Technical Note 126

SAMPLE PREPARATION BY NON-POLAR SPE USING ISOLUTE® SPE SORBENTS

This technical note describes the use of ISOLUTE non-polar SPE sorbents for the extraction of drugs from biological fluids such as plasma or urine.

Non-polar SPE is commonly used for extraction of acidic, neutral and basic drugs from biological fluids. It is particularly useful when a parent drug and metabolites of different functionalities are to be extracted simultaneously, and will provide cleaner extracts than alternative sample preparation techniques such as protein precipitation.

ISOLUTE non-polar SPE sorbents retain drugs from aqueous biological fluids through hydrophobic interactions. Endogenous compounds from the original sample matrix, such as proteins, are not retained. When the sorbent is rinsed, weakly retained compounds are eluted to waste, leaving strongly retained compounds to be eluted in the final elution solvent.

The strength of the non-polar interaction is a function of the sorbent chain length (for example ISOLUTE C2 is less retentive than ISOLUTE C18). If retention of unwanted compounds is minimized, a cleaner final extract will result.

EXTRACTION PROTOCOL

Screen ISOLUTE C2, C8 and C18, 25 mg/1 mL in the ISOLUTE Array® format using the procedure detailed below. Populate the plate with individual wells of each sorbent as required. Choose the SPE sorbent that combines high recoveries with the cleanest extract. Process using a VacMaster®-96 sample processing station or automated liquid handling system.

Vacuum settings

At all stages, use a short pulse (approx 1 second) of low vacuum (< -5" Hg), unless otherwise stated.

Sample volume

This procedure is optimized for a biological fluid sample volume of 100 µL. Sample should be diluted 1:1 (v/v) with appropriate buffer before applying to the column (total volume of buffered sample applied is 200 µL).

Note: Work in our R&D laboratory has shown that 25 mg ISOLUTE SPE columns have sufficient capacity for extraction of up to 1 mL plasma sample without analyte breakthrough. Test conditions: 1 mL plasma spiked at 0.1 mg/µL analyte concentration and diluted 1:1 with buffer before applying to the column (total volume of buffered sample applied is 2 mL).

Sample pre-treatment

For neutral analytes, dilute the sample (100 µL of plasma or urine), with ammonium acetate buffer, (0.05 M, pH 6.0, 100 µL) to give a 200 µL total sample volume at 1:1 dilution.

For ionizable analytes, adjust the sample pH to ensure neutralization of the analytes. This will maximize analyte recovery.

- *For acidic analytes, adjust the sample to 2 pH units below the pK of the analytes using an appropriate buffer (1:1, v/v dilution)*
- *For basic analytes, adjust the sample to 2 pH units above the pK of the analytes using an appropriate buffer (1:1, v/v dilution)*

Column conditioning

Place extraction plate on vacuum manifold. No collection plate should be used at this stage.

Condition each well with methanol (1 µL). Use gravity or apply a short pulse of vacuum to initiate flow. This will ensure efficient wetting of the hydrophobic frits, promoting even flow of sample through the wells.

Column equilibration

Rinse wells with buffer* (250 µL). Load all wells prior to applying a short pulse of vacuum to initiate flow.

*(*as used for sample pre-treatment)*

Sample loading

Apply 200 µL buffered sample. Load all wells prior to applying a short pulse of vacuum to initiate flow.

Interference elution

Elute weakly retained interferences with buffer*/methanol (95:5, v/v, 250 µL). Load all wells, and allow to soak for 1 minute.

*(*as used for sample pre-treatment)*

A higher concentration of methanol can be used to achieve cleaner extracts. However, the step must be checked for analyte breakthrough.

Following the soak step, apply vacuum for 30 seconds to dry the sorbent bed.

Analyte elution

Place collection plate in base of manifold. Ensure correct alignment (position A1 of collection plate directly underneath position A1 of extraction plate), and that extraction plate outlet Luer tips extend into the top of the collection plate. This will prevent sample cross contamination. Spacers are available to ensure optimum penetration.

Elute analytes with methanol/1M ammonium acetate (99.5 : 0.5, v/v, 2 x 100 µL).

- *For acidic drugs, evaluate the use of methanol containing 2% acetic acid to maximize recoveries*
- *For basic drugs, evaluate the use of methanol containing 2% ammonium hydroxide to maximize recoveries*

Apply the first 100 µL aliquot and allow to soak for 2–4 mins. If the aliquot has not reached the top frit at the end of the soak time, apply a short vacuum pulse.

Apply the second 100 µL aliquot and allow to soak for a further 2–4 mins. Apply low vacuum for 1 minute to complete elution.

Evaporate this elution solvent and re-constitute the sample in a solvent compatible with the analytical technique. For LC-MS the mobile phase is suggested.

Care should be taken to avoid losses of thermally labile or volatile analytes at this stage.

REAGENTS

1. Methanol

2. 0.05 M ammonium acetate buffer, pH 6
Ammonium acetate 97+% reagent, FW 77.08. Dissolve 3.854 g in 1 L of water and adjust pH using 1 M acetic acid (0.9635 g in 250 mL of water).

3. 0.05M Ammonium acetate buffer/methanol (95 : 5, v/v)
Add 5 mL methanol to a 100 mL volumetric flask, and make up to volume with ammonium acetate buffer, as described above.

4. Methanol/1 M ammonium acetate (99.5 : 0.5, v/v)
Ammonium acetate 97+% reagent, FW 77.08. Dissolve 19.27 g in 250 mL of water. Add 0.5 mL of 1 M ammonium acetate to a 100 mL volumetric flask, and make up to volume with methanol.

ORDERING INFORMATION

Description	Pack size	Part Number
ISOLUTE Array format		
ISOLUTE Array C2 25 mg/1 mL wells*	100	320-0025-R
ISOLUTE Array C8 25 mg/1 mL wells	100	290-0025-R
ISOLUTE Array C18 25 mg/1 mL wells	100	220-0025-R
ISOLUTE Array C2 25 mg/1 mL plate	1	320-0025-RP
ISOLUTE Array C8 25 mg/1 mL plate	1	290-0025-RP
ISOLUTE Array C18 25 mg/1 mL plate	1	220-0025-RP

* As with other Array products, loose wells can be processed on a standard VacMaster-10 or -20 Sample Processing Station equipped with Array Luer adaptors (p/n 120-1201). In order to process loose wells using a VacMaster-96 Sample Processing Station, a base plate (part number 120-1000-P01) and base plate sealing strips (part number 120-1200 for sealing unused positions) are required.

Description	Pack size	Part Number
ISOLUTE-96 format		
ISOLUTE-96 C2 25 mg plate	1	320-0025-P01
ISOLUTE-96 C8 25 mg plate	1	290-0025-P01
ISOLUTE-96 C18 25 mg plate	1	220-0025-P01
ISOLUTE column format		
ISOLUTE C2 25 mg/1 mL	100	320-0002-A
ISOLUTE C8 25 mg/1 mL	100	290-0002-A
ISOLUTE C18 25 mg/1 mL	100	220-0002-A
Tab-less ISOLUTE column format		
ISOLUTE C2 25 mg/1 mL (tab-less)	100	320-0002-AG
ISOLUTE C8 25 mg/1 mL (tab-less)	100	290-0002-AG
ISOLUTE C18 25 mg/1 mL (tab-less)	100	220-0002-AG

Other configurations are available, please contact Biotage for details.

VacMaster®-96 Processing Station

Description	Pack size	Part Number
VacMaster-96 manifold only*	1	121-9600
VacMaster-96 Vacuum Control Unit	1	121-9601
VacMaster-96 Vacuum Control Unit with integral vacuum source	1	121-9602
VacMaster-96 with Vacuum Control Unit (121-9601)	1	121-9603
VacMaster-96 with Vacuum Control Unit (121-9602)	1	121-9604

* Option does not include a vacuum control unit.

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