



Chiral Purity Determination of (+) - Camphor-10-Sulfonic Acid by Packed Column Supercritical Fluid Chromatography (pSFC)

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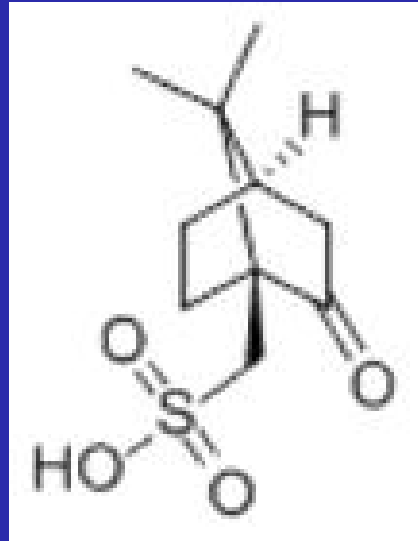


Determination of Chiral Salt by SFC

- Project Background:
 - Late Stage Oncology Project
 - Originally the API was a phosphate salt
 - Formulation switch from IV to solid form which triggered salt screen because phosphate salt easily hydrate/solvate
 - Campsylate salt (CSA) selected due to favorable solid form properties
 - Project team requires the BW (S-(+)-camsylate) salt Form A for the oral campaign.
 - Pilot Plant scaled API had a low level (2-5%) physical impurity peaks in PXRD
 - Team initiated work to identify these peaks – impurity is thought to be the R-(-)-camsylate leading to another crystalline form
 - Source of impurity narrowed down to optical purity of Camphor Sulfonic Acid (CSA) **BUT** project team was unable to determine chiral purity

Chiral Determination of CSA

- Camphor Sulfonic Acid (CSA)



- Commercially available small chiral acid
- No chromophore – therefore traditional HPLC-UV not an option
- GC is difficult without derivitization
- One published method by ESA Biosciences Inc. uses HPLC with CAD



Chiral method Screening


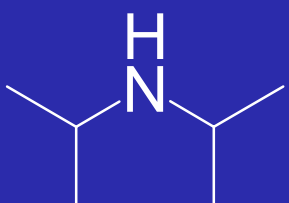


- Typical Screening conditions – Tier-1
- Chiral Columns
 - OD-H, AD-H, AS-H, OJ-H
- Modifiers
 - 0.1% TFA-MeOH
 - 0.1% IPAm or DEA – MeOH
 - 10 mM NH₄OAc - MeOH
 - MeOH
- Detectors
 - PDA
 - ELSD
 - MASS SPEC
- **Basic additive achieved the best recognition for the Sulphonic Acid – optimize conditions**



Chiral method optimization for low level determination of CSA

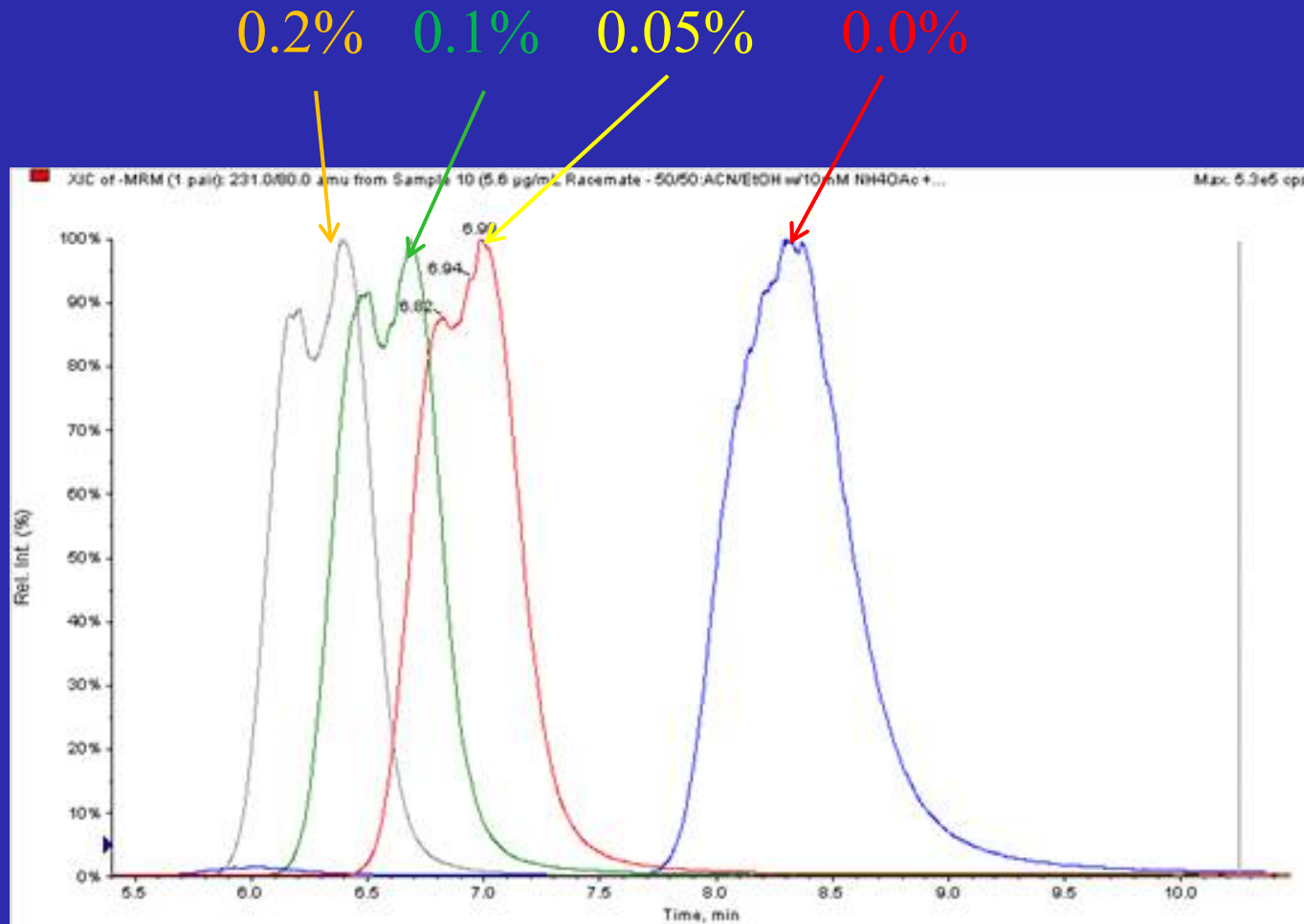
CHROMATOGRAPHIC CONDITIONS								
System:	Agilent 1100 with Aurora A5 and API 4000							
Column:	Chiralpak AD, 3 μ m, 4.6x250mm							
Column Temperature:	35C							
Back/Outlet Pressure:	140 bar							
Injection Volume:	5 μ L							
Flow Rate:	3.5 mL/min							
Detection:	API 4000							
Mobile Phase A:	CO ₂							
Mobile Phase B:	Various (Alcohol and/or Acetonitrile) + Basic Additive							
Dissolving solvent:	MeOH							
Gradient Conditions:	Time (min)	0.5	9.0	9.5	10.5	16	16.5	19.5
	% A	90	85	85	50	50	90	90
	% B	10	15	15	50	50	90	90
Run Time:	20 minutes							

Basic Additive Exploration

- Isopropylamine: 
- Diisopropylamine 
- Diethylamine: 
- Dibutylamine: 



Isopropylamine Additive in MeOH

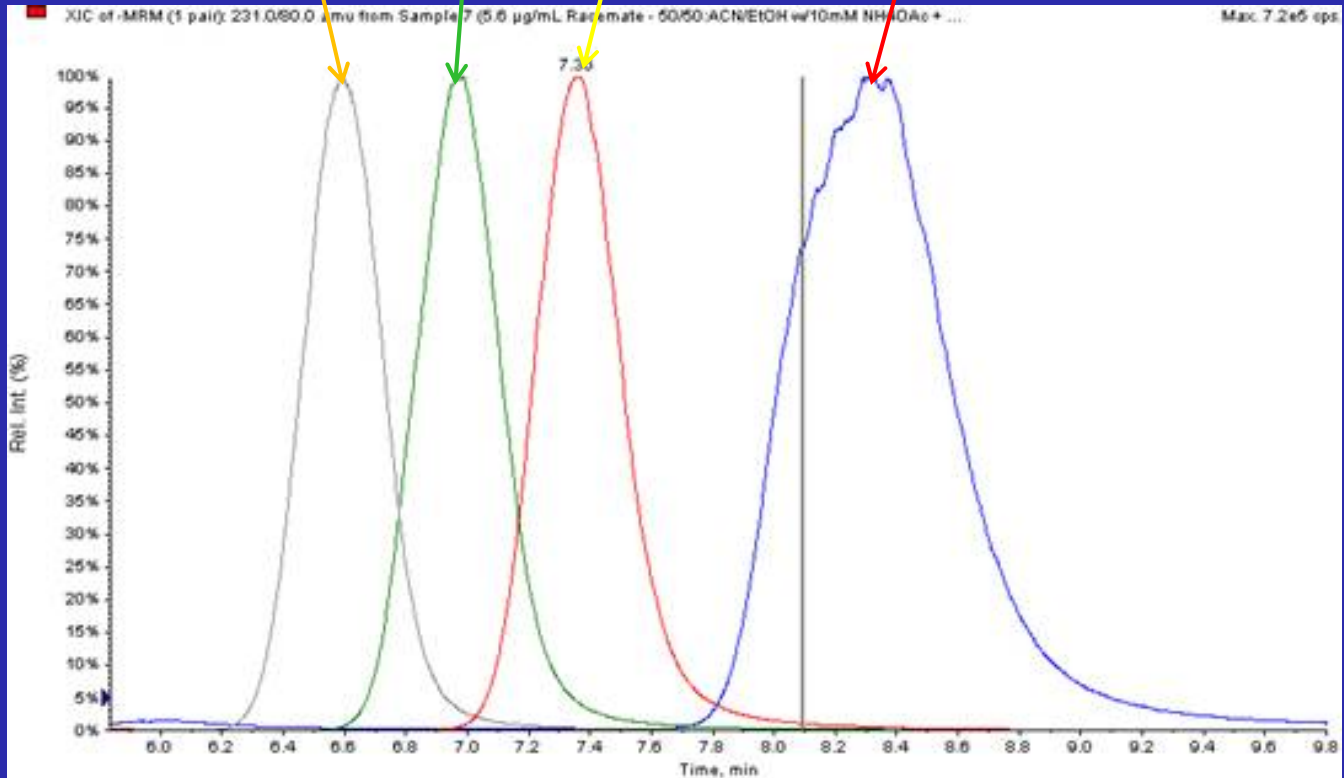


No/minor recognition exhibited



Diisopropylamine Additive in MeOH

0.2% 0.1% 0.05% 0.0%

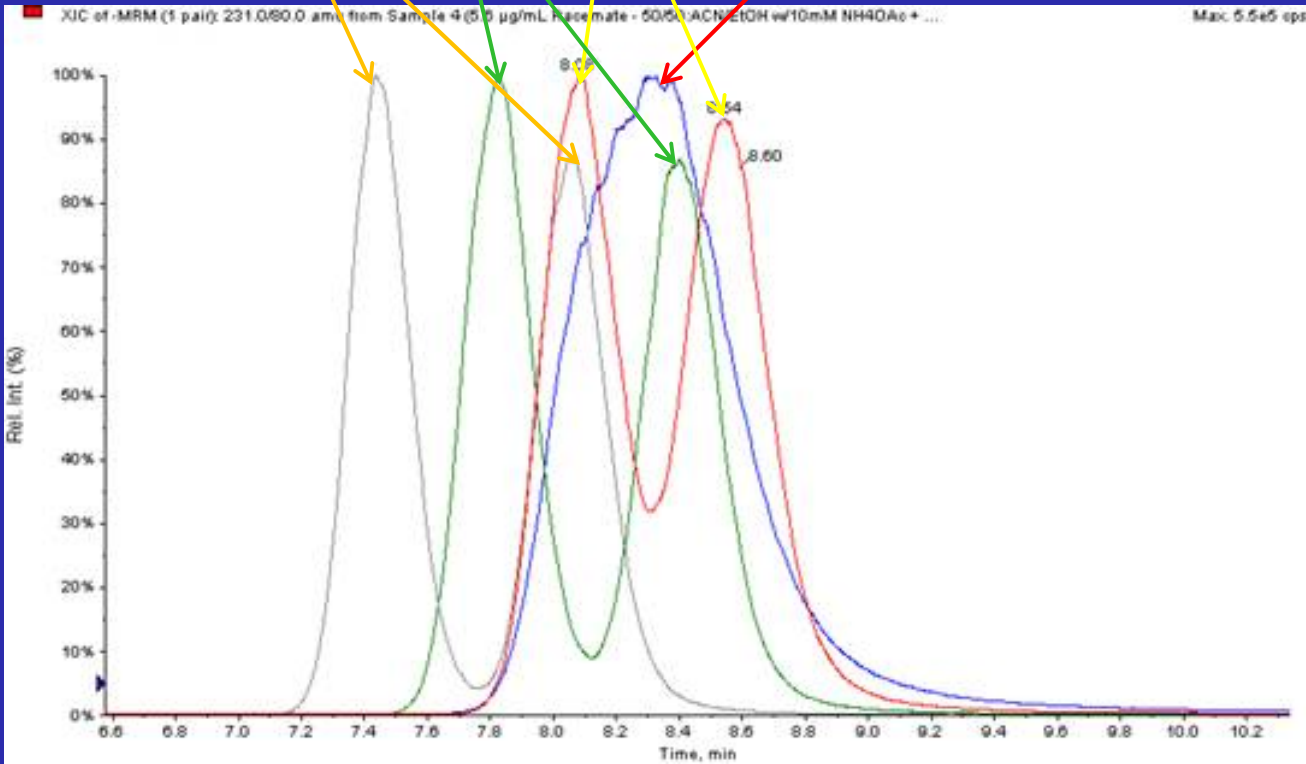


No recognition exhibited



Diethylamine Additive in MeOH

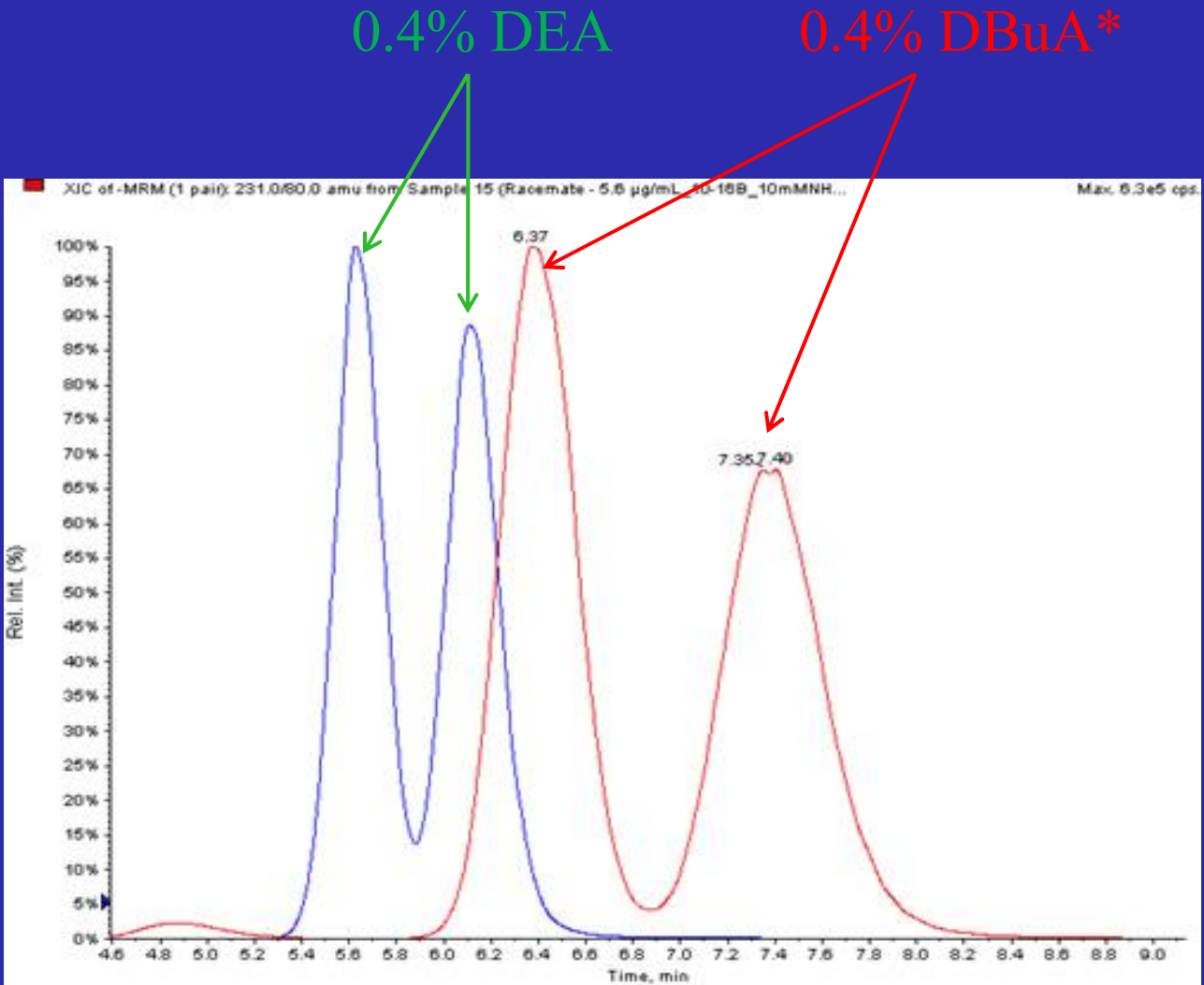
0.2%* 0.1% 0.05% 0.0%



0.2% DEA near baseline resolution



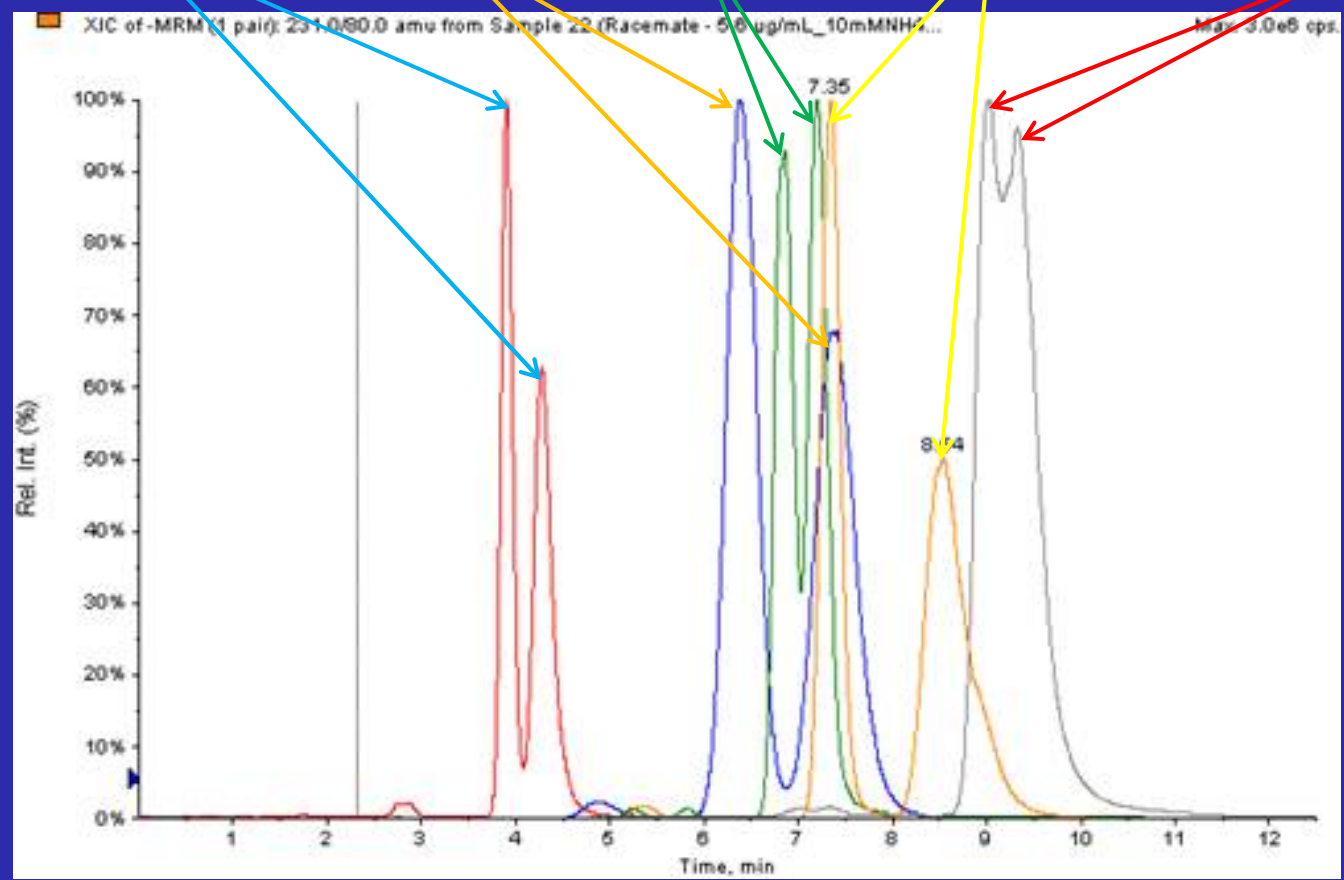
Diethylamine vs. Dibutylamine Additive in MeOH





0.4% Dibutylamine Additive - Solvent Combinations

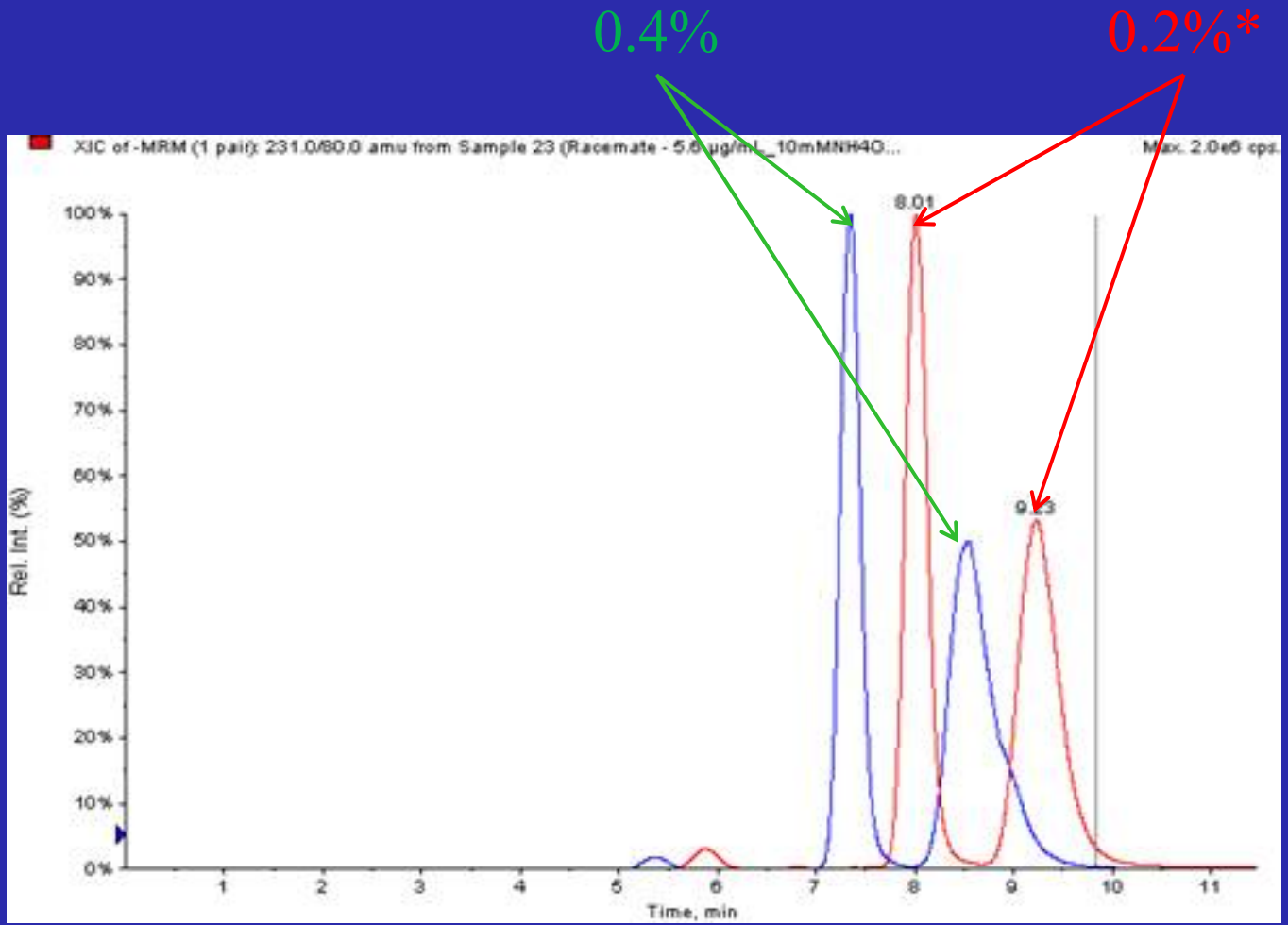
EtOH 1:1 MeOH:ACN iPrOH 1:1 EtOH:ACN* 1:1 iPrOH:ACN



1:1 EtOH:ACN achieved baseline resolution



Dibutylamine Concentration with 1:1 EtOH:ACN



0.2% DBuA selected as method of choice



Summary of CSA Application

- The use of longer-chain alkylamines was the most successful for the separation of the desired sulphonic acid.
- Separation is thought to be due to a combination of steric requirements for chiral recognition, and ion pairing between the CSA and DBuA.
- Able to detect 2-5% of the undesired R-(-)-camsylate in multiple lots of material purchased from vendor that claimed 99.8% S-(+).
- Project team was able to screen multiple suppliers and select vendor based on results provided.
- Continue to understand the mechanism of separation .
- Apply to other chiral acids such as Malic, Tartaric and Lactic .
- Discussions with Norbert Maier, CTI, is greatly appreciated.