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Improved LC/MS Methods for the Analysis of Anionic Analytes

Jordy J. Hsiao, Oscar G. Potter, Genevieve C. Van de Bittner, Te-Wei Chu, Hongfeng Yin

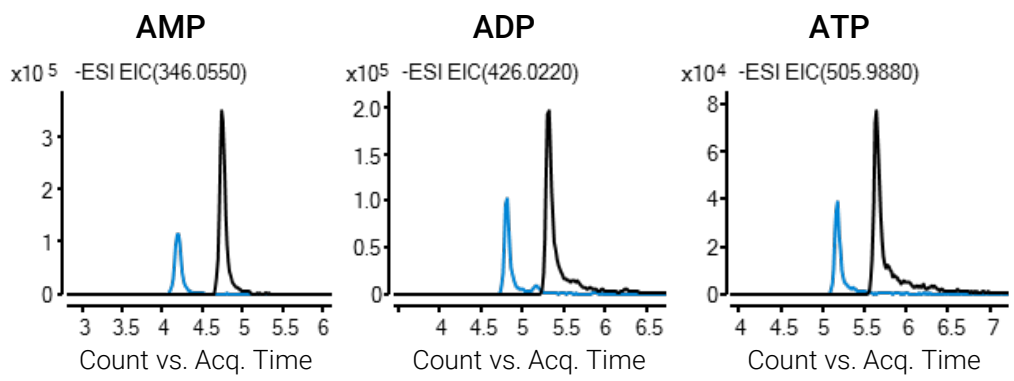
Agilent Technologies, Santa Clara , CA

Introduction

- Metal contamination in the LC/MS system can negatively impact the peak shape and detection limit of anionic compounds.
- Ethylenediaminetetraacetic acid (EDTA) is a strong metal chelator that enhances the detection of metabolites and phosphorylated analytes when used as a system flushing agent or as a mobile phase addtive[1].
- Unfortunately, EDTA is highly ionizable and causes ion suppression of target analytes[1] (Figure 1).
- Herein, medronic acid was discovered to enhance the chromatographic performance of metal-sensitive analytes with minimal ion suppression when used as a mobile phase additive.
- This method was utilized to monitor nutrient consumption and metabolic waste secretion in cell growth media.
- Furthermore, a metabolomics study was carried out to examine intracellular metabolite changes to K562 leukemia cells upon exposure to anticancer drug methotrexate (MTX).

Evaluating EDTA as a Mobile Phase Additive

Nucleotides



Organic acids

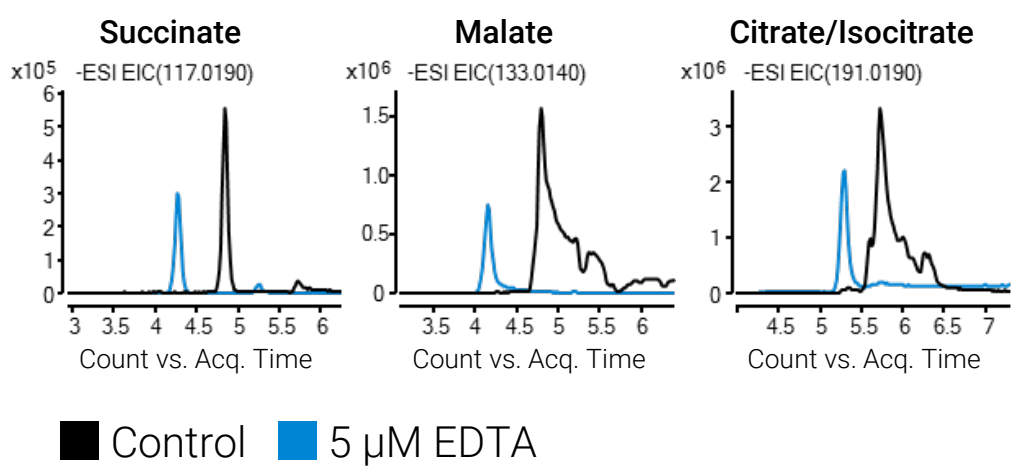


Figure 1. EDTA as a mobile phase additive improved the peak shapes of metal-sensitive analytes. However, signal intensity decreased for all metabolites due to ion suppression by EDTA.

Experimental

LC/MS Analysis

- Metabolite samples and pesticide standards were separated using Agilent's InfinityLab Poroshell 120 HILIC-Z columns on a 1290 LC system coupled on-line with a 6545 Q-TOF or a 6490 QQQ.
- Phosphopeptide standards were analyzed using Agilent's AdvanceBio Peptide Mapping column on a 1290 LC system coupled on-line with a 6550 iFunnel Q-TOF.

Instruments and Supplies

Agilent HPLC Columns (2.1 x 150 mm):

- InfinityLab Poroshell 120 HILIC-Z, PEEK-lined
- AdvanceBio Peptide Mapping Column

Agilent LC Instruments:

- 1290 Infinity Binary LC System

Agilent MS Instruments:

- 6545 Q-TOF LC/MS
- 6550 iFunnel Q-TOF LC/MS
- 6490 Triple Quad LC/MS with iFunnel technology

Agilent Mobile Phase Additive:

- InfinityLab Deactivator Additive (5 mM medronic acid)

Mobile Phase :

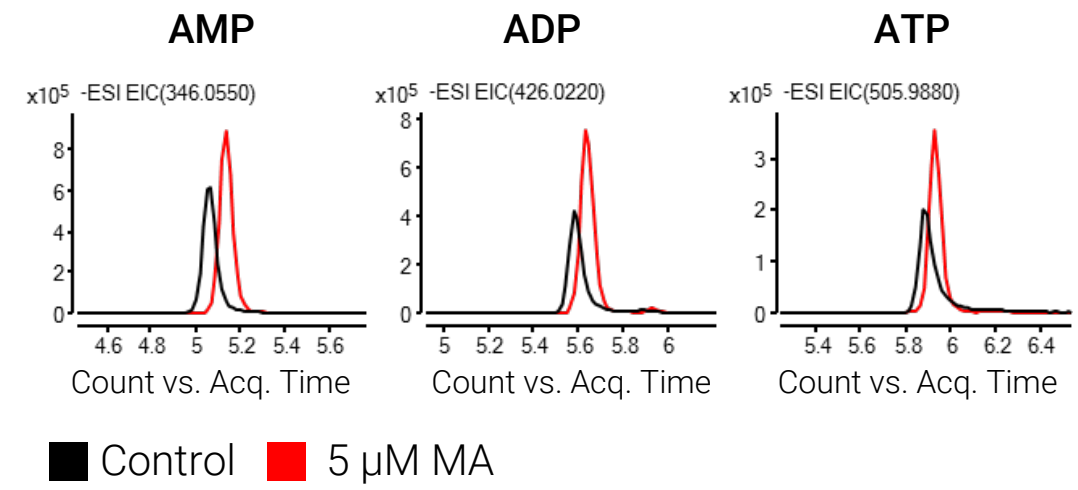
- A 100 mM ammonium acetate stock was made in water and adjusted to pH 9 with ammonium hydroxide.
- Mobile phase A = 10% (100 mM ammonium acetate in water at pH 9)/ 90% water.
- Mobile phase B = 10% (100 mM ammonium acetate in water at pH 9)/ 90% acetonitrile.

Cell Culture Samples

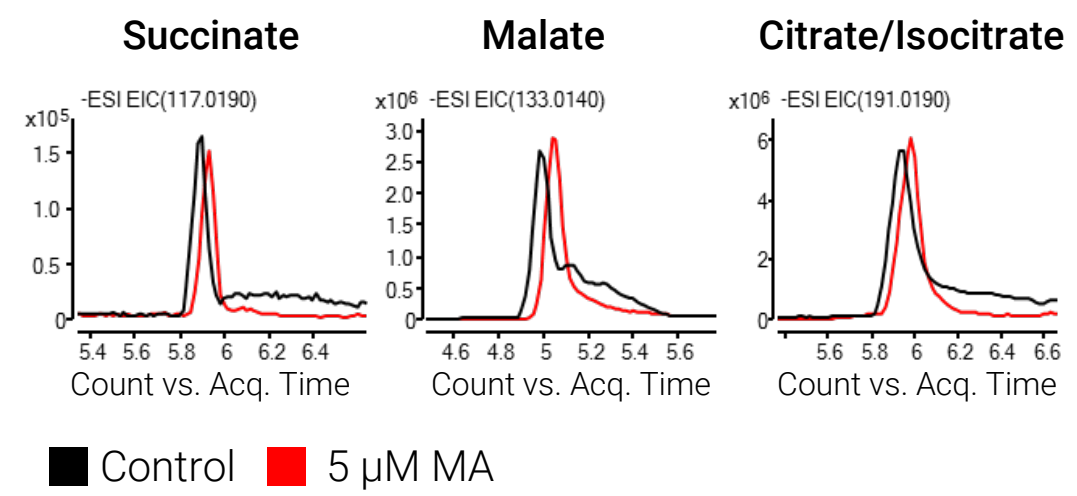
- K562 leukemia cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum. Growth media was collected from the culture dish on days 1, 2, 3, and 6 after splitting the cells into fresh growth media.
- Metabolites were extracted from K562 cells treated with 5 µM methotrexate (MTX) or vehicle (DMSO) for 16 hours. Metabolite samples were subjected to LC/MS analysis.

Evaluating Medronic Acid as a Mobile Phase Additive for Hydrophilic Interaction Chromatography (HILIC)

(A) Nucleotides



(B) Organic acids



(C) Polar Pesticides

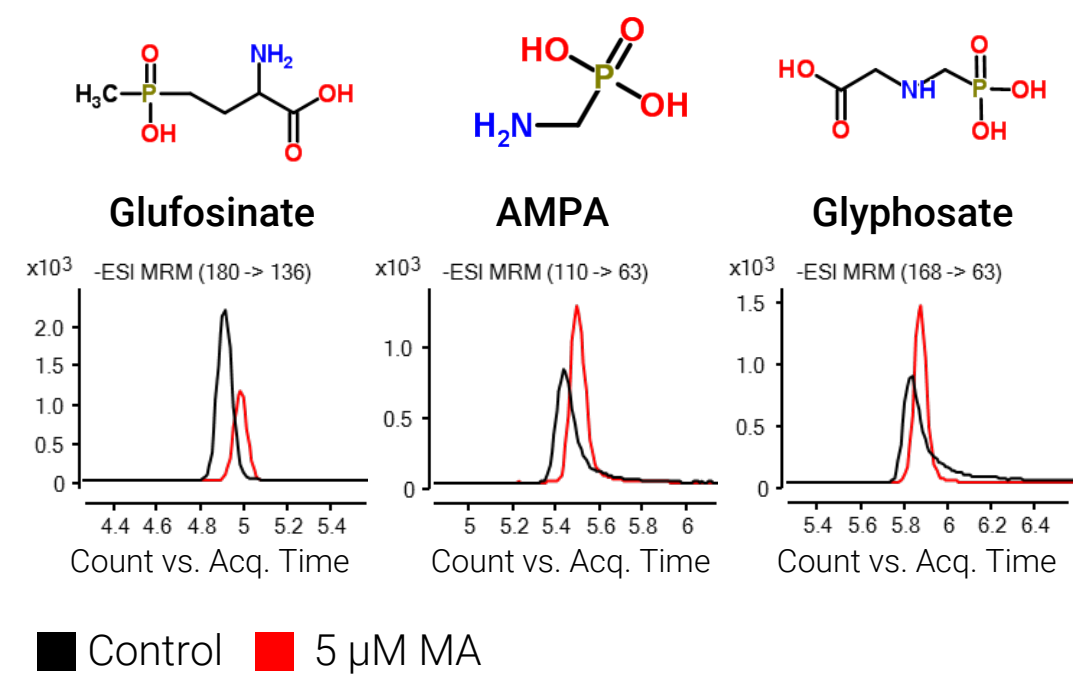
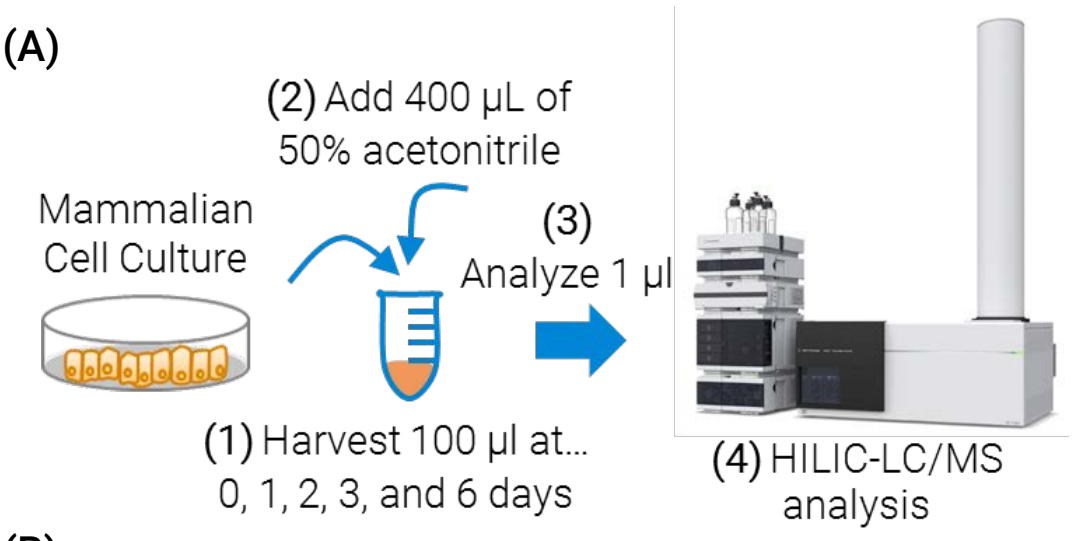


Figure 2. Medronic acid (MA) as a mobile phase additive improved the HILIC-LC/MS analysis of **(A)** phosphorylated nucleotides, **(B)** organic acids, and **(C)** phosphorylated polar pesticides with minimal ion suppression effects observed.

Monitoring Mammalian Cell Culture Media with HILIC-LC/MS

(A)



(B)

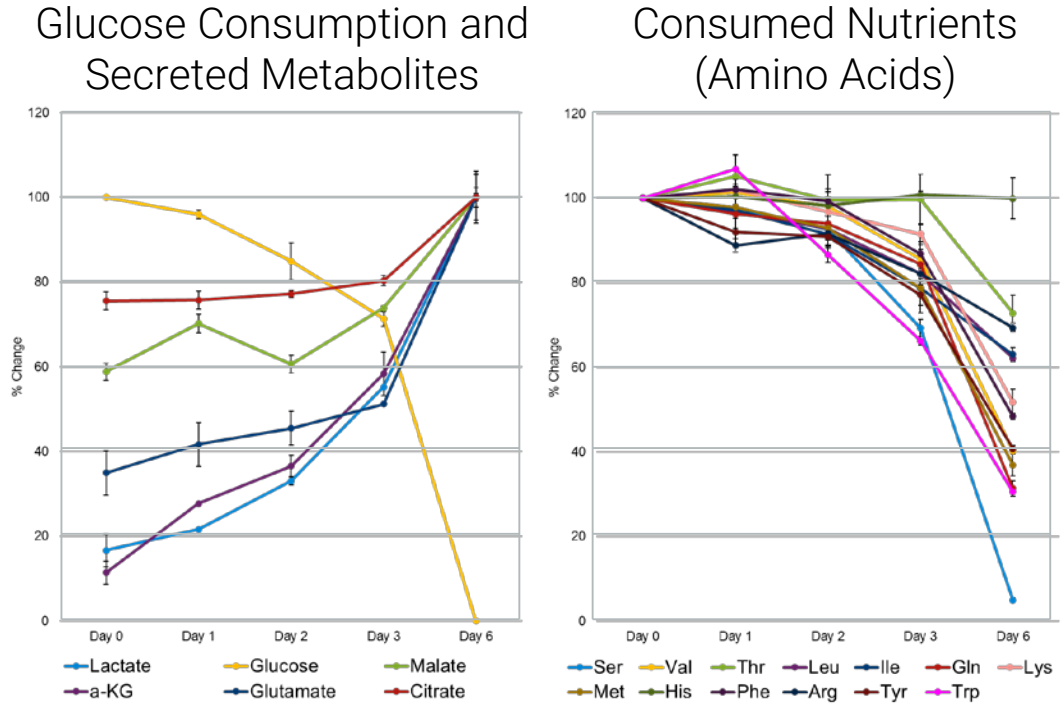


Figure 3 (A) A fast and simple approach to profile cell culture media. (B) Quantitative analysis of nutrient consumption and metabolite secretion from/into mammalian cell culture media.

Underivatized Amino Acid Analysis by HILIC-LC/MS

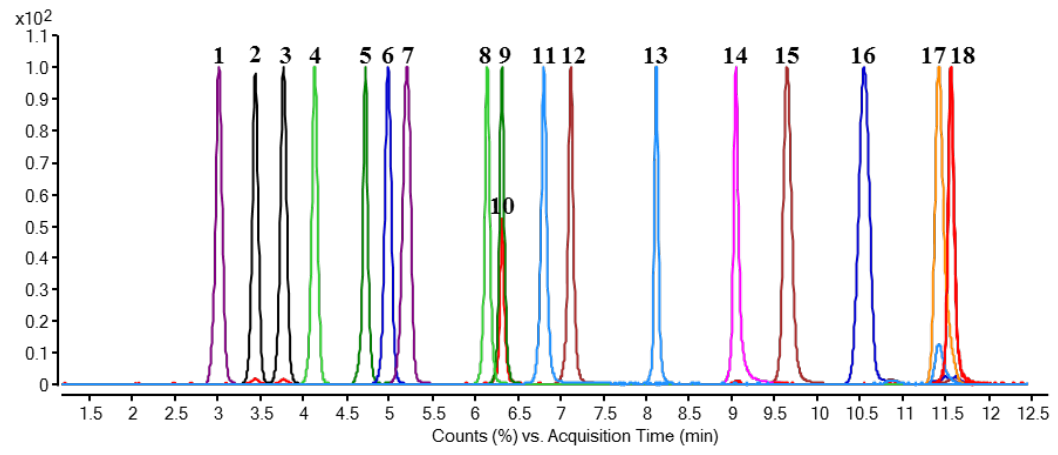


Figure 4. HILIC-LC/MS analysis of 18 underivatized amino acid mixture. Amino acids in order of elution: (1) Phe, (2) Leu, (3) Ile, (4) Met, (5) Tyr, (6) Val, (7) Pro, (8) Ala, (9) Thr, (10) Cys, (11) Gly, (12) Ser, (13) Glu, (14) Asp, (15) His, (16) Arg, (17) Lys, (18) 2-Cys (dimer).

Results and Discussion

Metabolomics Profiling of MTX-treated Leukemia Cells

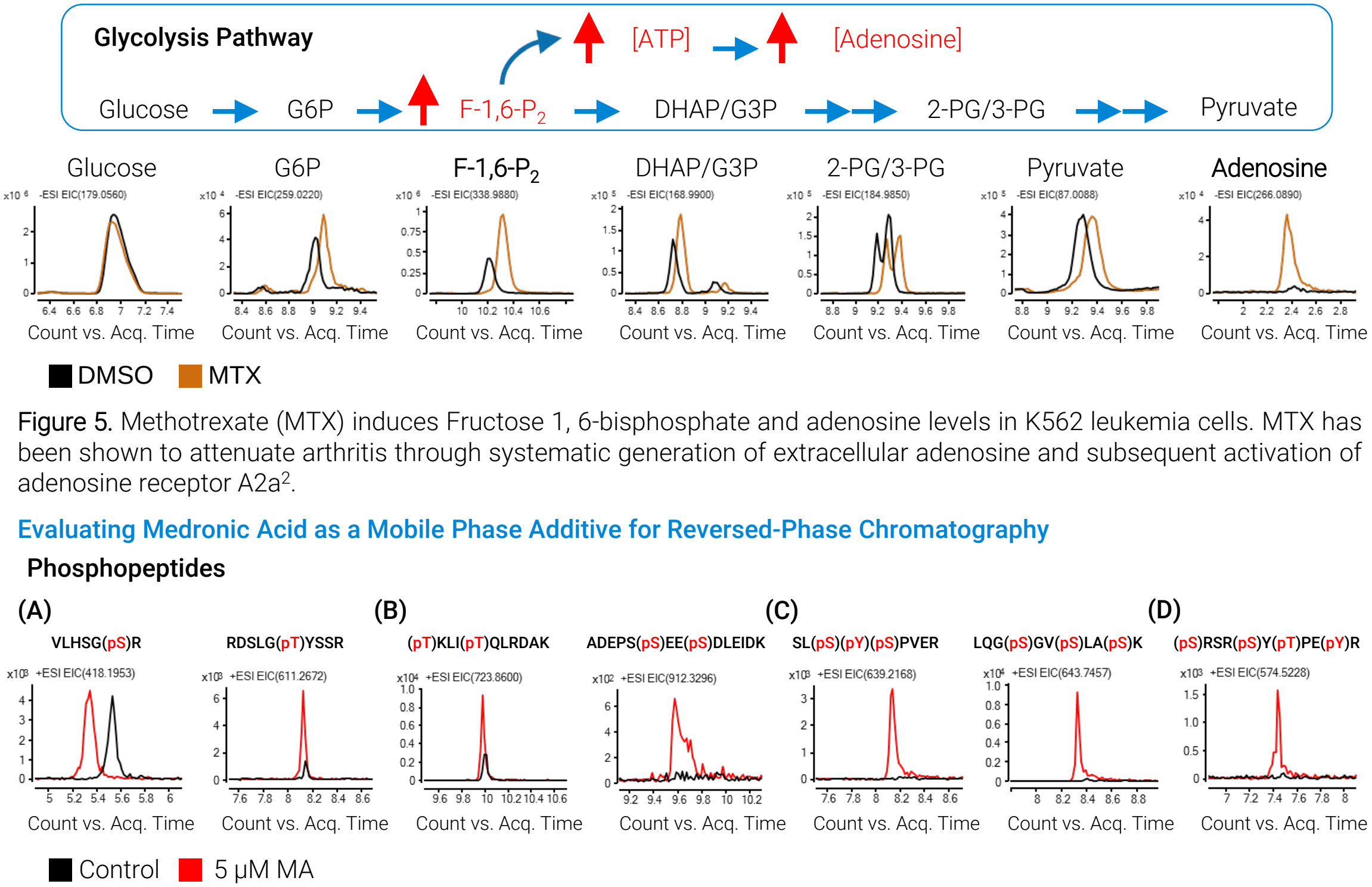


Figure 5. Methotrexate (MTX) induces Fructose 1, 6-bisphosphate and adenosine levels in K562 leukemia cells. MTX has been shown to attenuate arthritis through systematic generation of extracellular adenosine and subsequent activation of adenosine receptor A2a[2].

Evaluating Medronic Acid as a Mobile Phase Additive for Reversed-Phase Chromatography

Figure 6. Medronic acid (MA) as a mobile phase additive promoted the detection of phosphopeptides by reversed-phase chromatography. A panel of (A) singly, (B) doubly, (C) triply, and (D) tetra-phosphorylated peptides tested are shown. The phosphorylated sites on the peptide sequences are indicated in red. This finding represents a significant advancement in the phosphoproteomic field as enrichment steps and fractionation strategies are generally required for the detection and identification of phosphorylated peptides in the proteome.

Conclusions

- Medronic acid addition to mobile phase solvents facilitates the identification/quantification of metal-sensitive analytes and achieves lower detection limits.
- This method could also be applied to additional metal-sensitive applications utilizing ion-pairing reverse phase and ion exchange chromatography.

References

1. J.J. Pesek, M.T. Matyska, S.M. Fischer. “Improvement of peak shape in aqueous normal phase analysis of anionic metabolites”, J Sep Sci, 34(24):3509-16, 2011.
2. F.P. Veras, et al. “Fructose 1,6-bisphosphate, a high-energy intermediate of glycolysis, attenuates experimental arthritis by activating anti-inflammatory adenosinergic pathway”, Sci Rep, 5:15171. 2015.

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Trusted Answers