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Simultaneous determination of 33 amino acids and dipeptides in spent cell culture media by gas chromatography-flame ionization detection following liquid and solid phase extraction

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Abstract

A rapid, sensitive and reproducible gas chromatographic method with flame ionization detection is described for the simultaneous identification and quantification of 33 amino acids and dipeptides in spent cell culture media in under seven minutes. The method involves the use of the EZ:faast™ (Phenomenex) amino acid sample testing kit. Instrumental and assay precision, percent recovery, linear range, limit of detection and peak identity in highly complex cell culture media containing either soy hydrolysate or fetal bovine serum were validated using gas chromatography-flame ionization detector (GC-FID).

Keywords: Spent cell culture media; Amino acids derivatives; Dipeptide derivatives; EZ:faast kit; Liquid phase extraction; Solid phase extraction; Gas chromatography-flame ionization detector

1. Introduction

The development and optimization of cell culture media for the production of therapeutic proteins [1,2] and viral growth [3,4] is fundamental to the expanding biopharmaceutical industry. The optimization and utility of cell culture media requires a precise blend of nutrients such as essential and non-essential amino acids, carbohydrates, vitamins, lipids, growth factors, trace elements, minerals and many other compounds. The choice of culture medium and stoichiometric balance of complementing nutrients can have a significant and irremediable impact on the growth, function and relative phenotype of cells. However, product syntheses and metabolic shifts lead to ever changing external cellular conditions in batch culture which can inhibit cellular growth, metabolism and overall product synthesis. Hence, there is a vital need to optimize cell culture media formulations and control such dynamic environmental changes so as to efficiently maximize cell growth, viability and specific productivity.

Production of biopharmaceuticals is directly related to understanding the biochemical mechanisms of cells in cell culture media. One of the major challenges in the analysis of multicomponent media samples is the difficulty in completely separating individual or a group of constituents in a single chromatographic analysis without very involved sample preparation. The analysis of cell culture media and supernatants, as well as non-standard plant- or animal-derived protein hydrolysates such as fetuin, yeastolate, soy or collagens, has created the need for analytical techniques that are capable of accurately quantifying free amino acids as well as amino acids not normally found in virgin cell culture media. However, analysis of the amino acid and dipeptide constituents of cell culture media has been problematic due to the presence of many other interfering components. Moreover, the significant differences in the chemical structure of the functional groups, ranging from nonpolar to highly polar and acidic to alkaline side chains may interfere with the derivatization or detection of the amino acids and dipeptides derivatives.

Previously, physiologically free amino acid analysis has been performed by integrated pulsed amperometric detection (IPAD) [5,6], capillary electrophoresis (CE) [7], high-performance liquid chromatography (HPLC) [8], liquid chromatography–mass spectrometry (LC–MS) [9], gas chro-

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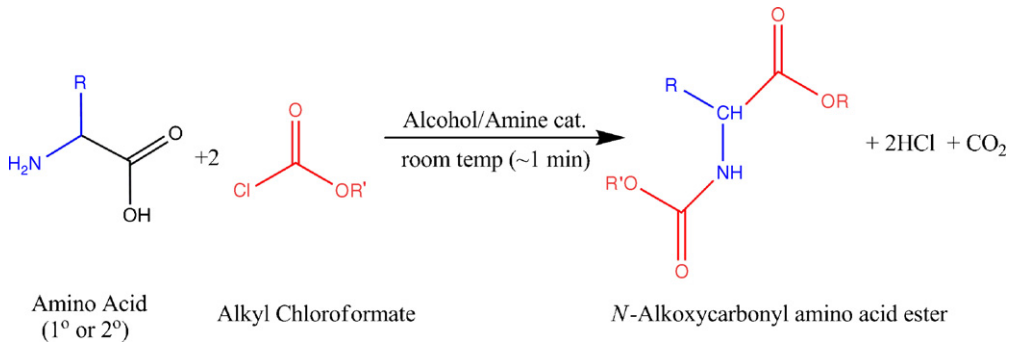

Fig. 1. Derivatization scheme of the alkylchloroformate esterification of amino acids.

matography (GC) [10–17] and gas chromatography–mass spectrometry (GC–MS) [16,18–20]. However, many of these methods have been impeded by labor intensive sample preparation, long analysis times, decreased resolution due to ion suppression, poor separation, low absolute sensitivity or dedicated and expensive instruments. Chromatographic methods of analysis have generally required a derivatization step to increase sensitivity and to improve selectivity of the analyte of interest. Both pre- and post-column derivatization have been used and their relative strengths and weaknesses discussed in detail [10,15,16,19–22]. Several liquid chromatographic methods have been developed for the determination of amino acid analysis. The most popular HPLC methods to date are *O*-phthalaldehyde (OPA) and 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) derivatized amino acids. These have several disadvantages. Most of these methods employ ultraviolet (UV) and fluorescent detection which either does not provide adequate sensitivity for the detection of amino acids or are limited to primary amines, especially, in cell culture media. The reaction of OPA with a primary amine and nucleophile, to produce a highly *N*-substituted isoindole has become a standard method for the trace analysis of amino acids. However, there are several

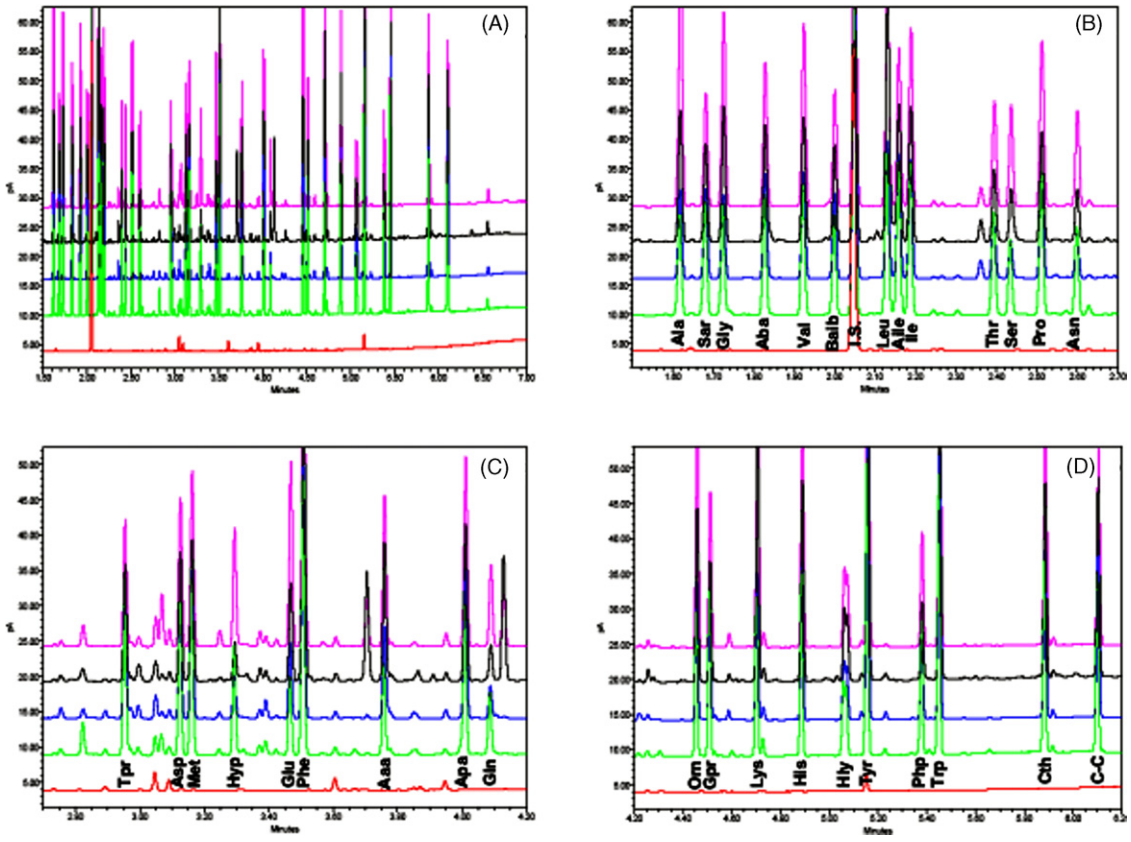

Fig. 2. Complete (A) and expanded ((B) 1.5–2.7 min, (C) 2.7–4.2 min and (D) 4.2–6.2 min) overlay chromatograms of a WFI blank (red trace) spiked with norvaline (I.S.), WFI blank with 10 nmol AA standard (green trace), blank media spiked with 10 nmol AA standard (blue trace), blank media spiked with 1% w/v hydrolysate and 10 nmol AA standard (black trace) and blank media spiked with 10% w/w FBS and 10 nmol AA standard (pink trace).