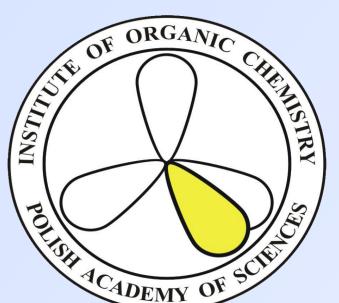
SEPARATION OF CATECHIN EPIMERS BY COMPLEXATION USING ION MOBILITY MASS SPECTROMETRY

Anna Troć, Magdalena Zimnicka, Witold Danikiewicz



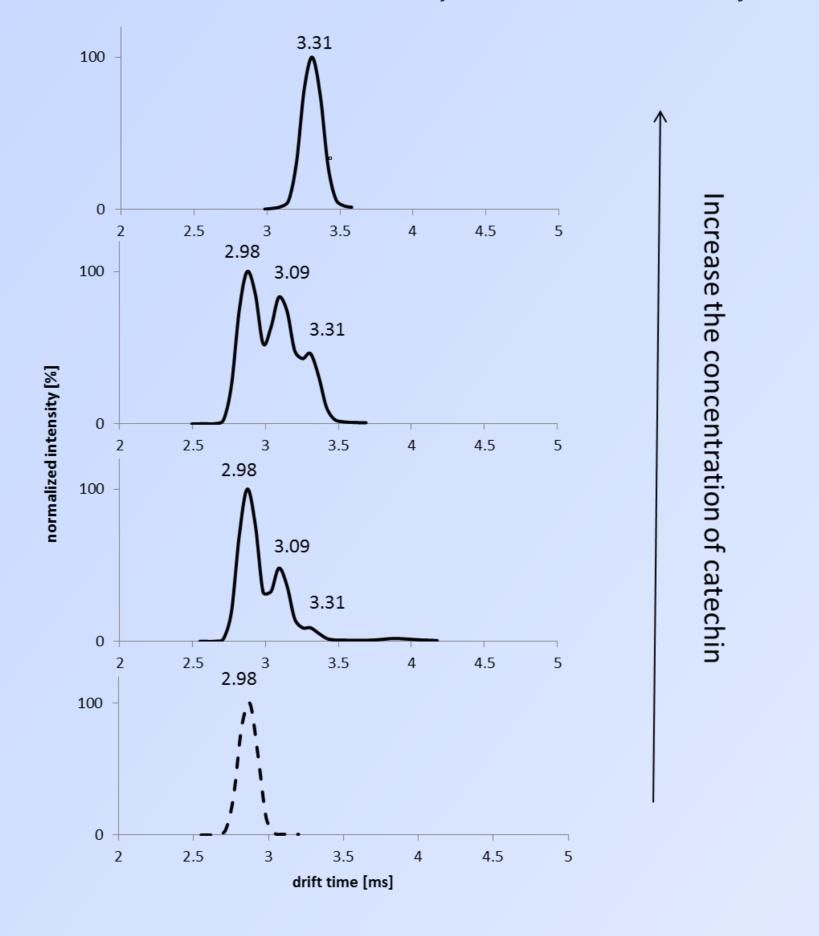
Institute of Organic Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224, Warsaw, Poland

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].

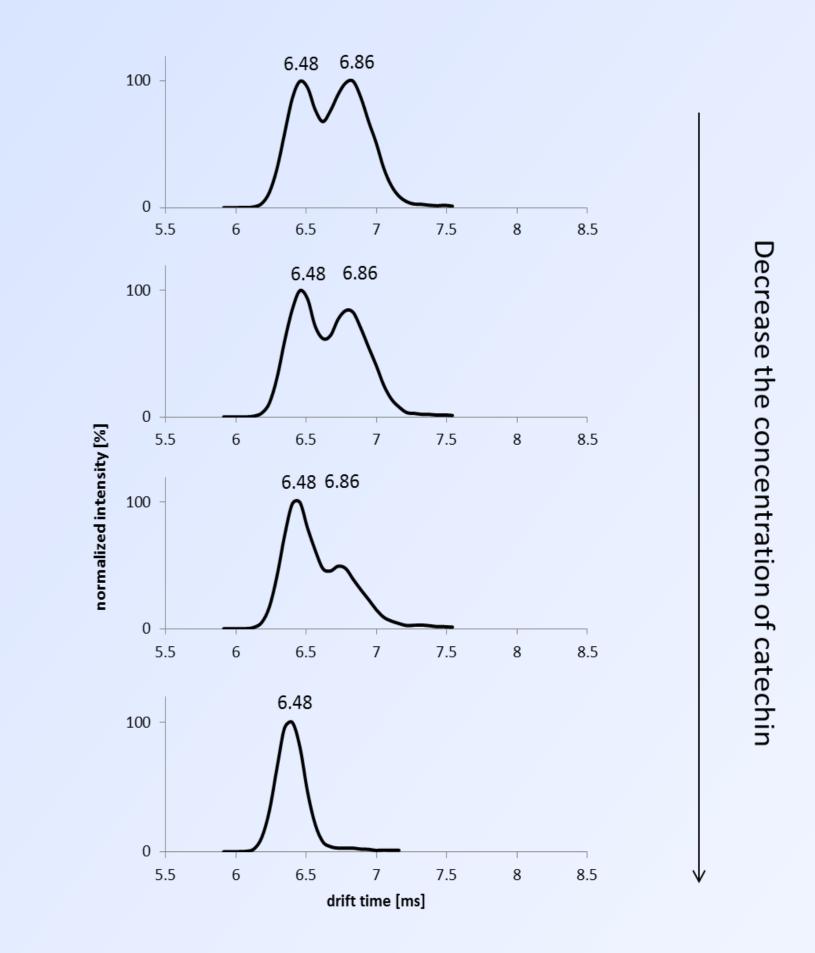
The aim of the study is to explore the potential application of the ion mobility technique coupled to mass spectrometry (IM-MS) to distinguish between two diastereoisomeric compounds: catechin and epicatechin. (Figure 1)

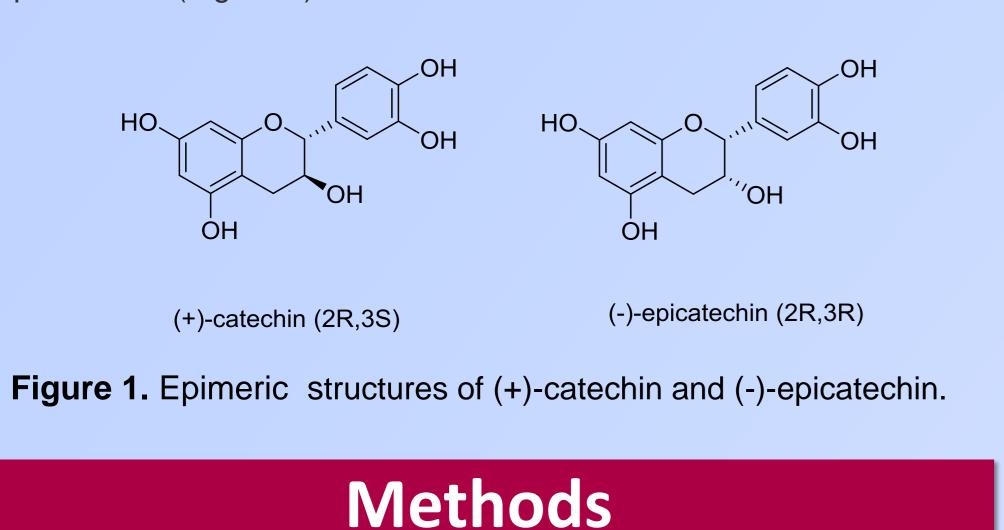
Figure 2. Ion mobility spectra of sodiated dimeric complex [2M + Na]⁺ at different solution compositions containing: a) epicatechin individually, **b)** equimolar mixture of epicatechin and catechin (1:1), **c)** epicatechin and catechin in the ratio 1:3 and d) catechin, individually.



Fiqure 4. Ion mobility spectra of $[2M + D-Leucine + Cu^{2+} - 3H]^{-1}$ complex containing different concentration of catechin.

Results





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.

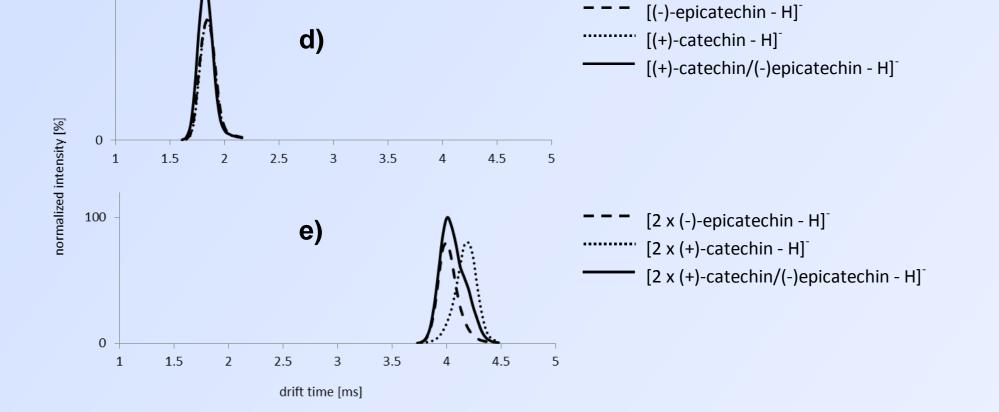
Table 1. Drift times [ms] for each individual epimer (M).

ions	m/z	drift time [ms]			
		(+)-catechin	(-)-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		

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

ter/methanol (1:1). The solutions of the metal salts were prepared at ca. 2.2 mM in water. The complexes were produced by mixing utions of the appropriate metal salt [MX ₂ and MX, where M= Cu(II), (I), Co(II), Pd(II) and X=CH ₃ COO, Cl], epimers of catechins and ution D-, L-amino acids . The concentration of the catechins in the Ution D-, L-amino acids . The concentration of the catechins in the	The colutions of the establing (0.0 mall) were increased in mathematic							
initial lattice water. The complexes were proportied at alls wore proportied by mighting on the appropriate at all (MX, where Me Coull). initial (H+H)* 2010 1.52 1.62	The amino acids were prepared at a concentration of 2.2 mM in			drift time [ms]		complex	n	R
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Implexes was 0.055 mM. Implemes was 0.055 mM. I	I(I), Co(II), Pd(II) and X=CH ₃ COO, CI], epimers of catechins and	[M+Na]+	313.0	1.79	1.74			
Results Image: part 1. Overlapped mobility separation spectra (raw data) of racemic mixture (plain line) and individual epimers according to the ion type ion abult separation spectra (raw data) of racemic mixture (plain line) and individual epimers. The curves show extracted ion chromatograms of [2M + D/L-amino add + Cu ²⁺ - 3H] ion type for a) D-, L-Alanine b) D-, L-Alanine b) D-, L-Leucine c) D-, L-Proline. Image: part 1. Curves show extracted ion chromatograms of [2M + D/L-amino add + Cu ²⁺ - 3H] ion type for a) D-, L-Alanine b) D-, L-Leucine c) D-, L-Proline. Image: part 1. Curves show extracted ion chromatograms of [2M + D/L-amino add + Cu ²⁺ - 3H] ion type for a) D-, L-Alanine b) D-, L-Leucine c) D-, L-Proline. Image: part 1. Curves show extracted ion chromatograms of [2M + D/L-amino add + Cu ²⁺ - 3H] ion type for a) D-, L-Alanine b) D-, L-Leucine c) D-, L-Proline. Image: part 1. Curves show extracted ion chromatograms of [2M + D/L-amino add + Cu ²⁺ - 3H] ion type for a) D-, L-Alanine b) D-, L-Leucine c) D-, L-Proline. Image: part 1. Curves show extracted ion chromatograms of [2M + D/L-amino add + Cu ²⁺ - 3H] ion type for a) D-, L-Alanine b) D-, L-Leucine c) D-, L-Proline. Image: part 1. Curves show extracted ion chromatograms of [2M + D/L-amino add + Cu ²⁺ - 3H] ion type for a) D-, L-Alanine b) D-, L-Leucine c) D-, L-Proline. Image: part 1. Curves show extracted ion chromatograms of [2M + D/L-amino add + Cu ²⁺ - 3H] ion type for a) D-, L-Alanine b) D-, L-Leucine c) D-, L-Proline. Image: part 1. Curves show extracted ion chromatograms of [2M + D/L-amino add + Cu ²⁺ - 3H] ion type for a) D-, L-Alanine b) D-, L-Proline. Image: part 1. Curves show extracted ion chromatograms of [2M + D/L-amino add + Cu ²⁺ - 3H] ion type for a) D-, L-Alanine b) D-, L-Leucine c) D-,	mplexes was 0.055 mM.	[2M+Na]+	603.1	3.31	2.98	[2M + D-alanine + Cu ²	- 3H] 1.05	0.61
Results pure 1. Overlapped mobility separation spectra (raw data) of racemic mixture (plain line) and individual epimers according to the ion type is the curves show extracted ion chromatograms of [2M + D/L-amino acid + Cu ²⁺ - 3H] in type for a) D ₁ , L-Alanine b) D ₂ , L-Leucine c) D ₂ , L-Mainine b) D ₂ , L-Leucine c) D ₂ , L-Mainine b) D ₂ , L-Leucine c) D ₂ , L-Mainine b) D ₂ , L-Leucine c) D ₂ , L-Mainine b) D ₂ , L-Leucine c) D ₂ , L-Mainine b) D ₂ , L-Mai		[M-H] ⁻	289.0	1.85	1.85			
Figure 1. Overlapped mobility separation spectra (raw data) of racemic mixture (plain line) and individual epimers according to the integration spectra (raw data) of racemic mixture (plain line) and individual epimers according to the integration spectra (raw data) of racemic mixture (plain line) and individual epimers according to the integration spectra (raw data) of racemic mixture (plain line) and individual epimers according to the integration spectra (raw data) of racemic mixture (plain line) and individual epimers according to the integration spectra (raw data) of racemic mixture (plain line) and individual epimers according to the integration spectra (raw data) of racemic mixture (plain line) and individual epimers according to the integration spectra (raw data) of racemic mixture (plain line) and individual epimers according to the integration spectra (raw data) of racemic mixture (plain line) and individual epimers according to the integration spectra (raw data) of racemic mixture (plain line) and individual epimers according to the integration spectra (raw data) of racemic mixture (plain line) and individual epimers according to the integration spectra (raw data) of racemic mixture (plain line) and individual epimers according to the integration spectra (raw data) of racemic mixture (plain line) and individual epimers according to the integration spectra (raw data) of racemic mixture (plain line) and individual epimers according to the integration spectra (raw data) of racemic mixture (plain line) and individual epimers according to the integration spectra (raw data) of racemic mixture (plain line) and individual epimers according to the integration spectra (raw data) of racemic mixture (plain line) and individual epimers according to the integration spectra (raw data) of racemic mixture (plain line) and individual epimers according to the integration spectra (raw data) of racemic mixture (plain line) and individual epimers according to the integration spectra (raw data) of racemic mixture (plain	Results	[2M-H] ⁻	579.1	4.20	4.00	[2M + D-proline + Cu ²⁺	– 3H] ⁻ 1.05	0.6
$\frac{1}{10} + \frac{1}{12} $	tonated [M+H] ⁺ (a), sodiated [M+Na] ⁺ (b), [2M+Na] ⁺ (c) or ions	+)-catechin/(-)-epicate	echin + D-amino acid + Cu [']	ions		-)-catechin/(-)-epicatechin + L-amino acids + Cu	Conclu	ciopo
$\frac{1}{1} + \frac{1}{15} + \frac{1}{2} + \frac{1}{25} + \frac{1}{3} + \frac{1}{35} + \frac{1}{3} + \frac{1}{25} + \frac{1}{55} + \frac{1}{5} + \frac$	$ \begin{array}{c} 100 \\ - \\ 0 \\ 1 \\ 1.5 \\ 2 \end{array} $ $ \begin{array}{c} a) \\ - \\ - \\ (-)-epicatechin + H]^{+} \\ - \\ (+)-catechin + H]^{+} \\ -$		5 7 7.5	8 8 8 [2 x (-)-epicatechin 8 8 8 9 10 <	D-, L-Ala + Cu ²⁺ - 3H] ⁻ x (-)epicatechin + D-, L-Ala + Cu ²⁺ - 3H] ⁻ n + D-, L-Leu + Cu ²⁺ - 3H] ⁻ D-, L-Leu + Cu ²⁺ - 3H] ⁻		 ⁸ We present the first to separate epimers We received the separation using IN 	et successful att of catechins. e improvement M-MS based or
	$ \begin{bmatrix} (+)-catechin/(-)epicatechin + Na]^{+} \\ \\ 100 \\ \\ \\ 0 \\ \\ \end{bmatrix}^{+} \\ \begin{bmatrix} (+)-catechin/(-)epicatechin + Na]^{+} \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\$	$ \begin{array}{c} 100 \\ $.5 7 7.5	[2 x (+)-catechin +	D-, L-Pro + Cu ²⁺ - 3H] ⁻	100	 ⁸ compounds and tran > The best separation 	nsition metals. In was achieve

 Table 2. Drift times [ms] of clusters having the form [2M + D-amino



acids + $Cu^{2+} - 3H$]⁻.

	drift time [ms]						
ions	epicatechin	catechin	⊿t _d	mixture)	⊿t _d	
	(t _{d1})	(t _{d2})		t _{d1}	t _{d2}		
[2M + D-Alanine + Cu ²⁺ – 3H] ⁻	5.86	6.18	0.32	5.86	6.29	0.43	
[2M + D-Leucine + Cu ²⁺ – 3H] ⁻	6.48	6.91	0.43	6.48	6.86	0.38	
[2M + D-Proline + Cu ²⁺ – 3H] ⁻	6.34	6.9	0.56	6.43	6.75	0.32	

References

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(4) Zhong, Y.; Hyung, S.-J.; Ruotolo, B. T. Expert Rev. Proteomics **2012,** *9*, 47.

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