cough–cold liquids. In addition, the results indicate that the methodology is applicable to a wide range of amine drugs commonly found in cough–cold liquids. The chromatographic system is capable of separating the amines of interest from the dyes, preservatives, and flavorings normally associated with a liquid cough–cold formulation. In all cases the sample preparation consisted of dissolution of the sample in the mobile phase and the total chromatographic run time was <20 min.

REFERENCES

(1) F. DeFabrizio, *J. Pharm. Sci.*, **69**, 854 (1980).
(2) R. E. Madsen and D. F. Magin, *ibid.*, **65**, 924 (1976).
(3) C. Hishta and R. G. Lauback, *ibid.*, **58**, 745 (1969).
(4) E. J. Kubiak and J. W. Munson, *ibid.*, **69**, 152 (1980).
(5) E. J. Kubiak and J. W. Munson, *ibid.*, **69**, 1380 (1980).
(6) W. O. McSharry and I. V. E. Savage, *ibid.*, **69**, 212 (1980).
(7) M. K. Chao, I. J. Holcomb, and S. A. Fusari, *ibid.*, **68**, 1463 (1979).
(8) N. Muhammad and J. A. Bodnar, *J. Liquid Chromatogr.*, **3**, 113 (1980).
(9) D. L. Massart and M. R. Detaevernier, *J. Chromatogr. Sci.*, **18**, 139 (1980).
(10) V. Das Gupta and A. G. Ghanekar, *J. Pharm. Sci.*, **66**, 895 (1977).
(11) L. R. Snyder and J. J. Kirkland, in "Introduction to Modern Liquid Chromatography," 2nd ed., Wiley, New York, N.Y., 1979, p. 457.
(12) C. G. Eckhart and T. McCorkle in "Analytical Profiles of Drug Substances," Vol 7, K. Florey, Ed., Academic, New York, N.Y., 1978, p. 43.

Precise High-Performance Liquid Chromatographic Procedure for the Determination of Cefsulodin, a New Antipseudomonal Cephalosporin Antibiotic, in Plasma

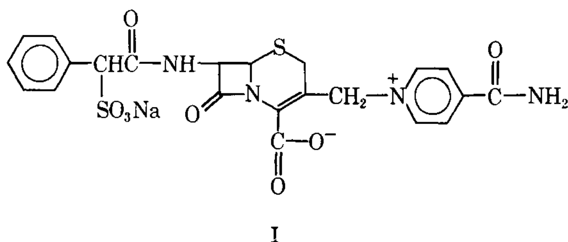
G. RICHARD GRANNEMAN and LAWRENCE T. SENNELLO [x]

Received August 25, 1981, from the *Drug Metabolism Department, Division of Drug Safety Evaluation, Abbott Laboratories, North Chicago, IL 60064.* Accepted for publication December 9, 1981.

Abstract ☐ A simple and precise high-performance liquid chromatographic (HPLC) procedure was developed for the determination of cefsulodin, a new antipseudomonal cephalosporin antibiotic, in plasma. The analytical procedure involved ultrafiltration of samples that were buffered to prevent cefsulodin degradation, followed by injection into an HPLC system, utilizing a C_{18} reversed-phase analytical column, a mobile phase of acetonitrile–modified aqueous acetate buffer, and a UV spectrophotometric detector. Because of the simplicity of the procedure, the intraassay (~2%) and interassay (~3–4%) coefficients of variation were extremely low. Recoveries of drug were essentially quantitative in freshly buffered specimens and in those stored buffered and frozen for nearly 3 months. Calibration curves were rectilinear from the limit of quantification (~0.2 μg/ml) to 200 μg/ml, as demonstrated by regression correlation coefficients averaging >0.999 during routine analyses.

Keyphrases ☐ High-performance liquid chromatography—procedure for the determination of cefsulodin in plasma, new antipseudomonal cephalosporin antibiotic ☐ Cefsulodin—new cephalosporin antibiotic, high-performance liquid chromatographic determination ☐ Ultrafiltration—high-performance liquid chromatographic determination of cefsulodin in plasma, new antipseudomonal cephalosporin antibiotic

Cefsulodin sodium [3-(4-carbamoyl-1-pyridiniomethyl) - 7β - (D - α -sulfophenylacetamido) - ceph - 3 - em-4-carboxylate monosodium salt] (I), a semisynthetic cephalosporin antibiotic (Fig. 1)[1], has been shown to have excellent antipseudomonal activity (1, 2).



I

Microbiological assays have traditionally been used for analysis of biological specimens containing antibiotics; however, such procedures are occasionally disadvantageous due to long analysis times, nonlinear calibration curves, inadequate specificity, and relatively poor precision. In addition, preliminary experiments suggested that problems might arise during microbiological assays for cefsulodin due to drug hydrolysis during incubation of inoculated analysis plates.

With these factors in mind, work was started to develop an alternate procedure which had adequate sensitivity, high precision, short analysis time, and which did not allow hydrolysis of the cefsulodin. High-performance liquid chromatography (HPLC) is ideally suited for the analysis of the relatively polar, nonvolatile cephalosporins. Since the therapeutic concentrations of these compounds are usually in the microgram per milliliter range, concentrating techniques are usually not required. However, the majority of the high molecular weight proteins and fibrin must be removed from plasma samples to prevent column filter and bed damage. Several HPLC procedures for cephalosporins, employing classical deproteination reagents such as tri-

Table I—Precision and Linearity of the HPLC Procedure for Cefsulodin

Concentration of Cefsulodin, μg/ml		Coefficient
Actual	Calculated[a]	of Variation, %
0.78	0.76 (0.74)[b]	4.5 (3.3)[b]
1.56	1.57 (1.57)	1.9 (1.7)
3.13	3.10 (3.13)	1.7 (0.4)
6.25	6.31 (6.44)	3.2 (2.1)
12.50	12.71 (12.70)	0.9 (0.6)
25.00	25.05 (25.17)	1.3 (0.4)
50.00	49.40 (50.41)	1.1 (0.4)
100.00	97.34 (98.87)	1.7 (0.3)

[a] Based on results of linear regression of means from quadruplicate determinations for each concentration using reciprocal variance weights ($r = 0.9999$). [b] Data in parentheses were obtained neglecting responses of the internal standard.

[1] Developed by Takeda Chemical Industries; also known as SCE-129. Currently under clinical investigation by Abbott Laboratories.

0022-3549/82/01000-1112$01.00/0

© 1982, American Pharmaceutical Association

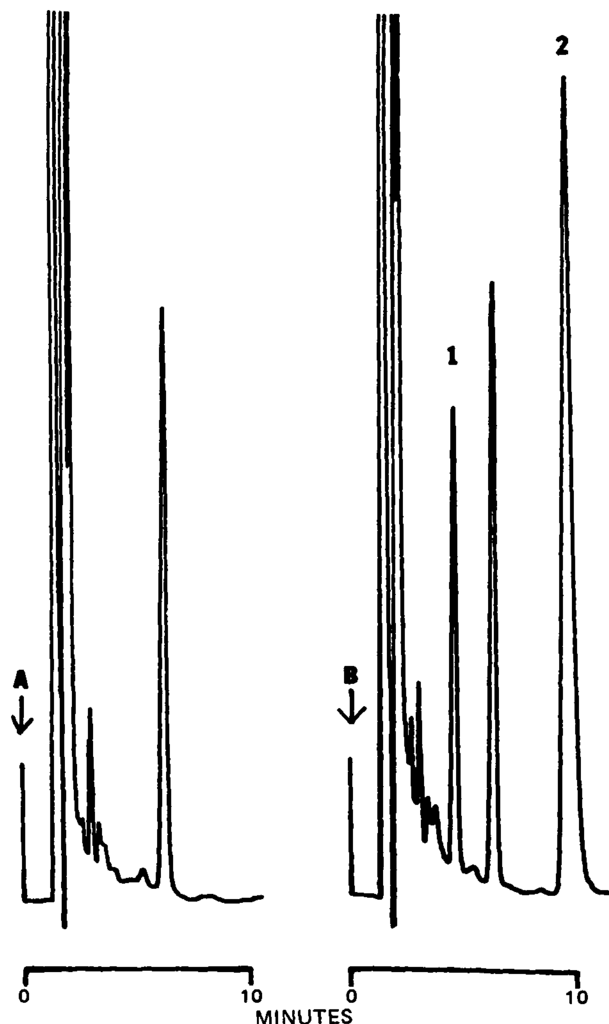


Figure 1—*Typical HPLC traces for ultrafiltrates of (A) predose and (B) postdose plasma from a subject who received a 500-mg dose of cefsulodin. The calculated concentration of cefsulodin was 4.3 μg/ml. Compound II was added to a final concentration in plasma of 400 μg/ml. Key: (1) cefsulodin; (2) II.*

chloroacetic acid (3, 4) or organic solvents (5–8) have been reported recently. The described HPLC procedure, involving removal of plasma proteins by ultrafiltration, offers the advantages of simplicity and high reproducibility, obviating the problems associated with the precipitation procedures, such as sample dilution, incomplete protein precipitation, drug coprecipitation, and acid catalyzed degradation of labile drugs. Furthermore, the ultrafiltration procedure would allow the determination of the free and total drug concentrations in plasma, if desired.

EXPERIMENTAL

Chromatography—HPLC analyses were conducted using a reciprocating pump[2] in conjunction with a UV detector[3] operated at an analytical wavelength of 254 nm. The mobile phase, consisting of 4.5% acetonitrile[4] in 0.02 *M* ammonium acetate, which was adjusted to pH 4.2 with glacial acetic acid, was maintained at a flow rate of 2.0 ml/min through a C_{18}[5] analytical column.

Ultrafiltration Apparatus—Ultrafiltrates of plasma samples were

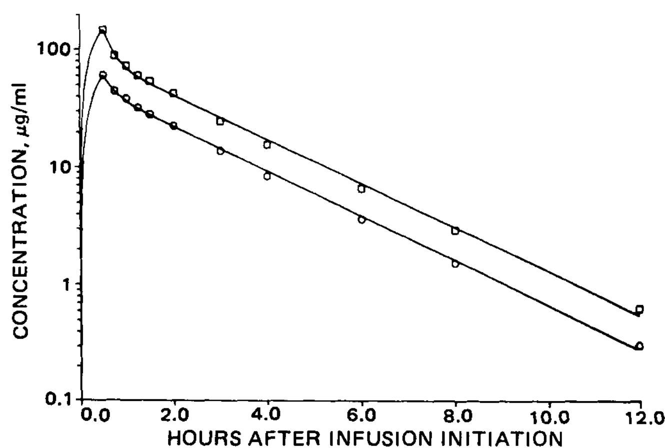


Figure 2—*Plasma cefsulodin levels determined for two subjects receiving 1000-mg (○) and 2000-mg (□), 30-min intravenous infusions of cefsulodin. Plotted curves represent nonlinear best-fit regressions.*

obtained using conical centrifuge tubes[6], conical supports[7], and membrane cones[8], with respective molecular weight cutoff values of 25,000 and 50,000. Use of the former cone model was slightly favored due to its higher apparent flux and protein retentivity. For reasons to be discussed, the two types of cones should not be mixed within an analytical run.

Sample Preparation—The stability of cefsulodin in plasma was demonstrated to be greatly improved by addition of phosphate buffer to bring the final pH to 6.5 or less. Thus, plasma samples should be mixed with an equal volume of 1.0 *M* phosphate buffer (pH 6) prior to freezing.

The 1.0 *M* phosphate buffer (pH 6) was prepared by addition of three parts (by weight) of 1.0 *M* $NaH_2PO_4 \cdot H_2O$ to one part 1.0 *M* $Na_2HPO_4 \cdot 7H_2O$, which was equivalent to 103.5 g of $NaH_2PO_4 \cdot H_2O$ and 67.05 g of $Na_2HPO_4 \cdot 7H_2O$/liter of solution. Mixture of this solution with an equal volume of plasma resulted in a solution with pH 6.1–6.3.

Standard Preparation—Since the plasma samples were diluted by the 1:1 mixture with phosphate buffer, standards had to be diluted similarly. Typically, a 200 μg/ml solution of the reference material, freshly prepared in 1.0 *M* phosphate buffer (pH 6) was mixed with an equal volume of fresh plasma. This standard thereafter was serially diluted with an equivolume mixture of phosphate buffer and plasma to provide the remaining standards.

Sample Workup—Sample processing involved ultrafiltration of a mixture of accurately pipeted aliquants of the sample and internal standard. The use of *p*-fluoro-α-methylbenzylamine hydrochloride (II) as an internal standard was found to be satisfactory. Typically, 0.05 ml of an 8.0 mg/ml solution of II hydrochloride was thoroughly mixed with 2.0 ml of the standards and unknowns. Control blanks and predose samples were processed without internal standard. After transfer to a clean filtration apparatus, the samples were centrifuged at the same relative centrifugal force for the same length of time. Centrifugation at 450×*g* for ~20 min produced ~1.0 ml of ultrafiltrate. Whenever possible, samples in the various stages of workup were refrigerated until all samples were processed. Thereafter, the ultrafiltrates could be stored frozen for several days prior to analysis.

Injection of 70 μl of these ultrafiltrates into the described HPLC system resulted in a nearly full-scale recorder reading for the internal standard (retention volume ~22 ml) at a detector attenuation of 0.02 aufs. Under the same conditions, the response for a sample containing 0.2 g of cefsulodin/ml would be two to three times greater than instrumental noise. To minimize the degradation of cefsulodin in the ultrafiltrates, they should not be allowed to stand at room temperature for more than 2 hr prior to analysis. Typical chromatograms are shown in Fig. 1.

RESULTS AND DISCUSSION

Method Development—*Cleanup Procedures*—Several of the classical procedures for the removal of protein from samples were investigated. Procedures involving deproteination with organic solvents, such as methanol, acetonitrile, and acetone, were all unsatisfactory due to coprecipitation of cefsulodin, chromatographic aberrations due to the

[2] Model 6000A, Waters Associates, Milford, Mass.
[3] Model 440, Waters Associates, Milford, Mass.
[4] HPLC Grade, Burdick and Johnson.
[5] μBondapak C_{18}, Waters Associates, Milford, Mass.
[6] Centriflo system, Amicon Corp., Lexington, Mass.
[7] Model CS1A, Amicon Corp., Lexington, Mass.
[8] Either model CF25 or CF50A, Amicon Corp., Lexington, Mass.

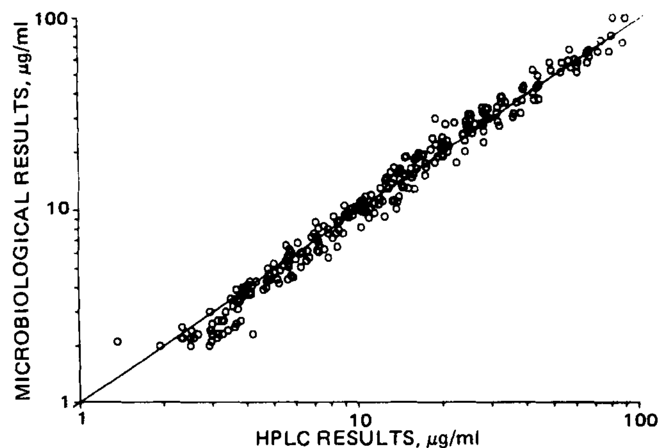


Figure 3—*Correlation of HPLC and microbiological assay results; 270 assays.*

high organic content of directly injected supernates, and degradation of cefsulodin during the evaporation of the supernates. Similarly, other deproteination reagents such as concentrated salt solutions (*e.g.*, sodium sulfate) and acids (*e.g.*, trichloroacetic, sulfosalicylic) were unacceptable due to coprecipitation of cefsulodin, incomplete protein precipitation, cefsulodin degradation, and chromatographic difficulties. Isolation of cefsulodin by column chromatographic techniques, such as anion and cation exchange, silica absorption, alumina absorption, and gel exclusion, was also not feasible due to inadequate protein removal, sample dilution, and the limited number of samples that could be analyzed per day.

Ultrafiltration proved to be ideally suited for the removal of protein from cefsulodin-containing plasma samples. Due to the low apparent protein binding of cefsulodin in the buffered plasma samples, recoveries were nearly quantitative. However, pressurized ultrafiltration techniques using cells or membrane tubing were slow, cumbersome, and involved variable sample dilution due to the necessity of prewetting the membrane. Alternatively, ultrafiltration by centrifugation with the apparatus described was found to be simple, fast, and highly reproducible.

Recovery—The recoveries of cefsulodin and II in the first 50–100 μl of ultrafiltrate were slightly lower than in subsequent fractions. Additionally, in some experiments, the peak height ratio from the initial ultrafiltrate was higher than the true ratio. Lower recoveries for the initial ultrafiltrate may be attributed to one or more of the following phenomena: (*a*) leaching of glycerin or other liquid materials from the membrane; (*b*) low-level binding to the membrane; and/or (*c*) molecular sieving. Regardless, when ultrafiltrate volumes approaching or exceeding 0.50 ml were collected, recoveries were nearly quantitative for both cefsulodin (>95%) and II (>93%), and their peak height ratio became constant.

Intersubject variability in ultrafiltration recovery was assessed by supplementing plasma samples from six subjects with cefsulodin and II. The intersubject coefficient of variation for cefsulodin recovery was 1.3% without II and 1.2% with II as the internal standard. Recovery also did not appear to be dependent on the concentration of cefsulodin in the sample. Mean recoveries for quadruplicate standards ranging from 0.8 to 100 μg/ml averaged 99.1%.

The 25,000 and 50,000 molecular weight cutoff membranes were compared for samples spiked with 2, 20, and 200 μg of cefsulodin/ml. Although the recoveries of cefsulodin were essentially the same for both types of membranes, recoveries of II were slightly lower for the lower cutoff cones. Thus, it is recommended that the two types of filters not be mixed within the same assay run.

Precision and Linearity—The precision and linearity of the procedure was assessed by quadruplicate analyses of buffered plasma samples supplemented with cefsulodin in the concentration range of 0.78–100 μg/ml. The results are summarized in Table I. With no correction made for the internal standard response, the mean assay coefficient of variation (*CV*) was 1.2 ± 1.1%. Correction of the data with the internal standard responses resulted in a mean *CV* of 2.0 ± 1.2%. The errors involved with either approach are uncharacteristically low for the analysis of drugs in biological matrixes. This is due to the simplicity of the workup procedure and the high recoveries.

Linear regression analyses were performed with the mean data from the above experiment, using reciprocal analytical variances as the weights (9). The same data were then refitted using 1, 1/concentration ($1/C$), and 1/concentration-squared ($1/C^2$) as the weighting schemes.

The high correlation coefficients from the regressions (~0.9999) demonstrate that the responses are rectilinearly related to the concentration of cefsulodin in the samples. The near-zero intercepts demonstrate that the blank plasma had negligible interferences for cefsulodin, and that recovery of drug in the ultrafiltrates was not concentration dependent. From careful consideration of the dispersion of the residual errors, the $1/C^2$ weighting scheme was found to best represent the reciprocal analytical variances when the internal standard was not used; hence, weighting by reciprocal squared concentrations should be used in regression analyses of peak height ratio data obtained during sample analyses. Alternatively, if an internal standard is not used, the best choice of the weighting scheme is $1/C$.

The interassay precision of the procedure was assessed from quality control data obtained during routine analyses. For the first application of the procedure to a clinical study, plasma was supplemented on-site at concentrations of 4 and 20 μg/ml of cefsulodin/ml. For the first group of subjects, the quality control standards averaged 3.9 ± 0.2 (n = 9) and 20.0 ± 0.7 (n = 9) μg/ml, respectively. In a second series of analyses, the references averaged 4.0 ± 0.2 (n = 5) and 19.9 ± 0.6 (n = 8) μg/ml. In the most recent use of the procedure, the mean value for a 10.0 μg/ml quality control pool, determined in 20 analytical runs performed over a 1-month period by two technicians, was 10.2 ± 0.3 μg/ml. The mean regression correlation coefficient for the 6–8 point calibration curves was 0.9996. Thus, under routine conditions, the interassay coefficient of variation is ~3–4%, and accuracy errors are negligible.

Stability—Preliminary experiments in this laboratory suggested that 15–40% of the cefsulodin activity in plasma or serum could be lost after storage at ~−17° for 7 weeks, and that the rate of degradation was dependent on the freshness of the plasma. The stability of the compound in buffer was considerably greater, especially in the pH range of 3–7. Degradation of cefsulodin in aqueous media is most pronounced under alkaline conditions, which facilitate isomerization, hydrolysis, and nucleophilic displacement at the various functional centers of the compound. As a result, acidification with phosphate buffer was evaluated as a means of enhancing the stability of cefsulodin in plasma. After incubation at 8° for 3 days, or 24° for 1 day, essentially quantitative recoveries of drug were realized from plasmas which were acidified within the pH range of 3–6.5 with 1.0 *M* phosphate buffer. In contrast, cefsulodin was ~50 and 80% inactivated when stored in unbuffered plasma under the same conditions.

Since sensitivity reduction of the microbiological assay, and protein denaturation were encountered in plasma samples buffered in the pH range of 3–5.5, a final pH of ~6 was found to be the most desirable. An equivolume mixture of plasma and buffer was deemed the most convenient and accurate acidification procedure for diverse clinical settings. Since the implementation of this stabilization procedure, degradation of cefsulodin during frozen storage or workup has not been encountered based on quality control data. In another experiment, the recovery of cefsulodin in frozen plasma was 101.4 ± 1.7% after 36 days and 98.5 ± 1.7% after 81 days; thus, prolonged frozen storage of cefsulodin-containing samples appears to present no significant problems.

Since the half-life of cefsulodin in unbuffered plasma at room temperature is roughly 8 hr, care must be taken to prevent appreciable degradation. Whole blood samples should be cooled prior to the harvest of serum or plasma and the subsequent acidification. After the specimens have been buffered, drug degradation is no longer a serious problem.

Sample Analysis—There are indications that cefsulodin may degrade in the ultrafiltrates at a slightly greater rate than in buffered plasma. Hourly analyses of an ultrafiltrate of a buffered plasma sample containing cefsulodin and internal standard showed that after 15 hr, ~11.7% of the cefsulodin had been lost, and that the concentration was declining at ~0.8%/hr.

Accordingly, on the day of analysis, ultrafiltrates are refrigerated before and after manual injection into the HPLC system, and are usually left at room temperature for <2–3 hr. For automated analyses of large numbers of samples, the ultrafiltrates can be loaded into the sampler in batches of 10–20. Alternatively, for long unattended analyses, standards allowing for compensation for degradation may be interspersed among the samples.

After analysis of over 2000 plasma samples with this procedure, the necessity of altering the described chromatographic conditions has been rare; however, column efficiency loss or chromatographic interference from atypical plasma samples, such as those from patients with severe renal impairment, has occasionally required alternate mobile phases. Generally, small changes in the pH of the eluent greatly affects the relative retention volume of cefsulodin and plasma components, providing flexibility for difficult separations. For extremely difficult separations, the use of an ion-pair reversed-phase system, employing tetrabutylam-

monium (III) as the counter ion, has been used successfully. To compensate for addition of 0.005 *M* III to the regular mobile phase, the acetonitrile content must be increased to ~12%. Since cefsulodin, by virtue of its sulfonic acid moiety, is more acidic than most compounds encountered in plasma, the III-containing mobile phases provide additional versatility. Although II cannot be used as the internal standard in such a system, analytical precision is not compromised, due to the extreme simplicity and quantitative recoveries of the ultrafiltration procedure.

Figure 2 shows the results of the analysis of plasma samples from two subjects who received single 1000- and 2000-mg, 30-min intravenous infusions of cefsulodin. The solid lines represent the nonlinear best-fit regressions calculated for these data.

Comparison to Microbiological Assay Procedures—In the first application of this HPLC procedure to a clinical study, 270 plasma samples were assayed both microbiologically, using *Pseudomonas aeruginosa* (NCTC 10490) as the test organism, and with HPLC as described. Figure 3 shows that results from the two procedures were highly correlated. Linear regression of the data yielded a correlation coefficient of 0.993.

The centrifugal ultrafiltration technique is a good alternative to classical deproteinization procedures because it is rapid and simple and requires no sample adulteration. For more highly protein-bound compounds, the ultrafiltration procedure allows direct quantification of unbound drug levels.

REFERENCES

(1) K. Tsuchiya, M. Kondo, and K. Nagatoma, *Antimicrob. Agents Chemother.*, **13,** 137 (1978).

(2) M. Kondo and K. Tsuchiya, *ibid.*, **14,** 151 (1978).

(3) E. Crombez, G. Van der Weken, W. Van den Bossche, and P. de Moerloose, *J. Chromatogr.*, **177,** 323 (1979).

(4) J. S. Wold and S. A. Turnipseed, *Clin. Chim. Acta*, **78,** 203 (1977).

(5) M. G. Torchina and R. G. Danzinger, *J. Chromatogr.*, **181,** 120 (1980).

(6) A. Suzuki, K. Nods, and H. Noguchi, *ibid.*, **182,** 448 (1980).

(7) J. G. Aziz, J. Gambertoglio, E. T. Lin, H. Grausz, and L. Z. Benet, *J. Pharmacokinet. Biopharm.*, **6,** 153 (1978).

(8) I. Nilsson-Ehle, T. T. Yoshikawa, M. C. Schotz, and L. B. Guze, *Antimicrob. Agents Chemother.*, **13,** 221 (1978).

(9) H. G. Boxenbaum, S. Riegelman, and R. M. Elashoff, *J. Pharmacokinet. Biopharm.*, **2,** 123 (1974).

ACKNOWLEDGMENTS

The authors thank Dr. Walton Grundy and Mrs. Barbara Zorc for their help in performing the microbiological analyses.

Simultaneous Assay of Hydrocodone Bitartrate and Acetaminophen in a Tablet Formulation

WARREN E. WALLO ˣ **and ANTHONY D'ADAMO**

Received September 1, 1981, from the *Analytical Research Department, Knoll Pharmaceutical Co., Whippany, NJ 07981.* Accepted for publication December 11, 1981.

Abstract □ A reversed-phase pressurized liquid chromatographic procedure is presented for the simultaneous quantitation of hydrocodone bitartrate and acetaminophen in a tablet formulation. The separation method was based on an octadecylsilane column with a buffered (pH 4.5) methanol-water mobile phase. Measurement was with a UV spectrophotometer set at 283 nm, compared to external standards. Assays for the active ingredients in tablet samples averaged 99.7% of the label claim for hydrocodone bitartrate and 100.3% for acetaminophen. The respective relative standard deviations of the retention time and precision were 2.2 and 1.75% for hydrocodone and 3.3 and 0.95% for acetaminophen. The range of interest studied was 0.035 to 0.065 mg/ml for hydrocodone bitartrate and 3.50 to 6.50 mg/ml for acetaminophen. The assay method was also compared to colorimetric and USP procedures for the active ingredients. The method was suitable for control, content uniformity, and stability-indicating use.

Keyphrases □ Hydrocodone bitartrate—simultaneous assay in a tablet formulation, acetaminophen □ Acetaminophen—simultaneous assay in a tablet formulation, hydrocodone bitartrate □ Tablet formulation—simultaneous assay of hydrocodone bitartrate and acetaminophen

The determinations of acetaminophen (I), hydrocodone (II), and related compounds have been reported by gas (1–3) and high-pressure liquid chromatographic (HPLC) methods (4–14). However, none of the methods has been applied to the simultaneous determination of these compounds.

Due to interferences, the pharmacopeial procedures for the individual drugs are also unsuitable for simultaneous analysis (15, 16). In addition, the authors are not aware of any published stability-indicating method for hydrocodone.

The present report presents an HPLC method for the quantitative analysis of both substances in a two-component tablet formulation. The content uniformity test of the minor component (II) in the tablet formulation is also feasible by this method. An analysis can be conducted in <13 min and separates possible impurities and degradation products.

EXPERIMENTAL

Reagents and Materials—Water and methanol were HPLC grade solvents. Hydrocodone bitartrate, acetaminophen, codeine phosphate, and hydromorphone hydrochloride were USP reference standards. Other materials were ACS grade or the highest quality commercial grade available.

The high-pressure liquid chromatograph[1] was connected to an automatic sampler[2], a variable wavelength UV detector[3], and an integrator/recorder[4]. A bonded reversed-phase C_{18} column[5] was used.

Chromatographic Conditions—The mobile phase consisted of 25% methanol and 75% of an aqueous solution containing 0.01 *N* monobasic potassium phosphate and 0.05 *N* potassium nitrate, adjusted to a pH of ~4.5 by dropwise addition of 3 *N* phosphoric acid solution. The mobile phase was degassed prior to use by vacuum. A flow rate of ~1.1 ml/min was established. The detector sensitivity was 2.0 aufs for acetaminophen and 0.010 aufs for hydrocodone, both measured at 283 nm. The chart speed was 0.7 cm/min.

External Standard Solutions—A two-component standard solution containing 5 mg/ml of I and 0.05 mg/ml of II bitartrate was prepared in water.

[1] Waters ALC 204, Waters Associates, Milford, MA 01757.
[2] WISP, 710B Waters Assoc., Milford, MA 01757.
[3] SF 770 Spectroflow Monitor, Schoeffel Instruments, Westwood, NJ 07675.
[4] DATA Module, Waters Assoc., Milford, MA 01757.
[5] μBondapak C_{18} column, Waters Assoc., Milford, MA 01757.

0022-3549/82/1000-1115$01.00/0

© 1982, American Pharmaceutical Association