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ScienceDirect
Forensic Science International 177 (2008) 11–16
Forensic Science International
www.elsevier.com/locate/forsciint

Drug intelligence based on MDMA tablets data I. Organic impurities profiling

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Received 23 May 2007; received in revised form 20 September 2007; accepted 5 October 2007
Available online 19 November 2007

Abstract

The main objectives of the European project ''Collaborative Harmonisation of Methods for Profiling of Amphetamine Type Stimulants'' (CHAMP) funded by the sixth framework programme of the European Commission, included the harmonisation of MDMA profiling methods and the creation of a common database in a drug intelligence perspective. In the preliminary stages of this project, the participating laboratories analysed the physical characteristics, the chemical composition and the organic impurities of MDMA tablets, using the previously harmonised methods. The aim of the present work was to apply statistical treatments to the recorded data in order to evaluate their potential in the fight against drug trafficking. Comparable working procedures were applied on the different types of data. The first part of this article deals with organic impurities data, while the second part focuses on the potential of the physical characteristics.

Organic impurities data were recorded by a harmonised Gas Chromatography/Mass Spectrometry (GC/MS) method previously developed. Statistical analysis provided a selection of pertinent variables among the 46 organic impurities identified in the chromatograms. Correlation coefficients were used to yield separation between populations of samples coming from the same synthesis batch and samples coming from different batches. It was shown that correlation measurements based on Pearson and cosine functions applied to the data pre-treated by normalisation to the sum of peak responses followed by the square root provided an excellent discrimination between the two populations. The statistical methods applied to organic impurities profiles proved to be excellent techniques to differentiate samples from different batches and to highlight operational links between samples.

Keywords: Drug profiling; Statistics; MDMA; Ecstasy; Organic impurities; GC/MS

1. Introduction

Since 1998, a strong increase of amphetamine type stimulants seizures has been recorded in the world and it therefore has become a major issue in the fight against drug trafficking in Europe [1]. While amphetamine type stimulants, and more particularly MDMA (3,4-methylenedioxymethamphetamine), are also produced in regions other than Europe, The Netherlands and Belgium remain the most important sources. Moreover, most of the MDMA tablets produced are trafficked in Europe, followed by North America and Oceania [2].

The main objectives of the European project Collaborative Harmonisation of Methods for Profiling of Amphetamine Type Stimulants (CHAMP) funded by the sixth framework programme (contract no. 502126) of the European Commission

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doi:10.1016/j.forsciint.2007.10.001

included the harmonisation of profiling methods and the creation of a common database in a drug intelligence perspective. Drug profiling represents the extraction of physical and/or chemical profiles from drug samples to collect strategic and operational intelligence [3,4]. A profile was defined as a set of specific characteristics selected as giving information on typical clandestine production stages.

1.1. MDMA tablets production

MDMA production confers physical and chemical properties to illicit drug. Two main stages can be distinguished in the production of MDMA tablets, also called Ecstasy. The first stage is the synthesis of the active substance. Several recipes and synthesis routes may be carried out, however the most common in Europe is reductive amination [5].

The chemical profile of the synthesized product is drastically affected by manufacturing parameters such as the synthesis pathway, the chemicals, or the quality of the precursors (it is also not rare that different synthesized batches are mixed together before the tabletting process). These parameters as well as the fact that the crystallisation, the drying and the seeding processes are not carried out in a controlled manner all contribute to the high variability of the final product. In this research, we considered that the final mixture prepared for the tabletting had a specific organic chemical profile, define as pre-tabletting batch (pre-TB).

In a second stage, the mixed powder is then compressed using a tabletting machine with interchangeable punches for the purpose of printing a logo and/or a score on the surface of the tablets [6]. The diameter is generally a fixed parameter for a given tabletting machine, while the thickness depends on the quantity of powder per tablet and the set pressure of the punches. Once compressed, the tablet cannot be altered anymore and it keeps its characteristics throughout the following trafficking stages until it is consumed or seized by police services. A post-tabletting batch (post-TB) is defined in this study as a set of tablets produced by a specific tabletting machine with given settings.

The different production stages (pre-B and post-TB) may be carried out at different locations and yield independent sets of characteristics. For this reason, these characteristics were analysed separately in order to investigate their individualizing potential of samples coming from a same pre-TB (first part) or post-TB (second part). When combined, these two sources of information bring an added value in the comparison process.

The obtained results can also be integrated in an operational or strategic drug intelligence perspective [3] regarding four possible scenarios (Fig. 1) that can be encountered in the comparison of illicit tablets samples. On an operational level, samples showing identical profiles may highlight connections between separate caseworks and provide links between ongoing inquiries. Strategic intelligence aims to obtain information in a political context and may highlight traffic tendencies among different countries. Profiles may then be characteristic of a distribution network, or a specific manufacturing process.

1.2. Hypotheses

The origin of the Ecstasy seized by police services is generally unknown, with the exception of dismantled clandestine laboratories. We therefore had to define the following hypotheses for the samples used in our study: we assumed that samples coming from one seizure and which could not be differentiated belonged to the same pre-TB and, respectively, samples coming from different and unrelated seizures were considered as coming from different pre-TB.

1.3. Workplan

A profiling method developed by Van Deursen et al. [7] for the analysis of organic impurities formed during the synthesis of MDMA was further harmonised among the partner

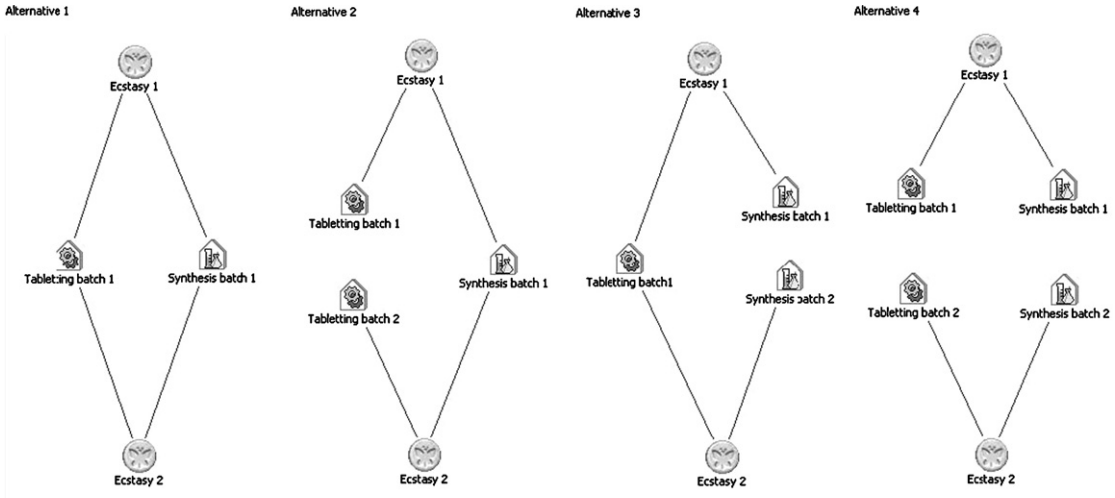

Fig. 1. There are four alternative combinations in the comparison of two samples: (1) tabletting and synthesis profiles both correspond; (2) only synthesis profiles correspond; (3) only tabletting profiles correspond; (4) neither of the profiles corresponds.

laboratories and used to collect profiling data on MDMA tablets. The potential of the analytical results will be evaluated in three main steps:

- A first set of reliable variables will be selected according to their reproducibility. Among these, a second selection will be carried out in order to highlight a subset of variables allowing the same discrimination between the samples as the first set (the number of variables is reduced in order to speed up and simplify the comparison process with a minimum loss of information).
- The degree of similarity between samples coming from the same pre-TB and samples coming from different pre-TB will be evaluated in order to determine if the populations of linked and unlinked samples can be discriminated.
- Finally, grouping of samples according to the country of seizure will be attempted to highlight potential traffic tendencies, and a search for links between samples from different countries will be performed.

2. Materials and methods

2.1. Sampling

Two types of MDMA samples were collected. The profiles of tablets of each seizure were considered homogeneous. On one hand, test samples consisted of 26 seizures originating from Finland and Germany. For each of the 26 samples, each partners received 10 g of homogenised powder (from ground tablets). The replicate analyses, performed on the test samples in four different laboratories, constituted the population of samples with corresponding profiles (same pre-TB). On the other hand, 80 street samples were collected by each laboratory among seizures made in their own country from 1996 to 2004 (Finland, The Netherlands, France and Switzerland) and constituted the population of samples (i.e. 320 samples) with different profiles (different pre-TB). Because of limited time resources, only one replicate analysis was carried out on each street sample.

2.2. Organic impurities

The samples were analysed using a Gas Chromatography/Mass Spectrometry (GC/MS) method that was developed in The Netherlands Forensic Institute (NFI). Three partner laboratories used exactly the same instrumentation, and one partner laboratory used a GC–MS from a different manufacturer. The mass spectra were recorded and the signal areas of the target ions were then integrated for each present impurity. This constituted the raw profile for a given sample. Detailed information about the method can be found in [7].

2.3. Statistical analysis

The numerical data obtained was statistically analysed with SPSS® 2000 (Mathsoft, Inc.), Microsoft® Excel (Microsoft Corporation) and The Unscrambler® 9.2 (Camo Process AS). Before any statistical treatments were done, the organic impurities target ion responses were normalised (i.e. divided by the summed area of all selected organic impurities).

The reproducibility of the replicate analysis of the test samples was calculated for each of the variables in order to carry out a first selection of target compounds. The variability of the selected variables was then determined in the population of street samples in order to achieve a second selection of variables.

A discriminant analysis was then performed to investigate the possibility of discriminating the 26 test samples (assumed to be different) on the basis of the selected sets of reliable and relevant variables. The correlation of the variables for each function was also calculated.

The square root and fourth square root were applied on the results as pre-treatments to reduce the influence of larger peaks. The correlation between the linked samples, as well as between the unlinked samples was calculated. Two correlation methods generally used in drug profiling were retained to process the normalised data: Pearson (r) [8] and square cosine [9]. The correlation coefficients were modified to fit a distance scale from 0 (maximum correlation between profiles) to 100 (minimum correlation between profiles):

$$\text{Modified Pearson} = \frac{1-r}{2} \cdot 100 \quad (1)$$

$$\text{Squared sinus} = 100 \cdot (1 - \cos^2) \quad (2)$$

In order to evaluate the separation between the distributions of linked and unlinked samples, Receiver Operating Characteristic (ROC) curves were used. ROC curves were built by plotting the true positive rate (sensitivity) as a function of the false positive rate (1 − specificity) at each correlation value. The area under the curve allows the quantification of the overlapping degree between the compared distributions. It theoretically ranges from 0.5 (distributions completely overlapped) to 1 (distributions entirely separated) [10].

A Principal Component Analysis (PCA) was carried out on all street samples to highlight potential grouping among and between countries.

3. Results and discussion

3.1. Selection of target compounds

The selection of variables was made from 46 organic impurities identified in the chromatograms. The reliability of these target compounds was tested on the test samples. A first selection was based on the reproducibility and 32 variables were selected (Table 1) according to RSD values under 20% [11]. A discriminant analysis was applied to observe if discrimination between the 26 test samples could be achieved by using these target compounds. The correct classification rate was 100%. No observation was then allocated to the wrong sample (one observation being defined as the set of measurements, i.e. 32 variables, made on a test sample by one partner laboratory). The variables mainly responsible for the discrimination between samples were MD-P2P-OH, MD-benzyl-MDMA, MD-DPIA (2), MD-DPIMA (1 + 2) according to the first discriminant function, and MD-P2P, MD-DPIA (2), MD-DPIMA (1 + 2) and MD-P2P-OH according to the second discriminant function.

A further selection was based on the inter-variability of the variables among the street samples. By selecting variables showing large inter-variability between samples, it was thus possible to decrease the set of target compounds from 32 to 8 (Table 1; bold). It was confirmed that large variations were not only due to the presence of outliers, but represented a general spread of the values. No strong correlation was found between the variables using the Spearman coefficient ($p < 0.01$). A new discriminant analysis was applied to check if the 8 variables offered the same discrimination as the 32 variables among different tests samples. The correct classification rate amounted to 99%. Only one observation was wrongly allocated. The variables mainly responsible for the discrimination between samples were MD-benzyl-MDMA, MD-DPIMA (1 + 2) and MD-P2P-OH according to the first discriminant function, and MD-P2P and MD-P2P-OH according to the second discriminant function. These results show that the choice of a reduced subset of variables was pertinent, as the four most discriminat-

Table 1
List of the 32 reproducible target compounds [7]

	Selected organic impurities
1.	1-(3,4-Methylenedioxyphenyl)propane
2.	3,4-Methylenedioxyphenylmethanol
3.	*N*-methyl-3,4-(methylenedioxy)benzylamine
4.	3,4-Methylenedioxyacetophenone
5.	*para*-Methoxymethamphetamine (PMMA)
6.	Unknown-176
7.	**3,4-Methylenedioxyphenyl-2-propanone (MD-P2P)**
8.	**3,4-Methylenedioxyphenyl-2-propanol (MD-P2P-OH)**
9.	3,4-Methylenedioxyphenyl-1-propanol
10.	3,4-Dimethoxy-benzylmethylketone
11.	**3-(3,4-Methylenedioxyphenyl)-3-buten-2-one (MD-P3B)**
12.	Trimethyl-3,4-methylenedioxychromane
13.	2-(3,4-Methylenedioxyphenyl)-1-methylethyl acetate
14.	3-(3,4-Methylenedioxyphenyl)-2-oxopropanoic acid
15.	4-(3,4-Methylenedioxyphenyl)-5-methyl-1,3-dioxolan-2-one
16.	3,4-Dimethoxymethamphetamine
17.	5-(3,4-Methylenedioxyphenyl)-4-methylpent-4-en-2-one
18.	4-(3,4-Methylenedioxy)but-3-en-2-one
19.	5-(3,4-Methylenedioxyphenyl)-2,2,3,4-tetramethyl-1,3-oxazolidine
20.	*N*-methyl-*N*-formyl-3,4-methylenedioxybenzylamine
21.	*N*-[2-(7-methoxy-3,4-methylenedioxyphenyl)-1-methylethyl]-*N*-methylamine
22.	*N*-methyl-*N*-acetyl-3,4-methylenedioxybenzylamine
23.	*N*-formyl-3,4-methylenedioxyamphetamine
24.	N-acetyl-3,4-methylenedioxyamphetamine
25.	***N*-formyl-*N*-methyl-3,4-methylenedioxyamphetamine (*N*-formyl-MDMA)**
26.	***N*-acetyl-*N*-methyl-3,4-methylenedioxyamphetamine (*N*-acetyl-MDMA)**
27.	***N*-(3,4-methylenedioxyphenylmethyl)-*N*-[2-(3,4-methylenedioxyphenyl)]-methylethyl]-*N*-methylamine (MD-benzyl-MDMA)**
28.	**di-[1-(3,4-Methylenedioxyphenyl)-2-propyl]amine (1) (MD-DPIA 1)**
29.	di-[1-(3,4-Methylenedioxyphenyl)-2-propyl]amine (2) (MD-DPIA 2)
30.	Unknown-192b
31.	**di-[1-(3,4-Methylenedioxyphenyl)-2-propyl]methylamine (1 + 2) (MD-DPIMA)**
32.	Unknown-218

The reduced subset of 8 variables is represented in bold.

ing variables (MD-P2P, MD-P2P-OH, MD-benzyl-MDMA and MD-DPIMA (1 + 2)) were actually selected in the subset of 8 variables.

3.2. Separation of linked and unlinked samples distributions

Both sets of 8 and 32 variables were then used to estimate their potential of separating populations of samples from the same batch, and samples from different batches. All combinations of the selected pre-treatments and correlation coefficients were carried out and ROC curves were built. The areas under the ROC curves were used to quantify the efficiency of each combination of statistical treatments in order to separate the two populations (Table 2). Both modified Pearson and square sinus coefficients yielded identical results whatever the pre-treatment or the number of variables used. The best pre-treatment method was the square root yielding areas above 0.990. Both sets of variables

Table 2
Values of area under the ROC curves: separation between populations of linked samples (distances between measurements made on a same test sample by different laboratories) and unlinked samples (distances between measurements made on different street samples by the same and different laboratories)

	N	*N* + square root	*N* + 4th square root
32 variables			
Modified Pearson	0.991	0.996	0.986
Square sinus	0.989	0.996	0.990
8 variables			
Modified Pearson	0.986	0.994	0.978
Square sinus	0.990	0.994	0.954

N: normalised data; Samples were characterized by 32 or by 8 variables.

gave very good separations of the populations, although not significantly better with 32 variables (area of 0.996) than with 8 variables (area of 0.994). Fig. 2 shows the distribution of distances between samples based on the 8 target compounds. These findings demonstrate that a search for links between samples can be done by comparing the 8 target compounds, which speeds up considerably the comparison process between samples. Nevertheless, it is advised to continue recording the whole set of 32 organic impurities, because they bring useful information about the synthesis route and may help track changes in the synthesis processes.

3.3. Drug intelligence

3.3.1. Strategic intelligence

A PCA was carried out on pre-treated data from street samples to highlight potential traffic tendencies. No distinct group of samples were observed (Fig. 3). The reason why no pattern could be distinguished between the four different countries can be explained by the fact that in Europe, MDMA is mainly synthesised via one specific synthesis route (i.e. reductive amination) with variations in the reduction process [5] and continues to be trafficked mostly in Europe [2]. However, there has been reports of large shipments towards non-European countries, such as the United States, Canada and Australia.

It must be kept in mind that street samples coming from different countries were collected at different times. The

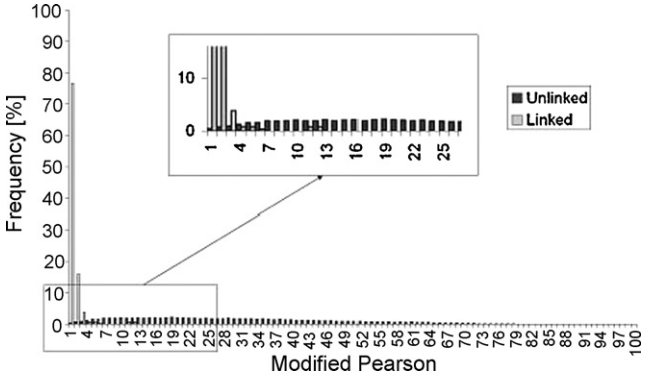

Fig. 2. Histogram representing modified Pearson distances between populations of linked (grey) and unlinked (black) samples using the subset of 8 variables. The square root was used to pre-treat the data.

Table 3
Values of peak areas extracted form the chromatograms, normalized to the sum and pre-treated for the subset of 8 variables in street samples NL 60 (seized in The Netherlands) and CH 2 (seized in Switzerland)

	VAR 7	VAR 8	VAR 11	VAR 25	VAR 26	VAR 27	VAR 28	VAR 31
NL 60	0.62	0.28	0.16	0.23	0.22	0.29	0.00	0.24
CH 2	0.52	0.30	0.15	0.21	0.22	0.27	0.00	0.22

Modified Pearson correlation coefficient for the comparison of these two samples is 0.52.

statistical treatment should be applied on samples collected at the same period of time in order to draw reliable conclusions regarding any traffic tendency.

3.3.2. Operational intelligence

A final aim of this work was to investigate the potential of the present method in pointing out links between samples. (A correlation value was fixed as threshold to decide whether two samples were linked or not. The threshold was chosen to tolerate a false positive rate of 2% (on the basis of the distances between street samples.) This value was found adequate for operational purposes (information for inquiries not for evidential purposes) [9]. The profiles of two samples were considered corresponding when the obtained modified Pearson value was under the given threshold that corresponded to a value of 2.69. A systematic search was carried out on street samples to highlight links based on organic impurities. Several links were highlighted within and between countries. This demonstrates the potential of these statistical tools to highlight links between samples seized and analysed in different European countries. For example corresponding profiles (Fig. 4) between two samples seized in The Netherlands (NL 60) and in Switzerland (CH 2) were discovered because their comparison yielded a modified Pearson value of 0.52 (Table 3). This link supported the hypothesis that the seized tablets came from the same pre-tabletting batch. The MDMA found in these tablets was probably synthesized in The Netherlands and then distributed in other countries of Europe, including Switzerland. This example was outlined because a post-tabletting link was also highlighted and will be detailed in the second part of this article.

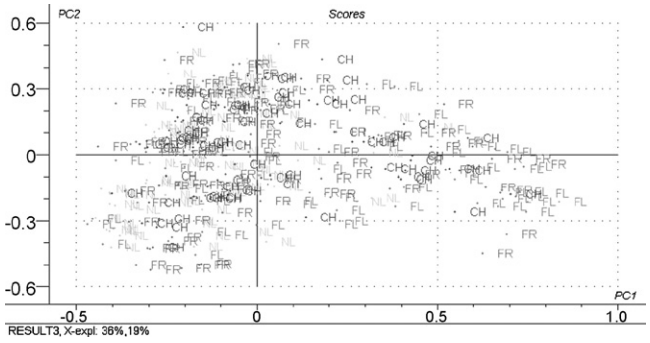

Fig. 3. Principal Component Analysis of 8 organic impurities in 320 MDMA street samples. The square root was applied on the previously normalized data. The first two principal components account for 55% of the variance. No pattern was distinguished between countries (The Netherlands: NL, Finland: FL, Switzerland: CH and France: FR).

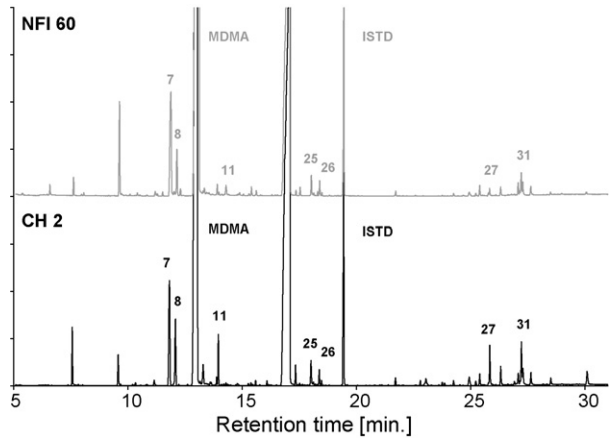

Fig. 4. Seven of the eight subset variables are present in the chromatograms of street samples NL 60 (seized in The Netherlands) and CH 2 (seized in Switzerland): 7: MD-P2P; 8: MD-P2P-OH; 11: MD-P3B; 25: *N*-formyl-MDMA; 26: *N*-acetyl-MDMA; 27: MD-benzyl-MDMA; 31: MD-DPIMA.

4. Conclusion

This work aimed to investigate the potential of organic impurities data in a drug intelligence perspective. It was assumed that every synthesis batch owns a specific organic impurities profile, and that different synthesis batches show different profiles. Based on this hypothesis, statistical analysis of organic impurities profiles of MDMA was carried out to provide a first selection of reproducible target compounds (i.e. variables) to compare samples. A further selection yielded a final set of 8 reproducible and pertinent target compounds. These 8 compounds presented approximately the same the discriminating power than the initial 32 compounds, and could thus be used to search for potential links between samples.

Both modified Pearson and square sinus correlation coefficients applied to normalised data pre-treated by the square root provide an excellent discrimination between populations of samples having corresponding profiles and samples having different profiles. On that basis, a search for corresponding organic impurities profiles within and between countries was then carried out on street samples and highlighted several links within and between countries.

Principal Component Analysis was carried out to visualize the data of all street samples. No distinct traffic tendencies were discovered, probably because MDMA tablets seized in Europe mainly come from the same region. The continuous filling of the harmonised data in the CHAMP common database will actually yield a large and representative sample pool available

as a support for drug investigations of European countries and partners.

Acknowledgements

The authors would like to thank the funding organisations the federal office of education and science in Switzerland (contract no. 04.0005) and the sixth framework programme of the European Commission (contract no. 502126).

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