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.

0022-3549/82/01000-1115$01.00/0

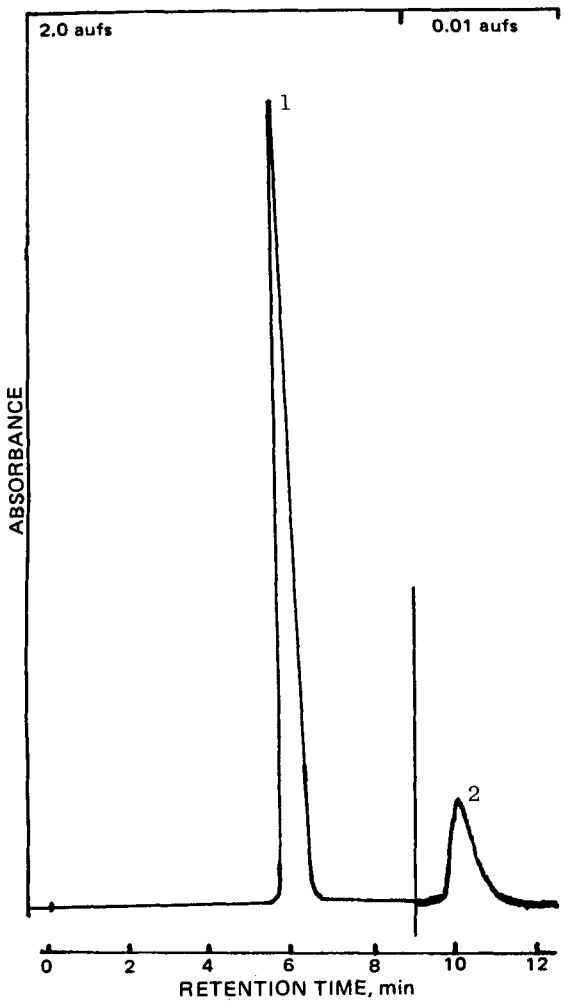

Figure 1—*HPLC chromatogram for standard preparation of (1) acetaminophen and (2) hydrocodone.*

Sample Preparation—For the content uniformity test and tablet composite assay, a tablet[6] or an amount equivalent to 1 average tablet was transferred to a 100-ml volumetric flask. About 95 ml of water was added and the flask was placed on a steam bath. After 15 min, the flask was removed, mixed for 15 min, sonicated, and allowed to stand. A portion of this solution was filtered[7] and placed in a sealed vial[8].

Assay Method—Equal volumes (~13 μl) of standard preparations and assay preparations were introduced into the HPLC operated at room temperature by means of the automatic injector.

Calculation—Quantitation was done by measuring peak heights electronically compared to external standards. To determine the number of milligrams per tablet of acetaminophen, the following equation was used:

$$\frac{Pha}{Phs} \times \frac{Ws}{5} \times \frac{ATW}{Wa} \qquad \text{(Eq. 1)}$$

Similarly, for the mg/tablet hydrocodone bitartrate:

$$\frac{Pha}{Phs} \times Ws \times 4 \times \frac{ATW}{Wa} \qquad \text{(Eq. 2)}$$

where *Pha* is the average peak height of the assay preparation; *Phs* is the average peak height of the standard preparation; *Ws* is the weight of the standard in milligrams; *Wa* is the weight of the assay sample in milligrams; and *ATW* is the average tablet weight in milligrams.

Accelerated Decomposition Studies—Accelerated, extreme degradation of hydrocodone bitartrate was accomplished by several methods and its peak height was measured by data module integration:

[6] Vicodin, Knoll Pharmaceutical Co., Whippany, NJ 07981.
[7] Waters Associates Aqueous Sample Clarification Kit part No. 26865, Milford, MA 01757.
[8] Waters Associates Standard 4-ml vial assembly part No. 73018, Milford, MA 01757.

Table I—Retention Time of Hydrocodone, Acetaminophen, and Related Compounds

Compound	Retention Time, min
p-Aminophenol	3.4
Hydromorphone	5.2
Acetaminophen	5.9
Codeine	7.3
Hydrocodone	10.0
p-Chloracetanilide	43.3

Table II—Reproducibility of Retention Times for Hydrocodone and Acetaminophen

Run	Retention Time, min: Acetaminophen (I)	Retention Time, min: Hydrocodone (II)	RRT (II)/(I)
1	5.2	10.4	2.00
2	5.1	10.1	1.98
3	5.5	10.1	1.84
4	5.2	9.9	1.90
5	5.5	10.4	1.89
6	5.1	9.9	1.94
7	5.3	10.3	1.94
8	5.1	9.9	1.94
$\bar{X}$	5.2	10.1	1.93
RSD, %	3.2	2.2	2.6

(a) Hydrocodone bitartrate powder was heated at 105° for 1-week.
(b) Hydrocodone bitartrate (1 mg/ml) was refluxed in 0.1 *N* NaOH for 24 hr.
(c) Hydrocodone bitartrate (1 mg/ml) was refluxed in 1.0 *N* HCl for 48 hr.
(d) Hydrocodone bitartrate (1 mg/ml) was dissolved in water, placed in a quartz cell, and irradiated with a 275-watt sunlamp for 2 hr.
(e) Hydrocodone bitartrate (1 mg/ml) was dissolved in 3% H_2O_2 and allowed to stand for 1 month.

These decomposition studies were compared with the following control samples:
(a) Hydrocodone bitartrate dissolved in water (1 mg/ml).
(b) Hydrocodone bitartrate refluxed in aqueous solution (1 mg/ml) for 24 hr.

Precision Analysis—Ten tablets were weighed then powdered by mortar and pestle. An amount of powder equivalent to about one average tablet was accurately weighed and transferred to each of eight separate 100-ml volumetric flasks. These samples were treated as described in *Sample Preparation*. Duplicate injections of each sample solution were made.

Accuracy Analysis—Known amounts at the 100% of tablet claim of hydrocodone bitartrate and acetaminophen were added to a placebo powder mixture dissolved in water. This sample was treated as described and six replicate injections were made into the high-pressure liquid chromatograph.

RESULTS

In a typical standard run, good separation of I and II can be seen (Fig. 1). The following related compounds were shown to be separable by this

Table III—Precision of HPLC Assay of Hydrocodone Bitartrate and Acetaminophen in Commercial Tablets [a]

Tablet Sample	Acetaminophen Found, %	Hydrocodone Bitartrate Found, %
1	98.4	96.4
2	97.0	96.4
3	98.2	100.4
4	97.8	98.2
5	100.2	101.0
6	99.0	98.6
7	98.0	98.2
8	98.4	100.0
Mean ($\bar{x}$)	98.4	98.6
SD ($n-1$)	0.93	1.73
RSD, %	0.95	1.75

[a] Vicodin tablets contain 5 mg of hydrocodone bitartrate and 500 mg of acetaminophen.

Table IV—Comparison of HPLC with USP Column Assays for Acetaminophen and Ion-Pairing Assays for Hydrocodone Bitartrate in Tablets [a]

Batch Number	Acetaminophen Found, %			Hydrocodone Bitartrate Found, %		
	HPLC	USP Column	Variation	HPLC	Ion-Pairing	Variation
1	100.4	99.1	1.3	100.3	102.8	2.5
2	99.9	99.3	0.6	97.1	98.2	1.1
3	101.3	99.9	1.4	98.2	98.4	0.2
4	97.6	101.8	4.2	97.8	95.6	2.2
5	97.7	97.2	0.5	97.2	96.8	0.4
6	97.6	94.5	3.1	98.8	101.8	3.0
7	99.8	94.6	5.2	99.8	99.0	0.8

[a] Vicodin tablets. [b] Based on method by Das Gupta (Ref. 17).

method: *p*-aminophenol, *p*-chloroacetanilide, codeine, and hydromorphone. The two compounds, *p*-aminophenol and *p*-chloroacetanilide, are possible hydrolysis and precursor compounds, respectively, of acetaminophen (17). Codeine and hydromorphone are narcotic drugs chemically related to hydrocodone. This separation demonstrates the specificity of the assay. The respective retention times of the related compounds are listed in Table I.

Linearity—Calibration standard solutions were prepared at 10% intervals between 70 and 130% levels in the expected range of analysis. A plot of peak heights *versus* the amount of the two components injected was linear as evidenced by a correlation coefficient of 0.998 for I between 3.5 and 6.5 mg/ml and 0.996 for II between 0.035 and 0.065 mg/ml.

Accuracy—In a study of a spiked placebo, results showed a mean accuracy of 100.3% for I and 99.7% for II. No interference due to the placebo ingredients could be detected in the chromatograms produced. Reproducibility of retention time was sufficiently precise to ensure separation and identity of I and II (Table II).

Precision—The relative standard deviations for eight samples of a commercial tablet were 0.95% for I and 1.75% for II (Table III). Comparison of this HPLC method and the bromothymol blue complex method for II and the USP method for I are shown in Table IV. HPLC assay results on these commercial tablet samples varied on an average of 2.3% from those of the USP assay for I and 1.45% from those of the dye complex assay for II.

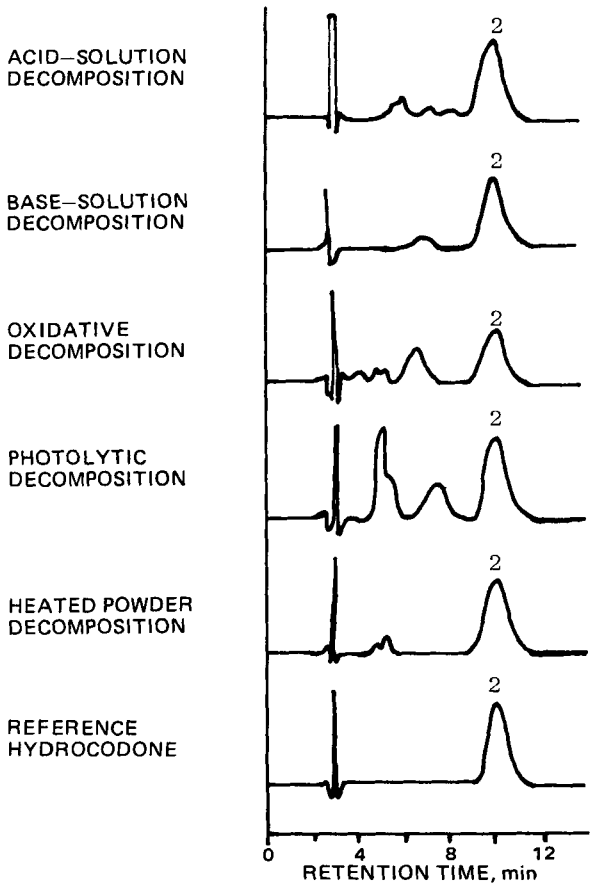

Figure 2—*Retention times of decomposition products resulting from extreme, accelerated decomposition studies of (Ref. 2) hydrocodone.*

Decomposition—No extraneous peak was detected in the analysis of the sample hydrocodone refluxed in water for 24 hr. Comparison of the chromatogram with USP reference standard hydrocodone bitartrate control solution showed a 100% quantitative recovery. Similar measurements of the main chromatographic peak from the decomposed solutions showed various amounts of residual drug depending on its treatment (Fig. 2).

The quantities remaining were found to be 81% after thermal, 79% after base hydrolysis, 87% after acid hydrolysis, 94% after UV photolysis, and 62% after oxidative treatment.

DISCUSSION

The proposed method requires less time than the USP method for acetaminophen tablets (15) which requires column preparation, elution, and absorbance measurements. A titration of an extract is reported for hydrocodone bitartrate tablets (16). For control assay purposes, a colorimetric ion-pairing assay has been adapted in this laboratory for determining II as described for codeine (17).

The chromatographic difficulty associated with the simultaneous quantitation of acetaminophen and codeine has been reported (12). The problem is even more severe in this dosage form. The quantitation of the 100:1 ratio of I to II is made possible by the electronic data module and optimum wavelength absorbance for II. For the tablet product, extracting the dosage form with water completely dissolved both components. Attention to detail in the chromatographic process was necessary so that the peak height of acetaminophen would be as large as possible without overloading the detector, thereby allowing the hydrocodone peak to be more easily and reproducibly quantitated. The elution times are also important, since band broadening increases with increasing elution time and subsequently decreases the peak height. In an additional analysis using the above method, a 10:1 weight ratio of acetaminophen to codeine was assayed simultaneously by measurements at the maximum wavelength of codeine.

The HPLC separation of degradation impurities that result when II is subjected to accelerated extreme decomposition is demonstrated in Fig. 2. The quantitative and qualitative content of unchanged II is easily determined, showing the stability-indicating nature of the analysis. To eliminate the possibility of an interference, no internal standard is employed. In this two-component solid dosage with many possible impurities and degradation products, this approach appears preferable.

It may be possible for a stress-produced degradation product of II to be within the peak for I. However, the response for I (>100:1) is so large

Table V—Comparison of Content Uniformity Assay for Hydrocodone Bitartrate by HPLC and by Ion-Pairing Method

Sample	Hydrocodone Bitartrate, %		
	HPLC	Ion-Pairing	Deviation
1	94.5	90.5	−4.0
2	95.9	96.1	+0.2
3	102.3	98.2	−4.1
4	95.4	91.8	−3.6
5	101.0	100.5	−0.5
6	96.1	94.3	−1.8
7	103.7	100.0	−3.7
8	98.4	98.6	+0.2
9	102.5	100.9	−1.6
10	103.4	102.3	−1.1
Average	99.3	97.3	−2.0

compared with II that the effect on I would be insignificant. In addition, the analysis for intact II is sufficiently specific and quantitative for stability purposes. The known degradation product, *p*-aminophenol, and the impurity of I have different retention times compared with II (Table I). Good assay data have been obtained for several 5-year-old tablet samples. Since there was only an interest in the strength of the hydrocodone, no attempt was made to identify the decomposition products formed under stress.

For the content uniformity run, aliquots of the same 10 tablet samples were assayed by the HPLC and ion-pairing methods for II as paired comparison. Assay data for the ion-pairing analysis are generally lower compared with HPLC (Table V).

Through the utilization of the selected wavelength of maximum absorbance for II, this procedure is being routinely used as an automated simultaneous determination. The HPLC method reported in this study allows for rapid, specific, stability-indicating, and simultaneous quantitation of both substances in a two-component tablet formulation.

REFERENCES

(1) E. Brochmann-Hanssen and A. Baerheim Svendsen, *J. Pharm. Sci.*, **51**, 1096 (1962).

(2) J. W. Barnhart and W. J. Caldwell, *J. Chromatogr.*, **130**, 243 (1977).

(3) F. M. Plakogiannis and A. M. Saad, *J. Pharm. Sci.*, **66**, 604 (1977).

(4) M. K. Chao, I. J. Holcomb, and S. A. Fusari, *ibid.*, **68**, 1463 (1979).

(5) E. J. Kubiak and J. W. Munson, *ibid.*, **69**, 152 (1980).

(6) A. Menyharth, F. P. Mahn, and J. E. Heveran, *ibid.*, **63**, 430 (1974).

(7) F. J. Sena, J. T. Piechocki, and K. L. Li, *ibid.*, **68**, 1465 (1979).

(8) S. K. Soni and S. M. Dugar, *J. Forensic Sci.*, **24** 437 (1979).

(9) V. Das Gupta, *J. Pharm. Sci.*, **69**, 110 (1980).

(10) D. Burke and H. Sokoloff, *ibid.*, **69**, 138 (1980).

(11) W. O. McSharry and I. V. E. Savage, *ibid.*, **69**, 212 (1980).

(12) C. Y. Ko, F. C. Marziani, and C. A. Janicki, *ibid.*, **69**, 1081 (1980).

(13) E. Townley and P. Roden, *ibid.*, **69**, 523 (1980).

(14) J. W. Munson and E. J. Kubiak, *Anal. Lett*, **13**(B8) 705 (1980).

(15) "The United States Pharmacopeia," 20th Rev., U.S. Pharmacopeial Convention, Rockville, Md., 1980, p. 12.

(16) *Ibid.*, p. 379.

(17) J. E. Fairbrother in "Analytical Profiles of Drug Substances," vol. 3, K. Florey, Ed., Academic, New York, N.Y., 1974 p. 34.

(18) V. Das Gupta, *Am. J. Hosp. Pharm.*, **28**, 55 (1971).

High-Pressure Liquid Chromatographic Assays for Ticarcillin in Serum and Urine

R. H. KWAN, S. M. MacLEOD, M. SPINO, and F. W. TEARE *x

Received April 6, 1981, from the **Faculty of Pharmacy, University of Toronto, Toronto, Ontario M5S 1A1, Canada*, and the *Department of Clinical Pharmacology, The Hospital for Sick Children, Toronto, Ontario M5S 1A1, Canada.* Accepted for publication December 9, 1981.

Abstract □ Rapid and sensitive high-pressure liquid chromatographic (HPLC) assays for ticarcillin in serum and urine have been developed. Sample pretreatment and optimized chromatographic conditions are presented for a C-18-bonded reversed-phase column used in an internal standard assay method. Ticarcillin has a retention time of ~5.3 min at a flow rate of 1.5 ml/min for a mobile phase of acetonitrile–aqueous 0.06 *M* sodium biphosphate, pH 2.05, (50.5:100). In a two-step extraction procedure, the ticarcillin extraction efficiencies from serum and urine were 76.1 ± 4.7 and 80.9 ± 3.2%, respectively. The assay sensitivity limit for ticarcillin in these fluids is ~1.0 μg/ml. A comparison is made of the HPLC and microbiological assay results for ticarcillin in 20 different but equally divided serum samples obtained from two volunteers.

Keyphrases □ Ticarcillin—high-pressure liquid chromatographic assays, serum and urine □ Urine—ticarcillin, high-pressure liquid chromatographic assays, serum □ Serum—ticarcillin, high-pressure liquid chromatographic assays, urine □ High-pressure liquid chromatography—ticarcillin in serum and urine

During the past decade, measurements of ticarcillin concentrations in clinical studies have been performed mainly by microbiological assays (1–9). A microtitration method (10, 11) has been applied to broth. Although numerous chemical and physical assay methods have been reported for the detection and assay of penicillins, they suffer from a variety of disadvantages. For instance, iodometric titration (12–15) cannot be applied to penicillins having unsaturated side chains. Spectrophotometric methods (16–18) are not very sensitive and a colorimetric assay (19–21) involving the reaction of hydroxylamine with penicillins in the presence of ferric ions to form a colored salt lacks both specificity and sensitivity, having a limit of ~20 μg/ml. A spectrofluorometric assay (22) has application only for fluorophoric penicillins. More recently, precise, convenient, and specific HPLC assays for various penicillins have been reported (23–29).

At the time the present study commenced there was, to the best of our knowledge, no HPLC assay reported for ticarcillin in biological fluids. This report presents a rapid, specific, and accurate HPLC assay for ticarcillin in human serum and urine.

EXPERIMENTAL

Materials—Powdered ticarcillin[1] (disodium salt) and carbenicillin[2], the internal standard, were used as received without further purification as standards. All solvents and reagents were analytical reagent grade, except for acetonitrile[3] which was HPLC grade, 190 nm cutoff.

Apparatus—The HPLC pump[4] was fitted with an injector[5] and variable wavelength detector[6] set at 210 nm and attenuated at 0.04 aufs. The signal was recorded either on a 10-mV recorder[7] at a speed of 0.5 cm/min or an integrator-plotter[8].

Columns—A prepacked 10- × 0.8-cm i.d. cartridge[9] containing a reversed-phase, C-18, chemically bonded to 10 μm of silica, and contained in a radial compression device[10] was employed at ambient temperature. A guard column[11] was placed between the injector and the column. Ti-

[1] Beecham Laboratories, Pointe Claire, Ontario, Quebec.
[2] Ayerst Laboratories Saint-Laurent, Quebec.
[3] Caledon Laboratories Ltd., Georgetown, Ontario, Canada.
[4] Waters Associates Inc., Milford, Mass., model 6000A.
[5] Waters Associates, model U6K.
[6] Waters Association, model 450.
[7] Beckman 10 inch Recorder, model 1005.
[8] Waters Associates, Data Module, model 730.
[9] Waters Associates, Radial-Pak-A.
[10] Waters Associates, RCM-100 Radial Compression System.
[11] Whatman Inc., Column Survival Kit with Co-Pell ODS.

0022-3549/82/01000-1118$01.00/0