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Applying a COVID-19 Sample-pooling Technique to Forensics Identification of Illicit Drugs

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ABSTRACT

This paper presents a method for materially speeding up the identification process of suspect illicit drugs by pooling samples that require GC-MS analysis. This method can be applied to samples seized from a single suspect that are similar in appearance and therefore meet the Israeli Dangerous Drug Ordinance requirements for sampling. A complementary test (GC, TLC, or FTIR) conducted separately on each of the sampled units can prove conclusively that all units contain the same drug. This study shows that even with large differences in relative weight of mixes in a pool, each drug is easily identifiable by GC-MS and dominant peaks do not overshadow minority substances. By using this method, a narcotics lab can improve its throughput of expert opinions in narcotics cases, and at the same time save resources, extend instrument life, and be more environment-friendly.

Keywords: Pooling, GCMS, Identification, Illicit drug analysis

1 Introduction

The Israeli National Narcotics Laboratory routinely analyzes large numbers of casework exhibits for suspected illicit drugs. Often these exhibits are composed of multiple individual units. The Dangerous Drug Ordinance regulations permit investigators to analyze a subset of these units (sampling) and apply the results of the analyzed samples to the entire seized exhibit. To qualify for sampling, the Ordinance requires that all units in the group be similar in size, color, and shape. Then, if there are 6-15 similar units, the required sample size is 5 units; if there are 16-50 similar units sample size is 6 units; and if there are 51 or more units the sample size is 7 units (Dangerous Drugs Ordinance, 2021). Compliant with the recommendations of the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG)—each unit is identified using two different methods. If both results are identical in each of the units, the laboratory can apply its legal opinion regarding the subset that is analyzed to the whole exhibit (Scientific Working Group, version 8). The difficulty is that the number of drug-related cases is large, and the pressure on the laboratory is compounded by the fact that narcotics cases often involve the arrest of suspects, making it quite important for the laboratory to issue a report rapidly.

Like many analytical and forensic laboratories, the Narcotics Laboratory's analytical method of choice is gas chromatography-mass spectrometry (GC-MS) (defined by the SWGDRUG as a category A method). Other identification methods used in our laboratory are FTIR (a category A method), and gas chromatography (GC) and thin layer chromatography (TLC) (both classified as category B methods) (Scientific Working Group, version 8). Given its wide application (Kranenburg et al., 2020), GC-MS analysis has become the bottleneck in our sample analysis schedule. To help reduce this problem, this study examined the possibility of pooling GC-MS samples, i.e., injecting a mixture composed of specimens from a subset of units (up to seven units, in compliance with sampling regulations in Israel). The laboratory

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would then apply an additional test to each of the units separately—either GC-FID (Mitrevski et al, 2011, Yuksel et al., 2018), FTIR (Hans et al, 2012, Kempfert et al., 1988) or TLC (Rajananda et al., 1985)—to confirm the presence of any illicit drug identified in the pool. Pooling samples in this way can shorten the time to identification by up to 86% (analyzing one specimen by GC-MS instead of seven saves 6/7 of the time), saving person-hours, consumables (solvents, vials, instrument life), and energy. So in addition to being able to provide service faster, by applying this method the forensic laboratory would also become more eco-friendly.

Sample-pooling is already in wide use in the analysis of diagnostic tests for COVID-19 (Wacharapluesadee et al., 2020, Shani-Narkiss et al., 2020, Garg et al., 2021, Quinn et al., 2000, Petkova et al., 2020, Ghosh et al., 2020) The current COVID-19 pandemic has created a demand for diagnostic tests well beyond what the responsible laboratories were able to process. Improving efficiency and reducing costs became imperative, so laboratories decided to pool samples: To test a pooled sample, specimens from several swabs were mixed, and the mixture was analyzed. If the pooled sample was negative, all the units in the mixture were confirmed negative. If the pooled sample was positive, each individual swab was then analyzed separately to identify which swab had made the mixture positive.

The efficiency of pooling coronavirus tests depends on the percentage of positive results. The reason for this is that if there are more positive individuals in the population, the number of positive pools will increase, and more tests will have to be repeated individually, which makes the system less effective. But in a narcotics laboratory, pooling samples can be consistently efficient. In the majority of drug cases (over 99% according to internal laboratory data), when multiple units that look sufficiently alike in color, shape, and dimensions were found in the possession of a single person and seized together the same drug was identified in all units sampled. Only rarely will there be a difference between units in a subset. Therefore, the assumption of this study is that, in most cases, a single GC-MS test of a pooled sample can be considered as equivalent to analyzing each of the seven units separately. A pooled result, together with an additional individual test of each of the units in the pool by another method of analysis (TLC, FTIR, GC) can allow the laboratory to identify and provide its report more promptly. Only in the rare cases where units are found to be different, will each unit in the subset be analyzed individually by GC-MS.

Street drugs seized by the police are rarely pure; unit volume is often inflated or adulterated by the addition of cutting agents such as sugars (e.g. mannitol), salts (e.g. magnesium sulfate), various anesthetics (e.g. lidocaine, benzocaine), and stimulants (e.g. caffeine). The instrument's manufacturer information about detection thresholds applies to pure samples with a known concentration of the tested sample. Seized drug exhibits pose a different challenge since they can be composed of various different substances in unknown concentrations. Therefore, a thorough study of the ability of the method to detect, differentiate and properly identify all components of this pooling is necessary to establish the methodology. The first step in the proposed method was to find the detection threshold of the GC-MS instrument used at the National Narcotics Laboratory (an Agilent 7890A) and then test the instrument's ability to identify the substances in street-drug samples that volume-wise are largely composed of additives. The pooled samples tested in this study were in powder form, blotter tabs, solutions, plant-derived substances, or extractions of substrate adsorbed on plant. All pooled samples were taken from street drug units seized by police officers in the field.

2 Materials and methods

Xylene and acetone were purchased from Gadot (Israel). Dichloromethane, dioxane, ammonium hydroxide, and ninhydrin were purchased from Bio-Lab (Israel). Ethanol was purchased from Frutarom (Israel). Potassium bromide was purchased from Merck (Germany).

Fifty pooled samples of specimens taken from street units were analyzed by GC-MS to test the method's ability to identify individual components of these pools. Mixtures prepared in the lab of pure cocaine and benzocaine, pure cocaine and creatine, and each pure substance separately (0.005g of each substance) were analyzed by GC-FID, TLC, and FTIR to understand the identification capabilities of each of these methods

when applied to such mixtures in conjunction with GC-MS. The characterization process is described below.

2.1 Gas chromatography mass spectrometry (GC-MS)

The 50 pooled samples were extracted in dichloromethane/ammonium hydroxide and analyzed on an Agilent 7890A (USA) GC coupled to a 5975C inert MSD under the following conditions: DB-5MS column (15.0 m, inner diameter 0.25 mm, and internal coating 0.25 μm; Agilent Technologies, USA); injector temperature – 220°C, oven temperatures for each sample are shown in Table 1; split ratio 1:25; transfer line temperature 280°C. Electron ionization (EI) spectra were recorded at 70 eV. Measurements were made at a range of 40–500 m/z. Data were analyzed using the MassHunter software.

Table 1: *List of 50 pooled samples components, their ratio and the injection conditions.*

Samp Num	Type of Material	Oven Temperature (ºC)	Pooling Components	Ratio	Identification yes/no
1	herbal	220-290 (25ºC/min)	0.1 gr MDMB 4-en PINACA - 0.6 gr 5F-MDMB BUTINACA	(1:6)	yes
2	blotter tabs	60-290 (25ºC/min)	6 LSD - 1 DOC	(6:1)	yes
3	blotter tabs	220-290 (25ºC/min)	6 LSD - 1 NBOME	(6:1)	yes
4	liquid	220-290 (25ºC/min)	6 drops ketamine - 1 drop LSD	(6:1)	partially identified
5	liquid	60-290 (25ºC/min)	6 drops LSD - 1 drop ketamine	(6:1)	yes
6	tablets	60-290 (25ºC/min)	0.0025 g 2CB - 0.0175 MDMA	(1:7)	yes
7	tablets	60-290 (25ºC/min)	0.0025 g amphetamine - 0.0187 MDMA	(1:8.5)	yes
8	powder	60-290 (25ºC/min)	0.002 g MMC - 0.014 g MDMA	(1:7)	yes
9	powder	60-290 (25ºC/min)	0.0235 g MMC - 0.0026 g MDMA	(9:1)	yes
10	powder	60-290 (25ºC/min)	0.0017 g cocaine - 0.0196 g methamphetamine	(1:11)	yes
11	powder	60-290 (25ºC/min)	0.0221 g cocaine - 0.0025 g methamphetamine	(9:1)	yes
12	herbal	190-290 (25ºC/min)	0.8 g MDMB 4-en PINACA - 0.1 g 5F-MDMB BUTINACA	(8:1)	yes
13	herbal	190-290 (25ºC/min)	0.7 g 5F-MDMB-PICA - 0.1 g 5F-MDMB BUTINACA	(7:1)	yes
14	herbal	190-290 (25ºC/min)	0.9 g Cannabis - 0.15 5F-MDMB BUTINACA	(6:1)	yes
15	herbal	190-290 (25ºC/min)	0.1 g Cannabis - 0.7 g 5F-MDMB BUTINACA	(1:7)	yes
16	tablets	60-290 (25ºC/min)	0.025 g 2C-B - 0.0045 g MDMA	(1:6)	yes

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Samp Num	Type of Material	Oven Temperature (ºC)	Pooling Components	Ratio	Identification yes/no
17	liquid	60-290 (25ºC/min)	10 drops THC - 1 drop CBD	(10:1)	yes
18	liquid	60-290 (25ºC/min)	1 drop THC - 10 drops CBD	(1:10)	yes
19	liquid	120-290 (25ºC/min)	10 drops GBL - 1 drop THC	(10:1)	yes
20	liquid	60-290 (25ºC/min)	1 drop THC - 10 drops GBL	(1:10)	yes
21	powder	60-290 (25ºC/min)	0.0236 methamphetamine 0.0022 2CB 0.0063 m-MMC 0.0232 MDMA 0.0115 amphetamine 0.0025 cocaine	(11:1:3:11:5:1)	yes
22	liquid	60-290 (25ºC/min)	10 drops GBL - 1 drop ketamine	(10:1)	yes
23	liquid	120-290 (25ºC/min)	1 drop GBL - 10 drops ketamine	(1:10)	yes
24	liquid	120-290 (25ºC/min)	10 drops ketamine - 1 drop deschloro ketamine	(10:1)	yes
25	liquid	120-290 (25ºC/min)	1 drop ketamine - 10 drops deschloro ketamine	(1:10)	yes
26	liquid	120-290 (25ºC/min)	10 drops ketamine - 1 drop deschloro ketamine – 1 drop CBD	(10:1:1)	yes
27	liquid	120-290 (25ºC/min)	10 drops CBD - 1 drop ketamine - 1 drop deschloro ketamine	(10:1:1)	yes
28	liquid	120-290 (25ºC/min)	1 drop ketamine - 10 drops deschloro ketamine – 1 drop CBD	(1:10:1)	yes
29	powder	120-290 (25ºC/min)	0.0061 g cocaine - 0.1161 g MDMA	(1:19)	yes
30	powder	120-290 (25ºC/min)	0.1855 g cocaine - 0.0088 g MDMA	(21:1)	yes
31	liquid	120-290 (25ºC/min)	1 drop CBD - 10 drops deschloro N-ethyl ketamine	(1:10)	yes
32	liquid	120-290 (25ºC/min)	10 drops CBD - 1 drop deschloro N-ethyl ketamine	(10:1)	yes
33	liquid	120-290 (25ºC/min)	1 drop CBD - 20 drops deschloro N-ethyl ketamine	(1:20)	yes
34	liquid	120-290 (25ºC/min)	20 drop CBD - 1 drop deschloro N-ethyl ketamine	(20:1)	yes
35	powder	120-290 (25ºC/min)	1 drop CBD - 30 drops deschloro N-ethyl ketamine	(1:30)	yes
36	powder	120-290 (25ºC/min)	30 drop CBD - 1 drops deschloro N-ethyl ketamine	(30:1)	yes
37	herbal	120-290 (25ºC/min)	0.3419 g 5F-MDMB-PICA & ADB-FUBINACA + 0.0403 5F-ADB	(8.5:1)	yes

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Samp Num	Type of Material	Oven Temperature (ºC)	Pooling Components	Ratio	Identification yes/no
38	herbal	120-290 (25ºC/min)	0.4083 g 5F-MDMB-PICA & ADB-FUBINACA + 0.0081 4F-ABUTINACA	(50.5:1)	yes
39	herbal	120-290 (25ºC/min)	0.3377 4F-ABUTINACA + 0.0154 g 5F-MDMB-PICA & ADB-FUBINACA	(22:1)	yes
40	herbal	120-290 (25ºC/min)	0.4051g 4F-ABUTINACA + 0.024g 5F-ADB	(17:1)	yes
41	powder	120-290 (25ºC/min)	0.0226g cocaine + 0.0003g MMC	(75.333:1)	yes
42	powder	120-290 (25ºC/min)	0.0052g cocaine + 0.0612g MMC	(1:11.77)	yes
43	powder	120-290 (25ºC/min)	0.0653g heroin + 0.0019g MMC	(34.37:1)	yes
44	powder	120-290 (25ºC/min)	0.0020g heroin + 0.0531g MMC	(1:26.55)	yes
45	powder	120-290 (25ºC/min)	0.0247g MMC + 0.0014 ketamine - MDMA	(1:17.64)	yes
46	powder	120-290 (25ºC/min)	0.0174g MMC + 0.0020 tiletamine-benzocaine-lidocaine	(8.7:1)	yes
47	powder	120-290 (25ºC/min)	0.0018g MMC + 0.0367 tiletamine-benzocaine-lidocaine	(1:20.39)	yes
48	powder	120-290 (25ºC/min)	0.0440g heroin + 0.0020 tiletamine-benzocaine-lidocaine	(22:1)	yes
49	powder	120-290 (25ºC/min)	0.01g heroin + 0.0028g ketamine - MDMA	(3.57:1)	yes
50	powder	120-290 (25ºC/min)	0.0029 euytlone + 0.0137 MDMA	(1:4.72)	yes

2.2 Gas chromatography – flame ionization detector (GC-FID)

GC system equipped with an FID detector (Agilent 7890B), injector (7683B), DB-5MS column (15.0 m, inner diameter 0.25 mm, and internal coating 0.25 μm; Agilent Technologies, USA); injector temperature, 250° C; oven temperature, 155°C for 1 min, 25°C/min up to 290°C and then at 290°C for 3.5 min; split ratio 1:25.

2.3 Thin Layer Chromatography (TLC)

TLC was performed using silica gel 60 F254 coated aluminum plates (Merck, Germany). Two drops of ethanol were added to each sample, and the samples were then applied to the plates using glass capillaries. Ethanol was spotted as a control. Each plate was developed using the following mobile phases: dioxane/xylenes/ethanol/ammonium hydroxide (20/15/2.5/2.5). When a run was completed, the plate was air-dried for 5 minutes and then oven dried for 10 minutes (120°C). Spots were located using ultraviolet (UV) light (254 nm). The plates were then sprayed with a ninhydrin solution (3 wt. % in acetone), air-dried for 5 minutes, and then oven dried for 10 minutes (120°C).

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2.4 Fourier transform infrared (FTIR) spectroscopy

Samples of pure cocaine, creatine, benzocaine, and mixtures of cocaine-creatine and cocaine-benzocaine were ground with KBr and compressed at a pressure of 5 tons in a Specac SA12 Millenium (England). FTIR spectra were recorded by ThermoFisher Nicolet 380 (USA) with 32 scans and spectral resolution of 7.7 cm^{-1}. Data were analyzed using Omnic 7.3 software.

3 Results and discussion

Each pool was prepared by mixing material from several seized units, and classified by type of material: powder, tablets, liquid, blotter tabs, suspect plant-derived material or suspect plant-derived substrate (suspected of being sprayed with an illicit drug). The injection conditions are shown in Table 1. The injection was configured for the category of material, by applying injection programs that are commonly used in the laboratory for different kinds of substances. For E.g., perforated blotter paper (tab) is usually used as a medium for LSD so the injection settings for blotter tab specimens were adjusted for LSD detection. The relative weight and quantities of components in a mixture in the initial pools that were prepared were compatible with the accepted quantities that the Narcotics Laboratory requires for GC-MS analysis. Next, we reduced the relative amount of one component and increased the relative amounts of one or more of the other components to test whether pooled samples that are compliant with the Drug Ordinance (up to 7 units) fall within the detection 'safe zone'.

As the Israeli legislation views the entire mixture of an illicit drug as illicit—including any additives such as sugar, starch, solvent—regardless of the drug's concentration in the mix (except for a few specific cases) (Dangerous Drugs Ordinance, 2021) it was sufficient to weigh each specimen before adding it to a pool and the concentration of the illicit drug was not measured. The relative composition of the mix was determined later based on the specimen's weight before analysis.

Since our specimens were taken from street drug units, which are mostly mixtures, the size of a specimen removed for analysis by GC-MS must be sufficient to ensure a quantity of drug that is higher than the instrument's limit of detection (LOD), otherwise the drug will not be identified even if it is present. Table 1 shows that in all 50 pools, the GC-MS identified all components in each mixture even when the weight ratio of the mixtures in the pool was much higher than 6:1 (according to Israeli sampling regulations the maximum sampling subset consists 7 units, thus in the extreme case in which only 1 unit consists a different substance the pool ratio will be 6:1). A good example is shown in Figure 1, which is a chromatogram of a pool composed of a mixture of 0.0226 g cocaine and 0.0003 g MMC. The two illicit components in the mixture were easily identifiable at mix weight ratio of 75.

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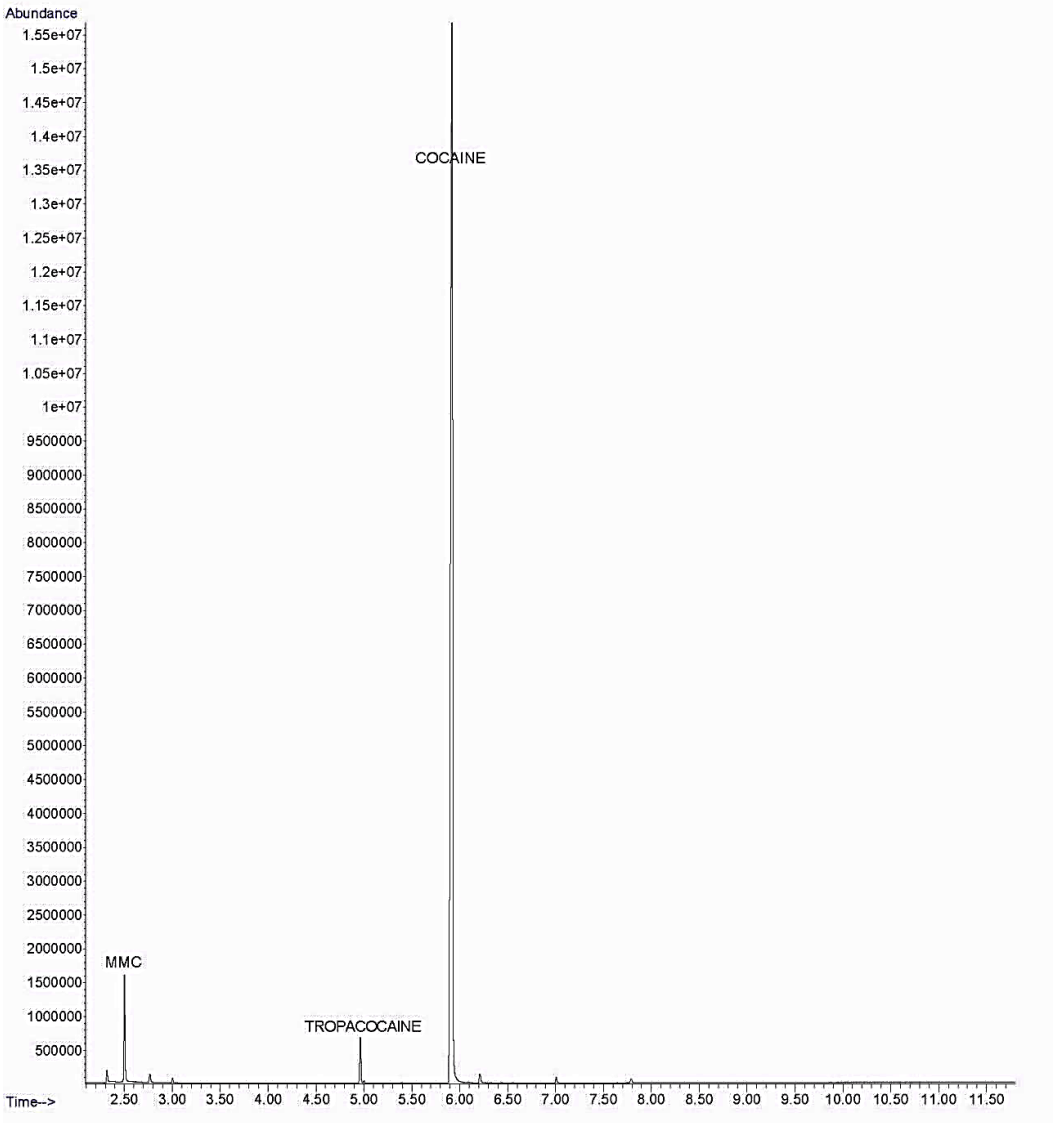

Figure 1: *GC chromatogram of a pooled sample created by pooling specimens of cocaine and MMC street units*

The differences seen in the peaks reflect the differences between the forms of the substances that were seized. Powders typically have higher drug content, and the exact content depends on the substance and on individual differences in mixing practices between distributors. Highly potent drugs are commonly distributed in tablet form, as solution soaked into blotting paper, or sprayed on plant-derived media. The percent weight of the drug in such mixes is very low. For example, LSD, a highly potent drug, is dissolved and soaked into blotting paper or diluted in solution to very low concentrations. The only pool which produced a partial identification (i.e. LSD was not identified) was a mixture of six drops of ketamine solution and one drop of LSD (Pool no. 4 in Table 1).

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Drugs in tablet form are either formulated by illicit drug distributors for use as a psychoactive substance (e.g. MDMA), or are pharmaceutical industry medicinal products in which case they contain a controlled amount of active substance. The latter (e.g. amphetamine) have even lower concentrations of active substance. The pool shown in Figure 2 contained 23.6 mg methamphetamine, 2.2 mg 2C-B, 6.3 mg MMC, 11.5 mg amphetamine, 23.2 mg MDMA, and 2.5 mg cocaine. The peaks for m-MMC and cocaine were higher than the amphetamine peak despite their lower relative weight (55% and 22%, respectively). An important note about pool sampling is that every peak on the chromatogram, however small, must be noted and identified. This is because in addition to the dominant peak, there could still be a substance of interest in any of the lower peaks (this is not the case with the regular un-pooled specimen).

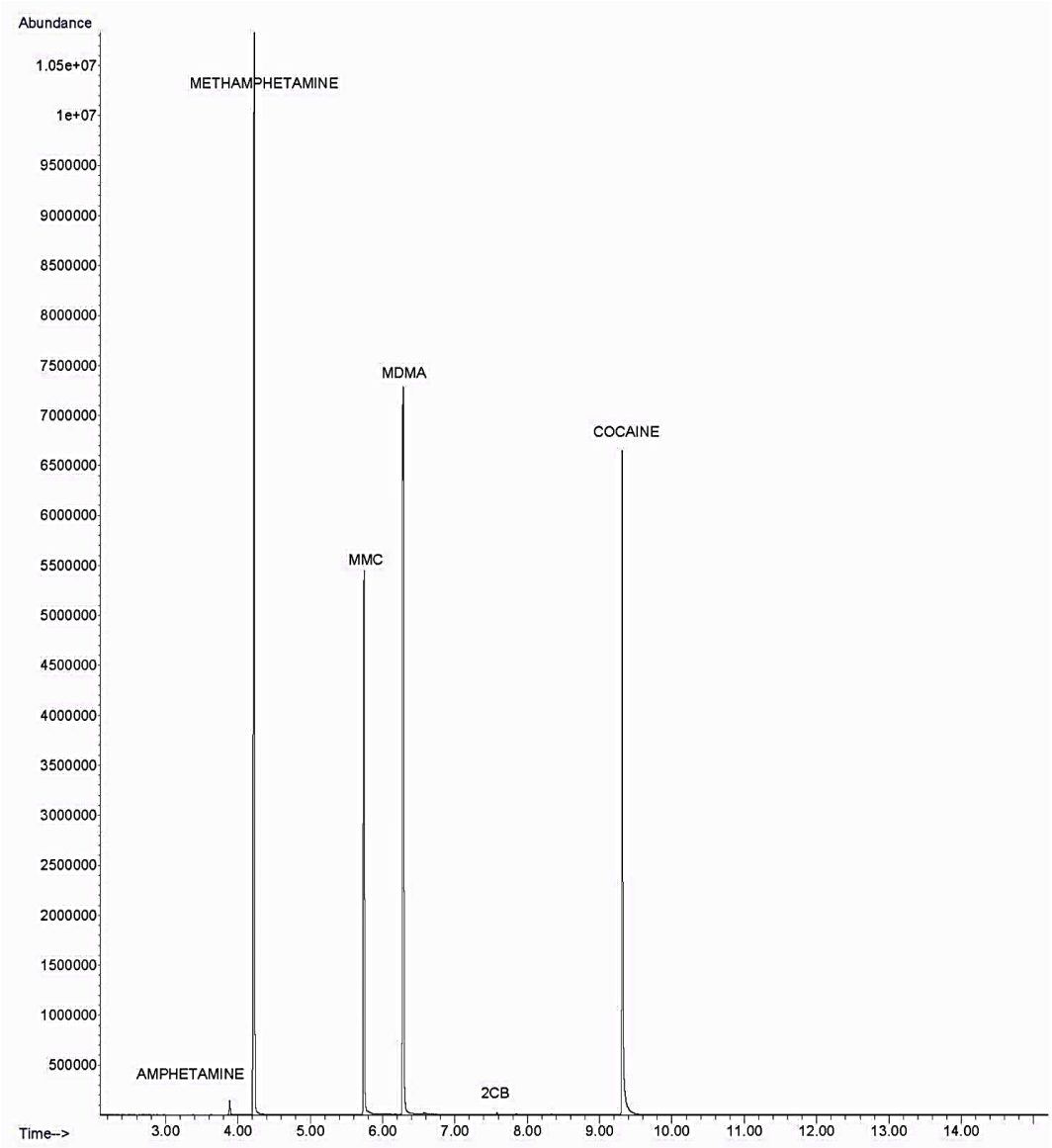

Figure 2: *GC chromatogram of pooled sample created by pooling specimens of amphetamine, methamphetamine, MMC, MDMA, 2C-B and cocaine street units*

In certain cases, pooling can produce an inconclusive result. The following examples demonstrate such cases and how they can be resolved:

3.1 Multiple substances identified in the pool:

When a GC-MS chromatogram shows two or more peaks the forensic expert should ask if this is because there are multiple substances in each of the units in the pool—each unit containing an identical mixture of substances—or if it is because each of the units placed in the pool contains a different mixture of substances. This question can be answered by analyzing each of the units separately to confirm whether or not the units are uniform in content. For example, in an exhibit of 55 units that were similar in appearance, seven units were randomly selected and pooled for analysis by GC-MS. GC-MS analysis of the pooled seven units showed a mix of cocaine and benzocaine. There are two possible explanations for this result—either all the units contain both cocaine and benzocaine, or some of the units contain one and the rest contain the other. A follow-up analysis by GC , TLC or FTIR can settle this question: If each of the pooled specimens contains a mixture of cocaine and benzocaine we can expect (1) two peaks in the individual GC analysis of each specimen at the retention times for cocaine and benzocaine (see Figure 3A), (2) two spots in TLC—one at the RF for cocaine and the other for benzocaine (see Figure 3B), and (3) the IR spectrum is expected to show an identical mix of the two substances in each of the specimens (see Figure 3C).

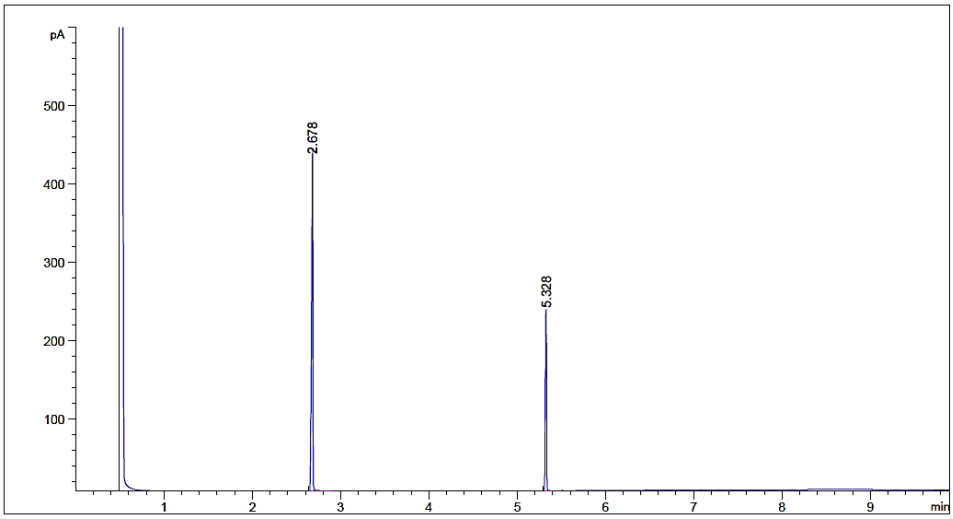

Figure 3A: *GC chromatogram of a sample created by pooling pure specimens of cocaine and benzocaine*

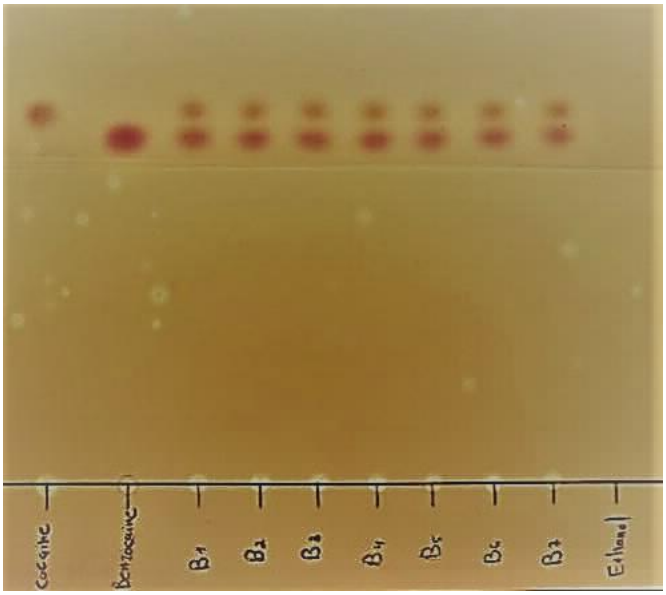

Figure 3B: *TLC plate of a sample created by pooling pure specimens of cocaine and benzocaine*

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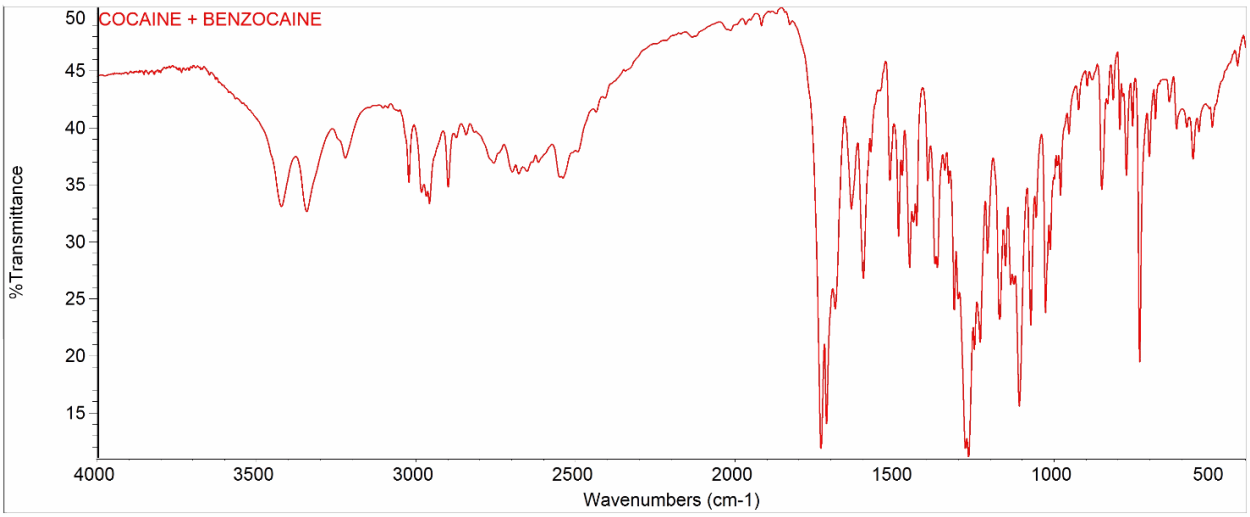

Figure 3C: *FTIR spectrum of a sample created by pooling pure specimens of cocaine and benzocaine*

If some units in the subset contain cocaine and others contain benzocaine, we can expect GC analysis to show peaks at different retention times (see Figure 4A), TLC to show a spot at a different RF (see Figure 4B), and IR to show a different spectrum—some units will look like cocaine and others like benzocaine (see Figure 4C).

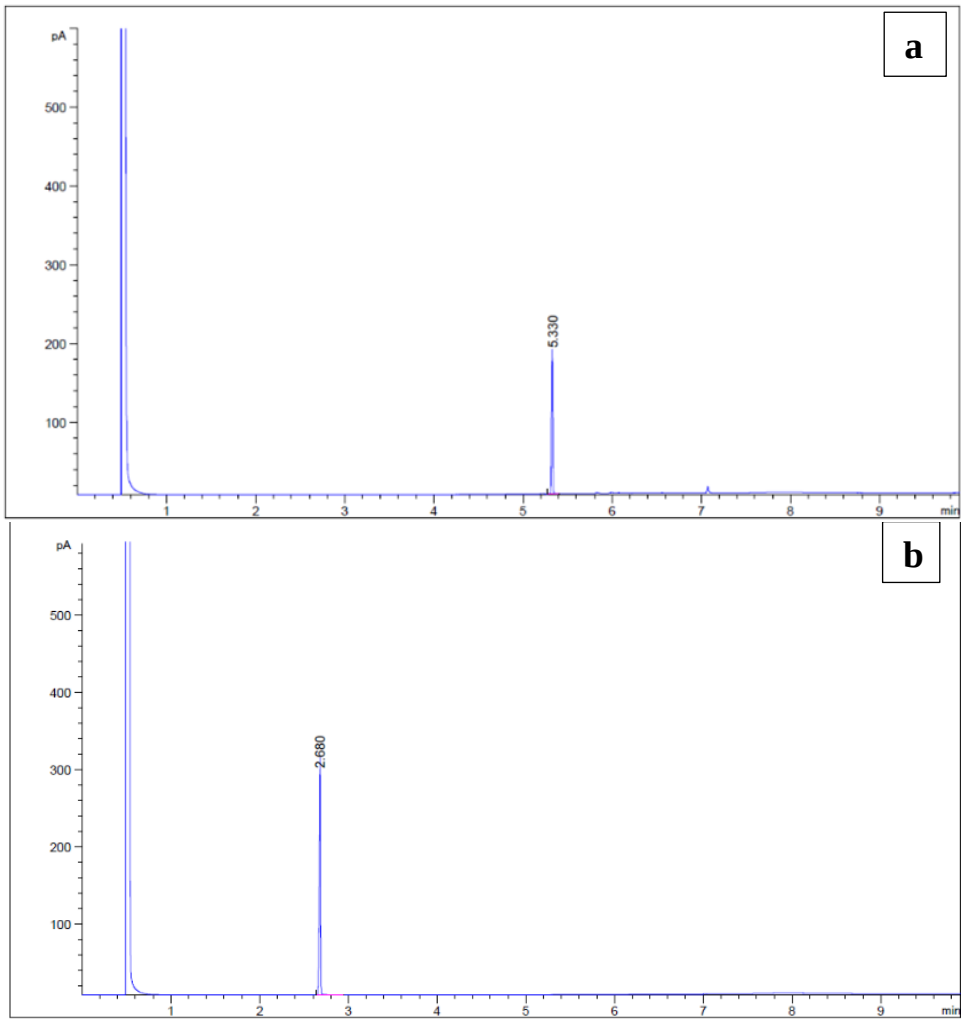

Figure 4A*: GC chromatogram of samples containing either pure cocaine (a) or pure benzocaine (b).*

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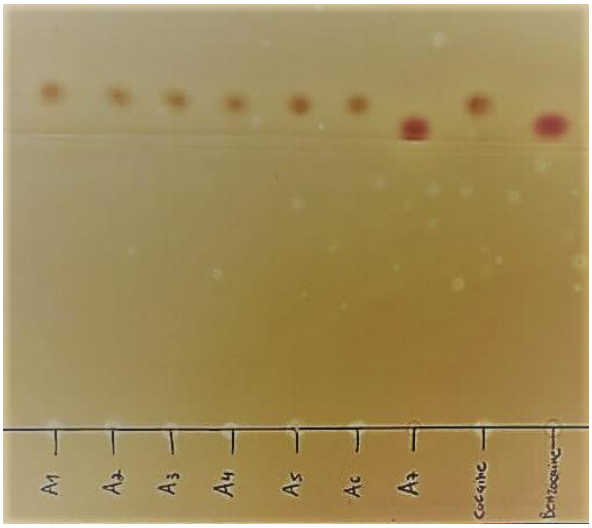
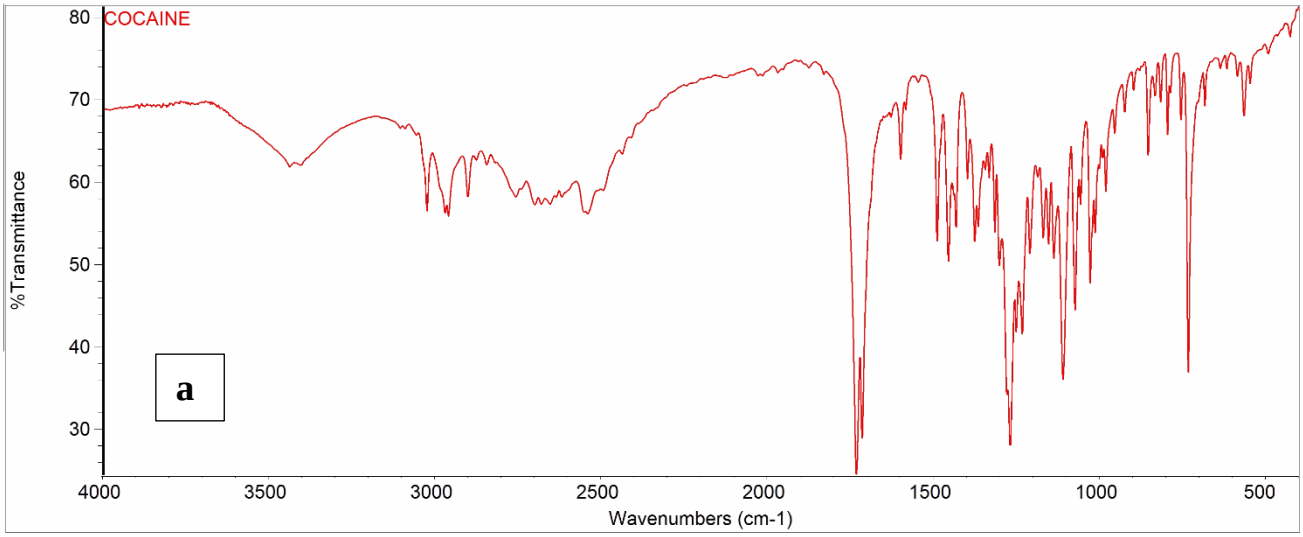


Figure 4B: *TLC plate of samples containing pure specimens of either cocaine (A1-6) or benzocaine (A7)*



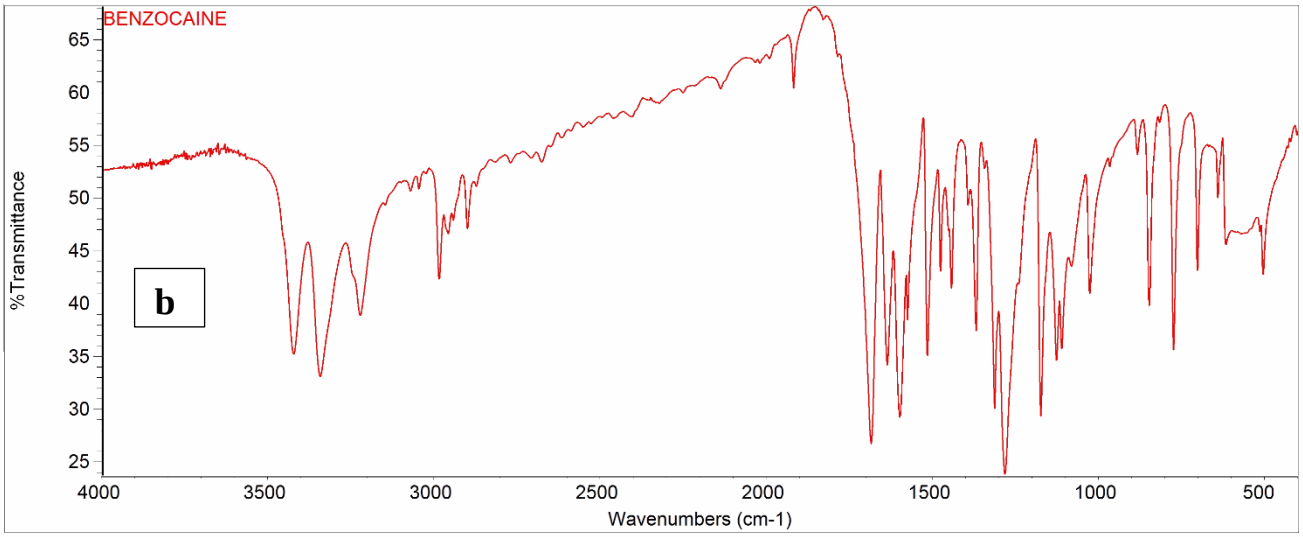


Figure 4C: *FTIR spectra of samples containing either pure cocaine (a) or pure benzocaine (b).*

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3.2 Not all substances in the pool are identified:

When a GC-MS chromatogram shows only one peak (for one substance) it is possible that some of the specimens in the subset contain a substance that is not visible on the chromatogram. This could be because some units contained a substance that is insoluble so it did not pass through the column, or is adsorbed by the column, or does run through the column but is out of range under the specific conditions used. For example, in an exhibit of 55 units that are similar in appearance, seven units were randomly selected and pooled for analysis by GC-MS. GC-MS analysis of the pooled seven units showed a single peak for cocaine. This result is still insufficient to confirm there is cocaine in all seven units. It is possible that some contained cocaine, and others contained creatine which is visually similar to cocaine. Unlike cocaine, creatine does not dissolve in dichloromethane (the solvent used in the analysis), so even if a specimen contained creatine it would still only show the peak for cocaine. A complementary analysis is required to distinguish between the two situations. In GC—when injecting a specimen from each unit separately—specimens that only contain a salt, or a sugar, or some other substance that is insoluble in the solvent used will not produce any peak. So GC can be used to find if there is a difference between the samples (see Figure 5A). If all the units are a cocaine-creatine mixture, each unit will show a single peak (for cocaine) (see Figure 5B).

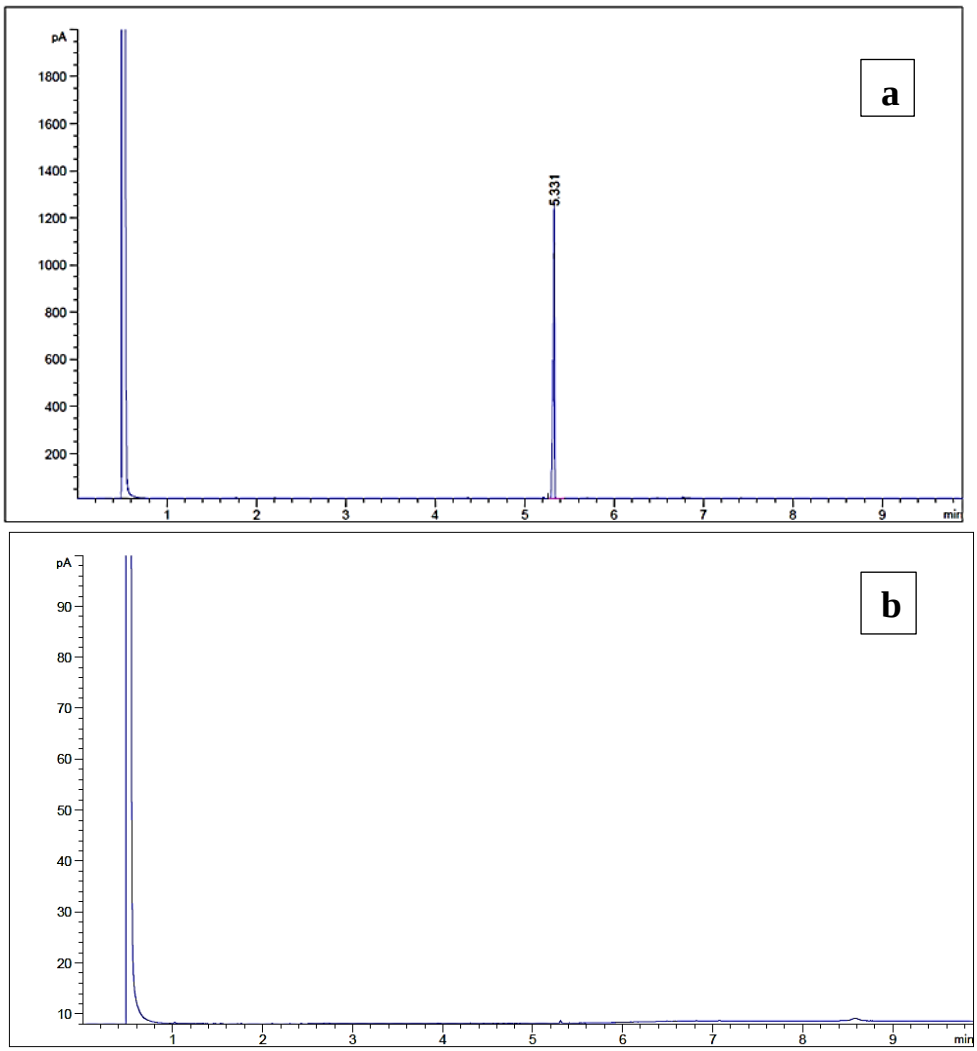

Figure 5A: *GC chromatogram of samples containing either pure cocaine (a) or pure creatine (b).*

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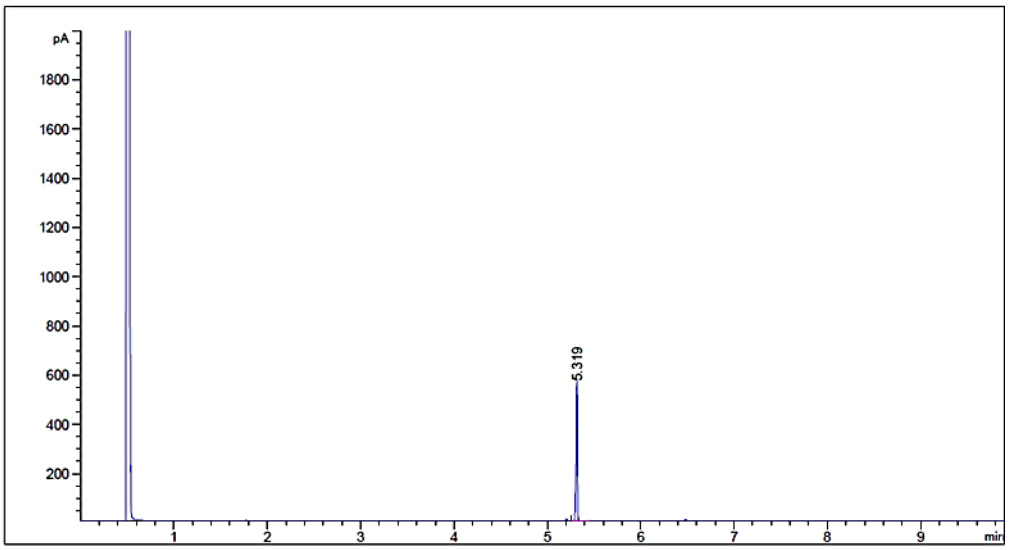
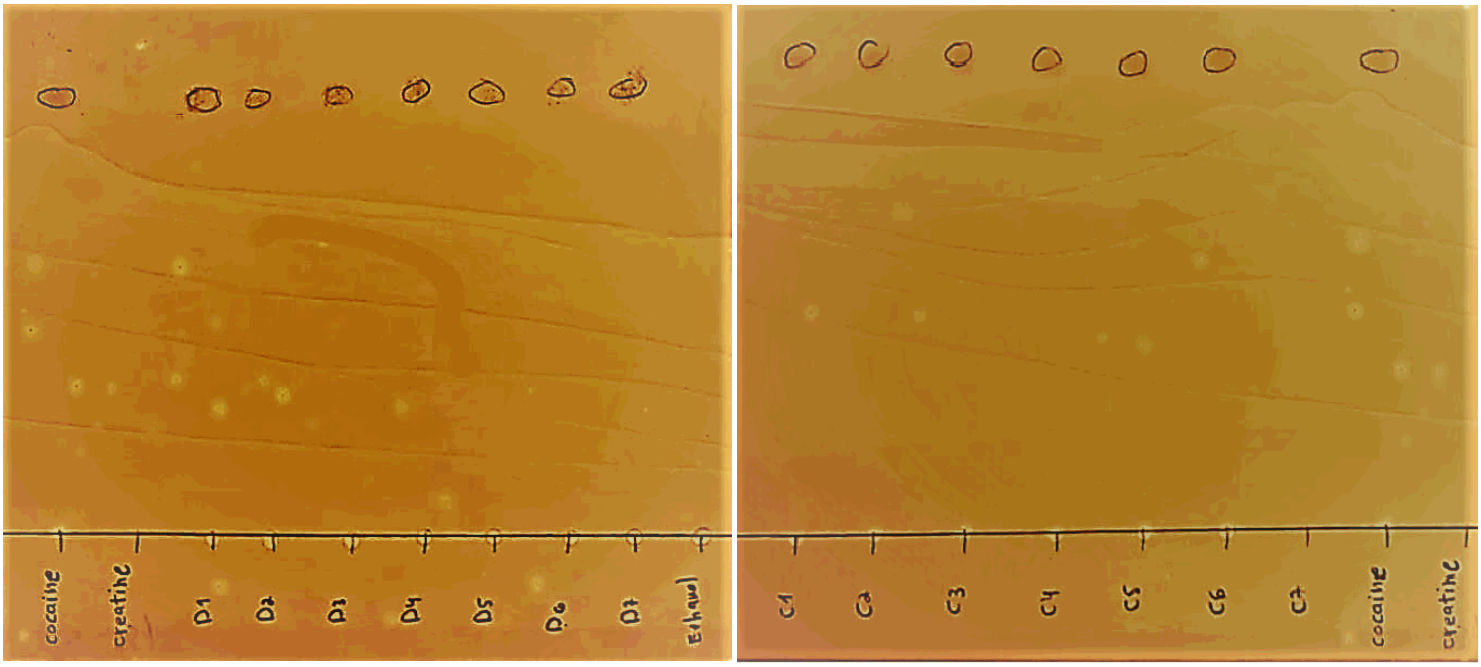


Figure 5B: *GC chromatogram of sample created by pooling pure specimens of cocaine and creatine*

Although this analysis will not reveal that creatine has been used as an additive, it is still sufficient to establish that all units contain an illicit drug. Also in TLC, if the units are different, units containing only an insoluble substance or one that is adsorbed on the silica will not produce a visible spot (see Figure 6A), but if all units contain the same mix of cocaine and creatine each unit will produce a colored spot indicating cocaine (see Figure 6B).



A **B**

Figure 6: *TLC plate of samples created by pooling pure specimens of cocaine and creatine (A) and pure specimens of either cocaine or creatine (B).*

If using FTIR as the secondary analysis, the spectrum obtained separately for each unit will be the same for all if the units contain the same mix (Figure 7A), and different if the samples are different (Figure 7B1,7B2).

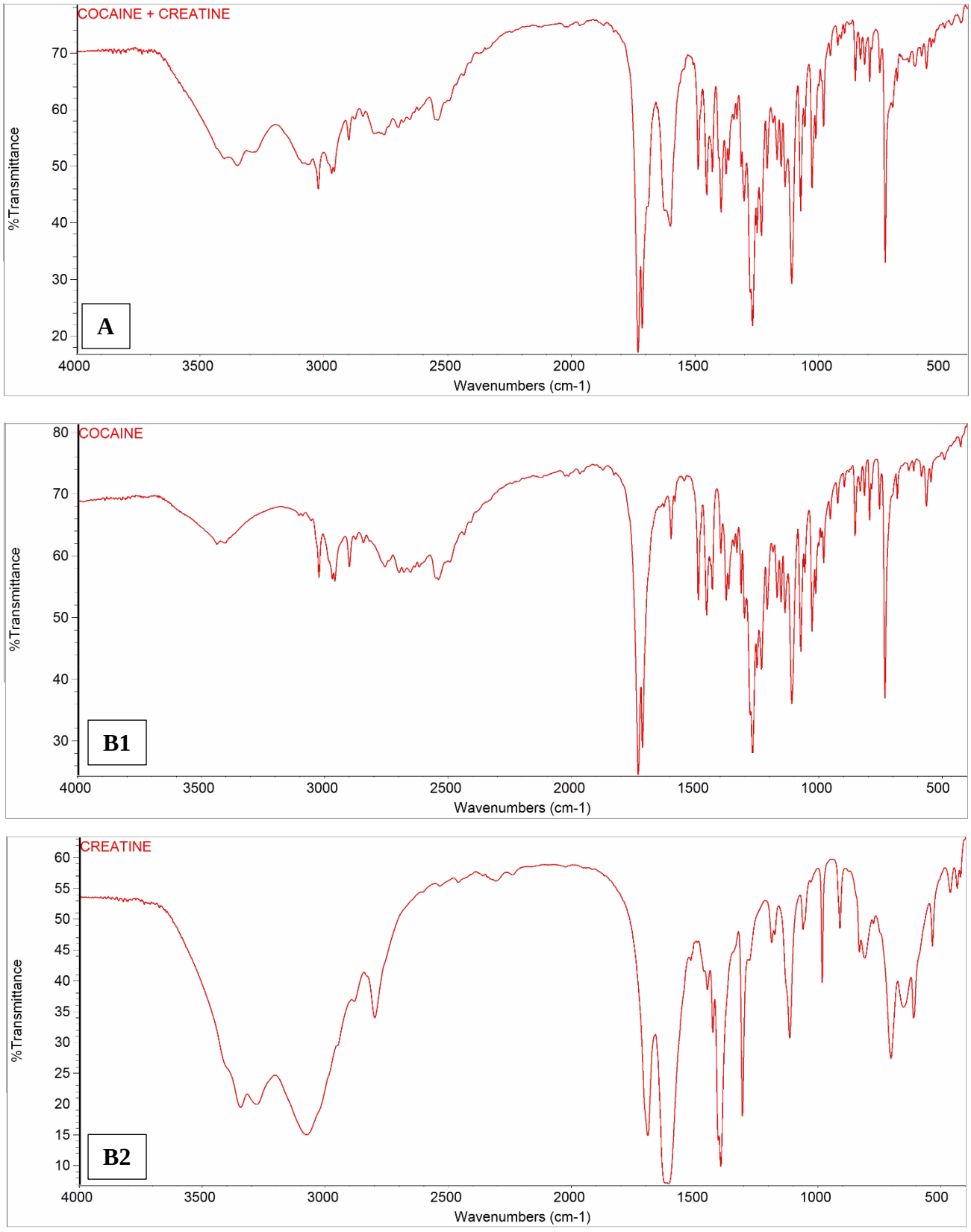


Figure 7: *FTIR spectrum of samples created by pooling pure specimens of cocaine and creatine (A) and of pure specimens of either cocaine or creatine (B1 and B2).*

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3.3 Several substances with the same retention time:

When a GC-MS chromatogram of a pooled sample shows only one peak (supposedly for one substance) a possible scenario could be a case of different substances with the same retention time exiting the column at the same time. In cases like this, MS analysis can be used to distinguish between a single-substance peak and a multiple-substance single peak that is the result of similar retention times. If the peaks of the two substances do not overlap perfectly, we may elicit a mass spectrum from the peak's shoulders to obtain a separate spectrum for each of the substances. Even with full overlap—in which a mixed spectrum of the two substances is obtained—if one of the substances is a known drug, its spectrum can be deducted and the second substance's spectrum can then be identified. Figure 8 shows how two peaks that almost merge represent two types of synthetic cannabinoids, MDMB-BUTINACA and XLR-11. When applying the routine injection conditions for samples of this sort (15M, 190-290°C (25D/M), injector temperature 220°C), their retention time is almost the same and they are visible as a nearly single peak. Until recently, exhibits sent to the lab contained only one of these substances either alone or with other drugs that were easily differentiated under standard lab injection practices. However, in the last few months the lab has been receiving exhibits that contain the two drugs together.

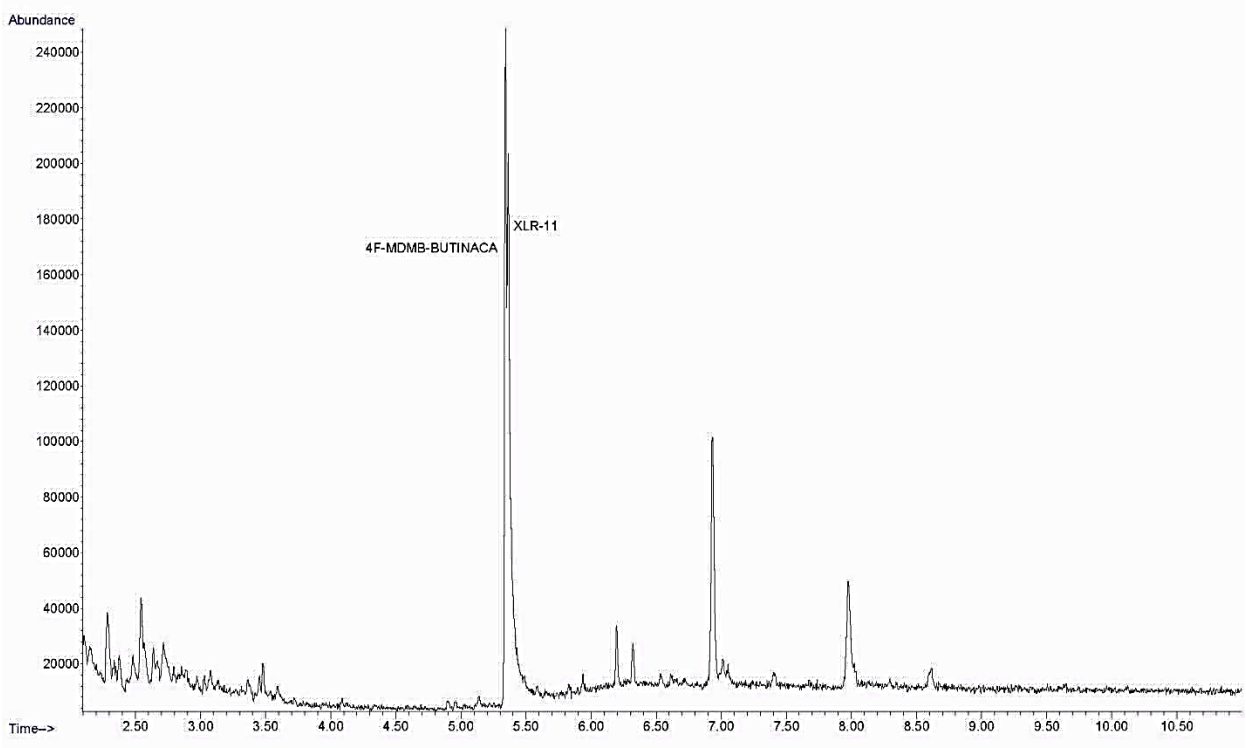

Figure 8: *GC chromatogram of a mixture sample created by pooling specimens of MDMB-BUTINACA and XLR-11 street units.*

The mass spectrum taken from the peak shoulders showed only one of the two substances each time (Fig. 9A – 4F-MDMB-BUTINACA, Fig. 9B XLR-11).

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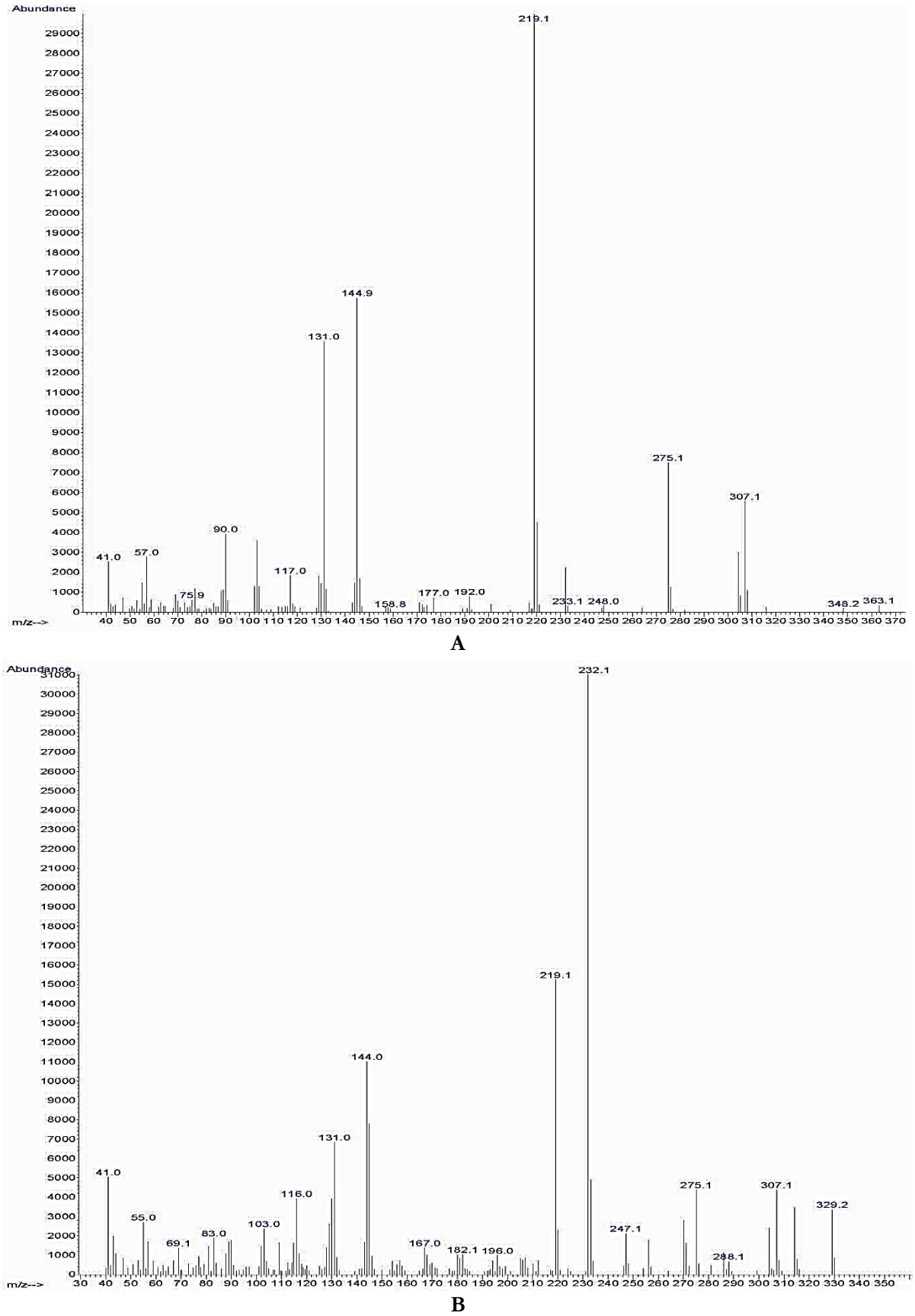

Figure 9: *Electron ionization mass spectra (EI-MS) of MDMB-BUTINACA (A) and XLR-11 (B) street units.*

The comparative methods of analysis are blind in cases like this one. Both GC and TLC will only show one peak or one spot, respectively, if the analyses are run under conditions that are unsuited to separation of the two substances. FTIR cannot be used to identify synthetic cannabinoids sprayed on a plant-derived medium. Analysis by GC-MS of a pooled sample will reveal the merge and the need to test each specimen separately after changing the injection conditions to allow separation of the substances.

4 Conclusions

The Israeli National Narcotics Laboratory requires two independent tests to identify a suspect substance as an illicit drug. This study proposes a method for reducing the laboratory's identification workload by using pooled samples. Pooling can be applied when an exhibit contains multiple units of similar color, shape and size, such that the selection of a subset of units for analysis is legally justified. The method was tested on specimens that required GC-MS analysis, which is the Narcotics Laboratory's most intensively used method of identification.

With one exception, GC-MS analysis of pooled samples identified the drugs present in each pool. Although the weight ratios used to create the pools in this study were higher than the maximum practical ratio of 6:1, it was still possible to identify minority substances. This test was repeated with 50 samples containing different seized substance mixtures. The results prove that the concentrations of active substances in the street drug units are sufficient for their identification in pools that are limited to the maximum of a 7-unit sampling subset.

Although the required complementary analysis by a second method proved to be very reliable with our available methods (GC, TLC or FTIR) it was clear that in certain cases these methods cannot adequately provide a confirmation. Such is the case when the sample contains two substances with identical GC retention time. However, we were able to demonstrate that analysis of the pooled sample by GC-MS can differentiate the 2 MS peaks and at the very least reveal the presence of the two substances in the mixture. Thus, we conclude that applying the specimen pooling strategy for GC-MS analysis will not only reduce material waste and instrument wear, but more importantly it can save up to 86% laboratory time, which means rapid substance identification and prompt laboratory reports.

5 Declarations

5.1 Competing Interests

The author declares no current or potential competing interests.

5.2 Publisher's Note

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