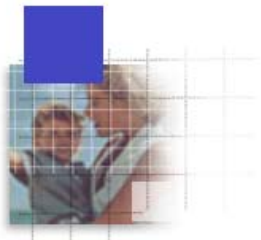


BENEFITS OF HPTLC IN ACTIVE PHARMACEUTICAL INGREDIENT MANUFACTURING SUPPORT

BY L. VICARD FROM AVENTIS PHARMA

Presentation of LAPS :
Site production support Laboratory

Objectives of LAPS

- Processes improvement,
- Quality improvement,
- Production workshops Support,
- Analytical standards preparation,
- In-process controls monitoring and implementation,
- Hygiene and environment monitoring on Water, Air and Atmosphere

Two examples of how HPTLC can help in manufacturing support

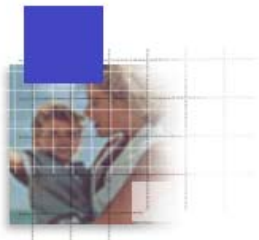
1. *Process monitoring of an esterification reaction*
2. *Determination of antibiotic in waste water after biological treatment.*

rocess monitoring of an esterification reaction

SCOPE: *To optimise, quality, yield, cycle time and reactants ratio, of an esterification reaction between an acid chloride with an excess of alcohol.*

ISSUES: *The reaction product is a complex heterogeneous compound with a molecular weight of about 500.*
The manufacturing cycle time is very long.
The acid chloride is not stable.

SOLUTION: *To find a method to follow the different compounds during the reaction.*



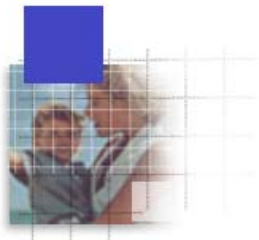
rocess monitoring of an esterification reaction

All three components of the mixture: remaining acid, alcohol and final product were to be determined simultaneously.

A special emphasis had to be put on the accuracy of the main component : the ester.

For all these reasons, TLC is considered as the best method due to it's rapidity, reliability and cost efficiency.

rocess monitoring of an esterification reaction



To avoid reaction between the reactants (acid and alcohol) onto the plate, we chose a CN stationary phase.

Samples were taken directly from the reaction mixture and the reaction was stopped by dilution.

The chromatography was perform using an auto-sampler, an automatic developing chamber (ADC) and densitometric evaluation.

OPERATING CONDITIONS

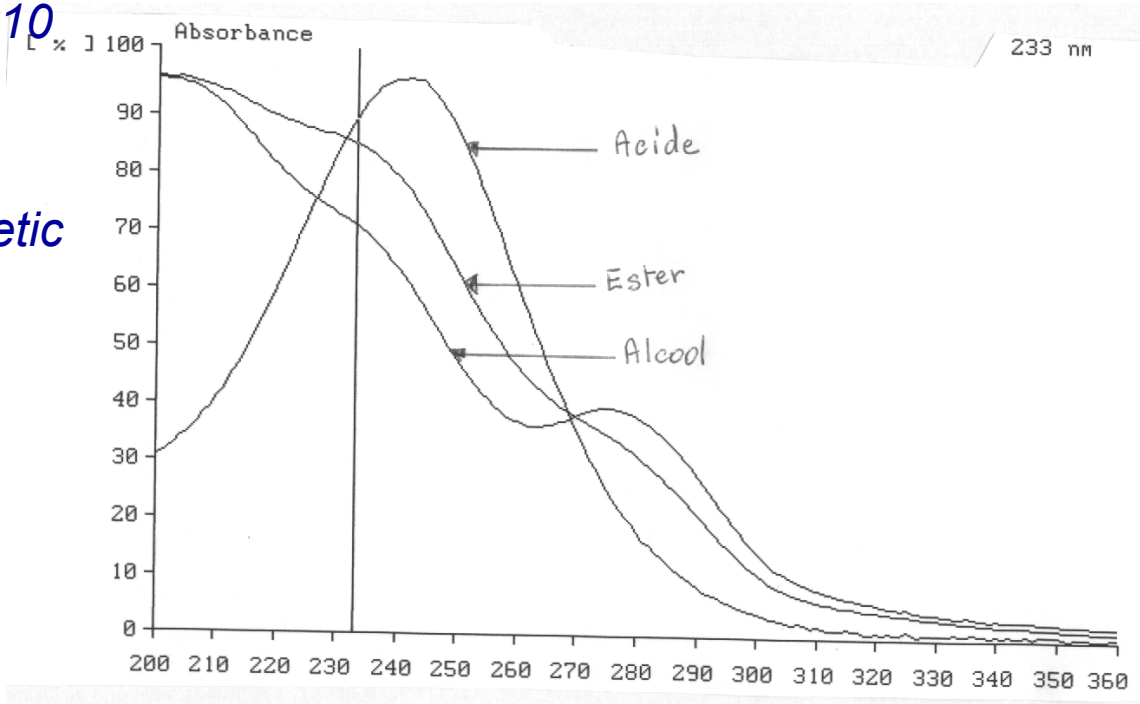
Plate *:*
HPTLC Merck CN F254s 20x10

Mobile phase *:*
No preconditioning
Cyclohexan/Ethyl acetate/Acetic acid 80:20:0.2

Developing distance *:*
40 mm

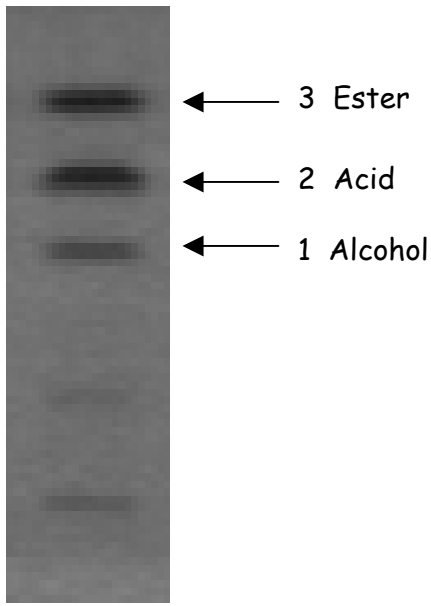
Absorption measurement *:*
UV 233 nm

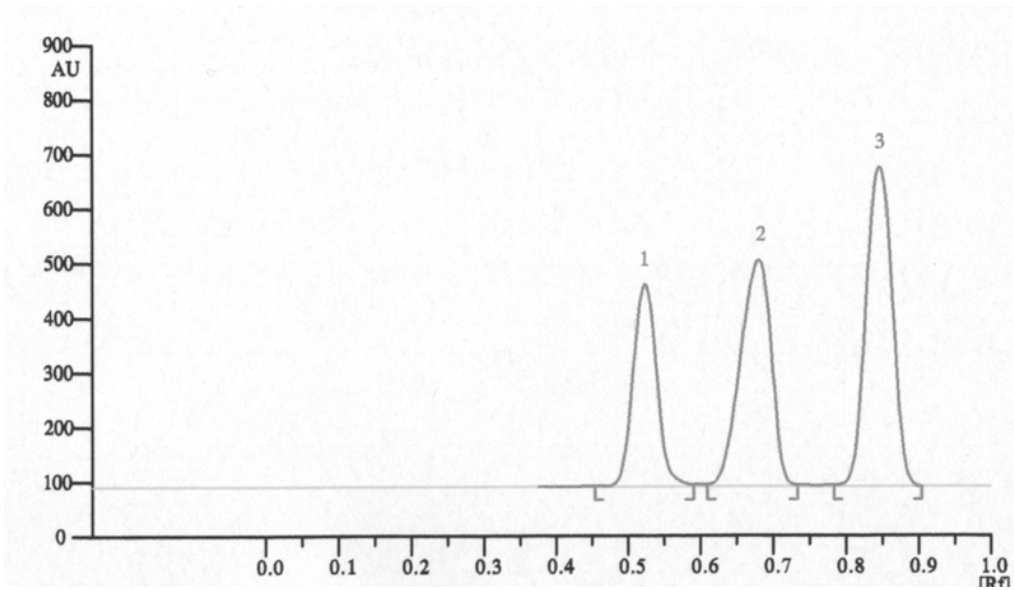
Time for development *:*
30 min including drying of the plate



Spectra of components

OPERATING CONDITIONS

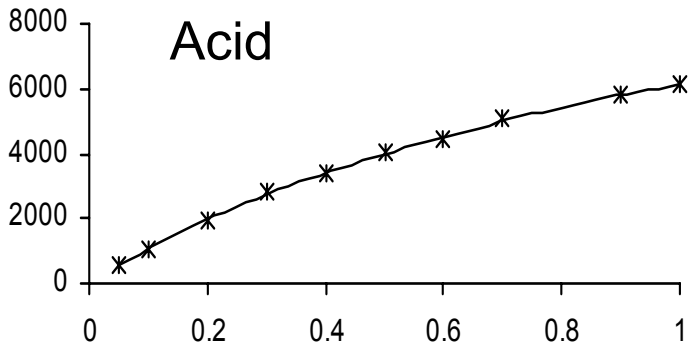


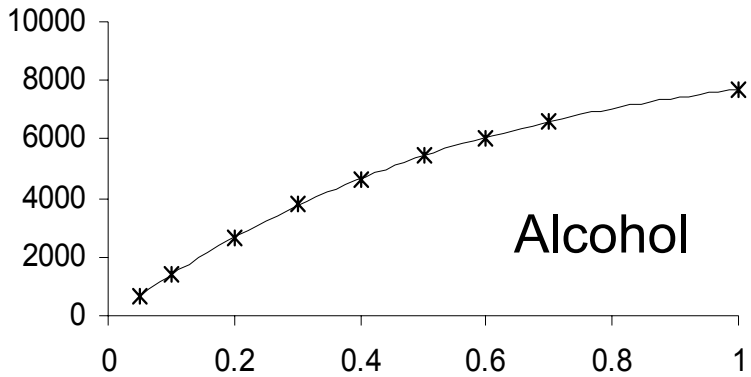


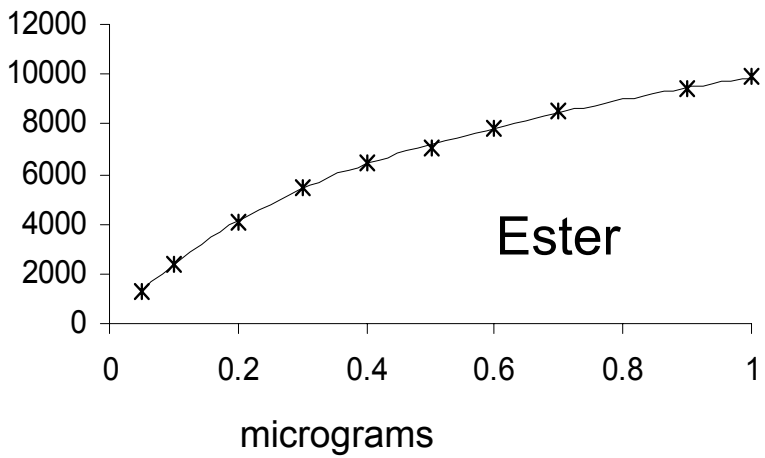
Picture taken at 254 nm

Densitogram at 233 nm

Calibration curves based on peak area







Calibration curve, with a polynomial regression, using both peak area and peak height (peak height gives better precision).

Linearity is satisfactory in the range of 50 to 250 ng (absolute).

CV (n=5) for Ester is between 1.5 and 3.2

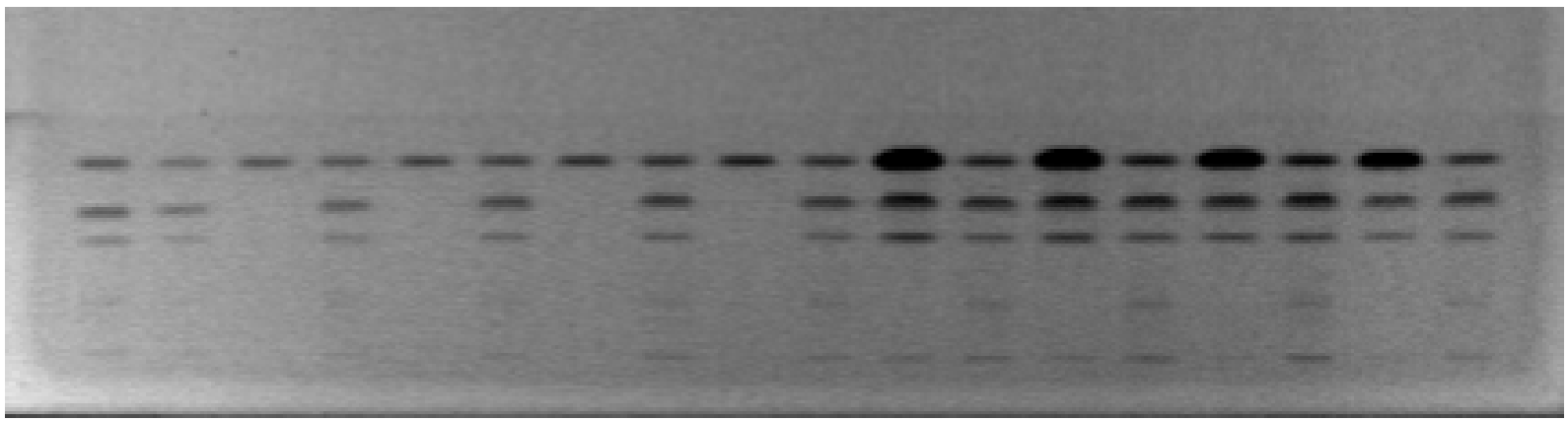
Acid is < 10
Alcohol below 5

ROUTINE ASSAY

Standards *: Solution at 0.01 g % ml of each component (alcohol, acid, ester) in CH_2Cl_2*
Application : 500 nl, 700 nl, 1000 nl, 1200 nl, 1500 nl, 2000 nl, 2500 nl

Evaluation for alcohol and acid *: Solution at 1.25 ml % ml of reaction mixture in CH_2Cl_2*
Application : 1000 nl, 1100 nl, 1200 nl, 1400 nl

Evaluation for ester *: Solution at 0.05 ml % ml of reaction mixture in CH_2Cl_2*
Application : 1000 nl, 1200 nl, 1500 nl, 2000 nl



Standards
2500 1500
Samples for Ester *1000 1200 1500 2000*
Samples for Alcohol and Acid *1400 1200*
1100 1000

CONCLUSION

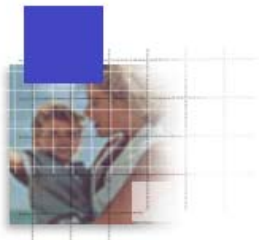
The most important information is the assay of the produced ester : it is a measure for the yield.

The acid content gives information about the stability of the acid chloride under reaction conditions.

The alcohol assay describes the purity of the alcohol used in excess as a reactant.

The method has proven to be reliable and suitable for monitoring the reaction.

Determination of antibiotic in waste water after biological treatment.



SCOPE *: Following the authorization of a new antibiotic production, the Authorities ask for the follow-up of this product in the effluents discharged after biological treatment.*

ISSUES *: The maximum allowed quantities are of the order of ppm and controls are to be carried out over one week of discharge once per quarter.*

SOLUTION *: Two kinds of methods are available*

1- Enrichment by extraction.

2- Chromatography.

Determination of antibiotic in waste water after biological treatment.

1- Extraction of enriched water with 50 mg/l of antibiotic :
- by various solvents,
- solid/liquid on filter cartridge

→ Insufficient extraction yield

2- Chromatography : TLC was chose because it is faster than HPLC and more robust against matrix.

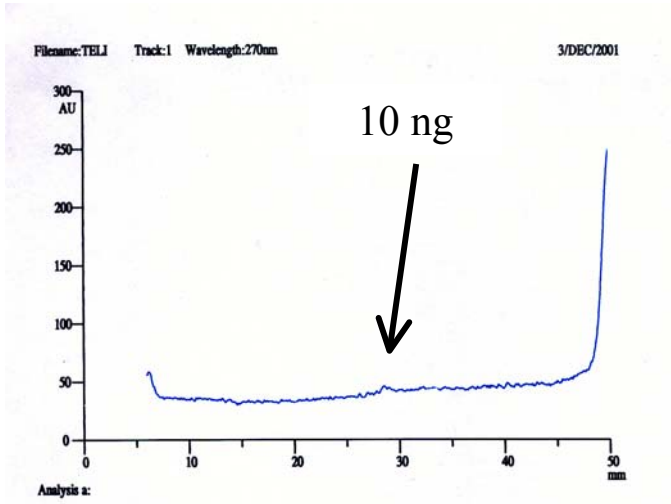
THIN LAYER CHROMATOGRAPHY

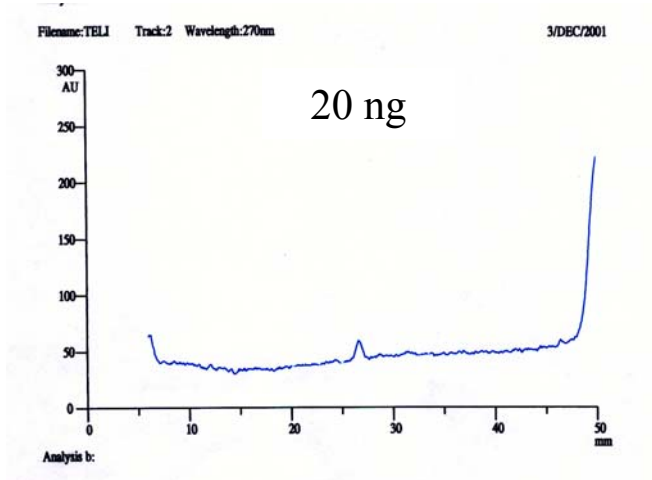
From the quality control procedure, lightly adapted :

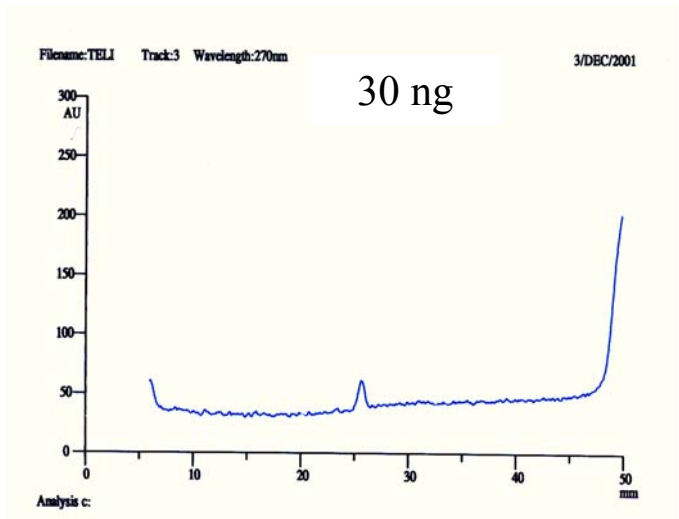
1 - Determination of the limit of detection and quantification

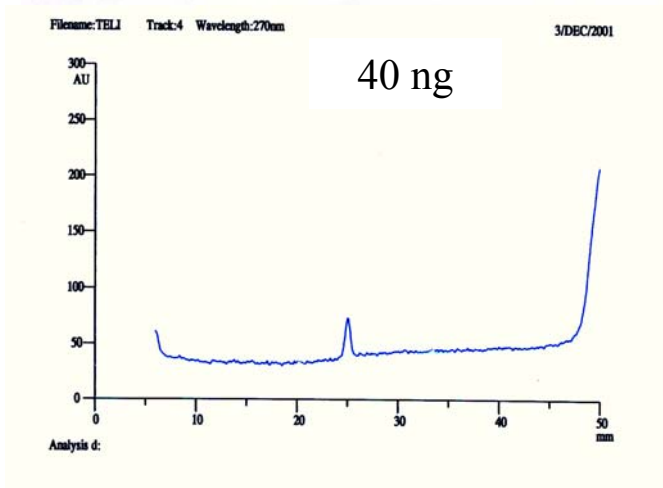
Conditions selected :

- *Standard solution of antibiotic at 0,04 g/l in CHCl3*
- *Application from 250 nl to 1500 nl → 0.01 µg to 0.05 µg*
- *Plate : silica gel HPTLC F254 20X10*
- *Mobile phase : CH_2Cl_2/MeOH/ NH_4OH 90/10/1*
- *Development in automated chamber on 50 mm (in 20 min)*
- *Absorption measurement : UV 270 nm*









Limit of detection is 10 ng and limit of quantification is 20 ng

THIN LAYER CHROMATOGRAPHY

2 - Water analysis :

Standard, 2 points calibration

Application : 1250 nl and 2500 nl of

0.04 g/l of antibiotic in $CHCl_3$

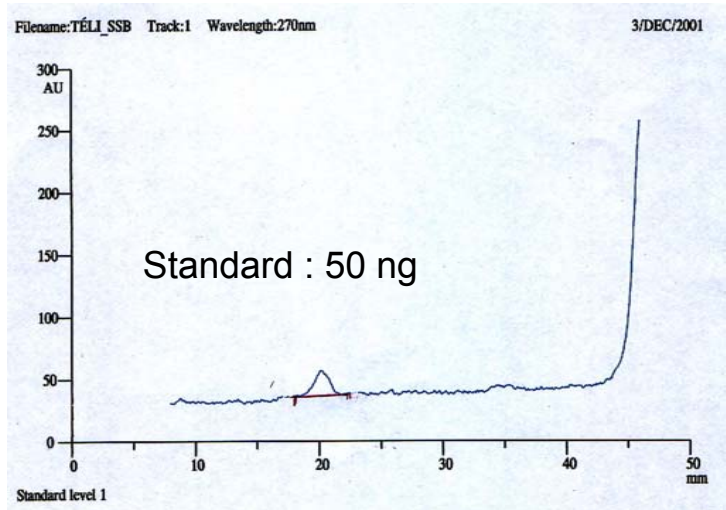
Analysis

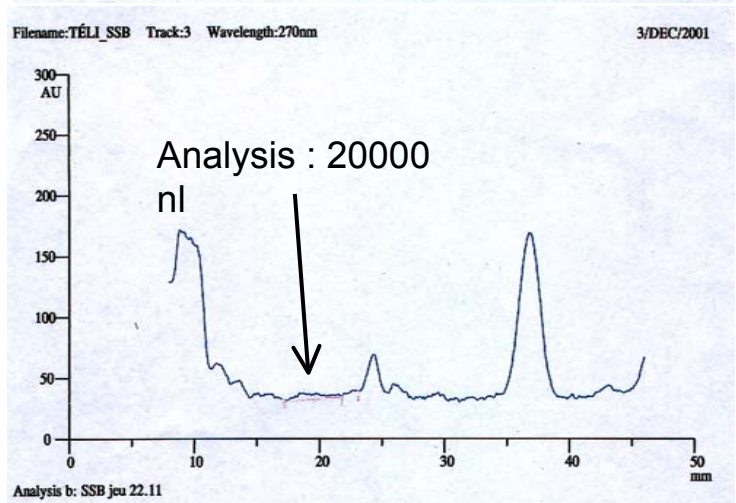
Application : 20 µl just as it is

Bandwidth : 5 mm

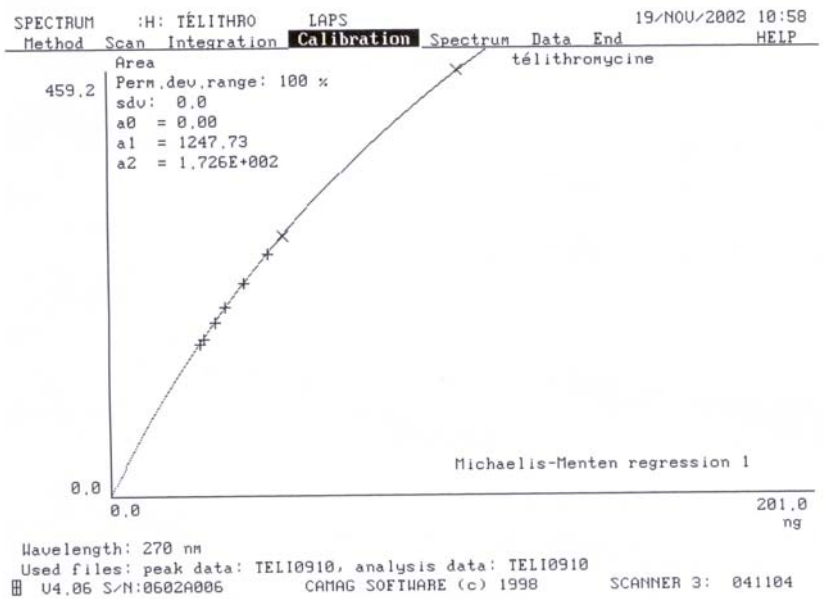
Application rate : 200 nl/s

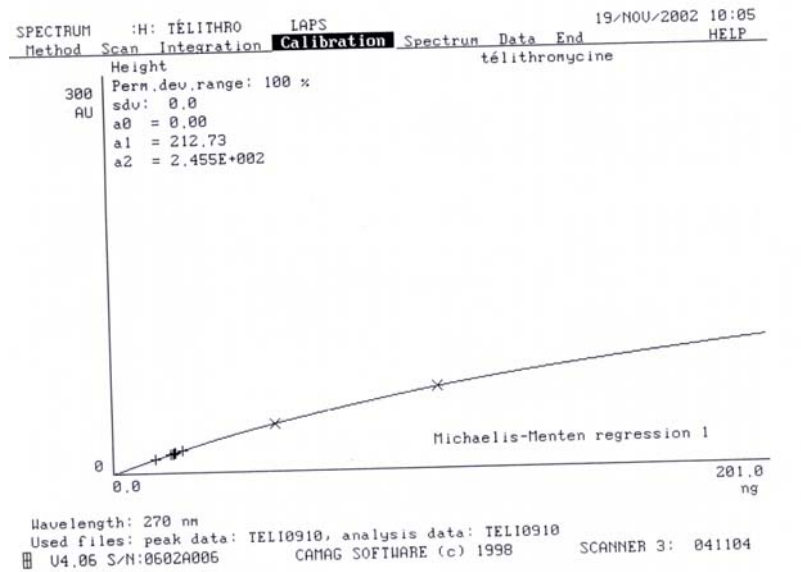
The standards represents 2.5 and 5 ppm





THIN LAYER CHROMATOGRAPHY





CONCLUSION

With TLC, sample preparation can be eliminated and the waste water applied directly onto the plate .

Cross contamination is avoided because the plate is used only once.

This analysis is routinely performed on a quarterly basis and we can control that the presence of the antibiotic is less than 2.5 ppm in the rejected water .