Journal of Pharmaceutical and Biomedical Analysis 102 (2015) 100–109

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Evaluation of enantiomeric purity of magnesium-L-aspartate dihydrate

Oliver Wahl, Ulrike Holzgrabe*

University of Würzburg, Institute for Pharmacy and Food Chemistry, Würzburg 97074, Germany

ARTICLE INFO

Article history:
Received 17 June 2014
Received in revised form 6 August 2014
Accepted 7 August 2014
Available online 17 August 2014

Keywords:
Pharmaceutical analysis
Capillary electrophoresis
Magnesium aspartate
Chiral separation
Racemization

ABSTRACT

Magnesium supplementation in form of organic magnesium salts is a very popular practice. We examined the enantiomeric purity of "Magnesium aspartate dihydrate" monographed in the European Pharmacopeia. A chiral capillary zone electrophoresis using (2-hydroxypropyl)-β-cyclodextrin coupled to laser induced fluorescence detection and a HPLC-fluorescence method with chiral derivatization using *o*-phthaldialdehyde and *N*-acetyl-L-cysteine as an orthogonal method were developed and validated. Two batch samples of this substance and three drug products containing the salt were analyzed by means of both methods. The concentration of the D-enantiomer of aspartic acid ranged from 0.03 to 0.12%. Simulations of the synthesis revealed that the D-aspartic acid content is elevated if the dissolution of L-aspartic acid was performed at acidic pH values.

1. Introduction

Innumerous magnesium salts for the sometimes controversially discussed treatment of hypomagnesaemia and cramp prophylaxis are currently available on the market [1,2]. The salts are classified according to the anion into organic and inorganic magnesium salts. Among the inorganic magnesium salts as drug products are magnesium oxide (MgO), magnesium carbonate ($MgCO_3$), magnesium hydroxide ($Mg(OH)_2$), magnesium hydrogen phosphate ($MgHPO_4$) and magnesium chloride ($MgCl_2$). The organic salts, mostly salts of carboxylic acids, possess a higher solubility in water and are therefore supposed to be better bioavailable [3,4]. Nevertheless, according to latest work, there is no special benefit of the organic salts for the reconstitution of the normal magnesium status compared to the inorganic salts [5,6]. At least from the pharmaceutical point of view, the better soluble organic salts seem to be more suitable for the production of effervescent tablets and powders because the resulting liquid dosage form is a solution and not a suspension. The organic salts are salts of citric, glucuronic, acetic, glutamic or aspartic acid. In the case of aspartic acid there are three relevant salts: magnesium aspartate hydrochloride trihydrate, magnesium bis(hydrogen-DL-aspartate) tetrahydrate and magnesium bis(L-hydrogenaspartate) dihydrate (Fig. 1). The latter one is the focus of this study. The substance is monographed in the European Pharmacopeia (Ph. Eur.) with the title "magnesium aspartate dihydrate" [7] and should contain the L-amino acid only. Among the amino acids, aspartic acid exhibits the highest racemization rate in peptide or protein structures [8]. That is why the D-aspartic acid content in aged tissue, fossil, tooth or bone samples is widely utilized for the age determination of those samples [9–13]. The mechanism of racemization in peptides, however, is different to that of the free amino acid because the amide moiety is required in the proposed mechanisms for peptides [14–16]. Because of its neuroexcitatory effect on NMDA (*N*-methyl-D-asparatate) receptors [17–20] and interference with several endocrinal and hormonal systems [21–25] the D-aspartic acid concentration has to be restricted and controlled by means of a sensitive CE or HPLC method.

Due to the highest daily dose of approx. 1.8 g magnesium bis(hydrogenaspartate) dihydrate, any impurity (in our case D-aspartic acid) above 0.06% (1 mg daily intake) has to be specified and qualified according to ICH guideline Q3A(R2) [26]. However, two challenges have to be faced: on the one hand the separation

Abbreviations: API, active pharmaceutical ingredient; CBQCA, 3-(4-carboxybenzoyl)quinolone-2-carboxaldehyde; DMSO, dimethyl sulfoxide; DOE, design of experiments; EDQM, European Directorate for the Quality of Medicines & HealthCare; FLD, fluorescence detection; HP-β-CD, (2-hydroxypropyl)-β-cyclodextrin; ICH, International Conference on Harmonisation; IEP, isoelectric point; IS, internal standard; KF, Karl-Fischer; LIF, laser induced fluorescence; MeCN, acetonitrile; NAC, *N*-acetyl-L-cysteine; NMDA, *N*-methyl-D-aspartate; OPA, *o*-phthaldialdehyde; PFP, pentafluorophenyl; Ph. Eur., European Pharmacopeia; PH, phenyl-hexyl; PVDF, polyvinylidene fluoride.

* Corresponding author at: Institute for Pharmacy and Food Chemistry, Am Hubland, 97074 Würzburg, Germany. Tel.: +49 931 31 85460; fax: +49 931 31 85494.
E-mail address: u.holzgrabe@pharmazie.uni-wuerzburg.de (U. Holzgrabe).

http://dx.doi.org/10.1016/j.jpba.2014.08.013
0731-7085/

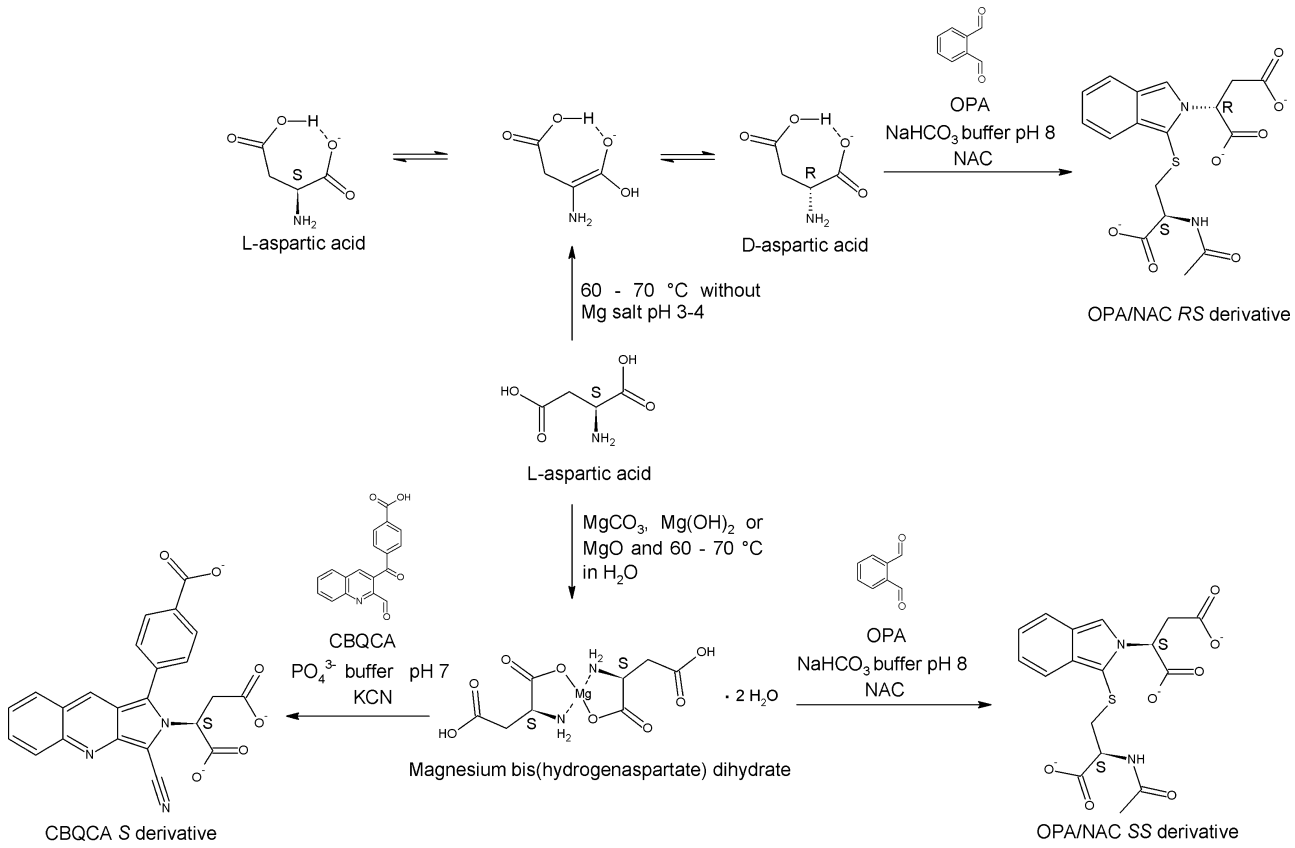

Fig. 1. Overview of magnesium bis(hydrogenaspartate) dihydrate synthesis, related substances and derivatization reactions; CBQCA, 3-(4-carboxybenzoyl)quinolone-2-carboxaldehyde; NAC, *N*-acetyl-L-cysteine; OPA, *o*-phthaldialdehyde.

of the enantiomers and on the other hand the fact that aspartic acid lacks a chromophore for UV detection. The enantiomeric resolution of amino acids can be achieved by many techniques. The chromatographic separation of D- and L-amino acids has been shown e.g. on chiral stationary crown ether phases [27–29], by chiral ligand exchange chromatography [30–32] or by chiral gas chromatography after derivatization [33]. However, the detection problem is not overcome by most of those techniques and in addition those stationary phases are relatively expensive. The key step therefore was to find an appropriate derivatization reaction that allowed for the detection and separation of the D- and the L-isomer of aspartic acid. A suitable derivatizing reagent with excellent characteristics for capillary electrophoretic separation coupled to laser induced fluorescence (LIF) detection is 3-(4-carboxybenzoyl)quinolone-2-carboxaldehyde (CBQCA) [34–37] (see Fig. 1) or naphthalene-2,3-dicarboxaldehyde [38]. The reactions lead to highly fluorescent derivatives easy to separate by means of CE.

The aims of this study were to evaluate the D-aspartic acid content in two active pharmaceutical ingredient (API) batch samples and three drug products obtained from the local market. Additionally, the reasons for elevated D-aspartic acid concentration were elucidated. For those purposes a robust CE separation of CBQCA derivatives using (2-hydroxypropyl)-β-cyclodextrin (HP-β-CD) as chiral selector was developed. The separation of diastereomeric *o*-phthaldialdehyde (OPA)/*N*-acetyl-L-cysteine (NAC) derivatives of aspartic acid (see Fig. 1) by means of an achiral HPLC separation [39] was elaborated as an orthogonal method in order to verify the CE results. The highly fluorescent isoindoles were detected by fluorescence detection (FLD) with excellent sensitivity (LOQ = 0.006%) and repeatability.

2. Experimental

2.1. Chemicals and materials

The magnesium aspartate dihydrate batch samples and L-aspartic acid were obtained from the European Directorate for the Quality of Medicines & HealthCare (EDQM) (Strasbourg, France). Magnaspart® 20 powder, Magnerot® A 500 granulate and Magnesium Verla® effervescent tablets were bought from a local pharmacy. HPLC grade acetonitrile, methanol, ammonium acetate and sodium hydrogen carbonate were purchased from VWR International S.A.S. (Fontenay-sous-Bois, France), hydrochloric acid 37%, (2-hydroxypropyl)-β-cyclodextrin with average MW = 1380 g/mol (HP-β-CD), sodium tetraborate decahydrate, acetic acid ≥ 99.9%, ammonium formate, hydrochloric acid 37%, HYDRANAL®-Titrant 5, HYDRANAL®-Solvent, *N*-acetyl-L-cysteine (NAC) 99%, L-glutamic acid > 99.5%, magnesium oxide, magnesium carbonate, magnesium hydroxide and *o*-phthaldialdehyde (OPA) from Sigma–Aldrich Chemie GmbH (Steinheim, Germany), D-aspartic acid from AlfaAesar (Karlsruhe, Germany), L-aspartic acid, disodium hydrogen phosphate dihydrate, sodium dihydrogen phosphate monohydrate, formamide, Acilit®, Neutralit® and Alkalit® indicator paper for pH measurement (catalog number: 109565) from Merck KGaA (Darmstadt, Germany), dimethyl sulfoxide 99.9% from Acros Organics (Geel, Beglium), CBQCA and potassium cyanide from Molecular Probes (Eugene, Oregon, USA), 2-propanol ≥ 99.7% from Bernd Kraft GmbH (Duisburg, Germany). All chemicals used were of analytical grade or even better. Ultrapure water was produced using the "MilliQ Synthesis" water purification system by Merck Millipore (Schwalbach, Germany).

HPLC columns tested during method development are summarized in Table 1.