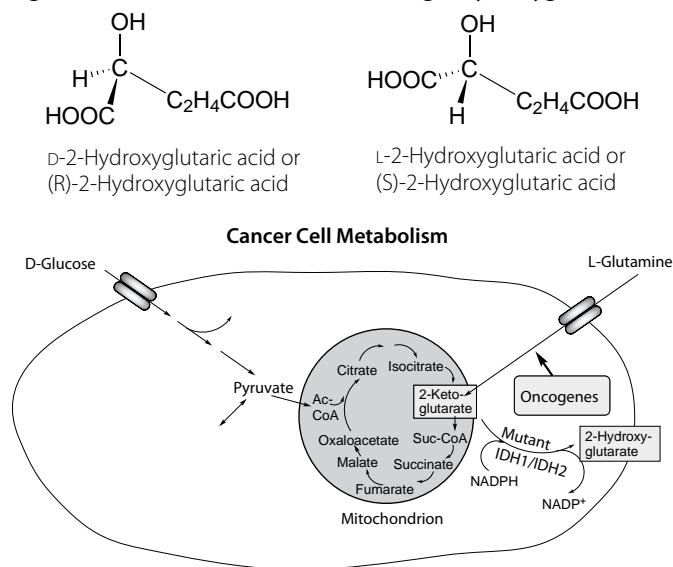


Chiral LC-MS/MS of D- and L-2-Hydroxyglutaric Acid Biomarkers

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Chiral 2-hydroxyglutarates (2-OHG) are important molecular signatures of their specific biochemical pathways and inborn errors of metabolism, for both healthy and diseased biological cells. For example, the TCA cycle of a cancer cell showing the buildup of 2-OHG is depicted in **Figure 1**. The chiral differentiation and quantification of D-2-OHG and L-2-OHG is key for characterizing neurometabolic disorders like the 2-hydroxyglutaric acidurias that cause neurological impairment early in life.¹ In patients with brain tumors, mutations in the enzyme cytosolic isocitrate dehydrogenase 1 (IDH1) are found in approximately 80% of grade II-III gliomas and secondary glioblastomas. The demonstration that cancer-associated IDH1 mutations result in a new ability of the enzyme to catalyze the NADPH-dependent reduction of α -ketoglutarate (2-ketoglutarate) to the oncometabolite D-2-OHG represents a milestone event in cancer biology (see **Figure 2**).² Cancer-associated IDH mutations in IDH1 and IDH2 across glioma as well as several hematologic malignancies have become of prognostic interest and for biomarkers and therapeutic opportunities.³

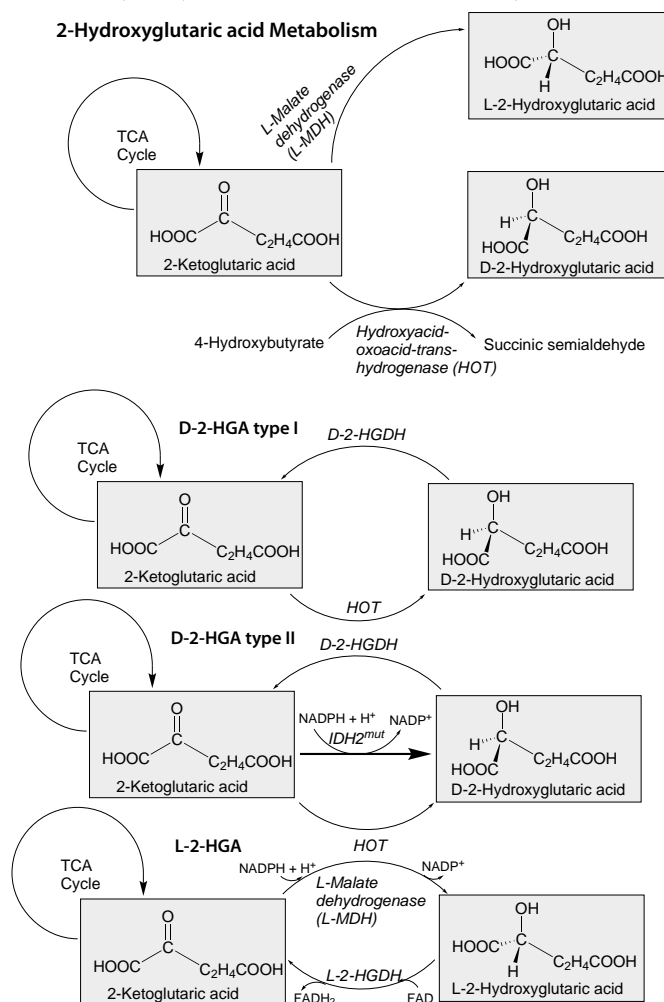
Figure 1. Cancer Cell Metabolism Involving 2-Hydroxyglutaric Acid



The ability to distinguish and quantify the enantiomers of 2-OHG is, therefore, important to be able to track its disposition throughout the metabolic pathway of normal vs. mutated cells (**Figure 2**). We describe here a rapid and sensitive method to separate and measure D-2-OHG and L-2-OHG using high resolution mass spectrometry (HRMS) detection. An Astec® CHIROBIOTIC® R (ristocetin chiral selector) column was run under polar ionic mobile phase conditions.

The separation conditions were based on the work of Rashed, et al, modified in an attempt to improve MS compatibility and separation efficiency.⁴

Figure 2. Hydroxyglutaric Acid Metabolic Pathway



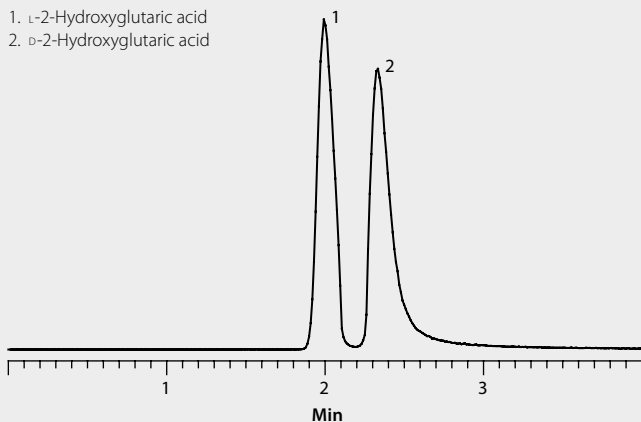
Using HRMS eliminated two fundamental analytical challenges: the lack of a UV-absorbing chromophore in the 2-OHG molecule and the abundance of competing low molecular weight acids in urine.

Figure 3 shows the separation of D-2-OHG and L-2-OHG obtained during method development. Besides providing the necessary enantioselectivity, Astec CHIROBIOTIC columns have the advantage of operating in aqueous and polar organic mobile phases that are amenable to polar drugs and metabolites. Retention and selectivity under such conditions promote analyte ionization which enhances sensitivity in ESI detection. In addition to the CHIROBIOTIC columns, Sigma-Aldrich also provides the high purity mobile phase agents, racemic and pure chiral 2-OHG enantiomers and other compounds in the isocitrate dehydrogenase (IDH) pathway.

Figure 3. LC-MS Analysis of 2-Hydroxyglutaric Acid Enantiomers on Astec CHIROBIOTIC R

column: Astec CHIROBIOTIC R, 10 cm x 4.6 mm I.D., 5 μ m (13022AST)
 mobile phase: 5:95, (water:methanol) with 0.3% acetic acid and 0.1% ammonium hydroxide
 flow rate: 1.0 mL/min
 pressure: 1700 psi (117 bar)
 column temp.: 25 $^{\circ}$ C
 detector: MS, ESI(-) TOF, SIM, m/z 147.03, split 100:1
 injection: 10 μ L
 sample: DL-2-Hydroxyglutaric acid, 1 mg/mL in 50:50, water:methanol

1. L-2-Hydroxyglutaric acid
2. D-2-Hydroxyglutaric acid



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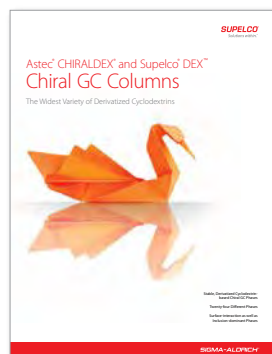
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Astec CHIROBIOTIC R, 10 cm x 4.6 mm I.D., 5 μ m particles	13022AST
Astec CHIROBIOTIC R, 25 cm x 4.6 mm I.D., 5 μ m particles	13024AST
Ethanol, ACS reagent, \geq 99.5% (200 proof), absolute	459844
Methanol, LC-MS Ultra CHROMASOLV, tested for UHPLC-MS, 1 L, 2 L	14262
Water, LC-MS Ultra CHROMASOLV, tested for UHPLC-MS, 1 L, 2 L	14263
Acetic acid, eluent additive for LC-MS, 50 mL	49199
Ammonium hydroxide solution, \geq 25% in H ₂ O, eluent additive for LC-MS, 10 x 1 mL, 100 mL	44273
DL-2-Hydroxyglutaric acid disodium salt	94577
D-2-Hydroxyglutaric acid disodium salt	H8378
L-2-Hydroxyglutaric acid disodium salt	90790
DL-Isocitric acid trisodium salt hydrate	I1252
α -Ketoglutaric acid sodium salt, BioUltra	K2010
IDH Activity Assay Kit	MAK062
Isocitrate Assay Kit	MAK061
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Isocitrate Dehydrogenase 1 (NADP+) human	I2411

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