



HPTLC as the choice tool for quality control in Chilean aquaculture industry

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International Symposium for High Performance
Thin Layer Chromatography

Salmon Production in Chile

Salmon was introduced into Chile in the early twentieth century.

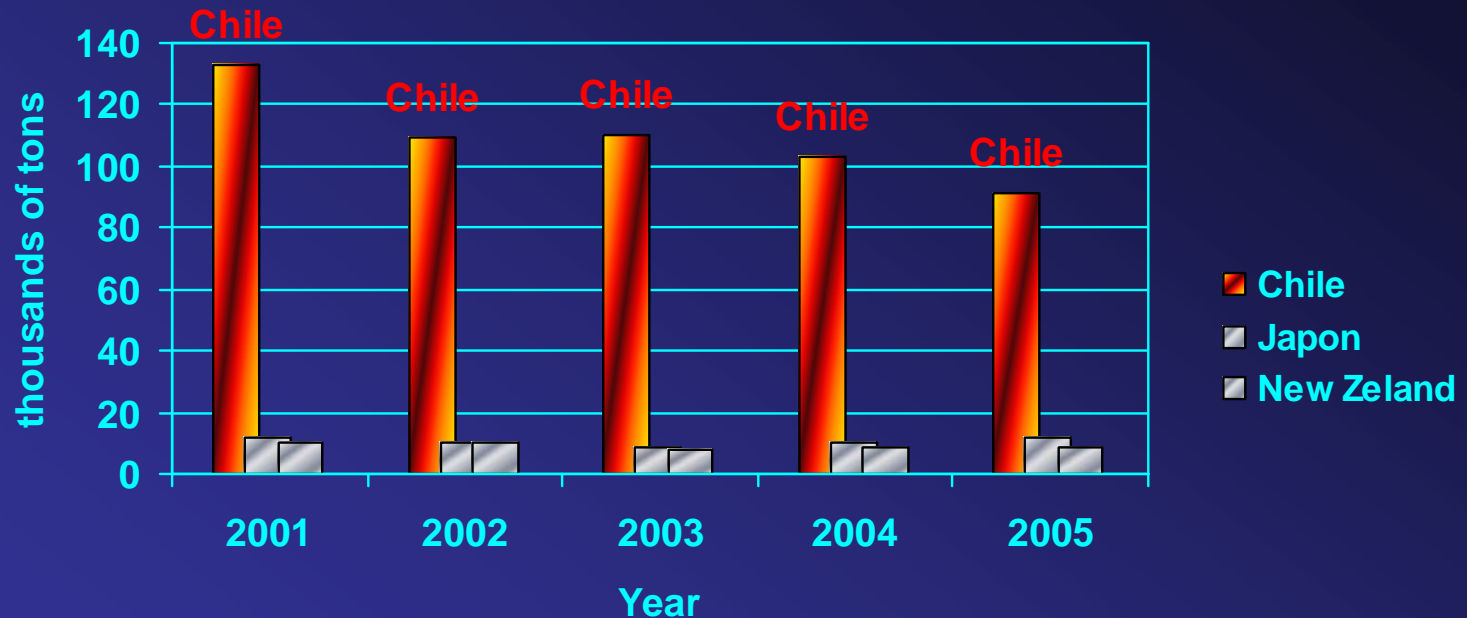
Since 80s it began to be farmed on a significant scale

The industry enjoys a number of natural advantages – like: Chile's cold uncontaminated sea - and the country's 4,300 kilometers of coastline mean there is plenty of physical room for growth.



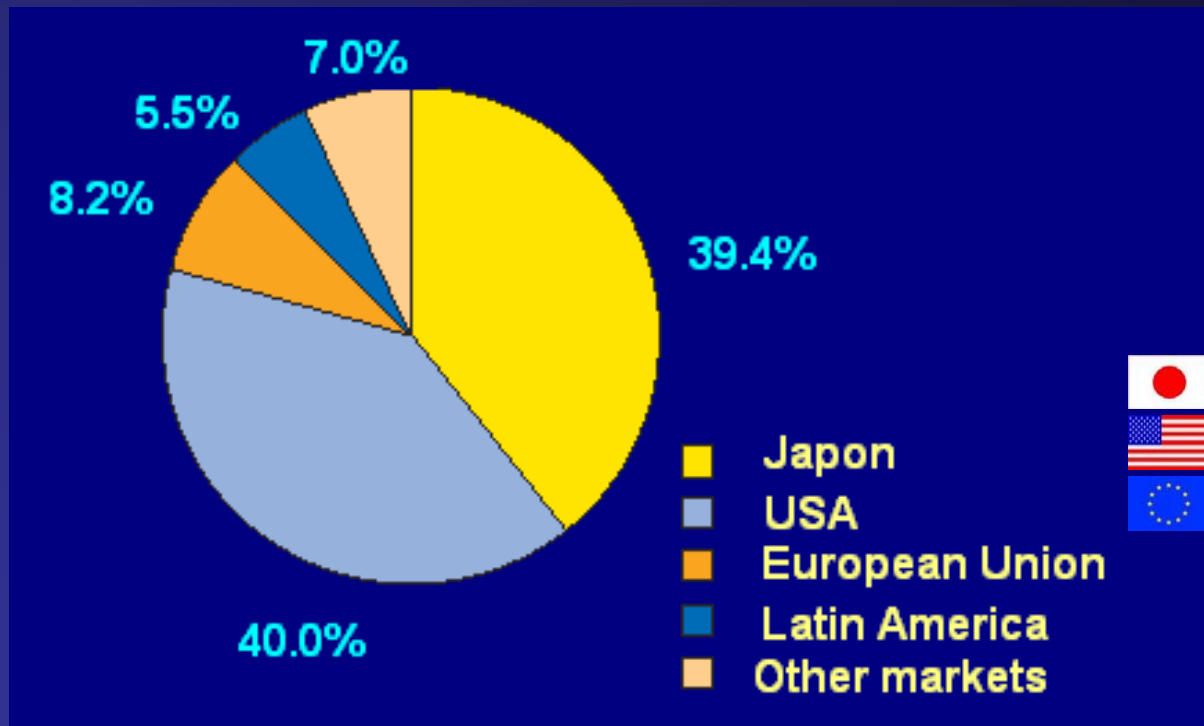
Salmon Production in Chile

Salmon coho Culture



Chile has become in the first Salmon Coho world producer and the second in Atlantic Salmon, representing 5.1% of Chilean exportations.

Chilean salmon markets



The main Chilean salmon markets are U.S. followed by Japan with a 39.4%

Salmon feeding

Fish feed is the vehicle not only for nutrients but also for pigments and medicines, this broad spectrum of analyze and the high throughput of samples required to cope with quality control parameters, makes the HPTLC as the choice tool for quality control of fish feed for salmon aquaculture industry



Composition of salmon feed



Nutrients



Antibiotics



Pigments



**Evaluation of the nutritional quality
in salmon feed.**

Torres del Paine, South of Chile

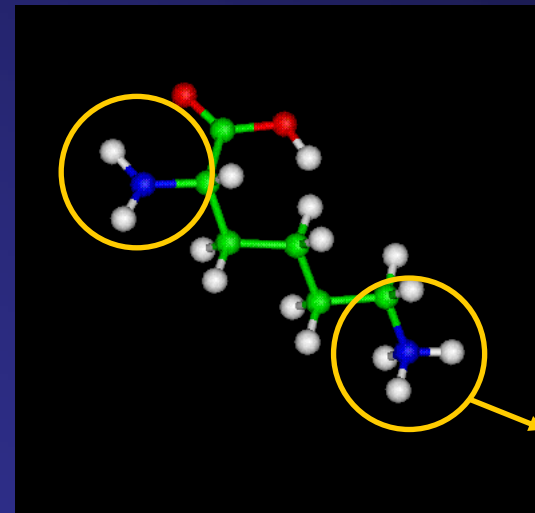
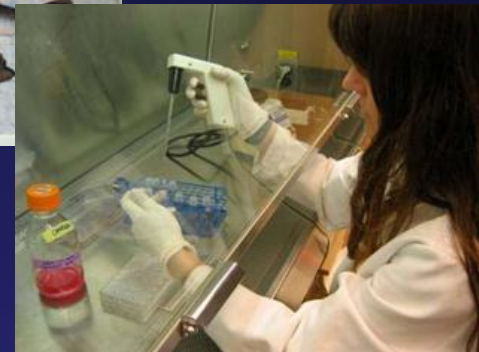
Determination of Available Lysine

Salmons need a requirement of 10 essential amino acids at list. One of them is Lysine.

A lysine deficit, it is related with a high alevins mortality

Under extreme conditions (extrusion process) the $-NH_2$ groups of lysine join carbonyl groups of other molecules, generate non digestible products.

To know the quantity of available lysine, gives us an estimation of the protein quality.



NH_2
group

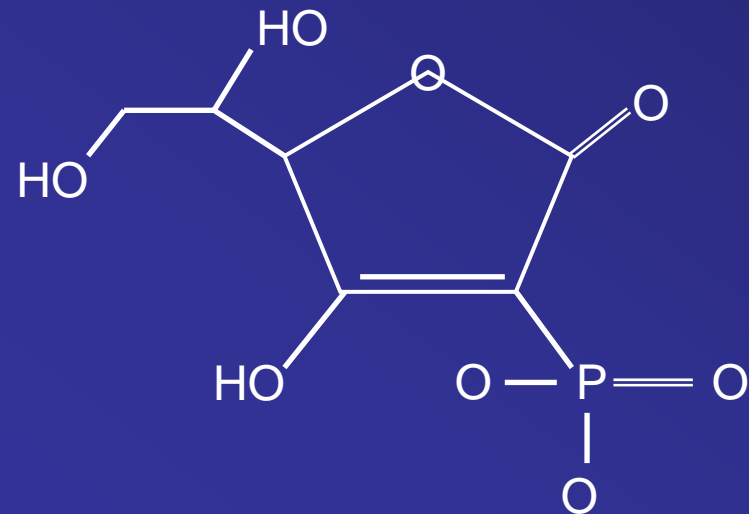
Determination of Acid L-ascobic-2-monophosphate



Salmons are one of the species that need Ascorbic acid as vitamin. Vitamin C.

A deficit of this vitamin produces symptoms such as alterations in bones and immune system.

The extreme conditions during extrusion process can reduce the level of vitamin C in salmon feed, for this reason is administrated as Vitamin C monophosphate



Methodology

Lysine



+

fluoro-2,4-
dinitrobenzene

100 mg defatted sample

Incubation 2 hrs 37°C

Hydrolysis with HCl 8N
24 hours at 100°C

Filtration and complete
to volume 50 ml with
methanol



available lysine

Vit C P04



+ 50 ml Methanol +
water 70 + 30 v/v

5 -10 g

Clean up
Petroleum ether

10 ml extract

+ 25 ml oxalic acid
+ 2.5 ml 2,6-DCPIP

20 ml

+

4 ml 2,4-DNPH

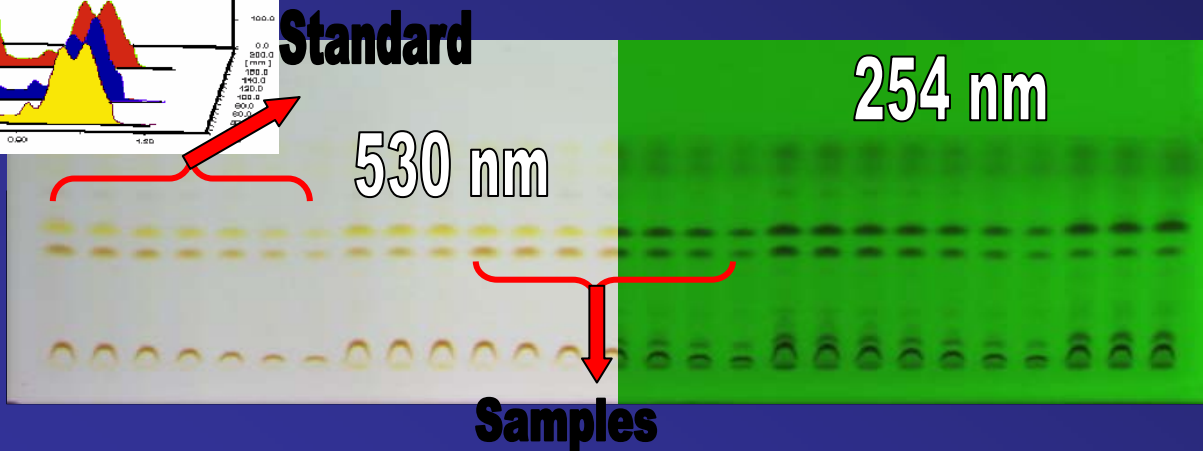
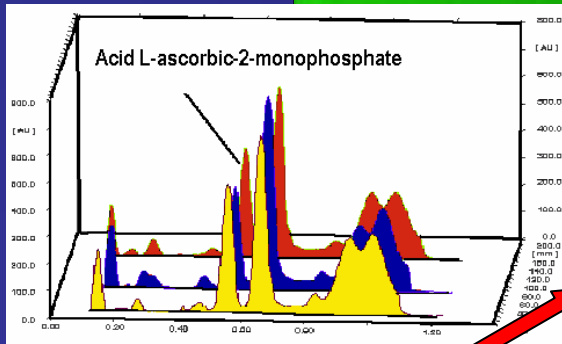
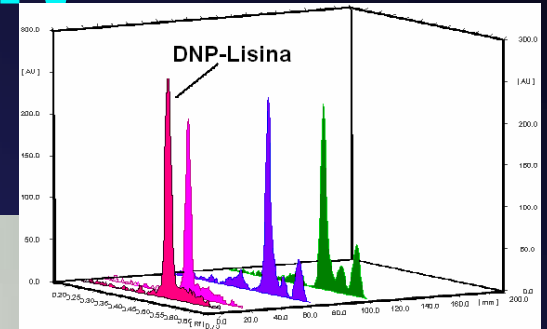
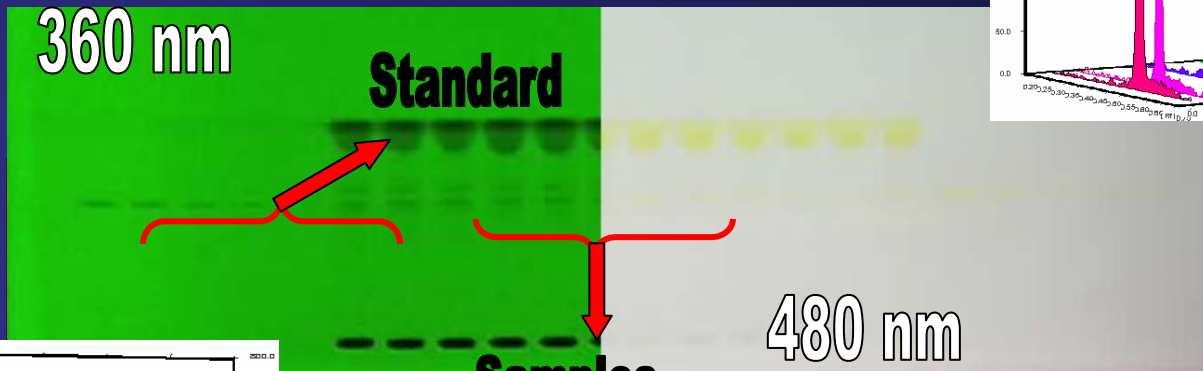
Incubation

(ozazone)
Colored derivate



Chromatography

Lysine



Marmol cathedral, South of Chile

**Antibiotics and antibacterial content in
salmon feed**



Salmon Illness

ILLNESS/ CONDITION	Host	Antibiotics for Treatments
E.R.M. Enteric Red Mouth disease	<i>S. salar</i>	Oxolinic acid, oxytetracycline.
Pseudomonas/ Aeromonas Septicemia	<i>S. salar</i>	Prevention/treatment: Oxytetracycline, Sulphas, Flumequine, Oxolinic acid.
Cold Water Disease	<i>S. salar</i>	Prevention/treatment: Oxytetracycline, Flumequine, Enrofloxacin,
SRS/UA Salmon Rickettsial syndrome	<i>S. salar</i>	Oxytetracycline, Oxolinic acid, Flumequine, (Enrofloxacin, Danofloxacin)
BKD Bacterial Kidney Disease	<i>S. salar</i> <i>S. trutta</i>	Erythromycin, Sulphas, Oxytetracycline.

Extraction



5 g sample

+

50 ml ACN + KCl/KOH
Nalidixic acid 0.2 mg/ml.
Internal Standard

Flumequine
Oxolinic acid



2-5 g sample

+

50 ml Methanol + HCl
(15%) 90 + 10 v/v

Oxytetracycline



5 g sample

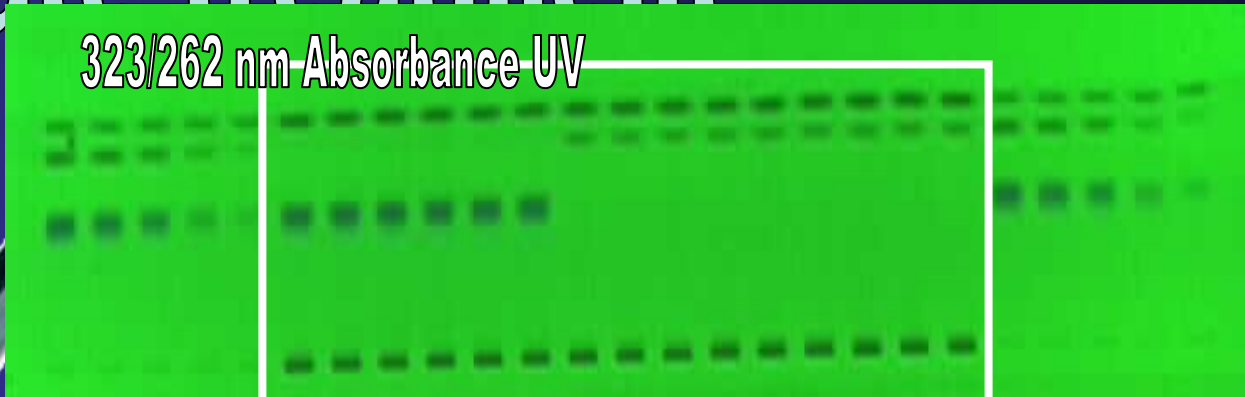
+

50 ml Methanol

Florfenicol

Chromatography

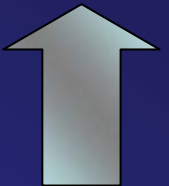
Plate treatment



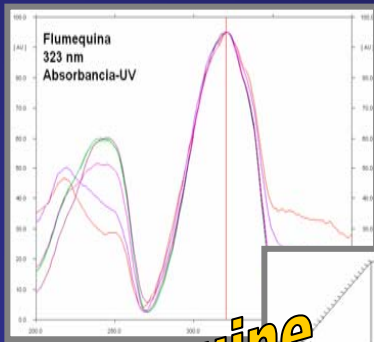
Samples



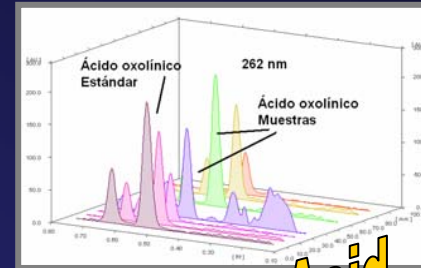
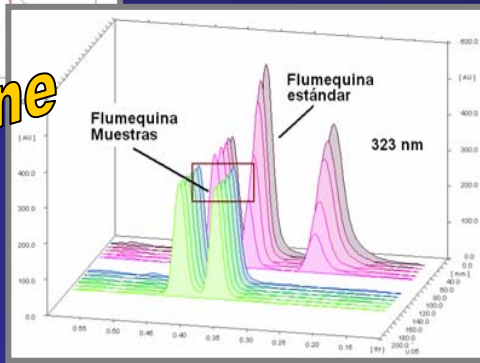
Samples



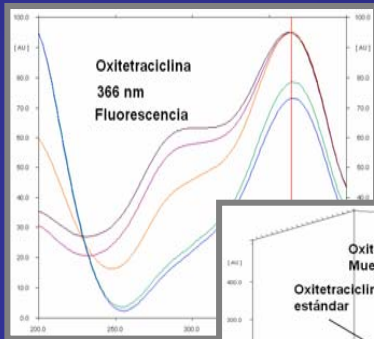
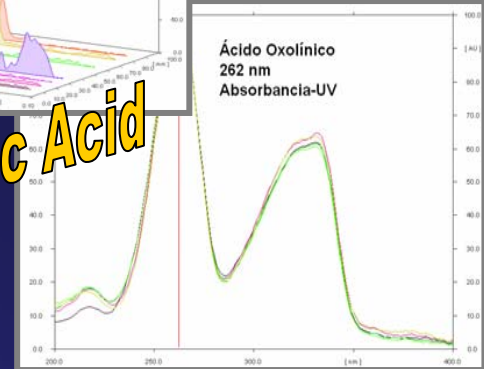
Chromatography



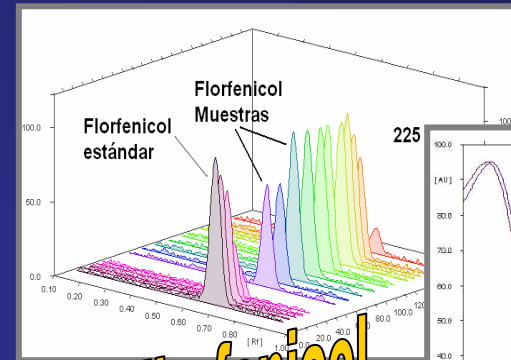
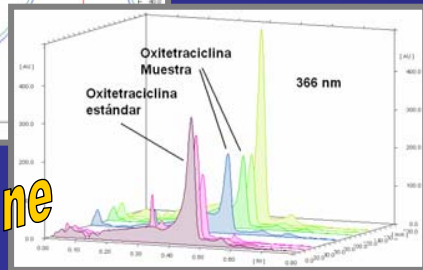
Flumequine



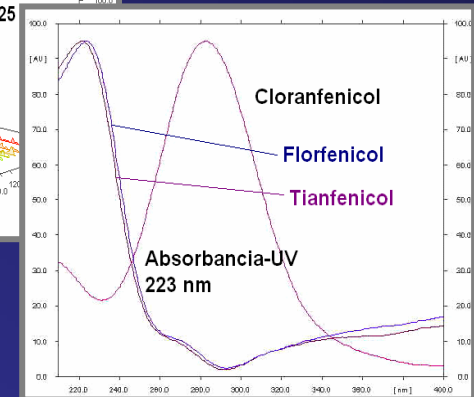
Oxolinic Acid



Oxytetracycline



Florfenicol





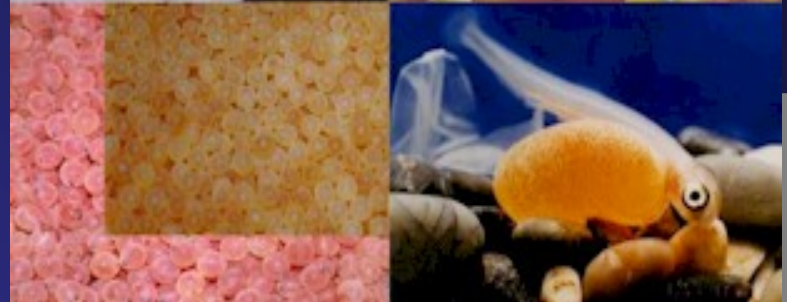
Salmon Color

Channels of Patagonia

Astaxanthin

Salmonids are a group of fishes able to keep carotenoid pigments in the skin and muscle.

Astaxanthin is a type of carotenoid administered through the feed. This coloration is a plus for different markets. By this reason it is important to control it before it is administered to the salmonids.



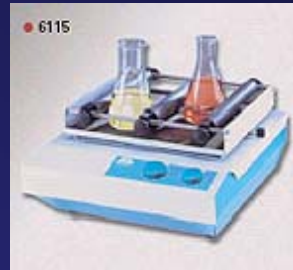
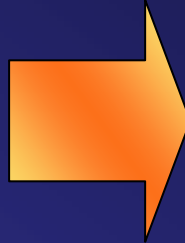
Extraction



2 g sample

+

Chloroform + acetone
8+2 v/v

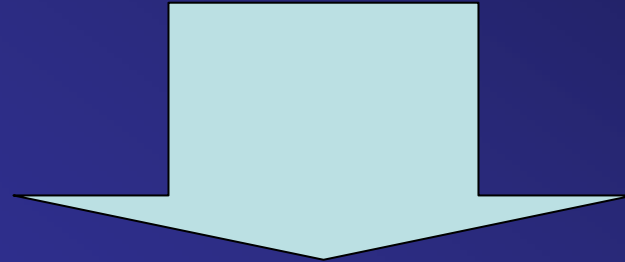
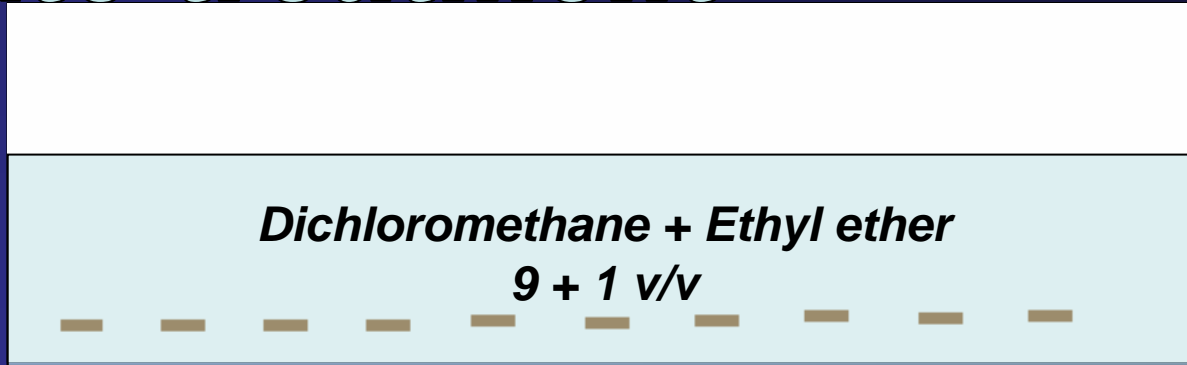


30 minutes



Chromatography

Plate treatment





Astaxanthin
Astaxanthin-Cis 13
Astaxanthin-Cis 9





Muchas Gracias