Structural moieties and tissue distribution of hepatoprotective lignin-derived agent determined by isotopic labeling mass spectrometry

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Introduction

Molecular complexity of biological active of natural polyphenols prevents thorough description of molecular mechanisms of their action and hampers pharmacokinetic studies. FTICR MS in combination with isotopic tags can be used to obtain structural information about individual components of complex organic mixtures. Data can be applied for an in silico search of molecular targets in biological activity databases [1]. Additional advantage of isotopic tagging is the possibility to distinct between metabolome and labeled material in physiological liquids and tissues. Here, we report on the first combination of several selective labeling techniques to enumerate functional groups and structural fragments of water-soluble product of oxidative degradation of lignin. D-labeled material was also used to study mouse tissue distribution after oral and intravenous administrations.

Experimental setup

Pharmacology-grade BP-Cx-1 sample and 10 other batches for comparison were provided by Nobel Ltd. To explore batch-to-batch consistency pairwise Tanimoto score were calculated. Carboxylic, carbonyl and phenolic moieties were determined by selective incorporation of deuterium tags via esterification, reduction and acetylation, respectively. Parent and labeled samples were analyzed on 7T FT MS Bruker Apex Ultra with harmonized cell (Bruker Daltonics). Enumeration of functional groups was performed semi-automatic for 200 abundant ions. FTICR MS data-mining was performed with ChEMBL 26. Confidence score based on the matching of functional groups was used for structures selecting along with their bioactivity data. For pharmacokinetics experiments H/D exchange of skeletal protons was performed and d-labeled sample was administrated orally and intravenously to mice followed by euthanasia. Liver was collected for extraction and determination of sample components and their metabolites by FTICR MS. Formulae were validated by the juxtaposition of mass-spectra of labeled and parent materials and extracted peaks composing HDX series.

Results

Formulae were calculated using browser-based application UltraMassExplorer created by Leefmann et al. (http://dockersrv1.awi.de:3838/ume) [2] with sensible chemical constraints. About 3000 formulae were resolved from the FTICR mass-spectrum of the parent material with the dominance of CHO-composition. In order to enhance applicability of suggested approaches batches were compared.

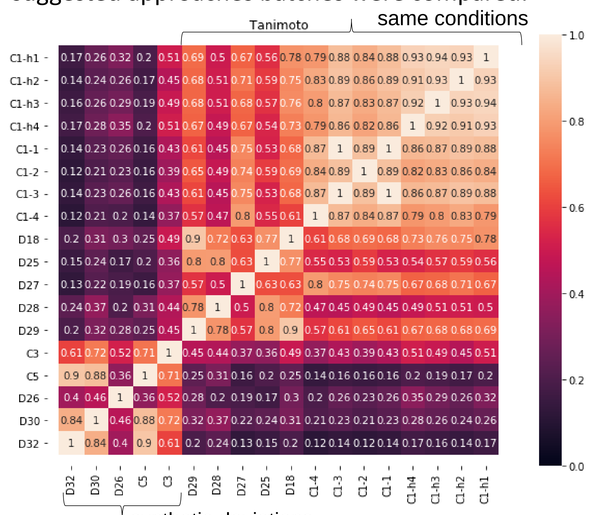


Figure 1. *Pairwise T-score for different batches of oxidized lignin hydrolizates obtained under similar and different synthetic conditions.*

Carboxyl, carbonyl and phenolic groups were enumerated by selective chemical reactions (Fig. 2). Reduction by $NaBD_4$ results in 1 exchangeable and 1 unexchangeable deuterons in the reduced form.

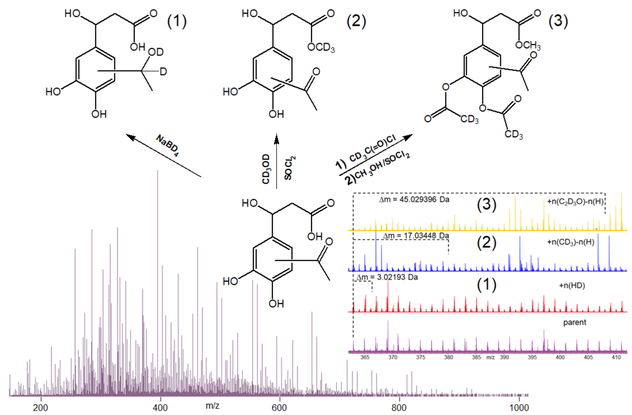


Figure 2. *Scheme of labeling experiments for BP-Cx-1 functional group determination*

Molecular mapping of functional groups was performed by plotting van Krevelen diagram (Fig. 3). The number of COOH-groups varied from 0 to 4. The number of carbonyl groups reached 3. Phenols were most abundant in case of aromatic relatively reduced compounds.

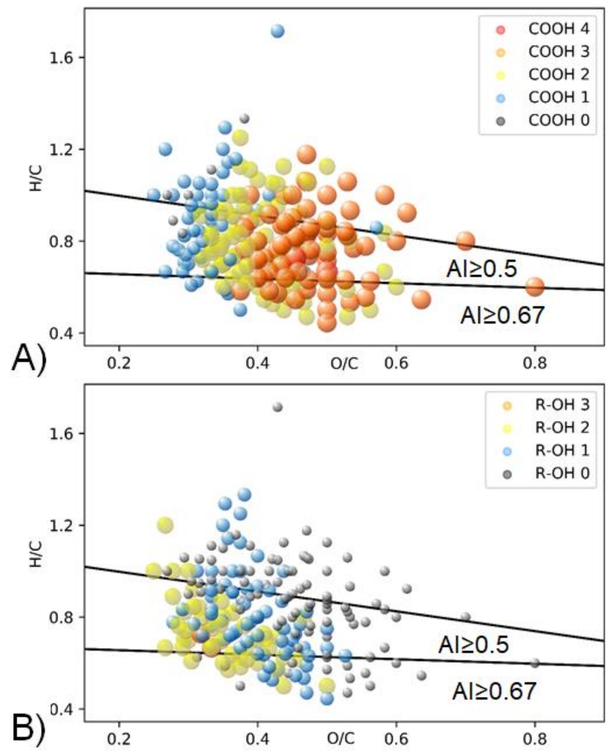
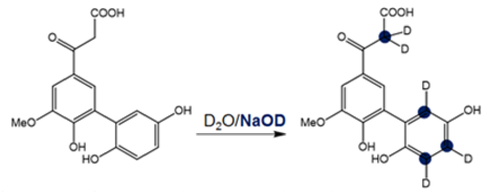


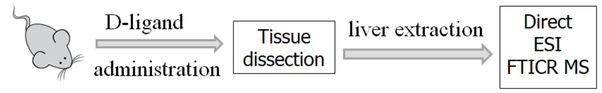
Figure 3. *Van Krevelen diagram with functional group mapping*

D-labeled material for pharmacokinetic study was obtained by H/D exchange of skeleton protons according to [3].

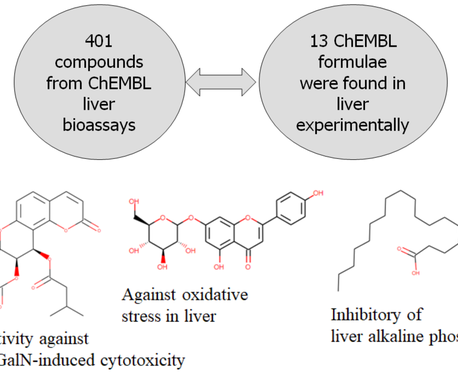


After purification in was administrated to the series of mice. After 6 hours tissues were dissected and livers was collected. To increase concentration half of liver samples were pulled together before the extraction.

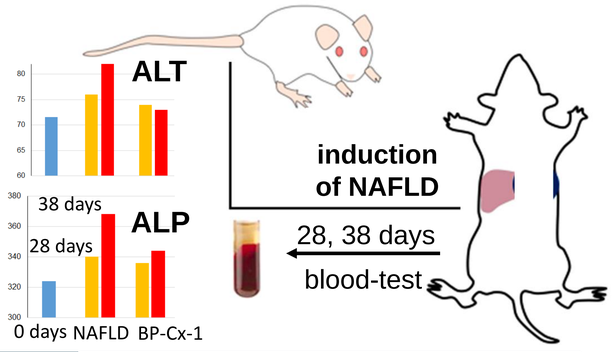
Hepatoprotective species



Spectra acquiring was performed with quadrupole isolation to increase S/N ratio of signals of interest. Only peaks presented in tissue extracts and labeled materials before administration were taken into account. 65 formulae were found in liver after extraction. Only 13 out of 65 formulae were found in ChEMBL data-base. Sill, they corresponded to 600 structures.



Examination of hepatoprotection on fructose-induced liver damage model



References

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[2] Leefmann, et al. "UltraMassExplorer: a browser-based application for the evaluation of high-resolution mass spectrometric data." Rapid Communications in Mass Spectrometry 33.2 (2019): 193-202.
[3] Zherebker et al. "Structural investigation of coal humic substances by selective isotopic exchange and high-resolution mass spectrometry." Faraday discussions 218 (2019): 172-190.

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