LAMPIRAN

Lampiran 1. Surat Keterangan Lolos Etik

UMY UNIVERSITAS MUHAMMADIYAH YOGYAKARTA
Unggul & Islami
FAKULTAS KEDOKTERAN DAN ILMU KESEHATAN

Nomor : 615.2/EP-FKIK-UMY/XII/2018

KETERANGAN LOLOS UJI ETIK
ETHICAL APPROVAL

Komite Etik Penelitian Fakultas Kedokteran dan Ilmu Kesehatan Universitas Muhammadiyah Yogyakarta dalam upaya melindungi hak asasi dan kesejahteraan responden/subyek penelitian, telah mengkaji dengan teliti protokol berjudul :

The Ethics Committee of the Faculty of Medicine and Health Sciences, University of Muhammadiyah Yogyakarta, with regards of the protection of human rights and welfare in research, has carefully reviewed the research protocol entitled :

"Uji Aktivitas Antagonisme Etil P- Metoksisnamat Senyawa Aktif Kencur (*Kaempferia galanga L*) terhadap Reseptor AcHM3 pada Organ Trakea *Cavia porcellus* Terisolasi : Studi *In Vitro* dan *In Silico*"

Peneliti Utama / *Principal Investigator* : Puguh Novi Arsito, Siska Nurjannah

Nama Institusi / *Name of the Institution* : Program Studi Farmasi FKIK UMY

Negara / *Country* : Indonesia

Dan telah menyetujui protokol tersebut diatas.
And approved the above-mentioned protocol.

Yogyakarta, 22 Desember 2018
Ketua
Chairperson

Dr. dr. Titiek Hidayati, M.Kes., Sp.Rad., FISPH., FISCM.

*Peneliti Berkewajiban :
1. Menjaga kerahasiaan identitas subyek penelitian
2. Memberitahukan status penelitian apabila :
   a. Setelah masa berlakunya keterangan lolos uji etik (1 tahun sejak tanggal terbit), penelitian masih belum selesai, dalam hal ini *ethical clearance* harus diperpanjang
   b. Penelitian berhenti di tengah jalan
3. Melaporkan kejadian serius yang tidak diinginkan (*serious adverse events*).
4. Peneliti tidak boleh melakukan tindakan apapun pada responden/subyek sebelum penelitian lolos uji etik.

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D.I.Yogyakarta 55183

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Lampiran 2. Hasil Determinasi Tanaman

LABORATORIUM BIOLOGI

FAKULTAS MIPA
UNIVERSITAS AHMAD DAHLAN
Jl. Prof. Dr. Soepomo, Yogyakarta Telp. (0274) 563515

SURAT KETERANGAN
Nomor : 007/Lab.Bio/B/I/2019

Yang bertanda tangan di bawah ini Kepala Laboratorium Biologi Universitas Ahmad Dahlan menerangkan bahwa :

Nama : Siti Lathifah R
NIM : 20150350083
Prodi, PT : Farmasi, Universitas Muhammadiyah Yogyakarta

Telah melakukan determinasi tanaman dengan bimbingan Hery Setiyawan, M.Si di Laboratorium Biologi Universitas Ahmad Dahlan, pada tanggal 21 Januari 2019

Tanaman tersebut adalah :
Kaempferia galanga Linn.

Demikian Surat Keterangan ini untuk dapat dipergunakan seperlunya.

Yogyakarta, 21 Januari 2019
Kepala Laboratorium Biologi

Drs. Hadi Sasongko, M.Si.

1b – 2b – 3b – 4b – 12b – 13b – 14b – 17b – 18b – 19b – 20b – 21b – 22b – 23b – 24b – 25b – 26b – 27a – 28b – 29b - 30b – 31a – 32a – 33a – 34b – 333b – 334b – 335a – 336a – 337b – 338a – 339b – 340a – Zingiberaceae

1a – 2a – 3b – 4a – 5b Kaempferia

1a *Kaempferia galanga L*

Flora of Java (Backer, 1965)

Lampiran 3. Perhitungan Larutan Agonis dan EPMS

1. **Perhitungan Agonis $AChM_3$**

   BM $AChM_3$ = 240,1 g/mol.

   Pembuatan larutan stok $2x10^{-1}$ M:

   Berat $AChM_3$ yang dibutuhkan → $M = \frac{berat\ (g)}{BM}\ x\ \frac{1000}{volume\ (mL)}$

   $2x10^{-1}$ M $= \frac{x}{240{,}1\ g/mol}\ x\ \frac{1000}{10\ mL}$

   X $= \frac{2\ x\ 10^{-1}\ M\ x\ 240{,}1 \frac{g}{mol}\ x\ 10\ mL}{1000}$

   X $= 480{,}2 \times 10^{-3}$ g.

   X = 480,2 mg.

   Dilakukan pengenceran dan dibuat seri kadar dari konsentrasi $2x10^{-2}$ hingga $2x10^{-8}$.

2. **Perhitungan Atropin Sulfat**

   BM Atropin Sulfat = 676,8 g/mol

   → Pembuatan larutan Atropin konsentrasi $2x10^{-6}$ M

   Berat Atropin Sulfat yang dibutuhkan:

   $2x10^{-6}$ M $= \frac{x\ (g)}{676{,}8\ g/mol}\ x\ \frac{1000}{10\ mL}$

   X $= \frac{2\ x\ 10^{-6}\ M\ x\ 676{,}8 \frac{g}{mol} x\ 10\ mL}{1000}$

   X $= 13{,}54 \times 10^{-6}$ g.

   X $= 13{,}54 \times 10^{-3}$ mg.

   Sediaan injeksi yang tersedia = 0,25 mg/mL.

→ Volume sediaan yang diambil =

V $= \frac{13{,}54\ x\ 10^{-3}\ g}{0{,}25\ g/mL}$ = 54,16 x 10^{-3} mL ~ 54,16 µL ad 10 mL.

→ Dosis Atropin volume 100 µL

$\frac{13{,}54 \text{ x } 10^{-3} \text{ mg}}{10 \text{ mL}} = \frac{13{,}54 \text{ µg}}{10.000 \text{ µL}} = 13{,}54 \text{ x } 10^{-4}$ µg/ µL.

13,54 x 10^{-4} µg/ µL x 100 µL = 0,1354 µg.

→ Konsentrasi larutan Atropin 2x10^{-6} M sebanyak 100 µL dalam buffer krebs 20 mL:

2x10^{-6} M x 0,1 mL = M_2 x 20 mL

$M_2 = \frac{2\ x\ 10^{-6} M\ x\ 0{,}1\ mL}{20\ mL}$

M_2 = 10^{-8} M ~ 10^{-2} µM.

→ Dosis Atropin volume 500 µL

$\frac{13{,}54 \text{ x } 10^{-3} \text{ mg}}{10 \text{ mL}} = \frac{13{,}54 \text{ µg}}{10.000 \text{ µL}} = 13{,}54 \text{ x } 10^{-4}$ µg/ µL.

13,54 x 10^{-4} µg/ µL x 500 µL = 67,7 x 10^{-2} µg.

→ Konsentrasi larutan Atropin 2x10^{-6} M sebanyak 500 µL dalam buffer krebs 20 mL:

2x10^{-6} M x 0,5 mL = M_2 x 20 mL

$M_2 = \frac{2\ x\ 10^{-6} M\ x\ 0{,}5\ mL}{20\ mL}$

M_2 = 5 x 10^{-8} M ~ 5 x 10^{-2} µM.

3. Perhitungan EPMS

BM EPMS = 260,2 g/mol

→ Pembuatan larutan stok EPMS konsentrasi $2x10^{-1}$ M

Berat EPMS yang dibutuhkan:

$2x10^{-1}$ M $= \frac{x\ (g)}{260{,}2\ g/mol}\ x\ \frac{1000}{5\ mL}$

$X = \frac{2\ x\ 10^{-1}\ M\ x\ 260{,}2\ \frac{g}{mol} x\ 5\ mL}{1000}$

X = 206,2 mg

Dilakukan pengenceran hingga mencapai konsentrasi $2x10^{-2}$ M.

→ Dosis EPMS volume 100 µL

$\frac{206{,}2\ mg}{5\ mL} = \frac{206.200\ \mu g}{5.000\ \mu L} = 41{,}24\ \mu g/\ \mu L$

41,24 µg/ µL x 100 µL = 4124 µg ~ 4,124 mg.

Dilakukan pengenceran 10x hingga mencapai konsentrasi $2x10^{-2}$ M dengan dosis yang didapatkan yaitu 0,4124 mg.

→ Konsentrasi larutan EPMS $2x10^{-2}$ M sebanyak 100 µL dalam buffer krebs 20 mL:

$2x10^{-2}$ M x 0,1 mL $= M_2$ x 20 mL

$M_2 = \frac{2\ x\ 10^{-2} M\ x\ 0{,}1\ mL}{20\ mL}$

$M_2 = 10^{-4}$ M ~ 100 µM.

→ Dosis EPMS volume 200 µL

$\frac{206{,}2\ mg}{5\ mL} = \frac{206.200\ \mu g}{5.000\ \mu L} = 41{,}24\ \mu g/\ \mu L.$

41,24 µg/ µL x 200 µL = 8248 µg ~ 8,248 mg.

Dilakukan pengenceran 10x hingga mencapai konsentrasi $2x10^{-2}$ M dengan dosis yang didapatkan yaitu 0,8248 mg.

→ Konsentrasi larutan EPMS $2x10^{-2}$ M sebanyak 200 µL dalam buffer krebs 20 mL:

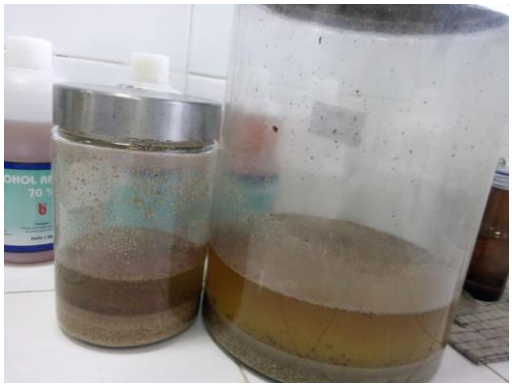
$2x10^{-2}$ M x 0,2 mL = M_2 x 20 mL

$M_2 = \frac{2\ x\ 10^{-2} M\ x\ 0{,}2\ mL}{20\ mL}$

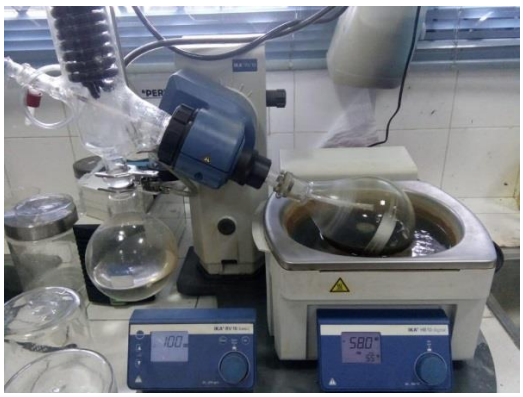
$M_2 = 2 \times 10^{-4}$ M ~ 200 µM.

Lampiran 4. Preparasi Bahan dan Organ

Gambar 1. Perajangan rimpang kencur



Gambar 2. Proses maserasi

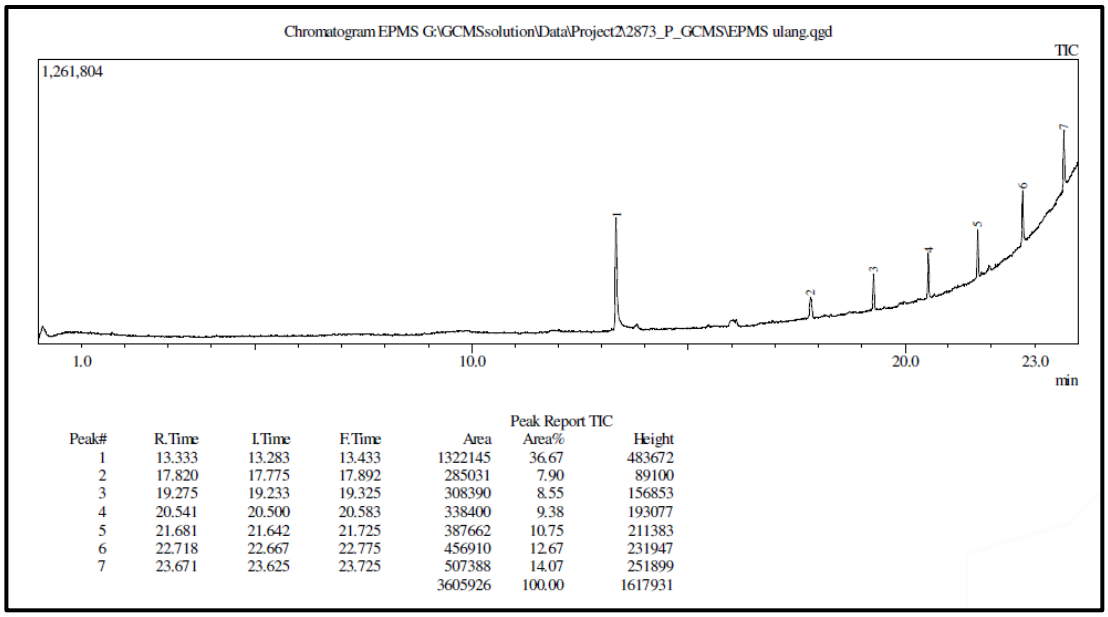


Gambar 3. Proses pemanasan menggunakan *Rotary Vacuum Evaporator* untuk memekatkan ekstrak

Gambar 4. Kristal Kencur setelah dilakukan pencucian

Gambar 5. Preparasi organ trakea

Lampiran 5. Hasil Uji GC-MS



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height
1	13.333	13.283	13.433	1322145	36.67	483672
2	17.820	17.775	17.892	285031	7.90	89100
3	19.275	19.233	19.325	308390	8.55	156853
4	20.541	20.500	20.583	338400	9.38	193077
5	21.681	21.642	21.725	387662	10.75	211383
6	22.718	22.667	22.775	456910	12.67	231947
7	23.671	23.625	23.725	507388	14.07	251899
				3605926	100.00	1617931

Gambar 6. Uji *Gas Chromatography* senyawa EPMS

Library

<< Target >>

Line#:1 R.Time:13.333(Scan#:1601) MassPeaks:319

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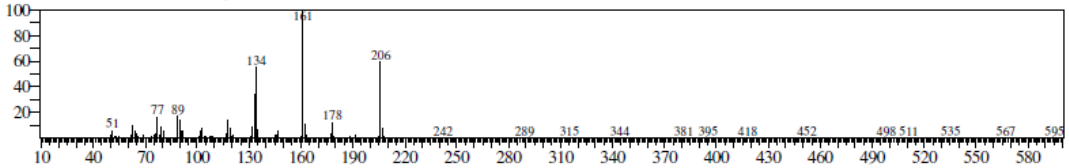
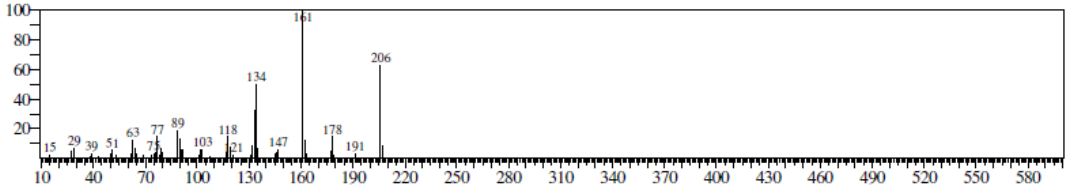
BG Mode:Calc. from Peak Group 1 - Event 1



Hit#:1 Entry:102430 Library:WILEY7.LIB

SI:97 Formula:C12 H14 O3 CAS:1929-30-2 MolWeight:206 RetIndex:0

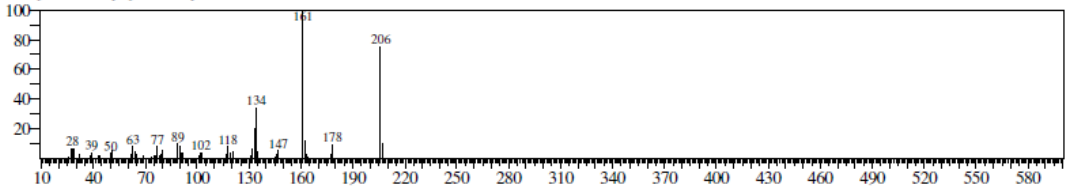
CompName:2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester $$



Hit#:2 Entry:103525 Library:WILEY7.LIB

SI:91 Formula:C12 H14 O3 CAS:24393-56-4 MolWeight:206 RetIndex:0

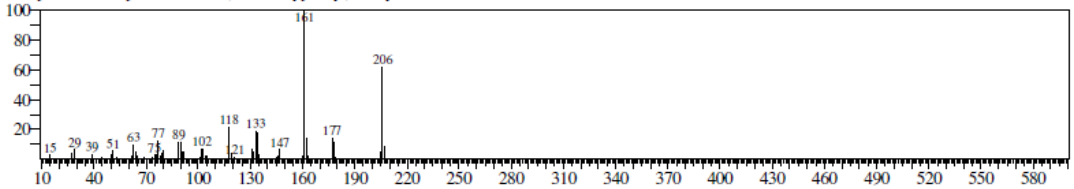
CompName:Ethyl p-methoxycinnamate $$



Hit#:3 Entry:102429 Library:WILEY7.LIB

SI:91 Formula:C12 H14 O3 CAS:33877-04-2 MolWeight:206 RetIndex:0

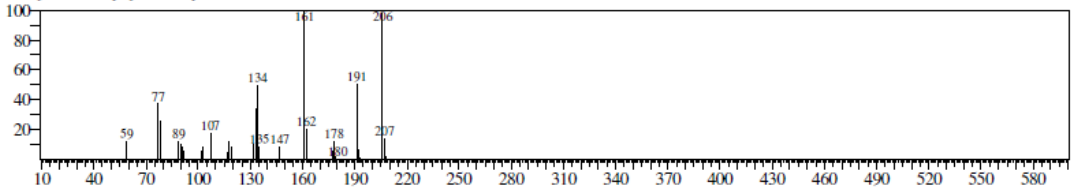
CompName:2-Propenoic acid, 3-(3-methoxyphenyl)-, ethyl ester $$



Hit#:4 Entry:103526 Library:WILEY7.LIB

SI:82 Formula:C12 H14 O3 CAS:24393-56-4 MolWeight:206 RetIndex:0

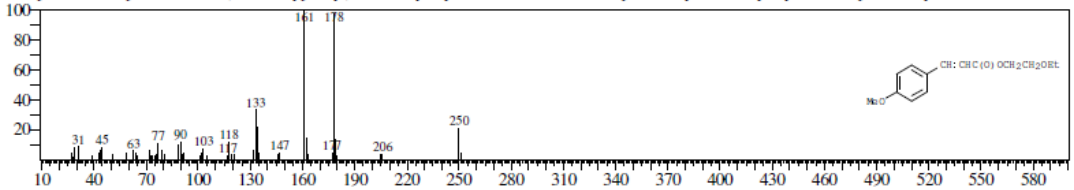
CompName:Ethyl p-methoxycinnamate $$



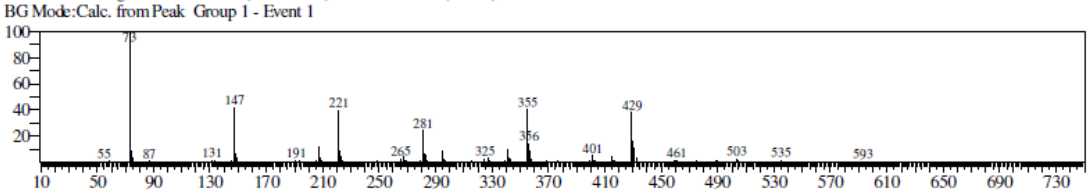
Hit#:5 Entry:157558 Library:WILEY7.LIB

SI:78 Formula:C14 H18 O4 CAS:104-28-9 MolWeight:250 RetIndex:0

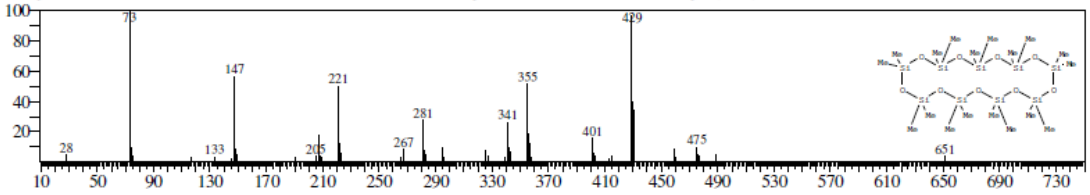
CompName:2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethoxyethyl ester $$ Cinnamic acid, p-methoxy-, 2-ethoxyethyl ester $$ p-Methoxycinnamic acid, 2-eth



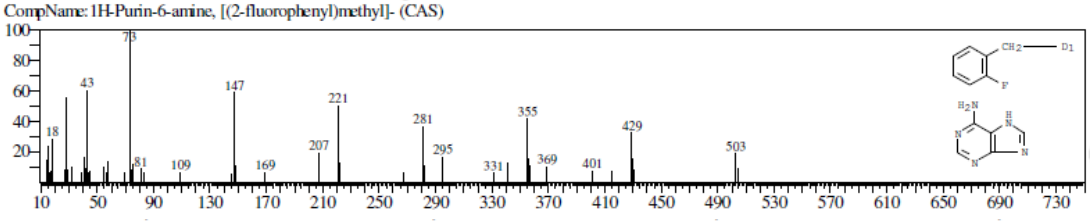
<< Target >>
Line#:2 R.Time:17.817(Scan#:2139) MassPeaks:366
RawMode:Averaged 17.808-17.825(2138-2140) BasePeak:73.00(15588)
BG Mode:Calc. from Peak Group 1 - Event 1



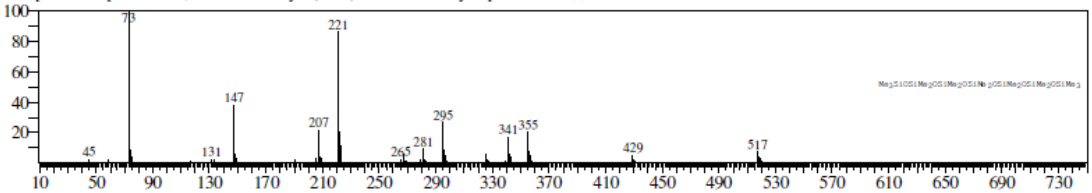
Hit#:1 Entry:332919 Library:WILEY7.LIB
SI:85 Formula:C18 H54 O9 SI9 CAS:556-71-8 MolWeight:666 RetIndex:0
CompName:OCTADECAMETHYLCYCLONONASILOXANE $$ Cyclononasiloxane, octadecamethyl- (CAS)



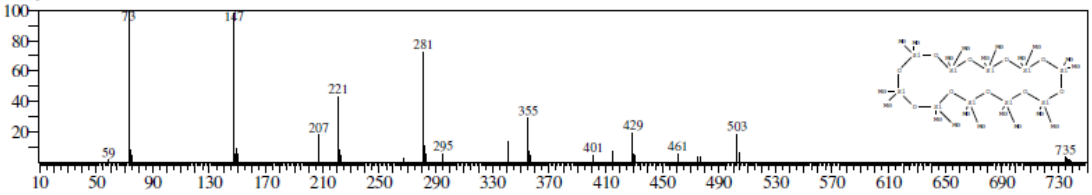
Hit#:2 Entry:148639 Library:WILEY7.LIB
SI:83 Formula:C12 H10 F N5 CAS:74421-44-6 MolWeight:243 RetIndex:0
CompName:1H-Purin-6-amine, [(2-fluorophenyl)methyl]- (CAS)



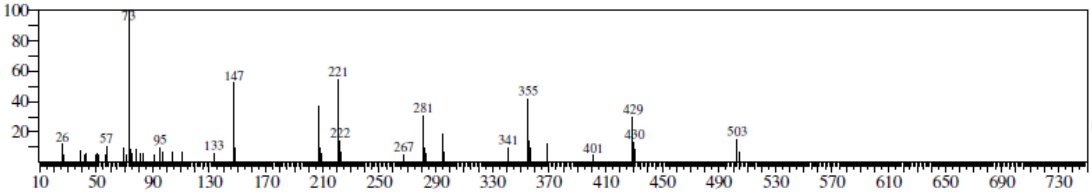
Hit#:3 Entry:321725 Library:WILEY7.LIB
SI:82 Formula:C16 H48 O6 SI7 CAS:541-01-5 MolWeight:532 RetIndex:0
CompName:Heptasiloxane, hexadecamethyl- (CAS) Hexadecamethylheptasiloxane $$



Hit#:4 Entry:335377 Library:WILEY7.LIB
SI:82 Formula:C20 H60 O10 SI10 CAS:18772-36-6 MolWeight:740 RetIndex:0
CompName:EICOSAMETHYLCYCLODECASILOXANE $$ CYCLODECASILOXANE, EICOSAMETHYL- $$



Hit#:5 Entry:338296 Library:WILEY7.LIB
SI:81 Formula: CAS:0-00-0 MolWeight:9999 RetIndex:0
CompName:SILIKONFETT SE30 (GREVELS) $$ Silicone oil $$



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Line#:3 R.Time:19.275(Scan#:2314) MassPeaks:365
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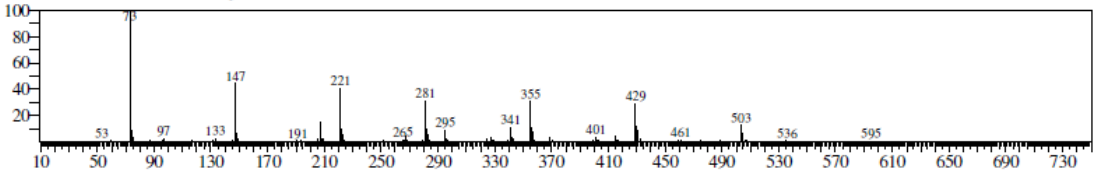
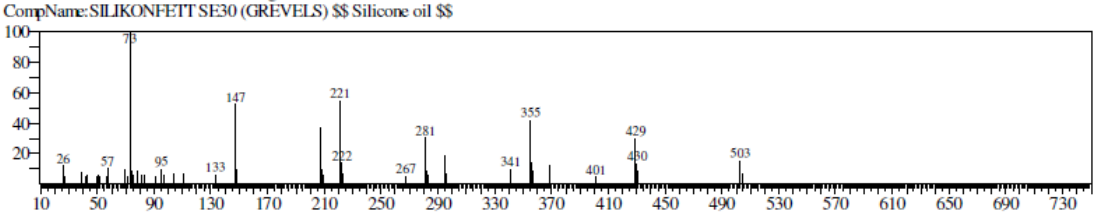
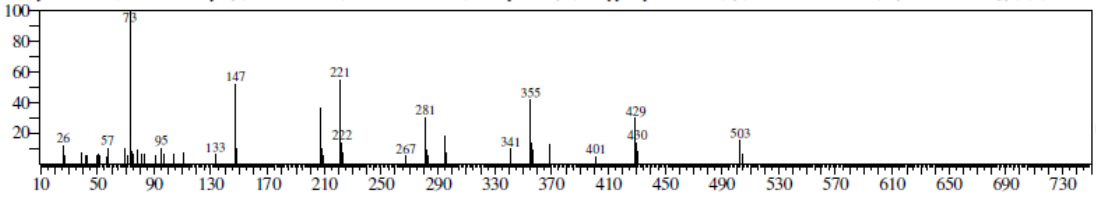
BG Mode:Calc. from Peak Group 1 - Event 1



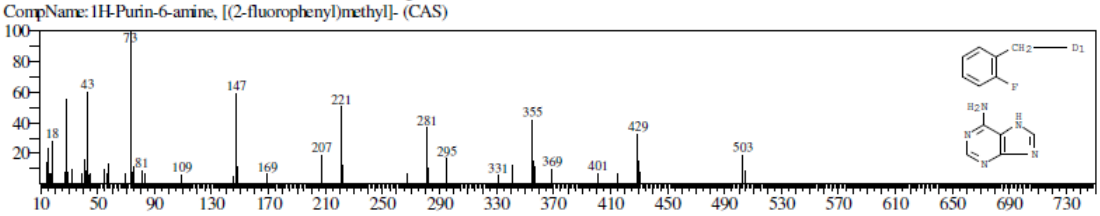
Hit#:1 Entry:338296 Library:WILEY7.LIB
SI:87 Formula: CAS:0-00-0 MolWeight:9999 RetIndex:0
CompName:SILIKONFETT SE30 (GREVELS) $$ Silicone oil $$



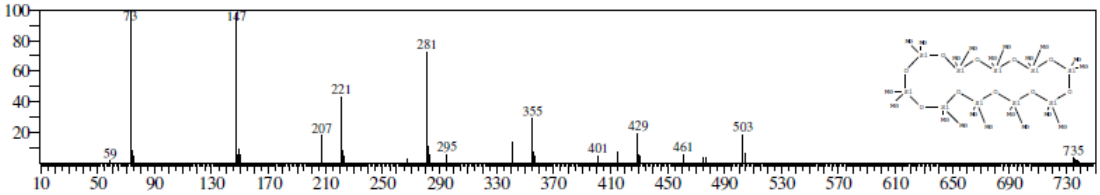
Hit#:2 Entry:300653 Library:WILEY7.LIB
SI:87 Formula:C21 H22 FE N2 O5 CAS:109007-87-6 MolWeight:438 RetIndex:0
CompName:Iron, monocarbonyl-(1,3-butadiene-1,4-dicarbonic acid, diethyl ester) a,a'-dipyridyl $$ IRON, (2,2'-BIPYRIDINE-N,N')CARBONYL[(2,3,4,5-.ETA



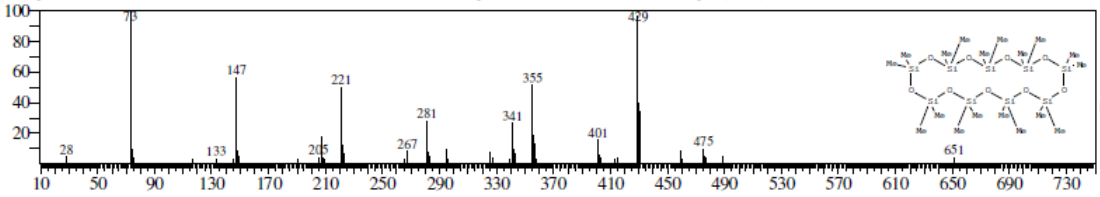
Hit#:3 Entry:148639 Library:WILEY7.LIB
SI:86 Formula:C12 H10 F N5 CAS:74421-44-6 MolWeight:243 RetIndex:0
CompName:1H-Purin-6-amine, [(2-fluorophenyl)methyl]- (CAS)



Hit#:4 Entry:335377 Library:WILEY7.LIB
SI:85 Formula:C20 H60 O10 SI10 CAS:18772-36-6 MolWeight:740 RetIndex:0
CompName:EICOSAMETHYLCYCLODECASILOXANE $$ CYCLODECASILOXANE, EICOSAMETHYL- $$



Hit#:5 Entry:332919 Library:WILEY7.LIB
SI:82 Formula:C18 H54 O9 SI9 CAS:556-71-8 MolWeight:666 RetIndex:0
CompName:OCTADECAMETHYLCYCLONONASILOXANE $$ Cyclononasiloxane, octadecamethyl- (CAS)



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Line#:4 R.Time:20.542(Scan#:2466) MassPeaks:358
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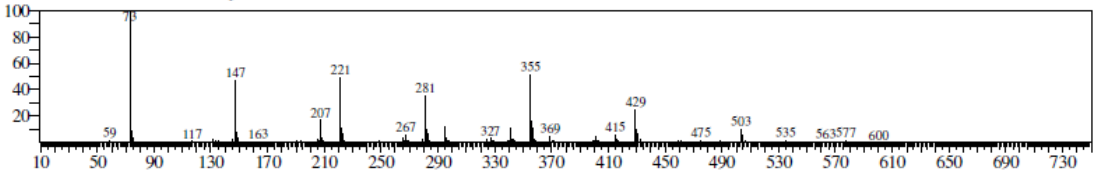
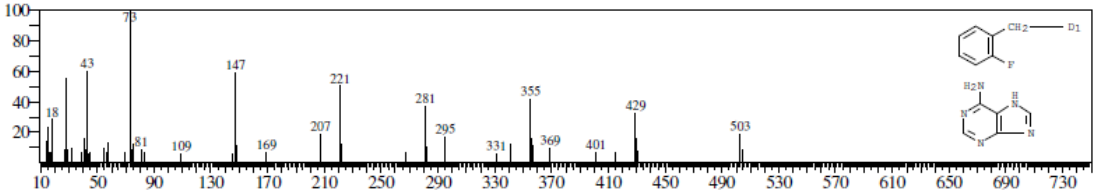
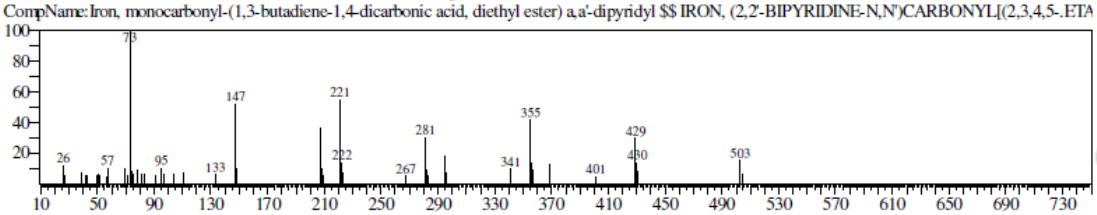
BG Mode:Calc. from Peak Group 1 - Event 1



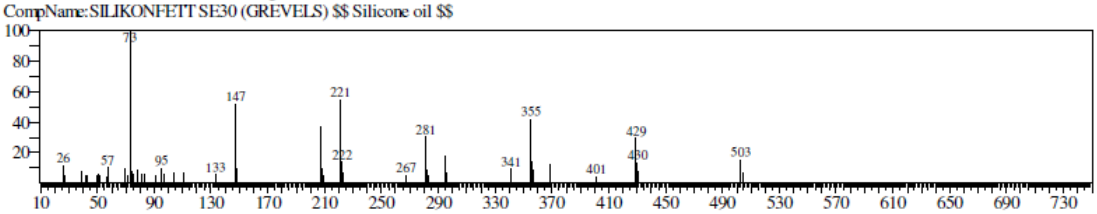
Hit#:1 Entry:148639 Library:WILEY7.LIB
SI:87 Formula:C12 H10 F N5 CAS:74421-44-6 MolWeight:243 RetIndex:0
CompName:1H-Purin-6-amine, [(2-fluorophenyl)methyl]- (CAS)



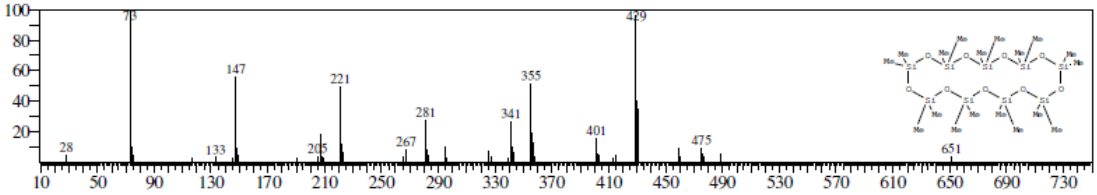
Hit#:2 Entry:300653 Library:WILEY7.LIB
SI:86 Formula:C21 H22 FE N2 O5 CAS:109007-87-6 MolWeight:438 RetIndex:0
CompName:Iron, monocarbonyl-(1,3-butadiene-1,4-dicarbonic acid, diethyl ester) a,a'-dipyridyl $$ IRON, (2,2'-BIPYRIDINE-N,N')CARBONYL[(2,3,4,5-.ETA



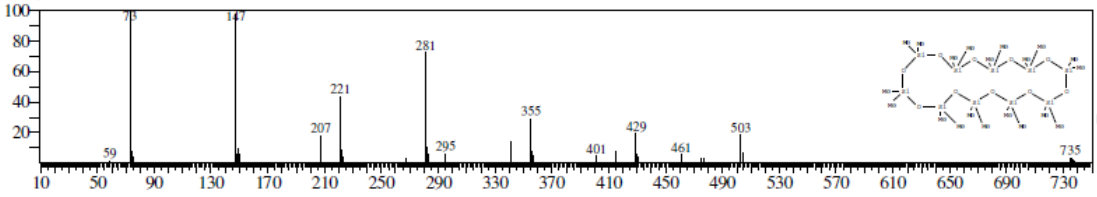
Hit#:3 Entry:338296 Library:WILEY7.LIB
SI:86 Formula: CAS:0-00-0 MolWeight:9999 RetIndex:0
CompName:SILIKONFETT SE30 (GREVELS) $$ Silicone oil $$



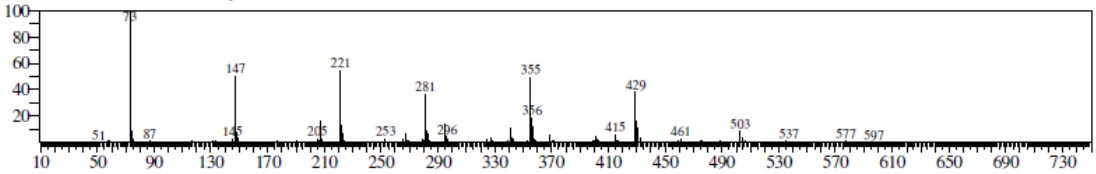
Hit#:4 Entry:332919 Library:WILEY7.LIB
SI:85 Formula:C18 H54 O9 SI9 CAS:556-71-8 MolWeight:666 RetIndex:0
CompName:OCTADECAMETHYLCYCLONONASILOXANE $$ Cyclononasiloxane, octadecamethyl- (CAS)



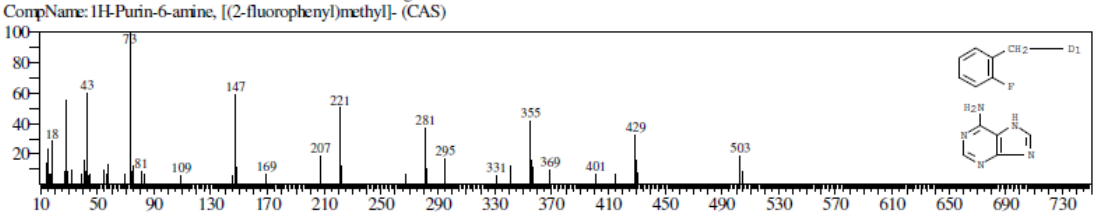
Hit#:5 Entry:335377 Library:WILEY7.LIB
SI:84 Formula:C20 H60 O10 SI10 CAS:18772-36-6 MolWeight:740 RetIndex:0
CompName:EICOSAMETHYLCYCLODECASILOXANE $$ CYCLODECASILOXANE, EICOSAMETHYL- $$



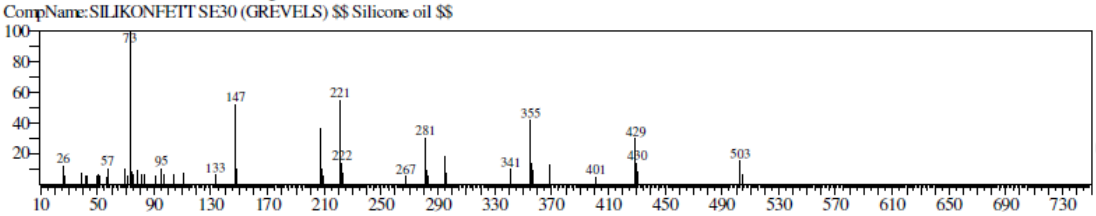
<< Target >>
Line#:5 R.Time:21.683(Scan#:2603) MassPeaks:376
RawMode:Averaged 21.675-21.692(2602-2604) BasePeak:73.00(28239)
BG Mode:Calc. from Peak Group 1 - Event 1



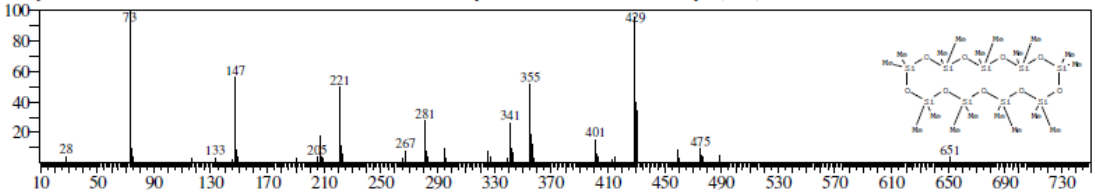
Hit#:1 Entry:148639 Library:WILEY7.LIB
SI:88 Formula:C12 H10 F N5 CAS:74421-44-6 MolWeight:243 RetIndex:0
CompName:1H-Purin-6-amine, [(2-fluorophenyl)methyl]- (CAS)



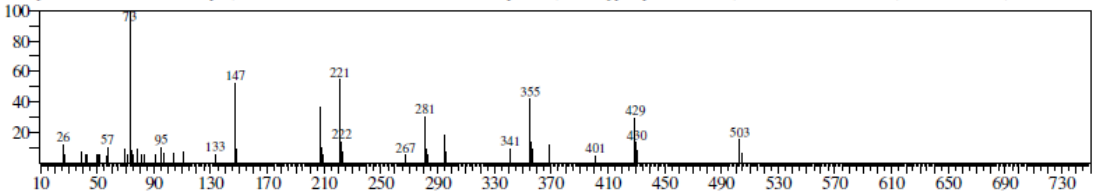
Hit#:2 Entry:338296 Library:WILEY7.LIB
SI:85 Formula: CAS:0-00-0 MolWeight:9999 RetIndex:0
CompName:SILIKONFETT SE30 (GREVELS) $$ Silicone oil $$



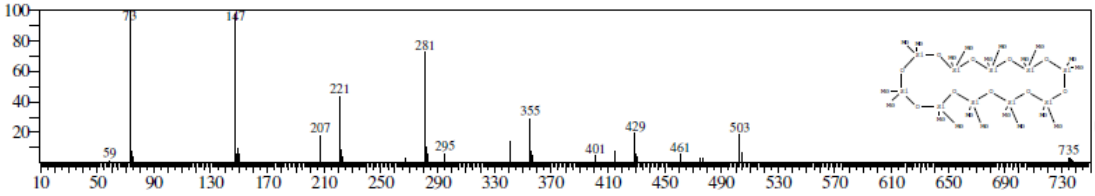
Hit#:3 Entry:332919 Library:WILEY7.LIB
SI:85 Formula:C18 H54 O9 SI9 CAS:556-71-8 MolWeight:666 RetIndex:0
CompName:OCTADECAMETHYLCYCLONONASILOXANE $$ Cyclononasiloxane, octadecamethyl- (CAS)



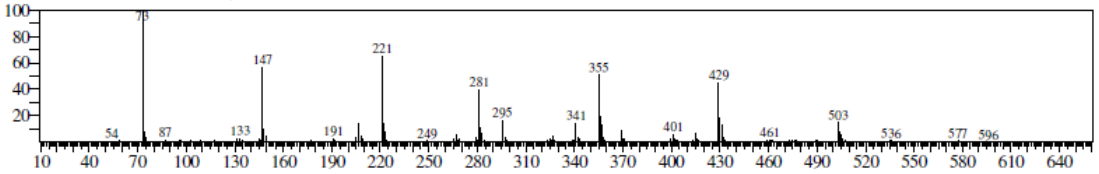
Hit#:4 Entry:300653 Library:WILEY7.LIB
SI:85 Formula:C21 H22 FE N2 O5 CAS:109007-87-6 MolWeight:438 RetIndex:0
CompName:Iron, monocarbonyl-(1,3-butadiene-1,4-dicarbonic acid, diethyl ester) a,a'-dipyridyl $$ IRON, (2,2'-BIPYRIDINE-N,N')CARBONYL[(2,3,4,5-.ETA



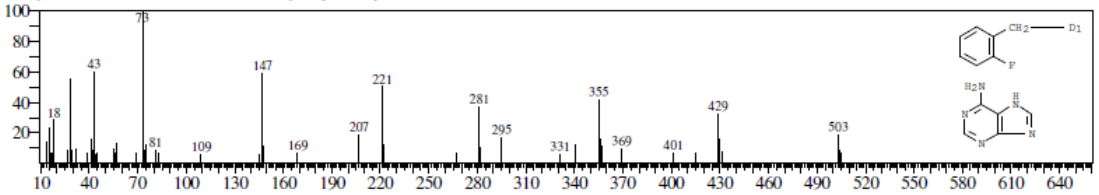
Hit#:5 Entry:335377 Library:WILEY7.LIB
SI:83 Formula:C20 H60 O10 SI10 CAS:18772-36-6 MolWeight:740 RetIndex:0
CompName:EICOSAMETHYLCYCLODECASILOXANE $$ CYCLODECASILOXANE, EICOSAMETHYL- $$



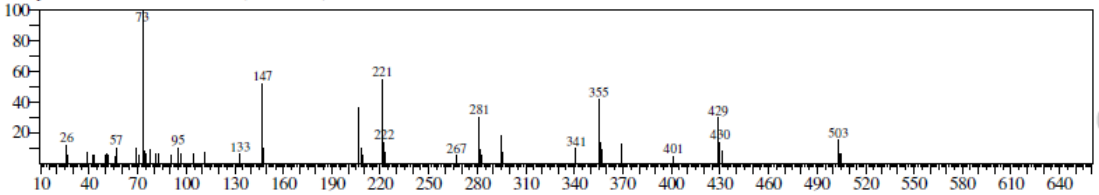
<< Target >>
Line#:6 R.Time:22.717(Scan#:2727) MassPeaks:376
RawMode:Averaged 22.708-22.725(2726-2728) BasePeak:73.00(27844)
BG Mode:Calc. from Peak Group 1 - Event 1



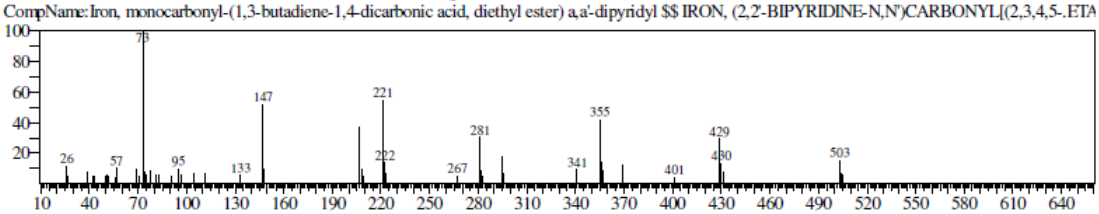
Hit#:1 Entry:148639 Library:WILEY7.LIB
SI:87 Formula:C12 H10 F N5 CAS:74421-44-6 MolWeight:243 RetIndex:0
CompName:1H-Purin-6-amine, [(2-fluorophenyl)methyl]- (CAS)



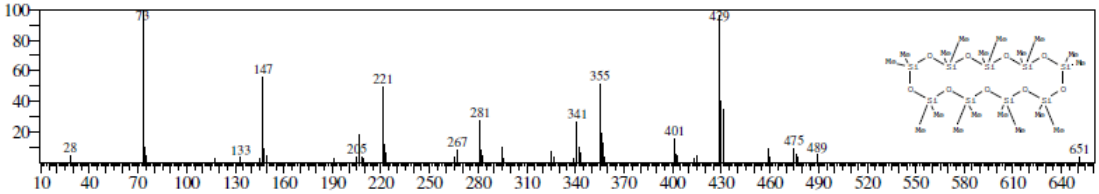
Hit#:2 Entry:338296 Library:WILEY7.LIB
SI:86 Formula: CAS:0-00-0 MolWeight:9999 RetIndex:0
CompName:SILIKONFETT SE30 (GREVELS) $$ Silicone oil $$



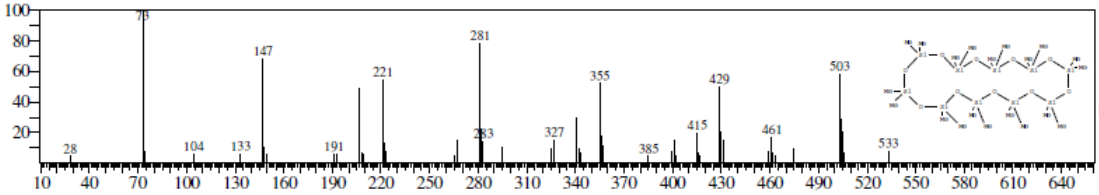
Hit#:3 Entry:300653 Library:WILEY7.LIB
SI:86 Formula:C21 H22 FE N2 O5 CAS:109007-87-6 MolWeight:438 RetIndex:0
CompName:Iron, monocarbonyl-(1,3-butadiene-1,4-dicarbonic acid, diethyl ester) a,a'-dipyridyl $$ IRON, (2,2'-BIPYRIDINE-N,N')CARBONYL[(2,3,4,5-.ETA

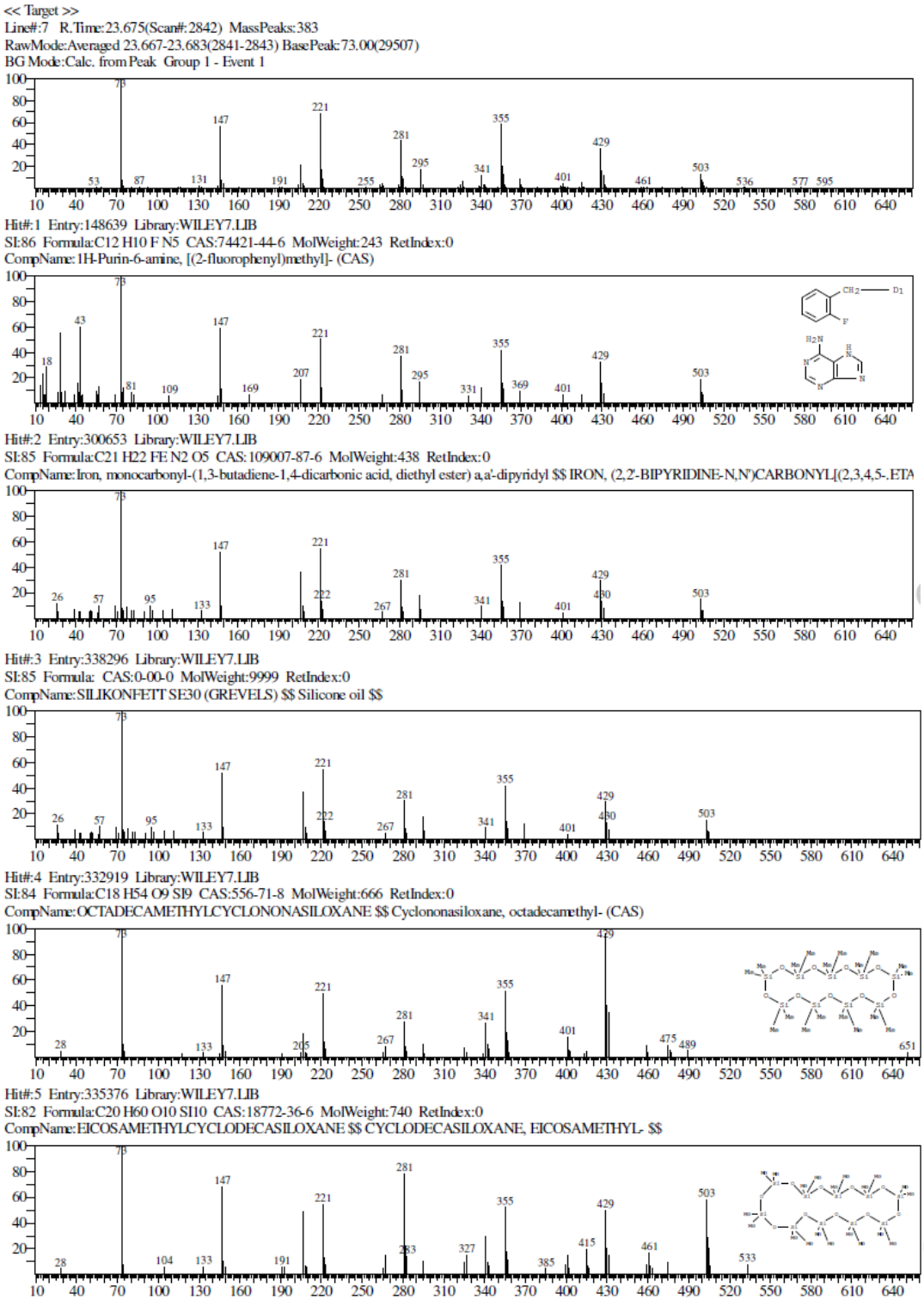


Hit#:4 Entry:332919 Library:WILEY7.LIB
SI:85 Formula:C18 H54 O9 SI9 CAS:556-71-8 MolWeight:666 RetIndex:0
CompName:OCTADECAMETHYLCYCLONONASILOXANE $$ Cyclononasiloxane, octadecamethyl- (CAS)



Hit#:5 Entry:335376 Library:WILEY7.LIB
SI:83 Formula:C20 H60 O10 SI10 CAS:18772-36-6 MolWeight:740 RetIndex:0
CompName:EICOSAMETHYLCYCLODECASILOXANE $$ CYCLODECASILOXANE, EICOSAMETHYL- $$





Gambar 7. Uji *Mass Spectrometry* senyawa EPMS

Lampiran 6. Data Hasil Uji *In-Vitro*

1. Uji Aktivitas Atropin terhadap kontraksi otot polos trakea terinduksi $AChM_3$

log	Respon kontraksi										Mean	SEM
	1	2	3	4	5	6	7	8	9	10		
-10,0	33,06	18,75	38,46	24,39	44,25	25,00	22,22	40,38	36,11	37,50	32,01	2,77
-9,5	38,02	25,00	38,46	29,27	54,87	31,25	25,00	44,23	43,06	51,56	38,07	3,32
-9,0	41,32	29,17	47,44	34,15	59,29	36,11	25,00	44,23	43,06	51,56	41,13	3,28
-8,5	56,20	29,17	52,56	40,24	61,95	50,69	38,89	55,77	43,06	56,25	48,48	3,23
-8,0	61,98	39,58	52,56	45,12	66,37	50,69	38,89	61,54	47,22	75,00	53,90	3,78
-7,5	80,99	50,00	73,08	51,22	69,03	56,25	38,89	61,54	47,22	75,00	60,32	4,37
-7,0	82,64	64,58	73,08	56,10	72,57	65,97	38,89	76,92	52,78	75,00	65,85	4,19
-6,5	95,87	79,17	74,36	68,29	80,53	75,00	38,89	82,69	54,17	75,00	72,40	5,01
-6,0	97,52	87,50	75,64	75,61	90,27	84,03	44,44	88,46	61,11	76,56	78,11	4,94
-5,5	100,00	91,67	84,62	90,24	97,35	88,89	68,06	90,38	88,89	87,50	88,76	2,71
-5,0	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	0,00

Gambar 8. Persentase kenaikan agonis sebelum dilakukan perlakuan

log	Respon kontraksi					Mean	SEM
	1	2	3	4	5		
-10,0	16,67	16,67	38,46	17,07	44,25	26,62	6,08
-9,5	16,67	20,83	38,46	18,29	21,24	23,10	3,93
-9,0	16,67	27,08	38,46	19,51	24,78	25,30	3,77
-8,5	16,67	29,17	38,46	20,73	24,78	25,96	3,75
-8,0	16,67	31,25	42,31	21,95	24,78	27,39	4,41
-7,5	16,67	33,33	52,56	25,61	24,78	30,59	6,09
-7,0	25,76	35,42	57,69	31,71	24,78	35,07	5,98
-6,5	36,36	37,50	58,97	40,24	25,66	39,75	5,41
-6,0	59,85	39,58	61,54	47,56	30,09	47,72	5,98
-5,5	73,48	50,00	61,54	74,39	51,33	62,15	5,21
-5,0	90,91	77,08	61,54	62,20	76,11	73,57	5,45

Gambar 9. Persentase kenaikan agonis setelah pemberian Atropin konsentrasi 0,01 µM

log	Respon kontraksi					Mean	SEM
	1	2	3	4	5		
-10,0	29,17	44,44	34,62	38,89	12,50	31,92	5,47
-9,5	29,17	44,44	34,62	38,89	23,44	34,11	3,66
-9,0	34,72	44,44	34,62	38,89	23,44	35,22	3,45
-8,5	35,42	44,44	34,62	38,89	23,44	35,36	3,45
-8,0	37,50	44,44	34,62	38,89	28,13	36,71	2,68
-7,5	40,28	44,44	38,46	40,28	32,81	39,25	1,89
-7,0	40,28	44,44	38,46	40,28	39,06	40,50	1,05
-6,5	40,97	44,44	38,46	41,67	46,88	42,48	1,45
-6,0	47,92	44,44	38,46	44,44	46,88	44,43	1,64
-5,5	49,31	44,44	38,46	55,56	48,44	47,24	2,83
-5,0	62,50	52,78	80,77	55,56	70,31	64,38	5,10

Gambar 10. Persentase kenaikan agonis setelah pemberian Atropin konsentrasi 0,05 µM

		pD2	A or A'	A'/A	x-1	log(x-1)	Kons M		Log M
							(uM)		
1	Kontrol	8,25	0,0000000056						
2	(+) Atropin 0,01 uM	6,22	0,00000060	107,1519	106,1519	2,025928	10	0,01	-2
3	(+) Atropin 0,05 uM	5,46	0,00000347	616,595	615,595	2,789295	50	0,05	-1,30103

Gambar 11. Perhitungan Parameter Antagonis (pA_2) Atropin terhadap Reseptor $AChM_3$

2. Uji Aktivitas EPMS terhadap kontraksi otot polos trakea terinduksi $AChM_3$

log	Respon kontraksi										Mean	SEM
	1	2	3	4	5	6	7	8	9	10		
-10,0	19,05	28,06	48,08	48,65	19,05	10,53	18,55	47,62	15,63	16,13	27,13	4,78
-9,5	24,60	33,09	51,92	56,76	19,05	10,53	18,55	57,14	25,00	19,35	31,60	5,49
-9,0	30,16	38,85	55,77	59,46	33,33	12,63	18,55	59,52	53,13	25,81	38,72	5,50
-8,5	34,13	43,17	57,69	62,16	47,62	12,63	18,55	61,90	78,13	41,94	45,79	6,43
-8,0	41,27	50,36	63,46	62,16	61,90	14,74	20,16	69,05	81,25	54,84	51,92	6,67
-7,5	48,41	58,27	67,31	64,86	76,19	23,16	30,65	73,81	87,50	64,52	59,47	6,38
-7,0	57,94	68,35	73,08	70,27	76,19	37,89	38,71	78,57	87,50	70,97	65,95	5,19
-6,5	70,63	75,54	75,00	70,27	85,71	58,95	50,00	85,71	87,50	77,42	73,67	3,80
-6,0	83,33	83,45	88,46	78,38	90,48	73,68	65,32	88,10	93,75	80,65	82,56	2,69
-5,5	93,65	92,09	88,46	89,19	100,00	93,68	85,48	90,48	93,75	93,55	92,03	1,25
-5,0	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	0,00

Gambar 12. Persentase kenaikan agonis sebelum dilakukan perlakuan

log	Respon kontraksi					Mean	SEM
	1	2	3	4	5		
-10,0	29,55	25,90	10,71	32,43	35,71	26,86	4,35
-9,5	33,33	28,78	12,50	32,43	35,71	28,55	4,16
-9,0	34,85	33,09	17,86	35,14	35,71	31,33	3,40
-8,5	37,12	35,97	19,64	35,14	35,71	32,72	3,28
-8,0	37,12	43,17	25,00	35,14	45,24	37,13	3,56
-7,5	40,91	47,48	28,57	40,54	50,00	41,50	3,72
-7,0	50,00	56,12	33,93	48,65	50,00	47,74	3,69
-6,5	62,12	64,75	42,86	56,76	57,14	56,73	3,78
-6,0	71,21	74,82	51,79	70,27	64,29	66,47	4,04
-5,5	80,30	82,73	57,14	75,68	85,71	76,31	5,07
-5,0	92,56	89,21	62,50	81,08	95,24	84,12	5,91

Gambar 13. Persentase kenaikan agonis setelah pemberian EPMS konsentrasi 100 µM

log	Respon kontraksi					Mean	SEM
	1	2	3	4	5		
-10,0	21,05	28,23	38,10	12,50	0,00	19,97	6,53
-9,5	25,26	28,23	42,86	12,50	9,68	23,70	5,97
-9,0	26,32	28,23	50,00	18,75	12,90	27,24	6,31
-8,5	30,53	28,23	52,38	37,50	32,26	36,18	4,33
-8,0	35,79	28,23	57,14	43,75	38,71	40,72	4,81
-7,5	41,05	28,23	61,90	43,75	45,16	44,02	5,38
-7,0	41,05	28,23	69,05	46,88	61,29	49,30	7,25
-6,5	43,16	32,26	73,81	50,00	70,97	54,04	8,02
-6,0	54,74	35,48	80,95	50,00	74,19	59,07	8,26
-5,5	54,74	43,55	85,71	59,38	74,19	63,51	7,42
-5,0	54,74	51,61	90,48	90,63	90,32	75,55	9,15

Gambar 14. Persentase kenaikan agonis setelah pemberian EPMS konsentrasi 500 µM

		pD2	A or A'	A'/A	x-1	log(x-1)	Kons M		Log M
							(uM)		
1	Kontrol	8,4	0,0000000040						
2	(+) EPMS 100	6,98	0,00000010	26,30268	25,30268	1,403167	10	0,01	-2
3	(+) EPMS 200	6,82	0,00000015	38,01894	37,01894	1,568424	50	0,05	-1,30103

Gambar 15. Perhitungan Parameter Antagonis (pA_2) EPMS terhadap Reseptor $AChM_3$

Lampiran 7. Hasil Uji Statistik

Tests of Normality

		Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	perlakuan	Statistic	df	Sig.	Statistic	df	Sig.
nilai PD2	kontrol	,186	10	,200(*)	,918	10	,339
	atropin 100	,299	5	,164	,819	5	,115
	atropin 500	,226	5	,200(*)	,969	5	,869
	epmc 100	,256	5	,200(*)	,851	5	,197
	epmc 200	,154	5	,200(*)	,990	5	,978

* This is a lower bound of the true significance.

a Lilliefors Significance Correction

Descriptives

	perlakuan			Statistic	Std. Error
pD2	kontrol asetilkolin	Mean		8,0600	,17572
		95% Confidence Interval for Mean	Lower Bound	7,6625	
			Upper Bound	8,4575	
		5% Trimmed Mean		8,0656	
		Median		8,1150	
		Variance		,309	
		Std. Deviation		,55568	
		Minimum		7,26	
		Maximum		8,76	
		Range		1,50	
		Interquartile Range		1,05	
		Skewness		-,114	,687
		Kurtosis		-1,692	1,334
	Atropin 100 uL	Mean		6,2180	,37302
		95% Confidence Interval for Mean	Lower Bound	5,1823	
			Upper Bound	7,2537	
		5% Trimmed Mean		6,1761	
		Median		5,9600	

	Variance		,696	
	Std. Deviation		,83410	
	Minimum		5,55	
	Maximum		7,64	
	Range		2,09	
	Interquartile Range		1,30	
	Skewness		1,750	,913
	Kurtosis		3,244	2,000
Atropin 500 uL	Mean		5,4600	,09566
	95% Confidence Interval for Mean	Lower Bound	5,1944	
		Upper Bound	5,7256	
	5% Trimmed Mean		5,4594	
	Median		5,4900	
	Variance		,046	
	Std. Deviation		,21389	
	Minimum		5,17	
	Maximum		5,76	
	Range		,59	
	Interquartile Range		,36	
	Skewness		,093	,913
	Kurtosis		1,088	2,000
EPMS 100 uL	Mean		6,9780	,23739
	95% Confidence Interval for Mean	Lower Bound	6,3189	
		Upper Bound	7,6371	
	5% Trimmed Mean		7,0028	
	Median		7,0900	
	Variance		,282	
	Std. Deviation		,53082	
	Minimum		6,10	
	Maximum		7,41	
	Range		1,31	
	Interquartile Range		,88	
	Skewness		-1,490	,913
	Kurtosis		2,279	2,000
EPMS 200 uL	Mean		6,8160	,61121
	95% Confidence Interval for Mean	Lower Bound	5,1190	
		Upper Bound	8,5130	
	5% Trimmed Mean		6,8028	
	Median		6,6300	
	Variance		1,868	

Std. Deviation	1,36670	
Minimum	5,10	
Maximum	8,77	
Range	3,67	
Interquartile Range	2,42	
Skewness	,383	,913
Kurtosis	,412	2,000

Test of Homogeneity of Variances

nilai PD2

Levene Statistic	df1	df2	Sig.
2,690	4	25	,054

ANOVA

nilai PD2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	26,185	4	6,546	11,410	,000
Within Groups	14,343	25	,574		
Total	40,528	29			

Lampiran 8. Hasil Uji *In-Silico*

1. Uji Validasi Native Ligand (Tiotropium) dengan Reseptor $AChM_3$

```
#################################################################
# If you used AutoDock Vina in your work, please cite:          #
#                                                               #
# O. Trott, A. J. Olson,                                        #
# AutoDock Vina: improving the speed and accuracy of docking    #
# with a new scoring function, efficient optimization and       #
# multithreading, Journal of Computational Chemistry 31 (2010)  #
# 455-461                                                       #
#                                                               #
# DOI 10.1002/jcc.21334                                         #
#                                                               #
# Please see http://vina.scripps.edu for more information.      #
#################################################################

Detected 2 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 441455400
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1        -6.4      0.000      0.000
   2        -6.2      3.637      6.384
   3        -6.1      2.153      3.033
   4        -6.1      3.426      7.019
   5        -5.9      1.769      2.191
   6        -5.9      4.039      5.340
   7        -5.8      2.939      5.769
   8        -5.8      3.490      6.371
   9        -5.8      2.955      6.871
Writing output ... done.
```

2. Uji Validasi EPMS dengan Reseptor $AChM_3$

```
#################################################################
# If you used AutoDock Vina in your work, please cite:          #
#                                                               #
# O. Trott, A. J. Olson,                                        #
# AutoDock Vina: improving the speed and accuracy of docking    #
# with a new scoring function, efficient optimization and       #
# multithreading, Journal of Computational Chemistry 31 (2010)  #
# 455-461                                                       #
#                                                               #
# DOI 10.1002/jcc.21334                                         #
#                                                               #
# Please see http://vina.scripps.edu for more information.      #
#################################################################

Detected 2 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -1922439776
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1        -5.5      0.000      0.000
   2        -5.2      1.613      1.819
   3        -5.0      4.410      6.316
   4        -4.8      3.757      3.909
   5        -4.8      7.198      8.692
   6        -4.8      5.988      7.167
   7        -4.5      2.706      3.400
   8        -4.5      2.952      3.634
   9        -4.4      5.861      6.282
Writing output ... done.
```

3. Uji Validasi Atropin dengan Reseptor AChM$_3$

```
#################################################################
# If you used AutoDock Vina in your work, please cite:          #
#                                                               #
# O. Trott, A. J. Olson,                                        #
# AutoDock Vina: improving the speed and accuracy of docking    #
# with a new scoring function, efficient optimization and       #
# multithreading, Journal of Computational Chemistry 31 (2010)  #
# 455-461                                                       #
#                                                               #
# DOI 10.1002/jcc.21334                                         #
#                                                               #
# Please see http://vina.scripps.edu for more information.      #
#################################################################

Detected 2 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 1003870420
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -6.2      0.000      0.000
   2         -6.2      1.438      2.617
   3         -6.1      4.578      6.414
   4         -6.1      4.470      6.276
   5         -6.1      1.227      1.894
   6         -6.1      5.015      6.366
   7         -6.0      4.574      6.451
   8         -5.9      2.694      6.303
   9         -5.9      4.901      6.697
Writing output ... done.
```