VALIDATED HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF FLAVOUR COMPOUNDS IN PLANT EXTRACTS

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QUANTITATIVE DETERMINATION OF ALCOHOLS AND ACETATES IN HAITIAN VETIVER EXTRACTS

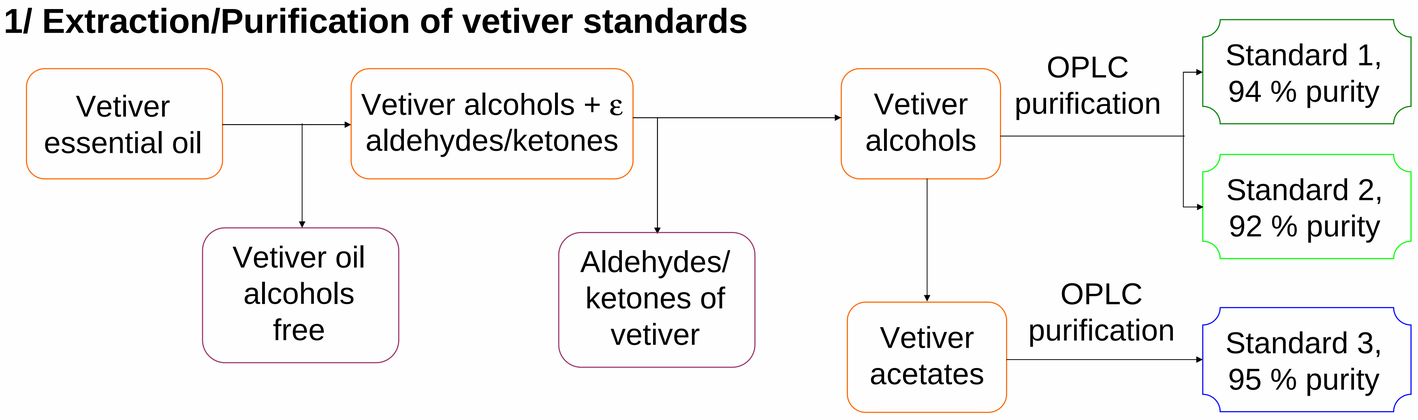
INTRODUCTION

Vetiver grass (*Vetiveria zizanioides*, Linn Nash) from the family *Poaceae* is a densely tufted grass growing throughout 70 tropical regions of the world, Java and Haïti being the largest suppliers [1]. Its roots, from which essential oils were obtained, diffuse a flavour that is used in perfumery and flavour industries. Haitian vetiver oil is one of the most complexes with more than 300 sesquiterpenoids [2]. Olfactory studies have shown that alcoholic fraction presents the most interesting flavour of the oil and the corresponding acetylated fraction is more appreciated.

Vetiver alcohols and acetates content are usually determined by GC/FID [3] and GC-MS [4] but precision of these methods are not well established and integration parameters depend on analysts (more than 30 alcohols or acetates). HPTLC analysis can separate compounds on the basis of their functional group (when compounds have the same molar mass); that is why it was decided to use this technique for vetiver alcohols and acetates quantification.

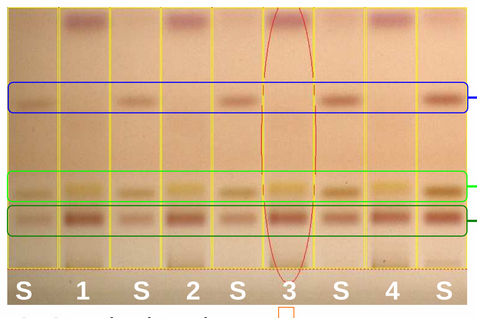
RESULTS

1/ Extraction/Purification of vetiver standards



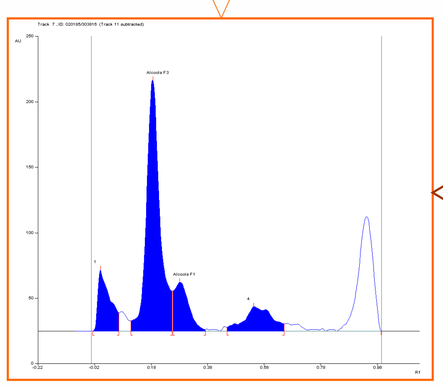
2/ Analysis of vetiver oils

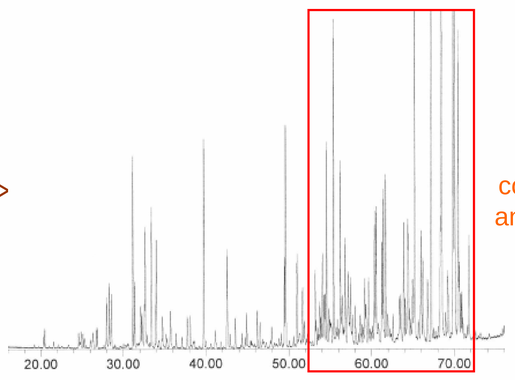
HPTLC plate silica gel 60F254
Hex/CHCL3/EtOAc (8:6:0.5) in ADC2 with 10 min saturation tank and plate conditionning
Derivatisation with vanillin/sulfuric acid reagent
Tracks scan at 530 nm



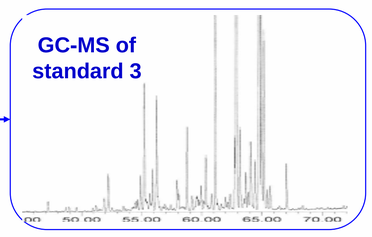
S: Standard tracks
1 – 4: Sample tracks

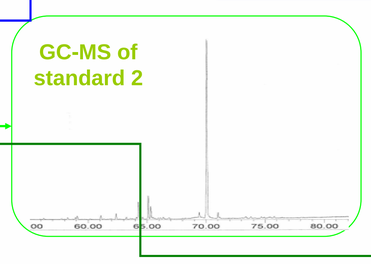
Scan 530 nm

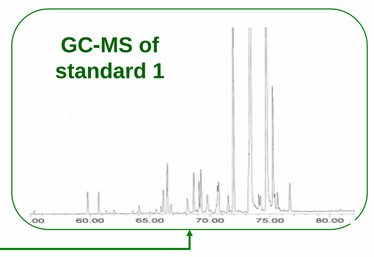




Eluting zone of major vetiver alcohols (containing carbonylated compounds, acids and hydrocarbons)







The developped method can be used to:

- ⇨ Quantifify vetiver alcohols in vetiver essential oils
- ⇨ Quantify vetiver acetates in acetylated vetiver essential oils
- ⇨ Determine the acetylation kinetic of vetiver alcohols

QUANTITATIVE DETERMINATION OF VANILLIN β-D-GLUCOSIDE AND FOUR MAJOR PHENOLIC COMPOUNDS IN VANILLA FRUITS, BEANS AND EXTRACTS

INTRODUCTION

Vanilla planifolia is a tropical aromatic orchid widely used in aroma industries for its flavour, mainly due to phenolic compounds [5]. Financial value depends on physical characters of the beans (colour, length and shape). Amount of aroma, especially vanillin, is important in beans quotation. Authenticity of vanilla beans and extracts is checked by determination of phenolic compounds ratio, according to DGCCRF specification [6]; that is why their content have to be determined. Development of vanilla fragrance appears during fruit curing, mainly due to glycosides hydrolysis, like vanillin β-D-glucoside. Its quantification in fruits can help to predict vanillin content in cured beans.

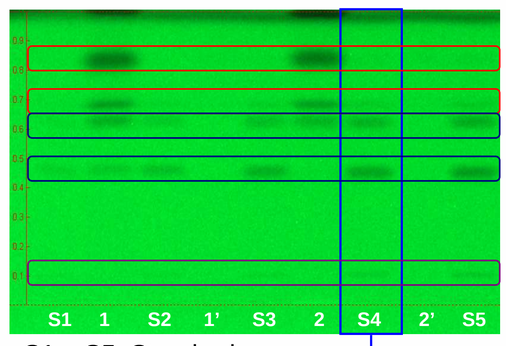
	Expected values
Vanillin/PHB	10 – 20
Vanillin/APHB	40 – 110
Vanillin/Vanillic acid	12 – 29
APHB/PHB	0.15 – 0.35
Vanillic acid/PHB	0.55 – 1.5

PHB: *p*-hydroxybenzaldehyde

APHB: *p*-hydroxybenzoic acid

RESULTS

HPTLC plate silica gel 60F254
Hex/CHCL3/MeOH/acetic acid (5:36:4:0.5) in ADC2 with 5 min saturation tank and plate conditionning
Visualisation at 254 nm
Tracks scan at 254 nm (APHB), 280 nm (vanillin β-D-glucoside, PHB, vanillic acid) and 313 nm (vanillin)

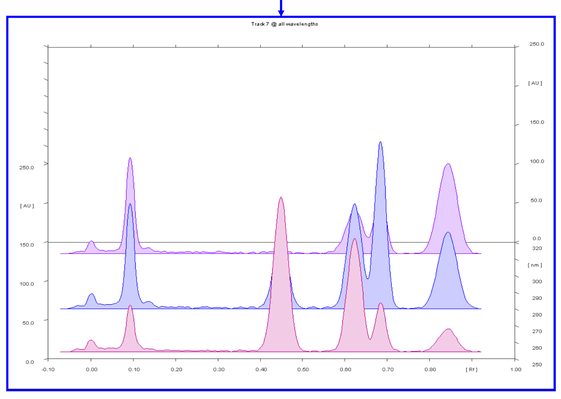


S1 – S5: Standards levels

1/1': Stock/diluted solutions of sample 1

2/2': Stock/diluted solutions of sample 2

Scan **254 nm**, **280 nm** and **313 nm**



The developped method can be used to:

- ⇨ Quantifify phenolic compounds in cured vanilla beans and vanilla bean extracts
- ⇨ Quantify vanillin β-D-glucoside in vanilla fruits

VALIDATION PARAMETERS

	Compounds	Rf	LOD (ng.spot^{-1})	LOQ (ng.spot^{-1})	Calibration range (ng.spot^{-1})	Recovery (%)
VETIVER	Standard 1	0.18	5	20	40 – 200	98.9
	Standard 2	0.28	10	40	40 – 200	102.0
	Standard 3	0.65	5	30	40 – 200	99.4
VANILLA	Vanillin β-D-glucoside	0.09	8	20	24 – 120	100.4
	APHB	0.42	6	20	21 – 106	98.8
	Vanillic acid	0.57	14	20	20 - 102	98.9
	PHB	0.62	2	6	6.5 – 33	99.5
	Vanillin	0.77	4	8	8 - 40	99.0

⇨ **Intra- and Inter-day variation of both quantitative HPTLC methods: % R.S.D. < 3 %**

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