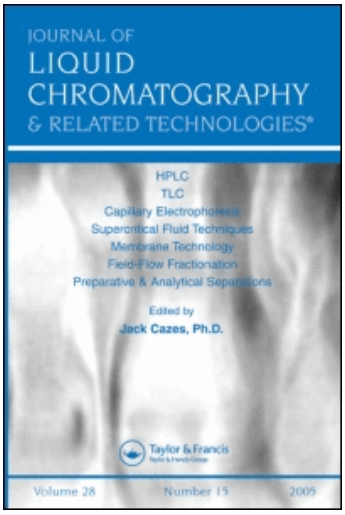

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR PROSTAGLANDIN E_2 DETERMINATION IN HUMAN GASTRIC JUICE WITHOUT DERIVATIZATION

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ABSTRACT

A rapid and practical method for the separation and quantitation of PGE_2 in human gastric juice by high performance liquid chromatography is described. Separation on a reversed-phase column and UV detection allows quantities as little as 20 nanograms of PGE_2 to be detected. Specificity, sensitivity, high yield and reproducibility make this method particularly suitable for prostaglandin determination in human gastric juice.

INTRODUCTION

Many prostaglandins (PGs) and their metabolites are present in gastric juice, indicating the relevant role that these substances play in gastric pathophysiology, such as modulation of acid secretion, cytoprotection and motility control.

Up till the present, various methods for specifically measuring PGs in the gastric juice and in other biological fluids have been reported; bio-assay (1), radioimmunoassay (RIA) (1-4) and gas chromatography - mass spectometry (GC/MS) (5) are the most frequently used.

Recently, high performance liquid chromatography (HPLC) was introduced to separate PGs (1,3,6,7) which were then quantified by RIA (2,3) or GC/MS (5,8).

Derivatization of PGs has also been carried out for fluorescence and UV detection by HPLC (9,10,11), but derivative proce-

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dures may have problems of specificity and recovery; in fact it is not clear if PGs give a single derivative under the conditions of analysis used and if the derivatization is completely carried out.

Terragno et al. recently used HPLC for the separation and quantitation of PGs; eleven standard PGs and metabolites were evaluated and a sensitivity of as little as 30 ng was obtained (12).

We therefore used HPLC to quantitate PGE_2 in human gastric juice without derivatization and aliquots as little as 20 ng were detected.

MATERIALS AND METHODS

Apparatus

A HPLC system was used (Perkin-Elmer) consisting of: a Model Series 4 solvent delivery system equipped with a Rheodyne 7120 injection valve with a 20 µl injection loop, a Model LC-85 variable wavelenght UV detector (with a 2.4 µl flowcell) operating at 192.5 nm (12) and a Sigma 15 Chromatography Data station. A reverse phase C-8 column (Perkin-Elmer, 10 cm x 4.6 mm i.d., 5 µm particle size) was used.

The mobil phase consisted of 17 mM orthophosphoric acid-acetonitrile (67.2: 32.8, v/v). The system was constantly kept at pH 3.5. Flow rate was 1.7 ml/min (12); column temperature 37°C.

Reagents and Chemicals

PGE_2 was purchased from Upjohn Co., Kalamazoo, Mi., U.S.A., PGB_1 from Sigma, Saint Louis, Mo. U.S.A. and Cortisone from Makor, Jerusalem, Israel. ^3H-PGE_2, specific activity 160 Ci/mmol and ^3H-PGB_1, specific activity 60 Ci/mmol, were purchased from New England Nuclear, Boston, Mass., U.S.A.

Acetonitrile LiChrosolv, methanol, chloroform, isopropanol, formic acid and absolute ethanol of HPLC grade were obtained from Merck, Darmstadt, F.R.G. Water for Chromatography was distilled three times and passed through a 0.20 µm pore size filter

(Sartorius). Solvents and other chemicals were of analytical grade.

Preparation of Biological Samples

Gastric juice was obtained from 12 healthy donors (6 males and 6 females) after a fasting period of 12 hours, at the same hour of the morning in order to avoid diurnal variations in PG levels.

The total volume (51±5.8 ml,mean value ± SE)of gastric juice collected during a single period of 30 minutes in basal conditions, was used for analysis. 10 µCi ^3H-PGB_1 were immediately added to each sample as an internal standard (IS) for total recovery evaluation of the method.

pH was controlled and eventually adjusted between 1.5 and 3 with 1N HCl.

All the samples were immediately centrifuged (2500 g) at 4 °C for 20 minutes and the supernatants extracted with ethyl acetate (5:1 ;v/v).

The organic phase , after filtration through a 0.20 µm pore size filter (Sartorius) was evaporated to dryness in a nitrogen atmosphere at 37 °C.

^3H-PGB_1 was chosen as IS for the total yield because: 1) it is not present in gastric juice (4,13); 2) it is stable and not metabolized in the gastric pH (14); 3) its HPLC retention time is very different from that of PGE_2.Unlabelled PGB_1 was used for collecting ^3H-PGB_1 from the column.

We did not use ^3H-PGE_2 as IS because PGE_2 is in part converted to PGA_2 in the gastric juice pH (13).

Thin Layer Chromatography (TLC)

Silica gel plates (Merck, 60; F 254, 20 x 20 cm) were used for ascending TLC in closed glass chambers. The following solvent system was employed: chloroform-isopropanol-ethanol-formic acid (45:5:0.5:0.3, v/v) (15). Dried samples were redissolved with 100 µl of methanol and applied on the silica gel plates as a single

spot using a Hamilton syringe. The plates were allowed to run at room temperature for 75 minutes. Aliquots of standard PGE_2 and PGB_1 were also spotted as markers at one corner of the plates and submitted to the same TLC development conditions. The entire TLC plate, except the standard PG channel, was covered with a glass plate and then exposed to iodine vapour in order to visualize standard spots. The areas of the plate containing biological samples, corresponding to the marker spots, were scraped off and eluted with methanol (4,16). Samples were then filtered (Sartorius, 0.20 μm pore size) and evaporated to dryness in nitrogen atmosphere.

HPLC Calibration Curve

Standard solutions of PGE_2 ranging from 40 μg/ml to 1.0 μg/ml were prepared in absolute ethanol containing 200 μg/ml of Cortisone used as HPLC external standard (ES) (7). 20 μl of each solution were injected into the column.

The calibration curve was obtained by calculating the ratio between the peak areas of standard PGE_2 and ES. These ratios were plotted against the concentrations. The curve was used to convert peak area ratios of unknown samples to PGE_2 concentrations.

HPLC Analysis

Dried samples were redissolved with 20 μl of absolute ethanol containing ES and injected into the chromatograph.

The sample preparation procedure is shown schematically in table 1.

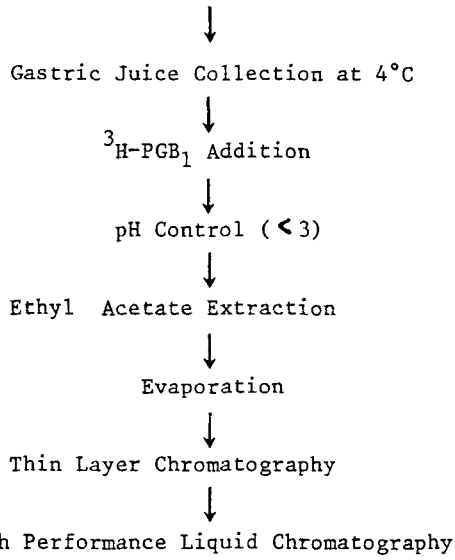
RESULTS

Separation of PGs

A typical chromatogram of biological samples processed by the method described is shown in Fig.1. A good separation of cortisone, PGE_2 and PGB_1 was obtained. Retention times of these compounds were 3.12, 5.62 and 18.06 minutes respectively.

TABLE 1

Scheme of the Sample Preparation Procedure



PG peaks were identified on the basis of their absolute and relative retention times and by adding known amounts of standard PGs to the samples.

Furthermore, in some sets of experiments, ^{3}H-PGE_2 was added to the samples, at various concentrations, immediately before injecting them into the chromatograph. A linear correlation(r=0.997) was obtained between the single amounts of ^{3}H-PGE_2, added to the samples and the radioactivity found in the fractions corresponding to the PGE_2 collected from the column.

In order to avoid the possibility of other compounds interfering with chromatographic separation of PGE_2, spectral scans of the fractions corresponding to PGE_2 peak of biological samples eluted from the column were performed in some sets of experiments by using a variable wavelength UV scanning spectrophotometer (Perkin-Elmer-Hitachi, mod.200). Spectral scans of the fractions eluted overlapped those of the corresponding standard preparations

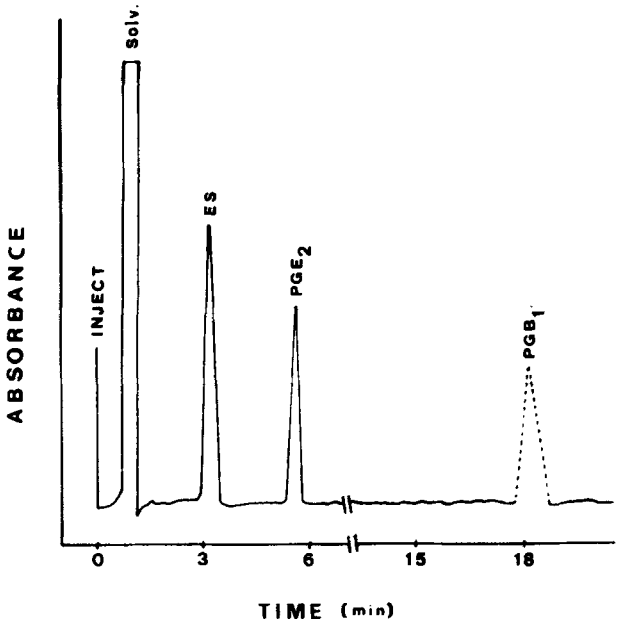


Figure 1. Representative HPLC chromatogram of a biological sample. Solv = Solvent; ES = Cortisone; PGB_1 = Area for collecting ^3H-PGB_1.

of PGE_2 at a maximum absorbance of wavelength between 192 and 193 nm.

Calibration Curve and Sensitivity

A linear calibration curve was obtained at concentrations of standard PGE_2 in the range used ($y = 0.00051940x - 0.003; r=0.999$).

The minimum detectable aliquot of PGE_2 in our samples was 20 ngin 20 µl injected into the chromatograph (S/N ratio=5).

Recovery

Total recovery of the method, obtained by evaluating the final cpm of ^3H-PGB_1 collected from the column in the biological samples, was 75 ± 2.08% (n=12). No radioactivity was recovered in the fra-

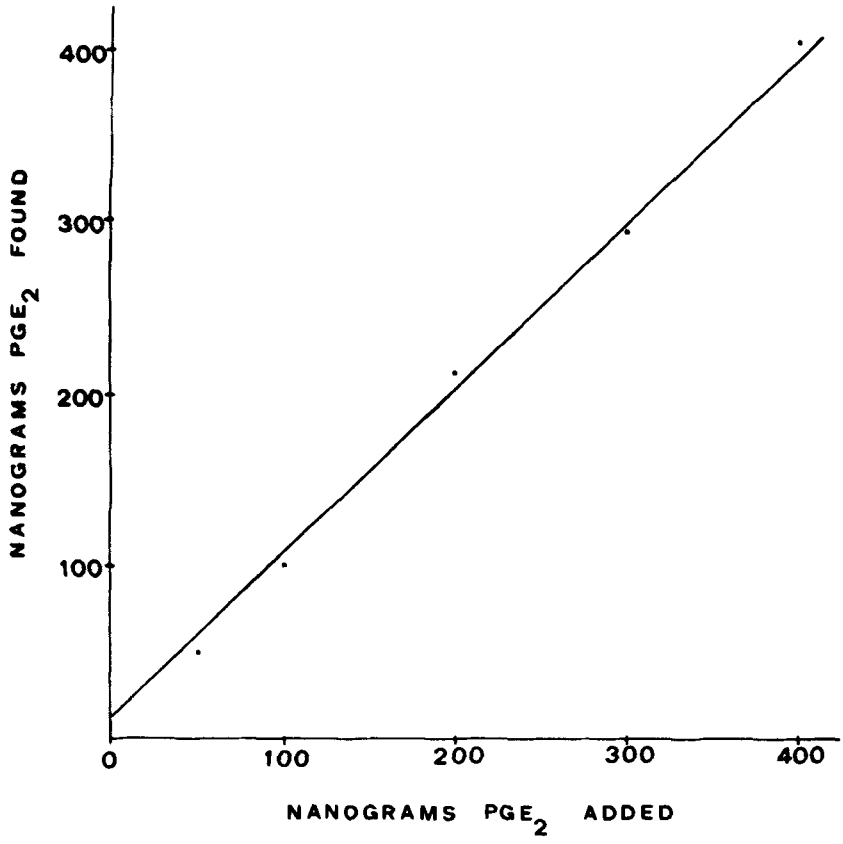


Figure 2. Recovery of PGE_2 added to gastric juice samples. All values are corrected for a recovery of 75% of PGE_2.

ctions collected before and after the one corresponding to the PGB_1 peak.

Accuracy

The accuracy of the method was checked by adding increasing amounts of PGE_2 (ranging from 25 ng to 400 ng) to 10 ml aliquots of gastric juice immediately before extraction (13). Figure 2 shows that the amounts of PGE_2 added can be reliably recovered from human gastric juice by the method described.

TABLE 2

Day-To-Day Precision Over Eight Weeks

PGE_2 Concentration	CV%
ng	
1600	0.36
400	1.26
200	2.78
100	4.50
50	0.35
25	1.50

n = 15

CV = Coefficient of variation

n = Number of assay

ng = Quantity injected

Precision

Day-to-day precision was evaluated by adding different amounts of standard PGE_2 to the same biological samples and processing them several times by the same method during a period of eight weeks. It was expressed as coefficient of variation (table 2).

PGE_2 Gastric Juice Concentration

The healthy donors studied showed a gastric juice basal secretion of 51±5.8 ml(mean value ± SE) during a period of 30 minutes. According to the HPLC determination, gastric juice concentration of PGE_2 was 504 ± 21 pg/ml (mean value ± SE).

DISCUSSION

The use of HPLC seems to be a very rapid and practical method for PG determination in the gastric juice. The specificity of the method is based on: 1) absolute and relative retention ti-

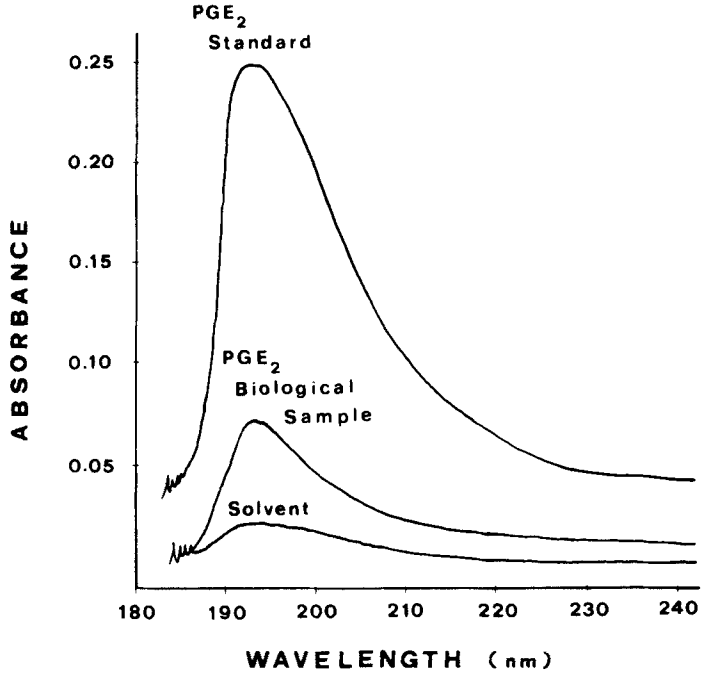


Figure 3. UV spectral scans of the fractions corresponding to PGE_2 peak of a biological sample and of a standard solution (800 ng/20 µl) eluted from the HPLC column. Solvents: 17mM H_3PO_4:CH_3CN(67.2:32.8,v/v).

mes of PGE_2; 2) enrichment of biological samples by standard preparation of PGE_2 and ^3H-PGE_2 immediately before the injection into the chromatograph; 3) performance of spectral scans of the eluted fractions corresponding to the PGE_2 (Fig.3).

Because of the very low UV wavelength (192.5 nm) used for detecting PGE_2 without derivatization, TLC pretreatment of the extracted samples provides a helpful procedure to reduce interference in the HPLC determination.

The sensitivity, although lower than that obtained by RIA and fluorescent derivative HPLC determination, is sufficient for detection of PGE_2 in the gastric juice. On the other hand, our procedure

offers a high specificity and the possibility of performing simultaneous analyses of PG mixtures.

The values reported in the study are similar to those obtained by Peskar at al. with RIA (4).

The high yield, accuracy and speed of chromatographic separation (less than 20 minutes) make this method particularly suitable and reliable for PGE_2 determination in the gastric juice in normal and pathological conditions.

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