ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
14 (1996) 1221–1227

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Automated multiple development thin layer chromatography of some plant extracts[1]

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Received for review 23 September 1995; revised manuscript received 15 November 1995

Abstract

The separation of ten plant extracts using automated multiple development thin-layer chromatography (AMD -TLC) is described. Alcoholic extracts were obtained from *Cinchona succirubra*, *Aesculus hippocastanum*, *Berberis vulgaris*, *Artemisia abrotanum*, *Carduus marianus*, *Thuja occidentalis*, *Baptisia tinctoria*, *Paulinia cupana*, *Lycopus europaeus* and *Echinacea angustifolia*. The separation was performed on silica plates (Sil G-50 UV 254 (Macherey–Nagel), 10 × 20 cm). AMD was achieved in 25 steps using methanol, ethyl acetate, toluene, 1,2-dichloroethane, 25% ammonia solution and anhydrous formic acid as modifiers. The chromatograms were evaluated with a Shimadzu CS-9000 dual-wavelength flying-spot scanner. Better separations were obtained using AMD than isocratic elution.

Keywords: Densitometry; Multiple development; Plant extracts; Thin layer chromatography

1. Introduction

Plant extracts have widespread applications in the drugs and cosmetics industries. Drug quality control needs selective analytical methods in order to identify the drug and to check the active substance content in the pharmaceutical form. All these methods are included in different pharmacopoeias, but for plant extracts there is a lack of information [1–4]. Most plant extracts are analysed using the "fingerprint" method by thin-layer chromatography (TLC) and usually a photograph can be attached to the analysis certificate [1,5]. Quantitative analysis is seldom applied in this field and only spectrophotometry was included in pharmacopoeias. Owing to the large number of compounds which can co-exist in a plant extract, TLC can give an answer to the different problems which occur: the separation of an unknown number of unidentified compounds must be sensitive to small structural changes and there may be wide differences between the polarities of the unknown compounds. Normal-phase TLC is a suitable technique for the separation of different plant extracts and a suitable gradient is required to provide separation over a wide polarity range.

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[1] Presented at the Fifth International Symposium on Drug Analysis, September 1995, Leuven, Belgium.

SSDI 0731-7085(95)01702-X

Table 1
Eluent compositions for isocratic elution of plant extracts according to the Homeopathic Pharmacopoeia [1]

No.	Plant extract	Applied to the plate (μl per spot)	Eluent composition (v/v)
1	*Paulinia cupana*	40	Not found
2	*Carduus marianus*	40	Chloroform–98% acetic acid–water (50:42:8)
3	*Baptisia tinctoria*	40	Toluene–acetone–ethanol–25% NH_3 (15:20:6:2)
4	*Cinchona succirubra*	20	Dichloromethane (double development)
5	*Aesculus hippocastanum*	20	1-butanol–98% acetic acid–water (50:10:40, upper phase)
6	*Lycopus europaeus*	20	Chloroform–methanol–water (52:42:8)
7	*Thuja occidentalis*	20	Dichloromethane
8	*Echinacea angustifolia*	20	Cyclohexane–diethyl ether–methanol (70:20:10)
9	*Artemisia abrotanum*	20	1-Butanol–98% acetic acid–water (68:16:16)
10	*Berberis vulgaris*	20	Ethyl acetate–formic acid–water (80:10:10)

Automated multiple development (AMD) is an instrumental technique which can be used to perform normal-phase chromatography with solvent gradients on HPTLC plates. Most of the AMD applications reported have used "universal" gradients: starting with a very polar solvent, the polarity is varied by means of "base" solvent of medium polarity to a non-polar solvent [6,7]. There are reported "universal" gradients with an abrupt change in eluotropic strength used for plant extract separations [8–11] and some solvent gradients with a linear eluotropic strength profile [12]. Instrumentation for AMD was introduced by Camag (Muttenz, Switzerland) and provides a means for normal-phase gradient development in HPTLC. A maximum number of 25 steps have to be performed to form an AMD gradient. The developing distances increase while the solvent polarity is decreasing. The repeated development compresses bands on the plate, resulting in increased sensitivity and resolution [13,14].

This paper reports a simultaneous AMD separation and a comparison with isocratic chromatography as described in the Homeopathic Pharmacopeia [1] for ten plant extracts which contain different classes of compounds: alkaloids (*Cinchona succirubra*, *Berberis vulgaris*), saponins (*Paulinia cupana*, *Aesculus hippocastanum*), terpenes (*Thuja occidentalis*, *Echinacea angustifolia*), flavonoides (*Carduus marianus*, *Lycopus europaeus*, *Baptisia tinctoria*) and coumarins (*Artemisia abrotanum*).

2. Experimental

2.1. Chemicals

Methanol, 1,2,-dichloroethane, ethyl acetate, dichloromethane, toluene 25% (v/v) ammonia solution and anhydrous formic acid were purchased from Roth (Karlsruhe, Germany). All solvents were of chromatographic grade and the modifiers (ammonia solution and formic acid) were of analytical grade.

The plant extracts are provided by Plantextrakt (Cluj, Romania) and are solutions in 50% ethanol.

2.2. Isocratic chromatography

The isocratic elution of the ten plant extracts was performed as described in the Homeopathic Pharmacopoeia [1]. The experimental conditions are given in Table 1.

Silica gel plates with fluorescent indicators and a layer width of 0.50 mm (Sil G-50 UV 254 from Macherey–Nagel, Düren, Germany) were used. Samples were applied to the plates using a Desaga AS-30 automated applicator as bands 1 cm from the bottom edge of the plate. The plates were developed in paper-lined chambers with 2 h presaturation and the development distance was 8 cm. When development was complete, the plates were scanned from the origin to the solvent front using a Shimadzu CS-9000 dual-wavelength flying spot scanner, in zigzag mode, at 254 nm. The

Table 2
Programme used for AMD development (drying time 3 min)

Step No.	Development time (min)	Gradient No.	Solvent composition:						
			From step No.	1	2	6	11	16	21
			Feed from bottle	1	2	3	4	5	6
1	0.2	1	Methanol	100	40	20	–	–	–
2	0.5		1,2-Dichloroethane	–	60	80	100	100	–
3	0.8		Ethyl acetate	–	–	–	–	–	20
4	1.0		Toulene	–	–	–	–	–	80
5	1.3		Anhydrous formic acid	0.1	0.1	0.1	0.1	0.1	–
6	1.6		25% NH_3	–	–	–	–	–	0.1
7	1.9								
8	2.3	2	Methanol	100	60	40	20	–	–
9	2.7		Ethyl acetate	–	40	60	80	40	20
10	3.1		Toluene	–	–	–	–	60	80
11	3.5		25% NH_3	0.1	0.1	0.1	0.1	0.1	0.1
12	4.0								
13	4.5	3	Methanol	100	80	40	–	–	–
14	5.0		Dichloromethane	–	20	60	100	100	100
15	5.7		Anhydrous formic acid	0.1	0.1	0.1	0.1	0.1	0.1
16	6.5								
17	7.2	4	Methanol	100	80	60	–	–	–
18	8.0		Ethyl acetate	–	20	40	80	60	–
19	9.0		Toluene	–	–	–	20	40	60
20	10.0		25% NH_3	0.1	0.1	0.1	0.1	0.1	0.1
21	11.0								
22	11.9								
23	12.8								
24	13.7								
25	14.6								

chromatograms were evaluated under UV radiation at 254 nm in order to establish whether the compact zones of unknown compounds obtained under isocratic conditions can be separated using multiple development.

2.3. Automated multiple development

AMD was performed under the same experimental conditions as isocratic elution, but obviously with the exception of the eluent composition. This means that the same plates were used, the same sample quantities were applied to the plate and the migration distances were the same in both cases. The plates were developed in a Camag AMD instrument. No specific gaseous phase was used for conditioning the development chamber, only compressed air. The corresponding densitograms were obtained as described for isocratic chromatography.

2.4. Solvent gradients

The term "universal" can be used to characterize a gradient which performs a separation of a mixture with a large polarity scale [15]. The four AMD gradients used in this study (the corresponding eluent composition and the time sequence) are shown in Table 2.

All solvents contain modifiers (anhydrous formic acid 25% ammonia solution in order to prevent peak tailing, which frequently occurs for this kind of sample.

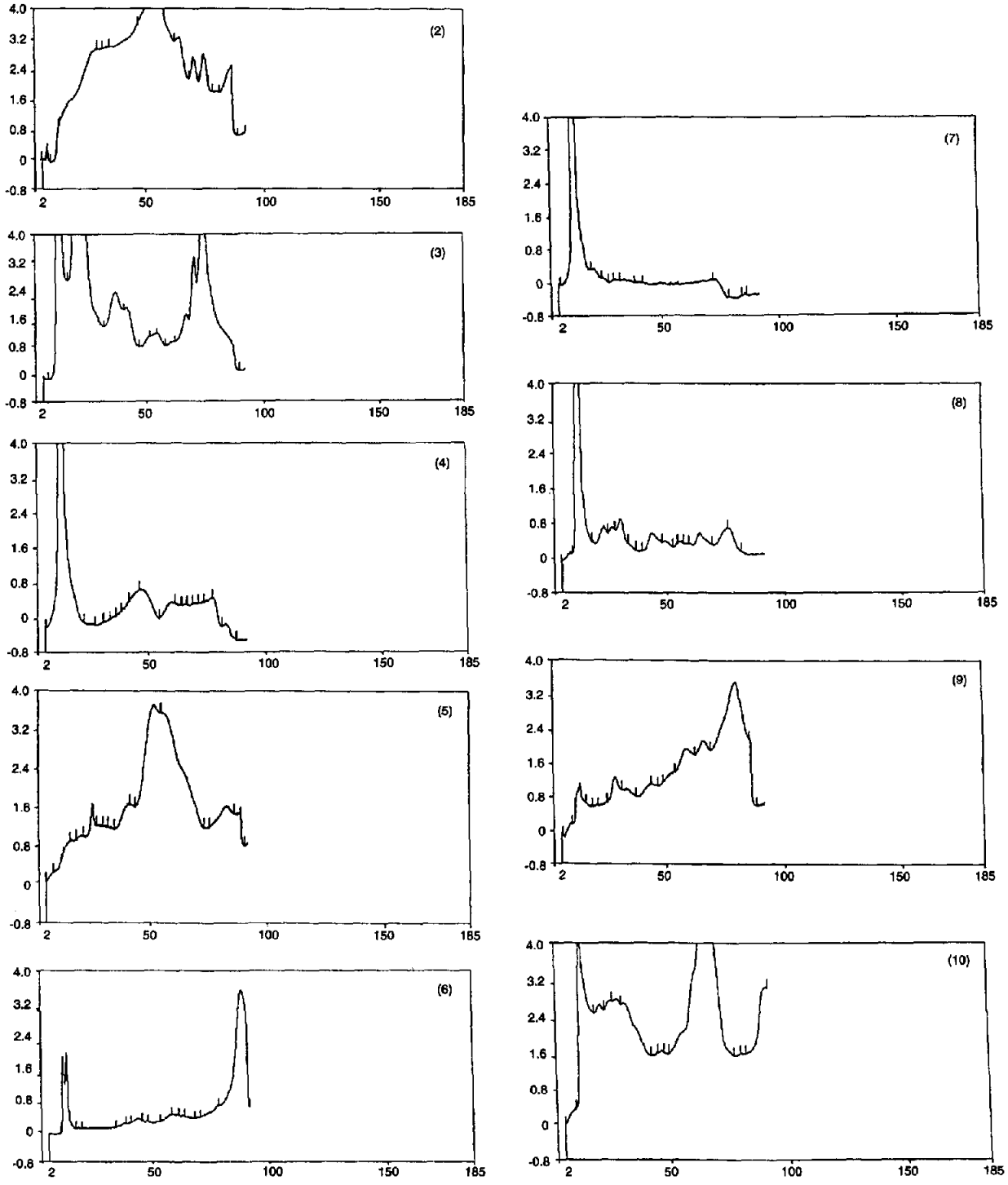

Fig. 1. Densitograms of the studied plant extracts using isocratic TLC. The numbers indicate the plant extracts in Table 1.

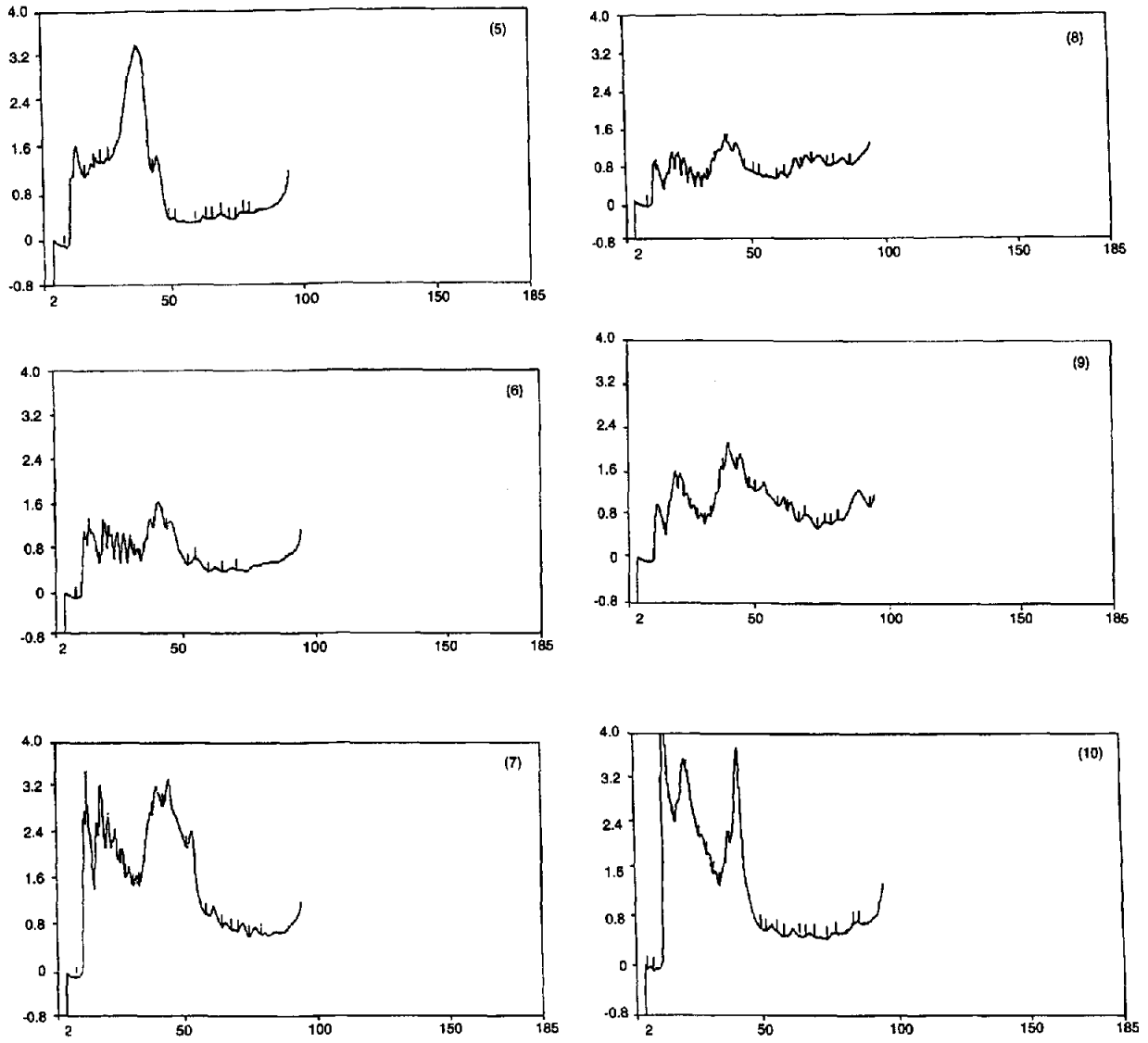

Fig. 2. Densitograms for six plant extracts obtained by AMD-TLC with gradient No. 2. The numbers indicate the plant extracts in Table 1.

3. Results and discussion

In AMD, the concept of R_f is inappropriate because of the multiple development steps and the continually changing distance traversed by the solvent. The elution distance (ED) measured as distance migrated from the bottom edge of the plate is often used [11,12,16]. There are two types of variable which have to be established for an AMD programme. The first includes all time-based parameters which control the operation of the AMD chamber. In this study, all gradients had the same execution programme in order to standardize the conditions for the simultaneous AMD of different plant extracts. The second relates to the optimization of sample resolution and concerns the selection of the appropriate composition of mobile phase for a given stationary phase.

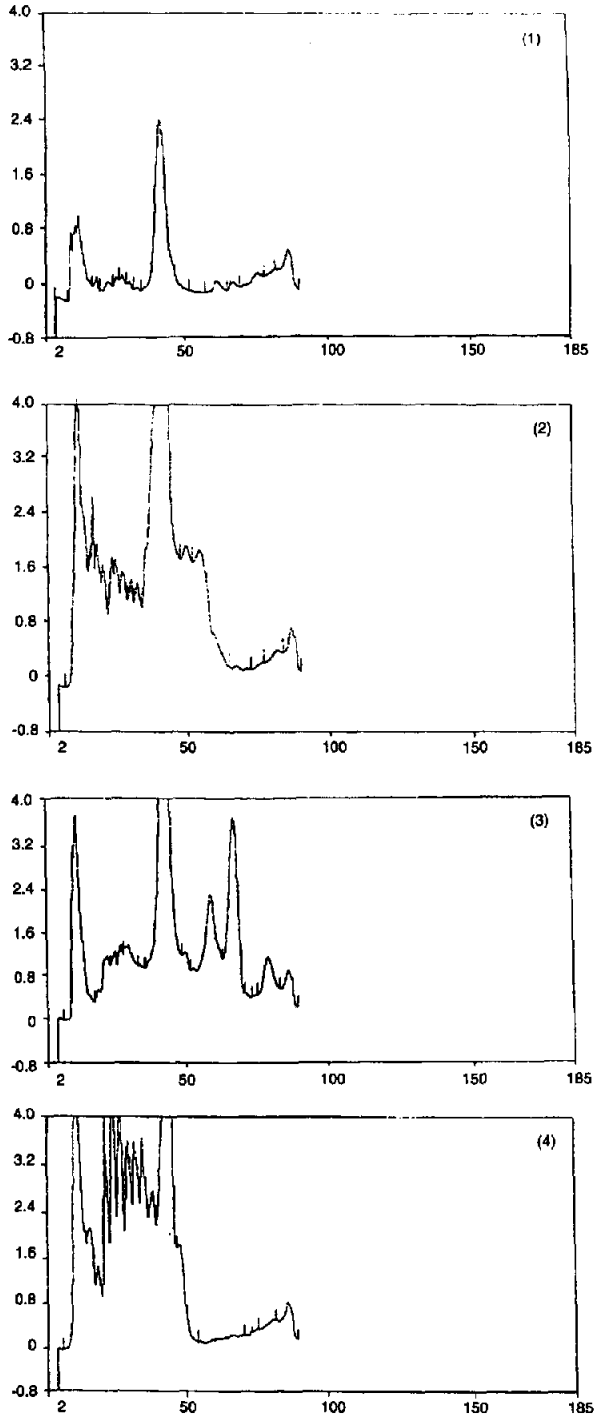

Fig. 3. Densitograms for four plant extracts obtianed by AMD-TLC with gradient No. 4. The numbers indicate the plant extracts in Table 1.

The "universal" gradients are suitable for the separation of mixtures with a wide polarity range and their use is suggested whenever unknown samples are under examination. A number of "universal" gradients suitable for separation of plant extracts (a complex mixture of non-polar and polar fractions) were checked, using dichloromethane as the main component of the eluent composition [15]. Gradients based on dichloromethane did not give an optimum separation for the studied plant extracts, especially for very polar fractions. In order to improve the separation, ethyl acetate was included in the eluent composition. Gradient 3 gave the best separation using the gradients based on dichloromethane. Gradient 1 improved the separation of the ten plant extracts owing to the ethyl acetate included in the eluent composition. The best separations were obtained using gradients 2 and 4. The corresponding densitograms obtained from the plates developed under isocratic conditions are shown in Fig. 1. For all ten plant extracts, the best separations were obtained using multiple development and the corresponding densitograms and AMD gradient are shown in Figs. 2 and 3.

Gradient chromatography is a powerful method which can have applications in biochemistry, especially for the separation of compounds with unknown structures from different mixtures. The "fingerprint" method described in the Homeopathic Pharmacopoeia [1] for plant identification can give only an enumeration of different coloured spots, related to some reference substances. In most cases, the reference substances are representative of groups of compounds with similar structures and they must be present in the analysed mixture. In some cases the reference substances are not present in the analysed mixture (plant extracts) and they are used as relative standards for the spots obtained by TLC [1]. Frequently, the very polar compounds remain at the start line and the use of more polar eluents can affect the quality of the separation. As shown in Figs. 1–3, AMD-TLC provides a good separation for polar substances in the lower part of the plate and for the less polar compounds in the upper

part. Finding an appropriate gradient is a semi-empirical method and some of the "universal" gradients have to be checked before performing an optimization for some of them.

4. Conclusion

AMD using solvent gradients is a powerful technique for separating mixtures with wide polarity range. Homoepathic Pharmacopoeia [1] recommends performing the identification of plant extracts using the "fingerprint" method by TLC. The AMD-TLC technique can be used to achieve better simultaneous separations of different plant extracts and development of the instrumentation may improve the reproducibility of the method.

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