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Development of immunosorbents coupled on-line to immobilized pepsin reactor and micro liquid chromatography-tandem mass spectrometry for analysis of butyrylcholinesterase in human plasma

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Highlights

- Comparison of two sorbents for the grafting of monoclonal antibodies
- On-line set-up for HuBuChE analysis including immunoextraction and digestion
- Fast, efficient, specific and sensitive method applied to plasma samples

Abstract

Human butyrylcholinesterase (HuBuChE) has been widely used as a biomarker of exposure to organophosphorus (OPs) warfare agents. Indeed, intoxication by OPs can be proven by LC-MS/MS analysis of a specific HuBuChE nonapeptide on which OPs covalently bind. Therefore, we developed a fast, selective and sensitive on-line set-up for the analysis of HuBuChE from plasma that combines immunoextraction by anti-HuBuChE antibodies, pepsin digestion on Immobilized Enzyme Reactors (IMER) and microLC-MS/MS analysis of the target nonapeptide, FGESAGAAS.

Two pepsin-based IMERs were prepared and characterized in terms of grafting and digestion yields and were coupled on-line to microLC-MS/MS analysis. In addition, immunosorbents were prepared by covalent grafting of three anti-HuBuChE antibodies on CNBr-sepharose and epoxy-polymethacrylate supports and packed in precolumns. The best antibody grafting yields were obtained with sepharose-based supports, with grafting yields up to 98%. B2 18-5 monoclonal antibody grafted on sepharose led to the best immunosorbent, with HuBuChE recovery close to 100%. The immunosorbent was introduced upstream of the on-line digestion set-up and immunoextraction of HuBuChE was achieved in 14 min while digestion was performed in 20 min, allowing detection of the target nonapeptide in less than 1 hour. The global recovery of the nonapeptide was higher than 42% using the best immunosorbent with a RSD value lower than 7% (n = 3). Finally, the limit of quantification evaluated in plasma sample was 2 fmol of nonapeptide. This value, corresponding to 0.5 fmol of HuBuChE tetramer, is well below the average amount of HuBuChE tetramer in 50 µL of plasma (590 fmol).

Keywords: Human butyrylcholinesterase; MicroLC-MS/MS; FGESAGAAS; Immunosorbents; Immobilized Enzyme Reactor; On-line coupling.

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