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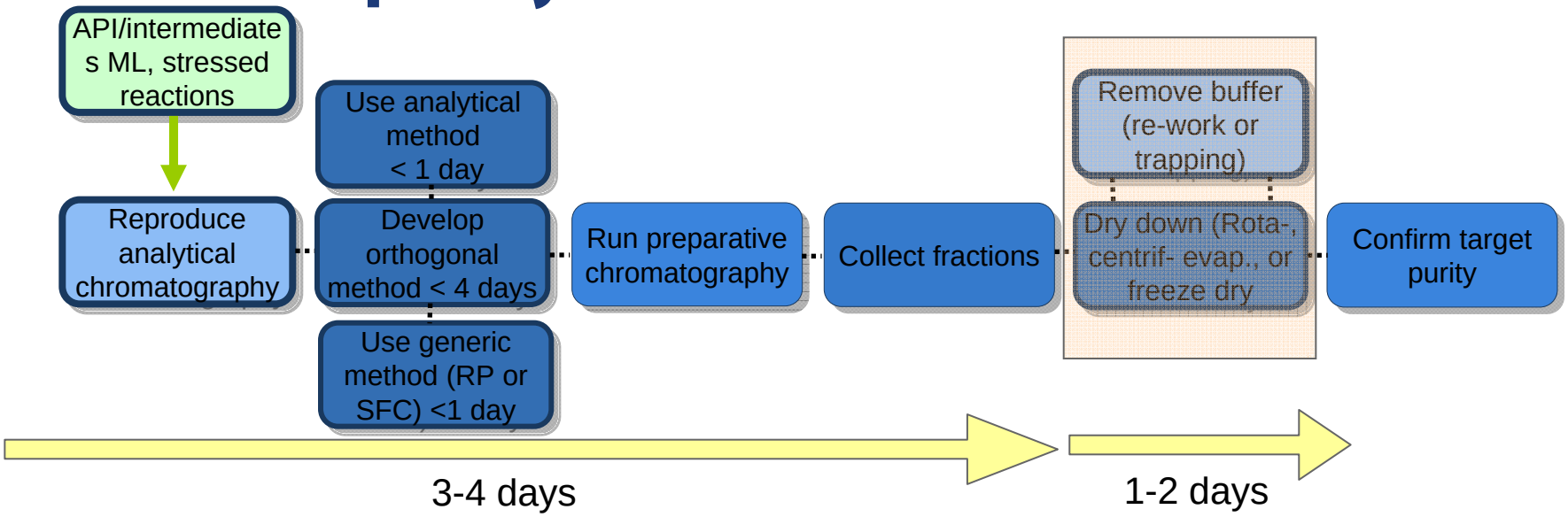
Abstract

Preparative chromatography is employed to separate and isolate the active compound. Preparative purification (50 x 150 mm, 75 mL/min, mass 0.3 – 15 gm) creates a fraction volume in the 20-120 mL range. The purified fraction collected requires obviously dry down to remove the mobile phase. Since the common preparative technique employed for purification is RP-HPLC the purified fraction will contain an aqueous and organic component including a modifier. Elimination of the solvent associated with the purified fraction requires vacuum/temperature increase. Concentration of the mobile phase modifier has been shown to cause hydrolysis of functional groups, possibly destroying the activity of the purified compound. In addition evaporation or dry-down is time consuming and requires manual intervention. In the case of large preparative fractions a specially designed polymer resin (patent pending) packed within a HPLC column is brought in-line through a switching valve in order to concentrate the fraction. The fraction loaded onto the trapping HPLC column via a high pressure/flow dilution pump trapping the sample on the head of the column. The concentrated fraction is then back extracted off the column in pure organic in a fraction of the initial volume. This process uses 2 trapping columns with a switching valve; as one of the columns is trapping a sample the other is being regenerated for the next sample, greatly increasing the number of samples processed per unit of time. In addition several organic solvents can be chosen (e.g. MeOH, ACN, THF) allowing for optimum back extraction of the compound into a small volume of organic. This trapping method allows for fractions collected to undergo transformation into 100% organic solvent without operator intervention, drastically reducing the dry down time and eliminating hydrolysis of the compound. The HPLC trapping column has been shown to be very robust and rugged stable for 1000s of fraction trapping samples.

Impurity Isolation Workflow



- Average substrate contains 6 impurities at 0.05% to 0.3%
- Timelines per substrate runs into weeks, average of 6 substrates per project, total project resource runs in to months
- Issues are debuffering, dry-down and slowness

Gilson FT-LC System

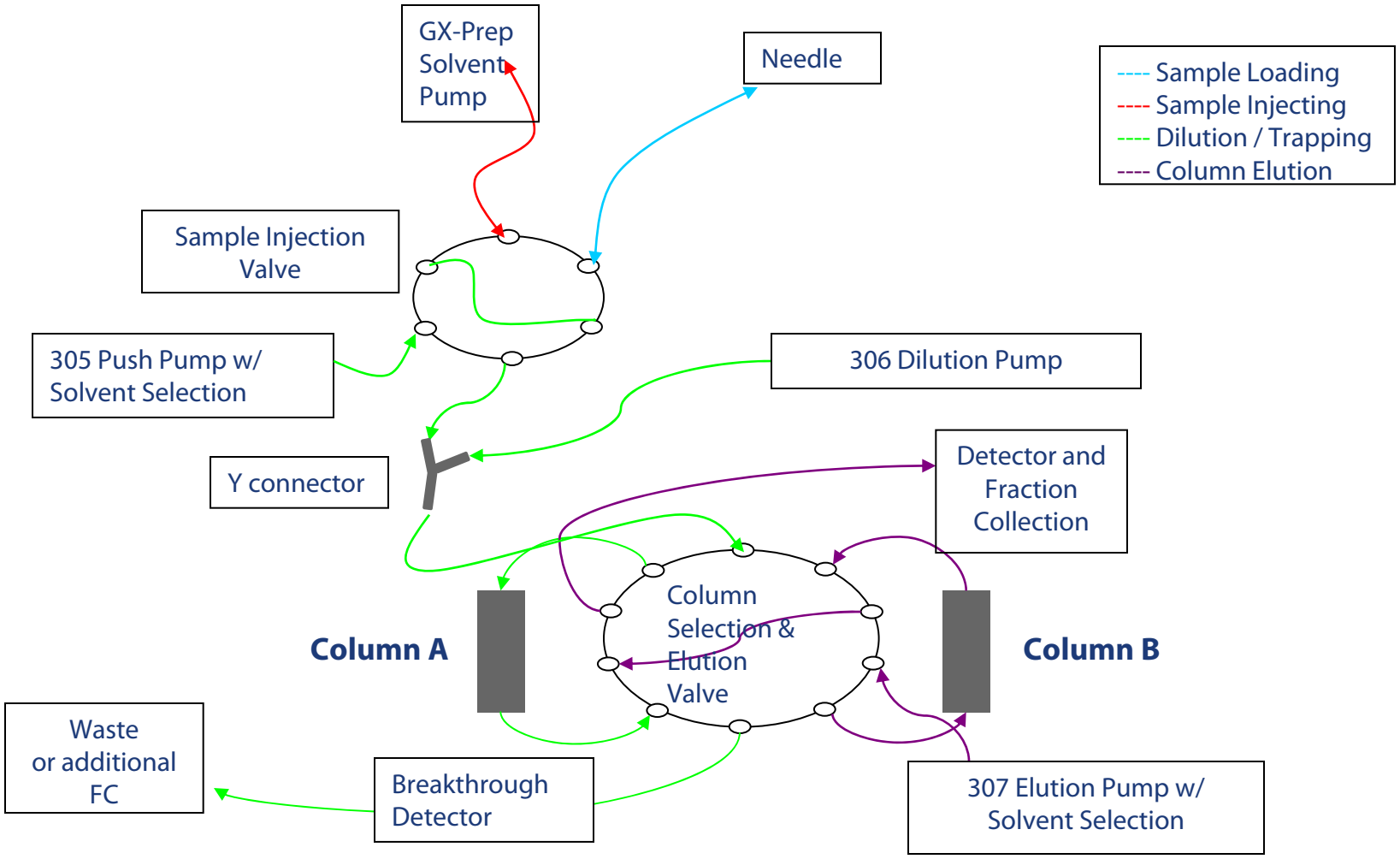
FT-LC System Components

- GX-271 Injector/Fraction Collector
  - Load Fractions up to 25 mL Fractions
  - 3 pump system:
    Loading Pump
    Dilution Pump
    Elution Pump
  - Two VALVEMATE II: 2 position 10 Port for dual column with regeneration; and 6 position Low Pressure for Solvent Selection
  - 155 UV/VIS Detector with High Pressure Flow Cell (SFC)

Optional Components

- Break Through Detector
  - Secondary Fraction Collector Connected to Break Through Detector
  - Additional Solvent Selection Valve on one of the loading pumps.

FTLC Schematic



Protocol

- Columns are a 45 micron Polymeric based material packed in a 4.6, 7, 10, or 21.2 mm ID column
  - Loading capacity of several 100 mg

1) Wash Column 1 and load sample in to sample loop

2) Load sample on to column 1 with loading Solvent A or B depending on Compound
   retention time. For early eluting compounds (<40% ACN) dilute with Water, for compounds that elute 40-75% use another solution.

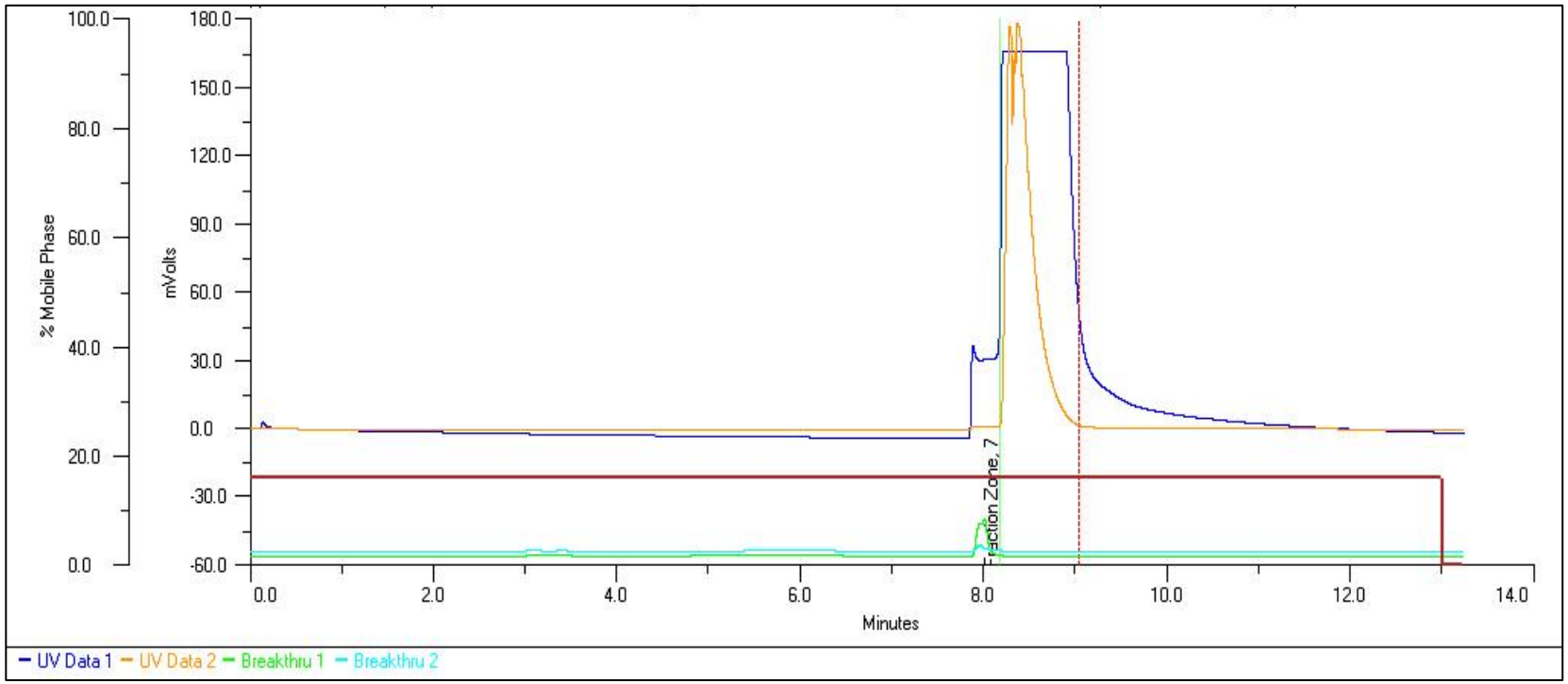
3) Elution of Column 1 with DCM/Methanol or Acetonitrile
   While simultaneously washing column 2 with Acetonitrile, DCM, THF & then Re-Equilibrium of Column 2 takes place

Total Time = 12-18 mins per cycle depending size of fraction and wash cycle.

FTLC Process

Column A	Column B
Contains Trapping Solvent (Trapping Solvent from Previous sample)	Contains Elution Solvent (DCM/MeOH)
Equilibrating with chosen Trapping Solvent	Wash with Wash Solvent A (THF/Acetone)
Load Sample into Loop	"
Switch Valve and Wait for Sample to Trap on trap column (Needle Rinse during this time)	Wash with Wash Solvent B (ACN)
Rinse Sample Loop and Valve	Switch to Elution Solvent for FC Baseline
Wash with Desalt Solvent or Water (with appropriate wait time)	
Column Switch to Start Trap Elution	
Sample elutes in Elution Solvent and FC occurs	Column Equilibrates with Trapping Solvent ready for trapping of next Sample

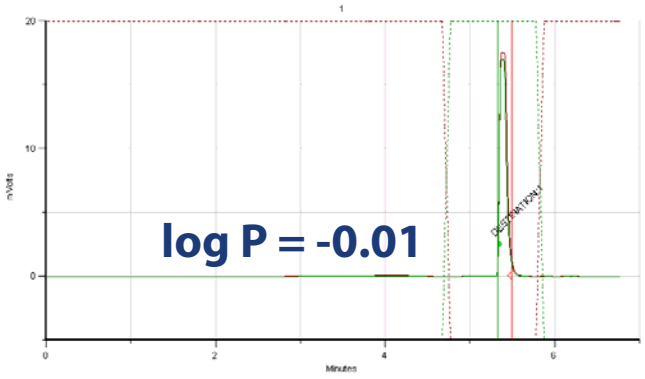
Propranolol



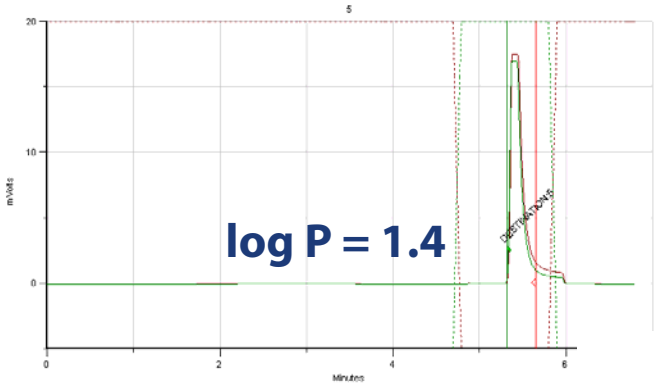
Load 40 mg Propranolol in 9mL containing 37% ACN, dilute 1:5 Water

Total loading flow rate 16 mL/min

Injection of Compounds with Different Lipophilicities onto 4.6 x 150 DVB column, 45 um

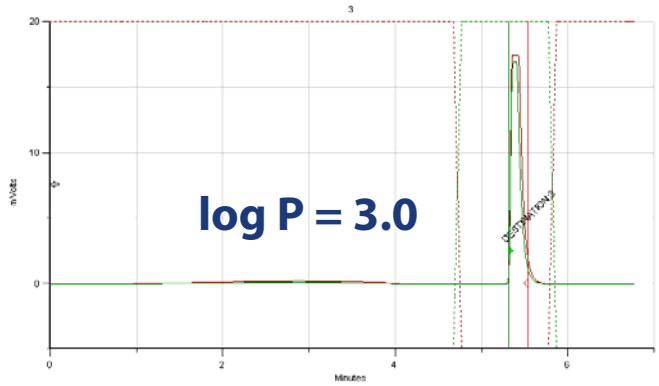


Metronidazole

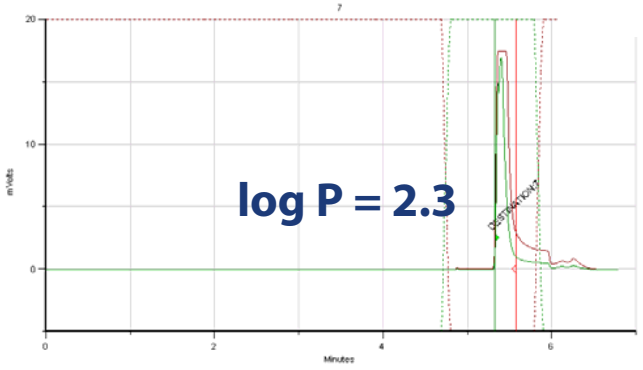


Cortisone

$$\log P_{oct/wat} = \log\left(\frac{[solute]_{octanol}}{[solute]^{un-ionized}_{water}}\right)$$

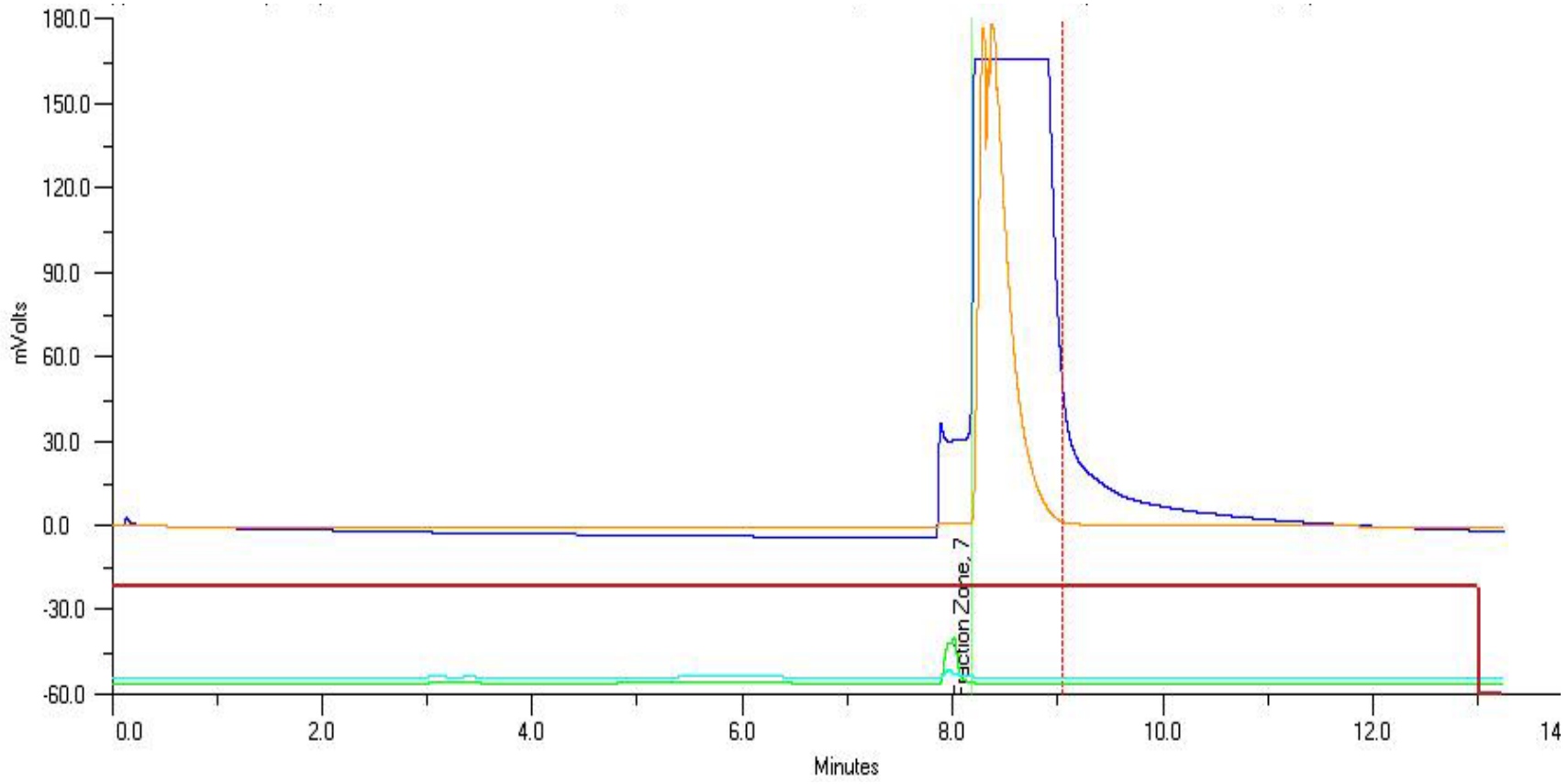


Amitriptyline



2-Hydroxybenzoic acid

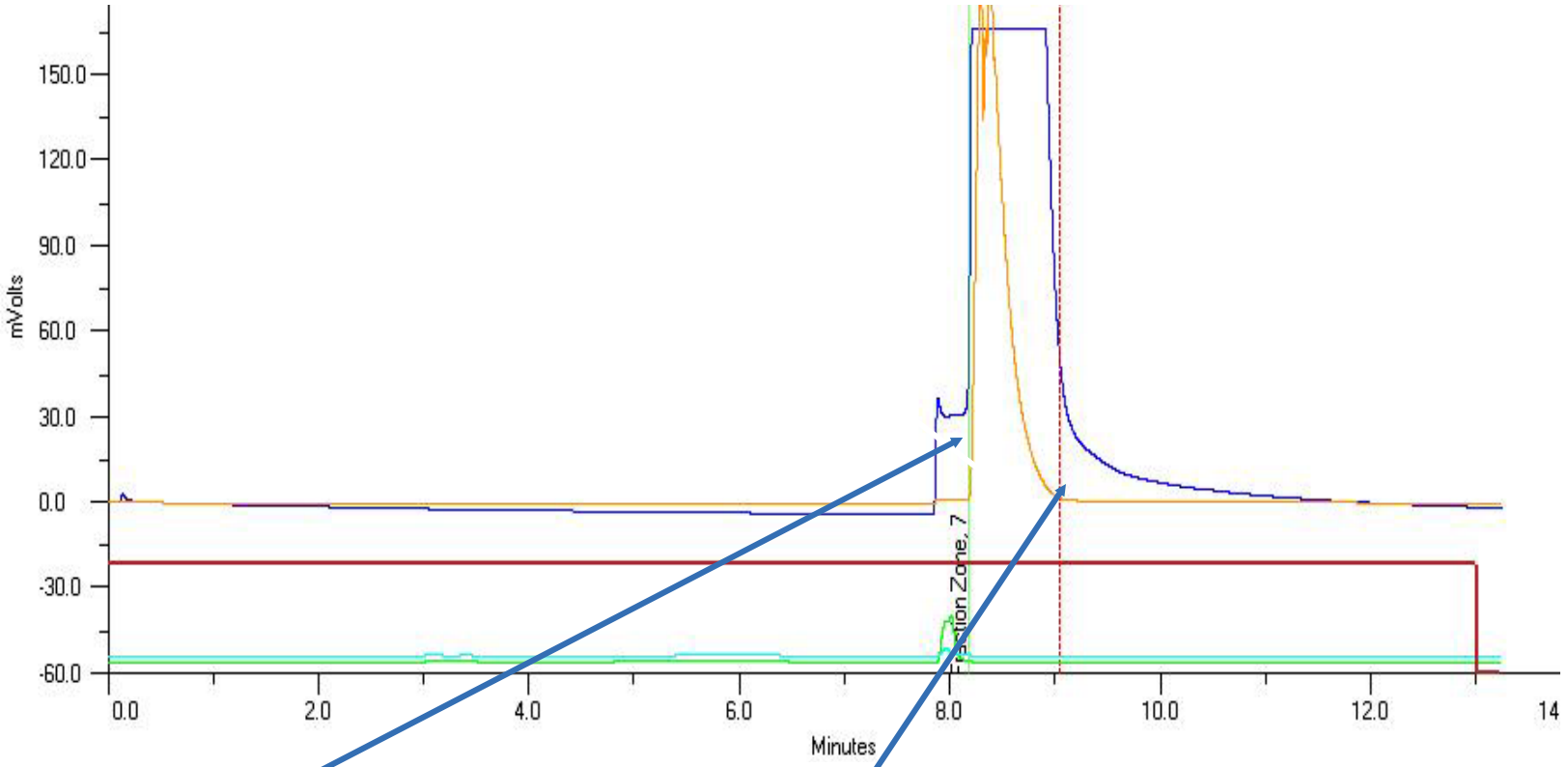
Water Content Methanol Elution



Load 40 mg in 9mL containing 37% ACN, dilute 1:5 Water.

Collected fractions across the peak in order to measured water content.

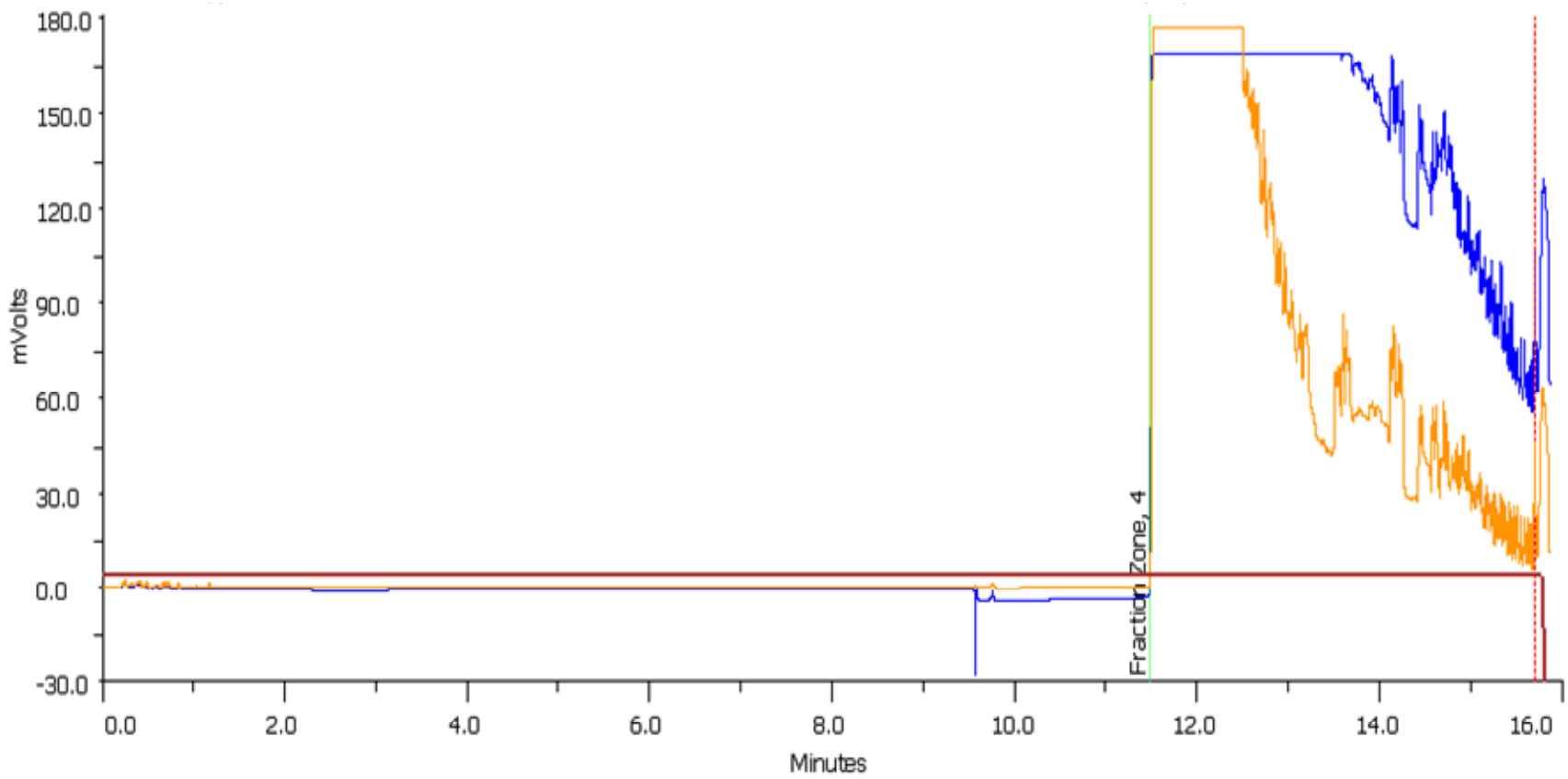
Water Content with Methanol



1) 84% water which reduced to 0.6%
2) The experiment was run again and collected the fraction measured the water content it was found to be < 4.5%
3) Recover was slightly reduced to 85% due to fraction collection parameters

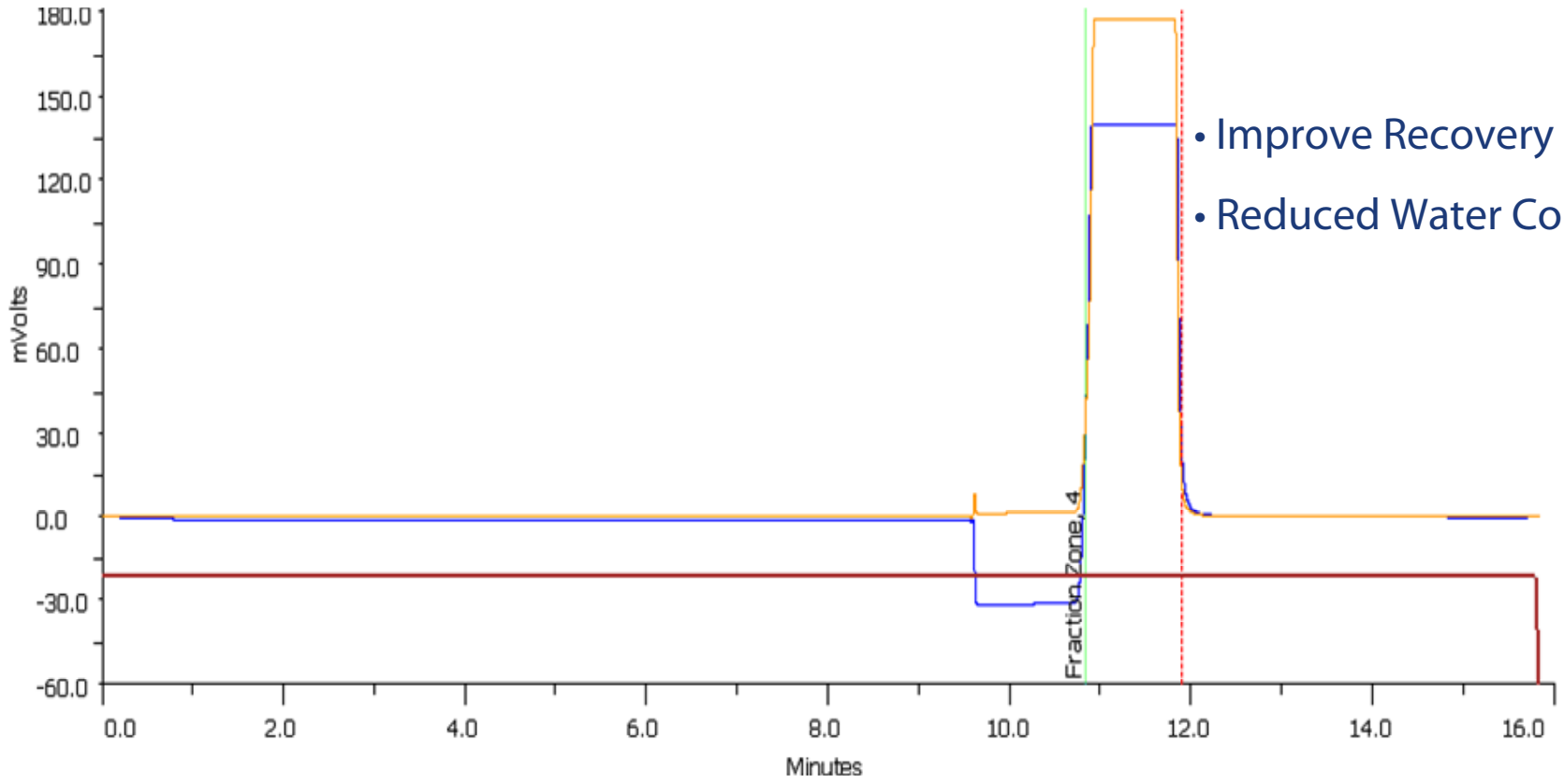
MeOH vs DCM/MeOH Elution

Warfarin 103mg, 18mL load, 10%MeOH/DCM eluted, 15mL fraction. 99.5% recovery



MeOH vs DCM/MeOH Elution

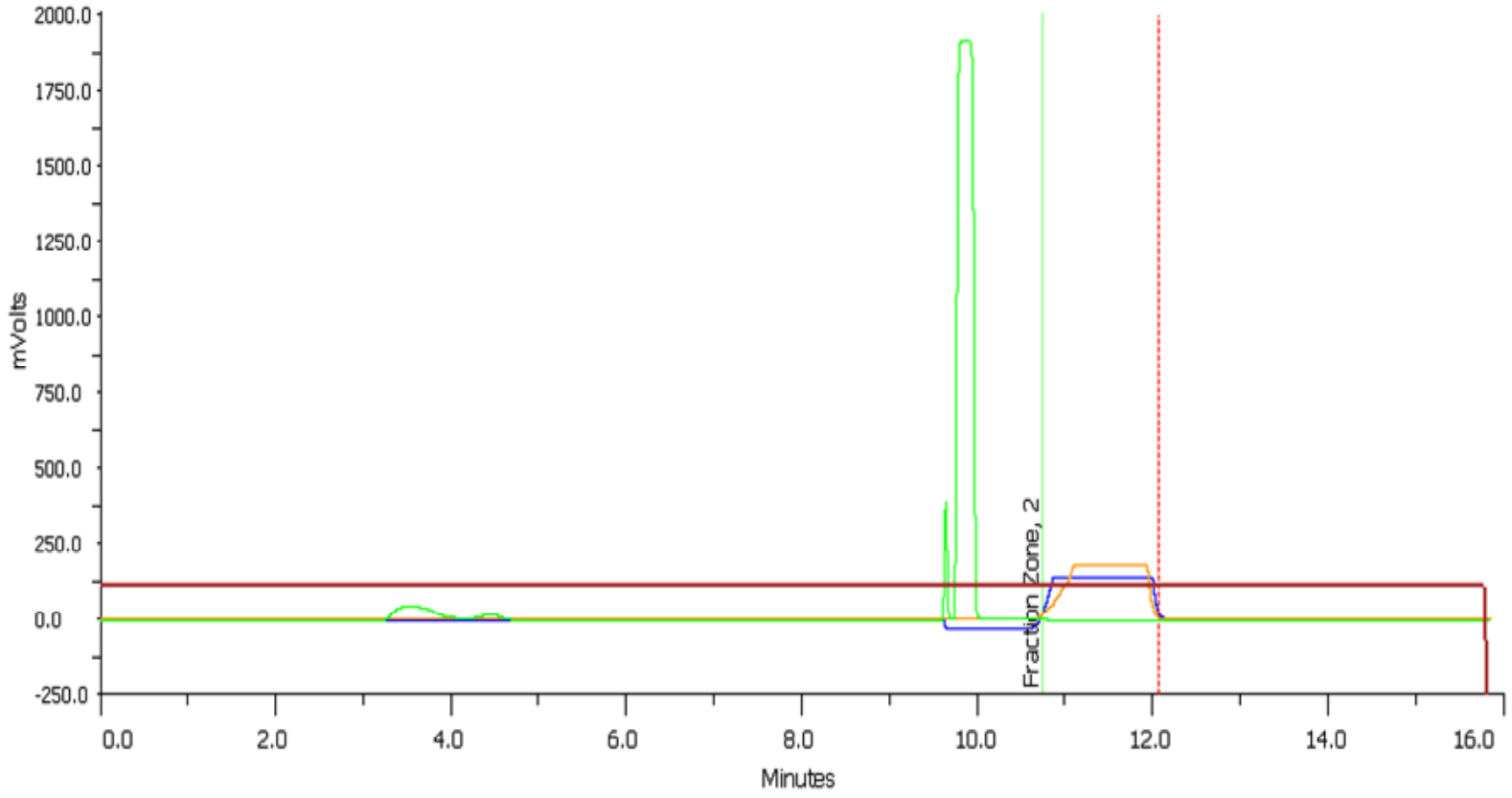
Bromoacetamido 99mg, 18mL load, 10%MeOH/DCM eluted 7mL fraction 94%recovery



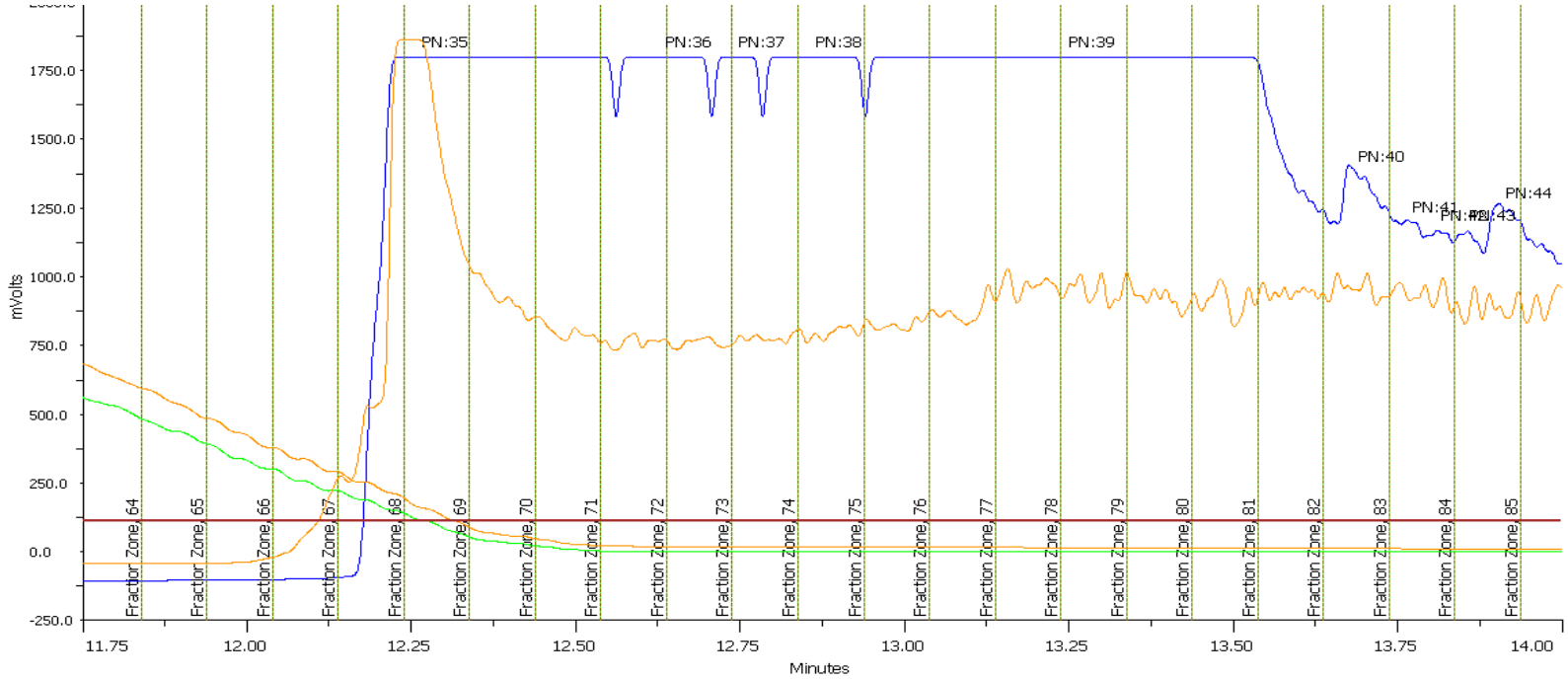
- Improve Recovery
- Reduced Water Content

DCM/MeOH Elution

Sample, with ‘breakthrough’ detector (green trace) AMME 103mg, 18mL load, 10%MeOH/DCM eluted, 95% recovery



Fraction Collection & Removal of Water



Elution profile match that of a blank elution and compounds diluted with Solvent 1. Fraction slices 0.5mL with NO fraction collection delay.

Frac 68 no product 100 water

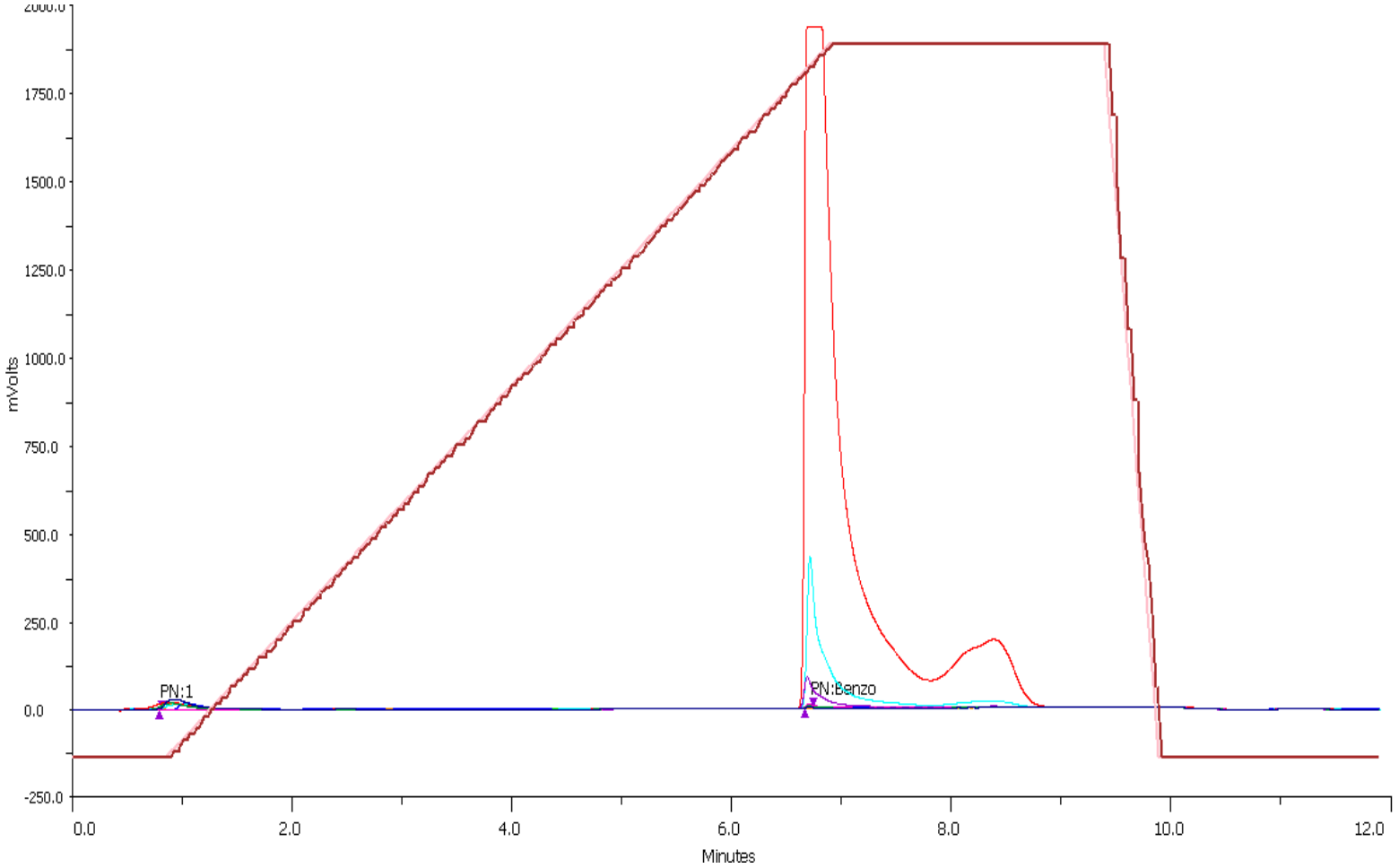
Frac 69 minimal sample maybe 1-2mg max just starting to elute

Frac 70 more significant amount of sample

Frac 71 EUREKA

Frac 75 not a lot eluting now similar

Fraction Collection & Removal of Water

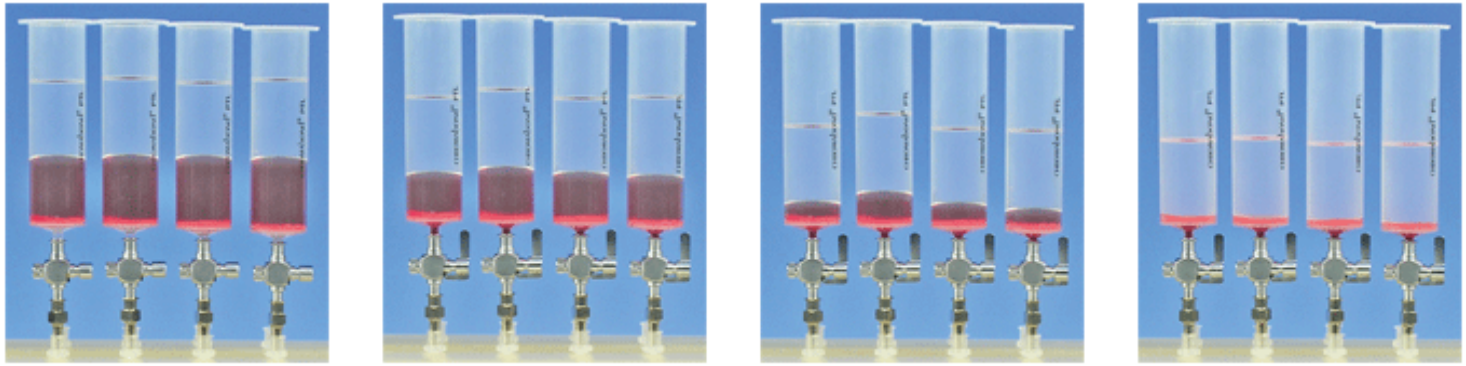


Analysis of the first fractions across the peak looking at 8 fractions across the peak looking at the water content and peak response

Phase Separation Columns

CHROMABOND® PTS / PTL

columns for phase separation



CHROMABOND® PTL in action: organic upper phase (colourless), aqueous lower phase (red)

Features

- **automatic separation of a two-phase mixture without separation funnel**
  two-phase mixtures are completely applied to the column and the phase boundary is determined without further work. The special membrane stops automatically and the interesting phase is separated.
  columns **must not** be run with vacuum or pressure
- **PTS**
  for solvents **heavier** than water, e. g. for trichloromethane, dichloromethane etc.
  maximum size 150 ml
- **PTL**
  for solvents **lighter** than water, e. g. for diethyl ether, hexane etc.
  maximum size 70 ml

Chromabond® Phase Separator

"Rules of Thumb".....Optimizing Trapping Conditions

$$log\ P_{oct/wat} = log\left(\frac{[solute]_{octanol}}{[solute]^{un-ionized}_{water}}\right)$$

- Early/mid eluters which are water soluble and elute from PrepLC with up to 40% ACN, e.g. Propranolol (log P 3.09)
  - *Should be trapped with 100% Water*
- Mid and late eluters which elute from PrepLC between 40-80% ACN e.g. Probenecid (log P 3.302) or Benzophenone (log P 3.18)
  - *Should be trapped with 20% ACN/Water*
- Very late eluters which elute from PrepLC later than 80% ACN e.g. Dibenzofuran (log P 4.12)
  - *May be trapped with 30-40% ACN*
  - *Although these compounds do not benefit greatly from FTLC*
  - *Limited studies*

Log P Calculations

There are many log P calculators or predictors available both commercially and via freeware

- Chemistry Development Kit
- JOELib
- ACD/LogP DB a commercial application that calculates Log P values and includes the largest commercially available database of experimental log P values with calculation of Rule-of-5 parameters
- ACD/LogP Freeware Download the free log P calculator [1]
- Simulations Plus - S+logP an application for calculating log P with high accuracy[1]
- ALOGPS Free online calculations and comparison of 10 log P methods
- Free online logP calculations using ChemAxon's Marvin and Calculator Plugins - requires Java
- miLogP free log P and Rule of Five calculator by
- an overview of on-line WWW resources for logP and other PhysProp calculations
- PreADMET Web-based logP/logS and ADME/Tox prediction program
- XLOGP3 a log P calculator by guiding an additive model with knowledge. Free for academy.

[1] Tetko, IV; Poda GI (2007). "Property-based logP prediction". Mannhold R *Molecular Drug Properties : Measurement and Prediction*, Weinheim, Germany: Wiley-VCH.

Solvents used in FTLC System

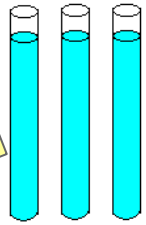
- Solvents available on Trapping Pump
  1) Water: trap solvent for early PrepLC eluters
  2) 20% ACN/Water: trap solvent for mid to late PrepLC eluters
  3) 2 M Ammonia/Water: De-salting solvent for de-salting trapped samples (if required)
  4) Formic Acid: De-salting solvent

Solvents Available on Elution Pump

1) 90% DCM/MeOH: eluting solvent that provides a tighter elution profile than ACN
2) 40% Acetone in THF: column wash 1
3) 100 ACN: column wash 2 helps aid the full column washing process

Methodology Evolution

Old Way – up to 2 days



New Way – up to half a day

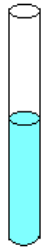
Buffer

No Buffer

MeCN removal

Complete Dry-down

Complete Dry-down



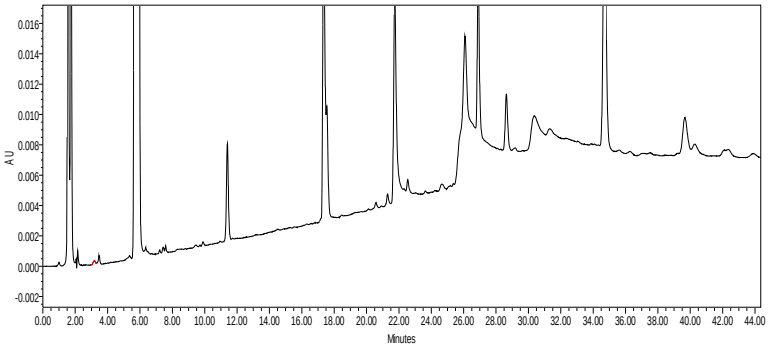
DCM/MeOH

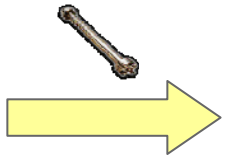
~ 10 mL

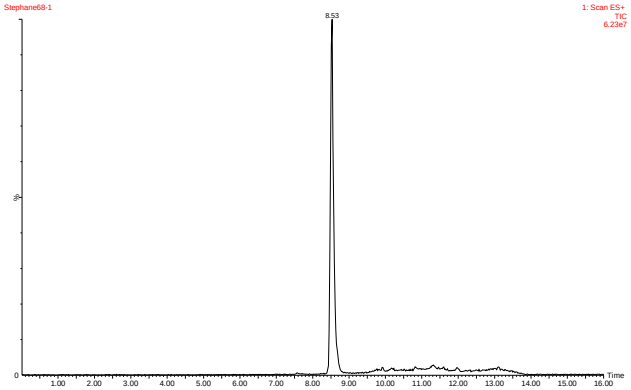
Dried-down and debuffered

Example 1: Low level impurity isolation

Sample containing 4 impurities to isolate for ID purposes



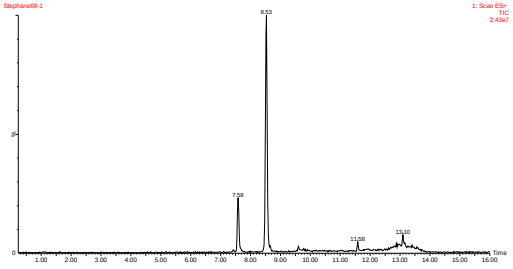


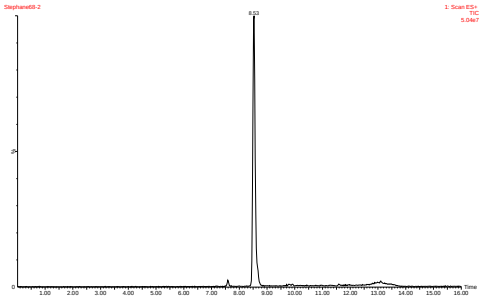


Old way

New way

- Minimal degradation
- No loss of product in waste line

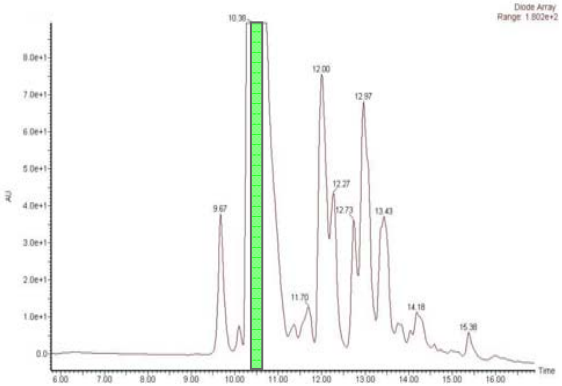


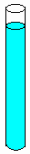


Debuffered – dried-down samples

Example 2: Marker isolation

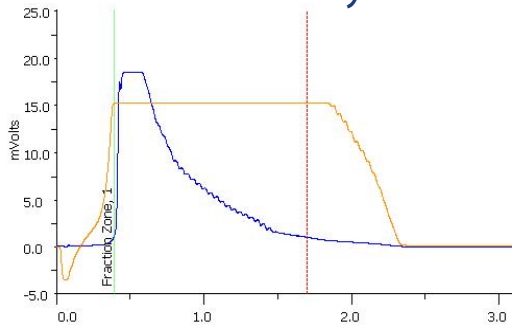
In Prep HPLC



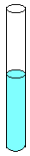


One HPLC fraction in Water/MeCN/HCOOH

In Fraction Trapping system



UV based collection



Target compound in DCM/MeOH mixture

50 mgs dried down and debuffered

Less than 1 day turn-around instead of 2 days

Summary

- FTLC is rapidly intuitive
- No product loss detected in waste lines
- Strongly impact researchers' work with minimal work on it
- Improved efficiency on impurity isolation (dry-down time cut by 50%)
- Milder drying-down conditions, preventing degradation
- De-buffer/salt for NMR

Conclusions

- The elution of fraction of samples from DCM/Methanol needs careful delay volume characterisation in order to optimise and reduce H20 Vs Recovery
- DCM/Methanol fraction now has a few 100's micro litres of water in a 18 mL fraction
- Carry over and blank issue now resolved with enhance column wash protocol
- Carry over in now eliminated with Instrument washing i.e. probe and loop between injections

Acknowledgements

Pfizer Sandwich	**Mark Taylor, Mike Stace, Stéphane Dubant, and Ben Matthews et al**
GSK Harlow & Stevenage	**Bob Boughtflower, Tim Underwood , Keith Brinded**
Polymer Labs	**Graham Cleaver et al**
Astra Zeneca	**Steve Norris , Mike Giles**