



Figure A.1: Liquid Chromatograph Tandem Mass Spectrometer (LC-MS/MS)



Figure A.2 Balance to weigh the sample



Figure A.3: Centrifuge



Figure A.4: Solvent Dispenser

Compound name: Udenafil

#	Sample Text	ID	Type	Std. Conc	RT	Area	IS Area	Response	ppm	%Dev
1	Spk Candy 10 ppb Udenafil		Standard	0.010	4.67	755.010		755.010	0.0	3.0
2	Spk Candy 50 ppb Udenafil		Standard	0.050	4.71	3300.955		3300.955	0.0	-12.4
3	Spk Candy 100 ppb Udenafil		Standard	0.100	4.67	8000.613		8000.613	0.1	5.6
4	Spk Candy 200 ppb Udenafil		Standard	0.200	4.67	15205.625		15205.625	0.2	0.2
5	Mobile Phase		Blank							
6	Spk Candy 10 ppb Udenafil (Rep 1)		Analyte		4.67	658.033		658.033	0.0	
7	Spk Candy 10 ppb Udenafil (Rep 2)		Analyte		4.71	787.324		787.324	0.0	
8	Spk Candy 10 ppb Udenafil (Rep 3)		Analyte		4.67	581.685		581.685	0.0	
9	Spk Candy 10 ppb Udenafil (Rep 4)		Analyte		4.67	790.032		790.032	0.0	
10	Spk Candy 10 ppb Udenafil (Rep 5)		Analyte		4.71	731.286		731.286	0.0	
11	Spk Candy 10 ppb Udenafil (Rep 6)		Analyte		4.67	665.132		665.132	0.0	
12	Spk Candy 10 ppb Udenafil (Rep 7)		Analyte		4.67	803.241		803.241	0.0	
13	Mobile Phase		Blank							
14	Spk Candy 10 ppb N-Desmethylsildenafil		Standard	0.010						
15	Spk Candy 50 ppb N-Desmethylsildenafil		Standard	0.050						
16	Spk Candy 100 ppb N-Desmethylsildenafil		Standard	0.100						
17	Spk Candy 200 ppb N-Desmethylsildenafil		Standard	0.200						
18	Mobile Phase		Blank							
19	Spk Candy 10 ppb N-Desmethylsildenafil (Rep 1)		Analyte							
20	Spk Candy 10 ppb N-Desmethylsildenafil (Rep 2)		Analyte							
21	Spk Candy 10 ppb N-Desmethylsildenafil (Rep 3)		Analyte							
22	Spk Candy 10 ppb N-Desmethylsildenafil (Rep 4)		Analyte							
23	Spk Candy 10 ppb N-Desmethylsildenafil (Rep 5)		Analyte							
24	Spk Candy 10 ppb N-Desmethylsildenafil (Rep 6)		Analyte							
25	Spk Candy 10 ppb N-Desmethylsildenafil (Rep 7)		Analyte							
26	Mobile Phase		Blank							
27	Spk Candy 5 ppb Gendenafil		Standard	0.005						
28	Spk Candy 20 ppb Gendenafil		Standard	0.020						
29	Spk Candy 100 ppb Gendenafil		Standard	0.100						
30	Spk Candy 200 ppb Gendenafil		Standard	0.200						
31	Mobile Phase		Blank							
32	Spk Candy 5 ppb Gendenafil (Rep 1)		Analyte							
33	Spk Candy 5 ppb Gendenafil (Rep 2)		Analyte							
34	Spk Candy 5 ppb Gendenafil (Rep 3)		Analyte							
35	Spk Candy 5 ppb Gendenafil (Rep 4)		Analyte							
36	Spk Candy 5 ppb Gendenafil (Rep 5)		Analyte							
37	Spk Candy 5 ppb Gendenafil (Rep 6)		Analyte							
38	Spk Candy 5 ppb Gendenafil (Rep 7)		Analyte							

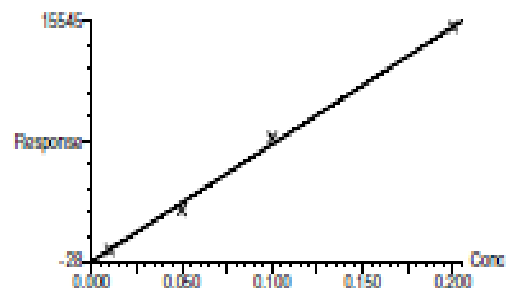
Compound name: Udenafil

Correlation coefficient: $r = 0.998106$, $r^2 = 0.996216$

Calibration curve: $76040.8 \cdot x + -28.1231$

Response type: External Std, Area

Curve type: Linear, Origin: Include, Weighting: 1/x, Axis trans: None



APPENDIX B

Compound name: Gendenafl

#	Sample Text	ID	Type	Std. Conc	RT	Area	IS Area	Response	ppm	%Dev
1	Spk Candy 10 ppb Udenafil		Standard	0.010						
2	Spk Candy 50 ppb Udenafil		Standard	0.050						
3	Spk Candy 100 ppb Udenafil		Standard	0.100						
4	Spk Candy 200 ppb Udenafil		Standard	0.200						
5	Mobile Phase		Blank							
6	Spk Candy 10 ppb Udenafil (Rep 1)		Analyte							
7	Spk Candy 10 ppb Udenafil (Rep 2)		Analyte							
8	Spk Candy 10 ppb Udenafil (Rep 3)		Analyte							
9	Spk Candy 10 ppb Udenafil (Rep 4)		Analyte							
10	Spk Candy 10 ppb Udenafil (Rep 5)		Analyte							
11	Spk Candy 10 ppb Udenafil (Rep 6)		Analyte							
12	Spk Candy 10 ppb Udenafil (Rep 7)		Analyte							
13	Mobile Phase		Blank							
14	Spk Candy 10 ppb N-Desmethylsildenafil		Standard	0.010						
15	Spk Candy 50 ppb N-Desmethylsildenafil		Standard	0.050						
16	Spk Candy 100 ppb N-Desmethylsildenafil		Standard	0.100						
17	Spk Candy 200 ppb N-Desmethylsildenafil		Standard	0.200						
18	Mobile Phase		Blank							
19	Spk Candy 10 ppb N-Desmethylsildenafil (Rep 1)		Analyte							
20	Spk Candy 10 ppb N-Desmethylsildenafil (Rep 2)		Analyte							
21	Spk Candy 10 ppb N-Desmethylsildenafil (Rep 3)		Analyte							
22	Spk Candy 10 ppb N-Desmethylsildenafil (Rep 4)		Analyte							
23	Spk Candy 10 ppb N-Desmethylsildenafil (Rep 5)		Analyte							
24	Spk Candy 10 ppb N-Desmethylsildenafil (Rep 6)		Analyte							
25	Spk Candy 10 ppb N-Desmethylsildenafil (Rep 7)		Analyte							
26	Mobile Phase		Blank							
27	Spk Candy 5 ppb Gendenafl		Standard	0.005	7.63	202.345		202.345	0.0	-2.3
28	Spk Candy 20 ppb Gendenafl		Standard	0.020	7.64	815.702		815.702	0.0	-6.3
29	Spk Candy 100 ppb Gendenafl		Standard	0.100	7.65	4617.922		4617.922	0.1	1.8
30	Spk Candy 200 ppb Gendenafl		Standard	0.200	7.65	9087.708		9087.708	0.2	-0.0
31	Mobile Phase		Blank							
32	Spk Candy 5 ppb Gendenafl (Rep 1)		Analyte		7.68	199.153		199.153	0.0	
33	Spk Candy 5 ppb Gendenafl (Rep 2)		Analyte		7.67	194.694		194.694	0.0	
34	Spk Candy 5 ppb Gendenafl (Rep 3)		Analyte		7.66	140.502		140.502	0.0	
35	Spk Candy 5 ppb Gendenafl (Rep 4)		Analyte		7.67	207.905		207.905	0.0	
36	Spk Candy 5 ppb Gendenafl (Rep 5)		Analyte		7.63	196.945		196.945	0.0	
37	Spk Candy 5 ppb Gendenafl (Rep 6)		Analyte		7.63	172.483		172.483	0.0	
38	Spk Candy 5 ppb Gendenafl (Rep 7)		Analyte		7.60	173.357		173.357	0.0	

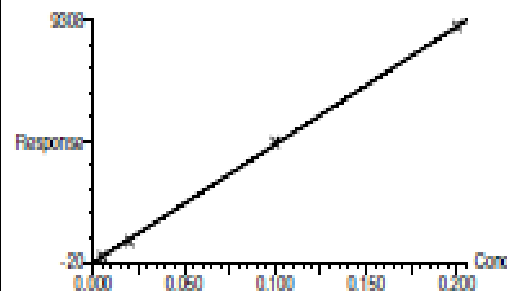
Compound name: Gendenafl

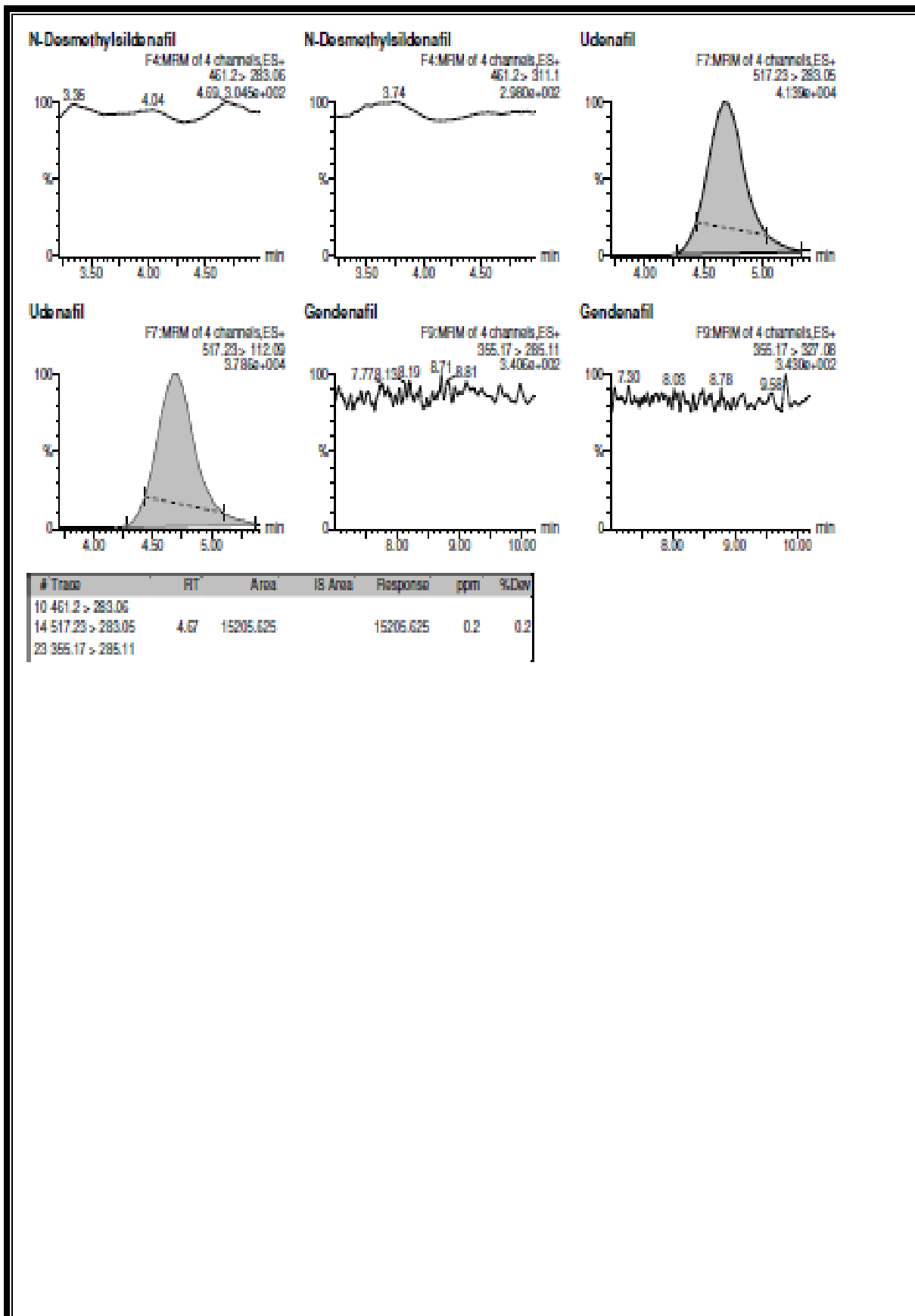
Correlation coefficient: $r = 0.999637$, $r^2 = 0.999274$

Calibration curve: $45550.6 * x + -20.0655$

Response type: External Std. Area

Curve type: Linear, Origin: Include, Weighting: 1/x, Axis trans: None





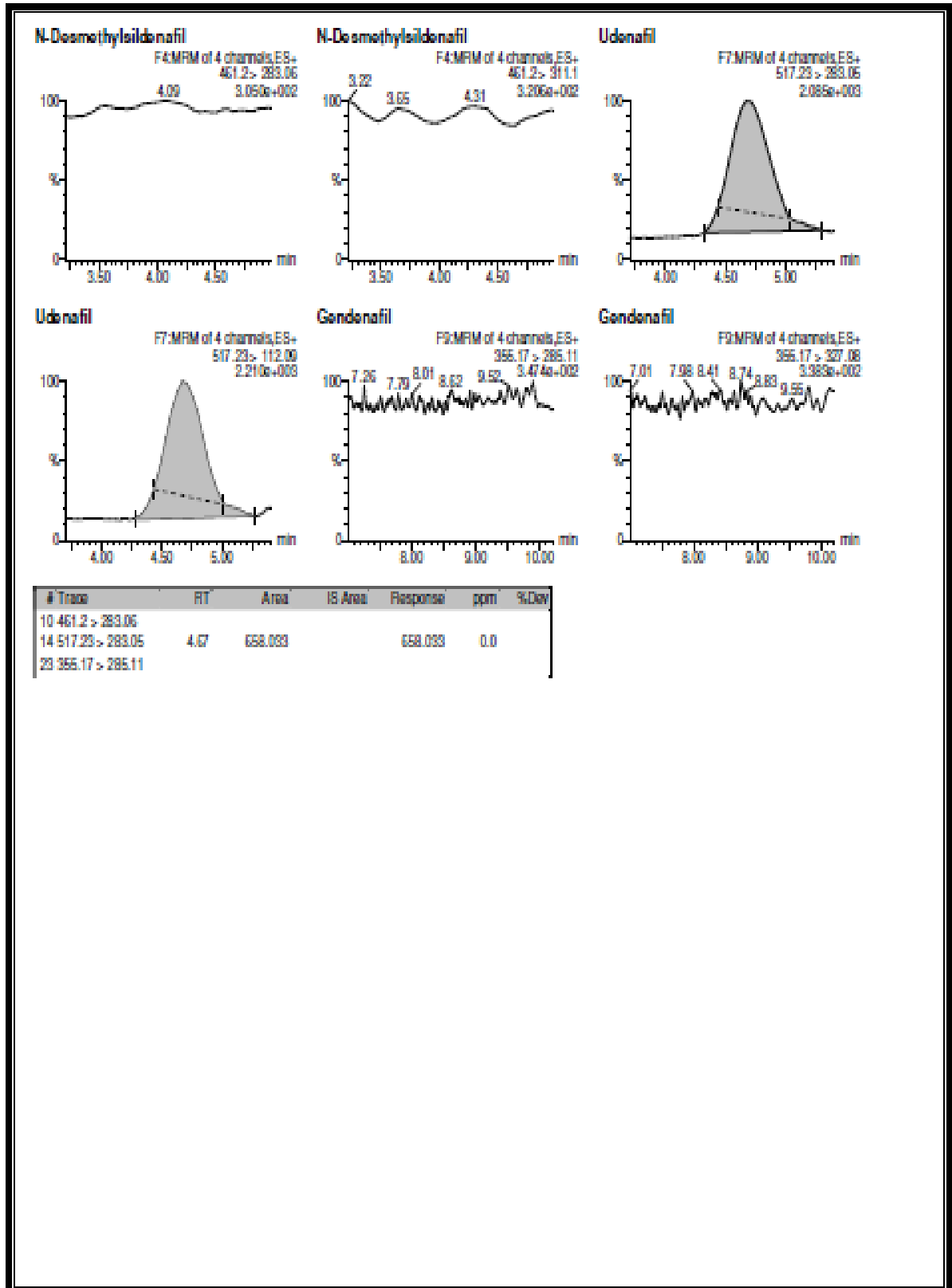


Figure B.1: Validation Study in Candy Matrix

Compound name: Thiosildenafil

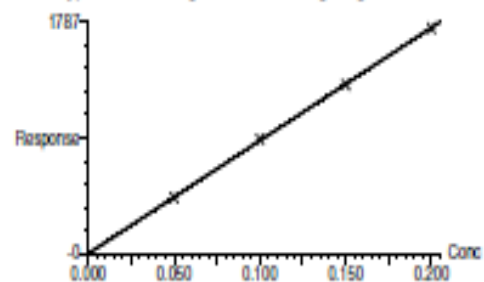
# Sample Text	ID	Type	Std. Conc	RT	Area	IS Area	Response	ppm	%Dev
1 Spk Herbal 10 ppb N-Desmetylsildenafil		Standard	0.010						
2 Spk Herbal 50 ppb N-Desmetylsildenafil		Standard	0.050						
3 Spk Herbal 100 ppb N-Desmetylsildenafil		Standard	0.100						
4 Spk Herbal 200 ppb N-Desmetylsildenafil		Standard	0.200						
5 Mobile Phase		Blank							
6 Spk Herbal 10 ppb N-Desmetylsildenafil (Rep 1)		Analyte							
7 Spk Herbal 10 ppb N-Desmetylsildenafil (Rep 2)		Analyte							
8 Spk Herbal 10 ppb N-Desmetylsildenafil (Rep 3)		Analyte							
9 Spk Herbal 10 ppb N-Desmetylsildenafil (Rep 4)		Analyte							
10 Spk Herbal 10 ppb N-Desmetylsildenafil (Rep 5)		Analyte							
11 Spk Herbal 10 ppb N-Desmetylsildenafil (Rep 6)		Analyte							
12 Spk Herbal 10 ppb N-Desmetylsildenafil (Rep 7)		Analyte							
13 Mobile Phase		Blank							
14 Spk Herbal 10 ppb Chloropretadalafl		Standard	0.010						
15 Spk Herbal 50 ppb Chloropretadalafl		Standard	0.050						
16 Spk Herbal 100 ppb Chloropretadalafl		Standard	0.100						
17 Spk Herbal 200 ppb Chloropretadalafl		Standard	0.200						
18 Mobile Phase		Blank							
19 Spk Herbal 10 ppb Chloropretadalafl (Rep 1)		Analyte							
20 Spk Herbal 10 ppb Chloropretadalafl (Rep 2)		Analyte							
21 Spk Herbal 10 ppb Chloropretadalafl (Rep 3)		Analyte							
22 Spk Herbal 10 ppb Chloropretadalafl (Rep 4)		Analyte							
23 Spk Herbal 10 ppb Chloropretadalafl (Rep 5)		Analyte							
24 Spk Herbal 10 ppb Chloropretadalafl (Rep 6)		Analyte							
25 Spk Herbal 10 ppb Chloropretadalafl (Rep 7)		Analyte							
26 Mobile Phase		Blank							
40 Spk Herbal 50 ppb Thiosildenafil		Standard	0.050	11.10	432.830		432.830	0.0	-0.7
41 Spk Herbal 100 ppb Thiosildenafil		Standard	0.100	11.07	881.778		881.778	0.1	1.1
42 Spk Herbal 150 ppb Thiosildenafil		Standard	0.150	11.07	1306.645		1306.645	0.1	-0.2
43 Spk Herbal 200 ppb Thiosildenafil		Standard	0.200	11.10	1741.471		1741.471	0.2	-0.2
44 Mobile Phase		Blank							
45 Spk Herbal 50 ppb Thiosildenafil (Rep 1)		Analyte		11.15	399.667		399.667	0.0	
46 Spk Herbal 50 ppb Thiosildenafil (Rep 2)		Analyte		11.15	397.264		397.264	0.0	
47 Spk Herbal 50 ppb Thiosildenafil (Rep 3)		Analyte		11.15	425.655		425.655	0.0	
48 Spk Herbal 50 ppb Thiosildenafil (Rep 4)		Analyte		11.24	403.453		403.453	0.0	
49 Spk Herbal 50 ppb Thiosildenafil (Rep 5)		Analyte		11.21	424.879		424.879	0.0	
50 Spk Herbal 50 ppb Thiosildenafil (Rep 6)		Analyte		11.21	402.428		402.428	0.0	
51 Spk Herbal 50 ppb Thiosildenafil (Rep 7)		Analyte		11.19	425.597		425.597	0.0	

Compound name: Thiosildenafil

Correlation coefficient: $r = 0.999968$, $r^2 = 0.999935$ Calibration curve: $8727.9 * x + -0.306416$

Response type: External Std, Area

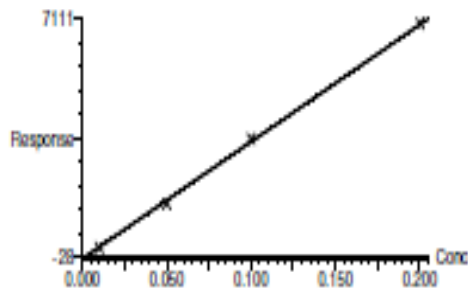
Curve type: Linear, Origin: Include, Weighting: 1/x, Axis trans: None

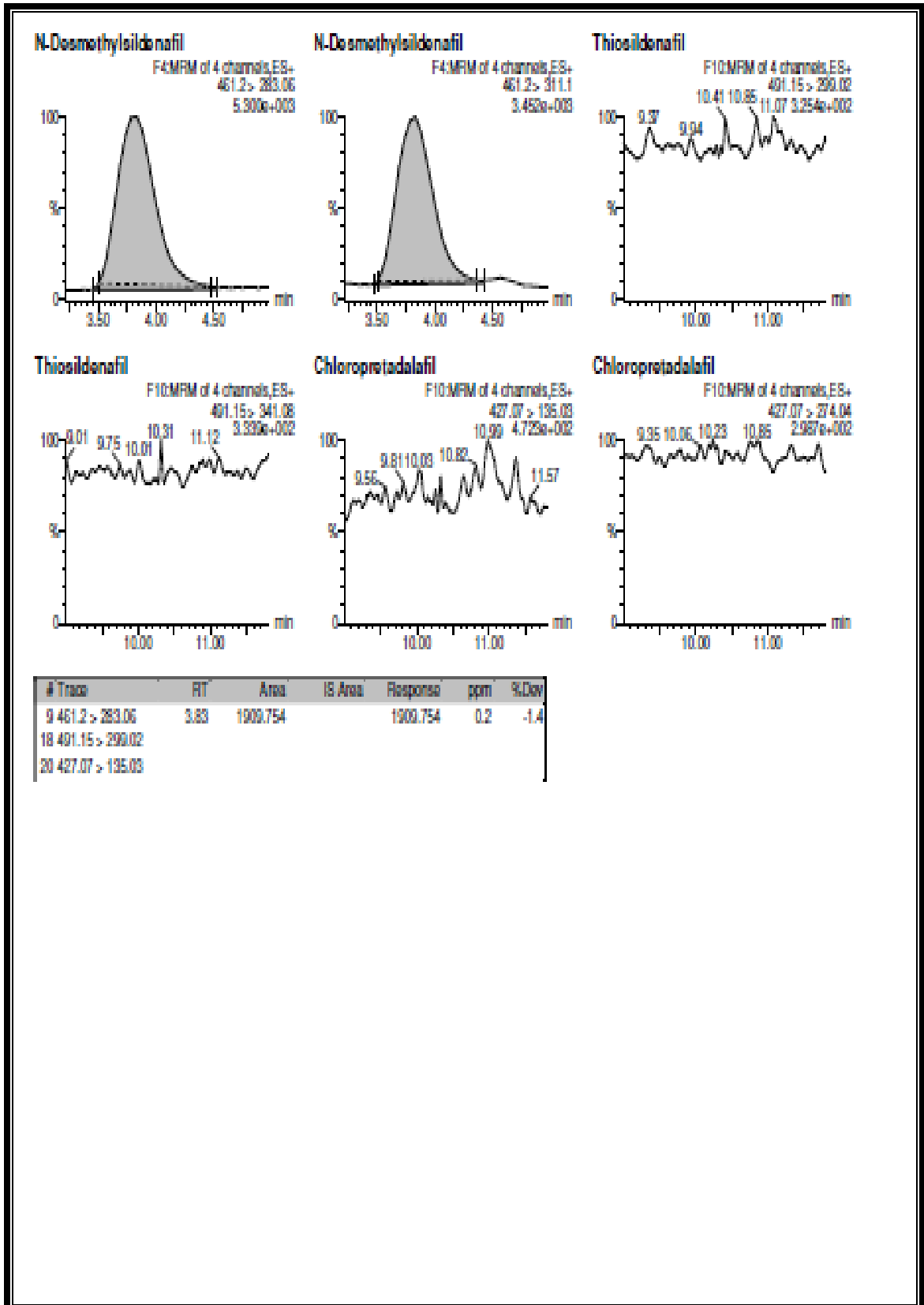


Compound name: Chloroprotadalaflil

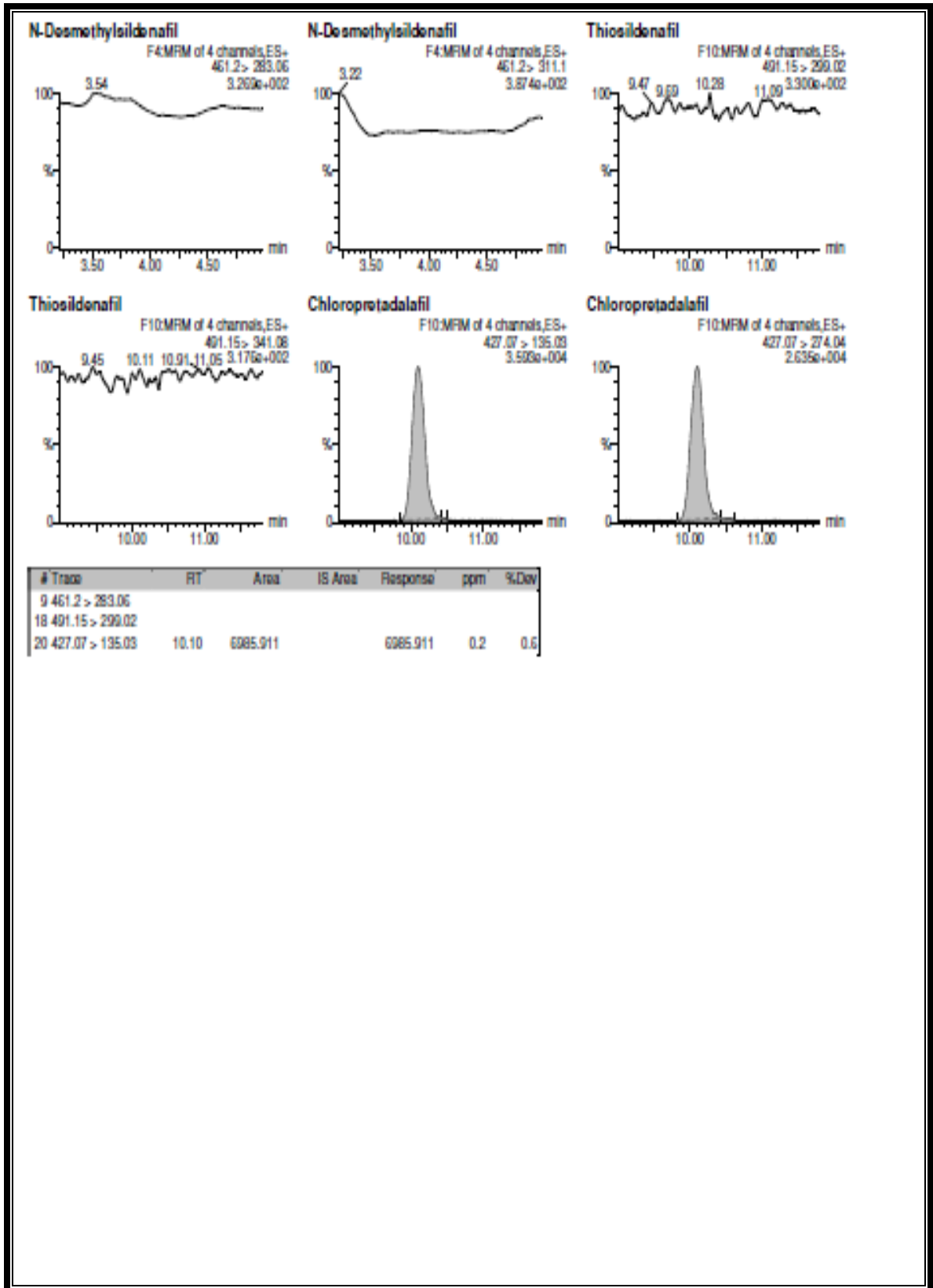
# Sample Text	ID	Type	Std. Conc	RT	Area	IS Area	Response	ppm	%Dev
1 Spk Herbal 10 ppb N-Desmetylsildenafil		Standard	0.010						
2 Spk Herbal 50 ppb N-Desmetylsildenafil		Standard	0.050						
3 Spk Herbal 100 ppb N-Desmetylsildenafil		Standard	0.100						
4 Spk Herbal 200 ppb N-Desmetylsildenafil		Standard	0.200						
5 Mobile Phase		Blank							
6 Spk Herbal 10 ppb N-Desmetylsildenafil (Rep 1)		Analyte							
7 Spk Herbal 10 ppb N-Desmetylsildenafil (Rep 2)		Analyte							
8 Spk Herbal 10 ppb N-Desmetylsildenafil (Rep 3)		Analyte							
9 Spk Herbal 10 ppb N-Desmetylsildenafil (Rep 4)		Analyte							
10 Spk Herbal 10 ppb N-Desmetylsildenafil (Rep 5)		Analyte							
11 Spk Herbal 10 ppb N-Desmetylsildenafil (Rep 6)		Analyte							
12 Spk Herbal 10 ppb N-Desmetylsildenafil (Rep 7)		Analyte							
13 Mobile Phase		Blank							
14 Spk Herbal 10 ppb Chloroprotadalaflil		Standard	0.010	10.05	303.614		303.614	0.0	-4.8
15 Spk Herbal 50 ppb Chloroprotadalaflil		Standard	0.050	10.08	1606.705		1606.705	0.0	-6.2
16 Spk Herbal 100 ppb Chloroprotadalaflil		Standard	0.100	10.10	3541.685		3541.685	0.1	2.4
17 Spk Herbal 200 ppb Chloroprotadalaflil		Standard	0.200	10.10	6985.911		6985.911	0.2	0.6
18 Mobile Phase		Blank							
19 Spk Herbal 10 ppb Chloroprotadalaflil (Rep 1)		Analyte		10.10	328.098		328.098	0.0	
20 Spk Herbal 10 ppb Chloroprotadalaflil (Rep 2)		Analyte		10.09	293.836		293.836	0.0	
21 Spk Herbal 10 ppb Chloroprotadalaflil (Rep 3)		Analyte		10.10	316.508		316.508	0.0	
22 Spk Herbal 10 ppb Chloroprotadalaflil (Rep 4)		Analyte		10.09	374.645		374.645	0.0	
23 Spk Herbal 10 ppb Chloroprotadalaflil (Rep 5)		Analyte		10.09	287.360		287.360	0.0	
24 Spk Herbal 10 ppb Chloroprotadalaflil (Rep 6)		Analyte		10.08	355.070		355.070	0.0	
25 Spk Herbal 10 ppb Chloroprotadalaflil (Rep 7)		Analyte		10.06	373.215		373.215	0.0	
26 Mobile Phase		Blank							
40 Spk Herbal 50 ppb Thiosildenafil		Standard	0.050						
41 Spk Herbal 100 ppb Thiosildenafil		Standard	0.100						
42 Spk Herbal 150 ppb Thiosildenafil		Standard	0.150						
43 Spk Herbal 200 ppb Thiosildenafil		Standard	0.200						
44 Mobile Phase		Blank							
45 Spk Herbal 50 ppb Thiosildenafil (Rep 1)		Analyte							
46 Spk Herbal 50 ppb Thiosildenafil (Rep 2)		Analyte							
47 Spk Herbal 50 ppb Thiosildenafil (Rep 3)		Analyte							
48 Spk Herbal 50 ppb Thiosildenafil (Rep 4)		Analyte							
49 Spk Herbal 50 ppb Thiosildenafil (Rep 5)		Analyte							
50 Spk Herbal 50 ppb Thiosildenafil (Rep 6)		Analyte							
51 Spk Herbal 50 ppb Thiosildenafil (Rep 7)		Analyte							

Compound name: Chloroprotadalaflil
 Correlation coefficient: $r = 0.999408$, $r^2 = 0.998816$
 Calibration curve: $34862.1 * x + -28.1133$
 Response type: External Std, Area
 Curve type: Linear, Origin: Include, Weighting: 1/x, Axis trans: None





APPENDIX B



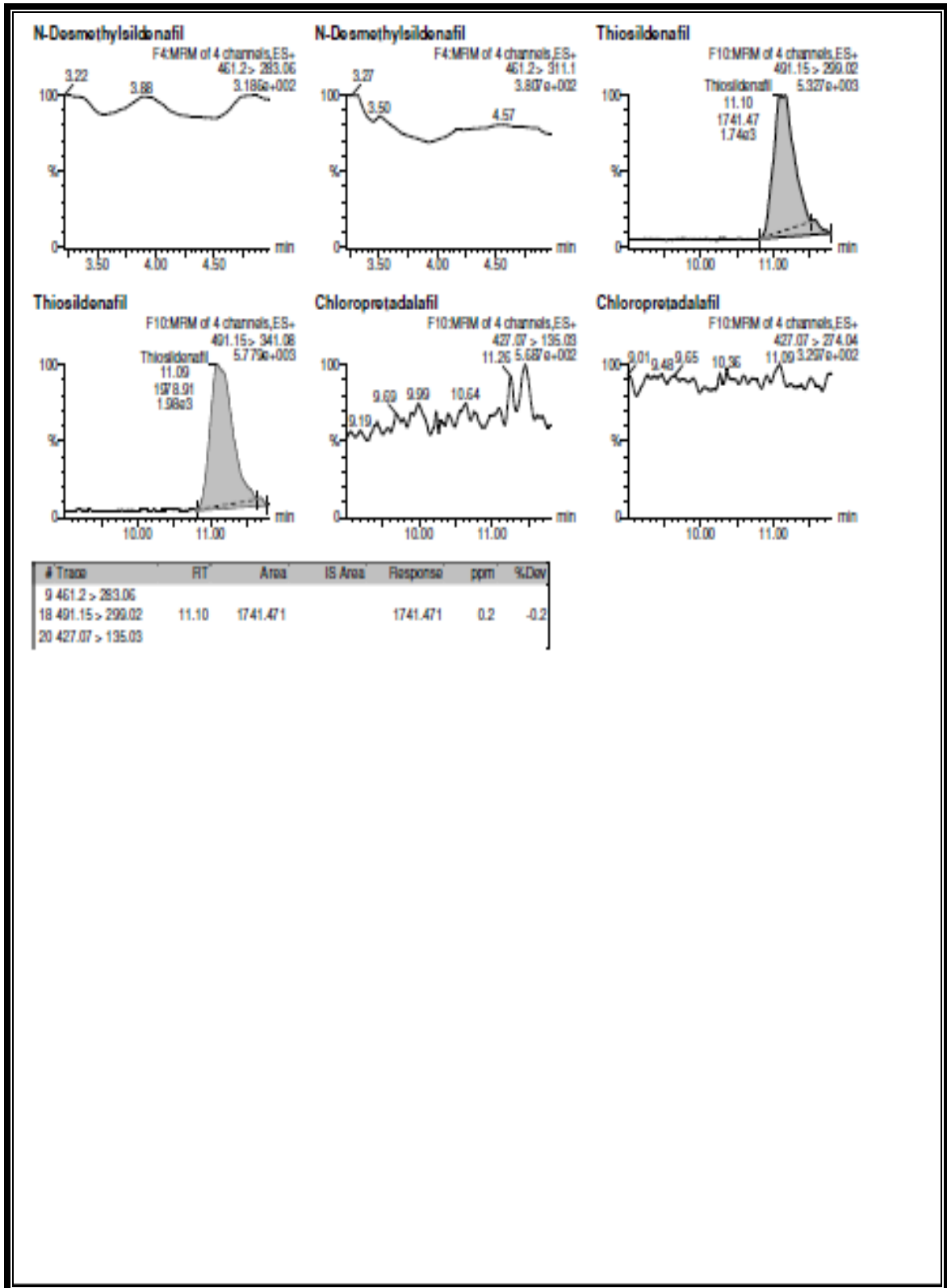


Figure B.2: Validation Study in Herbal Matrix

Compound name: Homosildenafil

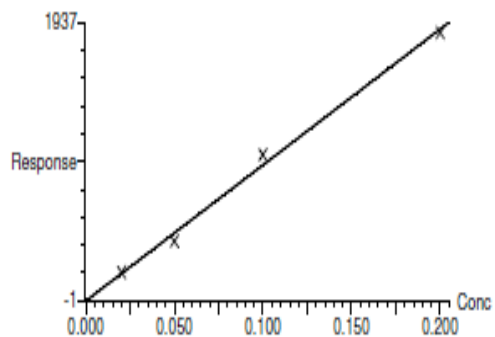
# Sample Text	ID	Type	Std. Conc	RT	Area	IS Area	Response	ppm	%Dev
1 Spk Coffee 20 ppb Sildenafil		Standard	0.020						
2 Spk Coffee 50 ppb Sildenafil		Standard	0.050						
3 Spk Coffee 100 ppb Sildenafil		Standard	0.100						
4 Spk Coffee 200 ppb Sildenafil		Standard	0.200						
5 Mobile Phase		Blank							
6 Blank Coffee		Analyte							
7 Mobile Phase		Blank							
8 Spk Coffee 20 ppb Sildenafil (Rep 1)		Analyte							
9 Spk Coffee 20 ppb Sildenafil (Rep 2)		Analyte							
10 Spk Coffee 20 ppb Sildenafil (Rep 3)		Analyte							
11 Spk Coffee 20 ppb Sildenafil (Rep 4)		Analyte							
12 Spk Coffee 20 ppb Sildenafil (Rep 5)		Analyte							
13 Spk Coffee 20 ppb Sildenafil (Rep 6)		Analyte							
14 Spk Coffee 20 ppb Sildenafil (Rep 7)		Analyte							
15 Mobile Phase		Blank							
16 Spk Coffee 20 ppb Homosildenafil		Standard	0.020	5.93	199.386		199.386	0.0	5.7
17 Spk Coffee 50 ppb Homosildenafil		Standard	0.050	5.84	414.017		414.017	0.0	-12.4
18 Spk Coffee 100 ppb Homosildenafil		Standard	0.100	5.84	1017.185		1017.185	0.1	7.6
19 Spk Coffee 200 ppb Homosildenafil		Standard	0.200	5.86	1868.058		1868.058	0.2	-1.3
20 Mobile Phase		Blank							
21 Blank Coffee		Analyte							
22 Mobile Phase		Blank							
23 Spk Coffee 20 ppb Homosildenafil (Rep 1)		Analyte		5.91	164.410		164.410	0.0	
24 Spk Coffee 20 ppb Homosildenafil (Rep 2)		Analyte		5.86	201.814		201.814	0.0	
25 Spk Coffee 20 ppb Homosildenafil (Rep 3)		Analyte		5.84	191.739		191.739	0.0	
26 Spk Coffee 20 ppb Homosildenafil (Rep 4)		Analyte		5.86	208.012		208.012	0.0	
27 Spk Coffee 20 ppb Homosildenafil (Rep 5)		Analyte		5.91	216.355		216.355	0.0	
28 Spk Coffee 20 ppb Homosildenafil (Rep 6)		Analyte		5.84	100.019		100.019	0.0	
29 Spk Coffee 20 ppb Homosildenafil (Rep 7)		Analyte		5.98	75.067		75.067	0.0	

Compound name: Homosildenafil

Correlation coefficient: $r = 0.997164$, $r^2 = 0.994337$ Calibration curve: $9463.04 * x + -0.669378$

Response type: External Std, Area

Curve type: Linear, Origin: Include, Weighting: 1/x, Axis trans: None



Compound name: Sildenafil

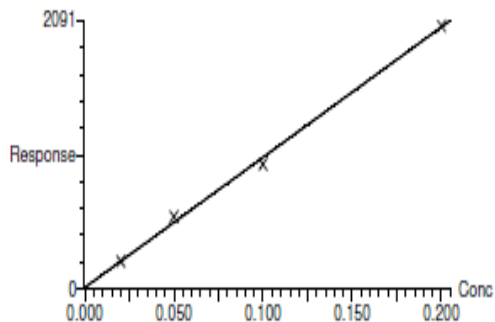
# Sample Text	ID	Type	Std. Conc	RT	Area	IS Area	Response	ppm	%Dev
1 Spk Coffee 20 ppb Sildenafil		Standard	0.020	5.61	215.487		215.487	0.0	1.1
2 Spk Coffee 50 ppb Sildenafil		Standard	0.050	5.61	562.743		562.743	0.1	8.8
3 Spk Coffee 100 ppb Sildenafil		Standard	0.100	5.58	972.485		972.485	0.1	-5.3
4 Spk Coffee 200 ppb Sildenafil		Standard	0.200	5.61	2049.435		2049.435	0.2	0.3
5 Mobile Phase		Blank							
6 Blank Coffee		Analyte							
7 Mobile Phase		Blank							
8 Spk Coffee 20 ppb Sildenafil (Rep 1)		Analyte		5.65	239.771		239.771	0.0	
9 Spk Coffee 20 ppb Sildenafil (Rep 2)		Analyte		5.65	217.051		217.051	0.0	
10 Spk Coffee 20 ppb Sildenafil (Rep 3)		Analyte		5.61	205.436		205.436	0.0	
11 Spk Coffee 20 ppb Sildenafil (Rep 4)		Analyte		5.71	180.015		180.015	0.0	
12 Spk Coffee 20 ppb Sildenafil (Rep 5)		Analyte		5.71	213.901		213.901	0.0	
13 Spk Coffee 20 ppb Sildenafil (Rep 6)		Analyte		5.58	182.789		182.789	0.0	
14 Spk Coffee 20 ppb Sildenafil (Rep 7)		Analyte		5.71	204.902		204.902	0.0	
15 Mobile Phase		Blank							
16 Spk Coffee 20 ppb Homosildenafil		Standard	0.020						
17 Spk Coffee 50 ppb Homosildenafil		Standard	0.050						
18 Spk Coffee 100 ppb Homosildenafil		Standard	0.100						
19 Spk Coffee 200 ppb Homosildenafil		Standard	0.200						
20 Mobile Phase		Blank							
21 Blank Coffee		Analyte							
22 Mobile Phase		Blank							
23 Spk Coffee 20 ppb Homosildenafil (Rep 1)		Analyte							
24 Spk Coffee 20 ppb Homosildenafil (Rep 2)		Analyte							
25 Spk Coffee 20 ppb Homosildenafil (Rep 3)		Analyte							
26 Spk Coffee 20 ppb Homosildenafil (Rep 4)		Analyte							
27 Spk Coffee 20 ppb Homosildenafil (Rep 5)		Analyte							
28 Spk Coffee 20 ppb Homosildenafil (Rep 6)		Analyte							
29 Spk Coffee 20 ppb Homosildenafil (Rep 7)		Analyte							

Compound name: Sildenafil

Correlation coefficient: $r = 0.998574$, $r^2 = 0.997151$ Calibration curve: $10162.5 * x + 10.0105$

Response type: External Std, Area

Curve type: Linear, Origin: Include, Weighting: 1/x, Axis trans: None



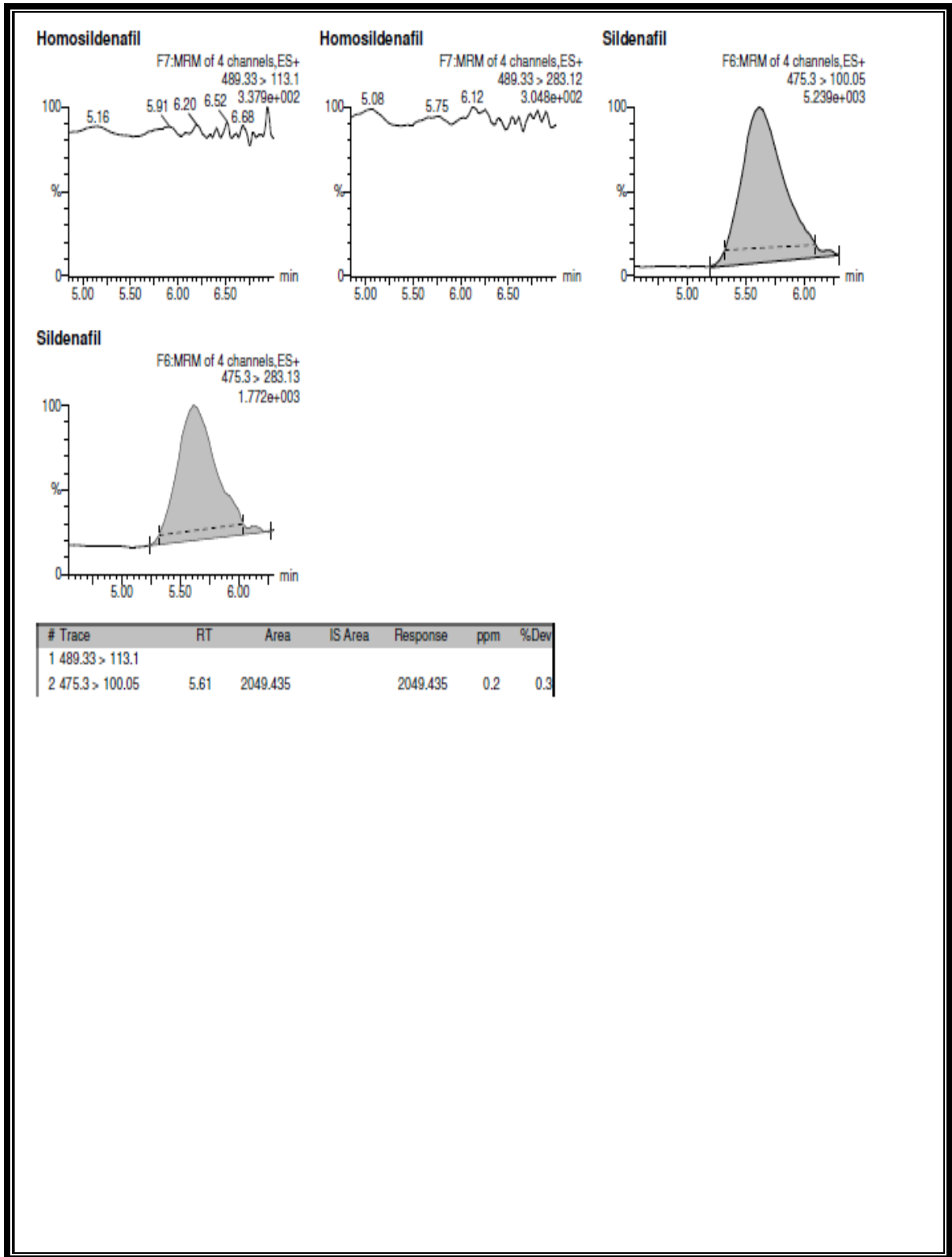


Figure B.3: Validation Study in Coffee Matrix

Compound name: Piperacetildenafil

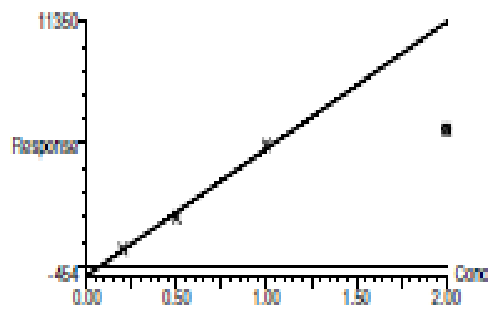
# Sample Text	ID	Type	Std. Conc	RT	Area	IS Area	Response	ppm	%Dev
1 Spk Coffee Piperacetildenafil 20ppb		Standard	0.200	4.12	783.762		783.762	0.2	5.1
2 Spk Coffee Piperacetildenafil 50ppb		Standard	0.500	4.12	2048.789		2048.789	0.5	-8.2
3 Spk Coffee Piperacetildenafil 1000ppb		Standard	1.000	4.08	5613.912		5613.912	1.0	3.1
4 Spk Coffee Piperacetildenafil 2000ppb		Standard	2.000	4.12	6345.198		6345.198	1.2	-42.3
5 Mobile Phase		Blank							
6 Spk Coffee Piperacetildenafil 20ppb (Rep 1)		Analyte		4.12	199.727		199.727	0.1	
7 Spk Coffee Piperacetildenafil 20ppb (Rep 2)		Analyte		4.12	172.796		172.796	0.1	
8 Spk Coffee Piperacetildenafil 20ppb (Rep 3)		Analyte		4.12	146.005		146.005	0.1	
9 Spk Coffee Piperacetildenafil 20ppb (Rep 4)		Analyte		4.08	120.368		120.368	0.1	
10 Spk Coffee Piperacetildenafil 20ppb (Rep 5)		Analyte		4.17	255.213		255.213	0.1	
11 Spk Coffee Piperacetildenafil 20ppb (Rep 6)		Analyte		4.17	78.329		78.329	0.1	
12 Spk Coffee Piperacetildenafil 20ppb (Rep 7)		Analyte		3.98	73.888		73.888	0.1	

Compound name: Piperacetildenafil

Correlation coefficient: $r = 0.995840$, $r^2 = 0.991698$ Calibration curve: $5887.13 * x + -453.867$

Response type: External Std, Area

Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



APPENDIX B

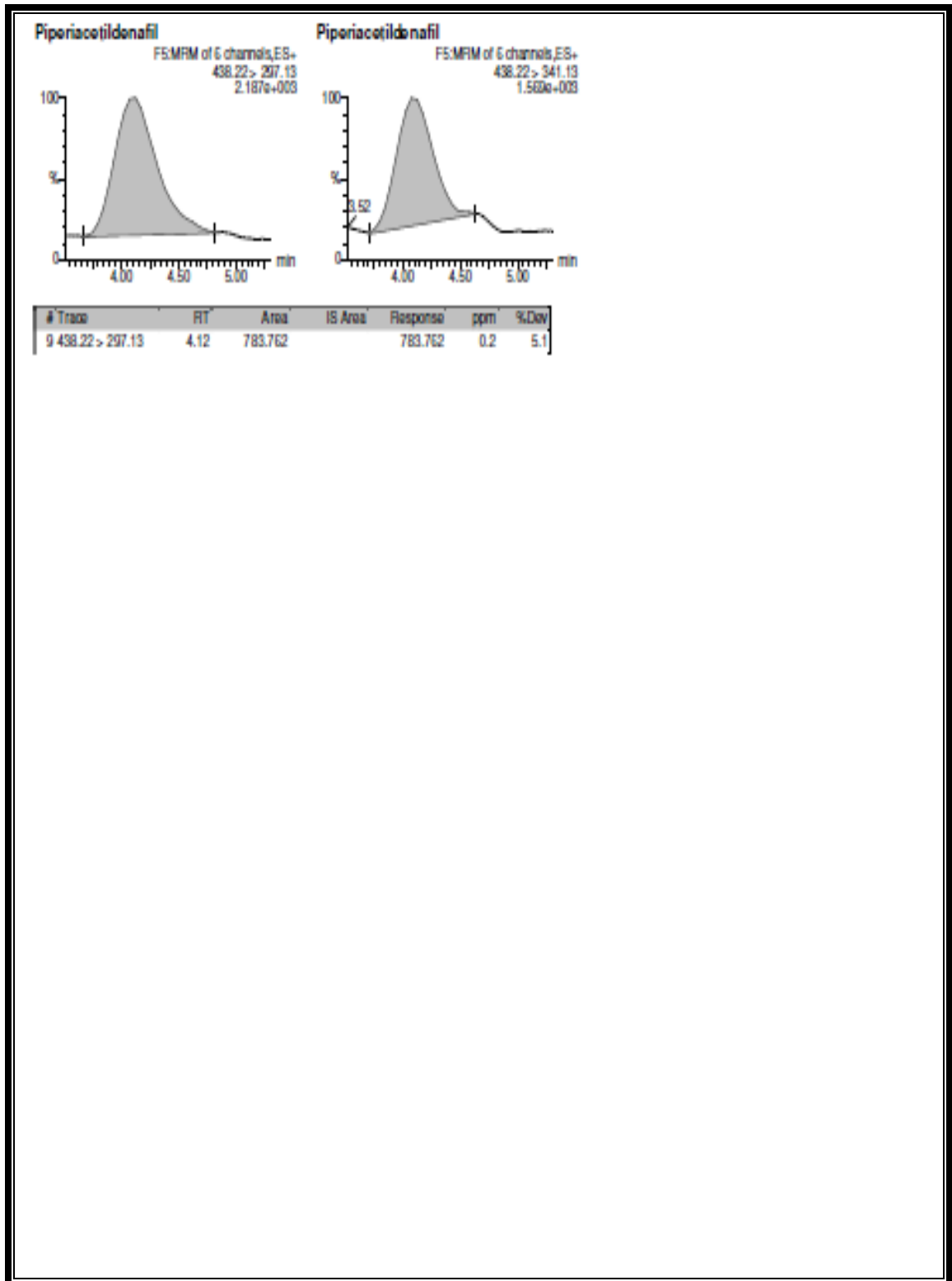


Figure B.4: Validation Study in Coffee Matrix-Piperacetildenafil

Determination of Sildenafil, Vardenafil and Tadalafil and their analogues in adulterated herbal medicinal preparations, herbal vitality products, supplements and foods and beverages by High Performance Liquid Chromatograph Tandem Mass Spectrometry (LC-MS/MS)

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HYDROXYTHIOHOMOSILDENAFIL

Expt: (ug/mL) Injected Volume:	Herbal 0.010
	0.009
	0.010
	0.010
	0.010
	0.010
	0.010
	0.010
	0.010
Mean	0.0099
Mean Recovery	0.9857
Std Dev	0.0004
R.S.D	0.0383
n	7.0

Mean Observation, C obs :	0.0099
Recovery, Rm :	0.9857

Standard Deviation, s :	0.0004
bias (1-Rm)	0.0143

The uncertainty μR_m due to method recovery R_m can be calculated from this formula :

$$uR_m = R_m \times \sqrt{\left(\frac{s^2}{n \times C_{obs}^2}\right) + \left(\frac{\mu C_{spike}}{C_{spike}}\right)^2} \rightarrow \text{ignored}$$

u(Rm)	0.014286
Significant testing :	
Calculated t-value, 1-Rm /u(Rm)	1.000000
<p>the calculated t-value is more than 2, the coverage factor. therefore, Rm is significant different from 1 correction of the expt value is necessary, u(Rm) is</p>	

<p>Correction uncertainty, $\bar{uR}_m = \frac{\bar{uR}_m}{R_m}$</p>

Figure C.1: Measurement Uncertainty-Recovery of Hydroxythiohomosildenafil

Determination of Sildenafil, Vardenafil and Tadalafil and their analogues in adulterated herbal medicinal preparations, herbal vitality products, supplements and foods and beverages by High Performance Liquid Chromatograph Tandem Mass Spectrometry (LC-MS/MS)

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HYDROXYTHIOHOMOSILDENAFIL

Expt: (ug/mL)	Coffee	Herbal	Candy
Injected Volume:	0.020	0.010	0.010
	0.020	0.009	0.010
	0.021	0.010	0.010
	0.020	0.010	0.010
	0.023	0.010	0.010
	0.020	0.010	0.011
	0.018	0.010	0.009
	0.020	0.010	0.009
Mean	0.0203	0.0099	0.0099
Mean Recovery	1.0143	0.9857	0.9857
Std Dev, u(Rs)	0.0165		
n	7	7	7

$$uR = \sqrt{u(Rm)^2 + u(Rs)^2}$$

$$RSD_{pooled} = \sqrt{\left(\frac{(n_1 - 1) \times RSD_1^2 + (n_2 - 2) \times RSD_2^2 + \dots}{(n_1 - 1) + (n_2 - 1) + \dots} \right)}$$

	std relative uncertainty
--	---------------------------------

u(Rm)	0.01429
u(Rs)	0.01650
combined relative uncertainty	0.0218

Figure C.2: Measurement Uncertainty-Sample Effect of Hydroxythiohomosildenafil

Determination of Sildenafil, Vardenafil and Tadalafil and their analogues in adulterated herbal medicinal preparations, herbal vitality products, supplements and foods and beverages by High Performance Liquid Chromatograph Tandem Mass Spectrometry (LC-MS/MS)

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Precision study**HYDROXYTHIOHOMOSILDENAFIL**

N	0.2ug/ml
1	0.208
2	0.207
3	0.209
4	0.200
5	0.208
6	0.194
7	0.227
8	0.220
9	0.210
10	0.203
Mean	0.2030
std Dev	0.0094
n	10
RSD	0.0462

$$RSD_{pooled} = \sqrt{\frac{(n_1 - 1) \times RSD_1^2 + (n_2 - 2) \times RSD_2^2 + \dots}{(n_1 - 1) + (n_2 - 1) + \dots}}$$

Parameter	Description	Value,x	Std uncertainty, (u(x))	Rel Std uncertainty(ux/x)
P	Precision	1	0.0462	0.0462

Rec	Recovery	1	0.0218	0.0218
Expanded uncertainty (k)	K	2		
Combined relative uncertainty				0.05111

$$u[\text{C Hydroxythiohomo}]/[\text{C Hydroxythiohomo}] = 0.05111 \quad (\text{Relative value})$$

The uncertainty of $_{\text{Hydroxythiohomo}}[\text{C Hydroxythiohomo}] = \frac{0.05}{\text{[Hydroxythiohomo]}} \times [\text{C Hydroxythiohomo}] = \frac{0.05111 \times \text{C}}{\text{[Hydroxythiohomo]}}$

At 95% Confidence level k=2 therefore expanded uncertainty, $U [\text{C}_{\text{Hydroxythiohomo}}] = u [\text{C}_{\text{Hydroxythiohomo}}] \times 2 =$

Example at 0.01 ppm = 0.01 ± 0.00102

Figure C.3: Measurement Uncertainty-Precision of Hydroxythiohomo



Figure D.1: Positive Samples