Sample Prep for Trace Analysis of Adulterants in Erectile Dysfunction Dietary Supplements

Application Note

Food Testing & Agriculture

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Introduction

The dietary supplement industry is rapidly growing at a 5.5% annual rate with annual sales of almost $5.6 billion for 2012 in the United States alone [1]. This has been largely driven by consumers interested in the health benefits associated with a wide variety of products ranging from dried botanicals to energy drinks fortified with vitamins, minerals, and natural products. Dietary supplements are also under increasing scrutiny as regulatory agencies such as the FDA enforce current good manufacturing practices (cGMPs) to ensure the safety and authenticity of consumable products. With this growing popularity, the number of products containing inauthentic, contaminated, and even pharmaceutically spiked materials has risen dramatically as suppliers seek to improve profit margins [2,3]. Erectile dysfunction (ED) supplements are among the products subject to adulteration by the addition of PDE-5 inhibitor pharmaceuticals such as sildenafil (Viagra), tadalafil (Cialis), vardenafil (Levitra), and ever-increasing numbers of their derivatives [4].

Detecting and measuring analytes of interest in these matrices presents many challenges due to their chemical complexity, and includes the elimination of chemical interferences, reduction in instrument contamination, and accounting for matrix effects. These issues are increasingly relevant for ED supplements that can include a single plant extract or a concoction of ingredients including Muira Puama, Korean ginseng, cinnamon, Ginkgo biloba, cordyceps, and so forth. For these reasons, efficient and comprehensive sample preparation is necessary when determining chemical adulterants at low concentrations. This work investigates the importance of adequate sample preparation for ED supplements in terms of matrix removal for the analysis of PDE-5 inhibitors and chemical adulterants at trace levels using LC/MS/MS instrumentation.

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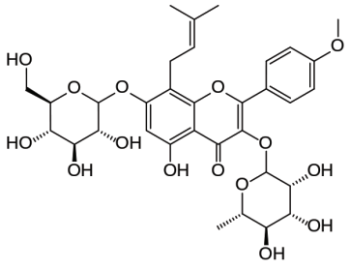
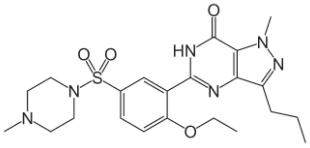
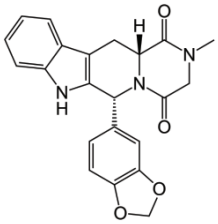
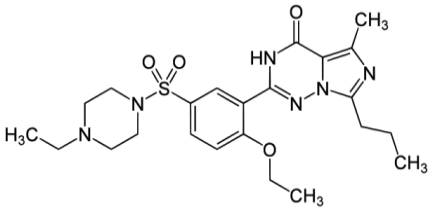
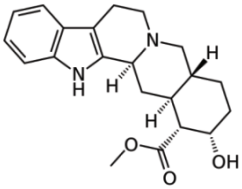
Materials and Methods

Methanol and acetonitrile were LC/MS grade, and water was filtered through a Milli-Q reverse osmosis purifier. Ammonium hydroxide, phosphoric acid, formic acid, and acetic acid were analytical grade. Standards were obtained from VWR International LLC and Sigma-Aldrich Corp. as pure solids, weighed out to 10 mg, dissolved in methanol, and diluted as necessary to make working standards. Samples were procured from local dietary supplement distributors in the form of tablets and capsules. An entire bottle of product was homogenized in a Robot Coupe food processor to ensure a representative sample. Chemical structures and properties of the analytes are given in Table 1.

Table 1. Chemical structures and properties of the analytes.

Name	Structure	LogP*	pKa*
Icariin		0.81	11.8
Sildenafil		1.65	5.97
Tadalafil		1.64	15.21
Vardenafil		1.33	6.21
Yohimbine		2.10	7.65

*values determined using Chemaxon software

Table 4 shows a list of the compounds and their MS/MS parameters.

LC Conditions

Columns:	Agilent Poroshell 120 EC-C18, 2.1 x 100 mm, 2.7 µm (p/n 695775-902), Agilent Poroshell 120 EC-C18, 2.1 × 5 mm, 2.7 µm guard (p/n 821725-911)
Eluent:	A, H_2O + 0.1% formic acid B, methanol + 0.1% formic acid
Injection volume:	10 µL
Flow rate:	0.4 mL/min
Gradient:	10% B for 0 to 0.5 min, then 10% B to 80% B for 0.5 to 9.0 min
Instrument:	Agilent 1290 Infinity LC System and Agilent 6410 Triple Quadrupole Mass Spectrometer

MS Conditions

Gas temperature:	300 °C
Gas flow:	8 L/min
Nebulizer:	35 psi
Capillary voltage:	3,500 V

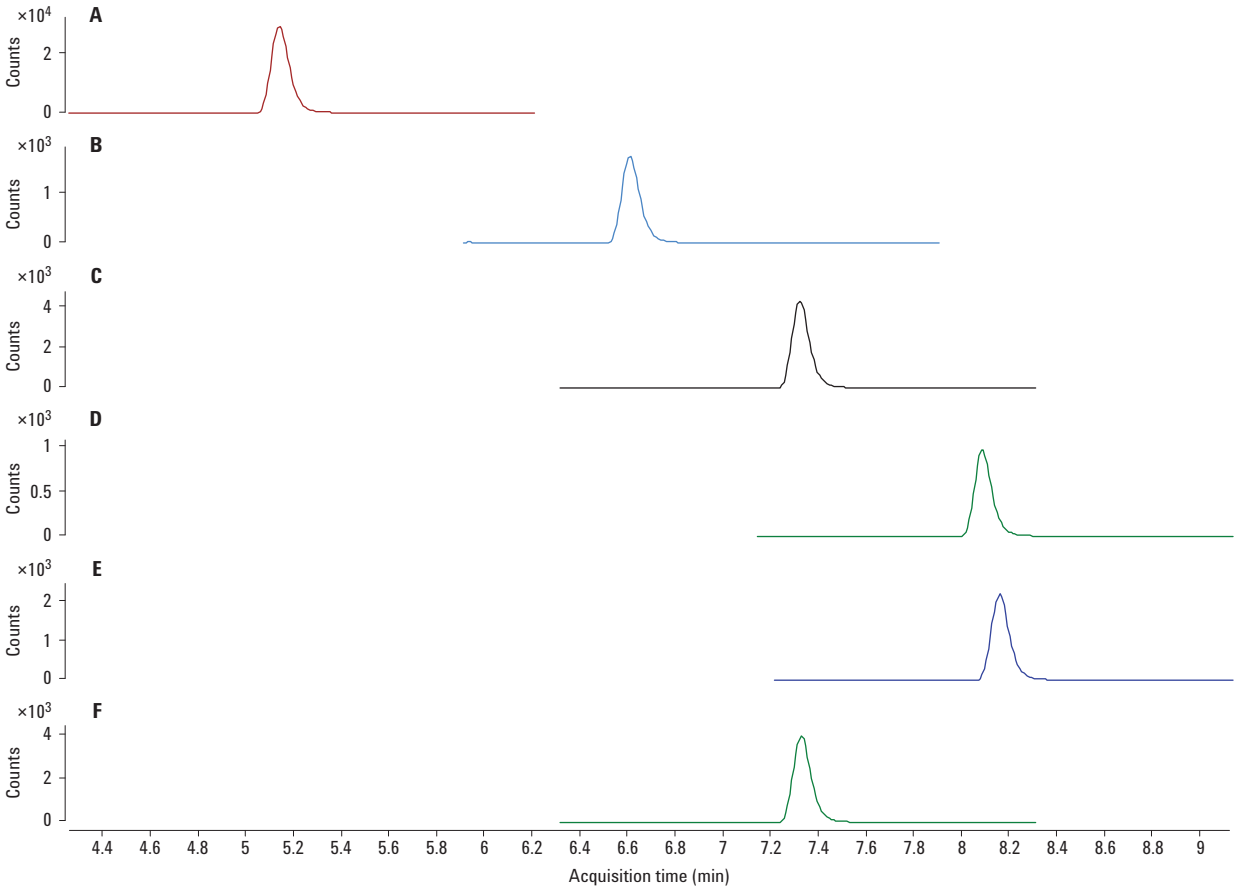


Figure 1. MRM chromatograms of primary transitions for 50 ng/g of A) yohimbine; B) vardenafil; C) sildenafil; D) icariin; E) tadalafil, and F) sildenafil-d8 (IS).

Sample preparation, modified QuEChERS

The first sample prep method used Agilent Bond Elut QuEChERS dispersive SPE for Drug Residues in Meat (p/n 5982-4956) and bulk carbon (p/n 5982-4482) (Figure 2).

Sample preparation, Bond Elut Plexa PC and Captiva

The second sample prep method used Agilent Bond Elut Plexa PCX, 1 mL, 60 mg (p/n 12108601) and Captiva ND 3 mL (p/n A5300063) (Figure 3).

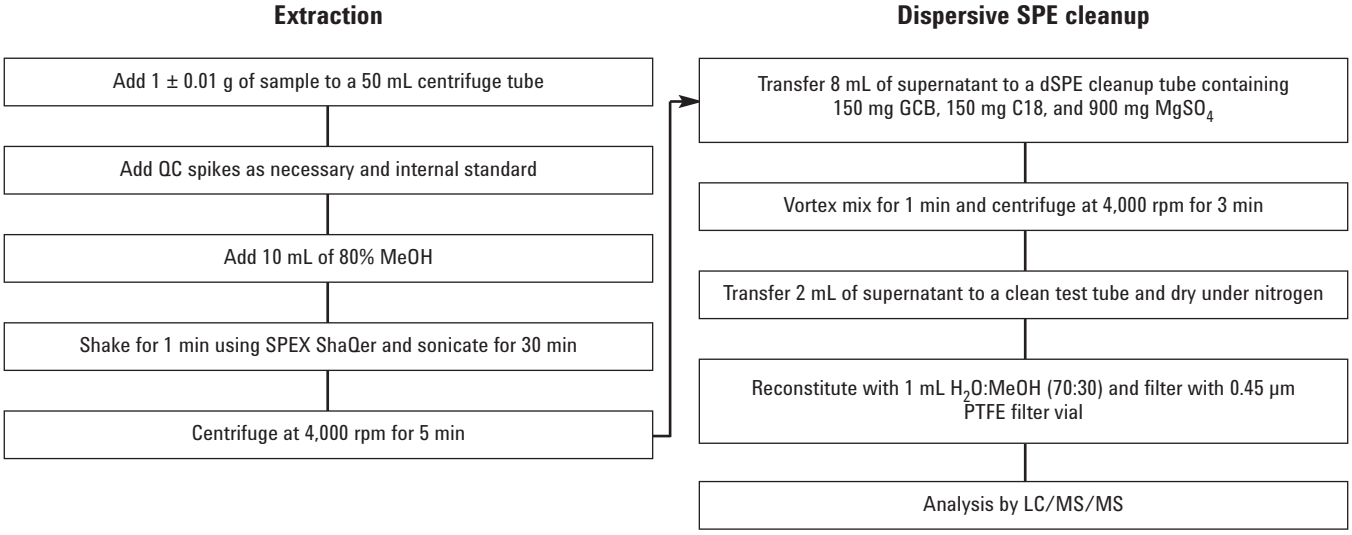


Figure 2. QuEChERS sample preparation workflow.

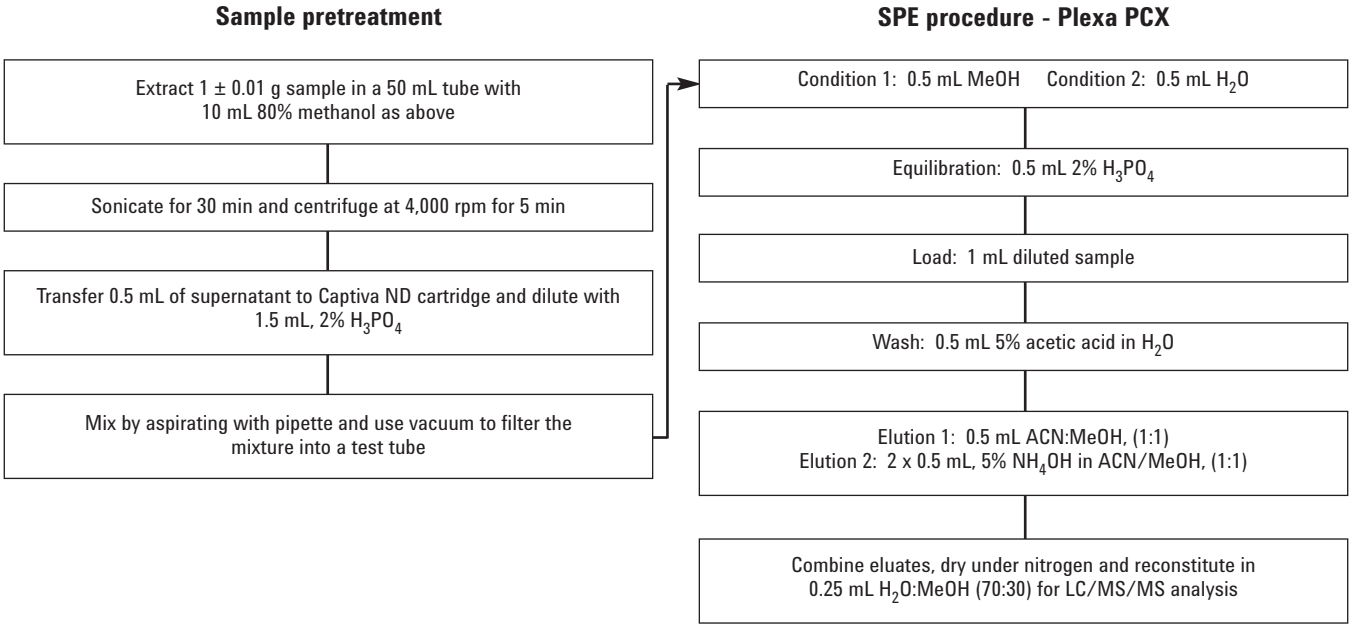


Figure 3. Sample preparation workflow for Agilent Bond Elut Plexa PCX.

Results and Discussion

Botanical dietary supplements are well known for being dirty due to their chemical complexity. Although QuEChERS showed excellent performance with Korean red ginseng, it is regarded as a, just good enough, sample preparation technique, and was unable to meet the lower detection limits in matrices containing multiple ingredients, even with the high sensitivity and selectivity of tandem mass spectrometry. To accommodate these matrices, Bond Elut Plexa PCX was chosen due to its greater analyte specificity and more thorough cleanup, delivering excellent performance for multi-ingredient ED supplements. Quantitation was performed to demonstrate sample preparation performance in terms of analyte recovery and reproducibility.

QuEChERS is traditionally applied to samples for pesticide residue analysis in fruits and vegetables, but the technique is increasingly popular for a number of nontraditional analytes and matrices. The ease of use, speed, and modular steps made QuEChERS a worthwhile sample preparation approach for the analysis of PDE-5 inhibitors. In this study, QuEChERS was modified by using an 80% methanol extraction solvent and by omitting the addition of salts. For cleanup, graphitized carbon black (GCB) was added to the Bond Elut QuEChERS Drug Residues in Meat dispersive kit to facilitate removal of pigments and other interfering compounds. In the Korean ginseng supplement, QuEChERS gave excellent recovery and reproducibility, as shown in Table 2. However, performance using QuEChERS dramatically decreased as the method was unable to detect the majority of these compounds in samples containing multiple ingredients.

Bond Elut Plexa PCX was optimized for matrices found to be unsuitable for QuEChERS and was selected based on the pKa of the analytes. Treatment with phosphoric acid formed the cationic species of the target analytes, resulting in a strong interaction with the stationary phase of the SPE cartridge. Ammonium hydroxide was applied during the elution step, resulting in deprotonation of the analytes of interest and changing their affinity for the stationary phase. Due to the enhanced selectivity of the ion exchange technique, a cleaner sample was achieved. Performance was good, with the exception of icariin as shown in Table 3. The enhanced sample cleanliness can be seen in Figure 4 through the comparison of chromatograms of the blank matrix and the clean sample extract spiked at 20 ppb.

Table 2. Recovery, reproducibility, and linearity of QuEChERS for analytes in Korean red panax ginseng (n = 3).

	20 ppb		50 ppb		100 ppb		
Analyte	**% Recovery**	**% RSD**	**% Recovery**	**% RSD**	**% Recovery**	**% RSD**	**R^2**
Sildenafil	84.1	4.4	91.4	7.4	103.6	3.7	0.9998
Vardenafil	58.5	15.6	80.5	16.6	95.9	11.1	0.9996
Tadalafil	80.2	10.4	85.5	10.6	103.5	4.5	0.9982
Icariin	103.5	5.5	111.8	8.9	127.5	6.4	0.9971
Yohimbine	99.1	5.5	105.8	6.0	115.2	3.4	0.9995

Table 3. Recovery, reproducibility, and linearity of Plexa PCX in complex ED supplement (n = 3).

	20 ppb		50 ppb		100 ppb		
Analyte	**% Recovery**	**% RSD**	**% Recovery**	**% RSD**	**% Recovery**	**% RSD**	**R^2**
Sildenafil	87.1	1.0	85.9	2.8	84.1	1.4	0.9999
Vardenafil	83.8	6.4	83.3	6.1	84.0	4.7	0.9999
Tadalafil	76.0	6.0	80.1	5.6	87.4	3.6	0.9974
Icariin	43.6	7.8	51.7	20.1	110.8	28.1	0.9994
Yohimbine	84.6	1.8	81.7	3.0	82.7	2.1	0.9967

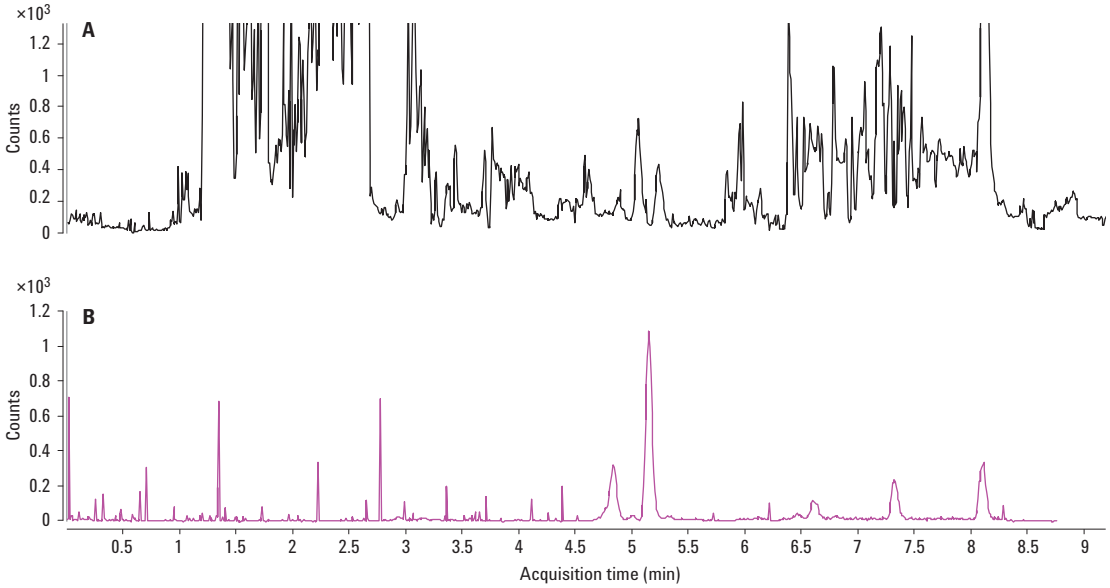


Figure 4. MRM TIC chromatograms of an ED supplement blank without cleanup (A) and a 20 ppb spike after cleanup using Agilent Bond Elut Plexa PCX (B).

Conclusions

Two sample preparation techniques were explored for the analysis of the adulterants sildenafil, vardenafil, tadalafil, icariin, and yohimbine in erectile dysfunction dietary supplements using LC/MS/MS. These matrices differed significantly in terms of complexity as they can contain single or multiple ingredients in a single capsule or tablet. While QuEChERS required only moderate method development, it did not provide sufficient cleanup to effectively measure these analytes at low concentrations in complicated matrices. For these complicated samples, the selectivity of cartridge-based SPE gave cleaner extracts resulting in excellent performance at these low concentrations. Although the selected analytes only represent the parent compounds of the growing library of PDE-5 pharmaceutical derivatives, this study provides preparation chemistries that can be directly applied and modified as necessary to accommodate larger analyte lists. The sample preparation selection process can be applied to other matrices of varying complexity, and be tailored towards different analytes to achieve improved sample cleanliness and method performance.

References

1. M. Blumenthal, A. Lindstrom, C. Ooyen, M. E. Lynch. *HerbalGram*, **99**, 60 (2013).
2. M. Nicoletti. *Int. J. Food Sci. Nutr.* **63**, 2 (2012).
3. P. A. Cohen, J. C. Travis, B. J. Venhuls. *Drug Test Anal*. (2013).
4. F. Song, A. El-Demerdash, S-J. S. H. Lee. *J. Pharma. Biomed. Anal.* **70**, 40 (2012).

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Appendix

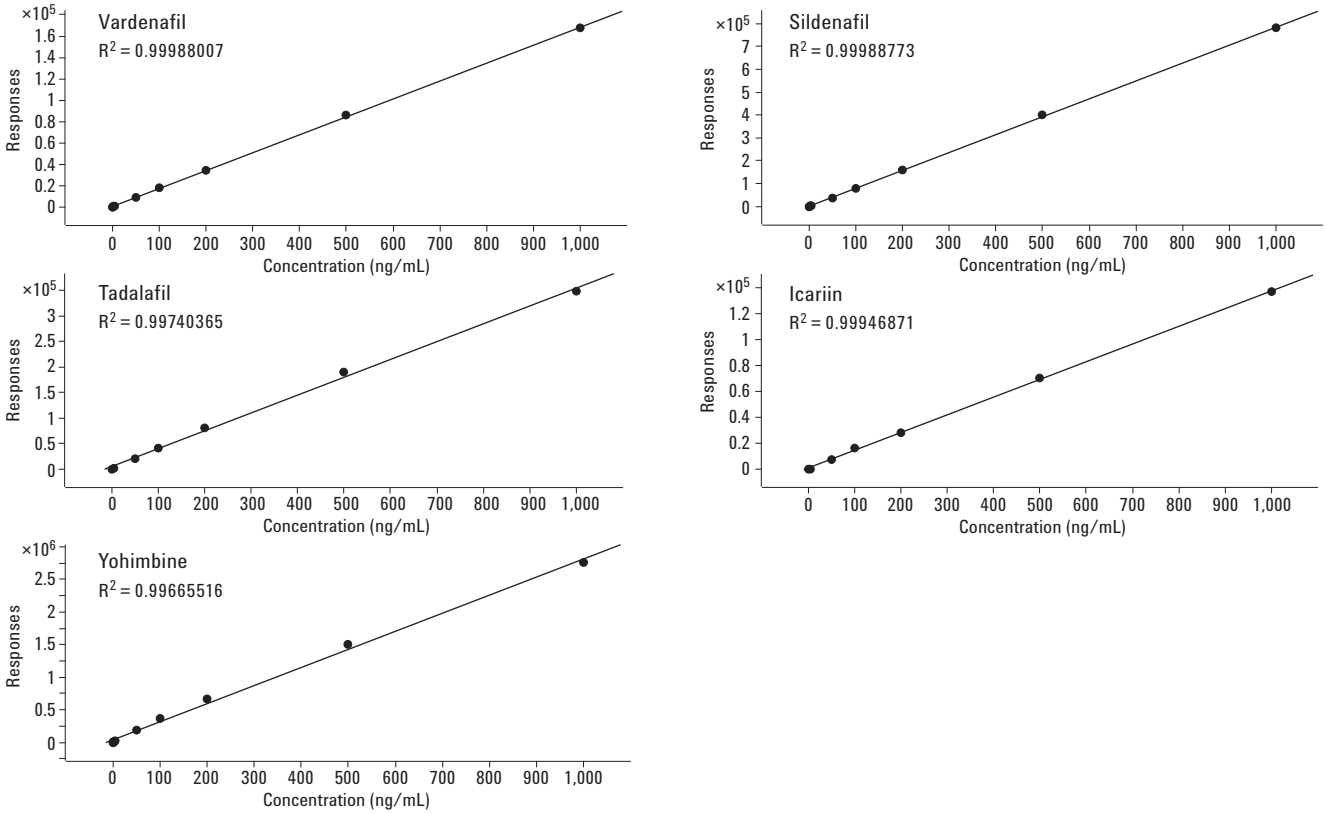

Figure 5. Calibration curves generated from 1 to 1,000 ppb for vardenafil, sildenafil, tadalafil, icariin, and yohimbine.

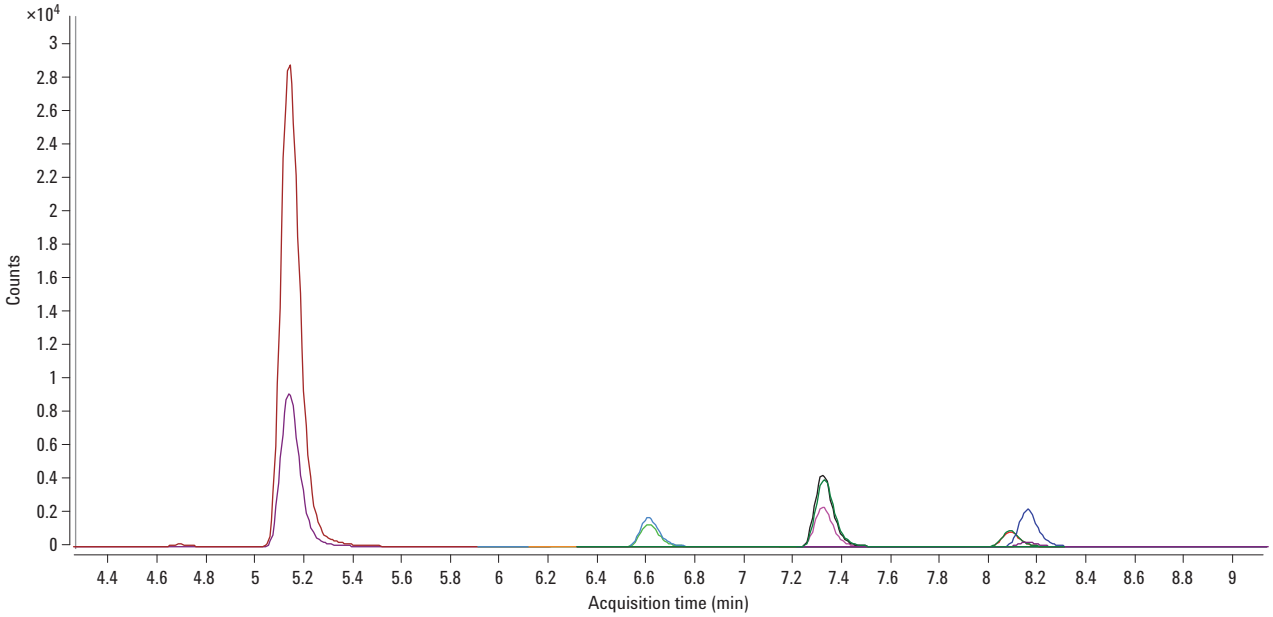

Figure 6. MRM chromatogram overlay of analyte primary and secondary transitions at 50 ppb.

Table 4. Analyte MS parameters and retention times.

Analyte	MRM 1, collision energy (V)	MRM 2, collision energy (V)	Fragmentor energy (V)	T_R (min)
Yohimbine	354.9 > 143.9 (25)	354.9 > 211.9 (20)	175	5.138
Vardenafil	489.0 > 151.0 (30)	489.0 > 311.8 (40)	135	6.610
Sildenafil	475.0 > 57.7 (50)	475.0 > 100.0 (30)	135	7.317
Sildenafil-d8	483.4 > 108.0 (30)	--	135	7.324
Icariin	676.9 > 368.6 (20)	676.9 > 530.6 (10)	125	8.082
Tadalafil	390.0 > 268.0 (5)	390.0 > 135.0 (10)	135	8.154

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Printed in the USA
February 3, 2014
5991-3638EN

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