MODULAR SFC™

Green Flash SFC

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Introduction

This poster describes instrumentation used for translating data from a TLC plate to a Supercritical Fluid Chromatography SFC system in order to create a mobile phase program that will separate the component(s) of interest from the other material in the sample.

Definition

Flash SFC – Utilization of TLC plates to elucidate mobile phase gradient programs for SFC which will allow separation of the material of interest from the other components in the sample.

Overview

Flash chromatography is the purification tool of choice for medicinal, synthetic and organic chemists (chemists hereafter) in the pharmaceutical as well as several other industries. During the drug development process, chemists synthesize molecules to test against certain targets for a given therapeutic area. The synthesis process could have several steps where different starting materials are reacted together to produce a final product. To speed up the process, many times the reactions are not left to go to completion. The goal is not to completely use all the starting products but rather to synthesize a small amount of the desired product as quickly as possible. By synthesizing the final product as soon as possible and with as little cost as possible, this allows chemists to rapidly determine if the materials will fail or pass the screening tests. The common industry phrase for this is "fail fast". Because reactions are not going to completion or because potential undesired products are created during the reaction process, chemists will use flash chromatography to isolate products of interest from the undesired products.

Flash SFC System

Flash SFC describes the process or instrumentation used for translating data from a TLC plate to the SFC system in order to separate the desired material in the sample from the undesired material and collect the desired material.

The Modular SFC Flash SFC system is composed of the following components:

CO_2 Pump - a pump modified to deliver liquefied gas to the flash sfc column.

Modifier Pump - a standard HPLC pump that withstands the system pressure.

Tee - combines the flow of the CO_2 pump and the modifier pump.

Injection Valve - Manual or automated for introducing the sample into the mobile phase before the column.

Solid Injection Module - introduces the sample pre-adsorbed onto some material, typically celite or silica (but could be the same material used in the flash SFC column) into the mobile phase.

Stationary Phase Column - provides the means of separating the components in the sample.

Detector - provides a response that indicates that material has passed through the detector flow cell.

Back Pressure Regulator - Device used to control, set or maintain the pressure necessary to keep the CO_2 or other material in a liquid, supercritical or liquid like state.

CFC-2 Series Fraction Collector - collects the fraction based on the detector response or a specific time.

Software Control - software to control system components.

Solid Injection Module

Back Pressure Regulator

CFC-2 Fraction Collector

CO_2 Pump Head Heat Exchanger

Experimental Data

A three component mixture was created with ibuprofen, caffeine and uracil. Saturated solutions were made of caffeine and uracil in ethanol. Ibuprofen was dissolved in ethanol to yield a solution with a concentration of approximately 10 mg/mL. Aliquots from each of these were diluted ten fold to make the mother solution. Each individual solution and the mother solution were spotted on the cyano propyl TLC plate. The spotted TLC plate was placed in a solution of 5% ethanol in hexanes. The flask was covered with a nitrile glove and the solvent front was monitored until it reached the desired point near the end of the plate. The plate was dried and then subjected to UV light at 254 nm. The migrated spots visible under the UV light were circled and the Rf values measured.

TLC plate spots from left to right: Caffeine, Uracil, Ibuprofen, and mixture of the three. Spots were computer enhanced to increase visibility.

Rf values - measured to the center of each spot:

Solvent front - 6.95 cm
Caffeine - 1.0 cm = 0.144 Rf
Uracil - 0.7 cm = 0.101 Rf
Ibuprofen - 5.0 cm = 0.719 Rf

Theoretical column volume values:

Caffeine - 6.94
Uracil - 9.92
Ibuprofen - 1.39

Column assumptions - appropriate data was not available from manufacturer so assumptions had to be made. These assumptions were based on data from similar sized columns.

1 column volume = 50 mL - instrument flow rate for experiment was 50 mL/min - 1 column volume = 1 minute

Theoretical elution times:

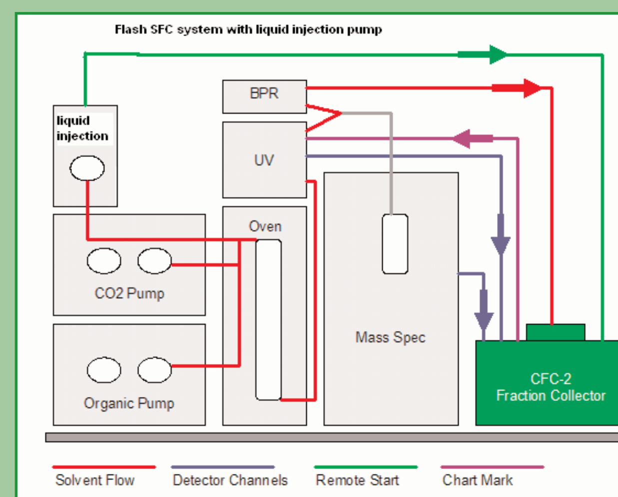
Caffeine - 6.94 min
Uracil - 9.92 min
Ibuprofen - 1.39 min (not a reliable data point because of lack of interaction with stationary phase; typically spots that migrate closely to the solvent front do not correlate with Rf to CV theory)
Each sample was injected using the solid loading method and then the mixture was injected using the same method.
Gradient: 2 to 10% Ethanol in CO2 over 12 minutes

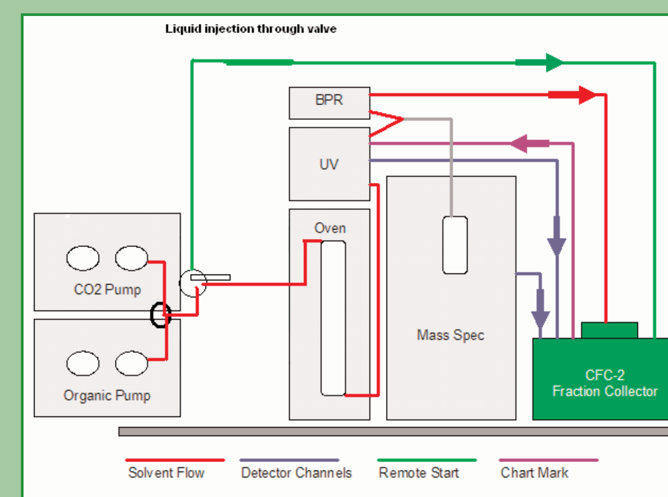
Flash SFC System Information

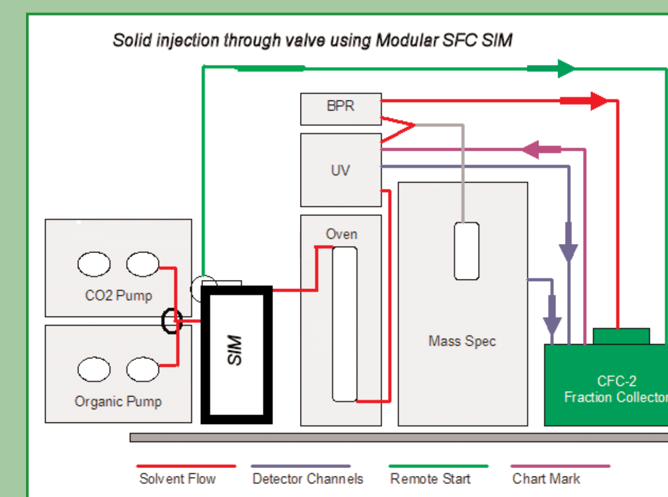
TLC Plates - Silica gel 60 CN HPTLC from VWR - 5 um particle size silica bonded with cyano propyl functional group
Column - Princeton Chromatography Cyano propyl - 6 um particle size
Pumps - Knauer K-1800
Heat Exchanger - Knauer
Chiller - VWR Scientific Products (Manufacturer: PolyScience Model: 1156)
Injection Pump - Knauer Smartline 100
Mixing tees - Valco
Manual Injector - Knauer
Solid Injection Module - Modular SFC
Detector - Knauer Smartline 200 (254nm)
Back Pressure Regulator - Modular SFC
Fraction Collector - Modular SFC CFC-2c
Software - Knauer Chromgate

SFC Methodology

The goal for Flash SFC is to correlate TLC data to the chromatogram mobile phase gradient in order to separate the desired components from the undesired ones and to collect the desired components. In this application, the appropriate separation of the sample components was achieved at 5% ethanol in hexanes on the TLC. Therefore, the SFC gradient should be made so that 5% ethanol is approximately the mid-point of the gradient.





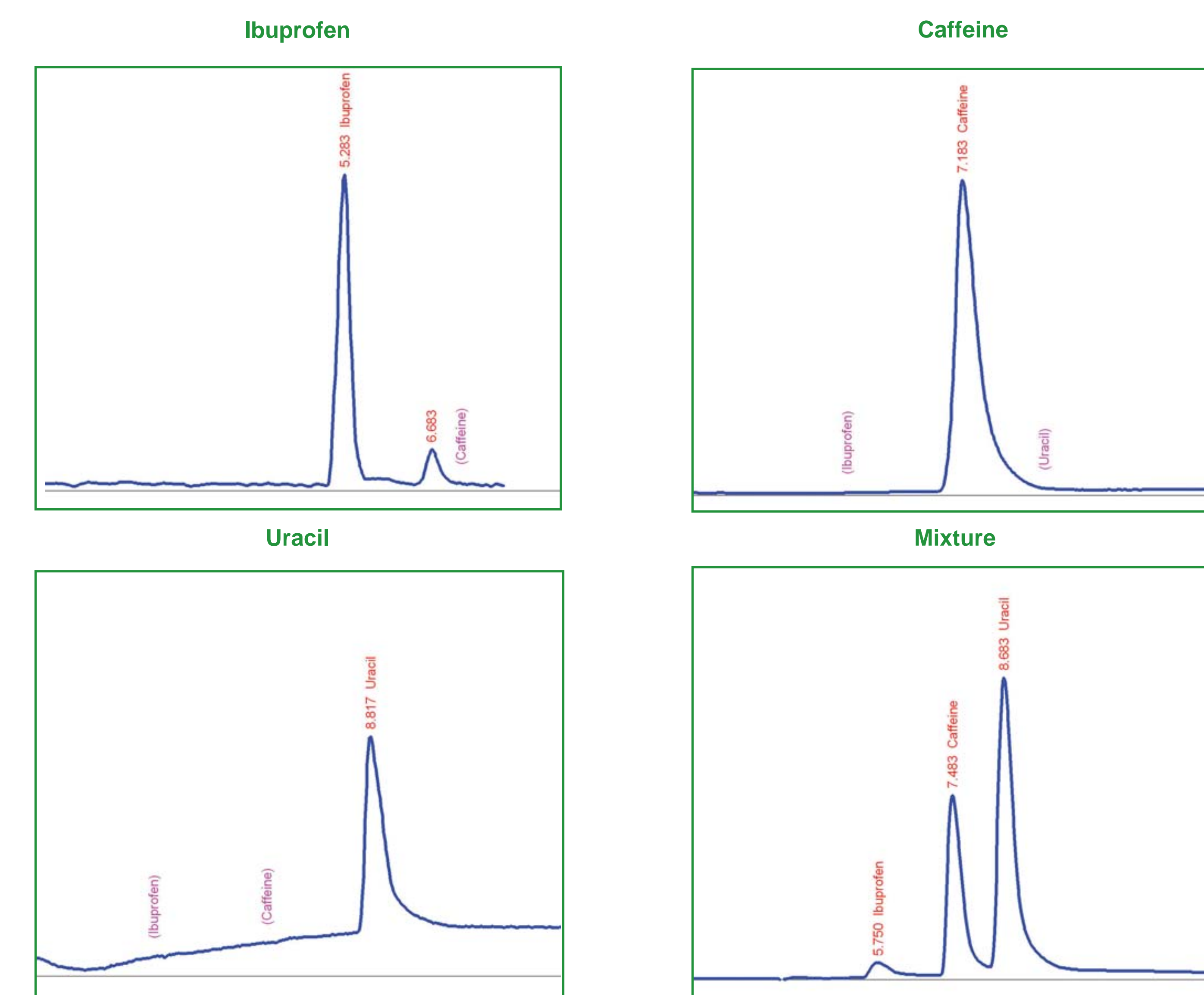


Flash SFC Method

Injection: Solid load method, sample pre adsorbed onto celite
Detector: 254 nm
Back Pressure: 100 bar
Collection: Threshold

Results and Discussion

Each sample was injected using the solid loading method and then the mixture was injected using the same method:



The data shows the appropriate correlation between the TLC and the chromatogram was obtained proving that SFC can be correlated to the TLC plate.

Conclusion

The data in the poster demonstrates the feasibility of Green Flash SFC which is a new paradigm in the small molecule purification process. Flash SFC will provide chemists with the means of isolating material of interest 3 to 5 times faster than traditional LC and with up to 90% less solvent. Taking the total purification process into account (including solvent removal), Flash SFC can produce pure material of interest as much as 10 times faster than traditional LC. The ultimate goal of Green Flash is to produce an SFC purification process that is as easy to use at Flash LC and that will bring SFC into the mainstream of pharmaceutical small molecule purification thus significantly reducing the carbon footprint for the purification process.

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