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An evaluation of paper chromatography for measuring the levels of radiochemical impurities in ^{99m}Tc Medronate Injection

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***Background*: The European Pharmacopoeia specifies Instant Thin-Layer Chromatography Silica Gel (ITLC-SG) as the stationary phase for measuring radiochemical impurities in ^{99m}Tc Medronate. The Pall Corporation has stopped manufacturing ITLC-SG. *Aim*: To investigate chromatography papers as alternatives to ITLC-SG. *Experimental*: The resolution of the main ^{99m}Tc Medronate and impurity peaks were compared on five Whatman papers (1CHR, 31ET, 3MM, 4CHR and 54SFC) using the pharmacopoeial mobile phases: methyl ethyl ketone for detecting ^{99m}Tc pertechnetate impurity and sodium acetate solution (136 g/l) for detecting hydrolysed reduced and colloidal ^{99m}Tc impurities. The effects of three sample spot treatments (wet, hot dried and air dried) on the distribution of the radiochemical impurities were compared. The levels of radiochemical impurities measured in samples of ^{99m}Tc Medronate were compared. *Results*: The highest resolutions were obtained with 1CHR, 4CHR and 54SFC. Sample spot drying resulted in the measurement of artifactually high levels of ^{99m}Tc pertechnetate impurity. When analysing ^{99m}Tc Medronate, erroneously high levels of colloidal and ^{99m}Tc pertechnetate impurities were measured with 1CHR and 4CHR. *Conclusions*: 54SFC paper is a suitable alternative to ITLC-SG for measuring radiochemical impurities in ^{99m}Tc Medronate. The sample spot should be wet when the paper strip is placed in the chromatography tank.**

Keywords: paper chromatography; ^{99m}Tc Medronate; radiochemical purity

Introduction

Skeletal scintigraphy is one of the most frequently performed nuclear medicine procedures. It is undertaken principally for the detection of skeletal metastases. Technetium-99m [^{99m}Tc] Medronate Injection is a radiopharmaceutical that is commonly used in this procedure. One of the factors that influences the quality of skeletal images is the presence of radiochemical impurities in the ^{99m}Tc Medronate that is injected into the patient. The typical impurities present in ^{99m}Tc Medronate concentrate in the stomach, thyroid, salivary glands and liver and these sites of extra-skeletal activity can interfere with interpretation of images. To assure the quality of skeletal images, the European Pharmacopoeia monograph for ^{99m}Tc Medronate specifies that the level of radiochemical impurities must be less than 5% and that not more than 2% can be ^{99m}Tc pertechnetate impurity.[1] The monograph specifies thin-layer chromatographic analyses on Instant Thin-Layer Chromatography Silica Gel impregnated glass fibre plates (ITLC-SG) for the isolation and quantitation of radiochemical impurities. In 2008, The Pall Corporation stopped manufacturing ITLC-SG. Once stocks of ITLC-SG are exhausted, radiopharmacies will be unable to use the pharmacopoeial methods for measuring the radiochemical purity of many ^{99m}Tc radiopharmaceuticals, including ^{99m}Tc Medronate.

In a previous study, we attempted to address this situation by investigating the use of Varian's Glass Microfibre Chromatography Paper impregnated with salicic acid (GMCP-SA) as a replacement for ITLC-SG.[2] Our results showed that this alternative is suitable for measuring the radiochemical impurities in all the ^{99m}Tc radiopharmaceuticals investigated with the exception of ^{99m}Tc Medronate. GMCP-SA was found to be unsuitable for use with ^{99m}Tc Medronate due to the measurement of erroneously high levels of impurities. An alternative to ITLC-SG is therefore still required for use with ^{99m}Tc Medronate.

The aim of this study was to investigate chromatography papers as alternatives to ITLC-SG for measuring radiochemical impurities in ^{99m}Tc Medronate.

Results and discussion

Whatman supplies a range of eleven chromatography papers. The five shown in Table 1 were chosen for initial evaluation for the following reasons: 1CHR is the world standard chromatography paper, 31ET has been suggested as being suitable for measuring ^{99m}Tc pertechnetate impurity in ^{99m}Tc Medronate[3] and 3MM, 4CHR and 54SFC have high flow rates and are recommended by Whatman for routine use.[4] To determine which of the five were worthy of further evaluation, a comparison of the resolution obtained with each was performed. Samples of ^{99m}Tc Medronate

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were analysed using the mobile phases specified in the pharmacopoeia for use with ITLC-SG: methyl ethyl ketone for the detection of ^{99m}Tc pertechnetate impurity and sodium acetate solution (136 g/l) for the detection of hydrolysed reduced and colloidal ^{99m}Tc impurities. Resolution was assessed by determining the percentage of the total activity in the chromatogram that was present in a region of interest that represented the trough between the positions at which the main and impurity peaks are expected. The regions of interest are shown in Figure 1(a) and the results in Table 1. With methyl ethyl ketone as the mobile phase, the chromatogram obtained with each of the five papers contained approximately 1% of the total activity in the region of interest. No significant differences were found between the papers. With sodium acetate solution as the mobile phase, the resolution was highest on 54SFC and lowest on 31ET and the difference is significant. The mobile phase migration times were recorded and are also shown in Table 1. The migration of sodium acetate solution on 3MM paper was much longer than on the others. On the basis of these results, we decided to reject the 31ET and 3MM papers and continue with comparisons of the other three.

Table 1. Comparison of migration times and peak resolution on five chromatography papers

Paper	Methyl ethyl ketone		Sodium acetate (136 g/l)	
	Migration time (min)	% ^{99m}Tc between Rf 0.4 and 0.8	Migration time (min)	% ^{99m}Tc between Rf 0.1 and 0.5
1CHR	18	0.7 ± 0.1	29	6.2 ± 2.3
31ET	18	0.8 ± 0.2	16	8.0 ± 1.0
3MM	15	0.9 ± 0.1	55	5.2 ± 0.9
4CHR	9	0.8 ± 0.2	18	6.4 ± 1.8
54SFC	12	0.7 ± 0.1	17	4.4 ± 1.4

When analysing ^{99m}Tc radiopharmaceuticals by thin-layer or paper chromatography, the treatment of the spot after it has been applied to the stationary phase can influence the result. To establish the best technique, samples of the two potential radiochemical impurities, ^{99m}Tc pertechnetate and colloidal ^{99m}Tc, were analysed on the three papers following three spot treatments. The results are shown in Table 2. With both mobile phases, ^{99m}Tc pertechnetate impurity is expected to migrate at or close to the solvent front—Figure 1(b). With methyl ethyl ketone this was true for all the three papers when the strips were put into the tank with the sample spots wet. In contrast, when the spots were dried either with hot air or at room temperature, most of the ^{99m}Tc was fixed at the origin and the results are significantly different from those obtained with the wet spots. This demonstrates that spot drying will produce an erroneously low result for the level of ^{99m}Tc pertechnetate impurity. In contrast, ^{99m}Tc pertechnetate migrated with the sodium acetate solution mobile phase regardless of the spot treatment and less than 0.5% of the activity was detected at the origin. With both mobile phases, colloidal ^{99m}Tc impurity is expected to remain at the origin. For all combinations of paper, mobile phase and spot treatment, at least 95% of colloidal ^{99m}Tc was detected at the origin—Figure 1(c). With the sodium acetate mobile phase that is used to determine the level of colloidal ^{99m}Tc impurity, 4CHR showed significantly lower retention at the origin than the other papers as did air drying of the spots on all the three papers. This series of measurements shows that detection of the radiochemical impurities is most efficient when the paper strips are put into the tank with the sample spots wet.

In the comparison of the papers with routine samples of ^{99m}Tc Medronate, 54SFC detected lower levels of both impurities than the other two. The differences are greatest in the levels of colloidal ^{99m}Tc impurity with 1CHR and 4CHR measuring significantly higher levels than 54SFC. The explanation for this is that the main ^{99m}Tc Medronate peak near the solvent front tails back to the origin to a greater extent on 1CHR

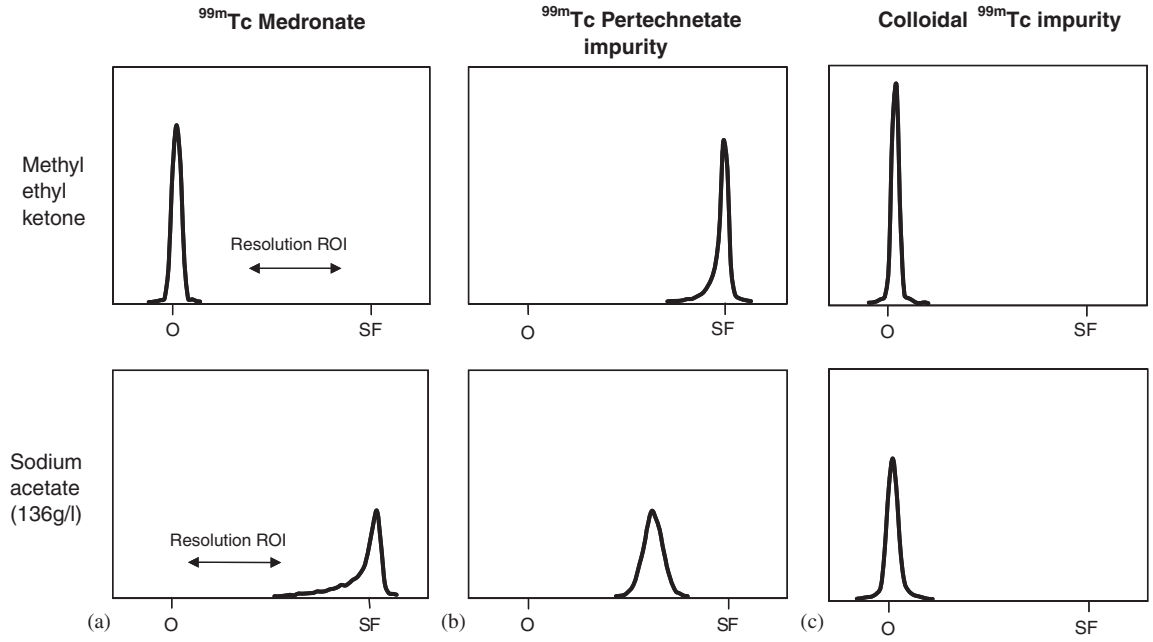

Figure 1. Chromatograms of ^{99m}Tc Medronate and two radiochemical impurities obtained on 54SFC paper. All chromatograms are presented with the same area under the curve.

Table 2. Effect of sample spot treatment on the migration of radiochemical impurities

Impurity/Paper	% ^{99m}Tc at expected position on strip					
	Methyl ethyl ketone			Sodium acetate (136 g/l)		
	Wet	Hot air	Air	Wet	Hot air	Air
Pertech						
1CHR	98.2 ± 0.4	6.5 ± 3.4	47.2 ± 13.9	99.8 ± 0.1	99.8 ± 0.1	99.7 ± 0.1
4CHR	98.8 ± 0.2	13.5 ± 11.0	26.3 ± 2.3	99.8 ± 0.1	99.8 ± 0.1	99.8 ± 0.1
54SFC	98.6 ± 0.2	1.4 ± 0.6	23.8 ± 3.4	99.8 ± 0.1	99.8 ± 0.1	99.8 ± 0.1
Colloid						
1CHR	99.2 ± 0.2	99.2 ± 0.4	98.1 ± 0.4	98.5 ± 0.5	98.2 ± 0.3	95.9 ± 0.6
4CHR	98.8 ± 0.1	99.2 ± 0.1	97.9 ± 0.4	97.8 ± 0.3	97.2 ± 0.5	94.9 ± 0.3
54SFC	98.6 ± 0.3	99.0 ± 0.2	98.3 ± 0.3	99.3 ± 0.1	99.2 ± 0.1	97.8 ± 0.5

and 4CHR. The colloidal ^{99m}Tc impurity peak sits on this 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. It is therefore safe to assume that the result from 54SFC is the truest measure of the colloidal ^{99m}Tc impurity. The 54SFC paper also produced lower values for ^{99m}Tc pertechnetate impurity than 1CHR and 4CHR although only the 1CHR results differed significantly. The reason for these differences is unclear as the earlier experiments demonstrate that almost 100% of ^{99m}Tc pertechnetate migrates to the solvent front on all the three papers. Three possible explanations can be postulated: (a) on 4CHR and 54SFC, not all the ^{99m}Tc pertechnetate impurity migrates with the solvent front when ^{99m}Tc Medronate is present in the sample, (b) there is a non-^{99m}Tc pertechnetate component in ^{99m}Tc Medronate that migrates on 1CHR but not on the others or (c) 1CHR has the ability to denature a small amount of ^{99m}Tc Medronate producing ^{99m}Tc pertechnetate that migrates with the solvent front. As ITLC-SG is the stationary phase specified in the pharmacopoeia, a reasonable assumption is that it gives the correct result. The ideal means of establishing which paper gives the true result would therefore have been to include ITLC-SG in the comparison. Unfortunately we no longer had any ITLC-SG plates with which to undertake this comparison. As an alternative means of producing a comparison, we reviewed historical results from 50 consecutive analyses undertaken with ITLC-SG during our routine quality assurance programme. These showed a ^{99m}Tc pertechnetate impurity level of 0.4 ± 0.3%, which is similar to the values obtained with 4CHR and 54SFC. This similarity along with the agreement between 4CHR and 54SFC leads us to conclude that values obtained with 1CHR are erroneously high.

Our findings demonstrate that either 4CHR or 54SFC is suitable for measuring ^{99m}Tc pertechnetate impurity but that 4CHR is not suitable for measuring colloidal ^{99m}Tc impurity. In practice, it is convenient to use the same stationary phase for both measurements. We have therefore selected 54SFC as the paper of choice. An additional advantage of a paper stationary phase is that it is inexpensive. Whatman 54SFC is approximately 10 and 40% of the costs of ITLC-SG and GMCP-SA, respectively.

Experimental

Preparation of samples

Sodium Pertechnetate [^{99m}Tc] Injection was eluted from a Drytec ^{99m}Tc generator (Code MCD45Z, GE Healthcare, Amersham, UK). Samples of ^{99m}Tc Medronate were prepared using Amerscan Medronate II Agent (Code N165K, GE Healthcare, Amersham, UK). Samples of colloidal ^{99m}Tc were prepared using a Kit for the preparation of ^{99m}Tc-Colloid (Code MTcK-2, Polatom, Otwock-Swierk, Poland). All samples were prepared at a radioactive concentration of 1.5 GBq/2.5 ml.

Chromatographic technique

A glass gas jar 45 × 200 mm was used as the chromatography tank. A 250 ml glass beaker was used as a lid. The mobile phase was added to a depth of 10 mm and allowed to equilibrate for at least 15 min. The mobile phases specified in the pharmacopoeia for use with thin-layer chromatography were used: methyl ethyl ketone for the detection of ^{99m}Tc pertechnetate impurity and sodium acetate solution (136 g/l) for the detection of hydrolysed reduced and colloidal ^{99m}Tc impurities. With methyl ethyl ketone, hydrolysed reduced and colloidal ^{99m}Tc impurities, and ^{99m}Tc Medronate remain at the origin while ^{99m}Tc pertechnetate impurity migrates with the solvent front. With sodium acetate solution (136 g/l), ^{99m}Tc hydrolysed reduced and colloidal impurities remain at the origin while ^{99m}Tc Medronate and ^{99m}Tc pertechnetate impurity migrate with the solvent front.

The chromatography papers were cut into 25 × 190 mm strips. The origin was marked 25 mm from the bottom of the strip and the solvent front was marked 100 mm from the origin. The top of the paper strip was attached to a clip, a sample spot 5–8 mm in diameter from a 0.5 × 16 mm hypodermic needle was applied at the origin mark. The strip was then suspended in the tank, ensuring that the paper did not touch the sides. When the mobile phase had migrated to the solvent front mark, the strip was removed from the tank and dried with hot air.

The distribution of ^{99m}Tc on the paper strip was recorded 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.[5] The % impurity was calculated by expressing the activity in the impurity peak as a percentage of the total activity on the strip.

Choice of papers

Five chromatography papers manufactured by Whatman (Maidstone, UK) were selected for the initial evaluation. The

Table 3. Comparison of three chromatography papers for measuring radiochemical impurities in routine samples of ^{99m}Tc Medronate Injection

Paper	^{99m}Tc pertechnetate impurity (%)	Colloidal ^{99m}Tc impurity (%)	Radiochemical purity (%)
1CHR	1.2 ± 0.5	5.2 ± 1.5	93.6 ± 1.5
4CHR	0.6 ± 0.2	4.7 ± 1.2	94.7 ± 1.2
54SFC	0.4 ± 0.1	1.5 ± 0.4	98.1 ± 0.4

papers are listed in Table 1. Samples of ^{99m}Tc Medronate were analysed on each paper with both mobile phases. As a measure of resolution, the counts in a region of interest that represented the trough between the positions of the main and impurity peaks were calculated. The regions of interest were between Rf 0.4 and 0.8 and between Rf 0.1 and 0.5 for chromatograms obtained with the methyl ethyl ketone and sodium acetate mobile phases, respectively. The regions of interest are shown in Figure 1(a). The counts in the region of interest were expressed as a percentage of the total counts in the chromatogram.

Effect of spot drying

The effects of three spot treatments on the chromatograms obtained with the two potential radiochemical impurities, ^{99m}Tc pertechnetate and colloidal ^{99m}Tc, were investigated. The three papers listed in Table 2 were tested with both mobile phases. After application of the spot to the paper, (a) the strip was placed in the tank immediately without any drying of the spot, (b) the spot was dried with hot air for 15 s and (c) the spot was allowed to dry at room temperature for 5 min.

Analysis of routine samples

The levels of ^{99m}Tc pertechnetate impurity and hydrolysed reduced and colloidal ^{99m}Tc impurities were measured in samples of ^{99m}Tc Medronate. After application of the spot, the paper strip was placed immediately in the tank. The three papers listed in Table 3 were used. The migration time for each paper/mobile phase combination was measured.

Statistical analysis

Data are mean ± standard deviation of five results. Statistical comparisons were performed using Statistica (Statsoft Inc., Tulsa, Oklahoma, USA). Results were compared by one-way analysis of variance and Fisher's post-hoc tests. Data were deemed to be statistically different if $P < 0.05$.

Conclusions

- Whatman 54SFC chromatography paper is suitable for measuring the levels of radiochemical impurities in ^{99m}Tc Medronate.
- The sample spot should be wet when the paper strip is placed in the chromatography tank.

References

[1] European Directorate for the Quality of Medicines, *European Pharmacopoeia*, 5th edn, European Directorate for the Quality of Medicines, **2004**.
[2] A. M. Millar, L. A. Beattie, F. Craig, L. M. O'Brien, *J. Label. Compd. Radiopharm*. **2009**, DOI: 10.1002/jlcr.1671.
[3] A. M. Zimmer, D. G. Pavel, *J. Nucl. Med.* **1977**, *18*, 1230.
[4] Cellulose chromatography papers, Whatman plc, Maidstone, UK, **2009**.
[5] L. A. Beattie, L. M. O'Brien, A. M. Millar, *Nucl. Med. Commun.* **2008**, *29*, 487.