



ISOLUTE®  
SLE+

USER GUIDE

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## SLE+ Ordering Information

Item	Quantity	Part Number
ISOLUTE SLE+ 200 Supported Liquid Extraction Plate	1	820-0200-P01
ISOLUTE SLE+ 400 Supported Liquid Extraction Plate	1	820-0400-P01

**Biotage Sample Preparation Products** provide a broad range of solutions for the bioanalytical, clinical, forensic, environmental and agrochemical markets. Our full range of products can be found on our website [www.biotage.com](http://www.biotage.com)

## Introduction

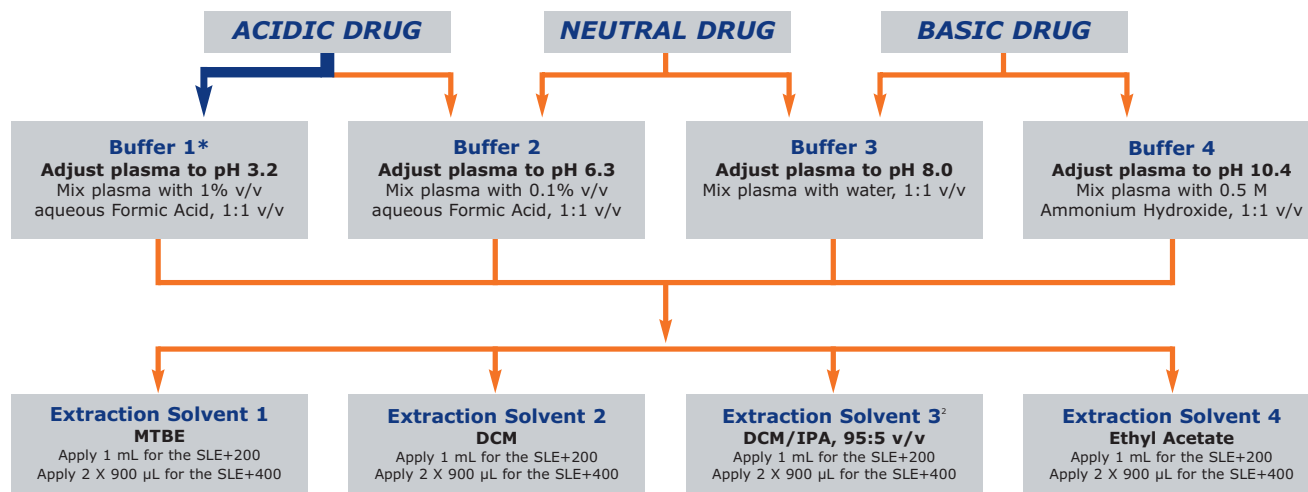
ISOLUTE SLE+ plates consist of 96 individual wells, each containing a modified form of diatomaceous earth. There are two ISOLUTE SLE+ plate sizes available, SLE+ 200 plate for a total aqueous sample load of up to 200  $\mu\text{L}$  and SLE+ 400 plate for 200–400  $\mu\text{L}$ . Narrower particle size distribution and advances in plate packing technologies have made ISOLUTE SLE+ plate a superior product for rapid and easy sample preparation. Excellent well-to-well reproducibility, greater loading capacity and a very simple method development process are the key features of this cost-effective product. This guide explains how easy good sample preparation can be with the ISOLUTE SLE+ plates.



## Method Development Chart

Biotage has examined a variety of acidic, basic and neutral compounds to determine recovery levels utilizing supported liquid extraction. Results are charted in Appendix 1. By screening 4 different loading pH's and 4 different extraction solvents, we were able to determine that recoveries were optimum when the pH was near the pKa of the analyte. Through these experiments there was no universal "best" solvent for elution discovered. While MTBE is widely used, ethyl acetate, DCM and mixed solvents also perform well. Depending on the drug's functionality, by simply screening 2 pHs, combined with 4 extraction solvents, you can develop a method in minutes.

## ISOLUTE SLE+ Supported Liquid Extraction Plates: Method Selection



This method selection guide is designed to minimize the choices required to optimize the Supported Liquid Extraction method. The buffer and solvent suggestions provide a range of pHs and solvent polarities for acidic, neutral and basic drugs of varying pKa and logP values. The selections are based on extensive work in Biotage's R&D Laboratories<sup>1</sup>.

\*Note that in some cases, such as the analysis of very polar compounds Biotage suggest more extreme pH control may be necessary to improve recoveries. Contact your local Biotage representative for additional recommendations.

**Matrix Effects:** Increasing the polarity of the extraction solvent can lead to increased matrix effects in LC-MS/MS analysis<sup>2</sup>.

<sup>1</sup> Simultaneous Extraction of Acidic, Basic and Neutral Drugs using 96-well Supported Liquid Extraction (SLE) and LC-MS/MS. L Williams et al. Presented at ASMS 2007.

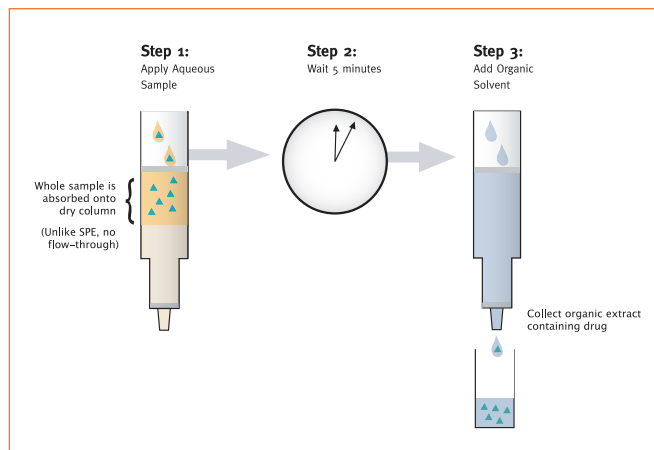
<sup>2</sup> Investigation of Phospholipid Removal using 96-well Supported Liquid Extraction. L Williams et al. Presented at Montreux Symposium 2007.



**NOTE:** A full sized copy of the **SLE+ Method Selection Chart** can be downloaded from our website at [www.biotage.com](http://www.biotage.com) or you can request a copy from your Biotage representative.

# Processing the Plates

SLE+ plates utilize flow-through technology, meaning the organic and aqueous layers are easily separated (see Figure 1). While most of this technique is performed under gravity, a pulse of vacuum is useful to initiate loading of the aqueous sample through the top hydrophobic frit and for a final draw of organic solvent after elution. Table 1 below shows the recommended procedure for workflows using SLE+ 200 and SLE+ 400 plates. Table 2 shows recommended buffer and elution volumes depending on initial sample volume.



**Figure 1.** Schematic of Supported Liquid Extraction Process

**TABLE 1**

	<b>ISOLUTE SLE+ 200 Procedure</b>	<b>ISOLUTE SLE+ 400 Procedure</b>
<b>1</b>	Dispense prebuffered sample (200 $\mu$ L)	Dispense pre-buffered sample (400 $\mu$ L)
<b>2</b>	Apply vacuum (-15"Hg / -0.5 bar) for 2-10 seconds to initiate loading.	Apply vacuum (-15"Hg / -0.5 bar) for 2-10 seconds to initiate loading.
<b>3</b>	Wait 5 minutes for sample to completely absorb.	Wait 5 minutes for sample to completely absorb.
<b>4</b>	Apply extraction solvent (1 x 1 mL).	Apply extraction solvent (2 x 900 $\mu$ L or 3 x 700 $\mu$ L).
<b>5</b>	Allow solvent to flow for 5 minutes under gravity.	Allow each solvent aliquot to flow for 5 minutes under gravity.
<b>6</b>	Apply vacuum (-15"Hg / -0.5 bar) for 2 minutes to complete elution.	Apply vacuum (-15"Hg / -0.5 bar) for 2 minutes to complete elution. Repeat for 2nd aliquot of solvent.
<b>7</b>	Evaporate to dryness. Reconstitute in mobile phase prior to analysis.	Evaporate to dryness. Reconstitute in mobile phase prior to analysis.

**TABLE 2**

<b>Sample Loading</b>	<b>Recommendation</b>	<b>Elution Volume Applied to ISOLUTE SLE+ Plate</b>	<b>Solvent Volume Collected*</b>
Sample volume is normally $\leq 100 \mu$ L	ISOLUTE SLE+ 200 Plate, with a 1:1 dilution of the plasma using the appropriate buffer.	SLE+ 200 Plate 1 x 1 mL	600 – 700 $\mu$ L
Sample volume is normally 100 – 200 $\mu$ L	ISOLUTE SLE+ 400 Plate with a 1:1 dilution of the plasma using the appropriate buffer.	SLE+ 400 Plate 2 x 900 $\mu$ L 3 x 700 $\mu$ L	1.2 – 1.3 mL 1.3 – 1.4 mL
Sample volumes are 200 $\mu$ L or more	The maximum capacity of the ISOLUTE SLE+ 400 Plate is 400 $\mu$ L; it is possible to load more than 200 $\mu$ L of original sample with a smaller dilution factor (the total 400 $\mu$ L load must not be exceeded). Please be aware that a smaller dilution factor may result in variable flow rates due to different sample viscosities.	SLE+ 400 Plate 2 x 900 $\mu$ L 3 x 700 $\mu$ L	1.2 – 1.3 mL 1.3 – 1.4 mL

**NOTE:** For the elution volume we assume the use of a  $\sim 2$  mL collection plate because only about 70% of the eluting solvent passes through the column bed. Up to 2.1 mL can be used in the elution step. The amount that passes through each well can vary from solvent to solvent and does not affect the results.

## Using SLE+ instead of traditional LLE

In a recent study<sup>1</sup>, Biotage examined the Limit of Quantitation (LoQ) of SLE+ 400 with 200 µL of plasma, compared with applying 500 µL of plasma using traditional LLE. The results illustrate that it is possible to obtain similar LoQs using smaller sample volumes compared with traditional LLE.

### Sample Preparation

#### Supported Liquid Extraction Procedure

**Plate:** ISOLUTE SLE+ 400 Supported Liquid Extraction Plate, part number 820-0400-P01

#### Sample pre-treatment

**Acidic analytes (NSAIDs):** Plasma (200 µL) pre-treated 1:1 v/v with 1% formic acid aq.

**Basic analytes (β-blockers):** Plasma (200 µL) pre-treated 1:1 v/v with 0.5M ammonium hydroxide.

**Sample Application:** The pretreated plasma (total 400 µL) was loaded onto the plate, a pulse of vacuum applied to initiate flow and the samples left to absorb for 5 minutes.

**Analyte Elution:** Addition of 2 x 900 µL of either MTBE (NSAIDs) or EtOAc (β-blockers).

#### Liquid-liquid Extraction Procedure

Plasma (500 µL) was pre-treated 1:1 v/v with: 1% formic acid aq and extracted with 1.8 mL of MTBE (NSAIDs); or 0.5M ammonium hydroxide and extracted with EtOAc (β-blockers). The layers were left to separate and the organic aliquot removed.

**Post Extraction:** The eluate was evaporated to dryness and the analytes reconstituted in 500 µL of appropriate H<sub>2</sub>O/MeOH mixtures prior to analysis.

#### Mass Spectrometry

**Instrument:** Ultima Pt triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK) equipped with an electrospray interface for mass analysis. Positive and negative ions were acquired in the multiple reaction monitoring mode (MRM). All β-blockers were analysed in positive ion mode, whereas, the NSAIDs required both positive and negative MRM transitions.

Analyte	Plasma		Urine	
	SLE+ LoQ (pg/mL)	LLE+ LoQ (pg/mL)	SLE+ LoQ (pg/mL)	LLE+ LoQ (pg/mL)
Atenolol	100	100	200	200
Nadalol	50	50*	50	50*
Metoprolol	<50 (25)	<50 (20)	<50 (25)	<50 (20)
Oxprenolol	<50 (25)	<50 (15)	<50 (25)	<50 (15)
Propranolol	<50 (25)	<50 (15)	<50 (25)	<50 (15)
Alprenolol	<50 (25)	<50 (15)	<50 (25)	<50 (15)

**Table 1.** LLE/SLE+ β-blocker LoQ comparison.

Analyte	Plasma		Urine	
	SLE+ LoQ (ng/mL)	LLE+ LoQ (ng/mL)	SLE+ LoQ (ng/mL)	LLE+ LoQ (ng/mL)
Acetaminophen	5	100	-	-
Sulindac	<1 (0.5)	<1 (0.5)	<1 (0.5)	<1 (0.3)
Ketoprofen	3	2	3	2
Naproxen	10	10	10	5
Flurbiprofen	10	10	10	5
Indomethacin	2	2	3	2
Diclofenac	1 (0.5)	1 (0.5)	1 (0.4)	1 (0.2)
Mefenamic Acid	<1 (0.2)	<1 (0.2)	<1 (0.1)	<1 (0.5)

**Table 2.** LLE/SLE+ NSAID LoQ comparison.

\*RSD > 10%. - acetaminophen detected in urine. Parentheses - estimated levels based on S/N at previous level

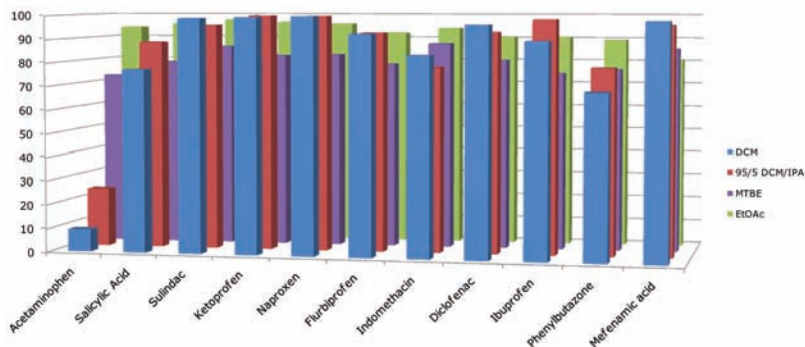
<sup>1</sup>Lee Williams, Rhys Jones, Steve Jordan, Richard Calverley, Claire Desbrow, Gary Dowthwaite & Joanna Caulfield ASMS 2009, (Philadelphia, PA), 2009, Biotage.

# Applying the Method Development Chart

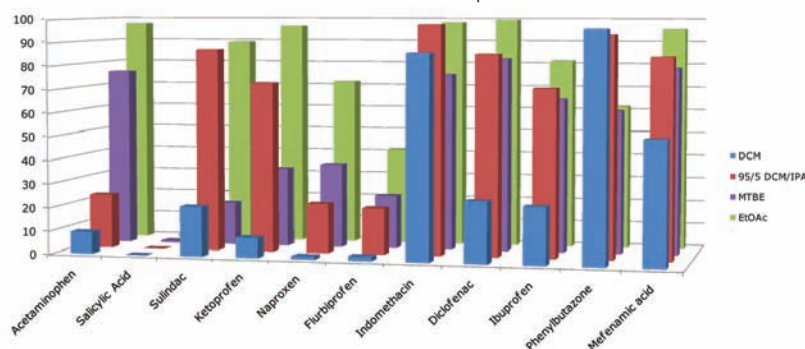
## Acidic Compound Extraction: NSAIDs

1. Dilute plasma 1:1 (v/v) with 1% (v/v) formic acid
2. Dispense sample (200  $\mu$ L) into each well
3. Apply vacuum (-15 "Hg / -0.5 bar) for 2-10 seconds to initiate loading
4. Wait 5 minutes for sample to completely absorb
5. Apply extraction solvent (1 mL)
6. Allow solvent to flow for 5 minutes under gravity
7. Apply vacuum (-15 "Hg / -0.5 bar) for 2 minutes to complete elution
8. Evaporate to dryness. Reconstitute in mobile phase prior to analysis

Various Extraction Solvents with a 1% formic acid: plasma load  
 pKa of mefenamic acid 4.2  
 pKa of naproxen 4.65  
 pKa acetaminophen 9.9



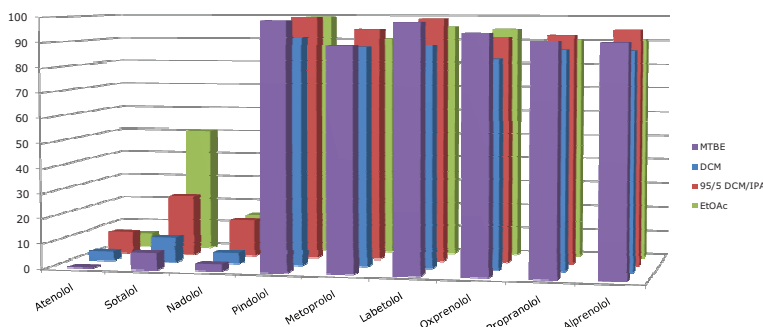
Various Extraction Solvents with a 0.1% formic acid: plasma load



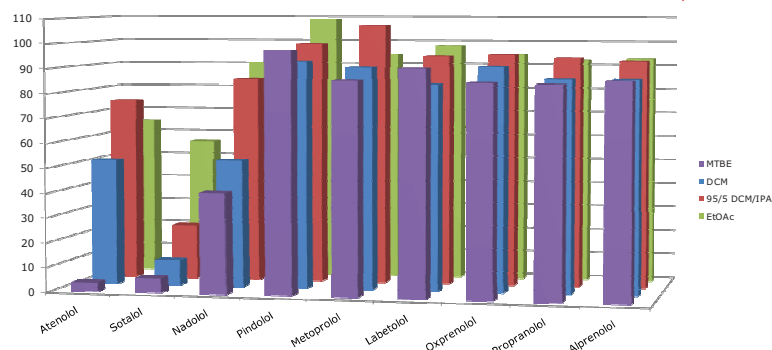
## Basic Compound Extraction: $\beta$ -Blockers

1. Dilute plasma 1:1 (v/v) with 0.5 M  $\text{NH}_4\text{OH}$  or  $\text{H}_2\text{O}$
2. Dispense sample (200  $\mu$ L) into each well
3. Apply vacuum (-15 "Hg / -0.5 bar) for 2-10 seconds to initiate loading
4. Wait 5 minutes for sample to completely absorb
5. Apply elution solvent (1 mL)
6. Allow solvent to flow for 5 minutes under gravity
7. Apply vacuum (-15 "Hg / -0.5 bar) for 2 minutes to complete elution
8. Evaporate to dryness. Reconstitute in mobile phase prior to analysis

$\beta$ -Blocker recovery chart comparing various extraction solvents with a  $\text{H}_2\text{O}$ : plasma load



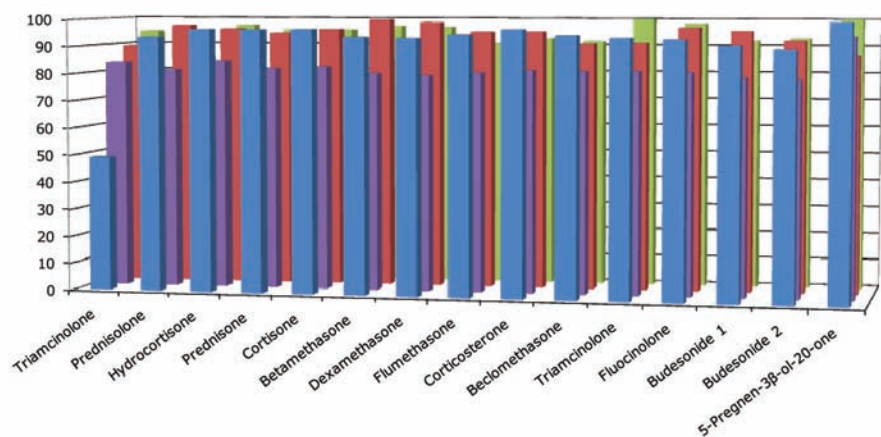
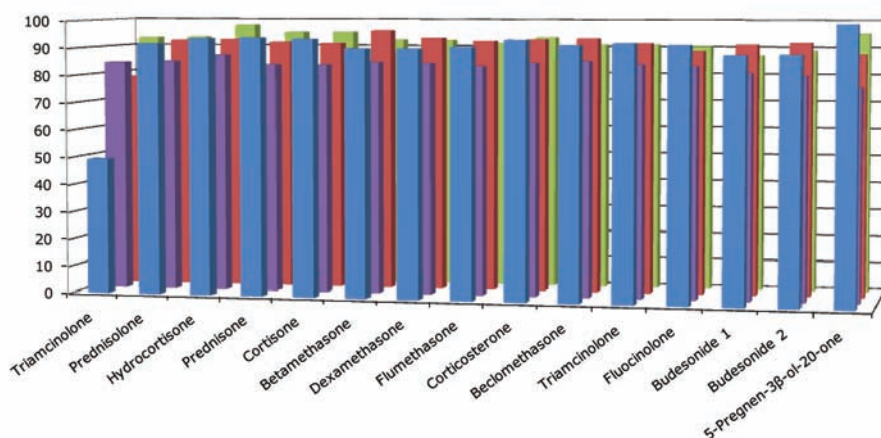
$\beta$ -Blocker recovery chart comparing various extraction solvents with a 0.5M  $\text{NH}_4\text{OH}$ : plasma load



# Applying the Method Development Chart

## Neutral Compound Extraction: Corticosteroids

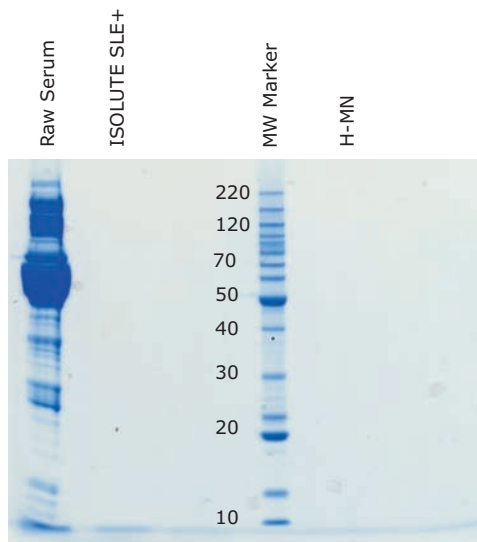
1. Dilute plasma 1:1 (v/v) with H<sub>2</sub>O
2. Dispense sample (200 µL)
3. Apply vacuum (-15 "Hg / -0.5 bar) for 2-10 seconds to initiate loading
4. Wait 5 minutes for sample to completely absorb
5. Apply extraction solvent (1 mL)
6. Allow solvent to flow for 5 minutes under gravity
7. Apply vacuum (-15 "Hg / -0.5 bar) for 2 minutes to complete elution
8. Evaporate to dryness. Reconstitute in mobile phase prior to analysis



# Cleaner Extracts

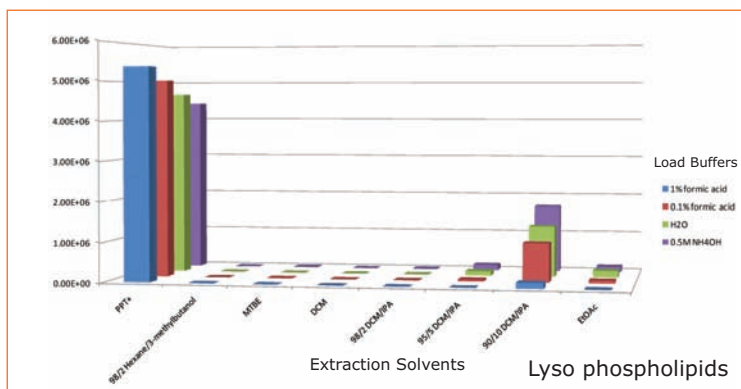
## Providing Clean Extracts

The extraction mechanism of ISOLUTE SLE+ completely removes phospholipids, salts, proteins and matrix interferences. In extensive studies we have found that water-immiscible organic solvents such as MTBE and ethyl acetate work effectively with a wide variety of compounds.<sup>1</sup> However, for molecules that do not elute well in these solvents, 5% of a polar modifier can be added to the extraction solvent to facilitate elution without sacrificing extract cleanliness.



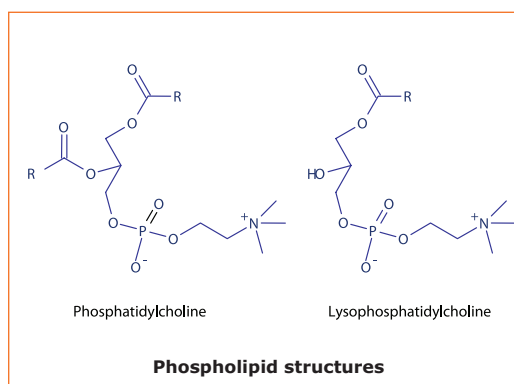
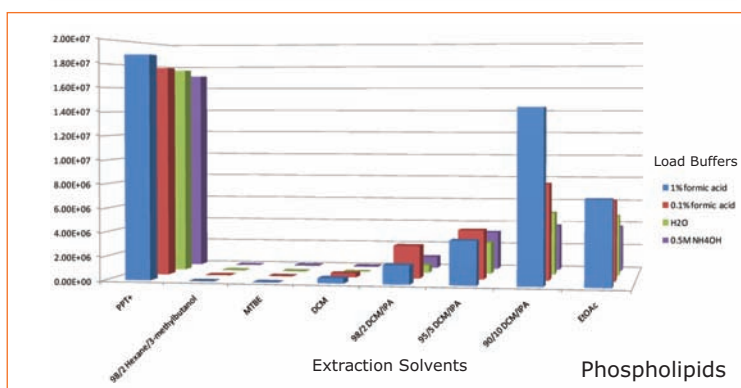
### Excellent removal of Proteins

- Rat serum was diluted 1:1 (v/v) with deionized H<sub>2</sub>O, loaded onto SLE+ and HM-N (200 mg) and extracted with MTBE
- Following sample preparation, the extracts were evaporated to dryness
- Electrophoresis was performed using NuPAGE Novex 12% Bis-Tris mini gels with MOPS SDS running buffer at 200 V, 120 mA and 12.5 W
- Gel Electrophoresis performed out using 5 µL serum equivalents and compared to 0.5 µL raw serum



### Excellent removal of Phospholipids

- Extraction conditions investigated:
  - Four load buffers covering the pH range 3.2 – 10.4
  - Eight common water immiscible extraction solvents
- Selected phospholipids monitored by LC-MS/MS analysis using electrospray ionization in the SIR operating mode



<sup>1</sup>Lee Williams, Rhys Jones, Steve Jordan, Richard Calverley, Claire Desbrow, Gary Douthwaite & Joanna Caulfield ASMS 2009, (Philadelphia, PA), 2009, Biotage.

## Processing stations for the ISOLUTE SLE+ Plates

ISOLUTE SLE+ plates are a standard 96-well configuration that can be used on liquid handlers or table-top manifolds. To the right is a picture of the VacMaster™-96 bench-top station. Positive pressure systems and centrifuge systems have also been used. For ordering information please visit [www.biotage.com](http://www.biotage.com) or request a catalog from your Biotage representative.



### Fully integrated 96-well system for high throughput sample processing.

From left to right: VCU-2 (vacuum control unit with integral vacuum generator), solvent trap, VacMaster-96 sample processing station with ISOLUTE-96 plate.



## Using a Partial Plate

Because of the low density of the diatomaceous earth, utilizing a mat to cover unused wells is important to ensure a pulse vacuum on the loading and final elution steps. Biotage recommend using the piercable sealing cap listed below.

## Ordering information for Cap Mats

Description	Qty	Part Number
Piercable Sealing Cap	50	121-5204



### SPE Dry™ 96 Dual Microplate sample concentrator systems

Designed for high throughput laboratories, the SPE Dry 96 (1 plate) and SPE Dry 96 Dual (2 plates) sample concentrator systems provide efficient solvent evaporation in microplate format, and are compatible with 24-, 96- and 384-well collection plates.

## Matrices

Plasma, serum and urine have most commonly been applied to this technique. When using whole blood it is still important to release the drugs from red blood cells utilizing buffers & sonication. Your Biotage representative can provide a recommended procedure if desired.

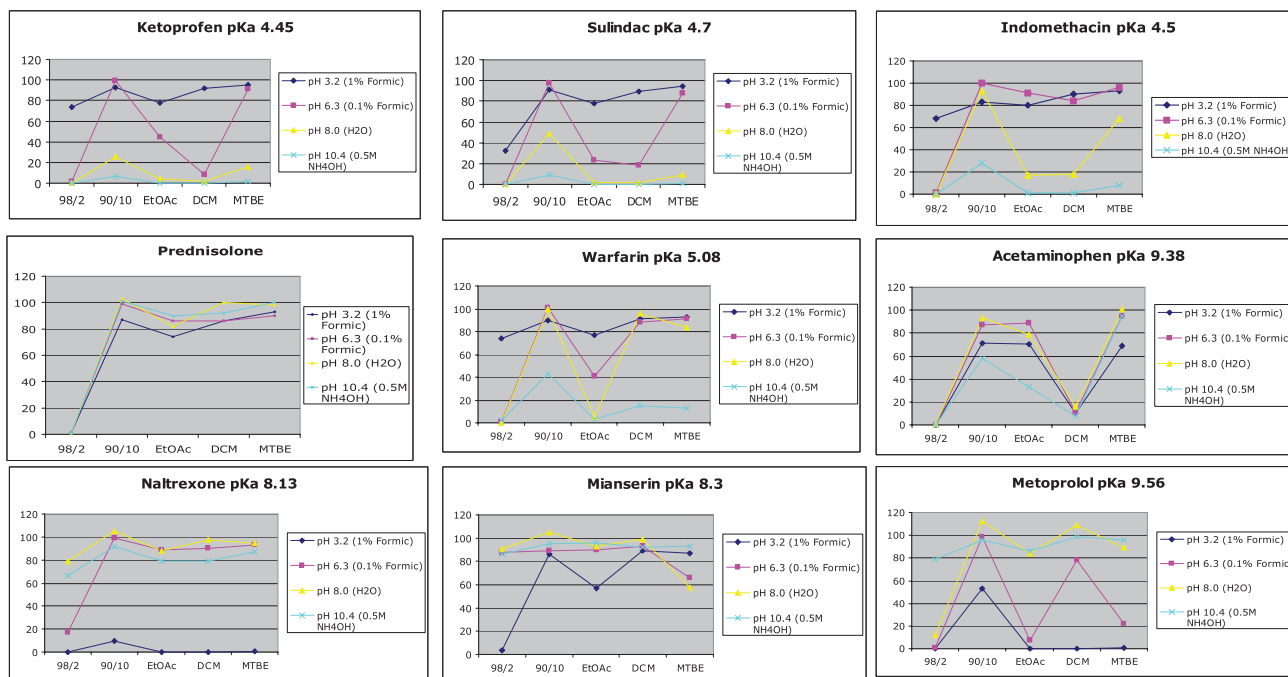
## Evaporating and Reconstitution

When faster evaporation is desired, the Biotage the SPE-Dry 96 is recommended as it accurately heats both the bottom and top of the plate for more rapid results. The unit provides efficient and safe evaporation of a variety of aqueous and organic solvents without causing well-to-well contamination. If using volatile solvents an evaporator may not be required.

# Results

## Appendix 1.

### Results of studies on pH and elution solvent optimization



Comparison of loading pH and extraction solvent on analyte recoveries using ISOLUTE SLE+ plates  
 Extraction solvents: 99.2 (v/v) hexane/3-methyl-1-butanol, 90:10 (v/v) DCM/IPA, Ethyl acetate, DCM and MTBE.

#### References:

Data taken from the following poster, presented at ASMS 2007:  
 Simultaneous Extraction of Acidic, Basic and Neutral Drugs using 96-well Supported Liquid Extraction (SLE) and LC-MS/MS  
 Matthew Cleeve, Lee Williams, Scott Merriman, Helen Lodder, Steve Jordan, Richard Calverley and Joanna Smith

## Appendix 2

### Posters and Presentations Available

*Full copies are available from your Biotage representative. See back page for territorial contacts.*

#### **Investigation of Phospholipid Removal using 96-well Supported Liquid Extraction;**

Lee Williams, Matthew Cleeve, Scott Merriman, Steve Jordan, Richard Calverly, Steve Plant, Joanna Smith, Montreaux LCMS 2007, 2007, Biotage.

#### **Revisiting Traditional Liquid-Liquid Extraction Techniques using SLE;**

P. Abram N. Brubaker; AAPS 2008 ; (Indianapolis, IN), 2008, MPI Research, Mattawan, MI.

#### **Streamlined Method Development using Supported Liquid Extraction (SLE+) prior to LC-MS/MS Analysis ;**

Lee Williams, Helen Lodder, Rhys Jones, Steve Jordan, Richard Calverly & Joanna Caulfield, Eastern Analytical Symposium 2008; AAPS 2008 ; (New Brunswick, NJ ; Atlanta, GA), 2008, Biotage.

#### **Sample Preparation and Protein Removal: A Comparison of Protein Removal with Various Sample Preparation Techniques using Gel Electrophoresis;**

Lee Williams, Rick Edmondson, Jason Taylor, Nathan Twaddle, Matthew Cleeve, Steve Jordan, Richard Calverly and Joanna Smith, ASMS 2008, (Indianapolis, IN), 2008, Biotage & National Centre for Toxicological Research.

#### **Comparison of Liquid-liquid Extraction (LLE) and Supported Liquid Extraction (SLE):- Equivalent Limits of Quantitation with Smaller Sample Volumes;**

Lee Williams, Rhys Jones, Steve Jordan, Richard Calverly, Claire Desbrow, Gary Dowthwaite & Joanna Caulfield, ASMS 2009 , (Philadelphia, PA), 2009, Biotage.

#### **Phospholipid Removal:- A Comparison between Traditional Liquid-liquid Extraction (LLE) and Supported Liquid Extraction (SLE) using LC-MS/MS Analysis;**

Lee Williams, Rhys Jones, Steve Jordan, Richard Calverly, Claire Desbrow, Gary Dowthwaite & Joanna Caulfield ASMS 2009 , (Philadelphia, PA), 2009, Biotage.

## Appendix 3

### Supported Liquid Extraction Recent Publications

#### *Journal Articles*

#### **Enantioselective determination of alprenolol in human plasma by liquid chromatography with tandem mass spectrometry using cellobiohydrolase chiral stationary phases.**

H. Jiang, X. Jiang and Q. Ji *Journal of Chromatography B* 2008, 872, p. 121-127.

#### **Determination of molindone enantiomers in human plasma by high-performance liquid chromatography-tandem mass spectrometry using macrocyclic antibiotic chiral stationary phases.**

H. Jiang, Y. Li, M. Pelzer, M. Cannon, C. Randlett, H. Junga, X. Jiang and Q. Ji *Journal of Chromatography A* 2008, 1192, p. 230-238.

#### **An improved LC-ESI-MS-MS method for simultaneous quantitation of rosiglitazone and N-desmethyl rosiglitazone in human plasma.**

G. O'Maille, S. Pai, X. Tao, G. Douglas and R. Jenkins *Journal of Pharmaceutical and Biomedical Analysis* 2008, 48, p. 934-939.

#### **Analysis of galanthamine-type alkaloids by capillary gas chromatography-mass spectrometry in plants.**

S. Berkov, J. Bastida, F. Viladomat and C. Codina *Phytochemical Analysis* 2008, 19, p.

#### **Supported liquid-liquid extraction of the active ingredient (3, 4-methylenedioxyamphetamine) from ecstasy tablets for isotopic analysis.**

A. De Korompay, J. Hill, J. Carter, N. NicDaeid and R. Sleeman *Journal of Chromatography A* 2008, 1178, p. 1-8.

#### **Simultaneous Quantification of Amphetamine and Methamphetamine in Meconium using ISOLUTE HM-N Supported Liquid Extraction Columns and GC-MS.**

J. Gunn, B. Sweeney, T. Dahn, S. Bell, R. Newhouse and A. Terrell *Journal of Analytical Toxicology* 2008, 32, p. 485-490.

Please note that Biotage offers two grades of Diatomaceous earth. ISOLUTE SLE+ is a finer grade, optimized for the smaller 96-well plate bed sizes. ISOLUTE HM-N is a much larger particle size material used in cartridges. Because the mechanism is similar, both have been included in this bibliography.

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**Part Number:**

UG\_SLE.0409