

Profiling nucleotides in low numbers of mammalian cells by sheathless CE-MS in positive ion mode: Circumventing corona discharge

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Gold statement

- A sensitive CESI-MS method was developed and validated for the sensitive analysis of nucleotides in limited numbers of HepG2 cells.
- This approach utilised a high pH CE separation coupled to MS operating in positive mode, and totally circumvented corona discharge.
- The established method can provide quantitative information of nucleotides for a prepared sample using as little as 500 HepG2 cells.

Introduction

Negative ion mode nano-ESI-MS is often considered for the analysis of acidic compounds, including nucleotides. However, under high aqueous separation conditions, corona discharge is frequently observed at emitter tips, which may result in low ion abundances and reduced nano-ESI needle emitter lifetimes.

Body

In this work, we introduce a sheathless CE-MS method for the highly efficient and sensitive analysis of nucleotides employing ESI in positive ion mode, thereby fully circumventing corona discharge. By using a background electrolyte of 16 mM ammonium acetate (pH 9.7) a mixture of 12 nucleotides, composed of mono-, di-, and tri-phosphates, could be efficiently analysed with plate numbers per meter above 220 000 and with LODs in the range from 0.06 to 1.3 nM, corresponding to 0.4 to 8.6 attomole, when using an injection volume of about 6.5 nL only. The utility of the method was demonstrated for the profiling of nucleotides in low numbers of mammalian cells using HepG2 cells as a model system. Endogenous nucleotides could be efficiently analyzed in extracts from 50 000 down to 500 HepG2 cells only. Moreover, apart from nucleotides, also some nicotinamide-adenine dinucleotides and amino acids could be detected under these conditions.

Conclusion

We developed a sheathless CE-MS method for the highly sensitive and efficient profiling of nucleotides in low numbers of mammalian cells. This work made use of the detection in positive MS mode to fully circumvent corona discharge. Sub-nanomolar detection limits were acquired for the selected group of nucleotides with an injection volume of roughly 6.5 nL. The usefulness of this method was exemplified for nucleotides in extracted from 50000 to 500 HepG2 cells only. One aspect not investigated in this work is the injection technique for material-limited samples, in order to get the relevant fraction/compounds of the sample effectively into the CE system.

References

- [1] Zhang W , Guled F , Hankemeier T , et al. Profiling nucleotides in low numbers of mammalian cells by sheathless CE-MS in positive ion mode: Circumventing corona discharge[J]. *Electrophoresis*, 2020, 41(5-6).