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Citation

Wang, Ketai, S. James Adelstein, and Amin I. Kassis. 2008. "DMSO Increases Radioiodination Yield of Radiopharmaceuticals." *Applied Radiation and Isotopes* 66 (1): 50–59. <https://doi.org/10.1016/j.apradiso.2007.07.026>.

Published version

<https://doi.org/10.1016/j.apradiso.2007.07.026>

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Published in final edited form as:

Appl Radiat Isot. 2008 January ; 66(1): 50–59.

DMSO Increases Radioiodination Yield of Radiopharmaceuticals

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Abstract

A high-yield radioiodination method for various types of molecules is described. The approach employs DMSO as precursor solvent, a reaction ratio of 2–5 precursor molecules per iodine atom, 5–10 µg oxidant, and a 10–25-µl reaction volume. The solution is vortexed at room temperature for 1–5 min and progress of the reaction is assessed by HPLC. Radioiodinated products are obtained in ≥95% yield and meet the requirements for radiotracer imaging, biodistribution studies, and molecular and cellular biology research.

Keywords

DMSO; radioiodination; no carrier added; high yield

1. Introduction

Radioactive isotopes of iodine possess excellent characteristics for noninvasive imaging (^{123}I , ^{124}I), radiotracer studies (^{125}I), tumor therapy (^{125}I , ^{131}I), and molecular biology research. For many types of molecules, radioiodination presents problems of yield, purification, and stability of the radiolabeled product (Prusoff, 1959; Mannan et al., 1991; Tjuvajev et al., 1993; Van den Abbeele et al., 1996; Kumar et al., 2005). In a standard procedure, radioiodide is oxidized and added to the molecule to be labeled. The iodine cation then reacts with the active position (e.g. phenol, hydroxyl, tributyltin, benzamino or vinyl moiety) on the molecule.

During our studies, in which radioiodination of water-insoluble precursors in aqueous medium often produces low yields, we observed that dissolving these compounds in DMSO improves the efficiency of radioiodination. While the original purpose for the addition of DMSO was the solubilization of water-insoluble compounds, we have since realized that the presence of DMSO dramatically increases the radioiodination yields of various water-soluble as well as water-insoluble organic and non-organic molecules.

2. Materials and methods

2.1. General methods

Reagents were obtained from Sigma Aldrich Chemical Company. HPLC separations were performed on a reversed-phase Zorbax SB C₁₈ column, 9.4 × 250 mm (Agilent Technology) at a flow rate of 3 mL/min with UV absorption (Waters 486 detector) and Y-ray detection

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(gamma-ram, IN/US Systems) used to analyze the eluates. Na¹²⁵I was purchased from GE Healthcare Life Sciences (17.4 Ci/mg [643.8 GBq/mg], 453 mCi/mL [16.76 GBq/mL] in 0.1 N sodium hydroxide, Na¹²³I from MDS Nordion as a no-carrier-added powder, and Na¹³¹I from PerkinElmer Life and Analytical Sciences (16.5 Ci/mg [610.5 GBq/mg], 10 mCi/mL [370 MBq/mL]) in 0.1 N sodium hydroxide.

2.2. Synthesis of radiolabeled (¹²³I/¹²⁵I) 5-iodo-2'-deoxyuridine (¹²³IUdR/¹²⁵IUdR)

A stock solution of tributylstannyl-2'-deoxyuridine (SnUdR, 20 µg/µL DMSO) was prepared. Phosphate buffered saline (PBS, 10 µL, 0.01 M, pH 7.4), SnUdR (1 µL, 1 µg/µL DMSO, methanol, or water), and Na¹²⁵I (0.5–3 µL) were placed in a reaction vial coated with 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril (Iodogen, 10 µg). When dichloromethane was used as precursor solvent, SnUdR (1 µg/20 µL solvent) also adhered to the vial, and the rest of the conditions were the same. The mixture was vortex mixed at ambient temperature for 1 min. Progress of the reaction was checked by C₁₈ HPLC with isocratic elution (3 mL/min) using 75% buffer A (phosphate buffer, 0.05 M, pH 2.5) and 25% buffer B (methanol) for 16 min.

For ¹²³IUdR, dry Na¹²³I (10 mCi) was dissolved in HCl (10 µL, 0.1 M) and sodium thiosulfate (1 µL, 1 µg/µL water) was added. The mixture was shaken for 1 min and NaOH (9 µL, 0.1 M) was then introduced, and the solution was vortex mixed for 10 sec. Radioiodination then proceeded as above.

2.3. Synthesis of radiolabeled (¹²³I/¹²⁵I/¹³¹I) 2-(2'-phosphoryloxyphenyl)-6-iodo-4-(3H)-quinazolinone (¹²³IQ_{2-P}/¹²⁵IQ_{2-P}/¹³¹IQ_{2-P})

A mixture of SnQ_{2-P(I)} and SnQ_{2-P} (0.5 mg) was synthesized (Ho et al., 2002). ESI-HRMS [M + H]⁺ calcd for SnQ_{2-P(I)}, 589.1296, found, 589.1296; calcd for SnQ_{2-P}, 609.1539, found, 609.1539; 31P NMR for SnQ_{2-P(I)}, -16.922 ppm, and for SnQ_{2-P}, -2.958 ppm. The mixture was dissolved in DMSO (100 µL), kept overnight until SnQ_{2-P(I)} was converted to SnQ_{2-P} (at which point 31P NMR for solution -2.958 ppm), and then diluted to a stock solution of SnQ_{2-P} (20 µg/µL DMSO).

Phosphate-buffered saline (10 µL, 0.01 M, pH 7.4) and SnQ_{2-P} (1 µL, 1 µg/µL DMSO, methanol, or water) were placed in a reaction vial coated with Iodogen (10 µg), and Na¹²³I/Na¹²⁵I/Na¹³¹I (2.0 µL) was added. The mixture was vortex mixed at ambient temperature for 3 min and analyzed by C₁₈ HPLC using phosphate buffer (0.05 M, pH 2.5) as buffer A and methanol as buffer B, going from 10% A to 100% B in 7 min and remaining at 100% B for an additional 9 min (3 mL/min).

2.4. Synthesis of iodinated (¹²⁵I/¹²⁷I) rhodamine 123 (¹²⁵I-Rhod/¹²⁷I-Rhod)

2.4.1. Synthesis of mon- and di-iodorhodamine 123—To a reaction vial containing rhodamine 123 (4.5 mg, 11.85 µmol) in ammonium acetate (0.5 mL, 0.2 M), peracetic acid (0.2 mL of 2%, 52.6 µmol) and NaI (2 mg/0.1 mL water, 13.3 µmol) were added. After vortex mixing for 2 h at room temperature, the reaction mixture was evaluated by HPLC and iodorhodamine 123 (I-Rhod) and diiodorhodamine 123 (I₂-Rhod) were identified by mass spectroscopy. ESI-HRMS calcd for C₂₁H₁₅N₂O₃I [M+H]⁺, 471.0212, found, 471.0206; ESI-HRMS calcd for C₂₁H₁₅N₂O₃I₂ [M+H]⁺, 596.9176, found, 596.9172.

2.4.2. Synthesis of radiolabeled (¹²⁵I) rhodamine 123 (¹²⁵I-Rhod)—A stock solution of I-Rhod (3 µg/µL DMSO, methanol, or water) was prepared. Copper chloride solution (2 µL, 50 µg/µL water, pH 4.0), I-Rhod (0.5 µL DMSO, methanol, or water, 3 µg/µL), and water (20 µL) were placed in a reaction vial. *N*-chloro-*p*-toluenesulfonamide sodium salt (chloramine-T [ChT], 1 µL, 10 µg/µL) and Na¹²⁵I (0.5–2 mCi) were added. The mixture was vortex mixed for 5 min and then analyzed by C₁₈ HPLC using phosphate buffer (0.05 M, pH

2.5) as buffer A and methanol as buffer B, going from 10% A to 100% B in 7 min, and remaining at 100% B for an additional 9 min (3 mL/min).

2.5. Synthesis of radiolabeled ($^{123}\text{I}/^{125}\text{I}/^{131}\text{I}$) 2-iodo-8-methyl-8H-quinolo[4,3,2-kl]acridine ($^{123}\text{I}\text{-Acr}/^{125}\text{I}\text{-Acr}/^{131}\text{I}\text{-Acr}$)

8,13-Dimethyl-2-(tributylstannyl)-8H-quinolino[2,3,4-mn]acridin-13-ium iodide (SnAcr) was kindly provided by C. A. Laughton, University of Nottingham, UK. A stock solution of SnAcr (20 $\mu\text{g}/\mu\text{L}$ DMSO) was prepared. Phosphate-buffered saline (8 μL , 0.1 M, pH 5.0) and SnAcr (1 μL , 2 $\mu\text{g}/\mu\text{L}$ DMSO, methanol, or water) were placed in a reaction vial and $\text{Na}^{123}\text{I}/\text{Na}^{125}\text{I}/\text{Na}^{131}\text{I}$ (2.0 μL) and ChT (2 μL , 5 $\mu\text{g}/\mu\text{L}$ water) were added. After vortex mixing at ambient temperature for 5 min, the reaction mixture was analyzed by C_{18} HPLC using phosphate buffer (0.05 M, pH 2.5) as buffer A and methanol as buffer B, going from 10% A to 100% B in 7 min, and remaining at 100% B for an additional 9 min (3 mL/min).

2.6. Synthesis of radiolabeled (^{125}I) Bolton-Hunter reagent ($^{125}\text{I}\text{-BH}$)

Crystalline *N*-succinimidyl 3-(4-hydroxyphenyl)propionate (Bolton-Hunter [BH] reagent) was dissolved in DMSO or methanol (1 $\mu\text{g}/\mu\text{L}$). To a reaction vial coated with Iodogen (5 μg), BH reagent in DMSO or methanol (1 μL) and PBS (10 μL , 0.01 M, pH 7.4) were added, followed by Na^{125}I (1 μL , 1 mCi/2.5 μL). When dichloromethane was used as precursor solvent, BH reagent (10 μL , 0.1 $\mu\text{g}/\mu\text{L}$ solvent) also adhered to the vial, and the rest of the conditions were the same. The mixture was vortex mixed at ambient temperature for 1 min and was analyzed by HPLC using phosphate buffer (0.05 M, pH 2.5) as buffer A and methanol as buffer B, going from 10% A to 100% B in 7 min, and remaining at 100% B for an additional 9 min (3 mL/min).

2.7. Radioiodination (^{125}I) of IgG ($^{125}\text{I}\text{-IgG}$)

IgG (20 $\mu\text{g}/2 \mu\text{L}$ DMSO or 20 $\mu\text{g}/4 \mu\text{L}$ water) was placed in a vial coated with Iodogen (20 μg), and sufficient PBS (0.01 M, pH 7.4) to make the reaction volume 24 μL and Na^{125}I (1 μL , 1 mCi/2.5 μL) were added. The mixture was vortex mixed at ambient temperature for 1 min, then transferred to another vial lacking Iodogen. The product was identified by HPLC (Bio-Sil column, 300 mm $\times$ 7.8 mm) with isocratic elution by phosphate buffer (0.05 M, pH 6.8) at 1 mL/min.

2.8. Radioiodination (^{125}I) of B72.3 monoclonal antibody ($^{125}\text{I}\text{-B72.3}$)

A stock solution of B72.3 (1.5 $\mu\text{g}/\mu\text{L}$ PBS) was prepared, and 11 μL of this solution was introduced into a vial coated with Iodogen (20 μg) and containing PBS (1 μL , 0.1 M, pH 7.4) and DMSO or water (2 μL). Na^{125}I (0.5 μL , 1 mCi/2.5 μL) was added. The mixture was vortex mixed at ambient temperature for 1 min, then transferred to another vial lacking Iodogen. The product was identified by HPLC (Bio-Sil column, 300 mm $\times$ 7.8 mm) with isocratic elution by phosphate buffer (0.05 M, pH 6.8) at 1 mL/min.

3. Results and discussion

3.1. Synthesis of radiolabeled ($^{123}\text{I}/^{125}\text{I}$) 5-iodo-2'-deoxyuridine ($^{123}\text{I}\text{UdR}/^{125}\text{I}\text{UdR}$)

Radioiodinated 5-iodo-2'-deoxyuridine, an effective agent in targeting radioactivity to nuclear DNA, has been used in exploring radiation effects in mammalian cells (Kassis et al., 1987) and in noninvasive scintigraphic detection and radiotherapy of tumors (Mariani et al., 1996; Kassis et al., 2004; Kassis, 2005). In the synthesis originally described by Prusoff et al. (Prusoff, 1959), in addition to IUdR, several radiolabeled byproducts are generated, and the overall yield of IUdR is approximately 50%. Specific activity is usually lowered by unidentified UV-absorbing impurities. Our group (Baranowska-Kortylewicz et al., 1994; Foulon et al., 1996)

developed a radiolabeling kit/generator for 5-radiohalogenated uridines; the reaction conditions include sodium iodide either in chloroform as solvent or in a heterogeneous mixture and test tubes coated with the precursor 5-trimethylstannyl-2'-deoxyuridine. The use of 25% hydrogen peroxide in glacial acetic acid (v/v) as oxidant simplifies the purification of the final product (radioIUdR). This method produces a 95% yield of $^{125}\text{IUdR}$.

In our rapid DMSO synthesis, the surface of the reaction vial is coated with the oxidant Iodogen; the precursor, 5-tributylstannyl-2'-deoxyuridine is dissolved in DMSO ($1\text{ }\mu\text{g}/\mu\text{L}$); the reaction medium is 0.01 M PBS; and the total reaction volume is maintained within $12\text{ }\mu\text{L}$ to keep concentrations high. Figure 1 shows the HPLC profiles of the reaction and Table 1 presents the reaction conditions and radioiodination yields. For $^{125}\text{IUdR}$, the retention time (t_R) of 8:24 min (gamma counter) (Fig. 1A) is consistent with that of 8:16 min (UV detector) for standard nonradiolabeled IUdR (Fig. 1D); the difference of 8 sec represents the transition time between the two detector cells. The radioiodination yield is greater than 99.8% (Fig. 1A). In this reaction, the amount of precursor should be twice that of radioisotope, $^{123}\text{I}/^{125}\text{I}$ (as NaI). For example, $1\text{ }\mu\text{g}$ SnUdR ($1.9 \times 10^{-9}\text{ mol}$) and Na^{125}I (74 MBq, 2 mCi, $0.9 \times 10^{-9}\text{ mol}$) give 99.8% $^{125}\text{IUdR}$. Only $0.5\text{ }\mu\text{g}$ SnUdR precursor ($0.95 \times 10^{-9}\text{ mol}$) and $0.3\text{ }\mu\text{g}$ tributyltin ($0.95 \times 10^{-9}\text{ mol}$) are left as impurities in the system, and the Iodogen ($10\text{ }\mu\text{g}$) remains attached to the surface of the vial. The whole process is simple and clean with no purification necessary to remove other radioiodinated products/species. Using this method, $^{125}\text{IUdR}$ has been prepared successfully for molecular biology, cell biology, genetic and imaging research. $^{125}\text{IUdR}$ has also been synthesized in the presence of methanol (yield 90.5% – Fig. 1B), dichloromethane (yield 82.1% – data not shown) and water (yield 18.9% – Fig. 1C), all other conditions remaining the same (Fig. 1B and C). These lower yields may be a consequence of the incomplete solubility of the tin precursor during the synthesis.

3.2. Synthesis of radiolabeled ($^{123}\text{I}/^{125}\text{I}/^{131}\text{I}$) ammonium 2-(2'-phosphoryloxyphenyl)-6-iodo-4-(3H)-quinazolinone ($^{123}\text{IQ2-P}/^{125}\text{IQ2-P}/^{131}\text{IQ2-P}$)

The radioiodinated quinazolinone derivative, ammonium 2-(2'-phosphoryloxyphenyl)-6-iodo-4-(3H)-quinazolinone has been developed as a new antitumor radiopharmaceutical (Ho et al., 2002; Chen et al., 2006; Chen et al., 2007; Pospisil et al., 2007; Wang et al., 2007). This is a water-soluble compound that is hydrolyzed by various phosphatases (overexpressed extracellularly by tumor cells) to a water-insoluble compound, 2-(2'-hydroxyphenyl)-6-iodo-4-(3H)-quinazolinone, which is trapped within the extracellular spaces of solid tumors.

The precursor $\text{SnQ}_{2-\text{P}}$ is dissolved in DMSO ($20\text{ }\mu\text{g}/\mu\text{L}$) and diluted to $2\text{ }\mu\text{g}/\mu\text{L}$ in DMSO, methanol, or water. When DMSO is the precursor solvent, the ^{125}I -labeled product $\text{IQ}_{2-\text{P}}$ ($t_R = 9.23\text{ min}$) is obtained in 99.3% yield (Fig. 2A). Similar high yields are obtained when the quinazolinone precursor is radioiodinated with ^{123}I or ^{131}I in the presence of DMSO (Table 1). With methanol as solvent, the labeling yield is only 11.6% (Fig. 2B) and, with water as solvent, the yield is 18.8% (Fig. 2C). Unlike SnUdR, the $\text{SnQ}_{2-\text{P}}$ derivative is soluble in methanol–water and appears to be a true solution; the explanation for the consequent low labeling yield is still under investigation. A turbid suspension is obtained when $\text{SnQ}_{2-\text{P}}$ is in water, and the low yield is, therefore, most likely due to the unequal distribution of the reactants.

3.3. Synthesis of radiolabeled (^{125}I) rhodamine 123 (^{125}I -Rhod)

Rhodamine 123, a lipophilic, permeant cationic fluorescent dye, has been used extensively as a mitochondrial stain in the study of cellular function. It is relatively nontoxic to healthy cells but displays selective toxicity in certain carcinoma cells in vitro and in vivo. These observations have prompted its radioiodination and evaluation of its tumor-targeting potential in vivo.

Moonen et al. (Moonen et al., 1987) first radioiodinated rhodamine 123 with Iodogen for biodistribution studies, and the overall radiochemical yield of the reaction was 20%. Kinsey et al. (Kinsey et al., 1987) found that electrophilic radioiodination of rhodamine 123 using Iodogen or ChT leads to formation of the iodide salt in which the radioiodine is not covalently bound to the xanthene ring of the dye, since electrophilic iodination is greatly inhibited by the presence of the delocalized positive charge on the ring. Harapanhalli et al. (Harapanhalli et al., 1998) selected peracetic acid as the oxidizing agent and optimized the conditions for synthesis, including buffer, pH, time, and temperature. This group reported radiolabeling yields of ^{125}I -rhodamine 123 between 40% and 45%.

Having analyzed the possible reasons for low radiolabeling yields of rhodamine 123, we have used Cu^{2+} to protect the amine groups and to inhibit salt formation and ChT as oxidant. When the precursor I-Rhod is dissolved in DMSO, ^{125}I -Rhod ($t_{\text{R}} = 11.0$ min) is obtained in 97.3% yield (Fig. 3A). Since the rhodamine molecule has two aryl rings, iodine labeling is favored at either or both positions that is/are ortho to the amine. During radioiodination, however, the concentration of precursor is much greater than that of radioisotope. Consequently, the probability for di-iodination, i.e. I_2 -Rho, is very low and the monoiodo-derivative is produced (Fig. 3A). Here again, when the solvents for the precursor are methanol and water, the labeling yields are only 29.9% (Fig. 3B) and 27.1% (Fig. 3C), respectively. Since methanol is a good solvent for I-Rhod, the cause of low yield with this solvent is unknown. Water is a poor solvent for I-Rhod, and this may have contributed to the low labeling yield.

3.4. Synthesis of radioiodinated ($^{123}\text{I}/^{125}\text{I}/^{131}\text{I}$) 2-iodo-8-methyl-8H-quino[4,3,2-kl]acridine (^{123}I -Acr/ ^{125}I -Acr/ ^{131}I -Acr)

Activation of the enzyme telomerase (hTERT), a reverse transcriptase responsible for annealing hexanucleotide telomeric repeats (TTAGGG) to the ends (telomeres) of chromosomes, has been shown to be an important step in human epithelial-tumor evolution. There is a burgeoning interest in the use of polycyclic acridines to stabilize the G-rich single-stranded telomeric overhang in G-quadruplex DNA polymorphic forms; these agents have also been shown to inhibit telomerase at the micromolar level (Heald et al., 2002). Acridine, therefore, is a potential antitumor agent, and its radioiodination provides a useful tool for the study of its biologic function and radiation efficiency in vitro and in vivo.

When iodination of SnAcr is carried out in the presence of DMSO, ^{125}I -Acr ($t_{\text{R}} = 8.46$ min) is obtained in 98.0% yield (Fig. 4A). Similar high yields (95.6% and 96.5%) are obtained when SnAcr is radiolabeled respectively with ^{131}I and ^{123}I (Table 1). Under similar conditions with methanol as solvent, the yield is 79.7% (Fig. 4B), and with water, the yield is only 40.1% (Fig. 4C).

3.5. Synthesis of radiolabeled (^{125}I) Bolton-Hunter reagent (^{125}I -BH)

Bolton and Hunter (Bolton, Hunter, 1973) developed a method of indirectly labeling proteins by conjugating them to a ^{125}I -containing acylating agent. This approach avoids contact between the protein and the oxidizing agent and preserves the biologic activity of the former. The method includes two steps: the first is radioactive labeling of BH and the second is the conjugation of BH and protein. The yield reported for the first step, however, is only 30% to 75% and a final absolute labeling yield of 13% to 53%.

When the BH reagent is dissolved in DMSO and the precursor solution is added to the radioiodination system, HPLC shows a t_{R} peak of 9.23 min and the yield is 97.3% (Fig. 5A). DMSO is, therefore, an excellent solvent for radioiodination of the reagent. With methanol as solvent, the yield is 66.3% (Fig. 5B). This low yield is not, however, a consequence of the presence of free iodine since the t_{R} of free iodine under these elution conditions is 3.22 min.

The radioiodination yield in the presence of dichloromethane is somewhat higher (86.3%) but not as high as that obtained in the presence of DMSO (Table 1).

3.6. Radioiodination (^{125}I) of immunoglobulin G (IgG)

Radioiodinated IgG has many uses in preclinical and clinical research. Various radiolabeling yields have been reported and this is usually ascribed to the radiolabeling conditions and/or the physical and chemical characteristics of the antibody molecule. In our current studies, we decided to determine whether the radioiodination yield of these proteins can also be improved by the presence of DMSO.

When mouse IgG is radioiodinated in the presence and absence of a small amount of DMSO (Fig. 6A and B), the radioiodination yield increases from 49.5% (no DMSO) to 99.7% (with DMSO). However, when we use the anti-mucin monoclonal antibody B72.3, known to target colon-adenocarcinoma cells grown subcutaneously in mice (Kassis et al., 1996), the radiolabeling yields with and without DMSO are both over 99% (Fig. 7A and B). The presence of DMSO during the radioiodination does not compromise the *in vitro* immunoreactivity of this antibody (data not shown).

4. Conclusion

Various types of precursors, including those with hydroxyl, tributylbenzene, and aminobenzene groups, were radioiodinated using DMSO, methanol, and water as alternative solvents. The data demonstrate that the addition of DMSO consistently led to quantitative radioiodination yields (95%–100%).

Acknowledgements

This work is supported by NIH Grant 5 R01 CA15523 (to A. I. K.)

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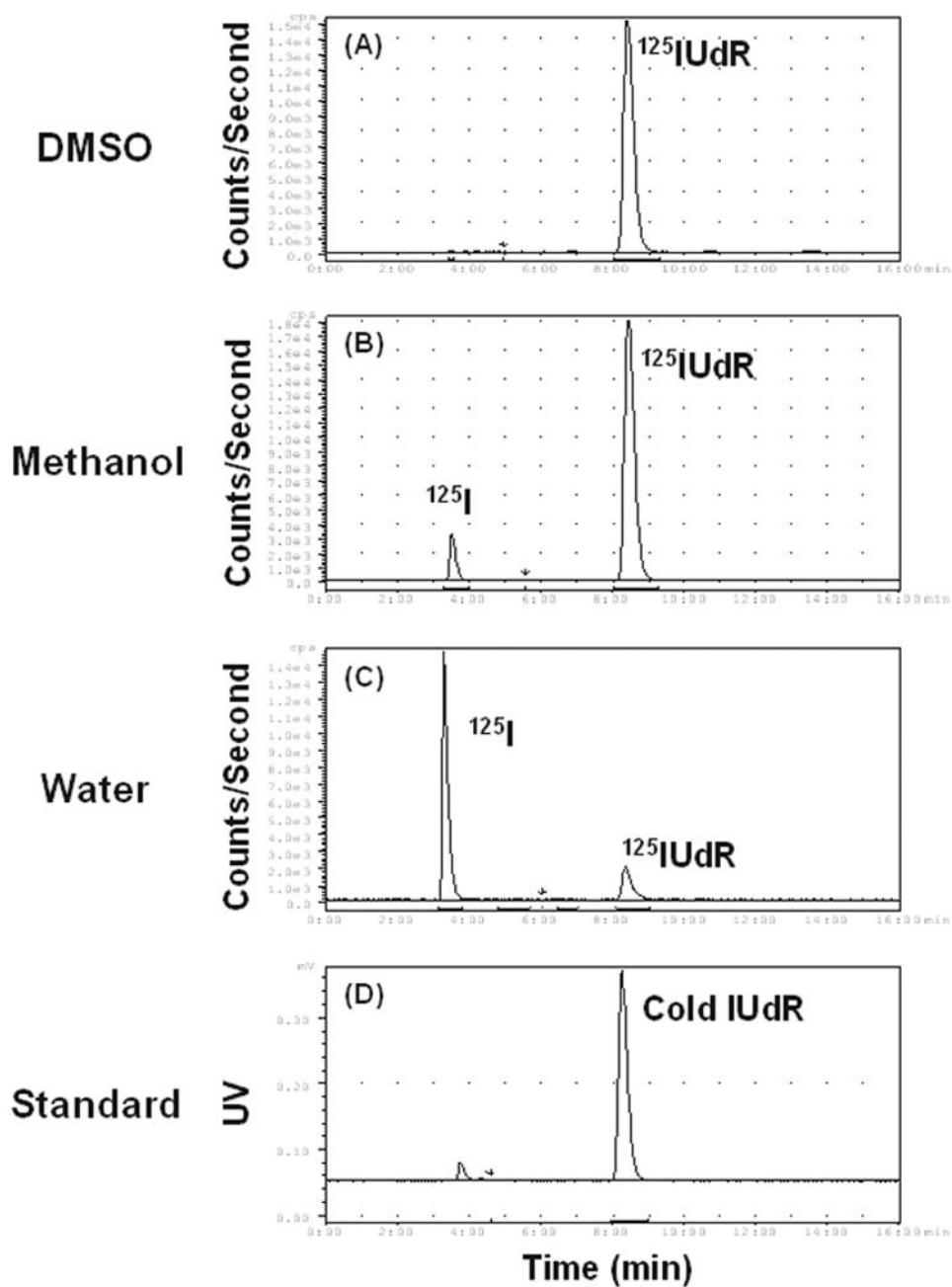


Fig. 1. HPLC of ^{125}I UdR synthesized with 1 μL precursor (1 μg SnUdR/ μL solvent): (A) DMSO, yield 99.8%; (B) methanol, yield 90.5%; (C) water, yield 82.1%; (D) standard.

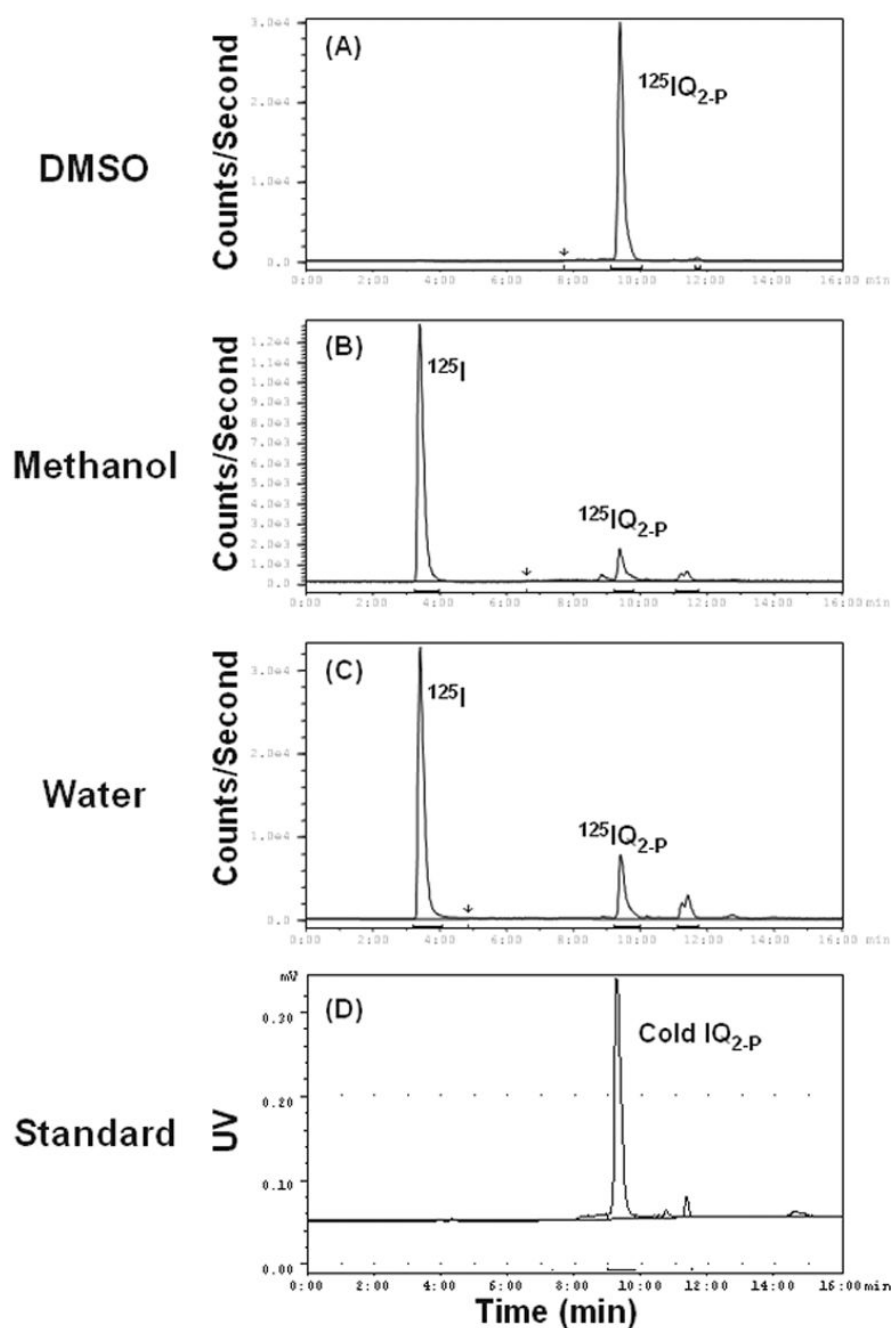


Fig. 2. HPLC of ^{125}I -Q₂-p synthesized with 1 μL precursor (1 μg SnQ₂-p/ μL solvent): (A) DMSO, yield 99.3%; (B) methanol, yield 11.6%; (C) water, yield 8.8%; (D) standard.

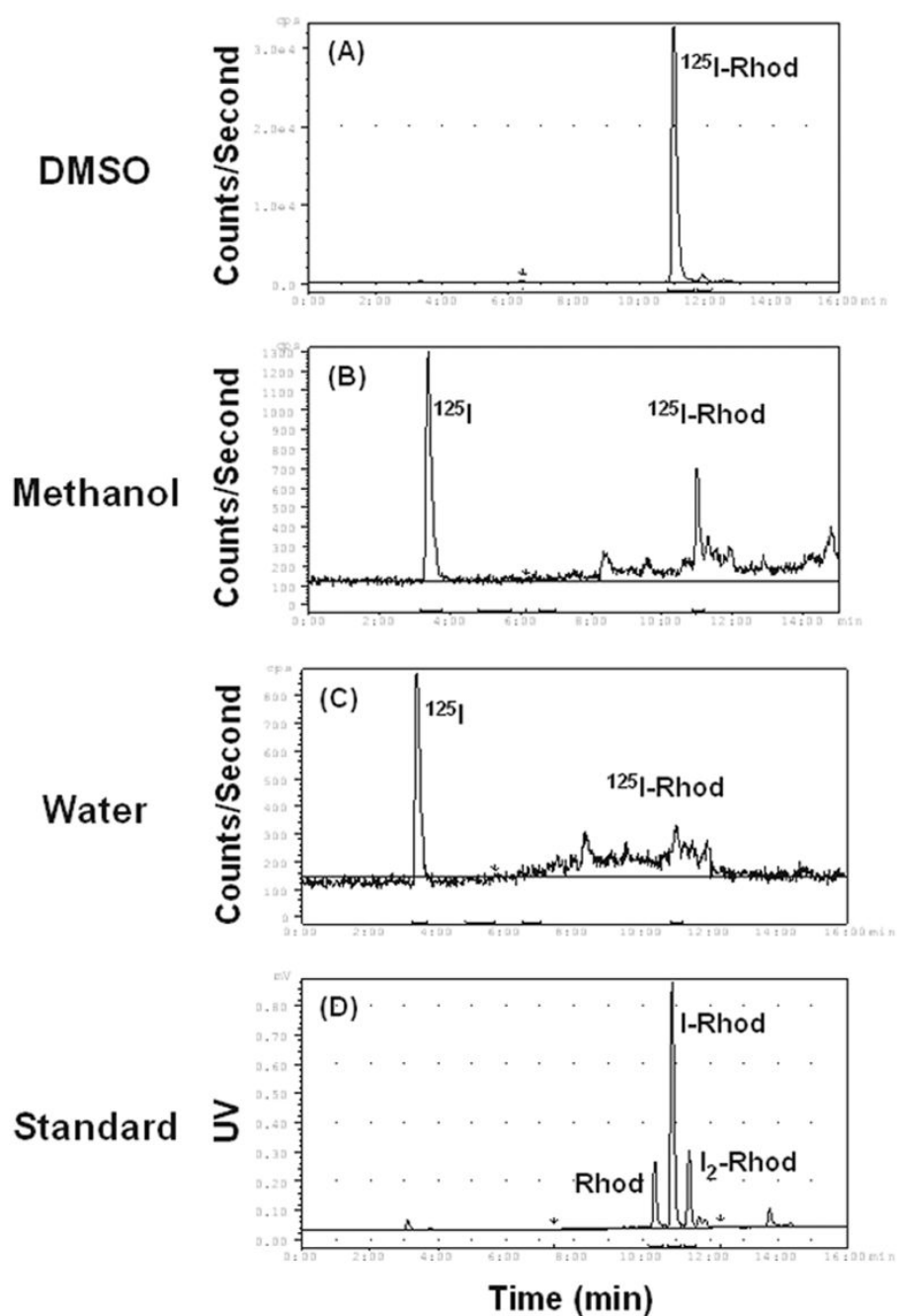


Fig. 3. HPLC of ^{125}I -Rhod synthesized with 0.5 μL precursor (3 μg I-Rhod/ μL solvent): (A) DMSO, yield 97.6%; (B) methanol, yield 29.9%; (C) water, yield 27.1%; (D) standard.

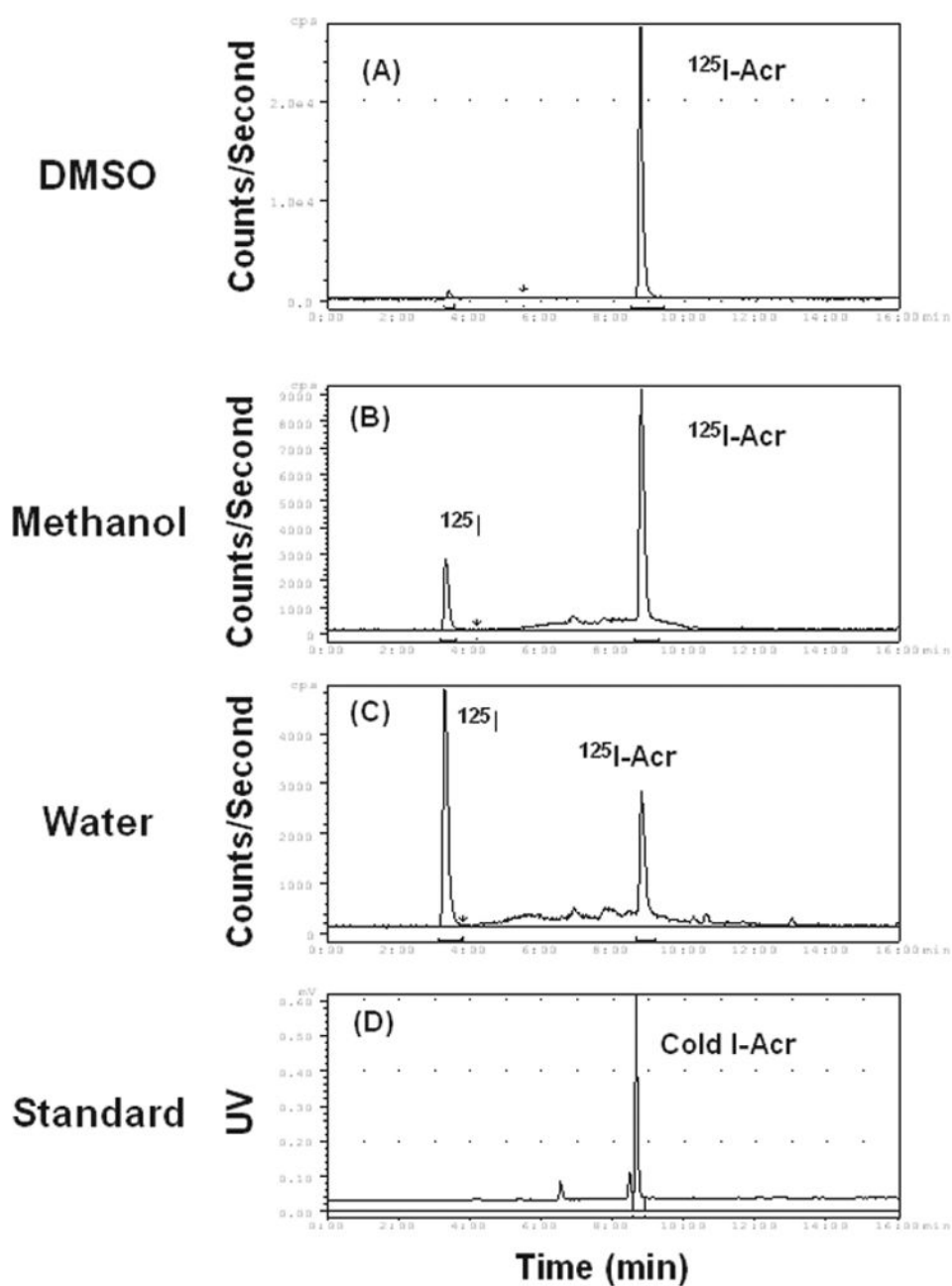


Fig. 4. HPLC of $^{125}\text{I-Acr}$ synthesized with 1 μL precursor (2 μg SnAcr/ μL solvent): (A) DMSO, yield 98.0%; (B) methanol, yield 79.9%; (C) water, yield 40.1%; (D) standard.

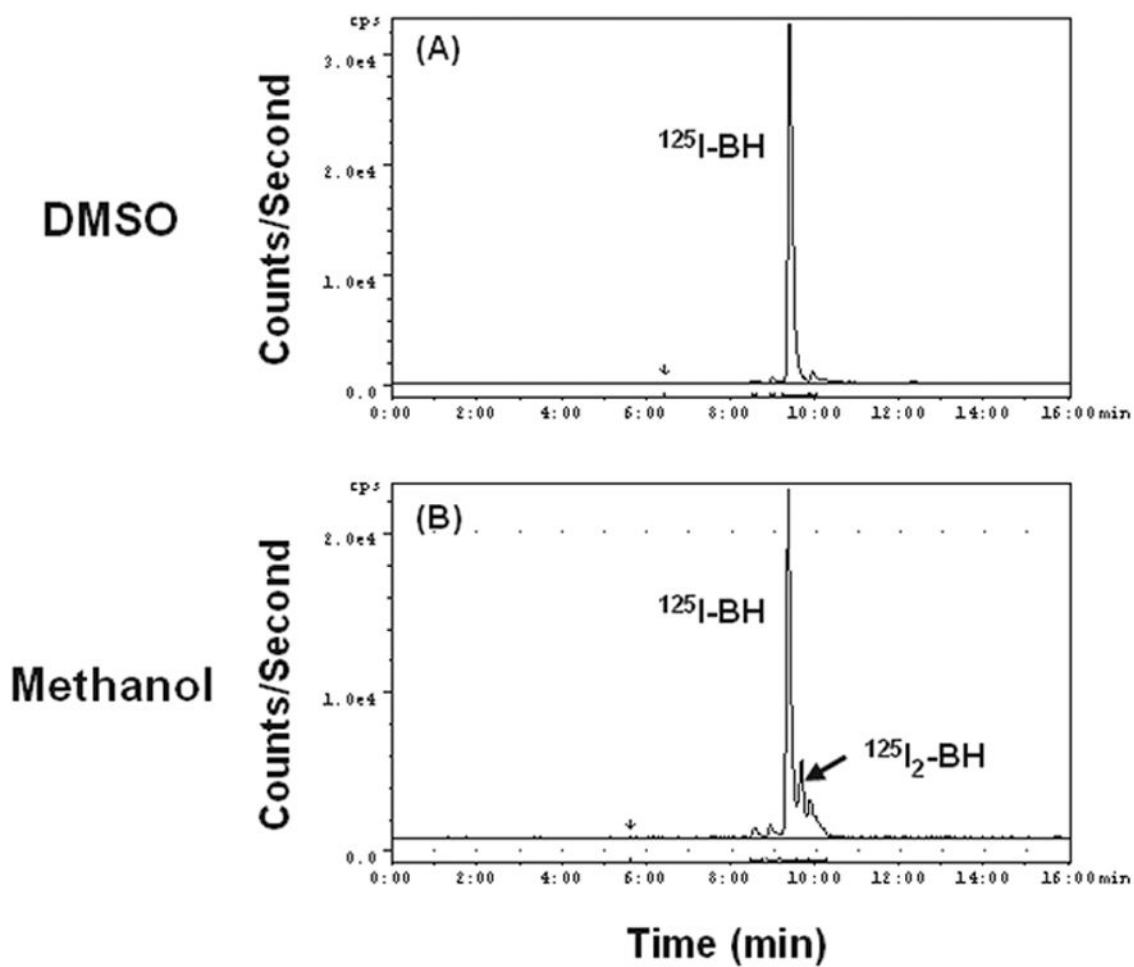


Fig. 5.

HPLC of ^{125}I -BH synthesized with 1 μL precursor solution (1 μg BH/ μL): (A) DMSO, yield 97.3%; (B) methanol, yield 66.3%.

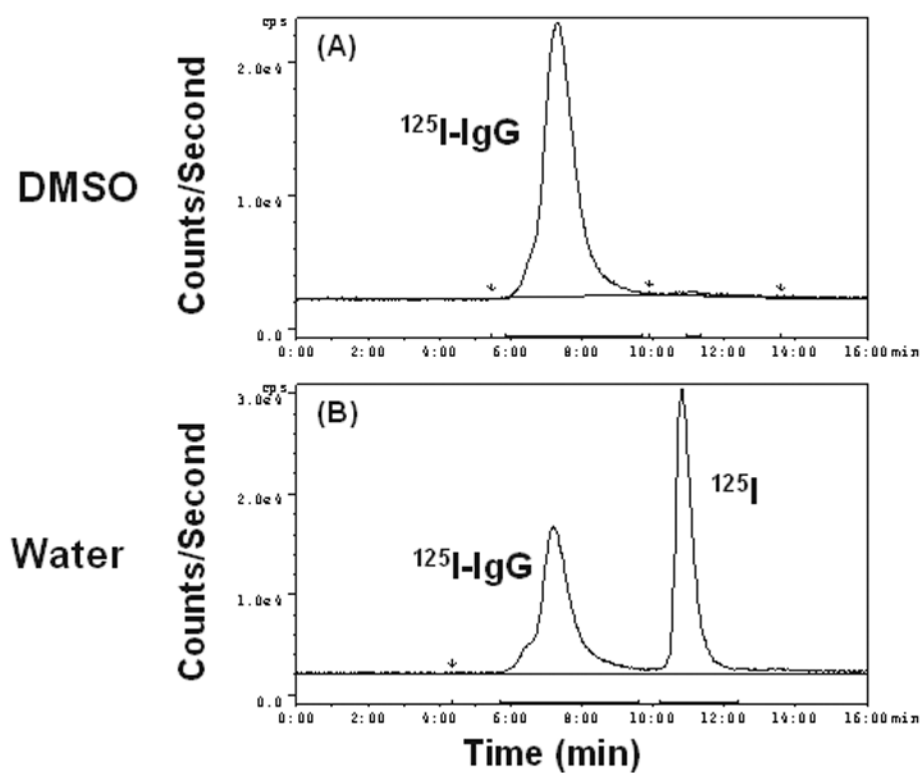


Fig. 6. HPLC of ^{125}I -IgG synthesized with 2 μL DMSO solution (20 μg IgG) or 4 μL water solution (20 μg IgG): (A) DMSO, yield 99.7%; (B) water, yield 49.5%.

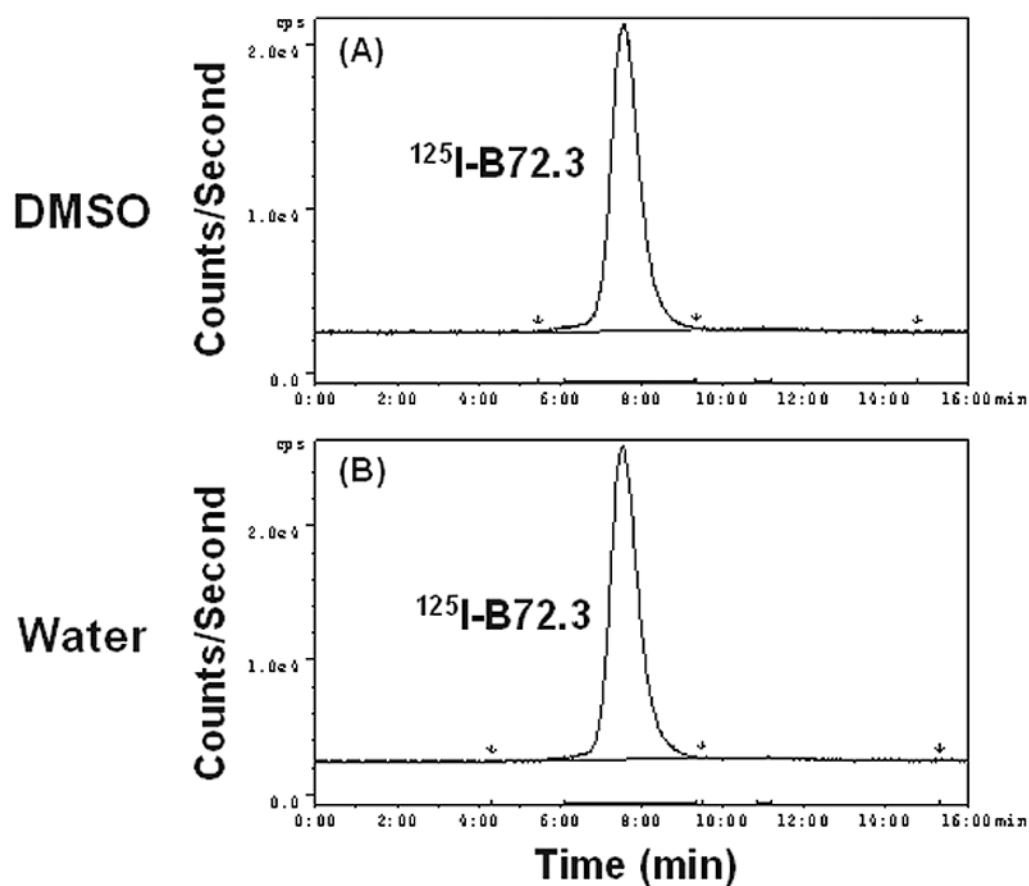


Fig. 7.
HPLC of ^{125}I -labeled B72.3 monoclonal antibody synthesized with 22 μL precursor solution (16.5 μg B72.3/2 μL solvent): (A) DMSO, yield 99.9%; (B) water, yield 99.9%.

Table 1

Radioiodination of various types of precursor

Compound	Precursor solvent	Reaction volume (μl)	pH/buffer	Oxidant	Vortex (min)	Yield (%)	Replicate number
¹²⁵ I-UdR	DMSO	12	7.4/PBS	Iodogen	3	99.8-0.2	35
	methanol	12	7.4/PBS	Iodogen	3	90.45-1.2	3
	dichloromethane	11	7.4/PBS	Iodogen	1	82.1-3.2	3
	water	12	7.4/PBS	Iodogen	3	18.9-6.5	3
¹²³ I-UdR	DMSO	12	7.4/PBS	Iodogen	3	100-0.1	8
¹²⁵ I-Q ₂ -P	DMSO	12	7.4/PBS	Iodogen	3	99.3-0.5	46
	methanol	12	7.4/PBS	Iodogen	3	116-4.8	3
	water	12	7.4/PBS	Iodogen	3	18.8-5.3	3
¹²⁵ I-Q ₂ -P	DMSO	12	7.4/PBS	Iodogen	3	100-0.1	5
¹²⁵ I-Q ₂ -P	DMSO	12	7.4/PBS	Iodogen	3	99.5-0.5	3
¹²⁵ I-Rhod	DMSO	25	4.0/CuCl ₂	ChT	5	97.6-1.5	5
	methanol	25	4.0/CuCl ₂	ChT	5	29.9-4.5	3
	water	25	4.0/CuCl ₂	ChT	5	27.1-5.2	4
¹²⁵ I-Acr	DMSO	13	7.4/PBS	ChT	5	98.0-1.2	6
	methanol	13	7.4/PBS	ChT	5	79.7-3.5	3
	water	13	7.4/PBS	ChT	5	40.1-3.7	3
¹²³ I-Acr	DMSO	13	7.4/PBS	ChT	5	95.6-1.3	4
¹³¹ I-Acr	DMSO	13	7.4/PBS	ChT	5	98.5-1.3	3
¹²⁵ I-BH	DMSO	12	7.4/PBS	ChT	5	97.3-1.6	5
	methanol	12	7.4/PBS	Iodogen	1	66.3-3.6	3
	dichloromethane	11	7.4/PBS	Iodogen	1	86.3-2.5	3
¹²⁵ I-IgG	DMSO	25	7.4/PBS	Iodogen	1	99.7-0.3	8
	water	25	7.4/PBS	Iodogen	1	49.5-2.6	3
¹²⁵ I-B72.3	DMSO	15	7.4/PBS	Iodogen	1	99.9-0.1	3
	water	15	7.4/PBS	Iodogen	1	99.9-0.1	6