Solid-Phase Extraction/Pre-Concentration Strategies for Drug Analysis

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Despite the fact that strong routine separation methodologies can give reliable specificity and validity at usual working pharmaceutical concentrations, they may fail at very low concentration levels. This poses considerable challenges for researchers investigating product purity and therapeutic drug monitoring. Sensitivity enhancement procedures are thus required to maximize the performance of separation techniques. Solid-phase extraction/solid-phase enrichment (SPE/SPEn) and pre-, post-, and in-column derivatization, as well as the use of sensitive detection devices, are the simplest strategies for improving sensitivity of separation-based analytical techniques. Large-volume injection of samples with online SPE/SPEn coupled with separation techniques increased sensitivity and improved detection as well as quantification limits without affecting peak shape and system performance.

separation-based analytical techniques large-volume injection solid-phase enrichment

1. Introduction

The development of a sufficiently sensitive procedure ensures the technology's viability for its intended purpose. Despite many advances in chromatographic technologies such as liquid chromatography (LC), gas chromatography (GC), and capillary electrophoresis (CE), sensitivity concerns continue to pose significant difficulties for compounds with poor detection limits. Highly sensitive analytical separation-based techniques play important roles in contamination assessments, environmental analysis, and therapeutic drug monitoring (TDM) [1][2]. In recent years, there has been a significant increase in the participation of researchers in the sensitivity improvement of quantitative techniques. In this area, a wide range of approaches has been described.

The analysis of pharmaceuticals in bio-fluids possesses numerous analytical challenges. The medications in a complex matrix such as serum or plasma are frequently bound to the protein's contents or are present as free forms in different ratios. Because it can be used to separate and isolate medications and their metabolites from biological fluids, high-performance liquid chromatography (HPLC) has emerged as a very helpful technology. Reversed-phase (RP) HPLC is widely used to separate various pharmaceuticals [3][4][5][6][7][8][9][10][11][12]; however, serum and plasma samples cannot be directly injected onto RP columns due to the presence of high-molecular-mass proteins. Biomolecules such as proteins may have many affinity mechanisms by which they are adsorbed onto surfaces they encounter because of their complex chemistry and amphoteric nature [13]. Each protein molecule generally contains a large number of hydrophobic moieties, as well as multiple positive and negative charges. Hence, there may be a variety of points of interaction that cause protein to adhere to surfaces. The

complexity of such macromolecules can lead to non-specific adhesion to surfaces. This can have an adverse effect on the chromatographic analysis in addition to carryover and instrument problems. As proteins can accumulate and plug the columns and instruments, they could make separation by HPLC troublesome. Thus, the LC system will certainly be affected after direct application of protein-rich complex matrices such as serum and plasma. For instance, the porosity of the columns will decrease if a lot of proteinaceous materials are absorbed onto their surfaces, resulting in increasing backpressure. The quick rise in pressure at the column's head is brought on by protein denaturation and precipitation. The high concentration of organic solvents in the mobile phases used to elute the tested analytes from the RP columns, particularly the ODS columns, causes protein denaturation and precipitation. Moreover, the analyte's distribution between the solid and mobile phases may be affected, resulting in alteration of the capacity factor. Thus, sample preparation is an essential component of analytical technique development, regardless of whether the analysis is for pharmaceutical, bio-analytical, environmental, or other applications. The fundamental purpose of sample preparation is to eliminate or reduce the impact of interferences caused by the many matrix components in complicated samples. Moreover, sample preparation can be utilized to simplify other elements of analysis, such as pre-concentration or derivatization, in order to improve sensitivity or selectivity.

In many circumstances, sample preparation consumes the most time and resources throughout the analytical process. Generally, analysts spend almost two-thirds of their time performing the sample preparation steps during the entire procedure [14]. Additionally, the offline sample preparation approach is anticipated to cause more than one-third of the analytical errors [15]. Conventional sample work-up processes in bio-analysis frequently entail a variety of pretreatment stages to purify the protein-rich samples before they are loaded into the LC, but three of the most frequent are solid-phase extraction (SPE) [3][4], liquid–liquid extraction (LLE) [16][17][18], and protein precipitation [19].

Despite their success, classical methods are generally regarded as having limited selectivity and/or poor recovery, as well as being more expensive for target analyte measurement. The equipment costs and development times of such approaches are only justifiable in situations where large sample throughput over an extended period of time is anticipated, despite the fact that using robotics can enable sophisticated sample preparation to be carried out with great precision and minimal labor cost. Thus, there is a huge need for simplified analytical procedures because the sample throughput in bio-analysis, for instance, in the TDM and pharmaceutical industry, has increased significantly. Therefore, developing new sample preparation procedures to accommodate the various types of samples and conditions encountered during analysis is a research field that requires continuing development.

2. Solid-Phase Extraction/Pre-Concentration Strategies for Drug Analysis

Because of the lower concentration levels of pharmaceuticals and the high levels of interferences present in bio-fluid samples, sample clean-up and enrichment processes are critical prior to chromatographic analysis to optimize technique sensitivity, recovery, and accuracy. It is difficult to justify extraction procedures that employ large amounts of hazardous organic solvents in the sample preparation steps in an era when it is recommended to

implement green chemistry principles in analytical laboratories. Of the different purification methods, SPE is a quick, low-cost, and extensively applicable technology. Furthermore, it is broadly applicable for the enrichment of pharmaceuticals and extraction of biological interferences with high removal effectiveness. SPE is seen as a good substitute for LLE because it moves past many of the shortcomings of LLE [16][17][18]. Moreover, the entire procedure can be automated. Besides that, SPE does not require phase separation, as LLE does, which eliminates errors related to inaccurately estimated extract volumes, one of the primary reasons for errors observed in the analysis of extracts obtained by LLE. Thus, major efforts have been made to design and evaluate innovative formats and efficient sorbent materials in order to improve their selectivity, specificity, and sorption capacity towards target analytes and enhance physicochemical or mechanical stability, among other SPE-related properties. Solventless sample preparation approaches based on analyte extraction and enrichment by online SPE, using phosphate buffers as washing solvents, have been proven to be viable and environmentally friendly alternatives to conventional solvent extraction techniques [20][21]. Fluconazole in serum has been directly quantified by trapping on a pre-column and subsequently separating on an analytical column, utilizing an elution mode by online SPE and HPLC methods (**Figure 1**) [21]. Because of its simplicity of use, flexibility, rapid extraction time, safety, minimal organic solvent consumption, and high enrichment factor, solid-phase microextraction (SPME) is a promising sample pretreatment approach [22]. To boost sensitivity in separation techniques, the large-volume injection approach and online sample pre-concentration have been widely employed. Highly sensitive and selective HPLC methodologies with less costly fluorescence (FL) detection systems are also required. Thus, the derivatization technique is necessary to enable sensitivity enhancement by converting non- or weakly native fluorescent compounds into highly fluorescence derivatives. The combination of pre- or post-column derivatization procedures with the chromatographic systems, as well as FL detection, made it possible for determining different medications at low concentration levels.

As stated, automation and miniaturization are two critical factors in developing greener derivatization techniques. When compared to conventional techniques, the amounts of chemicals needed and wastes generated are reduced. On-column/in-capillary [23][24] derivatization with HPLC and CE are two greener derivatization approaches. Because derivatization takes place during the separation process, these methodologies are superior to the most common pre-column and pre-capillary offline modes of derivatization, in that the sample and derivatizing agent consumption is low, and full automation arises without the need for additional equipment.

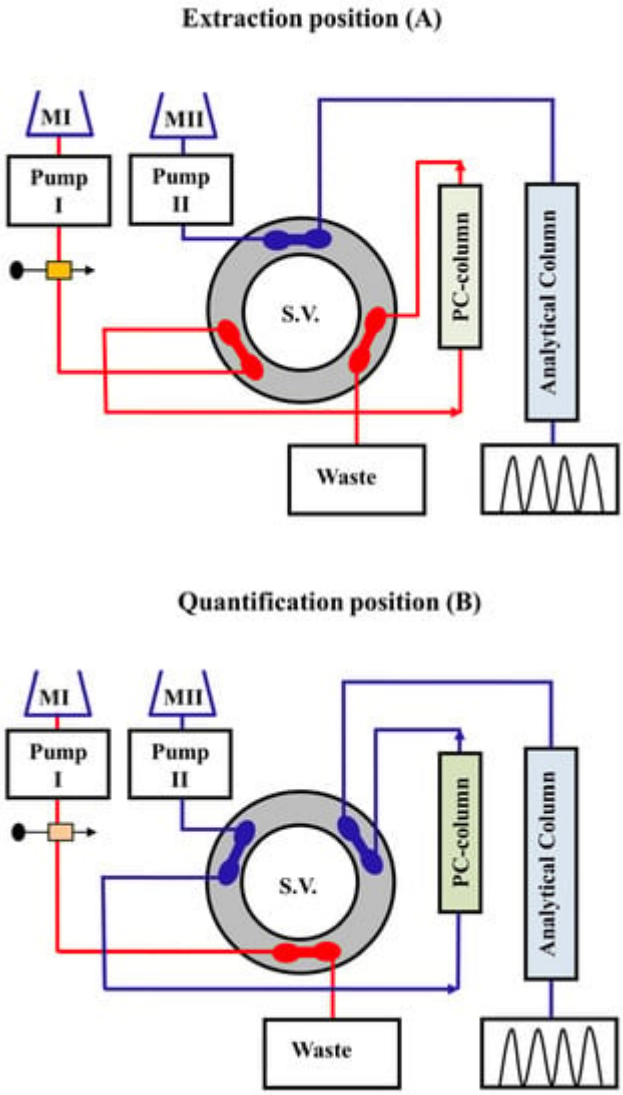

Figure 1. Manifold of on-through elution HPLC methodology for measuring fluconazole directly from serum samples: position (**A**) depicts the system set up for loading, washing, trapping, and pre-concentration of the analyte; position (**B**) shows the quantification step, ready for separation and measurement. HPLC circulation (separation and quantification) is segregated from the extraction position. A 6-port high pressure switching valve (S.V.) connects the PC column to an analytical column.

2.1. Offline Solid-Phase Extraction/Enrichment

Advanced sorbent technologies reorient SPE materials with various functionalities according to their structures, such as RP, normal-phase (NP), cation exchange (CEx), anion exchange (AEx), and mixed-mode types. Offline SPE in conjunction with the RP-HPLC approach for highly sensitive determination of ciprofloxacin, acetaminophen, caffeine, benzophenone, and irgasan in aquatic environments has been reported [25]. The SPE was conducted prior to the analysis using an RP-C18 cartridge to pre-concentrate the tested analytes from the ecological water samples. Dawson et al. reported a simple and reliable assay for nicotine and its main metabolite, cotinine, in plasma [26]. On the silica columns, an extraction/pre-concentration approach compatible with RP-HPLC separation was devised followed by quantification on ODS column using UV detection. Torre et al. simplified the SPE process

for determining risperidone and 9-hydroxyrisperidone in human plasma using polymeric RP sorbents [27]. The SPE-HPLC approach for assessing melamine in liquid milk has been reported to meet the detection demand for melamine-contaminated milk [28]. The developed method was validated using LC tandem mass spectrometry (LC-MS/MS) and has been successfully applied for routine melamine measurement in a variety of milk samples. Moreover, the CEx resin column is utilized for separation and pre-concentration purposes in LC-MS to analyze melamine in egg samples [29]. He et al. and Wang et al. described rapid and efficient SPE procedures followed by HPLC-UV methods for the determination of melamine in aqueous and milk formula samples, respectively [30][31].

2.2. Offline Solid-Phase Microextraction

The application of the twelve green chemistry principles to laboratory practice surely fueled the search for innovative methodological approaches to guarantee an improvement in results quality while enhancing environmental friendliness. Since the concept of SPE of target analytes was developed, substantial advancements in this technology have been noted, including the original concept's simplification, automation, and miniaturization. The method put forth in 1951 by Braus and colleagues, which was based on the insertion of up to 1.2–1.5 kg of granular activated carbon into an iron cylinder, is fundamentally different from the SPE formats currently employed in laboratory practice [32]. Because of undeniable advances in both the adsorption process and the large-scale production of new classes of materials, practical solutions for achieving high rates of recovery and enrichment while using significantly less sorbents and organic solvents have become possible for trapping various types of analytes. SPME is presently gaining popularity in a variety of fields of investigation, including dietary, biological, and pharmaceutical products [33]. SPME provides several advantages, including ease of use, low cost, compatibility with analytical systems, automation, and a solventless extraction procedure. In recent years, SPME has been employed prior to LC and CE, in addition to its application with GC. Enrichments of pharmaceuticals from various samples with complex matrices make it necessary to produce unique SPME fiber coatings such as metal organic frameworks, covalent organic frameworks, carbon, polymer, ionic liquids, metal/metal oxide nanoparticles, and silica [34][35].

The majority of pyrethroid metabolites are extracted from urine in the literatures using SPE or LLE, before being subjected to GC-MS or LC-MS [36][37][38]. As a pretreatment procedure to meet the demands for rapid and environmentally friendly extraction protocols, SPME is becoming more important. There have been various SPME methods reported in the literature [39][40], but they are not sensitive enough to measure the trace concentrations of pyrethroid metabolites that are important for evaluating environmental exposure. For the analysis of pyrethroid metabolites in urine samples, a green analytical method by a packed sorbent coupled to large-volume injection and GC-MS has been developed using an offline SPME technique [41]. With the aid of direct MS technology, quantification of cocaine, methamphetamine, 3,4-Methyl-enedioxy-methamphetamine, and lysergic acid diethylamide from oral fluid and urine samples has been conducted using offline SPME [42]. Quinine, naproxen, haloperidol, ciprofloxacin, and paclitaxel have all been extracted using multiple SPME [43]. After SPME, desorption was carried out offline, each drug was then analyzed using HPLC with UV or FL detection. Cantú et al. reported an offline SPME technique for analyzing anticonvulsants and tricyclic antidepressants in human plasma for TDM purposes by HPLC-UV [44]. For measuring various medicines in several matrices using offline SPE, different

separation-based analytical approaches have been proposed [45][46][47][48][49][50][51][52][53][54][55][56][57][58]. **Table 1** lists the LODs, LOQs, sample matrices, and offline extraction and separation processes as well as the detection systems used for the analysis of various pharmaceutics.

Table 1. Applications of offline SPEn approach coupled with different separation techniques for measuring various drugs in various matrices.

Analyte	Sample Matrix	Separation Technique	Detection System	LOD	LOQ	Ref.
- Ciprofloxacin - Acetaminophen - Caffeine - Benzophenone - Irgasan	Water	HPLC	UV-Vis	0.50 ppm 0.09 ppm 0.09 ppm 1.48 ppm 0.65 ppm	1.69 ppm 0.32 ppm 0.32 ppm 4.96 ppm 2.19 ppm	[25]
- Nicotine - Cotinine	Plasma	HPLC	UV-Vis	-	1.25 ng/mL 1.75 ng/mL	[26]
- Risperidone - 9-Hydroxyrisperidone	Plasma	HPLC	UV-Vis	1 ng/mL 1 ng/mL	2 ng/mL 2 ng/mL	[27]
- Melamine	Milk	HPLC	DAD	18 lg/kg	60 lg/kg	[28]
- Melamine	Water	HPLC	UV-Vis	0.1 ng/mL	0.5 ng/mL	[30]
- Melamine	Milk formula	HPLC	UV-Vis	0.01 µg/mL	0.033 µg/mL	[31]
- Cypermethrin - Deltamethrin - Permethrin	Urine	HPLC	MS/MS	0.015 ng/mL 0.015 ng/mL 0.015 ng/mL	0.025 ng/mL 0.025 ng/mL 0.020 ng/mL	[37]

Analyte	Sample Matrix	Separation Technique	Detection System	LOD	LOQ	Ref.
- Cyfluthrin				0.015 ng/mL	0.030 ng/mL	
- Quinine - Naproxen - Haloperidol - Ciprofloxacin - Paclitaxel	Urine	HPLC	Fluorescence	0.01 µg/mL 0.02 µg/mL 0.1 µg/mL 0.001 µg/mL 0.05 µg/mL	1 µg/mL 0.05 µg/mL 0.3 µg/mL 0.003 µg/mL 0.25 µg/mL	[43]
- Amitriptyline - Imipramine - Nnortriptyline - Desipramine - Carbamazepine - Phenobarbital - Phenytoin - Primidone	Plasma	HPLC	UV-Vis	-	75 ng/mL 75 ng/mL 75 ng/mL 75 ng/mL 5 ng/mL 6 ng/mL 5 ng/mL 75 ng/mL	[44]
- Clenbuterol	Urine	UHPLC	MS/MS	0.0125 ng/mL	0.1 ng/mL	[45]
- Andrographolide - Neoandrograholide -	Plasma	UPLC	MS/MS	0.04 µg/mL 01 µg/mL 0.02 µg/mL	0.15 ug/mL 0.32 ug/mL 0.06 ug/mL	[46]

Analyte	Sample Matrix	Separation Technique	Detection System	LOD	LOQ	Ref.
14-Deoxy-11,12-didehydroandrographolide						
- Valsartan - Hydrochlorothiazide	Plasma	HPLC	MS/MS	-	50.2 ng/mL 1.25 ng/mL	[47]
- Pantoprazole	Wastewater	UPLC	MS/MS	0.02 ng/mL	0.05 ng/mL	[48]
- Amisulpride	Saliva	UPLC	DAD	3 ng/mL	5 ng/mL	[49]
- Ciprofloxacin - Sparfloxacin - Moxifloxacin - Gatifloxacin	Wastewater	CE	UV-Vis	3 µg/mL 3 µg/mL 3 µg/mL 3 µg/mL	5 µg/mL 5 µg/mL 5 µg/mL 5 µg/mL	[50]
- Propionic acid - Butyric acid	Chicken feces	HPLC	DAD	0.14 mg/mL 0.14 mg/mL	0.45 mg/mL 0.43 mg/mL	[51]
- Amphetamine - Methamphetamine - 3,4-Methylenedioxymethamphetamine	Urine	CE	MS/MS	1 ng/mL 1 ng/mL 0.60 ng/mL	5 ng/mL 8 ng/mL 2 ng/mL	[52]
- Dimetridazole - Metronidazole - Secnidazole	Water	CE	DAD	0.016 µg/mL 0.040 µg/mL 0.097 µg/mL 0.037 µg/mL	0.05 µg/mL 0.14 µg/mL 0.33 µg/mL 0.13 µg/mL	[53]

Analyte	Sample Matrix	Separation Technique	Detection System	LOD	LOQ	Ref.
- Ornidazole - Tinidazole				0.037 µg/mL	0.13 µg/mL	
- Glyphosate - 3-(methylphosphinico) propionic acid - Glufosinate - Aminomethylphosphonic acid	Tea infusions	CE	Conductivity	0.80 ng/mL 0.56 ng/mL 1.56 ng/mL 0.54 ng/mL	2.68 ng/mL 1.87 ng/mL 5.19 ng/mL 1.82 ng/mL	[54]
- Tetracycline - Chlortetracycline - Oxytetracycline	Milk	CE	UV-Vis	19.93 ng/mL 23.83 ng/mL 18.60 ng/mL	59.79 ng/mL 71.49 ng/mL 55.8 ng/mL	[55]
- Sulfadimidine - Sulfathiazole - Sulfadiazine - Sulfachloropyridazine - Sulfamethoxazole - Sulfacetamide - Phthalylsulfathiazole - Succinylsulfathiazole	Milk	CE	UV-Vis	0.16 µg/mL 0.04 µg/mL 0.03 µg/mL 0.10 µg/mL 0.07 µg/mL 0.03 µg/mL 0.20 µg/mL 0.07 µg/mL	0.3 µg/mL 0.3 µg/mL 0.3 µg/mL 0.3 µg/mL 0.3 µg/mL 0.3 µg/mL 0.3 µg/mL 0.3 µg/mL	[56]
- Captopril	Plasma	GC	MS/MS	0.5 ng/mL	1 ng/mL	[57]

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Analyte	Sample Matrix	Separation Technique	Detection System	LOD	LOQ	Ref.
- Histimine	Foods	CE	UV-Vis	0.087 ng/mL	0.29 ng/mL	[58]

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