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ORIGINAL ARTICLE

GCMS analysis of *Cannabis sativa* L. from four different areas of Pakistan

Muhammad Tayyab *, **Durre Shahwar**

Department of Chemistry, Government College University Lahore, Pakistan

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KEYWORDS

GCMS;
Cannabis;
Chromatogram

Abstract Cannabis is the most frequently used drug of abuse not only in Pakistan but also in the whole world. Its use is increasing drastically every year. GCMS allows for the analysis of *Cannabis sativa* which shows the differences of the constituents of this plant. Prevalence of this plant can be identified through knowledge of its constituents. In this way we can obstruct the production if we know the region in which it is produced. GCMS is a useful technique for the comparison of constituents of this drug of abuse which will assist the investigator concerning the origin of plant. Comparison also aids in the understanding and acquaintance of similarities of different samples of cannabinoids.

1. Introduction

Cannabis products are the drugs most frequently used around the world, representing 65 percent of all global seizure cases (1.65 million cases) in 2011. 5200 tons of herbs and 1000 tons of resin were seized in 2006. Virtually all countries in the world are affected by cannabis trafficking. Similarly, cannabis remains the most widely used drug worldwide, with an estimated 166 million people who have used cannabis in 2006, equivalent to about 4 percent of the world's population aged 15–64 years. At the end of the last century, the production method for cannabis has become more complex, due to the availability of many illicit market hemp products with very different levels of the main psychoactive ingredient, delta-9-tetrahydrocannabinol (THC). Recently, there has also been a renewed debate about the increasing THC content (often referred to as "power") in illicit cannabis products.

All this requires analytical data that are comparable in laboratories and in time. However, most countries do not require a detailed analysis of the law of the THC content of different products, and such analyses are performed using various methods and experimental models, reducing the comparability of results.[1] *Cannabis sativa* derivates, like hashish and marijuana, are the most commonly consumed illegal drugs worldwide. The adverse health effects of cannabis have been debated, but cannabis has been considered to have low toxicity and abuse potential.[3,4] Nevertheless, accumulating and converging evidence during the last decade has, revealed that cannabis use may be a risk factor for psychotic symptoms.[5,6] The reason for the increased number of such reports is not clear. There has, however, been a great interest in whether the concentration of D9-tetrahydrocannabinol (THC), the psychoactive compound in cannabis, has increased in.[4,7,8]

* Corresponding author.
E-mail address: Dr.tayyabb@gmail.com (M. Tayyab).

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More than 400 chemical compounds have been isolated from *C. sativa* L. of which more than 60 are cannabinoids. Cannabinoids are a characteristic class of substances unique to Cannabis. The most abundant are D9-tetrahydrocannabinol or THC (the main psychoactive cannabinoid), cannabidiol (CBD), cannabigerol (CBG) and cannabichromene (CBC).[10]

The Cannabis herb, dried flower buds of the female cannabis plants is consumed not only in almost every country in the world, but also produced in most of the countries, cannabis resin is lim- ited to a much smaller number of countries, most of which are found in North Africa, the Middle East and Southwest Asia.[2]

In 2011, the main countries listed as a source country of hashish were Morocco, and Afghanistan and, to a lesser extent, India, Lebanon and Pakistan (Pakistan, it is estimated that the resin seized on its territory originated in Afghanistan). Afghanistan and Morocco were determined as countries of origin of hashish between 2009 and 2011, and thus may reflect the main markets for resin as Afghanistan and cannabis as Morocco. Morocco has been named as the source of hashish smuggled from 17 different countries, 11 of which are in Western and Central Europe Morocco seems to remain the main source of cannabis resin. Afghanistan, on the other hand, has been named the country of origin of the cannabis resin seized in neighboring countries and the countries of the North. The countries of the Middle East and Europe also named Afghanistan as a source of hashish seizures. Global distribution reflects Afghanistan as the main source, as well. In the period 2000–2011, global cannabis resin seizures were dominated by Spain, which is the main point of entry for Moroccan cannabis resin in Europe. In 2011, Spain accounted for 34 percent of global seizures, Pakistan 18 percent and Morocco 12 percent.[2]

2. Experimental

2.1. General

The rationale of experiment was to compare chemically and biologically active components of C. sativa from different regions of Pakistan. This study will make it possible to compare the divergence of normal and protein bound cannabis from different regions of Pakistan. For the intention of comparative analysis of different areas of cannabinoids, first of all we performed the extraction of cannabinoids in an organic solvent, *n*-hexane, so that the impurities could be detached and these impurities would not interfere with our results. After that we performed color test just to make sure the presence of cannabinoids. GCMS analysis of all four samples was carried out.

2.2. Equipment

Equipments used in this experiment include GCMS. GCMS was used for the comparison of samples from different areas of Pakistan.

3. Materials

Crude cannabis was obtained with the help of the Anti-Narcotic Force, Pakistan (ANF) from Bhakkar, Kashmir, Gujranwala and Chaman. The *n*-hexane, acetaldehyde, chloroform, vanillin, HCl, alcohol, acetone and ethyl ether were purchased from Merck. All of the solvents such as methanol, acetone, *n*-hexane and ethyl acetate were of analytical grade.

3.1. Extraction

25 gms of the plant was soaked in 500 ml *n*-hexane for 10 days. Then it was sonicated and solvent was evaporated using a rotary evaporator. Now the resultant sample is subjected to analysis.

3.2. GCMS

After that we conducted GCMS analysis so that we could compare the samples of Cannabis sativa from different areas. For the purpose of GCMS we needed to do sample preparation first.

3.2.1. Sample preparation

Gas chromatography–mass spectrometry requires derivatization of the samples for analysis. For this purpose, 500 microliters of the solution was transferred to a 2 ml GC vial. The vial was placed in a heating bath (150 °C) for 12 min, wherein the solvent was evaporated and decarboxylated. The residue was dissolved in 1.5 ml of ethanol (analytical grade), the vial was shaken well and the resulting solution was successfully analyzed by gas chromatography which is abbreviated as GC.

Following are the conditions used

- Column: 15 m × 0.25 mm, 0.25 micron;
- Phase: 5% diphenyl–95% dimethyl polysiloxane
- Vector: a hydrogen atom, 1.1 ml/min, constant flow
- Injector: split/inseparable, 280 °C
- Division ratio: 20:01
- Oven: 2 min at 200 °C, 10 C/min 200–240 °C, 2 min at 240 °C
- Detector: MS 300 °C, H
- 35 ml/min, air 350 ml/min
- Internal standard: tribenzylamine (TBA) in ethanol (0.5 mg/ml)
- Injection: 1.5 microliters, Split
- The order of elution: CBD, THC, CBN.[1]

4. Results

The results of GCMS analysis of the sample obtained from Kashmir region of Pakistan are as follows:

Fig. 1 reveals that there are five different constituents present in the cannabis plant obtained from the Kashmir region. By searching the library of American Academy of Forensic Science which is abbreviated as AAFS, the names of these constituents was obtained. The peaks indicates that the abundance of a constituent was different in each plant. It means the quantity of each constituent present in the plant is different from each other. The position of peaks shows the time of elution which is different for each constituent because of it's different structures.

Fig. 2 shows the names of different constituents present in the plant. There are five components which are present in the

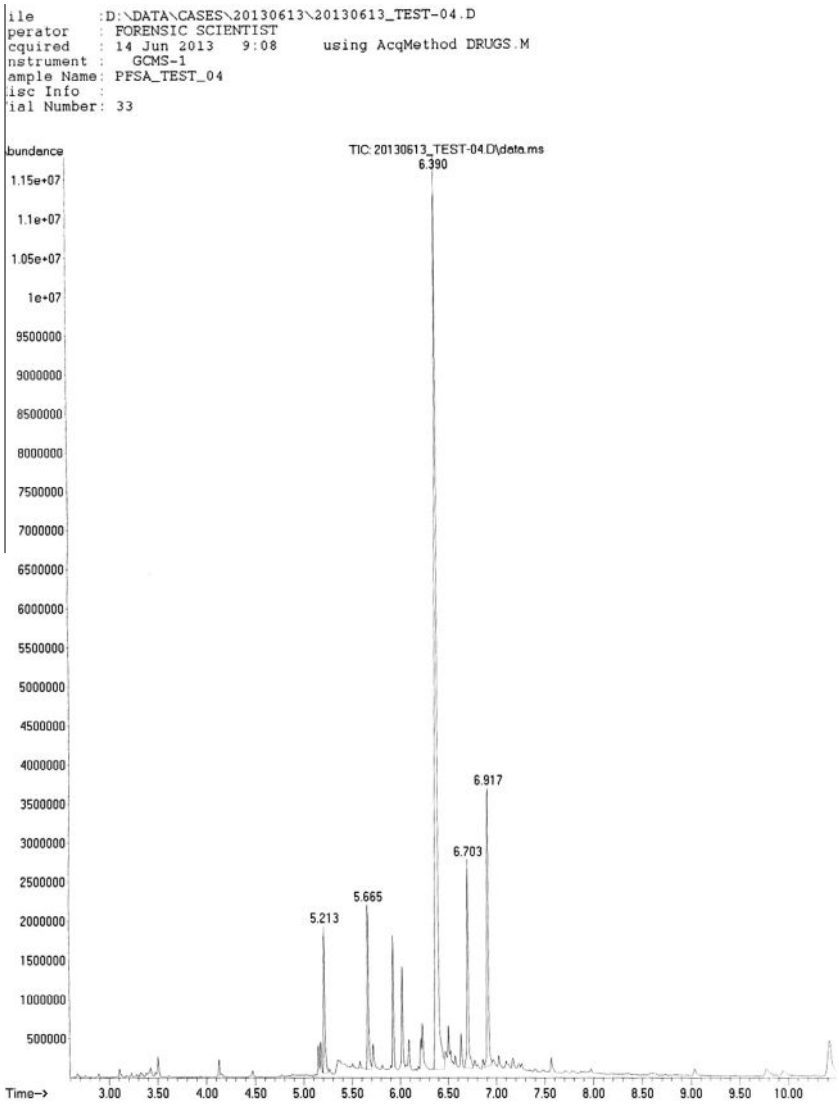
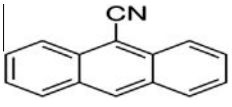
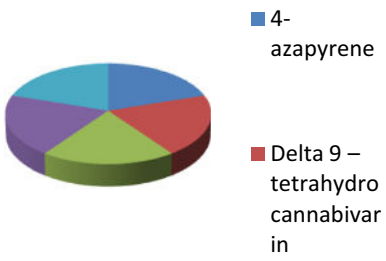


Figure 1 GCMS of Kashmir sample.

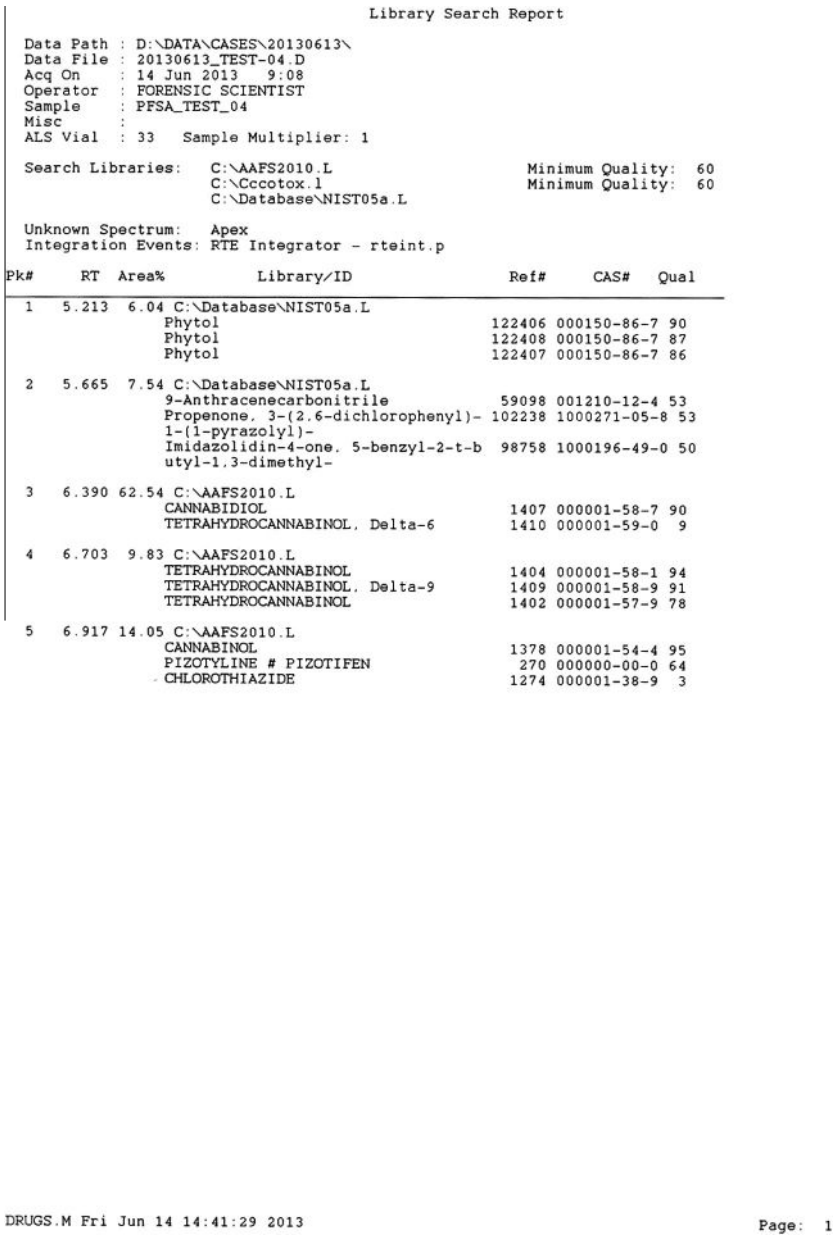
sample obtained from Kashmir region. The first constituent is phytol. It is an acyclic diterpene alcohol. Its molecular formula is $C_{10}H_{20}O$. This can be commercially used in the fragrance industry. The second constituent is 9-anthracenecarbonitrile.



is the structure of this compound. Its molecular formula is $C_{15}H_9N$. The third constituent is cannabidiol. It is one of the major constituents of the cannabis plant. It is supposed to have a wider scope than THC because of it is less psychoactive. Its molecular formula is $C_{21}H_{30}O_2$. It is used in the treatment of nausea, convulsions, bipolar disorders, etc. The fourth constituent is tetrahydrocannabinol also known as Δ^9THC which is considered the main or principal psychoactive constituent of the cannabis plant. It is classified as schedule 1 under the convention on psychotropic substances. It is available in a synthetic form as well, its brand name is Marinol. It is a toxic component and can cause death due to over dosing. The fifth constituent is cannabinol. It is usually present in or trace amounts in *C. sativa*. It may be a metabolite of THC. Its molecular formula is $C_{21}H_{26}O_2$.



Pie Chart of Kashmir Sample



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Misc :
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C:\Cccotox.l Minimum Quality: 60
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Unknown Spectrum: Apex
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			Phytol	122408	000150-86-7	87
			Phytol	122407	000150-86-7	86
2	5.665	7.54	C:\Database\NIST05a.L			
			9-Anthracenecarbonitrile	59098	001210-12-4	53
			Propenone, 3-(2.6-dichlorophenyl)- 1-(1-pyrazolyl)-	102238	1000271-05-8	53
			Imidazolidin-4-one, 5-benzyl-2-t-butyl-1.3-dimethyl-	98758	1000196-49-0	50
3	6.390	62.54	C:\AAFS2010.L			
			CANNABIDIOL	1407	000001-58-7	90
			TETRAHYDROCANNABINOL, Delta-6	1410	000001-59-0	9
4	6.703	9.83	C:\AAFS2010.L			
			TETRAHYDROCANNABINOL	1404	000001-58-1	94
			TETRAHYDROCANNABINOL, Delta-9	1409	000001-58-9	91
			TETRAHYDROCANNABINOL	1402	000001-57-9	78
5	6.917	14.05	C:\AAFS2010.L			
			CANNABINOL	1378	000001-54-4	95
			PIZOTYLINE # PIZOTIFEN	270	000000-00-0	64
			CHLOROTHIAZIDE	1274	000001-38-9	3

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Figure 2 Library search of Kashmir sample.

Following are the results of the GCMS analysis of the Cannabis extract from the District of Bhakkar of Pakistan.

Fig. 3 shows that the samples taken from Bhakkar region also contain five constituents. These Five constituents of Bhakkar region are similar to those of the Kashmir region. This was confirmed by the AAFS library which is shown in Fig. 4. All of the constituents showed similar RT values. From this, we can analyze that *C. sativa* of both areas have similar constituents.

Following are the results of the GCMS analysis of the Cannabis extract from the District of Gujranwala of Pakistan.

Fig. 5 shows that the samples taken from the Gujranwala region also contain five constituents. Fig. 6 shows the names of these constituents which were obtained by searching the library of American Academy of Forensic Science. The first constituent is acenaphthol. The second constituent is D^9tetrahydrocanna- bivarin. The third constituent is cannabidiol. It is one of the major constituents of the cannabis plant. It is supposed to have a wider scope than THC because it is less psychoactive. Its molecular formula is $C_{21}H_{30}O_2$. It is used in the treatment of nausea, convulsions, bipolar disorders, etc. The fourth constituent is tetrahydrocannabinol also known as Δ^9THC which is considered the main or principal psychoactive constituent of the cannabis plant. It is classified as schedule 1 under the convention on psychotropic substances. It is available in a synthetic form as well, its brand name is Marinol. It is a toxic component and can cause death due to over dosing. The fifth constituent is cannabinol. It is usually present in trace amounts in *C. sativa*.

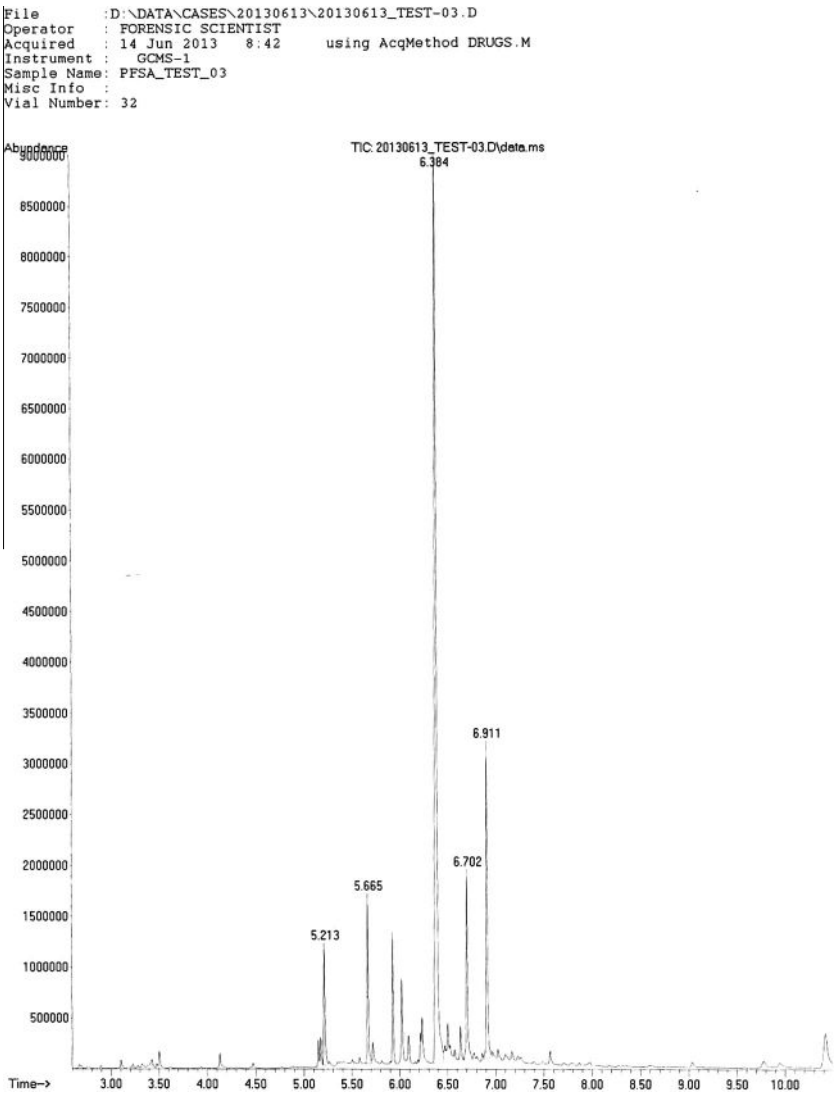
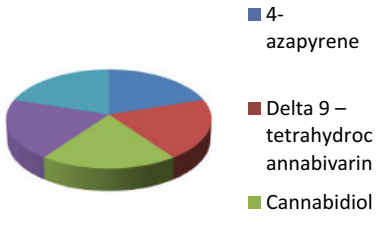
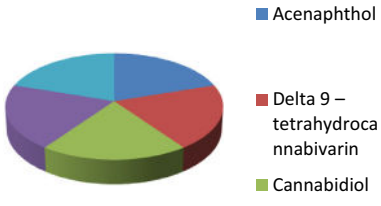


Figure 3 GCMS of Bhakkar sample.



Pie Chart of Kashmir Sample



Pie Chart of Kashmir Sample

Following are the results of the GCMS analysis of the Cannabis extract from the District of Chaman of Pakistan.

Fig. 7 reveals that the samples taken from the Chaman region also contain five constituents. Fig. 8 reveals the names of these constituents which were obtained by searching the library of the American Academy of Forensic Science. The first constituent is azapyrene. The second constituent is Δ^9tetrahydrocannabivarin. The third constituent is cannabidiol. It is one of the major constituents of the cannabis plant. It is supposed to have a wider scope than THC because it is less psychoactive. Its molecular formula is $C_{21}H_{30}O_2$. It is used in the treatment of nausea, convulsions, bipolar disorders, etc. The fourth constituent is tetrahydrocannabinol also known as Δ^9THC which is considered the main or principal psychoactive constituent of the cannabis plant. It is classified as schedule 1 under the convention on psychotropic substances. It is available in a synthetic form as well, its brand name is Marinol. It is a toxic component and can cause death due to over dosing. The fifth constituent is cannabinol. It is usually present in trace amounts in *C. sativa* (see Tables 1–5).

Library Search Report

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C:\Cccotox.l Minimum Quality: 60
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			Phytol	122409	000150-86-7	91
			Phytol	122406	000150-86-7	90
2	5.665	7.18	C:\Database\NIST05a.L			
			9-Anthracenecarbonitrile	59098	001210-12-4	53
			Propenone, 3-(2,6-dichlorophenyl)-1-(1-pyrazolyl)-	102238	1000271-05-8	53
			Imidazolidin-4-one, 5-benzyl-2-t-butyl-1,3-dimethyl-	98758	1000196-49-0	53
3	6.384	64.13	C:\AAFS2010.L			
			CANNABIDIOL	1407	000001-58-7	97
			TETRAHYDROCANNABINOL, Delta-6	1410	000001-59-0	9
4	6.702	8.90	C:\AAFS2010.L			
			TETRAHYDROCANNABINOL	1404	000001-58-1	94
			TETRAHYDROCANNABINOL	1402	000001-57-9	70
			TETRAHYDROCANNABINOL, Delta-9	1409	000001-58-9	53
5	6.911	14.41	C:\AAFS2010.L			
			CANNABINOL	1378	000001-54-4	92
			PIZOTYLINE # PIZOTIFEN	270	000000-00-0	64
			CHLOROTHIAZIDE	1274	000001-38-9	3

GS.M Fri Jun 14 14:42:11 2013 Page: 1

Figure 4 Library search of Bhakkar sample.

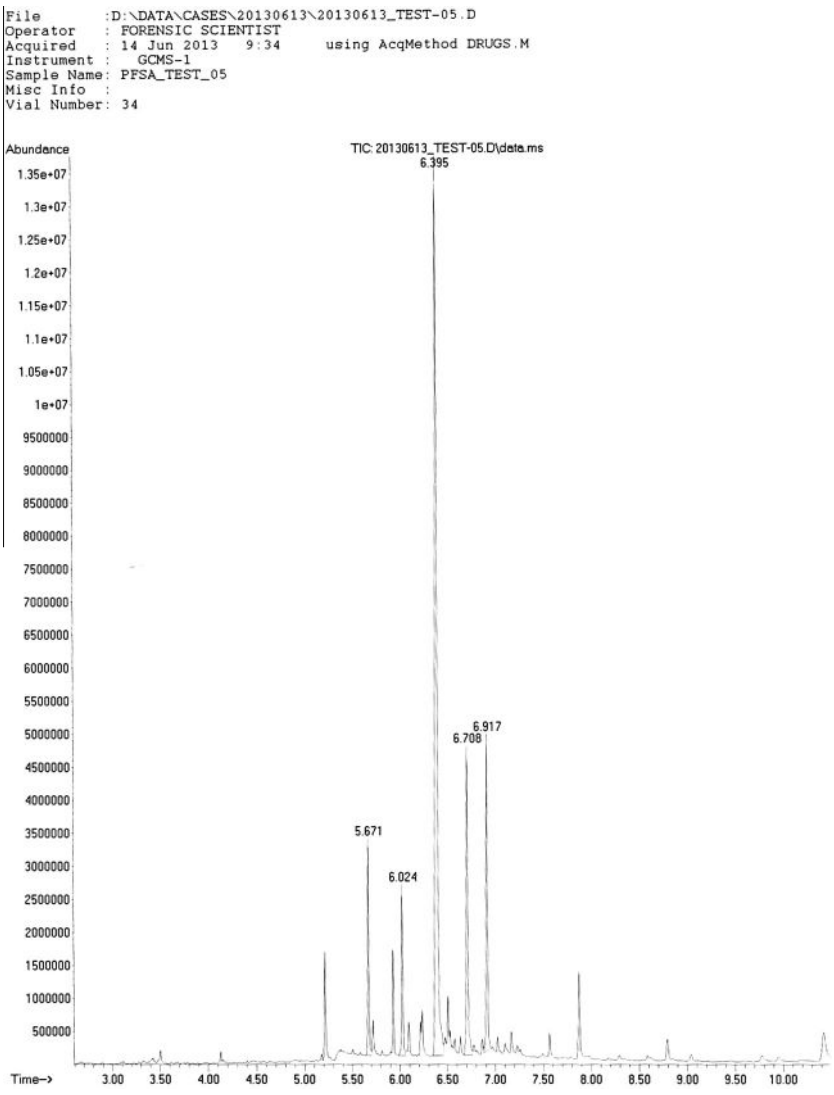
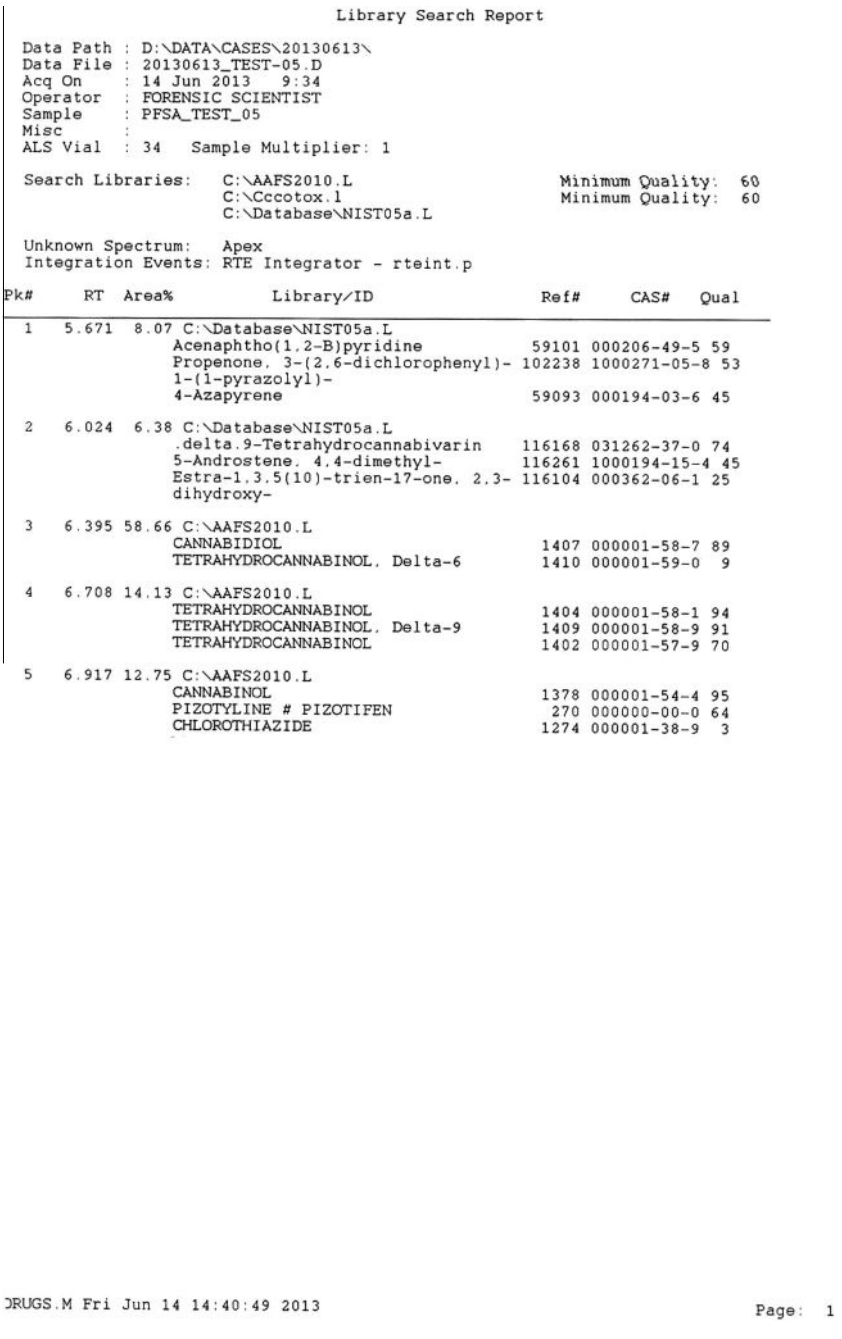


Figure 5 GCMS of Gujranwala sample.



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Unknown Spectrum: Apex
Integration Events: RTE Integrator - rteint.p

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			Propenone, 3-(2,6-dichlorophenyl)-1-(1-pyrazolyl)-	102238	1000271-05-8	53
			4-Azapyrene	59093	000194-03-6	45
2	6.024	6.38	C:\Database\NIST05a.L			
			.delta.9-Tetrahydrocannabivarin	116168	031262-37-0	74
			5-Androstene, 4,4-dimethyl-	116261	1000194-15-4	45
			Estra-1,3,5(10)-trien-17-one, 2,3-dihydroxy-	116104	000362-06-1	25
3	6.395	58.66	C:\AAFS2010.L			
			CANNABIDIOL	1407	000001-58-7	89
			TETRAHYDROCANNABINOL, Delta-6	1410	000001-59-0	9
4	6.708	14.13	C:\AAFS2010.L			
			TETRAHYDROCANNABINOL	1404	000001-58-1	94
			TETRAHYDROCANNABINOL, Delta-9	1409	000001-58-9	91
			TETRAHYDROCANNABINOL	1402	000001-57-9	70
5	6.917	12.75	C:\AAFS2010.L			
			CANNABINOL	1378	000001-54-4	95
			PIZOTYLINE # PIZOTIFEN	270	000000-00-0	64
			CHLOROTHIAZIDE	1274	000001-38-9	3

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Figure 6 Library search of Gujranwala sample.

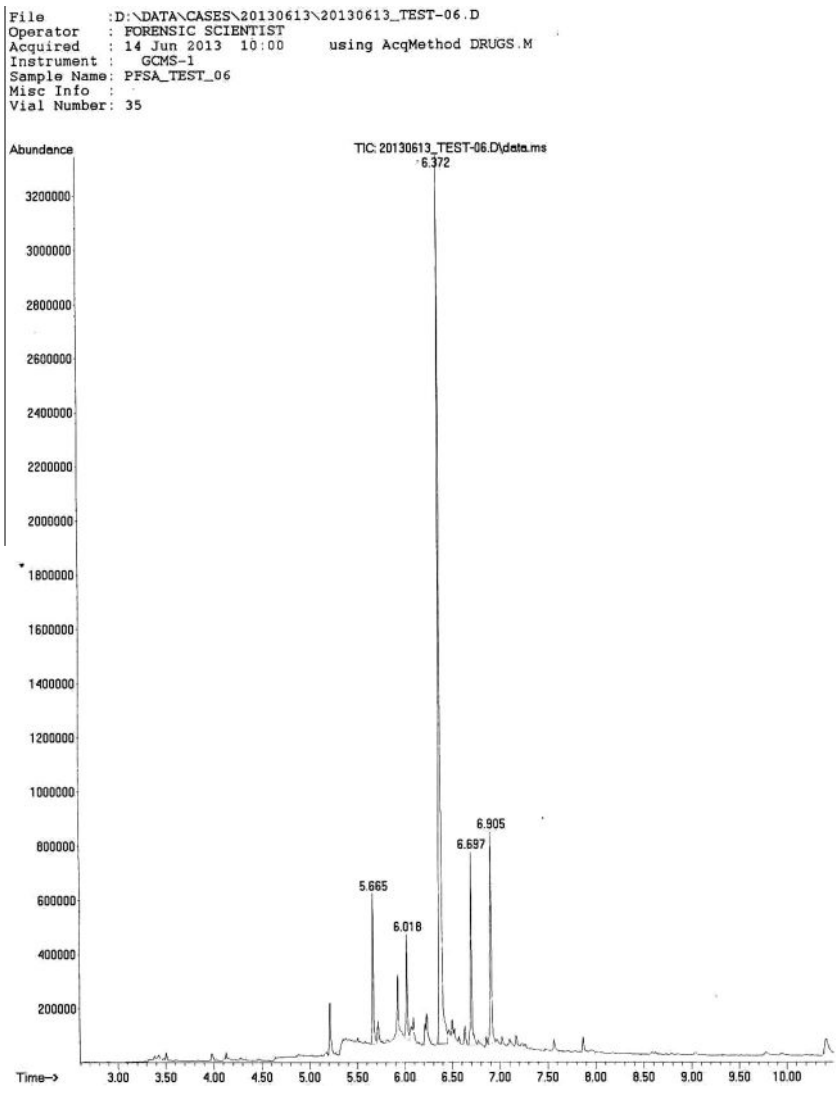

Figure 7 GCMS of Chaman sample.

Library Search Report

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Data File : 20130613_TEST-06.D
Acq On : 14 Jun 2013 10:00
Operator : FORENSIC SCIENTIST
Sample : PFSA_TEST_06
Misc :
ALS Vial : 35 Sample Multiplier: 1

Search Libraries: C:\AAFS2010.L Minimum Quality: 60
C:\Cccotox.l Minimum Quality: 60
C:\Database\NIST05a.L

Unknown Spectrum: Apex
Integration Events: RTE Integrator - rteint.p

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
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			4-Azapyrene	59093	000194-03-6	64
			Acenaphtho(1,2-B)pyridine	59101	000206-49-5	59
			Propenone, 3-(2,6-dichlorophenyl)-1-(1-pyrazolyl)-	102238	1000271-05-8	53
2	6.018	5.98	C:\Database\NIST05a.L			
			.delta.9-Tetrahydrocannabivarin	116168	031262-37-0	74
			Kaempferol	115752	000520-18-3	41
			Ethene, 1-[4-methoxyphenyl]-2,2-diphenyl-	116251	018648-74-3	35
3	6.372	62.04	C:\AAFS2010.L			
			CANNABIDIOL	1407	000001-58-7	92
			TETRAHYDROCANNABINOL, Delta-6	1410	000001-59-0	9
4	6.697	11.16	C:\AAFS2010.L			
			TETRAHYDROCANNABINOL	1404	000001-58-1	94
			TETRAHYDROCANNABINOL, Delta-9	1409	000001-58-9	91
			TETRAHYDROCANNABINOL	1402	000001-57-9	64
5	6.905	12.27	C:\AAFS2010.L			
			CANNABINOL	1378	000001-54-4	92
			CHLOROTHIAZIDE	1274	000001-38-9	3
			PIZOTYLINE # PIZOTIFEN	270	000000-00-0	2

DRUGS.M Fri Jun 14 14:38:51 2013 Page: 1

Figure 8 Library search of Chaman sample.

Table 1 GCMS results of Kashmir sample.

Peak number	Retention time	Name of constituent	Peak area
1	5.213	Phytol	6.04
2	5.665	9-Anthracenecarbonitrile	7.54
3	6.390	Cannabidiol	62.54
4	6.703	Delta-9-tetrahydrocannabinol	9.83
5	6.917	Cannabinol	14.05

Table 2 GCMS results of Bhakkar sample.

Peak number	Retention time	Name of constituent	Peak area
1	5.213	Phytol	5.38
2	5.665	9-Anthracenecarbonitrile	7.18
3	6.384	Cannabidiol	64.13
4	8.90	Delta-9-tetrahydrocannabinol	8.90
5	6.911	Cannabinol	14.41

Table 3 GCMS results of Gujranwala sample.

Peak number	Retention time	Name of constituent	Peak area
1	5.671	Acenaphthol	8.07
2	6.024	Delta-9-tetrahydrocannabivarin	6.38
3	6.395	Cannabidiol	58.66
4	6.708	Delta-9-tetrahydrocannabinol	14.13
5	6.917	Cannabinol	12.75

Table 4 GCMS results of Chaman sample.

Peak number	Retention time	Name of constituent	Peak area
1	5.665	4-Azapyrene	5.665
2	6.018	Delta-9-tetrahydrocannabivarin	6.018
3	6.372	Cannabidiol	62.04
4	6.697	Delta-9-tetrahydrocannabinol	6.69
5	6.905	Cannabinol	6.905

Table 5 Constituents of different samples.

Serial No.	Constituents	Samples
1	Cannabinol	All samples
2	Cannabidiol	All samples
3	Delta-9-tetrahydrocannabinol	All samples
4	Phytol	Bhakkar, Kashmir
5	9-Anthracenecarbonitrile	Bhakkar, Kashmir
6	4-Azapyrene	Chaman
7	Delta-9-tetrahydrocannabivarin	Gujranwala, Chaman
8	Acenaphthol	Gujranwala

5. Conclusion

The Cannabis samples obtained from the different areas of Pakistan are only slightly different from each other. All four samples contain the main psychoactive constituent Δ^9THC which is the most abundant in all four samples. The constituents of the samples obtained from the two regions, Bhakkar and Kashmir, are similar. Cannabinol and Cannabidiol are also present in all four samples. This study can help the researcher to identify the origin of the cannabis plant.

Funding

None.

Conflict of interest

None declared.

Ethical approval

Necessary ethical approval was obtained from the department of chemistry, Government college university Lahore, Pakistan.

References

1. UNODC, Recommended methods for the identification and analysis of cannabis and cannabis products; 2009.
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