HPLC INVESTIGATION OF TRIGLYCERIDES FROM PLANTS OF THE LAMIACEAE FAMILY

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The advantages and disadvantages of HPLC investigations of triglycerides of seed oil from plants of the Lamiaceae family were discussed. Special attention was paid to triglycerides formed from laballenic acid.

Key words: HPLC, triglycerides, Lamiaceae seed oil, laballenic acid.

Plants of the Lamiaceae family are common in the Belgorod flora. The large number of publications on the chemical composition of various plant parts [1], including the scientific periodical *Lamiales Newsletter*, is indicative of the great interest in plants of this family.

The present article reports results of using HPLC for direct investigation of the triglyceride composition of seed oils from Lamiaceae plants.

HPLC is of greatest interest for investigating oils containing laballenic (Lb) acid [2-4]. The chromatographic profile of the group of oils containing linolenic (Ln) acid is the same as that of ordinary linolenic oils. Many of these are more unsaturated than linoleic acid. To a certain extent, only the ratio of the signal areas changed. Figure 1 shows chromatograms of seed oils from three plants that were recorded on a microcolumn chromatograph. The data are difficult to interpret quantitatively owing to the increasing sensitivity of the UV detector for triglycerides as the number of unsaturated bonds in the molecule increases and to difficulties in calculating the content of pure components of "problem" triglycerides. Nevertheless, the increasing unsaturation of oils from *Mentha piperita* L. to *Ocimum basilicum* L. and *Majorana hortensis* Moench. is obvious from the relatively increasing size of the Ln_3 peak in the chromatogram. Table 1 gives the triglyceride composition of certain species, including two species of *Salvia* (*S. tesquicola* Klok. et Pobed. and *S. aethiopis* L.). It can be seen that both oils are linolenic (contain 56-60 mol% α-linolenic acid). They differ mainly only in the ratio of oleic (O) and linoleic (L) acids (18 mol% L and 14 mol% O in *S. aethiopis* oil and 31 mol% L and 7 mol% O in *S. tesquicola* oil). The principal acid in *Dracocephalum thymiflorum* L. oil is still linoleic (50 mol%) although α-linolenic (19 mol%) and oleic (27 mol%) are present.

There are no HPLC data for triglycerides formed by laballenic (Lb) acid. However, by using the trend in the change of retention time (t_R) of triglycerides [5, 6] formed from various fatty acids, it can be assumed that t_R should increase in the order L_3—L_2Lb—L_2O—LLbO (the formula indicates only the type and not the positional composition). In fact, the simple shift of the unsaturated bonds on going from α- to γ-linolenic acid and from oleic to petroselic (three methylene groups toward the glycerol) retains noticeably the corresponding triglycerides. A shift of the double bonds in the same direction, which corresponds to the transition from linoleic to laballenic acid, is accompanied by an additional migration of methylene groups and should lead to an even greater increase in t_R.

In fact, peak 1 appears in chromatograms of certain oils between peaks for L_3 and L_2O triglycerides (Fig. 2). This interval is very interesting because only triglycerides OdLO, OdLP, and Od_2S (where Od is octadecatrienoic acid) are observed in it. These are usually present in relatively small quantities, even if they are preceded by substantially stronger peaks with other combinations incorporating Od (Od_3, Od_2L, OdL_2, Od_2O, Od_2P). In our opinion, the only exception is seed oil of *Momordica charantia* L., which has a unique triglyceride composition. Its chromatogram has a main peak for Es_2S (Es is α-eleostearic acid, S is stearic acid). Seed oil of *Nigella sativa* L. is also notable because a triglyceride containing eicosadienoic acid appears in this interval. A careful analysis found that peak 1 and these triglycerides have different t_R values in Fig. 2.

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TABLE 1. Triglyceride Composition of Plant Seed Oils

Acid	*Salvia aethiopis* L.	*Salvia tesquicola* Klok. Et Pobed.	*Dracocephalum thymiflorum* L.	*Ballota nigra* L.	*Phlomis tuberosum* L.	*Stachys recta* L.	*Stachys palustris* L.	*Leonurus cardiaca* L.
Ln_3	24.8	23.3	1.8	Tr.	Tr.	Tr.	Tr.	Tr.
Ln_2L	15.4	23.8	5.8	Tr.	Tr.	Tr.	Tr.	Tr.
LnL_2+Ln_2O	20.8 (1:3)	19.9 (3:1)	19.9 (5:1)	2.8	2.3	2.2	1.6	3.9
Ln_2P	6.4	4.2	0.5	Tr.	Tr.	Tr.	Tr.	Tr.
L_3 +LnLO	9.7 (1:4)	10.9 (2:3)	17.2 (1:1)	24.2	26.1	38.9	30.2	31.1
LnLP+Ln_2S	5.7	6.1	2.1	0.6	0.6	0.5	0.2	0.4
L_2Lb	Tr.	Tr.	Tr.	8.0	4.1	6.1	4.9	8.5
L_2O+LnO_2	7.9 (1:3)	3.0 (3:1)	29.8	19.3	24.6	23.1	25.5	20.2
L_2P+LnLS	1.4	2.1	3.3	12.2	6	8.5	7.8	9.9
LnOP	3.5	2.7	0.6	Tr.	Tr.	Tr.	Tr.	Tr.
LLbO	Tr.	Tr.	Tr.	4.1	5	2.4	2.9	4.3
LO_2 +LLbP	Tr.	1.1	9.6	11.7	14.3	10.8	15.4	9.2
L_2S+LOP	1.4	1.0	3.4	8.5	6.5	4.4	5.6	6.5
LbO_2	Tr.	Tr.	Tr.	3.2	3.3	0.7	1.3	1.4
LbOP+O_3	Tr.	Tr.	3.8	2.2	3.3	1.4	2.9	1.8
Remaining	3.0	1.9	2.2	3.2	3.9	1.0	1.7	2.8

Ln, α-linolenic; L, linoleic; Lb, laballenic; O, oleic; P, palmitic; S, stearic. Ratios of pure triglycerides (in the order given in the first column) were obtained using an eluent containing CH_3CN (25 vol%) in CH_3COCH_3.

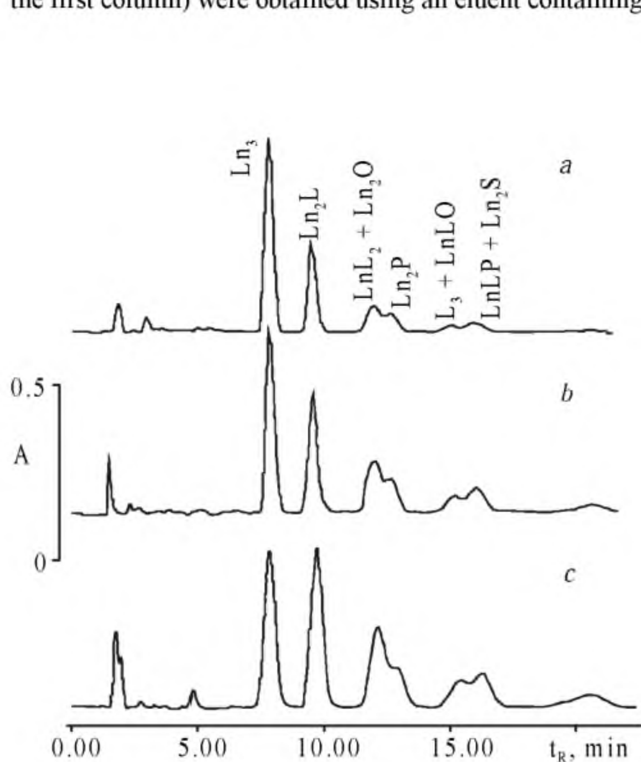

Fig. 1

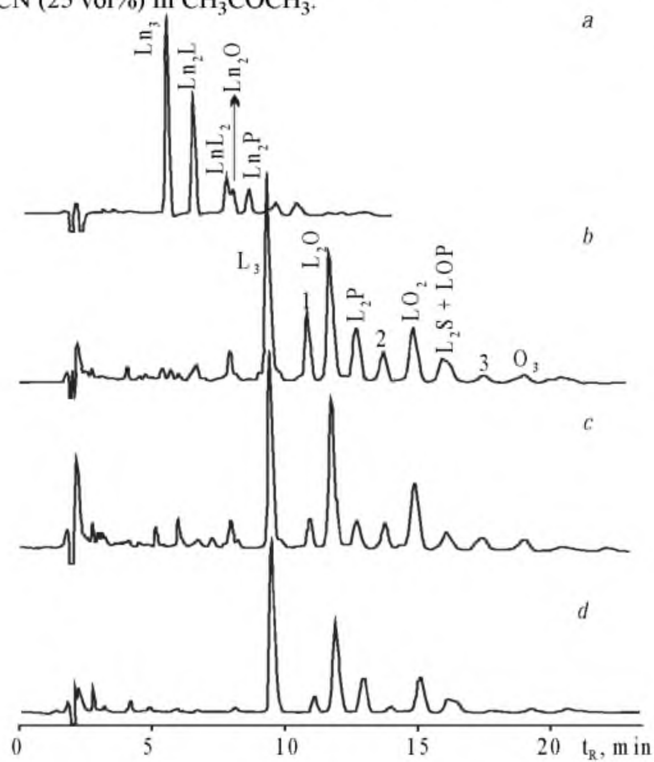

Fig. 2

Fig. 1. Chromatograms of seed oils of *Majorana hortensis* Moench (a), *Ocimum basilicum* L. (b), and *Mentha piperita* L. (c). Column: 80×2 mm, Diaspher-110-C18, 6 μm (BioKhimMak), eluent CH_3CN:$C_2H_5OC_2H_5$ (10:5 vol%), 100 μL/min, detector UV 210 nm.

Fig. 2. Chromatograms of seed oils from Lamiaceae plants: *Majorana hortensis* Moench (a), *Leonurus cardiaca* L. (b), *Phlomis tuberosa* L. (c), *Stachys lanata* L. (d). Column: 250×4 mm, Kromasil 100 C18, 5 μm (Dr. Maisch), eluent CH_3CN:CH_3COCH_3 (20:100 vol%), 1 mL/min; detector R-401 (Waters Millipore).

TABLE 2. Experimental and Calculated Retention Times of Triglycerides

Triglyceride composition	Data			
	experimental		calculated	
	t_R, min	log k	log k	t_R, min
L_3	**9.3**	**0.562**		
L_2Lb	**10.81**	**0.644**		
L_2O	**11.63**	**0.683**		
LLb_2			0.726	12.63
L_2P	**12.64**	**0.726**		
LLbO	13.65	0.765	0.764	13.62
LO_2	14.77	0.805	0.803	
Lb_3			0.807	14.83
LLbP			0.808	14.84
L_2S	**15.76**	**0.838**		
Lb_2O			0.846	16.03
LOP			0.846	16.04
Lb_2P			0.889	17.50
LbO_2	17.45	0.888	0.885	
LP_2			0.890	17.51
LLbS			0.919	18.61
O_3			0.925	18.85
LbOP			0.928	18.94
LOS			0.958	20.15
O_2P			0.969	20.61

Triglycerides used to calculate the increments are given in bold; log k is the logarithm of the capacity coefficients.

According to the literature, a system of cumulative double bonds has a weak absorption maximum at 230 nm [7]. However, chromatograms recorded on a microcolumn chromatograph at several wavelengths did not produce chromatograms of a fundamentally different profile, for example, for cherry seed oil at 210 and 280 nm [8]. Moreover, the relative heights of peaks 1, 2, and 3 increased on increasing the detection wavelength from 210 to 216, 220, and 226 nm compared with the predecessors. This is probably due to the small coefficient of molar absorption of the allene group and masking of the absorption by residual short-wavelength absorption of the esters, which is more pronounced in this solvent (eluent).

Having determined the increment [9] $\Delta(L \rightarrow Lb)$ from t_R for L_3 and peak 1 (Fig. 2), t_R of all possible combinations involving Lb can be calculated (Table 2 lists the calculations for some of these). By comparing the calculated and experimental t_R values, peaks corresponding to LLbO and LbO_2 can be found in the chromatogram of *Leonurus cardiaca*. However, there is some uncertainty for LbO_2 because LP_2, which is usually present in chromatograms of oils with a high content of palmitic acid, has a similar t_R. However, a peak with t_R = 13.6 min for seed oil of *Phlomis tuberosa* L. has an area comparable with that of the peak for LOP. This is possible only if LLbO makes a significant contribution to this signal. Furthermore, triglycerides containing oleic acid radicals typically have satellites resulting from substitution of oleic acid by palmitic. This explains the appearance of peaks for LLbP and LbOP before those of LO_2 and O_3, respectively, on increasing the content of CH_3CN in the eluent. These pairs could not be completely resolved. Unfortunately, it is practically impossible to determine completely and rigorously the relative quantities of triglycerides with different fatty-acid composition considering the ratio of increments that was found (Table 2). Therefore, the traditional method of preparing methyl esters should be used to determine the fatty-acid composition of the oils.

Thus, seed oil of *Leonurus cardiaca* (and not only Siberian *Leonurus* [2]) contains Lb radicals. This is consistent with unpublished data presented on the previously cited Internet site.

If the ratio of peak areas for $L_2Lb:L_3$ and $LLbO:L_2O$ (the difference between which is less than ±10 relative % for each specimen) is used as a measure of the ratio of contribution of Lb and L in these oils, then the data for *Leonurus cardiaca* L. (3.0:10) are similar to those for Siberian *Leonurus* (3.5:10) [2]. However, results for seed oil of *Ph. tuberosum* indicated half as less Lb (2.6:10 vs. 5.9:10). Table 1 shows that the contribution of Lb to the triglycerides is obviously notable.

EXPERIMENTAL

Samples were prepared from plant material grown in Belgorod region in 2004. HPLC conditions were the same as those previously published [8, 9].

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