Determination of ATP, ADP, AMP, and Adenosine Levels by Microchip Capillary Electrophoresis Coupled with High Resolution Mass Spectrometry

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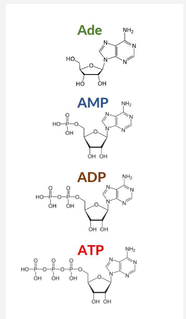
Highlights

- Rapid and robust method for analysis of adenosine, AMP, ADP and ATP
- No ion-pairing reagents necessary
- Method is tolerant towards complex biological matrices such as human plasma
- Accurate quantitation over 4-5 orders of magnitude dynamic range

Introduction

Mononucleotides such as adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), and adenosine 5'-monophosphate (AMP) play a vital role in controlling intra and extracellular energy metabolism. ATP is readily hydrolyzed to produce ADP through the enzymatic removal of one phosphate group, releasing energy. Further hydrolysis to AMP, after the removal of the second phosphate group, releases additional energy. RPLC/MS analysis of adenosine nucleotides is typically challenging, exhibiting poor sensitivity and susceptibility to the formation of adduct ions with ion-pairing agents.

In this study, we highlight a novel method for the analysis of these adenosine nucleotides using Microchip CE MS. The method is simple and fast, with excellent resolution of mononucleotides, high sensitivity, and free from ion pairing reagents. Adenosine (Ade), AMP, ADP, and ATP were spiked into human plasma extract at various concentrations and analyzed using the PATsmart™ ZipChip® System.



Materials & Methods

Materials: Adenosine (Ade), adenosine 5'-monophosphate monohydrate (AMP), adenosine 5'-diphosphate (ADP), and adenosine 5'-triphosphate disodium salt hydrate (ATP) were purchased from Millipore Sigma. Normal human plasma (3.2% NaCit gender pooled 0.2 µm filtered) was purchased from BioIVT.

Sample Preparation: Standards Stock solutions of Ade, AMP, ADP, and ATP were prepared by dissolving the standards in the Oligos Kit diluent (Repligen) to obtain individual solutions with a concentration of 500 000 pg/µL. Mixtures of standards were then prepared at the desired concentrations by further diluting the stock solutions using the Oligos diluent.

Plasma: Human plasma and LCMS grade IPA were cooled to 4°C and mixed in a 1:3 ratio to precipitate plasma proteins. The resulting precipitated solution was stored at 4°C for 1 hour and then centrifuged using Corning Co-star centrifuge filters (0.2 µm) for 5 minutes. To prepare working solutions spiked with a mixture of standards, 50 µL of plasma extract was added to 200 µL of standard mixtures, to obtain resulting spiked solutions ranging from 0.8 to 16,000 pg/uL.

Instrumentation: The ZipChip® Device was used for all analyses (Repligen).[1] The High Resolution Bare (HRB) Glass Chip and the Oligonucleotides BGE, both part of the Oligonucleotides kit (Repligen) were used. An on-chip injection volume was 1 nL with separation run at a field strength of 500 V/cm. MS analysis was performed on a Thermo Fisher QExactive HF mass spectrometer. MS data was acquired using the PRM mode. Data was visualized in QualBrowser (Thermo Fisher Scientific) and processed in Skyline Daily (22.2.1.488)

Mechanism of separation on BG chips: The HRB chips differ from standard polymer coated chips by having channels with charged bare silica surfaces and can be used for analysis of negatively charged species such as oligonucleotides and related molecules.[2] When a voltage potential is applied, the BGE moves unidirectionally due to electroosmotic flow (causing all analytes to migrate towards the ESI sprayer Positively charged species migrate first, followed by neutrals and negatively charged species, enabling electrophoretic separation of Ade, AMP, ADP and ATP based on varying charges.

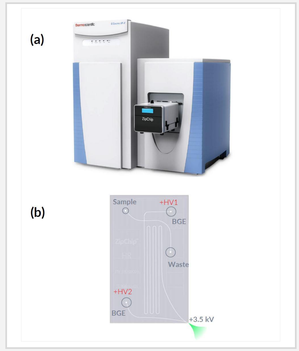


Figure 1. (a) ZipChip Interface coupled to Thermo QExactive HF mass spectrometer (b) Chip schematic

Separation of Ade, AMP, ADP, and ATP Standards on the ZipChip

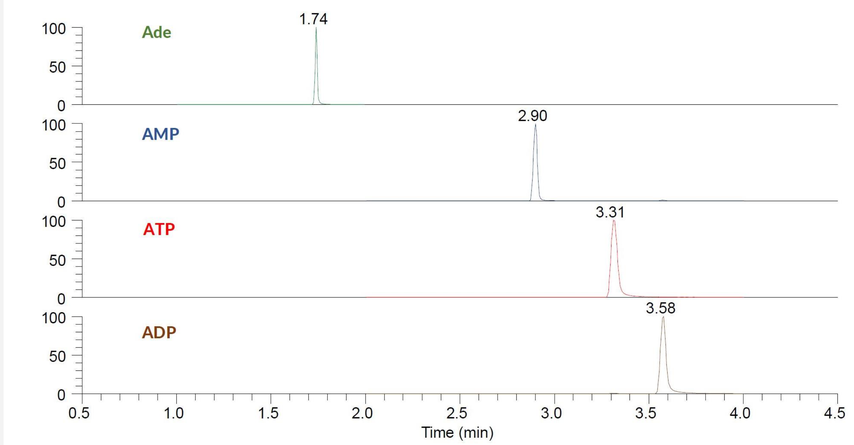
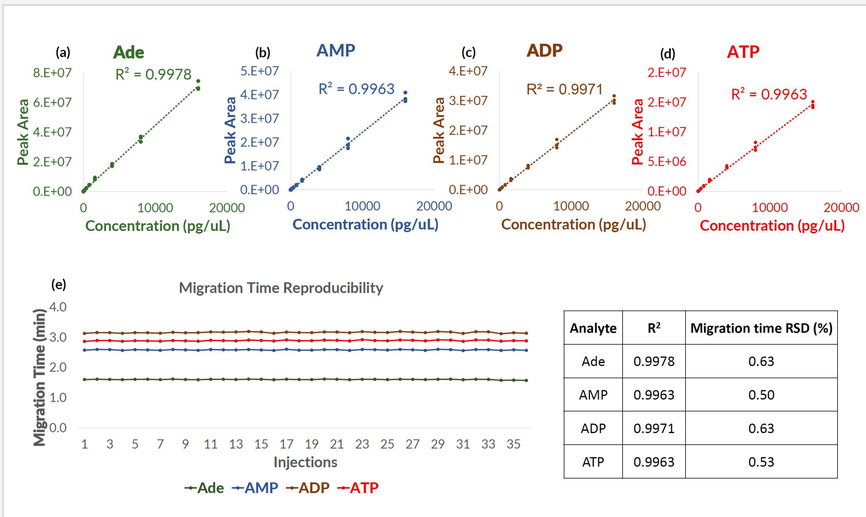


Figure 2. Extracted ion electropherograms for a standards of Ade, AMP, ATP, and ADP at 500 pg/uL showing baseline resolution of all analytes

Calibration Curves for Analytes Spiked in Human Plasma



Analyte	R^2	Migration time RSD (%)
Ade	0.9978	0.63
AMP	0.9963	0.50
ADP	0.9971	0.63
ATP	0.9963	0.53

Figure 3. Calibration curves for standard Ade, AMP, ATP, and ADP

Discussion

In this study, we present a novel ZipChip coupled with a QEHF for adenosine nucleotide analysis. To test the method's separation efficiency, a mixture of the four analytes was injected onto the ZipChip at a concentration of 500 pg/uL. A PRM method with optimized collision energies for each analyte was used. Preliminary data demonstrated narrow and symmetrical electrophoretic peaks The baseline peak width for adenosine was 2.4 seconds, while for AMP, it was 3.6 seconds.

One significant advantage of ZipChip MS analysis is its short analysis time. All four analytes, adenosine, AMP, ATP, and ADP, migrated within just 4 minutes. This allows for parameter optimization through multiple injections within a short timeframe. Interestingly, the migration order was Ade, AMP, ATP, and ADP. It is hypothesized that despite having three phosphate groups, ATP migrates faster than ADP, possibly due to ATP standard being available as a disodium salt.

Furthermore, no adduct formation was observed in the data obtained from CE-MS in positive ESI mode. The mass spectra showed significant improvement compared to IPRP LCMS due to the absence of adduct ions. Only M+H species were observed for all four analytes, indicating higher sensitivity compared to IPRP LC/MS. Detection limits of up to 1 ng/mL were achieved using a mSIM method.

To assess the effect of the sample matrix on separation performance, human plasma was chosen Mixtures of standards were spiked into human plasma at concentrations ranging from 0.8 to 16,000 pg/uL. Each sample was analyzed in triplicate The calibration curves generated from this data are shown in Figure 3 a-d, demonstrating excellent linearity (R^2 > 99%) for all analytes over a wide dynamic range. This indicates the method's ability to accurately quantify these analytes from a complex biological matrix with minimal sample preparation.

Figure 3e illustrates the reproducibility of migration times for all four analytes, with RSD values ranging from 0.50% to 0.63%.

Conclusion

- ZipChip offers a rapid method for analyzing adenosine nucleotides, providing a total analysis time of 4.5 minutes.
- Baseline resolution was achieved for adenosine, AMP, ADP, and ATP.
- The method is suitable for accurately quantifying adenosine nucleotides from complex biological matrices such as human plasma.
- Excellent linearity was observed for all four analytes when spiked into human plasma across a dynamic range spanning five orders of magnitude.
- The migration time reproducibility for all analytes was found to be highly robust.

References

1. Tech Note 1.0; 908 Devices; *ZipChip: What are they and how they work.*
2. Application Note 9.5; 908 Devices; Rapid characterization of oligonucleotides using microfluidic capillary electrophoresis mass spectrometry by ZipChip.

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