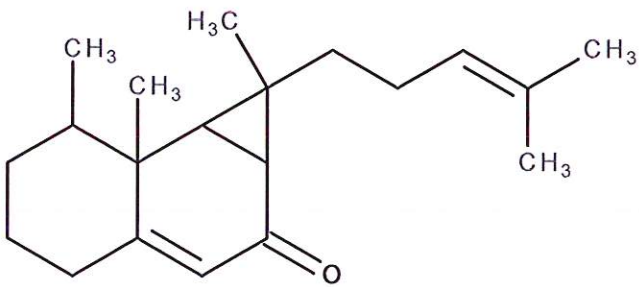


HBOI-381	
NICELLIN A	
$C_{20}H_{30}O$	286.45
<i>Nicella</i> sp.	

isolation scheme (Visio) attached

hplc method (trace attached)

Method: Vydac C18 Protein and Peptide, 4.6 x 250mm, 10 μ
 flow 1 ml/min, detection: PDA: UV (extracted at 220 nm, black); ELSD (green dotted)
 A: H₂O:CH₃CN (95:5, v/v), B: CH₃CN
 t=0 min A:B (90:10, v/v), t=20 min (100%B) , t=28 min (100%B)

LC-MS method

Method: Vydac C18 Protein and Peptide, 2.1x150 mm, flow 0.2 ml/min
 A: H₂O (0.1% formic acid), B:CH₃CN (0.1% formic acid)
 t= 0 min A:B (90:10, v/v), t=15 min (100%B), t=21 min (100%B), t=22.1 min A:B(90:10, v/v)
 using a linear gradient

¹H (600 MHz) CDCl₃ CD₃OD (Methanol-d₄) CDCl₃/CD₃OD

¹³C (150 MHz) CDCl₃ CD₃OD (Methanol-d₄) CDCl₃/CD₃OD

solubility CHCl₃/MeOH (9:1) MeOH DMSO

estimated purity >90%

sample weight 19.5 mg

For further information contact:

Amy E. Wright, PhD, HBOI@FAU, 5600 US 1, North, Fort Pierce, FL 34946
awrigh33@hboi.fau.edu, 772-242-2459

Date: 1/29/13

6-VI-93-1-002
NICELLA SPECIES
 100 g of sample extracted / 56 g of sample remaining
 Inner "bones" crushed & cut into manageable pieces for blender.
 Ground & extracted 2x w/250ml EtOAc:EtOH 9:1,
 2x w/250ml EtOH, 2x w/250ml MeOH
 GARJ 1-3-1 2.915 g

2.9 g partitioned 3x w/EtOAc : H₂O

GARJ 1-3-2
 EtOAc partition
 0.7632 g

~0.5942 g run on 12 g NP Rf Gold column, CV 16.8 ml, 30 ml/min
 CombiFlash Companion 50 psi system
 Heptane to DCM gradient; Wash 1: DCM to MeOH 75:25
 Wash 2: DCM to MeOH 1:1; Wash 3: MeOH

GARJ 1-3-
 Weight
 Tubes 6
 377.2 mg
 9-25

A portion of this fraction was brought to 73 mg/ml
 solution in MeOH for further purification via Waters Prep
 LC-MS system. Luna C18, 19 X 150mm column.

Flow: 23.5 ml/mn
 A: H₂O:ACN 95:5
 B: ACN 100%
 100% A for 0.5 min., 100% B by 8 min., hold at
 100%B to 12 min., collection stops at 12 min.,
 tube size 16/150 mm
 Five runs

05AW 19.2 20,44,68	04AW 0.5 19,43,67	03AW 0.7 18,42,66	02AW 0.9 17,41,65	01AW 0.8 16,40,64	Sam30-82- weight (mg) tubes	Day 1 runs
08AW 1.2 22-24,46-48,70-72		07AW 2.7 1-15,25-39,49-63		06AW 1.6 21,45,69	Sam30-82- weight (mg) tubes	
05AW 19.2 20,44	04AW 0.5 19,43	03AW 0.7 18,42	02AW 0.9 17,41	01AW 0.8 16,40	Sam30-83- weight (mg) tubes	Day 2 runs
08AW 1.2 22-24,46-48		07AW 2.7 1-15,25-39		06AW 1.6 21,45	Sam30-83- weight (mg) tubes	

Samples Sam30-82-05AW
 and Sam30-83-05AW
 were combined to give **Sam30-84-01AW**
 HPLC/ELSD, LC-MS, proton and Carbon NMR
 were performed on this sample

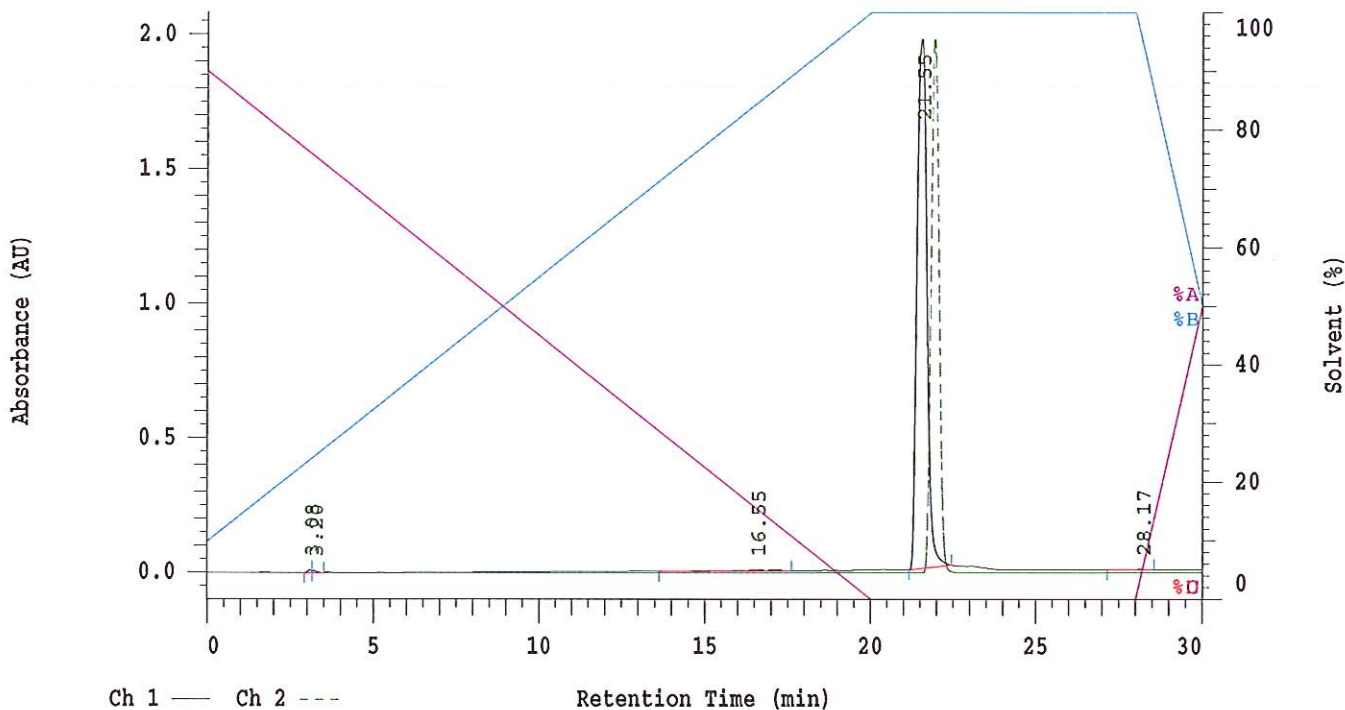
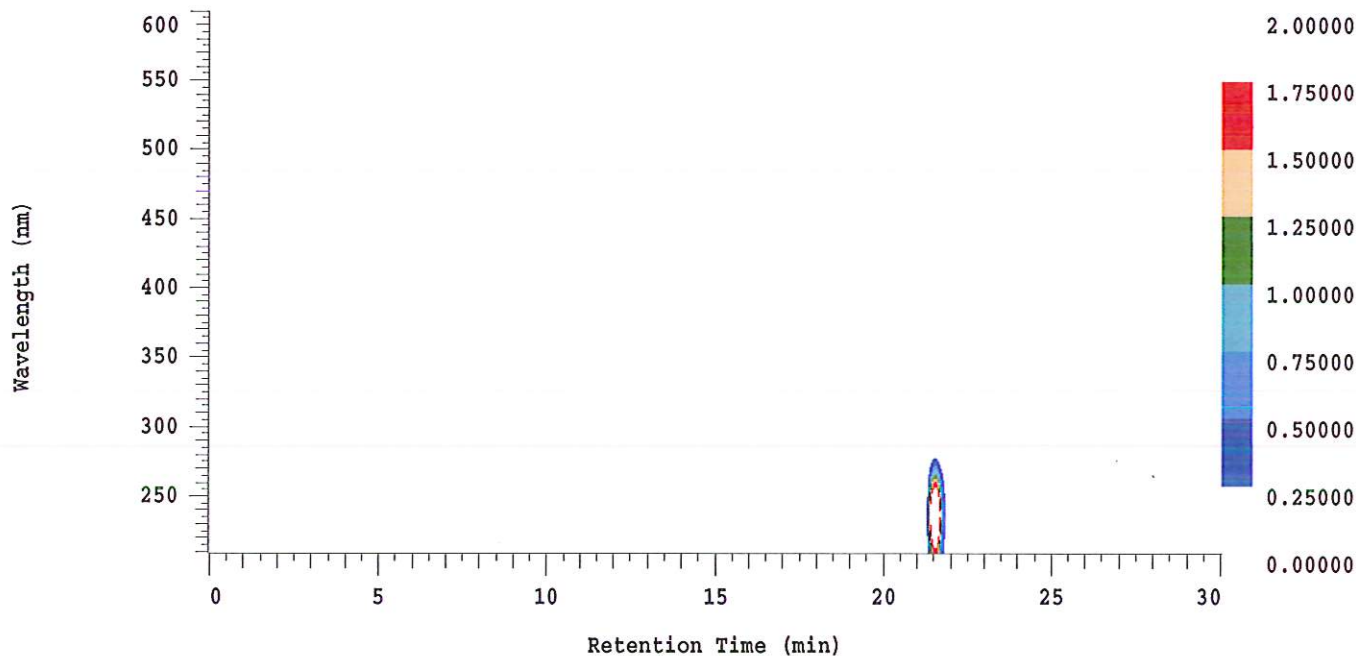
D-2000 Elite HPLC System Manager Report

Analyzed: 01/24/2013 03:18 PM

Reported: 01/24/2013 04:15 PM

Sample Name: HBOI-381

Sample Description: HBOI-381



Acquisition Method: autosamp_30mn_UV220_wELSD

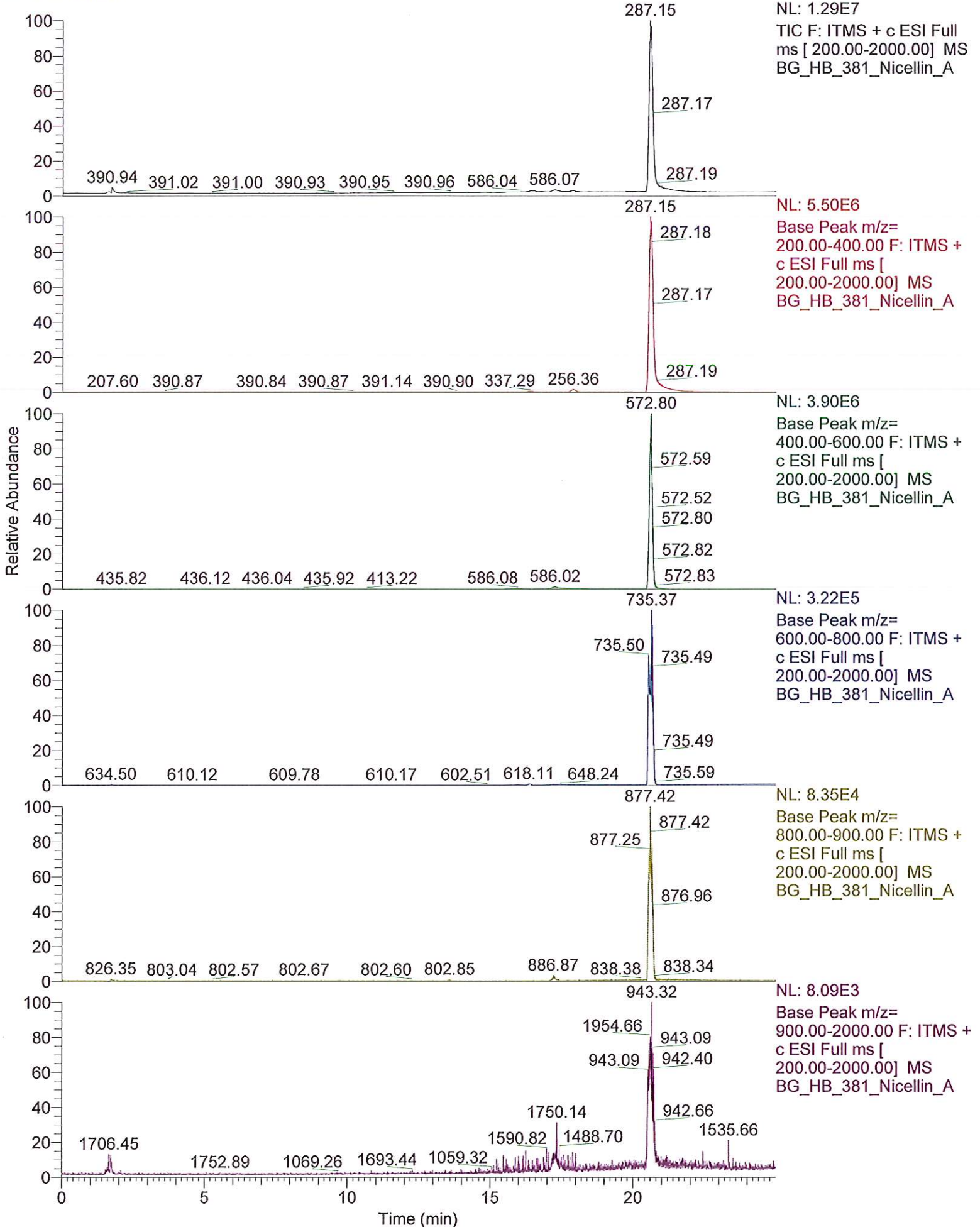
Column Type: Vydac C18

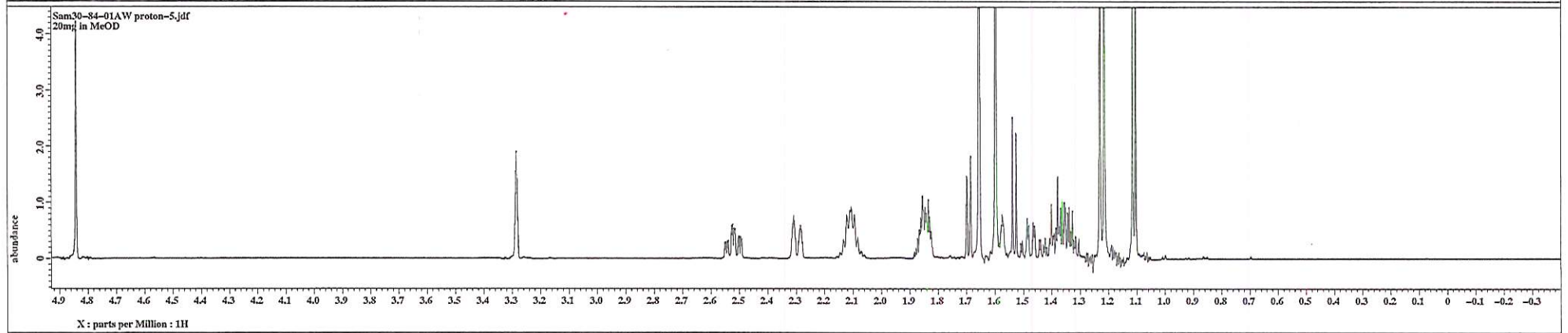
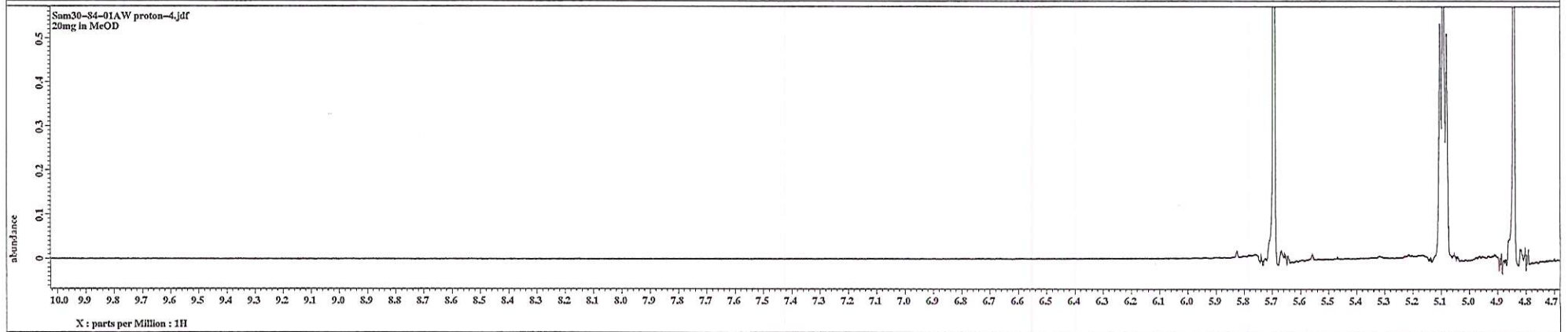
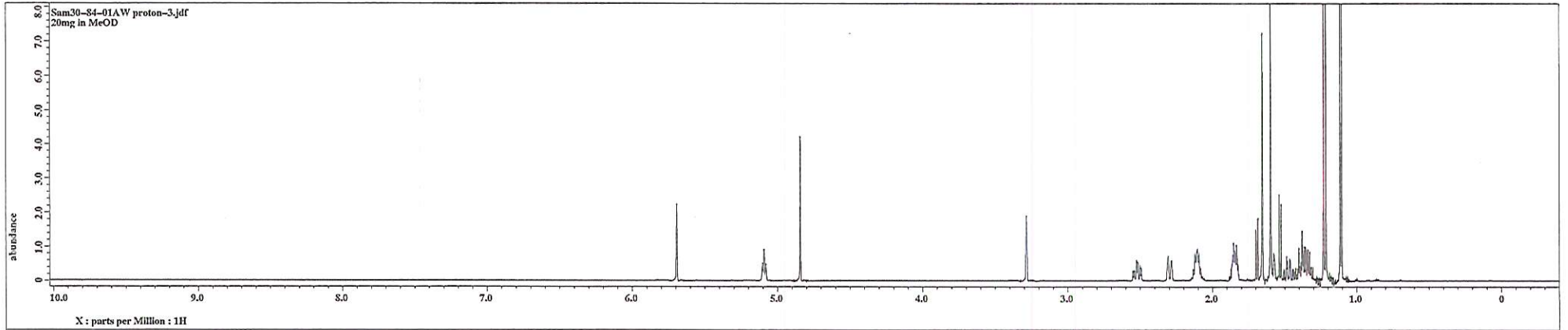
Pump A Solvent A: H2O/5% ACN

Pump A Solvent B: ACN

Method Description:

RT: 0.00 - 24.99





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