THESIS

The presence of adulterants and mislabeling of caffeine content in Australian sports supplements

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BSc Forensic Biology and Toxicology

This thesis is presented for the degree of
Master of Forensic Science (Professional Practice and Research) of Murdoch University

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2023

Thesis Declaration

I Joe Finan verify that in submitting this thesis;

the thesis is my own account of the research conducted by me, except where other sources are fully acknowledged in the appropriate format,

the thesis contains as its main content work which has not been previously submitted for a degree at any university,

the University supplied plagiarism software has been used to ensure the work is of the appropriate standard to be stored on the Murdoch University Research Repository,

that all necessary ethics and safety approvals were obtained, including their relevant approval or permit numbers, as appropriate, and

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Signed:

Acknowledgements

Thank you to both Dr. John Coumbaros and Dr. Garth Maker for all their assistance and guidance throughout the completion of my research. Without their help, this research would have never been completed, and I thank you both for your continued support throughout the year.

Table of Contents

Murdoch University

Sports supplement adulteration: A systematic review

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Date: June 11, 2023

1 Contents

2 Abstract

Background: Adulteration of sports supplements is an increasing issue that has changed over the years. Within the past few years, Australia has implemented new legislation that alters how supplements are regulated. Before this new regulation, little research had been conducted within the country surrounding the adulteration of sports supplements, predominately weight loss and pre-workout supplements. With the changes in adulteration being seen on a global scale, the characteristics and trends can be applied to the Australian context.
Methods: This systematic literature review investigated literature surrounding the adulteration of sports supplements, predominately weight loss and pre-workout supplements, from around the world. The review included an assessment of analysis methods utilised for the detection and analysis of adulterants such as sibutramine, DMAA, DMBA, sildenafil and others. Various databases were searched for relevant articles that included the testing of supplements relevant to the review, along with other inclusion criteria set out. The time frame in which it was searched ranged from 2013 until the present day, with the identification of 81 articles.
Results: Reviewing the included articles, it was found that current adulterants used in weight loss and pre-workout supplements have remained consistent over the ten-year period reviewed, with many adulterants banned in most countries. Common adulterants included sibutramine, phenethylamine, DMAA and phenethylamine. The regulation of supplements also affected the number of articles a country would publish due to the difference in how strict some countries regulation was compared to others; with the US topping the list for the highest number. The analysis techniques used showed two distinctive types, one being the methods of screening for specific adulterants. At the same time, the other techniques were for screening for the presence of unknown adulterants. The review revealed that most of the techniques utilised for screening would be unable to detect/quantify designer adulterants.
Conclusions: The need for more studies into adulteration within Australia was evident, including the need to conduct market surveys to evaluate the extent of adulteration within the supplement market. With the implementation of new legislation within the country, future research should be conducted to evaluate its effectiveness. Comparing legislation to other countries will help improve Australian regulation and is essential to stopping adulteration.

3 List of Figures/Tables

4 List of Abbreviations

1,4-DMAA	1,4-Dimethylamylamine
ANVISA	Brazilian Health Regulatory Agency, Agência Nacional de Vigilância Sanitária
ATR-FTIR	Attenuate total reflectance-Fourier transform infrared spectroscopy

C^4D	Coupled contactless conductivity detector
CE-QqQ-MS	Capillary electrophoresis triple quadrupole mass spectrometry
CNS	Central nervous system
CZE	Capillary zone electrophoresis
DART-QqQ-MS	Direct analysis in real time triple quadrupole mass spectrometry
DEPEA	N-*a*-diethylphenethylamine
DMAA	1,3-Dimethylamylamine
DMBA	1,3-Dimethylbutylamine
DSHEA	Dietary Supplement Health and Education Act
FDA	Food and Drug Administration
FI-QqQ-MS	Field ionization triple quadrupole mass spectrometry
FSANZ	Food Standards Australia New Zealand
GC-MS	Gas chromatography mass spectrometry
GC-MS/NPD	Gas chromatography mass spectrometry nitrogen phosphorus detector
GC-QqQ-MS	Gas chromatography triple quadrupole mass spectrometry
GC-Q-ToF-MS	Gas chromatography quadrupole time-of-flight mass spectrometry
GT-LC-Qq-LIT-MS	Graphene tip liquid chromatography triple-quadrupole linear trap mass spectrometry
HPLC	High performance liquid chromatography
HPLC-MS	High performance liquid chromatography mass spectrometry
HPLC-orbi-MS	High performance liquid chromatography orbitrap mass spectrometry
HPLC-PAD	High performance liquid chromatography pulsed amperometric detection
HPLC-PDAD	High performance liquid chromatography photodiode array detector
HPLC-QqQ-MS	High performance liquid chromatography triple quadrupole mass spectrometry
HPLC-QToF-MS	High performance liquid chromatography quadrupole time-of-flight mass spectrometry
HPLC-UV	High performance liquid chromatography ultra-violet
HPTLC	High performance thin layer chromatography
HPTLC-UV	High performance thin layer chromatography ultra-violet
IEC-CD	Ion exchange chromatography with conductivity
LC-MS	Liquid chromatography mass spectrometry
IPC	Ion pair chromatography
IS-MS	Ion-scan mass spectrometry
LC-IT/ToF	Liquid chromatography ion-trap time-of-flight
LC-MS	Liquid chromatography mass spectrometry
LC-MS/UV	Liquid chromatography mass spectrometry ultra-violet
LC-PDAD	Liquid chromatography photodiode array detector
LC-Qorbi-MS	Liquid chromatography quadrupole orbitrap mass spectrometry
LC-Qq-LIT-MS	Liquid chromatography triple quadrupole linear trap mass spectrometry

LC-QqQ-MS	Liquid chromatography triple quadrupole mass spectrometry
LC-QToF-MS	Liquid chromatography quadrupole time of flight mass spectrometry
m/z	Mass to charge
NADEP	N,*a*-Diethylphenethylamine
NMR	Nuclear magnetic resonance
NN-DMPPA	N,N-dimethyl-2-phenylpropan-1-amine
NNHPD	Natural and Non-prescription Health Products Directorate
NPD	Nitrogen phosphorus detector
PAD	Pulsed amperometric detection
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analyses
RP-HPLC-QqQ-MS	Reversed-phase high-performance liquid chromatography triple quadrupole mass spectrometry
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TLC	Thin layer chromatography
TGA	Therapeutics Goods Administration
UHPLC-MS	Ultra-high-performance liquid chromatography mass spectrometry
UHPLC-Qorbi-MS	Ultra-high-performance liquid chromatography quadrupole orbitrap mass spectrometry
UHPLC-ToF-MS	Ultra-high-performance liquid chromatography time-of-flight mass spectrometry
UHPLC-LTQ-orbi-ms	Ultra-high-performance liquid chromatography linear ion trap-orbitrap mass spectrometry
UHPLC-PDAD	Ultra-high-performance liquid chromatography photodiode array detector
UHPLC-QqQ-MS	Ultra-high-performance liquid chromatography tripe quadrupole mass spectrometry
UHPLC-QToF-MS	Ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry
WT-UHPLC-QqQ-MS	Wooden-tip ultra-high-performance liquid chromatography triple quadrupole mass spectrometry

5 Introduction

Modern-day health and well-being is a highly capitalised industry worldwide. Taking a pill or powder to help increase overall health is a widespread concept with roots leading back hundreds if not thousands of years (1). Today's health and well-being supplements fall under the guise of dietary supplements, an all-encompassing term relating to any pills, powders, or oils that a person may take to supplement part of their diet. The use of dietary supplements is based on the individual and can range from vitamins and minerals to essential amino acids; a user may take a particular supplement to improve their well-being or even increase their energy at a specific time (2). The market for supplementation is ever-expanding; supplement use is increasing yearly, with usage among US adults at 57.4% for the 2017-2018 year (3). This increase in usage to over half the US adult population alone means that the supplement industry is reportedly worth USD 353 billion as of 2019, which has only increased since then (4). Dietary supplements can be broken down into categories of supplements; one such category is sports supplements, which people engaged in some sports use to help improve their overall performance, whether it's by increasing energy or decreasing fat. Such supplements can then further be grouped into types, such as pre-workouts for the proposed enhancement in focus and energy, to fat burners that reportedly claim to contain ingredients to help burn more

unwanted body fat (5). It has been estimated that sports supplements make up 13.8% of all dietary supplements available (6), with estimations of the percentage of athletes using dietary supplements between 40 and 100% (5). With the proposed benefits of taking some of these sports supplements and a large amount of competition in such a saturated market, some manufacturers may turn to nefarious ways to achieve results better than their competition (7) . This review considers sports supplements and their adulteration within the last decade; with laws and regulations changing vastly over the past decade, the trends and patterns within the industry can be assessed, along with the impacts these new changes have on adulteration. Throughout this review, particular emphasis will be put on sports supplements that fall under the pre-workout category along with weight loss supplements; this is due to the prevalence of these supplements on the market and the number of reports surrounding their adulteration. This review will present a view of past, current and future trends of adulteration within the sports supplements industry and use these new insights, which can then be applied to the Australian supplement market. Whilst Australian supplement manufacturers and importers can have their products independently analysed for the presence of adulterants it is evident that currently there is a gap within Australian sports supplements with, a limited number of market surveys and/or articles published looking into locally owned and made supplements over the period of this review. Australia has experienced changes to its laws and regulations regarding supplements that may impact how adulteration may occur. With these newly implemented laws in place, this review aims to include articles highlighting different countries' regulations regarding sports supplements and how this may help deter adulteration. This information will be synthesised through the documented and systematic review of all currently available literature gathered using scientific databases and critical terms relating to the topic. With pre-determined inclusion and exclusion criteria known, only the most relevant information will be sorted and collected, forming relevant concepts and theories about adulteration within Australia.

5.1 Adulteration

Adulteration is the mixing of a substance within a food (8), or in this case a supplement, and is an ever-common practice that has plagued the supplement industry for decades, with laws and restrictions being unable to stop the practice entirely (7). It has been reported within Australia that one in five supplements contain a banned substance in the form of an adulterant (9); some of the compounds commonly used for adulteration included 1,3-dimethylamylamine (DMAA), sibutramine, higenamine HCl and more, leaving everyday Australians at risk of exposure to these drugs (10, 11). The reason for adulteration can be attributed to many factors, from the contamination of the product during production to the intentional addition of synthetic adulterants to supplements to ensure that an effect is experienced by the user (12). In relation to the adulteration of sports supplements, there two typical reasonings have been identified behind the practice, one being economic adulteration and the other pharmaceutical. Economic adulteration is based on adding a cheaper ingredient to replace a more expensive compound that might be stated on the label. In contrast pharmaceutical adulteration is when an active drug is added to a claimed herbal supplement or even into a product without listing the drug (13). Adulteration may benefit the manufacturer, but the potential adverse reactions a user may experience are significant. Due to the nature of adulteration, a manufacturer may add an amount they deem enough based on pre-existing literature about the substance and its use by others. The amount added will also be calculated to ensure that enough is given in each dose of the supplement; the issue that can proceed from this is if the user does not follow the dosage instructions from the manufacturer, they may end up over-dosing the adulterant leading to any number of issues. Along with this, if the adulterant is added with more nefarious thinking from the manufacturer, no amount of the supplement may be medically regarded as safe, leaving the user exposed to adverse effects after every use. The potential for over-dosing of commonly found adulterants is high, and the potential for life-altering adverse effects is great, too; due to this, many countries restrict these drugs to help protect the public (14).

5.2 Adulterants

Many different drug classes and individual compounds can be and are used for the adulteration of sports supplements (15). Depending on the promises made by the manufacturer regarding their product, such as general

medical/health benefits or specific performance outcomes, or the facility that the supplement is made in, the type of adulterant used can vary with the proposed effect the compound may have. When it comes to supplements intended to boost energy and reduce fatigue perception, it is seen that most of the adulterants used are stimulants as they increase activity associated with the brain and the central nervous system (CNS). Stimulants cover a wide range of drugs, from illegal compounds, such as amphetamines and cocaine, to everyday ingested drugs, such as caffeine (16). On the other side of sports supplements are weight loss supplements that are taken to aid with body fat reduction; these are often adulterated with drugs that fall under the classification of diuretics, laxatives, anorectics and stimulants, with stimulants used due to their appetite-suppressing characteristics (12). Within Australia, a large majority of laxatives, diuretics and anorectics are classified under the Therapeutic Goods Instrument 2023 (17) as Schedule 4 compounds, meaning they can only be accessed when prescribed by a doctor (17). These drugs work in different ways based on the class they belong to. Diuretics work by giving a false sense of weight loss as they induce increased water excretion leading to reducing body water content, not body fat (18). The mechanism of action for laxatives works by increasing the stimulation of intestinal mucosa and contraction of smooth muscle, which leads to increased defecation (19); like diuretics, laxatives do not achieve any reduction in body fat, but instead, they reduce overall body water content. Anorectic pharmacokinetics lay in the ability to increase the perceived level of satiety within the hypothalamus; unlike diuretics and laxatives, this will affect body fat, but anorectics are known to have side effects that may affect the user as they are structurally related to amphetamine (20). Finally, stimulants have the same effect as stated previously, but the desired action is their impact on reducing perceived hunger levels, thus helping to persist without eating (21). An issue that many manufacturers who intentionally adulterate their products with illicit substances face is how easily many adulterants are detected within sports supplements and any legal ramifications they may face as the manufacturer. With many of these drugs being easy to detect and countries stopping the importation of certain known adulterated products, manufacturers have started implementing designer drugs into their products to evade detection (22). Designer drugs are specially made to mimic other compounds' effects as closely as possible by slightly altering their structure to avoid easy detection. The main issue surrounding designer drugs is the lack of testing and human safety data, with the manufacturer dosing the drug at unknown levels that could potentially negatively affect the unknowing user (22). Over the past few years, it has been found that there has been an increase in the number of products adulterated with new designer drugs; among the highest recorded cases of adulteration with new synthetic drugs were weight loss supplements (22). These supplements are often tainted with analogues of sibutramine or other compounds, such as phenolphthalein, produced to elicit similar results to the parent compound. However, the potential safety of such drugs is still highly unknown, and therefore the damage these adulterants could create is great for an unknowing victim (23). This review hopes to highlight common adulterants currently found within the market and identify newly created designer drugs that are relatively new in discovery.

5.3 Australian supplement regulation

Before 2020, within Australia, all sports supplements were classified under the 'food' category by the Therapeutics Goods Administration (TGA) and therefore fall under the standards and regulations set out by the Food Standards Australia New Zealand (FSANZ). Under this classification, sports supplements are subjected to food safety standard 2.9.4 - formulated supplementary sports foods (24). This standard covers the composition of sports supplements regarding the vitamin, minerals, amino acids or any other substance that is used as a nutritive substance that may be added to the supplement, along with labelling information, nutritive substance claims, vitamin and mineral claims, prohibited representations and more specifically protein and energy content/provision (25). The enforcement of this standard and all the other standards created by FSANZ is carried out by each Australian State/Territory respective government department with the Australian Department of Agriculture, Water and Environment responsible for the inspection and sampling of imported food (24). Prior to 2020, sports supplements under standard 2.9.4 lay within this grey zone referred to as the food-medicine interface, which meant new products that were hitting the market at the time, could have been classified as medicine but were instead classed as food and therefore regulated with more lenient standards and codes; this discrepancy between what classifies as a medicine and what does not can inadvertently put the unknowing consumer at risk unless they are more educated on the product themselves (10). From the 23rd of September 2020, the TGA introduced a change to current regulations that now classified a large portion of sports supplements at the time as therapeutic goods (26). Under this new regulatory change, any

supplement that contains a specific substance based on its presence within the Therapeutic Goods Instrument 2023, is supplied in the form of a pill (from 2023) or is advertised for therapeutic use would now be classified as a therapeutic good (26); this applied to sports supplements as claims commonly made by manufactures and brands, would now classify their good as a therapeutic product and therefore be exposed to higher regulation and tighter controls. Under the classification of therapeutic good, the products would be exposed to manufacturing, formulation, labelling, evidence and advertising scrutiny to ensure that it meets all applicable legislation (26). Comparing this new legislation implemented to that of countries with similar regulation procedures will help determine how effective this new change will be in the fight against adulteration. With it now being close to 3 years since the implementation of this new legalisation, now is as good of a time as ever to conduct this research looking at the Australian market and determine if the incident rate of adulteration has changed for the better or worse.

5.4 Aim and objectives

The overarching aim of this review is to gain an understanding of the past, current and potential future trends within the adulteration of supplements, including

- The drugs used,
- The supplement typically adulterated with certain compounds,
- Any newly found drugs, adulterants used across multiple supplements and
- Drugs not considered relevant, being used as adulterants

This aim will predominately be applied to pre-workout and weight loss supplements. However, it will likely have other supplements included as researchers often test multiple supplements. The review will be correlated to the Australian supplement industry to better understand the Australian market, as it is evident that there has been limited research evaluating supplements within Australia.

Further to this, additional objectives are aimed to be achieved by conducting this review. One is to better understand the more commonly used adulterants and determine why they are used along with their popularity; this will help better understand common characteristics among the more popular adulterants. Another objective is to distinguish any new, emerging drugs that have been found as adulterants in the past couple of years and are currently becoming more popular globally, and this will help determine what to look out for in terms of drugs that are yet to hit Australia but can soon be expected. Another objective is to assess the effectiveness of supplement legislation to apply relevant other countries' regulations found in the review to the current regulation in Australia that was recently implemented; this would help determine how effective it may be, along with ideas for future improvements. The final objective was to determine the techniques and hyphenated variations that are most common when testing supplements for adulterants. This information will help future researchers decide which method is best suited for them based on their specific goals and ideas. With these aims and objectives in mind, a clear picture of what is hoped to be achieved within this review is presented.

6 Methods

6.1 Search Strategy

A systematic literature review was conducted to choose peer-reviewed publications related to the adulteration of sports supplements with illicit and prohibited compounds. The prohibited and illicit adulterants used in the search for relevant publications, were selected based on the more prevalent drugs used in adulteration from previous reviews (12). Searching was done over three databases: the National Library of Medicine's Medline database (PubMed), Scopus (Elsevier) and Google Scholar (Google). PubMed was last accessed on the 3rd of April 2023; Scopus was last searched on the 31st of March 2023, and Google Scholar was last utilised on the 5th of April 2023. Keywords were combined to create the subsequent search terms used in all databases; such terms included DMAA AND supplement, DMAA AND sport, methylhexanamine AND supplement, 1,3-dimethylamlyamine AND supplement, nutraceutical AND supplement AND illicit, Australia AND DMAA, 1,3-dimethylbutylamine AND supplement, phenethylamine AND supplement, phenethylamine AND supplement AND sport synthetic AND drugs AND supplement AND sports, designer AND drugs AND supplements AND sports, fat AND burner AND illicit, amphetamine AND supplement AND sports, sibutramine AND adulteration AND supplement, dietary AND supplement AND adulterant AND illicit, phenolphthalein AND adulteration, allintitle: DMAA in supplements, allintitle: presence of DMAA in sports supplements, allintitle: 1,3-

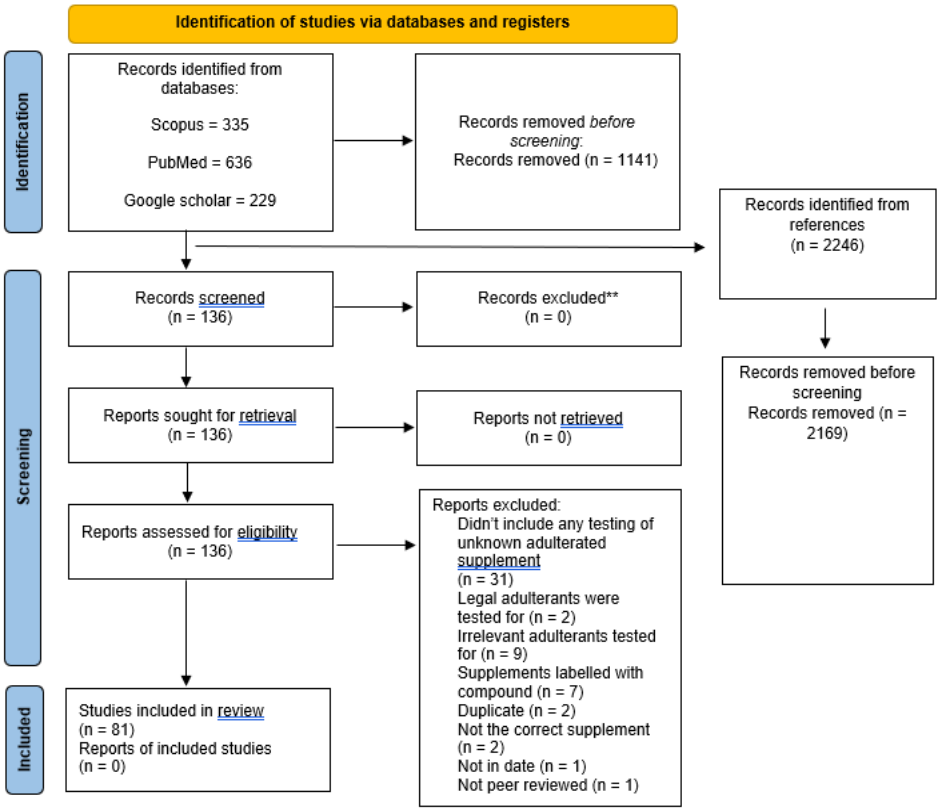

Figure 1 - PRISMA article identification process

dimethylamylamine presence in supplement, allintitle: phenethylamine adulteration, allintitle: sibutramine adulteration and allintitle: presence of sibutramine in sports supplements. In terms of search parameters, all databases were searched for articles from 2013 onwards. When searching on Scopus, the subject area was limited to chemistry, medicine, biochemistry, genetics, molecular biology, pharmacology, toxicology, pharmaceutics, agriculture, and biological science. The "related articles to" function on Google Scholar was also utilised. After all search terms were used across the three databases, each article's reference list was reviewed, and any relevant studies were noted. The search results for the literature review are shown in *Figure 1*, which is a Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flowchart.

6.2 Inclusion criteria

Relevant articles were examined, essential information from each article was noted in a Word document, and citations were uploaded into EndNote X9.

Inclusion criteria were as follows: testing of sports supplements had to be conducted during the review, testing was conducted on pre-workout or weight loss supplements suspected as being adulterated, the adulterants screened for are illegal within Australia, the article was written in English and the study was conducted between January 1st 2013 and current day.

Publications testing only supplements that did not include weight loss or pre-workout supplements were excluded. Articles testing for legal adulterants were excluded. Non-English language articles were also excluded from the review.

The rationale behind the inclusion criteria was first to ensure that weight loss or pre-workouts were included within the testing, as this review particularly emphasises these supplements. Another aspect of the reasoning was to ensure that the adulterants remained within the realm of illegality, as this was an integral part of the review. With different ways of adulterating supplements, ensuring that the adulterants screened for were illegally made it so that other forms of adulteration that are not relevant were not included. The English-speaking and time inclusions were made so that the reviewer could read the articles and that only recently published articles were used as evidence.

Articles were firstly screened by their titles for the inclusion of keywords that correlated to the review's aims. Relevant articles were then noted, and abstracts were read to ensure the article fit the selection criteria. If both the title and abstract met the requirements, the article was read, and essential information was noted. These articles were then subjected to further review and were either removed or included based on the article's relevancy to the aims.

6.3 Near miss cases

Near miss cases are those articles included throughout the entire screening and identification part of article screening but were cut at the included phase due to slight differences between objectives and aims and the review's inclusion criteria. One such article was by Lucas, et al. (27), in which the authors tested ten supplements for synthetically added yohimbine. Yohimbine HCl is a multi-use compound for adulteration; it has previous use tied to improving sexual performance and helping reduce body fat (27). The article in question did not specify the types of tested supplements; therefore, it is unknown if they were working on supplements claiming to help with sexual performance, ones that improved weight loss or even a mixture of both. Due to this discrepancy, it was determined that this paper would not be included in the final review. Another near-miss case was Wang et al. (28). This article met all inclusion criteria on first look, reporting on testing diet food supplements and testing for adulterants that were relevant. The issue with this article was that it originated from China, and therefore, there was no full article in English, only the abstract could be accessed in English. Due to there not being an entire article in English, the whole study was not assessed, and subsequently, it had to be excluded from this review.

A common point of exclusion that occurred many times was testing supplements previously known to be adulterated. In these cases, the decision was made to exclude these from the review as it was thought sampling procedures were not an accurate picture of the supplement industry and adulteration. Instead, it painted a picture that all supplements were tainted no matter the supplement bought, which was not the whole picture; this was especially important in a review that considered the number of supplements adulterated as a critical point. Most of the near-miss cases were like the examples stated here. They seemed upon first impression that they would fit this review and contribute to the overall conclusions. With slight deviations from the wanted criteria, these articles were less relevant than first thought.

7 Results

7.1 Study specifics

Eighty-two articles from the literature survey met the inclusion for this review. Of the 82, 76 included testing supplements that claimed to help improve weight loss (11, 13, 15, 18, 27, 29-99). The other six articles tested solely supplements marketed as pre-workouts (100-105). Other supplements tested included: memory boosters, athletic performance enhancers as well as sexual performance enhancers, improvement in mental clarity, ones that modulate hormones, tranquillizing products, traditional Chinese herbs, cannabinoids, protein shakes and St John's wart (15, 32-35, 37, 39-43, 48, 58-60, 66-69, 72, 75, 77, 80, 83, 89, 90, 95, 99).

Looking at the dates of publication, the oldest article included was published in January 2013 (30), while the newest article was from February 2023 (93). In terms of which years had the most and least publications, 2014 had the highest amount at 14 articles (40, 50, 56, 63, 68, 76, 82, 85, 91, 94, 100, 102-104), whereas 2023 had the lowest number of contributing articles at 1 (93)

The sampling size varied greatly from article to article; the smallest sample size of one occurred in four separate studies (35, 56, 74, 101) and 447 was the largest sample size (97) in one study. When looking at the total amount of supplements tested, 5203 were tested, with an average per article being 64 supplements.
Altogether, there were 81 different adulterants found in the articles included.

The most common adulterant detected was sibutramine and its analogues, with thirty articles claiming to have found sibutramine being used as an adulterant (13, 29, 30, 34, 36, 40, 44, 46, 54, 55, 57, 59, 61-65, 69, 71, 74, 76, 81, 84-86, 88, 91, 93, 95, 97). Following sibutramine, the second most common adulterant found was phenolphthalein, with sixteen instances of it being found (29, 30, 40, 46, 55, 59, 65, 69, 70, 75, 85, 86, 88, 91, 95, 97). Two compounds then both had the third most mentions within the articles being DMAA at 12 (11, 31, 33, 34, 41, 49, 62, 67, 68, 79, 85, 103) along with phenethylamine also at 12 (15, 31, 34, 41, 49, 50, 79, 82, 96, 98, 102, 104). Synephrine was the next most found adulterant, with ten articles finding it within their supplements (34, 35, 58, 77, 79, 80, 87, 90, 94, 98). Following this, yohimbine was another common adulterant, with nine articles observing it as an adulterant (31, 34, 35, 39, 69, 79, 90, 94, 95). Sildenafil (34, 46, 59, 66, 69, 72, 75, 85), fluoxetine (36, 40, 46, 52, 63, 65, 95, 97) and 1,3-Dimethylbutanamine (DMBA) (11, 31, 33, 41, 42, 49, 51, 67) were all found in eight different articles. The next most common adulterant was ephedrine, with six articles finding it as an adulterant (34, 49, 60, 63, 65, 95). Next, oxilofrine was found the most, with five articles finding the adulterant (15, 38, 41, 94, 98). Higenamine (34, 41, 53, 92), hordenine (31, 34, 58, 98), hydrochlorothiazide (18, 45, 78, 87) and sennosides (63, 69, 70, 95), were all found in 4 separate articles. Following this, deternol (41, 94, 98), furosemide (45, 78, 97), icariin (34, 69, 95), ostarine (58, 67, 68) and theophylline (46, 81, 94), all were found to be present in 3 separate articles each. Bisacodyl (44, 65), N-*a*-diethylphenethylamine (DEPEA) (100, 104, 105), doxepin (54, 59), methylstenbolone (58, 67), octopamine (34, 98), rauwolscine (43, 94), rimonabant (47, 85), tadalafil (59, 75) and testosterone (67, 68), all were detected within two studies each.

Finally, the remaining adulterants all occurred in one article each, these adulterants included: 5-HTP (90), 6-BROMO (58), acetaminophen (72), amfepramone (88), amphetamine (73), boldenone (68), canerone (93), chlordiazepoxide (59), chlorodehydromethyltestosterone (68), chlortalidone (87), chlorzoxazone (72), clomiphene (68), clonazepam (59), corynanthine (43), DHEA (89), diazepam (59), DNP (83), drostanolone (68), EAPB (105), ETH (50), GHRPs (68), hGH (68), ibuprofen (72), ibutamoren (58), levothyroxine (70), liothyronine (97), lorcaserin (56), melatonin (59), methandienones (68), methamphetamine (73), methasterone (67), methenolone (68), methyl-1-testosterone (67), methylphenidate (62), N,*a*-Diethylphenethylamine (NADEP) (102), nandrolone (68), N,N-dimethyl-2-phenylpropan-1-amine (NN-DMPPA) (101), norephedrine (54), orlistat (65), oxandrolone (68), oxymetholone (68), picamilon (32), piroxicam (60), pseudoephdrine (63), stanozolol (68), sulfamethoxazole (72), tamoxifen citrate (68), thyroxine (97), trenbalone (68), trendione (58), vardenafil (59), vinpocetine (32) and zopiclone (59).

The number of supplements found to be adulterated ranged from 0 (37, 48, 99) up to 266 (34). The total number of adulterated supplements was 1774, and the average number of adulterated supplements found per article was 22.

The articles included in this review were multinational, with 24 different countries represented in this review. USA had the highest number of articles at seventeen (11, 31-33, 38-43, 49, 50, 74, 82, 91, 98, 100), followed by South Korea at thirteen articles (37, 60, 63-66, 69, 70, 89, 90, 95, 96, 102), Brazil with ten (18, 44, 45, 47, 48, 62, 77-79, 87), China had nine (30, 36, 52, 54, 59, 61, 71, 72, 99), Netherlands had six (15, 34, 35, 85, 93, 94), following was France which had three (51, 55, 56), then Switzerland, Germany, Poland, Iran and Romania all had two articles (46, 53, 68, 73, 75, 76, 84, 88, 101, 103) and the remaining countries, Egypt, Indonesia, Norway, United Arab Emirates, Northern Ireland, Portugal, Italy, England, New Zealand, Serbia, Sweden, Australia and Singapore all had one article each (13, 29, 57, 58, 67, 80, 81, 83, 86, 92, 97, 104, 105).

Sampling techniques used in the articles reviewed included 45 differing techniques used to analyse supplements. Of the 45, the most commonly used techniques include: liquid chromatography triple quadrupole-mass spectrometry (LC-QqQ-MS), with eighteen articles using it (34, 35, 37, 51, 62, 63, 65, 68, 69, 73, 81-83, 90, 91, 96, 97, 99), followed by gas chromatography-mass spectrometry (GC-MS), which had fourteen articles incorporate it (15, 33, 40, 51, 58, 68, 72, 75, 79, 84, 88, 101, 102, 105), high-performance liquid chromatography photodiode array detector (HPLC-PDAD), having ten articles include it (29, 30, 47, 56, 59, 69, 86, 89, 95, 99) and finally, ultra-high-performance liquid chromatography photodiode array detector (UHPLC-PDAD), with seven inclusions (32, 56, 65, 69, 70, 86, 95).

The remaining techniques used were found in six or less articles. This included liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QToF-MS) (31, 39, 43, 47, 68, 81), nuclear magnetic resonance (NMR) (55, 56, 70, 74, 98, 103) and ultra-high-performance liquid chromatography mass spectrometry (UHPLC-MS) (11, 42, 53, 93, 95, 104), all having six articles. Followed by ultra-high-performance liquid chromatography triple quadrupole mass spectrometry (UHPLC-QqQ-MS) (13, 77, 80, 92, 95) and ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-QToF-MS) (33, 38, 41, 42, 60), being included in five articles each. Then with three inclusions, there was high performance thin layer chromatography (HPTLC) (33, 46, 76), liquid chromatography mass spectrometry (LC-MS) (15, 40, 68), ultra-high-performance liquid chromatography quadrupole orbitrap mass spectrometry (UHPLC-Q-orbi-MS) (34, 41, 70) and liquid chromatography-quadrupole orbitrap mass spectrometry (LC-Q-orbi-MS) (15, 51, 70).

With inclusions in two articles there was, attenuate total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) (75, 84), capillary zone electrophoresis (CZE) (18, 78), high-performance liquid chromatography triple quadrupole mass spectrometry (HPLC-QqQ-MS) (52, 89), high-performance liquid chromatography orbitrap mass spectrometry (HPLC-orbi-MS) (35, 74), high performance liquid chromatography pulsed amperometric detection (HPLC-PAD) (45, 87), high-performance liquid chromatography ultra-violet (HPLC-UV) (71, 73), raman spectroscopy (75, 86), ultra-high-performance liquid chromatography time-of-flight mass spectrometry (UPLC- ToF-MS) (64, 77) and finally, ultra-high-performance liquid chromatography linear ion trap-orbitrap mass spectrometry (UHPLC-LTQ-orbitrap-MS) (36, 100).

The remaining techniques, were all found in one article only capillary electrophoresis triple quadrupole mass spectrometry (CE-QqQ-MS), direct analysis in real time triple quadrupole mass spectrometry (DART-QqQ-MS), wooden tip ultra-high-performance liquid chromatography triple quadrupole electrospray ionization mass spectrometry (WT-UHPLC-QqQ-ESI-MS), field ionization triple quadrupole mass spectrometry (FI-QqQ-MS), gas chromatography mass spectrometry nitrogen phosphorus detector (GC-MS/NPD), gas chromatography-quadrupole time-of-flight mass spectrometry (GC-Q-ToF-MS), graphene tip liquid chromatography triple-quadrupole linear trap mass spectrometry (GT-LC-Qq-LIT-MS), high-performance liquid chromatography mass spectrometry (HPLC-MS), high-performance liquid chromatography quadrupole time-of-flight mass spectrometry (HPLC-Q-ToF-MS), high performance thin layer chromatography ultra violet (HPTLC-UV), ion exchange chromatography with conductivity detection (IEC-CD), ion-scan mass spectrometry (IS-MS), ion pair chromatography (IPC), liquid chromatography ion-trap time-of-flight (LC-IT-TOF), liquid chromatography mass spectrometry ultra-violet (LC-MS/UV), liquid chromatography photodiode array

detector (LC-PDAD), nano liquid chromatography tandem mass spectrometry (nanoLC-MS/MS), pulsed amperometric detection (PAD), reversed-phase high-performance liquid chromatography triple quadrupole mass spectrometry (RP-HPLC-QqQ-MS), sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gas chromatography triple quadrupole mass spectrometry (GC-QqQ-MS), liquid chromatography triple quadrupole linear trap mass spectrometry (LC-Qq-LIT-MS) and thin layer chromatography (TLC) (13, 44, 48, 50, 54, 57, 59, 61, 62, 68, 74, 76, 82, 85, 87, 91, 94, 96).

7.2 Assessment of scientific quality

Each article included in this review is published work, meaning that it has been sent to a journal, reviewed by the journal, and subsequently approved to be published. All articles included were published within peer-reviewed journals meaning that all articles are peer-reviewed. No grey literature or unpublished work were included.

Many of the papers included were written to develop a new analytical technique and applying this new technique to supplements. For these articles, validation of the technique was needed to ensure that the technique was appropriate to detect and quantify the presence of adulterants. Articles achieved this validity by determining selectivity, linearity, the limit of detection and the limit of quantification, among other validation techniques. Another way in which some articles validated results was the running of samples in duplicates or triplicates. This helps to ensure that no abnormalities are present within the results.

The final way results from articles were determined to be of scientific quality was the testing of sports supplements by multiple individual organisations. The supplements sent to separate individual organisations ensured that pre-conceived ideas from the authors did not determine the results; instead, the results came from scientific facts. These checks, used throughout the screening of potential articles, lead to the inclusion of scientifically valid and relevant articles.

Based on the results presented, heterogeneity was seen. The reasons behind this occurring include the different supplements each paper decided to test from pre-workouts (11, 32, 50, 51, 53, 58, 92, 100-105) and fat burners (11, 13, 15, 18, 29-99), which leads to the finding of differing adulterants. The different techniques used when testing the supplements such as LC-MS (34, 35, 40, 104) and GC-MS (32, 33, 40, 51, 58, 72, 75, 79, 84, 88, 101, 102, 105) also contributed to the heterogeneity. Heterogeneity was also seen in the results as different results were found throughout with some finding many adulterants present within their supplements (34, 97) to others finding no adulterants at all (37, 48). Along with the difference in number, the type of adulterant present was also different. The articles have a wide range of adulterants from stimulants (31, 33-35, 38, 39) to laxatives (29, 30, 40, 44, 46, 55). This clinical, and methodological heterogeneity leads to the data being further validated.

Taking a critical look at the results to determine inconsistencies, one that appeared was the testing of herbal and non-herbal weight loss supplements. The reason behind using articles that looked at herbal supplements was to ensure that a wide range of data was acquired for this review. Herbal and non-herbal weight loss supplements have the same goal; therefore, the adulterant used would likely be similar, if not the same and would further identify adulterants and trends.

Study and year	Supplement tested	Sample size	Adulterants found	Number of supplements adulterated	Location of study	Sampling technique
Ahmed N, et al. (2019)	Herbal weight loss supplements	6 but analysed in	Sibutramine, phenolphthalein, sildenafil	5	Egypt	HPLC-PDAD

		triplicates				
Ancuceanu R, Dinu M, Arama C (2013)	Herbal weight loss	2	Sibutramine and phenolphthalein	2	China	HPLC-PDAD
Avula B, et al. (2019)	Weight loss and ergogenic	27	B-PEA, R-B-methylphenethylamine, hordenine, yohimbine, DMAA, DMBA	16 – B PEA 5 – R-B 3 – hordenine 13 – yohimbine 3 – DMAA 1 – DMBA	USA	LCQToF-MS
Avula B, et al. (2016)	Memory booster, pre-workout, fat burner	31	Vinpocetine, picamilon	17 – vinpocetine 30 – picamilon	USA	UHPLC-PDAD
Avula B, et al. (2015)	Improve athletic performance, weight loss and mental acuity	13	DMBA, DMAA	11 – DMBA 2 - DMAA	USA	HPTLC and GC-MS and UHPLC-QToF-MS
Biesterbos JWH, et al. (2019)	Herbal sexual performance, physical performance, weight loss	416	Synephrine, higenamine, sibutramine, sildenafil, icariin, thiosildenafil, yohimbine, hordenine, phenethylamine, methyl-synephrine, DMAA, octopamine, ephedrine (seen over 10 times) and more	266	Netherlands	UHPLC-Q-orbi-MS and LC-QqQ-MS
Bovee TFH, et al. (2016)	Energy, mental focus, reduce appetite, anabolic state	1	Yohimbine alkaloids, synephrine	1	Netherlands	HPLC-ESI-orbi-MS and LC-QqQ-MS and ELISA
Cheng Q, et al. (2017)	Natural weight loss	120 batches of samples	Sibutramine, fluoxetine	27 – sibutramine 2 – fluoxetine	China	UHPLC-LTQ-MS
Choi JY, et al. (2015)	Enhance concentration, weight loss, sexual improvement	47	None detected	0	South Korea	LC-QqQ-MS

Cohen PA, et al. (2017)	Weight loss	27	Oxilofrine	14	USA	UHPLC-QToF-MS
Cohen PA, et al. (2016)	Weight loss, cognitive function, sports supplements	21	BMPEA	11	USA	LC-QTOF/MS
Cohen PA, et al. (2014)	Weight loss, sexual performance	27	Fluoxetine, sibutramine, phenolphthalein	18	USA	GCMS and LCMS
Cohen PA, et al. (2018)	Pre workouts and weight loss	7	DMAA, DMBA, octodrine	1 – octodrine 4 – DMAA 1 – DMBA	USA	UHPLC MS
Cohen PA, et al. (2021)	Weight loss, sport supplements	17	Deterenol, phenpromethamine, BMPEA, octodrine, oxilofrine, higenamine, DMAA, DMBA	4 had 2 2 had 3 2 had 4	USA	UHPLC – Q/Orb/MS and UHPLC-QToF
Cohen PA, et al. (2014)	Pre workout	3	DEPEA	3	USA	UHPLC-LTQ-Orbi-MS
Cohen PA, et al. (2015)	Weight loss, brain enhancer, sports supplement	14	DMBA	12	USA	UHPLC-QToF-MS and UHPLC-MS
Cohen PA, et al. (2016)	Sexual enhancement, weight loss	49	Yohimbine, rauwolscine and corynanthine	43	USA	LC-QToF-MS
Dal Molin TR, et al. (2019)	Weight loss	78	Sibutramine and bisacodyl	2	Brazil	IEC-CD
De Carvalho LM, et al. (2013)	Natural weight loss	26	Hydrochlorothiazide and furosemide	8	Brazil	HPLC-PAD
Do TTK, Reich E. (2019)	Weight loss,	15	Fluoxetine, phenolphthalein or sibutramine, sildenafil, theophylline	12	Switzerland	HPTLC
dos Santos JRMP, et al. (2021)	Weight loss	15	Rimonabant	1	Brazil	HPLC-PDAD and LC-Q-ToF

dos Santos VB, et al. (2016)	Phytotherapic medicine, weight loss	7	None detected	0	Brazil	CE-QqQ-MS
Duiven E, et al. (2021)	Modulate hormone regulation, muscle mass gain, weight loss and boost energy	66	Synthetic stimulants, oxilofrine, BMPEA	3 – synthetic stimulants 2 – oxilofrine and BMPEA 1 - oxilofrine	Netherlands	LC-Q-Orbi-MS and LC-MS and GC-MS
Eichner S, et al. (2016)	Weight loss	51	Ephedra, DMAA, BMPEA and DMBA	46	USA	N/A
ElSohly MA, Gul W. (2014)	Pre workout, weight loss,	19	ETH, phenethylamine	6 – ETH 8 – phenethylamine	USA	LC-Qq-LIT-MS
Fabresse N, et al. (2021)	Pre workout, weight loss	35 – DS	DMBA	1	France	GC-MS and LC-orbi-MS and LC-QqQ-MS
Gao J, et al. (2021)	Weight loss and lipid lowering	20 batches	Fluoxetine	2 batches	China	HPLC-QqQ-MS
Grucza K, et al. (2019)	Pre workout and weight loss	5	Higenamine	4	Poland	UHPLC-MS
Guo B, et al. (2015)	Anti-obesity, healthy blood pressure	113	Sibutramine, doxepin, norephedrine	30 – not all relevant	China	LCMS-IT/TOF
Hachem R, et al. (2016)	Weight loss, natural	160	Sibutramine, phenolphthalein	90	France	Proton NMR
Hachem R, et al. (2014)	Weight loss	1	Lorcaserin	1	France	UHPLC-PDAD and NMR
Hayun H, et al. (2016)	Weight loss, herbal	7	Sibutramine	6	Indonesia	TLC densitometric
Helle C, et al. (2019)	Pre workout, fat burning, muscle building, amino acids	93	Synephrine, hordenine, methylstenbolone, trendione, ostarine, ibutamorene, 6-BROMO	21	Norway	GC-MS

Hu B, et al. (2016)	Tranquilizer, aphrodisiac, weight loss	144	Doxepin, clonazepam, melatonin, diazepam, zopiclone, chlordiazepoxide, sildenafil, vardenafil, tadalafil, amino-tadalafil, sibutramine, phenolphthalein	98	China	Wooden tip ESI-MS and HPLC-PDAD
Hur J, et al. (2019)	Weight loss, cannabinoids	10	Ephedrine, piroxicam	5	South Korea	UHPLC-Q/TOF-MS
Jairoun AA, et al. (2021)	Weight loss	137	Sibutramine	23, 21 had sibutramine	UAE	RP-HPLC-QqQ-MS
Jin R, et al. (2017)	Weight loss	7	Sibutramine	4	China	GT-LC-Qq-LIT-MS
Kerpel dos Santos M, et al. (2018)	Weight loss	108	DMAA, sibutramine, methylphenidate	22	Brazil	Dart-QqQ-MS and LC-QqQ-MS
Kim HJ, et al. (2014)	Weight loss	188	Bisacdoyl, desmethylsibutramine, didesmethylsibutramine, ephedrine, fluoxetine, pseudoephedrine, sennoside A/B and sibutramine	62	South Korea	LC-QqQ-MS
Kim JW, et al. (2013)	Weight loss	7	Chlorosibutramine	7	South Korea	UHPLC-ESI-TOF/MS
Kim JY, et al. (2016)	Weight loss	193	Bisacodyl, chlorosibutramine, desmethylsibutramine, ephedrine, fluoxetine, orlistat, phenolphthalein, sibutramine	55	South Korea	UHPLC-PDAD and LC-QqQ-MS
Kim NS, et al. (2022)	Weight loss and sexual enhancement	18	Sildenafil precursors, no weight loss adulterants	4	South Korea	LC-QTOF-MS
Kimergard A, Walker C, Cowan D. (2015)	Weight loss, pro hormones	9	DMAA and DMBA, ostarine, methasterone, methylstenbolone, methyl-1-testosterone	9	Northern Ireland	N/A
Krug O, et al. (2014)	Weight loss, performance enhancers, muscle increasing	337	Test, tren, nandrolone, hGH and GHRPs, ostarine, boldenone, drostanolone, metandienone, methenolone, methydrostanolone, nandrolone, oxandrolone, oxymetholone, stanozolol, dehydrochloromethyltestoster	67	Germany	LC-QqQ-MS and LC-Q-ToF-MS and GC-Q-ToF-MS and SDS-PAGE and nanoLC-

			one, DMAA, clomiphene, tamoxifen-citrate			MS/MS and GC-MS/NPD
Kwaitkowska D, et al. (2015)	Pre workout	1	NN-DMPPA	1	Poland	GC-MS
Kwon J, et al. (2022)	Weight loss, sexual enhancement, muscular strength	51	Icariin, sibutramine, yohimbine, sildenafil, sennosides, phenolphthalein	45	South Korea	HPLC-PDAD and LC-QqQ-MS
Lee J, et al. (2014)	Pre workout	2	NADEP	2	South Korea	GC-MS
Lee JH, et al. (2019)	Weight loss	23	Levothyroxine, sennoside A and B, phenolphthalein	5	South Korea	UHPLC-PDAD and LC-Q-orbi-MS and NMR
Liang Q, et al. (2021)	Weight loss	2 60 batches	Sibutramine	36 batches	China	HPLC-UV
Lin YP, et al (2018).	Weight loss, sexual enhancement, traditional Chinese medicine	83	Acetaminophen, ibuprofen, chlorzoxazone, sulfamethoxazole, tadalfil and sildenafil	8	China	GC-MS with hydrogen
Lotfipoura F, Ghorbania NA, Farjamia A. (2022)	Weight loss	16	Amphetamine or methamphetamine with one containing both	2	Iran	HPLC-UV and LC-QqQ-MS
Mans DJ, et al. (2013)	Weight loss	1	Sibutramine and 11-desisobutyl-11-benzylsibutramine	1	USA	IONSCAN-MS and HPLC-Q-Orbi-MS and NMR
Mateescu C, et al. (2017)	Herbal weight loss, sexual enhancement	50	Sildenafil, tadalafil, phenolphthalein	11	Romania	Raman, ATR-FTIR and GC-MS
Mathon C, et al. (2014)	Weight loss	50	Sibutramine	26	Switzerland	HPTLC-UV

Monakhova YB, et al. (2014)	Pre workout	16	DMAA	9	Germany	1H NMR
Moreira APL, et al. (2013)	Weight loss herbal	26	Hydrochlorothiazide	3	Brazil	CZE with C^4D
Muller LS, et al. (2019)	Weight loss, thermogenic and meal replacement	128	Synephrine and caffeine	80	Brazil	UHPLC-QqQ-MS
Muller LS, et al. (2018)	Weight loss, fat burning, appetite reduction, metabolism increase	113	Hydrochlorothiazide, furosemide	15	Brazil	CZE-C^4D-UV
Neves DBdJ, Caldas ED. (2017)	Weight loss	213	Phenethylamine, synephrine, yohimbine, DMAA	92	Brazil (US made)	GC-MS
Paiga P, et al. (2017)	Weight loss, St John's wort	16	Synephrine	1	Portugal	UHPLC-QqQ-MS
Pascali JP, et al. (2018)	Weight loss natural	5	Sibutramine, theophylline	3	Italy	LC-QTOF-MS, LC-QqQ-MS
Pawar RS, et al. (2014)	Weight loss	21	Phenethylamines	20	USA	LC-QqQ-MS and GC-QqQ-MS
Petroczi A, et al. (2015)	Fat burners, protein shakes	98	DNP	14	England	LC-QqQ-MS
Popescu AM, Radu GL. (2015)	Weight loss herbal	10	Sibutramine	1	Romania	FTIR and GC-MS
Reeuwijk NM, et al. (2014)	Weight loss herbal	50	Sibutramine, DMAA, phenolphthalein, sildenafil, rimonabant	24	Netherlands	LC-MS/UV
Rooney JS, et al. (2015)	Weight loss herbal	35	Sibutramine, phenolphthalein	35	New Zealand	HPLC-PDA
Sabo Muller L, et al. (2018)	Weight loss	78	Hydrochlorothiazide, chlortalidone and synephrine	18	Brazil	HPLC-PAD and IPC
Shekari N, Vosough	Weight loss	9	Sibutramine, amfepramone, phenolphthalein	9	Iran	GC-MS

M, Tabar Heidar K. (2018)						
Shin D, et al. (2020)	Weight loss and sexual improvement	115	DHEA, kavain, magnoflorine	3	South Korea	HPLC-PDAD and HPLC-QqQ-MS
Shin D, Kim H, Moon G. (2020)	Weight loss, muscle strengthening	200	Synephrine, yohimbine and 5-HTP	27	South Korea	LC-QqQ-MS
Song F, et al. (2014)	Weight loss	17	Sibutramine, phenolphthalein	11, 9	USA	FI-QqQ-MS and LC-QqQ-MS
Stajic A, et al. (2017)	Pre workouts, weight loss	19	Higenamine	2	Serbia	UHPLC-QqQ-MS
Van de Koppel S, et al. (2023)	Weight loss, herbal	8	Canerone, sibutramine	8	Netherlands	UHPLC-MS
Venhuis B, et al. (2014)	Weight loss	2	Synephrine, oxilofrine, deterenol, yohimbine, rauwolscine and theophylline	2	Netherlands	HPLC-QToF-MS
Whalstrom R, Styles C, Hägglund G. (2014)	Pre-workout	2	N,β-DEPEA, a-DEPEA, N,N-DEPEA	2	Sweden	UHPLC-MS
Wójtowicz M, et al. (2015)	Pre-workout	16	EAPB	14	Australia	GC-MS
Yun J, et al. (2018)	Weight loss	370	Yohimbine, B-PEA, sibutramine, sennosides, BMPEA, fluoxetine, ephedrine, phenolphthalein, icariin	45, 12, 10, 7, 6, 3, 2, 1 (in order of drugs)	South Korea	HPLC-PDAD, UHPLC-QqQ-MS
Yun J, et al. (2017)	Weight loss, fat burning, energy enhancement, athletic performance	110	B-PEA, BMPEA	10	South Korea	LC-PDAD and LC-QqQ-MS
Zeng Y, et al. (2016)	Weight loss	447	Sibutramine and phenolphthalein, liothyronine, thyroxine, fluoxetine, furosemide	119	Singapore	LC-QqQ-MS

Zhao J, et al. (2018)	Weight loss	32	Phenethylamine, synephrine, oxilofrine, hordenine, b-methylphenethylamine, n-methyltyramine, octopamine, deterenol	15, 12, 6, 3, 2, 2, 1 (in order of drugs)	USA	1D and 2NMR spectra
Zhong Y, et al. (2017)	Weight loss, Chinese herbs	13	None detected	0	China	HPLC-PDAD, LC-QqQ-MS

Table 1 - Characteristics of the studies chosen to be included including, supplement tested, sample size, adulterants found, number of supplements adulterated, location of study and sampling technique used.

8 Discussion

8.1 Summary of findings

Using a documented and recorded research method, articles relating to the adulteration of sports supplements, including weight loss and pre-workout solutions, were identified along with the common adulterants used in this process. The amount of literature surrounding this topic was vast, and it was evident that this is an ongoing problem for the sports industry. Based on the level of adulteration identified in the reviewed articles over the period 2013 to 2023, it seems the situation is not improving. Since the peak in 2014, the number of articles produced surrounding the adulteration of supplements has gone through ebbs and flows, with there being a slight decrease in 2020 (potentially due to COVID and the overall reduction in studies outside of COVID (106)) before increasing again in 2021.

The overall compounds used for adulteration have not changed much over the ten-year period this review examined; some articles did find unusual adulterants, such as antibiotics (72) and depressants (39), but the most commonly found in the review, are the same adulterants over the ten year period. These common adulterants included sibutramine, which was found from 2013 up to 2023, phenolphthalein, which was observed in papers from 2013 to 2022, DMAA, which even after its ban in 2012 was still found as an adulterant in papers from 2014 up to 2021, and finally phenethylamine, which was seen as an adulterant from the articles in this review from 2014 and up to 2021. With little change in the adulterants used in sports supplements, it works to highlight the lack of effect at a legislative level many of these countries have had over the ten years and how in light of change, manufacturers turn to analogues of well-known adulterants to help combat the ever-changing legal landscape.

Of the 81 articles, three (37, 48, 99) found that the supplements examined did not include adulterants. The lack of adulterants present could have been a sign that due to changing laws and regulations surrounding adulteration, the number of supplements being adulterated had changed but due to the years that the articles were published along with one article being from a different country (48), it was instead an indication of three possible causes, the first being the number of adulterants that the authors tested for were not enough to cover the adulterants that may have been present in their samples, the second being the adulterants tested for were not common compounds found in the supplements they were testing. Finally, the supplements may not have been adulterated with anything in the first place. The inclusion of these articles helped to highlight the fact that not all supplements are adulterated. However, with only three articles reaching this conclusion based on the criteria set, it is more likely than not that these papers were a statistical anomaly. On the other end of the spectrum, there was a large number of articles that had 100% of their samples adulterated; 13 articles had this worrying statistic (30, 35, 56, 64, 67, 74, 86,

93, 94, 101, 102, 104). Of the 13 that had all supplements adulterated, all articles, excluding one (86), had less than ten supplements tested for, with a majority having two or even one supplement sample size. This small sample size can lead to a higher chance of all supplements being adulterated, especially if the supplements were previously thought to be adulterated but were not confirmed before testing.

An inclusion criterion for this review was that the articles tested supplements without prior knowledge of their adulteration status; if this were not the case, the number of articles that had all supplements adulterated would have been thought to have been greatly increased, leading to a skew in the perception of the supplements that were tested. Aside from the articles that either had 0% adulteration or 100% adulteration, the average rate of adulteration was 34.1%, meaning that, on average, the articles included found that 34.1% of the supplements they tested had adulterants present. With 5203 supplements having been tested across all the articles and 1774 confirmed as adulterated. This statistic could be extrapolated into the general sports supplement industry; this would give an idea going forward on the rates of adulteration at a global scale helping researchers have a realistic idea of what to expect when undertaking similar studies. In a previously cited source, it was said that within Australian one in five supplements were adulterated with a banned substance (9). The results from this review came out at a higher percentage but not drastically different; the time difference along with this review taking into consideration supplement adulteration on a global level, means that it's not possible to disprove that source.

As stated previously, the 24 different countries gave a wide base to compare legislative frameworks to Australia's newly implemented law. One article in the review came from Australia (105), while another came from New Zealand (86), which has very similar sports supplements laws to Australia. Both of these articles were published in 2015, well before the introduction of the new legislation; this makes the information found in these articles less relevant. With the inclusion of 22 other countries, with publication dates up to 2023, the subsequent analysis of these countries' sports supplements legislation may help determine the effect these new Australian laws may have on adulteration.

8.2 Pre-workout supplements

Based on the supplements tested and the adulterants detected, there was a clear pattern between the adulterants used and the supplements adulterated. The occurrence of specific drugs based on the supplement was thought to have occurred before the beginning of this review due to certain drugs having effects that some supplements would benefit from and others would not. From the 81 articles, pre-workout articles made up a small number of the supplements tested, with 13 articles testing them (11, 32, 50, 51, 53, 58, 92, 100-105). Of the adulterants found within these samples, a majority could be classed as stimulants as well as being analogues of amphetamine or methamphetamine, and these included NADEP, NN-DMPPA and DEPEA. These drugs were found within five articles (100-102, 104, 105), showing a clear trend with designer drugs as adulterants to potentially avoid detection and legality issues based on the amphetamine/methamphetamine structure and effects. DMAA and DMBA are other adulterants that can be classed as stimulants. Unlike NADEP, NN-DMPPA and DEPEA, these compounds were found within weight loss supplements as much as, if not more than, in pre-workout supplements. Their inclusion in weight loss supplements is likely due to stimulants' effect, with appetite suppression being a wanted side effect of intake (21); therefore, including such adulterants within weight loss supplements can help achieve more significant results.

8.2.1 Pre-workout adulterants

DMAA and its analogues have a structure similar to amphetamine and its derivates as shown in Figure 2 (107) and Figure 3 (108). However, the slight difference between the structures means that the effects experienced by the user are not the same.

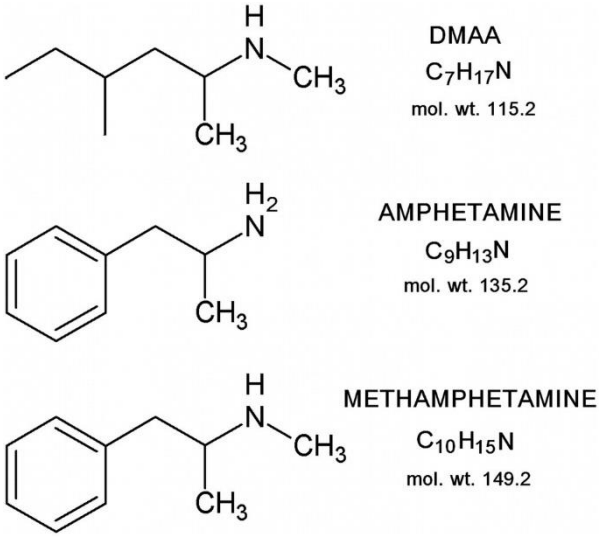

Figure 2 – 1,3-dimethylamylamine (DMAA), amphetamine and methamphetamine structure

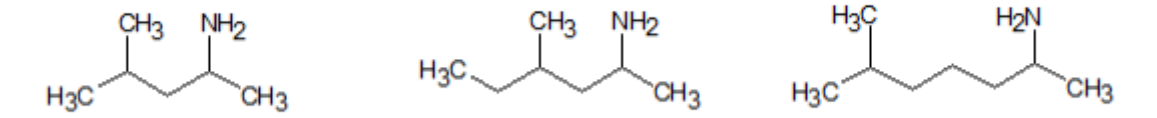

Figure 3 - 1,3-dimethylbutylamine (DMBA), 1,3-dimethylamylamine (DMAA) and 1,5-dimethylhexylamine (DMHA) structure

DMAA was first used in the 1940s as a nasal decongest before being removed by the FDA in the 1970s for unknown reasons (11). The ephedrine-like compound has similar physiological effects as amphetamine, increasing arterial blood pressure, vasoconstriction, tachycardia, and bronchodilation and is also a stimulant (109). These characteristics make it an ideal compound for pre-workout and weight loss supplements. The subsequent banning of DMAA in 2012 was said to be in response to reports of death from ingesting pills and powders containing the compound (109), but once the drug was banned, the illegal adulteration of supplements has not stopped. For the DMAA analogues, many of these compounds can be classified as experimental, meaning that they have never been thoroughly tested on humans, and the effects experienced by the user are not fully known. Before this review, DMAA and its analogues were thought to have been used predominately in pre-workout supplements. Based on the reviewed papers, it is clear to see that this is no longer the case as manufacturers have moved away from the commonly thought uses for these adulterants and into new alternatives. The reason for this is largely unknown; it might be due to increasing pressure from law enforcement that makes it harder to create and distribute pre-workouts with these adulterants present. Alternatively, it could be a case of manufacturers finding a better alternative for pre-workouts. Further study is needed to understand precisely why it is occurring, but it could lead to valuable information that might help combat adulteration. The use of methamphetamine or amphetamine was seen in only one article within this review (73). Even though only one found these compounds present, the article was published in 2022, meaning that currently, methamphetamine and amphetamine are still used as adulterants in supplements. Unlike most of the other adulterants found within this review, methamphetamine and amphetamine are classed as Schedule 8 compounds. Being a Schedule 8 compound, means they have known therapeutic uses and can be prescribed, but the potential for misuse and abuse is much higher than Schedule 4 drugs (110). Along with these known illicit compounds, the many analogues mentioned prior were found throughout the review, with the highest methamphetamine/amphetamine analogue present being DEPEA. N-*a*-diethylphenethylamine, or DEPEA, is a structural analogue of

methamphetamine, with its effects on humans after consumption entirely unknown. No previous literature has tested the compound within humans (100), yet three separate papers have quantified it in the adulteration of supplements. The compound has no previous uses in pharmacology and was first identified as a designer drug within urine amphetamine tests (100). This compound, along with the other analogues, is hazardous and malicious, yet manufacturers continue to put these drugs within their products and endanger many lives.

8.2.2 Trends

The trends apparent from the pre-workout articles show that the country of the study did not influence the type of adulterant found. Within the five articles (100, 102-105) that only looked at pre-workouts, all 5 had different countries of origin, with them being Sweden (105), Australia (104), Germany (103), South Korea (102) and the USA (100) yet, the majority of the papers found analogues of methamphetamine or amphetamine such as DMAA or DMBA, as an adulterants. This trend highlights the fact that pre-workouts are sold internationally, especially when it comes to big corporations; due to this, adulterants that are found in one country are more than likely present in others, putting more people at risk, especially when the adulterant is newly designed, and therefore testing is caught up yet. Additionally, the dates on which each article was published ranged from 2014 (100, 102-104) to 2015 (105). In such a short time, these countries found similar analogues in pre-workout supplements bought from their respective countries. These adulterants were common after these articles, as this review only looked at articles from 2013 onwards. However, these adulterants were not found in any subsequent searches. Without there being any recent articles within this review finding these adulterants, it can lead to suggestions that these designer drugs were used for a short period before testing caught up with the new design and rendered them useless, especially for people wanting an adulterant that can avoid detection, meaning that manufacturers scrapped the inclusion of these designer drugs and moved onto newer alternatives. Another cause is the nature of systematic literature reviews and how we navigate information. With an inclusion criterion each article must meet, articles reporting adulteration with these compounds may exist, but the criteria excluded them from this review. This factor of potentially leaving out important articles due to inclusion criteria needs to be considered, especially when making suggestions and theories surrounding supplement adulteration.

8.3 Weight loss supplements

75 of the 81 articles included in this review included weight loss supplements. With most of the supplements tested being weight loss, it shows a clear trend among the cases of adulteration. This theory that was developed before this review, as current research supports the idea that weight loss supplements are among the highest adulterated supplements (111). The review works to confirm this theory further.

8.3.1 Common adulterants

The most common adulterant found in the articles was sibutramine, with 30 separate articles reporting it (13, 29, 30, 34, 36, 40, 44, 46, 54, 55, 57, 59, 61-65, 69, 71, 74, 76, 81, 84-86, 88, 91, 93, 95, 97). Most of the articles that found this adulterant were testing weight loss supplements, where this drug is most commonly found as an adulterant (13, 29, 30, 36, 46, 55, 57, 61, 63-65, 74, 76, 84, 85, 88, 91, 93, 95, 97). Sibutramine has a long past with uses in weight loss dating back to the 90s when it was first approved by the American Drug Administration or FDA (112), where it was sold under the name Reductil here in Australia (113). *Reductil* is a prescription-only drug that was prescribed to obese patients who were not able to lose weight through conventional means (114) and had no other option other than to turn to medication. It was prescribed in Australia until 2010, when it was found after a re-evaluation that sibutramine increased the risk of threat to the consumers' cardiovascular health (115), subsequently removing the substance from Australian prescriptions. Looking at the time in which sibutramine was found as an adulterant, it was found throughout the ten-year duration of this review, starting in 2013 (30, 64, 74) and going up to 2023 (93). Over the ten years, sibutramine was found consistently over the time frame, with 2014 and 2016 having the highest number of articles finding the adulterant, at 5 (40, 55, 57, 59, 63, 65, 76, 85, 91, 97). This little to no change

displayed by the review shows that sibutramine is ever common as an adulterant, likely due to its ease of access for manufacturers to acquire and the known effects it has on weight loss.

Phenolphthalein was the second most common adulterant found within the articles, found in 16 of the 81 articles included. Like sibutramine, it has been chiefly used in the adulteration of weight loss supplements, with its original usage being a prescription-only drug used in Australia until the 1990s (116) for overweight individuals. Unlike sibutramine, phenolphthalein is classed as a laxative and therefore works by increasing the stimulation of intestinal mucosa and contractions of smooth muscle, which leads to increased defecation (19). In the 1990s, phenolphthalein was removed from the market due to research and subsequent classifying of the compound as a 2B carcinogen (117). With the drug removed from the market, it has since been detected in supplements as an adulterant and has seen use over the 10-year duration of this review (29, 30, 40, 46, 55, 59, 65, 69, 70, 75, 85, 86, 88, 91, 95, 97).

Aside from these two compounds, many of the remaining weight loss adulterants comprised of either anorexiants, diuretics, laxatives or stimulants (11, 13, 15, 29-31, 33-36, 38-42, 44, 46, 49-51, 54-59, 61-65, 67, 69, 71, 73, 74, 76, 77, 79, 82-86, 88, 89, 91, 93-97, 100-104). With there being such a wide range of potential adulterants that can be used, it is theorised that any number of drugs could be found as adulterants within weight loss supplements meaning testing will need to be updated at a consistent rate to ensure all newly designed and implemented drugs are accounted for when testing. A common characteristic seen with weight loss drugs is the addition of current or previously prescribed compounds used as adulterants (13). The use of prescription-only drugs may be due to the known effects such as inhibition of key systems helping reduce fat digestion, dopamine inhibition, opioid receptor antagonism and more (118), that these drugs have on the user, along with the dosages that it takes to elicit a response. Adding these drugs ensures that manufacturers know what the user will experience, and the chance of adverse reactions such as death (23), that may make the product look bad is decreased. However, the intentional addition of prescription-only drugs fails to address why they are prescription-only substances in the first place. Current Australian legislation puts all medicines and chemicals in classes based on the threat to public health (119). With prescription-only medicines, the chemicals that make up these drugs are classed as schedule 4 substances, meaning that only via prescription from a doctor can they be accessed as if taken haphazardly, which can lead to detrimental effects for the user. Adulterating supplements with prescription only drugs negates the reasoning behind why they are prescription-only drugs in the first place.

8.3.2 Designer drugs

An aspect of weight loss supplements is the adulteration of them with designer drugs making it harder to identify the drug used and also quantify the level of adulteration. An objective posed earlier was to identify any new emerging drugs that have yet to make an impact within Australia, and designer drugs tend to be the new drugs that are found. Looking at the articles within this review, twenty-eight (11, 15, 31, 33, 39, 41, 42, 49-51, 58, 59, 63-65, 67, 68, 74, 83, 94-96, 98, 100-102, 104, 105) papers found a designer drug present within their sampled supplements. Some of the designer drugs that were detected included DMAA, DMBA, NADEP and more. These articles ranged in the year starting from 2013 (64, 74) up until 2021 (15, 41, 51), showing that new drugs used are not necessarily designer drugs. The issue with designer drugs is that their existence is mainly unknown. Therefore, any current testing procedures would only be able to identify them in samples if they were previously known. It is also impossible to determine if the designer drugs found within the twenty-eight articles have made it into Australian supplements. The lack of research makes it challenging to come to any conclusions. Based purely on this review, it is likely that these drugs have made it into Australia as they are currently known as designer compounds. Therefore, the number of times these compounds have been previously detected is high. Looking at the articles that found designer drugs within weight loss supplements, the newer articles published in 2021 found the inclusion of designer stimulants more than designer weight loss drugs. These designer stimulants included 1,3-dimethybutylamine (DMBA), an analogue of DMAA (42) and have seen wide-scale use since the banning of its predecessor. Within the articles of this review, DMBA was first seen as an adulterant in 2015, which would be not long after the banning of DMAA. Different from DMAA, DMBA lasted only a short time before regulatory agencies like the FDA banned the compound from being added to supplements within the US in 2015 (120), with other countries following soon after. Even after its banning, DMBA can still be seen in adulterated supplements and other banned substances such as DMAA. The mechanism of action behind DMBA is unknown, as only a few tests have been done on animals to determine what occurs. These

tests, it was determined that the likely effect experienced by the user is similar to DMAA but less potent, suggesting that it has a similar mechanism of action to DMAA (42). DMBA is an example of the type of designer adulterant found within supplements, with little to no testing and the safety and efficacy also unknown; the potential side effects for the user are vast, but it is still being found in supplements as recently as 2021 (41, 51).

Overall, both sibutramine and phenolphthalein were the most common adulterants found in this review, and with weight loss supplements having the majority of articles testing them, it shows a distinct link between the two that has stayed the same over ten years. This slight change in the type of adulterant used can also be seen in other adulterants that were mentioned prior, such as DMBA, with it still being found even after the banning. The reason for little to no difference may be the lack of significant legislative change that has occurred worldwide over the past decade. With a known past of these adulterants being used as weight loss drugs, manufacturers have kept their methods the same in the current market. When theorising the future of weight loss adulteration, the current trajectory is on the idea that sibutramine and phenolphthalein are still among the most used adulterants, with little change to the level of adulteration found. Applying this theory locally to Australia poses more issues, as the current level of research needs to be more significant to make such blanket statements. With the introduction of new laws, future testing will need to be conducted on weight loss supplements to determine their effects on the level of adulteration in Australia.

8.4 Multi-national supplement regulation

As stated previously, 24 different countries are represented in this review. With such diversity in the countries, this is translated into the legislation surrounding supplements, with each country having different laws and regulations for sports supplements. Having the most common regions tested in the USA, South Korea and Brazil will be used to analyse the effect differing legislation has on adulteration when considering these are the most common countries found in a review pertaining to adulteration. To offer an alternate view on regulations from a country with no published studies, Canada will be used and compared to Australia.

8.4.1 United States of America

Within the US, the Food and Drug Administration (FDA) is responsible for regulating supplements found on the market. The FDA defines *dietary supplements* as a product that is “intended to add to or supplement the diet and are different from conventional food”, and any supplement or product that is labelled with intent to “treat, diagnose, cure or prevent diseases” is classified as a drug (121). Based on the classification given by the FDA, sports supplements would be classified under this exact definition and therefore be exposed to the same regulation. However, a regulatory framework was introduced in 1994 called the Dietary Supplement Health and Education Act of 1994 (DSHEA) (121). Under this framework, the FDA regulation of supplements was severely dampened, leaving the regulation of dietary supplements within the country mainly to the supplement company (12). Companies were tasked with ensuring that safety standards are met and are not in violation of any laws prior to being listed on the market, with the FDA having no impact on the approval of a product before it is available to consumers (12). The role of the FDA concerning sports supplements is the regulation of them after they have been listed on the market, not before, with that they monitor the manufacturer’s location along with labelling information and adverse event reports for the supplement that may indicate nefarious tactics used by the manufacturer (121). As stated by the official FDA website, pre-market regulation is not a tactic used by the agency; this is more than likely due to the number of new supplements that appear on the market each year, with an enormous number of resources to track each one before being sold to the public (12). This overwhelming reliance on reports of adverse effects to counter something like adulteration is a probable reason why the USA had the highest incident rate of adulteration out of all the articles included in this review. Comparing the current regulatory approach of the Australian TGA to that of the US FDA, it is clear that the previous Australian regulation standards were very similar to the US, with there being little in the way of regulation prior to release to the public and more emphasis placed on products after they have been put to market. With the introduction of this new legalisation (26), Australian regulation has altered slightly from the American model, but further analysis of currently available sports supplements is needed to determine the effect this has on adulteration.

Looking at the studies published from the US, the most commonly found adulterants were DMBA with six occurrences (11, 31, 33, 41, 42, 49), DMAA with five occurrences (11, 31, 33, 41, 49) and sibutramine with three (74, 91, 100). Looking at legislation surrounding DMBA use in sports supplements, currently, there are no indications that the compound has been classified as illegal. However, the compounds are banned by the FDA in sports supplements (122). Like DMBA, DMAA is also banned for use in sports supplements but, again, is not recognised as an illegal compound (109) with both having similar rates of use as adulterants and the same laws surrounding their usage. It highlights that the lack of strict scheduling of these compounds can lead to their use as adulterants. The final compound, sibutramine, is a Schedule 4 substance under Section 812 of the Controlled Substances Act (123), as it was formally used as a prescription weight loss drug before being pulled from the market (112). Even as a scheduled compound, sibutramine is still highly present within sports supplements; however, compared to both DMAA and DMBA, rates of adulteration using sibutramine are less, which may be due to the more rigid restrictions placed on compounds that are scheduled (124). Lower adulteration rates with sibutramine may offer a theory of how to restrict adulterants such as DMAA and DMBA, to protect users of sports supplements further.

8.4.2 South Korea

Looking at South Korean rules and regulations, like the US and Australia, South Korea classifies sports supplements as health functional food, which falls under the food category of their system. With this classification, products are subject to ingredient, manufacturing process and label regulation before being put on the market. The creation and enforcement of these regulations are carried out by the Ministry of Food and Drug Safety with help from the National Institute of Food and Drug Safety Evaluation, which runs the proposed supplements through tests to ensure they are confirmed before being sold to the public (125). The availability of information surrounding South Korea's regulatory system is not as comprehensive as in other countries, as many websites would lead to web pages in Korean, thus leading to less intricate knowledge of their system being acquired. Comparing South Korea's health functional food code to that of the US and Australia, it seems that this is by far the most in-depth code, with it covering manufacturing, processing, production, import, distribution and storage of supplements (125) with the critical difference being the regulation prior to public use. An alarming trend that this review found was that South Korea had the second-highest rate of adulteration (37, 60, 63-66, 69, 70, 89, 90, 95, 96, 102), even with these laws in place to help protect consumers. There are many possible reasons why this is the case, from having a higher rate of research looking into adulteration and quantifying it to there being a higher rate of clandestine supplement manufacturers bleeding into sports supplements due to the size of the Korean supplement industry; the exact reasoning behind why this is the case is complex and likely due to many different reasons. Contrasting the South Korean regulations to that of the Australian ones shows a lack of pre-market analysis of supplements in the Australian legislation, which leaves a potential for adulterated supplements to easily make it to market and potentially affect many unknowing users.

From the studies included that were South Korean based, the most common adulterants found were sennosides with four occurrences (63, 64, 70, 95), sibutramine with four (63, 64, 66, 69) and finally phenolphthalein also with four (64, 65, 70, 90). All three of these adulterants are weight loss drug adulterants, with at least one of them being a previously prescribed weight loss drug before being banned from the market (112). In this case, only reports of sibutramine being banned in weight loss drugs can be found (126). Sibutramine was banned in South Korea at the same time as in other countries as information was coming out about its potential long-term effects on the user (126). As it is banned, the adding of the compound into sports supplements would too be banned, but, with it having the joint highest rates of adulterant use in South Korea and the model of regulation that South Korea applies to their sports supplements, it questions the current system and how rigorous it is. For the other two adulterants, it was not possible to determine their legal standings as information in English surrounding drug scheduling is not commonly found.

8.4.3 Brazil

The final regulatory system being looked at is the Brazilian model, which like the Australian model, changed in 2018 (127). Within Brazil, the regulation of supplements is undertaken by the Brazilian Health Regulatory Agency (ANVISA) (128). ANVISA classifies sports supplements as food as long as it meets the classification of being orally administered, under pharmaceutical doses and intended to supplement a healthy individual's diet (128). Pre-market regulation is an adopted measure taken by ANVISA, with ingredient, label, claim and manufacturer facility checks

made before the ingredient makes it onto Brazilian store shelves (128). Food supplements were not recognised as a separate category in Brazil before 2018 (127); this gives a point of change that further investigation can determine if it was successful. Looking at the papers within this review, there is a clear difference between the number of articles included prior to 2018, with more included that were written before 2018. Out of the ten included, seven occurred before the implementation of the new legislation in 2018, while only three occurred after the fact. However, this is an encouraging sign for the implemented policy, it may indicate that the regulation of supplements helped reduce the number of adulteration cases, but it could also be due to external factors causing the reduction, it is impossible to gauge the cause based on the articles included in this review as that was not the intended purpose of this review and therefore valuable information and articles will be missing from the included information. When comparing the ANVISA regulation to that of the TGA, there is a clear difference between the two, with the ANVISA having pre-market checks for all supplements that fall under the food supplements category (128). In contrast, the TGA has a more relaxed regulation protocol for supplements unless that product is classified as a therapeutic good (26); at that point the regulation is similar to that of ANVISA.

Out of the articles published from Brazil, hydrochlorothiazide, sibutramine, and DMAA were the most common adulterants. Hydrochlorothiazide had four instances (18, 45, 78, 87), sibutramine had two (47, 77), and DMAA also had two (48, 77). Under the Brazilian Controlled Drugs and Substances Act, hydrochlorothiazide falls under class B1, whereas sibutramine is classified as B2 (129). Classification in the ‘B’ category means that these compounds are prescription only; therefore, the adulteration of sports supplements using them is illegal (129). Unlike hydrochlorothiazide and sibutramine, DMAA is not classified in the Act; this is more than likely due to the age of the article and with DMAA being a more recent finding (107). The Brazilian drug act has plenty of other classes that DMAA could easily fall under, but, like South Korea, the difference in language makes finding an up-to-date classification of the drug challenging.

8.4.4 Canada

Looking further afield, a country that is not represented at all in this review is Canada. With it being geographically beside the US and culturally very similar to other Westernised countries within this study, is there legislative reasoning behind the lack of adulteration reported in the scientific literature? Regulations in Canada are implemented by Health Canada, which is responsible for creating and enforcing supplement regulations (130). Sports supplements are classified as Natural and Non-prescription Health Products Directorate (NNHPD); this classification was first introduced in 2004 and was aimed to protect consumers from unsafe products (130). Health Canada regulates the supplement market by ensuring all products sold within the country have a product license given once the manufacturer meets proper measures, including specific labelling, packaging requirements, good manufacturing process, and safety and efficacy. These measures are taken before the product is sold on the market and apply to all products produced and imported into the country (130).

It is theorised that by the lack of inclusion of any articles written in Canada within this review, the regulatory system has a positive effect on the rate of adulteration within the country; this may also be due to other factors that affect the reporting of adulteration which must be considered when proposing this theory. This review negates the inclusion of articles looking at the adulteration of sexual performance enhancers, a large section of the supplement industry not accounted for. Along with excluding sexual performance enhancers, the inclusion criteria cut a large majority of articles that could present evidence showing the opposite of the theory proposed. When comparing the Australian and Canadian regulations, the Canadian version has a larger group size that it is applied to, with all supplements falling under the NNHPD classification and therefore subjected to regulations. With it applying to more supplements, the likelihood of a contaminated supplement making its way onto the market is reduced.

8.4.5 Australia

Considering the differing supplement regulation between these countries, Australia has an in-between approach with the same level of regulation for supplements that classify as foods that the US and FDA have with little to no pre-market regulation, just overall supplement surveillance (121). In contrast, when a supplement is classified as a therapeutic good, Australia has more in common with Brazil, South Korea and Canada regarding regulating similar product attributes before the public can access them (14).

The concept that the Australian version opens itself up to is a manufacturer slightly altering certain features of their product to make them fall under the food category before they adulterate their supplement with an illicit substance and with there being little to no regulation prior to market exposure (26), the product will have chance to reach the consumer for some time before the number of adverse effects is reported to the TGA and action is taken. This concept is only conceptualised based on the information provided by the TGA and Australian government; further confirming this concept would take research into supplements under the different classifications to see if this is the case.

With data now available from the USA, South Korea and Brazil, about which adulterants were most common. Relation to the Australian system to determine the scheduling of each compound can be done. One of the most common compounds was sibutramine. Under the Australian Therapeutic Goods Instrument 2023 (17), sibutramine is classified as a Schedule 4 substance, meaning it is a prescription-only medicine. Schedule 4 classification is also applied to hydrochlorothiazide and phenolphthalein. These compounds are classified under Schedule 4 due to the medical setting in which they are used. Under the Australian instrument, Schedule 4 is related to prescription-only medicines and prescription animal remedies (17) for the remaining relevant compounds, including DMAA, DMBA and sennosides. DMAA and DMBA are schedule 6 compounds used for the classification of poisons in the instrument (17), whereas sennosides are not listed in the scheduling instrument. Sennosides are used within an Australian medical setting (131). However, unlike the other medically used compounds, sennosides can be purchased without needing a prescription and, therefore, would not be classed as a Schedule 4 compound.

Looking at other countries' regulatory processes with sports supplements offers an idea of how effective the newly implemented Australian regulation may be for preventing adulterated products. In the circumstance in which further research finds that the implemented regulation is ineffective at curbing the rate of adulteration in Australia, the current Canadian system may offer direction for future improvements that are known to work effectively.

8.5 Techniques utilised for adulterant detection

Looking at Table 1 there are many different techniques used by the included articles in the identification of adulterants. The use of different techniques is due to the nature of the articles, in which many aimed to prove the utility of a new identification technique; therefore, validation was done for most papers that tested newly developed techniques. The validation procedures often undertaken included measuring the linearity, precision, accuracy, limit of detection and limit of quantitation which are then used to assess how suitable the technique is for the intended purpose (132). When deciding which method to use, the different techniques also depended on the adulterants and the number of adulterants that were being tested for. For most articles, similar analytical techniques were utilised as they tend to be the separation technique responsible for the separation of compounds of interest with compounds of no interest. The difference lies in the hyphenated portion of the method, which is the detection technology used to help determine the separated compounds of interest.

8.5.1 Liquid chromatography

Of the different analytical techniques, liquid chromatography (LC) had the highest number of variations, with triple quadrupole mass spectrometry being the most used technique. LC works by injecting a liquid sample that flows through a stationary phase known as the column; the column then separates the components of the sample into its constituents through chemical and physical characteristics. Once separated, a detector measures the analytes as they leave the column and displays the results for interpretation (133). Due to several reasons, including ease of use and cheap cost, LC is common when looking into supplements and the presence of adulterants, making it a strong option for researchers.

Triple quadrupole mass spectrometry (TQMS) was the most used detector out of all the LC-hyphenated techniques. This spectrometry technique utilises direct current and oscillating radio frequencies to filter out ions with the desired mass-to-charge ratio (m/z) before colliding these ions with neutral molecules forming fragments that are

then detected and analysed (134). TQMS is often used for the metabolic profiling of a few compounds and thus offers an advantage over other tandem mass spec techniques, as it can make the identification of fragments efficient as long as the methods are established and fragment pairs specific to the metabolite are known prior (135). This makes TQMS a viable option for supplement testing, as this review has shown that the adulterants used are commonly found amongst other supplements that make the same claims. The idea is further confirmed when looking at the articles within this review that used TQMS, as a majority of the papers tested supplements for adulterants that they decided upon based on the findings of other researchers with similar supplements (62, 65, 91). Many of the same articles also included testing with at least one other technique; this was the case for many reasons, including validation of their new technique, sending the supplements to separate testing facilities and using multiple testing methods to ensure all adulterants are detected (37, 65, 73, 81).

The issue arising from using TQMS is that if any adulterants not thought to be present prior to testing are used in the supplement, TQMS will not be able to quantify these compounds and with designer adulterants being an ever-present threat, leaving an issue of how to quantify newly synthesised drugs (134). This is where the second most common LC hyphenated technique becomes an alternative option, quadrupole time of flight (QToF).

QToF, like TQMS, works by filtering specific ions based on m/z ratio or radio frequency, then colliding these ions with the neutral molecules; fragments are then pulsed with an electric field and accelerated to their direction of origin. With this, an analyser detects the time it takes to reach the end of the flight, with lighter ions being faster than those heavier in mass (136). An incentive that researchers have when using QToF is the ability to determine precursor ions, which enables a researcher to gain an understanding of the compounds present within a sample without having prior knowledge; this is known as non-targeted screening (136). Along with the ability to run non-target screening, QToF can also run tests for specific compounds, known as targeted screening (136). With QToF being able to do both targeted and non-targeted screening, it makes it possible for researchers first to discover adulterants not previously thought to be used in their specific supplement and can also lead to them finding new designer drugs; the only issue with being the first to discover a new compound is the inability to validate the drug as no other current standards or information surrounding it exists. Looking at the papers that used QToF, it is clear that non-targeted screening was used, as most articles tried to determine the adulterants found within their samples (31, 47, 81).

LC-MS was tied with LC-Qorbi-MS (Qorbi) as the third most used technique of the remaining seven LC-hyphenated techniques. LC was explained prior, with the mass spectrometry detector responsible for interpreting results based on the ions m/z. LC-MS is an inexpensive and easy-to-use technique that has been proven countless times to produce accurate and precise results (133). The use of LC-MS for the articles included in this review also had to the inclusion of other techniques. LC-MS was often referred to as a low-level screening method with the implementation of higher-level techniques to identify adulterants further.

As for Qorbi, the orbitrap section of the technique works by combining the stable ion trajectories with rotation around a central electrode with harmonic oscillations; the oscillations depend on the ion's m/z charge and the instrument itself. The oscillating ions then produce a frequency detected by the outer electrode of the analyser, creating the mass spectra (137). This hybrid platform is quite recent in terms of commercially available, and therefore its use in articles included within this review only started in 2019 (137). Like QToF, Qorbi can detect non-targeted compounds, which is highly useful for such tasks as the ones carried out by researchers (70). However, due to it being such a new technology, it was not the only high-level technique used by researchers, with many of them opting to run the samples through various other high-level screening methods.

With the methods discussed, it is clear to see why TQMS was the most popular technique used. With many of the articles having pre-conceived theories about what adulterants are going to be found in certain supplements, and the ease-of-use along with cheaper cost TQMS has, compared to the other techniques, the ability for high-level identification (134), making it a sensible choice for determining adulteration of supplements.

8.5.2 Gas chromatography

Like LC, gas chromatography (GC) is a highly utilised separation technique used throughout many science disciplines (138). Within the articles included in this review, 14 articles used GC-MS, and four other hyphenated variations of the technique were used throughout. GC is used quite often in determining adulterants due to its ability to

separate mixtures based on boiling point/vapour pressure (138). GC works by injecting a sample into the injection port before the sample is taken by a carrier gas, also known as the mobile phase, and is sent through the column, which is the stationary phase (138). While going through the column in a temperature-enclosed oven, chromatographic separation occurs, with the separated components exiting the column and going past the detector, which produces an electronic signal based on the amount of eluting analyte that passes it (138). The addition of a mass spectrometer gives more information on the structure of chemical compounds by turning the chemical compounds into ions by fragmentation and ionisation; these ions are then sorted based on m/z producing a mass spectrum (138).

The advantages of using GC lie in its high efficiency, accurate quantitation, and is the gold standard in analytical methods. With it being so highly regarded, the techniques used are well-documented and known to researchers (138). GC does not come without its disadvantages, with its low sensitivity, and the compounds it can quantify have to be non-volatile and thermally stable, meaning that biological samples do not apply to this technique (139). The use of GC-MS in the articles included ranged in reasoning, with some articles using the technique as the compounds targeted were known to the researcher, meaning GC-MS was an applicable technique (33). In contrast, other papers used it along with other techniques to validate their findings (15).

After GC-MS, the remaining techniques had one article using them, with TQMS and QToF being hyphenated techniques talked about previously. The last GC method included was that of GC-MS/NPD (NPD), a variation on the detector used by the GC-MS. Nitrogen phosphorus detector (NPD) is selective to analytes containing either nitrogen or phosphorus (68). Therefore, applying such a detector would be particular and, in the case of adulterants screening, would be an applicable instance. The article that used this technique used GC-MS coupled with an NPD detector as a last screening step for supplements that returned negative for any adulterants present to confirm their negative status further.

Overall, GC is a valid identification procedure used throughout multiple articles. Its use often lies in its reliability in supporting or denying the validity of other techniques, as it is a cheap and easy-to-run technique. For future researchers, it offers a highly known and documented understanding and can be further enhanced by including TQMS or QToF.

8.5.3 UHPLC and HPLC

Both UHPLC and HPLC are of similar concept, with both systems working off the basic LC principle mentioned prior. The difference between these two techniques and LC, is the application of high pressure throughout the system to decrease the amount of time it takes for the sample to pass through the column (140). Looking more specifically at the difference between UHPLC and HPLC, UHPLC has further increases to speed, resolution and sensitivity of the analysis (140), this is due to the column used, with HPLC the usual particle size that is separated in the column ranges anywhere from 2.5µm to 10µm in size, whereas UHPLC can discern between particles <2µm but must maintain a high internal pressure while operating, which would be the upper limit for HPLC (141). Both offer a high-level technique that can be utilised in the screening of supplements.

Looking at the number of articles using these methods, a total of seven used either UHPLC or HPLC with just mass spectrometry, whereas the remaining hyphenated techniques made up the majority of uses for these methods. In both instances, coupled with photodiode array detector (PDAD), resulted in the highest number of uses suggesting a preference towards this technique. PDADs are detectors that analyse the light passed through a sample, and determines analytes based on comparison to the spectrum (142). The light is then converted into signals and outputted as a contour map (142). Using PDADs it gives the researcher an advantage as it can determine the whole spectrum for the samples inputted, eliminating the time that it takes to re-run a sample at different spectrums (142). Along with this, the use of PDADs can make the process of determining an unknown peak on a chromatogram easier and can also help determine the peak purity which tells the user if any analytes have not separated properly while going through the column (143). The use of this technique ranged from 2013 up to 2022 (30, 69), showing that it is a commonly used technique that has ample examples and documentation surrounding its usage for supplement adulteration. In a large portion of use cases, the technique was seen alongside others, this likely due to reasons mentioned prior, but the number of articles using solely UHPLC or HPLC with PDAD was higher than usual. The exclusive use suggests that it is reliable and accurate when it comes to the outputted results. Comparing both UHPLC

and HPLC with the hyphenated PDAD technique, both offer very similar results, but UHPLC has the increases in speed and resolution making it a more enticing option if a decision was to be made between the two.

One hyphenated technique that was seen with HPLC that wasn't seen with UHPLC, was pulsed amperometric detection (PAD). This technique is used for the analysis of carbohydrates and related substances. With the solution at a high pH level, carbohydrates can be broken down at the surface of a gold-plated electrode, the resulting reducing and non-reducing sugars, alditols and oligosaccharides are then measured (144). With the inclusion of the PAD, the results can remain stable and reproducible throughout the screening (144). The two articles that used this technique were both determining if HPLC with PAD is an appropriate method for detection of adulterants. Because of this aim, both were targeting a set number of known adulterants that they believed would be present in certain sports supplements; highlighting that the types of studies that this method is most appropriate for. With the technique working by breaking down carbohydrates, the adulterants would first have to be confirmed and noted to ensure that the application of PAD is not lost on a compound that would have no change on the results. Looking into HPLC and UHPLC with the hyphenated PAD technique applied, no information could be found pertaining to the inability to use UHPLC with PAD. Majority of the articles and publications read, used HPLC as their base technique and this review only had articles using PAD with HPLC; with the lack of information surrounding the use of PAD with UHPLC, it is not possible to determine why the two aren't used together.

Current day usage of HPLC sees the most common hyphenated technique used being UV and PDAD detectors (141), this review has proved this statement to be correct as the most common technique was PDAD whether it was with UHPLC or HPLC. With such high usage, it proves the validity of the results produced from articles using the technique, if proper procedures and protocol are followed.

8.5.4 Other techniques

Aside from the LC, GC and high-performance LC techniques used, other methods that do not fall under these categories were also implemented by some articles, once such technique was NMR. Nuclear magnetic resonance or NMR was seen in six separate articles making it the most common technique outside of the three previous stated methods. NMR works by introducing a sample into a magnetic field, once in this magnetic field the nuclei of the sample begin to spin, the sample is then irradiated with radio waves and the resulting signals produced by the sample are collected and outputted as a spectrum (145). With the outputted spectrum, it is then possible to determine the composition of the sample (146), this makes it a highly valuable research method when it comes to screening supplements for adulterants.

Within forensics, NMR has seen widespread use for the determination of new designer drugs as the technique has to the ability to elucidate the structure of a new compound (145). With this ability known by researchers, it is theorised that the use case for this technique would be for the identification of adulterants with no pre-determined ideas about which supplements contain which adulterants (145). Looking at the articles that used NMR, half of the articles used solely NMR, while the other half used NMR alongside other techniques. This works to neither confirm nor deny the previously proposed theory, but shows that NMR can used as either a screening technique for unknown compounds or for known compounds. Comparing NMR to other techniques that offer similar advantages, its clear that usage lacks. With it having the ability to determine new compounds never quantified prior (145), the reasoning behind the lack of use may lay more in its availability and less in its ability. NMR was conventionally used in high-end labs as the size and expense for a high field NMR machine is drastic (145). Over the past five-years, benchtop NMRs have become more popular and offer the advantages of an NMR technique at a smaller size and less cost, the only drawback being loss of dispersion and sensitivity (145). As these benchtop machines evolve and become more common place within laboratories, the availability of the technique to researchers will be greater.

After NMR, capillary zone electrophoresis (CZE) was another technique that was seen utilised in the included articles. CZE works in similar ways to LC, with the sample being injected into the system, it then flows through an electrolyte filled capillary that has a high voltage applied (147). The time it takes for the analytes of the sample to run through the capillary is based on three factors, these include the electrophoretic mobility of the analyte, the length of capillary and the applied potential (147). Once the analyte reaches the detector, it calculates the m/z ratio using the three variables and outputs an electropherogram that can then be interpreted by the user (147). The advantages when

using CZE include low sample size needed, minimal preparation of the sample, speed of analysis and low cost of consumables (148), makes using CZE easier and more efficient when comparing to other techniques.

Along with GC and LC, the hyphenated detector that CZE uses can be changed depending on what the user wants from the technique. The different detectors include ultraviolet (UV), diode array detection (DAD) and coupled contactless conductivity detector (C^4D) with the structure of the molecules in question being a contributing factor to the choice of detector (147). In the instance of supplement screening for adulterants, it was recently found that using both C^4D and UV is the best combination so that all adulterants, no matter the structure, will be detected meaning no other techniques are needed to find adulterants that would elude one detector (78). In both instances of use within this review, CZE was coupled with C^4D and UV in one article and in both instances CZE was the only technique used by researchers. This helps to prove that CZE is a valid option for the determination of adulterants, and there is enough documentation surrounding the use of it that makes it applicable to supplement screening.

For the screening of supplements, adulterants that are being screened for will firstly have to be identified and noted as able to be detected by the technique, this makes CZE a technique less for the quantification of new or unexpected compounds and more for the detection of expected and known compounds. The overall ability of CZE, makes it another compelling technique that could be used by researchers for the testing of supplements for adulteration.

8.5.5 Technique trends

With being able to look at the different techniques used by the included 81 articles; it shows that there are many different, valid techniques that a researcher can choose from. Looking at the techniques used they can be broken down into two broad categories, one being techniques that can quantify unknown compounds such as designer drugs and unexpected adulterants, such techniques include QToF, QOrbi, PDAD and NMR, and the other being techniques that can quantify adulterants that are believed to occur in specific supplements, these techniques include LC-MS, TQMS, GC-MS, UHPLC, HPLC, PAD and CZE. With there being more techniques present in the latter category, it shows that the majority of testing carried out on supplements is based on pre-existing inclinations about which adulterants are going to be found in certain supplements.

The overall range in potential techniques that a researcher can choose from, gives them the ability to pick a certain technique based on their own goals and objectives making it possible to further understand how much adulterations occurs within supplements.

8.6 Limitations

A limitation of this review was how up to date the articles included are. With the 5th of April 2023 being the last date that any database was searched, any research posted after that day would therefore not be included within this review. Along with this, the searching of three databases may not include all articles that would be relevant to this review making it a possibility that some articles were missed.

9 Experimental design

9.1 Aim

Going forward, the aim of the research project that will coincide with this review, is to determine the presence of illicit adulterants within Australian owned and made weight loss and pre-workout supplements.

9.2 Proposed Method

The way in which this research aim would be achieved, is firstly through a market sweep of currently available Australian supplements. When conducting the market sweep certain criteria will be in mind such as ensuring they are at least Australian owned supplements, with preference towards Australian made, if possible, along with this supplements that are advertised as either weight loss or pre-workout in some capacity will be the desired supplements to be acquired. Once the market sweep has been conducted, the process of buying and receiving the supplements is the next step in this research project. As the supplements are received testing can be started using gas chromatography mass spectrometry (GC-MS). For the initial identification of any illicit adulterants, five grams of the sample will be dissolved in 15mL of ethanol before being mixed thoroughly via vortexing. The solution will then be centrifuged at 5400rpm for 5 minutes. Once fully homogenized, the top layer will be pipette with a syringe filter and deposited into an autosampler vial which will then be run through the GC-MS. From these injected samples, a chromatograph will be developed; using this we can then compare the spikes indicating certain compounds, to that of a chromatograph library to determine any compounds of interest. Once an illicit adulterant is detected, further testing will be conducted via further GC-MS. The suspected samples will be dissolved in water based on manufacturer's instructions if physical form is powdered. For supplements in pill form, the pills will be split open, and contents removed before being dissolved in 100mL of water. All solutions will then be diluted with water 100 times, followed by extraction using either dichloromethane or acetonitrile. 0.2mL of the resulting solution along with 0.2mL of concentrated ammonia solution, will be mixed with 1mL of water and 5mL of cyclohexane solution. The cyclohexane solution will contain the standard at 100ng per 5mL, for the compound that is thought to be used as the adulterant in the sample. While mixing the solution, 75µL of pentafluorobenzoyl chloride solution will be added. Once all compounds are added into the solution, it will be centrifuged at 5400rpm for 5 minutes, once centrifuged the organic phase will be pipette from the mixed solution. The organic phase will then be evaporated to dryness using a vacuum before being reconstituted in 100µL of methanol before being injected into the GC-MS system.

9.3 Hypotheses

Through the testing of Australian sport supplements by GCMS, the presence of illicit substances will be determined.

10 Conclusion

In conclusion, the supplement industry has seen little improvement since 2013; with the compounds used for adulteration and the supplements adulterated, being similar throughout the 10-year period. This review works to highlight the lack of research within Australia surrounding adulteration within locally owned and made supplements. Along with this, the supplements adulterated, the adulterants used, the countries that adulterated products are commonly found in and the regulation of these countries have for adulteration are presented. This information helps to determine the past, current and future trends of adulteration and how it can be deterred within Australia. For future research, efforts should be turned to the further improvement in legislation surrounding supplement regulation, this may be achieved by the in-depth analysis of countries that have experienced low levels of adulteration which will help future law makers determine effective actions. Another aspect that must be continued to be explored, is the identification of new designer drugs, this will help inform other countries of the existence and may give time for the development of easier identification and potential banning. Legislative change has occurred within the past few years in Australia, but without the continued improvement and change surrounding the regulation of adulterants, adulteration will impact many lives and may cause irreversible damage.

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Sports supplement adulteration: A systematic review

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12 Abstract

Sports supplements are supplements taken with the purpose of promoting overall physical ability and well-being improvement. A prominent and long-lasting issue with sports supplements is the adulteration of products with illicit substances that can have dire effects on the user if taken unknowingly. This research looked into sports supplements purchased within Australia. It determined if any illegal adulterants were found within these, aiming to identify whether adulteration of these products occurred and, if so, the level of adulteration in the Australian market. Along with the inclusion of adulterants, the caffeine content of each product was investigated to determine the accuracy of label information, as this is an issue that consumers face with the potentially fatal effects excessive caffeine intake could have. These objectives were achieved by acquiring and analysing pre-workout and fat-burning supplements via gas chromatography-mass spectrometry (GC-MS). It was found that no samples had illegal adulterants present, and the caffeine content of six of the seven products examined was within the concentration levels specified by the manufacturer, with one product having slightly higher amounts than specified. The results present a positive image for the Australian supplement market. However, they will require constant and further testing, as well as expanding the range of the products evaluated, to ascertain the absence of adulteration and mislabelling comprehensively.

13 List of Figures/Tables

Figure 8 shows the caffeine standard curve produced using the peak normalised absorbance from running the caffeine standards. The figure also depicts the standard curve equation and R^2 value at 0.9661, which were used to determine the percentage of caffeine present within each sample compared to what was claimed on the product label.

Sample	Peak absorbance area mAU of caffeine	Claimed caffeine percentage	Determined caffeine percentage
1	4051687	13.34%	13.9%
2	245391	1.31%	1.27%
3	2993286	16.95%	10.4%
4	349142	1.75%	1.62%
5	933893	5.40%	3.56%
6	495106	2.38%	2.1%

7	251307	1.70%	1.3%

Table 5 - Sample number and caffeine peak normalised area, claimed caffeine percentage and determined caffeine percentage for one serving.**Error! Reference source not found.**

14 List of Abbreviations

DCM Dichloromethane

DMAA 1,3-Dimethylamlamine

DMBA 1,3-Dimethybutylamine

EAPB N, *a*-diethylphenethylamine

GC-MS Gas chromatography mass spectrometry

HPLC High performance liquid chromatography

LC-MS Liquid chromatography mass spectrometry

NIST The National Institute of Standards and Technology

TIC Total ion chromatogram

15 Introduction

Dietary supplements are a moniker given to products taken with the intended purpose of supplying specific nutrients that the user may be lacking and are typically consumed in pill, powder or liquid form (1). Consumption of dietary supplements, especially those classified as sports supplements, has increased worldwide over the past decade (2). Sports supplements are a form of dietary supplement used explicitly for the proposed increase in performance within a sporting context, whether it is for the rise in energy or the decrease in overall body fat (3). There are sports supplements for many different use cases. Pre-workout supplements are one such classification; these supplements promote the boosting of energy along with the introduction of other compounds proposed to help during physical performance, whereas fat-burning supplements are touted as being able to help reduce the user's body fat (3). The types of sports supplements within the market is vast, beyond just pre-workout and fat burner compounds, with many companies offering other sports supplement classes s that propose physiological improvements. The enormous number of products from different brands is indicative of a strong sports supplement market with, which in 2021 was estimated to have a value of $4.9 billion solely within Australia, continuing to increase since the time of publication (4). With sports supplements being so widely used and the increases that this brings with sales profit (2), however the market is very competitive leading to the introduction of illicit and substitute compounds that has become far too familiar within the industry (5). Previously, it has been estimated that one in five supplements sold within Australia contain a banned substance (6), with many of these substances being added unknowingly to the consumer. The reasoning behind the addition of prohibited substances can be linked to many different purposes, some of which can be theorised as cost-cutting measures, poor manufacturing quality or deliberate addition to help increase effects experienced by the user (5). No matter the reasoning behind the adulteration, it is an issue that plagues the supplement industry as a whole (7). With potential consequences as severe as death (8), the increased regulation of supplements is warranted. Along with the intentional or unintentional addition of adulterants, another issue that can have similar consequences to adulteration is the excessive amounts of caffeine present within a product. Mislabelling of sports supplements with the incorrect quantity of caffeine present is an issue observed in previous studies that is still prevalent today (9). With the acquisition of sports supplements to determine adulteration, it makes it possible to also determine the amount of caffeine present and to see how accurate Australian sports supplements are with labelling of caffeine content.

15.1 Current adulteration findings

After evaluating current literature research surrounding the adulteration of sports supplements, a lack of contemporary research into adulteration of sports supplements found within Australia was evident. This gap provides strong justification for the current study. Adding onto this are the recent changes in legislation within Australia leading to the proposed increase in scrutiny in manufacturing, formulation, labelling, evidence and advertising, not only to sports supplements but to all supplements (10). This new regulation change occurred in 2020, with outcomes still being implemented until the present day (2023) (10), meaning that the adulteration of sports supplements has changed over this time, with research being how we will determine if this was for better or worse. Within the last few years, Australian papers have been published surrounding the adulteration of supplements, but in many cases, this is related to supplements that are advertised under the guise of being herbal medicines (11, 12). Herbal medicines preoccupy another component of supplements that has little overlap with sports supplements, so to look at adulteration rates in herbal remedies and apply it to sports supplements would not represent an accurate picture for sports supplements. To date, the published Australian-based research surrounding sports supplement adulteration is limited and is dated (13, 14). For the two papers cited, both were published in 2015 with them finding adulterants such as the amphetamine-type derivatives β-methylphenylethylamines and N-methyl-β-methylphenylethylamine, and N, *a*-diethylphenethylamine (EAPB) in sports supplement samples (13, 14). This explicit use of amphetamine derivates in sports supplement adulteration is a theme that can be seen when looking at a broader scope of papers published that aren't just Australian-based (15). The use of these compounds in sports supplements, such as pre-workout and fat burners, is done because of the effect these compounds have on the user. With the use of amphetamine-type substances, effects of increased energy and decreased appetite can be felt by the user (16); these are the desired effects when taking either pre-workout or fat burner supplements and, therefore may be added by the manufacturer to elicit a greater response. Aside from amphetamine derivates being found in fat burner compounds, there have been many prescription weight loss drugs detected within the supplements (17-19). These are common due to the known effects they create and the dosage known to manufacturers; this can give their product an edge over fat burner supplements that may not use any restricted or illicit substances within. Compounds such as sibutramine, phenolphthalein, synephrine and more are all common weight loss prescription drugs found (18). Another type of adulterant compound that can be found within sports supplements is designer ones. Designer drugs are substances that have been specifically altered by a chemist such that they offer the same effects as the parent drug but are yet undetectable by a screening test and result in the drug existing in a grey area of the law (17). These drugs are often the most dangerous ones added as the potential side effects experienced by the user are unknown, and the efficacious dose is also something that is estimated by the manufacturer (20). The detection and determination of designer drugs is a challenging task, with them initially made to avoid detection and the majority of research not looking specifically for designer compounds; the number currently on the market is unknown. No matter the drug added, the adulteration of sports supplements is an issue that can not only put people's livelihoods at risk, but it can also put people's health on the line with the risk of adverse effects being so high (21).

15.2 Caffeine and sports supplements

Caffeine is a central nervous system stimulant widely consumed among people throughout the world. Caffeine is commonly found in highly consumed food and drinks such as tea, coffee and chocolate. Modern-day usage of caffeine-containing products is for the intended purpose of countering fatigue and increasing energy (22). Due to these commonly experienced effects, caffeine is beneficial in the sports supplement industry, especially in supplements such as pre-workout and fat loss, due to its ability to increase energy while also being linked to a suppression in appetite when consumed in high concentration (23). With the clinically backed effects of caffeine being widely known and understood (23), the dosage of caffeine within supplements is a variable that can be determined by the manufacturer and the level of impact that they want from their product. Within Australia, current regulation surrounding caffeine is laid out in the Food Standards Code 2.6.4, which restricts the amount that can be added to soft drinks and energy drinks and means that food containing caffeine must have a statement on the label warning the consumer. The code states that producers must add no less than 145 mg/L of caffeine to the drinks, and manufacturers can add a maximum amount of 320 mg/L (24). Code 2.6.4, which affects caffeine content in food, does not affect caffeine content within sports supplements. This gap in the code leaves the decision of what is safe and

acceptable to the manufacturer, which can often exceed what Australian guidelines suggest for a single minimum dose of 200 mg (25). Along with what can be deceptively high amounts of caffeine being added by manufacturers, mislabelling of caffeine content is also something that has been found to occur with sports supplements. Previous research has found as high as 41% of sports supplements have incorrect label information when it comes to caffeine content (9). Currently, Australian research on the erroneous labelling of caffeine content within sports supplements is limited. The differing laws and regulations surrounding sports supplements in Australia mean that research into the problem would be needed.

15.3 Aim and objectives

This research was aimed at determining the presence of illicit adulterants within sports supplements and the caffeine content within these supplements via gas chromatography-mass spectrometry (GC-MS). The first objective was the acquisition of four number of pre-workout supplements and three fat burner supplements to determine adulteration with prohibited and/or unlisted ingredients. The second objective was the quantitation of caffeine dosage within the supplements for comparison to the amount declared on the supplements' nutritional information label.

16 Methods

16.1 Samples

Seven samples were acquired from local Australian-owned supplement stores, four of which were explicitly advertised as pre-workout supplements, two as fat burner supplements, and one as an energy-boosting overall well-being supplement. The supplements were in powder form, either loose or encapsulated. The product name, brand and country of origin are shown in Table 1.

Name	Producer	Type of supplement	Country of Origin
1 Uppers	Faction Labs	Fat loss/energy booster	Australia
2 N.O. Xplode	BSN	Pre-workout	United States of America
3 Hydroxycut Hardcore Elite	MuscleTech	Fat burner	United States of America
4 Gold Standard Pre-Advanced	Optimum Nutrition	Pre-workout	United States of America
5 Melt	Legit	Pre-workout	Australia
6 Precision Pre	Precision Nutrition	Pre-workout	Unknown
7 ObliterX	Nexus	Fat burner	Australia

Table 2 - Name, producer, type, and origin of sampled supplements.

16.2 Chemicals and solutions

A caffeine standard powder was acquired from Lab Supply Australia (purity >95%). Standards and samples were prepared using high-performance liquid chromatography (HPLC) grade dichloromethane obtained from ChemSupply Australia.

16.3 Sample Preparation

16.3.1 Supplement Samples for Adulterant Analysis

Samples of each supplement were prepared by weighing 0.01g of the supplement into a 50 mL volumetric flask and diluted with dichloromethane (DCM). The flask was then inverted five times and shaken for 10 seconds. A 1 mL aliquot of this solution was transferred into a 2 mL gas chromatography mass spectrometry (GC-MS) vial.

16.3.2 Supplement Samples for Caffeine Determination

A subsample of each supplement was measured on the basis of what the manufacturer considers a single dose. For the encapsulated samples this involved opening the capsules and sampling and weighing the powder contents. For loose powder samples, this typically involved measuring out a scoop typically provided with the supplement. The weights measured for each supplement are presented in Table 3

Sample number and type of consumable	Recommended product weight per dose in grams	Measured product weight in grams
1 – pill	Not specified	0.75
2 – powder	18.5	15.21
3 – pill	Not specified	0.59
4 – powder	20	17.19
5 – powder	4	2.78
6 – powder	13	13.07
7 – powder	7	7.07

Table 3 - Sample name, type of consumable, product weight claimed by manufacturer and measured product weight.

16.3.3 Caffeine Standards

A stock solution was first prepared at a 2650ppm by first weighing out 200 mg of pure caffeine into a 100 mL volumetric flask and adding 75.5 mL of DCM before inverting three times until homogeneous. From the stock solution, the first standard created with at a concentration of 265 ppm, 1 mL of stock solution was first pipetted into a 10 mL volumetric flask before adding the remaining 9 mL of DCM. The next standard was a 132.5 ppm standard which was created by pipetting out 0.5 mL into a 10 mL volumetric flask before filling with 9 mL of DCM. For the 66.25 ppm standard, 250 µL was pipetted out of the stock solution before adding to a 10 mL volumetric flask and filling with 9 mL of DCM. For the 33.125 ppm standard, 125 µL of stock solution was pipetted into a 10mL volumetric flask and then filled with 9 mL of DCM. The final standard was a 13.25 ppm one, with 50 µL being pipetted out of the stock solution into a 10 mL volumetric flask and filling with 9 mL of DCM. For all the standards, once both stock solution and DCM were added, the flasks were inverted three times before pipetting out 1 mL of each standard into a 1.5 mL GC-MS vial.

16.4 GC-MS

Samples were analysed using a Shimadzu GC-2010 Plus connected to a Shimadzu TQ8040 GCMS (Shimadzu Scientific Instruments, Kyoto, Japan) and equipped with a Shimadzu AOC-20i Plus autoinjector (Shimadzu Scientific Instruments, Kyoto, Japan). The column used was a silica Restek Rxi-5ms (30 m x 0.25 mm ID x 0.25µm). The software used for analysis and quantitation was GCMS solution (Shimadzu Scientific Instruments, Kyoto, Japan). The

GC-MS operating parameters used are listed in Table 2. The peaks present within each sample were integrated and the resulting mass spectra were compared to that of known compounds from The National Institute of Standards and Technology (NIST) 2017 mass spectral library.

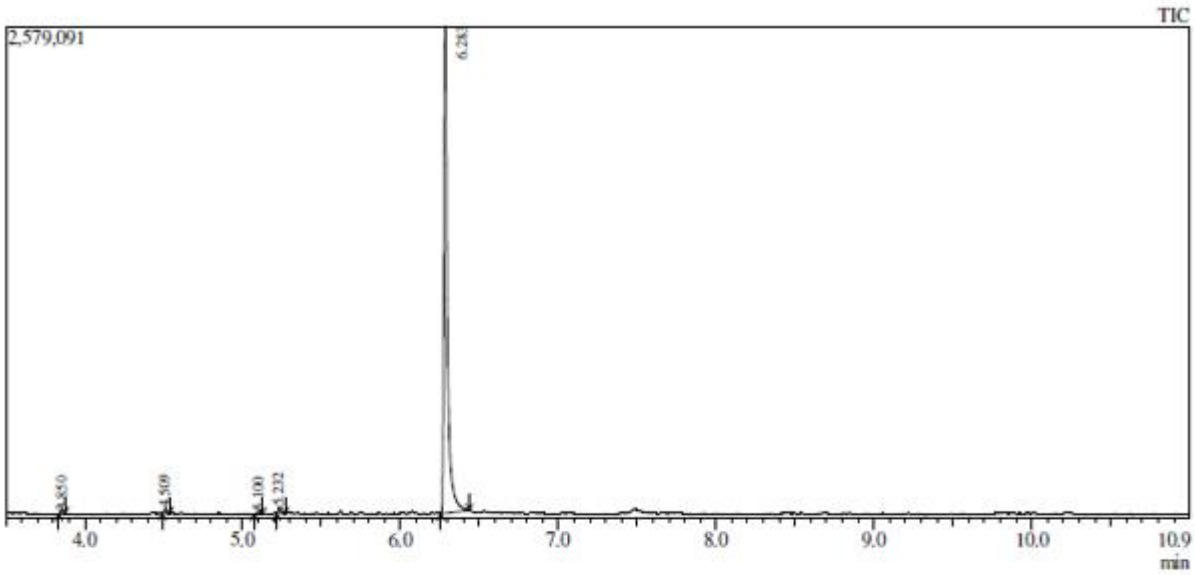
Variable	Setting
Carrier gas	Helium
Flow	1.50 mL/min
Pressure	87.7kPa
Injection mode	Split ratio of 5
Injection volume	1 µL
Injector temperature	250.0 ºC
Temperature ramp	50 ºC hold for 1 minute then increase at 40 ºC per minute until reaching 250 ºC and holding for 5 minutes
Acquisition mode	Q3 scan
Start time	3.5 minutes
Ion source temperature	200 ºC

Table 4 - Variables and settings of GC-MS.

17 Results

17.1 Illicit drug adulteration

The total ion chromatograms (TIC) for all seven sports supplements are presented in Figures 1 to 7. The peaks present in each chromatogram were integrated and mass spectra were collected for each peak and integrated against the NIST mass spectral database. Results of the library matches are presented in Figures 1 to 7. It was evident that in all seven samples, no illegal adulterants were present within the products.

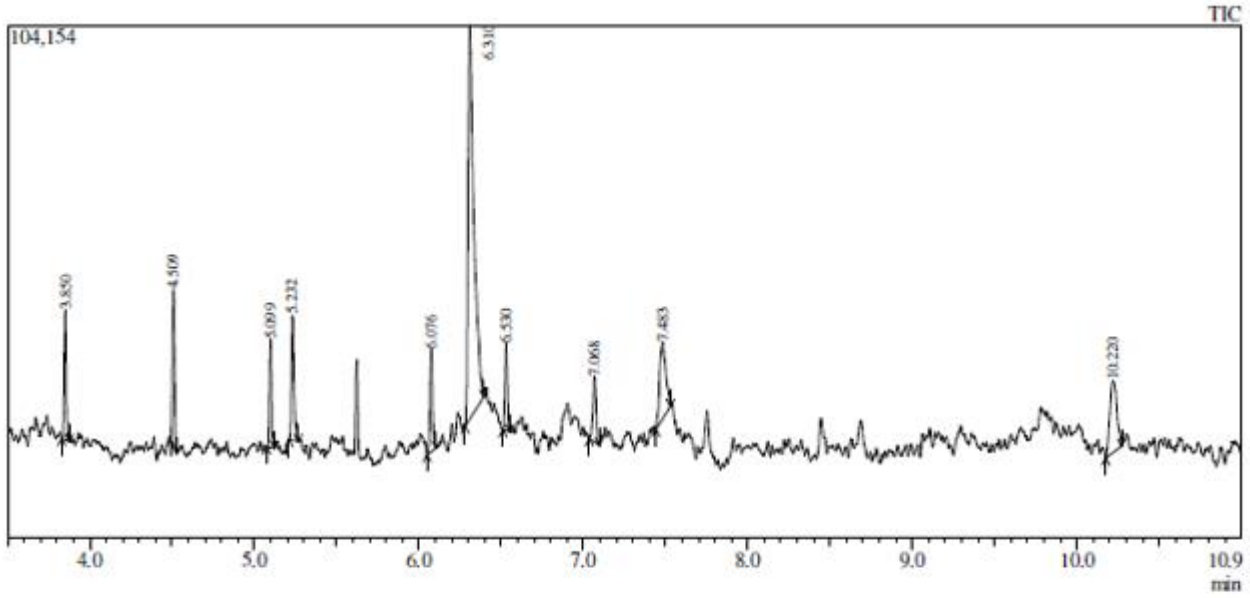


Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	3.850	3.835	3.875	19191	0.48	22098	0.84	0.87		Cyclopentasiloxane, decamethyl-
2	4.509	4.490	4.535	18636	0.47	21145	0.80	0.88		Cyclohexasiloxane, dodecamethyl-
3	5.100	5.075	5.125	15143	0.38	15627	0.59	0.97		Cycloheptasiloxane, tetradecamethyl-
4	5.232	5.215	5.270	35653	0.89	34484	1.30	1.03		Butylated Hydroxytoluene
5	6.283	6.255	6.435	3903728	97.78	2552210	96.47	1.53		Caffeine
				3992351	100.00	2645564	100.00			

Figure 4 - Mass spectra and peak report for sample 1.

Figure 1 shows the TIC and the peak report generated for sample 1, Uppers by Faction Labs. For sample 1, five peaks were designated based on the integration parameters, with caffeine identified at a retention time of 6.2 minutes. The other components that were identified were cyclopentasiloxane decamethyl-, cyclohexasiloxane dodecamethyl -, cycloheptasiloxane tetradecamethyl and butylated hydroxytoluene.

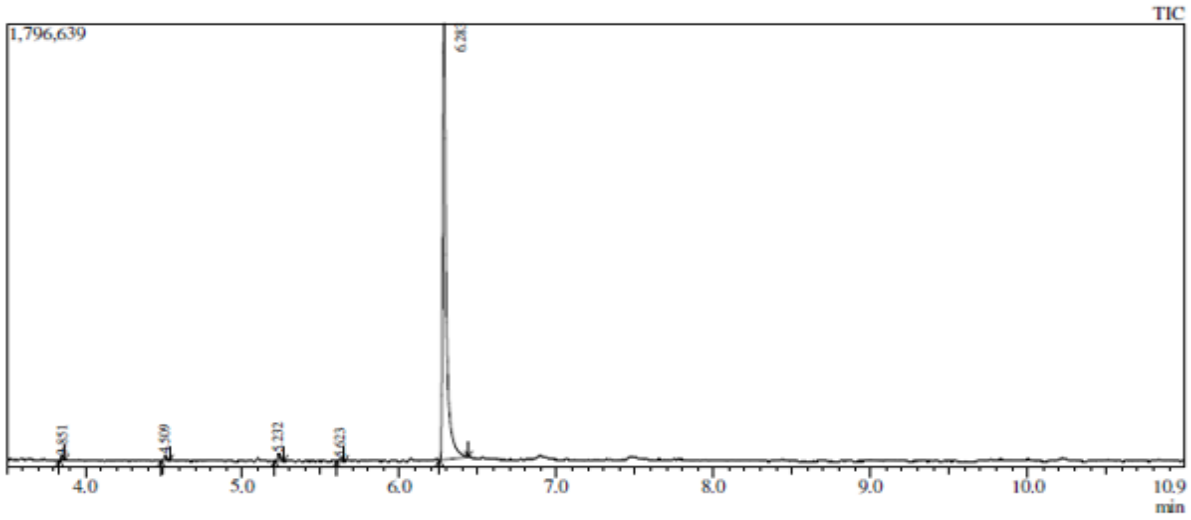


Peak Report TIC

R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
3.850	3.830	3.875	22413	5.17	26349	9.87	0.85		Cyclopentasiloxane, decamethyl-
4.509	4.490	4.530	26436	6.09	31607	11.84	0.84		Cyclohexasiloxane, dodecamethyl-
5.099	5.075	5.125	18705	4.31	22038	8.25	0.85		Cycloheptasiloxane, tetradecamethyl-
5.232	5.210	5.265	27523	6.34	25270	9.46	1.09		Butylated Hydroxytoluene
6.076	6.050	6.105	21598	4.98	20721	7.76	1.04		Cyclononasiloxane, octadecamethyl-
6.310	6.280	6.395	199194	45.91	79904	29.92	2.49		Caffeine
6.530	6.510	6.550	16973	3.91	17753	6.65	0.96		Cyclodecasiloxane, eicosamethyl-
7.068	7.035	7.095	17389	4.01	13376	5.01	1.30		Cyclooctasiloxane, hexadecamethyl-
7.483	7.440	7.530	41115	9.48	15489	5.80	2.65		Octadecanoic acid, 2-hydroxy-1,3-propanediyl
10.220	10.175	10.270	42580	9.81	14529	5.44	2.93		Hexadecanoic acid, 1-[[[(2-aminoethoxy)hydr
			433926	100.00	267036	100.00			

Figure 5 - Mass spectra and peak report for sample 2.

Figure 2 shows the TIC and peak report for sample 2, N.O. Xplode by BSN. Ten peaks were determined by the peak area, in which caffeine was identified at a retention time of 6.2 minutes. The remaining peaks included, cyclopentasiloxane decamethyl-, cyclohexasiloxane dodecamethyl-, cycloheptasiloxane tetradecamethyl-, butylated hydroxytoluene, cyclononasiloxane octadecamethyl-, cyclodecasiloxane eicosamethyl-, cyclooctasiloxane hexadecamethyl-, octadecanoic acid and hexadecanoic acid.

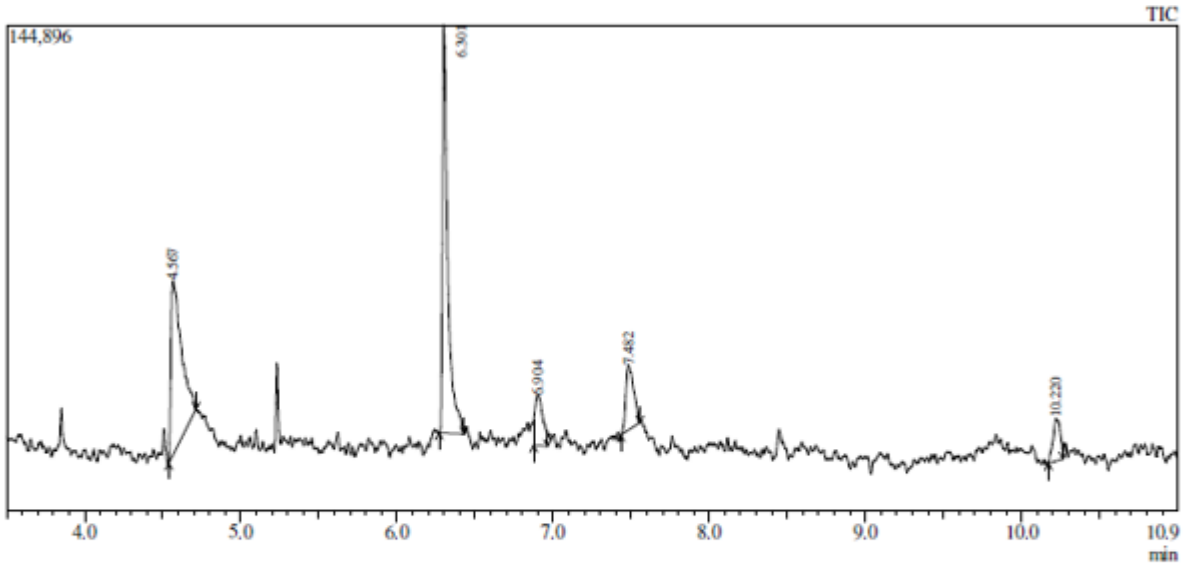


Peak Report TIC

Peak#	R.Time	LTime	FTime	Area	Area%	Height	Height%	A/H	Mark	Name
1	3.851	3.835	3.870	16543	0.58	20134	1.09	0.82		Cyclopentasiloxane, decamethyl-
2	4.509	4.485	4.535	19751	0.69	21298	1.15	0.93		Cyclohexasiloxane, dodecamethyl-
3	5.232	5.210	5.265	29983	1.04	28156	1.52	1.06		Butylated Hydroxytoluene
4	5.623	5.600	5.650	14527	0.51	13064	0.71	1.11		Cyclooctasiloxane, hexadecamethyl-
5	6.283	6.255	6.440	2790822	97.19	1765403	95.53	1.58		Caffeine
				2871626	100.00	1848055	100.00			

Figure 6 - Mass spectra and peak report for sample 3.

Figure 3 displays the TIC and peak report for sample 3, Hydroxycut Hardcore Elite by MuscleTech. Five peaks were determined via peak area, with caffeine being identified at the 6.2 minute mark. The remaining peaks included cyclopentasiloxane decamethyl-, cyclohexasiloxane dodecamethyl-, butylated hydroxytoluene, and finally cyclooctasiloxane hexadecamethyl-.

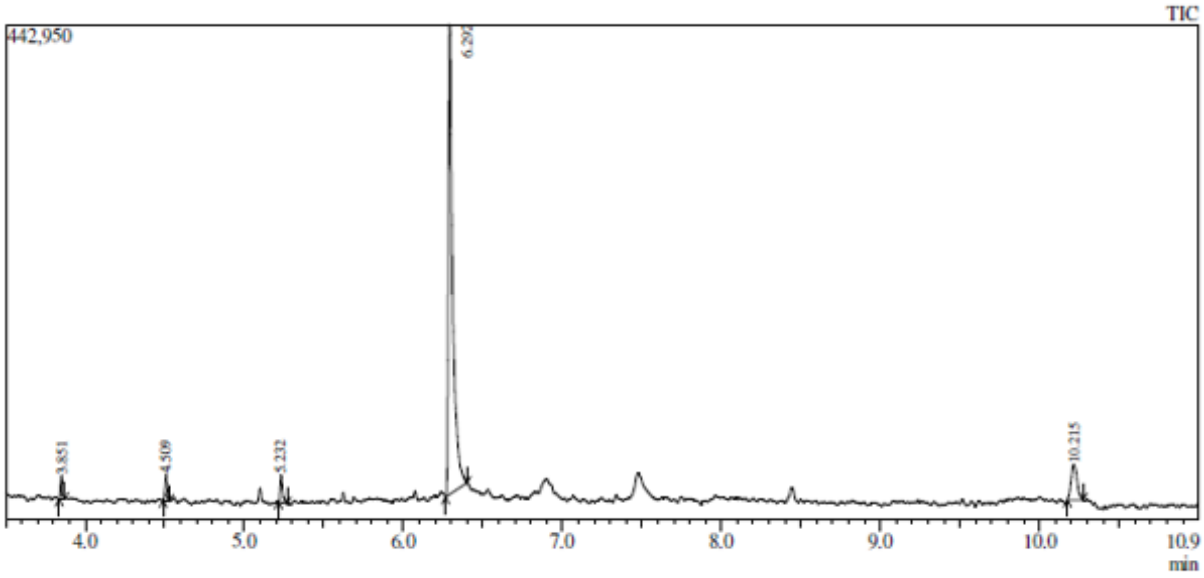


Peak Report TIC

Peak#	R.Time	LTime	FTime	Area	Area%	Height	Height%	A/H	Mark	Name
1	4.567	4.540	4.715	244563	35.12	51803	23.48	4.72		Ornithine
2	6.301	6.270	6.430	290446	41.71	121666	55.15	2.39		Caffeine
3	6.904	6.875	6.965	53358	7.66	15075	6.83	3.54	V	Palmitoyl chloride
4	7.482	7.435	7.555	66382	9.53	19430	8.81	3.42		Stearic acid chloride
5	10.220	10.170	10.265	41580	5.97	12641	5.73	3.29		Tricosanoic acid, 2-methoxy-, methyl ester
				696329	100.00	220615	100.00			

Figure 7 - Mass spectra and peak report for sample 4.

Figure 4 shows the TIC and peak report for sample 4, Gold Standard Pre-Advanced by Optimum Nutrition. Five peaks were determined based on peak area. At the 6.2 minute mark, caffeine was identified. The remaining compounds included ornithine, palmitoyl chloride, stearic acid chloride and tricosanoic acid.



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	3.851	3.835	3.870	18576	1.93	21165	4.06	0.88		Cyclopentasiloxane, decamethyl-
2	4.509	4.490	4.530	21132	2.20	23780	4.56	0.89		Cyclohexasiloxane, dodecamethyl-
3	5.232	5.215	5.270	26610	2.77	24375	4.67	1.09		Butylated Hydroxytoluene
4	6.292	6.265	6.400	811991	84.41	420283	80.60	1.93		Caffeine
5	10.215	10.175	10.275	83656	8.70	31821	6.10	2.63		Palmitoyl chloride
				961965	100.00	521424	100.00			

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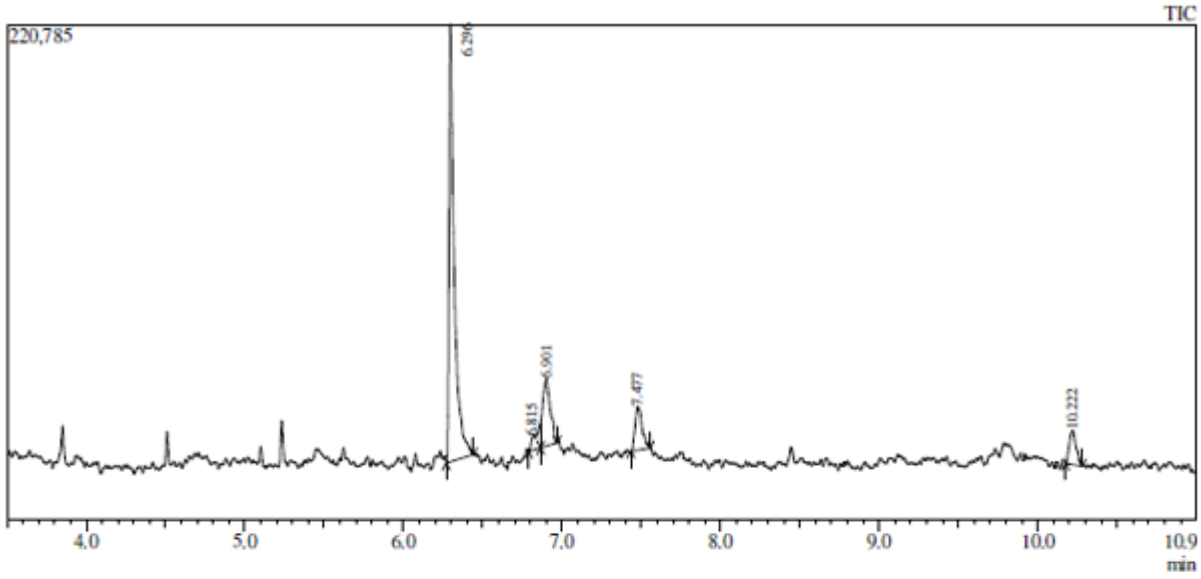
Figure 8 - Mass spectra and peak report for sample 5.

Figure 5 displays the TIC and peak report for sample 5 which was Melt by Legit. Five peaks were designated based on peak area and included caffeine with a retention time of 6.2 minutes. The other peaks included, cyclopentasiloxane decamethyl-, cyclohexasiloxane dodecamethyl-, butylate hydroxytoluene, and palmitoyl chloride.

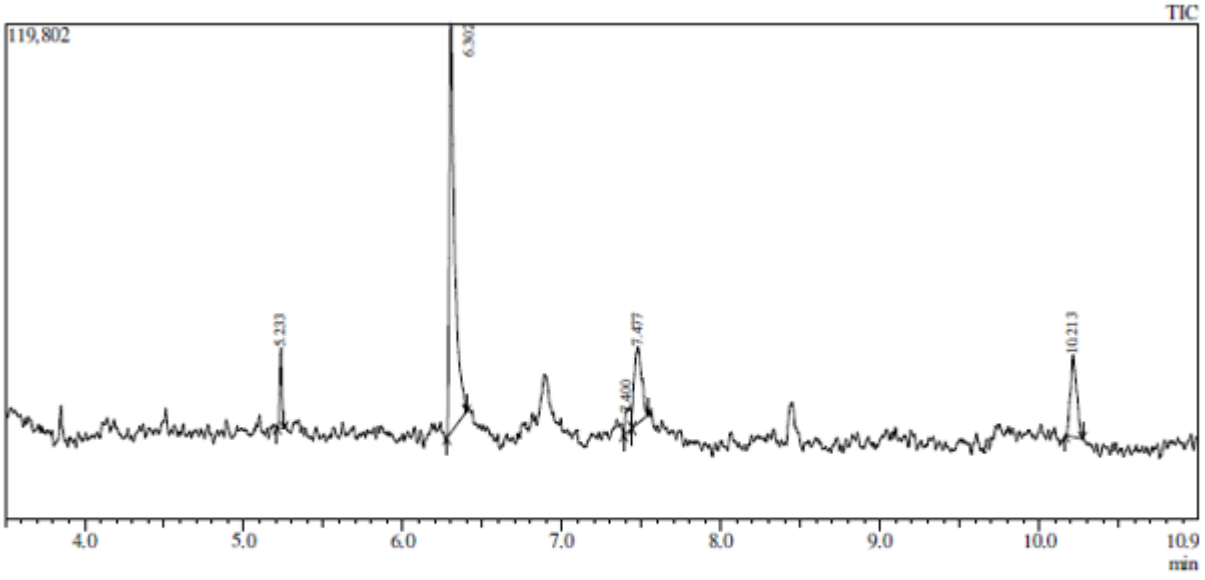


Peak Report TIC

ak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	6.296	6.270	6.440	447083	65.89	194820	73.44	2.29	V	Caffeine
2	6.815	6.790	6.865	27393	4.04	5848	2.20	4.68		2-Methyltetrahydrofuran-3-one oxime, o-[(pen
3	6.901	6.865	6.970	99671	14.69	30047	11.33	3.32	V	Palmitoyl chloride
4	7.477	7.435	7.555	59408	8.76	19288	7.27	3.08		Octadecanoic acid, 2,3-dihydroxypropyl ester
5	10.222	10.175	10.280	44946	6.62	15257	5.75	2.95		Palmitoyl chloride
				678501	100.00	265260	100.00			

Figure 9 - Mass spectra and peak report for sample 6.

Figure 6 shows the TIC and peak report for sample 6, Precision Pre by Precision Nutrition. The number of peaks designated by peak area was five and caffeine was identified at the 6.2 minute mark, The other peaks were identified as 2-methyltetrahydrofuran, palmitoyl chloride, octadecanoic acid and palmitoyl chloride.



Peak Report TIC

ak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	5.233	5.210	5.255	19124	5.01	19323	12.00	0.99		Butylated Hydroxytoluene
2	6.302	6.275	6.400	236095	61.85	98765	61.33	2.39		Caffeine
3	7.400	7.390	7.440	14413	3.78	4734	2.94	3.04		2- Bromopropionic acid, octadecyl ester
4	7.477	7.440	7.540	59198	15.51	18453	11.46	3.21	V	Octadecanoic acid, 2,3-dihydroxypropyl ester
5	10.213	10.165	10.270	52884	13.85	19756	12.27	2.68		Hexadecanoic acid, 1-[[[(2-aminoethoxy)hydr
				381714	100.00	161031	100.00			

Figure 10 - Mass spectra and peak report for sample 7.

Figure 7 shows the TIC and peak report for the final sample, sample 7, ObliterX by Nexus. This sample had five peaks appointed based on peak area and was similarly matched with caffeine found at 6.2 minutes. The remaining compounds included, butylated hydroxytoluene, 2-bromopropionic acid, octadecanoic acid and hexadecanoic acid.

17.2 Caffeine standards and samples

Caffeine peak area was compared to overall peak area to determine the amount of caffeine present. **Error! Reference source not found.** shows each sample and the corresponding peak area of the caffeine. With the use of the peak area, the percentage of caffeine was determined and compared to that of the claimed caffeine percentage for all samples in one serving. In six out of the seven cases, the amount of caffeine present was below what the manufacturer claimed to be present in their product. However, for sample 1 the amount of caffeine present was determined to be above the amount claimed by manufacturer with an increase of 0.56%. The peak absorbance was also used to determine the amount of caffeine present in each standard.

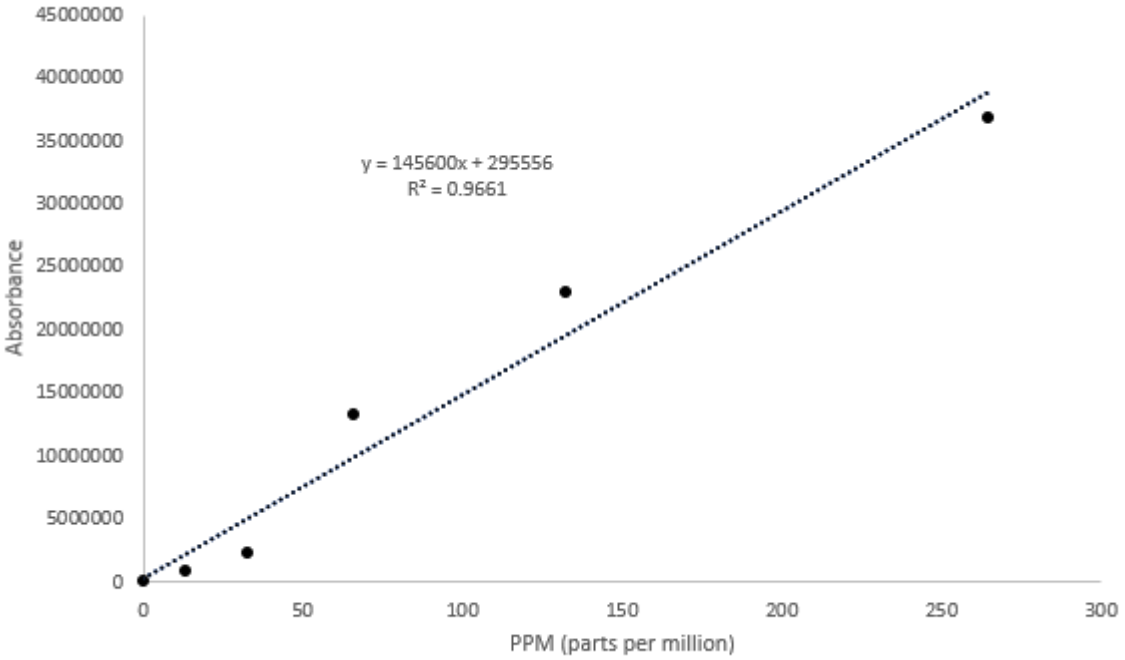


Figure 11 - Caffeine standard curve with equation and R2 value present.

Figure 8 shows the caffeine standard curve produced using the peak normalised absorbance from running the caffeine standards. The figure also depicts the standard curve equation and R^2 value at 0.9661, which were used to determine the percentage of caffeine present within each sample compared to what was claimed on the product label.

Sample	Peak absorbance area mAU of caffeine	Claimed caffeine percentage	Determined caffeine percentage
1	4051687	13.34%	13.9%
2	245391	1.31%	1.27%
3	2993286	16.95%	10.4%
4	349142	1.75%	1.62%
5	933893	5.40%	3.56%
6	495106	2.38%	2.1%
7	251307	1.70%	1.3%

Table 5 - Sample number and caffeine peak normalised area, claimed caffeine percentage and determined caffeine percentage for one serving.

18 Discussion

18.1 Supplement adulteration

No adulterants were found within samples 1 to 7. The lack of presence is a good indication for the Australian consumer as these supplements were acquired from an easily accessible Australian market. However, this does not rule out the possibility of illegal adulterants being present in supplements available in Australia. This study had a sample size of 7 supplements; with such a low sample size, problems arise when it comes to making implications based on the results of this research. It has been found that with the inclusion of a small sample size, the chance of assuming a false premise true increases (26). With that in mind, the idea that all supplements are free of any illegal adulterants within Australia is doubtful. Previous research would suggest that adulteration rates would be as high as 50% (7) with compounds such as sibutramine, DMAA, fluoxetine and higenamine. However, in this instance, none of the supplements tested were adulterated. It may be due to poor selection of supplements when buying, but out of the seven supplements, three were made in Australia, three were made in the US, and one had an unknown origin. The range of supplement origin gave a good selection of what is typically bought by the Australian consumer as the Australian market is predominately supplements made in these two countries. Along with the country of origin, in many manufacturing instances, sports supplements are made in batches, with one batch potentially containing adulterants and another not; this, too, can offer problems when testing only one product from one batch. To further this research topic and idea, these issues need addressing to ascertain adulteration rates properly.

18.1.1 Extraction issues

An issue faced with the preparation of the samples was the insolubility of large amounts of the samples. Pre-workouts and fat burners are typically filled with additives and fillers that are somewhat insoluble, especially when dissolved in a solvent like DCM. The fillers included in these products are often colourings, flavourings, sugar, lactose, rice flour and more, which often contribute a large portion of the product in the package (27). Insoluble fillers can cause issues as certain substances may bind to them and not dissolve when adding DCM. As adulterants could be any number of different substances, this is a potential issue when determining any illicit substances present within the samples. The changing of extraction technique to better dissolve these fillers, would help determine the actual amounts of caffeine and adulterants.

18.1.2 Presence of unknown compounds

When looking at the results of the TICs, other substances are present that, without prior research, would be typically thought to be present in sports supplements. The different cyclo-siloxanes were seen numerous times throughout most of the tested supplements. These compounds are often seen in cosmetic products to help improve various characteristics (28) and can be classified as fillers that were mentioned earlier. It is expected to see this type of filler within sports supplements and is not classified as an adulterant due to its abundant nature in food and cosmetics. Another compound seen numerous times throughout was butylated hydroxytoluene. This substance is a flavour enhancer and preservative; again, it can be added to the list of fillers present within these supplements. Butylated hydroxytoluene is a banned flavour enhancer in many countries, but in Australia and the US, it is a non-banned substance that is carcinogenic (29). Like the cyclo-siloxanes, butylated hydroxytoluene was not classified as an adulterant as it is a standard filler in many foods. In the instance in which the additive was banned in Australia, the compound would have been classified as an adulterant. The final, more commonly found compound was palmitoyl chloride. This substance is not added as a filler or food preservative but plays a role in the production of lipids and polymers (30). The source of this compound could not be determined. In the future, banned fillers could be investigated further to determine the amount of banned food additives found in sports supplements available for purchase in Australia.

18.1.3 Designer adulterants

An issue that was faced during the planning conception of this study was the determination and presence of adulterants explicitly designed to be undetected during testing. Designer adulterants 1,3-dimethylamylamine (DMAA) and 1,3-dimethybutylamine (DMBA) are becoming more of an issue as banning common adulterants leads to the creation of new, undetectable ones (31). It is often the case that these adulterants can be even more deadly to the consumer as they are usually created and used without proper regulatory testing that any registered compound used in humans would be put under before usage (32). Furthermore, with the lack of knowledge on how the drug affects humans, the dosage will be unknown. In this study, it was not possible to determine the presence of any designer drugs that may not be contained within the NIST 2017 mass spectral library. Due to this issue, it does not rule out the inclusion of these compounds in the samples tested. It would need further analysis with the inclusion of updated libraries or via other techniques, such as LC-MS, to rule out the possibility of any new designer compounds present. The creation and consumption of designer drugs is an issue not only in sports supplements but in other markets (32). The continual discovery and recording of designer drugs detected throughout industries will be a preventative measure for sports supplements.

18.2 Caffeine quantitation

As no illegal adulterants were found added to the samples, the focus was shifted from qualitative testing to quantitative testing for the amount of caffeine present compared to the claimed amount by the manufacturer. These quantitation results demonstrates lower caffeine amounts present in six samples. For sample one, a slight increase in caffeine concentration was observed; when further calculations were done, the amount of caffeine in milligrams (mg) equates to 104.2. This is an increase of 4.2 mg above the claimed amount of 100 mg made by the manufacturer. Due to the different reactions people have to caffeine (9), it is impossible to rule out that this slight increase in caffeine could lead to adverse effects in someone sensitive to it. Previous research has indicated an upper limit of 400mg/day for adults (9), which would put this sample below what would be classified as dangerous, even with the determined amount. Under Australian regulations for caffeine, which would fall under Standard 2.6.4 formulated caffeinated beverages (33), sample 1 would still fall within guidelines for caffeine content and what was deemed acceptable for sale to the Australian public.

With the many issues encountered along the way with extraction and creation of caffeine standards, higher amounts of caffeine could have been determined in the sample. Further testing would be needed to confirm or deny this. As only one sample had higher amounts of caffeine than claimed, it does not rule out the potential for some labels to be erroneous regarding caffeine content. It is a well-documented issue that occurs within sports supplements, with previous research suggesting that as high as 41% of sports supplements have mislabelled products (9). It would, therefore, be expected to see these rates occurring within the pool of samples used in this study, but that was not the case, as only one out of the seven was higher.

In six of the samples, there was lower caffeine content than specified by the manufacturer, making it plausible that the results were incorrect. The reasoning for this could be any number of issues surrounding the creation of the standard curve. When constructing the standard curve for calculating caffeine, the R^2 value was 0.9661. In the context of this study, the closer to 1 the R^2 value is, the more accurate the results gained using the standard curve equation would be (34). With that in mind, for this study, an R^2 value of between 0.98–0.99 was wanted to ensure that the results calculated from the equation were as accurate as possible. A value of 0.98-0.99 was not achieved, and therefore, results would have been less accurate than wanted. The reasoning behind using a 0.9661 R^2 value was that multiple runs had been attempted to achieve a higher value. However, it was unsuccessful in doing so, and with the limited time left to produce a result, it was decided to use this run as it had the highest coefficient out of all the runs. The inability to achieve a higher R^2 value was likely due to user error during the standards' creation, leading to peak area values being lower than expected when running through the GC-MS.

18.3 Future recommendations

This research topic needs continued work done; as stated previously, the likelihood of some sports supplements being adulterated is very high, with some estimates as high as 51% (7). With the Australian market constantly changing along with laws and regulations, research needs to be done into new and current adulterants in the supplements market. Due to this, the first recommendation would be to increase the number of samples used in the study. The increase in the number of samples helps further prove any points that may be reached; this would also encompass more of the sports supplement industry, mainly if supplements are acquired from more countries than what was done in this study. Some countries of origin that would be more appealing are the ones with fewer regulations surrounding sports supplements, such as Eastern Europe, China, and the USA. Including these countries' supplements could increase the chances of finding adulterants, but supplements should only be bought if easily accessible within Australia.

18.3.1 Sample and standard preparation

Another recommendation to be made is the change in sample extraction. It is clear that supplement fillers are not soluble in a solvent such as DCM, and therefore, loss of adulterants or caffeine could occur. With that in mind, sonification is one way in which articles of similar nature prepare their samples (35). Another alternative could be to heat the mixture while mixing, as some of the solids could be highly insoluble salts at room temperature. The standard preparation is another issue that could be resolved with the improvement of better preparation. Both methods work to improve mixing. Instead of just inverting the sample a few times, a more rigorous mixing technique would be implemented to ensure higher rates of homogeny. Changing the solvent could be one method to gain better results; DCM was a good choice as GC-MS work was involved, but it is known to be problematic when pipetting. Ethanol or methanol could be an alternative option, with many other papers of similar relevance using these solvents; along with that, the change of analytical instrument from GC-MS to liquid chromatography-mass spectrometry (LC-MS) would also benefit the research as water could be used as another alternative solvent and caffeine has a proven track record for quantitation via LC-MS. There are numerous potential for sample and standard preparation improvements; the research would need further investigation to determine which preparation methods best suit their objectives.

18.3.2 Internal standard

As stated, the standard curve creation and overall accuracy could have been better due to human error when creating the standards. Another aspect that could have impacted the results gained from the standards was the day-to-day variability of GC-MS results (36), along with other users using the machine between each run. Another recommendation to be made to negate both these issues would be the inclusion of an internal standard when running the samples. This internal standard would be caffeine; a set amount would be added to each sample and standard before running on the GC-MS. Then, using the internal standard and analyte, a ratio between the responses would be used in the calibration, nullifying any day-to-day variation that the machine may be experiencing. Applying an internal standard would help better produce accurate results to determine the amount of caffeine present along with any adulterants that may be found, as this method can be applied similarly to an illegal compound that may be present.

19 Conclusion

This paper aimed to determine the presence of any illegal compounds that were present as adulterants within sports supplements. No adulterants were found in the seven locally acquired samples. The lack of adulterants presents a good sign for the Australian consumer, as the presence of adulterants within sports supplements is an industry issue. However, due to the lack of samples and overall variability with sports supplements, it is impossible to conclusively say that all sports supplements available in Australia are free of illegal adulterants. Caffeine content is another aspect that was investigated, and from the results, six samples had lower than labelled caffeine content within the product. At the same time, one product had a higher caffeine content than what was specified by the manufacturer. Like the adulteration section, this also had issues, with lower than hoped R^2 value, to the issues encountered when using the

solvent DCM; there was potential for erroneous results. Even with the possibility of issues with the results, in the presence of adulterants or higher levels than what was found of caffeine, it would have been something that came up during testing, and with this in mind, with no overly problematic results occurring, presents a good sign for the average Australian consumer purchasing sports supplements. In conclusion, further work is needed on such topics. However, with the implementation of some of the recommendations presented and continuous improvement in laws and regulations to protect consumers, sports supplement adulteration can be monitored and stopped.

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