Sensitive and robust LC-MS analysis of polyisoprenoids and their phosphates in biological samples

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Abstract

Polyisoprenoids are hydrophobic long-chain polymers, consisting of several or tens of isoprene units, that play crucial physiological functions mediating membrane properties and cell metabolism. **Prenols** and **dolichols** (dihydro-prenols) are two classes of polyisoprenoids which are found in the cells. Due to their physico-chemical properties are less prone to undergo ionization in mass spectrometer's ion source, which hinders the sensitivity of the analysis. Moreover, prenol and dolichol with the same number of isoprene units show overlapping mass spectra, which makes separation of their signals difficult and gives false results on analyte content levels. Thus, we have developed sensitive and highly selective LC-MS analysis of both prenol and dolichol which enables quantitative estimation of their content in different biological matrices.

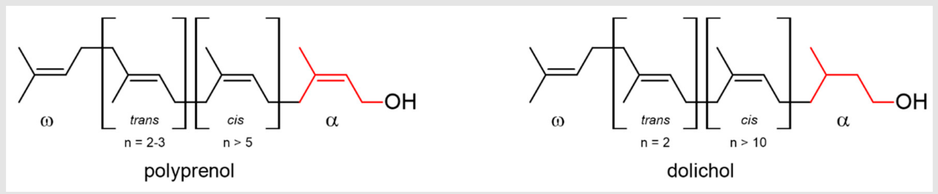

Figure 1. Structure of prenols and dolichols (Surowiecki *et al.*, 2019)

Methodology

Extraction of polyisoprenoids

Plant tissues were homogenized in a mixture of chloroform/methanol (2/1, v/v), after phase separation upper (methanol) phase was re-extracted and pooled chloroform phases were evaporated and dissolved in C/M 2/1 (v/v). For analysis of phosphates lipids were subjected to methylation.

Polyisoprenoid phosphates methylation

Prenyl and dolichyl monophosphates were derivatized using (Trimethylsilyl)diazomethane (TMSD) as in the protocol of Kale *et al.*

LC-MS method

Semi-quantification of polyisoprenoids and their phosphates was performed using ACQUITY UPLC chromatograph (Waters) coupled with Xevo TQ mass spectrometer (Waters) and Q-Exactive Orbitrap (Thermo Fisher Scientific) High Resolution Mass Spectrometer (HRMS).
Chromatographic separation was achieved using revesed phase Accucore C30 column (150 mm × 2.1 mm, 2.6 µm) from Thermo Fisher Scientific. Mobile phases consisted of:

Mobile phase A	Mobile phase B
10 mM ammonium formate 0.1 % formic acid ACN/MeOH/H_2O (2:1:1, v/v/v)	10 mM ammonium formate 0.1% formic acid IPA/ACN (9:1, v/v)

Spectra of 17 prenols and 17 dolichols were acquired in Single-Ion Reaction Monitoring mode (SRM) on Xevo TQ-MS and Data Dependent Acquasition mode (DDA) on HRMS. Both spectrometers operated with positive electrospray ionisation (ESI+). Ammonia adducts $[M+NH_4]^+$ were used for analytes identification. Spectra for selected monophosphates were acquired in Multiple-Reaction-Monitoring mode (MRM).

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Results

We have developed a robust LC-MS method for semi-quantification of **34 polyisoprenoids** of different chain length. Molecular mass of dolichols and prenols of the same chain length differ with ~2 Da. Isotopic envelopes of prenols and dolichols of the same length partially overlap, which causes prenol signal to falsely elevate dolichol abundance. Application of Accucore C30 column with dedicated composition of mobile phases resulted in efficient peak separation. This method ensures no signal overlapping, and thus, high specificity of the analysis.

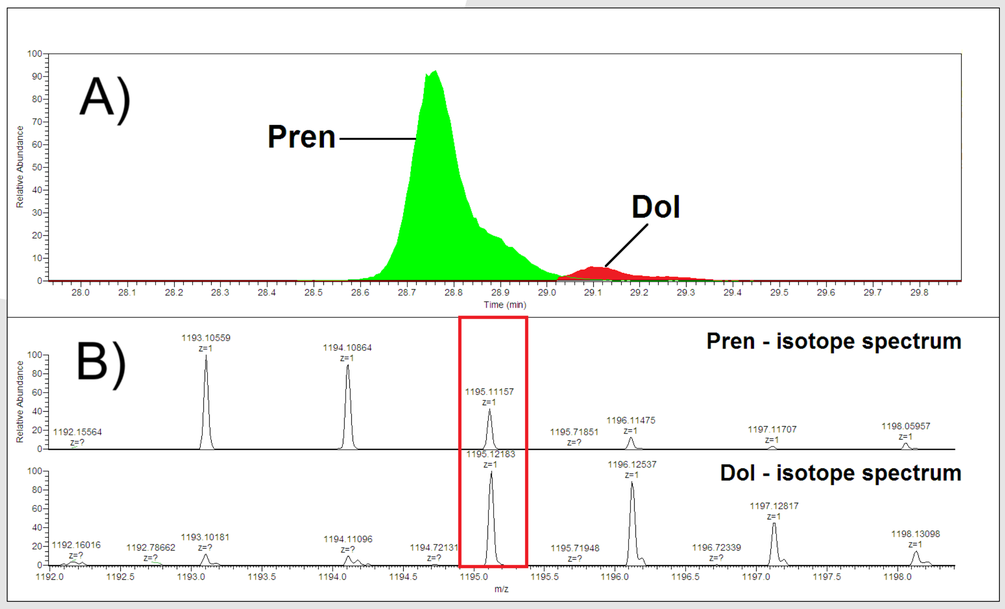

Figure 2. A – chromatogram of prenol-dolichol 17 pair (with seventeen isoprene units) showing signal separation between two analytes; **B** – isotope spectrum for prenol-17 (up) and dolichol-17 (down), a red frame shows overlapping m/z for both analytes

Methylation of prenyl and dolichyl monophosphates results in better sensitivity of the analytical method. Thanks to derivatization, phosphates can be analysed using MRM mode as they yield in production of specific daughter ions, such as the 127.0 (m/z) corresponding to the detached double-methylated phosphate group. We have established LC-MS conditions best for sensitive and rapid analysis of prenol/dolichol phosphates content.

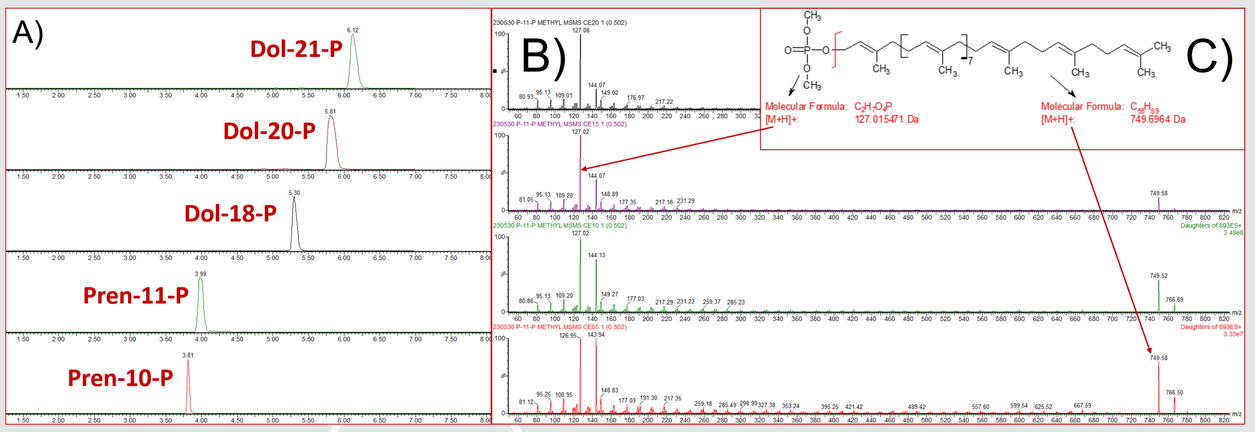

Figure 3. A - chromatograms for selected prenyl and dolichyl phosphates are presented; **B** - mass spectra acquired in MRM mode for increasing Collision Energy (CE) from 5 to 20 (V); **C** – a chemical structure of methylated prenyl monophosphate consisting of 11 isoprene units, arrows indicate signals for the respective daughter ions (ChemckSketch)

Conclusions

1. **Our LC-MS method results in sensitive and specific analysis of 34 polyisoprenoids**
2. **We have established chromatographic conditions for satisfactory prenol-dolichol separation**
3. **This method was already applied to various biological matrices from different organisms e.g.: human and mouse blood plasma, *Paramecium* sp. cells, mice retinas, *Arabidopsis thaliana* leaves and roots**
4. **Methylation of polyisoprenoid monophosphates increases method sensitivity and allows for high specificity thanks to establishing specific pairs of parental and daughter ions (in MRM mode)**

References

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