How to use Guide

Sample Preparation Products

TELOS® ENV

for Biological Fluid Samples

Introduction

This document provides comprehensive method development guidelines for the use of TELOS® ENV SPE Columns when extracting polar water soluble analytes from aqueous sample matrices. Each step of the SPE method is considered, with particular attention paid to aspects such as recommended solvents, flow rates and volumes for each column configuration. These guidelines will aid in creating the correct conditions for retention, interference removal and analyte elution to maximise the use of the TELOS ENV SPE Columns.

Structure	Styrene Divinylbenzene Copolymer
Surface	Surface Area ~900m²/g pH range 1–14 Water-wettable
Retention Mechanism(s)	Primary: Non-polar (hydrophobic) No secondary interactions
Particle Size Range	40–150µm
Applications	Water soluble analytes from aqueous samples

Sample Preparation Guidelines

This section comprises a general method that can be used as a starting point for new SPE methods, followed by detailed guidelines for each step of the procedure.

General Method: Biological Fluid Sample

The following method is recommended for more complex samples containing a variety of interferences, such as biological fluids. Considerations such as dilution and pH control are detailed on pages 2-4.

Column Selection	100mg/3ml Select configuration based on the application. See page 2 for guidelines.
Sample Pre-treatment	Dilute sample 1:1 v/v with a suitable buffer (typically 0.05–0.1M) to aid sample flow through the SPE column and to suppress ionisation of the analytes. See page 2 for details. Optimise dilution factor to achieve correct flow, since some samples are more viscous than others.
Column Conditioning	Rinse with methanol (3ml) at a flow rate of 3ml/min.
Column Equilibration	Rinse with buffer used in pre-treatment (3ml) at a flow rate of 3ml/min.
Sample Loading	Load sample (1ml) at a flow rate of 1ml/min. Optimise to a flow rate that does not cause analyte breakthrough.
Interference Elution	Rinse with buffer used in previous steps (3ml) at a flow rate of 3ml/min. Evaluate a rinse step containing up to 40% v/v water miscible organic solvent to elute polar interferences. Optimise each wash to a volume that maximises interference removal and a flow rate that does not cause analyte breakthrough.
Analyte Elution	Elute with methanol (2 x 1ml) at a flow rate of 1ml/min. Include a 1 minute soak step after the first aliquot. Optimise solvent composition, volume and flow rate to maximise recoveries.
Evaporation / Reconstitution	Evaporate solvent and reconstitute as required.

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Column Selection

The configuration required will depend on the sample volume and an estimated concentration of analytes and matrix components present in the volume of sample to be loaded. See page 4 for the configurations available.

It may be that all of, or an aliquot of the sample is required to meet detection and quantitation limits. The column selection should be based on the total volume of sample to be loaded onto the column.

Loading capacity of TELOS ENV is 10% w/w, which should be used to calculate the recommended maximum loading of the column. If other matrix components exhibit non-polar characteristics, it is likely that they will be retained on the column along with the analytes at sample loading. Therefore, it is important that the column has adequate capacity to retain the other components, even though they may be removed from the column prior to analyte elution.

Biological fluid samples usually contain high levels of matrix components that will compete with the analyte for retention on the SPE column. It is important to choose the correct SPE column format for these applications. See page 4 for recommendations.

Automation

If using automation, the solvent loading ability may be dependent on the volume of solvent that the automation equipment can handle.

Volumes and Flow Rates

The volumes and flow rates recommended for each configuration at each step of the method are for guideline purposes and should be optimised fully for a robust and efficient SPE procedure.

Sample Pre-treatment

Sample pre-treatment is necessary to create an ideal environment for retention of the analytes onto the SPE column. Depending on the sample type, a variety of parameters can be involved, such as pH control and dilution.

Biological fluid samples require dilution to aid correct flow through the SPE column. A dilution factor of 1:1 v/v is often adequate, but a higher ratio of sample/solvent may be necessary for more viscous samples.

For ionisable analytes, pH control to neutralise the charge on the molecule is advised in order to enhance retention via non-polar interactions. For acidic compounds, adjust the pH to 2 units below the pK_a of the analyte. For basic compounds, adjust the pH to 2 units above the pK_a of the analyte.

This approach can also be used to minimise the retention of polar ionisable interferences where the analytes of interest are non-ionisable. By adjusting the pH of the sample, non-ionisable analytes are unaffected, but the ionised interferences are less likely to be retained on the SPE column.

Column Conditioning

TELOS ENV SPE Columns are water-wettable, therefore can be used without a conditioning step. If conditioning is desirable, it should be carried out with a water miscible organic solvent. Methanol is recommended, but others such as acetonitrile are also suitable.

Conditioning should be 2–3 bed volumes* of solvent. *See Table 1* for recommendations on volumes and flow rates for the range of TELOS ENV SPE Columns. In the final method, solvent volumes can be optimised to reduce solvent consumption and flow rates can be adjusted for efficient processing.

**bed volume is based on a solvent volume of 250µl/100mg sorbent.*

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Table 1. Recommended Solvent Volumes and Flow Rates for Column Conditioning

Configuration	Solvent Volume	Flow rate
100mg/3ml	250-750µl	3ml/min
200mg/3ml	500µl-1.5ml	3ml/min
200mg/6ml	500µl-1.5ml	6ml/min

Column Equilibration

This is an important step following column conditioning, to remove excess solvent that would impair retention of the analytes onto the SPE column.

Water is often adequate, but if pH control is used in sample pre-treatment, it is useful to maintain that environment on the SPE column in order to maximise retention of the analytes.

Equilibration should be 2–3 bed volumes* of solvent. *See Table 2* for recommendations on volumes and flow rates for the range of TELOS ENV SPE Columns. In the final method, solvent volumes can be optimised to reduce solvent consumption and flow rates can be adjusted for efficient processing.

Table 2. Recommended Solvent Volumes and Flow Rates for Column Equilibration

Configuration	Solvent Volume	Flow rate
100mg/3ml	250–750µl	3ml/min
200mg/3ml	500µl-1.5ml	3ml/min
200mg/6ml	500µl-1.5ml	6ml/min

Sample Loading

Depending on the analytical requirements, an aliquot or the entire pre-treated sample should be loaded onto the column at the appropriate flow rate. Sample loading flow rates are typically lower than those used in column conditioning and equilibration, ensuring the analytes interact with the functional group(s) of the sorbent, which includes reaching into the porous areas that provide the surface area.

Gravity loading may be possible, but in most cases vacuum will be required for most biological fluids samples.

Flow rate should be optimised for the final SPE procedure, with the aim being to maximise efficiency and maintain high analyte recovery. Higher loading rates may be possible for some analytes and should be evaluated based on the application.

Interference Elution

Often, the use of water is sufficient during this step to remove water soluble polar interferences, while maintaining interactions between the analyte and sorbent.

The use of pH control may be important here to prevent analyte elution by providing the same environment as sample pre-treatment and column equilibration.

For some non-polar analytes, the addition of low % v/v of a water/water miscible organic solvent mixture can be used to remove weakly retained non-polar interferences. This should be optimised to provide the best extract cleanliness without causing analyte breakthrough.

For samples containing non-ionisable analytes and polar ionisable interferences, pH control can be used to enhance elution of the ionisable interferences, while retaining the analyte on the column.

Interference elution should be possible with 3–10 bed volumes* of the chosen solvent. Flow rate and volumes should be optimised for the final SPE method. See Table 3 for recommendations.

**bed volume is based on a solvent volume of 250µl/100mg sorbent.*

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Table 3. Recommended Solvent Volumes and Flow Rates for Interference Elution

Configuration	Solvent Volume	Flow rate
100mg/3ml	750–2.5ml	3ml/min
200mg/3ml	1–1.5ml	3ml/min
200mg/6ml	1–1.5ml	6ml/min

Analyte Elution

The solvent used for analyte elution must overcome the non-polar interactions between analyte and sorbent. For cases where interferences may be retained on the column following interference elution and after the analyte is recovered, the analyte elution solvent should be selected to elute the analytes but not the remaining interferences.

As TELOS ENV does not exhibit any secondary interactions, it is possible to use pure solvent such as methanol for analyte elution. In some cases, addition of a modifier (e.g. acid or base) or use of a solvent mixture (e.g. acetone/ethyl acetate) may be necessary for analyte solubility reasons. Mixed solvents (e.g. methanol or acetone/ethyl acetate) can be used to enhance recovery of analytes in multiple analyte suites.

If using a water immiscible organic solvent, the SPE column must be dried thoroughly before elution. See below for some recommended drying times for TELOS ENV SPE Columns.

Table 4. Recommended Drying Times for TELOS ENV SPE Columns

Configuration	Drying Time
100mg/3ml	5 min
200mg/3ml	10 min
200mg/6ml	10 min

Based on full vacuum at approx. -20”Hg.

The recommended solvent volume for analyte elution is typically 2–5 bed volumes*, although larger volumes may be necessary for certain analytes. Flow rate should be optimised to provide sufficient residence time of the solvent on the sorbent to maximise analyte recovery. Use of a solvent soak step can improve recoveries by allowing more time for the elution solvent to reach into the pores of the sorbent. Typically, this is a 1 minute soak for each aliquot of analyte elution solvent.

Table 5. Recommended Solvent Volumes and Flow Rates for Analyte Elution

Configuration	Solvent Volume	Flow rate
100mg/3ml	2 x 250-625µl	3ml/min
200mg/3ml	2 x 500µl-1.25ml	3ml/min
200mg/6ml	2 x 500µl-1.25ml	6ml/min

Ordering Information

Part Number	Description	Pack Size
690-100M-003T	TELOS ENV 100mg/3ml SPE Columns	50
690-200M-003T	TELOS ENV 200mg/3ml SPE Columns	50
690-200M-006T	TELOS ENV 200mg/6ml SPE Columns	30

TELOS ENV 100mg/3ml columns are the ideal configuration for biological fluids applications, including the extraction of polar drug metabolites from biological fluids such as urine and plasma. For larger sample volumes, the 200mg/3ml columns provide additional capacity. The 200mg/6ml configuration may also be useful for particularly viscous samples where additional sample dilution is necessary to provide optimised sample flow. Please contact Kinesis for additional information about other configurations.

**bed volume is based on a solvent volume of 250µl/100mg sorbent.*

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