Rapid log P determination of natural products in crude plant extracts from UHPLC-TOF-MS profiling data - an additional parameter for dereplication and bioavailability

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Philippe Eugster, Sophie Martel, Davy Guillarme, Pierre-Alain Carrupt, Jean-Luc Wolfender

School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Quai E-Ansermet 30, CH-1211 Geneva 4, Switzerland

Introduction

• In phytochemical analysis, HPLC metabolite profiling methods provide a large amount of data on the composition of a given crude plant extracts for both dereplication or rapid on-line structure determination of given natural products (NP’s) [1].

• Many physicochemical properties could be extracted from HPLC data, such as lipophilicity.

• Lipophilicity (described by log P) is a key-parameter involved in pharmacokinetic (absorption, distribution, metabolism, elimination and toxicity) and pharmacodynamic processes (ligand-target interactions) and has to be determined as early as possible.

• Liquid chromatography is a fast and low sample consuming technique fully used in log P determination. The method is based on the relationship existing between retention factors and log P using specific chromatographic conditions [2].

• The development of column packed with sub-2μm particles working at high pressure (Ultra High Pressure Liquid Chromatography) also allows higher throughput.

• NP’s retention factors extracted from UHPLC-MS metabolite profiling data could then provide log P of compounds of interest prior isolation.

Materials and Methods

• 42 NP’s were selected by cluster analysis based on 4 molecular descriptors : H-bond donor or acceptor properties (x₁, x₂), polarizability (x₃), and Mc Gowan volume from a database of 298 well-known NPs [3].

• According to previous works [2, 3], the 42 NP and 8 reference compounds (see below) were tested on Acquity UPLC BEH shield RP18 (100x2.1 mm, 1.7 μm) stationary phase.

• Retention times were measured using 2 generic gradients differing only in gradient time (3:1 ratio, i.e. 33 and 11 min).

• A test set of 42 NP were simultaneously injected in UHPLC system equipped with Aquity UPLC BEH shield RP18 100x2.1mm, 1.7mm stationary phase with 3 different mobile phase pH.

Iontionization

• Because retention factor of the neutral form is higher than log kw of the corresponding ionized form, some ionization information can be directly extracted from the retention behavior measured at different pH.

Relation log kw – log P

• In a previous work, a calibration curve was established on a shorter Aquity UPLC BEH shield RP18 (30 mm) using a test set of 38 compounds injected individually [2, 3].

• A mixture of 38 of reference compounds was injected in UHPLC system with the 100 mm stationary phase and log kw obtained were perfectly superimposed with calibration curve. No significant effect of column’s length and simultaneous injection was observed.

• Only the 29 NP existing under their neutral form at least at one of the 3 pH and the corresponding log kw were kept.

• log kw measured were generally overestimated and the differences between experimental log kw and expected log k depend on compound’s structure.

Conclusions & Perspectives

• The method previously described for small and simple reference compounds was not directly applicable to the log P determination of more complex natural compounds.

• The deviation between measured and expected log kw can be explained by intermolecular interactions.

• Therefore the model has been adapted and a new equation has been proposed including a linear combination of structural parameters.

• 3 outliers have been identified ; the reason of their deviation has to be investigated.

• A rapid NP’s structural parameters determination has to be developed in order to directly apply the new method on non isolated compounds with unknown structure.

• Therefore the new model will be applied to complex matrices such as crude plant extracts and the determined physicochemical properties would be of great value in the identification of new NPs of interest.

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References


