ILE™ Immobilized Liquid Extraction

Simplified Extraction of Drugs from Serum Using Immobilized Liquid Extraction 96-Well Plates

Immobilized Liquid Extraction (ILE) 96-Well Plates provide the extraction of drugs directly from serum in a high throughput environment.

The preparation of biological fluid samples (plasma, urine) prior to GC–MS/LC–MS analysis has traditionally been accomplished by two techniques: Liquid-liquid extraction (LLE) and Solid Phase Extraction (SPE). Each technique involves laborious procedures and steps that are difficult to automate, and both are frequently plagued by complications and therefore poorly suited to high throughput sample preparation.

ILE separations are fundamentally very similar to traditional liquid-liquid extractions. However, ILE extractions utilize a thin layer of polymer, rather than an organic solvent (as in LLE), to extract compounds from a sample. This extracting polymer, which exhibits the extractive characteristics of a solvent while maintaining complete matrix independence, is immobilized on the surface of an ILE device.

When an aqueous sample is directly exposed to the Immobilized Liquid (polymer), compounds partition between the sample and polymer based on their affinity for each. Compounds which partition into the polymer are back-extracted (eluted) into a small amount of GC or HPLC solvent to complete the extraction process.

A series of experiments was performed to determine the viability of ILE to extract a variety of drugs from normal serum samples. Each drug was extracted at a range of pHs (1.68 to 11) with a Polyacrylate phase - a very polar phase for extracting highly water-soluble compounds.

Experimental conditions

Analysis:
GC–MS analysis was performed using an HP-5971 in SIM mode and a 30m × 0.25 i.d. × 0.25 µm VB-5 column.
Analytes:
Imipramine, Flurazepam, Loratadine, Cocaine
Sample(s):
pH adjusted normal goat serum (Midland Bio), 100 µl
Back-extraction solvent:
Acetonitrile:Methanol mix (2:1), 150 µl
Phase:
Polyacrylate (Acrylate)

ILE Procedure:

1) Dispense sample (pH pre-adjusted)
2) Seal and agitate plate until determined equilibrium time (e.g., plate vortexer, orbital shaker, etc.)
3) Remove sample

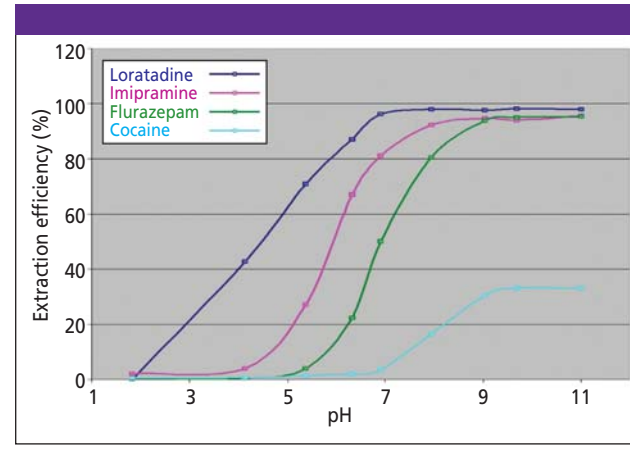

Figure 1: Extraction efficiency as a function of matrix pH using a Polyacrylate 96-Well Plate.

4) Dispense and remove rinse solution (optional interferant removal: e.g. water, buffer, etc.)
5) Dispense back-extraction solvent (elution solvent)
6) Seal and agitate plate until equilibrium
7) Resulting solvent extract is ready for analysis

Results

Extraction efficiencies for a range of polar drugs of different classes directly from whole (undiluted) serum were determined over a broad range of pHs. The results are depicted in Figure 1.

Conclusion

ILE extractions do not require many of the common sample preparation steps that tend to introduce variation to results. The simplified procedure requires minimal human interaction and is amenable to current trends toward fully automated high throughput applications. Consequently, ILE increases laboratory productivity while improving data precision.

The ability to extract over a wide range of pHs and with a variety of ILE phases ensures that optimal extraction conditions may be easily determined. The symmetry exhibited by plotting pH vs. extraction efficiency demonstrates the capability of ILE Well Plates to easily determine the pKa and partition coefficients of analytes in a variety of conditions.

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