Novel Method for Determination of Partition Coefficients of Penicillins and Cephalosporins by High-Pressure Liquid Chromatography

TSUKINAKA YAMANA ˣ, AKIRA TSUJI, ETSUKO MIYAMOTO, and OSAMU KUBO

Abstract □ Newly defined lipophilic indexes, log k', of a series of penicillins and cephalosporins were rapidly and reliably determined by reversed-phase high-pressure liquid chromatography (HPLC) on bonded octadecylsilane supports. The log k' values obtained from their retention times exhibited a linear relationship with methanol concentration (v/v %) in the mobile phase. The extrapolated log k' values to zero and those at 30% correlated well with the partition coefficients, log P, in 1-octanol–water and with R_m values from TLC. This HPLC technique provided some new log P and R_m values for highly ionizable β-lactam antibiotics. The HPLC method for the determination of partition coefficients of drugs has some advantages and is a useful alternative for the determination of log P and R_m.

Keyphrases □ Penicillins, various—high-pressure liquid chromatographic determination of lipophilic indexes, correlated to partition coefficients □ Cephalosporins, various—high-pressure liquid chromatographic determination of lipophilic indexes, correlated to partition coefficients □ High-pressure liquid chromatography—determination of lipophilic indexes of various penicillins and cephalosporins, correlated to partition coefficients □ Lipophilic indexes—log k' of various penicillins and cephalosporins determined by high-pressure liquid chromatography, correlated to partition coefficients □ Partition coefficients—various penicillins and cephalosporins, correlated to lipophilic indexes determined by high-pressure liquid chromatography □ Antibacterials—various penicillins and cephalosporins, high-pressure liquid chromatographic determination of lipophilic indexes, correlated to partition coefficients

Considerable effort has been expended to correlate biological properties of penicillins (1–7) and cephalosporins (5) with their partition coefficients. While the most useful and widely accepted lipophilic index is the logarithm of the partition coefficient, log P, determined in a 1-octanol–water system (8), such a measurement for penicillin molecules often presents practical difficulties because of their high instability in the acidic pH region near the pKa (~2.7). The log P values determined with a rapid pH-stat technique (9) seem to be the most accurate and reliable. Unfortunately, in spite of this technique, it is difficult to determine or compare the true log P values for β-lactam antibiotics because of two or more ionizable substituents in their side chains (*e.g.*, carbenicillin and sulbenicillin) and because of the zwitterion itself (*e.g.*, cyclacillin, ampicillin, amoxicillin, cephaloridine, cephaloglycin, cephalexin, and cephradine).

The R_m values, which are alternative lipophilic indexes, were determined from reversed-phase TLC for some penicillins (10) and cephalosporins (11). Recently, R_m values of penicillins were determined in another TLC system (9), but a difference between the actual pH on the plate and that of the mobile phase was observed (9). This pH difference is undesirable for acids such as β-lactam antibiotics, because the R_m values, in order to be an expression of the true partition coefficients, have to be corrected according to their degree of ionization at the mobile phase pH.

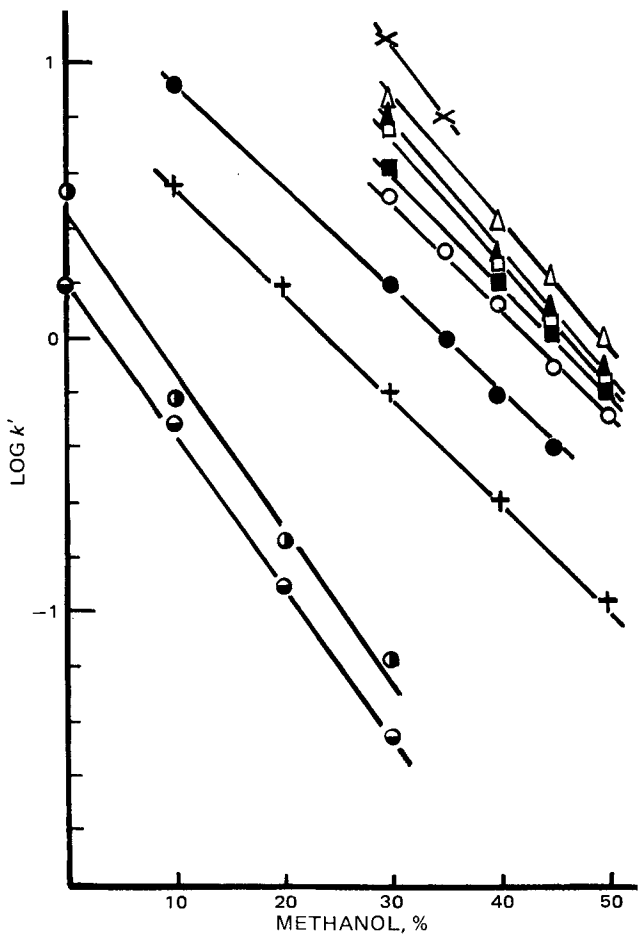

Figure 1—*Relationship between log* k' *values of penicillins and methanol concentration (v/v %) in the mobile phase. The compounds are numbered as in Table I. Key:* ×, *1;* △, *2;* ▲, *3;* □, *4;* ■, *7;* ○, *8;* ●, *9;* +, *11;* ◑, *12; and* ◒, *13.*

This paper reports a novel and useful method for the determination of partition coefficients of these β-lactam antibiotics by high-pressure liquid chromatography (HPLC). With the reversed-phase HPLC method developed recently (12), the lipophilic index, log k', can be defined as follows:

$$\log k' = \log [(t_R - t_0)/t_0] \quad \text{(Eq. 1)}$$

where t_R is the retention time of a retained peak, and t_0 is the retention time of an unretained peak. The term log k' is considered to be analogous to R_m.

EXPERIMENTAL

Materials—Carbenicillin phenyl sodium[1] (979 μg/mg), dicloxacillin

[1] Carfecillin (BRL 3475), Beecham Yakuhin Co., Tokyo, Japan.

Table I—Log k', Log P, and R_m Values of Penicillins and Cephalosporins

Number	Compound	Log k'[a] 30% Methanol	Log k'[a] Extrapolated[b]	Log $P_{octanol}$ Obs.[c]	Log $P_{octanol}$ Calc.[d]	$R_{m\,octanol}$ Obs.[e]	$R_{m\,octanol}$ Calc.[d]	$R_{m\,silicone}$ Obs.[f]	$R_{m\,silicone}$ Calc.[g]
1	Carbenicillin phenyl	1.09	2.68	3.10	3.14	—	0.77	—	2.05
2	Dicloxacillin	0.86	2.30	2.83	2.75	0.44	0.36	1.63	1.63
3	Floxacillin	0.79	2.14	2.67	2.58	—	0.19	—	1.45
4	Cloxacillin	0.77	2.04	2.44	2.48	0.01	0.08	1.34	1.33
5	Oxacillin	0.65	—	2.34	2.34	−0.15	−0.08	1.05	1.19
6	Propicillin	0.83	—	2.58	2.65	0.21	0.18	—	1.41
7	Phenethicillin	0.62	1.80	2.19	2.23	−0.23	−0.17	1.03	1.07
8	Penicillin V	0.51	1.62	2.01	2.05	−0.37	−0.37	0.89	0.86
9	Penicillin G	0.17	1.30	1.76	1.72	−0.66	−0.71	0.55	0.51
10	Cyclacillin	0.06	—	—	1.31	—	−0.93	—	0.46
11	Ampicillin	−0.19	0.94	—	1.35	—	−1.09	0.08	0.10
12	Amoxicillin	−1.14	0.48	—	0.87	—	−1.59	—	−0.41
13	Sulbenicillin	−1.50	0.20	—	0.59	—	−1.89	—	−0.73
14	Cephaloridine	0.37	—	—	1.85	—	—	0.98	0.85
15	Cephalothin	−0.08	—	0.94	1.07	—	—	0.40	0.29
16	Cephaloglyin	−0.10	—	—	1.04	—	—	0.29	0.27
17	Cephalexin	−0.32	—	—	0.65	—	—	—	0.00
18	Cephradine	−0.07	—	—	1.09	—	—	—	0.30
19	Cefazolin	−0.47	—	—	0.39	—	—	—	−0.19

[a] Log k' was defined in the text. A 2.1-mm × 25-cm stainless steel column was packed with Zorbax-ODS and eluted with 0.035 M NH_4Cl aqueous methanol solution of pH 7.4 at 150 kg/cm² and ambient temperature. [b] Extrapolated log k' value to 0% methanol from Fig. 1. [c] Data from Ref. 9, except those for Compounds 1, 3, and 15, which are averages of the values determined by employing the pKa values of 2.72 for Compounds 1 and 3 and of 2.22 for Compound 15 at four or more different pH's at 37° in this laboratory. [d] Calculated from corresponding Eqs. 2–4, except those for Compounds 5, 6, 10, and 14–19, which were calculated from Eqs. 5–7. [e] The R_m value determined at pH 4.0 (9); cellulose was the solid support, and 1-octanol was the stationary phase. [f] The R_m value determined at pH 7.4 (10, 11); silica gel was the solid support, and silicone oil was the stationary phase. [g] Calculated from Eq. 4 for the penicillins except Compounds 5, 6, and 10. Calculated from Eq. 7 for Compounds 5, 6, 10, and cephalosporins.

sodium[2] (900 μg/mg), floxacillin sodium[3] (893 μg/mg), cloxacillin sodium[2] (907 μg/mg), oxacillin sodium[4] (840 μg/mg), propicillin potassium[5] (993 μg/mg), phenethicillin potassium[2] (1444 units/mg), penicillin V potassium[2] (1490 units/mg), penicillin G potassium[2] (1600 units/mg), cyclacillin[5] (999 μg/mg), ampicillin sodium[5] (955 μg/mg), amoxicillin[3] (844 μg/mg), sulbenicillin disodium[5] (878 μg/mg), cephaloridine[6] (971 μg/mg), cephalothin sodium[6] (930 μg/mg), cephaloglycin[6] (864 μg/mg), cephalexin[6] (925 μg/mg), cephradine[7] (962 μg/mg), and cefazolin sodium[3] were used as supplied. Other chemicals[8] were of reagent grade and were used without further purification.

Procedures—A liquid chromatograph[9] equipped with a UV detector operating at 254 nm was used for HPLC. The stationary phase was totally porous silica gel bonded chemically with octadecyl chains prepacked into a 25-cm stainless steel column, 2.1 mm i.d.[10]. The mobile phase was a 0.035 M ammonium chloride–aqueous methanol solution adjusted to pH 7.4; ammonium salt was added to block the active silanol sites and to maintain the ionic strength. The instrument was operated at ambient temperature and 150 kg/cm². Solutions of penicillins and cephalosporins were prepared in the concentration range between 5×10^{-3} and 1×10^{-2} M. A 5-μl quantity of sample was injected into the column.

RESULTS AND DISCUSSION

Log k' values of the sample compounds were calculated from their t_R values by the application of Eq. 1, where the retention time of formamide was used for the t_0 value. The methanol concentration of the mobile phase was varied to obtain peaks with a reasonable retention time.

For the tested penicillins, plots of log k' *versus* methanol concentration (v/v %) showed a reasonable linear relationship (Fig. 1). Table I lists the log $k'_{(0\%)}$ values extrapolated to 0% methanol. The relationship between log $k'_{(0\%)}$ and the other reported lipophilic indexes (log P or R_m) (9, 10) is expressed by Eqs. 2–4 for penicillins and cephalosporins:

$$\log P_{octanol} = 1.03 \log k'_{(0\%)} + 0.38$$
$$n = 7 \quad r = 0.992 \quad s = 0.061 \qquad \text{(Eq. 2)}$$

$$R_{m\,octanol} = 1.07 \log k'_{(0\%)} - 2.10$$
$$n = 5 \quad r = 0.988 \quad s = 0.066 \qquad \text{(Eq. 3)}$$

$$R_{m\,silicone} = 1.12 \log k'_{(0\%)} - 0.95$$
$$n = 6 \quad r = 0.999 \quad s = 0.030 \qquad \text{(Eq. 4)}$$

where n, r, and s are number of experiments, correlation coefficient, and standard deviation, respectively. These equations show that log P and R_m in 1-octanol–water and silicone oil–water systems are satisfactorily correlated with log $k'_{(0\%)}$ values.

The slopes of Eqs. 2–4 are close to unity, which indicates that log $k'_{(0\%)}$ values should be regarded as those for the standard system in partition between a stationary phase and a mobile phase. However, such an extrapolation technique appears to be tedious and unreliable for highly lipophilic compounds, since one must extrapolate from a higher methanol concentration range. From the practical point of view, therefore, it would be desirable if log k' values at a suitable composition of the mobile phase could be used as an expression of the lipophilic character of the test compounds. For the present reference system, the log k' values at 30% methanol were selected because the 30% methanol concentration in the mobile phase provided a log $k'_{(30\%)}$ value in the range of maximum accuracy for most β-lactam antibiotics. The log $k'_{(30\%)}$ values (Table I) are considerably sensitive to the lipophilic balance of the substituents on the nucleus of penicillins and cephalosporins. Good correlations between log $k'_{(30\%)}$ values and log P or R_m are shown in Eqs. 5–7:

$$\log P_{octanol} = 1.74 \log k'_{(30\%)} + 1.21$$
$$n = 10 \quad r = 0.979 \quad s = 0.123 \qquad \text{(Eq. 5)}$$

$$R_{m\,octanol} = 1.45 \log k'_{(30\%)} - 1.02$$
$$n = 7 \quad r = 0.941 \quad s = 0.123 \qquad \text{(Eq. 6)}$$

$$R_{m\,silicone} = 1.23 \log k'_{(30\%)} + 0.39$$
$$n = 10 \quad r = 0.971 \quad s = 0.116 \qquad \text{(Eq. 7)}$$

The log P and R_m values calculated from Eqs. 2–7 with the corresponding log $k'_{(0\%)}$ or log $k'_{(30\%)}$ values are recorded in Table I. The results apparently confirm the usefulness of the log k' values as another expression of the lipophilic character and/or the conversion parameter for estimating unknown partition coefficients such as log P or Hansch π values (13) of β-lactam antibiotics.

For the determination of partition coefficients, Huber *et al.* (14) established a liquid–liquid chromatographic method; the HPLC method was recently proposed (12) as a useful alternative to the often tedious 1-octanol–water partition measurements. The HPLC method (12) is recommended for partition study of any compound because: (*a*) it is fast (*e.g.*, typical compounds can be eluted in less than 10 min) and repro-

[2] Meiji Seika Kaisha, Tokyo, Japan.
[3] Fujisawa Pharmaceutical Co., Osaka, Japan.
[4] Banyu Pharmaceutical Co., Tokyo, Japan.
[5] Takeda Chemical Industries, Osaka, Japan.
[6] Shionogi & Co., Osaka, Japan.
[7] Sankyo Co., Tokyo, Japan.
[8] Wako Pure Chemical Industries, Osaka, Japan.
[9] Shimadzu-DuPont 830.
[10] Zorbax-ODS, DuPont.

ducible, (*b*) the pH can be controlled, (*c*) it is applicable to compounds unstable in solution, (*d*) samples need not be pure, and (*e*) quantitative analytical methods need not be considered since any compound can be detected by a UV or refractive index detector.

However, an important unsolved problem remains. What system would produce the universal lipophilic index such as log *P*? A study is now in progress to develop a normalized method for correcting the differences in log *k'* determined with different columns from different sources, solvent compositions, pH's, and temperatures and to establish a general method for all kinds of chemical compounds.

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[x] To whom inquiries should be directed.

Rapid GLC Determination of Ibuprofen in Serum

D. J. HOFFMAN

Abstract □ A rapid procedure for the determination of ibuprofen in human serum was developed using a single extraction with carbon tetrachloride after deproteinization with perchloric acid. The internal standard, 3-methyl-3-phenylbutyric acid, was added directly to the serum. Gas chromatograms were free of interfering peaks. Calibration curves (0–40 μg/ml) were linear with a sensitivity of 0.5 μg of ibuprofen/ml of serum. Relative standard deviations ranged from 1.1 to 25%.

Keyphrases □ Ibuprofen—GLC analysis, human serum □ GLC—analysis, ibuprofen in human serum □ Anti-inflammatory agents—ibuprofen, GLC analysis in human serum

Ibuprofen is a 2-phenylalkylcarboxylic acid derivative possessing potent anti-inflammatory, antipyretic, and analgesic properties (1–3). Recently, a GLC method for determining ibuprofen in human plasma was reported (4). The assay involved an extraction into benzene, followed by evaporation of the extract to dryness and subsequent derivatization of ibuprofen to the methyl ester. A rapid GLC method for the anticonvulsant valproic acid in serum utilized the underivatized acid and a 5% FFAP column (5). By using similar conditions, a GLC method was developed for the assay of serum ibuprofen levels. This method is rapid and precise and does not require derivative formation.

EXPERIMENTAL

Reagents—Reagent grade perchloric acid[1] and carbon tetrachloride[2] were used without further purification. Ibuprofen and 3-methyl-3-phenylbutyric acid were used as supplied[3].

Instrumentation—The GLC analyses were performed on a gas chromatograph[4] equipped with an automatic sampler[5], computing integrator[6], and flame-ionization detector. The chromatograph was fitted with a 1.83-m (6-ft) U-shaped glass column (2 mm i.d.) packed with 5% FFAP[7] on Gas Chrom W (HP), 80–100 mesh[8]. The column was conditioned before use at 255° for 15 hr with a carrier gas (nitrogen[9]) at a flow rate of 30 ml/min.

The chromatographic conditions were: carrier gas, 35 ml/min; air, 260 ml/min; hydrogen, 37 ml/min; injection port and detector temperatures, 250°; and oven temperature, 220°. The attenuation of the computing integrator was ×8, and the electrometer[10] range was 10^2. Under these conditions, the internal standard and ibuprofen had retention times of 2.9 and 4.3 min, respectively (Fig. 1).

Assay—*Preparation of Standards*—A 2-mg/ml stock solution of ibuprofen was prepared by dissolving 100 mg of ibuprofen in a 50-ml volumetric flask with 2 ml of 0.5 *N* NaOH and adjusting to 50 ml with distilled water. A working serum ibuprofen standard at 40 μg/ml was prepared by adding 1.0 ml of the stock solution to a 50-ml volumetric flask and adjusting to volume with commercial human serum[11]. Other serum standards were prepared from serial dilutions (with serum) of the 40-μg/ml serum standard.

The internal standard stock solution (2 mg/ml) was prepared by dissolving 100 mg of 3-methyl-3-phenylbutyric acid in a 50-ml volumetric flask with 2 ml of 0.5 *N* NaOH and adjusting to 50 ml with distilled water. A working internal standard solution (100 μg/ml) was prepared by diluting 5 ml of the internal standard stock solution to 100 ml with distilled water.

Extraction Procedure—To a 15-ml conical glass centrifuge tube were added 0.5 ml of the internal standard solution, 1.0 ml of serum, and 0.5 ml of 10% perchloric acid. After vigorous mixing[12], 0.4 ml of carbon tetrachloride was added, followed again by vigorous mixing for 15 sec. The

[1] Mallinckrodt reagent grade, 60%.
[2] Mallinckrodt (low sulfur) reagent grade.
[3] Supplied by A. Geisler, Abbott Laboratories.
[4] Hewlett-Packard model 7610A.
[5] Hewlett-Packard model 7671A.
[6] Hewlett-Packard model 3380A.
[7] Varian Aerograph.
[8] Applied Science Laboratories.
[9] Linde Division, Union Carbide Corp.
[10] Hewlett-Packard model 7650A.
[11] K-N Enterprises.
[12] Maxi Mix model M-16715, Thermolyne, Sybron Corp.