Extraction of Non-steroidal Anti-inflammatory Drugs (NSAIDs) from Plasma using ISOLUTE SLE+ Supported Liquid Extraction Plates

Application Note

Introduction

This Application Note highlights the use of supported-liquid Extraction to isolate three common NSAIDs, Sulindac, Flurbiprofen and Ibuprofen, from human plasma.

Supported-liquid extraction (SLE) is a 96-well sample preparation technique that is similar to traditional liquid-liquid extraction (LLE). The extraction interface occurs between the buffered sample absorbed onto an inert solid support and a water immiscible solvent (see Figure 1). This provides excellent extraction efficiency while alleviating many of the liquid handling and emulsion formation issues associated with traditional LLE.

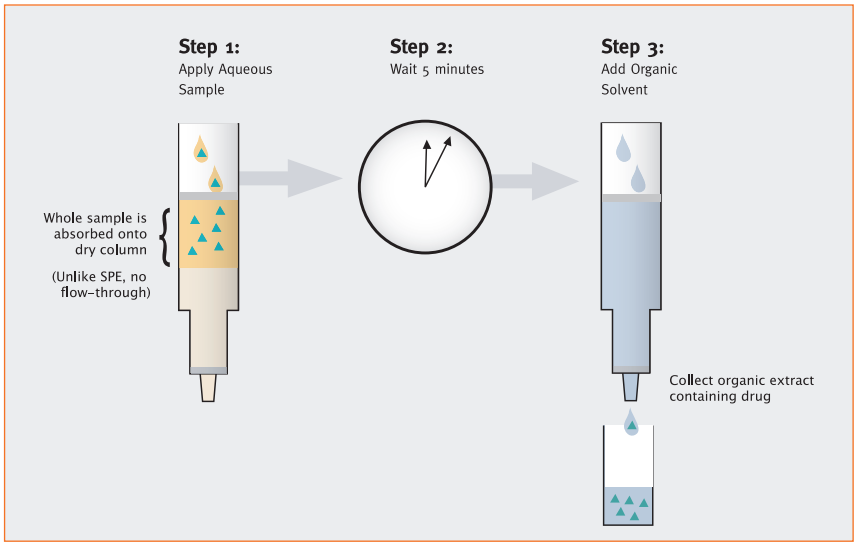


Figure 1. The supported-liquid extraction process using the ISOLUTE SLE+ supported-liquid extraction Plate (single well shown).

Method

Reagents

All analytes (see Table 2) and formic acid were purchased from Sigma Chemical Co. (Poole, UK). Blank human plasma was obtained through the Welsh Blood Service (Pontyclun, UK). All solvents were HPLC grade from Fisher Scientific (Loughborough, UK).

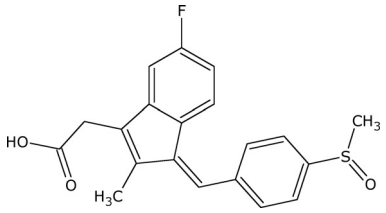
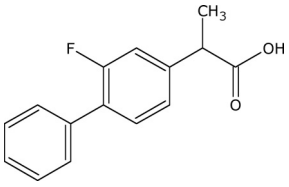
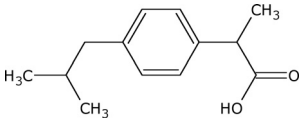
Supported Liquid Extraction Procedure

Plate:	ISOLUTE SLE+ 400 mg supported-liquid extraction plate (part number 820-0400-P01)
Sample:	Blank human plasma (200 µL) was spiked with the NSAIDs at 250 ng/mL. The plasma was then diluted 1:1 v/v with 1% formic acid prior to loading. This sample dilution results in approximate loading pH of 3.2.
Sample Application:	The pretreated plasma was loaded onto the plate, a pulse of vacuum (5 – 15 seconds) was applied to initiate flow and then the samples were left to absorb for 5 minutes.
Analyte Extraction:	MTBE (2 x 900 µL). Allowed solvent to flow for 5 minutes under gravity. Apply vacuum (-15 "Hg / -0.5 bar) for 2 minutes after each aliquot to complete elution.
Post Extraction:	The extracts were evaporated to dryness and the analytes reconstituted in 500 µL 60:40 (v/v) H_2O/MeOH prior to analysis.

Biotage

HPLC Conditons

Instrument:	Waters® 2795 Liquid Handling System (Waters Assoc., Milford, MA, USA).
Column:	Zorbax® Eclipse XDB C18 3.5 µm analytical column (100 x 2.1 mm id, 3.5 µm) (Agilent Technologies, Berkshire, UK).
Guard Column:	C8 Guard Column (Agilent Technologies, Berkshire, UK).
Mobile Phase:	50:50 (v/v) 0.1% (v/v) formic acid/acetonitrile at a flow rate of 0.25 mL/min.
Injection Volume:	25 µL
Temperature:	Ambient

Analyte	Structure
Sulindac	
Flurbiprofen	
Ibuprofen	

Results

Using the procedure described, recoveries for the NSAIDs is shown in Table 2 / Figure 2

Table 1. NSAID recoveries

Analyte	Recovery	RSDs
Sulidac	92	3
Flurbiprofen	94	3
Ibuprofen	91	10

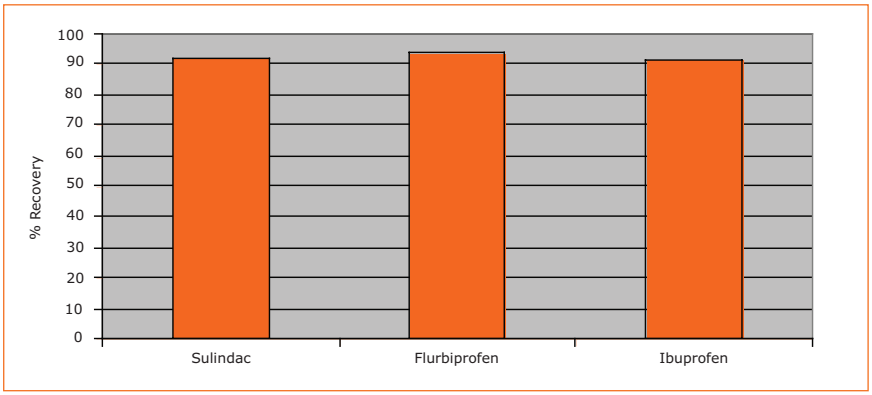


Figure 2. NSAID recovery

Conclusions

ISOLUTE SLE+ supported-liquid extraction plates can be used to extract Sulindac, Flurbiprofen and Ibuprofen from human plasma with quantitative recoveries and RSDs < 10%.

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