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**Note**

# Adsorption chromatographic separation of $^{125}$I-labelled prostaglandin $F_{2\alpha}$ and prostaglandin $E_2$ tyrosine methyl ester

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Radioiodine-labelled substances are often used as tracers in radioimmunoassay. With prostaglandins (PGs), radioiodine is usually introduced in the histamine, tyramine[1] or tyrosine[2] group, coupled to the carboxyl group of the prostaglandins. To produce $^{125}$I-labelled $PGF_{2\alpha}$ suitable for radioimmunoassay, *i.e.*, exhibiting high specific activity and radiochemical purity, at least three compounds, shown in Fig. 1, have to be separated. In addition, unreacted free radioiodine and unidentified labelled products are also formed in the course of the chloramine T labelling procedure.

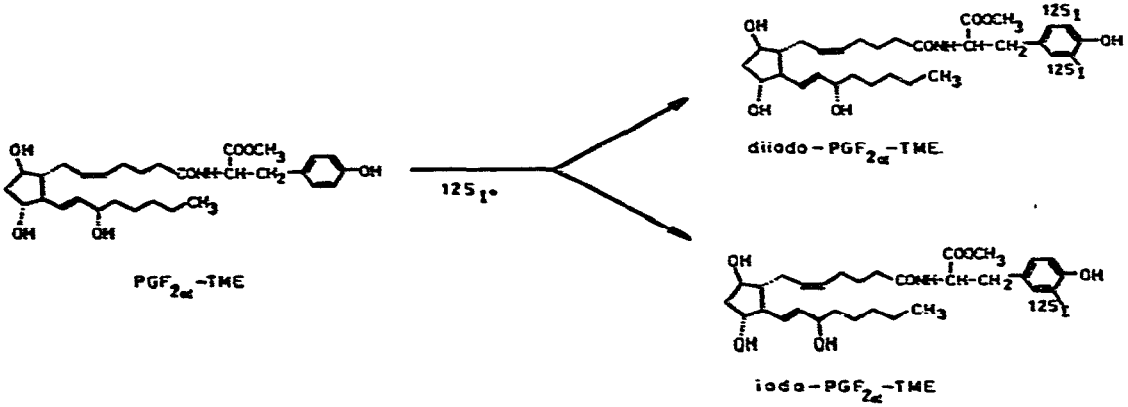


Fig. 1. Mono- and diiodotyrosine derivatives formed in the course of the chloramine T labelling.

The aim of this paper is to show that by the use of Sephadex LH-20 as adsorbent and aqueous ethanol as eluent the monoiodo derivatives of the tyrosine methyl esters (TMEs) of $PGF_{2\alpha}$ and $PGE_2$ of high radiochemical purity can be produced.

## MATERIALS AND METHODS

The chloramine T method was applied to label $PGF_{2\alpha}$-TME and $PGE_2$-TME with $^{125}$I. To 10–20 $\mu$g of $PGF_{2\alpha}$-TME or $PGE_2$-TME dissolved in 0.1–0.2 ml of

phosphate buffer (pH 7.6–8.0) was added 0.5–5.0 mCi of carrier-free iodine-125 in slightly alkaline solution, followed by 20–40 $\mu$g of chloramine T in 0.2–0.3 ml of phosphate buffer. The labelling reaction was quenched after 10–30 sec with 30–60 $\mu$g of sodium metabisulphite in a volume of 0.1–0.2 ml.

Sephadex LH-20 dextran gel, swollen in distilled water for 12–24 h, was used as the adsorbent. The gel was poured into a glass tube (length 130 mm, I.D. 10 mm), the bottom of which was fitted with a porous disc. The sample (volume 0.1–0.2 ml) from the chloramine T labelling procedure was placed on the top of the gel, together with tritium-labelled $PGF_{2\alpha}$-TME or $PGE_2$-TME, and was allowed to soak in. After 10–20 min, *i.e.*, when adsorption equilibrium had been attained, the elution was started with aqueous citrate buffer (pH 2). The effluent obtained was collected with a fraction collector and, as it contained the tritium-labelled $PGF_{2\alpha}$ or $PGE_2$ only, its radioactivity was determined by liquid scintillation counting.

After elution with the aqueous eluent was completed, elution of the $^{125}I$-labelled substances was carried out with aqueous ethanol, the effluent being passed over a NaI(Tl) scintillation crystal and the count rate being monitored by a rate meter and registered on an $X$–$Y$ plotter. The delivery of the eluents was carried out by the use of a peristaltic pump, the flow-rate of which was adjusted to 22–24 ml/h.

The distribution coefficient was calculated according to the equation

$$k = \frac{V_e - V_0}{W}$$

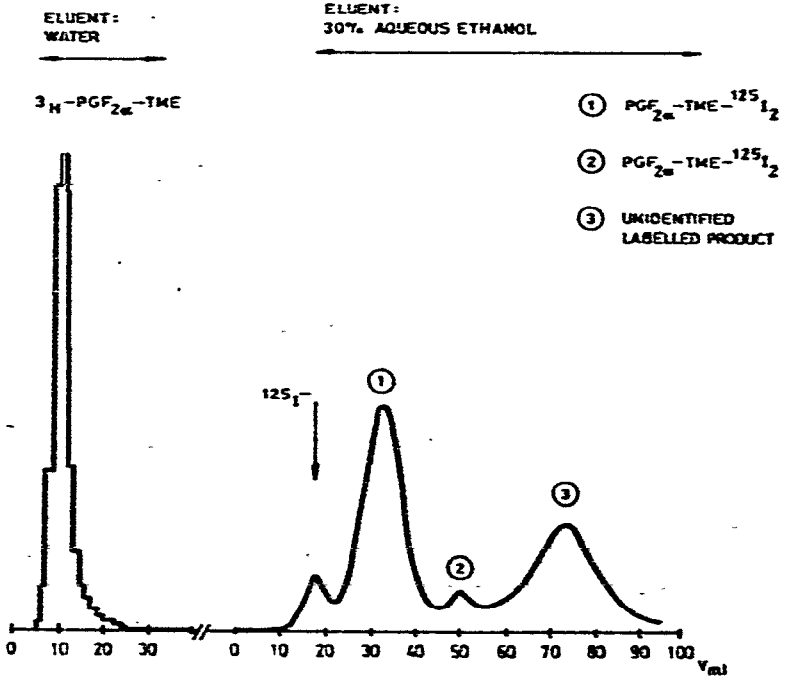


Fig. 2. Elution pattern obtained by the co-chromatography of tritium-labelled $PGF_{2\alpha}$-TME and the reaction mixture from the chloramine T labelling procedure.

where $V_e$, $V_0$ and $W$ are the elution volume, the dead volume and the weight of the adsorbent, respectively.

## RESULTS

Fig. 2 shows the elution pattern obtained by the co-chromatography of tritium-labelled $PGF_{2\alpha}$-TME and the labelling mixture from the chloramine T labelling procedure. It can be seen that tritium-labelled $PGF_{2\alpha}$-TME is eluted with water and the elution volume hardly exceeds the dead volume. During this process there is no substantial migration of the $^{125}I$-labelled products from the top of the gel.

Elution of the $^{125}I$-labelled substances, *e.g.*, mono- and diiodo-$PGF_{2\alpha}$-TME and -$PGE_2$-TME, can be performed with aqeous solutions of organic solvents, such as ethanol. Free radioiodine that has not been eluted with water appears first, followed by monoiodo-$PGF_{2\alpha}$-TME, an unidentified labelled product and diiodo-$PGF_{2\alpha}$-TME.

The elution volume ($V_e$) and the distribution coefficient ($k$) can be adjusted by choosing the appropriate ethanol concentration in the aqueous eluent.

TABLE I

ELUTION VOLUME ($V_e$) AND DISTRIBUTION COEFFICIENT ($k$) OF $^{125}I$-LABELLED $PGF_{2\alpha}$-TME AND $PGE_2$-TME AT DIFFERENT ETHANOL CONCENTRATIONS

| *Ethanol concentration* (%, v/v) | *$PGF_{2\alpha}$-TME-$^{125}I$* | | *$PGE_2$-TME-$^{125}I$* | |
|---|---|---|---|---|
| | *$V_e$ (ml)* | *k* | *$V_e$ (ml)* | *k* |
| 10 | 72 | 57 | 75 | 60 |
| 20 | 55 | 43 | 60 | 46 |
| 30 | 31 | 22 | 32 | 23 |
| 40 | 16 | 9 | 20 | 12.5 |

For the separation of $^{125}I$-labelled $PGF_{2\alpha}$-TME or $PGE_2$-TME it is recommended that free radioiodine and unlabelled $PGF_{2\alpha}$-TME or $PGE_2$-TME are first removed with aqueous eluent (pH 2), followed by the elution of monoiodo-$PGF_{2\alpha}$-TME or -$PGE_2$-TME aqueous ethanol.

## REFERENCES

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2 B. Tanácts, G. Tóth and I. Mucha, *Izotóptechnika*, in press.