Trace analysis of environmental matrices by large-volume injection and liquid chromatography-mass spectrometry

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Abstract The time-honored convention of concentrating aqueous samples by solid-phase extraction (SPE) is being challenged by the increasingly wide spread use of large-volume injection (LVI) liquid chromatography-mass spectrometry (LC-MS) for the determination of traces of polar organic contaminants in environmental samples. Although different LVI approaches have been proposed over the last 40 years, the simplest and most popular way of performing LVI is known as single column LVI (SC-LVI), in which a large-volume of an aqueous sample is directly injected into an analytical column. For the purposes of this critical review, LVI is defined as an injected sample volume that is ≥ 10% of the void volume of the analytical column. Compared to other techniques, SC-LVI is easier to set up, as it only requires small hardware modifications to existing autosamplers and, thus, will represent the main focus of the current review. Although not new, SC-LVI is gaining acceptance and the approach is emerging as a technology that will render SPE nearly obsolete for many environmental applications. In this review, we discuss 1) the history and development of various forms of LVI, 2) the critical factors that one needs to consider when creating and optimizing SC-LVI methods and 3) example applications that demonstrate the range of environmental matrices, to which LVI is applicable such as drinking water, groundwater and surface water including seawater as well as wastewater. Furthermore, we give responses to answer a set of 'frequently asked questions' typically encountered from audiences and we indicate future directions and areas that need to be addressed to fully delineate the limits of SC-LVI.

Keywords Large-volume injection • Direct injection • Liquid chromatography • LC-MS/MS • Soil • Solid-phase extraction • Sample preparation • Water • Wastewater

Abbreviations

LC-MS/MS	Liquid chromatography-tandem mass spectrometry
CC-LVI	coupled column large-volume injection
SC-LVI	single column large-volume injection
LOQ	Limit of quantification
LVI	Large-volume injection
ON-E	On-line enrichment
MS	Mass spectrometer
UV-Vis	Ultraviolet-visible absorption
SPE	Solid-phase extraction

Introduction

It is a widely held belief among chemists, instrument manufacturers and companies that produce solid-phase extraction media that solid-phase extraction (SPE) is needed to extract analytes from aqueous samples, to reduce the complexity of the matrix and to increase analyte concentrations in the final extract. The authors of this critical review represent three generations of analytical environmental chemists, all of whom have performed SPE during their careers (e.g.[1-5]). We too have held the belief that SPE is a 'necessary evil' that protects our analytical columns and sensitive mass spectrometers. By one way or the other, in some cases by serendipity, we have reached the conclusion that for many applications, large-volume injection (LVI) is chemically redundant with SPE. As a result, we posit that LVI will render SPE obsolete as a sample preparation step, especially for aqueous environmental samples.

Analytical chemists, by their very nature, tend to be cautious especially when it comes to protecting their valuable instrumentation. The desire to protect analytical columns and mass spectrometers has created, in our view, a 'folklore' that sample pre-treatment by SPE is needed to keep analytical columns and mass spectrometers clean and fully operational. Current opinion indicates that if SPE were eliminated one would experience shorter column lifetimes and the need for more frequent instrument cleaning and maintenance. In addition, SPE is thought to reduce matrix effects and many argue that SPE is required to avoid negative impacts on signal-to-noise and sensitivity. Such perceived advantages are the rationale for including SPE, which has many costs both time and financial to laboratories. The costs start from purchasing the SPE media that typically are used once and discarded. In addition, there are costs associated with solvent usage and disposal. However, one of the largest costs is for the labor required to perform SPE. The time required to add and optimize a SPE pre-concentration step is substantial. Additionally, if performed on a stand-alone SPE apparatus or by on-line SPE instruments, one also incurs costs due to the equipment and the additional labor to optimize, operate and maintain SPE instrumentation. SPE typically is performed with sample volumes usually ranging from milliliters to liters and the costs associated with shipping samples of such volumes increase the costs of SPE further.
Besides costs, the multi-step nature of SPE, which includes media preparation, sample application, wash steps, elution, evaporation and reconstitution is laborious and results in

variable accuracy and precision. Artifacts from SPE media and support material are problematic for those analyzing for analytes associated with common laboratory materials such as PTFE (e.g., fluorochemicals) and hydrophobic analytes that are prone to losses (negative artifacts) [6]. As we will discuss, the chemical processes occurring during SPE and LVI are equivalent and, thus, no net advantage in terms of column and instrument performance or reduction in matrix effects are realized by performing SPE. LVI can appear to have pitfalls when LVI performed without a thorough understanding of how it works and which factors must be controlled. Methods are developed faster without SPE and have similar or even improved accuracy and precision due to the simple nature of the process with the least amount of materials and handling involved. In the end, SPE uses time and resources that could be allocated elsewhere, while it is not offering the perceived advantages held by so many researchers in academia and in the analytical industry. The objectives of this critical review are:

1. To describe the history and development of LVI,
2. To discuss the factors that one needs to consider when creating and optimizing LVI methods,
3. To provide examples of applications for environmental matrices including surface, ground, drinking and waste water as well as for vegetables and soil,
4. To offer responses to answer a set of 'frequently asked questions' typically encountered from audiences and
5. To propose future directions and areas that need to be addressed for fully explore the limits of LVI and how environmental analytical chemistry can be drastically improved by LVI.

With this critical review, we offer the chance to challenge analytical chemists to think outside the 'classical chromatography' box and to put their instruments to full use without the labor and costs of SPE.

The history of large-volume or direct-injection

In the late 1970s, Little and Fallick [7] reported what they called "new ways" of using refractometers and ultraviolet-visible (UV-Vis) detectors coupled to modular LC systems

consisting of pumps and hand-operated injectors for analysis of trace organic pollutants (Table 1). For the first time, chemists were using LC systems as enriching devices to concentrate trace organics in place of other pre-extraction/concentration/clean-up procedures that existed at that time (e.g., liquid-liquid extraction). The "on-line enrichment" (ON-E) technique consisted of pumping a sufficient quantity of a filtered aqueous sample (200 mL) through a C18 column using an off-line or standalone pump. A solvent of greater elutropic strength was delivered by a second pump to elute the analytes for UV-VIS detection. The authors also pointed out that in cases where concentrations were sufficiently high that "only 0.5 to perhaps 2 mL of sample and pumping that across the column is sufficient to concentrate enough organics for detection" [7]. To the best of our knowledge, this is among one of the first publications demonstrating the possibility of analyzing samples through LC-UV-VIS without the need of a pre-concentration and/or a clean-up step. Since the early 1970s, several scientists started to look deeply into this attractive option and consequently were able to produce a number of reports on LVI methods for the analyses of a variety of chemicals in different matrices.

The second approach that developed in the late 1970s and early 1980s utilized a single analytical column (SC-LVI) was developed and consisted of simply injecting a large-volume of sample into an analytical column. Two early examples include Gloor and Johnson [8] who described the direct injection of 250 µL of wastewater for determination of linear alkylbenzene sulfonate (LAS) by ion-pair LC with UV-VIS detection (Table 1). Kiso et al. [9] reported a method employing 5 mL sample volumes for the analysis of 15 pesticides listed in the Japanese guidelines for potable water down to 40 – 500 ng/L levels (Table 1). Up to this point, UV-VIS was the detector of choice since routine and quantitative detection by mass spectrometry had not yet been developed.

In the 1980s throughout the 1990s, pesticides along with other organic contaminants were a major environmental concern. The urgency of developing fast and reliable analytical methods able to match environmental regulation without or with minimal sample preparation became soon clear. In the early 1990s, Hogendoorn and co-authors, published a series of papers [10-14]dealing with the analysis of various pesticide residues in a range of environmental samples using a coupled columns large-volume injection (CC-LVI) technique. The columns switching and on-line clean up approach they developed consisted of 1) pre-separation of the sample on

a low efficiency column, 2) diverting the analyte-containing fraction into a second column and 3) final analysis of the sample fraction containing the analyte by LC-UV-VIS. For example, Hogendoorn et al. analyzed methyl isothiocyanate at 1,000 ng/L levels by injecting 770 µL of aqueous sample onto a low efficiency column and then diverting the analyte-containing fraction into second analytical column followed by UV detection [11]. The elutropic strength of water allowed injection of large sample volumes into the column and the analyte's capacity factors (k') influenced the optimal injection volume. By correctly timing the divert valve that was positioned between the low efficiency column and the analytical column, only the analyte-containing fraction was sent to the analytical column. Other examples of CC-LVI include the injection of 2 [15-17] to 4 mL [13] of groundwater, drinking water and surface water containing pesticides and achieved limits of detection in the range < 20 -1,000 ng/L (Table 1).

Between the 1990s and the 2000s, LCs coupled to mass spectrometers emerged but the capacity of the vacuum systems permitted only low mobile phase flow rates. Low LC flow rates (µL/min rather than mL/min) required reduced LC column diameters (0.25-0.5 mm I.D. rather than 2.1-4.6 mm I.D.) and low injection volumes (nL rather than mL) [18]. Therefore, with the emergence of the first LC-MS systems, the development and application of LVI methods took a step backward. Limits of detection were confined by the restricted injection volumes that were compatible with narrow-bore columns used at that time [18, 19]. With the advent of commercialized mass spectrometers fitted with multiple stage vacuum systems and with more efficient atmospheric pressure ionization (API) sources, higher LC flow rates, larger column diameters and larger injection volumes were made possible. An increasing number of publications have since appeared in the scientific literature that describe SC-LVI for use with mass spectrometry for the determination of pesticides in vegetables [20], water [21-29], and soil [30-33]; fluorochemicals in wastewater, groundwater and surface water [33-37]; neurotoxins in surface water, groundwater and drinking water [38, 39]; pharmaceuticals (legal and/or illicit) in surface, ground, and waste water [25, 33, 40-44]; corrosion inhibitors in surface, ground, and waste water [33, 45]; chelating agents in surface, drinking, and waste water [46]; iodinated chemicals in waste water and treated water [47-49]; artificial sweeteners in ground, waste, and treated water [50]; biocides in surface and waste water [51]; bisphenol A in soil [33]; steroids in waste water [52]; and surfactants in seawater (unpublished) (Table

2). The success and popularity of SC-LVI compared to ON-E and CC-LVI are most likely due to its simplicity.

SC-LVI: factors for creating and optimizing methods

The purpose of this section is to walk readers through SC-LVI. For the purposes of this review, our working definition of SC-LVI is applied to those applications involving the direct introduction of sample volumes that are ≥ 10% of the void volume of the analytical column used for separations (Table 2). While reporting the % of void volume injected is convenient for comparing disparate LVI applications, the % of void volume injected cannot be used to predict analyte retention or capacity on a given column because additional factors are important such as particle size, column composition and sample solvent matrix. SC-LVI differs from ON-E and CC-LVI because SC-LVI is performed without the use of off-line or on-line sample pre-concentration steps or equipment and does not require additional pumps, sorbents, or analytical columns. In this section, a view of the factors involved in creating and optimizing SC-LVI methods is offered. The order and function of each step involved in SC-LVI are discussed relative to the analogous steps used in treating the sorbent in SPE (Table 3).

Enrichment and analytical column conditioning step

The first step in SPE is to condition or 'wet' the SPE media using μL to mL of non-aqueous solvents to ensure reproducible retention and sample flow [53] (Table 3). The organic solvent also serves to reduce or eliminate sorbent impurities. The analogous situation in SC-LVI occurs only when a new enrichment/separation column is first installed and must be conditioned. Once the columns are conditioned and properly stored after use, no subsequent 'wetting' steps are needed since the column is not allowed to dry and the normal operation of the LC re-equilibrates the enrichment/separation column.

Sample loading

The goal is to concentrate analytes onto both SPE media and reverse-phase analytical columns during SC-LVI from aqueous samples or samples composed of a solvent/water mixture of lower elutropic strength relative to the mobile phase. Although most of the

applications listed in Table 2 involve the direct injection of aqueous samples, several indicate that extracts of vegetables [20] or soil [30-32] are analyzed by SC-LVI (Table 2). The final organic solvent-based extracts were diluted with water and the injected samples ranged in composition from 25% organic solvent (acetonitrile or methanol)/75% up to 70% organic solvent/30% water and then analyzed by SC-LVI. These examples indicate that SC-LVI can be used with samples that are not 100% aqueous and that analytes can be focused on the analytical column in the presence of organic solvent. In SC-LVI, sample loading where the sample volume is the mobile phase is equivalent to isocratic separations with a very elutropically-weak solvent (e.g., 100% aqueous). The injected sample volume and flow rate determine the duration of the isocratic loading conditions. For example, a 4.5 mL injection at 1 mL/min would be equivalent to an isocratic separation of about 4.5 min.

Sample volumes for SPE and SC-LVI are selected as a function of analyte concentration and detector sensitivity. For SPE applications, the sample volumes processed range from < 1 mL to 1,000 mL while volumes up to 5 mL are employed in SC-LVI (Table 2). In both cases, sample volumes should not exceed the breakthrough volume of analytes [11, 53-56]. In the case of SC-LVI, maximum injection volumes are determined by the injection assemblies consisting of syringe plungers and sample loops. Syringes plungers and sample loops are matched by volume in order to ensure that the sample withdrawn by the syringe can be accommodated by the sample loop. For example, applications that consist of injecting 100 µL can be accomplished with a 100 µL analytical head and sample loop without hardware modification.

For sample volumes that exceed the analytical head capacity (e.g., 100 µL), it is necessary to make simple hardware modifications. For some commercial LCs, analytical heads can be exchanged for larger-volume models. For example, [35, 36] replaced 100 µL syringes for 900 µL syringe and installed a 1,400 µL sample loop. They operated the LC in a ‘multi-draw’ mode, which allowed for multiple injections to give a total volume that exceeds the syringe and sample loop volumes. To achieve injection volumes of 1,800 µL, two cycles of 900 µL injections are performed [41]. Backe et al. used a 5,000 µL sample loop and five cycles of 900 µL to achieve an injection volume of 4,500 µL for anabolic steroids in water [52].

Flow rates

The application of high pressure for SC-LVI maintains flow rates despite the small particle size while offering increased efficiency compared to SPE. SPE systems typically have a smaller number of theoretical plates and therefore efficiency owing to the relatively large particle size and short column length when compared to analytical columns that have smaller particle sizes and greater lengths. The optimum flow rate range during the application of samples processed by SPE is narrow and typically 1-10 mL/min. This upper flow rate is dictated by the small pressure drop than can be maintained by vacuum over the typically short SPE column beds (e.g., 1 cm) and larger particle sizes (e.g., 40 μm) of SPE media [53]. One can directly compare the minutes required to transfer large-volume sample to the analytical column during SC-LVI to the time and equipment is takes to prepare an extract by SPE, which ranges from minutes to hours (Table 3). This shift in sample preparation time onto the LC and away from laboratory personnel results in costs saving because labor costs are much greater than those associated with running the LC for a few extra minutes.

Programmed flow rates can be used in SC-LVI to load large-volumes of samples into the enrichment/separation column quickly in order to reduce analysis time [52]. For example, Backe et al. transferred sample from the sample loops to the analytical column at 1 mL/min after which the flow rate was reduced to 0.5 mL/min to separate anabolic steroids in wastewater. It is important to note that if the analytes of interest do not have a sufficiently high capacity factor in the sample solvent, changes in relative retention and resolution can occur when using a high flow rate to load the sample and a slower flow rate for separation.

Dwell volume: chromatographic control and minimizing run times

At the point where the analytical head/needle and sample loops are loaded (chromatographic 'time zero'), the total sample volume is then pushed onto the enrichment/separation column by the mobile phase flow. Note that it takes time to transfer the sample from the large-volume of injector tubing onto the column and this time must be taken into account when designing the gradient program. The initial gradient conditions, whether it is 100% aqueous or a solvent/water combination, will not reach the analytical column until the total sample volume in the injector sample loops are transferred onto the enrichment/separation column.

Sample load time can be calculated by using the mobile phase flow rate, the volume of the capillary and tubing before the column and the volume of the sample in the needle loop before the sample capillary. For example, at a flow rate of 0.5 mL/min, the column loading time for a 1,800 μL sample should be (1,400 μL + 900 μL total injection volume)/500 μL/min injection rate= 4.6 min total injection time. This simple calculation may not fully account for the entire volume of the injection system, therefore further experiments are typically necessary to determine the exact loading time. A more empirical method for determining sample load times is to map the pressure isotherm for samples, whose composition has a significantly different viscosity than the mobile phase. For example, water has a lower elutropic strength and higher viscosity than mobile phases containing methanol or acetonitrile. As aqueous samples are loading on the column, fluctuations occur in pressure. After initiating an injection, the return in pressure to the initial starting pressure indicates that the aqueous sample has completely passed through the guard/analytical column. Another method to calculate sample loading times is to monitor the presence of an unretained (k` = 0) analyte, such as thiourea or acetone [41, 57]. The arrival time of unretained peaks takes into account the time it takes for the sample to pass from the column to the detector, which is negligible for a well designed system.

All of these methods assume that the analytes in the sample are not interacting (e.g., retained) with the materials in the injection assembly. If this is the case, additional mobile phase may need to be run through the injection assembly to quantitatively transfer all the analytes to the column after the sample loading phase [52] . Understanding the time it takes for large-volumes (e.g., mLs) of sample to completely transfer into an analytical column is important when creating gradient programs and to minimize run times. In autosamplers, the system dwell volume, which is the volume the mobile phase occupies after the point of mixing to the head of the analytical column [58], can be quite large compared to normal systems due to increased volume associated with sample loops (e.g., hundreds of μLs). Initial attempts to control SC-LVI for sample volumes of 900 and 1,800 μL were conducted without taking advantage of the mainpass/bypass valve that is present in the Agilent 1100 [41]. SC-LVI systems may appear to be unresponsive to changes in gradient settings unless the dwell time and volume of the injector is taken into account. Further, six-port injection valves that are positioned after the sample loops (e.g., seat capillary) and before the guard/analytical column, can be used to direct mobile phase flow around the injection assembly after the sample has

been loaded into the column in order to significantly reduce dwell volumes and analysis times. In 'mainpass mode', the mobile phase is routed through the entire network of injector tubing and this mode is used to transfer sample onto the analytical column. 'Bypass" mode is used after the sample is transferred to the column and is achieved by rotating the valve so that mobile phase goes directly to the analytical column, thus bypassing the injector.

Washing SC-LVI columns

In SPE, the sorbent is often washed with a solvent or mixture of solvents of elutropic strength less than that required to elute the analytes of interest [56]. Wash steps are performed by incorporating a high percent aqueous isocratic wash step to eliminate salts followed by a wash with sufficient percent organic so as to elute all components from the SPE sorbent without eluting the analytes of interest. In SC-LVI, wash steps are potentially important. It is our experience that successful SC-LVI must include a wash step. For example, without a wash step, Chiaia et al. observed relative standard deviations (RSDs) of ≥ 30% for illicit drugs in wastewater [41]. Once a one min wash (at 0.5 mL/min) consisting of 90 % (0.1 % acetic acid in 5% aqueous methanol/acetonitrile 90:10 (v/v) reduced RSDs to < 12%. Analysts actually have more control over the wash step in SC-LVI compared to SPE because the retention of the analytes is simultaneously monitored. In addition, matrix components that elute earlier and later than the analytes of interest are discarded to waste by use of a post-column divert valve, which will be discussed in more detail. Note that while wash steps can eliminate salts and their potential for causing matrix effects, wash steps cannot eliminate co-eluting matrix components during either SPE or SC-LVI.

Post-column divert valve

If a mass spectrometer is used as a detector for LVI analysis, the authors of this paper recommend the addition of a post-column valve. The purpose of the post-column valve is to divert early eluting matrix components, like salts and highly polar organic interferences away from the mass spectrometer to waste. This is especially important for mass spectrometers, whose interface spray is not orthogonal or off axis to the capillary inlet. Diverting unwanted sample components protects the mass spectrometer from non-volatile sample components that might otherwise clog capillaries or eventually build up on optics. A divert-valve also reduces

the amount of non-dissolved aerosol droplets sprayed directly or indirectly onto capillaries and orifices by diverting to waste the high aqueous fraction of the mobile phase gradient, which is more resistant to desolvation.

Eliminated and redundant steps in SPE

No analogous steps are needed in SC-LVI that correspond to the drying of SPE sorbent beds and extract concentration. The elution of SPE media is accomplished as part of SC-LVI whereas in SPE the sorbent media is eluted and then the analytes are eluted again from the analytical column, effectively repeating the same task twice when performing SPE followed by LC-MS analysis. In addition, typically only a small portion of the SPE extract generated is actually injected. Therefore, much of the time, materials and labor required to generate the SPE extract is wasted. By contrast, SC-LVI is more cost and time efficient since the entire sample is used.

In conventional SPE, a typical volume of water to extract by SPE is 100 mL. If 100% of a 50 ng/L solution were extracted and ended up in a 1 mL final extract, the concentration would be increased 100 fold. However, when only 10 μL is injected of the final extract, 0.05 ng is injected, which is only 1% of the original mass isolated by SPE. In contrast, if 1,800 μL the same 50 ng/L solution of analyte were directly injected, 0.09 ng is injected onto the column and is a greater amount of mass introduced than that by the SPE approach.

Practical aspects of LVI

A good starting point would be to check the maximum allowable injection volume by the LC's syringe pump and sample loops (seat capillaries). Often larger analytical heads and seat capillaries can be purchased for use with existing systems that allow for multiple 'draws' of a single sample. The concentration ranges of the analytes in the crude samples, the required limits of detection for the specific application and MS sensitivity should be taken into account. One needs to consider the instrumental detection limits in terms of the total mass (e.g., pg or ng) injected onto analytical columns that are currently detected based on the injection of analytical standards in the system. This is providing information on 1) what volume to inject and 2) if an upgrade of the LC system with a multi-draw injection kit is needed. For the starting configuration, it is important to estimate or measure the system's

dwell time, which is the time it takes for a selected volume of sample to be transferred to the column.

Sample preparation for aqueous and solid samples

For aqueous samples, minimal sample preparation such as centrifugation is sufficient to prolong column life and avoid contamination from SPE or filtration materials. Samples to be injected by SC-LVI onto enrichment/separation columns still must be as particle free as possible to avoid plugging the system. Filtration is commonly used [23, 25, 26, 37, 45, 47, 48]. Centrifugation is simple, requires no specialized training, uses common equipment, generates no solid waste and samples can be treated in batches [35, 41, 52].

There are a limited number of cases where SC-LVI is used for the analysis of pesticides extracted using organic solvents such as methanol from soil [31, 32] and vegetables [20]. In each of these cases, the methanol extracts first are diluted to water to give sample compositions ranging from 25:75 to 70:30 (methanol:water). The diluted extracts are then analyzed by the injection of 900 to 1,000 µL (Table 2) with good retention and peak shape. The retention of analytes under SC-LVI conditions for organic solvent extracts diluted with water of varying elutropic strength is likely analyte dependent.

LC columns and MS conditions

If an LC separation uses 100% aqueous phase at the beginning of the chromatographic run, then fully end-capped stationary phases should be employed that are designed to not collapse at 100% aqueous phase and that favor retention of highly polar compounds. Column diameters, LC flow rates and mobile phase composition should be used that are compatible with the vaporization efficiency of the MS source. For example, most ESI sources can accommodate 100% aqueous phase at 200-300 µL/min for 2.1-3.0 mm I.D. columns. Chiaia et al. increased the ESI source and desolvation temperatures to 150 and 450 ^{o}C, respectively to accommodate the higher flow rates of 0.5 mL/min [41]. Alternatively, APCI can accommodate mL/min 100% aqueous phase flow rates with 4.6 mm I.D. columns. Increasing the percentage of organic phase in the initial mobile phase composition will increase desolvation efficiency and decrease LC backpressure, so that higher flow rates can be used.

At high flow rates, it should also be considered to “move away” the probe from the mass spectrometer entrance. This could result in increased desolvation efficiency due to an increase in the desolvation path.

Speed and injection speed

Samples draw speed and injection speed will affect the time that it takes to load sample onto the enrichment/separation column. For aqueous samples, draw speed and injection speed can be set up to 900 µL/min [52]. However, due to the viscosity of water, very high draw speed and injection speed are not recommended due to the possibility of creating suction during sample withdrawal. To check this, initial injection tests should be performed at low draw/injection speeds (e.g. 100 µL/min) on vials containing known amounts (by mass or volume) of water. The volume effectively injected by the system can be assessed by difference in weight and/or volume. If this test does not reveal any problem, then the draw/injection speeds should be increased to 500 µL/min or higher.

Operation of SC-LVI LC-MS methods under accredited conditions and long time performance

Several methods using SC-LVI LC-MS were successfully operated in an analytical laboratory accredited according to ISO 17025 [33]. According to this contract laboratory, rigorous requirements regarding method validation including quality assurance and quality control performance were met with by the SC-LVI LC-MS approach. Column life times were reported to be more than 300 days with 2,800 to 3,700 SC-LVI runs per column. In comparison to conventional LC-MS procedures, neither shorter column lifetimes nor a faster decrease of the MS sensitivity were observed.

Impact of matrix effects on SC-LVI LC-MS analysis

Matrix effect components present in contaminated water samples are known to be responsible for suppressing and, less frequently, for enhancing the absolute analyte response [59]. This often results in variable detection limits and, more importantly, erroneous quantitative results. It should be born in mind that matrix effects do exist both for SPE or SC-LVI LC-MS

methods [45]. Therefore, the 'tools' that are used to address matrix effects are the same for both approaches and include 1) tunable LC gradients for on-line clean-up [35, 41, 52]; 2) deuterated standards [43] 3) standard addition and matrix-matched calibration curves [25, 43, 47]; 4) sample dilution [60]. Although matrix effects are compound and sample dependent, it has been shown to be moderate to minimal for SC-LVI [25, 45, 47].

Instrumental background and LVI

Several reports indicate problems with instrumental background for fluorochemical analytes that is due to part of the LC systems such as PTFE frits, seals and tubing [35, 61-63] and for plasticizers in solvents [64]. For the purposes of this discussion, 'ghost' peaks are described as chromatographic peaks with retention times and MS transitions that correspond to the analyte of interest and increase the overall detection limits. Such 'ghost' peaks result from instrumental background contamination of the LC system and its parts [64] and are differentiated from peaks resulting from sample contamination (e.g., the presence of analytes within blank standards) or due to carryover from previously injected samples.

With the injection of large-volumes, background contamination arising from LC systems parts can be much more apparent than for smaller injection volumes due to the longer sample loading phase with high aqueous samples and resulting contact with LC systems parts that can lead to the buildup of hydrophobic analytes on the head of the analytical column. A 'no injection run', which simulates the entire injection sequence without actually introducing any sample, is a useful method for differentiating background contamination from contamination associated with samples [61]. If multiple no-injection runs give a constant ghost peak it then indicates background system contamination while decreasing peak areas indicate carryover from prior sample injections.

In the development of a SC-LVI method for the surfactants present in the oil dispersant use on the Gulf of Mexico oil spill, a ghost peak for the dioctylsulfosuccinate (DOSS) surfactant was observed that had an identical retention time MS transitions as those of the DOSS standard. For this method, 1,800 μL of seawater were injected onto a C18 column (Table 2) and the divert valve was used to direct the highly saline sample matrix to waste instead of to the mass spectrometer. The ghost DOSS peak was identified as originating from the LC

pump assembly. A second column was installed after the LC mixer but before the injector assembly as described in Powley et al. [61]. The presence of this column shifts the ghost peak in time so that the ghost peak is separated from the analyte peaks. In our experience, we have found that it is especially important that the column located between the LC pumps and the injector possesses equal or greater retentive capabilities than the analytical column to affect separation of ghost and sample analyte peaks. This finding indicates that a LVI can be performed for applications in which background contamination is present.

UPLC with LVI

The main reason for using a UPLC over HPLC is the speed advantage. Ultra performance liquid chromatography (uHPLC or UPLC) employs sub 2 μm particles to achieve improved speed of analysis (5-10 times faster) and separation efficiency. A net advantage of UPLC over HPLC is also an increased MS sensitivity. Because of sub 2 μm particles size and the mL/min flow rates that are typically adopted, the UPLC equipment must accommodate back pressures up to 1.03×10^8 Pa (15,000 psi). Smaller particles size (e.g. 1.8 μm and lower) are more prone to blockages as gaps between particles are extremely small. For this reason, a potential problem area for UPLC is the direct analysis of crude samples. To reduce columns plugging and for longer columns lifetime, ultra in-line filters are now sold with UPLC columns and sample filtration through filter membranes are strongly recommended. However, this may be problematic for analytes that are retained by filters or are artifacts of filter manufacture. As an alternative to UPLC, columns containing 2.5 μm fused core particles offer similar efficiencies to the smaller 1.8 μm UPLC columns without the high requisite high back pressure such that they can be used in conventional HPLC systems [43]. Mass spectrometers coupled with UPLC systems must deliver high speed acquisition rates for the narrow UPLC peaks. High speed acquisition rates are also desirable to allow screening of wider lists of known analytes and unknown analytes in a single chromatographic run without compromising sensitivity and sampling rate across chromatographic peaks.

Future perspectives

Despite the benefits of SC-LVI, there is an apparent reluctance within the environmental analytical chemistry community to adopt this practice. Although SC-LVI applications date

back to 1977 [8], the development of SC-LVI applications results from serendipity rather from guidance based in theory. Research is needed to systematically define the limits of SC-LVI for complex environmental and biological matrices to make SC-LVI more generalizable, which is likely to lead to a fundamental understanding of the technology and its widespread application.

Existing literature describes peak shapes as a function of injection volume, analyte physical chemical properties, column dimensions and sample composition under isocratic (loading and elution) conditions for clean water systems [65-68]. However, research is needed to determine if relationships that hold true under clean systems also apply to complex matrices, such as wastewater, urine and blood. For example, research is needed that relates the capacity (k') of chemicals under isocratic loading conditions to the maximum amount of a sample that can be injected for a specific analyte while maintaining acceptable peak shape. This is important as the capacity of an analyte under loading conditions is likely to be the limiting factor that determines the maximum injection volume for complex environmental sample, and therefore maximum attainable sensitivity. To date, research on maximum loading volume focuses on microbore columns and on how sample solvent composition and injection volume effect efficiency and area counts [68, 69]. As indicated earlier, LVI is compatible with the injection of extracts from environmental solids with appropriate dilution of extracts with water. However, this approach for the analysis of organic solvent-containing extracts has yet to be fully exploited.

Matrix components from environmental samples may limit the amount of sample volume that can be directly injected on the column. Matrix components may interact with analyte molecules in the column in a way that cannot be predicted by experiments in clean systems. Furthermore, matrix components may have the ability to displace analytes and adversely affect peak shape, especially analytes that co-elute with large amounts of matrix components. Further, more detailed experiments, such as those employing 2D chromatography, are needed to differentiate matrix effects due to column overloading from those associated with ionization. Direct comparisons of area counts for SPE extracts of environmental samples with those obtained by SC-LVI are needed to quantify any reduction or enhancement in matrix effects under SC-LVI conditions when compared to SPE. Undoubtedly, increased

mass spectrometer sensitivity can offset the need for large-volume injections so that direct injections of smaller volumes will provide equivalent or better sensitivity.

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Table 1. On-line enrichment (ON-E), coupled column (CC-LVI) and single-column (SC-LVI) applications for environmental matrices based on UV-Vis detection and early MS detectors. Entries are grouped by date of publication and then by analyte class and cover the years 1975 to 1999.

Chemical class	Sample matrix	Injection technique	Injected volume (μL)	Column void volume (μL)	% of Column void volume injected[a]	Detection	LODs	Reference
Polycyclic aromatic hydrocarbons	River water	ON-E	200,000	2,400	8,333	UV-Vis	NA	[7]
Surfactants	Waste water	SC-LVI	250	605	41	UV-Vis	NA	[8]
Pesticides	Surface water	SC-LVI	5,000	1,587-2,645	189-315	UV-Vis	40-500 ng/L	[9]
	Ground water	CC-LVI	200	1,058	19	UV-Vis	1,000 ng/L	[10]
	Surface water	CC-LVI	770	529-1,058	73-146	UV-Vis	1,000 ng/L 100 ng/L	[11]
	Surface water	CC-LVI	2,000	1,058	189	UV-Vis	100 ng/L	[12]
	Surface, ground, drinking water	CC-LVI	4,000	529-1,058	378-756	UV-Vis	100 ng/L	[13]
	Surface, drinking water	CC-LVI	6,000	1,587	378	UV-Vis	100 ng/L	[14]
	Surface water	CC-LVI	2,000	1,058	189	UV-Vis	100 ng/L	[15]
	Surface, drinking water	CC-LVI	2,000	317-1,058	189-630	UV-Vis	100 ng/L	[16]

Table 1. On-line enrichment (ON-E), coupled column (CC-LVI) and single-column (SC-LVI) applications for environmental matrices based on UV-Vis detection and early MS detectors. Entries are grouped by date of publication and then by analyte class and cover the years 1975 to 1999.

	Surface, drinking water	CC-LVI	2000	1,000	200	UV-Vis	500 ng/L	[17]
	Drinking water	SC-LVI	50	7.8	640	MS	<20 ng/L	[19]
Pharmaceuticals	Drinking water	SC-LVI	50	7.8	640	MS	<20 ng/L	[18]

ON-E: on-line enrichment; CC-LVI: coupled column large-volume injection; SC-LVI: single column LVI; NA not available

[a] Only applications are reported if the % of column void volume injected was ≥ 10%. The % of column void volume injected was calculated as (5 x 10^{-4}x column length (mm) x column ID $(mm)^2$ x 1000 [70].

Table 2. SC-LVI MS applications for environmental matrices (organized by earliest date of publication for individual classes of analytes that then occur by alphabetical order when the first year of publication is equivalent) covering the time from 2000 to 2010.

Chemical Class	**Matrix**	**Sample preparation**	**Maximum injected volume (μL)**	**Column void volume (μL)**	**% of Column void volume injected[a]**	**LODs (LOQs*)**	**Reference**
Pesticides	Vegetables	Solvent extraction, dilution with water	900	530	170	0.5-2 μg/kg	[20]
	Surface, ground, drinking water	Filtration	11,700	1,058	1,106	10-200 ng/L	[21]
	Drinking water	None	250-2,000	80-530	113-646	100 ng/L	[22]
	Surface, ground, drinking water	Filtration, acidification	1,000	1,590	63	10-20 ng/L	[23]
	Surface, ground, drinking water	Dilution	100	793	13	100 ng/L	[24]
	Surface, ground, drinking, waste water	Filtration	100	100	100	10-100 ng/L	[25]
	Surface, drinking water	Filtration	100	529	19	<15 ng/L	[26]

Table 2. SC-LVI MS applications for environmental matrices (organized by earliest date of publication for individual classes of analytes that then occur by alphabetical order when the first year of publication is equivalent) covering the time from 2000 to 2010.

	Mineral water	Degassed	100	100	100	100-1,000 ng/L	[27]
	Drinking water	Centrifugation	100	330	30	<100 ng/L	[28]
	Surface, ground, drinking water	None	50	220	23	10 ng/L	[29]
	Soil	Solvent extraction, centrifugation, evaporation	20	10	296	300-500 μg/kg	[30]
	Soil	Solvent extraction, dilution with water	1,000	1,000	100	300 μg/kg	[31]
	Soil	Solvent extraction, dilution with water	1,000	1,000	100	7-18 μg/kg	[32]
	Soil	Solvent extraction, dilution with water	100	221	45	0.23–0.69 μg/L*	[33]
Fluorochemicals	Ground water	Centrifugation	900	300	300	150-1,800 ng/L	[34]

Table 2. SC-LVI MS applications for environmental matrices (organized by earliest date of publication for individual classes of analytes that then occur by alphabetical order when the first year of publication is equivalent) covering the time from 2000 to 2010.

	Waste water	Centrifugation	500	300	167	0.5 ng/L*	[35]
	Surface, waste water	Centrifugation	900	331	272	1-300 ng/L	[36]
	Surface, waste water	Filtration	100	110	91	0.3-1.6 ng/L	[37]
	Surface, ground, drinking, waste water	Water: none Soil: extraction, activated carbon	500	221	227	4– 8 ng/L*	[33]
Neurotoxins	Drinking water	Filtration	500	2,000	25	200 ng/L	[38]
	Surface, ground, drinking water	Centrifugation	750	1,058	71	30 ng/L	[39]
Pharmaceuticals -illicit and/or legal	Surface, ground, drinking water	Filtration	25	0.8	3,125	50 ng/L	[40]
	Drinking, ground, surface, and waste waters	Filtration	100	100	100	10-100 ng/L	[25]
	Waste water	Centrifugation	1,800	1,587	113	2.5-10 ng/L	[41]

Table 2. SC-LVI MS applications for environmental matrices (organized by earliest date of publication for individual classes of analytes that then occur by alphabetical order when the first year of publication is equivalent) covering the time from 2000 to 2010.

	Surface water	Filtration, dilution	3000	44	6,803	2-10 ng/L	[42]
	Surface water	Filtration	100	300	33	0.2-100 ng/ L	[43]
	Waste water	Filtration	5,000	22	22,676	3-8,130 ng/L	[44]
	Waste water	filtration	500	221	227	9 – 18 ng/L*	[33]
Corrosion inhibitors	Surface, ground, drinking water	Filtration	60	300	20	33 ng/L	[45]
	Surface, ground, drinking, waste water	Filtration	500	221	227	32– 7 ng/L*	[33]
Chelating Agents	Drinking, surface, and waste waters	Filtration	50	300	17	(600-1,000) ng/L	[46]
Iodinated contrast media	Waste, post-membrane filtration and reverse osmosis water	Filtration	100	675	15	110-970 ng/L	[47, 48]}

Table 2. SC-LVI MS applications for environmental matrices (organized by earliest date of publication for individual classes of analytes that then occur by alphabetical order when the first year of publication is equivalent) covering the time from 2000 to 2010.

	Wastewater, post-reverse osmosis water	Filtration, dilution	100	675	15	<500 ng/L	[49]
Artificial sweeteners	Ground, waste, post-microfiltration reverse osmosis water	Filtration	100	330	30	0.7-2.4 µg/L	[50]
Biocide	Surface, waste, membrane flushing water	Filtration	2000	2910	69	30-100 ng/L	[51]
Bisphenol A	Soil	Solvent extraction	100	22	227	610 ng/L	[33]
Steroids	Surface, waste water	Centrifugation	1,800 – 4,500	1,587	113-284	1.2–360 ng/L	[52]
Surfactants	Sea water	Centrifugation	1,800	331	272	NA	Place et al. in preparation

∗Limit of quantification NA = not available

[a] Only applications are reported if the % of column void volume injected was ≥ 10%. The % of column void volume injected was calculated as (5 x 10^{-4}x column length (mm) x column ID $(mm)^2$ x 1000[70]

Table 3. Comparison of steps in solid-phase extraction (SPE) and in single column large-volume injection (SC-LVI)

Steps	SPE	SC-LVI
1. Conditioning	µL to mL organic solvent(s)	Performed by mobile phase
2. Sample volume; time	1 – 1,000 mL; minutes to hours	Up to 5 mL; minutes
3. Wash	µL to mL of water and/or solvent	Performed by mobile phase
4. Drying	often but not always included	NA
5. Elution	µL to mL of organic solvent	Performed by mobile phase
6. Extract concentration	Extracts typically concentrated under stream of N_2	NA
7. Extract injection	5-100 µL of final extract injected	NA

NA = not applicable