

Validation Study of Analysis of 1-Phenyl-2-Propanone in Illicit Methamphetamine Samples by Dynamic Headspace Gas Chromatography Mass Spectrometry

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Abstract

A new method based on dynamic headspace sampling (DHS) coupled to GC/MS analysis was developed, optimized and validated for the analysis of 1-phenyl-2-propanone (P2P) in illicit methamphetamine (MAMP) samples. The DHS parameters were investigated to reach the sensitivity suitable for this kind of analysis. The method showed of a good specificity, linearity, accuracy, precision and robustness. The analysis of ten MAMP samples seized by the judicial authority was carried out. P2P was found in all the seizure, confirming that P2P is the starting compound of the synthesis of amphetamines.

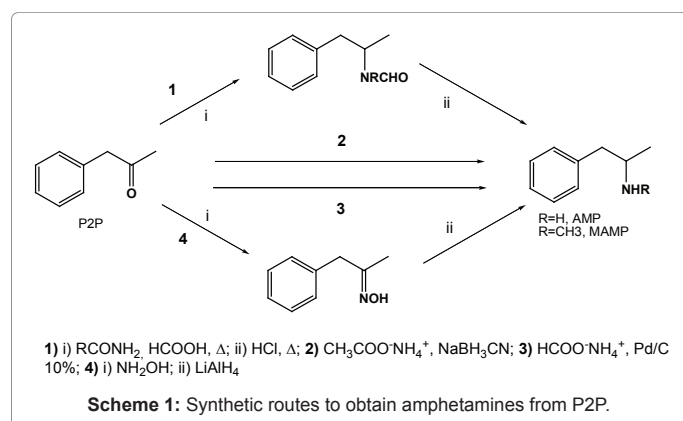
Keywords: 1-phenyl-2-propanone; P2P; Methamphetamine; DHS-GC/MS

Introduction

Amphetamine (AMP) and Methamphetamine (MAMP) are widely abused drugs all over the world [1,2]. In fact, the abuse of amphetamine-like compounds has increased continuously in the last years becoming a global problem in recent years [3]. For this reason, it is important to investigate how they are synthesized and where they are sold and diffused [4], analyzing their chemical impurity profiling. This information is useful for characterizing links between different samples originating from the same seizures, but also for determining the synthetic scheme [5,6], which gives details about the origin of the drug.

There are two major raw materials from which AMP and MAMP are prepared: ephedrine and pseudoephedrine and 1-phenyl-2-propanone (P2P). In order to prevent illicit drug manufacture, law enforcement authorities try to control illicit producers of the main drug precursor P2P (Scheme 1) [7,8].

P2P is colorless or slightly yellowish oil, with a density similar to that of water and a characteristic pleasant flavor [9]. P2P is listed in Table 1 of the United Nations Convention against Illicit Trafficking in Narcotic Drugs and Psychotropic Substances of 1988 and it is strictly controlled all over the world. It is prepared in clandestine laboratories starting from phenyl acetic acid [1]. The determination of P2P therefore is of utmost importance for determining the synthetic route by which MAMP is produced and its origin.



In a previous work we reported a method for determining P2P by static headspace gas chromatography (HS-GC/MS), which allowed us to resolve the case of a particular seizure. This finding was made of cornstarch soaked with P2P and it was sold as drug of abuse ("wet amphetamine") to deceive the consumer [10].

In this frame we were interested in applying the same method for the determination of P2P in seized methamphetamine samples, but we realized that it was not sensitive enough for this kind of analysis. So, we developed, optimized and validated a method based on dynamic headspace sampling [11-16], used for the first time in this field, which allowed us to obtain the optimal features for the analysis of impurities in illicit drug samples of MAMP. In this paper we report the results obtained from the analysis of samples containing MAMP seized by the judicial authority and delivered to our laboratory. In all these samples we detected traces of P2P as residual solvent.

Materials and Methods

Reagents and chemicals

All reagents were of analytical grade and were stored as indicated by the supplier. Propylene carbonate and acetophenone, chosen as internal standard (IS), were purchased from Sigma Aldrich (St. Louis, MO, USA). Water ($18.2 \text{ M}\Omega\cdot\text{cm}^{-1}$) was prepared by a Milli-Q System (Millipore, Darmstadt, Germany). Stock solutions of P2P (1 mg/mL) and IS (0.02 mg/mL) were prepared in propylene carbonate.

Synthesis of 1-phenyl-2-propanone: 1-Phenyl-2-propanol (1.36 g, 10 mmol) and a catalytic amount of PCC (5 mg) were added to a solution of periodic acid (2.28 g, 10 mmol) in acetonitrile (50 mL) at 0°C. The mixture was stirred for 30 min and then ethyl acetate (50 mL)

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was added. The organic phase was washed with brine (50 mL) and a saturated solution of sodium sulfite (50 mL) and dried with anhydrous sodium sulfate; and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (cyclohexane/ethyl acetate, 97/3, v/v). The yield was 90%. The structure of the synthesized compound was confirmed by ¹H-nuclear magnetic resonance (NMR) spectroscopy. The NMR spectrum was recorded in DMSO-*d*₆ at 300 MHz. The chemical shifts (data in δ ppm) were 2.15 (s, 3H); 3.70 (s, 2H); 7.18-7.22 (m, 2H); 7.22-7.38 (m, 3H).

Instruments

GC/MS: Analyses were carried on a HP5890 Series II GC system (Agilent, Santa Clara, CA), with a split-splitless injection system operated in a split mode and an Agilent MSD HP5971 Detector operated in electron impact mode (70 eV). The GC was equipped with a capillary column VF-624MS (60 m, 0.25 mm i.d., film thickness 1.4 μm) (Agilent, Santa Clara, CA) and a Master DHS autosampler (DANI Instruments, Milano, Italy) with 20 mL headspace vials.

The GC/MS system was operated under following conditions: split ratio, 60:1; solvent delay, 4.5 min; injector temperature, 280°C; interface transfer line, 300°C; ion source, 180°C; oven temperature program, from 100°C to 250°C, at 10°C/min, final isotherm, 10 min. Analysis time 25 min (Figure 1).

Helium was used as the carrier gas at a flow rate of 1.0 mL/min. For qualitative analysis the MS detector was operated in SCAN mode, mass range: 10 to 550 m/z. For quantitative determinations, the MS detector was operated in SIM mode. Solvent delay: 4.5 min. From 4.51 to 13.20 min only the m/z=51, 77, 105, 120 ions were acquired for the IS and the m/z=43, 65, 91, 134 ions were acquired for P2P. Quantitative analysis was performed integrating the peak at m/z 105 for the IS and 91 for P2P.

Dynamic headspace analysis: Incubation temperature: 150°C with slow shaking; Incubation time: 30 min; Stripping time: 5 min; Stripping flow: 100 mL/min; Stripping carrier: Nitrogen; Trap: Tenax® GR (70% porous polymers based on 2,6-diphenyl-p-phenylene oxide, 30% graphite carbon, Scientific Instruments Services, Inc Old York RD, Ringoes, NJ); Trap adsorption temp: 0°C; Dry step time: 10 min; Dry Step Flow: 30 mL/min; Dry Step Trap Temp: 40°C; Injection time: 1 min; Trap desorption temperature: 280°C; Dew Stop temperature: 200°C; Transfer line temperature: 280°C; Switching valve temperature: 250°C.

Samples and sample preparation

Ten samples seized by the judicial authority in 2014 were analyzed (Figure 2). The samples were characterized by different content of MAMP (Table 1) and different color and crystal shape.

In a 20 mL headspace vial, 25 mg of powder were weighed and added with 1 mL of IS and 2 mL of propylene carbonate. For the preparation of working Standard Samples (WSS) in a 20 mL headspace vial, different volumes of the stock solution of P2P (see 2.1) were made up to 2 mL with propylene carbonate and 1 mL of IS was added.

Results and Discussion

Applying the conditions previously developed [10], we were able to obtain peaks with a good shape and a good chromatographic response either for P2P or for the IS (acetophenone). On the other hand, we were interested in increasing sensitivity in order to reach a LOD suitable for the analysis of impurities in drugs of abuse. To this end, we studied and optimized the DHS parameters.

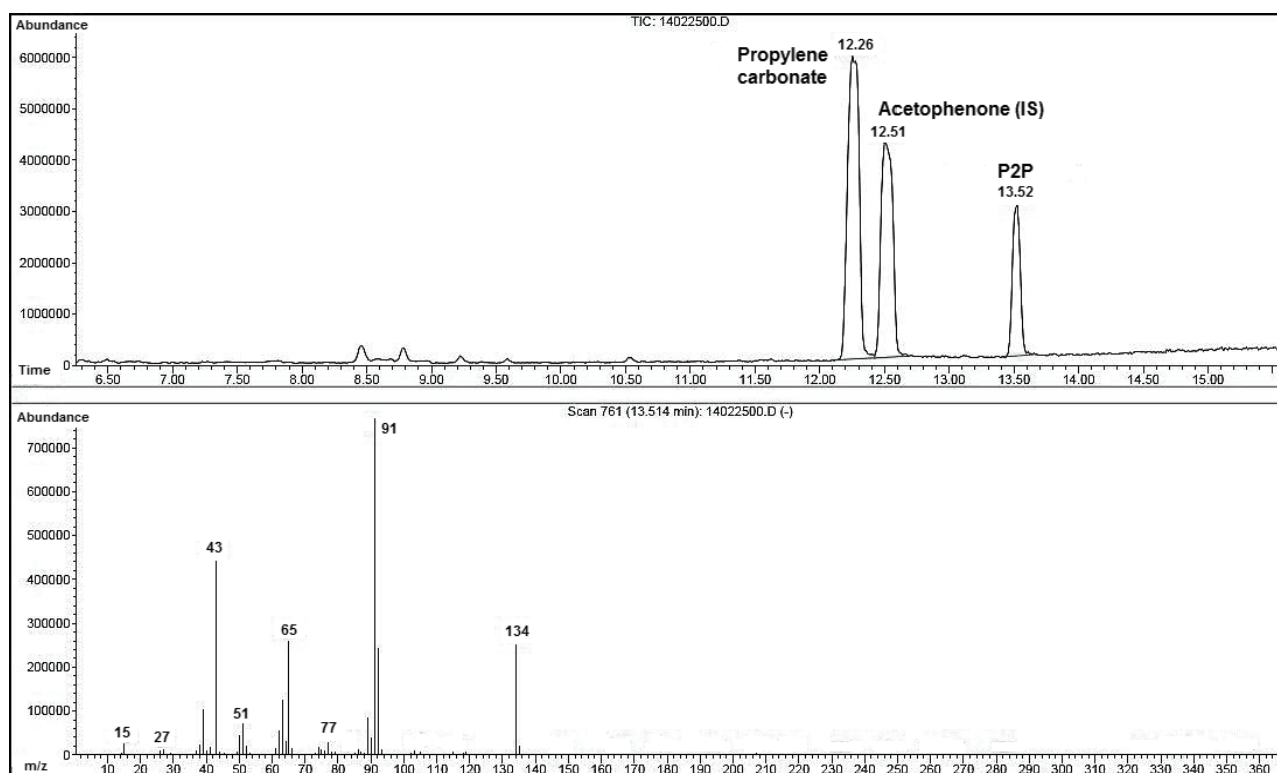


Figure 1: DHS-GC/MS SCAN chromatogram and spectrum of a working standard solution (WSS) of 1-phenyl-2-propanone (P2P).



Figure 2: Sample seized by the judicial authority.

Sample	% of MAMP	% P2P
1	83.8	0.287
2	88.2	0.359
3	89.9	0.188
4	90.4	0.426
5	77.6	0.398
6	98.3	0.335
7	91.8	0.277
8	95.8	0.299
9	96.8	0.123
10	95.5	0.619

Table 1: % of methamphetamine and P2P contained in the different seizures.

Optimization of DHS parameters

Incubation time and temperature: In the preliminary analysis [10], the incubation temperature was set at 80°C and the incubation time was 15 min. To favor the transition to the vapor phase of P2P, thus increasing extraction efficiency, we tested an increase of the incubation time (30 min) and an increase of the incubation temperature (100°C). As it is evident from Figure 3, increasing both the temperature and the incubation time led to a marked gain in extraction recovery. When analyzing a real sample of methamphetamine, on the other hand, the peak relative to P2P was not detected, so we decided to further increase the incubation temperature to 150°C. In this way, the chromatographic peak of P2P was evidenced.

Stripping: The duration of the stripping step is very important to transfer all the analyte to the vapor phase. We tested three different stripping times: 1, 5 and 10 min. Going from 1 to 5 min, there is a significant increase in extraction recovery either for P2P or for the IS, while with a stripping time of 10 min, there is a dramatic increase in the response of acetophenone (IS) but the peak related to P2P remains almost unchanged (Figure 4). So, the ideal stripping time resulted to be 5 min.

Trap adsorption temperature: Generally, the lower the trap's temperature, the greater is the adsorption of the analytes. Three different temperatures were tested: -10, 0 and 10°C. The worst result was obtained at 10°C; between 0 and -10°C there is not a big difference, but, at 0°C, the peaks have a sharper shape and propylene carbonate, the solvent, is less adsorbed (Figure 5).

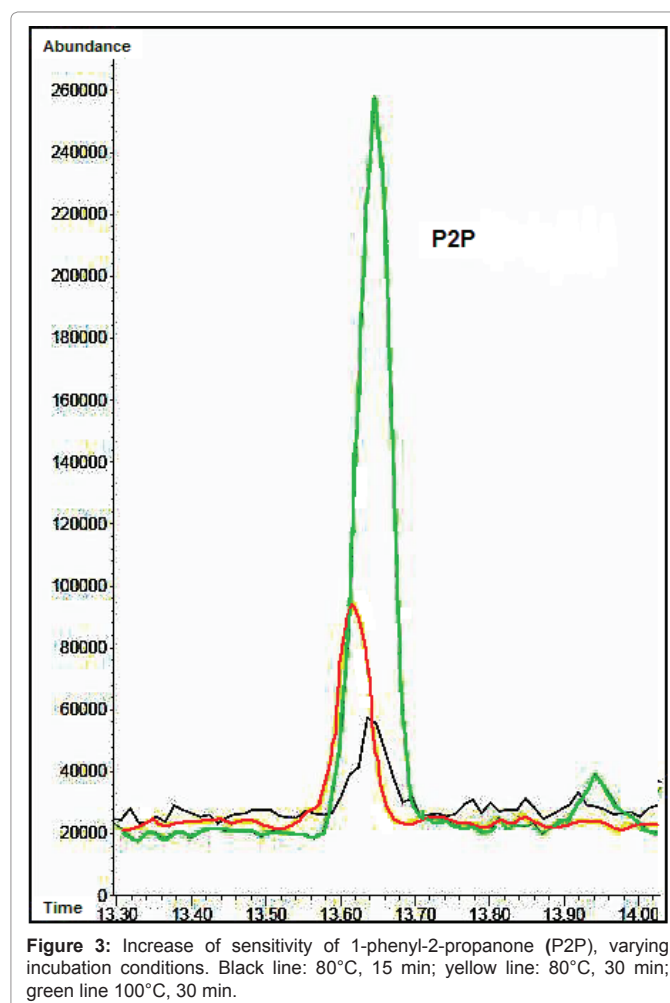


Figure 3: Increase of sensitivity of 1-phenyl-2-propanone (P2P), varying incubation conditions. Black line: 80°C, 15 min; yellow line: 80°C, 30 min; green line 100°C, 30 min.

Injection: The injection of the sample is a crucial step. The analyte has to be desorbed in the shortest time as possible, to obtain sharp peaks and increase sensitivity. We evaluated that the best results were obtained with 1 min of injection time and an inlet and transfer line temperatures of 280°C, in order to ensure the immediate volatilization of the analytes and to avoid the formation of condensate inside the instrument.

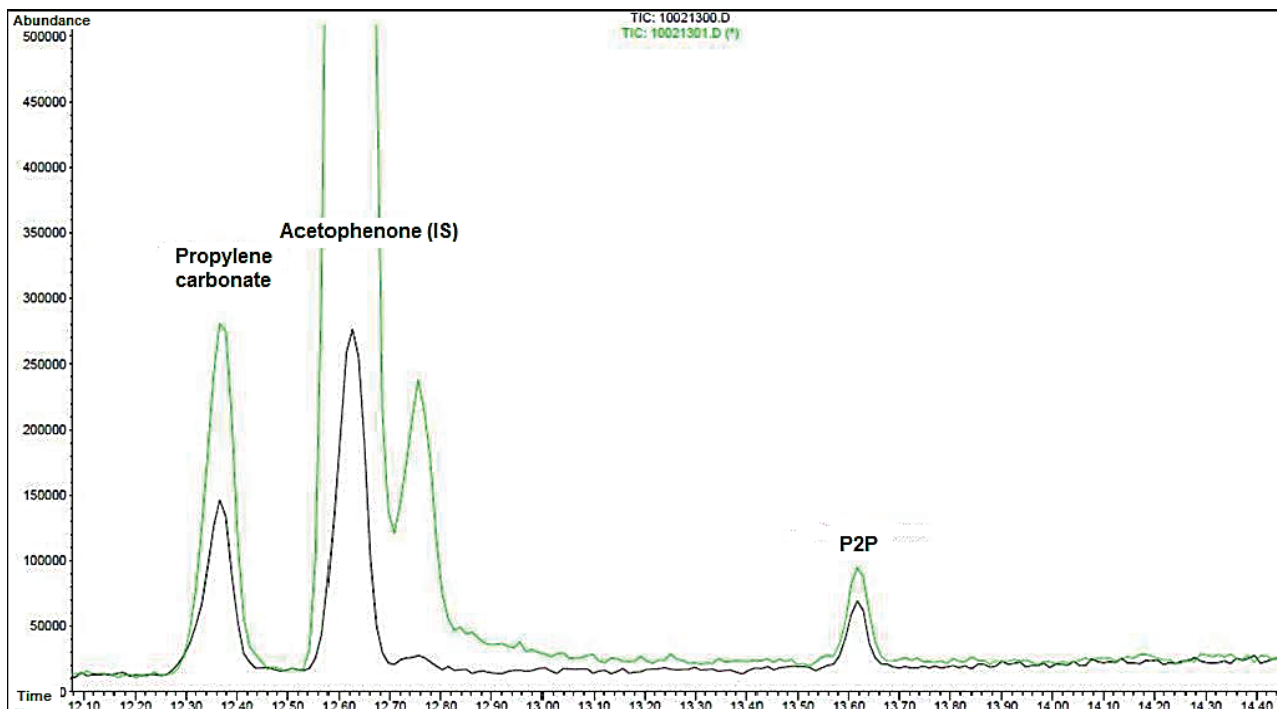


Figure 4: Increase of sensitivity for 1-phenyl-2-propanone (P2P) and acetophenone (IS), varying stripping time. Green line: 10 min; black line: 5 min.

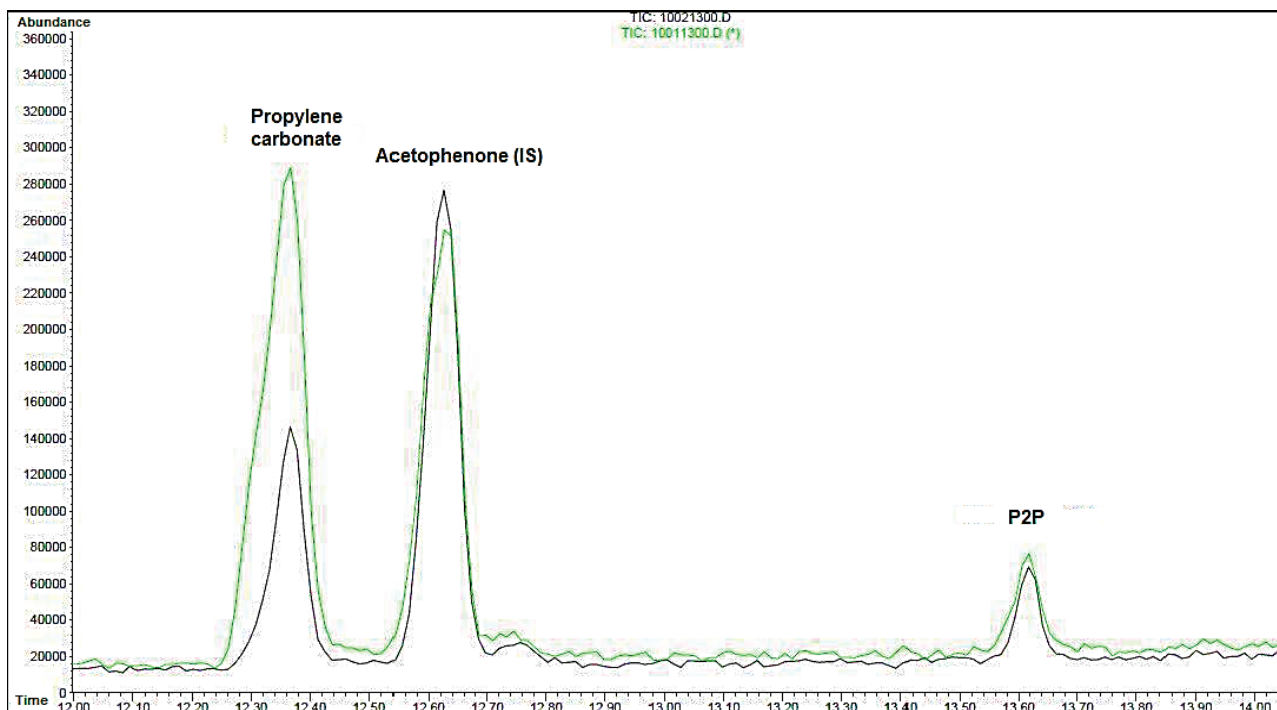


Figure 5: Peak shape at different trap adsorption temperatures. Green line: -10°C; black line: 0°C.

Validation of the DHS-GC/MS method

The DHS-GC/MS method was validated [17] for the identification and quantification of the analytes meeting the requirements of forensic analysis, analyzing WSS specifically prepared.

Specificity: When dealing with seized material synthesized in an illicit way, it is not possible to analyze a “matrix” identical to that of the seizures, which does not contain the analytes. Specificity is assessed if the retention time of standard P2P corresponds perfectly to that of the peak found in unknown samples and the ratio among the four

characteristic ions of P2P (m/z 43, 65, 91, 134) is found identical either in standard P2P or in seized MAMP samples.

Linearity: A series of WSS was prepared, made up of seven solutions at the following concentrations of P2P: 10, 50, 100, 150, 200, 250 and 300 μg_{tot} . Each solution was put in a 20 mL headspace vial, starting from the following volumes of the stock solution of P2P (1 mg/mL): 10, 50, 100, 150, 200 250 and 300 μL made up to 2 mL with propylene carbonate, then 1 mL of IS was added. Three series of WSS were analyzed.

The linearity of the method was adequate in all the range. The equation was $y=5.10 \times 10^{-3}-4.39 \times 10^{-2}$ ($R^2=0.9987$).

The calibration curve was built plotting the ratio between the area of the P2P peak and the IS peak against the ratio between the concentration of P2P and the concentration of the IS; the areas were obtained integrating the peaks related to one single ion for each analyte (m/z 105 for IS and 91 for P2P). In this way, it was possible to minimize the noise, increasing accuracy and precision.

LOQ: LOQ was considered as the lowest concentration in which linearity was still satisfied and it corresponded to 10 μg_{tot} of P2P. At this concentration, the signal to noise ratio was 10.83.

LOD: LOD was assessed progressively diluting the solution prepared for the determination of the LOQ, until a signal to noise ratio of 3 was reached. LOD for P2P resulted to be 5 μg_{tot} .

Accuracy: Three different WSS were analyzed in triplicate. In different headspace vials, 50, 100, 150 μL of stock solution of P2P were put and made up to 2 mL with propylene carbonated. Then, 1 mL of IS was added.

Accuracy was evaluated as % recovery according to the following formula:

$$\% \text{REC} = (\text{Analytical concentration} / \text{real concentration}) \times 100$$

Where the analytical concentration is the concentration of P2P calculated on the basis of the calibration curve and the real concentration is the effective concentration of P2P (μg_{tot}) present in each sample.

% REC ranged from 98.33 to 104.93%, showing a good degree of accuracy of the method.

Precision: In a headspace vial, 100 μL of a P2P stock solution (1 mg/mL) were added to 1 mL of IS and made up to 3 mL with propylene carbonate. Intra-day precision was assessed analyzing six samples in the same day in the same operative conditions.

The mean RA ($A_{\text{P2P}}/A_{\text{IS}}$), SD and %RSD were respectively $6.22 \cdot 10^{-2}$, $2.71 \cdot 10^{-3}$ and 4.35%. Inter-day precision was evaluated analyzing other six samples in a different day and combining the results with those of intra-day precision. The mean RA, SD and %RSD resulted respectively $6.08 \cdot 10^{-2}$, $3.05 \cdot 10^{-3}$ and 5.02%, confirming the good precision of the method.

Robustness: Robustness was determined analyzing a solution prepared as described in 3.2.6 with a 10% increase of the carrier gas flow (from 18.6 to 20.5 psi). The carrier gas flow directly affects the desorption of the analyte from the trap. % REC for P2P was 98.82%. The method therefore showed a good degree of robustness.

Quantitative determination of P2P in MAMP samples seized on the illegal market

The validated DHS-GC method was applied for the determination of P2P in ten methamphetamine samples seized by the judicial authority (Figure 2). The results are shown in Table 1. P2P was found in all the samples, demonstrating that P2P is at the moment the most frequently used precursor for the synthesis of MAMP [6,7]. In Figure 6, the chromatogram obtained for Sample 1 is shown. The large peak

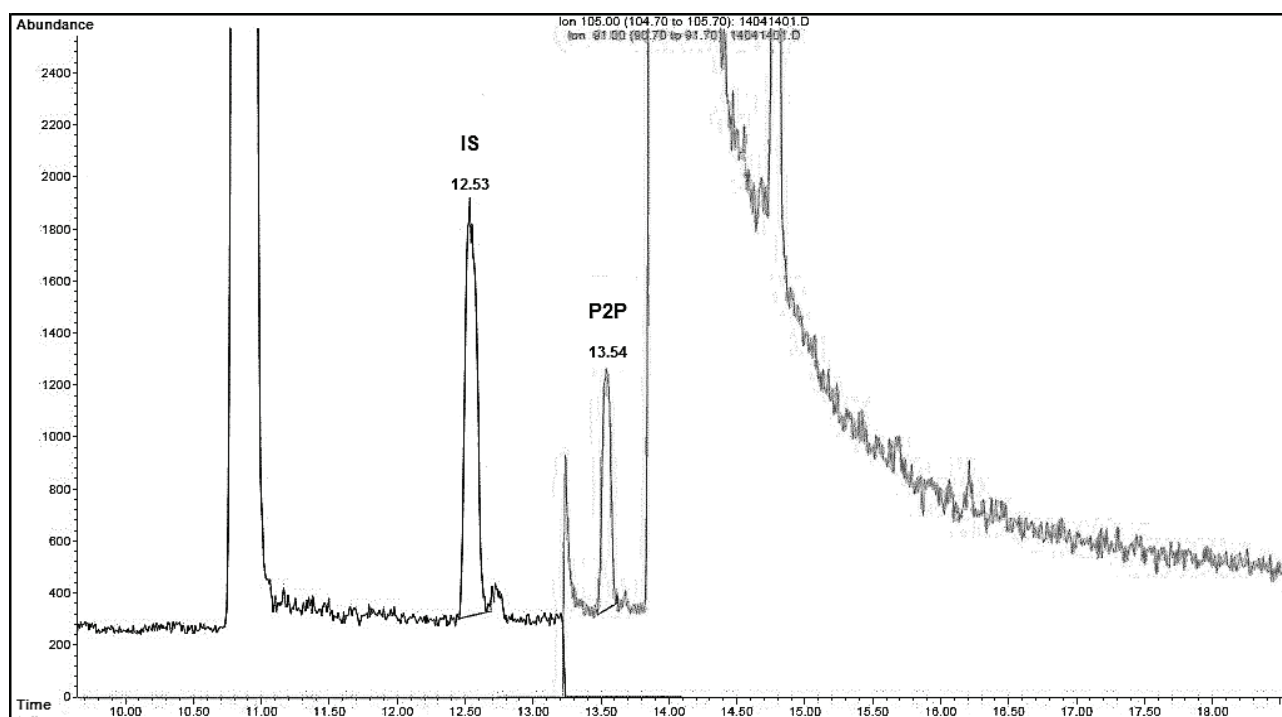


Figure 6: DHS-GC/MS chromatogram of the seized powder.

is due to methamphetamine that in the operative conditions passes to the vapor phase but it does not interfere with the analysis of P2P. It is interesting to note that the % of P2P is not correlated with the % of MAMP. There might have been expected that the more MAMP is pure the less is the concentration of P2P, but in Sample 1, which is less pure respect to Sample 10, the % of P2P instead of being higher resulted lower.

Concluding Remarks

A simple, sensitive and efficient dynamic headspace gas chromatography (DHS-GC/MS) method was optimized and validated for the analysis of P2P in MAMP samples seized by the judicial authority; P2P was found in all the seizure, confirming that P2P is the starting compound of the synthesis of amphetamines (Scheme 1). This method could be used to determine the impurity profile of a MAMP sample, thus establishing similarities among different production batches and laboratories in which the synthesis of the drug of abuse was carried out illegally.

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