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Rapid determination of sildenafil and its analogues in dietary supplements using gas chromatography–triple quadrupole mass spectrometry

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ABSTRACT

Application of gas chromatography–triple quadrupole mass spectrometry for identification, confirmation and quantification of 6 phosphodiesterase-5 (PDE-5) inhibitors (sildenafil, dimethylsildenafil, homosildenafil, thiosildenafil, thiodimethylsildenafil and thiohomosildenafil) in dietary supplements was investigated. The MS was operated in multiple reaction monitoring mode, for better sensitivity and selectivity. In this manner, the method is adequate to reduce background noise with less interference from co-eluting compounds in the samples. Two different ionisation techniques, electron ionisation (EI) and chemical ionisation (CI), were studied and compared. The chromatographic separation was performed on a short 10 m non-polar capillary column without any derivatisation step. This permitted fast analysis for all analogues with retention time less than 11 min, for both techniques. Use of backflushing can aid method retention time reduction and improves column maintenance. Evaluation of method validation included limit of detection (LOD), lower limit of quantitation (LLOQ), linearity, precision and recovery were performed for both EI and CI techniques. The LOD obtained varied from 0.03 to 1.50 μg/g and the LLOQ ranged from 0.10 to 5.00 μg/g. Good calibration linearity was obtained for all analogues for both techniques, with correlation coefficients (r^2) higher than 0.99. Mean recoveries of all analogues using CI show higher values (83.4–108.8%) than that of EI (61.9–91.1%). The intra- and inter-assay precisions were evaluated for all analogues at spiked concentration of 10 μg/g and the relative standard deviation was less than 15% for both methods. These methods were then successfully applied to dietary supplement samples without prior derivatisation, confirming that the samples were adulterated with sildenafil and/or its analogues.

1. Introduction

Sildenafil citrate is a synthetic phosphodiesterase type 5 enzyme inhibitor (PDE-5), used to treat male erectile dysfunction (ED) and pulmonary arterial hypertension (PAH). It is widely marketed under the name Viagra® (manufactured by Pfizer) [1]. The U.S Food and Drug Administration (FDA) has approved this drug, together with vardenafil (Levitra®, manufactured by Bayer) and tadalafil (Cialis®, developed and marketed by Lilly ICOS). However, several sildenafil analogues are increasingly found as adulterants in food and nutrient supplements outside the official health system. These analogues are structurally modified in the piperazine moiety or carbonyl in pyrazolopyrimidine moiety substituted with a thiocarbonyl group [2]. For instance, thioketone analogues may be synthesised by heating sildenafil with phosphorus pentasulfide (P_2S_5) and the C=O bond exchanged to C=S bond. Furthermore, such analogues are not generally registered as drug substances by the European Medicine Agency (EMA) and U.S Food and Drug Administration (FDA), but may be present as adulterants [3]. They can be considered as "designer drugs" and have no established safety profile. It is important to monitor these drugs because they can cause problems including adverse effects on cardiovascular function such as arterial systemic blood pressure reduction, headaches, facial flushing, dyspepsia, visual disturbances and back pain [4].

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Table 1
Significant peaks in EI and CI full scan MS for sildenafil and its analogues by GC–QQQMS with the relative ion abundances (%). Most abundant peak for each compound is shown in bold.

Analyte	Molecular ion, M^+[a]	Abundance peaks in full scan MS in order of relative abundance (%)	
		EI	CI
Sildenafil	474.2	**99.1** (100), 56.1 (30), 404.3 (10), 474.7 (0.1)	**475.3** (100), 313.2 (12.3)
Dimethylsildenafil	488.2	**113.2** (100), 70.1 (20), 488.4 (0.1)	**489.3** (100), 313.2 (18.1)
Homosildenafil	488.2	**113.1** (100), 70.1 (47.5), 404.3 (10), 488.4 (0.3)	**489.4** (100), 313.3 (14.9)
Thiosildenafil	490.2	**99.1** (100), 56.1 (32.5), 420.2 (7.5), 490.3 (0.8)	**491.3** (100), 223.1 (15.2), 299.2 (12.7)
Thiodimethylsildenafil	504.2	**113.2** (100), 70.1 (17.5), 504.3 (0.3)	**505.4** (100), 329.1 (8.4)
Thiohomosildenafil	504.2	**113.1** (100), 70.1 (32.5), 420.2 (5), 504.4 (1.2)	**505.3** (100), 313.3 (7.5), 223.2 (5.2)

[a] Molecular ion for each compound according to isotopic distribution (m/z).

Recently, numerous websites have been launched, offering various formulations for sale that potentially contain PDE-5. Interestingly, they often claim that the products (e.g., herbal products, dietary supplements or food products) are safe by labeling them as "all natural". Products may be sold at cheaper price, as compared to other health products, which have been approved by the FDA and other national regulatory bodies. They are adulterated by minor modification of the parent structure of approved PDE-5 inhibitors [5]. Development of a sensitive and accurate method to screen and confirm the illegal adulterants in dietary supplements, herbal and food products are of urgent priority.

Most studies reporting identification of sildenafil and its analogues have used liquid chromatography coupled with mass spectrometry (LC–MS) [6,7], high performance liquid chromatography (HPLC) [8,9], LC–tandem MS (LC–MS/MS) [9–12] and ultra-performance LC-time of flight MS [13]. Recently, high resolution benchtop quadrupole-Orbitrap (Q-Orbitrap) mass spectrometry was applied for the detection of illegal adulterants in herbal medicines and dietary supplements [14]. However, the conventional HPLC method has a limitation because the method cannot supply characteristic mass spectral library data, which can assist to discriminate between a wide range of analogues or chemicals. Application of analytical gas chromatography (GC) is largely restricted to low molecular mass, higher volatility and thermally stable compounds [5]. Thermal stability and volatility may be improved through derivatisation, but it is difficult to derivatise sildenafil analogues with standard silylation reagents [15]. Nevertheless, GC may be a preferred option because of simplicity, affordability, low maintenance and well defined MS library databases compared to LC–MS. GC with MS (GC–MS) has been applied to analyse sildenafil, tadalafil and vardenafil in several samples such as biological samples [16–20] food supplements [13,21], and herbal products [22] and pharmaceutical product [23]. However, few studies report determination of either thio or non-thio sildenafil analogues by GC–MS. Thus, further investigation is required because the safety of these analogues has not been clinically tested, and there is a lack of MS library data for sildenafil identification. Prior study identified several thioketone analogues of sildenafil by using GC–MS, focusing on fast identification of the analogues with characteristic mass fragmentations, without further investigation [2].

To improve the identification power of GC–MS, GC–MS/MS with a triple quadrupole instrument can be used to provide greater sensitivity and selectivity of identification because it allows the classic possibility to monitor only the analyte of interest through selection of appropriate ions. Operation in multiple reaction monitoring (MRM) mode (also termed selected reaction monitoring; SRM) is beneficial to accurate identification, confirmation and quantification of some components in a sample [24]. Using the first quadrupole for precursor ion selection, the second as collision cell, and the third quadrupole for product ion selection, high discrimination against background signals should be achievable [25].

Further, chemical ionisation (CI) can support ionisation of labile drugs, in contrast to electron ionisation (EI). The CI soft ionisation technique produces minimal fragmentation of molecular ions, so the molecular species can be more readily identified [26]. The CI mode is particularly useful in target analysis when the molecular weight is known, such as in determination of volatile fatty acids [27], amphetamines [28], amino acids [29] and others. Furthermore, use of CI and MRM mass spectra can be used as a supplemental database simultaneously with an EI mass spectrum database [30].

The aim of this study was to demonstrate rapid identification and quantification for sildenafil and its analogues without use of derivatisation, by using GC–QQQMS with both EI and CI techniques. The method was then applied to analysis of PDE-5 in dietary supplements. To date, little quantitative analysis has been applied to sildenafil and its analogues using GC.

2. Experimental

2.1. Chemicals and reagents

Standards of six sildenafil and its analogues (sildenafil, dimethylsildenafil, homosildenafil, thiosildenafil, thiodimethylsildenafil, and thiohomosildenafil) were obtained from TLC PharmaChem Inc. (Vaughan, Ontario, Canada). Their chemical structures are shown in Supporting information Fig. S1. Ethyl acetate was purchased from Merck kGaA (Darmstadt, Germany) while octacosane (C28) alkane used as internal standard (IS), was obtained from Sigma–Aldrich (St. Louis, USA). Stock solutions of each analyte were prepared as 100 μg/mL stock solutions in ethyl acetate and stored at −4 °C. Mixtures of lower concentration standard solutions were prepared via dilution of the stock solutions in ethyl acetate and prepared fresh daily when analysis was conducted.

2.2. Instrumentation

Analyses were determined using a Bruker Scion 456 TQ GC–MS/MS system (Bruker, Preston, Australia) operated by MS Workstation version 8. Separations were carried out using a BPX5 capillary column (10 m × 0.25 mm I.D × 0.1 μm film thickness (d_f); SGE Analytical Science, Australia). Under fast GC–MS/MS, the initial column temperature was set at 150 °C (0 min), increased to 320 °C at 20 °C/min and held for 5 min. An injection volume of 1 μL was employed in the splitless mode. Helium was used as carrier gas at a constant flow rate of 1.5 mL/min. Temperatures of the injector and the transfer line were held at 280 °C and 300 °C, respectively. The MS was operated in either full scan or MRM/SIM mode using electron ionisation (EI) energy of 70 eV with ion source temperature at 280 °C. The mass scan range for full scan mode was m/z 50–550 and the abundance ions obtained are listed in Table 1. The total run time was 13.50 min. MS/MS mode used argon (99.9999%