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Flow inconsistency: The evil twin of column switching—Hardware aspects

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ABSTRACT

Solvent flow, generated by HPLC pumps is consistent and accurate. This statement, while true for single column (one dimensional) liquid chromatography applications, may not apply to column switching applications. Connection of pumps and/or columns to one flow path may cause substantial pressure changes. Immediate post valve switch pressure differences between pumps can cause backflow where the mobile phase stored at higher pressure will temporary flow into the lower pressure area. A more common side effect of column switching is flow inconsistency during pump pressurization. For the duration of pump pressurization, liquid flow through the column will be smaller than expected since the HPLC column acts like a flow restrictor.

1. Introduction

One column/one pump is the standard hardware design for HPLC systems. Under such (1D) conditions all properly functioning HPLC pumps deliver a highly reproducible flow. Manufacturers have minimized flow pulsations and pressure ripples to very low levels and guaranteed a high degree of accuracy and precision of solvent flow for stand-alone pumps. In our experience however, we have observed that the expectation of flow consistency may be incorrect in the context of column switching applications and therefore, requires particularly close attention to such details during method development.

On-line two-dimensional liquid chromatography (2D chromatography) is an efficient solution for increasing peak capacity, improving data quality and enhancing assay sensitivity using LC/UV and especially LC/MS analysis. Implementation of 2D chromatography is advantageous for LC/MS analysis from complex biological matrixes such as urine, plasma or cell/tissue extracts. In contrast, LC/MS analysis of biological samples using conventional 1D chromatography can suffer from severe matrix effects and require careful assessment of off-line sample preparation strategies [1–4]. There is a general consensus in the field that for 1D (single column) LC/MS analysis, that solid phase extraction (SPE) is preferred to liquid–liquid extraction (LLE) or plasma crash for initial sample preparation; in contrast 2D LC/MS can be successfully implemented even with direct injection of biological samples. The ability of various 2D chromatographic platforms to maximize analyte purity and reduce background can dramatically increase instrumental sensitivity [5–9].

2D chromatography is based on the selective transfer of analyte from the first dimension column to the second dimension column (usually by using a 2 position switching valve) [10]. From the plumbing viewpoint, column switching results in the temporary on-line connection of 2 columns in one flow path. Any on-line 2D platform design will be influenced by HPLC pump design, flow rates, column characteristics (such as dimension and particle size), and even tubing dimensions and fitting material. All these factors during a column switching event can cause significant fluctuations in overall system backpressure (BKP). Abrupt pressure changes in turn can induce temporary large deviations in mobile phase flow rate from the expected value, and even can result in backflow. Such deleterious "side effects" of column switching complicate method development, optimization, validation and transfer to other HPLC systems. HPLC vendors assure uncompromised performance of pumps during typical single column (1D) applications; however, they cannot guarantee success with column switching applications. Although column switching is well known in the field, to our knowledge, flow inconsistency has only briefly been mentioned but not properly discussed [11]. In the present paper we describe in detail the phenomenon of flow inconsistency (FI) and its sources in column switching applications and discuss methods for qualitatively monitoring its effect on method performance.

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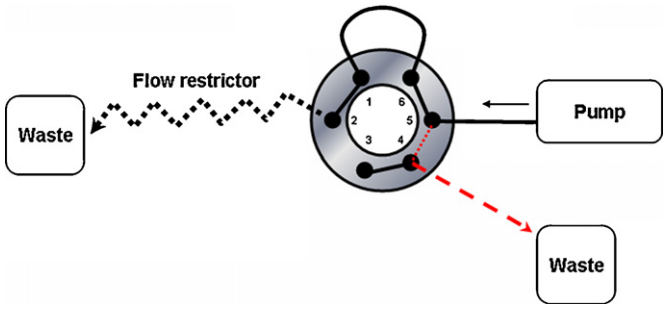

Fig. 1. Pump pressurization test. Pump at no pressure connected to the restriction capillary via switching valve. Slow pressurization occurs. See pressurization data in Table 1.

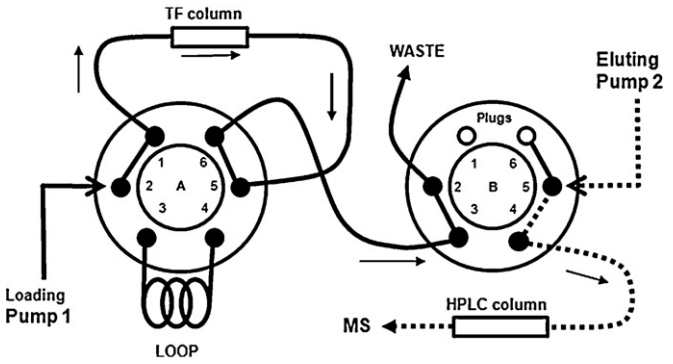

Fig. 2. Schematic design of TFC platform in Focus mode. Loading step. Solid line: matrix sample injected onto the TurboFlow column. Analyte is retained while the matrix debris are flushed out to waste. Dotted line: HPLC column awaiting analyte transfer.

2. Experimental

The investigation of flow consistency was performed using a Cohesive 2300 HTLC TurboFlow System (Thermo Fisher Scientific, formerly Cohesive Technologies, Franklin, MA, USA), which included a valve interface module (VIM) configured in focusing mode and two Agilent 1100 binary pumps G1312A, G1316A column compartment and G1367 well-plate autosampler and G1314 UV monitor (Agilent Technologies, Wilmington, DE, USA). ChemStation software was used for system control and for acquisition of pressure profiles. Positive electrospray SIM detection was achieved using an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Framingham, MA, USA) equipped with a Turboionspray source. In the current flow consistency experiments research samples were not injected; only blank runs were performed to monitor system parameters.

3. Results and discussion

3.1. Nature of the flow inconsistency

The essential part of multidimensional chromatography is column switching. Changes in the valve state result in changes in the flow path and system back pressure. Immediately after a valve actuation, flow inconsistency (FI) may appear as a result of a pressure drop and/or slow pump pressurization. A simple experiment can serve as a demonstration of the FI. A stand-alone 1100 Series pump (without a column) delivers mobile phase to the waste at almost no pressure. A low dead volume capillary tubing was then connected by means of the switching valve to the flow path (Fig. 1). This capillary tubing (65 μm ID × 150 cm from Rheodyne, Upchurch Scientific Division, Oak Harbor, WA, USA) acts as a flow restrictor. The pump pressurizes to the new effective system pressure, which is not an immediate process (Table 1) and may take a few minutes. As a result of this slow pressurization process, there is a deviation between the pre-set (programmed) flow rate and actual flow rate (which will be lower than expected in this case). This flow rate variability will be called flow inconsistency (FI). The most common appearance of FI occurs at the start of a HPLC method where the injection valve switches for the load position (where the sample loop is not pressurized) to the Inject position, where the sample loop is placed between pressurized pump and column. As a result, a brief pressure drop occurs followed by pump/system pressurization. During pressurization column flow is smaller than expected. Theoretically, the best way for absolute evaluation and monitoring of the FI is by using a mass flow meter. Most of these devices however, do not tolerate high pressure and must be installed after the HPLC column, while to monitor FI, the mass flow meter would have to be installed between the pump and column (or between the first and second dimension columns). Furthermore, we have been unable to find a flow meter device that meets the following experimental requirements: (a) able to tolerate high pressures common to HPLC applications; (b) have a low dead volume; (c) able to be synchronized with LC software.

3.2. Turbulent flow chromatography design and hardware aspects

While direct measurement of FI cannot be easily accomplished, indirect observation of the FI can still be achieved. Indirect control of flow inconsistency can be obtained from constant monitoring (and plotting) of the back pressure profile. As an example we will discuss the turbulent flow chromatography (TFC) platform developed by Thermo Fisher Scientific, formerly Cohesive Technologies. This 2D-based system provides an efficient tool for direct LC/MS analysis of biological fluids using TurboFlow technology with increased throughput via multiplexing (technical details available at http://www.thermo.com/turboflow). Briefly, the TFC system supports 2 main separation modes: Quick-Elute mode and Focus mode [12,13]. In the current paper we will discuss details of the Focus mode which is most suited for complex biological samples. The first dimension column (an Extraction or TurboFlow column) is made from large particle size material (50–60 μm) to enable high linear flow rates essential for turbulent flow chromatography. This allows small hydrophobic analytes to be retained on the extraction column, while large proteins from complex biological matrices are flushed out from the column to waste at the loading step (Fig. 2).

To perform on-line clean-up using a 1 mm ID extraction column, high flow rate (4–8 ml/min) is essential. A high dwell volume pump, such as an Agilent quaternary pump G1311A is often used as the loading pump. To avoid delays resulting from the ~1 ml dwell volume of this loading pump, analyte elution from the extraction column in Focus mode is carried out by a higher concentration of organic which is stored in the transfer loop (valve A).

During the transfer step (Fig. 3), the analyte-containing fraction from the extraction column is combined with a low organic mobile phase simultaneously delivered by the eluting Pump 2 via Valve B (inside of the special T rotor seal). As a result of the on-line dilution, the analyte(s) of interest are refocused on the second dimension analytical reversed phase column. In the final, eluting step (fig not shown), valve B is switched back and the analyte(s) of interest are eluted by a laminar flow gradient and detected (typically by MS/MS). Simultaneously the transfer loop is refilled by high organic during regeneration of the extraction column.

3.3. Flow inconsistency in turbulent flow chromatography

A few important details should be noted about the transfer step. A decade ago the C18 Hi-Res (second dimension) column