Metabolite Fingerprinting of Bioactive Compounds in *Echinacea pallida* by High-Performance Liquid Chromatography with Diode Array and Electrospray Ionization-Mass Spectrometry Detection

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In this study, a complete phytochemical characterization of *Echinacea pallida* (Nutt.) Nutt. root extracts and dietary supplements was carried out by developing advanced chromatographic techniques, based on HPLC with diode array (DAD) and electrospray ionization-mass spectrometry (ESI-MS) detection (with ion trap and triple quadrupole mass analyzers), for the simultaneous analysis of hydrophilic [1] and lipophilic secondary metabolites [2]. The HPLC analyses were carried out on an Ascentis C_{18} column (250 mm × 4.6 mm I.D., 5 μm), with a mobile phase composed by H_2O and ACN both containing 0.1% formic acid, under gradient elution [3].

The UV spectra, in combination with MS and MS/MS data, allowed the identification of fourteen compounds, including caffeic acids derivatives, polyacetylenes and polyenes, in the analyzed samples. MS and MS/MS data were discussed in detail and the typical fragmentation patterns of each class of secondary metabolites were identified. For the first time, a hydroperoxide intermediate was characterized as an oxidation product of one of *E. pallida* monocarbonilyc acetylenes.

The HPLC method was fully validated in agreement with ICH guidelines and then applied to real samples. The quantitative analysis indicated that there was a great variability in the amount of the active compounds in the dietary supplements containing *E. pallida* root extracts.

The developed method can be considered highly suitable for metabolite fingerprinting and quality control of *E. pallida* plant material and natural products.

References

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