

IRMS to study a common cocaine cutting agent: phenacetin

Virginie Ladroue,^{a*} Laurence Dujourdy,^b Fabrice Besacier^a and Patrick Jame^c

Phenacetin is a pharmaceutical closely related to acetaminophen that has been banned in France for a long time due to its nephritic and carcinogenic adverse effects. It frequently appears in cocaine seizures as a cutting agent. Following both sanitary and intelligence motivations, this molecule was chosen for this study, and stable isotopes seemed to be the most appropriate tool. A total of 228 seized samples were collected over a 6-year period, and 8 standards of known origin were purchased. They were submitted to gas chromatography (GC) or elemental analysis - isotope ratio mass spectrometry (EA-IRMS) measurements, depending on their complexity. Stable isotope ratios of carbon, hydrogen, and nitrogen for a part of the sample set, were acquired. The isotopic values of phenacetin standards acquired from various providers located worldwide are quite spread, which indicates that stable isotopes could be used to discriminate manufacturers. However, the measured values of most of the seized samples are concentrated in a narrow range, tending to demonstrate that phenacetin is smuggled from a single source or similar ones. Consequently, stable isotopes could only be used to exclude that several samples come from a common source. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: phenacetin; stable isotopes; ^{13}C ; ^{15}N ; ^2H

Introduction

Cocaine sold in Europe contains many cutting agents and recent studies have emphasized how crucial it is to investigate this aspect of the drug.^[1,2] For over two decades now,^[3] French police forensic laboratories have issued a yearly report on their analyzed samples, in an effort to monitor the illicit drugs market in France. It describes two kinds of cocaine-containing samples: airport seizures on the one hand, showing levels of cocaine between 60 and 80% (the remaining 20–40% being alkaloids from the coca paste and a few cutting agents added at the production site), and samples containing between 20 and 50 % of cocaine, on the other hand. Between wholesale (air import) and street cocaine, various cutting agents are added. Comparison of these two kinds of seizures indicates that some phenacetin is added in the country of origin, but most of it is added in Europe.^[4,5]

Among the usual cocaine cutting agents, phenacetin was chosen for this study as it features both strategic and sanitary interests.

Phenacetin is an analgesic and antipyretic, closely related to acetaminophen^[6] (Figure 1). Once widely used, it has been progressively banned since 1978^[7] due to its carcinogenic and nephritic adverse effects. In France, it was commercialized for pharmaceutical purposes until 1994^[8] and in 1995, phenacetin first appeared in cocaine samples.^[3] Today, phenacetin is present in 28% of the cocaine cases treated by the laboratories of the French Ministry of the Interior.^[4]

When found in cocaine samples, phenacetin never appears with pharmaceutical excipients. This led to the thinking that phenacetin used as a cutting agent is not diverted from a legitimate over-the-counter trade but smuggled from other sources. To feed the cocaine market (62 tons seized in 2014^[9]), large amounts of phenacetin must be smuggled into Europe every year. Considering the limited legitimate market for phenacetin and its wide use as a

cutting agent, networks in charge of this trade are likely closely interconnected with the cocaine supply chain. In that perspective, tracking phenacetin is expected to be a valuable complement to law enforcement intelligence.

As phenacetin is thought to originate from pharmaceutical production sites, profiling methods^[10] classically used by forensic scientists to map trafficking networks, such as synthetic impurities or residual solvents, can hardly be applied. Isotope ratio mass spectrometry (IRMS)^[11] therefore appears as a promising alternative to trace, and possibly source our molecule of interest.

In this work, the potential of stable isotopes to trace phenacetin was evaluated. For that purpose, phenacetin reference materials were purchased from various manufacturers located worldwide and seized samples submitted to the Police Forensic Laboratory of Lyon for analysis were collected. Pure phenacetin samples were submitted to elemental analysis - isotope ratio mass spectrometry (EA-IRMS) to determine their $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^2\text{H}$. Samples of cocaine cut with phenacetin, were measured by gas chromatography-combustion/pyrolysis-isotope ratio mass spectrometry (GC-C/P-IRMS) to retrieve the $\delta^{13}\text{C}$ and $\delta^2\text{H}$ of phenacetin.

* Correspondence to: Virginie Ladroue, Institut National de Police Scientifique, Laboratoire de Lyon, 31 avenue Franklin Roosevelt, 69134 Ecully Cedex, France. E-mail: virginie.ladroue@interieur.gouv.fr

a Institut National de Police Scientifique, Laboratoire de Lyon, 31 avenue Franklin Roosevelt, 69134 Ecully Cedex, France

b Institut National de Police Scientifique, Service Central des Laboratoires, 31 avenue Franklin Roosevelt, 69134 Ecully Cedex, France

c Institut des Sciences Analytiques, 5 rue de la Doua, 69100 Villeurbanne, France

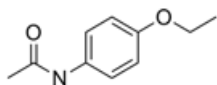


Figure 1. Structure of phenacetin.

Materials and methods

Samples and standards

A total of 169 seized cocaine samples cut with phenacetin were collected, together with 59 seized pure phenacetin samples. They issued from samples submitted to the Police Forensic Laboratory of Lyon (France) for routine analysis over a 6-year period. Eight chemical standards of pure phenacetin were purchased from different manufacturers and originated from different countries: Canada (CA), Germany (DE), USA (US), Switzerland (CH), and China (ZH). Three remained of unknown geographical origin.

To feature consistent results, an in-house phenacetin standard (provided by Sigma, part of Sigma-Aldrich Corporation, St Louis, MO, USA, batch #088 k1622, purity > 99.9%) was calibrated against IAEA reference materials in EA-C-IRMS (USGS-25, $\delta^{15}\text{N}_{\text{Air-N}_2} = -30.4\text{‰}$; IAEA-CH6, $\delta^{13}\text{C}_{\text{VPDB}} = -10.449\text{‰}$) and EA-P-IRMS (IAEA-CH7, $\delta^{2}\text{H}_{\text{VSMOW}} = -100.30\text{‰}$). The subsequent values assigned to the working in-house standard were $\delta^{13}\text{C} = -26.65\text{‰}$, $\delta^{15}\text{N} = -4.42\text{‰}$ and $\delta^{2}\text{H} = -163.52\text{‰}$. All reference materials originated from the International Atomic Energy Agency (IAEA, Vienna, Austria).

Sample preparation

All samples were homogenized in agate mortars prior to analysis. EA-C samples were prepared in tin capsules and EA-P samples in silver capsules from Elementar GmbH (Hanau, Germany). The amount of sample weighed was adapted so that the peak would be in the working linearity range of the instrument. GC samples were solubilized in EtOH/CHCl₃ (quality for analysis, provided by Carlo Erba Reagents, Milan, Italy) 50/50 v/v. For ¹³C measurements, 1 mg of powder was weighed; the analytical parameters were adjusted afterwards depending on the phenacetin content in the sample, so as to stay in the linearity range of the mass spectrometer. In contrast, the amount of powder weighed for ²H measurements was calculated in order to obtain a peak of acceptable intensity.

Instrumentation

The pure products were analyzed on a Vario Micro or a Vario Pyro cube elemental analyzer (Elementar GmbH, Hanau, Germany) coupled to an Isoprime JB isotope ratio mass spectrometer (Isoprime Ltd, Cheadle, UK). Carbon and nitrogen data were acquired in a single analysis using a dual CN method. The sample capsule was introduced into the combustion tube filled with chromium oxide heated at 950 °C in the presence of O₂. The copper packing of the reduction tube was held at 650 °C. For ²H measurement in EA-IRMS, the sample was thermally converted into elementary gases in a glassy carbon reactor heated at 1 450 °C.

Part of the H₂ data obtained on EA-IRMS presented here was acquired by our partner for this study, the Service Central d'Analyse (SCA). They were performed on a Thermo Flash EA-1112 HT (O/H-N/C) elemental analyzer coupled to a Thermo Delta V Plus isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). In this system, the pyrolysis unit was operated at a temperature of 1450 °C and was made of a vitreous glassy carbon tube half filled with black carbon granulates, incorporated into an

external ceramic tube. About 0.3 mg of sample was dropped into the pyrolysis unit in a stream of 80 mL/min of helium. Hydrogen was converted into H₂ gas and introduced in the source of the mass spectrometer owing to the Conflo IV interface. IAEA international reference materials IAEA-CH7 ($\delta^{2/1}\text{H}_{\text{VSMOW}}: -100.3\text{‰}$) and NBS 22 ($\delta^{2/1}\text{H}_{\text{VSMOW}}: -116.9\text{‰}$) were regularly analyzed for checking a non-deviation of the analytical system.

Comparability of the measurements was assessed via an inter-laboratory study over 8 phenacetin standards and 11 seized phenacetin samples (data not shown here for brevity). It demonstrated that values measured in both laboratories were consistent.

Cocaine samples were chromatographed on a 7890 gas chromatograph from Agilent (Santa Clara, CA, USA) coupled to a combustion/pyrolysis furnace prior to continuous flow measurement by the mass spectrometer mentioned above. Chromatography was performed on a J&W HP-5 capillary column from Agilent (Santa Clara, CA, USA) which dimensions were 30 m x 320 μm x 0.25 μm. The GC methods differed for ¹³C and ²H measurement.

For ¹³C measurements (Figure 2), the following gradient was applied starting at 75 °C for 2 minutes, the temperature was then raised at 15 °C/min up to 225 °C, then at 20 °C/min up to 290 °C and kept at 290 °C for 2 min. The injector temperature was set at 280 °C. A constant flow of helium at 1.20 mL/min circulated in the capillary column and by means of the heart-split device, directed either toward the FID (flame ionization detector) operated at 290 °C (H₂ flow 30 mL/min, air flow 400 mL/min), or to the combustion furnace. The combustion furnace was a quartz tube filled with CuO pellets held at 850 °C, silver wool was added at the IRMS end of the tube to trap halogens emanating from cocaine HCl samples. The interface was heated at 350 °C. A nafion membrane was placed after the combustion tube in order to remove the water from the gas stream by an osmosis process.

Measure of phenacetin's $\delta^{15}\text{N}$ by GC-C-IRMS was unsuccessfully attempted.

^{2/1}H measurements of complex samples were performed using a temperature gradient consisting of 7 steps, each one being reached at a 20 °C/min rate. Levels were at 75 °C during 2 min (initial temperature), 170 °C during 4 min, 180 °C during 6 min, 200 °C during 5 min, 210 °C during 5 min, 300 °C during 5 min and 320 °C during 0.75 min (final temperature). Carrier gas helium was introduced at a constant flow of 1.75 mL/min. The pyrolysis furnace was filled with chromium pellets and heated at 1050 °C. The interface was kept at 350 °C.

Data treatment

All samples were analyzed in triplicate. To validate a measure, the standard deviation between these values had to fall within a specification given by the instrument manufacturer (0.15 ‰ in EA ¹³C, 0.20 ‰ in EA ¹⁵N, 2.00 ‰ in EA ²H, 0.20 ‰ in GC ¹³C, and 4.00 ‰ in GC ²H). The average value was then assigned to the sample. Every three (GC ²H) or five (EA and GC ¹³C) samples, an in-house phenacetin standard was introduced. The in-house standards that bracketed the unknown samples were used to correct their data by a linear interpolation, to correct the natural magnet drift. Most of the data shown here have been acquired in 2010 and 2011, this is why the one-point-normalization was applied, and why USGS-25 was used as a reference material despite subsequent methodological recommendations.^[12,13]

^{2/1}H data shown here was not corrected for atmospheric hydrogen contribution.

Graphics were produced with Libre Office version 4.2.6.

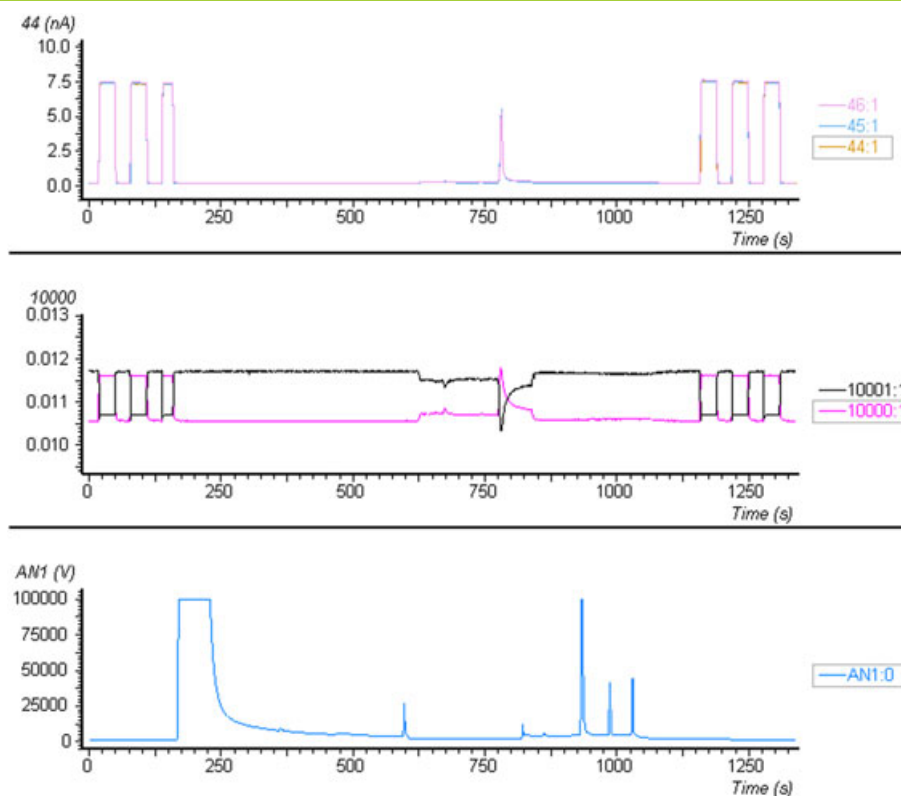


Figure 2. Chromatograms of a GC-C analysis. Plotted signals are, from top to bottom, beams of the masses 44, 45, 46, beam ratios and FID signal. IRMS signals show pulses of reference gas, and the CO₂ peak of phenacetin. FID signal shows the peaks of dissolution solvent, cocaine and alkaloids (this sample contained no other cutting agent than phenacetin - on the IRMS signal - and mannitol - not visible). [Colour figure can be viewed at wileyonlinelibrary.com]

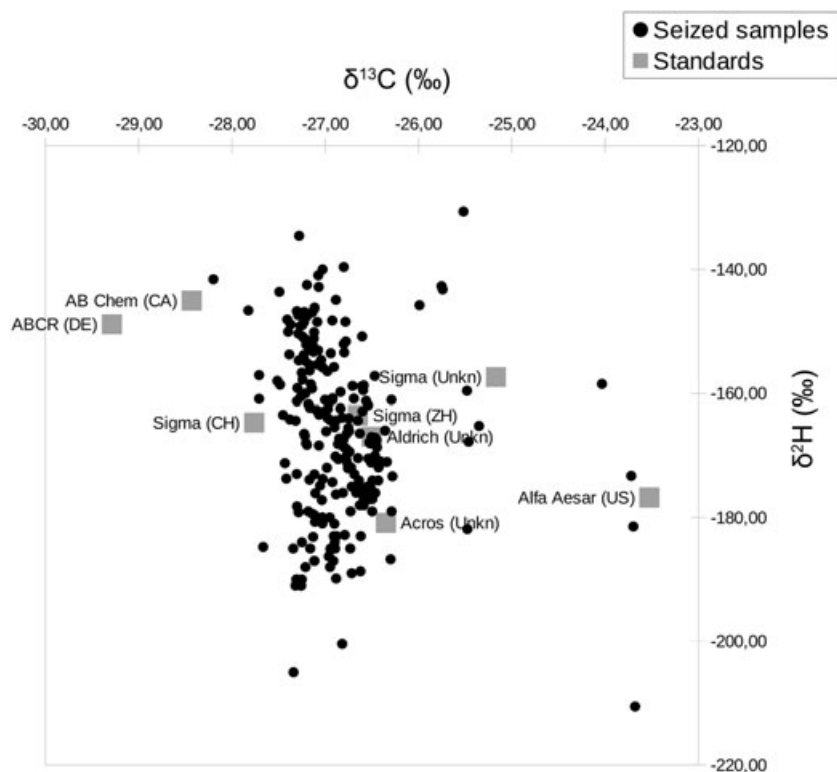


Figure 3. Hydrogen vs carbon data for all samples and standards (phenacetin from cut cocaine analyzed by GC, pure phenacetin analyzed by EA).

Results and discussion

For the 228 seized samples, the $\delta^{13}\text{C}$ values ranged from -28.20‰ to -23.68‰ , and the $\delta^2\text{H}$ from -210‰ to -130‰ . These values were measured either by GC (cocaine cut with phenacetin) or EA (pure phenacetin) coupled to IRMS. Only 59 of the 228 street samples were submitted to nitrogen measurements. These were the pure phenacetin samples analyzed by EA-IRMS. Their $\delta^{15}\text{N}$ varied between -5.28‰ and -0.11‰ . All the standards were analyzed by EA-IRMS, and their isotopic values spread from -29.29‰ to -23.52‰ in ^{13}C , from -16.5‰ to -2.5‰ in ^{15}N and from -181‰ to -145‰ in ^2H .

Plots of ^2H vs ^{13}C data and ^{15}N vs ^{13}C data are pictured in Figures 3 and 4, respectively. They show that most of the seized samples are concentrated in a narrow range, and that they do not fully reflect the diversity exhibited by the standards. This distribution leads to thinking that most of the samples are provided by a single manufacturer or similar ones.

Interestingly, Figures 3 and 4 also show that a few samples do not fall in the vicinity of one of the standards, which seems to show that the standard collection was not completely exhaustive. Unfortunately, most of the standards were purchased at the end of the study and it has not been possible to extend this collection to more manufacturers, or even various batches from the same provider.

The seized sample set consisted of 228 samples originating from 71 cases. To assess the potential of IRMS to track phenacetin, this collection was sub-divided into two populations: Linked and Not Linked samples.

Some cases contained multiple samples. Since some of them showed a great homogeneity in the carbon and hydrogen data, they were considered replicates of a single phenacetin production batch. The hypothesis was made that the traffickers had sub-

sampled their phenacetin stock, or that they had used the same batch to dilute a large amount of cocaine, which was later seized and analyzed as multiple doses. These cases were selected to determine the magnitude of intra-batch variability that can be expected in a population. A total of 12 cases containing between 3 and 27 samples were used for this. They constitute the Linked population.

To establish the Not Linked population, 35 samples were selected. They issued from seizures realized either 6 months apart in a given geographical area, or in very different locations. Similarly to illicit drugs, the hypothesis was made that it takes approximately six months for drug traffickers to disseminate a batch of phenacetin.

Correlations inside each population were calculated using the cosine function or Euclidian distance (for formulas, see Anderson *et al.*^[14]). The diagram of correlation based on the Euclidian distance (Figure 5) shows a systematic overlap between both populations in every calculated class. Whatever the threshold chosen, the amount of false positive and false negative results is considerable. For example, if the threshold is chosen at 0.39, which corresponds to the first two classes, 3.8 % of the Not Linked correlations are wrongly considered as Linked (false positives), and 49.9 % of the Linked correlations are labelled as Not Linked (false negatives). Alternatively, if the threshold is fixed at a higher value, 1.18 for instance, the false negatives dramatically fall to 2.6 % but the false positives rise to 26.5 %. In our experience of drug profiling, such poor performances are considered insufficient to validate the method as a valuable tool to trace phenacetin samples. Carbon and hydrogen alone therefore do not seem sufficient to discriminate phenacetin samples.

The same procedure was applied on samples for which ^{13}C , ^2H , and ^{15}N data were available. This time the sample sets were much smaller as only few samples had the three elements measured: 47 samples issuing from 5 different cases composed the Linked

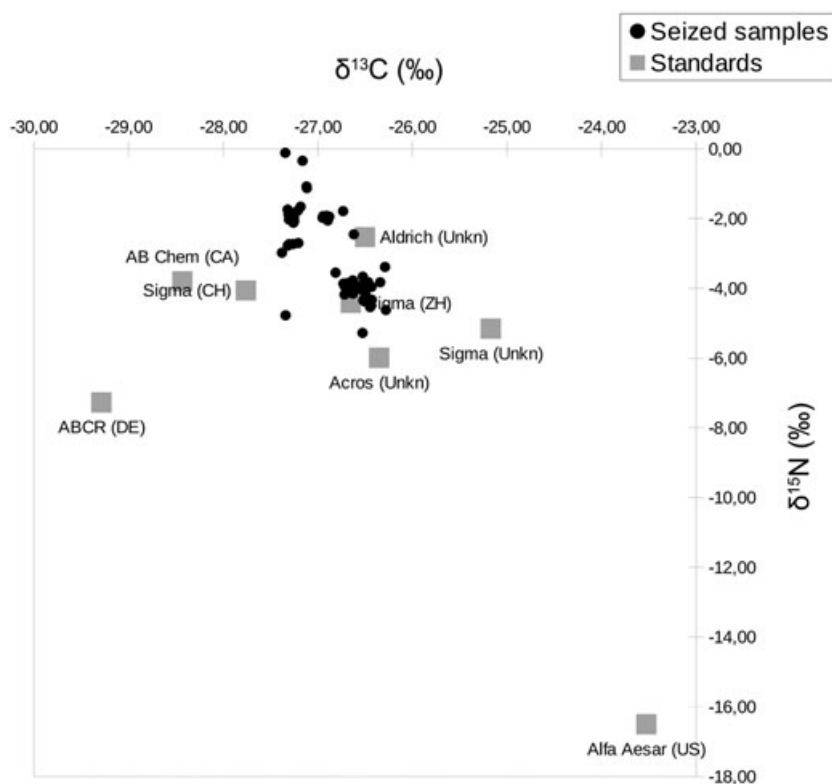


Figure 4. Nitrogen vs carbon data for phenacetin samples and standards analyzed in EA.

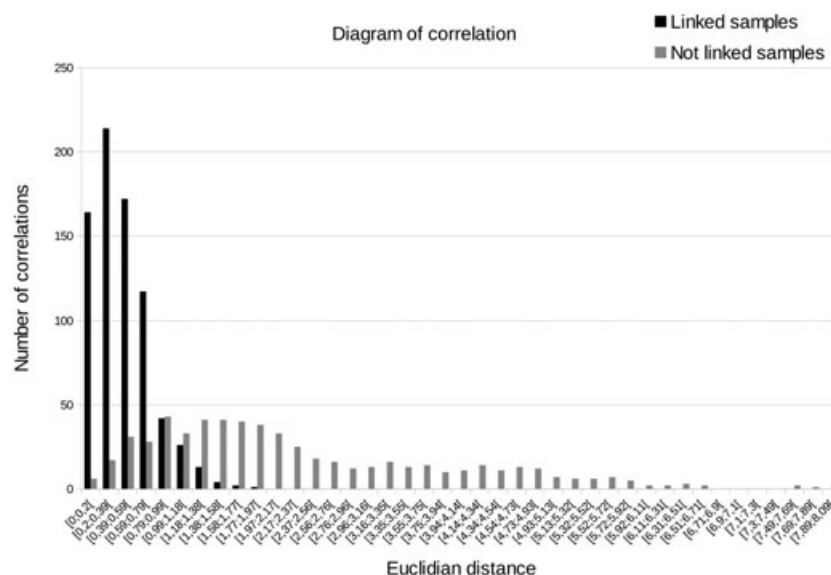


Figure 5. Diagram of correlation of Linked and Not linked samples based on Euclidian distance calculation over $\delta^{13}\text{C}$ and $\delta^2\text{H}$ data.

population, and 15 samples, the Not Linked population. Diagram of correlation based on Pearson, Euclidian, and Cosine metrics (for formulas, see Anderson *et al.*^[14]) were drawn, but none of them could demonstrate a satisfying discriminating power of the method.

Conclusion

The ability of stable isotopes to compare phenacetin in illicit drug samples was investigated. For that purpose, carbon, hydrogen, and nitrogen stable isotopes ratios of phenacetin were measured in a large number of samples. They could be mixtures of cocaine and phenacetin (measured by GC-C/P-IRMS) or pure phenacetin samples, either originating from seizures or purchased as standards of known origin (measured in EA-IRMS). Samples coming from a single seizure were used to constitute a population of Linked samples. The Not Linked population was composed of samples coming from seizures differing considerably in terms of date of seizure or location.

Results shown here tend to demonstrate that the three isotopic ratios studied are not discriminant enough to differentiate hundreds of samples. It is still possible to use them for case-to-case comparison, as different isotopic values mean different sources. But, to conclude on a common source, it would be necessary to combine isotope values with other parameters such as additional stable isotope ratios (nitrogen could be further investigated; oxygen which was not studied here due to laboratory constraints should be also considered), or their intra-molecular distribution, for example.

The apparent failure of this tool could also indicate that our starting hypotheses, based on our experience of cocaine profiling, cannot be transposed to phenacetin and should be reconsidered. For instance, it is possible that the time required to completely disperse a phenacetin batch is much longer than for cocaine (6 months). Besides, the inter-batch variability is likely much lower for phenacetin (probably diverted from pharmaceutical production sites) than for cocaine (which is issued from a large number of small and improvised clandestine facilities).

The extent of the data for the standards of known origin seems to show that, at some level, C, H, and N ratios can be exploited to

source samples, even though they cannot be applied to the seized samples encountered in our forensic lab. Collection of a larger number of standards from various origins is however required to validate this hypothesis.

Acknowledgments

We would like to thank Patrick Goetinck, Magali Batteau, Sylvie Guibert, and Hervé Casabianca from the Service Central d'Analyse, CNRS, who purchased commercial standards of known origin. They also developed the gradient for GC H₂ measurement shown here, and acquired part of the EA H₂ data.

References

- [1] J. Broséus, N. Gentile, P. Esseiva. The cutting of cocaine and heroin: A critical review. *Forensic Sci. Int.* **2016**, 262, 73.
- [2] J. Broséus, N. Gentile, F. Bonadio Pont, J. M. Garcia Gongora, L. Gasté, P. Esseiva. Qualitative, quantitative and temporal study of cutting agents for cocaine and heroin over 9 years. *Forensic Sci. Int.* **2015**, 257, 307.
- [3] L. Dujourdy, F. Besacier, V. Ladroue. Cocaine seized in France. Statistical data from National database of the National Forensic Institute. *Actual Chim.* **2010**, 342, 29.
- [4] Institut National de Police Scientifique. Stupéfiants 2014: Bilan du Système de Traitement Uniformisé des Produits Stupéfiants S.T.U. P.S. (C). Lyon, **2015**
- [5] European Monitoring Centre for Drugs and Drug Addiction – Europol. EMCDDA–Europol joint publications: Cocaine, a European Union perspective in the global context. Publications Office of the European Union, Lisbon, **2010**
- [6] M. J. O'Neil, P. E. Heckelman, C. B. Koch, K. J. Roman, C. M. Kenny, M. R. D'Arecca. *The Merck Index Fourteenth Edition*, Royal Society of Chemistry, London, **2006**.
- [7] International Agency for Research on Cancer (IARC). *Phenacetin*, IARC Monographs on the evaluation of carcinogenic risks to humans – pharmaceuticals, World Health Organisation Press, Geneva, **2012** 100A, 377.
- [8] Observatoire Français des Drogues et Toxicomanies. Phénacétine - Produit de coupe de la cocaïne en augmentation. *Note SINTES.* **2007**, 1, 1.
- [9] United Nations Office on Drugs and Crime (UNODC). World Drug Report 2016. United Nations publication, New York, **2016**.
- [10] United Nations Office on Drugs and Crime (UNODC). Drug characterization / impurity profiling - Background and Concepts. United Nations publication, New York, **2001**.

- [11] N. Gentile, R. T. W. Siegwolf, P. Esseiva, S. Doyle, K. Zollinger, O. Delémont. Isotope ratio mass spectrometry as a tool for source inference in forensic science: a critical review. *Forensic Sci. Int.* **2015**, 215, 139.
- [12] M. Coleman, W. Meier-Augenstein. Ignoring IUPAC guidelines for measurement and reporting of stable isotope abundance values affects us all. *Rapid Commun. Mass Spectrom.* **2014**, 28, 1953.
- [13] N. Gentile, M. J. Rossi, O. Delémont, R. T. W. Siegwolf. ^{15}N measurement of organic and inorganic substances by EA-IRMS: a speciation-dependent procedure. *Anal. Bioanal. Chem.* **2013**, 405, 159.
- [14] K. Andersson, E. Lock, K. Jalava, H. Huizer, S. Jonson, E. Kaa, A. Lopes, A. Poortman-van der Meer, E. Sippola, L. Dujourdy, J. Dahlén. Development of a harmonised method for the profiling of amphetamines VI. Evaluation of methods for comparison of amphetamine. *Forensic Sci. Int.* **2007**, 169, 86.