

## **Ion Pairing Free Mixed-Mode Chromatography for the Separation and Measurement of TCA Cycle Metabolites in Urine Samples of Breast Cancer Patients**

### **Mass Spectrometry**

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The Tricarboxylic acid (TCA) cycle is the ultimate fate of metabolism where Acetyl-CoA or other molecules are formed by the breakdown of carbohydrates, protein, and fats. Components of the TCA cycle, small polar organic carboxylic acids, are hard to retain under traditional reversed-phase LC conditions. Current LC methods used to analyze these compounds include: HILIC, ion-pairing, anion exchange. All of these methods have their unique challenges. HILIC methods give poor peak shape and insufficient separation, while the ion-pairing approach provides the chromatographic resolution but often limits the use of instrument due to the high background signal coming from lingering the ion pairing agent.

Here we present a MS compatible mixed-mode LC method for the analysis of TCA cycle analytes and other related compounds without the use of sample derivatization or ion-pairing reagents using the ACQUITY PREMIER CSH Phenyl-Hexyl column. This method allows separation the TCA cycle intermediates, including the critical pairs, for a comprehensive coverage of the TCA cycle. Biologically important critical pairs of citric acid and isocitric acid, for example, are isobaric at 191 m/z while malic acid undergoes degradation to have the same parent mass as fumaric acid, both sets of critical pairs are baseline resolved. We have used this method for the analysis of urinary TCA cycle metabolites on a time of flight mass spectrometer. Urine samples from control and breast cancer were analyzed. The multivariate analysis (PCA) of the data showed clear classification of the control and breast cancer samples. The distribution profile of the 16 TCA cycle and related metabolites were adequate for the classification of the study groups. This finding illustrates that analysis with a pathway focused approach may be used as an alternative approach to complete metabolomics profiling for a quick classification of breast cancer patient using urine samples.

In this work we showed the successful application of a mixed-mode chromatographic method for the analysis of TCA analytes and other related molecules without the need for ion-pairing reagents in the mobile phase. Excellent analytical reproducibility is achieved as shown by the tight cluster of the QC samples in the PCA scores plot.