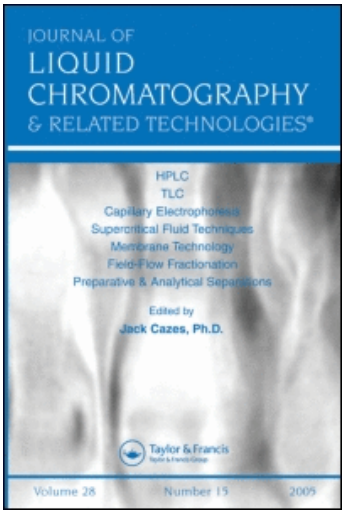

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Critical Considerations for High-Reliability Open Access LC/MS

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Critical Considerations for High-Reliability Open Access LC/MS

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ABSTRACT

The implementation of Open Access Liquid Chromatography Mass Spectrometry (OA-LC/MS) systems involved the investigation of various vendor's LC/MS systems, post-purchase LC and MS set-up involving theoretical and practical considerations, and the specific use of open access software. The targeted objectives were robustness, ease of use, chromatographic and mass spectral fidelity, long LC column life, and exceptional uptime. Numerous factors were addressed from initial instrument selection, user-friendliness, maintainability and operating costs, reliability, robustness through overall manageability, and finally, the impact on productivity. During the 24-months study, 12 systems were commissioned to meet the needs of 165 scientists and demonstrated benchmarks such as column durability, 6–8k injections/lifetime average, and >99% instrument uptime. The users' sample analysis rate is currently >130k samples/year at an average cost of <$2.50/sample analysis.

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This high-reliability OA-LC/MS facility is successful by virtue of the choice of instrumentation, management, and maintenance of all aspects of the operation by a single researcher.

Key Words: Open access LC/MS; Column selection; API-MS; HPLC; ESI-MS; High reliability.

INTRODUCTION

A study was conducted to assess the feasibility of Open Access Liquid Chromatography Mass Spectrometry[1,2] (OA-LC/MS) as being capable of meeting the low-resolution MS analytical needs of our department's synthetic organic medicinal chemists (subsequently called chemists or users). The study began with the investigation of various LC/MS systems, subsequent LC and MS setups involving theoretical and practical considerations, and the implementation of software specifically designed for open access use. Open Access refers to LC/MS systems that are available for use by investigators 24/7 without the intervention of MS (facility) personnel. Within the protocol the general targeted results were robustness, ease of use by the users, and exceptional uptime. During the installation and deployment of the department's 12 OA-LC/MS systems, the following key elements were considered: instrumentation, site preparation/geographical location, LC preparation, LC column selection, LC methods, flow injection analyses (FIA), mass spectrometer setup, open access software user training, preventative maintenance, and overall system management. All aforementioned elements were developed and implemented with a focus towards quality data, enhanced productivity, system(s) manageability, and near 100% instrument reliability.

EXPERIMENTAL

Instrumentation

Numerous vendors were visited in 1998 and their respective OA-LC/MS instruments were tested per our evaluation protocol. The system was required to be a bench-top, quadrupole model, with a robust atmospheric pressure ionization (API) source capable of handling flows up to 1 mL/min. Additional criteria employed were: mass range, type of detector, both electrospray ionization (ESI), and atmospheric pressure chemical ionization (APCI) available with quick change over capability. Mass spectrometry software must control the entire instrument for all standard methods, including the ability to

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perform batch analyses, and the system must have a proven, easy-to-use, open access software front-end. The number of instruments the manufacturer currently has in the field, product support, and cost of the complete package were also a consideration. An instrument from Waters (Waters Corp., 34 Maple St., Milford, MA 01757) satisfied our overall open access strategy.

Site Selection and Preparation

The geographical locations of the instruments within the department were considered. One instrument was located within close proximity to every 8–12 users. High-purity N_2 gas required for ESI source operation is obtained from the pressurized boiloff of a multi-thousand liter liquid nitrogen tank located outside the building that is automatically filled by the vendor, which eliminates the possibility of instrument downtime for the lack of nitrogen. Contamination from nitrogen sources and tubing is possible, therefore, copper, cleaned for oxygen, which is silver soldered under a nitrogen purge or stainless steel was used. Teflon tubing was used instead of polyethylene tubing to bring N_2 from the wall to the instrument. Adequate air conditioning capacity for the instrument environment was ensured. A 6-kVA (thousand Volt-Amp) uninterruptible power source (UPS) was installed on each system to guard against powerline fluctuations.

Liquid Chromatography Preparation (Waters' 2690/2695)

All LC lines were flushed with isopropyl alcohol overnight. All solvent reservoirs were triple washed with water, methanol, and lastly, acetonitrile (ACN). The current LC methods utilize the aqueous (A) solvent twice as quickly as the organic (B) solvent, therefore, the B reservoir is 2-L capacity and the A reservoir is a 5-L capacity. The maximum aqueous concentration used for an LC method is 94%. To make gradient programming easier, and to help eliminate microbial growth within the A reservoir and its respective solvent line, 6% ACN is added directly to the aqueous mobile phase. The C reservoir used for flow injection analyses, and the D reservoir used for periodic flushing of precipitated chemicals and residues from solvent flow paths and columns, are both filled with 100% methanol. As a source for refilling the reservoirs at the instruments, 20-L returnable ACN and methanol containers (Burdick and Jackson, 1953 S. Harvey St., Muskegon, MI 49442) are utilized and preferred over 4-L containers, because the solvent from 20-L containers exhibited lower blank total ion currents[3,4] (TIC). Water is produced in-house by a Millipore *Milli-Q Gradient* (80 Ashby Rd., Bedford, MA 01730) system. An internal UV lamp was added to destroy any residual

highly conjugated and aromatic carbon containing compounds. Biochemical grade trifluoroacetic acid (TFA) (Acros Organics—NJ) is used as an additive in the A (0.05%) and B (0.0425%) solvents. The Waters 2695's have two additional reservoirs filled with 100% methanol that supply solvent for a seal-wash and needle-wash. This set-up allows a 4-L bottle of methanol to reside near each installation, which is handy and allows quick filling of all reservoirs, except A and B reservoirs. Figure 1 illustrates the currently used flow path for the OA-LC/MS instruments.

The total peak broadening in an HPLC separation is the sum of several variances; $\sigma_{tot}^2 = \sigma_{col}^2 + \sigma_{inj}^2 + \sigma_{tub}^2 + \sigma_{fit}^2 + \sigma_{det}^2$ where σ_{tot}^2 is the total variance due to band spreading, σ_{col}^2, σ_{inj}^2, σ_{tub}^2, σ_{fit}^2, and σ_{det}^2 are the individual variances due to the column, injector, tubing, fittings, and detector, respectively. A reduction in the total variance will yield the least system band spreading (Fig. 2). Depending on flow rate, large dead-volumes create unusually long solvent delays and equilibration times. Because of variance considerations, the flow path from the injector to the MS is as short as possible creating low dead-volume. Stainless steel (SS) tubing, shorter than factory standard issue, has been inserted from the injector to the frit. A 2-μM frit filter assembly is attached to the end of the SS tubing. The frit may cause marginal band-broadening, however, since the increased variance is pre-LC column and the frit provides the vital function of protecting the LC column from solid material within the solvent stream, the marginal extra band-broadening is acceptable. PEEK tubing 0.005″ (red) is used from the frit until the Tee is reached, which directs flow post-LC-column (or FIA loop) to both the ultraviolet (UV) detector and to the MS. The PEEK tubing (Fig. 2) in the top

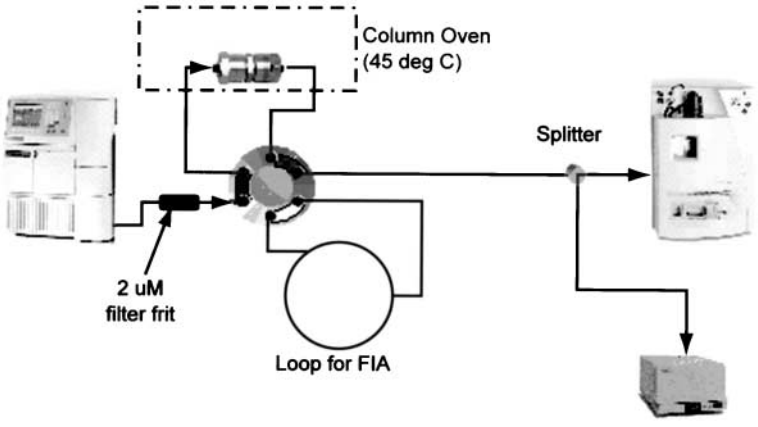

Figure 1. 2690 LC, flow path, ZQ MS, and 996 [or 2487] detector.

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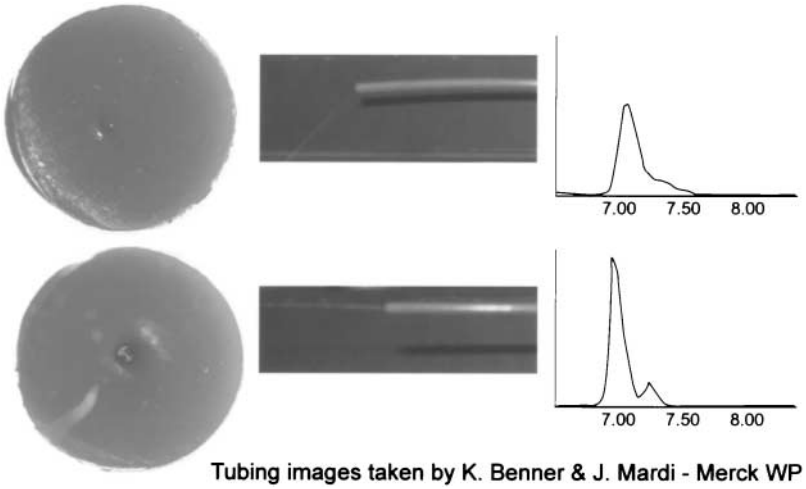

Figure 2. Top row: incorrectly cut PEEK tubing, skewed solvent stream, TIC displays deleterious effects of large system variance. Bottom row: correct PEEK opening, straight solvent flow, TIC displays improved efficiency with reduced system variance.

row was cut with a new blade, yet produced a deformed off-center hole, which resulted in skewed solvent flow. In the bottom, observe the head-on view of the same PEEK end-cut. A sharp pin was used to open up the hole and make it square with the tubing. The now centered opened hole produces a straight and direct solvent flow, further reducing variance. The TIC on the right side of each row (Fig. 2) indicates the effects of the increased tubing variance, causing excessive band spreading in the top row and minimal in the bottom row. A 75 or 100 μM fused silica tubing, 1.5–3 feet long, is functional between the Tee and the MS ES+ probe. One can adjust the split, or the fraction of solvent entering the UV detector and the MS, by varying the lengths ± diameters of tubing to either the UV detector or the MS. Flow to the MS, used in ES+ mode, was set to 50–100 μL/min. Immediate shutdown on error(s) is software enabled to prevent an MS solvent-flooding situation.

Column Selection

Target analysis time per sample, measured as injection to injection time (or total injection cycle time), and the subsequent LC column selection were the most critical issues to address. A 4-min analysis with a 5-min total injection cycle time was targeted. Limiting factors were considered: flow rate,

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efficiency (resolution), and backpressure. It was paramount for the LC system to provide a high peak capacity (PC) because samples may contain numerous analytes. [PC = total run time (sec)/baseline peak width (sec)]. For all experiments, a C18 reverse phase (RP) column was chosen. Liquid chromatography variables were manipulated such that the PC was within the optimal range (Fig. 3) area. Higher flow rates[5] are advantageous (Fig. 4) bearing in mind mass transfer kinetics, backpressure, and general practices of solvent conservation (i.e., cost savings). The more shallow the gradient per unit time the greater the PC. A simple method to "stretch out" the gradient for an LC analysis is to invoke a pre-column fill (or delayed injection)—in other words, when the gradient is started, hold the injection until a predetermined, i.e., system dwell, volume of solvent has been pumped into the LC system. The injection then occurs such that the analytes are deposited onto the column when initial solvent conditions still exist, but the gradient closely follows the injection plug. The analysis time has not changed, moreover, the gradient occurs over the entire analysis time (injection to injection time is increased). In the example using a monthly resolution and sensitivity test mix (Table 1, Fig. 5)—lower trace utilizes a pre-column fill (delayed injection) and the upper trace injects at time zero. Essentially all compounds are delayed without a

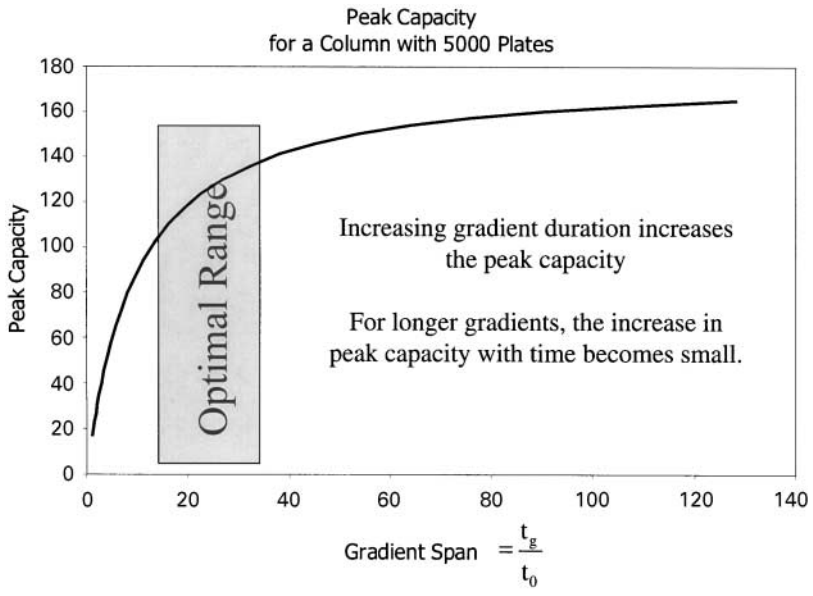

Figure 3. Peak capacity vs. gradient span. Optimal range highlighted.

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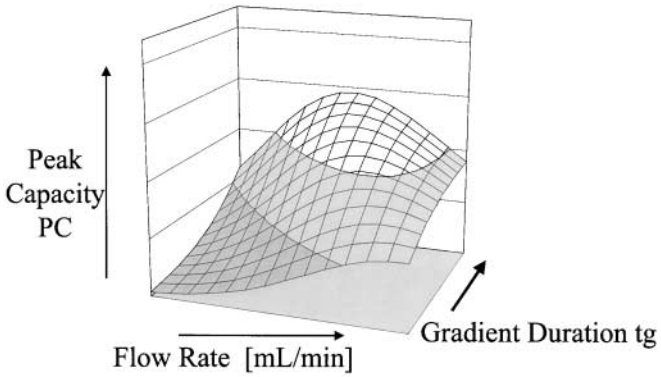

Figure 4. Peak capacity vs. flow rate and gradient duration.

pre-column fill invoked. Note that the last eluting analyte in the bottom trace does not elute in the top trace.

The equation for resolution is

$$\text{Res} = 0.25N^{0.5}(\alpha - 1)\frac{k}{k+1}$$

where N is the column plate number, α is the selectivity factor k_2/k_1, and k is the retention factor $[(t_r - t_0)/t_0]$, where t_r is the retention time of the peak and t_0 is the column dead time. The important point to this equation is that resolution is a function of the square root of the plate count. Figure 6 compares five column internal diameters (IDs) (1, 2, 3, 3.9, and 4.6 mm) each in four lengths[6–10] (20, 30, 50, and 150 mm). With normal LC instrumentation, one reaches the point of diminishing returns for small ID columns because the

Table 1. Standards mix, ~100 μg/mL per component, used as a monthly resolution and sensitivity check.

Compound	Neutral mass	~k'
Caffeine	194	2.25
2,4,5-Triphenylimidazole	296	5.6
Amitriptyline	277	5.85
Phenothiazine	199	10.1
4-(Phenylazo)diphenylamine	273	12.6
Monensin	693 (Na)	16.45

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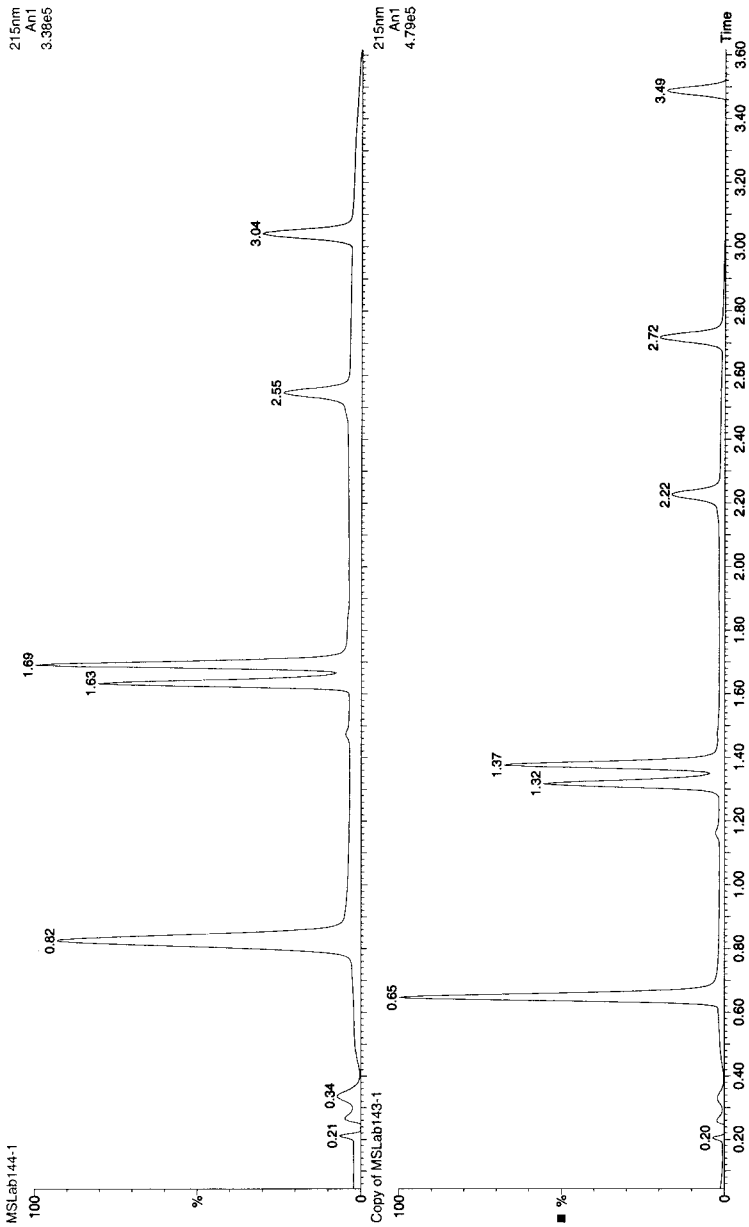

Figure 5. Upper UV trace—standard mix (Table 1) with normal injection at t_0 before gradient. Lower UV trace—standard mix (Table 1) with delayed injection.

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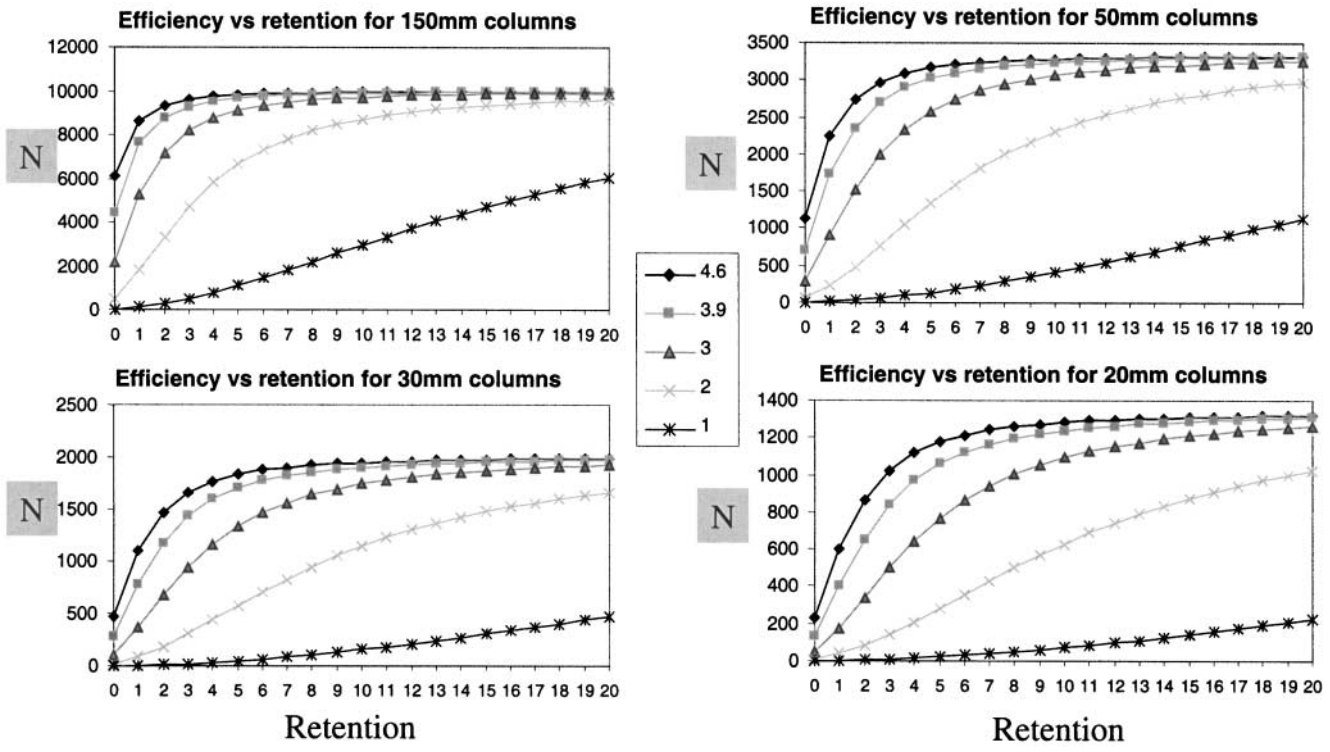

Figure 6. Efficiency vs. different column IDs for normal LC dead volume.

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dead volume of the system becomes the limiting factor (see low plate count for 1 and 2 mm ID columns). The 150-mm length columns create far too much backpressure at the flows needed to meet the desired injection cycle time. A higher plate count was desirable and 50-mm length column(s) appeared advantageous over the 20-mm and 30-mm length columns. The final decision was between the 3-mm and 4.6-mm ID columns. One additional factor in the LC column selection is that narrower LC columns typically produce narrower and, subsequently, taller peaks[7,9]—see Fig. 7. Well over 10 manufacturer's C18 chemistries in formats ranging from 1 to 4.6 mm ID and silica size ranging from 2 to 7 μM were tested (data not shown). The 2.1-mm, 3-mm, and 4.6-mm ID, 5-cm length, 5 μM YMC PRO (Waters Corp.) columns (Fig. 7) were compared [injection size (4-μL) was not scaled and the linear velocity of the mobile phase through each column was not equal, although close, vertical axes linked]. These tests were chosen to identify which column provided the better performance within the selected working situation and targeted event timings. The 4.6-mm ID column presented poorer efficiency and consumed substantially more solvent than the other columns. The reduced ID for the 2.1-mm ID column was observed to allow increased injection plug blow-through when injecting 100% organic samples. It was deemed that the maximum acceptable pressure would be less than 3000 psi for the equilibration cycle; the fastest flow rate through both the 2.1-mm and the 4.6-mm ID columns exceeded this value. The 3-mm ID column yielded good plate count for the entire chromatogram. Columns utilizing $\leq$ 3.5-μM silica were tested (data not shown) but necessitated a very short format to reduce back-pressure to an acceptable range, and typically produce fewer plates than a longer length (5 cm) format column containing particles 5-μM in size in the same pressure range. It was, therefore, decided the 4–5 μM silica in the 3 × 50-mm column would be used. This format column delivered high-performance and high-reliability qualities: good plate count, reduced blow-through possibility, acceptable solvent usage, rapid equilibration, acceptable back-pressures, and produced an increased injection lifetime vs. the sub-4 μM silica column packing. At elevated temperatures (45°C), LC system pressure was reduced and analyte/solid phase kinetics improved. Currently, the YMC PRO column is used. Typical baseline peak widths are about 3 seconds, yielding less than 2-second wide peaks at full width half maximum (FWHM).

Liquid Chromatography Methods

With a large department and numerous ongoing projects, the chemists produce a wide range of compounds from acidic to basic and polar to non-polar. A total gradient from 6% to 100% B was chosen to ensure elution of all

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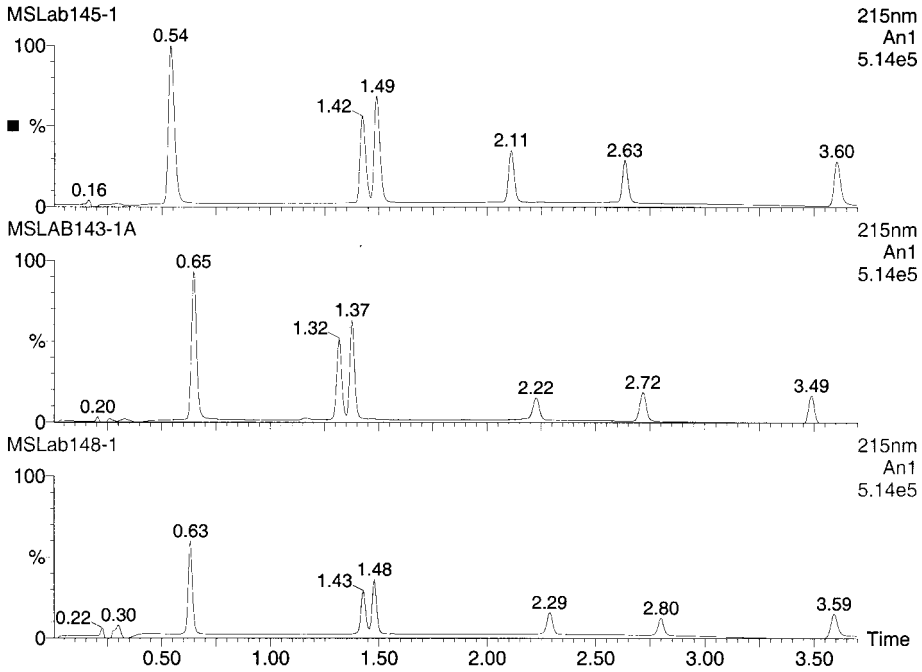

Figure 7. All 50 mm length, 5 μm particle size, YMC PRO (vertical axes linked).

Trace	Column ID	Flow (mL/min)	Pressure (initial)	Cross Sectional Area
Upper	2.1 mm	1.2–1.7	2300 psi	1.7 mm^2
Middle	3.0 mm	1.7–2.0	1850 psi	3.5 mm^2
Lower	4.6 mm	3.2–3.8	2650 psi	8.3 mm^2

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(a)

Gradient Table

Time	A	B	C	D	Flow	Curve
0.00	100.0	0.0	0.0	0.0	1.50	1
3.40	0.0	100.0	0.0	0.0	2.00	6
3.70	0.0	100.0	0.0	0.0	2.00	2
3.75	100.0	0.0	0.0	0.0	2.00	2
4.20	100.0	0.0	0.0	0.0	2.00	2

(b)

Time	A	B	C	D	Flow	Curve
0.00	100.0	0.0	0.0	0.0	1.50	1
4.10	0.0	100.0	0.0	0.0	2.00	6
4.70	0.0	100.0	0.0	0.0	2.00	6
4.75	100.0	0.0	0.0	0.0	2.00	2
5.20	100.0	0.0	0.0	0.0	2.00	2

(c)

Time	A	B	C	D	Flow	Curve
0.00	100.0	0.0	0.0	0.0	1.50	1
9.00	0.0	100.0	0.0	0.0	2.00	6
9.70	0.0	100.0	0.0	0.0	2.00	1
9.75	100.0	0.0	0.0	0.0	2.00	2
10.20	100.0	0.0	0.0	0.0	2.00	2

Figure 8. (a) Short LC method (4.2 min). (b) Medium LC method (5.2 min)—for extremely hydrophobic long retained peaks. (c) Long gradient LC method [10 min—night time].

compounds from the column. Three standard LC methods (Fig. 8) were created for each UV range (215 and 254 nm); a 4.2-min, a 5.2-min with slightly longer 100% B hold to drive off abnormally retained analytes, and a 10-min analysis offering exceptional resolution and PC. A 4.2 min LIBRARY (batch) analysis method (one method at 215 nm and one method at 254 nm) was also created, which utilizes a standard 4.2-min method, except that the user can log in any number of samples she or he desires, any time during the day, and the analyses will not commence till 20:00 hours. At the end of each method (Fig. 9), the solvent composition is changed to meet initial conditions and approximately one-half of the column equilibration process[10] is completed. The flow through the LC is 1.5 mL/min during the entire injection sequence and at initial solvent composition. This additional time, between when the injection sequence is started and injection occurs, is sufficient to finish the equilibration of the column. The Waters 2487 UV detector is set to zero upon injection (automatic on the 996 PDA). One instrument is located within the peptide and proteins group where the users typically have difficulty analyzing their samples on the YMC PRO LC column. A two position

switching valve, with bypass, was purchased to enable analyses of small compounds with using the YMC PRO column in position 1, peptides and proteins using a Vydac (Grace Vydac, 17434 Majave St. Hesperia, CA 92345) column in position 2, and flow injection analyses with the switch valve in the bypass position. The SS tubing was replaced with titanium and the frit is also titanium, which was thought to be more inert and less retentive than stainless steel.

Flow Injection Analysis

On some LC analyses, the analyte will present itself as $[M + H + ACN]^+$. Since analytes typically do not form clusters with methanol, and a wider range of analytes are soluble in methanol, a methanol-based FIA method is offered with a scan range lower limit of 100 m/z. Because of suppression issues, no modifier is used within the methanol. A LC switch method is used to convert the solvent in the entire LC and MS flow path from water/ACN used for LC analysis to methanol used for FIA, and vice versa. The short-duration switch method incorporates a high solvent flow rate.

Mass Spectrometer Setup

Ionization technique is largely a matter of personal preference. We have found that virtually all of our compounds, except small acids or weakly polar analytes, ionize utilizing ES+. (Often negative ion cannot be used on a system that has recently been exposed to TFA.) Infusion experiments were performed (data not shown) on typical sample analyte(s) with varying flow rates to tune the instrument. Each mass spectrometer demonstrated a "sweet spot" for voltages, ES probe position, and source and desolvation probe temperature, which was typically broad—i.e., "set and forget." Figure 9 is a random mass spectrum from a section of baseline for a ZMD when a blank was being analyzed by LC/MS. It demonstrates the low-baseline electrospray positive-ion (ES+) TIC from a clean LC system. As cone voltage is increased, hard-to-ionize analytes may become present, however, overall increased fragmentation may occur, which reduces pseudomolecular ion $[M + H]^+$ intensity and creates a mass spectrum that can be more difficult to interpret. The casual open access user typically prefers little fragmentation and an uncluttered $[M + H]^+$ spectrum. The MS is typically scanned from 145 to 900 Da in 0.5 sec w/0.1 sec reset time from t_0 to 3.8 min. Note the 3.8-min quadrupole scanning termination time, which is shorter than the 4.2-min total LC run time. Beyond 3.8 min 100% ACN conditions exist throughout the column and into the MS inlet. During that high organic condition, the ES+ TIC can reach elevated levels that dwarf a weak TIC. Since, all but extremely hydrophobic

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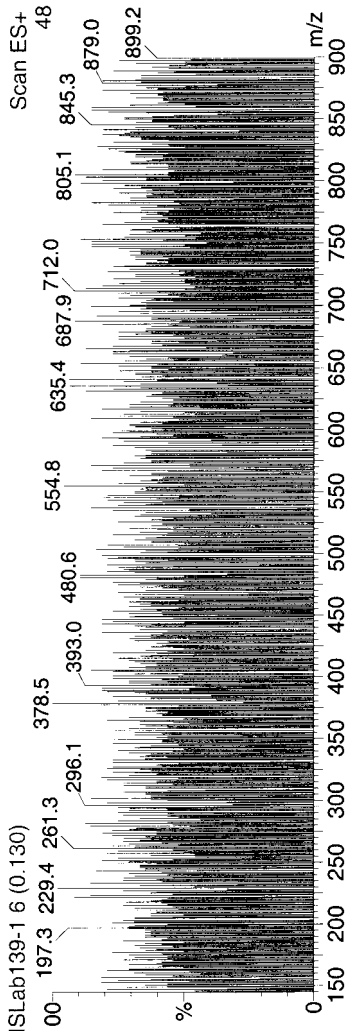

Figure 9. Random mass spectrum from a section of baseline, for a ZMD.

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extra-long retained analytes have eluted, the MS acquisition is terminated. The desired MS scan rate is based on the width of an LC eluted peak. A check is made to determine how many scans are present across each peak. If the number of scans is insufficient, or apparent resolution is poor, increase the scan rate bearing in mind the instrument's maximum scan rate. Typical quadrupole scanning parameters are 145–900 Da in 0.5 sec, 0.1-sec inter-scan reset time, ESP+ centroid mode from time 0 to 3.8 min.

In the ever increasing quest for a fast analysis where near-ballistic LC gradients deliver razor thin eluted peaks, the mass spectrometer and data system must be able to scan at increasingly rapid scan rates. Current LC conditions deliver peaks that are 3 seconds, full width at baseline, or no more than 2 seconds FWHM. Approximately 0.4 sec/scan, or 2000 AMU/sec, is close to the maximum scan rate on most quadrupoles without performance losses. Allowing for a 0.1 sec MS Quad reset time, only six scans maximum could exist across the total width of a peak. Considering the two outermost scans are at a region on the peak profile where, essentially, no TIC is present, only four scans are left to represent and define that peak. This resolution might be sufficient for qualitative experiments, albeit very poor chromatography, it would certainly be unacceptable for quantitative experiments. The following (Fig. 10) demonstrates the necessity for rapid scanning of the quadrupole. The MS scan rate for the top TIC trace is 1.2 sec/scan (+0.1 sec reset time) and for the bottom TIC trace is 0.4 sec/scan (+0.1 sec reset time). Note the apparent loss of resolution and lack of scans across an eluting analyte on the top, slow scan-speed, trace. At 1.2 sec/scan, barely two scans are recorded for a peak. Complete resolution was obtained at 0.4 sec/scan, which reflects actual ion

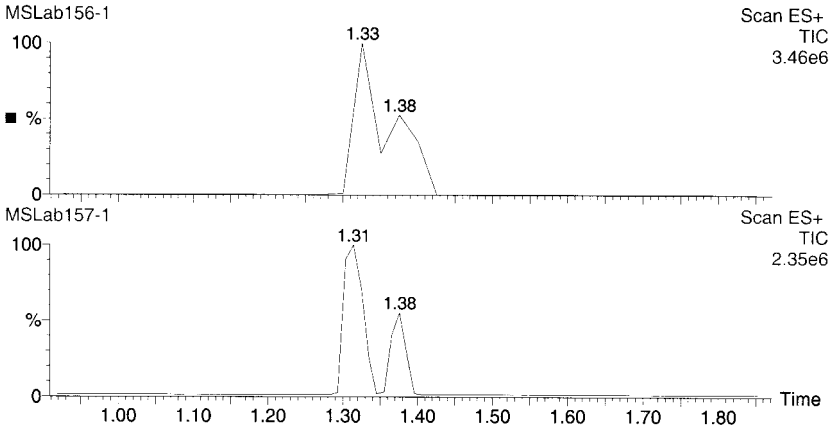

Figure 10. Top trace, 1.2 second per scan. Bottom trace, 0.5 second per scan.

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residence time within the source and was comparable in presence to the 10-Hz UV trace. This example also illustrates the necessity to calibrate the instrument such that the scan speed(s) used for all the LC/MS methods fall within the instrument calibration window.

Open Access Software Interface and User Training

OpenLynx Login software is robust and user-friendly and was utilized as the front-end for all OA-LC/MS systems. The open access software allows investigators to log-in and analyze samples using all available pre-programmed methods 24/7. Regardless of how well the hardware has been set-up the final important parameter is the end-user. As robust as modern OA-LC/MS systems are, they can be rendered inoperable with the introduction of one ill-prepared sample. All users were instructed in the areas of instrument operation, sample preparation, sample login procedure, and resultant API MS appearance. Listed are the critical guidelines to the users concerning sample preparation.

- Make every attempt to use some, even up to 50%, water in each sample vial.
- Filter samples if cloudy or displays visible precipitate (Fig. 11).

Figure 11. Vial 1 contains a sample dissolved in 100% methanol. Vial 2 is identical sample with 1 drop of water. Vial 3 contains a small particle of white precipitate in lower conical section. Vial 4 shows yellow ppt.

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- Do *NOT* use DMSO, THF, phosphates, methylene chloride, or concentrated acids.
- Maximum analyte concentration is 200 μg/mL.

One hundred percent of the vials and caps used in all the OA-LC/MS instruments are supplied to the users. This avoids problems with short vials or narrow ill-fitting glass inserts that tend to wreak havoc with injection needles. Three samples for LC/MS or five samples for FIA maximum per login are allowed.

Preventative Maintenance and Overall System Management

The implementation of a thorough PM program is necessary to ensure maximal uptime of instruments that produce the highest quality data. A spreadsheet of all PM activities, monthly resolution and response test mix (Table 1; Fig. 5 lower trace) results, and equipment failures were created that allows the system administrator to closely track the state of all instrumentation. A normal monthly PM involves rebooting the computer, elimination of old data, an LC system methanol wash to dissolve intractable analytes, and changing the filter frit and rough pump (RP) oil. A valve and petcock on the pump oil drain port (Fig. 12) enables rapid (<5 min) oil changes using vacuum-assisted oil draining. Rough pumps are ballast continually.

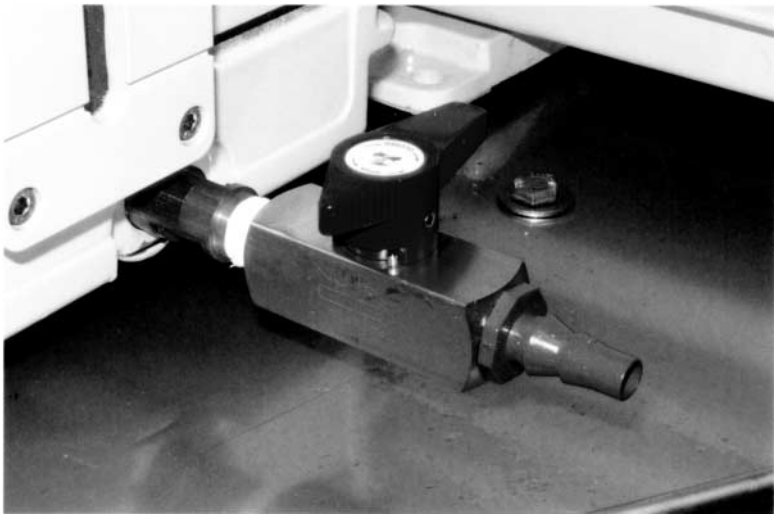

Figure 12. Petcock installed in RP drain port.

The Waters' Z-Spray interface typically delivers >10,000-sample lifetime before the cone and baffle plate requires cleaning (semi-annually). To minimize downtime, a large cache of spare parts is necessary. Inventoried spare parts: RP oil, filter frits, LC columns or LC (column) cartridges, all types of spare tubing and fittings, ferrules and nuts, all o-rings, source probe kits, UV lamps, source heaters, source cone and baffle, RP oil mist filters, vacuum tubing, and clamps. Inventoried extreme spare parts: E2M28 rough pump, both source and analyzer turbomolecular pumps and controllers, 2695 rebuild kits, MS source rebuild kits, and an LC switch valve.

RESULTS

Chemists are currently analyzing samples at a departmental annual rate of >130,000 (Table 2). During the week the instruments rarely need attention. On the rare occasion when a problem occurs, it is usually one of two issues: a user dissolved an LC sample in 100% methanol, which upon injection was insoluble in 94% aqueous solution (Fig. 11) and plugged the injector exit-line or a user inadvertently closed OpenLynx Manager. Judicious preparation of the entire OA-LC/MS system(s), combined with using an exceptional grade of solvents, followed by strict guidelines to the users has created instrumentation that is extremely reliable. Routine monthly maintenance rarely consumes more than 0.5 hour per instrument. Liquid chromatography column selection and operation was critical to ensure desired high peak capacity performance. The following summary details the final criteria: long gradient and higher flows, delayed injection, use a 3 mm ID × 5 cm length LC column (4–5 μM silica particle) at 2 mL/min max flow rate, and reduce all tubing lengths and diameters to a minimum with square-cut ends. To improve analyte/column kinetics set the LC column oven at 45°C. To operate the LC in a comfortable range, set maximum flow rate such that the pressure stays below 3000 psi at 94% aqueous. The YMC PRO is available in two formats; a complete column format and a currently used, cost-saving, cartridge format. That is where, one purchases a cartridge and upon removal of the old column from the LC the column end-caps are removed and placed on the new cartridge, thereby, creating a new column. Five-minute sample-to-sample analysis time does not break any speed records, however, given the chemists possible dirty sample a 5-min analysis offers high PC with 2-second FWHM eluted peak widths. This efficiency compares favorably to the typical user's stand-alone LC within his or her laboratory, that has a 15–30 min analysis method, utilizing a 10–25 cm length column. Under these high peak capacity conditions, the user rarely experienced overlapping peaks. Accurate UV integration on the OA-LC/MS system is desired because the chemist will use sample integration numbers to

Table 2. Chemists currently self analyze samples at a rate of ~130,000/yr.

Date (2001)	1	2	3	4	5	6	7	8	9	10	11	12	Total
Jan	799	1,761	914	688	1,720	604	957	760	690	838	1,205		10,936
Feb	865	1,670	798	670	1,801	234	1,086	580	579	584	1,003		9,870
Mar	809	1,715	760	774	1,355	119	1,330	906	417	323	1,110	584	10,202
Apr	637	1,662	662	596	1,272	495	845	993	550	609	896	513	9,730
May	745	1,994	741	582	1,385	749	1,069	975	479	595	1,014	623	10,951
Jun	853	1,776	1,079	548	1,358	585	1,080	854	411	628	708	456	10,336
Jul	795	1,713	948	704	1,378	704	1,238	863	499	990	797	523	11,152
Aug	998	1,888	980	875	1,214	669	1,441	857	404	1,413	1,046	513	12,298
Sep	898	1,650	1,025	656	985	617	1,221	774	492	1,042	1,039	537	10,936
Oct	1,113	1,978	1,324	960	1,524	791	1,477	1,250	521	1,282	1,270	814	14,304
Nov	688	1,566	1,215	827	1,139	931	1,100	949	384	999	1,280	342	11,420
Dec	555	1,101	779	783	759	627	855	698	405	765	854	273	8,454
Total	9,755	20,474	11,225	8,663	15,890	7,125	13,699	10,459	5,831	10,068	12,222	5,178	130,589

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report sample purity, which eliminates the need for a stand-alone LC system within the users' laboratory—which translates to a further cost savings. A limited portfolio of analyses, with quick and easy OpenLynx login, fulfills the users needs. Keeping injection volumes low with moderate column cross-sectional area and demanding that the maximum column back-pressure is held to <3000 psi delivers minimal sample "blow through" and exceptional LC column lifetimes. Keeping many of the solvent compositions identical (methanol in the two wash solvents and the C and D reservoirs) and being able to perform monthly routine maintenance in a short time span translates to maximal instrument uptime and low maintenance demands.

DISCUSSION

As with any analytical technique all users must be instructed in the use of the instrumentation as well as the meaning of the results, especially the peculiarities of the data such as $[M + Na]^+$, $[2M + H]^+$, $[M + H + ACN]^+$, and so on. The chemists now routinely perform multiple, and increasingly difficult reactions, because of the availability of virtually instantaneous LC/MS results. Reaction monitoring allows the users to rapidly adjust parameters ensuring more favorable reaction outcomes. Considering a meager 5-year instrument lifetime at approximately 1000 samples/mo, that amounts to including salaries and consumables, <$2.50/sample. One analytical chemist (who in this case has an electrical engineering degree) manages all of the systems hardware and software, solvent preparation, and PM activities. In discussion with colleagues who are located at companies that have similar instrumentation setups, where each OA-LC/MS system was managed by some bench chemist who happens to be in close proximity, the colleague related that scenario failed.

CONCLUSIONS

Proper instrumentation selection is imperative in allowing subsequent exercises to be fruitful. Initial LC setup, rigorous selection of column chemistry, and judicious analytical parameter selection creates high peak capacities and efficiencies in the front end. Proper MS setup provides quality mass spectra and data integrity. User training is imperative and cannot be overstated. Constant preventative maintenance keeps the instrumentation trouble free. All of these exercises will yield well-managed, cost-effective, and robust OA-LC/MS instrumentation that provides rapid-turnaround, high-quality data with greater than 99%+ uptime (data not shown). (Units are typically down 1 day annually for an annual PM and a cumulative total of

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1 additional day for all monthly PMs and minor problems). As an indication of reliability, the 17 LC columns that have been changed thus far average 7117 samples/column. Virtually all targeted parameters were achieved: good performance, ease-of-use, robustness, and reliability.

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