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# MEASUREMENT OF THE DISTRIBUTION COEFFICIENTS OF SEVERAL CLASSES OF DRUG USING REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY

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## SUMMARY

Using reversed-phase thin-layer chromatography, with octan-1-ol as stationary phase and phosphate buffer (pH 7.4) as mobile phase, the behaviour of different drugs at 37°C was studied. Three classes of drug were examined: β-adrenoceptor antagonists, non-steroidal anti-inflammatory agents and dihydropyridine calcium antagonists. As well as ranking these compounds in terms of their distribution coefficients, an attempt was also made to assign a quantitative value to each. For the β-adrenoceptor antagonists this was done by using a series of published values obtained using the shake-flask technique: for the non-steroidal anti-inflammatory agents a series of standard compounds was used. No good calibration data were available for the dihydropyridine calcium antagonists, but approximate values were assigned. The results obtained were compared with other published data and the applicability of the method discussed.

## INTRODUCTION

The magnitude of the partition coefficient of a drug is a major factor in determining its passage across membranes within the body for absorption, tissue penetration and elimination. Many drugs are weak acids or bases and their ionized and unionized forms exit in equilibrium within the body. When a drug is partially ionized, the apparent partition, or distribution coefficient is measured; this is referred to as $P'$, while the true partition coefficient is $P$. The relationship between the two can be simply expressed by the following, where $K_a$ is the dissociation constant:

for a base: $P = P' (1 + H^+/K_a)$

for an acid: $P = P' (1 + K_a/H^+)$

The standard method for determining the distribution coefficient of a drug is to partition it between a lipid-like organic phase and an aqueous phase, then measure the concentration of drug in both. Whether the classical "shake flask" technique or the more recent AKUFVE method is employed[1], this approach suffers from the

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disadvantage that quantitative measurements of drug in both phases must be carried out.

When it is desired to determine the distribution coefficients of a number of drugs, even closely related, it is rarely possible to use a single analytical method. Radiolabelled compounds can, of course, be used but are not always readily available. To overcome these disadvantages, several groups of workers have used reversed-phase chromatography where a lipid-like stationary phase is used in conjunction with an aqueous buffer as mobile phase.

High-performance liquid chromatography (HPLC) has been used[2], but thin-layer chromatography (TLC) is much cheaper and has been employed since the early studies on penicillins[3] and phenothiazines[4]. The method is simple and can be used even when the drug is impure.

We have applied the technique of reversed-phase thin-layer chromatography to the quinolone antibiotics[5] and now wish to report its application to β-adrenoceptor antagonists, non-steroidal anti-inflammatory agents and dihydropyridine (DHP) calcium antagonists.

## MATERIALS AND METHODS

### *Drugs and related chemicals*

The following drugs were used: penbutolol (Hoechst, U.K.), bevantolol (Warner Lamber, U.K.), propranolol and practolol (ICI, U.K.), labetalol (Duncan Flockhart, U.K.), alprenolol, metoprolol and felodipine (Hässle, Sweden), oxprenolol, diclofenac sodium and pirprofen (Ciba-Geigy, U.K.), pindolol and isradipine (Sandoz, U.K.), timolol, diflunisal, indomethacin and sulindac (MSD, U.K.), acebutolol, diacetolol and ketoprofen (May & Baker, U.K.), sotalol (Bristol-Meyers, U.K.), nadolol (Squibb, U.K.), atenolol (Stuart, U.K.), benoxaprofen (Dista, U.K.), fenbufen (Lederle, U.K.), flufenamic acid (Merrell Dow, U.K.), naproxen (Syntex, U.K.), flurbiprofen and ibuprofen (Boots, U.K.), tolmetin sodium (Ortho, U.K.), nisoldipine, nitrendipine, nimodipine and nifedipine (Bayer, U.K.). Antipyrine, 4-aminoantipyrine, mephenesin, phenacetin and salicylic acid were all purchased from Sigma, U.K.

### *Thin-layer chromatography*

Cellulose plates (CEL300, 20 × 20 cm) were obtained from Macherey-Nagel (Düren, F.R.G.) and octan-1-ol from Sigma. The plates were coated with octan-1-ol by placing them in a tank containing a solution of octan-1-ol (5%) in diethyl ether and allowing the organic phase to migrate up to plate until it had reached a few centimeters from the top. The plate was then removed from the tank and allowed to dry in air. Standard solutions of the drugs under investigation were made up in methanol, water or other suitable solvent to a concentration of 1 mg/ml and applied to the starting line, 1.5 cm from the bottom edge of the plate. The applied spots were dried in a gentle stream of air and the plate transferred to a glass tank containing the phosphate buffer (pH 7.4, 0.07 *M*), previously saturated with octan-1-ol. This developing tank was kept in an incubator at 37°C and development of the plate was allowed to continue until the mobile phase had travelled a distance of 10 cm (approximately 1 h). The plate was then removed, allowed to dry and the drugs visualized by examination under UV ligth or by spraying with a suitable reagent[6]. Many of the

drugs can be detected by simply observing the plate as it dries when they appear as white areas against a wet background. These spots disappear again as the plate continues to dry. The $R_F$ value of each drug is recorded and the $R_M$ calculated using the relationship

$$R_M = 1/R_F - 1$$

Relatively lipophilic drugs, such as penbutolol, show a concentration-dependent $R_F$ value and, in such cases, a range of concentrations were used and the $R_F$ extrapolated to zero-concentration.

The extremely lipophilic DHP calcium antagonists do not migrate at all when phosphate buffer is used as mobile phase. To overcome this, different amounts of acetone are added to the phosphate buffer and extrapolation made to zero acetone concentration. This method has previously been used to study the partition of a number of penicillins[7].

## RESULTS

Kielselguhr and silica gel layers were also examined initially but the former were too easily damaged while the latter were very slow to coat with octan-1-ol and took much longer to develop, once coated. The coating with octan-1-ol was reproducible and only very small variations in $R_F$ values were obtained from day to day. A study using 18 plates was performed over a period of several weeks and coefficients of variation of $R_F$ of 1.4–2.2% were obtained for the calibration compounds 4-aminoantipyrine, antipyrine, mephenesin and phenacetin. Three different classes of drug have been studied: β-adrenoceptor antagonists non-steroidal anti-inflammatory agents and dihydropyridine calcium antagonists. The $R_F$ and $R_M$ values for each class of compound are shown in Tables I–III, respectively. Examination of this data allows an

TABLE I

RETENTION DATA FOR β-ADRENOCEPTOR ANTAGONISTS

| *Drug* | *Abbreviation used in Fig. 1* | $R_F$ | $R_M$ |
|---|---|---|---|
| Penbutolol | | 0.10 | 9.00 |
| Bevantolol | | 0.12 | 7.33 |
| Propranolol | pr | 0.18 | 4.50 |
| Labetalol | la | 0.21 | 3.80 |
| Alprenolol | | 0.26 | 2.85 |
| Oxprenolol | ox | 0.45 | 1.20 |
| Pindolol | pi | 0.56 | 0.80 |
| Timolol | ti | 0.57 | 0.75 |
| Metoprolol | me | 0.61 | 0.65 |
| Acebutolol | ac | 0.66 | 0.51 |
| Diacetolol | | 0.80 | 0.25 |
| Sotalol | so | 0.85 | 0.17 |
| Nadolol | na | 0.87 | 0.15 |
| Atenolol | at | 0.90 | 0.11 |
| Practolol | | 0.91 | 0.10 |

TABLE II

RETENTION DATA FOR NON-STEROIDAL ANTI-INFLAMMATORY AGENTS

| *Drug* | $R_F$ | $R_M$ | *Drug* | $R_F$ | $R_M$ |
|---|---|---|---|---|---|
| Benoxaprofen | 0.33 | 2.03 | Naproxen | 0.74 | 0.35 |
| Diflunisal | 0.46 | 1.17 | Flurbiprofen | 0.79 | 0.27 |
| Fenbufen | 0.48 | 1.08 | Salicyclic acid | 0.80 | 0.25 |
| Indomethacin | 0.57 | 0.75 | Ibuprofen | 0.81 | 0.23 |
| Flufenamic acid | 0.62 | 0.61 | Pirprofen | 0.81 | 0.23 |
| Sulindac | 0.62 | 0.61 | Tolmetin sodium | 0.81 | 0.23 |
| Diclofenac sodium | 0.73 | 0.37 | Ketoprofen | 0.82 | 0.22 |

immediate ranking in terms of distribution coefficient and absolute values can be obtained using suitable calibration curves and the relationship

$$\log P' = A \log R_M + B$$

For the $\beta$-adrenoceptor antagonists, we used the data obtained by Woods and Robinson[8] who determined their distribution coefficients at pH 7.4 and 37°C using the "shake flask" technique. Their data are given in Table IV. A good fit was obtained when distribution coefficient was plotted against $R_M$ and this is illustrated in Fig. 1.

Unfortunately there is no similar set of data for the non-steroidal anti-inflammatory agents and calibration was performed using four compounds whose distribution coefficients have been measured under carefully controlled conditions: 4-aminoantipyrine, antipyrine, mephenesin and phenacetin. The calibration curve obtained is shown in Fig. 2. Some data has recently been published using a reversed-phase $C_{18}$ column[9] coated with octan-1-ol and this is shown in Table IV along with some quantitative structure–activity relationship (QSAR) data[10]. The distribution coefficients for flufenamic acid, flurbiprofen and ibuprofen are reasonably similar when the two chromatographic techniques are compared but large differences are observed for the other compounds studied. Agreement between both these sets and the QSAR data is also poor. Reasons for this are suggested in the discussion.

TABLE III

RETENTION DATA FOR DIHYDROPYRIDINE Ca ANTAGONISTS

| *Acetone in mobile phase (%)* | $R_M$ | | | | | |
|---|---|---|---|---|---|---|
| | *Nifedipine* | *Isradipine* | *Nimodipine* | *Nitrendipine* | *Nisoldipine* | *Felodipine* |
| 5 | 5.67 | 15.66 | 49.0 | 49.0 | 199.0 | 199.0 |
| 10 | 2.85 | 10.11 | 11.50 | 11.50 | 49.0 | 49.0 |
| 15 | 1.44 | 4.56 | 5.67 | 6.14 | 10.10 | 19.0 |
| 20 | 0.69 | 2.03 | 2.33 | 2.70 | 3.76 | 6.14 |
| 25 | 0.41 | 1.13 | 1.33 | 1.38 | 2.23 | 3.00 |
| 30 | 0.23 | 0.61 | 0.75 | 0.79 | 1.08 | 1.50 |
| 40 | 0.02 | 0.18 | 0.19 | 0.18 | 0.32 | 0.33 |

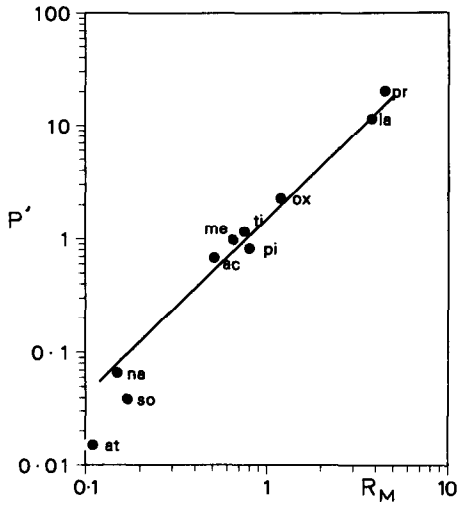


Fig. 1. Relationship between $R_M$ and distribution coefficient for a range of $\beta$-adrenoceptor antagonists.

The dihydropyridine calcium antagonists are all extremely lipid soluble and no good calibration compounds are available. The two most lipophilic compounds available to us with known distribution coefficients were penbutolol and diazepam and we used these to give us a two point calibration. The $R_M$ data were extrapolated to zero-acetone concentration (Fig. 3). We felt confident in doing this because a good linear relationship was apparent for the compounds nifedipine and isradipine. Extrapolation of the other data was far less certain but, nevertheless, we felt it would be an instructive exercise.

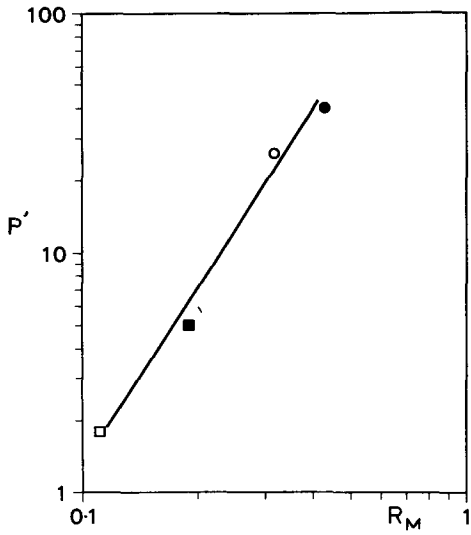


Fig. 2. Relationship between $R_M$ and distribution coefficient for antipyrine (□), 4-aminoantipyrine (■), mephenesin (○) and phenacetin (●).

TABLE IV

DISTRIBUTION COEFFICIENTS (OCTAN-1-OL–PHOSPHATE BUFFER, pH 7.4) AT 37°C

The values of other workers given here were obtained under a variety of conditions and some (ref. 10) were obtained from Hansch calculations.

| *β-Adrenoceptor antagonists* | | | *Non-steroidal anti-inflammatory agents* | | | | *DHP calcium antagonists* | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Drug* | *P'* | | *Drug* | *P'* | | | *Drug* | *P'* | | |
| | *This work* | *Ref. 8* | | *T his work* | *Ref. 9* | *Ref. 10* | | *This work* | *Ref. 11* | *Ref. 12* |
| Penbutolol | 44.5 | | Benoxaprofen | 1690 | 43.7 | | Felodipine | $11\,500 \cdot 10^3$ | | |
| Bevantolol | 32.2 | | Diflunisal | 480 | | | Nisoldipine | $11\,500 \cdot 10^3$ | | 38.0 |
| Propranolol | 15.0 | 20.2 | Fenbufen | 400 | | | Nitrendipine | $980 \cdot 10^3$ | 10.0 | |
| Labetalol | 11.5 | 11.5 | Indomethacin | 170 | 12.6 | | Nimodipine | $980 \cdot 10^3$ | | 730.0 |
| Alprenolol | 7.2 | | Flufenamic acid | 100 | 154.9 | | Isradipine | $19 \cdot 10^3$ | | |
| Oxprenolol | 1.9 | 2.3 | Sulindac | 100 | 2.0 | | Nifedipine | $2 \cdot 10^3$ | 2.6 | |
| Pindolol | 1.0 | 0.82 | Diclofenac Na | 31 | 11.0 | 6.4 | | | | |
| Timolol | 0.90 | 1.2 | Naproxen | 28 | 2.6 | 0.9 | | | | |
| Metoprolol | 0.72 | 0.98 | Flurbiprofen | 15 | 11.0 | 11.2 | | | | |
| Acebutolol | 0.49 | 0.68 | Salicylic acid | 12 | | | | | | |
| Diacetolol | 0.16 | | Ibuprofen | 10 | 13.8 | 31.3 | | | | |
| Sotalol | 0.09 | 0.04 | Pirprofen | 10 | | | | | | |
| Nadolol | 0.07 | 0.07 | Tolmetin Na | 10 | 1.0 | | | | | |
| Atenolol | 0.04 | 0.02 | Ketoprofen | 9 | 1.2 | | | | | |
| Practolol | 0.04 | | | | | | | | | |

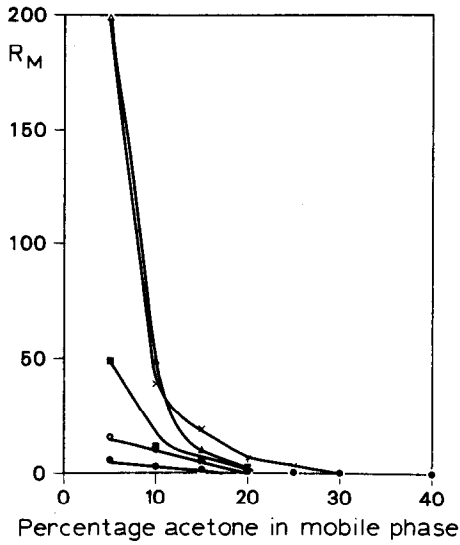


Fig. 3. Relationship between acetone concentration and $R_M$ for the DHP calcium antagonists nifedipine (●), isradipine (○), nimodipine (■), nitrendipine (□), nisoldipine (△) and felodipine (×).

A very limited amount of published data exists for the calcium antagonists and this is reproduced in Table IV.

## DISCUSSION

The good fit achieved with the β-adrenoceptor antagonists suggests that the present approach may be of some value. A number of other antagonists, not studied by Woods and Robinson[8], were examined using our method and the results are in general agreement with published values, bearing in mind that our measurements are carried out at 37°C.

The measured $R_F$ values of the β-adrenoceptor antagonists ranged from 0.1 (penbutolol) to 0.91 (practolol). In view of the good fit obtained for the compounds used to construct Fig. 1, we believe that $R_F$ values over this range can be used to give meaningful data. For very lipophilic compounds, with $R_F$ values less than 0.1, acetone can be added to the mobile phase as we described above for the dihydropyridine calcium antagonists. How useful the method is for compounds with $R_F$ values greater than 0.91 remains to be seen. An examination of this is planned.

Comparisons between different methods is difficult since most "shake-flask" determinations are carried out at 18–25°C; most drugs are weak acids or bases and their dissociation constants are temperature-dependent. We deliberately chose a working temperature of 37°C in order that our results would be more relevant to what happens *in vivo*.

As far as the non-steroidal anti-inflammatory agents are concerned, the poor agreement between the different studies obviously requires that further work be carried out. We ourselves plan a comparison of TLC with HPLC, as well as more comprehensive calculations of partition coefficients using Hansch analysis.

One property of the acidic anti-inflammatory drugs that may well be of relevance is their ability to form dimers in the octanol phase. This phenomenon is concentration-dependent and will, almost certainly, have an effect on the results obtained by different groups of workers.

We have carried out some simple Hansch calculations for the dihydropyridine calcium antagonists and our results suggest that the experimental ranking of lipophilicity that we have observed is largely correct, bearing in mind that the experimental extrapolation described above for the compounds other than nifedipine and isradipine can only be regarded as approximate. There is a large difference between our results and the limited published data. All the compounds we studied behaved chromatographically as though they had distribution coefficients much greater than diazepam ($P' = 661$)[13]. It is therefore very difficult to explain the very low values of other workers[11,12]. In support of our own observations, we have examined an internal report from Hässle[14], makers of felodipine. They measured the distribution coefficient for this compound between toluene and water and obtained a value for log $K_D$ of 4.52. We believe that our value of 7.06 ($P' = 11\,500 \cdot 10^3$) for the octan-1-ol–water system is sufficiently close, bearing in mind the technical difficulties and the extrapolation, to merit further study of the technique. This we intend to do. Comparison of distribution coefficients from different sources suffers from the disadvantage that they are often obtained under very different experimental conditions such as temperature, pH and mobile phase. We believe that reversed-phase TLC offers a versatile approach that can be applied to a wide range of drugs. When distribution coefficients are determined under a single set of conditions, more meaningful comparisons can be made to allow a greater understanding of processes such as tissue binding and penetration, metabolism and excretion.

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