ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
21 (1999) 895–900

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Application of numerical taxonomy techniques to the choice of optimum mobile phase in high-performance thin-layer chromatography (HPTLC)

C. Cimpoiu *, T. Hodisan

Faculty of Chemistry and Chemical Engineering, 'Babes-Bolyai' University, 11 Arany Janos, 3400 Cluj-Napoca, Romania

Received 17 November 1998; received in revised form 18 March 1999; accepted 4 May 1999

Abstract

A total of eight solvent systems used for the separation of 1,4-benzodiazepine mixtures were investigated. The classification of solvent systems was carried out by the numerical taxonomy technique. The selection of optimum solvent system was accomplished on the basis of information quantity and objective function. The method was found to be a rapid and efficient tool in the choice of optimum system solvent. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Numerical taxonomy; Optimization; Mobile phase; 1,4-benzodiazepines; High-performance thin-layer chromatography

1. Introduction

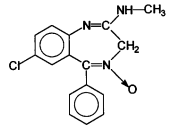
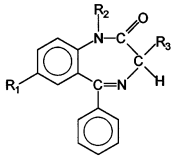
In high-performance thin-layer chromatography (HPTLC) some form of optimization is usually necessary if complete separation of all components in a sample is required. One of the problems with which the analyst can be confronted is the large number of possible separation systems from which to make a choice. The problem to be solved is how to choose the mobile phase so that this solvent system yields as much information as possible. One of the ways of achieving this using HPTLC is to classify the solvent systems into groups of systems with similar separation characteristics and to select one system from each group. A method that allows a formal classification is the numerical taxonomy technique [1,2].

In this paper, the efficiency of eight solvent systems for the separation of 1,4-benzodiazepine mixtures was investigated by the method of numerical taxonomy. The solvent systems are divided into groups with similar separation properties. The selection of the most efficient solvent system from each group is carried out according to the information quantity [3] and objective function [4,5].

* Corresponding author. Tel.: +40-64-193833; fax: +40-64-190818.

PII: S0731-7085(99)00209-5

Table 1
The chemical structures of studied compounds

Chemical formulae	No.	Compound	R_1	R_2	R_3
	1.	Chlordiazepoxide	-	-	-
	2.	Oxazepam	Cl	H	OH
	3.	Nitrazepam	NO_2	H	H
	4.	Medazepam	Cl	CH_3	H
	5.	Diazepam	Cl	CH_3	H

The 1,4-benzodiazepines are drugs with strong anticonvulsant and tranquilizing to hypnotic effects [6] and because they are widely used in therapy, great attention has been devoted to their analysis. The compounds studied were chlordiazepoxide, oxazepam, nitrazepam, medazepam and diazepam; the structures of the compounds are given in Table 1.

2. Experimental

2.1. Materials

All solvents were of analytical grade and were obtained from 'Reactivul' Bucharest, Romania. Solutions (1 mg ml^{-1}) of 1,4-benzodiazepines were prepared in methanol. Chromatography was performed on 5 × 10-cm glass HPTLC plates precoated with silica gel 60 F_{254} (Merck).

2.2. Chromatography

For every solvent system tested solutions (0.2 μl) of 1,4-benzodiazepines were applied to the plates by means of a capillary pipette. Plates were developed to a distance of 70 mm, at room temperature, by the ascending technique, in a saturated N-chamber. Detection were performed in UV light, $\lambda = 254$ nm.

3. Results and discussion

The numerical taxonomy used a variety of related mathematical techniques in order to classifying the solvent systems into groups based on the R_f values. The principle of this procedure is based on the formation of a matrix with columns representing the solvent systems and rows the 1,4-benzodiazepines. The taxonomic distance, $d_{j,k}$ characterizes the similarity between two solvent systems. The taxonomic distance is given by the following equation:

$$d_{j,k} = \sqrt{\sum_{i=1}^{n} (x_{i,j} - x_{i,k})^2 / n} \quad (1)$$

where $x_{i,j}$ and $x_{i,k}$ are the R_f values of the compound i in the solvent systems j and k, and n is the number of investigated compounds.

The smallest $d_{j,k}$ value is selected and the solvent systems j and k are the most similar solvent systems and they are considered to form a new group p'. The resemblance matrix is thereby reducing by one. The distance between the new group p' and all the other solvent systems, in the reduced resemblance matrix, is calculated as follows:

$$d_{j,p'} = \frac{1}{2}(d_{j,p'} + d_{j,q}) \quad (2)$$

All other $d_{j,k}$ values remain unchanged.

This process is repeated until all solvent systems are brought together in one classification system consisting of a hierarchy of non-overlapping groups and subgroups.

The optimal solvent system was selected using the following procedures:

1. classification of chromatographic system into groups with similar separation properties and selection of the most efficient system from each group;
2. determination and comparison of the information quantity and objective function for all chromatographic separations obtained with the studied solvent system.

To reflect the quality of a chromatogram by a single number, a function must be selected; such a function is denoted an objective function [5]. The conditions for the separation are selected such

Table 2
Thin-layer chromatographic systems studied

System number	Solvent system	Composition	Ref.
1	Chloroform-methyl acetate-methanol	70:25:5	[12]
2	Dichlorethan-methanol-water	95:5:0.2	[13]
3	Chloroform-i-propanol-acetic acid	17:2:1	[14]
4	Toluene-ethylic ether-methanol	15:20:0.7	[15]
5	Chloroform-methanol-ethylic ether	85:15:10	[16]
6	Benzene-i-propanol-aqueous ammonia 25%	85:15:1	[17]
7	Butanol-chloroform-aqueous ammonia 25%	50:50:1	[18]
8	Chloroform-acetone-i-propanol	80:15:5	–

that the numerical value of objective function becomes a maximum or possibly a minimum. A great number of objective functions have been designed and tested [7–10] because no single objective function will ever be entirely satisfactory in all cases. In our opinion, the preferred objective function is that which contains all the qualities of optimum chromatogram. The information quantity, I, illustrate the multi-component separation using discontinuities of probabilities related to some arbitrary 'groups' of retention parameter values. They are not affected by peak widths and therefore not very sensitive, especially for a small number of peaks.

The objective function used in this work was [5,11]:

$$F_{\text{obj}} = an + b\overline{IR_s} + 10c/IE + d/(I_p + \varepsilon) \quad (3)$$

where a, b, c and d are arbitrary weighting factors (in present work $a = 1$, $b = 1$, $c = 0.1$, $d = 10$); n is the number of components observed as peaks; I is quantity of information [3]; $\overline{R_s}$ is mean resolution; $\overline{IR_s}$is informational power [9];IE is informational energy [10]; I_p is performance index [8]; and ε is very small, arbitrary value (10^{-5}). These simple functions could be calculated with following equations:

$$I = -\sum_k p_k \log_2 p_k \quad (4)$$

$$IE = \sum_k p_k^2 \quad (5)$$

$$BI_p = \sqrt{\frac{\sum (\Delta h R_{f,i+1} - \Delta h R_f)^2}{n(n+1)}} \quad (6)$$

where p_k is the probability of finding a peak in a group; $\Delta h R_{f,i+1}$ is the measured interval between any two adjacent peaks and $\Delta h R_f$ is the measured interval for an ideal separation.

A total of eight solvent systems are used for the choice of the optimum one. Of the solvent systems, seven are taken from literature [12–18], and the eighth solvent system is a mixture of three solvents (chloroform (VIII), acetone (VI) and i-propanol (II)) and was chosen after preliminary chromatographic runs, using Snyder's classification [19]. The solvent systems are presented in Table 2 and the sets of R_f values of compounds are presented in Table 3.

The application of Eq. (1) allows the construction of a resemblance matrix (Table 4). The smallest $d_{j,k}$ value in this matrix is $d_{18} = 0.026$. This means that 1 and 8 are the most similar solvent systems and they can be combined into a group 1′. This leads to a new matrix (Table 5) with one column and one row less. In this matrix, the smallest $d_{j,k}$ is again combined into a new group and so on. The sequence of combinations is shown in Fig. 1. From this dendrogram it is can be seen that group 1‴ it can be distinguished from group 3′. In the first group, group 1″ can be distinguished from group 2″ and so on.

If there were no practical reasons for the choice of solvent system in a group, the choice in these groups was carried out using and comparing in

Table 3
The R_f values of 1,4-benzodiazepines obtained in the solvent systems presented in Table 1

Component	Solvent system							
	1	2	3	4	5	6	7	8
Medazepam	0.84	0.59	0.51	0.65	0.92	0.75	0.48	0.81
Oxazepam	0.46	0.23	0.69	0.36	0.69	0.30	0.32	0.49
Chlordiazepoxide	0.39	0.18	0.66	0.30	0.74	0.49	0.42	0.39
Nitrazepam	0.62	0.35	0.79	0.48	0.79	0.54	0.44	0.63
Diazepam	0.82	0.58	0.86	0.62	0.90	0.71	0.47	0.78

formation quantity and objective function. The values of information quantity and objective function are presented in Table 6.

The following solvent systems were chosen on the basis of values from Table 6:

- solvent system 5 is chosen from group 3′, because it offers more information than the other systems since the values of objective function are almost equal;
- solvent system 4 is chosen from group 2″, because for this system the value of objective function is the biggest one from the group and the information quantity is the same for these three systems from the group.
- solvent 8 is chosen from group 1″, because it offers more information than other systems and the value of objective function is less different from that for system 6.

Among systems 4, 5 and 8, the best system is system 8, which can be observed from the values of information quantity ($I_8 = I_5 > I_4$) and from values of objective function ($F_{obj8} > F_{obj4} > F_{obj5}$). It can be concluded that the optimum solvent system is system 8.

4. Conclusions

Very different chemometrical methods are often used in thin-layer chromatography, which permit the classification and combination of chromatographic systems.

Numerical taxonomy, which was used originally in biological research, allows ordering of a

Table 5
First reduced resemblance matrix

	1′	2	3	4	5	6	7
1′	0						
2	0.238	0					
3	0.223	0.380	0				
4	0.145	0.103	0.287	0			
5	0.207	0.431	0.188	0.332	0		
6	0.112	0.189	0.254	0.114	0.262	0	
7	0.240	0.140	0.307	0.117	0.385	0.171	0

Table 4
Resemblance matrix

	1	2	3	4	5	6	7	8
1	0							
2	0.241	0						
3	0.230	0.380	0					
4	0.151	0.103	0.287	0				
5	0.208	0.431	0.188	0.332	0			
6	0.112	0.189	0.254	0.114	0.262	0		
7	0.247	0.140	0.307	0.117	0.385	0.171	0	
8	0.026	0.236	0.217	0.140	0.207	0.112	0.233	0

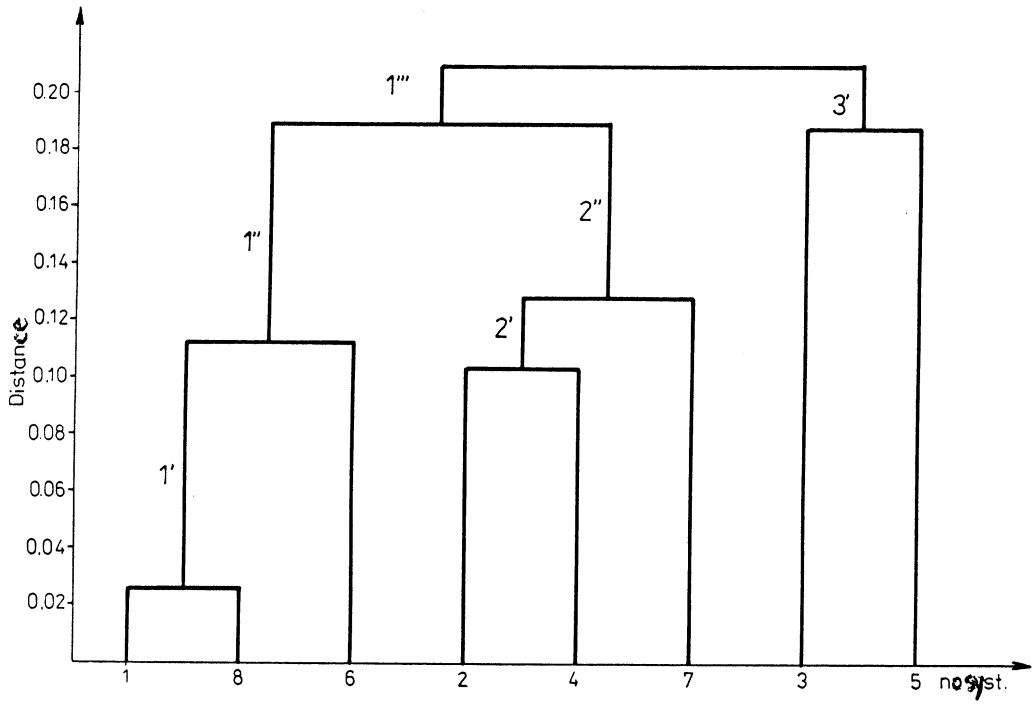


Fig. 1. Dendrogram for eight solvent systems.

Table 6
The values of information quantity, I, and objective function, F_{obj}, for each solvent system

	1	2	3	4	5	6	7	8
I	1.922	1.922	1.922	1.922	2.322	1.922	1.922	2.322
F_{obj}	14.984	14.134	14.761	15.167	14.734	18.662	11.509	18.510

formal classification of solvent systems. A rational and logical choice of optimum solvent system can be accomplished using information quantity and objective function as selection criteria. The method presented here is a rapid and efficient method and the optimum solvent system can be chosen without difficulty.

References

[1] D.L. Massart, H. De Clercq, Anal. Chem. 46 (1974) 1988.
[2] Z. Males, M. Medic-Saric, F. Bucar, Croat. Chem. Acta 71 (1998) 69.
[3] J. Souto, A.G. de Valesi, J. Chromatogr. 46 (1970) 274.
[4] H. Naşcu, T. Hodişan, C. Cimpoiu, Stud. Univ. B-B Chemia XXXIX (1994) 167.
[5] C. Cimpoiu, T. Hodisan, H. Nascu, J. Planar Chromatogr. 10 (1997) 195.
[6] J. Pasich, H. Wojdak, Zentralbl. Pharm. Pharmakother. Laboratoriumsdiagn. 119 (1980) 395.
[7] D.L. Massart, J. Chromatogr. 79 (1973) 157.
[8] S. Gocan, M. Mihaly, Stud. Univ. B-B Chem. XXVI (1981) 18.
[9] H. Nascu, C. Sarbu, E. Moraru, T. Hodisan, Rev. Chim. 33 (1982) 550.
[10] C. Sarbu, H. Nascu, Rev. Chim. 41 (1990) 271.
[11] C. Cimpoiu, L. Jantschi, T. Hodisan, J. Chromatogr. Rel. Techn. 22 (1999) 1429.

[12] S. Lauffer, E. Schmid, Arzneim.-Forsch. 20 (1970) 1232.
[13] H.J. Battista, H. Udermann, G. Henning, W. Vycudilik, Beitr. Gerichtl. Med. 37 (1979) 5.
[14] A.F. Fartushnii, E.B. Muzhanovskii, A.P. Sukhin, A.I. Sedov, E.V. Kvasov, Farm. Zh. 4 (1985) 45.
[15] A. De Souza, M. Hamon, Ann. Pharm. Fr. 48 (1990) 7.
[16] A.T. Howarth, G. Clegg, Clin. Chem. 24 (1978) 804.
[17] S. Ebel, H. Schutz, Arzneim.-Forsch. 27 (1977) 325.
[18] I. Bujor, P. Marcu, L. Roman, Clujul Med. 51 (1978) 351.
[19] L.R. Snyder, J. Chromatogr. Sci. 16 (1978) 223.