ISV Institut des
substances
végétales

19 rue Patrick Depailler
63000 Clermont-Ferrand – France

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Daniel JEAN PhD

Methods of quantification and identification of molecules in qRE

Herbal extracts are obtained mainly by simple aqueous or hydroalcoholic extraction of raw plant materials. These widely used methods lead to complex mixtures containing molecules from the primary metabolism and molecules from the secondary metabolism. Among the former, one finds mainly saccharides, organic acids, mineral salts and sometimes peptides and proteins.

The most important molecules, the ones that make a plant interesting and efficient, generally belong to the secondary metabolism and are most often specific to the plant species.

In quantified Reference Extracts all significant secondary metabolism molecules (those with the highest concentration or the ones most important for their activities) are identified by HPLC-ESIHR-MSMS. Among them, one or two molecules are quantified by a classical HPLC-UV detector from purified external standards whose purity is asserted by qNMR (incertitude ± 4.5 %).

An HPLC-CAD (charged Aerosol Detector) is also performed using the same HPLC method used for identification.
CAD is an evaporative detector similar to ELSD, but it is electrical charges that are measured here instead of scattered light. It produces a response which is similar but not fully identical from a non-volatile molecule to another, as shown by Robinson et al. 2017[1], and Schilling et al. 2019[2].

Our laboratory, the Institut des Substances Végétales, has developed a proprietary algorithm based on the relative response obtained from the molecules previously quantified by HPLC-UV and used in the HPLC-CAD method as internal standards. A corrective parameter is calculated for every molecule signal in order to obtain identical response coefficients between different molecules within a relative incertitude range of ± 7.5 %.
Quantifications are thus performed with an average 4.5 % incertitude for molecules used as internal standards (HPLC-UV) and 7.5 % for other identified molecules (HPLC-CAD).

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(1) Use of Calculated Physicochemical Properties to Enhance Quantitative Response When Using Charged Aerosol Detection.
Max W. Robinson, Alan P. Hill, Simon A. Readshaw, John C. Hollerton, Richard J. Upton, Sean M. Lynn, Steve C. Besley, and Bob J. Boughtflower.
Analytical Chemistry, 2017, 89, 1772–1777

(2) Quantitative structure-property relationship modeling of polar analytes lacking UV chromophores to charged aerosol detector response
Klaus Schilling & Jovana Krmar & Nevena Maljurić & Ruben Pawellek & Ana Protić & Ulrike Holzgrab.
Analytical and Bioanalytical Chemistry. 2019, 411, 13, 2945–2959