Supelco®
Analytical Products

Application Note

LC-MS analysis of plasma samples using Supel™ Swift HLB SPE cartridges

Introduction:

The preparation of biological samples can have a large impact on the reproducibility and confidence in the results[1]. Solid phase extraction (SPE) provides the opportunity to reduce matrix effects such as ion suppression to aid in the reliability of consistent results. Hydrophilic-Lipophilic Balanced (HLB) cartridges contain a sorbent which offers good wettability for hydrophilic compounds and the ability to provide reverse phase retention with the lipophilic phase[2]. These properties allow HLB cartridges to effectively handle a broad range of compounds with varying properties. In this study, a mixture of twenty compounds with ranging log P values of (-0.89 to 4.65) were analyzed from calf serum using a *Supel™ Swift HLB SPE cartridge*.

Experimental

A series of twenty analytes and sixteen internal standards as listed in **Tables 1** and **2** were spiked into calf-serum and allow to equilibrate for an hour. The calf-serum sample was diluted with an equal volume of 0.4% aqueous formic acid and mixed before the sample was loaded onto the *Supel™ Swift HLB SPE cartridges* (1 mL/30 mg), or a commercially available HLB cartridge (1 mL/30 mg).

Table 1. The twenty analytes analyzed in calf-serum by LC-MS/MS

	Analyte	Usage	Log P	Quantitative Transition	Qualitative Transition	Retention Time (min)	Collision Energy (V)	
1	Nizatidine	antacid	0.77	332.1/155.1	332.1/131.1	1.36	23.4	25.7
2	Amiloride	diuretic	-0.89	230.0/171.0	230.0/115.9	2.80	21	43
3	Benzoylecgonine	cocaine metabolite	-0.59	290.1/168.0	290.1/105.0	3.28	26.2	42
4	Imidacloprid	insecticide	0.87	256.0/208.9	256.0/175.0	4.16	22.3	22.4
5	Mirtazapine	antidepressant	3.21	266.2/195.1	266.2/166.9	4.60	33.9	60
6	Nevirapine	HIV antiviral	2.49	267.1/226.1	267.1/107.1	4.98	35.9	39.1
7	Methapyrilene	antihistamine	3.11	262.2/217.0	262.2/107.1	4.99	17	36
8	Imiquimod	anti-tumor	2.65	241.1/185.1	241.1/167.9	5.16	33.3	43.7
9	Buspirone	anxiolytic	1.78	386.2/122.0	386.2/95.1	5.36	39	65
10	Hydroquinidine	antiarrhythmic	2.82	327.3/172.2	327.3/160.2	5.40	52	42
11	Mesoridazine	neuroleptic drug	3.57	387.1/98.2	387.1/126.2	5.72	52	34
12	Mianserin	antihistamine	3.83	265.2/208.2	265.2/193.1	6.23	28.1	49.7
13	Haloperidol	antipsychotic	3.66	367.1/123.2	367.1/165.2	6.80	58.6	31.7
14	Imipramine	antidepressant	4.28	281.1/86.0	281.1/165.1	6.93	22	83
15	Atrazine	herbicide	2.2	216.1/174.0	216.1/68.0	7.13	22.4	50.4
16	Amitriptyline	antidepressant	4.81	278.1/191.0	278.1/233.2	7.17	31.8	22.5
17	Clarithromycin	antibiotic	3.24	748.5/590.1	748.5/158.0	7.50	24	32
18	Losartan	antihypertensive	4.06	423.2/207.2	423.2/235.2	7.50	33	22.7
19	Nefazodone	antidepressant	4.65	470.2/274.2	470.2/246.2	7.91	39.8	47.8
20	Loratadine	antihistamine	4.55	383.3/337.2	383.1/267.2	10.51	30	41.6

Table 2. The 16 internal standards analytes analyzed in calf-serum by LC-MS/MS

	Internal Standard Analyte	Corresponding Analyte	Monitored Transition	Retention Time (min)	Collision Energy (V)
1	Not Applicable	**Nizatidine**	-	-	-
2	5-(N,N-dimethyl) amiloride	**Amiloride**	258.0/199.0	5.24	23
3	Benzoylecgonine-D3	**Benzoylecgonine**	293.1/171.0	3.28	26.2
4	Imiacloprid-D4	**Imidacloprid**	260.0/213.0	4.16	22.3
5	N-desmethylmirtazapine	**Mirtazapine**	252.1/195.1	4.6	29
6	Abacavir	**Nevirapine**	287.2/191.1	3.56	26
7	Methapyrilene-dimethyl-D6	**Methapyrilene**	268.2/217.0	4.99	17
8	Not Applicable	**Imiquimod**	-	-	-
9	Buspirone-D8	**Buspirone**	384.2/122.0	5.36	39
10	Quinine	**Hydroquinidine**	325.2/172	5.12	52
11	Chlorpromazine	**Mesoridazine**	319.1/246.0	7.6	37
12	Mianserin-D3	**Mianserin**	268.2/208.2	6.23	28.1
13	Haloperidol-D4	**Haloperidol**	380.1/127.2	6.9	31.7
14	Imipramine-D3	**Imipramine**	284.1/89.0	6.93	22
15	Atrazine-D5	**Atrazine**	221.1/179.0	7.14	22.4
16	Amitriptyline-D3	**Amitriptyline**	281.1/191.0	7.17	31.8
17	Not Applicable	**Clarithromycin**	-	-	-
18	Not Applicable	**Losartan**	-	-	-
19	1-(3-Chlorophenyl)piperazine-D8 MCPP-D8	**Nefazodone**	205.2/157.8	4.21	37
20	Loratadine-D5	**Loratadine**	388.1/337.2	10.51	30

Recovery and Ion Suppression/Enhancement

The analytes were monitored at two different transitions, a quantifier and a qualifier (conformation), by a scheduled MRM method. Samples were analyzed against matrix-matched calibration curves. The external calibration used six concentrations of the analytes between 20 to 150 ng/mL and a fixed concentration of 50 ng/mL of the internal standards when applicable. Analytes were spiked in triplicates into calf-serum at concentrations of 100 ng/mL with a fixed internal standard of 50 ng/mL before processing by either the 3-step or 5-step method as shown in **Figure 1**. The samples were dried and resuspended in starting mobile phase before analysis in duplicates on an Agilent 1290 Infinity LC attached to a Sciex 3200 Q trap LC-MS/MS. The LC conditions including the starting mobile phase are listed in **Table 3**.

Figure 1. Processing of samples with the *Supel™ Swift HLB SPE cartridges* (30 mg/1mL) using the 5-step method and the 3-step method.

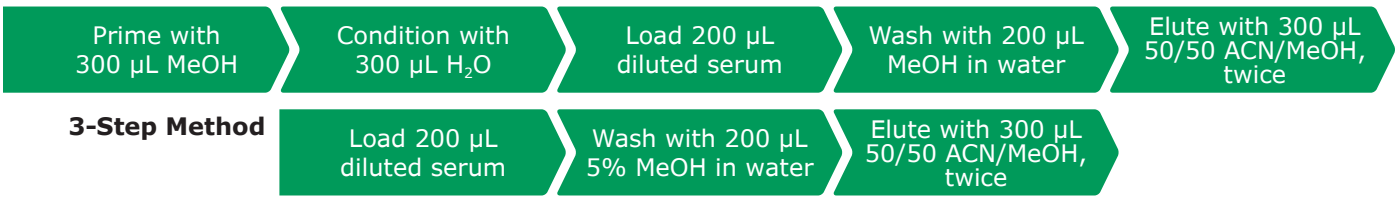


Table 3. LC-MS/MS analysis conditions for Recovery and Ionization Effects

Column	Ascentis® Express Phase RP-Amide 2.7 µm particle size 10 cm x 2.1 cm
Mobile Phase	[A] 5 mM ammonium acetate, 0.1% acetic acid in water [B] 5 mM ammonium acetate, 0.1% acetic acid in 95% acetonitrile and 5% water
Gradient	95% A, 5% B held for 1 min; to 43% B in 6 min; held at 43% B for 1 min; to 61.5% B in 2 min, to 95% A and 5% B in 1 min, held for 3 mins.
Flow Rate	0.4 mL/min
Column Temp	40 °C
Detector	MS, ESI(+) Scheduled MRM (See **Table 1** and **2**)
Injection	2 µL

Phospholipid Removal in Samples

Diluted calf serum with 0.4% formic acid was processed as described earlier by the 5-step process in triplicate. The samples were dried and resuspended in the starting mobile phase before submitting in duplicate.

For the comparison of no SPE cleanup, undiluted calf serum was mixed with three volume equivalents of acetonitrile and allowed to sit for 20 minutes before separation of the precipitated proteins and phospholipids by centrifugation. The samples conducted in triplicate were dried and resuspend in the starting mobile phase at two times the starting volume to equal the concentration of the diluted calf serum before submitting in duplicate.

The list of the fourteen phospholipids and their transitions are in **Table 4**. **Table 5** lists the liquid chromatography conditions. A representative chromatogram of analytes and internal standards is depicted in **Figure 5**.

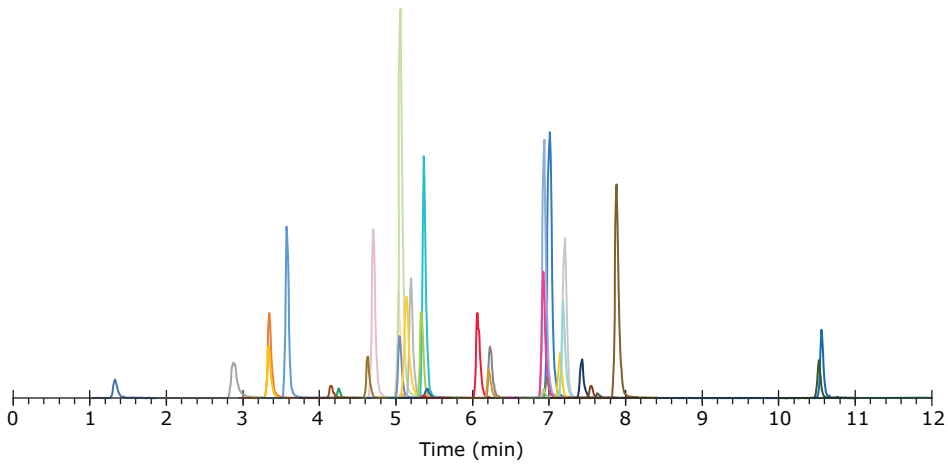
Table 4. Phospholipids monitored

Phospholipid	Precursor	Product	Dwell Time (msec)	Collison Energy (V)
1	184	104	65	80
2	496	184	65	80
3	524	184	65	80
4	704	184	65	80
5	758	184	65	80
6	786	184	65	80
7	806	184	65	80
8	520.4	184	65	80
9	522.4	184	65	80
10	782.6	184	65	80
11	810.7	184	65	80
12	760.4	184	65	80
13	784.4	184	65	80
14	808.4	184	65	80

Table 5. LC-MS/MS analysis conditions for Phospholipid Removal

Column:	Ascentis® Express C18 2.7 µM particle size, 5 cm x 2.1 mm
Mobile Phase:	[A] 5 mM ammonium formate in 90:10 (v/v) methanol/water
Run Time:	Isocratic for 30 minutes
Flow Rate:	0.5 mL/min
Column Temp:	40 °C
Detector:	MS, ESI(+) Scheduled MRM (See **Table 3**)
Injection:	2 µL

Figure 5. Representative chromatogram of analytes and internal standards for 5-Step Recovery showing individual analytes and internal standards.



Results & Discussion

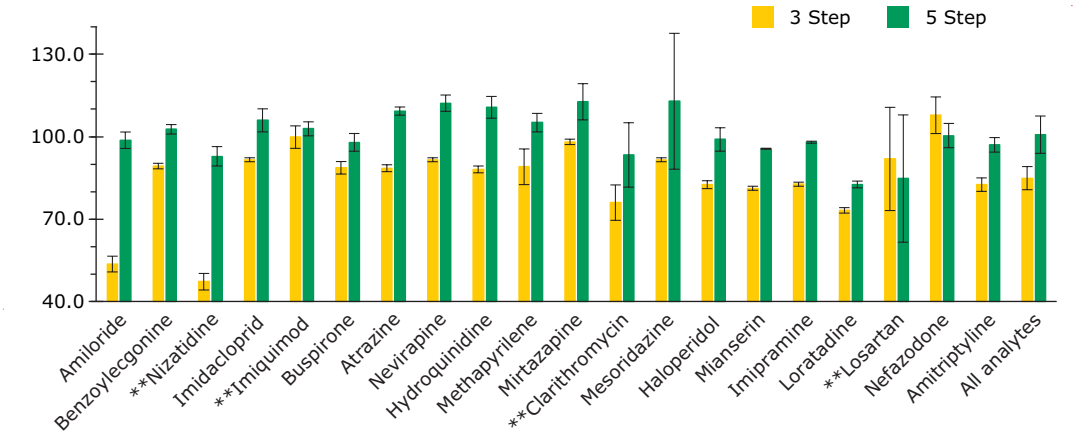
The percent recovery from both the 3-Step and 5-Step method of the *Supel™ Swift HLB SPE cartridges* are presented in **Table 6** and **Figure 2**. Overall, the 5-Step (100.7 ± 6.8%) had a slightly better recovery compared to the 3-Step process (85.1 ± 4.2%) by in part as a result of two earlier eluting compounds (amiloride and nizatidine, both below 70% recovery in 3-step method). Under the 5-Step process, all twenty analytes had recoveries between 80% and 120%. Eighty percent (16 of 20) of the analytes using the 3-Step process achieved recoveries in the 80% to 120% range.

Table 6. Summary of Recovery Results using the *Supel™ Swift HLB SPE cartridges*.

	5 Step		3 Step	
	AVE	RSD	AVE	RSD
Amiloride	98.7	3.0	53.6	2.9
Benzoylecgonine	102.7	1.7	89.3	1.0
*** Nizatidine**	92.9	3.5	47.2	3.0
Imidacloprid	105.9	4.2	91.6	0.7
*** Imiquimod**	102.9	2.5	96.9	4.1
Buspirone	97.9	3.2	88.7	2.3
Atrazine	109.3	1.5	88.5	1.3
Nevirapine	112.2	3.0	98	3.4
Hydroquinidine	110.7	4.0	88.1	1.2
Methapyrilene	105.1	3.4	89	6.5
Mirtazapine	112.7	6.6	98.1	1.0
***Clarithromycin**	93.4	11.7	76	6.4
Mesoridazine	112.9	24.7	91.6	0.7
Haloperidol	99	4.3	82.5	1.4
Mianserin	95.6	0.2	81.2	0.7
Imipramine	98	0.4	82.7	0.7
Loratadine	82.6	1.2	73.2	1.0
***Losartan**	84.8	23.2	91.9	18.8
Nefazodone	100.4	4.4	107.8	6.7
Amitriptyline	97	2.6	82.5	2.5
All analytes	100.7	6.8	85.1	4.2

*No internal standards used, represent absolute recoveries.

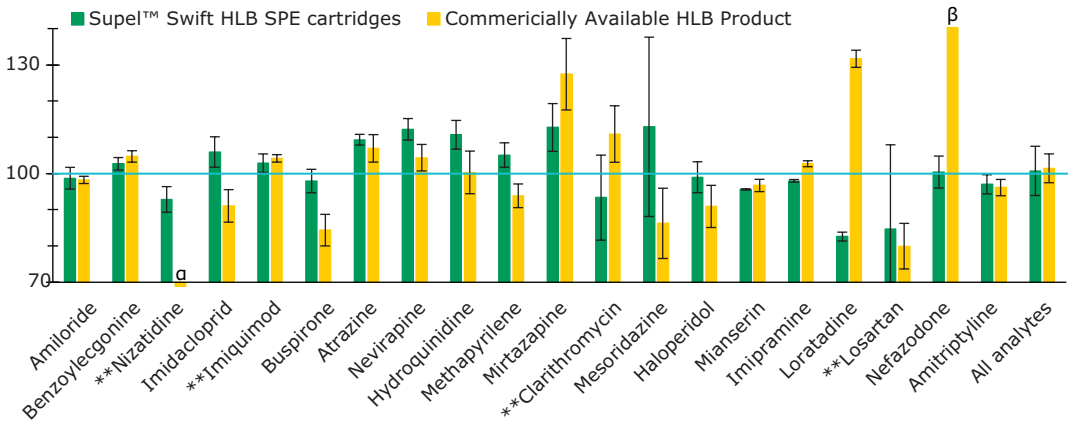
Figure 2: Summary of Recovery for the 3-Step and 5-Step Process using *Supel™ Swift HLB SPE cartridges*. Analytes are ordered by increasing log P values.



** Analytes did not have an internal standard.

In addition to evaluating the performance of the *Supel™ Swift HLB SPE cartridges*, there was interest in comparing the performance with a commercially available HLB cartridge. Both cartridges contained 30 mg of resin and had a max sample volume of 1 mL. To compare the effectiveness of these cartridges, the 5-Step method was employed under the same set of analytes and conditions. **Figure 3** and **Table 7** represent the recovery using the two cartridges. As previously mentioned, 100% of the analytes had recovery in the range of 80-120% using the *Supel™ Swift HLB SPE cartridges*. This is in contrast with the other commercially available cartridge, in which, only 80% (16 of 20 analytes) were recovered in the 80-120%.

Figure 3. Percent Recovery of 5-Step Method for *Supel™ Swift HLB SPE cartridges* and a commercially available HLB product. Analytes are in order of increasing log P values.



** Analytes did not have an internal standard.

α/β Commercially Available HLB (not shown, off scale): Nizatidine: 18.7 ± 1.3% and Nefazodone: 199.4 ± 16.8%

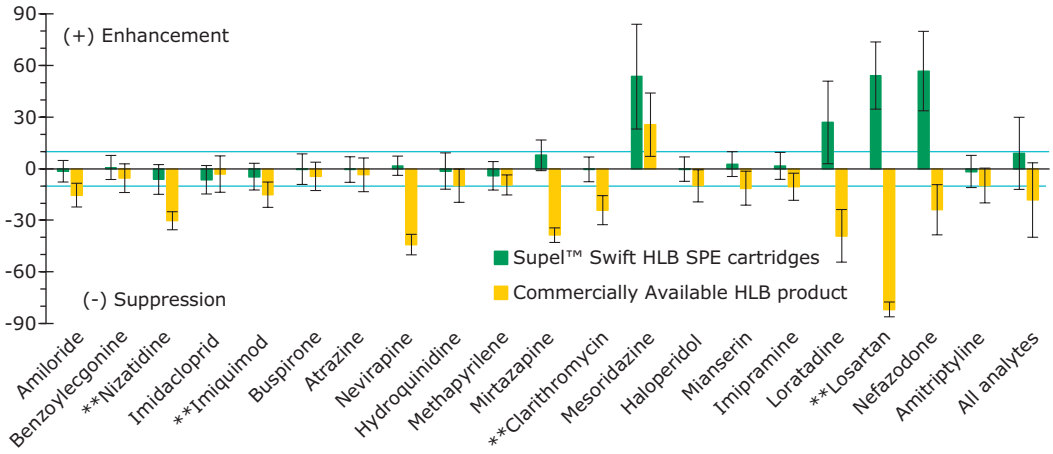
Table 7. Percent Recovery of 5-Step Method for *Supel™ Swift HLB SPE cartridges* and a commercially available HLB product. Analytes in order of log P value.

	Supel™ Swift HLB SPE cartridges		Commercially Available HLB product	
	AVE	RSD	AVE	RSD
Amiloride	98.7	3	98.2	1.0
Benzoylecgonine	102.7	1.7	104.7	1.6
***Nizatidine**	92.9	3.5	18.7	1.3
Imidacloprid	105.9	4.2	91.1	4.5
***Imiquimod**	102.9	2.5	104.2	1.0
Buspirone	97.9	3.2	84.4	4.4
Atrazine	109.3	1.5	107.0	3.8
Nevirapine	112.2	3	104.4	3.7
Hydroquinidine	110.7	4	100.3	5.9
Methapyrilene	105.1	3.4	93.9	3.3
Mirtazapine	112.7	6.6	127.4	9.9
***Clarithromycin**	93.4	11.7	110.9	7.8
Mesoridazine	112.9	24.7	86.2	9.7
Haloperidol	99	4.3	90.9	5.8
Mianserin	95.6	0.2	96.7	1.7
Imipramine	98	0.4	102.7	0.8
Loratadine	82.6	1.2	131.7	2.4
***Losartan**	84.8	23.2	80.0	6.3
Nefazodone	100.4	4.4	199.4	16.8
Amitriptyline	97	2.6	96.1	2.3
All analytes	100.7	6.8	101.4	4.0

*No internal standards used, represent absolute recoveries.

Beyond recovery rates, another important factor to consider when performing SPE is the impact on ion suppression and/or ion enhancement. This ion suppression/enhancement is an indication of how well the cartridge removes matrix components (in this case from calf-serum plasma). As seen in **Figure 4**, for 80% (16 of 20) analytes processed with *Supel™ Swift HLB SPE cartridges* had minimal impact on the ionization (+/- 10%) of the analytes. For the remaining 20% (4 of 20) analytes, an ion enhancement and no ion suppression were observed. This is in stark contrast to another commercially available cartridge where 70% (14 of 20) of analytes had an ionization suppression (13 of the 14) or enhancement (1 of 14) of more than 10%. The *Supel™ Swift HLB SPE cartridges* showed less ion suppression/enhancement when compared to other commercially available cartridges.

Figure 4. Signal suppression or enhancement effects for *Supel™ Swift HLB SPE cartridges* and another commercially available HLB product using the 5-Step method. Analytes are arranged in order of their increasing log P values.



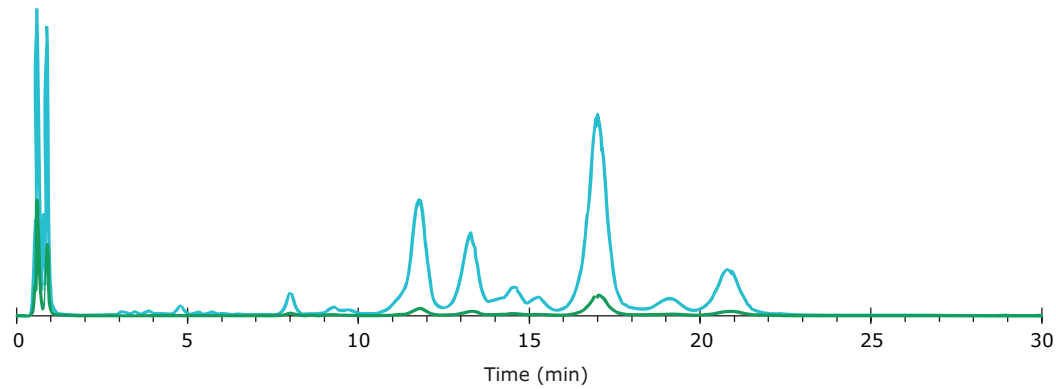
** Analytes did not have an internal standard.

A final investigation of the two cartridges was conducted to determine the amounts of phospholipids that were removed during SPE cleanup procedure in comparison to protein precipitation using acetonitrile. The *Supel™ Swift HLB SPE cartridges* showed similar removal of phospholipids (91.8%) compared to the commercially available cartridge (89.5%) as stated in **Table 8** and depicted in **Figure 6**.

Table 8. Percent of phospholipids removed for 5-Step process for the *Supel™ Swift HLB SPE cartridges* and another commercially available HLB product compared to acetonitrile precipitation

	Supel™ Swift HLB SPE cartridges	Commercially Available HLB product
% Phospholipid Removal	91.8%	89.5

Figure 6. Chromatogram of phospholipid removal compared by either protein precipitation (blue) or by using the *Supel™ Swift HLB SPE cartridges* (green).



Conclusion

Through the use of twenty analytes with various log P values, the *Supel™ Swift HLB SPE cartridges* was demonstrated to produce excellent recoveries (100% of analytes in the range of 80-120%) and minimal ion enhancement effects for 80% of the analytes.

The advantages of the *Supel™ Swift HLB SPE cartridges* was further demonstrated when comparing to a commercially available HLB cartridge under the same set of conditions.

Materials

Product	Description
C-112-1mL	1-(3-Chlorophenyl)piperazine-D8 hydrochloride solution
Z336777	3000 count 1.5 mL microcentrifuge tubes
27001-U	4 mL amber vial
A4562	5-(N,N-dimethyl) amiloride hydrochloride
PHR1256	Abacavir sulfate
45754	Acetic Acid for HPLC
34851	Acetonitrile LC-MS
A-7410	Amiloride
A-8404	Amitriptyline hydrochloride
A-121-1ML	Amitriptyline-D3 hydrochloride solution
73594	Ammonium Acetate for mass spectrometry
70221	Ammonium Formate eluent additive for LC-MS
53913-u	Ascentis Express Phase RP-Amide 2.7 um particle size 10 cm x 2.1 cm
53822-U	Ascentis® Express C18, 2.7 µm HPLC Column
90935	Atrazine
34053-R	Atrazine-D5
B-044-1ML	Benzoylecgonine solution 1 mg/mL
B-008-1ML	Benzoylecgonine-D3
B-055-1ML	Buspirone-D8 hydrochloride solution 100 ug/mL
PHR1038-500MR	Clarithromycin

Product	Description
57059	Disposable Liners for Visprep DL Manifolds, pk of 100
H1512	Haloperidol
H-002	Haloperidol-D4 solution
358343-5G	Hydroquinidine
34170	Imidacloprid-d4
I7379	Imipramine hydrochloride 5g
I-903-1ML	Imipramine-D3 maleate solution
PHR1376	Loratadine
646377	Methanol HPLC Plus
442641	Methapyrilene hydrochloride 1000 mg
M2525	Mianserin Hydrochloride
M-901-1ML	Mianserin-D3 solution 100 ug/mL
M-128	Mirtazapine solution 1 mL of 1 mg/mL
23187-U	MRQ30 Clear CD Vial™, Blue cap, Pre-slit TEF/ Silicone septa, volume 1.2 mL, clear glass vial, thread 9 mm pkg of 100 ea
D-086-1ML	N-Desmethylmirtazapine solution 1 mg/mL
N5536-10MG	Nefazodone Hydrochloride
57146-U	Visiprep Smanifold Replacement Flow Control Valve Stem
57030-U	Visiprep SPE Vacuum Manifold, standard 12-port model

References

1. Andrade-Eiora, A. et al (2016). Solid-phase extraction of organic compounds: A critical review (Part I). Trends in Analytical Chemistry, 641-654. doi:http://dx.doi.org/10.1016/j.track.2015.08.015

2. Michopoulos, F. L. et al (2009). UPLC-MS-Based Analysis of Human Plasma for Metabonomics Using Solvent Precipitation or Solid Phase Extraction. Journal of Proteome Research, 2114-2121. doi:https://doi.org/10.1021/pr801045q

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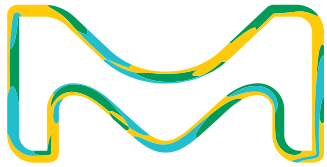

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