High-Performance Liquid Chromatographic Determination of Suprofen in Drug Substance and Capsules

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Abstract □ A rapid, simple, stability-indicating assay procedure for suprofen, a new analgesic agent, in suprofen drug substance and in capsules was developed using high-performance liquid chromatography. Suprofen was extracted from the sample matrix with methanol and diluted with internal standard solution, and an aliquot was chromatographed on a reversed-phase column using acetonitrile–pH 3.0 buffer solution as the mobile phase. The selectivity of the chromatographic system for intact suprofen was demonstrated by resolving suprofen from synthetic intermediates, potential impurities, and reaction products resulting from accelerated stress conditions. The method is linear, quantitative, and reproducible. Either peak height or peak area ratios can be used for quantitation.

Keyphrases □ Suprofen—high-performance liquid chromatographic determination in drug substance and capsules □ High-performance liquid chromatography—determination of suprofen in drug substance and capsules □ Analgesics—high-performance liquid chromatographic determination of suprofen in drug substance and capsules

Suprofen, α-methyl-4-(2-thienylcarbonyl)benzeneacetic acid (I), is a potent, new analgesic agent (1, 2) and inhibitor of prostaglandin synthetase (3, 4). Two analytical methods were previously reported for suprofen drug substance; a TLC method (5) was used to monitor suprofen stability and a high-performance liquid chromatographic (HPLC) method (6) was used to determine suprofen and its known metabolites in plasma.

This paper describes a rapid, simple, stability-indicating HPLC method for suprofen in suprofen drug substance and in capsules containing either 100 or 200 mg of suprofen.

EXPERIMENAL

Apparatus—The liquid chromatograph[1], equipped with a constant-displacement pump[2] and a UV detector[3] (254 nm), was operated at ambient temperature. Chromatograms were traced on a strip-chart recorder[4] or drawn by computer[5]. All analyses were performed using a 3.9-mm × 30-cm reversed-phase column[6]. Generally, an automatic sampler[7] was used to inject samples onto the column, but a septumless injector[8] or a fixed-loop injector[9] was also used. Peak height and peak area integrations and calculations were performed by computer[5].

Materials—All chemicals were reagent grade unless noted otherwise and were purchased from commercial sources. 4-Nitrobenzoic acid[10], acetonitrile[11], and methanol[11] were used without additional purification.

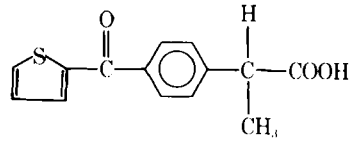
Chromatographic Conditions—The mobile phase was acetonitrile–pH 3.0 buffer solution (3:2). The pH 3.0 buffer solution was prepared by adding a 0.02 *M* dibasic sodium phosphate heptahydrate solution to a 0.01 *M* citric acid monohydrate solution until the solution pH was between 2.97 and 3.04. At least 500 ml of this solution was passed through a 0.22-μm filter[12], and 400 ml was thoroughly mixed with 600 ml of acetonitrile. The solution was sonified[13] for 15 min to degas the mixture and then equilibrated in the HPLC system at a rate of 0.5 ml/min.

Internal Standard Solution—The internal standard was 4-nitrobenzoic acid prepared as a 1.2 mg/ml solution in methanol.

Standard Solution—Suprofen standard, ~50 mg, was accurately weighed and transferred into a 25-ml volumetric flask; then it was dissolved and diluted to volume with methanol. Five milliliters of this solution was pipetted into a 10-ml volumetric flask. The flask was then diluted to volume with internal standard solution.

Standard Chromatogram—Two microliters of the standard solution were injected into the liquid chromatograph. The peak heights and peak areas obtained were used in the calculations for suprofen.

Suprofen Synthetic Intermediates, Impurities, and Reaction Products from Accelerated Stress Studies—α-Hydroxy-α-methyl-4-(2-thienylcarbonyl)benzeneacetic acid (II), 4-carboxy-α-methylbenzeneacetic acid (III), methyl-α-methyl-4-(2-thienylcarbonyl)benzeneacetic acid (IV), ethyl-4-(2-thienylcarbonyl)benzene (V), 4-acetyl-α-methylbenzeneacetic acid (VI), α-methyl-4-(2-thienylcarbonyl)benzene acetonitrile (VII), (1-bromoethyl)-4-(2-thienylcarbonyl)benzene (VIII), diethyl-2-methyl-2-[4-(2-thienylcarbonyl)phenyl]-1,3-propanedioate (IX), 4-fluorophenyl-2-thienylmethanone (X), α-methyl-4-(2-thienylcarbonyl)benzene acetamide (XI), 2-fluorophenyl-2-thienylmethanone (XII), 3-fluorophenyl-2-thienylmethanone (XIII),



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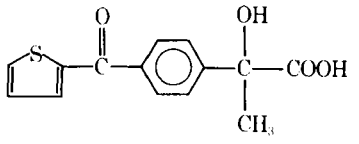

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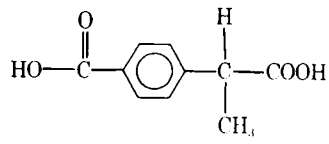

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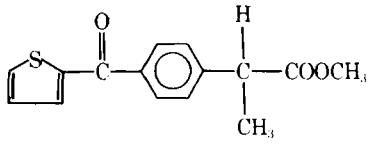

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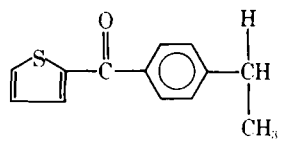

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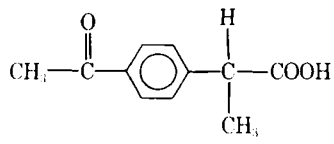

VI

[1] Model ALC 202, Waters Associates, Milford, Mass.
[2] Model M-6000, Waters Associates, Milford, Mass.
[3] Model 440, Waters Associates, Milford, Mass.
[4] Omniscribe recorder model B5117-IX, Houston Instruments, Austin, Tex.
[5] Model 3354C, Hewlett-Packard, Avondale, Pa.
[6] μBondapak C_{18}, Waters Associates, Milford, Mass.
[7] WISP autosampler model 710A, Waters Associates, Milford, Mass.
[8] Model U6K, Waters Associates, Milford, Mass.
[9] Model 7125, Rheodyne Inc., Cotati, Calif.
[10] Catalog No. N1179-5, Aldrich Chemical Co., Milwaukee, Wis.
[11] Distilled in glass, Burdick & Jackson, Muskegon, Mich.
[12] Millipore filter type GC.
[13] Model SC-200T, Sonicar Instrument Corp., Copiaque, N.Y.

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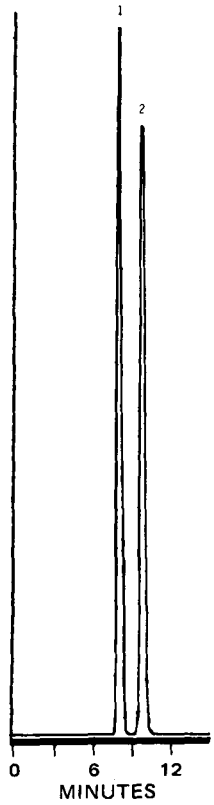


Figure 1—*Typical chromatogram obtained from a suprofen(I) capsule sample. Key: peak 1, internal standard; and peak 2, suprofen.*

α-methyl-4-(5-chloro-2-thienylcarbonyl)benzeneacetic acid (XIV), 2-[4-(2-thienylcarbonyl)phenoxy]propanoic acid (XV), methyl-4-(2-thienylcarbonyl)benzene (XVI), and 4,4-carbonylbis(α-methylbenzeneacetic acid) (XVII) were obtained[14] and chromatographed as methanolic solutions (~2 mg/ml).

Accelerated Stress Studies—Accelerated degradation of suprofen was accomplished by several methods:

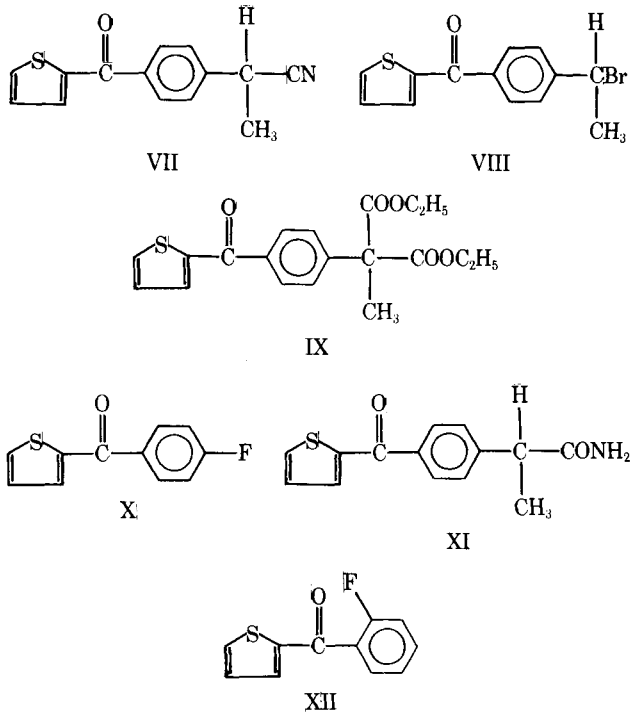
1. Suprofen drug substance (50.6 mg) was heated at its melting point of 124° for 1 hr in an oil bath.
2. Suprofen drug substance (50.5 mg) was dispersed in 5.0 ml of 1 *N* HCl and kept at 50° for 72 hr.
3. Suprofen drug substance (50.6 mg) was dispersed in 5.0 ml of 1 *N* NaOH and kept at 50° for 72 hr.
4. Suprofen drug substance (50.2 mg) was dispersed in 5.0 ml of 3% hydrogen peroxide solution and kept at 50° for 72 hr.

After 72 hr, the acid and base samples were neutralized and diluted to 25.0 ml with methanol. The excess peroxide was destroyed by gently heating the solution and then diluting to 25.0 ml with methanol. The sample heated at its melting point was made to 25.0 ml with methanol.

TLC Studies—TLC evaluation of the accelerated stress samples was carried out by spotting the equivalent of 100 μg of suprofen on silica gel plates[15] and developing them 15 cm in tanks lined with adsorbent paper in each of the following systems: System I, *n*-hexane–chloroform–methanol–strong ammonia (50:30:20:1), suprofen R_f 0.18; System II, *n*-hexane–dioxane–acetic acid (80:20:1), suprofen R_f 0.12; and System III, chloroform–methanol–methyl ethyl ketone (40:30:30), suprofen R_f 0.48. Visualization was by short wavelength UV light (254 nm).

Suprofen Recovery Studies—To simulate 100 mg of suprofen capsules, an accurately known amount of suprofen drug substance was transferred to a 50- ml volumetric flask containing 120 ± 2 mg of placebo. For 200-mg suprofen capsules, an accurately known amount of suprofen drug substance was transferred to a 100-ml volumetric flask containing 135 ± 2 mg of placebo. For both studies, 10 individual synthetic samples were prepared. The 200-mg study was conducted by two different operators on different days. Methanol was added to the flasks, which were then shaken[16] for 15 min. After the flasks were made to volume with methanol and thoroughly mixed, a portion of the solution was filtered[17]

[14] Janssen Pharmaceutica, Beerse, Belgium.
[15] Silica gel 60F-254, 0.25-mm thickness plates, E. Merck, Darmstadt, West Germany.
[16] Model 75 wrist action shaker, Burrel Corp., Pittsburgh, Pa.
[17] Millipore filter type FHLP, 0.22 μm pore size.



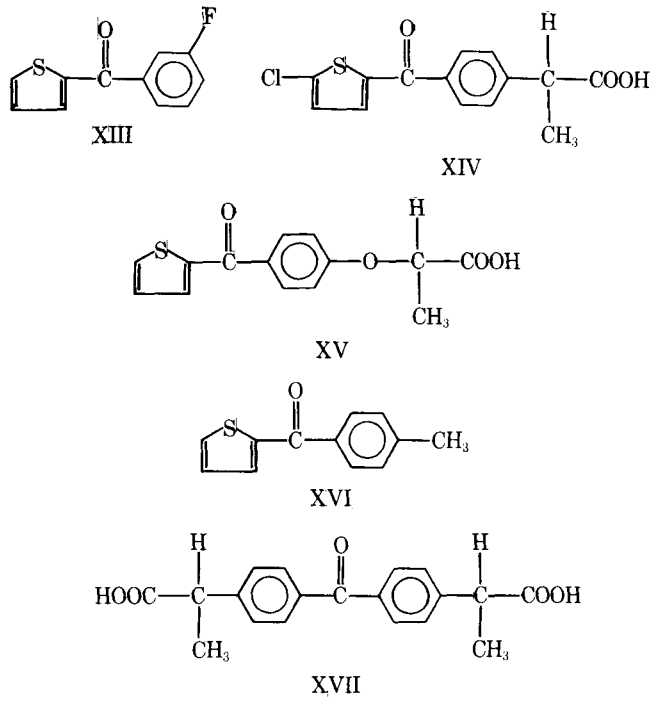
using a syringe equipped with a Swinny adapter. A 5.0-ml aliquot of the filtrate was pipetted into a 10-ml volumetric flask, which was then diluted to volume with internal standard solution. Then 2-μl portions were injected into the liquid chromatograph using either manual or automated injection techniques.

The suprofen recovery was calculated from:

$$\text{percent suprofen} = \frac{R_{sam}}{R_{std}} \times \frac{W_{std}}{W_{sam}} \times D \times 100 \qquad \text{(Eq. 1)}$$

where R_{sam} and R_{std} is the peak area or peak height ratio of suprofen to the internal standard for the sample and standard, respectively; W_{std} is the weight of suprofen standard; W_{sam} is the amount of suprofen taken; and D is a dilution factor, which is equal to 2 for the 100-mg spiked placebo capsules and 4 for the 200-mg spiked capsules.

Suprofen in Capsules—The contents of 20 suprofen capsules were weighed to determine the average capsule fill weight (C_{avg}) and were thoroughly mixed. For 100-mg suprofen capsules, duplicate 220 ± 3-mg



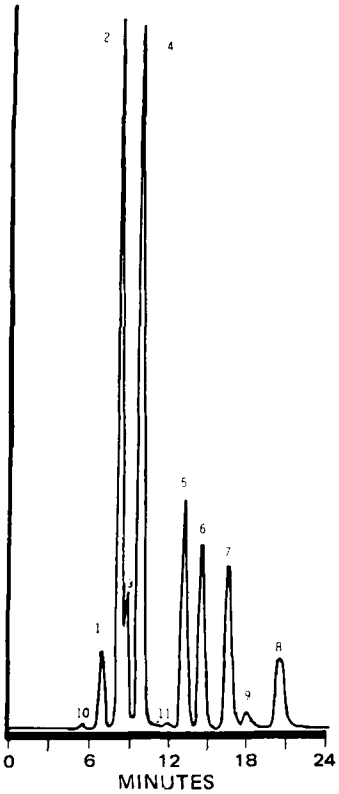


Figure 2—*Chromatogram showing separation of I from various structurally related compounds. Key: peak 1, III; peak 2, XV; peak 3, XVII; peak 4, I; peak 5, VII; peak 6, XVI; peak 7, X; and peak 8, IX. Peak 9 is an impurity in IX. Peaks 10 and 11 are unknown impurities.*

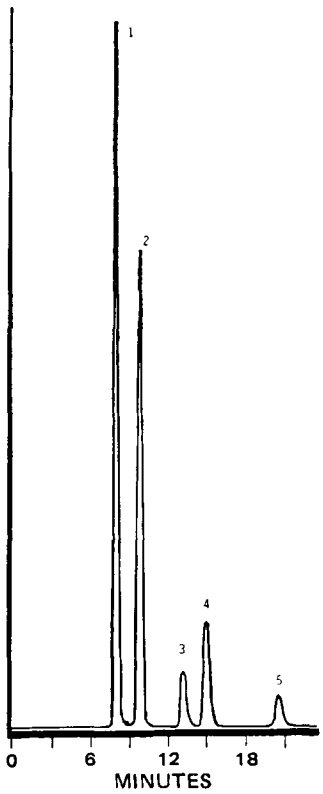


Figure 3—*Chromatogram showing separation of I from various structurally related compounds. Key: peak 1, VI; peak 2, I; peak 3, XIV; peak 4, XIII; and peak 5, VIII.*

samples were accurately weighed and transferred to a 50-ml volumetric flask. For 200-mg suprofen capsules, duplicate 335 ± 3-mg samples were accurately weighed and transferred to a 100-ml volumetric flask. The samples were assayed as already described and calculated as follows:

$$\text{milligrams of suprofen per capsule} = \frac{R_{sam}}{R_{std}} \times \frac{W_{std}}{W_{sam}} \times D \times C_{avg} \quad \text{(Eq. 2)}$$

Suprofen Drug Substance—The suprofen content of suprofen drug substance was determined by treating it like a suprofen standard solution. If samples are assayed for purity, the internal standard need not be added, although a fixed-loop injector should be used. The suprofen content may be calculated using Eq. 1 with a dilution factor (D) equal to 1.

System Suitability—The chromatographic system is considered to be performing satisfactorily if the internal standard has a retention time of 6.5–8.0 min, suprofen has a retention time of 9.0–11.0 min, and the calculated resolution between the two compounds is at least 2.0.

RESULTS AND DISCUSSION

The resolving power of the chromatographic system was demonstrated by chromatographing suprofen and a structurally related series of compounds that arise from various synthetic schemes as intermediates (V and VII–X), potential impurities (II, IV, and XI–XVII), or reaction products (III and VI) resulting from stress studies. The resolution of suprofen and the internal standard in a typical capsule sample is demonstrated in Fig. 1. Several mixtures of suprofen and the structurally related compounds were prepared and chromatographed to show the resolution obtained. The chromatograms resulting from these mixtures are shown in Figs. 2–4. Compounds II, VI, and XI were not totally resolved from the internal standard; if their presence in the sample is suspected, the internal standard should be deleted and the sample reinjected using a fixed-loop injector. Each compound was chromatographed with suprofen, and the relative retention time and resolution (R) were calculated with respect to suprofen (Table I).

Suprofen drug substance that was thermally stressed at its melting point afforded 97.8% recovery of suprofen with no extraneous peaks observed in its chromatogram. The sample subjected to peroxide oxidation afforded 97.4% recovery of suprofen. The chromatogram of this sample showed an extra peak with a retention time of 5.5 min. The suprofen sample subjected to acid hydrolysis afforded quantitative recovery of suprofen and did not show any extra chromatographic peaks. The sample subjected to base hydrolysis turned yellow during the 72-hr interval and afforded 92.8% recovery of suprofen. The chromatogram of this sample showed two extra small peaks at 6 and 7 min.

The stressed samples were qualitatively examined by TLC for any additional unknown spots using basic (System I), acidic (System II), and aprotic (System III) solvent systems. TLC evaluation was chosen because

Table I—Chromatographic Data for Suprofen and Structurally Related Compounds

Compound	Relative Retention Time	Resolution (R)
I	1.0	—
II	0.78	2.0
III	0.71	3.7
IV	1.54	5.3
V	2.12	8.6
VI	0.81	2.5
VII	1.34	3.4
VIII	2.11	8.3
IX	2.10	9.4
X	1.49	4.7
XI	0.86	1.8
XII	1.39	3.9
XIII	1.53	5.6
XIV	1.36	3.5
XV	0.91	1.1
XVI	1.71	6.1
XVII	0.84	1.9

Table II—Suprofen Recovery: 100-mg Dose Using Peak Height Ratio

Operator 1[a]			Operator 2[b]		
Suprofen Added, mg	Suprofen Found, mg	Recovery, %	Suprofen Added, mg	Suprofen Found, mg	Recovery, %
100.6	103	102	102.6	106	103
100.4	103	103	101.4	101	99.6
101.1	102	101	100.4	100	99.6
100.2	102	102	101.8	104	102
100.2	98.6	98.4	102.0	102	100

[a] Average recovery = 101 ± 1.76% (SD), and percent deviation = ±1.74%. [b] Average recovery = 101 ± 1.56% (SD), and percent deviation = ±1.55%.

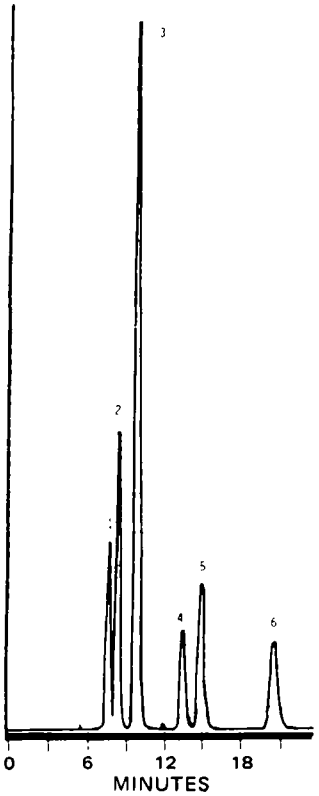


Figure 4—*Chromatogram showing separation of I from various structurally related compounds. Key: peak 1, II; peak 2, XI; peak 3, I; peak 4, XII; peak 5, IV; and peak 6, V.*

all substances present in the stressed sample would be carried through the TLC system. If an unknown UV-absorbing substance in the sample did not migrate in the system, it would still be detectable at the origin. Qualitatively, each of the three systems afforded identical results. The thermally stressed sample and the acid hydrolysis sample showed only the presence of suprofen. The peroxide-stressed sample, in addition to suprofen, showed a faint origin spot, which was in qualitative agreement with the HPLC results, which showed one extra peak. The base hydrolysis sample showed two extra spots, a small origin spot and a spot that was not totally resolved from suprofen. These results were also in qualitative agreement with the HPLC findings.

Since the HPLC system resolved suprofen from its synthetic intermediates, potential impurities, and reaction products from accelerated stress studies, showed drug loss due to decomposition in accelerated stress studies, and showed the presence of the same number of additional peaks from those studies compared to TLC, it was concluded that the method was stability indicating for suprofen.

Using peak height ratios, the detector response was demonstrated to be linear over the range of 0.0997–2.00 $\mu g/\mu l$ of suprofen (10–200% of label claim). Linear regression analysis of the data yielded a slope of 0.978, an intercept of −0.00995, and a coefficient of determination (r^2) of 0.9996. Using area ratios, the detector response was linear over the range of 0.200–1.50 $\mu g/\mu l$ of suprofen (20–150% of label claim). Linear regression analysis of the data yielded a slope of 1.01, an intercept of +0.00194, and coefficient of determination of 0.9999. Thus, the detector response was linear with either peak height ratios or peak area ratios.

The results of recovery studies conducted by two operators on 100 and 200-mg suprofen spiked placebo capsules are shown in Tables II and III,

Table III—Suprofen Recovery: 200-mg Dose Using Peak Height Ratio

Operator 1[a]			Operator 2[b]		
Suprofen Added, mg	Suprofen Found, mg	Recovery, %	Suprofen Added, mg	Suprofen Found, mg	Recovery, %
200.1	199	99.4	199.8	193	96.6
200.8	200	99.6	200.1	200	100
200.5	197	98.3	200.2	199	99.4
200.0	198	99.0	201.3	206	102
201.7	200	99.2	201.6	200	99.2
200.6	201	100	200.3	198	98.9
201.7	201	99.7	199.8	202	101
200.9	200	99.6	202.3	201	99.4
202.2	202	99.9	200.4	200	99.8
201.5	199	98.8	202.3	200	98.9

[a] Average recovery = 99.4 ± 0.53%, and percent deviation = ±0.53%. [b] Average recovery = 99.5 ± 1.42%, and percent deviation = ±1.42%.

Table IV—Analysis of Suprofen Capsules Using Peak Height and Peak Area Ratios

Capsule Sample, mg	Suprofen by Peak Height, mg	Suprofen by Peak Area, mg	Height/ Area[a]
100	96.9	96.7	1.002
	98.1	97.6	1.005
	97.2	96.5	1.007
	101	99.7	1.013
200	199	199	1.000
	196	196	1.000
	195	195	1.000
	197	197	1.000

[a] Theoretical height/area ratio = 1.000.

respectively, using peak height ratios to quantitate suprofen. Quantitative recovery of suprofen was obtained at both concentration levels. The overall recovery for the 30 samples was 100 ± 1.45%. The equivalence of quantitating suprofen by either peak area ratios or peak height ratios is shown in Table IV, where actual suprofen capsules were assayed and quantitated by both techniques. As shown, the ratio of the peak height result to the peak area result was essentially unity, indicating no difference between peak area and peak height results.

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