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Analysis of non-benzodiazepinic anxiolytic agents by capillary zone electrophoresis*

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Abstract: A simple capillary electrophoretic method was developed for the analysis of a new generation of serotonergic anxiolytics and their related substances: zalospirone, gepirone, ipsapirone and buspirone. All compounds run in a Tris/phosphate buffer at pH 3 as cations and the experimental conditions allowed good resolution of four drugs and their principal impurities. The analyses were made using two different kinds of capillary. The suitability of CZE and HPLC methods for the analysis of these non-benzodiazepinic anxiolytic agents and their impurities was compared.

Keywords: *Zalospirone; gepirone; ipsapirone; buspirone; related impurities; capillary zone electrophoresis; anxiolytic agent.*

Introduction

A new family of non-benzodiazepinic drugs belonging to the new generation of serotonergic anxiolytics has been developed, including zalospirone [1] (Z), 4,7-etheno-1H-cyclobut[f]-isoindole-1,3(2H)-dione,3*a*,4,4*a*,6*a*, 7,7*a*-hexahydro-2-{4-[4-(2-pyrimidinyl)1-piperazinyl]-butyl}-monohydrochloride; gepirone [2] (G), 2,6 piperidinedione,4,4'-dimethyl-1{4-[4-(2-pyrimidinyl)-1-piperazinyl]-butyl}-monohydrochloride; ipsapirone [3] (I), 1,2-benzisothiazol-3(2H)-one,2-{4-[4-(2-pyrimidinyl)-1-piperazinyl]-butyl}-1,1-dioxide, monohydrochloride. All these compounds have in common a [(2-pyrimidinyl)-1-piperazinyl] butyl moiety, such as their prototype buspirone (B), 8-azaspiro [4,5] decano-7,9 dione,8-[4-4-(2-pyrimidinyl)-1-piperazinyl]-monohydrochloride (Fig. 1) [4]. The main impurities of these drugs (Fig. 2) are all related to the synthetic procedures utilized for their production.

Of the few papers in the literature regarding this relatively new class of compounds, two gas chromatographic quantitative methods have been proposed for the analysis of buspirone; the first use flame ionization detection [5] and the second exploits electron capture detection [6]. Gepirone was analysed by a capillary GC–MS method [7] and ion-pair HPLC [8]. In a previous paper the present authors reported an RP–HPLC method for the analysis of gepirone, ipsapirone, zalospirone, their related substances and for specific assay at the therapeutic level in biological fluids [9]. Now a capillary zone electrophoretic (CZE) method is described to analyse the four compounds and their impurities. Since the suitability of capillary electrophoresis as a routine procedure for drug quality control is still an important issue of debate, the qualitative performance of CZE was compared with that of an established HPLC procedure.

Experimental

Apparatus

The electrophoretic analyses were performed with a SpectraPHORESIS 1000 apparatus (Thermo Separation Products, CA, USA) equipped with a multiwavelength SpectraFocus UV–Vis detector (190–800 nm) and a Peltier air-cooling system (15–60°C). Instrument con-

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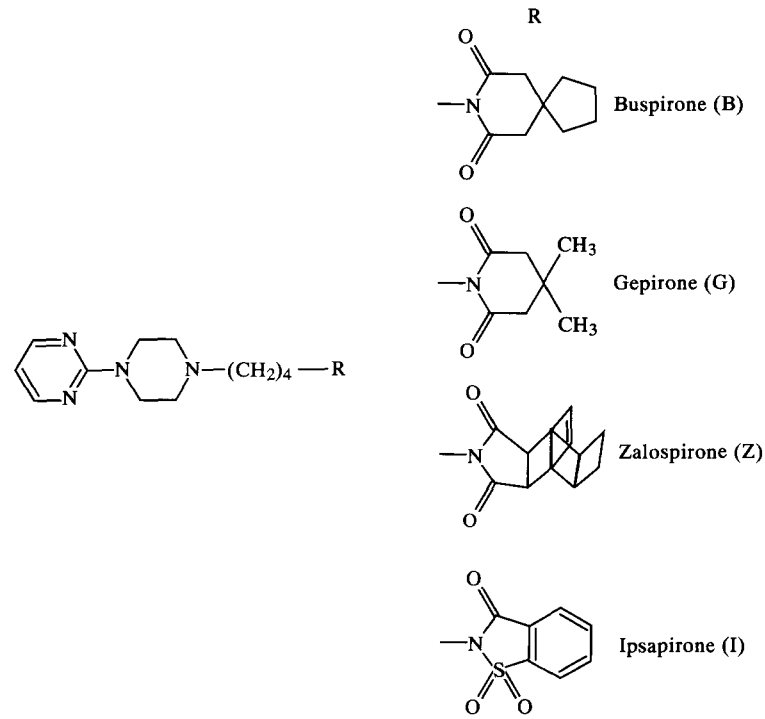

Figure 1
Chemical structures of drugs examined.

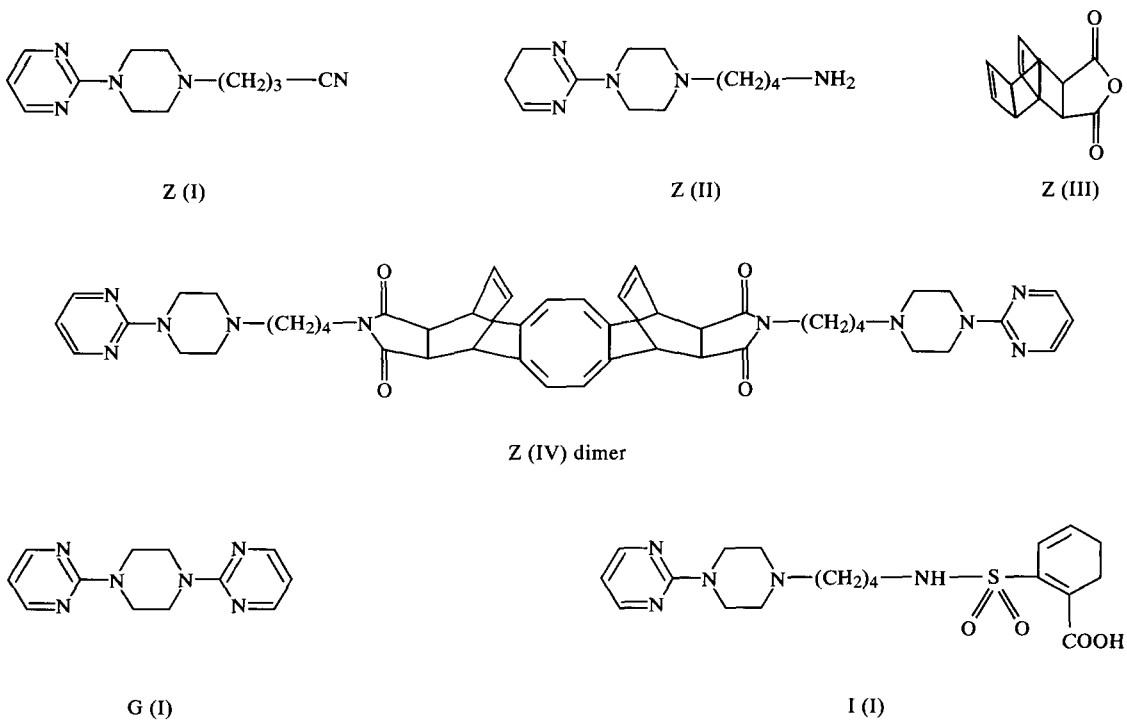

Figure 2
Chemical structures of the main impurities of: zalospirone, Z(I), Z(II), Z(III), Z(IV) dimer; gepirone; G(I); and ipsapirone, I(I).

trol and data collection were performed by IBM personal system/2 Model 70-386. On column UV detection was used at 240 nm.

Chemicals and reagents

Zalospirone (Wyeth), gepirone (Bristol–Myers, Squibb), ipsapirone (Bayer) and buspirone (Bristol) and pure samples of impurities were kindly supplied by their respective manufacturers.

Phosphoric acid, Tris(hydroxymethyl)-aminomethane (Tris) and sodium hydroxide were obtained from Merck (Darmstadt, Germany). The water used was of HPLC grade.

Analysis of serotonergic anxiolytics

Standards and working standards. Pure standard solutions of each compound were used to study the various electrolytes, pH, applied voltage current value and working temperature.

The following working standard solutions were prepared in distilled water: (1) 5×10^{-1} mg ml^{-1} of each drug to obtain the electrophoretic profile, adding 1×10^{-2} mg ml^{-1} of respective impurities (equivalent to 2% w/w); (2) different amounts of drug, from 0.5–2 mg ml^{-1}, all added with the same amount (1 mg ml^{-1}) of internal standard (IS) to obtain the calibration curves. As internal standards gepirone was used to determine zalospirone, zalospirone to determine ipsapirone and Gepirone or ipsapirone for buspirone. The calibration curves were obtained by plotting the peak area ratio (drug/IS) against concentration. The use of an internal standard was suggested in order to compensate the poor precision observed with the hydrodynamic injection mode.

Sample preparation. About 100 mg of each bulk sample of Z, G, I or B, accurately weighed, was dissolved in a 100-ml volumetric flask with 50 ml of the electrolyte solution, a fixed amount of internal standard added and then diluted to the mark with electrolyte to obtain a concentration of 1 mg ml^{-1}. This solution was used for the determination of the drug and its related impurities.

Electrophoretic conditions

The resolution of four drugs and their impurities was obtained with a Supelco HPE fused silica capillary (40 cm × 50 mm i.d., uncoated) and a Hewlett–Packard (HP) fused capillary with bubble cell (52 cm × 50 mm i.d., uncoated). The capillaries were filled with Tris/150 mM phosphate buffer or Tris/170 mM phosphate buffer at pH 3.

The applied voltage was 20 kV and the analyses were performed at 20°C.

Each day at the beginning of the analytical process the capillary was washed with 0.1 M sodium hydroxide for 30 min. The subsequent conditioning cycles were: washing with 0.1 M sodium hydroxide for 2 min followed by buffer for 3 min.

Standards or samples were introduced by the hydrodynamic mode for 2 s.

Results and Discussion

Performance of CZE method

Preliminary electrophoretic experiments showed that all compounds moved in a Tris/phosphate buffer at pH 3 as cations. The electropherograms of Figs 3 and 4 show that the selected electrophoretic conditions and the background electrolyte (BGE) were suitable for all four drugs and their related impurities. Only one impurity, Z(III), related to zalospirone, was not detected, this being a neutral compound.

The separation of charged compounds in an uncoated fused-silica capillary occurs principally through electroendosmotic flow, therefore the analysis of cations is generally rapid. As expected, the resolution of the impurities from each drug occurs in a short time (about 7 min). Only one impurity, I(I) related to ipsapirone, required about 20 min.

The identity of the impurities found in different batches of zalospirone, gepirone, ipsapirone was confirmed: (a) by comparison of the UV spectra obtained during the run with those obtained from the standard solutions of drug and impurities; (b) by the increase of respective peak heights when the bulk material test solution was sequentially enriched with a fixed amount of each impurity standard solution. No impurities were found in buspirone bulk material.

The repeatibility of migration time, the relationship between peak area and concentration of analytes and lower detection limits were measured. The relative standard deviation (RSD) of the migration time for each drug was about 0.4% (n = 30) and 0.7% (n =

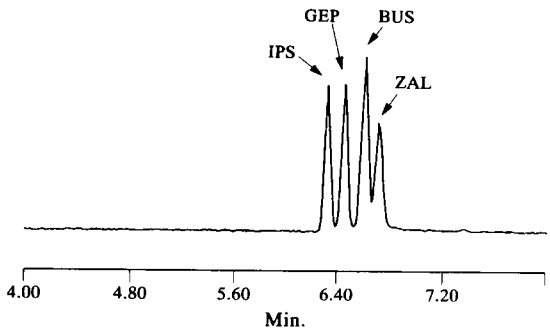

Figure 3
Electropherogram of a working standard mixture of ipsapirone, gepirone, buspirone, zalospirone (5×10^{-2} mg ml^{-1} of each drug). BGE: Tris/phosphoric acid 170 mM (pH 3). Applied voltage: 20 kV, 83.37 μA. Temperature 20°C. Injection by hydrodynamic mode for 2 s. Detection wavelength: 240 nm.

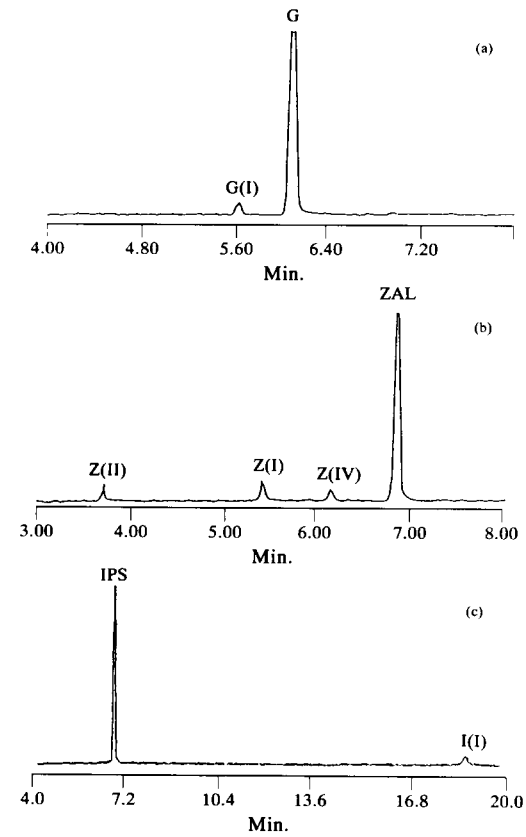

Figure 4
Electropherograms of a working standard mixture containing: (a) gepirone 0.1 mg ml^{-1} and 1 μg ml^{-1} of G (I) impurity; (b) zalospirone 0.1 mg ml^{-1}, Z(I) and Z(II) (1 μg ml^{-1}) and 5 μg ml^{-1} of Z(IV); (c) ipsapirone 0.1 mg ml^{-1} and I(I) 1 μg ml^{-1}. BGE: Tris/phosphoric acid 150 mM (pH 3). Applied voltage: 20 kV, 75.12 μA. The other conditions were as in Fig. 3.

20) for intraday and interday analysis, respectively.

The linearity of calibration graphs was tested over the range 0.5–2 mg ml^{-1} for each drug and over 2–20 μg ml^{-1} for each impurity. The correlation coefficients (six determinations) and corresponding relative standard deviation values were: 0.998 ± 1.2% for zalospirone; gepirone 0.989 ± 1.3%; ipsapirone 0.991 ± 1.0%; and buspirone 0.994 ± 0.8%. The corresponding data for all impurities varied between 0.979 ± 0.9% and 0.993 ± 0.7%.

Comparison of CZE method with HPLC

The qualitative and quantitative data obtained using the proposed method were compared with data obtained on the same samples by HPLC (Fig. 5, following an estab-

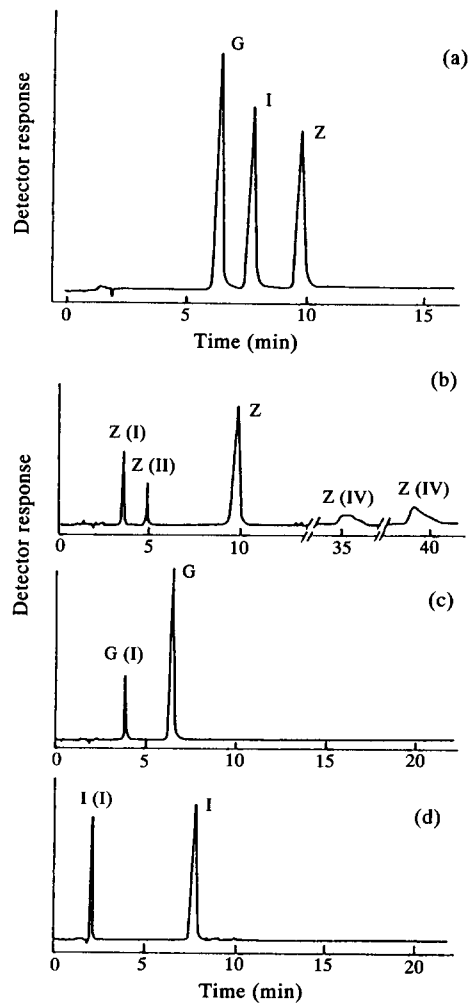

Figure 5
Chromatographic resolution of drugs and related substances [9]: (a) working standard mixture of gepirone, ipsapirone and zalospirone (1 mg ml^{-1} of each drug); (b), (c) and (d) drugs and related impurities. Column: Hypersil ODS, (150 × 4.6 mm i.d.), 5 μm. Mobile phase: 5 mM sodium lauryl sulphate in 50 mM potassium dihydrogenphosphate buffer (pH 4)–acetonitrile (55:45, v/v). Flow rate 1 ml min^{-1}; fluorescence detection at λ_{ex} 237 nm λ_{em} 374 nm.

lished method [9]). By comparison of the two techniques it can be noted that:

(a) the HPLC method enables the separation of gepirone, ipsapirone and zalospirone, but not of buspirone; the CZE method is suitable for separating all four drugs;

(b) both techniques allow good resolution of these drugs from their principal impurities. However, neither technique allows the detection of impurity Z(III), because it is a neutral and non-fluorescent compound. The resolution of the *cis* and *trans* form of impurity Z(IV) obtained by HPLC is interesting, but is not important in order to determine the quality of the drug;

(c) the detection limit by HPLC at a signal-to-noise ratio of 1:3 is about 0.15 ng for each compound, while it is about 0.36 ng using CZE;

(d) analysis by CZE requires less time than HPLC; The RSD of replicate test samples of drug at 1 mg ml^{-1} ($n = 6$) was: ±1.2% for zalospirone; ±1.3% for gepirone; ±1.0% for ipsapirone (for CZE using an IS). The corresponding RSD data obtained by HPLC (where no IS was used) were: ±0.7% for zalospirone; ±0.6% for gepirone; and ±1% for ipsapirone.

Comparison of these two separation techniques highlights their similar selectivity, linearity and repeatability. From the data reported above HPLC seems to be a little more precise than CZE. However, the differences in the volumes injected in HPLC and CE systems should be considered.

The major difference found for these two techniques was the detection limit. But in CZE this point can be readily improved using an uncoated capillary with a bubble cell. The bubble cell, located in the detection region, is an effective way to extend the optical path-length. When the zone front enters the bubble its velocity decreases and the zone concentrates or "stacks" in a manner similar to electrophoretic stacking during injection. As the sample zone expands radially (across the capillary) to fill the increased volume, it contracts longitudinally (along the capillary). Thus the sample concentration remains constant but the path-length increases. In fact repeating the analysis with the same solutions of drugs and impurities, the detection limit fell from 0.36 ng to about 0.12 ng, which is comparable with that observed for HPLC.

As reported in the literature [10, 11] the CZE method gave good performance in the analysis of pharmaceuticals and their related substances. The analyses of anxiolytic compounds and their impurities carried out by CZE and HPLC showed excellent agreement, demonstrating the complementary nature of the two techniques.

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