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LC–MS solvent composition monitoring and chromatography alignment using mobile phase tracer molecules

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Abstract

In the field of proteomics, reproducible liquid chromatographic description of analytes is often a key element for the differentiation or identification of proteins or peptides for clinical or biological research projects. However, analyte identification by retention time can be problematic in proteomics where lack of standardization can result in significantly different chromatography for the same analytes analyzed on different machines. Here we present a novel method of monitoring the mobile phase gradient of LC–MS/MS analyses by monitoring the ion current signal intensities of tracer molecules dissolved in the mobile phase solvents. The tracers' ion current signal intensities chronicled gradient fluctuations, did not adversely affect the number or quality of CID-based sequence identifications, and had lower run-to-run variance when compared to retention time.

Keywords: Liquid chromatography; Electrospray mass spectrometry; Proteomics; Peptide; Tracer molecules

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1. Introduction

The liquid chromatographic (LC) retention time of an analyte is often used to determine the analyte's identity and/or differentiate it from other analytes. For example, pharmaceutical and environmental compounds are commonly identified and characterized by their retention times relative to reference standards using HPLC analysis coupled with UV detection [1,2]. In the field of proteomics where samples are complex polypeptide mixtures, LC retention time is often paired with additional peptide and protein information to find differences in the patterns obtained from different samples. For example, retention time, the accurate mass, and MS peak signal intensity from LC–MS analyses have previously been used to create polypeptide maps for compositional comparison between normal and malignant breast epithelial cell lysates [3,4]. These maps were compared against each other to screen for differential protein patterns between the cell lines [3], and for differential protein patterns between cells treated with estrogen and control cells [4]. The additional peptide characteristic information gained from retention time has also been used to aide peptide mass fingerprinting identifications by providing an additional peptide identifying factor [5]. In addition, LC retention time coupled with accurate peptide mass has also been used to characterize peptides and proteins via global cataloging (a.k.a. tagging) values for subsequent identification of these sequences by their peptide tags [6,7].

Though chromatographic peak positions are often described by the peaks' retention times, there are several drawbacks to using retention time as a chromatography descriptor. First, retention time values are LC system specific. Identical analytes analyzed on different chromatographic columns, pumping systems or mobile phase gradients can have significantly different retention time values. In the pharmaceutical and environmental fields, this variability is minimized by standardized chromatography columns and automated HPLC systems. However, this standardization is not common in the field of proteomics where experiments between laboratories are often analyzed on columns of different dimensions and packing materials, and on systems with different gradient delay times. Thus, chromatographic retention times acquired in one proteomic laboratory can be difficult to replicate in other proteomic laboratories.

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Another drawback is that retention time can be affected by external factors that are not sample dependent such as changes in the ambient or column temperature, poor solvent mixing, small gas bubbles in the chromatographic systems, and other random events. Some of these factors are unpredictable and difficult to control and mathematically model.

LC retention time shifting is especially a problem when aligning multiple chromatography runs to each other. Methods to correct for these fluctuations and to align multiple chromatography runs have included calculating relative LC peak retention times with respect to reference peaks [1,2,8], and employing computer algorithms to shift peaks and warp chromatographic runs to fit target chromatographic patterns [9,10]. One problem with these methods is that they typically do not employ enough reference peaks to allow for continuous monitoring of the gradient. Thus, alignment in the region around a reference peak can be inaccurate if the reference peak is not detected. Even if all the reference peaks are identified, some chromatography alignment algorithms assume linear or simple polynomial relationships between reference peaks that do not account for unpredicted chromatography deviations that can cause alignment errors. Other methods that use direct chromatography mapping by regressive optimization can also fail if peaks are ill-defined and poorly aligned between runs.

Another way of describing peak positions in LC is to describe the chromatography using the mobile phase solvent composition to define the chromatographic positions. One advantage of directly monitoring the solvent composition is that we can detect gradient fluctuations and systemwide events as they occur. Monitoring the gradient can also simplify run-to-run chromatography alignment by removing the need for reference analyte peaks and mathematical models to describe the chromatography.

In this paper, we present a novel method of describing LC gradients of LC–MS and LC–MS/MS experiments using tracer molecules dissolved in the mobile phase solvents. We followed the signal intensities of the ion current from the MS analysis of two tracer molecules, one dissolved in solvent A and one dissolved in solvent B, and calculated the ratio of the signal intensities to describe the mobile phase solvent composition relative to each other throughout a chromatographic run. Using this method, we simultaneously monitored the LC gradient in real-time and identified peptide sequences by MS/MS. In addition, we show run-to-run chromatographic gradient alignment of peptide samples analyzed using different LC gradients and on different ion trap mass spectrometer systems by describing peak positions using the tracer molecules' signal intensity ratios to describe the chromatographic gradient position. From these results we demonstrate that the solvent composition, as determined from the ratio of tracer molecules is a better parameter for pattern alignment than peak retention time.

2. Experimental

2.1. Liquid chromatography mobile phase solvents

Buffer A, the aqueous phase solvent, consisted of 0.4% (v/v) acetic acid (Fisher Scientific, Fairlawn, NJ, USA) and 0.005% heptafluorobutyric acid (HFBA) (Fluka, Sigma–Aldrich, St. Louis, MO, USA) in HPLC quality water with 0.01 mg/ml maltitol (Fluka, Sigma–Aldrich, St. Louis, MO, USA) as the aqueous phase tracer molecule. Buffer B, the predominantly organic phase solvent, consisted of 0.4% acetic acid and 0.005% HFBA in 80% acetonitrile (Fisher Sci, Fairlawn, NJ, USA) and 20% water with 0.01 mg/ml lactose (Sigma, St. Louis, MO, USA) as the organic phase tracer molecule.

2.2. LC–MS/MS system

Two different LC–MS/MS systems were used for the study presented here. Both systems contained Agilent 1100 binary HPLC pumps. These pumps were fed into a reverse phase capillary column using the pre-column flow-splitting set-up previously described [11]. The LC reverse phase capillary column (Polymicron Technologies, Phoenix, AZ) was packed to a length of 12 cm with Magic C18 resin (Michrom BioResources, Auburn, CA). The pump flow rate was set to 0.12 ml/min. This flow was split to achieve an elution flow rate of 200 nl/min off the reverse phase column. Unless otherwise specified, the gradient was as follows (gradient number 1): 0 min, 5% buffer B; 5 min, 15% B; 65 min, 35% buffer B; 80 min, 100% buffer B; 87 min, 100% buffer B; 95 min, 5% buffer B. For the peak alignment experiment, gradient number 2 was used: 0 min, 4% buffer B; 5 min, 10% B; 60 min, 40% buffer B; 70 min, 100% buffer B; 85 min, 100% buffer B; 85 min, 5% buffer B. A 15 min equilibration time was used between analyses.

The two mass spectrometers used to analyze the chromatography eluent were a ThermoFinnigan LCQ Classic and a Finnigan LCQ-Deca XP ion trap tandem mass spectrometer. The peptides were ionized using inline ESI as previously described [11]. The instruments were set to acquire masses from 400 to 2000 Da in positive ion mode and the dynamic exclusion was set for 2 min. The characteristic masses from lactose and maltitol were put on the dynamic exclusion list so they would not be selected for CID analysis throughout the chromatographic run. The exclusion list window was set to ±2 Da.

MS/MS sequence identifications were obtained using the SEQUEST algorithm [12] and the quality of these identifications were assessed by computing the probability that the SEQUEST derived scores are typical of a correct identification using the Peptide Prophet and Protein Prophet algorithms [13,14]. Those sequences with Peptide Prophet scores greater than 0.9 were considered high-scoring identifications. This corresponded to a false positive rate of 0.7%.

2.3. Tracer molecule pre-testing for selection

Solubility of the potential tracer molecules was assessed by first dissolving 0.001, 0.01, 0.1, and 1 mg of the tracer compounds in 1 ml of 0.4% (v/v) acetic acid in water. Those compounds that resulted in a clear solution at all solubility concentrations were considered soluble. The soluble compounds were further tested for solubility in acetonitrile by dissolving 1 mg in 20 ml 0.4% (v/v) acetic acid in water and bringing the volume up to 100 ml with acetonitrile resulting in a solution