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Impact of reaction parameters on the chemical profile of 3,4-methylenedioxymethamphetamine synthesized via reductive amination: Target analysis based on GC-qMS compared to non-targeted analysis based on GC × GC–TOF-MS

M. Schäffer [a,b,1], S. Dieckmann [c,d,1], M. Pütz [c], T. Kohles [e], U. Pyell [d], R. Zimmermann [a,b,*]

[a] Comprehensive Molecular Analytics, Helmholtz Zentrum München – German Research Center for Environmental Health, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany
[b] Chair of Analytical Chemistry, Institute of Chemistry, University of Rostock, Dr.-Lorenz-Weg 1, 18059 Rostock, Germany
[c] Bundeskriminalamt – Federal Criminal Police Office, Forensic Science Institute, KT 34 – Toxicology, Äppelallee 45, 65193 Wiesbaden, Germany
[d] Chair of Analytical Chemistry, Institute of Chemistry, Philipps-University of Marburg, Hans-Meerwein-Straße 4, 35043 Marburg, Germany
[e] Pharmaceutical Institute, Eberhard-Karls University Tübingen, Auf der Morgenstelle 8, 72076 Tübingen, Germany

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ABSTRACT

The most common clandestine manufacturing procedure for the ecstasy derivative 3,4-methylenedioxymethamphetamine (MDMA), is the reductive amination of piperonylmethylketone (PMK) via platinum(IV) oxide/hydrogen. Deviations of the reaction conditions during the synthesis may result in different chemical profiles of the products. The chemical analysis of these profiles is an important objective for forensic drug intelligence. In this work we studied the impact of a systematic variation of the hydrogenation time, the reaction temperature and the precursor batch on the resulting organic chemical profiles of the MDMA bases and MDMA hydrochlorides. Target analysis was based on a gas chromatography mass spectrometry (GC–MS) method which was harmonized during the European project CHAMP.[2] In addition, samples were analyzed by comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC × GC–TOFMS) and subjected to non-targeted data analysis for a comprehensive analysis of the complete profiles. The reaction temperature, followed by the used precursor batch, revealed the highest impact on the chemical profile. The effect on individual impurity compounds is discussed in detail. With respect to the interpretation of the data, the profiles were compared to the profiles of MDMA samples obtained by reductive amination using sodium borohydride ("cold method") and aluminium/mercury amalgam as alternative reducing agents. Non-targeted analysis revealed that the discrimination according to the synthetic route and the batch of precursor used for the synthesis strongly depends on the selected target compounds.

1. Introduction

Different synthetic routes as well as different reaction conditions used during the synthesis of illicit psychoactive products result in divergences in the chemical profile of the product due to their impact on the precursor, intermediates and by-products of the reaction; the so-called synthesis impurities. The classification of seized illicit drug samples based on the quantitative analysis of their impurity compounds can provide important information for law enforcement and investigative authorities [1]. Studies on the reproducibility of the chemical profile of samples synthesized via a certain synthetic route provide important information which can be used to conclude on production cycles, batch size, precursors and synthesis conditions of seized samples.

The most common clandestine manufacturing process for the ATS[3]-derivative 3,4-methylenedioxymethamphetamine (MDMA), which is the predominant active ingredient in Ecstasy tablets, is

* Corresponding author. Tel.: +49 8931874544; fax: +49 8931873371.
E-mail address: ralf.zimmermann@helmholtz-muenchen.de (R. Zimmermann).
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[2] Collaborative Harmonisation of Methods for Profiling of Amphetamine Type Stimulants.
[3] Amphetamine type stimulant.

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the reductive amination of piperonalmethylketone (PMK) with methylamine. The prevalent reducing agents are sodium borohydride (cold method) or platinum(IV) oxide/hydrogen (PtO_2/H_2) (autoclave reaction). Profiling routines focus on the analysis of seized samples whose origin is often unknown. The target impurities upon which these routines are based on has been selected according to criteria such as frequency of occurrence, chemical stability, standard deviation of their peak areas and their discrimination potential. The mechanism of the impurity formation is often known. In contrast, little is known about the way in which the formation and the concentration of these impurities are affected by the reaction conditions. Such studies can only be conducted using samples which were synthesized under controlled conditions. Several studies focused on the investigation of route-specific impurities by analysing samples of in-house synthesis [2–6]. Buchanan et al. showed that the three common reductive amination routes (PtO_2/H_2, Al/Hg, $NaBH_4$) can be distinguished based on the stable isotope profiles (δ^2H) of the MDMA-hydrochloride batches [7]. In a second study they observed that varied reaction conditions during the Pt/H_2 reductive amination synthesis, namely the quantities of methylamine/PMK/catalyst (PtO_2), the time for the formation of the intermediate (imine), and the duration of hydrogenation, lead to differences in the isotopic ratios of the resulting MDMA-HCl [8]. In their most recent study they have published the investigation of the discrimination potential of different instrumental and pattern recognition techniques for the chemical profiling of MDMA-HCl [9]. They applied stable isotope ratio mass spectrometry and the harmonized CHAMP profiling routine via GC–MS which was originally established by van Deursen et al. [10]. Besides, the consequences of the results for the chemical profiling of MDMA-HCl are discussed [9].

In the present work we studied the impact of the reaction temperature, hydrogenation time and chemical origin of the precursor (different batches of PMK from seizures which are distinguishable by their impurities) on the organic chemical profiles of the MDMA bases and MDMA hydrochlorides resulting from the reductive amination via PtO_2/H_2. Therefore 9 samples of MDMA(-HCl) were synthesized via reductive amination using PtO_2/H_2 under controlled conditions (see Table 1), 3 samples of MDMA(-HCl) using sodium borohydride (“cold method”) and on sample using aluminium/mercury amalgam. 11 of these samples are based on the same batch of PMK used as starting material (see Table 1). The PtO_2/H_2 samples were synthesized during a collaborative project of the Bundeskriminalamt in Wiesbaden and the Eberhard-Karls-University Tübingen [11]. Parameters are based on enquiries to provide preferably representative conditions of a clandestine process. However, syntheses were carried out on a small scale compared to clandestine MDMA laboratories operating on a large scale. The three different batches of PMK used for the syntheses are derived from police seizures. Our study focused on the impact of each of the three investigated factors (origin of the precursor/reaction temperature/hydrogenation time) individually. Therefore in the experimental design only one factor per synthesis is varied, interactions between the factors are not considered. The impact of each parameter on the different impurity compounds is discussed in detail and the resulting chemical profiles were compared to the profiles obtained by reductive amination using $NaBH_4$ and Al/Hg amalgam as reducing agents.

Chemical profiling presupposes a precise analytical method. According to the literature, gas chromatography coupled to mass spectrometry is the method of choice for organic chemical profiling of MDMA [2–5,10–13]. The one-dimensional GC-qMS method used in this work was harmonized during the European project CHAMP to ensure the comparability of the obtained results and to allow results to be uploaded into a database for further comparison [14]. The CHAMP procedure comprises extraction, instrumental set-up and guidelines for data treatment. Comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry as an enhancement of conventional GC can significantly improve the separation power and the sensitivity due to the modulation process. Furthermore, the two-dimensional visualization of the chromatographic results facilitates the visual comparison of the chemical profiles. Preceding studies on batch-to-batch comparison of drug seizures based on GC × GC have been published recently [15,16]. The higher separation power of GC × GC compared to one-dimensional GC led to a reduction of overlapping peaks which facilitated deconvolution and peak integration and reduced incorrect peak assignment of the software. These factors limited the time required for the manual correction of data. Compared to the CHAMP profiling, which is based on target analysis of pre-defined compounds, a comprehensive non-targeted analysis of the obtained chemical profiles was carried out based on GC × GC analysis. Based on the target compounds of the CHAMP profiling method an accurate classification of all samples derived from the same synthetic route was prevented due to the impact of the precursor batches as well as the temperature on the chemical profile. Non-targeted analysis showed that discrimination according to the synthetic route and the used starting material strongly depends on the selected target impurities.

2. Materials and methods

2.1. Syntheses

Schematic reaction pathways of the different reductive amination reactions can be found in the literature [2,8].

2.1.1. Reductive amination with PtO_2/H_2 (autoclave reaction)

For the preparation of the methanolic methylamine solution, 230 g of methylamine-HCl were dissolved in 300 mL water. The solution was added drop-wise onto 400 g of sodium hydroxide pellets. The resulting gaseous methylamine was passed through a drying tube filled with potassium hydroxide pellets into a beaker with 200 mL of cooled methanol. A threefold excess of methylamine (in methanol, 32%) was added to 70 mmol of PMK which was then reduced in the presence of 0.300 g PtO_2/H_2O (1 mmol) under an inert gas atmosphere. The catalyst was filtered off and the solvents were removed by vacuum distillation. The MDMA base was distilled under vacuum. The resulting colourless MDMA base was added to 150 mL acetone and neutralized with concentrated hydrochloric acid. To complete the precipitation, the solution was placed into a refrigerator for 24 h. The resulting crystals were filtered off and dried.

Nine controlled syntheses have been carried out in which the parameters precursor origin, reaction temperature and hydrogenation time were systematically varied while the autoclave pressure was kept constant at 3.5 bar. The sample synthesized from PMK batch 1 at 50 °C with 2 h hydrogenation time served as the reference sample. Three replicates were prepared under the same conditions to

Table 1
Synthesis conditions and corresponding yields for the MDMA-HCl samples.

PtO_2/H_2										$NaBH_4$			Al/Hg
Precursor (batch of PMK)	1	1	1	**2**	**3**	1	1	1	1	1	1	1	1
Reaction temperature [°C]	50	50	50	50	50	**65**	**80**	50	50	0	−18	−18	–
Hydrogenation time [h]	2	2	2	2	2	2	2	**4**	**8**	–	–	–	–
Yield of MDMA-HCl [g]	7.6	7.2	7.2	6.2	7.6	8.0	7.4	8.0	7.6	11.8	10.8	7.3	9.6
Yield of MDMA-HCl [%]	47.3	44.8	44.8	38.6	47.3	49.8	46.1	49.8	47.3	60.6	55.5	37.5	55.9

verify the synthesis reproducibility. The different reaction conditions and the corresponding yields are shown in Table 1.

2.1.2. Reductive amination with $NaBH_4$ (cold method)

PMK from batch 1 was used as precursor for the following syntheses: 15.1 g of PMK (85 mmol) were mixed with 50 ml methanol and cooled to 0 °C. 20 ml methylamine (40% in water) was added and the solution was cooled to −18 °C. 1.3 g of $NaBH_4$ was added in four steps and the solution was stirred for a total of 24 h. Afterwards, the solvent was removed by distillation and the residue was distilled under vacuum. Precipitation was done in the same way as for PtO_2/H_2 samples. The synthesis was carried out at two different temperatures (−18 °C and 0 °C). Corresponding yields are shown in Table 1.

2.1.3. Reductive amination with Al/Hg

10 g aluminium and 250 mg $HgCl_2$ were placed into water for 15 min. The resulting aluminium/mercury amalgam was washed with water and 19 ml methylamine (40% in water), 36.25 g NaOH (25%), 13.25 g (75 mmol) of PMK batch1 and 130 ml isopropanol were added. The solution was stirred and kept at 35–45 °C for 2 h. The mixture was allowed to stand for another 12 h. The formed Al_2O_3 was filtered off and the solvent was removed by distillation. The residue was extracted with ether which was afterwards removed by distillation. The MDMA base was distilled under vacuum and precipitated as described above. Yields are shown in Table 1.

2.2. Extraction procedure

Prior to analysis, all samples were extracted according to the procedure which was optimized and harmonized during the European project CHAMP [14,17]. Details can be found elsewhere [10]. For preparation of the phosphate buffer KH_2PO_4 and $Na_2HPO_4 \times 2H_2O$ (Fluka, Buchs, Germany) and ultrapure water from a Millipore water system (Bedford, MA, USA) were used. The internal standard solution contained eicosane (Fluka, Buchs, Germany) in toluene (Acros, Geel, Belgium). MDMA bases were diluted with an internal standard solution prior to measurements due to the high abundance of 3,4-methylenedioxymethamphetamine. Since the extraction procedure was optimized for MDMA-HCl, the uncharged state led to enhanced solubility of the MDMA base.

2.3. Instrumentation

2.3.1. One-dimensional gas chromatography

GC-MS analysis was performed on an Agilent 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled with a 5975 quadrupole mass selective detector with an inert ion source (Agilent Technologies, Palo Alto, CA, USA). Injections were carried out with an auto injector (Agilent Technologies, Palo Alto, CA, USA).

For separation, a DB-1MS column (25 m × 0.2 mm i.d., 0.33 μm d_f) from Agilent Technologies (Palo Alto, CA, USA) was used connected to a deactivated fused silica pre-column with an inner diameter of 250 μm and a length of 2 m. The initial temperature of the GC oven was set to 90 °C for 1 min, followed by an increase of 8 °C/min to 300 °C (10 min hold). 2 μl-injections were done at 250 °C in the splitless mode. Helium was used as carrier gas at a constant flow rate of 0.6 mL/min. The transfer line and ion source temperatures were set on 310 °C and 230 °C, respectively. The mass range was set to 35–450 m/z. The GC method was retention-time locked (RTL) to the internal standard eicosane at 19.44 min. The GC-MS method was developed by Van Deursen et al. [10] and harmonized during the European project CHAMP [14,17]. Details on the reproducibility of the GC-MS method can be found elsewhere [14,17].

2.3.2. Comprehensive two-dimensional gas chromatography

GC × GC–TOFMS analysis was performed on an Agilent 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a LECO GC × GC system (Leco, St Joseph, MI, USA). The system consists of a dual-stage, four-jet cryogenic (liquid N_2) modulator and is coupled to a Pegasus III Time-of-Flight Mass Spectrometer (Leco Corporation).

A 1.5 m × 0.25 mm deactivated fused silica tubing combined with a 25 m × 0.22 mm (i.d.) × 0.25 μm d_f capillary column (BPX-5, SGE Analytical Science, Australia) were used in the first dimension and a 1.1 m × 100 μm i.d. × 0.1 μm d_f capillary column (BPX-50, SGE Analytical Science, Australia) was used in the second dimension. The initial temperature of the GC oven was set to 90 °C for 2 min followed by an increase of 10 °C/min to 340 °C. The modulation time was adjusted to 2 s. 1 μl injections were done at 300 °C in the splitless mode. MDMA base was injected in the split mode (split ratio 1:20) due to the higher abundance of 3,4-methylenedioxymethamphetamine. The head pressure was ramped up parallel to the oven temperature program up to 434 kPa. Helium was used as carrier gas. The transfer line and ion source temperatures were set on 300 °C and 250 °C, respectively. The mass range was set to 40–500 m/z at an acquisition rate of 100 Hz. Details on the reproducibility of the GC × GC method can be found elsewhere [16].

2.4. Software

For GC data acquisition and data analysis MSD ChemStation D.02.00.275 (Agilent Technologies, Palo Alto, CA, USA) was used. Data were normalized using Microsoft Office Excel 2003 and then imported into Matlab (R2008b, MathWorks, Inc, USA) for statistics. For GC × GC data acquisition ChromaTOF® software (2.0, Leco, St Joseph, MI, USA) was used. Raw data were imported into Matlab (R2008b, MathWorks, Inc, USA) via network common data format (NetCDF) for data processing and statistics. In addition the PLS_Toolbox (Eigenvector Research, Inc., USA) implemented in Matlab was utilized for statistical operations.

2.5. Data processing and statistical analysis

2.5.1. GC

The harmonized MDMA profiling routine of the CHAMP project also provides guidelines for data analysis. The resulting chemical profiles were analyzed based on pre-defined target compounds. In our study all initial 44 targets known from the CHAMP project were considered since we analyzed pure MDMA-HCl samples which were stored under the same conditions. This is not the case for illicit real samples, and thus, for the selection of target compounds the chemical stability is important. During the European project CHAMP the number of targets was in a first step reduced to 32 according to RSD values of the peak area below 20% and in a second step to only 8 targets which represented approximately the same discrimination power as the 32 targets [14,17].

The CHAMP profiling routine for comparative analysis of MDMA samples comprises of two steps for data pre-treatment. The first one is normalization of each peak response (peak area) based on individual target ions to the sum of all target responses present in the respective samples. This removes variation induced during sample preparation and analysis. Samples from the very same batch are expected to show an identical profile. To reduce the influence of larger peaks the normalized data was scaled to the fourth root. The processed data were imported into Matlab for further statistical evaluation.

2.5.2. GC × GC

The two-dimensional chromatogram can be handled as a picture in which the quantitative information is given within discrete values (pixels) of the raw signal. Data analysis can be performed on the basis of raw data instead of deconvoluted peak data and techniques from image processing can be applied. A routine for the pre-processing of data was generated for removal of sample-unspecific variations within the data set. Instead of the total-ion-chromatogram-signal (TIC) a sum-signal was generated by summing the most abundant masses known from target compounds. The response of the most abundant ions of sample-unspecific compounds, e.g. toluene (m/z 91), column bleed (m/z 73), etc. was thereby filtered out. Further pre-processing included baseline correction and alignment of data in the second dimension via the correlation optimized warping (cow) algorithm [18,19]. Details on data pre-processing are described elsewhere [16]. 3,4-Methylenedioxymethamphetamine as the major compound was excluded from statistical analysis as our work focuses solely on concomitant impurities.

(Un-) supervised discrimination techniques were used in order to determine compounds affected by the different modifications of the reaction conditions. Analysis of variance (ANOVA) combined with partial least square discriminant analysis (PLS-DA) has demonstrated its potential to identify discriminating compounds in complex samples such as analysis of tobacco smoke particular matter or MDMA samples from real seizures [16,20]. ANOVA was used as a filter algorithm for fast feature or variable selection. The significance level of ANOVA was set to 0.05 (95% probability) and all data points which met the criterion were selected, scaled by the square root and subjected to discriminant analysis. The vector of loading weights was extracted from the PLS-DA model and recalculated into the retention time plane for comparison with the original data and determination of corresponding compounds marked by high loading values. According to the algebraic sign of the loadings and the location of the sample in the scoring plot, class specific compounds can be identified.

Hierarchical cluster analysis (HCA) was applied to study the classification potential of the data set. Thereby, samples prepared via other reductive amination routes were considered. Mean-centering was applied on data prior to multivariate analysis. Both techniques were applied to GC-MS target data as well as non-targeted GC × GC data to verify if compounds, not considered as target compounds previously, provide additional information for the discrimination according to the synthetic route and/or the used precursor.

3. Results and discussion

The impact of the synthesis conditions on the target compounds is shown in Fig. 1. Normalized and scaled peak values for each target compound present in the MDMA-hydrochloride samples were used to set up the bar plots. Error bars represent the RSD value of each target. The first bar plot in Fig. 1 shows the samples prepared from different PMK batches, the second one shows the

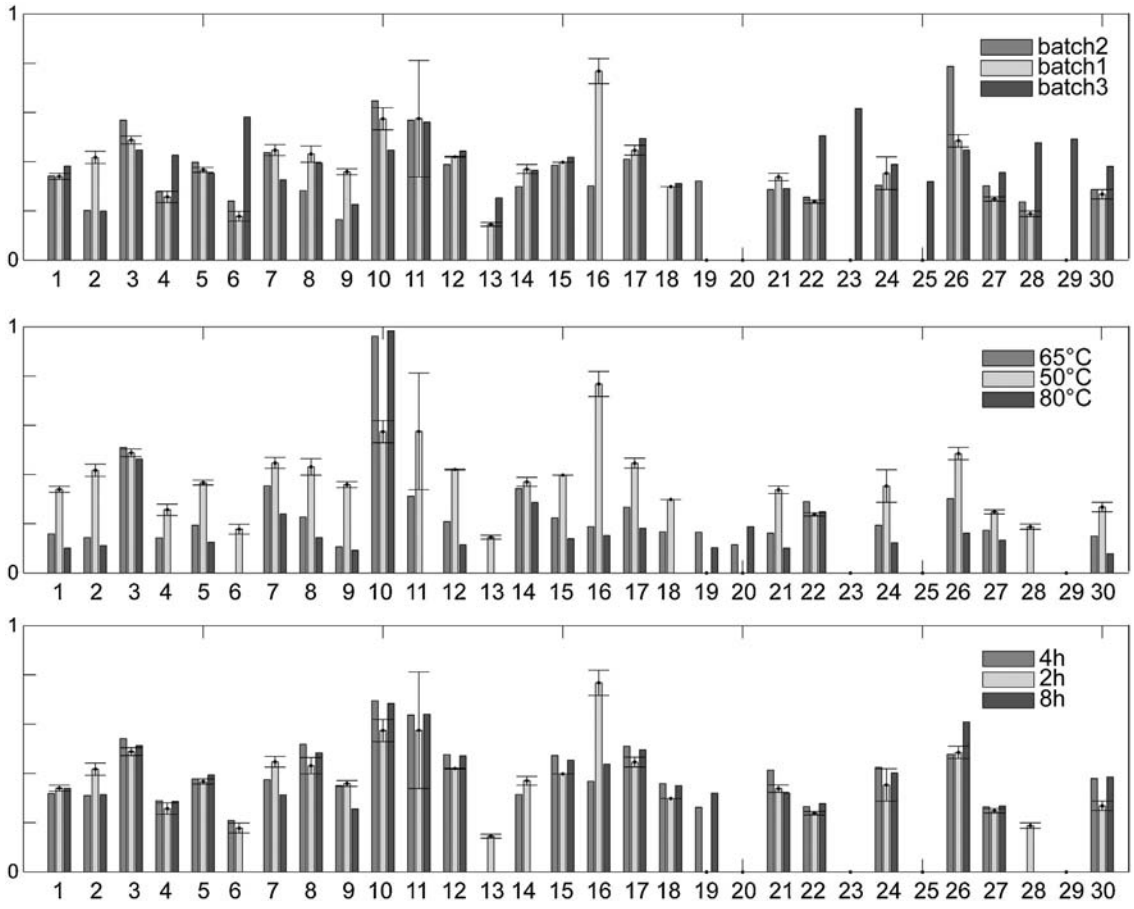

Fig. 1. Impact of varied reaction conditions combined with data pre-processing (norm. +4thrt) on target compounds. (**1**) safrole, (**2**) 1-(3,4-methylenedioxyphenyl)propane, (**3**) piperonal, (**4**) 3,4-methylenedioxyphenyl-methanol, (**5**) isosafrole, (**6**) N-methyl-3,4-(methylenedioxy)-benzylamine, (**7**) 3,4-methylenedioxy-acetophenone, (**8**) para-methoxymethamphetamine, (**9**) unknown-176, (**10**) 3,4-methylenedioxy-phenyl-2-propanone, (**11**) 3,4-methylenedioxyamphetamine, (**12**) 3,4-methylenedioxyphenyl-2-propanol, (**13**) 3,4-methylenedioxyphenyl-1-propanol, (**14**) 3-(3,4 methylenedioxyphenyl)-3-buten-2-one, (**15**) 3,4-methylendioxy-N-ethylamphetamine, (**16**) trimethyl-3,4-methylene-dioxychromane, (**17**) 3,4-methylenedioxydimethylamphetamine, (**18**) 3,4-dimethoxy-methamphetamine, (**19**) 3,4-(methylenedioxy)benzylmethylketoxime, (**20**) 5-(3,4-methylenedioxyphenyl)-4-methylpent-4-en-2-one, (**21**) N-[2-(3,4-methylenedioxy-phenyl)-1-methylvinyl]-N,N-dimethylamine, (**22**) 4-(3,4-methylenedioxy)but-3-en-2-one, (**23**) N-methyl-N-formyl-3,4-methylenedioxybenzylamine, (**24**) N-[2-(7-methoxy-3,4-methylendioxyphenyl)-1-methylethyl]-N-methylamine, (**25**) N-methyl-N-acetyl-3,4-methylenedioxybenzylamine, (**26**) N-formyl-methylenedioxymethamphetamine, (**27**) N-acetyl-methylenedioxymethamphetamine, (**28**) N-(3,4-methylenedioxyphenylmethyl)-N-[2-(3,4-methylenedioxyphenyl)-1-methylethyl]-N-methylamine, (**29**) unknown-192b, (**30**) di-[1-(3,4-methylenedioxyphenyl)-2-propyl]methylamine.

variation induced by the reaction temperature, and the third one the reaction time-dependency of individual target impurities.

GC × GC data were analyzed based on a non-targeted approach. The result of the previously mentioned multivariate analysis (Section 2.5.2) highlights differences between the different synthesis products and depicts impurity compounds which are affected by the respective modification of the synthesis conditions. Normalization based on the sum of responses of the target compounds, as it was done for GC data, could not be applied in this case.

3.1. Impact of varied reaction conditions on the MDMA-hydrochloride samples synthesized by reductive amination with PtO_2/H_2

3.1.1. Impact of the reaction temperature

Fig. 2 refers to GC × GC–TOFMS data and the extracted loading weights of the first latent variable separating the reference sample (50 °C) and the samples which had been prepared at 65 °C and 80 °C. The first latent variable explained already 88.56% of the variance. The scoring plot is shown in the lower right corner.

The precursor piperonylmethylketone (PMK) was affected most by the reaction temperature, resulting in a significant increase of

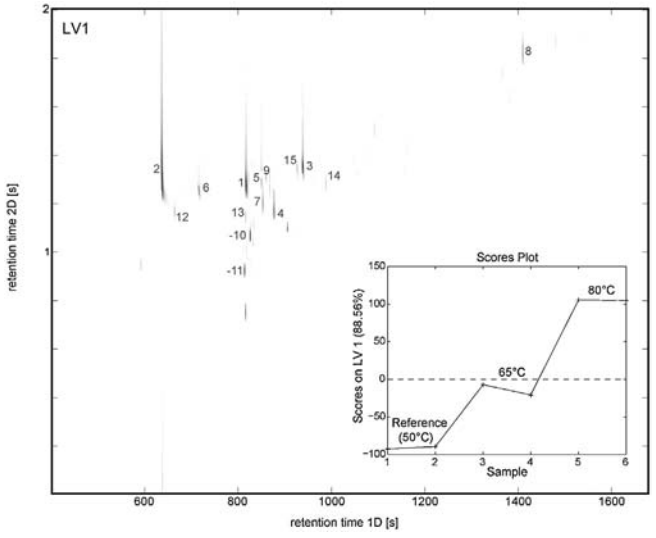

Fig. 2. Impact of the reaction temperature on the entire chemical profile of MDMA hydrochloride samples. Scoring plot of PLS-DA (sub-figure) and corresponding loading plot of the first LV. Positively assigned compounds are predominantly present in the sample prepared at 80 °C.

Table 2A
Compounds affected by the reaction temperature.

No.	Compounds
1	3-(3,4-Methylenedioxyphenyl)-3-buten-2-one
2	Piperonal
3	4-(3,4-Methylenedioxy)but-3-en-2-one
4	5-(3,4-Methylenedioxyphenyl)-4-methylpent-4-en-2-one
5	UNK-4 (bp43)
6	3,4-Methylenedioxyacetophenone
7	UNK-17 (bp 135)
8	UNK-18 (bp 209)
9	UNK-19 (bp 145)
-10	Trimethyl-3,4-methylenedioxychromane
-11	UNK-5 (bp 145)
12	UNK-20 (bp 135)
13	UNK-21 (bp 163)
14	UNK-22 (bp 200)
15	UNK-23 (bp 69)

Table 2B
Compounds affected by the hydrogenation time.

No.	Compounds
-1	Trimethyl-3,4-methylenedioxychromane
-2	UNK-5 (bp 145)
-3	1-(3,4-Methylenedioxyphenyl)propane
-4	UNK-4 (bp 43)
-5	UNK-8 (bp 148)
-6	3-(3,4-Methylenedioxyphenyl)-3-buten-2-one
-7	3,4-Methylenedioxyacetophenone
-8	UNK-11 (bp 149)
-9	UNK-7 (bp 86)
-10	UNK-12 (bp 145)
-11	UNK-13 (bp 97)[a]
12	UNK-10 (bp 91)
13	UNK-14 (bp 56)
-14	Unknown-176
15	UNK-15 (bp 135)

[a]2-(Dimethylamino)-2-methyl-3-(3,4methylenedioxyphenyl)-propanenitrile (Swist et al., [21])

its abundance which exceeded the dynamical range of the MS. Therefore PMK was excluded from analysis. The fifteen compounds associated with the highest loading values are enumerated in Table 2A. The given names refer to known target analytes used for chemical profiling of MDMA. Other compounds are labelled as unknowns (*UNK*). These unknown compounds must be also derived from the synthesis process since the presence of cutting agents can be excluded.

Analytes, indicated by high positive weights regarding the first LV (Fig. 2), correspond to compounds which are predominantly found in the 80 °C-sample. The abundance of these compounds (e.g. 1–5) increased considerably with a rising reaction temperature. For example, 5-(3,4-methylenedioxyphenyl)-4-methylpent-4-en-2-one (4) and one of the lower-volatile compounds with base peak 209 (UNK-18 (8)) were not detectable in the reference sample (50 °C), but were detected in trace amounts in the 65°-sample and in a comparably large amount in the 80 °C-sample. Several compounds could be determined which appeared only in those samples which had been prepared at a higher reaction temperature. In addition to the two compounds mentioned above, also *UNK-17* (7), *UNK-18* (8), *UNK-20* (12) and *UNK-23* (15) were only present in the samples prepared at 65 °C and 80 °C. On the contrary, trimethyl-3,4-methylenedioxychromane (-10) and *UNK-5* (-11) are assigned with negative weights and are primarily found in the reference sample (50 °C). Their abundance is considerably higher compared to the samples synthesized at 65 °C and 80 °C. As it becomes obvious from Table 2A, many unknown compounds are affected by the synthesis temperature. These compounds are not considered in the CHAMP routine which should restrict the impact of the temperature for profiling purposes.

From the target list, mostly 3-(3,4-methylenedioxyphenyl)-3-buten-2-one, piperonal, 4-(3,4-methylenedioxy)but-3-en-2-one, 5-(3,4-methylenedioxyphenyl)-4-methylpent-4-en-2-one and 3,4-methylenedioxyacetophenone showed a strong temperature dependency. This was also observed by one-dimensional GC-analysis. Compounds 4-(3,4-methylenedioxy)but-3-en-2-one and 5-(3,4-methylenedioxyphenyl)-4-methylpent-4-en-2-one for example are not present in the MDMA base but are derived during the precipitation step by reaction of PMK with acetone. The reaction scheme is shown in Fig. 3. The large abundance of PMK which resulted from the synthesis at a higher reaction temperature promoted the formation of these impurities.

The impact of normalization as part of the data analysis routine for the harmonized data became obvious when a comparison was done for the samples synthesized at different temperatures. Considering the samples prepared at 65 °C or 80 °C, the overall abundance of compounds increased significantly; in particular the observed response of PMK which was 700 times higher when compared to the reference sample. This led to normalization factors which were up to 80 times higher compared to the reference sample and consequently caused a decrease of the response of single targets when normalization was applied. From Fig. 1 it seems as if an increase of the reaction temperature leads to a decrease in the abundance of most of the target impurities, which is in fact exactly the opposite case. Qualitative differences are observed for 3,4-(methylenedioxy)benzylmethylketoxime and 5-(3,4-methylenedioxyphenyl)-4-methylpent-4-en-2-one, which only occurred at higher temperatures.

3.1.2. Impact of the hydrogenation time in the autoclave

As expected, the variations induced by modification of the hydrogenation time are significantly lower; visually all chromatograms appeared to be very similar. The first latent variable explained 81.63% of the variation and separates samples prepared at higher reaction times from the reference. The highest impact is attributed to the comparably large amount of trimethyl-3,4-methylenedioxy-chromane (-1) in the reference sample. *UNK-5* (-2), 1-(3,4-methylenedioxyphenyl)-propane (-3), *UNK-4* (-4) and *UNK-8* (-5) were also predominantly found in the reference sample. Only compounds *UNK-10* (12), *UNK-14* (13) and UNK-15 (15) revealed a higher abundance at higher hydrogenation times. LV2 covered 16.75% of the variation and the corresponding loading weights indicated two compounds, *UNK-15* (2) and *UNK-16* (1), which could only be found in the sample synthesized at 8 h. See Table 2B for the results.

Considering the one-dimensional GC-MS data it was also observed that the hydrogenation time had only slight effects on the target compounds. 3,4-(methylenedioxy)benzylmethylketoxime was only present at higher hydrogenation times (4 and 8 h) and some targets were only present at lower hydrogenation times, for example N-(3,4-methylenedioxyphenylmethyl)-N-[2-(3,4-methylenedioxyphenyl)-1-methylethyl]-N-methyl-amine and 3,4-methylenedioxyphenyl-1-propanol at 2 h and N-methyl-3,4-(methylenedioxy)benzylamine and 3-(3,4-methylenedioxyphenyl)-3-buten-2-one only at a hydrogenation time of 2 and 4 h. This might be due to the fact that some targets need more time for formation and some are already decomposed at a longer reaction time, such as N-(3,4-methylenedioxyphenylmethyl)-N-[2-(3,4-methylenedioxyphenyl)-1-methylethyl]-N-methylamine.

3.1.3. Impact of the precursor batch (PMK)

Impurities which were affected by the quality of the used precursor are shown in Table 2C. The samples have been prepared under the same synthesis parameters but the precursor piperonylmethylketone (PMK) was derived from three different

Table 2C
Compounds affected by the precursor quality.

No.	Compounds
1	N-methyl-3,4-(methylenedioxy)benzylamine
-2	Trimethyl-3,4-methylenedioxychromane
-3	UNK-1(bp 176)
4	N-methyl-N-formyl-3,4-methylenedioxybenzylamine
-5	3,4-Methylenedioxyphenyl-2-propanone
-6	Piperonal
-7	UNK-2 (bp 176)
8	4-(3,4-Methylenedioxy)but-3-en-2-one
9	UNK-3 (bp 148)
-10	N-formyl-methylenedioxymethamphetamine
-11	UNK-4 (bp 43)
-12	UNK-5 (bp 145)
-13	Trans-isosafrole
14	N-methyl-N-acetyl-3,4-methylenedioxybenzylamine
-15	UNK-6 (bp 83)
1	Trimethyl-3,4-methylenedioxychromane
-2	N-formyl-methylenedioxymethamphetamine
3	1-(3,4-Methylenedioxyphenyl)propane
4	UNK-7 (bp 86)
5	UNK-5 (bp 145)
-6	Trans-isosafrole
-7	3,4-Methylenedioxyphenyl-2-propanone
8	Unknown-176
9	UNK-8 (bp 148)
-10	Piperonal
-11	UNK-9 (bp 58)
-12	Safrole
-13	UNK-10 (bp 91)
-14	UNK-6 (bp 83)
-15	Cis-isosafrole (UNK)

batches. Latent variable 1 captured 66.29% of the variance and separated batch 3 from the two others. On LV2 (31.76%) all three samples are separated. N-methyl-3,4-(methylenedioxy)benzylamine (1), N-methyl-N-formyl-3,4-methylenedioxybenzylamine (4), 4-(3,4-methylenedioxy)but-3-en-2-one (8), *UNK-3* (9) and N-methyl-N-acetyl-3,4-methylenedioxybenzylamine (14) are characteristic compounds which were only found (9, 14), or predominantly found (1, 4 and 8), in the sample synthesized from batch 3. These compounds were indicated by strong positive loading weights. Trimethyl-3,4-methylenedioxychromane on the other side, indicated by a negative value, dominated the reference sample and was found in smaller amounts in the sample from batch 2 and was not detectable in the sample from batch 3. Additionally, the reference samples (batch 1) and sample batch 2 contained high amounts of *UNK-1* (-3), 3,4-methylenedioxyphenyl-2-propanone (-5), piperonal (-6) and *UNK-2* (-7) compared to batch 3. According to the second LV, trimethyl-3,4-methylenedioxychromane (1), 1-(3,4-methylenedioxyphenyl)propane (3), *UNK-7* (4) and *UNK-5* (5) are predominantly found in the reference samples and *N*-formylmethylenedioxymethamphetamine (-2) and isosafrole (-6) are compounds which are highly abundant in batch 2. Most of the impurity compounds, which are significantly affected by the quality of the precursor, are target compounds known from the CHAMP profiling routine.

The one-dimensional GC-MS data obtained for the MDMA hydrochloride sample, derived from PMK batch 3, also differed significantly from the MDMA hydrochloride samples derived from batches 1 and 2. This is mainly due to N-methyl-N-formyl-3,4-methylenedioxybenzylamine, N-methyl-N-acetyl-3,4-methylenedioxybenzylamine and UNK-192b which could not be detected in the two other samples. For compounds N-(3,4-methylenedioxyphenylmethyl)-N-[2-(3,4-methylenedioxyphenyl)-1-methylethyl]-N-methyl-amine and N-methyl-3,4-(methylenedioxy) benzylamine differences in their concentration was observed. Trimethyl-3,4-methylenedioxychromane was not present in the MDMA-HCl derived from batch 3. The sample derived from PMK batch 2 differed from the other samples regarding 3,4-dimethoxymethamphetamine which was not present in the sample derived from PMK batch 2 and 3,4-(methylenedioxy)benzylmethylketoxime which was only present in the sample derived from PMK batch 2. The differences might be due to the variations which already occurred in the PMK batches used for synthesis [11]. See Fig. 1 for the results.

The variation of the chemical profile of the MDMA-HCl samples induced by modified synthesis conditions is also visually apparent in the GC × GC data in Fig. 4. Baseline corrected two-dimensional chromatograms of the reference sample (A), the samples prepared of precursor batches 2 and 3 (C and D), and the sample synthesized at 80 °C (B) are shown for visual comparison. The most remarkable difference was observed for samples prepared at higher reaction temperatures as these samples exhibited a considerable increase in the abundance of PMK. Despite distillation and precipitation, the number of lower-volatile compounds observable in the chromatogram raised with an increasing reaction temperature. Possible reasons therefore can be ascribed to condensation reactions which occur more frequently at higher temperatures. Chromatograms of samples prepared from different batches of PMK, resulted in visually well-distinguishable profiles. In contrast, an increase in hydrogenation time showed visually only minor variations in the chromatograms (not shown).

3.2. Impact of varied reaction conditions on the MDMA-bases synthesized via reductive amination with PtO_2/H_2

Besides the MDMA-HCl samples, also the intermediate MDMA base was considered in our study. However, direct comparison of MDMA base and hydrochloride is not possible due to sample extraction which was developed for the hydrochlorides. Compared to the hydrochloride, the MDMA base is only minimally soluble in the buffer solution resulting in an increase of this compound in the organic layer. Therefore, the impact of varied reaction conditions on the bases is only discussed qualitatively. All results were observed for one-dimensional as well as two-dimensional GC. Fig. 5 presents chromatograms from GC-MS analysis of base extracts of samples synthesized at 50 °C and 80 °C.

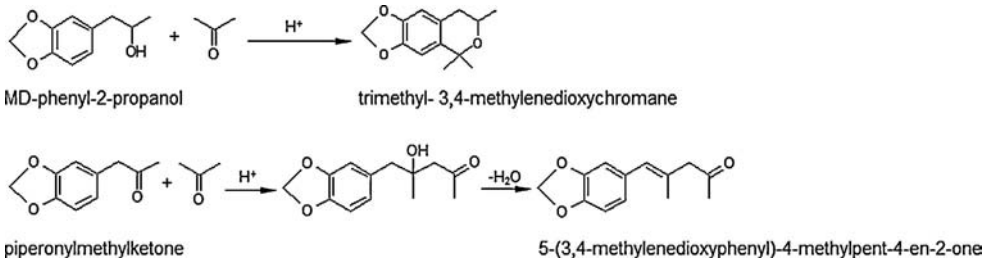

Fig. 3. Formation of trimethyl-3,4-methylendioxychromane and 5-(3,4-methylendioxyphenyl)- 4-methylpent-4-en-2-one.

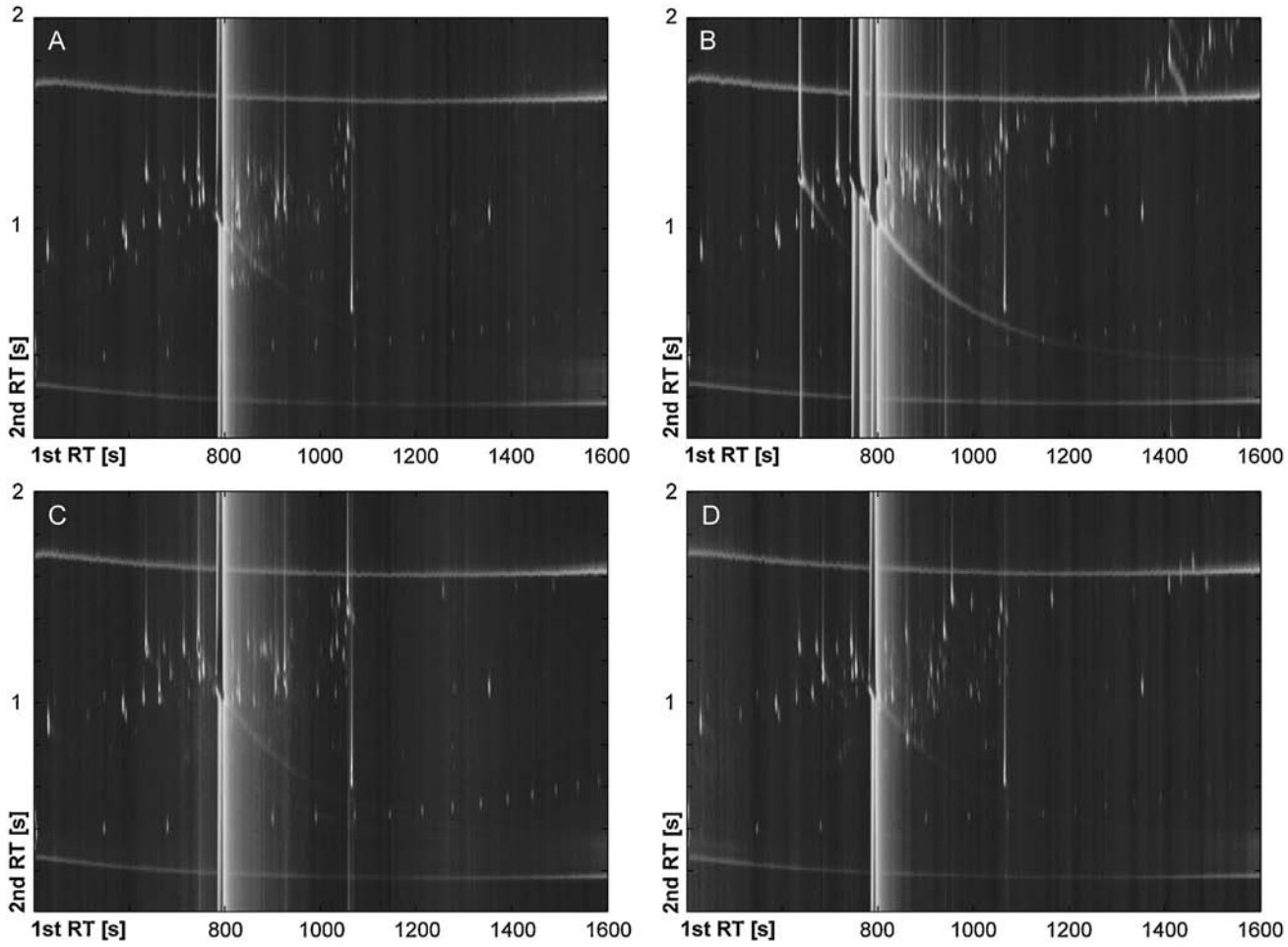

Fig. 4. Baseline corrected GC × GC chromatograms of MDMA-HCl samples derived from PtO_2/H_2 as reducing agent (A: Reference sample, B: reaction temperature of 80 °C, C: PMK batch 2 as starting material, D: PMK batch 3 as starting material.

At the top left part, the chromatogram of the MDMA base prior distillation is shown, below, the base past distillation, and on the right the corresponding MDMA-HCl-sample. In general, the distillation resulted in a decrease in the number of low-volatile compounds and in a significant reduction of the amount of N-formyl-methylenedioxymethamphetamine, N-acetyl-methylenedioxymethamphetamine, N-methyl-N-formyl-3,4-methylenedioxybenzylamine and 3,4-(methylenedioxy)benzyl-methylketoxime.

In general the number of impurities was higher for the MDMA bases compared to the MDMA-hydrochlorides because of the purification during the precipitation step. But nevertheless some target impurities only occurred in the MDMA-hydrochlorides. By example, compounds 4-(3,4-methylenedioxy)but-3-en-2-one and 5-(3,4-methylenedioxyphenyl)-4-methylpent-4-en-2-one were not present in the MDMA base, but are derived during the precipitation step from the reaction of PMK with acetone. The compound trimethyl-3,4-methylendioxychromane on the other hand originates from the reaction of MD-phenyl-2-propanol with acetone. The reaction schemes are shown in Fig. 3.

3.3. Comparison of one-dimensional and two-dimensional GC

Without extending the total run time, comprehensive two-dimensional GC significantly improved separation of the MDMA extracts. Co-elution which frequently occurred in one-dimensional analysis of MDMA could be prevented by the two-dimensional approach. Fig. 6 shows an example for MDMA-HCl. On the left, the peak of target compound N-formyl-methylenedioxymethamphetamine (N-formyl-MDMA) is shown. In one-dimensional analysis only one peak was observable even when depicting different masses. With two-dimensional chromatography three different compounds, N-formyl-methylenedioxymethamphetamine and two unknown compounds with main fragments of 97 and 162 respectively, were clearly separated. N-formyl-MDMA shared mass 162 with one of the unknowns. However, N-formyl-MDMA could be quantified with one-dimensional GC without difficulties since the target mass 86 is unique for this target compound. Another example is illustrated in Fig. 5. In this case, the large amount of PMK resulting from the reaction at higher temperatures prevented the detection of 3,4-methylenedioxyamphetamine (MDA) in these samples due to the absence of a unique mass. 3,4-Methylenedioxyphenyl-2-propanol was identified based on mass 180, although it could easily be missed due to the large PMK peak. On the right, the same part of the chromatogram for GC × GC is shown. The additional dimension allows the identification of MDA despite the large PMK peak. In general, overlapping of peaks was reduced significantly using GC × GC which facilitated deconvolution and peak integration, reduced incorrect peak assignment of the software and therefore limited the time needed for the manual correction of data. On the other hand one-dimensional GC-MS is the preferable instrument when it comes to harmonization of routinely applied chemical profiling methods for the characterization and classification of illicit psychoactive products as nearly all forensic laboratories are equipped with GC-MS.

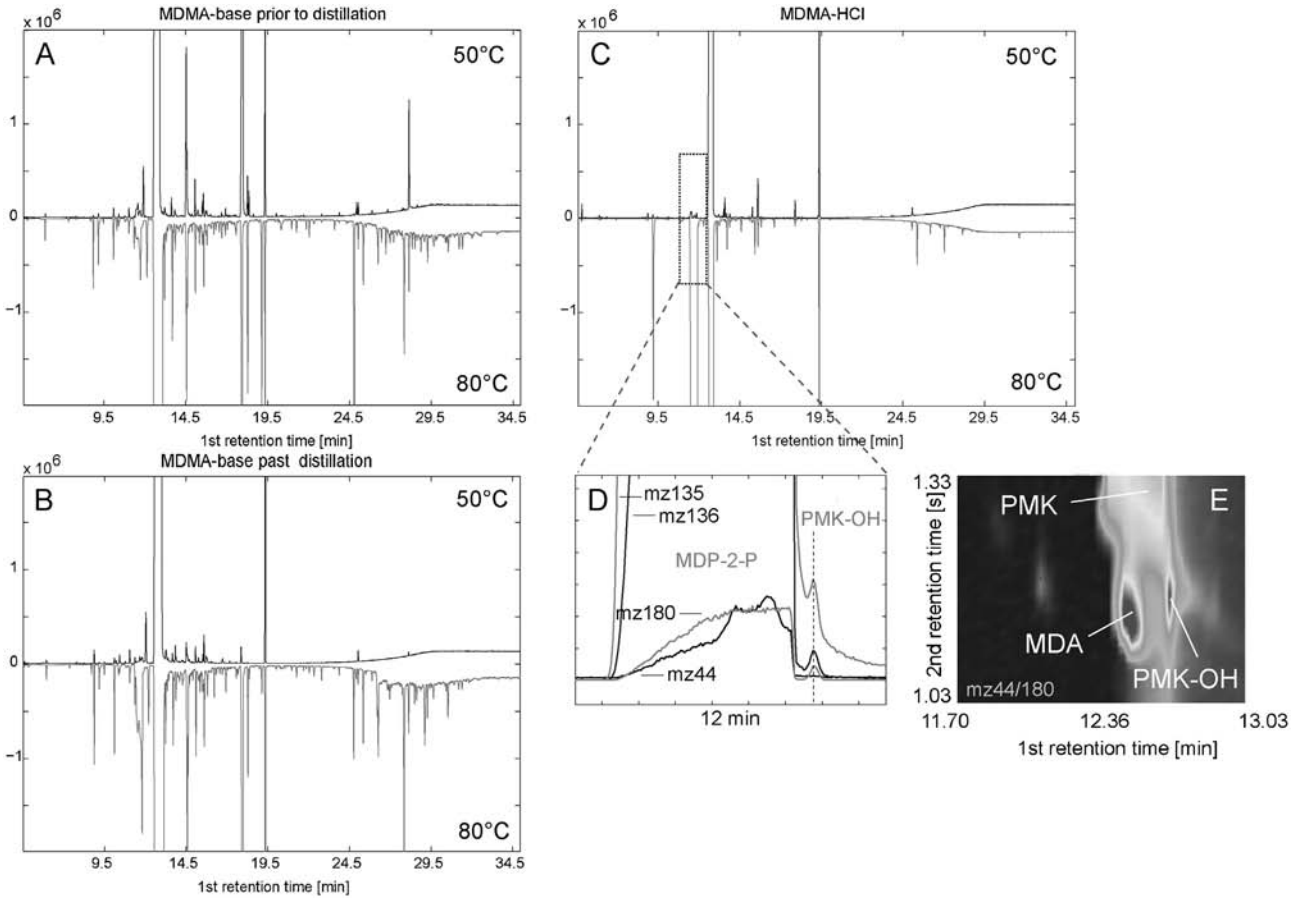

Fig. 5. MDMA synthesized at 50 °C compared to 80 °C. A: prior to distillation B: past distillation C: resulting MDMA-HCl D: expanded part of 1D chromatogram E: corresponding part of GC × GC chromatogram.

3.4. Potential significance of illicit product profiles in forensic science

The samples synthesized via reductive amination with PtO_2/H_2 were compared to the samples synthesized with sodium borohydride (“cold method”) and aluminium/mercury amalgam as reducing agents to evaluate the impact of the modified synthesis conditions regarding the discrimination potential based on the synthetic route or precursor batch used. Samples of the cold method ($NaBH_4$**) are derived from a replicate synthesis at −18 °C, and another $NaBH_4$ sample was synthesized at 0 °C. To compare the significance of the differences between these MDMA hydrochloride samples with regard to routinely applied chemical profiling strategies (CHAMP), the Euclidean distances were compared to the distances of an authentic MDMA control sample

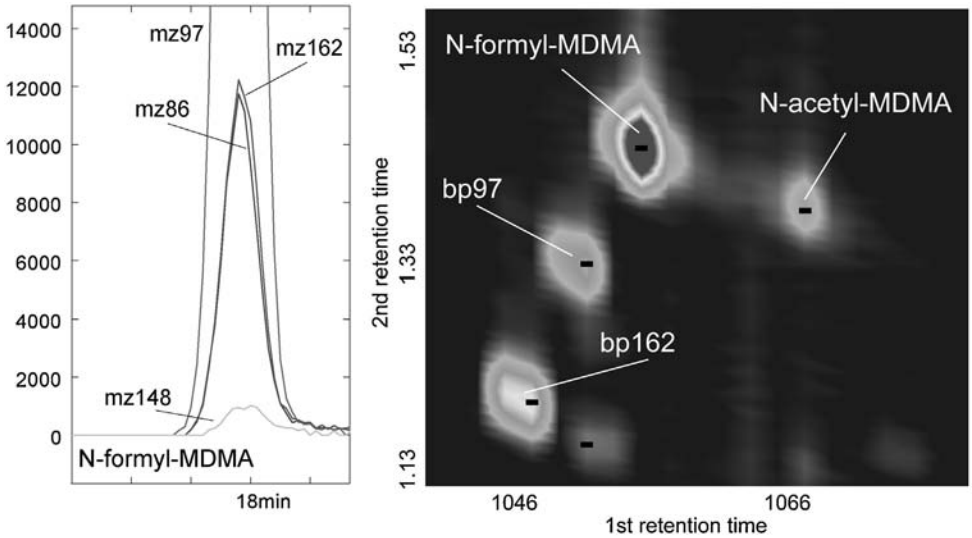

Fig. 6. Left part: unknown compounds with base peak 162 and mass 148 and bp 97 co-elute with N-formyl-MDMA in 1D GC. N-formyl-MDMA, sharing mass 162 with the unknown can still be quantified on its unique mass 86. Right part: GC × GC chromatogram – the three compounds are well-separated.

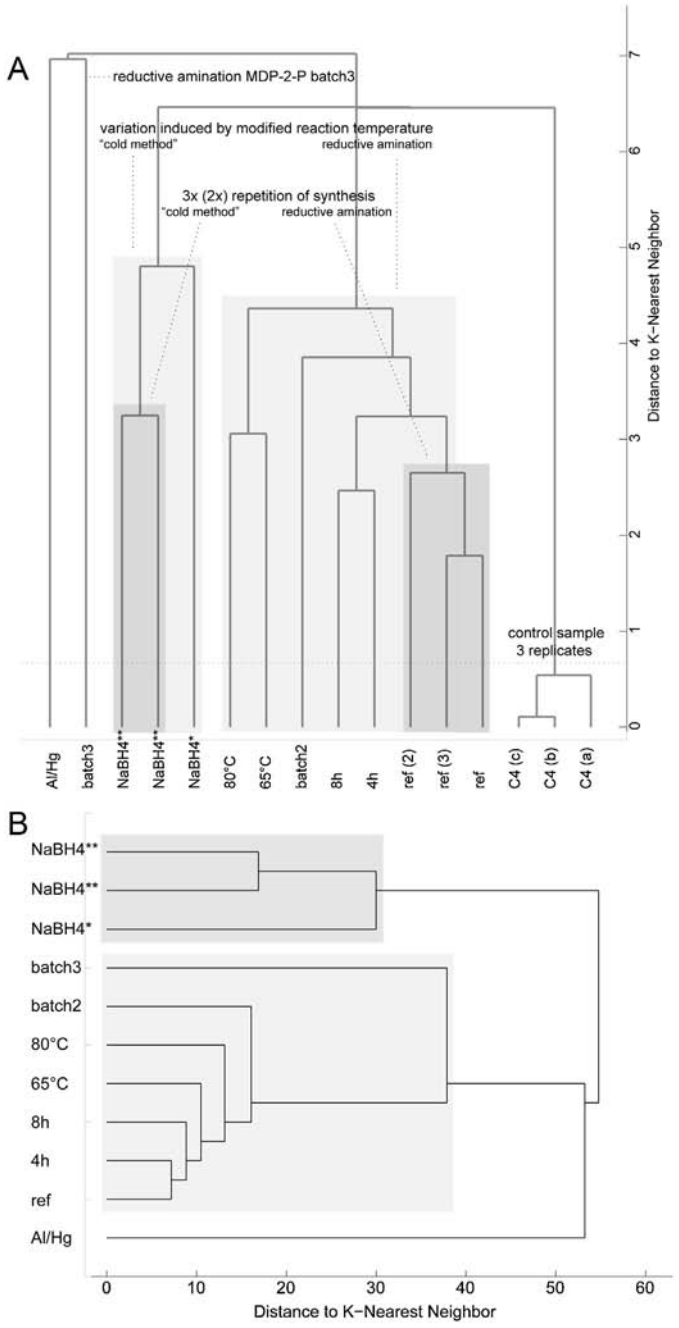

Fig. 7. A Hierarchical clustering based on target compounds (norm. +4thrt). Fig. 7B Hierarchical cluster analysis based on 23 compounds (peak responses of GC × GC data, un-normed, 4thrt) selected by ANOVA. ** Synthesized at −18 °C, *synthesized at 0 °C.

(C4) which was extracted and measured in triplicate (a–c). The distances were also compared to the distances of the three replicates of our reference samples. The three replicates (Ref 1–3) were synthesized under the same conditions (PtO_2/H_2, batch 1, 50 °C, 2 h). Data of on these two samples is only available for GC analysis. Peak areas of the chosen target compounds were normalized and the fourth root was taken. Fig. 7A shows the result of hierarchical cluster analysis using Euclidean distance and nearest neighbour linkage. The lowest fusion levels in the dendrogram were obtained for the set of control samples which were clearly distinguishable from the other samples and which represented the method reproducibility. The next fusion level was composed of the three synthesis replicates, which represented the reproducibility of the synthesis. The modification of the hydrogenation time showed the slightest effect on the resulting organic profile. The increase from 4 to 8 h caused approximately the same variation as it was observed for the replicate synthesis. Obviously the varied temperature has a higher impact on the resulting profile than the precursor batch. The sample prepared from PMK batch 3 differed significantly compared to the other samples synthesized via the same reducing agent (PtO_2/H_2). The impact on the chemical profile caused by the precursor batch prevents an accurate classification of all samples derived from the same synthetic route. Moreover, the high impact of the temperature variation on the organic chemical profiles prevented an accurate classification according to the precursor batch.

Table 3
Pearson correlation coefficients for all 16 MDMA-HCl samples.

	44 Targets	32 Targets	8 Targets
PtO_2/H_2 rep1(ref)	1.0000	1.0000	1.0000
PtO_2/H_2 rep2	0.9400	0.9241	0.8746
PtO_2/H_2 rep3	0.9752	0.9655	0.9436
PtO_2/H_2 (batch 2)	0.8341	0.8113	0.9031
PtO_2/H_2 (batch 3)	0.5590	0.3904	0.7350
PtO_2/H_2 (65 °C)	0.7374	0.7081	0.8027
PtO_2/H_2 (80 °C)	0.5319	0.5386	0.6629
PtO_2/H_2 (4 h)	0.9084	0.8992	0.9258
PtO_2/H_2 (8 h)	0.8716	0.8602	0.7960
$NaBH_4$ (0 °C)	0.4396	0.4624	0.3615
$NaBH_4$ (−18 °C)	0.6620	0.7534	0.4890
$NaBH_4$ (−18 °C)	0.6413	0.7219	0.4857
Al/Hg	0.4905	0.5075	0.6650
Control 1	0.4349	0.3725	0.4971
Control 2	0.4279	0.3568	0.4896
Control 3	0.4230	0.3545	0.4864

For all 16 samples, including all 44 targets, Pearson correlation coefficients were calculated using the normalized data from which the fourth root was taken. The calculated values refer to the reference sample (1). Pearson correlation coefficients were also calculated only for the 32 stable targets according to RSD values of the peak area below 20% which were selected during the CHAMP project [14,17]. The obtained results proved to be similar to the results for the 44 targets confirming the results described above (Fig. 7A). The Pearson correlation coefficients are listed in Table 3. Pearson correlation coefficients taking into account only the 8 targets which represented approximately the same discrimination power as the 32 targets [14,17] are also shown in Table 3. The results were similar; the variation of the reaction temperature still had a significant influence on the profile. However, the three samples synthesized under the same conditions showed lower values for the correlation coefficients.

Buchanan et al. also reported the high impact of the reaction conditions on the organic chemical profiles of the MDMA-hydrochloride samples synthesized via reductive amination with PtO_2/H_2 by analyzing the samples with the harmonized CHAMP GC-MS method taking all 44 targets as well as the 32 stable targets into account [9]. To obtain a classification of samples according to the synthetic route, they combined the harmonized GC-MS method with the data obtained by stable isotope ratio mass spectrometry. In contrast we assumed that the discrimination potential strongly depends on the selected target compounds. For the verification of this assumption, non-target analysis was applied to the GC × GC data using ANOVA. To afford discrimination according to the synthetic route but also to the used starting material, the samples synthesized via the three different routes were put into separated classes and the samples synthesized with PtO_2/H_2 as reducing agent, but starting from different precursor batches. A significance level of 0.01 was chosen and in addition a threshold was applied to eliminate contribution of compounds

Table 4
List of the 23 compounds selected by ANOVA.

1	3,4-(Methylenedioxy)toluene
2	Safrole
3	Cis-isosafrole
4	Trans-isosafrole
5	UNK-24 (bp 146)
6	UNK-25 (bp 121)
7	3,4-Methylenedioxyphenylmethanol
8	N-methyl-3,4-(methylenedioxy)benzylamine
9	UNK-26 (bp 126)
10	Unknown-176
11	3,4-Methylendioxy-N-ethylamphetamine
12	3,4-Methylenedioxydimethylamphetamine
13	UNK-7 (bp 86)
14	3,4-(Methylenedioxy)benzylmethylketoxime
15	UNK-1 (bp 176)
16	UNK-2 (bp 176)
17	UNK-14 (bp 56)
18	N-methyl-N-formyl-3,4-methylenedioxybenzylamine
19	N-formyl-methylenedioxymethamphetamine
20	N-acetyl-methylenedioxymethamphetamine
21	UNK-27 (bp 135)
22	N-(3,4-methylenedioxyphenylmethyl)-N-[2-(3,4-methylenedioxyphenyl)-1-methylethyl]-N-methylamine
23	Di-[1-(3,4-methylenedioxyphenyl)-2-propyl]methylamine

with a small signal to noise ratios due to their lower explanatory power. Corresponding compounds which showed a significant variation according to the pre-defined classes were identified subsequently. To verify the result, the peak areas of these compounds were determined in the GC × GC chromatograms by integration of their responses based on unique ions. The resulting peak table consisted of 23 compounds and was submitted to hierarchical clustering. Prior to HCA, data were scaled to the fourth square root. The corresponding dendrogram is shown in Fig. 7B. Based on this selection of compounds, a complete discrimination according to the synthetic route is given. In addition the strong impact of the temperature on the PtO_2/H_2 samples is reduced significantly and discrimination according to the batch of PMK is possible, although the variation between the sample synthesized from batch 2 and all PtO_2/H_2 – samples synthesized from batch 1 is not very pronounced. However, the targets selected here are effective for the samples investigated in this study and the results may not be transferable to arbitrary samples. The compounds selected include some of the finally chosen 8 targets from the CHAMP method (N-formylmethylenedioxymethamphetamine, N-acetylmethylenedioxymethamphetamine and di-[1-(3,4-methylenedioxyphenyl)-2-propyl]methylamine) as well as some targets such as safrole and isosafrole, which were rejected during the CHAMP project due to high RSDs. However, it shows that a large amount of information is provided by the organic chemical profile. Profiling results strongly depend on the focus of observation; in this case the pre-selection of target compounds. Table 4 lists the 23 compounds selected by ANOVA.

4. Conclusion

In our study, the reaction temperature as well as the precursor batch revealed a large impact on the resulting impurity compounds. Besides, the normalization procedure of the CHAMP method affected the interpretation of the resultant data. The high impact of the temperature and the starting material could be confirmed by a comparison of the chemical profiles of the reductive amination via PtO_2/H_2 to the profiles obtained by using sodium borohydride ("cold method") and aluminium/mercury amalgam as reducing agents. Based on the target compounds of the CHAMP profiling method, an accurate classification of all samples derived from the same synthetic route was prevented due to the impact of the precursor batches on the chemical profile. In addition, the reaction temperature revealed a higher impact on the profile than the variation which resulted from the impact of precursor batch 1 compared to batch 2. The effect of the temperature prevented an accurate classification according to the precursor. Buchanan et al. were able to achieve a classification of MDMA-hydrochloride samples according to the synthetic route by combining the results from the CHAMP GC-MS method with the information obtained from stable isotope ratio mass spectrometry [9]. A comprehensive and non-targeted analysis of the GC × GC profiles showed that additional information not considered previously is provided by the organic chemical profiles. Profiling results are constrained by the pre-selection of target compounds. Discrimination according to the synthetic route and the precursor batch could be achieved for our set of samples using a different selection of impurities which also takes unknown impurities into account.

Comprehensive two-dimensional GC significantly improved separation compared to one-dimensional GC and facilitated the visual comparison. Co-elution and over-lapping of peaks could be limited by the two-dimensional approach. Thus deconvolution and peak integration was facilitated considerably and incorrect peak assignments by the software as it was observed for the one-dimensional data could be avoided. On the other hand, one-dimensional GC-MS is the preferable instrument when it comes to harmonization of routinely applied chemical profiling methods for the characterization and classification of illicit psychoactive products as nearly all forensic laboratories are equipped with a GC-MS.

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