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APPLICATION NOTE

Comparison of Detection Techniques for Distribution of ^{14}C Residues by HPLC

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INTRODUCTION

Conduct of environmental fate, plant and animal metabolism studies with a ^{14}C radiolabeled test substance includes chromatographic analysis of extracts to elucidate the distribution of transformation products (metabolites) in a test system. The distribution of metabolites in extracts are commonly determined using chromatographic separation with HPLC.

Detection of radiolabeled components from a HPLC separation is performed either by collection of fractions followed by liquid scintillation counting of collected fractions, or using a flow through radioisotope monitor. Traditional liquid scintillation counters (LSC) detect one sample at a time. Introduction of microplate LSC (TopCount, MicroBeta) increased the throughput of detection to 12-samples simultaneously.

METHODS

General Procedure for ^{14}C Residue Detection

1 ^{14}C-treated animal or plant samples were extracted with an acetonitrile/water mixture and acetonitrile. Extracts were combined and concentrated prior to HPLC analysis.
2 HPLC separation of extracts were performed using a HPLC system equipped with a reverse phase column eluting with an aqueous to acetonitrile linear gradient.
3 Radioisotope flow monitor was a Beta-RAM (LabLogic) equipped with a 500 µL flow cell and auxiliary pump to mix scintillation cocktail with HPLC column eluent in a ratio of 3:1.
4 LSC was performed using Beckman LS 6000 or LS 6500 instruments or Perkin Elmer MicroBeta2 counter with 12-detectors. Counting time was 1 min per fractionated sample.

Instruments for ^{14}C Radioactive Detection after Chromatographic Separation

A Fractionation @ 30-sec. interval/Liquid Scintillation Counter: Beckman LS 6500 or LS 6000 IC liquid scintillation spectrophotometer.
B Flow-through Radio-HPLC Detection using a Beta-RAM by LabLogic with 500 µL liquid cell.
C Fractionation @ 15~20-sec. interval/MicroBeta Microplate Counter: PerkinElmer 2450 MicroBeta Microplate Counter including 12 detectors – (12 wells in a 96-well plate are counted simultaneously).
D Fractionation to vials and microplates: Gilson FC204 Fraction Collector.

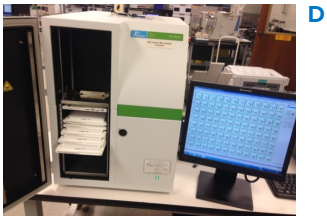
A

LSC

B

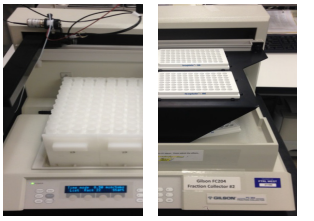
Flow-through radio-detector

C



MicroBeta Microplate Counter

D

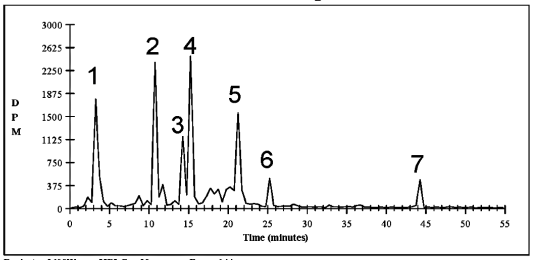


Fraction Collector to vials or microplates

RESULTS

In Case of Analysis of Low Sample Matrix: Similar Chromatographic Resolution

^{14}C-treated Radish Root, Concentrate of Combined ACN/H_2O Extracts



A HPLC/Typical fractionation (30-sec)/LSC:
- 16,300 dpm injected
- 100 µL injected

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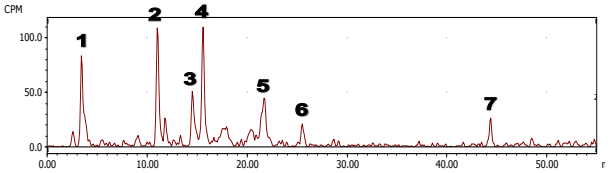
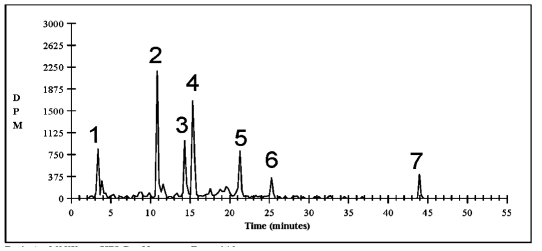
^{14}C-Treated Radish Root, Concentrate of Combined ACN/H_2O Extracts [Radio-HPLC (Non-stop)]



B HPLC/Flow-through radio detection beta RAM:
- 16,300 dpm injected
- 100 µL injected

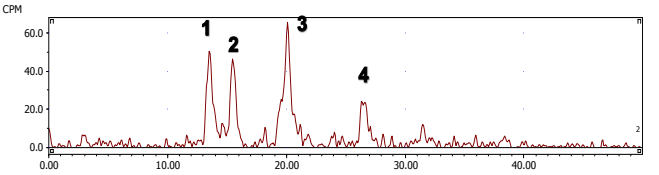
^{14}C-Treated Radish Root, Concentrate of Combined ACN/H_2O Extracts



C HPLC/96-well fractionation (15-sec)/ MicroBeta
- 16,300 dpm
- 100 µL injected

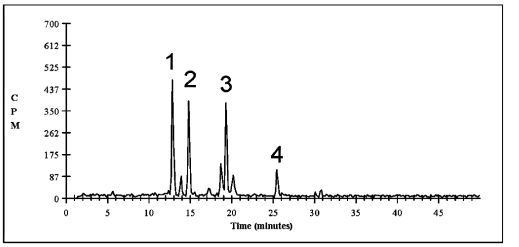
In Case of Analysis of Severe Sample Matrix: Enhanced Chromatographic Resolution

^{14}C-Treated Liver, Concentrate of Combined ACN/H_2O Extracts [Radio-HPLC (Non-stop)]



B HPLC/Flow-throchromatographic ugh radio detection beta-RAM: Poor resolution
- 15,600 dpm injected
- 500 µL injected

^{14}C-Treated Liver, Concentrate of Combined ACN/H_2O Extracts



C HPLC/96-well fractionation (15-sec)/Microbeta2 counting: Enhanced chromatographic resolution, compared to B.
- 3,000 dpm
- 200 µL injected

Comparison in terms of sensitivity, speed, cost, and chromatographic resolution

	HPLC/fractionation/LSC	HPLC/Flow-through beta-RAM	HPLC/fractionation/ MicroBeta
Operation			
[^{14}C]Metabolites separated by	HPLC column	HPLC column	HPLC column
Sample type	Concentrate of combined acetonitrile/water extracts	Concentrate of combined acetonitrile/water extracts	Concentrate of combined acetonitrile/water extracts
HPLC run time/sample	60 minutes	60 minutes	60 minutes
Typical fractionation interval	30 sec	-	20 sec
Total fraction numbers	120	0	180
Fraction volume	500 µL	0	333 µL
Fraction vial	7-mL single vial	-	350-µL 96-well plate
Concentration	Not required	-	Concentration to ~50 µL
Concentration time	-	-	< 60 minutes, max. 12 plates in a chamber
Scintillation cocktail volume (mL)/fraction	5 mL for vial	HPLC flow rate (mL/ min): cocktail flow rate (mL/min)=1:3	0.2 mL for one well
Individual fraction counting time	1 minute	-	1 minute
[14C]Detection type	single PMT using LSC vial	single PMT using flow-through beta-RAM	12 PMT using 96-well microplate

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	HPLC/fractionation/LSC	HPLC/Flow-through beta-RAM	HPLC/fractionation/ MicroBeta
Sensitivity			
Limit of Detection, 2 x Background level	70 dpm	250 dpm	20 dpm
Total scintillation volume	600 mL	180 mL	36 mL
Radioactive waste	High volume	Medium volume	Low volume
Speed including HPLC run			
for a sample	3 hours	1 hour	2 hours
for 4 samples	12 hours	4 hours	6 hours*
Cost for consumable materials	$55	$10	$15
Advantage	Sensitive	Automatic; Speedy; Cost effective	Sensitive; Speedy; High chromatographic resolution; Low radioactive waste
Disadvantage	Laborious; Time consuming; Less cost effective; Relative high radioactive waste	Less sensitive; Chromatographic resolution dependent on sample matrix; Relative high radioactive waste	Limited application to ^{14}C volatile material
*Including concentration time to 1 hour for 4 samples using SpeedVac system.			

CONCLUSIONS

1 Sensitivity: MicroBeta counter > LSC >> Flow-through beta-RAM

2 Speed:

- For single sample

  Flow-through beta-RAM > MicroBeta counter >> LSC

- For multiple samples

  Flow-through beta-RAM > MicroBeta counter >> LSC

3 HPLC Chromatographic Resolution:

- For low sample matrix analysis

  Flow-through beta-RAM = MicroBeta counter = LSC

- For high sample matrix analysis

  MicroBeta counter >> LSC > Flow-through beta-RAM

4 Cost Effective:

  Flow-through beta-RAM > MicroBeta counter >> LSC

5 Radioactive Waste Volume:

  MicroBeta counter > Flow-through beta-RAM >> LSC

- HPLC resolution using the traditional technique of fraction collection and liquid scintillation counter (LSC) equipped with a single detector is a function of fraction collection time (faster collection times results in higher resolution). The sensitivity by fraction collection is a function of counting time (longer counting time results in higher sensitivity). However, LSC of collected fractions is labor intensive and time consuming.
- HPLC equipped with a radioisotope flow-through detector has the benefit of providing real time monitoring of the radioactive components. Resolution of chromatographic peaks and sensitivity in radioisotope flow monitors are functions of the flow cell volume, flow rate through the counting cell and counting efficiency (liquid cell or solid cell). Detection using radioisotope flow monitors often requires the researcher to compromise between speed of analysis with lower sensitivity than the traditional fraction collection/LSC technique.
- Introduction of LSC instrument with capabilities to count 96-well plates with multiple detectors provides researchers improvements in counting time over the traditional fraction collection technique while maintaining high sensitivity.

ACKNOWLEDGEMENTS

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