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A solid-phase extraction and size-exclusion liquid chromatographic method for polyethylene glycol 25 *p*-aminobenzoic acid determination in urine: Validation for urinary excretion studies of users of sunscreens

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ABSTRACT

No previous publications about percutaneous absorption of polyethylene glycol 25 *p*-aminobenzoic acid (PEG-25 PABA) have been found in the literature and the expected levels to be found in human urine after sunscreens use are unknown. The method proposed here is suitable to determine PEG-25 PABA in the urine of sunscreens users in order to carry out studies on body accumulation/excretion. It is based on solid-phase extraction (SPE) with size-exclusion liquid chromatography determination. Solid-phase extraction allows the analyte to be retained and subsequently eluted for a clean-up, using a silica-based cartridge. The size-exclusion liquid chromatography of the eluted allows the rest of matrix interferences to be avoided. Fluorescence intensity was measured at $\lambda_{em} = 350$ nm ($\lambda_{exc} = 300$ nm). The sensitivity of the proposed method is in the order of 450 ± 5 mL ng^{-1} and the detection limit ($3\,S_{y/x}/b$) in the measured solutions is in the order of 13 ng mL^{-1}, that is 2.6 ng mL^{-1} in urine samples. The method enables PEG-25 PABA to be determined in both, spiked and unspiked human urine samples. Results obtained for spiked human urine samples (11–100 ng mL^{-1}) demonstrated the accuracy of the method. The mean relative standard deviation of the results was in the order of 3–10%. Three volunteers applied a sunscreen lotion containing a 8% PEG-25 PABA sunscreen cream and their urinary excretion was controlled from the moment of application until the excreted amounts were no longer detectable.

1. Introduction

Sunscreen products contain different chemicals that have high UV light absorbing properties, which are commonly referred to as UV filters. Authorized UV filters and their permitted contents depend on the legislation in force in each country. Different published articles can be found in analytical literature devoted to determine the content of UV filters in sunscreen formulations, as recently reviewed [1,2].

On the other hand, although UV filters should be designed to remain on the skin surface without any percutaneous absorption, it has recently been proven that the human body can absorb some organic UV filters contained in sunscreen formulations through the skin. Different methodologies have

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been applied to study this percutaneous absorption and excretion process in the human body, such as: *in vivo* or *in vitro* tape-stripping methods, *in vitro* methods using percutaneous diffusion cells, *in vivo* methods based on the analysis of biological tissues or *in vivo* methods based on the analysis of biological fluids, as can be seen in a recent review [3]. These studies will enable the most suitable UV filters to be selected, having high remaining on the skin and low percutaneous absorption, thus maximising sun protection efficacy and reducing possibilities of systemic side-effects. Most published articles refer to a few UV-filters, making it necessary to study all the compounds included in the lists of authorized UV filters.

Some interesting articles have been published and reviewed [3], devoted to determining the content of a few UV filters in urine from volunteers who applied sunscreen cosmetics to their skin. The INCI (International Nomenclature Cosmetic Ingredient) names of the UV filters determined until now are: 4-methylbenzylidene camphor [4,5], benzophenone-3 [5–11], ethylhexyl methoxycinnamate [5], terephthalylidene dicamphor sulphonic acid [12], *p*-aminobenzoic acid [13,14], phenylbenzimidazole sulphonic acid [15] and disodium phenyl dibenzimidazole tetrasulfonate [16].

Different analytical techniques have been proposed, such as liquid chromatography with UV detection [6–8,10,11], liquid or gas chromatography with mass spectrometry detection [4,9] or molecular fluorescence [15,16]. Preparation sample methods allowing clean-up and preconcentration steps have been based on liquid extraction and centrifugation, solid-phase extraction [15,16] or microextraction [9,10]. The levels of the studied UV filters in urine are reported to be between 10 and 990 ng mL^{-1}, showing they are percutaneously absorbed.

The cosmetic ingredient known as *p*-aminobenzoic acid (PABA) has been greatly used as a UV filter in sunscreen cosmetics for many years. It is a water-soluble compound that is especially useful for sun protection in the UVB range (290–320 nm). However, it causes allergic skin reactions in some people who are sensitive to it [17–20] and, moreover, it stains clothing. The maximum authorized content of PABA in cosmetics differs depending on each legislation, for instance in the European Union (EU) it is 5%. Because of the drawbacks of PABA, cosmetic chemists have proposed different PABA derivatives to be used as UV filters, such as esters, which can be formed by the reaction of PABA with long-chain alcohols. An example is polyethylene glycol 25 *p*-aminobenzoic acid (PEG-25 PABA) or ethoxylated ethyl 4-aminobenzoate with 25 mol polyoxyethylene average molar ratio (Fig. 1) which does not causes skin irritation or stain cloths, with a maximum authorized content in the EU twice that of PABA. No data have been found in the literature about percutaneous absorption of PEG-25 PABA or the expected levels to be found in human urine after sunscreen products use. However, an interesting article [21] should be cited, in which remnants of PEG-25 PABA and another UV filter (benzophenone-4) on skin, were studied using a stripping method.

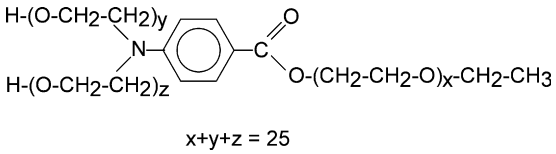

Fig. 1 – Chemical structure of PEG-25 PABA.

The aim of this work is to develop a selective and sensitive method that enables traces of PEG-25 PABA to be determined in urine without matrix interferences, in order to study excretion from the human body after the topical application of cosmetic products containing this UV filter. The proposed method is based on solid-phase extraction (SPE) followed by a size-exclusion liquid chromatographic separation with fluorescence detection.

2. Experimental part

2.1. Apparatus

Urine samples were filtered through a porous glass filter (10–16 μm porous size) from Schott Duran (Mainz, Germany) using an N86KT.18 KNF vacuum pump from Laboport (Trenton, NJ, USA).

1 mL Bond Elut cartridge packed with 100 mg of silica packing (SI) from Varian (Lake Forest, CA, USA) was used for SPE of urine samples.

A liquid chromatography system equipped with a 515 isocratic pump from Waters (Eschborn, Germany), an AS100 Spectra System automatic injector from Thermo (Allschwil, Switzerland), and a Series 200 fluorescence detector from Perkin Elmer (Norwalk, CT, USA), was employed for PEG-25 PABA determination. An exclusion PlGel Mixed-D (30 cm × 7.5 mm I.D., 5 μm particle size) column with a PlGel Guard precolumn (5 cm × 7.5 mm I.D., 5 μm particle size) from Polymer Laboratories (Church Stretton, United Kingdom) were employed for the chromatographic separation.

A F4500 spectrofluorimeter from Hitachi (Tokyo, Japan) and a 8453 UV/vis spectrometer from Agilent (Madrid, Spain) were used for the preliminary studies.

2.2. Reagents and samples

PEG-25 PABA (>99%) kindly provided by Basf Española S.A (Barcelona, Spain) was used as standard. The following reagents were also used: *N*,*N*-dimethylformamide (DMF) and ethanol (EtOH) LC grade from Scharlab Chemie S.A (Barcelona, Spain), dihydrogen sodium phosphate monohydrate from Merck (Darmstadt, Germany), sodium hydroxide from Scharlau Chemie S.A. (Sentmenat, Spain) and deionized water.

A home-made sunscreen cream was prepared according to a procedure provided by Guinama (Alboraya, Spain). This cream contained PEG-25 PABA (8%, w/w) and other commonly used ingredients (also purchased from Guinama) such as myristyl myristate, cetyl alcohol, glyceryl laurate, avocado oil, propylene glycol.

The urine samples were taken from three volunteers after applying approximately 2 mg cm^{-2} of sunscreen cream to their arms, thighs and upper part of the back. Their urinary excretion was controlled from the moment of application until the excreted amount of PEG-25 PABA was no longer detectable.

For the accuracy study, eight PEG-25 PABA free human urine samples were also taken from four volunteers and spiked