Development of LC-ESI-MS/MS Procedures for the Analysis of Neurosteroids in Rat Brain

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LC-ESI-MS is a powerful technique that combines the physical separation capabilities of liquid chromatography with the high sensitivity of the mass spectrometric detection. Since the ESI interface produces little fragmentation, LC-MS can't distinguish between analytes that elute at the same retention time and have the same molecular mass. Conversely, LC-ESI coupled to tandem mass spectrometry can provide characteristic fragmentation patterns and represents an extremely selective tool for the analysis of biological samples due to its superior specificity.

A sensitive LC-ESI-QqQ procedure has been developed and validated for the simultaneous determination in rat brain extracts of three endogenoous molecules, called neurosteroids (REF), i.e. pregnenolone sulphate (PS), dehydroepiandrosterone (DHEA) and allopregnanolone (AP), without any preliminary derivatisation, owing to the capability of LC to analyse directly thermally labile, nonvolatile and polar compounds. Quantitation was performed by multiple reaction monitoring, using deuterium-labelled analogues of the analytes as internal standards [1]. The proposed method provides a direct quantitative determination of PS (whithout hydrolisis) , DHEA and ALLO and can be applied to quantify these analytes in brain tissue to study their changes in pathological situation or after pharmacological treatments.

References

[1] Rustichelli, C., Pinetti, D., Lucchi, C., Ravazzini, F. Puia, G. Simultaneous determination of pregnenolone sulphate,dehydroepiandrosterone and allopregnanolone in rat brain areas by liquid chromatography–electrospray tandem mass spectrometry. *J.Chromatogr. B,* **2013**, *vol. 930*, 62-69.