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## Highlights

- IEX coupled to native MS was used for HSA isoforms characterization.
- HSA charge variants were efficiently separated by IEX with volatile salts.
- Cleaved, oxidized and glycated forms of HSA were monitored in a single analysis.
- High affinity Cu(II) binding to HSA was monitored at the isoform level.

### Abstract

Human Serum Albumin is the most abundant protein of the plasma and displays a wide range of non-oncotic properties such as antioxidant activity, distribution in tissues and organs of binding molecules and clearance of toxic compounds. Albumin is susceptible to numerous post-translational modifications and particularly related to its free thiol group at Cys<sub>34</sub> which is the main circulating scavenger of reactive oxygen species. The characterization of these modifications is of high interest for the diagnosis and treatment of patients with liver diseases and for the structural integrity assessment of albumin as a therapeutic protein.

In this study, an ion exchange chromatographic method coupled on-line to native mass spectrometry was developed in order to bridge an effective charge variants separation method with a powerful identification technique for a detailed characterization of albumin isoforms. The chromatographic performance of the method allows the separation of 9 different isoforms that were on-line characterized by MS as oxidized, glycated, deamidated and N/C-terminal truncated forms. The method is also able to detect Cu(II) ions binding to the N-terminal site of the protein which is an important antioxidant feature of albumin. Finally, the method showed preliminary good performance parameters in term of linearity, precision and sensitivity for characterization of purified albumin as well as albumin from raw plasma for clinical and pharmaceutical purposes.





## Abbreviations

HSA, Human Serum Albumin; RT, retention time; MS, mass spectrometry; IEX, ion exchange chromatography; FcRn, neonatal Fc receptor

#### Keywords

Mass spectrometry; Ion exchange chromatography; Oxidation; Charge variants; Human Serum Albumin

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