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Review

On-line solid phase extraction–liquid chromatography, with emphasis on modern bioanalysis and miniaturized systems

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ABSTRACT

On-line solid phase extraction (SPE)–liquid chromatography (LC) allows for automated, sensitive, precise and selective bioanalysis. It is a common feature in miniaturized- or nano LC systems, which are well suited for applications requiring high sensitivity and/or treatment of limited samples (laser micro-dissection samples, rare cancer stem cells, etc.). Traditionally, particles with reversed phase (RP) functional groups are used for the columns in SPE–LC systems. There is however an expanding diversity in SPE–LC combinations applied to meet today's bioanalytical challenges. Current online SPE–LC combinations employ, e.g. porous graphitic carbon (PGC) and hydrophilic interaction liquid chromatography (HILIC) materials for metabolomics and glycomics, restricted access media (RAM) columns coupled with nano LC for peptidomics, immunoaffinity trap columns for targeted proteomics and metal oxide affinity phases for phosphopeptide analysis. However, issues can arise when combining different phases in on-line SPE–LC, e.g. due to solvent incompatibilities between enrichment/separation principles and sample solvent requirements. Consequences can be low recovery and poor resolution, or need for additional instrumentation. On-line SPE–LC with very narrow columns (10–20 μm inner diameters) can be appropriate to obtain maximum sensitivity and information. In such highly miniaturized systems, non-particulate columns are arguably more suited (e.g. monolithic or porous layer open tubular (PLOT) columns) as e.g. hardware contributions resulting in extra column volumes are reduced. Basic SPE–LC systems can be configured/modified to perform quite complex analytical operations, and certain columns, configurations and hardware can improve robustness.

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1. Introduction

There is an increasing need in bioanalysis for sensitive and selective methodology for dealing with complex samples or analytes. Examples may be discovering and monitoring low abundant protein biomarkers [1], characterizing biopharmaceuticals [2], or mapping a metabolome as function of a medical treatment [3]. Along with the complexity of such tasks, a relatively large number of samples must often be analyzed, calling for automated instrumentation with high precision. As a result, liquid chromatography coupled with mass spectrometry (LC–MS) is increasingly becoming the tool of choice, often outclassing traditional assays in terms of selectivity and data output. Prior to LC–MS, analytes must often be enriched and isolated selectively from complex matrices. Also, samples of interest may be limited in availability. For such purposes, on-line solid phase extraction–liquid chromatography (on-line SPE–LC) is an effective approach. On-line SPE–LC is automatable, reduces manual preparation steps and lessons risk of human error. Additionally, it allows for microliter-scale injections in capillary- and nano LC, dramatically improving sensitivity [4]. On-line SPE–LC has been around for decades [5–7], and has primarily been used for drug and pollutant determination with non-miniaturized systems (see earlier reviews, e.g. [8,9,6,10]). However, online SPE–LC has grown in commercial availability and use, due to proteomics-related research using nano LC–tandem mass spectrometry (MS/MS). Typically, on-line SPE–LC systems consist of a reversed phase (RP) SPE column and an RP LC column (RP SPE–RP LC), allowing trapping and separation of relatively hydrophobic analytes. On the other hand, RP based SPE–LC is neither particularly selective nor applicable for all compounds (e.g. highly polar endogenous metabolites). Alternative chromatographic principles/phases may however be employed in SPE–LC to obtain the desired selectivity (for example, HILIC, PGC or MIPs).

In this review, we describe and discuss on-line SPE–LC combinations with emphasis on current bioanalytical challenges, including metabolomics, comprehensive- and targeted proteomics, peptidomics, glycomics and lipidomics. Although parts of the discussion can be related or relevant to e.g. comprehensive multidimensional chromatography [11,12], we focus here on "trap and separate" systems. Emphasis will be on capillary and nano LC systems, as bioanalysis is continuously moving towards miniaturization. Additionally, we discuss the use of using monolithic and porous layer open tubular (PLOT) columns in on-line SPE–LC systems, as these are arguably most suited for further downscaling (20 μm i.d. columns and less).

Pitfalls that can arise when combining two enrichment/separation principles will be discussed; these are often related to phase-to-phase mismatches or sample solution-trapping column incompatibility.

As on-line systems are arguably less used in many routine applications due to e.g. clogging, a presentation of on-line SPE–LC systems with enhanced robustness is included.

2. "BASIC" on-line SPE–LC systems for capillary/nano LC

Fig. 1 illustrates the two most common configurations in miniaturized online SPE–LC. For the most part, the systems described in this review are based on these configurations, sometimes with slight modifications or add-ons. The top figure shows a two-pump system; the loading pump transfers the injected sample on to the SPE column. Non-retained compounds are transferred to waste, while analytes are trapped. The analytical pump subsequently elutes the analytes to the LC column. This configuration is the basis for e.g. Agilent chip systems [13] as well as commercial "open" systems (see instrumentation in e.g. [14]). The bottom figure shows a one-pump system; the pump loads the sample on to the SPE, and non-retained compounds are transferred to waste (the path of least resistance). After loading, the waste position is closed and the solvent is forced to pass through the SPE to the LC column. A solvent gradient is applied, transferring the analytes from the SPE column to the LC column. The bottom configuration is also commercially available (see instrumentation in e.g. [15,16]). The bottom system is the simpler of the two (just one binary solvent pump). The top system is however more flexible, as it can incorporate two binary pumps. For both systems, a number of factors are of crucial importance for successful analysis. For example, analyte features (polarity, charge, functional groups), ability of stationary phase to

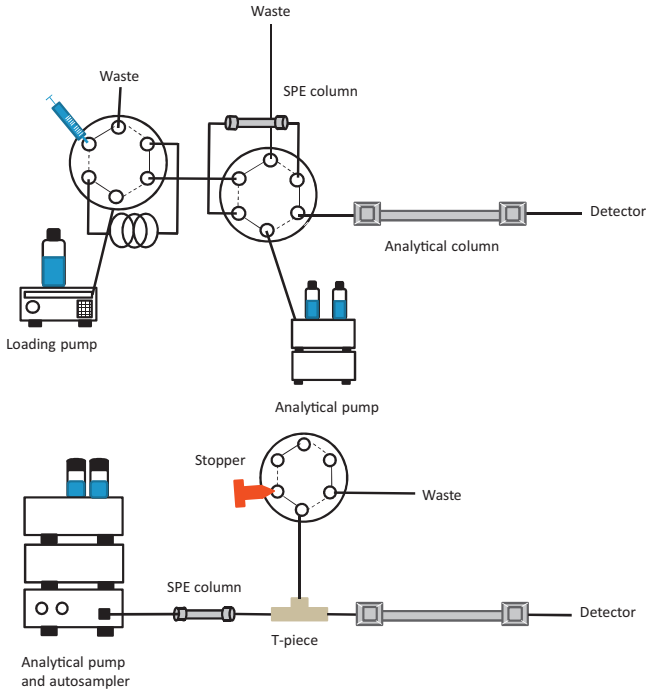

Fig. 1. Common on-line SPE–LC systems. Top: two pump system. Bottom: one pump system.