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.

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

$^{14}$C-treated Radish Root, Concentrate of Combined ACN/H$_2$O Extracts

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

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

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

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

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

#### HPLC/Flow-through chromatographic ugh radio detection beta-RAM: Poor resolution
- 15,600 dpm injected
- 500 µL injected

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

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

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

1. Sensitivity: MicroBeta counter > LSC >> Flow-through beta-RAM
   - For single sample: Flow-through beta-RAM > MicroBeta counter >> LSC
   - For multiple samples: Flow-through beta-RAM > MicroBeta counter >> LSC

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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