Optimisation of thin-layer chromatographic analysis of flavonoids and phenolic acids of Salviae folium

Maleš, Željan; Medić-Šarić, Marica

Source / Izvornik: **Acta Pharmaceutica, 1998, 48, 85 - 92**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:163:792960

Acta Pharm. 48 (1998) 85–92 Original scientific paper

Optimisation of thin-layer chromatographic analysis of flavonoids and phenolic acids of *Salviae folium**

ŽELJAN MALEŠ[1]**
MARICA MEDIĆ-ŠARIĆ[2]

[1]*Department of Pharmacognosy*
[2]*Department of Pharmaceutical Chemistry*

Faculty of Pharmacy and Biochemistry
University of Zagreb
10000 Zagreb, Croatia

The information content and values of discriminating power have been calculated for 11 systems used in thin-layer chromatographic (TLC) investigations of flavonoids and phenolic acids of *Salviae folium*. The TLC systems were classified by clustering methods. The results obtained by these numerical methods are similar. Most suitable chromatographic systems for the separation of investigated compounds are: ethyl acetate – formic acid – acetic acid – water (100:11:11:27 *V/V*) and ethyl acetate – formic acid – water (8:1:1 *V/V*).

Keywords: Salviae folium, thin-layer chromatography, flavonoids, phenolic acids, numerical methods, clustering

Received February 27, 1998
Accepted May 6, 1998

Salvia officinalis L. (Sage) is a *ca.* 70 cm tall subshrub belonging to the *Lamiaceae* family. It is widely spread in the Mediterranean region, especially in the Adriatic. The leaves of this plant are long-petiolate, 3–10 cm long and up to 3 cm wide, oval, oblong-ovate to lanceolate, olive-gray and densely pubescent on both surfaces. The flowers are *ca.* 2 cm long, mostly with a bluish violet corolla, arranged in whorls forming a loose spike (1). The drug (*Salviae folium*) contains the essential oil (1–2.5%), tannins (3–7%), flavonoids, phenolic acids, diterpenoid bitter substances and triterpenes (1–3). It is used as an antiphlogistic for the inflammation of mouth and throat, for gingivitis and stomatitis, mainly in the form of a gargle but also as a tea for digestive complaints, flatulence, inflammation of the intestinal mucosa, in diarrhoea and as an antihydrotic (1). Thin-layer chromatography (TLC) is more commonly used for the analysis of mixtures than for the isolation of pure flavonoids (4). The main value of TLC in flavonoid investigation is a rapid analytical method that requires very small amounts of material (5).

* Presented at the 57th International Congress of FIP, Vancouver, August 31–September 5, 1997, Canada

** Correspondence

In this paper, information theory and clustering methods have been used to evaluate the efficency of eleven TLC systems for the separation of flavonoids and phenolic acids identified in a methanolic extract of *Salviae folium* (6–10).

EXPERIMENTAL

Materials

Leaves of *Salvia officinalis* were collected in August 1995 in Nakovanj. A voucher specimen (No. 97252) was retained at the Department of Pharmacognosy, Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia.

All solvents were of analytical grade, purchased from Merck (Germany). Standards (apigenin, luteolin, caffeic acid and chlorogenic acid) were obtained from C. Roth (Germany).

Extract solution. – Air-dried and powdered leaves of *Salvia officinalis* (1.0 g) were refluxed with 10.0 mL methanol for 30 minutes, filtered and the filtrate concentrated under reduced pressure and then residue taken up in 5.0 mL methanol (11).

Standard solution. – Apigenin, luteolin, caffeic acid and chlorogenic acid (10 mg of each) were dissolved in 10 mL methanol.

Methods

Thin-layer chromatography (TLC). – TLC was performed on precoated 10 × 10 cm TLC silica gel 60 F_{254} sheets (thickness of layer 0.25 mm). The extract solution (5 μL) and the standard solution (5 μL) were applied 15 mm from the bottom of the sheet as 10 mm wide bands. The sheets were developed for 8 cm in paper-lined all-glass chambers (Desaga, Germany) previously left to equilibrate for at least 30 minutes. The eleven TLC systems used are given in Table I (11–17).

Visualisation of the flavonoids and phenolic acids was achieved by spraying the sheets with 1% methanolic diphenylboryloxyethylamine, followed by 5% ethanolic polyethylene glycol 4000. The chromatograms were evaluated in UV light at $\lambda = 366$ nm (flavonoids appeared as orange-yellow bands and phenolic acids as blue fluorescent bands) (11). The structures of the flavonoids and phenolic acids identified in the methanolic extract of *Salviae folium* are presented in Fig. 1.

Numerical methods

Calculation of the information content. – If the R_F values of the flavonoids and phenolic acids are distributed into groups with an error factor E (*e. g.* $E = 0.05$ or $E = 0.10$) in R_F units with the assumption of $n_k R_F$ values in the k-th groups, the entropy (average information content) is given by the Shannon's equation (18, 19):

$$I(X) = H(X) = -\sum_k \frac{n_k}{n} ld \frac{n_k}{n} \quad \text{(bit)} \qquad (1)$$

The entropy is the highest if there is only one R_F value, *i.e.* $H_m(X) = ldn$, within each group.

Table I. The thin-layer chromatographic systems studied

System No.	Solvents (*V/V*)	Ref.
1	Ethyl acetate:formic acid:water (8:1:1)	12
2	Ethyl acetate:formic acid:acetic acid:water (100:11:11:27)	11
3	Ethyl acetate:formic acid:water (65:15:20)	13
4	Ethyl acetate:formic acid:water (67:20:13)	14
5	Ethyl acetate:formic acid:water (88:6:6)	15
6	Ethyl acetate:formic acid:water (30:2:3)	16
7	1-Butanol:acetic acid:water (4:1:5), upper phase	11
8	1-Butanol:acetic acid:water (66:17:17)	14
9	Chloroform:methanol:water (6.5:3.5:1), lower phase	17
10	Ethyl acetate:methylethylketone:formic acid:water (5:3:1:1)	13
11	Ethyl acetate:formic acid:acetic acid:methylethylketone:water (50:7:3:30:10)	11

Determination of discriminating power (DP). – The discriminating power is a measure of the effectiveness of chromatographic systems. Two flavonoids or phenolic acids are chromatographically similar if the differences in their identification values do not exceed the error factor *E* (20).

The *DP* of a set of chromatographic systems is defined as a probability of identifying two randomly selected flavonoids (or phenolic acids) in at least one of the systems. It must be possible to discriminate all pairs of *N* compounds to compute the *DP* of *k*

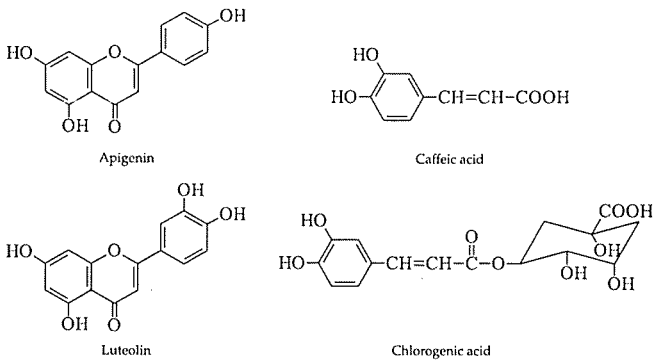

Fig. 1. Structures of the identified compounds.

chromatographic systems in which N flavonoids (or phenolic acids) are investigated. For the total number of matching pairs (M), the probability of random selection of chromatographically similar pairs is $2M/N\,(N-1)$. The DP of k systems is, therefore (21, 22):

$$DP_k = 1 - \frac{2M}{N(N-1)} \tag{2}$$

The average number of chromatographically similar flavonoids or phenolic acids (T) for the chromatographic systems considered can be calculated from the equation (23):

$$T = 1 + (N-1)\,(1 - DP_k) \tag{3}$$

Calculation of taxonomic distances, cluster formation and dendrogram. – Taxonomy is defined as the theoretical study of classification including its elementary principles, procedures and rules (6). Clustering deals with ways of classifying chromatographic systems into taxonomic groups based on R_F values. The mathematical basis of this procedure is the formation of a matrix where the columns represent the TLC systems and the rows the flavonoids (phenolic acids). The optimum combinations of two or more chromatographic systems for the separation of flavonoids and phenolic acids by TLC can be determined from the taxonomic distances (24). Taxonomic distance is inversely related to similarity. The distance $d_{j,k}$ between the systems j and k is equal to:

$$d_{j,k} = \left[\sum_{i=1}^{N} \frac{(X_{i,j} - X_{i,k})^2}{N}\right]^{1/2} \tag{4}$$

Chromatographic systems with high resemblance are grouped into clusters. Cluster formation in this paper was performed by a weighted pair group method using the arithmetic average (6).

The procedure for cluster formation is represented by a dendrogram (25–32). The three approaches were compared by an application of our computer search programme KT1 (25).

RESULTS AND DISCUSSION

Data set of R_F values for the separation of flavonoids and phenolic acids of the methanolic extract of *Salviae folium* by eleven different solvent systems was analysed (Table I).

Table II gives the input data (R_F values) for the flavonoids and phenolic acids investigated. Table III gives output data for the discriminating power and the information content for each TLC system. Table IV gives output data for combined systems $K = 2$ and $K = 3$ in a range of error factors. The error factors were $E = 0.05$ and 0.10, respectively.

Under the conditions most frequently used in chromatographic analyses, *i.e.* $E = 0.05$, the most suitable systems for separating the compounds studied are the systems 1 (ethyl acetate – formic acid – water 8:1:1 *V/V*) and 2 (ethyl acetate – formic acid – acetic

Table II. Input data: R_F *values of flavonoids and phenolic acids of* Salviae folium, *and development time* (t)

TLC system*	1	2	3	4	5	6	7	8	9	10	11
t (min)	27	36	38	46	30	28	110	97	25	32	30
Compound	R_F values										
Apigenin	0.95	0.95	0.95	0.96	0.93	0.90	0.86	0.89	0.99	0.99	0.98
Luteolin	0.94	0.94	0.91	0.95	0.90	0.88	0.82	0.85	0.98	0.94	0.96
Caffeic acid	0.89	0.89	0.89	0.93	0.88	0.84	0.75	0.79	0.95	0.93	0.94
Flavonoid 1	0.82	0.83	0.83	0.86	0.75	0.71	0.70	0.75	0.91	0.83	0.85
Flavonoid 2	0.75	0.75	0.75	0.83	0.59	0.57	0.70	0.75	0.90	0.80	0.70
Flavonoid 3	0.61	0.62	0.62	0.80	0.38	0.37	0.66	0.70	0.85	0.63	0.65
Flavonoid 4	0.50	0.50	0.50	0.75	0.30	0.30	0.66	0.67	0.82	0.50	0.54
Chlorogenic acid	0.42	0.38	0.38	0.65	0.23	0.23	0.61	0.63	0.75	0.47	0.47
Flavonoid 5	0.29	0.24	0.30	0.56	0.20	0.18	0.50	0.47	0.68	0.40	0.40
Flavonoid 6	0.13	0.10	0.10	0.45	0.11	0.10	0.37	0.35	0.50	0.25	0.25

* Copies of chromatograms can be obtained from the authors on request

Table III. Output data for DP *and* I *in a range of error factors* (E) *for each TLC system*

Error factor	$E = 0.05$		$E = 0.10$	
TLC-system	*DP*	*I* (bit)	*DP*	*I* (bit)
1	0.978	3.122	0.867	2.722
2	0.978	3.122	0.889	2.922
3	0.956	3.122	0.844	2.922
4	0.889	3.122	0.733	2.446
5	0.933	3.122	0.844	2.722
6	0.933	3.122	0.844	2.646
7	0.844	2.522	0.644	1.961
8	0.867	2.922	0.689	2.171
9	0.844	2.722	0.600	2.046
10	0.933	2.922	0.844	2.646
11	0.933	3.122	0.844	2.646

acid – water 100:11:11:27 *V/V*) because they have the largest discriminating power (*DP* = 0.978) and furnish high information content (*I* = 3.122). For *E* = 0.10, system 2 seems to be the most appropriate owing to its largest discriminating power (*DP* = 0.889) and high information content (*I* = 2.922).

Combining two chromatographic systems with the error factor *E* = 0.05, the combinations of systems 2 and 10, and 1 and 10 are the best, because they have the highest *DP* value (1.0000) and the smallest number of chromatographically similar compounds (*T* = 1.000). At *E* = 0.10, the first two combinations (*DP* = 0.9333; *T* = 1.600) contain system 2.

In a series of three systems, regardless of the error factor *E*, the TLC system 2 is also found in the first ten combinations.

The same results were obtained by cluster formation (Table V) and from the dendrogram (Fig. 2). The systems 1 and 2 are very similar, since the distance between them is

Table IV. Output data for DP *and* T *for combined TLC systems* – K = 2 *and* K = 3

	Combination sequence	E = 0.05 TLC-systems	*DP*	*T*	E = 0.10 TLC-systems	*DP*	*T*
K = 2	1.	2, 10	1.000	1.000	2, 6	0.933	1.600
	2.	1, 10	1.000	1.000	2, 5	0.933	1.600
	3.	10, 11	0.978	1.200	7, 10	0.911	1.800
	4.	8, 11	0.978	1.200	6, 7	0.911	1.800
	5.	7, 11	0.978	1.200	5, 7	0.911	1.800
	6.	7, 10	0.978	1.200	3, 7	0.911	1.800
	7.	6, 10	0.978	1.200	3, 6	0.911	1.800
	8.	6, 8	0.978	1.200	3, 5	0.911	1.800
	9.	6, 7	0.978	1.200	2, 11	0.911	1.800
	10.	5, 10	0.978	1.200	2, 10	0.911	1.800
K = 3	1.	8, 10, 11	1.000	1.000	3, 6, 7	0.956	1.400
	2.	7, 10, 11	1.000	1.000	3, 5, 7	0.956	1.400
	3.	6, 8, 10	1.000	1.000	2, 6, 7	0.956	1.400
	4.	6, 7, 10	1.000	1.000	2, 5, 7	0.956	1.400
	5.	5, 8, 10	1.000	1.000	7, 10, 11	0.933	1.600
	6.	5, 7, 10	1.000	1.000	6, 7, 11	0.933	1.600
	7.	3, 8, 10	1.000	1.000	6, 7, 10	0.933	1.600
	8.	3, 7, 10	1.000	1.000	5, 7, 11	0.933	1.600
	9.	2, 10, 11	1.000	1.000	5, 7, 10	0.933	1.600
	10.	2, 9, 10	1.000	1.000	4, 7, 10	0.933	1.600

Table V. Formation of clusters

Cluster	Solvent	Solvent	Distance
1	1	3	0.019
2	1	2	0.022
3	3	4	0.024
4	4	5	0.034
5	6	7	0.036
6	2	5	0.066
7	1	5	0.073
8	2	4	0.134
9	1	3	0.153
10	1	2	0.225

very small. If the criterion for the evaluation of systems is the development time, the systems 1 and 2 are the most favourable again due to their short development time.

CONCLUSION

Optimisation procedures are widely used in the development of different analytical methods. Thin-layer chromatography is one of the methods by which different chemometrical methods are used most frequently. In this paper, numerical methods are applied for evaluation and selection of optimum system and combinations of systems in

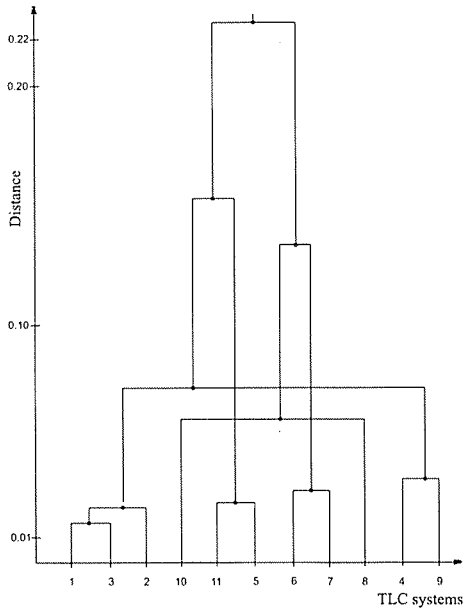

Fig. 2. Dendrogram for eleven TLC systems.

the TLC of flavonoids and phenolic acids of *Salviae folium*. The most suitable systems for TLC of investigated compounds are: ethyl acetate – formic acid – acetic acid – water (100:11:11:27 *V/V*) and ethyl acetate – formic acid – water (8:1:1 *V/V*).

REFERENCES

1. M. Wichtl, *Herbal Drugs and Phytopharmaceuticals*, Medpharm Scientific Publishers, Stuttgart 1994, 440.
2. E. Steinegger and R. Hänsel, *Lehrbuch der Pharmakognosie und Phytopharmazie*, Springer Verlag, Berlin 1988, 343.
3. H. Wagner, *Pharmazeutische Biologie –2. Drogen und ihre Inhaltsstoffe*, Gustav Fischer Verlag, Stuttgart-New York 1988, 64.
4. T. J. Mabry, K. R. Markham, and M. B. Thomas, *The Systematic Identification of Flavonoids*, Springer Verlag, Berlin-Heidelberg-New York 1970, 20.
5. K. R. Markham, *Techniques of Flavonoid Identification*, Academic Press, London 1982, 31.
6. P. H. A. Sneath and R. R. Sokal, *Numerical Taxonomy*, Freeman, San Francisco, 1973.
7. P. Cleij and A. Dijkstra, *Fresenius' Z. Anal. Chem.* **298** (1979) 97.
8. D. L. Massart, B. G. M. Vandeginste, S. N. Deming, Y. Michotte, and L. Kaufmann, *Chemometrics*, Elsevier, Amsterdam 1988.
9. G. J. Chaitin, *Algorithmic Information Theory*, Cambridge University Press, Cambridge 1987.
10. M. Quirynen, C. Dekeyser, and D. Vansteenberghe, *J. Periodontol.* **62** (1991) 100.
11. H. Wagner, S. Bladt, and E. M. Zgainski, *Drogenanalyse*, Springer Verlag, Berlin-New York 1983, 163.
12. M. Luckner, O. Bessler, and R. Luckner, *Pharmazie* **20** (1965) 681.

13. G. Willuhn and P. M. Röttger, *Dtsch. Apoth. Ztg.* **120** (1980) 1039.
14. M. Wichtl, B. Bozek, and T. Fingerhut, *Dtsch. Apoth. Ztg.* **127** (1987) 509.
15. M. Wichtl, *Teedrogen, Ein Handbuch für die Praxis auf wissenschaftlicher Grundlage*, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart 1989, 396.
16. R. Hänsel, K. Heller, H. Rimpler, and G. Schneider (Eds.), *Hagers Handbuch der Pharmazeutischen Praxis*, Springer Verlag, Berlin-New York 1994, Vol. 5, 474.
17. T. Kawasaki and K. Miyahara, *Chem. Pharm. Bull. (Tokyo)* **11** (1963) 1546.
18. C. Shannon and W. Weaver, *The Mathematical Theory of Communication*, University of Illinois Press, Urbana 1949.
19. H. De Clercq and D. L. Massart, *J. Chromatogr.* **115** (1975) 1.
20. A. C. Moffat, A. H. Stead, and K. W. Smalldon, *J. Chromatogr.* **90** (1974) 19.
21. P. Owen, A. Pendlebury, and A. C. Moffat, *J. Chromatogr.* **161** (1978) 195.
22. R. E. Kaiser (Ed.), *Planar Chromatography*, Hüthig, Heidelberg 1986, Vol. 1, 22.
23. A. C. Moffat, K. W. Smalldon, and C. Brown, *J. Chromatogr.* **90** (1974) 1.
24. D. L. Massart and H. De Clercq, *Anal. Chem.* **46** (1974) 1988.
25. M. Medić-Šarić, S. Šarić, and D. Maysinger, *Acta Pharm. Jugosl.* **39** (1989) 1.
26. A. Rotar, F. Kozjek, and M. Medić-Šarić, *Acta Pharm.* **43** (1993) 157.
27. Ž. Maleš, M. Medić-Šarić, and D. Kuštrak, *Acta Pharm.* **44** (1994) 183.
28. M. Medić-Šarić, A. Brantner, and Ž. Maleš, *Acta Pharm.* **46** (1996) 115.
29. M. Medić-Šarić, Ž. Maleš, G. Stanić, and S. Šarić, *Croat. Chem. Acta* **69** (1996) 1265.
30. M. Medić-Šarić, G. Stanić, Ž. Maleš, and S. Šarić, *J. Chromatogr. A.* **776** (1997) 355.
31. M. Medić-Šarić and Ž. Maleš, *J. Planar Chromatogr.* **10** (1997) 182.
32. Ž. Maleš, M. Medić-Šarić, J. Prstojević, and M. Puzović, *Pharm. Pharmacol. Lett.* **7** (1997) 50.

SAŽETAK

Optimiranje postupka za identifikaciju flavonoida i fenolnih kiselina lista kadulje (*Salviae folium*) tankoslojnom kromatografijom

ŽELJAN MALEŠ i MARICA MEDIĆ ŠARIĆ

Metoda tankoslojne kromatografije primijenjena je za odjeljivanje flavonoida i fenolnih kiselina lista kadulje (*Salviae folium*). U tu svrhu izračunati su srednji vlastiti sadržaj informacije (*I*) i koeficijenti razlikovanja (*DP*) za 11 kromatografskih razvijača. Slični razvijači svrstani su u skupine različitih identifikacijskih svojstava. Rezultati dobiveni primijenjenim numeričkim postupcima bili su vrlo slični. Najprikladniji razvijači za odjeljivanje istraživanih spojeva jesu: etilacetat – mravlja kiselina – ledena octena kiselina – voda (100:11:11:27 *V/V*) i etilacetat – mravlja kiselina – voda (8:1:1 *V/V*).

Ključne riječi: *Salviae folium*, tankoslojna kromatografija, flavonoidi, fenolne kiseline, numerički postupci, klasteri

Zavod za farmakognoziju i Zavod za farmaceutsku kemiju

Farmaceutsko-biokemijski fakultet Sveučilišta u Zagrebu, Zagreb