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ORIGINAL RESEARCH

Open access liquid chromatography/tandem mass spectrometry: implementation of a fully quantitative system in a drug discovery laboratory

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Abstract: High performance liquid chromatography with electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) has been utilized to produce fully open access (OA) quantitative systems. The systems described have the ability to optimize the mass spectrometer analysis method, stack these optimizations, create the mass spectrometry analytical methods and automatically process data for reporting. An in-house, Microsoft® Excel-based sample list generator in conjunction with two manufacturers' OA software packages have been implemented and are used exclusively for all *in vivo* and *in vitro* drug metabolism and pharmacokinetic assays within the department. We demonstrate that the OA systems have had a positive effect on the average turnaround times of all assays by utilizing the mass spectrometer time more effectively and by minimizing instrument downtime. Turnaround times have been monitored for 17 months post implementation of the OA systems as part of a Lean Six Sigma efficiency optimization project, and data are presented that support a sustained improvement throughout this period.

Keywords: open access, DMPK, quantitation, bioanalysis, discovery

Introduction

The aim of drug discovery is to nominate safe and effective candidate drugs (CDs) for progression through the drug development process. A study of drug attrition rates in later stages of clinical development[1] revealed that a significant number of CDs fail owing to absorption, distribution, metabolism, elimination (ADME) and toxicological issues. Lead optimization initiatives[2] in drug discovery are the processes that attempt to minimize these issues prior to the handover of CDs and reduce costly failures in development. Discovery drug metabolism and pharmacokinetics (DxDMPK) departments are now standard in the pharmaceutical industry and have gone someway to address the ADME attrition of CDs. DMPK departments utilize a wide variety of assays (eg, metabolic stability, permeability, time dependent inhibition) to explore the ADME profile of a particular chemical series of interest. Liquid chromatography coupled with mass spectrometry on triple quadrupole instruments operating in selected reaction monitoring (SRM) mode has become the analytical method of choice for DMPK analysis.[3] This method offers high sensitivity, specificity, selectivity and rapid turnaround in a high throughput environment making it ideal for quantitation of low levels of analyte in the presence of biological endogenous material.

The DxDMPK section within the Cardiovascular and Gastrointestinal research area at AstraZeneca, Alderley Park has utilized Lean Six Sigma analysis[4] (LSS) to

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improve assay turnaround times by removing non-value activities. LSS is data driven and as such a period of data collection known as the 'measure phase' is employed to build an understanding of the current process under review. Subsequent data are analyzed to find the root cause of any 'blockers' to efficiency which were highlighted in the measure phase. During our initial LSS project aimed at improving the process of gathering *in vivo* pharmacokinetic (PK) data it was evident that mass spectrometer use and robustness was a major 'blocker' to improving the efficiency of the process.

Open access (OA) mass spectrometry combined with generic chromatography systems and standardized sample preparation was identified as a way of managing sample throughput in a more efficient manner. OA has been used in high throughput chemistry laboratories for many years,[5,6] essentially employing walk–up single quadrupole or time of flight instruments that confirm mass and give some indication of purity and structural confirmation. OA systems in quantitative environments have been described in the literature[7] but fall short of 'true' OA in so much as they lack the ability for multiple users to optimize mass spectrometer methods, to stack optimization methods, create SRM methods and automatically process data for reporting. Two manufacturers now offer integrated OA software solutions that can be utilized in a quantitative way (Figure 1). Thermo Fisher Scientific (Hemel Hempstead, UK) market software called QuickQuan™ and QuickCalc™ that enables OA sample analysis on their series of triple quadrupole mass spectrometers. Waters Corporation (Elstree, UK) market software known as OpenLynx™ and QuanBrowser™ that enables OA sample analysis on their range of mass spectrometers. Both manufacturers OA platforms are in use in our laboratories and are used exclusively for all *in vivo* and *in vitro* analysis within the department.

Experimental

Liquid chromatography-mass spectrometry instrumentation

Four different liquid chromatography/tandem mass spectrometry (LC-MS/MS) systems are used within our laboratories.

Mass spectrometers I and II: TSQ Quantum Vantage and TSQ Quantum Ultra (Thermo Fisher Scientific, Hemel Hempstead, UK) triple quadrupole mass spectrometers using an electrospray ionization source (HESI2™, High Efficiency ESI).

Mass spectrometers III and IV: Quattro Ultima and Quattro Ultima Pt (Waters Corporation, Elstree, UK), using an electrospray ionization source.

Mass spectrometers I and II were both fitted with a Surveyor MS Pump Plus HPLC pump, (Thermo Fisher Scientific, Hemel Hempstead, UK) and a CTC Analytics HTS PAL autosampler (Presearch Ltd, Basingstoke, UK). Each autosampler was fitted with three Vici Cheminert valves (Vici AG International, Schenkon, Switzerland). Valve I is used for analytical batch injection, valve II is used for optimization infusion and valve III is used for analytical column selection. A further switching valve located on the mass spectrometer is used to divert the LC flow to waste for the initial one minute of each injection in order to protect the MS source from contamination. All valve positions and instrument parameters were controlled by ThermoFisher Xcalibur™ software, version 2.0.0 (Thermo Fisher Scientific, Hemel Hempstead, UK).

Mass spectrometers III and IV were both fitted with a degasser unit, HPLC pump and column oven Agilent 1100 series (Agilent Ltd, Cheadle, UK), comprising a degasser (model G1322A), binary pump (model G1312A) and column switching module (G1316A). The column switching module is used for analytical column selection. Samples are

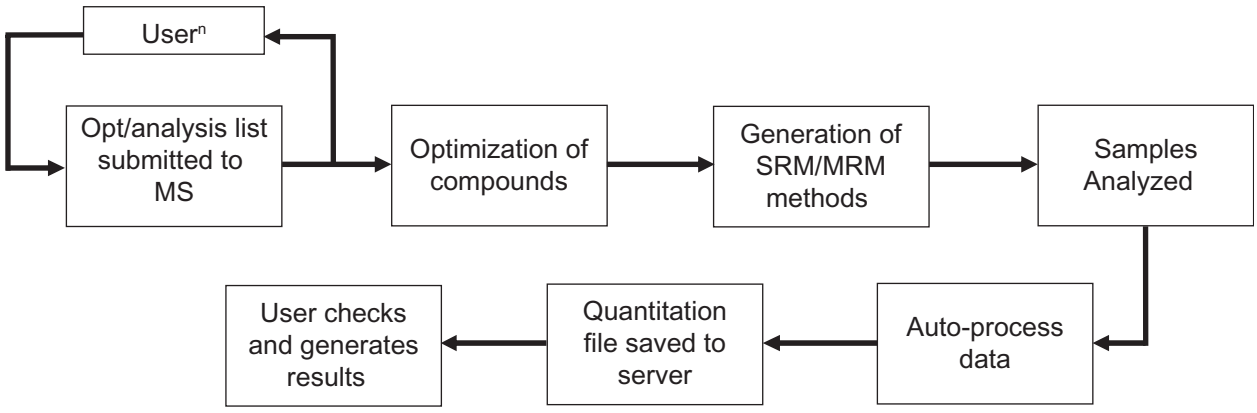


Figure 1 A schematic of the open access work flow. Each box shows a fully automated step in the open access process.

introduced into the system via a CTC Analytics HTS PAL autosampler (Presearch Ltd, Basingstoke, UK). A further switching valve located on the mass spectrometer is used to divert the LC flow to waste for the initial one minute of each injection in order to protect the MS source from contamination. All valve positions and instrument parameters were controlled by Waters MassLynx™ software, version 4.1 (Waters Corporation, Elstree, UK).

Open access software

Open Access sample login and data processing on the Thermo Fisher Scientific mass spectrometer systems was performed using the software packages Thermo Fisher Scientific QuickQuan™ (version 2.1) and QuickCalc™ (version 6.0.3). Open Access sample login and data processing on the Waters Corporation mass spectrometer systems was performed using the Waters Corporation software package OpenLynx™ (version 4.1), QuanLynx™ (version 4.0)

Open access liquid chromatography-mass spectrometry analysis methods

Four experimental methods (I to IV) are available by utilizing two linear gradient methods on two different columns: (1) Synergi Max RP (Phenomenex, Macclesfield, UK), 50 mm × 2.1 mm, 5 µm particle size, (2) Hypersil Gold C18 (Thermo Fisher Scientific, Loughborough, UK), 30 mm × 2.1 mm, 5 µm particle size. The methods offer the versatility to analyze a wide variety of drug candidates and maintain the balance between speed and chromatographic separation that is required in a high throughput environment.[8,9]

Gradient system I uses column (1) with mobile phases (A) 10 mM ammonium acetate in water and (B) 10 mM ammonium acetate in methanol. The linear gradient used is (T = minutes): at T = 0.0, 95% A:5% B, T = 3.0, 5% A: 95% B, T = 4.0, 5% A:95% B, T = 4.1, 95% A:5% B, T = 5.0, 95% A:5% B. This system is the preferred analytical method for all *in vivo* analysis within the department.

Gradient system II uses column (1) with mobile phases (C) 0.1% (v/v) formic acid in water and (D) 0.1% (v/v) formic acid in methanol. The linear gradient used is (T = minutes): at T = 0.0, 95% C:5% D, T = 3.0, 5% C: 95% D, T = 4.0, 5% C:95% D, T = 4.1, 95% C:5% D, T = 5.0, 95% C:5% D. This system is used for compounds that fail to give an adequate mass spectrometric response with gradient system I.

Gradient method III uses column (2) with mobile phase (C) and (D). The linear gradient used is (T = minutes): T = 0.0, 96% C:4% D, T = 1.0, 10% C:90% D, T = 1.9, 10% C:90% D, T = 2.0, 96% C:4% D, T = 2.5, 96% C:4% D. This system is the preferred analytical method for all *in vitro* analysis within the department.

Gradient method IV uses column (2) with mobile phase (A) and (B). The linear gradient used is (T = minutes): T = 0.0, 96% A:4% B, T = 1.0, 10% A:90% B, T = 1.9, 10% A:90% B, T = 2.0, 96% A:4% B, T = 2.5, 96% A:4% B. This system is rarely used but offers rapid analysis at pH 7 should this be required.

All of the systems use a flow rate of 0.75 mL/min and an injection volume of 50 µL are used.

Generic sample preparation

A generic sample preparation method has been introduced within the department with the intention of reducing variability, reducing instrument downtime and improving the quality of the data generated on the instruments.

50 µL of sample matrix from *in vivo* studies is protein precipitated with 200 µL of cold acetonitrile (4 °C) containing internal standard. The internal standard is project specific and chosen to represent the chemical series under investigation. The samples are then centrifuged at 3700 *g* for 10 minutes. 50 µL of the resulting supernatant is then diluted with 300 µL of deionized water prior to injection onto the OA systems.

Experiments involving *in vitro* incubations are halted by addition of cold acetonitrile (4 °C) containing internal standard. The resulting mixture is then centrifuged (3700 g) to remove any protein. 100 µL of supernatant is diluted with 100 µL of deionized water prior to injection onto the OA systems. All *in vitro* incubations were performed on a Tecan Genesis 200 (Tecan UK Limited, Reading, UK).

Quality control test solution preparation

A 1 mg weighing of erythromycin, lidocaine, propanolol and warfarin, (Sigma Aldrich, Poole, Dorset, UK) were individually dissolved in 1 mL of deionized water to form stock solutions. 50 µL of erythromycin, propanolol and warfarin stock solutions and 5 µL of lidocaine stock solution were then mixed and diluted with 1 L of deionized water to give a final concentration of 50 ng/mL erythromycin, propanolol, warfarin and 5 ng/mL lidocaine. 50 µL of this solution was injected onto the systems as a quality control between analytical batches.

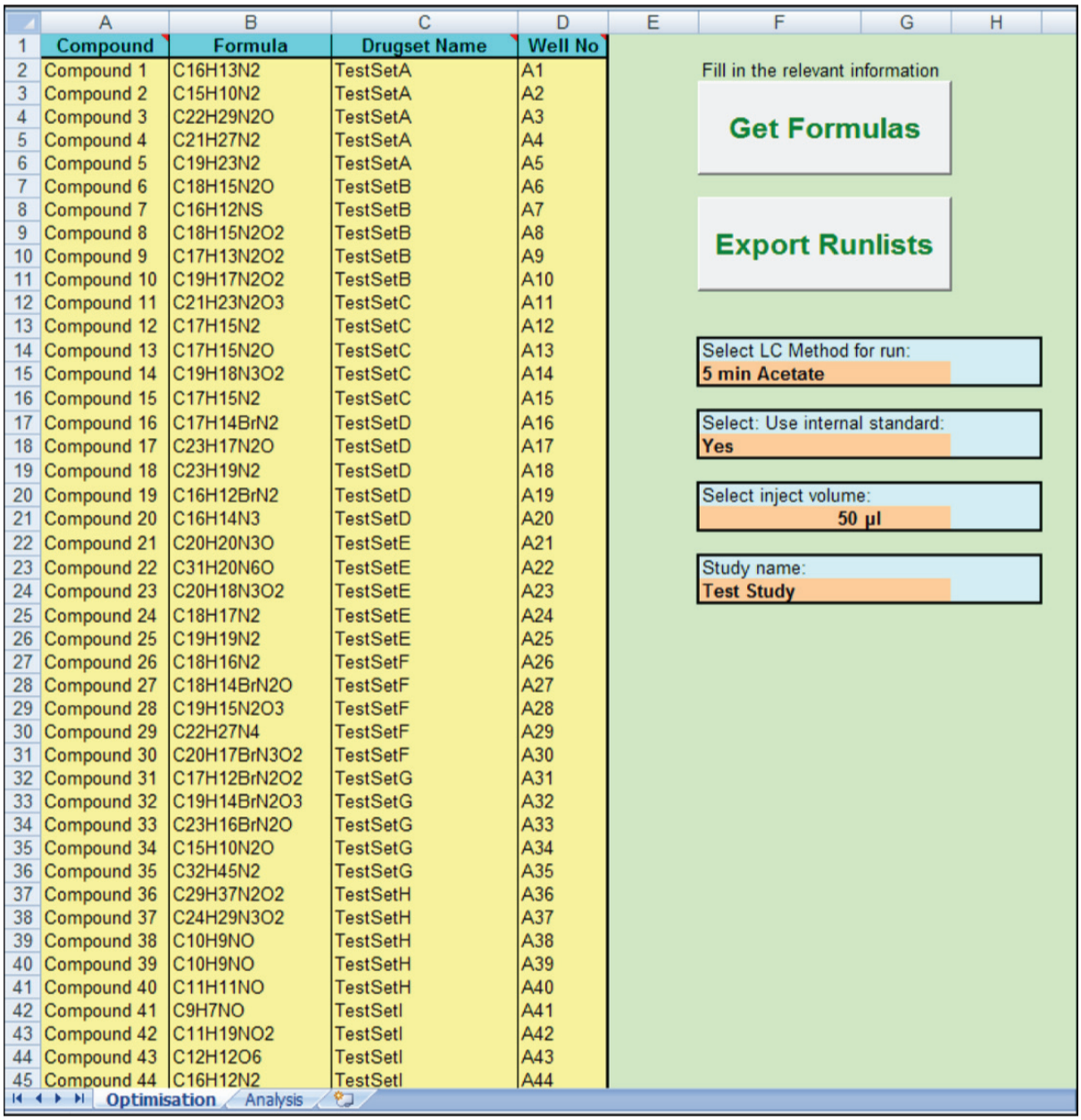
Results and discussion

OA using QuickQuan™ and QuickCalc™

The two Thermo Fisher Scientific TSQ Quantum mass spectrometers each function as identical OA systems. We have constructed a Microsoft® Excel 2003 (Microsoft

Corp, Seattle, USA) based workbook that is accessed via users office based workstations to enable easy construction of both optimization and analysis run lists. The workbook consists of two worksheets (Figure 2a and 2b). Optimization worksheet is populated with compound identification numbers. If compounds are in a cassette format they can be grouped into named drug sets which will be used to group SRM transitions post optimization. The optimization solution locations are also recorded on this worksheet (96-well plate format). The generic chromatography methods, injection volume and use of an internal standard are selected from drop down menus, the default settings are gradient system I with a 50 µL injection volume and inclusion of an internal standard as this is the most popular configuration and minimizes user input. The analysis worksheet is populated by the user with the sample injection order and other critical information such as drug set name, user specified

(A)



	A	B	C	D
1	Compound	Formula	Drugset Name	Well No
2	Compound 1	C16H13N2	TestSetA	A1
3	Compound 2	C15H10N2	TestSetA	A2
4	Compound 3	C22H29N2O	TestSetA	A3
5	Compound 4	C21H27N2	TestSetA	A4
6	Compound 5	C19H23N2	TestSetA	A5
7	Compound 6	C18H15N2O	TestSetB	A6
8	Compound 7	C16H12NS	TestSetB	A7
9	Compound 8	C18H15N2O2	TestSetB	A8
10	Compound 9	C17H13N2O2	TestSetB	A9
11	Compound 10	C19H17N2O2	TestSetB	A10
12	Compound 11	C21H23N2O3	TestSetC	A11
13	Compound 12	C17H15N2	TestSetC	A12
14	Compound 13	C17H15N2O	TestSetC	A13
15	Compound 14	C19H18N3O2	TestSetC	A14
16	Compound 15	C17H15N2	TestSetC	A15
17	Compound 16	C17H14BrN2	TestSetD	A16
18	Compound 17	C23H17N2O	TestSetD	A17
19	Compound 18	C23H19N2	TestSetD	A18
20	Compound 19	C16H12BrN2	TestSetD	A19
21	Compound 20	C16H14N3	TestSetD	A20
22	Compound 21	C20H20N3O	TestSetE	A21
23	Compound 22	C31H20N6O	TestSetE	A22
24	Compound 23	C20H18N3O2	TestSetE	A23
25	Compound 24	C18H17N2	TestSetE	A24
26	Compound 25	C19H19N2	TestSetE	A25
27	Compound 26	C18H16N2	TestSetF	A26
28	Compound 27	C18H14BrN2O	TestSetF	A27
29	Compound 28	C19H15N2O3	TestSetF	A28
30	Compound 29	C22H27N4	TestSetF	A29
31	Compound 30	C20H17BrN3O2	TestSetF	A30
32	Compound 31	C17H12BrN2O2	TestSetG	A31
33	Compound 32	C19H14BrN2O3	TestSetG	A32
34	Compound 33	C23H16BrN2O	TestSetG	A33
35	Compound 34	C15H10N2O	TestSetG	A34
36	Compound 35	C32H45N2	TestSetG	A35
37	Compound 36	C29H37N2O2	TestSetH	A36
38	Compound 37	C24H29N3O2	TestSetH	A37
39	Compound 38	C10H9NO	TestSetH	A38
40	Compound 39	C10H9NO	TestSetH	A39
41	Compound 40	C11H11NO	TestSetH	A40
42	Compound 41	C9H7NO	TestSetI	A41
43	Compound 42	C11H19NO2	TestSetI	A42
44	Compound 43	C12H12O6	TestSetI	A43
45	Compound 44	C16H12N2	TestSetI	A44

(B)

	A	B	C	D	E	I
1	Bracket Type=2					
2	Sample Type	Sample Name	Level	Position	L5	
3	Blank	wash	1	1:1	TestSetA	
4	Blank	wash		1:2	TestSetA	
5	Blank	wash		1:3	TestSetA	
6	Blank	wash		1:4	TestSetA	
7	Blank	wash		1:5	TestSetA	
8	Blank	Zero		1:6	TestSetA	
9	Std Update	Calibration 1	1	1:7	TestSetA	
10	Std Update	Calibration 5	5	1:8	TestSetA	
11	Std Update	Calibration 10	10	1:9	TestSetA	
12	Std Update	Calibration 50	50	1:10	TestSetA	
13	Std Update	Calibration 100	100	1:11	TestSetA	
14	Std Update	Calibration 500	500	1:12	TestSetA	
15	Std Update	Calibration 1000	1000	1:13	TestSetA	
16	Std Update	Calibration 2000	2000	1:14	TestSetA	
17	Std Update	Calibration 5000	5000	1:15	TestSetA	
18	Std Update	Calibration 10000	10000	1:16	TestSetA	
19	Blank	Blank		1:17	TestSetA	
20	Blank	Blank		1:18	TestSetA	
21	Unknown	Compound 1 IV1 1		1:19	TestSetA	
22	Unknown	Compound 1 IV1 2		1:20	TestSetA	
23	Unknown	Compound 1 IV1 3		1:21	TestSetA	
24	Unknown	Compound 1 IV1 4		1:22	TestSetA	
25	Unknown	Compound 1 IV1 5		1:23	TestSetA	
26	Unknown	Compound 1 IV1 6		1:24	TestSetA	
27	Unknown	Compound 1 IV1 7		1:25	TestSetA	
28	Unknown	Compound 1 IV1 8		1:26	TestSetA	
29	Blank	Blank		1:27	TestSetA	
30	Unknown	Compound 1 IV2 1		1:28	TestSetA	
31	Unknown	Compound 1 IV2 2		1:29	TestSetA	
32	Unknown	Compound 1 IV2 3		1:30	TestSetA	
33	Unknown	Compound 1 IV2 4		1:31	TestSetA	
34	Unknown	Compound 1 IV2 5		1:32	TestSetA	
35	Unknown	Compound 1 IV2 6		1:33	TestSetA	
36	Unknown	Compound 1 IV2 7		1:34	TestSetA	
37	Unknown	Compound 1 IV2 8		1:35	TestSetA	
38	Blank	Blank		1:36	TestSetA	
39	Unknown	Compound 1 PO1 1		1:37	TestSetA	
40	Unknown	Compound 1 PO1 2		1:38	TestSetA	
41	Unknown	Compound 1 PO1 3		1:39	TestSetA	
42	Unknown	Compound 1 PO1 4		1:40	TestSetA	
43	Unknown	Compound 1 PO1 5		1:41	TestSetA	
44	Unknown	Compound 1 PO1 6		1:42	TestSetA	
45	Unknown	Compound 1 PO1 7		1:43	TestSetA	
46	Unknown	Compound 1 PO1 8		1:44	TestSetA	
47	Blank	Blank		1:45	TestSetA	
48	Unknown	Compound 1 PO2 1		1:46	TestSetA	
49	Unknown	Compound 1 PO2 2		1:47	TestSetA	
50	Unknown	Compound 1 PO2 3		1:48	TestSetA	
51	Unknown	Compound 1 PO2 4		1:49	TestSetA	
52	Unknown	Compound 1 PO2 5		1:50	TestSetA	
53	Unknown	Compound 1 PO2 6		1:51	TestSetA	
54	Unknown	Compound 1 PO2 7		1:52	TestSetA	
55	Unknown	Compound 1 PO2 8		1:53	TestSetA	

Optimisation | Analysis

Figure 2A A representation of the Optimization worksheet in the Excel-based sample list generator. Figure **2B**: A representation of the Analysis worksheet in the Excel-based sample list generator.

sample location and nominal concentration. Samples are designated as blanks, standards, quality controls or unknowns. Once the analysis worksheet is constructed, the user clicks the 'Get Formulas' button which downloads the molecular formula from the company database. The user then clicks the 'Export Runlists' button on the optimization worksheet. The software checks for inconsistencies and population errors between the two worksheets, if any errors are detected they are highlighted on the worksheets and must be corrected before they can be saved. If no errors are detected the user is prompted for a unique file identifier and the optimization and analysis worksheets are saved as text (.txt) and comma delimited (.csv) file formats respectively.

Submission to the instrument sample queue is performed by the user via QuickQuan™. In the optimization view (Figure 3a) the optimization list can be imported, populating the database with compound identifiers (name and mass), drugset and the location of the compound optimization solution. This is visualized on a virtual 96-well plate. The analysis list is imported into the acquisition view (Figure 3b), here an internal standard can be specified and an output path for storage of the acquired data files can be input. The user clicks the 'go' button which opens a final option window where parameters such as ionization method, polarity, instrument settings and any post analysis processing methods are selected. Clicking 'Run' switches on the mass spectrometer and all peripherals and initiates the optimization sequence.

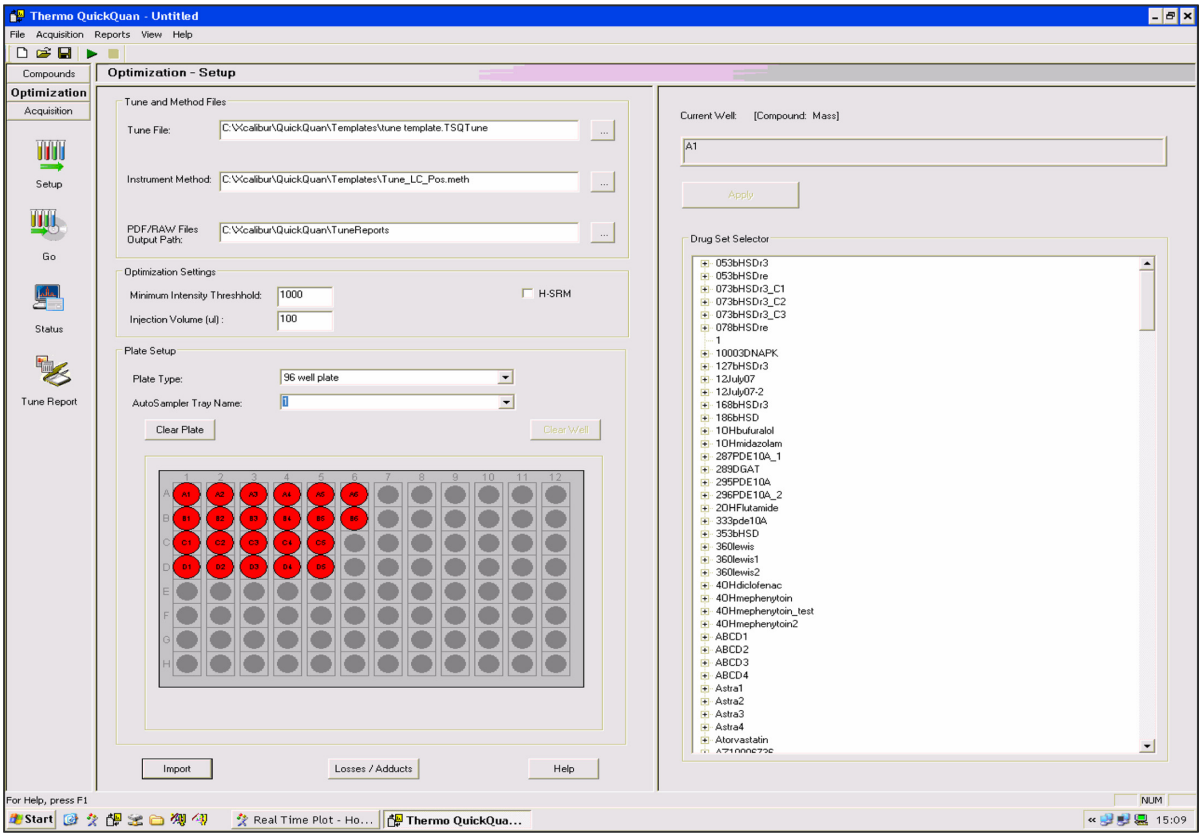
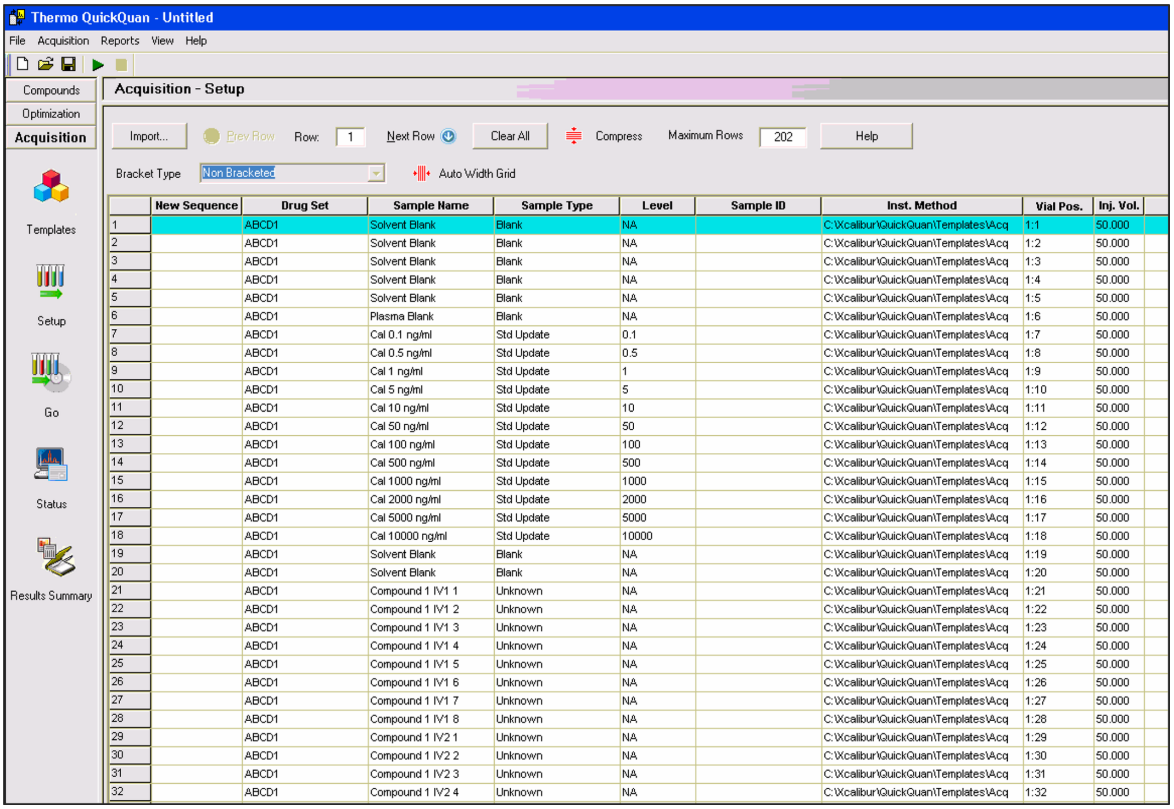


Figure 3A A screenshot of the Optimization view within the QuickQuan™ software.



	New Sequence	Drug Set	Sample Name	Sample Type	Level	Sample ID	Inst. Method	Vial Pos.	Inj. Vol.
1		ABCD1	Solvent Blank	Blank	NA		C:\Xcalibur\QuickQuan\Templates\Acq	1:1	50.000
2		ABCD1	Solvent Blank	Blank	NA		C:\Xcalibur\QuickQuan\Templates\Acq	1:2	50.000
3		ABCD1	Solvent Blank	Blank	NA		C:\Xcalibur\QuickQuan\Templates\Acq	1:3	50.000
4		ABCD1	Solvent Blank	Blank	NA		C:\Xcalibur\QuickQuan\Templates\Acq	1:4	50.000
5		ABCD1	Solvent Blank	Blank	NA		C:\Xcalibur\QuickQuan\Templates\Acq	1:5	50.000
6		ABCD1	Plasma Blank	Blank	NA		C:\Xcalibur\QuickQuan\Templates\Acq	1:6	50.000
7		ABCD1	Cal 0.1 ng/ml	Std Update	0.1		C:\Xcalibur\QuickQuan\Templates\Acq	1:7	50.000
8		ABCD1	Cal 0.5 ng/ml	Std Update	0.5		C:\Xcalibur\QuickQuan\Templates\Acq	1:8	50.000
9		ABCD1	Cal 1 ng/ml	Std Update	1		C:\Xcalibur\QuickQuan\Templates\Acq	1:9	50.000
10		ABCD1	Cal 5 ng/ml	Std Update	5		C:\Xcalibur\QuickQuan\Templates\Acq	1:10	50.000
11		ABCD1	Cal 10 ng/ml	Std Update	10		C:\Xcalibur\QuickQuan\Templates\Acq	1:11	50.000
12		ABCD1	Cal 50 ng/ml	Std Update	50		C:\Xcalibur\QuickQuan\Templates\Acq	1:12	50.000
13		ABCD1	Cal 100 ng/ml	Std Update	100		C:\Xcalibur\QuickQuan\Templates\Acq	1:13	50.000
14		ABCD1	Cal 500 ng/ml	Std Update	500		C:\Xcalibur\QuickQuan\Templates\Acq	1:14	50.000
15		ABCD1	Cal 1000 ng/ml	Std Update	1000		C:\Xcalibur\QuickQuan\Templates\Acq	1:15	50.000
16		ABCD1	Cal 2000 ng/ml	Std Update	2000		C:\Xcalibur\QuickQuan\Templates\Acq	1:16	50.000
17		ABCD1	Cal 5000 ng/ml	Std Update	5000		C:\Xcalibur\QuickQuan\Templates\Acq	1:17	50.000
18		ABCD1	Cal 10000 ng/ml	Std Update	10000		C:\Xcalibur\QuickQuan\Templates\Acq	1:18	50.000
19		ABCD1	Solvent Blank	Blank	NA		C:\Xcalibur\QuickQuan\Templates\Acq	1:19	50.000
20		ABCD1	Solvent Blank	Blank	NA		C:\Xcalibur\QuickQuan\Templates\Acq	1:20	50.000
21		ABCD1	Compound 1 IV1 1	Unknown	NA		C:\Xcalibur\QuickQuan\Templates\Acq	1:21	50.000
22		ABCD1	Compound 1 IV1 2	Unknown	NA		C:\Xcalibur\QuickQuan\Templates\Acq	1:22	50.000
23		ABCD1	Compound 1 IV1 3	Unknown	NA		C:\Xcalibur\QuickQuan\Templates\Acq	1:23	50.000
24		ABCD1	Compound 1 IV1 4	Unknown	NA		C:\Xcalibur\QuickQuan\Templates\Acq	1:24	50.000
25		ABCD1	Compound 1 IV1 5	Unknown	NA		C:\Xcalibur\QuickQuan\Templates\Acq	1:25	50.000
26		ABCD1	Compound 1 IV1 6	Unknown	NA		C:\Xcalibur\QuickQuan\Templates\Acq	1:26	50.000
27		ABCD1	Compound 1 IV1 7	Unknown	NA		C:\Xcalibur\QuickQuan\Templates\Acq	1:27	50.000
28		ABCD1	Compound 1 IV1 8	Unknown	NA		C:\Xcalibur\QuickQuan\Templates\Acq	1:28	50.000
29		ABCD1	Compound 1 IV2 1	Unknown	NA		C:\Xcalibur\QuickQuan\Templates\Acq	1:29	50.000
30		ABCD1	Compound 1 IV2 2	Unknown	NA		C:\Xcalibur\QuickQuan\Templates\Acq	1:30	50.000
31		ABCD1	Compound 1 IV2 3	Unknown	NA		C:\Xcalibur\QuickQuan\Templates\Acq	1:31	50.000
32		ABCD1	Compound 1 IV2 4	Unknown	NA		C:\Xcalibur\QuickQuan\Templates\Acq	1:32	50.000

Figure 3B A screenshot of the Acquisition view within the QuickQuan™ software.

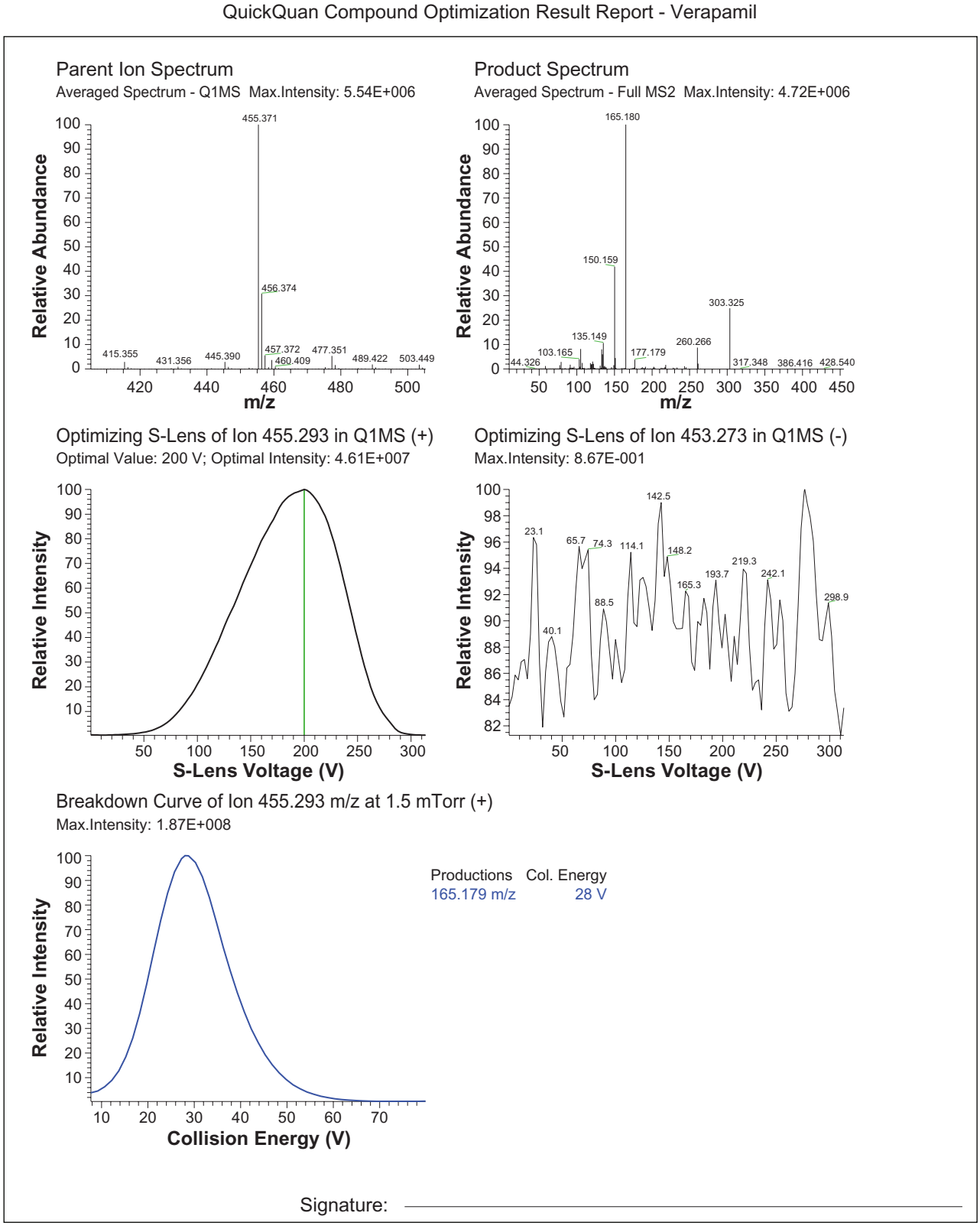


Figure 4 An example of a QuickQuan™ Optimization Tune Report for verapamil (*Continued on p. 8*).

QuickQuan Compound Optimization Result Report - Verapamil

Compound optimization result in raw file:
C:\Xcalibur\QuickQuan\Optimization\Verapamil.raw

RT (min)	Message
0.01	Tune S-Lens of Tune Mass (m/z) 455.293 in positive polarity
0.10	Tune S-Lens of Tune Mass (m/z) 453.273 in negative polarity
0.15	Negative Adduct -1.010 gives the intensity of 8.41e-01
0.15	Positive Adduct 1.010 gives the best intensity of 4.61e+07
0.23	Optimizing collision energy at 1.5 mTorr
0.23	Waiting for the collision gas to stabilize
1.39	Product Ion: 165.179 Maximum Intensity: 1.87e+08
1.39	Finish compound optimization.

Signature:_____

Figure 4 (*Continued*).

Any subsequent users open a new instance of QuickQuan™ and repeat the process.

Thermo Fisher Scientific instrument optimization procedure

The QuickQuan™ optimization procedure infuses a standard 1 µg/mL methanolic solution of each compound (or a mixture of compounds) via the syringe on the autosampler into the mobile phase eluent (250 µL/min) directed to the mass spectrometer source. The infusion lasts for a total of 1 min during which an optimization algorithm is run. QuickQuan™ uses the molecular formula uploaded by the user to calculate a monoisotopic molecular mass (M) and scans a mass window M ± 50 amu. Parent ions are scanned in positive and negative ion mode against a tube lens ramp (0 to 300 volts). The algorithm selects polarity and tube lens voltage giving the most intense parent; it then records the measured molecular ion. Parent mass and tube lens voltage are applied to quadrupole one and a breakdown curve is acquired across a collision energy ramp (5 to 80 volts) with the collision cell at a pressure of 1.5 mTorr argon. The most intense product ion and corresponding collision voltage is recorded. Polarity, parent ion, tube lens voltage, product ion and collision energy are saved to the QuickQuan™ database for future use. A detailed report is also constructed (Figure 4) showing parent/product ion spectra, useful for investigating failed optimizations. The database storing all of the optimized parameters is located on a company server accessible from all TSQ Quantum instruments in the laboratory, removing the need to optimize compounds more than once.

QuickQuan™ uses the saved parameters to construct SRM methods for a compound post optimization for use in the analytical batch.

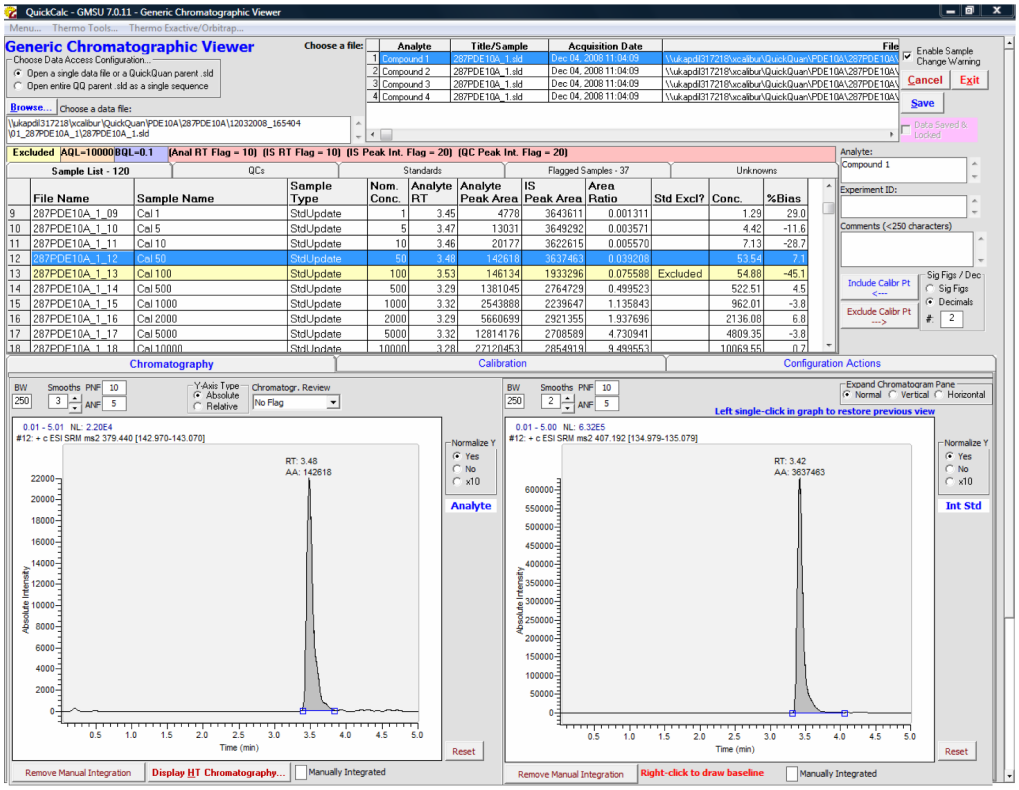
Thermo Fisher Scientific instrument data processing

QuickQuan™ saves all of the data captured for a batch in a user identified output path. Each log in procedure is given a time/date stamp and an Xcalibur sequence file (.sld file) is created for the analysis. Users access the .sld file at their office workstations using QuickCalc™. QuickCalc™ is a suite of programs specifically designed for high throughput DMPK mass spectrometric analysis.[10] It encompasses software solutions for hepatic clearance, permeability, drug-drug interactions and pharmacokinetics, although at this time the authors only use the generic chromatography viewer that allows quantification of analytical data. QuickCalc™ auto-processes the analytical batch; integration is based on a user specified standard within the run and can be easily checked by the user (Figure 5a and 5b). Designated standards, quality controls, blanks, unknowns and nominal concentrations are imported into QuickQuan™ at the beginning of the log on process, these are embedded in the raw data and are used in QuickCalc™ for quantification. Standards can be included or excluded from the calibration curve and different analytes in a drug set can be selected for processing.

Results are reported through a report generator that can be custom formatted to the users requirements, result summaries are displayed in Excel. The different software suites within QuickCalc™ have the scope to report DMPK specific data as a finished product.

Open access using OpenLynx™ and Quan Browser™

Our department has two Quattro Ultima mass spectrometers (one Quattro Ultima, one Quattro Ultima Pt) each functioning as identical OA systems and running the same analytical methods as the Thermo equipment. The department uses a similar custom in-house Excel based 'run list generator' to that used with the QuickQuan™ software. Files are saved to the same server formatted (as .txt files) for import into the



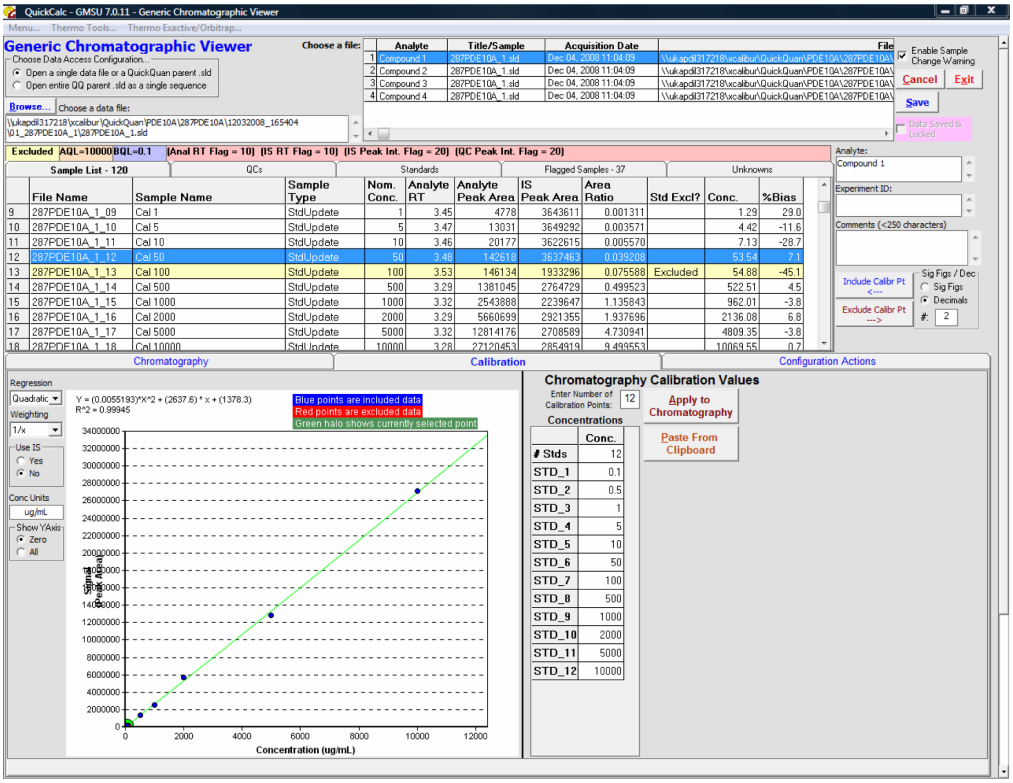


Figure 5A and 5B The QuickCalc™ generic chromatography viewer user interface developed by Gubbs Inc. The software auto processes data acquired on the thermo Fisher Scientific instruments across the computer network.

OpenLynx™ software. The log in procedure is administrated through an OpenLynx™ log in console on the desktop of the instrument linked workstation. The console is left open along with a batch manager that monitors the sample queue.

The console acts as a wizard prompting for different information at each step (Figure 6). Initially the user is prompted for details such as project name that is used to construct a unique log in identification, an output path can also be designated so any data generated can be sent to a server location. The user is then prompted to choose analysis conditions (eg, polarity, chromatographic conditions) from a list of available methods, this is used to run the submitted analytical batch. Optimization and analytical run lists can then be imported into the console from an external server. OpenLynx™ instructs the user to place sample plates in a specific tray in the autosampler prior to finishing the log in procedure. Additional users simply start the process again.

Waters instrument optimization procedure

The OpenLynx optimization procedure is performed via loop injections of a standard solution of each compound onto the chromatography system used for sample analysis (5 min per injection). OpenLynx™ uses the molecular formula imported at log in to create a mass window for a parent ion of interest (± 25 amu). The software optimizes cone voltage (via a voltage ramp) on the parent mass of the compound of interest in both positive and negative ion mode. The polarity and cone voltage in which the parent ion gives the most intense response is saved by the software and a product ion scan is run across a collision energy voltage ramp. Parent ion, cone voltage, product ion and collision energy are all saved as a MassLynx™ MS method file for future analysis. The optimization process requires three injections of the standard solution totaling 15 minutes per compound. However, optimization can be done in a single injection[11] by instructing the system to only operate in a single ionization polarity and by relying on the molecular formula imported at log in to yield a M ± 1 amu parent ion for positive and negative ion mode respectively. The software then performs a product ion scan in the appropriate polarity with a generic cone voltage and optimizes collision energy; this reduces optimization time to 5 minutes. A further time saving can be made by removing the column from the chromatographic system and making a loop injection straight into the source. It has been found that injection through the chromatographic system yields more data points throughout the 'chromatographic' peak, this allows the data system to characterize the peak better and gives rise to less optimization failures than loop injection straight to the mass spectrometer source. Three injections per optimization is standard. The analytical systems are exposed to a diverse range of chemical series, optimization success rate is increased by offering a choice of ionization polarity.

OpenLynx™ uses the parameters derived from instrument tuning to construct multiple reaction monitoring (MRM) methods for use in the analytical batch.

Waters Corporation instrument data processing

OpenLynx™ auto-processes all analytical batches prior to saving them on an external server. It uses a predefined calibration standard (identified by the user in the analytical batch upload) to set peak integration parameters and applies them to the entire batch. It then saves the processed data as a QuanLynx™ datafile (.qld file) in the location specified at log in.

Users can access the .qld file at office workstations via a software package called QuanLynx™ browser (Figure 7). QuanLynx™ browser is similar in style and operation to QuickCalc™ and offers the usual versatility needed to perform quantitative bioanalytical analysis.

Quantitative results are exported to Excel prior to any secondary data processing (pharmacokinetic data analysis) and subsequent reporting of data.

System suitability and quality control

The open access systems normally run 24 hours a day 7 days a week. It is necessary to run quality control samples to monitor the mass spectrometers response and the chromatographic peak shape. Four commercially available compounds (Table 1) were selected that encompass the chemical scope that our samples fall into covering both positive and negative ionization modes. The quality control test solution is run daily and monitored to ensure consistent instrument performance (chromatographic retention time and mass spectrometer sensitivity). Instrument failures can be spotted prior to total system failure enabling preventative maintenance and planned downtime.

Limitations of the open access systems

Compounds do fail to optimize on the open access systems due a variety of reasons including, most commonly poor mass spectral sensitivity, adduct formation or in-source

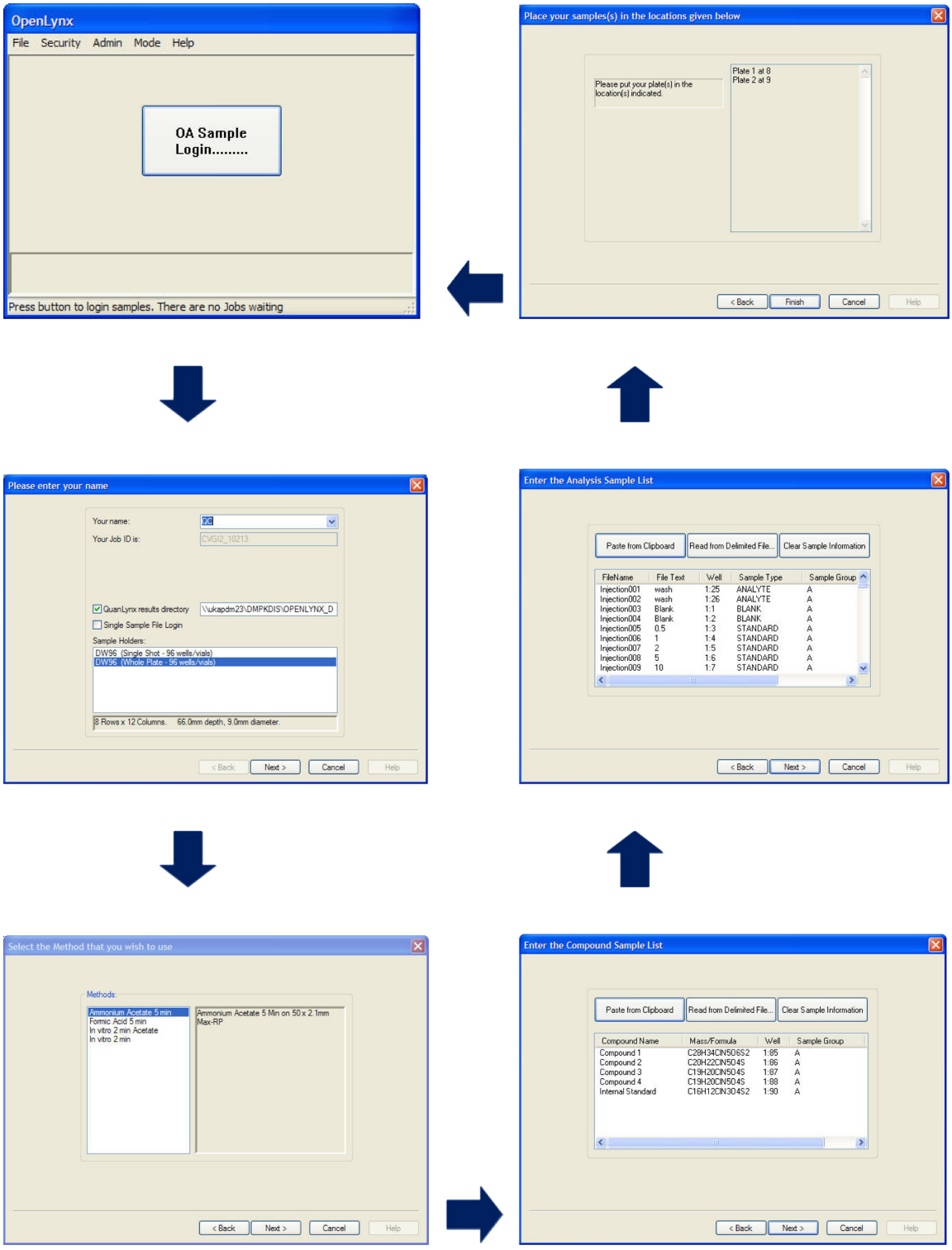


Figure 6 A schematic of the Openlynx™ log in process. **A**) The Openlynx™ welcome screen, **B**) Batch identification and output screen, **C**) Analytical method selection screen, **D**) Optimization run list import screen, **E**) Analysis run list import screen, **F**) 96 well plate location instructions (generated by the software).

fragmentation. Failures can more often than not be corrected by running in an alternative solvent system, although on occasion it is necessary to consider alternative approaches, such as monitoring for an adduct or a in source fragment loss.

Chromatographic system blockages were initially a persistent problem. Preventative maintenance measures were introduced to minimize the problems, these included introducing in-line post injection filters that are changed twice

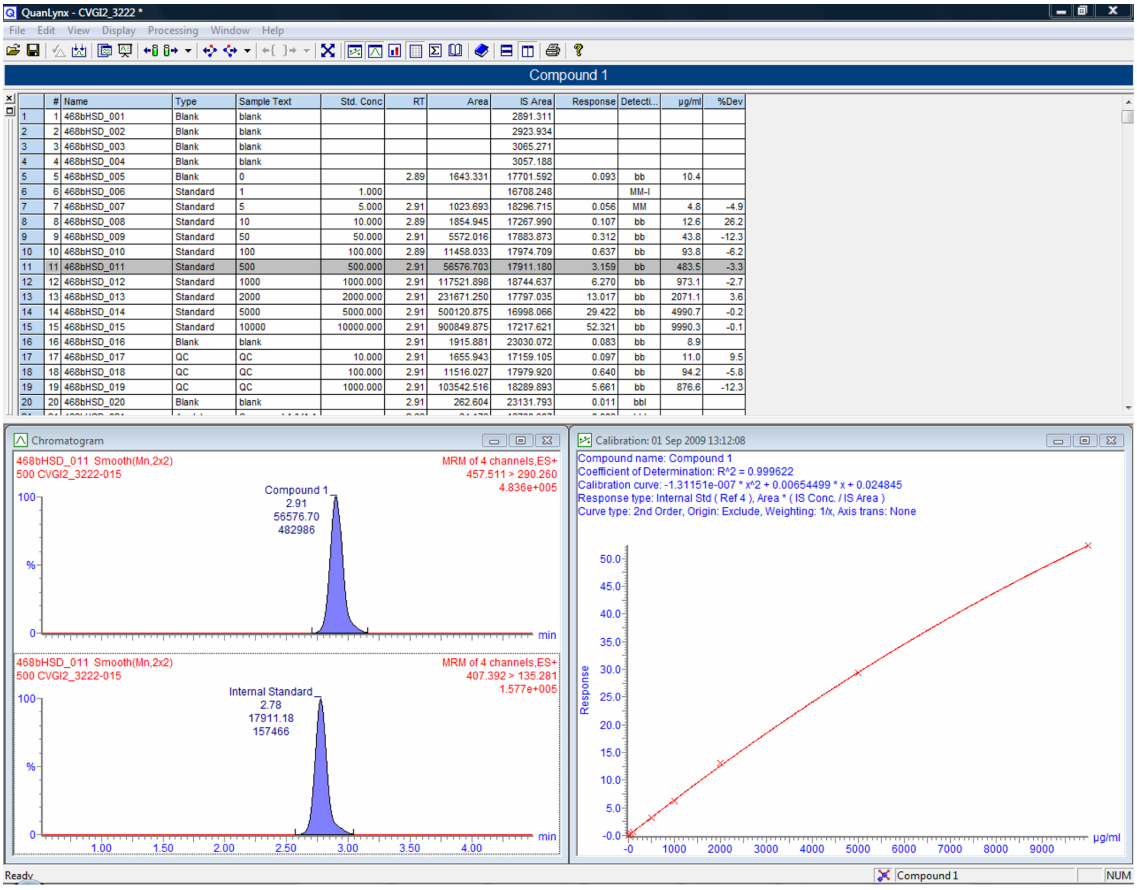


Figure 7 The QuanLynx™ browser user interface used to auto process data from the waters corporation instruments.

weekly and guard column changes on a weekly basis. Planned maintenance cycles are carried out on the mass spectrometer hardware and peripherals twice yearly to ensure consistent performance.

Chromatographic peak co-elution could occur occasionally during analysis of cassette samples, as a consequence of the non-optimized generic gradient conditions. Problems with ion suppression caused by co-eluting peaks have been largely eliminated by the dilution of the samples with water (1:5) as part of our generic sample preparation method. Typical lower limits of quantitation are at a level of 1 ng/mL which is sufficient for pharmacokinetic profiling.

The two OA platforms discussed here both have different strengths and weaknesses. The OpenLynx™ platform instructs the operator to locate the injection plate in a specific position in the autosampler. QuickQuan™ does not specify the plate position, this must be carefully managed to ensure the same sample location is not used by different users. The QuickQuan™ platform offers a significantly faster and more comprehensive optimization procedure compared to OpenLynx™ as polarity and all tune parameters are optimized in 1 minute infusion. A full optimization on the OpenLynx™ platform requires three injections, one for each source polarity and one for product ion parameters. As mentioned earlier the number of injections can be reduced by selecting a single polarity and reducing the number of instrument parameters tuned.

Table 1 Quality control compounds used to monitor chromatographic and mass spectrometer performance between analytical batches

Compound	Polarity (+/–)	Parent mass (M ± 1)	Production mass	Retention time (mins)	Typical area (arbitary units)
Erythromycin	+	734.5	158.1	2.9	8613
Lidocaine	+	235.2	86.1	2.8	24477
Propanolol	+	260.2	116.1	2.7	169614
Warfarin	+	309.1	163.0	2.5	121070
Warfarin	–	307.1	161.0	2.5	385625

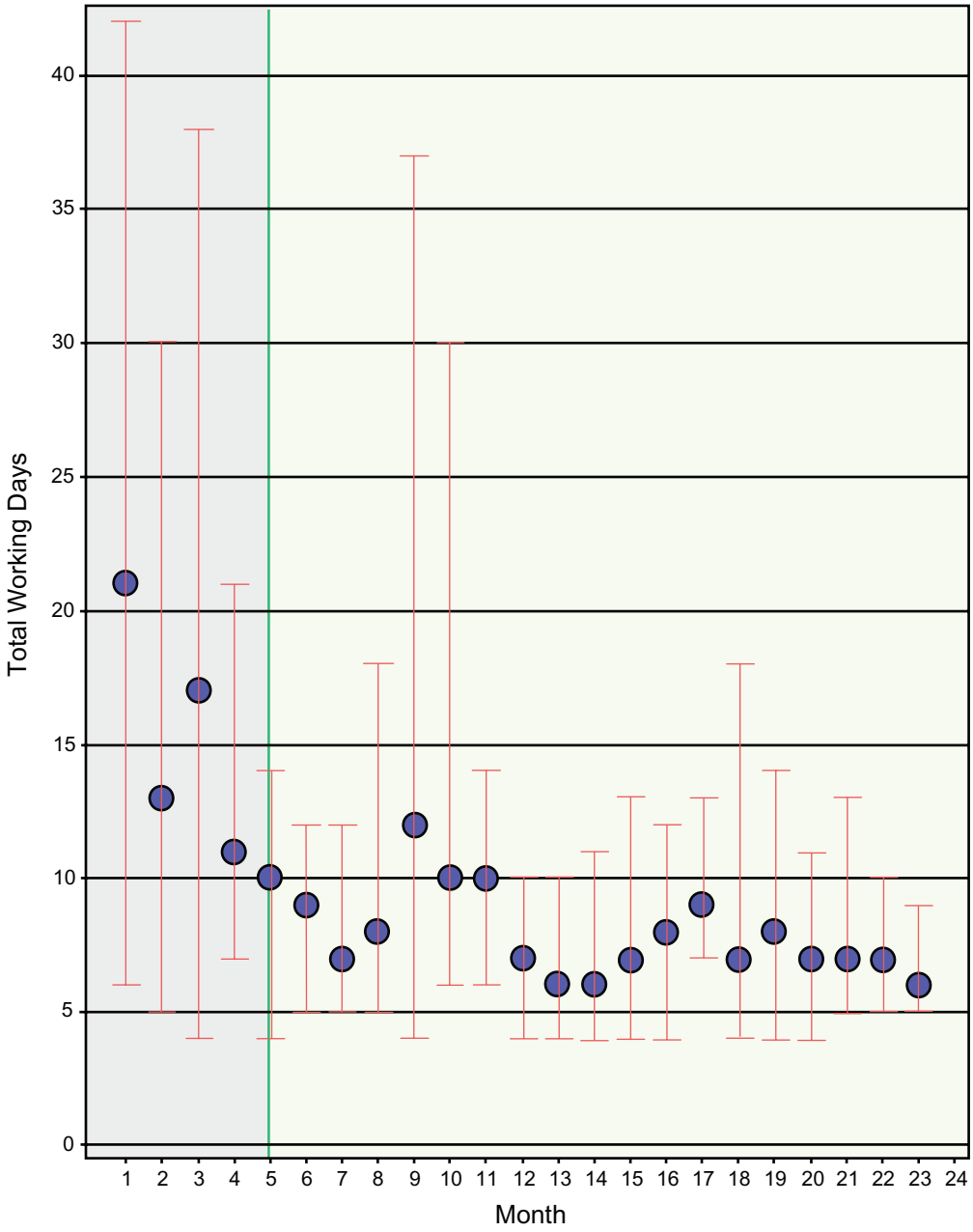


Figure 8 The average rat pharmacokinetic study turnaround times before (grey area) and after (green area) the introduction of the open access instruments (green line).The error bars denote the range of turnaround times within each dataset.

Impact on pharmacokinetic study turnaround times

The data gathered throughout the LSS project aimed at lowering rat pharmacokinetic study turnaround times demonstrated that implementation of the changes to the process, including the introduction of the open access systems, showed an immediate and marked improvement. Between 10 to 16 studies were performed each month within the 'measure' phase (1 to 5 months) and the 'control' phase (6 to 23 months). The average turnaround time for these studies was reduced from an initial range of between 4 to 44 working days to a desired target mean value of 10 working days. The variability was also observed to be reduced (Figure 8). The data collected during the 'control' phase shows that the results have been sustained for 17 months post implementation.

Conclusions

The four quantitative open access instruments have created a powerful LC-MS/MS platform. They are used for a wide variety of *in vitro* and *in vivo* DMPK assays, typically yield-

ing specific and sensitive data in a high throughput (2 to 5 minutes per injection) environment. In addition to this they are capable of analyzing compounds with a wide range of physical properties enabling support of lead optimization projects across our research area.

We have demonstrated that open access systems have had a positive effect on the turnaround times of both *in vivo* and *in vitro* studies by utilizing mass spectrometer time more effectively and by minimizing instrument downtime. The Lean Six Sigma control phase data supports this sustained improvement.

The open access philosophy adopted by the department has been shown to provide the level of MS resource needed to perform discovery DMPK analysis in a harsh economic climate. In terms of performance metrics, during a 2 month period one open access system optimized 192 compounds and analyzed 15,294 injections, this equated to the mass spectrometer being used for 58 days out of a possible 67 (or 87% of the available time). The OA systems have allowed the DMPK scientists to spend less time on laboratory activities and more time on data interpretation and project support activities.

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Disclosures

All authors are employees of AstraZeneca.

References

1. Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates. *Nat Rev Drug Discov*. 2004;3(8):711–716.
2. Borman S. Solid-phase synthesis, informatics, and high-throughput screening advances help combinatorial chemistry find leads. *Chem Eng News*. 1999;77(10):33–48.
3. Gilbert JD, Olah TV, Mcloughlin DA. In: Snyder AP, ed. *Biological and Biotechnological Applications of Electrospray Ionization MS*. New York, USA: The American Chemical Society; 1996:330–350.
4. Hammond C, O'Donnell CJ. Lean six sigma: its application to drug discovery. *Drug Discovery World*. 2008; Spring:9–18.
5. Coddington A, Van Antwerp J, Ramjit H. Critical considerations for high-reliability open access LC/MS. *J Liq Chromatogr Relat Technol*. 2003;26(17):2839–2859.
6. Mallis LM, Sarkahian AB, Kulishoff JM, Watts WLJ. Open-access liquid chromatography/mass spectrometry in a drug discovery environment. *Mass Spectrom*. 2002;37:889–896.
7. Wright P, Chassaing C, Cussans N, et al. Evolution of an open-access quantitative bioanalytical mass spectrometry service in a drug discovery environment. *Biomed Chromatogr*. 2006;20:585–596.
8. Ayrton J, Dear GJ, Leavens WJ, Mallett DN, Plumb RS. Use of generic fast gradient liquid chromatography-tandem mass spectroscopy in quantitative bioanalysis. *J Chromatog B*. 1998;709:243–254.
9. Jemal M, Xia YQ. The need for adequate chromatographic separation in the quantitative determination of drugs in biological samples by high performance liquid chromatography with tandem mass spectrometry. *Rapid Commun Mass Spectrom*. 1999;13:97–106.
10. Brockman A, Yan Z, Lu C, Wu JT, Elvebak L. Validation of a high-throughput absorption, distribution, metabolism and excretion (ADME) system and results for 60 literature compounds. *Rapid Commun. Mass Spectrom*. 2005;19:1191–1199.
11. Hiller DL, Zuzel TJ, Williams JA, Cole RO. Rapid scanning technique for the determeniation of optimal tandem mass spectrometric conditions for quantitative analysis. *Rapid Commun Mass Spectrom*. 1997;11:593–597.

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