



Open Access Purification Utilizing Mass Directed Supercritical Fluid Chromatography

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Introduction

In recent years, the intrinsic nature of drug discovery has placed tremendous emphasis on the discovery chemists ability to deliver high purity novel molecular entities while at the same time maintaining aggressive synthesis goals. In an effort to assist in the purification phase of this process, the Discovery Problem Solving Purification Group, in conjunction with the ARD Structure Elucidation Group, has expanded its vision past traditional Reverse Phase and Normal Phase Open Access purification and looked to SFC Open Access Purification. With the goal in mind that Medicinal Chemists want to readily purify small scale reactions as part of their daily work, in the least amount of time and using a minimum of solvent.

Thar SFC 100 mass directed purification platform

The Thar 100 SFC performs the Pre-Purification Analytical, Purification, and Post-Purification Analysis utilizing a single hardware platform operating within an open-access environment. The system is optimized through a series of pumps, a phase separator, and the FractionLynx software.



AutoPurify Software by Waters Corp.

AutoPurify is an intelligent software package that automates the sample purification process by providing fraction collection control based on target molecular weight, as well as effortless tracking of samples, fractions and associated data operating from within the FractionLynx environment. AutoPurify processing lets you automate the process of initial analytical screening, purification, and fraction reanalysis through a series of three stages, which ultimately increases the overall quality and throughput of collecting fractions for complex mixtures.

Stage 1: Pre-Purification Analytical: A small aliquot is injected to determine whether the compound target mass is present and to measure the UV purity of the sample prior to purification. The confirmation of target mass being found and the sample purity determine whether a sample moves to the preparative stage. This then determines which narrow preparative method, based on target retention time, will be automatically selected for the purification process.

Stage 2: Preparative: The entire sample is injected and the target mass extracted using fraction collection based on the narrow method identified in Stage 1. The target compound is collected into the collection bed. The aim is to extract the target mass from other compounds, thus purifying the sample.

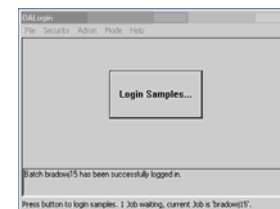
Stage 3: Post-Purification Analytical: A small aliquot is injected from each tube of collected fraction material and the final sample purity is measured along with mass confirmation of the target compound.

Acknowledgements:

Laurence Philippe¹, Bill Farrell¹, and Christine Aurigemma¹ for the advancement of the SFC 100.

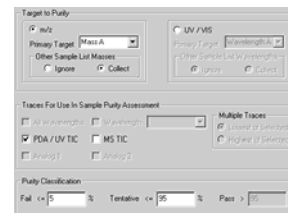
Open Access Log-in

OA Login allows users to purify samples without having an extensive background or training in mass based purification techniques.



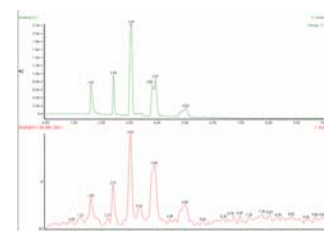
Purification:

Stage 1: Analytical Interpretation is the portion of the software where the administrator establishes the analytical criteria for purity determination. "Target to Purify" allows the admin to identify the collection mode, "Purity Classification" allows limits to be set for the purification strategy.



Pre-Purification Analysis\purification strategy selection

Target Peak: Carbamazepine



Analytical Pre-Purification, 20µL injection
Rt 2.4min: Flavone
Rt 3.0min: Carbamazepine
Rt 3.8min: Amcinonide
Rt 3.9min: Ketoprofen
Rt 5.0min: Sulfamethazine

Strategy Selection

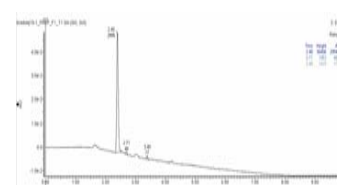
| TARGET | PURITY | PURIFICATION STRATEGY |
|-----------|-----------|-----------------------|
| Not Found | N/A | [None] |
| Found | No Peak | [Narrow] |
| Found | Fail | [Narrow] |
| Found | Tentative | [Narrow] |
| Found | Pass | [Narrow] |

The System takes the results and matches it with the administrator defined purification strategy

Purify Using Narrow Gradient C

Retention time of the target compound determines the method of choice

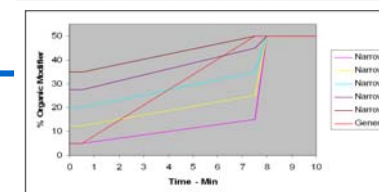
Post-Purification Fraction Analysis of Carbamazepine



Analytical re-analysis, Carbamazepine, 100µL injection

Narrow Method Settings

| Run | Time | Flow | Name | Description | Use Method | Search | Flow Rate Method | Purge Flow Method |
|-----|------|------|----------|-------------|-------------|-------------|------------------|-------------------|
| A | 1.00 | 2.00 | Narrow A | Narrow | JP_Narrow_A | JP_Narrow_A | JP_Narrow_A | JP_Narrow_A |
| B | 1.00 | 3.00 | Narrow B | Narrow | JP_Narrow_B | JP_Narrow_B | JP_Narrow_B | JP_Narrow_B |
| C | 1.00 | 4.00 | Narrow C | Narrow | JP_Narrow_C | JP_Narrow_C | JP_Narrow_C | JP_Narrow_C |
| D | 1.00 | 5.00 | Narrow D | Narrow | JP_Narrow_D | JP_Narrow_D | JP_Narrow_D | JP_Narrow_D |
| E | 1.00 | 6.00 | Narrow E | Narrow | JP_Narrow_E | JP_Narrow_E | JP_Narrow_E | JP_Narrow_E |
| F | 6.00 | 9.00 | Narrow F | Narrow | JP_Narrow_F | JP_Narrow_F | JP_Narrow_F | JP_Narrow_F |



Narrow methods as programmed, independent inlet files