostated cell, it was thermostated at 25.0 ± 0.1 °C.

Velnacrine solutions of different ionic strengths were obtained by KCl addition. No influence of the ionic strength on the fluorescence was observed in the KCl range studied (0–3 M).

Velnacrine fluorescence was also measured in the presence of some metal ions occurring typically in serum and urine samples (Ca^{2+}, K^{+}, Mg^{2+} and Na^{+}); no influence was observed at biological levels. The fluorescence of velnacrine in the presence of other metal ions (Al^{3+}, Be^{2+}, Ga^{3+}, Mg^{2+}, Ni^{2+}, Ti^{3+} and Zn^{2+}) was studied and no interference was observed at molar ratios of metal/velnacrine ≤ 10.

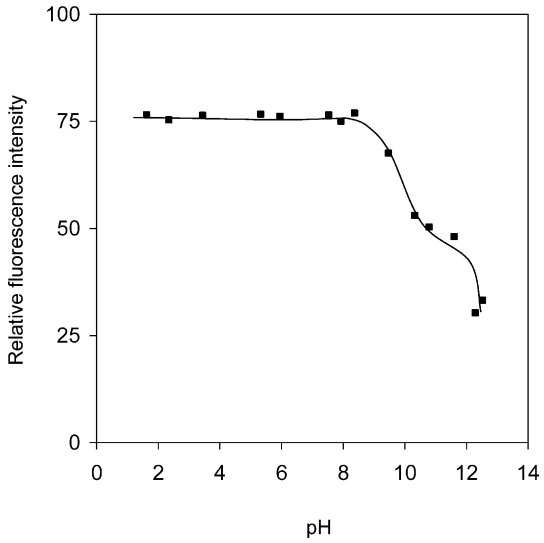

Fig. 4. Influence of the pH on velnacrine fluorescence intensity. $C_{velnacrine}$: 1.5 μg ml^{-1}.

In order to enhance the fluorescence, cationic (hexadecyltrimethylammonium bromide), anionic (sodium dodecylsulfate and dodecylbenzenesulfonic acid sodium salt) and no ionic (Nonidet® P-40 and Brij®-96) surfactants were added to velnacrine solutions. The fluorescence of dodecylbenzenesulfonic acid sodium salt and Nonidet® P-40 do not allow the determination of velnacrine. The presence of hexadecyltrimethylammonium bromide, sodium dodecylsulfate or Brij®-96 has no influence on velnacrine fluorescence at weight ratios of surfactant/velnacrine ≤ 5000 and acidic, neutral or basic pH values.

Lactose, sucrose, glucose and fructose do not cause interference at weight ratios of excipient/velnacrine $\leq$ 10000.

3.1. Linearity

Four series of fifteen standard solutions of velnacrine (from 5 to 240 ng ml^{-1}) were prepared following the procedure described in the experimental section. A linear relationship between fluorescence intensity and concentration was found in the concentration range 5–100 ng ml^{-1} ($y = (1.2 \pm 0.1) + (0.934 \pm 0.003)x$; $r = 0.9998$).

3.2. System precision

Ten replicates of 20 and 40 ng ml^{-1} velnacrine solutions were consecutively prepared and measured. The precision expressed as a coefficient of variation was 0.6 and 0.3%, respectively.

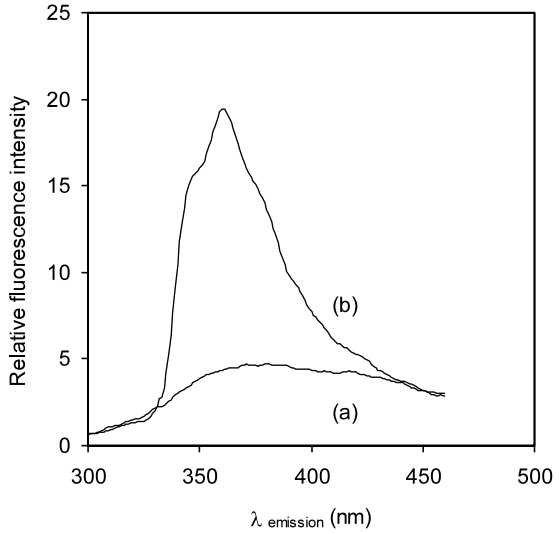

Fig. 5. Spectra of a blank serum sample (a) and a 50 ng ml^{-1} spiked serum sample (b).

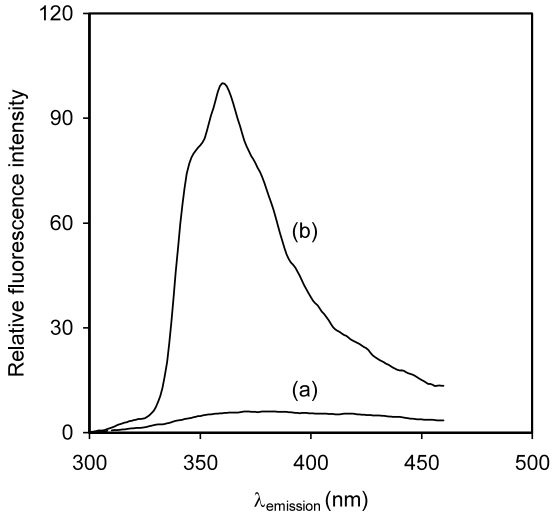

Fig. 6. Spectra of a blank urine sample (a) and of a 900 ng ml^{-1} spiked urine sample (b).

3.3. Method precision

Ten replicates of 20 and 40 ng ml^{-1} velnacrine solutions were prepared and measured by using the proposed procedure on different days within a month. The coefficients of variation were 1.0 and 0.7%, respectively.

3.4. Detection and quantitation limits

The limit of detection (LOD), expressed as the concentration at a signal-to-noise ratio $S/N = 3$, was 1.7 ng ml^{-1} and the limit of quantitation (LOQ), expressed as the concentration at a signal-to-noise ratio $S/N = 10$, was 4.5 ng ml^{-1}.

3.5. Determination of velnacrine in serum

Fig. 5 shows spectra of blank and 50 ng ml^{-1} spiked serum samples obtained after processing them through the method described above. As can be seen, blank serum samples exhibit low signal but the low velnacrine levels in serum make necessary the use of the standard addition method. Serum samples were spiked with velnacrine at levels found after drug administration at normal clinical doses (25, 40 and 50 ng ml^{-1}), the standard addition method was applied and samples were treated according to the procedure described above. The application of Student's t-test indicates that the method is accurate (null hypothesis accepted) [16]. Recoveries are presented in Table 1. Samples were also analysed by a described HPLC method [11] using fluorimetric detection obtaining recoveries (96 ± 3%) similar to those obtained by these authors (94–99%) and those obtained by the proposed spectrofluorimetric method (94–96%).

3.6. Determination of velnacrine in urine

Urine samples were spiked with velnacrine to obtain concentrations found after drug administration at normal clinical doses (450, 900 and 1150 ng ml^{-1}) and treated according to the procedure described above. Fig. 6 shows spectra of blank and 900 ng ml^{-1} spiked urine samples obtained after processing them through the method described above. As can be seen, blank urine sample do not cause significant interference in the determination, so external calibration was applied. The application of Student's t-test indicates that the method is accurate (null hypothesis accepted) [16]. Recoveries

Table 1
Accuracy/recovery results of the determination of velnacrine in serum and urine

Amount added (ng ml^{-1}) serum	Amount added (ng ml^{-1}) urine	Amount obtained [a] (ng ml^{-1})	Recovery [a] (%)
25		23.4 ± 1.9	94 ± 8
40		38 ± 3	96 ± 8
50		48 ± 3	96 ± 5
	450	428 ± 22	95 ± 5
	900	884 ± 27	98 ± 3
	1150	1135 ± 31	99 ± 3

[a] Average of five determinations ±SD.

are presented in Table 1. Samples were also analysed by a described HPLC method [11] using fluorimetric detection obtaining recoveries ($85 \pm 5\%$) similar to those obtained by these authors (71–93%) but lowers than those obtained by the proposed spectrofluorimetric method (95–99%).

4. Conclusions

A simple, accurate and sensitive spectrofluorimetric method for quantification of velnacrine has been developed and validated with parameters such as precision, linearity, detection and quantitation limits. The spectrofluorimetric method allows velnacrine determination in human serum and urine samples spiked at levels found after drug administration at normal clinical doses. The recommended procedure is a less time consuming and cheaper alternative to conventional determinations by HPLC with better recoveries and accuracy and equivalent sensitivity.

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