Electronic Supplementary Information

East Indian sandalwood (*Santalum album* L.) oil confers neuroprotection and geroprotection in *Caenorhabditis elegans* via activating SKN-1/Nrf2 signaling pathway

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Table 1

Genotype	Gene description	Phenotype
N2		Wild type
mev-1 (*kn1*)	*mev-1* encodes succinate dehydrogenase cytochrome b560 subunit of mitochondrial respiratory chain complex II, which is essential for oxidative phosphorylation	Stress and oxygen sensitive. Short life span
isp-1 (*qm150*)	*isp-1* encodes a Rieske iron sulphur protein (ISP) which is a subunit of the mitochondrial complex III	Low oxygen consumption, resistance to stress, slow development, and long lived
clk-1 (*e2519*)	*clk-1* encodes demethoxyubiquinone (DMQ) hydroxylase that is necessary for the biosynthesis of ubiquinone	Low oxygen consumption, resistance to stress, slow development, and long lived
daf-2 (*e1370*)	*daf-2* encodes a receptor tyrosine kinase that is the *C. elegans* insulin/IGF receptor ortholog. DAF-2 regulates various biological process	Long lived, Temperature sensitive dauer constitutive
akt-1 (*ok525*)	*akt-1* encodes an ortholog of the serine/threonine kinase Akt/PKB and downstream of IIS pathway	Long lived
daf-16 (*mgDf50*)	*daf-16* encodes *C. elegans* homologue of forkhead box O (FOXO) transcription factor regulates dauer formation, longevity, fat metabolism, stress response, and innate immunity in a IIS dependent manner	Dauer defective. Short lived
lin-45 (*n2520*)	*lin-45* encodes an ortholog of the RAF family of serine/threonine kinases (MAPKKK) and is necessary for larval viability and vulval differentiation. LIN-45 is an upstream regulator of ERK MAPK	
mek-2 (*n1989*)	*mek-2* encodes a MAP kinase kinase and it function as core regulator of Ras-mediated signaling in the regulation of multiple biological processes	

mpk-1 (*ku1*)	*mpk-1* encodes a *C. elegans* ortholog of ERK MAPK	Susceptibility to pathogens
skn-1 (*zu67*)	*skn-1* encodes basic-leucine zipper transcription factor orthologous to the mammalian Nrf	
wdr-23 (*tm1817*)	*wdr-23* ncodes a WD40 repeat-containing protein; WDR-23 functions as part of a CUL-4/DDB-1 ubiquitin ligase complex that negatively regulates accumulation and hence, transcriptional activation activity of the SKN-1 transcription factor in intestinal nuclei.	Long lived, resistance to stress
egl-1 (*n1084n3082*)	*egl-1* encodes a novel protein that contains a region similar to the BH3 (Bcl-2 homology region 3) domain of mammalian cell death activators	
ced-9 (*n1950*)	*ced-9* encodes the sole *C. elegans* homolog of the mammalian cell-death inhibitor Bcl-2.	Dominant maternal effect. Survival of cells which normally undergo programmed cell death.
ced-4 (*n1162*)	*ced-4* encodes a novel protein; along with CED-3, CED-4 is required for the initiation of programmed cell death	
ced-3 (*n717*)	*ced-3* encodes a caspase, a cysteine-aspartate protease; CED-3 activity is required for execution of apoptosis and functions in a conserved genetic pathway with CED-4, CED-9, and EGL-1 to regulate apoptosis during *C. elegans* development.	Abnormal cell death. Cells that normally die survive
oxIs12 (*unc-47p::GFP + lin-15*)	*unc-47p::GFP*	Expression of GFP in all eight GA neurons.
ldIs7 (*skn-1::GFP*)	*skn-1 b/c::GFP*	Stress-inducible expression of GFP in intestinal nuclei and ASI neurons, roller
dvIs19 (*gst-4::GFP*)	*gst-4p::GFP*	Oxidative stress-inducible/SKN-1 dependent GFP expression in whole body
ldIs3 (*gcs-1::GFP*)	*gcs-1p::GFP*	Oxidative stress-inducible/SKN-1 dependent GFP expression in pharynx and mid-body

wuIs151 (*ctl-1,2,3::GFP*)	*ctl-1,2,3p::GFP*	Expression of GFP in pharynx region, slow growing
bcIs39 (*lim-7p::ced-1::GFP + lin-15*)	*ced-1::GFP*	Expression of functional CED-1::GFP fusion protein in the sheath cells.
opIs3 (*hus-1::GFP*)	*hus-1::GFP*	Nuclear expression of GFP in germ cells and embryos
pkIs2386 (*unc-54p::alpha-synuclein::YFP+unc-119*)	*unc-54p::alpha-synuclein::YFP*	Expression of YFP fused α-synuclein expression in body wall muscle cells
egIs1 (*dat-1p::GFP*)	*dat-1p::GFP*	GFP observable in all eight DA neuron soma and processes
Gene and phenotype description of worm strains used also available at wormbase (www.wormbase.org), and Caenorhabditis Genetics Centre (www.cgc.umn.edu).		

Table. 1. Genotypic and phenotypic description of *C. elegans* strains used.

Table 2

	Treatment	EISO	Treatment	α-Santalol	β-Santalol
		% Survival		% Survival	% Survival
Acute Exposure (24 h from L4 stage)	Control	100	Control	100	100
	3 µg/mL	98.86±0.58	2 µM	100	99.54±0.46
	6 µg/mL	99.34±0.44	4 µM	99.68±0.32	98.63±0.95
	12 µg/mL	100	8 µM	99.70±0.30	100
	24 µg/mL	100	16 µM	99.18±0.55	99.72±0.28
	48 µg/mL	98.75±0.63	32 µM	99.12±0.61	99.07±0.92
			64 µM	99.13±0.58	99.35±0.43
Chronic Exposure (120 h from L1 stage)	Control	88.41±2.13	Control	88.96±1.93	86.05±1.71
	3 µg/mL	87.90±2.64	2 µM	87.76±2.34	85.84±2.34
	6 µg/mL	87.97±2.17	4 µM	87.22±1.55	86.32±2.23
	12 µg/mL	90.60±2.28	8 µM	86.03±1.82	90.98±1.86
	24 µg/mL	92.48±2.14	16 µM	92.81±0.82	92.73±1.23
	48 µg/mL	89.70±1.67	32 µM	93.98±1.19	90.07±1.96
			64 µM	90.51±1.52	89.08±1.92

Table 2. Toxicity assessment of EISO, α- and β-santalols on wild type *C. elegans*. Acute and chronic exposure to various pharmacological doses/molarities of EISO and its principal components did not significantly affect the survival of nematodes (n=20~25/replicate) compared to control at 20°C. Data are presented as mean±SEM of three independent runs, and analyzed by one way analysis of variance (ANOVA) followed by Bonferroni post-hoc test.

Table 3

Strain	Treatment		Brood size (Mean±SEM)	% Change	Body length (mm)	% Change
N2	EISO	Control	257.78±4.29		1.19±0.03	
		3 µg/mL	258.00±6.15	(+) 0.09	1.21±0.03	(+) 1.68
		6 µg/mL	257.22±6.16	(-) 0.22	1.18±0.04	(-) 0.84
		12 µg/mL	254.78±4.23	(-) 1.16	1.19±0.03	0
		24 µg/mL	258.33±3.36	(+) 0.21	1.20±0.03	(+) 0.42
		48 µg/ml	254.00±5.40	(-) 1.47	1.19±0.05	0
	α-Santalol	Control	259.89±3.81		1.22±0.03	
		2 µM	255.89±6.77	(-) 1.54	1.20±0.04	(-) 1.65
		4 µM	256.44±5.18	(+) 0.21	1.21±0.04	(+) 1.26
		8 µM	255.44±4.46	(-) 1.71	1.20±0.05	(-) 1.23
		16 µM	256.44±4.43	(-) 1.33	1.19±0.04	(-) 2.47
		32 µM	254.00±5.40	(-) 2.27	1.20±0.05	(-) 3.29
		64 µM	258.00±6.54	(-) 0.73	1.21±0.06	(-) 0.82
	β-Santalol	Control	257.56±3.90		1.20±0.05	
		2 µM	257.00±4.83	(-) 0.22	1.20±0.04	(+) 0.42
		4 µM	262.11±5.32	(+) 1.77	1.18±0.04	(-) 1.26
		8 µM	259.56±4.03	(+) 0.78	1.20±0.03	0
		16 µM	259.33±2.44	(+) 0.69	1.27±0.02	(+) 6.28
		32 µM	258.33±6.09	(+) 0.30	1.20±0.04	0
		64 µM	256.00±8.93	(-) 0.61	1.19±0.03	(-) 0.42
NL5901	EISO	Control	205.11±4.42		1.19±0.03	
		3 µg/mL	200.11±4.09	(-) 2.44	1.16±0.03	(-) 2.94
		6 µg/mL	206.33±7.01	(+) 0.60	1.14±0.03	(-) 4.62
		12 µg/mL	231.22±4.49*	(+) 12.73	1.16±0.04	(-) 2.52
		24 µg/mL	240.22±5.98**	(+) 17.12	1.14±0.07	(-) 3.87
		48 µg/ml	212.78±6.91	(+) 3.74	1.09±0.07	(-) 8.49
	α-Santalol	Control	203.89±4.88		1.17±0.04	
		2 µM	208.33±4.63	(+) 2.18	1.17±0.04	(+) 0.43
		4 µM	208.56±8.68	(+) 2.29	1.16±0.03	(-) 0.43
		8 µM	209.78±6.86	(+) 2.89	1.14±0.03	(-) 2.15
		16 µM	231.89±2.93*	(+) 13.73	1.18±0.07	(+) 1.20
		32 µM	240.89±4.16**	(+) 18.15	1.12±0.03	(-) 3.86
		64 µM	224.00±7.63	(+) 9.86	1.19±0.05	(+) 1.71
	β-Santalol	Control	209.00±4.99		1.12±0.03	
		2 µM	210.78±4.00	(+) 0.85	1.14±0.04	(+) 1.79
		4 µM	209.56±6.21	(+) 0.27	1.12±0.07	(-) 0.09
		8 µM	236.00±3.99*	(+) 12.92	1.17±0.03	(+) 4.46
		16 µM	254.44±3.74**	(+) 21.74	1.24±0.04	(+) 10.27
		32 µM	228.22±5.33	(+) 9.20	1.11±0.03	(-) 1.34
		64 µM	229.56±3.97	(+) 9.84	1.14±0.04	(+) 1.79

Table 3. Effect of EISO, α- and β-santalol on the reproduction and growth of wild type or NL5901 *C. elegans*. For reproduction assay, L1 stage N2 worms (30~35 worms/treatment) were raised on to NGM plates with and without EISO or santalol isomers for 72 h. Then, worms were individually shifted to fresh treatment plates each day until the reproduction period becomes ceased. Total eggs and offspring were counted at the L2/L3 stage. Growth was determined by measuring body length of the nematode of control and treated groups. L1 stage worms (30~40 worms/treatment) were treated with and without EISO or santalol isomers for 96 h. After exposure, day 1 worms subjected to analysis for body length measurement using Optika IsView image analyzing system. Data are presented as mean±SEM of three independent runs, and analyzed by one way analysis of variance (ANOVA) followed by Bonferroni post-hoc test. Asterisks (* and **) indicate statistically significant difference ($p<0.05$ and $p<0.001$).

Table 4

Treatment		Mean survival (days±SEM)	% Change	Maximum lifespan (days)	Sample Size (N)	Censored	*p* value
EISO	Control	10.383±0.350		14	99	9	
	3 µg/mL	10.089±0.361	(-) 2.83	14	93	10	0.8463
	6 µg/mL	10.142±0.351	(-) 2.32	15	94	11	0.7075
	12 µg/mL	11.863±0.342	(+)14.25	17	105	8	0.0003
	24 µg/mL	13.491±0.361	(+) 29.93	18	94	8	0.0001
	48 µg/ml	9.950±0.323	(-) 4.1	14	93	9	0.1804
α-Santalol	Control	10.253±0.320		14	96	8	
	2 µM	10.080±0.335	(-) 1.69	14	88	3	0.6057
	4 µM	10.121±0.342	(-) 1.29	14	88	6	0.8528
	8 µM	10.502±0.326	(+) 2.43	15	90	5	0.8683
	16 µM	10.751±0.315	(+) 4.86	15	98	5	0.2110
	32 µM	12.532±0.359	(+) 22.23	19	102	0	0.0001
	64 µM	10.967±0.360	(+) 6.96	15	95	2	0.1558
β-Santalol	Control	10.922±0.329		15	99	5	
	2 µM	10.366±0.340	(-) 5.09	15	97	6	0.3819
	4 µM	10.259±0.339	(-) 6.07	15	91	4	0.0976
	8 µM	11.308±0.342	(+) 3.53	18	112	8	0.1700
	16 µM	14.086±0.408	(+) 28.97	20	94	0	0.0001
	32 µM	10.846±0.408	(-) 0.70	17	104	2	0.7590
	64 µM	10.867±0.309	(-) 0.50	16	105	2	0.9194

Table 4. Lifespan analysis of NL5901 *C. elegans* cultured at 20°C supplemented with various pharmacological doses of EISO, α- and β-santalol. The mean lifespan of NL5901 worms were calculated using Kaplan–Meir survival analysis and significance levels were estimated by long-rank test in Medcalc statistical tool.

Table 5

Genotype	Treatment	Mean survival (Mean±SEM)	Maximum lifespan (days)	Sample size (N)	Censored	% Change	*p* value
Wild (From late L4 stage)	EISO						
	Control	15.305±0.423	23	110	12		
	3 µg/mL	15.449±0.454	24	98	11	(+) 0.94	0.7267
	6 µg/mL	16.620±0.502	25	115	14	(+) 8.59	0.0025
	12 µg/mL	17.922±0.593	28	112	14	(+) 17.10	<0.0001
	24 µg/mL	19.339±0.586	31	100	8	(+) 26.36	<0.0001
	48 µg/mL	15.951±0.528	24	94	6	(+) 4.22	0.9699
	α-Santalol						
	Control	15.486±0.449	24	121	13		
	4 µM	15.577±0.422	23	118	8	(+) 0.63	0.8914
	8 µM	15.878±0.475	23	103	9	(+) 2.57	0.6466
	16 µM	16.151±0.528	24	119	10	(+) 4.33	0.0901
	32 µM	17.076±0.478	26	113	7	(+) 10.31	<0.0074
	64 µM	15.626±0.493	24	120	11	(+) 0.94	0.4619
	β-Santalol						
	Control	17.550±0.425	24	130	14		
	4 µM	17.071±0.470	24	119	13	(-) 2.73	0.4249
	8 µM	18.016±0.458	26	129	14	(+) 2.66	0.3892
	16 µM	19.754±0.515	30	142	9	(+) 12.56	<0.0001
	32 µM	18.214±0.487	26	123	7	(+) 3.78	0.0975
	64 µM	18.364±0.511	26	125	15	(+) 4.64	0.0311
Wild (From embryo)	Control	15.086±0.379	23	114	10		
	EISO (24 µg/mL)	19.811±0.577	30	125	8	(+) 31.32	<0.0001
	α-Santalol (32 µM)	17.807±0.513	26	106	5	(+) 18.04	<0.0001
	β-Santalol (16 µM)	18.915±0.642	30	113	9	(+) 25.38	<0.0001

Table 5. EISO and santalol isomers mediated longevity in wild-type *C. elegans*. Survival rate of wild-type *C. elegans* cultured on NGM plates supplemented with various pharmacological doses of EISO, α- and β-santalol at 20°C from late L4 stage or embryo. The mean lifespan of *C. elegans* were calculated using Kaplan–Meir survival analysis and significance levels were estimated by long-rank test in Medcalc statistical tool. For the analysis of statistical significance, treated groups were compared with control group.

Table 6

Transgene	Treatment	CED-1::GFP clusters around cell corpses
ced-1::GFP	Control	0.44±0.08
	6-OHDA	3.42±0.27 #
	6-OHDA/EGCG	1.24±0.15 **
	6-OHDA/EISO	1.16±0.13 **
	6-OHDA/α-Santalol	1.52±0.13 **
	6-OHDA/β-Santalol	1.06±0.14 **
	EISO	0.33±0.21
	α-Santalol	0.33±0.21
	β-Santalol	0.17±0.17

Table 6. Influences of EISO, α- and β-santalol on CED-1::GFP expression in *C. elegans*. The 6-OHDA-induced elevation in clustering of CED-1::GFP around cell corpses was greatly attenuated with EISO, α- and β-santalol treatment in MD701 *C. elegans*. Data were pooled from biological triplicates in experimental duplicates and presented as mean±SEM. A hash (#) mark indicates significant differences (p<0.001) between 6-OHDA exposed and unexposed (control) worms, an asterisk (**) indicate significant difference between 6-OHDA exposed and 6-OHDA/EGCG, EISO, α- or β-santalol treated groups (p< 0.001).

Table 7

Genotype	Treatment	Mean survival (Mean±SEM)	Maximum lifespan (days)	Sample size (N)	Censored	% Change	*p* value
daf-16	Control	15.230±0.429	21	100	9		
	6-OHDA	12.468±0.418	18	84	6	(-) 18.14	<0.0001
	6-OHDA/EISO	16.257±0.482	24	104	5	(+) 30.39	<0.0001
	6-OHDA/α-Santalol	15.300±0.466	24	106	8	(+) 22.71	<0.0001
	6-OHDA/β-Santalol	17.382±0.482	25	100	8	(+) 39.41	<0.0001
daf-2	Control	27.815±0.834	46	122	11		
	6-OHDA	25.229±0.820	40	113	8	(-) 9.30	0.0199
	6-OHDA/EISO	32.049±0.973	50	117	10	(+) 27.03	<0.0001
	6-OHDA/α-Santalol	30.886±0.898	47	114	6	(+) 22.42	<0.0001
	6-OHDA/β-Santalol	34.426±1.021	54	119	9	(+) 36.45	<0.0001
akt-1	Control	23.032±0.705	32	89	9		
	6-OHDA	20.567±0.622	27	80	2	(-) 10.70	<0.0001
	6-OHDA/EISO	19.635±0.645	29	84	8	(-) 4.53	0.3276
	6-OHDA/α-Santalol	18.876±0.592	28	101	10	(-) 8.22	0.6825
	6-OHDA/β-Santalol	20.302±0.747	29	84	4	(-) 1.29	0.2410
mev-1	Control	9.77±0.272	14	76	4		
	6-OHDA	7.440±0.255	10	70	7	(-) 23.85	<0.0001
	6-OHDA/EISO	11.194±0.292	17	72	1	(+) 50.46	<0.0001
	6-OHDA/α-Santalol	10.323±0.325	16	74	5	(+) 38.75	<0.0001
	6-OHDA/β-Santalol	10.268±0.161	18	80	5	(+) 38.01	<0.0001
isp-1	Control	20.733±0.660	32	85	6		
	6-OHDA	17.425±0.507	22	66	10	(-) 15.96	<0.0001
	6-OHDA/EISO	24.638±0.715	35	99	9	(+) 41.39	<0.0001
	6-OHDA/α-Santalol	22.724±0.778	35	89	6	(+) 30.41	<0.0001
	6-OHDA/β-Santalol	25.702±0.788	36	91	8	(+) 47.50	<0.0001
clk-1	Control	21.244±0.626	28	69	7		
	6-OHDA	14.144±0.572	22	63	7	(-) 33.24	<0.0001
	6-OHDA/EISO	15.179±0.570	21	52	4	(+) 7.32	0.3911
	6-OHDA/α-Santalol	14.822±0.644	20	46	5	(+) 4.79	0.5101
	6-OHDA/β-Santalol	15.596±0.324	21	51	3	(+) 10.27	0.2443
lin-45	Control	13.086±0.409	17	65	4		
	6-OHDA	11.389±0.388	15	60	5	(-) 12.97	<0.0001
	6-OHDA/EISO	11.327±0.335	15	68	7	(-) 0.54	0.6423
	6-OHDA/α-Santalol	10.699±0.310	15	72	0	(-) 6.06	0.6556
	6-OHDA/β-Santalol	11.533±0.170	14	64	7	(+) 1.26	0.0178
mek-2	Control	8.234±0.302	12	61	5		
	6-OHDA	7.143±0.231	9	49	2	(-) 13.25	<0.0001
	6-OHDA/EISO	7.707±0.214	10	56	6	(+) 7.90	0.0469
	6-OHDA/α-Santalol	7.182±0.226	10	63	5	(+) 0.55	0.6810
	6-OHDA/β-Santalol	6.932±0.211	9	59	3	(-) 2.95	0.4600
mpk-1	Control	13.832±0.429	22	116	9		
	6-OHDA	11.443±0.312	18	87	7	(-) 17. 27	<0.0001
	6-OHDA/EISO	11.929±0.348	19	103	10	(+) 4.25	0.1164
	6-OHDA/α-Santalol	11.977±0.369	18	107	9	(+) 4.67	0.0854

	6-OHDA/β-Santalol	11.459±0.293	19	98	8	(+) 0.14	0.9061
skn-1	Control	15.635±0.348	19	62	3		
	6-OHDA	12.333±0.289	16	65	4	(-) 21.12	<0.0001
	6-OHDA/EISO	11.683±0.224	15	63	0	(-) 5.27	0.0097
	6-OHDA/α-Santalol	11.771±0.283	15	66	4	(-) 4.56	0.1591
	6-OHDA/β-Santalol	12.268±0.344	16	65	5	(-) 0.53	0.9686
wdr-23	Control	22.491±0.595	32	95	7		
	6-OHDA	22.702±0.580	32	86	5	(+) 0.94	0.7979
	6-OHDA/EISO	21.878±0.581	31	81	4	(-) 3.63	0.2867
	6-OHDA/α-Santalol	22.078±0.531	31	107	8	(-) 2.75	0.3084
	6-OHDA/β-Santalol	23.126±0.509	32	99	8	(+) 1.87	0.7585

Table 7. Genetic requirement for EISO, α- and β-santalol-mediated geroprotection against 6-OHDA-induced toxicity in *C. elegans*. The mean lifespan of *C. elegans* mutants were calculated using Kaplan–Meir survival analysis and significance levels were estimated by long-rank test in Medcalc statistical tool. For the analysis of statistical significance, 6-OHDA alone treated group was compared with untreated control, and 6-OHDA/EISO, α- or β-santalol treated groups were compared with 6-OHDA alone treated group.

Table 8

Compound	donorHB[1]	accptHB[2]	QPPCaco[3]	QPlogBB[4]	QPPMDCK[5]	QPlogKp[6]
Acceptable range	0.0 to 6.0	2.0 to 20.0	<25 poor, >500 great	–3 to -1.2	<25 poor, >500 great	-8 to -1
α-Santalol	1	1.70	3943.57	-0.088	2179.9	-1.775
β-santalol	1	1.70	3371.88	-0.158	1840.421	-1.819

Compound	QPlogKhsa[7]	% Human oral absorption[8]	Human oral absorption[9]	Lipinski's rule of five[10]
Acceptable range	–1.5 to 1.5	>80% is high <25% is poor	1-low, 2-medium, 3-high	< 5
α-Santalol	0.365	100	3	0
β-santalol	0.404	100	3	0

Table 8. Predicted ADME properties of santalol isomers using QikProp module.

[1]Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution
[2]Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution
[3]Predicted apparent Caco-2 cell permeability in nm/sec
[4]Predicted brain/blood partition coefficient
[5]Predicted apparent MDCK cell permeability in nm/sec
[6]Predicted skin permeability, log Kp
[7]Prediction of binding to human serum albumin
[8]Predicted human oral absorption on 0 to 100% scale
[9]Predicted qualitative human oral absorption
[10]Number of violations of Lipinski’s rule of five

Figure 1

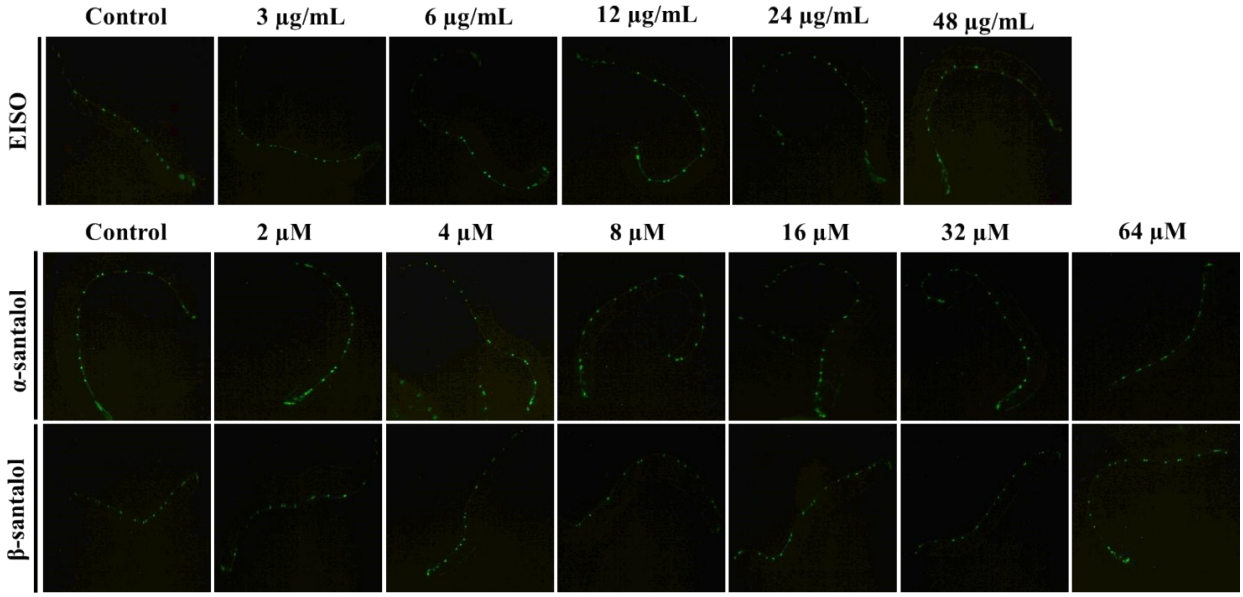


Fig. 1. Effect of EISO, α- and β-santalol on the development and morphology of D-type GABAergic motor neurons. Exposure was performed from L1 stage to adult stage.

Figure 2

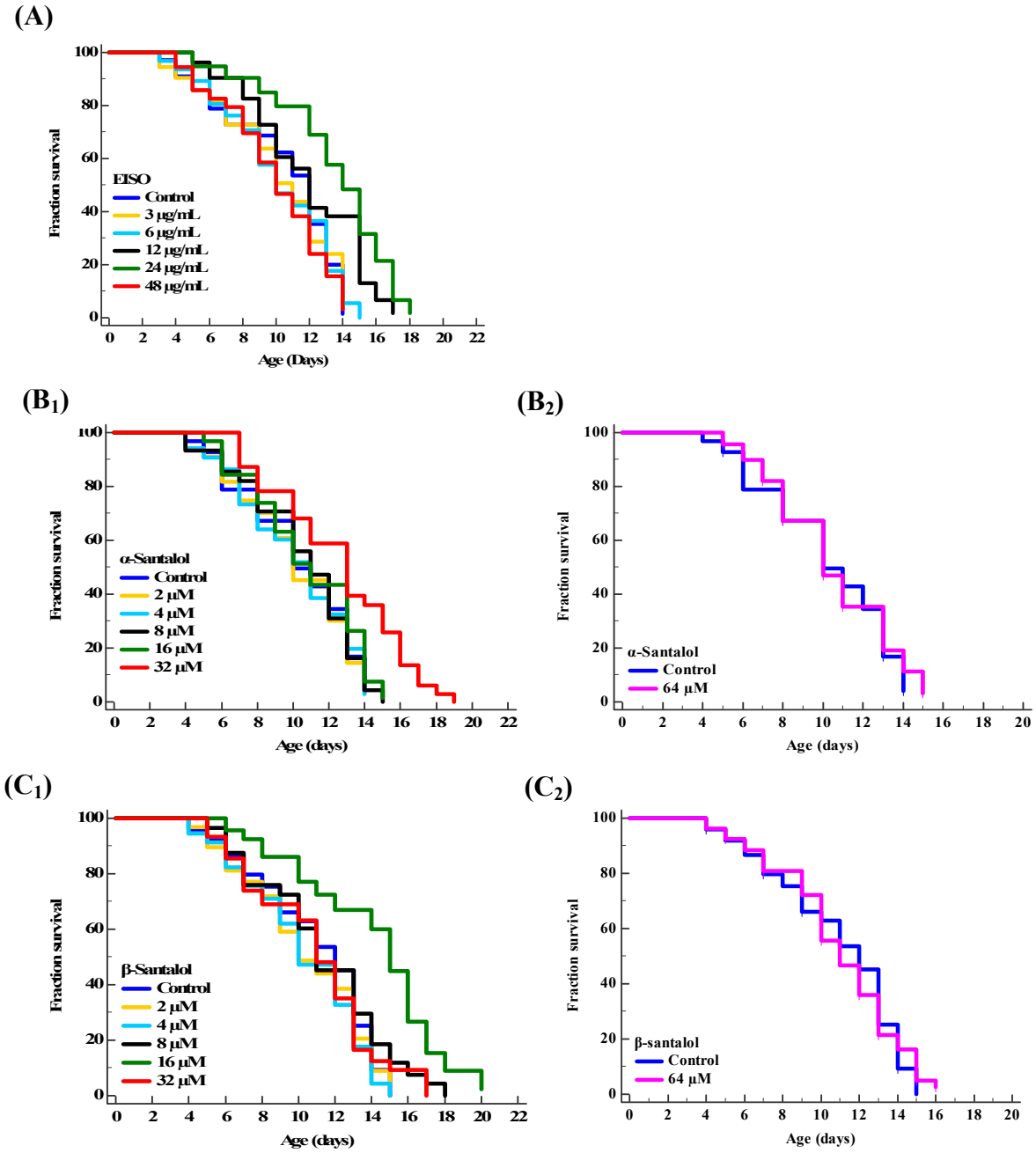


Fig. 2. Beneficial effect of EISO and its principal components on the lifespan of NL5901 *C. elegans*. The survival graphs showed the effect of dietary supplemented (**A**) EISO (**B1-2**) α-santalol and (**C1-2**) β-santalol on the lifespan of transgenic *C. elegans* strain NL5901 constitutively express human α-synuclein (a Parkinson's related protein) in body wall muscles. The survival curves were plotted using Kaplan-Meier survival analysis and significance levels were estimated by long-rank test in Medcalc statistical software v.14. The representative Kaplan-Meier survival curves from three independent runs were presented.

Figure 3

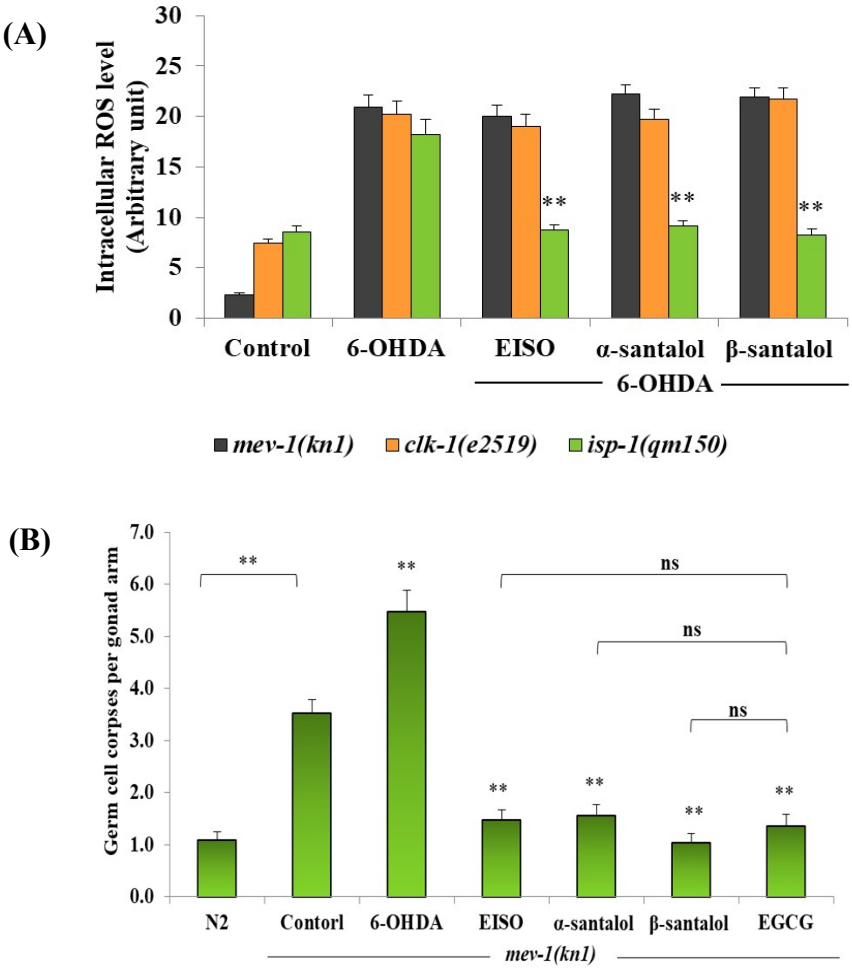


Fig. 3. Histogram represents the changes in (**A**) intracellular ROS level and (**B**) germ cell death/apoptosis in mitochondrial ETC pathway mutants treated with 6-OHDA and 6-OHDA/EISO, α- or β-santalol. Data pooled from three independent biological experiments with appropriate replicates. Probability levels of $p<0.05$ were considered as statistically significant. $^{**}p<0.01$, ^{ns}not significant.

Figure 4

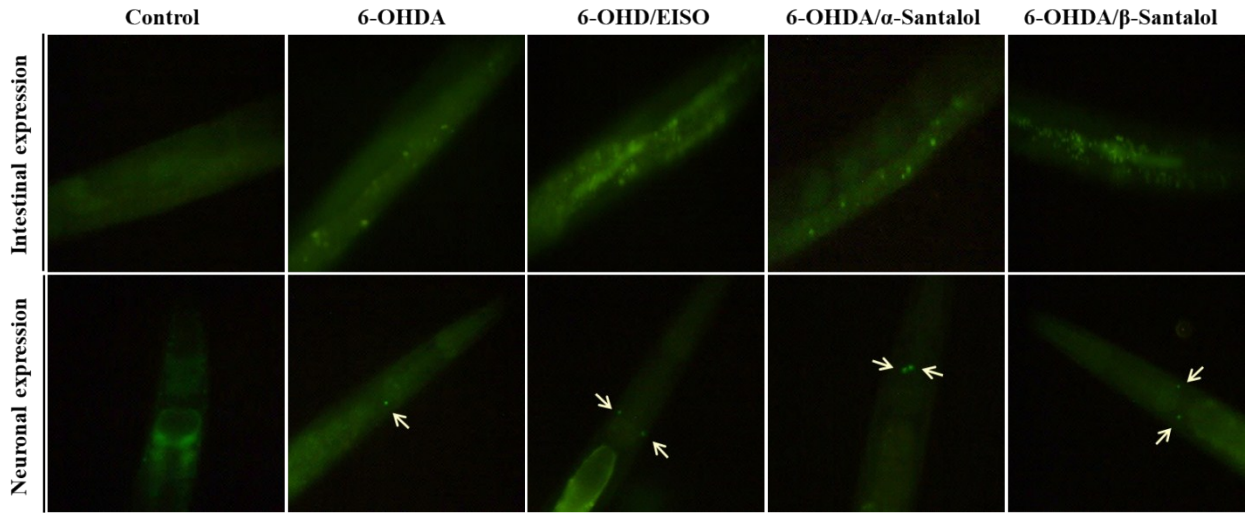


Fig. 4. Effect of EISO, α- and β-santalol on the expression of *skn-1::gfp* transgene in LD1 *C. elegans*. Intestinal and ASI neuronal expression of *skn-1 b/c::GFP* was observed in adult *C. elegans* after treated with 6-OHDA and 6-OHDA/EISO, α- or β-santalol. *skn-1 b/c::GFP* were visualized in under fluorescence microscope and classified into low, medium and high based on expression pattern (see **Fig. 16** in main article). Expression of *skn-1 b/c::GFP* in ASI neurons are indicated by white arrow(s).

Figure 5

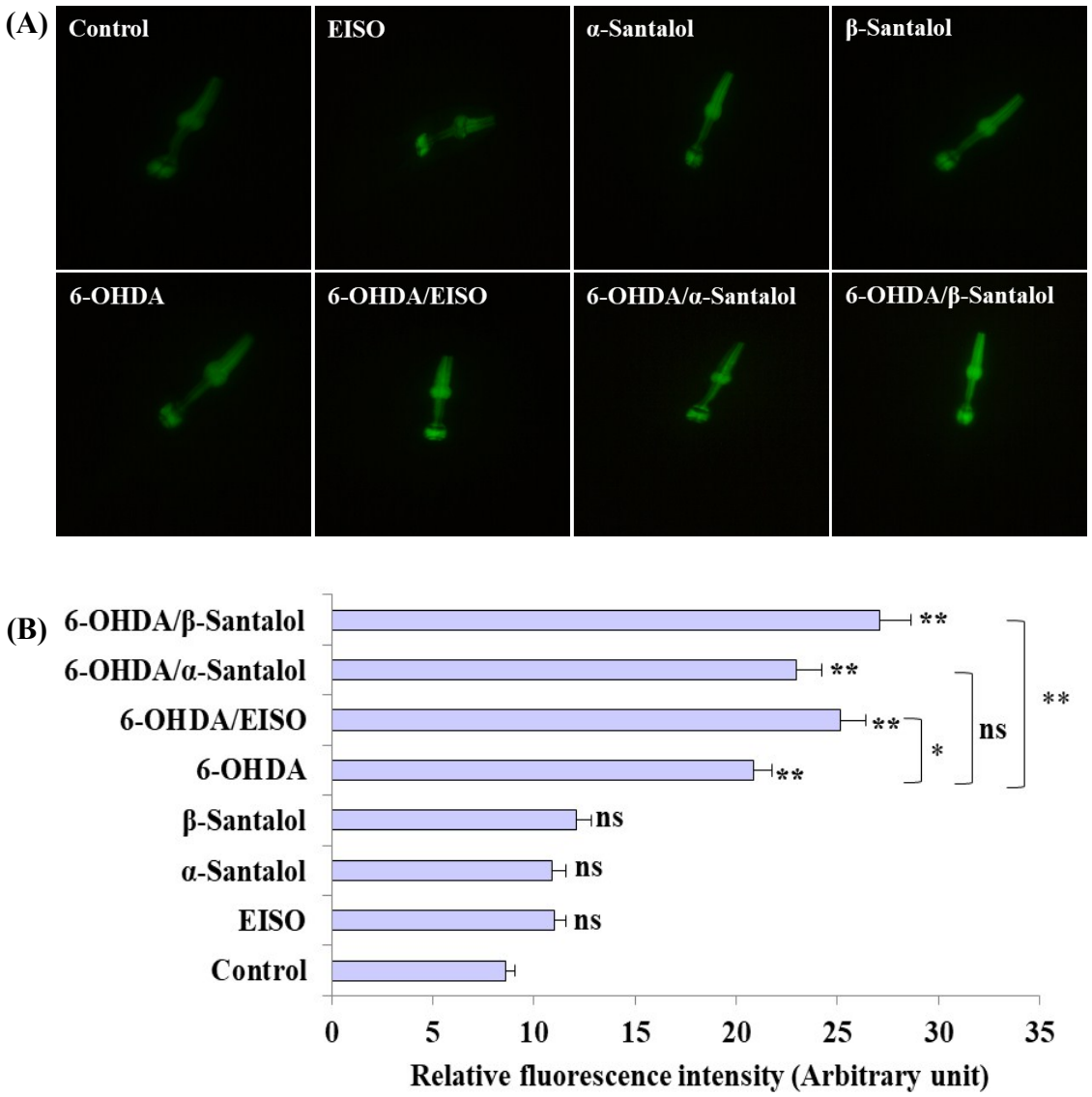


Fig. 5. (**A**) Relative expression of *ctl-1,2,3p::GFP* transgene in GA800 *C. elegans* treated with 6-OHDA, EISO, α-santalol, β-santalol and 6-OHDA/EISO, α- or β-santalol at 20°C. (**B**) Histogram represents the changes in expression across various treatments. Data shown here are mean±SEM, the probability levels of $p<0.05$ were considered as statistically significant. *$p<0.05$, **$p<0.01$ and ^{ns}not significant.

Figure 6

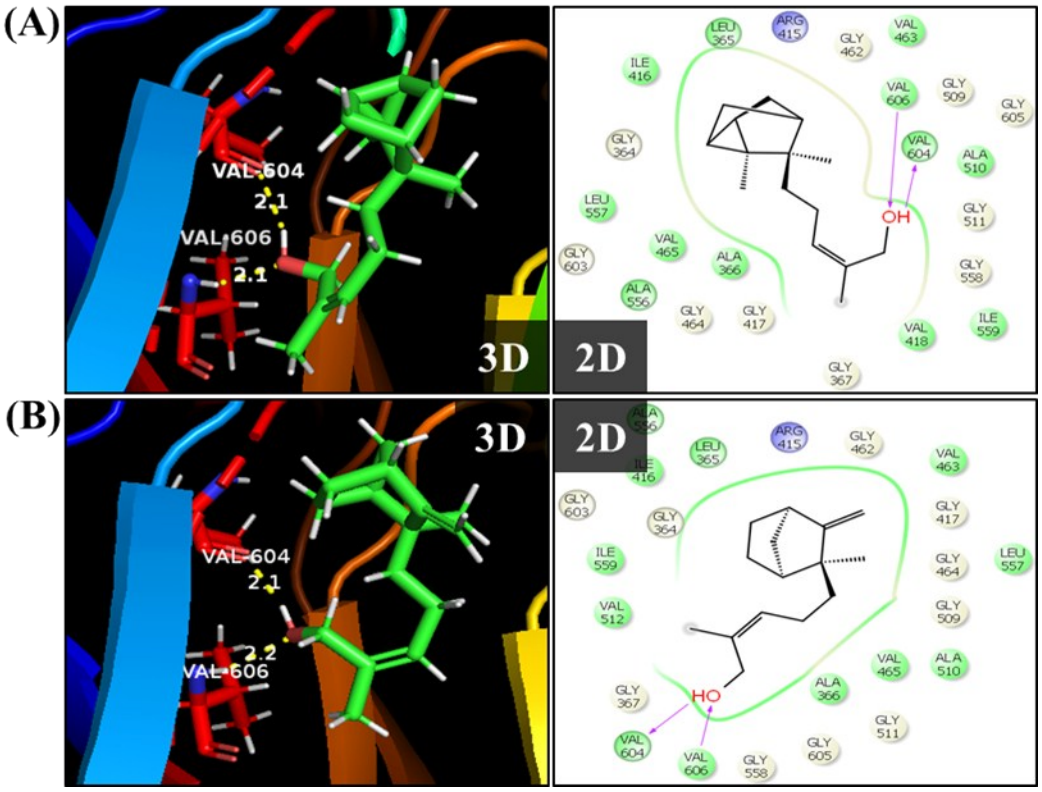


Fig. 6. Proposed interaction of (**A**) α-santalol and (**B**) β-santalol on the binding cleft of Klech domain of Keap1. The representative three-dimensional and two-dimensional interaction map shows the binding mode of α- and β-santalol (stick style in a green color) through hydrogen bonds (yellow dashed lines) with Keap1 (shown in ribbon style and colored shape). The pink arrow in two-dimensional image indicates potential interactions between amino acid residues and α- or β-santalol.

Figure 7

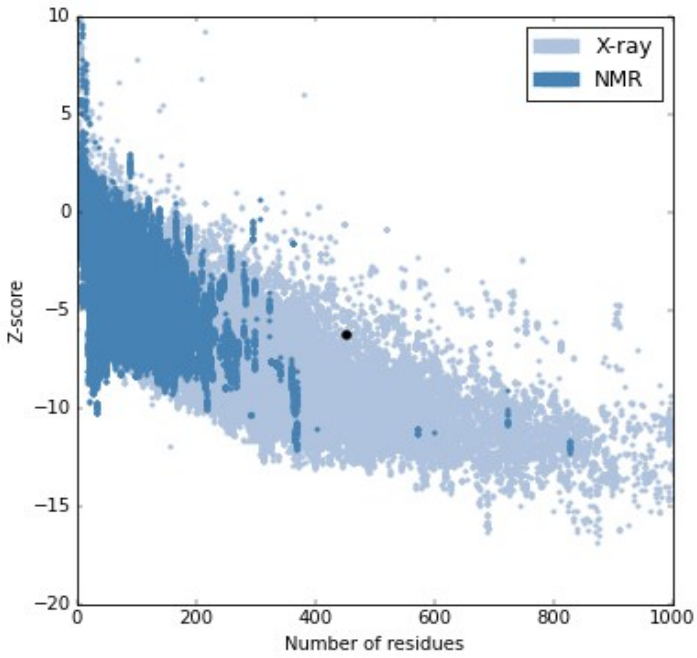
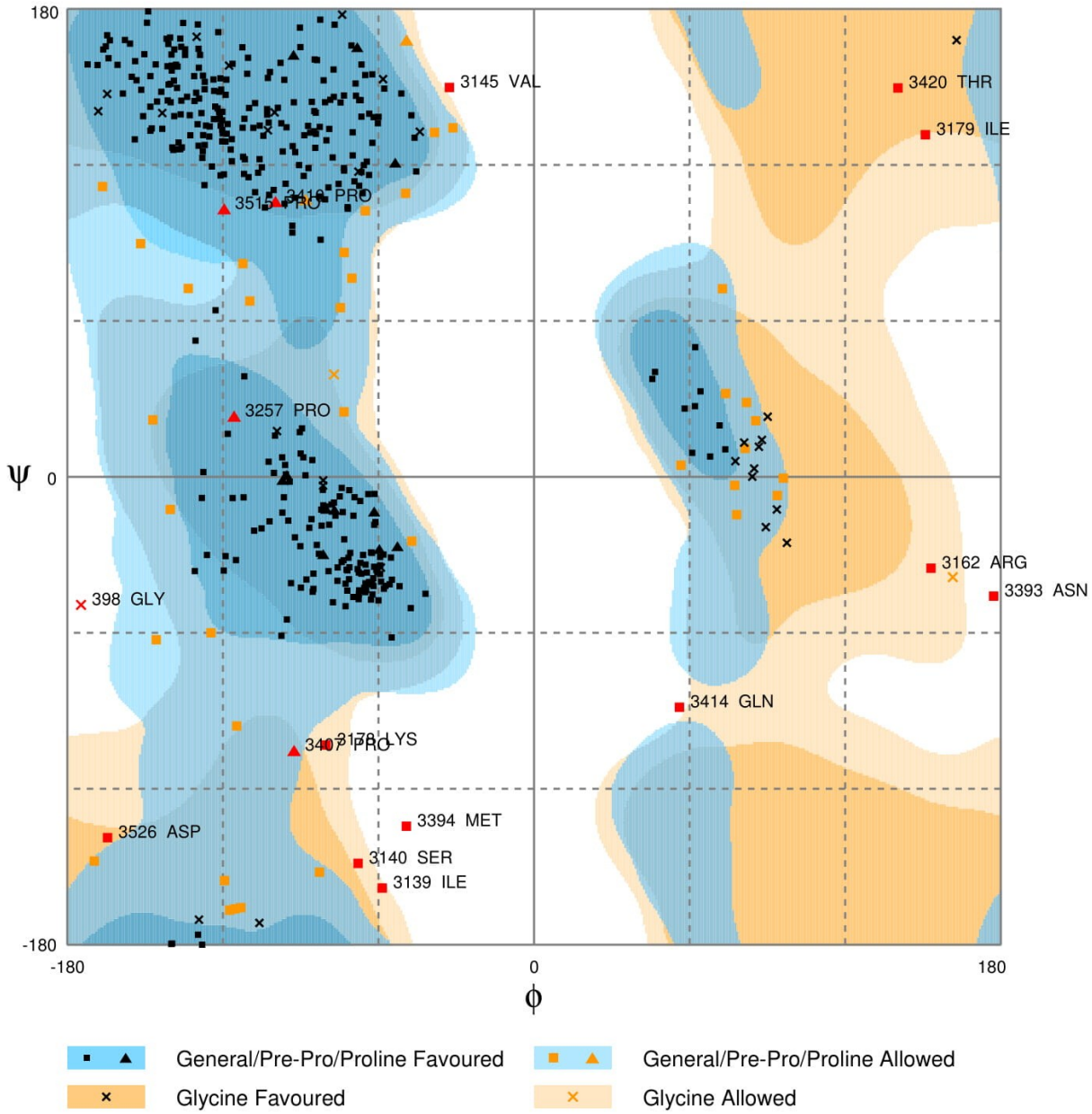


Fig. 7. Investigation of the modeled WDR-23 protein structure. The Z-score (black dot) of this model is -6.22 which is closer to the experimentally resolved X-ray and NMR structures.

Figure 8A



Number of residues in favoured region (~98.0% expected)	: 397 (87.8%)
Number of residues in allowed region (~2.0% expected)	: 39 (8.6%)
Number of residues in outlier region	: 16 (3.5%)

Figure 8B

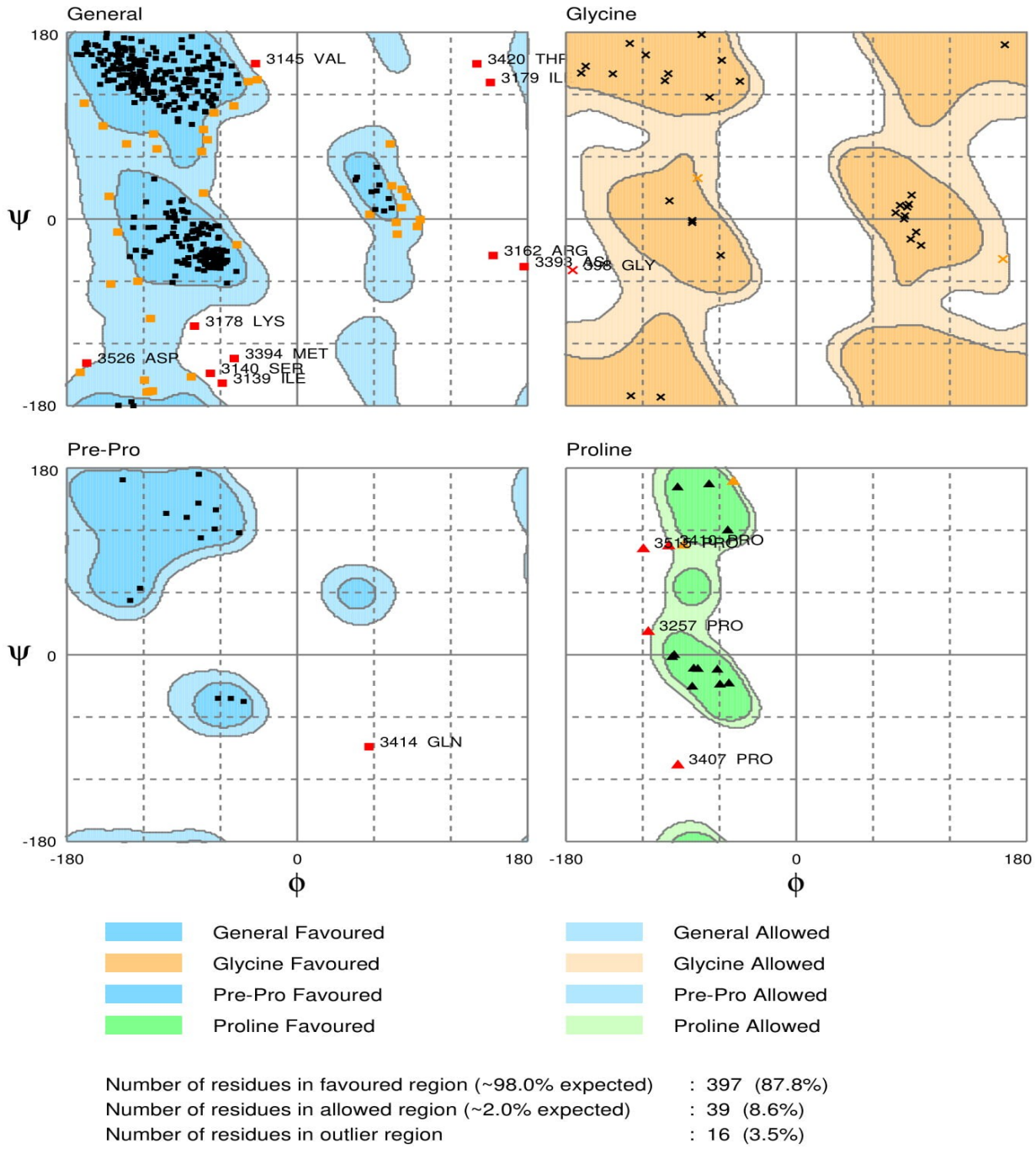


Fig. 8. (**A**, **B**) Ramachandran plot of modeled WDR-23 protein.

Figure. 9. Certificate of analysis (α-Santalol)

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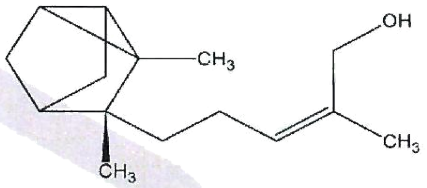
Certificate of Analysis

Cerilliant Quality
ISO GUIDE 34
ISO/IEC 17025
ISO 13485
ISO 9001
GMP/GLP

cis-α-Santalol

(2Z)-5-[(3R)-2,3-dimethyltricyclo[2.2.1.0^{2,6}]hept-3-yl]-2-methylpent-2-en-1-ol



Reference Number:	CSQ-15208
Lot Number:	AV12066
Retest Date:	June 2013
CAS Number:	115-71-9
Chemical Formula:	$C_{15}H_{24}O$
Molecular Weight:	220.35
Chromatographic Purity:	98.2%
Purity Factor [1]:	96.8%
Storage:	Refrigerate.
Handling:	As specified in the manufacturer MSDS.
Intended Use:	For R&D/ analytical purposes only.

[1] Purity Factor = (100 - wt% residual solvent - wt% residual water) x Chromatographic Purity/100

Spectral and Physical Data

Analytical Test	Method	Results
Appearance	Visual Inspection	Clear oil
Chromatographic Purity by GC/FID Analysis	SP10-0101	98.2% [2]
Identity by LC/MS Analysis	SP10-0107	Consistent with Structure
Identity by FTIR Analysis	SP10-0108	Consistent with Structure
Identity by ^{1}H-NMR Analysis	USP <761>, SP10-0116	Consistent with Structure
Identity by ^{13}C-NMR Analysis	USP <761>, SP10-0116	Consistent with Structure
Identity by 2D-NMR Analysis	USP <761>, SP10-0116	Consistent with Structure

[2] Purity value is the average of two independent analyses.

Cerilliant certifies the analyses detailed in this certificate are true and accurate. Retest date assigned at the request of the Customer. Cerilliant has not collected stability and makes no claims or warranty regarding shelf life or stability of material.

Authorized Signature: Lara Sparks

Lara Sparks, Quality Assurance Director

July 16, 2012
Date

Cerilliant Corporation 811 Paloma Drive, Suite A, Round Rock, TX 78665 800-848-7837 / 512-238-9974

Spectral and Physical Data (cont.)

Analytical Test	Method	Results		
Residual Solvent Analysis by GC/FID Headspace	AM1087 [3]	1.22%		
Residual Water Analysis by Karl Fischer Coulometry	USP <921>, SP10-0103	0.21%		
Specific Rotation	SP10-0133	$[\alpha]^{20}_{D} = 21.004°$		
Elemental Analysis	SP10-0117		Calculated	Analyzed
		C	81.12%	80.92%
		H	11.00%	10.83%
		O	7.89%	7.33%
Inorganic Content by ICP	Outsourced	Refer to results on page 7		
Thermogravimetric Analysis	SP10-0136	2.31%		
Refractive Index Analysis	Outsourced	1.4965 at 25 °C		

[3] Validated analytical method

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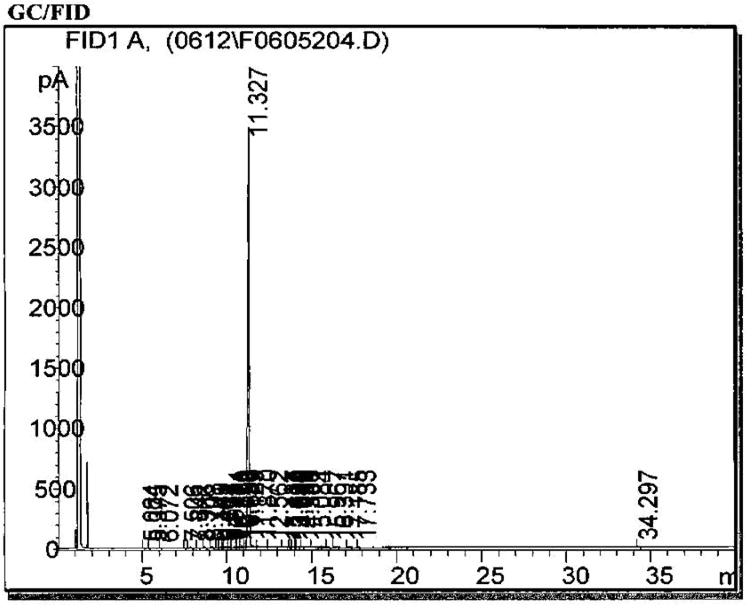
Spectral and Physical Data (cont.)

GC/FID



Column: DB-5ms, 30 m x 0.53 mm ID, 1.5 µm film thickness
Temp Program: 40°C to 160°C at 40°C/min
160°C to 210°C at 2°C/min
210°C to 280°C at 10°C/min (hold 5 min)
Injector Temp: Cool-on-Column
Detector Temp: 325°C

Data File Name: S:\GC\GC6\2012\0612\F0605204.D
Operator: RPC
Instrument: GC#6
Sample Name: AV12066
Method File: CISSANTA.M
Acquired: June 5, 2012 11:48 AM

Peak #	Ret Time	Area	Height	Area %
1	5.06	0.30	0.12	0.00
2	5.38	0.74	0.32	0.00
3	6.07	0.89	0.26	0.00
4	7.51	0.38	0.11	0.00
5	7.84	12.92	3.00	0.06
6	8.31	0.60	0.15	0.00
7	8.71	2.76	0.65	0.01
8	9.15	7.25	1.49	0.04
9	9.46	0.61	0.15	0.00
10	9.61	7.93	1.60	0.04
11	9.75	2.33	0.44	0.01
12	9.97	6.88	1.07	0.03
13	10.18	2.46	0.46	0.01
14	10.50	2.91	0.37	0.01
15	10.58	1.19	0.30	0.01
16	10.73	2.05	0.46	0.01
17	10.90	93.63	16.52	0.46

Peak #	Ret Time	Area	Height	Area %
18	11.13	4.97	1.06	0.02
19	11.33	19935.90	3472.82	98.29
20	11.87	140.45	9.40	0.69
21	12.56	26.65	1.95	0.13
22	13.44	3.33	0.35	0.02
23	13.69	0.18	0.05	0.00
24	13.79	0.39	0.09	0.00
25	13.85	0.77	0.12	0.00
26	14.01	0.18	0.07	0.00
27	14.05	0.35	0.09	0.00
28	14.39	0.35	0.05	0.00
29	15.08	4.89	0.69	0.02
30	15.97	4.54	0.65	0.02
31	16.39	7.28	0.99	0.04
32	17.19	1.75	0.17	0.01
33	17.73	0.66	0.14	0.00
34	34.30	3.84	1.08	0.02

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AV12066
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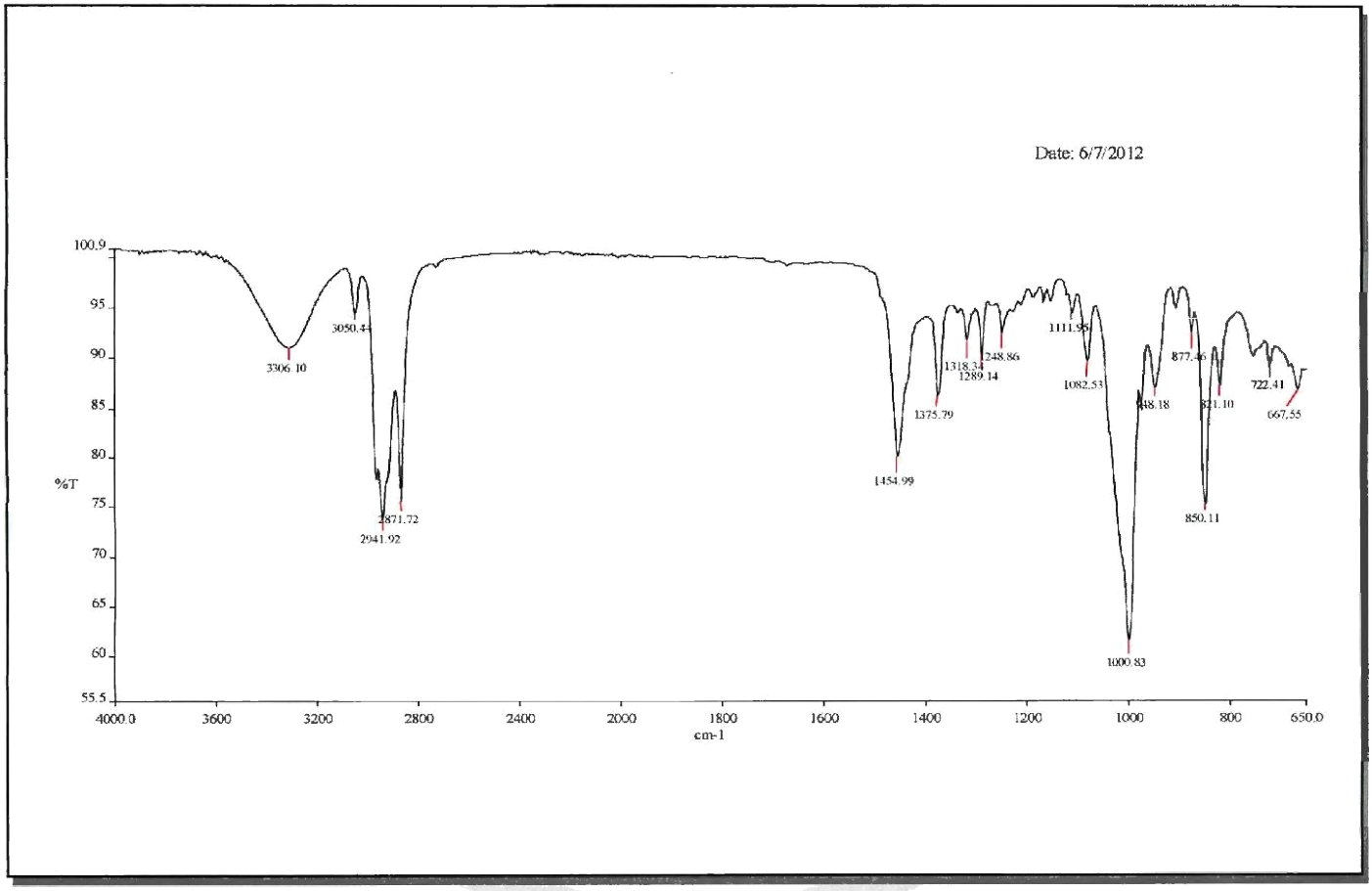
Spectral and Physical Data (cont.)

Identity by FTIR Analysis

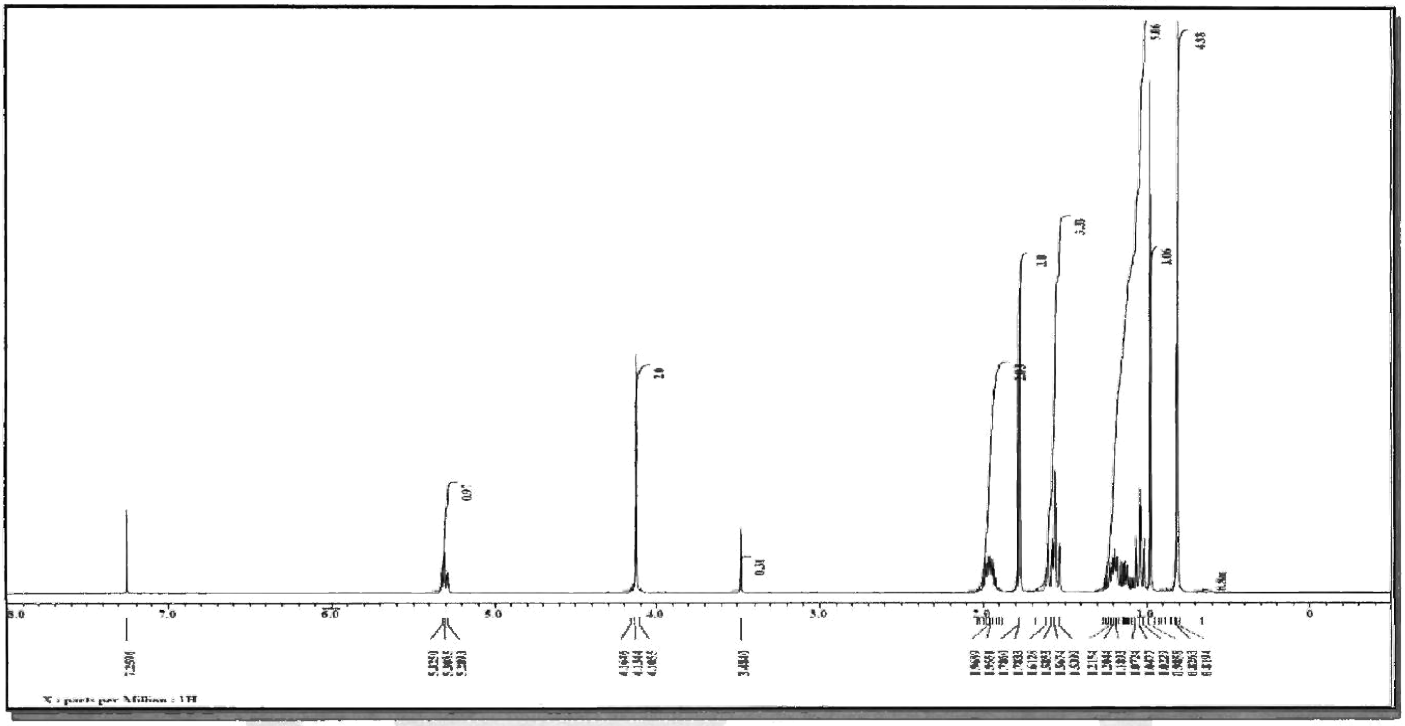
Instrument:	Perkin Elmer Spectrum One
Matrix:	Neat
Scans:	32



Spectral and Physical Data (cont.)

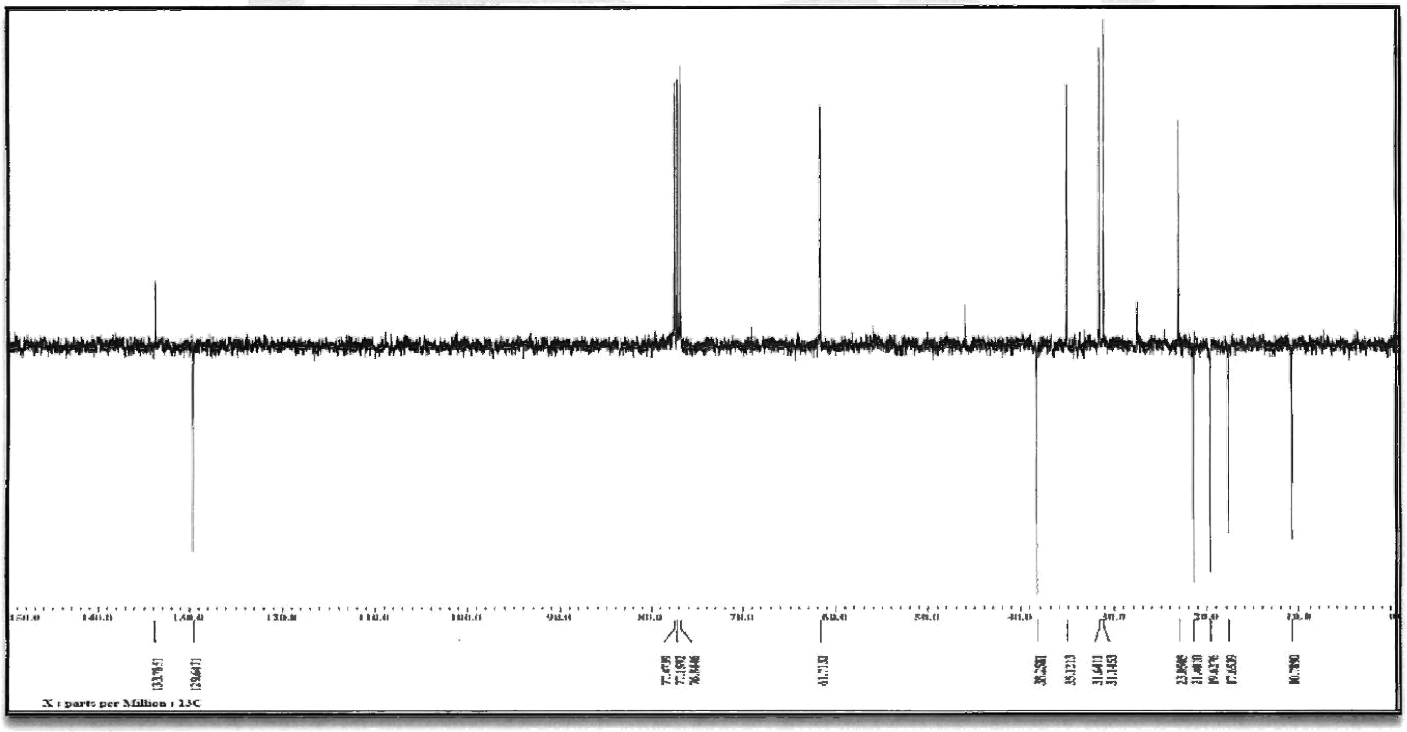
^{1}H NMR

Instrument: JEOL ECS 400
Solvent: Chloroform-D



^{13}C NMR

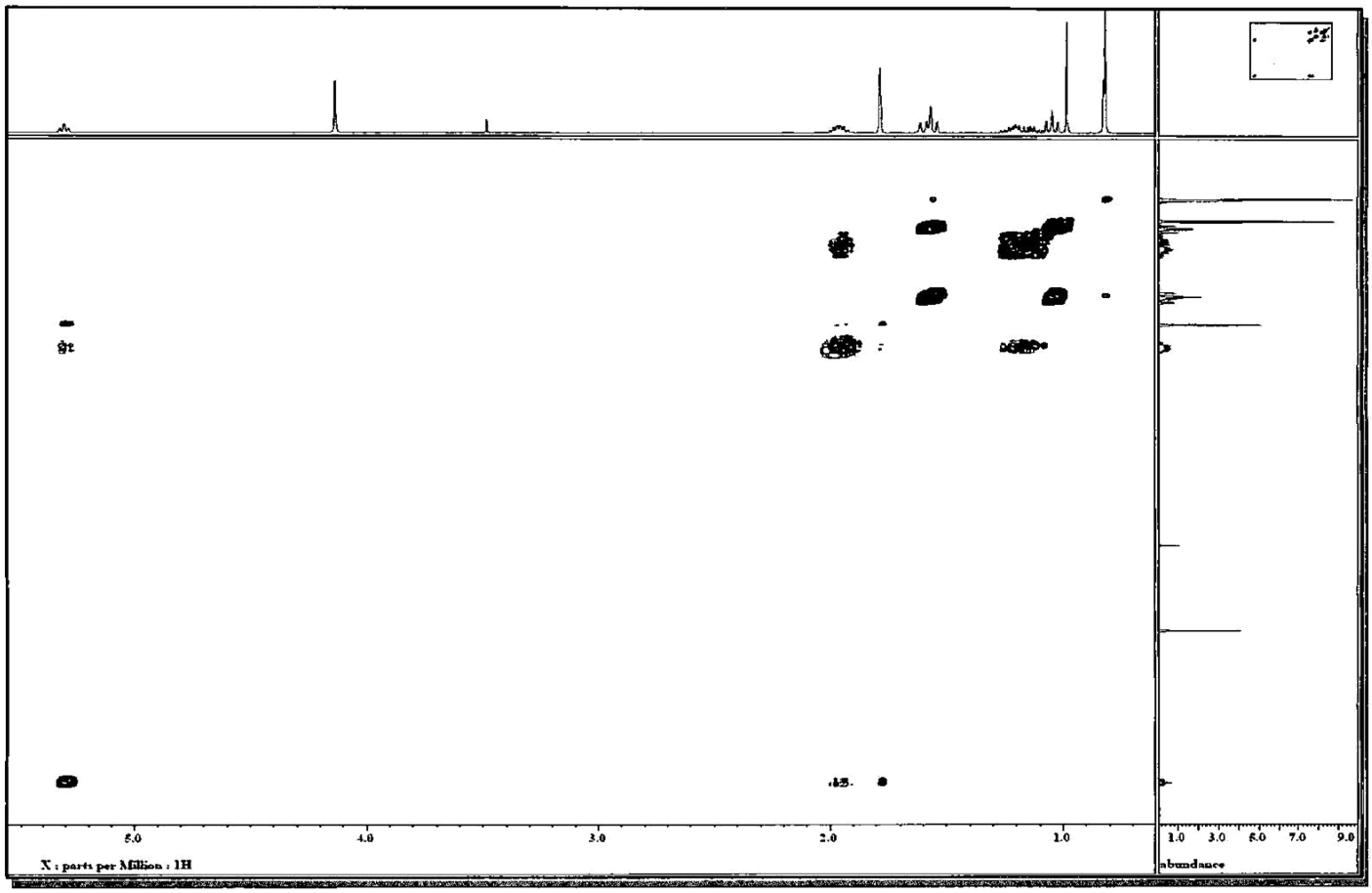
Instrument: JEOL ECS 400
Solvent: Chloroform-D



Spectral and Physical Data (cont.)

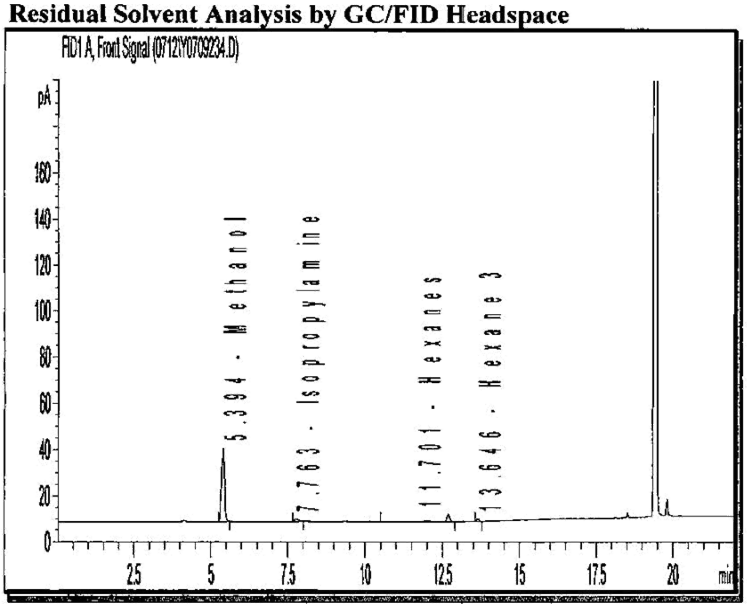
2D NMR

Instrument: JEOL ECS 400
Solvent: Chloroform-D



Spectral and Physical Data (cont.)

Residual Solvent Analysis by GC/FID Headspace



Column:	DB-ALC1 30 m x 0.53 mm, 3 µm film thickness
Temp Program:	40°C (12 min) to 220°C at 40°C/min (5.5 min)
Carrier Gas:	Helium
Flow Rate:	2.0 mL/min
Detector Heater Temp:	250°C
Injector:	Headspace Sampler
HS Oven Temp:	60°C
Vial Equilibration:	10 minutes
Data File Name:	S:\GC\GC-HS11\2012\0712\Y0709234.D
Operator:	RPC
Instrument:	GC#11
Sample Name:	AV12066
Acquired:	July 11, 2012 12:13 PM

Peak	Compound	Area	Weight %
1	Methanol	219.03149	1.21
2	Isopropylamine	11.26934	0.01
3	Hexanes	31.51361	BQL
4	NMP	NA	NA
Total			**1.22**

BQL - Below Quantitation Limit

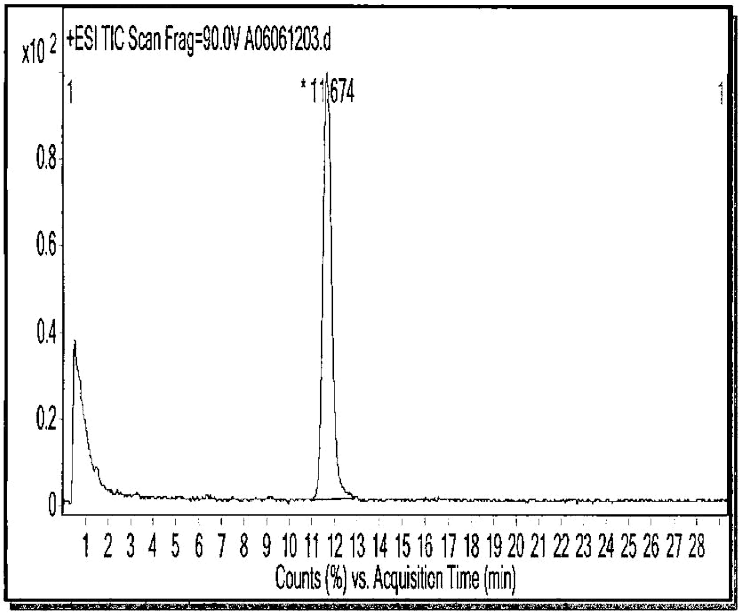
ICP Analysis

Semi Quantitative Scan for Trace Metals

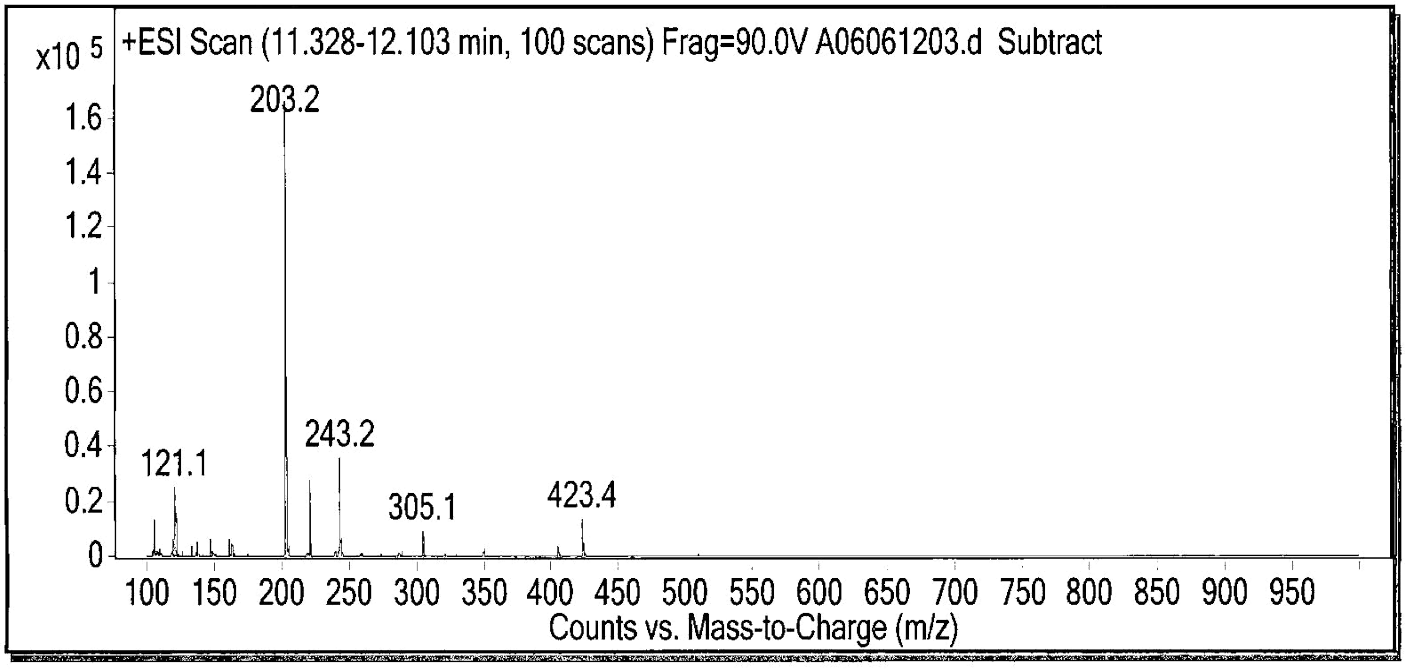
Element	Result	Element	Result	Element	Result	Element	Result		
Ag	4.3 ppm	Eu	< 0.1 ppm	Mo	< 0.1 ppm	Se	0.2 ppm	Zn	9.4 ppm
Al	1.9 ppm	Fe	8.9 ppm	Na	0.16 %	Si	491.5 ppm	Zr	< 0.1 ppm
As	< 0.1 ppm	Ga	< 0.1 ppm	Nb	< 0.1 ppm	Sm	< 0.1 ppm		
Au	< 0.1 ppm	Gd	< 0.1 ppm	Nd	< 0.1 ppm	Sn	0.2 ppm		
B	0.6 ppm	Ge	< 0.1 ppm	Ni	0.2 ppm	Sr	< 0.1 ppm		
Ba	0.5 ppm	Hf	< 0.1 ppm	P	6.3 ppm	Ta	< 0.1 ppm		
Be	< 0.1 ppm	Hg	N/A	Pb	< 0.1 ppm	Tb	< 0.1 ppm		
Bi	< 0.1 ppm	Ho	< 0.1 ppm	Pd	< 0.1 ppm	Th	< 0.1 ppm		
Ca	36.9 ppm	In	< 0.1 ppm	Pr	< 0.1 ppm	Ti	< 0.1 ppm		
Cd	< 0.1 ppm	Ir	< 0.1 ppm	Pt	< 0.1 ppm	Tl	< 0.1 ppm		
Ce	< 0.1 ppm	K	0.4 ppm	Rb	< 0.1 ppm	Tm	< 0.1 ppm		
Co	< 0.1 ppm	La	< 0.1 ppm	Re	< 0.1 ppm	U	< 0.1 ppm		
Cr	4.1 ppm	Li	< 0.1 ppm	Rh	< 0.1 ppm	V	< 0.1 ppm		
Cu	0.9 ppm	Lu	< 0.1 ppm	Ru	< 0.1 ppm	W	< 0.1 ppm		
Dy	< 0.1 ppm	Mg	< 0.1 ppm	Sb	< 0.1 ppm	Y	< 0.1 ppm		
Er	< 0.1 ppm	Mn	0.1 ppm	Sc	0.4 ppm	Yb	< 0.1 ppm		

Spectral and Physical Data (cont.)

LC/MS



Column:	Kinetex 2.6µ C_{18}, 2.1 x 50 mm
Mobile Phase:	Acetonitrile::0.1% Formic acid in Water (40::60)
Flow Rate:	0.4 mL/min
Scan Range:	100-1000 amu
Ionization:	Electrospray, Positive Ion
Data File Name:	A06061203.d
Operator:	BBM
Instrument:	LC/MS/MS
Sample Name:	AV12066
Method File:	46060C1P.m
Acquired:	June 6, 2012 2:19 PM



Spectral and Physical Data (cont.)

Thermogravimetric Analysis

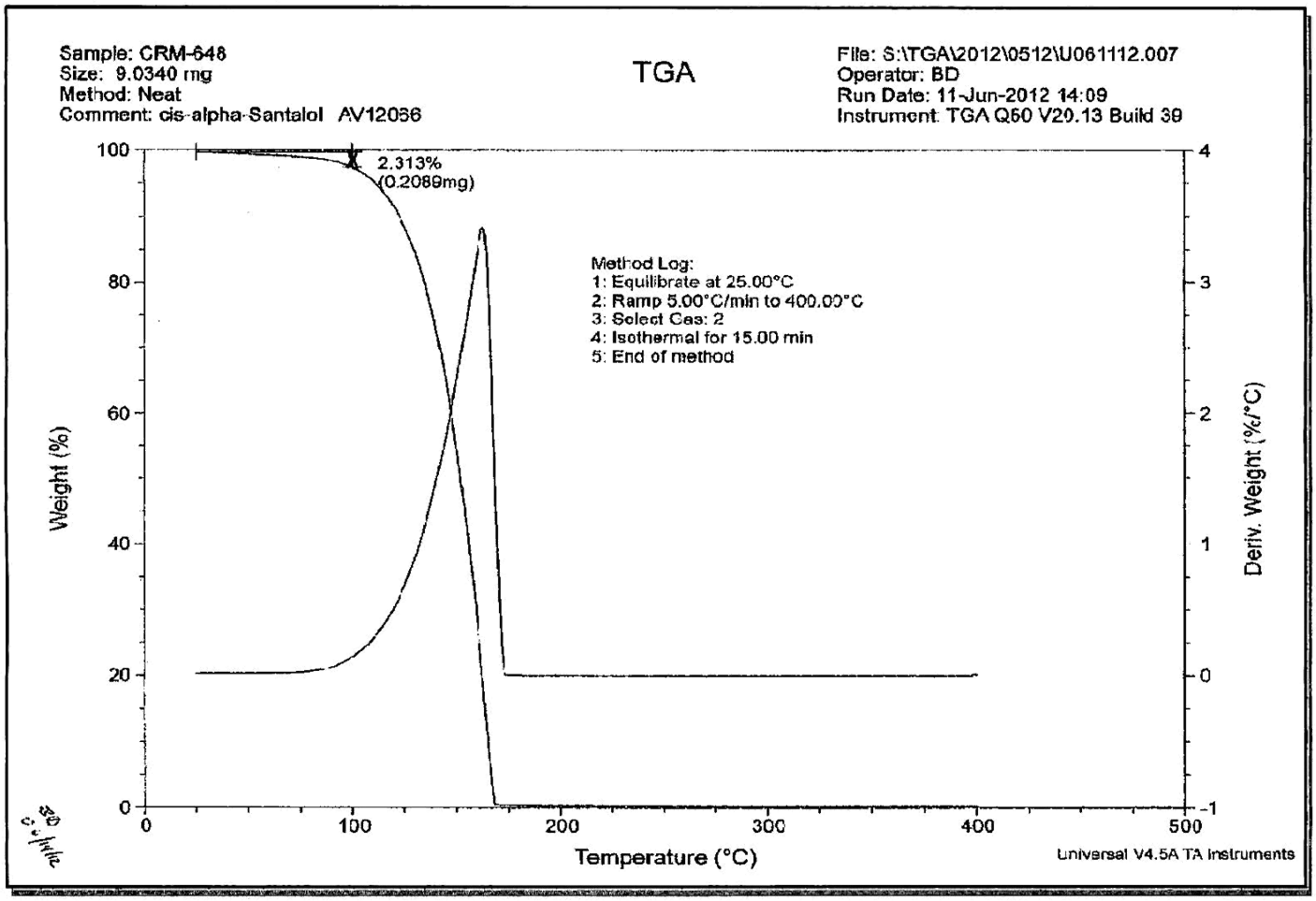


Figure. 10. Certificate of analysis (β-Santalol)

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Analytical Reference Standards
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CSQ-15209
AV12136
Revision 0
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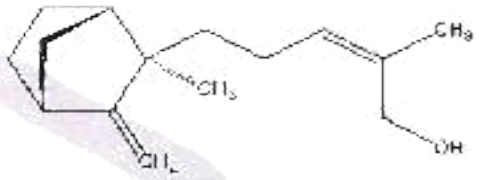
ISO GUIDE 34
ISO/IEC 17025
ISO 13485
ISO 9001
GMP/QMP

Certificate of Analysis

cis-β-Santalol

(2Z)-2-methyl-5-[(1S,2R,4R)-2-methyl-3-methylenebicyclo[2.2.1]hept-2-yl]pent-2-en-1-ol

Reference Number:	CSQ-15209
Lot Number:	AV12136
Retest Date:	June 2013
CAS Number:	77-42-9
Chemical Formula:	$C_{15}H_{24}O$
Molecular Weight:	220.35
Chromatographic Purity:	93.2%
Purity Factor [1]:	91.3%
Storage:	Refrigerate.
Handling:	As specified in the manufacturer MSDS.
Intended Use:	For R&D analytical purposes only.



[1] Purity Factor = (100 - wt% residual solvent - wt% residual water) x Chromatographic Purity/100

Spectral and Physical Data

Analytical Test	Method	Results
Appearance	Visual Inspection	Clear oil
Chromatographic Purity by GC/FID Analysis	SP10-0101	93.2% [2]
Identity by LC/MS Analysis	SP10-0107	Consistent with Structure
Identity by FTIR Analysis	SP10-0108	Consistent with Structure
Identity by ^{1}H-NMR Analysis	USP <761>, SP10-0116	Consistent with Structure
Identity by ^{13}C-NMR Analysis	USP <761>, SP10-0116	Consistent with Structure
Identity by 2D-NMR Analysis	USP <761>, SP10-0116	Consistent with Structure

[2] Purity value is the average of two independent analyses.

Cerilliant certifies the analyses detailed in this certificate are true and accurate. Retest date assigned at the request of the Customer. Cerilliant has not collected stability and makes no claims or warranty regarding shelf life or stability of material.

Authorized Signature: Lara Sparks

Lara Sparks, Quality Assurance Director

July 16, 2012
Date

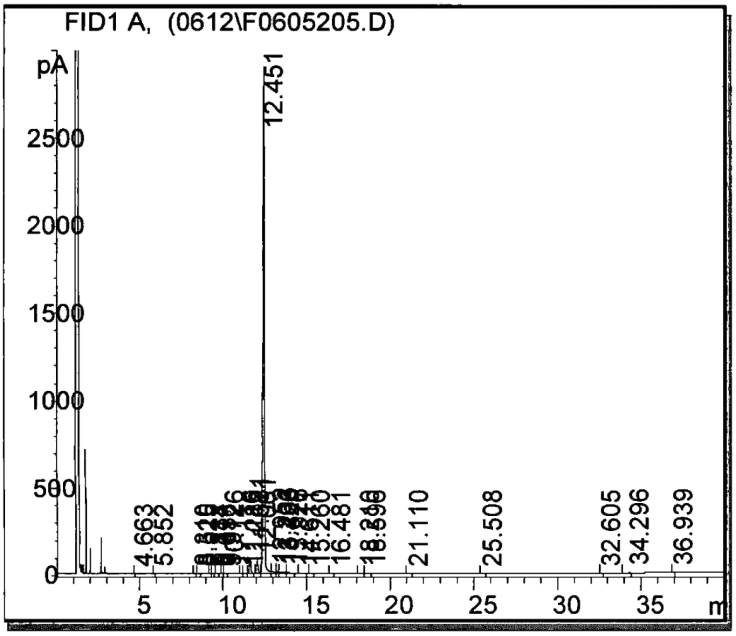
Spectral and Physical Data (cont.)

Analytical Test	Method	Results		
Residual Solvent Analysis by GC/FID Headspace	AM1087 [3]	1.63%		
Residual Water Analysis by Karl Fischer Coulometry	USP <921>, SP10-0103	0.26%		
Specific Rotation	SP10-0133	$[\alpha]^{20}_{D}$ = -95.238°		
Elemental Analysis	SP10-0117		Calculated	Analyzed
		C	81.76%	80.96%
		H	10.98%	10.68%
		O	7.26%	7.06%
Inorganic Content by ICP	Outsourced	Refer to results on page 7		
Thermogravimetric Analysis	SP10-0136	3.92%		
Refractive Index Analysis	Outsourced	1.5012 at 25 °C		

[3] Validated analytical method

Spectral and Physical Data (cont.)

GC/FID



Column: DB-5ms, 30 m x 0.53 mm ID, 1.5 µm film thickness
Temp Program: 40°C to 160°C at 40°C/min
160°C to 210°C at 2°C/min
210°C to 280°C at 10°C/min (hold 5 min)
Injector Temp: Cool-on-Column
Detector Temp: 325°C

Data File Name: S:\GC\GC6\2012\0612\F0605205.D
Operator: RPC
Instrument: GC#6
Sample Name: AV12136
Method File: CISSANTA.M
Acquired: June 5, 2012 12:39 AM

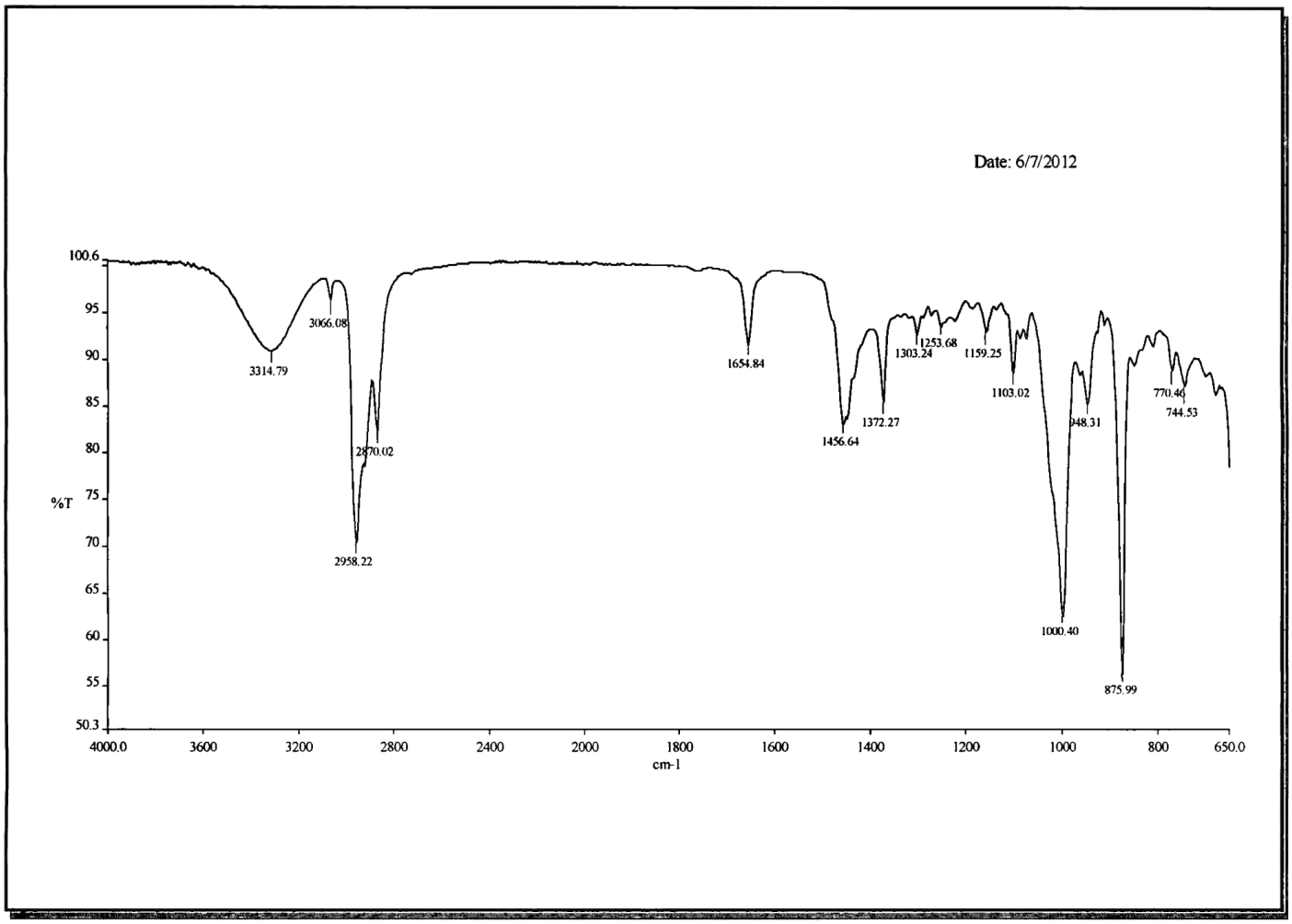
Peak #	Ret Time	Area	Height	Area %
1	4.66	0.11	0.08	0.00
2	5.85	0.45	0.15	0.00
3	8.31	1.78	0.44	0.01
4	8.53	2.11	0.51	0.01
5	9.23	1.03	0.25	0.01
6	9.38	2.02	0.43	0.01
7	9.63	1.02	0.21	0.01
8	9.97	1.12	0.26	0.01
9	10.13	5.24	0.82	0.03
10	11.12	2.04	0.38	0.01
11	11.29	11.78	2.06	0.06
12	11.63	442.24	78.39	2.35
13	12.06	428.62	71.14	2.27
14	12.45	17599.20	2846.98	93.40

Peak #	Ret Time	Area	Height	Area %
15	12.95	142.87	10.13	0.76
16	13.27	76.05	8.14	0.40
17	13.43	82.70	5.69	0.44
18	13.93	27.22	1.71	0.14
19	14.61	1.95	0.20	0.01
20	15.26	1.45	0.21	0.01
21	16.48	2.09	0.27	0.01
22	18.21	0.69	0.07	0.00
23	18.60	0.53	0.06	0.00
24	21.11	1.06	0.11	0.01
25	25.51	0.43	0.04	0.00
26	32.61	0.85	0.19	0.00
27	34.30	6.49	1.88	0.03
28	36.94	0.43	0.09	0.00

Spectral and Physical Data (cont.)

Identity by FTIR Analysis

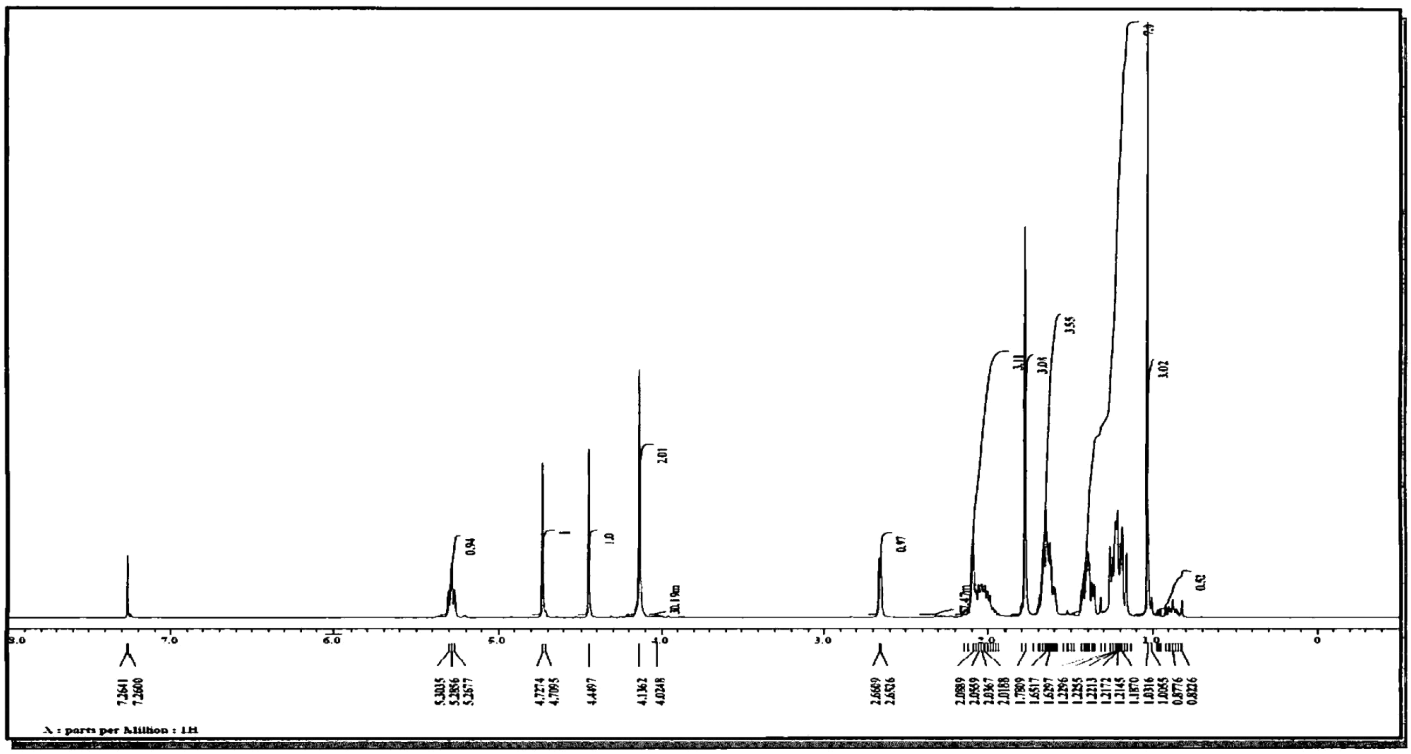
Instrument:	Perkin Elmer Spectrum One
Matrix:	Neat
Scans:	32



Spectral and Physical Data (cont.)

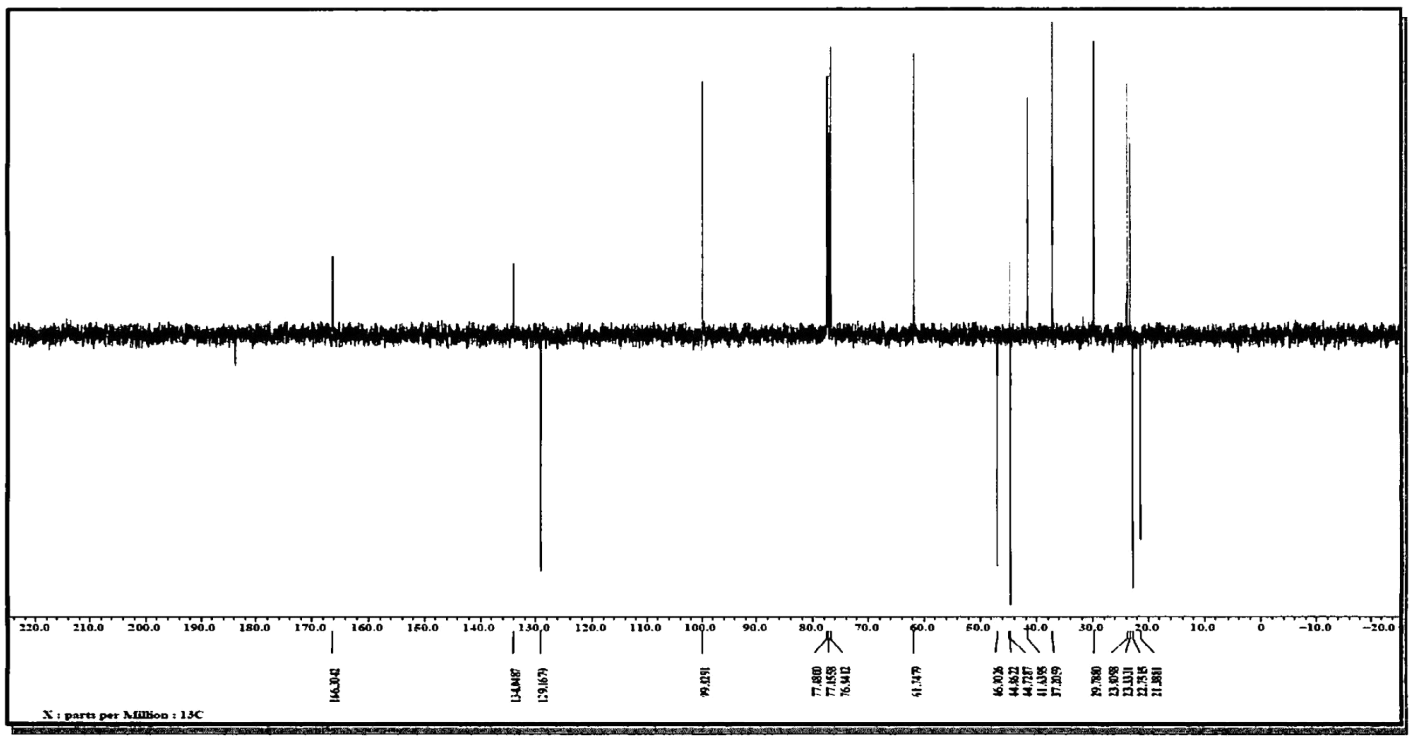
^{1}H NMR

Instrument: JEOL ECS 400
Solvent: Chloroform-D



^{13}C NMR

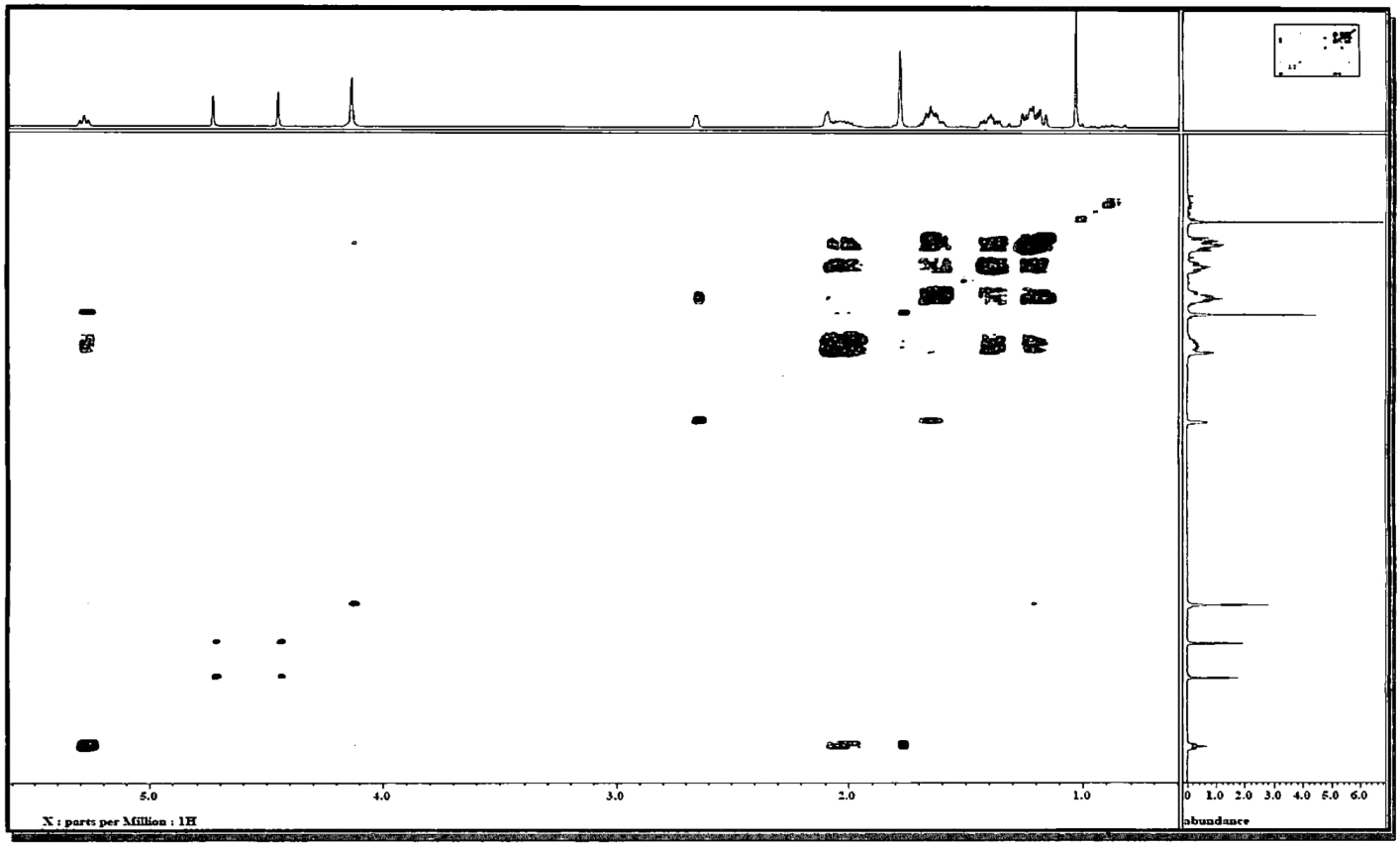
Instrument: JEOL ECS 400
Solvent: Chloroform-D



Spectral and Physical Data (cont.)

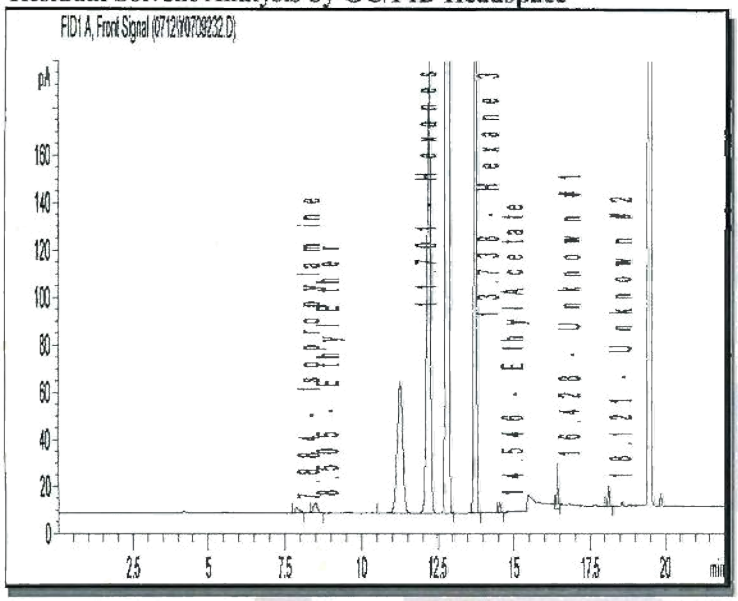
2D NMR

Instrument: JEOL ECS 400
Solvent: Chloroform-D



Spectral and Physical Data (cont.)

Residual Solvent Analysis by GC/FID Headspace



Column:	DB-ALC1 30 m x 0.53 mm, 3 µm film thickness
Temp Program:	40°C (12 min) to 220°C at 40°C/min (5.5 min)
Carrier Gas:	Helium
Flow Rate:	2.0 mL/min
Detector Heater Temp:	250°C
Injector:	Headspace Sampler
HS Oven Temp:	60°C
Vial Equilibration:	10 minutes
Data File Name:	S:\GC\GC-HS11\2012\0712\Y0709232.D
Operator:	BD
Instrument:	GC#11
Sample Name:	AV12136
Acquired:	July 11, 2012 11:15 AM

Peak	Compound	Area	Weight %
1	Isopropylamine	19.82406	0.01
2	Ethyl ether	37.21335	BQL
3	Hexanes	11448.65283	1.60
4	Ethyl acetate	15.53636	0.03
5	Unknown # 1	65.3055	BQL
6	Unknown # 2	31.47961	BQL
7	NMP	NA	NA
Total			**1.63**

BQL - Below Quantitation Limit

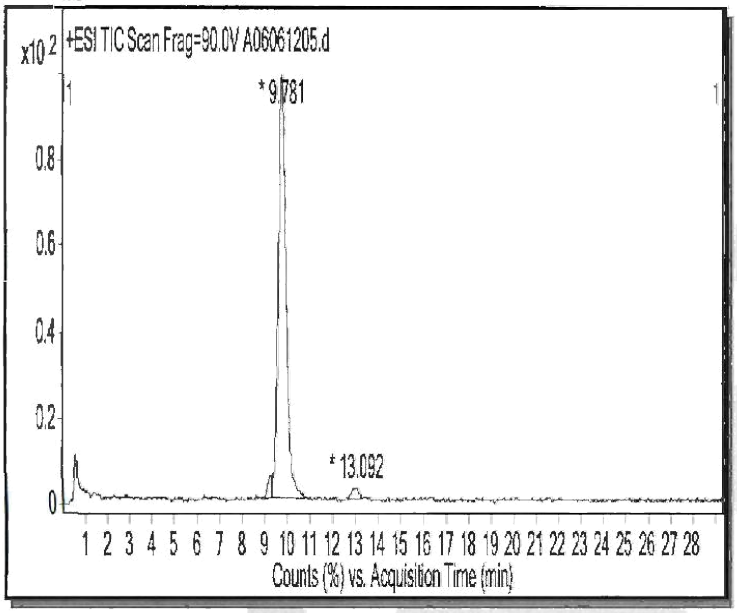
ICP Analysis

Semi Quantitative Scan for Trace Metals

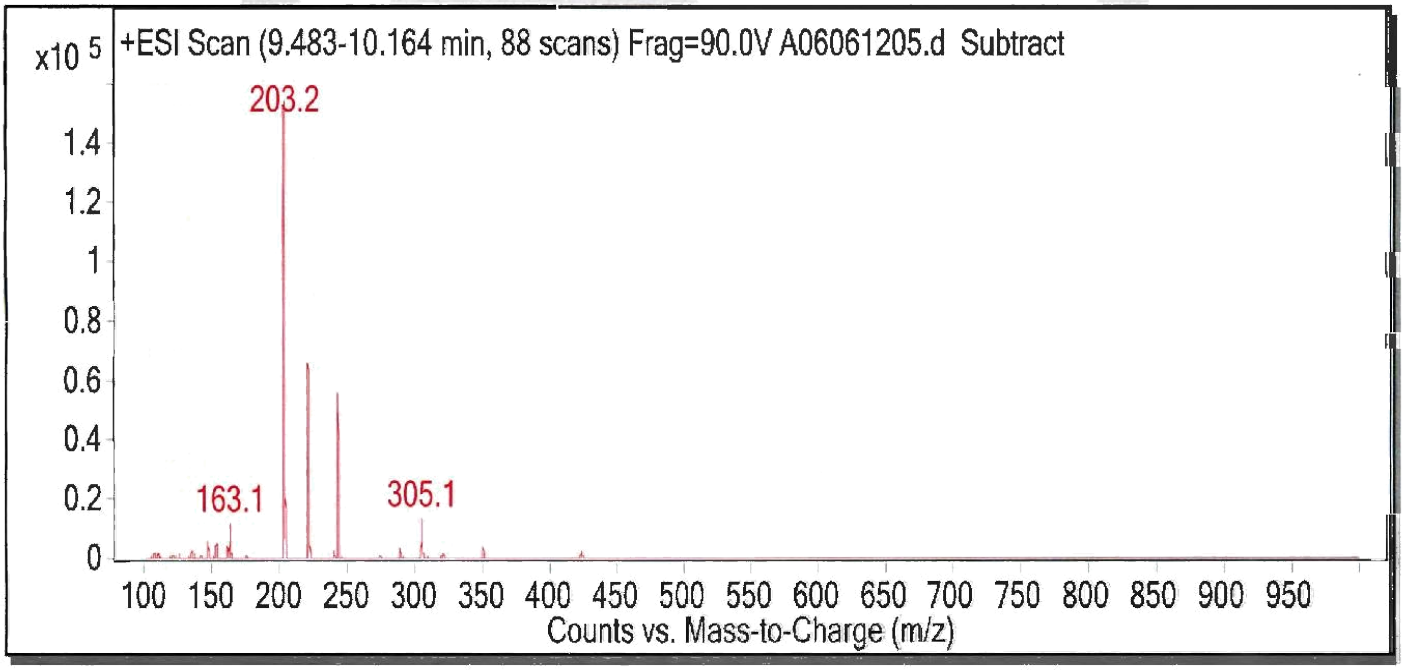
Element	Result	Element	Result	Element	Result	Element	Result		
Ag	0.6 ppm	Eu	< 0.1 ppm	Mo	< 0.1 ppm	Se	0.1 ppm	Zn	11.3 ppm
Al	2.3 ppm	Fe	40.2 ppm	Na	774.1 ppm	Si	310.0 ppm	Zr	< 0.1 ppm
As	< 0.1 ppm	Ga	< 0.1 ppm	Nb	< 0.1 ppm	Sm	< 0.1 ppm		
Au	< 0.1 ppm	Gd	< 0.1 ppm	Nd	< 0.1 ppm	Sn	0.3 ppm		
B	0.9 ppm	Ge	< 0.1 ppm	Ni	0.1 ppm	Sr	< 0.1 ppm		
Ba	0.6 ppm	Hf	< 0.1 ppm	P	17.6 ppm	Ta	< 0.1 ppm		
Be	< 0.1 ppm	Hg	N/A	Pb	< 0.1 ppm	Tb	< 0.1 ppm		
Bi	< 0.1 ppm	Ho	< 0.1 ppm	Pd	< 0.1 ppm	Th	< 0.1 ppm		
Ca	16.0 ppm	In	< 0.1 ppm	Pr	< 0.1 ppm	Ti	0.3 ppm		
Cd	< 0.1 ppm	Ir	< 0.1 ppm	Pt	< 0.1 ppm	Tl	< 0.1 ppm		
Ce	< 0.1 ppm	K	9.9 ppm	Rb	< 0.1 ppm	Tm	< 0.1 ppm		
Co	< 0.1 ppm	La	< 0.1 ppm	Re	< 0.1 ppm	U	< 0.1 ppm		
Cr	2.0 ppm	Li	< 0.1 ppm	Rh	< 0.1 ppm	V	< 0.1 ppm		
Cu	1.2 ppm	Lu	< 0.1 ppm	Ru	< 0.1 ppm	W	< 0.1 ppm		
Dy	< 0.1 ppm	Mg	< 0.1 ppm	Sb	< 0.1 ppm	Y	< 0.1 ppm		
Er	< 0.1 ppm	Mn	< 0.1 ppm	Sc	0.3 ppm	Yb	< 0.1 ppm		

Spectral and Physical Data (cont.)

LC/MS



Column:	Kinetex 2.6µ C_{18}, 2.1 x 50 mm
Mobile Phase:	Acetonitrile::0.1% Formic acid in Water (40::60)
Flow Rate:	0.4 mL/min
Scan Range:	100-1000 amu
Ionization:	Electrospray, Positive Ion
Data File Name:	A06061205.d
Operator:	BBM
Instrument:	LC/MS/MS
Sample Name:	AV12163
Method File:	46060C1P.m
Acquired:	June 6, 2012 4:27 PM



Spectral and Physical Data (cont.)

Thermogravimetric Analysis