Testing of Various Solvents for the Preparation of Lipid Samples for Use in Thin Layer Chromatography

Antonia Odagiu – Gheorghe Sălăjan – Daniel Mierliță – Bogdan Georgescu – Mariana Dinea
University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca

SUMMARY

We analyzed the muscular tissue of 6 sheep. Lipid extraction was performed using the Handson and Olley method. TLC separation was performed using 4 separation systems and the best separation method was determined. Visualization was done using a mixture copper acetate and phosphoric acid, at 150 ^oC.

INTRODUCTION

The number of thin layer chromatography (TLC) techniques used for various lipids separation increased over the last decade. These new TLC techniques allow the separation of almost all major types of polar lipids through a single migration. In this way, it is now possible not only to qualitatively analyze polar lipids but, using densitometry *in situ* from chromatography plates, it is possible to perform very accurate quantitative analyses.

These new methods are very rapid and even more sensible than the classic ones that use, for example, bidimensional chromatography followed by phosphorus and galactose determinations. The lipid structure of muscle tissue in Turcana sheep from Romania has not received proper study as yet. The aim of our paper is to emphasize those systems that allow polar lipid separation and to establish an improved solvents system for TLC separation of polar lipids from Turcana sheep muscular tissue.

MATERIAL AND METHOD

A single sheep muscular tissue sample (dry matter) was used from five Turcana sheep individuals. We performed the extraction of the total lipids using the Handson and Olley method (1962) – extraction with the mixture chloroform – methanol – water, 2:2:1, v/v – (Hamilton and Rossel, 1986). The TLC separation was realized in a chromatographic chamber, using kieselgel TLC plates (produced by Merck, 10 x 20 cm), activated for 30 minutes at 100 ^oC.

The solvents systems used were:

A: metil acetate – n – propanol – chloroform – methanol – KCl aqueous solution, 0,25% (25:25:28:10:7, v/v); [Bratt and Akerluna, 1994]

B: chloroform – methanol – acetate – water (85:15:10:3,5, v/v); [Heape, et al., 1985]

C: metil acetate – izopropanol – chloroform – methanol – KCl aqueous solution, 0,25% (25:25:28:10:7, v/v); [Vittelo and Zanetta, 1978]

D: metil acetate – izopropanol – chloroform – methanol – KCl aqueous solution, 0,25% (25:25:25:10:7, v/v); [Vittelo and Zanetta, 1978].

In order to determine the main types of polar lipids, we used the R_f values as a control for the above mentioned systems of solvents (*Table 1*).

Visualization was realized using a mixture copper acetate and phosphoric acid, at 150 ^oC.

RESULTS AND DISCUSSION

The use of the four separation systems gave the best results only after a previous TLC separation of the polar lipids from the neutral lipids. As a separation system the mixture petroleum ether – ethyl ether – acetate (10:10:0.1, v/v) was used.

The results show that a higher proportion of chloroform in a mixture of solvents led to a lower value for R_f, for almost all polar lipids. The R_f values obtained for the analysed samples (*Table 2*) are closer to those mentioned in the literature (*Table 1*) for the C solvents system. The method has a high repeatability.

CONCLUSIONS

1. The TLC separation of polar lipids is a rapid method for their qualitative analysis, allowing simultaneous performance of an accurate quantitative analysis, using densitometry.

2. The analysed Turcana sheep muscular tissue contains a high proportion of neutral lipids, emphasized by the necessity to separate them using a mixture of solvents with low polarity, in order to permit a clear separation of the remained polar lipids.

3. The best solvents system for qualitative separation of the polar lipids seems to be in our case (Turcana sheep muscular tissue) the C system.

REFERENCES

Bratt, C. E.-Akerluna, M. E. (1994): Journal of Plant Physiology. 32. 2. 313-315.

Folch, I.-Lees, M.-Sloane-Stanley, G. A. (1957): Journal of Biology and Chemistry. 24. 1. 226, 497.

Hamilton, R. J.-Rossel, J. B. (1986): Analysis of oils and fats, London: Elsevier Applied Science Publishers. 22-50.

Heape, A. M.-Jaquelin, M.-Boiron, F.-Cassagne, C. (1985): Journal of Chromatography. 49. 3. 322, 391-395.

Vittelo, F.-Zanetta, I. P. (1978): Journal of Chromatography. 42. 1. 166, 637.

Table 1: **R_f for the main polar lipids**

Polar lipids	Solvents system			
	A	**B**	**C**	**D**
SF	0,08	0,22	0,09	0,07
PC	0,10	0,28	0,11	0,09
PA	0,32	0,52	0,40	0,38
PG	0,38	0,37	0,45	0,43
PE	0,42	0,45	0,51	0,50

R_f = resolution factor; SF = sphingolipids; PC = phosphatidil-coline; PA = phosphatidil-acetilamine; PG = phosphatidil – glicerolamine; PE = phosphatidil – etanolamine

Table 2: **R_f for polar lipids from analysed Turcana sheep muscular tissue (average values)**

Sample	Polar lipids	Solvents system			
		A	**B**	**C**	**D**
1	SF	0,11	0,16	0,09	0,10
	PC	0,14	0,28	0,11	0,15
	PA	0,40	0,54	0,38	0,28
	PG	0,54	0,40	0,44	0,54
	PE	0,59	0,48	0,50	0,59
2	SF	0,11	0,16	0,09	0,10
	PC	0,14	0,28	0,11	0,15
	PA	0,40	0,54	0,38	0,28
	PG	0,54	0,40	0,44	0,54
	PE	0,59	0,48	0,50	0,58
3	SF	0,11	0,16	0,09	0,10
	PC	0,17	0,28	0,11	0,15
	PA	0,40	0,54	0,38	0,28
	PG	0,54	0,40	0,45	0,54
	PE	0,59	0,48	0,50	0,59
4	SF	0,11	0,16	0,09	0,10
	PC	0,14	0,27	0,11	0,13
	PA	0,41	0,54	0,38	0,29
	PG	0,54	0,40	0,44	0,54
	PE	0,58	0,47	0,50	0,57
5	SF	0,12	0,16	0,09	0,10
	PC	0,14	0,28	0,11	0,13
	PA	0,40	0,54	0,38	0,30
	PG	0,54	0,40	0,44	0,54
	PE	0,59	0,48	0,50	0,59