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Short communication

High performance liquid chromatographic determination of prozapine in pharmaceutical formulations

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1. Introduction

Prozapine (hexadiphane), 1,1-diphenyl-3-hexamethyleneimino propane (**I**) is a papaverine-like compound with weak anticholinergic activity [1]. It is used as an antispasmodic in the form of the hydrochloride, in combination with sorbitol, in biliary and gastro-intestinal disorders.

The analysis of (**I**) is normally based on the reactivity of the imino moiety. In particular, the classical methods are those used for basic nitrogen compounds, such as titrations in non-aqueous solvents, titrations with anionic surfactants or acid dyes which form stable addition compounds with (**I**), and titrations involving precipitate formation (e.g. picrate, reineckate, iodomercurate) [2]. Such methods are accurate but they lack specificity and are time-consuming as they often require complicated extractions.

Chromatographic techniques present the characteristics of specificity and rapidity without the loss of precision and accuracy. A gas–liquid chromatography (GLC) method [3] developed for the purity control of (**I**) has been applied to the analysis of pharmaceutical formulations. The method proved to be rapid and accurate. To the authors' knowledge no report has been published on a high performance liquid chromatography (HPLC) method for the determination of (**I**). Such a method might be of use for the analysis of (**I**) in the course of its synthesis, which involves decyanation of the parent compound, in order to evaluate the completeness of the reaction.

A simple HPLC method has been developed utilizing both isocratic and linear gradient elution for the determination of (**I**) and the method has been applied to the analysis of a liquid pharmaceutical formulation. The proposed method is based on reversed-phase ion-pair chromatography with perchlorate as the counter ion and a moderately polar stationary phase (a cyanopropyl bonded phase). The only sample preparation nec-

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essary for the analysis is its dilution with the mobile phase.

2. Experimental

2.1. Chemicals

Prozapine hydrochloride was kindly supplied by Rhône Poulenc (France); the pharmaceutical preparation was obtained on the Italian market. All chemicals were of analytical-reagent grade and were used as obtained (Carlo Erba, Milan, Italy). Acetonitrile was of HPLC-grade. Water was deionized, distilled with glass apparatus and passed through a Milli Q system (Millipore). All solvents and solutions for HPLC analysis were filtered through a 0.45 μm nylon Millipore filter and vacuum-degassed by sonication before use.

2.2. Apparatus

Chromatography with gradient elution was performed with a Varian 9010 pump (Varian, Zug, Switzerland) equipped with a 10 μl Valco external loop injector and a Varian Polychrom 9065 photodiode-array detector (DAD) connected to a Vectra 486/33N computer. For isocratic elution, the analyses were carried out by means of a Varian 2510 pump coupled to a Varian 2550 variable-wavelength UV detector (VWD) and a Varian 4290 printer–plotter. The analytical column (250 mm × 4.6 mm i.d.) was packed with 5 μm LiChrosorb® CN (Merck, Darmstadt, Germany).

2.3. HPLC conditions

For isocratic elution, the mobile phase A was acetonitrile 10^{-3} M perchloric acid and 0.02 M sodium perchlorate in water (50:50, v/v). Linear gradient elution conditions were 30% to 95% (v/v) acetonitrile in 20 min. The flow rate was 1.0 ml min^{-1}, the injection volume was 10 μl, the column temperature was 25°C and the detection wavelength (Varian 9050) was 254 nm. The range of wavelengths examined by means of the DAD was 190–367 nm.

2.4. Calibration standards

A stock solution of prozapine was prepared by dissolving 250 mg of the pure compound in 100 ml of the mobile phase A. Working standard solutions were prepared by diluting aliquots of the stock solution to give concentrations of 5×10^{-3}–5×10^{-1} mg ml^{-1}. The peak areas were plotted against the corresponding amounts (μg) injected to obtain the calibration graph.

2.5. Sample preparation

An accurately measured amount of the liquid pharmaceutical preparation was transferred to a volumetric flask and diluted to volume with the mobile phase A; usually, the dilution factor was 2–5. After filtration through a 0.45 μm nylon filter, the solutions were injected into the liquid chromatograph.

3. Results and discussion

Figs. 1A and 1B show the chromatograms of standard solutions containing 0.5 and 0.25 mg ml^{-1} of prozapine respectively. The former was obtained by gradient elution and the latter by isocratic elution. The retention times of the analyte were 10.01 min and 7.70 min respectively. These times were reproducible under the analytical conditions used: the relative standard devia-

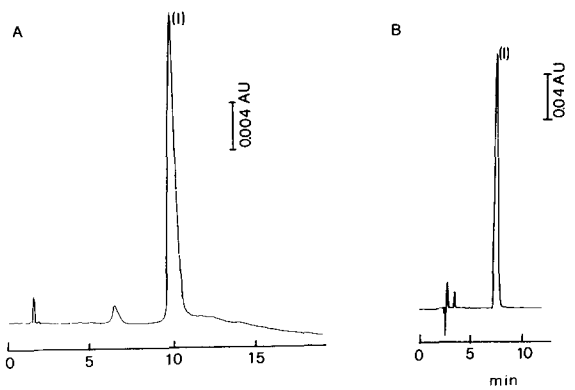

Fig. 1. Typical chromatograms of standard solutions of prozapine (I) obtained by (A) gradient and (B) isocratic elution. Chromatographic conditions are reported in Section 2.

tion (RSD, $n = 10$) ranged from 1.2%–2.1% for gradient elution and from 0.7%–1.3% for isocratic elution.

Peak purity analysis was performed by means of the spectra recorded by the DAD; the value of the purity parameter [4], calculated over the wavelength range 190–367 nm, was 223.03 nm.

Six-point calibration graphs were constructed from the results of five consecutive injections and were rectilinear over the studied range of concentrations. For the VWD the concentration range was 0.005–0.25 mg ml^{-1}; for the DAD the range was 0.05–0.5 mg ml^{-1}. Since the VWD is at least one order of magnitude more sensitive than the DAD, the working concentrations used for calibration were lower when the elution was isocratic.

The equations obtained by the least-squares regression fit were: $y = (19.00 \pm 0.18) \times 10^{-4}x + (0.24 \pm 0.09) \times 10^{-4}$ for isocratic elution; and $y = (19.12 \pm 0.20) \times 10^{-3}x + (0.29 \pm 0.08) \times 10^{-3}$ for gradient elution where y is the peak area and x is the amount of prozapine injected (μg). The corresponding correlation coefficients were 0.9995 and 0.9996 respectively.

The absolute detection limit, defined as the lowest concentration of prozapine resulting in a signal-to-noise ratio of 3:1, was 10 ng (for the VWD) and 100 ng (for the DAD).

The within-day and between-day precisions, as indicated by the RSDs of the peak areas obtained from replicate ($n = 10$) analyses of prozapine standard solutions (0.1 mg ml^{-1}) were satisfactory; RSD values were 1.4% and 1.9% respectively. The applicability of the proposed method for the determination of prozapine was tested by analyzing a commercial liquid preparation containing (**I**) in a concentration of 20 mg per 100 ml. The calculated concentration was 20.57 mg for 100 ml with an RSD of 1.8% ($n = 6$).

Figs. 2A and 2B show chromatograms obtained from the analysis of this pharmaceutical formulation by gradient and isocratic elution respectively. Both chromatograms display a very big peak close to the solvent peak, which can be ascribed to methyl *p*-hydroxybenzoate (**II**) present in the sample as an antimicrobial agent. Since this preservative is very often added to pharmaceutical formulations, standard solutions of (**II**) have been

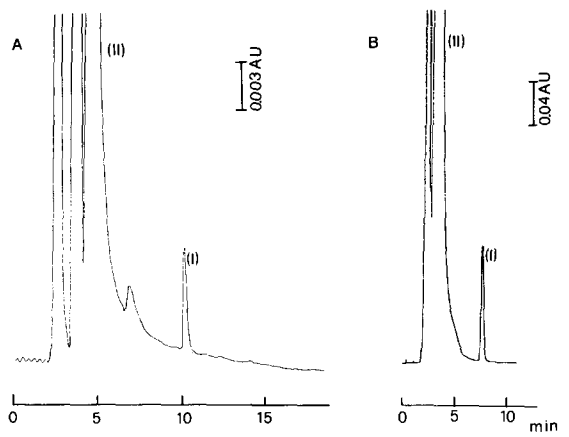

Fig. 2. Chromatograms obtained after injecting the liquid pharmaceutical formulation containing prozapine hydrochloride (**I**) as active principle and methyl *p*-hydroxybenzoate (**II**) as preservative: (A) gradient elution; (B) isocratic elution.

submitted to HPLC using both gradient and isocratic elution. The resulting retention times were 4.20 and 3.50 min respectively.

The other components of the formulation (sorbitol, tartaric acid, vanillin and lemon essence) did not interfere since they display insignificant absorption at the detection wavelength. The chromatograms obtained by gradient analysis (Figs. 1A and Fig. 2A) show the presence of a small peak eluting at 6.9 min; since this peak is also present in the chromatogram of the standard solution as well as that of the formulation, it might be attributable to the presence of a synthesis impurity.

The analytical results lead to the conclusion that both the isocratic and gradient techniques proposed for the LC separation can be adopted for the routine analysis of (**I**) in liquid pharmaceutical formulations. However, gradient elution may be more suitable for the analysis of samples containing UV-absorbing components of a wide range of polarities because under these conditions propazine is eluted later.

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