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Kinetic determination of nortriptyline in pharmaceutical samples by use of photometric and fluorimetric detection

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Abstract: A fast kinetic method is proposed for the photometric and fluorimetric determination of nortriptyline hydrochloride. The method involves measuring the rate of formation of an adduct with 4-chloro-7-nitrobenzofurazan, which exhibits light absorption and fluorescent properties, and the use of a stopped-flow mixing technique, which facilitates application to automatic routine analyses. The reaction rate is measured within only 30 s. The detection limit is 0.12 μg ml^{-1} (photometry) and 0.18 μg ml^{-1} (fluorimetry) and the calibration graph is linear up to 60 μg ml^{-1} in both cases. The precision (as RSD) is less than 1.5%. The proposed method was satisfactorily used for direct analysis of pharmaceutical preparations and a mean recovery near 100% was obtained with both photometric and fluorimetric detection.

Keywords: *Nortriptyline; kinetic method; stopped-flow method; photometry; fluorimetry.*

Introduction

Nortriptyline belongs to the family of tricyclic antidepressants, a group of drugs widely used for treating depressive diseases. A variety of HPLC methods have been described for the routine clinical determination of this compound; all involve sample pretreatment such as liquid–liquid extraction [1, 2], solid-phase extraction [3, 4] or column switching [4, 5]. Detection is always achieved with a UV detector. Homogeneous enzyme immunoassay (EMIT) has also been widely used for the determination of nortriptyline although the results are generally greater than those provided by HPLC [6].

HPLC and EMIT methods have adequate sensitivity for determining the typically low therapeutic levels of nortriptyline in biological fluids (50–150 ng ml^{-1}) although phenothiazine tranquillizers [3, 7–10] frequently interfere. Use of these methods is justified when the sample matrix is rather complex and the nortriptyline concentration low, as is usually the case with clinical samples. However, in pharmaceutical analysis, where the sample matrix is usually less complex and analyte concentration levels are fairly high, the main aim is to develop fast, simple, inexpensive methods that can readily be adapted for routine analyses without the need for laborious techniques or expensive reagents. One still unexplored alternative to the determination of this compound is kinetic methodology based on the stopped-flow mixing technique. This technique facilitates automation because it allows sample and reagent solutions to be automatically mixed and kinetic data from the mixed solution to be acquired for immediate processing in order to obtain the desired information.

Few photometric and fluorimetric methods have been reported for the determination of nortriptyline. They usually involve ion-pair extraction into dichloromethane or chloroform, and use Chrome Azurol S [11], anthracene-2-sulphonate [12] or tetrabromosulphonefluorescein [13] as the reagent with photometric [11] or fluorimetric [12, 13] detection.

With the aim of developing a fast, direct method for the determination of nortriptyline, its reaction with 4-chloro-7-nitrobenzofurazan (NBD-Cl) to give the corresponding 4-dialkylamino-7-nitrobenzofurazan was studied kinetically using photometric and fluorimetric detection. This reagent is widely used for the derivatization of aliphatic thiols [14] and primary and secondary aliphatic amines [15, 16] but has never been used as a reagent for the

determination of nortriptyline. The reaction products are usually determined fluorimetrically [16, 17] although photometric detection has also occasionally been used for this purpose [18, 19]. The detection limits obtained by using fluorimetry are generally lower than those provided by photometry although this depends on the compound concerned. Thus, a systematic study of low-molecular weight alkylthiols [14] provided very similar detection limits whether absorbance or fluorescent detection was used although the latter reportedly gives higher limits.

Experimental

Reagents

All chemicals were of analytical-reagent grade. A 200 μg ml^{-1} stock solution of nortriptyline hydrochloride (the Sigma Chemical Company) was prepared in water. Of NBD-Cl, 0.1 M was made daily by dissolving the appropriate amount of reagent (Sigma) in ethanol. A 0.1 M bicarbonate buffer (pH 8.2) was also used.

Instrumentation

A Pye-Unicam 8625 UV-visible spectrophotometer and a Perkin–Elmer LS-50 luminescence spectrometer were used for photometric and fluorimetric measurements, respectively. Both instruments were fitted with a stopped-flow module [20] supplied by Quimi-Sur Instrumentation and controlled via an interfaced computer. The observation cell of the stopped-flow module had a path length of 10 mm. The solutions in the module and cell compartment were kept at a constant temperature (50°C) by circulating water from a thermostated tank.

Procedure

One of the two 10-ml reservoir syringes of the stopped-flow module was filled with a previously prepared solution containing 1.8 ml of 0.1 M NBD-Cl in ethanol, 4.5 ml of ethanol, 1.5 ml of bicarbonate buffer and 2.5 ml of distilled water. The other syringe was filled with a premixed solution containing 6 ml of ethanol, 1.5 ml of bicarbonate buffer, a volume of nortriptyline hydrochloride standard solution containing a final concentration of 0.6–60 μg ml^{-1} and distilled water to 10 ml. After the two 2-ml drive syringes had been filled, equal volumes (0.15 ml) of the two solutions were mixed in the mixing chamber in each run. Reaction changes were monitored by measuring the variation of the absorbance (λ 470 nm) or fluorescence intensity (λ_{ex} 470, λ_{em} 535 nm) with time. All measurements were made at 50°C. The absorbance or fluorescence values obtained were processed by linear regression using the microcomputer, furnished with software for application of the initial-rate method. The reaction rate was determined within *ca* 30 s and each sample was assayed in triplicate. The blank signal was subtracted from all measured values.

Determination of nortriptyline in pharmaceutical preparations

Each sample was analysed by weighing a tablet, which was finely powdered and dissolved in 500 ml of distilled water. The solution was shaken for 5 min in an ultrasonic bath and filtered. An adequate volume of the filtrate was then treated as described above.

Results and Discussion

Like other secondary alkylamines, nortriptyline reacts with NBD-Cl to form an adduct with light absorption (λ 470 nm) and fluorescent (λ_{ex} 470, λ_{em} 535 nm) properties (Fig. 1) that can be exploited to develop a simple method for the determination of this antidepressant. The reaction takes 10–15 min to reach equilibrium. Thus, use of kinetic methodology is advantageous as it allows analytical results to be obtained in a shorter time. In addition, the use of a stopped-flow mixing technique facilitates adaptation for routine analyses as it expedites data acquisition time and minimizes reactant manipulation, which results in a high sample throughput and

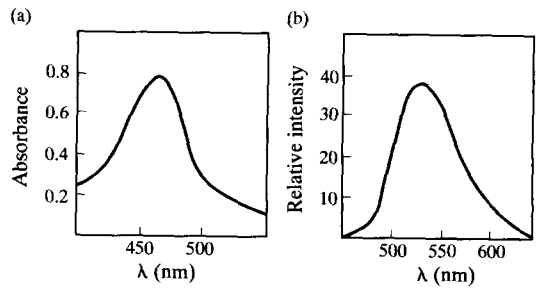

Figure 1
(A) Absorption and (B) emission (λ_{ex} 470 nm) spectra of nortriptyline (4 μg ml^{-1}) in the presence of NBD-Cl (1.8×10^{-2} M) and 60% (v/v) ethanol (apparent pH = 9.5, time = 10 min).

low reagent consumption. Figure 2 shows a typical kinetic curve obtained by this approach, as well as the corresponding blank signal. The reaction rate thus obtained was *ca* twice that provided by the batch technique. A reaction time of *ca* 30 s was long enough for analytical measurements to be obtained.

Optimization of variables

The effect of variables affecting the performance of the proposed kinetic method for the determination of nortriptyline was investigated by the univariate method and both photometric and fluorimetric measurements. All concentrations given are initial concentrations in the syringes (twice the actual concentrations in the stopped-flow cuvette at time zero after mixing). Each kinetic result was the mean of three measurements.

The system required a high ethanol content to avoid formation of a precipitate. The highest reaction rate was obtained by including 60% (v/v) ethanol in each solution in each syringe, which ensured that the mixture remained clear throughout the reaction.

The pH dependence of the system was studied in the range 7–11 using sodium hydroxide and hydrochloric acid solutions. The optimum apparent pH was 9.0–10.0. Two buffers of pH 8.2 (bicarbonate and borate) were assayed to adjust the sample pH. In both cases, the apparent pH of the reaction mixture was 9.5 as measured in the waste. The reaction rates were very similar with both buffers, although slightly higher for bicarbonate. The best results were obtained at a bicarbonate concentration of 1.5×10^{-2} M in the solutions held in each syringe. Increasing temperatures in the range 20–50°C resulted in increased reaction rates as measured by the variation of the absorbance or the fluorescence intensity with time. A temperature of 50°C was thus selected for both types of detection.

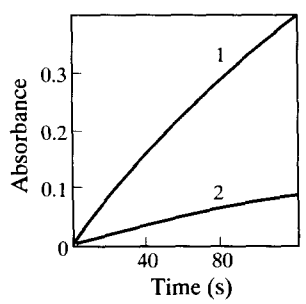

Figure 2
(1) Kinetic curve obtained for nortriptyline (10 μg ml^{-1}) in the presence of NBD-Cl (1.8×10^{-5} M) and 60% (v/v) ethanol (apparent pH = 9.5); (2) blank signal.

Figure 3 shows the effect of the NBD-Cl concentration on the reaction rate values obtained by measuring the variation of the absorbance with time. This parameter was independent of the reagent concentration over the range $1.5–2.0 \times 10^{-2}$ M. Similar reaction rate values were obtained by measuring the variation of the fluorescence intensity with time. The decrease in the reaction rate at a high reagent concentration could be attributed to a physical effect caused by the low solubility of NBD-Cl in the hydroalcoholic medium. Thus, excess reagent can result in slight turbidity in the mixing chamber on mixing with the sample solution, thereby decreasing the absorbance or fluorescence signal and giving rise to an apparent decrease in the reaction rate.

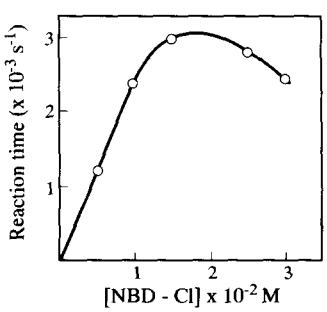

Figure 3
Variation of the reaction rate with the reagent concentration, [nortriptyline] = 10 μg ml^{-1}.

Figures of merit of the proposed method

The kinetic curves obtained at different concentrations of nortriptyline hydrochloride by measuring the variation of the absorbance or fluorescence intensity as a function of time were processed by using the initial-rate method. As can be seen in Table 1, the figures of merit of the photometric and fluorimetric variant are very similar. The detection limits were calculated according to IUPAC's recommendations [21]. The Pearson's correlation coefficients obtained indicate very good calibration linearity. One of the most salient features of the proposed method is speed; since the time required to obtain analytical data is only *ca* 30 s, the method is a useful, simple alternative to automatic routine procedures for the determination of nortriptyline.

The selectivity of the method was assessed by assaying several phenothiazines, including

Table 1
Figures of merit of the proposed method

Detection system	Linear range ($\mu g\ ml^{-1}$)	Detection limit* ($\mu g\ ml^{-1}$)	Slope ± SD†	Intercept ± SD	r‡	SEE	RSD§ %
Photometry	0.4–60	0.12	$(1.40 \pm 0.02) \times 10^{-4}$	$(1.3 \pm 0.4) \times 10^{-4}$	0.9995	1.04×10^{-4}	1.1,‖ 1.4¶
Fluorimetry	0.6–60	0.18	$(4.03 \pm 0.06) \times 10^{-4}$	$(1.0 \pm 0.5) \times 10^{-4}$	0.9997	9.31×10^{-5}	1.3, 1.3

*3σ.
†Units, photometry: A — $ml\ s^{-1}\ \mu g^{-1}$; fluorimetry: I_F — $ml\ s^{-1}\ \mu g^{-1}$ (A = absorbance, I_F = relative intensity).
‡$n = 6$.
§$n = 10$.
‖4 $\mu g\ ml^{-1}$.
¶10 $\mu g\ ml^{-1}$.

Table 2
Determination of nortriptyline hydrochloride in pharmaceutical preparations

	Nortriptyline hydrochloride content (mg)				Recovery			
		Found*			A		B	
Sample†	Labelled	A	B	Added ($\mu g\ ml^{-1}$)	Found* ($\mu g\ ml^{-1}$)	Recovery (%)	Found* ($\mu g\ ml^{-1}$)	Recovery (%)
1	10	9.75	9.85	1.5	1.5	100.0	1.45	96.7
				3	3.1	103.3	3.05	101.7
				6	5.8	96.7	6.2	103.3
2	10	9.75	9.60	1.5	1.45	96.7	1.42	94.7
				3	3.1	103.3	2.8	93.3
				6	6.3	105.0	5.7	95.0
3	25	25	24.8	1.5	1.5	100.0	1.4	93.3
				3	3.1	103.3	3.0	100.0
				6	6.1	101.7	5.9	98.3
4	12.5	12.2	12.3	1.5	1.6	106.7	1.6	106.7
				3	3.0	100.0	3.2	106.7
				6	6.1	101.7	6.05	100.8

*Mean of three determinations. (A) Photometric detection; (B) fluorimetric detection.
†Trade mark, manufacturer's name and composition of samples: (1) Martimil, Alonga, S.A., nortriptyline hydrochloride (10 mg), excipient (lactose and others); (2) Norfenazin, Alonga, S.A., nortriptyline hydrochloride (10 mg), perphenazine (2 mg), excipient (lactose and others); (3) Paxtibi, Dista, S.A., nortriptyline hydrochloride (25 mg), excipient (lactose and others); (4) Tropargal, Alonga, S.A., nortriptyline hydrochloride (12.5 mg), diazepam (2.5 mg), excipient (lactose and others).

perphenazine, chlorpromazine and promethazine, as well as other tricyclic antidepressants such as amitriptyline, imipramine, desipramine and trimipramine. A substance was considered not to interfere at a given concentration if the reaction rate obtained in its presence was within one standard deviation of the value obtained with the analyte alone. The phenothiazines were tolerated in a two-fold excess, amitriptyline in a 30-fold excess and imipramine and trimipramine in a 90-fold excess relative to the analyte. On the other hand, desipramine interfered at the same concentration level as nortriptyline as it is also a secondary alkylamine.

Applications

In order to validate the proposed stopped-flow method for nortriptyline, the technique was applied to various pharmaceutical samples using photometric and fluorimetric reaction-rate measurements. The results obtained are summarized in Table 2. Recoveries were determined by adding three different amounts of nortriptyline hydrochloride standard to each sample after weighing, fine grinding and dissolution in 500 ml of distilled water. The mean recoveries obtained using photometric and fluorimetric reaction-rate measurements were 101.5 and 99.2%, respectively.

Conclusions

The proposed method is the first reported application of kinetic methodology in conjunction with the stopped-flow mixing technique to the development of a fast method for the determination of nortriptyline. The method can readily be adapted for automatic quality control of the analyte in pharmaceutical preparations. The features of the adduct formed with NBD-Cl allow photometric or fluorimetric measurement of the reaction rate with very similar results. Although the detection limit is higher than those achieved by HPLC and EMIT, the proposed method is very simple, inexpensive and tolerates moderate levels of phenothiazine derivatives, some which interfere with alternative methods.

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