Alturas Analytics, Inc.

The LC-MS Experts

Alturas Analytics, Inc.
1324 Alturas Drive
Moscow, ID 83843

208.883.3400

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Developing a rugged chiral assay for the biomarker 2-hydroxyglutaric acid for use in routine clinical analysis of plasma samples from oncology studies

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Jennifer Zimmer, PhD
Alturas Analytics, Inc.

Background

- Isocitrate dehydrogenase (IDH) enzymes normally catalyze the conversion of isocitrate to alpha-ketoglutarate (α-KG), and reduce $NADP^+$ to NADPH
- In some cancers, such as certain types of gliomas and acute myeloid leukemia, mutations in the gene encoding the IDH protein cause a loss of its native function and a gain of an enzymatic function that allows IDH to convert α-KG into 2-hydroxyglutarate (2-HG)
- 2-HG accumulates in large amounts in tumor cells and can interfere with normal enzyme activities in the cells

Background

- In addition to high levels in cells, plasma concentrations of 2-HG have been shown to be elevated
- This conversion has been found to be stereospecific for the D- enantiomer of 2-HG
- Monitoring the reduction of D-2-HG could be an effective way to measure effectiveness of IDH-targeted cancer chemotherapy.

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Bioanalytical Method Requirements

1. Measure 2-HG from plasma samples
2. Chiral method to quantify D-2HG and L-2HG separately
3. LLOQ under 100 ng/mL for each enantiomer
4. Ability to analyze samples with a quick turnaround
5. Surrogate matrix due to endogenous levels of 2-HG

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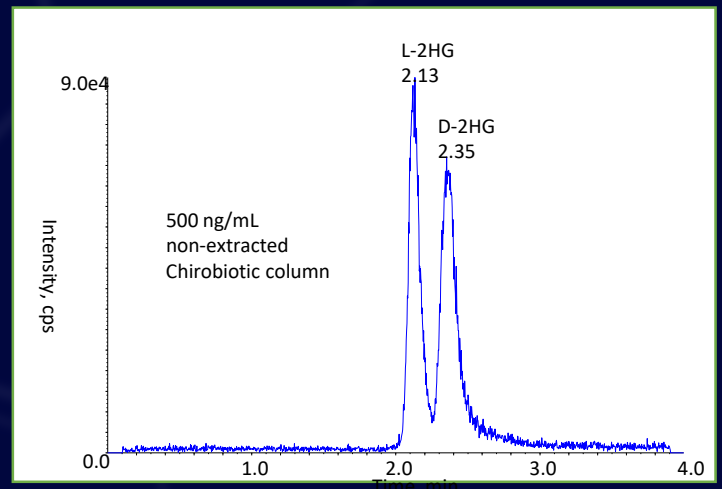
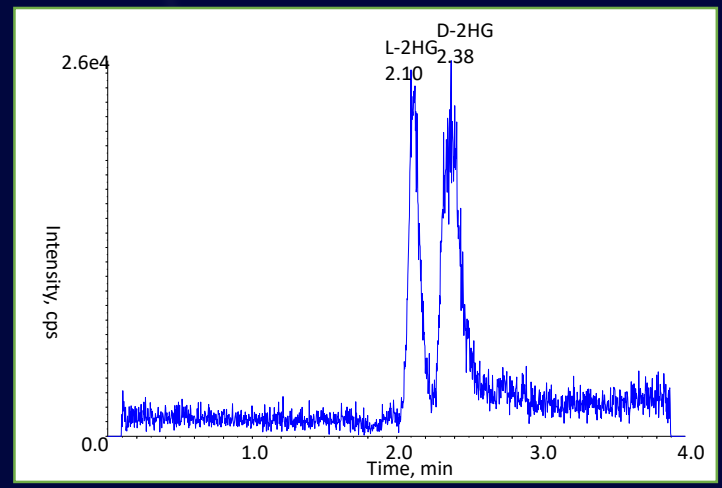
Method Development

Separation of enantiomers using Chirobiotic R column

1. No derivatization necessary
2. MS compatible LC solvents
3. HPLC run time <5 minutes
4. No data published on LLOQ or extraction
5. Suppression when applied to extracted samples





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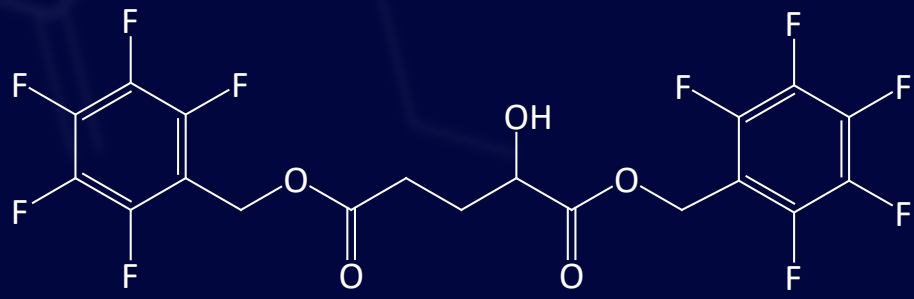
Method Development

Derivatization with pentafluorobenzyl bromide

1. Overnight derivatization
2. 40 minute normal phase HPLC method
3. Sub-ng detection in $1X10^6$ cells



Worth AJ, Gillespie KP, Mesaros C, et al. Rotenone Stereospecifically Increases (S)-2-Hydroxyglutarate in SH-SY5Y Neuronal Cells. *Chem Res Toxicol*. 2015;28(5):948-954. doi:10.1021/tx500535c

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Derivatization with TSPC (N-(p-toluenesulfonyl)-L-phenylalanyl chloride)

1. Reported high sensitivity (~0.3 ng/mL) in water
2. Easy extraction and fast derivatization
3. Separation requires >30 min
4. Unsuccessful in reducing analysis time

Bao X, Wu J, Shuch B, LoRusso P, Bindra RS, Li J. Quantitative Profiling of Oncometabolites in Frozen and Formalin-Fixed Paraffin-Embedded Tissue Specimens by Liquid Chromatography Coupled with Tandem Mass Spectrometry. Sci Rep. 2019;9(1):11238. Published 2019 Aug 2. doi:10.1038/s41598-019-47669-5

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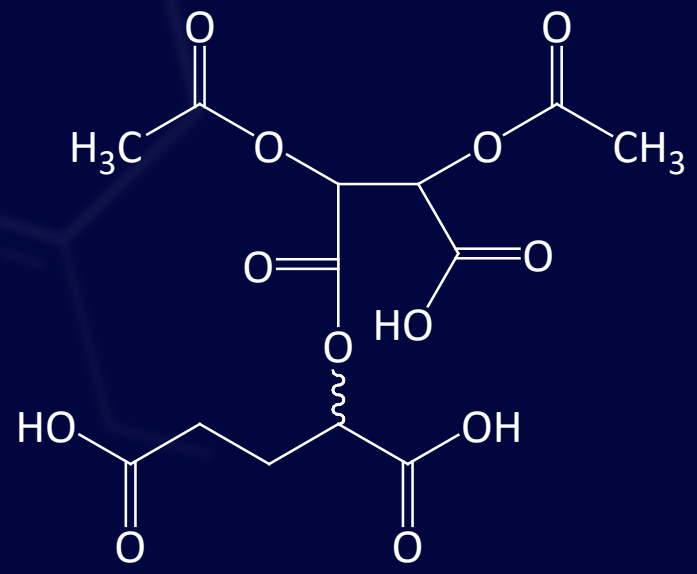
Derivatization with DATAN (diacetyl-L-tartaric anhydride)

1. Lower sensitivity (~50 ng/mL) in water/serum
2. SPE extraction and longer derivatization time
3. Separation requires ~10 minutes using RP-HPLC



Poinsignon V, Mercier L, Nakabayashi K, et al. Quantitation of isocitrate dehydrogenase (IDH)-induced D and L enantiomers of 2-hydroxyglutaric acid in biological fluids by a fully validated liquid tandem mass spectrometry method, suitable for clinical applications. J Chromatogr B Analyt Technol Biomed Life Sci. 2016;1022:290-297. doi:10.1016/j.jchromb.2016.04.030

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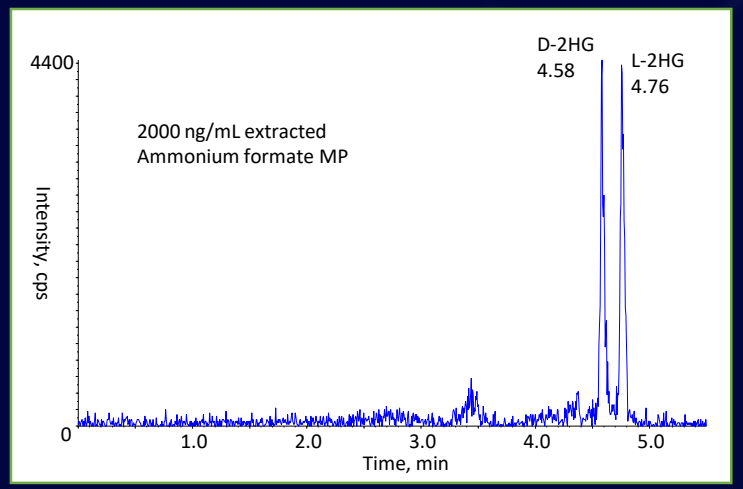
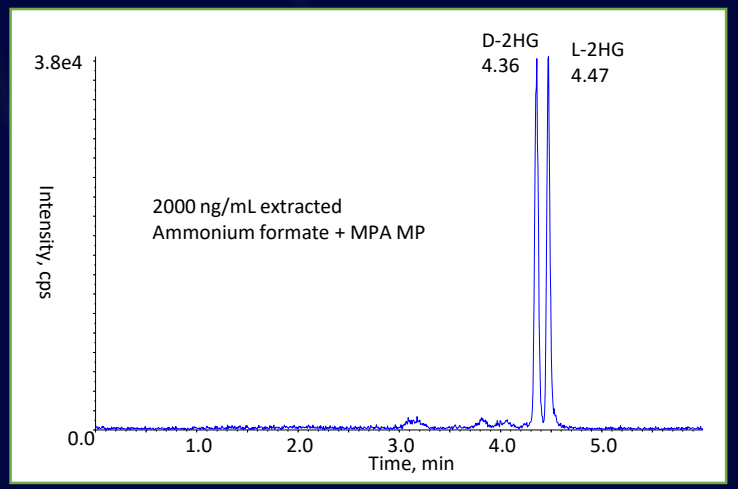
Method Development - DATAN

Optimized LC method

- HILIC-Z column
- Isocratic method with ammonium formate mobile phase
- Methyl phosphonic acid added for peak shape and signal enhancement





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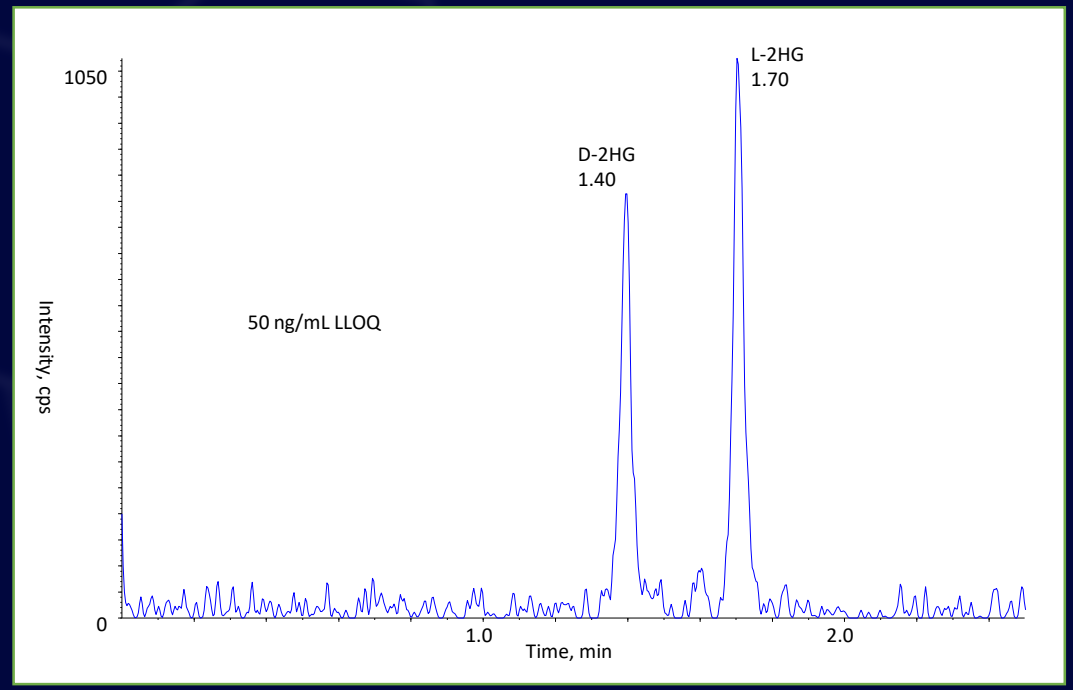
Validated Method

- Surrogate matrix
- 50-50000 ng/mL
- 25 µL sample volume
- Methanol precipitation assay
- DATAN derivatization
- Poroshell HILIC-Z column
- Isocratic HPLC method using ammonium acetate with MPA
- 1-2 µL injection volume
- Sciex API-4000



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Validated Method

- D-2HG
  - Intrabatch accuracy -2.8 – 8.0%; precision 1.3 – 4.8%
  - Interbatch accuracy -1.3 – 5.4%; precision 3.6 – 9.4%
  - Matrix effects within 9.6% accuracy & precision
  - Quantitative recovery
  - 6h ambient benchtop stability in plasma
  - 4 freeze/thaw cycles (-70°C) in plasma
  - 160 hours reinjection stability

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Validated Method

- L-2HG
  - Intrabatch accuracy -7.3 – 0.8%; precision 1.5 – 4.5%
  - Interbatch accuracy -4.7 – 2.2%; precision 3.8 – 7.1%
  - Matrix effects within 12.5% accuracy & precision
  - Quantitative recovery
  - 6h ambient benchtop stability in plasma
  - 4 freeze/thaw cycles (-70°C) in plasma
  - 160 hours reinjection stability

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Summary

- Developed surrogate matrix assay that met client specifications
- Able to separate enantiomers from plasma samples using small sample volume
- Sample preparation takes ~2 hours including derivatization
- Assay is in current use to evaluate efficacy of IDH chemotherapy

Acknowledgements

- Chad Christianson
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