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P.33 OPTIMIZATION OF THE DETERMINATION OF TOCOPHEROLS IN LAMIACEA SP. BY A NORMAL PHASE LIQUID CHROMATOGRAPHIC METHOD

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Many wild plants gathered from the scrubland were preserved and used for medicinal and food purposes in the north-eastern region of Portugal. Since they are important ingredients of the folk pharmacopoeia and traditional cuisine some of these wild botanicals have been semi-domesticated and are still cultivated in homegardens and present in every homesteads. Several ethnobotanical surveys conducted in this Portuguese region have highlighted the use of three particular *Lamiaceae*¹⁻³. Ground ivy (*Glechoma hederaceae*), oregano (*Origanum vulgare* subsp. *virens*) and mastic thyme (*Thymus mastichina*) are widespread Mediterranean perennial herbs widely considered as medicinal plants, although it has also been reported some other common uses.

Epidemiological and experimental studies have consistently shown an inverse association between consumption of vegetables and fruits and the risk for chronic diseases related to oxidative stress, such as cardiovascular diseases, arthritis, chronic inflammation and cancers⁴. Therefore, there is a growing interest in natural antioxidants for their potential role in the prevention of those oxidative stress-related diseases; furthermore, synthetic antioxidants are being questioned due to their potential carcinogenic activity.

In the present study, the tocopherols (powerfull antioxidants) contents of three *Lamiaceae* often used in Portuguese folk medicine were determined. The individual profiles were obtained by a normal-phase high-performance liquid chromatography (NP-HPLC). The linearity and sensitivity of the HPLC analysis were determined and the method was validated by the repeatability and accuracy, using *Thymus mastichina*.

It was used a simple solid-liquid extraction procedure without saponification, in which a BHT solution (100µL) and IS solution (250µL) were added to the sample prior to the extraction procedure. The samples (~500mg) were homogenized with methanol (4mL) by vortex mixing (1min). Subsequently, hexane (4mL) was added and again vortex mixed for 1min. After that, saturated NaCl aqueous solution (2mL) was added, the mixture was homogenized (1min), centrifuged (5min, 4000g) and the clear upper layer was carefully transferred to a vial. The sample was re-extracted twice with hexane. The combined extracts were taken to dryness under a nitrogen stream, redissolved in 1mL of n-hexane, dehydrated with anhydrous sodium sulphate, filtered through a 0.22µm disposable LC filter disk, transferred into a dark injection vial and analysed by HPLC.

The chromatographic separation was achieved using a HPLC equipment integrated with a Smartline pump 1000, a degasser system Smartline manager 5000, an AS-2057 auto-sampler and a 2500 UV detector at 295nm connected in series with a FP-2020 fluorescence detector programmed for excitation at 290nm and emission at 330nm. Data were analysed using Clarity 2.4 Software. The chromatographic separation was achieved with a Polyamide II (250x4.6mm) normal-phase column from YMC Waters operating at 35°C. The mobile phase used was a mixture of n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1mL/min, and the injection volume was 20µL. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response, using the internal standard method (0.075 to 16.0µg/mL; α-tocopherol $y=2.12899x$; β-tocopherol $y=0.51248x$; δ-tocopherol $y=0.7359x$; γ-tocopherol $y=0.65148x$). Tocopherol contents in the samples are expressed in µg per g of dry matter.

For each compound, 7-level calibration curve was constructed using the peak-area ration between the tocopherol and tocol versus concentration of the standard (ng/mL). The average of triplicate determinations for each level was used. The correlation coefficients were always higher than 0.999 for all the compounds. The limits of detection (LOD), calculated as the concentration corresponding to three times the calibration error divided by the slope, ranged from 14.79 and 25.82ng/mL. The limits of quantification (LOQ) were calculated using the concentration corresponding to ten times the calibration error divided by the slope, and ranged from 49.32 to 86.07ng/mL.

In order to evaluate the instrumental precision, the sample extract was injected six times. The chromatographic method proved to be precise (CV% between 0.22 and 2.36%). Repeatability was evaluated by applying the whole extraction procedure 6 times to the same sample. All the obtained values were low (CV% ranging from 2.18 to 4.45%). The accuracy of the method was evaluated by the standard addition procedure (% of recovery) with three addition levels (0.5, 1.0 and 2.5µg/mL of each one in duplicate). The standard mixture was added to the sample, and all the extraction procedure was carried out. The results demonstrate good recovery for the compounds under study (ranging from 89 to 95%).

Glechoma hederacea revealed higher content of tocopherols (369mg/100g), while the other *Lamiaceae* presented the opposite. The four tocopherols (α , β , γ , δ -tocopherols) were quantified in all the samples. α -Tocopherol was the major tocopherol vitamer for *Glechoma hederacea* (272mg/100g) and *Origanum vulgare* (10.1mg/100g). For *Thymus mastichina*, γ -tocopherol was the most abundant vitamer (3.8mg/100g), revealing this species the lowest total tocopherols content (4.1mg/100g).

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