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Blood Doping Detection – A New Analytical Approach with Capillary Electrophoresis

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Blood doping is defined by the World Anti-Doping Agency (WADA) as the use of products and methods that enhance the uptake, transport, or delivery of oxygen to the blood. One approach uses artificial oxygen carriers, known as hemoglobin-based oxygen carriers (HBOC). These products are made of bovine or human hemoglobin (Hb) and were developed to treat some types of severe anaemia. They are claimed to provide a threefold more efficient oxygen transport and consequently to potentially improve sports performance, particularly in endurance disciplines such as long-distance running, cycling or swimming.

Capillary electrophoresis (CE) appears to be a promising technique for HBOC analysis in the context of doping control, since different CE protocols have already been developed for

the analysis of Hb variants. In addition, the online combination of CE with mass spectrometry (MS) is an attractive option for intact protein analysis (*i.e.* no digestion, no derivatization step required). On the one hand, CE offers features such as high speed, great efficiency, and low solvent and sample consumptions. On the other hand, MS provides selectivity and specificity.

In this context, a complete analytical strategy based on CE was developed to detect intact HBOC in plasma samples. This methodology includes four distinct steps:

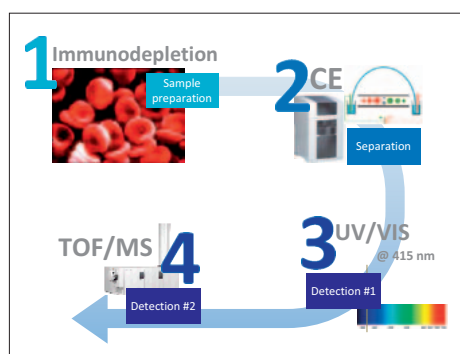
- Plasma samples preparation based on immunodepletion to remove the most abundant proteins (*e.g.* albumin and immunoglobulin) that can interfere with CE separation and alter electrospray ionization.
- CE separation to obtain sufficient electrophoretic resolution between HBOC and Hb that could be released from mechanical hemolysis.
- Online UV-visible detection at 415 nm to selectively detect hemoproteins such as HBOC and Hb.
- TOF/MS detection to provide accurate mass on analytes and unambiguous determination of HBOC uptake.

The limits of detection were in agreement with doping control requirements. This methodology thus appears suitable for implementation as a doping control screening method for HBOC analysis.

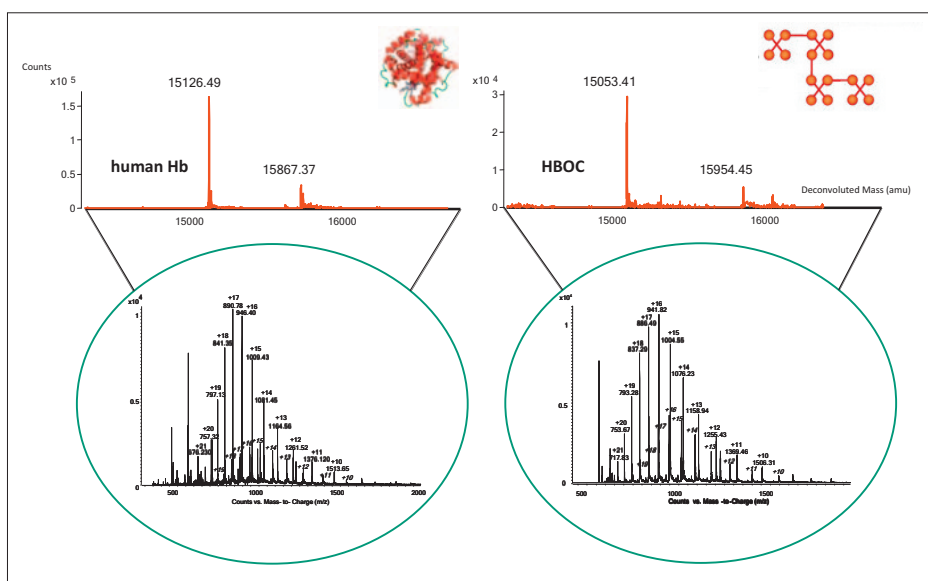
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A. Staub, J. Schappler, S. Rudaz, J. L. Veuthey, *Electrophoresis* **2009**, *30*, 1610.



The four selectivity levels obtained with our method.



Mass spectra and deconvoluted mass spectra of Hb and HBOC by CE-TOF/MS.

Can you show us your analytical highlight?

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