NMR spectra database for on-flight identification of HPLC-SPE-NMR data

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NMR spectra database for on-flight identification of HPLC-SPE-NMR data

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Introduction

Current developments within sensitivity of NMR spectroscopy combined with hyphenated methods such as HPLC-SPE-NMR have eased the work of natural product chemists. The fact that we are now capable of automatically acquiring NMR data of even minor metabolites in the ng-range [1] makes dereplication based on structure elucidation viable. This work presents such a dereplication tool based on the information obtained with a rapidly acquired 1H NMR spectrum.

HPLC-MS-SPE-NMR

Advances within the area of NMR hyphenation have led to implementation of an on-line solid phase extraction (SPE) step between the outlet of HPLC and the NMR. By lowering the eluotropic strength of the eluent through addition of water, trapping of metabolites onto SPE cartridges can be triggered by, e.g., ion count (mass detection) or absorption (UV detection). After drying, the metabolites can be transferred with deuterated solvents to either a flow probe or into NMR tubes in automation.

One of the numerous advantages of the HPLC-SPE-NMR setup over other hyphenated NMR techniques [2][3][4] is the increased sensitivity obtainable by doing repeated trapppings. Our lab has recently been equipped with a cryogenically cooled 1.7 mm probe installed in our 600 MHz magnet, making it possible to acquire NMR data of a vast majority of even minor metabolites in a crude extract in automation. Although the hyphenated system is equipped with a high resolution mass spectrometer - traditionally used for dereplication - we propose the use of the easily acquired, structurally information-rich 1H spectra instead.

Challenges with a 1H dereplication tool

The strength of NMR as a dereplication tool is that a signal not only tells something about a given proton, but also about its chemical environment. This strength is unfortunately also a weakness since many factors such as solvent, temperature, ionic strength, shimming, and concentration can cause changes in chemical shift values or signal appearances as illustrated below with strychnine.

Spectral matching

The dereplication tool was developed in the MATLAB environment, which offers great flexibility. The tool is still under development and therefore currently lacks a graphical user interface. It divides the chosen reference spectra into subspectra, calculates the best match for each signal and returns an overall match factor as well as a graphical presentation of each matched signal for visual inspection. The dereplication tool currently accepts user inputs regarding the maximum allowed shift of resonances, maximum amount of data points used for matching, and which databases to match against.

Results and perspective

In this study, we show how 1H NMR data hold a great potential for automatically dereplicating crude extracts of natural products. The dereplication tool we have developed in the MATLAB environment is based on a subspectra match routine that takes resonance shifts, often encountered in NMR spectroscopy, into account. With the amount of structural information obtainable from a 1H spectrum, we believe that, with a growing database size, this tool will be able to not only dereplicate but also be a valuable help as a structure elucidation tool or for classification of compounds into compound classes.

References


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