

Analytical Methods

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

Revised, *Analytical methods*

Release of toxic ammonia and volatile organic compounds by heated cannabis and their relation to tetrahydrocannabinol content

David Smith^{*a}, Roger Bloor^b, Claire George^c, Andriy Pysenenko^d and Patrik Španěl^{a,d}

^aInstitute for Science and Technology in Medicine, School of Medicine, Keele University, Thornburrow Drive, Hartshill, Stoke-on-Trent ST4 7QB, UK

^bAcademic Psychiatry Unit, Keele University Medical School, Academic Suite, Harplands Hospital, Hilton Road, Harpfields, Stoke-on-Trent, UK

^cAlere Toxicology Plc, 92 Park Drive, Milton Park, Abingdon, Oxfordshire, OX14 4RY, UK

^dJ. Heyrovsky Institute of Physical Chemistry of Science, Academy of Science of the Czech Republic, Dolejškova 3, 18223 Prague 8, Czech Republic

Abstract

Studies have been carried out of the compounds generated from heated “street” cannabis in the commercial device known as the “Volcano” using the selected ion flow tube mass spectrometry (SIFT-MS) analytical method. Such vaporising devices are preferred for the delivery of the cannabis active ingredients for pain relief and therapeutic purposes since they remove from the smoke the harmful high molecular weight compounds such as tars and polycyclic aromatic hydrocarbons. Whilst it is known that smoking cannabis is associated with adverse health effects, little is known about risks of its inhalation of volatile compounds from vaporizers. In the present study, the concentrations of the volatile lower molecular weight compounds ammonia, methanol, acetic acid, methyl acetate, monoterpenes and sesquiterpenes present in the trapped air/vapour volume of the “Volcano” have been determined directly by SIFT-MS obviating sample collection/pre-concentration and delayed off-line analysis as used by most other analytical techniques. The concentrations of these compounds are compared to the tetrahydrocannabinol (THC; expected to be largely Δ -9-THC) content of the cannabis plant material as assayed using standard extraction/derivatisation/GC-MS analysis. The observed high concentrations of ammonia were strongly correlated with the THC which is largely contained in the buds of the cannabis plant, whereas the identified volatile organic compounds were predominantly released by the leaves. Whilst the “Volcano” device removes some toxic compounds from the smoke and reduces their inhalation by its user, it likely leads to enhanced ingestion of toxic ammonia known to result in neurobehavioural impairment.

1. Introduction

The risks to respiratory and general health of smoking both tobacco and cannabis are well known¹⁻³. Cannabis, or marijuana, has been used for medicinal purposes for many years⁴. Whilst smoking remains the most common mode of use for medical cannabis, vaporization of cannabis is becoming increasingly popular due to its perceived reduction of the release of noxious chemicals⁵ and ingestion as a cake could also be a vehicle of administration. Vaporisation devices ostensibly remove the higher molecular weight compounds, such as tars and polycyclic aromatic hydrocarbons that are especially damaging to the lung tissues, yet deliver the sought-after active species tetrahydrocannabinol (THC). Some such devices utilize an electrically heated plate on which the cannabis plant material is pyrolysed and partially combusted; others use a hot airstream that heats the cannabis to a temperature below which combustion occurs. The gaseous products are collected into a sealed container from which they are inhaled, usually through an attached tube.

Some years ago, we carried out an analytical study⁶ of the low molecular mass volatile compounds that were generated by the heating of “street cannabis” in two readily available commercial devices “Blue Meanie” and “Volcano”⁷. Direct analysis and quantification of the vapours emitted by the heated cannabis, as collected into glass (“Blue Meanie”) and inflated plastic bags (“Volcano”), were carried out using selected ion flow tube mass spectrometry (SIFT-MS). These revealed that copious amounts of ammonia and low molecular weight volatile organic compounds (VOCs) were present in the “smoke”, including methanol, acetone, acetic acid and terpenes. It was not possible to detect the parent analyte ions of THC (molecular weight 314) using the *Profile 3* SIFT-MS instrument, because the mass-to-charge ratio, m/z , of these parent ions exceeds that accessible to the current instrument⁸.

The toxicity of these low molecular weight compounds has not often been addressed, the focus being placed on the tars and higher molecular weight compounds. But acetaldehyde is a probable carcinogen⁹ and ammonia inhaled at high concentrations can result in neurobehavioural impairment¹⁰. The health consequences of transient repeated exposure to high ammonia concentrations during inhalation of heated cannabis remain unknown; however, the acute exposure to ammonia during inhalation of heated cannabis plant may have cerebral effects¹¹. Some of these health implications of the inhalation of ammonia from heated cannabis have been addressed in⁶. We also reported that ammonia can build up in the contained volumes of the “Volcano” device to concentrations greater than those inhaled by mainstream smoking of cannabis cigarettes and also that ammonia was at much higher concentrations in sidestream smoke than in mainstream smoke.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

The same differences were observed in a systematic study of the compounds emitted by combusting tobacco and cannabis cigarettes¹². This differentiation between the ammonia content of mainstream and sidestream cannabis cigarette smoke may be relevant to the interpretation of the results presented in this study. The present study extends the previous work by including the analysis of the cannabis plant samples for their THC content. Correlations between the THC levels and the volatile compounds generated in the “Volcano” device, especially ammonia, are perhaps of some significance in the safety assessment of vapourised cannabis.

2. Materials and methods

2.1 Cannabis samples

Samples of cannabis often obtained by users, ‘street cannabis’, were held under a UK Home Office licence and supplied to us from material seized by the local police force. Five samples were selected randomly from recent seizures and were stored in sealed plastic enclosures to minimize the loss of moisture. Samples were prepared by finely chopping and mixing the plant material, some predominantly being leaf and others the plant bud; each sample weighed 200 mg.

2.2 Analyses of THC content

The analysis of the THC in the cannabis material was performed using a fully validated GC-MS method. A known quantity of sample (typically 500 mg) was prepared in methanol and derivatised with bis(trimethylsilyl)trifluoroacetamide (BSTFA) prior to analysis. The GC-MS system comprised a Hewlett Packard HP 6890 gas chromatograph interfaced to a Hewlett Packard HP 5972 mass selective detector. A 30-m HP5MS capillary column, with a film thickness of 0.25 μm and an internal diameter of 0.25 mm was operated in the splitless mode at an initial temperature of 150 $^{\circ}\text{C}$ held for 1 min, increasing by 30 $^{\circ}\text{C}$ per min to 240 $^{\circ}\text{C}$ held for 0.5 min, then increasing by 3 $^{\circ}\text{C}$ per min to 280 $^{\circ}\text{C}$ held for 1 min finally increasing by 30 $^{\circ}\text{C}$ per min to 300 $^{\circ}\text{C}$ with a final hold time of 1 min (total run time 20.5 min). The carrier gas was helium at a constant flow of 1.5 mL/min. The m/z of the ions monitored after electron ionisation to analyse the eluted THC derivative were 386 (target ion), 371, 315, 303, 343 and 330. Accurate quantification was carried out using a 10 ng/mL deuterated internal standard solution (THC D3, target ion 374) added to each THC/methanol sample. The concentrations of THC in the cannabis given in Table 1 are expressed as %w/w.

2.3 Selected ion flow tube mass spectrometry, SIFT-MS

The SIFT-MS analytical technique has been described and reviewed previously¹³⁻¹⁵. This technique facilitates detection and quantification of trace gases in air, exhaled breath and liquid headspace at parts-per-billion by volume (ppbv) levels in several seconds of analysis time. The trace gases are analysed in real time, obviating sample collection, pre-concentration and the removal of water vapour, as is required for most other analytical methods. SIFT-MS relies on the chemical ionization of the trace gases by selected reagent ions (H_3O^+ , NO^+ , O_2^+) in a flow tube reactor. These reactions produce characteristic analyte ions that identify the trace compounds present in the air sample and their count rates provide the concentrations of the compounds in the sample. The air/heated cannabis products sample collected in the inflated plastic bag is sampled into the SIFT-MS instrument by puncturing the plastic surface by a hypodermic needle connected directly to the input port and heated line of the instrument. SIFT-MS can be operated in the full scan mode (FS) where the downstream mass spectrometer scans and detects the precursor and product ions to form a mass spectrum that is used to identify the particular trace gases that are present; sample FS spectra are shown in Figure 1. The multi-ion monitoring mode (MIM), in which the analytical mass spectrometer is switched rapidly between chosen m/z values to target particular trace gases, is used for more accurate quantification. A thorough validation of the SIFT-MS method has been carried out using standard techniques¹⁶, and it is shown that the simultaneous quantification of several organic vapours in air samples can be achieved to an accuracy of 10% at the 100 ppbv level as described in detail in a more recent paper¹⁷.

2.4 Analyses of vaporized cannabis plant material

A commercial “Volcano” hot-air ‘drug vaporizer’ was used which consists of a ceramic heater with a heat vent and a removable chamber into which the cannabis sample is placed and positioned above the heat vent. Hot air is blown through the chamber to release vapours from the sample without initiating combustion. The vapour inflates a disposable plastic bag that when filled can be detached and sealed for gas analysis. The temperature of the heater is adjustable and was set to its highest reachable value of 218°C at the heater screen and 155°C at the sample surface following the suggestion in¹⁸. However, it is likely that a different heater temperature could result in different concentrations of the volatile compounds, including the THC. The “Volcano” vaporizer⁷ heater was set to maximum for 3–5 minutes after which it reached its highest temperature above 200°C. Then 200 mg of the chopped and mixed cannabis was introduced and the air flow initiated. The collection bag was inflated totally with the heated air containing the released compounds (loosely called cannabis smoke) in about 45 seconds. The trace gases in the samples were analysed using all three

available precursor ions as described in the previous section using the FS mode over an m/z range of 10 to 250. Additionally, some MIM data were also taken to provide more accurate data, in particular using $O_2^{+\bullet}$ reagent ions targeting ammonia analyte ions ¹⁹. The FS spectra obtained comprised more than 50 different ion peaks (see Figure 1), but focus was placed on the readily recognisable analyte ion peaks due to water vapour, ammonia, methanol and total monoterpenes and total sesquiterpenes obtained using H_3O^+ reagent ions; the readily recognisable ion peaks obtained using NO^+ reagent ions are due to acetone, acetic acid, methyl acetate, total monoterpenes and total sesquiterpenes [7]. Because of the unusually high concentrations of ammonia detected in some samples, the sample gas flow rate into the SIFT-MS instrument had to be reduced below the value used normally for trace gas analysis. This was achieved using an in-line metering valve together with a flow meter as the simplest practical option. The concentrations of the identified compounds were obtained from the ratios of the count rates of their analyte ions to the count rate of the reagent ions in relation to the sample air/vapour mixture flow rate, as outlined in detail in previous papers ^{14, 15, 17}.

3. Results

As can be seen in Figure 1a and Figure 1b, the FS spectra obtained for both H_3O^+ and NO^+ reagent ions are very complex, analyte ion peaks being present at most integer values of m/z , even though the ion/molecule reactions that produce the analyte ions are not very energetic. However, the spectra obtained using $O_2^{+\bullet}$ reagent ions are even more complex and difficult to interpret, because the reactions of the radical ion $O_2^{+\bullet}$ with most polyatomic VOCs are sufficiently exothermic to result in serious fragmentation of the nascent parent ions produced in these reactions. This can be seen in Figure 1c where the molecular sesquiterpene radical cation at m/z 204 partially fragments to m/z 189 (by loss of CH_3^\bullet), m/z 161 (by loss of $C_3H_7^\bullet$). Similarly, the parent monoterpene cation at m/z 136 loses hydrocarbon radical moieties, producing m/z 121 (CH_3^\bullet loss) and m/z 93 ($C_3H_7^\bullet$ loss). As can be seen, groups of fragmentation ions from C_7 to C_{15} are present. However, the $O_2^{+\bullet}$ reaction with ammonia molecules, NH_3 , has often been shown to only result in $NH_3^{+\bullet}$ analyte ions at m/z 17 and so this analytical reaction is very valuable for the analysis of ammonia and is exploited in this study¹⁹. It should be noted that the reaction of H_3O^+ and its hydrates $H_3O^+(H_2O)_{1,2,3}$ with ammonia result in both H_3O^+ and NH_4^+ ions clustered with H_2O and NH_3 molecules, especially for humid samples and at high NH_3 concentrations¹⁹, and so ammonia analysis using H_3O^+ reagent ions are avoided in favour of the much simpler use of $O_2^{+\bullet}$ reagent ions. No further attempt is made to interpret in detail the spectra obtained using $O_2^{+\bullet}$ reagent ions and to use them for the analysis of the cannabis smoke.

1
2
3
4 The major product ions in the H_3O^+ and NO^+ spectra can be “read” to identify the neutral analyte
5 molecules present in the cannabis smoke and these are indicated in the spectra in Figure 1. Using
6 H_3O^+ reagent ions (Figure 1a), ammonia, methanol, acetic acid, monoterpenes and sesquiterpenes
7 are recognised and quantified by utilizing the SIFT-MS kinetics library entries for these
8 compounds. Using NO^+ reagent ions, acetic acid, methyl acetate, monoterpenes and sesquiterpenes
9 are recognised and analysed. Even so, the analytical reactions of the H_3O^+ and NO^+ reagent ions are
10 not always simple, because more than a single product (analyte) ion can be produced in some
11 reactions, overlaps of product ions of their reactions producing ions with the same m/z value but of
12 different character can occur and hydrates of the analyte ions can be produced when humid samples
13 are being analysed. These complications must be recognised and accounted for to obtain accurate
14 analyses [15, 17](#).

21
22
23 However, in the present SIFT-MS analyses there are few complications involved in the analysis of
24 the major compounds present in the cannabis smoke. One possible overlap is of protonated isoprene
25 C_5H_9^+ formed in the H_3O^+ reaction with the second hydrate of protonated methanol
26 $\text{CH}_3\text{OH}_2^+(\text{H}_2\text{O})_2$ both being at m/z 69. Fortunately, NO^+ ions do not react at a significant rate with
27 methanol molecules, whereas they do react rapidly with isoprene molecules producing parent
28 radical cations $\text{C}_5\text{H}_8^{+\bullet}$ at m/z 68 [20](#). This ion is not present in the spectra obtained when analyzing
29 the cannabis smoke using NO^+ reagent ions and so we can confidently label the m/z 69 ions (and its
30 hydrates) on Figure 1a as being due to methanol only. When using H_3O^+ reagent ions, protonated
31 monoterpenes and sesquiterpenes molecules appear in the analytical mass spectrum at m/z 137 and
32 205 respectively, but it must be appreciated that both of these nascent protonated molecules
33 partially dissociate losing a C_4H_8 moiety, the m/z 137 ion realizing a fragment ion at m/z 81 and the
34 m/z 205 ion realizing a fragment at m/z 149 [21](#). A weak signal at m/z 223 is present that is the
35 hydrate of the m/z 205 ion. Using the NO^+ reagent ions the molecular cations of these two terpenes
36 appear at m/z 136 and 204 respectively; the latter ion is also seen to partially hydrate producing m/z
37 222, but this is a relatively small fraction of the m/z 204 signal at the low absolute humidity of the
38 smoke sample which in all the experiments was relatively dry and close to 1%, as derived routinely
39 in SIFT-MS experiments [15, 17, 22](#).

50
51
52 The protonated molecules at m/z 61 and its hydrates at m/z 79 and 97 seen on the H_3O^+ reagent ion
53 spectrum (Figure 1a) have three possible identities viz. acetic acid, propanol and methyl formate.
54 Fortunately, we have carried out a detailed study of this potentially confusing situation and
55
56
57
58
59
60

1 discovered that the use of NO^+ reagent ions in parallel with H_3O^+ reagent ions can distinguish these
2 three compounds. Thus, it was found that NO^+ ions react with both acetic acid and methyl formate
3 by ion/molecule association producing adduct ions at m/z 90, whereas propanol molecules do not ²³.
4 Further to this, and very significantly, the acetic acid adduct ion also hydrates producing the ion at
5 m/z 108 whereas methyl formate adduct does not, which provides the vital information to
6 distinguish these two compounds. Additionally, the ratio of the count rate m/z 108 to the sum of
7 count rates at m/z 90 and m/z 108 increases with sample humidity and this variation has been
8 defined by previous experimental work (using the same SIFT-MS instrument) ²³. Analysis of the
9 “Volcano” cannabis smoke mass spectra shows that the absolute humidity of the smoke samples is
10 close to 1%, which from the previous work equates to a m/z 108/(m/z 90+ m/z 108) ratio of 0.2,
11 which is precisely the value obtained for the cannabis smoke. This is the final confirmation that the
12 compound detected is surely acetic acid. Such additional investigations are often required in SIFT-
13 MS studies to establish the exact nature of the analyte ions and hence of the precursor analyte
14 neutral molecules⁸. The concentrations of ammonia (determined using $\text{O}_2^{+\bullet}$ and m/z 17 product ion),
15 methanol (H_3O^+ , m/z 33, 51 and 69), acetic acid (NO^+ , m/z 90 and 108), methyl acetate (NO^+ , m/z
16 104), monoterpenes (H_3O^+ , m/z 81 and 137) and sesquiterpenes (H_3O^+ , m/z 109, 149 and 205) are
17 given in Table 1.
18
19
20
21
22
23
24
25
26
27
28
29
30
31

32 4. Discussion

33
34 Whilst recognising that many volatile compounds are present in the smoke, the most abundant
35 amongst those quantified by SIFT-MS are ammonia, methanol, acetic acid, methyl acetate,
36 monoterpenes and sesquiterpenes. This discussion is concerned with the significance of their
37 concentrations in the cannabis smoke and their relation to the THC content of the heated cannabis
38 material as determined in a parallel assay to be in the range from 0.26 to 8.47%, typical of seized
39 street cannabis (recently quoted as 1.9% for cannabis without flowers and 8.0% for cannabis with
40 flowers²⁴).
41
42
43
44
45
46
47

48 It is no surprise to find that **methanol** is present in the smoke since it is commonly released from
49 most plant material even at room temperature^{25, 26}. In these smoke samples it was at concentrations
50 ranging from about 1 to 16 parts-per-million by volume, ppmv. Whilst the ingestion of methanol is
51 toxic at doses greater than a reference dose of 160 mg/day (for an 80 kg person according to the
52 2013 revision of U.S. Environmental Protection Agency Integrated Risk Information System), the
53 absorption of methanol into the blood stream at the alveolar interface by inhaling the cannabis
54 smoke produced by the “Volcano” device is likely to be small at the above vapour concentrations.
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Acetic acid also is released from all the samples, but appears in the smoke at much lower concentration, typically at a few hundred parts-per-billion by volume, ppbv, and is unlikely to be toxic since acetic acid appears naturally in exhaled breath ²⁷. Because of the presence of methanol and acetic acid it is not surprising that **methyl acetate** is present in the smoke, albeit at relatively low concentrations. The appearance of the **terpenes** in the smoke is also not surprising, but it is surprising that isoprene is barely present when the **monoterpenes** and **sesquiterpenes** are so obviously present. Many different isomers of the latter compounds exist, running into hundreds, and they are responsible for the often pleasant odours of plants and fruits ^{28, 29} and, to some individuals, the very pleasant odour of combusting cannabis (anecdotal!). In these mass spectrometry observations the specific monoterpene and sesquiterpene isomers can not be identified; they are presumably mixtures of the various structural and optical isomers ^{21, 30, 31}, which at concentrations of typically a few ppmv they are not toxic. However, the very high concentration of **ammonia** in the smoke is of some concern, ranging from about 5 ppmv to greater than 200 ppmv; this needs special attention.

The physiological effects of smoking cannabis have been discussed in many texts ¹⁻⁵ and need not be re-iterated here except to focus on the high ammonia concentrations. The use of the closed “Volcano” system that operates as a lower temperature vaporizer is intended as a safer methods of delivery of medicinal cannabis by removing the toxic high molecular weight compounds from the smoke ^{7, 18}, but it is now clear that the closed volume of the vaporizer allows the capture of the lower molecular weight compounds that are mostly lost from the inhaled mainstream smoke of a burning cannabis cigarette ¹² by their relatively rapid diffusion into the sidestream smoke. For comparison with results given in Table 1, it is useful to note that the ammonia concentration measured in the mainstream of a cannabis cigarette was 10 ppmv ⁶ whilst that in the sidestream cannabis cigarette smoke was 250 ppmv ⁶. The ammonia concentrations in the air in the “Volcano” enclosed volumes, can greatly exceeding the short term (15-minute) upper limit occupational exposure limit, which is given as 35 ppmv. ³². Some of the neurological implications of ammonia ingestion are given in our previous paper ⁶.

Now we focus on the relationship between the ammonia concentration in the smoke and the THC content of the cannabis material. A very revealing plot is shown in Figure 2 where it is seen that a strong correlation exists between these parameters ($R = 0.92$, $R^2 = 0.85$). It is well recognised that the flowering tops, or "buds", of the cannabis plant have the highest concentrations of THC, followed in decreasing order by the leaves, then the stalks and seeds³³. The strong correlation

1 shown in Figure 2 confirms this trend in potency also to ammonia generation. However, it has to be
2 noted that this correlation is largely due to the two samples with THC content above 4% and it is
3 certainly a limitation of this study that more samples in this range were not included. Interestingly,
4 it has been suggested the amount of ammonia produced by combustion of cannabis is related to the
5 use of nitrate fertilizer during growth¹². A possible explanation for the very high concentrations of
6 ammonia found in marijuana smoke is that street cannabis is often grown hydroponically using
7 water soluble fertiliser containing both nitrate and ammoniacal nitrogen, but we have no
8 information on the growing conditions of the present cannabis samples we acquired from the local
9 police. It is also reported that the heating/combustion temperature can also influence the production
10 of ammonia³⁴ and so the relatively low temperature at which the cannabis material is heated in the
11 “Volcano” (non-combusting) may favour ammonia production.
12
13
14
15
16
17
18
19

20
21
22 What are the generation sites in the cannabis plant material of the other major volatile compounds?
23 Simple analysis of the data shown in Table 1 reveals that the concentrations in the smoke of
24 methanol, acetic acid and both terpenes are highest for those samples for which the ammonia
25 concentration in the smoke and the THC level in the cannabis material are the lowest. The results of
26 pairwise correlation analyses of all combinations of compounds, given in Table 2, confirm this
27 observation, indicating that there are inverse correlations between the concentrations of THC and
28 methanol and between ammonia and methanol. However, considering multiple comparisons
29 correction these correlations are not highly significant. The apparent negative correlations of the
30 terpenes with THC and ammonia are also not statistically significant, but these terpenes most
31 probably do mostly originate in the leaves of the plants. It is no surprise that there are highly
32 significant positive correlations between the concentrations of the monoterpenes and sesquiterpenes,
33 the methanol and acetic acid and the methyl acetate and acetic acid, as they are linked by the
34 chemistries and their common origins in the cannabis leaves.
35
36
37
38
39
40
41
42
43
44

45 5. Concluding remarks

46
47 By these experiments on the analysis of volatile compounds released by the heating of “street”
48 cannabis plant material in the commercially available device “Volcano”, we have shown that high
49 concentrations of ammonia are present in the trapped volume of the device that are available for
50 inhalation by the users. So, whilst this device is intended to mitigate the toxic effects due to high
51 molecular weight compounds in burning cannabis cigarette smoke, it inadvertently enhances the
52 ingestion of toxic ammonia. Effects of acute exposure to ammonia on the respiratory tract are well
53 documented and include asthma provocation at ammonia concentrations of 12 ppmv³⁵. This risk to
54
55
56
57
58
59
60

1 health has not been openly acknowledged and so further research is needed to ascertain the relative
2 safety of these devices. It is the buds that contain most of the sought-after THC and release most
3 toxic ammonia, but the other VOCs we have identified are largely released by the cannabis leaves,
4 albeit with lower THC, so choices can be made. Clearly, many more VOCs are generated by
5 heating the cannabis in the “Volcano” device, which we have not identified and quantified;
6 presumably many will be toxic. Other analytical techniques, especially gas chromatography mass
7 spectrometry (GC-MS), have been used to identify many of these compounds [18](#). However, GC-MS
8 cannot be used to quantify ammonia whereas SIFT-MS is ideal for this. The powerful combination
9 of GC-MS and SIFT-MS [27, 36-38](#) could be very effective in positively identifying and accurately
10 quantifying more of the compounds present in the cannabis smoke generated by the vaporisation
11 devices.
12

13 **Acknowledgements.**

14 We thank Dr. Tianshu Wang for his help in some aspects of the laboratory preparations for the
15 experimental work.
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1 Concentrations of THC (%) in the cannabis material, as determined by GC-MS, and the major volatile compounds (parts-per-billion by volume, ppbv), as determined by SIFT-MS, present in the smoke of heated cannabis.

THC (%)	ammonia (ppbv)	methanol (ppbv)	acetic acid (ppbv)	methyl acetate (ppbv)	monoterpenes (ppbv)	sesquiterpenes (ppbv)
0.26	14770	4150	307	56	1044	251
0.31	11010	4756	304	40	773	391
0.32	14630	8153	403		1381	140
0.49	31170	9682	523	81	6665	2501
0.50	18400	10740	365	81	3317	289
0.50	14490	12680	947	272	1421	249
0.52	5608	8436	779	294	6225	3435
0.58	11530	7012	308	131	2566	295
0.66	25030	8395	331	54	3180	1056
0.82	21600	5345	353	79	2353	1655
0.89	20770	16240	928	137	1895	212
0.92	21250	8079	355	81	1097	870
1.42	14480	6169	644	135	2509	555
1.43	57350	6462	49	18	423	4
1.72	20860	6005	441	83	1730	370
1.75	14010	4375	271	60	1474	671
2.18	15620	4102	367	77	1299	812
3.69	43290	7790	400	72	844	11
4.79	152250	1376	123	37	395	788
8.47	213050	1230	137	26	1187	23

Table 2 Pearson's *R* scores for all possible pair-wise correlations of the compound concentrations given in Table 1.

	<i>THC</i>	<i>Ammonia</i>	<i>methanol</i>	<i>acetic acid</i>	<i>methyl acetate</i>	<i>monoterpenes</i>	<i>sesquiterpenes</i>
THC	1.00						
ammonia	*0.92^a	1.00					
methanol	-0.55	-0.52	1.00				
acetic acid	-0.41	-0.47	*0.76	1.00			
methyl acetate	-0.36	-0.40	0.54	*0.84	1.00		
monoterpenes	-0.34	-0.28	0.35	0.41	0.47	1.00	
sesquiterpenes	-0.25	-0.20	0.07	0.28	0.44	*0.82	1.00

^a Asterisks indicate that the statistical significance of correlation for the 20 pairs of concentrations is $p < 0.002$ ($R > 0.6$) that corresponds to $p = 0.05$ corrected for multiple comparisons. Where the absolute value of *R* is < 0.45 , the correlation is not **significant even for a single comparison** ($p > 0.05$).

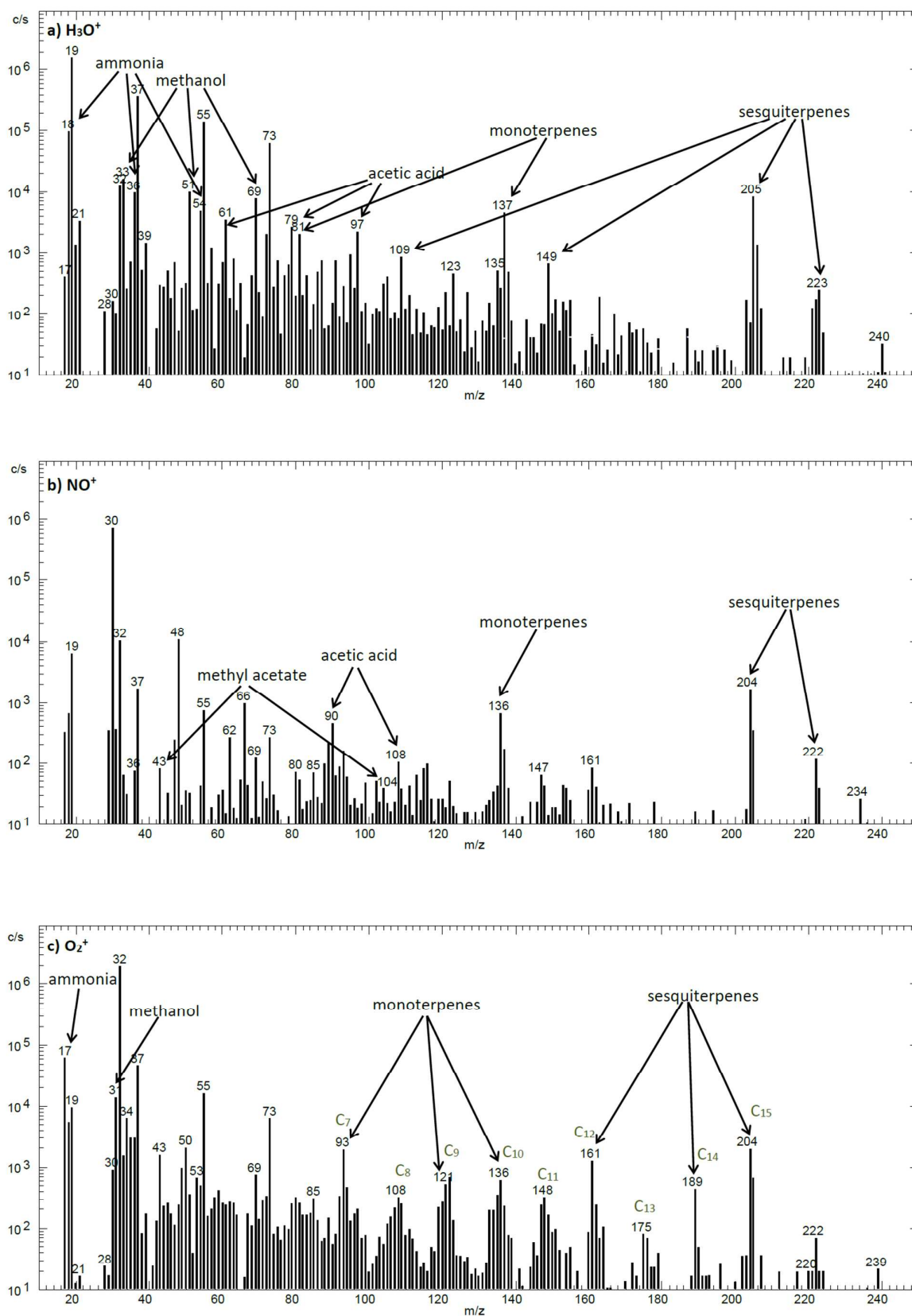


Figure 1. SIFT-MS spectra obtained when smoke released from heated cannabis (0.92 % THC) is introduced into the SIFT-MS instrument and ionised using a) H_3O^+ , b) NO^+ and c) O_2^+ reagent ions. The characteristic analyte ions of the identified compounds are indicated. Groups of fragment ions observed on the O_2^+ spectrum are indicated by the numbers of C atoms in the ions.

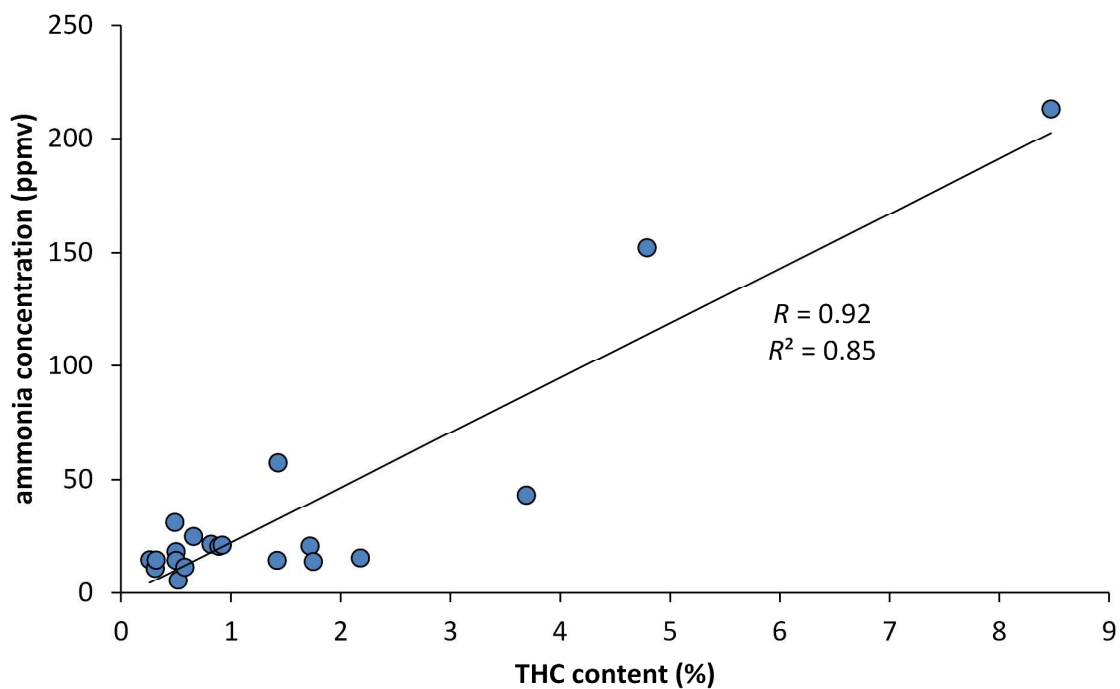
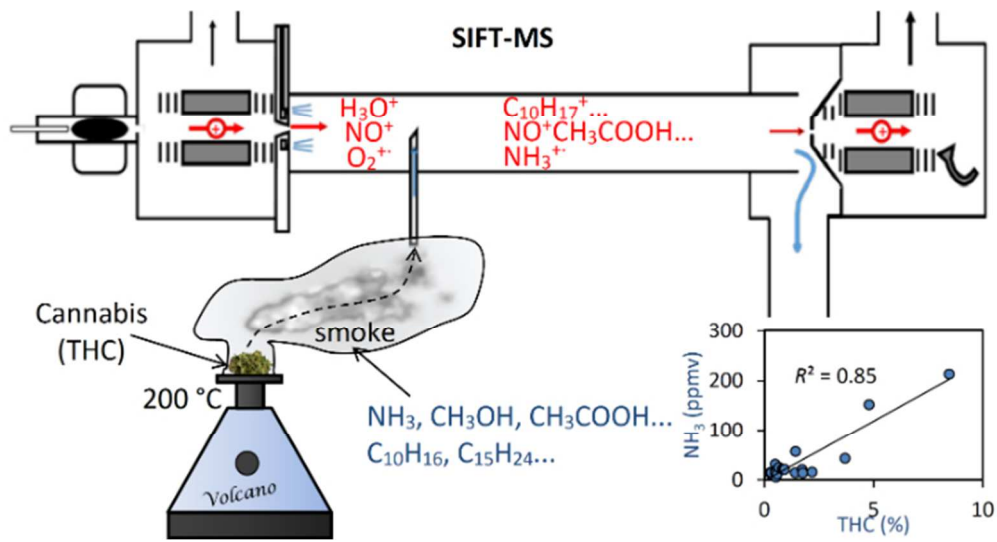


Figure 2 Dependence of the concentration of ammonia (parts-per-million by volume, ppmv) in the cannabis smoke on the THC concentration in the cannabis material (%) as plotted from the data in Table 1.

References.

1. British Lung Foundation, The impact of cannabis on your lungs, 2012, <http://www.blf.org.uk/Page/Special-Reports>, Accessed February, 2015.
2. W. Hall, *Addiction*, 1998, 93, 1461-1463.
3. M. H. S. Lee and R. J. Hancox, *Expert Rev. Resp Med*, 2011, 5, 537-547.
4. L. M. Borgelt, K. L. Franson, A. M. Nussbaum and G. S. Wang, *Pharmacotherapy*, 2013, 33, 195-209.
5. C. Reinerman, H. Nunberg, F. Lanthier and T. Heddlestone, *J. Psychoactive Drugs*, 2011, 43, 128-135.
6. R. N. Bloor, T. S. Wang, P. Španěl and D. Smith, *Addiction*, 2008, 103, 1671-1677.
7. A. Hazekamp, R. Ruhaak, L. Zuurman, J. van Gerven and R. Verpoorte, *Journal of pharmaceutical sciences*, 2006, 95, 1308-1317.
8. D. Smith and P. Španěl, *Mass Spectrom. Rev.*, 2005, 24, 661-700.
9. B. Secretan, K. Straif, R. Baan, Y. Grosse, F. El Ghissassi, V. Bouvard, L. Benbrahim-Tallaa, N. Guha, C. Freeman, L. Galichet, V. Coglianò and W. I. A. R. C. Monograph, *Lancet Oncol*, 2009, 10, 1033-1034.
10. K. H. Kilburn, *Environmental Management and Health*, 2000, 11, 239-250.
11. B. Hindfelt and B. K. Siesjö, *Scandinavian Journal of Clinical & Laboratory Investigation*, 1971, 28, 365-374.
12. D. Moir, W. S. Rickert, G. Levasseur, Y. Larose, R. Maertens, P. White and S. Desjardins, *Chemical Research in Toxicology*, 2008, 21, 494-502.
13. P. Španěl and D. Smith, *Mass Spectrom. Rev.*, 2011, 30, 236-267.
14. D. Smith and P. Španěl, *Analyst*, 2011, 136, 2009-2032.
15. P. Španěl and D. Smith, *Curr. Anal. Chem.*, 2013, 9, 525-539.
16. D. Smith, P. Španěl, J. M. Thompson, B. Rajan, J. Cocker and P. Rolfe, *Applied occupational and environmental hygiene*, 1998, 13, 817-823.
17. P. Španěl, K. Dryahina and D. Smith, *Int. J. Mass Spectrom.*, 2006, 249, 230-239.
18. D. Gieringer, J. St. Laurent and S. Goodrich, *Journal of Cannabis Therapeutics*, 2004, 4, 7-27.
19. P. Španěl, S. Davies and D. Smith, *Rapid Commun. Mass Spectrom.*, 1998, 12, 763-766.
20. P. Španěl, S. Davies and D. Smith, *Rapid Commun. Mass Spectrom.*, 1999, 13, 1733-1738.
21. F. Dhooche, C. Amelynck, N. Schoon, E. Debie, P. Bultinck and F. Vanhaecke, *Int. J. Mass Spectrom.*, 2008, 272, 137-148.
22. P. Španěl and D. Smith, *Rapid Commun. Mass Spectrom.*, 2001, 15, 563-569.
23. A. Pysanenko, P. Španěl and D. Smith, *Int. J. Mass Spectrom.*, 2009, 285, 42-48.
24. L. Ambach, F. Penitschka, A. Broillet, S. König, W. Weinmann and W. Bernhard, *Forensic Sci Int*, 2014, 243, 107-111.
25. Y. L. Dorokhov, T. V. Komarova, I. V. Petrunia, V. S. Kosorukov, R. A. Zinovkin, A. V. Shindyapina, O. Y. Frolova and Y. Y. Gleba, *Plos One*, 2012, 7, e36122.
26. R. Fall and A. A. Benson, *Trends in Plant Science*, 1996, 1, 296-301.
27. K. Dryahina, V. Pospisilova, K. Sovova, V. Shestivska, J. Kubista, A. Spesyvyi, F. Pehal, J. Turzikova, J. Votruba and P. Španěl, *J. Breath Res.*, 2014, 8, 037109.
28. P. Španěl and D. Smith, *Rapid Commun. Mass Spectrom.*, 1999, 13, 585-596.
29. S. D. Maleknia, T. L. Bell and M. A. Adams, *Int. J. Mass Spectrom.*, 2007, 262, 203-210.
30. N. Schoon, C. Amelynck, L. Vereecken, H. Coeckelberghs and E. Arijs, *Int. J. Mass Spectrom.*, 2004, 239, 7-16.
31. T. S. Wang, P. Španěl and D. Smith, *Int. J. Mass Spectrom.*, 2003, 228, 117-126.
32. Health and Safety Executive, *EH40/2005 Workplace exposure limits*, The Stationery Office, Norwich, Second edn., 2011.
33. J. McLaren, W. Swift, P. Dillon and S. Allsop, *Addiction*, 2008, 103, 1100-1109.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
34. W. R. Johnson, R. W. Hale and S. C. Clough, *Nature*, 1973, 244, 51-52.
 35. H. Lee, C. Chan, K. Tan, T. Cheong, C. Chee and Y. Wang, *Singapore medical journal*, 1993, 34, 565-566.
 36. J. Kubišta, P. Španěl, K. Dryahina, C. Workman and D. Smith, *Rapid Commun. Mass Spectrom.*, 2006, 20, 563-567.
 37. V. Shestivska, K. Dryahina, J. Nunvář, K. Sovová, D. Elhottová, D. Smith and P. Španěl, *Journal of Breath Research*, 2015, In press.
 38. V. Shestivska, P. Španěl, K. Dryahina, K. Sovová, D. Smith, M. Musilek and A. Nemeč, *J. Appl. Microbiol.*, 2012, 113, 701-713.



75x39mm (244 x 244 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60