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Amino acid analysis for pharmacopoeial purposes

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ABSTRACT

The impurity profile of amino acids depends strongly on the production process. Since there are many different production methods (e.g. fermentation, protein hydrolysis or chemical synthesis) universal, state of the art methods are required to determine the impurity profile of amino acids produced by all relevant competitors. At the moment TLC tests provided by the Ph. Eur. are being replaced by a very specific amino acid analysis procedure possibly missing out on currently unknown process related impurities. Production methods and possible impurities as well as separation and detection methods suitable for said impurities are subject to this review.

Contents

Abbreviations: AA, amino acid; AAA, amino acid analysis; APCI, atmospheric pressure chemical ionization; APPI, atmospheric pressure photo ionization; C4D, capacitively coupled contactless conductivity detector; CAD, corona charged aerosol detector; CBQCA, 3-(4-carboxybenzoyl)quinolone-2-carboxaldehyde; CE, capillary electrophoresis; CLND, chemiluminescent nitrogen detector; CZE, capillary zone electrophoresis; CNLSD, condensation nucleation light scattering detector; ECD, electrochemical detector; ELSD, evaporative light scattering detector; ESI, electrospray ionization; FID, flame ionization detector; FLD, fluorescence detector; FMOC-Cl, fluorenylmethoxycarbonyl chloride; FQ, 3-(furan-2-carbonyl)quinolone-2-carbaldehyde; HILIC, hydrophilic interaction liquid chromatography; HPLC, high performance liquid chromatography; IEC, ion-exchange chromatography; IPC, ion-pairing chromatography; LED, light emitting diode; LIF, laser induced fluorescence; LOD, limit of detection; MEKC, micellar electrokinetic chromatography; MOPS, 3-(*N*-morpholino)propanesulfonic acid; NAC, *N*-acetylcystein; NQAD, nano quantity analyte detector (see CNLSD); OPA, *o*-phtalaldehyde; PGC, porous graphitic carbon; Ph. Eur., European Pharmacopoeia; PITC, phenylisothiocyanate; QqQ, triple quadrupole; RID, refractive index detector; TLC, Thin-layer chromatography; TOF, time-of-flight; UHPLC, ultra-high performance liquid chromatography

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1. Introduction

Amino acids (AA) belong to the most important chemical structures on earth. They are building blocks of vital proteins and peptides, neurotransmitters, nourishment, poison, antibiotics, and transporters [1]. Thus, they are subject to great scientific interest [2]. In the pharmaceutical industry AA are used as starting material for therapeutic proteins, peptides and small molecules (e.g. Methotrexate, *N*-acetylcystein, pemetrexed, captopril, etc), as components of total parenteral nutrition, excipients [3–6], and active pharmaceutical ingredients (API), e.g. L-DOPA, Carbocisteine, Gabapentin, etc.

The quality and the safety of the applied AA for the intended purpose have to be assured by adequate analytical procedures and tests. The challenging part in purity assessment is in most cases the separation and detection of related substances. Since amino acids (natural and synthetic) are rather hydrophilic compounds and are lacking a chromophore, separation as well as detection by HPLC is very difficult. The nature of the impurities present in AA depends highly on their origin (e.g. recombinant, synthetic or other) and on possible modification reactions such as acylation or alkylation. Often, the related compounds are other AA, degraded AA, and precursors, causing the same challenges regarding separation and detection. Since the second half of the 20th century [7,8] the detection of AA was facilitated by numerous derivatization reactions each coming along with particular advantages and disadvantages (see Fig. 1 for some examples). During the last decades, great effort has been made to find ways to separate and detect amino acids without time-consuming and fault-prone derivatization steps. Recent trends in AA analytics use HILIC or mixed-mode columns combined with CNLSD, CAD, C4D or LC-MS, respectively. The application of those comparatively new techniques enjoys increasing popularity in the field of research and discovery, whereas official regulatory authorities are more reluctant in the adoption of new ways to analyze AA and rather rely on comparatively old techniques like TLC and amino acid analysis (AAA) with post-column derivatization. Thus, this review focuses on content determination and impurity profiling of AA by liquid chromatography without using derivatization reactions.

2. Preparation of amino acids

Nowadays AAs are produced via various ways. Since the production pathway determines the impurity profile the manufacturing is summarized below. For the monographed AA the most common production pathways are summarized in Table 1.

2.1. Chemical synthesis

Since Adolph Strecker developed the first chemical synthesis of α-amino acids in the middle of the 19th century [9] many different variants of this reaction and other ways of producing amino acids have been established in order to obtain enantiomerically pure products and/or to improve yields. There are also specific syntheses e.g. for Lys: Toray and DSM procedure [10]. The question rises whether all impurities from all commercially used synthesis variants using different precursors and/or reagents are covered by a given test [11–17]. Besides Strecker synthesis another way to obtain racemic α-amino acids is the amination of α-hydroxy carboxylic acids [18].

2.1.1. Chiral resolution

Although asymmetric syntheses are available, they often do not provide enantiomerically pure D-and L-forms, respectively, are expensive, elaborate and thus often not profitable. Nevertheless, some approaches of racemate separation found their way into the commercial production of amino acids. E.g. the conversion of racemic methionine comprising acetylation and specific enzymatic deacetylation of the L-form is today an important and elegant way to obtain L-methionine [19]. Chiral resolution using additional steps like fractional crystallization of diastereomeric precursors [20] is essential to convert racemic AAs into one enantiomer after chemical synthesis.

2.2. Recombinant synthesis by microorganisms and fermentation

The biochemical synthesis of AA using microorganisms involves several advantages over chemical total synthesis, e.g. the enantiomeric

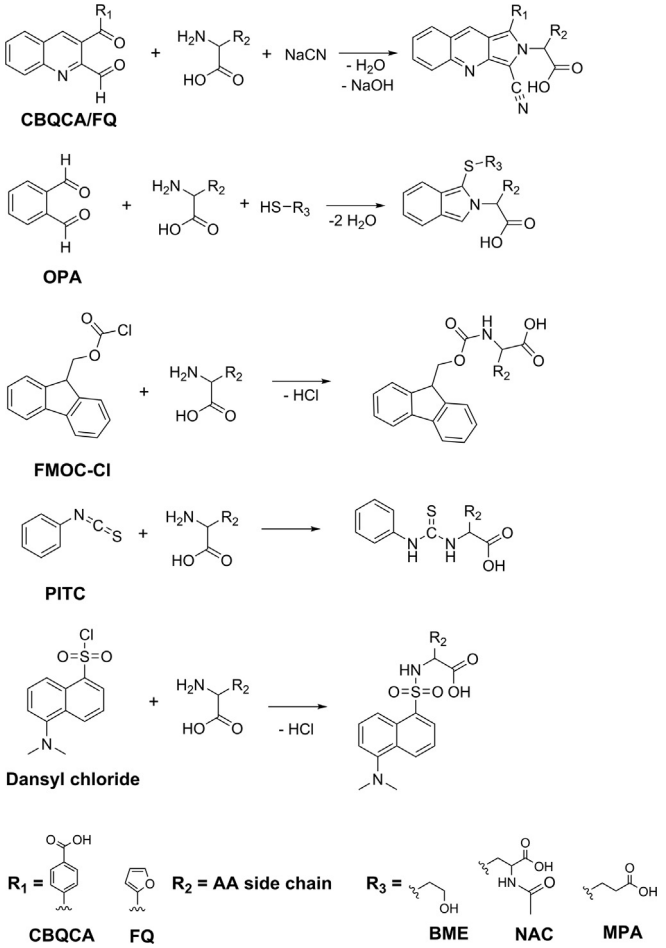

Fig. 1. Some derivatization reactions used in AAA [70,72,155–160]; BME, β-mercaptoethanol; NAC, *N*-acetylcysteine; MPA, 3-mercaptopropionic acid.