Journal of Labelled Compounds and Radiopharmaceuticals
J Label Compd Radiopharm 2007; **50**: 543–544.
Published online in Wiley InterScience
(www.interscience.wiley.com). DOI: 10.1002/jlcr.1255

JLCR

Short Research Article

Applications of LC-ARC™ stop-flow radiochemical detection†

DAVID J. SCHENK*, JONATHAN Z. HO, JOSEPH P. SIMEONE, MICHAEL A. WALLACE, BRIAN P. CATO, STEVEN J. STASKIEWICZ, ALLEN N. JONES and DAVID G. MELILLO

Department of Drug Metabolism, Merck Research Laboratories, RY80R-104, 126 E. Lincoln Ave, P.O. Box 2000, Rahway, NJ 07065, USA

Received 9 October 2006; Revised 8 January 2007; Accepted 9 January 2007

Keywords: Radio-HPLC; LC-ARC; stop-flow; fraction collection; scintillation counting

Introduction

Determining the radiochemical purity of low specific activity tracers (ca. 1 μCi/mg) can be challenging due to low signal to noise in the radiochromatogram. The result can be an overestimated radiochemical purity. Often a compromise must be made between the impurity detection limit and the chromatographic resolution because overloading the HPLC column can increase the signal to noise but can also decrease the resolution of closely eluting impurities. Another option is manual fraction collection with off-line scintillation counting[1] but this process involves the tedious fractionation of the HPLC eluent, filling each of the ~140 scintillation vials (45 min collection with 3 fractions/min) with cocktail and counting each fraction for an extended time (5–20 min). After counting, each data point must be entered into a program to correct for the background count and to calculate the percent abundance of each peak.

Liquid chromatography-accurate radioisotope counting (LC-ARC™)[2] removes the need to physically fractionate each portion of the HPLC flow. The system software stops and starts the HPLC pump and through the operation of a gas-actuated value, keeps the HPLC column held at operating pressure during the stop/counting phase. Each section of the flow is mixed with scintillation cocktail and the amount of radioactivity is measured with a typical radiochemical detector. Before returning the eluent flow to the column for the next section of the chromatogram, the radiochemical detector cell is flushed with scintillation cocktail to remove any radiochemical contamination. Previously this system has been used to study the bacterial culture and activated sludge biodegradation of a ^{14}C-labeled perfluorodecanol[3] and was used along with MS detection to detect tritiated propranolol metabolites.[4] Here we give a few examples of typical LC-ARC™ use, both successful and unsuccessful.

Results and discussion

The radiochemical purities of ^{14}C- and ^{35}S-labeled compounds were determined using a commercially available AIM Research LC-ARC™ stop-flow system, a PerkinElmer® Radiomatic™ flow analyzer and an Agilent 1100 series HPLC system. In the initial evaluation and setup of the system a cursory set of experiments using a ^{35}S-labeled tracer verified that the detection limit of the LC-ARC™/Radiomatic™ stop-flow combination was ~25 times better than the detection limit observed using non-stop flow conditions with the Radiomatic™ detector.[4] In these experiments the LC-ARC™ results measured at a 25 times lower concentration were very similar to those obtained from higher concentration runs with only a radiomatic detector except that a minor area of elevated baseline (<0.3%) was not observed in the LC-ARC™ chromatogram. In support of a human ADME study, LC-ARC™ analysis of a carrier-diluted ^{14}C-labeled tracer (~1 μCi/mg, Figure 1(a)) gave 98.7% radiochemical purity which was very similar to the result received for the prediluted material (~56 μCi/mg) when employing only the radiomatic detector. However when only the radiomatic detector with normal HPLC flow was used on the carrier-diluted tracer, the signal to noise was not high enough to observe the minor impurities

*Correspondence to: David J. Schenk, Department of Drug Metabolism, Merck Research Laboratories, RY80R-104, P.O. Box 2000, Rahway, NJ 07065, USA. E-mail: david_schenk@merck.com
†Proceedings of the Ninth International Symposium on the Synthesis and Applications of Isotopically Labelled Compounds, Edinburgh, 16–20 July 2006.

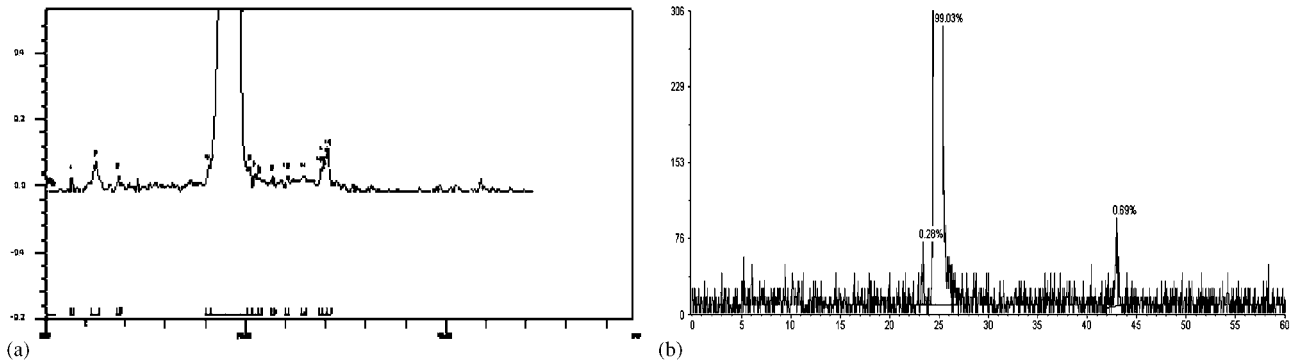

Figure 1 (a) LC-ARC™ chromatogram of **1** after carrier dilution and (b) radiomatic chromatogram of **1** after carrier dilution.

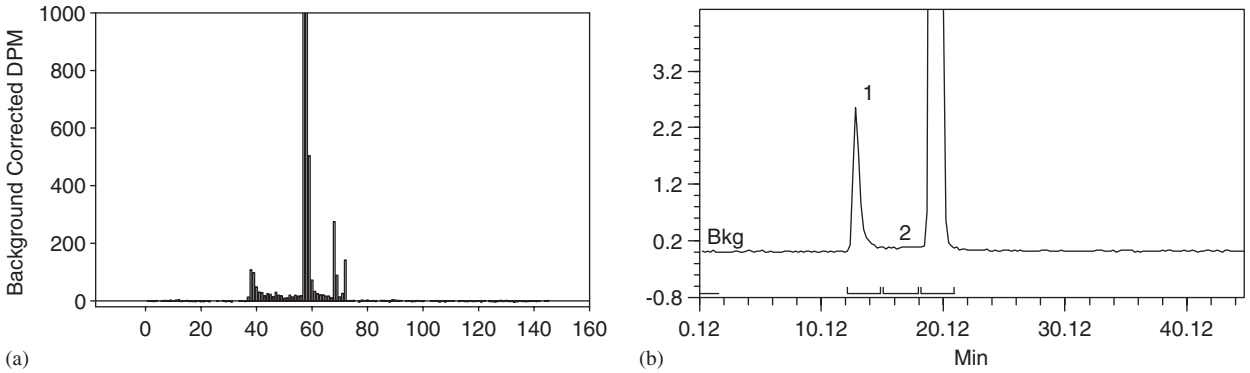

Figure 2 (a) Simulated chromatogram produced from manually fraction collected counts of **2** and (b) LC-ARC™ chromatogram of **2**.

(Figure 1(b)) and the resulting radiochemical purity was overestimated (~99%).

Certain compounds are inappropriate for LC-ARC analysis. Duplicate manual fraction collection runs with off-line scintillation counting followed by background correction gave peak percentage data and simulated chromatograms for ^{14}C-labeled compound **2** (~0.9 μCi/mg, Figure 2(a)). The relatively long LC-ARC™ analysis time contributed to the acid-induced degradation of the oxadiazole ring in **2** as seen by the ~1.7% impurity peak eluting before the main peak (Figure 2(b)). This impurity peak was observed at ~0.4% abundance in the manual fraction collection runs. Significantly modifying the LC-ARC™ method conditions including only measuring the radioactivity in the areas where the majority of the radioactivity eluted and thus limiting the total HPLC run time gave data that were more similar to the manual fraction data (not shown). In the cases where a compound has limited stability to the method conditions LC-ARC should not be used unless there is another overriding factor such as compound or impurity volatility.

Besides the outcome discussed above in all other cases the LC-ARC™ and manual fraction collection results are comparable. The LC-ARC™ system is currently used along with manual fraction collection until it has been documented that the compound is appropriate for LC-ARC analysis. The amount of sample throughput, and the difficulty of any sample preparation required for HPLC analysis, should be considered along with the use of alternate techniques such as TLC analysis or techniques such as HPLC with off-line microplate scintillation counting.[5]

REFERENCES

1. Elmarakby SA, Supplee D, Cook R. *J Agric Food Chem* 2001; **49**: 5285–5293.
2. Several references are on the company's website (last viewed January 2007). http://www.stopflow.com/applications.htm
3. Wang N, Szostek B, Buck RC, Folsom PW, Sulecki LM, Capka V, Berti WR, Gannon JT. *Environ Sci Technol* 2005; **39**: 7516–7528.
4. Nassar AEF, Bjorge SM, Lee DY. *Anal Chem* 2003; **75**: 785–790.
5. Kiffe M, Jehle A, Ruembeli R. *Anal Chem* 2003; **75**: 723–730.

DOI: 10.1002.jlcr