

Hands-free SPE method development in under two hours

US-based PhenoLogix is offering a new paradigm in SPE method development for CROs and diagnostic labs. Combining the Freedom EVO® SPE workstation with the Phenomenex Strata™-X 96-Well SPE Method Development Plates, this approach reduces development time from six to seven days to under two hours.



PhenoLogix, located in Torrance, California, is a division of global separation sciences company Phenomenex. Initially part of the parent company's R&D department, it has grown into a standalone analytical support service providing chromatography method development and optimization support to customers around the world. SPE method development is an integral part of LC-MS workflows for most CROs and diagnostic labs, but is traditionally extremely time consuming and error prone. Sean Orłowicz, PhenoLogix Manager, explained: "SPE method development is often the first and most laborious step for CROs performing studies on biological samples such as plasma or serum. It involves careful screening of various SPE sorbents, solvent concentrations and pH ranges – for both loading and elution – to ensure maximum recoveries and clean samples ready for LC-MS analysis. Thorough exploration of various conditions is crucial for the success of their client's studies, as well as ensuring maximum lifetime for expensive analytical columns and minimizing the time and money spent on maintenance of mass spectrometers. Unfortunately, this process usually takes around six or seven days to complete, and so any reduction in the SPE method development time would offer savings in operational costs and reduce turnaround times."

To help address these issues, Phenomenex has developed the Strata-X 96-Well SPE Method Development Plate (MD Plate). Packed with four unique polymer-based SPE sorbents – reversed phase, mixed-mode strong cation exchanger, mixed-mode weak cation exchanger and mixed-mode weak anion exchanger – the MD Plate can be combined with the Freedom EVO SPE workstation to provide a faster, more structured approach to screening. Sean continued: "Using the four different Strata-X sorbents, we have developed a walkaway protocol to screen acidic, basic and neutral conditions for each step of the SPE process (load, wash and elution). This allows customers to determine the best conditions for retention of the analyte of interest in a single hands-free SPE experiment lasting under two hours. Processing the MD plate is completely automated, requiring no analyst intervention, with all pipetting and vacuum steps controlled through the platform's Freedom EVOware® software. We typically pipette volumes of between 50 and 750 µl, and the platform's reproducibility in this range is excellent – in the region of 2-3 %. The flexibility of the Freedom EVO SPE workstation also means that, once the most appropriate sorbent has been identified, you can quickly and easily switch to high throughput processing without reconfiguring the workdeck, which is a real benefit for our customers."

"We chose four structurally diverse analytes to test our assay set-up – amitriptyline and metoprolol (both basic), diclofenac (acidic), and

prednisone (neutral) – allowing us to identify the optimal sorbents and load/wash conditions for good recovery of all four analytes (Table 1). To reduce the background as much as possible – maximizing column life – we used an aggressive 70 % organic wash, however this caused some issues with the moderately polar prednisone (logP = 2.07), leading to some loss of the drug during washing. We are confident that, by decreasing the organic content of the wash, the analyte recovery can be improved further."

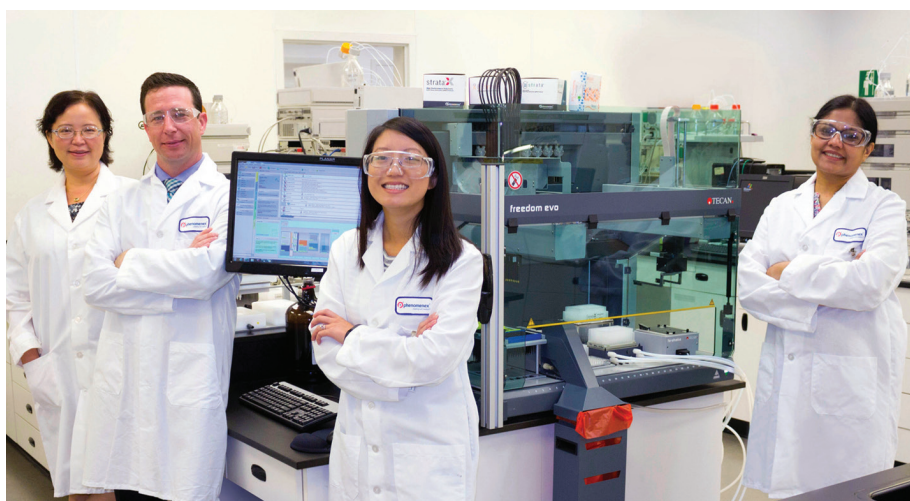
"As a method development lab, the Freedom EVO's versatility is another major benefit to us. We implement different products on a regular basis – switching between simple serial dilutions, protein precipitations, and liquid and solid phase extraction protocols – and it is very easy to reconfigure the workdeck for new applications. Programming the instrument through Freedom EVOware is straightforward – it's very easy to write new scripts or edit existing ones – and the TouchTools™ graphical user interface is self-explanatory for selecting, setting up and starting existing applications."

"To date, our primary focus has been on biological fluids – plasma, serum and urine – as well as some work with tissue samples. Moving forward, we see the potential to develop environmental, waste water and food applications, and there is considerable demand for whole blood applications for forensic toxicology. We also plan to develop applications in tube format, particularly for clinical diagnostics and clinical toxicology,

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implementing positive pressure SPE on the Freedom EVO workstation. This will offer customers even more versatility, allowing them to choose between the 96-well format for maximum throughput and tube-based assays for lower sample numbers.”

“Overall, our automated approach offers CROs and diagnostic laboratories a rapid and systematic way of performing SPE method development. Working together with Tecan to demonstrate the potential gains in productivity and turnaround times – while reducing operational costs – has been a pleasure, creating the ideal basis for future collaborations,” Sean concluded.



The PhenoLogix team

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To learn more about PhenoLogix, visit www.phenomenex.com/home/phenologix

1) Phenomenex TN-0078. Automated SPE Method Development Using Strata™-X 96-Well SPE Method Development Plates In Conjunction with a Tecan Freedom EVO Liquid Handling System. Shahana Wahab Huq, Sean Orłowicz, and Matthew Brusius. Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA.

Analyte	Sorbent/ conditions	Recovery (%)	Reproducibility (%CV)
Amitriptyline	Strata-X-CW Loading – basic pH Elution – acidic pH	98.8	3.2
Metoprolol	Strata-X-CW Loading – basic pH Elution – acidic pH	98.5	9.6
Diclofenac	Strata-X-AW Loading – acidic pH Elution – basic pH	72	6.7
Prednisone	Strata-X Loading – basic pH Elution – acidic pH	50	1.8

Table 1: Example analyte recovery rates for four diverse drug analytes using polymer-based Strata-X sorbents (Strata-X, reversed phase; Strata-X-CW, mixed-mode weak cation exchanger; Strata-X-AW, mixed-mode weak anion exchanger).¹