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Short communication

Application of numerical methods to thin-layer chromatographic investigation of the main components of chamomile (*Chamomilla recutita* (L.) Rauschert) essential oil[1]

Marica Medić-Šarić[a,*], Gordana Stanić[b], Željan Maleš[b], Slavko Šarić[c]

[a]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Biochemistry, University of Zagreb, 10000 Zagreb, Croatia*
[b]*Department of Pharmacognosy, Faculty of Pharmacy and Biochemistry, University of Zagreb, 10000 Zagreb, Croatia*
[c]*Faculty of Traffic Science, University of Zagreb, 10000 Zagreb, Croatia*

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Abstract

The efficiency of eleven TLC systems for separating the main components of chamomile essential oil has been tested. The information theory and the methods of numerical taxonomy have been applied for this purpose. The design of the most effective series of chromatographic systems is discussed in terms of discriminating power and mean information content. The series of systems with the highest discriminating power is shown to produce the best overall separations for a large specified population of components of chamomile essential oil. It leads to the identification of an unknown component using a minimum number of systems. It has been established that the most favourable mobile phases for TLC of chamomile essential oil are chloroform–toluene (75:25) and chloroform–toluene–ethyl acetate (65:30:5) mixtures. © 1997 Elsevier Science B.V.

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1. Introduction

Chamomile (*Chamomilla recutita* (L.) Rauschert, Asteraceae) is undoubtedly one of the most representative medicinal plants. It is an annual herb which grows wild, but is also cultivated in many countries because of the great interest of pharmaceutical, cosmetic and food industries. The flower heads are gathered when plants are in full bloom and dried.

Dried flower heads of aromatic odour are an ancient drug that has been used in therapy since the 5th century before Christ until now. The drug is used in modern phytotherapy primarily for its spasmolytic [1–5] and antiphlogistic properties [6–9]. In addition, bacteriostatic and fungistatic activities of some chamomile components [10,11] contribute to its medicinal value.

It is well known that the main active principles are some components of essential oil and flavonoids as well. Therefore, the majority of the research interest concerns these compounds.

The drug *Matricariae flos* or *Chamomillae flos* is official in many pharmacopoeas. Quality control of the drug is based on the determination of essential oil content as well as on identification of its main components by thin-layer chromatography (TLC).

*Corresponding author.

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TLC is an ideal technique for the screening of drugs, because of low cost, easy maintenance and selectivity of detection reagents. A method for the determination of effectiveness for a series of chromatographic systems which uses the concept of discriminating power, and calculations of information content for each mobile phase is described [12–21]. The main components of chamomile essential oil are sesquiterpenes: matricin/chamazulene, (−)-α-bisabolol, bisabololoxides A and B and en-in-dicycloethers.

Searching the literature, one can find various mobile phases for the chromatographic separation of these compounds [22–26]. As it is unreliable to select the best chromatogram with at least ten separated compounds by visual observation, we applied for this purpose the methods of numerical taxonomy [27–31].

2. Experimental

2.1. Materials

2.1.1. Test solution

The essential oil of dried chamomile flower heads was obtained by distillation in an apparatus as described by Clevenger [32]. The oil was dissolved in toluene (1:10) for TLC analysis.

2.1.2. Reference solution

(−)-α-Bisabolol (BASF, Ludwigshaven, Germany) was dissolved in toluene (1:30).

2.1.3. Thin-layer chromatography

Precoated 20×20 TLC silica gel 60 F254 sheets (Merck, Darmstadt, Germany) were used. Five-μl volumes of essential oil solution and reference solution were applied as bands. Developments were performed by the linear ascending mode at room temperature in a saturated and unsaturated mobile phase. All solvents were of analytical grade, purchased from Kemika (Zagreb, Croatia) and Merck. The chromatograms were dried in a stream of cold air. Visualisation of the components of essential oil was achieved by spraying the sheets with the vanilin–sulfuric acid reagent [22] and drying in an oven at 105°C.

2.2. Methods

2.2.1. Calculation of the information content

Extensive information has been calculated for eleven TLC systems the by Shannon formula. Calculation of the information content will become possible if the uncertainties before and after the analysis can be expressed in a quantitative way.

The distribution of R_F values into groups with the error factor E (e.g. $E=0.03$ or $E=0.05$) with respect to R_F units and the assumption of n_k R_F values in the k-th groups, the average information content (entropy) is given by the following Shannon equation [13]:

$$I(X) = H(X) = -\sum_k [n_k/n] ld [n_k/n] \text{bit} \quad (1)$$

The R_F range is divided into m classes of a given class-width (e.g. $E=0.03$ or $E=.05$). For each of the m classes the probability that an unknown compound will appear to have an R_F value within the limit of this class equals r_k/n for a group containing r_k members of the n comprising the total class. The information content, expressed in bit, is thus [12]:

$$I = -\sum_{k=1}^{m} \frac{r_k}{n} \cdot \log_2\left(\frac{r_k}{n}\right) \quad (2)$$

It is also assumed that the compounds with R_F values within one group cannot be identified. It is obvious that the entropy is at its highest level if there is only one R_F value, i.e. $H_m(X) = ld\ n$ within each group.

2.2.2. Determination of discriminating power (DP)

The DP of a set of chromatographic systems is defined as the probability of identifying two randomly selected compounds in at least one of the systems [15–20]. It must be possible to discriminate all pairs of N in order to compute the DP of k chromatographic systems in which N compounds are investigated. For the total number of matching pairs (M) the probability of a random selection of chromatographically similar pairs is $2M/N(N-1)$. Therefore, the DP of k systems is

$$DP_k = 1 - 2M/N(N-1) \quad (3)$$

The average number of chromatographically similar compounds (T) for the chromatographic systems

considered can be calculated from the following equation [15]:

$$T = 1 + (N - 1)(1 - DP_k) \quad (4)$$

2.2.3. Calculation of taxonomic distances, cluster formation and dendrogram

Taxonomy is defined as the theoretical study of classification including its elementary principles, procedures and rules [21]. Numerical taxonomy deals with ways of classifying chromatographic systems into taxonomic groups based on characteristic values (R_F values). The mathematical principle of this procedure is established on the formation of a matrix where the columns represent the mobile phases and the lines of the substances. Classification is carried out with respect to resemblances between the mobile phases. Dissimilarity, expressed as the complement of similarity, is proportional to the distances of the mobile phases in the given metric space. The greater the differences in properties of the mobile phases, the larger their spatial distances are. Taxonomic distance is inversely related to similarity. The distance $d_{j,k}$ between the mobile phases j and k is defined as

$$d_{j,k} = \left[\sum_{i=1}^{N} (X_{i,j} - X_{i,k})^2 / N \right]^{1/2} \quad (5)$$

where $X_{i,j}$ and $X_{i,k}$ are the R_F values of investigated compound i in the mobile phases j and k and N is the number of R_F values taken into account.

In the classification by taxonomy a resemblance matrix containing either the correlation coefficient or the taxonomic distance is constructed. The reduction of this matrix is carried out by a weighted pair group method using the arithmetic average [21]. The smallest distance $d_{i,k}$ or the highest correlation coefficient is selected: i and k are the most similar mobile phases and are therefore considered to form one group p′. The similarity coefficient between the new group p′ and all other phases (e.g. q), is calculated, e.g. for the distance, as follows:

$$d_{j,p'} = 1/2(d_{j,p'} + d_{j,q}) \quad (6)$$

The total number of rows and columns in the resemblance matrix is therefore reduced to one. This process is repeated until all chromatographic systems

Table 1
Mobile phases for TLC of the components of chamomile essential oil

No.	Mobile phase	Ref.
1	Toluene–ethyl acetate (93:7, v/v)	[22]
2	Toluene–ethyl acetate (90:10, v/v)	
3	Toluene–ethyl acetate–acetic acid (93:6:1, v/v/v)	
4	Dichlormethane	
5	Dichlormethane–toluene (75:25, v/v)	[23]
6	Dichlormethane–ethyl acetate (98:2, v/v)	
7	Chloroform	[24]
8	Chloroform–toluene (75:25, v/v)	[25]
9	Chloroform–toluene–ethyl acetate (75:20:5, v/v/v)	
10	Chloroform–toluene–ethyl acetate (65:30:5, v/v/v)	
11	Chloroform–toluene–ethyl acetate (50:45:5, v/v/v)	

Table 2
Input data: R_F values of the components of chamomile essential oil obtained in mobile phases tested (**1–11**)

Components	Mobile phase[a]										
	1	2	3	4	5	6	7	8	9	10	11
Bisabololoxide B	0.18	0.24	0.13	0.10	0.08	0.16	0.19	0.12	0.24	0.20	0.20
Bisabololoxide A	0.18	0.24	0.13	0.10	0.08	0.16	0.19	0.12	0.28	0.24	0.20
Terpene 1	0.26	0.32	0.19	0.18	0.18	0.22	0.24	0.17	0.35	0.30	0.26
Terpene 2	0.26	0.35	0.19	0.18	0.18	0.27	0.24	0.20	0.35	0.32	0.29
Terpene 3	0.33	0.39	0.21	0.22	0.21	0.32	0.32	0.31	0.46	0.43	0.38
Bisabolol	0.35	0.41	0.25	0.23	0.24	0.35	0.29	0.26	0.41	0.40	0.36
Terpene 4	0.41	0.52	0.34	0.33	0.31	0.42	0.38	0.37	0.51	0.47	0.44
Trans-en-in-dicycloether	0.41	0.52	0.34	0.33	0.48	0.42	0.38	0.41	0.51	0.47	0.44
cis-en-in-dicycloether	0.46	0.52	0.38	0.57	0.54	0.56	0.51	0.49	0.55	0.52	0.48
Chamazulene	0.68	0.71	0.68	0.75	0.79	0.73	0.67	0.69	0.69	0.69	0.68
Farnesene	0.72	0.75	0.67	0.80	0.84	0.77	0.69	0.74	0.74	0.74	0.73

[a] Copies of chromatograms can be obtained from the authors on request.

Table 3
Output data of *DP* values and mean information contents (*I*) for each mobile phase

Mobile phase	Error: $E=0.03$		Error: $E=0.05$	
	DP	*I*	DP	*I*
1	0.9273	2.732	0.9091	2.732
2	0.9091	2.845	0.8545	2.482
3	0.9091	2.664	0.8545	2.732
4	0.9273	2.732	0.8545	2.732
5	0.9273	2.845	0.8909	2.914
6	0.9636	3.096	0.9091	2.914
7	0.9273	2.914	0.8000	2.732
8	0.9818	3.278	0.9455	3.096
9	0.9636	3.096	0.8727	2.845
10	0.9636	3.096	0.8545	3.278
11	0.9455	2.914	0.8909	2.732

are comprised in one non-overlapping hierarchic system of groups and subgroups (clusters). The procedure for cluster formation is presented by a dendrogram [27–31].

The three approaches were compared applying our computer search program KT 1 [27].

3. Results and discussion

The efficiency of eleven mobile phases for TLC separation of the components of chamomile essential oil was tested (Table 1). Some of these mobile phases were taken from the literature, while the others were created by modifying the polarity of known mobile phases. Chromatographic procedure was carried out in a saturated chamber.

In order to chose the most optimal mobile phases numerical taxonomy methods were applied. Table 2 presents input data for R_F values of separated components of chamomile essential oil in eleven chromatographic systems. Table 3 gives the output data of discriminating power (*DP*) and mean information content (*I*) for each mobile phase in a range of two error factors (0.03 and 0.05 respectively). The measurement of the discriminating power for a series of chromatographic systems allows the effectiveness of the series to be expressed as a single value, the series with the highest discriminating power being the most effective.

Table 4
Output data of *DP* values and *T* values for: (a) combinations of two mobile phases (error 3% or 5%); (b) combination of three mobile phases (error 3% or 5%)

Combination sequence	Error: $E=0.03$			$E=0.05$		
	Mobile phase	DP	*T*	Mobile phase	DP	*T*
(a)						
1	8,10	1.0000	1.000	6,9	0.9636	1.364
2	8,9	1.0000	1.000	6,8	0.9636	1.364
3	10,11	0.9818	1.182	5,9	0.9636	1.364
4	9,11	0.9818	1.182	5,8	0.9636	1.364
5	8,11	0.9818	1.182	8,11	0.9455	1.545
6	7,8	0.9818	1.182	8,10	0.9455	1.545
7	6,10	0.9818	1.182	8,9	0.9455	1.545
8	6,9	0.9818	1.182	7,9	0.9455	1.545
9	6,8	0.9818	1.182	7,8	0.9455	1.545
10	5,11	0.9818	1.182	6,11	0.9455	1.545
(b)						
1	8,10,11	1.0000	1.000	5,6,9	0.9818	1.182
2	8,9,11	1.0000	1.000	5,6,8	0.9818	1.182
3	8,9,10	1.0000	1.000	6,9,11	0.9636	1.364
4	7,8,10	1.0000	1.000	6,9,10	0.9636	1.364
5	7,8,9	1.0000	1.000	6,8,11	0.9636	1.364
6	6,8,10	1.0000	1.000	6,8,10	0.9636	1.364
7	6,8,9	1.0000	1.000	6,8,9	0.9636	1.364
8	5,10,11	1.0000	1.000	6,7,9	0.9636	1.364
9	5,9,11	1.0000	1.000	6,7,8	0.9636	1.364
10	5,8,10	1.0000	1.000	5,9,11	0.9636	1.364

Table 5
Cluster formation

Cluster	Mobile phase	Mobile phase	Distance
1	1	1	0.0245
2	2	9	0.0286
3	2	9	0.0315
4	1	7	0.0371
5	1	6	0.0433
6	4	5	0.0505
7	1	5	0.0517
8	1	4	0.0720
9	1	3	0.0814
10	1	2	0.1268

The selection of the most suitable mobile phases is based on the highest values of *DP* and *I*. Thus, as it is seen from Table 3, mobile phases 8 (chloroform–toluene, 75:25 v/v) and 10 (chloroform–toluene–ethyl acetate, 65:30:5) are the most favourable.

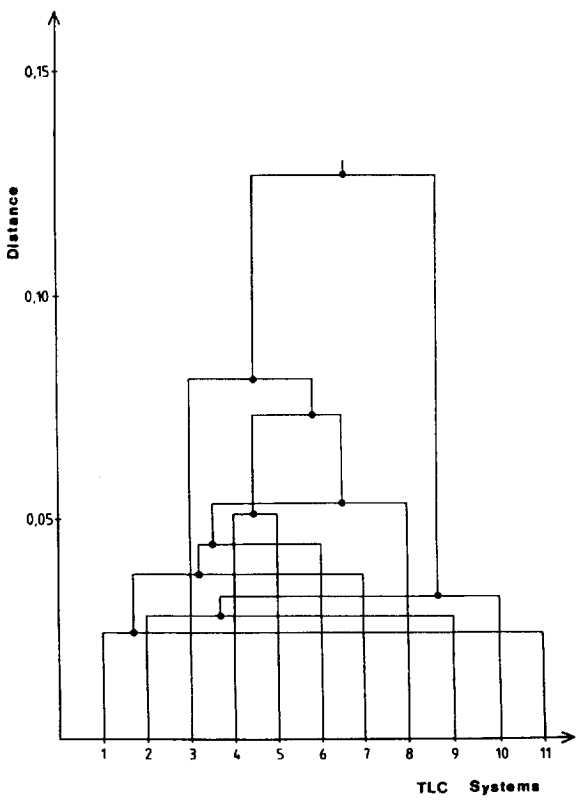

Fig. 1. Dendrogram for 11 TLC systems.

Subsequently, in the combination of two mobile phases (Table 4a, error 0.03) the highest value of *DP* and the smallest value of *T* refer again to mobile phases 8 and 10. In a series of three mobile phases (Table 4b) all the mobile phases have the maximum discriminating power ($DP = 1.0000$) and the number of chromatographically similar compounds is minimal ($T = 1.000$). However, each combination contains mobile phase 8 and/or mobile phase 10.

The same results were obtained by the cluster analysis (Table 5) of chromatographically similar mobile phases. According to the dendrogram (Fig. 1.) mobile phase 8 should be chosen from cluster 1 and mobile phase 10 from cluster 2.

4. Conclusions

The numerical taxonomic procedures based upon calculation of discriminating power (*DP*) and cluster formations were applied to select the most efficient chromatographic systems for TLC separation of the components of chamomile essential oil. The numerical techniques presented herein allow the rational classification and selection of separating systems in TLC. The similarity between the results obtained by these two procedures shows that our previous choice of chromatographic systems was reasonable. It is useful to calculate the discriminating power since a single numerical value expresses the effectiveness of chromatographic systems whether used alone or in combination.

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