An Automated SPE Procedure and Analysis for the Determination of Ibuprofen and Ketoprofen in Plasma

Application Note 214

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Introduction

Prostaglandins are produced by the cells of the body and have several important functions. They promote inflammation, pain, fever, support the function of platelets that are necessary for the clotting of blood, and protect the lining of the stomach from the damaging effects of acid. Prostaglandins are produced within the cells by the enzyme cyclooxygenase (Cox). Nonsteroidal anti-inflammatory drugs (NSAIDs) block the Cox enzymes and reduce prostaglandins throughout the body. As a consequence, ongoing inflammation, pain, and fever are reduced. Since the prostaglandins that protect the stomach and support the platelets and blood clotting also are reduced, NSAIDs can cause ulcers in the stomach promote bleeding and intensify the effects of kidney disease/damage. Frequent use of NSAIDs can effect kidney function in patients of all ages, it may be particularly damaging in older adults due to declining renal function that accompanies age.

Two of the more common NSAIDs are Ketoprofen and Ibuprofen. Everyone has their aches and pains that haunt them at one time or another. For those with chronic pain or the above mentioned conditions it is helpful to monitor the effectiveness of NSAIDs in the body. This application will describe an automated solid phase extraction method coupled with HPLC analysis to determine the various levels of Ibuprofen and Ketoprofen in human plasma.

In today's laboratory, results must be achieved more accurately, with more speed and less effort. Automation of SPE is an effective tool in achieving all three of these goals. The SPE 215 is an automated solid phase extraction instrument that offers the flexibility and versatility required in order to automate most SPE methods. The application presented here will, also, demonstrate these facts.

Materials & Methods

Chemicals and Reagents

Methanol, HPLC Grade
Acetonitrile , HPLC Grade
Triethylamine(TEA), HPLC Grade
Isopropyl Alcohol(IPA), HPLC Grade
Formic Acid, 88%
Ketoprofen
Ibuprofen

System Components

322 Pump; H2 pump head, flow rates up to 30mL/min, pressure up to 4300psi

215 Liquid Handler; 125 mm arm, 5 ml dilutor syringe, 819 injection module, 100 uL sample loop

ZORBAX SB-C18 5um, 4.6 x 150mm column

SPE 215 System; 175mm 4 Probe Z arm, 5mL syringes

Bakerbond SPE Octadecyl(C_{18}), 3.0mL, 200mg, tabless for SPE extraction

156 UV wavelength detector with analytical flow cell 5mm path length, sensitivity 0.01

UniPoint Version 3.3

735 Sampler Software v5.1

506C interface

Pentium 4, > 2 GHz, 512 MB RAM, 9.5 GB Harddrive computer running both Unipoint and 735

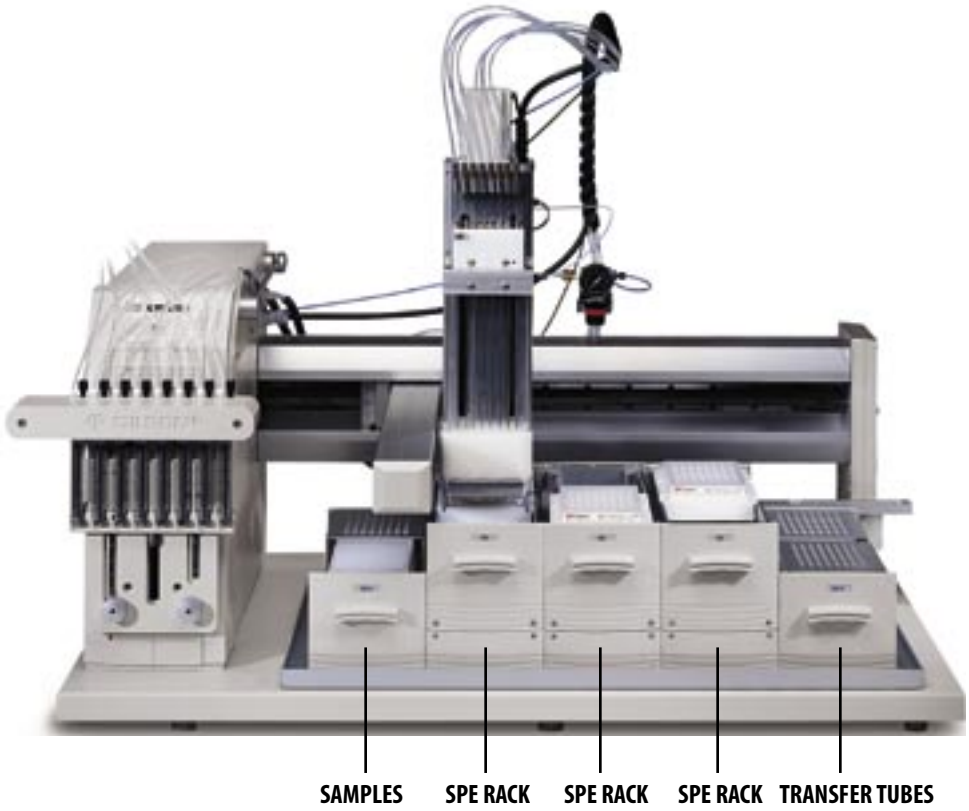

Photo 1: SPE 215

Description of Solutions

4% IPA in 0.25M Formic Acid

2% Formic Acid in methanol

Ketoprofen Stock(1.0mg/mL) in methanol

Ibuprofen Stock(1.0mg/mL) in methanol

Sample Preparation(50ug/mL):
40mL plasma, 2.0mL Ketoprofen Stock(1.0mg/mL), 2.0mL Ibuprofen Stock(1.0mg/mL), 336uL Formic Acid(88%)

Standard Preparation(50ug/mL):
40mL 4% IPA in 0.25M Formic Acid, 2.0mL Ketoprofen Stock(1.0mg/mL), 2.0mL Ibuprofen Stock (1.0mg/mL)

Step	Solvent	Volume
Condition*	2%Formic in Methanol	1.5mL
Condition*	Water	1.0mL
Load	Sample	1.0mL
Wash	4%IPA in 0.25M Formic	1.0mL
Wash	4%IPA in 0.25M Formic	1.0mL
Elution	2%Formic in Methanol	0.5mL
Elution	Acetonitrile	0.5mL
Dry Down	Dry Eluent for 60min	na
Reconstitute	4%IPA in 0.25M Formic	1.0mL

*Column was not allowed to dry during condition step

Table 1: Sample Preparation

Column: ZORBAX SB-C18 5um, 4.6 x 150mm
Flow: 2.0mL/min
Mobile Phase: 0.1%TFA in Water, 0.1% TFA in Acetonitrile
Detector: UV 221 nm(Sensitivity = 0.01)

Time(min)	Water(%)	Acetonitrile(%)
0.00	90	10
0.50	90	10
6.50	15	85
7.50	15	85
8.50	90	10
9.50	90	10

Table 2: Analytical HPLC Conditions

Procedure

Solid Phase extractions were run manually to evaluate the individual steps, prior to being run on the automated SPE 215. A Baker SPE-10 with 5-7 psi vacuum was used for the manual extractions.

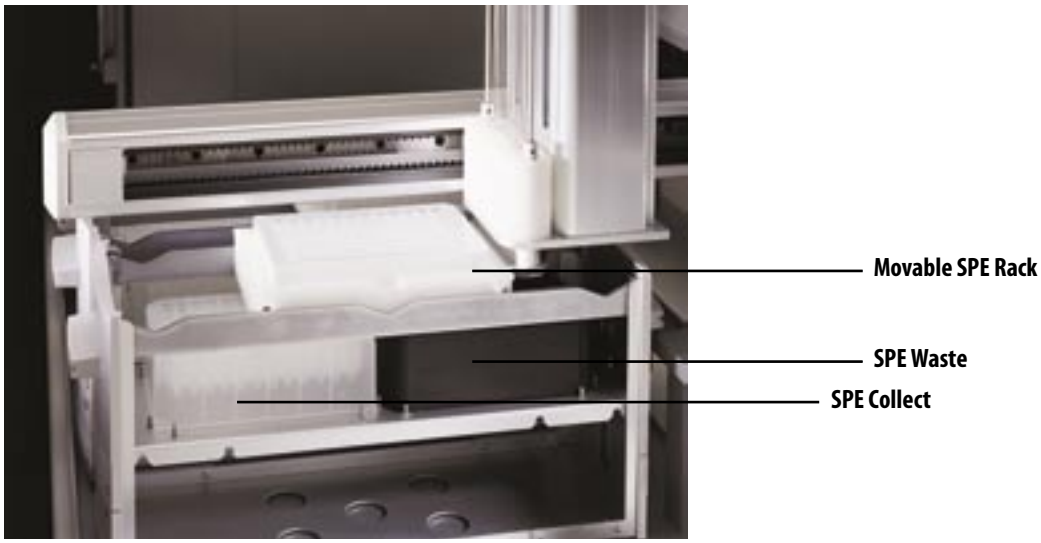

Photo 2: SPE 215

The automated procedure for the NSAID extractions were run on consecutive days to determine the day-to-day variance and therefore the method and instrument ruggedness. All samples were analyzed by the previously described analytical method.

System Controllers

The SPE 215 is controlled under 735 Sampler software. The software is a user friendly drag and drop package that allows for changes and modifications in the method, racks and trays on the fly. These modifications will be seen upon initializing the applications list. The 735 Sampler Software allows complete control of rates for aspiration and dispensing of the solutions/samples onto the SPE cartridges, including the time associated with the air push(15-20psi) and pressure equilibrium. Thus, total optimization of the solid phase extraction of the NSAIDs from the plasma was achieved.

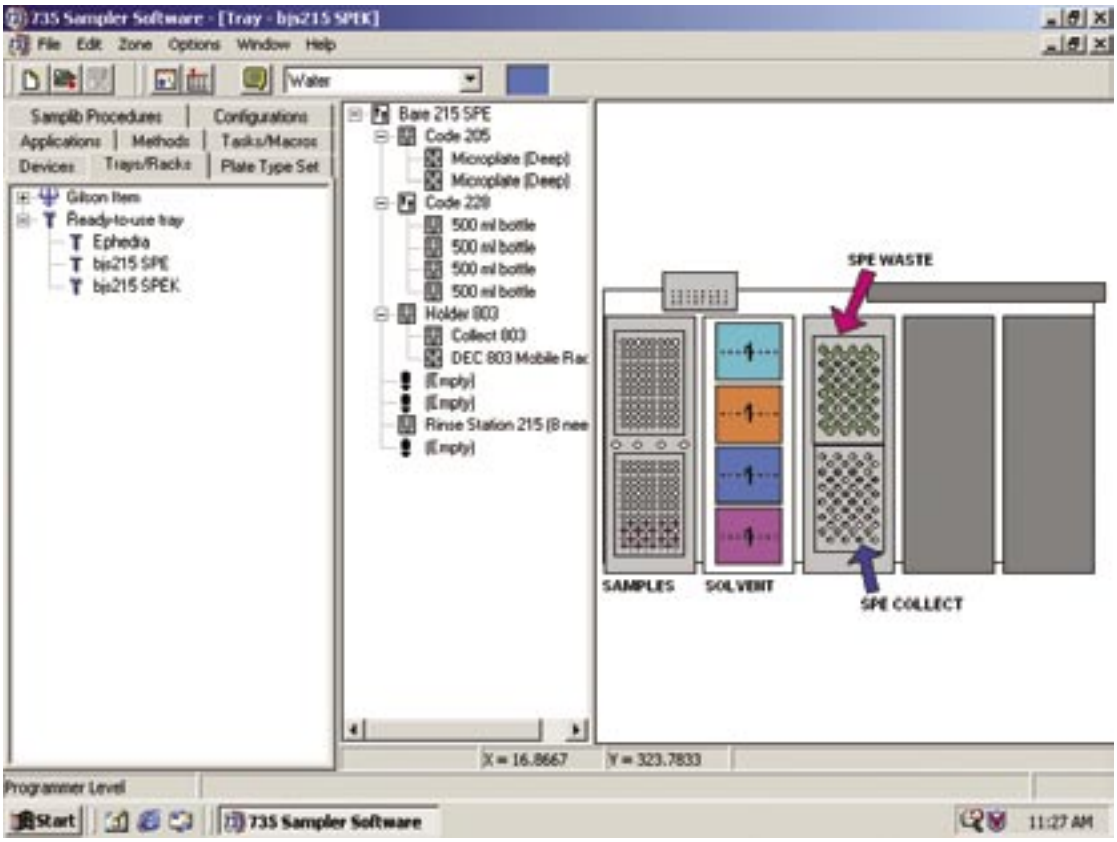

Photo 3: 735 Tray

From the Task list the SPE method is created by activating the individual Tasks in the required series. Each Task can then be optimized for the specific method. For example the time of the air push was critical for the conditioning of the column and the final elution of solvent from the column and was set accordingly.

Once the final elution had been dried down and reconstituted it was then transferred to a 215 Liquid Handler based HPLC system for analysis. Unipoint was used to control and acquire data. The samples could also be analyzed on-line using the SPE 215 with a 849(4-probe) injection modules in parallel with additional HPLC systems for higher sample throughput. This approach wasn't taken based on the amount of time required to dry samples down. Therefore, instead of employing the SPE 215 for dry down and limiting SPE throughput the samples were analyzed off line.

Manual	%Recovery	
50ug/mL	Ketoprofen	Ibuprofen
AVE(%)	90.37	75.66
CV(%)	8.7	7.9

Automated	%Recovery	
50ug/mL	Ketoprofen	Ibuprofen
AVE(%)	90.41	90.65
CV(%)	4.5	4.1

Table 7&8: Overall

Note: Results for Manual and Automated SPE were collected over a number of days to show reproducibility

The quantitative level of detection was determined to be 1ug/mL for Ketoprofen and Ibuprofen.

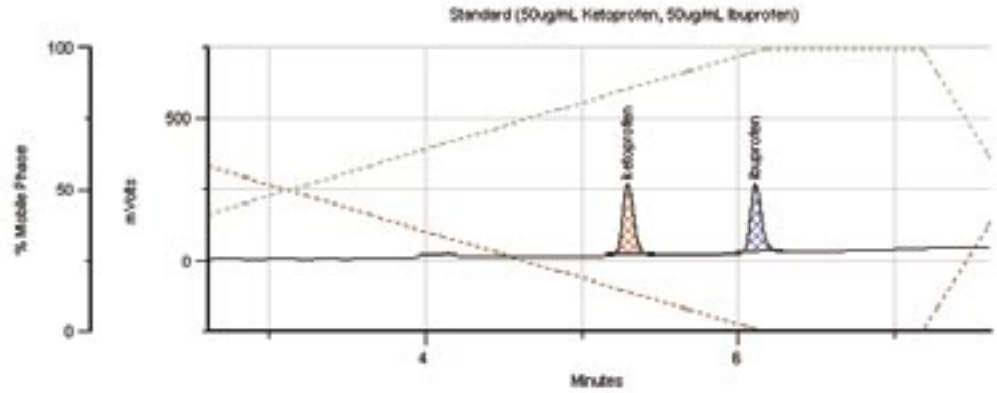

Figure 1: NSAIDs Standard

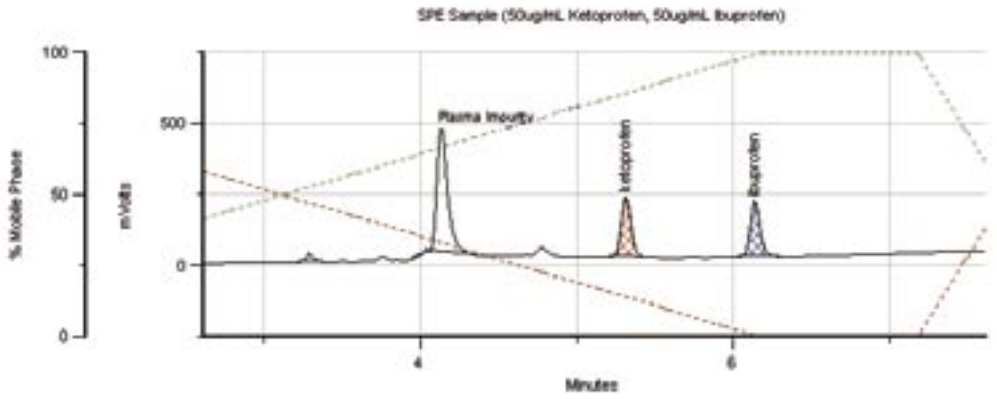

Figure 2: NSAIDs SPE Sample

Sample	%Recovery	
Concentration	Ketoprofen	Ibuprofen
25ug/mL	83.42	66.94
100ug/mL	74.29	89.87

Table 9: Samples were spiked at a lower and higher concentration to show variance in the chromatography. Samples were extracted using the automated SPE 215 at 25ug/mL and 100ug/mL.

Summary

Automating the extraction of NSAIDs from plasma on the SPE 215 was superior to the manual extraction procedure. The SPE 215 yielded CV's in the 4.1-4.5% range. While the CV's for the manual process were 7.9-8.7%. Automating the procedure yielded a significant increase and more consistent recoveries for Ibuprofen. Automation on the SPE 215 offers a degree of consistency and efficiency that is not attainable via the manual method.

Using a fully automated SPE 215(11min 20sec/4 samples), the amount of time required by the analyst to complete the extraction is greatly reduced. The manual solid phase extractions performed in 20min for 4 samples. In addition to, the manual extraction procedure requiring 100% of the analyst's time..

The automated SPE 215 allows the analyst to set rates, equilibration time, volumes and choosing between air or pressurized air push. This yields more consistent result within and between batches. The amount of time required by the analyst to complete the extraction is greatly reduced, freeing the analyst to concentrate on other tasks or duties. Plus Gilson's 735 and Unipoint generate a log file, that records each step, an added security that the samples are being run correctly.

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