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Short communication

Enantioselective analysis of amisulpride in pharmaceutical formulations by means of capillary electrophoresis

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Abstract

A capillary electrophoretic method has been developed for the enantioselective analysis of amisulpride in pharmaceutical formulations, using β-cyclodextrin sulfate as the chiral selector. Several parameters, such as cyclodextrin type and concentration, buffer concentration and pH and capillary temperature were investigated for method optimisation. Baseline enantioseparation of the racemic compound was achieved in less than 10 min using a fused silica capillary (50 μm i.d. and 33.0, 8.5 cm, total and effective length, respectively), filled with a background electrolyte consisting of a 10 mM citrate buffer at pH 3.5 supplemented with 0.22% (w/v) β-cyclodextrin sulfate at 20 °C and applying a voltage of +15 kV. Formulation analysis was carried out after analyte extraction by methanol. The method was fully validated, with good results in terms of precision, selectivity, accuracy and amount of drug found with respect to the label claim. Thus, the method seems to be suitable for the enantiomeric analysis of amisulpride in pharmaceutical formulations.

Keywords: Amisulpride; Enantiomers; Capillary electrophoresis; Chiral separation; Cyclodextrin; Quality control

1. Introduction

Amisulpride ((±)-4-amino-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-(ethylsulfonyl)-2-methoxybenzamide, AMI, Fig. 1) is an atypical antipsychotic drug with benzamidic structure, that is active against both positive (hallucinations, delusions) and negative (anergia, flat affectivity) symptoms of schizophrenia [1–3]. AMI is usually associated with fewer extrapyramidal side-effects [4] than "classical" neuroleptics; its most common adverse events are insomnia, hyperkinesia, anxiety and mild extrapyramidal symptoms [3,5].

AMI possesses an asymmetrically substituted carbon atom, thus it exists as an enantiomer pair. The drug is usually administered as a racemic mixture in the form of uncoated (Deniban®, Solian® or Sulamid®) or coated (Solian®) tablets. However, binding studies [6] have found that *S*-(−)-AMI is twice more potent than the racemic form and 19–38 times more potent than *R*-(+)-AMI as a D_2 and D_3 ligand. Thus, it has been hypotesised that *S*-(−)-AMI is the enantiomer responsible for the pharmacological activity of the drug [6].

Several analytical methods dealing with the determination of amisulpride have been published. Most of them have been applied to the simultaneous determination of several drugs for, e.g., screening, toxicological and forensic purposes [7–9]. The analysis of amisulpride alone (or together with other benzamides) in biological fluids has been carried out using liquid chromatographic methods [10–14]; another paper deals with the analysis of AMI in formulations [15]. To the best of our knowledge, only one method based on HPLC can be found in the literature for the enantioselective analysis of AMI [16]. The aim of the present work is the development of a reliable capillary electrophoretic method for the enantioseparation and the analysis of amisulpride in pharmaceutical formulations. In fact, capillary electrophoresis is a very versatile and highly efficient technique, obtaining reliable chiral separations of drugs [17,18]

Abbreviations: AMI, amisulpride; S-β-CD, β-cyclodextrin sulfate sodium salt; CD, β-cyclodextrin

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in short times and using minute amounts of expensive chiral selectors [19–21].

2. Experimental

2.1. Chemicals and solutions

Racemic standard amisulpride was kindly provided by Sanofi Synthelabo (Paris, France). Lamotrigine, used as the internal standard (I.S., Fig. 1), was kindly provided by GlaxoSmithKline (Stevenage, UK).

All chemicals were analytical grade or better. β-Cyclodextrin sulfate sodium salt (S-β-CD) was purchased from Fluka (Buchs, Switzerland). Phosphoric acid (85%, w/w), citric acid, methanol, 2 M sodium hydroxide were from Carlo Erba (Milan, Italy).

Ultrapure water (18.2 MΩ cm) was obtained by means of a Millipore (Bedford, MA, USA) MilliQ apparatus.

Stock solutions of AMI (1 mg mL^{-1}) were prepared by dissolving suitable amounts of the pure substance in methanol. Standard solutions were obtained by diluting stock solutions with ultrapure water. The stock solutions were stable for at least 2 months when stored at –20 °C (as assessed by electrophoresis); standard solutions were prepared every day.

The background electrolyte (BGE) was a pH 3.5, 10 mM citrate buffer containing 0.22% (w/v) S-β-CD.

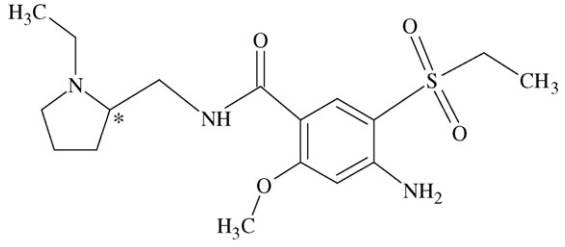
2.2. Apparatus and electrophoretic conditions

All assays were carried out on an Agilent (Palo Alto, CA, USA) ^{3D}CE apparatus equipped with a diode array detector. The separation was achieved on an uncoated fused silica capillary (Composite Metal Services Ltd., Hallow, UK; 33.0 cm total length, 8.5 cm effective length, 50 μm i.d., 375 μm o.d.). Peak detection was carried out at 227 nm.

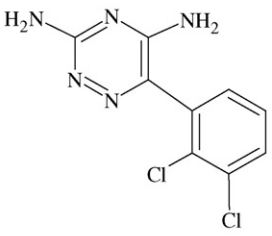
A constant voltage of +15 kV was applied and the sample was injected by pressure (50 mbar × 5 s) at the cathodic end of the capillary. The capillary was thermostatted at 20 °C.

Before use, new capillaries were washed with water (10 min), 1 M NaOH (10 min) and water again (20 min), then conditioned with the BGE for 15 min before injecting. Between each electrophoretic run the capillary was conditioned with: HCl (2 min), water (1 min), MeOH (2 min), water (1 min), NaOH (30 s), water (2 min) and finally BGE (3 min).

For storage overnight, the capillary was rinsed with water (5 min), 0.1N NaOH (5 min) and water (10 min) and was then air dried (2 min).



Amisulpride



Lamotrigine

Fig. 1. Chemical structures of the analytes and the I.S.

2.3. Analysis of formulations

Tablets of Deniban® (Sanofi-Synthelabo) with a declared content of 50 mg of racemic AMI were analysed. The excipients were sodium starch glycolate type A, lactose, microcrystalline cellulose, hypromellose, magnesium stearate.

Twenty tablets were accurately weighed, then ground to a fine powder in a mortar and thoroughly mixed. An amount equivalent to 50 mg (declared) of racemic AMI was weighed and transferred into a 50-mL volumetric flask and about 40 mL of MeOH and a suitable amount of I.S. were added. The mixture was then sonicated for 10 min, allowed to rest for 10 min before bringing it up to volume and finally filtered (filter pore size: 0.20 μm) This solution (nominal racemic concentration: 1 mg mL^{-1}) was then suitably diluted with water and analysed.

2.4. Method validation

2.4.1. Calibration curves

Six point calibration curves were set up for each AMI enantiomer in the 2.5–25 μg mL^{-1} concentration range, by plotting the analyte/I.S. peak area ratios (pure numbers) as a function of the injected concentration (expressed as μg mL^{-1}).

2.4.2. Amount of drug found of label claim

The analyte/I.S. peak area ratios of each enantiomer, obtained by injecting the diluted extract (at the nominal racemic concentrations of 5.0, 25.0 and 40.0 μg mL^{-1}), were analysed and the concentrations found were compared to those declared by the manufacturer.

2.4.3. Precision assays

AMI standard and tablet solutions were prepared at three different levels (racemic concentrations corresponding to 5.0, 25.0 and 40.0 μg mL^{-1}) and analysed six times within the same day to obtain repeatability and in six different days to obtain intermediate precision according to the United States Pharmacopeia requirements [22]. The quantitation limit (LOQ) and the detection limit (LOD) were calculated according to USP 28 guidelines [22] as the AMI concentrations whose peak height