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HPLC-ESI-MS/MS Assay for Quantitative Metabolic Profiling in Fermentation Broths from β -Lactam Antibiotics Production

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Recent efforts for the optimization of biotechnological production processes such as for β -lactam antibiotics comprise, amongst others, metabolomics-based methodologies. In metabolic profiling approaches it is aimed at measuring quantitatively time-dependent concentration profiles of intra- and extracellular metabolites, nutrients, target product compounds and their intermediates. It is the goal to monitor concentration variations of as many substances as possible in the course of fermentation processes with adequate sampling at regular reasonably short time intervals, allowing to draw conclusions on the progression of the fermentation. Information gained from such analytical measurements should ultimately lead to better understanding of the production process and improvements in the productivity.

In this contribution, the development and validation of a HPLC-ESI-MS/MS assay for the quantitative metabolic profiling of amino acids, organic acids, vitamins, β -lactam antibiotics and their intermediates in fermentation broths of penicillin and cephalosporin production will be presented. The hydrophilic nature of the target analytes resulted in insufficient retention on reversed-phase columns which is usually associated with a strong susceptibility for matrix effects such as ion-suppression. Therefore, a hydrophilic interaction chromatography (HILIC) mode was utilized. For this purpose, a ZIC-HILIC column (SeQuant, Merck) was employed which allowed the retention of the polar metabolites with acetonitrile-rich mobile phases and separation of critical peak pairs. Moreover, the HILIC mode is also favourable in terms of ionization efficiency and sensitivity. MS detection was performed in the multiple reaction monitoring (MRM) mode with characteristic quantifier transitions for the target solutes. Various methodologies were tested for calibration (external calibration with neat standard solutions, calibration employing internal standards, matrix-matched calibration with standard addition in various matrices with and without internal standards). The results in terms of linearity, sensitivity (quantitation limit), precision and accuracy for the individual calibration methodologies will be compared. Challenges, peculiarities, problems and shortcomings will be discussed.