Assay of Amino-propanol in dermatological products.

HPTLC Method.

International Symposium for HPTLC – Berlin – October 2006

Caroline PETITTI – Bayer Santé Familiale - ITC

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I – Bayer Group and Bayer Santé Familiale : quick overview

II - Aminopropanol HPTLC Method

III - Validation of the method

IV - Method comparisons

V - Occurred problems

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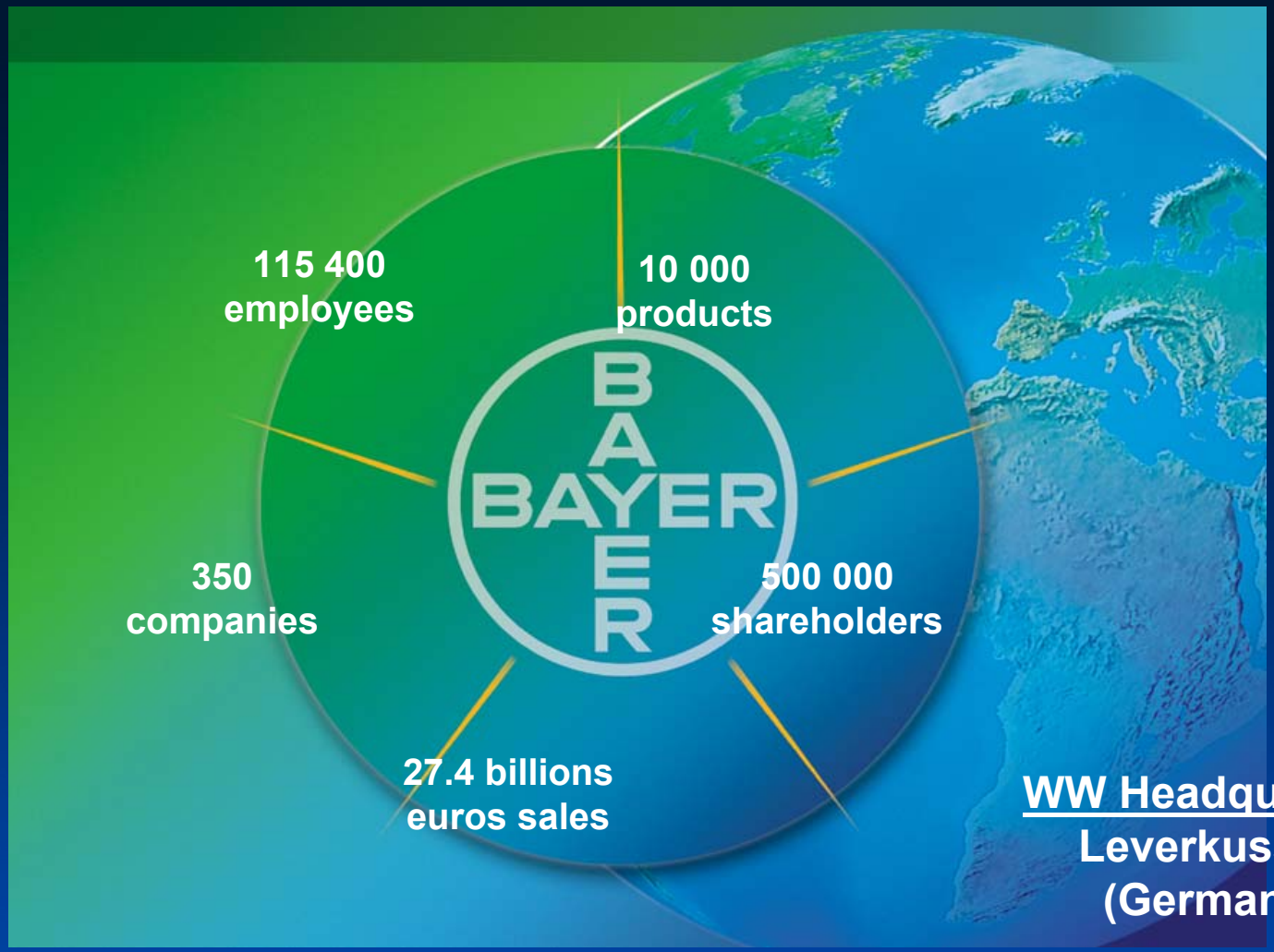

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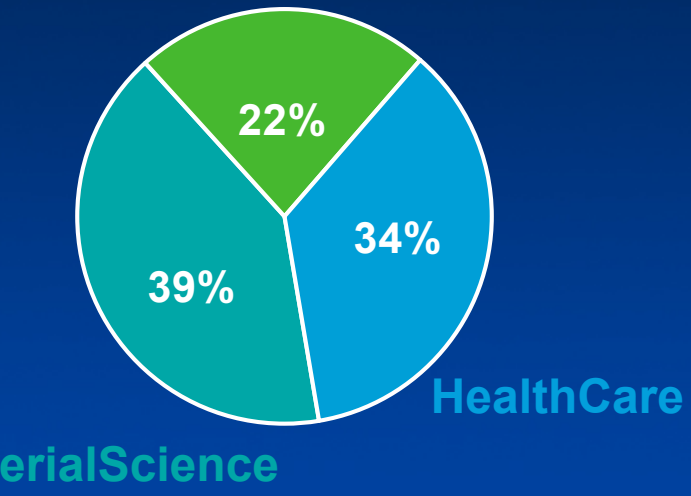

HealthCare

9,4 billions €
33 800 employees

CropScience

5,9 billions €
18 800 employees

MaterialScience

10,7 billions €
18 800 employees

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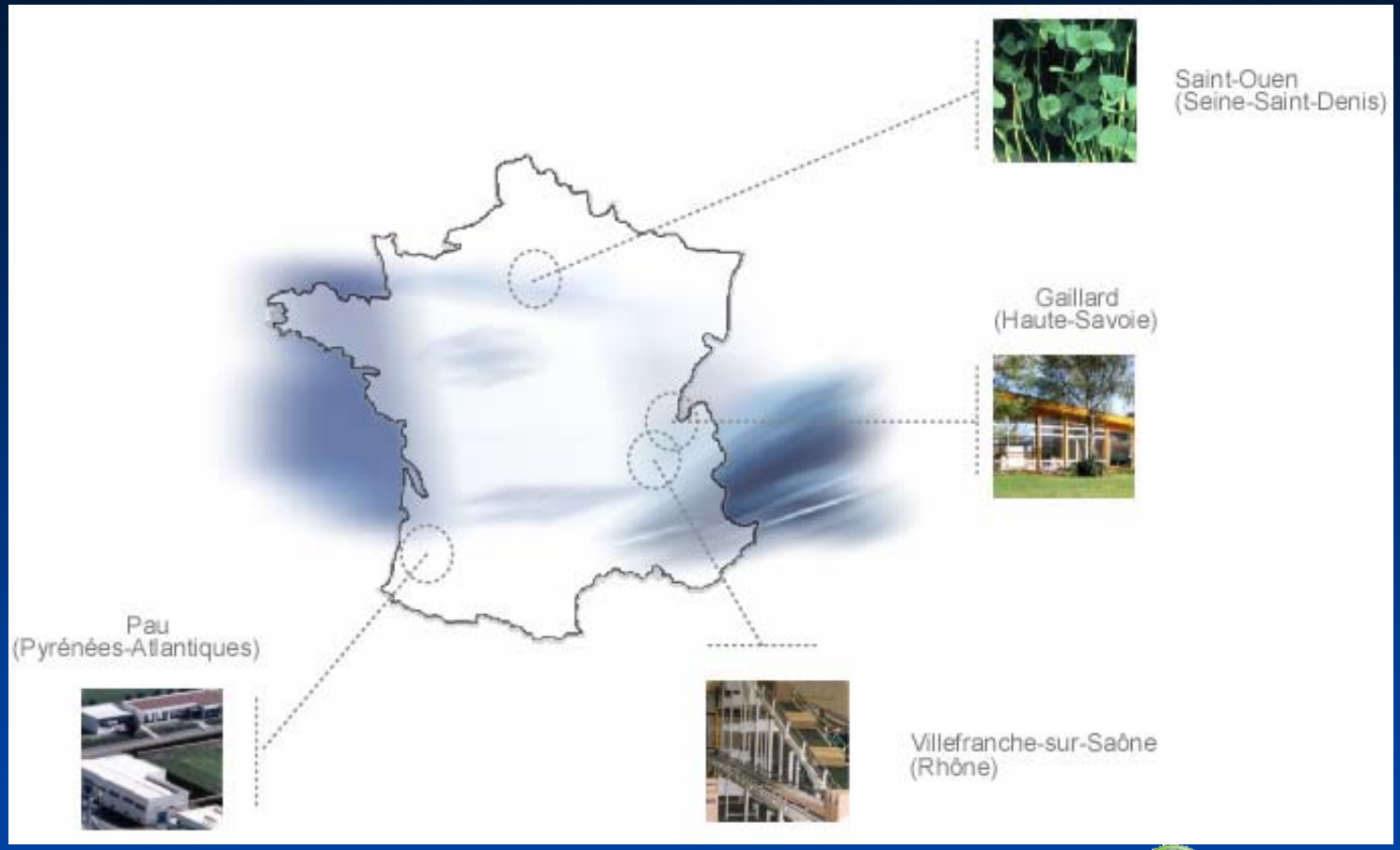
Geographic location :



Bayer Santé Familiale

- Bayer Health Care – Consumer Care (2005)
- Approx. 700 employees
- Gaillard : General Administration, Production unit , International Technical Center (ITC)
  - formulation, scale-up
  - packaging development
  - regulatory affairs group
  - analytical development

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Our OTC Products

.***Analgesic / cough and cold***:

Bayer Aspirine ®, Aleve®, Aspro®, Alka Seltzer®, Actron®, Claradol ®

.***Multivitamins and dietary supplement:***

Supradyn®, Berocca®, One-a-day®, Elevit®, Redoxon®, CalDVita®, Vital 50 plus®

.***Dermatological & topical products :***

Bepanthen®, Canesten®, Dermaspray®, Biseptine®, Bepanthol® , Gyn Hydralin®

.***Gastro-intestinal products*** *:*

Rennie®, Talcid®, Transipeg®

.***Phytotherapy*** *: Euphytose®*

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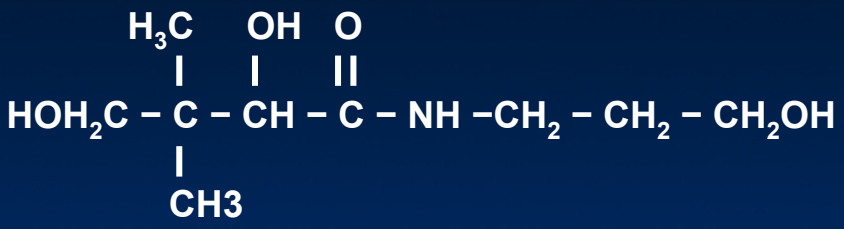
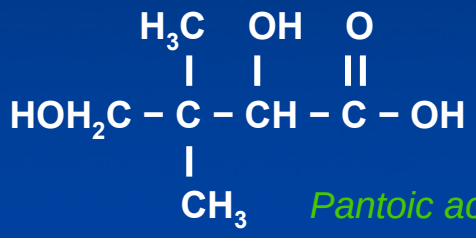
II. AMINO-PROPANOL ASSAY

Amino-3-propan-1-ol is the degradation product of dexpanthenol (pro-vitamin B5, active ingredient)



Dexpanthenol

Hydrolysis



Pantoic acid

Amino-3-propan-1-ol

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II. Aminopropanol dosage - Method

Why a HPTLC method ?

- Existing method : HPLC
- Sample preparation, mobile phase preparation : long and “boring” (included internal standard, fluorimetric detection…)
  - ⇨ Wining time by HPTLC

II. Aminopropanol dosage - Method

↳ SAMPLE PREPARATION

- Sample (2g) dissolved into 20,0 ml of ethanol.

-For ointment :

Heat in a water bath (70°c) until dissolution (5min)

-Centrifuge 10 min at 10 000 rpm

↳ STANDARDS PREPARATION

5 points range calibration :

1 concentrated solution , 5 dilutions : 12.5, 25, 50, 100, 200 µg/ml

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II. Aminopropanol dosage - Method

↳ Chromatographic conditions

- HPTLC plate Silica Gel 60F254

- Application volume : 2µL

- *Mobile Phase* : (80 V of ethanol / 15 V H2O / 5 V CH_3COOH) + 0.5% ninhydrine

- Development : 4 cm (approx. 30 minutes)

↳ Detection : Heat the plate at 105°C

- Rf value 0.5 (pink)

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II. Aminopropanol dosage - Method

- Quantification :

  CAMAG SCANNER III

Wavelength :

- 486 nm

Calculation :

- peak area

Calibration method :

- Michaelis Menten regression (2nd degree)

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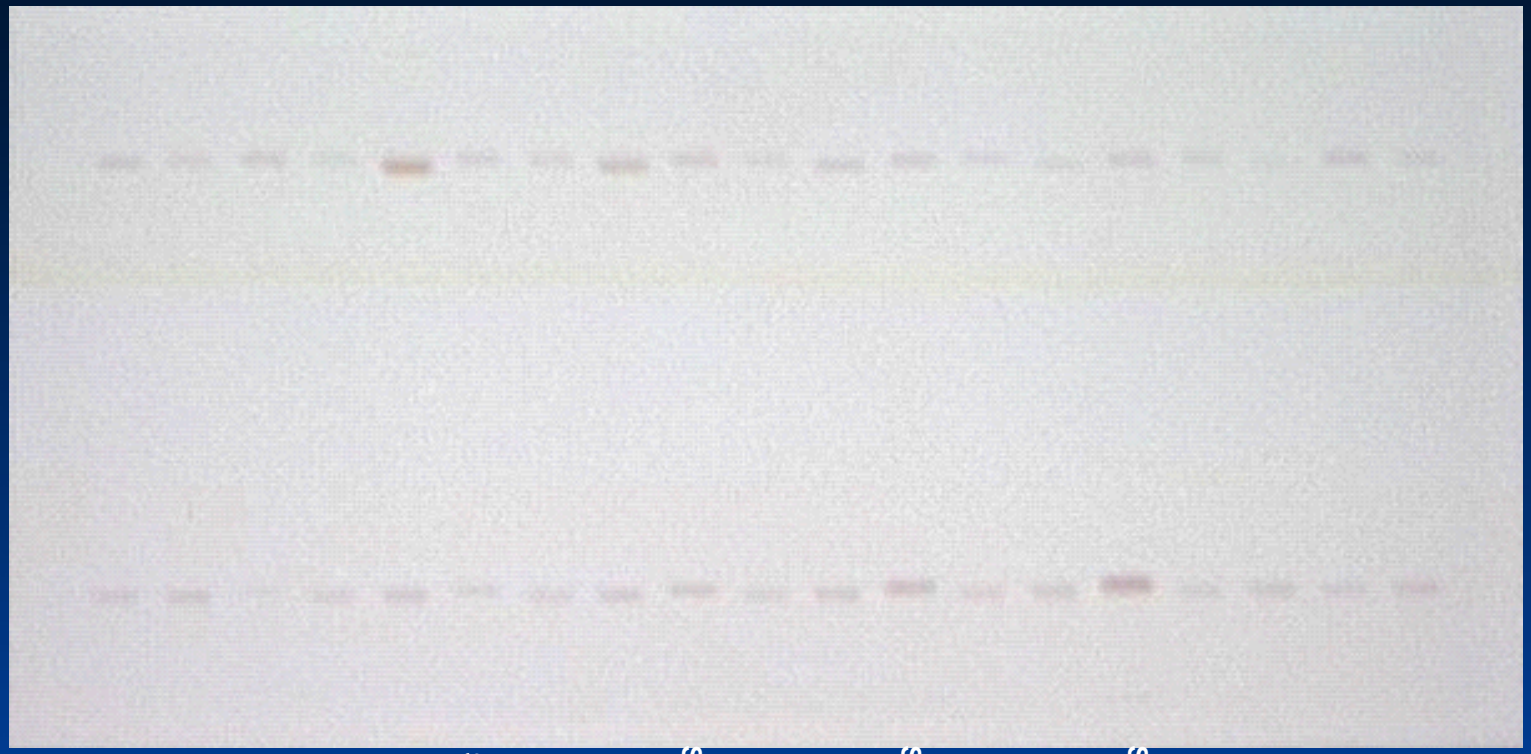
Plate example :



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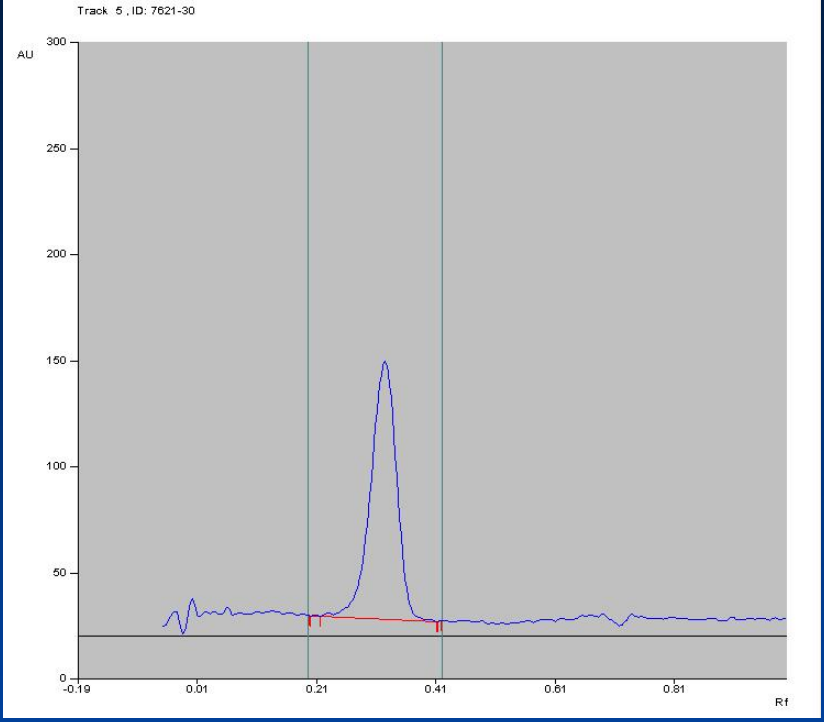
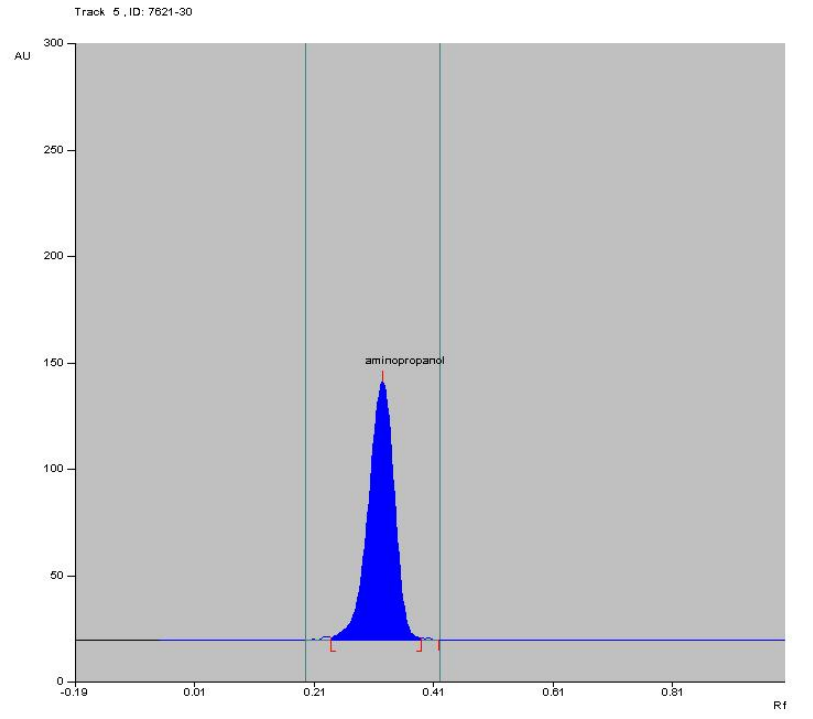
Peak example :

sample solution : ~50µg/ml (= 0.05% m/m ointment)





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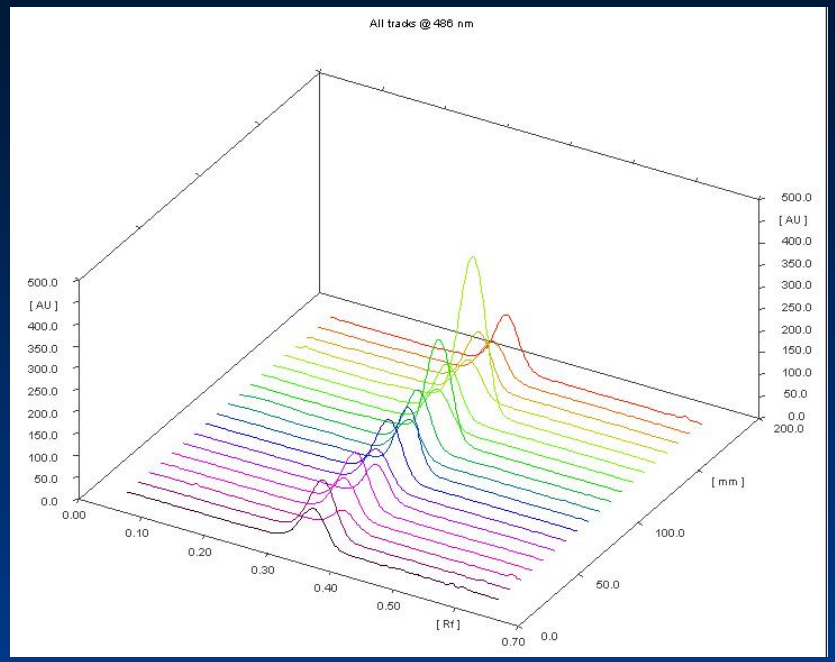
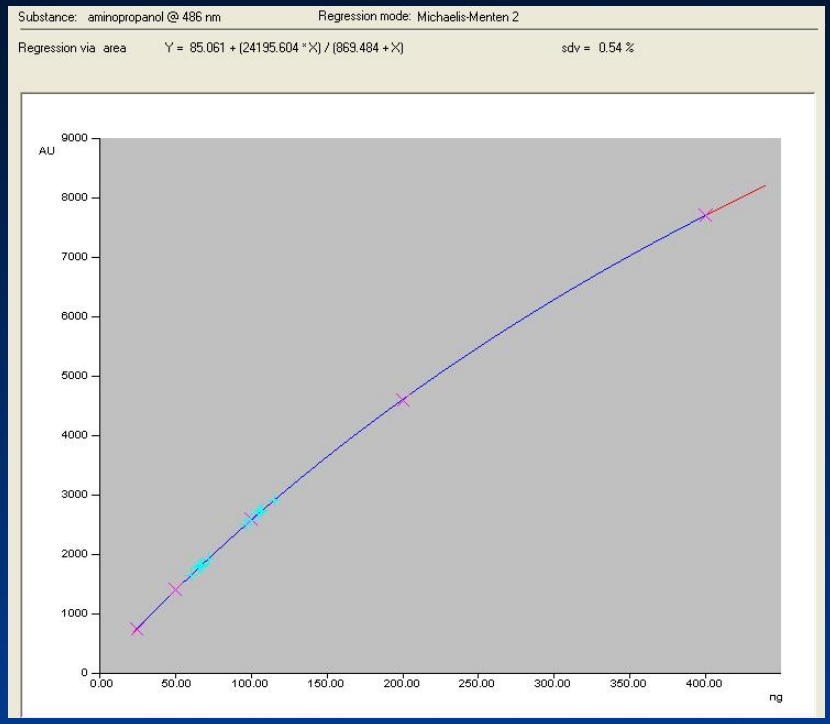
3D and calibration curve :





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III. Validation of the method :

III . 1) LOD / LOQ Determination

- Noise calculation : integrate all peaks from Rf=0.1 to Rf=0.9
- LOD = 3 x noise
- LOQ = 10 x noise
- Result : LOD = 4.5µg/ml (applied solution)
  corresponding to a 0.0045% (m/m) product

  LOQ = 15µg/ml (applied solution)
  corresponding to a 0.015% (m/m) product

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III. Validation of the method :

III . 2) Specificity

- According to the method described, prepare and apply an amino-propanol free reconstituted sample.

➔ No spot appears

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III. Validation of the method :

III . 3) Linearity / exactitude

- linearity range : 10 to 130 % of maximal authorized value in product.
- Preparation of a range in 5 points : placebo + added solutions
  - Each point : 3 preparations

Results :

Coefficient of correlation : 0.9979

Mean recovery : 102 %

Repetability : 4.9%

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III. Validation of the method :

III - 4) Intermediate precision

- 3 Technicians analyze the same batch, for 3 days in succession, each preparing one sample and one calibration range each day

- Reagents are different, tanks are different (horizontal tank, vertical tank and ADC)

Result :

Intermediate precision relative standard deviation : 5.7%

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IV. Method Comparisons

IV– 1 Comparison with immersion

① *Mobile phase* : 80v Et-OH / 15v H_2O / 5v CH_3COOH

Reagent : Ninhydrine : 0.5%(m/v) in Et-OH

↳ *Immersion* : speed : 5 – time : 0

② *Mobile phase* : [80v Et-OH / 15v H_2O / 5v CH_3COOH]
+ 0.5% Ninhydrine

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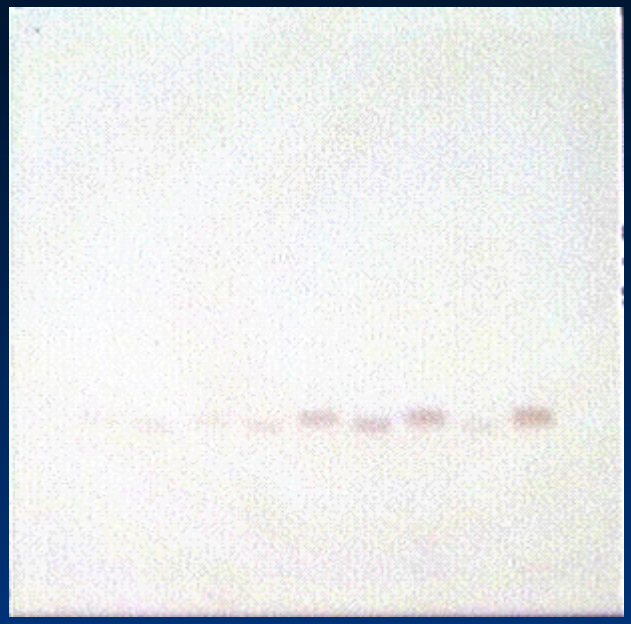
Results :

1



Calibration : Sdv : 1.4%

Results : Sample1 : 18 µg/ml

Sample 2 : 20 µg/ml

Sample 3 : 46 µg/ml

Sample 4 : 17 µg/ml

2

Calibration : Sdv : 0.2%

Results : Sample1 : 18 µg/ml

Sample 2 : 21.5 µg/ml

Sample 3 : 52.5 µg/ml

Sample 4 : 18 µg/ml

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IV. Method Comparisons

IV – 2 HPLC /HPTLC Comparison

HPLC Method :

- Internal standard solution : aminocaproïc acid solution
- Fluorescence solution : fluorescamine in Acetonitrile
- Mobile phase : Borate buffer (70v) / Methanol (30v)pH 8.0
- Standard solution : aminopropanol in ethanol.

Final solution (in mobile phase) : aminopropanol solution containing fluorescamine and internal standard

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IV. Method Comparisons

↳ Sample preparation :

Approx. 1g of cream into 50.0 ml of ethanol.

Final solution : 10mg/ml + IStd and fluo reagent.

↳ Chromatographic conditions :

Octadecylsilyl phase bonded to silica gel 5µ

Wavelength : λ excitation : 390 nm - λ emission : 480 nm

Run time : 3 min.

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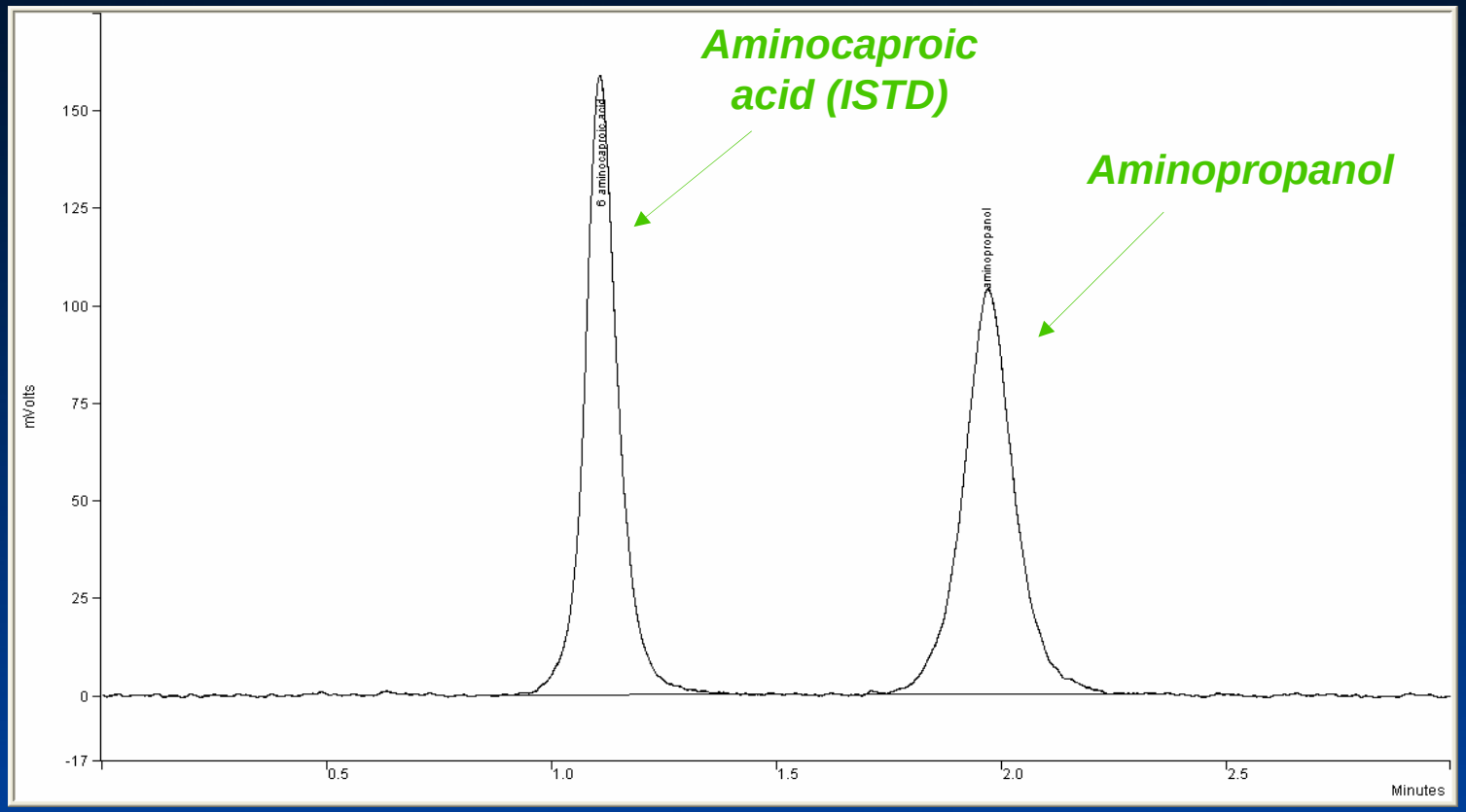
Chromatogram example :



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Results comparison :

9 samples : each method

↳ Comparison of variances pass the acceptance limit

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IV. Method Comparisons

Time Comparison

- Time to analyse 10 samples :

HPTLC	HPLC
5 hours	8 hours (1 day)

- Time to analyse 30 samples :

HPTLC	HPLC
1 day	3 days

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V - Some problems

- Optimization of sample preparation : filtration problems
- Optimization of sample application parameters (“fat” solutions)

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Conclusions

- alternative HPTLC Method / existing HPLC method.
- Method is fast : possibility to analyze 36 samples at once (anti-parallel)
- Method which save us plate immersion. We don't need lot of reagent

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