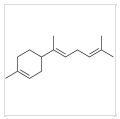

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(E)-α-bisabolene GC sample preparation

Dennis Dienst[1], Pia Lindberg[1], João Rodrigues[1]

[1]Department of Chemistry - Microbial Chemistry, Ångström Laboratory, Uppsala, Sweden

1 Works for me dx.doi.org/10.17504/protocols.io.mwdc7a6

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Dennis DD. Dienst
Department of Chemistry - Microbial Chemistry, Ångström Labo...

ABSTRACT

This is a quick guide for the preparation of (*E*)-α-bisabolene 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 (*E*)-α-bisabolene. 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 (*E*)-α-bisabolene in the samples of interest.

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

MATERIALS

NAME	CATALOG #	VENDOR
β-Caryophyllene ≥80%, FCC, FG	W225207	Sigma Aldrich
(−)-α-Bisabolol; analytical standard	95426	Alfa Aesar
HPLC/GC Vials 1.5 mL clear glass	548-1488	Vwr
Dodecane Reagent Grade ≥99%	D221104	

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 α-bisabolene standard stocks

2

	α- bisabolene standard
Stock I 200 µL	1:100 dilution from original Stock (⇒ 8.9 µg/µL in Stock C)
	⇓
Stock II 2 mL	179.6 µL **Stock I** + 1820.4 µL **Stock C** (⇒ 800 µg * mL^{-1})

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Preparation of α-bisabolene calibration curve

3 **Example: α-bisabolene external standard (ES) dilution series**:

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

High Range Dilution Series:			
	Vol. Stock 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	609,375	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	1229,6875	2610,9375	

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Preparation of biological α-bisabolene samples (in dodecane)

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

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