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Comparison of planar chromatographic methods (TLC, OPLC, AMD) applied to essential oils of wild thyme and seven chemotypes of thyme☆

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Abstract

Essential oils analysis is more often realized by gas chromatography. However, thin-layer chromatography (TLC) remains the reference for Pharmacopoeia. Nevertheless classical TLC has its own limitations but it is always a good technique because it is simple, rapid and less expensive. Actually the reproducibility, the separation quality and the possibility to obtain good and reproducible quantitative determinations have been improved significantly with automated sample applicator, scanner densitometers and two new chromatographic planar methods: the optimum performance laminar chromatography (OPLC) and automated multiple development (AMD). In this work, we show and compare the performance of these methods and TLC through a study of seven thyme chemotypes and wild thyme essential oil. © 2001 Éditions scientifiques et médicales Elsevier SAS

Keywords: Thymes; Wild thyme; Essential oil; Thin-layer chromatography (TLC); Optimum performance laminar chromatography (OPLC); Automated multiple development (AMD)

1. Introduction

Essential oils analysis is generally realized by gas chromatography [1–3], however the reference method for Pharmacopoeia (and specially the European one) is always the thin-layer chromatography (TLC) because it is rapid, easy to use and less expensive.

During the last years, TLC has taken a modern trend with new commercially available adsorbents, HPTLC plates (CN, NH_2, diol, etc.), automated sample applicators and introduction of new techniques: automated multiple development (AMD), and optimum performance laminar chromatography (OPLC) and the high-performance densitometric scanners for quantitative evaluation. So planar chromatography is now a modern technique of analysis.

In this work we report the TLC, OPLC and AMD analysis of the essential oils of wild thyme and thymes in order to compare the performances of each chromatographic method.

2. Experimental

2.1. Chemicals and samples

Authentic samples: thymol and carvacrol were commercially pure products (Extrasynthèse, 69726 Genay, France).

Thymes and wild thyme essential oils were obtained from Myrtéa, 63122 St. Genès Champanelle, France. The samples studied were seven chemotypes of *Thymus vulgaris*: *T. vulgaris* b.s. (thymol), *T. vulgaris* b.s. (carvacrol), *T. vulgaris* b.s. (linalool), *T. vulgaris* b.s. (geraniol), *T. vulgaris* b.s. (thuyanol), *T. vulgaris* b.s. (limonene), *T. vulgaris* b.s. (borneol) and *T. serpyllum* (Fig. 1).

2.2. Apparatus

The following instruments were used: Linomat IV (Camag, Muttenz, Switzerland) for sample applications; TLC-MAT (automated development in TLC) Desaga (Bionisis, 92350 Le Plessis-Robinson, France); optimum performance laminar chromatography (OPLC) (Bionisis, 92350 Le Plessis-Robinson, France); and automated

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multiple development (AMD) (Camag, Muttenz, Switzerland).

2.3. Procedure

2.3.1. Preparation of the standard solutions

Thymol and carvacrol were prepared through dissolution of 10 mg/10 ml of pure ethanol (96°). A mixture of thymol and carvacrol was prepared through dissolution of 10 mg of thymol and 10 mg of carvacrol/10 ml in pure ethanol (96°).

2.3.2. Preparation of the sample solutions

All the essential oils samples were prepared by dilution of 0.1 ml/1 ml in pure toluene.

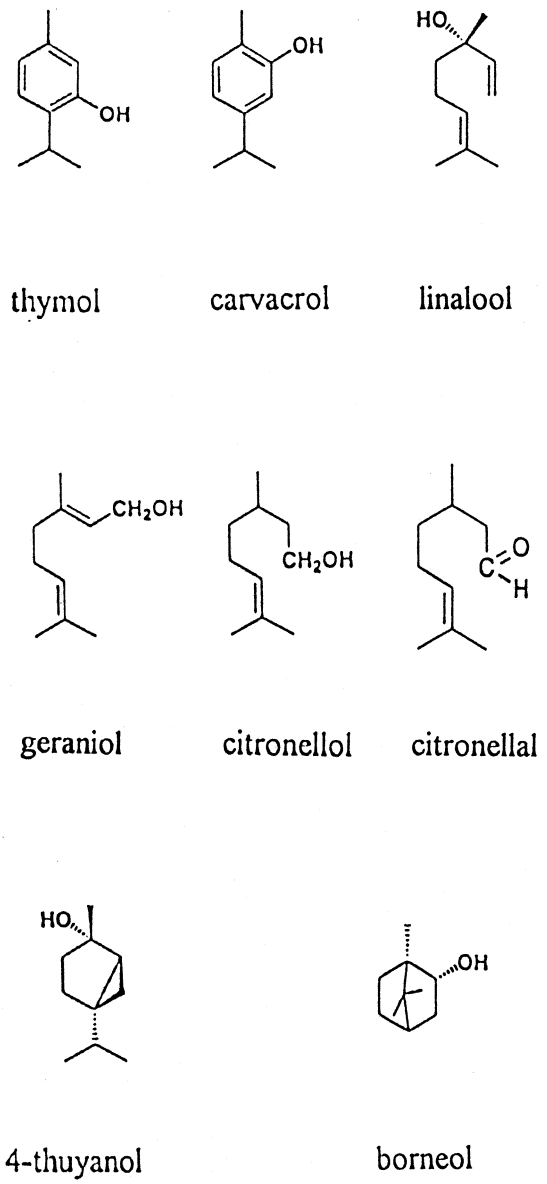

Fig. 1. Structures of principal constituents of the seven thyme chemotypes studied.

2.3.3. Solvents

All solvents were of analytical grade and filtered through a 0.45 μm Millipore membrane after sonication.

2.3.4. Chromatography

For TLC, plates used are silica gel 60 F_{254} 20 × 20 cm on glass Merck, Germany, Art: 5715. Eluent: toluene–ethyl acetate 95:5 (v/v). For OPLC, the plates used are silica gel 60 F_{254} 20 × 20 cm on aluminium sheet, Merck, Germany, Art: 5554, sealed by OPLC-NIT Co. Ltd., Budapest, Hungary. External pressure: 50 bar, start flash volume 300 μl, flow rate 300 μl, elution volume 600 μl, total elution time 1210 s. Eluent: hexane–ethyl acetate 95:5 (v/v). For AMD, plates used are silica gel 60 F_{254} 10 × 20 cm on glass, Merck, Germany, Art: 5729. Elution gradient: chloroform 100, chloroform–cyclohexane 50:50, cyclohexane 100, cyclohexane 100, hexane 100; number of steps 20.

2.3.5. Reagents

Anisaldehyde–sulfuric acid and vanillin–sulfuric acid were prepared according to Wagner and Bladt [4].

3. Results and discussion

The *Thymus* genus, which belongs to the family of Lamiaceae is the main representative of aromatic plants found in the Mediterranean countries [5]. There are numerous varieties and biochemical specificities in the *Thymus* genus. According to its biochemical specificity, the essential oil of any given species will have some individual characteristics of its own [6]. This biochemical specificity shows that one or many components provide the particular therapeutic action of the essential oil though such components are not necessarily dominant [7].

The concept of biochemical specificity finds its full meaning with these species depending on the biotope in which they grow, the biochemical composition of the essences varies in a marked way [6].

The use of planar chromatography (TLC, OPLC, AMD) highlights the main compound in each chemotype of thyme. The anisaldehyde–acid reagent [4] gives the best results because the shades are different (orange for thymol and light brown for carvacrol) while with vanillin–sulfuric acid thymol and carvacrol are both pink–violet.

These techniques provide information that help identify the position of different compounds included in the essential oils of the seven chemotypes of thymus studied.

For TLC, the eluents generally used for essential oil separation are hexane–ethyl acetate in proportions (v/v) 95:5, 94:6, or 93:7 [8] and toluene–ethyl acetate in

Table 1
hR_f values of the principal constituents present in essential oil of the thyme chemotypes studied

Compounds	hR_f ($100R_f$)			
	Classical TLC	TLC-MAT[a]	OPLC	AMD
Borneol	22	20	30	21
Carvacrol	45	44	55	36
Citronellal	64	66	85	51
Citronellol	19	17	23	14
Geraniol	16	16	19	16
Linalool	29	27	44	27
Thymol	48	48	60	38

[a] TLC-MAT = TLC automated tank.

the same proportions, particularly 93:7 v/v [9]. Under these conditions with vanillin–sulfuric reagent the *T. vulgaris* and the chemotypes *Thymus* b.s. (thymol) and *Thymus* b.s. (carvacrol) show thymol and its isomer carvacrol as a red zone at hR_f ($100 \times R_f$) near 55 but these two compounds are not separated. A separation of the isomers thymol and carvacrol is achieved by two-dimensional TLC with toluene–ethyl acetate 93:7 v/v in the first and toluene–carbon tetrachloride–*o*-nitrotoluene 33:33:33 v/v in the second dimension [10].

The TLC technique is limited because the compounds of essential oils are volatile; so at the top of the plate a diffusion can be observed as an edge effect. So, it is important to work with a small tank like the automatic TLC tank (Desaga) in which the evaporation is weaker in comparison with a TLC plate in a classical tank.

In our work we have studied the seven chemotypes of thymus previously determined by classical TLC and automated tank, Desaga. The results obtained are listed in Table 1 and shown in Figs. 2 and 3.

The observation of the two TLC chromatograms shows numerous compounds with very different shades of colours; so it is easy to determine unambiguously to which chemotype each belongs. Particularly when we have the reference compound, except for the *Thymus* b.s. (thuyanol), because the standard is not marketed, in this case we have only the chromatographic profile of the essential oil.

The volatility of these substances involves a character of diffusion which limits the performance of classical TLC; so, we have studied the chromatographic behaviour of these compounds with two techniques: OPLC and AMD.

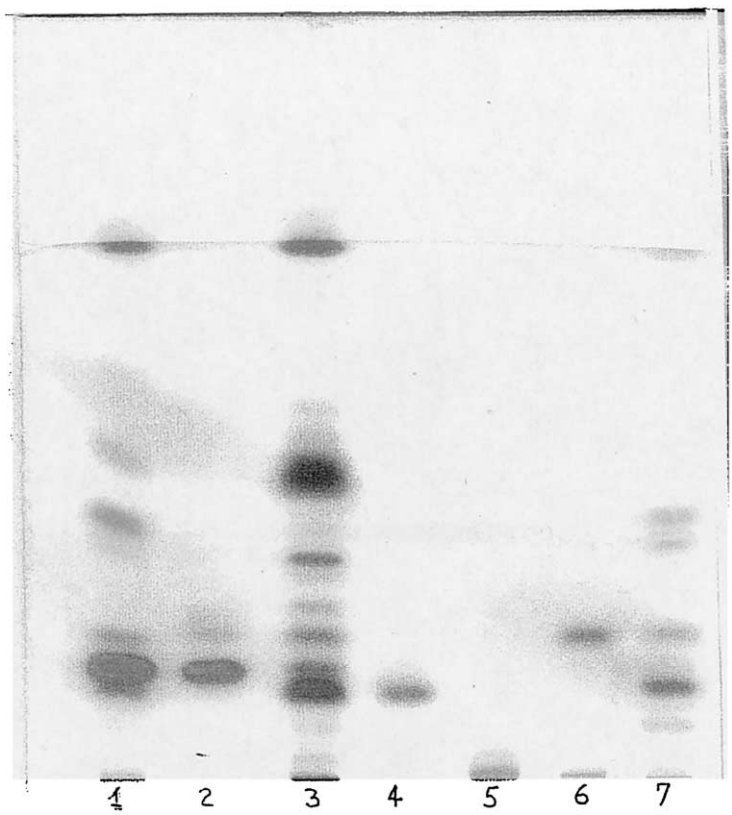

Fig. 2. Chromatogram of essential oils of thyme chemotypes with their typical standard by TLC-MAT Desaga. 1–thyme b.s. borneol, 2–borneol, 3–thyme b.s. geraniol, 4–geraniol, 5–thyme b.s. linalool, 6–linalool, 7–thyme b.s. thuyanol.

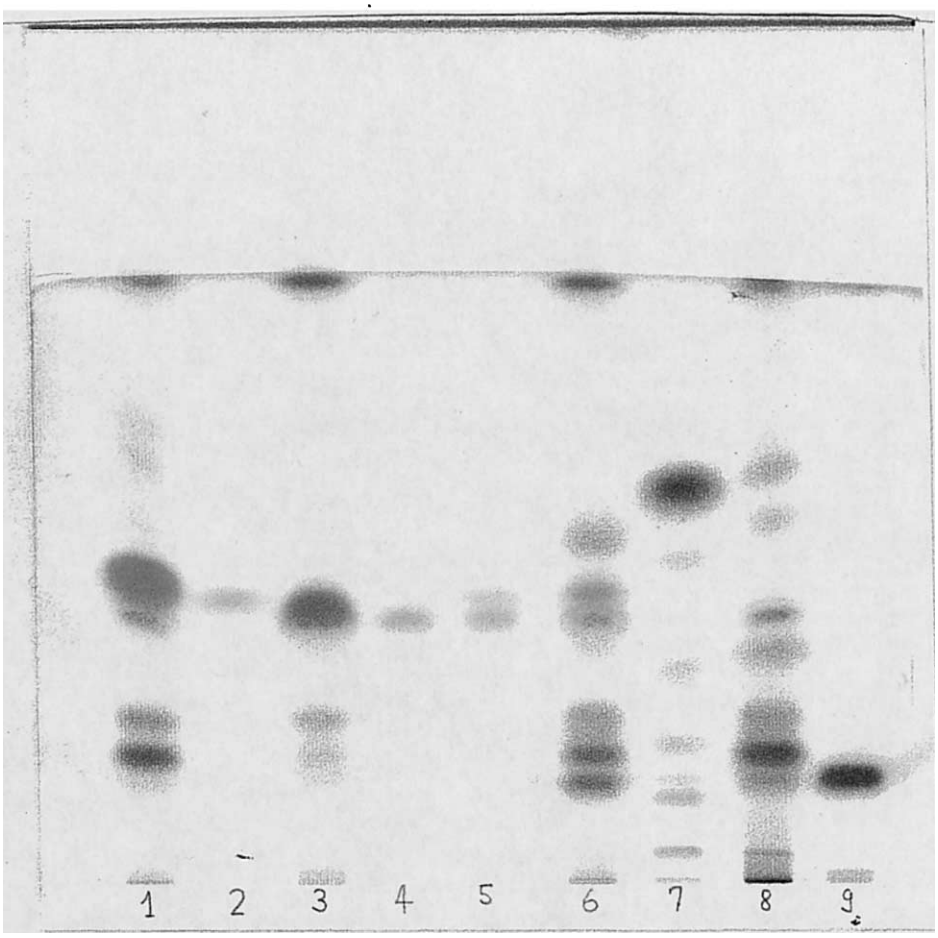

Fig. 3. Chromatogram of essential oils of thyme chemotypes with their typical standard by TLC-MAT Desaga. 1–thyme b.s. thymol, 2–thymol, 3–thyme carvacrol, 4–carvacrol, 5–thymol and carvacrol in mixture, 6–wild thyme, 7–citronellal, 8–lemony thyme, 9–citronellol.

OPLC [11,12] combines the underlying principles of HPLC and TLC, being both an analytical and preparative tool. It is a planar chromatographic method performed using a pressurised chamber in which the vapour phase above the sorbent layer is virtually eliminated; a continuous development can be performed by a pump, and the eluent is pushed through the sorbent layer. The analysis of chromatograms shows that the quickness of the migration limits the diffusion effect, thereby making the separation cleaner than in classical TLC.

On the other hand, OPLC also permits the analysis of numerous samples; the quantity of eluent for each development is low (3 or 4 ml) and the linearity in time allows to envisage the semi-preparative mode and collect each substance by direct elution on the way to structural analysis [13].

However, this technique provides good information highlighting the position of the different compounds included in the essential oils of the seven chemotypes of thyme studied. With the eluent hexane–ethyl acetate 95:5 v/v the results obtained are better than in classical TLC and the two phenol isomers are cleanly separated

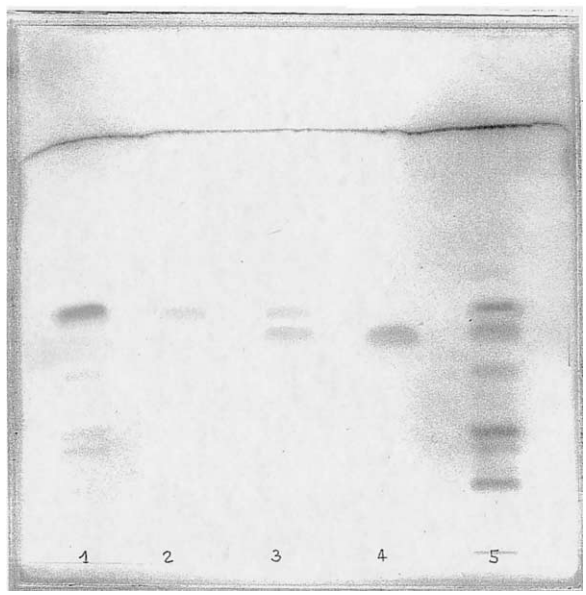

Fig. 4. Chromatogram of essential oils of thyme and wild thyme with the two phenol isomers standard: thymol and carvacrol by OPLC. 1–E.O. of thyme, 2–thymol, 3–thymol + carvacrol, 4–carvacrol, 5–E.O. of wild thyme.

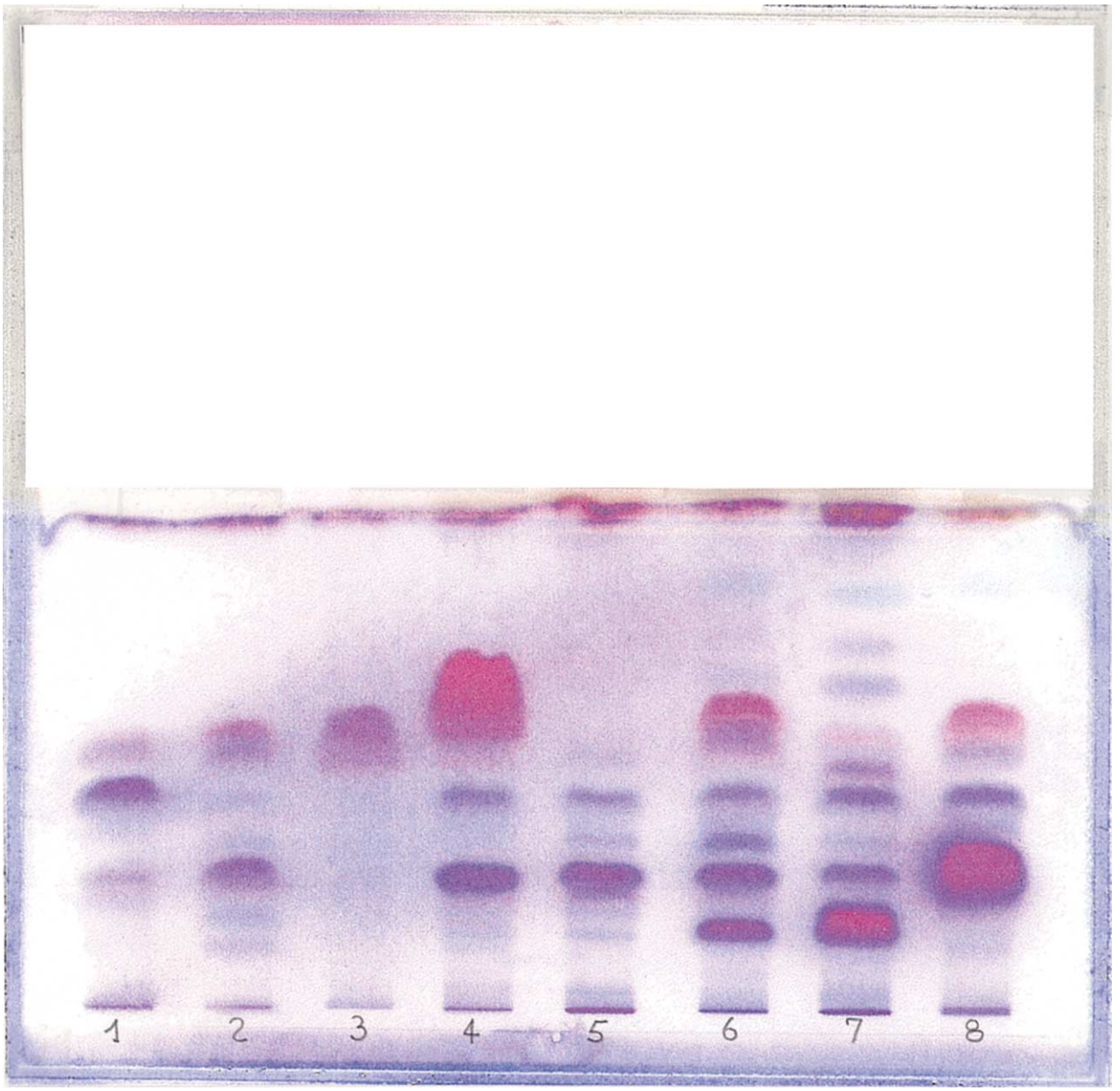

Fig. 5. Chromatogram of essential oils of seven chemotypes of thyme by OPLC. 1–thyme b.s. linalool, 2–thyme b.s. thuyanol, 3–thyme b.s. carvacrol, 4–thyme b.s. thymol, 5–lemony thyme, 6–wild thyme, 7–thyme b.s. geraniol, 8–thyme b.s. borneol.

(carvacrol $hR_f = 55$ and thymol $hR_f = 60$), We note that the mixture of thymol and carvacrol and other constituents of thyme and wild thyme extracts are clearly separated without diffusion; so OPLC is an interesting method of analysis for these compounds (Table 1; Figs. 4 and 5).

AMD [14–16] consists of a developing module and a microprocessor control unit. The mobile phases are prepared by a pump and gradient mixer from pure solvents contained in six storage bottles. The plate is subjected to multiple (up to 25) linear developments higher every time (3–5 mm distances) with solvents of decreasing strength (i.e. decreasing polarity for silica gel). The solvent is removed and the layer is dried under vacuum between runs. Before the next development, the plate is reconditioned by pumping a vapour phase from a reservoir into the chamber. With the AMD technique, the diffusion on the plate is reduced and the evaporation is also very limited. Besides, the successive migrations progress into a nitrogen atmosphere. Oxidation is thus prevented. Therefore, it is possible to study essential oils that are thermolabile and easily oxidizable. With this technique (see Section 2) we have obtained the separation of thymol and carvacrol,

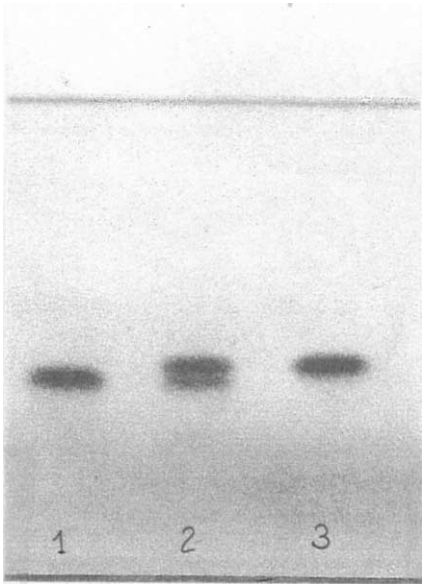

Fig. 6. Chromatogram in AMD of two phenol isomers thymol, carvacrol and thymol–carvacrol in a mixture. 1–carvacrol, 2–thymol + carvacrol, 3–thymol.

and with fine spots. This is the reason why AMD is useful for chromatography of essential oils because with very small quantities of sample the resolution is satisfactory and it is possible to envisage a quantitative analysis by scanner densitometry (Table 1; Figs. 6–8).

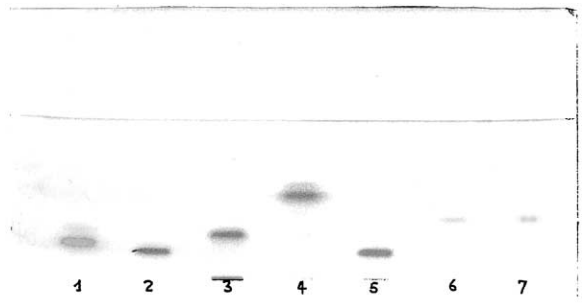

Fig. 7. Chromatogram of the seven standards in AMD. 1–borneol, 2–geraniol, 3–linalool, 4–citronellal, 5–citronellol, 6–thymol, 7–carvacrol.

4. Conclusion and perspectives

In our work the results obtained with OPLC are better than those currently reported by Pharmacopoeia. With this method it is possible to separate cleanly the two phenol isomers *thymol* and *carvacrol* with a classical binary eluent (hexane–ethyl acetate 95:5 v/v). A single chromatographic plate permits the analysis of about ten samples within 15 min and also the easy identification of the different chemotypes of thymus.

With AMD it is also possible to correctly separate thymol and carvacrol. This technique has the advantage of working with small quantities of samples. This method could be finalized in the view to permit the identification of the typical compound of the chemotype and the quantization of the different constituents of the essential oils studied.

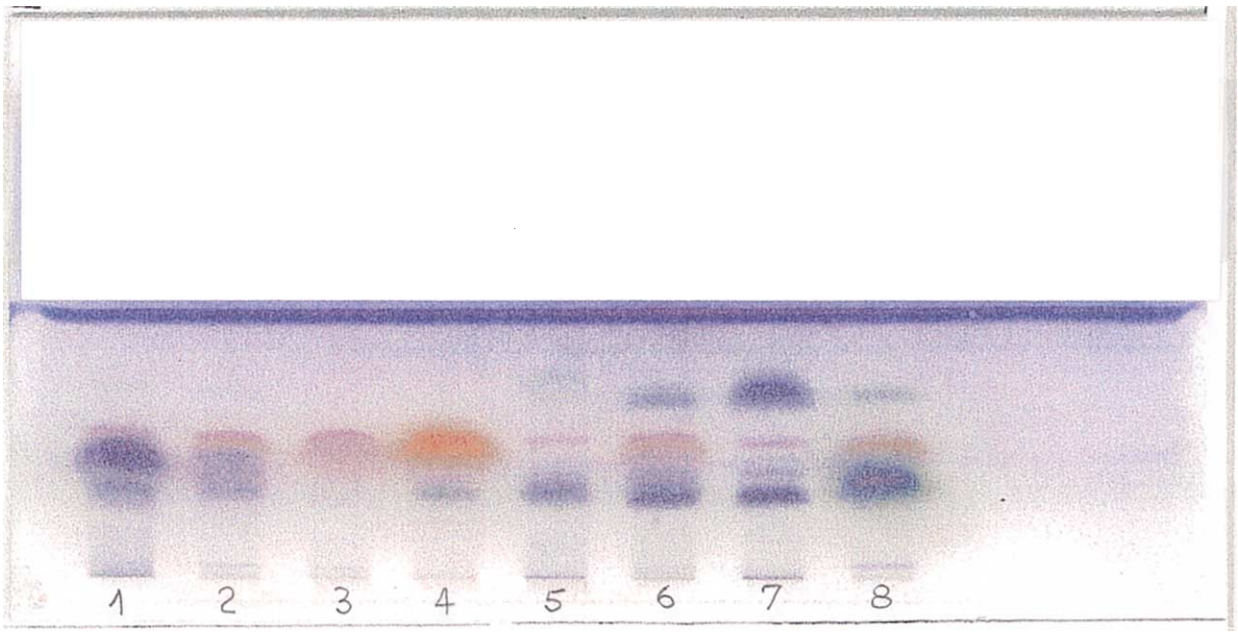

Fig. 8. Chromatogram of essential oils of seven chemotypes of thyme by AMD. 1–thyme b.s. linalool, 2–thyme b.s. thuyanol, 3–thyme b.s. carvacrol, 4–thyme b.s. thymol, 5–lemony thyme, 6– wild thyme, 7– thyme b.s. geraniol, 8–thyme b.s. borneol.

References

[1] Anonymous, La chromatographie en phase gazeuse sur colonne remplie — Méthode générale. Norme AFNOR, NFT 75-400.

[2] Anonymous, La chromatographie en phase gazeuse sur colonne capillaire — Méthode générale. Norme AFNOR, NFT 75-401.

[3] P. Teisseire, Quelques aspects de la chromatographie en phase gazeuse dans l'industrie des matières odorantes utilisées en parfumerie, Recherches 17 (1969) 37.

[4] H. Wagner, S. Bladt, Plant Drug Analysis, 2nd ed., Springer, Berlin, 1996, p. 359, 364.

[5] J. Bruneton, Pharmacognosy, Phytochemistry, Medicinal Plants, 2nd edition, Technique et Documentation, Paris, 1999, pp. 545–547.

[6] J.-M. Soulier, The thymus genus, Les cahiers de l'Aromathérapie (Aromather. Records) 1 (1995) 38–49.

[7] J.-M. Soulier, *Thymus vulgaris*: properties and indications, Les cahiers d'Aromathérapie (Aromather. Records) 1 (1995) 50–53.

[8] E. Stahl, Analyse chromatographique et microscopique des drogues, Entreprise Moderne d'Edition et Technique et Documentation, Paris, 1975, pp. 141–145.

[9] H. Wagner, S. Bladt, Plant Drug Analysis, 2nd ed., Springer, Berlin, 1996, pp. 151–152.

[10] H. Wagner, S. Bladt, Plant Drug Analysis, 2nd ed., Springer, Berlin, 1996, p. 176.

[11] E. Tyihák, E. Mincsovics, H. Kalász, New planar liquid chromatographic technique: over pressured thin-layer chromatography, J. Chromatogr. 174 (1979) 75–81.

[12] B. Fried, J. Sherma, Thin-Layer Chromatography — Techniques and Applications, Marcel Dekker, New York, 1994, pp. 21–22, 123–124.

[13] J. Pothier, N. Galand, S. Fouchécourt, C. Viel, Isolation of alkaloids by overpressured thin-layer chromatography, Fitoterapia 68 (1997) 42–44.

[14] K.D. Burger, H. Tengler, Automatic multiple development: A new technique of thin-layer chromatography, in: R.E. Kaiser (Ed.), Planar Chromatography, vol. 1, Dr Alfred Huethig Verlag, Heidelberg, 1986, pp. 193–205.

[15] C.F. Poole, M.T. Bellay, Progress in automated multiple development, J. Planar Chromatogr. 4 (1991) 345–359.

[16] B. Fried, J. Sherma, Thin-Layer Chromatography — Techniques and Applications, Marcel Dekker, New York, 1994, pp. 115–117.