

Short communication

Adsorption of technetium-99m [^{99m}Tc] in the sample loop of an HPLC injection valve

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1. Introduction

Technetium [^{99m}Tc] Succimer Injection Ph Eur is used in imaging of the kidneys [1]. The images obtained with this radiopharmaceutical are of value in the diagnosis, localisation and evaluation of kidney disorders such as calculi, obstructive disease, pyelonephritis, tumours and cysts. The pharmacopoeial monograph for Technetium [^{99m}Tc] Succimer Injection specifies a thin-layer chromatographic technique for analysing radiochemical purity. This is less than ideal as it is not specific for the ^{99m}Tc -succimer complex. It provides a value for the level of only one potential radiochemical impurity— ^{99m}Tc -pertechnetate ion, and assumes that all the remaining ^{99m}Tc exists in the form of ^{99m}Tc -succimer. A more satisfactory technique for measuring radiochemical purity would therefore be of value in assuring the quality of this radiopharmaceutical.

Moretti et al. have used high performance liquid chromatography (HPLC) to show that a high level of ^{99m}Tc in the Sodium Pertechnetate [^{99m}Tc] Injection Ph Eur that is used to prepare ^{99m}Tc -

succimer can result in the formation of two radio-labelled species in the radiopharmaceutical [2]. HPLC has also been used to demonstrate that pentavalent $^{99m}\text{Tc(V)}$ -dimercaptosuccinic acid complexes [3] can be present as impurities in ^{99m}Tc -succimer [4]. These reports demonstrate that under particular conditions, several radiochemical species can be present in Technetium [^{99m}Tc] Succimer Injection and that the pharmacopoeial thin-layer chromatographic technique is therefore inadequate for measuring the radiochemical purity of the radiopharmaceutical.

A search for an HPLC technique that could be used to measure the radiochemical purity of Technetium [^{99m}Tc] Succimer Injection has been undertaken but has, to date, been unsuccessful due to adsorption of the radiolabelled species onto a range of stationary phases under a range of chromatographic conditions. During the course of this work, recoveries of ^{99m}Tc from an HPLC column were measured as greater than 100%. After investigation of several possible causes, this interesting phenomenon was traced to adsorption of ^{99m}Tc in the sample loop during loading of the injection

valve. When the valve was switched to the inject position, the adsorbed ^{99m}Tc was washed from the loop by the mobile phase and subsequently appeared in the eluate from the column. This paper describes how the artefact was detected and resolved.

2. Experimental

Technetium [^{99m}Tc] Succimer Injection was prepared at a radioactive concentration of 150 MBq per 2.5 ml by a wet labelling method [5]. Briefly, a DMSA kit (Product Code N107, Amersham International) was reconstituted with 10.5 ml Sodium Chloride Injection Ph Eur. A 1 ml aliquot of this solution and 1.5 ml Sodium Pertechnetate [^{99m}Tc] Injection Ph Eur (100 MBq per ml) were injected into a sterile 10 ml vial containing a nitrogen atmosphere (Product Code N46, Amersham International). The resulting solution has a pH in the range 2.3–3.5. The radiopharmaceutical was stored at room temperature.

The high performance liquid chromatograph was equipped with a gamma-radiation detector. Samples were injected using a Rheodyne Model 7125 valve fitted with a 20 μl stainless-steel loop. In the experiments in which recoveries of greater than 100% were measured, a 250 \times 4.6 mm PLRP-S column (Polymer Labs) was eluted with a mobile phase of acetonitrile-ammonia (100 mM) (70:30, v/v) (pH 11.0) at a flow rate of 1 ml min^{-1} .

Recovery from the column was measured as follows. After injection of the sample onto the column, the eluate was collected in a vial. At the end of the analysis, the vial was removed from the instrument and its contents were mixed thoroughly. A standard was prepared by pipetting a 20 μl aliquot of the radiopharmaceutical into an identical vial. The standard was diluted to a volume equal to the volume of eluate and mixed thoroughly. The count-rate from the solution in each vial was measured by placing it 15 cm from a sodium iodide crystal detector that was connected to a counter-ratemeter. A counting time that resulted in the collection of at least 10 000 counts was chosen. After subtraction of back-

ground, recovery from the column was calculated by expressing the count-rate from the eluate as a percentage of the count-rate from the standard.

Adsorption of ^{99m}Tc in the valve was investigated by measuring the radioactive concentration of a sample of the radiopharmaceutical before and after it had been passed through the sample loop. This procedure was performed as follows. The loop was flushed with water. A 1 ml syringe was filled with Technetium [^{99m}Tc] Succimer Injection and the needle supplied for loading the valve was fitted. Approximately 0.5 ml of the technetium [^{99m}Tc] succimer solution was injected into a weighed sample tube. With the valve in the 'Load' position, the remaining 0.5 ml was passed through the loop and collected in a second weighed sample tube. Both tubes were reweighed and the weight of solution in each was calculated. The count-rate from each tube was measured using the sodium iodide scintillation detector described above. The count-rate per gram of liquid in each tube was then calculated. The adsorption in the loop was calculated from the difference between the radioactive concentrations.

In an attempt to overcome adsorption, the stainless-steel sample loop was replaced by one made from a 40 cm length of polyetheretherketone (PEEK) tubing of 0.25 mm internal diameter and the experiment was repeated.

3. Results

Analysis of Technetium [^{99m}Tc] Succimer Injection with the acetonitrile-ammonia mobile phase gave a chromatogram with two peaks at retention times of 88 and 158 s. The percentages of the total activity in the peaks were 10 and 90%, respectively. The first of the peaks had the retention time of an unretained solute. The mean recovery from the column was 112% (range 107–115%, $n = 5$). Under these chromatographic conditions, the retention time for the pertechnetate [^{99m}Tc] ion was found to be 178 s.

In the comparison of the radioactive concentrations of ^{99m}Tc before and after passage through the stainless-steel sample loop, the mean count-rate per g from the samples that had not been

passed through the loop was 13% (range 10–15%, $n = 5$) higher than those that had. After the stainless-steel loop was replaced with one made from PEEK, the mean count-rate per g from the samples that had not been through the loop was only 2% (range 0–5%, $n = 5$) higher.

4. Discussion

An important aspect of the quality of a radiopharmaceutical is its radiochemical purity. This is defined in the European Pharmacopoeia as ‘The ratio, expressed as a percentage, of the radioactivity of the radionuclide concerned that is present in the source in the chemical form declared to the total radioactivity of that radionuclide present in the source’. For the majority of radiopharmaceuticals, including Technetium [^{99m}Tc] Succimer Injection, the Pharmacopoeia stipulates a radiochemical purity of not less than 95%. The consequence of low radiochemical purity is that the ^{99m}Tc does not behave in the required manner after the radiopharmaceutical has been administered to the patient. This can result in an unsatisfactory investigation in which the patient receives a worthless exposure to ionising radiation.

HPLC has been widely used in the analysis of radiopharmaceuticals, mainly during development to demonstrate the composition of the product. For the technique to be of value in the determination of radiochemical purity, there must be complete recovery from the HPLC column. When incomplete recovery occurs due to an impurity in the radiopharmaceutical being irreversibly adsorbed in the column, the impurity will remain undetected. Consequently, a value for radiochemical purity that is calculated using data from an on-line radiation detector will be erroneously high. Conversely, if incomplete recovery is a consequence of a proportion of the radiopharmaceutical species being adsorbed, calculation of the radiochemical purity will give an erroneously low value.

In the assay of non-radioactive pharmaceuticals by HPLC, the problem of adsorption is overcome by comparing the chromatogram obtained from the test material with one obtained from a pure

reference sample of the compound. With this technique, adsorption on the column of a proportion of the compound does not affect the result since test and reference samples undergo adsorption to the same extent. The adsorption on the column of an impurity in the test material is of no consequence since the purity is calculated from a comparison of the size of the peaks obtained with the test and reference samples. When dealing with radiopharmaceuticals, there are no reference samples available due to the inherent instability of these substances—a consequence of their radioactive nature and, in the case of most ^{99m}Tc radiopharmaceuticals, their susceptibility to oxidation. An assay procedure similar to that used for non-radioactive pharmaceuticals is not therefore feasible. In view of these limitations, successful analysis of the radiochemical purity of radiopharmaceuticals by HPLC is dependent upon complete recovery from the column.

A phenomenon encountered frequently when attempting to use HPLC to analyse ^{99m}Tc radiopharmaceuticals is incomplete recovery from the column. The measurement of recoveries greater than 100% was therefore a surprise finding. As these high recoveries were seen only when a mobile phase of high pH was used, a possible explanation for the phenomenon is that at the low pH of Technetium [^{99m}Tc] Succimer Injection, adsorption of ^{99m}Tc onto the sample loop occurs during loading of the valve. When the loop is switched into the flow of high pH mobile phase, the adsorbed ^{99m}Tc is washed off the loop and into the column along with the technetium [^{99m}Tc] succimer that is in solution in the loop. More ^{99m}Tc than would be in 20 μl of sample is therefore applied to the column.

The results presented in this paper support this explanation for the phenomenon. The finding that the radioactive concentration of the solution flowing from the vent tube during loading of the sample loop was lower than that of the original sample demonstrates that ^{99m}Tc is adsorbed on the internal surface of the loop. The results also show that replacing the stainless steel loop with one made from PEEK is a satisfactory method of minimising the phenomenon. However, although the PEEK loop resolved the artefact in the recov-

ery measurements, it did not result in a satisfactory technique for measuring the radiochemical purity of Technetium [^{99m}Tc] Succimer Injection. The reasons for this conclusion are (a) that one of the peaks has the retention time of an unretained solute—not a satisfactory basis for an analytical technique and (b) the technique does not have the ability to identify the three peaks of pentavalent $^{99m}\text{Tc(V)}$ -dimercaptosuccinic acid [3] which, under certain conditions, can be present in Technetium [^{99m}Tc] Succimer Injection [4].

Although this artefact was observed under alkaline conditions, it might be of greater significance when acidic mobile phases are used. Under acidic conditions, any ^{99m}Tc adsorbed in the sample loop will not be washed off when the mobile phase passes through. In this situation, adsorption will result in incomplete recovery which might be attributed to adsorption in the column. This could lead to the conclusion that the technique is unsatisfactory for analysis of the radiopharmaceutical. In practice, the use of PEEK tubing could eliminate the artefact. The use of PEEK tubing should therefore be considered when attempting to measure the radiochemical purity of Technetium [^{99m}Tc] Succimer Injection by HPLC. The adsorp-

tion of ^{99m}Tc onto the loop of the injection valve is a potential problem in any HPLC system that is used to measure the radiochemical purity of a radiopharmaceutical and should be excluded during validation of the technique.

5. Conclusions

^{99m}Tc can adsorb to the stainless-steel loop of the valve injector when HPLC is used to analyse Technetium [^{99m}Tc] Succimer Injection. The use of a PEEK loop minimises this unwanted effect.

References

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