Identification of specific markers for amphetamine synthesised from the pre-precursor APAAN following the Leuckart route and retrospective search for APAAN markers in profiling databases from Germany and the Netherlands

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Abstract
α-Phenylacetoacetonitrile (APAAN) is one of the most important pre-precursors for amphetamine production in recent years. This assumption is based on seizure data but there is little analytical data available showing how much amphetamine really originated from APAAN. In this study, several syntheses of amphetamine following the Leuckart route were performed starting from different organic compounds including APAAN. The organic phases were analysed using gas chromatography–mass spectrometry (GC–MS) to search for signals caused by possible APAAN markers. Three compounds were discovered, isolated, and based on the performed syntheses it was found that they are highly specific for the use of APAAN. Using mass spectra, high resolution MS and nuclear magnetic resonance (NMR) data the compounds were characterised and identified as 2-phenyl-2-butenenitrile, 3-amino-2-phenyl-2-butenenitrile, and 4-amino-6-methyl-5-phenylpyrimidine. To investigate their significance, they were searched in data from seized amphetamine samples to determine to what extent they were present in illicitly produced amphetamine. Data of more than 580 cases from amphetamine profiling databases in Germany and the Netherlands were used for this purpose. These databases allowed analysis of the yearly occurrence of the markers going back to 2009. The markers revealed a trend that was in agreement with seizure reports and reflected an increasing use of APAAN from 2010 on. This paper presents experimental proof that APAAN is indeed the most important pre-precursor of amphetamine in recent years. It also illustrates how important it is to look for new ways to identify current trends in drug production since such trends can change within a few years.

KEYWORDS
amphetamine, APAAN, clandestine laboratories, impurity profiling, pre-precursor

INTRODUCTION

The substance class of amphetamine-type stimulants (ATS) covers structurally closely related synthetic drugs including the widely known compounds amphetamine and methamphetamine. ATS are the second most prevalent drug class after cannabis used worldwide and dominate the European market for synthetic drugs in terms of both production and use. The production of amphetamine mainly takes place in the Netherlands, Belgium, Poland, and Germany. Especially the production in the Netherlands is important since it supplies markets throughout Europe and is often conducted by organised crime groups. Several countries have reported on seized amphetamine freebase that originated from the Netherlands which is converted to its consumer form, the amphetamine sulphate, on their territories. This shows that the market is dominated by large-scale producers with the required resources and access to precursor chemicals. This way of operation makes it more and more difficult to interpret trends in amphetamine production because of decreasing numbers of production facilities caused by an increase of production capacity of these laboratories. The most popular synthetic route for...
the clandestine manufacture of amphetamine is, due to its simplicity, the Leuckart reaction.\(^6\) It starts from the internationally controlled precursor benzyl methyl ketone (BMK, often also referred to as P2P, the abbreviation for phenyl-2-propanone) which is reductively aminated in a first step to form N-formylanphetamine (N-FA).\(^7\) The second step of the Leuckart reaction is a hydrolysis of the formed N-FA using a strong acid which results in the corresponding amphetamine salt.\(^7\) By adding an alkaline compound like sodium hydroxide, the salt is converted into the amphetamine freebase which can easily be transported to a different location. The final step is to precipitate the amphetamine as sulphate salt. In theory, 1 L of BMK can yield around 1.4 kg of pure amphetamine sulphate but in practice this amount is well under 1 kg due to the low yield of the Leuckart reaction caused by several side reactions.\(^1\)

The precursor BMK is usually produced from sources outside the EU including the Russian Federation and China.\(^3\) According to the International Narcotics Control Board (INCB) 11738 L of BMK seizures were reported between 2010 and 2014 in the EU.\(^8\) However, as a result of greater international cooperation, BMK becomes more difficult to source which leads illicit manufacturers to look for alternatives. One alternative is to use non-scheduled chemicals which can easily be converted into BMK and are therefore defined as pre-precursors.\(^3\) One of the most important pre-precursors in recent years is α-phenylacetacetonitrile (APAAN) which, looking at the European market, was first related to illicit amphetamine production in 2009.\(^5\) However, the synthesis of BMK starting from APAAN has been known for much longer.\(^9\) Enormous amounts of APAAN have been seized in Europe with more than 43.5 tons being registered in 2013 alone.\(^6\) APAAN can be produced by condensing benzylcyanide with ethyl ethanoate in the presence of sodium ethoxide.\(^10\) The conversion of APAAN into BMK is simple and requires only an acid (hydrochloric, phosphoric or sulphuric acid), water and a heat source.\(^3,11\) APAAN confiscated in Europe originated primarily from China with the main destination being the Netherlands which resulted in about 50 tons of APAAN being seized there alone between 2011 and 2013.\(^3\) It is likely that producers in recent years heavily relied on APAAN to produce amphetamine which is also indicated in a decline of BMK seizures in Europe from approximately 5500 L in 2010 to 61 L in 2013.\(^8\) Because of this development APAAN was placed under control in the EU at the end of 2013 and under international control at the end of 2014.\(^2\) These control measurements could explain why BMK seizures in Europe went up again from 61 L in 2013 to 2640 L in 2014 with APAAN seizures going down from 43.5 tons to 11 tons at the same time.\(^8\) However, it is likely that the seized BMK originated from APAAN to some extent due to the fact that the BMK was typically seized in illicit APAAN conversion laboratories.\(^8\) Despite the implemented control measures, APAAN is still being seized and seizure data suggest that large quantities of APAAN were stockpiled before it was placed under control.\(^3\) Due to this situation, it is difficult to get reliable estimates of the actual use of APAAN as a pre-precursor.

Amphetamine sulphate generally contains different quantities of impurities which can originate from the following sources: (1) impurities present in the starting material, (2) compounds formed from impurities in the starting material, (3) by-products from side reactions, and (4) intermediates or starting material that were not completely converted. The use of these impurities for forensic investigations was already suggested in 1975 by Stömberg.\(^12\) Such contaminants can be used to differentiate between synthetic routes, the origin of precursors, and reaction conditions or to link samples to a common production location.\(^13,14\) A harmonised method to perform the profiling of amphetamine was presented from 2005 on, in a series of 6 publications.\(^15-20\) In addition, several papers have identified possible marker substances which indicate a certain route of production if found in a sample.\(^7,10,13,21-25\) However, Stojanovska et al pointed out that such studies are often relatively limited in scope due to the small number of samples involved.\(^13\) Profiling could also be implemented for APAAN to get reliable data on the use as a pre-precursor. Therefore, specific marker compounds have to be identified which will only be present if APAAN was used for production. At the moment, the only research in this direction has been performed by Power et al, who have published 4 papers about this topic.\(^11,26-28\) In their studies they proposed 4,6-dimethyl-3,5-diphenylpyridin-2-one, 2-methyl-1-phenyl-1,3-dicarbonitrile-1H-indene, and recently 4-amino-6-methyl-5-phenylpyrimidine as possible route-specific markers indicating that APAAN was used. Since these are the only studies looking at APAAN markers with only a very limited number of investigated samples, there is a great need for further work on this topic.

The aim of this paper is to identify and characterise marker compounds which indicate, with a high degree of certainty, that APAAN was used as a pre-precursor for the production of amphetamine. Three possible marker compounds were identified and full characterisation data are given for all of them. To determine the significance of these markers, they were searched in profiling databases dating back several years. Amphetamine profiling data from the Federal Criminal Police Office in Germany and the Netherlands Forensic Institute in the Netherlands were therefore compared resulting in a total of 585 cases. Other marker compounds identified by Power et al were also included to have a comparison of relevant markers that have been proposed so far. To our knowledge this is the first study presenting a retrospective analysis of the use of APAAN based on already proposed marker compounds as well as newly identified ones. It is also the first time such a large sample pool was used to determine the use of a pre-precursor for the production of amphetamines dating back several years in general.

## 2 | MATERIALS AND METHODS

### 2.1 | Chemicals

Formamide and hydrochloric acid (37%) of analytical grade and sulphuric acid and sodium hydroxide of technical grade were purchased from Carl Roth (Karlsruhe, Germany). Formic acid of analytical grade, methanol of analytical grade and phenylacetone (99%) were purchased from Sigma-Aldrich (St Louis, MO, USA). Methanol of technical grade and benzylcyanide (99%) were purchased from Merck (Darmstadt, Germany). Chloroform-D1 and Tetramethylsilane were purchased from Deutero GmbH (Kastellaun, Germany) and of
analytical grade. Seized APAAN and APAA samples were used for the syntheses. Only deionised water was used for the experiments.

2.2 | Syntheses

To investigate the formation of APAAN markers, several controlled syntheses following the Leuckart route were conducted. This route is well known and several instructions are available from Internet sources and publications which were adapted for this study. First a complete 4-step Leuckart synthesis of amphetamine sulphate starting from APAAN was performed. After each synthesis step, a part of the organic phase was collected and analysed using gas chromatography–mass spectrometry (GC–MS). To determine the specificity of these possible marker substances it was crucial to perform additional experiments to exclude other sources for the formation of these markers. Starting from Leuckart Step 1, 5 different variations were performed using the following compounds as organic phase:

1. 100% BMK
2. Mixture of 50% BMK and 50% APAAN (molar ratio)
3. 100% APAAN
4. 100% benzyl nitrite
5. 100% α-phenylacetoacetamide (APAA)

The reactions were performed on a small scale using the same molar ratios and reaction conditions used for the complete synthesis which is now described.

Step 1. APAAN conversion

APAAN (250 g) was mixed with water (250 mL) and heated to 85°C. Conc. H₂SO₄ (500 mL) was added dropwise, the temperature was held at 100°C for 10 min and then cooled to room temperature before adding 1.25 L of water. The mixture was heated to 100°C and held there for 2 hours. The organic phase was collected after cooling down to room temperature.

Step 2. Leuckart Step 1

One mol organic phase (= 133 mL BMK) was mixed with 5 mol formamide and 3.7 mol formic acid. For the 5 different experiments mentioned before the organic phase was replaced with the corresponding compound at the same molar ratios. The mixture was refluxed for 4 hours and then cooled to room temperature. The mixture was made basic using aqueous sodium hydroxide solution and the formed phases were separated.

Step 3. Leuckart Step 2

The organic phase from Leuckart Step 1 was mixed with the same volume of hydrochloric acid (37%) and refluxed for 2 hours. After cooling down to room temperature, the mixture was made alkaline using an aqueous sodium hydroxide solution and the 2 formed phases were separated.

Step 4. Precipitation of amphetamine

The organic phase from Leuckart Step 2 was mixed with methanol and sulphuric acid (50%) was added. The amphetamine precipitated as sulphate salt during this process, was filtered and then dried at 50°C.

2.3 | GC–MS analyses

GC–MS measurements of the synthesis samples were performed using a 7890 A GC system combined with a 5975C inert XL MSD as detector (both from Agilent Technologies, Santa Clara, CA, USA). The used column was a 30 m TG-5MS with 0.25 mm inner diameter and film thickness of 0.25 μm (Thermo Fisher Scientific, Waltham, MA, USA). Data were analysed using the MSD ChemStation software. System parameters were helium carrier gas at 1.5 mL/min, a split ratio of 10:1, 70 eV ionisation energy, mass range 40–600 m/z and a temperature programme starting at 60°C for 1 minute, ramping at 10°C/min to 65°C, hold there for 15 minutes ramping at 15°C/min to 300°C and, finally, hold there for 2 minutes resulting in a total run time of 34 minutes. High resolution masses of the markers were collected using a Scion GC-456 coupled to a compact™ quantum time-of-flight (QToF) mass spectrometer using a GC-APCI II source (all from Bruker Daltonics, Billerica, MA, USA). Amphetamine profiling was performed following the harmonised method for the profiling of amphetamines. The used column was a 30 m DB-35MS with 0.25 mm inner diameter and film thickness of 0.25 μm (Thermo Fisher Scientific, Waltham, MA, USA) with a 2.5 m DB-35MS pre-column with 0.25 mm inner diameter and 0.1 μm film thickness.

2.4 | Marker isolation

Marker references were purified from the organic phase of batch 3 (100% APAAN) by using a Sepacore® flash system X50 from Büchi Labortechnik AG (Flawil, Switzerland). Ethanol and water as eluent combined with a Revelis C18 reversed-phase cartridge with 4 g sorbens material were used for separation. Marker 185 was purified from the same phase by using an aqueous acid extraction.

2.5 | NMR analysis

Nuclear magnetic resonance (NMR) spectra were collected on an Advance 500 spectrometer equipped with a 5 mm 500 MHz BBI SB probe with Z-gradient at 25°C (both from Bruker, Billerica, MA, USA). Approximately 10–20 mg of each purified marker was dissolved in CDCl₃. Chemical shifts were referenced to tetramethylsilane (TMS) as internal standard which signals were assigned 0 ppm. One dimensional ¹H and ¹³C experiments and 2-dimensional COSY, HSQC, and HMBC experiments were performed for each marker substance. Spectra were processed using the software Mnova version 11 (Mestrelab Research, Santiago de Compostela, Spain).

Chemical structures were drawn using Chemsketch (Advanced Chemistry Development, Inc. (ACD/Labs)).

2.6 | Retrospective analysis

The APAAN marker signals were searched in amphetamine profiling databases going back as far as 2009 to determine their occurrence in
seized amphetamine sulphate samples. Databases from both Germany and the Netherlands were compared in this study. Due to the enormous number of samples in these databases the following procedure was utilised. The first sample of each individual case was used for comparison which lead to a total number of 227 cases in Germany and 358 in the Netherlands. The markers were identified based on both retention time and mass spectrum.

3 | RESULTS AND DISCUSSION

3.1 | Syntheses

3.1.1 | Complete 4-step synthesis

Figure 1 shows a summary of the 4-step reaction that was used in this work to synthesise amphetamine sulphate from APAAN. The organic phase of the APAAN conversion step contained large amounts of BMK but also considerable amounts of unreacted APAAN. Seven APAAN conversion experiments applying different reaction parameters were performed in addition to the one mentioned earlier and unreacted APAAN was found in each organic phase. This led to the conclusion that APAAN is a very likely contaminant found in BMK as proposed by Power et al,26 were also present. Their second marker 4,6-dimethyl-3,5-diphenylpyridin-2-one could not be searched since GC–MS derivatization is required to observe this compound and therefore it was not suitable for the amphetamine profiling employed.27

The organic phase was used without further purification for Leuckart Step 1 where it was mixed with formamide and formic acid. After reaction, the main component of the organic phase was identified as N-formylamphetamine (N-FA) as shown in the chromatogram in Figure 2. 4-Methyl-5-phenylpyrimidine (4M5PP) was also present which is a route specific marker for the Leuckart reaction.24 Other impurities like N,N-di-(β-phenylisopropyl)amine (DPIA), which are known compounds resulting from the Leuckart reaction, were found as well. Benzyl nitrile was identified which was an impurity of the used APAAN. In addition to these known signals, 4 peaks were detected which were unknown at that time. Their corresponding base peaks were at 143, 185, and 2 times 158, respectively, with their mass spectrum and it was therefore assumed that they were caused by isomeric forms of the same compound. This resulted in 3 possible marker compounds which were initially named marker 143, 158, and 185 based on their assumed molecular masses. Marker 2-methyl-1-phenyl-1,3-dicarbonitrile-1H-indene was still present but with an area over 2500 times less abundant compared to marker 185 when comparing their base peaks.

The N-FA containing phase was used without further purification in Leuckart Step 2 where it was mixed with concentrated hydrochloric acid. After the reaction, amphetamine was the main component of the organic phase as shown in Figure 3. The 4M5PP signal was still visible together with other known Leuckart impurities. Two of the previously found signals, namely marker 185 and 143, were still present. The 2 peaks of compound 158 were missing, leading to the assumption that this compound is unstable in an acidic environment. This assumption was later confirmed by experiments where the marker disappeared after hydrochloric acid was added. 2-Methyl-1-phenyl-1,3-dicarbonitrile-1H-indene was still detectable but almost indistinguishable from the background with the intensity of the base peak being 3200 times lower compared to marker 185.

The final synthesis step was to precipitate the amphetamine free-base as sulphate using sulphuric acid. After the precipitate was dried, it was dissolved in methanol and analysed using GC–MS. The analysis showed a typical profile of an amphetamine sample synthesised using the Leuckart route. Additional signals from compounds 185 and 143 were present, which showed that under the applied conditions these substances can be carried over into the final product.

Based on this experiment, it was assumed that unreacted APAAN from the APAAN conversion step formed 3 characteristic compounds during Leuckart Step 1. Two of these compounds were carried over into the final amphetamine sulphate where they were detected. The next step was to confirm these assumptions using syntheses with differing amounts of APAAN.

3.1.2 | BMK/APAAN experiment

The first batch was prepared by mixing pure BMK with formamide and formic acid. After the reaction N-FA was the main compound but no peaks of the markers 143, 158, and 185 were found. The main impurities found were 4M5PP and DPIA. The organic phase was used for the second Leuckart synthesis step and after the reaction with hydrochloric acid, amphetamine was found to be the main compound; 4M5PP and DPIA were still present as the main impurities. Again, no peaks for markers 143, 158, and 185 were present.
FIGURE 2 GC chromatogram of the organic phase from Leuckart step 1 of the controlled synthesis with the mass spectra of the newly found marker compounds

FIGURE 3 GC chromatogram of the organic phase from Leuckart step 2 of the controlled synthesis
APAAN and BMK with a molar ratio of 50:50 were used for the second batch to determine if the proposed markers could originate from a reaction involving both BMK and APAAN. After reaction, all 4 marker peaks appeared in addition to the signals of 4M5PP and DPIA. The organic phase was used for the second Leuckart synthesis step and amphetamine was again the main compound with smaller peaks for 4M5PP and DPIA being present. From the 4 marker peaks only the signals of 143 and 185 remained. This supported the previous finding that marker 158 is converted in an acidic environment. In addition, significant signals of BMK and APAAN were found which indicated that marker 158 might be converted into these compounds since only trace amounts were present after the first Leuckart step. This was proven by adding hydrochloric acid to a small amount of purified marker 158. The GC–MS analysis showed that the signal of marker 158 disappeared and a signal for APAAN appeared. During Leuckart Step 2 this APAAN will then react with the high concentration of hydrochloric acid to form BMK which explains the increase of both signals.

The third batch contained only APAAN to determine if the markers were also formed if no BMK is present. The resulting organic phase showed only trace amounts of N-FA revealing that, as expected, APAAN is unlikely to be converted into BMK under these conditions. The main signal was derived from the APAAN contaminant benzylnitrile. Large amounts of the marker substances 143, 158, and 185 were found but no 4M5PP and DPIA. It confirmed that the proposed markers were formed from APAAN alone. The organic phase was then used for the second Leuckart step and as with batch 2, the 2 peaks of marker 158 were missing after the reaction with hydrochloric acid. The main signals were from benzylnitrile and the markers 143 and 185. BMK was found in considerable amounts showing that under these conditions the APAAN formed from marker 158 was converted into BMK.

Since equal molar amounts were used for all 3 batches a comparison of the signal intensities was performed. The results are given in Table 1. From this table, it is clear that the occurrence of the marker compounds correlated with the used amount of APAAN. Benzylantite as a contamination of the used APAAN was directly proportional to the used amount of APAAN and marker 185 showed a similar behaviour. N-FA on the other hand demonstrated a negative proportional trend because there was less BMK present to be converted to N-FA.

This series of experiments proved that the proposed markers are formed during Leuckart Step 1. An increase in the amount of APAAN during this step leads to an increase in signal intensity of the markers.

**TABLE 1** Comparison of 3 batches of Leuckart step 1 with either 0% APAAN (batch 1), 50% APAAN (batch 2) or 100% APAAN (batch 3) as starting material. Shown are the main compounds with the relative signal intensities of each batch. The APAAN markers were most abundant in batch 3 whereas N-FA and the route specific marker 4M5PP were dominant in batch 1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Batch 1 [%]</th>
<th>Batch 2 [%]</th>
<th>Batch 3 [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzylnitrile</td>
<td>0</td>
<td>54.8</td>
<td>100</td>
</tr>
<tr>
<td>4M5PP</td>
<td>100</td>
<td>83.3</td>
<td>0</td>
</tr>
<tr>
<td>N-FA</td>
<td>100</td>
<td>56.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Marker 143</td>
<td>0</td>
<td>27.4</td>
<td>100</td>
</tr>
<tr>
<td>Marker 158</td>
<td>0</td>
<td>31.3</td>
<td>100</td>
</tr>
<tr>
<td>Marker 185</td>
<td>0</td>
<td>46.1</td>
<td>100</td>
</tr>
</tbody>
</table>

This means that for these markers to be present, the used BMK must contain traces of unreacted APAAN. It was pointed out earlier that it is very likely that some APAAN will be present after the conversion to BMK. Therefore, it is highly probable to find the proposed markers in an amphetamine sample that originated from the use of APAAN as pre-precursor.

### 3.1.3 Additional syntheses

Since the used APAAN contained benzylnitrile as contaminant an additional experiment was performed to eliminate this compound as source of the markers. Therefore, pure benzylnitrile was used instead of BMK for a Leuckart Step 1 reaction and the produced organic phase was analysed. Not one of the 4 marker peaks was present proving that the marker compounds are not formed from benzylnitrile under these conditions.

Another synthesis was performed using the structurally very similar compound α-phenylacetoacetamide (APAAN). This compound is an intermediate of the APAAN conversion into BMK and recent reports show that it is also used as pre-precursor since 2015. APAA was therefore used instead of BMK during Leuckart Step 1 and there were again no marker peaks present in the organic phase.

These additional syntheses were evidence that the proposed markers are highly specific for APAAN.

### 3.2 Marker identification

To identify each marker a combination of electron ionisation (EI) fragmentation patterns, high resolution masses and $^1$H/$^13$C NMR spectra was used. After confirming the structure of each marker possible mechanisms for their formation were proposed.

#### 3.2.1 Structure elucidation/characterisation

EI fragmentation patterns of each marker can be seen in Figure 2 and show that the compounds were likely of a low molecular mass below 200 g/mol. To determine the sum formula of each marker the high-resolution mass was determined using HR-GC–MS with APCI ionisation. NMR analyses of the purified markers were used to perform $^{13}$C and $^1$H as well as COSY, HSQC, and HMBC experiments which were used to elucidate the structure. The results on the structures of marker 143, 158, and 185 are given in Tables 2, 3, and 4, respectively.

Marker 143 was identified as 2-phenyl-2-butenenitrile which can exhibit 2 isomeric forms. During analysis, the (Z) isomer was found with a 10 times higher intensity. The same compound was analysed in 2008 by Kosjek et al, who also provided NMR data which match our measurements.

Marker 158 was identified as 3-amino-2-phenylbut-2-enenitrile which exhibits 2 isomeric forms. Looking at the NMR spectra it was found that (E) and (Z) configuration occurred in a ratio of 3:1 and therefore only data for the more abundant (E) isomer are given in Table 3.

The compound was described in 2009 by Li et al, who also provided NMR data which match our measurements. The structure explained the behaviour of this compound when coming into contact with an acid. We propose that this enamine is hydrolysed in an aqueous acidic environment since enamines are easily converted back to their...
carbonyl precursor by acid-catalysed hydrolysis. In this case APAAN will be formed which was confirmed by the experimental data.

Marker 185 was identified as 4-amino-6-methyl-5-phenylpyrimidine which is the same compound recently proposed by Power et al.\textsuperscript{28} This compound was mentioned in 1952 in a US patent but not related to APAAN or the production of amphetamine.\textsuperscript{36} It shows a structure similar to the route specific marker 4M5PP. It has a molar mass of 185.23 g/mol and the only difference to 4M5PP is an additional amino group which causes a high solubility in acidic water which was used to extract it from the organic phase.

### 3.2.2 Proposed mechanisms

Mechanisms for the formation of each marker are proposed based on the experimental data and are shown in Figure 4. Power et al.\textsuperscript{28} have proposed a mechanism for the formation of 4-amino-6-methyl-5-phenylpyrimidine but we propose an alternative one. Their mechanism involves the addition of 2 formamide molecules to 1 APAAN molecule before formaldehyde is eliminated during ring closure. The last step is a rearrangement which involves the elimination of water. Our mechanism is based on the formation of marker 158 which then reacts with a single formamide molecule to form marker 185 after a cyclization reaction.

### 3.3 Retrospective analysis

The occurrence of each marker in amphetamine profiling samples from Germany and the Netherlands was investigated to determine their prevalence in the timeframe 2009–2016. The used databases contained GC–MS impurity profiles of amphetamine sulphate preparations that were sent to the forensic laboratories for analysis. Only seizures with more than 500 g (the Netherlands) and 1000 g (Germany) are usually profiled which means that no street samples are included in these databases. Profiling was conducted following the harmonised method for amphetamine profiling.\textsuperscript{15–20}

First the German database was searched and the following compounds were included: 4-methyl-5-phenylpyrimidine as route specific marker for the Leuckart synthesis according to several studies.\textsuperscript{13,21,24} It was important to determine if the analysed amphetamine samples...
were synthesised using the Leuckart route because this is a requirement for the formation of the markers proposed in this paper. APAAN itself was included to determine how likely it is to find this pre-precursor in the final product. 2-methyl-1-phenyl-1,3-dicarbonitrile-1H-indene (Marker 256) as proposed by Power et al. and, finally, the 3 proposed markers of this work were included and the results are given in Table 5.

4M5PP showed a very high and constant occurrence over the years which confirmed that the Leuckart route is by far the most frequently used way to clandestinely synthesise amphetamine in Europe. Looking at the raw data it was visible that a missing 4M5PP signal also resulted in the missing of all investigated APAAN markers. This happened in only 2 cases and suggested that either purification step was implemented or that another synthesis route than the Leuckart method was used. Both reasons could lead to missing signals of 4M5PP and APAAN markers. APAAN was not found in any sample which led to the conclusion that this pre-precursor will not be carried over into the final product increasing the demand for reliable marker compounds. Two reasons for the missing APAAN signal are the conversion of APAAN into BMK during the 2 steps where strong acids are used (APAAN conversion; Leuckart Step 2) as well as an increased solubility of APAAN in alkaline water which is used after Leuckart Step 2 to separate the amphetamine freebase. Several samples contained small amounts of 2-methyl-1-phenyl-1,3-dicarbonitrile-1H-indene. The evaluation showed that this marker was present in small amounts in several samples from 2011 on and had a maximum occurrence in 2013 at 22%. However, the other markers showed a much higher prevalence demonstrating that this compound has only a limited usability as a reliable APAAN marker on its own.

As was shown during the syntheses experiments, marker 158 will likely be converted to APAAN during Leuckart Step 2 due to the acidic environment. Therefore, it was surprising that some samples contained this marker which could mean that the reaction conditions were not ideal during production. A possibility is also that the marker was formed under certain circumstances from APAAN which originated from the hydrolysis of marker 158 in the first place. This could probably happen during the neutralisation step if the organic phase is not immediately removed since the acid-catalysed hydrolysis of the enamine is reversible. Another explanation could be that dirty equipment was used during the synthesis which would lead to a contamination of the final product with marker 158.

Out of the 3 markers characterised in this paper, it was found that the markers 185 and 143 were the most significant. Marker 143 was found in samples from 2010 on and had its maximum occurrence in 2013 with 86%. Marker 185 showed even higher percentages going as high as 97% in 2013. Both markers showed a similar

<table>
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<th>Year</th>
<th>Sample size</th>
<th>4M5PP [%]</th>
<th>APAAN [%]</th>
<th>Marker 256 [%]</th>
<th>Marker 143 [%]</th>
<th>Marker 158 [%]</th>
<th>Marker 185 [%]</th>
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<th>4M5PP [%]</th>
<th>Marker 143 [%]</th>
<th>Marker 158 [%]</th>
<th>Marker 185 [%]</th>
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trend starting from 2010 and reaching a maximum in 2013. Looking at the signal intensities it was found that from 144 samples which contained either one of these 2 markers, 120 had marker 185 as the stronger signal.

Both markers were then also searched in the Dutch database to determine if there were similarities in the occurrence. The data of 358 different cases from 2009 to 2015 are given in Table 6. There were no data available for the second half of 2015 and 2016 which is the explanation for the smaller case number in 2015 and the missing data for 2016. A combination of both markers (143 + 185) seemed to give the most reliable results which is the reason the remaining interpretation was based on this.

Both markers first appeared in 2010 which correlated with the first reports of APAAN seizures in Europe in 2009. The first APAAN labs in the Netherlands were discovered in early 2011. Figure 5 shows the combined occurrence of markers 143 and 185 in amphetamine samples from the last few years in Germany and the Netherlands. It illustrates an increasing trend from 2010 on until it reaches a maximum in 2013 at around 100%. Both databases show a strong correlation indicating that these compounds are indeed reliable markers for the use of APAAN as pre-precursor. The years 2014 and 2015 were rather stable at around 90–100% occurrence indicating that the control of APAAN from 2013 on did not have an immediate effect on the use of APAAN. This stable trend is also in agreement with seizure reports since APAAN kept on being seized. It also supports the view that large quantities of APAAN might have been stockpiled before the control mechanisms were applied which were then used in the following years to keep the production going. This is the first time that analytical data of seized amphetamine showed that APAAN is by far the most important pre-precursor for the production of amphetamine in recent years.

Another observation was that looking at the German data the marker occurrence in amphetamine seems to decline which is indicated by a decrease of around 15% from 2015 to 2016. This could indicate that additional purification steps are suddenly implemented during the synthesis which seems to be very unlikely. A more plausible explanation is that APAAN is being replaced by other pre-precursors that are currently not under control leading to a decreased use of APAAN. As mentioned before one such pre-precursor is α-phenylacetocacetamide (APAAN) which is closely related to APAAN. As was shown in the syntheses section this compound does not form the mentioned marker substances when being used for amphetamine production. Further research will be required in the coming years to confirm this declining trend.

4 | CONCLUSION

In this study, 3 markers were isolated and characterised that indicate if amphetamine was produced from the pre-precursor APAAN. Two of them are proposed for the first time as APAAN markers. The compounds were identified as 2-phenyl-2-butenenitrile, 3-aminophenyl-2-butenenitrile, and 4-aminophenyl-5-phenylpyrimidine and all 3 are formed from unreacted APAAN during Leuckart Step 1. It was found that 3-aminophenyl-2-butenenitrile is not stable under acidic conditions and will therefore be hydrolysed during Leuckart Step 2. 2-phenyl-2-butenenitrile and 4-aminophenyl-5-phenylpyrimidine however will be carried over into the final product where they can be detected. Using a retrospective analysis of amphetamine sulphate profiling data from databases in Germany and the Netherlands it was shown that the proposed markers gave reliable information about the use of APAAN. A combination of 4-aminophenyl-5-phenylpyrimidine and 2-phenyl-2-butenenitrile was found to give the most reliable results for the use of APAAN and we propose to use both when determining if a sample originated from APAAN. Both reflected an increasing trend of APAAN use from 2010 on in both German and Dutch samples reaching a maximum between 2013 and 2015. Currently this trend seems to decline indicating that other pre-precursors are currently replacing APAAN. This research shows how important it is to identify new marker substances and to combine them with statistical data to draw conclusions about developments on the drug market.

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REFERENCES


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