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Short communication

Validation of an enantioselective analysis for (L)-pidolic acid by chiral gas chromatography with derivatization

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ABSTRACT

A sensitive and rapid analytical method has been validated for the enantiomeric purity determination of L-pidolic acid, a biological lactam and metabolite of glutamic acid commonly found in urine, skin, bones, brain and is available commercially as a food supplement. An efficient, two-step achiral derivatization was implemented which consisted of an alkylation step (using HCl-IPA) followed by an acylation step (using TFAA) of the carboxy and amide functional groups. This allowed detection with high sensitivity using gas chromatography with flame ionization detection. The described procedure employs a CP-Chiralsil-L Val column (25 m × 0.25 mm) at a constant flow rate of 1.5 mL min^{-1}, a gradient temperature program from 80 °C to 160 °C and an injector and detector temperature of 250 °C. The proposed method was validated according to ICH Q2 standards and included such parameters as specificity, system precision, analyst repeatability, intermediate precision, accuracy, linearity, LOD/LOQ and solution stability.

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1. Introduction

It has been over 130 years since Haitinger first discovered pidolic acid (PCA) as the dehydration product of glutamic acid [1]. Similar to naturally occurring amino acids, PCA is a chiral molecule with an asymmetric carbon in the absolute L stereochemical configuration. It is a well-known synthetic and biological derivative frequently explored in the medicinal, organic, materials and pharmaceutical chemistry fields.

In medicinal chemistry, L-PCA can be formed from glutamic acid or γ-glutamyl amino acids by γ-glutamylcyclotransferase and is found in urine, bone and other tissues [2,3].[1] It is marketed as a beauty and dietary supplement and is believed to be associated with the production of acetylcholine and γ-aminobutyric acid from the cerebral cortex [4]. It has even been reported to be present in human plasma [5]. In organic chemistry, (L)-PCA is routinely used as a starting material to explore the synthesis of new artificial amino acids [6].

More recently, (L)-PCA is being used as a coformer for the pharmaceutical candidate ertuglflozin, a sodium glucose cotransporter 2 (SGLT2) inhibitor currently in Phase 3 studies [7]. A cocrystalline complex of the amorphous ertugliflozin was formed with L-pidolic acid to improve the physical properties for manufacturing and to ensure appropriate drug substance quality. This is an emerging field with widespread application to pharmaceutical science and crystal engineering [8]. As L-PCA is a raw material in the ertugliflozin process, the analytical chiral control of this material provided the substrate for this manuscript.

The separation of enantiomers of common amino acids by HPLC or GC has been well reported in the literature [9–11]. Typically a derivatization reaction is employed on the carboxy and (or) amino functional groups of the amino acid, which chemically modifies the species appropriately to allow resolution and detection using various achiral or chiral platforms (i.e., HPLC or GC). Although there are numerous publications on the separation and identification of (L)- and (D)-amino acids, there are few available analytical reports concerning (L)-PCA. Global (L)-PCA suppliers rely on optical rotation analysis to control the enantiomeric purity of (L)-PCA rather than a more distinguishable and specific analysis to determine enantiomeric excess. Although PCA in the γ-glutamyl cycle consists of the single (L) enantiomer, the (D) enantiomer has been reported

Abbreviations: (L)-PCA, L-pidolic acid; (D)-PCA, D-pidolic acid; PCA, pidolic acid; (L)-PGA, L-pyroglutamic acid; (D)-PGA, D-pyroglutamic acid; PGA, pyroglutamic acid; TFAA, trifluoroacetic anhydride; HCl-IPA, hydrochloride 2-propanol solution; CPF, chiral purity factor; Wt, weight.

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[1] The IUPAC designation of L-pidolic acid is 2-(S)-(−)-5-oxoproline.

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in the urine of healthy individuals [12]. It is therefore beneficial to have a reproducible, accurate and discriminatory analysis for the quantitation of the enantiomeric purity of (L)-pidolic acid.

In this study, a sensitive and efficient GC method coupled with a two-step achiral derivatization was optimized and validated to ICH Q2 standards to determine the enantiomeric purity of (L)-pidolic acid. To our knowledge, this is the first report of a validated chiral analysis for the enantioseparation of (D)-PCA and (L)-PCA. A previous study reported the derivatization and subsequent separation of pidolic acid enantiomers using methyl chloroformate but the method was not validated [12]. Further, the exothermic nature of that reaction would not be suitable for routine analysis in a pharmaceutical laboratory.

2. Materials and methods

2.1. Reagents and materials

(L)-pidolic acid was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Anhydrous 2-propanol, anhydrous dichloromethane, racemic pidolic acid and (D)-pidolic acid were purchased from Alfa Aesar (Lancashire, UK). A 1.25N Hydrogen chloride—2-propanol solution (HCl-IPA) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Anhydrous trifluoroacetic anhydride (TFAA) was purchased from Chem-Impex International (Wood Dale, IL, USA).

2.2. Chromatographic equipment and conditions

Analyses were conducted using a gas chromatograph (Agilent 6890) coupled with a flame ionization detector (Agilent Technologies, Santa Clara, CA, USA). Split mode was used (30:1) with an injector and detector temperature of 250 °C. The chromatographic separation was performed on a 25 m CP-Chirasil-L Val (Agilent Technologies, Santa Clara, CA, USA) with 0.25 mm internal diameter and a 0.12 μm film thickness, at a constant helium flow of 1.5 mL min^{-1}. The column temperature started at 80 °C and then increased at a rate of 4 °C/min until reaching 160 °C. All results were collected on TotalChrom version 6.3.1 (PerkinElmer, Waltham, MA USA). Under these chromatographic conditions the retention times for the (D) and (L) enantiomers were 16.5 and 17.3 min, respectively.

2.3. Sample preparation

Samples of L-pidolic acid were prepared by weighing approximately 10 mg into a 2 mL vial and adding 1 mL of the 1.25 N hydrogen chloride-2-propanol solution. The sample vial was then crimped and heated at 100 °C for 2 h to ensure full reaction. After 2 h, the sample was removed, cooled and evaporated to dryness using nitrogen. To this residue, 0.5 mL of trifluoroacetic anhydride was added and the vial was allowed to stand at ambient temperatures for 30 min. The vial was then evaporated to dryness using nitrogen. To this residue, 1 mL of anhydrous methylene chloride was added and then injected for GC analysis. The derivatization reaction can be found in Fig. 1.

2.4. Chiral purity validation

The optimized purity method was validated for several parameters consistent with ICH Q2 guidelines. Specificity was evaluated by comparing the chromatograms of blank solvent, blank sample preparation, system suitability solution, and sample solution. The resolution between enantiomers was calculated within the presence of the derivatization matrix. Accuracy was assessed by spiking a stock solution of (D)-PCA at 1%, 2%, 5% and 5% relative to (L)-PCA. Linearity was assessed by plotting the experimental accuracy results versus the theoretical values. A simple linear regression analysis by the least squares was applied to determine the correlation coefficient, y-intercept and slope. System precision was measured for six replicate injections of the racemate. Analyst repeatability was demonstrated by a single analyst performing sample analysis of three samples lots in triplicate. The intermediate precision was demonstrated by a second analyst performing a second Analyst repeatability experiment (testing the same three sample lot in triplicate). Quantitation and detection limits were derived from signal to noise calculations for a representative (L)-PCA sample containing 0.1% (D)-PCA.

3. Results and discussion

3.1. Development of the method

Derivatization reactions are routinely used in analytical chemistry as a way to chemically modify a species to a more desirable form for analysis. Achiral derivatization of functional groups is routinely used for GC analysis as a way to improve volatility, decrease absorption and improve resolution and stability [13]. A silylation reaction is a common and versatile achiral derivatization technique for compounds with polar protic functional groups (e.g., —OH, =NH, —NH2, —SH, —COOH), which allows the compound to be analyzed by GC–FID (or GC–MS) [14]. Other reactions may include employing a derivatization agent to form diastereomeric pairs that can either be separated by HPLC or GC. Ilisz provides an in depth review of chiral derivatizion agents used in the separation of amino acid enantiomers by HPLC with 136 references [10]. As an alternative option, NMR spectroscopy has been used as an orthogonal approach to determine enantiomeric excess and has been thoroughly reviewed by Wenzel [15].

Like most derivatization reactions, the development of the reaction conditions was a time consuming challenge to optimize sensitivity, reaction time and work-up procedures [16]. For this study, a two-step achiral derivatization was implemented which consisted of an alkylation step (using HCl-IPA) followed by an acylation step (using TFAA). The enantiomer derivatives were then separated using chiral gas chromatography with flame ionization detection. This approach was first reported by Pizzarello and Cronin [17] to determine enantiomeric excess of pyroglutamic acid in water extracts from meteorites and was found to most effective in reducing the exothermic nature of the reaction while ensuring complete derivatization. Further, the stationary phase employed by Pizzarello and Cronin eluted the minor (D) enantiomer in front of the major (L) enantiomer, which allows a more sensitive and reproducible quantification [18,19].

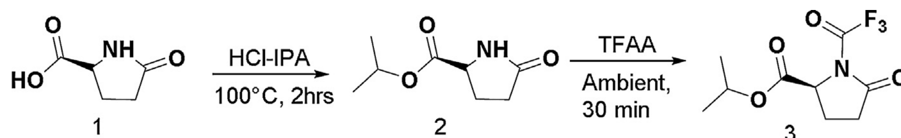

Fig. 1. Derivatization of L-pidolic acid.

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