

This article was downloaded by:

On: 24 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597273>

### CHROMATOGRAPHIC DATA-TOPOLOGICAL INDEX DEPENDENCE FOR SELECTED STEROIDS

A. Pyka<sup>a</sup>

<sup>a</sup> Faculty of Pharmacy, Academy of Medicine, Sosnowiec, Poland

Online publication date: 28 February 2001

**To cite this Article** Pyka, A.(2001) 'CHROMATOGRAPHIC DATA-TOPOLOGICAL INDEX DEPENDENCE FOR SELECTED STEROIDS', *Journal of Liquid Chromatography & Related Technologies*, 24: 4, 453 — 460

**To link to this Article:** DOI: 10.1081/JLC-100103385

**URL:** <http://dx.doi.org/10.1081/JLC-100103385>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

## CHROMATOGRAPHIC DATA–TOPOLOGICAL INDEX DEPENDENCE FOR SELECTED STEROIDS

A. Pyka

Faculty of Pharmacy, Academy of Medicine,  
4, Jagiellońska Street, PL-41-200 Sosnowiec, Poland

### ABSTRACT

Selected steroids have been separated by reversed phase thin layer chromatography and reversed phase high performance liquid chromatography. The chromatographic data ( $R_M$  and  $\log k'$ ), and partition coefficient ( $\log P$ ) values of the compounds have been correlated with the numerical values of topological indices. The most accurate predictions of the  $R_M$ ,  $\log k'$ , and  $\log P$  values of the selected steroids were achieved by using monoparametric equations employing the topological indices  $^{\circ}B$ .

### INTRODUCTION

Steroids have a cyclopentanoperhydrophenanthrene skeleton. Steroids have a biological significance.<sup>1-4</sup> Studies were proven by analysis of steroids content and composition in food.<sup>5-10</sup> Studies of the correlation between structure and retention time and hydrophobic properties are particularly important in the case of biologically active substances.<sup>11-17</sup>

Optimization of chromatographic systems and the possibility of predicting the course of TLC and HPLC separation has been the subject of many papers.<sup>15,16,18-22</sup>

Steroids, considering their biological properties, are the subjects of intensive studies. In this connection, the aim of the work reported herein was to apply topological indices and to evaluate the correlation between these topological indices, and chromatographic data ( $R_M$  and  $\log k'$  values), and the partition coefficient of the selected steroids separated by RP-TLC and RP-HPLC. The aim of these investigations were to also compare the significance of particular topological indices in discussed dependences.

## EXPERIMENTAL

### Reversed Phase Thin Layer Chromatography

The steroids: corticosterone acetate (**I**), 11-dehydrocorticosterone acetate (**II**), corticosterone (**III**), 11-dehydrocorticosterone (**IV**), hydrocortisone (**V**), and cortisone (**VI**) (E. Merck, Darmstadt, Germany) were investigated. RPTLC was performed on precoated kieselguhr and silica gel plates (#5567, E. Merck, Germany), which were impregnated with a 10% solution of paraffin oil in hexane. Steroids were separated using methanol-water (65:35, V/V) as the mobile phase. The development distance was 14 cm. The chromatograms were visualized in UV light (=254 nm).

### Reversed Phase High Performance Liquid Chromatography

RP-HPLC was performed on 150 x 3.3 mm (i.d.) Separon Six C18 column using a Knauer instrument with UV detection (=254 nm). Column void time was determined using the peak derived from a solution of sodium nitrite in methanol. The mobile phase was prepared from analytical grade methanol and redistilled water (3:2, V/V). Earlier, this mobile phase was applied by Śliwiok et al.<sup>23</sup>

### Calculation of Topological Indices Described in the Literature

The Randić ( $^0\chi$ ,  $^0\chi_v$ ,  $^1\chi$ ,  $^1\chi_v$ ),<sup>24-26</sup> Wiener (W),<sup>24,25,27</sup> Balaban ( $I_B$ ),<sup>24,25,28</sup> and Pyka (A,  $^0B$ )<sup>24,29</sup> indices were calculated for the investigated compounds. The Wiener, Balaban, and Pyka indices were calculated by building the distance matrix and determining its elements by means of values given by Barysz et al.<sup>30</sup>

Calculation of Partition Coefficients, logP

Partition coefficients logP were calculated from fragmental constants, according to the method of Rekker,<sup>31</sup> using eq.:

$$\log P = \sum_n a_n f_n \tag{1}$$

using the following numerical values of  $f_n$  where  $n$  denotes a given structural fragment:

$$\begin{aligned} f_{-CH_3} &= 0,702; f_{-CH_2} = 0,527; f_{-CH} = 0,236; f_{-C=} = 0,140; f_{-CH=C=} = 0,51; \\ f_{-C=O} &= -1,69; f_{-OH} = -1,44; \text{ and } f_{-COO} = -1,28. \end{aligned}$$

RESULTS AND DISCUSSION

$R_p$ ,  $R_M$  and logk' values; logP values by Rekker; and numerical values of the topological indices  $^0\chi$ ,  $^0\chi^v$ ,  $^1\chi$ ,  $^1\chi^v$ , W,  $I_B$ , A,  $^0B$  are listed in Table 1 for the investigated steroids.

The computer program, Statgraphics, was then used to perform correlation analysis, by a least squares method on all the topological indices and values listed in Table 1. The correlation coefficients for simple linear relationships between the topological indices,  $R_M$  and logk', and logP are listed in Table 2.

The best relationships for chromatographic parameters ( $R_M$  and logk') and hydrophobic parameter logP were obtained as:  $R_M$  vs. logk';  $R_M$  vs. logP; logk' vs. logP;  $R_M$  vs.  $^0B$ ; logk' vs.  $^0B$ ; and logP vs.  $^0B$ . These correlation have good linearity, usually with  $r > 0.96$ :

$$\begin{aligned} R_M &= -0.7210 (\pm 0.0495) + 0.8211 (\pm 0.0717) \log k' & (2) \\ n=6; \quad r &= 0.9851; \quad s=0.031; \quad F= 131.05; \quad P=0.0003 \end{aligned}$$

$$\begin{aligned} R_M &= -0.2974 (\pm 0.0134) + 0.1442 (\pm 0.0101) \log P & (3) \\ n=6; \quad r &= 0.9904; \quad s=0.025; \quad F= 205.7; \quad P=0.0001 \end{aligned}$$

$$\begin{aligned} \log k' &= 0.5196 (\pm 0.0227) + 0.1713 (\pm 0.0170) \log P & (4) \\ n=6; \quad r &= -0.9808; \quad s=0.042; \quad F= 101.34; \quad P=0.0005 \end{aligned}$$

$$\begin{aligned} R_M &= 14.9756 (\pm 1.4002) - 5.9486 (\pm 0.5498) ^0B & (5) \\ n=6; \quad r &= -0.9833; \quad s=0.033; \quad F= 111.07; \quad P=0.0004 \end{aligned}$$

$$\begin{aligned} \log k' &= 18.5859 (\pm 2.2622) - 7.0362 (\pm 0.8882) ^0B & (6) \\ n=6; \quad r &= -0.9696; \quad s=0.053; \quad F= 62.75; \quad P=0.0014 \end{aligned}$$

$$\begin{aligned} \log P &= 106.454 (\pm 3.332) - 41.464 (\pm 1.308) ^0B & (7) \\ n=6; \quad r &= -0.9980; \quad s=0.078; \quad F= 1004.23; \quad P=0.0000 \end{aligned}$$

**Table 1.** Numerical Values of  $R_F$ ,  $R_M$ , and  $\log k'$ , and  $\log P$  by Rekker and the Topological Indexes  $\chi^0$ ,  $\chi^1$ ,  $\chi^2$ ,  $W$ ,  $I_B$ ,  $A$ , and  ${}^{\circ}B$

| No. of Steroid | Retention Data <sup>a</sup> |            |                    | Topological Indexes |          |          |                |                     |              |               |        |
|----------------|-----------------------------|------------|--------------------|---------------------|----------|----------|----------------|---------------------|--------------|---------------|--------|
|                | By RP-TLC                   | By RP-HPLC | $\log P$ by Rekker | Randić Indexes      |          |          | Wiener Index W | Balaban Index $I_B$ | Pyka Indexes |               |        |
|                | $R_F$                       | $R_M$      | $\log k'$          | $\chi^0$            | $\chi^1$ | $\chi^2$ | ${}^1\chi$     | ${}^1\chi'$         | A            | ${}^{\circ}B$ |        |
| <b>I</b>       | 0.475                       | 0.043      | 0.925              | 18.949              | 17.500   | 13.077   | 11.563         | 1786.5              | 1.705        | 689.50        | 2.3171 |
| <b>II</b>      | 0.525                       | -0.043     | 0.810              | 18.579              | 17.085   | 13.108   | 11.613         | 1776.4              | 1.658        | 685.94        | 2.5250 |
| <b>III</b>     | 0.580                       | -0.141     | 0.759              | 15.332              | 17.035   | 11.870   | 10.562         | 1248.5              | 1.717        | 508.36        | 2.5350 |
| <b>IV</b>      | 0.610                       | -0.141     | 0.590              | 16.665              | 15.216   | 12.050   | 10.613         | 1239.5              | 1.718        | 504.36        | 2.5450 |
| <b>V</b>       | 0.675                       | -0.317     | 0.500              | 17.956              | 15.702   | 12.238   | 10.654         | 1360.9              | 1.758        | 542.75        | 2.5741 |
| <b>VI</b>      | 0.710                       | -0.388     | 0.420              | 17.588              | 15.585   | 12.269   | 10.705         | 1351.5              | 1.748        | 539.27        | 2.5837 |

<sup>a</sup> Average of 10 measurements.

Table 2. Correlation Matrix for  $R_M$ ,  $\log k'$ ,  $\log P$ , and Topological Index Values

| $R_M$        | $\log k'$ | $\log P$ | ${}^0\chi$ | ${}^0\chi^y$ | ${}^1\chi$ | ${}^1\chi^y$ | $W$    | $I_B$   | $A$     | ${}^0B$ |
|--------------|-----------|----------|------------|--------------|------------|--------------|--------|---------|---------|---------|
| 1            | 0.9851    | 0.9904   | 0.2927     | 0.8289       | 0.6521     | 0.7670       | 0.7140 | -0.7945 | 0.7272  | -0.9833 |
| $\log k'$    | 1         | 0.9808   | 0.2306     | 0.9049       | 0.6103     | 0.7331       | 0.6909 | -0.7569 | 0.7038  | -0.9696 |
| $\log P$     |           | 1        | 0.1611     | 0.8124       | 0.5507     | 0.6834       | 0.6196 | -0.7976 | 0.6346  | -0.9980 |
| ${}^0\chi$   |           |          | 1          | 0.2432       | 0.8793     | 0.7786       | 0.8300 | -0.2564 | 0.8209  | -0.1328 |
| ${}^0\chi^y$ |           |          |            | 1            | 0.6044     | 0.7086       | 0.7056 | -0.6380 | 0.7136  | -0.7910 |
| ${}^1\chi$   |           |          |            |              | 1          | 0.9828       | 0.9906 | -0.6516 | 0.9891  | -0.5333 |
| ${}^1\chi^y$ |           |          |            |              |            | 1            | 0.9904 | -0.7588 | 0.9923  | -0.6703 |
| $W$          |           |          |            |              |            |              | 1      | -0.6734 | 0.9998  | -0.5992 |
| $I_B$        |           |          |            |              |            |              |        | 1       | -0.6846 | 0.8212  |
| $A$          |           |          |            |              |            |              |        |         | 1       | -0.6147 |
| ${}^0B$      |           |          |            |              |            |              |        |         |         | 1       |

where  $n$  is the number of compounds used in the derivation of the regression equation;  $r$  is the correlation coefficient;  $F$  is the value of the Fisher test;  $P$  is the significance level of  $F$  value; and  $s$  is the standard error of the estimate.

The relationships between the  $R_M$  values and the topological indices indicate that topological index  ${}^0B$  has an important significance in the research of this relationship. The identical observation refers to the dependence  $\log k'$  vs. topological index  ${}^0B$ . The high correlation coefficient was observed for the relationship between  $\log P$  and  ${}^0B$ , also. This fact indicates that this discussed dependence has significance in the partition process in an octanol - water system. The values of correlation coefficients have slight noticeable differences for the above-mentioned relationships. These differences are contained in the limit of error of estimation of the research method. Significantly lower values of correlation coefficient for relationship  $\log k'$  vs.  ${}^0B$  ensues from the work of the column, among other things.

The high correlation coefficients and significance levels of the equations presented in this paper, are indicative of the special physicochemical importance of topological index  ${}^0B$  from amongst all investigated topological indices. Index  ${}^0B$  shows important significance on the scale of investigated correlations. This fact ensues from the establishment of calculation of topological index  ${}^0B$ .<sup>24,29</sup>

One test of a predictive equation like Eqn (5) is how well it predicts values of a compound not included in the training set. One compound, hydrocortisone, was removed from the training set, and a monoparametric equation was re-calculated as:

$$R_M = 15.5795(\pm 1.7269) - 6.1878(\pm 0.6796){}^0B \quad (8)$$

$n=5; \quad r = -0.9824; \quad s=0.035; \quad F= 82.91; \quad P=0.0028$

Table 3 gives the  $R_M$  and  $R_F$  values, calculated from Eqn (8), of the hydrocortisone deleted from the training set. As would be expected, the  $R_M$  and  $R_F$  values predicted by Eqn (8) are accurate. The correlations obtained indicate that they are robust and can be used for reasonably good predictions of the  $R_M$  and  $\log k'$  values, and  $\log P$  values.

**Table 3.**  $R_M$  and  $R_F$  Values of Hydrocortisone Omitted from Equation (8) and Predicted by That Equation

| $R_M$     | $R_F$        |           | $\Delta R_F$ | % Error |
|-----------|--------------|-----------|--------------|---------|
|           | Experimental | Predicted |              |         |
| Predicted |              |           |              |         |
| -0.348    | 0.675        | 0.690     | 0.015        | 2.2     |

These experiments indicate that the topological index  ${}^0B$  is connected in a special way with the hydrophobic parameter of a compound between immiscible phases. This fact was confirmed by high correlation coefficients.

Further investigations concerning the physicochemical significance of the topological index  ${}^0B$ , in connection with chromatographic data, are being continued.

## REFERENCES

1. Smejkal, G.B.; Hoppe, G.; Hoff, H.F. *Anal. Biochem.* **1996**, *239*, 115–117.
2. Zinder, O.; Dar, D.E. *Acta Physiol. Scand.* **1999**, *167*, 181–188.
3. Rishani, R.; El-Khatib, M.; Mroueh, S. *Pediatr. Pulmonol.* **1999**, *28*, 451–453.
4. Finn, D.A.; Gallaher, E.J.; Crabbe, J.C. *J. Pharmacol. Exp. Ther.* **1000**, *292*, 394–405.
5. Valdenebro, M.S.; Leon-Camacho, M.; Pablos, F.; Gonzalez, A.G.; Martin, M.J. *Analyst* **1999**, *124*, 999–1002.
6. Fried, B. *Thin-Layer Chromatography*; Marcel Dekker, Inc.: New York, 1999.
7. Aitzetmueller, K.; Bruehl, L.; Fiebig, H.J. *Fett / Lipid* **1998**, *100*, 429–435.
8. Bodzek, D.; Bakowski, W.; Wielkoszyński, T.; Janoszka, B.; Jaremczuk, B.; Tarnawski, R.; Tyrpień, K. *Acta Chromatogr.* **1998**, *8*, 122–143.
9. Reina, R.J.; White, K.D.; Jahnggen, E.G.E. *J. AOAC Int.* **1997**, *80*, 1272–1280.
10. Van Boven, M.; Daenens, P.; Maes, K.; Cokelaere, M. *J. Agric. Food Chem.* **1997**, *45*, 1180–1184.
11. Śliwiok, J.; Kocjan, B. *La Rivista delle Sostanze Grasse* **1988**, *LXV*, 197–199.
12. Śliwiok, J.; Kocjan, B. *J. Planar Chromatogr. - Mod. TLC* **1994**, *7*, 327–328.
13. Dimov, N.; Moskovkina, M. *J. Chromatogr.* **1991**, *552*, 59–69.
14. Lafont, R.; Morgan, E.D.; Wilson, I.D. *J. Chromatogr. A* **1994**, *658*, 31–53.
15. Różyło, J.K.; Żabińska, A.; Matysiak, J.; Niewiadomy, A. *Chem. Environ. Res.* **1998**, *7*, 65–75.
16. Różyło, J.K.; Żabińska, A.; Matysiak, J.; Niewiadomy, A. *J. AOAC Int.* **1999**, *82*, 31–37.
17. Cîmpan, G.; Bota, C.; Coman, M.; Grinberg, N.; Gocan, S. *J. Liq. Chrom. & Rel. Technol.* **1999**, *22*, 29–40.
18. Różyło, J.K.; Matysiak, J.; Gumieniak, A.; Niewiadomy, A. *Pol. J. Environ. Stud.* **1998**, *7*, 35–38.
19. Niewiadomy, A.; Matysiak, J.; Żabińska, A.; Różyło, B.; Senczyna, B.; Józwiak, K. *J. Chromatogr. A* **1998**, *828*, 431–438.



20. Matysiak, J.; Niewiadomy, A.; Żabińska, A.; Różyło J.K.. J. Chromatogr. A **1999**, *830*, 491–496.
21. Różyło, J.K.; Niewiadomy, A.; Żabińska, A.; Matysiak, J. J. Planar Chromatogr. - Mod. TLC **1998**, *11*, 450–456.
22. Różyło, J.K.; Janicka, M.; Żabińska, A.; Niewiadomy, A. J. Planar Chromatogr. - Mod. TLC **1998**, *11*, 433–437.
23. Śliwiok, J.; Podgórnny, A.; Siwek, A.; Witkowska, B. J. Planar Chromatog. - Mod. TLC **1990**, *3*, 410–412.
24. Pyka, A. Wiad. Chem. **1997**, *51*, 783–802.
25. Balaban, A.T.; Motoc, I.; Bonchev, D.; Mekenyan, O. Top. Curr. Chem. **1983**, *114*, 21–55.
26. Randić, M. J. Am. Chem. Soc. **1975**, *97*, 6609–6615.
27. Wiener, H. J. Am. Chem. Soc. **1947**, *69*, 2636–2638.
28. Balaban, A.T. Chem. Phys. Lett. **1982**, *89*, 399–404.
29. Pyka, A. J. Planar Chromatogr. - Mod. TLC **1991**, *4*, 316–318.
30. Barysz, A.; Jashari, G.; Lall, R.S.; Srivastava, V.K.; Trinajstić, N. Chemical Applications of Topology and Graph Theory. Studies in Physical and Theoretical Chemistry **1983**, *28*, 222–230.
31. Rekker, R.F. *The Hydrophobic Fragmental Constant*; Elsevier: Amsterdam, 1977.

Received May 1, 2000

Manuscript 5294

Accepted September 7, 2000