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Are supplements supplemented? Evaluating the composition of complementary and alternative medicines using mass spectrometry and metabolomics

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Declaration

I declare that:

i. The thesis is my own account of my research, except where other sources are acknowledged.

ii. The extent to which the work of others has been used is clearly stated in each chapter and certified by my supervisors.

iii. The thesis contains as its main content, work that has not been previously submitted for a degree at any other university.

Abstract

The complementary and alternative medicines (CAM) industry is worth over US$110 billion globally. Products are available to consumers with little medical advice; with many assuming that such products are 'natural' and therefore safe. However, with adulterated, contaminated and fraudulent products reported on overseas markets, consumers may be placing their health at risk. Previous studies into product content have reported undeclared plant materials, ingredient substitution, adulteration and contamination. However, no large-scale, independent audit of CAM has been undertaken to demonstrate these problems in Australia.

This study aimed to investigate the content and quality of CAM products on the Australian market. 135 products were analysed using a combination of next-generation DNA sequencing and liquid chromatography-mass spectrometry. Nearly 50% of products tested had contamination issues, in terms of DNA, chemical composition or both. 5% of the samples contained undeclared pharmaceuticals.

Increasing reports of adulteration with novel drug analogues led to the development of a high-throughput untargeted method for pharmacovigilance. Rapid direct sample analysis coupled to mass spectrometry was used to screen products, this time for hundreds of compounds in minutes with minimal sample preparation. The data correlated well with previous analyses, with the added benefit of detected additional compounds including phytochemicals and vitamins.

Finally, metabolomics was used to assess the compositional diversity of finished herbal products on the market and how they compare to standard reference materials. The analysis

showed that, despite all products stating the same ingredients, there was a clear difference in biochemical profile between products and also the reference materials.

The combined techniques and analyses used in this project provide an audit and quality control toolkit which will allow for stronger regulation of CAM products. The data collected has shown that such regulation is needed to improve product quality and to protect consumer safety.

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Authorship Declaration

Statement of Contribution of Others

This thesis has been developed in the format of Thesis by Publication. Chapters Two, Three and Five within this thesis have been published or are currently in review or draft for submission to scientific journals. These chapters represent collaborative works; however, the PhD candidate for which this thesis represents has completed the majority of the study design, data collection, data analyses and interpretation, and drafting of the manuscript.

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Confirmation of contribution

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Cartoon from Matt Golding, "Supplements may contain traces of frog - but will they make you croak?" *The Age*, 6/9/19

1. Background to the regulation, safety and quality control of complementary and alternative medicines

This thesis explores the composition, safety and quality of complementary and alternative medicines (CAM) on the Australian market from 2014 to 2017. CAM can be defined as any treatment, product, drug or supplement which is used in combination with, or in place of, conventional medicine and is not considered to be a part of an evidence-based medicinal treatment [1]. The use of CAM products has grown substantially in the last two decades [2-5], with a global market valued at US$83 billion per year in 2010 [6], to now being estimated at over US$150 billion by 2025 [7]. The market is only expected to grow. The topic of CAM is controversial for several reasons. Ongoing questions about the effectiveness of treatments using herbal medicines, homoeopathy, naturopathy and traditional medicines ignite strong debate from both supporters of CAM and advocates of evidence-based medicines. Debate continues on topics including the use of funding, appropriateness of scientific methods, ethical considerations and conflicts of interest, bias in data and interpretation and the overall scientific consensus. Controversy also exists as to the best way to approach CAM use and demand in patients and general consumers, the possible benefits and risks of 'integrative' medicines [8], the advertisement of CAM products to consumers and the claims of benefit and efficacy of CAM, often without strong scientific evidence. This thesis does not directly address these topics (indeed, the above-mentioned areas would fill many books [9-12]), and nor does it discuss the potential efficacy of CAM products. Instead, I argue that discussions regarding efficacy should be surpassed by urgent issues of safety and quality.

Key to any discussion of CAM safety are matters regarding regulation and the debate over how much 'red tape' is needed to ensure consumer safety, without unnecessarily impeding consumer access to CAM products. This subject of regulation is, therefore at the heart of this thesis. In the debate about efficacy, demand, acceptance and integration of CAM into mainstream medicine, potential issues of quality and safety are often overlooked. It is only due to the substantial increase in use that attention has been drawn to the regulation and safety of CAM, in particular to the safety of herbal medicines and dietary supplements, their side effects and possible interactions with conventional medication. These products are already on the market and are being regularly taken by over 50% of the population [13]. In many countries, regulation of CAM is relatively lax, due to a perception of lower risk compared to prescription and over-the-counter drugs. In Australia, the Therapeutic Goods Administration (TGA) is the regulatory body for medicines and medical devices, with products requiring an entry in the Australian Register of Therapeutic Goods (ARTG). Under this system, CAM products are 'listed medicines' (AUST L) and post-market monitoring is generally less rigorous than registered medicines (AUST R), such as prescription drugs, which are considered high-risk and whose efficacy must be demonstrated along with safety and quality [6,14]. The unique AUST L number for each CAM remedy must be displayed on the packet as part of the regulation for therapeutic products. The number denotes that the CAM remedy is considered 'low risk', and that the product is manufactured in accordance with Good Manufacturing Practices (GMP) and *complies* with safety and quality criteria. An AUST L number does not permit any therapeutic claims for the indications of use [6]. In spite of this system, there is a perception within the general public that CAM remedies have been tested for efficacy, safety and quality before going onto the market [15]. The ideal of 'natural' CAM products equating to safe products is not only wrong; it is a key belief and explanation for the vast industry growth

[16,17]. Issues with adulterated, contaminated and mislabelled products further complicate the problem with the increased use of CAM. The presence of these adulterants and contaminants may not be immediately known to treating doctors and other healthcare workers; leading to delays in diagnosis or even misdiagnosis [18].

Outline of Thesis

This project on CAM products on the Australian market is part of a larger study, in which we have sought to answer the question posed by Byard*, et al.* [19]: "What are the risks to the Australian community from herbal medicines?". This project follows on from a preliminary study in 2013 (paper in Appendix) which found that 92% of the products tested had some form of contamination, adulteration and/or substitution [20]. In particular, this thesis explores the use of advanced mass spectrometry to investigate the composition of CAM products and better understand the safety implications of these products. The thesis is in two parts; a) a selective look at the risk of adulteration, followed by b) a wider focus on the overall composition of CAM products.

Part A concerns the screening and detection of possible adulterated and contaminated CAM products, with the aim of developing new methods by which this could be achieved. Part A starts with a review of published methods for adulterant detection in CAM products, with an emphasis on how adulterated products are a clinically relevant problem (Chapter 1). Chapter 2 is the first experimental section focusing on "Toxicological screening and DNA sequencing detects contamination and adulteration in regulated herbal medicines and supplements for diet, weight loss and cardiovascular health" [21]. Following on from the data in Chapter 2, Chapters 3 and 4 detail the development and application of a screening method utilising a

new direct mass spectrometry platform and the application of that method for the rapid analysis of CAM products.

Part B focuses on the overall composition of herbal CAM products and advocates for the use of a metabolomic approach to assess quality and safety. Part B starts with Chapter 5, an essay on the potential application of metabolomics for herbal medicine pharmacovigilance. Chapter 6 uses a metabolomic approach to assess the compositional similarities of commercial herbal products (green tea and Korean ginseng).

Significance, rationale and research questions

No large-scale, independent audit of CAM has been undertaken in Australia. This means that there is no clear picture of whether the widespread problems of adulteration, contamination and mislabelling that are seen overseas are present in Australia. To gain a true picture of the extent of the problem and therefore the risk to consumers, a large-scale screen of CAM products available in Australia must be undertaken, which is what this the first half of this project aimed to do. There is also a gap in the literature in regard to fast, high-throughput screening methods which could be adapted to commercial use and also pre- and post-market audits and reviews for regulatory bodies. All of this led to the research question for part A:

What is the occurrence of adulteration/contamination in CAM products available in Australia?

The second aim of this project was to assess the compositional diversity among CAM products on the market. As the results for part A show, product labels are often inaccurate at best, leaving us to wonder just what is in the products tested. By using metabolomics, we can investigate the quality differences in CAM products as well as safety. For example, are all green tea products the same? Metabolomics is the identification and quantification of the

small molecules within a biological system under specific conditions. As reviewed in Chapter 5, metabolomics can provide detailed insights, or 'snapshots' of the effect of environmental, genetic and processing factors on herbal medicines. The application of untargeted methods such as metabolomics and metabolite profiling provides yet another set of methods for quality control and pharmacovigilance of formulated products. This led to the part B question:

What is the compositional diversity /similarity of formulated herbal products?

The potential risk to consumers in Australia from CAM is currently ill-defined. It appears from the preliminary study [20] and a review of the literature that current regulation is failing to ensure the safety and quality of CAM products on the Australian and overseas markets, and that the potential risks from CAM, notably herbal medicines, may outweigh any benefits of use. There is also clear economic motivation to 'improve' products with the use of undisclosed ingredients [22]. With the questions outlined above, the overall aim of this thesis was to answer the following questions:

What is in these herbal products and does the content match the label?

What is the risk posed to consumers by CAM products?

The data from this project will give weight to the arguments on whether Australia (and other countries) need stronger CAM regulations, guide future research into CAM products and herbal medicine composition and develop and apply an audit and quality control method toolkit.

Definitions of common terms

The following terms have been used throughout the thesis. Below outlines the definition and criteria as to when this nomenclature is used. In some cases, it is not possible to distinguish between adulteration, contamination, or ingredient substitution.

Adulteration: The addition of an undeclared substance to a product. For example, when a pharmaceutical detected cannot be explained by the products ingredients list, it is possibly adulterated.

Contamination: The inadvertent addition of a substance to a product. For example, when DNA from plants or animals not listed o related to ingredients is found. Pharmaceutical and heavy metal contamination is also possible.

Substitution: Where an ingredient listed on the package has instead been switched for possibly cheaper material [23].

Undeclared ingredients: An umbrella term for substituted ingredients, adulterants and/or contaminants.

Note to the reader

Certain details such as industry worth, product usage, etc. vary from chapter to chapter based on the information that was available at the time of publication, with the most recent figures quoted in the main introduction.

Contamination, adulteration and toxicity concerns with Complementary and Alternative medicines.

Abstract

Complementary and alternative medicines (CAM) are becoming more popular choices for consumers worldwide, and are utilised for a broad range of diseases. Consumer use is generally in a 'self-treatment' manner, with such products assumed to be 'natural' and therefore safe. However, light regulation and these wrongful assumptions of safety have led to alarming outcomes and adverse effects for consumers, with numerous case studies demonstrating how adulterated products have led to overdose or poisoning. Adulterated and contaminated CAM products are known problems for quality control and pharmacovigilance. Pharmaceutical adulterants are generally found in herbal medicines that have matching indications. However, this is not always the case and screening methods, therefore, need to be able to detect both expected and unexpected compounds for pharmacovigilance purposes. A range of mass spectrometry-based methods for adulterant detection in CAM products have been developed to help ensure safety and quality and for continued pharmacovigilance. Such methods must be rapid and high-throughput if they are to be adapted for regulatory use, with LC-MS/MS methods being the preferred choice due to the sensitivity and range of application to pharmaceuticals. New, un-targeted screening methods have a strong potential for detecting drug analogues and novel compounds. This would allow for more effective regulation, the establishment of more rigorous standardised methods and the reduction of adverse health outcomes for consumers. While under-reported, adulterated

products are a clinically relevant problem for consumers, with adverse effects ranging from headaches and rashes to cognitive impairment and death.

Introduction

Complementary and alternative medicines (CAM) can be defined as 'procedures and products excluded from mainstream medicine' [1], and commonly include traditional medicines, herbal medicines and homoeopathic remedies. Natural health products such as vitamins and supplements are also included under the umbrella of CAM. Recently, there has been a marked increase in the use of CAM, with products typically used in self-treatment and self-selected manner by consumers, often seen as 'low risk' [6]. Associated with this increase in use, there has been an increase in concern about the safety and quality of CAM products in the literature, as reviewed below. With the regulation of CAM often 'soft-touch' and industry-based [6], adulterated and contaminated products can more easily make their way onto the market and to consumers. This has resulted in overdoses [24,25], adverse effects from pharmaceuticals which the patient was unaware they were even taking [26,27] and poisoning from contaminants such as heavy metals [28].

There have been many reports on the adulteration and contamination of herbal medicines, and several extensive reviews on the subject [2,29]. However, with regard to the regulatory control of CAM, safety issues are often insufficient, and with the increasing use of CAM, there is pressure for such issues to be addressed [19,30]. Cases of adulteration can sometimes be seen as economically motivated fraud, which arises for financial advantage [31]. This leads to a clear conflict of interest between compliance and financial gain in the largely industry-based regulatory system, leaving some companies with little motivation to follow regulations, both

in Australian and internationally [32]. An excellent example of conflict of interest is given by Li*, et al.* [8] on the inclusion CAM at medical institutions in the USA due to the financial incentives but without providing evidence-based care. In some countries, such as Australia, there is also a lack of enforcement in cases of non-compliance [33]. A comprehensive summary of the literature on the adulteration and contamination of herbal medicinal products can be found in Posadzki*, et al.* [29], and the need for better regulation of CAM has been strongly emphasised by Ernst [2]. The regulations in Australia, New Zealand, the USA and Canada have been summarised by Barnes*, et al.* [34] and Job*, et al.* [35].

The purpose of this review is to support the argument for stronger regulation and pharmacovigilance of CAM products by highlighting clinically relevant outcomes of adulteration and contamination. Additionally, a summary of recently published methods for the screening of CAM is included to demonstrate that relevant screening and detection methods have been developed and are already available for regulatory use.

Adulteration screening studies and mass spectral analysis

Adulteration of herbal medicines with pharmaceuticals is not a recent problem. In 1997, Huang*, et al.* [36] found that nearly 24% of traditional Chinese medicines (TCM) in Taiwan were adulterated with pharmaceuticals. Since then there have been numerous reports of pharmaceutical adulteration of herbal medicines, summarised in Table 1-1. The literature presented in Table 1-1 shows that herbal products for male health and sexual function, along with slimming products and remedies for general health are often adulterated with pharmaceuticals that suit their intended purpose and indications for use. This problem can be addressed using targeted screening of known or likely adulterants for certain indications or specific cases. Adulterants are generally found in herbal medicines with indications

matching the therapeutic class of the pharmaceutical [36,37], although in some instances there appears to be no logical reason for the adulteration [36,38]. This is demonstrated by Poon*, et al.* [38], where the addition of glibenclamide would have had no effect on the indication for 'sexual enhancement'.

This 'adulterant to match indication' also highlights a possible bias in adulteration studies, due to the selection of samples for analysis, as well as the range of potential pharmaceutical adulterants screened. Many of the studies presented in Table 1-1 considered only a specific set of products, such as those indicated to 'enhance sexual performance' [39,40]. There were also methods developed to detect only certain classes of pharmaceutical, such as those by Savaliya*, et al.* [41] and Kim*, et al.* [42,43]. The studies by Bogusz*, et al.* [44], Wang*, et al.* [45] and Huang*, et al.* [36], where upwards of 60 drugs were screened in each study, give a much broader view of the magnitude of the adulteration problem.

A number of techniques have been used for the detection of undisclosed pharmaceuticals in herbal medicines, including high performance liquid chromatography (HPLC) [40,46-48], nuclear magnetic resonance (NMR) spectroscopy [49,50], direct mass spectrometry (MS) [51-54] and various hyphenated methods such as gas chromatography-mass spectrometry (GC-MS) [46], liquid chromatography-mass spectrometry (LC-MS) [40] and liquid chromatography-tandem mass spectrometry (LC-MS/MS) [44,48,55,56]. Haneef*, et al.* [57] and Vaclavik*, et al.* [58] discuss the various methods used for adulteration studies in great detail. LC-MS/MS is arguably the most popular technique, as it is applicable to a wide range of pharmaceuticals [59], as well as being sensitive, quantitative, highly selective and usually requires only simple sample preparation [57]. In addition, the MS fragmentation patterns give structural information, allowing for the identification of unknown analytes. Table 1-2 summarises LC-MS methods that have been

used in screening studies for adulterants in herbal medicines since 2000. The majority of the methods were quantitative and used only a simple solvent extraction with sonication and filtration before instrumental analysis. For a method to be easily adopted for regulatory purposes, it needs to be rapid, simple and preferably cheap.

Not all adulterants are approved pharmaceuticals, as analogues and new compounds have also been detected in CAM products (see Table 1-1 and Table 1-3). Two studies by Cohen*, et al.* [60,61] found analogues of methamphetamine and the stimulant 1,3-dimethylamylamine (DMAA) in workout supplements. After several athletes failed drug tests due to a positive result from the methamphetamine analogue N,α-diethyl-phenylethylamine (N,α-DEPEA), their workout supplement was investigated as the possible source. Three samples of the supplement from different batches were tested, and in each case, N,α-DEPEA was detected. N,α-DEPEA is a known stimulant, but its addictiveness and possible adverse effects are unknown [60]. In the other case, an analogue of DMAA, 1,3-dimethylbutylamine (DMBA) was found in 12 out of 14 supplements tested. This is highly concerning as DMBA has never been tested in humans and has no safety profile, while DMAA is banned in many countries due to possible serious health risks including stroke and heart failure [61]. The majority of the methods in Table 1-1 and Table 1-2 did not include analogues amongst their targets; those included were mostly known PDE-5 inhibitor analogues. Only Wang*, et al.* [45] actively looked for unknown adulterants using a HPLC-HRMS method. The detection of such novel compounds would remain a challenge even with the more comprehensive methods listed.

More studies are needed that screen for diverse products and indications, as well as the use of untargeted methods which profile hundreds of pharmaceuticals and other potentially problematic compounds [45]. These are particularly important for cases where a product has

been adulterated with an unexpected drug that has no obvious benefit for the product indications. This type of approach creates a clear distinction between non-quantitative screening for the presence of pharmaceuticals and quantification of any adulterants found. An ideal approach would be an initial screen using an untargeted method, followed by confirmation and quantification with a targeted method. Screening has the benefit of being a rapid process by which hundreds of samples can be tested in the minimum time, where a quantitative method is not necessary. If a positive sample is detected, confirmation and quantification against reference standards can follow, but only for those positive samples. A two-step process would ensure a high throughput of samples without unnecessary quantification and confirmation for negative samples. There is a potential for other MS platforms, such as direct MS, to be implemented for a two-step process [51-54]. These screening approaches need to be implemented on an ongoing basis, as batch-to-batch variation means that while one batch may be safe and free of possible adulterants, the next batch may not [62].

Clinical relevance and case studies

The clinical relevance of adulterated products can be hard to define. This is often due to the fact that use of herbal medicines is not reported to a patient's general practitioner or pharmacist, providing little or no record of product use [18,63,64]. One case study [63] reported a patient who was taking 24 CAM remedies along with one prescription medication. While there was no adverse outcome, the total number of individual ingredients from the 24 different remedies was greater than 55, and these remedies were taken without any disclosure to the patient's general practitioner or pharmacist.

A second factor is that any side effects from the product or the adulterant might not be associated with the herbal medicine due to the preconceived idea that herbal medicines are

'natural' and harmless, and therefore could not cause side effects [26,64-66]. Effective systems for reporting such side effects and adverse reactions are generally not in place, hindering the collection of data and, even with such systems, consumers may not report side effects from CAM products [64,66-68]. These limitations are often noted in case studies or reports, and there is an assumption that the incidence of side effects and adverse reactions is repeatedly underestimated [2,26,67,68]. Yet it is clear from the below studies (Table 1-1 and Table 1-2) that consumers are being exposed to adulterated products. Many of the detected pharmaceuticals in Table 1-1 are common, well-tolerated drugs, such as paracetamol, sildenafil and caffeine, raising the possibility the adulterant's therapeutic actions are being attributed to the efficacy of the CAM product, resulting in the adulterated products not being reported [20].

Table 1-1: Summary of adulteration studies from 1997 to 2015. Literature search terms included herbal medicines, traditional Chines medicines, Complementary and alternative medicines, adulteration and contamination. NSAIDs= Nonsteroidal anti-inflammatory drugs, PDE5= phosphodiesterase type 5.

Author	Samples	Indications	Results	Comments
Bogusz, *et al.* (2006)	Various herbal remedies and products	Various, including male sexual function, slimming remedies, natural general health and diabetes.	Sildenafil, tadaladil, glibenclamide, fenfluramine, phentermine, caffeine, phenylbutazone, dipyrone and testosterone decanote.	80 drugs from various classes were screened in this method.
Campbell, *et al.* (2013) [39]	91 samples of herbal products were collected from locations in Atlanta and Baltimore, USA. 7 samples were from the USA. Customs seizures. There were 58 different products.	Naturally enhance sexual performance.	81% of the samples contained one or more PDE5 inhibitor (tadalafil and sildenafil) or an analogue of a PDE5 inhibitor.	14 samples warned against the concurrent use with nitrates. 18 of the 40 samples that contained a PDE5 inhibitor had a contraction of a greater than 110% of the highest approved dose.

Author	Samples	Indications	Results	Comments
Cho, *et al.* (2014) [55]	294 food and dietary supplements collected over 4 years in South Korea.	Arthritis, bone ache and joint pain.	Around 30% of the samples were positive for steroids. 45.1% of the positive samples contained dexamethasone.	35 different steroid compounds were screened for; including dexamethasone, betamethasone, prednisolone, prednisone, cortisone and derivatives.
Gratz, *et al.* (2004) [40]	Around 40 different herbal products.	Sexual performance formulas.	19 of the products contained therapeutic levels of a PDE5 inhibitor.	Only sildenafil and tadalafil were detected, not vardenafil.
Huang, *et al.* (1997) [36]	Over a year, 2609 samples of traditional Chines medicines were collected from hospitals in Taiwan.	Various indications.	Caffeine, paracetamol, indomethacin, hydrochlorothiazide, prednisolone were the most frequently detected.	618 samples (23.7%) had been adulterated with 69 different substances
Kim, *et al.* (2014) [42]	188 food and dietary supplements from South Korea collected between 2009 and 2012.	Not given.	Bisacodyl, desmethylsibutramine, didesmethylsibutramine, ephedrine, fluoxetine, pseudoephedrine, sennoside A, sennoside B and sibutramine were detected in 62 samples.	Samples were screened for 29 'weight-loss' compounds.

Author	Samples	Indications	Results	Comments
Kim, *et al.* (2014) [43]	126 food and 88 dietary supplements from South Korea (total = 214).	Not given.	52 samples were adulterated with paracetamol, diclofenac, ibuprofen, indomethacin, naproxen and piroxicam.	Samples were screened for paracetamol and 16 NSAIDs.
Ko (1998) [62]	260 Asian patent medicines from retail herbal stores in California, U.S.A.	Not given.	At least 83 products contained undeclared pharmaceuticals or heavy metals. 23 products had more than one adulterant. Most common adulterants were ephedrine, chlorpheniramine, methyltestosterone, and phenacetin.	14 products were labelled declaring pharmaceuticals. Authors noted that due to batch to batch inconsistency the remaining products could not be assumed to be safe.
Li, *et al.* (2010) [56]	30 samples of dietary supplements and Chinese medicines.	Not given.	14 samples were positive for 9 different adulterants, including chlorpropamide, glenclamide, gliclazide, glimepiride, metformin, mitiglinide, nateglinide, phenformin and rosiglitazone.	Method was to identify 14 anti-diabetic drugs.
Lau, *et al.* (2003) [69]	Indonesian sachet of powdered herbs. The product had been recalled and banned in Singapore in 2002.	Rheumatism, body and bone aches, muscle and joint pain, giddiness, toothache, backache and chronic numbness	Caffeine, phenylbutazone and oxyphenbutazone	The doses of caffeine and phenylbutazone were below the daily therapeutic dose if the remedy was taken as recommended.

Author	Samples	Indications	Results	Comments
Liang, *et al.* (2006) [37]	Over 200 products were analysed after being confiscated by the State Drug Administration (SDA) of China.	Not given	74 products were positive for sildenafil, famotidine, ibuprofen, promethazine, diazepam, nifedipine, captopril, amoxicillin and dextromethorphan.	Sildenadil and famotidine were the two most commonly detected pharmaceuticals.
Liu, *et al.* (2000) [47]	AsthmaWan herbal capsules	Anti-asthmatic.	Small amounts of codeine were detected (61.8 μg/capsule)	
Miller and Stripp (2007) [46]	90 Chinese herbal medicines from New York's Chinatown.	Various: Eczema, antihistamine, male sexual performance enhancer, antihypertensive.	Five products contained nine pharmaceuticals: Promethazine, chlormethiazole, chlordiazepoxide, hydrochlorothiazide, chlorpheniramine, diclofenac, diphenhydramine and sildenafil.	
Panusa, *et al.* (2007) [48]	Homeopathic products	For inflammation.	None of the products were positive for pharmaceuticals.	Diclofenac, ibuprofen, ketoprofen, naproxen, nimesulide, paracetamol and piroxicam were investigated as possible adulterants.

Author	Samples	Indications	Results	Comments
Reeuwijk, *et al.* (2014) [27]	50 herbal food supplements sold on the Dutch market between 2004 and 2013.	Weight loss.	24 samples contained pharmaceuticals (sibutramine, desmethylsibutramine, didesmethylsibutramine, phenolphthalein, sildenafil and rimonabant) with 11 samples having more than one adulterant.	Sibutramine was the most commonly detected.
Ren, et al. (2012) [70]	16 herbal medicines from markets and the internet.	Male sexual potency.	9 samples contained PDE5 inhibitor and/or an analogue.	Method was designed to screen for yohimibine and PDE5 inhibitors and analogues.
Savaliya, et al. (2009) [41]	58 Ayurvedic herbal medicines where purchased in India.	Not given.	12 samples were positive for dexamethasone, diclofenac and/or piroxicam.	Method was developed to detect steroidal and NSAIDs adulteration.
Wang, et al. (2008) [71]	Around 50 samples of health food and herbal medicines from local Chinese suppliers or the internet.	Various.	14 samples were positive for pharmaceuticals and/or analogues.	

Table 1-2: Summary of the methods used in analytical adulteration studies. ACN = Acetonitrile, DAD = Diode-array detector, ESI = Electrospray ionization, EtOH = Ethanol, HRMS = High resolution mass spectrometry, MeOH = Methanol, MRM = Multiple reactions monitoring, MTSF = Mass spectral tree similarity filter, SRM = Selected reaction monitoring, TLC = Thin-layer chromatography, TOF = Time of fight, UFLC = Ultra-fast liquid chromatography, UHPLC = Ultrahigh-performance liquid chromatography, UPLC = Ultra-performance liquid chromatography.

Author	Sample Matrix	Extraction	Instrumentation	Quantitative
Bogusz, *et al.* (2006)	Herbal remedies, ground to a homogenous material	Extracted with MeOH for 30min. Samples with sugar (e.g. honey) were extracted with dichlormethane-isopropanol (9:1) and then evaporated with N_2 and reconstituted with MeOH.	LC-ESI-MS/MS in MRM	Yes
Cho, *et al.* (2014) [55]	Samples were classes as liquid, powder, pill, tablet, capsule or granules.	Dissolved in 70% MeOH and sonicated for 30 min. Filtered before analysis.	LC-ESI-MS/MS	Yes
Gratz, *et al.* (2004) [40]	Herbal matrices products	Extracted with 50/50 ACN and water and then sonicated for 15 min. Filtered and diluted with ACN before analysis.	LC-ESI-MS LC-UV	Yes
Guo, et al. (2014) [72]	Herbal medicines and dietary supplement in the form of tablets, pellets or capsules.	Samples pulverised to a homogeneous powder. ACN and water (v/v 60:40) was added and the samples were shaken for 30 secs and sonicated for 15 min at 20°C and then diluted with ACN and water. Extracts were left to stand for 10 min before the supernatant was filtrated.	UHPLC-Quadrupole-Orbitrap MS	Yes

Author	Sample Matrix	Extraction	Instrumentation	Quantitative
Kim, *et al.* (2014) [43]	Various forms; powder, capsules, pills, tablets, granules.	Extracted with 70% MeOH and sonicated for 30min. Filtered before analysis.	LC-ESI-MS/MS	Yes
Lau, *et al.* (2003) [69]	Powdered herbal sachet	EtOH was added to the sample and then boiled and filtered. Extraction was repeated 3 times. The extract was evaporated and reconstituted in MeOH.	LC-MS/MS in SRM.	Yes
Li, *et al.* (2010) [56]	Chinese medicines in the form of tablets, pills, granules and capsules.	Samples grounded to a homogeneous powder, extracted in MeOH and sonicated for 20 min	UPLC-MS/MS	Yes
Liang, *et al.* (2006) [37]	Products were mostly in capsule, tablet or oral solution form	Mobile phase was added to the samples and then extracted in an ultrasonic washer. Samples were centrifuged and supernatant was diluted for analyses.	LC-MS/MS in MRM	No
Liu, *et al.* (2000) [47]	Herbal capsules, with between 9 to 11 ingredients. Tested for the presence of codeine.	Capsules dissolved in 10mM sodium phosphate buffer and EtOH. The mixture was then extracted using chloroform and evaporated. Reconstituted in MeOH.	Detection and Quantification by RP-HPLC-UV Confirmation of codeine by LC-MS/MS in SRM	Yes

Author	Sample Matrix	Extraction	Instrumentation	Quantitative
Lu, et al. (2010) [73]	35 samples of dietary supplements and traditional Chinese medicines in the forms of either capsules or tablets.	Samples 'smashed' and mixed homogeneously. Extracted with MeOh-water (v/v 1:1) by sonication for 30 min.	HPLC-ESI-MS/MS	Yes
Miller and Stripp (2007) [46]	Chinese herbal medicines in various forms; pills, teas, capsule, creams, etc.	Pills, tablets and capsules were ground and extracted with MeOH. Samples were then vortexed, centrifuged and filtered. Teas were brewed according to packaging instructions. Samples were then centrifuged and filtered. Creams were dissolved in methylene chloride, vortexed and then centrifuged and filtered. All samples were then adjusted to an acidic (pH2 using HCl), basis (pH 12 using NaOH) or neutral pH.	Screening: TLC Confirmation: GC-MS in SIM and HPLC	No
Panusa, *et al.* (2007) [48]	Homeopathic products such as 'mother' tinctures, solutions, tablets, granules, creams, and suppositories.	All samples were diluted with MeOH-water (v/v 80:20), spiked, homogenised, sonicated and then filtered before analysis.	HPLC-UV HPLC-ESI-MS in SIM	Yes

Author	Sample Matrix	Extraction	Instrumentation	Quantitative
Reeuwijk, *et al.* (2014) [27]	Herbal supplements, mostly in the forms of capsules, tablets and sachets	The samples were sonicated with MeOH, and then diluted 100 fold with MeOH/0.1% formic acid. Extracts where filtered before analysis.	HPLC-DAD-MS/MS	Yes
Ren, et al. (2012) [70]	Herbal medicines in the forms of capsules or tablets	Samples ground to a homogeneous powder. MeOH was added and samples were extracted by vortexing and sonication. The extracts were diluted before analysis.	UFLC-ESI-MS/MS	Yes
Savaliya, et al. (2009) [41]	Ayurvedc herbal medicines from India	Samples extracted with 80% MeOH, sonicated and centrifuged.	LC-MS/TOF using both APCI and ESI.	Yes
Wang, et al. (2008) [71]	Health foods and herbal medicines in tablet or capsule forms.	Samples homogenised, sonticated for 15 min with MeOH. Extract was centrifuged and supernatant diluted with MeOH.	HPLC-HRMS with data processing via MTSF	Yes

Table 1-3: Summary of case studies concerning adverse effects from adulterated products.

Author	Product used	Adulterant/ Contaminant	Adverse Effects	Comments
Amster, *et al.* (2007) [28]	Kelp Supplement	Arsenic (8.5 mg/kg)	Alopecia, memory loss, diarrhoea, nausea, vomiting, weakness, fatigue, headache and rash.	The patient had originally increased the dose of the supplement to help treat her alopecia and memory loss. Occurred in California, USA.
Chen, et al. (2010) [65]	20 herbal slimming products	Sibutramine, *N*-desmethyl-sibutramine, *N*-bisdesmethyl-sibutramine and Fenfluramine.	Psychotic features included auditory and (63%) visual hallucinations (38%), persecutory ideas (38%), delusive thoughts (25%) and suicidal ideation (13%).	14 of the products contained more than one adulterant. The amount of sibutramine was determined for some of the products with a range from 2.8 to 19.6 mg. Cases occurred in Hong Kong, with products purchased locally, over the internet, and overseas.
Corns and Metcalfe (2002) [15]	Chinese herbal weight loss products	Fenfluramine hydrochloride (20 mg)	Hypertension, weight loss, abdominal pain, nausea, visual hallucinations.	Occurred in the UK. Three cases of patients taking various herbal weight loss products after seeing the same herbalist.

Author	Product used	Adulterant/ Contaminant	Adverse Effects	Comments
Jung, *et al.* (2006) [24]	Chinese herbal medicine for weight reduction	Sibutramine	Headache, vertigo and numbness	One capsule contained 27.4 mg of sibutramine; approximately double the highest single dose (12.55 mg). Occurred in Germany with the product purchased over the internet.
Lim, *et al.* (2011) [26]	Multiple ingredient herbal product	Steroids	Not detailed.	Product consumed by a child (age between 0 to 16 yrs.) Occurred in Australia.
Poon, *et al.* (2009) [38]	Numerous (n= 25) erectile dysfunction products	Sildenafil and glibenclamide	Coma, convulsion, confusion, cerebral oedema, sweating, drowsiness, light-headedness, cognitive impairment, death	Occurred in Hong Kong, products coursed from various places including pharmacies in Hong Kong and China.
Tait, *et al.* (2002) [74]	Ayurvedic tablets	Lead and mercury	Chronic lead poisoning of a preterm infant via mother.	The mother's intake of lead was greater 50 times the average weekly lead intake of Western populations. Occurred in Australia with Ayurvedic tablets prescribed from India.

Author	Product used	Adulterant/ Contaminant	Adverse Effects	Comments
Tang, *et al.* (2011) [75]	81 slimming products.	Fenfluramine, sibutramine, mazindol, phenolphethalein, anthraquinones, bisacodyl, hydrochlorothiazide and spironolactone, drug analogues (*N*-desmethyl-sibutramine, *N*-bisdesmethyl-sibutramine and *N*-Nitrofenfluramine) and animal thyroid tissue.	Palpitation, tremor, insomnia, dizziness, myocardial infarction, supraventricular tachycardia, hypertension, weakness, sweating, liver failure, pulmonary hypertension, hypokalaemia, diarrhoea, NSAID-related acute renal failure, subclinical and clinical thyrotoxicosis, tremor, irritability, thyrotoxic periodic paralysis and right heart failure resulting in death.	Multiple adulterants (up to 6) were found in single products. Occurred in Hong Kong with products purchased from the internet, China, over the counter, herbalists or friends

Due to the perception that CAM remedies and herbal products are 'natural' and therefore harmless, they are consumed by both adults and children. Over a 36-month period in Australia, there were 39 reports of CAM associated adverse reactions in children (aged from birth to 16 years old) [26]. In 56% of these cases (n =22), the adverse events were associated with the use of medicinal CAM. In one of the cases, the cause of the adverse reaction was linked steroid contamination of herbal products[26].

In this next section, case studies of adverse effects from adulterated products will be summarised (Table 1-3) to highlight that, while under-reported, adulterated products are a clinically relevant problem for consumers, health professionals and government agencies.

Weight loss supplements

There are many case studies and reports of adverse reactions resulting from the consumption of adulterated and contaminated herbal medicines, including a systematic review by Ernst [2]. A major area of CAM use is for weight loss, and case studies and reviews have identified numerous incidents of adverse reactions to adulteration (Table 1-3). A retrospective review in Hong Kong looked at psychosis associated with herbal slimming products and identified 16 patients from 2004 to 2009 [65]. The products were found to be adulterated with fenfluramine, sibutramine and its analogues *N*-desmethyl-sibutramine and *N*-bisdesmethyl-sibutramine. More than one adulterant was detected in 14 of the products. While psychosis is not a common adverse reaction to sibutramine [27], the authors of this case review suggested that the patients may have inadvertently overdosed themselves, resulting in the adverse reaction, by using the products liberally under the belief that the products were purely herbal and therefore could not cause harm [65]. Another case study involving adulteration with sibutramine saw a healthy 20-year old woman develop severe headache, vertigo and

numbness after taking a Chinese herbal medicine purchased over the internet to support weight reduction [24]. Quantification showed that one capsule contained 27.4 mg of sibutramine; approximately double the highest single dose approved for use in Germany [24].

Tang*, et al.* [75] looked at herbal slimming medicines in Hong Kong, analysing 81 weight-loss and slimming products after 66 cases of poisoning from 2004 to 2009. In the cases presented by Tang*, et al.* [75], two involved pulmonary hypertension from fenfluramine adulteration, one of which was fatal (heart failure). Fenfluramine was a widely used appetite suppressant in the 1980s and 1990s until its withdrawal from the market due to the fact that it can cause pulmonary hypertension and valvular heart disease [15,75]. The products also contained a number of other adulterants, including sibutramine, mazindol, phenolphethalein, anthraquinones, bisacodyl, hydrochlorothiazide and spironolactone, drug analogues and animal thyroid tissue. Multiple adulterants (up to 6) were found in single remedies. The adverse reactions were consistent with the adulterant(s) found in the product and included cardiovascular effects such as palpitation, tachycardia, myocardial infarction and hypertension.

Fenfluramine has been found in a number of different Chinese herbal medicines, particularly unnamed yellow pills for weight loss in studies presented by Corns and Metcalfe [15]. The first patient had developed hypertension, weight loss and abdominal pain after taking four yellow pills per day over four months for 'weight loss and appetite suppression'. This patient was also taking a number of other herbal medicines, in total 45 pills a day; another instance of a patient taking multiple herbal products, which can itself lead to drug interactions and complicate any diagnosis [38,75]. In the second case, the patient became ill with rapid heart palpitation, nausea and visual hallucinations. The patient had been given no dosage

information by the herbalist and took about eight capsules, believing them 'herbal' and 'harmless'. In the third case study, the pregnant and breastfeeding patient had been assured by the same herbalist that the tablets were pure herbs and could not cause harm. After media reporting of the case studies, 14 further people came forward having taken either the same or similar herbal slimming products. In total 19 herbal slimming products, all imported from China, were collected and it was confirmed that the unnamed yellow pills contained 20 mg fenfluramine hydrochloride [15].

Diabetes-related supplements

While Lim*, et al.* [26] found that the biggest risk of CAM remedies was a rejection of conventional medicines in favour of CAM, Wood*, et al.* [76] present a different view. A 48-year old man with Type 2 diabetes had poor weight and glycaemic control, despite twice daily insulin and oral metformin. Complications included ischaemic heart disease needing coronary bypass surgery. After a year of no follow-up in which time the patient travelled to India and stopped all diabetic medication, the patient returned to the UK with excellent glycaemic control. While in India, the patient had taken three different herbal balls, three times a day with meals. During this time there were no hypoglycaemic episodes and no side effects from the herbal balls. Analysis of the herbal balls revealed that one of them contained chlorpropamide. Chlorpropamide is no longer recommended for use in the management of diabetic patients, particularly those with a history of cardiac disease (like this patient), due to the risk of serious side effects [76]. However, in this case, there was clearly a benefit to the patient.

There was a cluster of hypoglycaemia in Hong Kong in male patients from 2007 to 2008 [38]. The outcomes for 68 patients ranged from full recovery to 3 fatalities, with one patient remaining in a vegetative state and another with permanent cognitive impairment. The

hypoglycaemia was suspected to have been drug-induced, and urine testing showed the presence of sildenafil and glibenclamide (or metabolites). Many of the patients denied the use of the drugs, but 56% did admit to using non-prescription erectile dysfunction products, and 14 of these patients surrendered 25 unused products for analysis. In all, there were seven distinct CAM products. Both sildenafil and glibenclamide were detected in the products at various levels; both sub-therapeutic and at several times the maximum recommended daily dose. In some cases, one capsule/tablet contained enough glibenclamide to cause hypoglycaemia. It was strongly noted in this study that the reluctance of the patients to reveal their use of the CAM products hindered determination of the scale of the cluster. Patients with milder symptoms may not have sought treatment [38], meaning that the true extent of the incident remains unknown.

Heavy metal contamination

Contamination with pesticides or heavy metals [20] can also be the cause of adverse reactions to CAM remedies. Lead and mercury contamination of Ayurvedic tablets taken by a pregnant mother resulted in the chronic lead poisoning of herself and her infant in Australia with the products prescribed in India [74]. At the time of publishing, the case study by Tait*, et al.* [74] reported neonatal blood lead level were the highest recorded for a surviving infant, with lead concentrations of 11.8 µmol/L. The mother had been taking several tablets periodically for nice years, with the lead content being 4.5% to 8.9% in two tablets. Her blood lead concentrations were 5.2 µmol/L, the recommended public health levels are ≤ 0.48 µmol/L [74].

Amster*, et al.* [28] presented a case of arsenic toxicity in a 54-year-old woman in California, USA. For two years, the patient had been suffering from worsening alopecia and memory loss to the point that she could no longer remember her home address. With no clear diagnosis, the

patient had taken to self-treatment with kelp supplements, fish oil, ginkgo biloba and grape seed extract. The symptoms were thought to be related to menopause, however, hormonal treatment had no effect. The patient increased her dose to at least four pills of kelp supplement per day, and her symptoms worsened to include diarrhoea, nausea, vomiting and daily pressure headaches. A urine test showed elevated arsenic levels, and analysis of the kelp supplement revealed an arsenic concentration of 8.5 mg/kg. The patient was advised to stop taking the supplement, and within three weeks, there was a near complete resolution of symptoms. The patient's urine arsenic levels had returned to normal within two months. The intensity of the patient's symptoms was most likely due to a long duration of exposure [28].

The case by Amster*, et al.* [28] is another key example of a patient self-treating with herbal medicines and increasing dosages without any medical supervision. Not only does this case raise the issue of 'natural' does not equal harmless, but it also shows how such contamination or adulteration can complicate and dramatically lengthen the time of diagnosis and accessing the correct treatment for a patient, a point also apparent in Poon*, et al.* [38]. The clinical relevance of adulteration and contamination needs to be further publicised, and more education is needed for the general public to build awareness of the potential health risks surrounding herbal medicines.

Conclusion

The call for stricter regulation of herbal medicines and dietary supplements has been repeated throughout the literature [38,64,77]. Effective pre- and post-market screening and surveillance of CAM products is one method which would allow for the detection of adulterated or contaminated products and the withdrawal of products from the market [5], hopefully before adverse outcomes for consumers. The ideal outcome is the establishment

of more rigorous standardised methods for the analysis of CAMs that can be implemented in any regulatory jurisdiction. So what is next? Adulteration of herbal products is not a new problem, yet it is one for which there appears to be little political will to combat via regulation. While the issue is partly about misleading consumers, adulteration can have serious clinical implications, including mortality. The question is no longer 'if' tougher regulation and pre- and post-market screening are needed; but rather 'how' we can achieve these needs. Methodologies to screen herbal medicines have been developed, with numerous published studies outlining high-throughput and rapid methods. Screening approaches for regulation could be adapted from the existing literature, although refinement would be needed. Beyond science and technology approaches, further research is also needed into regulatory effectiveness in preventing these problems. At the end of the day, so-called 'low risk' products used every day for self-treatment by consumers, should actually be low risk.

2. Toxicological screening and DNA sequencing detects contamination and adulteration in regulated herbal medicines and supplements for diet, weight loss and cardiovascular health

Crighton, E., Coghlan, M. L., Farrington, R., Hoban, C. L., Power, M. W. P., Nash, C., Mullaney, I., Byard, R. W., Trengove, R., Musgrave, I. F., Bunce, M. & Maker, G. Toxicological screening and DNA sequencing detects contamination and adulteration in regulated herbal medicines and supplements for diet, weight loss and cardiovascular health. *Journal of Pharmaceutical and Biomedical Analysis* **176**, 112834, doi:10.1016/j.jpba.2019.112834 (2019).

Link:

There is a lack of accessible data on the quality of CAM products on the Australian market. This chapter forms a part of research into the problems of adulteration, contamination and mislabeling of products on purchased in Australia by the authors. Novel to this research is the use of a combination of DNA metabarcoding and mass spectrometry techniques to investigate the contents of the products.

Abstract:

Use of herbal medicines and supplements by consumers to prevent or treat disease, particularly chronic conditions continues to grow, leading to increased awareness of the minimal regulation standards in many countries. Fraudulent, adulterated and contaminated herbal and traditional medicines and dietary supplements are a risk to consumer health, with adverse effects and events including overdose, drug-herb interactions and hospitalisation. The scope of the risk has been difficult to determine, prompting calls for new approaches, such as the combination of DNA metabarcoding and mass spectrometry used in this study. Here we show that nearly 50% of products tested had contamination issues, in terms of DNA, chemical composition or both. Two samples were clear cases of pharmaceutical adulteration, including a combination of paracetamol and chlorpheniramine in one product and trace amounts of buclizine, a drug no longer in use in Australia, in another. Other issues include the undeclared presence of stimulants such as caffeine, synephrine or ephedrine. DNA data highlighted potential allergy concerns (nuts, wheat), presence of potential toxins (Neem oil) and animal ingredients (reindeer, frog, shrew), and possible substitution of bird cartilage in place of shark. Only 21% of the tested products were able to have at least one ingredient corroborated by DNA sequencing. This study demonstrates that, despite current monitoring approaches, contaminated and adulterated products are still reaching the consumer. We suggest that a better solution is stronger pre-market evaluation, using techniques such as that outlined in this study.

Keywords:

Pharmacovigilance, mass spectrometry, toxicology, DNA metabarcoding, next generation DNA sequencing, complementary and alternative medicine, diet supplements, herbal medicine, contamination, adulteration.

Background:

Complementary and alternative medicine (CAM) therapies include acupuncture, aromatherapy, chiropractic, homoeopathy, traditional medicines, herbal and botanical medicines and dietary or food supplements. In the United States (USA) and Australia, at least 50% of the population uses some form of CAM, often without the knowledge or support of their primary practitioner [78]. In spite of increased usage and awareness of CAM, there persists the idea among consumers that, as many of these CAM treatments and remedies are promoted as 'natural', they are also harmless and essentially considering 'natural' to be equivalent to 'safe' [16]. These ideals have proven to be false, with reports of drug-herb interactions, adverse effects and hospitalisation [79]. An Australian survey on the use of complementary and alternative medicines (CAM) in people with Type 2 diabetes and/or cardiovascular disease (CVD) found that the average annual expenditure on CAM products was A$360 per person [80]. Bailey*, et al.* [81] stated that consumers are moving towards a more integrated approach to medicine, with the implied aim to prevent or treat disease. In many cases, treatment for such diseases focuses on management rather than 'cure', motivating patients to look for alternative therapies. Cohen and Ernst [82] effectively summarised the concerns for CVD patients using herbal supplements, finding that there is a broad range of potential risks posed to such patients with under-reporting of adverse effects and drug-herb

interactions. Since there is an assumption of safety, patients will often not indicate supplement use and need to be directly asked about their herbal supplement consumption[82].

In addition to safety concerns, there are well-documented quality control problems with CAM [29]. Australia currently has some of the most stringent regulations of CAM products [83]. Yet, post-market compliance reviews conducted by the Australian Department of Health have shown that during the 2016-17 period, 79% of products reviewed breached the current regulations, consistent with previous data [84]. Notably, the 2016-17 performance report states that the 'increase in the number of compliance reviews in recent years has not driven any improvement in compliance rates' [84]. These consistent levels of regulatory non-compliance prompted a review [85], which made several recommendations that have been accepted by the Australian Government, including changes to indications for use and advertisement of CAM products, and increased post-market surveillance [86] of the estimated 11,000 CAM products currently listed on the Australian Register of Therapeutic Goods (ARTG).

Regardless of these proposed changes, there remains an established level of regulatory non-compliance for CAM products, with a reliance on manufacturers making truthful and accurate declarations regarding the composition and manufacturing conditions of their products. Assumptions of regulatory compliance contradict data from post-market testing, as well as the current literature examining the wider CAM industry [20,23,29]. In Australia, natural health products were the largest selling over-the-counter products in 2015-16 (A$1.4 billion) as well as the fastest growing [87]. With the CAM industry expected to be worth an estimated US$180 billion by 2020 [88], there is a strong economic motivation to 'improve' products, often with the use of undisclosed ingredients [22]. Previous studies into discrepancies between product

content and listed ingredients have reported undeclared plant materials, ingredient substitution, pharmaceutical adulteration and contamination with heavy metals [20,23,29].

It was the aim of this study to investigate these quality control issues in CAM samples indicated for cardiovascular health, general wellness/wellbeing, and diet issues, including weight loss, gastrointestinal health and metabolism support, using a combination of DNA metabarcoding and advanced mass spectrometry techniques (LC-MS). One hundred thirty-five products purchased in Australian capital cities and online were analysed using this multi-faceted biomolecular survey.

Methods:

Samples:

135 CAM products were purchased from pharmacies, health food stores, traditional herbal retailers and online in Australian capital cities from 2014 to 2017. The samples existed in a variety of formulations including tablets, capsules, gel capsules, tea, chewable tablets and gummies (or jubes), honey, liquids and powders, and were from various origins such as traditional Chinese medicine (TCM), Ayurvedic and Western herbal medicines and supplements. The products were aliquoted for analysis using aseptic techniques and allocated random identification numbers. Duplicate samples (for Samples 78, 186 and 253) were two different batches of the same product, noted by "1" and "2". Samples 296 and 307 each consisted of two separate tablets or pills, which were analysed separately.

DNA extraction and quantification:

The DNA extraction procedure followed methods previously described [20,89]. Quantification of the extracted DNA was carried out through polymerase chain reaction (PCR) amplification of a plastid gene region using the universal primers *trn*Lgh and a mammalian mitochondrial DNA

gene region targeting a fragment of the 16S rRNA (see Coghlan*, et al.* [20] for primer details). All PCRs were carried out using an ABI StepONE Plus qPCR platform (Applied Biosystems, USA) in a 25 µL volume including: 2 mM $MgCl_2$ (Applied Biosystems, USA), 1 × Taq polymerase buffer (Applied Biosystems, USA), 0.4 µM dNTPs (Astral Scientific, Australia), 0.1 mg bovine serum albumin (Fisher Biotec, Australia), 0.4 µM of each primer, 0.2 µL of AmpliTaq Gold DNA polymerase (Applied Biosystems, USA) and SYBR-Green dye. Each sample was amplified using the undiluted extract and two dilution points (1:10 and 1:100) to gauge template copy number and identify if PCR inhibitors were present. The qPCR conditions were: 50 cycles of 95°C for 30 s, annealing at primer specific temperature for 30 s, and extension at 72°C for 30 s.

Amplicon generation:

Previously described in [20,89], fusion primers with unique 6-8 bp multiplex identifier (MID) tags were designed for the same plant and mammal primer sets as used for the qPCR above, but with the inclusion of a second chloroplast gene region, *rbc*L (see Coghlan*, et al.* [20] for primer details). Fusion tag PCR was carried out using the same cycling conditions, with duplicate reactions for each DNA extract. Amplicons were blended into a library pool in equimolar amounts, as determined by amplicon concentrations on a Labchip GX Touch HT instrument (PerkinElmer, MA, USA). The pooled library was size selected on a Pippin Prep (Sage Sciences) using a 2% agarose gel with ethidium bromide cassette to exclude any primer dimer and cleaned over a QIAquick PCR Purification kit column (Qiagen, USA) following manufacturer's instructions. The final purified library was again quantified on the Labchip GX Touch HT to determine the optimal amount of library required for sequencing. Amplicon sequencing was carried out on an Illumina MiSeq (Illumina, USA) according to manufacturer's protocols using a 300-cycle V2 kit with a nano flow cell.

Bioinformatic analysis:

The data analysis has been previously described in [20,89]. The sequencing output files were retrieved, filtered and processed using Geneious (v8.1). Samples were deconvoluted according to their unique MID tags, with reads that did not match exactly to the primers and MID tag sequences at both amplicon ends being filtered out. Reads were dereplicated in Geneious and exported for chimera removal in USEARCH using the UCHIME *de novo* method, along with singletons, and uploaded onto a high performance computer (Magnus - Pawsey Supercomputing Facility, Perth, Australia) where a BLASTn search was conducted against the National Centre for Biotechnology Information (NCBI) GenBank NR database. The resultant BLAST files were imported into the program MEtaGenome ANalyzer (MEGAN v4.7) for taxonomic analysis. The lowest common ancestor parameters for sequence assignments were: min score of 65, top percent of 5, and min support of 1. Taxonomic assignments for each sample were evaluated using available databases, and conservative estimates of families, genera and species were made.

Toxicological analysis:

Toxicological analysis for contaminants and adulterants such as conventional pharmaceuticals and pesticides was carried out using methods previously detailed in Hoban*, et al.* [89]. Samples were crushed, and 50 mg was extracted by two methods, ethanol and basic ammonia extractions. Ethanol (EtOH) extraction: 1 mL of absolute EtOH (analytical reagent grade; Univar, Sydney AU) with 25 µL of internal standard mixture (see Supplementary Table 9-1) was added to the crushed sample, sonicated and centrifuged. The supernatant was evaporated to dryness and reconstituted with 100 µL of absolute EtOH before analysis. Basic extraction: 1 mL of glass distilled water and 250 µL of 30% ammonia (analytical reagent grade; Chem-Supply, Adelaide AU) were added to the crushed sample and vortexed. 5 mL of high

purity butyl chloride (BuCl; Honeywell, Adelaide AU) was added, and samples further extracted for 15 min using a mechanical roller and then centrifuged. The BuCl layer was evaporated to dryness and reconstituted with 100 µL of absolute EtOH before analysis.

Sample extracts were analysed on an Agilent 1200 HPLC-6510 quadrupole time of flight-mass spectrometer (LC-QTOF-MS) (Agilent Technologies, USA) in positive ESI, auto-MS/MS mode with an Acquity BEH C_{18} column (1.7 µm, 3.0 x 50 mm; Waters, USA) and a Phenomenex C_{18} 4.0 x 3.0 mm guard cartridge (Phenomenex, USA). Mobile phases A and B were acetonitrile and aqueous 0.1% formic acid, respectively, with the following gradient: 0-0.5 min: 90% B, 0.5-8 min: 90-50% B, 8-10 min: 50-5% B, 10-12 min: 5-0% B, 4-min post-gradient equilibration at 90% B. Total run time was 12 min with a 0.35 mL/min flow rate, increased to 0.4 mL/min for the last 2 min. An injector program was utilised which allowed mixing of 2 µL of the ethanolic sample extract with formic acid buffer prior to injection. The source temperature was set to 350°C, capillary voltage was 3000 V, and gas flow was 10 L/min. Skimmer and fragmentor voltages were 65 and 125 V, respectively. The analysis was repeated on an Agilent 1100 HPLC with diode array detector (LC-UV), using an Agilent Eclipse Plus C_{18} column (1.8 µm, 4.6 x 50 mm) with C_{18} guard cartridge (4.0 x 3.0 mm; Phenomenex, USA). Sample extracts underwent further analysis on an Agilent 7890 gas chromatograph with nitrogen-phosphorous and mass spectrometer detectors (GC-NPD/MSD).

Results were processed using MassHunter Qualitative Analysis (vB.07.00) for LC-QTOF-MS data, ChemStation for LC (vB.04.01) for LC-UV data and MSD ChemStation (vE.02.00.493) software for GC-NPD/MSD data. Peaks were matched against Forensic Science SA's in-house retention time, accurate mass and MS/MS spectral library of approximately 350 compounds,

and a commercial database containing 3,490 MS/MS spectra (Forensic and Toxicology Personal Compounds Database and Library, Agilent Technologies).

Caffeine and paracetamol were quantified using the ethanol extraction described above with analysis by LC-UV. Buclizine and chlorpheniramine were quantified using the basic extraction with LC-QTOF-MS analysis, as described above. For quantitation of synephrine, ephedrine and the ephedrine analogues, samples were extracted with 2 mL of sodium acetate buffer (pH 5.7), sonicated and vortexed. The supernatant was passed through a solid phase extraction (SPE) cartridge (200 mg/3 mL XTRACT; UCT, USA) and eluted with CH_2Cl_2/propan-2-ol. The eluent was evaporated to dryness and reconstituted with methanol. These samples were analysed using an Agilent 1200 HPLC coupled to a Sciex 4000 QTRAP-MS system (Sciex, USA) in positive ESI multiple reaction monitoring (MRM) mode. Mobile phases A and B were methanol and aqueous 0.1% formic acid. Total run time was 9 min with a 0.80 mL/min flow rate. Source temperature was set to 650°C, ion spray voltage was 4000 V, ion spray gas was 70 psi, and curtain gas was 30 psi. Calibration curves from reference standards (run in parallel with samples) were used to determine adulterant/contaminant concentrations.

Results:

Figure 2-1 shows the comparison between the samples listed/not listed on the ARTG, while detailed individual sample results can be found in Supplementary Table 9-2. Overall, 86 samples (63%) were listed with the TGA and could be found on the ARTG. Of the total sample pool, data were obtained for 99 samples (72%), from either DNA or toxicological analysis, or both.

Genetic analyses:

Of the 137 samples, 40% (n = 55) were found to contain no amplifiable DNA. Of those samples which did contain DNA (n = 82), 51% (n = 42) had additional plant DNA, 30% (n = 25) contained commonly used 'filler' ingredients such as soybean, rice, oat, wheat or grasses, 4% (n = 3) had additional animal DNA (DNA from non-domestic animals not listed or related to ingredients) and 21% (n = 17) had animal contamination (DNA from domestic species such as dog, pig, rat, mouse or cow).

Figure 2-1 B and C show the difference in DNA results between ARTG listed and non-listed samples. In the ARTG listed products for which DNA data could be generated, 44% (n = 21) had stated ingredients confirmed, 65% contained additional plant DNA, 27% contained filler ingredients, 2% contained additional animal DNA, and 31% had animal contamination. For the products *not* listed on the ARTG (n = 34), only 24% had stated ingredients confirmed, 32% had additional plant DNA, 35% contained filler ingredients, 6% contained additional animal DNA and 6% contained animal contaminants.

Overall, only 22% (n = 30) of the samples could be considered to have had ingredients confirmed by this analysis, with taxonomic assignment corroborating the presence of an ingredient listed on the sample packaging. However, some of these samples also contained fillers or additional DNA, and not all stated ingredients were identified through DNA metabarcoding.

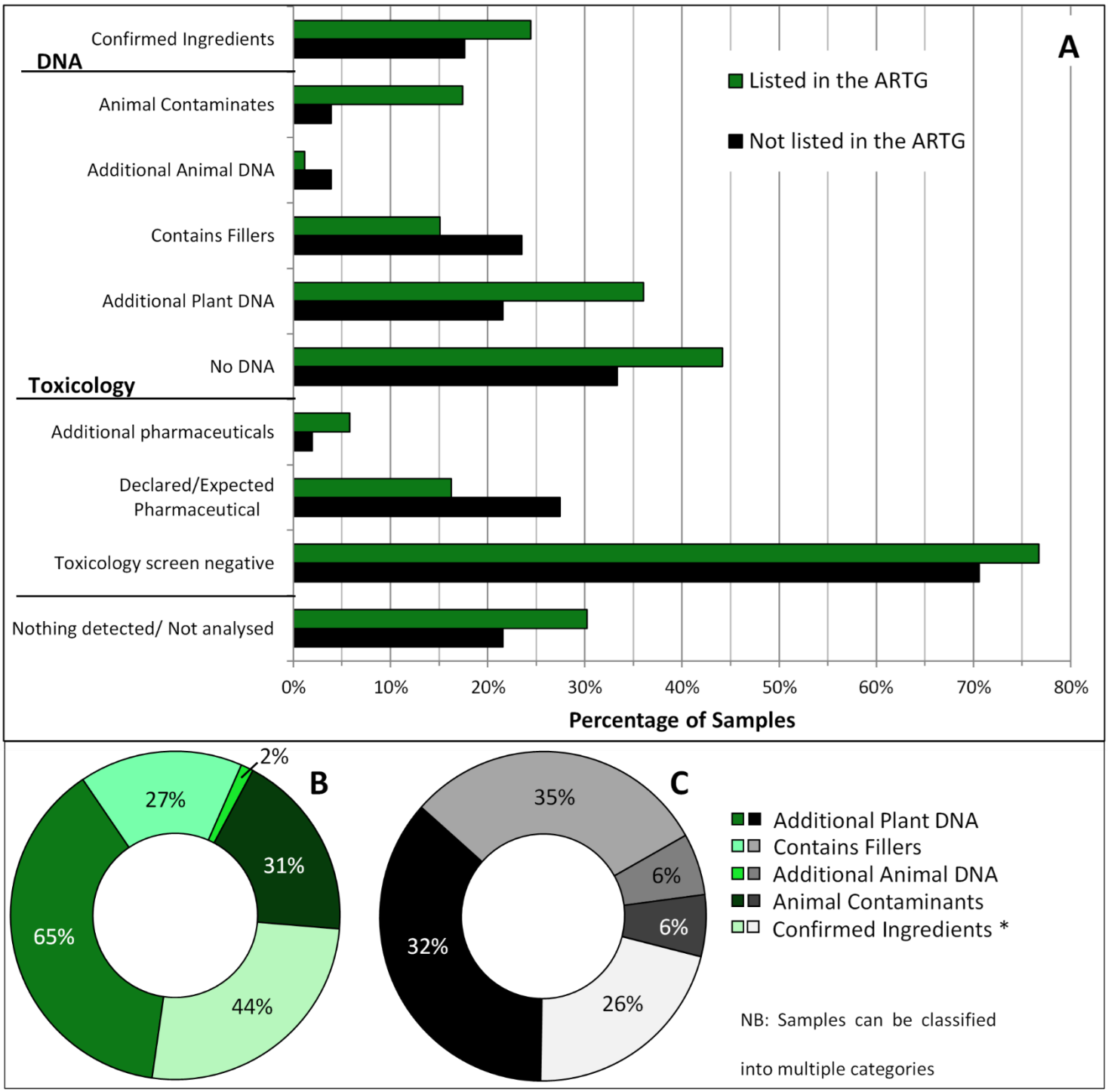

Figure 2-1: A) Compassion between the categories of adulteration, contamination and undeclared ingredients of the samples listed/ not listed on the ARTG. B and C) The DNA results from the samples listed (B, green) and *not* listed (C, black) on the ARTG which contained DNA. Percentage of samples in each category is shown. *DNA from plants or animals listed as main ingredients found. Samples may still contain fillers or additional DNA.

Toxicological data:

Most of the samples were negative for pharmaceuticals (74%, n = 101). Of those samples which did contain pharmaceuticals, 20% (n = 27) were declared on the label or explained by the ingredient list, such as caffeine from green tea or synephrine from bitter orange. 19% (n = 26) of samples contained caffeine, 14 of which were listed and 12 not listed on the ARTG. 5% (n = 7) of the samples contained additional unexplained pharmaceuticals, including caffeine, synephrine, ephedrine and related alkaloids, paracetamol, chlorpheniramine and trace amounts of mycophenolic acid and buclizine (Table 2-1). Of these seven samples, six were listed on the ARTG.

Table 2-1: Samples which contain additional unexplained pharmaceuticals as detected by LC-QTOF.

Sample # ID	In ARTG database (Yes/No)	Pharmaceuticals	Amount (mg/ pill)	Dose per day	Maximum total daily intake (mg)
44	Y	Synephrine	0.69	Take 3 capsules, three times a day	6.2
86	Y	Buclizine	0.0002	Take 1-2 tablets daily or 'for a quick energy boost' take 2-4 tablets	0.0008
93	Y	Ephedrine	0.0001	Not Given	N/A
162	Y	Mycophenolic acid	Trace levels	Take 1 tablet daily	N/A
296 B	N	Paracetamol	0.16	Take 1, twice a day	0.32
		Chlorpheniramine	0.0017		0.0034
322	Y	Ephedrine	0.0016	Take 1 capsule once or twice a day	0.0032
		Pseudoephedrine	0.0004		0.0008
		Methylephedrine and norephedrine	Trace levels		N/A

Discussion:

The data generated in this study continues to highlight a number of concerns with herbal CAM products [20,89]. The large number of products containing undeclared plant ingredients indicates either widespread ingredient substitution or that current methods used to identify plant ingredients are inadequate. Many products were contaminated with animal DNA that cannot be explained based on the ingredient list and may speak to poor manufacturing processes. 5% of the samples contained undeclared pharmaceuticals, at least two of which are clear cases of adulteration (samples 296 B and 322).

Genetic analysis:

As found in our previous study [20] and elsewhere [23], the ingredient list for a product does not always reflect what is actually contained within that product. Of the 137 samples tested in this study, only 21% (n = 29) had at least one of the listed ingredients corroborated by DNA barcoding. Furthermore, DNA from plants not listed or related to the product ingredients was found in 31% of the samples, making it impossible for consumers and their medical professionals to determine exactly what an individual has ingested. This lack of regulatory compliance demonstrates serious shortfalls in quality control and/or manufacturing processes. DNA from the cashew nut (*Anacardium*) and the walnut (*Juglandaceae*) families were detected in samples 68 and 319, and 79, respectively, presenting possible nut allergy concerns. DNA from black walnut (*Juglans nigra*) was also detected [23] in single-herb products containing ginkgo. Further to this, 18% of samples contained filler ingredients, such as rice (*Oryza*) or soybean (*Glycine*). The presence of undeclared fillers is of particular concern to people with allergies (e.g. celiac disease).

Unknown ingredients and inaccurate labels increase the potential for adverse and allergic reactions because consumers cannot make informed choices [90]. Not only this but in cases where an adverse reaction does occur, it will be very difficult to determine the causal agent if the ingredient list does not accurately reflect the contents. To highlight this point, sample 80, indicated for assisting 'healthy people to maintain cholesterol and triglycerides within the normal range', contained DNA from the Neem tree (*Azadirachta indica*). Not declared on the label as an ingredient, the Neem tree has been widely used in Ayurvedic medicine [91] and has many potential bioactive properties. However, Neem oil has also been the cause of poisonings (mostly in children), causing vomiting, metabolic acidosis, and toxic encephalopathy [92]. Products sold in Australia containing Neem oil for topical application need to be labelled with the warnings 'not to be taken' and 'keep out of reach of children'.

Not only was additional plant DNA detected, but in a small number of samples, additional animal DNA or contamination was detected. Contamination from species such as rat, dog, goat, pig and other domesticated animals is potentially from manufacturing deficiencies or transportation and could be seen as inadvertent [20]. The detection of DNA from non-domesticated animals is harder to explain. In sample 69, a supplement powder for general wellbeing, reindeer DNA (*Rangifer tarandus*) was detected, while in sample 245, a tea for 'slimming' and 'detox', DNA was found from frog (subfamily Rhacophorinae) and the Asian Highland Shrew (*Suncus montanus*). Neither of these products declared animal ingredients, and it is possible that this was also inadvertent contamination. However, frog has previously been found in herbal products [20] and is a potential deliberate addition for zootherapy [93].

Sample 70 raises questions of ingredient substitution or fraud [93]. Listed only to contain shark cartilage (no species information given) and indicated for general wellbeing, as an anti-

inflammatory and for joint health, the product had animal contamination from rat, dog and pig, as well as shark DNA from tiger shark (*Galeocerdo cuvier*) and *Carcharhinus* sp., indicating that the product was at least partly accurate in its ingredient declaration. However, there is also the possibility of ingredient substitution for bird cartilage rather than shark, with DNA found from *Anatidae* sp. and the subfamily Phasianinae. Both bird taxa were not reflected on the ingredient list.

DNA barcoding provides vital information about the composition of a sample, but it does have limitations, particularly when no DNA is detected in a sample, as was the case with 40% of the samples in this study. This can be due to various factors, including the nature of the product ingredients (e.g. herbal extract), the form of the product itself (e.g. oil capsule) or the manufacturing process, where DNA can be totally degraded and therefore unable to be detected [94]. Alternatively, it is possible some formulations contain no biological material so would never yield a DNA profile. The use of PCR methods for commercial testing has also been recently discussed in Newmaster*, et al.* [90], arguing for a transparent system and supply chain with well-defined common incidental DNA limit (such as the DNA from common domestic species detected in this study). Nevertheless, DNA degradation and its loss are key drivers for our suggested multi-tiered approach to pharmacovigilance for CAM products [20,94].

Toxicological analysis:

Caffeine was the most commonly detected compound in this study, in 19% of samples (n= 26). The presence of caffeine is chiefly explained by ingredients such as green tea (*Camellia sinensis*), coffee (*Coffea canephora*) and Yerba Mate *(Ilex paraguariensis)*. Caffeine has a long history of use in weight loss and diet products [95,96], and is one of the most widely consumed drugs worldwide [95]. Due to the widespread and generally safe consumption of moderate

doses of caffeine (up to 400 mg/day) [95], Australian regulations only require a product to declare that it contains caffeine [97]. Of the 27 products which were found to contain caffeine, the content was declared on the labels of 14, of which 12 were listed on the ARTG, fulfilling the labelling requirements. For samples on which this declaration was made or when caffeine was expected due to the ingredients, caffeine was not quantified in this study. Sample 243 contained a small amount of caffeine (0.55 mg/g) which could not be explained by the listed ingredients, indicting possible contamination.

Occurring naturally, Ephedrine and its related alkaloids are produced by plants in the *Ephedra* genus, also known as *Ma Huang* [98], which has a long history of traditional use. Recently, it has become more likely to be abused due to its effectiveness in weight loss and performance enhancement, and its stimulant effects. Adverse effects from taking herbal medicines which contain ephedrine are well documented and include cardiovascular and cerebrovascular complications [99]. This has led to stricter regulations for ephedrine in most countries, including a 2004 ban in the USA [35]. Ephedrine taken in combination with other stimulant drugs, such as caffeine or synephrine, is another potential risk to consumers [96], although, in this study, no products contained this combination.

Sample 322 was positive for DNA from *Ephedra* and many other plant families and genera including Anthemideae, Astereae, Solanoideae, Acalypheae, *Linum*, Triticeae and *Medicago*. The sample indicated to 'balance and support normal male physiology and function, enhance stamina and endurance during intense physical activity and maintain the production of testosterone levels in the body', also contained DNA from the family Tribuloideae, which includes the only stated ingredient for this product, *Tribulus terrestris* extract. In Australia, *Ephedra* is restricted to prescription only, unless the total ephedrine content is less than 10

mg/kg or 0.001% [97]. With 3 mg/kg or 0.0003% of ephedrine content, Sample 322 did not exceed the regulatory limits for ephedrine, but in other countries, the detection of *Ephedra* DNA alone would make this product illegal for sale. While the concentration of ephedrine was at trace levels, the larger concern with Sample 322 is that it is listed on the ARTG, indicating that it claims to comply with safety, quality and manufacturing requirements, despite clear evidence of non-compliance with labelling and quality requirements by the contamination of other plant species and no declaration of ephedrine content.

Synephrine is another naturally occurring compound that was detected in this study, in 5% (n = 7) of samples. Structurally similar to ephedrine, synephrine occurs in many citrus species such as bitter orange (*Citrus aurantium*), and, like caffeine, has become popular in weight loss and diet assistance products. In the case of sample 44, none of the plants listed or found in the DNA screen (see Supplementary Table 9-2) are from genera known to contain synephrine and the levels that would be consumed in recommended doses of Sample 44 (6.2 mg) would be a threshold dose for pharmacological activity only. While the presence of the compound without the associated botanicals indicates was likely to have been added exogenously. Defining this addition as either contamination or adulteration with near-active concentrations is however difficult.

Sample 296B contained paracetamol and chlorpheniramine (Table 2-1), suggesting deliberate adulteration of this product. Paracetamol is a common over-the-counter analgesic, however, dosage information must be followed, as overdose will result in hepatotoxicity. In cold and flu medication, paracetamol is often combined with an antihistamine such as chlorpheniramine. While chlorpheniramine is an over-the-counter medicine in Australia, the packet must carry a sedation warning, as impairment occurs even at low doses [100]. As a first-

generation antihistamine, the most common side effects are drowsiness, sedation, fatigue, coordination disturbances and impaired memory [101]. Stated to be 'suitable for people with high cholesterol levels, skin problems, simple obesity and for improvement of general health', there appears to be no obvious reason for Sample 296B to contain this combination. Consumed in recommended doses, the intake of paracetamol and chlorpheniramine (0.32 and 0.0034 mg, respectively) would be at or below the threshold for pharmacological activity, yet the presence of these synthetic drugs, even at sub-therapeutic amounts, again raises concerns about quality and manufacturing conditions.

Trace amounts of buclizine and mycophenolic acid were detected in Samples 86 and 162, respectively, and were most likely contaminants from manufacturing. Both products were listed on the ARTG, and the presence of these drugs implies failings in manufacturing standards. Buclizine is a first-generation antihistamine that has been superseded by more successful drugs of its class. To the best of the authors' knowledge, buclizine is not currently used in Australia, Europe or the USA, and is only available in the UK in combination with paracetamol and codeine (brand name Migraleve Pink) for treatment of migraine. The detection of trace amounts (0.2 μg/g) of this drug in Sample 86, a product purportedly manufactured in Australia and marketed for 'increased energy, stress resistance, improved immunity, sexual function and healthy heart' is of some concern. Mycophenolic acid, detected in trace amounts (below LOQ and not quantified) in Sample 162, is a prescription-only immunosuppressant medicine. Once again, this drug should not have been found in any concentration in this product.

The use of routine screening methods did limit the findings of this study. Samples were only analysed using positive ionisation and only therapeutically relevant LOQ values are achieved.

Despite these limited restrictions from the use of routine methods in this study, if safety and quality control measures of CAM products were sufficient, this study would report zero cases of undeclared pharmaceuticals in the ARTG listed products. Instead, clear cases of adulteration and the presence of pharmaceuticals of indeterminate (and often inexplicable) origin were demonstrated in both listed and non-listed products.

Conclusion:

Of the samples analysed in this study, 63% were listed on the ARTG (n = 86), meaning that these products have been assessed for safety and quality under current regulation, and are only allowed to contain approved ingredients [97]. Those samples which were not listed with the TGA were purchased online, exempt from regulation (e.g. some homoeopathic products) or should not have been able to be purchased on the Australian market. The fact that the authors could purchase these products suggests a failure in regulatory control, demonstrating a clear need to strengthen the regulation of these products to improve consumer safety. While a worthy effort, increasing post-market testing will not keep non-compliant and potentially dangerous products from being sold in the first place. This study has shown that contaminated and adulterated products are not minor, niche-market remedies, but are sold in some of the biggest health food stores and pharmacies in Australia. Pre-market evaluation using the methods described here and in our previous studies [20,89] would provide an audit toolkit that could better prevent contaminated and adulterated products from reaching the consumer.

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Author Contributions:

E.C., G.M., R.T, I.M., M.L.C., C.H., R.F., R.W.B, I.F.M., and M.B. contributed to the design of experiments in this study. M.L.C., C.H., R.F., C.N., M.W.P.P., conducted the experiments. E.C., M.L.C., C.H., R.F., C.N., M.W.P.P., analysed the experimental data. EC wrote the manuscript. All authors contributed to the editing and approved the final copy of the manuscript.

3. Exploring the application of the DSA-TOF, a direct, high resolution time-of-flight mass spectrometry technique for the screening of potential adulterated and contaminated herbal medicines

Crighton E, Weisenseel J, Bunce M, Musgrave IF, Trengove R, Maker G. Exploring the Application of the DSA-TOF, a Direct, High-resolution Time-of-Flight Mass Spectrometry Technique for the Screening of Potential Adulterated and Contaminated Herbal Medicines. *J Am Soc Mass Spectrom* **30**, 1713-1719, doi:10.1007/s13361-019-02256-w (2019).

Link:

Following on from chapter 2, where contamination and adulterated products were found to be present in products on the Australian market, this study and Chapter 4 present the development and application of a rapid mass spectrometry screening method for herbal medicines to complement the methods detailed in Chapter 2 by expanding to an untargeted profiling analysis.

Abstract

Global consumption of complementary and alternative medicines, including herbal medicines, has increased substantially, and recent reports of adulteration demonstrate the need for high throughput and extensive pharmacovigilance to ensure product safety and quality. Three different standard reference materials and five previously analysed herbal medicines have been used as a proof of concept for the application of adulteration/contamination screening using a DSA ion source with TOF MS on the Perkin Elmer AxION 2 TOF. This technique offers the advantages of minimum sample preparation, rapid analysis and mass accuracies of 5 ppm. The DSA TOF analysis correlates well with the previous analysis on the initial sample set (which found undeclared herbal ingredients), with the added advantage of detecting previously untargeted compounds, including species-specific flavonoids and alkaloids. The rapid analysis using the DSA-TOF facilitates screening for hundreds of compounds in minutes with minimal sample preparation, generating a comprehensive profile for each sample.

Keywords

Herbal medicines, pharmacovigilance, adulteration, contamination, direct mass spectrometry

Introduction

With the increasing use of complementary and alternative medicines (CAMs), the quality and safety of such products have been called into question. The 2017 global market for herbal medicines alone is currently estimated to be US$107 billion [29], and expected to reach US$115 billion by 2020 [17]. The reasons behind this increased use are numerous, but the popular belief

that CAM products are 'natural' and therefore less harmful, with fewer side effects than conventional pharmaceuticals, has been suggested as a key reason for the observed growth [17,102,103]. There is now considerable data to challenge this assumption of safety, and CAM are estimated to cause 23,000 emergency department visits per year in the USA [13]. Adulteration and contamination with pharmaceuticals, heavy metals and lower quality ingredients are not uncommon and should be a serious safety concern for consumers [20].

Despite these concerns, regulation of CAM is often trust-based, so there is a pressing need for new tools to rapidly assess the safety of herbal CAM both pre- and post-market. Rather than using a multi-target analysis, the screening and detection of pharmaceutical adulterants in complex herbal matrices have mostly been focused on specific pharmaceuticals being added to herbal products with similar indications [20,44] (e.g., the adulteration of male sexual health products with sildenafil [39]). This focused screening can miss contamination of products with pharmaceuticals of unrelated indications [20], drug isomers or novel compounds [60,61,104]. The data [20,44,60,61,104] gathered to date clearly demonstrates the need for a rapid, multi-target or untargeted screening method. Until such a method is developed and a comprehensive screen of products on the market is undertaken, the scope of adulteration and contamination of herbal CAM products will remain unquantified.

In this study we explore the use of a rapid, high-throughput screen for pharmaceutical adulterants using the AxION 2 Direct Sample Analysis-Time of Flight (DSA-TOF) mass spectrometer. This analysis needs minimal sample preparation and employs rapid, ambient ionization mass spectrometry via the DSA source coupled to a TOF for high resolution spectral data [105]. This allows for tentative identification of possible adulterated or contaminated products through accurate mass and isotopic pattern matching without the requirement for

separation by chromatography. The resulting matches can then be rated in confidence, with subsequent confirmation and quantification of positive results by Liquid Chromatography-Mass Spectrometry (LC-MS), such as employed in our previous work [20] and that by Bogusz*, et al.* [44]. The benefits of this two-stage approach are that the more complex and time-consuming confirmatory analysis has a smaller sample load, the stratified scoring system allows for prioritising of samples, and the analysis is carried out over two platforms, giving greater confidence in the results. It was the aim of the study to determine if the DSA-TOF could be appropriate as a rapid screening tool for CAM products. We used NIST standard reference materials (SRMs) and five previously analysed CAM products as a proof of concept for testing the efficacy of DSA-TOF to detect adulteration and contamination in herbal CAMs and provide a rapid method for pharmacovigilance.

Methods

Materials

Drug standards amoxicillin, aspirin, brucine, caffeine, chlorpheniramine, cyproheptadine, dexamethasone, diphenhydramine, ephedrine, ibuprofen, lignocaine, mianserin, paracetamol, prednisolone, ranitidine, salicylic acid, sildenafil, simvastatin, strychnine, tadalafil and warfarin were purchased from Sigma-Aldrich (Sydney, Australia) in the highest purity available. Codeine, diazepam, digoxin, sibutramine, streptomycin, tamoxifen, vardenafil, verapamil, yohimbine and the internal standard diazepam-D5 were purchased from Novachem (Melbourne, Australia) in the highest purity available. LC-MS grade methanol and water were purchased from Fisher Scientific (Hampton, USA).

The NIST standard reference materials (SRM) used were *Camellia sinensis* (green tea) powder (SRM 3254), multivitamin tablets (SRM 3280) and *Ginkgo*-containing powder (SRM 3248). The

five CAM products (tablets, capsules and tea) were purchased in Australia in 2014 from pharmacies, health food stores and traditional herbal retailers. These products had been previously analysed [89] for heavy metal content, pharmaceutical adulteration and undeclared plant content via next generation DNA sequencing. The samples were indicated for various uses such as anxiety and depression, blood circulation, stress, 'energy', 'brain function', 'mental clarity' and 'mood'.

Sample preparation

The NIST SRM and CAM samples were ground to a fine homogenised powder using a Precellys lysing kit consisting of CKMix 50-R 2 mL tubes (Bertin Technologies, France). The sample extraction was based on our previous work [20]. The powdered samples were transferred to 2 mL lysis tubes and stored at -80°C until use. For methanol extraction, approximately 5 mg of each sample was weighed out in triplicate 2 mL lysis tubes. 1 mL of methanol was added, and samples were then shaken for 80 s at 6500 rpm in a Precellys tissue lyser (Bertin Technologies, France). After shaking, the extracts were centrifuged for 15 min at 13.2 x 10^4 x *g*. The supernatant was transferred to fresh 1.5 mL tubes and dried by evaporation using an Eppendorf Concentrator Plus rotary vacuum concentrator (Eppendorf South Pacific Pty. Ltd., Sydney, Australia). Dry extracts were stored at -80°C until use. For analysis, 1 mL of 10% methanol with 0.1% formic acid was added to the dried extracts and the samples shaken for 10 min. 10 µL of reconstituted sample was then spotted for analysis.

A standard mix of 30 drugs from various pharmaceutical classes (listed in 2.1 above) was used as a positive control and run in triplicate at the beginning of each day. The concentration of each compound was 100 ng/ml with a mass range of 138 to 780 Da.

DSA-TOF analysis

The DSA is a combined ambient ionisation source and sampling platform previously described in Botch-Jones*, et al.* [106] and Winter*, et al.* [105]. DSA-TOF (PerkinElmer, Waltham, USA) conditions were as follows: positive acquisition mode, 350°C probe temperature, 60 psi nebuliser gas (N_2) pressure, 4 L/min auxiliary gas flow and drying gas at 3 L/min and 25°C. The corona current was 4 µA, scan rate was 10 spectra/s, capillary entrance was set to 800 V, endplate heater was 200 V, and the capillary exit was 100 V. The total method run time was 0.55 min., with a sample spot time of 30 s. The Agilent APCI-L low concentration tuning mix (Agilent Technologies, Santa Clara, USA) was used to calibrate the DSA-TOF before analysis each day and was also used as the internal 'lock-mass' calibrant during sample acquisition.

Data analysis

Spectral data were viewed and analysed using AxION SOLO software, and R. Raw spectra were scanned by AxION SOLO for matches to exact monoisotopic masses and isotope ratios of targeted compounds based on compound formula. Over 350 pharmaceuticals, analogues and phytochemicals were screened against an in-house library in this first step (Supplementary Table 9-3). After initial analysis in SOLO, the individual spectra of each sample were processed and analysed in R using locally developed code (see appendix for code). The enviPat R package [107] (v2.2) was used to compile the isotopic patterns of the targeted compounds to compare with the mass spectral data.

Results

Standard compound mix

Of the 30 pharmaceuticals in the standard mix, 21 were consistently detected (Figure 3-1): brucine, caffeine, chlorpheniramine, codeine, cyproheptadine, diazepam, diphenhydramine, ephedrine, lignocaine, mianserin, paracetamol, ranitidine, sibutramine, sildenafil, strychnine, tadalafil, tamoxifen, vardenafil, verapamil, warfarin, and yohimbine. Compounds not detected were: amoxicillin, aspirin, dexamethasone, digoxin, ibuprofen, prednisolone, salicylic acid; simvastatin and streptomycin; reasons for these non-detections are discussed below.

NIST standard reference materials

Table 3-1 shows the data from DSA-TOF analysis of the three NIST SRMs: *Camellia sinensis* (green tea) powder, multivitamin tablet and ginkgo powder. The certified composition is compared to that detected by the analysis.

Camellia sinensis (green tea) powder

The expected content of the SRM was caffeine, catechin, epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, gallic acid, gallocatechin, gallocatechin gallate, L-theanine and theobromine. Caffeine, catechin/epicatechin, and epigallocatechin/ gallocatechin were all detected with errors less than 5 ppm for the respective molecular ions [M+H]+. The diastereoisomers catechin/epicatechin and epigallocatechin/gallocatechin cannot be distinguished using this method, so are referred to collectively. Gallic acid and theanine were also putatively identified, but with higher errors of 10.52 and 9.71 ppm, respectively. Kaempferol and quercetin were also detected, but are not listed on the certificate of analysis for this NIST standard. These compounds, however, are known to occur in green tea, and several other plants [108]. Theobromine was also detected, but with a high

ppm error (>10 ppm). The gallic acid esters, epicatechin gallate, epigallocatechin gallate and gallocatechin gallate were not detected.

Multivitamin tablet

The NIST multivitamin tablet was certified to contain the following vitamins and carotenoids: α-tocopherol, ascorbic acid, biotin, *cis*-β-carotene, cyanocobalamin, ergocalciferol, folic acid, lutein, nicotinamide, pantothenic acid, phylloquinone, pyridoxine hydrochloride, retinol, riboflavin, thiamine hydrochloride and *trans*-β-carotene. In the DSA analysis, pantothenic acid and pyridoxine were identified with 4.69 and 4.51 ppm error, respectively. Nicotinamide, thiamine and riboflavin were also identified, but had errors >10 ppm. None of the fat-soluble vitamins were detected, however these compounds were not expected to be extracted or detected using the methodologies employed here.

Ginkgo powder

The ginkgo powder was listed to contain bilobalide, ginkgolides A, B, C and J, isorhamnetin, kaempferol and quercetin. Isorhamnetin (5.05 ppm error), kaempferol (1.98 ppm error) and quercetin (5.17 ppm error) were all detected by the DSA-TOF analysis. However, none of the terpene lactones (ginkgolides and bilobalide) were identified. There was also an alkaloid-like compound that was detected, but not definitively identified.

CAM products

Table 3-2 shows the data from the DSA-TOF analysis compared with a previous investigation using next generation DNA sequencing (NGS) and LC-MS [89]. The previous analysis of the samples had shown no contamination or adulteration with heavy metals or pharmaceuticals. Due to the processing of the products, which are mostly extracts, DNA material was not expected to be recovered in 3 of the 5 samples. These *Ginkgo biloba* products, samples 92

and 157, showed the same contents as the ginkgo SRM with isorhamnetin, kaempferol and quercetin all detected. The Skullcap tea, sample 175, also contained isorhamnetin, kaempferol and the flavonoid wogonin. All the compounds in samples 92, 157 and 175 had errors ≤ 5 ppm.

The ingredients listed for sample 73 were vitamins ascorbic acid, biotin, calcium pantothenate, cyanocobalamin, nicotinamide, pyridoxine hydrochloride, riboflavin sodium phosphate, thiamine hydrochloride, *Eleutherococcus senticosus* (Siberian Ginseng) and *Paullinia cupana* (Guarana). Caffeine was detected in both analyses of sample 73; nicotinamide, pantothenic acid, pyridoxine and trigonelline were also detected. All the compounds detected in sample 73 had errors < 5 ppm.

The multivitamin, sample 100, was listed to contain several B vitamins including calcium pantothenate, nicotinamide and pyridoxine hydrochloride, along with *Rhodiola rosea*, calcium hydrogen phosphate, choline bitartrate, chromium picolinate, inositol, potassium sulphate and zinc sulphate monohydrate. Similar to the SRM multivitamin, only nicotinamide, pantothenic acid and pyridoxine were detected, with errors of approximately 9 ppm.

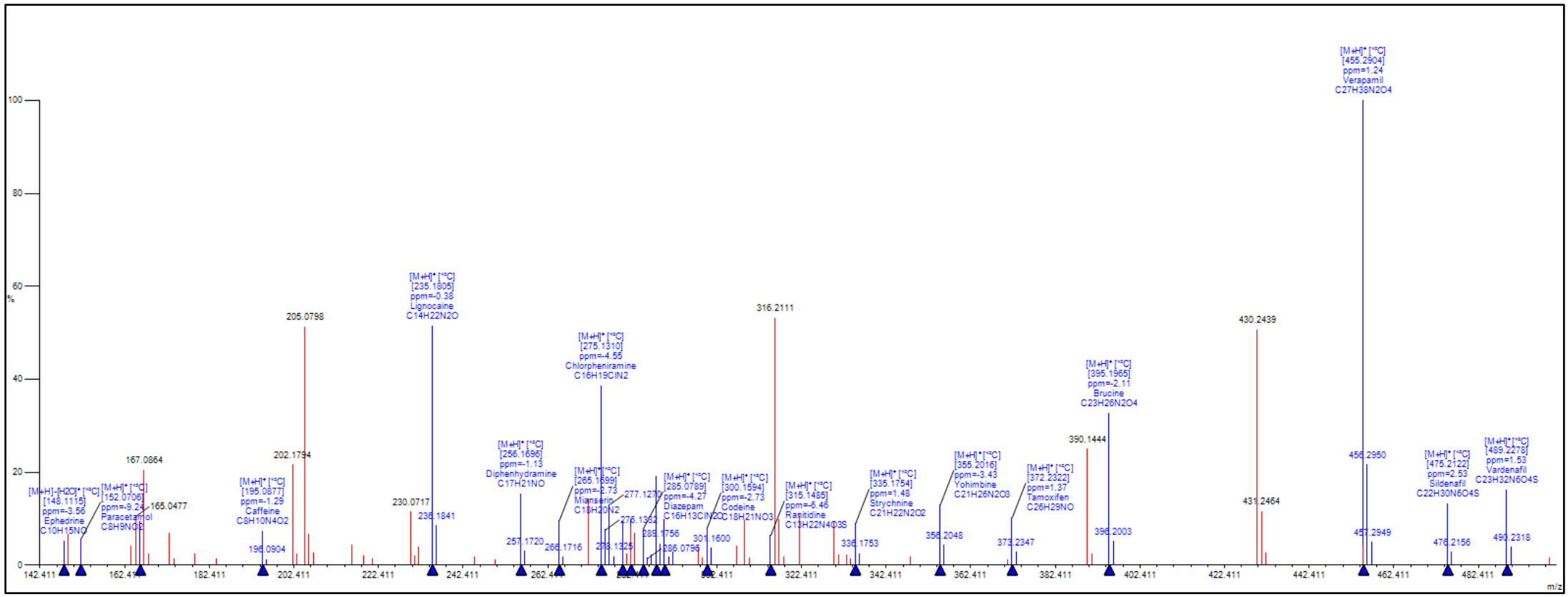

Figure 3-1: DSA spectra and ppm error for 18 of 21 detected pharmaceutical standards. From left to right: Ephedrine, paracetamol, caffeine, lignocaine, diphenhydramine, mianserin, chlorpheniramine, diazepam, codeine, rantidine, strychnine, yohimbine, tamoxifen, brucine, verapamil, sildenafil, vardenafil. Not highlighted in this spectra are cyproheptadine, sibutramine, tadalafil, and warfarin**.**

Table 3-1: Data from DSA-TOF analysis of the three NIST SRMs. Ingredients detected by the DSA analysis are listed in bold. # Indicates compounds that were detected with error rates above 10 ppm.

NIST #	Certified composition (vitamin)	DSA ID (error)	Comment
1 *Camellia sinensis* (green tea) powder	**Caffeine** **Catechin** **Epicatechin** Epicatechin gallate **Epigallocatechin** Epigallocatechin gallate **Gallic acid** **Gallocatechin** Gallocatechin gallate **Theanine** Theobromine	Caffeine (4.96 ppm) Catechin /Epicatechin (2.18 ppm) Epigallocatechin/Gallocatechin (1.63 ppm) Gallic acid (10.52 ppm) Kaempferol (2.32 ppm) Quercetin (1.10 ppm) Theanine (9.71 ppm) Theobromine#	
2 Multivitamin tablets	Ascorbic acid (C) Biotin (B7) Folic acid (B9) Nicotinamide (B3) **Pantothenic acid (B5)** **Pyridoxine hydrochloride (B6)** Riboflavin (B2) Thiamine hydrochloride (B1)	Pantothenic acid (4.69 ppm) Pyridoxine (4.51 ppm) Nicotinamide, Thiamine, Riboflavin #	Alpha-Tocopherol, beta-carotene, cyanocobalamin, ergocalciferol, lutein, phylloquinone and retinol are also a part of the SRM composition. However, these compounds were either gel encapsulated fat-soluble vitamins or outside of the mass range and were not expected to be extracted or detected with this method.
3 Ginkgo powder	Bilobalide Ginkgolides A, B, C and J **Isorhamnetin** **Kaempferol** **Quercetin**	Isorhamnetin (5.05 ppm) Kaempferol (1.98 ppm) Quercetin (5.17 ppm)	Catecholamine and alkaloid-like compounds were detected but not definitively identified.

Table 3-2: Comparison of analytes detected in five CAM samples using DSA-TOF analysis against that found using a combination of LC-MS and next generation DNA sequencing. Ingredients and previously detected compounds and DNA which agree with the DSA analysis are listed in bold. * Indicates that catecholamine and alkaloid-like compounds were detected but not definitively identified.

CAM #	Ingredients (vitamin)	Previous analysis	Comment on previous analysis	DSA ID (error)
73	Ascorbic acid (C) Biotin (B7) **Calcium pantothenate (B5)** Cyanocobalamin (B12) *Eleutherococcus senticosus* **Nicotinamide (B3)** *Paullinia cupana* **Pyridoxine hydrochloride (B6)** Riboflavin sodium phosphate (B2) Thiamine hydrochloride (B1)	**Caffeine** *Homo sapien* DNA *Rutaceae sp.* (citrus) DNA	Caffeine expected based on ingredient list (***Paullinia cupana)***	**Caffeine (2.73 ppm)** Nicotinamide (4.06 ppm) Pantothenic acid (3.33 ppm) Pyridoxine (2.55 ppm) Trigonelline (3.38 ppm)
92	***Ginkgo biloba***	Nothing detected	DNA detections not expected due to use of extracted ingredients.	Isorhamnetin (1.79 ppm) Kaempferol (5.11 ppm) Quercetin (3.08 ppm)*

CAM #	Ingredients (vitamin)	Previous analysis	Comment on previous analysis	DSA ID (error)
100	Biotin (B7) Calcium hydrogen phosphate **Calcium pantothenate (B5)** Choline bitartrate Chromium picolinate Cyanocobalamin (B12) Folic acid (B9) Inositol **Nicotinamide (B3)** Potassium sulphate **Pyridoxine hydrochloride (B6)** *Rhodiola rosea* Riboflavin (B2) Thiamine nitrate (B1) Zinc sulphate monohydrate	Nothing detected	DNA detections not expected due to use of extracted ingredients.	Nicotinamide (9.75 ppm) Pantothenic acid (9.54 ppm) Pyridoxine (9.02 ppm)
157	***Ginkgo biloba* extract**	Nothing detected	DNA detections not expected due to use of extracted ingredients.	Kaempferol (3.37 ppm) Quercetin (2.64 ppm) Isorhamnetin (1.05 ppm)*
175	**Organic Skullcap tea**	DNA detected: *Canis lupus familiaris* *Homo sapiens* Poeae (*PACMAD clade)* ***Scutellaria* sp.**	Undeclared herbal ingredient (grass species) and animal/human contamination.	Isorhamnetin (2.63 ppm) Kaempferol (2.56 ppm) **Wogonin (1.17 ppm)**

Discussion

This paper aimed to assess the appropriateness of the DSA-TOF as a rapid, high-throughput screening method for herbal medicines/CAMs. A high-throughput approach has been advocated by the authors [19] to aid the implementation of a more thorough approach to pre- and post-market auditing and overall regulation of herbal CAMs. Until such a method is developed and a comprehensive screen of products on the market is undertaken, we will not have a clear picture of the scope of contamination and adulteration of herbal CAM products. The obvious benefits of the DSA-TOF method are rapid analysis and minimal sample preparation, as seen in this study where simple methanol extracts were each analysed in less than 1 min.

Another benefit of this method is the collection of untargeted data; with the method only becoming 'targeted' when a specific library is applied during data analysis. In this study, the target database consisted of over 300 pharmaceuticals, vitamins, phytochemicals and analogues of these compounds. This broad coverage, coupled with the ability for retrospective analysis beyond the original target database, is a substantial benefit of the DSA-TOF analysis. In some cases, the DSA-TOF was unable to detect certain standards: amoxicillin, aspirin, dexamethasone, digoxin, ibuprofen, prednisolone, salicylic acid, simvastatin and streptomycin (see Supplementary Table 9-4 for predicted detections). As the analysis was only carried out in positive ion mode, detection of aspirin, ibuprofen and salicylic acid was not expected, as these compounds are typically analysed in negative ion mode [41]. As the DSA is a modified APCI source [105], the inability to detect amoxicillin, dexamethasone, prednisolone and streptomycin was possibly due to the low volatility or high polarity of these compounds. However, this method still has the potential to give a good indication of what a product does

or does not contain with 70% of the target pharmaceutical standards detected with less than a 1-min analysis. With the use of negative mode, the coverage of this method could be improved further. More crucially the common adulterants sibutramine, sildenafil and tadalafil can be detected. In the first 6 months of 2018, these three drugs have been responsible for 14 regulator health alerts in Australia after being detected in counterfeit products [109].

The absence of chromatographic separation reduces the level of assurance and confidence in the identification of compounds. Analysis of the multivitamin SRM detected only 25% of the contents within an acceptable ppm range (Figure 3-2), highlighting the challenges posed by ion suppression or saturation, due to the wide dynamic range of the complex sample matrices and analyte concentrations. For example, the apparent detection of paracetamol in the multivitamin SRM was based on a false positive match to the $[M+H-H_2O]^+$ fragment of pyridoxine (Figure 3-2). Isobaric compounds can also not be distinguished between using this method. Accurate mass was the primary basis of identification for this study. As discussed by De Vijlder*, et al.* [110], the accuracy of an the isotope pattern is dependent on instrument type and resolving power, the mass of the ion of interest and potential inference of chemical and noise in the spectra. Subsequently, isotopic patterns have not been used as a primary basis for identification, instead the calculated pattern of the suspected compound was used to help rule out possible matches or to substantiate the potential identification.

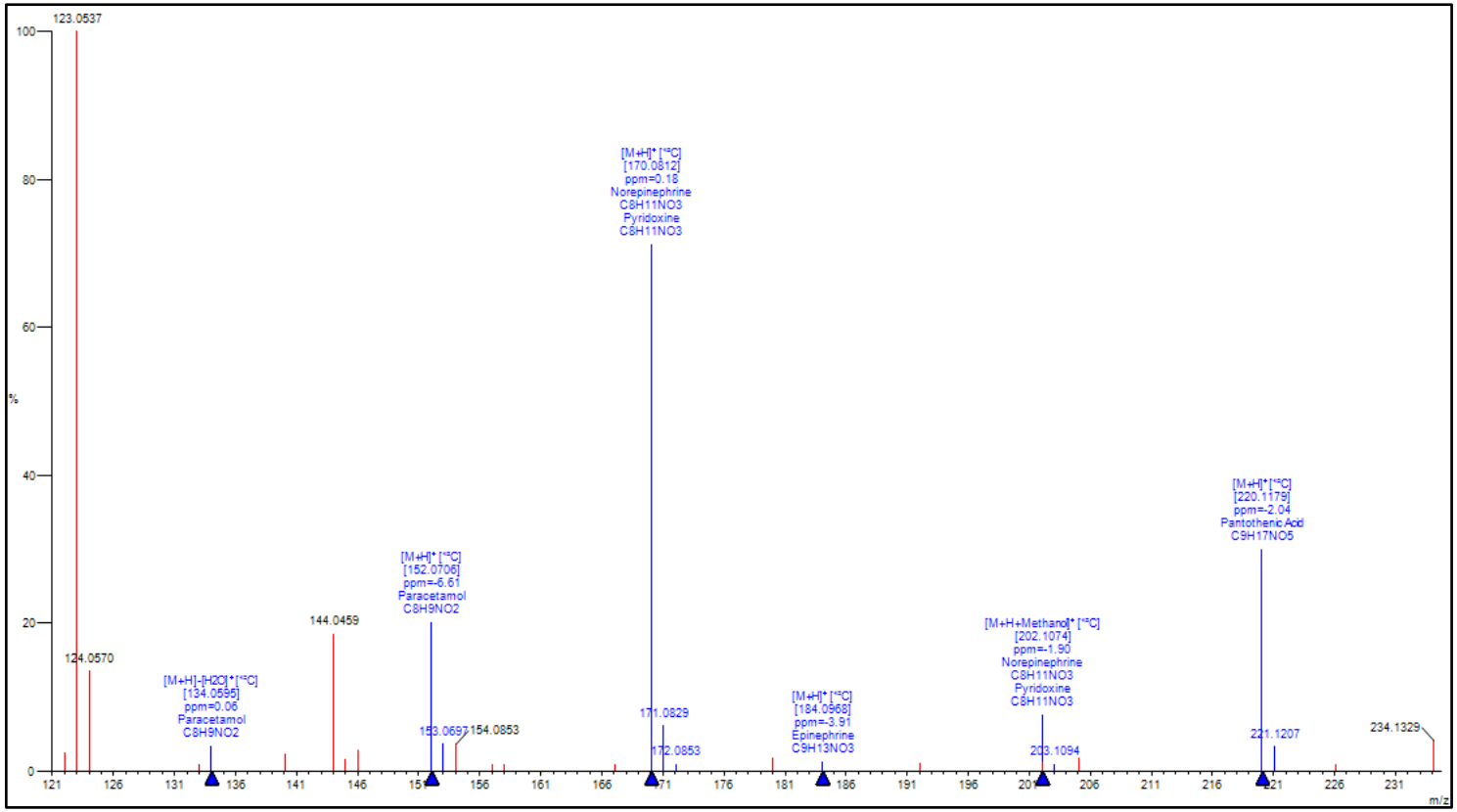

Figure 3-2: DSA spectra of the NIST multivitamin SRM. The false positive match for paracetamol, $[M+H]^+$ 152.0706 m/z, is based on the $[M+H - H_2O]^+$ fragment of pyridoxine (also labelled as norepinephrine). The $[M+H + Methanol]^+$ adduct of pyridoxine and $[M+H]^+$ of pantothenic acid are also highlighted.

Overall, analysis of the CAM samples confirmed the samples' listed ingredients. For example, B vitamins listed as ingredients in Sample 100 were detected by this analysis. The B vitamins and the alkaloid trigonelline found in sample 73 were also consistent with both listed ingredients and the DNA data. The DSA analysis correlated well with the previous toxicological LC-MS screen, for example, caffeine in sample 73 was detected in both the previous and current analyses.

It is important to highlight that this method does not intend to confirm the presence/absence or quantity of compounds within these products, but rather to serve as a rapid screening tool for pre- and post-market monitoring of herbal products, to give a preliminary snapshot of the composition. For example, the flavonoid wogonin is known to occur specifically in *Scutellaria baicalensis* [111], and its detection is in agreement with the DNA analysis of sample 175, which showed the product contained *Scutellaria* sp., as well as undeclared grass species and human and dog DNA contamination. We continue to advocate that a combination of mass spectrometry compound detection coupled with DNA analysis provides key insights into ingredients, substitutions and adulterations.

The analogous results between the *Ginkgo biloba* samples and the NIST Ginkgo powder further demonstrate the potential for the DSA-TOF technique. Both the samples and the NIST standard contained isorhamnetin, kaempferol and quercetin, as well as other catecholamine and alkaloid-like compounds. While no specific Ginkgo phytochemicals were detected, the detection of other phytochemicals does suggest that there was plant material in the products. As no DNA material was detected and the previous analysis did not screen for such phytochemicals, the DSA results are the first to support the products' claim of plant-based contents.

The correlation between the previous LC-MS and DNA analyses and the current DSA-TOF analysis, as well as the detection of non-specific plant alkaloids and flavonoids, gives strength to this rapid analysis for its ability to profile relevant phytochemicals and possible undeclared ingredients for quality control purposes. While there are limitations to this approach, DSA analysis allows screening of hundreds of compounds with minimal sample preparation, generating an outline profile of a product's composition. For products where DNA cannot be extracted, or where interest in the composition is beyond a standard toxicological screen, such analysis can aid in a zero-tolerance approach to contaminated and adulterated products. Given that many regulatory agencies face the challenge of limited resources, with thousands of samples to be screened, but only a proportion needing comprehensive confirmation and quantification of possible adulterants and contaminants, DSA analysis has the potential to substantially improve efficiency.

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Author Contributions

E.C., G.M., J.W. and R.T. contributed to the design of experiments in this study. E.C. and J.W. conducted the experiments. E.C. analysed the experimental data. E.C., G.M., R.T., J.W., I.F.M. and M.B. contributed to the preparation and writing of the manuscript. M.B., I.F.M., E.C., G.M. and R.T. contributed to the previous study.

4. Direct, high-resolution time-of-flight mass spectrometry analysis provides additional data for adulterated and contaminated herbal medicines and supplements previously analysed by LC-MS.

Abstract

Complementary and alternative medicines continue to grow in popularity in both Australia and overseas markets. The safety and quality of such products has been repeatedly questioned in numerous studies. In this study, samples previously analysed by LC-MS have been reanalysed using the direct sample analysis-time of flight mass spectrometry method previously described in this thesis. The new screening method offers minimum sample preparation and rapid analysis time, along with an untargeted approach to compound ID. Using the data from the new method combined with the previous results, 40% of samples were able to have at least one of the ingredients listed on the product packaging corroborated. However, a further 25% of products now require further investigation to confirm the contents. Overall, nearly 70% of products tested now have potential adulteration or contamination concerns, compared to 50% identified from the previous analysis.

Introduction

Consumers are becoming more health conscious, with a substantial rise in 'wellness' trends and interest in 'natural' therapies, including complementary and alternative medicines (CAM). This increased use of CAM products has emphasised the current state of regulatory standards in many countries, finding them to be less strict with the assumption that the use of CAM products is 'low-risk' [112,113]. CAM products are very popular for Australian and overseas consumers [87,113], yet a large proportion of these products have been found to be non-compliant with the current regulations [21,29,114,115].

With many CAM products claiming efficacy for weight loss or weight/metabolism/diet support along with heart health, they appear attractive to the consumer who is looking to improve their overall health. In our recent study using a multi-tiered approach to pharmacovigilance with a combination of DNA metabarcoding and small molecule analysis by mass spectrometry, 135 CAM products (tablets, capsules, gel capsules, tea, chewable tablets and gummies, honey, liquids, and powder) indicated for cardiovascular health and diet issues, including weight loss, gastrointestinal health and metabolism support, were investigated for quality control issues and pharmaceutical adulteration. The study found that nearly 50% of products tested had contamination issues, in terms of DNA, chemical composition or both. Pharmaceutical adulteration and undeclared stimulants were detected in 5%, and only 21% of the tested products were able to have at least one ingredient corroborated by DNA sequencing [21]. Consumers are, therefore, at risk of exposure to fraudulent, adulterated and contaminated products.

All of this leads to the question that, if the label of a product does not match the contents, what is actually in the product? As discussed in Chapter 3 [116], while useful as a confirmatory

method, LC-MS can be time-consuming. Detection of unknown drug analogues and novel compounds is also difficult using targeted methods. One approach which has the potential to better determine the composition of CAM products would be a rapid untargeted method. Generating a 'compositional snapshot' of a product would allow screening for pharmaceuticals, vitamins, phytochemicals and analogues of these compounds. Using the direct sample analysis-time of flight (DSA-TOF) mass spectrometer method developed previously [116], a subset of the original 137 samples (n = 102) were re-analysed, including a number (n = 11) which were unable to be analysed with the original method due to sample type.

Methods

Samples

Full samples details can be found in Chapter 2 [21]. In summary, samples (n = 137) were purchased from various retailers and online in Australian capital cities from 2014 to 2017 and allocated random identification numbers. The samples existed in a variety of formulations (including tablets, capsules, tea, liquids and honey) and were from various origins such as traditional Chinese medicine (TCM), herbal medicines and supplements. Due to time constraints on the analysis, only a subset (n = 102) of the original 137 samples were able to be analysed.

Sample preparation

Sample preparation, extraction and analysis were the same as previously reported in Chapter 3 [116]. Briefly, samples were ground to a fine homogenised powder using a Precellys lysing kit consisting of CKMix 50-R 2 mL tubes (Bertin Technologies, France) and 5 mg was extracted in triplicate with 1 mL of methanol. Samples were then shaken for 80 s at 6500 rpm. After

shaking, the extracts were centrifuged for 15 min at 13.2 x 10^4 x *g*. The supernatant was transferred to fresh 1.5 mL tubes and dried by evaporation using an Eppendorf Concentrator Plus rotary vacuum concentrator (Eppendorf South Pacific Pty. Ltd., Sydney, Australia). Dry extracts were stored at -80°C until use. For analysis, 1 mL of 10% methanol with 0.1% formic acid was added to the dried extracts and the samples shaken for 10 min. 10 µL of the reconstituted sample was then spotted for analysis.

DSA-TOF analysis

DSA-TOF (PerkinElmer, Waltham, USA) conditions were as follows: positive acquisition mode, 350°C probe temperature, 60 psi nebuliser gas (N_2) pressure, 4 L/min auxiliary gas flow and drying gas at 3 L/min and 25°C. The corona current was 4 µA, the scan rate was 10 spectra/s, the capillary entrance was set to 800 V, endplate heater was 200 V, and the capillary exit was 100 V. The total method run time was 0.55 min., with a sample spot time of 30 s. The Agilent APCI-L low concentration tuning mix (Agilent Technologies, Santa Clara, USA) was used to calibrate the DSA-TOF before analysis each day and was also used as the internal 'lock-mass' calibrant during sample acquisition.

Data analysis

Spectral data were viewed and analysed using AxION SOLO software, and R. Over 350 pharmaceuticals, analogues and phytochemicals were screened against an in-house library (see Supplementary Table 9-3). Raw spectra were scanned by AxION SOLO for matches to exact monoisotopic masses and isotope ratios of targeted compounds based on the compound formula. The individual spectra of each sample were then processed and analysed in R using locally developed code (see appendix for code).

Results and discussion

Figure 4-1 presents a comparison between the results for the original study (n=137) [21], and the cumulative results of both the original and the new DSA-TOF analysis (n=102). Detailed sample results can be found in Supplementary Table 9-5.

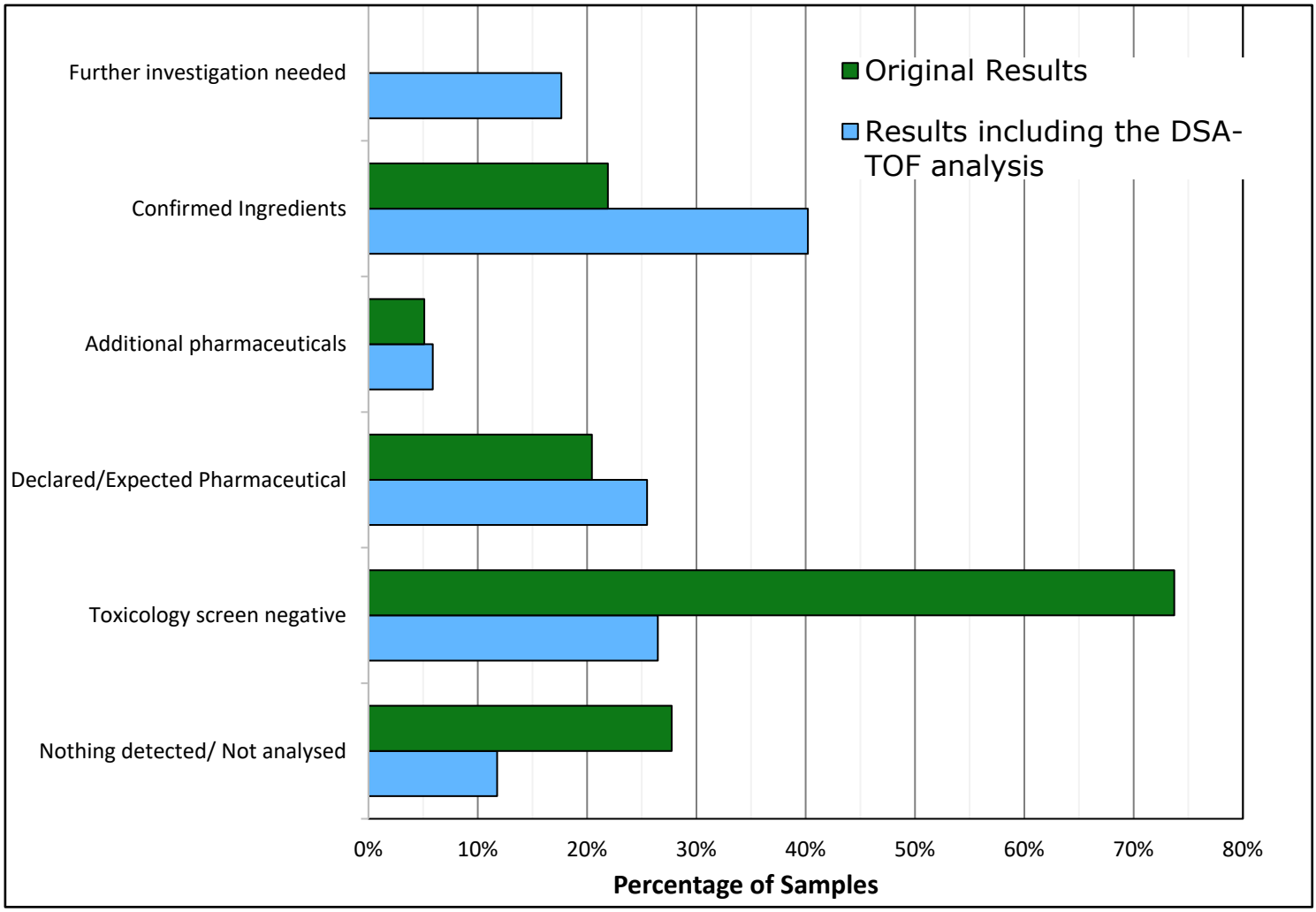

Figure 4-1: Evaluation of adulteration, contamination and presence of undeclared ingredients in CAM samples. Green is data from Chapter 2 [21] only, while blue includes data from both the original [21] and DSA-TOF analyses combined.

Overall, the data from the DSA-TOF analysis is in keeping with the previous findings in Chapter 2, offering initial validation to the rapid analysis method, corroborating the results of adulterated products previously found. For example, paracetamol and chlorpheniramine

were detected in sample 296B in the original study, and the DSA-TOF analysis detected both paracetamol and chlorpheniramine, along with the new preliminary result of ephedrine (7.02 ppm error). The detected of ephedrine now necessities further investigation into sample 296, continuing from the original LC-MS analysis in Chapter 2.

Caffeine was the most common compound detected in both studies, found in 19% (n = 26) of samples in the original study and 18% (n = 18) of samples in the rapid analysis. Synephrine was another common detection, but there was disagreement in the detection of synephrine between the two analyses. In Chapter 2, synephrine was detected in samples 24, 44, 47, 128, 224 and 227 at concentrations of 1.1 mg/g or less. Sample 323 also contained synephrine at a higher concentration of 7.4 mg/g. The DSA-TOF analysis did not detect the compound in the majority of these samples (24, 47, 128, 224 and 227). This was most likely due to the low concentrations and the detection limits in complex sample matrices [116]. In the case of sample 44, the rapid analysis matched synephrine with a high mass error (> 15 ppm). The higher concentration in sample 323 resulted in positive detection of synephrine with a mass error of 1.76 ppm. There were also cases where synephrine was detected in the rapid DSA-TOF analysis, but not found in the original study. Samples 4, 10, 59, 241, 244, 293 and 253-2 all had the compound tentatively identified, however, only samples 4 and 293 have ingredients listed that could explain the presence of synephrine. The original analysis did not detect synephrine in any of these samples, noting that samples 10 and 59 were unable to be analysed by LC-MS. The results from this study and the previous chapter [116] suggest that higher limits of detection and analyte volatility and polarity are limitations of the DSA-TOF method. Further development of the DSA-TOF method will require full validation to determine the limits of detection, matrix effects and analyte recovery.

While the original analysis focused on conventional pharmaceuticals, the DSA-TOF analysis screened for several phytochemicals, vitamins (such as pyridoxine, alpha-tocopherol, niacinamide and pantothenic acid), as well as pharmaceuticals. This extended compound screening helped confirm the presence of ingredients listed on the sample packaging for some samples by putatively identifying indicative phytochemicals. For example, in sample 218 (which could not be analysed in the previous method) with the listed ingredients of lecithin, *Piper nigrum* and ubidecarenone, piperine, an alkaloid of the *Piper nigrum* (pepper) plant, was tentatively identified with a mass error of 5.47 ppm. Samples 77, 81 and 317 all had alpha-tocopherol putatively identified by the DSA-TOF analysis, confirming the ingredients listed on the packaging. Overall, addition of the DSA-TOF data to the original data set increased the percentage of the samples tested that had at least one ingredient corroborated by either the DNA analysis or detection of an indicative phytochemical from 22% (n = 30) to 40% (n= 41) (Figure 4-1) [21].

Another notable difference between the two analyses was the increased amount of positive data generated by the rapid DSA-TOF analysis. The cause behind this shift is the reduction in negative results for the toxicological analysis, where the majority of samples (74%, n = 101) were negative in Chapter 2. Instead, only 38% (n= 39) of samples were negative when screened with the DSA-TOF. 18% (n= 18) of samples had a putative compound ID which, if correct, indicates that further investigation is needed to confirm the product's contents and if the labelled ingredients and warnings meet the current criteria. A potential limitation of the DSA-TOF analysis is that it is not a confirmatory method, with compounds only tentatively identified by accurate mass and isotopic patterns [116]. This is demonstrated by the results for samples 6, 244 and 292, which were listed as containing *Caralluma adscendens*, but the DSA-TOF analysis identified delta-9-tetrahydrocannabinol (THC) in all three samples. As the

samples were negative for THC in the original analysis, this result is likely a false positive for THC. It can be deduced that there is an interfering compound, with the same molecular weight as THC, present in samples containing *Caralluma adscendens.* The nature of this false positive needs further investigation.

Applying the rapid DSA-TOF analysis developed in Chapter 3 [116] to the broader sample set of Chapter 2 [21] has offered further clarification regarding the contents of the products. 40% of the tested samples have had labelling claims at least partially supported by the combined results of DNA analysis and the DSA-TOF analysis. However, 18% of samples now require further investigation into their contents after the DSA-TOF analysis identified a large variety of compounds not explained by the product's ingredients. When the DSA-TOF results are taken into consideration with the previous DNA and LC-MS results from Chapter 2, 71% of samples are possibly adulterated and/or contaminated. This compares to a figure of 50% based solely on the data from the original analysis. The results from this study further support our hypothesis that the contents of the products are exceedingly heterogeneous and not accurately reflected on the label. This chapter also demonstrates that new approaches are available to investigate the composition of these products and assess the risks to consumer safety. The DSA-TOF analysis has the potential to fill the gap between DNA and targeted toxicological analysis with its application of extended compound screening for unpredicted adulterants.

5. The application of metabolomics for herbal medicine pharmacovigilance: A case study on ginseng.

Crighton, E., Mullaney, I., Trengove, R., Bunce, M. & Maker, G. The application of metabolomics for herbal medicine pharmacovigilance: a case study on ginseng. *Essays In Biochemistry* **60**, 429-435, doi:10.1042/ebc20160030 (2016).

Link:

The data in the preceding chapters has demonstrated that pre-market quality control measures need to be improved. Metabolomics for pharmacovigilance is a rapidly emerging application which can contend with the complex matrix of herbal medicines. This literature review focuses on ginseng to demonstrate the possibilities of using metabolomics for quality control.

Abstract:

Herbal medicines are growing in popularity, use and commercial value; however there remain problems with the quality and consequently safety of these products. Adulterated, contaminated and fraudulent products are often found on the market, a risk compounded by the fact that these products are available to consumers with little or no medical advice. Current regulations and quality control methods are lacking in their ability to combat these serious problems. Metabolomics is a biochemical profiling tool that may help address these issues if applied to quality control of both raw ingredients and final products. Using the example of the popular herbal medicine, ginseng, this essay offers an overview of the potential use of metabolomics for quality control in herbal medicines and also highlights where more research is needed.

Introduction:

It has been observed by many authors that the use of complementary and alternative medicines (CAM) is increasing [2-5,20,29]. A major component of this rise in CAM has been an increase in the use and commercialisation of herbal medicines. For example, sales of complementary medicines in Australia are currently worth a conservative estimate of A$2 billion annually [117], while global figures for herbal medicines alone are expected to reach US$107 billion by 2017 [29]. In many countries, herbal medicines are classified under the regulatory guidelines for other CAM treatments, which are often 'lightly' regulated and largely based on 'self-assessment' by manufacturers and providers, with little to no post-market monitoring [6,118]. As summed up by Cohen*, et al.* [119], "supplements are often sold as medicine, but regulated as food". This type of regulation can result in a lower standard of quality and

efficacy assessment of the products before they are launched onto the market, due to the perceived low risk that is ascribed to the use of CAM [6,32]. This approach can lead to numerous problems for consumers, health professionals and regulatory bodies, including adulteration with active pharmaceuticals, or the substitution of active botanical ingredients with cheaper, lower quality ingredients, such as rice or wheat [5,20]. Contamination with heavy metals or pesticides is another issue which could have serious, long-term health effects [2,20,29]. Despite these concerns, herbal medicines continue to be a popular choice for consumers, with the best way to regulate this industry left as a question yet to be adequately addressed. Efficacy arguments aside, consumers deserve access to safe, quality controlled products. The problems highlighted above, which are going largely unchecked by the industry, demonstrate a compelling need to research more effective methods of quality control, both in terms of the methods utilised, and the overall system. The scope of this essay seeks to summarise the potential of metabolomics in the regulation of the herbal medicine sector. We refer readers to other review articles for descriptions of instrumentation and methodologies [120-122]

Formulations classed as 'herbal medicines' are variable, ranging from single plant products, such as ginseng, Echinacea or St. John's wort (*Hypericum perforatum*), to complex, multiple ingredient products, such as those used in traditional Chinese medicine (TCM). Most products on the large international commercial market are standardised based on the anticipated bioactive components of botanical ingredients, such as hypericin in St. John's wort [123]. In other cases, extracts are normalised to the quantity of the dry herb which they contain [124]. However, when the bioactive component of a herbal medicine is unknown, or there are multiple possible active compounds (such as the ginsenosides found in ginseng), quality control becomes a far more complex problem. This complexity increases further when multiple ingredient products, each with their own bioactive components and mechanisms of action,

are considered. The chain of production is another important factor, as growing conditions, time of day and year when the plant was harvested, storage conditions, extraction process and the formulation used in the final preparation can all affect the bioactive compounds present, both in terms of composition and concentration [123-125].

Current, and arguably inadequate, quality control procedures utilise various methods, including plant morphology [126,127], chemical analysis [128,129] and DNA assays [127]. In some cases, a pharmacopoeia monograph may exist for a single, specific herbal medicine and outline the expected quality parameters, such as morphological tests, chromatography profiles and quantified tests for active ingredients [130]. In cases where a monograph does not exist, quality control methods are open to interpretation and potential bias. Even when monographs are available, they are often out-dated or limited. For example, there may be a monograph for the raw plant but not an extracted form. Plant morphology is difficult, particularly when dealing with dried or processed material, or only specific parts of a plant [131]. Such techniques often lack the specificity required to differentiate closely related species with similar features. Chromatographic profiles, or fingerprints, can be used for plant identification and can quantify suspected active ingredients. However, these profiles rely on phytochemicals that have been previously identified and the availability of reference compounds or herbal medicine/ botanical reference standards for comparison. In cases where there are limited studies on the phytochemical composition of a particular herbal medicine, the lack of data will greatly restrict the utility of chromatographic analysis [131,132]. The use of chromatographic fingerprinting also does not account for different experimental conditions and cannot be used to compare between plant species, growing locations and conditions, harvesting times and methods, or different extraction processes [129]. DNA barcoding has been successful in identifying the presence of endangered species, product substitution and the use of

dangerous or toxic plants, but there are limits to this single approach. Degradation of DNA during processing of products is a significant problem, and detection of DNA from a toxic or bioactive plant species does not indicate the presence of the toxic or bioactive components of that plant [3,20].

Recently, the growing technology of metabolomics has been proposed as part of the solution to the current limitations surrounding herbal medicine quality control and safety [129]. A domain of the 'omics group of technologies, metabolomics has been widely employed for drug discovery, identification of biomarkers of disease [133,134] and studies of animal and plant biochemistry and toxicology [125,135-137]. Metabolomics allows the identification and quantification of the small molecule metabolites within a given sample and therefore provides a detailed insight into the biochemical composition of that sample. Metabolomics can be considered as essentially providing a 'snapshot' of the biochemistry at a given time and under specific conditions. This systematic approach allows for change to be observed and distinguished under different conditions, such as disease state, stress conditions and seasonal variations [125,129,135,137]. Plant metabolomics has been successfully used to distinguish between plant parts [138], age [139] and species [126,135], processed and unprocessed products [125], and also different regions of cultivation [140,141].

To highlight the possible use of metabolomics for quality control in herbal medicines, its application to the popular herbal medicine ginseng will be discussed. Several studies will be considered as an example of what can be achieved using metabolomics, and to emphasise what further development is needed across the herbal medicine sector.

Ginseng and metabolomics:

Ginseng has been used for over 2000 years, with current production estimated at 8000 tons per year and a global market of approximately US$2 billion [135]. Quality control factors of primary concern, such as species composition, region of origin and methods of processing, have all been assessed using metabolomic analysis of ginseng, making it an excellent case study of the potential applicability of metabolomics in quality control of herbal medicines in general.

Herbal products labelled as 'ginseng' are usually the root of the *Panax* species, including *Panax ginseng* C. A. Meyer (Asian or Korean ginseng), *P. japonicus* (Japanese ginseng) and *P. quinquefolius* L. (American ginseng) [126,142]. *P. notoginseng* is another species which also contains many of the same active ginsenosides [143]. The bioactive components of ginseng are numerous, including polysaccharides, saponins, polyacetylenes, sesquiterpenes and fatty acids [140,142]. However, the dammarane saponins, more commonly known as ginsenosides [141] have been attributed to most of the possible therapeutic effects of the plant [139,140]. More than 40 ginsenosides have been identified from the root of *P. ginseng* [139,140], with reported therapeutic effects including antioxidant, anti-tumor, immunostimulatory, anti-hyperglycaemic [126], anti-aging, vasodilatory, and memory enhancement effects [141]. It is due to its continued popularity, considerable market size and value, and numerous possible pharmacological effects that fraudulent ginseng products are a notable problem [125,141]. Particular issues include fraudulent declaration of the area of cultivation and the 'blending' of wild-type ginseng with the cheaper alternatives; both of which are associated with a lower cost or pharmacological effects [141].

Zhao*, et al.* [126] compared commercially sourced *P. ginseng* and both wild and cultivated *P. quinquefolius* and found that sugar (glucose vs. sucrose) content was a major difference between species. When sugars were excluded from the principal component analysis (PCA), *P. ginseng* was shown to contain significantly higher levels of arginine, choline, malate and 2-oxoglutarate than both types of *P. quinquefolius* ($P < 0.01$). A downside to this study, as noted by the authors, is that these samples were commercially purchased. As such, factors which would affect the metabolite composition, such as the age and cultivation of the samples, could not be controlled. Despite this, not only was the ^{1}H NMR metabolomic analysis was successful at distinguishing the three types of ginseng analysed, with three distinct metabolite profiles generated. A sample claiming to be cultivated *P. quinquefolius* was found to have a different profile from all other sample types. This suggests that an inferior plant with less commercial value may have been sold as a substitute for cultivated *P. quinquefolius.*

Nguyen*, et al.* [141] compared a metabolomic approach to a DNA-based approach using chloroplast intergenic space regions for distinguishing area of cultivation of *P. ginseng*. They found that all sixty ginseng samples had very little genetic diversity between cultivation regions [141], whereas the metabolomic approach was effective at distinguishing between cultivation regions in Korea and China. It was hypothesised that changes in the concentration of carbohydrates and other metabolites involved in energy generation pathways are distinct based on the different environmental stresses and cultivation conditions between Korea and China. Notably, the change in harvesting time from before winter (Korea) to after winter (China) resulted in a change in carbohydrate levels, with higher levels of the primary metabolites detected in the Korean samples [141]. In identifying these changing metabolites, their respective biochemical pathways, and how they interact with the environment, both growing conditions and harvesting methods may be further optimised [141]. The authors also

constructed a statistical model to predict the ratio between blends of ginseng from the different regions, using a constrained least squares method. The model was modestly accurate at estimating the percentage amounts of Korean ginseng in a blend when compared to the known true amount (R^2 of 0.8343). The ability to provenance herbal products based on metabolomics profiles is a promising area of research that warrants further investigation.

It has been established that processing and extraction of plant material alters the composition of the final medicinal product [124,125]. A metabolomic study conducted by Chan*, et al.* [125] using ultra-performance liquid chromatography coupled to time-of-flight mass spectrometry (UPLC-TOF-MS) sought to measure biochemical differences, in particular ginsenoside content, between raw and steamed *P. notoginseng* root. There was a large variation in the metabolite profiles of the raw extracts, even though all samples were extracted in the same manner. The steamed extracts showed a much smaller level of variation, suggesting that the steaming process may, in altering the biochemical composition, result in a greater consistency in the final product. Chan*, et al.* [125] detected approximately 200 metabolites in raw and steamed *P. notoginseng* root and attempted to identify possible biomarkers for each processing technique. This aspect of the study was limited by the small sample size (n=6), but did demonstrate the potential of metabolomics to identify differently processed botanical material.

Another potential quality control factor that has been investigated using metabolomics is the age of the plant at the time of harvesting, with older plants being higher in value and market demand [139]. Kim*, et al.* [139], analysed *P. ginseng* root with cultivation ages of one to six years. Using UPLC-TOF-MS, 1361 metabolites were identified from sixty *P. ginseng* samples. Despite few chromatographic differences, the ginseng samples were able to be grouped by age using

the identified metabolites. The metabolite selection was based on several classification methods: random forest, prediction analysis of microarray, and partial least squares discriminant analysis [139]. With this selective approach, age discrimination was achieved, particularly of the older ginseng root (> 4 years).

The studies outlined above have shown that metabolomics is a potentially powerful tool in addressing numerous quality control issues for herbal medicines, being able to distinguish between species, age and areas of cultivations, with a substitute product identified in one study [126]. In addition, the methods can be rapid and high-throughput (>1000 samples per day) [144], critical advantages when dealing with a high volume of samples. One such high-throughput approach is as the rapid evaporative ionisation mass spectrometry (REIMS) technology, which has recently been applied to investigation of authenticity and fraud in meat products [145].

Moving forward:

The purpose of this review is to demonstrate whether metabolomics can be an effective tool for the quality control of herbal medicines. Metabolomics clearly has the potential to improve numerous aspects of the pipeline used in the herbal medicine industry, and data collected to date has demonstrated that a deeper look into the biochemistry of the botanical ingredients needs to be a strong focus of future research. The question then becomes whether metabolomics is a better, more rigorous test for quality compared to the current methods, such as monographs, or if it is likely to remain a secondary tool. Current methods of quality control can become out-of-date as more is learned about the botanical ingredients of herbal medicines. For example, in the case of St. John's wort where, as mentioned above, the extract if often standardised based on the amount of hypericin. Recent findings now suggest that

there are numerous other active compounds in the plant [146], none of which are covered by the existing quality control approach. This demonstrates that methods using a single compound as a standard are no longer appropriate.

There any many advantages to the 'top-down' approach of metabolomics; the most notable one being that metabolomics is the closest measurement to a system's phenotype [147]. In the case of untargeted metabolomics, another advantage is that retrospective data analysis is possible; allowing for data to be re-analysed for a different purpose (such as biomarker identification or metabolite fingerprinting) without the need to re-analyse a sample. However, metabolomics is not without potential limitations, and these have been thoroughly reviewed [120,122,148,149]. Multiple platforms such as gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry, and nuclear magnetic resonance spectroscopy are needed to cover the broad range of chemical properties of possible analytes [139]. While this range could be overcome with advances in the technology platforms, being certain of the identification of the analytes is another matter [122,148]. The most assured method of identification is comparison with an authentic standard under the same experimental conditions, as used by Gao*, et al.* [131]. However, such comparison may not always be possible, in which case the confidence of analyte identification needs to be made clear based on a systematic scoring system [148]. The studies summarised above are promising initial steps, but there needs to a concerted effort to comprehensively compare the metabolite profiles of the many plants used in herbal medicines. A limitation of the studies considered above is the fact that analysis is often limited to a subset of the metabolites present, as identified by multivariate analysis of the data. Many studies have focused only on the possible major active phytochemicals, in this case ginsenosides, which limits the power of metabolomics. Exceptions to this narrow focus include the studies by Kim*, et al.* [139] and Nguyen*, et al.* [141].

Another limitation is the data analysis methods that have been applied. While PCA and other multivariate statistical methods such as hierarchical clustering analysis are acceptable for pattern recognition and determining the major components that result in sample variability, digging further into the biochemical composition of herbal medicines is crucial [131]. The use of metabolomics for the optimisation of the production chain could allow producers to achieve the best quality plants for commercial purposes, by determining which combination of conditions will produce the best yield of high-quality components in particular species [123,124]. This approach would also generate a deeper understanding of the botanical ingredients, which will allow for more comprehensive and guided quality control of the final products. To know exactly which metabolites, and therefore possible active phytochemicals, are responsible for variation in the herbal medicine of interest would be invaluable, potentially providing novel biomarkers for quality control [131], and allowing properly-designed evidence-based efficacy studies of herbal medicines or identifying putative pharmacologically active compounds. This use of metabolomics to map the underlying biology of botanical ingredients goes beyond routine quality control and approaches the more comprehensive techniques used in pharmaceutical discovery and development. It is worth noting that the application of pharmacometabolomics and toxicometabolomics for studies on the bioactivity of herbal medicines is another area where 'omics shows great promise [150-152].

One proposed solution to the issue of quality control, beyond the utilisation of good manufacturing practices (GMP), is for a combined DNA and metabolomic testing approach. Such an approach is needed for the simple reason the intentional adulteration is not avoided by GMP alone [153]. This combined approach, similar to the one presented in Coghlan*, et al.* [20], would involve the use of DNA barcoding and mass spectrometry to screen for adulterants and contaminants, and metabolomics to scrutinise quality and safety. This combined approach

must be applied to both raw ingredients and final quality checks of market-ready products [132] to ensure that processing methods do not reduce the quality and content of desired phytochemicals. Continuous regulatory assessment of post-market products should also be implemented to provide higher quality commercial products. The added benefits of such rigorous testing, including the involvement of regulatory evaluation and possible third-party analysis is that the issues of contamination and adulteration can also be confronted without the reliance on a trust-based industry system. The set up costs associated with metabolomics and mass spectrometry would surely be offset by the benefits to health systems [13]. A key next step will be to undertake comparative research on traditional quality control methods and metabolomics [135].

Conclusion:

To improve compliance and safety, and therefore public confidence, the herbal medicine industry needs new processes for sufficient and effective quality control. The numerous reports of adulterated, contaminated and fraudulent products have demonstrated this need for change. Metabolomics has the potential to offer a solution to many of the quality control problems with herbal medicines. As demonstrated above, metabolomics can effectively determine product pipeline changes, including differences in regional cultivation, and distinguish between plant species and age, processing methods and blended products. A combined -omics' approach with DNA testing would move further to build a new standard of testing. However, as outlined above, further research into the biochemistry of the numerous ingredients in herbal medicines is clearly needed. It is an ambitious undertaking to develop high-fedelity and cost-effective –omics' toolkit, but the approach offers an pathway to address the numerous problems currently facing the herbal medicine industry.

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6. Metabolomic analysis of commercially purchased herbal products (green tea and Korean ginseng) to assess quality and safety.

Link:

Following on from the review in Chapter 5, an untargeted GC-MS metabolomics workflow was employed to compare the compositional diversity of green tea and Korean ginseng products. Linking with the DSA screening method developed in Chapters 3 and 4, where 18% of samples now require further investigation into their contents due to the large variety of unidentified compounds detected, metabolite profiling would allow comparison to standard reference materials. This may possibly lead to new quality and safety control methods for these complex matrices. Green tea and Korean ginseng products were chosen due to the overall popularity of these products on the market.

Abstract

The use of herbal medicines is growing throughout the world, with various levels of regulation in place. Raw plant ingredients are known to change in biochemical composition based on growth conditions, extraction and processing. The compositional diversity of finished products is not, however, routinely assessed in many countries. Using an untargeted metabolomics approach, green tea and Korean ginseng commercial products, purchased between 2015 and 2017, were analysed by GC-MS and compared to standard reference materials. The green tea products showed significant compositional differences, both from each other and the reference standard, while the ginseng products showed large intra-sample variation. The analysis showed that, despite all products stating the same ingredients, there was a clear difference in biochemical profile between products and the reference materials.

Introduction

Herbal medicines are becoming increasing popular throughout the world [154]. From use as primary health care and folk medicines to commercial and highly marketed products, herbal medicines can range from simple single ingredient teas to complex, multi-ingredient extracts and pills. The higher the complexity in the ingredients and formulation of a product, the more difficult it is to ensure quantity and safety. Factors in herbal medicine quality control include longer production chains for commercial products, issues with plant morphological identification, use of substitute and filler ingredients, adulteration and contamination of both raw and final products, batch to batch variation, and the natural variation of phytochemicals affecting the potential bioactive constituents. Despite the many factors that affect the final

composition of a product, quality control measures often rely on single content assays and standardisation to potential bioactive constituents (e.g., ginsenosides in *Panax ginseng*).

Recently, a more comprehensive approach to the characterisation of herbal products has been taken using metabolomics [152,155-158]. The advantage of such an approach is that many of the factors affecting composition such as plant age [139], growth conditions [156], processed and raw material [141], the use of fillers and species substitution [141,159,160] can be identified. Beyond quality control applications, metabolite profiling of herbal medicines has been of increasing interest [124,152,158,160] for potential outcomes in identifying active phytochemicals and optimisation of the production chain for commercial plant products. Metabolomics for comparison of compositional diversity has been applied to several herbal products such as St. John's wort [123,159] and chamomile tea [161]. Metabolomics and chemometrics have also been used to discriminate counterfeit medicines and authenticate raw herbal materials [141,162,163] with varied success.

Green tea and ginseng are some of the most popular herbal medicines used today [141,164]. Ginseng has been used a medical herb for over 2000 years with *Panax ginseng,* also known as Korean ginseng, one of the most commonly used herbal medicines [135,141], for its professed strengthening and rejuvenating powers [165]. There are numerous bioactive components of ginseng, with the group of dammarane saponins or ginsenosides exhibiting numerous pharmacological effects including antioxidant, immunostimulatory [126], vasodilatory, and memory enhancement effects [141]. Green tea, *Camellia sinensis,* is also a popular medicinal herb and one of the most popular drinks worldwide, second only to water [166], and an increasingly popular weight-loss supplement [167]. Components of green tea such as the polyphenols, including the catechins and gallic acid, have been shown to exhibit antioxidant

activity [166]. Epigallocatechin gallate (EGCG) is thought to have a variety of beneficial effects [168], but has also been implicated in liver and kidney toxicity, particularly when taken in the high doses found in 'green tea extract' products [167].

A better understanding of the compositional variation of products will allow us to better assess the safety and quality of these products. This study used an untargeted metabolomic workflow and multivariate data analysis to assess the composition of commercial single ingredient herbal products (green tea and Korean ginseng) on the Australian market.

Method

Materials and sample details

LC-MS grade methanol and water were purchased from Fisher Scientific (Hampton, USA). D-$^{13}C_6$-sorbitol (internal standard), methoxyamine hydrochloride, *n*-alkanes (C_{10}, C_{12}, C_{15}, C_{19}, C_{22}, C_{28}, C_{32}, C_{36}), N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and pyridine were all purchased from Sigma-Aldrich in the highest purity available. CAM and herbal medicine (HM) products labelled to contain green tea (GT) (n = 5) or Korean ginseng (KG) (n = 7) were purchased between 2015 and 2017 from various Australian retailers (Table 6-1). NIST standard reference material (SRM) *Camellia sinensis* (GT) (SRM 3254) and European Pharmacopoeia Ginseng reference standard were included as reference standards.

Table 6-1: Stated ingredients and indications of herbal medicines (HM) and CAM samples analysed in this study.

Sample group	Sample ID #	In ARTG database? (Yes/No)	Ingredients on package	Indications on package
Korean ginseng	HM_6	Y	Panax ginseng	Enhances stamina and endurance, supports energy levels, reduces fatigue, enhances concentration and work efficiency, helps with convalescing after illness.
	HM_7	Y	Panax ginseng	Help promote general endurance and assist stamina. Best used in times of physical exertion. Aid in the maintenance of general wellbeing.
	HM_8	Y	Panax ginseng	Increase stamina, endurance and vitality. Relieve nervous tension and stress, support busy, active lifestyles.
	HM_9	Y	Panax ginseng root powder	Helps to improve stamina and endurance, may be beneficial during times of stress, helps to maintain the immune system.
	HM_10	Y	Panax ginseng	Energise and invigorate the body. Strengthen the immune system. Improve physical performance, endurance, stamina and fitness. Of benefit during times of stress.
	HM_11	N	Fermented Korean ginseng extract, Korean ginseng extract	None given.
	HM_18	Y	Panax ginseng root extract	May assist in maintaining general well-being and help to provide increased endurance.
Green tea	HM_19	Y	Camellia sinensis, Coffea canephora	Aids healthy weight loss. May help people who've lost weight avoid putting it back on. Activates thermogenesis. Supports heart and blood vessel health. Helps neutralise potentially damaging free radicals.
	HM_20	Y	Camellia sinensis	Assist with healthy weight management. Help to maintain healthy body weight. Enhance the body's antioxidant defences. Increase energy and promote detoxification. Maintain normal health of the skin and liver.

Sample group	Sample ID #	In ARTG database? (Yes/No)	Ingredients on package	Indications on package
	CAM_239	Y	Camellia sinensis, Coffea canephora	Helps burn fat and supports healthy weight loss. Activates thermogenesis to help burn stored fat for energy. Helps improve body composition and reduce body fat percentage and body mass index (BMI). Supports normal burning of calories for fat loss and assists in maintaining healthy metabolic function. Helps regulate absorption and utilisation of glucose and fat from the diet. May also assist weight loss by supporting normal glucose metabolism and blood sugar levels in healthy individuals. May reduce the oxidation of LDL 'bad' cholesterol. Provides antioxidant benefits.
	CAM_245 and B_245 as a brewed tea	N	Cinnamon, Ganoderma mushroom, Green tea, Peppermint, Chicory root	2 in 1 Slimming and Detox. Strong antioxidant formula.
	CAM_305	Y	Camellia sinensis	Provide antioxidant support, cardiovascular health, healthy metabolism.

Sample preparation and extraction

10 tablets or tea bags from each product were ground to a fine homogenised powder using Precellys CKMix 50-R 2 mL lysing tubes (Bertin Technologies, France). The NIST SRM GT product was sampled twice, with one set being brewed for extraction. Powdered samples were transferred to 2 mL lysis tubes and stored at -80°C until use. Approximately 5 mg of each sample was extracted multiple times with methanol and water. The first extraction used 0.5 mL methanol containing $^{13}C_6$-sorbitol internal standard (1.11 µg/mL). The samples were shaken for 40 s at 6500 rpm in the Precellys tissue lyser. After shaking, extracts were centrifuged for 10 min at 13.2 x 10^4 x *g* and 0.35 mL of supernatant was collected. Samples were then extracted again with 0.5 mL of methanol and shaken and centrifuged as above, with 0.4 mL of supernatant collected. The final extraction was 0.5 mL of 50% methanol, shaken and centrifuged as above, with 0.45 mL of supernatant collected.

To brew the tea samples (CAM_245), 5 mg of ground tea was prepared with 1 mL of water, heated to 95°C and shaken at 400 rpm for 10 min. The tea was then centrifuged for 10 min at 13.2 x 10^4 x *g* and 0.9 mL of supernatant was collected and dried by evaporation using an Eppendorf Concentrator Plus rotary vacuum concentrator (Eppendorf South Pacific Pty. Ltd., Sydney, Australia). The brewed tea was then triple extracted as described above.

QC samples were made from a pooled sample of the extracts. Extracts were diluted 1:10 and dried by evaporation in vial inserts. Dry extracts (both neat and 1:10) were stored at -80°C until derivatisation. Derivatisation was carried out by treating the freeze-dried extracts with 20 µl of methoxyamine hydrochloride solution (20 mg/ml in pyridine) and agitated using an Eppendorf Thermomixer Comfort at 1200 rpm and 30°C for 90 min. Extracts were then centrifuged for 1 min at 13.2 x 10^4 x *g*. 40 µl of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) was added and the extracts were further agitated for 30 min at 300 rpm at 75°C. 5 µl of alkanes in *n*-hexane was added and samples were centrifuged for 1 min at 13.2 x 10^4 x *g* for a final time before analysis within 12 h. The GT and KG samples were analysed separately with only the 1:10 dilutions being analysed.

GC-QTOF instrumentation

An Agilent 7200 QTOF-GC-MS (Agilent Technologies, Santa Clara, USA) was used for untargeted metabolomic analysis with electron ionisation. 1 µL of derivatised sample was injected into the inlet at 270°C in splitless mode, with the carrier gas of ultra-high purity helium at a constant flow rate of 0.85 mL/min. An Agilent VF-5-ms fused silica capillary column was used (0.25 mm ID, 30 m length, 0.25 µm film, and 10 m EZ-guard). The chromatographic method was 23.5 min and included a 10-min solvent delay. Initial oven temperature was 70°C before a temperature ramp of 15°C/min for 6 min and 40 s until the final temperature of

325°C was reached. The transfer line was set at 300°C and the ion source to 280°C. Ionisation was achieved with a 70 eV electron beam. The MS has a scan rate of 10 spectral scans/s and scanned ion masses in the range of *m/z* 50 to 700. For both sample sets, samples were randomised and the run sequence started with three extraction blanks, then eight QC samples for conditioning. A QC sample was included every sixth injection, as well as at the start and end of the derivatisation batches.

Data analysis and statistics

GC-MS data was processed and deconvoluted using AnalyzerPro v5.5.1, (SpectralWorks, Runcorn, UK). Data pre-treatment included zero-filling using MetImp [169], with 50% filtering of missing values and random forest (RF) imputation applied. Further data treatment and statistical analysis of the data matrix was carried out using MetaboAnalyst [170], with filtering (RSD >30% based on QC samples), cube root or log transformation (for the GT and KG data sets, respectively) and quantile normalisation carried out. The principal component analysis (PCA) and heatmaps were generated by MetaboAnalyst while EZInfo, v3.0 (Umetrics) was used for ANOVA comparisons between sample groups.

Results

Green tea

Overall, 1301 components were detected over the analysis of the five different GT products and SRM. After filtering and processing, 84 components (noted as M_1 to 84) were imported into MetaboAnalyst. The GT group comprised of three distinct product types: green tea tablets (n=2), green tea + green coffee tablets (n=2) and green tea leaves , sample CAM_245/ B_245 (n=1, raw vs brewed).

Figure 6-1 shows the PCA scores plot for all of the green tea products analysed compared to the SRM, with the different samples types then compared separately. All of these comparisons (Figures 6-2 to 6-4) show that samples of the same product type group together, but separate from the SRM. This may be expected for samples containing green tea + green coffee (Figure 6-2), but is more surprising for green tea tablets (Figure 6-3) and green tea leaves (Figure 6-4), which would be expected to be more similar to the SRM. Figure 4 also shows that raw and brewed tea leaves had similar metabolic profiles. Figure 6-5 comprises the group means for the top 25 components. The difference between samples and the SRM is most prominent for components M_28, M_40, M_54, M_49, M_68 and M_20. These components are also responsible for the separation of the groups on the PCA. The concentration of these components are also responsible for the disbursement seen in some of the samples replicates. For example, M_28 and M_40 are responsible for the wide scatter of HM_20 in Figure 6-3 due to a larger concentration range in HM_20 than CAM_305.

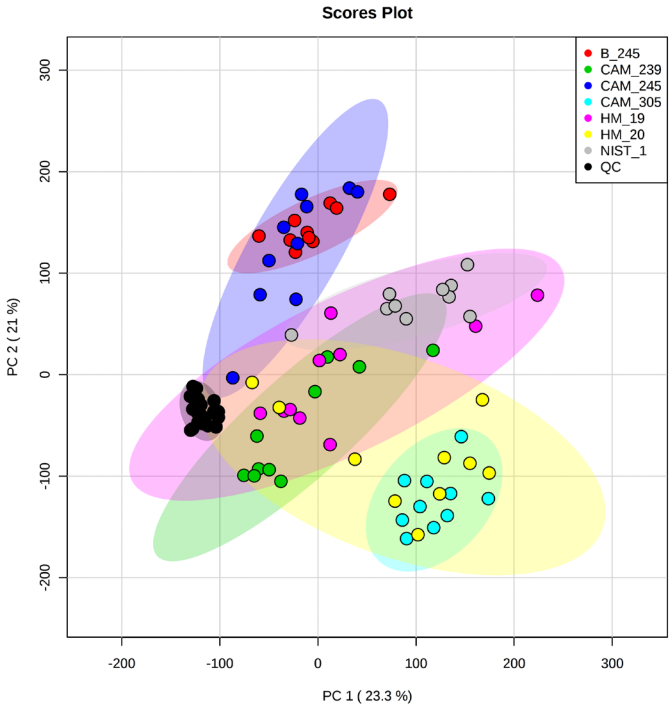


Figure 6-1: PCA scores plot of GC-MS data from all GT products and the NIST reference standard.

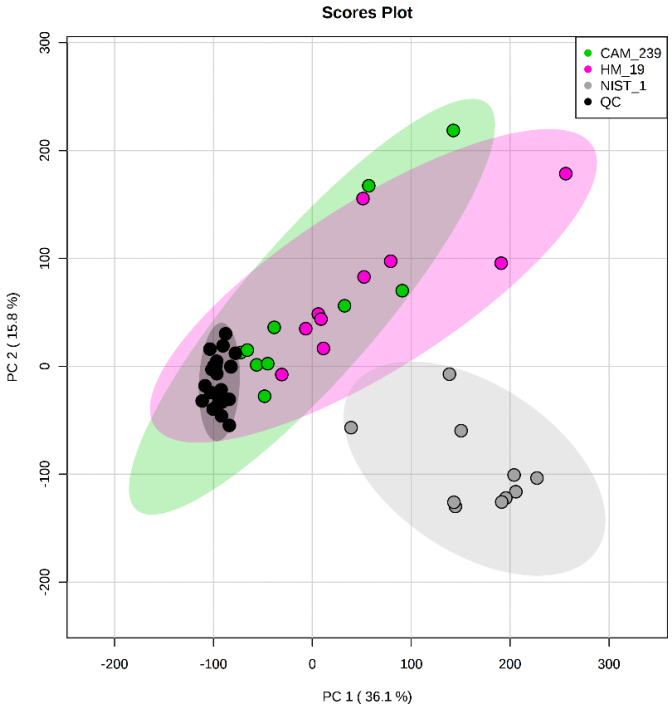


Figure 6-2: PCA scores plot of GC-MS data from green tea + green coffee products (CAM_239 and HM_19) and the NIST reference standard.

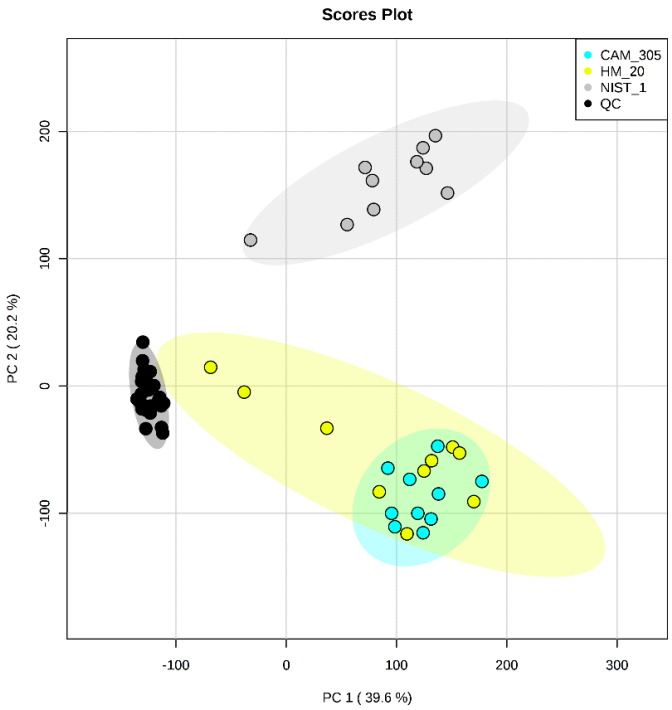


Figure 6-3: PCA scores plot of GC-MS data from green tea tablet products (CAM_305 and HM_20) compared to the NIST reference standard.

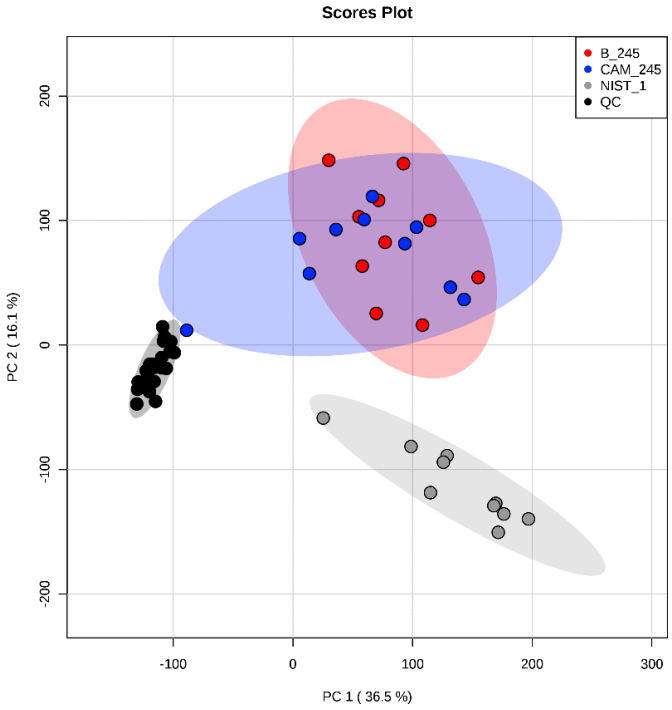


Figure 6-4: PCA scores plot of GC-MS data from green tea leaves, raw (CAM_245) and brewed (B_245) compared to the NIST reference standard.

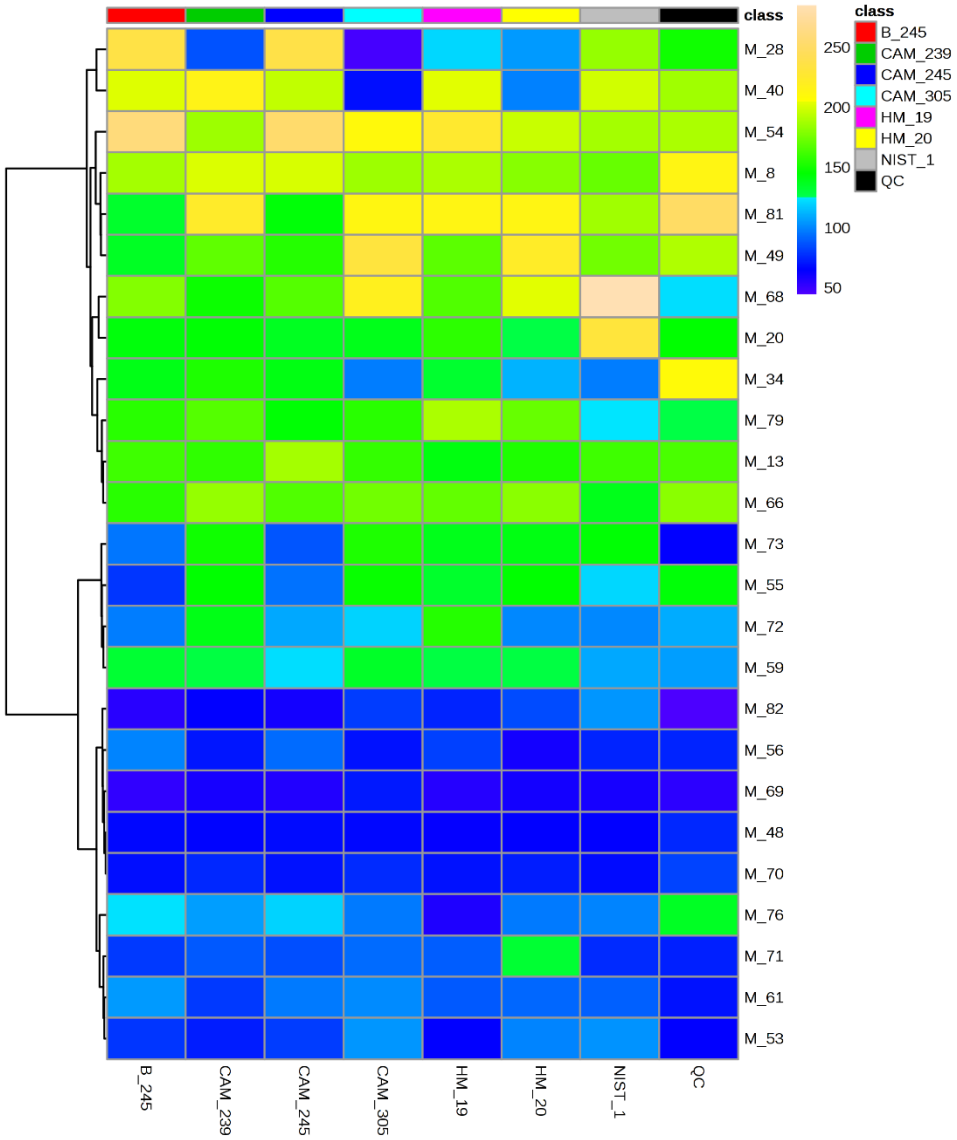


Figure 6-5: Heatmap of the group means for the top 25 components as determined by PCA between all samples.

Ginseng

For KG, 2126 components were detected for the analysis of seven *Panax ginseng* samples and the reference standard. After filtering and processing, 103 components were imported into MetaboAnalyst. Four sample replicates were removed as outliers based on RF and not included in the PCA or heatmap analysis. HM samples 6 to 10 were tablets or capsules, HM11

was a ginseng tea and HM18 was a liquid sample. The products were stated to be standardised by either ginsenoside content or the amount of dried ginseng in the extract. Due to the same single ingredient, samples would be expected to be similar, as reflected in Figure 6-6, which shows the PCA scores plot for all of the ginseng products analysed. However, there is a large variation within the KG sample groups and separation of the reference standard from the majority of the samples. When compared using a heatmap (Figure 6-7), there are a number of components with significant difference ($p < 0.001$) which correlate to the PCA separation; M_2078, M_2078, M_1181, M_2046, M_460 and M_1146.

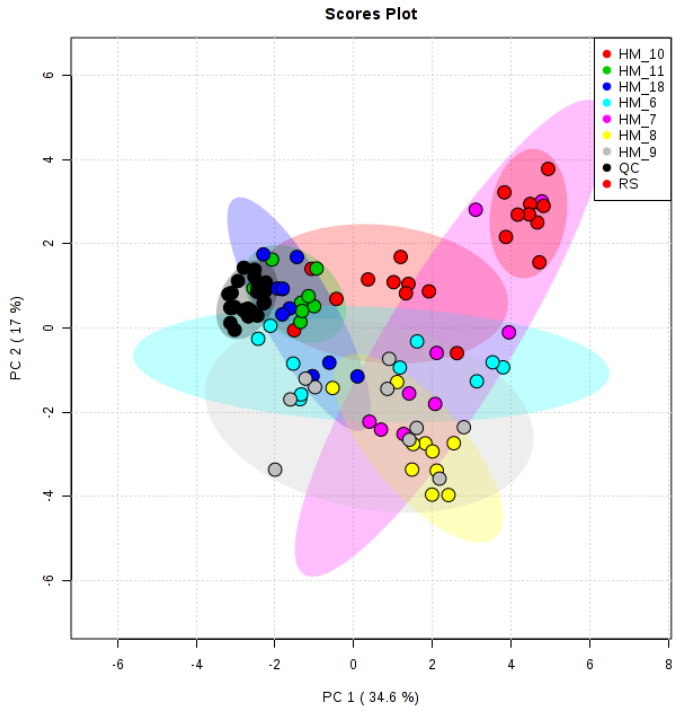


Figure 6-6: PCA scores plot of GC-MS data from KG products and the European Pharmacopoeia Ginseng reference standard (top right red group).

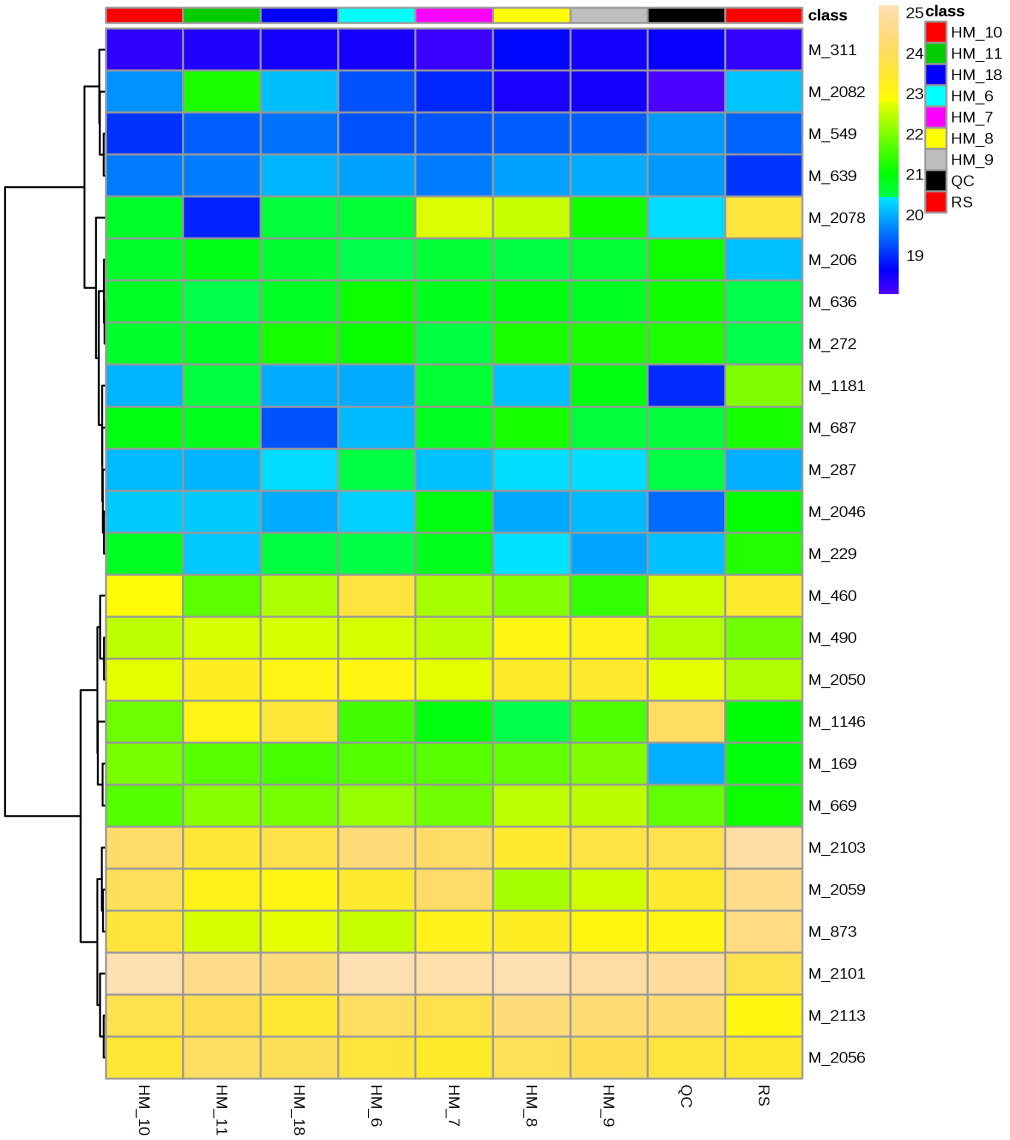


Figure 6-7: Heatmap of the group means for the top 25 components identified by PCA of all *Panax ginseng* samples.

Discussion

The key observation from this study is the variation within, and between, sample groups and corresponding reference standard. All products tested showed significant compositional differences, both from each other and the reference standards. While some of this diversity is likely due to different processing methods between brands and products, the reference

standard in both sample sets was distinct in the PCA analysis. This indicates that the composition of the products varied, even though they all contained the same ingredient. Currently in Australia, only pre-approved ingredients which have been deemed low risk are allowed to be marketed and sold [34]. With such compositional diversity, even when the products contain the same ingredient (as per the label), the difference between pre-approval of ingredients and pre-approval of an individual product becomes crucial to assessing a product's safety. Assuming that the diversity observed is from the advertised ingredient, there are a multitude of factors that would affect the final chemical composition of a herbal product. All stages of production will affect the biochemical profile of a herbal product, from the growth conditions of the plants, to the harvesting, storage and processing of raw materials, extraction of potential active ingredients and production of the final product, [124,171], as reviewed in Chapter 5.

Green tea is an increasingly popular weight loss supplement, with all products tested having indications such as 'weight management or loss, detox, and metabolism support' (Figure 6-1). Green tea products indicated for weight loss have been linked to liver damage in several case studies [167,168,172,173]. The major compounds of green tea attributed to the bioactivity are catechins [166] with the most abundant, epigallocatechin gallate, along with caffeine, thought to account for the potential weight loss effects [164]. A 2012 Cochrane review found green tea had no significant effect on weight maintenance [164]. Figure 6-3 shows how distinctly different the green tea tablets (CAM_305 and HM_20) were to the NIST SRM in terms of GC-MS profile. Figure 6-4 shows a similar separation between the SRM and green tea leaves, raw or brewed. Such compositional differences between products and the SRM raise the question of how representative SRMs are to the marketed products, as well as the usefulness of such standards for QC. Furthermore, with such diversity, large variation of the potential

pharmacologically active compounds, such as catechins, cannot be ruled out. It was noted by Jurgens*, et al.* [164] that inconsistency in product content may account for the variation in results obtained in the systematic Cochrane review. To challenge this inconsistency and variation in products, quality control techniques must move beyond quantifying only the known or suspected active compound. By profiling the complete composition of raw and processed ingredients and finished products, reliable and consistent herbal products can hopefully be produced [154,159,174,175].

Unlike the GT analysis, the KG products showed greater similarly to the reference standard (Figure 6-6), although there was a larger variation within sample replicates compared to the reference standard. Ginsenosides are the attributed active compounds for the *Panax* species, and products are often standardised by ginsenoside content. However, with over 40 ginsenosides identified from the root of *Panax ginseng* [140], standardising to the vague description of 'ginsenosides' does not reveal much about the total composition of a product. The products analysed fell into two groups: standardised to ginsenoside content or standardised to amount of dried product in the extract.

As previously reviewed in Chapter 5 [171], there have been numerous studies into the use of metabolomics in characterising quality control factors such as species, age and region of origin for raw ingredients. In this study, the different standardisation methods and known variation in raw ingredients did not translate into large variability between sample groups in the PCA (Figure 6-6 and Figure 6-8), although there were still components that were significantly different ($p < 0.001$) (Figure 6-7). Contrasting the KG data with the GT results, the ginseng reference standard appears to be more representative of commercial products, which could result from the way that the KG products were standardised.

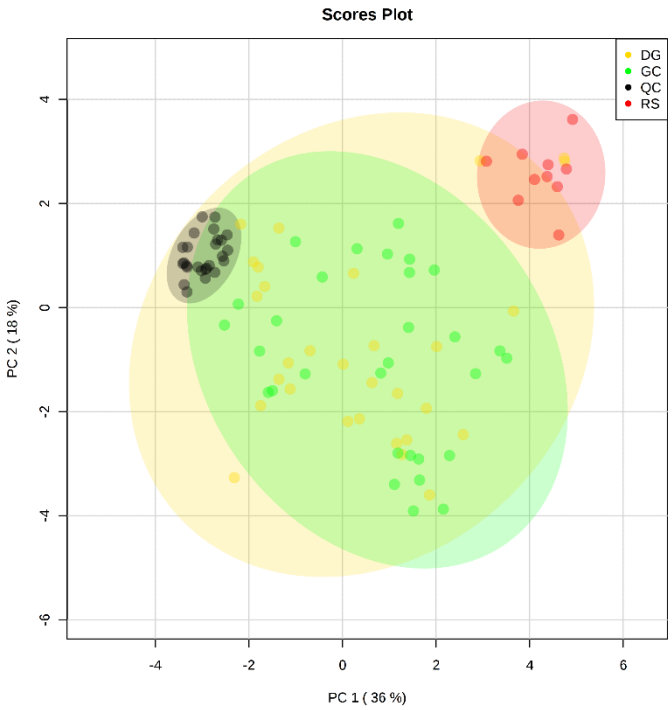


Figure 6-8: PCA scores plot of GC-MS data from KG products based on the standardisation methods: ginsenoside content (GC) or standardised to amount of dried product (DC). The amounts ranged from 4 mg to over 40 mg of ginsenosides or 400 mg to 2 g of dry ginseng.

Three of the samples had been analysed in our previous study by DNA barcoding and LC-MS analysis [116]. Samples CAM 239, 245 and 305 all contained caffeine. Of the samples not previously analysed, the green tea + green coffee tablets (HM_19) and green tea tablets (HM_20) also declared caffeine content on the product labels. CAM 245 was found to have animal contamination (dog, camel and ox) and additional animal DNA (frog and Asian highland shrew) and CAM 305 also contained additional plant DNA (*Apiaceae* sp.). Further work is needed to better characterise the chemical components of these products to determine if the

diversity encountered in this study arose from the declared ingredients, the formulation or undeclared botanicals and contaminants.

The key next step in this research is the extensive process of identifying these components. Approaches to identifying components include comparison to in-house libraries of authentic standards where available or searching against publicly accessible spectra libraries and databases. However, due to incompleteness of libraries, matrix background and noise, these approaches do not always lead to structural identification of the compounds of interest [176]. Structure elucidation of small molecules requires excellent mass accuracy for chemical formula computation alone, often leaving many possible compound formula matches to components at the higher masses ranges [177,178]. Currently levels or scoring systems are used to assess putative identification, with comparison to authentic standards at the highest level, and fragmentation pattern analysis, isotopic patterns and accurate mass scoring lower [148].

Conclusion

This study presents a potential new approach to understanding herbal product composition using metabolomics. The results from this study and others [123,141,159,161] show that, notwithstanding the use of standardised methods such as ginsenoside content, there is still large compositional diversity among finished herbal products on the market. What is still unclear is whether this compositional diversity has any safety implications, with unknown bioactive phytochemicals possibly accounting for the variation. The use of metabolomics and similar techniques to screen for adulterated and fraudulent products is one which needs further research and time, but approaches that allow the full chemical composition of a herbal product are clearly needed to produce more consistent and safer products.

7. General discussion

The first part of this thesis, Chapters 1 to 4, investigated methods for detection of adulteration/contamination in CAM products, and used these methods to examine the occurrence of such issues. Chapter 2 showed that 50% of samples indicated for diet, cardiovascular health and general wellness/wellbeing contained undeclared ingredients. The original research presented in Chapter 2 is part of a larger body of work studying the quality of CAM products available on the Australian market [89,179] (manuscripts included in the Appendix). Hoban*, et al.* [89] details the testing of a further 59 products indicated for psychiatric conditions such as insomnia or sleeplessness, anxiety and depression, finding that 29 products (49%) contained substances not declared as listed ingredients. This included heavy metals such as lead and cadmium at levels above the TGA guidelines [89]. For 49 products indicated for analgesic and anti-inflammatory use, 26 (53%) were adulterated or contaminated with undeclared ingredients [179]. Combined, these studies suggest that, despite the different indications, the level of undeclared ingredients remains mostly consistent at approximately 50%.

The individual methods employed in this research are not novel for testing CAM and herbal medicine product. As reviewed in Chapter 1, there are numerous LC-MS methods for the targeted screening of pharmaceuticals in herbal matrices, including high resolution LC-MS methods such as those employed by Jin*, et al.* [180] and Guo*, et al.* [181]. However, the novel combination of the techniques of DNA barcoding and LC-MS analysis has provided a more comprehensive picture on a product's contents, allowing the ingredient claims to be ascertained, as demonstrated in Chapter 2. However, in spite of the scope of data obtained,

there is not one universal method for quality control for complex products. Questions can remain regarding the total content and compositions of products, particularly when only targeted analysis has been carried out.

Chapters 3 and 4 detail the development and application of the DSA-TOF, a rapid and direct sampling MS screening method. This method, while not as comprehensive as LC-MS analysis, was able to screen for the majority of a selection of pharmaceuticals in an analysis time of less than one minute. Applying the method in Chapter 4, 18% of samples had a variety of unidentified compounds detected and require further investigation into their contents. When the results were combined with those from Chapter 2, 71% of samples tested were either adulterated or contaminated. Only 40% of the tested samples had their labelling claims somewhat substantiated by the combined results of the different analyses.

Following on from the questions raised in the first part of the thesis, the second part of this thesis was concerned with the content and composition of herbal products on the Australian market and how untargeted metabolomics can be used for as a quality control technique. Chapters 5 and 6 focused on single ingredient herbal products, reviewing and applying metabolomics and metabolite profiling to green tea and Korean ginseng CAM products. As reviewed in Chapter 5, raw Korean ginseng products can be differentiated using metabolomics by characterising features such as species, age and region of origin of the plant products. Other herbal products have also been similarly profiled and evaluated [123,132,161,182]. Metabolomics can also be used to help find new biomarkers for quality control [131]. However, using metabolomics to validate the final composition and therefore quality of commercial products is only starting to be explored [154]. Chapter 6 profiled commercially available green tea and Korean ginseng products and compared them to standard reference materials (SRM)

using PCA. The results found that there were significant compositional differences between products and SRM, as well as intra-sample variation. Despite stating that they contained the same ingredients, there was dissimilarity in the biochemical profiles of the product tested. The implications and causes of this diversity remain to be investigated. The possibility of using metabolomics to help develop quality control standards by profiling the complete composition of products at all stages of production is another strong area of research[124,154,183].

Limitations and improvements

There are many limitations within this body of work. The many mass spectrometry techniques used each have strengths and weaknesses, and not one of the employ methods gives a complete picture on its own. Chapter 2 used a routine LC-MS toxicology screening method in positive mode only. The LOQ of this method were developed for therapeutic levels of pharmaceuticals only [21]. As a targeted screen, novel drug analogues and relevant phytochemicals would not be detected. It was these limitations in mind that the rapid, untargeted DSA-TOF analysis was explored as an application. However, detailed method validation for the DSA-TOF analysis is still required, with the assessment of matrix and ion suppression, in-source fragmentation, quantitative capacity, and negative mode analysis (only positive mode was used in the current analyses). The DSA-TOF is also capable of direct analysis of solid samples rather than the solvent extractions utilised in this research. Chapters 3 and 4 have shown that this direct analysis methodology is appropriate for analysis of herbal medicines, mainly where the form of products such as oils, gels and pastes are more challenging for traditional methods. The major limitation for chapter 6 is that the components of interest remain unidentified, which is the next step in this research. Similarity, the use GC-MS for the metabolomics analysis, while helpful for the use of spectra libraries for the future

identification of components, does have the limit of requiring derivatisation for thermally labile and non-volatile compounds. Future use of both GC-MS and LC-MS based metabolomics would give a more comprehensive view of product composition.

These limitations should not however take away from the novelty of this work. The combination of DNA, multiple MS techniques, on commercially purchased products is current the only published investigation of Australian products, giving a clearer insight to the character of what are very popular commercial health products. The combination of techniques also strengthens the conclusions overall, helping to offset of each method's individual limitations. All methods: DNA testing, LC-MS, direct MS, or GC-MS each raise questions about the true content of herbal products.

Conclusion:

This thesis set out to answer four questions:

1. What is the occurrence of adulteration/contamination in CAM products available in Australia?

Approximately 50% of the samples tested contained additional plant (31%) and animal DNA (2%), animal contaminants (12%), or additional pharmaceuticals (5%) when tested using a combined method of targeted LC-MS and DNA barcoding. This occurrence conceivably increased when untargeted screening using the DSA-TOF was combined with the targeted approaches.

2. What is the compositional diversity /similarity of formulated herbal products?

There are consistent, significant compositional differences between products and reference standards, which means that regardless of having the consistent ingredients, products are diverse enough to challenge the idea of 'pre-approved' ingredients rather than products.

3. What is in these herbal products and does the content match the label?

This study has demonstrated that the label is accurate in only a proportion of CAM products and that validating the label requires a multi-tiered and multi-method approach. GC and LC-MS, DNA testing and also direct MS methods can all be used in combination to give a holistic overview of product content and composition.

4. What is the risk posed to consumers by CAM products?

The risk to consumers is still undefined; however, many risks have been identified in this work. Contaminated and adulterated products, possibly inaccurate labels and products not listed with the relevant regulatory bodies could all pose a risk for consumers. As people cannot know precisely what they are consuming, the idea that these products are 'low-risk' needs to be contested.

There are various future lines of inquiry that can develop from this work. Broadly focusing on the topics of composition and safety, further research is needed into the differences in herbal products and any relation to differences in toxicity. To enable this research, supporting the development of rapid screening and profiling methods would facilitate evaluation of compositional diversity. Metabolomics and mass spectrometry can be utilized in all areas of this future research as demonstrated in this thesis, complementing other specialities such as DNA barcoding. Research collaboration between regulation bodies, industry and academia would be of further benefit to ensure the highest quality products. Such research would carry

substantial costs, however it is worthy of funding, as this research and thesis has shown that the pre-approval of ingredients by Australian regulators does not necessarily generate compliant products. Despite the numerous studies undertaken and the different methods employed in this research, we still cannot with absolute certainty specify the contents of the products tested. It is only through the work presented here and future work that herbal medicines and other CAM products can safely take a place in consumer health choices.

8. References:

1 Smith, J. M., Sullivan, S. J. & Baxter, G. D. Complementary and alternative medicine: contemporary trends and issues. *Physical Therapy Reviews* **16**, 91-95 (2011).

2 Ernst, E. Adulteration of Chinese herbal medicines with synthetic drugs: a systematic review. *J Intern Med* **252**, 107-113, doi:10.1046/j.1365-2796.2002.00999.x (2002).

3 Coghlan, M. L., Haile, J., Houston, J., Murray, D. C., White, N. E., Moolhuijzen, P., Bellgard, M. I. & Bunce, M. Deep sequencing of plant and animal DNA contained within traditional Chinese medicines reveals legality issues and health safety concerns. *PLoS genetics* **8**, e1002657, doi:10.1371/journal.pgen.1002657 (2012).

4 Cheung, F. TCM Made in China. *Nature* **480**, S82-S83 (2011).

5 Yee, S. K., Chu, S. S., Xu, Y. M. & Choo, P. L. Regulatory control of Chinese Proprietary Medicines in Singapore. *Health Policy* **71**, 133-149, doi:10.1016/j.healthpol.2003.09.013 (2005).

6 ANAO, A. N. A. O. *Therapeutic Goods Regulation: Complementary Medicines*, <https://www.anao.gov.au/work/performance-audit/therapeutic-goods-regulation-complementary-medicines> (2011-2012).

7 *Global Complementary and Alternative Medicine Market Is Set For Swift Growth, To Reach Around USD 163.3 Billion by 2025*, <https://www.zionmarketresearch.com/news/complementary-and-alternative-medicine-market> (2020).

8 Li, B., Forbes, T. L. & Byrne, J. Integrative medicine or infiltrative pseudoscience? *Surgeon* **16**, 271-277, doi:10.1016/j.surge.2017.12.002 (2018).

9 Offit, P. A. *Do You Believe in Magic? Vitamins, Supplements, and All Things Natural: A Look Behind the Curtain*. (New York, NY : Harper, 2013).

10 Hurley, D. *Natural Causes: Death, Lies and Politics in America's Vitamin and Herbal Supplement Industry*. (Broadway Books, 2006).

11 Singh, S. & Ernst, E. *Trick or Treatment: The Undeniable Facts about Alternative Medicine*. (W. W. Norton & Company, 2009).

12 Goldacre, B. *Bad Science: Quacks, Hacks, and Big Pharma Flacks*. (Farrar, Straus and Giroux, 2010).

13 Geller, A. I., Shehab, N., Weidle, N. J., Lovegrove, M. C., Wolpert, B. J., Timbo, B. B., Mozersky, R. P. & Budnitz, D. S. Emergency Department Visits for Adverse Events Related to Dietary Supplements. *N Engl J Med* **373**, 1531-1540, doi:10.1056/NEJMsa1504267 (2015).

14 Blasche, R. E. C., Worsley, A. & Lawrence, M. The effects of an apparent breach of therapeutic regulations on consumers: the Pan Pharmaceuticals recall. *Critical Public Health* **18**, 367-379, doi:10.1080/09581590802075679 (2008).

15 Corns, C. & Metcalfe, K. Risks associated with herbal slimming remedies. *The journal of the Royal Society for the Promotion of Health* **122**, 213-219 (2002).

16 Klepser, T. B., Doucette, W. R., Horton, M. R., Buys, L. M., Ernst, M. E., Ford, J. K., Hoehns, J. D., Kautzman, H. A., Logemann, C. D., Swegle, J. M., Ritho, M. & Klepser, M.

E. Assessment of patients' perceptions and beliefs regarding herbal therapies. *Pharmacotherapy* **20**, 83-87, doi:10.1592/phco.20.1.83.34658 (2000).

17 Gabay, M., Smith, J. A., Chavez, M. L., Goldwire, M., Walker, S., Coon, S. A., Gosser, R., Hume, A. L., Musselman, M., Phillips, J. & Abe, A. M. White Paper on Natural Products. *Pharmacotherapy* **37**, e1-e15, doi:10.1002/phar.1874 (2017).

18 Byard, R. W. & Musgrave, I. Herbal medicines and forensic investigations. *Forensic Sci Med Pathol* **6**, 81-82, doi:10.1007/s12024-010-9157-x (2010).

19 Byard, R. W., Musgrave, I., Maker, G. & Bunce, M. What risks do herbal products pose to the Australian community? *Med J Aust* **206**, 86-90, doi:10.5694/mja16.00614 (2017).

20 Coghlan, M. L., Maker, G., Crighton, E., Haile, J., Murray, D. C., White, N. E., Byard, R. W., Bellgard, M. I., Mullaney, I., Trengove, R., Allcock, R. J., Nash, C., Hoban, C., Jarrett, K., Edwards, R., Musgrave, I. F. & Bunce, M. Combined DNA, toxicological and heavy metal analyses provides an auditing toolkit to improve pharmacovigilance of traditional Chinese medicine (TCM). *Sci Rep* **5**, 17475, doi:10.1038/srep17475 (2015).

21 Crighton, E., Coghlan, M. L., Farrington, R., Hoban, C. L., Power, M. W. P., Nash, C., Mullaney, I., Byard, R. W., Trengove, R., Musgrave, I. F., Bunce, M. & Maker, G. Toxicological screening and DNA sequencing detects contamination and adulteration in regulated herbal medicines and supplements for diet, weight loss and cardiovascular health. *J Pharm Biomed Anal* **176**, 112834, doi:10.1016/j.jpba.2019.112834 (2019).

22 Wheatley, V. M. & Spink, J. Defining the Public Health Threat of Dietary Supplement Fraud. *Comprehensive Reviews in Food Science and Food Safety* **12**, 599-613, doi:10.1111/1541-4337.12033 (2013).

23 Newmaster, S. G., Grguric, M., Shanmughanandhan, D., Ramalingam, S. & Ragupathy, S. DNA barcoding detects contamination and substitution in North American herbal products. *BMC Med* **11**, 222, doi:10.1186/1741-7015-11-222 (2013).

24 Jung, J., Hermanns-Clausen, M. & Weinmann, W. Anorectic sibutramine detected in a Chinese herbal drug for weight loss. *Forensic Sci Int* **161**, 221-222, doi:10.1016/j.forsciint.2006.02.052 (2006).

25 Lam, P. K., Leung, K. S., Wong, T. W., Lee, H. H., Tang, M. H. & Mak, T. W. Serotonin syndrome following overdose of a non-prescription slimming product containing sibutramine: a case report. *Hum Exp Toxicol* **31**, 414-417, doi:10.1177/0960327111431704 (2012).

26 Lim, A., Cranswick, N. & South, M. Adverse events associated with the use of complementary and alternative medicine in children. *Archives of disease in childhood* **96**, 297-300, doi:10.1136/adc.2010.183152 (2011).

27 Reeuwijk, N. M., Venhuis, B. J., de Kaste, D., Hoogenboom, R. L., Rietjens, I. M. & Martena, M. J. Active pharmaceutical ingredients detected in herbal food supplements for weight loss sampled on the Dutch market. *Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment* **31**, 1783-1793, doi:10.1080/19440049.2014.958574 (2014).

28 Amster, E., Tiwary, A. & Schenker, M. B. Case report: potential arsenic toxicosis secondary to herbal kelp supplement. *Environ Health Perspect* **115**, 606-608, doi:10.1289/ehp.9495 (2007).

29 Posadzki, P., Watson, L. & Ernst, E. Contamination and adulteration of herbal medicinal products (HMPs): an overview of systematic reviews. *Eur J Clin Pharmacol* **69**, 295-307, doi:10.1007/s00228-012-1353-z (2013).

30 Gilbert, J. D., Musgrave, I. F., Hoban, C. & Byard, R. W. Lethal hepatocellular necrosis associated with herbal polypharmacy in a patient with chronic hepatitis B infection. *Forensic Sci Int* **241**, 138-140, doi:10.1016/j.forsciint.2014.05.021 (2014).

31 Everstine, K., Spink, J. & Kennedy, S. Economically motivated adulteration (EMA) of food: common characteristics of EMA incidents. *J Food Prot* **76**, 723-735, doi:10.4315/0362-028X.JFP-12-399 (2013).

32 TGA, A. G. D. o. H. a. A. T. G. A. TGA reforms: A blueprint for TGA's future. (2011)

33 Wardle, J., Weir, M., Marshall, B. & Archer, E. Regulatory and legislative protections for consumers in complementary medicine: Lessons from Australian policy and legal developments. *European Journal of Integrative Medicine* **6**, 423-433, doi:10.1016/j.eujim.2014.03.008 (2014).

34 Barnes, J., McLachlan, A. J., Sherwin, C. M. & Enioutina, E. Y. Herbal medicines: challenges in the modern world. Part 1. Australia and New Zealand. *Expert Rev Clin Pharmacol* **9**, 905-915, doi:10.1586/17512433.2016.1171712 (2016).

35 Job, K. M., Kiang, T. K., Constance, J. E., Sherwin, C. M. & Enioutina, E. Y. Herbal medicines: challenges in the modern world. Part 4. Canada and United States. *Expert Rev Clin Pharmacol* **9**, 1597-1609, doi:10.1080/17512433.2016.1238762 (2016).

36 Huang, W. F., Wen, K. C. & Hsiao, M. L. Adulteration by synthetic therapeutic substances of traditional Chinese medicines in Taiwan. *J Clin Pharmacol* **37**, 344-350, doi:10.1002/j.1552-4604.1997.tb04312.x (1997).

37 Liang, Q., Qu, J., Luo, G. & Wang, Y. Rapid and reliable determination of illegal adulterant in herbal medicines and dietary supplements by LC/MS/MS. *J Pharm Biomed Anal* **40**, 305-311, doi:10.1016/j.jpba.2005.07.035 (2006).

38 Poon, W. T., Lam, Y. H., Lee, H. H., Ching, C. K., Chan, W. T., Chan, S. S., Lai, C. K., Tse, M. L., Chan, A. Y. & Mak, T. W. Outbreak of hypoglycaemia: sexual enhancement products containing oral hypoglycaemic agent. *Hong Kong medical journal = Xianggang yi xue za zhi / Hong Kong Academy of Medicine* **15**, 196-200 (2009).

39 Campbell, N., Clark, J. P., Stecher, V. J., Thomas, J. W., Callanan, A. C., Donnelly, B. F., Goldstein, I. & Kaminetsky, J. C. Adulteration of purported herbal and natural sexual performance enhancement dietary supplements with synthetic phosphodiesterase type 5 inhibitors. *J Sex Med* **10**, 1842-1849, doi:10.1111/jsm.12172 (2013).

40 Gratz, S. R., Flurer, C. L. & Wolnik, K. A. Analysis of undeclared synthetic phosphodiesterase-5 inhibitors in dietary supplements and herbal matrices by LC-ESI-MS and LC-UV. *J Pharm Biomed Anal* **36**, 525-533, doi:10.1016/j.jpba.2004.07.004 (2004).

41 Savaliya, A. A., Prasad, B., Raijada, D. K. & Singh, S. Detection and characterization of synthetic steroidal and non-steroidal anti-inflammatory drugs in Indian ayurvedic/herbal products using LC-MS/TOF. *Drug testing and analysis* **1**, 372-381, doi:10.1002/dta.75 (2009).

42 Kim, H. J., Lee, J. H., Park, H. J., Cho, S. H., Cho, S. & Kim, W. S. Monitoring of 29 weight loss compounds in foods and dietary supplements by LC-MS/MS. *Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment* **31**, 777-783, doi:10.1080/19440049.2014.888497 (2014).

43 Kim, H. J., Lee, J. H., Park, H. J., Kim, J. Y., Cho, S. & Kim, W. S. Determination of non-opioid analgesics in adulterated food and dietary supplements by LC-MS/MS. *Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment* **31**, 973-978, doi:10.1080/19440049.2014.908262 (2014).

44 Bogusz, M. J., Hassan, H., Al-Enazi, E., Ibrahim, Z. & Al-Tufail, M. Application of LC-ESI-MS-MS for detection of synthetic adulterants in herbal remedies. *J Pharm Biomed Anal* **41**, 554-564, doi:10.1016/j.jpba.2005.12.015 (2006).

45 Wang, Z., Wu, C., Wang, G., Zhang, Q. & Zhang, J. Novel strategy for the determination of illegal adulterants in health foods and herbal medicines using high-performance liquid chromatography with high-resolution mass spectrometry. *J Sep Sci* **38**, 925-935, doi:10.1002/jssc.201401134 (2015).

46 Miller, G. M. & Stripp, R. A study of western pharmaceuticals contained within samples of Chinese herbal/patent medicines collected from New York City's Chinatown. *Leg Med (Tokyo)* **9**, 258-264, doi:10.1016/j.legalmed.2007.04.001 (2007).

47 Liu, S. Y., Woo, S. O., Holmes, M. J. & Koh, H. L. LC and LC-MS-MS analyses of undeclared codeine in antiasthmatic Chinese proprietary medicine. *J Pharm Biomed Anal* **22**, 481-486, doi:10.1016/s0731-7085(99)00309-x (2000).

48 Panusa, A., Multari, G., Incarnato, G. & Gagliardi, L. High-performance liquid chromatography analysis of anti-inflammatory pharmaceuticals with ultraviolet and electrospray-mass spectrometry detection in suspected counterfeit homeopathic medicinal products. *J Pharm Biomed Anal* **43**, 1221-1227, doi:10.1016/j.jpba.2006.10.012 (2007).

49 Wiest, J., Schollmayer, C., Gresser, G. & Holzgrabe, U. Identification and quantitation of the ingredients in a counterfeit Vietnamese herbal medicine against rheumatic diseases. *J Pharm Biomed Anal* **97**, 24-28, doi:10.1016/j.jpba.2014.04.013 (2014).

50 Gilard, V., Balayssac, S., Tinaugus, A., Martins, N., Martino, R. & Malet-Martino, M. Detection, identification and quantification by 1H NMR of adulterants in 150 herbal dietary supplements marketed for improving sexual performance. *J Pharm Biomed Anal* **102**, 476-493, doi:10.1016/j.jpba.2014.10.011 (2015).

51 Musah, R. A., Domin, M. A., Walling, M. A. & Shepard, J. R. Rapid identification of synthetic cannabinoids in herbal samples via direct analysis in real time mass spectrometry. *Rapid Commun Mass Spectrom* **26**, 1109-1114, doi:10.1002/rcm.6205 (2012).

52 Wong, M. Y., So, P. K. & Yao, Z. P. Direct analysis of traditional Chinese medicines by mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* **1026**, 2-14, doi:10.1016/j.jchromb.2015.11.032 (2016).

53 Yang, Y. & Deng, J. Analysis of pharmaceutical products and herbal medicines using ambient mass spectrometry. *TrAC Trends in Analytical Chemistry* **82**, 68-88, doi:10.1016/j.trac.2016.04.011 (2016).

54 Huang, Y. Q., You, J. Q., Zhang, J., Sun, W., Ding, L. & Feng, Y. Q. Coupling frontal elution paper chromatography with desorption corona beam ionization mass spectrometry for rapid analysis of chlorphenamine in herbal medicines and dietary supplements. *J Chromatogr A* **1218**, 7371-7376, doi:10.1016/j.chroma.2011.08.067 (2011).

55 Cho, S. H., Park, H. J., Lee, J. H., Kim, H. J., Cho, S., Yoon, C. Y. & Kim, W. S. Monitoring of 35 illegally added steroid compounds in foods and dietary supplements. *Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment* **31**, 1470-1475, doi:10.1080/19440049.2014.946100 (2014).

56 Li, N., Cui, M., Lu, X., Qin, F., Jiang, K. & Li, F. A rapid and reliable UPLC-MS/MS method for the identification and quantification of fourteen synthetic anti-diabetic drugs in adulterated Chinese proprietary medicines and dietary supplements. *Biomed Chromatogr* **24**, 1255-1261, doi:10.1002/bmc.1438 (2010).

57 Haneef, J., Shaharyar, M., Husain, A., Rashid, M., Mishra, R., Siddique, N. A. & Pal, M. Analytical methods for the detection of undeclared synthetic drugs in traditional herbal medicines as adulterants. *Drug testing and analysis* **5**, 607-613, doi:10.1002/dta.1482 (2013).

58 Vaclavik, L., Krynitsky, A. J. & Rader, J. I. Mass spectrometric analysis of pharmaceutical adulterants in products labeled as botanical dietary supplements or herbal remedies: a review. *Analytical and bioanalytical chemistry* **406**, 6767-6790, doi:10.1007/s00216-014-8159-z (2014).

59 Peters, F. T. Recent advances of liquid chromatography-(tandem) mass spectrometry in clinical and forensic toxicology. *Clin Biochem* **44**, 54-65, doi:10.1016/j.clinbiochem.2010.08.008 (2011).

60 Cohen, P. A., Travis, J. C. & Venhuis, B. J. A methamphetamine analog (N,alpha-diethyl-phenylethylamine) identified in a mainstream dietary supplement. *Drug testing and analysis* **6**, 805-807, doi:10.1002/dta.1578 (2014).

61 Cohen, P. A., Travis, J. C. & Venhuis, B. J. A synthetic stimulant never tested in humans, 1,3-dimethylbutylamine (DMBA), is identified in multiple dietary supplements. *Drug testing and analysis* **7**, 83-87, doi:10.1002/dta.1735 (2015).

62 Ko, R. J. Adulterants in Asian patent medicines. *N Engl J Med* **339**, 847, doi:10.1056/nejm199809173391214 (1998).

63 Necyk, C., Barnes, J., Tsuyuki, R. T., Boon, H. & Vohra, S. How well do pharmacists know their patients? A case report highlighting natural health product disclosure. *Can Pharm J (Ott)* **146**, 202-209, doi:10.1177/1715163513493387 (2013).

64 Giveon, S. M., Liberman, N., Klang, S. & Kahan, E. Are people who use "natural drugs" aware of their potentially harmful side effects and reporting to family physician? *Patient education and counseling* **53**, 5-11, doi:10.1016/S0738-3991(03)00241-6 (2004).

65 Chen, S. P., Tang, M. H., Ng, S. W., Poon, W. T., Chan, A. Y. & Mak, T. W. Psychosis associated with usage of herbal slimming products adulterated with sibutramine: a case series. *Clin Toxicol (Phila)* **48**, 832-838, doi:10.3109/15563650.2010.517208 (2010).

66 Barnes, J., Mills, S. Y., Abbot, N. C., Willoughby, M. & Ernst, E. Different standards for reporting ADRs to herbal remedies and conventional OTC medicines: face-to-face interviews with 515 users of herbal remedies. *Br J Clin Pharmacol* **45**, 496-500, doi:10.1046/j.1365-2125.1998.00715.x (1998).

67 Braun, L. A., Tiralongo, E., Wilkinson, J. M., Poole, S., Spitzer, O., Bailey, M. & Dooley, M. Adverse reactions to complementary medicines: the Australian pharmacy experience. *Int J Pharm Pract* **18**, 242-244, doi:10.1111/j.2042-7174.2010.00036.x (2010).

68 Vohra, S., Cvijovic, K., Boon, H., Foster, B. C., Jaeger, W., LeGatt, D., Cembrowski, G., Murty, M., Tsuyuki, R. T., Barnes, J., Charrois, T. L., Arnason, J. T., Necyk, C., Ware, M. & Rosychuk, R. J. Study of natural health product adverse reactions (SONAR): active surveillance of adverse events following concurrent natural health product and

prescription drug use in community pharmacies. *PLoS One* **7**, e45196, doi:10.1371/journal.pone.0045196 (2012).

69 Lau, A.-J., Holmes, M. J., Woo, S.-O. & Koh, H.-L. Analysis of adulterants in a traditional herbal medicinal product using liquid chromatography–mass spectrometry–mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis* **31**, 401-406, doi:10.1016/s0731-7085(02)00637-4 (2003).

70 Ren, Y., Wu, C. & Zhang, J. Simultaneous screening and determination of 18 illegal adulterants in herbal medicines and health foods for male sexual potency by ultra-fast liquid chromatography-electrospray ionization tandem mass spectrometry. *J Sep Sci* **35**, 2847-2857, doi:10.1002/jssc.201200280 (2012).

71 Wang, J., Chen, B. & Yao, S. Analysis of six synthetic adulterants in herbal weight-reducing dietary supplements by LC electrospray ionization-MS. *Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment* **25**, 822-830, doi:10.1080/02652030801946553 (2008).

72 Guo, C., Shi, F., Jiang, S., Gong, L., Zhao, Y., Zhang, J. & Zeng, S. Simultaneous identification, confirmation and quantitation of illegal adulterated antidiabetics in herbal medicines and dietary supplements using high-resolution benchtop quadrupole-Orbitrap mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* **967**, 174-182, doi:10.1016/j.jchromb.2014.07.032 (2014).

73 Lu, Y. L., Zhou, N. L., Liao, S. Y., Su, N., He, D. X., Tian, Q. Q., Chen, B. & Yao, S. Z. Detection of adulteration of anti-hypertension dietary supplements and traditional Chinese medicines with synthetic drugs using LC/MS. *Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment* **27**, 893-902, doi:10.1080/19440040903426710 (2010).

74 Tait, P. A., Vora, A., James, S., Fitzgerald, D. J. & Pester, B. A. Severe congenital lead poisoning in a preterm infant due to a herbal remedy. *Med J Aust* **177**, 193-195 (2002).

75 Tang, M. H., Chen, S. P., Ng, S. W., Chan, A. Y. & Mak, T. W. Case series on a diversity of illicit weight-reducing agents: from the well known to the unexpected. *Br J Clin Pharmacol* **71**, 250-253, doi:10.1111/j.1365-2125.2010.03822.x (2011).

76 Wood, D. M., Athwal, S. & Panahloo, A. The advantages and disadvantages of a 'herbal' medicine in a patient with diabetes mellitus: a case report. *Diabet Med* **21**, 625-627, doi:10.1111/j.1464-5491.2004.01202.x (2004).

77 Ching, C. K., Lam, Y. H., Chan, A. Y. & Mak, T. W. Adulteration of herbal antidiabetic products with undeclared pharmaceuticals: a case series in Hong Kong. *Br J Clin Pharmacol* **73**, 795-800, doi:10.1111/j.1365-2125.2011.04135.x (2012).

78 Xue, C. C., Zhang, A. L., Lin, V., Da Costa, C. & Story, D. F. Complementary and alternative medicine use in Australia: a national population-based survey. *J Altern Complement Med* **13**, 643-650, doi:10.1089/acm.2006.6355 (2007).

79 Hoban, C. L., Byard, R. W. & Musgrave, I. F. A comparison of patterns of spontaneous adverse drug reaction reporting with St. John's Wort and fluoxetine during the period 2000-2013. *Clin Exp Pharmacol Physiol* **42**, 747-751, doi:10.1111/1440-1681.12424 (2015).

80 Spinks, J., Hollingsworth, B., Manderson, L., Lin, V. & Canaway, R. Costs and drivers of complementary and alternative medicine (CAM) use in people with type 2 diabetes or cardiovascular disease. *European Journal of Integrative Medicine* **5**, 44-53, doi:10.1016/j.eujim.2012.08.003 (2013).

81 Bailey, R. L., Gahche, J. J., Miller, P. E., Thomas, P. R. & Dwyer, J. T. Why US adults use dietary supplements. *JAMA Intern Med* **173**, 355-361, doi:10.1001/jamainternmed.2013.2299 (2013).

82 Cohen, P. A. & Ernst, E. Safety of herbal supplements: a guide for cardiologists. *Cardiovasc Ther* **28**, 246-253, doi:10.1111/j.1755-5922.2010.00193.x (2010).

83 TGA, A. G. D. o. H. a. A. T. G. A. *Complementary medicine regulation basics*, <https://www.tga.gov.au/complementary-medicine-regulation-basics> (

84 TGA, A. G. D. o. H. a. A. T. G. A. *Annual Performance Statistics Report July 2016 to June 2017*, <https://www.tga.gov.au/annual-performance-statistics-report-july-2016-june-2017> (2017).

85 Australian Government: Department of Health and Ageing, D. *Expert Review of Medicines and Medical Devices Regulation*, <http://www.health.gov.au/internet/main/publishing.nsf/content/expert-review-of-medicines-and-medical-devices-regulation> (2017).

86 TGA, A. G. D. o. H. a. A. T. G. A. *Complementary medicines reforms*, <https://www.tga.gov.au/complementary-medicines-reforms> (2018).

87 Australian Institute of Health and Welfare. *Australia's health series no. 16. AUS 221.*, <https://www.aihw.gov.au/getmedia/7c42913d-295f-4bc9-9c24-4e44eff4a04a/aihw-aus-221.pdf.aspx?inline=true%20%3E> (2018).

88 Research, P. M. *Dietary supplements market will reach US$ 179.8 Billion globally in 2020*, <https://www.persistencemarketresearch.com/mediarelease/dietary-supplements-market.asp> (2015).

89 Hoban, C. L., Musgrave, I. F., Coghlan, M. L., Power, M. W. P., Byard, R. W., Nash, C., Farrington, R., Maker, G., Crighton, E., Trengove, R. & Bunce, M. Adulterants and Contaminants in Psychotropic Herbal Medicines Detected with Mass Spectrometry and Next-Generation DNA Sequencing. *Pharmaceutical Medicine* **32**, 429-444, doi:10.1007/s40290-018-0252-8 (2018).

90 Newmaster, S. G., Shanmughanandhan, D., Kesanakurti, P., Shehata, H., Faller, A., Noce, I. D., Lee, J. Y., Rudzinski, P., Lu, Z., Zhang, Y., Swanson, G., Hanner, R. & Ragupathy, S. Recommendations for Validation of Real-Time PCR Methods for Molecular Diagnostic Identification of Botanicals. *J AOAC Int*, doi:10.5740/jaoacint.18-0321 (2019).

91 Alzohairy, M. A. Therapeutics Role of Azadirachta indica (Neem) and Their Active Constituents in Diseases Prevention and Treatment. *Evid Based Complement Alternat Med* **2016**, 7382506, doi:10.1155/2016/7382506 (2016).

92 Mishra, A. & Dave, N. Neem oil poisoning: Case report of an adult with toxic encephalopathy. *Indian J Crit Care Med* **17**, 321-322, doi:10.4103/0972-5229.120330 (2013).

93 Yang, F., Ding, F., Chen, H., He, M., Zhu, S., Ma, X., Jiang, L. & Li, H. DNA Barcoding for the Identification and Authentication of Animal Species in Traditional Medicine. *Evid Based Complement Alternat Med* **2018**, 5160254, doi:10.1155/2018/5160254 (2018).

94 Raclariu, A. C., Heinrich, M., Ichim, M. C. & de Boer, H. Benefits and Limitations of DNA Barcoding and Metabarcoding in Herbal Product Authentication. *Phytochem Anal* **29**, 123-128, doi:10.1002/pca.2732 (2018).

95 Nawrot, P., Jordan, S., Eastwood, J., Rotstein, J., Hugenholtz, A. & Feeley, M. Effects of caffeine on human health. *Food Addit Contam* **20**, 1-30, doi:10.1080/0265203021000007840 (2003).

96 Greenway, F. L. The safety and efficacy of pharmaceutical and herbal caffeine and ephedrine use as a weight loss agent. *Obesity Reviews* **2**, 199-211, doi:10.1046/j.1467-789x.2001.00038.x (2001).
97 TGA, A. G. D. o. H. a. A. T. G. A. Substances that may be used in Listed medicines in Australia. (2007)
98 Barnes, J., Anderson, L. A. & Phillipson, J. D. *Herbal Medicines*. 2nd edn, (Pharmaceutical Press, 2002).
99 Haller, C. A. & Benowitz, N. L. Adverse cardiovascular and central nervous system events associated with dietary supplements containing ephedra alkaloids. *N Engl J Med* **343**, 1833-1838, doi:10.1056/NEJM200012213432502 (2000).
100 Kay, G. G. The effects of antihistamines on cognition and performance. *J Allergy Clin Immunol* **105**, S622-627, doi:10.1067/mai.2000.106153 (2000).
101 Church, M. K., Maurer, M., Simons, F. E., Bindslev-Jensen, C., van Cauwenberge, P., Bousquet, J., Holgate, S. T., Zuberbier, T., Global, A. & Asthma European, N. Risk of first-generation H(1)-antihistamines: a GA(2)LEN position paper. *Allergy* **65**, 459-466, doi:10.1111/j.1398-9995.2009.02325.x (2010).
102 Rocha, T., Amaral, J. S. & Oliveira, M. B. P. P. Adulteration of Dietary Supplements by the Illegal Addition of Synthetic Drugs: A Review. *Comprehensive Reviews in Food Science and Food Safety* **15**, 43-62, doi:10.1111/1541-4337.12173 (2016).
103 Ritchie, M. R. Use of herbal supplements and nutritional supplements in the UK: what do we know about their pattern of usage? *Proc Nutr Soc* **66**, 479-482, doi:10.1017/S0029665107005794 (2007).
104 Cohen, P. A., Bloszies, C., Yee, C. & Gerona, R. An amphetamine isomer whose efficacy and safety in humans has never been studied, beta-methylphenylethylamine (BMPEA), is found in multiple dietary supplements. *Drug testing and analysis* **8**, 328-333, doi:10.1002/dta.1793 (2016).
105 Winter, G. T., Wilhide, J. A. & LaCourse, W. R. Characterization of a Direct Sample Analysis (DSA) Ambient Ionization Source. *J Am Soc Mass Spectrom* **26**, 1502-1507, doi:10.1007/s13361-015-1175-1 (2015).
106 Botch-Jones, S., Foss, J., Barajas, D., Kero, F., Young, C. & Weisenseel, J. The detection of NBOMe designer drugs on blotter paper by high resolution time-of-flight mass spectrometry (TOFMS) with and without chromatography. *Forensic Sci Int* **267**, 89-95, doi:10.1016/j.forsciint.2016.08.008 (2016).
107 Loos, M., Gerber, C., Corona, F., Hollender, J. & Singer, H. Accelerated isotope fine structure calculation using pruned transition trees. *Anal Chem* **87**, 5738-5744, doi:10.1021/acs.analchem.5b00941 (2015).
108 Bhagwat, S. & Haytowitz, D. *USDA Database for the Flavonoid Content of Selected Foods, Release 3.2*, <Nutrient Data Laboratory Home Page: http://www.ars.usda.gov/nutrientdata/flav> (2015).
109 TGA, A. G. D. o. H. a. A. T. G. A. *Current year alerts*, <https://www.tga.gov.au/current-year-alerts> (2018).
110 De Vijlder, T., Valkenborg, D., Lemiere, F., Romijn, E. P., Laukens, K. & Cuyckens, F. A tutorial in small molecule identification via electrospray ionization-mass spectrometry: The practical art of structural elucidation. *Mass Spectrom Rev* **37**, 607-629, doi:10.1002/mas.21551 (2018).

111 Boyle, S. P., Doolan, P. J., Andrews, C. E. & Reid, R. G. Evaluation of quality control strategies in Scutellaria herbal medicines. *J Pharm Biomed Anal* **54**, 951-957, doi:10.1016/j.jpba.2010.11.019 (2011).
112 TGA, A. G. D. o. H. a. A. T. G. A. *An overview of the regulation of complementary medicines in Australia*, <https://www.tga.gov.au/overview-regulation-complementary-medicines-australia> (2013).
113 Cohen, M. & Hunter, J. Complementary medicine products: interpreting the evidence base. *Internal Medicine Journal* **47**, 992-998, doi:10.1111/imj.13534 (2017).
114 Cohen, P. A., Avula, B., Venhuis, B., Travis, J. C., Wang, Y.-H. & Khan, I. A. Pharmaceutical doses of the banned stimulant oxilofrine found in dietary supplements sold in the USA. *Drug testing and analysis* **9**, 135-142, doi:10.1002/dta.1976 (2017).
115 Navarro, V. J., Khan, I., Björnsson, E., Seeff, L. B., Serrano, J. & Hoofnagle, J. H. Liver injury from herbal and dietary supplements. *Hepatology* **65**, 363-373, doi:10.1002/hep.28813 (2017).
116 Crighton, E., Weisenseel, J., Bunce, M., Musgrave, I. F., Trengove, R. & Maker, G. Exploring the Application of the DSA-TOF, a Direct, High-resolution Time-of-Flight Mass Spectrometry Technique for the Screening of Potential Adulterated and Contaminated Herbal Medicines. *J Am Soc Mass Spectrom* **30**, 1713-1719, doi:10.1007/s13361-019-02256-w (2019).
117 Health Technologies Section, A. T. B., Department of Industry, Innovation and Science *Pharmaceuticals Industry Profile*, <http://www.industry.gov.au/industry/IndustrySectors/PharmaceuticalsandHealthTechnologies/Pharmaceuticals/Pages/PharmaceuticalsIndustryProfile.aspx> (2015).
118 Gilbert, N. Regulations: Herbal medicine rule book. *Nature* **480**, S98-99, doi:10.1038/480S98a (2011).
119 Cohen, P. A., Venhuis, B. J. & Brandt, S. D. Advancing supplement science: challenges and solutions. *Drug testing and analysis* **8**, e1-3, doi:10.1002/dta.1953 (2016).
120 Dettmer, K., Aronov, P. A. & Hammock, B. D. Mass spectrometry-based metabolomics. *Mass Spectrom Rev* **26**, 51-78, doi:10.1002/mas.20108 (2007).
121 Dunn, W. B. Current trends and future requirements for the mass spectrometric investigation of microbial, mammalian and plant metabolomes. *Phys Biol* **5**, 011001, doi:10.1088/1478-3975/5/1/011001 (2008).
122 Dunn, W. B., Erban, A., Weber, R. J. M., Creek, D. J., Brown, M., Breitling, R., Hankemeier, T., Goodacre, R., Neumann, S., Kopka, J. & Viant, M. R. Mass appeal: metabolite identification in mass spectrometry-focused untargeted metabolomics. *Metabolomics* **9**, 44-66, doi:10.1007/s11306-012-0434-4 (2012).
123 Farag, M. A. & Wessjohann, L. A. Metabolome classification of commercial Hypericum perforatum (St. John's Wort) preparations via UPLC-qTOF-MS and chemometrics. *Planta Med* **78**, 488-496, doi:10.1055/s-0031-1298170 (2012).
124 Heinrich, M. Quality and safety of herbal medical products: regulation and the need for quality assurance along the value chains. *Br J Clin Pharmacol* **80**, 62-66, doi:10.1111/bcp.12586 (2015).
125 Chan, E. C., Yap, S. L., Lau, A. J., Leow, P. C., Toh, D. F. & Koh, H. L. Ultra-performance liquid chromatography/time-of-flight mass spectrometry based metabolomics of raw and steamed Panax notoginseng. *Rapid Commun Mass Spectrom* **21**, 519-528, doi:10.1002/rcm.2864 (2007).

126 Zhao, H., Xu, J., Ghebrezadik, H. & Hylands, P. J. Metabolomic quality control of commercial Asian ginseng, and cultivated and wild American ginseng using (1)H NMR and multi-step PCA. *J Pharm Biomed Anal* **114**, 113-120, doi:10.1016/j.jpba.2015.05.010 (2015).
127 Xin, G. Z., Hu, B., Shi, Z. Q., Zheng, J. Y., Wang, L., Chang, W. Q., Li, P., Yao, Z. & Liu, L. F. A direct ionization mass spectrometry-based approach for differentiation of medicinal Ephedra species. *J Pharm Biomed Anal* **117**, 492-498, doi:10.1016/j.jpba.2015.09.032 (2016).
128 TGA, A. G. D. o. H. T. G. A. *Standards, guidelines & publications (complementary medicines)*, <https://www.tga.gov.au/node/4238> (2016).
129 Li, X., Chen, H., Jia, W. & Xie, G. A metabolomics-based strategy for the quality control of traditional chinese medicine: shengmai injection as a case study. *Evid Based Complement Alternat Med* **2013**, 836179, doi:10.1155/2013/836179 (2013).
130 Bilia, A. R. Science meets regulation. *J Ethnopharmacol* **158 Pt B**, 487-494, doi:10.1016/j.jep.2014.06.036 (2014).
131 Gao, W., Yang, H., Qi, L. W., Liu, E. H., Ren, M. T., Yan, Y. T., Chen, J. & Li, P. Unbiased metabolite profiling by liquid chromatography-quadrupole time-of-flight mass spectrometry and multivariate data analysis for herbal authentication: classification of seven Lonicera species flower buds. *J Chromatogr A* **1245**, 109-116, doi:10.1016/j.chroma.2012.05.027 (2012).
132 Mari, A., Lyon, D., Fragner, L., Montoro, P., Piacente, S., Wienkoop, S., Egelhofer, V. & Weckwerth, W. Phytochemical composition of Potentilla anserina L. analyzed by an integrative GC-MS and LC-MS metabolomics platform. *Metabolomics* **9**, 599-607, doi:10.1007/s11306-012-0473-x (2013).
133 Wishart, D. S. Emerging applications of metabolomics in drug discovery and precision medicine. *Nat Rev Drug Discov* **15**, 473-484, doi:10.1038/nrd.2016.32 (2016).
134 Patel, S. & Ahmed, S. Emerging field of metabolomics: big promise for cancer biomarker identification and drug discovery. *J Pharm Biomed Anal* **107**, 63-74, doi:10.1016/j.jpba.2014.12.020 (2015).
135 Park, H. W., In, G., Kim, J. H., Cho, B. G., Han, G. H. & Chang, I. M. Metabolomic approach for discrimination of processed ginseng genus (Panax ginseng and Panax quinquefolius) using UPLC-QTOF MS. *J Ginseng Res* **38**, 59-65, doi:10.1016/j.jgr.2013.11.011 (2014).
136 Bouhifd, M., Hartung, T., Hogberg, H. T., Kleensang, A. & Zhao, L. Review: toxicometabolomics. *J Appl Toxicol* **33**, 1365-1383, doi:10.1002/jat.2874 (2013).
137 Francki, M. G., Hayton, S., Gummer, J. P., Rawlinson, C. & Trengove, R. D. Metabolomic profiling and genomic analysis of wheat aneuploid lines to identify genes controlling biochemical pathways in mature grain. *Plant Biotechnol J* **14**, 649-660, doi:10.1111/pbi.12410 (2016).
138 Lv, M., Sun, J., Wang, M., Huang, W., Fan, H., Xu, F. & Zhang, Z. GC-MS based metabolomics study of stems and roots of Ephedra sinica. *J Pharm Biomed Anal* **114**, 49-52, doi:10.1016/j.jpba.2015.04.035 (2015).
139 Kim, N., Kim, K., Choi, B. Y., Lee, D., Shin, Y. S., Bang, K. H., Cha, S. W., Lee, J. W., Choi, H. K., Jang, D. S. & Lee, D. Metabolomic approach for age discrimination of Panax ginseng using UPLC-Q-Tof MS. *J Agric Food Chem* **59**, 10435-10441, doi:10.1021/jf201718r (2011).

140 Lee, D. Y., Kim, J. K., Shrestha, S., Seo, K. H., Lee, Y. H., Noh, H. J., Kim, G. S., Kim, Y. B., Kim, S. Y. & Baek, N. I. Quality evaluation of Panax ginseng roots using a rapid resolution LC-QTOF/MS-based metabolomics approach. *Molecules (Basel, Switzerland)* **18**, 14849-14861, doi:10.3390/molecules181214849 (2013).

141 Nguyen, H. T., Lee, D. K., Choi, Y. G., Min, J. E., Yoon, S. J., Yu, Y. H., Lim, J., Lee, J., Kwon, S. W. & Park, J. H. A 1H NMR-based metabolomics approach to evaluate the geographical authenticity of herbal medicine and its application in building a model effectively assessing the mixing proportion of intentional admixtures: A case study of Panax ginseng: Metabolomics for the authenticity of herbal medicine. *J Pharm Biomed Anal* **124**, 120-128, doi:10.1016/j.jpba.2016.02.028 (2016).

142 Attele, A. S., Wu, J. A. & Yuan, C. S. Ginseng pharmacology: multiple constituents and multiple actions. *Biochem Pharmacol* **58**, 1685-1693, doi:10.1016/s0006-2952(99)00212-9 (1999).

143 Yu, C., Wang, C.-Z., Zhou, C.-J., Wang, B., Han, L., Zhang, C.-F., Wu, X.-H. & Yuan, C.-S. Adulteration and cultivation region identification of American ginseng using HPLC coupled with multivariate analysis. *Journal of Pharmaceutical and Biomedical Analysis* **99**, 8-15, doi:http://dx.doi.org/10.1016/j.jpba.2014.06.031 (2014).

144 Fuhrer, T. & Zamboni, N. High-throughput discovery metabolomics. *Curr Opin Biotechnol* **31**, 73-78, doi:10.1016/j.copbio.2014.08.006 (2015).

145 Balog, J., Perenyi, D., Guallar-Hoyas, C., Egri, A., Pringle, S. D., Stead, S., Chevallier, O. P., Elliott, C. T. & Takats, Z. Identification of the Species of Origin for Meat Products by Rapid Evaporative Ionization Mass Spectrometry. *J Agric Food Chem* **64**, 4793-4800, doi:10.1021/acs.jafc.6b01041 (2016).

146 Butterweck, V. & Schmidt, M. St. John's wort: role of active compounds for its mechanism of action and efficacy. *Wien Med Wochenschr* **157**, 356-361, doi:10.1007/s10354-007-0440-8 (2007).

147 Fiehn, O. Metabolomics - the link between genotypes and phenotypes. *Plant Mol Biol* **48**, 155-171, doi:10.1023/a:1013713905833 (2002).

148 Creek, D. J., Dunn, W. B., Fiehn, O., Griffin, J. L., Hall, R. D., Lei, Z., Mistrik, R., Neumann, S., Schymanski, E. L., Sumner, L. W., Trengove, R. & Wolfender, J.-L. Metabolite identification: are you sure? And how do your peers gauge your confidence? *Metabolomics* **10**, 350-353, doi:10.1007/s11306-014-0656-8 (2014).

149 Lei, Z., Huhman, D. V. & Sumner, L. W. Mass spectrometry strategies in metabolomics. *J Biol Chem* **286**, 25435-25442, doi:10.1074/jbc.R111.238691 (2011).

150 Tian, P. Convergence: Where West meets East. *Nature* **480**, S84-86, doi:10.1038/480S84a (2011).

151 Ouedraogo, M., Baudoux, T., Stevigny, C., Nortier, J., Colet, J. M., Efferth, T., Qu, F., Zhou, J., Chan, K., Shaw, D., Pelkonen, O. & Duez, P. Review of current and "omics" methods for assessing the toxicity (genotoxicity, teratogenicity and nephrotoxicity) of herbal medicines and mushrooms. *J Ethnopharmacol* **140**, 492-512, doi:10.1016/j.jep.2012.01.059 (2012).

152 Liu, H., Wang, J., Zhou, W., Wang, Y. & Yang, L. Systems approaches and polypharmacology for drug discovery from herbal medicines: an example using licorice. *J Ethnopharmacol* **146**, 773-793, doi:10.1016/j.jep.2013.02.004 (2013).

153 Sarma, N., Giancaspro, G. & Venema, J. Dietary supplements quality analysis tools from the United States Pharmacopeia. *Drug testing and analysis* **8**, 418-423, doi:10.1002/dta.1940 (2016).

154 Mattoli, L., Burico, M., Fodaroni, G., Tamimi, S., Bedont, S., Traldi, P. & Stocchero, M. New frontiers in pharmaceutical analysis: A metabolomic approach to check batch compliance of complex products based on natural substances. *Journal of Pharmaceutical and Biomedical Analysis* **126**, 156-162, doi:https://doi.org/10.1016/j.jpba.2016.04.010 (2016).

155 Guo, L.-X., Li, R., Liu, K., Yang, J., Li, H.-J., Li, S.-L., Liu, J.-Q., Liu, L.-F. & Xin, G.-Z. Structural characterization and discrimination of Chinese medicinal materials with multiple botanical origins based on metabolite profiling and chemometrics analysis: Clematidis Radix et Rhizoma as a case study. *Journal of Chromatography A* **1425**, 129-140, doi:http://dx.doi.org/10.1016/j.chroma.2015.11.013 (2015).

156 Liu, J., Liu, Y., Wang, Y., Abozeid, A., Zu, Y.-G. & Tang, Z.-H. The integration of GC–MS and LC–MS to assay the metabolomics profiling in Panax ginseng and Panax quinquefolius reveals a tissue- and species-specific connectivity of primary metabolites and ginsenosides accumulation. *Journal of Pharmaceutical and Biomedical Analysis* **135**, 176-185, doi:https://doi.org/10.1016/j.jpba.2016.12.026 (2017).

157 Wang, M., Lamers, R. J., Korthout, H. A., van Nesselrooij, J. H., Witkamp, R. F., van der Heijden, R., Voshol, P. J., Havekes, L. M., Verpoorte, R. & van der Greef, J. Metabolomics in the context of systems biology: bridging traditional Chinese medicine and molecular pharmacology. *Phytotherapy research : PTR* **19**, 173-182, doi:10.1002/ptr.1624 (2005).

158 Wang, X., Zhang, A., Zhou, X., Liu, Q., Nan, Y., Guan, Y., Kong, L., Han, Y., Sun, H. & Yan, G. An integrated chinmedomics strategy for discovery of effective constituents from traditional herbal medicine. *Scientific Reports* **6**, 18997, doi:10.1038/srep18997 (2016).

159 Booker, A., Agapouda, A., Frommenwiler, D. A., Scotti, F., Reich, E. & Heinrich, M. St John's wort (Hypericum perforatum) products – an assessment of their authenticity and quality. *Phytomedicine* **40**, 158-164, doi:https://doi.org/10.1016/j.phymed.2017.12.012 (2018).

160 Booker, A., Jalil, B., Frommenwiler, D., Reich, E., Zhai, L., Kulic, Z. & Heinrich, M. The authenticity and quality of Rhodiola rosea products. *Phytomedicine* **23**, 754-762, doi:http://dx.doi.org/10.1016/j.phymed.2015.10.006 (2016).

161 Avula, B., Wang, Y. H., Isaac, G., Yuk, J., Wrona, M., Yu, K. & Khan, I. A. Metabolic Profiling of Hoodia, Chamomile, Terminalia Species and Evaluation of Commercial Preparations Using Ultrahigh-Performance Liquid Chromatography Quadrupole-Time-of-Flight Mass Spectrometry. *Planta Med*, doi:10.1055/s-0043-109239 (2017).

162 Deconinck, E., Sacré, P. Y., Courselle, P. & De Beer, J. O. Chemometrics and chromatographic fingerprints to discriminate and classify counterfeit medicines containing PDE-5 inhibitors. *Talanta* **100**, 123-133, doi:http://dx.doi.org/10.1016/j.talanta.2012.08.029 (2012).

163 Custers, D., Van Hoeck, E., Courselle, P., Apers, S. & Deconinck, E. Clustering and diagnostic modelling of slimming aids based on chromatographic and mass spectrometric fingerprints. *Drug testing and analysis* **9**, 230-242, doi:10.1002/dta.1964 (2017).

164 Jurgens, T. M., Whelan, A. M., Killian, L., Doucette, S., Kirk, S. & Foy, E. Green tea for weight loss and weight maintenance in overweight or obese adults. *Cochrane Database of Systematic Reviews*, doi:10.1002/14651858.CD008650.pub2 (2012).

165 Miao, X.-S., Metcalfe, C. D., Hao, C. & March, R. E. Electrospray ionization mass spectrometry of ginsenosides. *Journal of Mass Spectrometry* **37**, 495-506, doi:10.1002/jms.309 (2002).
166 Navratilova, K., Hrbek, V., Kratky, F., Hurkova, K., Tomaniova, M., Pulkrabova, J. & Hajslova, J. Green tea: Authentication of geographic origin based on UHPLC-HRMS fingerprints. *Journal of Food Composition and Analysis* **78**, 121-128, doi:https://doi.org/10.1016/j.jfca.2019.02.004 (2019).
167 Hu, J., Webster, D., Cao, J. & Shao, A. The safety of green tea and green tea extract consumption in adults – Results of a systematic review. *Regulatory Toxicology and Pharmacology* **95**, 412-433, doi:https://doi.org/10.1016/j.yrtph.2018.03.019 (2018).
168 Ramachandran, B., Jayavelu, S., Murhekar, K. & Rajkumar, T. Repeated dose studies with pure Epigallocatechin-3-gallate demonstrated dose and route dependant hepatotoxicity with associated dyslipidemia. *Toxicology Reports* **3**, 336-345, doi:https://doi.org/10.1016/j.toxrep.2016.03.001 (2016).
169 Wei, R., Wang, J., Su, M., Jia, E., Chen, S., Chen, T. & Ni, Y. Missing Value Imputation Approach for Mass Spectrometry-based Metabolomics Data. *Scientific Reports* **8**, 663, doi:10.1038/s41598-017-19120-0 (2018).
170 Xia, J. & Wishart, D. S. in *Current Protocols in Bioinformatics* (John Wiley & Sons, Inc., 2016).
171 Crighton, E., Mullaney, I., Trengove, R., Bunce, M. & Maker, G. The application of metabolomics for herbal medicine pharmacovigilance: a case study on ginseng. *Essays In Biochemistry* **60**, 429-435, doi:10.1042/ebc20160030 (2016).
172 Federico, A., Tiso, A. & Loguercio, C. A case of hepatotoxicity caused by green tea. *Free Radical Biology and Medicine* **43**, 474, doi:http://doi.org/10.1016/j.freeradbiomed.2007.05.010 (2007).
173 Isomura, T., Suzuki, S., Origasa, H., Hosono, A., Suzuki, M., Sawada, T., Terao, S., Muto, Y. & Koga, T. Liver-related safety assessment of green tea extracts in humans: a systematic review of randomized controlled trials. *Eur J Clin Nutr* **70**, 1221-1229, doi:10.1038/ejcn.2016.78 (2016).
174 Buriani, A., Garcia-Bermejo, M. L., Bosisio, E., Xu, Q., Li, H., Dong, X., Simmonds, M. S. J., Carrara, M., Tejedor, N., Lucio-Cazana, J. & Hylands, P. J. Omic techniques in systems biology approaches to traditional Chinese medicines research: Present and future. *Journal of Ethnopharmacology* **140**, 535-544 (2012).
175 Dan, M., Su, M., Gao, X., Zhao, T., Zhao, A., Xie, G., Qiu, Y., Zhou, M., Liu, Z. & Jia, W. Metabolite profiling of Panax notoginseng using UPLC–ESI-MS. *Phytochemistry* **69**, 2237-2244, doi:http://dx.doi.org/10.1016/j.phytochem.2008.04.015 (2008).
176 Styczynski, M. P., Moxley, J. F., Tong, L. V., Walther, J. L., Jensen, K. L. & Stephanopoulos, G. N. Systematic Identification of Conserved Metabolites in GC/MS Data for Metabolomics and Biomarker Discovery. *Analytical Chemistry* **79**, 966-973, doi:10.1021/ac0614846 (2007).
177 Kind, T. & Fiehn, O. Seven Golden Rules for heuristic filtering of molecular formulas obtained by accurate mass spectrometry. *BMC Bioinformatics* **8**, 1-20, doi:10.1186/1471-2105-8-105 (2007).
178 Kind, T. & Fiehn, O. Metabolomic database annotations via query of elemental compositions: Mass accuracy is insufficient even at less than 1 ppm. *BMC Bioinformatics* **7**, 1-10, doi:10.1186/1471-2105-7-234 (2006).

179 Hoban, C. L., Musgrave, I. F., Byard, R. W., Nash, C., Farrington, R., Maker, G., Crighton, E., Bunce, M. & Coghlan, M. Combined Liquid Chromatography-mass Spectrometry and Next-generation DNA Sequencing Detection of Adulterants and Contaminants in Analgesic and Anti-inflammatory Herbal Medicines. *Pharmaceut Med* **34**, 49-61, doi:10.1007/s40290-019-00314-y (2020).

180 Jin, P., Xu, S., Xu, W., He, X., Kuang, Y. & Hu, X. Screening and quantification of fourteen synthetic antidiabetic adulterants in herbal pharmaceuticals and health foods by HPLC and confirmation by LC-Q-TOF-MS/MS. *Food Additives & Contaminants: Part A* **37**, 11-18, doi:10.1080/19440049.2019.1675910 (2020).

181 Guo, C., Gong, L., Wang, W., Leng, J., Zhou, L., Xing, S., Zhao, Y., Xian, R., Zhang, X. & Shi, F. Rapid screening and identification of targeted or non-targeted antitussive adulterants in herbal medicines by Q-Orbitrap HRMS and screening database. *International Journal of Mass Spectrometry* **447**, 116250, doi:https://doi.org/10.1016/j.ijms.2019.116250 (2020).

182 Murch, S. J., Rupasinghe, H. P. V., Goodenowe, D. & Saxena, P. K. A metabolomic analysis of medicinal diversity in Huang-qin (*Scutellaria baicalensis* Georgi) genotypes: discovery of novel compounds. *Plant Cell Rep* **23**, 419-425 (2004).

183 Booker, A., Johnston, D. & Heinrich, M. Value chains of herbal medicines—Research needs and key challenges in the context of ethnopharmacology. *Journal of Ethnopharmacology* **140**, 624-633, doi:http://dx.doi.org/10.1016/j.jep.2012.01.039 (2012).

184 Crighton, E., Coghlan, M. L., Farrington, R., Hoban, C. L., Power, M. W. P., Nash, C., Mullaney, I., Byard, R. W., Trengove, R., Musgrave, I. F., Bunce, M. & Maker, G. Toxicological screening and DNA sequencing detects contamination and adulteration in regulated herbal medicines and supplements for diet, weight loss and cardiovascular health. *Journal of Pharmaceutical and Biomedical Analysis* **176**, 112834, doi:10.1016/j.jpba.2019.112834 (2019).

9. Appendix:

Supplementary Table 9-1: Internal standards used for LC-QTOF analysis at FSSA.

Compound	Concentration (mg/L)
Olanzapine D8	0.125
Oxycodone D3	0.050
Alprazolam D5	0.050
BFPMP	0.333
Quetiapine D8	0.125
Amphetamine D5	0.125
7-Aminoflunitrazepam D7	0.063
Diazepam D5	0.150
Oxazepam D5	0.175
Methamphetamine D5	0.100
Atropine D3	0.100
13C6 Amlodipine	0.097
ODM-Tramadol D6	0.200
Prazepam	0.250

Supplementary Table 9-2: Adulterants, contamination, undeclared ingredients, plant and animal families and genera detected in the CAM products tested in this study. Samples 78, 186 and 253 are same product but different batches, noted by "1" and "2". Samples 296 and 307 contained multiple tablets or pills. 'T.S. Neg.' stands for Negative Toxicology Screen.

Sample # ID	In ARTG database (Yes/ No)	Ingredients On Package	Toxicological data	DNA Plant	DNA Animal	Comments
1	Y	Euphausia superba				Nothing detected
4	N	Apium graveolens, Astragalus membranaceus, Avena sativa, Barosma betulina, Berberis vulgaris, Betacarotene, Biotin, Calcium ascorbate dihydrate, Calcium citrate, Calcium pantothenate, Camellia sinensis, Carica papaya, Centella asiatica, Cholecalciferol, Choline bitartrate, Chromium picolinate, Citrus bioflavonoids extract, Copper gluconate, Crataegus monogyna, Cyanocobalamin, Cynara scolymus, d-alpha tocopheryl acid succinate, Equisetum arvense, Ferrous fumarate, Foeniculum vulgare, Folic acid, Ginkgo biloba, Inositol, Lycopersicon esculentum, Lysine hydrochloride, Magnesium oxide-heavy, Manganese amino acid chelate, Nicotinamide, Panax ginseng, Petroselinum crispum, Potassium iodide, Potassium sulfate, Pyridoxine hydrochloride, Riboflavin, Selenomethionine, Serenoa repens, Silybum marianum, Smilax officinalis,	Caffeine	Apiaceae, Asteraceae, Araliaceae (includes Ginseng), Ericales (includes Tea), Ixoroideae (includes Coffee), Solanoideae, Rutaceae (includes Citrus), Pooideae (inculde Oats)		Expected Pharmaceutical, Confirmed Ingredients

Sample # ID	In ARTG database (Yes/ No)	Ingredients On Package	Toxicological data	DNA Plant	DNA Animal	Comments
		Spearmint oil, Thiamine hydrochloride, Turnera diffusa, Tyrosine, Ubidecarenone, Vaccinium myrtillus, Vitis vinifera, Zinc amino acid chelate, Zingiber officinale				
6	Y	Caralluma adscendens var. fimbriata		Asterids (includes C. fimbriata), Cyamopsis tetragonoloba (Guar plant), Acalypheae		T.S. Neg., Additional Plant DNA, Contains Fillers, Confirmed Ingredients
8	Y	Fig dry, Phoenix dactylifera, Senna alexandrina extract		Arecaceae (includes P. dactylifera), Fabaceae (includes S. alexandrina), Ficus (fig), Prunus, Brassicaceae, Poaceae (grass)	Canis lupus familiaris, Capra hircus, Sus scrofa scrofa	T.S. Neg., Animal Contaminants, Additional Plant DNA, Confirmed Ingredients
9	N	Fish Oil, Food Acids, Gelatin, Glucose, Natural Colours, Natural Flavours, Sugar, Vitamin E, Water				Nothing detected
10	Y	Alisma ago aquatica, Comus officinalis, Dioscorea opposita, Ophiopogon japonicus, Paeonia suffruticosa, Poria cocos, Rehmannia glutinosa, Schizandra chinensis		Paeonia (includes P. suffruticosa), PACMAD clade, Anthemideae, Apiaceae, Pteridium	Bos	T.S. Neg., Animal Contaminants, Additional Plant DNA, Confirmed Ingredients
15	Y	Ulmus rubra		Ulmus, Brassicaceae		T.S. Neg., Additional Plant DNA
22	N	Creosote, Citris Unshiu Peel, Gambir, Glycyra, Phellodendron	Synephrine 0.4 mg/g	Rutaceae, Glycyrrhiza		Expected Pharmaceutical, Confirmed Ingredients

Sample # ID	In ARTG database (Yes/ No)	Ingredients On Package	Toxicological data	DNA Plant	DNA Animal	Comments
23	N	Calcium Carbonate, Chromium Polynicotinate, Garcinia cambogia extract, Green coffee extract, Magnesium Stearate, Microcrystalline Cellulose, Moringa oleifera, Potassium Citrate, Silicon Dioxide	Caffeine	Amaranthaceae, Apiaceae, Poaceae		Expected Pharmaceutical, Additional Plant DNA, Contains Fillers
40	Y	Ascophyllum nodosum, Fucus vesiculosus, Iodine		Rosoideae, Holcus		T.S. Neg., Additional Plant DNA, Contains Fillers
43	Y	Angelica archangelica, Carum carvi, Chelidonium majus, Glycyra glabra, Iberis amara, Matricaria chamomilla, Melissa officinalis, Mentha X piperita, Silybum marianum				Nothing detected
44	Y	Abrus cantoniensis, Bupleurum falcatum, Curcuma longa, Cyperus rotundus, Desmodium styracifolium, Gardenia florida, Glycyra uralensis, Paeonia veitchii, Rheum palmatum, Scutellaria baicalensis	Synephrine 1.1 mg/g	Cedrus, Apiaceae (includes B. falcatum), Artemisia, Oleaceae, Solanoideae, PACMAD clade		Additional pharmaceutical, Additional Plant DNA, Confirmed Ingredients
47	N	Angelica polymorpha, Atractylodes macrocephala, Bupleurum falcatum, Citris aurantium, Glycyra uralensis, Leonurus sibiricus, Mentha haplocalyx, Paeonia lactiflora, Paeonia suffruticosa, Poria cocos, Spatholobus suberectus, Trichosanthes kirilowii	Synephrine 0.5 mg/g	Panax, Ericale, Dalbergieae		Expected Pharmaceutical, Additional Plant DNA
50	Y	Docosahexaenoic acid, Eicosapentaenoic acid				Nothing detected
51	Y	Docosahexaenoic acid, Eicosapentaenoic acid, Euphausia superba				Nothing detected

Sample # ID	In ARTG database (Yes/ No)	Ingredients On Package	Toxicological data	DNA Plant	DNA Animal	Comments
52	Y	d-alpha tocopherol, Euphausia superba, Fish Oil - Natural, Glucosamine Hydrochloride				Nothing detected
53	Y	Docosahexaenoic acid, Eicosapentaenoic acid				Nothing detected
54	Y	Docosahexaenoic acid, Eicosapentaenoic acid, Euphausia superba				Nothing detected
55	Y	Euphausia superba, Omega-triglycerides				Nothing detected
59	Y	Astragalus complanatus, Calcium sulfate, Euryale ferox, Nelumbium speciosum, Oyster Shell		Nymphaeaceae, Nelumbo, Glycyrrhiza, Rutaceae, Paeonia, Asterids	Bos, Capra hircus	T.S. Neg., Animal Contaminants, Additional Plant DNA, Confirmed Ingredients
62	N	Unknown		Danthonia, Panicoideae, Maleae, Plantago, Gynostemma, Rutaceae, Papilionoideae (including Fabeae, Glycyrrhiza, Vigna)		T.S. Neg., Contains Fillers
66	Y	Amorphophallus konjac		Araceae (includes A. konjac)	Bos, Sus scrofa	T.S. Neg., Animal Contaminants, Confirmed Ingredients
68	Y	Coffea canephora		BEP clade, Saliceae, Fabeae, Apiaceae, Anacardium	Bos	T.S. Neg., Animal Contaminants, Additional Animal DNA, Contains Fillers
69	N	Acerola Fruit Powder, Alfalfa Powder, Apple Powder, Ascorbic Acid, Astragalus Extract, Barley Powder, Beta Glucan, Betacarotene, Bilberry		Papilionoideae (inlcuding Fabeae, Triticeae, Oryza, Hordeum) Bambusoideae, Glycyrrhiza, Linum	Rangifer tarandus (Reindeer), Sus crofa, Mus	T.S. Neg., Animal Contaminants, Additional Animal

Sample # ID	In ARTG database (Yes/ No)	Ingredients On Package	Toxicological data	DNA Plant	DNA Animal	Comments
		Fresh Fruit Extract, Biotin, Broccoli Powder, Burdock Extract, Calcium Carbonate, Calcium Citrate, Calcium Pantothenate, Calcium Phosphate, Carica papaya, Carica Papaya Powder, Carrot Powder, Chlorella Powder, Citric Acid, Citrus Bioflavanoids Extract, Cocoa Bean Polyphenol Extract, Colloidal Anhydrous Silica, Copper Gluconate, Cyanocobalamin, Dandelion Extract, Dibasic Phosphate, Ergocalciferol, Flaxseed Powder, Folic Acid, Ginger ome Powder, Globe Artichoke Extract, Gotu kola Extract, Grapeseed Extract, Green Tea Extract, Hawthorne Fruit Powder, Inulin, Kelp Powder, Lecithin, Licorice Powder, Magnesium Citrate, Manganese Amino Acid Chelate, Milk Thistle Extract, Nicotinamide, Nicotinic Acid, Pea Protein Isolate, Picolinate, Pineapple Flavour, Pineapple Fruit Extract, Pyridoxine Hydrochloride, RS-Alpha Lipoic Acid, Red Beet Powder, Reishi Mushroom Powder, Resveratol, Riboflavin, Rice Bran Powder, Rosehip Fruit Extract, Rosemary Extract, Selenomethione, Shitake Mushroom Powder, Slippery Elm Powder, Spinach Powder, Spirulina, Thaumatin, Thiamine Hydrochloride, Ubidecarenone, Vanilla Flavour, Wheatgrass Powder, Withania somnifera Extract, Wolfberry (Goji) Fruit Powder, Zinc Amino Acid Chelate		usitatissimum, Rhodiola, Beta vulgaris, Brassicaceae, Zingiberaceae, Heliantheae, Elsholtzieae, Apiaceae, Asteraceae	musculus, Rattus, Capra	DNA, Confirmed Ingredients

Sample # ID	In ARTG database (Yes/ No)	Ingredients On Package	Toxicological data	DNA Plant	DNA Animal	Comments
70	Y	Shark cartilage		Glycine (soybean)	Sus scrofa, Rattus, Canis lupus familiaris, Anatidae, Phasianinae, Galeocerdo cuvier, Carcharhinus	T.S. Neg., Animal Contaminants, Additional Animal DNA, Contains Fillters
71	Y	Colostrum powder - bovine		Fabeae, Helianthease, Triticeae, Citrus, Glycine (soybean)	Bos	T.S. Neg., Contains Fillers
72	N	Flavour, Glucose, Goats Milk Poweder, Lactose, Natural Colour		Cucurbitaceae, Theobroma, Magnolids, Glycine (soybean)	Capra hircus	T.S. Neg., Contains Fillers
74	Y	Retinyl Acetate, Lutein, Lycopene, Betacarotene, Thiamine Nitrate, Riboflavin, Nicotinamide, Pyridoxine Hydrochloride, Cyanocobalamin, Ascorbic Acid, Cholecalciferol, d-alpha-tocopherol Acetate, Phytomenadione, Biotin, Folic Acid, Calcium Pantothenate, Calcium Carbonate, Calcium Hydrogen Phosphate, Potassium Sulfate, Chromic Chloride, Copper Sulfate, Potassium Iodide, Ferrous Fumarate, Magnesium Oxide, Manganese Sulfate, Sodium Selenate, Zinc Oxide		Citrus, Apioideae		T.S. Neg., Confirmed Ingredients
77	Y	d-alpha Tocopherol, Ubidecarenone, Zinc Amino Acid Chelate		Oryza sativa		T.S. Neg., Contains Fillers
78_1	Y	Selenomethionine, Silybum marianum, Taurine		Oryza, PACMAD clade, Brassicaceae, Asteraceae (includes S. marianum)		T.S. Neg., Additional Plant DNA, Contains Fillers, Confirmed Ingredients

Sample # ID	In ARTG database (Yes/ No)	Ingredients On Package	Toxicological data	DNA Plant	DNA Animal	Comments
78_2	Y	Selenomethionine, Silybum marianum, Taurine		Asteraceae (includes S. marianum)	Sus scrofa	T.S. Neg., Confirmed Ingredients
79	Y	Ascorbic Acid, Vaccinium macrocarpon		Rutaceae, Maleae, Poaceae, Juglandaceae	Bos, Camelus	T.S. Neg., Additional Plant DNA, Animal Contaminants
80	Y	Citris bioflavonoids extract, Tocotrienols complex - palm		Rutaceae (includes Citrus), Azadirachta indica		T.S. Neg., Additional Plant DNA
81	Y	d-alpha-Tocopherol				Nothing detected
82	N	Pure New Zealand Manuka Honey		Podocarpaceae, Loganiaceae, Anthospermeae, Pseudocarpidium, Corynocarpus, Ulex, Melicytus, Phormium tenax, Laurales, Asteraceae		T.S. Neg., DNA from pollen in the honey
84	Y	Globe artichoke (Cynara scolymus), St Mary's Thistle, Tumeric		Cynara cardunculus var. scolymus, Medicago sativa, Zingiberales (includes Curcuma), Asparagales, Quercus, Brassicaceae, Citrus, Triticeae		T.S. Neg., Additional Plant DNA, Contains Fillers, Confirmed Ingredients
86	Y	Cordyceps sinensis, Eleutherococcus senticosus, Withania somnifera, Ganoderma lucidum, Astragalus membranaceus, Panax ginseng, Panax quinquefolium	Buclizine 0.2 □g/g	Astragalus, Morus, Maleae, Brassicaceae, Araliaceae		Additional pharmaceutical, Additional Plant DNA
88	N	Ginger, Ginseng, Guarana, Spirits	Caffeine, theobromine			Expected Pharmaceutical
91	N	Burdock, Dandelion, Milk Thistle, Nettle, Spirits				Nothing detected
93	Y	Isatis tinctoria, Taraxacum mongolicum, Viola yedoensis	Ephedrine 0.3 □g/g			Additional pharmaceutical

Sample # ID	In ARTG database (Yes/ No)	Ingredients On Package	Toxicological data	DNA Plant	DNA Animal	Comments
94	Y	Curcumin phospholipid complex		Papilionoideae		T.S. Neg.
99	Y	Chromic chloride, Cinamomum cassia, Coleus forskohlii, Gymnema sylvestre, Hydroxycitrate complex				Nothing detected
105	Y	Biotin, Chromium pioclinate, Cinnamomum cassia, L-Leucine, Manganese amino acid chelate, Momordica charantia				Nothing detected
107	N	Gymnema				Nothing detected
113	Y	Cunara scolymus (globe artichoke), Silybum marianum (Milk Thistle) seed, Taraxacum officinale (Dandelion)				Nothing detected
116	Y	Alisma ago aquatic, Bupleurum falcatum, Cornus officinalis, Dioscorea opposita, Paconia suffruticosa, Poria cocos, Rhemania glutinosa, Schizandra chinensis				Nothing detected
121	Y	Achyranthes bidenta, Eucommia ulmoides, Gardenia floridia fruit, Gastrodia elata tuber, Leonurus sibiricus herb, Oyster shell powder, Polygonatum multiflorum , Poria cocos fruit , Scutellaria baicalensis, Uncaria rhyncophylla , Viscum coloratum herb		Asteraceae, Lamiales (includes S.baicalensis and L. sibiricus), Boraginaceae, Glycyrrhiza, Pooideae, Nymphaeaceae, Apiaceae, Ornithogaloideae, Campanulaceae, Anthemideae, Lonicera, Galium, Boraginaceae, Convolvulaceae, Solanum, Thermopsis, Prunus, Rosoideae, Ulmaceae, Brassicaceae, Avena, Triticeae, Zingiberaceae		T.S. Neg., Additional Plant DNA, Contains Fillers, Confirmed Ingredients
124	Y	Angelica polymorpha, Carthamus tinctorius, Ligusticum wallichii, Paeonia laciflora, Prunus persica, Rehmannia glutinosa		Paeonia (includes P. laciflora), BEP clade, Rutaceae, Apiaceae (includes A. polymorpha and L. wallichii)		T.S. Neg., Confirmed Ingredients

Sample # ID	In ARTG database (Yes/ No)	Ingredients On Package	Toxicological data	DNA Plant	DNA Animal	Comments
125	Y	Arctium lappa, Cynara scolymus, Schisandra chinensis, Silybum marianum, Taraxacum officinate				Nothing detected
128	Y	Achyranthes bidenta, Angelica polymorpha, Bupleurum falcatum, Carthamus tinctorious, Citrus aurantium, Glycyra uralenses, Ligusticum sinense, Paeonia lactiflora, Platycodon grandiflorum, Prunus persica, Rehmannia glutinosa	Synephrine 0.32 mg/g	Amaranthaceae (includes A. bidenta), Apisceae (includes A. polymorpha and B. falcatum), Paeonia (includes P. laciflora), Sapindales (includes C. aurantium), Poaceae, Polycarpon, Polygonoideae		Expected Pharmaceutical, Confirmed Ingredients, Additional Plant DNA
134	Y	Alisma orientale, Angelica polymorpha, Bupleurum falcatum, Clematis armandii, Gardenia florida, Gentiana scabra, Glycra uralensis, ago asiatica, Rehmannia glutinosa, Scutellaria baicalensis		Apiaceae (includes A. polymorpha), Gentiana, Glycyrrhiza, Astragalus	Sus scrofa	T.S. Neg., Animal Contaminants, Additional Plant DNA, Confirmed Ingredients
135	Y	Aloe Ferox powder		Calocedrus decurrens		T.S. Neg., Additional Plant DNA
152	Y	Urtica dioica				Nothing detected
158	Y	Iodine, Kelp				Nothing detected
160	N	Acai extract, Gelatin		Arecaceae (includes Acai)		T.S. Neg., Confirmed Ingredients
161	Y	Trigonella foenum-graecum		Trigonella, Anthemideae, Lonicera, Lamioideae, Capsium, BEP clade, Allium, Rutaceae, Peganum nigellastrum		T.S. Neg., Additional Plant DNA, Contains Fillers
162	Y	polygonum cuspidatum dry extract, vitis vinifera dry extract, ascorbic acid	Mycophenolic acid			Additional pharmaceutical
167	N	Cat's Claw		Asterids (includes U. tomentosa)		T.S. Neg., Confirmed Ingredients

Sample # ID	In ARTG database (Yes/ No)	Ingredients On Package	Toxicological data	DNA Plant	DNA Animal	Comments
168	N	Wormwood (Artemisia)		Triticeae, Phragmites, Asteroideae (includes Artemisia)		T.S. Neg., Additional Plant DNA, Confirmed Ingredients
177	N	Organic Stinging Nettle		Urtica dioica, Pooideae, Lamiaceae, Bromus		T.S. Neg., Additional Plant DNA
178	N	Devil's Claw		Harpagophytum procumbens		T.S. Neg., Confirmed Ingredients
185	Y	Ascorbic acid, Sambucus nigra				Nothing detected
186_1	Y	Allium sativum		Glycine	Bos	T.S. Neg., Animal Contaminants
186_2	Y	Allium sativum		Glycine		T.S. Neg.
187	Y	Magnesium, Viburnum opulus, Zingiber officinale		Oryza, Brassicaceae, Roscoea, Zingiberaceae	Sus scrofa	T.S. Neg., Animal Contaminants, Additional Plant DNA, Contains Fillers, Confirmed Ingredients
198	N	Calcium hydrogen phosphate, Cellulose fibre, Gelatine, Maltodextrin, Processing aids (E, E), Sheep Placenta concentrate		BEP clade (Oryza)	Ovis	T.S. Neg., Contains Fillers
200	Y (under a different AUST L)	Bifidobacterium lactis, Lactobacillus acidophilus, Lactobacillus rhamnosus		Triticeae, Salvia		T.S. Neg., Additional Plant DNA
201	N	sorbitol, maltitol, gum base, maltitol syrup, vitamins- C E, niacin B, pantothenic acid B, B, A, D, B, flavour, vegetable gum, colour, food acid, humectant glazing agent, sweetener				Nothing detected

Sample # ID	In ARTG database (Yes/ No)	Ingredients On Package	Toxicological data	DNA Plant	DNA Animal	Comments
202	N	Bacopa, BHT, Carnuba Wax, Ginkgo biloba, Guarana, Gum Arabic, Gum Base, Maltitol, Monniera, Natural Flavours, Resinous Glaze, Sorbitol, Soy Lecithin, Sucralose, Titanium Dioxide, Vinpocetine, Xylitol				Nothing detected
204	N	Yerba Mate, Spirits	Caffeine, theobromine			Expected Pharmaceutical
207	N	Ginkgo biloba, Maca, Reishi Mushroom, Siberian Ginseng, Yerba mate, Spirits	Caffeine, theobromine			Expected Pharmaceutical
212	N	Chamomill, Colocynthis, Dioscorea		Theaceae, Fabaceae		T.S. Neg., Additional Plant DNA
217	Y	Achillea millefolium, Arctium lappa, Berberis aquifolium, Frangula purshiana, Handroanthus heptaphyllus, Rhamnus frangula, Rumex crispus, Smilax officinalis, Taraxacum officinale, Trifolium pratense, Zanthoxylum clava-herculis		Zanthoxylum, Rhamnus, Trifolium, Asteroideae (includes A. millefolium)	Canis lupus familiaris, Bos, Cervinae (deer)	T.S. Neg., Animal Contaminants, Confirmed Ingredients
218	Y	Lecithin, Piper nigrum, Ubidecarenone				Nothing detected
219	Y	Astaxanthin, d-alpha Tocopherol, Haematoccocus pluvialis, Linoleic acid, Linolenic acid, Linseed oil, Oleic acid				Nothing detected
220	Y	Betacarotene, Retinyl palmitate, Thiamine hydrochloride, Riboflavin, Nicotinamide, Calcium pantothenate, Pyridoxine hydrochloride, Folic acid, Cyanocobalamin, Biotin, Ascorbic acid, Chlecalciferol, d-alpha-Tococpheryl acid succinate, Calcium carbonate, Chromium picolinate, Iron, Magnesium, Manganese, Potassium, Selenium, Zinc,		BEP clade (Oryza), PACMAD clade, Citrus	Sus scrofa	T.S. Neg., Animal Contaminants, Contains Fillers

Sample # ID	In ARTG database (Yes/ No)	Ingredients On Package	Toxicological data	DNA Plant	DNA Animal	Comments
		Glucosamine sulphate, Choline bitartrate, Inositol, Citrus bioflavonoids extract, Papain,Lecithin powder, Enriched soy, Fucus vesiculosus (Kelp) extract				
221	Y	Aesculus hippocastanum, Ginkgo biloba, Ruscus aculeatus, Vaccinium myrtillus				Nothing detected
224	Y	Camellia sinensis, Chitosan, Chromic chloride, Chromium picolinate, Citris aurantium, Fucus vesiculosus, Gymnema sylvestre, Pyridoxine hydrochloride, Riboflavin, Thiamine hydrochloride	Synephrine, Caffeine			Expected Pharmaceutical
225	Y	Coffea canephora	Caffeine			Expected Pharmaceutical
226	Y	Garcinia gummi-gutta, Nicotinamide, Pyridoxine hydrochloride, Thiamine hydrochloride				Nothing detected
227	Y	Camellia sinensis, Capsicum annuum, Chromic chloride, Citris aurantium, Garcinia quaesita, Pyridoxine hydrochloride, Taurine, Zingiber officinale	Synephrine, Caffeine			Expected Pharmaceutical
228	Y	Camellia sinensis, Cocos nucifera, Coffea canephora, Garcinia gummi-gutta	Caffeine			Expected Pharmaceutical
229	Y	Coffea canephora	Caffeine	Oryza, Triticeae, Apocynaceae, Platanus orientalis, Solanaceae		Additional Plant DNA, Contains Fillers, Expected Pharmaceutical

Sample # ID	In ARTG database (Yes/ No)	Ingredients On Package	Toxicological data	DNA Plant	DNA Animal	Comments
230	N	Acai berry, Ascorbic acid, Black tea powder, Capsicum annuum, Citric acid, Elderberry, Flavours, Goji berry, L-Carnitine, Pomegranate powder, Red grape powder, Schisandra berry, Stevia, Xylitol	Caffeine	Lythraceae		Expected Pharmaceutical
231	N	Acidophilus, Aloe vera, Bertonite Clay, Buckthorn, Calcium carbonate, Cape aloe, Cascara sagrada, Cayenne Pepper, Cirus pectin, Fennel Seed, Flax Seed Oil, Gelatin, Ginger, Irvingia gabonesis, Licorice, Magnesium stearate, Oat Bran, Prune Juice, Pumpkin Seed, Rhubarb, Senna		Quercus		T.S. Neg.
232	Y	Hydoxycitrate complex		Musaceae		T.S. Neg., Additional Plant DNA
233	Y	Hydroxycitrate complex				Nothing detected
234	N	African Mango Seed Extract, Calcium Phosphate, Hypromellose, Magnesium Stearate, Purified Water, Silicon Dioxide		Avena		T.S. Neg., Contains Fillers
235	Y	Premium Garcinia Extract (Garcinia quaesita)		Fagaceae		T.S. Neg., Additional Plant DNA
236	N	Hydroxy Citric Acid		Oryza		T.S. Neg., Contains Fillers
237	Y	Potassium Iodide, Rubus idaeus				Nothing detected
238	Y	Hydroxycitrate complex				Nothing detected
239	Y	Camellia sinensis, Coffea canephora	Caffeine			Expected Pharmaceutical
240	N	Chromic chloride, Cinnamomum cassia, Coleus forskohlii, Garcinia quaesita,				Nothing detected

Sample # ID	In ARTG database (Yes/ No)	Ingredients On Package	Toxicological data	DNA Plant	DNA Animal	Comments
		Gymnema sylvestre, Hydroxycitrate complex, Piper nigrum				
241	Y	Chromium chloride, Coffea canephora, Encapsulating aids	Caffeine			Expected Pharmaceutical
242	Y	Coffea canephora	Caffeine			Expected Pharmaceutical
243	Y	Crataegus pinnatifida, Dioscorea opposita, Nelumbium speciosum, Poria cocos	Caffeine 0.55 mg/g	Plantago		Additional Pharmaceutical, Additional Plant DNA
244	Y	Caralluma adscendens, Crocus sativus	Caffeine			Expected Pharmaceutical
245	N	Cinnamon, Ganoderma mushroom, Green Tea, Peppermint, Chicory	Caffeine	Mentha, Theaceae	Canis lupus familiaris, Bos, Camelus dromedarius, Rhacophorinae , Suncus montanus (Asian highland shrew)	Animal Contaminants, Additional Animal DNA, Expected Pharmaceutical
246	Y	Citrus aurantifolia, Crataegus pinnatifida, Hordeum vulgare, Nelumbo nucifera, ago asiatica, Poria cocos, Rheum officinale, Senna alexandrina, Vigna umbellate		Fabaceae (includes S. alexandrina)	Canis lupus familiaris, Bos,	Animal Contaminants, Confirmed Ingredients
247	N	Raspberry Ketone				Nothing detected
248	Unknown	Alfalfa Extract, Barley Grass Powder, Broccoli Powder, Calcium Hydrogen Phosphate, Chlorella Powder, Green Kale Powder, Green Tea Extract, Kelp Extract, Magnesium Stearate, Nori Powder, Pea Protein, Povidone, Shitake	Caffeine, theobromine	Hordeum, Brassicaseace, Pooideae, Fabeae		Expected Pharmaceutical, Confirmed Ingredients

Sample # ID	In ARTG database (Yes/ No)	Ingredients On Package	Toxicological data	DNA Plant	DNA Animal	Comments
		Mushroom Powder, Silica Colloidal Anhydrous, Spinach Powder, Spirulina, Wheat Grass Powder				
253_1	Y	Trigonella foenum-graecum		Trigonella, Parietaria, Musaceae, Apocynaceae		T.S. Neg., Additional Plant DNA, Confirmed Ingredients
253_2	Y	Trigonella foenum-graecum		Plantago, Asteraceae	Canis lupus	T.S. Neg., Animal Contaminants, Additional Plant DNA
286	Y	Coleus forskohlii		Theaceae, Ocimeae		Additional Plant DNA, Confirmed Ingredients
287	N	Garcinia cambogia extract, Garcinia cambogia Gelatin Magnesium stearate microcrystalline cellulose		Apiaceae		Additional Plant DNA
288	Y	Coleus forskohlii, Chromium picolinate		Trigonella, Piper	Ovis (sheep)	Animal Contaminants, Additional Plant DNA
289	N	Vitamin B, Chromium picolinate, Green tea extract, Cha De Bugre powder, Guarana seed extract, Caffeine anhydrous, Hoodia chinese extract	Caffeine	Salicaceae		Additional Plant DNA, Expected Pharmaceutical

Sample # ID	In ARTG database (Yes/ No)	Ingredients On Package	Toxicological data	DNA Plant	DNA Animal	Comments
290	N	African mango extract, Green tea extract, Solathin Non-GMO potato	Caffeine			Expected Pharmaceutical
291	Y	Garcinia cambogia, Hydroxycitric acid, Chromium chloride		Musaceae		Additional Plant DNA
292	Y	Caralluma adscendens var. fimbriata extract				Nothing detected
293	Y	Astragalus membranaceous, Crataegus pinnatifida, Polygonum multiform, Codonopsis pilosula, Atractylodes macrocephala, Nelumbium speciosum, Pinellia temata, Poria cocos, Alisma ago aquatica, Morus alba, Cassia tora, Citrus reticulata, Zizyphus jubata		Asteraceae		T.S. Neg.
294	N	Garcinia Cambogia with HCA, Calcium phosphate, Hypromellose, maltodextrin, Silicon dioxide, Magnesium stearate, Purified water				Nothing detected
295	N	Green tea extract (Camelia sinensis), Hordenine, N-Methyltyranine, Octopamine, Tyramine, Ginger (Gingerols), L-tyrosine, Grape seed extract, Quercetin, Vitamin C, Vitamin B (as pyridoxine), Pantothenic acid, Magnesium carbonate		BEP clade, Crotonoideae		T.S. Neg., Contains Fillers
296 A	N	Unknown		Araliaceae		T.S. Neg.

Sample # ID	In ARTG database (Yes/ No)	Ingredients On Package	Toxicological data	DNA Plant	DNA Animal	Comments
296 B	N	Unknown	Paracetamol 0.46 mg/g, Chlorpheniramine 0.005 mg/g			Additional pharmaceutical
297	N	Garcinia cambogia, potassium, Calcium, Chromium		Poaceae		Contains Fillers
298	N	Green coffee bean extract	Caffeine	Ericales, Fabaceae, Apiaceae		Additional Plant DNA, Contains Fillers, Expected Pharmaceutical
300	N	chromium picolinate, cobalamin (vit B), thiamine, riboflavin, niacinamide, pantothenic acid, pyridoxine HCl, green coffee bean, chlorogenic acid, gardenia cambogia, HCA, raspberry ketones, white kidney bean extract, green tea extract.		BEP clade (Oryza)		Contains Fillers
301	Y	paullinia cupana extract, citrus aurantium extract, zingber officinale extract, panax ginseng extract, camellia sinensis extract, psyllium husk powder, chromium picolinate, potassium iodide, pyridoxine hydrochloride, thiamine nitrate, riboflavin, cyanocobalamin	Caffeine	Plantago, Brassicaceae, Rosids		Expected Pharmaceutical, Confirmed Ingredients
302	N	calcium, iodine, chromium polynicotinate, sodium, garcinia cambogia extract, panax ginseng extract, gelatin, magnesium stearate, stearic acid				Nothing detected

Sample # ID	In ARTG database (Yes/ No)	Ingredients On Package	Toxicological data	DNA Plant	DNA Animal	Comments
303	N	cellulose, gelatin, african mango seed powder, silicon dioxide, magnesium stearate, water, maltodextrin		Apiaceae		Additional Plant DNA
304	N	acacia rigidula P.E., B-phenylethylamine,,-dimethoxytramine, -methyl--pyridinamine, -cyclohexylethylamine, tyramine, white tea, green tea, kuding tea, dextrose, caffeine, hordnine, konjac, cissus quadrangularis extact, rhodiola rosea extract, dextrose	Caffeine			Expected Pharmaceutical
305	Y	Camellia sinensis	Caffeine	Apiaceae		Additional Plant DNA, Expected Pharmaceutical
306	N	Unknown		Apiaceae		T.S. Neg.
307 A	N	Acacia arabica, Argyreia speciose, Asparagus adescendens, Asparagus racemosus, Capparis aphylla, Convolvulus pluricaulis, Curculigo orchioides, Emblica officinalis, Glycyrrhiza glabra, Ipomoea digitate, Tinospora cordifolia, Vitex trifolia, Withania somnifera		Cycadales, Apocynaceae, Anthemideae, Moltkia, Convolvulaceae (includes I. digitate), Solanum, Cicer, Glycyrrhiza, Asparagus, Hypoxidaceae (includes C. orchioides), Solanoideae (includes W. somnifera), Ranunculales, Euphorbiaceae, Rutaceae, Apocynaceae, Asparagoideae (includes A. adescendens and A. racemosus)		T.S. Neg., Additional Plant DNA, Confirmed Ingredients
307 B	N	Centella asiatica, Cinnamomum zeylanicum, Convolvulus pluricaulis, Curcuma longa, Cyperus rotundus, Cyperus scariosus, Elettaria cardamomum, Embelia ribes, Ghee, Glycyrrhiza glabra, Honey, Meua ferrea,				Nothing detected

Sample # ID	In ARTG database (Yes/ No)	Ingredients On Package	Toxicological data	DNA Plant	DNA Animal	Comments
		Phyllanthus emblica, Piper longum, Santalum album, Sugar, Terminalia chebula				
313	Unknown	Unknown				Nothing detected
314	Y	Ascorbic acid, Biotin, Calcium pantothenate, Calcium hydrogen phosphate anhydrous, Cholecalciferol, Chromic chloride, Cupric sulfate pentahydrate, Cyanocobalamin, d-alpha-tocopheryl acid succinate, Dunaliella salina, Eleutherococcus senticosus, Ferous fumerate, Folic acid, Inositol, Magnesium oxide-heavy, Manganese amino acid chelate, Nicotinamide, Potassium iodide, Pyridoxine hydrochloride, Riboflavin, Selenomethionine, Silybum marianum, Thimine nitrate, Zinc amino acid chelate				Nothing detected
316	Y	Ascorbic Acid, Folic Acid, Ferrrous Fumerate, Cyanocobalamin		Beta vulgaris		T.S. Neg.
317	Y	Natural vitamin E (d-alpha Tocopherol)				Nothing detected
319	N	Creatine Monohydrate, Dextrose, Peak ATP		PACMAD clade, Ocimum, Theobroma, Papilionoideae, Anacardium		T.S. Neg., Additional Plant DNA, Contains Fillers
320	N	Unknown		Amaranthaceae, Papilionoideae, Ulmaceae, Triticeae		T.S. Neg.
321	Y	Camellia sinesis extract, fallopia japonica extract, standardised to contain resveratrol	Caffeine			Expected Pharmaceutical

Sample # ID	In ARTG database (Yes/ No)	Ingredients On Package	Toxicological data	DNA Plant	DNA Animal	Comments
322	Y	Tribulus terrestrial extract	Ephedrine 0.003 mg/g, Pseudoephedrine 0.7 □g/g, methylephedrine, norphedrine	Ephedra, Anthemideae, Astereae, Solanoideae, Acalypheae, Linum, Triticeae, Medicago, Tribuloideae (includes T. terrestrial)		Additional pharmaceutical, Additional Plant DNA, Contains fillers
323	Y	Citrus aurantium fruit, coleus forskohlii, paullinia cupana seed, capsicum frutescens fruit, zingiber officinate ome, camelia sinesis, RS-alpha lipoic aicd, levocarnitine, potassium iodide	Caffeine, Synephrine 7.4 mg/g	Platycladus orientalis, Capsicum, Glycyrrhiza, Rosaceae, PACMAD clade, Theaceae		Expected Pharmaceutical, Additional Plant DNA, Confirmed Ingredients
324	N	Calcium Sulphate		Pinus		T.S. Neg., Contains Fillers

Supplementary Table 9-3: The pharmaceuticals, analogues, and phytochemicals which were included in the DSA screen.

Compound	Formula
2,5-Dimethoxy-4-iodoamphetamine	C11H16I1N1O2
2,5-Dimethoxy-4-methylamphetamine	C12H19N1O2
3,4-Dimethoxyamphetamine	C11H17N1O2
3-Methoxyamphetamine	C10H15N1O1
4-Bromo-2,5-dimethoxyamphetamine	C11H16Br1N1O2
4-Methylmethamphetamine	C11H17N1
4-Methylthioamphetamine	C10H15N1S1
5-methoxy-alpha-methyltryptamine	C12H16N2O1
5-methoxy-di isopropyl tryptamine_(5-MeO-DIPT)	C17H26N2O1
5-methoxy-diallyltryptamine	C17H22N2O1
Acetylmethadol	C23H31N1O2
Agomelatine	C15H17N1O2
Alfentanil	C21H32N6O3
Aliskiren	C30H53N3O6
Alpha_Thujone	C10H16O1
Alpha-PVP	C15H21N1O1
Alpha-Tocopherol	C29H50O2
Amidopyrine	C13H17N3O1
Amiodarone	C25H29I2N1O3
Amisulpride	C17H27N3O4S1
Amitriptyline	C20H23N1
Amlodipine	C20H25Cl1N2O5
Amoxicillin	C16H19N3O5S1
Amphetamine	C9H13N1
Anabasine	C10H14N2
Anastrozole	C17H19N5
Aripiprazole	C23H27Cl2N3O2
Aristolochic_Acid_1	C17H11N1O7
Ascorbic acid	C6H8O6
Aspirin	C9H8O4
Astaxanthin	C40H52O4
Atenolol	C14H22N2O3
Atomoxetine	C17H21N1O1
Atropine	C17H23N1O3
Avanafil	C23H26Cl1N7O3
Azatadine	C20H22N2

Compound	Formula
Azelastine	C22H24Cl1N3O1
Benzhexol	C20H31N1O1
Benzocaine	C9H11N1O2
Benztropine	C21H25N1O1
Beta_Thujone	C10H16O1
Beta-Carontene	C40H56
Biperiden	C21H29N1O1
Bisoprolol	C18H31N1O4
Bromazepam	C14H10Br1N3O1
Bromhexine	C14H20Br2N2
Brompheniramine	C16H19Br1N2
Brucine	C23H26N2O4
Buprenorphine	C29H41N1O4
Buspirone	C21H31N5O2
BZP_(1-Benzylpiperazine)	C11H16N2
Caffeine	C8H10N4O2
Calcidiol	C27H44O2
Calcipotriol	C27H40O3
Calcitriol	C27H44O3
Camazepam	C19H18Cl1N3O3
Cantharidin	C10H12O4
Carbamazepine	C15H12N2O1
Carfentanil	C24H30N2O3
Chlorotetracycline	C22H23Cl1N2O8
Chlorpheniramine	C16H19Cl1N2
Chlorpromazine	C17H19Cl1N2S1
Chlorpropamide	C10H13Cl1N2O3S2
Cholesterol	C27H46O1
Cinnaminc_Acid	C9H8O2
Cisapride	C23H29Cl1F1N3O4
Citalopram	C20H21F1N2O1
Clobazam	C16H13Cl1N2O2
Clomethiazole	C6H8Cl1N1S1
Clomipramine	C19H23Cl1N2
Clonazepam	C15H10Cl1N3O3
Clonidine	C9H9Cl2N3
Clopidogrel	C16H16Cl1N1O2S1
Clozapine	C18H19Cl1N4
Cocaine	C17H21N1O4
Codeine	C18H21N1O3
CPCPP_(1-(3-chlorophenyl)-4-(3-chloropropyl) piperazine)	C13H18Cl2N2

Compound	Formula
Curcumin	C21H20O6
Cyanocobalamin	C63H88Co1N14O14P1
Cyclizine	C18H22N2
Cyproheptadine	C21H21N1
Dehydroepiandrosterone	C19H28O2
Delta-9-tetrahydrocannabinol	C21H30O2
Demeclocycline	C22H19Cl1N2O6S1
Desacetyl_diltiazem	C20H24N2O3S1
Desipramine	C18H22N2
Dexamethasone	C22H29F1O5
Dextromethorphan	C18H25N1O1
Dextromoramide	C25H32N2O2
Diazepam	C16H13Cl1N2O1
Diazepam-D5	C16H8ClD5N2O
Diclofenac	C14H11Cl2N1O2
Diethylcathinone	C13H19N1O1
Digoxin	C41H65O14
Dihydrocodeine	C18H23N1O3
Diltiazem	C22H26N2O4S1
Diphenhydramine	C17H21N1O1
Dothiepin	C19H21N1S1
Doxepin	C19H21N1O1
Doxylamine	C17H22N2O1
Droperidol	C22H22F1N3O2
Ephedrine	C10H15N1O1
Epicatechin	C15H14O6
Epicatechin_Gallate	C22H18O10
Epigallocatechin	C15H14O7
Epigallocatechin_Gallate	C22H18O11
Epinephrine	C9H13N1O3
Ergocalciferol	C28H44O1
Ergosterol	C28H44O1
Famotidine	C8H15N7O2S3
Felodipine	C18H19Cl2N1O4
Fenfluramine	C12H16F3N1
Fentanyl	C22H28N2O1
Ferulic_Acid	C10H10O4
Flecainide	C17H20F6N2O3
Fluconazole	C13H12F2N6O1
Flunitrazepam	C16H12F1N3O3
Fluoroamphetamine	C9H12F1N1

Compound	Formula
Fluoromethamphetamine	C10H14F1N1
Fluoxetine	C17H18F3N1O1
Flupentixol	C23H25F3N2O1S1
Fluphenazine	C22H26F3N3O1S1
Flurazepam	C21H23Cl1F1N3O1
Fluvoxamine	C15H21F3N2O2
Folic_Acid	C19H19N7O6
Forskolin	C22H34O7
Gabapentin	C9H17N1O2
Gallic_Acid	C7H6O5
Gallocatechin	C15H14O7
Gallocatechin_Gallate	C22H18O11
Ginkgolide_A	C20H24O9
Ginkgolide_B	C20H24O10
Ginkgolide_C	C20H24O11
Ginkgolide_J	C20H24O10
Ginsenoside_Rb1	C54H92O23
Ginsenoside_Rb2	C53H90O22
Ginsenoside_Rb3	C53H90O22
Ginsenoside_Rc	C53H90O23
Ginsenoside_Rd	C48H82O18
Ginsenoside_Re	C48H82O18
Ginsenoside_Rf	C42H72O14
Ginsenoside_Rg1	C42H72O14
Ginsenoside_Rg2	C42H72O13
Glibenclamide	C23H28Cl1N3O5S1
Gliclazide	C15H21N3O3S1
Glucosamine	C6H13N1O5
Glutethimide	C13H15N1O2
Haloperidol	C21H23Cl1F1N1O2
Harpagoside	C24H30O11
Hydrocodone	C18H21N1O3
Hydrocortisone	C21H30O5
Hydroxybupropion	C13H18Cl1N1O2
Hydroxychloroquine	C18H26Cl1N3O1
Hydroxycitric_Acid	C6H8O8
Hyoscine	C17H21N1O4
Hyperforin	C35H52O4
Hypericin	C30H16O8
Ibuprofen	C13H18O2
Imipramine	C19H24N2

Compound	Formula
Isorhamnetin	C16H12O7
JWH-018	C24H23N1O1
JWH-073	C23H21N1O1
JWH-200	C25H24N2O2
JWH-201	C22H25N1O2
Kaempferol	C15H10O6
Ketamine	C13H16Cl1N1O1
Lamotrigine	C9H7Cl2N5
Laudanosine	C21H27N1O4
Levetiracetam	C8H14N2O2
Lidocaine	C14H22N2O1
Lodenafil	C23H32N6O5S1
Loratadine	C22H23Cl1N2O2
Lorazepam	C15H10Cl2N2O2
LSD	C20H25N3O1
L-Theanine	C7H14N2O3
Lutein	C40H56O2
Malonyl_Ginsenoside_Rb1	C57H94O26
Malonyl_Ginsenoside_Rb2	C56H92O25
Malonyl_Ginsenoside_Rb3	C56H92O25
Malonyl_Ginsenoside_Rc	C56H92O25
Malonyl_Ginsenoside_Rd	C51H84O21
Malonyl_Ginsenoside_Re	C51H84O21
Malonyl_Ginsenoside_Rf	C45H74O17
Malonyl-Ginsenoside_Rg1	C45H74O17
MDA_(Methylenedioxyamphetamine)	C10H13N1O2
MDEA_(3,4-Methylenedioxyethamphetamine)	C12H17N1O2
MDMA_(Methylenedioxymethamphetamine)	C11H15N1O2
MDPBP_(3,4-methylenedioxy-alpha-pyrrolidinobutiophenone)	C15H19N1O3
MDPV_(3,4-methylenedioxypyrovalerone)	C16H21N1O3
Memantine	C12H21N1
Mephedrone_(4-methylmethcathinone)	C11H15N1O1
Mephentermine	C11H17N1
Mescaline	C11H17N1O3
Mescaline-NBOMe	C19H25N1O4
Metformin	C4H11N5
Methadone	C21H27N1O1
Methamphetamine	C10H15N1
Methaqualone	C16H14N2O1
Methcathinone	C10H13N1O1
Methdilazine	C18H20N2S1

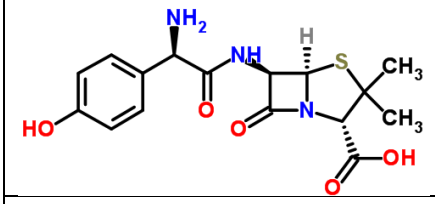
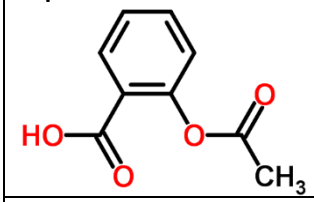
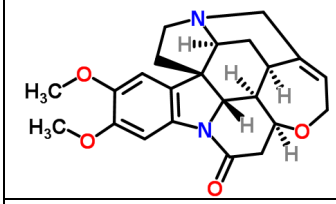
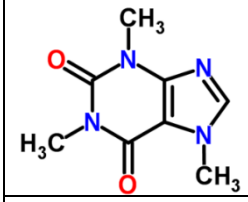
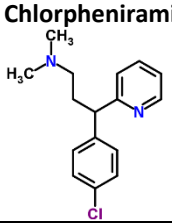
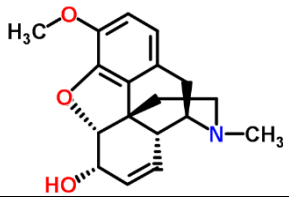
Compound	Formula
Methorphan	C18H25N1O1
Methoxyphenamine	C11H17N1O1
Methylcobalamin	C63H91Co1N13O14P1
Methylephedrine	C11H17N1O1
Methylone_(3,4-Methylenedioxymethcathinone)	C11H13N1O3
Methylphenidate	C14H19N1O2
Methylpseudoephedrine	C11H17N1O1
Metoclopramide	C14H22Cl1N3O2
Metoprolol	C15H25N1O3
Metronidazole	C6H9N3O3
Mianserin	C18H20N2
Midazolam	C18H13Cl1F1N3
Mirodenafil	C26H37N5O5S1
Mirtazapine	C17H19N3
Moclobemide	C13H17Cl1N2O2
Morphine	C17H19N1O3
N,N-dimethylamphetamine	C11H17N1
N,N-dimethyltryptamine	C12H16N2
Naratriptan	C17H25N3O2S1
NDM-Venlafaxine (presumptive)	C16H25N1O2
Nefazodone	C25H32Cl1N5O2
N-ethylamphetamine	C11H17N1
N-ethylcathinone	C11H15N1O1
Nevirapine	C15H14N4O1
Niacinamide	C6H6N2O1
Nicotine	C10H14N2
Nifedipine	C17H18N2O6
Nitrazepam	C15H11N3O3
Norclozapine	C17H17Cl1N4
Norepinephrine	C8H11N1O3
Norfluoxetine	C16H16F3N1O1
Norketamine	C12H14Cl1N1O1
Normethadone	C20H25N1O1
Noroxycodone	C17H19N1O4
Norpethidine	C14H19N1O2
Norsertraline	C16H15Cl2N1
Nortriptyline	C19H21N1
Norverapamil	C26H36N2O4
Notoginsenoside_R1	C47H80O18
Notoginsenoside_R2	C41H70O13
ODM-Tramadol	C15H23N1O2

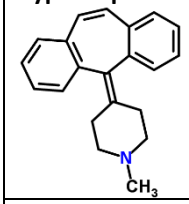
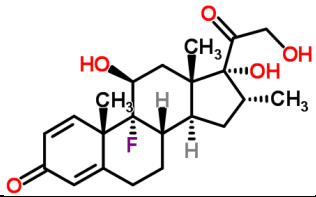
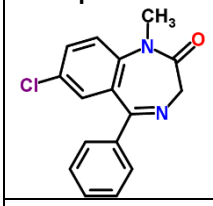
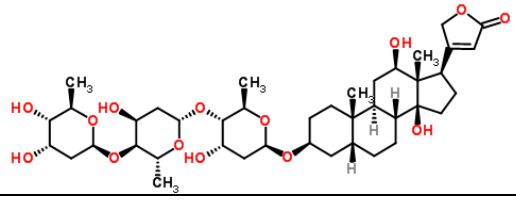
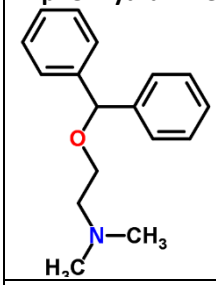
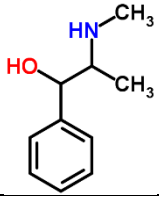
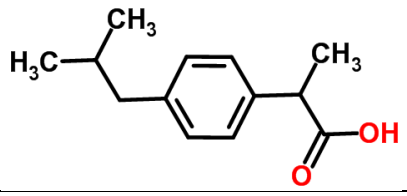
Compound	Formula
ODM-Venlafaxine	C16H25N1O2
Olanzapine	C17H20N4S1
Ondansetron	C18H19N3O1
Orphenadrine	C18H23N1O1
Oxazepam	C15H11Cl1N2O2
Oxpentifylline	C13H18N4O3
Oxycodone	C18H21N1O4
Oxymetazoline	C16H24N2O1
Oxymorphone	C17H19N1O4
Pantothenic_Acid	C9H17N1O5
Paracetamol	C8H9N1O2
Paroxetine	C19H20F1N1O3
PCP_(Phencyclidine)	C17H25N1
Penicillin_G	C16H18N2O4S1
Pentazocine	C19H27N1O1
Perhexiline	C19H35N1
Pericyazine	C21H23N3O1S1
Pethidine	C15H21N1O2
Phenazepam	C15H10Br1Cl1N2O1
Phendimetrazine	C12H17N1O1
Phenethylamine	C8H11N1
Pheniramine	C16H20N2
Phenmetrazine	C11H15N1O1
Phenolphthalein	C20H14O4
Phentermine	C10H15N1
Phenylbutazone	C19H20N2O2
Phenylephrine	C9H13N1O2
Pholcodine	C23H30N2O4
Phylloquinone	C31H46O2
Pimozide	C28H29F2N3O1
Pioglitazone	C19H20N2O3S1
Piroxicam	C15H13N3O4S1
Pizotifen	C19H21N1S1
PMA_(Paramethoxyamphetamine)	C10H15N1O1
Prazepam	C19H17Cl1N2O1
Prednisolone	C21H28O5
Prilocaine	C13H20N2O1
Procaine	C13H20N2O2
Prochlorperazine	C20H24Cl1N3S1
Procyclidine	C19H29N1O1
Promazine	C17H20N2S1

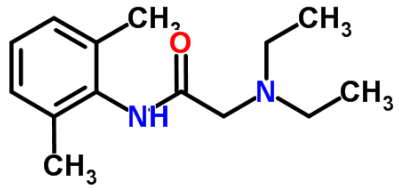
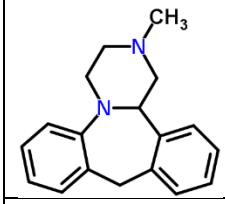
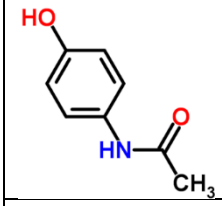
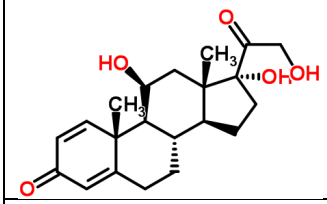
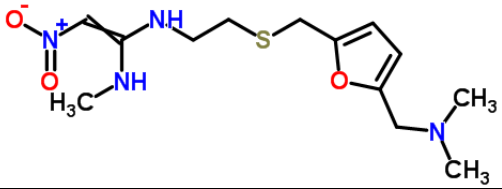
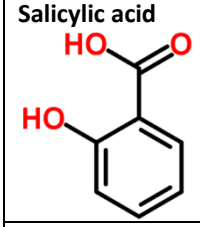
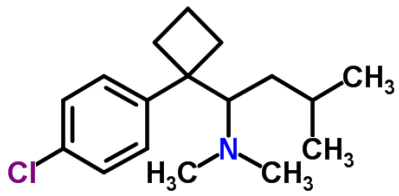
Compound	Formula
Promethazine	C17H20N2S1
Propoxyphene	C22H29N1O2
Propranolol	C16H21N1O2
Protodioscin	C51H84O21
Protopanaxadiol	C30H52O3
Protopanaxatiol	C30H52O4
Protriptyline	C19H21N1
Pyridoxine	C8H11N1O3
Pyridoxine_Hydrochloride	C8H12Cl1N1O3
Quercetin	C15H10O7
Quinic_Acid	C7H12O6
Quinidine	C20H24N2O2
Quinine	C20H24N2O2
Quinquenoside_R1	C56H94O24
Ranitidine	C13H22N4O3S1
Reboxetine	C19H23N1O3
Retinol	C20H30O1
Riboflavin	C17H20N4O6
Rizatriptan	C15H19N5
Ropivacaine	C17H26N2O1
Roxithromycin	C41H76N2O15
Rutin	C27H30O16
Salicylic_Acid	C7H6O3
Scopoletin	C10H8O4
Selegiline	C13H17N1
Sertraline	C17H17Cl2N1
Sibutramine	C17H26Cl1N1
Sildenafil	C22H30N6O4S1
Simvastatin	C25H38O5
Sotalol	C12H20N2O3S1
Streptomycin	C21H39N7O12
Strychnine	C21H22N2O2
Sumatriptan	C14H21N3O2S1
Synephrine	C9H13N1O2
Tadalafil	C22H19N3O4
Tamoxifen	C26H29N1O1
Tapentadol	C14H23N1O1
Taurine	C2H7N1O3S1
Temazepam	C16H13Cl1N2O2
Tetrabenazine	C19H27N1O3
Tetrahydrozoline	C13H16N2

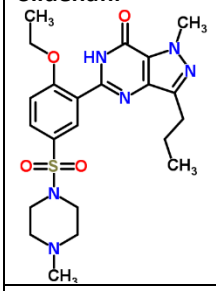
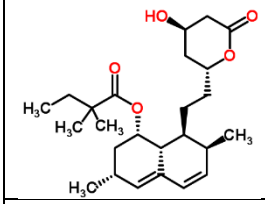
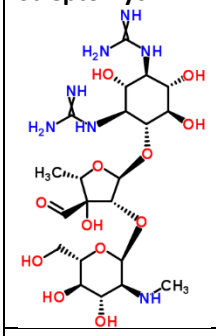
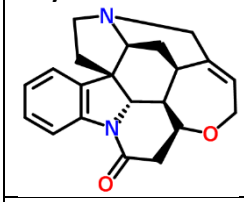
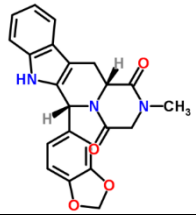
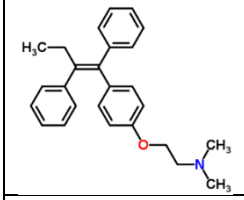
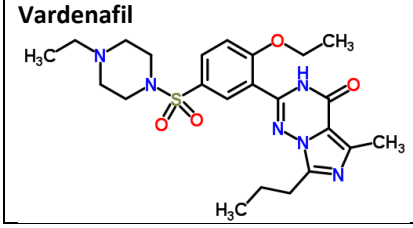
Compound	Formula
TFMPP_(N-(alpha,alpha,alpha-trifluoromethylphenyl)piperzaine)	C11H13F3N2
Thebaine	C19H21N1O3
Theobromine	C7H8N4O2
Theophylline	C7H8N4O2
Thiamine	C12H17N4O1S1
Thiamine_Hydrochloride	C12H18Cl2N4O1S1
Thioridazine	C21H26N2S2
Tiotixene	C23H29N3O2S2
Tolbutamide	C12H18N2O3S1
Tramadol	C16H25N1O2
Tranylcypromine	C9H11N1
Triazolam	C17H12Cl2N4
Trifluoperazine	C21H24F3N3S1
Trigonelline	C7H7N1O2
Trimeprazine	C18H22N2S1
Trimethoprim	C14H18N4O3
Trimipramine	C20H26N2
Triprolidine	C19H22N2
Tropisetrone	C17H20N2O2
Tryptamine	C10H12N2
Tyramine	C8H11N1O1
Udenafil	C25H36N6O4S1
Uncarine	C21H24N2O4
Uracil_Mustard	C8H11Cl2N3O2
Vardenafil	C23H32N6O4S1
Varenicline	C13H13N3
Venlafaxine	C17H27N1O2
Verapamil	C27H38N2O4
Vildagliptin	C17H25N3O2
Warfarin	C19H16O4
Wogonin	C16H12O5
XLR-11	C21H28F1N1O1
Yohimbine	C21H26N2O3
Zaleplone	C17H15N5O1
Ziprasidone	C21H21Cl1N4O1S1
Zolpidem	C19H21N3O1
Zopiclone	C17H17Cl1N6O3
Zuclopenthixol	C22H25Cl1N2O1S1

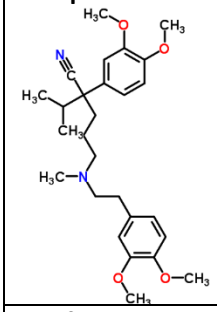
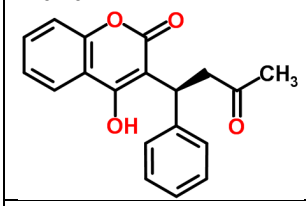
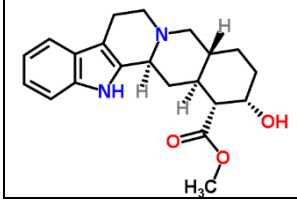
Supplementary Table 9-4: The standard mix compounds structure and expected detection results. The predicted detections are based on previous in-house work on an LC-MS/MS using both ESI and APCI ionization (unpublished) and the compounds chemical properties (e.g. polarity) given the DSA is a modified APCI source and a literature search.

Compound	Expected to be detected in on DSA source (Positive Mode)	DSA-TOF detected (Positive Mode)	LC-ESI-MS Mode
Amoxicillin 	N	N	+
Aspirin 	N	N	-
Brucine 	Y	Y	+
Caffeine 	Y	Y	+
Chlorpheniramine 	Y	Y	+
Codeine 	Y	Y	+

Cyproheptadine 	Y	Y	+
Dexamethasone 	Possibly, based on Savaliya *et. al* [41].	N	+
Diazepam 	Y	Y	+
Digoxin 	N	N	+
Diphenhydramine 	Y	Y	+
Ephedrine 	Y	Y	+
Ibuprofen 	N	N	-

Lignocaine 	Y	Y	+
Mianserin 	Y	Y	+
Paracetamol 	Y	Y	+
Prednisolone 	Possibly, based on Savaliya *et. al* [41].	N	+
Ranitidine 	Y	Y	+
Salicylic acid 	N	N	-
Sibutramine 	Y	Y	+

Sildenafil 	Y	Y	+
Simvastatin 	Possibly	N	+
Streptomycin 	N	N	+
Strychnine 	Y	Y	+
Tadalafil 	Y	Y	+
Tamoxifen 	Y	Y	+
Vardenafil 	Y	Y	+

Verapamil 	Y	Y	+
Warfarin 	Y	Y	+
Yohimbine 	Possibly	Y	+

R Code:

```
# Library ------------------------------------------------------------------------------------------------------
##DSA-TOF RAW DATA ANALYSIS##
setwd("C:/Users/ellyc/Documents/PhD/R/")
.libPaths("C:/Users/ellyc/Documents/PhD/R/Library")
library(enviPat)
library(plyr)
library(dplyr)
library(data.table)
library(xlsx)
library(ggplot2)
library(readr)
library(tidyr)

# Functions ----------------------------------------------------------------------------------------------------

#Functions for error values
mass_error<-function(a,e) {
  a-e
}#a= measured accurate mass, e=exact mass
ppm_error<-function(a,e) {
  ((a-e)/e)*(10^6)
} #a= measured accurate mass, e=exact mass

#this works for individual data frames
Matching_function<-function(RD,tolerance){
  SI<-data.table(
    Compound =SF$Compound,
    Formula = SF$Formula,
    Mass = as.numeric(SF$m.z),
    Start = as.numeric(SF$m.z) - tolerance,
    End = as.numeric(SF$m.z) + tolerance,
    Rel.Ab = SF$Abundance,
```

```
   key= c("Start", "End"))
  foverlaps((data.table(RD)), SI)%>%
   subset(select = c(Sample,Compound, Formula, Mass, Start, End, Rel.Ab, m.z, Intensity))
}

#same as above but with ppm error
Matching_function_ppm<-function(RD,tolerance){
  SI<-data.table(
   Compound =SF$Compound,
   Formula = SF$Formula,
   Mass = as.numeric(SF$m.z),
   Start = as.numeric(SF$m.z) - tolerance,
   End = as.numeric(SF$m.z) + tolerance,
   Rel.Ab = SF$Abundance,
   key= c("Start", "End"))
  R<-foverlaps((data.table(RD)), SI)%>%
   subset(select = c(Sample,Compound, Formula, Mass, Rel.Ab, m.z, Intensity))
  Error<-as.data.frame(mass_error(R$m.z,R$Mass))
  ppm<-as.data.frame(ppm_error(R$m.z,R$Mass))
  cbind.data.frame(R,Error,ppm)
}

# Required Information ----------------------------------------------------------------------------------

###Information import for data analysis###
#See Isotope pattern code on how to compile a data.frame of isotopic patterns
data(adducts)
data(isotopes)
#Write own list using http://www.envipat.eawag.ch/index.php or the enviPat package.
#import standard substance istopic patterns as SF (standard formula).
Standard_Patterns <- read.csv("C:/Users/ellyc/Documents/PhD/R/Standard_Centroid.csv",
                              stringsAsFactors = FALSE)
SF<-Standard_Patterns
#now have a list of compound isotope patterns to match against own data.
```

```
#import raw data as CSV files with columns:
#m.z, start, end (duplicates of the m.z columns) Abundance or Intensity and sample name.
#see Isotope matching code for examples

# Sample data import section ----------------------------------------------------------------------------

#The code below works as an import loop for the triplicate samples
dataR<-Sys.glob("C:/Users/ellyc/Documents/PhD/CAM samples/Category D/CAM_296 B/SP_*.csv") %>%
  lapply(read.csv,stringsAsFactors=FALSE)

for(i in 1:length(dataR)) {
  assign(paste0("Raw.", i), dataR[[i]])
}

# Analysis section ---------------------------------------------------------------------------------------

#single working line for DSA-ToF searching function
Raw.1<-select(Raw.1,m.z,Start, End, Intensity, Sample)
Raw.1$Sample=Raw.1[1,5]
Raw.1<-drop_na(Raw.1)
R1<-Matching_function_ppm(Raw.1,0.01)
write.xlsx(R1,path.expand("C:/Users/ellyc/Documents/PhD/CAM samples/Category D/CAM_296 B/Raw_1.xlsx"))

Raw.2<-select(Raw.2,m.z,Start, End, Intensity, Sample)
Raw.2$Sample=Raw.2[1,5]
Raw.2<-drop_na(Raw.2)
R2<-Matching_function_ppm(Raw.2,0.01)
write.xlsx(R2,path.expand("C:/Users/ellyc/Documents/PhD/CAM samples/Category D/CAM_296 B/Raw_2.xlsx"))

Raw.3<-select(Raw.3,m.z,Start, End, Intensity, Sample)
```

```
Raw.3$Sample=Raw.3[1,5]
Raw.3<-drop_na(Raw.3)
R3<-Matching_function_ppm(Raw.3,0.01)
write.xlsx(R3,path.expand("C:/Users/ellyc/Documents/PhD/CAM samples/Category D/CAM_296 B/Raw_3.xlsx"))

##END PROCESSING##

# Processed Data Import ----------------------------------------------------------------------------
#code for dealing with processed data:
#import loop for the triplicate samples
dataP<-Sys.glob(
  "C:/Users/ellyc/Documents/PhD/CAM samples/Category D/CAM_296 B/Raw_*.xlsx")%>%
  lapply(read.xlsx2,sheetIndex=1,stringsAsFactors=FALSE)

for(i in 1:length(dataP)) {
  assign(paste0("Processed.", i), dataP[[i]])
}
#NEXT

# Formatting ---------------------------------------------------------------------------------------
N<-
c("X.","Sample","Compound","Formula","Mass","Rel.Ab","m.z","Intensity","mass_error","ppm_error")
specify_decimal <- function(x, k) trimws(format(round(x, k), nsmall=k))
for(i in 1:length(Processed.1)){
  Processed.1$Sample =Processed.1[1,2]
  Processed.1[Processed.1=="ERROR"]<-NA
}
colnames(Processed.1)<-N
Processed.1$X.<-NULL
P1<-drop_na(Processed.1)
P1$mass_error<-specify_decimal(as.numeric(P1$mass_error),4)
P1$ppm_error<-specify_decimal(as.numeric(P1$ppm_error),2)
```

```
P1$ppm_error<-as.numeric(P1$ppm_error)
P1<-filter(P1,P1$ppm_error >-15 & P1$ppm_error <15)
P1<-arrange(P1,(as.numeric(P1$Mass)))
write.xlsx(P1,path.expand("C:/Users/ellyc/Documents/PhD/CAM samples/Category D/CAM_296 B/Processed_1.xlsx"))

for(i in 1:length(Processed.2)){
  Processed.2$Sample = Processed.2[1,2]
  Processed.2[Processed.2=="ERROR"]<-NA
}
colnames(Processed.2)<-N
Processed.2$X.<-NULL
P2<-drop_na(Processed.2)
P2$mass_error<-specify_decimal(as.numeric(P2$mass_error),4)
P2$ppm_error<-specify_decimal(as.numeric(P2$ppm_error),2)
P2$ppm_error<-as.numeric(P2$ppm_error)
P2<-filter(P2,P2$ppm_error >-15 & P2$ppm_error <15)
P2<-arrange(P2,(as.numeric(P2$Mass)))
write.xlsx(P2,path.expand("C:/Users/ellyc/Documents/PhD/CAM samples/Category D/CAM_296 B/Processed_2.xlsx"))

for(i in 1:length(Processed.3)){
  Processed.3$Sample =Processed.3[1,2]
  Processed.3[Processed.3=="ERROR"]<-NA
}
colnames(Processed.3)<-N
Processed.3$X.<-NULL
P3<-drop_na(Processed.3)
P3$mass_error<-specify_decimal(as.numeric(P3$mass_error),4)
P3$ppm_error<-specify_decimal(as.numeric(P3$ppm_error),2)
P3$ppm_error<-as.numeric(P3$ppm_error)
P3<-filter(P3,P3$ppm_error >-15 & P3$ppm_error <15)
P3<-arrange(P3,(as.numeric(P3$Mass)))
```

```
write.xlsx(P3,path.expand("C:/Users/ellyc/Documents/PhD/CAM samples/Category D/CAM_296 B/Processed_3.xlsx"))

#NEXT

# Comparing tripicates ----------

P4<-inner_join(P1,P2, by="Mass")

P5<-full_join(P4,P3, by="Mass")

P6<-P5 %>% distinct(Mass, .keep_all=TRUE)

average_PPM<-(as.numeric(P6$ppm_error.x) + as.numeric(P6$ppm_error.y) + as.numeric(P6$ppm_error))/3

average_PPM<-specify_decimal(average_PPM,2)

P7<-cbind(P6,average_PPM)

write.xlsx(P7,path.expand("C:/Users/ellyc/Documents/PhD/CAM samples/Category D/CAM_296 B/CAM_296 B.xlsx"))

##END DATA CLEAN UP##
```

Supplementary Table 9-5: Data from the original study [184] with the addition of the DSA ID. 'Further investigation needed' is noted when a wide variety of putative compounds have been identified, but adulteration or contamination cannot be confirmed. Samples 78, 186 and 253 include different batches of the same product, noted by "_1" and "_2". Sample 296 contained multiple tablets or pills. 'T.S. Neg.' indicates negative for toxicology screen.

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
4	N	Apium graveolens, Astragalus membranaceus, Avena sativa, Barosma betulina, Berberis vulgaris, Betacarotene, Biotin, Calcium ascorbate dihydrate, Calcium citrate, Calcium pantothenate, Camellia sinensis, Carica papaya, Centella asiatica, Cholecalciferol, Choline bitartrate, Chromium picolinate, Citrus bioflavonoids extract, Copper gluconate, Crataegus monogyna, Cyanocobalamin, Cynara scolymus, d-alpha tocopheryl acid succinate, Equisetum arvense, Ferrous fumarate,	Caffeine	Phenylephrine/ Synephrine (5.15 ppm) Niacinamide (4.88 ppm) Pyridoxine (7.84 ppm) Pantothenic acid (7.87 ppm) Epinephrine (7.97 ppm) Thiamine (5.64 ppm) Riboflavin (1.77 ppm)	Apiaceae, Asteraceae, Araliaceae (includes Ginseng), Ericales (includes Tea), Ixoroideae (includes Coffee), Solanoideae, Rutaceae (includes Citrus), Pooideae (inculde Oats)		Expected Pharmaceutical, Confirmed Ingredients

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
		Foeniculum vulgare, Folic acid, Ginkgo biloba, Inositol, Lycopersicon esculentum, Lysine hydrochloride, Magnesium oxide-heavy, Manganese amino acid chelate, Nicotinamide, Panax ginseng, Petroselinum crispum, Potassium iodide, Potassium sulfate, Pyridoxine hydrochloride, Riboflavin, Selenomethionine, Serenoa repens, Silybum marianum, Smilax officinalis, Spearmint oil, Thiamine hydrochloride, Turnera diffusa, Tyrosine, Ubidecarenone, Vaccinium myrtillus, Vitis vinifera, Zinc amino acid chelate, Zingiber officinale					
6	Y	Caralluma adscendens var. fimbriata		Trigonelline (3.62 ppm), Delta-9-tetrahydrocannabinol (2.75 ppm)	Asterids (includes C. fimbriata), Cyamopsis tetragonoloba (Guar plant), Acalypheae		Confirmed Ingredients, T.S. Neg., Additional Plant DNA, Contains Fillers, Further investigation needed

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
8	Y	Fig dry, Phoenix dactylifera, Senna alexandrina extract		Trigonelline (4.34 ppm) Pyridoxine (3.53 ppm)	Arecaceae (includes P. dactylifera), Fabaceae (includes S. alexandrina), Ficus (fig), Prunus, Brassicaceae, Poaceae (grass)	Canis lupus familiaris, Capra hircus, Sus scrofa scrofa	Animal Contaminants, Additional Plant DNA, Confirmed Ingredients
9	N	Fish Oil, Food Acids, Gelatin, Glucose, Natural Colours, Natural Flavours, Sugar, Vitamin E, Water		Scopoletin (3.80 ppm) Ferulic acid (7.42 ppm) Alpha thujone (3.48 ppm) Prazepam (4 ppm)			Further investigation needed
10	Y	Alisma ago aquatica, Comus officinalis, Dioscorea opposita, Ophiopogon japonicus, Paeonia suffruticosa, Poria cocos, Rehmannia glutinosa, Schizandra chinensis		Trigonelline (3.86 ppm) Pyridoxine (10 ppm) Phenylephrine/ Synephrine (3.17 ppm) Thiamine (5.01 ppm)	Paeonia (includes P. suffruticosa), PACMAD clade, Anthemideae, Apiaceae, Pteridium	Bos	Animal Contaminants, Additional Plant DNA, Confirmed Ingredients, Further investigation needed
15	Y	Ulmus rubra		Pyridoxine (3.92 ppm) Cantharidin (9.13 ppm) Curcumin (7.31 ppm) Ginkgolide A (6.35 ppm)	Ulmus, Brassicaceae		Additional Plant DNA, Confirmed Ingredients, Further investigation needed
22	N	Creosote, Citris Unshiu Peel, Gambir, Glycyra, Phellodendron	Synephrine 0.4 mg/g		Rutaceae, Glycyrrhiza		Expected Pharmaceutical, Confirmed Ingredients

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
23	N	Calcium Carbonate, Chromium Polynicotinate, Garcinia cambogia extract, Green coffee extract, Magnesium Stearate, Microcrystalline Cellulose, Moringa oleifera, Potassium Citrate, Silicon Dioxide	Caffeine	Caffeine (3.59 ppm)	Amaranthaceae, Apiaceae, Poaceae		Expected Pharmaceutical, Additional Plant DNA, Contains Fillers
40	Y	Ascophyllum nodosum, Fucus vesiculosus, Iodine		Pyridoxine (6.47 ppm)	Rosoideae, Holcus		T.S. Neg., Additional Plant DNA, Contains Fillers
43	Y	Angelica archangelica, Carum carvi, Chelidonium majus, Glycyra glabra, Iberis amara, Matricaria chamomilla, Melissa officinalis, Mentha X piperita, Silybum marianum		Trigonelline (5.55 ppm) Kaempferol (7.55 ppm) Retinol (3.13 ppm)			Confirmed Ingredients
44	Y	Abrus cantoniensis, Bupleurum falcatum, Curcuma longa, Cyperus rotundus, Desmodium styracifolium, Gardenia florida, Glycyra uralensis, Paeonia veitchii, Rheum palmatum, Scutellaria baicalensis	Synephrine 1.1 mg/g	Wogonin (6.78 ppm) Tyramine (8.45 ppm) Synephrine#	Cedrus, Apiaceae (includes B. falcatum), Artemisia, Oleaceae, Solanoideae, PACMAD clade		Additional pharmaceutical, Additional Plant DNA, Confirmed Ingredients

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
47	N	Angelica polymorpha, Atractylodes macrocephala, Bupleurum falcatum, Citris aurantium, Glycyra uralensis, Leonurus sibiricus, Mentha haplocalyx, Paeonia lactiflora, Paeonia suffruticosa, Poria cocos, Spatholobus suberectus, Trichosanthes kirilowii	Synephrine 0.5 mg/g	Amisulpride (4.77 ppm)	Panax, Ericale, Dalbergieae		Expected Pharmaceutical, Additional Plant DNA, Further investigation needed
50	Y	Docosahexaenoic acid, Eicosapentaenoic acid					Nothing detected
51	Y	Docosahexaenoic acid, Eicosapentaenoic acid, Euphausia superba					Nothing detected
52	Y	d-alpha tocopherol, Euphausia superba, Fish Oil - Natural, Glucosamine Hydrochloride					Nothing detected
53	Y	Docosahexaenoic acid, Eicosapentaenoic acid					Nothing detected
54	Y	Docosahexaenoic acid, Eicosapentaenoic acid, Euphausia superba					Nothing detected
55	Y	Euphausia superba, Omega- triglycerides					Nothing detected
59	Y	Astragalus complanatus, Calcium sulfate, Euryale ferox, Nelumbium speciosum, Oyster Shell		Pyridoxine (7.84 ppm) Phenylephrine/ Synephrine (10.31 ppm) Trigonelline (3.14 ppm)	Nymphaeaceae, Nelumbo, Glycyrrhiza, Rutaceae, Paeonia, Asterids	Bos, Capra hircus	Animal Contaminants, Additional Plant DNA, Confirmed

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
				Tyramine (8.93 ppm)			Ingredients, Further investigation needed
62	N	Unknown		Salicylic acid (4.32 ppm) Cinnaminc acid (4.86 ppm) Ferulic acid (5.54 ppm) Catharidin (7.27 ppm) Norketamine (9.67 ppm) Nifedipine (8.55 ppm)	Danthonia, Panicoideae, Maleae, Plantago, Gynostemma, Rutaceae, Papilionoideae (including Fabeae, Glycyrrhiza, Vigna)		Contains Fillers, Further investigation needed
66	Y	Amorphophallus konjac			Araceae (includes A. konjac)	Bos, Sus scrofa	T.S. Neg., Animal Contaminants, Confirmed Ingredients
68	Y	Coffea canephora		Pantothenic Acid (2.88 ppm)	BEP clade, Saliceae, Fabeae, Apiaceae, Anacardium	Bos	T.S. Neg., Animal Contaminants, Contains Fillers
69	N	Acerola Fruit Powder, Alfalfa Powder, Apple Powder, Ascorbic Acid, Astragalus Extract, Barley Powder, Beta Glucan, Betacarotene, Bilberry Fresh Fruit Extract, Biotin, Broccoli Powder, Burdock Extract, Calcium Carbonate, Calcium Citrate, Calcium Pantothenate, Calcium Phosphate, Carica papaya, Carica Papaya			Papilionoideae (inlcuding Fabeae, Triticeae, Oryza, Hordeum) Bambusoideae, Glycyrrhiza, Linum usitatissimum, Rhodiola, Beta vulgaris, Brassicaceae, Zingiberaceae, Heliantheae, Elsholtzieae, Apiaceae, Asteraceae	Rangifer tarandus (Reindeer), Sus crofa, Mus musculus, Rattus, Capra	T.S. Neg., Animal Contaminants, Additional Animal DNA, Confirmed Ingredients

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
		Powder, Carrot Powder, Chlorella Powder, Citric Acid, Citrus Bioflavanoids Extract, Cocoa Bean Polyphenol Extract, Colloidal Anhydrous Silica, Copper Gluconate, Cyanocobalamin, Dandelion Extract, Dibasic Phosphate, Ergocalciferol, Flaxseed Powder, Folic Acid, Ginger ome Powder, Globe Artichoke Extract, Gotu kola Extract, Grapeseed Extract, Green Tea Extract, Hawthorne Fruit Powder, Inulin, Kelp Powder, Lecithin, Licorice Powder, Magnesium Citrate, Manganese Amino Acid Chelate, Milk Thistle Extract, Nicotinamide, Nicotinic Acid, Pea Protein Isolate, Picolinate, Pineapple Flavour, Pineapple Fruit Extract, Pyridoxine Hydrochloride, RS-Alpha Lipoic Acid, Red Beet Powder, Reishi Mushroom Powder, Resveratol, Riboflavin,					

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
		Rice Bran Powder, Rosehip Fruit Extract, Rosemary Extract, Selenomethione, Shitake Mushroom Powder, Slippery Elm Powder, Spinach Powder, Spirulina, Thaumatin, Thiamine Hydrochloride, Ubidecarenone, Vanilla Flavour, Wheatgrass Powder, Withania somnifera Extract, Wolfberry (Goji) Fruit Powder, Zinc Amino Acid Chelate					
70	Y	Shark cartilage			Glycine (soybean)	Sus scrofa, Rattus, Canis lupus familiaris, Anatidae, Phasianinae, Galeocerdo cuvier, Carcharhinus	T.S. Neg., Animal Contaminants, Additional Animal DNA, Contains Fillters
71	Y	Colostrum powder - bovine			Fabeae, Helianthease, Triticeae, Citrus, Glycine (soybean)	Bos	T.S. Neg., Contains Fillers

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
74	Y	Retinyl Acetate, Lutein, Lycopene, Betacarotene, Thiamine Nitrate, Riboflavin, Nicotinamide, Pyridoxine Hydrochloride, Cyanocobalamin, Ascorbic Acid, Cholecalciferol, d-alpha-tocopherol Acetate, Phytomenadione, Biotin, Folic Acid, Calcium Pantothenate, Calcium Carbonate, Calcium Hydrogen Phosphate, Potassium Sulfate, Chromic Chloride, Copper Sulfate, Potassium Iodide, Ferrous Fumarate, Magnesium Oxide, Manganese Sulfate, Sodium Selenate, Zinc Oxide		Niacinamide (2.17ppm)	Citrus, Apioideae		Confirmed Ingredients
77	Y	d-alpha Tocopherol, Ubidecarenone, Zinc Amino Acid Chelate		Alpha-Tocopherol (1.24 ppm)	Oryza sativa		T.S. Neg., Contains Fillers, Confirmed Ingredients
78_1	Y	Selenomethionine, Silybum marianum, Taurine		Kaempferol (4.53ppm) Quercetin (0.99 ppm)	Oryza, PACMAD clade, Brassicaceae, Asteraceae (includes S. marianum)		T.S. Neg., Additional Plant DNA, Contains Fillers, Confirmed Ingredients

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
78_2	Y	Selenomethionine, Silybum marianum, Taurine		Kaempferol (2.44ppm) Quercetin (1.54ppm) Taurine (1.42 ppm)	Asteraceae (includes S. marianum)	Sus scrofa	T.S. Neg., Confirmed Ingredients
80	Y	Citris bioflavonoids extract, Tocotrienols complex - palm			Rutaceae (includes Citrus), Azadirachta indica		T.S. Neg., Additional Plant DNA
81	Y	d-alpha-Tocopherol		Alpha-Tocopherol (9.19 ppm)			Confirmed Ingredients
82	N	Pure New Zealand Manuka Honey			Podocarpaceae, Loganiaceae, Anthospermeae, Pseudocarpidium, Corynocarpus, Ulex, Melicytus, Phormium tenax, Laurales, Asteraceae		T.S. Neg. (DNA from pollen in the honey)
84	Y	Globe artichoke (Cynara scolymus), St Mary's Thistle, Tumeric		Quercetin (0.77 ppm), Kaemperol (5.69 ppm)	Cynara cardunculus var. scolymus, Medicago sativa, Zingiberales (includes Curcuma), Asparagales, Quercus, Brassicaceae, Citrus, Triticeae		T.S. Neg., Additional Plant DNA, Contains Fillers, Confirmed Ingredients
86	Y	Cordyceps sinensis, Eleutherococcus senticosus, Withania somnifera, Ganoderma lucidum, Astragalus membranaceus, Panax ginseng, Panax quinquefolium	Buclizine 0.0002 mg/g (0.2ug/g)		Astragalus, Morus, Maleae, Brassicaceae, Araliaceae		Additional pharmaceutical, Additional Plant DNA

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
88	N	Ginger, Ginseng, Guarana, Spirits	Caffeine, theobromine	Caffeine (8.03 ppm)			Expected Pharmaceutical
91	N	Burdock, Dandelion, Milk Thistle, Nettle, Spirits		Trigonelline (8.45ppm)			Further investigation needed
93	Y	Isatis tinctoria, Taraxacum mongolicum, Viola yedoensis	Ephedrine 0.0003 mg/g (0.3ug/g)				Additional pharmaceutical
94	Y	Curcumin phospholipid complex			Papilionoideae		T.S. Neg.
99	Y	Chromic chloride, Cinamomum cassia, Coleus forskohlii, Gymnema sylvestre, Hydroxycitrate complex					Nothing detected
121	Y	Achyranthes bidenta, Eucommia ulmoides, Gardenia floridia fruit, Gastrodia elata tuber, Leonurus sibiricus herb, Oyster shell powder, Polygonatum multiflorum , Poria cocos fruit , Scutellaria baicalensis, Uncaria rhyncophylla , Viscum coloratum herb		Wogonin (2.34 ppm) Trigonelline (7 ppm) Cinnamic Acid (6.48 ppm) Pyridoxine (1.57 ppm) Clobazam/ Temazepam (6.86 ppm)	Asteraceae, Lamiales (includes S.baicalensis and L. sibiricus), Boraginaceae, Glycyrrhiza, Pooideae, Nymphaeaceae, Apiaceae, Ornithogaloideae, Campanulaceae, Anthemideae, Lonicera, Galium, Boraginaceae, Convolvulaceae, Solanum, Thermopsis, Prunus, Rosoideae, Ulmaceae, Brassicaceae, Avena, Triticeae, Zingiberaceae		T.S. Neg., Additional Plant DNA, Contains Fillers, Confirmed Ingredients, Further investigation needed

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
124	Y	Angelica polymorpha, Carthamus tinctorius, Ligusticum wallichii, Paeonia laciflora, Prunus persica, Rehmannia glutinosa			Paeonia (includes P. laciflora), BEP clade, Rutaceae, Apiaceae (includes A. polymorpha and L. wallichii)		T.S. Neg., Confirmed Ingredients
128	Y	Achyranthes bidenta, Angelica polymorpha, Bupleurum falcatum, Carthamus tinctorious, Citrus aurantium, Glycyra uralenses, Ligusticum sinense, Paeonia lactiflora, Platycodon grandiflorum, Prunus persica, Rehmannia glutinosa	Synephrine 0.32 mg/g		Amaranthaceae (includes A. bidenta), Apisceae (includes A. polymorpha and B. falcatum), Paeonia (includes P. laciflora), Sapindales (includes C. aurantium), Poaceae, Polycarpon, Polygonoideae		Expected Pharmaceutical, Additional Plant DNA, Confirmed Ingredients
178	N	Devil's Claw			Harpagophytum procumbens		T.S. Neg., Confirmed Ingredients
186_1	Y	Allium sativum			Glycine	Bos	T.S. Neg., Animal Contaminants
186_2	Y	Allium sativum			Glycine		T.S. Neg.
217	Y	Achillea millefolium, Arctium lappa, Berberis aquifolium, Frangula purshiana, Handroanthus heptaphyllus, Rhamnus frangula, Rumex crispus, Smilax officinalis, Taraxacum officinale, Trifolium pratense,			Zanthoxylum, Rhamnus, Trifolium, Asteroideae (includes A. millefolium)	Canis lupus familiaris, Bos, Cervinae (deer)	T.S. Neg., Animal Contaminants, Confirmed Ingredients

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
		Zanthoxylum clava-herculis					
218	Y	Lecithin, Piper nigrum, Ubidecarenone		Piperine (5.47 ppm)			Confirmed Ingredients
221	Y	Aesculus hippocastanum, Ginkgo biloba, Ruscus aculeatus, Vaccinium myrtillus		Kaempferol (7.78 ppm)			Confirmed Ingredients
224	Y	Camellia sinensis, Chitosan, Chromic chloride, Chromium picolinate, Citris aurantium, Fucus vesiculosus, Gymnema sylvestre, Pyridoxine hydrochloride, Riboflavin, Thiamine hydrochloride	Synephrine Caffeine 0.28 mg/g	Riboflavin (1.59 ppm) Epicatechin (4.92 ppm)			Expected Pharmaceutical, Confirmed Ingredients
225	Y	Coffea canephora	Caffeine 50.59 mg/g	Pyridoxine (0.78 ppm) Caffeine (4.79 ppm)			Expected Pharmaceutical
226	Y	Garcinia gummi-gutta, Nicotinamide, Pyridoxine hydrochloride, Thiamine hydrochloride		Niacinamide (7.59 ppm) Pyridoxine (6.27 ppm) Thaimine# Trimethoprim (3.55 ppm)			Confirmed Ingredients
227	Y	Camellia sinensis, Capsicum annuum, Chromic chloride, Citris aurantium, Garcinia quaesita, Pyridoxine hydrochloride, Taurine, Zingiber officinale	Synephrine, Caffeine 4.12 mg/g	Pyridoxine (4.90 ppm) Gallic acid (3.90 ppm) Methcathinone (8.33 ppm) Scopoletin (7.60 ppm)			Expected Pharmaceutical, Confirmed Ingredients, Further investigation needed

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
228	Y	Camellia sinensis, Cocos nucifera, Coffea canephora, Garcinia gummi-gutta	Caffeine 1.69 mg/g	Pantothenic Acid# Kaempferol (7.31 ppm) Pyridoxine (7.64 ppm)			Expected Pharmaceutical
229	Y	Coffea canephora	Caffeine 0.43 mg/g	Pantothenic Acid#	Oryza, Triticeae, Apocynaceae, Platanus orientalis, Solanaceae		Additional Plant DNA, Contains Fillers, Expected Pharmaceutical
230	N	Acai berry, Ascorbic acid, Black tea powder, Capsicum annuum, Citric acid, Elderberry, Flavours, Goji berry, L-Carnitine, Pomegranate powder, Red grape powder, Schisandra berry, Stevia, Xylitol	Caffeine 3.33 mg/g	L-Theanine (1.90 ppm) Caffeine (4.27 ppm) Quercetin (6.16 ppm)	Lythraceae		Expected Pharmaceutical
231	N	Acidophilus, Aloe vera, Bertonite Clay, Buckthorn, Calcium carbonate, Cape aloe, Cascara sagrada, Cayenne Pepper, Cirus pectin, Fennel Seed, Flax Seed Oil, Gelatin, Ginger, Irvingia gabonesis, Licorice, Magnesium stearate, Oat Bran, Prune Juice, Pumpkin Seed, Rhubarb, Senna			Quercus		T.S. Neg.
232	Y	Hydoxycitrate complex			Musaceae		T.S. Neg., Additional Plant DNA

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
233	Y	Hydroxycitrate complex					Nothing detected
234	N	African Mango Seed Extract, Calcium Phosphate, Hypromellose, Magnesium Stearate, Purified Water, Silicon Dioxide		Pyridoxine (5.68 ppm) Ephedrine (10.64 ppm)	Avena		Contains Fillers
235	Y	Premium Garcinia Extract (Garcinia quaesita)			Fagaceae		Additional Plant DNA
236	N	Hydroxy Citric Acid			Oryza		Contains Fillers
237	Y	Potassium Iodide, Rubus idaeus		Pyridoxine (7.05 ppm), Trigonelline (5.55 ppm)			Further investigation needed
238	Y	Hydroxycitrate complex					Nothing detected
239	Y	Camellia sinensis, Coffea canephora	Caffeine 6.01 mg/g	Pyridoxine (1.57 ppm) Quinic acid (9.84 ppm) Kaempferol (1.74 ppm) Epicatechin (3.55 ppm) Quercetin (2.31 ppm) Epigallocatechin (5.43 ppm) Ginkolide A or Amlodipine (3.34 ppm)			Expected Pharmaceutical, Confirmed Ingredients
240	N	Chromic chloride, Cinnamomum cassia, Coleus forskohlii, Garcinia quaesita, Gymnema sylvestre, Hydroxycitrate complex, Piper nigrum		Piperine (4.54 ppm)			Confirmed Ingredients
241	Y	Chromium chloride, Coffea canephora, Encapsulating aids	Caffeine 3.22 mg/g	Pyridoxine (9.80 ppm) Phenylephrine/ Synephrine (3.17 ppm)			Expected Pharmaceutical

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
				Caffeine (7.53 ppm) Panthothenic Acid# Levetiracetam (1.36 ppm)			
242	Y	Coffea canephora	Caffeine 2.73 mg/g	Caffeine#			Expected Pharmaceutical
243	Y	Crataegus pinnatifida, Dioscorea opposita, Nelumbium speciosum, Poria cocos	Caffeine 0.55 mg/g	Alpha-Thujone (11.67 ppm) Gallic Acid (2.34 ppm) Caffeine (6.84 ppm) Kaemperol (2.09 ppm) Epicatechin (4.24 ppm) Quercetin (3.74 ppm)	Plantago		Additional Pharmaceutical, Additional Plant DNA
244	Y	Caralluma adscendens, Crocus sativus	Caffeine 0.04 mg/g	Trigonelline (3.14 ppm) Ephedrine (9.23 ppm) Phenylephrine/ Synephrine (0.99 ppm) Delta-9-tetrahydrocannabinol (5.08 ppm) Prednisolone (5.45 ppm) Levetiracetam (5.26 ppm) Methcathinone (5.68 ppm)			Expected Pharmaceutical, Further investigation needed
245	N	Cinnamon, Ganoderma mushroom, Green Tea, Peppermint, Chicory	Caffeine 3.63 mg/g	Caffeine (4.27 ppm) Kaempferol (4.88 ppm) Quercetin (3.19 ppm)	Mentha, Theaceae	Canis lupus familiaris, Bos, Camelus dromedarius, Rhacophorinae , Suncus montanus (Asian highland shrew)	Animal Contaminants, Additional Animal DNA, Expected Pharmaceutical, Confirmed Ingredients
246	Y	Citrus aurantifolia, Crataegus pinnatifida, Hordeum vulgare, Nelumbo nucifera, ago		Kaempferol (8.01 ppm) Isorhamnetin (3.99 ppm) Ginkgolide A (11.49 ppm)	Fabaceae (includes S. alexandrina)	Canis lupus familiaris, Bos,	Animal Contaminants, Confirmed Ingredients

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
		asiatica, Poria cocos, Rheum officinale, Senna alexandrina, Vigna umbellate					
247	N	Raspberry Ketone					Nothing detected
248	Unknown	Alfalfa Extract, Barley Grass Powder, Broccoli Powder, Calcium Hydrogen Phosphate, Chlorella Powder, Green Kale Powder, Green Tea Extract, Kelp Extract, Magnesium Stearate, Nori Powder, Pea Protein, Povidone, Shitake Mushroom Powder, Silica Colloidal Anhydrous, Spinach Powder, Spirulina, Wheat Grass Powder	Caffeine, theobromine	Kaempferol (3.72 ppm) Epicatechin (4.47 ppm) Caffeine (2.56 ppm)	Hordeum, Brassicaseace, Pooideae, Fabeae		Expected Pharmaceutical, Confirmed Ingredients
253_1	Y	Trigonella foenum-graecum		Trigonelline (5.07 ppm) Methylone (9.29 ppm) Fluoxetine#	Trigonella, Parietaria, Musaceae, Apocynaceae		T.S. Neg., Additional Plant DNA, Confirmed Ingredients, Further investigation needed
253_2	Y	Trigonella foenum-graecum		Trigonelline (4.34 ppm) Phenylephrine/ Synephrine (4.56 ppm)	Plantago, Asteraceae	Canis lupus	Animal Contaminants, Additional Plant DNA, Further investigation needed

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
286	Y	Coleus forskohlii		Forskolin (5.67 ppm) Azatadine (4.47 ppm)	Theaceae, Ocimeae		Additional Plant DNA, Confirmed Ingredients
287	N	Garcinia cambogia extract, Garcinia cambogia Gelatin Magnesium stearate microcrystalline cellulose			Apiaceae		Additional Plant DNA
288	Y	Coleus forskohlii, Chromium picolinate		Forskolin (3.65 ppm) Flupentixol (1.53 ppm)	Trigonella, Piper	Ovis (sheep)	Animal Contaminants, Additional Plant DNA
289	N	Vitamin B, Chromium picolinate, Green tea extract, Cha De Bugre powder, Guarana seed extract, Caffeine anhydrous, Hoodia chinese extract	Caffeine 141 mg/g	Pyridoxine (1.37 ppm) Caffeine (4.95 ppm) Kaempferol (0.12 ppm) Quercetin (5.61 ppm)	Salicaceae		Additional Plant DNA, Expected Pharmaceutical, Confirmed Ingredients
290	N	African mango extract, Green tea extract, Solathin Non-GMO potato	Caffeine 0.12 mg/g	Caffeine#			Expected Pharmaceutical
291	Y	Garcinia cambogia, Hydroxycitric acid, Chromium chloride			Musaceae		Additional Plant DNA
292	Y	Caralluma adscendens var. fimbriata extract		Delta-9-tetrahydrocannabinol (2.33 ppm)			Further investigation needed

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
293	Y	Astragalus membranaceous, Crataegus pinnatifida, Polygonum multiform, Codonopsis pilosula, Atractylodes macrocephala, Nelumbium speciosum, Pinellia temata, Poria cocos, Alisma ago aquatica, Morus alba, Cassia tora, Citrus reticulata, Zizyphus jubata		Trigonelline (4.11 ppm) Phenylephrine/ Synephrine (3.77 ppm)	Asteraceae		Further investigation needed
294	N	Garcinia Cambogia with HCA, Calcium phosphate, Hypromellose, maltodextrin, Silicon dioxide, Magnesium stearate, Purified water					Nothing detected
295	N	Green tea extract (Camelia sinensis), Hordenine, N-Methyltyranine, Octopamine, Tyramine, Ginger (Gingerols), L-tyrosine, Grape seed extract, Quercetin, Vitamin C, Vitamin B (as pyridoxine), Pantothenic acid, Magnesium carbonate		Pyridoxine (0.78 ppm) Theobromine/ Theophylline (4.05 ppm) Adrenaline (2.72 ppm) Caffeine (7.35 ppm) Pantothenic Acid (3.03 ppm)	BEP clade, Crotonoideae		Expected Pharmaceutical, Contains Fillers

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
296 A							T.S. Neg.
296 B				Paracetamol (10.52 ppm) Chlorpheniramine (3.51 ppm) Ephedrine (7.02 ppm)			Additional pharmaceutical, Further investigation needed
297	N	Garcinia cambogia, potassium, Calcium, Chromium			Poaceae		Contains Fillers
298	N	Green coffee bean extract	Caffeine 0.06 mg/g	Caffeine (8.71 ppm)	Ericales, Fabaceae, Apiaceae		Additional Plant DNA, Contains Fillers, Expected Pharmaceutical
300	N	chromium picolinate, cobalamin (vit B), thiamine, riboflavin, niacinamide, pantothenic acid, pyridoxine HCl, green coffee bean, chlorogenic acid, gardenia cambogia, HCA, raspberry ketones, white kidney bean extract, green tea extract.		Pyridoxine (3.92 ppm) Pantothenic Acid (6.81 ppm) Trigonelline (6.04 ppm) Niacinamide (2.98 ppm) Riboflavin (1.24 ppm) Kaempferol (3.14 ppm) Quercetin (3.41 ppm)	BEP clade (Oryza)		Contains Fillers, Confirmed Ingredients
301	Y	paullinia cupana extract, citrus aurantium extract, zingber officinale extract, panax ginseng extract, camellia sinensis extract,	Caffeine 27.43 mg/g	Caffeine (5.64 ppm) Pyridoxine (1.76 ppm) Epicatechin (6.64 ppm)	Plantago, Brassicaceae, Rosids		Expected Pharmaceutical, Confirmed Ingredients

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
		psyllium husk powder, chromium picolinate, potassium iodide, pyridoxine hydrochloride, thiamine nitrate, riboflavin, cyanocobalamin					
302	N	calcium, iodine, chromium polynicotinate, sodium, garcinia cambogia extract, panax ginseng extract, gelatin, magnesium stearate, stearic acid					Nothing detected
303	N	cellulose, gelatin, african mango seed powder, silicon dioxide, magnesium stearate, water, maltodextrin		Caffeine (8.37 ppm)	Apiaceae		Additional Plant DNA
305	Y	Camellia sinensis	Caffeine 2.85 mg/g	Caffeine (2.73 ppm) Epicatechin (4.47 ppm) Epigallocatchin (9.63 ppm) Epigallocatechin Gallate (6.75 ppm) Kaempferol (2.79 ppm) L-theanine (1.71 ppm) Quercetin (3.19 ppm) Scopoletin (6.91 ppm)	Apiaceae		Additional Plant DNA, Expected Pharmaceutical, Confirmed Ingredients
313	Unknown	Unknown		Niacinamide (2.44 ppm) Pantothenic Acid (6.51 ppm) Riboflavin (1.95 ppm) Pyridoxine (5.49 ppm)			Confirmed Ingredients

Sample # ID	In ARTG database (Yes/ No)	Ingredients on package	Toxicological data	DSA ID (error)	DNA plant	DNA animal	Comments
317	Y	Natural vitamin E (d-alpha Tocopherol)		Alpha-Tocopherol (1.31 ppm)			Confirmed Ingredients
319	N	Creatine Monohydrate, Dextrose, Peak ATP			PACMAD clade, Ocimum, Theobroma, Papilionoideae, Anacardium		T.S. Neg., Additional Plant DNA, Contains fillers
320	N	Unknown			Amaranthaceae, Papilionoideae, Ulmaceae, Triticeae		T.S. Neg.
321	Y	Camellia sinesis extract, fallopia japonica extract, standardised to contain resveratrol	Caffeine	Caffeine (5.98 ppm) Quercetin (3.19 ppm)			Expected Pharmaceutical
322	Y	Tribulus terrestrial extract	Ephedrine 0.003 mg/g, Pseudoephedrine 0.0007 mg/g, methyephedrine, norphedrine	Ephedrine (7.22 ppm) Trigonelline (7 ppm) Promazine or Promethazine (5.62 ppm) Calcitriol (1.92 ppm)	Ephedra, Anthemideae, Astereae, Solanoideae, Acalypheae, Linum, Triticeae, Medicago, Tribuloideae (includes T. terrestrial)		Additional pharmaceutical, Additional Plant DNA, Contains fillers
323	Y	Citrus aurantium fruit, coleus forskohlii, paullinia cupana seed, capsicum frutescens fruit, zingiber officinate ome, camelia sinesis, RS-alpha lipoic aicd, levocarnitine, potassium iodide	Caffeine, Synephrine 7.4 mg/g	Ephedrine (8.43 ppm) Phenylephrine/ Synephrine (1.78 ppm) Caffeine (3.76 ppm)	Platycladus orientalis, Capsicum, Glycyrrhiza, Rosaceae, PACMAD clade, Theaceae		Expected Pharmaceutical, Additional Plant DNA, Confirmed Ingredients, Further investigation needed
324	N	Calcium Sulphate		Pyridoxine (4.51 ppm)	Pinus		T.S. Neg., Contains Fillers

Co-author papers

Coghlan, M. L., Maker, G., Crighton, E., Haile, J., Murray, D. C., White, N. E., Byard, R. W., Bellgard, M. I., Mullaney, I., Trengove, R., Allcock, R. J., Nash, C., Hoban, C., Jarrett, K., Edwards, R., Musgrave, I. F. & Bunce, M. Combined DNA, toxicological and heavy metal analyses provides an auditing toolkit to improve pharmacovigilance of traditional Chinese medicine (TCM). *Sci Rep* **5**, 17475, doi:10.1038/srep17475 (2015).

Hoban, C. L., Musgrave, I. F., Coghlan, M. L., Power, M. W. P., Byard, R. W., Nash, C., Farrington, R., Maker, G., Crighton, E., Trengove, R. & Bunce, M. Adulterants and Contaminants in Psychotropic Herbal Medicines Detected with Mass Spectrometry and Next-Generation DNA Sequencing. *Pharmaceutical Medicine* **32**, 429-444, doi:10.1007/s40290-018-0252-8 (2018).

Hoban, C. L., Musgrave, I. F., Byard, R. W., Nash, C., Farrington, R., Maker, G., Crighton, E., Bunce, M. & Coghlan, M. Combined Liquid Chromatography-mass Spectrometry and Next-generation DNA Sequencing Detection of Adulterants and Contaminants in Analgesic and Anti-inflammatory Herbal Medicines. *Pharmaceutical Medicine* **34**, 49-61, doi:10.1007/s40290-019-00314-y (2020).

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