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RP-HPLC PHENOLIC COMPOUNDS PROFILE OF OLIVE LEAVES, EXTRA VIRGIN OLIVE OIL AND OLIVE MILL WASTE WATER FROM MOROCCO USING A DESIGN OF EXPERIMENTS (DoE) APPROACH

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Phenolic compounds are a large class of plant secondary metabolites comprising a great number of heterogeneous structures that range from simple molecules to highly polymerized compounds, which are commonly bound to other molecules, frequently to sugars, although phenolic compounds in free form also occur in plant tissues. These compounds form an integral part of human diet, contributing to the sensory properties of plant-based aliments and to their beneficial effects on human health. The phenolic compounds, as well as many other plant secondary metabolites, have also a remarkable position as bioactive components in medicinal plants and have evidenced to exhibit numerous biological activities and a variety of health benefits against chronic and degenerative human diseases. A variety of instrumental analytical separation techniques are employed to identify and quantify each of the main phenolic compounds occurring in plants and plant-derived food products. Among them, high performance liquid chromatography, mostly in reversed phase separation mode (RP-HPLC), is the techniques of choice [1]. This communication discusses the results of a study carried out to investigate a variety of factors that influence the RP-HPLC separation of phenolic compounds occurring in olive leaves, extra virgin olive oil (EVOO) and oil mill waste water (OMWW). The study has been carried out in the framework of an EU Project (OliveNet 734899) aimed at the valorization and marketing of new products based on bioactive compounds from Olea europaea, with particular attention to phenolic compounds.

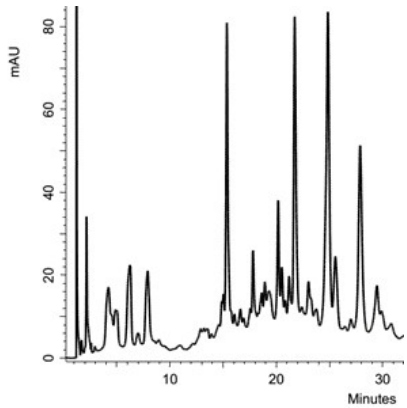

Figure 1. RP-HPLC separation of phenolic compounds extracted from leaves of Olea europaea, variety Koroneiki.

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After the processing of olives for olive oil extraction, less than 1–2% of the phenolic compounds are found in EVOO, whereas the majority are lost either in the pomace or in the OMWW. Significant content of phenolic compounds are also occurring in the leaves of the Olea europaea trees, which form a huge bulk of solid wastes produced by the cultivation of olive trees (see Figure 1). Therefore, both the olive processing industry and the cultivation of olive trees are responsible for the production of large quantities of by-products, which might either create major environmental problems when are not correctly disposed in nature or become a source of novel active ingredients that could be used in the pharmaceutical, nutraceutical and cosmetic industry.

This presentation evaluates the influence of pH and mobile phase composition, column temperature and gradient elution program on the RP-HPLC separation of phenolic compounds occurring in olive leaves, EVOO and OMWW. Appropriate selection of the mobile phase in RP-HPLC involves the evaluation of the equilibrium in solution that might take place between the analytes and the components of the liquid phase. The ionogenic nature of most of the considered compounds requires the control of the protonic equilibrium in solution, which is performed by incorporating proper additives into the mobile phase, such as an organic acid or a buffer. The constituents of the mobile phase do not limit their action at controlling the protonic equilibrium. They also might interact with the analytes, for examples by an ion-pairing mechanism, with the result of altering their chromatographic retention, which is modulated by the chemical composition and concentration of the organic solvent, which progressively increases during the analysis in gradient elution mode.

In this study, the appropriate selection of the separation conditions employed in RP-HPLC has been conducted by a Design of Experiments (DoE) approach, which has allowed the simultaneous optimization of pH and composition of the hydro-organic mobile phase, shape and duration of the binary gradient elution program, and column temperature on the basis of the retention times and peak areas of the analytes of interest, obtained by a limited number of experiments. The presentation describes the use of the above experimental data to construct 3-D resolution maps, which have been used to evaluate the influence of column temperature, duration and shape of the elution gradient, pH and composition of the mobile phase on the retention and resolution of a variety of analytes. In our study, 3-D resolution maps were constructed using either mixtures of standard phenolic compounds or samples of these compounds extracted from EVOO, OMWW and olive lives. The goal of our study was to model the variations that can help for the better selection of the mobile phase composition and gradient elution mode, in order to improve peak resolution and to reduce the analysis time, using a limited number of experiments and, consequently, reduced amounts of expensive and environmentally harmful chemicals. Excellent correlation between simulated and experimental separations of phenolic compounds are demonstrated. The application of the above DoE approach to study the phenolic compounds profile of EVOO, OMWW and olive lives from Morocco is illustrated and discussed.

References

[1] Corradini D., Orsini F., De Gara L., Nicoletti I. LC GC Europe, 2018, 31, 480.