Chromatographic and/or Electrophoretic methods development and validation for correlating Tamoxifen resistant MCF-7 cell line changes with biomarkers (lactate, pyruvate, glucose and glutamine) level

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Abstract

HPLC-MS/MS and CE-C^4D are powerful analytical techniques used to separate, identify, and quantify components in complex mixtures. Because of their selectivity and sensitivity to wide range of analytes, these techniques are currently widely employed in metabolomics especially for breast cancer metabolomics. In this study, two analytical techniques were developed and validated to track the changes in the metabolites production including lactate and pyruvate and the substrates consumption including L-glutamine in cell culture supernatants of MCF-7 cell line as a result of developing acquired Tamoxifen resistance.

HPLC-MS/MS validation results for all analytes showed very good linearity (R^2 = 0.9972, 0.9963, 0.9988), high sensitivity with LOD values of 0.16, 0.28, 4.6 µM and LOQ values of 0.49, 0.849, 13.95 µM for lactate, pyruvate and L-glutamine, respectively. While for CE-C^4D the validation results show very good linearity (R^2 = 0.9912), with LOD and LOQ for lactate of 11 and 21 µM, respectively. As a result of acquiring resistance to Tamoxifen on the cellular level of MCF-7 cells, there was a significant transition in morphological shape from epithelial phenotype into mesenchymal phenotype. Also, a significant change is the metabolic pathways were detected by the increase of lactate and pyruvate production and a decline in L-glutamine consumption.

Keywords: HPLC-MS/MS, CE-C^4D, metabolomics, resistance MCF-7 cells, breast cancer, method development