Data in brief 25 (2019) 104234

Contents lists available at ScienceDirect

Data in brief

journal homepage: www.elsevier.com/locate/dib

Data Article

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

Ahmad Yusri Mohd Yusop [a, b], Linda Xiao [a], Shanlin Fu [a, *]

[a] Centre for Forensic Science, University of Technology Sydney, Ultimo, NSW, 2007 Australia
[b] Pharmacy Enforcement Division, Ministry of Health, Petaling Jaya, Selangor, 46200 Malaysia

ARTICLE INFO

Article history:
Received 21 May 2019
Received in revised form 23 June 2019
Accepted 1 July 2019
Available online 8 July 2019

Keywords:
LC-HRMS
PDE5 inhibitors
Adulteration
Instant coffee premix
Optimisation
Validation

ABSTRACT

This paper presents the 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 and their analogues as adulterants in instant coffee premixes. The method development data covered chromatographic optimisation for better analyte separation and isomeric resolution, mass spectrometry optimisation for high sensitivity and sample preparation optimisation for high extraction recovery (RE) and low matrix effect (ME). The validation data covered specificity, linearity, range, accuracy, limit of detection, limit of quantification, precisions, ME, and RE. The optimisation and validation data presented here is related to the article: "Determination of phosphodiesterase 5 (PDE5) inhibitors in instant coffee premixes using liquid chromatography-high-resolution mass spectrometry (LC-HRMS)" Mohd Yusop et al., 2019.

DOI of original article: https://doi.org/10.1016/j.talanta.2019.05.078.
* Corresponding author.
E-mail address: shanlin.fu@uts.edu.au (S. Fu).

https://doi.org/10.1016/j.dib.2019.104234

Specifications table

Subject	Chemistry
Specific subject area	Analytical chemistry
Type of data	Table and extracted ion chromatograms (EICs)
How data was acquired	Liquid chromatography-high-resolution mass spectrometry (LC-HRMS) Agilent Technologies (Santa Clara, CA, USA) 1290 Infinity II LC system coupled to Agilent Technologies 6510 quadrupole time of flight-mass spectrometer (QTOF-MS)
Data format	Extracted and analysed LC-HRMS data
Parameters for data collection	Extraction of analyte-free blank instant coffee premix using dilute and shoot technique with methanol and modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) procedure
Description of data collection	Liquid chromatography-high-resolution mass spectrometry (LC-HRMS) for the determination of phosphodiesterase 5 (PDE5) inhibitors and their analogues as adulterants in instant coffee premixes
Data source location	Institution: University of Technology Sydney City/Town/Region: Ultimo, NSW, 2007 Country: Australia Latitude and longitude for collected data: 33.8832° S, 151.2005° E
Data accessibility	Data are available with this article
Related research article	A.Y. Mohd Yusop, L. Xiao, S. Fu, Determination of phosphodiesterase 5 (PDE5) inhibitors in instant coffee premixes using liquid chromatography-high-resolution mass spectrometry (LC-HRMS), Talanta 204 (2019) 36–43. https://doi.org/10.1016/j.talanta.2019.05.078

Value of the data

- The comprehensive optimisation procedures presented in this paper are valuable for those working on LC-HRMS method development, especially for complex matrices.
- Optimisation of chromatography, sample extraction, and sample dilution are the key to the minimisation of matrix effect.
- The validation data would serve as a reference for forensic drug testing laboratories working on a similar aim of combating adulterated consumable products.

1. Data

Fig. 1 shows the chromatographic separation of four different groups of structural isomers of phosphodiesterase 5 (PDE5) inhibitors displaying the extracted ion chromatograms (EICs) of the protonated molecule ($[M+H]^+$) precursor ions. Table 1 presents the comparison of matrix effect (ME) for instant coffee premix matrix using different extraction technique while Tables 2–4 summarise the validation data of the analytical method. The optimisation of the analytical method additionally described in this paper.

2. Experimental design, materials, and methods

The detail on the chemicals and reagents, standard solution and blank matrix preparation, and data analysis are described in [1].

2.1. Sample preparation

The whole content of an instant coffee premix sachet was weighed into a 50 mL polypropylene tube and recorded. Then, 100 mg of the powder was weighed into a 15 mL polypropylene tube and dissolved in 5 mL of acetonitrile and methanol (50:50, v/v), followed by vortex mixing for 1 minute, sonication for 20 minutes, and centrifugation for 5 minutes at 2500×*g*, successively. The whole upper solution was then transferred into another 50 mL polypropylene tube prefilled with half of a sachet of the Restek Q-sep QuEChERS salts. Immediately, the sample solution was vortexed for 1 minute followed by centrifugation for 5 minutes at 2500×*g* to separate the solid extraction salts. The upper layer was filtered using a 0.22 μm PTFE syringe filter and then diluted with methanol at 1:10 dilution level for analysis. The blank instant coffee premix was treated in the same manner as the steps described for the sample analysis. For quantification purpose, whenever the analyte concentration was beyond the linear range,

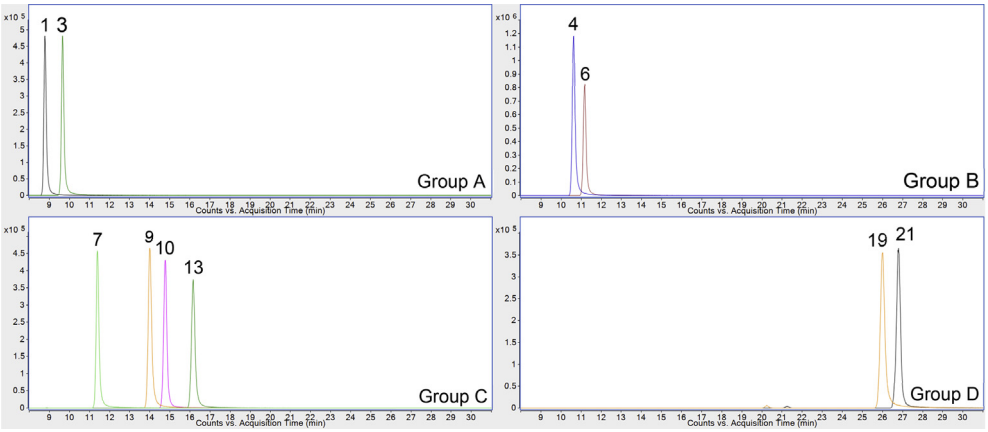

Fig. 1. Chromatographic separation of structural isomers with EICs of the $[M+H]^+$ precursor ions of: Group A (*m/z* 439.2452): desmethylcarbodenafil (1) and N-desethylacetildenafil (3); Group B (*m/z* 467.2765): acetildenafil (4) and dimethylacetildenafil (6); Group C (*m/z* 489.2279): vardenafil (7), homosildenafil (9), dimethylsildenafil (10), and propoxyphenyl-sildenafil (13); and Group D (*m/z* 505.2050): thiohomosildenafil (19) and thiodimethylsildenafil (21).

the sample solution was further diluted with methanol to fit the resulting concentration within the range of the constructed external calibration curve.

2.2. LC-HRMS conditions

The chromatographic separation was performed using an Agilent Technologies (Santa Clara, CA, USA) 1290 Infinity II LC system coupled to an Agilent Technologies 6510 quadrupole time of flight-mass

Table 1
ME for instant coffee premix using D&S technique with methanol and modified QuEChERS procedure at three levels of dilution.

No.	Analytes	ME (%) (n = 9)					
		Dilution 1:2		Dilution 1:10		Dilution 1:100	
		D&S	QuEChERS	D&S	QuEChERS	D&S	QuEChERS
1	Desmethylcarbodenafil	−19.7	−9.8	−6.4	+1.2	−0.9	−0.2
2	Carbodenafil	−21.1	−9.7	−5.1	+1.0	+0.3	0.0
3	N-desethylacetildenafil	−95.4	−23.3	−62.3	+0.1	+1.7	+1.5
4	Acetildenafil	−100.0	−36.7	−73.0	−5.2	−1.7	+0.3
5	Hydroxyvardenafil	−3.4	−6.1	+4.8	+5.3	+0.7	+2.7
6	Dimethylacetildenafil	−96.7	−22.7	−63.9	−2.9	−3.3	+1.2
7	Vardenafil	−2.9	−8.9	+1.5	+0.2	−1.1	−0.4
8	Sildenafil	−19.3	−11.8	−4.4	+1.3	0.0	+1.9
9	Homosildenafil	−23.0	−9.5	−8.4	+1.4	−1.9	+0.7
10	Dimethylsildenafil	−18.2	−6.0	−5.6	+5.3	−2.2	+3.1
11	Propoxyphenyl-hydroxyhomosildenafil	−12.9	−6.7	−2.7	+5.6	+0.1	+4.4
12	Udenafil	−15.2	−10.5	−4.3	+2.1	−0.5	+1.0
13	Propoxyphenyl-sildenafil	−20.7	−13.1	−8.3	−0.5	−3.1	+1.1
14	Hydroxythiovardenafil	−14.0	−10.1	−1.9	+5.4	−1.5	+3.4
15	Tadalafil	−23.3	−9.4	−10.2	+3.4	−1.9	+3.2
16	Mirodenafil	−14.6	−9.4	−3.9	+1.0	−2.2	+1.4
17	Mutaprodenafil	−11.8	−10.1	−3.5	+2.1	−1.7	+0.2
18	Thiosildenafil	−15.9	−7.1	−3.2	+5.1	−3.0	+3.6
19	Thiohomosildenafil	−21.4	−12.1	−5.4	+5.6	−2.9	+3.0
20	Dithiodesmethylcarbodenafil	−10.6	−8.9	−2.9	−4.9	−0.2	+0.2
21	Thiodimethylsildenafil	−24.0	−11.3	−4.2	+8.7	−2.6	+3.8
22	Propoxyphenyl-thiohydroxyhomosildenafil	−9.0	−2.8	+3.6	+7.3	−1.4	+5.8
23	Propoxyphenyl-thiodimethylsildenafil	−22.7	−6.9	−4.8	+6.4	−2.9	+4.2

Table 2
Retention time, accurate mass of $[M+H]^+$ precursor ion, mass error, and fragment ions of 23 targeted PDE5 inhibitors.

No.	Analytes	Retention time (min)	Accurate mass of $[M+H]^+$ precursor ion (*m/z*)		Mass error (ppm)	Fragment ion 1 (*m/z*)	Fragment ion 2 (*m/z*)
			Theoretical	Observed			
1	Desmethylcarbodenafil	8.78	439.2452	439.2452	0.00	311.1139	339.1452
2	Carbodenafil	9.23	453.2609	453.2600	−1.96	311.1139	339.1452
3	N-desethylacetildenafil	9.65	439.2452	439.2454	0.46	325.1295	297.1346
4	Acetildenafil	10.62	467.2765	467.2764	−0.21	127.1230	111.0917
5	Hydroxyvardenafil	10.80	505.2228	505.2222	−1.19	312.1581	151.0866
6	Dimethylacetildenafil	11.17	467.2765	467.2763	−0.43	325.1295	127.1230
7	Vardenafil	11.39	489.2279	489.2276	−0.61	312.1581	151.0866
8	Sildenafil	13.38	475.2122	475.2119	−0.63	283.1190	100.0995
9	Homosildenafil	13.99	489.2279	489.2274	−1.02	283.1190	113.1073
10	Dimethylsildenafil	14.77	489.2279	489.2279	0.00	283.1190	113.1073
11	Propoxyphenyl-hydroxyhomosildenafil	15.74	519.2384	519.2391	1.35	129.1022	283.1190
12	Udenafil	15.99	517.2592	517.2594	0.39	283.1190	112.1121
13	Propoxyphenyl-sildenafil	16.16	489.2279	489.2286	1.43	100.0995	283.1190
14	Hydroxythiovardenafil	18.36	521.1999	521.2000	0.19	328.1352	167.0637
15	Tadalafil	20.63	390.1448	390.1444	−1.03	169.0760	268.1081
16	Mirodenafil	21.59	532.2588	532.2588	0.00	312.1343	296.1394
17	Mutaprodenafil	21.81	630.2275	630.2275	0.00	113.1073	142.0070
18	Thiosildenafil	24.96	491.1894	491.1886	−1.63	100.0995	299.0961
19	Thiohomosildenafil	25.99	505.2050	505.2049	−0.20	299.0961	113.1073
20	Dithiodesmethylcarbodenafil	26.26	471.1995	471.1994	−0.21	343.0682	371.0995
21	Thiodimethylsildenafil	26.81	505.2050	505.2048	−0.40	113.1073	299.0961
22	Propoxyphenyl-thiohydroxyhomosildenafil	27.48	535.2156	535.2155	−0.19	129.1022	299.0961
23	Propoxyphenyl-thiodimethylsildenafil	30.36	519.2207	519.2210	0.58	113.1073	299.0961

spectrometer (QTOF-MS) equipped with a dual electrospray ionisation (ESI) nebuliser. The LC system was fitted with 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 binary mobile phase composition comprised solvent A (10 mM ammonium formate in ultrapure water) and solvent B (acetonitrile). Both solvents were acidified with 0.1% v/v formic acid. The gradient elution was initiated at 5% B and held for 1 minute, followed by a linear boost for 1 minute to 25% B. It was then slowly ramped up to 50% B for 30 minutes followed by a linear boost to 95% B for 1 minute. For the isocratic hold at high organic solvent, 95% B was held for 1 minute before immediately returning the system at 34.01 minutes to the initial gradient of 5% B for 6 minutes. The flow rate was maintained at 0.4 mL/min for the first 34 minutes before immediately ramping it to 1 mL/min between 34.01 and 40 minutes. Post-run equilibration was maintained at 0.4 mL/min for 5 minutes before the next injection. The injection volume was set for 5 μL with the autosampler compartment temperature maintained at 10 °C.

The QTOF-MS was operated at a low mass range of *m/z* 1700, calibrated before each chromatographic run to achieve an excellent mass accuracy. ESI in positive ionisation mode was employed with flow-dependent source parameters set at 300 °C for gas temperature, 12 L/min for drying gas flow, and 32 psig for nebuliser pressure. The compound-dependent source parameters were set at 3500 V for capillary voltage and 175 V for fragmentor voltage. Other common source parameters were maintained at 65 V for skimmer voltage and 750 V for OCT 1 RF Vpp. An auto MS/MS acquisition was selected for simultaneous MS and MS/MS experiments within a mass range of *m/z* 100 to 1100. The acquisition rates were set at 1 and 3 spectra/s for the MS and MS/MS experiments, respectively, with a narrow isolation width of *m/z* ~1.3. For the fragmentation of the $[M+H]^+$ precursor ion, the collision energy (CE) was fixed at 10, 20, and 40 eV in a separate scan with nitrogen as the collision gas. The reference mass solution was continuously infused through the reference nebuliser at a steady pressure of 5 psig.

Table 3
Coefficient of determination, accuracy, limit of detection (LOD), and limit of quantification (LOQ) of 23 targeted PDE5 inhibitors.

No.	Analytes	Coefficient of determination (r^2)	Accuracy (Mean ± SD, %) (n = 3)			LOD (ng/mL)	LOQ (ng/mL)
			Low	Med	High		
1	Desmethylcarbodenafil	0.9979	116.5 ± 3.4	102.9 ± 1.9	108.8 ± 2.2	10	80
2	Carbodenafil	0.9982	119.3 ± 1.1	100.4 ± 2.8	107.8 ± 1.6	10	80
3	N-desethylacetildenafil	0.9980	105.8 ± 2.5	96.6 ± 4.1	104.1 ± 2.8	30	80
4	Acetildenafil	0.9969	106.3 ± 0.4	106.8 ± 6.4	100.6 ± 1.7	20	80
5	Hydroxyvardenafil	0.9985	103.5 ± 2.2	108.8 ± 3.2	108.6 ± 1.5	10	80
6	Dimethylacetildenafil	0.9960	117.5 ± 3.0	94.8 ± 3.8	109.2 ± 1.9	20	80
7	Vardenafil	0.9972	94.8 ± 1.2	110.3 ± 1.4	107.0 ± 1.2	20	80
8	Sildenafil	0.9989	110.9 ± 1.3	101.0 ± 1.6	109.3 ± 1.5	30	80
9	Homosildenafil	0.9993	108.3 ± 1.0	105.8 ± 1.1	107.3 ± 1.2	40	80
10	Dimethylsildenafil	0.9989	116.5 ± 0.8	103.5 ± 1.2	107.7 ± 0.8	30	80
11	Propoxyphenyl-hydroxyhomosildenafil	0.9984	116.8 ± 3.6	103.2 ± 1.4	107.9 ± 2.4	70	80
12	Udenafil	0.9994	109.2 ± 1.9	105.8 ± 1.2	108.0 ± 1.4	10	80
13	Propoxyphenyl-sildenafil	0.9988	106.2 ± 1.0	107.4 ± 2.4	107.3 ± 1.6	10	80
14	Hydroxythiovardenafil	0.9984	98.5 ± 1.6	107.8 ± 1.0	107.5 ± 0.7	20	80
15	Tadalafil	0.9983	119.2 ± 4.4	106.5 ± 1.8	107.0 ± 3.1	60	80
16	Mirodenafil	0.9963	117.3 ± 1.4	101.6 ± 2.4	106.5 ± 0.7	10	80
17	Mutaprodenafil	0.9977	88.1 ± 1.6	108.4 ± 1.6	107.3 ± 1.4	20	80
18	Thiosildenafil	0.9972	97.5 ± 1.9	109.0 ± 1.6	107.6 ± 1.4	30	80
19	Thiohomosildenafil	0.9972	97.5 ± 1.5	109.6 ± 1.7	106.9 ± 1.9	10	80
20	Dithiodesmethylcarbodenafil	0.9960	90.7 ± 2.5	109.9 ± 1.3	106.7 ± 2.0	10	80
21	Thiodimethylsildenafil	0.9992	107.4 ± 2.2	105.2 ± 2.1	106.8 ± 0.8	20	80
22	Propoxyphenyl-thiohydroxyhomosildenafil	0.9986	104.3 ± 2.8	109.2 ± 1.2	108.4 ± 0.6	20	80
23	Propoxyphenyl-thiodimethylsildenafil	0.9989	106.9 ± 4.9	105.1 ± 1.6	108.6 ± 1.2	10	80

2.3. Optimisation of chromatographic separation

The simultaneous separation of a multi-analyte analysis can be a difficult task. Critical attention must be given for each target analyte to achieve an excellent chromatographic separation for reliable screening, identification, and quantification. The presence of four different groups of structural isomers with their $[M+H]^+$ precursor ions, i.e. Group A (*m/z* 439.2452): desmethylcarbodenafil (1) and N-desethylacetildenafil (3); Group B (*m/z* 467.2765): acetildenafil (4) and dimethylacetildenafil (6); Group C (*m/z* 489.2279): vardenafil (7), homosildenafil (9), dimethylsildenafil (10), and propoxyphenyl-sildenafil (13); and Group D (*m/z* 505.2050): thiohomosildenafil (19) and thiodimethylsildenafil (21) among the 23 targeted PDE5 inhibitors need to be addressed to achieve, if possible, an acceptable baseline chromatographic separation. Furthermore, matrix components from the instant coffee premix may cause a significant ME and impair the overall analytical method performance. Thus, the chromatographic separation will be optimised to resolve these issues apart from achieving a good peak shape and resolution, and reproducible retention time.

During the early stage of method development, the suitability and performance of two different columns were assessed using the initial scouting method. Both columns are of reverse phase but with slightly different specifications. The first column tested was Nucleoshell RP 18 (100 × 4.6 mm, 2.7 μm) produced a very broad peak with severe peak tailing for most target analytes. The second column, Chromolith® High Resolution RP-18 end-capped (100 × 4.6 mm, 2.0 μm), displayed an exceptional narrow peak for almost all target analytes with peak asymmetry factor of less than 1.2 based on the symmetrical shape of a Gaussian peak. Although both columns have identical length and internal diameter, the reduction in the particle size of the Chromolith® column led to higher peak efficiencies and thus was chosen for the final methodology.

Organic solvents such as acetonitrile and methanol [2,3] are widely utilised in the analysis of PDE5 inhibitors using LC-MS/MS technique, were initially assessed. Acetonitrile produced a good chromatographic separation for most target analytes compared to methanol and thus selected as the organic mobile phase. Due to the presence of multiple basic amine groups within PDE5 inhibitors,

Table 4
Precisions, ME, and RE of 23 targeted PDE5 inhibitors.

No.	Analytes	Precisions (%RSD) (n = 9)						ME (%) (n = 9)	RE (Mean ± SD, %) (n = 3)		
		Repeatability			Intermediate						
		Low	Med	High	Low	Med	High		Low	Med	High
1	Desmethylcarbodenafil	4.4	2.0	2.1	3.1	2.9	3.0	+1.2	117.4 ± 6.1	112.5 ± 1.8	97.6 ± 1.1
2	Carbodenafil	1.4	3.1	1.5	5.3	4.1	1.7	+1.0	115.1 ± 2.2	111.4 ± 0.9	100.3 ± 0.3
3	N-desethylacetildenafil	2.7	4.4	2.7	3.0	1.5	2.0	+0.1	53.8 ± 0.8	65.1 ± 2.0	95.1 ± 0.6
4	Acetildenafil	0.4	6.2	1.7	6.7	7.7	9.1	−5.2	87.3 ± 0.1	84.7 ± 0.4	111.0 ± 4.5
5	Hydroxyvardenafil	2.5	3.1	1.4	6.1	1.5	2.6	+5.3	116.7 ± 2.6	116.6 ± 1.2	106.4 ± 0.4
6	Dimethylacetildenafil	3.4	4.3	1.8	1.4	2.1	1.0	−2.9	85.1 ± 4.6	83.9 ± 4.4	89.1 ± 4.4
7	Vardenafil	1.2	1.2	1.1	2.3	2.6	2.9	+0.2	123.0 ± 8.6	115.5 ± 1.8	108.9 ± 1.1
8	Sildenafil	1.7	1.7	1.4	5.6	3.1	2.6	+1.3	113.7 ± 4.5	109.5 ± 1.2	103.9 ± 1.8
9	Homosildenafil	1.2	1.1	1.2	3.6	1.8	1.4	+1.4	116.0 ± 5.4	110.1 ± 1.8	103.5 ± 0.8
10	Dimethylsildenafil	1.1	1.3	0.8	6.9	2.7	4.1	+5.3	115.3 ± 6.0	109.0 ± 1.6	103.1 ± 0.5
11	Propoxyphenyl-hydroxyhomosildenafil	4.5	1.5	2.3	7.2	7.3	5.5	+5.6	117.3 ± 6.7	114.3 ± 0.6	99.1 ± 11.8
12	Udenafil	2.1	1.2	1.3	0.6	1.2	2.2	+2.1	113.3 ± 2.8	109.4 ± 1.2	102.3 ± 1.6
13	Propoxyphenyl-sildenafil	1.1	2.3	1.5	6.2	3.2	1.6	−0.5	123.5 ± 0.2	120.4 ± 2.2	108.5 ± 0.6
14	Hydroxythiovardenafil	1.6	1.0	0.6	4.0	2.2	2.3	+5.4	111.1 ± 1.4	109.4 ± 1.8	107.7 ± 0.3
15	Tadalafil	7.2	1.9	3.1	6.7	1.4	1.1	+3.4	103.5 ± 8.3	101.5 ± 4.0	99.3 ± 2.2
16	Mirodenafil	2.0	2.6	0.7	2.3	2.7	3.1	+1.0	111.0 ± 3.4	109.7 ± 1.5	103.5 ± 1.3
17	Mutaprodenafil	1.7	1.4	1.3	1.2	1.3	0.5	+2.1	119.6 ± 5.6	112.0 ± 1.5	105.9 ± 0.9
18	Thiosildenafil	2.3	1.5	1.3	3.6	1.9	0.9	+5.1	114.2 ± 4.6	108.8 ± 0.6	107.1 ± 1.4
19	Thiohomosildenafil	1.6	1.6	1.8	1.7	1.6	1.1	+5.6	114.1 ± 3.7	106.7 ± 0.9	110.1 ± 1.9
20	Dithiodesmethylcarbodenafil	2.4	1.1	1.9	6.6	1.3	0.5	−4.9	111.9 ± 2.1	105.6 ± 5.2	107.2 ± 5.2
21	Thiodimethylsildenafil	3.1	2.2	0.8	1.7	1.9	1.7	+8.7	113.4 ± 3.9	107.0 ± 0.4	112.9 ± 0.5
22	Propoxyphenyl-thiohydroxyhomosildenafil	3.9	1.2	0.6	5.7	2.1	4.1	+7.3	111.0 ± 4.0	110.6 ± 1.5	109.4 ± 1.6
23	Propoxyphenyl-thiodimethylsildenafil	7.3	1.7	1.2	1.0	0.6	1.4	+6.4	111.0 ± 4.2	107.1 ± 0.8	104.8 ± 1.2

pH-dependent chromatographic problems are expected to be observed as these analytes may exist in both neutral and ionised forms. Therefore, the pKa values of these target analytes were first evaluated using ChemAxon (Budapest, Hungary) software which revealed some significant variations depending on their chemical structure. Taking all these pKa values into consideration led to the selection of ammonium formate as a matrix modifier attributable to its pKa values and useful pH range, and its superior volatility, which is essential for LC-MS/MS analysis. The ammonium formate was assessed at three different concentrations of 5, 10, and 20 mM. The 10 mM buffer solution proved to be adequate to achieve reproducible retention and improved separation with excellent peak shape and resolution for all target analytes in the presence of 0.1% v/v formic acid in the mobile phases.

The elution profile covered 5%–95% of the organic solvent to account for the suspected-target and non-targeted screening approaches. Also, the chromatographic gap at the first quarter of the runtime permitted any highly polar and unretained matrix components to be eluted first, minimising the possibilities of co-elution between matrix components and target analytes. The same principle applied in the last quarter of the chromatographic run where strongly retained matrix components eluted. These strategies were adopted to minimise any interference from the instant coffee premix matrix which may lead to a significant ion suppression or ion enhancement of target analytes.

The chromatographic elution profile incorporated an extensive column re-equilibration segment in the developed method. Immediately after conditioning at 34.01 minutes, the re-equilibrium time was maintained at the initial gradient percentage for about 6 minutes by ramping up the flow rate to 1 mL/min. Consequently, the column was flushed with approximately five times of the column volume before the next sample injection. Apart from achieving a good separation and a constant retention time for all target analytes, quantitative variability was minimised, and the carry-over effect was not observed in subsequent analysis. Besides, all four different groups of structural isomers were separated down to a baseline level, ensuring the specificity of each target analyte as illustrates in Fig. 1.

2.4. Optimisation of MS conditions

The first MS issue encountered during the method development phase was the presence of sodium adducts, especially with analytes containing carbonyl groups. Although this phenomenon often observed in previous studies, it has not been acknowledged and addressed. It is widely known that one of the most common sources of this metal adduct contamination is the laboratory glassware [4]. Plasticware, therefore, was utilised throughout the whole experimental processes. High drying gas temperature may also contribute to the formation of sodium adducts; thus, the temperature was lowered from 350 °C to 300 °C. These approaches led to the absence of metal adducts, particularly single charge sodium adducts.

All other MS source parameters were adjusted based on the flow- and compound-dependent parameters. The flow-dependent source parameters, which include nebuliser pressure and drying gas flow, were adjusted based on the flow rate of 0.4 mL/min. The compound-dependent source parameters, i.e. capillary voltage, fragmentor voltage, and CE, were adjusted based on the mass range of *m/z* 100 to 1100. The continuous infusion of two reference masses viz. purine (*m/z* 121.050873) and hexakis(1H, 1H, 3H-tetrafluoropropoxy)phosphazine (*m/z* 922.009798) during each chromatographic run led to the mass accuracy of less than 2 ppm for $[M+H]^+$ precursor ions and less than 5 ppm for fragment ions.

2.5. Optimisation of sample preparation

Even though the chromatographic separation and MS conditions have been fully optimised, the widely used dilute and shoot (D&S) technique initially applied on instant coffee premixes with either methanol, acetonitrile, or in combination with ultrapure water often end up in severe and moderate MEs for most target analytes. During the early stage of method development, methanol was chosen as the solvent for the D&S technique on instant coffee premixes as it produced the least number of target analytes exhibiting ME.

The optimisation of sample preparation was mainly evaluated based on the ME and extraction recovery (RE) efficiency. As the D&S technique using methanol is very simple, a dilution approach was further attempted to minimise any possible ME that may arise from the instant coffee premix matrix. The matrix was assessed at three levels of dilution at 1:2, 1:10, and 1:100, while maintaining the concentration of target analytes at low, medium, and high quality control (QC) levels.

As expected, the D&S technique at the lowest dilution level of 1:2 had caused severe and moderate ionisation suppressions for three and seven analytes, respectively. Even at 1:10 dilution, severe MEs were still prominent for the same three analytes that belong to the acetyl-bonded analogue of sildenafil. The MEs for the D&S technique were found to be reduced in proportion to the matrix dilution and turn out to be insignificant at 1:100 dilution. However, one major concern at this dilution level was the ability of the developed procedure to detect target analytes at trace concentrations.

Consequently, another sample extraction technique was assessed in a review of the first attempted D&S technique. The quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction technique was selected primarily based on its time-saving advantage of sample preparation. To the best of our knowledge, only two previous studies had employed the AOAC official 2007.01 QuEChERS method to determine PDE5 inhibitors in herbal dietary supplements [5] and in Chinese tonic liquors [6]. On the contrary, in this study, the EN 15662 QuEChERS salts were chosen due to its claimed capacity in removing polar interferences, sugars, and fats that might be present in the sample matrix. Unfortunately, the official buffered EN 15662 method using acetonitrile and hydrated instant coffee premix give rise to poor RE for some target analytes. Therefore, several modified QuEChERS procedures were attempted on a trial-and-error basis with varying solvent mixtures, sample loads and QuEChERS salts quantities. The final methodology is given in detail in Section Sample preparation.

The modified QuEChERS extraction procedure was also evaluated at three dilution levels like the D&S technique discussed above. The instant coffee premix matrix at 1:10 dilution exhibited an insignificant ME for all target analytes with the percentage of within −5.22 to +8.67. Equally, the RE proved to be excellent for all target analytes at low, medium, and high QC levels within ±25% except for N-desethylacetildenafil at low (53.8%) and medium (65.1%) QC levels. The analysis of real samples had

evinced that any trace analyte was highly unlikely to produce false negative identification at this dilution level as opposed to a higher level of dilution. Table 1 displays the comparison of the ME for all 23 targeted PDE5 inhibitors using the D&S technique with methanol against the modified QuEChERS procedure on the instant coffee premix at different dilution levels.

2.6. Method validation

The analytical method validation was performed in accordance with the described procedure in Section Method validation of Ref. [1]. The data on each validation parameter are presented in Tables 2–4

Acknowledgements

This work was supported by the University of Technology Sydney, Higher Degree by Research Student Project Funding.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] A.Y. Mohd Yusop, L. Xiao, S. Fu, Determination of phosphodiesterase 5 (PDE5) inhibitors in instant coffee premixes using liquid chromatography-high-resolution mass spectrometry (LC-HRMS), Talanta 204 (2019) 36–43. https://doi.org/10.1016/j.talanta.2019.05.078.
[2] F. Shi, C. Guo, L. Gong, J. Li, P. Dong, J. Zhang, P. Cui, S. Jiang, Y. Zhao, S. Zeng, Application of a high resolution benchtop quadrupole-Orbitrap mass spectrometry for the rapid screening, confirmation and quantification of illegal adulterated phosphodiesterase-5 inhibitors in herbal medicines and dietary supplements, J. Chromatogr. A 1344 (2014) 91–98. https://doi.org/10.1016/j.chroma.2013.12.030.
[3] S. Strano-Rossi, S. Odoardi, E. Castrignano, G. Serpelloni, M. Chiarotti, 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. 106 (2015) 144–152. https://doi.org/10.1016/j.jpba.2014.06.011.
[4] N.B. Cech, C.G. Enke, Practical implications of some recent studies in electrospray ionization fundamentals, Mass Spectrom. Rev. 20 (6) (2001) 362–387. https://doi.org/10.1002/mas.10008.
[5] L. Vaclavik, A.J. Krynitsky, J.I. Rader, Targeted analysis of multiple pharmaceuticals, plant toxins and other secondary metabolites in herbal dietary supplements by ultra-high performance liquid chromatography-quadrupole-orbital ion trap mass spectrometry, Anal. Chim. Acta 810 (2014) 45–60. https://doi.org/10.1016/j.aca.2013.12.006.
[6] B. Ding, Z. Wang, J. Xie, G. Zeng, W. Chen, Phosphodiesterase-5 inhibitors in Chinese tonic liquors by liquid chromatography coupled with quadrupole time of flight mass spectrometry, Food Addit. Contam. Part B Surveill. 11 (3) (2018) 214–222. https://doi.org/10.1080/19393210.2018.1488769.