

Improve Sample Prep Selectivity through 96-well SPE Method Development Plates

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In pharmaceutical bioanalysis, researchers are charged with the responsibility of developing and running assays to quantitate drugs, pharmaceutical candidates, and their metabolites in biological fluids such as serum and plasma. With recent advances in combinatorial chemistry, genomics and proteomics, knowledge of drug mechanisms are increasing resulting in drug designs structurally catered to endogenous biomolecules. Such drugs are often more potent allowing for smaller dosages which results in smaller concentrations of the drugs and their metabolites in biological fluids. Although advances in LC-MS technology have reaped overwhelming benefits in terms of increased throughput and sensitivity, good sample preparation has and continues to become more critical.

Bioanalytical scientists are often asked to detect drug levels in the parts-per-trillion to parts-per-quadrillion range. Because solid phase extraction (SPE) technology is based on chromatographic separation, analysts can develop robust methods that offer high analyte recoveries. More importantly, SPE offers the selectivity necessary to specifically target the retention and elution of analytes of interest in the presence of complicated biological matrix components.

Even with SPE technology's advantages, many analysts do have reservations for using the technology. The most common disadvantage is the wide perception that SPE is

overly complex. The wide selection of phase chemistries coupled with the large number of potential reagents/solvents used for each step of the SPE process makes method development and troubleshooting a daunting and time consuming task. In effect, many researchers find it difficult to develop rugged SPE methods that meet their analytical objectives.

96-well SPE MD (Method Development) Plate – BAN For extracting basic, acidic, and neutral compounds (BAN)

To address this widespread concern, we have developed a 96-well SPE platform to ease the method development process. Our new 96-well SPE MD (Method Development) Plate – BAN contains a selection of 8 different SPE chemistries commonly used in the extraction of basic, acidic, and/or neutral compounds from biological fluids (Figure 1). The mix of phase chemistries contained within this 96-well SPE plate allows researchers to screen for the phase(s) that offer the best analyte recovery, selectivity, and reproducibility when using the generic methods described in Table 1.

The Benefits of Evaluating Multiple Phase Chemistries

Most method developers often focus their method development efforts on popular reversed-phase chemistries such as C18 and hydrophilic polymer phases. Such phases often offer good retention of a broad range of analytes and can typically yield high recoveries under

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Figure 1. Phase Chemistry Template for 96-well SPE MD Plate-BAN, 25 mg/well (577522-U)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Discovery DSC-PS/DVB (polystyrene divinyl benzene) ¹											
B	Discovery DSC-18 (tC18) ¹											
C	Discovery DSC-8 (C8) ¹											
D	Discovery DSC-CN (cyanopropyl) ¹											
E	Discovery DSC-MCAX (mixed-mode cation exchange) ²											
F	Discovery DSC-WCX (weak cation exchange) ²											
G	Discovery DSC-SAX (strong anion exchange) ³											
H	Discovery DSC-NH ₂ (aminopropyl weak anion exchange) ³											

- ¹ Reversed-phase
- ² Cation-exchange
- ³ Anion-exchange



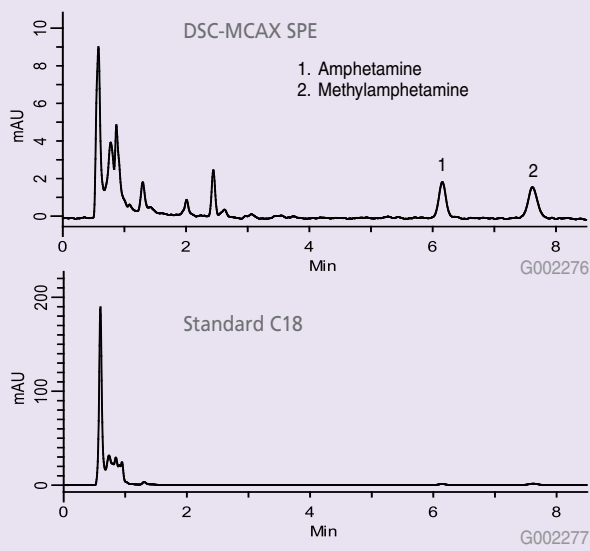
Table 1. Recommended Generic Methods for 96-well SPE MD Plate-BAN

SPE Step	Reversed-phase	Cation-exchange	Anion-exchange
1. Sample Pre-Treatment	Dilute biological sample 1:1 with 10-50 mM buffer (phosphate, ammonium acetate, or ammonium formate) at 2 pH units above analytes' pKa for basic analytes, or 2 pH units below pKa for acidic analytes.	Dilute biological sample 1:1 with 10-50 mM buffer (phosphate, ammonium acetate, or ammonium formate), pH 3 for basic analytes.	Dilute biological sample 1:1 with 10-50 mM buffer (phosphate, ammonium acetate, or ammonium formate), pH 10 for acidic analytes.
2. Condition/Equilibrate	Condition with methanol. Equilibrate with DI water or buffer used in sample pre-treatment.	Condition with methanol. Equilibrate with DI water or buffer used in sample pre-treatment.	Condition with methanol. Equilibrate with DI water or buffer used in sample pre-treatment.
3. Sample Load	Load pre-treated sample from step 1.	Load pre-treated sample from step 1.	Load pre-treated sample from step 1.
4. Wash	Wash off co-retained interferences with 5-20% methanol diluted in DI water or buffer used in sample pre-treatment.	Wash off co-retained interferences with low pH buffer used in sample pre-treatment, followed by 1M acetic acid and 100% methanol.	Wash off co-retained interferences with high pH buffer used in sample pre-treatment, followed by 100% methanol.
5. Elution	Elute with methanol or acetonitrile. pH modification may be necessary to facilitate elution. Use 2% acetic acid in methanol or acetonitrile for basic analytes; or 2% ammonium hydroxide in methanol or acetonitrile for acidic analytes.	Elute basic analytes with 2-5% ammonium hydroxide in methanol or acetonitrile.	Elute acidic analytes with 2-5% acetic acid in methanol or acetonitrile.
6. Evaporate/Reconstitute	Evaporate SPE eluate and reconstitute with analytical mobile phase		

Figure 2. DSC-MCAX SPE vs. C18 SPE for the Extraction of Amphetamine and Methylamphetamine in Urine

SPE tube: Discovery DSC-MCAX, 100 mg/3 mL, standard C18, 100 mg/3 mL
 cat. no.: 52783-U
 HPLC column: Discovery HS F5, 15 cm x 4.6 mm I.D., 5 µm particle size
 cat. no.: 567516-U
 mobile phase: 10 mM ammonium acetate, pH 4.5:MeCN (35:65)
 flow rate: 2 mL/min.
 temp.: 40 °C
 det.: 210 nm, UV
 injection: 10 µL

- Note the Y-axis scale difference between DSC-MCAX and C18 SPE. DSC-MCAX SPE offered a maximum background height of ~9 mAU.
- In contrast, standard C18 background levels were 20 times greater than DSC-MCAX.
- Also, on DSC-MCAX absolute recovery averaged at 100.3 and 101.7%, for amphetamine and methylamphetamine, respectively.
- On standard C18, absolute recovery averaged at 48 and 79% for the two compounds.



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generic methodology. However, because of this broad affinity, matrix interferences can often co-retain and elute with analytes of interest.

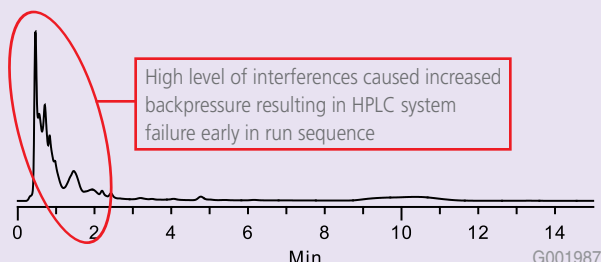
For example, in this application, we compare the extraction of 2 µg/mL amphetamine and methyl amphetamine spiked in human urine on both a standard C18 SPE and Discovery DSC-MCAX using the generic protocols described in Table 1 (reversed-phase protocol for C18 and cation-exchange protocol for MCAX) followed by subsequent LC-UV analysis (Figure 2). The MCAX phase offered ~100% absolute recovery, whereas the C18 phase offered 79 and 48% recovery for the compounds tested. Also note that the C18 background was 20 times greater than the MCAX phase.

In this second application, four corticosteroids (0.5 and 1.0 µg/mL) were extracted from urine on both Discovery DSC-CN and standard C18 (100 mg/well) using the reversed-phase procedure described in Table 1 followed by LC-UV analysis. Note that the background level on C18 was so high, HPLC system failure occurred early in the run (Figure 3). DSC-CN also offered excellent recovery for the corticosteroids tested (Figure 4).

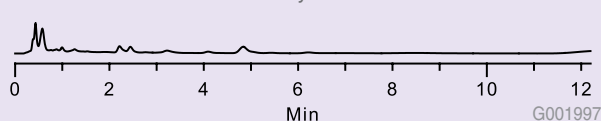
Figure 3. Background Comparison of Blank Urine Sample

column: Discovery HS F5, 5 cm x 4.6 mm I.D., 3 µm particles
mobile phase: 40:60 methanol:deionized water
flow rate: 1.5 mL/min.
temp.: 35 °C
det.: UV at 240 nm
injection: 5 µL

Blank urine extract on conventional C18



Blank urine extract on Discovery DSC-CN



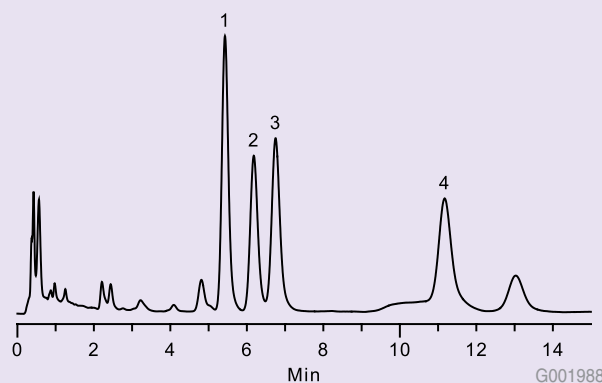
Conclusion

In pharmaceutical bioanalysis, sample prep selectivity and recovery is of vital importance when achieving very low limits of quantitation in difficult sample matrices. Supelco's new 96-well SPE MD Plate-BAN offers a convenient format for researchers to screen an array of SPE phase chemistries during SPE method development. Once one or more phase chemistries are selected, further

Figure 4. Recovery and Example Chromatogram for DSC-CN Extraction of Corticosteroids

Compound	% Recovery ± RSD (n=3)	
	0.5 µg/mL spike level	1.0 µg/mL spike level
1. Hydrocortisone	123.3±1.4%	95.9±1.7%
2. Prednisilone	107.2±1.1%	91.9±1.1%
3. Prednisone	103.2±1.0%	88.4±1.8%
4. Corticosterone	102.0±1.2%	93.1±5.6%

1 µg/mL spiked urine extract on Discovery DSC-CN



method optimization can be conducted to offer maximum assay selectivity, recovery, and accuracy/precision. In this report, we demonstrate the importance of evaluating multiple phase chemistries during method development.

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For more information or to inquire about products, please contact Supelco Technical Service at 800-359-3041/814-359-3041, or email techservice@sial.com

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Description	25 mg/well	50 mg/well	100 mg/well
96-well SPE MD Plates			
96-well SPE MD Plate-BAN (Basic, Acidic, Neutral Compounds)	577522-U	Inquire	Inquire
Standard 96-well SPE Plates			
Discovery DSC-18	575601-U	575602-U	575603-U
Discovery DSC-18Lt	575604-U	575605-U	575606-U
Discovery DSC-8	575629-U	575628-U	575627-U
Discovery DSC-Ph	575632-U	575631-U	575630-U
Discovery DSC-CN	575626-U	575625-U	575624-U
Discovery DPA-6S (polyamide)	Inquire	Inquire	-
Discovery PS/DVB	575610-U	575611-U	-
Discovery DSC-MCAX (C8/SCX)	575639-U	575640-U	575641-U
Discovery DSC-SCX	575623-U	575622-U	575621-U
Discovery DSC-WCX	575635-U	575634-U	575633-U
Discovery DSC-MANX (C8/SAX)	Inquire	Inquire	Inquire
Discovery DSC-SAX	575620-U	575619-U	575618-U
Discovery DSC-NH ₂	575617-U	575616-U	575615-U
Supelclean PSA	Inquire	Inquire	Inquire
Supelclean LC-4 (wide pore)	Inquire	Inquire	Inquire

TRADEMARKS: Agilent - Agilent Technologies; Ascentis, CHROMASOLV, Discovery, Equity, SLB, Supelclean, Supelco - Sigma-Aldrich Co.; Carbowax - Union Carbide Chemical & Plastics Technology Corp.; CombiPAL - CTC Analytics; FocusLiner - SGE International Pty Ltd.; GERSTEL - Gerstel GmbH; Microseal - Merlin Instrument Company; PerkinElmer - PerkinElmer Corp.; Shimadzu - Shimadzu Corp.; SIR - Airgas; Thermo - Thermo Electron Corp.; Varian - Varian Associates Corp.; Waters - Waters Associates, Inc.

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