pharmacological action of the parent drug and possibly the metabolite. This finding is consistent with our previous observation of no significant alterations in the action potentials of rabbit sinoatrial node, atrioventricular node, and atrial fibers after a 1-h superfusion with therapeutically meaningful concentrations of amiodarone suspended in homologous plasma (19). Although similar studies with microelectrodes will be needed to establish the electrophysiological properties of the metabolite, our preliminary data on ECG changes suggest that the behavior of the metabolite is similar to that of the parent drug under acute conditions. However, this lack of the acute effect does not exclude the possibility that the accumulation of metabolite during chronic therapy may contribute significantly to the pharmacological and electrophysiological action of amiodarone. Thus, more definitive electrophysiological studies in rabbits treated chronically with amiodarone and its metabolite will be required to estimate the contribution of the metabolite to the observed pharmacological action of amiodarone during chronic administration.

In summary, both amiodarone and its major metabolite, the deethyl analogue, have similar serum kinetics after intravenous administration in rabbits and are rapidly taken up by the myocardium. Neither the parent drug nor its metabolite produces significant electrophysiological changes under acute conditions in concentrations that are therapeutically relevant. The data do not exclude the possibility that the metabolite exerts pharmacological effects that are similar to those of the parent compound during chronic dosing.

REFERENCES

(1) R. E. Kates and P. Jaillon, *J. Pharmacol. Exp. Ther.*, **214**, 31 (1980).

(2) N. Benowitz, R. P. Forsyth, K. L. Melmon, and M. Rowland, *Clin. Pharmacol. Ther.*, **16**, 87 (1974).

(3) Y. W. Cho, *Am. Heart J.*, **85**, 648 (1973).

(4) D. L. Keefe and R. E. Kates, *J. Pharmacol. Exp. Ther.*, **220**, 91 (1982).

(5) E. Patterson, P. Stetson, and B. R. Lucchesi, *J. Cardiovasc. Pharmacol.*, **5**, 541 (1979).

(6) A. Yacobi, H. F. Stampfli, C. M. Lai, and B. L. Kamath, *Drug Metab. Dispos.*, **9**, 193 (1981).

(7) K. Nademanee, J. A. Hendrickson, R. Kannan, and B. N. Singh, *Am. Heart J.*, **103**, 950 (1982).

(8) B. N. Singh, K. Nademanee, N. Ikeda, and R. Kannan, in "New Aspects in the Medical Treatment of Tachyarrhythmias," G. Breithardt and F. Loogen, Eds., Urben and Schwarzenberg, Munich, Federal Republic of Germany, 1983, p. 46.

(9) R. Kannan, K. Nademanee, J. A. Hendrickson, H. Rostami, and B. N. Singh, *Clin. Pharmacol. Ther.*, **31**, 438 (1982).

(10) G. C. A. Storey, D. W. Holt, P. Holt, and P. V. L. Curry, *Ther. Drug Monit.*, **4**, 385 (1982).

(11) R. J. Flanagan, G. C. A. Storey, and D. W. Holt, *J. Chromatogr.*, **187**, 391 (1980).

(12) C. M. Metzler, NONLIN, Upjohn Co. Tech. Rep. 7292/69/7291/005, 1974.

(13) M. Gibaldi and D. Perrier, in "Pharmacokinetics," Dekker, New York, N.Y., 1982, p. 412.

(14) L. Harris, W. J. McKenna, E. Rowland, G. C. A. Storey, D. M. Krikler, and D. W. Holt. *Circulation*, **64**, Suppl. IV, 263 (1982).

(15) F. Andreason, H. Agerback, P. Bjergaard, and H. Hotzche, *Eur. J. Clin. Pharmacol.*, **19**, 293 (1981).

(16) R. Latini, S. Conolly, R. Kernoff, and R. E. Kates, *Circulation*, **66**, Suppl. II, 223 (1982).

(17) R. Latini, S. J. Conolly, and R. E. Kates *J. Pharmacol. Exp. Ther.*, **224**, 603 (1982).

(18) E. Riva, M. Gerna, P. Neyroz, R. Urso, I. Bartozek, and A. Guaitani, *J. Cardiovasc. Pharmacol.*, **4**, 270 (1982).

(19) R. Kannan, N. Ikeda, K. Prasad, M. Ookhtens, and B. N. Singh, *Circulation*, **64**, Suppl. IV, 69 (1981).

ACKNOWLEDGMENTS

This work was supported by a grants-in-aid award from the American Heart Association, Los Angeles affiliate (756 G1-2) and by the Medical Research Service of the Veterans Administration. M. Drachenberg was a summer Research Trainee of the American Heart Association. We thank Dr. Keith Muir of the University of Southern California for helpful suggestions.

Analysis of Carprofen Dosage Forms and Drug Substance by High-Performance Liquid Chromatography

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Received August 19, 1983, from the *Pharmaceutical Research Products Section, Quality Control Department, Hoffmann-La Roche, Inc., Nutley, NJ 07110.* Accepted for publication September 26, 1983.

Abstract □ A high-performance liquid chromatographic method for the analysis of carprofen in solid dosage forms and as the bulk drug substance was developed. The simple, accurate, reproducible, and stability-indicating method was shown to be applicable to drug substance and dosage form stability studies, as well as the quality control of carprofen dosage forms.

Keyphrases □ Carprofen—analysis of dosage forms and drug substances, HPLC □ HPLC—analysis of carprofen dosage forms and drug substance

Carprofen [(±)-6-chloro-α-methylcarbazole-2-acetic acid (I)], under development as a nonsteroidal anti-inflammatory agent (1–9), has been formulated as a solid oral dosage form. Analytical procedures for the analysis of carprofen in biological fluids have appeared in the literature (10–16). However, since carprofen is a relatively new drug entity, analytical procedures suitable for the analysis and control of carprofen drug substance and dosage forms have not appeared in the literature or the USP.

The purpose of this work was to provide specific, accurate, and reproducible liquid chromatographic (HPLC) methods which would be applicable to various quality control functions, *i.e.*, drug substance stability studies, potency and content uniformity assays of dosage forms, and dosage form stability studies. To satisfy these criteria, it was necessary to develop a method which would separate I from all known impurities and potential degradation products and separate the impurities and potential degradation products from each other.

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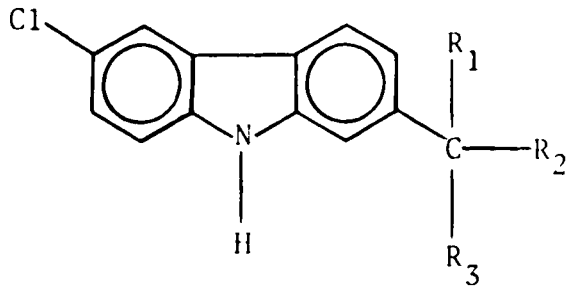

I: $R_1 = CH_3$, $R_2 = COOH$, $R_3 = H$
II: $R_1 = CH_3$, $R_2 = R_3 = CO_2C_2H_5$
III: $R_1 = CH_3$, $R_2 = CO_2C_2H_5$, $R_3 = H$
IV: $R_1 = CH_3$, $R_2 = OH$, $R_3 = H$
V: $R_1 = CH_3$, $R_2 = O$
VI: $R_1 = CH_3$, $R_2 = R_3 = H$

The dosage form assay procedure was designed to be amenable to either manual or automated injection. It was also designed so that quantification could be done either by a computer data acquisition system or by manual calculation of peak response.

EXPERIMENTAL SECTION

Materials—Benzophenone[1], anthraquinone[2], and glacial acetic acid[3] were all analytical reagent grade and were used as received. The methanol[4] and acetonitrile[4] used were distilled in glass. Water used in the preparation of mobile phases was doubly distilled.

Mobile Phase—The mobile phases used were prepared by mixing appropriately measured volumes of methanol and 1% acetic acid. The mobile phase selected for the assay was methanol-water (1% acetic acid) (65:35). All mobile phases were filtered before use.

Instrumentation—Portions of the present study were conducted with several different HPLC systems. The early developmental work on the dosage form assay was done with a constant-pressure single-piston pump[5], a fixed-loop injector[6], and a UV detector[7] set at 254 nm. The early developmental work on the drug substance stability assay was done with an HPLC system consisting of two constant-flow solvent-metering pumps[8], a solvent programmer[9], an injector[10], and an UV detector[11]. The major portion of the work was done with a 10-μm particle size octadecylsilane column[12]. Quantifications associated with developmental work on the dosage form assay were done with manual peak height measurements. Those associated with the drug substance stability study were done using peak area measurements obtained with an electronic integrator[13]. Subsequent to the earlier work, both dosage form and drug substance analyses were automated with an autosampler[14] and a more sophisticated computer data acquisition system[15].

Methods Development—Carprofen (I) and its known impurities (II and III) were chromatographed under various conditions until a suitable column–mobile phase combination was obtained that was capable of separating all compounds of interest. Samples from accelerated degradation studies were also chromatographed in this initial system to make certain that the system was also capable of separating potential degradation products. Compounds IV, V, and VI were isolated and identified as the major potential decomposition products[16]. These compounds were formed only under accelerated storage conditions. A study was also conducted in which the mobile phase composition was varied to determine the sensitivity of the separation to changes in mobile phase composition.

Dosage Form Assay Procedures—All solutions containing I were prepared by using low-actinic glassware and acetonitrile-water (50:50) as the diluent.

Internal Standard Solution—Benzophenone (380 mg) was accurately weighed into a 2-L volumetric flask and dissolved in 1 L of acetonitrile. Once dissolved, the resulting solution was diluted to volume with distilled water.

Standard Solution—Approximately 40 mg of carprofen reference standard was accurately weighed into a 100-mL volumetric flask. The standard was then dissolved and diluted to volume with the internal standard solution.

Sample Preparation for Potency Assay—Twenty tablets were combined and finely powdered with a mortar and pestle. From the composite, a tablet mass equivalent to 40 mg of I was accurately weighed into a 100-mL volumetric flask. The sample was dissolved in the internal standard solution by shaking for 15 min. After shaking, the sample was diluted to volume with the internal standard solution and filtered through a 5-μm filter[17].

System Suitability Test—Six injections of the standard solution were chromatographed, and the carprofen–internal standard response ratio was calculated for each injection. The system precision was considered to be adequate if the *RSD* for the six response ratio values did not exceed 1.5%.

The resolution factor (R^1) was calculated for the separation between the benzophenone and carprofen peaks according to the USP. The resolution was acceptable if R^1 was >3.5.

Potency Assay Procedure—The sample solutions were injected into the chromatograph using 20-μL injections. The carprofen content per tablet was then calculated by using the standard response ratio from the system suitability test, the respective peak heights in the sample chromatogram, the sample weight, and the appropriate dilution factor. Alternatively, peak areas obtained from a suitable computer system can be substituted for peak heights throughout the calculations.

Content Uniformity Sample Preparations—The same assay procedure as described above was applied to conduct content uniformity tests. For content uniformity measurements, the following sample preparation was substituted. One tablet was placed in each of ten 100-mL volumetric flasks. To each flask, 50 mL of the internal standard solution was added, and the flasks were shaken on a mechanical shaker for 1 h. Samples were then diluted to volume with internal standard solution and filtered as described above. Depending on the dosage strength, the samples were then appropriately diluted to obtain a final carprofen concentration of ~0.4 mg/mL. The final sample solutions were then chromatographed as described above, and the carprofen content per tablet was calculated.

Drug Substance Stability Assay Procedure—The same HPLC system was applied to drug substance stability testing. Several modifications in terms of sample preparation, amount of carprofen injected, and quantification techniques were necessary when the assay was to be used in bulk substance stability studies. For these assays, ~10 mg of carprofen was accurately weighed into a 10-mL volumetric flask, dissolved, and diluted to volume with the internal standard solution. Anthraquinone was used as an internal standard in some studies. During subsequent studies, it was discovered that use of benzophenone as an internal standard would shorten the analysis time. Either internal standard gave acceptable results. The carprofen concentration was determined by using an internal standard quantification technique, and the impurity and degradation product levels were estimated by using an area percent technique corrected for the presence of the internal standard in the chromatogram.

Validation—Both the dosage form assay and the drug substance stability assay were validated with respect to specificity, linearity, precision, and accuracy. The methods were also validated with both manual and automated

Table I—Method Precision of the Carprofen Dosage Form Assay

Sample Weighing	Assay Value, mg/Tablet[a]
1	150.6
2	149.3
3	149.5
4	150.7
5	150.0
6	150.3
7	151.3
8	150.1
9	149.7
10	149.3
11	150.9

[a] Mean, 150.2 mg per tablet; 101% of claim; *RSD*, 0.45%. Determined by internal standard quantification techniques; manual (loop) injection and manually measured peak heights.

[1] Fluka A. G., Buchs S. G., Switzerland.
[2] Pfaltz and Bauer Inc., Stamford, Conn.
[3] J. T. Baker Chemical Co., Phillipsburg, N.J.
[4] Burdick and Jackson, Muskegon, Mich.
[5] LDC mini-pump; Laboratory Data Control, Riviera Beach, Fla.
[6] Model 7010; Rheodyne, Inc.
[7] Model 1285; Laboratory Data Control.
[8] Model 6000A; Waters Associates, Milford, Mass.
[9] Model 660; Waters Associates.
[10] Model U6K; Waters Associates.
[11] Model 440 set at 254 nm; Waters Associates.
[12] Chromegabond C-18; E. S. Industries, Marlton, N.J.
[13] Model 3385A; Hewlett-Packard, Avondale, Pa.
[14] WISP Model 710B, Waters Associates.
[15] Computer Inquiry Systems, Waldwick, N.J.
[16] The full details of the isolation and identification of these compounds and studies on the degradation pathway of carprofen are the subject of a separate report.
[17] Type LS5; Millipore Corp., Bedford, Mass.

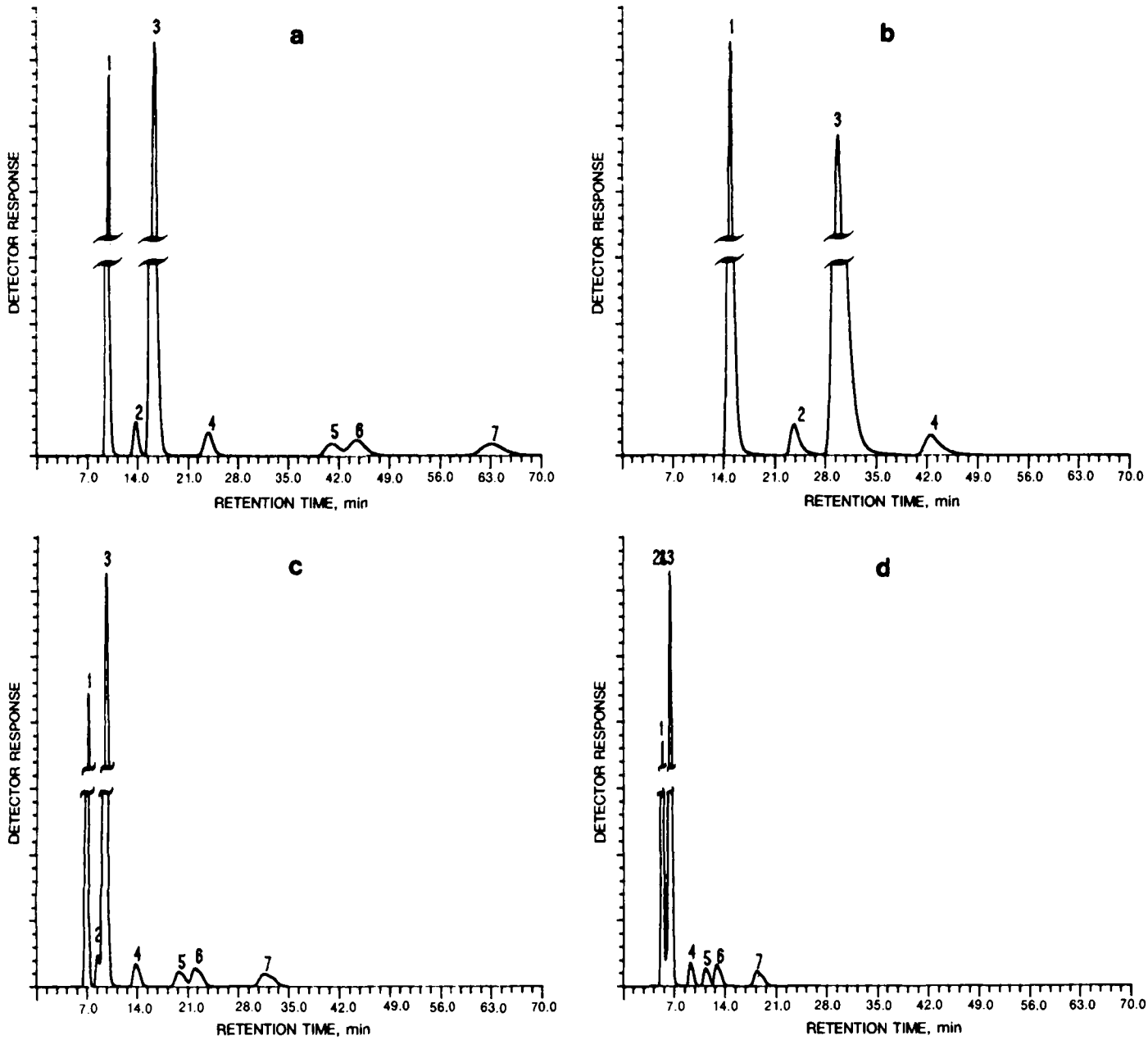

Figure 1—*Chromatograms of carprofen and related compounds* versus *the following percent methanol–water (1% acetic acid) in the mobile phase: (a) 65:35; (b) 60:40; (c) 70:30; (d) 75:25. Key: (1) benzophenone; (2) IV; (3) carprofen; (4) V; (5) III; (6) II; (7) VI.*

injection devices and by using both internal and external standard techniques.

RESULTS AND DISCUSSION

During preliminary methods development experiments, the carprofen peak exhibited marked tailing when chromatographed on a number of reverse-phase columns. This was believed to be due to partial ionization of the carboxylic acid function of the molecule in the mobile phase. Consequently, acetic acid was added to the mobile phase to suppress ionization and improve peak shape.

Since the intended purpose of the method was to supply a reliable and reproducible procedure suitable for assay, content uniformity, and stability studies, various columns and mobile phase compositions were carefully studied to achieve baseline resolution between carprofen, the internal standard, known impurities, and potential degradation products. The column[12] originally selected for the dosage form assay and used in the validation of the method was one that provided the desired separation within a reasonable analysis time and was commercially available at the time the method was developed. A chromatogram showing the separation between all possible components obtained with this column and the specified mobile phase composition is given in Fig. 1a. The sensitivity of that separation to variations in the mobile phase composition was studied with the same column (Fig. 1b–d). The data obtained were used to design an appropriate system suitability test for the dosage form assay. The separation most adversely affected by variations in mobile phase composition and most critical to the specificity of the dosage form assay is the separation between IV and carprofen (Fig. 1). Direct assurance of adequate separation between these two components could be obtained by specifying a minimum resolution factor between these two compounds in a system suitability test. However, this would require that all laboratories have access to a reference standard of IV. To make the method generally applicable for any laboratory and to eliminate the need for a reference standard of IV, an alternative approach was taken. The resolution factors R^1 (carprofen–internal standard) and R^2 (IV–carprofen) were obtained and plotted as a function of mobile phase composition (Fig. 2). For symmetrical peaks, minimum baseline resolution between two peaks should yield a resolution factor >1.0. By using the plot in Fig. 2, the appropriate resolution factor (R^1) which would simultaneously yield an R^2 value ≥ 1.0 was determined. The value for R^1 selected and included in the system suitability test was $R^1 \geq 3.5$. With the column specified, all mobile phase compositions which resulted in an R^1 value ≥ 3.5 also produced an R^2 value > 1.0 (Fig. 2). Therefore, by performing the system suitability test with the specified column and adjusting the mobile phase

Table II—System Precision of the Carprofen Dosage Form Assay

Injection	RR/Internal Standard[a]	RR/Internal Standard[b]	RR/Internal Standard[c]	External Standard[d]	External Standard[e]
1	0.4478	0.9177	1.683	429.3	23664
2	0.4445	0.9239	1.683	434.6	23744
3	0.4468	0.9220	1.683	436.2	23793
4	0.4449	0.9241	1.681	438.3	23816
5	0.4474	0.9237	1.682	437.3	23838
6	0.4470	0.9259	1.682	438.9	23737
Mean	0.4464	0.9234	1.682	435.8	23765
RSD	0.31%	0.39%	0.05%	0.81%	0.27%

[a] Response ratio (RR) by internal standard technique; manual (loop) injections and manually determined peak heights. [b] By internal standard technique; automatic injection and computer-generated peak heights. [c] By internal standard technique; automatic injection and computer-generated peak areas. [d] By external standard technique; automatic injection and computer-generated peak heights. [e] By external standard technique; automatic injection and computer-generated peak areas.

composition to achieve a resolution factor $R^1 \geq 3.5$ between internal standard (commercially available) and carprofen, any laboratory can perform the assay and be assured of adequate resolution between all possible components.

The specificity of the method was examined in several ways. The method was shown to separate all known synthetic impurities. Solutions of carprofen subjected to accelerated degradation studies were chromatographed, and the method was shown to be capable of separating several potential degradation products. To establish the carprofen peak integrity in these accelerated degradation study chromatograms, several injections were made, and the carprofen peak was collected as it eluted from the column. After removal of the mobile phase under a stream of nitrogen followed by lyophilization, NMR analysis demonstrated that the peak assigned to carprofen appeared to be only one substance. The collected carprofen fractions also showed one spot on TLC.

The precision of the method was examined by repeating the entire assay procedure with several weighings of the same composite sample (Table I). The method was shown to be reproducible, with an *RSD* of 0.45%, by manual injection, manual peak height measurements, and the internal standard quantification technique. The system precision was evaluated by making several injections of the same standard solution. The *RSD* of the response ratios obtained was 0.31% (Table II). Subsequent to the initial validation, the method was automated. The system precision was examined using automated injection by both peak areas and peak height measurements and both internal standard and external standard quantification techniques (Table II). The system precision was acceptable by any of the quantification methods described above.

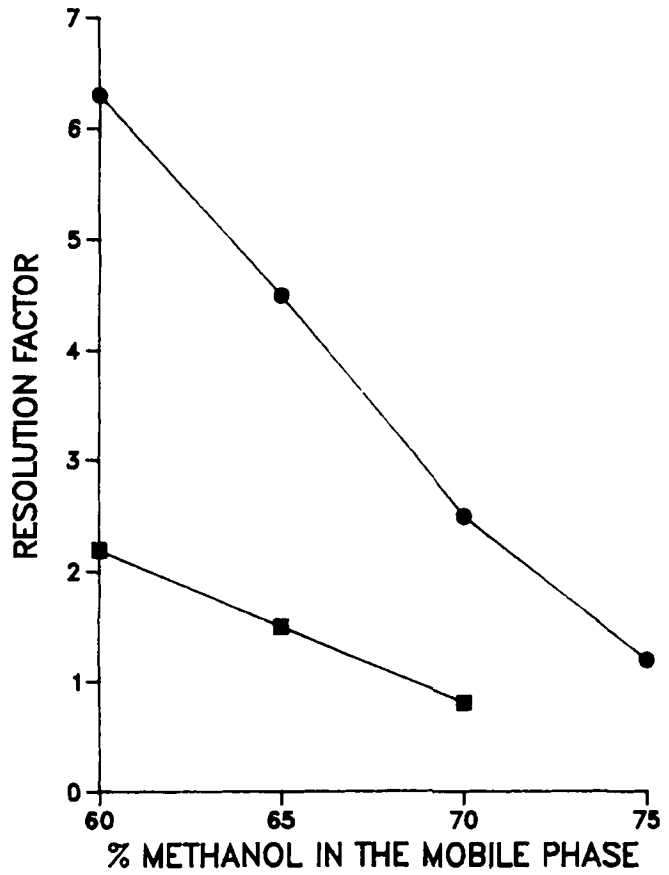

Figure 2—*Plot of resolution factors* versus *percent methanol in the mobile phase. Key: (●) resolution factor (R^1) between carprofen and internal standard; (■) resolution factor (R^2) between carprofen and IV.*

The linearity of the method was also studied. For the dosage form assay, samples containing carprofen concentrations spanning 50–150% of the expected working range were analyzed by the method. Plots of peak response ratios (carprofen–internal standard) *versus* carprofen concentration were generated. Similar plots were generated by using both peak heights (measured manually) and computer-calculated peak areas. The linearity of each plot was determined by using a computer program, LIN, to fit the observed data to a curve of the form $y = mx^n + b$. For perfect linearity, $n = 1.0$. The program also calculated the maximum error to be expected by the deviations from perfect linearity (17). When the observed data from the linearity plots was subjected to the LIN program, the value of n was between 0.95 and 1.0 in all cases. The maximum relative percent error due to nonlinearity over the assay range was also calculated by use of the LIN program and was found to be <0.3% in all cases.

The accuracy of the dosage form assay was also examined. Synthetic samples of carprofen tablets were prepared, covering 50–133% of the theoretical dosage range. The samples were prepared in such a way as to approach the average tablet weight of 365 mg. Appropriate weights of placebo tablet mass were mixed with known weights of carprofen spanning the range given above. These samples were analyzed by the described method. The recovery of carprofen from the tablet matrix is presented in Table III; the data show that excellent recoveries were obtained at all levels.

This HPLC system was also applied to drug substance stability testing, with several aforementioned modifications. For this application, emphasis was placed on the estimation of degradation products with reasonable accuracy, as well as quantification of intact carprofen in the stability study samples. Because of these joint objectives, the sample preparation was adjusted so that the concentration of carprofen in the sample injected was as high as possible without exceeding the linear range of the detector. This allowed accurate determination of the carprofen content and, at the same time, maximized the detector response for any impurities or degradation products present. For this purpose, a separate linearity study was conducted. The results show that carprofen concentrations as high as 2.0 mg/mL (20-μL injections) could be used while remaining within the linear range of the detector.

As mentioned above, various quantification techniques were studied for use with this method. The appropriate quantification technique depends both on the intended use of the method and on the instrumental and data acquisition systems available to the analytical laboratory.

Since the original developmental work on the dosage form assay was carried out by using manual injection and manual peak height measurements, it was believed that an internal standard quantification technique was appropriate. Since that time, state-of-the-art autosamplers and computer data acquisition systems have continually become more readily available. Because of this, the system precision of the dosage form assay was revalidated by other quantification techniques so that the assay procedure could be performed by any laboratory, regardless of the degree of sophistication of its automation and

Table III—Percent Recovery and Dosage Form Assay for Synthetic Tablet Mix

Placebo, mg	Carprofen, mg	Carprofen Found, mg	Recovery, %
340.35	26.08	27.02	103.6
314.90	49.80	50.26	100.9
264.45	101.45	101.88	100.4
214.70	150.36	145.92	97.0
165.42	203.25	203.93	100.3

data acquisition. The data with which the system precision of the dosage form analysis was compared by various quantification techniques is presented in Table II. The precision obtained by any of the quantification techniques was quite acceptable. Since external standard techniques require fewer calculations and are more convenient in terms of sample preparation, those laboratories in which automated equipment is used may select this as the preferred quantification technique.

Two different quantification techniques were used when the method was applied to drug substance stability studies. In such studies, the amount of carprofen in each sample was determined by using internal standard calculations similar to those used in the dosage form assay. However, the quantifications performed to estimate the impurity and degradation product levels were carried out by using an area percent calculation method corrected for the presence of the internal standard in the chromatogram. This technique has been found to be particularly useful when conducting drug substance stability studies on compounds in early phases of development. An underlying assumption with such a quantification technique is that potential degradation products and impurities have the same absorptivity as the parent compound at the chosen detector wavelength. Although this assumption may not always be valid, in many cases it is reasonable. It eliminates the need for reference standards of all impurities and degradation products, which are usually not available during the early stages of development. Considering this restriction, the technique described above is certainly acceptable for demonstrating stability trends. When reference materials become available, the data generated by using the area percent method can be refined or recalculated to take into account any differences in absorptivity. The area percent technique is also useful if an outside laboratory (without access to reference standards of potential impurities and degradation products) is conducting the assay.

The method described has been shown to be accurate, precise, and broadly applicable to all control and analysis laboratories and functions. The sample preparations are simple, and the method can easily be automated.

REFERENCES

(1) L. O. Randall and H. Baruth, *Arch. Int. Pharmacodyn. Ther.*, **220,** 94 (1976).

(2) T. F. Yu and J. Perel, *J. Clin. Pharmacol.*, **20,** 347 (1980).

(3) R. A. Dickey, A. Wasserman, E. Evans, and J. Proctor, Abstracts of the International Meeting on Inflammation, Verona, Italy, 1979, p. 75.

(4) R. A. Dickey and R. L. Lipson, Abstracts of the International Meeting on Inflammation, Verona, Italy, 1979, p. 85.

(5) H. A. Silverman, T. G. Lawrence, and J. D. Holloman, 14th International Congress on Rheumatology, San Francisco, Calif., 1977, p. 258.

(6) R. A. Dickey and J. A. Huleatt, Abstracts of the International Symposium on Rheumatoid Arthritis, Verona, Italy, 1980, p. 113.

(7) E. M. Jensen, J. Fossgreen, B. Kirchheiner, J. Kryger, P. Holm, and K. Mollenbach, *Curr. Ther. Res. Clin. Exp.*, **28,** 882 (1980).

(8) B. Kirchheiner, J. Fossgreen, E. M. Jensen, J. Kryger, P. Holm, and K. Mollenbach, *Curr. Ther. Res. Clin. Exp.*, **28,** 875 (1980).

(9) A. Lussier, L. Rouleau, M. Caron, and L. Tetreault, *Int. J. Clin. Pharmacol. Ther. Toxicol.*, **18,** 482 (1980).

(10) J. A. F. de Silva, N. Strojny, and M. A. Brooks, *Anal. Chim. Acta*, **73,** 283 (1974).

(11) N. Strojny and J. A. F. de Silva, *J. Chromatogr. Sci.*, **13,** 583 (1975).

(12) C. V. Puglisi, J. C. Meyer, and J. A. F. de Silva, *J. Chromatogr.*, **136,** 391 (1977).

(13) B. J. Hodshon, W. A. Garland, C. W. Perry, and G. J. Bader, *Biomed. Mass Spectrom.*, **6,** 325 (1979).

(14) G. Palmskog and E. Hultman, *J. Chromatogr.*, **140,** 310 (1977).

(15) W. A. Garland and M. L. Powell, *J. Chromatogr. Sci.*, **19,** 392 (1981).

(16) J. K. Stoltenborg, C. V. Puglisi, F. Rubio, and F. M. Vane, *J. Pharm. Sci.*, **70,** 1207 (1981).

(17) E. Debesis, J. P. Boehlert, T. E. Givand, and J. C. Sheridan, *Pharm. Tech.*, **1982,** p. 120.

ACKNOWLEDGMENTS

The authors thank Dr. P. Bhattacharyya and Ms. A. Grant for help in obtaining and interpretation of NMR spectral data. The authors also thank Ms. F. Manalili and Mr. G. Clark for technical assistance, and Ms. J. Comment for the typing of this manuscript.

Development and Evaluation of Enteric-Coated Penicillamine Tablets

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Received February 18, 1983, from the *Department of Pharmaceutics, University of Mississippi, University, MS 38677.* Accepted for publication September 26, 1983. * Present address: Chief, Pharmaceutical Development Services, Building 10, Room 1D37, Clinical Center, National Institutes of Health, Bethesda, MD 20205.

Abstract □ Commercially available 250-mg penicillamine tablets were converted into enteric-coated tablets. Based on *in vitro* dissolution and disintegration tests, tablets coated with five layers of a cellulose acetate phthalate formulation by a modified pan coating technique were judged to be superior to other coated tablets. These tablets resisted disintegration in simulated gastric fluid over a 4-h period and disintegrated in an average of 21 min in simulated intestinal fluid. Enteric-coated penicillamine tablets were tested *in vivo* in nine weanling pigs divided into three groups: a negative control group, a test group dosed with enteric-coated penicillamine tablets, and a positive control group dosed with uncoated tablets. The incidence of GI tract bleeding, as determined by daily occult blood tests of the stools, was significantly less in the animals receiving the enteric-coated tablets when compared with the positive control group. The enteric-coated dosage form appeared to decrease GI tract irritation caused by penicillamine. Plasma concentration–time curves for penicillamine in the pigs were similar in shape to those reported in humans. Atypical double peaks occur in both species. Relative bioavailability of the enteric-coated tablet was found to be 67% when compared with the uncoated tablet. This apparent reduction is probably due to a large intrasubject variation in areas under the plasma concentration–time curves and not to a dosage form effect.

Keyphrases □ Penicillamine—development and evaluation of enteric-coated tablets □ Formulations—penicillamine, development and evaluation of enteric-coated tablets

Penicillamine has been used for many years in the treatment of Wilson's disease and cystinuria. More recently, the drug has been approved for the treatment of rheumatoid arthritis. Due to severe adverse reactions, penicillamine therapy in the arthritic patient is normally limited to those who have been previously treated unsuccessfully by conventional therapy (1).

It has been estimated that up to one-third of the patients

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