DETERMINATION OF SPECIFIC ACTIVITY OF RADIOHALIDE PREPARATIONS (^{75}Br, ^{77}Br, ^{123}I, ^{131}I) BY HPLC-UV DETECTION FOLLOWING CHEMICAL DERIVATIZATION TO 1-HALONAPHTHOL-2

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Summary

Specific activity is an important parameter for receptor-binding radiopharmaceuticals. We present here a method for determination of specific activities of radiobromide (^{75}Br,^{77}Br) and radioiodide (^{123}I,^{131}I) using naphthol-2 and chloramin T as reagents for derivatization. Subsequent hplc determination of the mass of 1-halo-naphthols-2 on a RP 18 column is highly sensitive due to their high UV extinction coefficient in the range of 232 nm (detection limit at 220 nm: 5 x 10^{-13} mol) and linear over 6 orders of magnitude. It can be rapidly and conveniently performed in parallel to the actual radiopharmaceutical preparation using only a small part of the radioactive halide (less than 1 mCi for all isotopes). Dilution factors (carrier atoms per radioactive atom) found for the radiohalide using this method were 21-100 for ^{75}Br, 2-58 for ^{77}Br, 3-6 for ^{123}I and 120-130 for ^{131}I, respectively.

Introduction

In recent years considerable interest has evolved in nuclear medicine for the design and use of receptor-binding radiopharmaceuticals (for a review see 1). In order to be useful for in-vivo application, potential receptor-binding radiopharmaceuticals have to be prepared at a specific activity

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of larger than 1oo-1ooo Ci/mmol (2). These specific activities can theoretically be obtained with many short-lived positron emitting (^{11}C, ^{18}F, ^{75}Br) of γ emitting (^{77}Br, ^{123}I) radionuclides that can be covalently incorporated into organic molecules. However, even though no carrier is added intentionally, all of these radionuclides normally contain various amounts of non-radioactive isotopes of the same element (no carrier added [n.c.a.] instead of carrier-free [c.f.]). The amount of carrier present in n.c.a. preparations of radionuclides or radiopharmaceuticals is usually (except for ^{11}C) less than the detection limit of analytical procedures available in radiopharmaceutical laboratories that can be employed without using up the total sample. Since this institute has great interest in bromine-75 and bromine-75 labelled radiopharmaceuticals (3,4), we have developed a method for the high performance liquid chromatographic (hplc)-UV detection of the specific activity of radiohalide preparations (^{75}Br: $T_{1/2}$ = 1.6 h; ^{77}Br: $T_{1/2}$ ▫ 56 h; ^{123}I: $T_{1/2}$ = 13.3 h; ^{131}I: $T_{1/2}$ = 8.o2 d) that can be used parallel to the actual labelling procedure.

Specific activities of short-lived c.f. radiohalides are very high (> $1o^4$ Ci/mmol), and consequently, mass per mCi is very low. In order to detect these small masses on hplc, substances with large extinction coefficients ($\varepsilon \sim 1o^5$) have to be used. The halide ions themselves have only negligible extinction coefficients. Therefore, derivatives have to be prepared having high extinction coefficients. The highest extinction coefficients are found in the series of polycyclic aromatic compounds.

Furthermore, a high-yield reaction is mandatory for derivatization in order to leave the greater part of the radionuclide for radiopharmaceutical preparation. The labelling of phenols or aromatic amines with radiohalide ion and chloramin T is such a high-yield reaction (6,7,8), even at n.c.a. concentrations of radiohalide.

Combining the two conditions, we chose naphthol-2 as the compound to be labelled, since on halogenation only the 1-halo-naphthol-2 is formed (5). Thus, no other positional isomers have to be expected.

Materials and Methods

Radionuclides: $^{75}Br^-$ and ^{77}Br were prepared at the Jülich compact cyclotron CV 28 by the $^{75}As(^3He,3n)^{75}Br$- or the $^{75}As(^4He,2n)^{77}Br$-reaction, respectively (3). $^{123}I^-$ (Squibb-von Heyden, München, FRG) and $^{131}I^-$ (Amersham-Buchler, Braunschweig, FRG) were obtained commercially.

Chemicals: Naphthol-2 and chloramin T were obtained from Merck, Darmstadt, FRG. Naphthol-2 was purified by hplc before using the chromatographic conditions shown in figure 1. 1-Chloro-naphthol-2, 1-bromo-naphthol and 1-iodo-naphthol-2 were prepared according to literature procedures (5) and characterized by nmr spectroscopy and melting point. All solvents used were analytical grade.

Radiohalogenation: 1oo µg freshly purified naphthol-2 in 1oo µl methanol:water 55:45 (v/v) were added to 1oo µl water containing the radiohalide ($^*X^-$). To this mixture 1o µl of trifluoroacetic acid (neat) and 1o mg of chloramin T in 1o µl water were added. This mixture was allowed to react for 3o min at room temperature. After this time it was analyzed by hplc using the conditions detailed in figure 1.

Chromatography: The hplc-system consisted of a Waters M 6ooo A pump, a 25 x o.4 cm column filled with Lichrosorb RP 18 1o µm, and a Knauer UV/vis-detector with 22o nm filter (fixed wavelength). The eluent, methanol/water 55:45 (v/v), was pumped through the column at a rate of 2.8 ml/min. The recorder was run at 1 cm/min, the width being 25 cm full scale.

Results

Chromatography: The different 1-halo-naphthols-2 formed after bromination or iodination of naphthol-2 (N2) with halide and chloramin T can be completely separated, as shown in figure 1. 1-Chloro-naphthol-2 (Cl-N2) is always formed during radiohalogenation of N2 from chloramin T; since chloramin T and N2 are in excess over the radiohalide, unreacted N2 and Cl-N2 are the main products in this reaction. Under the conditions of radiobromination, however, the baseline separation between Cl-N2 and 1-bromo-naphthol-2 (Br-N2) is not

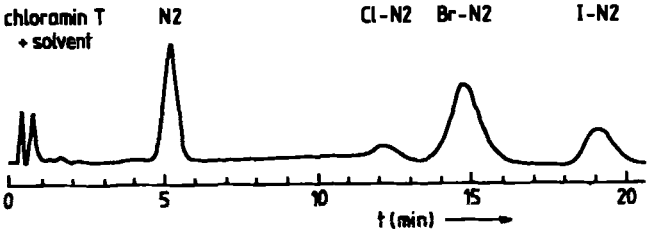

Figure 1: Hplc separation of a standard mixture containing naphthol-2, 1-chloro-naphthol-2, 1-bromo-naphthol-2 and 1-iodo-naphthol-2. Conditions: column: LiChrosorb RP 18, 1o µ, 25 x o.4 cm; solvent: methanol:water 55:45 (v/v); detector: UV 22o nm; flow rate: 2.8 ml/min.

possible on one hplc column due to the large excess of Cl-N2 over Br-N2. Therefore, a second identical column (in series) was used for the final separation of Cl-N2 and Br-N2. The material eluting from the first column between 13 and 17 min was directly led onto the second column; after this time, the second column only was developed giving a baseline separation between residual Cl-N2 and Br-N2. In contrast, due to the efficient separation of 1-iodo-naphthol-2 (I-N2) and Cl-N2, the analysis of radioiodination mixtures was performed on a single column.

Extinction Coefficients

For both Br-N2 and I-N2, UV spectra were recorded in methanol From these, the molar extinction coefficients (ε) were calculated at different wavelengths. Both compounds have their maximum absorption at 23o-232 nm; the ε for Br-N2 is 5965o and that for I-N2 1o7ooo at this wavelength. At the wavelength of the detector used (22o nm) the molar extinction coefficients are 3728o for Br-N2 and 63o25 for I-N2.

Calibration Curve and Detection Limits

With standard solutions of Br-N2 and I-N2, a calibration curve was generated relating mass of the sample (in moles) with normalized peak areas (peak height x peak width x extinction units full scale). Since this curve covers many orders of magnitude, it was plotted using logarithmic axes (figure 2). A least square linear regression analysis of the

data yielded the equation log (mass) = 1.050 log (peak area) - 13.75 with a correlation coefficient r^2 = 0.9852. Since the factor of log (peak area) is very near to one, there is also a linear correlation between mass and peak area, if both variables are plotted linearly.

The detection limit of the hplc-system used was found to be 4.8×10^{-13} mol for Br-N2 and I-N2. (Hplc conditions as detailed in Materials and Methods).

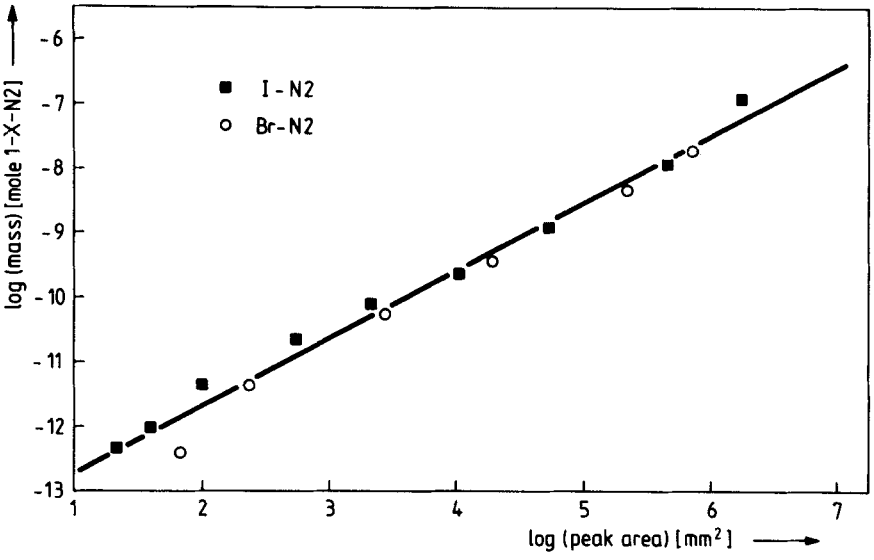

Figure 2: Calibration curve. Mass of 1-halo-naphthol-2 (log scale) is plotted versus peak area detected at 220 nm, normalized for detector response (log scale). For details of hplc conditions see Materials and Methods and Figure 1.

Blank Values

Blank values obtained employing the conditions detailed for radiohalogenation (see Material and Methods) without adding the radiohalide solution consistently showed the absence of a UV peak for Br-N2 and I-N2, respectively, on hplc-analysis. Thus, they are less than the detection limit of the system.

Reproducibility

One sample each of ^{77}Br-bromide and ^{123}I-iodide was split into three aliquots; these aliquots were used for radiohalogenation. Hplc analyses of these aliquots resulted in the following masses: 1.3×10^{-9}, 1.3×10^{-9} and 0.9×10^{-9} mole for ^{77}Br and 4.0×10^{-11}, 4.9×10^{-11} and 2.4×10^{-11} mole for ^{123}I, respectively. Thus, the variation is rather large at $\pm$ 25 - 30 %, but this seems to be tolerable for determination of specific activities of n.c.a. bromide or iodide.

Minimum Amounts of Radioactivity Needed

From the detection limits of the hplc system and the mass of c.f. radionuclides, the minimum amount of radioactive material can be calculated that has to be present in the Br-N2 or I-N2 peaks to allow the detection of a carrier-free preparation. These data are shown in Table 1. Radiochemical yields for the radiohalogenation reaction were usually higher than 50 % and often as high as 80 - 85 %. Furthermore, as can be seen below, we have not yet encountered a c.f. ^{75}Br-bromide preparation. Thus, less than 3 mCi of activity are

Table 1: Amounts of radioactivity needed to detect by hplc carrier-free radiohalide after derivatization of naphthol-2

	^{75}Br	^{77}Br	^{123}I	^{131}I
Detection limit (µCi)	923	26	118	7.5

(Hplc conditions as detailed in Materials and Methods and Figure 1).

needed for ^{75}Br-bromide and less than 1 mCi for the other isotopes investigated for an efficient determination of radiohalide specific activities.

Carrier Content of Radiohalides

A number of ^{77}Br-bromide and ^{75}Br-bromide preparations were used to determine the carrier content of these n.c.a. preparations. For both isotopes used, the mean mass detected was identical at $4x10^{-10}$ mol. Since for both isotopes identical targets (Cu_3As) were irradiated (3), this seems to be the bromide content of these targets. Specific activities, however, were different for both isotopes: the dilution factor at EOB (number of atoms detected via UV/number of atoms detected via radioactivity) varied from 2 to 58 for ^{77}Br-bromide and from 21 to 100 for ^{75}Br-bromide, respectively.

In the same way, dilution factors of 3-6 were found for commercial ^{123}I-iodide at calibration time. For ^{131}I-iodide, also obtained commercially, the dilution factor was higher at 120-130 at calibration time.

Table 2: Specific activities for various radiohalides ions in the carrier-free state and dilution factors found in n.c.a. preparations.

	^{75}Br	^{77}Br	^{123}I	^{131}I
Spec. activity Ci/mmol	$1.91x10^{6}$	$5.6x10^{4}$	$2.35x10^{5}$	$1.6x10^{4}$
dilution factor found (carrier atoms per radioactive atom)	21-100	2-58	3-6	120-130

Discussion

For short-lived cyclotron-produced radionuclides, except ^{11}C, the determination of specific activities of n.c.a. preparations has been very difficult due to the low carrier content. For ^{11}C, due to the large amounts of ubiquitous ^{12}C present in most materials, the specific activity of radiopharmaceuticals labelled with this radionuclide can usually be calculated from the amount of radioactivity prepared and the mass of the carrier peak seen on chromatographic systems used to purify these radiopharmaceuticals. For the halogen-radionuclides used for the metabolic analogue approach (9) or for preparation of receptor-binding radiopharmaceuticals (1), namely ^{75}Br, ^{77}Br and ^{123}I, this method has usually failed. This seems to be due both to the rather low amount of carrier present in these radiohalide preparations and to the insufficient molar extinction coefficients of the radiopharmaceuticals labelled with these radionuclides. Therefore, minimal specific activities were often quoted (for example: 1o) derived from the mass detection limit of the chromatographic system used. Depending on the molar extinction coefficient of the labelled compound in question, this is more or less unsatisfactory, especially if one is dealing with aliphatic or cycloaliphatic compounds.

The method presented here, in contrast, allows the determination of the specific activity of the starting radiohalide preparation by a hplc method using chemical derivatization. We used a high-yield reaction, namely the halogenation using chloramin T and radiohalide (6), on a polycyclic aromatic compound, namely naphthol-2. Thus, 1-halo-naphthol-2 was obtained as the sole product. As can be seen from the data presented in the Results section, as little as $5x1o^{-13}$ mole of Br-N2 or I-N2 can be detected using a fixed-wavelength detector at 22o nm.

Using a detector system operating at the maximum absorption of Br-N2 and I-N2 (230 nm), this limit can be lowered by a factor of 1.6. A linear response between mass and peak area was observed covering 6 orders of magnitude. Due to the low mass detection limit of the chromatographic system, it is possible to observe the mass peak derived from a truely carrier-free radiohalide preparation using as little as 3 mCi of ^{75}Br-bromide and correspondingly less for the other radiohalogen isotopes. Thus, less than 5 % of the starting radioactivity is needed for the method of analysis presented here. This is a particular advantage, since the analysis can be performed parallel to or directly after the preparation of the desired radiopharmaceutical with a negligible portion of the total activity. Furthermore, all the equipment needed for this analysis is normally available in a laboratory engaged in preparation of radiopharmaceuticals from cyclotron-produced radiohalide isotopes. These advantages are in clear contrast to the conditions necessary for possible competing methods of analysis. Gas chromatographic methods using non-destructive detector (thermal conductivity) have detection limits far too high to be useful for determining specific activities of short-lived radiohalide preparations. Destructive detector systems (flame ionization, electron capture or mass spectrometric detectors) use up most, if not all of the available radioactive material. Furthermore, an electron capture detector can only be employed after the radioactivity in the sample has decayed considerably. The same is true, if the determination of carrier content is done by activation analysis. So these methods either use up most of the available sample or can only be performed after several half-lives of the isotope have gone by. A further competing method for determination of specific activities of special compounds, namely brominated estrogen derivatives, has been described (11,12,13). Here, specific activity was determined using the binding to preparations rich in estrogen receptors. Using this method, not only true carrier is assayed, but also pseudo-carrier, i.e. receptor-

binding substances different from the actual radiopharmaceutical that could not be chromatographically separated from it (for example-Cl in a -Br preparation). This binding assay seems to be superior for certain radiopharmaceuticals, but its disadvantage is that different method of analysis have to be used for different radiopharmaceuticals. In contrast, the method described in this publication can be used for every bromine or iodine radiopharmaceutical, since it only needs halide as starting material.

In a strict sense, the method detailed here provides only data on the specific activity of the radiohalide starting material. It can, however, be used to derive the specific activity of the final radiopharmaceutical prepared from this radiohalide. To achieve this, two conditions have to be fulfilled: a) the carrier halide content of reagents used in radiopharmaceutical preparation has to be determined by activation analysis, and b) the chromatographic system used for purification of the radiopharmaceutical must be capable of separating pseudo-carrier compounds. Provided these two conditions are fulfilled specific activity of the final radiopharmaceuticals is clearly defined.

Conclusion:

A highly sensitive assay method for the determination of specific activities of radiobromide and radioiodide preparations was developed using derivatization with chloramin T and naphthol-2. In contrast to competing analysis methods like gas chromatography or activation analysis, the method presented here can be performed with a minimum amount of radioactive material (about 5 % of that needed for radiopharmaceutical production) in a reasonable time (1 hr) parallel to the actual radiopharmaceutical preparation. Due to the low mass detection limit of our method, even truely carrier-free radiobromide or radioiodide specific activities can be determined.

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