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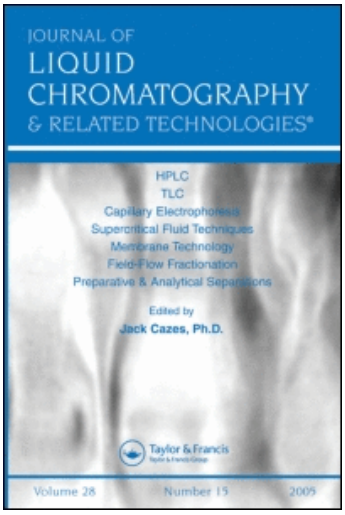

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
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To cite this Article Kenley, Richard , Lee, Kathleen M. and Mahoney II, T. Randall(1986) 'Stability-Specific HPLC Analysis of the Antiulcer Prostaglandin, Enprostil, in a Soft Elastic Gelatin Capsule Formulation', Journal of Liquid Chromatography & Related Technologies, 9: 16, 3577 — 3595
To link to this Article: DOI: 10.1080/01483918608077805
URL: http://dx.doi.org/10.1080/01483918608077805

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JOURNAL OF LIQUID CHROMATOGRAPHY, 9(16), 3577-3595 (1986)

STABILITY-SPECIFIC HPLC ANALYSIS OF THE ANTIULCER PROSTAGLANDIN, ENPROSTIL, IN A SOFT ELASTIC GELATIN CAPSULE FORMULATION

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ABSTRACT

This report describes a reverse-phase HPLC assay for the antiulcer drug, enprostil, formulated as a 0.3 mM solution (in propylene carbonate) filled into soft elastic gelatin capsules. The method uses: a 5 μm C18 column, a ternary (THF-methanol-phosphate buffer) mobile phase and 220 nm spectrophotometric detection to provide a sensitive and specific assay for enprostil and its major degradation products. The method satisfies the usual statistical criteria (recovery efficiency, response linearity, precision, lower quantitation limit) and performs well under various operating conditions as demonstrated by system suitability tests (analyte capacity factor dependence on column type, mobile phase composition, flow rate, and column temperature). We also demonstrate how a diode-array spectrophotometric detector

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facilitates: 1) identifying an optimal detection wavelength, and 2) verifying HPLC peak purity in samples containing intact drug plus degradation products.

INTRODUCTION

Enprostil (trademark GARDRIN) is an E-type prostaglandin analog (see Figure 1, structure I) that exhibits potent antiulcer activity (1). For oral administration, enprostil is formulated as a 0.3 mM propylene carbonate solution filled into soft elastic gelatin capsules. Although the conversion of E-type prostaglandins to the corresponding PGA and PGB analogs is generally facile (2-6), the soft elastic gelatin capsule formulation with propylene carbonate as solvent drastically retards enprostil chemical degradation (7). To directly demonstrate the satisfactory storage stability of the soft elastic gelatin capsule formulation, however, required a sensitive and specific assay for enprostil and its principal degradation products. Accordingly we undertook to develop an HPLC method for enprostil and its PGA and PGB analogs (structures II and III) in the soft elastic gelatin capsule formulation with the objective of quantitatively determining the percentage of intact drug and the product mass balance in samples exposed to different environmental conditions.

Representing challenges to method development were: the low enprostil concentration, the (even lower) concentrations of II and III present after partial enprostil degradation, the requirement to resolve enprostil from structurally-related compounds (IV through VI) present in the drug raw material as trace impurities, and the stereochemistry of enprostil. In the latter regard, the synthetic route to enprostil leaves the stereochemistry at two centers (the allenic carbon, C5, and the

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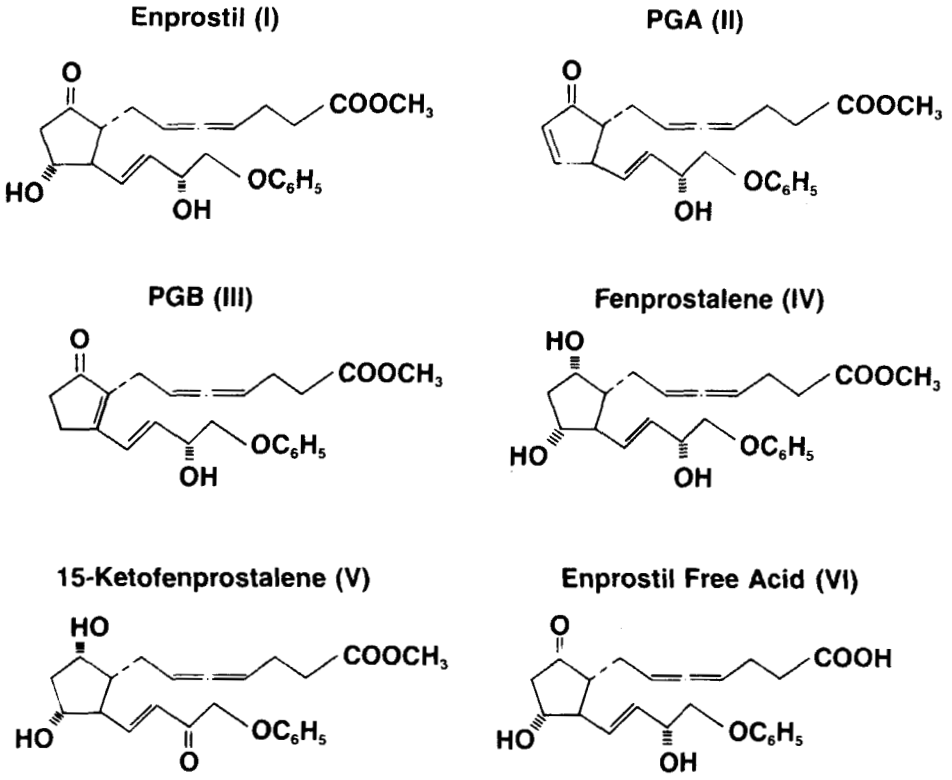

Figure 1. Structural Representations of Enprostil (I) and Related Compounds.

cyclopentanone ring carbon at C8) unresolved. Thus enprostil exists as two diastereomeric pairs of enantiomers that retain slightly differently during reverse-phase chromatography. The stereochemistry of enprostil dictates an optimal mobile phase composition because selective diastereomer retention causes peak broadening or peak splitting under chromatographic conditions that otherwise satisfactorily resolve enprostil from compounds IV and V.

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In the following, we report a specific procedure that resolves enprostil from known or potential interferences while preserving the peak symmetry necessary for quantitative analysis. We also present statistical indicators of method performance and the results of tests for method ruggedness. Finally, we demonstrate how diode-array spectrophotometric detection facilitates method development by identifying an optimal detection wavelength and by verifying enprostil peak purity in partially degraded samples.

EXPERIMENTAL DETAILS

Materials.

Compounds I-VI were provided by the Syntex Institute of Organic Chemistry. Structures and purities were determined by the usual spectroscopic and elemental analyses. Tetrahydrofuran (UV grade, glass distilled) and methanol (glass distilled) were from Burdick and Jackson Laboratories. Propylene carbonate was N.F. grade (Texaco). Reagent grade phosphoric acid (85%) and ammonium phosphate dibasic were provided by Mallinckrodt, Inc. A Barnstead filtration system was used to purify water for mobile phase and sample preparation. Soft elastic gelatin capsule samples (0.28-mL fill) were prepared by R.P. Scherer, Inc.

Apparatus.

The following components comprised the HPLC instrumentation: model 6000A pump, model 710B autosampler, model 730 data system (Waters Associates); model SF769 variable

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peak broadening or peak splitting under chromatographic conditions that otherwise satisfactorily resolve enprostil from compounds IV and V.

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The following components comprised the HPLC instrumentation: model 6000A pump, model 710B autosampler, model 730 data system (Waters Associates); model SF769 variable wavelength detector (Kratos Analytical Instruments); model 748C column oven (Spectra-Physics). Some experiments also employed a

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model 1040A diode-array spectrophotometric detector (Hewlett-Packard).

Conditions.

The 100 x 4.6 mm analytical column was a 5-μm particle size Partisil 5 ODS-3 RACII, from Whatman, Inc. Mobile phase was a 15:30:55 mixture of THF, methanol, and 1 mM phosphate buffer adjusted to apparent pH = 6.5. At a 1.0-mL/min flow rate and with the column maintained at 40°C, the back pressure was approximately 1500 psi. The detection wavelength was 220 nm, and the detector range was 0.02 AUFS.

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Procedures.

For sample preparation, five soft elastic gelatin capsules were cut open and the contents combined in a scintillation vial. A 0.5-mL aliquot of sample was pipetted into a 5-mL volumetric flask. The flask was filled to volume with dilution solvent (a 15:30:55:1 mixture of THF, methanol, water, and 8.5% phosphoric acid) and a 50-μL aliquot (=625 ng of enprostil) was filtered and injected onto the analytical column. Enprostil and compounds II and III were quantitated using peak height measurements versus external standards and the results expressed as a percentage of dosage form labeled strength (=0.3 mM). The standards were dissolved in methanol, and further diluted into a 1:10 mixture of dilution solvent (described above) plus propylene carbonate. Injected standard solutions and sample solutions thus contained equal concentrations of propylene carbonate. This procedure was necessary to correct for the influence of injected propylene carbonate on chromatographic peak shapes.

Suitable control experiments establish that enprostil, II, and III in dilution solvent are stable overnight when

refrigerated. Methanolic solutions may be stored for 1 mo at -20°C without appreciable degradation of the prostaglandin analogs.

Statistical Performance Indicators.

Recovery efficiency and response linearity were determined by spiking six placebos (in duplicate) with 70 to 120% of 0.3 mM enprostil and 5 to 20% of 0.3 mM II and III. Observed versus added concentrations were then used to calculate recovery and linearity, according to equations (1) and (2) respectively:

$$\% \text{ Recovery} = 100 \times [\% \text{ Found}]/[\% \text{ Added}] \quad (1)$$

$$[\% \text{ Found}] = \text{Intercept} + \text{Slope}^*[\% \text{ Added}] \quad (2)$$

Two analysts on two different days evaluated the method precision by assaying twelve samples total of the soft elastic gelatin capsules.

Method sensitivity was evaluated by spiking placebos with 0 to 25% of 0.3 mM enprostil and 0 to 5% of 0.3 mM II and III. The spiked placebos were then assayed and the observed response plotted versus [% Added] according to equation (3):

$$\text{Peak Height (mm)} = \text{Intercept} + \text{Slope}^*[\% \text{ Added}] \quad (3)$$

These same method sensitivity chromatograms were used to determine the lower quantitation limits for enprostil, II and III. We define the quantitation limit as the analyte concentration providing a signal-to-noise ratio of 2. Our conservative estimate of quantitation limit uses the intersection of the lower 95% confidence interval about the

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slope in equation (3) with a line representing twice the baseline noise.

System Suitability Tests.

To ensure method specificity and ruggedness, enprostil standards were spiked with compounds II through VI at 10% of 0.3 mM and assayed under a range of operating conditions (mobile phase composition, flow rate, column temperature, and so forth). Chromatograms were used to calculate the following performance parameters: tailing factor at 10% of peak height (T_s), theoretical plate count (N), height equivalent to theoretical plate (HETP), resolution factor (R), and capacity factor (k'). These five parameters were calculated according to the usual (8) equations.

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RESULTS AND DISCUSSION

Representative Chromatograms.

Figure 2 shows chromatograms of enprostil standard solution and an enprostil soft elastic gelatin capsule sample. Enprostil eluted at approximately 8 min with compounds II and III eluting at 13 and 16 min, respectively. The early-eluting peaks are the solvent artifact and propylene carbonate. Placebo chromatograms (not shown) demonstrated no significant interferences from the capsule material and propylene carbonate. Figure 3, a chromatogram of enprostil standard solution spiked with compounds II through VI, shows adequate resolution of the drug and its degradation products from potential interferences. Enprostil and compounds II through VI all exhibited rather broad peaks, reflecting the partial resolution of analyte stereoisomers (vide supra). As described below, however, the selected chromatographic

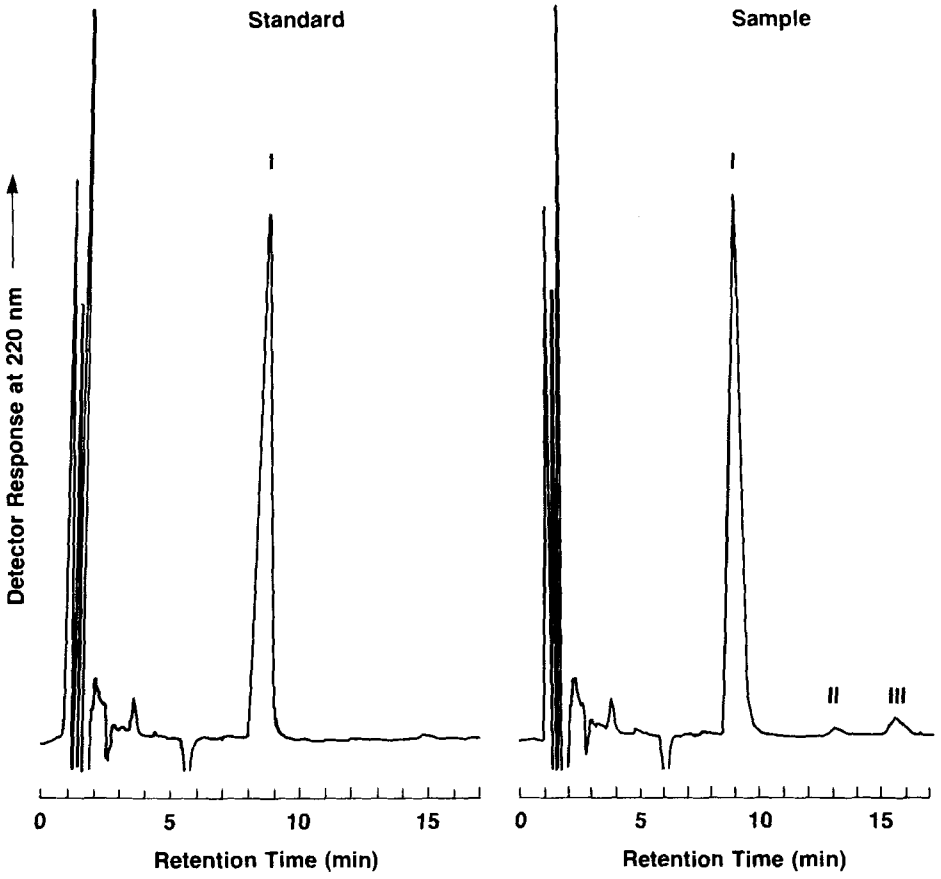

Figure 2. Representative Chromatograms for Enprostil Standard Solution (Left Chromatogram) and Enprostil Soft Elastic Gelatin Capsule Sample (Right Chromatogram). Roman Numerals Correspond to Structures Given in Figure 1.

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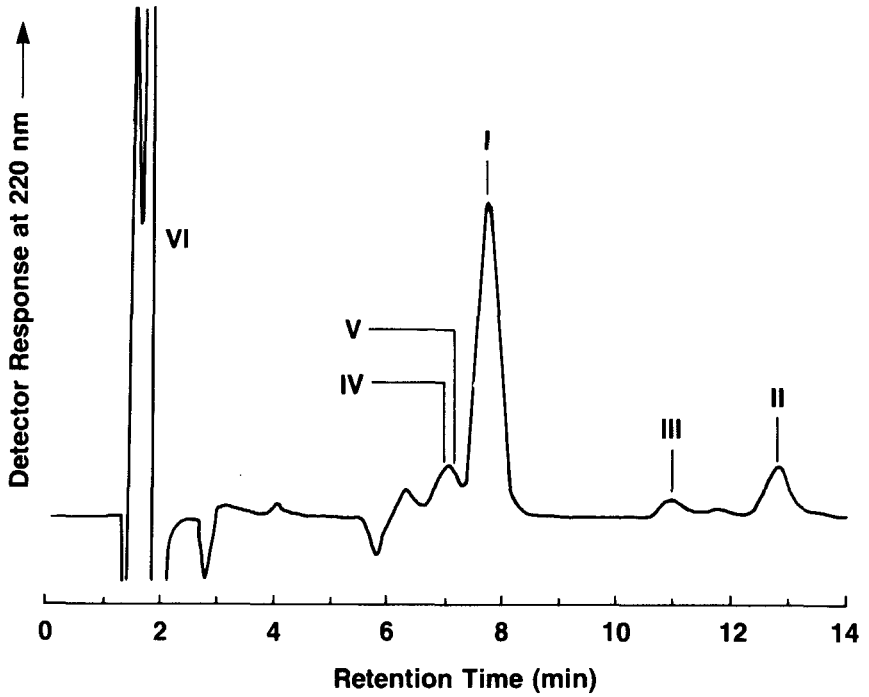

Figure 3. Representative Chromatogram of Enprostil Standard Solution Spiked With Known or Potential Interfering Compounds. Roman Numerals Correspond to Structures Given in Figure 1.

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conditions adequately resolved all injected solutes while maintaining satisfactory peak symmetry.

Spectral Determinations.

To choose a a detection wavelength suitable for quantitating low concentrations of enprostil plus compounds II and III, we injected a standard solution containing all three analytes and used a diode-assay spectrophotometer to record UV spectra during chromatographic elution. Figure 4

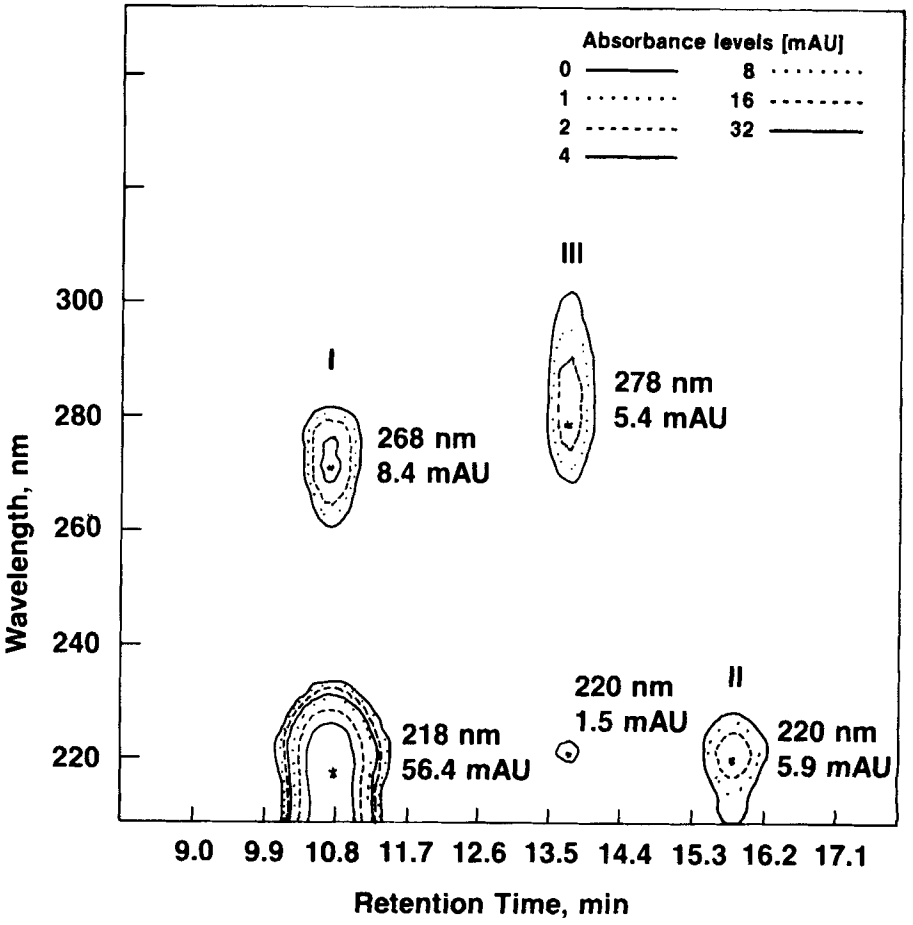

Figure 4. Isoabsorbance Contour Plot of Enprostil Standard Solution Spiked With Compounds II and III. Diode-array Spectra Captured During Chromatographic Elution of the Indicated Compounds.

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presents the data as isoabsorbance contours (9) for each HPLC peak, and demonstrates that intense, long-wavelength absorption maxima appeared for enprostil (268 nm) and compound III (278 nm), but not for compound II. Accurately quantitating all three analytes required a common wavelength with high extinction, and Figure 4 clearly shows 220 nm to be a wavelength satisfying this requirement.

The diode-array spectrophotometric detector also helped to establish the method specificity. Soft elastic gelatin capsules were heated until enprostil degraded to 90% of 0.3 mM and the samples were assayed. We then recorded UV spectra of the enprostil peak as it eluted from the column. Superimposing the 'upslope', 'downslope' and 'apex' spectra (Figure 5) established the spectral purity of the HPLC peak and verified specificity for enprostil.

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Statistical Performance Indicators.

Table I summarizes the method statistical validation (see Experimental Details Section) and shows that the method performs very well as measured by these common criteria.

System Suitability.

To establish the degree to which system operating conditions influence critical separations we measured chromatographic performance parameters (T_s, N, HETP, R, and k') as a function of: column type and source, column temperature and mobile phase composition and flow rate. Table II shows the dependence of chromatographic performance parameters on column type and manufacturer. Each column tested satisfactorily resolved enprostil from compounds IV and V, and compounds II and III from each other. The data in Table II suggest that N should exceed approximately 1700 and T_s

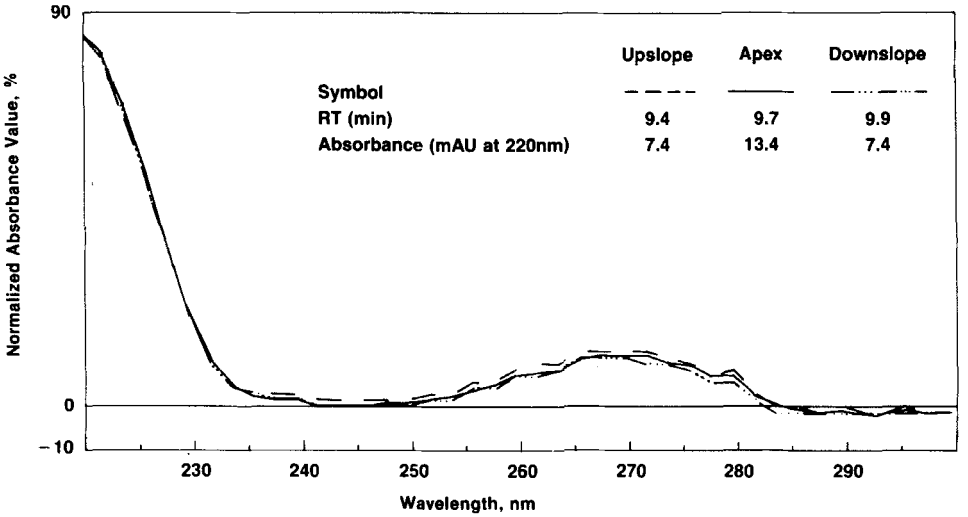

Figure 5. Superimposed Diode-Array Spectra Captured During Chromatographic Elution of Enprostil. Injected Sample Was from a Soft Elastic Gelatin Capsule Containing Enprostil at 90% of 0.3 mM.

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should remain below approximately 1.3 to maintain critical separations.

Of the six other system components examined, three showed no influence on retention of enprostil or compounds II through VI. Thus, analyte capacity factors were invariant with the following system changes: buffer concentration (0 to 2 mM), mobile phase pH (6 to 7), and flow rate (0.9 to 1.4 mL/min). As shown in Table III, however, analyte retention did strongly depend on: mobile phase methanol-to-THF ratio (at constant total organic fraction), mobile phase organic fraction (at constant methanol-to-THF ratio), and column temperature. The table demonstrates the influence of mobile phase THF

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TABLE I. STATISTICAL INDICATORS OF METHOD PERFORMANCE FOR ENPROSTIL ASSAY

Indicator	Statistic	Units[b]	Enprostil	II[a]	III[a]	Equation
Recovery	Mean	%	101.0	103	100	1
	Std. Dev.	%	0.57	4.5	3.5	
Linearity	Slope (+/- 95% Conf. Inter.)	-	1.01 (0.01)	0.990 (0.03)	0.980 (0.03)	2
	Inter. (+/- 95% Conf. Inter.)	%	0.08 (0.77)	0.26 (1.3)	0.14 (0.035)	
	Corr. Coeff.	-	0.9999	0.9995	0.9994	
Precision	Mean	%	97.6	3.2	5.2	See Experimental Details
	Std. Dev.	%	1.40	0.27	0.15	
Sensitivity	Slope (+/- 95% Conf. Inter.)	mm/%	1.18 (0.01)	1.59 (0.13)	0.841 (0.069)	3
	Inter. (+/- 95% Conf. Inter.)	mm		0.68 (0.34)	0.0033 (0.19)	
Lower Quantitation Limit	Limit	%	1.35	0.56	1.55	See Experimental Details
	Signal Level	mm	0.50	0.50	0.55	

a. See Figure 1 for structures.
b. % of 0.3 mM.

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TABLE II. SYSTEM SUITABILITY TESTS FOR DEPENDENCE OF CHROMATOGRAPHIC PERFORMANCE PARAMETERS ON COLUMN TYPE

Column #	Column Type	Performance Parameters[a] for Indicated Compounds[b]											
		k'					R				N	HETP	T
		IV	V	I	III	II	I-IV	I-V	I-III	II-III	I	I	I
1	Partisil-5 ODS-3 RAC II (Whatman, 10 cm)	5.47	5.77	6.30	9.61	11.5	1.46	0.829	4.57	2.38	1944	0.0514	1.03
2	Partisil-5 ODS-3 RAC II (Whatman, 10 cm)	6.13	6.42	7.22	10.8	13.0	1.90	1.24	4.92	2.82	2408	0.0415	1.00
3	Partisil-5 ODS-3 RAC II (Whatman, 10 cm)	5.54	5.77	6.34	9.59	11.4	1.58	1.00	5.23	2.66	2496	0.0401	1.02
4	Partisil-5 ODS-3 (Whatman, 25 cm)	5.46	5.68	6.21	9.44	11.1	1.82	1.11	6.70	3.12	3562	0.0702	1.26
5	Ultrasphere ODS-5 μ (Beckman, 25 cm)	4.96	5.02	5.58	7.75	9.47	1.84	1.39	5.56	4.19	4003	0.0624	1.14
6	Spherisorb ODS-5 μ (ASI, 25 cm)	4.76	4.79	5.19	7.51	8.82	1.18	0.97	5.43	2.93	3457	0.0723	1.10
7	μBondapak ODS-10 μ (ASI, 30 cm)	4.49	4.52	5.00	6.76	8.05	1.23	1.05	3.71	2.59	2483	0.121	1.46

a. See Experimental Details Section for parameter definition.
b. See Figure 1 for structures.

TABLE III. SYSTEM SUITABILITY TESTS FOR DEPENDENCE OF CHROMATOGRAPHIC PERFORMANCE PARAMETERS ON MOBILE PHASE COMPOSITION AND TEMPERATURE

System Parameter	System Parameter Value	k' Values for Compound[a]				
		IV	V	I	II	III
Methanol:THF Ratio[b]	45:0	c	c	c	c	c
	40:5	12.9	11.8	13.8	20.7	25.5
	35:10	8.41	8.27	9.37	14.1	17.2
	30:15	5.47	5.77	6.30	9.61	11.5
	25:20	3.94	4.38	4.74	7.36	8.72
Organic:Buffer Ratio[d]	39:61	11.1	11.6	13.0	20.4	25.1
	42:58	7.70	8.10	8.90	13.8	16.8
	45:55	5.47	5.77	6.30	9.61	11.5
	48:52	4.04	4.23	4.80	7.02	8.34
	51:49	3.01	3.18	3.41	5.17	6.00
Column Temperature (°C)	Ambient (23-25°C)	8.77	8.90	10.5	15.6	19.6
	30	7.51	7.79	8.83	13.2	16.2
	35	6.52	6.77	7.69	11.5	14.0
	40	5.88	6.16	6.85	10.4	12.5
	43	5.27	5.54	6.06	9.20	11.0
	45	5.16	5.44	5.99	9.02	10.7

a. See Figure I for structures.
b. Mobile phase composition = 45:55 mixture of organic phase (various methanol:THF ratios as shown) and buffer.
c. Retention time greater than 30 min.
d. Mobile phase composition = methanol:THF ratio of 2 with various organic:buffer ratios as shown.

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fraction on solute capacity factors and shows that THF:methanol ratios in the range 38:7 to 32:13 maintained satisfactory resolution. Similarly, total organic:buffer ratios in the range 39:61 to 48:52 maintained critical separations. Operating outside these mobile phase composition ranges resulted in peak broadening (or splitting) with attendant decreased resolution between components. Column temperature strongly affected analyte capacity factors, but satisfactory retention obtained at all temperatures in the investigated range (23 to 45°C).

Representative Determinations.

A principle objective in developing the method described herein was to accurately quantitate enprostil and its two degradation products (compounds II and III) at the low (<15%) conversions representative of a drug product maintained under realistic storage conditions. To demonstrate the utility of the method for these determinations, we maintained soft elastic gelatin capsule samples at 23°C and assayed capsules at timed intervals for enprostil, II and III content. At each timepoint we also calculated the mass balance normalized to initial enprostil concentration according to equation (4):

$$NMB = \{[enprostil]_t + [II]_t + [III]_t\}/[enprostil]_{t=0} \quad (4)$$

Table IV shows that (within the limits of experimental uncertainty) the NMB did equal 100% for all samples assayed and that the method satisfactorily accounted for enprostil loss and accumulation of compounds II and III even at conversions below 5%.

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Table IV.

Accumulation of Compounds II and III in Enprostil Soft Elastic Gelatin Capsules Maintained at 23°C

Time mo	Observed Amount[a] of Indicated Compound Enprostil	II	III	NMB[b]
0	106	0	0	100
6	102	2	c	96
12	103	2	c	100
18	100	3	2	100
24	99	5	3	100

a. Expressed as a percentage of 0.3 mM.
b. Normalized mass balance calculated according to equation (4).
c. Products not detected or detected at concentrations below the lower quantitation limit (~2% of 0.3 mM).

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CONCLUSIONS

We developed a reverse-phase HPLC analysis for soft elastic gelatin capsules that is sensitive and specific for enprostil and its degradation products. The method is convenient to use with respect to: sample preparation, run time, column backpressure, and absence of an internal standard. The method is rugged and performs well under a defined range of operating conditions. Quantitating enprostil and its degradation products in actual capsule samples demonstrated that the method satisfactorily measures product mass balance even at low extents of enprostil degradation. Thus, the method permits the direct demonstration of product integrity for samples maintained under realistic storage conditions.

Diode-array spectrophotometers have many applications in pharmaceutical analysis. In our laboratory, the diode-array technique to considerably shortens method development time by identifying optimal detection wavelengths and by verifying HPLC peak spectral purity.

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