Improved Clean Up and Recovery of Pharmaceutical Compounds From Plasma using Strata™-X Solid Phase Extraction (SPE) vs. Traditional Liquid-Liquid Extraction Methods

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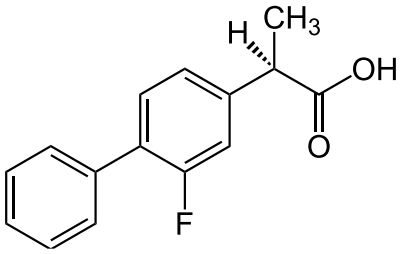
Liquid-liquid extraction (LLE) is perhaps the most established clean up technique used in the chromatography field. Although it has been used for years, newer techniques with improved specificity towards particular analytes have allowed analysts to improve recovery and reproducibility of their samples. This work explores the benefits of solid phase extraction (SPE) as compared to LLE in a pharmaceutical setting. It was found that SPE provides cleaner extracts, higher recoveries, and better reproducibility which can greatly improve results.

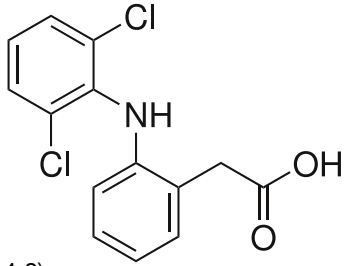
Introduction

Diclofenac (**Figure 1**) is a slightly acidic (pK_a = 4.0) non-steroidal anti-inflammatory drug (NSAID) that has been used as a post operative pain reliever in adult and pediatric patients. As a pain reliever, diclofenac is purported to act via the cyclooxygenase (COX) pathway by inhibition of prostaglandins. However, it also acts as a NSAID that inhibits the lipooxygenase pathway via a process that diminishes the formation of pro-inflammatory hormones. The subsequent quantization of diclofenac from the small volumes of biological matrices, such as plasma, has been of significant concern. Therefore, this article explores two popular extraction methods, solid phase extraction (SPE) and liquid-liquid extraction (LLE), for the isolation of diclofenac from plasma, using a water matrix as the control.

Figure 1.
Structure of Internal Standard Flurbiprofen and Diclofenac



Flurbiprofen (pK_a = 4.2)



Diclofenac (pK_a = 4.0)

Materials and Methods

The plasma pre-treatment step was the same for SPE and LLE and was comprised of filtration through a gauze cloth. Afterwards, 500 µL of diclofenac, which was dissolved in 5 % Methanol, was added to 500 µL of plasma, and the solution mixture was then acidified with 600 µL of 1M Phosphoric acid.

Solid Phase Extraction

The pre-treated plasma samples were further cleaned up and concentrated using SPE.

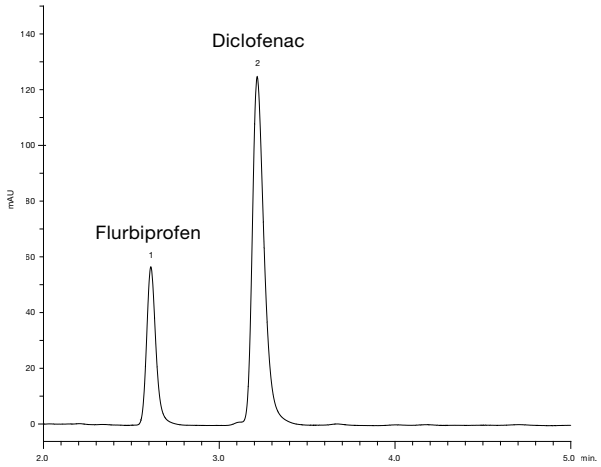
Cartridge:	Strata-X 30 mg/ 1 mL
Part No.:	8B-S100-TAK
Condition:	1 mL Methanol
Equilibrate:	2 mL Water
Load:	1.6 mL Pre-treated plasma
Wash:	1 mL 5 % Methanol
Dry:	1 minute under vacuum at 10 inches Hg
Elute:	1 mL Methanol
Dry down:	Dry down @ 53 °C under a stream of nitrogen for 20 minutes
Reconstitute:	Reconstitute in 500 µL of mobile phase

Liquid-Liquid Extraction

After pre-treatment, 5 mL of Hexane:IPA (95:5) was added to the pre-treated solution, which was followed by 1 minute of vortexing, and 10 minutes of centrifugation at 2,000 rpm. Subsequently, 4 mL of the top organic layer was transferred to a clean glass centrifuge tube and then evaporated to dryness under a stream of nitrogen at 53 °C for 20 minutes.

LC/UV

Chromatogram of Diclofenac and IS after SPE extraction from a plasma matrix.



App ID 19701

Diclofenac spiked plasma sample (50 µg/mL) after extraction with Strata™-X. Flurbiprofen (IS) was added post-extraction at a concentration of 160 µg/mL. Note: the flurbiprofen was added post blow down, which is also post-extraction

Column: Kinetex® 2.6 µm C18
Dimensions: 75 x 4.6 mm
Part No.: 00C-4462-E0
Mobile Phase: Water (adjusted to pH 3.3 with phosphoric acid): Methanol (63:37)
Gradient: Isocratic hold for 5 min
Flow Rate: 1.5 mL/min
Temperature: 30 °C
Detection: UV @ 254 nm
Sample: 1. Flurbiprofen (IS)
2. Diclofenac

Results and Discussion

Traditionally, liquid-liquid extraction (LLE) has been used as the standard clean up procedure for a variety of pharmaceutical samples. Although the technique has been used for many decades it could stand to be improved upon. While performing this work, it was discovered that solid phase extraction provided many benefits over this traditional method including improved recoveries, time and solvent savings, as well as more consistent results.

Plasma samples spiked with diclofenac were used to compare extraction efficiencies of both SPE (using a polymer-based SPE sorbent, Strata-X) and LLE. It was found that SPE on the Strata-X sorbent yields approximately 86 % absolute recovery of 15 µg/mL of diclofenac in the plasma matrix as opposed to 46 % for LLE (**Table 1**). This accounts for almost a two-fold decrease in recovery when using LLE. Furthermore, while this procedure for LLE involved one extraction step, as opposed to multiple extraction steps with the hexane:IPA mixture, in order to have obtained a greater yield using LLE, a greater amount of solvent would have been required for LLE. This would not have only increased the time to obtain a higher extraction yield, but would also increase the total time required for the evaporation of the solvent.

Table 1.
% Absolute Recovery for Diclofenac

	Spiked concentration	Diclofenac	Mean % RSD
SPE	15 µg/mL	86 % (n=4)	10
LLE	15 µg/mL	46 % (n=4)	35

In addition to SPE providing a greater absolute percent recovery by two-fold over LLE, the Strata-X sorbent procedure shows less variability between cartridges, with a mean % RSD of 10 % while the LLE procedure produced more variability with a mean % RSD of 3 times as much, at 35 % (**Figures 2** and **3**). According to the % RSD values for SPE and LLE, SPE is more precise and reproducible than LLE for the extraction of pharmaceutical compounds.

Figure 2.
Diclofenac Extracted Reference Curve: Solid Phase Extraction in Plasma Matrix

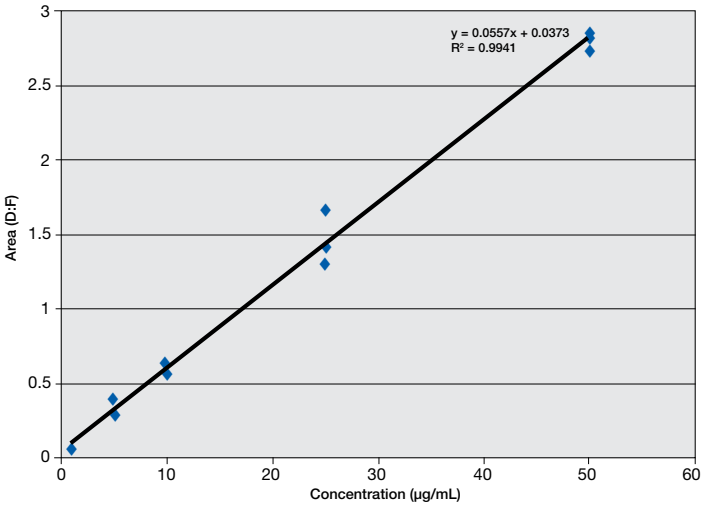
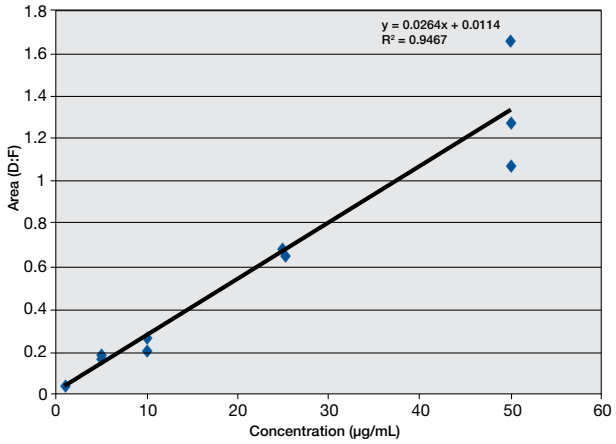


Figure 3.
Diclofenac Extracted Reference Curve: Liquid-Liquid Extraction in Plasma Matrix

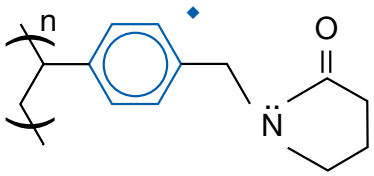


Conclusion

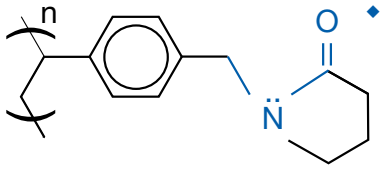
The Strata™-X SPE sorbent utilizes the chemical bonding properties of a pyrrolidone ligand, which makes it a prime target for the retention of hydrophobic neutral compounds, while also retaining basic and acidic compounds under strong organic wash conditions. With the presence of the phenyl ring in the pyrrolidone ligand, pi-pi interactions are prevalent for compounds with aromatic structures, and acidic and basic compounds are able to bind to the nitrogen of the secondary amine and the carbonyl carbon, respectively (**Figure 4**).

Figure 4.
Binding Properties of Strata-X

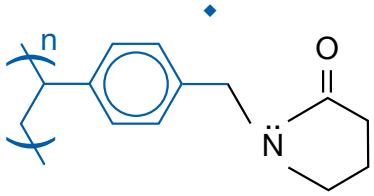
Pi-Pi Bonding



Hydrogen Bonding,
Dipole-Dipole Interactions



Hydrophobic Interactions



♦ Analyte-sorbent interaction site highlighted in blue.

LLE instead utilizes two immiscible solvents that compete for interaction with the analyte of interest. While LLE has been a universal choice of extraction, SPE poses many advantages over LLE for the extraction of diclofenac, via the existence of multiple binding sites on the Strata-X sorbent. Consequently, this data shows that SPE provides greater absolute recovery of diclofenac when compared to LLE; and is less time-intensive, consumes less solvent than traditional LLE procedures, and provides better reproducibility, thereby demonstrating that the extraction method of choice for pharmaceuticals, such as diclofenac, is SPE.

APPLICATIONS

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Ordering Information

Strata™-X

Sorbent Mass	Part No.	Unit
Tube		
10 mg	8B-S100-AAK	1 mL (100/box)
30 mg	8B-S100-TAK*	1 mL (100/box)
30 mg	8B-S100-TBJ	3 mL (50/box)
60 mg	8B-S100-UBJ*	3 mL (50/box)
100 mg	8B-S100-EBJ	3 mL (50/box)
100 mg	8B-S100-ECH	6 mL (30/box)
200 mg	8B-S100-FBJ†	3 mL (50/box)
200 mg	8B-S100-FCH	6 mL (30/box)
500 mg	8B-S100-HBJ	3 mL (50/box)
500 mg	8B-S100-HCH	6 mL (30/box)
Giga™ Tube		
200 mg	8B-S100-FDG	12 mL (20/box)
500 mg	8B-S100-HDG	12 mL (20/box)
1 g	8B-S100-JDG†	12 mL (20/box)
1 g	8B-S100-JEG	20 mL (20/box)
2 g	8B-S100-KEG	20 mL (20/box)
5 g	8B-S100-LFF	60 mL (16/box)
96-Well Plate		
10 mg	8E-S100-AGB	2 Plates/Box
30 mg	8E-S100-TGB	2 Plates/Box
60 mg	8E-S100-UGB	2 Plates/Box

* Tab-less tube
† Available in Teflon® tubes
Additional sizes available. Contact your Phenomenex Sample Preparation Specialist for additional information.

Kinetex® Core-Shell HPLC/UHPLC Columns

1.7 µm Minibore Columns (mm)

	50 x 2.1	100 x 2.1	150 x 2.1
C18	00B-4475-AN	00D-4475-AN	00F-4475-AN

2.6 µm Minibore Columns (mm)

	50 x 2.1	75 x 2.1	100 x 2.1	150 x 2.1
C18	00B-4462-AN	00C-4462-AN	00D-4462-AN	00F-4462-AN

2.6 µm Solvent Saver MidBore™ Columns (mm)

	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0
C18	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0

2.6 µm Analytical Columns (mm)

	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6
C18	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0

More dimensions and phases available, please inquire.

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Strata-X is patented by Phenomenex, Inc. U.S. Patent No. 7,119,145

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