Journal of Pharmaceutical & Biomedical Analysis
Vol. 8, Nos 8-12, pp. 877-880, 1990
Printed in Great Britain

0731-7085/90 $3.00 + 0.00
Pergamon Press plc

Chromatographic retention relationships between aliphatic tertiary amines and their putative *N*-oxide metabolites — preliminary results*

PETER BALLARD† and BRIAN LAW

ICI Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK

Abstract: The chromatography of a series of tertiary amines and the corresponding *N*-oxides have been studied and the Functional Group Contribution Approach used to describe retention correlations between them. Good correlations are observed between the behaviour of compounds on HPLC and with the exception of some alicyclic nitrogen compounds also on thin layer chromatography. The models produced are used to predict k' and R_f values for ranitidine *N*-oxide and tamoxifen *N*-oxide based upon those for the parent molecule. The deviation between actual and predicted values was larger than expected, presumably due to the structural or physico-chemical differences of ranitidine and tamoxifen compared to the model compounds.

Keywords: *Functional group contribution approach; metabolite identification;* N-*oxides; tertiary amines; HPLC; TLC.*

Introduction

The formation of *N*-oxides is an important metabolism route for many tertiary amino compounds in animals and man. Certain *N*-oxides act as possible inducers of cancer [1]. *N*-oxides have been identified generally after isolation by chromatography followed by mass spectrometry or simply co-chromatography with authentic reference standards. Direct analysis of *N*-oxides is problematical due to their thermal instability, when they are converted back to the parent amine or other pyrolysis products. They are also highly water soluble, making solvent extraction difficult.

Reversed-phase HPLC retention data have been reported to correlate well with partition coefficients (log P) for structurally similar compounds. Consequently, a particular substituent might be expected to provide a constant change in log P, and a corresponding constant change in retention for a given set of compounds, providing the chromatographic system is modelling the octanol–water partitioning correctly — the so-called functional group contribution approach (FGCA) [2].

This paper presents an investigation into the chromatographic behaviour of putative *N*-oxide metabolites relative to the parent amino compounds with the aim of predicting retention data for *N*-oxides. A set of model compounds were evaluated by reversed-phase HPLC and standard thin layer chromatography (TLC) systems and the data generated were used to predict k' and R_f values for ranitidine *N*-oxide and tamoxifen *N*-oxide based upon those of the parent molecules.

Experimental

Reversed-phase HPLC was carried out using a 150 × 4.6 mm i.d. Zorbax Rx C8, 5 μm column. The eluent was methanol–0.02 M phosphate buffer, pH 5.0 (80:20, v/v), giving a measured pH of 6.6, delivered at 1 ml min^{-1}. The 0.02 M, phosphate buffer pH 5.0 was prepared by dissolving 2.70 g of potassium dihydrogen orthophosphate and 0.023 g of disodium hydrogen orthophosphate in 1 l of distilled water. Lithium nitrate was used as void volume marker (retention time = t_o). Retention times were converted to capacity factors, k', according to the equation: $k' = (t_r - t_o)/t_o$, where t_r is the retention time of the peak of interest.

The silica TLC plates (SG 60 F-254) were supplied by Merck. The retention of solutes was expressed in terms of R_m values [3], where:

$$R_m = \log\left[\frac{1}{R_f} - 1\right],$$

*Presented at the "Second International Symposium on Pharmaceutical and Biomedical Analysis", April 1990, York, UK.

†Author to whom correspondence should be addressed.

R_f = ratio of the distance travelled by the analyte from the origin relative to the solvent front.

A test set of tertiary amino compounds and the corresponding *N*-oxides were obtained from ICI Pharmaceuticals and commercial sources. The *N*-oxides used in the investigation are shown in Fig. 1. The amines studied possessed pK_a values between 5.7–9.9 and log *P* values between (1.2–4.9).

Results and Discussion

Reversed-phase HPLC

Log *P* values are related to the partition coefficients of compounds in their unionized form. In the HPLC eluent, where the pH is below the pK_a, the compound will be ionized and the effective lipophilic character, log *D*, is related to log *P* by the expression

$$\log P = \log D + \log [1 + 10^{(pK_a - pH)}] \quad (1)$$

In an ideal chromatographic system log *D* is proportional to the logarithm of the capacity factor (log *k'*) and log *P* is proportional to log k'_p (the logarithm of the capacity factor of the unionized compound, i.e. when eluent pH > pK_a). Hence, equation (1) can be expressed as:

$$\log k'_p = \log k' + \log [1 + 10^{(pK_a - pH)}]. \quad (2)$$

For the set of test compounds, a good correlation ($R^2 = 0.92$) was observed for log *k'* values of *N*-oxide against the tertiary amines (Fig. 2).

One pair of compounds, tribenzylamine and its *N*-oxide (Fig. 1), did not fit this correlation. This anomaly was probably due to a combination of the steric effects of the three benzyl groups around the nitrogen and the relatively low pK_a (5.7) for the amine.

Using the model described in Fig. 2, the capacity factor of ranitidine *N*-oxide was predicted from that of the parent compound. The

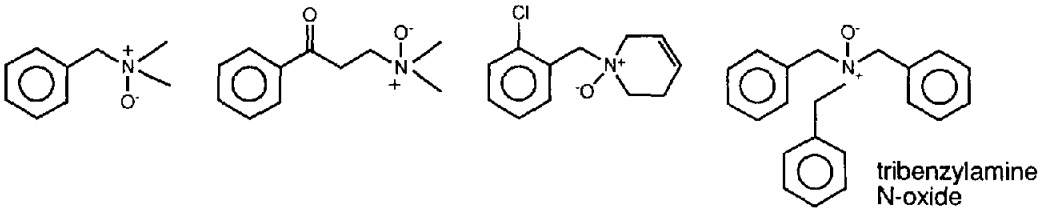

Figure 1
Some typical *N*-oxides used as model compounds.

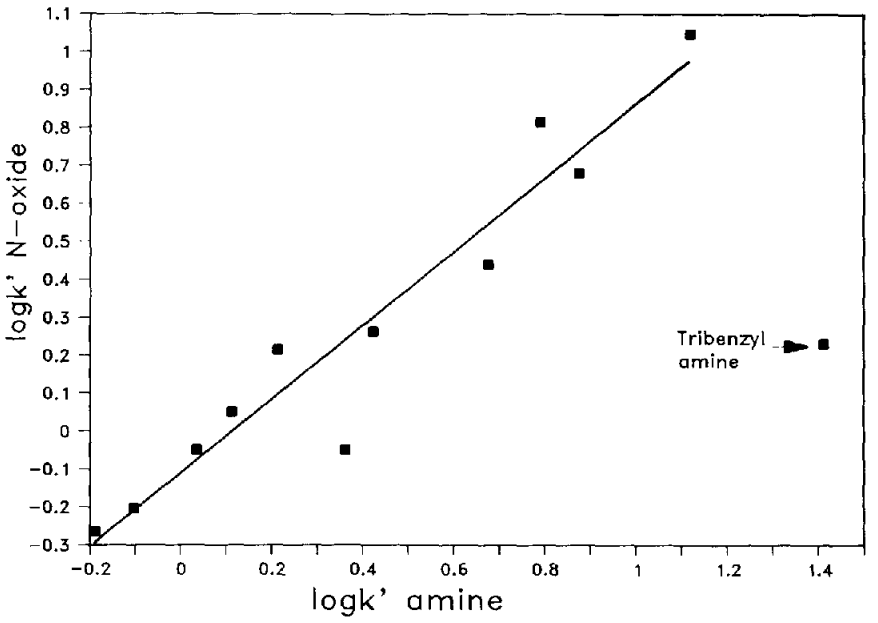

Figure 2
Log *k'* *N*-oxide versus log *k'* amine. HPLC column: Zorbax Rx C8, 150 × 4.6 mm i.d. Eluent: 70% methanol–0.02 M buffer (pH 5); measured pH 6.6. Linear regression: log *k'* (*N*-oxide) = 0.97. Log *k'* (amine) − 0.11.

Table 1
Predicted and actual k' for ranitidine based on the model HPLC data

	Predicted k'	Actual k'
Ranitidine	—	1.0
Ranitidine *N*-oxide	0.78	0.47

predicted and actual k' values for ranitidine *N*-oxide are not as close as would have been expected from the data for the model compounds (see Table 1). This may be due to ranitidine having a measured log *P* of 0.27 and consequently it is outside the range of log *P* values for the set of model compounds.

Correcting for the state of ionization, by converting log k' values to log k'_p should result in a better correlation. However, these data showed poor correlation, although they could be split into two sets: compounds where the amine log *P* values were greater or less than 2. The poor correlation was further exemplified by comparison of log k'_p and calculated amine log *P* values which again showed little correlation. This suggests that there is an inconsistency in the calculation of log k'_p probably due to the pK_a values calculated for the compounds. It has been reported [4] that pK_a calculations are subject to an error of ±0.25 when in an aqueous environment. The HPLC eluent, containing 70% methanol, will presumably add to the inaccuracies associated with pK_a.

Thin layer chromatography

Examination of previously published TLC data [5] on aromatic tertiary amines and their *N*-oxides showed good correlation (R^2 = 0.92) with one system in particular as illustrated by Fig. 3. However, the same system applied to the model set of alkyl and alicyclic tertiary amines, and *N*-oxides gave very poor results with the *N*-oxides typically remaining at the origin.

Many other TLC systems were evaluated with generally limited success. Of these, the highest correlation (R^2 = 0.90) of *N*-oxide against tertiary amine R_m values was observed with the system of methanol–0.1 M sodium chloride [6] (Fig. 4). However, this correlation was restricted to the alkyl compounds. The alicyclic compounds did not show a good correlation.

From the regression based upon the data for the alkyl series, the R_f values for tamoxifen and ranitidine were used to predict the R_f of their respective *N*-oxide metabolites (see Table 2).

The predicted and actual R_f values for tamoxifen *N*-oxide show reasonable agreement, although the agreement for ranitidine is not as good as expected. This latter feature may be attributed to the quite marked struc-

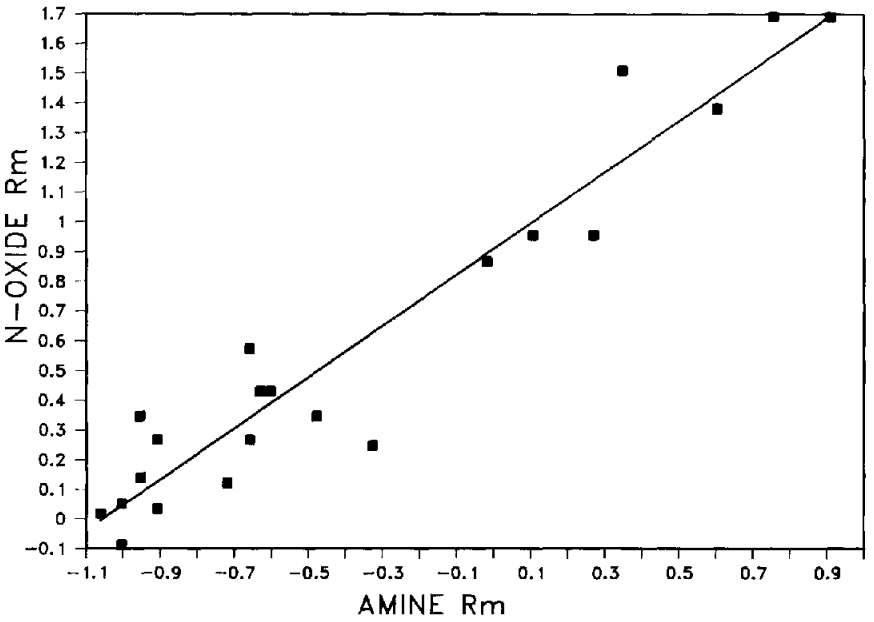

Figure 3
N-oxide versus amine R_m values for a series of aromatic compounds TLC system: silica plates with solvent $CHCl_3$–EtOH–NH_3 (95:4:1, v/v/v).

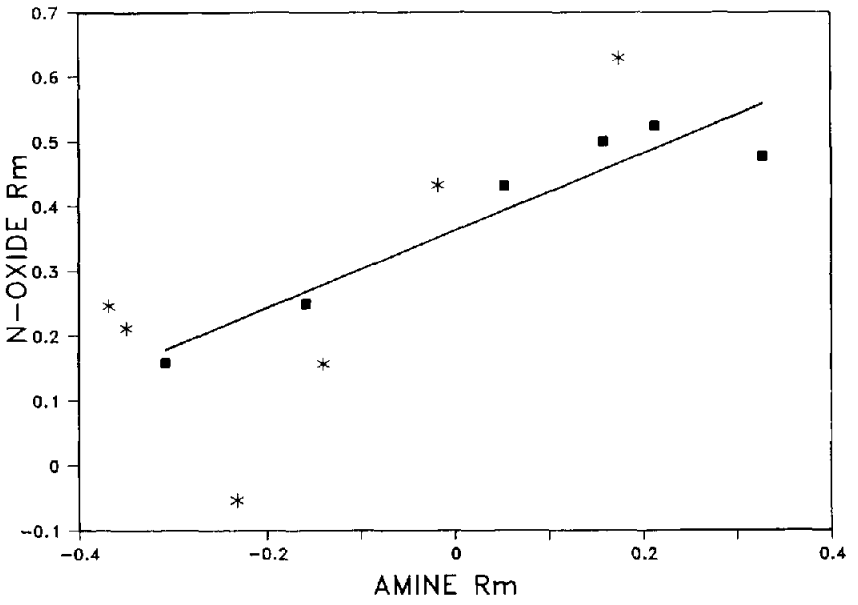

Figure 4
R_m values for *N*-oxide versus amine for the model set of alkyl (■) and alicyclic (*) compounds. TLC system: silica plates with solvent methanol–0.1 M sodium chloride. Linear regression: R_m (*N*-oxide) = 0.60. R_m (amine) + 0.36.

Table 2
Predicted and actual R_f values for tamoxifen *N*-oxide and ranitidine *N*-oxide based on the model TLC data

Compound	Predicted R_f	Actual R_f
Tamoxifen *N*-oxide	0.24	0.19
Ranitidine *N*-oxide	0.19	0.10

tural and physico-chemical differences between this compound and the set of model compounds used in the present study.

Conclusions

The FGCA, which has been the basis of previously successful studies [7, 8] did not work as well as was expected, i.e. addition of oxygen to an amine to produce the *N*-oxide did not result in a constant change in R_m or k' under all circumstances.

However, in certain limited cases the *N*-oxide metabolites of alkyl and alicyclic tertiary amines could be characterized by their retention characteristics relative to the parent molecule on both reversed-phase HPLC and standard TLC systems. This approach could be useful in identifying *N*-oxides from relatively impure samples.

On TLC the two sets of compounds (alkyl and alicyclic) cannot be described by a single relationship. This is in contrast to the aromatic amines and their *N*-oxides [3], where their more fixed steric and electronic environment probably makes the FGCA more applicable.

References

[1] M.H. Bickel, *Xenobiotica* **1**, 313–319 (1971).
[2] B.-K. Chen and C. Horvath, *J. Chromatogr.* **171**, 15–28 (1979).
[3] E.C. Bate-Smith and R.G. Westall, *Biochem. Biophys. Acta* **4**, 427–440 (1950).
[4] D.D. Perrin, pK_a *Calculations for Organic Acids and Bases*. Chapman and Hall, London (1980).
[5] L.A. Damani, L.H. Patterson and J.W. Gorrod, *J. Chromatogr.* **155**, 337–348 (1978).
[6] R.A. De Zeeuw, F.J.W. Van Mansvelt and J.E. Greving, *J. Chromatogr.* **148**, 255–263 (1978).
[7] R. Gill, S.P. Alexander and A.C. Moffat, *J. Chromatogr.* **218**, 639–646 (1981).
[8] B. Law, *J. Chromatogr.* **407**, 1–18 (1987).

[Received for review 4 April 1990; revised revision received 14 June 1990]