Forensic Science International 232 (2013) e24–e27

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Case report

Chemical profiling of different hashish seizures by gas chromatography–mass spectrometry and statistical methodology: A case report

Liv Cadola, Julian Broséus *, Pierre Esseiva

Institut de Police Scientifique, School of Criminal Sciences, Batochime, University of Lausanne, 1015 Lausanne-Dorigny, Switzerland

ARTICLE INFO

Article history:
Received 11 June 2012
Received in revised form 17 May 2013
Accepted 9 August 2013
Available online 19 August 2013

Keywords:
Cannabis
Cannabis resin
Illicit drugs chemical profiling
Gas chromatography–mass spectrometry
Similarity measures

ABSTRACT

Limited information is available regarding the methodology required to characterize hashish seizures for assessing the presence or the absence of a chemical link between two seizures. This casework report presents the methodology applied for assessing that two different police seizures were coming from the same block before this latter one was split.

The chemical signature was extracted using GC–MS analysis and the implemented methodology consists in a study of intra- and inter-variability distributions based on the measurement of the chemical profiles similarity using a number of hashish seizures and the calculation of the Pearson correlation coefficient. Different statistical scenarios (i.e., a combination of data pretreatment techniques and selection of target compounds) were tested to find the most discriminating one.

Seven compounds showing high discrimination capabilities were selected on which a specific statistical data pretreatment was applied. Based on the results, the statistical model built for comparing the hashish seizures leads to low error rates. Therefore, the implemented methodology is suitable for the chemical profiling of hashish seizures.

1. Introduction

This paper presents the development of a methodology for the comparison of hashish samples for chemical profiling. This methodology was developed to answer a question raised by a Prosecutor concerning a criminal case: *Can we infer that two different police seizures of hashish come from one unique block?* In other words, is it possible to establish that these two seizures of hashish were previously part of the same block and thus also differentiate seizures coming from different blocks of hashish?

The majority of the literature available focuses on the extraction of the chemical profile of cannabis seizures [1–16] but limited information is available regarding the methodology developed for interpreting hashish profiles showing similar characteristics [17–24].

This study uses methodology already applied to other illicit drugs such as cocaine and heroin. The method is based on the measurement of chemical profile similarity using Pearson correlation and the study of the distribution of intra (samples coming from the same source) and inter-variability (samples coming from different sources) [25]. In our case the source level refers to one hashish block which has been divided.

This article firstly describes the case circumstances and secondly the methodology applied to solve the problem raised during the police investigation. The sampling, analytical method and statistical model used are then explained. Finally, the main results are presented and discussed.

2. Case circumstances

During a police investigation, a hashish block was found in the flat of a suspect. After questioning, police suspected a connection between this case and a hashish dealer. Police seized one block of hashish at the flat of the dealer. Law enforcement authorities asked our laboratory to answer the following question: *Do the hashish seizures found at the flat of the suspect and the flat of the dealer come from one block?*

3. Materials, analytical methods and software

3.1. Sampling

The statistical model was built using 2 hashish blocks seized in this case and 8 others coming from different police seizures (i.e., different police investigations). Intra-variability was established by analyzing 4 replicas for each of the 10 hashish blocks and inter-variability was estimated by comparing the 8 hashish seizures known not to be linked.

* Corresponding author. Tel.: +41 21 692 46 46; fax: +41 21 692 46 05.
E-mail address: julian.broseus@unil.ch (J. Broséus).

0379-0738/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.forsciint.2013.08.014

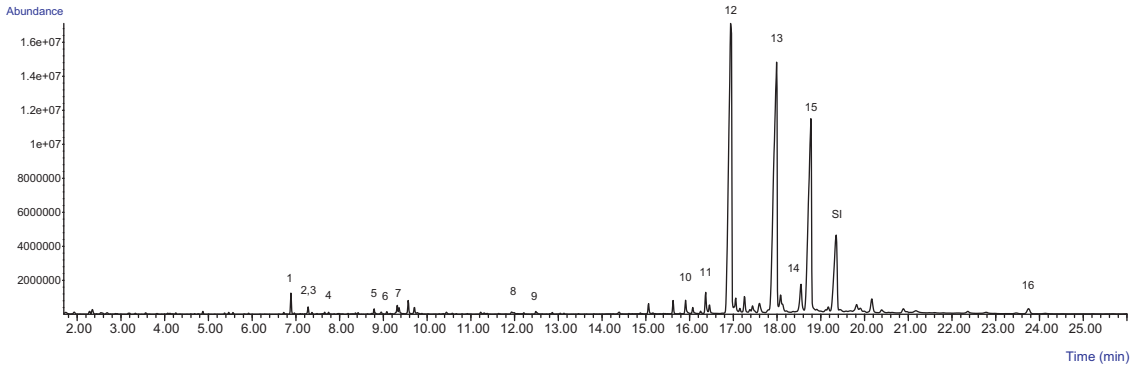

Fig. 1. TIC chromatogram of a hashish sample. Number on the chromatogram refers to Table 1.

3.2. Analytical methods

In order to extract a chemical profile of each hashish specimen, the approach proposed by Broséus et al. [22] for Cannabis leaves was selected. Due to a difference in raw material, some preliminary tests were performed to check the consistency of the analytical method. The sample preparation consisted of grinding the hashish to a fine powder and then drying it for 24 h at room temperature. Four 50 mg replica for each block were extracted using the protocol described in Ref. [22]. An internal standard of 5 mL of hexane containing 35 mg squalane per 100 mL was added. The tube containing the sample and the extraction solution were placed in an ultrasonic water bath for 15 min and then in a rotating beater for 60 min. After centrifugation (4 min) 1 mL of the supernatant was transferred to a vial. Each sample was then individually analyzed by GC–MS in order to determine its chemical profile.

An Agilent HP 6890 gas chromatograph interfaced with an Agilent 5973 mass selective detector was used for the analysis. Separation was accomplished on a HP-5ms capillary column (30 m length, 0.25 mm i.d. and 0.25 μm film thickness). Injections were carried out in split mode using a general-purpose split/splitless liner packed with glass wool. The temperature program started at 100 °C, increased to 260 °C (at 10 °C/min) and held for 10 min for a total run of 26 min. 2 μL of each sample was injected with helium as the carrier gas (constant flow mode, 1 mL/min) using a split ratio of 1:10. Temperatures applied were 280 °C for the injector, 250 °C for the transfer line, 230 °C for the ion source and 150 °C for the quadrupole. Electron multiplier voltage was fixed to 1200 V. Data was acquired in full scan mode (10–450 *m/z* mass range) with a sampling rate of 3 (1.77 scans/s) and was analyzed using MSD Enhanced ChemStation v.D.02.00.275 (Agilent Technologies).

Compounds found were characterized using a mass spectrum database (NIST05) and data from the literature [1–14].

The THC standard was purchased from Lipomed AG and the hexane and squalane from Sigma Aldrich.

3.3. Target compounds for the determination of a hashish chemical profile

In this case the attention was focused on the overall set of compounds present in detectable amounts in the hashish block. Specifically, compounds present in all samples and in relatively high quantity were selected. Fig. 1 shows a typical chromatogram obtained for a hashish analysis.

Table 1
List of the targeted ions for each compound extracted for the determination of a hashish chemical profile.

Peak number/compound name	RRT (min)	**Target ion**, qualifiers (*m/z*)
1. Caryophyllene	0.356	**93**,133,69,79
2. Trans alpha bergamotene	0.359	**119**,93,69,41
3. Alpha-caryophyllene	0.376	**93**,80,121,147
4. Beta-selinene	0.396	**105**,93,161,79
5. Caryophyllene oxyde	0.454	**79**,93,43,69
6. Humulene epoxyde	0.469	**109**,138,96,67
7. Caryophylla-3	0.484	**136**,91,69,41
8. 4,4,8 trimethyltricyclo [6.3.1.0(1,5)]dodecane-2, 9 diol	0.617	**164**,135,107,93
9. n-Hexadecanoic acid	0.654	**73**,60,43,129
10. Cannabichromene	0.846	**231**,174,314,299
11. Cannabivarin	0.850	**267**,282,238,223
12. Cannabidiol	0.876	**231**,174,314,246
13. Δ^9-THC	0.927	**299**,314,231,271
14. Cannabigerol	0.957	**93**,231,123,316
15. Cannabinol	0.968	**295**,238,310,223
16. Nonacosane	1.227	**57**,71,85,43

Table 1 summarizes target compounds with their respective retention times (relative to the internal standard) and their specific target ions and qualifiers.

3.4. Data statistical treatment

Numerous statistical scenarios including different normalization techniques, choice of variables and data pretreatment techniques were tested in order to optimize the discriminating power of the approach. Two types of normalization were used: internal standard normalization (the area of each variable is divided by the area of the internal standard) and total sum normalization (the area of each variable is divided by the sum of areas of all the compounds). Two pretreatments (the square root and the log of each normalized value) were also tested in order to reduce the influence of larger peaks. This ensures that all the variables are on a comparable scale and can contribute equally to the differentiation of the two populations. The combination of total sum normalization followed by a log pretreatment based on a selection of variables described below produced the best results and will now be presented in more detail. Data processing was performed using Microsoft Excel 2007, R version 2.11.1 and The Unscrambler X 10.1.

4. Results and discussion

4.1. Variable selection

For each target compound the areas under the peaks of target ions found in the chromatograms were integrated. Area values were normalized as described above and boxplots were plotted in order to select the most relevant variables. The selected variables are those which show good repeatability when analyzing replicas of the same material and large variability (discrimination) when analyzing samples from different materials (see Figs. 2 and 3). Following these criteria, 7 variables were selected: Cannabichromene, Cannabivarin, Cannabidiol, Δ^9-THC, Cannabigerol, Cannabinol and Nonacosane.

4.2. Model performance evaluation

To choose the best performing model the procedure presented in Esseiva et al. [25] was followed. First the intra- and the inter-variability were modeled for each scenario. The distribution of the intra-variability was evaluated by calculating the Pearson correlation between replicas of samples coming from the same block (60 comparisons). The inter-variability was evaluated by modeling the distribution of the Pearson correlation obtained by comparing pairs of samples from unrelated seizures (336 comparisons).

ROC curves were measured in order to evaluate the performance of the different scenarios. ROC curves allow for the measurement of the false positive (FP) rate and true positive (TP) rate; two factors which are essential for defining a threshold. ROC curve methodology graphically represents the overlapping zone between linked (intra-variability) and non-linked (inter-variability) sample distributions. This overlapping rate is calculated using the Area Under the Curve (AUC), the best model being the one with the biggest AUC (i.e., AUC equals to 100). By maximizing