The Use of Capillary Electrophoresis Coupled to Mass Spectrometry (CESI-MS) for Quantitation of Nucleotides and Nucleosides

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OVERVIEW

OBJECTIVE

Develop a simple methodology for separation and quantitation of nucleotides using CESI-MS.

METHODS

- Standards are dissolved in water
- Background electrolyte is a 12.5 mM ammonium acetate adjusted to pH 9.7
- Separation voltage is 30 kV assisted with 5 psi forward pressure
- Data acquisition was done using CESI 8000 interfaced to SCIEX TripleTOF® 5600 system. The quantitative data analysis was performed using MultiQuant™ software (SCIEX, Framingham, MA).

RESULTS

- A method, that does not required derivatization, for separation of some nucleotides was developed for CESI with a short time of analysis (< 10 min), and minimum required sample volume.

INTRODUCTION

- Nucleotides and nucleosides are charged particles that are vital compounds in the biological processes. These polar compounds are usually difficult to analyze by traditional chromatography techniques, needing an extra derivatization step.
- Capillary Electrophoresis (CE) is a separation method that is ideal for highly charged and polar molecules, which can be notoriously challenging for liquid or gas chromatography. In addition, CE offers advantages of nano- and picoliter sample-volume, high separation efficiency, and fast injection-to-injection times.
- CESI is a technology that integrates capillary electrophoresis (CE) and electrospray ionization (ESI) into a single process in a single device (see Figure 1). With CESI, a stable ESI spray is generated at flow rates in the range of 5-30 nL/min. Operating at these ultra-low flow rates, it helps reduce ion suppression and increases assay sensitivity for optimal results.
- In this study, we present a workflow using CESI-MS for the quantitative analysis of selected nucleotides using minimal sample volume, demonstrating baseline separation, reproducibility and detection at nanoMolar levels.

MATERIALS AND METHODS

Chemicals:

All chemicals were Reagent Grade from Sigma-Aldrich. The method was set up for detection of 7 nucleotides.

Name	Formula	$[M+H]^+$
Cytidine 5'-triphosphate	$C_9H_{16}N_3O_{14}P_3$	483.99
Cytidine 5'-diphosphate	$C_9H_{15}N_3O_{11}P_2$	404.03
Cytidine monophosphate	$C_9H_{14}N_3O_8P_1$	324.06
Adenosine triphosphate	$C_{10}H_{16}N_5O_{13}P_3$	508.00
Guanosine triphosphate	$C_{10}H_{16}N_5O_{14}P_3$	524.00
Uridine triphosphate	$C_9H_{15}N_2O_{15}P_3$	484.98
Thymidine triphosphate	$C_{10}H_{17}N_2O_{14}P_3$	483.00

CE Conditions:

CESI-MS Silica Surface Cartridge:	30 µm ID x 90 cm total length
Background Electrolyte (BGE):	Ammonium acetate (12.5 mM, pH = 9.7)
Separation Voltage:	30 kV, assisted with 5 psi of forward pressure

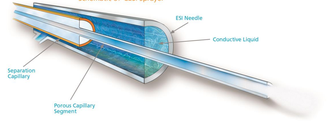

Figure 1. CESI Sprayer Interface

MS Conditions:

CESI 8000 (sold through SCIEX Separations) was coupled to a TripleTOF® 5600+ mass spectrometer (SCIEX) operating in positive ion mode with Analyst 1.7™ software

Mass Spectrometer:	SCIEX TripleTOF® 5600+
ToF acquisition mode:	200-600 *m/z* values
Ionization mode:	Positive, $[M+H]^+$

Data Analysis

Analysis of the mass spectra were carried out using Peak View® software (SCIEX, Framingham, MA). The quantitative data analysis was performed using MultiQuant™ software (SCIEX, Framingham, MA).

RESULTS

Establishment of experimental conditions: Background Buffer Electrolyte, pH, voltage and pressure

Several Background Electrolytes (BGE) are recommended in the literature[1], ammonium acetate was selected because it was demonstrated its use in positive ionization mode. Several experimental conditions were tested to achieve the best separation using Adenosine triphosphate (ATP) as model analyte. Figure 2 shows the Extracted Ion Electropherogram (XIE) for ATP at the final experimental conditions.

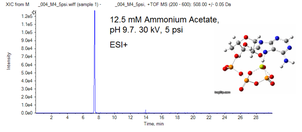

Figure 2. Extracted Ion Electropherogram (XIE) of ATP.

To evaluate analytical parameters as peak shape and migration times, samples were injected individually (see Figure 3) , then a standard mixture was also injected to check
Separation performance (see Figure 4). All compounds showed migration times below 10 min, high reproducibility and good peak shapes.

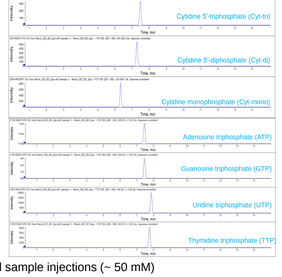

Figure 3. XIE of individual sample injections (~ 50 mM)

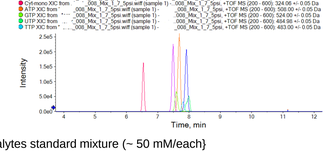

Figure 4. XIE of analytes standard mixture (~ 50 mM/each}

RESULTS (continued)

Quantitative Analysis

Linearity and limits of detection (LODs) were evaluated using analyte sample mixtures from 50 nM to 50 mM for each compound. Calibration curves and integration parameters were adjusted using MultiQuant™ software (SCIEX, Framingham, MA). The analytical method exhibits a quantitative linear response (r > 0.99) and LODs between 100 nM and 200 nM for all analyzed compounds. (see Figure 5).

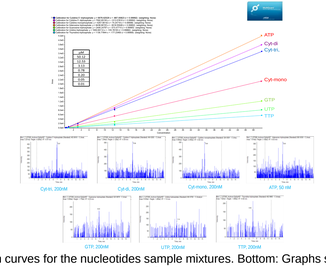

Figure 5. Top: Calibration curves for the nucleotides sample mixtures. Bottom: Graphs show the lowest concentration where the analytes can be distinguished from noise.

CONCLUSIONS

- A method for separation of some nucleotides was developed for CESI with a short analysis time (< 10 min) and minimum required sample volume. In addition it does not require derivatization for the analytes.
- Separation of challenging charged molecules: cytidine phosphates, ATP, GTP, UTP and, TTP has been demonstrated using the CESI-MS system.
- CESI could be applied for quantitative analysis with low detection limits (~ 200 nM) for the analyzed compounds.
- Application of this CESI-method is promising for metabolomics in biological areas (e.g. neurosciences, pharmaceutical industry) where almost all the samples are obtained at low volume and concentration.

REFERENCES

[1]Liu, Charles C., et al *Electrophoresis* 26.7-8 (2005): 1424-1431
ATP Image from: http://en.wikipedia.org/wiki/Adenosine_triphosphate

TRADEMARKS/LICENSING