

Automated Rapid and Robust SPE Method Development Using strata™ X 96-Well Plates



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Part 1 – Theory

1.1 Introduction

Sample preparation is a critical step prior to LC/MS-MS analysis for quantifying drugs and their metabolites in complex biological matrices such as plasma, whole blood, urine or saliva in the drug discovery process. Solid phase extraction (SPE) has been the method of choice for sample preparation, owing to its environmental friendliness, ease of operation and automation. In state-of-the-art analytical laboratories, polymeric sorbents, especially those carrying polar or ion exchange functionalities, are preferred over silica based materials, owing to the tolerance of the former for pH extreme conditions, retention of polar analytes, accidental dry out, larger surface areas and reproducibility of sorbent chemistry. Until a decade ago, sample pretreatment was performed in the syringe barrel shaped cartridge format using a vacuum manifold. With the advent of the high throughput combinatorial chemistry era came the need for rapid analysis of thousands of samples from **DMPK** or **ADME** studies. Sample clean up on 96 or even higher well plate formats have taken over the sample preparation arena owing to their capability to handle hundreds of samples within a short time and their amenability for automation [1,2,3]. In spite of all these advances, method development for sample preparation still remains a tedious task due to the time/personnel factors involved in screening each sorbent individually. Phenomenex has recently introduced the strata™X multi-sorbent 96-Well plate format, which in tandem with an automated liquid handling system, vastly simplifies the SPE method development process. In this communication, a protocol for achieving rapid optimization of an SPE method for any analyte from a biological matrix is described.

Part 2 – Set Up

2.1 Materials and Equipment Used in the Current Work for Automated SPE

The **Polymeric SPE Method Development Plate**, supplied by Phenomenex, contains four sorbents in three columns each, furnishing a total of 24 wells for each material. These consist of **strata-X** (a neutral polar functionalized styrene-divinylbenzene based polymer), **strata-X-C** (a strong cation exchange sorbent with sulfonic acid groups on polystyrene-divinylbenzene base), **strata-X-CW** (a styrene-divinylbenzene polymer carrying carboxylic acid moieties) and **strata-X-AW** (a styrene-divinylbenzene polymer with primary and secondary amine functionalities). Each sorbent is packed at a bed mass of 30 mg (plates with bed mass of 10 mg of each sorbent are also available). Single sorbent 96-Well plates packed with one of the above four sorbents (bed mass 30 mg) are used in stages 2 and 3 of the SPE method development process.

Part 2 – Set Up – Continued

2.1 Materials and Equipment Used in the Current Work for Automated SPE - Continued

The automated liquid handling system consists of a MultiPROBE® II, equipped with a movable eight pipette liquid transfer assembly mounted perpendicularly to the plane of the deck (or platform) carrying the hardware for reagent/sample solutions, a six-way valve capable of delivering six different liquids/reagents and a vacuum pump connected to a vacuum manifold with the 96-Well Method Development Plate. All liquid handling (from reservoirs or test tube racks or well plates or reagent bottles to SPE plate or dilution plate) are software controlled, as well as the volumes of reagents and/or sample solutions being transferred. The duration and amount of vacuum application to the Method Development Plate is also software controlled. One of the liquid delivery channels transports water (de-ionized) used for flushing the system or for dilution purposes. The other five lines are available for reagents including buffers and elution solvents. The six-way valve pumps these liquids (water or reagents) into the syringes, which deliver the desired volumes to the Method Development Plate or other desired apparatus mounted on the horizontal deck (**Figure 2**).

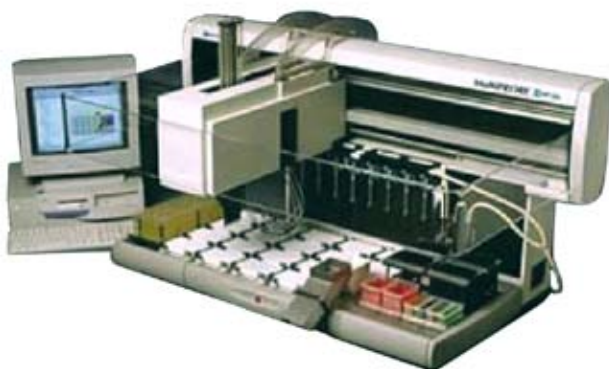


Figure 1: Packard Multiprobe II Liquid Handling System



Figure 2: Deck view of Step 1 Experiment from WinPREP™ Liquid Handler Operating Software

2.2 Solvents & Reagents

The following solutions (besides water) were used for method development

Solvent Type	Solution Composition
Buffer solution 1:	Ammonium formate (25 mM in water), adjusted to pH 2.5 with formic acid (called the acidic buffer)
Buffer solution 2:	Ammonium acetate (25 mM in water), adjusted to pH 5.5 with acetic acid (called the basic buffer)
Elution solvent 1:	Methanol containing 5 % formic acid (acidic elution)
Elution solvent 2:	Methanol containing 5 % (by weight) of ammonia (basic elution)
Elution solvent 3:	Pure methanol (neutral elution)

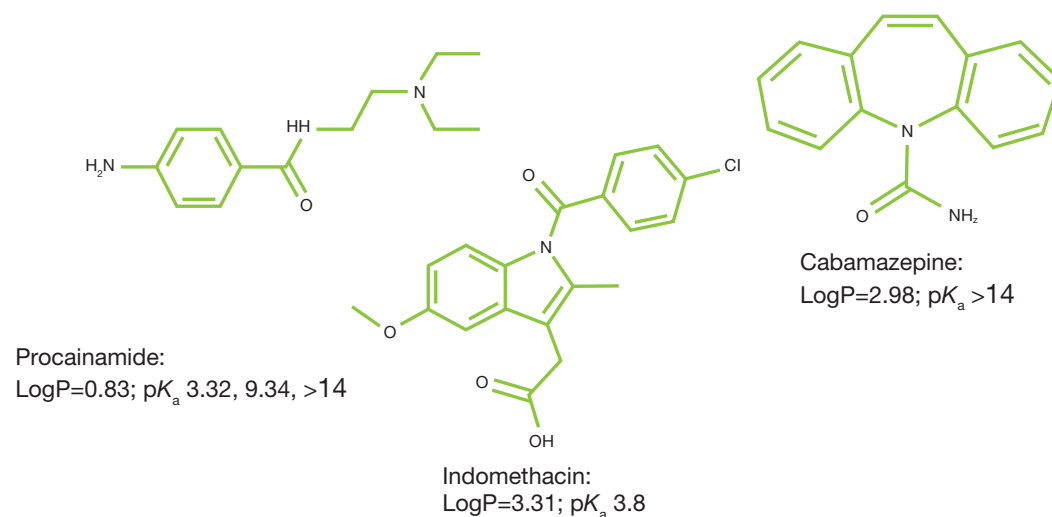
Sample solutions: The analytes procainamide, carbamazepine and indomethacin (from Sigma, see **Figure 3** for structures) are spiked into human plasma in two concentrations: ULOQ (upper limit of quantitation) concentration of 10 µg per mL and LLOQ (lower limit of quantitation) at 100 ng per mL, after final dilution of the SPE extracts. For SPE method development, the ULOQ samples are primarily utilized since they permit analyses by UV detection in Steps 1 and 2. However, if desired, LC/MS-MS detection can be used to cater to both extremes of concentrations needed for validation studies. In the current study, samples of concentrations lower than 50 ng were used in Step 3 for linearity and other studies, with detection by LC/MS-MS.

Human plasma (Steps 1, 2 and 3) with sodium EDTA as anticoagulant (500 µL), diluted with water or buffers 1 or 2 (1 mL) containing appropriate concentration of the analytes was used for loading. Thus, the total volume of diluted plasma loaded is 1.5 mL.

Reagent volumes used for various stages of the automated method development protocol:

Method STEP	Solvent
Conditioning 1	Methanol (400 µL)
Conditioning 2	Water or Buffer 1 or 2 (400 µL)
Load solution:	Plasma (500 µL) diluted with water or Buffer 1 or 2 (1mL) containing appropriate concentration of the analytes (total diluted plasma volume 1.5 mL).
Washing	Water or Buffer 1 or 2 (800 µL) (for Step 1)/ Water or buffer 1 or buffer 2 in methanol (800 µL) (for Step 2)
Elution	Methanol (with or without 5% formic acid or ammonium hydroxide) (single elution with 400 µL in Step 1 or with two 200 µL fractions of water or buffer 1 or buffer 2 in methanol at various concentrations in step 2)

Figure 3: Structures of Probes Used (with LogP and pK_a values)



Part 3 – Goals and Solutions

3.1 Goals and Solutions for SPE Method Development for High Throughput Analysis

Goals:

- Simultaneous screening of multiple sorbents
- Comprehensive, but robust, method for screening the sorbents within a short time frame
- Universal applicability of the method
- Automation of operations
- Rapid evaluation of linearity, precision, robustness and matrix effects

Solutions:

- Design a 96-Well format to accommodate multiple sorbents
- Develop a protocol that would enable SPE method development through optimization of sorbent, wash and elution conditions in minimal time
- Utilization of an automated liquid handling system for automation of the protocol
- Simultaneous evaluation of several probes for verification of universality of the method
- Rapid evaluation of quantitation parameters such as linearity, precision, accuracy and matrix effects using the optimized sorbent and SPE conditions

3.2 Detailed Description of Solutions

The protocol designed consists of three steps. The first step consists of optimization of the sorbent and the pH for load/wash/elution. In the second stage, optimization of the organic percentage for the wash and elution stages, along with the pH of the buffer needed, is carried out. In the third step, the linearity, limit of detection, quantitation of recovery and matrix effects are probed using a stable isotope labeled analyte as internal standard.

Step 1: This step utilizes the four sorbent 96-Well Polymeric Method Development Plate. It facilitates the determination of which sorbent is the most suitable for retaining and eluting an analyte and what pH conditions are most appropriate for this. These parameters are determined by employing three sets of load and elution conditions – neutral (water) load with 100 % pure solvent elution (designated NN); loading in an acidic buffer with elution in basified organic solvent (designated AB); and loading in basic buffer with elution in acidified organic solvent (designated BA). In each case, the wash is performed with the same medium in which the loading took place. Of the 24 wells available for each sorbent, four are used for each condition (NN, AB and BA) at the ULOQ concentration (total 12 wells) and two are used at the LLOQ concentration (total 6 wells). For each condition, one well is used for reference (called E-Ref), wherein the blank plasma is extracted with the sorbent and the analyte spiked into the extract and this sample for each condition serves as the reference point for determining the recovery under this condition. Three other wells serve as blanks, wherein the unspiked plasma extract is analysed. The well plate design is illustrated pictorially in **Figure 4**.

strata-X			strata-X-C			strata-X-CW			strata-X-AW		
ULOQ X NN	ULOQ X AB	ULOQ X BA	ULOQ C NN	ULOQ C AB	ULOQ C BA	ULOQ CW NN	ULOQ CW AB	ULOQ CW BA	ULOQ AW NN	ULOQ AW AB	ULOQ AW BA
ULOQ X NN	ULOQ X AB	ULOQ X BA	ULOQ C NN	ULOQ C AB	ULOQ C BA	ULOQ CW NN	ULOQ CW AB	ULOQ CW BA	ULOQ AW NN	ULOQ AW AB	ULOQ AW BA
ULOQ X NN	ULOQ X AB	ULOQ X BA	ULOQ C NN	ULOQ C AB	ULOQ C BA	ULOQ CW NN	ULOQ CW AB	ULOQ CW BA	ULOQ AW NN	ULOQ AW AB	ULOQ AW BA
ULOQ X NN	ULOQ X AB	ULOQ X BA	ULOQ C NN	ULOQ C AB	ULOQ C BA	ULOQ CW NN	ULOQ CW AB	ULOQ CW BA	ULOQ AW NN	ULOQ AW AB	ULOQ AW BA
EREF X NN	EREF X AB	EREF X BA	EREF C NN	EREF C AB	EREF C BA	EREF CW NN	EREF CW AB	EREF CW BA	EREF AW NN	EREF AW AB	EREF AW BA
BLK X NN	BLK X AB	BLK X BA	BLK C NN	BLK C AB	BLK C BA	BLK CW NN	BLK CW AB	BLK CW BA	BLK AW NN	BLK AW AB	BLK AW BA
LLOQ X NN	LLOQ X AB	LLOQ X BA	LLOQ C NN	LLOQ C AB	LLOQ C BA	LLOQ CW NN	LLOQ CW AB	LLOQ CW BA	LLOQ AW NN	LLOQ AW AB	LLOQ AW BA
LLOQ X NN	LLOQ X AB	LLOQ X BA	LLOQ C NN	LLOQ C AB	LLOQ C BA	LLOQ CW NN	LLOQ CW AB	LLOQ CW BA	LLOQ AW NN	LLOQ AW AB	LLOQ AW BA

Figure 4: Illustration of the well plate arrangement for Implementing Step 1
(**X**=strata-X; **C**=strata-X-C; **CW**=strata-X-CW; and **AW**=strata-X-AW)

- NN** = neutral load/ neutral elution
- AB** = acid pH load/basic pH elution
- BA** = basic load/acidic elution)
- BLK** = blank (unspiked plasma extract)
- EREF** = plasma extract spiked with analytes

Part 3 – Goals and Solutions – Continued

3.2 Detailed Description of Solutions - Continued

Figure 5 (see page 10) exemplifies how the above Step 1 protocol furnishes the optimized sorbent and pH conditions on the basis of the results obtained from this Step 1 experiments for the polar basic drug procainamide and the medium-polar acidic drug indomethacin. The top bar graph of **Figure 5** shows the data for procainamide. The neutral load/neutral solvent elution conditions (NN) and the basic pH buffer load/acidified methanol elution condition (BA) yield the maximum recoveries with strata-X, while the acidic load/basic elution (AB) conditions give much lower recovery yields. On the other hand, near quantitative recoveries are obtained with the cation exchange sorbents strata-X-C and strata-X-CW only under the acidic pH buffer load/basic methanol elution conditions (AB). With the weak anion exchanger strata-X-AW, quantitative recoveries are observed under the BA conditions only, with only negligible recoveries under the AB conditions.

The Step 1 experimental data for the acidic probe indomethacin is shown in the lower bar graph in **Figure 5**. With all sorbents (strata-X, -X-C, X-CW and X-AW), only the acidic load/basic methanol elution (AB conditions) gave good recoveries. Under the other conditions, recoveries were in the 0-30 % range.

To rationalize the behavior of the sorbents, we need to consider all possible modes of interaction of the basic and acidic probes with the surface chemistries. For example, with the neutral polar functionalized polymer strata-X, the polar basic drug procainamide can be visualized to interact not only through hydrophobic mechanism, but also through hydrogen bonding and dipolar interactions as well. Thus, methanol by itself is able to disrupt these interactions and elute the procainamide off strata-X in good recovery yields. When procainamide is loaded under the basic pH buffer conditions, it is still in the neutral form and all these interactions are still operative and hence it is retained on strata-X under these load conditions and can be eluted off with acidified methanol in quantitative yields. When acidic pH buffer is used to load procainamide, protonation causes a reduction in the hydrophobic interactions and some breakthrough occurs on strata-X, resulting in reduced recoveries. On the other hand, with the strong and weak cation exchangers, strata-X-C and -X-CW, respectively, basic methanol elution is needed to neutralize the ionic interactions and thus, only the AB conditions show good recoveries. The lower yields under the BA conditions with strata-X-CW indicate that the 5 % formic acid in methanol in one pass (elution volume for all experiments is 400 μ L) is not sufficient to neutralize the ionic interactions (it was found subsequently that two passes of the acidified methanol significantly improved the recoveries of basic drugs). In the case of the weak anion exchanger strata-X-AW, both NN and BA conditions furnish good recoveries, as expected, but the AB conditions showed very low recovery yields and this is most likely due to protonation of both the sorbent (which leads to a reduction of surface hydrophobicity) and the analyte (causing repulsion from the sorbent) under these acidic load conditions, resulting in breakthrough during load/wash steps.

A similar rationalization applies to the acidic drug indomethacin. In this case, the drug exists in the non-ionized (free carboxylic) form under neutral (water) or acidic load conditions. Further its hydrophobic interactions are a lot stronger than the polar procainamide and can significantly contribute to its retention under neutral or acidic load conditions on strata-X, -X-C and X-CW sorbents. Also, indomethacin can form multiple hydrogen bonds (through its carboxyl, amide, ether and chloro functionalities) with the polar segments of strata-X or the acidic moieties on strata-X-C and -X-CW and these can add to retention under neutral or acidic load conditions. Hence, one elution with methanol (400 μ L) was not sufficient to elute this acidic probe from strata-X, -X-C and -X-CW under the NN conditions. Recoveries are good under basic elution conditions from neutral and acidic sorbents, since the drug is converted into its ionized carboxylate form, which lowers its hydrophobicity considerably and minimizes H-bonding interactions. On the other hand, basic load conditions ionize the carboxylic group of indomethacin as well as the sulfonic (strata-X-C) and carboxylic (strata-X-CW) and hence breakthrough can occur under the BA load conditions.

strata-X also is expected to behave similarly under the BA conditions. On the other hand, strata-X-AW is protonated under both BA and AB load conditions and ionic interactions must be favored with indomethacin. However, only basic elution gives good recoveries with this sorbent. Both NN and BA conditions give low recoveries on strata-X-AW. As with the other sorbents, incomplete elution under NN conditions may contribute to low recoveries. But, the protonated X-AW surface retains indomethacin under the BA conditions, contributing to incomplete elution.

In contrast to procainamide and indomethacin, the neutral drug carbamazepine (see **Figure 3** for structures) interacts with all four sorbents through hydrophobic mechanism exclusively under both pH conditions used for load, wash and elution. This uniform behavior with all four sorbents presents more options for generating cleaner extracts and to develop more robust methods. From the traditional approach viewpoint, one can use strata-X as the basis for evaluation of step 2; however, this option does not permit utilization of all levers for enhancing cleanliness of the extracts. On the other hand, if strata-X-C is employed for the clean up of neutrals like carbamazepine from plasma, one can load and wash under the basic pH conditions and elute under acidic pH conditions, since the recovery of this drug is unaffected by pH. Although this protocol is opposite to that of the normal procedure used for this strong cation exchanger (which is, load and wash under acidic pH and elute under basic pH), it has a double advantage. On the one hand, loading/washing under basic pH conditions would remove all acidic (negatively charged) impurities; on the other, elution under acidic pH would enable retention of basic (positively charged) impurities on strata-X-C and at the same time elute off the desired neutral compound. Thus, all kinds of charged impurities from plasma can be eliminated and more cleaner carbamazepine extract can be obtained. This is not possible with the uncharged strata-X surface.

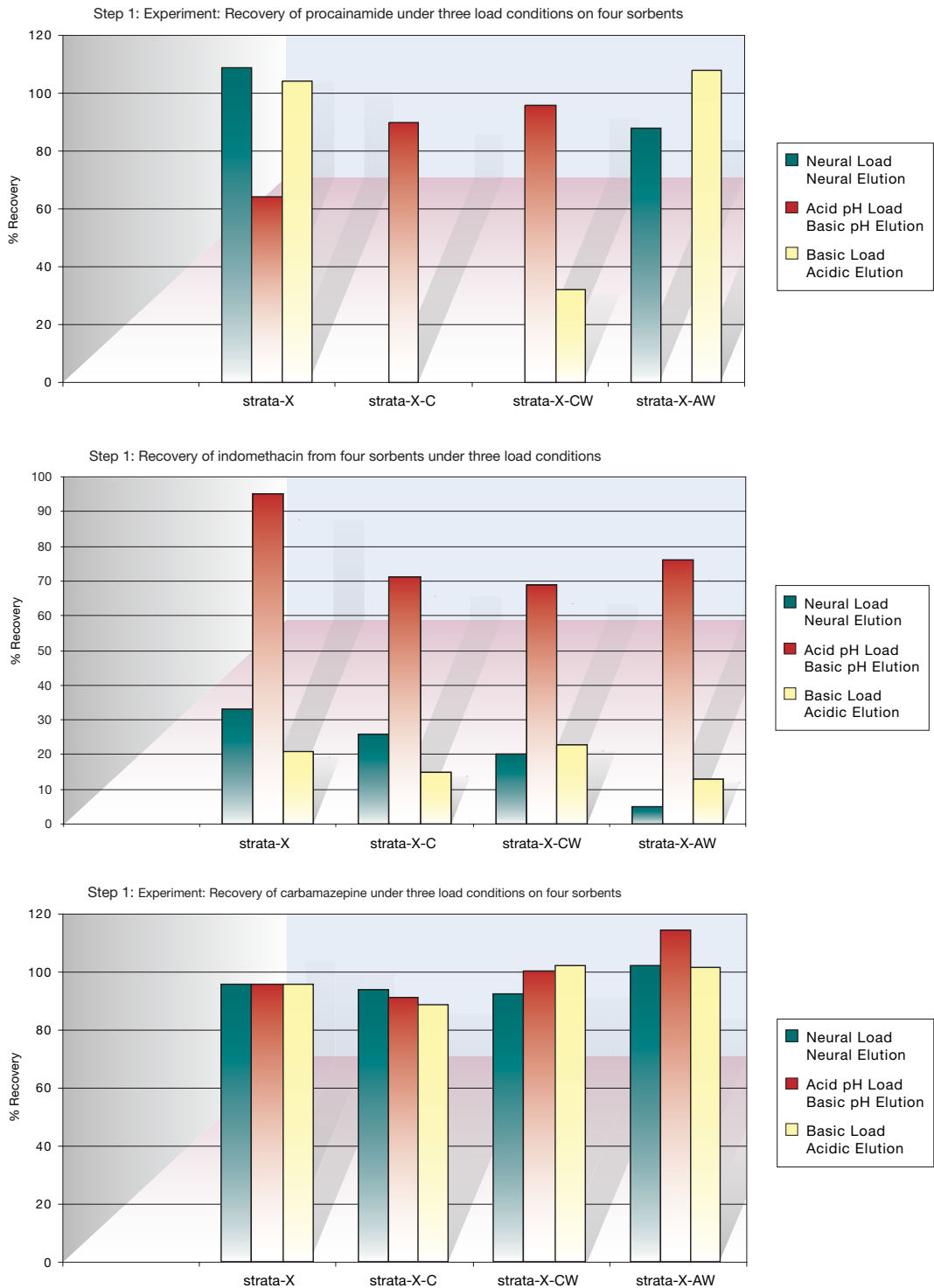


Figure 5: Recoveries of procainamide, carbamazepine and indomethacin from four sorbents under three sets of loading conditions (Step 1 Experiment)

Part 3 – Goals and Solutions – Continued

3.3 Step 2 Experiments for SPE Method Development

This protocol is intended to provide information about the percent organic that a probe can tolerate on a particular sorbent during the wash step(s) of the SPE and also about the percent of organic needed to elute the probe from the same sorbent. The sample may be loaded under two conditions - neutral (water) or in a buffer (acidic or basic) and then washed with the same load condition solvent (either water or load buffer). In the first option (Option A), after the initial water wash, a second wash with a range of organic (methanol) concentrations (for example, 10 to 100 %) is included. In the second option (Option B), after the initial buffer wash, the first six columns are eluted with the load pH (acidic or basic) buffer/organic at six concentrations, while the second six columns are eluted with the opposite pH to that of the load pH along with the organic at six concentrations. In summary, Option A (see **Figure 6**) uses methanol/water (10 / 90 to 100 / 0) for elution, while Option B (see **Figure 7**) uses methanol/acid (6 columns) or methanol/base (6 columns) as eluent (with methanol concentrations ranging from 10 to 100 %). A single sorbent 96-Well plate is used for this step 2.

ULOQ 10	ULOQ 15	ULOQ 20	ULOQ 25	ULOQ 30	ULOQ 40	ULOQ 50	ULOQ 60	ULOQ 70	ULOQ 80	ULOQ 90	ULOQ 100
ULOQ 10	ULOQ 15	ULOQ 20	ULOQ 25	ULOQ 30	ULOQ 40	ULOQ 50	ULOQ 60	ULOQ 70	ULOQ 80	ULOQ 90	ULOQ 100
ULOQ 10	ULOQ 15	ULOQ 20	ULOQ 25	ULOQ 30	ULOQ 40	ULOQ 50	ULOQ 60	ULOQ 70	ULOQ 80	ULOQ 90	ULOQ 100
ULOQ 10	ULOQ 15	ULOQ 20	ULOQ 25	ULOQ 30	ULOQ 40	ULOQ 50	ULOQ 60	ULOQ 70	ULOQ 80	ULOQ 90	ULOQ 100
E REF 10	E REF 15	E REF 20	E REF 25	E REF 30	E REF 40	E REF 50	E REF 60	E REF 70	E REF 80	E REF 90	E REF 100
BLK 10	BLK 15	BLK 20	BLK 25	BLK 30	BLK 40	BLK 50	BLK 60	BLK 70	BLK 80	BLK 90	BLK 100
LLOQ 10	LLOQ 15	LLOQ 20	LLOQ 25	LLOQ 30	LLOQ 40	LLOQ 50	LLOQ 60	LLOQ 70	LLOQ 80	LLOQ 90	LLOQ 100
LLOQ 10	LLOQ 15	LLOQ 20	LLOQ 25	LLOQ 30	LLOQ 40	LLOQ 50	LLOQ 60	LLOQ 70	LLOQ 80	LLOQ 90	LLOQ 100

Figure 6: Plate configuration for **Option A** protocol in Step 2 of SPE Method Development

(numerical figures represent % of methanol in elution solvent) (BLK, EREF convey the same meaning as in Figure 3)

ULOQ 10 L	ULOQ 20 L	ULOQ 40 L	ULOQ 60 L	ULOQ 80 L	ULOQ 100 L	ULOQ 10 E	ULOQ 20 E	ULOQ 40 E	ULOQ 60 E	ULOQ 80 E	ULOQ 100 E
ULOQ 10 L	ULOQ 20 L	ULOQ 40 L	ULOQ 60 L	ULOQ 80 L	ULOQ 100 L	ULOQ 10 E	ULOQ 20 E	ULOQ 40 E	ULOQ 60 E	ULOQ 80 E	ULOQ 100 E
ULOQ 10 L	ULOQ 20 L	ULOQ 40 L	ULOQ 60 L	ULOQ 80 L	ULOQ 100 L	ULOQ 10 E	ULOQ 20 E	ULOQ 40 E	ULOQ 60 E	ULOQ 80 E	ULOQ 100 E
ULOQ 10 L	ULOQ 20 L	ULOQ 40 L	ULOQ 60 L	ULOQ 80 L	ULOQ 100 L	ULOQ 10 E	ULOQ 20 E	ULOQ 40 E	ULOQ 60 E	ULOQ 80 E	ULOQ 100 E
E REF 10 L	E REF 20 L	E REF 40 L	E REF 60 L	E REF 80 L	E REF 100 L	E REF 10 E	E REF 20 E	E REF 40 E	E REF 60 E	E REF 80 E	E REF 100 E
BLK 10 L	BLK 20 L	BLK 40 L	BLK 60 L	BLK 80 L	BLK 100 L	BLK 10 E	BLK 20 E	BLK 40 E	BLK 60 E	BLK 80 E	BLK 100 E
LLOQ 10 L	LLOQ 20 L	LLOQ 40 L	LLOQ 60 L	LLOQ 80 L	LLOQ 100 L	LLOQ 10 E	LLOQ 20 E	LLOQ 40 E	LLOQ 60 E	LLOQ 80 E	LLOQ 100 E
LLOQ 10 L	LLOQ 20 L	LLOQ 40 L	LLOQ 60 L	LLOQ 80 L	LLOQ 100 L	LLOQ 10 E	LLOQ 20 E	LLOQ 40 E	LLOQ 60 E	LLOQ 80 E	LLOQ 100 E

Figure 7: Plate Configuration for **Option B** protocol in Step 2 of the SPE Method Development process design

(numerical figures represent % methanol; L = elution at load pH and E = elution at pH opposite that of load)

Part 3 – Goals and Solutions – Continued

3.3 Step 2 Experiments for SPE Method Development - Continued

The wash/elution profiles of procainamide on strata-X-C and indomethacin on strata-X-AW from human plasma under Option B conditions are shown in **Figures 8 and 9**, respectively. From **Figure 8**, it can be inferred that even a 100 % organic wash under acidic load conditions will not elute procainamide and this drug can be eluted with basified methanol completely with about 80 % content of methanol. This behavior is in agreement with the strong cation exchange character of the sulfonic acid functionalized strata-X-C. By a similar token, it can be inferred from **Figure 9** that indomethacin in biological samples can be cleaned up efficiently using strata-X-AW by washing with either 80 % of methanol in acidic buffer or 40 % methanol in basic buffers and eluted in more than 90 % recovery yields with 100 % methanol containing 5 % ammonium hydroxide. This is also in agreement with the weak cation exchange properties of strata-X-AW. On the other hand, both drugs start to elute off with 40 % methanol from the neutral polymer strata-X (see **Figure 10**) even under neutral aqueous conditions.

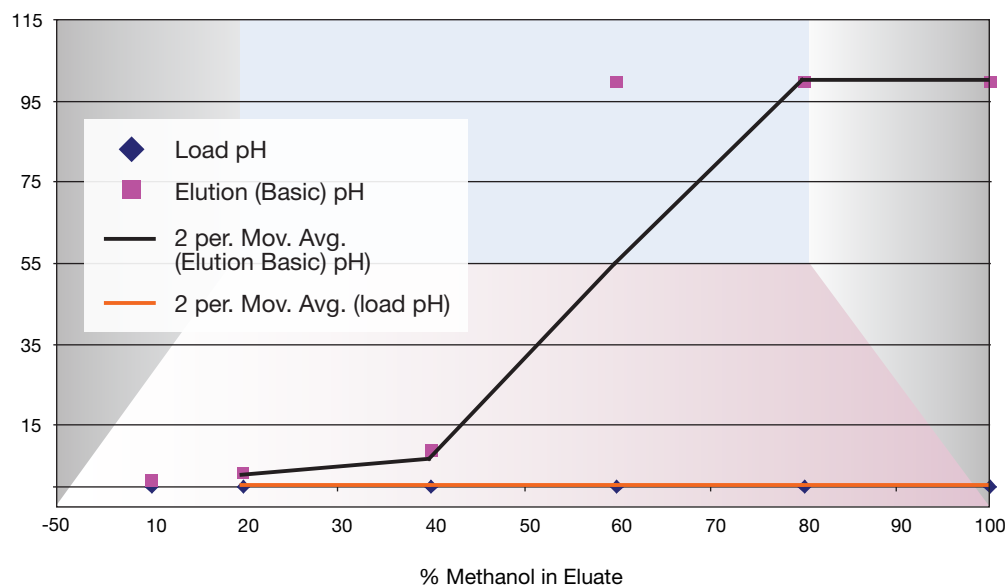


Figure 8: Wash/Elution Profile of procainamide on strata-X-C in Step 2 of SPE process design using option B (acidic and basic methanol elution comparison)

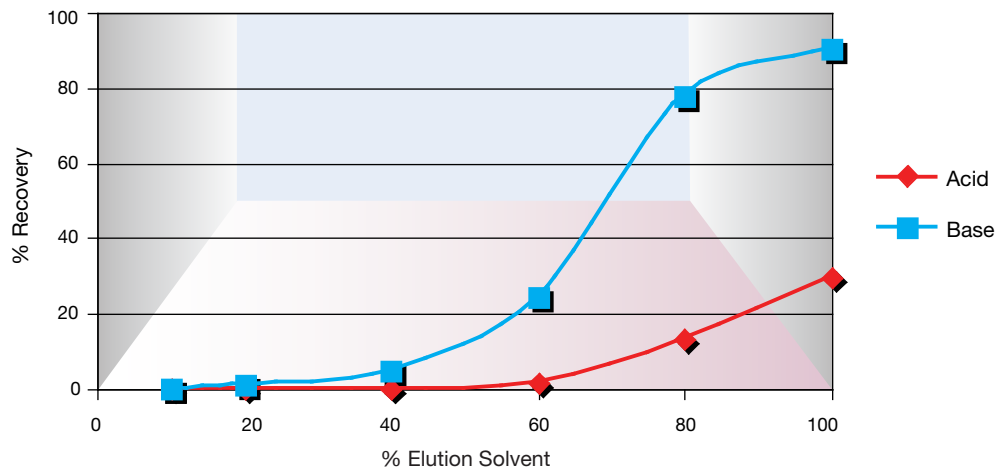


Figure 9: Wash/Elution Profile of indomethacin from Step 2 of SPE method development process design on strata-X-AW (X-axis numbers are % of methanol in binary elution solvent) using Option B

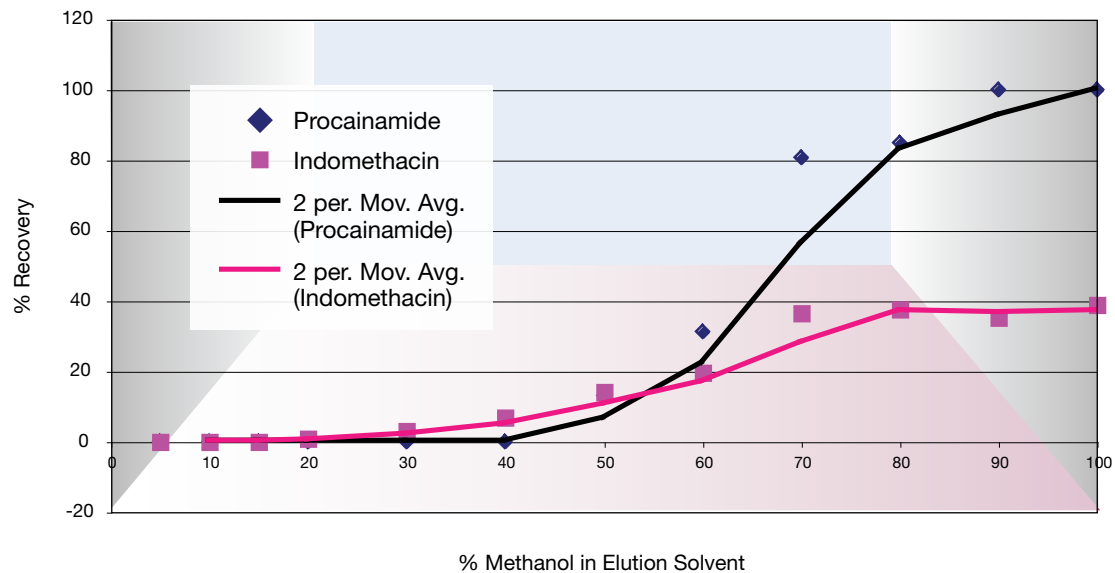


Figure 10: Wash/ Elution Profile of indomethacin from step 2 of SPE method development process design on strata-X (X-axis numbers are % of methanol in binary elution solvent) using Option A

Part 3 – Goals and Solutions – Continued

3.4 Step 3 of the SPE Method Development Process Design

From the data in Step 1 and Step 2, one can easily pick the sorbent most suitable for the SPE of the analyte under consideration and also gain information about what percent concentration of organic solvent in a wash solution the analyte can tolerate without breakthrough on this sorbent and under what eluting solvent conditions it can be eluted off this optimized sorbent. The purpose of Step 3 is to use this knowledge to determine the precision and accuracy of the optimized method, as well as to ascertain matrix effects from the components of biological matrices if they are not eliminated during the wash steps completely. Step 3 utilizes a single sorbent 96-Well plate and a range of concentrations of the analyte (for example, from 1 nanogram to 1 microgram per mL of plasma sample) can be loaded for determining the linearity of the SPE method. The experimental design of the 96-Well plate in Step 3 is governed by the need of an analyst with respect to the concentration ranges he/she is looking for and how many replicate experiments are desired, as well as the number of blank extracts he/she wants to use for quantitating matrix effects. Quantitation of the analyte in this Step 3 is done using a stable labeled isotope of the analyte under investigation as internal standard and detection is performed by LC/MS-MS to furnish sensitivity of detection at very low concentrations. This is the typical procedure followed by the pharmaceutical industry.

As an example of Step 3, the extraction of indomethacin from plasma is carried out on the Multiprobe II using strata-X-AW as the sorbent. This sorbent is chosen because it can be protonated under acidic load conditions and the resulting cationic surface can hold indomethacin by hydrophobic/ion exchange mechanisms during the load and wash steps, as was demonstrated from results of Step 1 experiments. Also, washing can be carried out efficiently with 60 % methanol in an acidic buffer, as can be inferred from Step 2 experiments, which also show that indomethacin can be eluted in basic methanol at any concentration of the organic over 80 %.

Matrix effects were compared by peak area ratios for indomethacin and its d4-labeled analog, for extracted blank samples spiked with the drugs in comparison with reference standards of the same drugs in the mobile phase used for LC/MS. The peak area data for indomethacin extracts from strata-X-AW shows less than 5-12 % difference from the standards, demonstrating excellent cleanliness of the extracts.

Part 4 – Results

4.1 Conclusions

1. The whole SPE method development process can be completed **within one day** by using a three step protocol that enables the optimization of the sorbent, load, wash and elution conditions.
2. The four sorbent SPE Polymeric Method Development Plate is universal for neutral, acidic and basic drugs and covers all possible interaction modes of analytes contributing to their retention and enabling optimization of the SPE clean up on one of these sorbents.
3. The protocol enables a rapid evaluation of all quantitation parameters for validation, such as linearity, precision, accuracy and matrix effects.
4. The sorbent/protocol utilizes reagents/buffers compatible with mass spectrometric detection.
5. The process is automatable on any state-of-the-art liquid handling system.

4.2 References

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May not be representative of all applications shown. The following applications is to be used as a starting point, some optimization may be required. strata-X is a trademark of Phenomenex, Inc. Multiprobe is a registered trademark of Perkin Elmer, Winprep is a trademark of Perkin Elmer. Phenomenex is in no way affiliated with Perkin Elmer. © Phenomenex, Inc. 2006. All rights reserved.



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