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Review

Advances in the *o*-phthalaldehyde derivatizations Comeback to the *o*-phthalaldehyde–ethanethiol reagent

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Abstract

The main aims of this work were (a) to present the characteristics and stability of the *o*-phthalaldehyde (OPA)–ethanethiol (ET) derivatives of 22 amino acids, including the believed-to-be less stable OPA derivatives providing glycine, γ-aminobutyric acid, β-alanine, histidine, ornithine, lysine and the C_1–C_5 aliphatic amines; (b) to compare the stability properties of the most common amino acids and amines as OPA–ET-fluorenylmethyl chloroformate (FMOC) derivatives to the corresponding ones obtained from OPA reagents containing various (SH)-additives; (c) to show the molar responses of alanine and lysine depending on the OPA reagent's composition; as well as (d) to prove the practical utility of these basic researches, by the simultaneous HPLC separation of 22 amino acids and 15 amines as their OPA–ET–FMOC derivatives. Investigations have been carried out by varying the composition of the reagents, the molar ratios of reactants and the reaction time, applying diode array and fluorescence detections simultaneously. Average reproducibility of quantitations, characterized with the relative standard deviations (RSDs) based on the fluorescence intensities of derivatives, in the order of listing, proved to be 1.2–5.9% for amino acids and 1.1–8.7% for amines. The practical utility of the method is demonstrated by the analysis of the amino acid and amine contents of mouse tissues, with an average reproducibility of 3.5%.
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Keywords: Amino acids; Amines; OPA derivatives; Ethanethiol; FMOC; Comparison studies; HPLC/UV/FL detections

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1. Introduction

1.1. Literature overview as a function of the o-phthalaldehyde reagent's (SH)-additive

The relevancy and continued interest towards OPA derivatizations can be characterized by the huge amount of papers dealing with the quantitation of the primary amino group containing compounds as OPA derivatives: mainly, by means of high-performance liquid chromatography (HPLC). On the basis of our exhaustive literature evaluation (2000–2006), almost 800 papers applying HPLC of amino acids as various OPA derivatives – representing ∼40% of all – have been selected [1]. It was surprising to realize that ethanethiol as SH-additive, in this period of time, has not been used, except in our proposals [1–5]. In the overwhelming part of recent publications in 62.9% of the publications that applied OPA derivatization, the OPA–2-mercaptoethanol (MCE) reagent was preferred [1]. Data of our recent studies revealed that the OPA–ET derivatization is an exceptionally promising technique in respect of the HPLC analysis of amino acids and amines, equally [1–15].

1.2. Evaluation of derivatizations, applying the o-phthaldehyde–ethanethiol reagent

Coming back to the pioneer period of OPA derivatizations,

(a) subsequently to the introduction of the Roth's principle [17], and,
(b) in synchrony with the first identification of the reaction product's structure – confirming its isoindole character, on the basis of NMR evidences [18,19] – a detailed study was commenced to examine and overcome the instability of the very welcomed, however unstable, OPA–MCE derivatives. Primarily, the OPA–ET reagent [18–30] was reported as an alternative that provides more stable derivatives than the initially described OPA–MCE ones (Table 1). Later on, OPA–MPA and OPA–alkyl cysteines were applied as preferred substitutes of the OPA–MCE reagent [31,32].

The first use of the OPA–ET derivatization [18] resulted in all cases examined (i) in higher stability of the fluorescent isoindoles, and (ii) also in higher fluorescent intensity compared to the corresponding OPA–MCE derivatives: particularly in a medium of high ethanol content.

Further experiences from the same laboratory [18,19] revealed that (i) the fluorescent products transform to give another fluorescent adduct [18], as well as (ii) the fluorescence intensity of monomer versus dimer isoindoles do not differ considerably; it was also demonstrated that the second isoindole moiety influenced the overall fluorescent intensity that was inversely proportional to the distance between the two rings. The lower stability of the OPA–MCE derivatives compared to the OPA–ET ones was assumed to be associated with the intramolecular reaction of the hydroxyl group belonging to the MCE moiety of the OPA–MCE derivatives [19].

The first HPLC separation of the OPA–ET amino acid derivatives, on reversed phase column, was optimized in order to quantify the amino acids in human serum at the picomole level [20].

A reaction temperature optimization study was performed for OPA–MCE, OPA–ET and OPA–methanethiol (MT) derivatives of primary amines containing a fully substituted carbon atom adjacent to the NH_2 group (isovaline, α-aminoisobutyric acid, 2-methylnorvaline, etc.) [21]. By varying the reaction temperature between 25 °C and 100 °C, it turned out that the fluorescence yield of these types of compounds can be considerably increased, up to the response level of the classical protein amino acids by substituting MCE either by ET or by MT, as well as, by increasing the reaction temperature from 25 °C to 100 °C.

The early chromatographic optimization study [22] relating to two different separations of the OPA–ET derivatives of

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