The Association for Mass Spectrometry: Applications to the Clinical Lab

Proteins and Proteomics

Separation of Intact Parathyroid Hormone and Variants Using a Highly Sensitive Sheathless CE-ESI-MS/MS Method

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Introduction

Parathyroid hormone (PTH), an 84 amino acid protein, is a common clinical marker. Its quantification relies on immunoassays which give variable results as batch, brand, or target epitope changes. Moreover, immunoassays may cross-react with PTH variants such as C-terminal fragments that stem from PTH catabolism. These reliability issues make it difficult to compare results obtained in different laboratories. A reference quantification method is necessary to harmonize PTH assays, both sensitive and selective enough to detect PTH at low concentrations among a variety of closely related compounds.

In this study, our main goal was to reach a very high sensitivity (pg/mL range) for the analysis of PTH and its variants. Two variants were selected, namely 7-84 PTH as C-terminal fragment and 1-34 PTH as related peptide, but also as potential internal standard for future works.

To achieve our goal, we developed a sheathless CE-ESI-MS method for the separation of 1-34 PTH, 7-84 PTH, and 1-84 PTH.

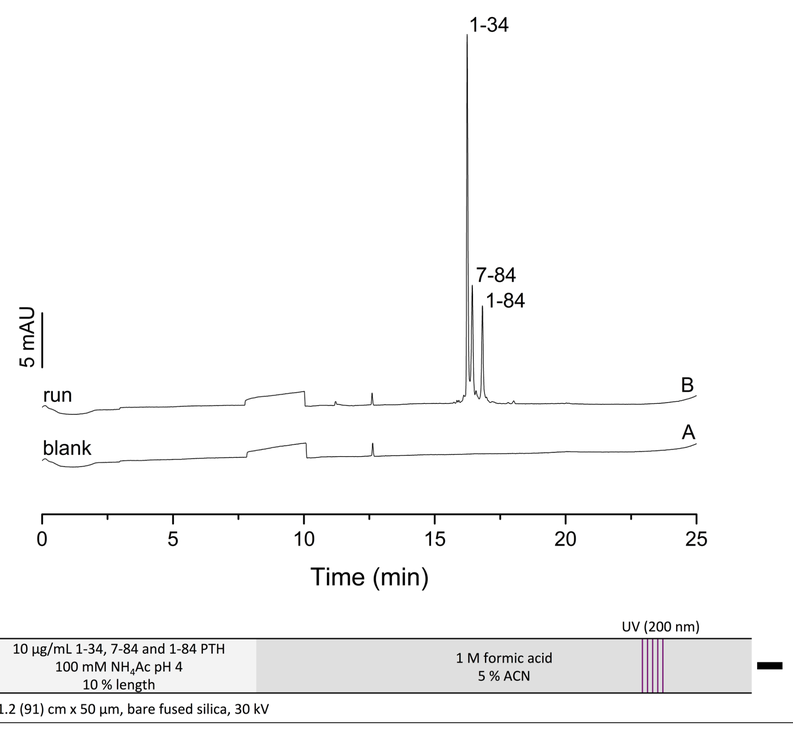
Methods

Experiments were conducted on a CESI 8000 CE system, first equipped with a UV detector and a fused silica capillary. An acidic background electrolyte (BGE) containing acetonitrile was used to reduce analyte adsorption onto the capillary wall, and ammonium acetate was used as sample medium to improve sensitivity through transient isotachophoresis (t-ITP). Then, the method was adapted for coupling with a QT 6500 mass spectrometer through a sheathless ESI interface. Briefly, the capillary outlet is made porous and passes through a metal needle, whose lumen is filled with the BGE and connected to the outlet BGE reservoir through an auxiliary capillary. Solvent and small ions pass through the porous wall, ensuring electrical continuity between CE electrodes, while ESI takes place at the tip using only inner capillary flow. BGE conductivity was reduced to preserve MS-compatible capillaries. Fused silica and neutral coated capillaries were investigated, as well as preconcentration methods such as t-ITP, field-amplified sample injection (FASI) and electrokinetic supercharging (EKS).

Results and discussion

Figure 1 shows a typical electropherogram of a sample containing 10 µg/mL of 1-84 PTH, 7-84 PTH and 1-34 PTH dissolved in 100 mM NH_4Ac pH 4, using t-ITP-CZE-UV with a 10 % injection volume. All compounds were separated.

Figure 1.



For CE-MS on fused silica capillary, BGE formic acid and sample medium NH_4Ac concentrations were reduced to lower the current below the recommended limit (10 µA). Only samples containing at least 10 µg/mL of 1-84 PTH, 7-84 PTH and 1-34 PTH could be analyzed, probably due to the adsorption of proteins on the silica wall and the formation of ammonium adducts, which reduces parent ion intensity (data not shown).

Then, the use of neutral coated capillaries was investigated to reduce analyte adsorption onto the capillary wall. A BGE made up of 50 mM formic acid was used, giving rise to a current of ± 3 µA. Considering the ammonium adducts observed when applying t-ITP, FASI was evaluated as sample preconcentration method. For this purpose, a small volume of water was injected before performing the electrokinetic injection (EKI) and the sample medium was made up of a mixture of acetonitrile and water (50:50; v/v). A high acetonitrile percentage in the sample medium was used not only to decrease possible adsorption but also to perform stacking through the use of gradually more conductive mediums and EKI. Under these conditions, the three compounds, each at 100 ng/mL, could be detected and separated (see **Figure 2**).

The application of a separation pressure to both inlet and outlet was also investigated. The BGE consisted of 50 mM formic acid and 5 % (v/v) acetonitrile. A significant increase in sensitivity was observed since analyte concentrations in the low ng/mL range could be detected (data not shown). Nevertheless, it is detrimental to the separation efficiency since the three biomolecules comigrated.

In order to further improve the sensitivity, the use of EKS was investigated, where EKI is performed after the injection of NH_4Ac and water plugs, allowing t-ITP conditions. Under these conditions, there was no signal anymore for 7-84 PTH; it is worth noting that the fragmentation pattern of 7-84 PTH is different compared to the two other compounds. Nonetheless, concentrations as low as 100 pg/mL could be detected for 1-84 PTH and 1-34 PTH, with S/N values equal to 13 and 27, respectively (see **Figure 3**). Assuming that the LODs correspond to a S/N ratio of 3, they are estimated at 25 pg/mL for 1-84 PTH and 10 pg/mL for 1-34 PTH.

Figure 2.

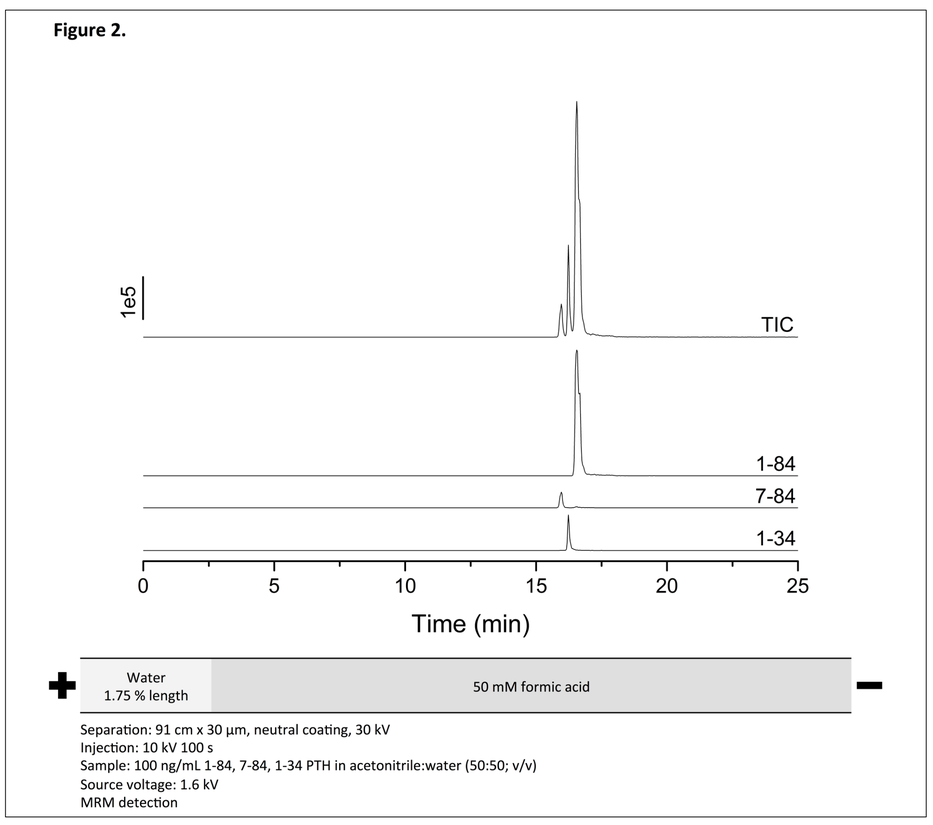
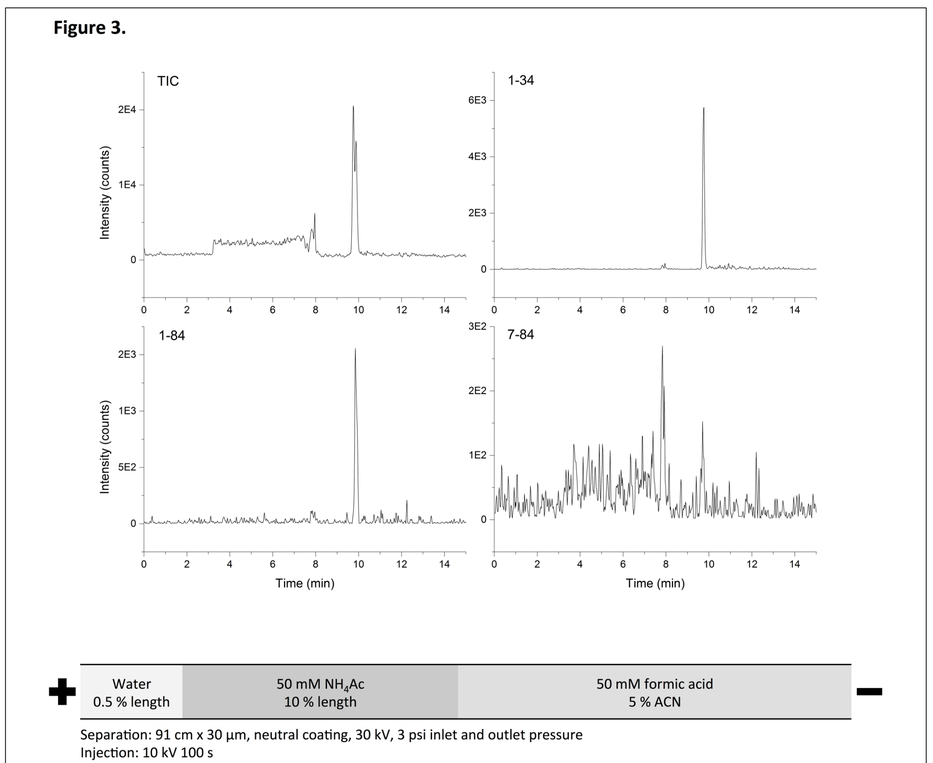


Figure 3.



Conclusion

A CE-UV method able to separate 1-84 PTH, 7-84 PTH and 1-34 PTH forms was developed and coupled with MS. Even if it is well known that the coupling of CE with MS is not easy to be implemented, it is very promising for the quantification of intact proteins. In this context, our preliminary study has allowed to find reliable sheathless CE-ESI-MS/MS conditions for the analysis of very low levels of proteins. We think that this method has the potential to reach the low pg/mL range in biological samples after the optimization of the sample preparation method.

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