# Development of Mass Spectroscopy assay for Nε-(1-Carboxymethyl)-L-Lysine, Nε-(1-Carboxyethyl)-L-lysine in human plasma

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## Introduction

Advanced glycation end-products (AGEs) are complex compounds formed during nonenzymatic reactions of reducing sugars or lipid oxidation products with amino groups of proteins by so called Maillard reaction [1]. AGEs are associated with various pathological states, i.e. atherosclerosis, diabetes neurodegeneration, inflammation, tumors, age-related pathologies and septic shock[2]. Nε-(Carboxymethyl) lysine (CML), Nε-(carboxyethyl) lysine (CEL) are typical AGEs, which have been associated with sepsis and sepsis shock. AGEs are mediated as signaling compounds by receptor for advanced glycation end-products (RAGE) considered as a crucial component innate immune response in sepsis. Many analytical methods have been proposed for analysis of CML, CEL. They are based either on chromatographic techniques including gas chromatography-mass spectrometry (GC-MS)[1,3], liquid chromatography-mass spectrometry (LC-MS)[4–6] and liquid chromatography coupled with fluorescence detector (HPLC-FLD) or analysis using enzyme immunoassays (ELISA)[7,8]. ELISA techniques are associated with limitations such as lack of specificity, risk of matrix interference[7,8] . Methods which overcomes this limitations are those utilizing LC-MS technique. Due to high polarity of CML and CEL this compounds are difficult to retain on reversed phase columns. Wherefore many methods utilize chromatographic separation on HILIC columns [4,9], for reversed phase chromatography commonly nonafluoropentanoic acid (NFPA)[5,6] has been used as an ion-pairing agent or derivatization with 9-fluorenylmethyl chloroformate has been employed [2]. Here, we present LC-MS method of for the simultaneous analysis of CML and CEL in human plasma with propyl chloroformate as derivatization reagent.

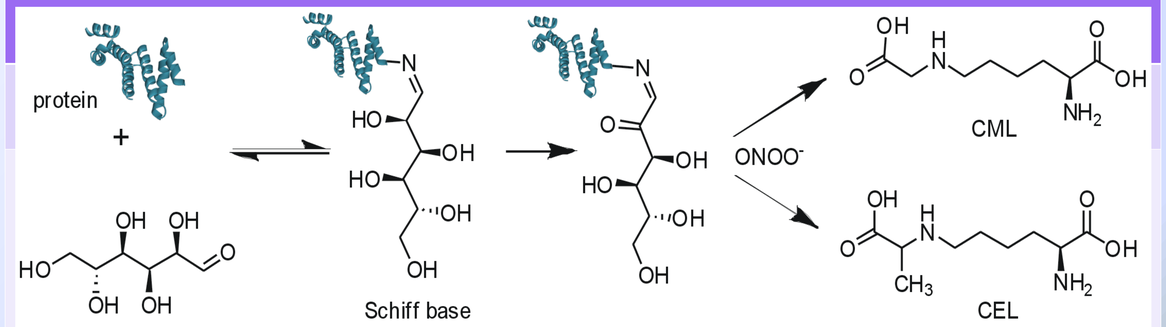


Figure 1. Formation of Nε-(Carboxymethyl) lysine (CML) and Nε-(carboxyethyl) lysine (CEL) as Maillard reaction products

## Aim

The aim of of this studies was to develop a method of the simultaneous extraction and derivatization of circulating CML and CEL from human plasma and application of the developed method to assess the concentrations of these metabolites in sepsis in relation to its severity.

## Materials and methods

For the analysis of association of plasma glycation levels with severity in sepsis we have used plasma of 10 patients with sepsis and 10 healthy controls. Healthy controls consisted of blood donors, whose blood samples were kindly provided by the Regional Center of Blood Donation and Therapeutics in Wroclaw, Poland. Mass spectrometric based method described here uses a process of simultaneous extraction and derivatization of the CML and CEL using the propyl chloroformate as derivatization reagent. Chromatographic separation was achieved using a nanoAcquity UPLC system. The MS/MS measurements were carried out using a Xevo G2 Q-TOF mass spectrometer in the positive ion mode. For the qualitative and quantitative analysis MassLynx and QuanLynx software from Waters were used.

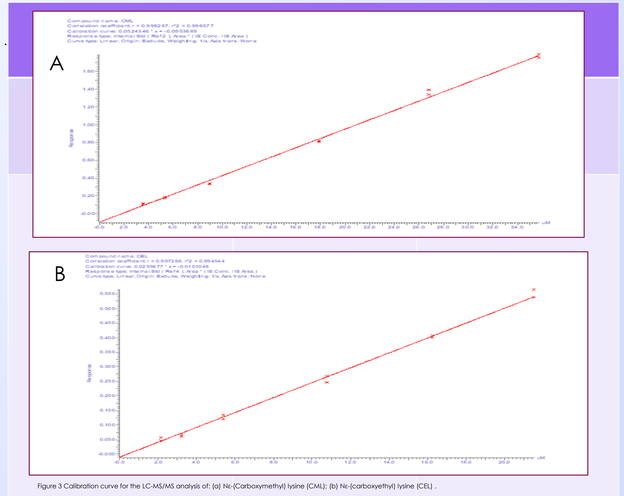


Figure 3 Calibration curve for the LC-MS/MS analysis of: (a) Nε-(Carboxymethyl) lysine (CML); (b) Nε-(carboxyethyl) lysine (CEL) .

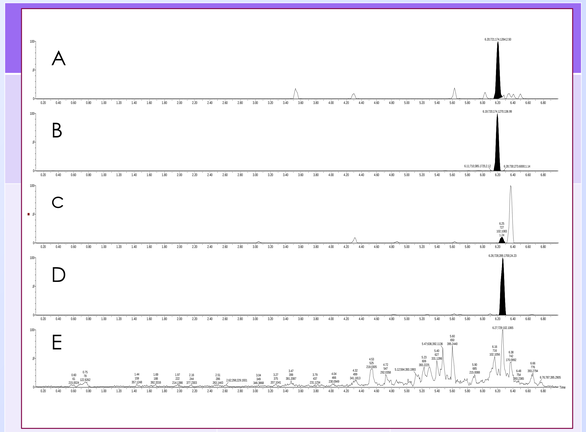


Figure 2. Ion chromatograms of (a) Nε-(Carboxymethyl) lysine (CML); (b)D4- Nε-(Carboxymethyl) lysine (D4-CML); (c) Nε-(carboxyethyl) lysine (CEL); (d) D4-Nε-(carboxyethyl) lysine (D4-CEL); € total ion current of patients plasma extract:

## Results and Discussion

The purpose of this studies was to develop a method of the simultaneous extraction and derivatization of CML and CEL from biological samples. Achieving this method allowed us to obtain a large range of linearity, high stability of extracted substances from the samples and the appropriate level of limits of detection (LOD) and quantification (LOQ). The use of high resolution Q-TOF mass spectrometer coupled with UPLC system provides accurate and comprehensive qualitative data necessary for the proper results analysis. Significantly lower concentrations of CEL in sepsis and significantly higher concentrations of CML have been demonstrated

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