Validation of the BioScan Mini-Scan thin-layer radiochromatogram scanner and associated LabLogic Laura software for the QA of 99mTc radiopharmaceuticals

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Introduction
Thin layer chromatography (TLC) is a common means of measuring the radiochemical purity (RCP) of radiopharmaceuticals. Validation of our equipment and experience using the Bioscan Mini-Scan has allowed us to modify our technique to achieve accurate results. The purpose of this work was to determine the ideal scanning conditions for measuring RCP of 99mTc radiopharmaceuticals using TLC and the Mini-Scan.

Equipment
The Mini-Scan (Bioscan Inc, Washington DC, USA) consists of a moving table that passes the TLC plate below a lead collimator with a 3 mm slit. Above the collimator is a sodium iodide crystal detector (B-FC-3200, Bioscan). The detector is controlled by a Flow-Count unit (B-FC-1000, Bioscan). The instrument is connected to a PC via a LabLogic interface box (LabLogic, Sheffield, UK). Plates are scanned from 50 mm below the origin line to 50 mm above the solvent front line. Chromatograms are acquired using LauraLite software (LabLogic).

Effect of dead-time
The relationship between count-rate and detector dead-time was determined by measuring a high activity 99mTc source continuously over 5 days. The resulting observed counts were plotted against predicted counts and the loss at different count rates calculated.

Dead-time makes the RCP lower than is correct by reducing the counts in the main peak to a greater extent than the counts in the impurity peak. A value that is greater than the RCP limit always therefore indicates a satisfactory product regardless of dead-time. Similarly, a product with a measured RCP below the limit may be satisfactory due to the artificially reduced counts in the main peak. For example, a value of 94.8% from a chromatogram with a maximum count-rate of 10,000 cps is really 95.0% once a 0.2% correction for dead-time has been applied. This correction can therefore be applied to borderline results. An alternative to correcting for dead-time would be to reduce the count-rate by decreasing the width of the collimator slit.

It is worth noting that the loss due to dead-time will not be as great as the estimated values shown above as the count-rate is not as high as 10,000 cps throughout the peak.

Conclusions
1. The count-rate should not exceed 10,000 counts per second if correction for dead-time is to be avoided.
2. A background region should be placed at the end of the chromatogram away from the main peak to avoid a falsely high value for RCP.
3. Reproducibility is excellent.
4. The following ideal scanning conditions for 99mTc radiopharmaceuticals of different radioactive concentrations have been established:

Reproducibility
Reproducibility of the scanning technique was calculated from the repeated scans performed for the background experiments and was found to be excellent with no coefficient of variation >0.3%.

Background correction
The optimum method for background correction was determined by preparing a 99mTc–Medronate (MDP) TLC plate with a 5% pertechnetate impurity. This was achieved by placing spots of 99mTc–MDP and 99mTc–pertechnetate of appropriate concentrations on an ITLC-SG plate and developing it in butanone over 10 cm. The plate was scanned 10 times at 1mm/s with a dwell time of 2s per channel. The chromatogram contained a main peak at the origin and a pertechnetate "impurity" peak at the solvent front. Each chromatogram was analysed with four background correction techniques:

- no background region
- one background region at the end of the plate closest to the main peak
- one background region at the end of the plate furthest from the main peak
- two background regions, one at either end of the chromatogram

The RCPs from the 99mTc–MDP chromatograms analysed using the four different background correction methods

<table>
<thead>
<tr>
<th>No Bgd region</th>
<th>1 Bgd region close to main peak</th>
<th>1 Bgd region away from main peak</th>
<th>2 Bgd regions</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCP</td>
<td>94.8 ± 0.1%</td>
<td>96.2 ± 0.3%</td>
<td>95.0 ± 0.1%</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>0.1%</td>
<td>0.3%</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

Each value of RCP is the mean ± standard deviation of 10 results.

Laura uses two methods to correct for background depending on the number of regions set. When only one region is set an average value for this region is calculated and the resulting value is used as a flat background and deducted from each channel in the chromatogram. When two regions are set, one at either end of the chromatogram, an average value for each region is calculated. Using these averages, a graduated linear background is plotted between the two background regions. When a single background region is placed at the end of the chromatogram closest to the main peak, scatter from the peak increases the average background value. Similarly, when two background regions are set, the region at the main peak end gives a higher than true background value. This also results in an increase in the average background value. Both of these methods lead to over correction and result in a RCP that is higher than the true value.

To avoid over correction due to scatter, our recommendation is to set one background region at the end of the chromatogram that is furthest from the main peak.

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Observed counts (kCPS) vs Predicted counts (kCPS)