(trimethylsilyl)acetamide, or *N,N*-bis(trimethylsilyl)trifluoroacetamide, were totally unsatisfactory. The disappearance of trimethylsilylsalicylic acid with respect to trimethylsilyl-*p*-toluic acid over time was most demonstrable under the cork-stoppered vessel conditions. Polyseal screw-capped and ground-glass-stoppered vessels were satisfactory.

To determine the difficulty with the cork-stoppered tubes, pieces of cork were suspended in three screw-capped tubes, each containing a sample to be silylated along with one silylating reagent. A polyseal cap was used to seal off the tube, and incubation and analysis were performed as described. The chromatograms showed the same pattern as was found for the cork-stoppered vessels for each reagent. The cork probably absorbed the trimethylsilyl derivatives and thereby reduced the amount of measurable derivative.

Regarding the optimal time for injection of samples, the most reliable time for consistent results for salicylic acid was between 1 and 4 hr after incubation. On the other hand, aspirin was reported to be silylated almost instantaneously (4), and its trimethylsilyl derivative probably should be analyzed as soon as possible to reduce the extent of hydrolysis by the presence of moisture. If both salicylates are being analyzed simultaneously, a prescribed time for injection of the sample should be followed closely, recognizing the variables involved for optimizing each determination.

In conclusion, certain recommendations can be made pertaining to the routine assaying of salicylates by GLC. For the qualitative and quantitative analysis of the silylated derivative of salicylic acid, *N,N*-bis(trimethylsilyl)trifluoroacetamide is the silylating reagent of choice. Polyseal screw-capped stoppered tubes are also recommended as the reaction vessel due to their ease and reliability of use. In addition, injection should be performed between 1 and 4 hr after incubation for optimal and reliable results for salicylic acid.

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High-Performance Liquid Chromatographic Separation of C-15-Epimers of 15-Methylprostaglandin E_2 Methyl Ester and 15-Methylprostaglandin E_2

R. K. LUSTGARTEN

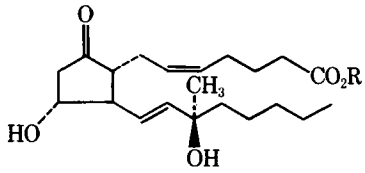
Abstract □ A high-performance liquid chromatographic system that permits quantitation of 15-methylprostaglandin E_2 and its methyl ester is described. Separation from the corresponding C-15 epimers is effected by adsorption chromatography on microparticulate silica with refractive index detection.

Keyphrases □ 15-Methylprostaglandin E_2 and methyl ester—high-performance liquid chromatographic analysis and separation from C-15-epimers □ High-performance liquid chromatography—analysis, 15-methylprostaglandin E_2 and methyl ester, separation from C-15-epimers □ Prostaglandins—15-methylprostaglandin E_2 and methyl ester, high-performance liquid chromatographic analysis and separation from C-15-epimers

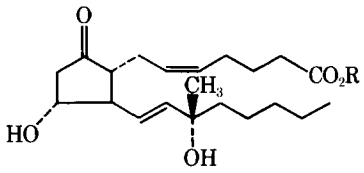
The methyl ester of 15-(*R*)-methylprostaglandin E_2 (I*a*) and its corresponding acid (I*b*) are potentially pharmacologically active in the treatment of gastric hyperacidity (1, 2). Procedures are known that would separate them from possible degradation products, *e.g.*, the prostaglandin A_2 and prostaglandin B_2 analogs (3–5), but there has been no efficient way of quantitating them in the presence of their 15-epimers (II*a* and II*b*). This paper presents a precise, rapid, and efficient method for doing so. The technique and data are discussed for the I*a*–II*a* system. Except for the composition of the mobile phase, the procedure and results for the I*b*–II*b* system directly parallel those for the esters, so this system will not be discussed in detail.

EXPERIMENTAL

Materials—Prostaglandin[1] samples, methyl acetate[2], and all other materials were obtained from commercial sources and used directly.



I*a*: R = CH_3
I*b*: R = H



II*a*: R = CH_3
II*b*: R = H

[1] The Upjohn Co.
[2] Fisher Certified grade.

High-Performance Liquid Chromatographic (HPLC) Procedure—The instrumentation was a modular setup consisting of a solvent delivery system[3], a loop injector[4], a 30-cm microparticulate silica gel column[5], and a differential refractometer for detection (attenuation = 8)[6] linked to a strip-chart recorder[7]. The mobile phases were 100% methyl acetate for I*a*–II*a* and 99.5% methyl acetate–0.5% acetic acid for I*b*–II*b*. The flow rate was 1.25 ml/min (~450 psi), and the injection volume was 8–10 μl.

Internal Standard Solution—This solution was prepared by dissolving sufficient 1,3-butanediol in the mobile phase to obtain a concentration of about 25 mg/ml. 1,6-Hexanediol, at 30 mg/ml, is a satisfactory alternative internal standard.

Reference Standard Solution—This solution was prepared by accurately weighing about 6.6 mg of the reference material and dissolving it in 1.0 ml of the internal standard solution.

Sample Solution—This solution was prepared in the same way as the reference standard solution.

Computation—Peak heights were measured and expressed in three significant figures. The following equation for the percent purity of the sample was then applied:

$$\text{percent purity} = \left(\frac{H_{is}W_r}{H_r}\right)\left(\frac{H_s}{H_{is}W_s}\right)(P) \qquad \text{(Eq. 1)}$$

where:

W_r = weight of reference material (milligrams)
W_s = weight of sample (milligrams)
H_r = peak height of reference material
H_s = peak height of sample
H_{is} = peak height of internal standard
P = percent purity of reference material

RESULTS AND DISCUSSION

Figure 1 shows a chromatogram of a simulated mixture of I*a*, II*a*, and the internal standard. Chloroform was added to mark the solvent front. Essentially baseline resolution of the epimers was achieved. The time from injection to complete elution of internal standard was about 7 min. Since dehydration products of I (*e.g.*, prostaglandin B and A analogs) are considerably less polar, they would appear on the solvent front and would not interfere with quantitation (3–5). Furthermore, products due to deesterification, *e.g.*, I*b* and II*b*, would remain on the column. Thus, the chromatography is highly selective for the compounds of interest. Moreover, the prostaglandin $F_{2\alpha}$ analogs of I*a* and II*a* have significantly longer retention times than II*a* under the conditions of Fig. 1.

The refractive index detector is inherently less sensitive than other popular forms of detection. This creates no problem in the present case, since there is virtually no limitation on sample size. The amount of sample injected corresponds to about 70 μg of material. Up to several hundred micrograms may be injected without loss of efficiency. The absolute limit of detection is about 1 μg. The sensitivity with respect to trace contamination by II*a* was studied separately (*vide infra*); about 1% could be detected. This limit could presumably be reduced if larger quantities of sample are injected.

The experimental response curve was measured over a range corresponding to about 40–85 μg of I*a*. (The concentration range was ~3–9 mg of drug/ml of internal standard solution.) Linear regression analysis revealed excellent linearity over this range. The correlation coefficient was 0.9998. Within experimental error, the response curve passed through the origin. Indeed, no interfering peaks were detected in a sample blank. Therefore, the procedure contains no systematic bias on accuracy.

Table I demonstrates the precision for the assay of an impure sample *versus* the reference standard. Satisfactory results were obtained: *RSD* = 0.87%.

Table II demonstrates the precision in measuring less than 1% of II*a* in I*a*. The sample was a synthetic mixture of the two epimers. The peak height for II*a* was measured by decreasing the detector attenuation by a factor of four relative to the attenuation of I*a*.

In quantitating II*a* relative to I*a*, a relative response factor was needed. This factor was determined by using the well-known base-catalyzed conversion of prostaglandin E_2 systems to the corresponding prostaglandin B_2 chromophore (6–8). The procedure was as follows. First, a peak height ratio was carefully determined for several synthetic mixtures of I*a* and II*a*. The mobile phase was then changed to ethyl acetate. This less polar mobile phase gives even better resolution of the two epimers and allows the quantitative collection of each peak.

The samples were reinjected, and each peak was collected in a small vial. The mobile phase was evaporated, and the residue was dissolved in 10.0 ml of a solution of 0.2 *N* KOH in 90% methanol. The solutions were allowed to stand for 0.5 hr, and the UV absorbance was determined at the maximum (278 nm) (6). The ratio of the absorbances was taken to be the true ratio of the two epimers. (It was shown independently that the absorptivities of the corresponding prostaglandin B_2 epimers are equal within experimental error.)

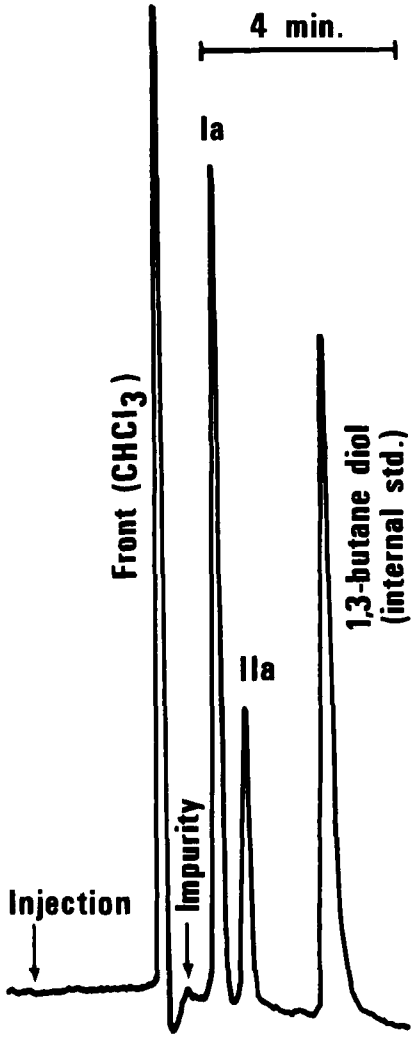


Figure 1—*HPLC chromatogram of synthetic mixture. Chromatographic conditions are given in the text.*

Table I—Precision of Assay

Sample Weight, mg	Assay Result, mg		Purity, %
3.855	3.687		95.6
5.994	5.600		93.4
7.088	6.704		94.6
5.233	4.930		94.2
7.892	7.388		93.6
4.351	4.127		94.9
		Average	94.4
		RSD	0.87

[3] LDC Corp.
[4] Waters model U6K.
[5] μ-Porasil, Waters.
[6] Waters model 401.
[7] Hewlett-Packard model 7127A.

Table II—Precision of Assay for Trace Quantity of IIa in a Simulated Mixture

Measurement	IIa Found, %[a]
1	0.86
2	0.78
3	0.82
4	0.82
5	0.72
6	0.83
7	0.84
Average	0.81
RSD	5.7

[a] Relative to Ia + IIa = 100% and computed with a correction for detector response as described in the text.

The original chromatographic peak height ratio could then be adjusted accordingly. The net result was that the Ia peak height had to be divided by 1.25; *i.e.*, Ia showed an apparent enhancement relative to IIa by a factor of 1.25 under the assay conditions. Equivalent experiments with the acids were performed, and IIa was enhanced relative to IIb by a factor of 1.23. The reason for the difference in detector response between the epimers is not known.

Sample and reference preparations are equivalent, quick, and routine. Consecutive chromatographic injections may be repeated every 7 min, or the injections may be staggered between the drug and internal standard to effect even shorter intervals.

The silica gel column appears to be quite stable. After several weeks of constant use, it gave chromatograms insignificantly different from the one shown in Fig. 1. This finding was also true for Ib, indicating that a small amount of acetic acid in the mobile phase does not cause significant deterioration of column performance. Some variability was noted, however, between different columns. For best results, the use of a column with 4000 or more plates/0.305 m, as measured and reported by the manufacturer, is recommended. Alternatively, ethyl acetate may be substituted for methyl acetate in the mobile phase. Because the former solvent is less polar than the latter, it gives better resolution of the epimers, albeit with a longer analysis time.

Preliminary experiments showed that the chromatographic system described here has good potential for separating various prostaglandin pairs, each characterized by being epimeric at C-15. Interestingly, the 15-(*R*)-epimer always was eluted before the 15-(*S*)-epimer. This trend was noted previously for some naturally occurring prostaglandins and their C-15-epimers (4).

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Comparison of Observed and Predicted First-Pass Metabolism of Nortriptyline in Humans

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Abstract □ The extent of first-pass metabolism of nortriptyline, calculated by comparing the areas under the plasma concentration–time curves following intravenous and oral dosing in six individuals, varied from 41 to 54%. Theoretically predicted values ranged from 41 to 61% based on a plasma flow model, indicating that the clearance takes place mainly from the plasma, which does not represent the whole blood concentration.

Keyphrases □ Nortriptyline—first-pass metabolism predicted using plasma flow rates, compared to observed rates □ Metabolism, first pass—nortriptyline, predicted using plasma flow rates, compared to observed rates □ Pharmacokinetics—nortriptyline, first-pass metabolism predicted using plasma flow rates, compared to observed rates □ Antidepressant agents—nortriptyline, first-pass metabolism predicted, compared to observed rates

The systemic availability of nortriptyline in humans was recently reported to vary from 46 to 59% (1). This low bioavailability compared to intravenous dosing was attributed to the first-pass metabolism of nortriptyline, assuming complete absorption from the GI tract and no extrahepatic metabolism (1). It was also postulated (1) that the plasma concentration represents the whole blood concentration.

The purpose of this report is to show that the last assumption may not be correct, since a theoretical prediction of first-pass metabolism can be made if plasma flow rates are considered instead of total blood flow, indicating restriction of nortriptyline to plasma or slow partitioning between plasma and blood cells (2).

DISCUSSION

Theoretical prediction of the first-pass metabolism of imipramine based on the equation of Gibaldi *et al.* (2) was reported (3):

$$\% FP = \frac{(\text{dose}/AUC)(100)}{\text{flow rate} + (\text{dose}/AUC)} \qquad \text{(Eq. 1)}$$

where % *FP* is the percent of drug metabolized during each pass through the liver, *AUC* is the area under the plasma concentration–time curve following oral administration, and flow rate is the blood or plasma flow rate through the liver.

It was shown (3) that good correlations can be obtained for im-