Exemples d'application de la CCM

Laboratoire de développement analytique,
Bayer Santé Familiale.

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Bayer HealthCare
Consumer Care

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2➢ Le laboratoire de développement analytique

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Du point de vue industriel et R & D

Pharma

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R & D

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Segments de marché/principales marques France

Vitamines et Multivitamines

Supradyn(e)®, Berocca®, Laroscorbine® …

Dermatologie & Soins Gynécologiques

Bepanthen®, Biseptine®, Hydralin® …

Analgésiques

Aspirine du Rhône®

Aspro®

Médicaments à visée gastro-intestinale

•Rennie®, Transipeg® …

Phytothérapie

Euphytose®

Antimoustiques

Cinq sur cinq®

Localisation géographique

PUTEAUX
Administration Serdex

GAILLARD
Administration, R&D, Marketing, Visite médicale, Production, Logistique international

PAU
Fabrication Serdex

SAINT GEORGES
Logistique France, Conseillers clientèle pharmacie, Administration des ventes

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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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Le laboratoire de développement analytique

1 responsable de service, 2 chefs de projets, 1 responsable Matières premières , 14 techniciens, 1 agent de laboratoire

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Le laboratoire de développement analytique

- Missions principales :

- Développer & valider des méthodes d'analyse de produits finis qui seront transférées sur les différents sites Bayer Consumer Care
- Analyser les produits pour essai clinique/consommateur
- Suivre les échantillons en stabilités

- Principales méthodes analytiques :

HPLC ✧ ICP - Absorption Atomique ✧ Potentiométrie ✧ Etude de Dissolution ✧ TLC ✧ GC

⇨ 16 systèmes HPLC pour 1 équipement CCM ! ☹

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Le laboratoire de développement analytique

- Produits analysés :

- Comprimé : * multi-vitamines + minéraux (+extrait de plante ou autre actif)
  * anti-acide

  Effervescent, filmé, dragéifié, à croquer
- Crème, pommade, gel, lotion *(cométique ou dermatologique)*
- Matière première (excipient ou PA)

- Nos contraintes:

- Adapter nos méthodes aux équipements du futur site de production
- Matrices importantes (excipients + nombreux PA)
- Convaincre nos interlocuteurs que la CCM peut être performante !

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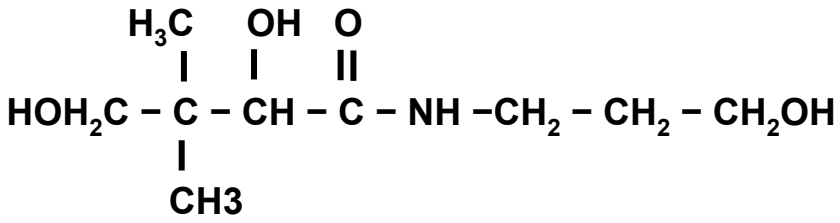
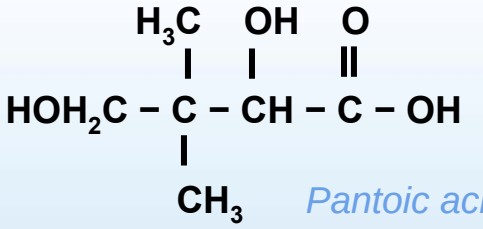
AMINO-PROPANOL ASSAY

Amino-3-propan-1-ol is dexpanthenol (pro-vitamin B5) degradation product

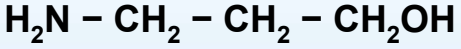


Dexpanthenol

Hydrolysis



Pantoic acid



Amino-3-propan-1-ol

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Why a HPTLC method ?

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

  ⇨ Wining time by HPTLC

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 :

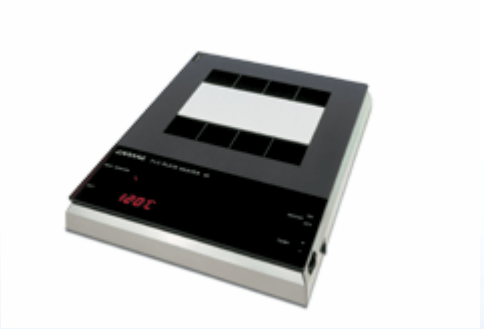
1 concentrated solution , 5 dilutions : 12.5, 25, 50, 100, 200 µg/ml

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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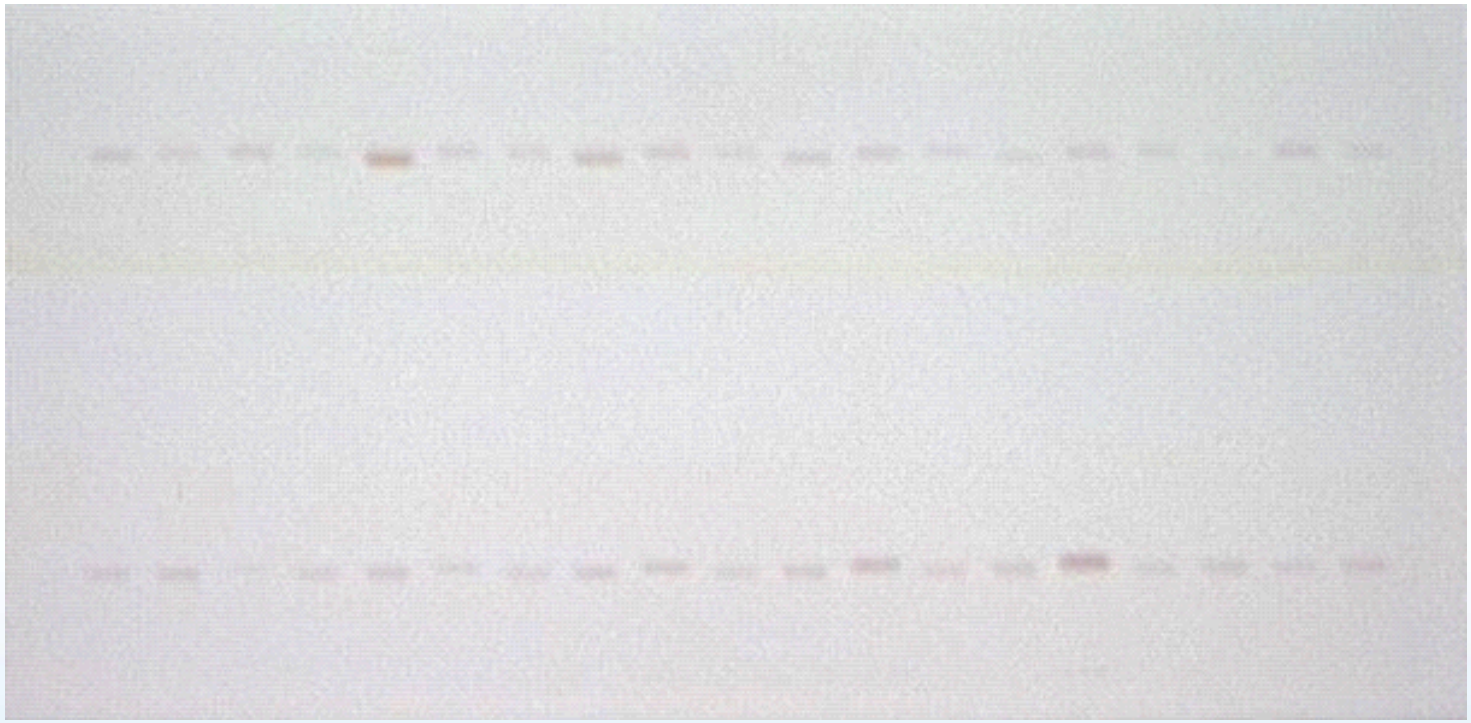
Quantification :

CAMAG SCANNER III

- Wavelength : ↳ 486 nm
- Calculation : ↳peak area
- Calibration method : ↳ Michaelis Menten regression (2nd degree)

Plate example :



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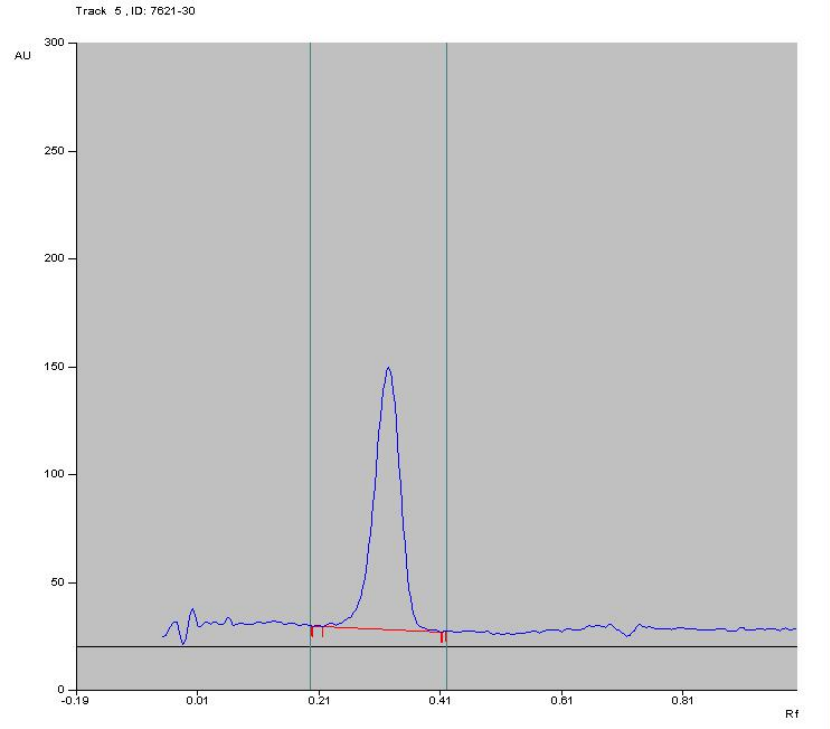
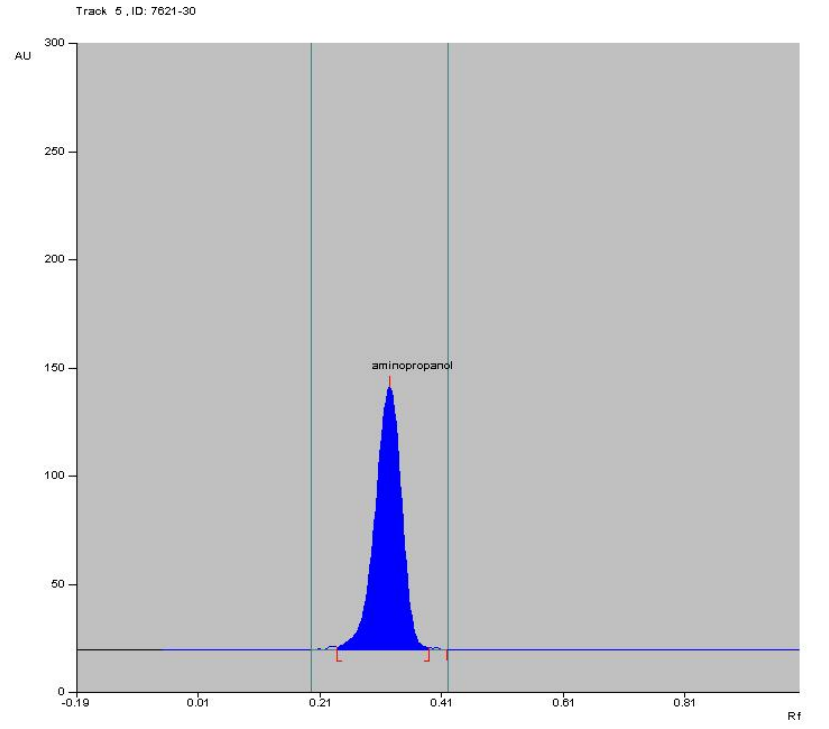
Peak example :

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





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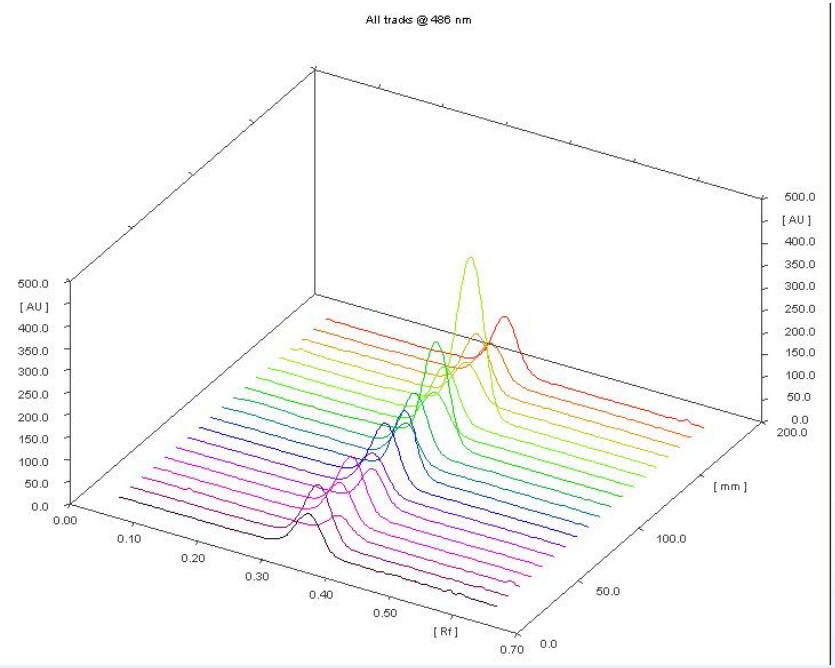
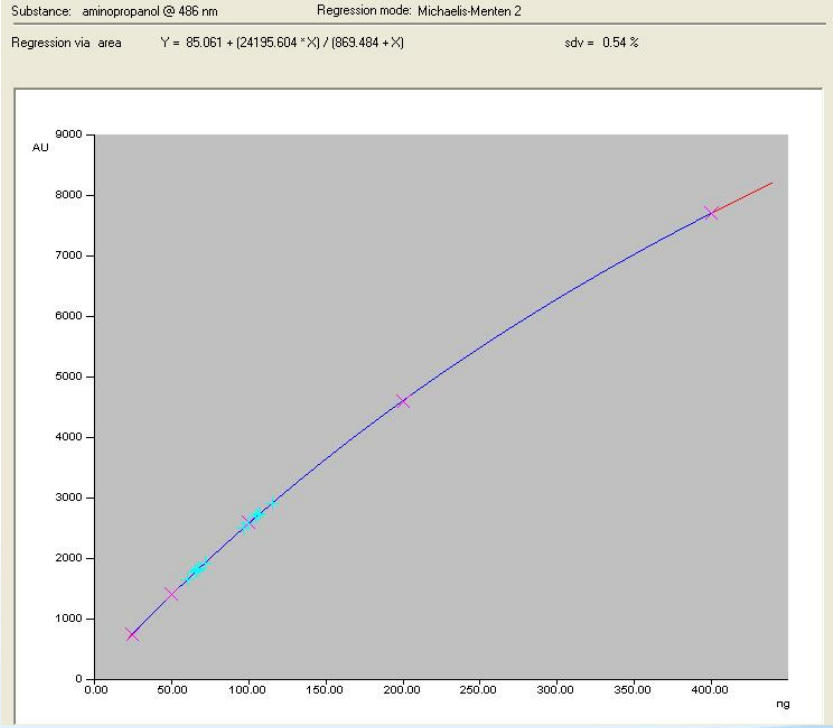
3D and calibration curve :





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Validation of the method :

- 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

Validation of the method :

- 2) Specificity
- According to the method described, prepare and apply a free amino-propanol reconstituted sample.
- ➔ No spot appears
- 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

VALIDATION OF ANALYTICAL METHOD

PRODUCT: Bepanthen Onguent Date: 15/09/2005

METHOD: Amino-3-propan-1-ol by HPTLC

Test product content: 3 Units: % Specification : ± 10% (Impurity)

LINEARITY - ACCURACY - REPEATABILITY

Input data

Index	Data			Predicted	Residuals		Recovery	
	Spiked	Found	Found	Fit		100(Yi-<Y>i)		100.Yi
i	Xi	Yi	Yim	<Y>i	Yi-<Y>i	<Y>i	Yi-Xi	Xi
1		3,300E-01		2,910E-01	3,897E-02	1,339E+01	3,000E-02	110,0%
2	3,000E-01	3,400E-01	3,200E-01	2,910E-01	4,897E-02	1,683E+01	4,000E-02	113,3%
3		2,900E-01		2,910E-01	-1,025E-03	-3,522E-01	-1,000E-02	96,7%
4		9,000E-01		8,844E-01	1,558E-02	1,762E+00	2,000E-02	102,3%
5	8,800E-01	9,300E-01	8,767E-01	8,844E-01	4,558E-02	5,154E+00	5,000E-02	105,7%
6		8,000E-01		8,844E-01	-8,442E-02	-9,545E+00	-8,000E-02	90,9%
7		1,760E+00		1,775E+00	-1,450E-02	-8,174E-01	1,000E-02	100,6%
8	1,750E+00	1,810E+00	1,763E+00	1,775E+00	3,550E-02	2,000E+00	6,000E-02	103,4%
9		1,720E+00		1,775E+00	-5,450E-02	-3,072E+00	-3,000E-02	98,3%
10		2,970E+00		3,084E+00	-1,141E-01	-3,698E+00	-6,000E-02	98,0%
11	3,030E+00	3,110E+00	3,023E+00	3,084E+00	2,594E-02	8,411E-01	8,000E-02	102,6%
12		2,990E+00		3,084E+00	-9,406E-02	-3,050E+00	-4,000E-02	98,7%
13		3,960E+00		4,056E+00	-9,599E-02	-2,367E+00	-2,000E-02	99,5%
14	3,980E+00	4,320E+00	4,107E+00	4,056E+00	2,640E-01	6,509E+00	3,400E-01	108,5%
15		4,040E+00		4,056E+00	-1,599E-02	-3,943E-01	6,000E-02	101,5%
	%	%	%	%	%	%	%	%

Range: 10 % to 133 % of product content

Linearity Summary

Coefficient of correlation:	0,9979
Regression line:	y=1,02E+00 x - 1,59E-02
Residual sum of squares:	rss = 1,20E-01
Residual standard error:	9,59E-02
Confidence interval of the Y-intercept:	- 9,37E-02 +6,19E-02 (p=0.05)
Confidence interval of the slope:	+9,91E-01 +1,06E+00 (p=0.05)
Error Probability for Lack of Fit in %:	55%

5 points

% recovery

Coefficient of correlation : 0.9979

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Linearity assessment

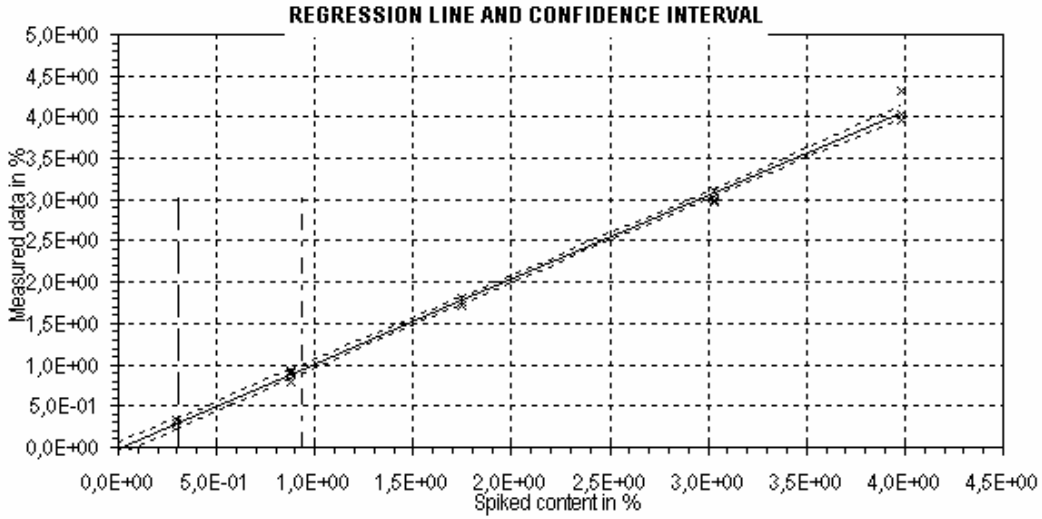
The coefficient of correlation corresponds to the acceptance limit (>=0.9900)
There is no significant deviation from linearity (P = 95 %).

Accuracy Summary

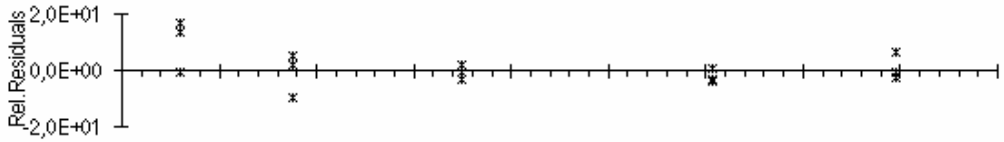
Mean recovery (%):	102,00	
Standard deviation of recovery (%):	5,68	
Confidence interval of the mean recovery (%):	92,04 to 111,96	(p=0.05)

Accuracy assessment

The mean recovery corresponds to the acceptance limits (90 to 110 %).



Crosses = Measured Data, Straight Line = Fit, Dotted lines = Confidence Limits
The vertical dashed and dash-dotted lines mark the LOD and LOQ respectively.



Mean Recovery : 102%

Repetability : 4.9 % < 10 %

Repeatability Summary

Mean:	2,013E+00	%
Repeatability standard deviation:	9,883E-02	%
Repeatability relative standard deviation:	4,9%	
Confidence interval of the mean (p=0.05):	1,97E+00 to 2,07E+00	%

Repeatability Assessment

The Repeatability relative standard deviation corresponds to the acceptance limit (<= 10 %).

Validation of the method :

- 4) Intermediate precision
- ↳ 3 chemists 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)

INTERMEDIATE PRECISION

3 operators

Input data

X(operator)

Operator 1			Operator 2			Operator 3		
apparatus	day	result	apparatus	day	result	apparatus	day	result
1	1	1,900E+00	1	1	1,810E+00	1	1	2,120E+00
1	2	1,980E+00	1	2	1,920E+00	1	2	1,820E+00
1	3	1,820E+00	1	3	1,830E+00	1	3	2,030E+00
Mean:		1,900E+00			1,853E+00			1,990E+00
Rel. standard deviation:		4,21%			3,16%			7,74%

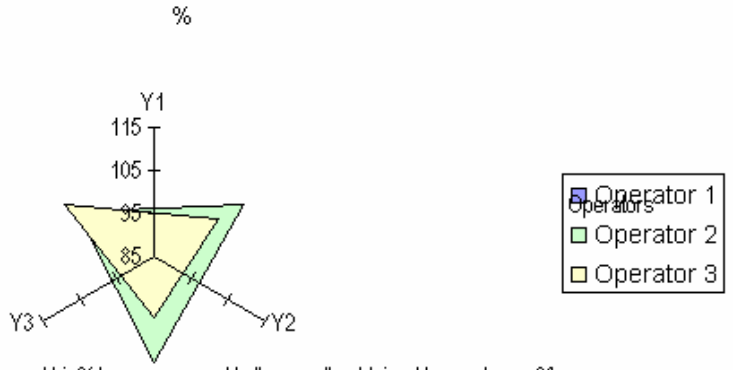
Intermediate Precision Summary

Mean:	1,914E+00	%
Intermediate precision standard deviation:	1,096E-01	%
Intermediate precision relative standard deviation:	5,72%	
Confidence interval of the mean (p=0.05):	1,84E+00 to 1,99E+00	%

Intermediate precision relative standard deviation = 5.7% < 10 %

Intermediate Precision Assessment

The Intermediate Precision relative standard deviation corresponds to the acceptance limit (<= 10 %).



The summits of the coloured triangles correspond to the results obtained by each operator.
A triangle centered on the graph indicates that the results are apparently independant from Y(apparatus,day).
An off-centre triangle points toward a possible interaction Y(apparatus,day).
Equal triangles indicate that the result is apparently independant from the operators.

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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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Identification du Guarana dans un comprimé (multi-vitamines + minéraux + guarana)

- Standard solution : Catechin standard in ethyl acetate.
- Sample solution : Into a 250 ml erlen flask, introduce 24 g of sample. Extract with ethyl acetate
- Analytical and chromatographic conditions :
- ↳ Plate material — silica gel 60 F254
- ↳ Application volume — 2 µl of each solution (spray, 6mm band)
- ↳ Solvent — anhydrous formic acid R / Toluene / Acetone (1 / 3 / 3)
- ↳ Development mode — vertical in a satured chamber / 5cm
- ↳ Operate in 4 times (to avoid matrix elution problem) :
  - 1st develop on 3.5 cm, dry 2min
  - 2nd develop on 4 cm, dry 2 min
  - 3rd develop on 4.5 cm, dry 2 min
  - 4th develop on 5 cm, dry 2 min

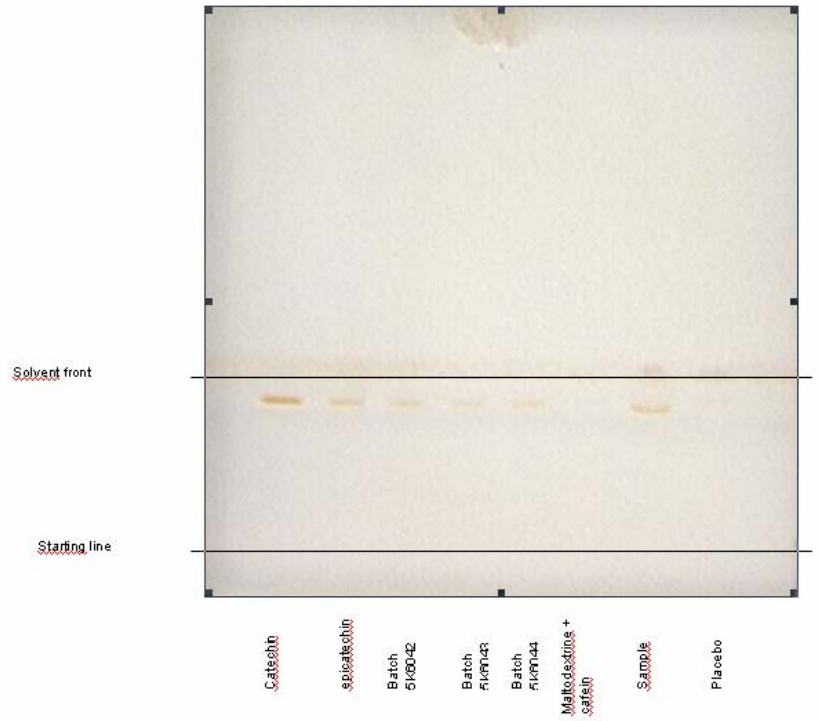
Derivatization :

Spray a solution of sulphuric anisaldehyde.
Dry the plate at 100-105 °C during 5 min.
Examine under day light

- Result :

Observe the presence of a brown-orange band with a Rf of approx.0.8

TYPE CHROMATOGRAM



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Identification de plantes dans un comprimé Euphytose® (aubépine & passiflore)

- **Standard solutions :**
- ➢ Hyperoside and vitexin solution
- ➢ Hawthorn extract solution
- ➢ Passion Flower extract solution.
- **Analytical and chromatographic conditions :**

	Ancienne méthode	Nouvelle Méthode optimisée
Plate material	TLC Si 60 F254	HPTLC Licrospher Si 60 F254
Preconditionning	X	10 min in NH3 vapors (10% NH3 in MeOH)
Application volume	20µl (spary band 10mm band)	4 µl of each solution (spray 6mm band)
Development mode	in a satured chamber / 17cm	in a satured chamber / 8cm

- ↳ Solvent : Water R / anhydrous formic acid R / methylethylcetone R / ethyl acetate R
- ↳ Dry the plate at 100-105 °C during 5 min.
- ↳ Derivatization : Spray profusely a solution of diphenylboric acid aminoethyl ester R and 50 g/l macrogol 400 R in methanol R.
- ↳ Allow to dry in the air then examine under UV light at 366 nm.

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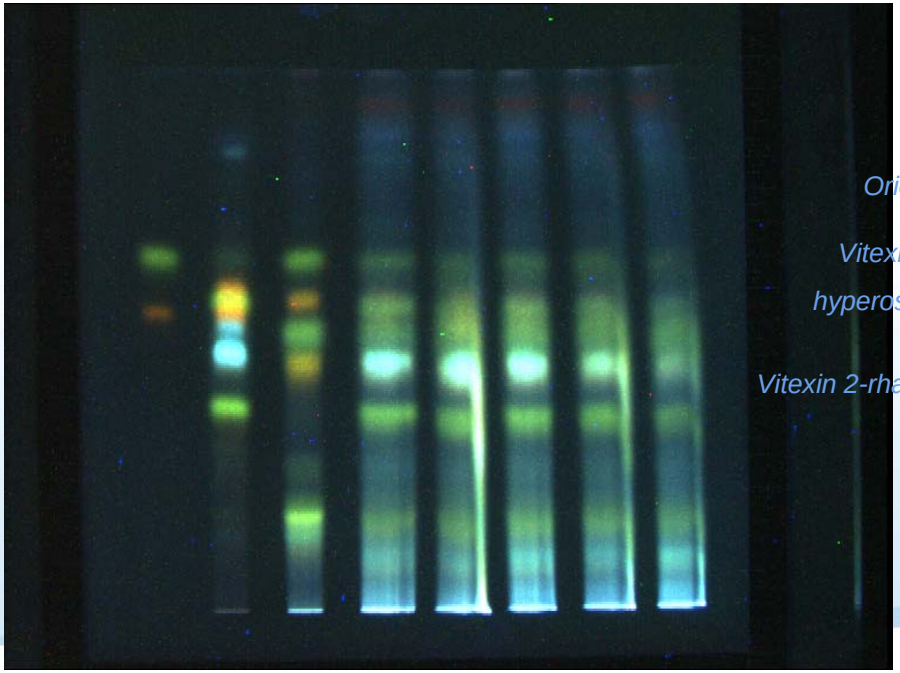
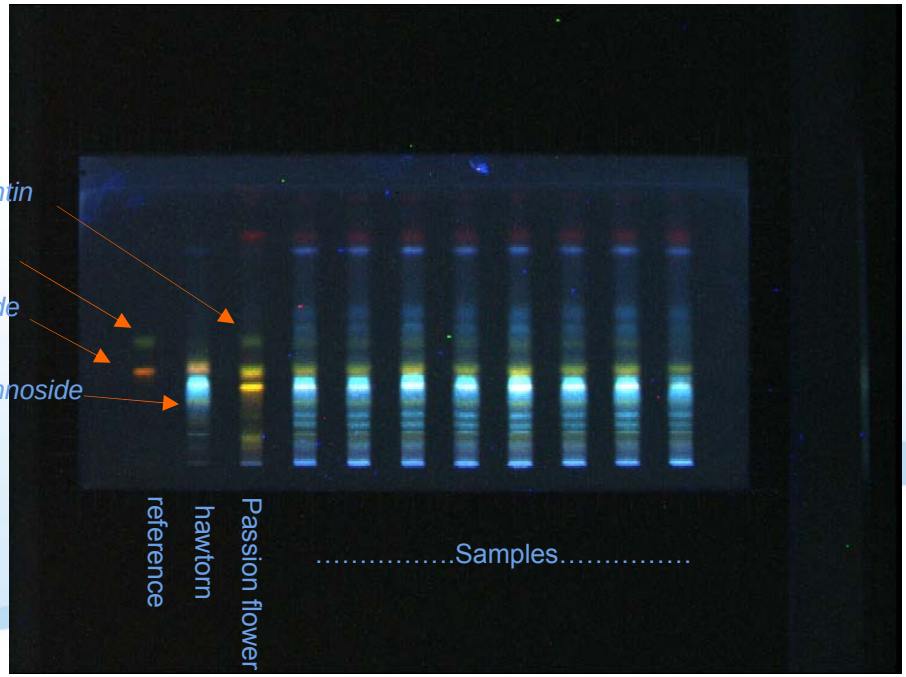
Results :

- Typical bands of each plant extract should be present in the chromatogram of the sample :
  - Vitexin as a green fluorescent band (Passion Flower)
  - Orientin as a greenish yellow fluorescent band (Passion Flower)
  - Hyperoside* as a yellowish orange fluorescent band (Hawthorn)
  - Vitexin 2-rhamnoside as a greenish yellow fluorescent band (Hawthorn)
  - *Hyperoside band may sometimes be hidden by other fluorescent bands.





Identification de plantes dans un comprimé Euphytose® (ballote)

- **Standard solutions :**
- ➢ horehound solution
- **Analytical and chromatographic conditions :**

	Ancienne méthode	Nouvelle Méthode optimisée
Plate material	TLC Si 60 F254	HPTLC Licrospher Si 60 F254
Preconditionning	X	15 min in NH3 vapors (10% NH3 in MeOH)
Application volume	20µl (spary band 10mm band)	4 µl of each solution (spray 6mm band)
Development mode	in a satured chamber / 15cm	in a satured chamber / 8cm

- ↳ Solvent : Water R / anhydrous formic acid R / anhydrous acetic acid R / ethyl acetate R
- ↳ Dry the plate at 100-105 °C during 5 min.
- ↳ Derivatization : Spray profusely a solution of diphenylboric acid aminoethyl ester R and 50 g/l macrogol 400 R in methanol R.
- ↳ Allow to dry in the air then examine under UV light at 366 nm.

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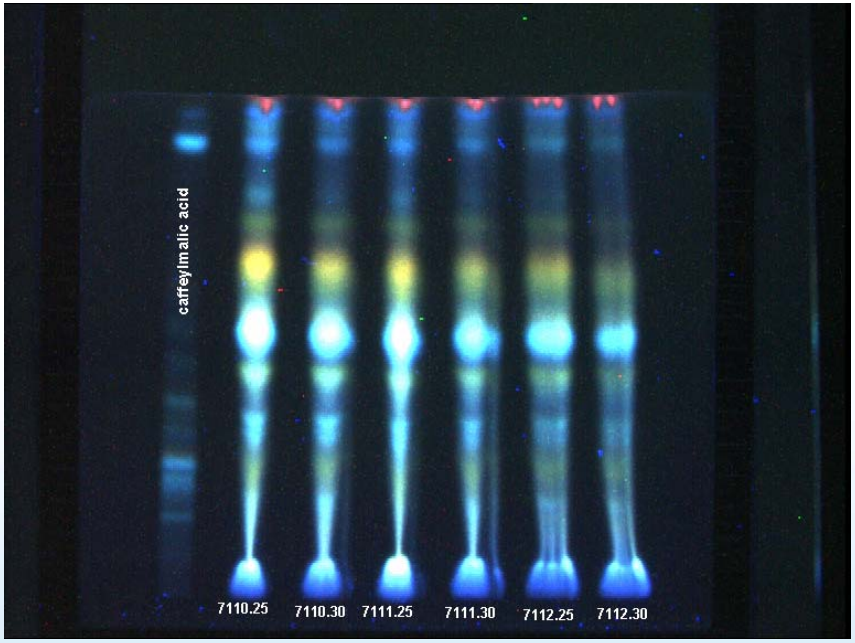
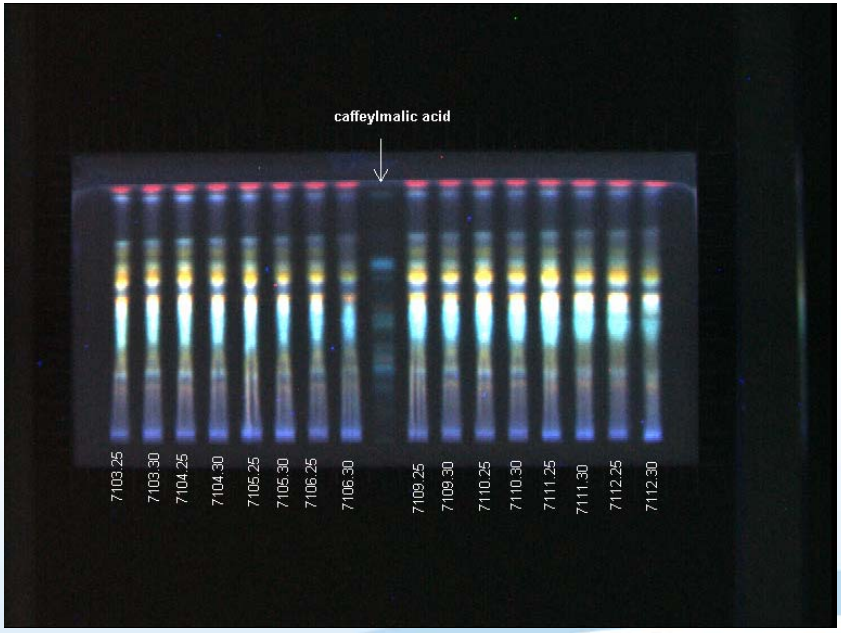
- Result : observe on both plates a blue fluorescent band with a Rf of approx. 0,75 (caffeylmalic acid)





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Merci pour votre attention.

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