

# A Novel Approach to Concentration of Reversed Phase Semi-Prep LC Fractions Using ISOLUTE® 103

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## Introduction

Reversed phase semi-prep LC followed by mass directed fraction collection is widely used for the purification of target compounds and intermediates. Compounds purified using this technique are subsequently required in 100% organic solvent or as the solid or liquid compound. Evaporation is the most commonly used method for isolation of these compounds from the collected fractions.

However, this approach has significant disadvantages:

- High aqueous component of fractions - often 90% v/v water
- Fraction volume - often up to 50 mL of sample

These factors mean evaporation can take several hours (particularly if limited to low temperatures by the stability of the compounds involved) and is a bottleneck in the purification process.

This poster investigates the use of ISOLUTE® 103 solid phase extraction columns as an alternative to evaporation, for compound isolation.

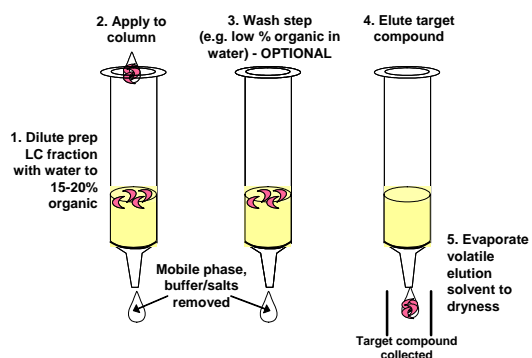
The following factors were investigated in this work:

1. Optimum fraction dilution to ensure compound retention on ISOLUTE 103
2. Requirement for column conditioning
3. Elution solvent selection

## Features of ISOLUTE 103:

- Hydroxylated polystyrene-divinylbenzene resin based sorbent with high surface area and high compound capacity
- Retains compounds from aqueous or semi-aqueous solutions by a hydrophobic retention mechanism

## Typical procedure using ISOLUTE 103



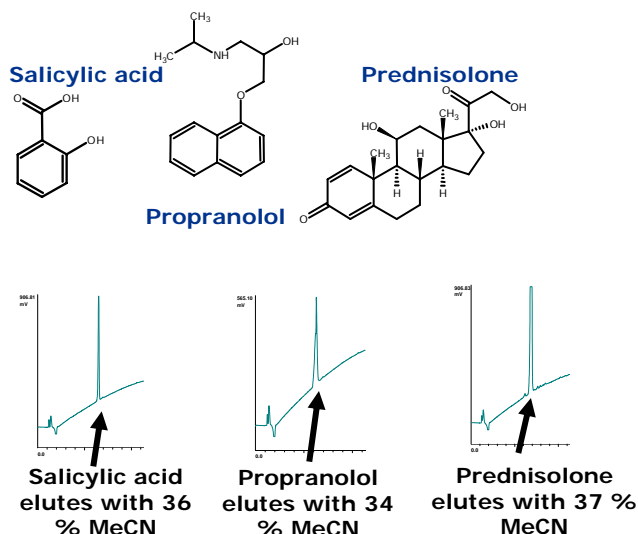
## Analytical HPLC Conditions

Column: RP C18 150 x 4.6 mm

Gradient: A: Acetonitrile B: Water (0.2% Formic acid, v/v)

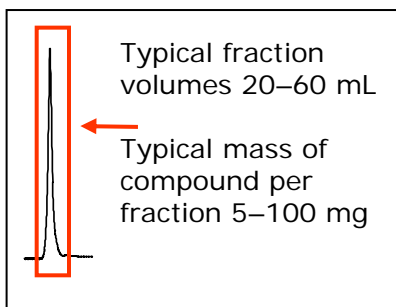
Time (min)	% A	% B	Flow Rate (mL/min)
0	5%	95%	1.5
10	95%	5%	1.5

## Test Compounds



Analytical HPLC is directly scaleable to semi-prep LC

# 1. Optimum Fraction Dilution to Ensure Compound Retention on ISOLUTE 103



## Experimental Section

### Procedure:

1. Collect Fraction containing probe analyte
2. Dilute to 15, 20 or 30% MeCN (v/v)
3. Load onto unconditioned ISOLUTE 103 1g / 6mL at flow rate of 1-2 mL/minute
4. Elute with MeCN

Samples were analysed using a Hitachi UV2000 UV/VIS Spectrophotometer at a  $\lambda_{\text{max}}$  of 303nm

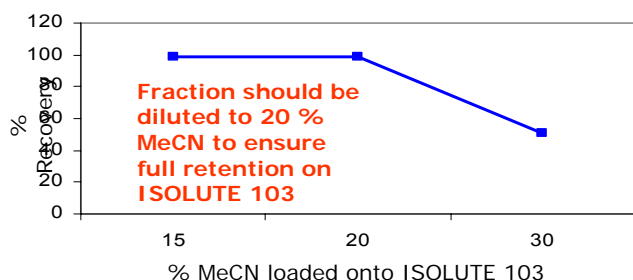
## Results Section

### Salicylic acid – Elutes at 36 % MeCN

50 mg salicylic acid / 20 mL fraction

Fraction diluted to:	Mean Recovery	Elution volume MeCN
15% MeCN	99.2 %	5 mL
20% MeCN	99.1 %	5 mL
30% MeCN	50.7 %	5 mL

**Note: 20 mL AQUEOUS fraction reduced to 5 mL of VOLATILE solvent**



100 mg salicylic acid / 20 mL fraction

Fraction Diluted to:	Mean Recovery	Elution volume MeCN
15% MeCN	95.3 %	10 mL
20% MeCN	95.8 %	10 mL

**Note: 20mL AQUEOUS fraction reduced to 10 mL of VOLATILE solvent**

### Prednisolone – Elutes at 37% MeCN

50 mg prednisolone / 54 mL fraction

Fraction Diluted to:	Mean Recovery	Elution volume MeCN
20% MeCN	98.3%	15 mL

**Note: 54 mL AQUEOUS fraction reduced to 15 mL of VOLATILE solvent**

### Propranolol – Elutes at 34% MeCN

10 mg propranolol / 20 mL fraction

Fraction Diluted to:	Mean Recovery	Elution Volume MeOH / NH <sub>3</sub> (95:5)
15 % MeCN	94.2 %	20 mL
20 % MeCN	67.7 %	20 mL

25 mg propranolol / 20 mL fraction

Fraction Diluted to:	Mean Recovery	Elution Volume MeOH / NH <sub>3</sub> (95:5)
10 % MeCN	67 %	20 mL
15 % MeCN	43 %	20 mL

## Optimum Fraction Dilution - Conclusions

- Fractions require dilution to reduce MeCN organic content prior to retention on ISOLUTE 103.
- Maximum recovery obtained with dilution to between 15 and 20% MeCN.
- Elution from ISOLUTE 103 is achieved in  $\leq 20$  mL volatile solvent which can be evaporated in  $\leq 60$  minutes.
- ISOLUTE SCX-2 can be used as an alternative approach for basic compounds (see page 3).

## Alternative approach for BASIC compounds

BASIC compounds can also be extracted using silica based ion exchangers e.g. ISOLUTE SCX-2.

### Method:

**Column Configuration:** 1g/6 mL ISOLUTE SCX-2

1. Column Conditioning: Methanol (3 mL)
2. Column Equilibration: 34% MeCN, 66% water (0.2% formic acid, v/v) (3 mL)
3. Sample Application: Propranolol (50 mg) in 20 mL 34% MeCN, 66% water (0.2% formic acid, v/v). Flow rate 1-2 mL/min
4. Product Elution: 5 mL Methanol / 1 M NH<sub>3</sub> (v/v)

Mean Recovery	Elution volume MeOH / NH <sub>3</sub> (1 M)
94%	5 mL

**Fraction does NOT require dilution prior to loading on ISOLUTE SCX-2**

## Comparison Between ISOLUTE 103 and ISOLUTE SCX-2 for Concentration of Aqueous Flash or Preparative HPLC Fractions

### ISOLUTE 103: Hydroxylated PS-DVB    ISOLUTE SCX-2:

- |   |   |
|---|---|
| ▪ Hydrophobic retention<br>> Extracts acidic, basic and neutral compounds | ▪ Cation exchange retention<br>> Extracts basic compounds |
| ▪ Dilute to 15-20% MeCN   | ▪ No dilution required                                    |
| ▪ Elute with methanol   | ▪ Elute with ammonia/methanol                             |
|   | ▪ Affords free amine                                      |

## Summary of Product Recovery from Reverse Phase Chromatography Fractions using ISOLUTE 103 and ISOLUTE SCX-2 SPE Columns (see complete data on page 2)

Compound	% MeCN in fraction	Fraction Composition	%MeCN for SPE	Elution Volume MeOH (mL)	% Recovery
Salicylic acid <sup>1</sup>	36	50 mg/20 mL	15	5	95
Salicylic acid <sup>1</sup>	36	100 mg/20 mL	15	10	96
Prednisolone <sup>1</sup>	37	50 mg/55 mL	20	15	98
Propranolol <sup>1</sup>	34	10 mg/20 mL	15	20	94
Propranolol <sup>2</sup>	34	50 mg/20 mL	34	5 <sup>3</sup>	94

<sup>1</sup> Using ISOLUTE 103 1 g/6 mL SPE column

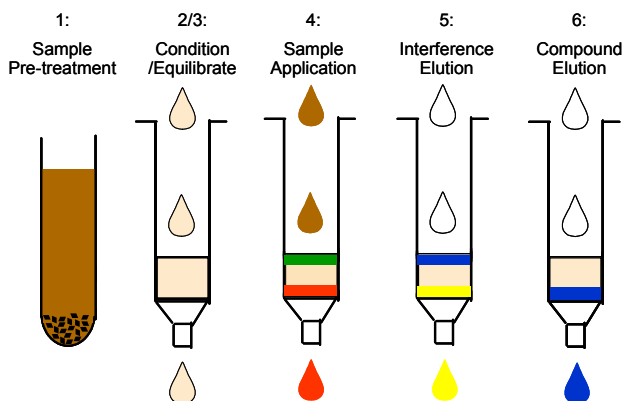
<sup>2</sup> Using ISOLUTE SCX-2 1 g/6 mL SPE column

<sup>3</sup> Elution solvent is 2 M ammonia/methanol

## 2. Requirement for Column Conditioning

In a typical 6-step SPE procedure (see figure opposite), column conditioning is usually necessary to prepare the SPE column for the extraction process and ensure high compound recovery. In this process an organic solvent e.g. methanol or acetonitrile is passed through the column to 'wet' the sorbent.

However, for certain modified resins e.g. ISOLUTE 103, conditioning may not be necessary since the sorbent is classified as 'water-wettable' i.e. can retain compounds from aqueous samples without conditioning.



6-step SPE procedure

## Experimental Section

Compound retention by ISOLUTE 103 was compared when the columns were **conditioned** with acetonitrile (**Method 1**) and **unconditioned** (**Method 2**).

**Column Configuration:** ISOLUTE 103 1 g/6 mL

**Method:**

Method 1 - SPE Conditioned	Method 2 - SPE Unconditioned
1. Condition: 5 mL MeCN	1. Load (Fraction diluted to 20% MeCN, v/v)
2. Equilibrate: 5 mL 20% MeCN: water, v/v	2. Elution: 20 mL MeCN
3. Load (Fraction diluted to 20% MeCN, v/v)	
4. Elution: 20 mL MeCN	

## Results Section

50 mg Prednisolone / 54 mL fraction (37% MeCN)

Fraction diluted to:	% Recovery Conditioned	% Recovery Unconditioned
20% MeCN	98.4	99.6
	99.4	97.7
	97.7	97.6
	<b>MEAN – 98.5</b>	<b>MEAN – 98.3</b>

### Column Conditioning - Conclusions

ISOLUTE 103 gives good recovery without requiring column conditioning.

**Therefore:**

ISOLUTE 103 SPE columns can be used without any pre-treatment, so reducing the number of processing steps in the procedure. Prep LC fractions can be diluted to 15-20% organic and loaded directly onto the SPE column.

### 3. Elution Solvent Selection

Compounds solubility and functionality can affect the choice of elution solvent used. The effect on compound recovery and required elution volume was investigated using prednisolone and propranolol.

#### Results Section

##### Prednisolone

Sample: 50 mg/54 mL fraction (containing 37% MeCN), diluted to 20% (v/v) with water

Elution Solvent	Elution Volume (mL)	Recovery (%)
MeOH	10	93
MeCN	15	98.5

**Prednisolone is more soluble in MeOH than MeCN – elution volume is lower with MeOH**

##### Propranolol

10 mg/20 mL fraction (containing 34% MeCN), diluted to 20% MeCN with water

Elution Solvent	Elution Volume (mL)	Recovery (%)
MeOH	20	1.3
MeOH / NH <sub>3</sub> (95:5, v/v)	20	94.2

**Basic compounds require the addition of a basic modifier (e.g. NH<sub>3</sub>) to ensure complete elution from ISOLUTE 103**

#### Elution Volume Selection - Conclusions

Elution volume can be reduced by choosing the optimal elution solvent. Lower elution volumes further reduce the overall purification time.

Basic compounds require the addition of a volatile basic modifier (e.g. NH<sub>3</sub>) to ensure complete elution from ISOLUTE 103.

The elution volume can be further optimized by including a soak step as part of the elution profile.

#### Overall Conclusions

- Acidic, basic and neutral compounds can be extracted from prep LC fractions containing a high percentage of aqueous component using ISOLUTE 103 SPE columns.
- Dilution of fractions to approx. 15-20% organic ensures optimum retention by the ISOLUTE 103 column, whilst eluting in a much smaller volume of volatile solvent. Elution from ISOLUTE 103 can be achieved in ≤ 20 mL volatile solvent which can be evaporated in ≤60 minutes.
- ISOLUTE 103 columns can be processed without conditioning, further reducing the number of processing steps and enhancing productivity.
- An alternative approach for basic compounds is the use of silica-based strong cation exchange sorbents e.g. ISOLUTE SCX-2.
- This work provides 'proof of principle' that a broad range of compound types can be extracted from semi-prep LC fractions using SPE columns. In this work, sample concentration is achieved manually in an 'off-line' mode, however further work is being carried out in this area.

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