Poster Note PN-30

Analysis of propolis extracts and isomeric flavonoid mixtures using trapped ion mobility spectrometry (TIMS)

Bruker's new timsTOF instrument extends high performance QTOF mass spectrometry with high resolution ion mobility separation. Here, the novel platform is used for analysis of isomeric flavonoid mixtures as well as spiked and non-spiked propolis extracts.

Introduction

Flavonoids are secondary metabolites produced by plants. Several subclasses of flavonoids are known, with a total number of published structures exceeding 8,000 compounds. Many of them show significant biological activity.

Propolis is a natural bee resin that contains high levels of flavonoids, because the raw material stems from plants. Due to the enormous structural diversity of flavonoids, the analysis of complex samples, such as Propolis, is very challenging.

Ion mobility separation coupled to high performance mass spectrometry (LC-IMS-MS/MS) introduces an additional analytical dimension for the identification of co-eluting isomers, based on the collisional cross section of the compounds. For very complex mixtures conventional ion mobility devices often do not have enough resolving power.

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Flavonoids	timsTOF
Isomeric compounds	Data Analysis 5.0
Ion mobility resolution	OTOF Control 5.0
Separation	
CCS values	
LC-IMS-MS/MS	

Methods

We used a high resolution QTOF mass spectrometer coupled to trapped ion mobility spectrometry (Bruker timsTOF™) for analysis of propolis extracts and isomeric flavonoid mixtures. Propolis samples were collected from different bee yards across Germany (Fig. 1) and extracted with 70% EtOH using an ultrasonic bath. The supernatants were used as complex blank matrices. Propolis extracts and isomeric flavonoid mixtures were additionally spiked with mixtures of isomeric flavonoids at a concentration of 1 µg/mL (apigenin, galangin, quercetin, morin, orientin, isoorientin, quercitrin, kaempferol, fisetin, scutellarein and luteolin; Sigma Aldrich).

The standard mixtures and the complex samples were separated by standard HPLC prior to analysis. The QTOF mass spectrometer was tuned for a mass range of 50-1000 m/z. For ion mobility separation, TIMS was enabled to separate ions according to their collisional cross sections. The mobility separated ions were investigated with HR-QTOF-MS and –MS/MS methods.

Fig. 1: Sampling at a bee hive.

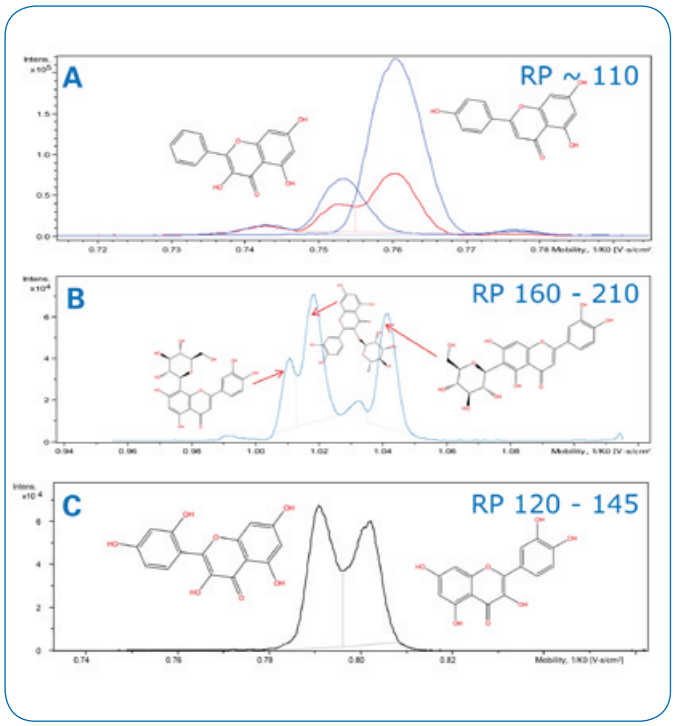

Fig. 2: Mobilograms of isomeric compounds showing ion mobility separation of mixtures. **A** Galangin and Apigenin, **B** Orientin,Isoorientin and Quercitrine, **C** Quercetin and Morin.

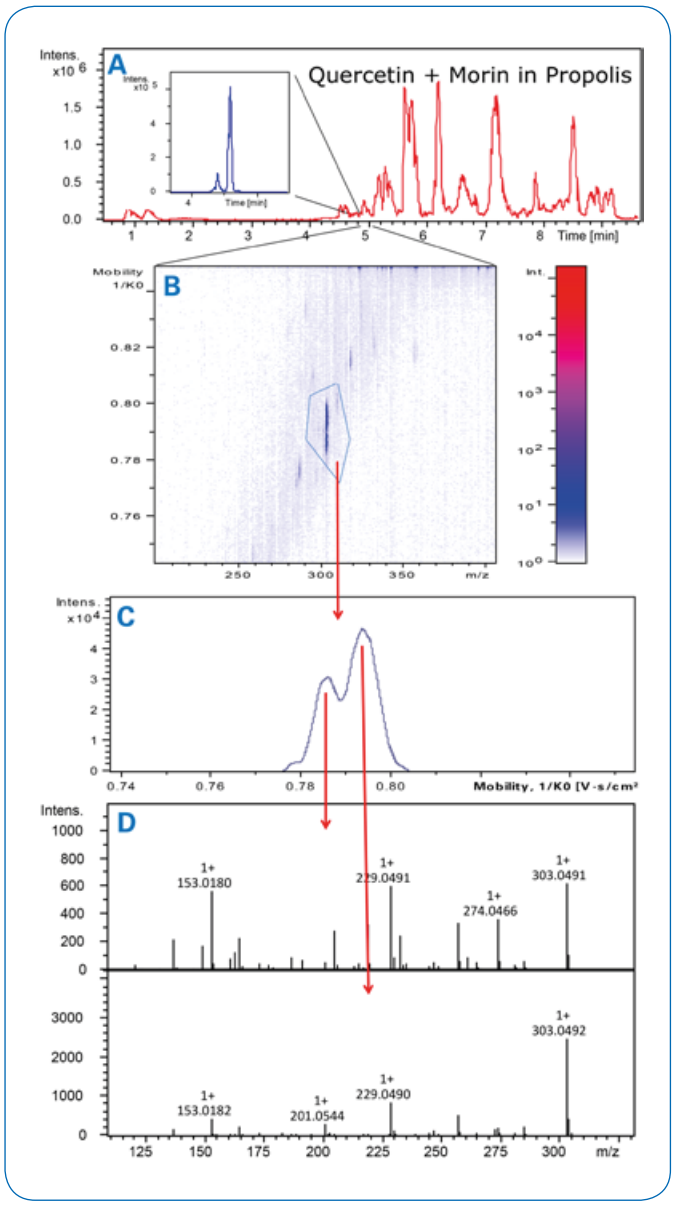

Fig. 3: A Base Peak Chromatogram and Extracted Ion Chromatogram of quercetin and morin of a propolis sample. **B** Heatmap of the area of interest. **C** Mobilogram revealing two isomeric compounds. **D** Clean MS/MS spectra after ion mobility separation.

Results

Several mixtures of isobaric flavonoids were investigated in direct infusion and LC-MS experiments complemented by high resolution ion mobility separation (Bruker timsTOF™). This workflow is particularly beneficial for co-eluting isobaric compounds and we analyzed compound mixtures consisting of galangin and apigenin ($C_{15}H_{10}O_5$, Fig. 2A), orientin, isoorientin and quercitrine ($C_{21}H_{20}O_{11}$, Fig. 2B), kaempferol, fisetin, scutellarein and luteolin ($C_{15}H_{10}O_6$) as well as quercetin and morin ($C_{15}H_{10}O_7$, Fig. 2C). High resolution ion mobility in the range of 150-250 often provided baseline separation of the analytes and thus allowed acquisition of clean MS/MS spectra of the respective isomers (example: quercetin and morin, Fig. 3). Based on the mobility data ($1/K_0$), collision cross section (CCS) values for the respective compounds were calculated and compared to published CCS values (Table 1). Together, clean MS/MS spectra and CCS values greatly increase the confidence of identification of the respective flavonoid isomers.

Compound	$1/K_0$ [Vscm^{-2}]	CCS value (Å^2)	% RSD
Galangin	0.754	158.599	0.1
Apigenin	0.760	159.861	0.2
Quercetin	0.801	167.650	0.2
Morin	0.792	165.767	0.1
Orientin	0.971	200.416	0.1
Isoorientin	0.981	202.480	0.2
Quercitrine*	1.009	207.974	0.2
Kaempferol	0.777	163.010	0.1
Fisetin	0.783	164.269	0.2
Scutellarein	0.773	162.171	0.2
Luteolin	0.784	164.479	0.2

Table 1: CCS values for $[M+H]^+$ adducts.
(* refers to the respective Na-adduct)

Summary

- Several mixtures of isomeric flavonoids were investigated in direct infusion and LC experiments with trapped ion mobility spectrometry (TIMS) coupled to high performance QTOF
- Acquisition of MS/MS spectra following TIMS separation provided clean MS/MS spectra of isobaric compounds for unambiguous compound identification
- Accurate CCS values provided orthogonal information to confirm compound identification.

Conclusions

High resolution ion mobility separation of isobaric flavonoids was investigated for complex sample analysis using the Bruker timsTOF instrument. Accurate CCS values and clean MS/MS spectra based on an ion mobility resolution > 200 were achieved.

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