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An analysis of legal highs – do they contain what it says on the tin?

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In recent years the availability of so-called legal highs over the Internet has hugely increased. Numerous online legal-high retailers market a broad variety of products which are advertised as research chemicals, bath salts, or plant food although clearly intended for human consumption as recreational drug replacements. No guidelines exist as to what is sold and in what purity. Consumers are led to believe that purchased goods are entirely legal.

In this study, several legal-high products were purchased and analyzed for their content. The powdered products were screened with attenuated total reflectance – Fourier Transform Infrared (ATR-FTIR) followed by gas chromatography-mass spectrometry (GC-MS) analysis of methanol extracts. Spectra were compared to reference standards and the NIST library.

Results showed that 6 out of 7 products did not contain the advertised active ingredient. Moreover, five samples contained the controlled substances benzylpiperazine and 1-[3-(trifluoromethyl)phenyl]piperazine combined with caffeine.

Keywords: legal highs; MDAI; 6-APB; FTIR; GC-MS 3-TFMPP

Introduction

There has been a recent explosion in the number of substances that can be purchased from the Internet marketed as legal highs. A survey of Internet availability published in April 2009 found 39 individual UK online legal-high retailers selling a total of 1308 products.[1] These substances are sold as 'not for human consumption' and for a variety of uses, such as research chemicals, plant food, and bath salts, but it is clear that they are intended for human consumption and that the intended market is as a replacement for either 3,4-methylenedioxymethamphetamine (MDMA) or cannabis on the recreational drugs scene. In fact, recent figures suggest that there has been a decrease in the use of MDMA with the suggestion that this is not due to a decrease in recreational drug use but simply a switch to more readily available legal highs.[2] The term 'legal high' carries with it a perception of safety and acceptability and although many of the compounds advertised as being the active ingredient in the currently marketed products are not controlled, many of these have as yet not been tested on humans and the purity of the products is unknown. This is an unregulated area and is simply being driven by the market.

2009 and 2010 saw a significant increase in legislation in the UK to try and curb the availability and use of legal highs with a number of modification orders to the Misuse of Drugs Act. These controlled a number of cathinones[3] including the much publicised 4-methylmethcathinone (mephedrone), piperazines, and synthetic cannabinoids[3,4] using generic controls. On the face of it, these controls have been effective in removing products claiming to contain these substances from website retailers; however, it has been seen that so-called new legal products do in fact contain controlled substances.[5] Analysis of 24 products claiming to be legally purchased from 18 UK-based websites obtained in a six-week period following the April 2010 ban of cathinones revealed that 62.5% of the products contained these controlled substances.[6] Clearly this is unknown to the user and may well be unknown to the online retailer. Users are left to evaluate new substances on their experience of use and through communicating their experiences in online forums.

As substances are controlled, new substances quickly appear and are marketed as being better than previous products. The synthesis of many of the active ingredients of these second-generation legal highs such as 5,6-methylenedioxy-2-aminoindane (MDAI), 5-iodo-2-aminoindane (5-IAI), and 6-(2-aminopropyl)benzofuran (6-APB) (Figure 1) have been previously published;[7–9] however, little is known about their effects on humans. Another problem highlighted is that substances labelled as the same product may in fact be structural isomers of the expected active ingredient which are unknown within the literature.[10]

There is also little reported on methods of analysis for these new compounds with a need to use techniques not routinely used in forensic drugs labs, such as nuclear magnetic resonance (NMR) and high resolution mass spectrometry.[11] This is due to the lack of good quality reference standards required by routine analytical methods to update spectroscopic databases and for calibration. Some are available[12] but these tend to be expensive for a few mgs and a full range for current products is not available. Identification of previously unreported cathinones was possible as a follow-up to the study carried out by Brandt *et al*.[6] because the authors were able to synthesise the reference standards required.[13]

We report here the analysis of legal highs purchased in the second half of 2010 from several Internet sites using a standard approach of Fourier Transform Infrared (FTIR) and gas chromatography-mass spectrometry (GC-MS) of methanol extracts. We were interested in the chemical composition of these

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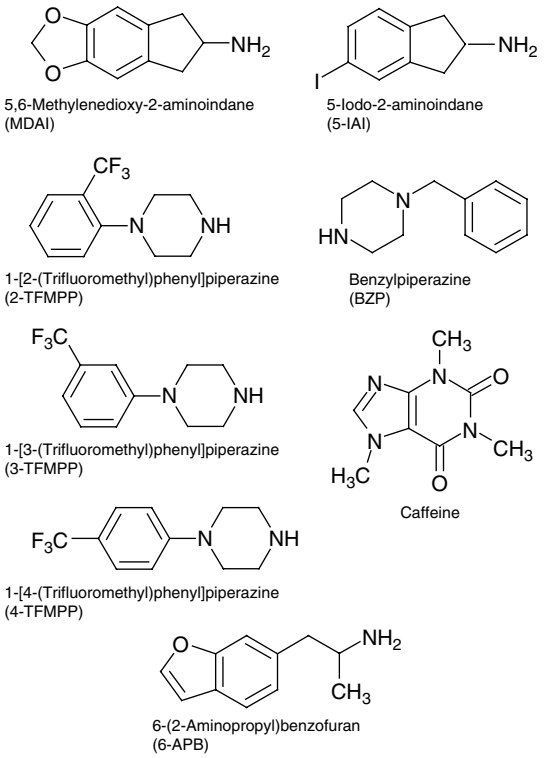

Figure 1. Chemical structures of selected legal high related substances.

products to test the previous findings that legal-high products are not always what they are advertised to be. We were also interested in seeing if these products are in fact illegal as reported in previous investigations; if so, suppliers and users would be committing an offence even if they were ignorant of this fact.

Materials and methods

Chemicals and materials

5,6-Methylenedioxy-2-aminoindane was purchased from LGC GmbH (Luckenwalde, Germany), 1-(2-trifluoromethylphenyl) piperazine and 1-(4-trifluoromethylphenyl) piperazine from FluoroChem (Hadfield, UK), mephedrone from ReseaCHEM (Burgdorf, Switzerland), and caffeine from Sigma-Aldrich (Gillingham, UK). Methanol (HPLC-grade) was obtained from Fisher Scientific (Loughborough, UK). Chemical structures are shown in Figure 1.

Four legal-high samples were purchased online in August 2010 as MDAI, 5-IAI, Benzo Fury, and NRG-3 including a free sample of E2 from www.benzofury.me.uk; two more MDAI-labelled samples were purchased in October 2010 from www.VIPlegals.com and www.wide-mouth-frog.com (Table 1).

FTIR conditions

Fourier-transform infrared (FTIR) spectroscopy was performed on a Perkin Elmer Spectrum 100 FT-IR spectrometer using the attenuated total reflectance (ATR) attachment (Specac Golden Gate). The instrument was operated with Perkin Elmer Spectrum software (2006). The scan range was 4000–450 cm^{-1} with a scan number of 64, a resolution of 4 cm^{-1} and a scan speed of 0.5 cm s^{-1}.

Standards and samples were applied in their powdered form. Spectra were compared to an in-house drugs library which contained a range of drugs of abuse and legal highs apart from 5-IAI and 6-APB as genuine standards were not available at the time of analysis. Finally, a cross correlation of all Internet samples was performed using the Euclidean algorithm.

Sample preparation for GC-MS

All standards were prepared in methanol at concentrations ranging between 0.1 and 1 μg mL^{-1} and filtered through 0.45 μm pore size syringe filters from Chromacol (Herts, UK) prior to injection.

The legal-high samples were extracted into methanol at a concentration of 10 mg powder per 1 ml methanol. After vortexing for 1 min the samples were centrifuged at 4000 rpm for 2 min using a Beckman Coulter Allegra X-22 centrifuge. The supernatant was filtered through Chromacol syringe filters with a pore size of 0.45 μm. Samples were diluted 1000 times in methanol prior injection.

GC-MS conditions

Gas chromatography was performed using a Perkin Elmer Clarus 600 gas chromatograph equipped with an autosampler. Standards and samples were run on an Agilent Technologies DB-1 MS column (30 m × 0.25 mm × 0.25 μm). The oven temperature program was an initial 4-min plateau at 150 °C increasing to a final temperature of 350 °C at 32 °C min^{-1} giving a total run time of 10.25 min. The temperature programmable injector port was held at 250 °C for 4 min and then increased to 300 °C at 50 °C min^{-1}. The final temperature was held for 5.25 min to correlate with the total run time of 10.25 min. The carrier gas was helium at 1 ml min^{-1} with vacuum compensation and the injection volume was 1 μl.

Mass spectrometry was performed using a Perkin Elmer Clarus 600 mass spectrometer operated with Perkin Elmer TurboMass (2008) software. The transfer line temperature was held at 300 °C. Positive ionization was achieved using an Electron Impact (EI^+) source at 200 °C with electron energy of 70 eV. The multiplier was set to 300 V. After 2 min solvent delay, the peaks were observed in total ion count (TIC) mode. The scan range was 40–300 *m/z* with 1 scan per second and an interscan delay of 0.01 s. Mass spectra of selected peaks were compared to the National Institute of Standard and Technology library (TurboMass NIST 2008 Library, version 2.2.0) and in-house run reference standards.

Results and discussion

Samples obtained from the benzofury.me.uk site were white powders in simple, plastic, re-sealable bags. The content was marked in black felt-tip pen on the bag as the abbreviations MDAI, 5IAI and NRG3. The benzo fury product was contained in two capsules. No other information was provided. The MDAI product obtained from www.wide-mouth-frog.com was a white powder in a sealed metallic bag. The full name was given along with the warnings 'Strictly not for human consumption' and '1 g feeds 10 plants'. The MDAI product obtained from www.VIPlegals.com was also a white powder in a re-sealable plastic bag. A printed label was attached to the bag with the abbreviation MDAI and a warning 'Not for human consumption'. An accompanying certificate of analysis sheet was also provided giving the full name of the product as 5,6-methylenedioxy-2-aminoindane hydrochloride and giving a purity as measured by HPLC of 99.55%.

Table 1. Overview of analyzed legal-high samples

Date purchased	Product	Price	Expected active ingredient
August 2010	MDAI (Benzofury.me.uk)	£14.50 per 1 g	5,6-Methylenedioxy-2-aminoindane
	5-IAI	£26.99 per 2 g	5-Iodo-2-aminoindane
	Benzo fury	£16.00 per 2 pellets	6-(2-aminopropyl)benzofuran (6-APB)
	NRG-3	£27.99 per 2 g	n/a
	E2	free	n/a
October 2010	MDAI (VIPlegal.com)	£15.00 per 1 g	5,6-Methylenedioxy-2-aminoindane
	MDAI (Wide-mouth-frog.com)	£15.00 per 1 g	

Table 2. Results of FTIR search of in-house drugs library

Internet product	Library search results
5-IAI	Caffeine 0.635
Benzo fury	Caffeine 0.681
NRG-3	Caffeine 0.648
E2	Caffeine 0.996
MDAI (Benzofury.me.uk)	Caffeine 0.677
MDAI (VIPlegal.com)	MDAI 0.723
MDAI (Wide-mouth-frog.com)	Caffeine 0.617

FTIR studies

The legal-high reference standards and Internet-bought samples were screened using ATR-FTIR and a search carried out against an in-house 'drugs of abuse' library.

These results showed that the benzofury.me.uk products all appeared to contain caffeine which was the best match found in the library for all the spectra (Table 2). The difference spectra obtained by subtraction of the caffeine spectrum from each of these product spectra did not result in any subsequent library matches. This was expected for the 5-IAI and benzo fury products as the active ingredients, 5-IAI and 6-APB (the active ingredient in benzo fury) were not in the library due to unavailability of the standards. However it was expected that the MDAI product would match with the MDAI standard spectrum (Figure 2). This was not the case suggesting this product did not contain the advertised active ingredient, at least as a major constituent. Care was taken in interpreting the FTIR spectral data as it can be difficult to detect substances in mixtures below about 10%. When comparing the spectra of MDAI and caffeine it can be seen that caffeine as the major component in a mixture would almost completely obscure the spectrum of MDAI except for the 3345, 3288 (corresponding to the N-H stretch of the aliphatic primary amine), 1143 (related to the C-O stretch of the alkyl substituted ether C-O-C), and 944 cm^{-1} (resulting from one of the aromatic C-H in-plane bends) peaks (Figure 3).[14] Despite these features being unique to MDAI when compared to caffeine, a 10% MDAI mixture in caffeine gave a library search result of 0.995 for caffeine, proving these MDAI-only featured peaks were not strong enough for the MDAI to be picked up by the algorithm.

The sample from VIPlegals.com gave a good match with MDAI indicating that this was a genuine product at least in that it contained the active ingredient as advertised. This was not the case with the product from wide-mouth-frog.com which had a poor correlation and gave a similar match to caffeine observed with the benzofury.me.uk products. The free E2 sample gave the best match with caffeine although this does not demonstrate the absence of any active ingredient as discussed earlier. Correlations are summarized in Table 2.

Cross-correlation of the benzofury.me.uk products all appeared to be well matched as do these products with the wide-mouth-frog.com MDAI product (Table 3). This suggests that the same mixture was simply being sold as different products and that although the new legal-high products are being actively marketed by Internet retailers, they aren't necessarily the products that are being sold. It also suggests that different Internet retailers are selling the same mixtures as the same or different advertised products. This may point to the fact that retailers are simply packaging samples and are not blending mixtures.

GC-MS studies

Gas chromatography coupled with mass spectrometry was used to separate the various compounds present in the legal-high samples and to identify any active ingredients present. The methanol extracts were injected underivatized into a temperature-programmable injector port where the initial temperature 250 °C was held constant to accommodate the plateau in the oven program but then ramped up to 300 °C to ensure full vaporization of the injected sample.

Retention times and mass spectra profiles of the standards MDAI, mephedrone, 2-TFMPP, 4-TFMPP, and caffeine were established (Table 4). Caffeine did not elute as a sharp peak but tailed off and merged into the column bleeding which expectedly occurred towards the end of the run. The molecular ion peak for mephedrone could not be seen due to high ionization energies applied.

After analysis of the seven legal-high samples purchased online, chromatograms were scrutinized for peaks which matched retention times with the tested standards. Mass spectra profiles of these peaks were compared to the standards to ensure correct identification. Unidentified peaks were searched through the NIST library. Finally, ions of known psychoactive substances (legal and illegal) were extracted from the recorded TIC spectra.

Results (Table 5) showed that only one sample contained the label-described substance (MDAI); all other samples contained caffeine in a mix of BZP and 3-TFMPP. Although quantitative work was not carried out in this study, the relative amounts (based on peak areas) of the two piperazines were similar, suggesting a similar product. This confirmed the FTIR results.

The inaccuracy of product labels has been reported by other chemical analysis studies of Internet products.[5,6] Of interest is that an MDAI product was analyzed in both of these studies. In one product the active ingredient was found to be the controlled substance methylone, whereas the other product simply consisted

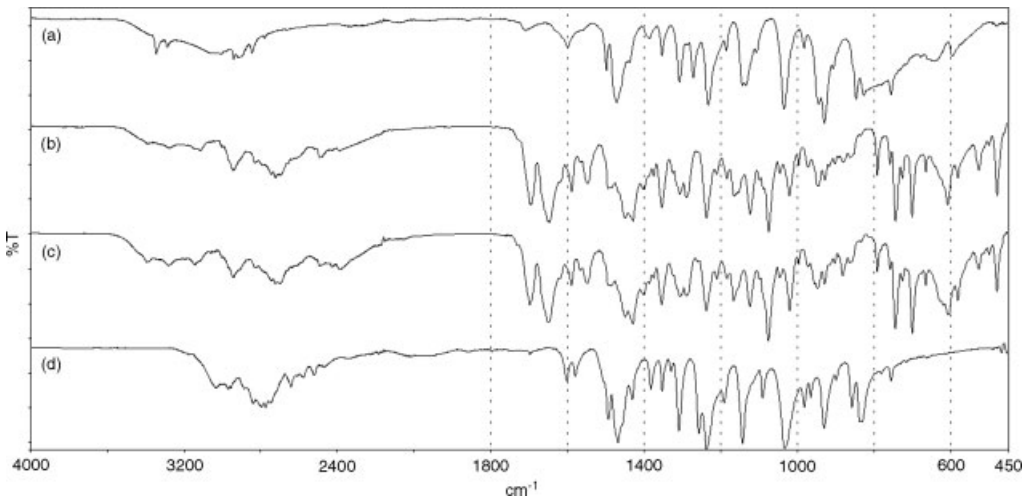

Figure 2. FTIR spectra of (a) MDAI LGC standard, (b) Benzofury.me.uk sample, (c) Wide-mouth-frog.com sample, and (d) VIPlegal.com sample.

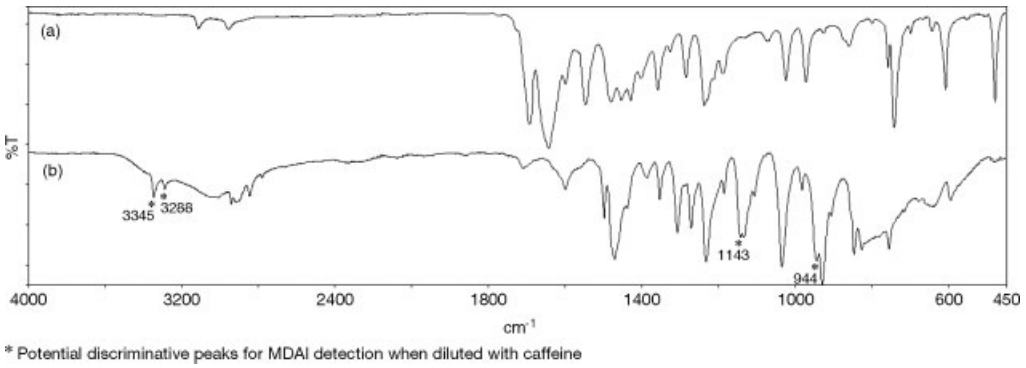

Figure 3. FTIR spectra of (a) caffeine and (b) MDAI.

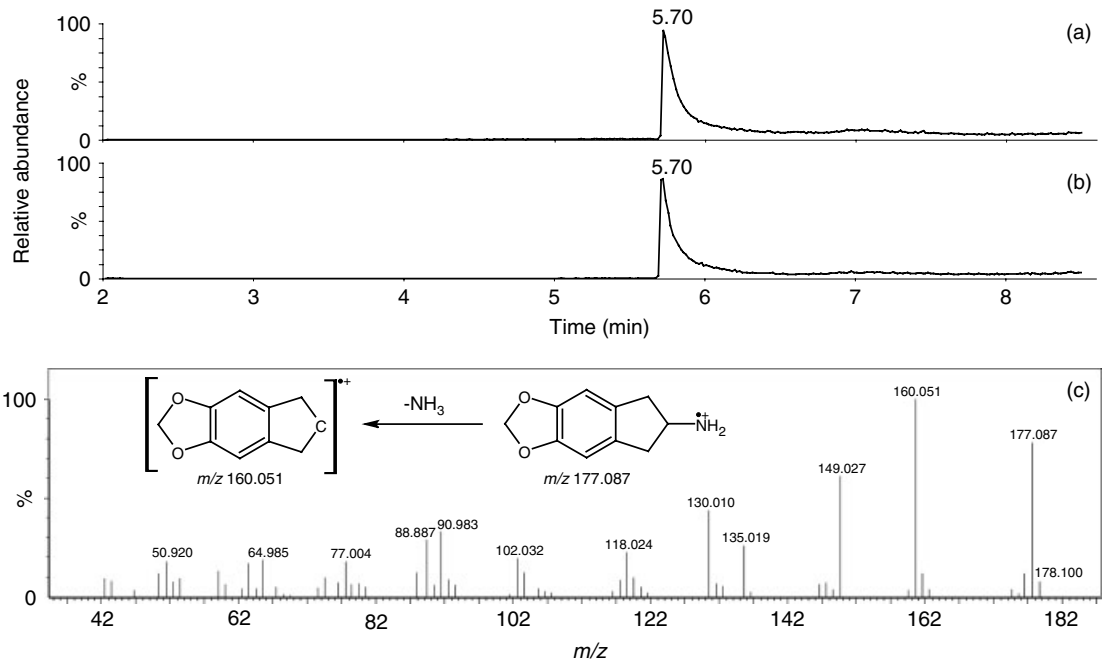

Figure 4. Extracted chromatograms of (a) MDAI standard and (b) VIPlegal.com sample (*m/z* 177, 160, 149, 130, 91); (c) EI^+ mass spectrum of MDAI and related molecular ion and base peak chemical structures.

Table 3. Correlation table of the FTIR spectra of all Internet products

	5-IAI	Benzo fury	NRG-3	E2	MDAI (Benzo fury.me.uk)	MDAI (VIPlegal.com)	MDAI (Wide-mouth-frog.com)
5-IAI	–	0.9977	0.9980	0.5824	0.9974	0.0629	0.9705
Benzo fury	0.9977	–	0.9975	0.6219	0.9990	0.0618	0.9668
NRG-3	0.9980	0.9975	–	0.5953	0.9975	0.0654	0.9625
E2	0.5824	0.6219	0.5953	–	0.6125	0.0569	0.5520
MDAI (Benzo fury.me.uk)	0.9974	0.9990	0.9975	0.6154	–	0.0635	0.9659
MDAI (VIPlegal.com)	0.0629	0.0618	0.0654	0.0569	0.0635	–	0.0463
MDAI (Wide-mouth-frog.com)	0.9705	0.9668	0.9625	0.5520	0.9659	0.0463	–

Table 4. Key ions for the compounds of interest for this study

Compound	Retention time (min)	EI^+ generated ions (*m/z*)
Mephedrone	4.7	147, 119, 58 (base peak)
2-TFMPP	4.8	230 (molecular ion), 188 (base peak)
4-TFMPP	5.7	230 (molecular ion), 211, 188 (base peak)
MDAI	5.7	177 (molecular ion), 160 (base peak), 149, 130, 91
Caffeine	7.1	194 (molecular ion and base peak), 109, 82, 67, 55
BZP[1]	5.0	176 (molecular ion), 91 (base peak), 134
3-TFMPP[1]	5.3	230 (molecular ion), 188 (base peak)

[1] Identified by NIST library, retention times deduced from the products.

Table 5. Overview of GC-MS results of Internet-bought legal-high samples

Product	Active substances identified	Product	Active substances identified
MDAI (Benzofury.me.uk)	BZP 3-TFMPP Caffeine	Benzo fury	BZP 3-TFMPP Caffeine
MDAI (VIPlegals.com)	MDAI	5-IAI	BZP 3-TFMPP Caffeine
MDAI (Wide-mouth-frog.com)	BZP 3-TFMPP Caffeine	NRG-3	BZP 3-TFMPP Caffeine
		E2	Caffeine

of inorganic material. This is consistent with our results that show MDAI was the active ingredient in one product but that the MDAI label was also used as a cover for other substances, which may be controlled.

The free sample labelled 'E2' simply consisted of caffeine as an active ingredient and so suggests the absence of other low-concentration active ingredients that could not be ruled out by FTIR alone.

At the time of this study, we did not have a 3-TFMPP standard which is the controlled psychoactive isomer sold in Internet products; however, the mass spectrum was available in the NIST database. Identification of 3-TFMPP in the products was achieved by comparing retention times of the non-psychoactive isomers 2-TFMPP and 4-TFMPP combined with an NIST library search of the mass spectra in question. The library matched the unknown peak at 5.3 min to 3-TFMPP. All mass spectra of the three structural isomers appeared similar but retention times varied greatly with peaks being perfectly separated at 4.8 min for 2-TFMPP, 5.3 min for 3-TFMPP, and 5.7 min for 4-TFMPP therefore allowing a definitive identification of the 3-isomer. This highlights the importance of the GC separation and use of retention times when discriminating between structural isomers with similar mass spectra.[10]

The combination of BZP and 3-TFMPP is not unusual as it is commonly found in party pills under names such as Charge and Bliss, depending on the relative amounts.[15]

The MDAI MS was not in the NIST database; however, it was determined from the commercial standard (Figure 4) which showed an expected molecular ion with *m/z* 177.087 with its corresponding isotope with *m/z* 178.100, their relative abundance matching closely the expected natural occurrences of 89.01% and 9.99%, respectively. The base peak was *m/z* 160.051 which corresponds to a loss of NH_3 from the molecular ion (Table 4).

It was established that 5-IAI and APB were not present in the products in which they were expected by a selected ion search for their respective molecular ions of *m/z* 259 and 174.

It is possible that non-volatile active ingredients could also be present in these products and so a derivatization method with GC-MS or further analysis using LC-MS might yield more ingredients.

Conclusions

Seven samples were purchased online as legal highs and characterized by FTIR and GC-MS. The FTIR study revealed that 6 out of 7 samples did not contain the claimed drug but large quantities of caffeine. The presence of large amounts of caffeine was confirmed by GC-MS. Moreover, it was found that only one of the purchased samples contained MDAI as claimed. Five samples were a mixture of the controlled substances BZP and 3-TFMPP combined with caffeine. As BZP and 3-TFMPP are both controlled substances, users of these Internet products are in possession of illegal substances having purchased them assuming them to be legal. Another issue of concern, previously highlighted by others, is the lack of consistency between products of the same name. If purchasers use different suppliers for the same-named product, it is also highly likely that they could be using products with different active ingredients.

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