# TRITIUM LABELED PROSTACYCLIN SODIUM SALT

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## SUMMARY

Crystalline [11β-$^3$H]prostacyclin ($PGI_2$) sodium salt (*5*) was prepared from prostaglandin $D_2$ ($PGD_2$) methyl ester (*1*). Reduction of $PGD_2$ methyl ester with sodium borotritide afforded [11β-$^3$H]prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) methyl ester (*2a*) in 57% radiochemical yield. [11β-$^3$H]$PGF_{2\alpha}$ methyl ester was converted *via* (5R, 6R)- and (5S, 6S)-5-iodo-[11β-$^3$H]prostaglandin $I_1$ methyl ester (*3*) to [11β-$^3$H]-prostacyclin methyl ester (*4*). Carefully controlled hydrolysis of *4* produced crystalline [11β-$^3$H]$PGI_2$ sodium salt which was shown to be >95% pure by means of high performance liquid chromatographic (HPLC) analysis. The overall yield from [11β-$^3$H]$PGF_{2\alpha}$ methyl ester to [11β-$^3$H]$PGI_2$ sodium salt was 53%. [11β-$^3$H]$PGI_2$ sodium salt with a specific activity of 502 μCi/mg has only limited radiochemical stability. Its radiochemical purity declined 15% after three weeks of storage at -20°C in the crystalline state.

Key Words: Tritium, Prostacyclin Sodium Salt, Preparation, Purity, Stability

## INTRODUCTION

Prostacyclin (1,2) is a potent natural inhibitor of blood platelet aggregation (3). The substance is under development for clinical application as its sodium salt ($PGI_2Na$). Because of the submilligram dosage presaged by the high potency, high specific activity tritium labeled $PGI_2Na$ (*5*) is required to facilitate detection and quantitation of the intact drug and drug-related materials during *in vivo* metabolism studies with prostacyclin in test animals and man. Prostacyclin sodium salt and most of the synthetic intermediates leading to it are of limited stability, even in the crystalline state. $PGI_2Na$ is particularly unstable (4) in protic solvents and undergoes very facile hydrolysis at <pH 9.3

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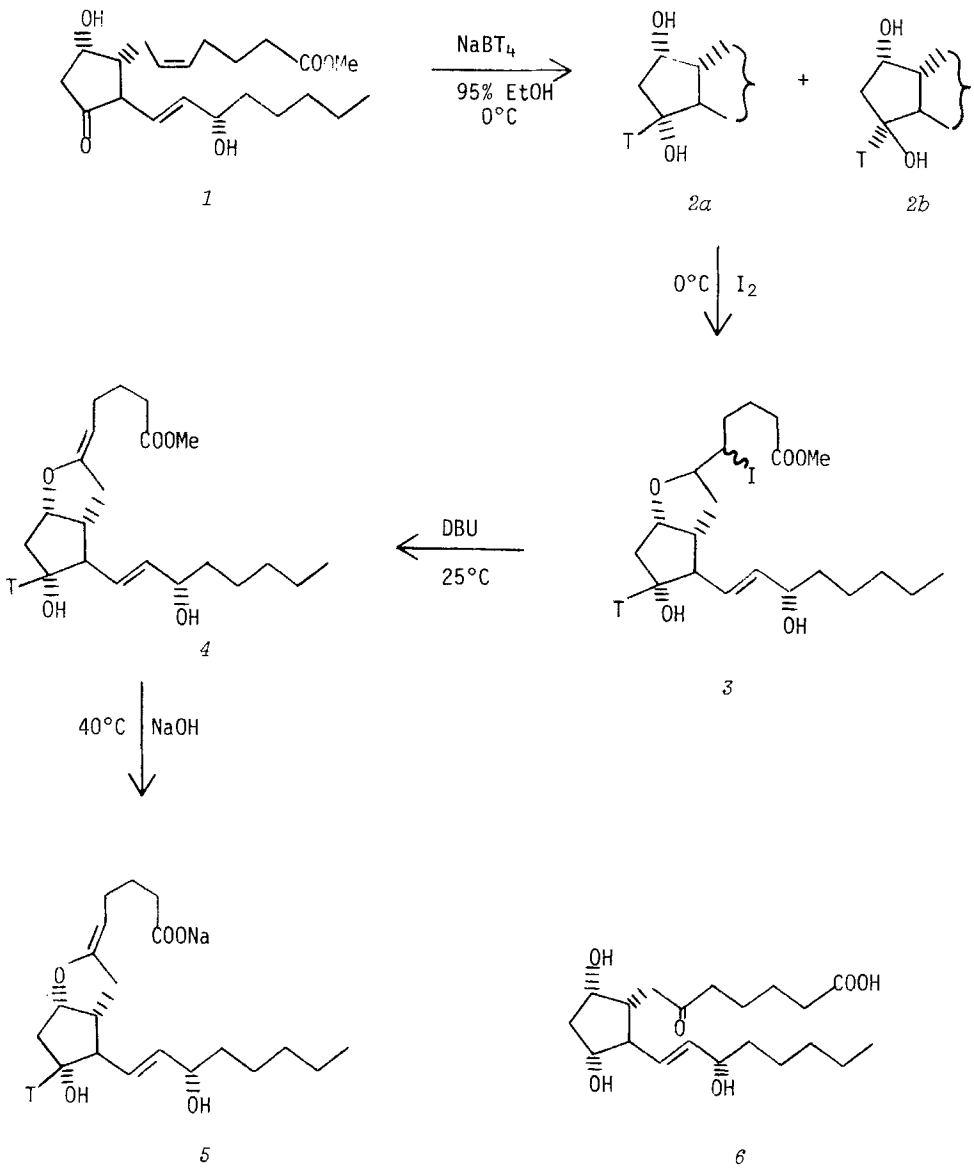
Preparation of [11β-$^3$H]$PGI_2Na$ from $PGD_2$ Methyl Ester



to produce 6-oxo-prostaglandin $F_{1\alpha}$ (*6*) (5). High specific activity tritium labeled $PGI_2Na$ should be stored at high dilution in a suitable solvent to suppress autoradiolytic decomposition. Instability of $PGI_2Na$ in protic solvents precludes the use of such solvents, which are most compatible with the ionic nature of $PGI_2Na$. Polar organic solvents which would solubilize $PGI_2Na$, on the other hand, are incompatible with *in vivo* metabolism studies. Because of these constraints, we determined at the outset that tritium labeled $PGI_2Na$ must be prepared in the crystalline form and analyzed with rapid procedures, and the radioactive material must then be administered to test subjects immediately following completion of its synthesis and analysis.

## DISCUSSION AND RESULTS

The more readily accessible positions in prostaglandins for incorporation of a tritium label are at $C_9$, $C_{11}$, and $C_{15}$.* A pre-positioned ketone function at one of these points, *e.g.*, in $PGE_2$ (7), $PGD_2$ (8), and 15-keto-$PGF_{2\alpha}$ (9), can be reduced with sodium borotritide to introduce tritium into the molecule. Labeling at $C_{11}$ is preferred over $C_9$ and $C_{15}$, since $C_{11}$ is the most metabolically stable position because of the presence *in vivo* of 9- and 15-hydroxyprostaglandin dehydrogenases (10,11). Reduction of $PGD_2$ methyl ester (*1*) with sodium borotritide in 95% ethanol afforded [11β-$^3$H]$PGF_{2\alpha}$ methyl ester (*2a*) in 56.6% radiochemical yield. Also obtained in the process was a 15.4% radiochemical yield of 11-epi-[11α-$^3$H]$PGF_{2\alpha}$ methyl ester (*2b*) of the same specific activity. We note that although a higher chemical yield (~95% *vs.* ~60%) and more favorable isomer ratio of $PGF_{2\alpha}$ to 11-epi-$PGF_{2\alpha}$ (~16:1 *vs.* ~4:1) was achieved when the reduction was carried out in methanol rather than 95% ethanol, the use of the latter solvent provided a higher radiochemical yield (~72% *vs.* ~35%) and produced a product of higher specific activity. We observed that sodium borohydride reacted more rapidly with methanol than with 95% ethanol with evolution of gases. Sodium borotritide presumably behaves likewise, and thus in methanol more of its tritium is lost to the reaction as tritium gas.

*See Ref. 6 for nomenclature and numbering conventions for prostaglandins.

Treatment of [11β-$^3$H]$PGF_{2\alpha}$ methyl ester with iodine afforded the iodoether *3*. Dehydrohalogenation of *3* with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was best carried out without solvent at ambient temperature. The reaction under these conditions required only one hour for completion, and a rapid purification of the crude product by means of chromatography on silica gel led to [11β-$^3$H]$PGI_2$ methyl ester (*4*) which readily crystallized from an ether-hexane mixture in 61% yield based on *2a*. Hydrolysis of *4* with slightly less than one equivalent of 1N sodium hydroxide solution in methanol at 40°C led to [11β-$^3$H]$PGI_2$ sodium salt (*5*), obtained in 86.5% yield in the crystalline state. Reverse phase HPLC analysis of this material, sp. act. 502 μCi/mg, originally >95% radiochemically pure, showed a decline in purity of 5% and 15% after storage in the crystalline state at -20°C for one week and three weeks, respectively. Freshly prepared [11β-$^3$H]$PGI_2$ sodium salt, however, was administered to test animals (dogs and rats) within four hours after completion of its synthesis.

Blair *et al.* (12) have independently completed the synthesis of *5* of sp. act. ~18 μCi/mg, although no direct evidence for the radiochemical and chemical purity of this material was provided. The synthesis of [9β-$^3$H]$PGI_2$ was recently reported (13).

## EXPERIMENTAL SECTION

Radioactivity determinations were made with a Packard Tri-Carb Model 2425 liquid scintillation spectrometer. The external standard method was used, with Diotol (Burdick-Jackson Laboratories, Muskegon, Michigan, U.S.A.) as the scintillation solvent. Thin-layer chromatography (TLC) analyses were done on 2.5 x 10 cm glass plates precoated with a 250 μm layer of silica gel GF (Analtech, Newark, Delaware, U.S.A.), plain or impregnated with boric acid.* Developed zones were visualized by vanillin-$H_3PO_4$ spray and heating. Radioactive zones were detected with a Packard Model 7220 Radiochromatogram Scanner equipped with

*The boric acid impregnated silica gel plates were prepared by dipping neutral silica gel plates in a mixture of 50 ml of saturated solution of $H_3BO_3$ in MeOH, 495 ml of MeOH, and 660 ml of $CHCl_3$, and drying the plates under vacuum at room temperature. The impregnated plates must be kept dry to be effective.

Model 7222 Thin-Layer Plate Scanner. High performance liquid chromatographic (HPLC) analyses were carried out as described by Fitzpatrick, *et al.* (14). For radiochemical purity determinations by means of HPLC, the mobile phase flow rate was reduced to half the normal rate and the effluent was collected in 0.5 minute fractions for counting.

[11β-$^3$H]PGF$_{2\alpha}$ Methyl Ester (*2a*)

An ice cold solution of 733 mg of $PGD_2$ methyl ester (2.0 mmols) in 10 ml of 95% ethanol was added to sodium borotritide*, nominally 12.1 mg, 0.30 mmol, 20 Ci. The solution was stirred at 0°C for 1.5 hour and 76 mg of sodium borohydride (2.0 mmols) was added to complete the reduction. The mixture was stirred at 0°C for another hour. The reaction flask was fitted with a silicon rubber syringe bottle cap through which nitrogen gas inlet and outlet tubes were inserted. To the reaction mixture was added dropwise with stirring from a syringe 1 ml of 1:1 (v/v) acetic acid:water to destroy the excess $NaBH_4$. After the evolution of gases had subsided, the reaction flask was purged with a stream of nitrogen gas. The reaction mixture was partitioned with 25 ml of saturated $NaHCO_3$ solution and 50 ml of ethyl acetate. The aqueous phase was extracted with 50 ml of ethyl acetate twice. The combined organic phases were twice washed with saturated brine and dried over anhydrous sodium sulfate. The colorless residual oil obtained after removal of solvent was applied onto a column of 100 g of Silic AR CC-4 (Mallinckrodt Corp., St. Louis, MO, U.S.A.), 60-100 mesh, impregnated with boric acid**, which was packed in and eluted with boric acid-saturated 3.5% (v/v) methanol in chloroform***. After 550 ml, the eluent, collected in 18 ml fractions at 5.5 minutes per fraction, was changed to boric acid-saturated 7.0% (v/v) methanol in chloroform***. A total of sixty fractions were collected. The residue from the pooled fractions which contained

*Supplied by New England Nuclear Corp., Boston, MA, U.S.A. as purplish solids.

**This packing material was prepared by slurrying 250 g of Silic AR CC-4 with a mixture of 50 ml of a saturated solution of boric acid in MeOH, 495 ml of methanol and 660 ml of chloroform. Excess solvent was filtered and solids were dried *in vacuo* at 50-60° for 18 hours.

***Boric acid-saturated X% (v/v) methanol in chloroform was prepared by mixing 1000-10X ml of chloroform with 10X ml of a saturated solution of boric acid in methanol. Precipitates were removed by filtration through a medium frit sintered glass disc Buchner funnel to give approximately one liter of eluent.

$PGF_{2\alpha}$ methyl ester (fractions 19-32) was dissolved in 50 ml of ethyl acetate and the solution was twice washed with 25 ml of saturated $NaHCO_3$ solution. The aqueous wash was extracted with 50 ml of ethyl acetate. The combined ethyl acetate solution was washed with saturated brine and dried over anhydrous sodium sulfate. The residual oil obtained after removal of solvent was chromatographed on 125 g of plain Silic AR CC-4, which was packed in ethyl acetate and eluted with 2% (v/v) methanol in ethyl acetate. The eluate was collected in 10 ml fractions at 3 minutes per fraction. Fractions 39-60 were pooled and concentrated to give 343 mg of [11β-$^3$H]$PGF_{2\alpha}$ methyl ester (*2a*), 11.32 Ci. Thin-layer chromatographic analyses of this material showed it contained a single radioactive component with identical mobility as a standard sample of $PGF_{2\alpha}$ methyl ester (silica gel, 5% (v/v) methanol in ethyl acetate, $R_f$ 0.48: silica gel, 10% (v/v) methanol in chloroform, $R_f$ 0.41; $H_3BO_3$-silica gel, 10% (v/v) methanol in chloroform, $R_f$ 0.42).

Fractions 46-57 from the boric acid-impregnated Silic AR CC-4 column were pooled and worked up in the same manner as fractions 19-32 (see above) to give 97 mg of 11-epi-[11α-$^3$H]$PGF_{2\alpha}$ methyl ester (*2b*) which spontaneously crystallized without further purification, 3.07 Ci, 96% radiochemically pure by TLC (silica gel, 10% (v/v) methanol in chloroform, $R_f$ 0.38; $H_3BO_3$-silica gel, 10% (v/v) methanol in chloroform, $R_f$ 0.31; identical to a standard sample of 11-epi-$PGF_{2\alpha}$ methyl ester). Both *2a* and *2b* were stored as solutions in methanol (~5 mCi/ml) at -20°C.

(5R, 6R)- and (5S, 6S)-5-Iodo-[11β-$^3$H]$PGI_1$ Methyl Ester (*3*)

To a solution of 17.8 mg (nominal, calculated from total radioactivity) of *2a*, 586 mCi, and 965 mg of $PGF_{2\alpha}$ methyl ester (2.67 mmols in total) in 20 ml of dichloromethane was added 20 ml of saturated aqueous $NaHCO_3$ solution. The mixture was well stirred into an emulsion and cooled in an ice bath. To the cold stirred emulsion was added a solution of 762 mg of iodine crystals (3.00 mmols) in 50 ml of dichloromethane in 10 minutes. The mixture was stirred at 0°C for 50 minutes and the layers were allowed to separate. The aqueous phase was extracted with 30 ml of dichloromethane. The dark red organic phases were combined and shaken

with 25 ml of water containing 6 g of sodium sulfite. The colorless dichloromethane solution was washed with saturated brine and dried over anhydrous sodium sulfate. The solution was evaporated at reduced pressure to give *3* as a pale straw-colored oil, >95% radiochemically pure by TLC (silica gel, 50% (v/v) acetone in hexane, $R_f$ 0.47; ethyl acetate, $R_f$ 0.48; identical to a standard sample of (5R, 6R)- and (5S, 6S)-5-iodo-$PGI_1$ methyl ester). This material was used immediately without further purification in the next step.

[11β-$^3$H]$PGI_2$ Methyl Ester (*4*)

Compound *3* from above was dissolved in 20 ml of ether, and 3 ml of DBU (Aldrich Chemical Co., Milwaukee, WI, U.S.A.) was added. The mixture was concentrated at reduced pressure, and the residue was kept at room temperature for 1 hour. The mixture was partitioned with 40 ml of 50% saturated NaCl solution and 80 ml of 50% (v/v) ether in hexane containing 0.8 ml triethyl amine*. The organic phase was washed with saturated brine and dried over anhydrous sodium sulfate. The solution was concentrated at reduced pressure and <30°C to give crude *4* as a pale yellow oil, which was immediately chromatographed on 60 g of neutral silica gel (60-100 mesh, EM Laboratories, Elmsford, NY, U.S.A.) packed in 35% (v/v) acetone in hexane containing 1% triethyl amine (10 ml per liter of solvent mixture). The column was eluted with 35% (v/v) acetone in hexane containing 0.2% triethyl amine (2 ml per liter of eluent). The eluate was collected in 10 ml fractions at 3 minutes per fraction for 60 fractions. Fractions 27-50, which contained [11β-$^3$H]$PGI_2$ methyl ester were pooled and concentrated at reduced pressure. The residual oil was dissolved in 3 ml of ether and to the solution was added dropwise with stirring ~5 ml of hexane. The slightly hazy mixture was seeded sparingly with crystalline $PGI_2$ methyl ester. As crystallization progressed with stirring at room temperature, hexane was added in small portions until a total of 20 ml was added in 2 hours. The copious fine white crystals were filtered, washed briefly with 10% (v/v) ether in hexane followed by hexane, and dried under vacuum to give 609 mg of *4*, 61% yield based on *2a*, m.p. 57-8°C,

*Presence of triethyl amine is essential to the stability of $PGI_2$ methyl ester in any solvent used in the procedures described in this report.

sp. act. 539 μCi/mg, single radioactive component by TLC, identical in mobility to a standard sample of $PGI_2$ methyl ester (silica gel, 50% v/v acetone in hexane, $R_f$ 0.49; ethyl acetate, $R_f$ 0.49; system A IX*, $R_f$ 0.19). Although unlabeled crystalline $PGI_2$ methyl ester exhibits good stability, its tritium labeled form at 539 μCi/mg proved highly susceptible to autoradiolytic decomposition. Radiochemical purity of *4* declined 38% after three weeks of storage at -20°C. We carried out the conversion of *4* to the corresponding sodium salt *5* within one hour after preparation of *4* was completed.

[11β-$^3$H]$PGI_2$ Sodium Salt (*5*)

A solution of 370 mg of *4* (1.01 mmol) in 2 ml of methanol** and 1.00 ml of 1.00N NaOH** was stirred under a nitrogen atmosphere at 40°C for 3 hours. The mixture was diluted with 5 ml of methanol, filtered, and concentrated at reduced pressure and room temperature (~5 minutes). The aqueous residual paste was dissolved in 1 ml of water and 30 ml of acetonitrile was added in small portions with efficient stirring. The resulting fluffy white crystals were filtered, washed with acetonitrile, and dried under vacuum to give 324 mg of *5*, 86.5% yield, sp. act. 502 μCi/mg; direct HPLC analysis (14) of this material showed it was >95% radiochemically pure (Figure 2) and contained no discrete, known prostaglandin-related materials at detectable (UV detector, 214 nm) levels (Figure 1). Freshly prepared [11β-$^3$H]$PGI_2$ sodium salt was administered to test animals for metabolism studies within four hours after completion of synthesis. HPLC analysis of *5*, 502 μCi/mg, after storage in the crystalline state at -20°C for one and three weeks, showed that its purity declined 5% and 15%, respectively.

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*Upper phase of 90:20:50:100 (v/v) ethyl acetate:acetic acid:isooctane:water.
**Degassed by sonication immediately prior to use to remove any dissolved $CO_2$.

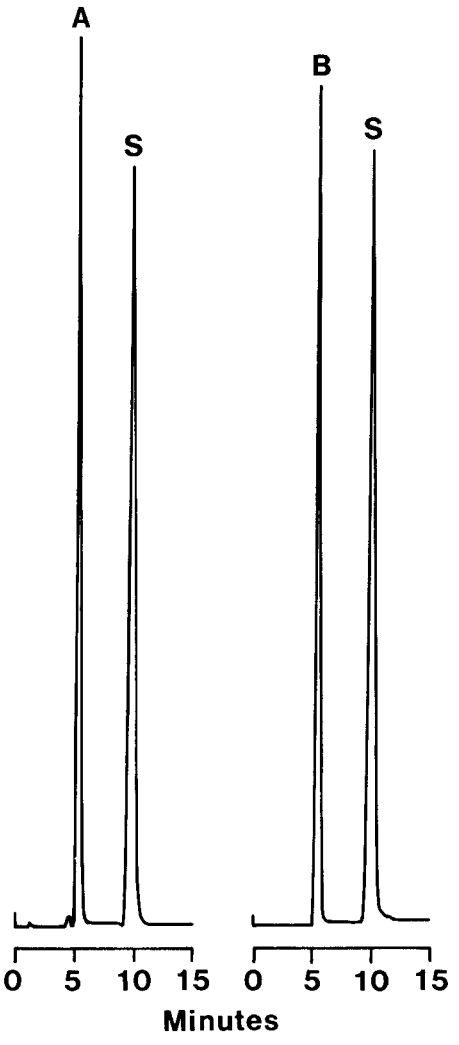


Figure 1. Chromatogram of authentic $PGI_2Na$ (A) and [11β-$^3$H]$PGI_2Na$ (B). Chromatography was performed on a 30 cm x 3.9 mm I.D. μBondapak $C_{18}$ column with a mobile phase of 21:79 (v/v) acetonitrile:water, buffered at pH 9.3 with boric acid (0.75 g per liter) and sodium borate (1.14 g per liter). The flow rate was 1.8 ml $min^{-1}$ at 2,200 p.s.i.g. Propyl p-hydroxybenzoate was used as the internal standard (S). Compounds were detected by ultraviolet light absorption at 214 nm.

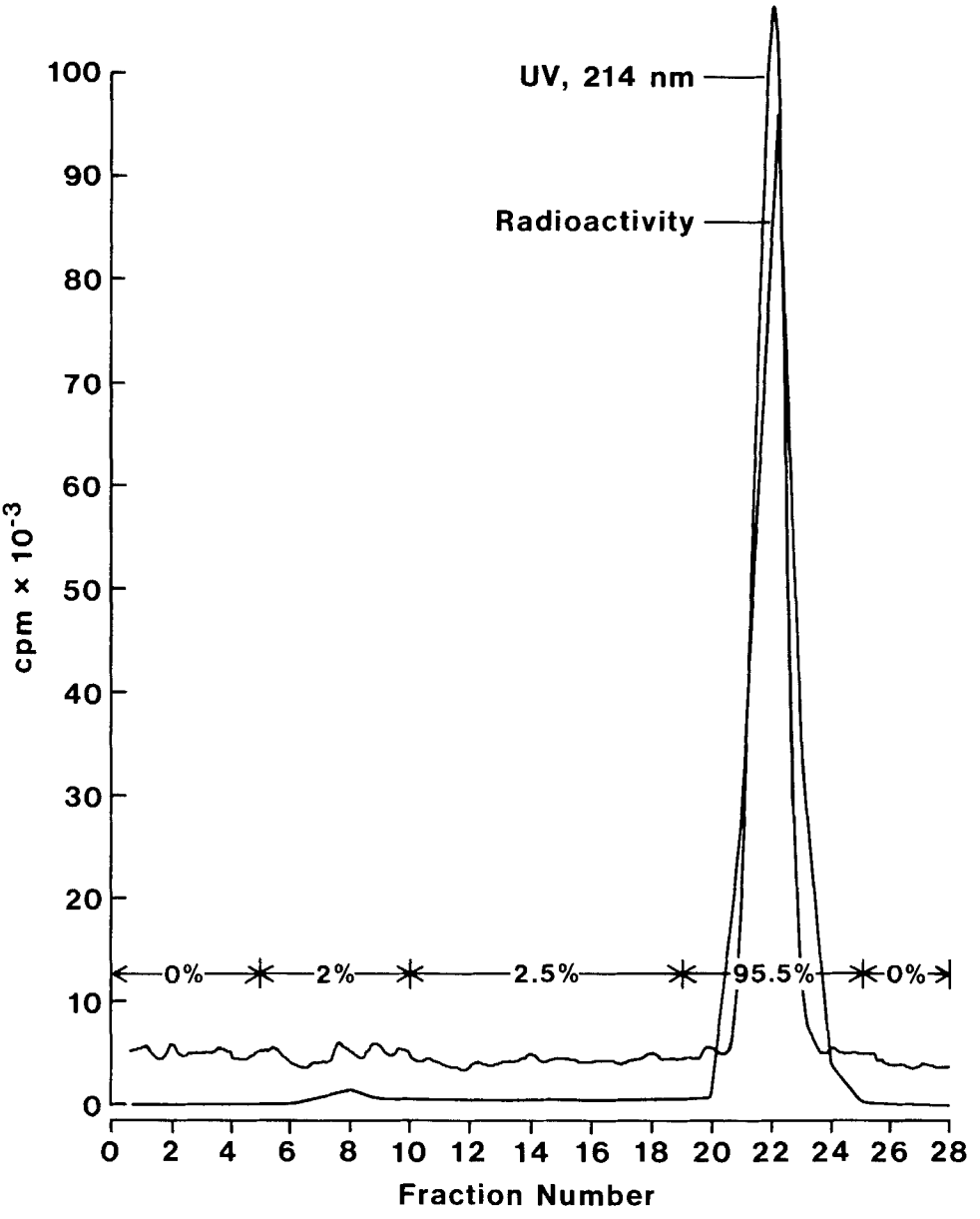


Figure 2. Radiochromatogram of [11β-$^3$H]$PGI_2$Na. Chromatogram was obtained with a mixture of 0.206 mg of [11β-$^3$H]$PGI_2$Na and 2.370 mg of authentic $PGI_2$Na using borate buffered 21:79 (v/v) acetonitrile:$H_2O$ (pH 9.3) as the mobile phase at a flow rate of 0.9 ml $min^{-1}$ on a 30 cm x 3.9 mm I.D. μBondapak $C_{18}$ column. Detection was by ultraviolet light absorption at 214 nm. The effluent was collected in 40 0.5 min fractions, each of which was counted for radioactivity in 15 ml of Diotol. No radioactivity was found in fractions 29-40.

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