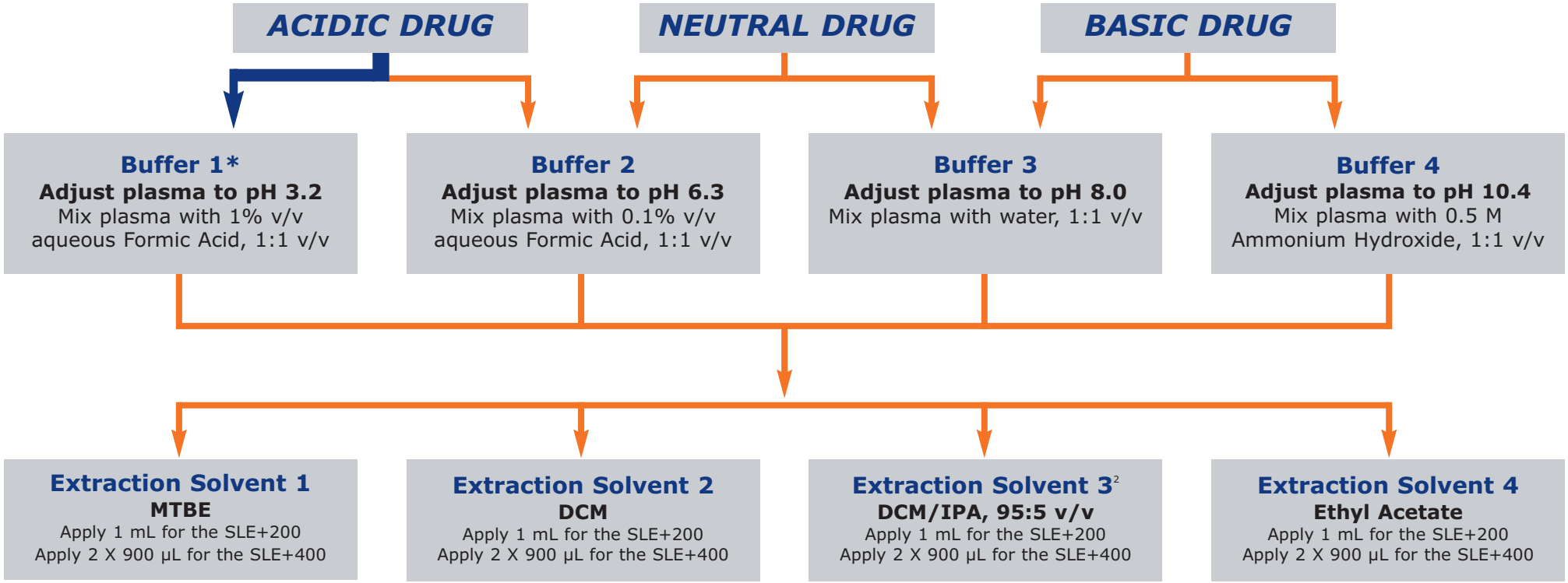
ISOLUTE SLE+ Supported Liquid Extraction Plates: Method Selection



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 pK_a and logP values. The selections are based on extensive work in Biotage's R&D Laboratories[1].

*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[2].

Biotage

[1] 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.

[2] Investigation of Phospholipid Removal using 96-well Supported Liquid Extraction. L Williams et al. Presented at Montreux Symposium 2007.

Generic Method/Instruction for Use

Description	Part Number	Maximum Aqueous Load
ISOLUTE SLE+ 200mg Supported Liquid Extraction Plate	820-0200-P01	200 µL
ISOLUTE SLE+ 400mg Supported Liquid Extraction Plate	820-0400-P01	400 µL

1. Sample Pre-treatment

To promote even flow, biological fluid samples should be diluted 1:1 v/v with aqueous buffer prior to loading.
For ionizable analytes, extraction efficiency can be enhanced by the use of pH control to suppress ionization.

IMPORTANT: Maximum aqueous capacity
ISOLUTE SLE+ Supported Liquid Extraction plates contain either 200 or 400 mg support media per well. For the 200 and 400 mg formats the maximum aqueous load is 200 and 400 µL respectively. **It is important that the total aqueous load volume (biological fluid plus diluent) does not exceed the recommended volume.**

Recommended dilution factor: To promote even flow we recommend that samples are diluted 1:1 with aqueous buffer prior to loading.

To increase the amount of biological fluid sample loaded, a lower dilution factor may be used. However, samples of varying viscosity may not flow consistently from well to well.

Note: The minimum practical volume of sample per well (i.e. biological fluid plus diluent) is 150 µL.

2. Sample Load

Place the ISOLUTE SLE+ Plate on a suitable sample processing manifold, with collection plate in position. Apply aqueous sample to each well. Sample may percolate through frit at this stage. Apply vacuum (-15" Hg / -0.5 bar) for 5-15 seconds to initiate loading. Sample should be fully loaded within 1 minute (200 and 400 mg plate), depending on sample viscosity.

Note: There should be no sample breakthrough at the sample load stage. The entire aqueous volume is absorbed onto the support.

3. Sample Soak

Once loaded wait 5 minutes for sample to completely absorb.

4. Analyte Elution/Extraction

Ensure collection plate is in place. Some extraction solvents (e.g. MTBE) will flow immediately under gravity. Others (e.g. hexane) will require vacuum to complete elution. We recommend the following procedure for all solvents:

ISOLUTE SLE+ 200 mg: 1 x 1 mL using gravity flow for 5 minutes followed by -15" Hg / -0.5 bar for 2 minutes

ISOLUTE SLE+ 400 mg: 2 x 900 µL using gravity flow for 5 minutes. For maximum recovery apply a vacuum of -15" Hg / -0.5 for 2 minutes after each aliquot

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