ELSEVIER

Journal of Chromatography A, 926 (2001) 43–55

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Applications of new liquid chromatography–tandem mass spectrometry technologies for drug development support

Liyu Yang, Ning Wu, Patrick J. Rudewicz*

Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033-1300, USA

Abstract

We have evaluated (i) a multiplexed electrospray interface, (ii) serial sample introduction, and (iii) a quadrupole time-of-flight mass spectrometer for quantitative bioanalysis in compliance with good laboratory practice. These evaluations were done using a 96-well plate liquid chromatography–tandem mass spectrometry method for the quantitation of loratadine and its metabolite, descarboethoxyloratadine. The assay has a dynamic range of 1–1000 ng/ml with 5.56 pg of each analyte being injected on-column at the limit of quantitation. For the four-channel multiplexed electrospray experiments, one-run validations were performed simultaneously in rat, rabbit, mouse and dog plasma. In the four-stream serial experiments, the total run time of the assay was reduced from 3.5 to 0.35 min, resulting in a net acquisition time of 11 s. Four simulated validation runs with standard and quality control solutions were analyzed. Precision and accuracy for standards and quality control samples met US Food and Drug Administration recommended criteria for both the drug and the metabolite using those two approaches. In addition, a quadrupole time-of-flight mass spectrometer was used as a detector in the tandem mass spectrometry mode for the loratadine assay. Our results demonstrated that a dynamic range of three orders of magnitude could be achieved using the quadrupole time-of-flight mass spectrometer, making it useful for quantitation in preclinical toxicology studies.

Keywords: Liquid chromatography–mass spectrometry; Quality control; Instrumentation; Multiplexed electrospray ionization; Interfaces, LC–MS; Electrospray ionization; Loratadine; Descarboethoxyloratadine

1. Introduction

In an effort to reduce the time required to get a new drug on the market, bioanalytical laboratories are continually investigating new strategies for improving sample preparation, chromatography, and mass spectrometric detection. Very often, samples are prepared using a robotic system with 96- or 384-well microplates [1–4]. With microplates, various methods have been used for sample clean-up, including protein precipitation [5], liquid–liquid [6] and solid-phase extraction [7]. Microplate sample preparation has also been combined with fast chromatography to dramatically increase sample throughput in liquid chromatography–tandem mass spectrometry (LC–MS–MS) analyses [8]. For example, with a total run time of less than 30 s, over 2000 human plasma samples were analyzed within a 24-h period [9].

Apart from the off-line 96-well plate format, on-line sample processing has also been used to increase sample throughput in LC–MS–MS laboratories. Traditionally, this has been accomplished using column-switching high-performance liquid chromatography (HPLC) techniques [10]. Another approach is

*Corresponding author. Tel.: +1-908-740-6513; fax: +1-908-740-4474.

E-mail address: patrick.rudewicz@spcorp.com (P.J. Rudewicz).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)00963-3

the use of the Prospekt system (Spark-Holland, Emmen, Netherlands) in which disposable extraction columns are utilized for sample clean-up [11]. Turbulent flow chromatography (TFC) (Cohesive Technologies, Franklin, MA, USA) has also been used for the on-line extraction of samples. The TFC column may serve as both the sample purification and analytical column. TFC has been used for the analysis of multiple analytes in support of drug discovery [12]. TFC has also been compared to a traditional liquid–liquid extraction turboionspray LC–MS–MS assay for good laboratory practice (GLP)-toxicokinetic support [13].

In our laboratory, we have investigated several approaches to increasing the speed and efficiency of GLP-compliant quantitative LC–MS–MS analyses to support drug development studies. We have explored parallel analysis using a four-channel multiplexed electrospray ion source (MUX) on a triple quadrupole mass spectrometer to increase throughput in GLP quantitative analysis [14]. With this interface, the effluents from four HPLC columns are introduced into the mass spectrometer simultaneously. A four-channel multiplexed electrospray ion source interfaced to a time-of-flight mass spectrometer for the qualitative analysis of a mixture of four test compounds has been reported [15].

We have also evaluated a multi-stream serial LC approach to increase bioanalytical throughput. Serial LC–MS–MS can provide high throughput capability, particularly in cases where the useful run time is a fraction of the total analysis time. When used in conjunction with high-speed chromatographic separation, serial LC–MS–MS provides not only higher throughput but also increased sensitivity afforded by narrower chromatographic peaks.

In addition, we have evaluated a quadrupole time-of-flight (Q-TOF) mass spectrometer for use in GLP-quantitative bioanalysis. Q-TOF-MS generates high-resolution and accurate mass product ion spectra, which are critical for trouble-shooting matrix or metabolite interference during assay development or routine sample analysis. The enhanced full scan sensitivity of a time-of-flight mass analyzer makes it useful in drug development where sample amount or concentration is often limited. Moreover, Q-TOF-MS could also provide an alternative to the triple quadrupole mass spectrometer multiple reaction monitoring (MRM) approach for quantitation of low-molecular-mass drugs and their metabolites in biological matrices with acceptable sensitivity and superior selectivity. The quantitative properties of Q-TOF-MS have been investigated using 3,4-methylenedioxymethamphetamine [16]. The results compared favorably with a validated HPLC method with fluorescence detection.

In this paper, we describe our evaluation of (i) four-channel parallel (MUX) analysis, (ii) multi-stream serial LC analysis, and (iii) the use of a quadrupole time-of-flight mass spectrometer for drug development quantitation. In our laboratory, sample analysis is carried out in a GLP-compliant manner and therefore the LC–MS–MS methods need to be validated according to currently accepted US Food and Drug Administration (FDA) guidelines [17]. These three techniques were evaluated using the same assay, a 96-well plate electrospray LC–MS–MS method for the quantitative determination of loratadine and its metabolite, descarboethoxyloratadine.

Loratadine is a long-acting tricyclic antihistamine with selective peripheral histamine H_1-receptor antagonistic activity. To support clinical studies, an LC–MS–MS method with 96-well plate solid-phase extraction was developed and validated for both loratadine and its metabolite, descarboethoxyloratadine [18]. This assay had a quantitative range of 0.025–10 ng/ml for both the drug and the metabolite. As part of our efforts to evaluate new LC–MS–MS technology, we changed the concentration range of the loratadine assay and evaluated each of these three techniques by performing validation experiments from 1 to 1000 ng/ml. The results of these experiments as well as the advantages and disadvantages of each approach are discussed.

2. Experimental

2.1. Materials and reagents

The analytes, SCH 29851 (loratadine) and SCH 34117 (descarboethoxyloratadine), and their isotopically labeled I.S. [$^2H_4$]SCH 29851 and [$^2H_4$]SCH 34117 (Fig. 1), were synthesized by Schering-Plough Research Institute (Kenilworth, NJ, USA). Rat,

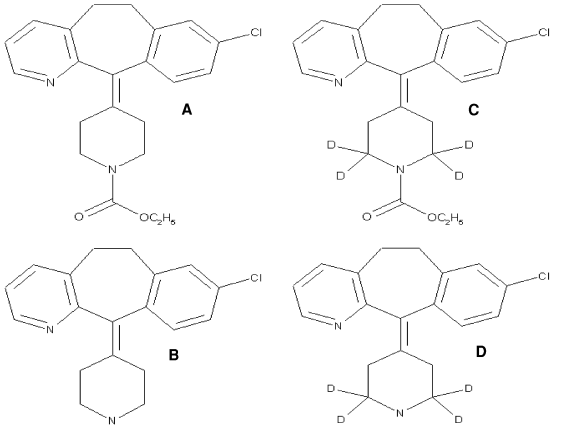

Fig. 1. Chemical structures of (A) SCH 29851 (loratadine), (B) SCH 34117 (descarboethoxyloratadine) and (C, D) their respective isotopically labeled internal standards.

mouse, rabbit, and dog plasma, with tetra sodium salt ethylenediaminetetraacetic acid (EDTA) as anticoagulant, were purchased from Bioreclamation (Hicksville, NY, USA). Optima grade (99.9%) acetonitrile and optima grade (99.9%) methanol were obtained from Fisher Scientific (Pittsburgh, PA, USA). Formic acid (minimum 95%, ~5% water and 0.5% acetic acid) was obtained from Sigma (St. Louis, MO, USA). ReagentPlus grade (99.99%) ammonium acetate and glacial acetic acid (99.99%) were obtained from Aldrich (Milwaukee, WI, USA). Milli-Q water used in this study was purified in the laboratory with an A10 Millipore water purification system (Millipore, Bedford, MA, USA).

2.2. Sample preparation

Analyte stock solution containing SCH 29851 and SCH 34117 at 100 μg/ml each was prepared in methanol. Internal standard (I.S.) stock solution containing [$^2H_4$]SCH 29851 and [$^2H_4$]SCH 34117 at 100 μg/ml each was also prepared in methanol. Calibration curve standards (STD) were prepared at ten concentrations: 1, 2, 5, 10, 20, 50, 100, 200, 500 and 1000 ng/ml. Quality control (QC) samples were prepared at four concentrations: QC limit of quantitation (LOQ) at 1 ng/ml, QC low at 3 ng/ml, QC medium at 400 ng/ml, and QC high at 800 ng/ml. STD 10 at 1000 ng/ml was prepared by diluting 250 μl of the analyte stock solution into 25 ml of blank plasma. Standards 1–9 were prepared by serial dilutions of STD 10 with blank plasma. Similarly, QC high at 800 ng/ml was prepared by diluting 200 μl of the analyte stock solution into 25 ml of blank plasma. QC samples at the LOQ, low, and medium levels were prepared by serial dilutions of QC high with blank plasma. Internal standard working solution (I.S.W.S.) at 40 ng/ml in 100 m*M* ammonium acetate pH 6.0 was prepared by diluting 200 μl of I.S. stock solution (100 μg/ml) into 500 ml of 100 m*M* ammonium acetate solution at pH 6.0 in a 500-ml volumetric flask.

2.3. Extraction procedure

A 500-μl volume of each plasma sample was aliquoted into a Coster cluster tube (Fisher Scientific, Fair Lawn, NJ, USA) and arranged into the 96-well format. The extraction procedure was carried out using a TomTec Quadra 96 Model 320 liquid handling system (TomTec, Hamden, CT, USA) and 3M Empore C_{18} SD 96-well extraction disk plate (Varian Associates, Sugar Land, TX, USA). A 250-μl volume of [$^2H_4$]SCH 29851 and [$^2H_4$]SCH 34117 internal standard working solution (I.S.W.S.) (40 ng/ml) was added to each sample tube, except for the double blanks in which 250 μl of 100 m*M* ammonium acetate pH 6.0 was added instead of I.S.W.S. The C_{18} disk plates were conditioned with 0.8 ml of methanol and 0.8 ml of Milli-Q water. Each sample was loaded onto the plates in two aliquots. After the sample had completely flowed through the plate, the plate was washed with 0.8 ml of Milli-Q water and 0.8 ml solution B (2 m*M* ammonium acetate, 0.1% acetic acid, 0.1% formic acid in acetonitrile–methanol (50:50, v/v))–solution A (2 m*M* ammonium acetate, 0.1% acetic acid, 0.1% formic acid in water) (20:80). The analytes and their respective I.S. were then eluted into a 1-ml 96-well collection plate (Fisher Scientific) using 150 μl of solution B. The C_{18} disk plates were then washed with 300 μl of solution A. This yielded ~450 μl of processed sample solution. After mixing, 5 μl was injected directly into the LC–MS–MS system. At the LOQ, this represented 5.56 pg of each analyte injected onto the HPLC column.

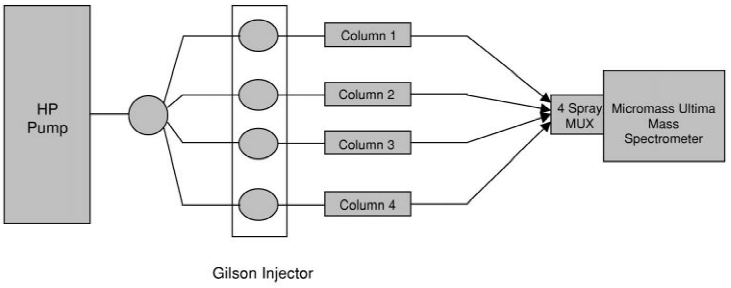

Fig. 2. Schematic diagram of the four-channel multiplexed electrospray LC–MS–MS experimental set-up.

2.4. Liquid chromatography–mass spectrometry for parallel analysis using MUX

A schematic of the parallel LC–MS–MS system is shown in Fig. 2. The LC–MS–MS system consisted of a Gilson 215/889 Multiple Injection Module (Gilson, Middleton, WI, USA), an HP 1100 pump system (Hewlett-Packard, Palo Alto, CA, USA), four HPLC columns and guard columns (BDS-C_8, 100×2 mm and 20×2 mm, 5 μm, Keystone Scientific, Bellefonte, PA, USA), and a Micromass Quattro Ultima triple quadrupole equipped with a MUX-technology four-channel multiplexed electrospray ion source (Micromass UK, Manchester, UK). Fig. 3 shows a schematic diagram of the four-channel multiplexed electrospray ion source. Although the

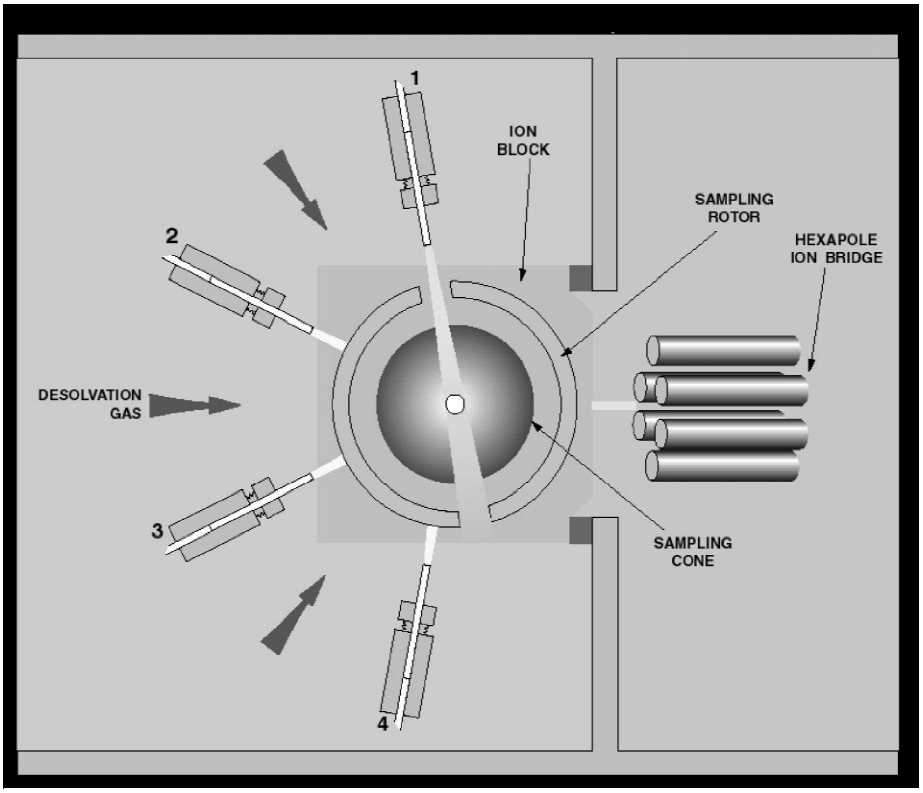

Fig. 3. Schematic diagram of the four-channel multiplexed electrospray ion source.

effluents from four HPLC columns were continuously introduced into the mass spectrometer through four sprayers, at any one time, the position of the sampling rotor allows only the spray from one probe to be admitted into the sampling cone of the mass spectrometer. A programmable stepper motor, which is controlled by the Micromass Masslynx data system, controls the position of the sampling rotor. This allows the data system to track the data from each of the four sprays separately. Detailed descriptions of the parallel LC–MS–MS system with MUX interface and the experimental condition are available elsewhere [14].

2.5. Liquid chromatography–mass spectrometry for multi-stream serial analysis

The goal of the experiment was to develop a multi-stream LC–MS system using readily available LC devices, apply the concept of serial LC to the loratadine assay, and then optimize the timing and chromatography for maximum throughput. For this experiment, extracts were not prepared, rather the extracts were simulated by manually preparing solutions and using a Gilson 2158-probe liquid handler to create duplicate plates from the stock solutions.

Fig. 4 is a schematic of a four-stream LC system showing the connections necessary to achieve synchronization and serial introduction to the mass spectrometer. The external run output from pump 4 is wired to the ready signal of AS1 to start the system. A four-stream isocratic LC system that consisted of Perkin-Elmer 200 Micro pumps and autosamplers (Perkin-Elmer, Norwalk, CT, USA) was used. The HPLC columns were Betasil-C_8, 3 μm, 20×2 mm (Keystone Scientific). Isocratic conditions of methanol–25 m*M* ammonium formate, pH 3.5 (85:15, v/v) at a flow rate of 1.35 ml/min were

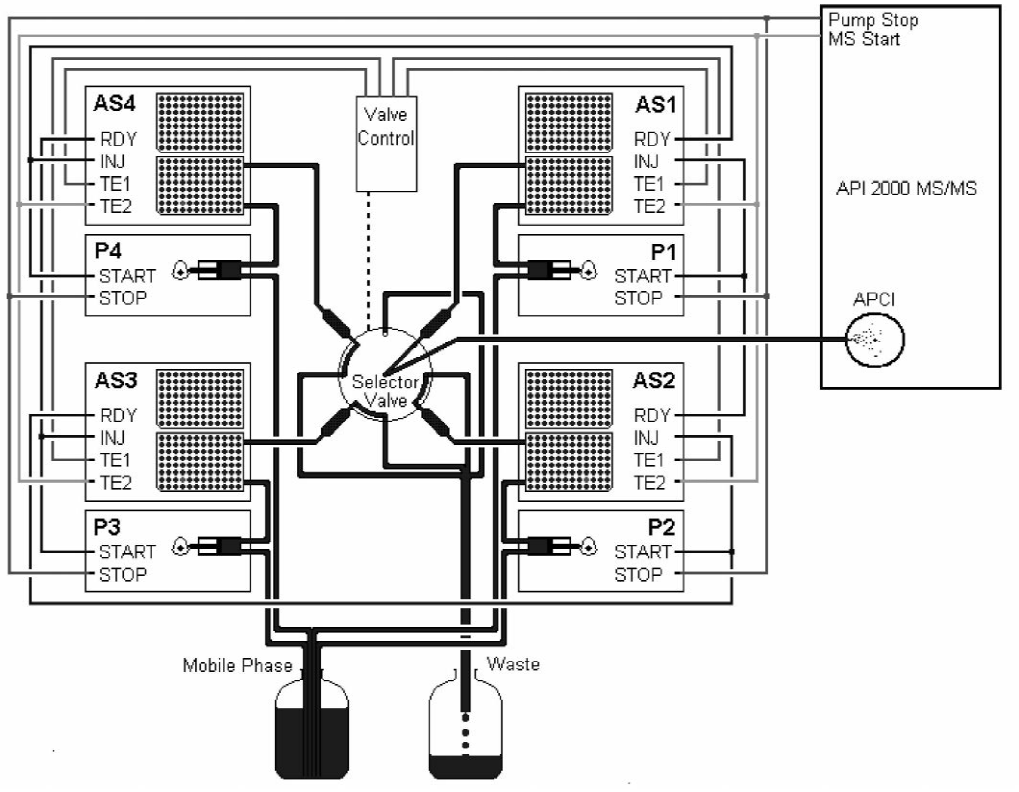

Fig. 4. Schematic of a four-stream LC system showing the connections necessary to achieve synchronization and serial introduction to the mass spectrometer.

used. A PE-Sciex API 2000 triple quadrupole mass spectrometer (PE Sciex Instruments, Concord, Ont., Canada) was used in the positive ion MRM mode. The following MRM transitions were monitored for the analytes and internal standards: SCH 34117, m/z 311 to 259; [$^2H_4$]SCH 34117, m/z 315 to 263; SCH 29851, m/z 383 to 337; [$^2H_4$]SCH 29851, m/z 387 to 341. These are the same ion transitions monitored in the MUX experiment. The dwell time was 60 ms.

2.6. Liquid chromatography–mass spectrometry for the quadrupole time-of-flight

The LC system consisted of a Shimadzu LC-10AD VP liquid chromatograph pump (Shimadzu, Columbia, MD, USA) and a Perkin-Elmer 200 Series autosampler. Separation of SCH 29851 and SCH 34117 was achieved using a Keystone BDS-C_8 column and guard column (100×2 mm and 20×2 mm, 5 μm) (Keystone Scientific) and isocratic elution with methanol–25 m*M* ammonium formate, pH 3.5 (85:15, v/v) as the mobile phase. The flow rate was 0.3 ml/min and the total run time was 3.5 min.

The analytes and I.S. were detected using a PE-Sciex QSTAR Pulsar quadrupole orthogonal acceleration time-of-flight mass spectrometer in the MS–MS mode, with TurboIonSpray ionization. The collision energy was 20 eV for all the analytes and internal standards.

The time-of-flight was operated with a pulsing frequency of 10 kHz. Calibration was achieved using CsI at m/z 133 and a peptide, ALILTLVS, at m/z 829. The resolution of the time-of-flight was 8000, full width half maximum (FWHM) at m/z 829. The quadrupole mass analyzer was set to unit resolution.

3. Results and discussion

3.1. MUX

For the MUX interface evaluation, a one-run method validation over the concentration range of 1–1000 ng/ml was performed simultaneously in four preclinical species: dog, rat, mouse and rabbit plasma. Representative ion chromatograms for LOQ QC samples for SCH 29851 and SCH 34117 extracted from rat plasma are shown in Fig. 5. The chromatograms show good signal-to-noise ratios for both analytes at the LOQ. The statistics for the QCs are summarized in Tables 1 and 2.

The advantage of using the MUX interface is the four-fold increase in throughput. However, certain aspects of MUX technology present challenges to the analyst, including cross-talk between the sprayers,

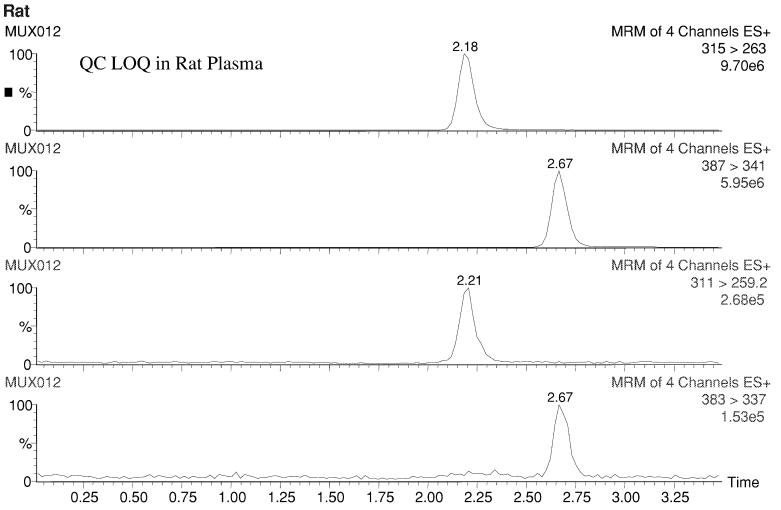

Fig. 5. Multiple reaction ion chromatograms for limit of quantitation quality control sample in rat plasma showing from bottom trace to top trace: SCH 29851, SCH 34117, [$^2H_4$]SCH 29851 and [$^2H_4$]SCH 34117. Time scale in minutes.

Table 1
Quality control sample concentrations of SCH 29851 (within-run precision and accuracy obtained from MUX interface)

Statistics	QC LOQ 1.00 (ng/ml)	QC low 3.00 (ng/ml)	QC medium 400 (ng/ml)	QC high 800 (ng/ml)
Rabbit plasma				
Mean	0.982	2.82	409	829
RSD (%)	6.9	3.2	1.2	1.8
n	6	6	6	6
Mean diff. (%)	−1.8	−5.9	2.2	3.6
Mouse plasma				
Mean	1.06	3.10	414	833
RSD (%)	3.8	2.8	1.1	2.7
n	6	6	6	6
Mean diff. (%)	5.8	3.3	3.4	4.2
Rat plasma				
Mean	1.11	2.75	409	836
RSD (%)	16	2.3	1.0	1.2
n	6	6	6	6
Mean diff. (%)	11	−8.4	2.3	4.4
Dog plasma				
Mean	0.952	2.90	417	823
RSD (%)	11	2.2	0.97	2.1
n	6	6	6	6
Mean diff. (%)	−4.8	−3.4	4.2	2.9

Table 2
Quality control sample concentrations of SCH 34117 (within-run precision and accuracy obtained from MUX interface)

Statistics	QC LOQ 1.00 (ng/ml)	QC low 3.00 (ng/ml)	QC medium 400 (ng/ml)	QC high 800 (ng/ml)
Rabbit plasma				
Mean	1.05	2.95	422	851
RSD (%)	6.5	2.1	1.5	2.0
n	6	6	6	6
Mean diff. (%)	4.7	−1.7	5.6	6.4
Mouse plasma				
Mean	1.02	3.08	407	816
RSD (%)	8.5	2.5	1.7	2.1
n	6	6	6	6
Mean diff. (%)	2.2	2.6	1.8	2.0
Rat plasma				
Mean	1.05	2.73	401	810
RSD (%)	10	2.6	0.68	2.2
n	6	6	6	6
Mean diff. (%)	4.8	−9.1	0.26	1.3
Dog plasma				
Mean	0.973	2.90	413	821
RSD (%)	11	2.3	0.72	1.3
n	6	6	6	6
Mean diff. (%)	−2.7	−3.4	3.4	2.6

decrease in sensitivity relative to a single spray ion source, and increase in total cycle time [14]. Since four LC effluents are sprayed simultaneously into the mass spectrometer, cross-talk exists between the sprayers. We found that the cross-talk was ~0.01% at 100 ng/ml and 0.08% at 1000 ng/ml for loratadine and its metabolite, descarboethoxyloratadine. This may present limitations in the dynamic range of the assay. Second, sensitivity of the MUX interface was found to be about three times lower than the single sprayer interface. This is because with the MUX interface, the position of the sprayers could not be optimized as with the single sprayer interface. In addition, the electrospray desolvation efficiency in the MUX interface may be lower than the single sprayer interface. Third, total cycle time with MUX is longer than with a single sprayer interface. This will limit the number of analytes and I.S. that may be monitored in an assay.

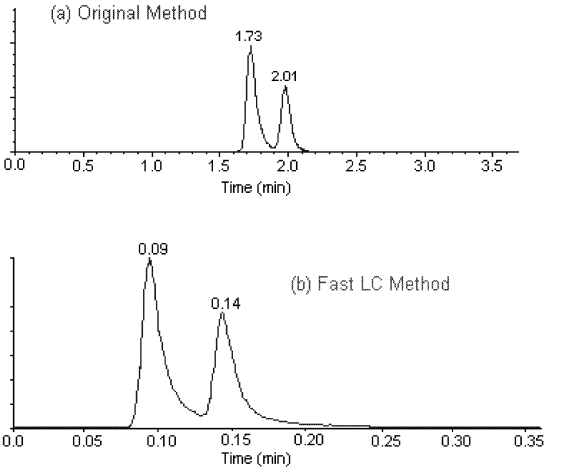

Fig. 6. (a) Chromatogram obtained using the four-stream LC system and the original LC method showing a net analysis time of 52 s. (b) Chromatogram obtained using the fast LC method showing a net analysis of 11 s.

3.2. Multi-stream serial

Even with the selectivity of a triple quadrupole mass spectrometer as a detector, LC–MS–MS methods still have relatively long run times, the length of which is dictated by several factors. First, whether using isocratic or gradient elution, time is required for separation of the analytes from matrix components to reduce ion suppression. When separation is achieved with a fast gradient, the column needs to be equilibrated. When combined with the inevitable overhead (dead volume, autosampler rinse, etc.) associated with LC devices, the actual fraction of run time which provides useful information is frequently less than 25% of the total analysis time. The result of these steps is to add ''useless'' acquisition time to the run. The application of serial chromatography in a multi-stream LC system, will for most simple assays, provide a significant boost in throughput, with no change to the existing method.

Using the original isocratic loratadine method, the total run time was 3.5 min and the net run time was 52 s (Fig. 6a). To further enhance sample throughput, a fast LC method was developed with a total run time of 0.35 min and a net acquisition time of 11 s (Fig. 6b). This was achieved using a 2-cm rather than a 10-cm HPLC column and increasing the flow rate from 0.3 to 1.35 ml/min. Fig. 7 shows the synchronization of the four-stream LC–MS–MS system using the 11-s net acquisition time method.

Using the fast LC method, a preliminary evaluation of the multi-stream serial methodology was done by analyzing solutions at four QC levels ($n=6$ at each level) and calibration curve solutions at ten calibration curve standard concentrations in duplicate. The QCs and calibration curve standards were prepared in the 96-well plate format. The QC results for SCH 29851 and SCH 34117 are listed in Tables 3 and 4, respectively.

These data demonstrate that multi-stream serial analysis is a viable approach to high-throughput LC–MS–MS analysis. There are several advantages to this approach. Serial LC–MS–MS can provide higher throughput capability than parallel introduction, particularly in cases where the useful run time is a fraction of the total analysis time. In addition, serial LC is not limited to slower chromatography. There is no loss of fidelity at faster chromatographic speeds. In fact, the performance of the mass spectrometer is enhanced by narrower peak widths. Furthermore, a multi-stream serial LC system is easily interfaced to existing mass spectrometers and software.

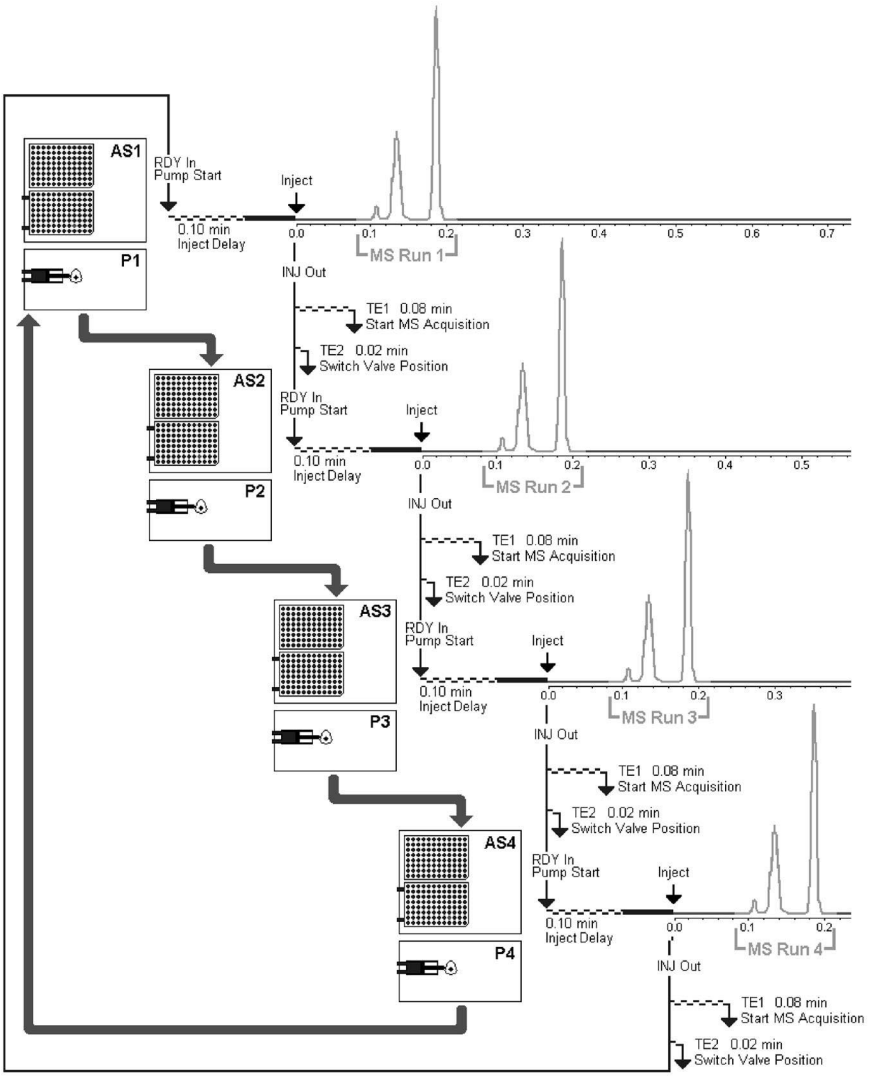

Fig. 7. Synchronization of a four-stream LC system used to provide a net analysis time of 11 s per sample.

3.3. QSTAR Pulsar

The workhorse of the LC–MS bioanalytical laboratory is the triple stage quadrupole mass spectrometer (TSQ). Usually MRM experiments are used for GLP quantitation. In the MRM experiment, a precursor or parent ion is selected in the first quadrupole (Q1), collisionally dissociated in the second quadrupole (Q2) and one product ion is monitored with the third quadrupole (Q3). Hence, in the MRM experiment, we have three layers of selectivity: chromatographic, precursor ion, and product ion selectivity. Normally this works well; however, specificity issues may still arise with metabolite or matrix interference.

We are particularly interested in the ability to obtain full scan product ion spectra using a Q-TOF system in development bioanalytical assays, especially in preclinical assays when definitive radiolabeled metabolism studies may not have yet been

Table 3
Quality control sample concentrations of SCH 29851 (within-run precision and accuracy obtained from serial LC approach)

Statistics	QC LOQ 1.00 (ng/ml)	QC low 3.00 (ng/ml)	QC medium 400 (ng/ml)	QC high 800 (ng/ml)
Plate 1				
Mean	0.920	3.89	396	807
RSD (%)	8.6	2.7	1.7	1.9
n	6	6	6	6
Mean diff. (%)	−8.1	−2.7	−1.0	0.88
Plate 2				
Mean	0.945	3.83	397	775
RSD (%)	8.0	4.7	2.7	3.3
n	6	6	6	6
Mean diff. (%)	−5.5	−4.3	−0.67	−3.1
Plate 3				
Mean	0.908	3.89	393	794
RSD (%)	3.9	2.9	1.9	2.0
n	6	6	6	6
Mean diff. (%)	−9.3	−0.33	−1.8	−0.77
Plate 4				
Mean	0.919	3.87	392	796
RSD (%)	9.0	5.6	2.1	2.2
n	6	6	6	6
Mean diff. (%)	−8.1	−3.3	−2.1	−0.45

Table 4
Quality control sample concentrations of SCH 34117 (within-run precision and accuracy obtained from serial LC approach)

Statistics	QC LOQ 1.00 (ng/ml)	QC low 3.00 (ng/ml)	QC medium 400 (ng/ml)	QC high 800 (ng/ml)
Plate 1				
Mean	0.989	3.89	394	786
RSD (%)	3.2	2.1	2.0	1.5
n	6	6	6	6
Mean diff. (%)	−1.0	−2.9	−1.5	−1.7
Plate 2				
Mean	0.984	3.94	394	801
RSD (%)	5.8	4.1	2.0	1.1
n	6	6	6	6
Mean diff. (%)	−1.6	−1.4	−1.4	0.17
Plate 3				
Mean	1.00	3.74	385	759
RSD (%)	4.8	3.9	5.0	5.1
n	6	6	6	6
Mean diff. (%)	0.15	−6.7	−2.8	−5.2
Plate 4				
Mean	1.02	3.87	398	795
RSD (%)	5.5	5.6	1.4	1.7
n	6	6	6	6
Mean diff. (%)	2.0	−2.8	−0.42	−0.56

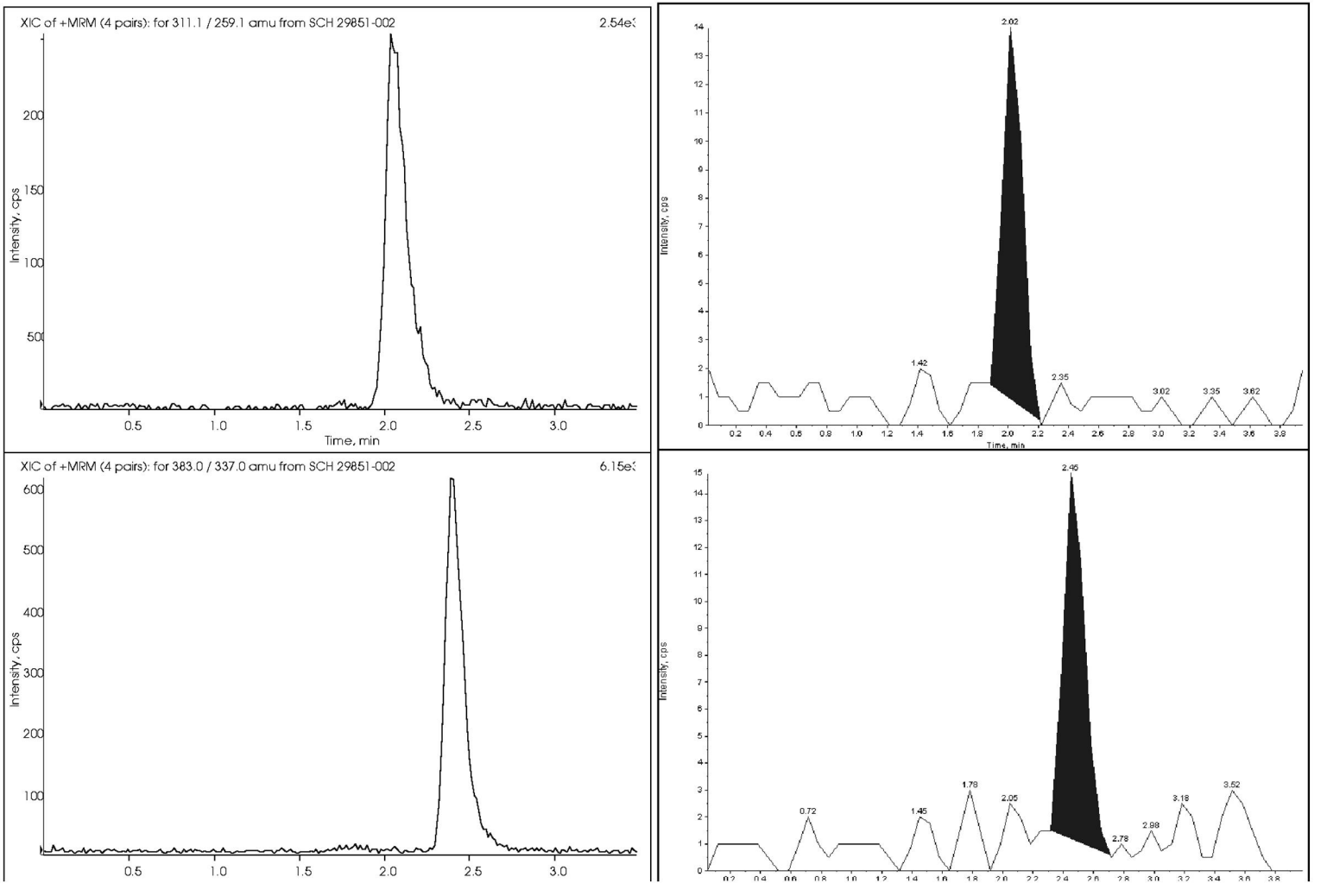

Fig. 8. Reconstructed ion chromatograms for a 1-ng/ml (LOQ) sample in dog plasma obtained from API 3000 (left) and QSTAR Pulsar (right). From bottom trace to top trace: SCH 29851, SCH 34117.

Table 5
Comparison of the within-run precision and accuracy obtained from API 3000 and QSTAR Pulsar

Instrument	Statistics	QC low 3.00 (ng/ml)	QC medium 400 (ng/ml)	QC high 800 (ng/ml)
API 3000	*SCH 29851*			
	Mean	3.03	415	844
	RSD (%)	2.1	2.8	3.2
	n	6	6	6
	Mean diff. (%)	1.0	3.8	5.5
	SCH 34117			
	Mean	2.99	407	841
	RSD (%)	3.2	1.6	1.8
	n	6	6	6
	Mean diff. (%)	−0.33	1.8	5.1
QSTAR Pulsar	*SCH 29851*			
	Mean	2.72	410	896
	RSD (%)	11	4.6	9.5
	n	6	6	6
	Mean diff. (%)	−9.3	2.5	12
	SCH 34117			
	Mean	3.30	443	864
	RSD (%)	11	6.9	7.7
	n	6	6	6
	Mean diff. (%)	10	11	8.0

done. Although we realized that a Q-TOF system in the MS–MS mode is not as sensitive as a TSQ in the MRM mode, we wanted to explore using a Q-TOF system for quantitative analysis to support GLP toxicology studies where the LOQs are often in the ng/ml range or higher. For this purpose, we used the assay for SCH 29851 and its metabolite, SCH 34117, with a dynamic range from 1 to 1000 ng/ml, a typical range used to support preclinical toxicology studies.

A one-run method validation over the concentration range of 1–1000 ng/ml was performed in dog plasma. The run contained duplicate calibration curve standards at ten concentrations, QC samples at three concentrations ($n=6$ at each concentration) and four matrix blanks. The concentrations for the calibration curve standards and QC samples were determined for SCH 29851 and SCH 34117 using weighted quadratic regressions.

Reconstructed ion chromatograms for SCH 29851 and SCH 34117 at the LOQ of 1 ng/ml using the QSTAR Pulsar are compared to those obtained with a Sciex API 3000 in Fig. 8. The signal-to-noise ratio at this level was ~10–20 times less than that obtained on a Sciex API 3000 in the MRM mode. Nevertheless, a complete product ion spectrum is obtained at a resolution of 8000 (FWHM) using the QSTAR Pulsar, providing more information than the triple quadrupole in the MRM mode. With the QSTAR Pulsar, quality control samples at 3, 400, and 800 ng/ml had acceptable (within 15%) precision and accuracy and the results compared favorably with those obtained using an API 3000 (Table 5).

4. Conclusions

Several new LC–MS–MS approaches were evaluated that can be utilized to increase the speed and efficiency in GLP-compliant bioanalytical laboratories. With MUX, simultaneous validation experiments were done in four preclinical species, decreasing the analysis time by a factor of four. By combining fast chromatography and serial LC–MS–MS, net acquisition time was reduced to 11 s, substantially increasing sample throughput. With the quadrupole time-of-flight, a working dynamic range of three orders of

magnitude was demonstrated, making it useful for quantitation in preclinical toxicology studies where sensitivity requirements are generally not too demanding.

Acknowledgements

The authors would like to acknowledge Yves LeBlanc, Takeo Sakuma, Byron Kieser and Tanya Gamble of PE-Sciex as well as David Little, Thierry Mann and Donald Mason of Micromass for their contributions to this work.

References

[1] J. Henion, E. Brewer, G. Rule, Anal. Chem. 70 (1998) 650A.
[2] B. Kaye, W.J. Herron, P.V. Macrae, S. Robinson, R. Stopher, F. Venn, W. Wild, Anal. Chem. 68 (1996) 1658.
[3] J.P. Allanson, R.A. Biddlecombe, A.E. Jones, S. Pleasance, Rapid Commun. Mass Spectrom. 10 (1996) 811.
[4] H. Simpson, A. Berthemy, D. Buhrman, R. Burton, J. Newton, M. Kealy, D. Wells, D. Wu, Rapid Commun. Mass Spectrom. 12 (1998) 75.
[5] P. Watt, D. Morrison, K.L. Locker, D.C. Evans, Anal. Chem. 72 (2000) 979.
[6] L. Ramos, R. Bakhtiar, F.L.S. Tse, Rapid Commun. Mass Spectrom. 14 (2000) 740.
[7] S.X. Peng, S.L. King, D.M. Bornes, D.J. Foltz, T.R. Baker, M.G. Natchus, Anal. Chem. 72 (2000) 1913.
[8] J. Zweigenbaum, J. Henion, S. Steinborner, T. Wachs, J. Henion, Anal. Chem. 71 (1999) 2294.
[9] J. Zweigenbaum, J. Henion, Anal. Chem. 72 (2000) 2446.
[10] Y. Xia, D. Whigan, M. Powell, M. Jemal, Rapid Commun. Mass Spectrom. 14 (2000) 105.
[11] F. Beaudry, J.C. Yves Le Blanc, M. Coutu, N.K. Brown, Rapid Commun. Mass Spectrom. 12 (1998) 1216.
[12] J. Wu, H. Zeng, M. Qian, B.L. Brogdon, S.E. Unger, Anal. Chem. 72 (2000) 61.
[13] D. Zimmer, V. Pickard, W. Czembor, C. Muller, J. Chromatogr. A 854 (1999) 23.
[14] L. Yang, T.D. Mann, D. Little, N. Wu, R.P. Clement, P.J. Rudewicz, Anal. Chem. 73 (2001) 1740.
[15] V. de Biasi, N. Haskins, A. Organ, R. Bateman, K. Giles, S. Jarvis, Rapid Commun. Mass Spectrom. 13 (1999) 1165.
[16] K. Clauwaert, J. Van Bocxlaer, H.J. Major, J.A. Claereboudt, W.E. Lambert, E. Van den Eeckhout, C. Van Peteghem, A. De Leenheer, Rapid Commun. Mass Spectrom. 13 (1999) 1540.
[17] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pittman, S. Spector, Pharm. Res. 9 (1992) 588.
[18] L. Yang, R. Clement, F. Beaudry, C. Grandmaison, L. Di Donato, R. Masse, P. Rudewicz, Poster presented at the 48th American Society for Mass Spectrometry and Allied Topics, Long Beach, CA, 11–15 June, 2000.