Département
Chimie

DOSAGE PAR HPTLC DE LA VANILLINE ET DE L'ÉTHYLVANILLINE DANS DES SUCRES AROMATISÉS DANS LE CADRE DE TP DE MASTER CHIMIE

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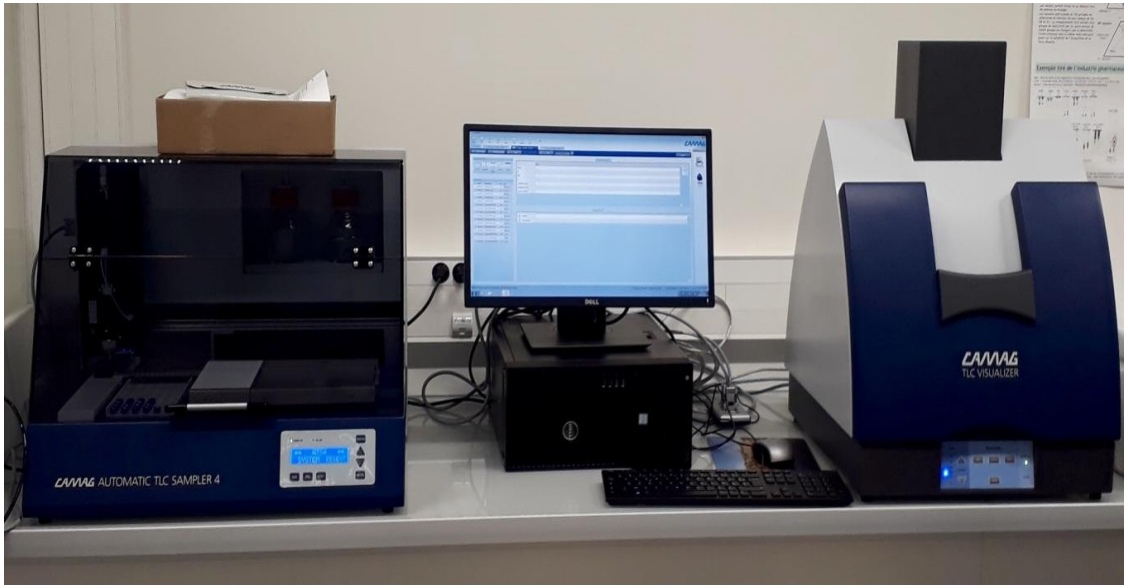
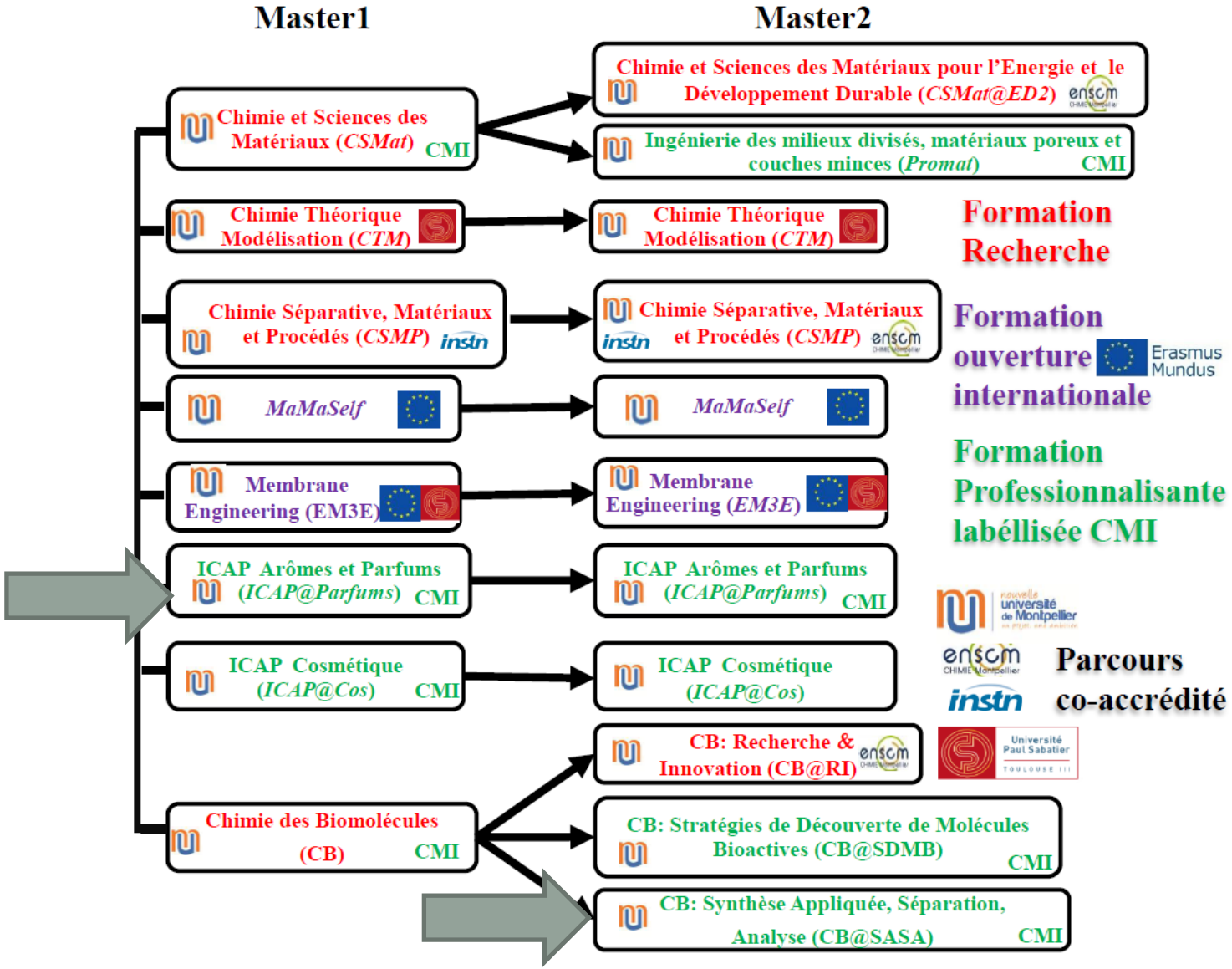
Club de CCM 20ème anniversaire – 27-28 juin 2018, Montpellier

Appareillage HPTLC Du Dept. Enseignement Chimie (avril 2017)



Lumière blanche
254 nm
366 nm

PARCOURS MASTER CHIMIE DE LA FACULTÉ DES SCIENCES



Parcours Synthèse Appliquée, Séparation, Analyse (M2)

Responsable: Pr. Alain MORERE

Débouchés:

- Industrie pharmaceutique
- Chimie fine, synthèse à façon
- Industrie agrochimique
- Laboratoires d'analyse et qualité
- Organismes de recherche publics (CNRS, INSERM, Universités, …)
- Thèse

Métiers:

- Recherche et développement
- Contrôle – qualité
- Ingénieur d'études
- Technico-commercial

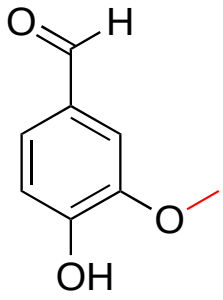
→ TP de HMCH365 Méthodes séparatives (12h CM + 12hTP)

HPLC, LC-MS, GC-MS, SPE … HPTLC

Objectifs Du TP

Il s'agit de doser, par HPTLC, **la vanilline** et **l'éthylvanilline** contenues dans des sucres utilisés en pâtisserie : le **sucre vanillé** (arôme naturel de vanille) et le **sucre vanilliné** (arôme artificiel).

Ce dosage ne nécessite pas d'étape préalable d'extraction sur phase solide. Les résultats obtenus seront comparés à ceux obtenus par HPLC-UV (David Egron).

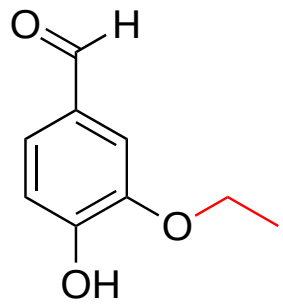


Vanilline

Ingrédients :

- Sucre
- **Extrait naturel de vanille**
- Antiagglomérant : amidon de maïs

4 g de gousses au minimum mises en œuvre pour 100 g de sucre vanillé



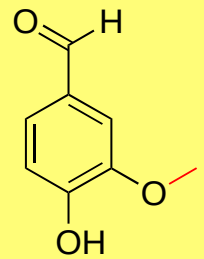
Ethylvanilline

Ingrédients :

- Sucre
- **Arôme artificiel : éthyl vanillline**

VANILLE : ORIGINE ET COMPOSITION

Vanilla planifolia

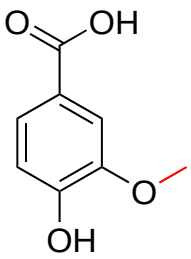


vanilline

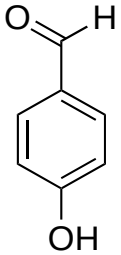
Expected values of phenolic compounds ratios.

Ratios	Expected values
Vanillin/PHB	10–20
Vanillin/APHB	40–110
Vanillin/vanillic acid	12–29
APHB/PHB	0.15–0.35
Vanillic acid/PHB	0.53–1.5

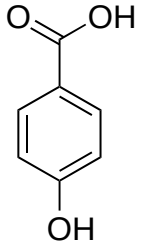
Paillat L. et al.. *J. Planar. Chromatogr.* **2012**,25, 295-300.



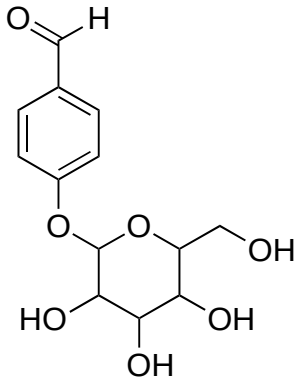
Acide vanillique



para-hydroxy benzaldéhyde (PHB)



Acide para-hydroxybenzoïque (APHB)



vanilline β-D-glucoside

BIBLIOGRAPHIE : ARTICLE 1

J. Sep. Sci. 2007, *30*, 3174–3180

Upendra Kumar Sharma
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Original Paper

RP-HPTLC densitometric determination and validation of vanillin and related phenolic compounds in accelerated solvent extract of *Vanilla planifolia**

A simple, fast and sensitive RP-HPTLC method is developed for simultaneous quantitative determination of vanillin and related phenolic compounds in ethanolic extracts of *Vanilla planifolia* pods. In addition to this, the applicability of accelerated solvent extraction (ASE) as an alternative to microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) and Soxhlet extraction was also explored for the rapid extraction of phenolic compounds in vanilla pods. Good separation was achieved on aluminium plates precoated with silica gel RP-18 F_{254S} in the mobile phase of methanol/water/isopropanol/acetic acid (30:65:2:3, by volume). The method showed good linearity, high precision and good recovery of compounds of interest. ASE showed good extraction efficiency in less time as compared to other techniques for all the phenolic compounds. The present method would be useful for analytical research and for routine analysis of vanilla extracts for their quality control.

Keywords: Accelerated solvent extraction / HPTLC / Phenolic compounds / *Vanilla planifolia*

ARTICLE 1

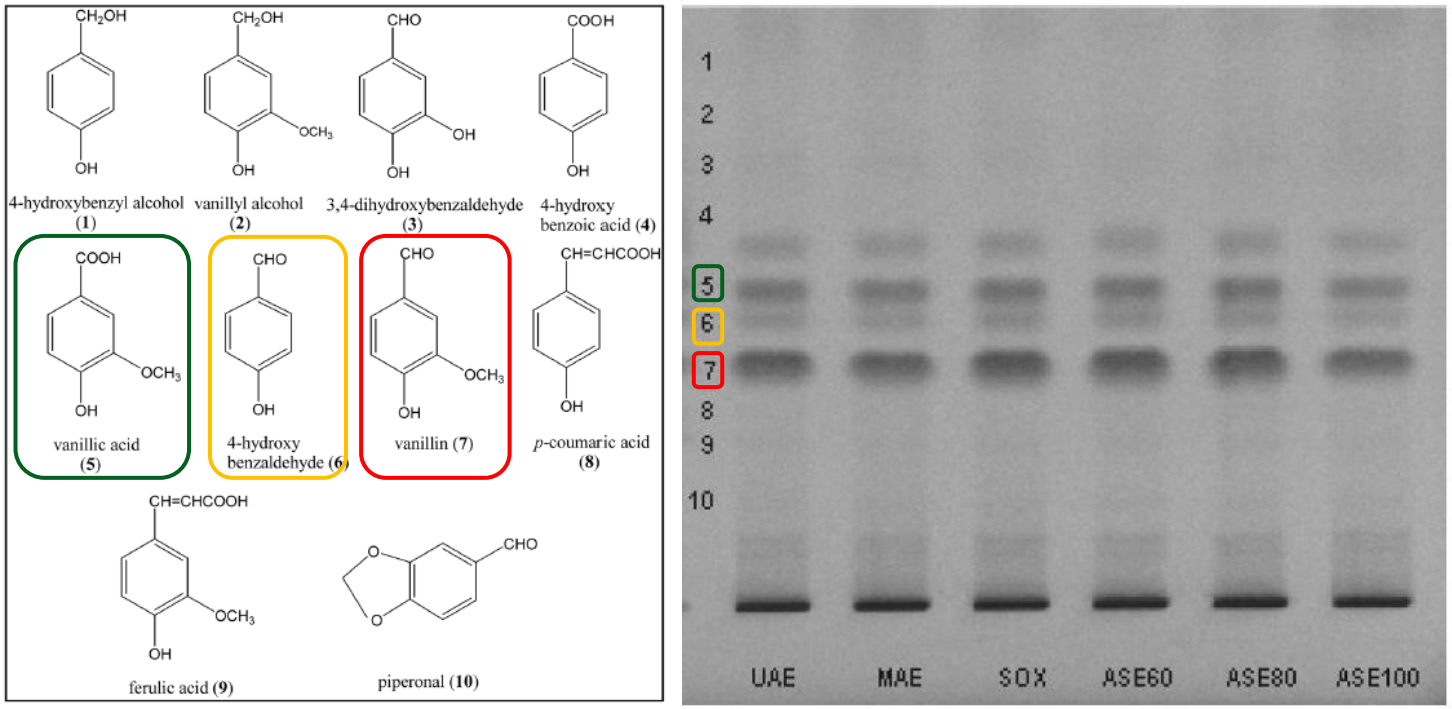


Figure 3. RP-TLC plate of different ethanolic extracts of *Vanilla planifolia* at 254 nm.

BIBLIOGRAPHIE : ARTICLE 2

Journal of Planar Chromatography 25 (2012) 4, 295–300

Validated High-Performance Thin-Layer Chromatography (HPTLC) Method for Quantification of Vanillin β-D-Glucoside, and Four Major Phenolic Compounds in Vanilla *(Vanilla planifolia)* Fruits, Beans, and Extracts

Lionel Paillat, Christine Périchet, Sophie Lavoine, Uwe J. Meierhenrich, and Xavier Fernandez*

Key Words

Vanilla planifolia
HPTLC
Vanillin β-D-glucoside
Phenolic compounds

Summary

A simple, sensitive, selective, precise, and robust high-performance thin-layer chromatography (HPTLC) method was developed and validated for the quantification of vanillin β-D-glucoside, *p*-hydroxybenzoic acid (APHB), vanillic acid, *p*-hydroxybenzaldehyde (PHB), and vanillin in vanilla fruits, beans, and extracts. The analysis was performed on HPTLC glass plate precoated with silica gel $60F_{254}$ as stationary phase. Vertical development was carried in an automated vertical developing chamber (ADC2), saturated, and preconditioned 5 min with the developing solvent *n*-hexane–chloroform–methanol–acetic acid (5:36:4:0.5, vol). A thin-layer chromatography scanner was used for spectrodensitometric scanning and analysis in absorbance mode at 254 nm, 280 nm, and 313 nm. The calibration plots showed good linear relationships in the concentration ranges 24–120 ng per spot for vanillin β-D-glucoside, 20–100 ng per spot for acids, and 8–40 ng per spot for aldehydes. The method was validated for precision, accuracy, and robustness. Detection and quantification limits were determined. Statistical analysis of the data revealed that the method is reproducible and selective for the determination of vanillin β-D-glucoside, APHB, vanillic acid, PHB, and vanillin in vanilla fruits, beans, and extracts.

ARTICLE 2

Analytical characteristics of the validated HPTLC method for the quantification of vanillin β-D-glucosides, APHB, vanillic acid, PHB, and vanillin.

Parameters	Vanillin β-D-glucosides		APHB	Vanillic acid	PHB Vanillin
R_F value	0.09 ± 0.02	0.42 ± 0.03	0.57 ± 0.02	0.62 ± 0.04	0.77 ± 0.03
Densitometric linear relationship					
Working concentration range (ng band^{-1})	24–121	21–106	20–102	6.5–33	14–70
Regression equation	$y = 141.7 + 11.0x$	$y = 123.6 + 23.1x$	$y = -83.8 + 15.9x$	$y = 212.8 + 57.1x$	$y = 201.7 + 31.7x$
Correlation coefficient	0.998	0.999	0.999	0.999	0.997
F_{exp} ($F_{th} = 3.36$)	2.66	2.33	2.26	1.96	2.29
Sensitivity					
LOD (ng band^{-1})	8	6	14	2	4
LOQ (ng band^{-1})	20	20	20	6	8
Precision					
Intra-day (% RSD) ($n = 6$)	0.87	0.93	1.23	0.97	1.05
Inter-day (% RSD) ($n = 6$)	1.09	1.14	1.42	1.33	1.27
Robustness (% RSD) ($n = 3$)					
Mobile phase composition	0.40	0.14	0.27	0.32	0.42
Mobile phase volume	0.52	0.17	0.24	0.29	0.29

Déroulement Du TP

Dosage par HPTLC de **la vanilline** et de **l'éthylvanilline** contenues dans le **sucre vanillé** (arôme naturel de vanille) et le **sucre vanilliné** (arôme artificiel).

- 3 trinômes
- 1 séance de 4h/ trinôme
  - Présentation de la technique et de l'appareil
  - Introduction au TP
  - Préparation des solutions étalon et des échantillons
  - CCM
  - Dépôt des échantillons sur plaque HPTLC (≃ 30 min)
  - Migration
  - Révélation
  - Traitement des données

MATERIEL & METHODES

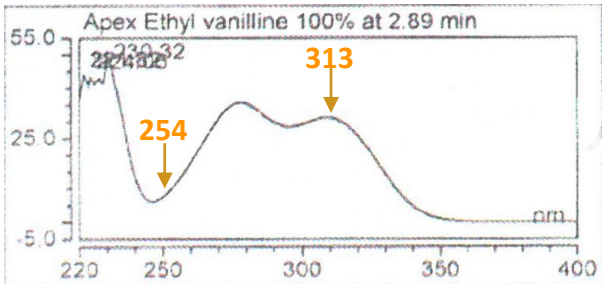
- Echantillons
  - PHB 1 g.L^{-1} (MeOH)
  - Vanilline 0,2 g. L^{-1} (MeOH)
  - Ethylvanilline 0,2 g. L^{-1} (MeOH)
  - Sucre vanillé 100 g. L^{-1} (H_2O/MeOH 1/1 *v*/*v*)
  - Sucre vanilliné 100 g. L^{-1} (H_2O/MeOH 1/1 *v*/*v*)

- HPTLC Silica gel 60F254 (Merck) 20 x 10 cm
- Dépôt par aires de 8 x 3 mm
- Solvant de développement

  n-Hexane–Chloroforme–Méthanol–Acide acétique 10/85/4/1

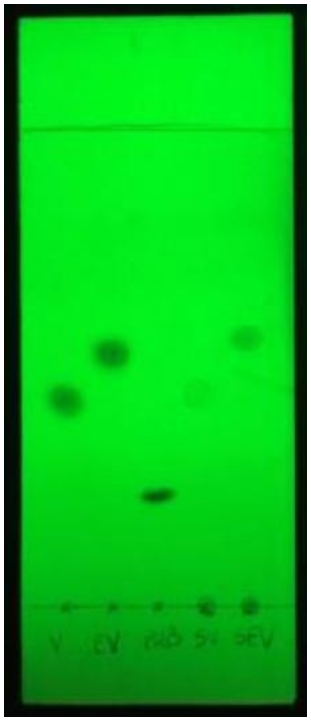
Paillat L., Périchet C., Lavoine S., Meierhenrich U. J., Fernandez X. *J. Planar. Chromatogr.* **2012**,25, 295-300.

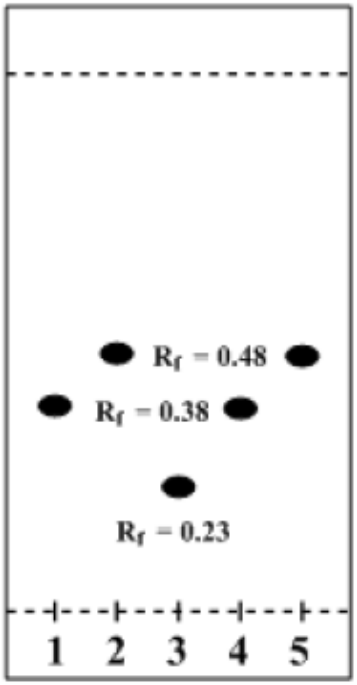
- Cuve verticale préalablement saturée
- Révélation : 254 nm



Chromatographie Sur Couche Mince

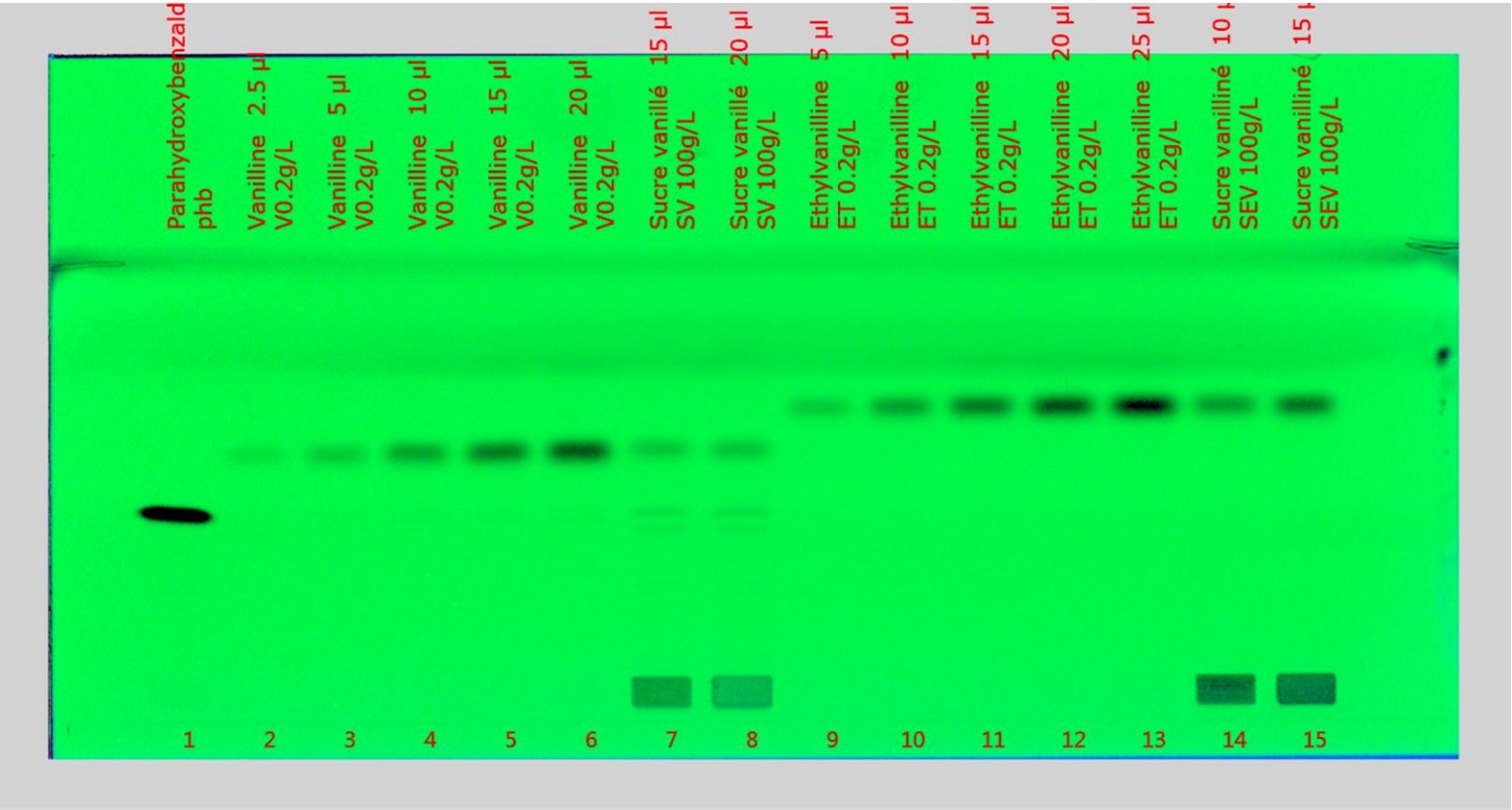
- Séparation des substances
- Identification des substances présentes dans les sucres





1. Vanilline 0,2 g. L^{-1}
2. Ethylvanilline 0,2 g. L^{-1}
3. PHB 1 $g.L^{-1}$
4. Sucre vanillé 100 g. L^{-1}
5. Sucre vanilliné 100 g. L^{-1}

Révélation De la Plaque



PHB

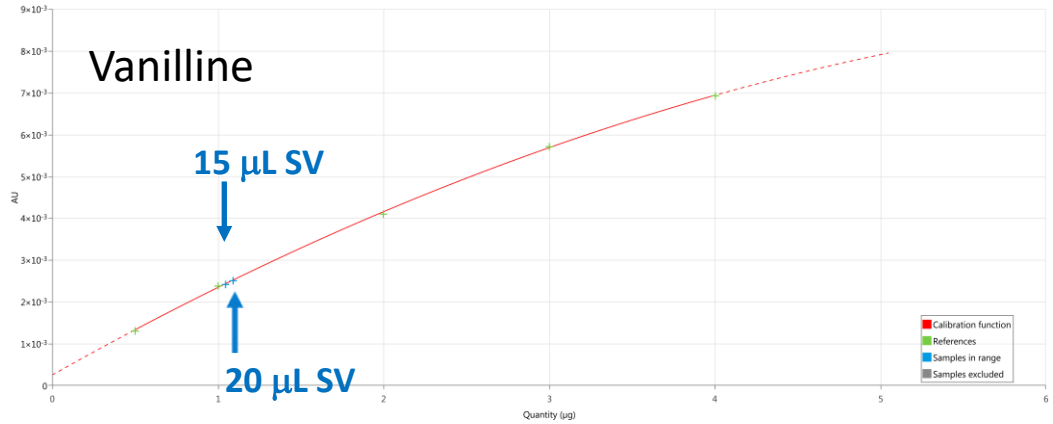
Vanilline
0,5-4 µg

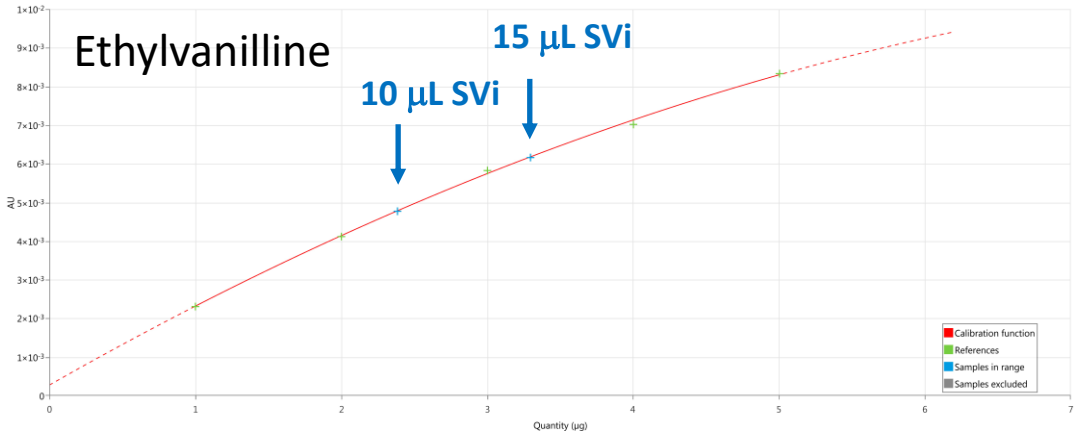
Sucre
vanillé
(100 g.L^{-1})

Ethylvanilline
1-5 µg

Sucre
vanilliné
(100 g.L^{-1})

RÉSULTATS

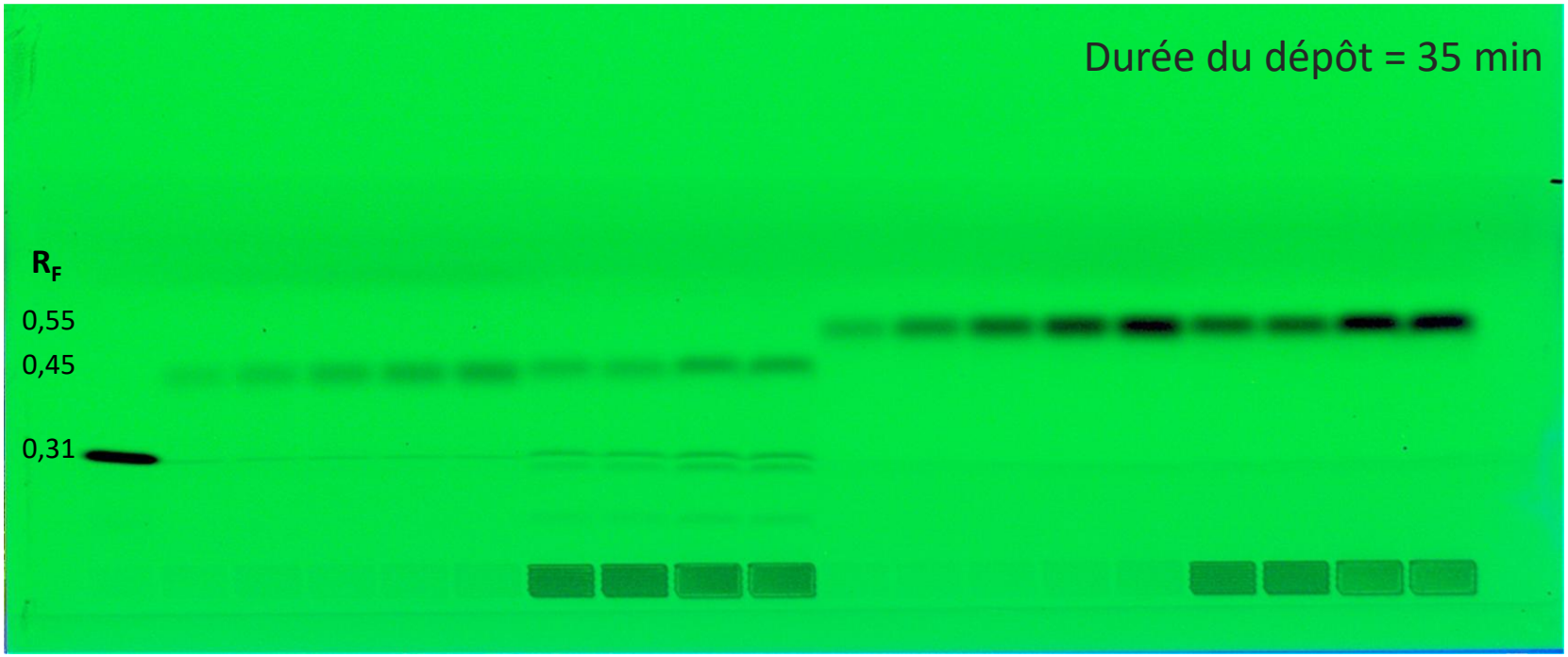




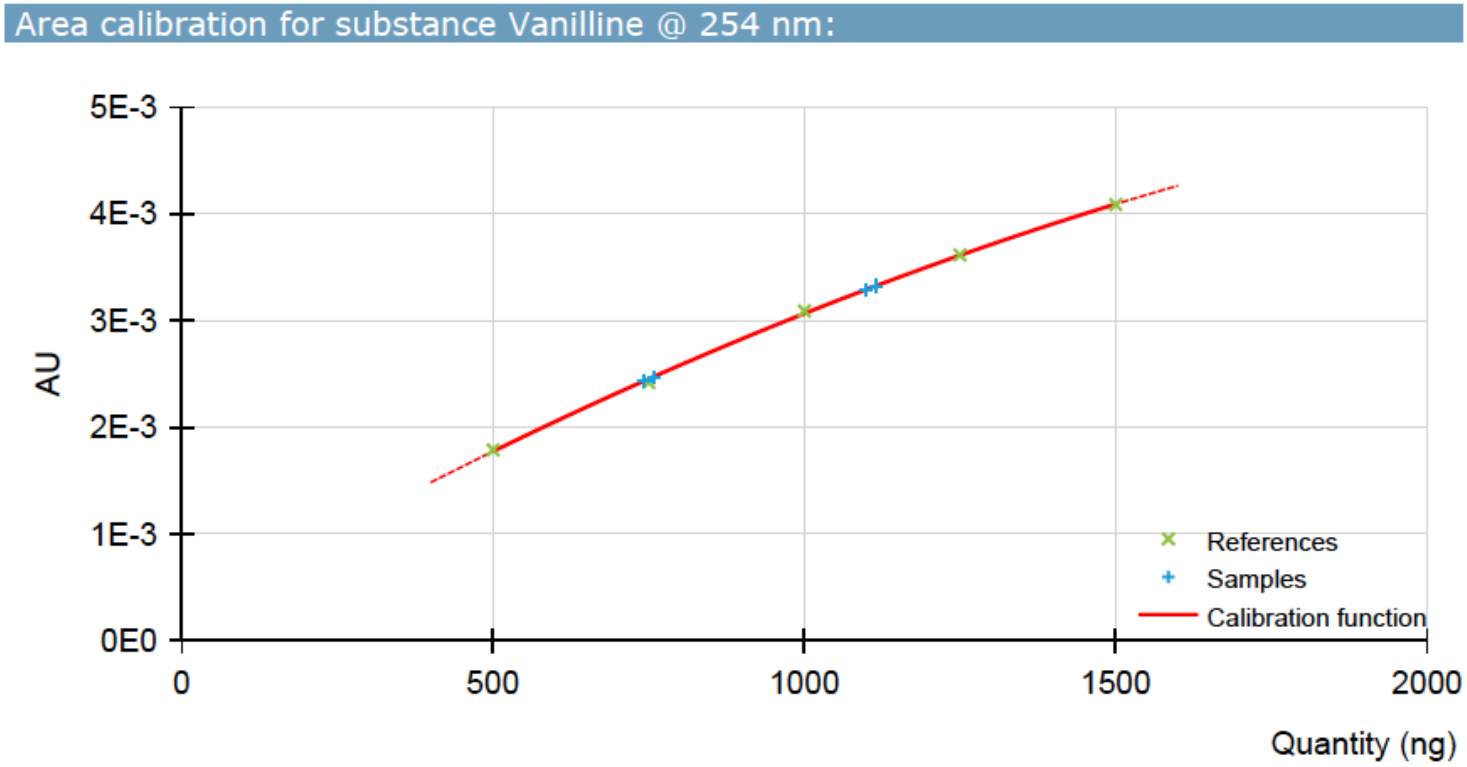
	HPTLC	SPE + HPLC-UV
Vanilline dans la solution de sucre vanillé (µg.mL^{-1})		
Ethylvanilline dans la solution de sucre vanilliné (µg.mL^{-1})		

Optimisation Des Conditions

Arnaud Folliard, David Egron, Département Chimie, FdS

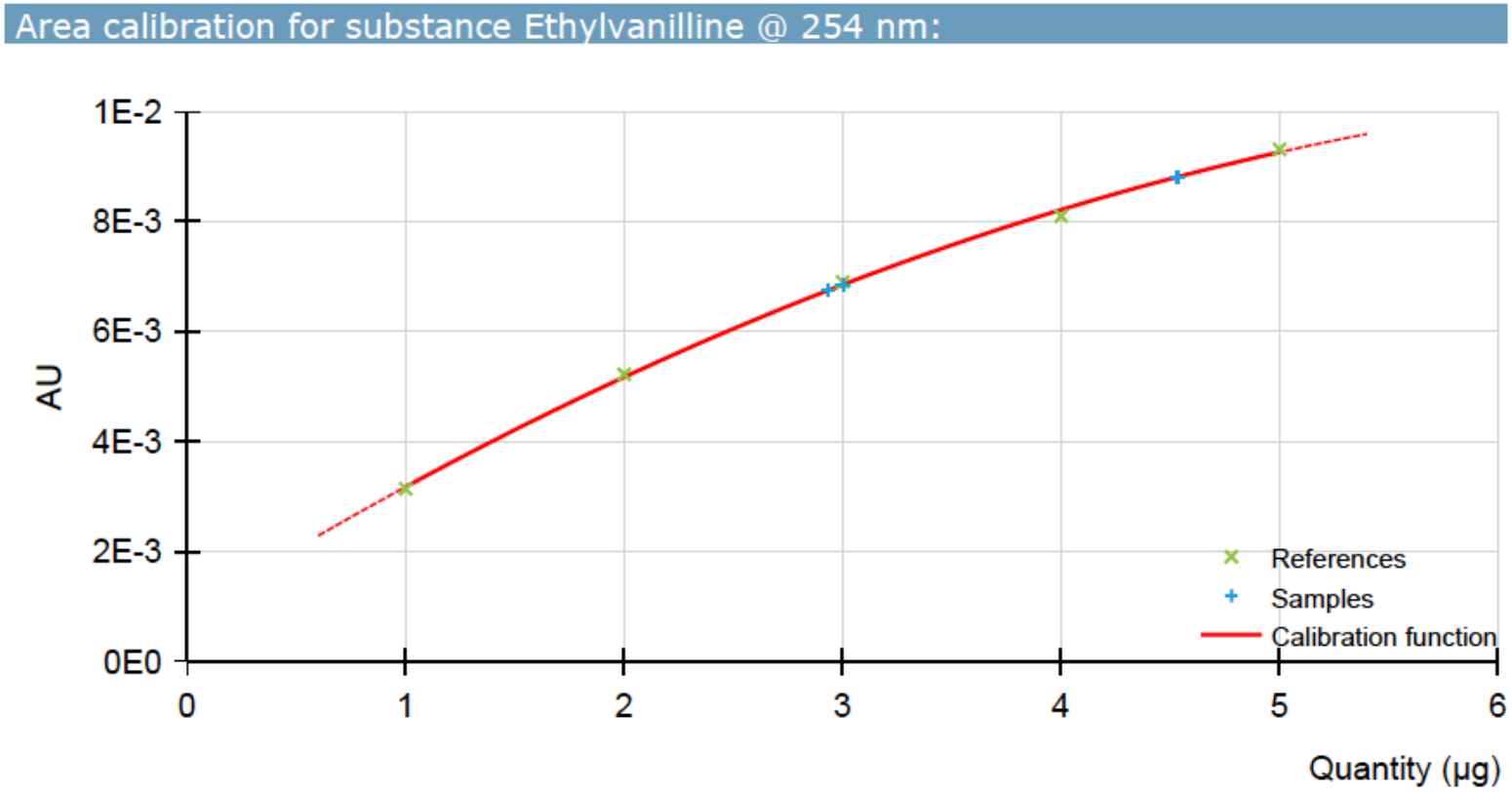


VANILLINE : COURBE DE CALIBRATION



Regression mode	Polynomial
Range deviation	10.00 %
Number of references	5
Calibration function	$y=-5.331\times10^{-16}x^2+3.384\times10^{-9}x+2.158\times10^{-4}$
Coefficient of variation	CV=0.6556 %
Correlation coefficient	R=0.999713

ETHYLVANILLINE : COURBE DE CALIBRATION



Regression mode	Polynomial
Range deviation	10.00 %
Number of references	5
Calibration function	$y=-1.58\times10^{-16}x^2+2.469\times10^{-9}x+8.662\times10^{-4}$
Coefficient of variation	CV=1.0130 %
Correlation coefficient	R=0.999534

Bilan Dosages Par HPTLC

	Vanilline dans le sucre Vanillé		Ethylvanilline dans le sucre vanilliné	
Manipulateur	Solution mère ($\mu g.mL^{-1}$)	Teneur (% masse)	Solution mère ($\mu g.mL^{-1}$)	Teneur (% masse)
1				
2				
3				
MOYENNE				

Normes Iso

4 g de gousses au minimum mis en œuvre pour 100 g de sucre vanillé

Teneur en vanilline des gousses de vanille = 2 % (*Vanilla planifolia*)
1,5 % (*Vanilla tahitensis*)

→ Teneur dans le sucre vanillé = 0,06-0,08 %

CONCLUSION AND PERSPECTIVES

- Développement d'un nouveau TP basé sur l'HPTLC
- Méthode facile à mettre en œuvre : pas de prétraitement de l'échantillon, analyse simultanée de 15 échantillons
- Identification et dosage
- Pas de validation de méthode
- Amélioration du protocole

→ Application lors des prochains TP de M2 SASA

→ JOURNAL OF CHEMICAL EDUCATION — ACS Publications Most Trusted. Most Cited. Most Read.

High performance thin layer chromatography

Samuel J. Costanzo

J. Chem. Educ., **1984**, *61* (11), p 1015

Publication Date: November 1984 (Article)

DOI: 10.1021/ed061p1015

Considers the various ways in which high performance has been achieved in thin layer chromatography, including the new TLC plates, sample application, plate development, and instrumental techniques.

Merci pour votre Attention !

Remerciements :

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- David Egron, Maître de Conférences, Faculté des Sciences