Development of an Automated Solid Phase Extraction of Procainamide in Serum Samples

Sikander S Gill, Mo S Wang, Rajwant K Gill, Thomas Lum, and Dong C Liang

Aurora Biomed Inc., Vancouver, BC, Canada

Aurora
ILLUMINATING SOLUTIONS

I. Abstract

Background: In determining patient compliance and assessing dosage of antiarrhythmic drugs like Procainamide (4-amino-*N*-(2-diethylaminoethyl) benzamide), quantification in the patient serum or plasma samples is essential. As the demand on sample processing and analysis increases, laboratory automation solutions become necessary for a testing facility. Automated solutions not only facilitate greater sample processing throughput, but these solutions also minimize the variation and error between samples. However, robotic systems can be subject to positional or spatial biases - intra-performance differences between positions on the deck of the system. Here we investigated potential positional biases of solid phase extraction (SPE) column positions on the VERSA 10 SPE deck.
Methods: A fully automated assay on VERSA 10 SPE Workstation was used for water and serum samples spiked with procainamide using 3mL columns (C8 / SCX). A set of 12 columns were simultaneously processed for activations, wash and elution steps of the protocol. The samples and reagents were mediated through the columns by an automated 12 channel positive pressure module. The eluted samples were subjected to drying with an automated nitrogen dryer in combination with automated heating and shaking. The dried pellet was reconstituted in 1mL of mobile phase and transferred to HPLC vials for high performance liquid chromatographic (HPLC) analysis. The reconstituted procainamide HCl samples (10µL) were analyzed on a Plastisil ODS column.
Results: Positional biases were investigated on the VERSA 10 SPE by examining the recovery of 20µg of procainamide HCl serum samples after SPE on all column positions. Four batches of twelve samples were tested (11 procainamide samples and 1 negative control). The average procainamide HCl recovery per batch (n=11) shows a recovery range between 87.86% - 98.35%. Analysis of Variance (ANOVA) and Tukey-Kramer HSD analysis on post SPE procainamide HCl recovery indicated no statistically differences between column positions on the VERSA 10 SPE deck. Furthermore, the average coefficient of variance (CV%) across column positions was 4.05% (±0.859%).
Conclusion: The presented data and analysis show that there are no positional biases in column positions across the deck of the VERSA 10 SPE automation system. The overall coefficient of variance within each batch was low indicating high precision across each column position that suggests a well-suited solution for increasing throughput and reproducibility while minimizing hands-on sample preparation time.

II. Introduction

Measurement of drug levels in serum is becoming increasingly popular to optimize the dosage of the drugs[1]. Procainamide (4-amino-*N*-(2-diethylaminoethyl) benzamide) a pharmaceutical antiarrhythmic agent is one of such drugs whose serum concentration needs to be monitored for its very narrow therapeutic window (Figure 1). However, an error-free, and high throughput monitoring is essential for clinical samples on routine basis. Laboratory automation solutions can facilitate drug monitoring by allowing higher sample processing throughput, while minimizing the variation and error among samples[2]. In view of the SPE methods being time consuming and complex, and to provide a simplified, robust and less time consuming way, extraction of this drug from serum samples was automated on VERSA 10 SPE Workstation (www.aurorabiomed.com). The workstation is capable of fully automating the SPE protocols for 12 columns, simultaneously. Wash and elution buffers are delivered by a 4-channel air displacement robotic arm (<2% CV, 100µL) with disposable tips. User-defined wash and elution rates are mediated by a 12-channel N_2 positive pressure module controlled by software.

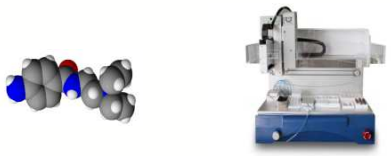

Figure 1: (a) Chemical Structure of Procainamide (b) VERSA 10 SPE Workstation

III. Objectives

- Validation of VERSA 10 Solid Phase Extraction (SPE) Workstation
- Preparation of ready-to-use melamine sample for LCMS determination
  - Automation of SPE process
  - Hands-free extraction
  - Excellent recovery
- Walk-away automation system

IV. Materials & Methods

The validation of the VERSA 100 SPE Workstation was carried as follows:

I.Materials:

1. **VERSA 10 SPE Workstation & deck equipment**: The deck of the VERSA 10 SPE Workstation (LxWxH cm: 60x60x45), was equipped with SPE column holder (1, and 3mL), positive pressure module, N2 dryer, shaker-heater, and deck slots for samples, and HPLC vials (Figure 2a).
2. **SPE columns**: 3mL SPE columns containing 20mg of C8/SCX matrix (200mg/mL, 74.1 x OD10.7 mm column. Biocomma, Shenzhen, China, Figure 2a).
3. **Samples**: Pig serum samples were spiked with Procainamide (CAS No.614-39-1)
4. **Software programming**: VERSAware was used to define the automation workflow.

II.Extraction process: The workflow of automation (Figure 2b) was carried as follows:

1. **Condition**: Column were condition with addition of 3mL CH_3OH (99%) followed by addition of 3mL distilled water and then by 0.02M KH_2PO_4 pH 3.0 with flow rate @ ~10mL/min by applying positive pressure on each column.
2. **Loading of samples**: 2mL Serum samples spiked with Procainamide [(1mL serum +960 µl KH_2PO_4 0.02M pH 3.0 + 40 µl of stock solution (1mg/mL Procainamide)] were loaded onto each SPE column with flow rate @ ~2mL/min by applying positive pressure.
3. **Washing**: The columns were washed by adding 3mL of methanol - water (95:5) with flow rate @ ~10mL/min by applying positive pressure on each column.
4. **Elution:** Procainamide HCl was eluted with methanol - ammonia (95:5).
5. **Sample drying and reconstitution**: The eluted samples (3mL) were then heated at 40°C for 2 minutes and dried with N_2 gas for 30 minutes (10L/min.). The dried samples were reconstituted in 1mL of mobile phase (0.02M Methanol - KH2PO4 (15:85 v/v) and transferred 800 µl to HPLC vial.

III.HPLC conditions: The HPLC conditions were set as follows:

1. **HPLC column:** HPLC Model:STI501 (Science Technology (Hangzhou) Co., Ltd. China) and Plastisil ODS column (150X4.6mm, 5µm; 1.5mL/minute, 25°C) Dimma Science and Technology, China.
2. **Mobile phase**: Methanol /0.02M KH2PO4 buffer solution =15/85 (pH6.0), flow rate of 1.5mL/min, column temperature 25°C, sample 10uL.
3. **Detection wavelength**: 220nm.
4. **Standard curve**: Procainamide HCl standards of 0, 1, 10, 20 , 40, and 80µg

IV.Data analysis: Recovery of Procainamide HCl (%) was determined by comparing pre-SPE concentrations to post-SPE concentrations and normalized to the mean of all recoveries. Normalized recoveries at each column position were analyzed by ANOVA to determine statistical significance across all positions. A Tukey-Kramer HSD test was used to do a pair-wise comparison between all column positions to identify any difference among columns or the runs. Statistical analyses were completed using JMP 11 software (SAS Institute, Inc., USA).

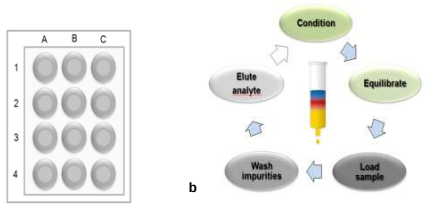

Figure: 2: (a). A 12 SPE column holder with layout (1mL or 3mL), and (b). Steps of solid phase extraction

V. Results & Discussion

Results from both the automated and manual SPE process are presented as follows. There was no carryover between samples or clogging of columns was observed during the process.

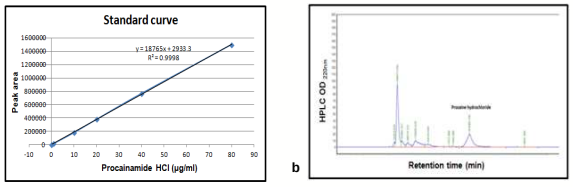

Figure 3: (a). Standard curve of Procainamide, (b). HPLC retention time of Procainamide

1. **Linearity studies:** Standard curve of Procainamide with coefficient of determination, R^2=0.9998 indicating the regression line approximates the real data points with high confidence, was used to calculate the concentration (Figure 3a).

2. **Accuracy**: Analysis of Variance (ANOVA) and Tukey-Kramer HSD analysis on post SPE Procainamide HCl recovery indicates no statistically differences between column positions on the VERSA 10 SPE deck ($F(10,33)$ = 0.967, p = 0.4892). Furthermore, the average coefficient of variance (CV%) across column positions is 4.05% (±0.859%) presented in Table 1. These results indicate consistent performance and low variance in SPE sample processing.

3. **SPE column positions in SPE rack**: Ordered differences from the Tukey-Kramer HSD analysis provides a pair-wise comparison of each SPE column position of Procainamide recovery after automated SPE. Differences are examined between each column position. The standard error of difference uses a pooled estimate of error variance. The lower and upper confidence limits (CL) are generated with α = 0.05. Values of difference between two column positions that fall outside of the lower and upper CL ranges are statistically significant. The step-wise p-Values are much greater than 0.05, indicating an absence of statistical significance between any two column positions.

The statistics show that there are no significant differences across cartridge positions (Tukey data; a = 0.05) as shown in Table 2. However, a significant difference between runs was observed where the batch 2 had significantly different from batch 1 and 3 (a = 0.05; p <.0001). This block effect was most likely human error. Positional biases were investigated on the VERSA 10 SPE by examining the recovery of 20µg of procainamide HCl serum samples after SPE on all column positions. Samples were loaded at every position with the exception of B1 that was used as a negative control (Figure 1). Four batches of twelve samples were tested (11 Procainamide samples and 1 negative control). The average Procainamide HCl recovery per batch (n=11) shows a recovery range between 87.86% - 98.35%. We found a statistical batch effect when comparing the recovery of Procainamide in all four batches ($F(3,40)$ = 17.6853, p < 0.0001). However, batch effects between experiments are not surprising. Prob > F = 0.4892 indicates an absence of statistical differences between column position.

Table 1: The average recovery of Procainamide from each replicated experiment (n=11).

	Average Recovery	Standard Deviation	%CV
Batch 1	88.51%	2.47%	2.79%
Batch 2	98.35%	4.62%	4.70%
Batch 3	87.86%	3.82%	4.35%
Batch 4	92.99%	4.06%	4.37%

Table 2 - Analysis of Variance of Procainamide recovery across column positions on the deck.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column Position	10	0.01326726	0.001327	0.967	0.4892
Error	33	0.0452753	0.001372		
C. Total	43	0.05854256			

VI. Advantages of VERSA 10 SPE Workstation

1. Four channel liquid handling on 1mL, and 3mL columns.
2. Positive pressure applied on 12 columns simultaneously.
3. User-defined flow rates for optimization.
4. Automated N_2 drying.
5. Equipped with heater-shaker for derivatization.
6. Transfer of eluted or derivatized samples to HPLC or LC-MS vials or plate.
7. Ensures the accuracy, reproducibility, and minimize human error.

VII. Acknowledgements

Technical support from Cathy Xu, and Denis Poultove, hardware and software engineers , respectively, is acknowledged.

VIII. References

1. Fenster, PE - Comess, KA: Marsh, R; Katzenberg, C; Hager, W.D: "Conversion of atrial fibrillation to sinus rhythm by acute intravenous procainamide infusion". *American Heart Journal* 1983; 106 (3):501–504.
2. Matsumoto, K, Kikuchi H, Kano S, Iri H, Takahashi H, and Umino M - Automated determination of drugs in serum by liquid chromatography with column-switching. I. Separation of antiepileptic drugs and metabolites. *Clin Chem.* 1988;34(1):141-144.

*Correspondence should be addressed to Aurora Biomed Inc., 1001 E. Pender St., Vancouver, BC, Canada, V6A 1W2, Tel: 1-604-215-8700, Fax: 1-604-215-9700, info@aurorabiomed.com, www.aurorabiomed.com