Liquid Chromatography Problem Solving and Troubleshooting

Question

Recently, I have been experiencing significant quantitative variability each time I inject a sample. Prior to this everything was working well with the assay and equipment. I have tried a number of things in terms of the procedure and have not been able to find the cause of the problem.

Answer

The difficulty you have described does not sound like it is associated with either the column or detector but is a sample injection problem. Two common causes of quantitative variability are either poor injection technique or mechanical malfunctions associated with your injection valve. It appears from your question that poor injection technique can be ruled out and valve problems are a likely cause of the variability you are observing because you have been obtaining satisfactory results until recently. In order to help you understand the cause of your problem and how to solve it, it is important to briefly review the construction and operation of manually operated injection valves.

Injection valves are constructed such that during the loading cycle the sample introduction syringe slides into a small opening in the valve body. A common design of one manufacturer is to place this inlet opening in the center of the switching handle. As the syringe needle is inserted into the inlet opening it passes through a sealing sleeve just as it seats itself within the valve. It should be noted that the inlet opening is sometimes lined with a Teflon collar, which can be seen protruding slightly. This collar is not the sealing sleeve but is simply used to guide the syringe needle into the valve. The purpose of the sealing sleeve, which is within the valve assembly, is to make a liquid tight seal.

In the load position, sample liquid passes from the syringe through a very low dead volume path inside the valve, through the fill loop, back though the outlet path within the valve assembly, and finally to waste through a small exit line. Most often the exit line is 1/16 tubing either stainless steel or an inert polymer such as Teflon. In order to properly fill the valve a threefold to fivefold excess of liquid is used. A typical size for the injection loop is 20 µL, and a typical syringe is 100 µL and has a blunt needle tip. The use of excess sample serves two purposes: first it flushes or cleans the sample loop and second it assures that it is completely filled. Once the loop is filled, the syringe is left in the valve assembly until the valve is switched to the inject position. In many cases a small bottle is used to catch any excess injection waste, and often this bottle is positioned such that it is lower than the valve-assemble.

During operation, as long as the seal around the syringe needle is tight it will prevent liquid from syphoning out of the loop as the result of excess liquid continuing to drip from the waste line. However, if the needle is removed prior to injection or there is an incomplete seal around the needle, liquid within the loop can be drawn out of the loop via syphoning action as excess liquid within the exit line drips into the waste bottle. For cases in which the internal seal is severely damaged, you may be able to see the liquid continue to drip from the exit line even though you have stopped introducing liquid into the injection valve.

The purpose of *Chromatography Problem Solving and Troubleshooting* is to have selected experts answer chromatographic questions in any of the various separation fields (GC, GC–MS, HPLC, TLC, SFC, HPTLC, open column, etc.). If you have questions or problems that you would like answered, please forward these to the *Journal* editorial office with all pertinent details: instrument operating conditions, temperatures, pressures, columns, support materials, liquid phases, carrier gas, mobile phases, detectors, example chromatograms, etc. In addition, if you would like to share your expertise or experience in the form of a particular question accompanied by the answer, please forward it to: JCS Associate Editor, *Chromatography Problem Solving and Troubleshooting,* P.O. Box 48312, Niles, IL 60714. All questions/answers are reviewed to ensure completeness. The *Journal* reserves the right not to publish submitted questions/answers.

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Associate Editor

In order to correct a faulty seal problem you will need to either send the valve back to the manufacturer for refurbishing or repair the valve yourself. In the event you decide on the latter option and have never replaced any of the valve internal parts, you should be extremely cautious in two important aspects of valve disassembly and servicing. First, you should be sure that you only remove the retaining screws that hold the valve body together and not loosen any spacing screws that are present in order to properly position the valve's internal parts in the *x*-, *y*-, and *z*-directions. Should the spacing screws be accidentally turned or removed, it is extremely difficult to reposition these correctly. Secondly, you should be extremely careful in observing (i.e., recording) how the internal parts are positioned, because in common valve designs these can be reassembled in more than one configuration. Although both of these precautions may seem obvious, over the years I have observed many graduate students and postdoctoral fellows attempt to replace a faulty valve part only to be frustrated after reassembling the valve incorrectly as the result of making one or both of these mistakes.