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Accelerating high quality bioanalytical LC/MS/MS assays using fused-core columns☆

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ABSTRACT

High quality, ultra-fast bioanalytical LC/MS/MS methods were developed using short columns packed with fused-core particles and high (1.0–3.0 mL/min) flow rates. For more than two years, at flow rates up to 3.0 mL/min, using 0.33 min non-ballistic gradients, these methods were shown to provide comparable or better performance than slower assays for accuracy, precision, sensitivity, specificity, and ruggedness, and met all criteria required by the bioanalytical regulatory guidance.

1. Introduction

It is often assumed that one must perform "quick and dirty" bioanalytical assays to achieve fast results. Does this mean that quality must be sacrificed for speed? With recent advances in HPLC columns and LC/MS/MS technology, high quality bioanalytical assays can be achieved at fast speeds.

Over the past decade, various groups have made advances in accelerating bioanalysis using LC/MS/MS. These include using sub-2 μm particle columns [1,2], ballistic gradients [3,4], fast isocratic LC/MS runs [5], increased flow rates using shorter columns [6], and the use of monolithic [7] or fused-core [8–12] HPLC columns. With the recent advent of direct atmospheric pressure sampling techniques for MS, various groups have also explored assays that may require little or no sample preparation, and introduce samples to the MS with no chromatography, using methods such as DESI [13,14] and DART [15,16].

Regardless of the approach taken, various criteria must be considered to develop bioanalytical LC/MS/MS methods that are fast, and still meet the quality required by the regulatory bioanalytical guidance [17,18]. The major criteria to consider for LC/MS/MS methods are: accuracy, precision, specificity, sensitivity, ruggedness and reproducibility. The HPLC components that have the greatest impact on LC/MS/MS throughput are (1) autosampler, (2) HPLC pump, and (3) HPLC columns. The mass spectrometer does not typically limit the speed of a method and, therefore, it was not considered a speed limiting factor. The dwell times of modern mass spectrometers can be set to acquire enough data points across a narrow LC peak. To properly describe the throughput of the method, one must consider the total time from one injection to the next. This "total time" between injections is made up of two components: the "LC/MS time" and the "overhead time." The LC/MS time is the actual time required for HPLC gradient, MS data acquisition, column washing and equilibration. The overhead time is required for the autosampler and software initialization for the next sample.

The goal of this work was to reduce run times for typical LC/MS/MS bioanalytical methods from 3–5 min to 1 min or less, while maintaining the assay quality. New methods were developed using standard LC/MS/MS equipment, without the need for any specialized equipment; therefore, the transfer of these methods to any laboratory is straightforward. For this work, two types of methods were considered: (1) bioanalytical methods for early discovery studies for which a generic HPLC method is most efficient and (2) bioanalytical methods that must meet the regulatory guidance requirements [15,16]. The generic method typically used for early discovery studies can be optimized further if the compound moves into development.

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2. Materials and methods

2.1. Mass spectrometry

All mass spectrometry was performed on triple quadrupole instruments (API-4000, Applied Biosystems, Foster City, CA) using a TurboIonSpray source. The instrument was operated using Analyst 1.4.2 software. Scan functions consisted of selected reaction monitoring (SRM) experiments using unit mass resolution. Conditions for declustering potential (DP), collision energy (CE) and exit cell potential (CXP) for each analyte were obtained using the Quantitative Optimization function in Analyst software. Unless otherwise specified, ESI source conditions were: gas flows (GS1, GS2) = 80, and gas temperature (TEM) = 650. Dwell times were set to record approximately 15 points across each chromatographic peak (for the fastest runs with multiple analytes this was typically 5 ms).

2.2. Autosampler

A single arm autosampler (PAL, CTC Analytics, Swingen, Switzerland), with cooled tray holder was used for all experiments. Standard 50 μL autosampler syringes with polyethylene-tipped plungers (Leap Technologies Inc., Carrboro, NC) were used. Washing of the syringe and injection valve was performed using two solvents. Wash #1 is typically 100% ethanol, except when necessary for reducing autosampler carryover; Wash #2 was methanol/water (50/50%).

In order to ensure that syringe and injection valve washing was performed to minimize carryover and that washing times did not exceed the faster LC/MS times, the syringe and injection valve were washed independently. Syringe washing was performed in the PAL wash station without injecting the wash solvent into the injection valve. Washing of the injection valve was performed using a separate valve wash (Valve Self Wash 2, Leap Technologies Inc., Carrboro, NC), consisting of a pump that flushes the wash solvents through the area between the injection port and the waste position of the injection valve. Independent software (Launchpad, Leap Technologies Inc., Carrboro, NC) was used to program the pump to deliver the necessary wash volume at a proper flow rate. It was determined that 0.5 mL of each wash solvent at a flow rate of 5.0 mL/min was optimal for all cases shown. The device was programmed to trigger approximately 3 s after the sample was injected.

2.3. HPLC pumps

HPLC was performed using a binary pump rated to a maximum pressure of 600 bar (1200SL, Agilent Technologies, Santa Clara, CA) and a heated column compartment (G1316A, Agilent Technologies, Santa Clara, CA). Flow rates ranged from 0.4 to 3.0 mL/min, with the column temperatures at 50 °C. Mobile phases were water/acetonitrile (95/5%) with 0.1% acetic acid (mobile phase A) and acetonitrile with 0.1% acetic acid (mobile phase B). For some of the pressure measurements, mobile phase B contained methanol with 0.1% acetic acid. Stainless steel tubing (0.005″ ID) was used for all connections from the HPLC pump to the column; PEEK-coated fused-silica (0.004″ ID) was used from the column exit to the mass spectrometer inlet.

2.4. HPLC columns

During the course of these studies, a number of columns were tested. Data are presented from three HPLC columns for small molecule work: (1) 2.1 mm × 50 mm XTerra C_{18}, 5 μm (Waters, Milford, MA); (2) 2.1 mm × 20 mm XDB Eclipse C_{18} 1.8 μm (Agilent Technologies, Santa Clara, CA); and (3) fused-core (Ascentis Express): 2.1 mm × 30 mm and 2.1 mm × 20 mm C_{18}, 2.7 μm (Sigma–Aldrich/Supelco, St. Louis, MO). For the peptide method a 300 Å pore size, 2.1 mm × 30 mm C_4, 3 μm ACE column (Mac-Mod Analytical Inc., Chadds Ford, PA) was used. The XDB Eclipse and 30 mm fused-core columns were used in the initial experiments (data not shown), but the only data presented here are related to the pressure and flow rate comparisons. Unless otherwise stated (e.g. for peptides or comparison purposes), all data shown were obtained using the 20 mm fused-core column.

2.5. Generic sample extraction for discovery studies

For early discovery studies, a generic extraction approach was developed to use with the generic LC gradients described in Section 3.1. Plasma or tissue homogenate samples (25 μL) are protein precipitated in a 96-well block with 8 volumes of acetonitrile. After vortexing and centrifugation, 100 μL of supernatant are transferred to an injection block and 200 μL of Milli-Q water are added. After vortexing and centrifugation, 2–10 μL are injected onto the LC/MS/MS system.

This generic extraction procedure provides suitable sensitivity for an LLOQ of 2–5 ng/mL and a 1000-fold dynamic range that is typically used for these studies, and with the MS instrumentation being used. For later-stage discovery studies and method validation (see Section 3.8), liquid–liquid extraction or on-line extraction is used and the range is changed as required for the specific analyte.

3. Results and discussion

3.1. Small molecule analysis

To demonstrate the performance of various HPLC methods, four small molecule pharmaceutical compounds with molecular weights between 300 and 500 Da were analyzed at various speeds. The chromatographic settings and the results obtained are shown in Table 1. The chromatograms for the following three types of methods (a)–(c) are shown in Fig. 1:

a. Standard method: 5 μm, 2.1 mm × 50 mm column at 0.4 mL/min.
b. Fast method: 2.7 μm fused-core, 2.1 mm × 20 mm column at 1 mL/min.
c. Fastest method: 2.7 μm fused-core, 2.1 mm × 20 mm column at 3 mL/min.

The peaks in all chromatograms in Fig. 1 are baseline resolved. To compare the chromatographic performance of the methods, the peak capacity for each method was calculated by dividing the gradient time by the average peak width for all four peaks [19]. The peak capacity of the standard assay (Fig. 1a) is 19, while for the two fast assays at 1 mL/min and 3 mL/min (Fig. 1b and c) it is 13 and 14, respectively. This result shows a small loss in peak capacity for the faster runs compared to the slower run, but in general, chromatographic performance is maintained in the faster runs, even at the very high flow rates that may not be optimal for the column.

In choosing the column size for the new, faster assay, the column length-to-particle size ratio was maintained as closely as possible, which should retain LC performance while enabling faster run times. Despite the fact that the ratios of column length to *nominal* particle size are different in these experiments, similar performance was expected for these columns based on previous reports that the 2.7 μm fused-core particles have an effective particle size of ~2 μm because they are not completely porous like the 5 μm particles [8]. Gradients used for the fast LC/MS methods (Table 1) were based on scaled versions of the standard method. Direct scaling of

Table 1
Comparison of chromatographic conditions for various LC/MS/MS methods.

	Standard method	Fast method	Fastest method
C_{18} column	2.1 mm × 50 mm, 5 μm	2.1 mm × 20 mm 2.7 μm, fused-core	2.1 mm × 20 mm 2.7 μm, fused-core
Flow rate (mL/min)	0.4	1.0	3.0
Gradient time (min): 30–95% organic	3.25	1.0	0.33
LC/MS time (min)	4.2	1.3	0.4
Overhead time (min)	0.5	0.5	0.5
Total time (min)	4.7	1.8	0.9
Throughput increase	1	2.6	5.2

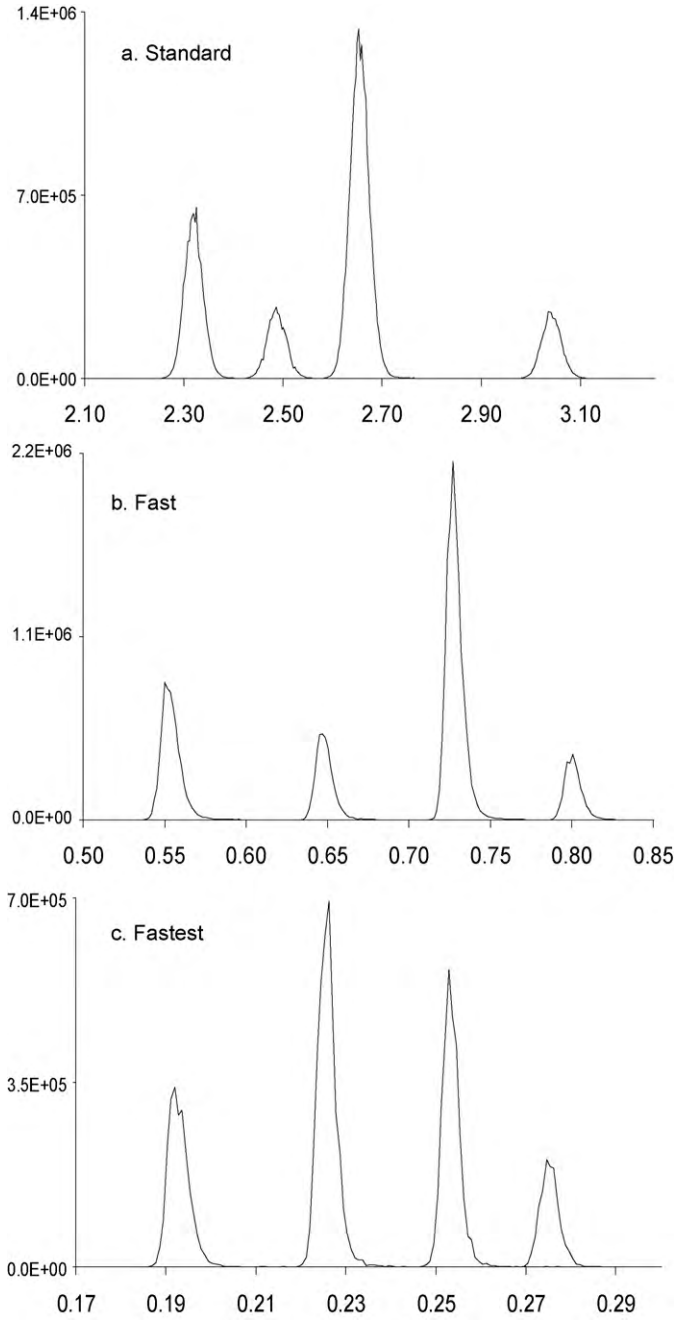

Fig. 1. Comparison of various LC/MS/MS methods: (a) standard method using 2.1 mm × 50 mm, 5 μm column; 3.25 min gradient at 400 μL/min, (b) fast method using 2.1 mm × 20 mm, 2.7 μm fused-core column; 1.0 min gradient at 1.0 mL/min, (c) fastest method using 2.1 mm × 20 mm, 2.7 μm fused-core column; 0.33 min gradient at 3.0 mL/min.

the 3.25 min gradient from the standard method would result in a 0.17 min gradient, accounting for both the change in column length (50 vs. 20 mm) and flow rate (0.4 vs. 3 mL/min). In order to compensate for any column inefficiencies at extremely high flow rates [9], the gradient time was slowed further by a factor of two for the faster methods. This enables both increased throughput while still maintaining similar chromatographic performance to the standard method as shown in Fig. 1.

The observed peaks in these chromatograms also demonstrate an improved sensitivity at 1.0 mL/min (Fig. 1b) relative to the slower run at 0.4 mL/min (Fig. 1a) and fastest run at 3.0 mL/min (Fig. 1c). The increase in sensitivity at 1.0 mL/min relative to 0.4 mL/min is due to the narrower peaks obtained at higher flow rates. The decrease in sensitivity at 3.0 mL/min (obtained without a post-column split) is due to sub-optimal ESI source performance at a high flow rate. Further discussion of flow rate effects on sensitivity is in Section 3.3.

3.2. Pressure, flow rates, and assay time

The advantage of the fused-core column comes primarily from the substantially decreased operating pressure as compared to traditional small particle columns at the same flow rate. Table 2 shows the resulting maximum pressures of fast gradients at various flow rates using both methanol and acetonitrile mobile phases with the 20 mm fused-core column. In this example, the gradients (from 30 to 95% mobile phase B) were scaled based on the flow rate, and the maximum average pressure was determined at a column temperature of 50 °C using replicate injections. As has been described in the literature about fused-core columns [8,9], the pressure is substantially lower than a 1.8 μm, 2.1 × 20 mm column. For example, the pressure of the 20 mm fused-core column is only 340 bar at 2.0 mL/min (the 30 mm column results in a pressure of 375 bar at 1.5 mL/min), while that same pressure is reached at approximately 1 mL/min with the 20 mm 1.8 μm column; thus, giving nearly a 2-fold flow rate (and time) advantage to the fused-core column. Table 2 also shows that, by using the fused-core column with acetonitrile mobile phase, it is possible to use a 1.1 min total time while still maintaining pressures below the 400 bar limit of traditional HPLC pumps. Thus, it is possible to have fast methods without the need to upgrade to newer, high pressure or UPLC pumps; however, with the use of higher pressure pumps, methods can be further accelerated.

Experimentally observed total times shown in Table 2 include the overhead time, which averages about 30 s for the PAL autosampler and Analyst software processing. Because of this overhead time, equilibration for the faster runs occurs during the overhead time and no explicit step is incorporated into the method. The total time decreases down to approximately 1.0 min for both the methods at 2.0 and 3.0 mL/min. At 3.0 mL/min, the decreased LC/MS time is not significant compared to the overhead time; therefore, no significant gain in overall throughput is achieved. This demonstrates the current limitation in speed achievable with this approach, and the need for an improvement in autosampler cycle time, such as with a "look-ahead" function, or through the use of a dual-arm

Table 2
Experimental maximum pressures and total times using fused-core columns (2.1 mm × 20 mm, 2.7 μm).

Flow rate (mL/min)	LC/MS time (min)	Maximum pressure with acetonitrile (bar)	Maximum pressure with methanol (bar)	Total time (min)
1.0	1.3	180	270	1.8
2.0	0.6	340	500	1.1
3.0	0.4	500	N/A	0.9

autosampler, both of which are not currently supported by the Analyst software.

3.3. Effects of high flow rates on ESI performance

While it has been demonstrated that the HPLC hardware can be used at flow rates up to 3.0 mL/min for bioanalytical methods, it is necessary to consider the performance of electrospray ionization (ESI) under these conditions, specifically the TurboIonSpray source. To test the effects of flow rate on ionization, experiments were conducted using the 20 mm fused-core column at various flow rates between 0.40 and 3.0 mL/min with the gradients scaled proportionally to the flow rate. At all flow rates studied, the maximum sensitivity was achieved using "hot" source conditions, using high flow rates for nebulizing and drying gases, and hot drying gas temperatures, as given in Section 2.1.

An additional observation was made that at flow rates greater than approximately 1.5 mL/min, "ghost" peaks (sidebands) often appeared in the MRM channels of the analytes. This phenomenon was observed for some analytes (data from one analyte is shown), but does not occur with all analytes. To understand this effect, which appeared to be flow rate dependent, the experiment was also performed by incorporating a flow splitter between the column and the ESI source. These results show that the effect is related to the ability of the ESI source to accept high flow rates. The "ghost" peaks are observed only when the eluent is directed to the ESI source at flow rates greater than approximately 1.5 mL/min. This phenomenon has been observed for both small molecules and peptides.

Fig. 2a shows a chromatogram for a single analyte at 1 mL/min that exhibits normal chromatography. Fig. 2b is the same analyte but with a method at a flow rate of 1.5 mL/min. This chromatogram clearly shows multiple ghost peaks including a second peak and noise spikes that appear at retention times later than the main analyte peak. When the method from Fig. 2b is run with a 50% post-column split so that only 0.75 mL/min are delivered to the MS, the chromatogram returns to normal (Fig. 2c). The "ghost" peaks also become more prominent when the source conditions are made cooler (lower gas flows and lower temperature) at flow rates where the "ghost" peaks are present.

This phenomenon is likely a result of incomplete desolvation and entrainment of the ESI droplets in the source [20]. Droplets may remain in the source region and then later be pulled in and register as an additional peak in the MRM channel. This theory fits with the experimental observations, because no peaks have ever been observed at retention times before the main analyte peak, only afterwards, and the retention times of the ghost peaks are not consistent.

As a rule, any assays with flow rates greater than 1.5 mL/min are run with a splitter to ensure that the ESI source operates in an acceptable flow range of approximately 1.0 mL/min. This approach has eliminated the observation of any "ghost" peaks in routine use of the fast methods. It should be noted, however, that for many analytes, it is possible to run at flow rates of 3 mL/min, without post-column splitting, and still observe no "ghost" peaks (see Fig. 1c). This effect is the result of differences in droplet composition and desolvation processes for analytes with varying chemical and physical properties.

In addition to the ESI performance shown above, sensitivity at flow rates greater than 1.0 mL/min was also examined. Replicate injections for a number of analytes were run at 1.0, 2.0 (without a split and also split to 1.0 mL/min), and 3.0 mL/min (split to 1.0 mL/min). In general, for the analytes examined, 1.0 mL/min flow without a split into the ESI source provided the best sensitiv-

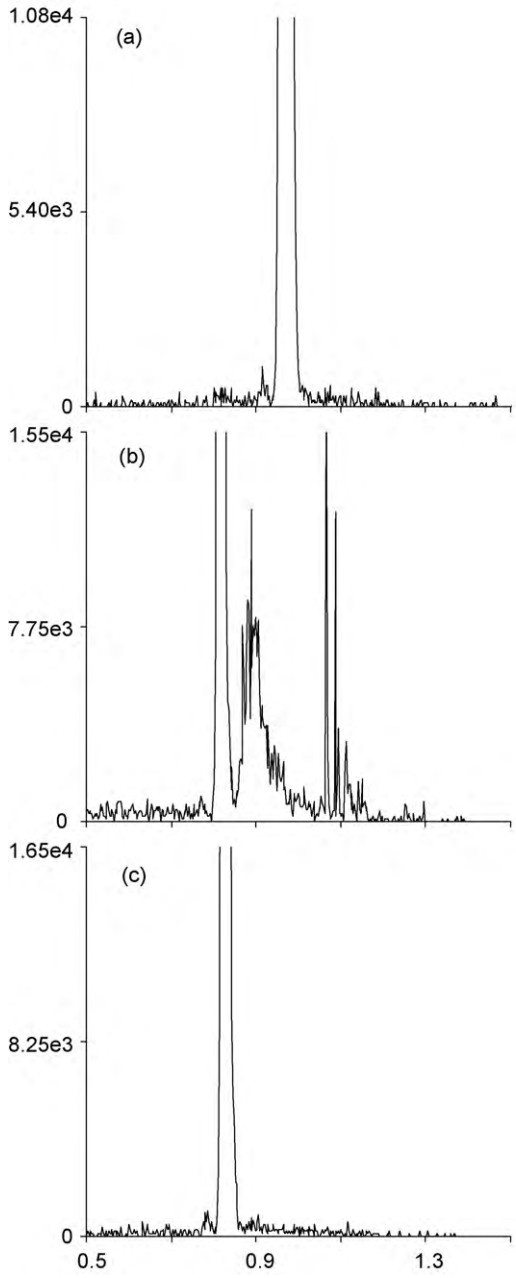

Fig. 2. Observation of additional "ghost" peaks in MRM chromatograms at high flow rates. (a) 1 mL/min where ghost peaks are not expected, (b) 1.5 mL/min showing multiple ghost peaks at retention times after the analyte, (c) 1.5 mL/min with 50% post-column split, showing only the analyte peak.

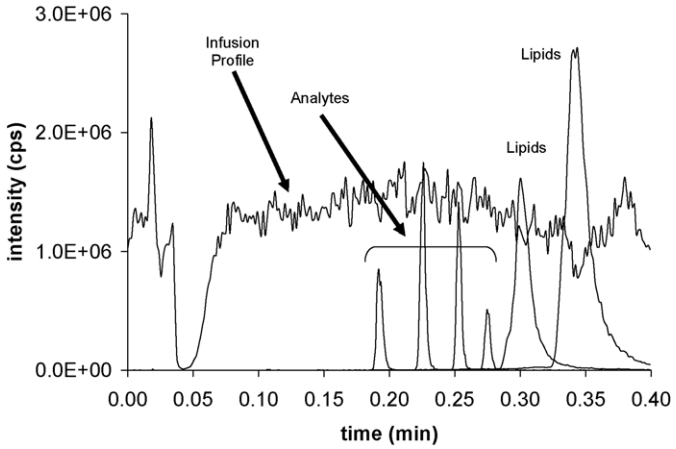

Fig. 3. Evaluation of areas of common matrix interference in a generic method at 3.0 mL/min with a 0.33 min gradient from 30 to 95% mobile phase B (acetonitrile). Overlaid plots include a representative infusion profile, two major early-eluting phospholipid components of plasma (MRM transitions: 496 → 184 and 524 → 184), and four example analytes (as shown in Fig. 1c).

ity. All other conditions gave lower sensitivity by about 15–25%. This loss in sensitivity may not be considered significant given the assay requirements and typical MS performance. Use of 3.0 mL/min directly into the ESI source was not tested because of previous results showing reduced performance and the "ghost" peak effect.

3.4. Matrix effects

Another crucial issue to consider for high-throughput bioanalysis is the separation of analytes from common matrix interferents. Although it is not possible to ensure separation of all analytes from all possible matrix interferents, it is useful to verify the regions of the generic fast method's chromatogram where the matrix effects would be minimized. This can be done by examining the regions where the void volume and major plasma phospholipid peaks elute. This information is especially important for discovery methods where little method development is done, and protein precipitation is the standard sample preparation technique. This will ensure that the generic methods will be generally reliable for early phase studies. Users can modify the methods to incorporate more specific extraction methods (e.g. liquid–liquid or on-line extraction) for later phase studies or when problems are observed in early studies.

One way of examining the effect of matrix components is through post-column infusion profiling [21]. In this experiment, a constant flow of analyte is added into the flow path between the column outlet and the ESI source, while blank extracted plasma is injected onto the column and the normal LC/MS/MS method is run. The perturbation of the analyte signal from a smooth profile indicates ionization suppression or enhancement due to the matrix components.

An example of examining the matrix effect is shown in Fig. 3. It shows overlaid chromatograms of the four analytes shown in Fig. 1c, the major earliest-eluting phospholipid components of plasma, and a post-column infusion profile of a representative analyte. Phospholipids were monitored using MRM transitions of 496 → 184 and 524 → 184 [22]. These data were obtained using a 0.33 min gradient from 30 to 95% mobile phase B at 3.0 mL/min. The plasma sample was prepared using the generic acetonitrile precipitation method from Section 2.5. Ion suppression from the first major lipid component occurs near 0.33 min. These data provide a reasonable first estimate that the region between 0.05 and 0.33 min should generally be acceptable for the elution of analytes, while still minimizing potential matrix effects. This information is generally useful in developing a generic gradient for discovery studies.

3.5. Autosampler washing and carryover

In most cases, the standard autosampler injector port wash using the VSW2 with independent syringe washing was sufficient to achieve acceptable carryover (<20% of the LLOQ for a 1000-fold dynamic range method) within the time of the method. One major advantage of using the VSW2 is the speed with which the injector can be washed. Washing occurs within approximately 20 s, including a programmed 3 s delay after the sample injection to make sure the syringe has pulled out. Interestingly, in cases where carryover was a problem, changing the VSW2 parameters (e.g. increasing the wash volume or changing the flow rate) made no significant difference. Only changing the extent and duration of syringe washing achieved the reduction in carryover, demonstrating that syringe washing is usually the limiting factor for carryover in this system.

One particularly sticky compound (a fluorinated anion) demonstrated a limitation of high-throughput LC/MS/MS using this experimental setup. A fast method was used, but the syringe washing necessary for acceptable levels of carryover was longer than the LC/MS time. In this case, the LC/MS time was 1.55 min, while the assay time was 2.5 min, due to the additional time for syringe washing and the need to perform a solvent chase with the injection to reduce the carryover to acceptable levels. A dual-arm autosampler system would alleviate this problem by enabling one syringe to be washed while the other was injecting a sample, and therefore provide a significant improvement in overall throughput.

3.6. Peptide analysis

In addition to small molecule methods, fast LC/MS/MS methods have been developed for peptides. As an example, a 0.7 min gradient from 20 to 40% mobile phase B was run at 1.5 mL/min with an LC/MS time of 1.3 min. Because this peptide required a large pore C_4 column to maintain good peak shape, a 300 Å, 2.1 mm × 30 mm, 3 μm column was used. At this time, a column using traditional porous particles was used because no short, large pore fused-core columns were available. The gradient was shallower than for small molecules because of the known potential for close-eluting interferents from *in vivo* samples. Fig. 4 shows the results for the peptide (MW 2257 Da) in mouse plasma prepared using acetonitrile protein precipitation. Fig. 4a shows the results for a mid QC of 150 ng/mL. Fig. 4b shows the lower limit of quantitation (LLOQ) at 2 ng/mL and a measure of the carryover from an extracted plasma blank sample that immediately followed a standard at the upper limit of quantitation (1000 ng/mL). The accuracy range for standards and QCs over a linear range of 2.0–1000 ng/mL was 89–109%.

3.7. Bioanalysis of discovery PK samples

Generic bioanalytical assays with gradients of 1 min or less were routinely used in our laboratory to analyze samples from discovery pharmacokinetic studies. These assays were performed using the 20 mm fused-core column, without internal standards and included multiple analytes per assay. Quality control samples (QCs) at three concentrations in duplicate were added to each study. Results from a total of approximately 2000 QCs showed that 88% of QCs were within ±15% accuracy. These statistics give an idea of how successful the fast assays have been, even when using generic HPLC gradients, or with completely new analytes, as well as using the generic protein precipitation (Section 2.5) for sample preparation.

A closer look at a single project, consisting of multiple studies containing many different analytes, provides more information about the quality of the data. For 107 QCs analyzed across multiple

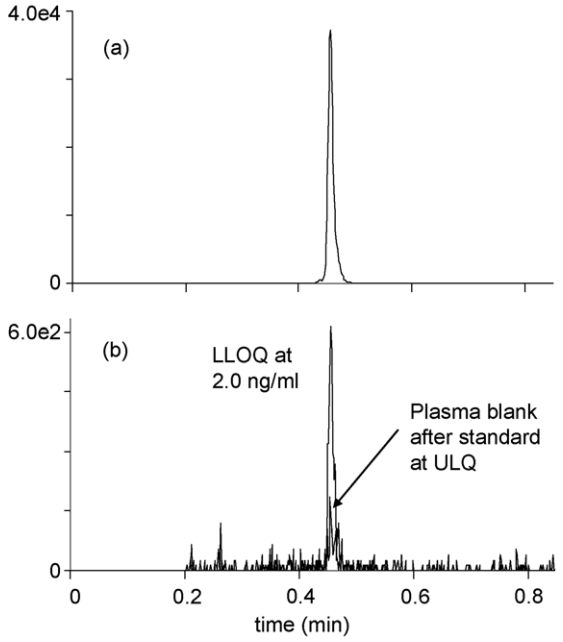

Fig. 4. A fast method for a peptide. (a) 150 ng/mL, (b) 2.0 ng/mL (LLOQ) and extracted plasma blank injected immediately following high standard at the upper limit of quantitation (ULQ = 1000 ng/mL). Data obtained using the 300 Å pore size, 2.1 mm × 30 mm C_4, 3 μm ACE column.

studies in a single discovery project using protein precipitation for sample preparation, the mean accuracy was 98.1% with a % CV of 6.9%. These data for this project are representative of other projects and were not chosen as the best case scenario.

Additional evaluation was also performed by comparing results from standard methods with those from fast methods for a number of studies. These studies involve many different classes of compounds. For comparison, calibration standards, QCs, and sample results were examined. In general, the fast assays provided similar or better chromatographic resolution and increased sensitivity, and no difference in standard/QC statistics or concentrations for samples were ever observed. These measurements provided added confidence in these types of assays, especially for users more comfortable with slower methods.

3.8. Method validation

Assays using fast chromatography with the 20 mm fused-core column, similar to that shown above, have been validated for GLP use. For a typical method validation, the gradient is made shallower (using a 30–50% gradient change in 1 min) in order to improve selectivity for the specific analyte, and the flow rate is reduced to 1 mL/min to enable the method to be transferred to other laboratories if necessary.

As an example, one validated method used liquid–liquid extraction with MTBE and consisted of a 1 min gradient with acetonitrile from 25 to 50% organic, with an LC/MS time of 1.5 min. Table 3 shows the combined QC statistics for two GLP toxicology studies in two different species. Incurred sample reanalysis (ISR) data was also excellent. For 86 total ISRs, the range of assay variability was −11 to 16%, with a mean of 0.4% and a % CV of 10%. Assay variability % is calculated as:

$$\left[\frac{(\text{repeat analysis concentration} - \text{original concentration})}{\text{average concentration}}\right] \times 100.$$

Table 3
Results from GLP studies using a validated method using a 20 mm fused-core column with a 1 min gradient.

	Low QC	Mid QC	High QC
Number of QCs	33[a]	34	34
% Relative error	−2.8	−0.7	−3.3
% CV	5.3	5.1	2.9

[a] 1 QC was a missed injection.

4. Conclusions

Results from extended use of fast LC/MS/MS assays and over 2 years of using fused-core columns have been shown. All the criteria necessary for high quality bioanalytical data have been evaluated and have been found to be achievable with methods less than 1 min LC/MS times, even with traditional 400 bar LC pumps. Because of their lower back pressure and analytical performance, fused-core columns have been chosen as a standard column for these methods. In addition, a single fused-core column typically has withstood more than one thousand injections without reduced performance. Generic HPLC methods developed with these columns and described here have been very useful for a wide range of analytes in early discovery studies when method development is undesirable or often unnecessary. These generic methods have also been adopted for all LC/MS/MS screening assays used in drug metabolism studies such as microsome stability, CYP induction, and cytochrome P450 inhibition. Generic bioanalytical methods have been further developed into validated methods for a number of compounds.

Current limitations of this approach include the lack of widespread availability of higher pressure pumps for running assays at flow rates higher than 2.0 mL/min. Also, in cases of extreme carryover, syringe washing time may exceed the LC/MS time. In cases where carryover is manageable, however, autosampler overhead time may ultimately limit throughput.

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