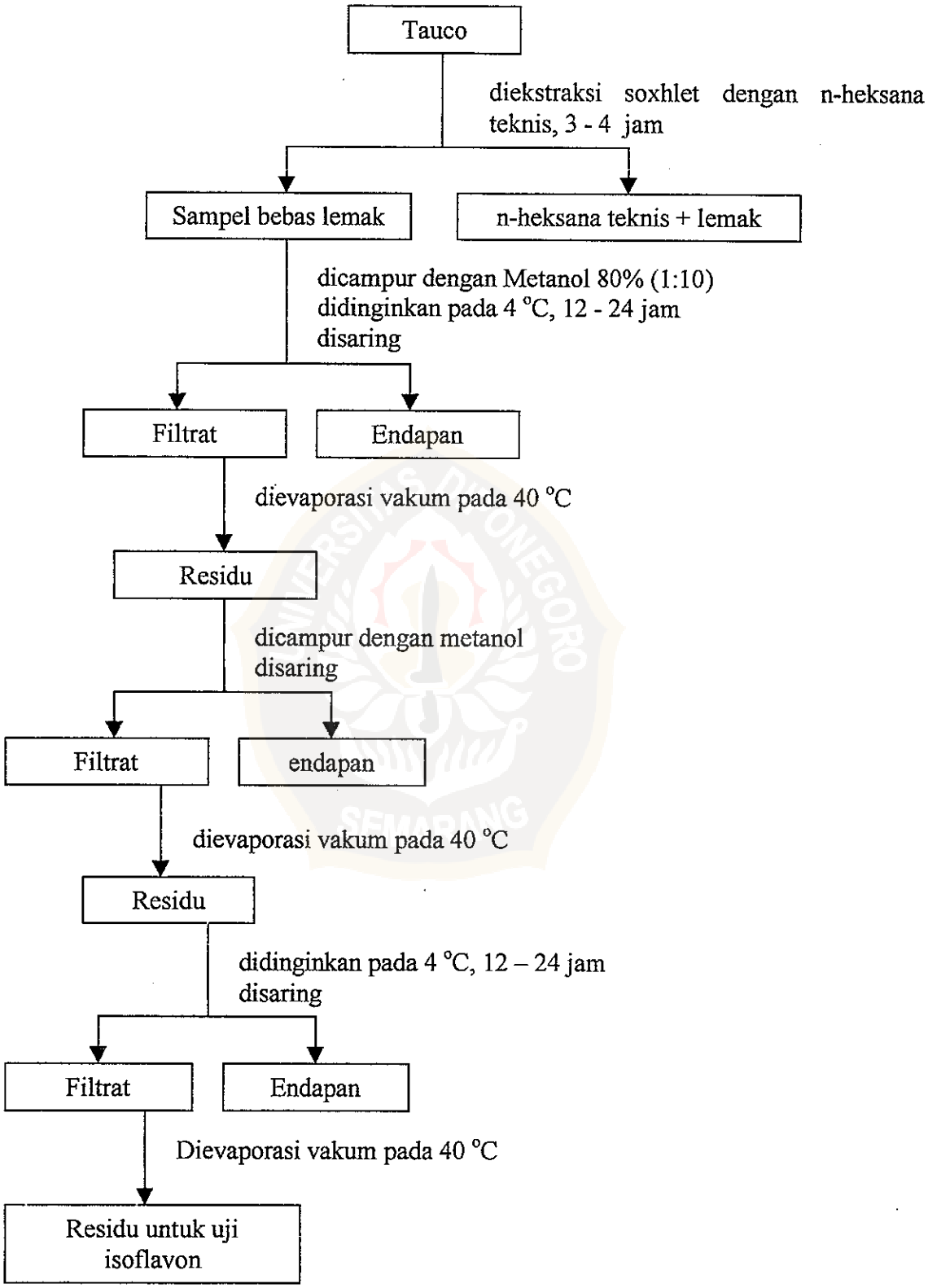
Lampiran 1: Prosedur Ekstraksi Isoflavon dari Tauco



Lampiran 2: Analisis kuantitatif Isoflavon dengan KCKT

Perhitungan konsentrasi daidzein dan genestein dalam 100 g sampel

A_x = area rata-rata yang didapatkan dari puncak isoflavon

A_s = area rata-rata dari standar isoflavon

m_s = massa isoflavon standar (μg)

m_x = massa isoflavon (μg)

C = konstanta volume terhadap jalur kerja

Perhitungan yang dipakai adalah:

$$m_x = C x \frac{A_x}{A_s} x\ m_s$$

Konsentrasi isoflavon adalah m_x/100 g sampel

Perubahan volume sampel terhadap jalur kerja:

a mL → b mL ⇒ c mL

Keterangan:

Tanda (→) artinya diencerkan sampai b mL dari a mL

Tanda (⇒) artinya diambil c mL dari b mL

A. Konsentrasi daidzein dan genestein dalam 100 g tempe

Perubahan volume sampel terhadap jalur kerja:

1 mL → 10 mL ⇒ 1 mL → 5 mL ⇒ 2 mL → 4 mL ⇒ 2,3 mL → 40 mL ⇒ 20 μL

a. Konsentrasi daidzein

$A_{daidzein}$ = 63750 satuan luas

$A_{s,\ daidzein}$ = 651876,5 satuan luas

$m_{s,\ daidzein}$ = 0,2 μg

$m_{daidzein}$ = [(10/1) x (10/1) x (5/1) x (5/2) x (4/2) x (4/2,3) x (40/2,3) x (40000/20)] x (63750/651876,5) x 0,2 µg

$m_{daidzein}$ = 2957870,548 µg

$m_{daidzein}$ = 2957,8705 mg

Konsentrasi daidzein adalah 2957,8705 mg/100 g tempe

b. Konsentrasi genestein

$A_{genestein}$ = 33284,5 satuan luas

$A_{s,\ genestein}$ = 2069868 satuan luas

$m_{s,\ genestein}$ = 0,2 µg

$m_{genestein}$ = [(10/1) x (10/1) x (5/1) x (5/2) x (4/2) x (4/2,3) x (40/2,3) x (40000/20)] x (33284,5/2069868) x 0,2 µg

$m_{genestein}$ = 486366,5 µg

$m_{genestein}$ = 486,3665 mg

Konsentrasi genestein adalah 486,3665 mg/100 g tempe

B. Konsentrasi daidzein dan genestein dalam 100 g tauco

Perubahan konsentrasi sampel terhadap jalur kerja:

1mL → 12 mL ⇒ 2 mL → 4 mL ⇒ 3,3 mL → 60 mL ⇒ 20 µL

a. Konsentrasi daidzein

$A_{daidzein}$ = 103660,5 satuan luas

$A_{s,\ daidzein}$ = 651876,5 satuan luas

$m_{s,\ daidzein}$ = 0,2 µg

$m_{daidzein}$ = [(12/1) x (12/2) x (4/2) x (4/3,3) x (60/3,3) x 60000/20)] x (103660,5/651876,5) x 0,2 µg

$m_{daidzein}$ = 302792,5 µg

$m_{daidzein}$ = 302,7925 mg

Konsentrasi daidzein adalah 302,7925 mg/100 g tauco

b. Konsentrasi genestein

$A_{genestein}$ = 52620 satuan luas

$A_{s,\ genestein}$ = 2069868 satuan luas

$m_{s,\ genestein}$ = 0,2 µg

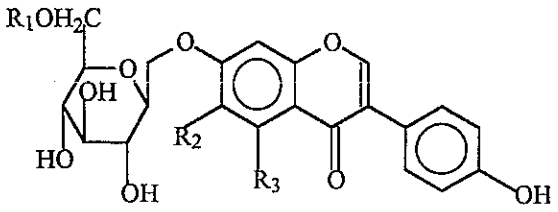
$m_{genestein}$ = [(12/1) x (12/2) x (4/2) x (4/3,3) x (60/3,3) x (60000/20)] x (52620/2069868) x 0,2 µg

$m_{genestein}$ = 48406,7 µg

$m_{genestein}$ = 48,4067 mg

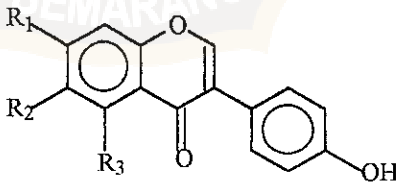
Konsentrasi genestein adalah 48,4067 mg/100 g tauco

Lampiran 3: Isoflavon dalam Kedelai[6]



(a)

No.	Senyawa Isoflavon	R_1	R_2	R_3
1	Daidzin	H	H	H
2	Glisitin	H	OCH_3	H
3	Genistin	H	H	OH
4	6"-O-Malonil daidzin	$COCH_2COOH$	H	H
5	6"-O-Malonil glisitin	$COCH_2COOH$	OCH_3	H
6	6"-O-Malonil genistin	$COCH_2COOH$	H	OH
7	6"-O-Asetil daidzin	$COCH_3$	H	H
8	6"-O-Asetil glisitin	$COCH_3$	OCH_3	H
9	6"-O-Asetil genistin	$COCH_3$	H	OH



(b)

Daidzein: R_1 = OH; R_2 = H; R_3 = H; R_4 = OH
Genestein: R_1= OH; R_2 = H; R_3 = OH; R_4 = OH

(a) Struktur isoflavon glikosida; (b) Struktur isoflavon aglikon

t 026

D-7000 HSM: SAMPLES Series: 0022 Report: original System: Sys 1

D-7000 HPLC System Manager Report

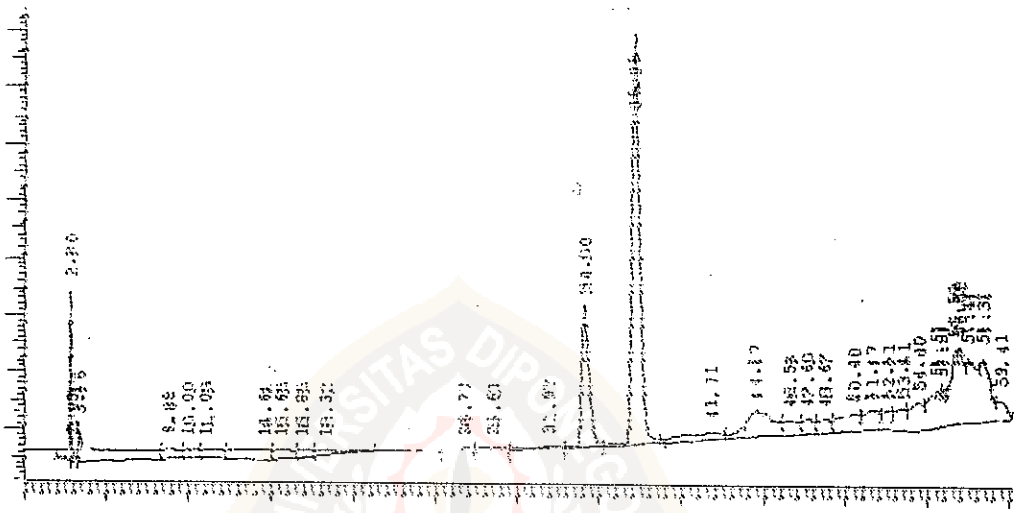
Analyzed: 10/31/00 10:46 AM
Reported: 10/31/00 11:46 AM
Processed: 10/31/00 11:46 AM

Data Path: C:\WIN32APP\HSM\samples\DATA\0022\
Processing Method: tmh

System(acquisition): Sys 1
Application: SAMPLES
Sample Name: tmh001
Injection from this vial: 1 of 1
Sample Description:

Series:0022
Vial Number: 1
Vial Type: UNK
Volume: 20.0 ul

Chrom Type: Fixed WL chromatogram, 260 nm



Acquisition Method: tmh
Column Type:
Developed by: ratna
Pump A Type: L-7100
Solvent A: air/acet
Pump B Type: L-7100
Solvent A: meoh/acet
Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1	BC
1	2.80	115060	1.359	BB
2	2.93	28000	0.331	BB
3	3.36	520796	6.152	BV
4	8.86	115869	1.369	VV
5	10.00	79565	0.940	VV
6	11.09	112766	1.332	VV
7	14.64	164718	1.946	VV
8	15.65	61595	0.728	VV
9	16.85	42870	0.506	VV
10	16.32	75577	0.893	VB
11	26.77	12903	0.152	BB
12	28.61	19236	0.227	BB
13	31.97	30671	0.362	BB
14	34.00 V	649008	7.667	BB
15	36.96 V	2059721	24.333	BV

026

D-7000 HSM: SAMPLES		Series: 0022	Report: original	System: Sys 1	
16	41.71	211346	2.497	VV	
17	44.37	531175	6.275	VV	
18	46.53	159270	1.882	VV	
19	47.60	123414	1.458	VV	
20	48.67	122647	1.448	VV	
21	50.40	252065	2.978	VV	
22	51.47	205866	2.432	VV	
23	52.51	115667	1.366	VV	
24	53.41	161934	1.913	VV	
25	54.40	223876	2.645	VV	
26	55.52	310043	3.663	VV	
27	55.64	122301	1.445	VV	
28	56.59	334251	3.949	VV	
29	56.85	405877	4.795	VV	
30	57.44	505859	5.976	VV	
31	58.32	452731	5.348	VV	
32	59.41	136653	1.636	VB	
		8464810	100.000		

Peak rejection level: 0

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D-7000 HSM: SAMPLES | Series: 0023 | Report: original | System: Sys 1

D-7000 HPLC System Manager Report

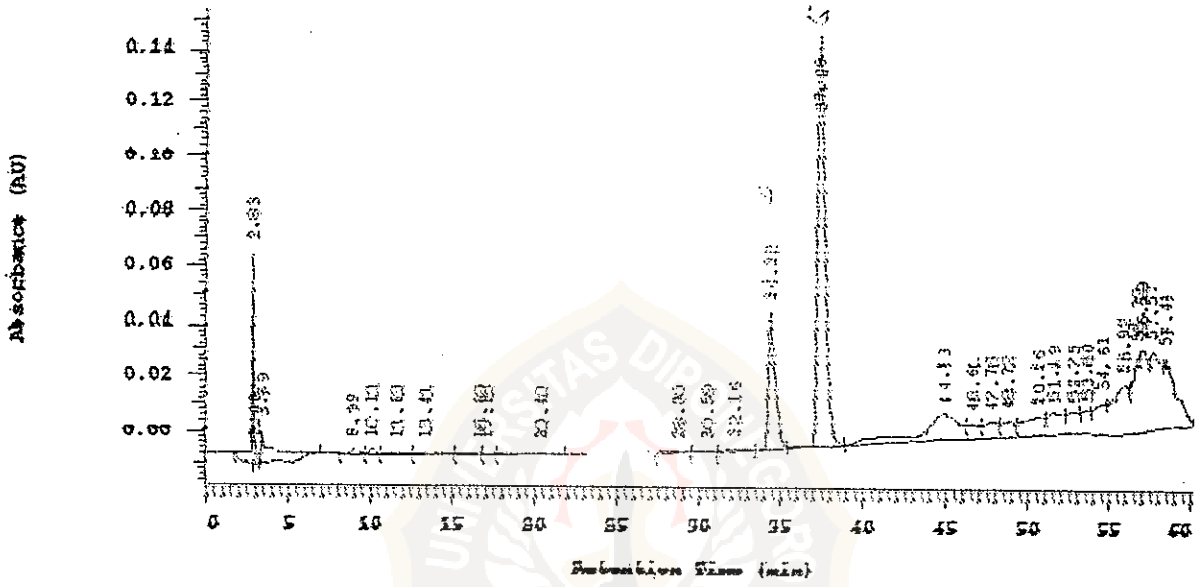
Analyzed: 10/31/00 12:16 PM
Reported: 10/31/00 01:28 PM
Processed: 10/31/00 01:16 PM

Data Path: C:\WIN32APP\HSM\samples\DATA\0023\
Processing Method: tmh

System(acquisition): Sys 1
Application: SAMPLES
Sample Name: tmh002
Injection from this vial: 1 of 1
Sample Description:

Series:0023
Vial Number: 2
Vial Type: UNK
Volume: 20.0 ul

Chrom Type: Fixed WL Chromatogram, 260 nm



Acquisition Method: tmh
Column Type:
Developed by: ratna
Pump A Type: L-7100
Solvent A: air/acet
Pump B Type: L-7100
Solvent A: meoh/acet
Peak Quantitation: AREA
Calculation Method: AREA%

NO.	RT	Area	Conc 1	BC
1	2.83	144360	1.709	BB
2	2.96	31502	0.373	BB
3	3.39	253217	2.997	BB
4	8.99	22400	0.265	BB
5	10.11	15640	0.185	BB
6	11.63	23538	0.279	BB
7	13.41	33263	0.394	BB
8	16.69	14109	0.167	BB
9	17.15	11469	0.136	BB
10	20.40	29916	0.354	BB
11	26.80	11500	0.136	BB
12	30.59	11380	0.135	BB
13	32.16	31220	0.369	BB
14	34.32	654745	7.749	BB
15	37.26	2060015	24.616	BB

D-7000 HSM: SAMPLES		Series: 0023	Report: original	System: Sys 1	
16	44.83	800107	9.470	BV	
17	46.61	131527	1.557	VV	
18	47.76	179926	2.129	VV	
19	48.72	139789	1.654	VV	
20	50.56	296304	3.507	VV	
21	51.49	254872	3.016	VV	
22	52.75	182654	2.162	VV	
23	53.60	151663	1.795	VV	
24	54.61	266383	3.153	VV	
25	55.92	534158	6.322	VV	
26	56.72	394145	4.665	VV	
27	56.99	466684	5.526	VV	
28	57.57	666916	7.893	VV	
29	58.43	615643	7.286	VB	
		8449269	100.000		

Peak rejection level: 0

TC 11

D-7000 HPLC System Manager Report

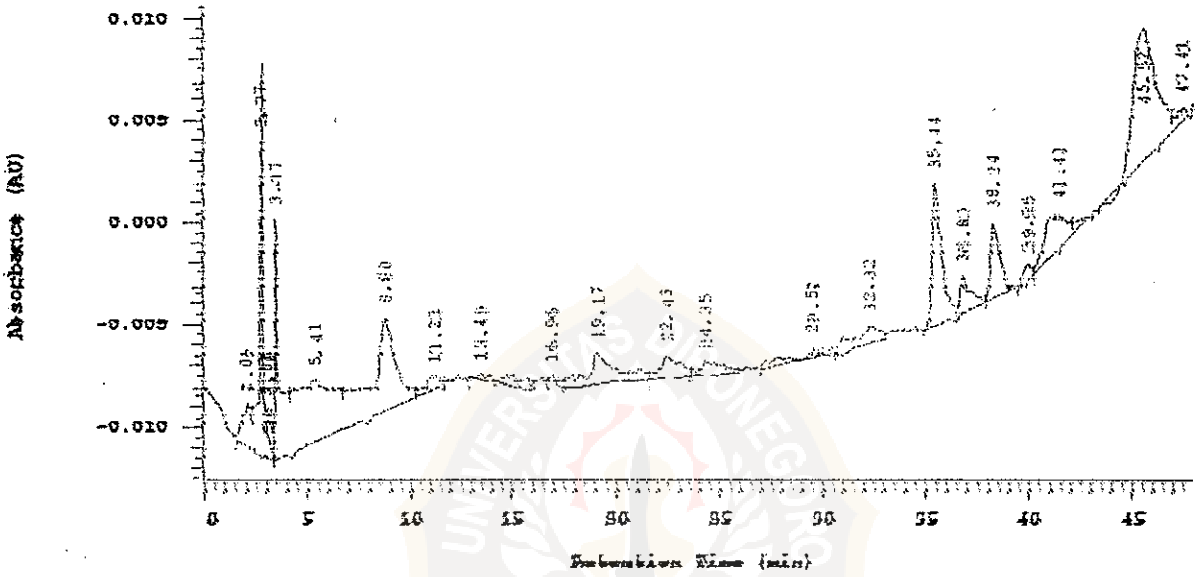
Analyzed: 10/31/00 02:40 PM
Reported: 10/31/00 03:28 PM
Processed: 10/31/00 03:26 PM

Data Path: C:\WIN32APP\HSM\samples\DATA\0025\
Processing Method: tmh

System(acquisition): Sys 1
Application: SAMPLES
Sample Name: tmh001
Injection from this vial: 1 of 1
Sample Description:

Series:0025
Vial Number: 1
Vial Type: UNK
Volume: 20.0 ul

Chrom Type: Fixed WL Chromatogram, 260 nm



Acquisition Method: tmh
Column Type:
Developed by: ratna
Pump A Type: L-7100
Solvent A: air/acet
Pump B Type: L-7100
Solvent A: meoh/acet
Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1	BC
1	2.05	28867	2.260	BV
2	2.77	74552	5.872	VV
3	3.01	3662	0.287	TBV
4	3.20	9677	0.758	TVB
5	3.47	76167	5.965	BV
6	5.41	200503	15.698	VV
7	8.80	190218	14.893	VB
8	11.23	14845	1.163	BB
9	13.49	10640	0.833	BB
10	16.96	3460	0.271	BB
11	19.17	58763	4.601	BB
12	22.43	34311	2.686	BB
13	24.35	24725	1.936	BB
14	29.52	17660	1.383	BB
15	32.32	32200	2.521	BB

D-7000 HSM: SAMPLES		Series: 0025	Report: original	Sysrem: Sys 1
16	35.44	103970	8.140	BB
17	36.60	30569	2.393	BB
18	38.24	52520	4.112	BB
19	39.95	8800	0.689	BB
20	41.41	60776	4.758	BV
21	45.52	226994	17.773	VV
22	47.41	12869	1.008	VB
		1277212	100.000	

Peak rejection level: 0

Channel 1 Noise: Not Measured
Channel 1 Drift: Not Measured

Configuration parameters:
Interface Module: D-7000 Gradient Mode: High
Channel 1 Detector: L-7450 Channel 2 Detector: None
Column Oven: None Autosampler: Manual
Pump A: L-7100 Pump B: L-7100
Pump C: None External Instrument Software: None
Column Name:

Method Information:
Method Name: tmh Developed by: ratna
Description:
analisadas

Pump Setup:
All Pumps Pressure Limit: 0 to 420 kgf/cm2

Pump A (L-7100):
Solvent A: air/acet
Pump B (L-7100):
Solvent B: meoh/acet

Pump Table

Time (min)	%air/a	%meoh/	Total Flow (ml/min)	Event 1	Event 2	Event 3	Event 4
0.0	100.0	0.0	1.000				
5.0	100.0	0.0	1.000				
55.0	0.0	100.0	1.000				
60.0	100.0	0.0	1.000				

Channel 1 Detector (L-7450):
Spectral Bandwidth: 4 nm Spectral Interval: 1000 msec
Absorbance Mode: Normal(2.0AU) Auto Zero before Injection: YES
Wavelength Range: 190 to 590 nm Monitoring Wavelenth: 260 nm
Start Time: 0.00 min Stop Time: 60.00 min

Method DP for channel 1

Calculation Method: Peak Quantitation: Area
Calculation Method: AREA# Concentration data from method.
Peak Identification Window: % Time
UNK peaks identification rule: Closest peak
Update RT in Component Table: NO Do blank subtraction: NO
Do library search: NO

Component Table

RT (min)	Window (%)	Func1	Func2	Func3	E-Conc	Tolerance (%)
5.00	10.00					

Integration Table

Time (min)	Function	Value/Status
0.00	NOISE	10
0.00	SMOOTHING	OFF
0.00	SENSITIVITY	20
0.00	N-METHOD	0

DAD Processing Setup:
  Peak spectrum integration enabled: NO
  Chromatogram to create: Fixed at 260 nm
Peak purity check enabled: NO
Delete DAD data after reporting: NO

DAD Display Format:
  Time range: 0.00 to 60.00 min
  Offset: 0.0
  Auto Mark Peak WL: NO
  3-D resolution: Medium
  3-D rotation: 30
  Display spectra only: NO
Absorbance scale (Au): Auto
Wavelength range: 190 to 390 nm
Spectrum display: Absorbance
Auto BG Subtraction: NO
3-D tilt: 30
3-D mirror: NO
Report spectra: Peak top and sides.

Perform system suitability test : NO
Perform module performance test : NO
Perform data diagnosis : NO

Chromatogram Display Format:
  Autoscale Time Range: 0.00 to 60.00 min
  Use alternate scale: NO
  Scale to Full Chrom Time Range: YES
  Baseline overlay: YES
  Marker-in signals: NO
  Show integration time table: NO
  Show gradient curves: NO
  Report channel 1 labels in the chromatogram overlay graph.
  Picture in picture: None
Autoscale: YES
Auto zero: NO
Peak rejection level: 0 uV * sec
Peak start-end markers: YES
Peak labels: Time, None
Show sampling period time table: NO

Report Format:
  Use primary layout: YES
  Print primary layout report: YES
  Acquisition DDE: NO
  Reprocess DDE: NO
  Concentration 1 Unit: Other
  Concentration 1 scale factor: 1.0000
  Concentration 1 divide by sample amount: NO
  Concentration 2 Unit: Other
  Concentration 2 scale factor: 1.0000
  Concentration 2 use component multiplier: NO
  Injection report column 1 header: PK-NUM
  Injection report column 2 header: RT
  Injection report column 3 header: AREA
  Injection report column 4 header: CONC1
  Injection report column 5 header: BC
Reported peaks: All Peaks
Use secondary layout: NO
Print secondary layout report: NO
Acquisition macro name:
Reprocess macro name:
Concentration 1 name:
Concentration 2 name:

D-7000 HSM: SAMPLES | Series: 0028 | Report: original | System: Sys 1

D-7000 HPLC System Manager Report

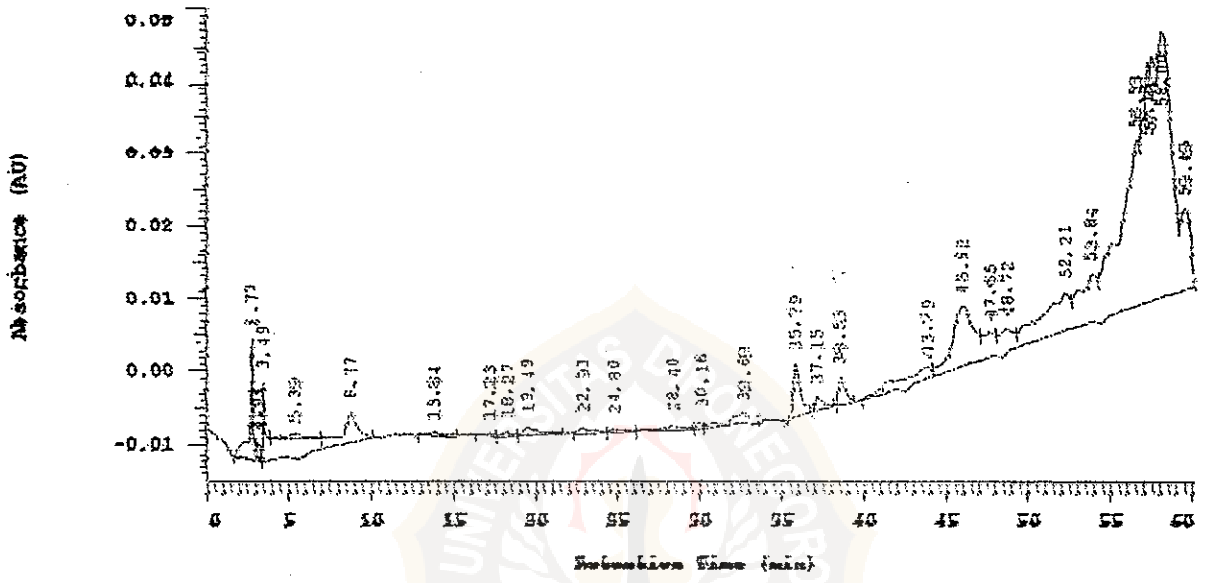
Analyzed: 11/01/00 11:01 AM
Reported: 11/01/00 12:02 PM
Processed: 11/01/00 12:02 PM

Data Path: C:\WIN32APP\HSM\samples\DATA\0028\
Processing Method: tmh

System(acquisition): Sys 1
Application: SAMPLES
Sample Name: tmh001
Injection from this vial: 1 of 1
Sample Description:

Series:0028
Vial Number: 1
Vial Type: UNK
Volume: 20.0 ul

Chrom Type: Fixed WL Chromatogram, 260 nm



Acquisition Method: tmh
Column Type:
Developed by: ratna
Pump A Type: L-7100
Solvent A: air/acet
Pump B Type: L-7100
Solvent A: meoh/acet
Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1	BC
1	2.77	93360	1.776	BV
2	3.04	2729	0.052	TBB
3	3.23	8790	0.167	TBB
4	3.49	247454	4.708	BV
5	5.39	22060	0.420	TBB
6	8.77	142905	2.719	VB
7	13.84	16280	0.310	BB
8	17.23	5540	0.105	BB
9	18.27	9636	0.183	BB
10	19.49	36243	0.690	BB
11	22.91	22560	0.429	BB
12	24.80	18780	0.357	BB
13	28.40	32160	0.612	BB
14	30.16	8443	0.161	BB
15	32.69	52556	1.000	BB

16	35.79	103351	1.967	BB
17	37.15	26648	0.511	BB
18	38.53	52720	1.003	BB
19	43.79	121072	2.304	BV
20	45.52	361930	6.887	VV
21	47.65	92284	1.756	VV
22	48.72	90562	1.724	VV
23	52.21	332176	6.320	VV
24	53.84	243765	4.638	VV
25	56.53	905592	17.231	VV
26	57.17	497144	9.459	VV
27	57.44	456159	8.337	VV
28	58.00	1045656	19.896	VV
29	59.49	224812	4.278	VB
		5255589	100.000	

Peak rejection level: 0

Channel 1 Noise: Not Measured
Channel 1 Drift: Not Measured

Configuration parameters:

Interface Module: D-7000	Gradient Mode: High
Channel 1 Detector: L-7450	Channel 2 Detector: None
Column Oven: None	Autosampler: Manual
Pump A: L-7100	Pump B: L-7100
Pump C: None	External Instrument Software: None
Column Name:	

Method Information:

Method Name: tmi	Developed by: ratna
Description:	
analisaDaG	

Pump Setup:
All Pumps Pressure Limit: 0 to 420 kgf/cm2

Pump A (L-7100):
Solvent A: air/acet
Pump B (L-7100):
Solvent B: meoh/acet

Pump Table

Time (min)	%air/a	%meoh/	Total Flow (ml/min)	Event 1	Event 2	Event 3	Event 4
0.0	100.0	0.0	1.000				
5.0	100.0	0.0	1.000				
55.0	0.0	100.0	1.000				
60.0	100.0	0.0	1.000				

Channel 1 Detector (L-7450):

Spectral Bandwidth: 4 nm	Spectral Interval: 1600 msec
Absorbance Mode: Normal(2.0AU)	Auto Zero before Injection: YES
Wavelength Range: 190 to 390 nm	Monitoring Wavelenth: 260 nm
Start Time: 0.00 min	Stop Time: 60.00 min

Method DP for Channel 1

Calculation Method:

Calculation Method: AREA#	Peak Quantitation: Area
Peak Identification Window: % Time	Concentration data from method.
UNK peaks identification rule: Closest peak	
Update RT in component Table: NO	Do blank subtraction: NO
Do library search: NO	

Component Table

RT (min)	Window (%)	Func1	Func2	Func3	E-Conc	Tolerance (%)
5.00	10.00					

Integration Table

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D-7000 HSM: SAMPLES | Series: 0029 | Report: original | System: Sys 1

D-7000 HPLC System Manager Report

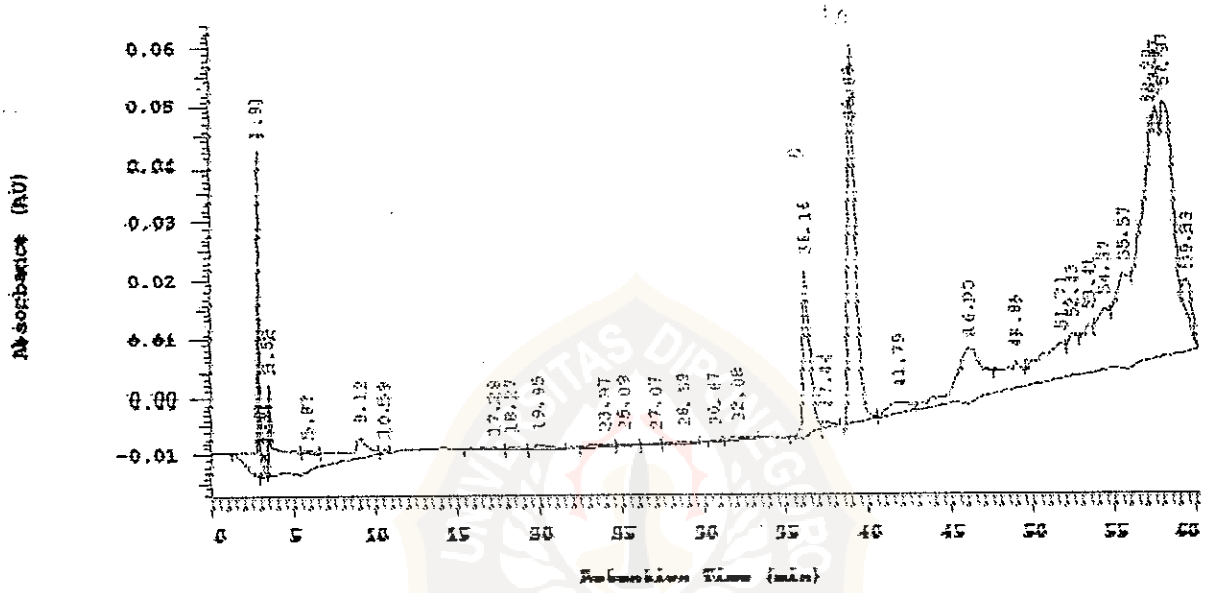
Analyzed: 11/01/00 12:12 PM
Reported: 11/01/00 03:52 PM
Processed: 11/01/00 01:13 PM

Data Path: C:\WIN32APP\HSM\samples\DATA\0029\
Processing Method: tmh

System(acquisition): Sys 1
Application: SAMPLES
Sample Name: tmh002
Injection from this vial: 1 of 1
Sample Description:

Series:0029
Vial Number: 2
Vial Type: UNK
Volume: 20.0 ul

Chrom Type: Fixed WL Chromatogram, 260 nm



Acquisition Method: tmh
Column Type:
Developed by: ratna
Pump A Type: L-7100
Solvent A: air/acet
Pump B Type: L-7100
Solvent A: meoh/acet
Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1	BC
1	2.91	178340	2.383	BB
2	3.01	22063	0.295	BV
3	3.31	14157	0.189	VB
4	3.52	315272	4.213	BV
5	5.67	8440	0.113	TBB
6	9.12	194437	2.598	VB
7	10.59	7789	0.104	BB
8	17.28	13340	0.178	BB
9	18.27	10840	0.145	BB
10	19.95	27319	0.365	BB
11	23.97	20971	0.280	BB
12	25.09	13466	0.180	BB
13	27.07	8539	0.114	BB
14	28.69	25820	0.318	BB
15	30.67	5120	0.066	BB

D-7000 HSM: SAMPLES | Series: 0029 | Report: original | System: Sys 1

16	32.08	23660	0.316	BB
17	36.16	353240	4.720	BB
18	37.44	12460	0.166	BB
19	38.88	934600	12.488	BB
20	41.79	84072	1.123	BV
21	46.00	518305	6.925	VV
22	48.65	221091	2.954	VV
23	51.71	341267	4.560	VV
24	52.43	165585	2.213	VV
25	53.41	195974	2.619	VV
26	54.37	282021	3.768	VV
27	55.57	521439	6.967	VV
28	57.25	1011955	13.522	VV
29	57.47	558040	7.456	VV
30	57.97	1292426	17.265	VV
31	59.39	103920	1.389	TBB
		7484012	100.000	

Peak rejection level: 0

tmh

D-7000 HPLC System Manager Report

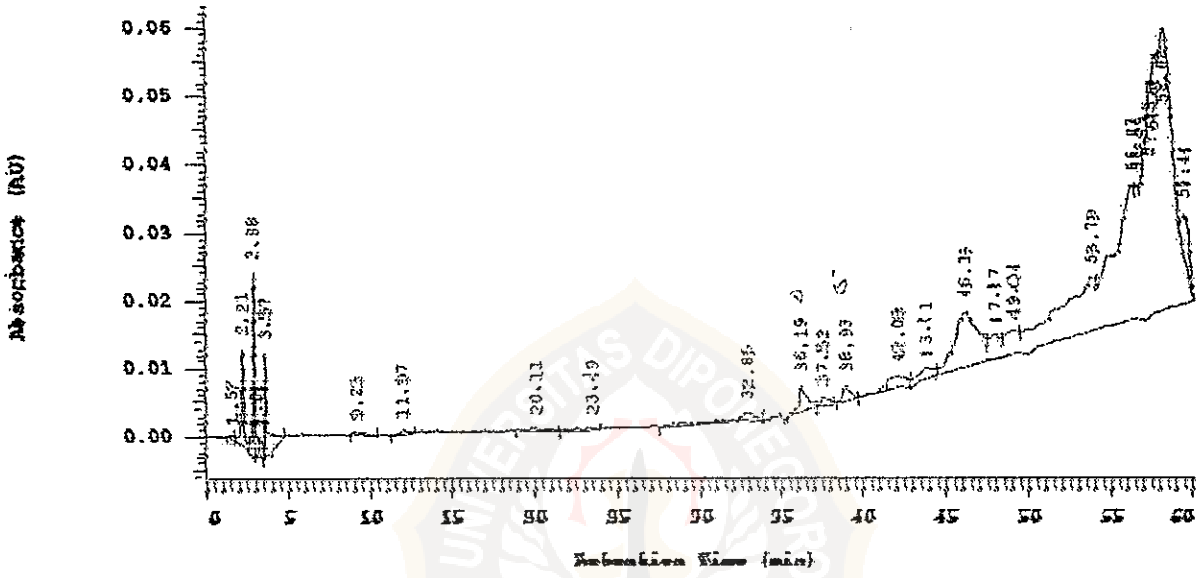
Analyzed: 11/01/00 01:23 PM
Reported: 11/01/00 02:24 PM
Processed: 11/01/00 02:24 PM

Data Path: C:\WIN32APP\HSM\samples\DATA\0030\
Processing Method: tmh

System(acquisition): Sys 1
Application: SAMPLES
Sample Name: tmh010
Injection from this vial: 1 of 1
Sample Description:

Series:0030
Vial Number: 10
Vial Type: UNK
Volume: 20.0 ul

Chrom Type: Fixed WL Chromatogram, 260 nm



Acquisition Method: tmh
Column Type:
Developed by: ratna
Pump A Type: L-7100
Solvent A: air/acet
Pump B Type: L-7100
Solvent A: meoh/acet
Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1	BC
1	1.57	8226	0.157	BV
2	2.21	59211	1.133	VV
3	2.86	51822	0.992	VB
4	3.01	13292	0.254	BV
5	3.33	14106	0.270	VB
6	3.57	81040	1.551	BB
7	9.23	9600	0.184	BB
8	11.97	7520	0.144	BB
9	20.11	9920	0.190	BB
10	23.49	14360	0.275	BB
11	32.85	39720	0.760	BB
12	36.19	63400	1.214	BB
13	37.52	19560	0.374	BB
14	38.93	34280	0.656	BB
15	42.08	67855	1.299	BV

D-7000 HSM: SAMPLES | Series: 0030 | Report: original | System: Sys 1

16	43.71	46898	0.898	VV
17	46.13	397776	7.615	VV
18	47.67	86366	1.654	VV
19	49.04	101570	1.944	VV
20	53.79	643226	12.313	VV
21	56.27	877953	16.806	VV
22	56.56	153161	2.932	VV
23	57.33	553252	10.591	VV
24	57.60	319370	6.114	VV
25	58.08	1441325	27.591	VV
26	59.44	109040	2.087	TBB
		5223693	100.000	

Peak rejection level: 0

Channel 1 Noise: Not Measured
Channel 1 Drift: Not Measured

Configuration parameters:
Interface Module: D-7000 Gradient Mode: High
Channel 1 Detector: L-7450 Channel 2 Detector: None
Column Oven: None Autosampler: Manual
Pump A: L-7100 Pump B: L-7100
Pump C: None External Instrument Software: None
Column Name:

Method Information:
Method Name: tmh Developed by: ratna
Description:
analisaDBG

Pump Setup:
All Pumps Pressure Limit: 0 to 420 kgf/cm2

Pump A (L-7100):
Solvent A: air/acet
Pump B (L-7100):
Solvent B: meoh/acet

Pump Table

Time (min)	%air/a	%meoh/	Total Flow (ml/min)	Event 1	Event 2	Event 3	Event 4
0.0	100.0	0.0	1.000				
5.0	100.0	0.0	1.000				
55.0	0.0	100.0	1.000				
60.0	100.0	0.0	1.000				

Channel 1 Detector (L-7450):
Spectral Bandwidth: 4 nm Spectral Interval: 1600 msec
Absorbance Mode: Normal(2.0AU) Auto Zero before Injection: YES
Wavelength Range: 190 to 390 nm Monitoring Wavelenth: 260 nm
Start Time: 0.00 min Stop Time: 60.00 min

Method DP for channel 1

Calculation Method: Peak Quantitation: Area
Calculation Method: AREA% Concentration data from method.
Peak Identification Window: % Time
UNK peaks identification rule: Closest peak
Update RT in component Table: NO Do blank subtraction: NO
Do library search: NO

Component Table

RT (min)	Window (%)	Func1	Func2	Func3	E-Conc	Tolerance (%)
5.00	10.00					

Integration Table

Time (min)	Function	Value/Status
0.00	NOISE	10
0.00	SMOOTHING	OFF
0.00	SENSITIVITY	20
0.00	N-METHOD	0

DAD Processing Setup:
Peak spectrum integration enabled: NO
Chromatogram to create: Fixed at 260 nm
Peak purity check enabled: NO
Delete DAD data after reporting: NO

DAD Display Format:
Time range: 0.00 to 60.00 min
Offset: 0.0
Auto Mark Peak WL: NO
3-D resolution: Medium
3-D rotation: 30
Display spectra only: NO
Absorbance scale (Au): Auto
Wavelength range: 190 to 390 nm
Spectrum Display: Absorbance
Auto BG Subtraction: NO
3-D tilt: 30
3-D mirror: NO
Report spectra: Peak top and sides.

Perform system suitability test : NO
Perform module performance test : NO
Perform data diagnosis : NO

Chromatogram Display Format:
Autoscale Time Range: 0.00 to 60.00 min
Use alternate scale: NO
Scale to Full Chrom Time Range: YES
Baseline overlay: YES
Marker-fn signals: NO
Show integration time table: NO
Show gradient curves: NO
Report channel 1 labels in the chromatogram overlay graph.
Picture in picture: None
Autoscale: YES
Auto zero: NO
Peak rejection level: 0 uV * sec
Peak start-end markers: YES
Peak labels: Time, None
Show sampling period time table: NO

Report Format:
Use primary layout: YES
Print primary layout report: YES
Acquisition DDE: NO
Reprocess DDE: NO
Concentration 1 Unit: Other
Concentration 1 scale factor: 1.0000
Concentration 1 divide by sample amount: NO
Concentration 2 Unit: Other
Concentration 2 scale factor: 1.0000
Concentration 2 use component multiplier: NO
Injection report column 1 header: PK-NUM
Injection report column 2 header: RT
Injection report column 3 header: AREA
Injection report column 4 header: CONC1
Injection report column 5 header: BC
Reported peaks: All Peaks
Use secondary layout: NO
Print secondary layout report: NO
Acquisition macro name:
Reprocess macro name:
Concentration 1 name:
Concentration 2 name:

TMH

D-7000 HPLC System Manager Report

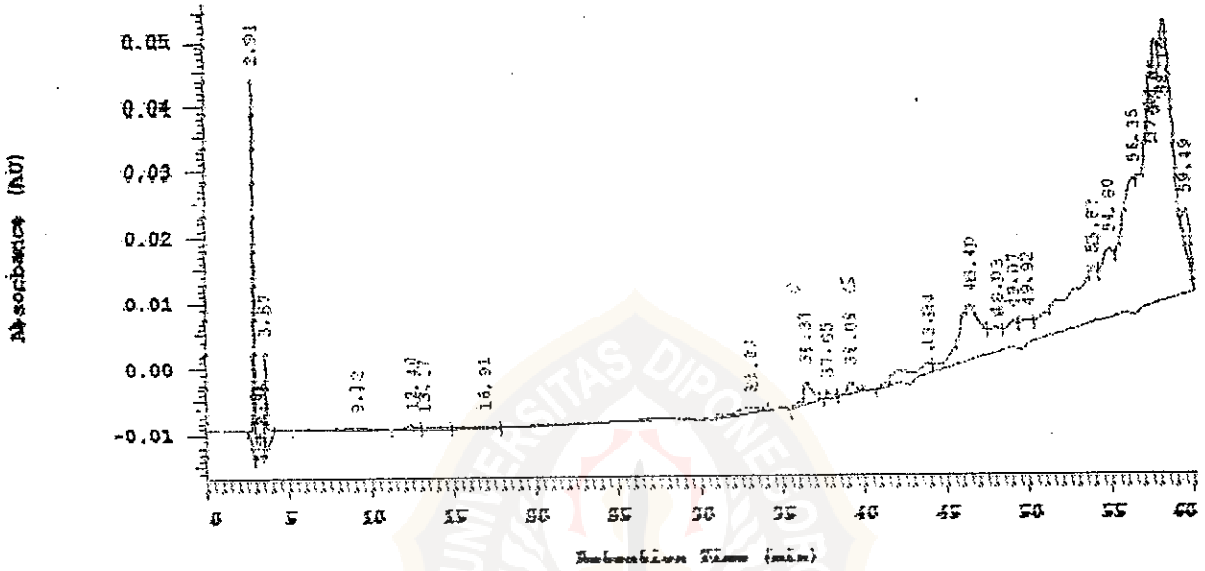
Analyzed: 11/01/00 02:29 PM
Reported: 11/01/00 03:29 PM
Processed: 11/01/00 03:29 PM

Data Path: C:\WIN32APP\HSM\samples\DATA\0031\
Processing Method: tmh

System(acquisition): Sys 1
Application: SAMPLES
Sample Name: tmh001
Injection from this vial: 1 of 1
Sample Description:

Series:0031
Vial Number: 1
Vial Type: UNK
Volume: 20.0 ul

Chrom Type: Fixed WL Chromatogram, 260 nm



Acquisition Method: tmh
Column Type:
Pump A Type: L-7100
Solvent A: air/acet
Pump B Type: L-7100
Solvent A: meoh/acet
Peak Quantitation: AREA
Calculation Method: AREA

Developed by: ratna

No.	RT	Area	Conc 1	BC
1	2.91	95440	1.796	BB
2	3.04	20840	0.392	BV
3	3.33	15560	0.293	VB
4	3.57	44880	0.845	BB
5	9.12	20060	0.378	BB
6	12.40	18050	0.340	BB
7	13.17	22360	0.421	BB
8	16.91	30029	0.565	BB
9	33.07	38660	0.727	BB
10	36.37	64100	1.206	BB
11	37.65	13350	0.251	BB
12	39.09	32289	0.608	BB
13	43.84	109685	2.064	BV
14	46.40	397260	7.475	VV
15	48.03	104266	1.962	VV

D-7000 HSM: SAMPLES | Series: 0051 | Report: original | System: Sys 1

16	49.07	96934	1.824	VV
17	49.92	91340	1.719	VV
18	53.67	602191	11.331	VV
19	54.80	309820	5.830	VV
20	56.35	592780	11.154	VV
21	57.41	723642	13.617	VV
22	57.65	523657	9.854	VV
23	58.16	1254260	23.602	VV
24	59.49	92840	1.747	TBB
		5314313	100.000	

Peak rejection level: 0

Channel 1 Noise: Not Measured
Channel 1 Drift: Not Measured

Configuration parameters:
Interface Module: D-7000 Gradient Mode: High
Channel 1 Detector: L-7450 Channel 2 Detector: None
Column Oven: None Autosampler: Manual
Pump A: L-7100 Pump B: L-7100
Pump C: None External Instrument Software: None
Column Name:

Method Information:
Method Name: tmh Developed by: ratna
Description:
analisadsg

Pump Setup:
All Pumps Pressure Limit: 0 to 420 kgf/cm2

Pump A (L-7100):
Solvent A: air/acet
Pump B (L-7100):
Solvent B: meoh/acet

Pump Table

Time (min)	%air/a	%meoh/	Total Flow (ml/min)	Event 1	Event 2	Event 3	Event 4
0.0	100.0	0.0	1.000				
5.0	100.0	0.0	1.000				
55.0	0.0	100.0	1.000				
60.0	100.0	0.0	1.000				

Channel 1 Detector (L-7450):
Spectral Bandwidth: 4 nm Spectral Interval: 1600 msec
Absorbance Mode: Normal(2.0AU) Auto Zero before Injection: YES
Wavelength Range: 190 to 590 nm Monitoring Wavelenth: 260 nm
Start Time: 0.00 min Stop Time: 60.00 min

Method DP for Channel 1

Calculation Method: Peak Quantitation: Area
Calculation Method: AREA3 Concentration data from method.
Peak Identification Window: % Time
UNK peaks identification rule: Closest peak
Update RT in Component Table: NO Do blank subtraction: NO
Do library search: NO

Component Table

RT (min)	Window (%)	Func1	Func2	Func3	E-Conc	Tolerance (%)
5.00	10.00					

Integration Table

me nj	function	value/status
00	NOISE	10
00	SMOOTHING	OFF
00	SENSITIVITY	20
00	N-METHOD	0

Processing setup:
eak spectrum integration enabled: NO
hromatogram to create: Fixed at 260 nm

Peak purity check enabled: NO
Delete DAD data after reporting: NO

Display Format:
ime range: 0.00 to 60.00 min
ffset: 0.0
uto Mark Peak WL: NO
-D resolution: Medium
-D rotation: 30
isplay spectra only: NO

Absorbance scale (AU): Auto
Wavelength range: 190 to 390 nm
Spectrum Display: Absorbance
Auto BG Subtraction: NO
3-D tilt: 30
3-D mirror: No
Report Spectra: Peak top and sides.

form system suitability test : NO
form module performance test : NO
form data diagnosis : NO

omatogram Display Format:
utoscale Time Range: 0.00 to 60.00 min
se alternate scale: NO
cale to Full Chrom Time Range: YES
aseline overlay: YES
arker-in signals: NO
how integration time table: NO
how gradient curves: NO
eport channel 1 labels in the chromatogram overlay graph.
icture in picture: None

Autoscale: YES
Auto zero: NO
Peak rejection level: 0 uV * sec
Peak start-end markers: YES
Peak labels: Time, None
Show sampling period time table: NO

ort Format:
se primary layout: YES
rint primary layout report: YES
cquisition DDE: NO
eprocess DDE: NO
oncentration 1 Unit: Other
oncentration 1 scale factor: 1.0000
oncentration 1 divide by sample amount: NO
oncentration 2 unit: other
oncentration 2 scale factor: 1.0000
oncentration 2 use component multiplier: NO
njection report column 1 header: PK-NUM
njection report column 2 header: RT
njection report column 3 header: AREA
njection report column 4 header: CONC1
njection report column 5 header: BC

Reported peaks: All Peaks
Use secondary layout: NO
Print secondary layout report: NO
Acquisition macro name:
Reprocess macro name:
Concentration 1 name:
Concentration 2 name:

D-7000 HPLC System Manager Report

lyzed: 11/02/00 08:20 AM

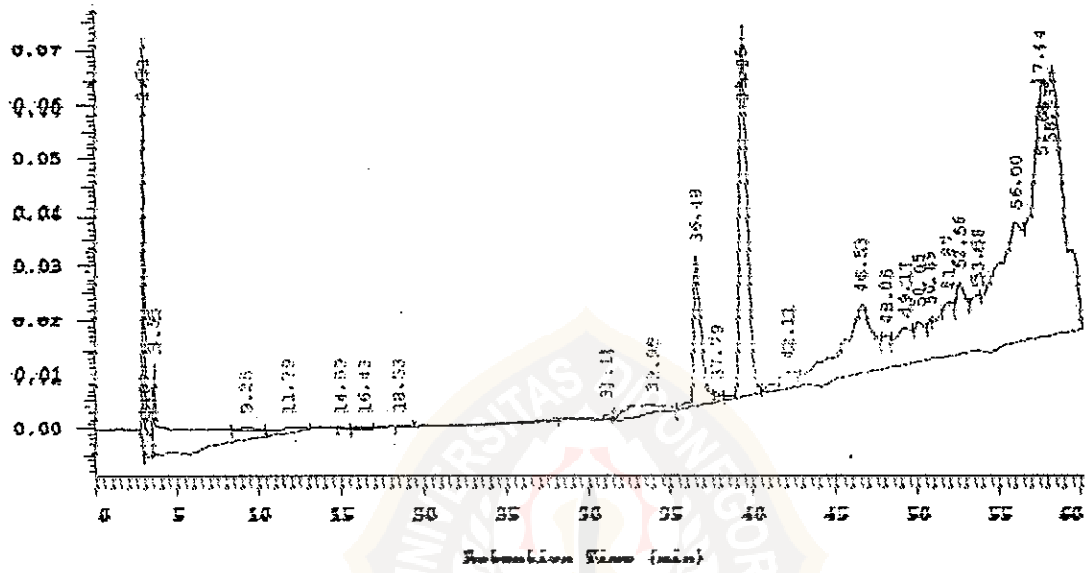
Reported: 11/02/00 10:45 AM
Processed: 11/02/00 09:20 AM

a Path: C:\WIN32APP\HSM\samples\DATA\0032\
cessing Method: tmh

tem(acquisition): Sys 1
lication: SAMPLES
ple Name: tmh010
ection from this vial: 1 of 1
ple Description:

Series:0032
Vial Number: 10
Vial Type: UNK
Volume: 20.0 ul

Chrom Type: Fixed WL Chromatogram, 260 nm



quisition Method: tmh
lumn Type:
p A Type: L-7100
Solvent A: air/acet
p B Type: L-7100
Solvent A: meoh/acet
.K Quantitation: AREA
.culation Method: AREA%

Developed by: ratna

	RT	Area	Conc 1	BC
	2.91	103320	1.132	BB
	3.07	50761	0.556	BV
	3.55	629339	6.898	VV
	9.25	20460	0.224	TBB
	11.79	59756	0.655	VB
	14.99	4500	0.049	BB
	16.43	9660	0.106	BB
	18.53	4760	0.052	BB
	31.15	22680	0.249	BB
	33.95	96400	1.057	BB
	36.48	416803	4.569	BV
	37.79	6120	0.067	TBB
	39.25	1022679	11.209	VB
	42.11	123301	1.351	BV
	46.53	819168	8.979	VV

16	48.05	109015	1.195	VV
17	49.17	245766	2.694	VV
18	50.05	159960	1.754	VV
19	50.69	68940	0.756	VV
20	51.87	351531	3.853	VV
21	52.56	283441	3.107	VV
22	53.66	215596	2.362	VV
23	56.00	1287085	14.108	VV
24	57.44	882637	9.674	VV
25	57.68	406061	4.451	VV
26	58.13	1723386	18.890	VB
		9123331	100.000	

Peak rejection level: 0