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Good Practices for Successful High-Throughput LC-MS Bioanalysis

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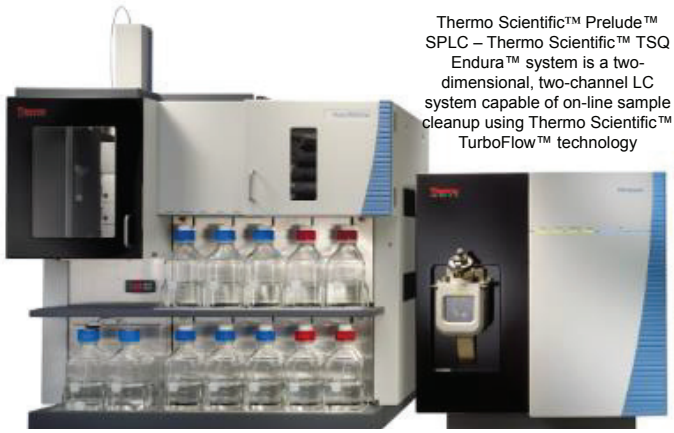
ABSTRACT

As LC-MS systems are designed to measure part-per-trillion amounts of analytes in biological samples, steps must be taken to prevent sample and system contamination and degradation to ensure reliable results. Precautions include dusting off reagent bottles before use, adding antimicrobial reagents to aqueous mobile phases, preventing contact of solvent lines and filters with contaminated surfaces, protecting columns from buildup of interfering sample components and avoiding residue buildup in MS ion sources. The application of these and other good practices to conventional, multi-dimensional and multi-channel LC-MS systems used for high-throughput bioanalysis (Figure 1) are summarized in this presentation.

Figure 1. Example high-throughput LC-MS system.



Thermo Scientific™ Prelude™ SPLC – Thermo Scientific™ TSQ Endura™ system is a two-dimensional, two-channel LC system capable of on-line sample cleanup using Thermo Scientific™ TurboFlow™ technology

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Ensure clean systems and reagents

Prevent dust buildup

Injection ports and LC-MS-grade solvents are easily contaminated by dust, which can contain fragments of dead skin cells and hair. The analytes we measure in biological samples are often found in dust. Avoid topping-off solvent bottles, which promotes accumulation of dust and other particles.

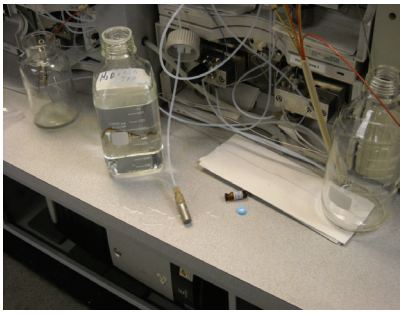
Prevent contamination of solvent filters and lines

Avoid letting them touch contaminated surfaces when changing solvents. Aluminum foil can help, as shown in Figure 2. Too frequently, samples spill on surfaces. Also, your hands and fingers may be contaminated with analytes, such androgens, estrogens, nicotine, cannabinoids and cocaine. Be careful what you touch, wash frequently and put on fresh disposable gloves before making fresh solvents.

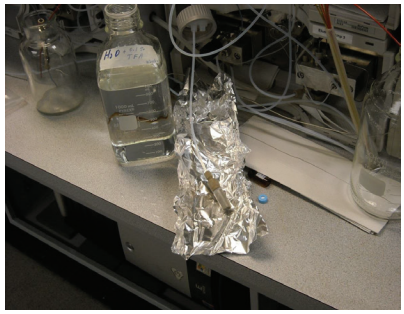
Also, use appropriate caps for solvent bottles to secure solvent lines in and keep dust out of bottles. Never use polymer films to cap solvent bottles.

Figure 2. Changing solvent lines.

Bad way



Good way



Prevent microbial growth

Aqueous solutions of acetic acid and especially ammonium acetate support microbial life. Addition of either 2% acetonitrile or 5% methanol will prevent growth. Fortunately, formic acid and ammonium formate do not support microbial life.

Although phosphate-buffered saline is not used in mobile phases, it is often used to make calibrators. Figure 3 shows the consequences of not having a preservative in such solutions.

Figure 3. Phosphate-buffered saline with and without anti-microbial reagent.

Phosphate-buffered saline supports microbial growth. Left bottle had no preservative and became cloudy after several days. Right bottle had 2% acetonitrile and remained clear for years.

Use highest purity solvents, gases and reagents

Even “HPLC-grade” solvents have caused high backgrounds in certain applications. LC-MS-grade solvents and reagents should be used for applications with the lowest limits of quantitation.

Ensure consistently good system performance

Perform preventative maintenance diligently on each module and computer

- Pump piston seals & solvent filters – replace after pumping 200 liters of solvents
- Pump cam lubrication – run purge procedure every 6 weeks
- Injector needles & needle seals – replace if scratched or bent or after 5,000 injections
- Injector syringe or syringe plunger – replace when carryover noticeably increases or after 2,000 injections
- Valve rotor seals – replace after 15,000 injections
- TurboFlow™, HPLC, UHPLC columns – replace after 2,000 injections
- Mass spectrometer – keep it clean and calibrated
  - Clean ion source surfaces and ion-transfer tube at least weekly, clean ion optics at least annually
  - Check/perform mass calibration and detector gain at least quarterly
- Computer – reboot daily, delete temp files weekly, archive/change data folders monthly (no folder should have more than 200 files), defragment hard drive quarterly
  - Computer should not utilize power-saving settings other than screen-saver
  - Disable automatic software updates during data acquisition.

Ensure consistently good system performance

Perform all system-startup checklist tasks and allow the system to equilibrate

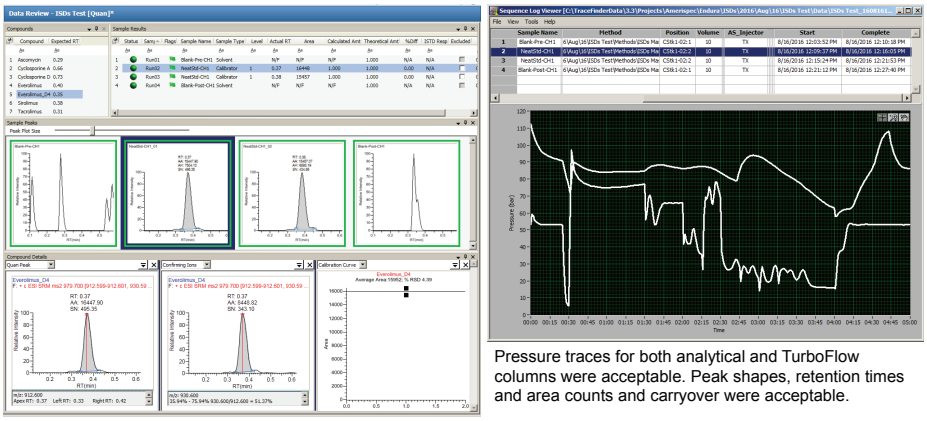
- Check/refill all solvents & wash solutions, purge all lines to remove bubbles
- Verity that solvent degassers are working
- Check/empty all waste containers
- Check/replace/service nitrogen & argon gas cylinders/generators
- Start up instrument control software and verify communication/control for each module
- Start up MS with ion source parameters of 1st method, equilibrate for at least 30 minutes
- Verify stable ion source temperatures and gas flows, verify acceptable MS or MS/MS background signals
- Start pump(s) with parameters of 1st method, equilibrate for at least 5 minutes
- Verify acceptable startup pressures and check for leaks

Run suitability/preview test batches before large sample batches

Run a pre-blank (verify functionality of the system and acceptable background signals) followed by neat solution of standards or internal standards (IS) at least twice (verify acceptable peak shapes, retention times & area counts) followed by post-blank (verify acceptable carryover).

Example test-batch results from a research method for immunosuppressant drugs are shown in Figure 4.

Figure 4. Suitability/preview batch results – immunosuppressant drugs (ISDs) example.



Pressure traces for both analytical and TurboFlow columns were acceptable. Peak shapes, retention times and area counts and carryover were acceptable.

Ensure good sampling

Perform best sample preparation technique to minimize matrix interferences

Evaluate protein precipitation (PPT), solid-phase extraction (SPE), liquid-liquid extraction (LLE), supported-liquid extraction (SLE), etc. to maximize recovery while minimizing ion suppression and other interferences.

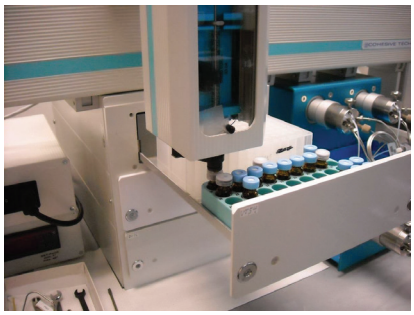
Verify particle-free and bubble-free sample vials/wells before making injections

Avoid aspirating precipitates and bubbles when sampling.

Verify proper autosampler-needle penetration, sample-volume aspiration and injection

Be sure that autosampler vial trays and micro-titer plates are positioned properly (see Figure 5).

Figure 5. Autosampler objects – calibrate all positions



Optimize X, Y, Z and needle penetration for

- Injection valves
- Sample trays
- Wash stations

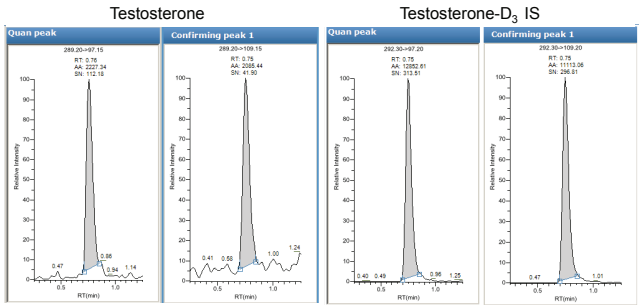
Position trays properly

Ensure reliable peak processing

Verify peak retention time and purity – peak shape and ion-ratio confirmation

Figure 6 shows example for testosterone peaks extracted from blood serum for research purposes

Figure 6. Example peak shape & ion-ratio confirmation results



Ion Ratio: 94% (100% average) Ion Ratio 86% (80% average)

Testosterone calculated amount was 21 ng/dL

Verify channel-to-channel reproducibility of processing peaks

Table 1 shows example data from IQ/OQ test results for replicate injections of caffeine standard with (TX) and without (LX) TurboFlow on-line extraction.

Table 1. Example IQ/OQ test results for Transcend II TLX-2 system.

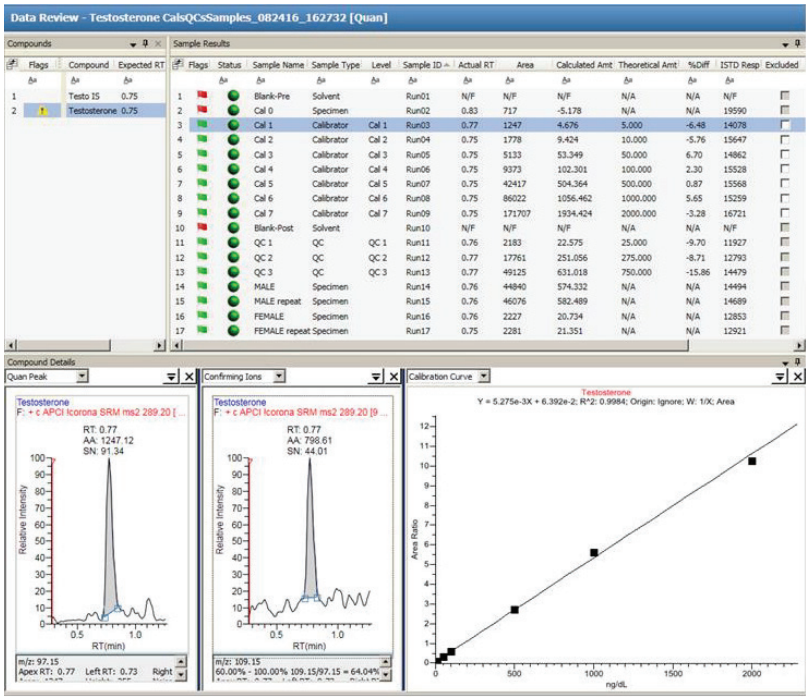
Component Name	Curve Index	Weighting Index	Origin Index	Equation			
Caffeine	Average RF	Equal	Ignore	Average Response Factor = 24446.1			
Filename	Sample Name	Area	RT	Filename	Sample Name	Area	RT
Repeat_CH1_LX_002	Caffeine 80	1953448	0.66	Repeat_CH2_LX_002	Caffeine 80	2019594	0.66
Repeat_CH1_LX_003	Caffeine 80	1929760	0.66	Repeat_CH2_LX_003	Caffeine 80	2019006	0.66
Repeat_CH1_LX_004	Caffeine 80	1956965	0.66	Repeat_CH2_LX_004	Caffeine 80	2017899	0.66
Repeat_CH1_LX_005	Caffeine 80	1956648	0.66	Repeat_CH2_LX_005	Caffeine 80	2015078	0.66
Repeat_CH1_LX_006	Caffeine 80	1972124	0.66	Repeat_CH2_LX_006	Caffeine 80	2029134	0.66
Repeat_CH1_LX_007	Caffeine 80	1965164	0.66	Repeat_CH2_LX_007	Caffeine 80	2030565	0.66
	Average:	1955685.1	0.66		Average:	2021879.3	0.66
	StdDev:	14420.64	0.00		StdDev:	6382.04	0.00
	%CV:	0.74	0.38		%CV:	0.32	0.38
Filename	Sample Name	Area	RT	Filename	Sample Name	Area	RT
Repeat_CH1_TX_002	Caffeine 80	1985087	0.72	Repeat_CH2_TX_002	Caffeine 80	2041419	0.73
Repeat_CH1_TX_003	Caffeine 80	1986108	0.73	Repeat_CH2_TX_003	Caffeine 80	2047173	0.73
Repeat_CH1_TX_004	Caffeine 80	1993398	0.72	Repeat_CH2_TX_004	Caffeine 80	2044071	0.73
Repeat_CH1_TX_005	Caffeine 80	1982425	0.72	Repeat_CH2_TX_005	Caffeine 80	2042243	0.73
Repeat_CH1_TX_006	Caffeine 80	1969915	0.72	Repeat_CH2_TX_006	Caffeine 80	2037637	0.73
Repeat_CH1_TX_007	Caffeine 80	1988777	0.73	Repeat_CH2_TX_007	Caffeine 80	2034522	0.73
	Average:	1984284.8	0.73		Average:	2041177.5	0.73
	StdDev:	7964.27	0.00		StdDev:	4524.47	0.00
	%CV:	0.40	0.47		%CV:	0.22	0.24

Verify performance of quantitation and quality-control (QC) results:

- %Difference between calculated and accepted concentrations of calibrators and QCs
- Regression statistics of calibration plot
- Reproducibility of internal standard (IS) peak areas throughout batches

Figure 7 shows examples of such data for total testosterone measured in blood serum for research purposes

Figure 7. Typical quantitative results for total testosterone research method



%Differences < 10% (+/-) for Calbrators & QCs, good linearity using 1/X weighting, IS peak areas averaged 15,315, < 6% RSD among Calibrators. QC & Specimen IS peak areas were within (+/-) 25% of average

Shutdown the system properly

- Automatically have the system go into Standby after completion of the last submitted batch or run a Shutdown method, which cleans the column(s) and then turns off the pump(s) and places the MS ion source in standby condition – low temperature, low nitrogen flow, no collision-gas flow.
- Prevent power outages, especially for vacuum pumps – use uninterruptable power supply (UPS).

Document all of the above practices and outcomes

SOPs should be clearly written in order to properly

- Start up, operate and shut down the system each work period
- Report and get resolution to any issues that occur during each work period
- Qualify batch results to be released/reported
- Enable and facilitate quality-assurance auditing
- Plan future system maintenance, service, upgrades, etc.

CONCLUSIONS

Our good HPLC practices ensure compliance with system suitability tests and quality control specifications as large sample batches are reliably completed.

For research use only. Not for use in diagnostic procedures.

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