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# Determination of hydroxytyrosol and tyrosol by liquid chromatography for the quality control of cosmetic products based on olive extracts

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ABSTRACT

An analytical method for the simultaneous determination of hydroxytyrosol and tyrosol in different types of olive extract raw materials and cosmetic cream samples has been developed. The determination was performed by liquid chromatography with UV spectrophotometric detection. Different chromatographic parameters, such as mobile phase pH and composition, oven temperature and different sample preparation variables were studied. The best chromatographic separation was obtained under the following conditions: C18 column set at 35 °C and isocratic elution of a mixture ethanol: 1% acetic acid solution at pH 5 (5:95, v/v) as mobile phase pumped at 1 mL $min^{-1}$. The detection wavelength was set at 280 nm and the total run time required for the chromatographic analysis was 10 min, except for cosmetic cream samples where 20 min runtime was required (including a cleaning step). The method was satisfactorily applied to 23 samples including solid, water-soluble and fat-soluble olive extracts and cosmetic cream samples containing hydroxytyrosol and tyrosol. Good recoveries (95–107%) and repeatability (1.1–3.6%) were obtained, besides of limits of detection values below the μg $mL^{-1}$ level. These good analytical features, as well as its environmentally-friendly characteristics, make the presented method suitable to carry out both the control of the whole manufacture process of raw materials containing the target analytes and the quality control of the finished cosmetic products.



## 1. Introduction

Hydroxytyrosol (2-(3,4-di-hydroxyphenyl)ethanol) and tyrosol (2-(4-hydroxyphenyl)ethanol) are phenolic compounds naturally present in olive trees (*Olea europaea* L.), fruits, olive oil and olive mill waste ('alperujo'), both in their molecular form or as part of more complex molecules, mostly as esters of elenolic acid [1–5]. The chemical structures of hydroxytyrosol and tyrosol are shown in Fig. 1.

Several studies have reported various biological activities of hydroxytyrosol and tyrosol, such as antimicrobial [6,7], anti-carcinogenic [8,9] and anti-inflammatory [10], but especially as powerful antioxidant agents [11–14]. In fact, the antioxidant capacity of hydroxytyrosol is higher than that of other phenolic compounds with similar structures and other natural antioxidants such as vitamin C, vitamin E or resveratrol [15–17]. Therefore, in addition to its applications as food additive and as pharmaceutical active substance, hydroxytyrosol has a great potential as a cosmetic ingredient.

The use of natural plant products in cosmetics dates back to ancient times. However, indicating that a cosmetic product contains natural ingredients is nowadays still a claim. Although some natural plant products are banned by the current European Union Regulation on cosmetic products [18], the number of natural products that can be used is really immense.

Due to the beneficial properties of these compounds, the use of olive extracts as raw material in the manufacture of cosmetic products is an interesting way of innovation for cosmetic industries. As consequence of the olive oil extraction, a wet solid waste ('alperujo') is produced. This by-product from the olive oil extraction is so rich in hydroxytyrosol and tyrosol [19], that it can be used to produce olive extracts with high content of these compounds. In the subsequent steps to refine and concentrate this product, different by-products containing significant amounts of hydroxytyrosol and tyrosol are obtained, such as dry solid wastes and fat-soluble liquid extracts. The cosmetic raw material to be obtained is a water-soluble liquid extract used as ingredient in the manufacture of cosmetic products. Additionally, the water-soluble olive extracts

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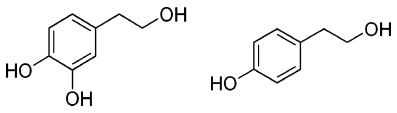


**Fig. 1.** Chemical structures of hydroxytyrosol (left) and tyrosol (right).

can be treated to obtain solid extracts, where hydroxytyrosol and tyrosol have been purified and concentrated. Therefore, it is necessary to develop analytical procedures to control the industrial processes to obtain the required olive extracts and to assess the quality of cosmetic products containing them.

Different analytical methods for the characterization of phenolic compounds in olive leafs [20], olive oil [21,22], olive extracts [23] and olive mill wastes [24,25] have been published. Most of these methods provide qualitative or semi-quantitative information, so they are not suitable for routine determination of hydroxytyrosol and tyrosol contents in these samples. Besides, several methods for the determination of the target compounds in olive related samples have been published; such as the determination of oleuropein, hydroxytyrosol and tyrosol in olive stems and roots by liquid chromatography [26], the determination of hydroxytyrosol and tyrosol in olive oil by liquid chromatography [27,28] or by capillary electrophoresis [29–32] and the determination of hydroxytyrosol in olive extracts by micellar liquid chromatography [33].

To the best of our knowledge, this is the first work addressing a reliable and useful analytical method which allows the simultaneous determination of hydroxytyrosol and tyrosol in all the stages of the whole manufacture process of cosmetics based on olive extracts. The method is applied to different samples, from the olive mill wastes to the finished cosmetic products, using simple instrumentation (LC–UV system) with the same conditions for all types of samples, just slightly modifying the sample preparation according to their nature.

## 2. Experimental

### 2.1. Apparatus

An Agilent 1220 Infinity LC system including a degasser, a binary pump, an autosampler with up to 100 μL injection volume, a thermostated column oven and a UV/vis detector was employed. The column used was a Purospher® STAR RP-18 endcapped (12.5 cm length, 4 mm I.D., 5 μm particle size) from Merck (Darmstadt, Germany).

An ultrasound bath (50 Hz, 360 W) from J.P. Selecta S.A. (Barcelona, Spain) was used for the lixiviation of the target compounds from solid samples.

A ZX3 vortex mixer (40 Hz) from VELP Scientifica (Usmate Velate, Italy) was used to ease the liquid–liquid extraction in the preparation of fat-soluble and cosmetic cream samples and an EBA 21 centrifuge from Hettich (Tuttlingem, Germany) was used for phase separation.

A Basic 20 pH meter from Crison (Alella, Spain) was used for the pH adjustments.

### 2.2. Reagents and samples

Hydroxytyrosol (2-(3,4-di-hydroxyphenyl)ethanol) ≥98% from Extrasynthese (Genay, France) and tyrosol (2-(4-hydroxyphenyl)ethanol) 98% from Aldrich (Steinheim, Germany) were used as standards.

LC-grade absolute ethanol (EtOH) and LC-grade n-hexane 96%, both from Scharlab Chemie (Barcelona, Spain), were used as solvents to prepare the sample and standard solutions. EtOH and LC-grade acetonitrile, also from Scharlab Chemie, were tested as mobile phases.

Glacial acetic acid extra-pure from Scharlab Chemie (Barcelona, Spain) and deionized water obtained using a NANOpure II ultrapure water system from Barnstead (Boston, USA) were used to prepare the acetic acid solutions used as solvent in the sample and standard solutions preparation and as aqueous mobile phase. Sodium hydroxide, also from Scharlab Chemie, was used to adjust the pH of these solutions.

Twenty three samples including the wet solid waste from olive oil extraction 'alperujo' (samples A, B), dry solid wastes (sample C), fat-soluble liquid extracts (samples D–F), water-soluble liquid extracts (samples G–M), solid concentrated extracts (samples N, O) and cosmetic creams (samples P–W) were provided by Extractia Health (Valencia, Spain).

Additionally, a home-made cosmetic cream (sample X) based on an olive extract containing the target compounds was fabricated at laboratory scale.

### 2.3. Proposed method

#### 2.3.1. Preparation of sample solutions and standards

*2.3.1.1. Solid samples and water-soluble liquid extracts.* Samples (A–C, G–O) were prepared by weighing 0.01–0.10 g into a 10 mL volumetric flask and adding 1% acetic acid solution adjusted to pH 5 as solvent. This solution was placed in an ultrasonic water bath for a few minutes in order to lixiviate the target compounds and facilitate the dissolution of the sample. After this, it was filtered through a 0.45 μm nylon membrane filter and 1 mL of this solution was placed into a 1.5 mL injection vial.

A 500 $\mu g\,mL^{-1}$ standard stock solution containing the target compounds was prepared employing a 1% acetic acid solution adjusted to pH 5 as solvent. From this solution, working standard solutions from 1 to 50 $\mu g\,mL^{-1}$ were daily prepared in the same solvent.

*2.3.1.2. Fat-soluble liquid extracts and cosmetic creams.* In order to clean-up the sample matrix, avoid chromatographic overlaps and obtain an aqueous solution appropriate for the chromatographic analysis, a liquid–liquid extraction was performed. Samples (D–F, P–X) were prepared by weighing 0.01–0.10 g into a 15 mL polypropylene conical-bottom tube (Falcon-type tube) and diluted with 2 mL of n-hexane and 1 mL of EtOH. This solution was mixed using a vortex mixer (40 Hz) for 10 s. Then, 1.5 mL of 1% acetic acid solution adjusted to pH 8 were added and mixed again using the vortex mixer for another 10 s. After this, the mixture was centrifuged at 3500 rpm for 2 min. Finally, 1 mL of the hydro-ethanolic phase (denser phase) was placed into a 1.5 mL injection vial.

A 250 $\mu g\,mL^{-1}$ standard stock solution containing the target compounds was prepared in EtOH. From this solution, standard solutions were prepared daily from 0.5 to 25 $\mu g\,mL^{-1}$ in the same solvent. In order to proceed in the same way as in preparation of samples, 1 mL of the standard solution was placed into a 15 mL polypropylene conical-bottom tube (Falcon-type tube) containing 2 mL of n-hexane, and mixed using a vortex mixer (40 Hz) for 10 s. Then, 1.5 mL of 1% acetic acid solution adjusted to pH 8 were added and mixed again using the vortex mixer for another 10 s. After this, the mixture was centrifuged at 3500 rpm for 2 min and 1 mL of the hydro-ethanolic phase was placed into a 1.5 mL injection vial.

#### 2.3.2. Chromatographic analysis

20 μL of the standard or sample solution were injected into the column set at 35 °C. The elution was performed at 1 mL $min^{-1}$ flow rate with an EtOH: 1% acetic acid solution at pH 5 (5:95, v/v) mixture as mobile phase, in isocratic elution mode. Detection wavelength