

Simple, Fast Methods for Preparing Biological Fluids for LC/MS Analysis

Technical Overview

Improve Liquid/Liquid Extraction for Bioanalysis Studies with Agilent Chem Elut Solid Supported Liquid Extraction (SLE) Products.

- Improve high-throughput metabolism, pharmacokinetic, and bioanalytical studies
- Get reproducible organic phase separation – every time
- Use simple, general methods for all sample types

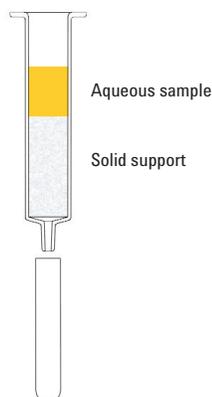
High-throughput bioanalysis demands fast, ready-to-go methods that provide reliable results. Bioanalysts often turn to LLE extraction for low cost, simple method development and overall effectiveness for MS analysis [1]. Chem Elut SLE products improve your liquid/liquid extraction by streamlining methods for all sample types, and eliminating phase separation and emulsion problems. With Chem Elut, simply apply your sample to the gravity-flow cartridge and extract with solvent. Unlike traditional liquid/liquid extraction, shaking is not required and reproducibility is improved. Your blood, plasma, urine, or other biofluid samples are prepared for analysis quickly, reproducibly, and without emulsions. Choose from a 96-well plate or individual cartridges to fit any sample size, and start improving your sample prep.



Extraction Procedure for Aqueous and Biofluid Samples

Step 1

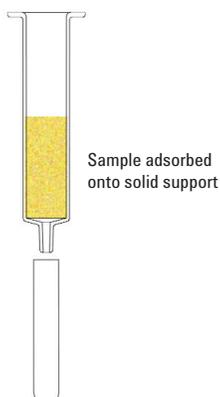
Add the aqueous sample to the dry cartridge.



Chem Elut cartridges are packed with specially cleaned and sized diatomaceous earth.

Step 2

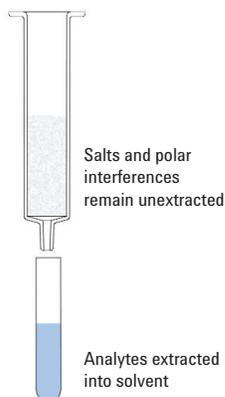
Wait 3-5 minutes.



The aqueous sample is adsorbed and distributed into a thin film over the solid support.

Step 3

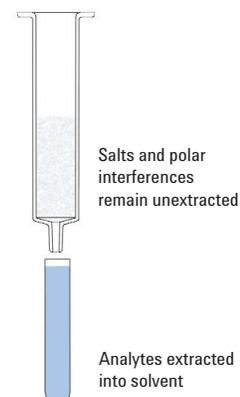
Add the first aliquot of organic extraction solvent. Collect.



As the solvent passes through the cartridge, analytes are extracted from the aqueous layer with recoveries comparable to liquid/liquid extraction. The gravity flow process prevents the formation of emulsions.

Step 4

Add the second aliquot of organic extraction solvent. Collect.



Elution with two aliquots instead of one improves extraction efficiency and recovery. The collected eluents can be analyzed directly, or dried and reconstituted.

General Sample Preparation Methods

The general methods below can be used for many biological fluids, including blood, plasma, serum, urine, and bile. Select the method based on the acid/base properties of your analyte.

Neutral Analytes

1. Select the Agilent Chem Elut cartridge based on total sample volume. Apply sample to appropriate Chem Elut product by gravity flow only. Note: sample will adsorb onto the adsorbent material, rather than flow through the cartridge. Allow 3-5 minutes for complete adsorption to take place.
2. Apply water-immiscible extraction solvent to the Chem Elut under gravity flow. Use two aliquots, with each aliquot equal to the original sample volume. Typical choices are dichloromethane, ethyl acetate, methyl t-butyl ether (MTBE), methyl ethyl ketone (MEK), and butyl acetate. For polar analytes with poor solubility in these solvents, the solvent can be diluted with isopropanol. Ensure that the final extraction solvent is water-immiscible.

3. Collect the extraction solvent as it passes through the Chem Elut cartridge. The extract can now be analyzed directly, or dried down and reconstituted in an LC mobile phase or a GC-amenable solvent.

Basic Analytes

Dilute the sample with an equal volume of basic buffer. For amines and other weakly basic analytes, 1 M ammonium buffers, pH 9-10, are recommended. Follow the procedure for Neutral Analytes above, selecting the Chem Elut product based on the total sample volume including buffer.

Acidic Analytes

Dilute the sample with an equal volume of acidic buffer. For carboxylic and other weakly acidic analytes, 1 M phosphate buffers, pH 2-3, are recommended. Follow the procedure for Neutral Analytes above, selecting the Chem Elut product based on the total sample volume including buffer.

Agilent Chem Elut improves 96-well sample preparation with fast parallel processing and no phase separation problems.

A 96-well Chem Elut (Combilute) can be used for determination of basic drug concentrations in biological fluids for pharmacokinetic studies [2]. The Combilute plate adapts easily to automation, eliminates phase boundary guesswork, and the entire extraction can be performed on automated liquid handling apparatus without manual intervention.

1. Pipet plasma samples (0.2 mL), internal standard solution (500 nM, 0.05 mL), and 1% (w/v) ammonium carbonate solution, pH 9.5 (0.2 mL) into a 96-well collection plate.
2. Vortex-mix (manual method) or agitate with several aspirate and dispense cycles of liquid handler pipette-tips (automated method).
3. Transfer the sample mixtures to a Combilute 96-well plate positioned on top of a 2 mL 96-well collection plate. Allow samples to adsorb for 3-5 minutes. Note: the samples will not flow through the sorbent bed, but become adsorbed onto the sorbent material.
4. Elute with 2.5 mL MTBE by allowing the solvent to gravity-flow through each well. A very slight (< 1 in Hg) vacuum can be used to speed the flow slightly. Higher recoveries are often obtained if multiple aliquots of smaller volumes are used (that is, 2 × 1.25 mL aliquots of MTBE or other solvent).
5. Evaporate the extracts to dryness under nitrogen at 25 °C.
6. Reconstitute the residue with 0.1 mL mobile phase. Inject into LC/MS system for analysis.

Agilent Chem Elut is ideal for fast processing of blood or blood products.

Extraction of drugs from blood is fast and easy with Chem Elut. Extraction of 33 benzodiazepines, metabolites, and similar substances from whole blood has been reported with average recoveries >75%, average RSDs of 9.2% and average limits of detection (LOD) and limits of quantitation (LOQ) values of 1.7 and 5.7 ng/mL, respectively [3].

1. Add 1 mL whole blood, spiked with a 50 mL internal standard solution in methanol, to a 3 mL Chem Elut cartridge buffered to pH 9 (item # 12198005). Add 1.5 mL distilled water to the cartridge. Wait 3-5 minutes for all liquid to adsorb onto the sorbent.
2. Elute with 3 × 4 mL methyl t-butyl ether (MTBE).
3. Evaporate the eluant at 50 °C. Reconstitute in 100 mL mobile phase and inject into LC/MS.

Agilent Chem Elut can be used for a variety of biological sample types with no method modification.

In this example, melatonin is extracted from either plasma or cerebrospinal fluid [4], giving high recoveries (>90%), good reproducibility (C.V. <6%), with low detection limits (0.5 pg/mL, based on 1 mL samples).

1. Add TCA (60 mM, 300 µL) to plasma or cerebrospinal fluid samples (1 mL). Cool in an ice bath for 10 minutes, centrifuge (5,000 g, 10 minutes). Adjust the pH of supernatant to 7.4 by adding NaOH (1 M, 20 µL).
2. Apply an aqueous sample to the top of a 3 mL Chem Elut cartridge. Allow the sample to adsorb onto the sorbent for 3-5 minutes.
3. Extract the melatonin with 2 × 4 mL dichloromethane.
4. Evaporate the combined eluants in a water bath at 37 °C. Reconstitute in 60 µL mobile phase, inject 40 µL.

References

1. "Liquid/liquid extraction is generally recognized to provide extracts containing fewer interferences which contribute to ion suppression than protein precipitation and SPE". See, for example, Bonfiglio, R., King, R. C., Olah, T. V. Merkle, K. *Rapid Commun. Mass Spectrom.*, 13, 1175-1185 (1999).
2. This is a typical method. For variations, see also Peng S.X., Branch, T.M. King, S.L., *Anal Chem.* 73.708-714 (2001).
3. Smink, B.E., *et al. Journal of Chromatography B*, 811 (2004) 13 20.
4. Rizzo, V. Porta, C. Moroni, M. Scoglio, E. Moriatti, R. *J. Chrom. B*, 774, 17-24 (2002).

Ordering information

Part number	Description	Units/package
65401507	Combitute 96-well plate 200 mg Hydromatrix per well	1
12198001	Agilent Chem Elut , 0.3 mL aqueous capacity	100
12198002	Agilent Chem Elut, 0.1 mL aqueous capacity	100
12198003	Agilent Chem Elut, 3 mL aqueous capacity	100
12198004	Agilent Chem Elut, 3 mL aqueous capacity, pre-buffered to pH 4.5	100
12198005	Agilent Chem Elut, 3 mL aqueous capacity, pre-buffered to pH 9	100
12198006	Agilent Chem Elut, 5 mL aqueous capacity	100
12198007	Agilent Chem Elut, 10 mL aqueous capacity	100
12198008	Agilent Chem Elut, 20 mL aqueous capacity	100
12198009	Agilent Chem Elut, 50 mL aqueous capacity	50
12198010	Agilent Chem Elut, 100 mL aqueous capacity	25

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