Determination of Partition Coefficients of Glucocorticosteroids by High-Performance Liquid Chromatography

J. C. CARON ˣ and B. SHROOT

Received April 7, 1983, from the *Centre International de Recherches Dermatologiques, Sophia Antipolis, 06565 Valbonne, France.* Accepted for publication February 24, 1984.

Abstract □ Two high-performance liquid chromatographic (HPLC) techniques have been used for the determination of the lipophilicity of 50 glucocorticosteroids. For a log P range of 1 to 3, we used a persilylated octadecylsilane phase saturated with 1-octanol, which was eluted with 1-octanol saturated phosphate buffer (pH = 7). For log P values ≥3.00, the same phase was used without 1-octanol saturation and was eluted with different methanol-buffer mixtures. These two procedures allowed the determination of log P values ranging from 1.00 to >5.00. Good agreement was found between these procedures and others described in the literature. The effect of different structural changes in the glucocorticosteroid molecule on the lipophilicity is discussed.

Keyphrases □ Glucocorticosteroids--partition coefficients, determination by two HPLC methods □ HPLC--partition coefficients of glucocorticosteroids determination with and without 1-octanol □ Partition coefficients--1-octanol-water, glucocorticosteroids

The determination of 1-octanol-water partition coefficients is of particular interest in drug design for several reasons, but primarily because it governs absorption and transport to the site of action (1). Attempts have been made to calculate the partition coefficient of a given compound (2–4), but these theoretical approaches cannot take into account all the physicochemical properties of the molecule, especially the stereochemistry. It is thus advantageous to experimentally determine the lipophilicity of the compounds of interest. For this purpose, several methods have been proposed, of which the shake-flask technique (5) and the TLC method (6) are the most widely used. More recently, high-performance liquid chromatographic (HPLC) has been proposed for this purpose (7, 9) and we selected reverse-phase HPLC as a rapid and reliable method for the determination of log P. Log P is defined as the logarithm of the partition coefficient, $P = C_s/C_w$, where C_s and C_w are the equilibrium concentrations of a solute in the organic (usually 1-octanol) and aqueous (usually a buffer) phase, respectively.

EXPERIMENTAL SECTION

Materials - Hydrocortisone and its derivatives[1], triamcinolone acetonide[2], and other glucocorticosteroids[3] were used as received. Phenol, aniline, acetanilide, quinoline, anisole, benzene, *p*-chlorophenol, 4-methylquinoline, 3-bromoquinoline, phenazine, and acridine were analytical grade[4]. Pyridine, pyridine-*N*-oxide, 1-octanol, ethanol, trimethylchlorosilane, and hexamethyldisilazane were also analytical grade[5]. The HPLC columns used (stainless steel, 4.6 mm id) were of different lengths (5, 10, 15, 20, and 30 cm)[6], and packed with C_{18} Corasil[7] (particle size, 37–50 μm). The HPLC apparatus consisted of a pump[8], a valve injector[9], a UV-visible detector[10] fixed at 254 nm, and a strip chart recorder[11].

[1] Gist-Brocades.
[2] Léderlé.
[3] Glaxo.
[4] Aldrich.
[5] Fluka.
[6] Chrompack.
[7] Waters Associates.
[8] Model 6000A; Waters Associates.
[9] 7000 psi; Valco.
[10] Model M440; Waters Associates.
[11] Omniscribe.

Methods—Two systems have been used for the determination of log P, depending on the lipophilicity of the compound studied.

Log P Range, 0.93 to 3.00 (a)--The method of Mirrless *et al.* (7) was used with minor modifications. The HPLC column was dry packed with a persilylated octadecylsilane phase and saturated with 1-octanol by successive injections of 250 μL of alcohol under pressure until droplets appeared at the outlet of the column. The column was then flushed with 1-octanol-saturated phosphate buffer (0.01 M, pH 7) until the eluate appeared clear. The column was then attached to the detector and elution was continued until a stable baseline was obtained (1–2 h).

Samples were dissolved in ethanol at a concentration of 0.2% (w/v) and 10 μL was injected *via* the valve injector. Pyridine-*N*-oxide was used as a nonretained solute for the determination of the void volume of the column, measured as the elution time on the recorder.

We determined the correlation between log P and log k' for 11 standards of known log P (9) (aniline, acetanilide, quinoline, benzene, anisole, *p*-chlorophenol, phenol, 4-methylquinoline, 3-bromoquinoline, phenazine, and acridine) for a given flow rate and length of column. The capacity factor, k', is defined as: $(t_r - t_0)/t_0$ where t_r is the retention time of the compound and t_0 is the retention time of a nonretained solute.

The calibration curve obtained by the HPLC 1-octanol saturation method, for a given column length and flow rate, is shown in Fig. 1. There is good linear correlation between log P obtained by the shake-flask method (7) and log k' ($r = 0.994$) which is expressed as: $\log P_{SF} = 1.024 \log k' + 0.775$. The slope is near unity and indicates that the HPLC elution process emulates a true 1-octanol-buffer partition.

To determine the log P of steroids, three standards appropriate to the log P range under investigation were run twice a day. The reproducibility between days appeared to be >2% in the k'. For each unknown compound, the average of three determinations of the elution time was used to determine k', and again the *SD* was >2%. The log P of steroids ≤3.00 log P units was determined in this way.

The use of a 5-cm column with a flow rate of 4 mL/min results in retention times of 1–30 min for a log P range of 1–3 using the octanol saturation method. Under these conditions, it is calculated that a compound with a log P = 4 would have a retention time of 280 min; if log P = 5, a retention time of 2670 min would be anticipated. Long retention times for log P values >3 under these experimental conditions lead to peak broadening with a resultant loss in resolution, accuracy, and sensitivity. In addition, high flow rates lead to a rapid

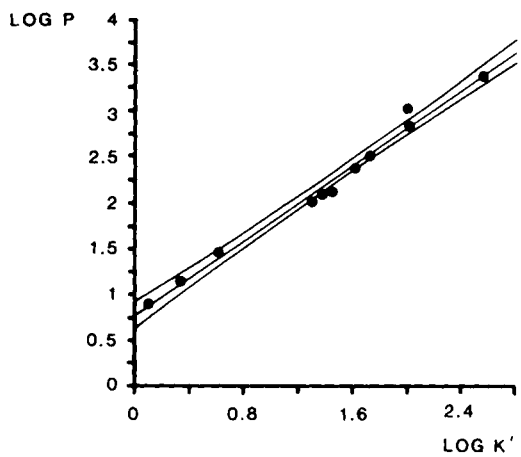


Figure 1 - *Standard calibration curve. Conditions: column length, 20 cm; flow rate, 1 mL/min. The curved lines show the 95% confidence limits.*

0022-3549/84/1200-1703$01.00/0

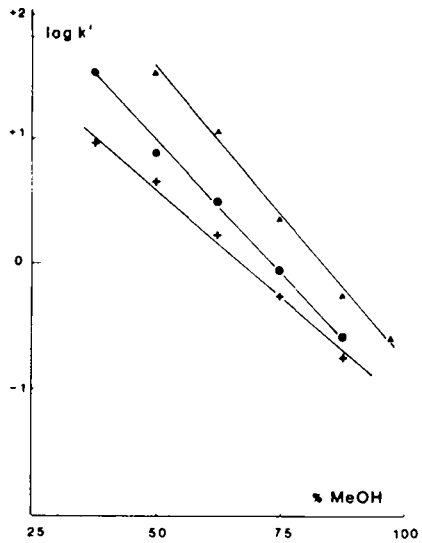


Figure 2—*Correlation between log* k' *and percent methanol (v/v). Key: (●) triamcinolone acetonide; (▲) hydrocortisone-21-butyrate; (+) prednisolone.*

wash out of the 1-octanol coating of the column, the frequent replacement of which considerably reduces the convenience of the procedure. These points led us to the idea of the solvent mixture method for the determination of log P values >3.00 (9, 10).

Log P Values of 3.00 and Above (b)—For the measurement of these log P values, a 20-cm column, filled with the same persilylated octadecylsilane phase, was used but without the 1-octanol coating. Elution was performed with at least five different methanol–buffer mixtures, and the retention time for any given solvent composition was determined for each compound (standards and unknown). An extrapolation of the logarithm of the capacity factor to 0% of organic solvent gives an estimation of the lipophilicity of the molecules studied (Fig. 2).

The proportions of methanol for each compound were chosen to keep the log k' <1.5 so that retention times could be precisely determined ($t_r \leq 45$ min). These proportions were usually kept between 45 and 95% of methanol. For five different methanol buffer mixtures, the correlation between log k' and the proportion of methanol was linear; the correlation coefficient was always >0.98 (for the majority of the steroids, $r \approx 0.99$). Figure 2 shows examples of such correlations for prednisolone, triamcinolone acetonide, and hydrocortisone-21-butyrate, which cover two decades of log P.

RESULTS AND DISCUSSION

By the first procedure, we determined the log P of 12 steroids in the range 1.21–2.88 for which the log P was reported in the literature (11). A good correlation was obtained ($r = 0.951$): $\log P_{SF} = 1.011 \log P_{HPLC} + 0.072$.

Using the second procedure, extrapolation to 0% methanol gave a measure of the partition between the buffer and the octadecylsilane phase, which is

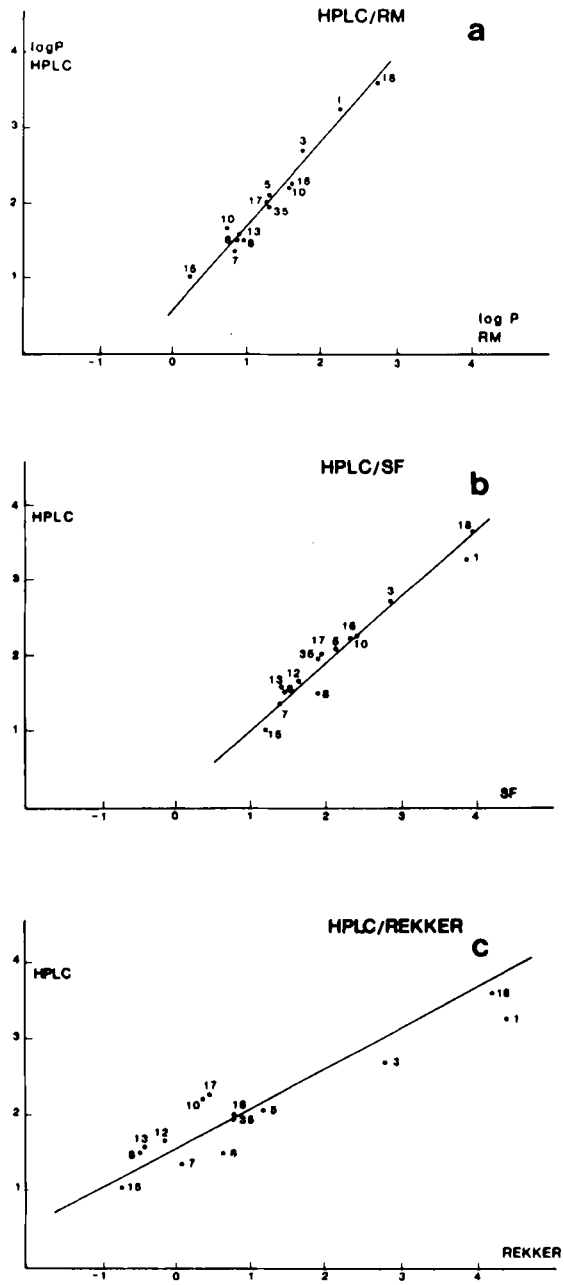


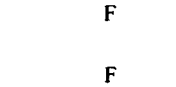
Figure 3—*Correlation between log P determined by HPLC*$_{1\text{-octanol}}$ *and (a) the* R_m *method (r = 0.983); (b) the shake-flask method (r = 0.980); (c) the Rekker method (r = 0.914).*

Table I—Log P Values for the Glucocorticosteroids Obtained by Different Methods

Steroid	$HPLC_{1\text{-octanol}}$	R_m[a]	Shake Flask[b]	Rekker[c]
Cortisone	1.50	0.88	1.47	0.64
Triamcinolone	1.02	0.24	1.21	−0.90
Prednisone	1.36	0.84	1.46	0.07
Hydrocortisone	1.50	0.96	1.93	−0.47
Prednisolone	1.58	0.90	1.42	−0.41
9α-F-hydrocortisone	1.67	0.73	1.68	−0.14
Dexamethasone	1.95	1.30	1.93	0.78
Betamethasone	2.01	1.26	1.98	0.78
Corticosterone	2.06	1.27	2.17	1.17
Hydrocortisone-21-acetate	2.21	1.58	2.37	0.39
Triamcinolone acetonide	2.27	1.63	2.44	0.46
Deoxycorticosterone	2.70	1.78	2.88	2.79
Progesterone	3.26	2.28	3.87	4.40
Betamethasone-17-valerate	3.60	2.77	3.94	3.22

[a] Ref. 10. [b] The correlation between the shake-flask and R_m method was 0.963; between the shake-flask and Rekker method, 0.914. Ref. 9. [c] Ref. 3.

Table II—Log P of Glucocorticosteroids Determined by the Solvent Mixture Method [a]

	Log P by Procedure *b*	$\Delta^{1,2}$ [b]	R_1	R_2	R_3	R_4	R_5
Progesterone	3.26	−	H	H	H	H	H
17α-Hydroxyprogesterone	2.74	−	H	H	H	OH	H
Deoxycorticosterone	2.70	−	H	H	H	H	OH
Cortexolone	2.04	−	H	H	H	OH	OH
Corticosterone	2.06	−	H	OH	H	H	OH
Cortisone	1.50	−	H	=O	H	OH	OH
Prednisone	1.36	+	H	=O	H	OH	OH
Hydrocortisone	1.50	−	H	OH	H	OH	OH
Hydrocortisone-17-butyrate	3.18	−	H	OH	H	OCOPr	OH
Hydrocortisone-21-acetate	2.21	−	H	OH	H	OH	OCOMe
Hydrocortisone-21-butyrate	2.91	−	H	OH	H	OH	OCOPr
9α-Fluorohydrocortisone	1.67	−	F	OH	H	OH	OH
Prednisolone	1.58	+	H	OH	H	OH	OH
9α-Fluoroprednisolone	1.51	+	F	OH	H	OH	OH
Triamcinolone	1.02	+	F	OH	OH	OH	OH
Triamcinolone acetonide	2.27	+	F	OH			OH
Betamethasone	2.01	+	F	OH	Me	OH	OH
Betamethasone-17-valerate	3.60	+	F	OH	Me	OCOBu	OH
Betamethasone-21-valerate	3.87	+	F	OH	Me	OH	OCOBu
Betamethasone-17,21-divalerate	5.26	+	F	OH	Me	OCOBu	OCOBu
21-Deoxybetamethasone	2.35	+	F	OH	Me	OH	H
21-Deoxybetamethasone-17-propionate	3.09	+	F	OH	Me	OCOEt	H
Clobetasol	2.48	+	F	OH	Me	OH	Cl
Clobetasol-17-propionate	3.18	+	F	OH	Me	OCOEt	Cl
Clobetasol-17-butyrate	3.63	+	F	OH	Me	OCOPr	Cl
Clobetasol-17-propionate (Cl)	3.28	+	Cl	OH	Me	OCOEt	Cl
Clobetasol-17-propionate (F)	3.34	+	H	OH	Me	OCOEt	Cl
Clobetasone	2.61	+	F	=O	Me	OH	Cl
Clobetasone-17-propionate	3.46	+	F	=O	Me	OCOEt	Cl
Clobetasone-17-butyrate	3.76	+	F	=O	Me	OCOPr	Cl
Clobetasone-17-propionate (F)	3.30	+	H	=O	Me	OCOEt	Cl
Clobetasone-17-propionate (H)	3.09	+	F	=O	Me	OCOEt	H
Clobetasone-17-propionate (Br)	3.55	+	F	=O	Me	OCOEt	Br
9α-Fluorocortisone	1.37	−	F	=O	H	OH	OH
Dexamethasone	1.95	+	F	OH	Me	OH	OH
Nandrolone	2.62	−	H	H	H	OH	—
Betamethasone-21-butyrate	3.55	+	F	OH	Me	OH	OCOPr
Hydrocortisone-17-valerate	3.79	−	H	OH	H	OCOBu	OH
Hydrocortisone-17-propionate	2.70	−	H	OH	H	OCOEt	OH
Prednisone-17-acetate	2.43	+	H	=O	H	OCOMe	OH
Cortisone-17-acetate	2.45	−	H	=O	H	OCOMe	OH
Hydrocortisone-21-propionate	2.80	−	H	OH	H	OH	OCOEt
Hydrocortisone-17-acetate	2.30	−	H	OH	H	OCOMe	OH
Hydrocortisone-21-valerate	3.62	−	H	OH	H	OH	OCOBu
Prednisone-17-valerate	3.82	+	H	=O	H	OCOBu	OH
Betamethasone-21-propionate	3.06	+	F	OH	Me	OH	OCOEt
Betamethasone-21-acetate	2.77	+	F	OH	Me	OH	OCOMe

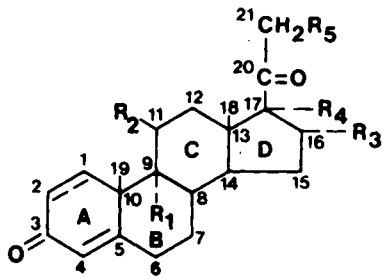
[a] These values are the means calculated from our experimental data and are not to be used to predict the lipophilicity changes induced by structure modification. [b] 1,2 refers to an olefinic bond between C_1 and C_2.

not, in fact, a true 1-octanol–buffer partition. When we compared these log k' 0% with the log P of the steroids determined by the saturation method, we obtained a linear relationship expressed as: log P (procedure *b*) = 0.939 log k' (procedure *a*) + 0.147 which validates the postulate that this procedure can be used for log P determinations >3 units, if closely related standards are used for the calibration. The main advantages of this method are the rapidity and ease of use, compared with the saturation method, but it also may allow us to determine log P values up to 6.

Listed in Table I are the log P values obtained by the 1-octanol saturation HPLC method, the shake-flask technique (11), the R_m values of Biaggi *et al.* (12), and hydrophobicity calculated by the Rekker method (3). R_m is defined as $R_m = \log (1/R_f - 1)$ and is related to the lipophilicity by log P = log K + R_m where K is a constant for the system. R_f is the usual measure of the migration of a compound in TLC.

Figure 3 shows the correlation between $HPLC_{1\text{-octanol}}$ and the 3 other methods for the 14 steroids of Table I. The agreement between $HPLC_{1\text{-octanol}}$ and shake flask and R_m methods is better than the correlation with the calculated hydrophobicity (Rekker). This is due to the fact that the Rekker method gives only an approximation and, like all results based upon calculations, it cannot take into account all the physicochemical properties of a molecule which are responsible for its lipophilicity (3).

We have used these 14 steroids as standards to determine, by the solvent mixture method, the log P of the glucocorticosteroids listed in Table II. The effect of the different structural changes on the lipophilicity of the steroids



A ring = $\Delta^{1,2}$
R_1 = H, F
R_2 = H, OH, =O
R_3 = H, OH, CH_3
R_4 = H, OH, ester
R_5 = H, OH, ester, Cl

can be determined by examining pairs of compounds differing by only one substituent.

The changes induced by the introduction of a double bond at C_1—C_2, the substitution of the hydrogen atom by a fluorine at C_9, or by changing from hydroxyl to carbonyl at C_{11} have only little effect on lipophilicity. However, changes at C_{16}, C_{17}, and C_{21} have a more significant effect on lipophilicity. The substitution of the C_{16} hydrogen atom by a methyl group induces a small increase in the lipophilicity (±0.50). At C_{17}, the substitution of H by OH only slightly modifies the lipophilicity, but the esterification of the hydroxyl group leads to an increase of the lipophilicity. Changing from OH to acetate increases the log P by 0.89 ± 0.15 (n = 4). A further increase in the chain length of the ester increases the log P with an increment of 0.46 ± 0.12 (n = 5) per carbon

atom. The C_{21} position shows the same tendency. Here the log P value changes by 0.42 ± 0.22 (n = 6) per carbon atom with increasing chain length. The introduction of a chlorine atom in place of OH causes only a slight increase in the log P value.

The two HPLC methods described for the determination of the lipophilicity of glucocorticosteroids are more rapid than the shake-flask method, and are more convenient than the TLC procedure.

REFERENCES

(1) E. J. Lien, *Ann. Rev. Pharmacol. Toxicol.*, **21**, 31 (1981).

(2) C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, **86**, 1616 (1964).

(3) R. F. Rekker, in "The Hydrophobic Fragmental Constant", W. Th. Nauta and R. F. Rekker, Eds., Elsevier, Amsterdam-Oxford-New York, 1977.

(4) P. Ahmad, C. A. Fyfe, and A. Mellors, *Biochem. Pharmacol.*, **24**, 1103 (1975).

(5) C. Hansch, *Acc. Chem. Res.*, **2**, 232 (1969).

(6) C. B. Boyce and B. V. Milborrow, *Nature (London)*, **5010**, 537 (1965).

(7) M. S. Mirrless, S. J. Moulton, C. T. Murphy, and P. J. Taylor, *J. Med. Chem.*, **19**, 615 (1976).

(8) T. Hanai and J. Hubert, *J. Chromatogr.*, **239**, 527 (1982).

(9) S. F. Unger, J. R. Cook, and J. S. Hollenberg, *J. Pharm. Sci.*, **67**, 1364 (1978).

(10) T. Yamana, A. Tsuji, E. Miyamoto, and O. Kubo, *J. Pharm. Sci.*, **66**, 747 (1977).

(11) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, **71**, 521 (1971).

(12) G. L. Biaggi, A. M. Barbaro, O. Gandolfi, M. C. Guerra, and G. Cantelli-Forti, *J. Med. Chem.*, **18**, 873 (1975).

Stability-Indicating Assay for Phenylbutazone: High-Performance Liquid Chromatographic Determination of Hydrazobenzene and Azobenzene in Degraded Aqueous Phenylbutazone Solutions

H. FABRE ˣ, N. HUSSAM-EDDINE, and B. MANDROU

Received December 30, 1982, from the *Laboratoire de Chimie Analytique, Faculté de Pharmacie, 34060 Montpellier Cedex, France.* Accepted for publication February 2, 1984.

Abstract □ A high-performance liquid chromatographic method was developed for the simultaneous determination of azobenzene, hydrazobenzene, and four other decomposition products in phenylbutazone injectable formulations. Separation was achieved on a C_{18} column, with 0.1 M Tris-citrate buffer (pH 5.25) and acetonitrile (52:48), at a flow rate of 2 mL/min and a detection wavelength of 237 nm. Diphenylamine was used as an internal standard. The limit of quantitation is 0.5% (with respect to phenylbutazone) of each degraded product. The detectability is 2.4×10^{-3} μg for azobenzene and 1.5×10^{-3} μg for hydrazobenzene. The limit of quantitation may be lowered to 0.1% (with respect to phenylbutazone) for azobenzene and hydrazobenzene in the presence of the two major decomposition products, which have been determined in commercially available injectable formulations. A higher sensitivity was obtained for azobenzene using the mobile phase 0.1 M Tris-citrate buffer (pH = 5.25) and acetonitrile (40:60) with detection at 314 nm. Under these conditions, 0.025% (with respect to phenylbutazone) of azobenzene is quantitated.

Keyphrases □ Phenylbutazone—HPLC, stability-indicating assay, degradation products □ Azobenzene—HPLC determination □ Hydrazobenzene—HPLC determination

The sodium salt of phenylbutazone (I) in aqueous solution undergoes hydrolysis and, to a small extent, oxidation and decarboxylation according to degradation Scheme I (1). Among these degradation products, some are of particular interest because of their deleterious effects: 4-butyl-4-hydroxy-1,2-diphenyl-3,5-pyrazolidinedione (II) may be involved in allergic reactions (2); hydrazobenzene (VIII) and azobenzene (IX) are suspected carcinogens (3). Despite their toxicity, no analytical method has been proposed for the identification and quantification of VIII and IX in phenylbutazone formulations. Only separation on chromatographic columns and identification by TLC have been reported as evidence for the presence of IX in commercial injections after prolonged storage (4). The present study was undertaken to complete preliminary work (5, 6); the conditions used previously in the high-performance liquid chromatographic (HPLC) procedure (6) did not allow the determination of VIII and IX within acceptable time. In this report, an improved, rapid, and sensitive HPLC procedure is presented that allows simultaneous determination of trace levels of II, III, IV, VI, VIII, and IX and a monitoring of the stability of I in injections.

EXPERIMENTAL SECTION

Materials and Reagents—Compounds I, II, III, IV, and VI were used as received[1]. Compounds VIII and IX, diphenylamine (DPA), Tris, and citric acid were analytical reagent grade. HPLC-grade acetonitrile, distilled-in-glass grade methanol, and water were also used. Injectable formulations were commercial formulations[2] (600 mg of the sodium salt of phenylbutazone, 6 mg of dibucaine, propylene glycol, and water per 3 mL of solution).

The HPLC[3] was equipped with a variable-wavelength UV detector[4]. The separation was carried out on a laboratory-made column (15 cm × 4.0 mm) containing microsilica particulate-bonded (5-μm) octadecylsilane[5]. The mobile phase A was 0.1 M Tris-citrate buffer (pH 5.25) and acetonitrile (52:48). The citrate buffer was filtered through a 0.45-μm filter[6], and the mixture was deaerated before use.

Standard Solutions—An initial mixed stock solution was prepared in the mobile phase using I (200 μg/mL) and II, III, IV, VI, VIII, and IX (100 μg of each/mL). This solution was suitably diluted in the mobile phase to give standard solutions. An internal standard solution (diphenylamine, 500 μg/mL in the mobile phase) was added to each diluted standard solution to give a 100-μg/mL concentration of diphenylamine. The final concentration range was from 5 to 160 μg/mL for I and from 2.5 to 80 μg/mL for II-IV, VI, VIII, and IX.

Test Solutions—Recovery studies were carried out on laboratory-prepared injections similar to commercial formulations with the following amounts

[1] Gifts from Geigy Laboratories, Basel, Switzerland.
[2] Butazolidine; Geigy.
[3] SP 8000; Spectra Physics.
[4] SF 770; Schoeffel.
[5] Lichrosorb RP-18; Merck, Darmstadt, W. Germany.
[6] Sartorius.

0022-3549/84/1200-1706$01.00/0