

Supplementary Material

Method development for isolation and purification of Z-Guggulsterone, Dihydroguggulsterone, and Progesterone from guggul resin using RP-HPLC

Rohit Singh,^{a,b,c} Astbhuja Mishra,^{a,b} Ramanand Prajapati,^{a,d} and T. Narender^{a,b}

^aMedicinal & Process Chemistry, CSIR-Central Drug Research Institute, Lucknow (U.P)-226031, India

^bAcademy of Scientific & Innovative Research (AcSIR), Ghaziabad (U.P)-201002, India

^cDepartment of Pharmaceutical Chemistry, College of Pharmacy, JSS Academy of Technical Education, C-1/A, Sector-62, Noida, (U.P)-201309, India

^dChemistry Innovation Research Center, Jubilant Biosys Ltd, Greater Noida, Uttar Pradesh-201310, India

Email: t_narendra@cdri.res.in

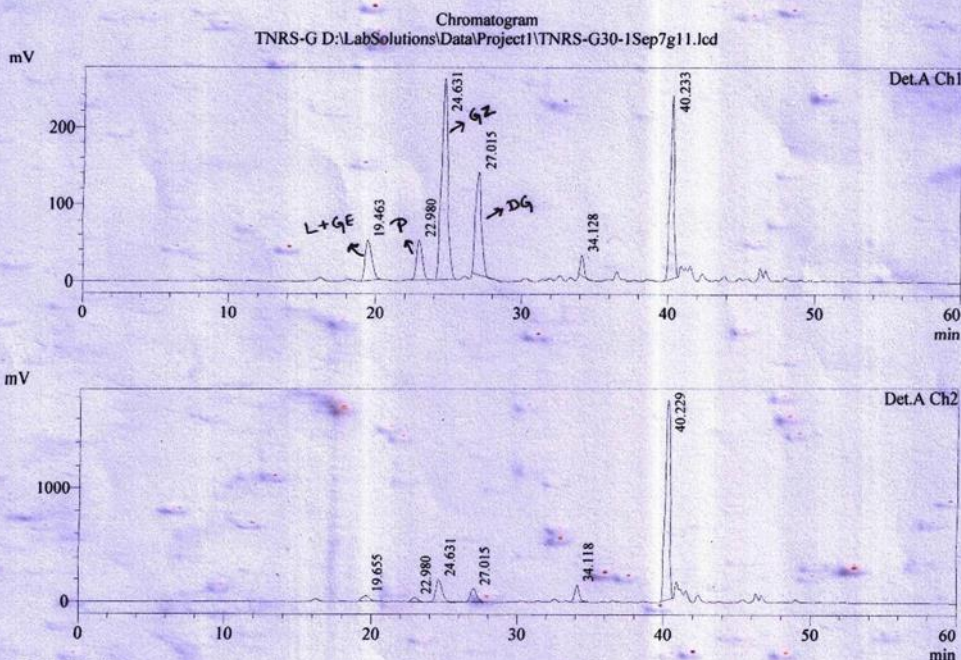
Table of Contents

NMR and LCMS spectra of the isolated compounds	S2
--	----

MPC Division, C D R I

Sample Information

Acquired by : Ramjeet
 Sample Name : TNRS-G
 Data Filename : TNRS-G30-1Sep7g11.lcd
 Method Filename : RAMJEET-TN.lcm
 Date Acquired : 9/7/2017 12:31:05 PM



1 Det.A Ch1 / 255nm
 2 Det.A Ch2 / 225nm

Detector A Ch1 255nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	19.463	1576998	50113	8.236	6.674
2	22.980	1335866	49611	6.976	6.607
3	24.631	7684776	257714	40.133	34.323
4	27.015	3709114	132626	19.370	17.663
5	34.128	409143	24935	2.137	3.321
6	40.233	4432447	235850	23.148	31.411
Total		19148343	750849	100.000	100.000

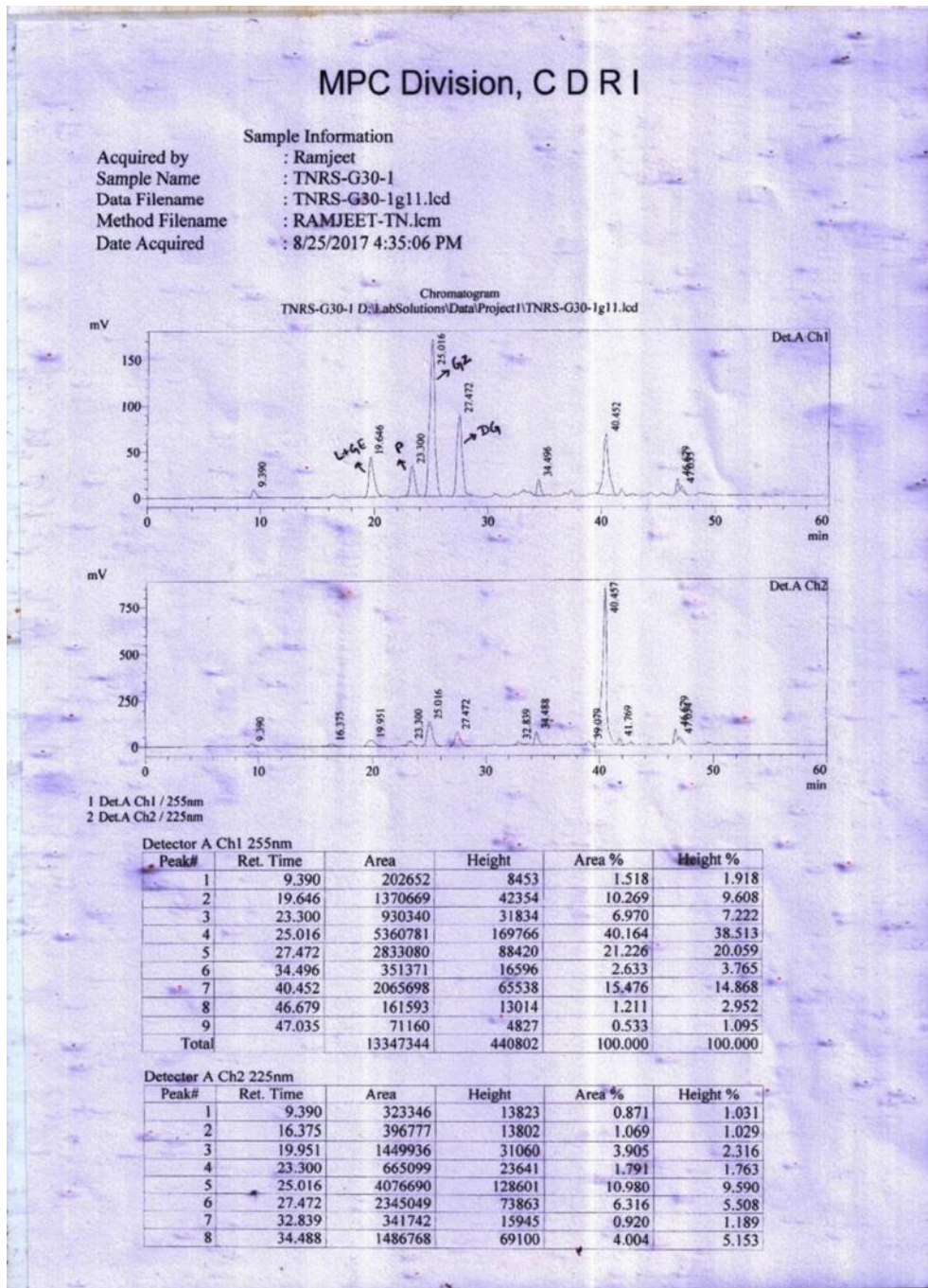
Detector A Ch2 225nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	19.655	1401614	44464	2.607	1.962
2	22.980	946185	36495	1.760	1.610
3	24.631	5771190	193209	10.734	8.523
4	27.015	3073663	109562	5.717	4.833
5	34.118	3007688	140822	5.594	6.212
6	40.229	39564466	1742291	73.588	76.860
Total		53764806	2266844	100.000	100.000

HPLC chromatogram of Enriched fraction (Batch 1st)

(Note: L+GE: 3',3''-dimethoxysesamin and *E*-Guggulsterone isomer; P: Progesterone; GZ: *Z*-Guggulsterone isomer; DG: Dihydroguggulsterone)

1



2

3

4

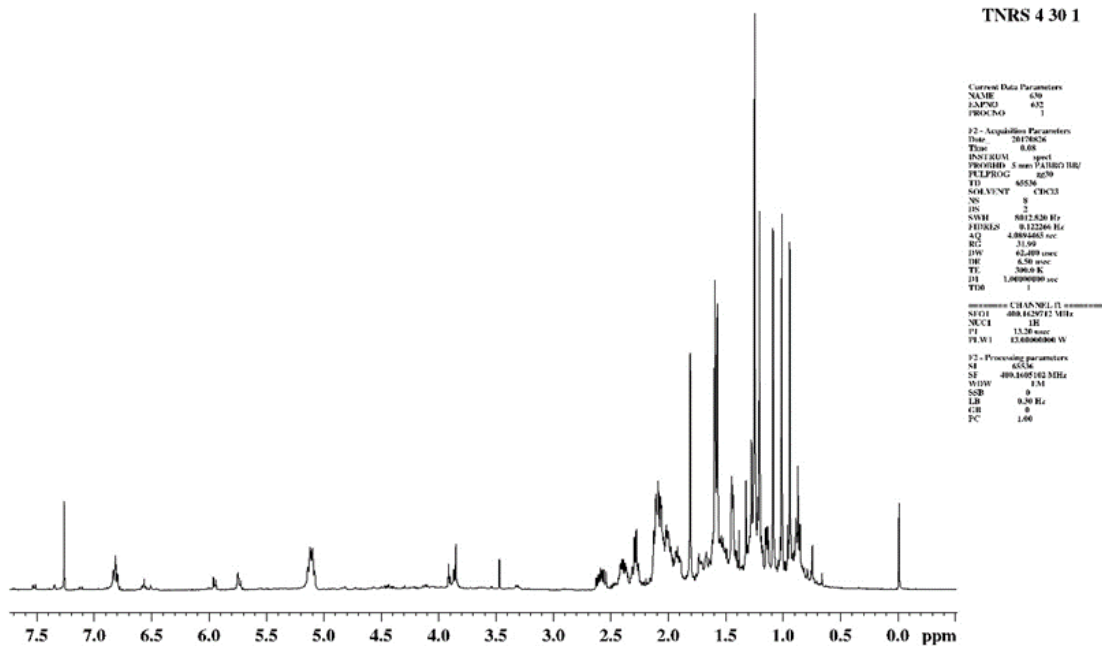
5

Peak#	Ret. Time	Area	Height	Area %	Height %
9	39.079	308773	15651	0.832	1.167
10	40.457	24042595	839872	64.753	62.634
11	41.769	568761	31847	1.532	2.375
12	46.679	814438	63413	2.194	4.729
13	47.034	309480	20301	0.834	1.514
Total		37129453	1340918	100.000	100.000

HPLC chromatogram of Enriched fraction (Batch 2nd)

(Note: L+GE: 3',3''-dimethoxysesamin and E-Guggulsterone isomer; P: Progesterone; GZ: Z-Guggulsterone isomer; DG: Dihydroguggulsterone)

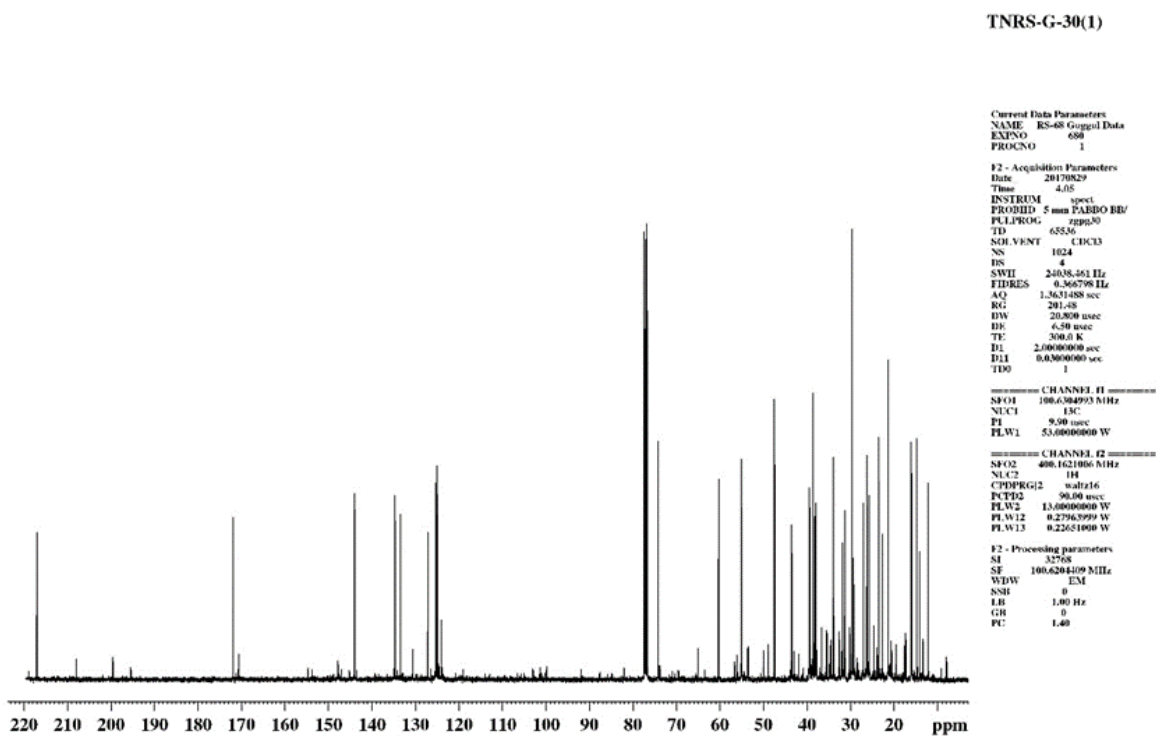
6

¹H-NMR Spectra of Enriched Fraction (CDCl₃, 400 MHz)

7

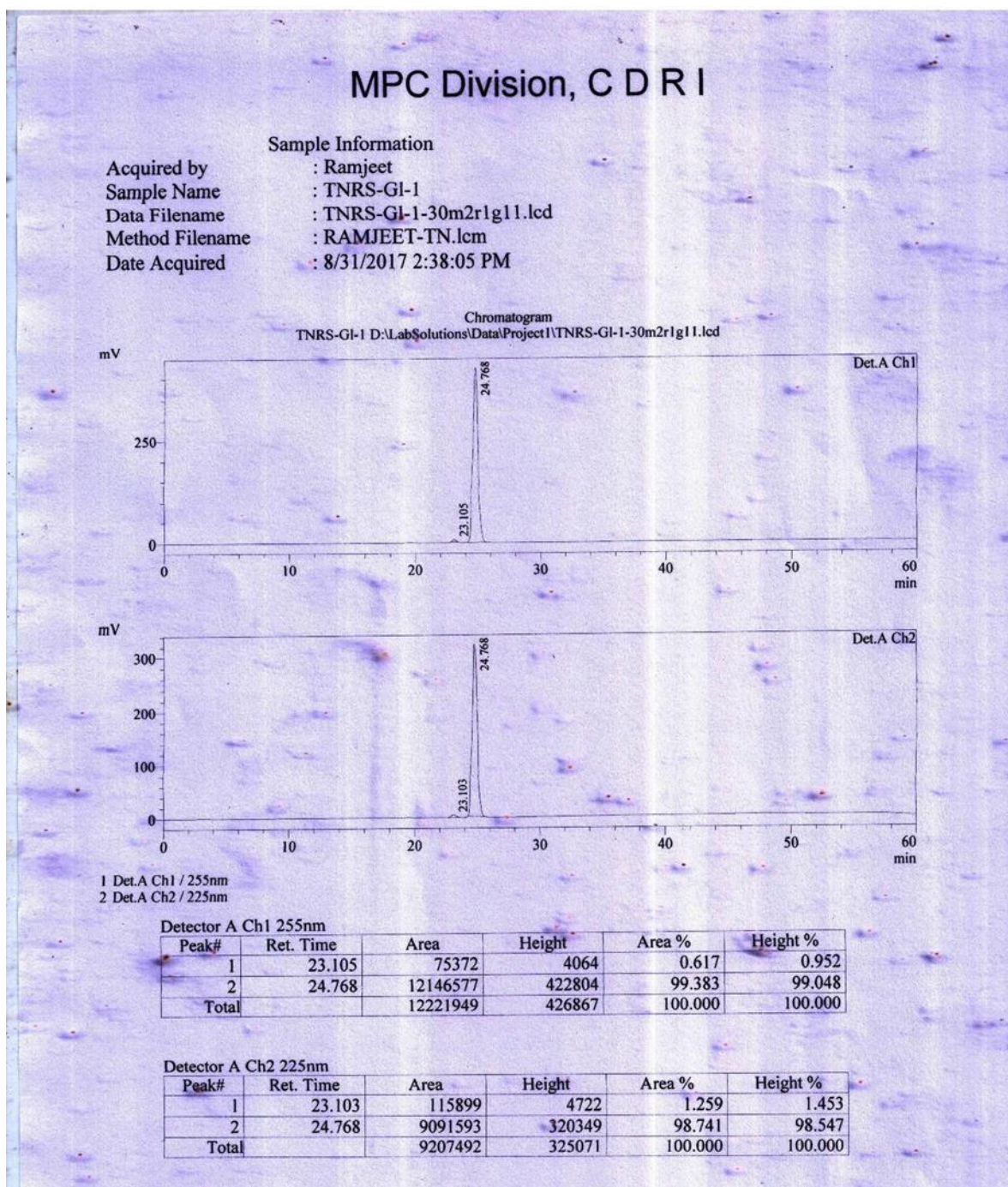
8

9

¹³C-NMR Spectra of Enriched Fraction (CDCl₃, 100 MHz)

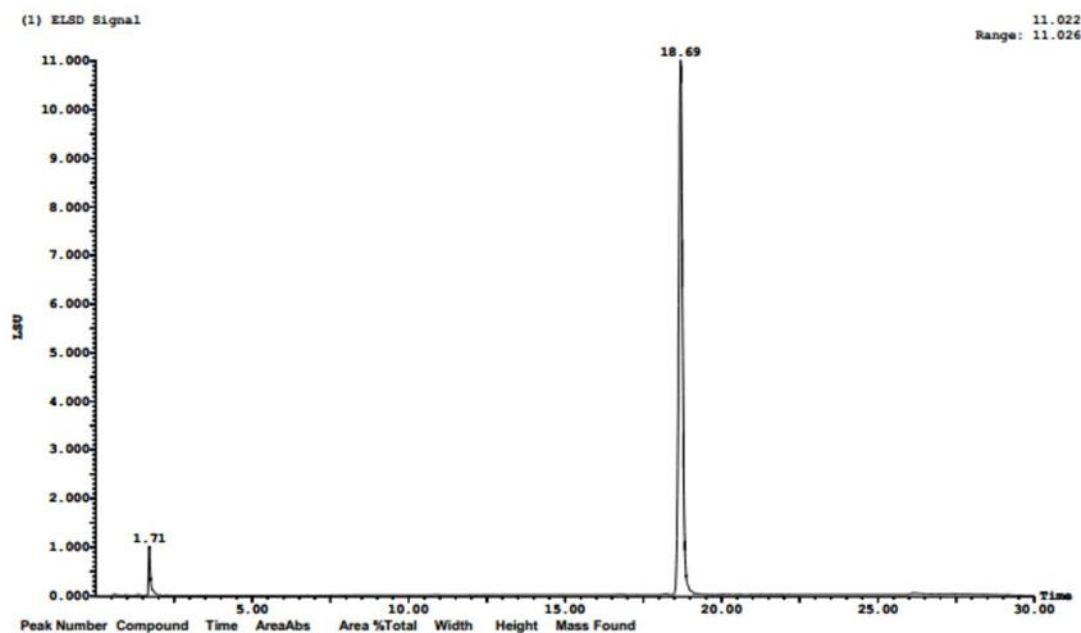
10

11



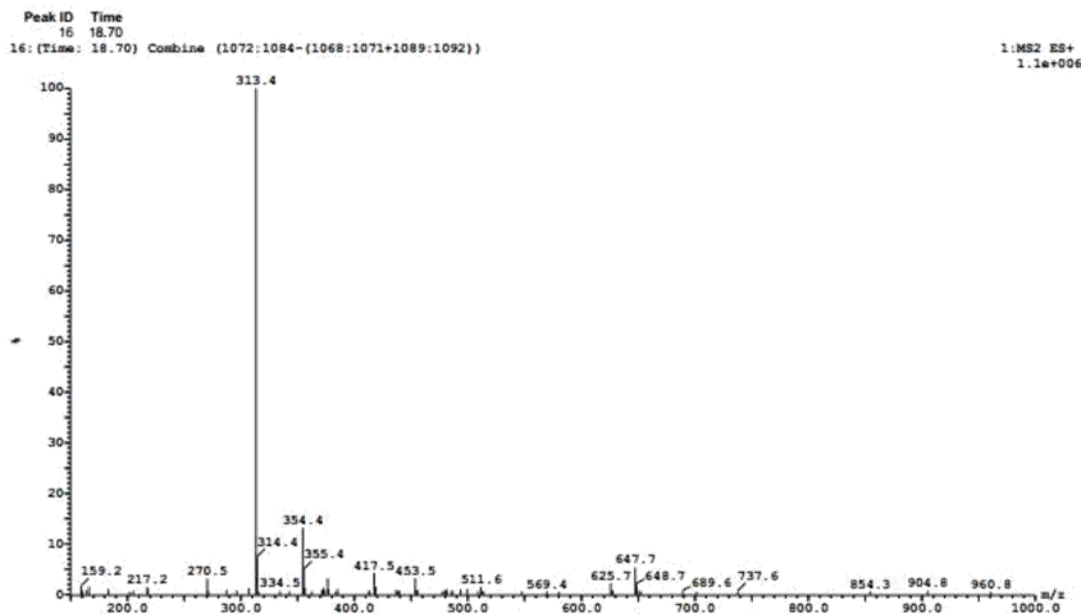
HPLC chromatogram of Z-Guggulsterone isomer

Openlynx Report SAIF, CSIR-CDRI, Lucknow
 Sample: 3 Vial:2:19 ID:TNR5-G-M2 [T.NARENDRA] Page 1
 File:17ISEP03 Date:04-Sep-2017 Time:16:17:45
 Description:SUNFIRE C-18, 250X4.6, 5um
 Printed: Tue Sep 05 09:43:47 2017

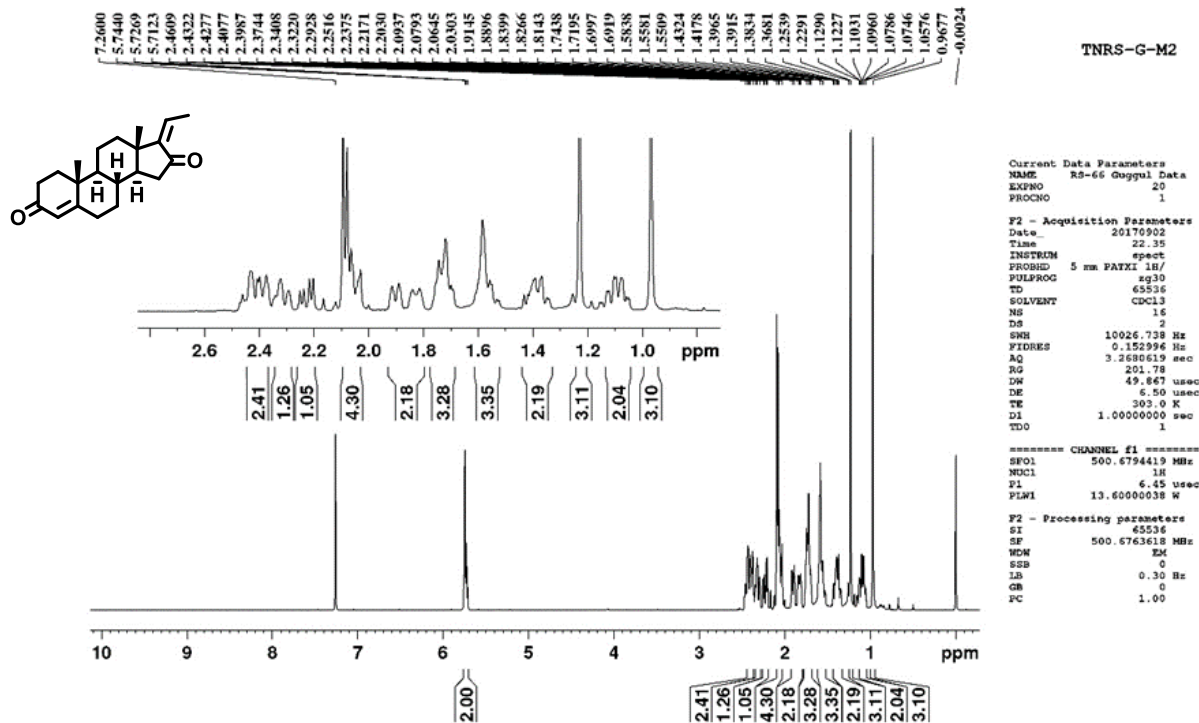


LC-MS chromatogram of Z-Guggulsterone isomer

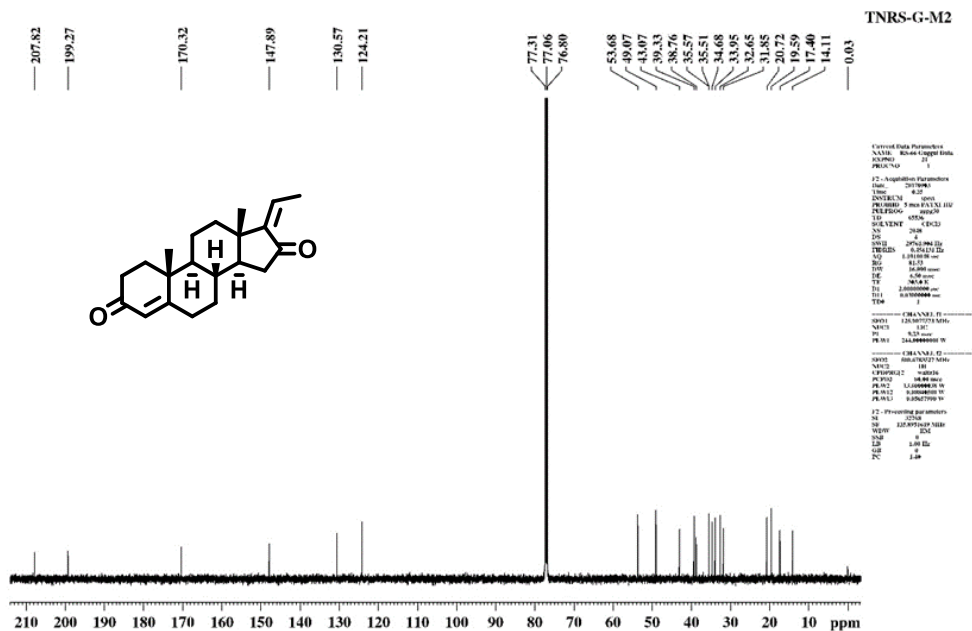
Openlynx Report SAIF, CSIR-CDRI, Lucknow
 Sample: 3 Vial:2:19 ID:TNR5-G-M2 [T.NARENDRA] Page 19
 File:17ISEP03 Date:04-Sep-2017 Time:16:17:45
 Description:SUNFIRE C-18, 250X4.6, 5um
 Printed: Tue Sep 05 09:43:47 2017



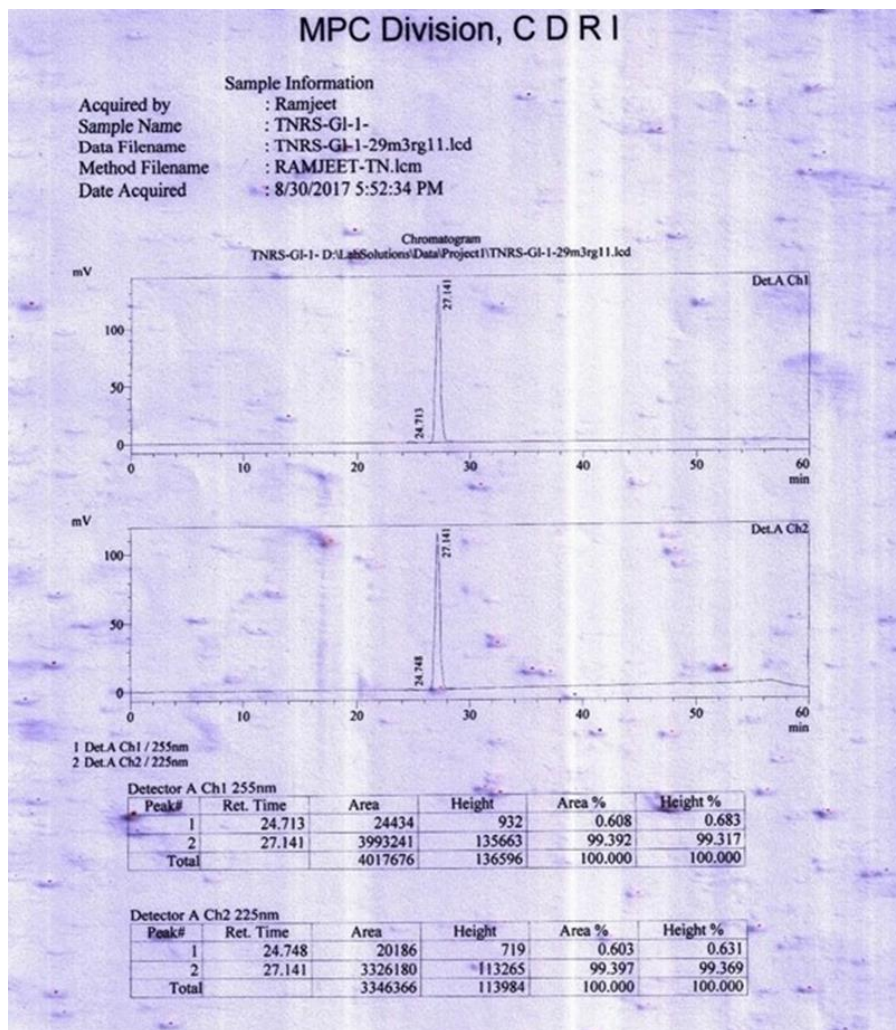
LC-MS chromatogram of Z-Guggulsterone isomer



¹H-NMR Spectra of Z-Guggulsterone (CDCl₃, 501 MHz)



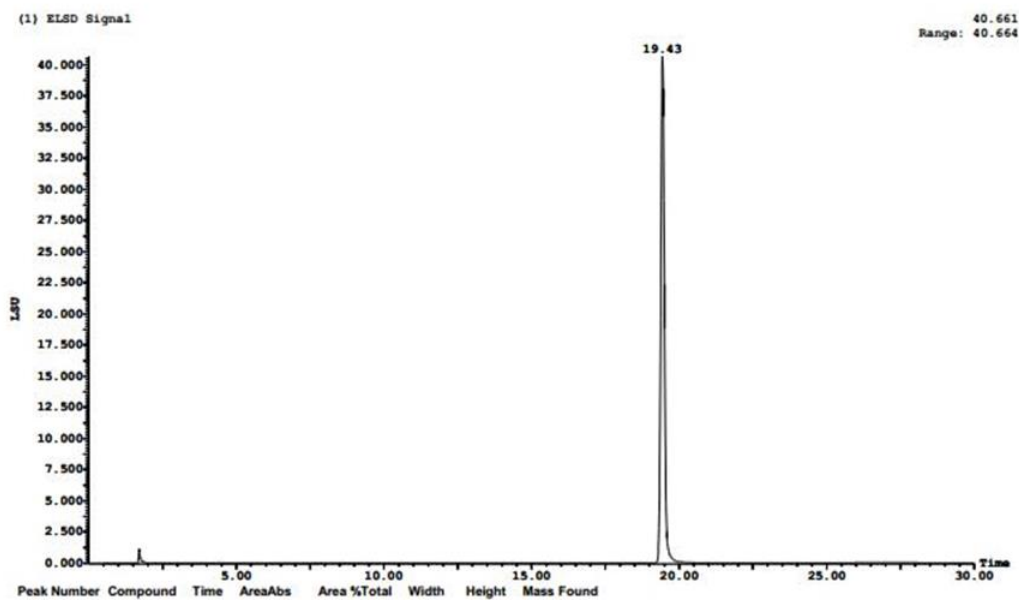
¹³C-NMR Spectra of Z-Guggulsterone (CDCl₃, 126 MHz)



HPLC Chromatogram of Dihydroguggulsterone

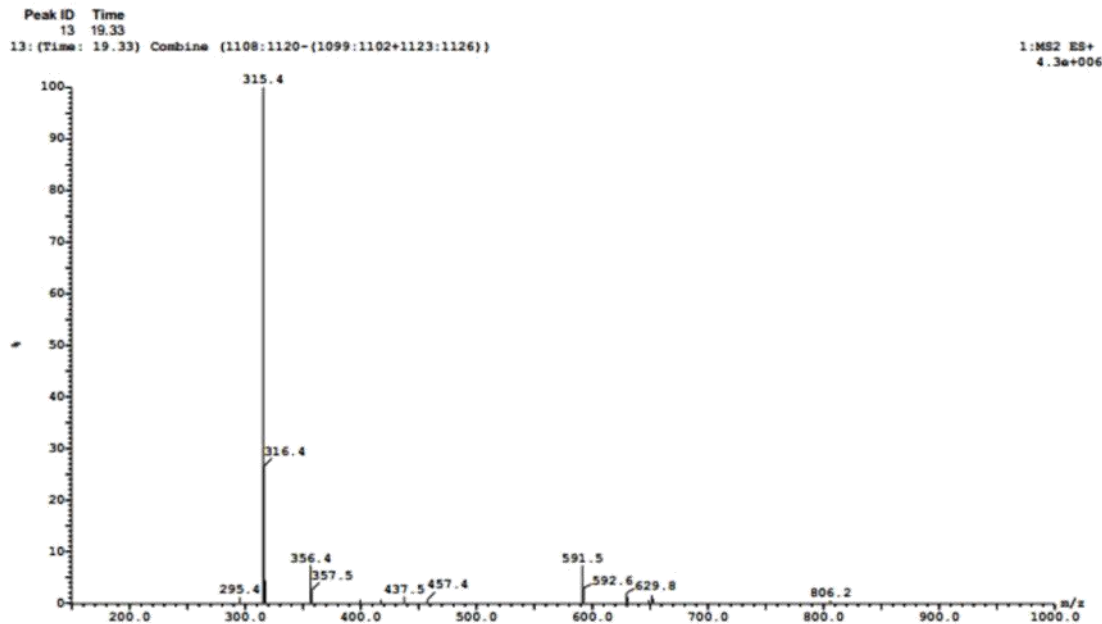
22
23

Openlynx Report SAIF, CSIR-CDRI, Lucknow
 Sample: 4 Vial:2:20 ID:TNRS-G-M3 [T.NARENDRA] Page 1
 File:17ISEP04 Date:04-Sep-2017 Time:16:49:05
 Description:SUNFIRE C-18, 250X4.6, 5um
 Printed: Tue Sep 05 09:44:29 2017

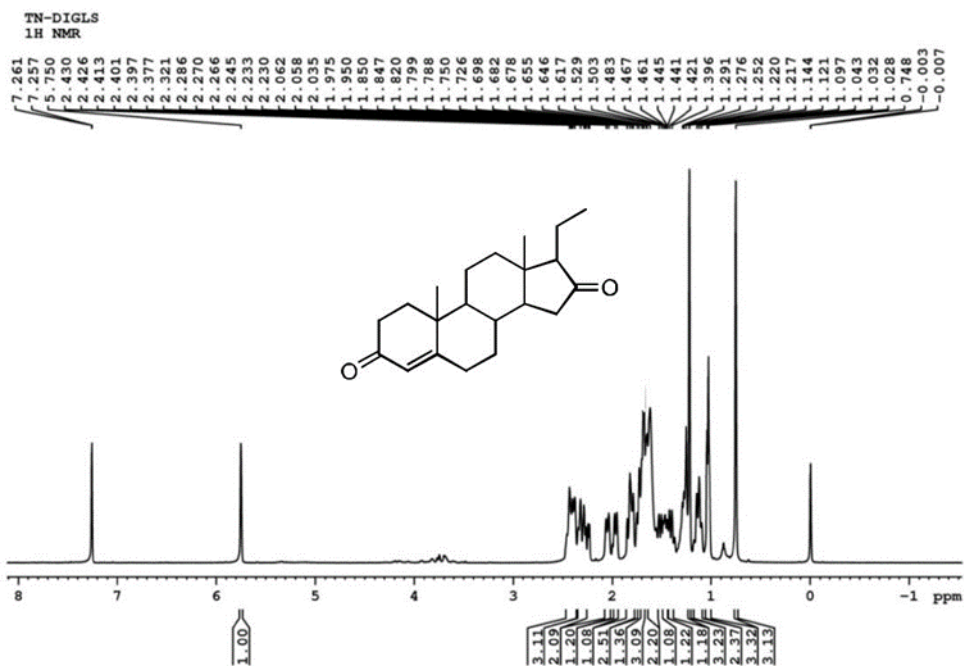


LC-MS chromatogram of Dihydroguggulsterone

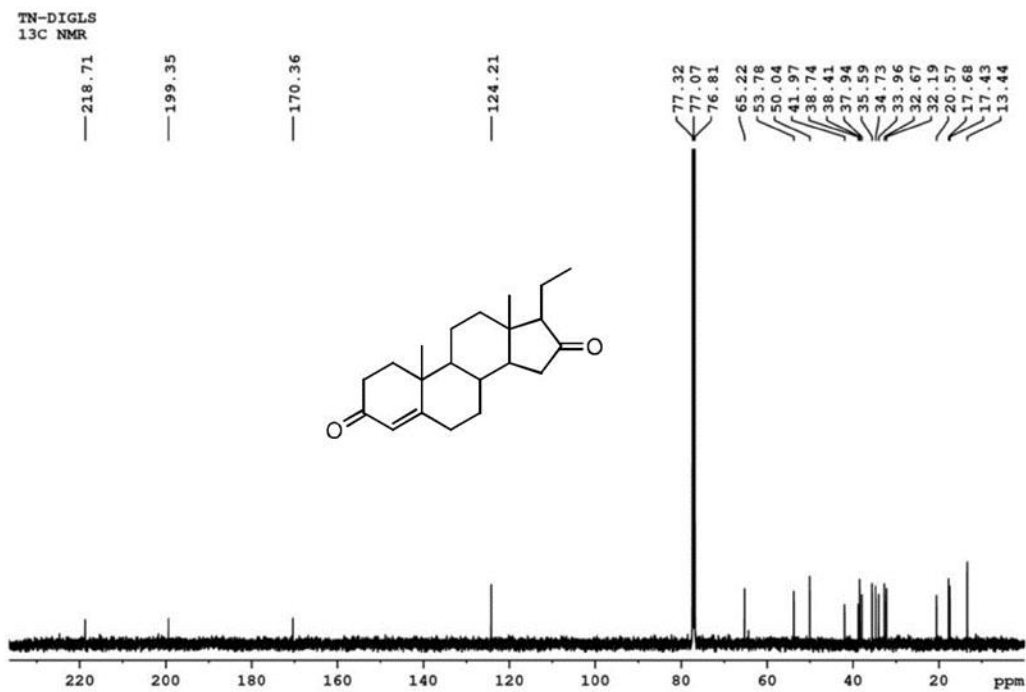
Openlynx Report SAIF, CSIR-CDRI, Lucknow
 Sample: 4 Vial:2:20 ID:TNRS-G-M3 [T.NARENDRA] Page 16
 File:17ISEP04 Date:04-Sep-2017 Time:16:49:05
 Description:SUNFIRE C-18, 250X4.6, 5um
 Printed: Tue Sep 05 09:44:29 2017



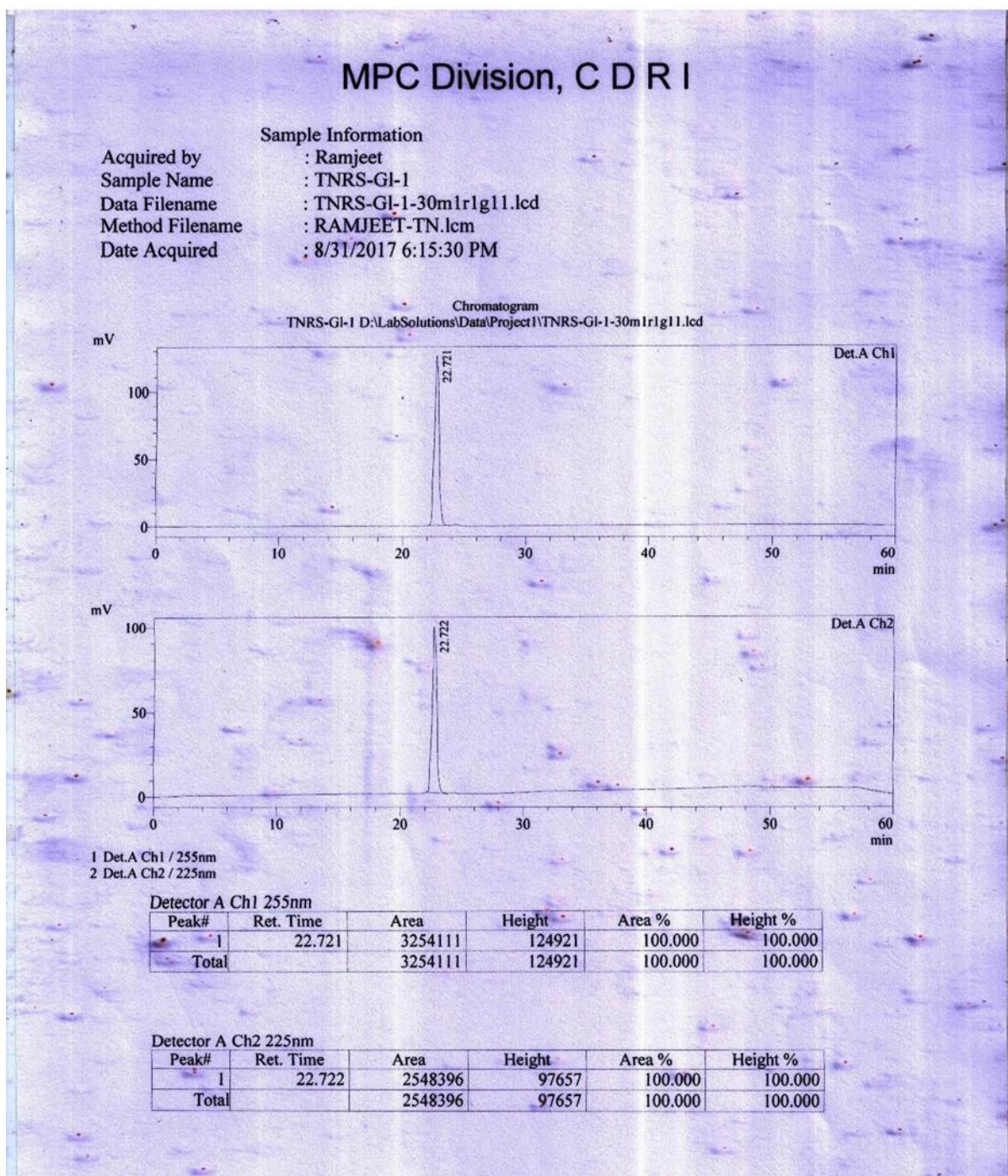
LC-MS chromatogram of Dihydroguggulsterone



¹H NMR Spectra of Dihydroguggulsterone (CDCl₃)



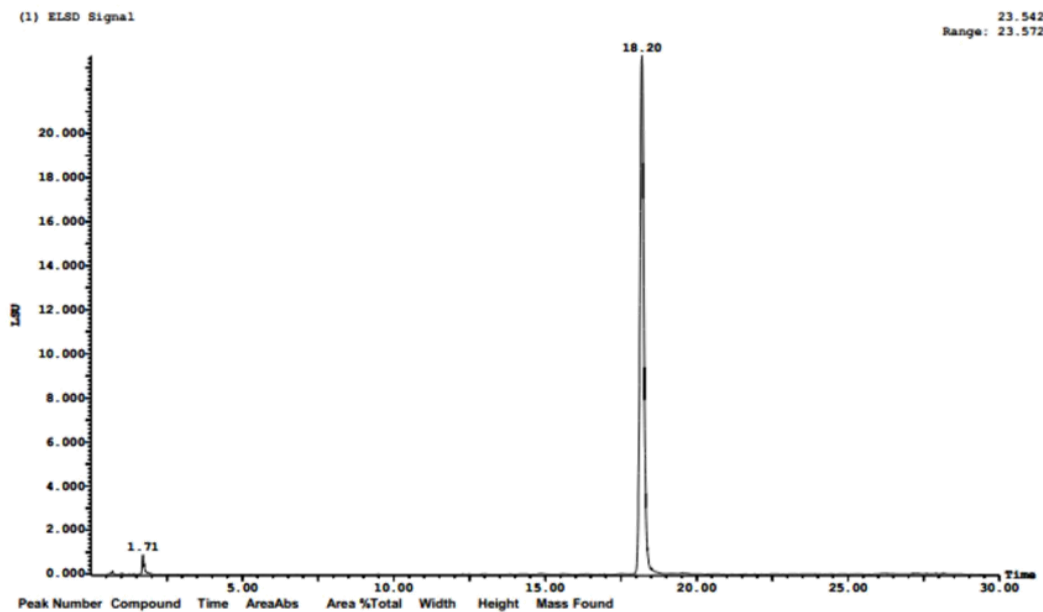
¹³C NMR Spectra of Dihydroguggulsterone (CDCl₃)



HPLC chromatogram of Progesterone

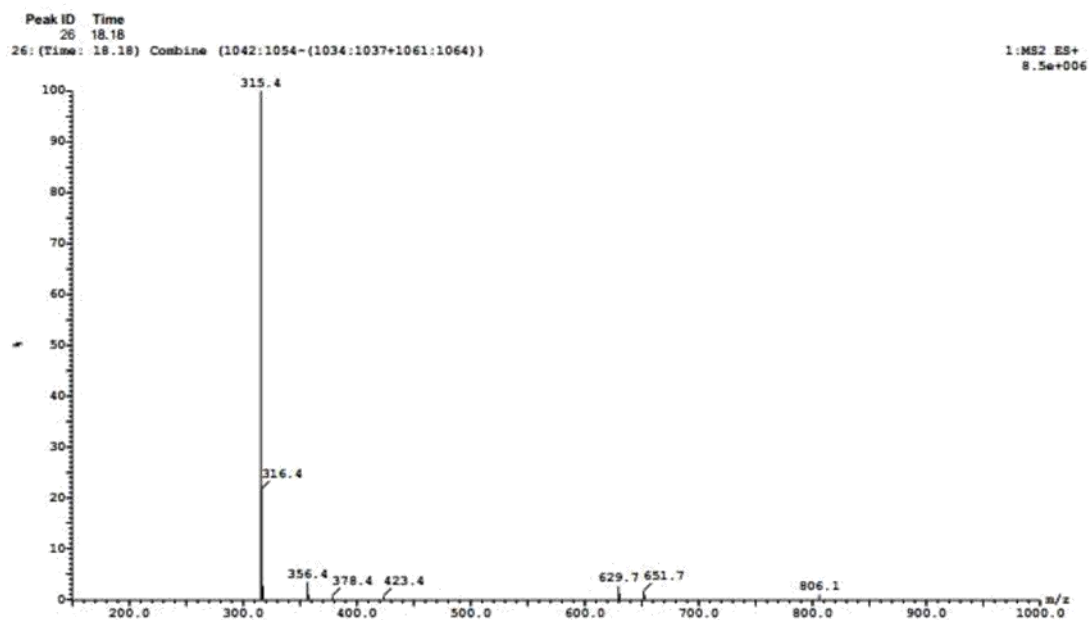
32
 33

Openlynx Report SAIF, CSIR-CDRI, Lucknow
 Sample: 2 Vial:2:18 ID:TNRSG-M1 [T.NARENDRA] Page 1
 File:17ISEP02 Date:04-Sep-2017 Time:15:40:42
 Description:SUNFIRE C-18, 250X4.6, 5um
 Printed: Tue Sep 05 09:41:01 2017

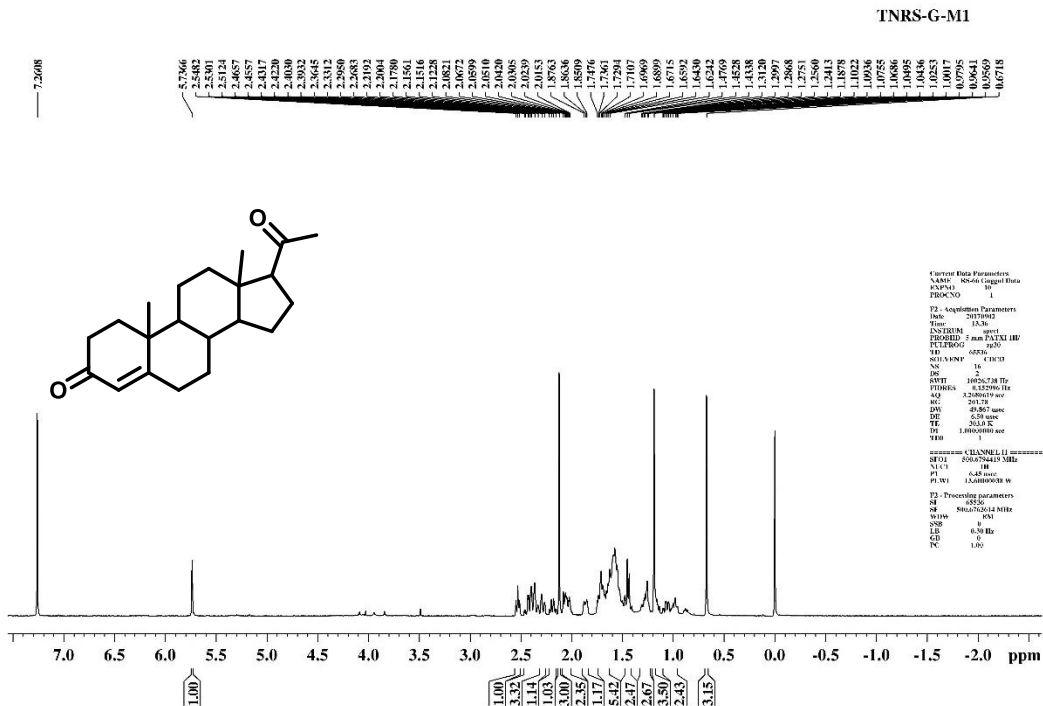


LC-MS chromatogram of Progesterone

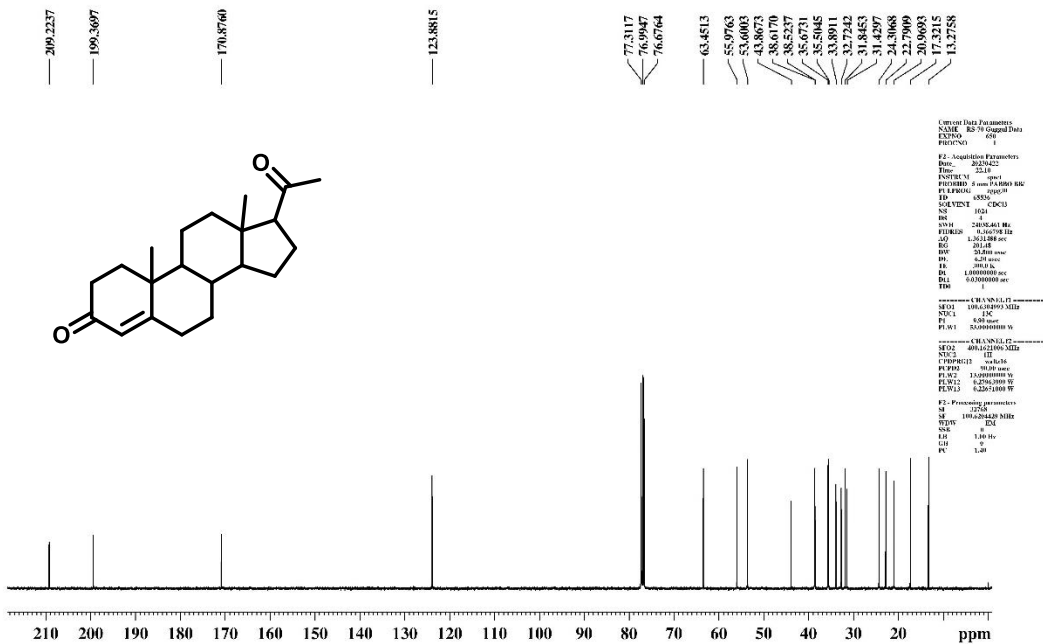
Openlynx Report SAIF, CSIR-CDRI, Lucknow
 Sample: 2 Vial:2:18 ID:TNRSG-M1 [T.NARENDRA] Page 23
 File:17ISEP02 Date:04-Sep-2017 Time:15:40:42
 Description:SUNFIRE C-18, 250X4.6, 5um
 Printed: Tue Sep 05 09:41:01 2017



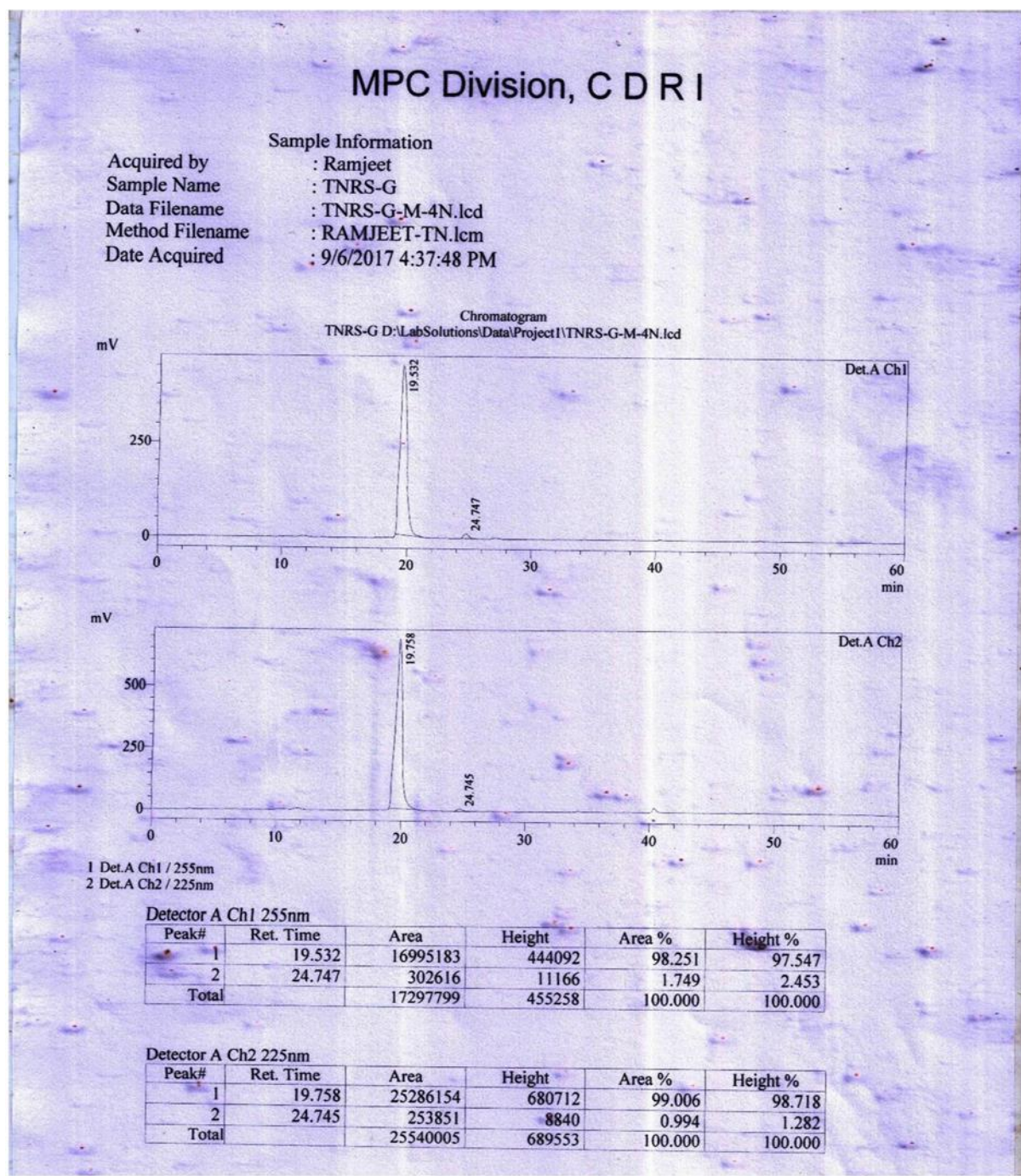
LC-MS chromatogram of Progesterone



¹H NMR Spectra of Progesterone (CDCl₃)

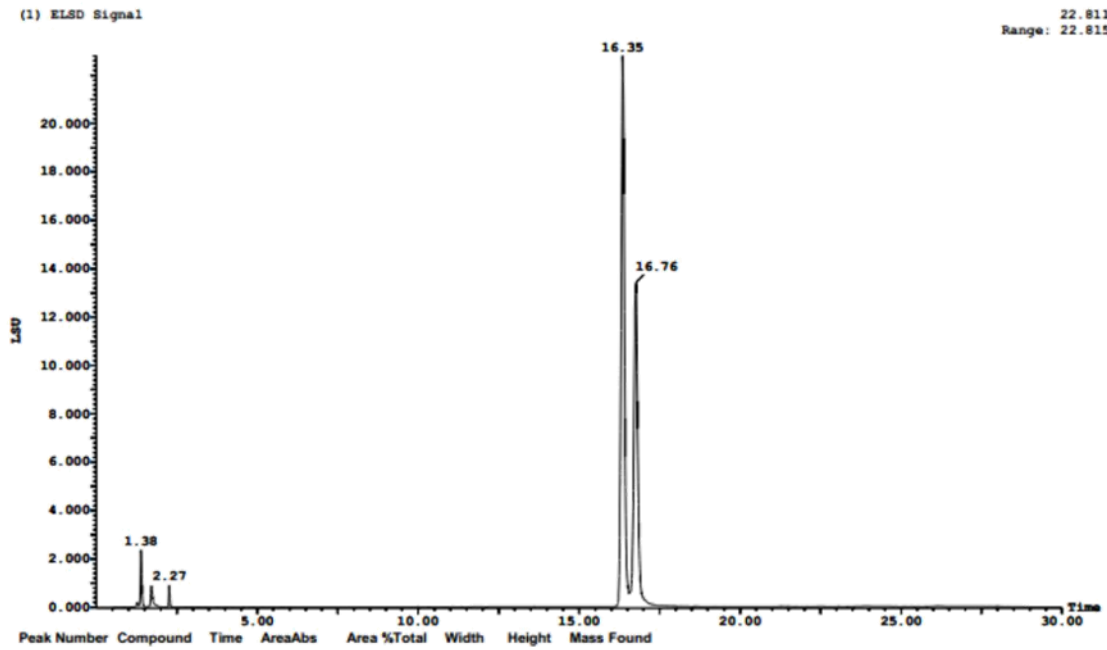


¹³C NMR Spectra of Progesterone (CDCl₃)



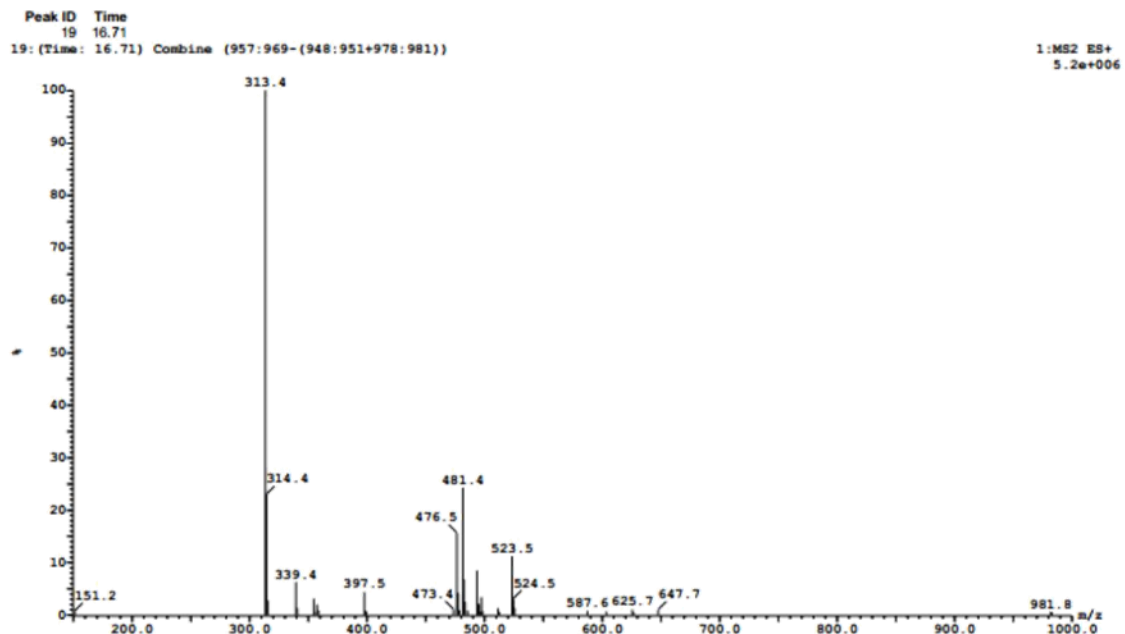
HPLC chromatogram of *E*-Guggulsterone isomer and 3',3''-dimethoxysesamin mixture

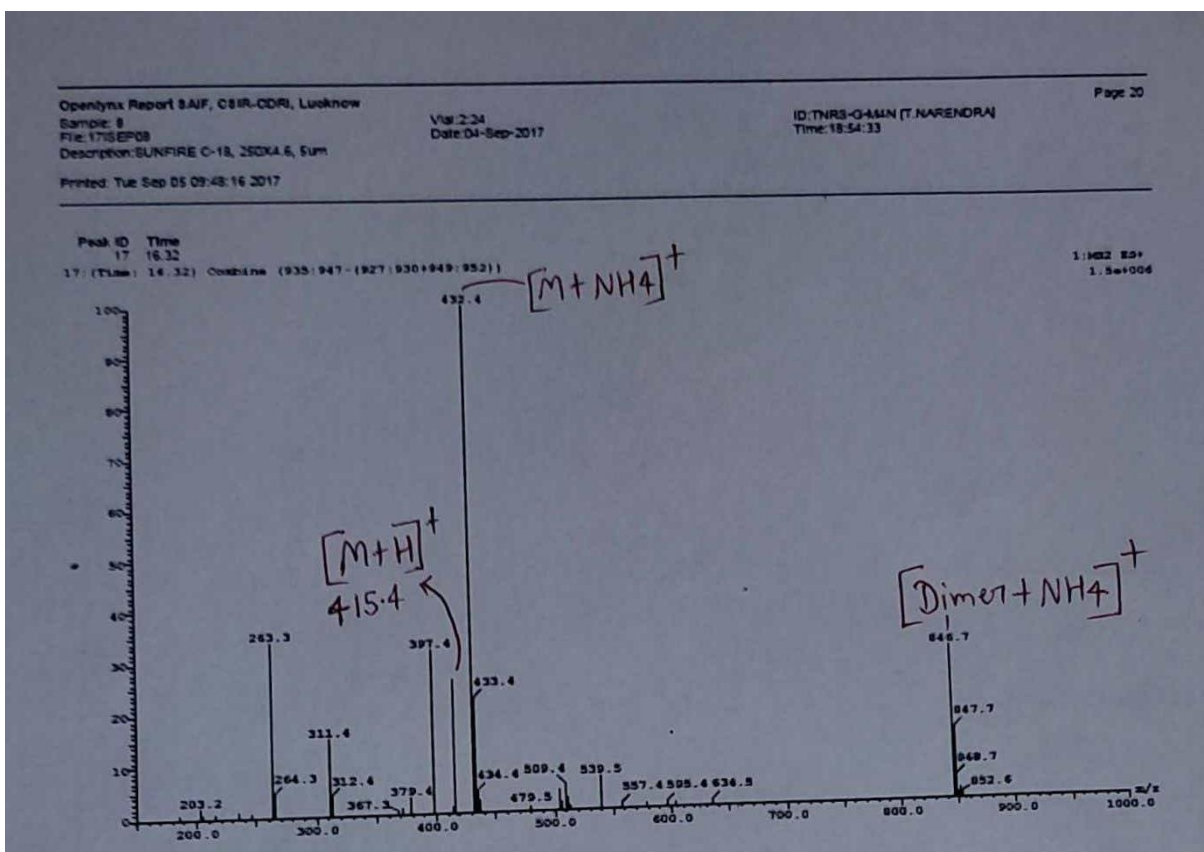
Openlynx Report SAIF, CSIR-CDRI, Lucknow
 Sample: 8
 File:17ISEP08
 Description:SUNFIRE C-18, 250X4.6, 5um
 Vial:2:24
 Date:04-Sep-2017
 ID:TNR5-G-M4N [T.NARENDRA]
 Time:18:54:33
 Page 1
 Printed: Tue Sep 05 09:48:16 2017



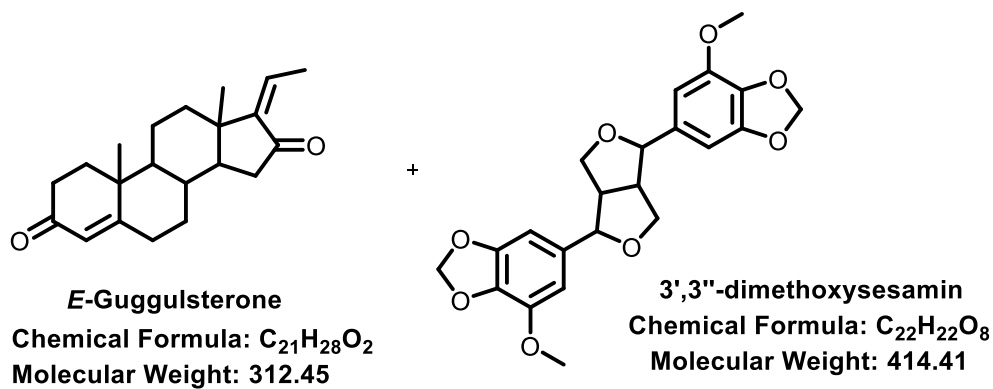
LC-MS of *E*-Guggulsterone and 3',3''-dimethoxysesamin mixture

Openlynx Report SAIF, CSIR-CDRI, Lucknow
 Sample: 8
 File:17ISEP08
 Description:SUNFIRE C-18, 250X4.6, 5um
 Vial:2:24
 Date:04-Sep-2017
 ID:TNR5-G-M4N [T.NARENDRA]
 Time:18:54:33
 Page 22
 Printed: Tue Sep 05 09:48:16 2017

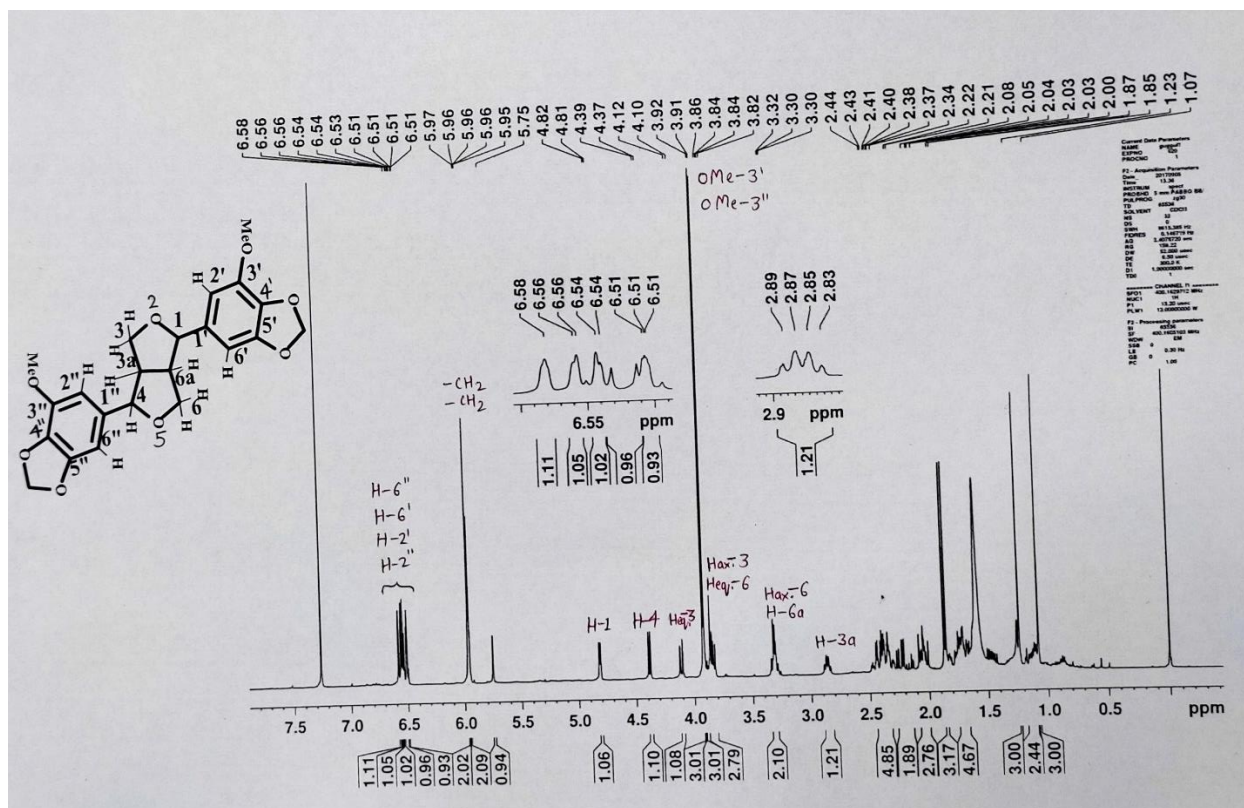




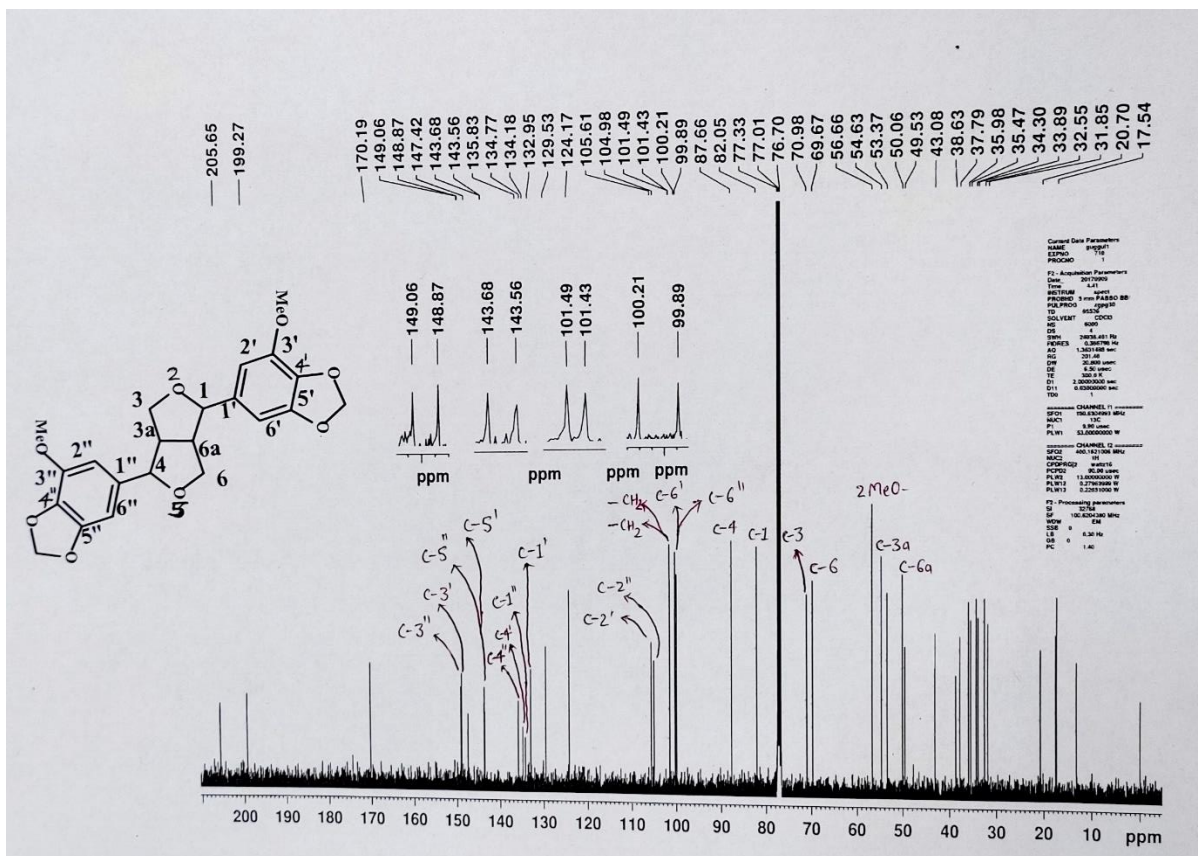
LC-MS of *E*-Guggulsterone and 3',3''-dimethoxysesamin mixture



E-Guggulsterone and 3',3''-dimethoxysesamin mixture



¹H-NMR Spectra of *E*-Guggulsterone and 3',3''-dimethoxysesamin mixture (CDCl₃)



¹³C-NMR Spectra of *E*-Guggulsterone and 3',3''-dimethoxysesamin mixture (CDCl₃)