An Automated SPE Procedure and Analysis for the Extraction of Chlorpromazine and Thioridazine from Plasma

Application Note 213

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Introduction

In the 1950's neuroleptic drugs began flooding the state mental hospitals. The term "neuroleptic" comes from the Greek "neur" for nerve or nervous system and "lepsis" for taking hold. Literally, "taking hold of the nervous system". These drugs are most often used to treat psychotic disorders and symptoms such as hallucinations, delusions and hostility. They can also be used to prevent and treat nausea and vomiting, and behavior problems in children. These drugs need to be very closely regulated in the body at normal serum levels of 10–1300 ng/mL. Symptoms of an overdose include uncontrollable movements, agitation, seizures, severe dizziness or fainting, coma, very deep sleep, irregular heartbeats and high or low body temperature

The application presented here will describe an automated solid phase extraction method coupled with HPLC analysis to determine the levels of Chlorpromazine and Thioridazine in human plasma. Chlorpromazine (Thorazine) is one of the more common neuroleptic agents, used in the treatment of many disorganized thinking disorders, while Thioridazine (Mellaril) is a very powerful neuroleptic agent used only as a last resort and needs to be very closely monitored.

Materials & Methods

Chemicals and Reagents

Methanol, HPLC grade
Acetonitrile , HPLC grade
Triethylamine(TEA), HPLC grade
Phosphoric Acid, 85%
Trifluoroacetic Acid, 99%+ spectrophotometer grade
Chlorpromazine
Thioridazine

System Components

SPE 215 System

- 4-probe Z arm
- 5-mL syringes
- Bakerbond SPE Octadecyl (C_{18}), 1.0 mL, 100 mg, tables

215 HPLC System

- 215 Liquid Handler
- 819 Injection Module
- 156 Dual-Wavelength UV/VIS Detector, 5 mm flow cell
- 322 Pump, H2 pump head(3–20 mm ID columns, up to 30 mL/min, up to 4,300 psi)
- 735 Sampler Software version 5.1
- UniPoint™ System Software version 3.3, 506C System Interface

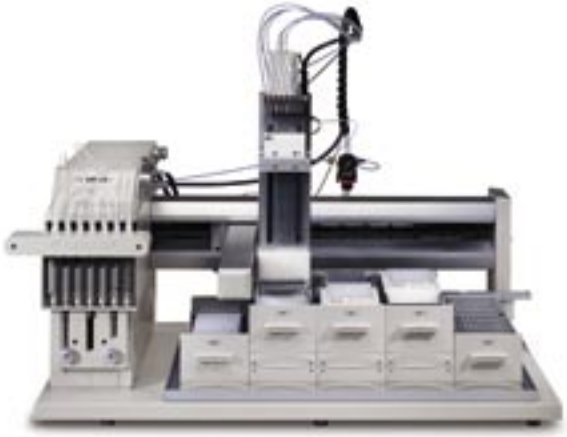

Photo 1. Gilson's SPE 215 System.

Description of Solutions

Solid Phase Extraction:

Buffer A: Dilute 4.5mL phosphoric acid(85%) and 4.5mL TEA to 1000mL HPLC-grade water.

Eluent: Combine methanol, acetonitrile and buffer A(1:2:1)

Wash Acetonitrile/Water (1:1): Combine equal parts of acetonitrile and water

Sample Preparation:

Biological: Combine 500 µL plasma (spiked 40 µg/mL chlorpromazine and 40 µg/mL thioridazine) and 500 µL 0.2M phosphoric acid, shake thoughly and allow to settle for 5 min (approx. 20 µg/mL)

Aqueous/Standard: Dissolve 20 mg chlorpromazine and 30 µg thioridazine in 20 mL eluent, dilute 200 µL to 10 mL eluent (approx. 20 µg/mL chlorpromazine and 30 µg/mL thioridazine)

Sample Preparation Steps

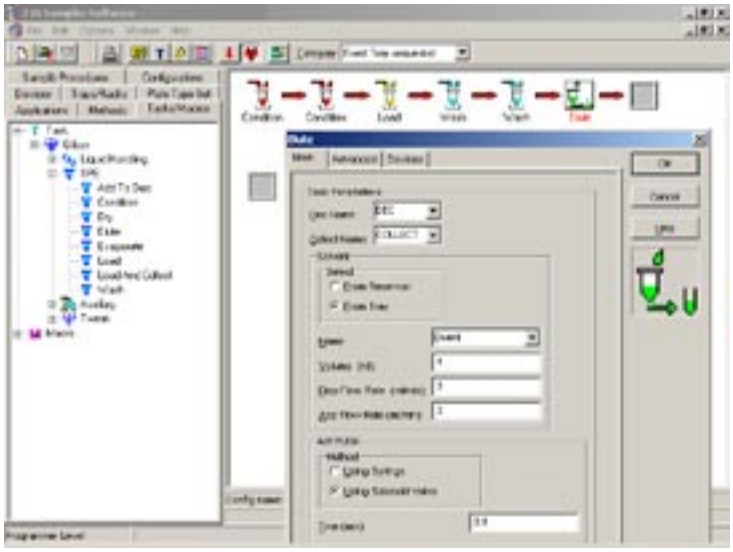

Figure 1. 735 SPE Method screen.

Step	Solvent	Volume
Condition*	Methanol	1.0 mL
Condition*	Water	1.0 mL
Load	Sample	0.5 mL
Wash	Water	1.0 mL
Wash	CAN/water	0.5 mL
Elution	Eluent	1.0 mL

*Column should not be allowed to dry during condition steps.

Table 1. SPE method.

Analytical:

Column: YMC C_{18} ,4.6 x 5 cm, 5 µ

Flow: 2.0 mL/min

Mobile phase: water, 0.1%TFA in acetonitrile

Detector: UV 254 nm(sensitivity = 0.01)

Time (min)	Water (%)	Acetonitrile
0.00	95	5
0.50	95	5
8.00	5	95
9.00	5	95
10.00	95	5
10.50	95	5

Table 2. Analytical HPLC gradient conditions.

Procedure

Solid Phase extractions were run manually to evaluate the individual steps, prior to being run on the SPE 215. A Baker SPE-10 with 5–7 psi vacuum was used for the manual extractions. All samples were analyzed by the previously described analytical method.

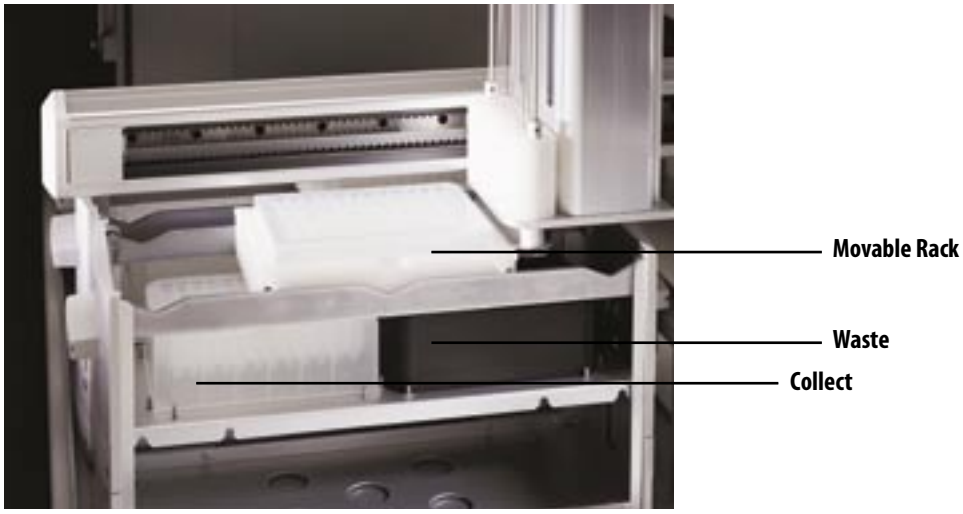

Photo 2. SPE 215 movable rack.

The SPE 215 allowed 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, we achieved total optimization of the solid phase extraction of the neuroleptic agents from the plasma. The automated procedure for the neuroleptic 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.

Photo 3. SPE 215 sample aspiration.

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. Simulation mode is available and allows the user to review the method prior to its actual running, at that point adjustments can be made. Customization of all aspects of the software is available.

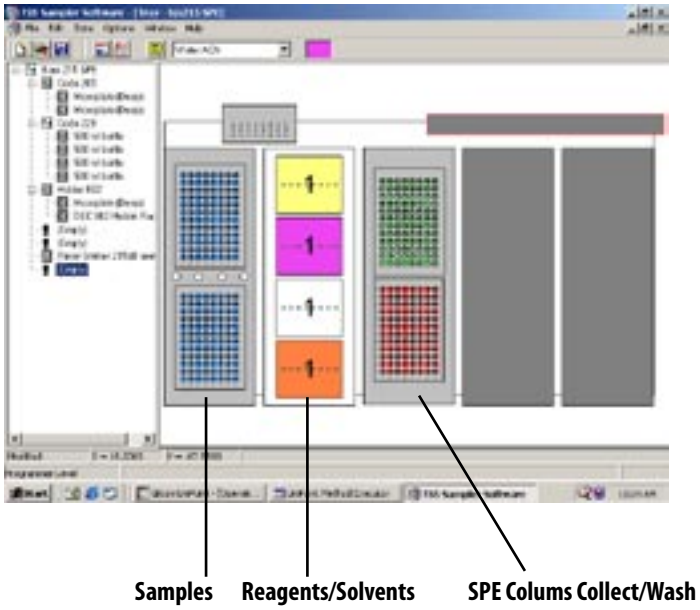

Figure 2. 735 SPE tray file.

Up to 5 racks can be accommodated on the SPE 215 bed. From the Task list the SPE method is created by activating the individual Tasks in the required series. Each Task can then be customized 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.

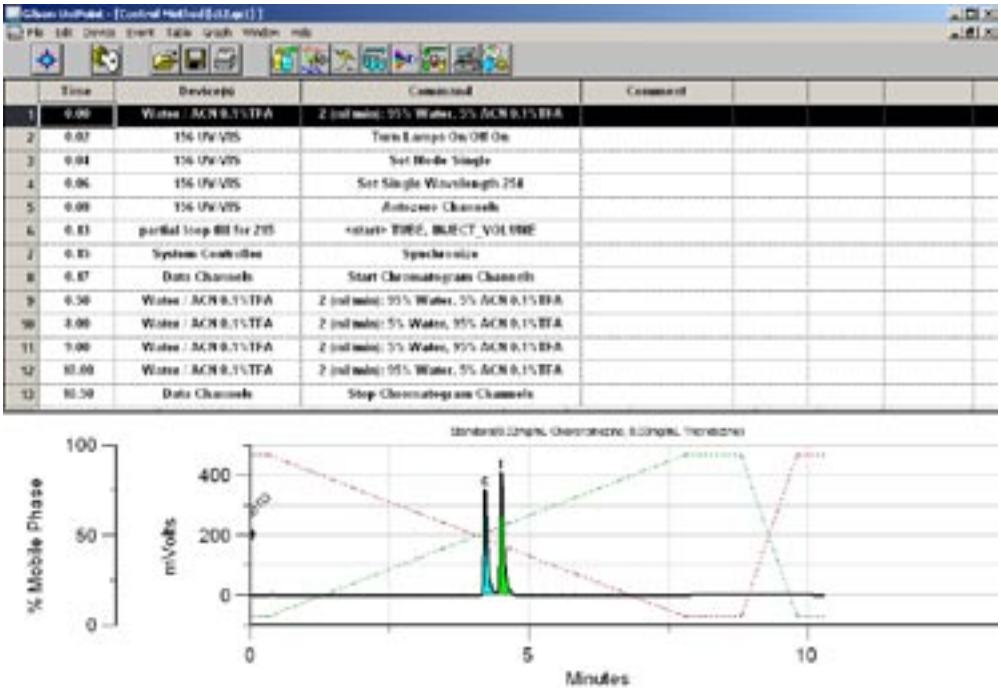

Figure 3. UniPoint Control Method and chromatogram.

Once the elution was collected it was then transferred to a 215 Liquid Handler based HPLC system for analysis. UniPoint was used to control and aquire data. The samples could also be analyzed on-line using the SPE 215 with a 849 (4-probe) or 889 (8-probe) injection modules either in series or in parallel with additional HPLC systems for higher sample throughput.

Results

Manual	% Recovery	
	Chlorpromazine	Thioridazine
AVE (%)	89.1	80.0
CV (%)	8.7	7.1

Automated	% Recovery	
	Chlorpromazine	Thioridazine
AVE (%)	90.2	80.3
CV (%)	3.8	4.1

Tables 3 & 4. Day 1 results.

Manual	% Recovery	
	Chlorpromazine	Thioridazine
AVE (%)	89.2	82.3
CV (%)	6.3	5.6

Automated	% Recovery	
	Chlorpromazine	Thioridazine
AVE (%)	95.6	82.9
CV (%)	2.5	2.6

Tables 5 & 6. Day 2 results.

Manual	% Recovery	
	Chlorpromazine	Thioridazine
AVE (%)	89.0	81.1
CV (%)	7.1	6.1

Automated	% Recovery	
	Chlorpromazine	Thioridazine
AV E(%)	92.9	81.6
CV (%)	4.2	3.6

Tables 7 & 8. Overall results.

Note: Data for Day 1 and Day 2 were collected on consecutive days; however, Manual Day 1 and Automated Day 1 are not the same day.

Through development of the analytical analysis it was found that with this set up it is possible to quantitate down to 2000 ng/mL of both Chlorpromazine and Thioridazine.

Summary

There are three main reasons to choose the automation of solid phase extraction; more consistent results, quicker results and less time involved for the analyst.

The data shows that we can achieve the same results using the automated SPE 215 system that we did when we performed the Solid Phase Extractions manually. The automated SPE 215 allows the analyst to set rates, equilibration time volumes and choosing between air or pressurized air push. This gives more consistent results within and between batches.

Using a fully automated SPE 215 with HPLC, the amount of time required by the analyst to complete this task is greatly reduced. With the system up and running, you would only have to check it periodically, if at all, during the run.

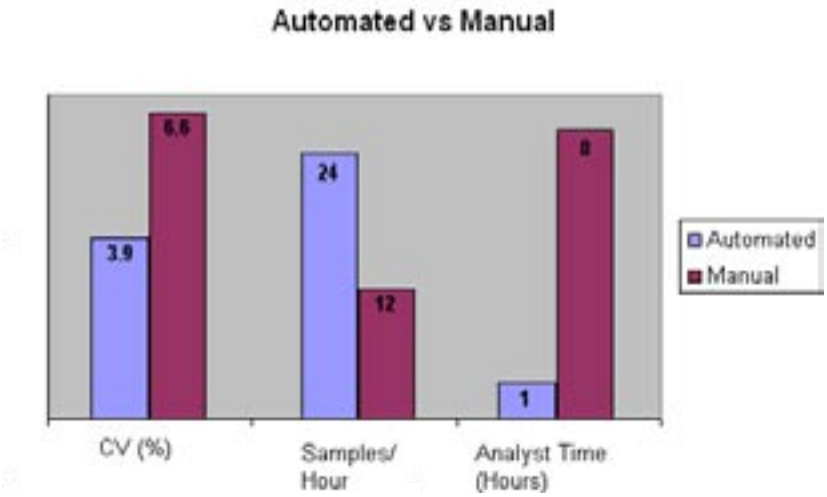

Figure 4. Automated vs. manual SPE results.

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