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Determination of fenticonazole and its impurities by capillary electrophoresis and high performance liquid chromatography

Fenticonazole, a topical antifungal agent containing a stereogenic centre, is used in therapy as a racemic mixture. Five related compounds can be found as impurities in the drug. We propose a HPLC method using as stationary phase a RP-8 column eluted with different gradients of acetonitrile/phosphate buffer (pH 6) for simultaneous determination of the drug and these impurities. We also studied a high performance capillary electrophoresis (HPCE) method for quality control of fenticonazole. The separation of fenticonazole from all its impurities by HPCE was obtained in a relative short capillary (40 cm, effective length 34 cm, 50 μm ID) with a running buffer of 30 mM phosphate (pH 3) containing 8 mM trimethyl-β-cyclodextrin. Under these experimental conditions very good separation of fenticonazole from each individual impurity is obtained in less than 20 minutes. The choice of cyclodextrin added to the running buffer was dictated by the chiral nature of fenticonazole; trimethyl-β-cyclodextrin is mainly used as chiral selector. The optimised HPLC and HPCE methods are compared.

Key Words: High performance liquid chromatography; Capillary electrophoresis; Quality control; Fenticonazole

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1 Introduction

Fenticonazole, [(*RS*)-1-[2-(2,4-dichlorophenyl)-2-[4-(phenylthio)phenyl)methoxy]-ethyl-1*H*-imidazole] nitrate, is a chiral azoic antifungal agent used in therapy as racemic mixture. Generally five impurities can be present in the fenticonazole raw material (**Figure 1**). These impurities are (*RS*)-1-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethanol (A), [(*RS*)-1-[2-(2,4-dichlorophenyl)-2-[4-(phenylsulphinyl)benzyloxy]ethyl]-1*H*-imidazole (B), [(*RS*)-1-[2-(2,4-dichlorophenyl)2-[4-(phenylsulphonyl)-benzyloxy]ethyl]-1*H*-imidazole (C), [(*RS*)-1-[2-(2,4-dichlorophenyl)-2-hydroxyethyl]-3-[4-(phenylthio)phenylmethyl]imidazolium nitrate (D), and [(*RS*)-1-[2-(2,4-dichlorophenyl)-2-[4-(phenylthio)phenyl)methoxyl]ethyl]-3-[4-(phenylthio)phenylmethyl]imidazolium nitrate (E). Over the last decade many spectroscopic [1–3], chromatographic [4–8], and capillary electrophoretic [9, 10] methods for different azoic derivatives have been published, but none of these papers was about fenticonazole. Only European Pharmaco-

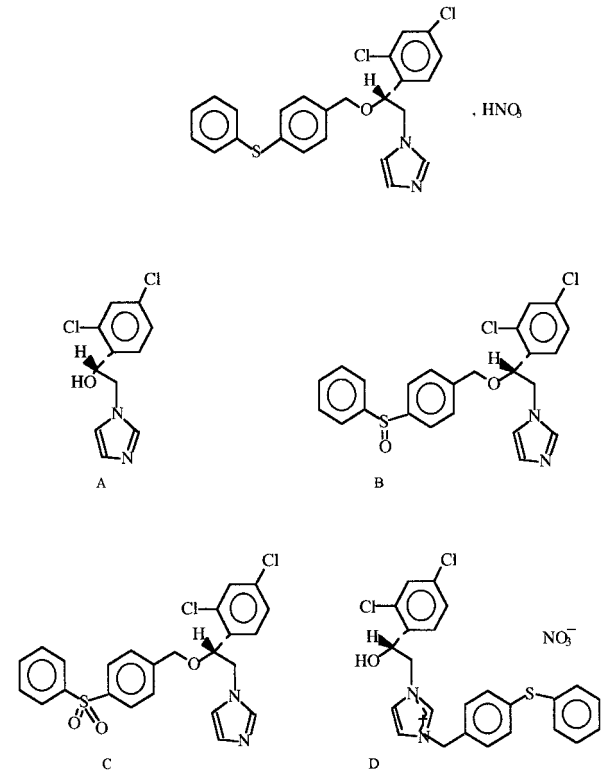

Figure 1. Molecular structures of fenticonazole and impurities A, B, C, D, and E.

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peia – Supplement 2000 [11] suggests a RP18-HPLC method for recognition of the fenticonazole impurities.

The quality control of a drug is generally performed by HPLC, but sometimes, as in this case, the separation requires a long analysis time. Therefore we turned our attention to HPCE which could be a good choice for the determination of a drug and its related impurities. The separation of fenticonazole from all impurities by HPCE was accomplished with a phosphate running buffer (pH 3) containing trimethyl-β-cyclodextrin. To compare the suitability of HPCE and HPLC techniques for the quality control of fenticonazole, we also studied a chromatographic method which, using a RP-8 column eluted with different gradients of phosphate buffer/acetonitrile mixture, allows a good separation of the drug from each one of the impurities. The data obtained from HPLC and HPCE analyses were compared.

2 Experimental

2.1 Equipment

High Performance Liquid Chromatography

The chromatographic separation of fenticonazole from its five impurities was carried out using a Merck-Hitachi Series L-7000 chromatograph, equipped with a Merck-Hitachi Series L-7450 photodiode array detector. The chromatograph was controlled and the data evaluated by a Flyer Pentium computer, interface D-7000.

The HPLC separation was performed on a Lichrosphere RP-8 column (250 × 4 mm ID, 5 μm).

Standard and sample solutions were injected via a Rheodyne Model 7725 valve using a 20 μL sample loop.

The detector conditions were: acquisition rate of spectra 1600 ms; spectral bandwidth for each channel, 4; wavelength range: 200–380 nm, λ = 220 nm.

High Performance Capillary Electrophoresis

Standards and samples were analysed by a Hewlett-Packard^{3D} CE Instrument (Palo Alto, CA, USA) equipped with an autosampler and a linear diode array detector. The instrument was controlled and the data evaluated by a ChemStation and a computer HP KAYAK XM 600 Pentium 3. The analyses were carried out in an uncoated fused silica capillaries (Hewlett-Packard CE capillaries).

2.2 Chemicals

Solvents and chemicals were of analytical HPLC grade (Merck, Darmstadt, Germany). Econazole, used as internal standard, was obtained from Sigma-Aldrich (Italy), while fenticonazole and related compounds, standards, and raw material were kindly supplied by Recordati Laboratories (Campoverde-Latina, Italy).

2.3 Analysis conditions

Fenticonazole and impurities standards were used to prepare reference solutions, while fenticonazole raw material was used to prepare the sample solutions.

To prepare the reference solution, about 25 mg of fenticonazole standard, exactly weighed, was dissolved in a 25 mL volumetric flask with methanol and diluted up to the mark with the same solvent.

About 10 mg, exactly weighed, of each individual impurity standard (A, B, C, D, E) was transferred into a 10 mL volumetric flask, dissolved in methanol, and then diluted up to the mark with the same solvent.

A working standard solution was prepared by transferring 5 mL of drug reference solution and 5 mL of each individual impurity reference solution into a 50 mL volumetric flask and diluting to volume with methanol.

The sample solution was prepared by exactly weighing about 25 mg of fenticonazole raw material and dissolving the drug in methanol up to 25 mL.

2.4 Analytical procedures

The solutions prepared as described above were used for both HPLC and CE analyses.

HPLC analyses of fenticonazole and its impurities were performed using a mixture of solvent A (acetonitrile) and solvent B (10 mM KH_2PO_4, titrated with 1 M NaOH until pH 6) mixed in different ratios (**Table 1**) as mobile phase. The flow rate was 0.8 mL min^{-1}.

The chromatograms were recorded at a λ value of 220 nm using external standards. The concentrations of the impurities were determined from the impurity to fenticonazole peak area ratios.

The HPCE analyses were carried out in an uncoated capillary (40 cm total length and 34 cm effective length, ID 50 μm). The running buffer was a 30 mM NaH_2PO_4 solution, titrated until pH 3 with 30 mM H_3PO_4, admixed with

Table 1. Mobile phase conditions used in HPLC experiments.

Time (min)	% A	% B
0	60	40
7	60	40
25	90	10
80	80	20

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6 mM trimethyl-β-cyclodextrin solution. The determination of fenticonazole and its impurities was carried out using econazole as internal standard.

Standard and sample solutions were injected for 4 s in the hydrodynamic mode. The working parameters were: pressure 0.8 psi; applied voltage 15 kV; capillary temperature 25°C.

The electropherograms were recorded at a λ value of 200 nm.

3 Results and discussion

A RP 18 stationary phase, eluted in the isocratic mode with acetonitrile/phosphate buffer (70/30, pH 3), failed to give a good separation of the impurities from the drug. Actually impurity B coeluted with impurity C. Therefore we studied a more robust HPLC method, while simultaneously also turning our attention to HPCE, which could be an alternative route to control the quality of this drug.

3.1 High performance liquid chromatography

To improve the separation among fenticonazole and impurities we increased the polarity of stationary phase using a Lichrosphere RP-8. As mobile phase a gradient of 10 mM phosphate buffer (pH 6) and acetonitrile was used (see Section 2.3 of the Experimental Part). **Figure 2** shows the chromatogram, obtained by injecting the working standard solution. The method allowed a good separation of fenticonazole from impurities, but it required a long analysis time of about 60 minutes. Using this method we undertook quality control of some raw material samples in which only impurity A was found (0.12% on average).

3.2 High performance capillary electrophoresis

With the aim of developing a method for certifying the purity of fenticonazole, we analysed this drug by Capillary Electrophoresis. As stated above, five fenticonazole impurities are reported in *Eur. Pharm.* This requires a method able to rapidly separate six compounds. Initially we used a long capillary (total length 64 cm and effective length 50 cm), filled with 50 mM phosphate buffer (pH 3). Under these conditions the analysis time was shorter than HPLC analysis (about 15 min), but only four peaks were observed in the electropherogram. These peaks were related to impurity A, impurities B and C, impurity D, and fenticonazole with impurity E (**Figure 3**). Moreover, using a long capillary fenticonazole seems to interact with the internal capillary wall, making the washing cycle between the runs more difficult.

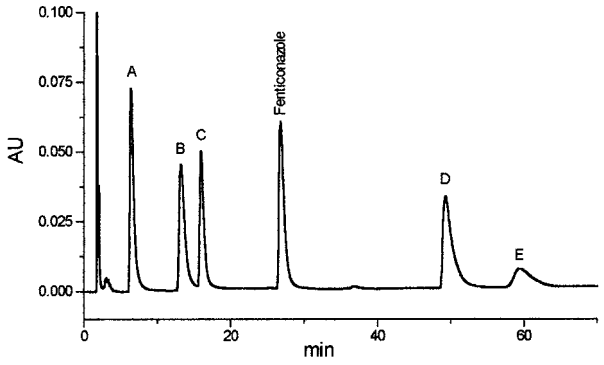

Figure 2. RP-C8 chromatogram of a working standard solution containing fenticonazole (0.5 mg × mL^{-1}) and impurities A, B, C, D, E (0.5 mg × mL^{-1}).

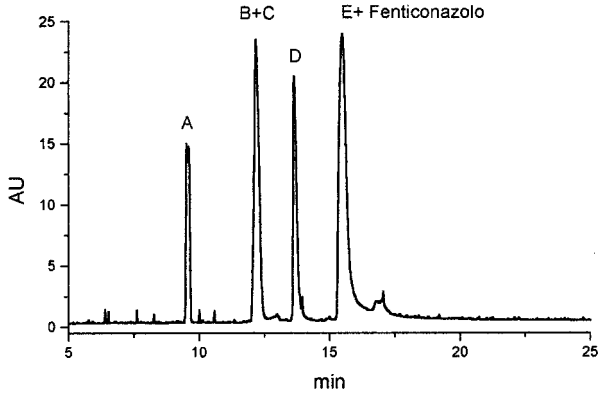

Figure 3. Electropherogram of a working standard solution containing fenticonazole and impurities A, B, C, D, E. Capillary: ID 50 μm, total length 64 cm, effective length 50 cm; BGE: 50 mM phosphate buffer (pH 3); applied voltage: 15 kV capillary temperature: 25°C.

The HPCE experiments were repeated in a shorter capillary (40 cm total length and 34 cm effective length). The running buffer was 50 mM phosphate (pH 3) containing 10% of methanol as organic modifier. Under these conditions impurity A and fenticonazole were well resolved, impurities B and C were not baseline resolved, while the impurity D and E were coeluted (**Figure 4**). Undoubtedly, the resolution of the mixture has been improved and the analysis time has been reduced to 12 minutes, but the method was still not satisfactory.

Resolution of fenticonazole from each one of the impurities was accomplished by reducing the ion strength of the phosphate buffer (30 mM) and adding 8 mM trimethyl-β-cyclodextrins in the background electrolyte. The drug-CD complex formation increased the analysis time from 12 min to 20 min, but allowed a very good resolution of all compounds of interest (**Figure 5**).

The addition of trimethyl-β-cyclodextrin to the buffer solution requires great care. Actually fenticonazole and impurities are chiral compounds and trimethyl-β-cyclodextrins

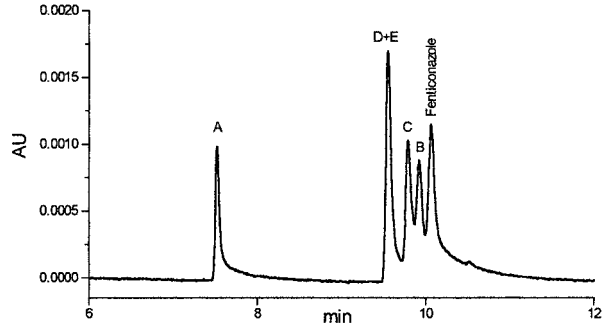

Figure 4. Electropherogram of a working standard solution containing fenticonazole and A, B, C, D, E impurities. Capillary: ID 50 μm, total length 40 cm, effective length 34 cm; BGE: 50 mM phosphate buffer (pH 3) containing 10% methanol; applied voltage: 15 kV; capillary temperature: 25°C.

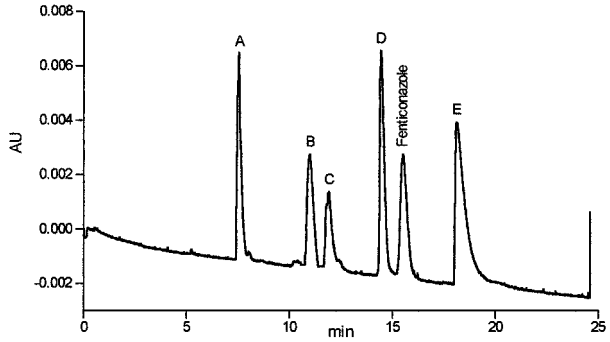

Figure 5. Electropherogram of a working standard solution containing fenticonazole and impurities A, B, C, D, E. Capillary: ID 50 μm, total length 40 cm, effective length 34 cm. BGE: 30 mM phosphate buffer (pH 2.5) with 8 mM trimethyl-β-cyclodextrin; applied voltage: 15 kV; capillary temperature 25°C.

are very suitable chiral selectors for a lot of drugs, including the compounds of present interest. Therefore, if the concentration of trimethyl-β-cyclodextrin is increased from 8 mM to 10 mM, a partial enantioseparation of fenticonazole starts.

3.3 Linearity, precision and accuracy, LOD, LOQ

To verify the chromatographic and electrophoretic performances of proposed methods, a working standard mixture, containing a known amount of fenticonazole and impurities, was prepared. This solution was analysed using both techniques.

3.3.1 HPLC

A calibration curve obtained by plotting five increasing concentrations of fenticonazole standard solution *versus* peak areas (external standardization) allowed determination of the drug. All experiments were performed twice and the individual data points were the mean of two determinations. The drug concentration range observed was between 0.05 mg mL^{-1} and 1.5 mg mL^{-1} ($r = 0.998$), while the impurity concentrations range examined was between 0.0025 mg mL^{-1} – 0.15 mg mL^{-1}. The impurity concentrations were expressed as the percentage ratio between impurities and drug peak areas.

The accuracy and precision of the method were 98.9% and 1.8%, respectively (mean of six determinations). Calculation of the signal to noise (*S/N*) ratio gave the limits of detection (LOD) and quantitation (LOQ). The medium LOD and LOQ for the impurities were 5×10^{-5} mg and 1.5×10^{-4} mg, respectively. In all raw material samples examined only the impurity A (about 0.12%) was found.

3.3.2 HPCE

Quantitative analyses of fenticonazole and its impurities by HPCE were performed using econazole as internal standard. To evaluate the suitability of econazole as internal standard, the calibration curves of drug and internal standard were plotted on the same graph. The concentration range examined was between 0.01 mg mL^{-1} and 0.15 mg mL^{-1} for fenticonazole and internal standard and between 0.0025 mg mL^{-1} and 0.01 mg mL^{-1} for each individual impurity. The accuracy and precision of the method were 97.4% and 2.2%, respectively (mean of six determinations). The signal to noise (*S/N*) ratio allowed determination of the limits of detection (LOD) and quantitation (LOQ). The medium LOD for the fenticonazole impurities was 1.2×10^{-8} mg while the medium LOQ was 3.6×10^{-8} mg.

In the fenticonazole raw material samples examined only the impurity A was found (about 0.115%).

4 Conclusion

HPCE can be regarded as an alternative and complementary technique for quality control of pharmaceuticals. The simplicity and speed of the HPCE method described and the low cost of capillaries and solvents underline the suitability of this technique. Both the HPLC and the HPCE techniques showed a comparable sensitivity and precision. The repeatability, on the basis of the peak area ratios for six replicated injections of each sample, was 0.75–1.40% for HPLC and 0.89–1.98% for HPCE. Therefore CE is a valuable alternative to the HPLC technique.

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