

## Introduction

The gall bladder imaging agent <sup>99m</sup>Tc-Mebrofenin is prepared using Cholediam, a radiopharmaceutical kit supplied by Mediam (Faches-Thumesnil, France). The instructions for the preparation of <sup>99m</sup>Tc-Mebrofenin specify that the kit should be reconstituted in a volume of 1-5 ml and incubated at room temperature "for about 30 minutes" [1].

The European Pharmacopoeia (EP) has published a new method for measuring the radiochemical purity (RCP) of <sup>99m</sup>Tc-Mebrofenin [2]. This method uses thin-layer chromatography to quantify the level of Impurity A and liquid chromatography to quantify the levels of other impurities and <sup>99m</sup>Tc-Mebrofenin. Using this new method, we have observed that the minimum RCP of 94% is not always achieved 30 minutes after reconstitution of Cholediam.

Our experience of <sup>99m</sup>Tc-dimercaptosuccinic acid injection is that the highest rate of radiolabelling is achieved with a small reconstitution volume [unpublished data]. Our investigation was undertaken to determine if the same is true for Cholediam.

## Aims

To investigate the significance of reconstitution volume on the radiochemical purity of <sup>99m</sup>Tc-Mebrofenin and establish the preparative conditions that produce a radiopharmaceutical of the highest quality.

## Material and Methods

### Preparation of <sup>99m</sup>Tc-Mebrofenin

Cholediam kits were reconstituted with 500 MBq <sup>99m</sup>Tc-pertechnetate in a volume of 1 ml or 5 ml. The 1 ml kits were diluted to 5 ml after incubation for 25 minutes.

### Thin-layer chromatography

Stationary phase: ITLC-SG plates, 25 x 200 mm (Pall Corporation, New York, USA)  
Mobile phase: acetonitrile : water (60 : 40)  
Origin: 25 mm from the bottom  
Solvent front: 150 mm above the origin  
Sample volume: 5 µl  
Scanner: Mini-Scan (Bioscan Inc, Washington DC, USA) with Laura Lite software (LabLogic, Sheffield, UK)

### Liquid chromatography

Column: HyperClone 5 µ BDS C18 130A, 250 x 4 mm (Phenomenex, Torrance, USA)  
Mobile phase: ammonium acetate (3.85 g/l) : acetonitrile (70 : 30) for 22 minutes then a linear gradient over 5 minutes to acetonitrile 100 % then acetonitrile 100 % for 3 minutes  
Flow rate: 1 ml / minute  
Sample volume: 20 µl  
Detector: Sodium iodide crystal scintillation detector with Laura Lite software

### Measurement of RCP

RCP was measured at 0.5, 1, 1.5, 4 and 6 hours post reconstitution. Each experiment was performed 5 times.

## References

1. Mediam. *Cholediam product leaflet*. Faches-Thumesnil, France; July 2006.
2. Pharmeuropa 2007; Vol 19 No2: 342-344.

## Results

Typical chromatograms are shown in figure 1.

The RCP values are shown in table 1. At 30 minutes, the RCPs of kits reconstituted in 1 ml and 5 ml were  $97.2 \pm 0.7\%$  and  $93.6 \pm 0.7\%$  respectively. The difference was statistically significant ( $P < 0.05$ ). All other results were greater than the EP limit of 94% with no statistical differences between the RCPs obtained with the two volumes.

Figure 1. Thin-layer and liquid chromatograms of <sup>99m</sup>Tc-Mebrofenin

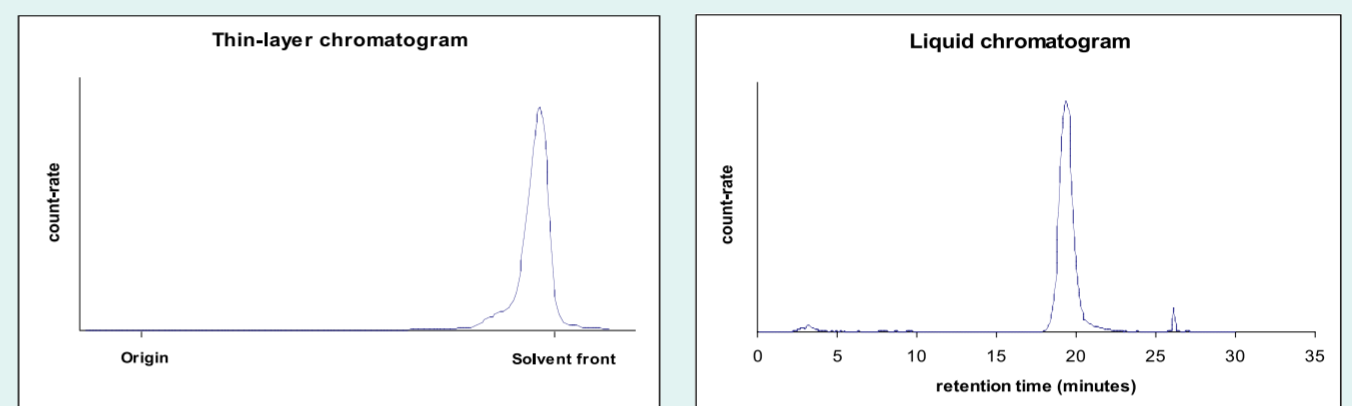


Table 1. The effect of reconstitution volume on the RCP of <sup>99m</sup>Tc-Mebrofenin injection

Time after reconstitution (h)	Radiochemical purity (%)	
	1ml	5ml
0.5	$97.2 \pm 0.7$	$93.6 \pm 0.7$
1.0	$96.6 \pm 0.4$	$96.1 \pm 0.8$
1.5	$96.6 \pm 0.6$	$96.3 \pm 0.6$
4.0	$96.4 \pm 1.1$	$96.0 \pm 0.2$
6.0	$96.5 \pm 0.4$	$96.2 \pm 0.8$

Each value is the mean  $\pm$  SD of 5 results

## Discussion

When using the proposed European Pharmacopoeia method for measuring the RCP of <sup>99m</sup>Tc-Mebrofenin injection, we observed that the minimum value of 94% was not always reached within the recommended incubation time. Mediam, the supplier of the kit, specifies that it should be reconstituted in a volume of between 1 and 5 ml and should be incubated "for about 30 minutes". Our observations of low RCP were made on products prepared in 5 ml. We decided to investigate this phenomenon to identify the reconstitution and incubation conditions that produce <sup>99m</sup>Tc-Mebrofenin of high RCP. If <sup>99m</sup>Tc-Mebrofenin behaves in a similar fashion to other <sup>99m</sup>Tc radiopharmaceuticals such as <sup>99m</sup>Tc-Dimercaptosuccinic Acid Injection, then reconstitution and incubation in the minimum volume of 1 ml might accelerate the attainment of high RCP. Our results confirm this to be true for Cholediam with the 5 ml reconstitution volume producing low RCP at 30 minutes but satisfactory RCP thereafter.

Our findings may be of relevance to radiopharmacies located within nuclear medicine departments where radiopharmaceuticals can be prepared and administered within a short period of time. Similarly, they are relevant to situations where <sup>99m</sup>Tc-Mebrofenin is requested at short notice.

## Conclusion

Cholediam kits should be reconstituted in 1ml and incubated for 30 minutes before dilution.