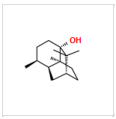

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(-)-patchoulol GC sample preparation

Forked from (-)-α-Bisabolol GC sample preparation

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1 Works for me dx.doi.org/10.17504/protocols.io.xivfke6

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ABSTRACT

This is a quick guide for the preparation of (-)--patchoulol samples and external standards in dodecane for GC analysis. This protocol has been established in the Lindberg lab at Ångström laboratory (Uppsala University) for direct analysis of dodecane-based *ex-situ* extracts from cyanobacterial strains producing (-)-patchoulol. The protocol includes the preparation of stock solutions for the internal standard β-caryophyllene (BCP) and the external standard. The preparation of a fresh external standard series is suggested for each sample analysis. The calibration curve is required for quantification of (-)-patchoulol in the samples of interest.

This protocol is used in combination with the following GC protocol:
dx.doi.org/10.17504/protocols.io.kj2cuqe

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

[1] Bähr, L., Wüstenberg, A. & Ehwald, R. J Appl Phycol (2016) 28: 783. https://doi.org/10.1007/s10811-015-0614-5

MATERIALS

NAME	CATALOG #	VENDOR
β-Caryophyllene ≥80%, FCC, FG	W225207	Sigma Aldrich
HPLC/GC Vials 1.5 mL clear glass	548-1488	Vwr
Dodecane Reagent Grade ≥99%	D221104	
Patchouli alcohol primary reference standard	5986-55-0	Sigma Aldrich

Preparation of BCP (β-caryophyllene) internal standard (IS) stocks

1

	BCP Standard		
Stock A	1: 10 Dilution from Original BCP Stock (⇒ 89 mg *mL^{-1})		
	⇓		
Stock B	281 µL Stock A + 719 µL dodecane (⇒ 25 mg *mL^{-1})	⇒	1: 100 to samples (2 µL + 198 µL sample)
	⇓		
Stock C	200 µL Stock B ad 19,8 mL Dodecane (⇒ 250 µg * mL^{-1})	⇒	use for preparation of α-bisabolene external standard (ES) series

Store Stocks in the fridge at ~ 4 °C

Dodecane is toxic! Wear protective gloves and goggles. Work under the fume hood, or use a respirator!
https://pubchem.ncbi.nlm.nih.gov/compound/dodecane#section=Handling-and-Storage

Preparation of (-)-patchoulol standard stocks

2

Patchoulol standard	
Resuspend 10 mg stock powder in 100 µL Dodecane	10 mg/ 100 µL = 100 µg/µL
Stock P-I 200 µL	20.57 µL Stock solution (100 µg/µL) + 179,43 µL Stock C ⇒ 10.284 µg/µL
	⇓
Stock P-II 2 mL	155,6 µL **Stock P-I** + 1844.4 µL **Stock C** (⇒ 800 µg * mL^{-1})

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Preparation of patchoulol calibration curve

3 **Example: Patchoulol external standard (ES) dilution series**:

0; 25; 50; 100; 200; 400; 800 µg * μL^{-1}

High Range Dilution Series:			
	Vol. **Stock P-II**	Vol. **Stock C**	Transfer to vial (3 x)
800 µg*mL-1	600	0	200
400 µg*mL-1	325	325	200
200 µg*mL-1	162,5	487,5	200
100 µg*mL-1	81,25	568,75	200
50 µg*mL-1	40,625	600	200
25 µg*mL-1	20,3125	629,6875	200
12.5 µg*mL-1	10,15625	639,84375	200
0 µg*mL-1	0,0	600	200
Sum	1218,75	2581,2	

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Preparation of biological patchoulol samples (in dodecane)

4
- pipette 198 µL sample to GC vial
- add each 2 µL **Stock B**

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