Evaluation of Active Counting Mode option in LabLogic β-RAM Model 5 and Laura software

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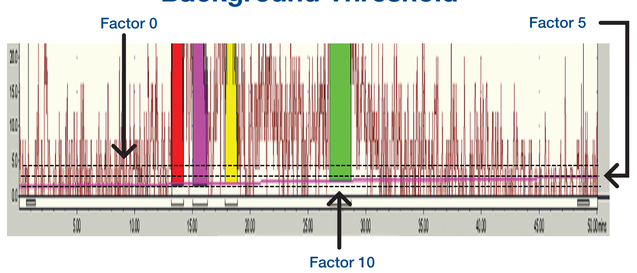
Introduction

LabLogic have recently introduced the β-RAM® Model 5 radio-HPLC detection system, which includes the option of Active Counting Mode (ACM™), a new online counting system with "unrivalled limits of detection" and "improved resolution and peak definition" compared to older systems. ACM enhances counting conditions in real time. Following the detection of a peak, ACM optimises scintillant flow rate, which impacts upon analyte residence time and improves signal-to-noise. We have evaluated this new technology by assessing the comparability of online detection between standard radio-HPLC and ACM radio-HPLC, as well as standard radio-UPLC and ACM radio-UPLC.

ACM Parameters

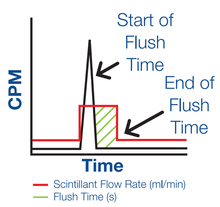
Background Threshold



----- Background subtraction (calculated pre-injection)

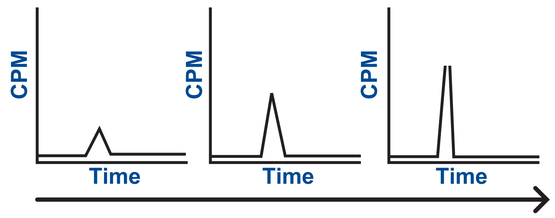
Flush Time

A short period of a run in which the scintillant flow rate is increased.



Set Flush Time prior to run
↓
β-RAM detects start of peak
↓
Scintillant Flow Rate increases
↓
β-RAM detects end of peak
↓
Flush Time (s)
↓
Scintillant Flow Rate returns to original value

Scintillant Flow Rate



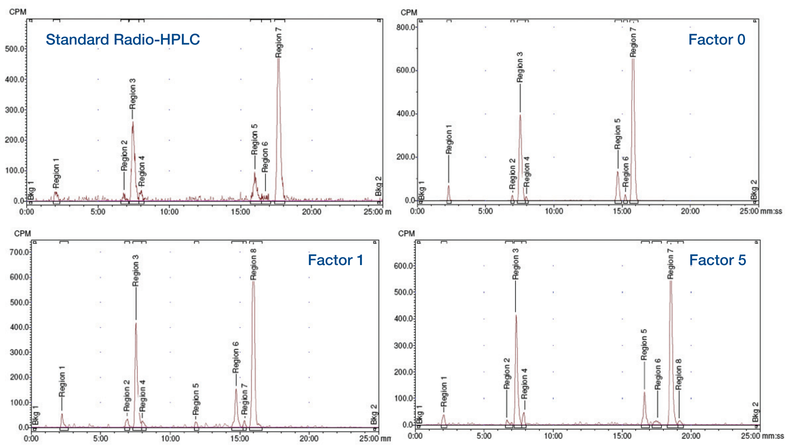
Scintillant (ml/min)
(Decreasing residence time in flow cell)

Method

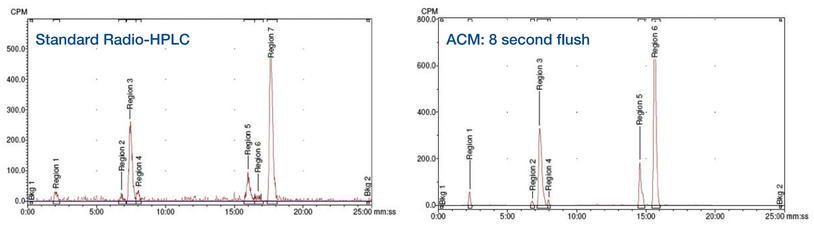
A pooled human urine sample with nominal ^{14}C concentrations of 18,000 DPM/75µL (HPLC) and 2,400 DPM/10µL (UPLC) was utilised as a source of radioactivity for all analyses. Sub-aliquots of this sample were concentrated by freeze-drying or diluted where appropriate, prior to Liquid Scintillation Counting (LSC) to confirm ^{14}C content. HPLC: Waters Xterra MS C18 column; UPLC: Acquity UPLC® C18 column; HPLC & UPLC: gradient elution using ammonium bicarbonate and acetonitrile:methanol with 2:1 scintillant:eluate flow rate. The following parameters and settings were assessed: radio-HPLC & radio-UPLC: Background Threshold (0,1,2,3,5 & 10), Scintillant Flow Multiplier (x2 and x3) and Flush Time (4, 8 & 16 seconds); radio-UPLC only: flow-cells (200, 500 & 1000 µL); radio-HPLC: 500 µL flow-cell only.

Results

HPLC-ACM: Background Threshold



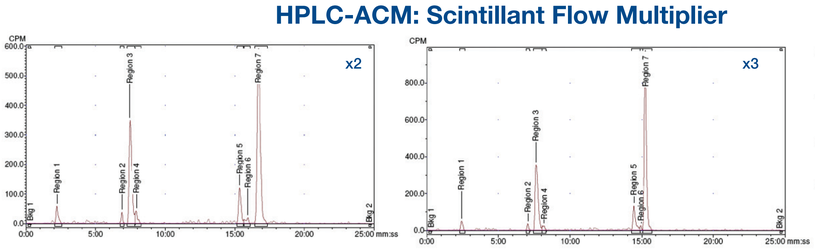
HPLC-ACM: Flush Time



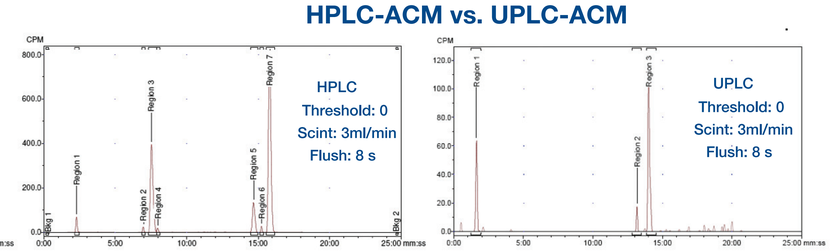
UPLC-ACM: Flow Cells

As expected, increasing flow-cell size resulted in broadened peaks and decreased peak resolution due to a lower residence time of the analyte

HPLC-ACM: Scintillant Flow Multiplier



HPLC-ACM vs. UPLC-ACM



Discussion

- Reproducibility was generally good, although detection of minor peaks varied between some replicates.
- Flush time increased peak height but may merge neighbouring peaks into one peak.
- UPLC-ACM requires less sample and displays better limits of detection than HPLC-ACM.
- No observable difference between Background Threshold 2 and above.
- Scintillant flow rate setting should be optimised to the methodology.
- Further investigations into the comparability of ACM and off-line radioactivity counting, as well as ACM analysis of ^{3}H are required.

Conclusions

- ACM should be used with optimised chromatography.
- Peak reject criteria needed for smaller, insignificant peaks.
- Background threshold could be scaled down from 0-10 to 0-2.

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