SEPARATION OF CATECHIN EPIMERS BY COMPLEXATION USING ION MOBILITY MASS SPECTROMETRY

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Introduction

Catechins belongs to the group of flavan-3-ols (or flavanols), part of the chemical family of flavonoids. They are widely distributed in plant derived foods including red wine, green tea, chocolate and many fruits [1]. The flavanols show also large strong pharmacological properties, including of anticarcinogenic [2], antibiotic [3] and antiatherogenic effects [4].

The aim of the study is to explore the potential application of the ion mobility technique coupled to mass spectrometry (IM-MS) to distinguish between two diastereoisomeric compounds: catechin and epicatechin. (Figure 1)

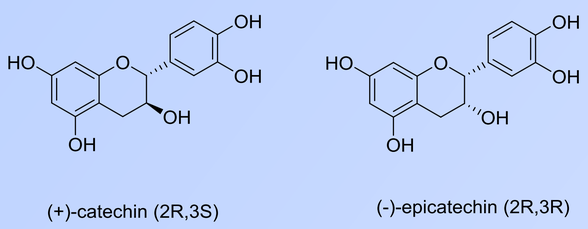


Figure 1. Epimeric structures of (+)-catechin and (-)-epicatechin.

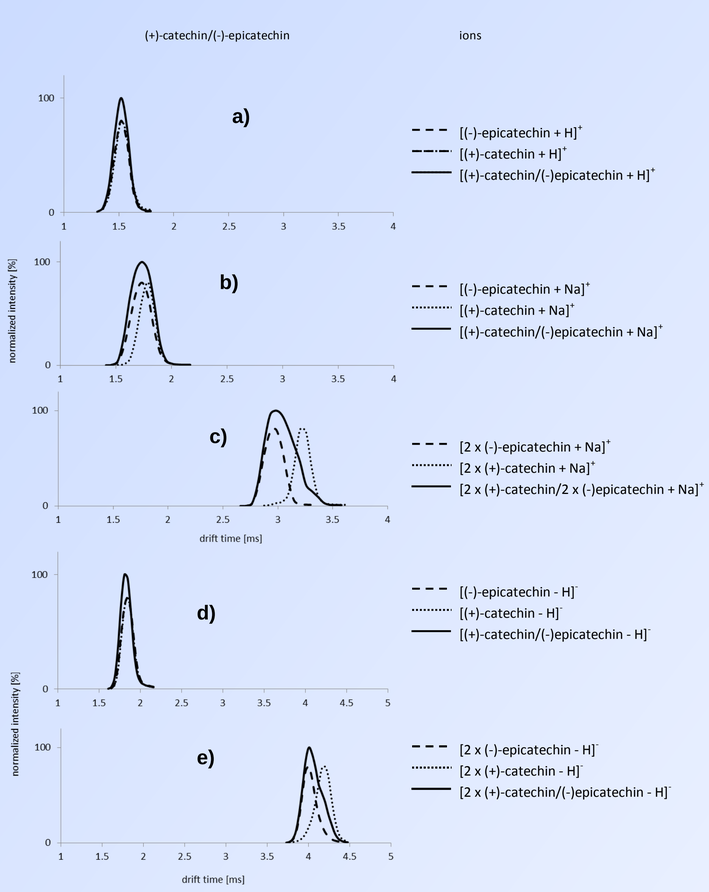
Methods

Traveling-wave IM-MS experiments were performed on a quadrupole ion mobility time-of-flight instrument (Synapt G2-S HDMS, Waters). The IM-MS spectra in the positive and negative ion mode were recorded. The nitrogen was used as neutral drift gas.

The solutions of the catechins (2.2 mM) were prepared in methanol. The amino acids were prepared at a concentration of 2.2 mM in water/methanol (1:1). The solutions of the metal salts were prepared at a ca. 2.2 mM in water. The complexes were produced by mixing solutions of the appropriate metal salt [MX_2 and MX, where M= Cu(II), Cu(I), Co(II), Pd(II) and X=CH_3COO, Cl], epimers of catechins and solution D-, L-amino acids . The concentration of the catechins in the complexes was 0.055 mM.

Results

Figure 1. Overlapped mobility separation spectra (raw data) of racemic mixture (plain line) and individual epimers according to the ion type i.e. protonated $[M+H]^+$ **(a)**, sodiated $[M+Na]^+$ **(b)**, $[2M+Na]^+$ **(c)** or ions resulting from hydrogen lost $[M-H]^-$ **(d)** and $[2M-H]^-$ **(e)**.



Results

Figure 2. Ion mobility spectra of sodiated dimeric complex $[2M + Na]^+$ at different solution compositions containing: **a)** epicatechin individually, **b)** equimolar mixture of epicatechin and catechin (1:1), **c)** epicatechin and catechin in the ratio 1:3 and **d)** catechin, individually.

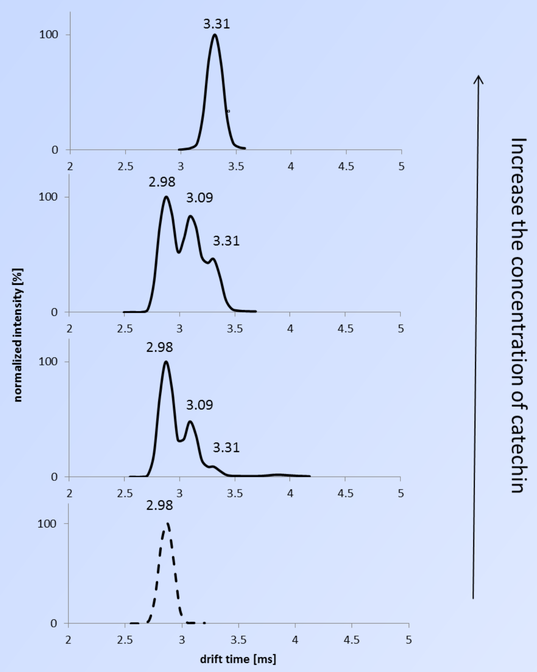


Table 1. Drift times [ms] for each individual epimer (M).

ions	m/z	drift time [ms] (+)-catechin	drift time [ms] (-)-epicatechin
$[M+H]^+$	291.0	1.52	1.52
$[M+Na]^+$	313.0	1.79	1.74
$[2M+Na]^+$	603.1	3.31	2.98
$[M-H]^-$	289.0	1.85	1.85
$[2M-H]^-$	579.1	4.20	4.00

Figure 3. Overlapped mobility separation spectra (raw data) of racemic mixture (plain line) and individual epimers. The curves show extracted ion chromatograms of $[2M + D/L\text{-amino acid} + Cu^{2+} – 3H]^-$ ion type for **a)** D-, L-Alanine **b)** D-, L-Leucine **c)** D-, L-Proline.

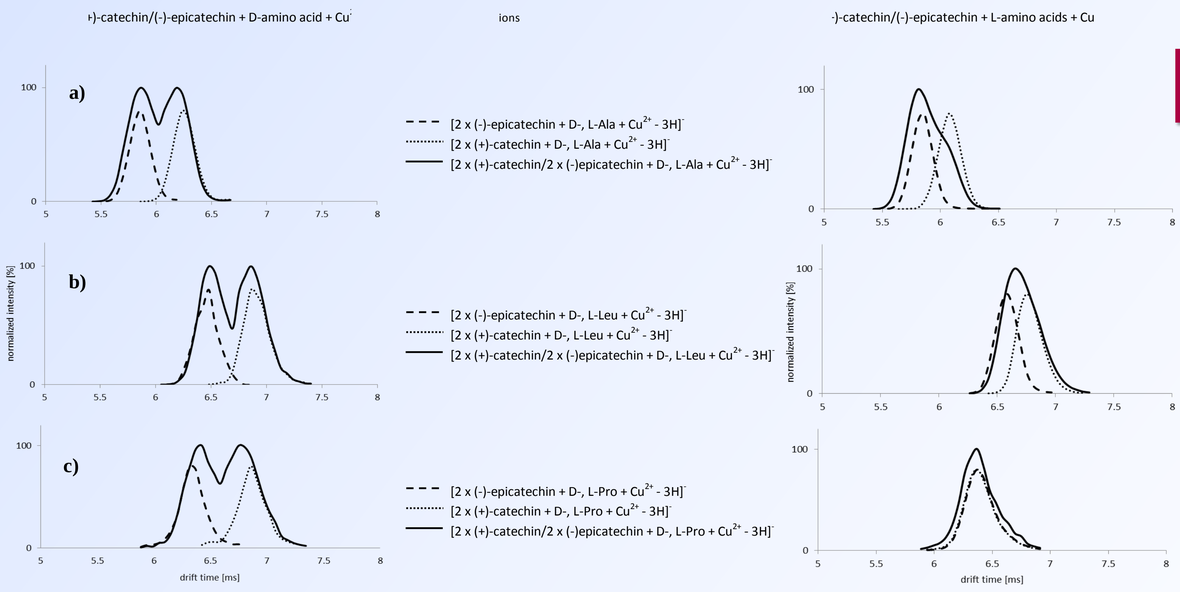


Table 2. Drift times [ms] of clusters having the form $[2M + D\text{-amino acids} + Cu^{2+} – 3H]^-$.

ions	drift time [ms] epicatechin (t_{d1})	catechin (t_{d2})	Δt_d	mixture t_{d1}	mixture t_{d2}	Δt_d
$[2M + D\text{-Alanine} + Cu^{2+} – 3H]^-$	5.86	6.18	0.32	5.86	6.29	0.43
$[2M + D\text{-Leucine} + Cu^{2+} – 3H]^-$	6.48	6.91	0.43	6.48	6.86	0.38
$[2M + D\text{-Proline} + Cu^{2+} – 3H]^-$	6.34	6.9	0.56	6.43	6.75	0.32

Figure 4. Ion mobility spectra of $[2M + D\text{-Leucine} + Cu^{2+} – 3H]^-$ complex containing different concentration of catechin.

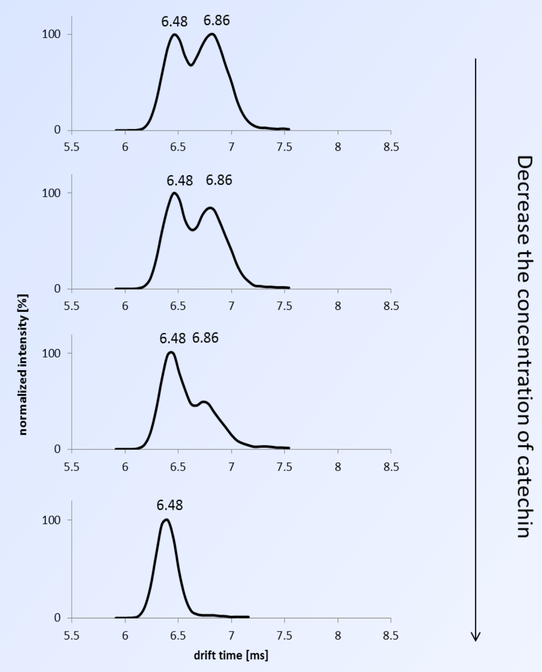


Table 3. Separation factor (α) and peak-to-peak resolution ($R_{p\text{-}p}$) for complexes.

complex	α	$R_{p\text{-}p}$
$[2M + D\text{-leucine} + Cu^{2+} – 3H]^-$	1.06	0.71
$[2M + D\text{-alanine} + Cu^{2+} – 3H]^-$	1.05	0.61
$[2M + D\text{-proline} + Cu^{2+} – 3H]^-$	1.05	0.6

Conclusions

- We present the first successful attempt to separate epimers of catechins.
- We received the improvement of separation using IM-MS based on the stereospecific interactions with chiral compounds and transition metals.
- The best separation was achieved for $[2M + D\text{-leucine} + Cu^{2+} – 3H]^-$ complex.

References

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Acknowledgements

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„**The structural studies of the selected organic compounds with the use of ion mobility-mass spectrometry method”**