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Short communication

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

Alessandro Musenga [a], Roberto Mandrioli [a], Emanuele Morganti [a], Salvatore Fanali [b], Maria Augusta Raggi [a,*]

[a] *Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy*
[b] *Institute of Chemical Methodologies, National Council of Research, Research Area of Rome, 00016 Monterotondo Scalo, Rome, Italy*

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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

* Corresponding author. Tel.: +39 051 2099700; fax: +39 051 2099740.
E-mail address: mariaaugusta.raggi@unibo.it (M.A. Raggi).

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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.

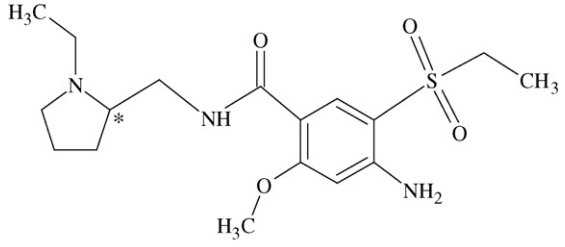
2.2. Apparatus and electrophoretic conditions

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.

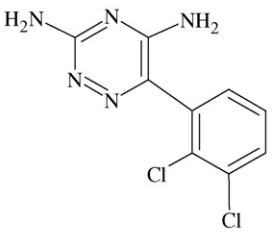
A constant voltage of +15 kV was applied and the sample was injected by pressure (50 mbar × 5 s) at the cathodic end of the capillary. The capillary was thermostatted at 20 °C.

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

corresponded to 10 times and 3 times the background noise, respectively.

2.4.4. Accuracy

The accuracy of the method was evaluated by means of recovery assays, by adding a known amount of the reference powder to a certain amount of the pharmaceutical formulation, in order to obtain three different levels of addition (racemic concentrations: 5, 10 and 25 μg mL^{-1}). The samples were analysed and accuracy was calculated as the mean recovery percentage.

3. Results and discussion

3.1. CZE conditions

Amisulpride is sparingly soluble (solubility = 0.14 g L^{-1}, see footnote 1) in unbuffered water. However, it has one ionisable amino group, which can be charged at acidic pH values, making the molecule more soluble (solubility = 160 g L^{-1} at pH 3.0 (see Footnote 1)). For this reason, it was decided to use a pH 2.5, 50 mM phosphate buffer as a starting point. As regards the chiral selector, cyclodextrins, especially when modified with charged or chargeable groups, are well known for their capability to separate amino compounds [21,23–25]. Different neutral and negatively charged cyclodextrins were tested. As expected, considering the positive charge presented by the analytes, negatively charged cyclodextrins (carboxymethyl-β-cyclodextrin and β-cyclodextrin sulfate, S-β-CD) gave the best results, as no separation was observed employing unmodified β-cyclodextrin and only a slight chiral separation with heptakis(2,6-di-*O*-methyl)-β-cyclodextrin up to 2% (w/v) in the BGE.

3.1.1. Separation in normal polarity mode

As a first trial, normal polarity mode conditions were investigated, using carboxymethyl-β-cyclodextrin and S-β-CD. Only a partial enantiomeric separation was obtained with carboxymethyl-β-cyclodextrin at pH values between 2.0 and 4.5, while no separation was obtained at pH 5.5. S-β-CD showed stronger interaction than carboxymethyl-β-cyclodextrin with both enantiomers, however baseline separation was not obtained, even if resolution improved when increasing S-β-CD concentrations. A combination of S-β-CD and carboxymethyl-β-cyclodextrin was not successful either.

3.1.2. Separation in reversed polarity mode

According to our previous experience [25], efficient enantiomeric separations can be obtained employing a negatively charged cyclodextrin at high concentration and a reversed polarity mode.

In this case, the electroosmotic flow (EOF) is opposed to the migration of the analytes; this is useful in order to increase the analyte resolution, however longer migration times have to be expected when the EOF is too high.

Therefore, the pH of the BGE has to be maintained in the acidic range. Moreover, using a low concentration (10 mM) BGE, anodic migration of AMI enantiomers was observed even at low S-β-CD concentrations (e.g. 0.15% (w/v)). Thus, the ionic concentration of the buffer was maintained constant at 10 mM and several S-β-CD concentrations were tested, between 0.15 and 0.50% (w/v), keeping the voltage at −25 kV. As expected, resolution increased when reducing the S-β-CD concentration, but with longer migration times and considerable peak tailing. Higher pH values were also investigated: pH 3.0 phosphate and pH 3.5 and pH 4.0 citrate buffers. An increase of EOF was observed when raising the buffer pH, together with longer migration times and an increased enantiomeric resolution. Best results (highest chiral resolution in the shortest time) were obtained at pH 3.5 with 0.2% S-β-CD. The effect of the applied voltage and the capillary temperature on resolution was studied: best results and complete enantiomeric separation were obtained at 20 °C, applying a −15 kV voltage.

Finally, in order to reduce the analysis time, a shorter effective capillary length (8.5 cm instead of 24.5 cm) was used, reversing both the voltage and the injection side; consequently, the instrumental voltage was set to a value of +15 kV, which still corresponds to a reversed polarity mode, due to the short-end injection. The S-β-CD concentration was finely tuned under these new conditions, in order to reduce analysis times as much as possible while maintaining complete enantioresolution of AMI. This result was obtained with 0.22% (w/v) S-β-CD. The electropherogram of a standard AMI solution (racemic concentration 25 μg mL^{-1}; I.S. concentration 10 μg mL^{-1}) is reported in Fig. 2a. As can be seen, complete enantiomeric separation is obtained within a 10-min run.

3.2. Method validation and formulation analysis

3.2.1. Extraction and analysis of amisulpride from pharmaceutical formulations

The extraction procedure is based on a single extraction with methanol; this simple procedure was sufficient to quantitatively extract AMI from Deniban® tablets. In order to verify that a complete extraction was obtained, higher volumes of methanol (75 and 100 mL) were tested, keeping the amount of weighed Deniban® constant. The solutions obtained were analysed and compared to those obtained by extracting with 50 mL of methanol: no difference was observed, confirming that 50 mL were sufficient for a complete extraction.

An electropherogram obtained by analysing a Deniban® extract solution (nominal racemic concentration 25 μg mL^{-1}) spiked with the I.S. (10 μg mL^{-1}) is reported in Fig. 2b. No interference from the matrix can be observed and the electropherogram has the same profile as the one in Fig. 2a. AMI peaks were confirmed by comparison of their migration times and UV spectra with those obtained analysing standard solutions.

3.2.2. Linearity

Good linearity for each enantiomer (r_c always higher than 0.999) was obtained within the 2.5–25.0 μg mL^{-1} concentration range. Calibration equations were $y = 0.0897x + 0.0175$ (AMI 1)

[1] Calculated using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris.

Table 1
Enantiomeric quality control of amisulpride in Deniban® tablets

Racemic concentration ($\mu g\,mL^{-1}$)	Amount found of the label claim (%)		Repeatability (R.S.D.%)[a]				Intermediate precision (R.S.D.%)[a]			
	AMI 1	AMI 2	Standard solutions		Deniban® extracts		Standard solutions		Deniban® extracts	
			AMI 1	AMI 2	AMI 1	AMI 2	AMI 1	AMI 2	AMI 1	AMI 2
5.0	95.7	96.2	6.8	7.4	7.3	7.4	6.4	7.3	7.4	6.3
25.0	98.0	98.8	2.9	3.7	3.1	4.6	3.6	5.1	5.3	5.5
40.0	96.4	96.0	2.6	3.0	3.7	5.1	4.4	4.1	5.3	5.6

[a] $n = 6$.

and $y = 0.0861x + 0.0496$ (AMI 2). LOQ and LOD values for each enantiomer corresponded to 2.5 and 1.0 $\mu g\,mL^{-1}$, respectively.

3.2.3. Amount of drug found of label claim

The amount of drug found with respect to the label claim was calculated for both enantiomers by analysing Deniban® extracts after dilution in water at three different nominal concentration levels: 5.0, 25.0 and 40.0 $\mu g\,mL^{-1}$ (concentration of the racemate). The results obtained were expressed as w/w percentages and are reported in Table 1.

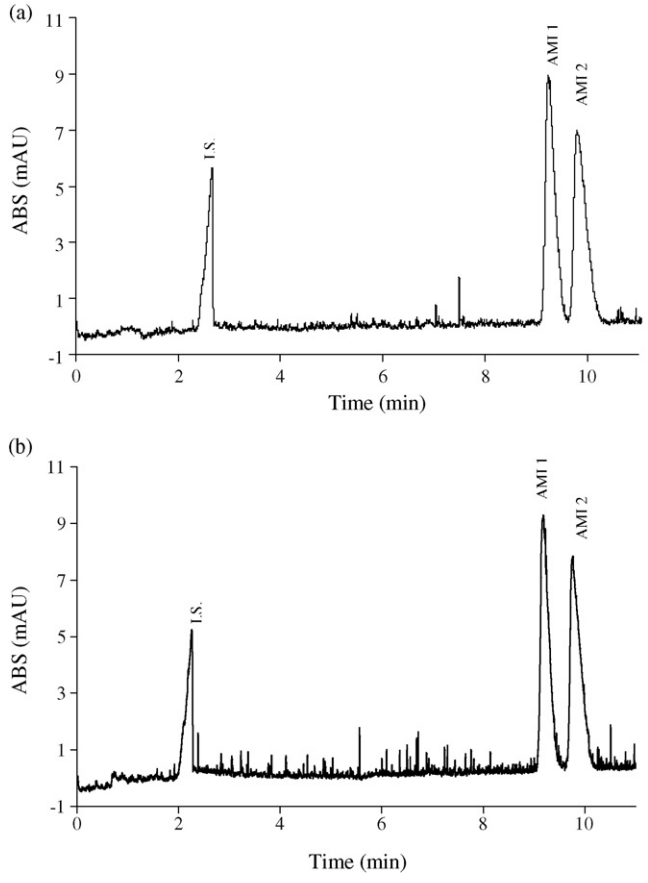


Fig. 2. Electropherograms of: (a) an AMI standard solution (racemic concentration 25.0 $\mu g\,mL^{-1}$; I.S. concentration 10 $\mu g\,mL^{-1}$); (b) a Deniban® extract (racemic concentration 25.0 $\mu g\,mL^{-1}$; I.S. concentration 10 $\mu g\,mL^{-1}$). CE conditions: capillary, T.L. 33.0 cm, E.L. 8.5 cm; BGE, 10 mM, pH 3.5 citrate buffer containing 0.22% (w/v) S-β-CD; voltage, +15 kV; injection, pressure (50 mbar × 5 s); *T*, 20 °C.

3.2.4. Precision assays

Repeatability and intermediate precision were assessed on both AMI standard solutions and Deniban® extracts, as described in Section 2. The results obtained are reported in Table 1.

3.2.5. Accuracy

Accuracy was also evaluated and the results, expressed as recovery percentage values, were always between 90.0 and 95.6%.

4. Conclusions

A capillary electrophoretic method has been developed for the enantioselective analysis of AMI. The effect of several parameters, such as pH of the BGE, cyclodextrin type and concentration, applied voltage and temperature, on the enantiomeric separation was studied. A complete separation of AMI enantiomers was achieved within a 10 min electrophoretic run. The method has been fully validated, and it was then successfully applied to the determination of AMI enantiomer content in Deniban® tablets. To the best of our knowledge, this is the first method which analyses AMI enantiomers by means of capillary electrophoresis.

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