

Investigation of fundamental aspects of supercritical fluid chromatography tandem mass spectrometry (SFC-MS-MS) and its application in the enantioselective analysis of ketamine and its metabolites in the biological samples.

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Supercritical Fluid Chromatography (SFC) is a powerful separation technique. This technique offers certain advantages in terms of separation efficiency, analysis speed, sensitivity and is environmentally friendlier compared to high-performance liquid chromatography (HPLC). The use of supercritical CO₂ as the major component of a mobile phase in the analysis provides low viscosity and high diffusion coefficient, enabling the potential for achieving higher theoretical plates at higher flow rates compared to HPLC. Furthermore, the combination of CO₂ with polar mobile phases (organic modifiers) allows the technique to effectively analyze both polar and non-polar compounds simultaneously.

SFC coupled with tandem mass spectrometry (MS/MS) is one of the most powerful and advantageous techniques in modern science for both separation and identification, as the minimal amount of polar mobile phase used significantly increases sensitivity compared to other chromatographic methods.

Additionally, SFC is better compatible with Electrospray Ionization (ESI) techniques, as the use of CO₂ in the mobile phase facilitates the desolvation of droplets generated in the ion source, increasing the likelihood of ionization. SFC coupled with tandem mass spectrometry does not require base-acid additives to enhance ionization degree, as the use of methanol (and also ethanol and isopropanol) in the mobile phase leads to a reaction between the organic modifier and CO₂, resulting in the formation of methoxycarbonyl acid, which represents good proton donor, thus ensuring effective ionization without the need for additives from ionizers.

This study explores a comparison between HPLC and SFC in the analysis of ketamine and its metabolites in human saliva samples, evaluating separation efficiency, sensitivity, and various fundamental aspects of these techniques.

Keywords: Supercritical fluid chromatography-mass spectrometry (SFC-MS-MS), Liquid chromatography-mass spectrometry (LC-MS-MS), enantioselective analysis, toxicology analysis.