



ISOLUTE® SLE+ Supported Liquid Extraction Plates for the Extraction of Drugs from Biological Fluids

This Chemistry Data Sheet describes the use of ISOLUTE SLE+ Supported Liquid Extraction Plates for the isolation of acidic, basic and neutral drugs from biological fluids.

Introduction

Liquid-liquid extraction (LLE) is a commonly used sample preparation technique for the isolation of drugs from biological fluids. While the technique provides clean extracts, time consuming off-line steps such as capping, mixing, shaking and de-capping, along with possible emulsion formation do not make the technique amenable to high throughput sample preparation.

Supported Liquid Extraction (SLE) is a high throughput alternative to LLE. The technique uses an inert support to mimic the LLE process and does not have many of the problems associated with the traditional technique.

ISOLUTE SLE+ 200 and 400 mg Supported Liquid Extraction Plates contain a modified form of diatomaceous earth. The support provides an inert interface between the two liquids, allowing rapid transfer of the drugs into the extraction solvent.

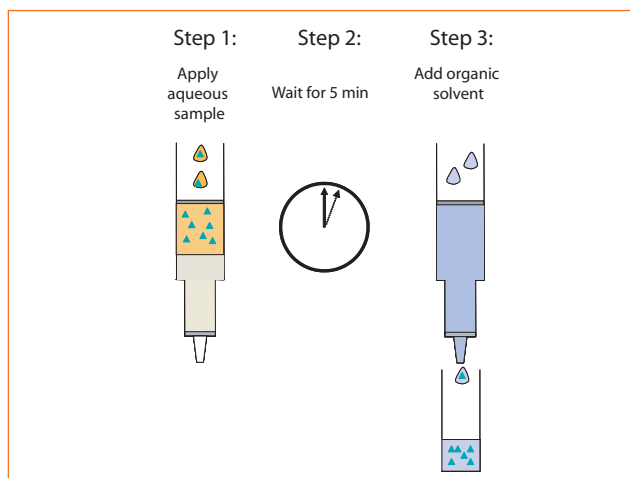


Figure 1. Schematic of the ISOLUTE SLE+ Supported Liquid Extraction procedure. A single well of a 96-well plate is illustrated.

It is important the maximum aqueous load volume (biological fluid plus diluent) does not exceed the recommended volume for 200 and 400 mg ISOLUTE SLE+ Plates.

Product	Maximum Aqueous Load
ISOLUTE SLE+ 200 mg Plate	200 μ L
ISOLUTE SLE+ 400 mg Plate	400 μ L

Generic approach to Method Development using ISOLUTE SLE+ Supported Liquid Extraction Plates.

Biotage has developed a method selection approach using ISOLUTE SLE+ Plates.

The generic procedure takes three key factors into consideration:

- Analyte functionality
- Sample pH
- Extraction solvent

Using this systematic approach, methods can be rapidly developed, thereby minimizing method development time and increasing productivity.

Protocol for Acidic Drug Extraction

Evaluate the following procedure for acidic analytes:

Procedure / Step	200 mg Plate ¹	400 mg Plate ²
Sample Pre-treatment	Dilute sample (100 µL) ³ 1:1 (v/v) with aqueous buffer. For acidic drugs evaluate pH 3.2 and pH 6.3 ⁴ .	Dilute sample (200 µL) ³ 1:1 (v/v) with aqueous buffer. For acidic drugs evaluate pH 3.2 and pH 6.3 ⁴ .
Sample Load	Apply sample. Apply vacuum pulse (-15 "Hg / -0.5 bar) for 5-15 seconds to initiate loading; sample should typically load within 1 minute.	
Soak Step	Once loaded, wait 5 minutes to allow the aqueous phase to be fully adsorbed.	
Analyte Extraction	Apply extraction solvent ⁷ (1 mL) using gravity flow for 5 minutes. For maximum recovery apply a vacuum of -15 "Hg / -0.5 bar for 2 minutes.	Apply extraction solvent ⁷ (2 x 900 µL) using gravity flow for 5 minutes. For maximum recovery apply a vacuum of -15 "Hg / -0.5 bar for 2 minutes after each aliquot.

Protocol for Neutral Drug Extraction

Evaluate the following procedure for neutral analytes:

Procedure / Step	200 mg Plate ¹	400 mg Plate ²
Sample Pre-treatment	Dilute sample (100 µL) ³ 1:1 (v/v) with aqueous buffer. For neutral drugs evaluate pH 6.3 and pH 8 ⁵ .	Dilute sample (200 µL) ³ 1:1 (v/v) with aqueous buffer. For neutral drugs evaluate pH 6.3 and pH 8 ⁵ .
Sample Load	Apply sample. Apply vacuum pulse (-15 "Hg / -0.5 bar) for 5-15 seconds to initiate loading; sample should typically load within 1 minute.	
Soak Step	Once loaded, wait 5 minutes to allow the aqueous phase to be fully adsorbed.	
Analyte Extraction	Apply extraction solvent ⁷ (1 mL) using gravity flow for 5 minutes. For maximum recovery apply a vacuum of -15 "Hg / -0.5 bar for 2 minutes.	Apply extraction solvent ⁷ (2 x 900 µL) using gravity flow for 5 minutes. For maximum recovery apply a vacuum of -15 "Hg / -0.5 bar for 2 minutes after each aliquot.

Protocol for Basic Drug Extraction

Evaluate the following procedure for basic analytes:

Procedure / Step	200 mg Plate ¹	400 mg Plate ²
Sample Pre-treatment	Dilute sample (100 µL) ³ 1:1 (v/v) with aqueous buffer. For basic drugs evaluate pH 8 and pH 10.4 ⁶ .	Dilute sample (200 µL) ³ 1:1 (v/v) with aqueous buffer. For basic drugs evaluate pH 8 and pH 10.4 ⁶ .
Sample Load	Apply sample. Apply vacuum pulse (-15" Hg / -0.5 bar) for 5-15 seconds to initiate loading; sample should typically load within 1 minute.	
Soak Step	Once loaded, wait 5 minutes to allow the aqueous phase to be fully adsorbed.	
Analyte Extraction	Apply extraction solvent ⁷ (1 mL) using gravity flow for 5 minutes. For maximum recovery apply a vacuum of -15 "Hg / -0.5 bar for 2 minutes.	Apply extraction solvent ⁷ (2 x 900 µL) using gravity flow for 5 minutes. For maximum recovery apply a vacuum of -15 "Hg / -0.5 bar for 2 minutes after each aliquot.

Supporting Notes

¹Maximum aqueous load (biological fluid and diluent) for the 200 mg plate is 200 µL.

²Maximum aqueous load (biological fluid and diluent) for the 400 mg plate is 400 µL.

³To increase the volume of biological fluid loaded, a lower dilution factor may be used. However, samples of increased viscosity may not flow evenly across the plate.

⁴Recommended buffers to evaluate for acidic analytes are pH 3.2: 1% aqueous formic acid and pH 6.3: 0.1% aqueous formic acid.

⁵Recommended buffers for neutral analytes are pH 6.3: 0.1% aqueous formic acid and pH 8: water.

⁶Recommended buffers for basic analytes are pH 8: water and pH 10.4: 0.5 M aqueous ammonium hydroxide.

⁷Evaluate the following solvents: MTBE, DCM, 5:95 (v/v) IPA/DCM and ethyl acetate.

Buffers and Solvents

1% (v/v) Formic Acid

Used to adjust sample to ~pH 3.2. Take concentrated formic acid (1 mL) and add HPLC grade water (99 mL). Mix thoroughly.

0.1% (v/v) Formic Acid

Used to adjust sample to ~pH 6.3. Take concentrated formic acid (100 µL) and add HPLC grade water (99.9 mL). Mix thoroughly.

0.5 M NH₄OH

Used to adjust sample to ~pH 10.4. Take 28% aqueous ammonium hydroxide (3.33 mL) and add deionized water (96.6 mL). Mix thoroughly.

5:95 (v/v) IPA/DCM

Take IPA (5 mL) and add DCM (95 mL). Mix thoroughly.

For further information download the **ISOLUTE SLE+ Method Selection Guide** from www.biotage.com

Recovery Data

ISOLUTE SLE+ Supported Liquid Extraction Plates extract acidic, neutral and basic analytes from biological fluids. The following sections show typical recoveries for different drug classes.

Basic Drugs

Analytes:	Trimipramine, Imipramine and Nortriptyline.
Concentration:	1 ng/mL.
Product:	ISOLUTE SLE+ 200 mg Supported Liquid Extraction Plate (part number 820-0200-P01).
Sample Pre-treatment:	To human plasma (100 µL) add 0.5 M NH ₄ OH (100 µL) and mix thoroughly.
Sample Load:	Load diluted sample (200 µL) to each well. Initiate flow with a pulse of vacuum (-15 "Hg / - 0.5 bar for 5 – 15 seconds).
Soak Step:	Wait for five minutes when all samples are fully loaded onto the plate.
Extraction:	Elute with 98:2 (v/v) hexane / 3-methyl-1-butanol (1 mL) under gravity for 5 minutes and then apply vacuum (-15 "Hg / -0.5 bar) for 2 minutes.

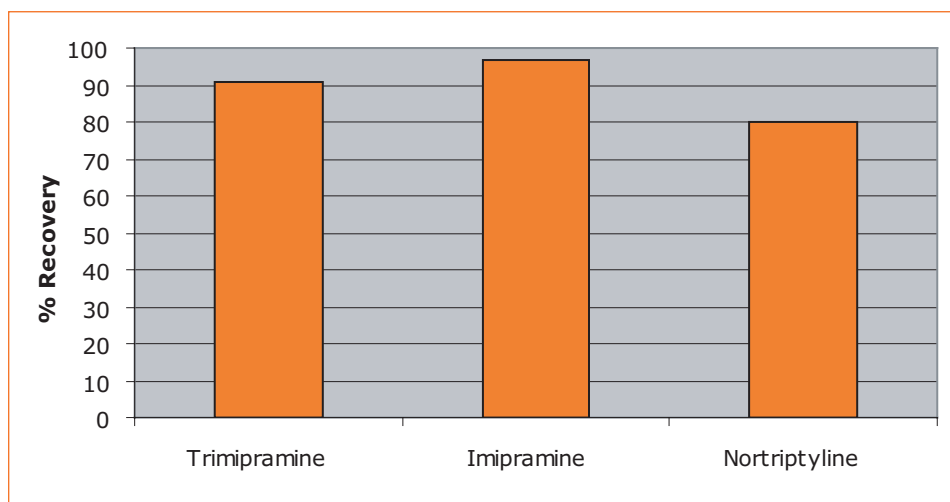


Figure 2. Recovery of tricyclic antidepressants from plasma using ISOLUTE SLE+ 200 mg Supported Liquid Extraction Plate (< 10% RSD, n = 7).

Acidic Drugs (NSAIDs)

Analytes:	Sulindac, Flurbiprofen and Ibuprofen.
Concentration:	500 ng/mL.
Product:	ISOLUTE SLE+ 400 mg Supported Liquid Extraction Plate (part number 820-0400-P01).
Sample Pre-treatment:	To human plasma (200 µL) add 1% aqueous formic acid (200 µL) and mix thoroughly.
Sample Load:	Load diluted sample (400 µL) to each well. Initiate flow with a pulse of vacuum (-15 "Hg / -0.5 bar for 5 – 15 seconds).
Soak Step:	Wait for five minutes when all samples are fully loaded onto the plate.
Extraction:	Elute with MTBE (2 x 900 µL) under gravity for 5 minutes. Apply vacuum (-15 "Hg / -0.5 bar) for 2 minutes after each aliquot.

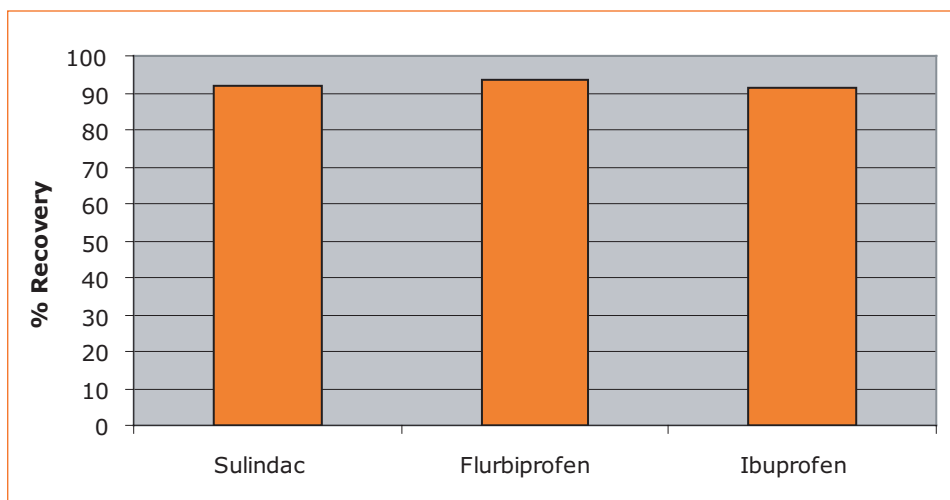


Figure 3. Recovery of NSAIDs from plasma using ISOLUTE SLE+ 400 mg Supported Liquid Extraction Plate (< 10% RSD, n = 7).

Neutral Drugs (Corticosteroids)

Analytes: Triamcinolone, Prednisolone, Hydrocortisone, Prednisone, Cortisone, Betamethasone, Dexamethasone, Flumethasone, Corticosterone, Beclomethasone, Triamcinolone Acetonide, Fluocinolone Acetonide, Budesonide and 5-Pregnen-3 β -ol-20-one.

Concentration: 200 ng/mL.

Product: ISOLUTE SLE+ 200 mg Supported Liquid Extraction Plate (part number 820-0200-P01).

Sample Pre-treatment: To human plasma (100 μ L) add HPLC grade water (100 μ L) and mix thoroughly.

Sample Load: Load diluted sample (200 μ L) to each well. Initiate flow with a pulse of vacuum (-15 "Hg / -0.5 bar for 5 – 15 seconds).

Soak Step: Wait for five minutes when all samples are fully loaded onto the plate.

Extraction: Elute with 5:95 (v/v) IPA/DCM (1 mL) under gravity for 5 minutes and then apply vacuum (-15 "Hg / -0.5 bar) for 2 minutes.

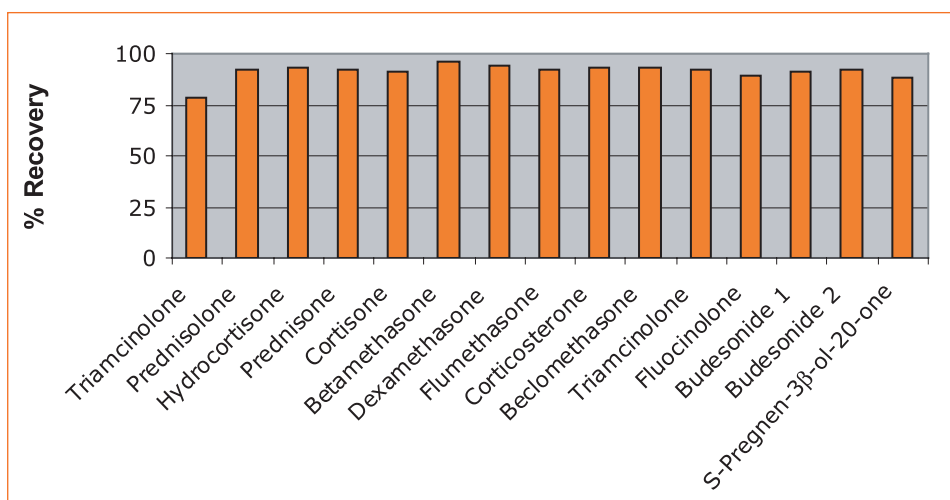


Figure 4. Recovery of corticosteroids from plasma using ISOLUTE SLE+ 200 mg Supported Liquid Extraction Plate (< 5% RSD, n = 7).

Phospholipid Removal

The removal of phospholipids from biological fluid extracts prior to drug quantitation is a key component of sample preparation. Phospholipid interferences do not generally chromatograph as sharp discrete peaks, often taking several minutes to elute from the analytical column. As a result, analyte quantitation can be adversely affected when the drug co-elutes with these interferences.

ISOLUTE SLE+ Supported Liquid Extraction Plates provide extremely clean extracts and in addition remove significant levels of phospholipids when using typical combinations of sample pH and common extraction solvents. The level of phospholipid in the final extract can increase when more polar solvents are used.

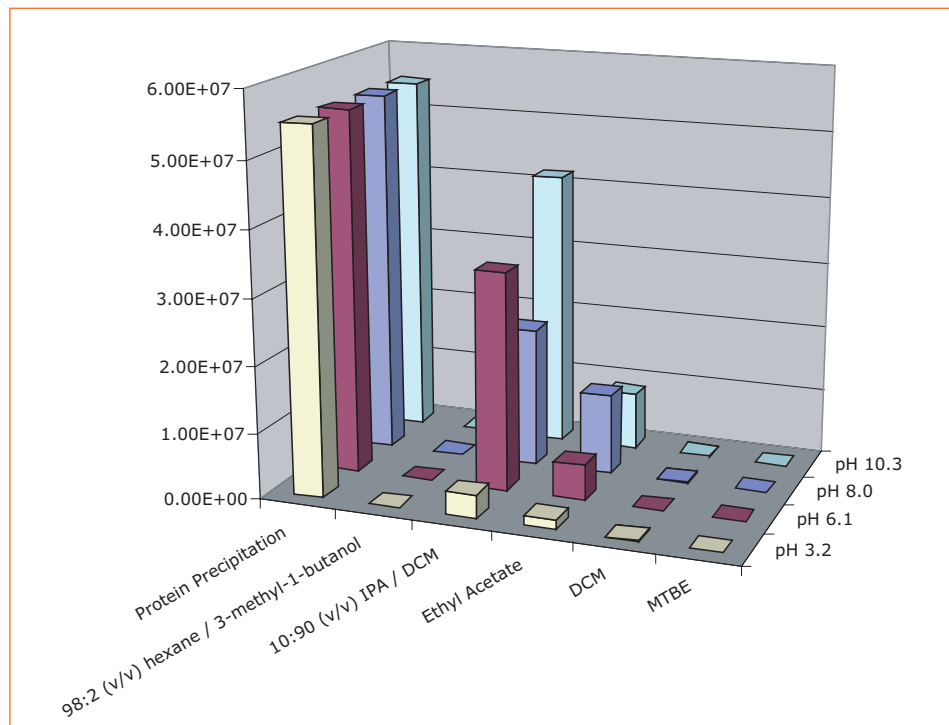


Figure 5. Phospholipid removal (SIR at 496, 520, 522 & 524 Da) with different sample pHs and extraction solvents using ISOLUTE SLE+ Supported Liquid Extraction Plates.

Processing Options

ISOLUTE SLE+ Supported Liquid Extraction Plates are compatible with manual and automated sample processing. Contact Biotage for details on the range of VacMaster™-96 Sample Processing Manifold options for manual processing, along with a range of 96-well plate accessories.

ORDERING INFORMATION

Description	Quantity	Part number
ISOLUTE SLE+ 200 mg Supported Liquid Extraction	1	820-0200-P01
ISOLUTE SLE+ 400 mg Supported Liquid Extraction	1	820-0400-P01

www.biotage.com

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