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Note

Improved sample preparation for the quantitative mass spectrometric determination of prostaglandins in biological samples*

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The simultaneous analysis of various prostaglandins in biological samples is difficult because the prostaglandins have to be extracted from a complex matrix, and because of the presence of many other prostaglandins and their metabolites.

We have developed a sample preparation procedure which uses both reversed-phase octadecylsilyl (C_{18}) silica and normal-phase silica cartridges (Sep-Pak^{TM}, Waters Assoc., Milford, MA, U.S.A.) to selectively extract and purify prostaglandins from biological samples. The procedure is simple and rapid. Small volumes of clear eluates are obtained which do not contain biological contaminants which would otherwise overburden the subsequent high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Deuterium- and tritium-labelled 7α-hydroxy-5,11-diketo-tetranorprostane-1,16-dioic acid (PGE-M) were a generous gift of Dr. W.J.A. VandenHeuvel, Merck Sharp & Dohme Research Labs., Rahway, NJ, U.S.A. The other deuterium-labelled prostaglandins were a generous gift of Dr. U. Axen, The Upjohn Company, Kalamazoo, MI, U.S.A.; the other tritium-labelled prostaglandins were purchased from Amersham Buchler, Braunschweig, G.F.R.

The derivatization procedures, HPLC and SE-30 glass capillary gas chromatography—mass spectrometry (GC—MS) were all carried out as previously described [1, 2].

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Sample preparation for the simultaneous determination of prostaglandins in urine or serum

The sample preparation procedure is shown schematically in Fig. 1.

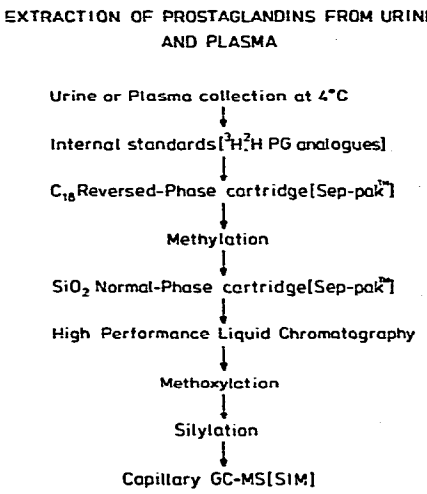

Fig. 1. Scheme of the sample preparation procedure.

Tritium- and deuterium-labelled prostaglandin analogues were added to samples of urine (10—40 ml) or plasma (5—50 ml) and the samples were then acidified to pH 3.2 with formic acid. A reversed-phase silica cartridge (Sep-Pak C_{18}) was prepared by rinsing it with 10 ml of methanol followed by 10 ml of water. The acidified sample was passed through the cartridge. The cartridge was washed with water (10 ml) to remove polar components, and the prostaglandins were then quantitatively eluted with chloroform (20 ml). Non-polar components remained on the cartridge. After methylation with diazomethane, the sample was passed through a normal-phase silica cartridge (Sep-Pak) which was then washed with chloroform (10 ml) to remove non-polar components; the prostaglandin methyl esters were eluted with chloroform—methanol (98 : 2, 20 ml). Polar components remained on the cartridge. This extract was subjected to preparative HPLC using a normal-phase silica (10 μm) column (μPorasil, Waters Assoc.). Individual prostaglandin fractions were treated to give the methoxime and silyl ether derivatives and the isotope ratios were determined with a GC—MS system equipped with a glass capillary column [1].

RESULTS

The HPLC separation of various tritiated prostaglandins added to human urine or plasma is shown in Fig. 2. Sharp separations of the prostaglandin methyl esters were obtained; the various prostaglandins occurred in fractions of usually 2 ml or, at most, 3 ml. The methyl esters of these prostaglandins span a smaller range of polarity than their parent acids, so that a HPLC gradient can be used with only a moderate change in the polarity of the eluents. These chromatographic characteristics, together with the clarity of the extract,

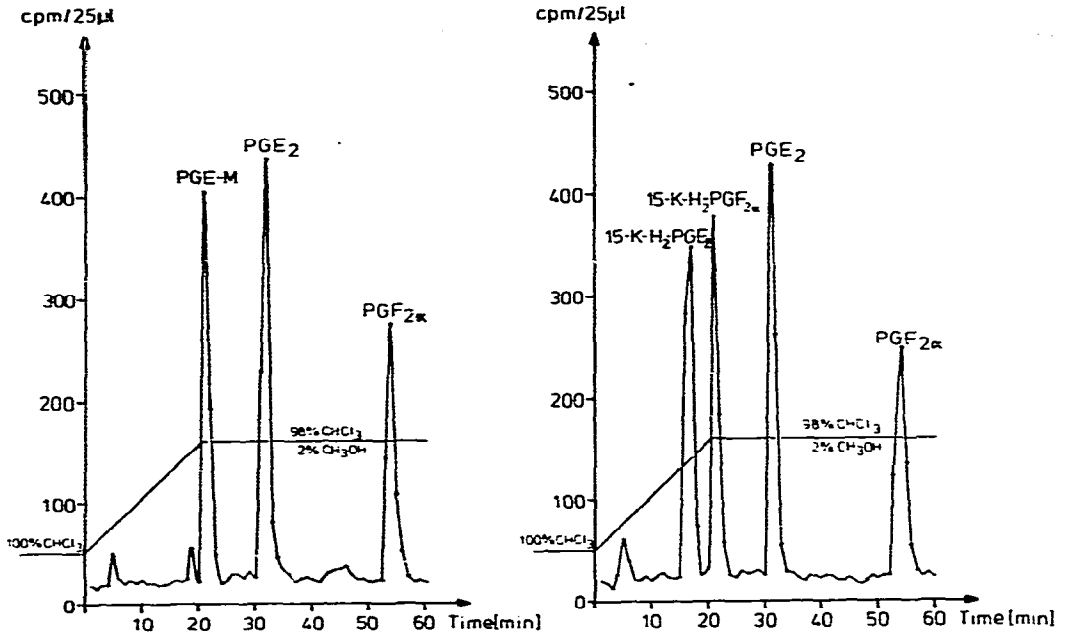

Fig. 2. HPLC separation of the tritiated methyl esters of PGE-M, PGE_2 and $PGF_{2\alpha}$ added to human urine (left), and 15-keto-13,14-dihydro-PGE_2 (15-K-H_2-PGE_2), 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (15-K-H_2-$PGF_{2\alpha}$), PGE_2 and $PGF_{2\alpha}$ added to human plasma (right). A 10-µm µPorasil column (250 mm × 4.0 mm I.D.) was used. Two M6000A pumps (Waters Assoc.) were controlled by an M660 gradient controller (Waters Assoc.). The initial eluent was chloroform and the final eluent, after 20 min, was chloroform—methanol (98 : 2) (program No. 6, linear). The flow-rate was 1 ml/min. Fractions of 1 ml were collected; the radioactivity of 25-µl samples was determined by liquid scintillation spectrometry.

help make this HPLC separation a reliable and reproducible part of the analysis.

The recovery after HPLC of tritium-labelled prostaglandins (34,500 to 40,000 cpm) added to human urine was (mean ± S.D.): PGE-M, 65.7 ± 9.5% (n = 10); PGE_2, 63.8 ± 7.0% (n = 7); and $PGF_{2\alpha}$, 49.8 ± 4.9% (n = 8). The reproducibility of the method was demonstrated by adding 50 ng of each of PGE-M, PGE_2 and $PGF_{2\alpha}$ to four 40-ml samples of urine. The amounts recovered were (mean ± S.D.): PGE-M, 48.0 ± 4.8 ng; PGE_2, 53.8 ± 1.3 ng; and $PGF_{2\alpha}$, 49.0 ± 0.9 ng.

A sample preparation procedure designed to measure a number of prostaglandins in one sample will also extract other prostaglandins and other substances of similar polarity. Some of these interfering substances will also be present in individual fractions of the HPLC separation. The selected-ion chromatograms of derivatized PGE_2, $PGF_{2\alpha}$, PGE-M and 6-keto-$PGF_{1\alpha}$ from biological samples (Fig. 3) show that subsequent capillary GC—MS is needed. This separation gives accurate quantitation which is not always achieved with packed GC columns [1, 3].

DISCUSSION

The introduction of Sep-Pak C_{18} cartridges has greatly simplified the

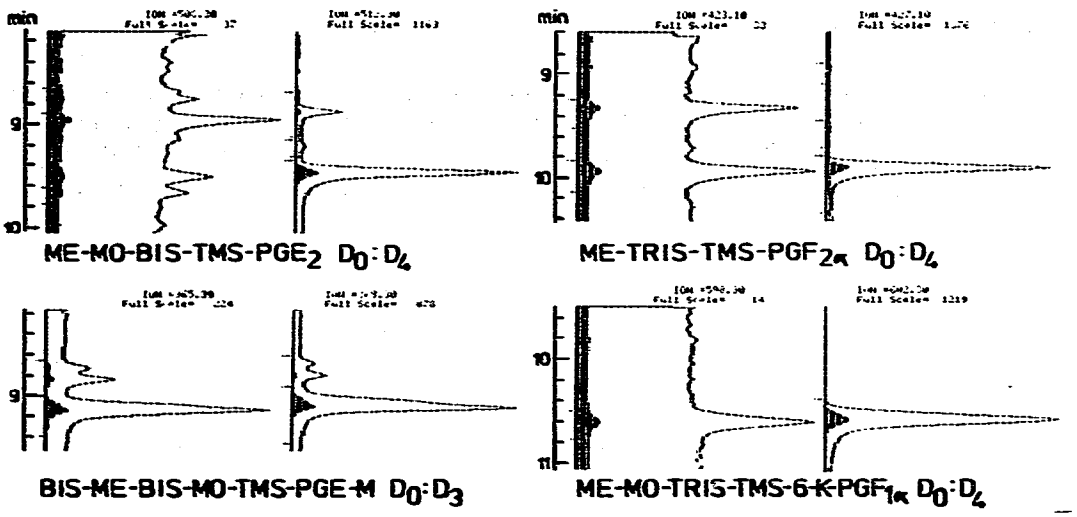

Fig. 3. Portions of typical selected-ion chromatograms of derivatized endogenous and deuterated PGE_2, $PGF_{2\alpha}$, PGE-M, and 6-keto-$PGF_{1\alpha}$ from biological samples. 6-Keto-$PGF_{1\alpha}$ was extracted from plasma, the other prostaglandins were extracted from urine. The gas chromatographic separation was obtained on an SE-30 glass capillary column 20 m long. Dwell times were 150 msec and 50 msec for the ions of endogenous and deuterated prostaglandins, respectively.

analytical extraction of drugs from plasma (see, for example, ref. 4), of natural and synthetic steroids from urine [5] and, indeed, of prostaglandins from urine [6]. All of these procedures use the C_{18} cartridge to obtain an extract from the aqueous biological medium in a polar organic solvent such as methanol or methyl formate.

In our procedure, the biological liquid is passed through the C_{18} cartridge and washed free of strongly polar components with water. Elution with chloroform, which is a very weak eluent of C_{18} silica, selectively extracts prostaglandins and, presumably, other substances of similar lipophilicity and polarity.

Our improved sample preparation procedure using Sep-Pak cartridges is simpler, more rapid and more reliable than our previous procedure which involved extraction and chromatography on open silica columns. We used the previous procedure to quantitate various prostaglandins in the urine of infants and children [2], in the urine of tumour-bearing rats [7] and in the perfusates of isolated rat kidneys [8], and to identify PGE_2 and 15-keto-13,14-dihydro-PGE_2 in human gastric juice [9].

The sample preparation procedure should also be suitable as a preliminary to radioimmunoassay or bioassay, although the methylation step has then to be excluded and the free acids separated on a suitable HPLC system (see ref. 10).

In conclusion, the extraction of biological samples for the analysis of prostaglandins is simplified and improved by the use of reversed-phase silica (C_{18}) and silica Sep-Pak cartridges. Considerable time is saved and the small volumes of eluates are free of contaminants which could overburden the subsequent HPLC.

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