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Note

Combined capillary column gas chromatography—mass spectrometric method for the quantitative analysis of urinary prostaglandins

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The development of accurate, sensitive, and highly specific methods for the quantitative analysis of prostaglandins in biological materials has received considerable attention recently [1—5]. Of particular interest is the determination of urinary prostaglandins [6]. It has been postulated that urinary prostaglandin excretion is a reflection of renal prostaglandin production and it has been used as a tool to study renal prostaglandin physiology and pathology.

In 1970 Samuelsson and coworkers employed for the first time the stable-isotope dilution technique of selected ion monitoring for prostaglandin analysis [1]. Since then gas chromatography—mass spectrometry (GC—MS) has been successfully applied to the quantitative analysis of prostaglandins, prostaglandin metabolites, and thromboxanes [3, 5], and is considered to be the most accurate method for prostaglandin determination. However, one of the main difficulties in the use of this methodology is caused by the very low concentration of prostaglandins in complex biological samples such as urine. This analytical problem may be improved by increasing the poor chromatographic resolution usually achieved with conventional packed columns.

This paper describes the quantitative analysis of PGE_2, $PGF_{2\alpha}$ and 7α-hydroxy-5,11-diketotetranor-prostane-1,16-dioic acid (PGE-M), the major urinary metabolite of the E-prostaglandins, using a glass capillary column combined with a mass spectrometer, which was operating in the selective ion monitoring mode. Compared to packed columns, resolution, sensitivity, and specificity were greatly improved by the capillary column. Similar results have been reported by Maclouf et al. [7] and by Fitzpatrick [8], although they did not consider quantitative aspects in their prostaglandin analysis.

EXPERIMENTAL

Materials

All solvents were purchased from the Prochem Company (Wesel, G.F.R.). The tetradeutero (D_4) analogs of PGE_2 and $PGF_{2\alpha}$ were a generous gift from Dr. U. Axen, The Upjohn Company. Tritium-multilabelled PGE_2 and $PGF_{2\alpha}$ (specific activity 120—170 Ci/mol) were purchased from Amersham/Buchler Cooperation (Braunschweig, G.F.R.). Heptatritio and heptadeutero PGE-M were provided by Dr. W.J.A. VandenHeuvel, The Merck, Sharp and Dohme Research Laboratories. Methoxyamine·HCL and N,O-bis(trimethylsilyl)trifluoroacetamide were purchased from Serva (Heidelberg, G.F.R.) and Fluka AG (Buchs, Switzerland), respectively. Diazomethane was prepared as described previously [4].

Extraction of prostaglandins from urine

Tritium- and deuterium-labelled PGE_2, $PGF_{2\alpha}$ and PGE-M were added to urine samples of different patients as internal standards and tracers. Extraction and separation were carried out essentially as described previously [9] by high-performance liquid chromatography (HPLC) on a microparticulate silicic acid column [10].

Gas chromatography—mass spectrometry

A Hewlett-Packard HP 5992A microprocessor-controlled GC—MS system was used. The system was alternatively equipped with a 1% Dexsil 300 packed column (1.8 m × 2 mm I.D.), and an SE-30 glass capillary column (30 m × 0.3 mm I.D.) with a Grob-type splitless injector [11]. The GC—MS system included an HP 5990A quadrupole mass spectrometer coupled to a 9825 HP computer and a 9885 flexible disk. The interface for the packed column was a one-stage jet separator. The capillary column was coupled directly via an open split connection with glass capillary restriction basically designed as the interface described by Henneberg et al. [12]. Helium flow-rates were 20 and 2 ml/min, respectively. Operation conditions were: injection port, 260°; interface, 240°; ion source, 170°; electron impact energy, 70eV. The electron multiplier voltage was 2.4 kV.

RESULTS AND DISCUSSION

Sensitivity

Selected ion chromatograms of *m/e* 512 from the tetradeutero PGE_2-methyl ester-methyloxime-bis-trimethylsilyl ether (Me-Mo-bis-TMS) and *m/e* 508 from the protium form of the PGE_2 derivative are shown in Fig. 1. Unlabelled PGE_2 added to a constant amount of tetradeutero PGE_2 ranged from 0 to 1% of the tetradeutero PGE_2 derivative. Approximately 50 ng of tetradeutero PGE_2 were injected either into the packed column (upper tracings of Fig. 1) or into the glass capillary column (lower tracings of Fig. 1). Using the selected ion chromatograms obtained with the capillary column, the lower detection limit was 50 pg unlabelled PGE_2 with a signal-to-noise ratio of 2:1. After subtraction of the blank value the constructed standard line of ten different unlabelled standards

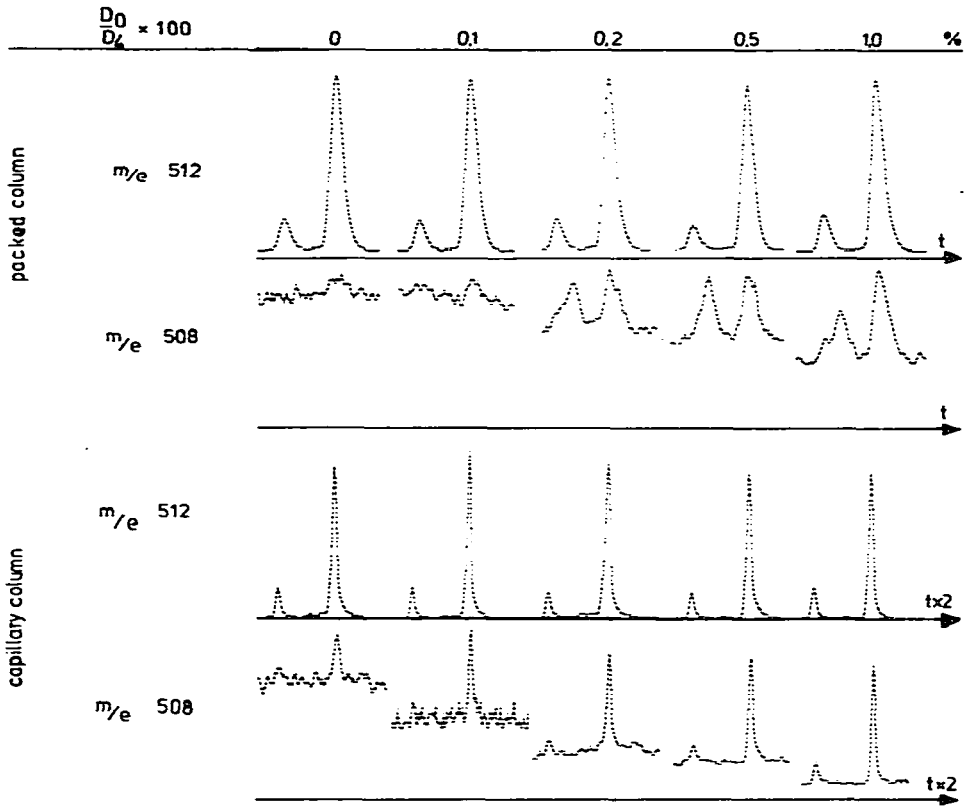

Fig. 1. Comparison of selected ion chromatograms obtained with a 1% Dexsil 300 packed column (upper tracings) and an SE-30 glass capillary column (lower tracings). The vertical axis represents abundance, the horizontal axis time (t). The recording speed was doubled by means of dwell time for the tracings obtained with the capillary column. Selected ions of the Me-Mo-bis-TMS derivatives are *m/e* 512 for tetradeutero PGE_2 (D_4) (full-scale 180 for the packed column and 1000 for the capillary column) and *m/e* 508 for unlabelled PGE_2 (D_0) (full-scale from 2 to 4 for the packed column and from 4 to 28 for the capillary column). The amount of D_4-PGE_2 injected was approximately 50 ng.

(from 50 pg to 50 ng unlabelled PGE_2) had a slope of 0.996 with an intercept of −0.249 and a correlation coefficient of 0.9997. Using the packed column 1 ng of unlabelled PGE_2 was the minimum amount which could be detected quantitatively. This also applies to $PGF_{2\alpha}$ and PGE-M.

Detection of urinary prostaglandins

Some examples of selected ion chromatograms of PGE_2-Me-Mo-bis-TMS, $PGF_{2\alpha}$-methyl ester-tris-trimethylsilyl ether (Me-tris-TMS) and PGE-M-bis-methyl ester-bis-methyloxime-trimethylsilyl ether (bis-Me-bis-Mo-TMS) after extraction and separation from human urine are shown in Fig. 2. Despite intensive purification and isolation procedures using organic solvent extraction, open column chromatography, HPLC and GC, the selected ion chromatograms of the protium forms show a remarkably high background and peaks with different retention times from those of the corresponding deuterated prosta-

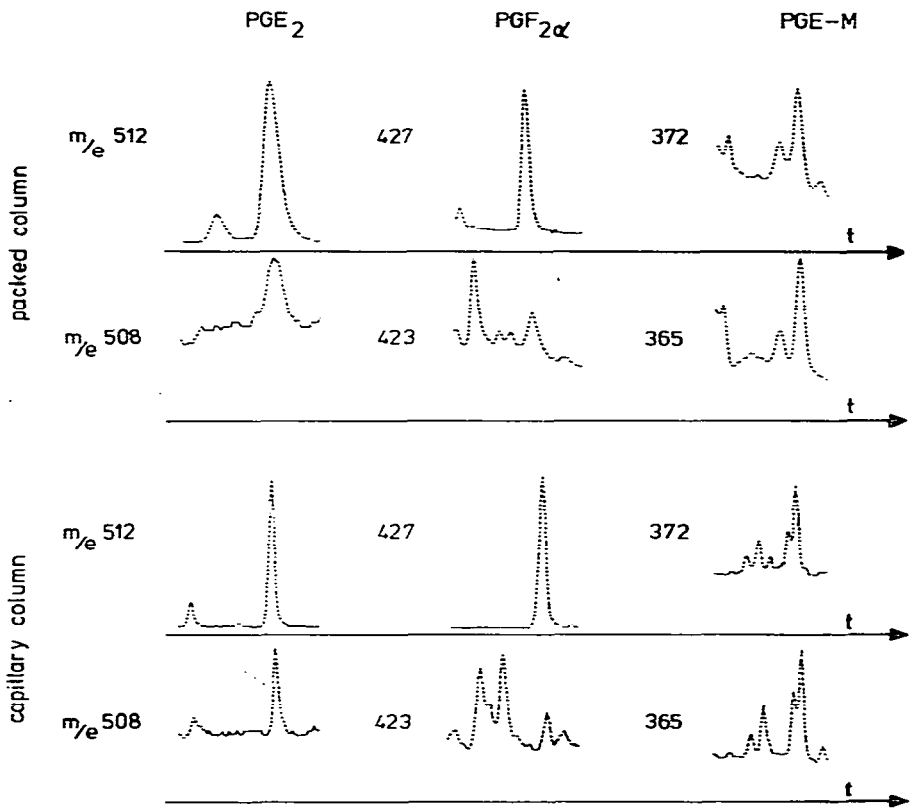

Fig. 2. PGE_2, $PGF_{2\alpha}$ and PGE-M from human urine. The vertical axis represents abundance, the horizontal axis time (t). The recording speeds for the capillary chromatograms (lower tracings) were increased two- to five-fold compared to the chromatograms of the packed column (upper tracings). The recorded ion pairs for Me-Mo-bis-TMS of unlabelled and tetradeutero PGE_2 were *m/e* 508 (full-scale 8 for the packed column and 6 for the capillary column) and *m/e* 512 (full-scale 85 for the packed column and 58 for the capillary column); the ion pairs for Me-tris-TMS of unlabelled and tetradeutero $PGF_{2\alpha}$ were *m/e* 423 (full-scale 35 for the packed column and 13 for the capillary column) and *m/e* 427 (full-scale 86 for the packed column and 109 for the capillary column); the ion pairs for bis-Me-bis-Mo-TMS of unlabelled and heptadeutero PGE-M were *m/e* 365 (full-scale 57 for the packed column and 33 for the capillary column) and *m/e* 372 (full-scale 62 for the packed column and 24 for the capillary column). The amounts of deuterated prostaglandin analogs injected as internal standard were approximately 50 ng for PGE_2 and $PGF_{2\alpha}$ but only 20 ng for PGE-M. The columns used were the same as described in the legend of Fig. 1.

glandin analogs. The poor resolution of packed columns may then lead to a low signal-to-noise ratio as shown by the SIM tracings of the unlabelled PGE_2 derivative. Using the packed column, the peak area of *m/e* 508 was only about half of that obtained with the capillary column. The measured values were 3.1 ± 1.5 versus 5.3 ± 0.5% unlabelled PGE_2 ($\bar{x}$ ± S.D. of four injections), which corresponds to an excretion rate of 718.6 ± 348.8 versus 1234.4 ± 111.6 ng/day. This discrepancy is explained in part by an automatic integration device triggered by slope sensitivity which results in distorted values when integrating only partially resolved peaks. The opposite was the case in the example of

$PGF_{2\alpha}$. Contaminating peaks at the nominal mass 423, which were unresolved from the signal of the unlabelled $PGF_{2\alpha}$ derivative, gave a false value of 12.1 ± 1.3% unlabelled $PGF_{2\alpha}$ with the packed column, while the percentage of unlabelled $PGF_{2\alpha}$ was only 1.7 ± 0.3 when the capillary column was used. The excretion of $PGF_{2\alpha}$ would be 499.0 ± 54.2 instead of 67.8 ± 10.7 ng/day. The four *syn* and *anti* isomers of the bis-methyloxime derivative of PGE-M did not show up in the mass chromatograms of the packed column. Quantitative analysis of PGE-M was distorted by unresolved isomers and a falling base-line. The PGE-M excretion rate would be either 2.7 or 3.8 μg/day depending which gas chromatographic system was used, either packed or capillary column.

These studies have demonstrated a successful attempt to introduce capillary columns into the quantitative analysis of prostaglandins. The determinations of urinary prostaglandins were more accurate when a glass capillary column was used instead of a conventional packed column because of the much higher resolution of the capillary column. This technique should be used for the quantitative analysis of other arachidonic acid metabolites in complex biological samples. Such work is currently under way in our laboratory.

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