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# Solid Phase Extraction for HPLC-MS/MS Clinical Analysis: Finding a Needle in a Haystack

**Jennifer Knaack***

*Department of Pharmaceutical Sciences, College of Pharmacy & Health Sciences, Mercer University, 3001 Mercer University Drive, Atlanta, GA 30341, USA*

Easily obtained clinical specimens, such as blood or urine, are vital components of clinical analysis the can be used for pharmacokinetic studies, chemical and biological exposure studies, and diagnostics of disease states. Methods for clinical analysis of biological specimens benefit greatly from high performance liquid chromatography and tandem mass spectrometry (HPLC-MS/MS) that, together, provide a high degree of sensitivity and specificity that are rarely matched by other detection techniques. Unfortunately, complex matrices, including blood and urine, contain many contaminants that are incompatible with HPLC-MS/MS like high levels of salts or proteins. Additionally, finding a specific analytical target in such a complex matrix can be like finding the proverbial needle in a haystack. For these matrices, sample clean up procedures are highly recommended to removed interferents and concentrate analytical targets for improved sensitivity. Although some analytical methods suggest "dilute and shoot" methodology, where specimens are simply diluted prior to HPLC-MS/MS analysis, this practice does not remove salts or proteins which can alter chromatography, plug HPLC columns, and cause contamination or clogging of the MS source and quadrupoles. Solid phase extraction (SPE) is an ideal technique for removing these interferents while specifically extracting the analytes of interest and allowing for concentration of the analytical targets and is suggested for chemical analysis of clinical specimens.

Commonly associated with extractions from environmental samples, SPE takes advantage of the chemical characteristics associated with specific analytes to preferentially isolate these analytes in a controlled fashion. In a typical SPE method, a sample is passed over a syringe cartridge containing a specific media, or sorbent, which is chosen to compliment the chemical attributes of the target analyte. The target analyte binds to the sorbent while other matrix components are eluted. The sorbent can then be washed to remove other potential interfering compounds before eluting the target analyte using appropriate solvents. Additionally, the target analyte can be concentrated by evaporation of the elution solvent. Sorbents are traditionally available in three classes: reversed-phase, normal phase, and ion exchange. However, new "multi-mode" sorbents containing combinations of two or three sorbent types are now being produced and can be used to extract multiple target analytes in a single extraction. A thoughtful study of the target analyte should be performed prior to choosing an appropriate SPE sorbent and should result in more successful extractions.

SPE has many advantages over other traditional sample clean up procedures. "Dilute and shoot" is by far the simplest and most direct of all sample preparation methods and requires only simple dilution of the sample with solvent or buffer before direct injection into the HPLC-MS/MS system. While this method has proven useful for some specific analytes, it generally results in high levels of background interferences and high salt or protein concentrations that can surpass the capacity of an HPLC column or overwhelm a MS source or detector. Furthermore, clinical analyses often require high throughput formatting for processing hundreds or thousands of specimens in a day. Without appropriate sample clean up, frequent HPLC column changes and MS source cleaning is necessary which make high throughput analysis difficult. Liquid-liquid extractions are also commonly used for clinical sample preparation and require that the sample be mixed with an immiscible organic solvent under conditions in which the target analyte will extract into the organic solvent. After allowing the two phases to settle, the organic phase containing the target analyte is collected. The extraction is typically repeated to ensure maximal recovery of the target analyte. While liquid-liquid extractions can provide excellent isolation of target analytes when carefully planned, they are not usually amenable to high throughput formatting and tend to generate large amounts of organic solvent waste. A third technique, affinity extraction, provides the most specific extraction of target analytes based on specific recognition of the analytes by antibodies, molecularly imprinted polymers, or similar affinity compounds. In some cases, affinity extraction can be used to extract entire classes of analytes. However, antibodies and other recognition compounds are not commercially available for all compounds and these extractions often require custom development. In contrast to these extraction techniques, SPE is flexible and can be tailored to most analytical targets, is amenable to high throughput formatting, and a variety of sorbents are available requiring little, if any, custom production.

Innovative technologies are routinely being developed for SPE sorbents allowing for more specific and flexible extractions. Of the newest technologies, mixed-mode sorbents are one of the more successful. These sorbents can contain mixtures of hydrophobic and ionic characteristics to allow specific separation of multiple analytes in a single extraction. For example, acidic drugs can be extracted from biological fluids using a sorbent containing both non-polar and strong anion exchange chemistries. Using this type of mixed mode sorbent, only analytes with non-polar and acidic characteristics can be retained. In contrast, mixed mode sorbents containing non-polar and strong cation exchange chemistries are ideal for extracting basic drugs. For further reading, Fontanals et al. provide a comprehensive review of multi-mode SPE (TrAC-Trend. Anal. Chem. 29 (2010), pp. 765–779).

It should be noted that, for proper analysis, the type of sorbent used during solid phase extraction should differ from the chromatography method used during HPLC separation. For example, a method relying

***Corresponding author:** Jennifer Knaack, Department of Pharmaceutical Sciences, College of Pharmacy & Health Sciences, Mercer University, 3001 Mercer University Drive, Atlanta, GA 30341, USA, Tel: (+1) 678-547-6737; E-mail: Knaack_JS@Mercer.Edu

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on a C-18 reversed-phase sorbent for sample extraction should utilize a different type of reversed-phase chromatography, such as phenyl- or cyano-based, for separation. By using two different separation techniques, better specificity and removal of interfering compounds can be obtained ultimately resulting in rugged, specific, and sensitive analytical methods.

HPLC-MS/MS is an excellent tool for the detection and quantification of drugs, metabolites, and biomarkers of disease from common aqueous clinical specimens. These techniques benefit greatly from specific sample preparation. SPE is flexible for various analyte chemistries, amenable to high throughput formatting, and is an excellent method for extracting target analytes from specimens. When performed correctly, SPE can effectively remove salts, proteins, lipids, and other interferents from aqueous specimens leaving a relatively clean sample containing concentrated analyte that can be easily analyzed by HPLC-MS/MS.