

# CURRENT TRENDS IN MASS SPECTROMETRY AND CHROMATOGRAPHY

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## A simple and efficient approach for recovery estimation during reverse phase prep purification

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Reversed phase high performance liquid chromatography (RP-HPLC) is an indispensable purification technique in drug discovery. Usually the LC/MS short run data is submitted for purification but many times it does not provide the real purity information and after the prep method development the profile changes drastically and the purity drops down. Usually the recovery calculation in purifications is based on crude HPLC profile and dry weight estimation before and after the purification. Such assumption can be off significantly when the crude material contains UV inactive impurities and inorganic salts. In such cases poor recovery of the compound after the prep purification becomes a point of concern and there was no proven method to address this concern for real samples. We developed a simple and efficient method for calculating the recovery of each sample purified by preparative HPLC. It is based on the ratio of the HPLC/UV peak area of the compound of interest in the crude solution to that in the final collected fraction with both accounted for their volumes. This approach eliminates not only the need for drying of the collected fraction to calculate recovery but also the inaccuracy associated with the true content in the crude sample using the traditional dry weight method. A systematic study was conducted to verify this method using caffeine mixed with various UV-active and inactive impurities. The calculated recoveries using this approach were found to be consistent within 2% with the true recoveries based on dry weight estimation. The approach has been successfully applied for our in-house purifications. Furthermore, the approach was extended to library purifications to get the tentative recovery in heart-cutting fractions.

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