

Standard Test for determining Labelling Efficiency in the quality control of *no carrier added* Y-90Cl₃

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Abstract

Particle emitting radionuclides (e.g. β -emitters Y-90 and Lu-177; α -emitter Tb-149, Auger electron emitter Er-165 or positron emitter Y-86) are more frequently used in research and clinical practice for imaging and radionuclide targeted therapy in nuclear medicine. These radiometals, altogether trivalent lanthanides or actinides with high specific radioactivity, coupled to biomolecule carriers (peptides or monoclonal antibodies) through chelating link (e.g. DTPA or DOTA) bind to specific antigens and/or receptors of diseased tissues, which enables the imaging (positron emitters) or destruction (β -, α -, and Auger electron emitter) of the diseased tissue releasing the antigens or carrying the receptors. The radionuclide precursor Y-90 Cl₃ (solution of hard β -emitter Y-90 in diluted HCl) with high purity and specific activity is already commercially produced and successfully used in nuclear medicine, e.g. for radioimmunotherapy (RIT) of Lymphoma. Specification and purity of our product obtained using extraction ⁹⁰Sr/⁹⁰Y generator (using technology of centrifuge extractors with di-2-ethylhexylphosphoric acid, D2EHPA) is examined and compared to other similar products in this paper. A standard method for determination of labelling efficiency of the Y-90 Cl₃ precursor based on its reaction with DOTA-Tyr³-Octreotide (DOTA-TOC) and ITLC-SG chromatographic separation is described and proposed for the quality control

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Introduction

The internal targeted radionuclide therapy for treatment of various diseases in clinical oncology has now reached the age of maturity. In February 2002, Y-90 Ibritumomab Tiuxetan (Zevalin; IDEC Pharmaceuticals Corp., San Diego, CA) monoclonal antibody (mAb) received the final approval by the Food and Drug Administration (FDA) as the first commercially available radiolabeled antibody for cancer treatment. Schering received the European approval for Zevalin on January 22, 2004. Zevalin has been proven to be highly efficient in the treatment of relapsed or refractory non-Hodgkin's lymphoma of B-cells in clinical trials (1). An overall response rate of 80 % and a complete response of 30 % have been observed compared to the 56 % and 16 % response for immunotherapy with Rituximab, unlabelled mAb alone. Since that time, very intensive research on various biomolecules labelled with different therapeutic radionuclides, targeted against the most significant tumor antigens and receptors, has started.

For diagnostics of chosen types of tumors, radioactive labelled octreotide derivatives are often used. Octreotide is a synthetic somatostatin analogue with a longer biological half-life. It is metabolised in the liver. Much like somatostatin, octreotide binds to the somatotropin release inhibiting factor (SRIF) 1-class SSRT. Y-90 DOTA-d-Phe¹-Tyr³-octreotide (Y-90 DOTATOC) has recently been used for treatment of patients with somatostatin receptor positive tumors. A pilot study with 44 patients with advanced somatostatin receptor positive tumors showed that only 29 of the 44 patients could be treated with Y-90 DOTATOC. Taking into account the radiotoxicity of Y-90, a reliable diagnostic tool for examination of the Y-90 DOTATOC affinity to the somatostatin receptor positive tumor is essential to avoid any sort of empirical "trial and error" practice. Patients with a cumulative radiation dose of

7400 MBq/m² showed no severe renal or haematological toxicity, while patients having received a cumulative radiation dose of > 7400 MBq/m² developed renal and/or haematological toxicity(2). Therefore it is essential to develop and optimize a standard method for determining the labelling efficiency for the synthesis and quality control of ^{86/90}Y-DOTATOC derivatives. The biochemical and biophysiological properties of the octreotide derivatives are not influenced by the used yttrium isotope.

Very high demands are imposed on purity of radionuclide precursors used for labelling of biomolecules in nuclear medicine. There are only few β -emitting radionuclides

Product (Company)	RCH Purity ($^{90}\text{YCl}_3$,%)	^{90}Sr impurity (kBq·GBq $^{-1}$ of ^{90}Y)	Main chemical impurities
Ytracis (CIS)	97.0	20	Fe 10 $\mu\text{g}\cdot\text{ml}^{-1}$
Yttrium-90 (Perkin-Elmer)		< 2.5	Fe < 20 $\mu\text{g}\cdot\text{Ci}^{-1}$
Yttriga (QSA Global GmbH)	> 99	< 10	DTPA-binding Test > 80 %
Yttrium-90 (MDS Nordion)			
- Canada		20	(Fe+Zn+Cu+Cd+Pb)
- Belgium	95	2.5	30 $\mu\text{g}\cdot\text{Ci}^{-1}$

Table 1. Commercial products survey of no carrier added $^{90}\text{YCl}_3$ radionuclide precursors

Batch No.	Volume activity (GBq·ml $^{-1}$)	RCH Purity ($^{90}\text{YCl}_3$,%)	^{90}Sr impurity (kBq·GBq $^{-1}$ of ^{90}Y)	Fe impurity ($\mu\text{g}\cdot\text{ml}^{-1}$)
060807-075zk	0.97	98.3	1.81	1.04
060814-076zk	0.64	99.9	1.53	1.23
060821-077zk	1.42	99.9	0.99	1.54
060904-079zk	1.23	99.6	0.98	0.55
060925-080zk	1.58	99.4		40.8
061002-081zk	1.45	99.5		36.3

Table 2. Summary of individual $^{90}\text{YCl}_3$ production batches used for development of SLT

Batch No.	RD (YYYY/MM/DD-hh:mm)	Volume activity (GBq·ml $^{-1}$)	RCH Purity ($^{90}\text{YCl}_3$,%)	Fe impurity ($\mu\text{g}\cdot\text{ml}^{-1}$)
083zk	2006/10/18-13:30	0.5	99.8	2.39
086zk	2006/11/06-16:30	1.08	99.8	1.79
Yttriga	2006/11/07-12:00	2.0	99.9	2.61

Table 3. Main quality parameters of [^{90}Y]yttrium chloride produced in INP ASCR and Yttriga

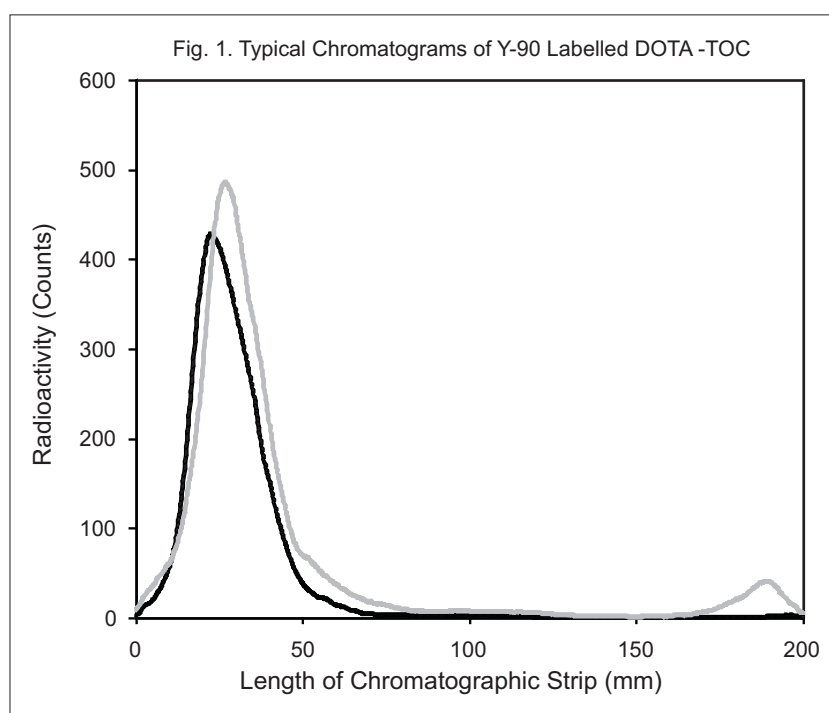
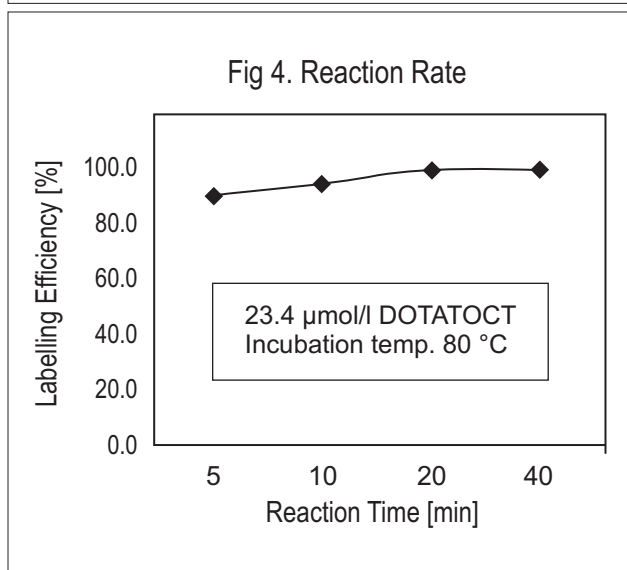
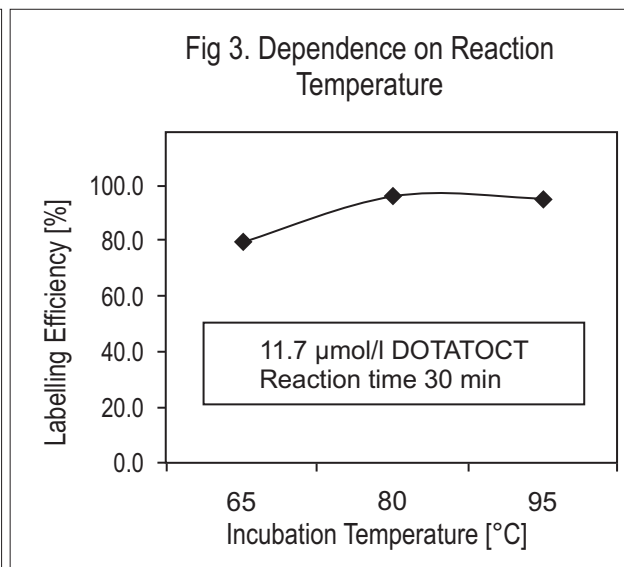
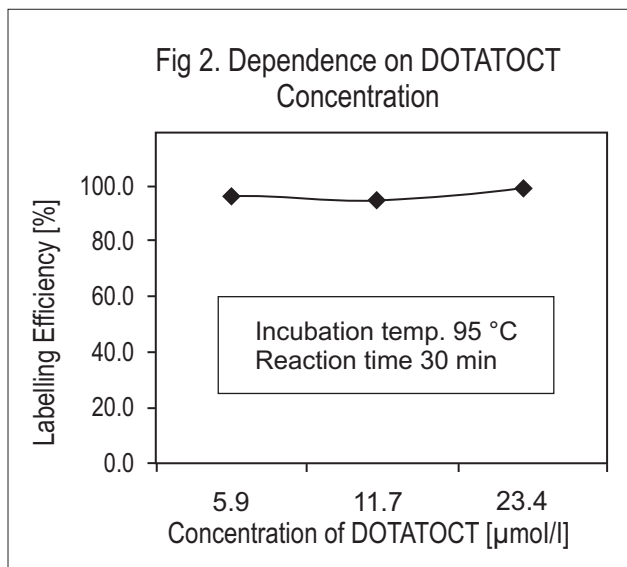


Figure 1. Typical chromatograms of Y-90 DOTA-TOC



Figures 2-4. Conditions affecting the optimal labelling of Y-90 with DOTATOC. These include concentration of DOTATOC, Temperature and Reaction time, etc.

commercially produced in corresponding quality. Among them, no carrier added Y-90 Cl_3 radionuclide precursor in diluted $0.04 - 0.05 \text{ mol}\cdot\text{l}^{-1}$ hydrochloric acid is currently the most important because of the very high Y-90 specific radioactivity (theoretically about $500 \text{ Ci}\cdot\text{mg}^{-1}$) being reached in the production and the Y-90 decay properties (pure β^- -emission; average energy 0.935 MeV , maximum 2.284 MeV ; penetration in tissue $5-9 \text{ mm}$; physical half-life 64.1 h).

Some of the commercially produced and marketed Y-90 radionuclide preparations, including their main properties as stated in producer's specifications, are presented in Table 1. The quality of the latest Y-90 Cl_3 commercial product approved in Europe as radiopharmaceutical precursor (Yttriga, AEA Technology, QSA Global GmbH, Braunschweig, Germany) is estimated using the binding efficiency test with DTPA instead of chemical impurities definition. This test seems to be much more predicative and crucial for quality control of preparations than estimating the admissible limits of individual chemical impurities. Determination of all trace impurities and estimation of their

acceptable concentrations are nearly impossible. Therefore, a Standard Labelling Test (SLT) using DOTATOC, one of the DOTA conjugated peptides frequently used in RIT preclinical and clinical experiments has been developed in our laboratory. The results are presented in this submission.

Materials and Methods

High pure Y-90 Cl_3 radionuclide precursor was prepared in our laboratory using the experimental pilot facility with two-stage Centrifugal Semicounterflow Extractors (3). The separation of Y-90 from the $^{90}\text{Sr}/^{90}\text{Y}$ mixture in $0.5 \text{ mol}\cdot\text{l}^{-1}$ nitric acid was accomplished by extracting it with di-2-ethylhexylphosphoric acid in n-dodecane. The extraction was followed by washing steps (e.g. with n-hexane) and re-extraction of Y-90 into $5 \text{ mol}\cdot\text{l}^{-1}$ hydrochloric acid. Vacuum evaporation was used for the final concentration. Subsequently, the product was dissolved in a small volume of $0.05 \text{ mol}\cdot\text{l}^{-1}$ HCl. The characteristics of individual production batches used in development of Standard

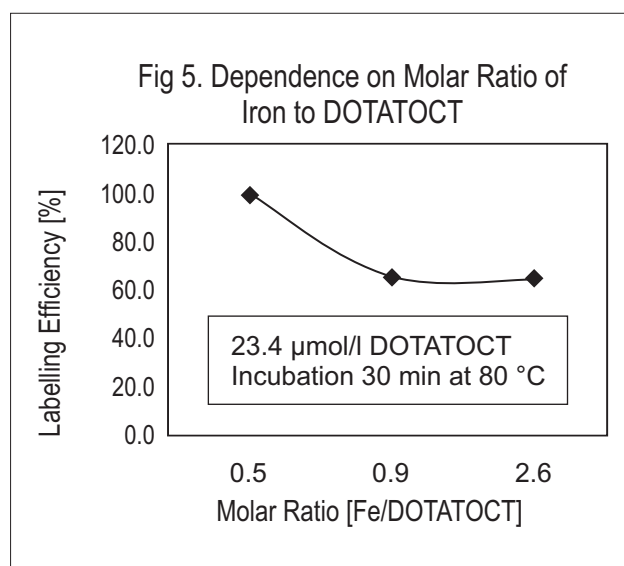


Figure 5. Maximum permissible limit for Fe impurity in the labelling of Y-90 with DOTATOCT. This limit corresponds to stoichiometric saturation of DOTATOCT. Acceptable labelling yield was obtained up to molar ratio [Fe/DOTATOCT] of 0.5.

Labelling Test are summarized in Table 2. The two last batches significantly contaminated by Fe were used to determine the level of Fe resulting in rapid decrease of Y-90 labelling efficiency.

The main quality parameters of our preparation in comparison to the Yttriga product (AEA Technology, QSA Global GmbH, Braunschweig, Germany) are presented in Table 3.

DOTA-Tyr3-Octreotide, DOTA-TOC (MW 1421.64 g·mol⁻¹), was supplied by piCHEM, Austria (white lyophilisate, purity >95 % by HPLC).

Labelling Protocol for analytical use (4): About 2.5–5 MBq of Y-90 Cl₃ in 300 µl of 0.05 mol·l⁻¹ HCl was mixed with 5–20 µg of DOTA-TOC in 300 µl of 0.4 mol·l⁻¹ sodium acetate (NaAc – supra pure) and heated in water bath for 5–40 min at 60–95 °C in order to find the optimal labelling conditions.

Chromatographic method for determination of Y-90 fraction incorporated in DOTA-TOC complex: About 2 µl of the solution after termination of Labelling Protocol was applied on the start (about 10 mm at the margin) of ITLC chromatographic strips (15x200 mm, ITLC-SG, Pall Life Sciences, PALL Corporation). Subsequently, the strips were dipped in 0.1 mol·l⁻¹ sodium citrate (Na₃Cit) up to about 10 mm below the strip margin. The strips were analyzed on Y-90 distribution using β-scanner after being air dried. Each sample was analyzed in 3 parallels.

Instruments: Linear scanning of radiochromatograms for ⁹⁰Y distribution was done by TLC Scanner RAYTEST, Minigita with Proportional Gas Flow (Argon 90 %-Methane 10 %) Beta Counting Tube (Isotopenmeßgeräte GmbH).

Results

Figure 1 demonstrates the typical chromatogram appearance of SLT (Batch No. 060904-079zk) of our production (Table 2). Separation of Y-90 DOTATOC

complex from free yttrium-90 is quantitatively presented in the chromatographic system. Only two components appear in solutions after the labelling procedure described above, thereby making the analytical evaluation of results much easier. More precise determination of optimal labelling conditions (labelling kinetics, incubation temperature, and concentration of DOTATOC) is illustrated in Figures 2-4. Obviously, the optimal incubation temperature (maximum yield) is 80 °C. Higher temperature may cause degradation of the peptide resulting in decreased labelling yields. The optimal reaction time seems to be 25-30 minutes because of slower formation kinetics of DOTA complexes.

Two production batches of Y-90 Cl₃ radionuclide precursor with higher Fe content (080zk and 081zk) (Table 2) were analyzed using this method (SLT) in order to determine maximum permissible limit for Fe impurity. This limit corresponds to stoichiometric saturation of DOTATOCT (Figure 5). Acceptable labelling yield was obtained up to molar ratio [Fe/DOTATOCT] of 0.5.

The SLT developed in our laboratory and described above was used to estimate the labelling efficiency of two Y-90 Cl₃ radionuclide precursor products compiled in Table 3, namely; Yttriga-06/11/07 (QSA Global GmbH) and our product Batch No.086zk-06/11/06. Labelling efficiency (mean ± SD, n=3) for Yttriga was 99.5 ± 0.1 %; while it was 96.1 ± 0.8 % for our product (Batch No.086zk-06/11/06).

Discussion

Very high demands are imposed on purity of radionuclide precursors used for labelling of biomolecules in nuclear medicine. There are only few β-emitting radionuclides which are available commercially for therapeutic purposes. Carrier free Y-90 Cl₃ radionuclide precursor is probably one of the most important in the field of radionuclide therapy, because of the very high Y-90 specific radioactivity being reached in the production, and the physical and radiation properties of Y-90 (pure β-emission; average energy 0.935

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MeV, maximum 2.284 MeV; penetration in tissue 5-9 mm; physical half-life 64.1 h).

A standard method for determination of labelling efficiency of the Y-90 Cl₃ precursor based on its reaction with DOTA-Tyr³-Octreotide (DOTA-TOC) and ITLC-SG chromatographic separation is described and proposed for quality control. It may be noted that bifunctional chelators such as 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA) and diethylenetriaminepentaacetic acid (DTPA)(5) are widely used to bind radiometals, namely trivalent lanthanides and actinides, onto biomolecules. The DOTA bioconjugates demonstrate much higher *in vivo* stability than the DTPA analogues. However, the kinetics of their synthesis (e.g. chelation of Y-90 by DOTA) is significantly slower and requires temperature range up to 100 °C. Nevertheless, they are preferred for use in nuclear medicine, and, thereby, they represent suitable examples for developing standard labelling test (SLT). Such tests of labelling efficiency reflect credibly the labelling power of Y-90 Cl₃ radionuclide precursor in majority of its applications. Very high attainable labelling efficiency despite the presence of chemical impurities (e.g. metal interferents like iron) in low concentrations is an added reason for choosing the Y-90 DOTATOC system for SLT.

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