L C T R O U B L E S H O O T I N G

Recognizing and Eliminating Noise Problems in Liquid Chromatography

LYNDA H. FLEMING*, JEFFREY P. MILSAP, and NORMAN C. REYNOLDS, JR.

Identifying and correcting problems related to spurious noise, both chemical and electronic, can be a difficult task. This comprehensive case study describes the sources found for and methods used to eliminate specific noise problems that the authors encountered during their use of an LC system equipped with an electrochemical detector. While it is unlikely that all of these problems will occur in one lab at one time, you may encounter many of them from time to time. These simple diagnostic tools and correction procedures can be useful when these laboratory "gremlins" do cause problems.

Lynda H. Fleming and Norman C. Reynolds are currently part of the University of Wisconsin Medical School, Milwaukee Clinical Campus; Jeffrey P. Milsap is an electrical engineer at Ohmeda Anesthetic Systems, Madison, Wisconsin. Their research interests are in the areas of chromatography and electrochemistry of endorphins.

John W. Dolan
LC Troubleshooting Editor

During our research into the use of liquid chromatography (LC) with electrochemical detection (ECD) for analyzing endogenous opioid peptides (endorphins), we experienced interferences with peak quantification and excessive baseline noise. This noise was not related to the commonly known factors that reduce the sensitivity of ECD, such as pump pulsations, an unpassivated chromatographic system, or scratched electrodes. The chromatographic literature did not provide any insights into identifying these interferences, so resolving them was time-consuming and frustrating. It became obvious that these problems were not unique to ECD and that other electronic detectors or electronic equipment might also be susceptible. This susceptibility was confirmed through discussions with other chromatographers who use different detection systems, yet experience similar disturbances. In this installment of "LC Troubleshooting," we address each of these disturbances, which contribute to baseline noise, and the approaches we used to attenuate or eliminate them.

Erratum: In the September "LC Troubleshooting" installment ("Troubleshooting LC Fittings, Part I," *LC•GC* 6(9), 788–792 [1988]), some thread types were misidentified. In the first column on p. 792, line 16 of the second paragraph should read: "However, the 1/4–28 and M6 nuts are close enough in size . . . ." Similarly, lines 21 and 22 of the same paragraph should read "... it is important to clearly label 1/4–28 and M6 fittings. . . ."

EXPERIMENTAL SYSTEM

Endorphin standards (1) were methionine–enkephalin (ME) and [L-Ala^2]-methionine–enkephalin (L-Ala^2–ME) (Sigma Chemical Co., St. Louis, Missouri). All water used for chemical solutions and LC mobile phases was prepared by adding activated charcoal (Sigma) to fresh glass-distilled water. After standing overnight, the water was filtered through a 0.2-μm nylon-66 filter (Rainin, Woburn, Massachusetts) and degassed. All LC equipment was powered by an electrical line conditioned by a Powermark frequency converter (Topaz, San Diego, California). The chromatographic system included model 6000A pumps, a model U6K sample injector (Waters Chromatography Div., Millipore Corp., Milford, Massachusetts), and a model LC-4 or LC-4B electrochemical detector (Bioanalytical Systems, West Lafayette, Indiana). A thin-layer single glassy carbon electrode was used with a stainless steel auxiliary electrode directly opposed. An applied potential of +1.05 V versus Ag/AgCl was used for the detection of endorphins. The guard column was either a Soft Sealguard column (1) packed with 10-μm Ultrapack octyl (Beckman Instruments, Altex Division, San Ramon, California) or a Phantom microbore C8 column (C.-M. Labs, Nutley, New Jersey). An in-line filter was used with an 8 cm × 6.2 mm Zorbax Golden series C8 column (Du Pont, Wilmington, Delaware). The isocratic mobile phase was 14.8 vol % acetonitrile in 27.8 m*M* KH_2PO_4 and 78 μ*M* glycylglycine; the mobile phase reservoir was suspended in a circulating water bath at 27 °C. Stainless steel solvent filters were omitted from mobile phase reservoirs. The mobile phase flow rate for all separations was 1.0 mL/min.

CONTAMINATION

Electrochemistry at high applied potentials is less selective than at low potentials because more substances are oxidizable. Commonly used chromatographic supplies for sample preparation (filters, precolumns, frits, etc.), which are advertised as being chemically resistant and free of extractables, can leach substances that are electroactive at high applied potentials. Some of these contaminants have chromatographic properties similar to those of the endorphins and therefore complicate endorphin detection and quantification (Figure 1). To avoid this problem, each piece of labware was tested with the solutions used for sample preparation or sample processing, and the solutions were injected into the chromatograph. In some cases when leaching occurred, washing the labware with acetonitrile removed the contaminating substance. If necessary, PTFE or siliconized glass labware was substituted in the sample-preparation protocol.

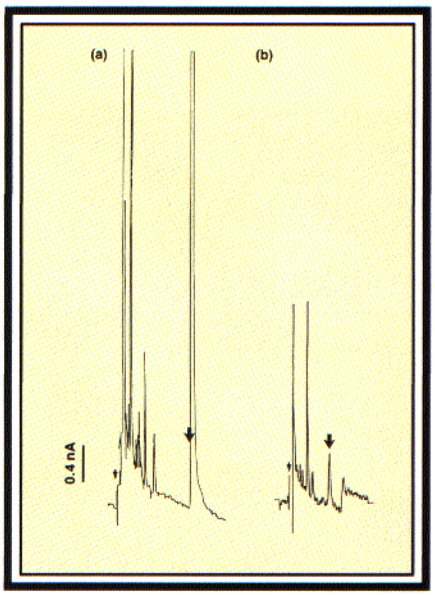

FIGURE 1: This is a chromatogram of contaminating substances leached from (a) a commercially available disposable extraction column holder and (b) an O-ring from a microfilter holder. The column holder was treated with the same series of solutions as brain extracts, and an aliquot was injected into the LC. The O-ring was placed in HPLC-grade water, and an aliquot of the water was injected. The large arrow denotes the position where ME would elute under these chromatographic conditions.

STATIC ELECTRICITY

The buildup and subsequent discharge of static electricity in the environment can contribute to a noisy baseline. There are several simple solutions to this problem. Technicians' clothes can be washed, dried, or sprayed with an antistatic agent. Dry air can be moisturized with an ultrasonic humidifier, and static mats can be used on the floor near the equipment to minimize static charge buildup.

ELECTRICAL POWER

Disturbances in electrical power (sags, surges, spikes, and dropouts) can also contribute to baseline noise. We rented an electrical power line disturbance monitor to record fluctuations in the main power source to our laboratory, and then correlated the time of each disturbance and its magnitude with baseline noise. This information provided useful documentation to building management and to power company officials that the problem existed; furthermore, improvement in instrument performance with clean conditioned power could also be documented.

Air conditioners, freezers, or other equipment that require a large amount of power and cycle on and off can also increase baseline noise if they use the same transformer as the electrochemical detector uses. To eliminate power-related problems, the current source for the chromatograph was changed to an isolated electrical line conditioned by a Powermark frequency converter. The frequency converter changes the incoming power first to direct current and then, using a crystal frequency-controlled, high-quality inverter, changes it back to a pure 60-Hz alternating current. Although an inexpensive power inverter can be used to condition electrical lines, the quality of the current is sometimes quite poor, with more noise and frequency deviations than the original power source.

ELECTROMAGNETIC INTERFERENCES (EMI)

Pumps, line voltage regulators, stirrers, or other equipment containing electromagnetic coils (that is, motors), if not properly shielded, can produce oscillating or rotating magnetic fields, which are possible sources of EMI. Any interruption of the electromagnetic field between its source and the electrochemical detector will cause a deflection on the chart recorder. We used a magnetic compass as a quick and inexpensive way to check for a stationary magnetic field in or around the chromatograph. Fields created by line-powered equipment are too fast to make a compass react and therefore required the use of a magnetic probe. This probe, essentially a Helmholtz coil attached to an amplifier or oscilloscope, was purchased from a company that provides shielding equipment (Perfection Mica Company, Bensenville, Illinois).

Because EMI decreases exponentially with distance, a small change in the position of an LC component, specifically separating the EMI emitter from the detector, can eliminate EMI as a contributor to baseline noise. If moving the equipment is not an option, magnetic shielding can be used to isolate either the EMI emitter or the EMI-sensitive detector system.

Other sources of deflections or "spikes" on chromatograms include ferrous perturbations of electromagnetic fields and direct effects from the technician's body. If the deflections occur as the technician walks past the electrochemical detector, iron-containing jewelry, name tags, or other objects should be removed systematically as an initial effort to resolve the interference.

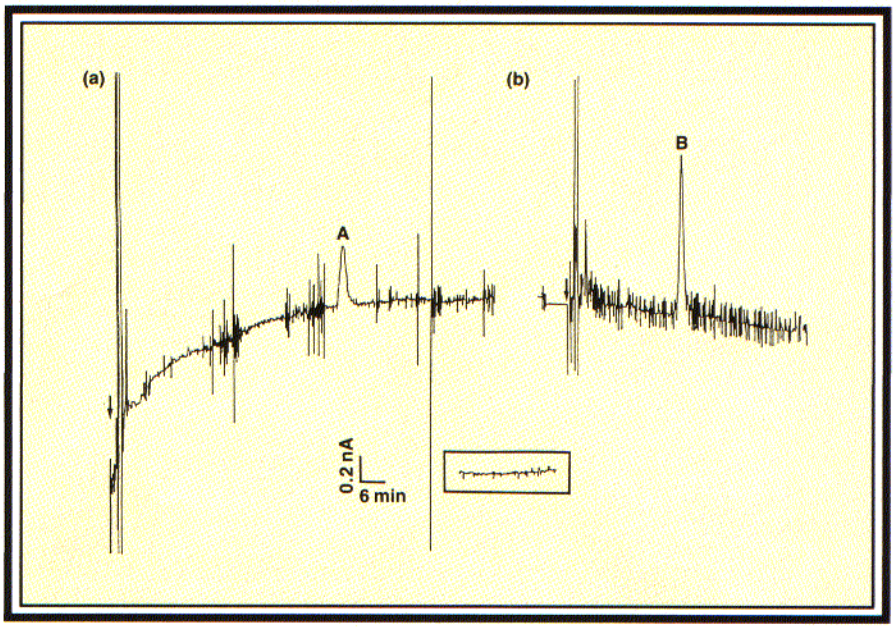

FIGURE 2: (a) RFI from citizens band radios, taxicab radios, walkie-talkies, etc. These interferences showed a random pattern of spikes of varying amplitude. (b) RFI possibly from a base station or paging system showing a regular series of spikes. Inset box: Baseline after shielding. Conditions for (a) and (b) were filter: 0.1 Hz; chart speed: 10 cm/h; applied potential: +1.05 V vs. Ag/AgCl; sensitivity: 5 nA/V. Peaks: A = 10 ng L-Ala^2-ME, B = 9 ng ME.

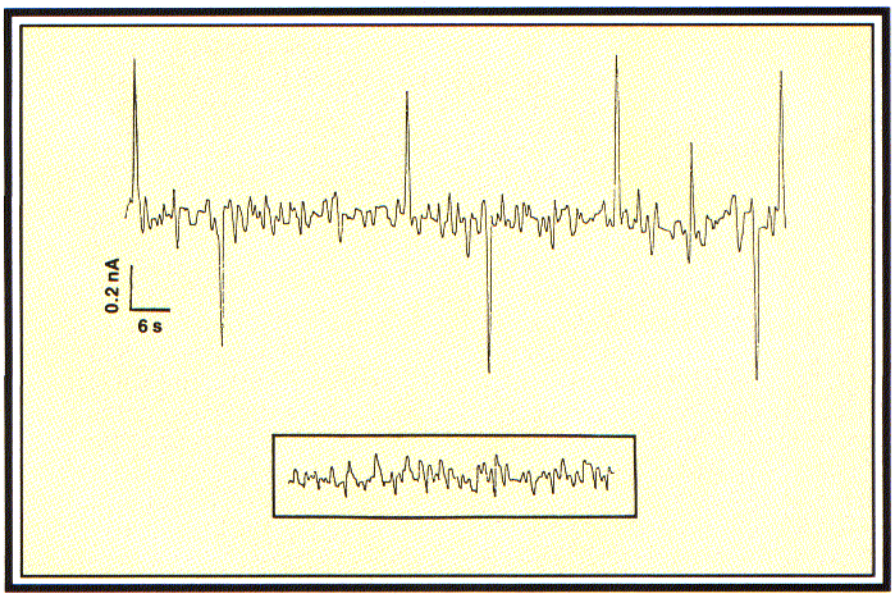

FIGURE 3: Decreasing the filtering to 1.0 Hz and increasing the chart speed to 10 cm/min revealed the characteristics of the waveform from Figure 2b. Inset box: Waveform characteristics after shielding.

RADIO-FREQUENCY INTERFERENCE (RFI)

The most difficult interference to identify and correct was RFI. A random pattern of spikes of varying amplitude (Figure 2a) appeared on the chromatogram with intermittent frequency throughout the day and into the night. To more fully characterize these interferences, we turned off the pumps, used the lowest filtering setting on the electrochemical detector, and increased the sensitivity of detection and the chart speed (see Figure 3). The waveform

showed a characteristic repeating up–down pattern, which was suggestive of an on–off pattern. To determine the source of this noise, we used a portable frequency modulation (FM) radio with a directional antenna as an RFI detector. We tuned the radio between major broadcast frequencies, so that we could hear the background static, and pointed the antenna at various pieces of equipment. An RFI source was identified if the presence or absence of tones or buzzing noises above the background static correlated with turning equipment on and off. For each suspected RFI emitter, the procedure was repeated at multiple frequency settings. Although this radio procedure is not a definitive test, it is an inexpensive first approach to troubleshooting RFI. If it is necessary to determine the exact frequencies and relative strengths of the RFI, a radio-frequency spectrum analyzer can be rented.

To provide an experimental RFI source, we parked a vehicle with a citizens band (CB) radio outside the laboratory. Using an oscilloscope connected to an FM antenna, we found that the spikes on the chromatogram (Figure 2a) corresponded to the oscilloscope deflections caused by keying the CB microphone. RFI sources that were then identified by the frequencies of their transmissions included CB radios, taxicabs, snow-removal equipment, and security guards' walkie-talkies. The range of broadcast voltages that we detected on the oscilloscope during several days of monitoring was 100 mV to 2 V. Some time later, we experienced another interference, which displayed a continuous pattern of spikes of similar amplitude (Figure 2b). The characteristic nature of this RFI (Figure 3) led us to believe that it was emitted from a single stationary source — possibly a base station or a paging system.

The solution to the RFI problem was relatively inexpensive but required multiple shielding and grounding efforts. The RFI-sensitive equipment (electrode box, controller, and injectors) was placed inside a cabinet above the chromatograph but well separated from the pumps. An electrical outlet with isolation plugs was also placed inside the cabinet, and the detector was plugged into that outlet. The entire cabinet was lined with continuous sheets of heavy-duty aluminum foil, making an inexpensive Faraday cage. Electrical continuity of all sides of the cage was checked with a multimeter. All signal-carrying lines from the detector were connected to the cage via a metal plate. These signal-carrying lines were coaxial cables with BNC connectors. On the other side of the metal plate were BNC connectors and coaxial cables, which were then connected to strip-chart recorders. An electrical connection was made from a BNC connector on the cage side of the metal plate to a ground hole in the electrical outlet located inside the Faraday cage. This metal plate system accomplished two purposes: grounding the entire cage through the electrical connection to the outlet ground, and preventing RFI from entering the cage via a "skin effect" along the outer sheath of the coaxial cable.

TABLE I: REDUCTION IN NOISE LEVEL BY SIMPLE FARADAY CAGE AND GROUNDING PROCEDURES*

	Before Shielding (nA)	After Shielding (nA)	Improvement
LC-4: Baseline noise	0.53–0.58	0.11–0.12	80%
LC-4: Spikes	0.88	0.18	80%
LC-4B: Baseline noise	0.22–0.32	0.06–0.08	75%
LC-4B: Spikes	0.38–0.98	0.09–0.12	77–88%

*applied potential: +1.05 V vs. Ag/AgCl

All solvent-carrying stainless steel tubing entered the cage through one hole, which was filled with aluminum foil. This made a connection between the stainless steel lines and the cage, draining off any RFI. We replaced a glass cabinet door with Plexiglas and cut a small door in it that was attached to the cage with a metal hinge. A Velcro patch was used to hold the door open for access to the injector. This door allowed the shielding to remain intact during sample injections. To view the LED readouts from the detectors, we cut small windows in the cage and covered them with copper screening in contact with the aluminum foil of the cage.

If the Plexiglas doors that made up the outside wall of the cage were slid past each other several times throughout the day, an electrostatic charge built up on the outside of the doors. To prevent charge buildup, we sprayed the doors daily with an antistatic spray or rubbed them with fabric softener sheets. We also installed an RFI shunt on each of the detectors, which consisted of a capacitor on the input lines to the amplifier portion of the electrochemical detector. It acted as a short circuit to radio-frequency signals without affecting the low-frequency signals coming from the electrode.

Although the shielding did not completely eliminate RFI contributions to noise, it did significantly reduce them. Table I shows that noise levels, both baseline and spikes, were decreased by 75% or more through this shielding approach. The inset boxes in Figures 2 and 3 show baselines after shielding. This decrease in interferences allowed us to operate the detector at considerably improved sensitivities.

CONCLUSIONS

In addition to the common causes of baseline noise, several other potential interferences require special attention. Although electrochemical detectors are especially sensitive to these interferences, other instruments or detectors may be affected if the interference is large, especially with RFI and EMI. Because the origin of these interferences is not always apparent, troubleshooting them can be difficult and time-consuming. Our problem-solving approach can help simplify the process.

- Deal with the most obvious problems first, systematically eliminating one problem at a time.
- Test for a problem using commonly available equipment, such as a magnetic compass, a multimeter, an FM radio, or an oscilloscope.
- Use the most simple and inexpensive solution to the problem (such as an aluminum foil Faraday cage).
- Run controls to check for leaching of contaminants from sample-processing supplies.
- Finally, if these attempts fail and you know the nature of the problem, then go to the experts, such as an instrumentation or electrical engineer or a magnetic shielding company.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the guidance and expertise provided by Dr. Robert Bishke for RFI shielding. We also thank Mr. Alan M. Gresch for his electronic and mechanical technical assistance and Ms. Sharon A. Neuman for preparing this manuscript.

REFERENCE

(1) L.H. Fleming and N.C. Reynolds, *J. Chromatogr.* **375,** 65–73 (1986).

"LC Troubleshooting" editor John W. Dolan is president of LC Resources Inc. of Lafayette, California, USA, and is a member of the Editorial Advisory Board of LC•GC.

Bulletin

DeVries receives Palmer Award. The Minnesota Chromatography Forum (MCF) has awarded the 1988 Palmer Award to Dr. Jonathan DeVries. DeVries currently is section leader of analytical methods development at General Mills and is author of numerous papers on chromatographic techniques in food and feed analysis. He has also taught courses on liquid and gas chromatography at the St. Paul Technical Vocational Institute. A member of MCF since its inception, DeVries is a past president of the forum and has served on many of its committees. He also served as chairman for the National ASTM Meeting in October 1987. The Palmer Award recognizes and encourages MCF members' involvement in the art and science of chromatography.