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An evaluation of GMCP-SA as a replacement for ITLC-SG when measuring the levels of radiochemical impurities in ^{99m}Tc radiopharmaceuticals by thin-layer chromatography

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***Background*: The European Pharmacopoeia and radiopharmaceutical manufacturers specify Instant Thin-Layer Chromatography Silica Gel impregnated glass fibre (ITLC-SG) as the stationary phase for measuring the radiochemical purity (RCP) of most technetium-99m (^{99m}Tc) radiopharmaceuticals. During 2008, The Pall Corporation stopped manufacturing ITLC-SG. A replacement is required. *Aim*: To evaluate Varian's Glass Microfibre Chromatography Paper impregnated with salicic acid (GMCP-SA) as a replacement. *Experimental*: Samples of six ^{99m}Tc radiopharmaceuticals (albumin nanocolloid, colloidal tin, exametazime, medronate, pentetate and succimer) were prepared containing levels of radiochemical impurities between 0 and 10%. Samples were tested using ITLC-SG and GMCP-SA with the mobile phase specified in the pharmacopoeia or by the manufacturer. Analysis was performed by thin-layer radiochromatogram scanning. *Results*: For measuring the RCP of albumin nanocolloid, colloidal tin, exametazime and succimer, GMCP-SA is a suitable replacement for ITLC-SG. For medronate, GMCP-SA is suitable for measuring colloidal ^{99m}Tc impurity despite producing slightly higher values than ITLC-SG, but unsuitable for measuring ^{99m}Tc pertechnetate impurity due to significant over-estimation. For pentetate, GMCP-SA is suitable for measuring colloidal ^{99m}Tc impurity but gives slightly higher values for ^{99m}Tc pertechnetate impurity than ITLC-SG. *Conclusion*: GMCP-SA is a suitable alternative to ITLC-SG for analysing some ^{99m}Tc radiopharmaceuticals but is not a universal replacement.**

Keywords: ITLC-SG; ^{99m}Tc radiopharmaceuticals; radiochemical purity; thin-layer chromatography

Introduction

During 2008, The Pall Corporation stopped the manufacture of Instant Thin-Layer Chromatography Silica Gel impregnated glass fibre plates (ITLC-SG). This was a serious event for radiopharmacy as ITLC-SG is a thin-layer chromatographic stationary phase that is widely used to measure the radiochemical purity of radiopharmaceuticals prepared in hospital radiopharmacies. A replacement for ITLC-SG is urgently required to ensure that the quality assurance programmes in hospital radiopharmacies are not compromised.

Radiochemical purity is an important indicator of the quality of a radiopharmaceutical as it determines the biodistribution of the radionuclide after administration to a patient. A simple definition of radiochemical purity is 'the percentage of the radionuclide that is present in the stated chemical form'. For many ^{99m}Tc radiopharmaceuticals, it is determined by quantifying the proportion of the radionuclide that is present as radiochemical impurities and assuming that the remaining radionuclide is present in the stated chemical form. Thin-layer chromatography is a technique that is commonly used to measure the levels of radiochemical impurities. ITLC-SG was proposed in the 1970s as being ideal for this technique and has become the most commonly used stationary phase.[1–3] Since then, ITLC-SG has been specified in monographs of the European Pharmacopoeia as the stationary phase to be used for measuring radiochemical impurities in many ^{99m}Tc radiopharmaceuticals.[4] Manufacturers also commonly specify ITLC-SG for use with ^{99m}Tc radiopharmaceuticals prepared using their kits. With so many routine radiochemical purity determinations being based on ITLC-SG and few alternative methods having been validated for use with ^{99m}Tc radiopharmaceuticals, an alternative is needed. Varian supplies Glass Microfibre Chromatography Paper impregnated with salicic acid (GMCP-SA), a stationary phase that appears similar to ITLC-SG. The aim of this work was to determine whether the Varian product is a suitable replacement for ITLC-SG.

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Results

The results of the comparisons between ITLC-SG and GMCP-SA are shown in Table 1. For ^{99m}Tc albumin nanocolloid, ^{99m}Tc colloidal tin, ^{99m}Tc exametazime and ^{99m}Tc succimer, the results obtained with the two stationary phases show no significant differences over the ranges of radiochemical purities tested.

In the analysis of ^{99m}Tc medronate, comparison of the results obtained with both mobile phases shows statistical differences. GMCP-SA grossly overestimates the level of ^{99m}Tc pertechnetate impurity. In the determination of colloidal ^{99m}Tc impurity, GMCP-SA gives slightly higher values than ITLC-SG. The difference is most likely caused by tailing of the principal ^{99m}Tc medronate peak back to the origin. The colloidal ^{99m}Tc sits on top of this tail, resulting in higher values. This is illustrated in Figure 1(a), which shows chromatograms from a sample of ^{99m}Tc medronate that contains approximately 3.5% colloidal ^{99m}Tc impurity.

With ^{99m}Tc pentetate, GMCP-SA also gives higher values for ^{99m}Tc pertechnetate impurity. The difference is small but statistically significant. The results from the two stationary phases show no significant difference when sodium chloride (9 g/l) was used as the mobile phase for the measurement of colloidal ^{99m}Tc impurity.

For most of the radiopharmaceuticals, the chromatograms obtained using GMCP-SA showed a higher peak resolution than those obtained using ITLC-SG. Figure 1(b) shows chromatograms of ^{99m}Tc exametazime that contains approximately 6.5% ^{99m}Tc pertechnetate impurity. The chromatogram obtained with ITLC-SG shows a broad peak close to the origin whereas with GMCP-SA the corresponding peak is sharp. Figure 1(c) shows chromatograms of ^{99m}Tc succimer that contains approximately 3% ^{99m}Tc pertechnetate impurity. The impurity peak at the solvent front is much sharper with GMCP-SA. Conversely, Figure 1(a) shows chromatograms of ^{99m}Tc medronate that contains approximately 3.5% colloidal ^{99m}Tc impurity. In this situation, the chromatogram obtained with GMCP-SA has a much poorer shape than the one obtained with ITLC-SG. In an attempt to improve the shape of the chromatogram obtained with GMCP-SA, plates were activated before use by heating at 110C for 10 min. This pre-treatment of the plates produced no improvement in the shape of the chromatogram.

Discussion

The end of ITLC-SG manufacture was a serious event for radiopharmacy as it is the stationary phase specified for many routine methods of measuring radiochemical purity. A possible alternative material is the glass microfibre chromatography paper impregnated with salicic acid (GMCP-SA) that is manufactured by Varian. GMCP-SA has several favourable characteristics. It is supplied in large sheets that can be cut into plates of the required dimensions, it is a rigid material that needs no support in a chromatography tank and it is approximately 25% of the cost of ITLC-SG. Its disadvantages are that the mobile phases migrate approximately 50% slower than on ITLC-SG and there is an absence of data on its suitability for use with ^{99m}Tc radiopharmaceuticals. GE Healthcare has issued guidance on the use of GMCP-SA for measuring the radiochemical purity of ^{99m}Tc tetrofosmin[5] but to date, no information exists on its suitability for use with other radiopharmaceuticals.

The GMCP-SA plate dimensions of 22 mm × 152 mm were chosen to make efficient use of the material. These dimensions provide 10 plates from each sheet. Our standard technique for ITLC-SG plates is to mark the origin 25 mm from the bottom. To minimize the effect of the slower migration on GMCP-SA, the origin was marked 15 mm from the bottom and a run length of 100 mm was maintained. In view of the problems that are known to be caused by heat activation of ITLC-SG,[6] GMCP-SA plates were used without activation.

A comparison of analytical techniques for measuring radiochemical purity should not be restricted to the analysis of

Table 1. Comparison of the level of impurity measured using ITLC-SG and GMCP-SA

Radiopharmaceutical	Manufacturer	Impurity	Mobile phase	Impurity position on plate[a]	Impurity level %[b]		*P*
					ITLC-SG	GMCP-SA	
^{99m}Tc albumin nanocolloid	GE[c]	Pertechnetate	Methanol, water (85:15)	SF	4.1 ± 3.4	4.4 ± 3.5	0.33
^{99m}Tc colloidal tin	Polatom[d]	Pertechnetate	Sodium chloride (9 g/l)	SF	5.1 ± 3.4	5.8 ± 4.5	0.22
^{99m}Tc exametazime	GE	Pertechnetate	Sodium chloride (9 g/l)	SF	4.3 ± 4.0	4.0 ± 4.1	0.10
		Colloid	Methyl ethyl ketone	O	8.9 ± 4.1	9.8 ± 3.8	0.14
		2° complex	Methyl ethyl ketone	O	6.4 ± 2.5	7.3 ± 1.8	0.10
^{99m}Tc medronate	GE	Pertechnetate	Methyl ethyl ketone	SF	5.7 ± 2.8	10.6 ± 1.4	0.00[g]
		Colloid	Sodium acetate (136 g/l)	O	5.3 ± 4.1	5.8 ± 4.0	0.01[g]
^{99m}Tc pentetate	Mallinckrodt[e]	Pertechnetate	Methyl ethyl ketone	SF	4.5 ± 4.0	5.6 ± 4.6	0.03[g]
		Colloid	Sodium chloride (9 g/l)	O	4.7 ± 3.2	4.7 ± 3.2	0.82
^{99m}Tc succimer	CIS bio[f]	Pertechnetate	Methyl ethyl ketone	SF	5.1 ± 3.8	5.0 ± 3.7	0.39

[a]SF = solvent front, O = origin.
[b]Each value is the mean ± standard deviation of 6 results.
[c]GE Healthcare, Amersham, UK.
[d]Polatom, Otwock-Swierk, Poland.
[e]Mallinckrodt Medical, Petten, The Netherlands.
[f]CIS bio, Gif-sur Yvette Cedex, France.
[g]Statistically different ($P < 0.05$).

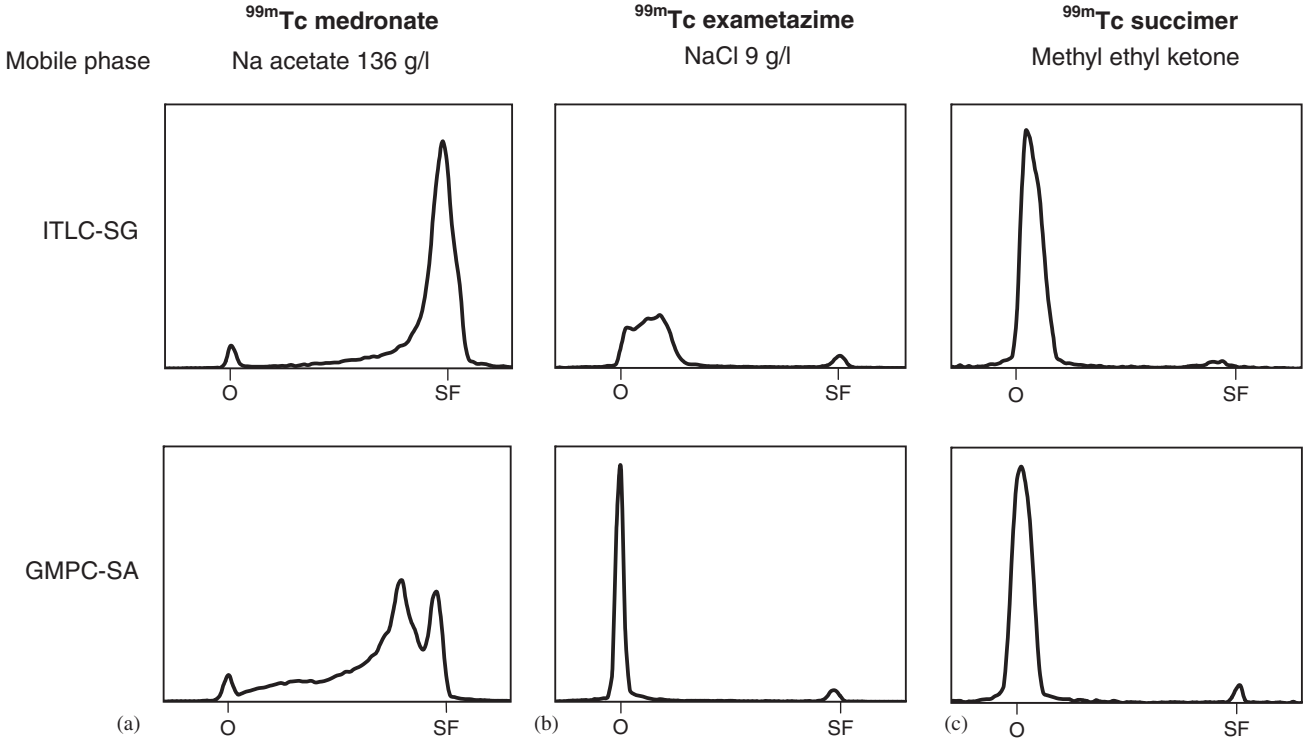

Figure 1. Comparison of chromatograms obtained with ITLC-SG and GMCP-SA.

radiopharmaceuticals prepared routinely as these will probably contain low levels of impurities. Under such conditions, the results from a comparison are likely to show good agreement because the presence of only low levels of impurities may be inadequate to demonstrate differences. Also, the value of most importance when measuring radiochemical purity is the limit of acceptability. For most ^{99m}Tc radiopharmaceuticals this is 95%. This is the point where confidence in the value provided by a technique is essential as it is where the decision to pass or fail a product is most important. Demonstration of agreement over a range of radiochemical purities is therefore a means of establishing confidence in the validity of results around the limit of acceptability. For this reason we compared the results over a range of impurity levels at approximately 2% intervals between 0 and 10%. To achieve this, a number of techniques were devised to obtain the samples of ^{99m}Tc radiopharmaceuticals that contained impurities: making use of a slow rate of labelling or natural decomposition, addition of an oxidant to generate ^{99m}Tc pertechnetate impurity and addition of colloidal ^{99m}Tc.

Our findings show that when measuring the radiochemical purity of ^{99m}Tc albumin nanocolloid, ^{99m}Tc colloidal tin, ^{99m}Tc exametazime and ^{99m}Tc succimer, GMCP-SA is a suitable replacement for ITLC-SG. This is not true when measuring ^{99m}Tc pertechnetate impurity in ^{99m}Tc pentetate where the values obtained with GMCP-SA are approximately 1% higher than those obtained with ITLC-SG. In practice, this difference may not be significant. Our experience of ^{99m}Tc pentetate is that the levels of ^{99m}Tc pertechnetate impurity are extremely low at typically less than 0.5%. The pharmacopoeia specifies a maximum level of 5% for ^{99m}Tc pertechnetate impurity in ^{99m}Tc pentetate. A small increase in the typical level that is measured will give a value that is well within the limit. Also, as the difference is due to an over-estimate of ^{99m}Tc pertechnetate impurity, no product that would have failed a test performed with ITLC-SG will pass if GMCP-SA is used.

A similar over-estimate has been shown when GMCP-SA is used to measure ^{99m}Tc pertechnetate impurity in ^{99m}Tc medronate. In this case, the over-estimate is so great that GMCP-SA is unsuitable and the evaluation of other stationary phases is required to find a suitable replacement for ITLC-SG. Paper chromatography is a possible alternative as previous work has shown that Whatman 31ET paper with acetone as the mobile phase is suitable for measuring ^{99m}Tc pertechnetate in ^{99m}Tc medronate.[2] When measuring the level of colloidal ^{99m}Tc in ^{99m}Tc medronate, GMCP-SA has also produced higher values than ITLC-SG. The difference is small, typically 0.5% regardless of the level of colloidal impurity. The reason for this is that on GMCP-SA the ^{99m}Tc medronate peak near the solvent front tails back to the origin more than on ITLC-SG. The impurity peak sits on the tail and the values are greater because the region of interest used to quantify the impurity contains the tail of the main peak plus the impurity peak. With ITLC-SG, the tail is much less pronounced, leading to lower values. This effect is demonstrated in Figure 1(a). The pharmacopoeia specifies that the ^{99m}Tc pertechnetate impurity in ^{99m}Tc medronate must be less than 2% and the sum of the ^{99m}Tc pertechnetate and colloidal impurities must be less than 5%. This means that the permissible level of ^{99m}Tc colloidal impurity is 3–5%, depending on the level of ^{99m}Tc pertechnetate impurity. As discussed above for ^{99m}Tc pentetate, the small difference between the values obtained with GMCP-SA and ITLC-SG may not be significant in practice, as most products will pass the test regardless of the stationary phase used. Also, in common with ^{99m}Tc pentetate, no product that fails when analysed with ITLC-SG will pass if GMCP-SA is used.

The ideal method for analysing chromatography plates is scanning as it provides a visual display of the distribution of ^{99m}Tc on the plate. This allows regions of interest to be defined around the peaks of the chromatogram and quantitation to be based on the counts in the regions. In the absence of a scanner, quantitation can be achieved using cut-and-count. In this technique, the chromatographic plate is cut into pre-defined sections, each of which should contain a peak. The count-rate from each section is then measured in a gamma-counter and quantitation is performed by comparing the count-rates. The accuracy of this technique depends upon the ^{99m}Tc being distributed on the plate as expected. If it is not, the wrong result is obtained. Many of the chromatograms obtained using GMCP-SA showed higher peak resolution than the corresponding ones obtained using ITLC-SG. For analyses in which GMCP-SA is a suitable replacement for ITLC-SG, better resolution should lead to more accurate and reproducible results regardless of whether analysis is performed by scanning or cut-and-count.

Experimental

Preparation of radiopharmaceuticals

The ^{99m}Tc radiopharmaceuticals that are prepared in this hospital radiopharmacy and for which thin-layer chromatography is the method for measuring radiochemical purity were included in this study. They are shown in Table 1. Samples of each radiopharmaceutical containing different levels of known impurities were prepared. The level of impurity in each sample was measured simultaneously using the two thin-layer stationary phases and the appropriate mobile phase for the detection of the impurity. The impurities and mobile phases used for their detection are shown in Table 1. Measurements were performed over a range of impurity levels, at approximately 2% intervals between 0 and 10%. The following techniques were used to create impure samples of each radiopharmaceutical.

An albumin nanocolloid kit was reconstituted with ^{99m}Tc generator eluate that had been diluted with sodium chloride injection from a plastic ampoule that had been exposed to light. In previous work we have shown that this slows the rate of labelling.[7] Samples containing the required levels of ^{99m}Tc pertechnetate impurity were obtained by withdrawing aliquots from the kit over a period of 2 h.

Samples of ^{99m}Tc medronate, ^{99m}Tc pentetate, ^{99m}Tc colloidal tin and ^{99m}Tc succimer with varying levels of ^{99m}Tc pertechnetate impurity were created by the addition of hydrogen peroxide solution to reconstituted kits. Typically, addition of 0.1–0.5 ml of a 0.03% solution of hydrogen peroxide was required to achieve the desired oxidation of the labelled compound and the consequent generation of ^{99m}Tc pertechnetate impurity. A series of samples taken from each kit over a period of a few hours provided results at the required levels of impurity.

Samples of ^{99m}Tc exametazime containing levels of ^{99m}Tc pertechnetate impurity between approximately 1 and 10% were obtained by reconstituting an exametazime kit with ^{99m}Tc generator eluate that had been eluted 1 h previously. Under these conditions, the level of ^{99m}Tc pertechnetate impurity in ^{99m}Tc exametazime has been shown to increase over a few hours.[8–11] A series of aliquots taken from the kit over a period of 3 h provided samples containing the required levels of impurity.

Samples of ^{99m}Tc exametazime, ^{99m}Tc medronate and ^{99m}Tc pentetate containing varying levels of colloidal ^{99m}Tc impurity were obtained by adding ^{99m}Tc colloidal tin to the radiopharmaceuticals.

Samples of ^{99m}Tc exametazime containing various levels of secondary complex were obtained by reconstituting an exametazime kit according to the manufacturer's instructions[12] and withdrawing a series of aliquots over 2.5 h. The level of secondary ^{99m}Tc exametazime complex has been shown to increase with time.[8,11]

Thin-layer chromatography

The thin-layer chromatographic methods were based on the techniques specified in the monographs of the European Pharmacopoeia.[4] As no monograph exists for ^{99m}Tc albumin nanocolloid, the method recommended by the manufacturer of the radiopharmaceutical kit was used.[13]

Instant Thin-Layer Chromatography Silica Gel impregnated glass fibre (ITLC-SG, Pall Corporation, Ann Arbor, USA) is supplied as 50 mm × 200 mm sheets. Each sheet was cut to provide two plates 25 mm × 200 mm. The origin was marked 25 mm from the bottom of the plate. The solvent front was marked 100 mm above the origin. Where specified in the pharmacopoeial monograph, plates were activated by heating in an oven at 110C for 10 min immediately before use.

Glass microfibre chromatography paper impregnated with salicic acid (GMCP-SA, Varian, Palo Alto, USA) is supplied as 114 mm × 304 mm sheets. Each sheet was cut to provide 10 plates 22 mm × 152 mm. The origin was marked 15 mm from the bottom of the plate. The solvent front was marked 100 mm above the origin. No GMCP-SA plates were heat activated before use.

A glass gas jar 45 mm × 200 mm with a ground glass lid was used as the chromatography tank. The mobile phase was added to a depth of not greater than 3 mm and allowed to equilibrate for at least 10 min.

Analysis of chromatograms

The distribution of ^{99m}Tc on the thin-layer plates was measured with a scintillation detector on a Mini-Scan radiochromatogram scanner (Bioscan, Washington, USA). Chromatograms were recorded and analysed with LauraLite 3 radiochromatography software (LabLogic, Sheffield, UK). Background subtraction was applied using a region of interest away from the main peak as described previously.[14] The percentage impurity was calculated by expressing the activity in the impurity peak as a percentage of the total activity on the plate.

In the comparison of results, ITLC-SG was assumed to provide the correct result, as it is the stationary phase specified in the European Pharmacopoeia. Data obtained with the two stationary phases were compared using the paired *t*-test and deemed to be statistically different if $P < 0.05$.

Conclusion

When measuring the radiochemical purity of ^{99m}Tc albumin nanocolloid, ^{99m}Tc colloidal tin, ^{99m}Tc exametazime, ^{99m}Tc pentetate and ^{99m}Tc succimer, GMCP-SA is a suitable replacement for ITLC-SG, although slightly higher levels of ^{99m}Tc pertechnetate impurity may be detected in ^{99m}Tc pentetate. GMCP-SA is unsuitable for measuring the radiochemical purity of ^{99m}Tc medronate.

References

[1] M. W. Billinghurst, *J. Nucl. Med.* **1974**, *14*, 793.
[2] A. M. Zimmer, D. G. Pavel, *J. Nucl. Med.* **1977**, *18*, 1230.
[3] P. J. Robbins, *Chromatography of Technetium-99m Radiopharmaceuticals – A Practical Guide*. The Society of Nuclear Medicine, New York, **1984**.
[4] European Directorate for the Quality of Medicines, *European Pharmacopoeia,* 5th ed., European Directorate for the Quality of Medicines, **2004**.
[5] *Procedure for Alternative Method for Quality Control of Myoview Using Varian SA Chromatography Paper*, GE Healthcare Ltd, Amersham, UK, **2009**.
[6] D. Graham, A. M. Millar, *Nucl. Med. Commun.* **1999**, *20*, 439.
[7] F. Craig, L. A. Beattie, L. M. O'Brien, A. M. Millar, *Nucl. Med. Commun.* **2009**, submitted.
[8] V. J. Bayne, A. M. Forster, D. A. Tyrell, *Nucl. Med. Commun.* **1989**, *10*, 29.
[9] J. A. Ponto, *Am. J. Hosp. Pharm.* **1990**, *47*, 2511.
[10] A. M. Millar, *Am. J. Hosp. Pharm.* **1993**, *50*, 103.
[11] P. S. Weisner, G. R. Bower, L. A. Dollimore, A. M. Forster, B. Higley, A. E. Storey, *Eur. J. Nucl. Med.* **1993**, *20*, 661.
[12] Ceretec Technical Information, GE Healthcare Ltd, Amersham, UK, **2006**.
[13] Nanocoll Technical Leaflet, GE Healthcare Srl, Milan, Italy, **2006**.
[14] L. A. Beattie, L. M. O'Brien, A. M. Millar, *Nucl. Med. Commun.* **2008**, *29*, 487.