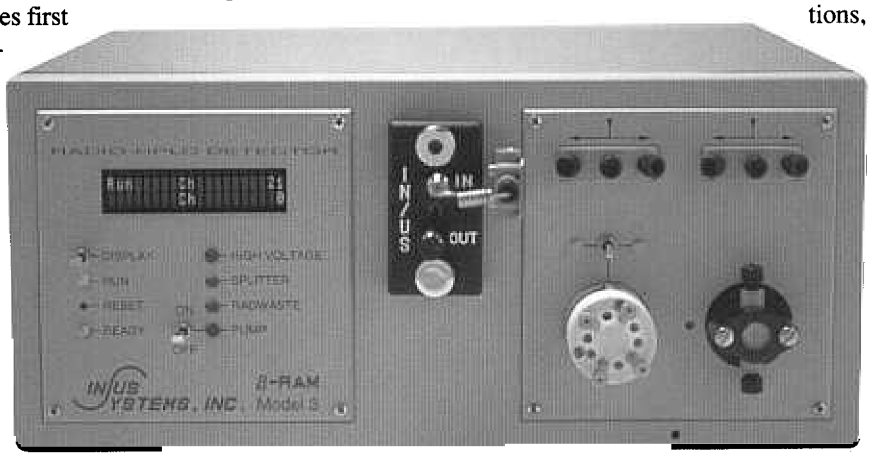
New Radio-HPLC Detector Solves Old Problems

Radio-HPLC, on-line measurement of radioactivity in HPLC eluates, has gained acceptance as a replacement for fraction collection and discrete sample counting. New instruments have made the benefits of radio-HPLC clear — low instrument operating and disposal cost, real-time results, and high resolution. Fraction collection and longer counting may have an edge in sensitivity, but most samples do not test the limits. With those that do, a little more sample often provides comparable sensitivity.

By Edward Rapkin, Ph.D.

The more serious problem with radio-HPLC is that as the field has grown, control of instrument performance, stability, and reproducibility has been neglected. Instruments are plumbed into and made part of an HPLC system and there is a reluctance to routinely disassemble such systems in order to periodically test the detection element with a standardized sealed radioactive source. That reluctance derives first from the fact that the instruments are generally reliable and known to be so, but also from concern that these detectors, all coincidence-type liquid scintillation (LS) counters, are negatively affected by high voltage interruption and by exposure of the photomultipliers and some counting cells to ambient light.

The alternative, testing the detector in place under flow conditions, is equally problematic. Radioactivity detection is so sensitive that trace amounts of sample are often retained within the HPLC column packing and sometimes are even sequestered on stainless steel tubing, and then later eluted. A standard material subjected to chromatography may act differently than a composite sample. Is the activity seen during standard measurement all that is present in, and only from, the standard?



A ten-port motorized injector

The IN/US Model 3 β-RAM Radio-HPLC Detector addresses these problems. Its 10-port motorized injector's primary function is to simplify and encourage instrument calibration. Operators can make frequent injections of a stock sample, even immediately before and after critical runs, to monitor performance. Chromatography does not enter the picture, counting conditions are those of the actual experiment, and there is no fear of affecting the photomultipliers or the measurement cells. Statistical treatment, included as part of system software, provides numerical checks on reproducibility and long-term stability. With serial dilution and repetitive injections, instrument linearity is easily and quickly determined.

Once the instrument is known to be properly operating, perhaps the most important parameter is counting efficiency. For each 100 decay events that occur within the measurement cell, how many are actually detected and recorded? Radioactivity counters almost never see them all. Many radio-HPLC detector users, recognizing that it is no simple thing to determine counting efficiency, never bother; they make relative measurements. But, when absolute activity is required, users have been faced with questionable choices. Under comparable conditions, counting efficiency is largely independent of chemical form and primarily related to isotope energy. However, subjecting a standard to chromatography and counting presumes that unknowns will act similarly in the HPLC column. We have seen that this is not always so. It is preferable to manually collect a peak from an unknown sample at the radio-HPLC detector exit, standardize it in a LS counter, and compare that result to the result given by the radio-HPLC detector.

That approach, too, is fraught with problems. Counters, settings and concentrations all differ, and the radio-HPLC cell has different optics than the LS vial. The injector valve overcomes these objections in the same way it did when checking the instrument. But, here, an accurately known level of activity must be used, most usually derived from NIST-traceable sources. Efficiencies (and spillover for double-isotope counting) are established in minutes, at minimal cost, and with more confidence than ever before.

Determining % Recovery is another problem simplified by the multiport injector valve. With mixtures of well-defined components together with degradation products, all the activity that enters the system may not appear during the run, while traces of activity from previous runs might. Taken together with background, a summation of measured activity possibly represents considerable error. To report each peak as a percent of that sum compounds the error while measuring the composite starting sample in a LS counter brings us back to the differences already recited – the counter, the counting mixture, the sample container, the background. Calibration and correction are possible, and usual, but are time-consuming. And, the LS counter costs more than the radio-HPLC detector.

The injector valve eliminates all of this. A sample aliquot is directly passed into the radio-HPLC detector without chromatography. It is in and out in a few seconds, giving a single peak. With the same mobile phase, scintillator composition, detector cell, flow rate, etc., it isn't necessary to translate numbers from one system to another. Background is inconsequential both because the composite peak is so narrow and the background of radio-HPLC detectors is inherently low. Each peak from the separation run is then reported as a percent of the composite. Results are more accurate than ever before because many complicating factors are eliminated.

"Quenching" is a topic that receives more lip service than real attention. Is counting performance affected during a chromatography run by a changing gradient? Most users assume that it is not and mostly they are correct. But, few do check; it has not been convenient. While it is possible to remove samples of mobile phase throughout a run, pipette activity into them, and test performance in another counter, that is almost never done. Now it needn't be. The multiport injector valve makes it simple to add an activity reference – it needn't even be calibrated – at various points throughout a run to see how one injection compares with another, and to make corrections if warranted.

Conclusion

The multiport injector valve makes the radio-HPLC detector essentially independent of the LS counter. This lowers costs and leads to more accurate results at a fast pace and with great convenience.

More information about the β-RAM radioactivity detector is available from:

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