EMPLOYMENT OF A GROUP OF CATECHINS AND FLAVONOIDS AS SCAVENGERS OF HYDROXYL RADICAL; APPLICATION APPROACH IN CHRONOAMPEROMETRIC DETECTION IN LIQUID CHROMATOGRAPHY

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Today, it is known that virtually every disease state involves free radicals, particularly the most reactive of all, the hydroxyl radical (•OH), a well-known molecule of great physiological and pathophysiological importance. The in vivo half-life of the •OH, though difficult to measure directly by conventional analytical techniques, is estimated to be around $10^{-9}$ second. Therefore, a confident analytical approach is needed to ascertain the importance of the quantification of the •OH.

An HPLC-ECD method is described for the indirect determination of •OH. Fenton’s reaction is used to produce •OH, which simultaneously attacks polyphenols (phenol derivatives, catechins or flavonoids) to form the adducts by hydroxylation of the optimum position. Thus, •OH quantification is based on the separation and detection of the corresponding adducts by an isocratic HPLC-ECD method. All used chemicals were of HPLC-grade or better. The liquid chromatographic system (Agilent 1100 series) supplied by SEM Company (Izmir/Turkey) was equipped with an electrochemical detector (HP 1049 A Programmable Electrochemical Detector), a pump (HP 1100 series G1310A isocratic pump), a thermostatted column unit (HP 1100 series G1316A thermostatted column compartment), a manual injector (HP 1100 series G1328A Rheodyne 7725i) with 20 µL loop and a chromatographic data processing software (HP ChemStation for LC Rev. A. 06. 03 [509]). The separation was performed using an octadecyl (C18) analytical column (Hichrom 5 C18, 7.75 x 300 mm, 5µm particle size). Separation of phenolic adduct(s) was performed with a flow rate of 1 mL/min for 80 min. Amperometric detection was carried out at +1.20 V (vs. Ag/AgCl, 0.5 µA detector fullscale) in the electrochemical flow cell. The solvents used and their proportions were as follows: methanol / 0.01 M phosphoric acid (30/70 v/v). Each compound was tentatively identified by its unique retention time under the same conditions. Quantitative determinations were carried out by the external standard method based on peak heights. For example, the tR values assigned for pyrogallic acid and pyrocatechol were 13.38 min and 24.63 min, respectively. The calculated concentration of •OH during incubation (0.626 ppm) can be detected with negative errors by the HPLC-ECD (for example 0.595 and 0.615 ppm with the errors −5.2 and −1.8%, respectively for pyrogallic acid and pyrocatechol). Analytical method validation data is displayed as tables for both detectors.