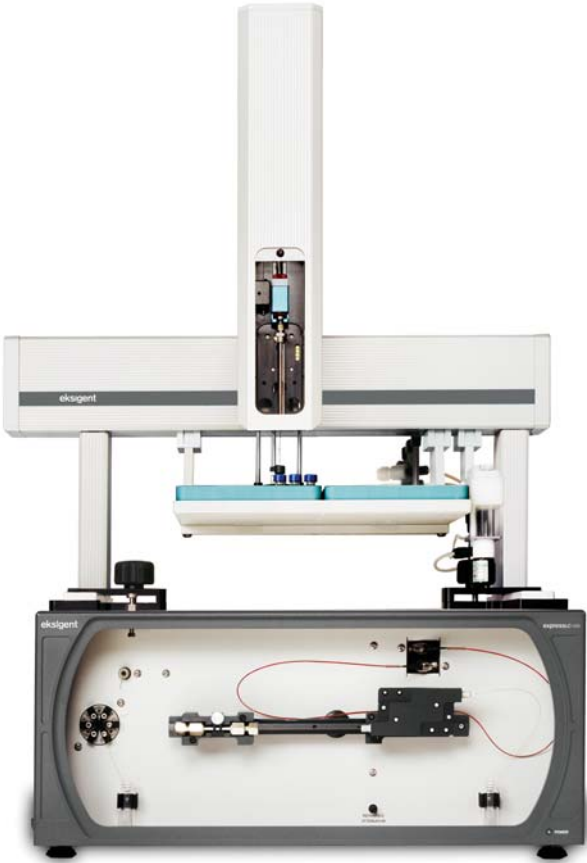


eksigent
rethinkHPLC



expressLC systems

a new generation of performance with the expressLC™ systems

The ExpressLC system is a fully integrated HPLC system that combines advances in gradient delivery, sample injection, and optical detection to deliver dramatically increased levels of chromatographic resolution and speed. Sample throughput can be increased by as much as 6 times compared to conventional HPLC, making the ExpressLC-100 single-channel system ideal for high throughput applications in drug discovery and development. The ExpressLC-100 single-channel and ExpressLC-800 8-channel parallel systems provide binary solvent delivery, high pressure gradient mixing, variable volume injection, UV absorbance detection, and PC-based instrument control in a compact, integrated package.

microfluidic flow controlled pumps and advanced technology

Optimized for 300 μm i.d. columns at flow rates of 0.20 to 30 $\mu\text{L}/\text{min}$, the ExpressLC system provides speed and instrumental performance not achievable with conventional HPLC equipment.

The ExpressLC system incorporates a new pumping principle (Figure 1). Rapid, precise adjustment of a variable-pressure pumping source is achieved using Eksigent's microfluidic flow controller (MFC). In-line flow meters actively measure the actual flow rate in each mobile phase and provide immediate adjustments to deliver an accurate, consistent and precise gradient, run after run (Figure 2).

short analysis times

Eksigent's innovative system design dramatically reduces analysis times. While conventional HPLC systems have gradient delay times of 1 minute or more, the first peak in Figure 3 elutes in under 5 seconds. Figure 4 demonstrates the ExpressLC system's capability to fully resolve 7 compounds in 16 seconds.

expressLC-100 system for single-channel HPLC

Achieve high performance separations in a single-channel format with the ExpressLC-100 system. The ExpressLC-100 is ideal for sample-limited applications requiring speed, high quality quantitative data, and separation efficiency. You can obtain retention time precision and accuracy with real-time flow rate control and reduce mobile phase consumption by more than 99% compared to conventional HPLC systems.



expressLC-800 system for 8-channel parallel HPLC

Eight independent HPLC channels and a high-capacity autosampler are provided in the integrated ExpressLC-800 system. The ExpressLC-800 is ideal for high-throughput applications such as ADMETox screening and analytical method development. The ExpressLC-800 offers true parallel HPLC for dramatic savings in analysis time, labor, and it occupies less than 25% the bench space of conventional HPLC systems.



figure 1.

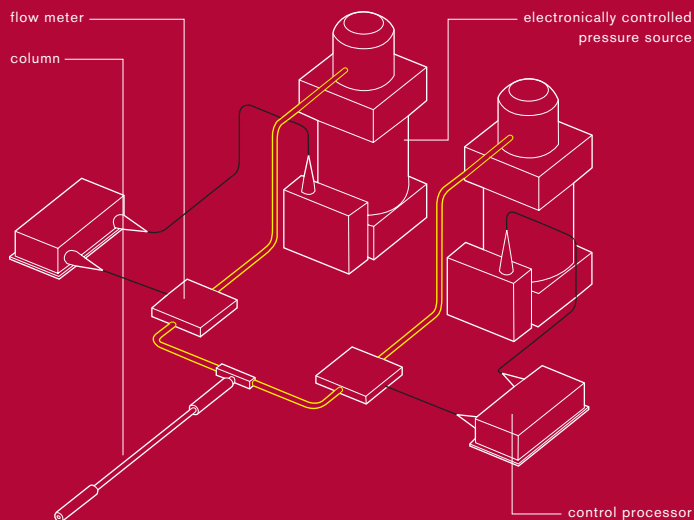


Figure 1. Microfluidic Pumping Technology. Flow meters continuously monitor the flow rate of each mobile phase with microprocessor feedback to a precise, rapid pressure source. Using MFC you can maintain precise nanoscale flow rates as low as 20 nL/min without flow-splitting.

figure 2.

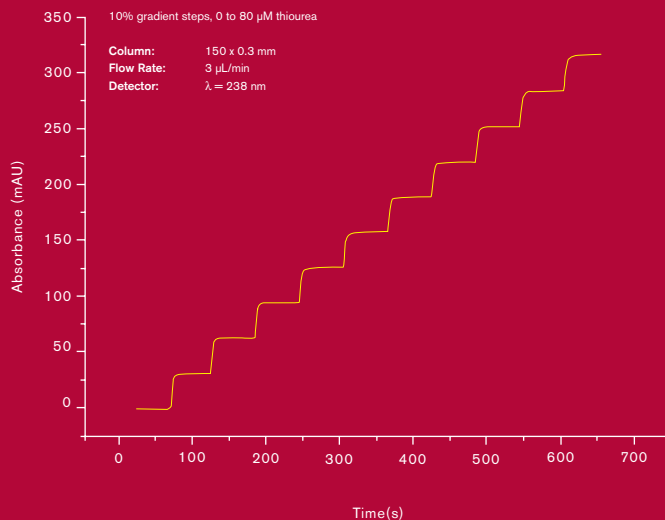


Figure 2. Repeated step gradients on the ExpressLC system demonstrate the precise and rapid gradient formation not achievable with conventional mixers.

Figure 3. Gradient separation of uracil and phenones in 60 seconds on the ExpressLC system. The first peak elutes in under 5 seconds, compared to 1 minute on conventional HPLC systems.

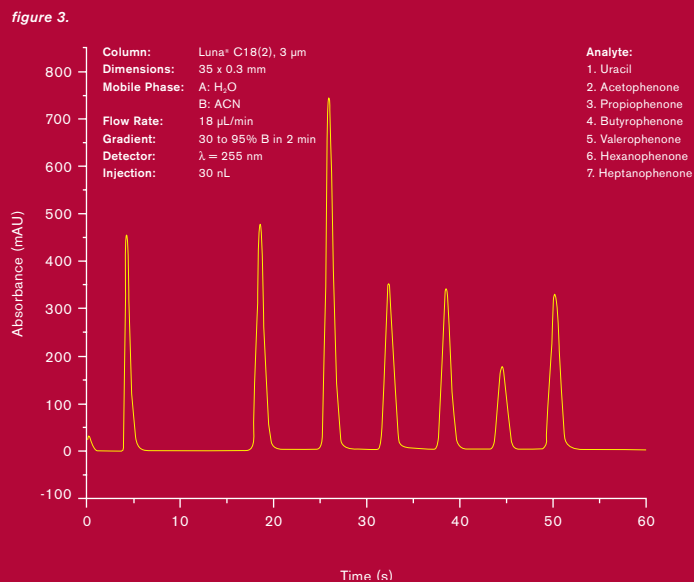
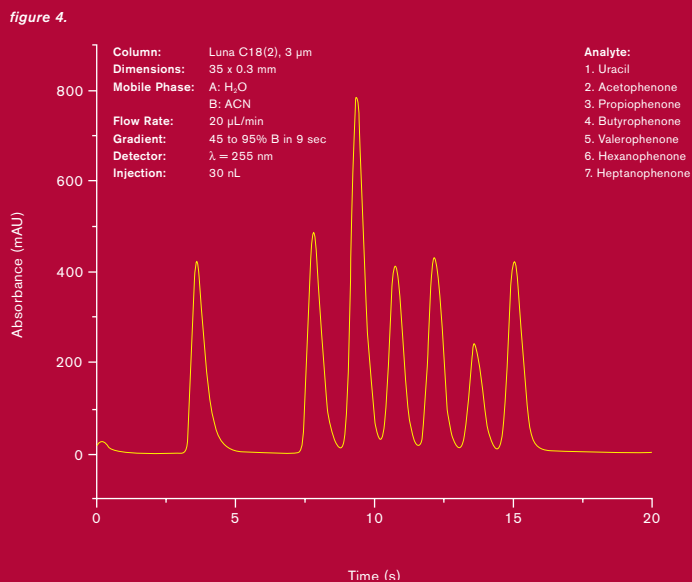


Figure 4. Separation of Uracil in 20 seconds on the ExpressLC system using a 9 second gradient.

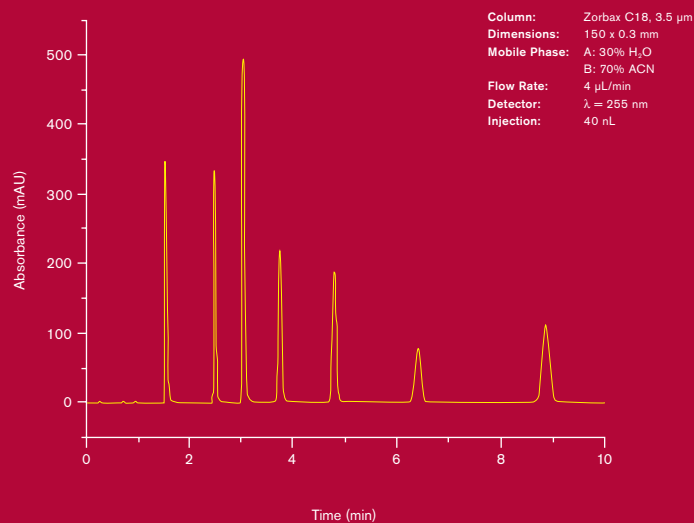


efficient, precise and sensitive chromatography



Figure 5. ExpressLC system achieves high separation efficiencies even for early-eluting peaks.

figure 5.



no-compromise chromatography

Achieve short cycle times without sacrificing efficiency. The ExpressLC system doesn't rely on positive displacement pumps and large volume mixers to generate gradients, so speed and resolution need not be compromised. Get efficient, high-speed chromatography with the ExpressLC system (Figure 5).

- Low dispersion system design
- Fully integrated components, including injector, column, and detector
- Rapid gradient change response
- Fast re-equilibration with an instrumental void volume of only 300 nL from mixer to column

gradient precision

Eksigent's low-volume, high-efficiency mixing combined with outstanding flow rate control provide the gradient repeatability required for separating complex matrices. Figure 6, and its associated table, indicates the performance typical of an ExpressLC system.

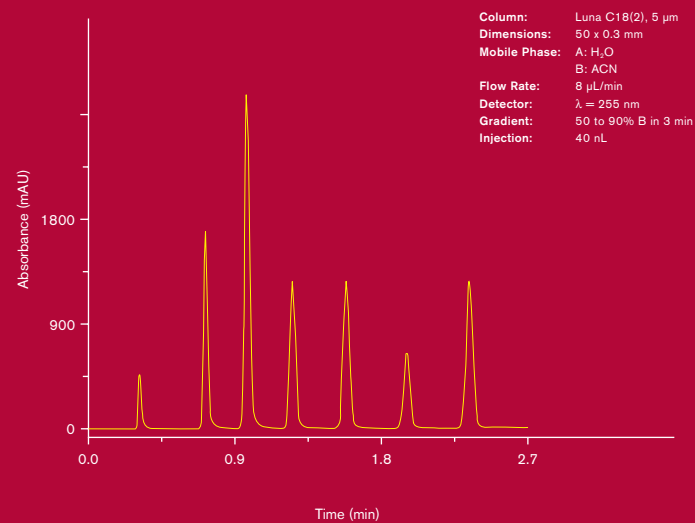
isocratic precision

The ExpressLC system's revolutionary flow control ensures high retention time repeatability. High retention time precision translates into higher confidence in peak identification and quantitation. The results shown in Figure 7, and its associated table, demonstrate the system's isocratic precision.



Figure 6. Ten overlaid chromatograms of uracyl and six phenones demonstrating the high peak area, peak height, and retention time precision of Eksigent's ExpressLC system.

figure 6.



exceptional detection sensitivity in the femtomole range

Achieve exceptionally low detection limits without increasing injection volume or sample concentration. Eksigent's ExpressLC system achieves high sensitivity using a low dispersion, patented microfabricated 45 nL flow cell with array-based UV detection in the range of 200–380 nm (pictured at right). Figure 8 illustrates the levels of detection achievable. Minimum detection limits (DL) at a signal-to-noise ratio (S/N) = 3 were calculated from the chromatogram to be less than 15 ng/mL (< 15 femtomoles). The minimum quantitation limit (QL) was determined to be less than 50 femtomoles.



fully integrated control software

Eksigent's Control Software makes the ExpressLC system easy to learn, operate, and maintain (at right).

- Automated and manual operation
- Program gradients graphically or in table format
- View chromatogram and gradient composition in real-time
- Bar graphs provide at-a-glance pressure, flow rate, and temperature characteristics of the system
- Built-in automated diagnostics
- Real-time spectral information and spectral contour plots

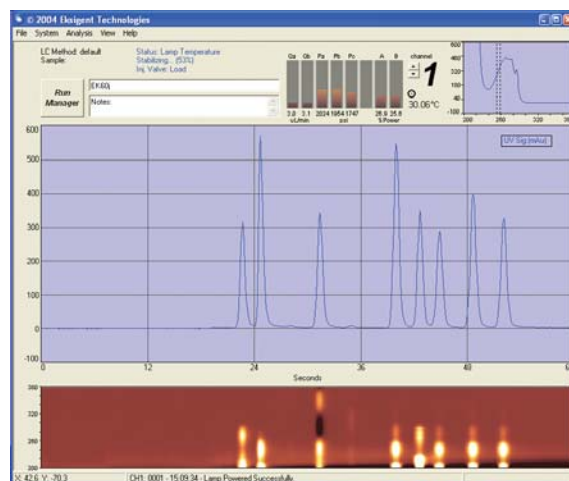


Figure 7. Ten overlaid chromatograms of uracil and three phenones demonstrating high quantitative precision during a high-speed isocratic chromatographic analysis

| Analyte | Retention Time | RSD (%) | Area Precision | RSD (%) |
|--------------|----------------|---------|----------------|---------|
| uracil | 0.15 | 0.30 | | |
| acetophenone | 0.18 | 0.36 | | |
| propiofenone | 0.21 | 0.37 | | |
| butyrofenone | 0.23 | 0.51 | | |

figure 7.

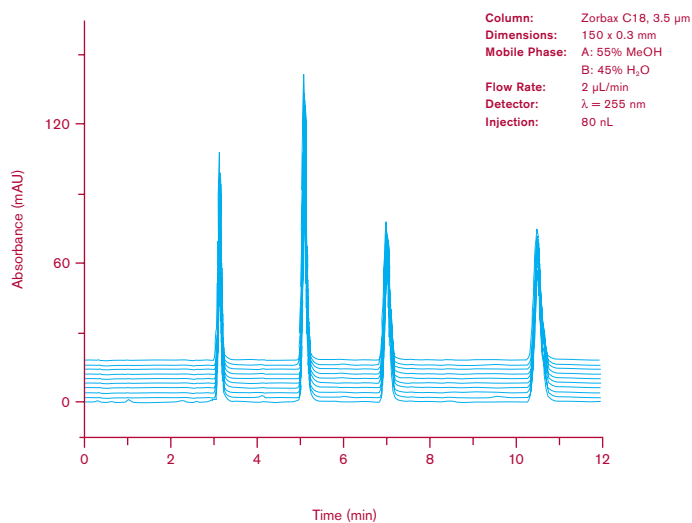
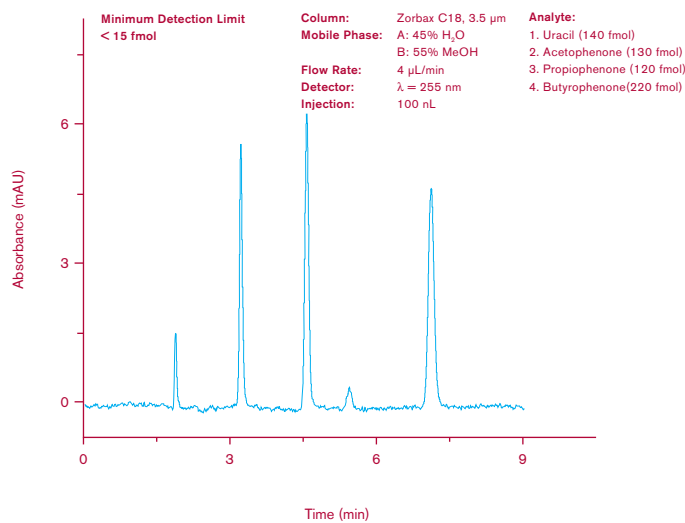


Figure 8. Chromatogram of uracil and three phenones used for the determination of the detection limits of uracil and three phenones on the ExpressLC system.

figure 8.



applied performance applications using the expressLC systems

pharmaceutical applications

solution degradation of an indomethacin formulation

Whether it's degradation studies to challenge the specificity of a drug substance, analysis of toxicological dosing solutions, robustness studies for an NDA submission, or long term stability studies (LTSS)-the number of samples can be overwhelming. For specificity and LTSS studies, sensitivity is a crucial aspect of analyses. With the ExpressLC system, consistent and high-quality data can be achieved routinely (Figure 9).

- Detect low-level impurities before they become regulatory issues
- Reduce time and resource intensive activities with high throughput and excellent sensitivity
- Make 10–15 injections from the same sample volume required for one conventional HPLC analysis
- Eliminate embarrassing "insufficient sample for analysis" data entries

chiral drug analysis

Increased sophistication of chiral HPLC separations has come to the forefront of pharmaceutical studies, including analysis of atropisomeric (hindered rotation around a single bond) species. The ExpressLC system improves chiral analyses by reducing the volume of costly stationary phase, shortening analysis times, and providing rapid system conversion from reversed phase to normal phase solvents.

The ExpressLC system performs the chiral analysis of 2-benzoyl-5-norbornene in less than 90 seconds, with efficient peaks and very low sample size requirements (Figure 10). The system's inherent low volume design and automated purge feature facilitate rapid conversion to normal-phase eluants.

Figure 9. Thermal degradation of indomethacin.

| Analyte | Area % |
|--------------|--------|
| 1 | 3.02 |
| 2 | 0.13 |
| 3 | 5.82 |
| 4 | 82.85 |
| Indomethacin | 0.41 |
| 5 | 0.98 |
| 6 | 6.80 |

Figure 10. Chiral separation of 2-benzoyl-5-norbornene. Chiral analysis of 2-benzoyl-5-norbornene is performed in less than 90 seconds.

figure 9.

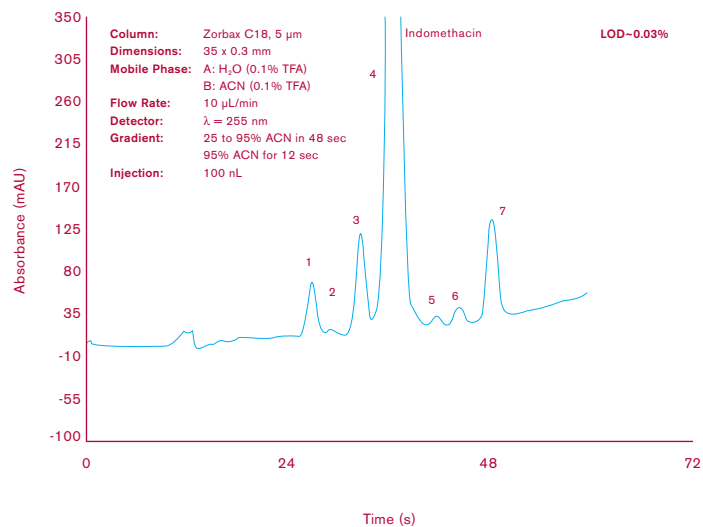
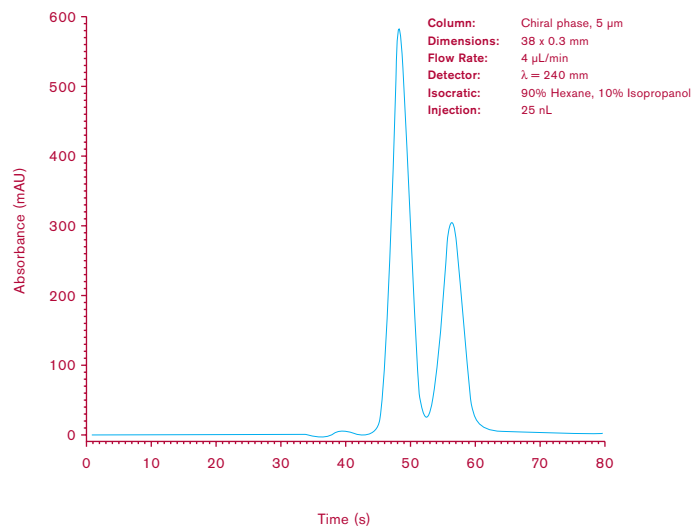


figure 10.



biotechnology applications

peptide standards and a peptide map of cytochrome C

Peptide maps are particularly useful for comparison of different lots of the same protein and for use as the first dimension in inference of the protein's amino acid sequential structure, degrees and sites of glycosylation, and extent of post-translational modification. After hydrolysis of the protein, the resulting peptides (ranging in size from a few to several hundred amino acid residues) are then separated by HPLC and the resulting chromatogram (peptide map) is analyzed. Crucial to the protocol is the ability to separate the peptides with high retention time precision so that comparison of maps does not regularly require an identification step, such as mass spectrometry, cross-reaction with antibodies, or partial sequencing. Figure 11 illustrates how the ExpressLC system, with its characteristic high-accuracy pumping profiles, is ideal for fast and repeatable peptide standards and samples.

food/feed applications

acid-induced degradation of steroidal compounds in rat chow

Rat chow is a good representation of a complex matrix with residue present even after sample preparation, and has the potential to clog the injector, column, and optical cell. The ExpressLC system's ability to analyze rat chow with no degradation of chromatographic efficiency of fluoxetine-related peaks is demonstrated in Figure 12.

Figure 11. Anti-factor IX digest.

figure 11.

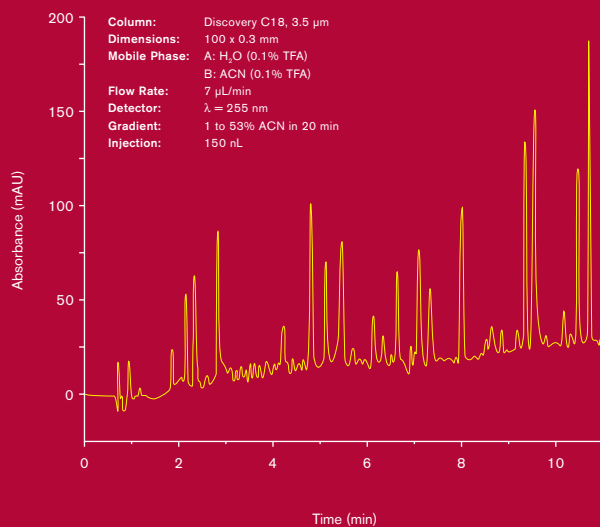
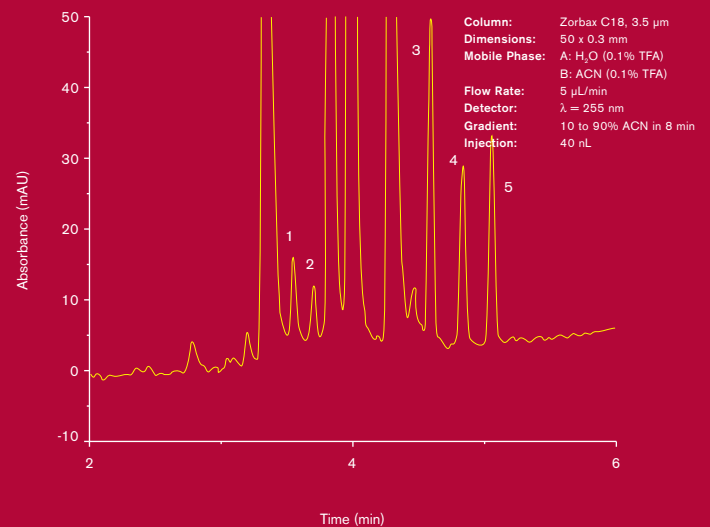


Figure 12. Assay of steroid in rat chow.

| Analyte | Area % |
|--------------------------|--------|
| Hydrocortisone | 36.8 |
| Degradent | 0.56 |
| Degradent | 0.35 |
| Dexamethasone | 18.4 |
| Corticosterone | 18.5 |
| Hydroxycortisone acetate | 22.2 |
| Degradent | 1.44 |
| Degradent | 0.83 |
| Degradent | 0.94 |

figure 12.





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expressLC system specifications

configuration

expressLC-100 single-channel system: Includes binary gradient pump, electronic injection valve, column temperature control, and array-based UV detection system. Optional high-speed autosampler available.

expressLC-800 8-channel parallel system: Includes 8 binary gradient pumps, 8 electronic injection valves, 8 column temperature control compartments, an array-based UV detection system and high-throughput autosampler.

flow rate range

0.20–30 $\mu\text{L}/\text{min}$.

pump type

Microfluidic direct pumping system with independent flow control feedback for each mobile phase. Retention time RSD < 0.5%.

gradient formation

High pressure gradient mixing. System can run full gradients as rapidly as 8 seconds. Maximum gradient length 2 hrs. at 5 $\mu\text{L}/\text{min}$.

delay volume

< 500 nL from mixer to column.

mobile phase compatibility

All mobile phases compatible with 316 stainless steel, PEEK, and silica.

injection valve

Eksigent Variable-Volume Injection System (software selectable). Standard injection volume 10–250 nL (larger injection volumes available).

columns

System optimized for use with 2.5–15 cm, 300 μm i.d. capillary LC columns

column temperature control

Software selectable from 27–40°C; stability within $\pm 0.1^\circ\text{C}$

detection

UV absorbance detection from 200–380 nm using linear CCD array detector. Detector drift $\leq 4 \times 10^{-4}$ AU/hr Non-linearity $\leq 5\%$ @ 2 AU.

flow cell

45 nL microfabricated flow cell with integral fiber optics, 4 mm path length

autosampler

High-throughput CTC autosampler available

system control

Computer with graphical user interface for control of all system parameters. Software allows import of run tables and creates CDF, text, and Excel files for data export and analysis. Tracking of instrument runtime, column usage, total injections, solvent usage, lamp hours, and error codes. System drivers available for Thermo Electron Xcalibur, Agilent EZChrom Elite and Applied Biosystems/MDS SCIEX Analyst 1.4.1 mass spectrometer software.

report features

Generates reports that include method conditions, chromatograms, peak retention times and areas, and spectral absorbance map.

dimensions

expressLC-100 system: 21" (53 cm) wide, 20" (51 cm) deep, 18" (46 cm) high
expressLC-100 autosampler: Additional 14" (36 cm) in height and 6" (15 cm) in width

expressLC-800 system: 30" (76 cm) wide, 34" (86 cm) deep, 40" (102 cm) high
expressLC-800 autosampler: Additional 16" (41 cm) in height and 16" (41 cm) in width

computer

Additional lab space needed for keyboard, mouse and monitor