Journal of Chromatography, 588 (1991) 303–305
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2375

Quantitative planar chromatography of phospholipids with different fatty acid compositions☆

G. Lendrath

Department of Pharmacognosy, Hamburg University, Bundesstrasse 43, W-2000 Hamburg 13 (Germany)

A. Bonekamp

Lucas Meyer GmbH & Co., Ausschläger Elbdeich 62, W-2000 Hamburg 26 (Germany)

Lj. Kraus*

Department of Pharmacognosy, Hamburg University, Bundesstrasse 43, W-2000 Hamburg 13 (Germany)

(First received September 24th, 1990; revised manuscript received June 25th, 1991)

ABSTRACT

A method was developed for the determination of phospholipids irrespective of their origin.

INTRODUCTION

Lecithin is a mixture of different phospholipids including phospatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidic acid (PA), and it is used as an emulsifier in a wide variety of food, in pharmaceuticals and in technical applications. The origin, soybean, rapeseed, sunflower and egg yolk, influences the fatty acid composition. Phospholipids have different fatty acid compositions in lecithin. The determination of individual phospholipids is commonly based either on spectrophotometric determination of fatty acids (high-performance liquid chromatography with UV detection at 206 nm [1]) or on densitometric determination {thin-layer chromatographic (TLC) separation and detection with lipid-specific reagents [2–8]}.Therefore, is it necessary to use phospholipid standards separated from the same origin. We report here a TLC method for the determination of phospholipids independent of their origin.

EXPERIMENTAL AND RESULTS

Fatty acid composition of phospholipids

We separated individual phospholipid standards (PC, PE, PI, PA) from different sources (soybean, rapeseed, sunflower, egg yolk) by using various chromatographic procedures (Table I). Based on the known fatty acid composition of individual phospholipids, it is possible to calculate their average molecular weights (Table II).

Detection of phospholipids

For our purpose we used the group-specific Dittmer–Lester reagent, which reacts only with the

☆ Presented at the *18th International Symposium on Chromatography, Amsterdam, September 23–28, 1990*. The majority of the papers presented at this symposium have been published in *J. Chromatogr.*, Vols. 552 and 553 (1991).

0021-9673/91/$03.50

TABLE I

FATTY ACID COMPOSITIONS OF PHOSPHOLIPIDS

Phospholipid class	Source	Fatty acid					
		16:0	18:0	18:1	18:2	18:3	Others
PC	Soybean	14.5	4.2	8.9	59.6	6.3	4.5
	Rapeseed	9.6	1.2	44.5	28.5	4.1	8.6
	Sunflower	11.1	3.7	12.3	63.3	5.5	3.5
	Egg	26.9	9.4	24.3	24.2	2.1	12.0
PE	Soybean	19.1	3.0	7.7	58.9	6.4	3.1
	Rapeseed	9.5	0.7	45.1	37.8	4.4	2.2
	Sunflower	13.3	3.4	9.2	71.2	0.3	2.5
PI	Soybean	32.7	5.7	5.6	47.0	6.8	1.9
	Rapeseed	17.2	1.6	37.7	35.1	5.6	2.7
	Sunflower	32.6	4.7	6.9	47.2	5.1	2.9
PA	Soybean	20.4	3.6	10.8	55.9	5.7	3.1
	Rapeseed	9.0	0.9	48.3	35.5	4.1	1.5
	Sunflower	12.3	3.8	10.3	67.8	0.9	4.7

phosphorus that is common to all phospholipids [9]. In order to be able to use the reagent as dip-in reagent and thus to enhance the quantitative evaluation, we added ethanol. Impregnation of the silica gel layer with phosphoric acid enhanced the separation of phospholipids. It also improved the stability of the chromogenic response with the Dittmer–Lester reagent [4,10].

We detected the same relative peak areas at 720 nm for phosphatidylcholines from soybean, rapeseed, sunflower and egg lecithin. Similar quantitative results were obtained for PE, PI and PA (Table III).

Quantitative analysis of lecithin samples

For the quantitative analysis of different lecithin samples, Merck silica gel high-performance TLC plates (5 × 5 cm) were impregnated with phosphoric acid. For the separation a Desaga H-chamber was used [11]. Detection was effected with modified Dittmer–Lester reagent, with measurement at 720 nm. Calibration was carried out by using either standards (weight ranges) from the same origin as the sample or from the other origins. The results are given in Table IV.

TABLE II

AVERAGE MOLECULAR WEIGHTS OF PHOSPHOLIPIDS

Phospholipid class	Source			
	Soybean	Rapeseed	Sunflower	Egg
PC	770	768	778	771
PE	724	735	736	
PI	834	850	843	
PA	687	691	695	

TABLE III

RELATIVE PEAK AREAS OF PHOSPHOLIPIDS (n = 20)

Phospholipid class	Source			
	Soybean	Rapeseed	Sunflower	Egg
PC	100	101.4	98.1	99.6
PE	100	97.0	97.7	–
PI	100	96.0	95.4	–
PA	100	104.0	101.5	–

CONCLUSIONS

Phospholipids have different fatty acid compositions according to their origin. For densitometric determination phospholipids the Dittmer–Lester reagent was selected, which reacts with the phosphate group that is component of all phospholipids. The signal obtained by this method for each class of

TABLE IV

PHOSPHOLIPID DETERMINATION IN LECITHIN SAMPLES: COMPARISON BETWEEN STANDARDS OF DIFFERENT ORIGIN (%)

Lecithins	Sample	Standard[a]												
		Soybean				Rapeseed				Sunflower				Egg
		PC	PE	PI	PA	PC	PE	PI	PA	PC	PE	PI	PA	PC
Soybean	a	12.6	10.8	7.4	7.1	11.8	11.5	8.6	6.4	12.9	11.2	7.3	5.7	13.1
		12.3	9.9	7.0	6.5	12.0	11.6	9.1	6.3	13.3	10.6	7.8	6.2	13.2
	b	12.5	11.7	8.6	6.1	13.5	11.9	9.5	6.6	13.8	11.1	8.9	5.9	13.2
		12.9	12.1	7.8	6.0	12.9	11.5	9.0	6.6	13.6	11.9	8.1	6.3	13.6
Rapeseed	c	8.7	4.9	6.9	6.9	9.3	5.1	7.7	5.6	10.1	5.1	8.3	8.5	8.5
		8.8	4.5	7.0	6.5	9.4	5.3	7.6	5.3	9.9	5.3	8.5	8.6	8.5
	d	7.7	5.2	6.6	8.3	7.4	5.2	7.6	7.4	8.5	5.0	7.8	8.8	8.1
		8.1	5.3	6.8	8.5	7.5	5.2	7.9	7.8	8.3	4.7	7.2	8.5	8.1
Sunflower	e	2.8	1.4	2.4	3.7	2.7	1.2	2.6	3.9	3.3	1.3	2.6	3.8	3.2
		2.8	1.4	2.6	3.9	2.8	1.2	2.7	3.9	3.5	1.2	2.5	3.8	3.1
	f	5.4	2.9	3.9	5.5	5.1	2.5	4.2	4.9	5.1	2.7	4.5	4.9	5.1
		5.3	3.0	3.9	5.4	5.2	2.6	4.4	5.1	5.3	2.9	4.4	4.9	5.2
Egg	g	39.8	–	–	–	42.8	–	–	–	40.6	–	–	–	38.3
		40.2	–	–	–	42.0	–	–	–	38.4	–	–	–	39.2
	h	41.0	–	–	–	39.6	–	–	–	41.9	–	–	–	41.0
		41.0	–	–	–	40.7	–	–	–	42.5	–	–	–	40.3

[a] Standards are from the same origin as the sample or from the other origins.

phospholipids is independent of the origin of the lecithin and of the fatty acid composition, because the same phospholipid classes of different origin have similar molecular weights. By application of this method it is possible to determine individual phospholipids using standards not obtained from the same origin as the samples.

REFERENCES

1 A. Nasner, *Dissertation*, Hamburg University, Hamburg, 1983.
2 A. Nasner and Lj. Kraus, *Dtsch. Apoth.-Ztg.*, 122 (1982) 2407–2415.
3 G. Klatt, A. Nasner and Lj. Kraus, *Planta Med.*, 5 (1986) 415–416.
4 G. Lendrath, *Dissertation*, Hamburg University, Hamburg, 1989.
5 J. Sherma and S. Bennet, *J. Liq. Chromatogr.*, 6 (1983) 1193–1211.
6 M. Goppelt and K. Resch, *Anal. Biochm.*, 140 (1984) 152–156.
7 J. C. Touchstone, S. S. Levin, M. F. Dobbins and P. C. Beers, *J. Liq. Chromatogr.*, 6 (1983) 179–192.
8 E. Hedegaard and B. Jensen, *J. Chromatogr.*, 225 (1981) 450–454.
9 J. C. Dittmer and R. L. Lester, *J. Lipid Res.*, 5 (1964) 126–127.
10 G. Lendrath, A. Nasner and Lj. Kraus, *J. Chromatogr.*, 502 (1990) 385–392.
11 Lj. Kraus, *Kleines Praktikumsbuch der Dünnschichtchromatographie*, Desaga, Heidelberg, 3rd ed., 1990.