INTRODUCTION
The adulteration of herbal/dietary supplements with erectile dysfunction (ED) drugs and their analogues is reported worldwide and is an increasing problem13. The sale of so-called 100% ‘all natural’ products has become a highly profitable business for online pharmacies, however these products can pose a serious threat to consumers owing to the undisclosed presence of approved/ prescription drugs in the unsafe and toxicity profile of unapproved ED drugs.

The Drug Control Laboratory, Qatar, has been involved in the testing of adulterated and counterfeit products for a number of years. Each year brings countless new warnings and alerts over the adulteration of products which are illegally advertised for the enhancement of male sexual performance. Consequently the identification of ED drugs and their analogues in these products is of great interest.

OBJECTIVE
The aim of this study was to develop a comprehensive method for screening, confirmation and quantification of illegally added ED drugs and their analogues in herbal and dietary products that are marketed to improve male sexual performance, and imported to Qatar.

RESULTS AND DISCUSSION
SCREENING: A spectral library for 32 compounds including 26 ED drugs and their analogues was prepared. Owing to recent reports of increased availability of all-in-one/combination herbal products, we included tentatively the sub-classes: PDE5 inhibitors, the 5α-reductase inhibitors (used for prostatic hyperplasia) and testosterone. The library was created according to a previously described approach14. i.e., dfc was conducted and all compounds were then screened by multiple cone voltages conditions (in-source neutralization-inducement dissociation, CID). To generate both spectral and retention time (RT) information, the Waters Chromatography Manager software was instrumental (Figure 2).

Chromatographically relevant (co-eluting) chromatograms were held at each cone voltage, which the compounds and calculate the average spectral match factor (FM) against the library. When performing in both S+ and S- modes, under multiple cone voltage conditions, along with RT provides high confidence in the identification.

The developed method was applied to 43 suspected samples, 18 of which were found to be adulterated with ED analogues (Figure 4, Table 1). Two samples that screened positive for thiodimethylsildenafil also showed the presence of sildenafil. Similarly, under these conditions, separation permitted clear differentiation between these two substances. Moreover, sildenafil and vardenafil were also detected as minor compounds due to the hydrolysis of this analogue ofildenafil (e.g., thiodimethylsildenafil)13.

CONFIRMATION: For subsequent quantitative analyses, a MRM method was also developed and validated for three alternative matrices (i.e., cap- sicle, h办理al, and plasma). Calibration curves were con- structed over the range of 5 - 10,000 ng/mL. The coefficient of determination (R²) for all compounds in this study was ≥ 0.995. The precision, measured as coefficient of variation (% CV), was < 11% for 26 compounds and < 10% at 10 and 100 ng/mL concentrations for all compounds when the standard mixture was spiked into herbal matrix. The limit of quantification (LOQ) was 1.0 ng/mL for 29 compounds based on a signal-to-noise ratio of ≥ 10 for both quantifier and qualifier ions.

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CONCLUSION
• We have developed a novel screening method that is suitable for both the detection of known and unknown ED drugs and analogues. It is the first description of a single analytical method with this capability.
• Full scan data is collected simultaneously in both S+ and S- positive modes, under multiple energy conditions, providing comprehensive spectral data which are automatically compared to a spectral library of known drugs.
• The high energy fragmentation patterns generated in negative S- mode are used specifically to facilitate identification of new and currently unknown analogues of ED drugs.
• Furthermore, a quantitative confirmatory method for 32 compounds including 26 ED drugs and their analogues has been developed and validated. This UPLC-MS/MS method is sensitive, accurate and demonstrates excellent linearity.

These procedures have been applied to the analysis of 43 samples received by our laboratory between the periods 10/2010 - 06/2011. Eighty samples were found to contain unauthorised substances.

METHODOLOGY
Instrumentation Waters Acquity-UPLC system in combination with the TQ Detector Mass Spectrometer (Figure 1).

UPLC conditions
Column Waters HSS C18 2.1 x 100mm, 1.8µm
Column Temp. 45°C
Injection Volume 7 µL
Mobile Phase A 10mM Ammonium Formate pH 2.9
Mobile Phase B Aqueous with 0.1% Formic acid
Flow Rate 0.50 µL/min

Sample preparation
Liquid or solid herbal/dietary supplements were extracted with acetonitrile (7 to 9:3 to 1 volume ratio) at 0°C for 10 minutes, followed by centrifugation at 12,000 rpm for 10 minutes. The supernatant was diluted with acetonitrile to 0.25% acetic acid aqueous solution to a final concentration of 0.25% acetic acid.

Sample analysis
Samples were manually injected into the UPLC system with a 50 µL loop and a 10 µL loop (Figure 1) for screening and quantitative analysis, respectively. Blank samples were used for extraction and instrumental blanks. The standard curves were generated using linear regression analysis. Each sample was injected in triplicate and the average peak area was used in the analysis. The final concentrations were calculated using the standard curve provided by Waters Corporation.

REFERENCES


