

**UNIVERSITY OF PÉCS**

DOCTORAL SCHOOL OF CHEMISTRY

**Characterization of lipid-A molecules by tandem mass spectrometry  
combined with chromatography**

PhD Thesis

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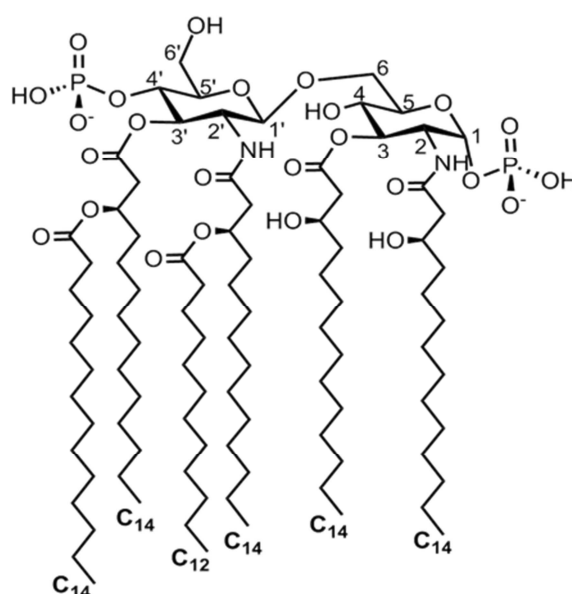
**Pécs, 2018**

## 1. INTRODUCTION

The outer leaflet of the outer membrane of Gram-negative bacteria is composed of a distinctive type of macromolecules called lipopolysaccharides (LPS) that cover up to 80% of the cell surface. LPS molecules generally comprise three defined regions distinguished by their genetics, structure and function. These are the *lipid-A* which is a special phosphoglycerolipid, the *core-oligosaccharide* and the *O-polysaccharide chain* (also called O-antigen). In some Gram-negative bacteria, LPS can terminate with the core portion to form lipooligosaccharide (LOS). The highly ordered amphiphilic LPS/LOS monolayer is essential for the survival of Gram-negative bacteria since it protects them from their surroundings, helps them to resist both hydrophilic and hydrophobic antimicrobial agents. Besides, in consequence of their external position, LPS/LOS molecules act like antennas of the cells and are involved in several host-bacterium interactions such as recognition, colonization as well as in virulence. LPSs are also termed as *endotoxins*, which is more of a medical origin. This denomination primarily expresses their toxic principle, on the other hand, the prefix “endo” reflects on their membrane integrity. Once they are released from the membrane during proliferation or as the result of the death of the bacterial cells, the immune system of the host organism begins to evade the Gram-negative bacterial infection after the endotoxins have been recognized by special pathogen pattern recognition receptors called Toll-like receptors (TLR). The bioactivity, the immune-stimulatory effect of endotoxins, including the capacity to activate the receptors of the immune system is strongly influenced by the primary structure of their lipid-A portion as the connecting part of endotoxins to the TLR4 receptor. Unfortunately, the immune responses can be even so highly aggressive and self-destructive, that, in the worst case, the patient can die in septic shock.

The lipid-A moiety is a rather conserved part of endotoxins (compared to the polysaccharide part), however lipid-A structures widely vary among the different bacterial species, and even within a species if the growth conditions have been changed. Moreover, the lipid-A composition of a single cell/a single colony is never homogenous, but it has an extremely heterogeneous molecular pattern comprising several different lipid-A molecules. Lipid-A possesses a unique structure characterized mostly by  $\beta(1',6)$ -linked diglucosamine (di-GlcN) backbone, which can be further modified at two specific sites (C1 and/or C4' positions) by phosphate group(s) and other phosphate-linked substituents (e.g., phosphate,

phosphoetanolamine, aminoarabinose, etc). To this hydrophilic backbone, primary ester- and amide-linked acyl chains ( $\beta$ -OH fatty acids in great majority) are attached, which can be further esterified by secondary fatty acids (or rarely by other substituents). The major determinants that influence the endotoxicity of lipid-A are the number, the type and the distribution of acyl chains between the two glucosamine monomers, as well as the phosphorylation pattern. For example, the most immunologically potent lipid-A is the *Escherichia coli*-type hexa-acylated, C1-/C4'-bis-phosphorylated species, whereon the distribution of acyl chains is asymmetric (the two secondary fatty acids are present on the non-reducing glucosamine residue, Fig. 1).



**Figure 1:** Structure of the *Escherichia coli*-type lipid-A

Thus, the overall analysis of the lipid-A part of the bacterial endotoxins is of high importance to understand the structural background of the effects. Currently, our understanding of the lipid-A structure and function is limited, because mostly heterogeneous bacterial extracts have been used for physiological investigations. However, separation, isolation of pure molecular forms, as well as, the extension of tandem mass spectrometry, especially the collision-induced dissociation rules of different lipid-A structures are paramount for the exact structural characterization and hence for the understanding of the mechanisms of Gram-negative infections at the molecular level.

## 2. AIMS

The overall aim of the work was to develop a new reversed phase-high performance liquid chromatography (RP-HPLC) method combined with electrospray ionization quadrupole time-of-flight tandem mass spectrometry (ESI-QqTOF MS/MS) for the comprehensive analysis of heterogeneous lipid-A extracts and for the accurate determination of the structure of every lipid-A molecule present in such extracts. We aimed to perform the comparative study of three lipid-A samples isolated from different strains of the *Enterobacteriaceae* family by using the developed method. For this, our ideas and challenges were:

1. To develop a universally applicable HPLC-ESI-MS method for the satisfactory separation of the lipid-A components present in very heterogeneous samples and for their efficient detection in both the negative- and positive-ionization modes.
2. To study the collision-induced dissociation (CID) of the differently acylated and/or phosphorylated lipid-A components by negative-ion mode ESI-QqTOF tandem mass spectrometry, with special attention to the immunologically important, however far less studied non-phosphorylated and C1-monophosphorylated compounds.
3. To study the collision-induced dissociation (CID) of the differently acylated and/or phosphorylated lipid-A components by positive-ion mode ESI-QqTOF tandem mass spectrometry, which could provide complementary information for the results obtained by the negative-ion mode measurements.
4. To perform energy-resolved mass spectrometry (ERMS) measurements to confirm and extend our interpretation of the low-energy CID processes of lipid-A precursors both in the negative- and positive-ion mode. Moreover, ERMS measurements may allow an opportunity to differentiate isobaric lipid-A compounds based on the variations in their fragmentation pathways and patterns as a function of collision energy.

### **3. MATERIALS AND METHODS**

#### **3.1. Chemicals and reagents**

Methanol, isopropyl alcohol, water (LC-MS Chromasolv grade), dichloromethane (Chromasolv Plus, for HPLC,  $\geq 99.9\%$ ), triethylamine ( $\text{Et}_3\text{N}$ ) and acetic acid (AcOH) (eluent additive for LC-MS) were purchased from Sigma-Aldrich (Steinheim, Germany).

#### **3.2. Bacterial strains**

*Proteus morganii* O34 (*P. morganii*), *Escherichia coli* O111 (*E. coli*) and *Salmonella adelaide* O35 (*S. adelaide*) strains were cultured at 37°C in a laboratory fermenter on Mueller-Hinton broth at pH 7.2 and then collected by centrifugation. The bacterial LPSs were extracted from acetone-dried organisms by the traditional hot phenol/water procedure and were lyophilized.

#### **3.3. Lipid-A isolation and sample preparation**

Lipid-A was released from each lipopolysaccharide by mild acid hydrolysis with 1% (v/v) acetic acid (pH 3.9) at 100°C for 1 hour, then the solution was centrifuged (8000 $\times$ g, 4°C, 20 min). The sediment (lipid-A) was washed 4 times with distilled water and lyophilized. The sample was dissolved in methanol/dichloromethane (95:5, v/v) at a final concentration of 0.5 mg/mL, and filtered through a 0.22- $\mu\text{m}$  pore size PVDF syringe filter.

#### **3.4. HPLC-ESI-QqTOF MS/MS**

Separation and mass spectrometric detection of lipid-A samples were performed with an Infinity 1290 UHPLC system (Agilent Technologies, Waldbronn, Germany) coupled to a 6530 Accurate Mass QqTOF LC/MS system (Agilent Technologies, Singapore). Chromatographic separations were achieved using a fully porous Hypersil BDS C18 column (100 mm  $\times$  4.6 mm, 3  $\mu\text{m}$  particle size, Thermo Scientific) and a core-shell Kinetex™ C18 column (100 mm  $\times$  4.6 mm, 2.6  $\mu\text{m}$  particle size, Phenomenex), respectively. Mobile phase A consisted of methanol/water (95:5, v/v) with 0.1% (v/v)  $\text{Et}_3\text{N}$  and 0.1% (v/v) AcOH, and mobile phase B consisted of isopropyl alcohol with 0.1% (v/v)  $\text{Et}_3\text{N}$  and 0.1% (v/v) AcOH. Injection volumes of the sample solutions were 3  $\mu\text{L}$ . Separation was performed at a constant flow rate of 0.5 mL/min at 50°C. A linear gradient of 50 min started at 0% mobile

phase B and ramped to 50% mobile phase B before holding at 50% mobile phase B for 2 min. Between the separations, the column was equilibrated for 10 min with 100% mobile phase A. Positive-ion and negative-ion mass spectra of the column eluate were recorded in the range of  $m/z$  100–2500 at a measuring frequency of 10000 transients/s and a detection frequency of 4 GHz. The Agilent Jet Stream ion source was operated using the following conditions: pressure of nebulizing gas ( $N_2$ ) was 30 psi; temperature and flow rate of drying gas ( $N_2$ ) was 325°C and 8 L/min, respectively; temperature and flow rate of sheath gas was 300°C and 11 L/min, respectively. The capillary voltage was set to 4 kV, the nozzle voltage to 2 kV, the fragmentor potential to 100 V, and the skimmer potential to 70 V. For the targeted MS/MS analysis, the following parameters were used: mass range,  $m/z$  20–2500; acquisition rate, 333.3 ms/scan; quadrupole isolation width, 1.3  $m/z$ ; maximum precursor ions/cycle, 4. Tandem mass spectra were recorded at various collision energies between 5 and 100 eV in the positive-ion mode and between 5 and 110 eV in the negative-ion mode.

## 4. RESULTS AND DISCUSSION

### 4.1. Development of HPLC–MS method for analysis of lipid-A preparations

Crude lipid-A preparations obtained from the hydrolyzed LPSs from *P.morganii* O34, *E. coli* O111 and *S. adelaide* O35 bacteria were subjected to reversed-phase HPLC separation with ESI-QqTOF MS detection. The chromatographic conditions were optimized by varying the mobile phase composition, temperature and flow rate. The best separation was obtained using a linear gradient with increasing concentration of isopropyl alcohol (with 0.1% Et<sub>3</sub>N and 0.1% AcOH) in 95:5 methanol/water (with 0.1% Et<sub>3</sub>N and 0.1% AcOH) up to 65%, at an increased temperature of 50°C and a flow rate of 0.5 mL/min for both C18 columns. Using these variables, lipid-A mixtures of the three cross-reacting bacteria could be effectively separated with reasonable retention times on both applied C18 columns.

We found that all lipid-A samples expressed extreme (unexpected extent of) structural heterogeneity, where the number of separated components was 41 in the *E. coli* and *S. adelaide* isolates and 56 in the *P. morganii* sample. Mainly monophosphorylated, and a few diphosphorylated and nonphosphorylated lipid-A species (containing the common di-GlcN backbone) were detected, and some lipid-A partial structures (containing only one GlcN monosaccharide in the backbone) were also verified in the three mixtures. In all samples, numerous isobaric lipid-A species could be observed (even non-separated ones), in which the length and/or position of the acyl chains, as well as the position of phosphoryl groups varied. Generally, the elution order of the separated compounds was consistent with their relative hydrophobicity, defined predominantly by the total number and length of hydrophobic acyl chains, followed by the number of polar phosphate groups. In addition, we described the rules with respect to the relative position of secondary fatty acids that influences the elution order of acylation isomers, as follows: no separation was achieved for the lipid-A isomers differing in the two secondary fatty acids at the C3' and C2' positions, or in which the two primary fatty acids at the C2' or C3 positions were interchanged. On the other hand, the chromatographic behaviour was typical for the lipid-A species having an unsaturated C3' fatty acid (instead of a C3' hydroxy fatty acid) or a single secondary fatty acyl group located at the C3' site (instead of the C2' site), as these regularly appeared at higher retention times compared with their counterparts. These rules proved to be valid for both stationary phases, despite the significant difference between their separation efficiency.

## 4.2. CID study of deprotonated lipid-A species

### 4.2.1. C4'-monophosphorylated precursors

Collision-induced dissociation of several differently acylated (the degree of their acylation varied from tri to hepta) C4'-monophosphorylated lipid-A species was studied and the well-known fragmentation rules of the *E. coli*-type hexa-acylated species have been extended to lipid-A precursors possessing higher or lower degree of acylation. It has been found that the directing effect of the negative charge – which has the same location for all the examined anions with a C4' phosphate group – results in the following consecutive dissociation order: C3' secondary > C3 primary > C3' primary (both as an acid and as a ketene loss) > C2 secondary > C2' secondary acyl chains. The lack of any acyl-chain substituent has no significant effect on the subsequent eliminations. Moderately abundant cross-ring cleavages resulting in  $^{0,4}A_2$ - and  $^{0,2}A_2$ -type diagnostic product ions were also observed, which could thus be used to confirm the described elimination sites of fatty acids. Loss of any primary amide-linked acyl-chain was not detected.

### 4.2.2. C1-monophosphorylated precursors

The negative-ion mode low-energy CID fragmentation processes of the C1-monophosphorylated lipid-A species were also studied, in order to explore and compare their fragmentation pathways with that of the well-known C4'-monophosphoryl isomers. In contrary to the hepta-acylated C4'-phosphorylated compound, we observed a more complex fragmentation pattern for its C1-phosphorylated isomer. The competition between the elimination processes of the ester-linked fatty acyl chains was much higher for the C1-, than for the C4'-phosphorylated isomer. Based on the relative intensity of the product ions resulting from the secondary fatty acid losses for the C1-phosphorylated isomer, the preference of eliminations was deduced: C2' secondary > C3' secondary > C2 secondary side chains. There was a lack of any diagnostic intra-ring product ions, as well as of the acid/ketene loss-pairs as the diagnostic elimination products of the C3' primary acyl-chain.

### 4.2.3. Non-phosphorylated precursors

We have reported the structural characterization of non-phosphorylated lipid-A species as deprotonated  $[M - H]^-$  ions by negative-ion mode MS/MS. The position of the deprotonation site on a non-phosphorylated lipid-A precursor or on its different product ions



was not obvious, because they did not possess any functional group of clearly noticeable acidic character. Evidently, the position of the negative charge had to be different from that of the phosphorylated lipid-A anions, because of the lack of phosphate groups.

Charge-driven stepwise dissociation processes occurring under low-energy CID conditions could be assumed for the deprotonated, non-phosphorylated lipid-A ions,  $[M - H]^-$ , similarly as for the extensively studied deprotonated, C4'-monophosphorylated lipid-A ions  $[M_p - H]^-$ . In both cases, intermediate ion-molecule complexes (by internal proton transfer) could be involved in the stepwise fragmentations, which were rationalized by the serial release of the ester-linked acyl chains, however, (because of the different location of the charge) the order of the losses was different for each lipid-A class. The observed elimination order of fatty acids from the deprotonated non-phosphorylated lipid-A species was as follows: C3 primary > C3' secondary > C2 secondary > C2' secondary > C3' primary acyl chains. In contrary to C4'-monophosphorylated precursors, no significant C3'-diagnostic acid/ketene loss-pairs were observed and there was a lack of  $^{0,2}A_2$ -type intra-ring product ions, however dominant  $^{0,4}A_2$ -type and its complementary  $^{0,4}X_0$ -type diagnostic cross-ring fragments, as well as intensive carboxamide ions (diagnostic for the C2 position) were detected.

### 4.3. CID study of triethylammonium adduct lipid-A species

We found that both, phosphorylated and nonphosphorylated lipid-A molecules can be readily ionized in the positive-ion mode by adduct formation with triethylamine added to the eluent. Comparison of the positive-ion tandem mass spectra of the bisphosphorylated, monophosphorylated (either at C1 or C4'), and nonphosphorylated lipid-A triethylammonium adducts revealed similar type of fragment ions, indicating that the fragmentation pathways of the differently phosphorylated lipid-A compounds were very similar. This similarity could be attributed to the same location of the positive charge (i.e., the protonation site) that was assumed to be one of the two amide groups, both with similar probabilities within a protonated lipid-A molecule. Thus, contrary to the low-energy CID analysis of deprotonated lipid A  $[M - H]^-$  in the negative-ion mode, the position of the phosphoryl group(s) in terms of the charge-driven fragmentation processes was not relevant in the positive-ion mode. To be specific, the first step in the low-energy CID fragmentation of every  $[M + H + Et_3N]^+$  precursors was the competitive formation of  $[Et_3N + H]^+$  and  $[M + H]^+$ .

The relative abundances of these two primary fragment ions depended on two factors: (1) the differences in their gas phase basicity and (2) their lability under CID conditions. This latter resulted that the  $[M + H]^+$  ion could hardly be detected in most tandem mass spectra due to its rapid decomposition by further fragmentation, whereas the protonated triethylamine gave the base peak in all tandem mass spectra.

The fragmentation profiles – in contrary to the negative-ion mode profiles – could be used to reveal the presence of phosphorylation isomers of monophosphorylated lipid A species and gave sufficient information to assign the phosphorylation sites (i.e., at C1 or C4'). The tandem mass spectra of the lipid-A triethylammonium adduct ions showed several product ions corresponding to interring glycosidic cleavages of the sugar residues, as well as consecutive and competitive eliminations of fatty acids, phosphoric acid, and water following the neutral loss of triethylamine. Although, the fragmentation pathways of the differently phosphorylated (and non-phosphorylated) lipid-A species significantly differed in the negative-ion mode, they were very similar in the positive-ion mode. The complementary use of positive-ion and negative-ion mode tandem mass spectrometry was found to be essential for the full structural characterization of the C1-monophosphorylated lipid A species.

#### **4.4. Energy-resolved mass spectrometry study of lipid-A precursors in the negative- and positive-ion modes.**

The breakdown curves were determined for acylation and/or phosphorylation isomer precursors as well as product ions detected in both ionization modes. These plots show the relative intensities of the ions versus the applied collision energy. We observed that the breakdown curves of acylation isomer precursors – in which the secondary fatty acids at the C3' and C2' positions were interchanged – overlapped in both ionization modes, respectively. We found the similar coincidence of the breakdown curves of the corresponding product ions of the same precursors, thus we conclude on that such structural difference has no effect on the pathway of their CID fragmentation. It means that the comparison of the fragmentation of such isomers could be performed in any collision energy, and still the MS/MS spectra will be comparable.

The inflexion point of the sigmoidal breakdown curves of the C1 and C4' phosphorylation isomer precursors was found to be shifted by 25 eV from each other,

consequently the collision energy needed to obtain 50% fragmentation of each precursors is different. Thus, it is advisable to perform the CID fragmentation measurements of such compounds at adjusted collision energies to get the same dissociation ratio of the precursors, thereby making them comparable.

## 5. THESIS POINTS

1. We developed a new HPLC-ESI-QqTOF MS/MS method for the efficient separation, ionization – in both, negative- and positive-ionization modes – and detection of a wide variety of lipid-A molecules obtained by mild acid hydrolysis of bacterial lipopolysaccharides. Two partially different chromatographic columns containing reversed-phase stationary phases were compared for the separation performance of the differently acylated and phosphorylated lipid-A isomer molecules. We determined the structural properties of lipid-A molecules (isomers) having influence on the retention order.
2. Using the developed method, we screened and compared the lipid-A isolates from three enterobacteria (*P. morganii* O34, *E. coli* O111 and *S. adelaide* O35) showing serological cross-reactivity. We showed that the lipid-A composition of the *E. coli* and *S. adelaide* is very similar (both quantitatively and qualitatively), however the lipid-A pattern of the *P. morganii* isolate is significantly different. Furthermore, we detected some lipid-A compounds that have never been identified, despite extensive study of the *E. coli* O111 sample.
3. The elimination order of the ester-linked acyl chains of deprotonated, tri-, tetra-, penta-, hexa- and hepta-acylated, C4'-monophosphorylated lipid-A precursors has been determined by low-energy collision-induced dissociation fragmentation in a QqTOF MS instrument.
4. The fragmentation processes – including the elimination order of the ester-linked acyl chains and the dissociation of the di-GlcN backbone – of deprotonated, penta- to hepta-acylated, non-phosphorylated lipid-A precursors has been determined by low-energy collision-induced dissociation fragmentation in a QqTOF MS instrument.
5. The fragmentation processes of positively charged triethylammonium adduct lipid-A species with different degree of phosphorylation (C1-mono-, C4'-mono-, C1/C4'-bis- and non-phosphorylated) and with different acylation patterns have been determined. Our investigations pointed at the uniqueness of the method in the simplicity and reliability for proving the phosphorylation isomerism (C1 and C4') of lipid-A species in their

triethylammonium adduct form, even in case of the lack of their chromatographic separation.

6. Energy-dependent investigations of different lipid-A precursors in the negative- and positive-ionization modes have been used to prove our statements about the fragmentation processes, which we determined for the acylation and phosphorylation isomers. We determined the CID energy ranges for the reliable and effective structural analysis of chromatographically separated lipid-A precursors (taking into consideration that each is detected only for a short period of time), which energies may even be used with instruments that utilizes high-speed polarity switching between positive and negative ionization modes.

## 6. LIST OF PUBLICATIONS

### Publications related to the PhD Thesis:

**Sándor Viktor**, Kilár Anikó, Kilár Ferenc, Kocsis Béla, Dörnyei Ágnes  
Characterization of complex, heterogeneous lipid A samples using HPLC-MS/MS technique III. Positive-ion mode tandem mass spectrometry to reveal phosphorylation and acylation patterns of lipid A.  
**JOURNAL OF MASS SPECTROMETRY** 53: pp. 146-161. (2018) **IF.: 2.112**

Kilár Anikó, Dörnyei Ágnes, **Sándor Viktor**, Kilár Ferenc, Kocsis Béla  
Phosphoglycolipid profiling of bacterial endotoxins  
**HUNGARIAN JOURNAL OF INDUSTRY AND CHEMISTRY** 46: pp. 7-11. (2018)

**Sándor Viktor**, Kilár Anikó, Kilár Ferenc, Kocsis Béla, Dörnyei Ágnes  
Characterization of complex, heterogeneous lipid A samples using HPLC-MS/MS technique II. Structural elucidation of non-phosphorylated lipid A by negative-ion mode tandem mass spectrometry  
**JOURNAL OF MASS SPECTROMETRY** 51: pp. 615-628. (2016) **IF.: 2.422**

**Sándor Viktor**, Dörnyei Ágnes, Makszin Lilla, Kilár Ferenc, Péterfi Zoltán, Kocsis Béla, Kilár Anikó  
Characterization of complex, heterogeneous lipid A samples using HPLC-MS/MS technique I. Overall analysis with respect to acylation, phosphorylation and isobaric distribution.  
**JOURNAL OF MASS SPECTROMETRY** 51: pp. 1043-1063. (2016) **IF.: 2.422**

### Poster presentations related to the PhD Thesis:

**Viktor Sándor**, Ágnes Dörnyei, Anikó Kilár, Béla Kocsis, Ferenc Kilár  
Energy-resolved mass spectrometry of lipid-A structures in negative-ionization mode  
*36<sup>th</sup> Informal Meeting on Mass Spectrometry, Kőszeg, 2018. 15.6-2018.05.09.*

Anikó Kilár, **Viktor Sándor**, Ágnes Dörnyei, Béla Kocsis, Ferenc Kilár  
Comparison of LC-MS and CE-MS for bacterial lipid-A samples  
*36<sup>th</sup> Informal Meeting on Mass Spectrometry, Kőszeg, 2018. 15.6-2018.05.09.*

Ágnes Dörnyei, **Viktor Sándor**, Anikó Kilár, Nikolett Erős, Béla Kocsis, Ferenc Kilár  
Structure elucidation of lipid-A by energy-resolved tandem mass spectrometry in positive-ionization mode  
*36<sup>th</sup> Informal Meeting on Mass Spectrometry, Kőszeg, 2018. 15.6-2018.05.09.*

Anikó Kilár, Ágnes Dörnyei, **Viktor Sándor**, Szandra Schwarcz, Ferenc Kilár, Béla Kocsis  
Direct detection of microbial membrane lipooligosaccharides by MALDI-TOF MS  
*29<sup>th</sup> MassSpec Forum Vienna, Bécs, Ausztria, 2018.02.21-2018.02.22.*

Ágnes Dörnyei, **Viktor Sándor**, Anikó Kilár, Laura Deutsch-Nagy, Ferenc Kilár, Béla Kocsis  
Energy-resolved mass spectrometry of lipid-A structures in positive-ionization mode  
*29<sup>th</sup> MassSpec Forum Vienna, Bécs, Ausztria, 2018.02.21-2018.02.22.*

**Sándor Viktor**, Dörnyei Ágnes, Kilár Anikó, Kilár Ferenc, Kocsis Béla: A Novel Structure Elucidation Strategy of Non- Phosphorylated Lipid A Variants Applying HPLC-MS/MS  
*11<sup>th</sup> Balaton Symposium on High-Performance Separation Methods, Siófok, 2017.09.06-2017.09.08.*

Dörnyei Ágnes, **Sándor Viktor**, Kilár Anikó, Kilár Ferenc, Kocsis Béla: A Novel Structure Elucidation Strategy of Monophosphorylated Lipid A Variants Applying HPLC-MS/MS  
*11<sup>th</sup> Balaton Symposium on High-Performance Separation Methods, Siófok, 2017.09.06-2017.09.08.*

**Sándor Viktor**, Kilár Ferenc, Kocsis Béla, Kilár Anikó, Dörnyei Ágnes: Endotoxinok foszfolipid régiójának vizsgálata HPLC-ESI-MS/MS technikával negatív- és pozitív-ionizációs módban  
*Elválasztástudományi Vándorgyűlés, Kecskemét, 2016.11.08-2014.11.11.*

Dörnyei Ágnes, Kilár Anikó, **Sándor Viktor**, Kocsis Béla, Kilár Ferenc: Tömegspektrometriás stratégiák bakteriális endotoxinok szerkezetmeghatározására  
*Elválasztástudományi Vándorgyűlés, Egerszalók, 2014.10.12-2014.10.14.*

**Sándor Viktor**, Kilár Ferenc, Kocsis Béla, Kilár Anikó, Dörnyei Ágnes: Keresztkapcsolatú baktériumokból származó endotoxinok foszfoglikolipid mintázata  
*Elválasztástudományi Vándorgyűlés, Egerszalók, 2014.10.12-2014.10.14.*

**Sándor Viktor**, Kilár Ferenc, Kocsis Béla, Dörnyei Ágnes, Kilár Anikó: Novel CZE-MS and LC-MS methods for the analysis of the lipid-A part of bacterial endotoxins  
*9<sup>th</sup> Balaton Symposium on High-Performance Separation Methods, Siófok, 2013.09.04.-2013.09.06.*

Dörnyei Ágnes, Kilár Anikó, **Sándor Viktor**, Kocsis Béla, Kilár Ferenc: Bakteriális endotoxinok glikolipid régiójának tömegspektrometriás jellemzése  
*Elválasztástudományi Vándorgyűlés, Tapolca, 2010.11.10-2010.11.12.*

### Oral presentations related to the PhD Thesis:

Anikó Kilár, Ágnes Dörnyei, **Viktor Sándor**, Ferenc Kilár, Béla Kocsis: Phosphoglycolipid profiling of bacterial endotoxins  
*CECE 2017, the 14th International Interdisciplinary Meeting on Bioanalysis, Veszprém, Magyarország, 2017.10.08-2017.10.11.*

Ágnes Dörnyei, **Viktor Sándor**, Anikó Kilár, Béla Kocsis, Ferenc Kilár: A Novel Structure Elucidation Strategy of Bacterial Lipid-A Applying HPLC-MS/MS for Future Vaccine Adjuvant Bioanalysis  
*33<sup>rd</sup> International Symposium on Microscale Separations and Bioanalysis, Noordwijkerhout, Hollandia, 2017.03.26-2017.03.29.*

Anikó Kilár, Ágnes Dörnyei, **Viktor Sándor**, Béla Kocsis, Ferenc Kilár  
Novel Structure Elucidation Strategy of Bacterial Endotoxins Applying HPLC-MS/MS  
*28<sup>th</sup> MassSpec-Forum, Bécs, Ausztria, 2017.02.16.-2017.02.17.*

Kilár Anikó, **Sándor Viktor**, Dörnyei Ágnes, Kilár Ferenc, Kocsis Béla: Endotoxikus foszfoglikolipidek szerkezetmeghatározása.  
*MKE Tömegspektrometriai Társaság Szakmai Napja, Budapest, Magyarország, 2016.12.01.*

Ferenc Kilár, Lilla Makszin, Anikó Kilár, Ágnes Dörnyei, **Viktor Sándor**, Béla Kocsis  
Challenges and solutions in the structure elucidation of lipopolysaccharides  
*Nordic Separation Science Society 7<sup>th</sup> Conference, Stockholm, Svédország, 2014.06.09-2014.06.12.*

**Viktor Sándor**, Anikó Kilár, Ágnes Dörnyei, Béla Kocsis, Ferenc Kilár  
Challenges and solutions in the structure elucidation of lipopolysaccharides with CE-MS and UHPLC-MS  
*21<sup>st</sup> international Symposium on Electro and Liquid Phase- separation Techniques, Natal, Brazília, 2014.10.04-2014.10.08.*

### Publications related to other research topics:

Ivanova-Petropulos V, Naceva Z, **Sándor V**, Makszin L, Nagy L, Berkics B, Stafilov T, Kilár F  
Fast determination of lactic, succinic, malic, tartaric, shikimic and citric acids in red Vranec wines by CZE-ESI-QTOF-MS.  
**ELECTROPHORESIS** 39:(13) pp. 1597-1605. (2018) **IF.: 2.569**

Csilla Páger, Nikoleta Biherczová, Roland Ligetvári, Balázs Berkics, Tamás Pongrácz, **Viktor Sándor**, Anita Bufa, Viktória Poór, Andrea Vojs Staňová, Ferenc Kilár  
Advanced on-line mass spectrometry detection of proteins separated by capillary isoelectric focusing after sequential injection  
**JOURNAL OF SEPARATION SCIENCE** 40:(24) pp. 4825-4834 (2017) **IF.: 2.415**

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### **Poster and oral presentations related to other research topics:**

Anikó Kilár, Szandra Péter, **Viktor Sándor**, Ágnes Dörnyei, Ferenc Kilár, Béla Kocsis  
Characterization of Bacterial Endotoxins Directly from a Few Bacterial Colonies by MALDI-TOF MS  
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Deli József, Papp Nóra, Varga Erzsébet, Tatár Tímea, **Sándor Viktor**, Nagy Laura, Kilár Ferenc  
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Fenyvesiné Páger Csilla, Biherczova Nikoleta, Ligetvári Roland, Berkics Balázs Viktor, **Sándor Viktor**, Kilár Ferenc  
Kapilláris izoelektromos fókuszálás alkalmazása tömegspektrometriás detektálással fehérjekeverék elválasztására  
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Novel Results with Capillary Electrophoresis Coupled to Mass Spectrometry

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Violeta Ivanova - Petropulos, Zaneta Naceva, **Viktor Sándor**, Balázs Berkics, Trajce Stafilov, Ferenc Kilár

Determination of Organic Acids in Wines Using Capillary Zone Electrophoresis - Electropray Ionization / Quadrupole - Time - of - Flight - Mass Spectrometry (CZE - ESI/QTOF - MS)

16<sup>th</sup> CEEPUS Symposium and Summer School on Bioanalysis, Varsó, Lengyelország, 2016.07.06-2016.07.15.

Anikó Kilár, Ágnes Dörnyei, **Viktor Sándor**, Ferenc Kilár, Béla Kocsis

Mass spectrometry of endotoxins from whole-cell lysates

32<sup>nd</sup> Informal Meeting on Mass Spectrometry, Balatonszárszó, 2014.05.11-2014.05.14.

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Csilla Páger, Ágnes Dörnyei, Viktória Poór, **Viktor Sándor**, Ferenc Kilár

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21<sup>st</sup> international Symposium on Electro and Liquid Phase- separation Techniques, Natal, Brazília, 2014.10.04-2014.10.08.

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Novel LC-MS/MS method for the analysis of intact R-type bacterial endotoxins

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**Viktor Sándor**, Ferenc Kilár, Béla Kocsis, Anikó Kilár, Ágnes Dörnyei

Novel LC-MS/MS method for the analysis of intact R-type bacterial endotoxin

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Analysis of Cavitand Derivatives with High Performance Liquid Chromatography – Mass Spectrometry

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