APPLICATIONS

A Fast Approach for Determination of Ibuprofen in Human Plasma Using Strata® DE Supported Liquid Extraction Plate and LC-MS/MS

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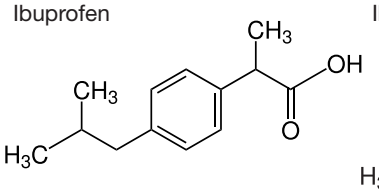
In the treatment of rheumatoid arthritis, inflammatory diseases, and for the relief of mild to moderate pain, ibuprofen is a commonly used over-the-counter (OTC) medication. Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) of the 2-arylpropionic acid class, which is available in several oral dose forms including tablets, capsules, chewables and oral suspension. Consistent with common bioequivalence (BE) studies used by generic pharmaceutical drug companies, we will demonstrate a fast analytical method including both sample preparation and LC-MS/MS analysis of ibuprofen in human plasma. The method was evaluated for sensitivity, linearity, precision, accuracy, and recovery of the extraction method using Strata DE supported liquid extraction (SLE) 96-well plate for fast sample preparation to minimize potential matrix interferences. A Kinetex® 2.6µm C18 50 x 2.1mm analytical column was utilized for rapid LC-MS/MS analysis, which results in time savings for the modern analytical laboratory.

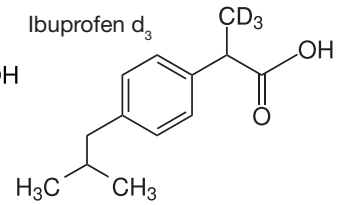
Materials

Standards and all other reagents and chemicals were obtained from Sigma Aldrich®.
Human plasma was purchased from BioIVT® (Westbury, NY).

Ibuprofen



Ibuprofen d_3



Experimental Conditions

Sample Extraction

An eight-point calibration curve was generated by preparing standards, in duplicate, at 10, 20, 50, 100, 250, 500, 800, 1000 ng/mL in human plasma. Three levels of QC samples (n = 6) were prepared at 50, 400, 800 ng/mL (QCL, QCM, and QCH, respectively). These standards and QC samples were then extracted using a supported liquid extraction (SLE) 96-well plate (Strata DE, 200 µL, 96-well plate, P/N: 8E-S325-FGB) as outlined in the sample extraction procedure below.

For the assay recovery experiment, six replicates of pre-extraction spiked samples, equivalent to QCM (400 ng/mL ibuprofen) were prepared by spiking human plasma sample matrix solution containing 100 µL of working internal standard (IS) solution. These samples were processed per the sample extraction procedure.

Post-extraction samples (recovery samples, n = 4) consisted of human plasma blank samples containing 100 µL of working internal standard (IS) solution that were spiked with an equivalent of 400 ng/mL ibuprofen **after** SLE extraction and **before** sample dry down.

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Sample Extraction Procedure

1. Transfer a 50 µL aliquot of human plasma sample into a 13 x 100 mm glass tube
2. Add 100 µL of working internal standard (IS) solution (200 ng/mL of Ibuprofen-d3 in 0.1 % Formic acid in Water)
3. Mix (vortex) sample for 30 seconds
4. Load sample into an unused well on the Strata DE 96-well plate
5. **Wait for 5 min**
6. Elute the sample twice with 600 µL of 95:5 Dichloromethane/Isopropanol, and collect sample in 2 mL 96 deep well plate (P/N AH0-7194 or AH0-8635)
7. Dry down at 40 °C under N_2
8. Reconstitute with 200 µL of 70:30 0.1 % Formic acid/Acetonitrile
9. Inject 10 µL

LC-MS Conditions	
Column:	Kinetex 2.6 µm C18 50 x 2.1 mm (P/N: 00B-4462-AN)
Sample Preparation:	Strata DE 200 µL 96 well plate (P/N: 8E-S325-FGB)
Mobile Phase:	A = 0.1 % Formic Acid in Water B = Acetonitrile
LC Condition:	Isocratic: 40:60 (A/B)
Flow Rate:	500 µL/min
Run Time:	2 min
Temperature:	40 °C
Pressure:	~150 bar
Injection Volume:	10 µL
HPLC System:	Agilent® 1260 Infinity (Agilent Technologies, Santa Clara, CA, USA)
Mass Spectrometer:	SCIEX® 4500 Triple Quad™, ESI negative (AB SCIEX Pte. Ltd.)

Table 1.
MRM Transitions

ID	Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	DP	CE
Ibuprofen	205	161	100	-60	-10
Ibuprofen-d3	208	164	100	-80	-10

Table 2.
Accuracy and precision

ID	QCL	QCM	QCH
Nominal Concentration (ng/mL)	50	400	800
1	50.4	405	828
2	48.3	379	762
3	49.8	348	769
4	47.3	398	746
5	49.5	384	793
6	43.7	381	744
Mean	48.2	383	774
S.D.	2.46	19.8	27.7
% CV	5.10	5.17	3.58
% Theoretical	96.3	95.6	96.7
N	6	6	6

Table 3.
Extraction recovery

Sample ID	Area Ratio (Ibuprofen / Ibuprofen-d_3)	Mean	STDV	CV (%)	Extraction Recovery (%)
QCM 1	1.82E+00				
QCM 2	1.70E+00				
QCM 3	1.56E+00				
QCM 4	1.78E+00				
QCM 5	1.72E+00				
QCM 6	1.71E+00	1.72	0.09	5.19	77.3
Recovery 1	2.32E+00				
Recovery 2	2.37E+00				
Recovery 3	2.01E+00				
Recovery 4	2.17E+00	2.22	0.16	7.32	

Figure 1.
Representative of a chromatogram of QCM (400 ng/mL) in human plasma

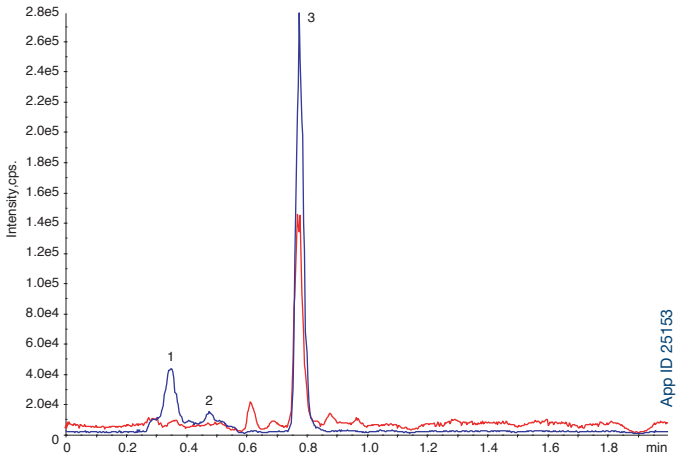
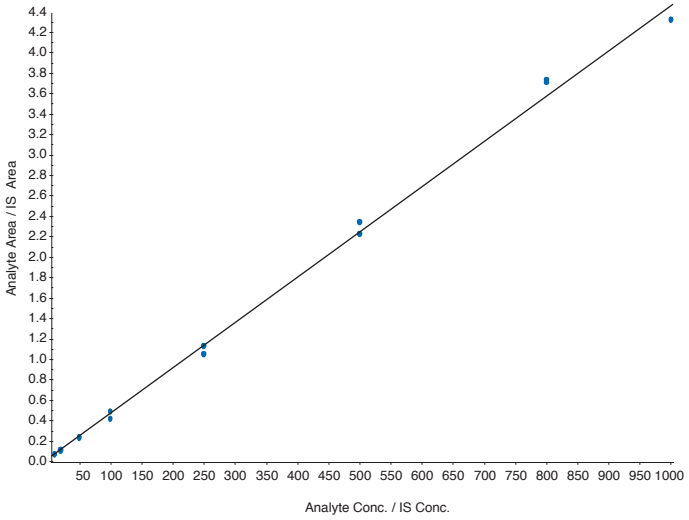


Figure 2.
Representative of calibration curve range



Results and Discussion

Table 1 shows the mass transitions monitored for ibuprofen and the internal standard (ibuprofen-d_3) and the mass spectrometry settings used for ESI, negative mode. **Table 2** shows the mini assay evaluation run results to demonstrate the accuracy and precision of the assay. Three QC levels (QCL, QCM and QCH) were used in the run, with accuracy and precision across all QC samples ranging from 95.6 – 96.7 % with CV% 3.58 -5.17 %, respectively. **Table 3** gives the sample extraction recovery results at 77.3 % (QCM) with CV% at 5.19 (n=6) using Strata® DE SLE 96-well plate. A representative chromatogram of the extracted QCM at 400 ng/mL is shown in **Figure 1**, which shows good chromatographic separation of ibuprofen from observed matrix interference peaks present in the human plasma matrix. (Note that if a cleaner sample is desired or required, an alternative sample preparation technique such as solid phase extraction (SPE) should be explored). The linear dynamic range of this method was tested with eight calibrators, in duplicate, from 10-1000 ng/mL with acceptable linearity (r = 0.9990) and the calibration curve is shown in **Figure 2**.

The use of the Strata DE 96-well plate format for SLE sample preparation allows for the processing of multiple samples simultaneously, thereby reducing the overall time usually devoted to sample preparation. To further minimize sample analysis time, a short (50 x 2.1 mm) Kinetex C18 column was utilized to resolve the ibuprofen analyte from residual sample matrix interferences not completely removed during sample preparation.

Conclusions

For a new generic drug product, several time points across multiple patient samples would be required for analysis to demonstrate bio-equivalence. These experiments are a significant undertaking for any company. In order to be effective, an efficient method is highly preferred. In this technical note, we demonstrate an assay with a total run time of only 2 minutes on a LC-MS/MS system using Kinetex C18 2.6 µm, 50 x 2.1 mm column. In addition, the sample extraction with Strata DE supported liquid extraction 96-well plate is an automation friendly format. The fast analysis time demonstrated for the assay of ibuprofen in human plasma would be ideally suited for a bio-equivalence study. A fast analytical method as demonstrated here is ideal for reducing costs in high-throughput analysis in research and production environments, without compromising analytical results.

Ordering Information

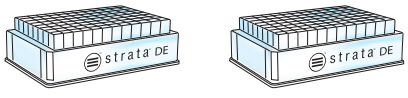
Kinetex® Core-Shell LC Columns

2.6 µm Analytical Columns (mm)						SecurityGuard™ ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	75 x 2.1	100 x 2.1	150 x 2.1	3/pk
C18	00A-4462-AN	00B-4462-AN	00C-4462-AN	00D-4462-AN	00F-4462-AN	AJ0-8782
						for 2.1 mm ID

‡SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

Strata® DE (Diatomaceous Earth SLE) 96-Well Plates

Ideal for smaller volume, high-throughput cleanups such as bioanalytical samples.



Strata DE 96-Well Plates	200 µL	400 µL
Maximum Sample Volume (after dilution)	200 µL	300 µL
Recommended Elution Volume	2x 600 µL	3x 600 µL
Part No.	8E-S325-FGB	8E-S325-5GB
Unit	2/pk	2/pk

APPLICATIONS

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