Microcolumn Chromatographic Cleanup and GLC Determination of 11-Methyl-16,16-dimethylprostaglandin E_2 in Polyethylene Glycol 400 Solutions

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Abstract □ An analytical procedure is described for the GLC determination of 11-methyl-16,16-dimethylprostaglandin E_2 in aqueous polyethylene glycol 400 solutions. Because of the nature of the carrier matrix, sample cleanup was required prior to GLC separation. Separation was achieved using a diethylaminoethylcellulose microcolumn. This procedure has proven to be amenable to routine analysis.

Keyphrases □ Prostaglandin analogs—GLC analysis, pre-GLC sample cleanup procedure, polyethylene glycol carrier matrix □ GLC—prostaglandin analog determination, sample cleanup prior to GLC, polyethylene glycol carrier matrix □ Polyethylene glycol—carrier matrix in aqueous prostaglandin analog solutions, GLC analysis, pre-GLC sample cleanup procedure

Research into the synthesis of prostaglandin analogs that provide increased metabolic and chemical stability over naturally occurring prostaglandins has focused on the prostanoid 11-methyl-16,16-dimethylprostaglandin E_2[1] (I), the structure of which differs only slightly from that of natural prostaglandin E_2 (II). Previous studies (1, 2) showed that I is a potent inhibitor of gastric acid secretion in humans and dogs with no appreciable side effects.

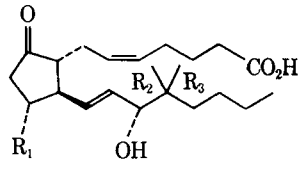
In compliance with the Good Laboratory Practices (GLP) regulations, aqueous solutions of I were analyzed as part of an analytical monitoring program for rat and dog toxicity studies. Many GLC and high-performance liquid chromatographic procedures have been published for the analysis of prostaglandin E_2 (3–13), but none was suitable for the present application because of interferences from excipients in the sample solutions.

This paper describes a sensitive and specific procedure for the analysis of I in aqueous media containing polyethylene glycol 400, butylated hydroxyanisole, and ascorbyl palmitate (III). The procedure involves the elimination of water and polyethylene glycol 400 by utilizing a diethylaminoethylcellulose microcolumn, conversion of I to the trimethylsilyl derivative, and GLC determination.

EXPERIMENTAL

Reagents—All solvents and chemicals were reagent grade unless otherwise specified.

Instrumentation—The gas chromatograph[2] was equipped with a hydrogen flame-ionization detector connected to an electronic chromatographic automation system[3] for peak area integration. The stainless steel column, 2.5 m × 2 mm i.d., was packed with 3% OV-1 on Chromosorb W-HP[4] (80–100 mesh) and conditioned at 320° for 24 hr under nitrogen at a flow rate of 30 ml/min. The instrument parameters were: injector temperature, 280°; detector temperature, 300°; carrier gas (nitrogen) flow rate, 30 ml/min; hydrogen flow rate, 30 ml/min; and air flow rate, 300 ml/min.



I: $R_1 = R_2 = R_3 = CH_3$
II: $R_1 = OH$, $R_2 = R_3 = H$

The column temperature was programmed stepwise isothermally from 0 to 12.5 min at 290°, followed by flash heating to 310° to elute III. Electrometer sensitivity was adjusted to give ~60% full-scale deflection for 8 μl of injected standard solution.

Sample Preparation—Aliquots of the sample solution (2.0 ml for I concentrations of >0.005% and 5.0 ml for those having <0.005%) were diluted to 50 ml with methanol.

Standard Preparation—Standard solutions were prepared by diluting I in methanol to give a final concentration of 8×10^{-3} mg/ml.

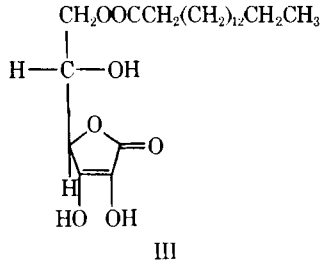
Internal Standard Preparation—Stock solutions of *n*-triacontane (IV), the internal standard, were prepared by dissolving 6 mg of IV in 50 ml of hexane. A 1.0-ml aliquot of this solution was added to a 12-ml conical flask and evaporated to dryness under a dry nitrogen stream. The residue was dissolved in 10 ml of trimethylsilylating reagent, which was a mixture of 60% hexamethyldisilazane[5] and 10% trimethylchlorosilane[5] in acetonitrile.

Microcolumn Cleanup Procedure—A diethylaminoethylcellulose[6] slurry was prepared by adding 300 ml of methanol to ~6.0 g of the ion-exchange medium. The slurry was degassed under vacuum for 30 min and transferred to a glass-stoppered solution bottle.

A glass column (15 cm × 6 mm i.d.) was used for the pre-GLC cleanup. The column, fitted with a hydrophilic polyethylene disk tapered to 1.65 mm i.d., was filled with the cellulose slurry to a gel height of 8 ± 1 mm (Fig. 1).

The column bed was washed with 2 ml of methanol, and the effluent was discarded. Based on the concentration of I, various volumes of the sample or standard solution (standard solution, 2.0 ml; samples with I concentrations of >0.02%, 2.0 ml; samples with I concentrations of <0.005%, 4.0 ml; and placebo, 4.0 ml) were applied to the top of the gel and allowed to absorb into the bed; the resulting effluent was discarded.

Interfering excipient substances were washed from the column with 4.0 ml of distilled water applied to the top of the gel bed, and the effluent was discarded. Residual water was removed from the gel by applying a



III

[1] Hoffmann-La Roche Inc., Nutley, N.J.
[2] Varian Aerograph model 3700, Varian Associates, Walnut Creek, Calif.
[3] Model 3385A, Hewlett-Packard, Palo Alto, Calif.
[4] Alltech Associates, Arlington Heights, Ill.
[5] Aldrich Chemical Co., Milwaukee, Wis.
[6] Preswollen DE-52, Whatman Inc., Clifton, N.J.

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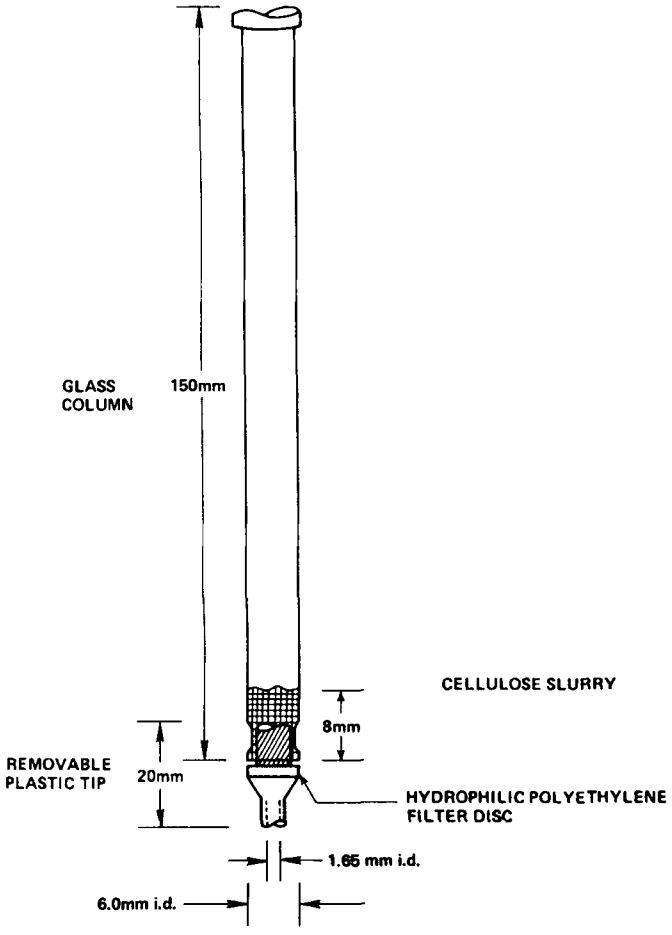

Figure 1—*Diethylaminoethylcellulose microcolumn utilized in pre-GLC sample cleanup procedure.*

gentle nitrogen stream to the top of the column for ~1 min. Compound I was eluted from the column with 7.0 ml of anhydrous ether, and the eluate was evaporated to dryness at room temperature under a nitrogen stream.

Preparation of Trimethylsilyl Derivatives—After the eluate was evaporated carefully to dryness, 400 μl of the trimethylsilylating reagent containing the internal standard (IV) was added, and the solution was left for 30 min at room temperature. This solution was injected onto the GLC column.

Calculations—The concentration of I in 40% polyethylene glycol 400 solutions was determined from:

$$\frac{\text{milligrams of I}}{\text{milliliters of solution}} = \left(\frac{X_{spl}}{Y_{spl}}\right)\left(\frac{Y_{std}}{X_{std}}\right)(C_{std})\left(\frac{DF}{V_I}\right) \qquad \text{(Eq. 1)}$$

where X_{spl} and X_{std} are the peak areas of I in the sample and standard preparations, respectively; Y_{spl} and Y_{std} are the peak areas of IV for the sample and standard, respectively; V_I is the volume of the initial sample aliquot (milliliters); DF is the sample dilution factor; and C_{std} is the concentration of I in the standard solution (milligrams per milliliter).

RESULTS AND DISCUSSION

The samples studied were aqueous solutions containing 40% polyethylene glycol 400, 0.05% ascorbyl palmitate, 0.02% butylated hydroxyanisole, and 0.001–0.02% (0.01–0.2 mg/ml) of I. In all cases, pre-GLC sample cleanup was necessary to eliminate sample matrix interferences.

Column chromatographic techniques have been used widely for sample preparation and cleanup procedures, but few applications employing ion-exchange resins have been reported where the adsorptive properties of the resin matrix were a factor in the separation. By utilizing ion-exchange resins and either nonpolar solvents or electrolyte-free water–alcohol mixtures as eluents, separations have been achieved for sugars (14), alcohols (15), organic acids (16), analgesics (17), and benzene derivatives (18, 19). In each of these applications, the dominant mechanism was adsorption onto the resin matrix with little or no ion exchange occurring.

With this technique, a microcolumn sample cleanup procedure was developed that eliminated matrix interferences in aqueous solutions of I. It was found that I could be separated selectively from polyethylene glycol 400 and isolated from water (water had to be eliminated before silylation reagents could be used) using a microcolumn containing diethylaminoethylcellulose (free base form). Columns were prepared by adding a degassed methanolic slurry of diethylaminoethylcellulose to a gel height of 8 ± 1 mm to a 15-cm × 6-mm i.d. glass column outfitted with a fritted disk. The small void volume of this column, a departure from most conventional procedures, was especially advantageous since only a small volume of solvent was needed for complete elution of I.

Aliquots of sample solutions of I were diluted with methanol and applied onto the microcolumns. Polyethylene glycol 400, I, and III were retained on the diethylaminoethylcellulose due to a combination of adsorption onto the cellulose matrix backbone and ion exchange with the diethylaminoethyl functional groups bound to the cellulose. The ion-exchange interaction was confirmed by attempting the same separation using cellulose without the diethylaminoethyl functional groups. With this medium, definitive separation of polyethylene glycol 400 and I was not accomplished, and the results were not reproducible.

With diethylaminoethylcellulose, solvent systems were investigated

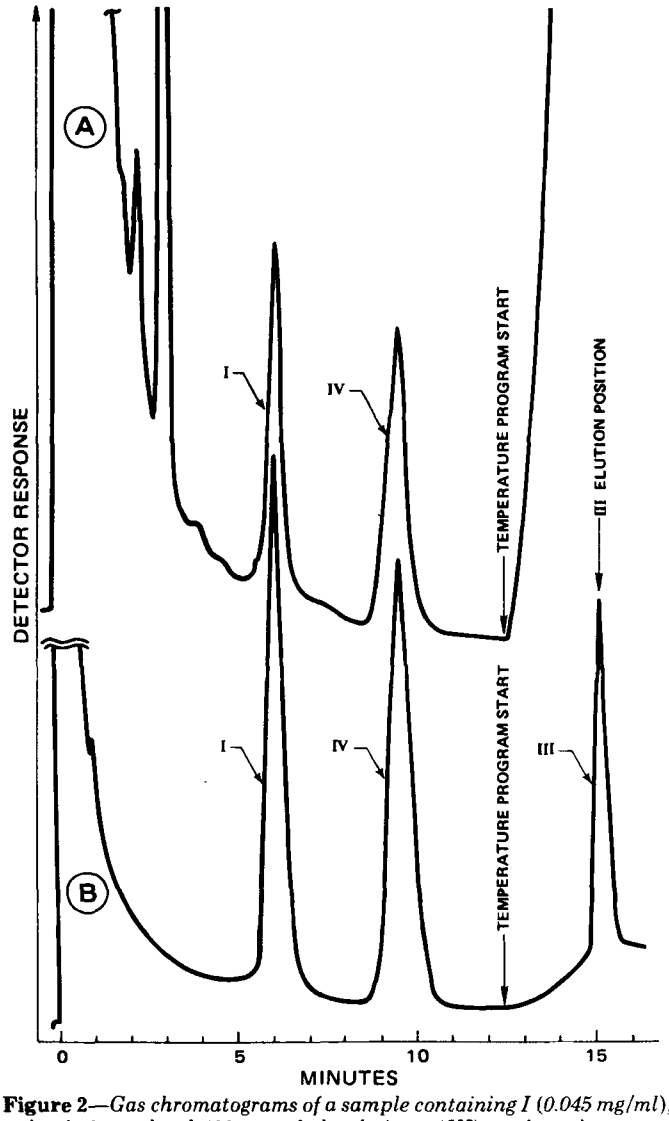

Figure 2—*Gas chromatograms of a sample containing I (0.045 mg/ml), polyethylene glycol 400, ascorbyl palmitate (III), and n-triacontane (IV) as the internal standard after pre-GLC sample cleanup for which detector response was 2 × 10^{-11} amp/sec full scale (A); and a standard stock solution containing I, ascorbyl palmitate (III), and n-triacontane (IV) for which the detector response was 16 × 10^{-11} amp/sec full scale (B).*

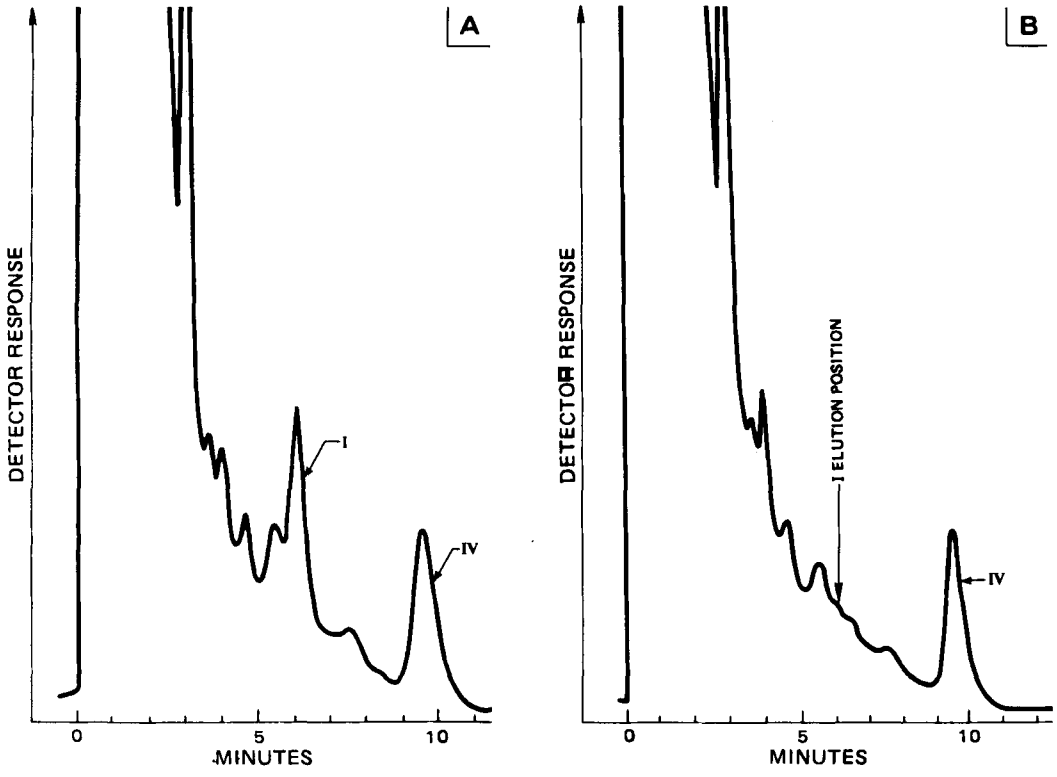

Figure 3—*Gas chromatograms of a sample containing I (0.01 mg/ml), polyethylene glycol 400, and* n*-triacontane (IV) as the internal standard after pre-GLC sample cleanup (A); and a placebo containing* n*-triacontane (IV) as the internal standard, after pre-GLC sample cleanup, prepared at the same excipient concentration level as Sample A (B). The detector response was 2 × 10^{-11} amp/sec full scale.*

Table I—Assay Results for Samples Containing I, Polyethylene Glycol 400, Ascorbyl Palmitate, and Butylated Hydroxyanisole

Sample Claim of I, mg/ml	*n*	Average Result ± *SD*, mg/ml	Percent of Claim	*RSD*, %
0.200	6	0.207 ± 0.00115	103	5.6
0.044	5	0.0469 ± 0.00530	106	11.3
0.010	3	0.00974 ± 0.00042	97.4	4.3

in which I would be retained on the column while polyethylene glycol 400 and III would be eluted; however, no system could be found with these properties that also would provide 100% recovery of I. For this reason, efforts were directed to the development of a system to remove polyethylene glycol 400 and water, leaving the separation of I and III by GLC as the next step.

Polyethylene glycol 400 was removed during the wash step by partitioning with water; I and III, having little affinity for water, remained bound to the column. The latter compounds then were eluted with anhydrous ether. The resulting eluate was evaporated to dryness under a nitrogen stream, and the residue was reacted with silylating reagent.

Baseline separation of I and III was achieved in 16 min by GLC using a 2.5-m × 2-mm i.d., 3% OV-1 on Chromosorb W-HP column for samples containing >0.045 mg of I/ml (Fig. 2). However, artifacts of polyethylene glycol 400 were found in those samples having <0.045 mg of I/ml because of the need to apply significantly more sample to the microcolumn for quantitative detection. Even at low levels, quantitation of I was not affected (Fig. 3).

The trimethylsilyl derivative of I was formed by reaction with a hexamethyldisilazane–trimethylchlorosilane mixture in acetonitrile, with no loss in potency of I detected after 24 hr. In earlier studies with stronger silylating reagents, I degradation was observed by GLC as a peak eluting prior to I.

Results of validation studies have shown that the procedure is accurate, precise, and suitable for routine analysis. The loading capacity of the microcolumns was linear over a range of 7.50–22.5 μg of I, with a least-squares correlation coefficient of 0.997. Results of a recovery study using the standard addition method yielded a mean value of 101%. A method precision study, conducted with 15 replicate samples, yielded a coefficient of variation of 7.3%. The analysis of actual samples over a concentration range of 0.01–0.2 mg of I/ml yielded results with acceptable variation (Table I).

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