

"This is the peer reviewed version of the following article: [Drug testing and analysis, 2020] which has been published in final form at [<https://onlinelibrary.wiley.com/doi/abs/10.1002/dta.2861>] purposes in accordance with [Wiley Terms and Conditions for Self-Archiving](#)."



Mohd Yusop Ahmad Yusri (Orcid ID: 0000-0001-7735-856X)

Fu Shanlin (Orcid ID: 0000-0002-6238-3612)

RESEARCH ARTICLE

Special issue - Non-targeted screening of drugs

Suspected-target and non-targeted screenings of phosphodiesterase 5 (PDE5) inhibitors in herbal remedies by liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTOF-MS)

Ahmad Yusri Mohd Yusop^{1,2}, Linda Xiao¹, Shanlin Fu¹

¹Centre for Forensic Science, University of Technology Sydney, Ultimo, NSW, 2007
Australia

²Pharmacy Enforcement Division, Ministry of Health, Petaling Jaya, Selangor, 46200
Malaysia

Correspondence

Shanlin Fu, Centre for Forensic Science, University of Technology Sydney, Ultimo,
NSW, 2007 Australia

Email: shanlin.fu@uts.edu.au

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/dta.2861

Abstract

The lucrative market of herbal remedies spurs rampant adulteration, particularly with pharmaceutical drugs and their unapproved analogues. A comprehensive screening strategy is, therefore, warranted to detect these adulterants, and accordingly, to safeguard public health. This study utilises the data-dependent acquisition of a liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) to screen phosphodiesterase 5 (PDE5) inhibitors in herbal remedies using suspected-target and non-targeted strategies. The suspected-target screening employed a library comprising 95 PDE5 inhibitors. The non-targeted screening adopted top-down and bottom-up approaches to flag novel PDE5 inhibitors analogues based on common fragmentation patterns. The LC-QTOF-MS was optimised and validated for capsule and tablet dosage forms using 23 target analytes, selected to represent different groups of PDE5 inhibitors. The method exhibited excellent specificity and linearity with limit of detection and limit of quantification of <math><40\text{ ng/mL}</math> and 80 ng/mL, respectively. The accuracy ranged from 79.0%–124.7% with precision of <math><14.9\% \text{RSD}</math>. The modified QuEChERS extraction provided insignificant matrix effect within -9.1%–8.0% and satisfactory extraction recovery of 71.5%–105.8%. These strategies were utilised to screen 52 herbal remedy samples that claimed to enhance male sexual performance. The suspected-target screening resulted in 33 positive samples, revealing ten target analytes and two suspected analytes. Systematic MS and tandem MS interrogations using the non-targeted screening returned insignificant signals, indicating the absence of potentially novel analogues. The target analytes were quantified from 0.03–121.31 mg per dose of each sample. The proposed strategies ensure all PDE5 inhibitors are comprehensively screened, providing a useful tool to curb the widespread adulteration of herbal remedies.

Keywords: LC-QTOF-MS, suspected-target screening, non-targeted screening, herbal remedies, PDE5 inhibitors

1. INTRODUCTION

Globally, people consume an array of health products to treat minor ailments, prevent illnesses, and boost their health and well-being¹. Herbal remedies have recently surged to be a substantial part of this market due to the many side effects associated with modern medicines. At present, the herbal industry is one of the most rapidly growing sectors with annual sales over several billion dollars worldwide². Herbal remedies are typically marketed in pharmaceutical dosage forms and apportioned into specific doses.

Herbal remedies commonly claim to be of natural origin, giving the perception of being effective and safe³. However, this lucrative market often tempts intentional adulteration with pharmaceutical drugs and their unapproved analogues, aimed to provide the desired efficacy which may pose severe health and life-threatening risks to consumers^{2,4}. Among the most prevalent include products adulterated with phosphodiesterase 5 (PDE5) inhibitors and their analogues, frequently marketed to enhance male sexual performance⁵.

Novel PDE5 inhibitors analogues used as adulterants pose a challenge to forensic drug testing laboratories, as they may evade detection during routine screening⁶. As a result, the adulterated herbal remedies may be distributed in the market undetected, putting the consumers at absolute risk, owing to the unknown safety and toxicology profiles⁷. Thus far, the literature has identified more than 90 unapproved PDE5

inhibitors analogues as adulterants⁸. From 2015 to 2019, the United States Food and Drug Administration (USFDA) had reported that 260 out of 390 adulterated products contain PDE5 inhibitors and their analogues⁹.

The literature described several analytical methods to determine PDE5 inhibitors in various matrices, for instance, thin layer chromatography (TLC)¹⁰, gas chromatography-mass spectrometry (GC-MS)¹¹, Raman spectroscopy¹², and nuclear magnetic resonance (NMR) spectroscopy¹³. More frequently, liquid chromatography (LC) coupled with mass spectrometry (MS) detection in tandem mode has demonstrated to be an indispensable tool in the analysis of PDE5 inhibitors. The analyses used both low-resolution MS¹⁴ and high-resolution MS (HRMS)⁸. However, HRMS has proven to be superior as it delivers full-spectral information for both MS and tandem MS mode simultaneously¹⁵, which provides an unrivalled specificity.

In recent years, researchers are getting more interested in HRMS techniques such as quadrupole time-of-flight MS (QTOF-MS), as they can use the full-spectral information to develop targeted, suspected-target, and non-targeted analysis^{15,16}. However, the widely used targeted analysis is limited, depending on the availability of certified reference materials (CRMs)¹⁷. In the case of PDE5 inhibitors identification, it would not be financially viable for forensic drug testing laboratories to acquire all the available CRMs. Therefore, suspected-target screening provides extended coverage of known analytes without the need for CRMs. Additionally, non-targeted screening can address the growing concerns of the novel PDE5 inhibitors analogues found as adulterants. This strategy plays a pivotal role to discover those novel analogues based on the common fragmentation patterns of the known PDE5 inhibitors.

This study utilised the data-dependent acquisition (DDA) of an LC-QTOF-MS for comprehensive screening of PDE5 inhibitors and their analogues in herbal remedies. The screening procedure was carefully developed using suspected-target and non-targeted strategies. The analytical method was optimised and validated using 23 target analytes, ensuring robust and reliable performance to determine PDE5 inhibitors in different herbal remedies' matrices. These strategies were then employed to screen 52 distinct samples of herbal remedies that claimed to enhance male sexual performance. The highlighted significant results showcased the applicability of the developed screening strategies.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

In total, the 23 CRMs of PDE5 inhibitors purchased from TLC Pharmaceutical Standards Ltd (Aurora, Ontario, Canada) were as follows: (1) desmethylcarbodenafil, (2) carbodenafil, (3) N-desethylacetildenafil, (4) acetildenafil, (5) hydroxyvarodenafil, (6) dimethylacetildenafil, (7) vardenafil, (8) sildenafil, (9) homosildenafil, (10) dimethylsildenafil, (11) propoxyphenyl-hydroxyhomosildenafil, (12) udenafil, (13) propoxyphenyl-sildenafil, (14) hydroxythiovarodenafil, (15) tadalafil, (16) mirodenafil, (17) mutaprodenafil, (18) thiosildenafil, (19) thiohomosildenafil, (20) dithiodesmethylcarbodenafil, (21) thiodimethylsildenafil, (22) propoxyphenyl-thiohydroxyhomosildenafil, and (23) propoxyphenyl-thiodimethylsildenafil. Each of the CRM was carefully selected as target analytes to represent different groups of PDE5 inhibitors based on the structural similarities, as presented in Tables S1–S6 (supplementary data).

The vendor for methanol and acetonitrile of LC-MS grade was Chem-Supply Pty Ltd (Gillman, SA, Australia); while Sigma Aldrich Pty Ltd (Castle Hill, NSW, Australia) supplied the formic acid of LC-MS grade and ammonium formate of analytical grade. Ultrapure water (18.2 M Ω -cm) was collected from a Sartorius arium® pro ultrapure water system (Goettingen, Germany); and LECO Australia Pty Ltd (Castle Hill, NSW, Australia) supplied the quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction salt (EN 15662). Each sachet of the QuEChERS extraction salt is composed of 4 g magnesium sulphate, 1 g sodium chloride, 1 g trisodium citrate dihydrate, and 0.5 g disodium hydrogen citrate sesquihydrate.

2.2. Standard solution preparation

The stock solution of each CRM was prepared in methanol at 1 mg/mL and stored in the dark at 4°C. A mixture of all CRMs (working solution) was freshly prepared for each analysis from the stock solutions by further dilution in methanol to make up to 25 μ g/mL concentration.

2.3. Sample collection and storage

Altogether, the 52 distinct herbal remedy samples in capsule and tablet dosage forms were obtained from Malaysia (44 samples) and Australia (8 samples). These suspected samples were selected based on brand names, label claims, images, herbal ingredients, or advertising materials related to male sexual performance. The Pharmacy Enforcement Division, Ministry of Health Malaysia, kindly donated most of these samples which were confiscated at the international airport (10 samples) and international seaport (16 samples), including those from routine market surveillance activities (17 samples). The remainder of the samples were purchased from various

online shopping platforms based in Malaysia (1 sample) and Australia (8 samples). Each sample was labelled as SPC001 to SPC032 for capsule samples and SPT001 to SPT020 for tablet samples. These samples were deposited in a plastic zip-lock bag individually and then stored in an airtight container in the dark.

A representative blank matrix of capsule or tablet, free from any analyte of interests, was sourced from a local pharmacy in Australia and used for method optimisation and validation. The compositions of the capsule utilised as a blank matrix were as follows: *Epimedium sagittatum*, *Eleutherococcus senticosus*, *Tribulus terrestris*, *Dulacia inopiflora*, zinc oxide, and encapsulating aids. Meanwhile, the tablet employed as a blank matrix was composed of *Morinda officinalis*, *Epimedium sagittatum*, *Panax ginseng*, *Schisandra chinensis*, *Serenoa repens*, lycopene, zinc amino acid chelates, calcium hydrogen phosphate, carnauba wax, microcrystalline cellulose, chlorophyllin-copper complex, croscarmellose sodium, magnesium stearate, silica, and tablet coating ingredients. The constituents of these two blank matrices were stated on the products' labels.

2.4. Sample preparation

The initial weight of each sample was recorded according to the recommended dose on its label. Then, using an electric grinder for capsules or a mortar and pestle for tablets, the entire recommended dosage was homogenised. For instrumental analysis, 100 mg of the homogenised sample was weighed in a polypropylene tube and then extracted with 5 mL of acetonitrile and methanol (1:1, v/v) by 1-min vortex mixing, 20-min sonication, and 5-min centrifugation at $2500 \times g$, successively. The resulting mixture was then transferred into another polypropylene tube prefilled with half of a

sachet of the QuEChERS extraction salt (2 g magnesium sulphate, 0.5 g sodium chloride, 0.5 g trisodium citrate dihydrate, and 0.25 g disodium hydrogen citrate sesquihydrate); and vortexed for 1 min, followed by centrifugation for 5 min at 2500 × g to separate the solid residues. The upper layer was filtered using a 0.22 µm PTFE syringe filter and diluted with methanol at 1:10 dilution level for analysis. The blank matrices were treated in the same manner as the sample analysis. For quantification purpose, the sample solution was further diluted with methanol whenever the target analyte concentration was beyond the linear range of the external calibration curve.

2.5. LC-QTOF-MS conditions and data analysis

This study employed an Agilent Technologies (Santa Clara, CA, USA) 1290 Infinity II LC system coupled to an Agilent Technologies 6510 QTOF-MS using our previously developed methodology^{8,18}. The chromatographic separation was carried out using a reverse-phase high-performance LC column from Merck KGaA (Darmstadt, Germany) Chromolith® High-Resolution RP-18 end-capped (100 × 4.6 mm, 2.0 µm) with column compartment temperature maintained at 20°C.

The injection volume was set at 5 µL with the autosampler compartment temperature maintained at 10°C. The mobile phases were acidified with 0.1% v/v formic acid and consisted of solvent A (10 mM ammonium formate in ultrapure water) and solvent B (acetonitrile). The settings of the gradient elution were as follows: 5% B for 0–1 min, 5%–25% B for 1–2 min, 25%–50% B for 2–32 min, 50%–95% B for 32–33 min, and 95% B for 33–34 min at 0.4 mL/min. The elution was immediately returned to the initial gradient at 34.01 min for 6 min at 1 mL/min. Post-run equilibration was set for 5 min at 0.4 mL/min before the next injection.

The QTOF-MS, equipped with a dual electrospray ionisation (ESI) nebuliser was calibrated at a low mass range of m/z 1700 before each chromatographic run to achieve a typically attainable mass accuracy within ± 5 ppm for precursor ion and ± 20 ppm for product ion. ESI in positive ionisation mode was employed using the following experimental parameters: 300°C for gas temperature, 12 L/min for drying gas flow, 32 psig for nebuliser pressure, 3500 V for capillary voltage, 175 V for fragmentor voltage, 65 V for skimmer voltage, and 750 V for OCT 1 RF Vpp.

A DDA (auto MS/MS) mode was selected for simultaneous MS and tandem MS experiments within a mass-to-charge range of m/z 100 to 1100. The acquisition rates' settings were 1 and 3 spectra/s for the MS and tandem MS experiments, respectively, within a narrow isolation width of m/z ~ 1.3 . The collision-induced dissociation experiments were performed at fixed collision energies (CEs) of 10, 20, and 40 eV in a separate scan with nitrogen as the collision gas. The reference mass solution containing purine (m/z 121.050873) and hexakis (1H, 1H, 3H-tetrafluoropropoxy) phosphazine (m/z 922.009798) were continuously infused throughout the chromatographic run at a steady pressure of 5 psig.

Agilent Technologies Mass Hunter workstation software version B.07.00, Mass Hunter qualitative analysis software version B.07.00, and personal compound database and library (PCDL) manager software version B.04.00 were used to process all qualitative and quantitative data. All other calculations were done using Microsoft (Redmond, WA, USA) Excel 2016 (Microsoft Office).

2.6. Analytical method validation

The analytical method validation was performed for specificity, linearity, limit of detection (LOD), and limit of quantification (LOQ)¹⁹. The accuracy, precision, matrix effect (ME), and extraction recovery (RE) were also evaluated for each target analyte in each of the blank matrices of capsule and tablet at low (0.1 µg/mL), medium (0.4 µg/mL), and high (1 µg/mL) quality control (QC) levels following the recommended procedures²⁰. All validation parameters were analysed in triplicate.

Each target analyte was evaluated for specificity based on (1) the chromatographic separation and (2) the high-resolution mass of the protonated molecule ($[M+H]^+$). The tandem MS experiment was then used to establish the presence of two product ions corresponding to each target analyte. The average intensity ratio between the first and the second product ion at average CEs was compared to those obtained from the matrix-matched QC analytes within $\pm 30\%$ and thus, confirming the target analytes' identity. The extent of interfering components from the extracted blank matrices was also ascertained at the retention time of each target analytes.

An external calibration curve was constructed using the peak areas of each target analyte from the $[M+H]^+$ precursor ion versus their concentrations. The linearity was then determined based on the coefficient of determination (r^2), and the regression equation was used to calculate the QC analytes and samples concentrations. The lowest and highest concentrations of target analytes expected in adulterated herbal remedies were applied for the linear range.

The LOD was determined experimentally by tapering down each point of 10 ng/mL of the working solution concentration starting from 100 to 10 ng/mL. The LOD was then selected based on the lowest concentration of target analyte that can be reliably identified as defined in the specificity assessment. The LOQ was established at the lowest concentration of the external calibration curve with acceptable accuracy and precision. Whenever a background noise is present, the signal-to-noise ratio was ascertained at >3 for LOD and >10 for LOQ.

The accuracy and precision were established at three QC levels. The extracted blank matrices were spiked with the working solution and submitted to analysis. The observed target analyte concentration versus the expected concentration at the same QC level was expressed as a percentage of accuracy with an acceptable value of $\pm 25\%$. Precision was determined using the same QC analytes at intra-day for repeatability and inter-day for intermediate precision. The results were then expressed as a percentage of the relative standard deviation (%RSD) of the peak areas of the $[M+H]^+$ precursor ion with an acceptable value of $<20\%$.

Using the same QC levels, the ME was evaluated based on the post-extraction addition method⁸. The slopes of the matrix-matched calibration curve versus those of the external calibration curve were compared to determine the percentage of ionisation suppression (negative value) or ionisation enhancement (positive value). The ME categories for each target analyte in both matrices were as follows: insignificant (0% to $\pm 10\%$), acceptable ($\pm 10\%$ to $\pm 20\%$), moderate ($\pm 20\%$ to $\pm 50\%$), and severe ($-50\% < >+50\%$). In contrast, the comparison of the peak areas of the $[M+H]^+$ precursor ion of target analytes spiked into the blank matrices before extraction versus those spiked

into an extracted blank matrix at the same QC level, generated the percentage of RE with an acceptable value of $\pm 25\%$.

2.7 Suspected-target and non-targeted screenings of herbal remedies

The screening of PDE5 inhibitors and their analogues as adulterants in herbal remedies was performed using suspected-target and non-targeted strategies. For the suspected-target screening, a library was constructed using the PCDL manager software. The library comprised of 95 PDE5 inhibitors and their analogues that are presently known as adulterants (listed in Table S7 of the supplementary data). Specific details such as compound name, molecular formula and structure, and exact mass were manually stored in this library. Also, extended details from the LC-QTOF-MS analysis of the 23 target analytes were imported from the Mass Hunter qualitative analysis software into the same library, which includes the retention time and tandem MS spectra at different CEs.

The suspected-target screening was employed to match the observed accurate mass of the $[M+H]^+$ precursor ion of the sample to those theoretical ones in the library within ± 5 ppm mass tolerance. The matching scores of the observed mass, isotopic abundance distribution, and isotopic spacing for each analyte were also ascertained to be $>80\%$. Based on these findings, a list of matched analytes was generated. Next, the observed tandem MS spectra of the sample were compared to those of target analytes within ± 20 ppm mass tolerance. Subsequently, the identity of each target analyte was confirmed by comparing the observed retention time to those of the CRMs within ± 0.25 min tolerance.

At this stage, there are two possible outcomes for positive samples, where: (1) sample matched with target analytes and (2) sample matched with suspected analytes. Only samples in outcome number (1) were submitted to quantification. Whereas, a list of suspected analytes was generated for outcome number (2). The negative samples were tentatively categorised as possible non-adulterated samples and were submitted to the non-targeted screening.

The non-targeted screening was performed using comprehensive top-down and bottom-up approaches to screen visible and non-visible chromatographic peak, respectively. The screening was adapted and modified following a critical review by Pasin et al.¹⁵ to flag novel PDE5 inhibitors analogues based on common fragmentation patterns of target analytes. The visible peaks within a base peak chromatogram (BPC) were integrated and extracted using the top-down approach to reveal the mass spectra. Each of this spectrum was then interrogated for the $[M+H]^+$ precursor ions. Using the product ion scan of the Mass Hunter qualitative analysis software, the link between the pre-determined $[M+H]^+$ precursor ions and the product ions of target analytes was established at the specific retention time of each chromatographic peak.

In contrast, with the bottom-up approach, all generated $[M+H]^+$ precursor ions during the chromatographic run were considered to establish if there is any link to the product ions of target analytes. Therefore, additional investigations are required to ultimately establish the correct $[M+H]^+$ precursor-product ion pair. In both approaches, any two tandem MS signals observed belonging to the same group of PDE5 inhibitors within ± 20 ppm mass tolerance would reveal the presence of a novel analogue.

3. RESULTS AND DISCUSSION

3.1. Method optimisation

The simultaneous separation of multiple PDE5 inhibitors with structural similarities is critical for a reliable determination of these adulterants, particularly from complex matrices such as herbal remedies. The mobile phases, matrix modifier, and chromatographic column were initially selected based on the physical and chemical properties of target analytes to obtain optimum chromatographic resolution. The chromatographic separation was then optimised by varying the LC parameters such as injection volume, flow rate, column temperature, elution gradient, and elution time. The MS conditions were tuned according to the flow- and compound-dependent parameters to improve the method sensitivity. The chromatographic separation and MS conditions were optimised following the previous literature¹⁸.

Although the LC-QTOF-MS via ESI is superior in detecting analytes from complex matrices, its performance is often hindered by the presence of ME, which often lead to errors in quantification²¹. Therefore, the presence of either ionisation suppression or ionisation enhancement needs to be addressed to minimise the possibilities of false-negative and false-positive results. Consequently, two extraction techniques were compared and assessed based on the ME and RE efficiency to resolve this issue. The ME was also evaluated at three levels of matrix dilution while maintaining the target analytes concentration at three QC levels.

The widely used dilute-and-shoot (D&S) technique was initially performed to analyse the PDE5 inhibitors in the blank matrices. Methanol was chosen as the solvent for this technique based on previous literature^{22,23}. The capsule matrix produced moderate

ME for two and acceptable ME for eight target analytes at 1:2 matrix dilution. The remaining 13 target analytes showed insignificant ME. However, non-detection of several target analytes at the same matrix dilution was observed for the tablet matrix at low and medium QC levels. Therefore, the ME cannot be determined for seven target analytes, particularly those with pyrazolopyrimidine-7-thione and imidazotriazine-4-thione moiety. The same problem persisted for the tablet matrix at a higher 1:10 matrix dilution. In general, the ME was minimised to insignificant percentages with increasing matrix dilution from 1:2 to 1:100 for all target analytes in both matrices.

From the D&S ME assessment, the presence of the tablet matrix had resulted in a complete loss of MS signals which subsequently led to false-negative results of the seven target analytes. Consequently, another sample extraction technique was assessed to overcome this problem. After several trial-and-error, a modified QuEChERS procedure was developed specifically to resolve the ME issue. The application of the modified QuEChERS extraction in combination with appropriate matrix dilution had resulted in insignificant ME percentages for both matrices at 1:10 and 1:100 matrix dilution. Finally, the 1:10 matrix dilution was selected and submitted to RE assessment. Table S8 (supplementary data) presents the full ME assessment results of the blank matrices using D&S technique and modified QuEChERS extraction at three levels of matrix dilution.

3.2. Analytical method validation

Table S9 (supplementary data) shows the specificity, linearity, and sensitivity results of the analytical method. The presence of each target analyte was ascertained using

the optimised chromatographic separation and the full-scan MS data of the $[M+H]^+$ precursor ion. Isomeric analytes were chromatographically resolved, qualifying their specificity. Furthermore, the presence of two product ions from the tandem MS experiment confirmed the target analytes' identities. The effects of interferences from the extracted blank matrices were established to be trivial. Moreover, the carry-over effect was not observed in the subsequent analysis using the optimised chromatographic separation as the reverse-phase high-performance LC column was flushed with approximately five times of the column volume starting from 34.01–40 min at 1 mL/min before the next sample injection. The linear relationship between the peak areas of target analytes and their concentrations was verified by r^2 of >0.9870 within the selected range of 0.08–1.2 $\mu\text{g/mL}$. The LOD was determined between 10–40 ng/mL, while the LOQ was fixed at 80 ng/mL for all target analytes.

Supplementary Table S10 and S11 respectively, present the accuracy and precision data. Excellent accuracy was obtained for both capsule and tablet matrices. The capsule matrix produced the percentage of accuracy ranged from 90.8%–123.1% at low; 94.4%–104.9% at medium; and 95.6%–103.4% at high QC level. The percentage of accuracy for tablet matrix at low, medium, and high QC levels were within 79.0%–124.7%; 93.8%–109.9%; and 90.8%–103.9%, respectively. The precision was also satisfactory with the %RSD of $<14.9\%$. The repeatability and intermediate precision for both matrices were calculated within 0.3%–8.6% and 0.1%–14.9% of RSD, respectively, at all QC levels.

As mentioned in Section 3.1, the ME was within insignificant percentages for all target analytes. The MEs for capsule and tablet matrices were within -9.1%–1.7% and -

3.7%–8.0%, respectively. Table S12 (supplementary data) shows the RE results of the modified QuEChERS extraction. The RE was satisfactory within 72.6%–105.8% for capsule matrix and 71.5%–102.3% for tablet matrix.

3.3 Screenings of herbal remedies for PDE5 inhibitors

A total of 52 distinct herbal remedy samples obtained from Malaysia and Australia were comprehensively screened using suspected-target and non-targeted strategies. The top three countries of origin based on the products' label are Malaysia, China (Hong Kong), and Indonesia. Most of these samples claimed to contain *Eurycoma longifolia*, *Tribulus terrestris*, and *Panax ginseng*, which are usually regarded as herbal aphrodisiacs. Table 1 compiles the analysis results of the adulterated herbal remedy samples.

The suspected-target screening generated a list of 12 matched analytes from 33 samples. The tandem MS and retention time matching subsequently confirmed the identity of each analyte, particularly distinguishing those with isomeric configurations. Based on these findings, ten target analytes were identified from 32 samples and quantified. Just two suspected analytes, i.e. aminotadalafil and hydroxythiohomosildenafil, were detected from 3 samples (2 samples contained a combination of target analytes and suspected analytes while another 1 sample contained only suspected analyte). The remaining 19 of possibly non-adulterated samples were submitted to the non-targeted screening. Systematic MS and tandem MS interrogations using top-down and bottom-up approaches returned insignificant signals, and no novel PDE5 inhibitors analogues were detected; thereby, confirming the negative results.

Fig. 1A summarises the identification of target analytes and the detection of suspected analytes in adulterated herbal remedy samples. The active ingredient of Viagra®: sildenafil, was identified in nearly half of the samples. It was found in 19 samples as a sole adulterant and 6 samples in combinations with other PDE5 inhibitors. Other target analytes identified in this study were as follows: propoxyphenyl-sildenafil, tadalafil, and thiodimethylsildenafil (4 samples each); dimethylsildenafil and thiosildenafil (3 samples each); and propoxyphenyl-hydroxyhomosildenafil, propoxyphenyl-thiodimethylsildenafil, propoxyphenyl-thiohydroxyhomosildenafil, and vardenafil (1 sample each). These target analytes can either be present as a sole adulterant or in combinations of up to four different adulterants in any one sample.

These findings indicated that sildenafil is the most prevalent adulterant detected among the 33 adulterated samples. Similarly, analogues of sildenafil are frequently detected compared to those of tadalafil and vardenafil. The trends may be attributed to the easily accessible and inexpensive cost of raw materials to obtain or synthesise the adulterants²⁴. Furthermore, the synthesis steps are readily available from the patent literature, which could yield hundreds of active sildenafil analogues²⁵.

The total target analytes quantified for each recommended dose ranged from 0.03–121.31 mg per sample. These findings were then categorised based on the recommended dose of the approved PDE5 inhibitors (i.e. 25–100 mg for sildenafil and 5–20 mg for vardenafil and tadalafil)²⁶, summarised in Fig. 1B. The quantification of target analytes was indicative of suprathreshold level for 10 samples. Sildenafil (7 samples) and tadalafil (2 samples), in particular, were quantified exceeding their

maximum therapeutic dose of 100 mg and 20 mg, respectively. Sample SPC027 notably contains four distinct analogues of sildenafil, combined to produce a suprathereapeutic level of PDE5 inhibitors. The high dose of these adulterants generally increases the incidence of side effects which could easily jeopardise consumers' health and well-being. In some cases, concurrent consumption with nitrates or α -blockers may lead to life-threatening hypotension²⁷. The remainder of the samples were categorised as follows: therapeutic level (13 samples), subtherapeutic level (8 samples), and trace level (1 sample).

Table 2 compiles the suspected analytes detected using the developed screening strategies. Samples SPC005 and SPT003 comprised of target analytes and suspected analytes with a total of four analytes in each sample. In contrast, sample SPT017 contained only one suspected analyte. For example, with the suspected-target screening, an unidentified BPC peak at the retention time of 23.92 min from sample SPC005 was initially matched with two analytes, namely hydroxythiohomosildenafil and hydroxythiovaridenafil based on the theoretical $[M+H]^+$ precursor ion of m/z 521.1999. As these two isomeric analytes belong to different groups of PDE5 inhibitors analogues, it can be clearly distinguished using the tandem MS spectra. Furthermore, the differences in the retention time of both analytes confirmed the presence of hydroxythiohomosildenafil, which was similarly detected from sample SPT003. The observed retention time can be tentatively assigned to hydroxythiohomosildenafil to match it with the specific CRM when available, and thus, identify the same analyte in the future.

The non-targeted screening did not detect any novel PDE5 inhibitors analogues among the study samples. Its effectiveness and validity, however, can be demonstrated by the detection of aminotadalafil (a known analyte) in sample SPT003 (Fig. 2). By using the top-down approach, an unidentified BPC peak at 17.75 min was integrated and extracted, revealing the observed $[M+H]^+$ precursor ion of m/z 391.1391. The link between the pre-determined $[M+H]^+$ precursor ion and the product ions of target analytes was established using the product ion scan of the Mass Hunter qualitative analysis software. As a result, the tandem MS signals at the same retention time were specific to the common fragmentation pattern of tadalafil within ± 20 ppm mass tolerance at m/z 135.0441 and 169.0760. Based on these findings, the “novel” analogue (aminotadalafil) can be flagged and narrowed down into the tadalafil group of analogues.

Contrarily, the bottom-up approach utilised all generated $[M+H]^+$ precursor ions during the chromatographic run to establish if there is any link to the product ions of target analytes. For sample SPT003, two tandem MS signals were detected using the product ion scan at different retention times for both product ions of tadalafil. These findings indicated the presence of two different analytes belonging to the tadalafil group of analogues. Indeed, one of the analytes was tadalafil based on the matching of the retention time at 21.12 min. Thorough investigations established the link between the observed $[M+H]^+$ precursor ion of the “novel” analogue (aminotadalafil) at m/z 391.1391 and the product ions of tadalafil. From these findings, the chemical formula or structure of the “novel” analogue (aminotadalafil) can be predicted based on tadalafil and tentatively assigned before further structural elucidation. Fig. 3 presents the proposed common fragmentation pattern shared by the tadalafil group of

analogues. Although sample SPT003 contained other analytes such as vardenafil and hydroxythiohomosildenafil, no interferences of the tandem MS signals were observed at the m/z of tadalafil product ions.

The suspected analytes detected in this study, i.e. aminotadalafil and hydroxythiohomosildenafil, had been initially reported as adulterants in herbal remedy capsules²⁸⁻³⁰. The USFDA had also warned consumers on the dangers of these unapproved PDE5 inhibitors analogues, detected in 26 products marketed to enhance male sexual performance^{31,32}. As exhibited by sample SPT003, these two analytes had previously detected in pairs either with³³ or without³⁴ other PDE5 inhibitors.

4. CONCLUSION

This study explored the applicability of an LC-QTOF-MS for comprehensive screening of PDE5 inhibitors and their analogues in herbal remedies using suspected-target and non-targeted strategies. The method was fully optimised and validated for 23 target analytes to screen 52 herbal remedy samples in capsule and tablet dosage forms. The screening strategies revealed 33 positive samples, identifying ten target analytes and detecting two suspected analytes. The target analytes were quantified from 0.03–121.31 mg for each of the recommended dose of the samples. The DDA provides cleaner spectra where the observed product ions could be easily linked to their $[M+H]^+$ precursor ion. The screening strategies discussed in this study would be beneficial to curb the widespread of adulterated herbal remedies, particularly those with PDE5 inhibitors and their analogues. It is vital to ensure that herbal remedies do not pose any health risks to consumers and thus, protecting their safety.

ACKNOWLEDGEMENTS

The authors acknowledge and thank the University of Technology Sydney for their Higher Degree by Research Student Project Funding.

The authors greatly appreciate the contribution of the Pharmacy Enforcement Division, Ministry of Health Malaysia, in providing samples used in this study.

REFERENCES

1. Boon H, Bozinovski N. A systematic narrative review of the evidence for labeling of natural health products and dietary supplements. *J Altern Complement Med* 2019;25(8):777-788. doi:<https://doi.org/10.1089/acm.2018.0533>
2. Skalicka-Wozniak K, Georgiev MI, Orhan IE. Adulteration of herbal sexual enhancers and slimmers: the wish for better sexual well-being and perfect body can be risky. *Food Chem Toxicol* 2017;108:355-364. doi:<https://doi.org/10.1016/j.fct.2016.06.018>
3. Haneef J, Shaharyar M, Husain A, et al. Analytical methods for the detection of undeclared synthetic drugs in traditional herbal medicines as adulterants. *Drug Test Anal* 2013;5(8):607-613. doi:<https://doi.org/10.1002/dta.1482>
4. Primpray V, Chailapakul O, Tokeshi M, Rojanarata T, Laiwattanapaisal W. A paper-based analytical device coupled with electrochemical detection for the determination of dexamethasone and prednisolone in adulterated traditional medicines. *Anal Chim Acta* 2019;1078:16-23. doi:<https://doi.org/10.1016/j.aca.2019.05.072>

5. Fibigr J, Šatínský D, Solich P. Current trends in the analysis and quality control of food supplements based on plant extracts. *Anal Chim Acta* 2018;1036:1-15. doi:<https://doi.org/10.1016/j.aca.2018.08.017>
6. Marcus DM. Dietary supplements: What's in a name? What's in the bottle? *Drug Test Anal* 2016;8(3-4):410-412. doi:<https://doi.org/10.1002/dta.1855>
7. Rocha T, Amaral JS, Oliveira MBPP. Adulteration of dietary supplements by the illegal addition of synthetic drugs: a review. *Compr Rev Food Sci Food Saf* 2016;15(1):43-62. doi:<https://doi.org/10.1111/1541-4337.12173>
8. Mohd Yusop AY, Xiao L, Fu S. Determination of phosphodiesterase 5 (PDE5) inhibitors in instant coffee premixes using liquid chromatography-high-resolution mass spectrometry (LC-HRMS). *Talanta* 2019;204:36-43. doi:<https://doi.org/10.1016/j.talanta.2019.05.078>
9. United States Food and Drug Administration. Tainted products marketed as dietary supplements 2019. https://www.accessdata.fda.gov/scripts/sda/sdNavigation.cfm?filter=&sortColumn=1d&sd=tainted_supplements_cder&page=1. Accessed March 21, 2020.
10. Do TT, Theocharis G, Reich E. Simultaneous detection of three phosphodiesterase type 5 inhibitors and eight of their analogs in lifestyle products and screening for adulterants by high-performance thin-layer chromatography. *J AOAC Int* 2015;98(5):1226-1233. doi:<https://doi.org/10.5740/jaoacint.14-285>
11. Mokhtar SU, Chin ST, Kee CL, Low MY, Drummer OH, Marriott PJ. Rapid determination of sildenafil and its analogues in dietary supplements using gas chromatography-triple quadrupole mass spectrometry. *J Pharm Biomed Anal* 2016;121:188-196. doi:<http://dx.doi.org/10.1016/j.jpba.2016.01.034>

12. Minh DTC, Thi LA, Huyen NTT, Van Vu L, Anh NTK, Ha PTT. Detection of sildenafil adulterated in herbal products using thin layer chromatography combined with surface enhanced Raman spectroscopy: “double coffee-ring effect” based enhancement. *J Pharm Biomed Anal* 2019;174:340-347. doi:<https://doi.org/10.1016/j.jpba.2019.05.043>
13. Gilard V, Balayssac S, Tinaugus A, Martins N, Martino R, Malet-Martino M. Detection, identification and quantification by ^1H NMR of adulterants in 150 herbal dietary supplements marketed for improving sexual performance. *J Pharm Biomed Anal* 2015;102:476-493. doi:<http://dx.doi.org/10.1016/j.jpba.2014.10.011>
14. Lee JH, Park HN, Park OR, Kim NS, Park S-K, Kang H. Screening of illegal sexual enhancement supplements and counterfeit drugs sold in the online and offline markets between 2014 and 2017. *Forensic Sci Int* 2019;298:10-19. doi:<https://doi.org/10.1016/j.forsciint.2019.02.014>
15. Pasin D, Cawley A, Bidny S, Fu S. Current applications of high-resolution mass spectrometry for the analysis of new psychoactive substances: a critical review. *Anal Bioanal Chem* 2017;409(25):5821-5836. doi:<https://doi.org/10.1007/s00216-017-0441-4>
16. Álvarez-Ruiz R, Picó Y. Sequential window acquisition of all theoretical fragments versus information dependent acquisition for suspected-screening of pharmaceuticals in sediments and mussels by ultra-high pressure liquid chromatography-quadrupole time-of-flight-mass spectrometry. *J Chromatogr A* 2019;1595:81-90. doi:<https://doi.org/10.1016/j.chroma.2019.02.041>

17. von Eyken A, Ramachandran S, Bayen S. Suspected-target screening for the assessment of plastic-related chemicals in honey. *Food Control* 2020;109:106941. doi:<https://doi.org/10.1016/j.foodcont.2019.106941>
18. Mohd Yusop AY, Xiao L, Fu S. Data on the optimisation and validation of a liquid chromatography-high-resolution mass spectrometry (LC-HRMS) to establish the presence of phosphodiesterase 5 (PDE5) inhibitors in instant coffee premixes. *Data Brief* 2019;25:104234. doi:<https://doi.org/10.1016/j.dib.2019.104234>
19. ICH harmonized tripartite guideline: validation of analytical procedures: text and methodology Q2(R1). In: International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use; 2005.
20. Krueve A, Rebane R, Kipper K, et al. Tutorial review on validation of liquid chromatography-mass spectrometry methods: part II. *Anal Chim Acta* 2015;870:8-28. doi:<https://doi.org/10.1016/j.aca.2015.02.016>
21. Krueve A, Rebane R, Kipper K, et al. Tutorial review on validation of liquid chromatography-mass spectrometry methods: part I. *Anal Chim Acta* 2015;870:29-44. doi:<https://doi.org/10.1016/j.aca.2015.02.017>
22. Lee S, Ji D, Park M, Chung KH. Development of a comprehensive spectral library of sildenafil and related active analogues using LC-QTOF-MS and its application for screening counterfeit pharmaceuticals. *Forensic Sci Int* 2015;257:182-188. doi:<http://dx.doi.org/10.1016/j.forsciint.2015.08.016>

23. Strano-Rossi S, Odoardi S, Castrignano E, Serpelloni G, Chiarotti M. Liquid chromatography-high resolution mass spectrometry (LC-HRMS) determination of stimulants, anorectic drugs and phosphodiesterase 5 inhibitors (PDE5I) in food supplements. *J Pharm Biomed Anal* 2015;106:144-152. doi:<https://doi.org/10.1016/j.jpba.2014.06.011>
24. Patel DN, Li L, Kee C-L, Ge X, Low M-Y, Koh H-L. Screening of synthetic PDE-5 inhibitors and their analogues as adulterants: analytical techniques and challenges. *J Pharm Biomed Anal* 2014;87:176-190. doi:<http://dx.doi.org/10.1016/j.jpba.2013.04.037>
25. Bell AS, Brown D, Terrett NK. Pyrazolopyrimidinone antianginal agents. In. United States: Google Patents; 1993.
26. Bruzziches R, Francomano D, Gareri P, Lenzi A, Aversa A. An update on pharmacological treatment of erectile dysfunction with phosphodiesterase type 5 inhibitors. *Expert Opin Pharmacother* 2013;14(10):1333-1344. doi:<https://doi.org/10.1517/14656566.2013.799665>
27. Ventimiglia E, Capogrosso P, Montorsi F, Salonia A. The safety of phosphodiesterase type 5 inhibitors for erectile dysfunction. *Expert Opin Drug Saf* 2016;15(2):141-152. doi:<https://doi.org/10.1517/14740338.2016.1131818>
28. Zou P, Hou P, Oh SS-Y, Low M-Y, Koh H-L. Electrospray tandem mass spectrometric investigations of tadalafil and its analogue. *Rapid Commun Mass Spectrom* 2006;20(22):3488-3490. doi:<https://doi.org/10.1002/rcm.2752>
29. Zou P, Hou P, Low M-Y, Koh H-L. Structural elucidation of a tadalafil analogue found as an adulterant of a herbal product. *Food Addit Contam* 2006;23(5):446-451. doi:<https://doi.org/10.1080/02652030500479690>

30. Li L, Low MY, Aliwarga F, et al. Isolation and identification of hydroxythiohomosildenafil in herbal dietary supplements sold as sexual performance enhancement products. *Food Addit Contam Part A* 2009;26(2):145-151. doi:<http://dx.doi.org/10.1080/02652030802368757>
31. United States Food and Drug Administration. Tainted products marketed as dietary supplements 2019. https://www.accessdata.fda.gov/scripts/sda/sdNavigation.cfm?filter=aminotadalafil&sortColumn=1d&sd=tainted_supplements_cder&page=1. Accessed January 11, 2020.
32. United States Food and Drug Administration. Tainted products marketed as dietary supplements 2019. https://www.accessdata.fda.gov/scripts/sda/sdNavigation.cfm?filter=hydroxythiohomosildenafil&sortColumn=1d&sd=tainted_supplements_cder&page=1. Accessed January 11, 2020.
33. United States Food and Drug Administration. Public notification: Alpha Male contains hidden drug ingredient 2013. <https://www.fda.gov/drugs/medication-health-fraud/public-notification-alpha-male-contains-hidden-drug-ingredient>. Accessed January 11, 2020.
34. United States Food and Drug Administration. Tainted products marketed as dietary supplements 2013. https://www.accessdata.fda.gov/scripts/sda/sdNavigation.cfm?filter=rezzrx&sortColumn=1d&sd=tainted_supplements_cder&page=1. Accessed January 11, 2020.

Table 1: Identification of target analytes and detection of suspected analytes in adulterated herbal remedy samples.

Sample	Target analytes identified	Total average weight per recommended dose in mg - quantification level	Herbs claimed on the label (top 3 herbs)	Product origin claimed on the label
SPC001	1. Sildenafil	0.11 - SUB	<i>Panax ginseng</i> , <i>Tribulus terrestris</i> , <i>Ginkgo biloba</i>	Canada
SPC003	1. Sildenafil	33.50 - THR	<i>Panax ginseng</i> , <i>Cordyceps sinensis</i> , <i>Epimedium</i>	Not stated
SPC005	1. Thiodimethylsildenafil 2. Thiosildenafil 3. Dimethylsildenafil *Suspected analyte 1. Hydroxythiohomosildenafil	20.05 - SUB	<i>Mulberry leaves</i> , <i>yam roots</i> , <i>Rhodiola rosea</i>	Norway
SPC008	1. Sildenafil	116.96 - SPR	<i>Myristica fragrans</i> , <i>Pausinystalia yohimbe</i> , <i>Eurycoma longifolia</i>	Indonesia
SPC010	1. Sildenafil	35.73 - THR	Unspecified herbs	Malaysia
SPC011	1. Tadalafil	53.96 - SPR	<i>Tribulus terrestris</i> , <i>Lepidium meyenii</i> , <i>Eurycoma longifolia</i>	Malaysia
SPC012	1. Sildenafil	73.18 - THR	<i>Epimedium grandiflorum</i> , <i>Eurycoma longifolia</i> , <i>Serenoa repens</i>	United States

SPC015	1. Sildenafil 2. Propoxyphenyl-sildenafil (<LOQ)	74.98 - THR	<i>Eurycoma longifolia</i> , <i>Ginkgo biloba</i> , <i>Tribulus terrestris</i>	Malaysia
SPC017	1. Propoxyphenyl-thiohydroxyhomosildenafil 2. Propoxyphenyl-hydroxyhomosildenafil	4.07 - SUB	<i>Eurycoma longifolia</i> , <i>Panax ginseng</i> , <i>Cordyceps sinensis</i>	Malaysia
SPC019	1. Sildenafil	111.27 - SPR	<i>Tribulus terrestris</i> , <i>Paullinia cupana</i> , <i>Citrus aurantium</i>	Not stated
SPC021	1. Sildenafil 2. Propoxyphenyl-sildenafil (<LOQ)	116.31 - SPR	<i>Curcuma longa</i> , <i>cactus extract</i>	Thailand
SPC022	1. Sildenafil 2. Propoxyphenyl-sildenafil (<LOQ)	71.02 - THR	<i>Crocus sativus</i> , <i>Cordyceps sinensis</i> , <i>snow lotus flower</i>	Hong Kong
SPC023	1. Tadalafil	34.77 - SPR	<i>Eurycoma longifolia</i> , <i>Lepidium meyenii</i> , <i>Lycium barbarum</i>	Malaysia
SPC027	1. Thiodimethylsildenafil 2. Dimethylsildenafil 3. Thiosildenafil 4. Propoxyphenyl-thiodimethylsildenafil	121.31 - SPR	<i>Eucommia ulmoides</i> , <i>Cynomorium songaricum</i> , <i>Ganoderma lucidum</i>	Malaysia
SPC028	1. Thiodimethylsildenafil 2. Dimethylsildenafil 3. Thiosildenafil (<LOQ)	23.84 - SUB	<i>Avena sativa</i> , <i>Okra mucilage</i> , <i>Desert cistanche</i>	Not stated
SPC029	1. Sildenafil 2. Thiodimethylsildenafil	24.68 - SUB	<i>Eurycoma longifolia</i> , <i>Ginkgo biloba</i> , <i>Tribulus terrestris</i>	Not stated

SPC030	1. Sildenafil	99.24 - THR	<i>Pausinystalia yohimbe, Eurycoma longifolia, Tribulus terrestris</i>	Indonesia
SPC031	1. Sildenafil	24.75 - SUB	<i>Cornus officinalis, Turnera diffusa, Ptychopetalum olacoides</i>	Indonesia
SPC032	1. Sildenafil	0.18 - SUB	<i>Eurycoma longifolia, Rehmanniae preparata, Eucommia ulmoides</i>	Not stated
SPT001	1. Sildenafil	0.03 - TRC	<i>Panax quinquefolius, Epimedium, Rhodiola rosea</i>	Hong Kong
SPT002	1. Sildenafil	0.18 - SUB	<i>Pausinystalia yohimbe, Tribulus terrestris, Panax ginseng</i>	Canada
SPT003	1. Vardenafil 2. Tadalafil *Suspected analytes 1. Aminotadalafil 2. Hydroxythiohomosildenafil	6.89 - THR	<i>Rhodiola rosea, dodder seed, Angelica</i>	China
SPT005	1. Sildenafil	67.93 - THR	Unspecified herbs	Not stated
SPT007	1. Sildenafil 2. Propoxyphenyl-sildenafil (<LOQ)	107.17 - SPR	Unspecified herbs	Hong Kong
SPT008	1. Sildenafil 2. Tadalafil	66.33 - THR	Unspecified herbs	Hong Kong
SPT012	1. Sildenafil	112.42 - SPR	<i>Boschniakia rossica, ginseng, medlar</i>	Hong Kong

SPT013	1. Sildenafil	100.20 - SPR	<i>Cordyceps, Tianshan snow lotus, Cistanche</i>	Not stated
SPT015	1. Sildenafil	88.43 - THR	Unspecified herbs	Hong Kong
SPT016	1. Sildenafil	113.94 - SPR	<i>Boschniakia rossica, ginseng, medlar</i>	Hong Kong
SPT017	Not detected *Suspected analyte 1. Aminotadalafil	-	<i>Cynomorium songaricum, kudzu roots, Cordyceps</i>	United States
SPT018	1. Sildenafil	91.90 - THR	<i>Boschniakia rossica, ginseng, medlar</i>	Hong Kong
SPT019	1. Sildenafil	59.49 - THR	<i>Cordyceps sinensis, ginseng</i>	Hong Kong
SPT020	1. Sildenafil	73.35 - THR	Unspecified herbs	Hong Kong

Note: TRC: trace, SUB: subtherapeutic, THE: therapeutic, SUP: suprathematic, *suspected analyte

Table 2: Suspected analytes detected from herbal remedy samples.

Sample	Suspected analytes	Retention time (min)	Theoretical accurate mass of [M+H] ⁺ (m/z)	Mass error (ppm)	Product ion 1 (m/z)	Mass error (ppm)	Product ion 2 (m/z)	Mass error (ppm)
SPC005	1. Hydroxythiohom sildenafil	23.92	521.1999	1.0	129.1022	-3.9	299.0961	-1.7
SPT003	1. Aminotadalafil	17.75	391.1401	-2.6	135.0441	-17.0	169.0760	-3.0
	2. Hydroxythiohom sildenafil	24.20	521.1999	2.3	129.1022	-7.7	299.0961	-6.0
SPT017	1. Aminotadalafil	17.57	391.1401	0.5	135.0441	-2.2	169.0760	-1.8

Accepted Article

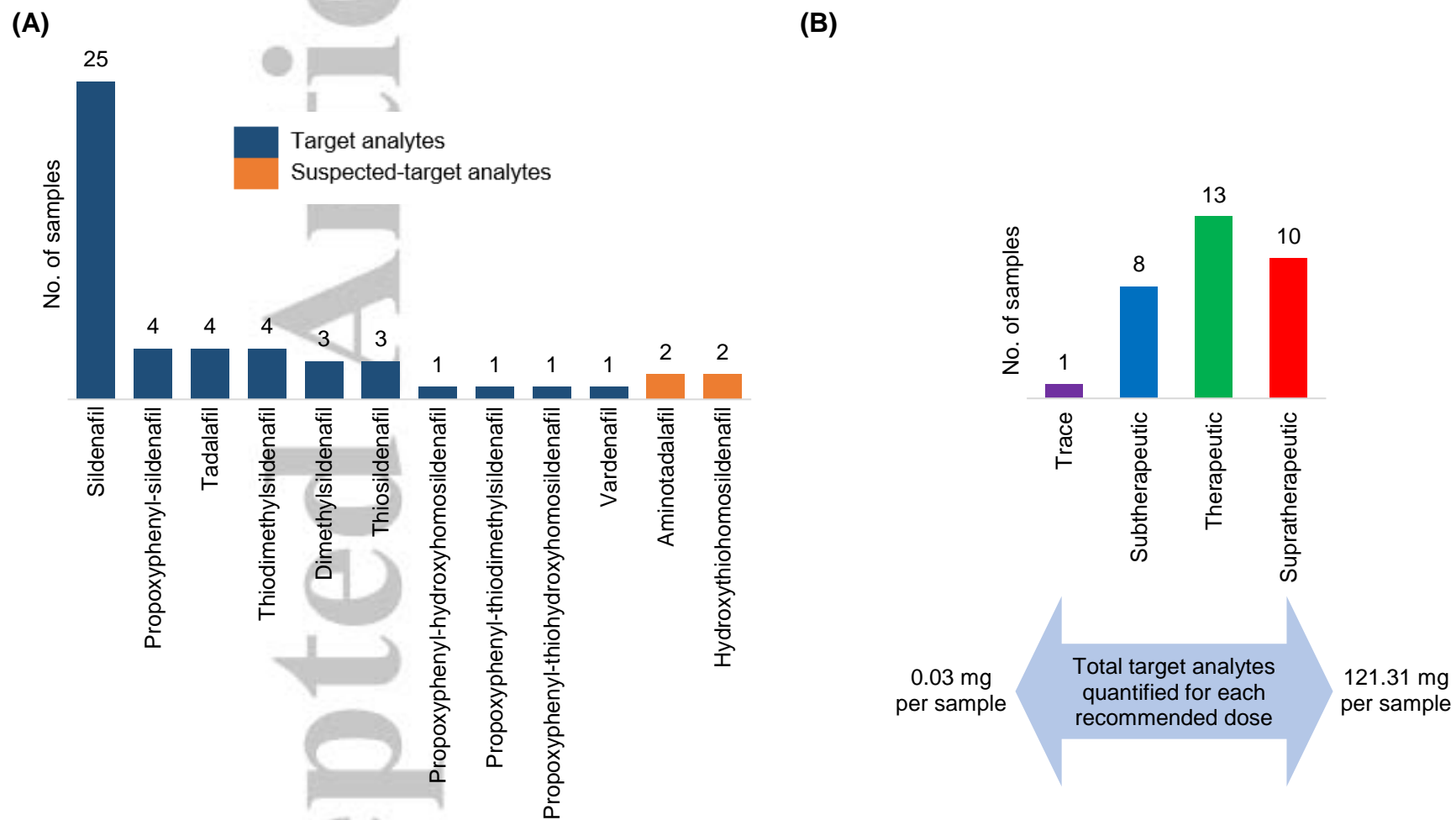


Fig. 1: Results summary of PDE5 inhibitors and their analogues in adulterated herbal remedy samples. (A) Identification of target analytes and detection of suspected analytes. (B) Quantification level of target analytes.

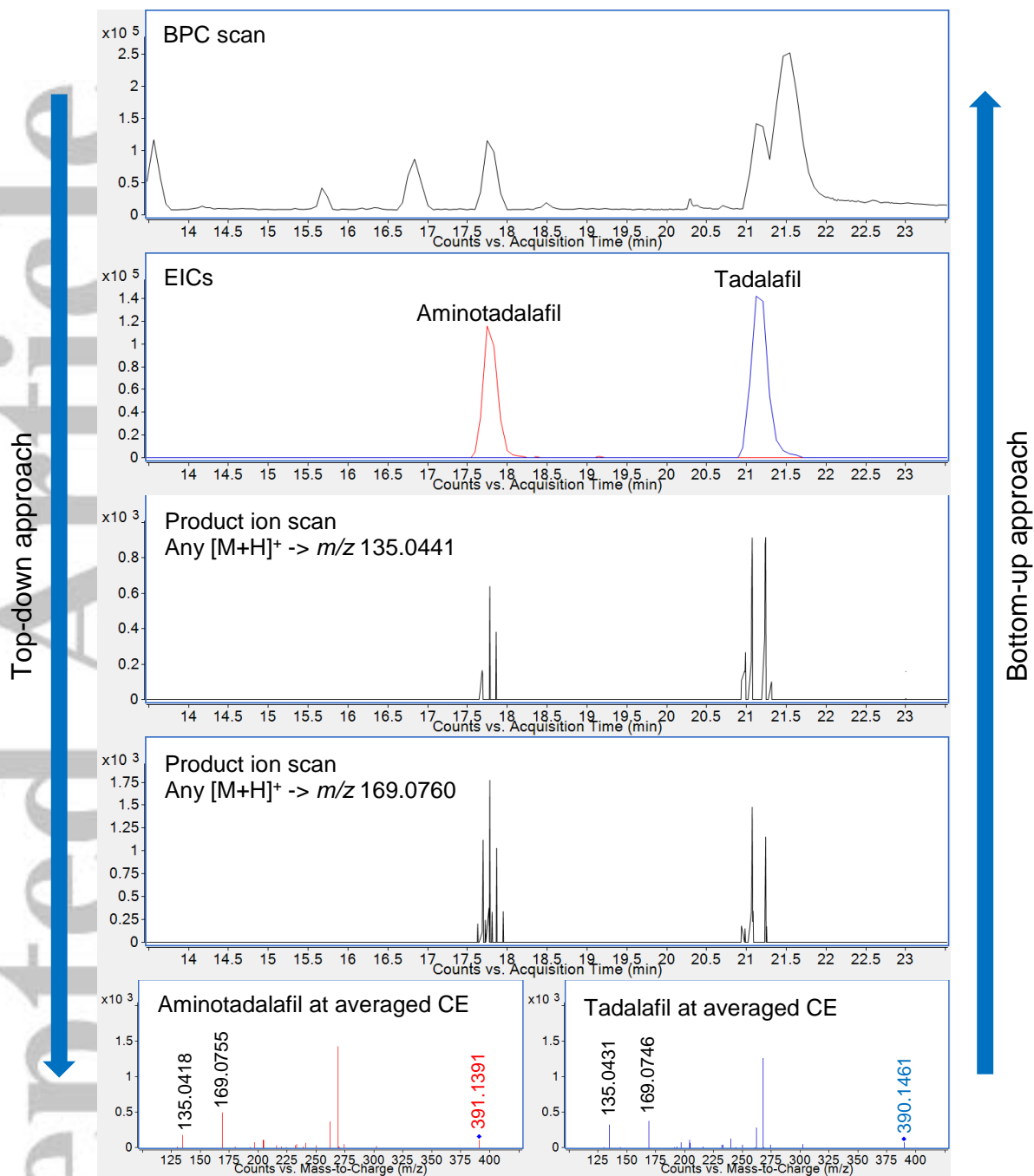


Fig 2: Representative base peak chromatogram (BPC); overlaid extracted ion chromatograms (EICs) of aminotadalafil and tadalafil; and tandem MS spectra of sample SPT003 demonstrating the non-targeted screening based on top-down and bottom-up approaches.

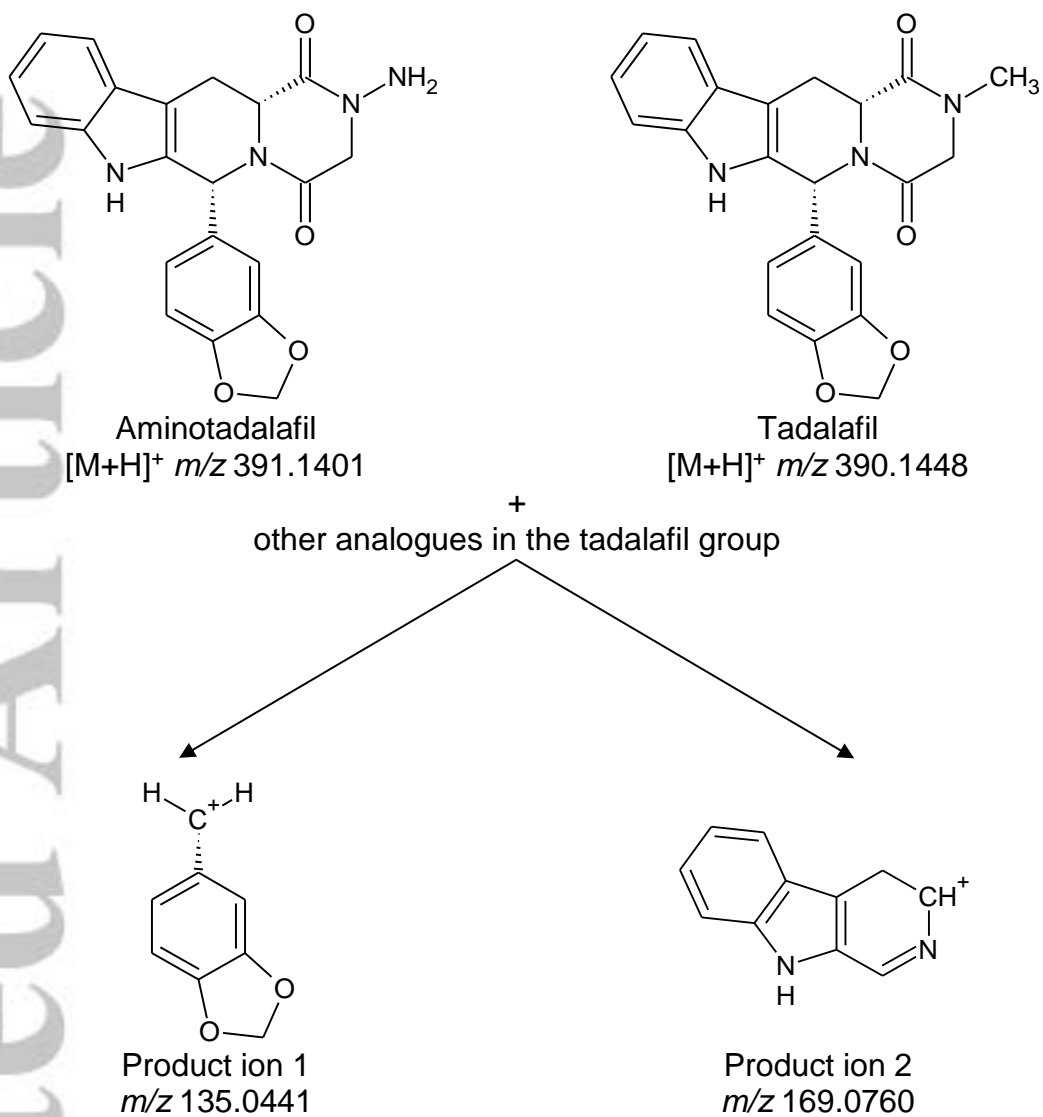


Fig. 3: The proposed common fragmentation pattern shared by the tadafafil group of analogues.

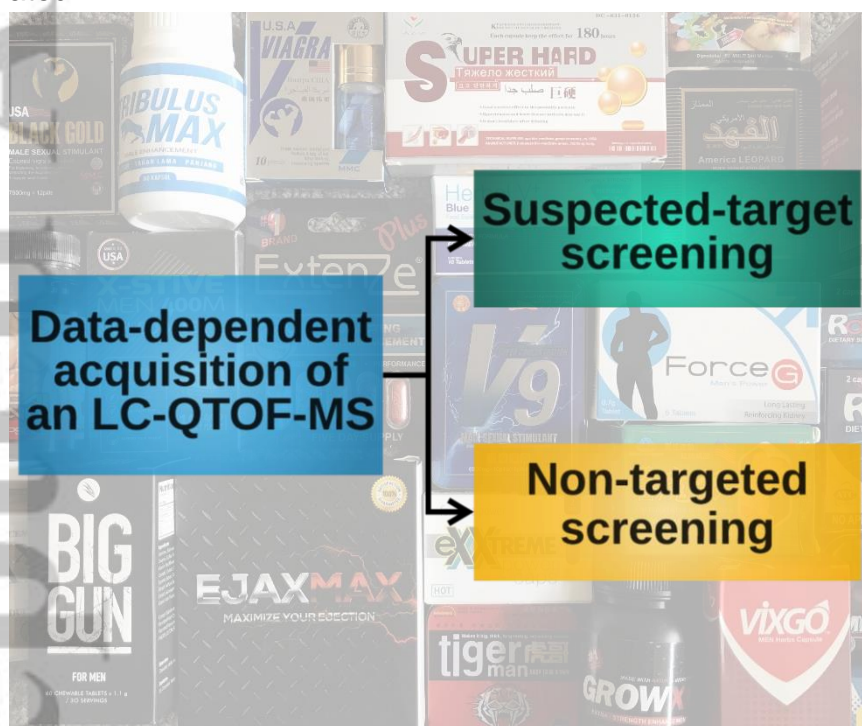
Graphical table of contents

RESEARCH ARTICLE

Special issue - Non-targeted screening of drugs

Drug Testing and Analysis

The data-dependent acquisition of an LC-QTOF-MS was optimised and validated to comprehensively screen PDE5 inhibitors in 52 herbal remedy samples, using suspected-target and non-targeted strategies. The suspected-target screening employed a library comprising 95 PDE5 inhibitors; resulted in 33 positive samples. Systematic MS and tandem MS interrogations of the non-targeted screening, performed using top-down and bottom-up approaches returned insignificant signals, indicating the absence of potentially novel analogues; thus, verifying the 19 non-adulterated samples.



Suspected-target and non-targeted screenings of phosphodiesterase 5 (PDE5) inhibitors in herbal remedies by liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTOF-MS)

Ahmad Yusri Mohd Yusop, Linda Xiao, and Shanlin Fu*