Title: THE CHEMISTRY BEHIND "KROKODIL": STREET-LIKE SYNTHESIS AND PRODUCT ANALYSIS

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- "Krokodil" was produced mimicking street synthesis followed by addicts
- Desomorphine was synthesized using the hydriodic acid/red phosphorous reduction
- Qualitative and quantitative analysis of desomorphine was performed by GC-EI/MS
- Dihydromorphine-3,6-dideoxy and morphinan-4,5-epoxy-3-ol were also obtained

Full Title: THE CHEMISTRY BEHIND "KROKODIL": STREET-LIKE SYNTHESIS AND PRODUCT ANALYSIS

Running Head: "krokodil" synthesis and analysis

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ABSTRACT

"Krokodil" is the street name for a drug, which has been attracting media and researchers attention due to its increasing spread and extreme toxicity. "Krokodil" is a homemade injectable mixture being used as a cheap substitute for heroin. Its use begun in Russia and Ukraine, but it is being spread throughout other countries. The starting materials for "krokodil" synthesis are tablets containing codeine, caustic soda, gasoline, hydrochloric acid, iodine from disinfectants and red phosphorus from matchboxes, all of which are easily available in a retail market or drugstores. The resulting product is a light brown liquid that is injected without previous purification. Herein, we aimed to understand the chemistry behind "krokodil" synthesis by mimicking the steps followed by abusers. The successful synthesis was assessed by the presence of desomorphine and other two morphinans. An analytical gas chromatography-electron impact/mass spectrometry (GC-EI/MS) methodology for quantification of desomorphine and codeine was also developed and validated. The methodologies presented herein provide a representative synthesis of "krokodil" street samples and the application of an effective analytical methodology for desomorphine quantification, which was the major morphinan found. Further studies are required in order to find other hypothetical byproducts in "krokodil" since these may help to explain signs and symptoms presented by abusers.

Keywords: Opioid abuse; desomorphine; "krokodil" synthesis; GC-EI/MS analysis.

INTRODUCTION

"Krokodil" is the street name for an injectable mixture that has been used as a cheap substitute for heroin and is attracting media and researchers attention due to its spreading and extreme toxicity [1-5]. "Krokodil" first appeared around 2002/3 in Russia and Ukraine [1-4]. It is obtained from codeine tablets after a homemade process aimed to synthesize desomorphine, as a low cost option for heroin addicts. Data about the homemade synthesis of "krokodil" are related to a Nagai and "Moscow" methods, both commonly used for methamphetamine [1, 6-8]. Thereby, precursors are chemical products easily purchased in supermarkets, pharmacies and hardware stores [4]. As refereed, the morphinan starting material is codeine usually extracted from analgesic and antitussive medicines sold in the form of tablet or syrup, which may also contain other substances such as paracetamol, acetylsalicylic acid and caffeine [1]. The other chemicals used in the synthesis, iodine and red phosphorus, are readily available as components of medical tinctures and matchboxes, respectively [1]. The process requires very little equipment and is usually undertaken in unsanitary conditions.

The manufacture of "krokodil" involves two steps [6, 8]. Firstly, a simple acid-base extraction of codeine from the tablets, using gasoline as organic solvent is performed. The second step is the reduction reaction of codeine to desomorphine, using iodine and red phosphorus [8]. The resulting mixture is light brown liquid and has a strong acidic pH. Some users reported the use of cigarette ashes or sodium bicarbonate to increase the pH value of the mixture [4]. The obtained product is filtered, using cotton wool or a cigarette filter, to remove suspended particles. After filtration, the resulting mixture is usually directly injected without further purification.

By definition, in "krokodil", desomorphine is believed to be the main active opioid [1, 2, 9-11]. However, descriptions of possible by-products in the catalytic reduction from codeine to desomorphine at different conditions of their synthesis was previously described in samples from syringes and biological fluids of Russian users [12] .

The availability of real samples of "krokodil" is very scarce, which hampers analytical studies and the elucidation of the toxicity of this drug. Therefore, this study aims to follow a procedure for the synthesis of "krokodil", by mimicking the street conditions used in its preparation as reported by abusers, namely the raw materials and home equipment. The process of synthesis was filmed and reproduced in laboratory.

Moreover, considering the absence of documented validation method for detection and quantification of desomorphine and codeine in "krokodil" samples, a gas chromatography-mass spectrometry with electron impact ionization (GC-EI/MS) method was fully developed and validated. In addition, this methodology was applied for the identification of sub-products of the synthesis.

MATERIAL AND METHODS

Reagents and standards

For "krokodil" synthesis gasoline, alkali solutions for cleaning pipes and matchboxes were purchased from local retail stores in Porto, Portugal. Hydrochloric acid 37% was purchased from VWR Prolabo®. Codeine-containing capsules, iodine tinctures, hydrogen peroxide and, commercial ethanol 96% were purchased from local pharmacies in Porto, Portugal.

For GC-EI/MS analysis, ethyl acetate and sodium sulphate were purchased from Carlo Erba (Milan, Italy), *N*-methyl-*N*-trimethylsilytrifluoroacetamide (TMSFA) was purchased from Sigma-Aldrich (St Louis, MO, USA). Phenacetin (internal standard for GC-EI/MS, IS), codeine and desomorphine were purchased from Lipomed AG (Arlesheim, Switzerland). Helium C-60 (99.99%) was obtained from Gasin (Portugal). Nitrogen was supplied by AirLiquid (Algés, Portugal). For GC-EI/MS analysis, all the reagents used were of analytical grade of from the highest available grade. For High-Performance Liquid Chromatography with Diode-Array (HPLC-DAD) analysis, methanol, hexane, ethyl acetate and triethylamine (TEA) of HPLC grade were obtained from Sigma-Aldrich.

Synthesis of "krokodil"

Extraction of codeine

Codeine was extracted from analgesic capsules containing 30 mg of codeine phosphate, using an alkali solution obtained from commercial pipe cleaning products in proportion of 20% (m/v), gasoline and hydrochloric acid (37%). The solvents were

chosen according to clandestine synthesis information obtained by inquiring abusers. For each extraction, the entire content of five codeine-containing capsules was mixed with 20 mL of alkali solution and 200 mL gasoline, respectively. The organic phase was transferred to another bottle and hydrochloric acid 37% and water (30 mL) were added until pH 1, and mixture was agitated for 5 minutes. The aqueous phase was then removed with a syringe, transferred into a plate and evaporated using a water bath.

Extraction of iodine from iodine tincture

Iodine tincture (30 mL, 6%), hydrochloric acid 37% (15 mL,) and hydrogen peroxide 10 volumes (30 mL, 3%) were mixed together with swirling. The mixture was left to stand for 30 minutes. The mixture was filtered to obtain the iodine crystals.

Extraction of red phosphorus from matchboxes

Matchboxes sides were soaked in ethanol 96% (2 mL) and scrapped with fingers. The mixture was stored at ambient temperature and left to dry.

Nagai type reaction

A mixture of codeine hydrochloride obtained from the extraction and red phosphorus was transferred to an injection flask containing iodine and heated in a candle flame. Water was added on the final step and the obtained solution was filtered using a syringe filter and stored.

Preparation of blank "krokodil" samples

Blank "krokodil" samples were obtained performing the above procedures in the absence of capsules containing codeine.

Quantitative and qualitative analysis of "krokodil"

Preparation of stock and working standard solutions of codeine and desomorphine

Stock solutions of the desomorphine and codeine and IS were prepared in methanol at the concentration of 1 mg/mL. Desomorphine and codeine concentrations of working standard solutions for the calibration curve were prepared at different concentrations by diluting stock solutions in ethyl acetate (0.625, 1.25, 2.5, 5.0 and 10.0 µg/mL). A

working solution of the IS at 4 µg/mL was also prepared in ethyl acetate. Working solutions were prepared fresh daily and stock solutions were stored at -80ºC prior use. "Krokodil" blank samples were spiked with different standards working solutions to validation curves.

Sample Preparation

Synthesized "krokodil" samples (100 µL) were diluted in deionized water (1:10) and basified using one drop of NaOH 0.1N (Fig. 1). An aliquot of 200 µL of the diluted solution was extracted with 600 µL of ethyl acetate. The organic layer was dried over Na_2SO_4, transferred to another vial and evaporated to dryness under a gentle stream of nitrogen (Fig. 1). 60 µL of *N*-methyl-*N*-trimethylsilytrifluoroacetamide (TMSFA) was added and samples heated at 80º for 30 min to accomplish derivatization (silylation). An aliquot of 1 µL of the derivatized extract was injected into the GC-EI/MS system (Fig. 1).

Gas chromatography-mass spectrometry conditions

Quantitative and qualitative GC-EI/MS analyses were performed on a Trace GC 2000 Series ThermoQuest gas chromatography equipped with ion-trap GCQ Plus ThermoQuest Finnigan mass detector. Chromatographic separation was achieved using a capillary column (30m × 0.25 mm × 0.25 µm, cross-linked 5% diphenyl and 95% dimethyl polysiloxane) from Restek® and high-purity helium C-60 was used as carrier gas maintained at 1.0 mL/min. An initial temperature of 80ºC was maintained for 1 min, increased to 300ºC at 10ºC/min, and held for 5 min giving a total run time of 28 min. The injector temperature was set at 280ºC. Quantitative analyses were performed in selected ion monitoring mode with splitless injection (1 µL). The designated ions were *m/z* 148, 286 and 271 for desomorphine, 178, 229 and 280 for codeine, and 162, 236 and 251 for IS. Qualitative analyses were performed in the full-scan mode in the range of *m/z* 50-650.

Liquid chromatography conditions

Qualitative HPLC-DAD analyses were performed on a Finnigan Surveyor (Thermo Electron Corporation, USA) equipped with an AutoSampler Plus and a diode array detector TSP UV6000LP (Thermo Separation Products, USA). The separation was

carried out on a 250 × 4.6 mm i.d. Hypersil silica 3 mm, pore size 120 Å, (Hichrom, UK). LC analysis was performed by gradient elution, with mobile phase consisting of hexane as solvent A, methanol as solvent B and ethyl acetate with 0.005% of triethylamine as solvent C. The gradient elution program was as follows: 100% of A from 0 to 30 min, 0% to 100% C from 30 to 90 min, isocratic 100% C from 90 to 100 min, 1:1 (B:C) from 100 to 140 min, isocratic on this condition from 140 to 150 min, 50% to 100% C from 150 to 180 min. The injected volume was 20 mL and the elution was monitored in UV/Vis at 254nm. Chromeleon 7.1 SR2 software Thermo Fisher Scientific managed chromatographic data. Prior to use, mobile phase solvents were degassed in an ultrasonic bath for 15 min. The identification of desomorphine was established based on the comparison with standard retention time under the same chromatographic conditions and UV/Vis spectrum.

^{31}P NMR conditions

^{31}P liquid state NMR spectra were performed and recorded on a Brücker DRX-300 spectrometer, using deuterated methanol (CD_3OD) or deuterium oxide (D_2O) as solvents from Deutero GmbH®.

Method validation

The validation of the method was performed accordingly to European Medicines Agency [13] and other authors [14-16]. The evaluated parameters were selectivity, limit of detection (LOD), lower limit of quantification (LLOQ), precision, accuracy, recovery and linearity of the method. The calibration curves were prepared by spiking blank "krokodil" samples with proper volumes of standard solutions of desomorphine and codeine as described above.

Stability of "krokodil"

In order to evaluate the stability of the synthesized "krokodil" samples, desomorphine was quantified after freezed/thawed three times in different moments (in the first three consecutive days, one week and one month after the synthesis). Moreover, each sample was submitted to three different storage temperatures (*i.e.* room

temperature, 4ºC and -20ºC) to evaluate the thermal stability of the products and the mixture obtained.

RESULTS AND DISCUSSION

Synthesis of "krokodil"

The need of samples for analytical purposes and toxicological analysis was the main reason for the synthesis of "krokodil" in the present work. The method used for the synthesis was based on what is known to be followed by "krokodil" users in Georgia. Preparation was recorded and later reproduced in our laboratory. The synthesis method resemblances Nagai route, firstly used to synthesize methamphetamine from ephedrine or pseudoephedrine. Indeed, both syntheses use the hydriodic acid (HI) formed *in situ* by the reaction between red phosphorus and iodine as catalyst of the reduction reaction to obtain the final product. Therefore, data regarding the by-products formed during Nagai route [8, 17, 18], could be useful to hypothesize which impurities may be present in "krokodil" samples.

Usually, codeine is commercially available as a phosphate salt. Its extraction was performed by liquid-liquid extraction, based on acid-base chemistry. 65.13% of codeine was recovered, which is acceptable once the extraction process was totally homemade. Firstly, a strong base was added to form the codeine free base, which was dissolved in the organic phase. The strong base was usually obtained from commercial available products that contain sodium hydroxide and are used to clean pipes. The organic solvent was usually gasoline, although some addicts also reported using paint thinner [4]. The water-soluble compounds associated with codeine in the tablets were washed away in this step. Subsequently, the organic extract was acidified with hydrochloric acid, obtained from industrial products found in supermarkets, and then water was added. After the separation of the phases, codeine was back-extracted into the aqueous layer as its hydrochloride salt. The aqueous solution containing codeine can be used directly or evaporated. Even though, being aware of the street procedure limitations, our protocol mimics precisely the steps undertaken by "krokodil" abusers.

After these extraction steps, the reduction of codeine to desomorphine was performed. Taking into account the reagents used in the manufacture of "krokodil", the proposed mechanism of its synthesis is described in Figure 2. Iodine and red phosphorus react to form phosphorus triiodide (PI_3) [19]. PI_3 usually reacts, by a SN_2 mechanism, with primary and secondary alcohols, displacing the hydroxyl group which is replaced by iodine, to produce an alkyl iodide [20]. We propose that PI_3 promotes the nucleophilic substitution of the hydroxyl group at C(6) of codeine forming α-iodocodeine (6-iodocodeine) [19]. Subsequently, in the presence of water, PI_3 is converted to HI, which promotes the acidic ether cleavage [21] of the methoxyl group and the dehalogenation of 6-iodocodeine to 6-deoxymorphine (or dehydro-desomorphine) [19]. HI is also responsible for the reduction of the double bond at C(7) and C(8) [22], leading to the formation of desomorphine. Iodine released in the dehalogenation of 6-iodocodeine reacts again with red phosphorus, initiating a new redox cycle [19].

In Figure 2 is also showed the formation of phosphorous acid (H_3PO_3) as a product of the nucleophilic substitution of the alcohol and as a product of the reaction of PI_3 with water. Phosphorous acid produces phosphoric acid (H_3PO_4) and phosphine (PH_3), once the reaction is conducted at high temperature (a gas flame is used for heating, T > 200 ºC).

Approximately 45 minutes after the beginning of the extraction, "krokodil" is ready to be injected. The obtained product has a dark brown color, probably a result of iodine formed during the reaction of a strong acid with iodide ion [23], and a characteristic acid odor. The measured pH was 1.15±0.30. The physical appearance and the found pH values are in accordance with literature [1]. Usually, each user injects 1 mL of "krokodil" into different parts of body. The most common route of administration is intravenous, but intramuscular or even intradermal was also reported.

Method validation

The analytical parameters of the developed method were discussed in the following topics.

Selectivity. Six blank samples were analyzed to evaluate chromatographic interferences. No interference peaks were detected, either in the retention times of desomorphine or codeine or in the IS retention time (Fig. 3A).

Carry-over. During the validation process, injections of calibration standards containing more than 10 times the concentration correspondent to the limit of quantification were followed by blank sample injections of ethyl acetate, to ensure that there was no carry-over from one injection to the next one. The obtained carry-over results were <20% of the LLOQ and <5% for the IS, which were within the proposed acceptance limits for this parameter [13].

Linearity. The weighted least squares linear regression equations and coefficients of determination were calculated using three different curves of each analyte obtained from independent sets of standards. The results obtained were showed in Table 1. The determination coefficients (r^2) were >0.990 over the concentration range, showing good linearity for all the analytes.

Limit of Detection and Limit of Quantification. LOD and LLOQ were determined as following: LOD=3.3σ/m and LOD=10σ/m where σ is the standard deviation of the response and *m* the slope of the calibration curve. Detection limits were 0.150±0.002 µg/mL for desomorphine and 0.170±0.002 µg/mL for codeine in normal autotune conditions. The quantification limit for was 0.490±0.002 µg/mL for desomorphine and 0.570±0.002 µg/mL for codeine. The values of LOD and LLOQ are listed on Table 1.

Precision, Accuracy and Recovery. The results obtained are showed in Table 1. The %CV for desomorphine and codeine did not exceed 15% and the developed method was considered precise for both analytes. Accuracies in the range of 101.3 – 110.2% for desomorphine and 101.3 – 106.6% for codeine were obtained, which are within the proposed acceptance limits for this parameter (100±15%, [13]). The recoveries were 89.42% and 92.88% for desomorphine and codeine, respectively. Associated with lower %CV (0.49 – 11.6%), these results suggest that extraction was efficient for the three different concentrations evaluated.

Qualitative Analysis

Our aim was to obtain "krokodil" real samples. The performed synthesis mimics the street procedure and leads to a crude product that was not submitted for further purification. Therefore, the gas chromatographic profile of the synthesized "krokodil" was complex, revealing the presence of several compounds (Fig. 3B). Many of these

compounds belong to the raw materials used for the synthesis (excipients of the formulation, plastics, gasoline etc.) or could arise from reactions between them. Our focus was on the compounds with the morphinan nucleus.

Desomorphine was the predominant morphinan presented. Its identification was established by the retention time and co-injection with a reference standard, as well as by the interpretation of the mass spectral fragmentations (Fig. 1S). The mass spectral fragmentations of desomorphine showed the molecular ion (*m/z* = 343), which was the base peak, consistent with the molecular mass of the trimethylsilyl derivative (Fig. 1S). The ion with *m/z* = 328 $[M-15]^+$ corresponded to the loss of the methyl group and the ion *m/z* = 286 $[M-57]^+$ is consistent with a possible loss of C_3H_7N fragment, which resulted from the cleavage of the piperidine ring. The ion *m/z* = 271 $[M-72]^+$ corresponded to the loss of the trimethylsilyl group followed by protonation.

The work developed by Savchuk, Barsegyan [12] pointed out that the procedure and raw materials adopted in "krokodil" preparation would reflect the final chemical composition, not only in terms of the amount of desomorphine, but also on the presence/absence of other morphinans. Therefore, in order to assess how well the followed procedure mimicked real samples, a further analysis was undertaken. The synthesis method applied in the present study, revealed trace amounts of dihydromorphine-3,6-dideoxy and morphinan-4,5- epoxy-3-ol in the "krokodil" samples. These morphinans had also been found in street "krokodil" samples analyzed previously by Savchuk, Barsegyan [12]. Their mass spectra (Fig. 1S) are in agreement with NIST library for these compounds (please see the following website: http://webbook.nist.gov/cgi/cbook.cgi?ID=C427009&Units=SI&Mask=200#Mass-Spec). Taking into account chemical structure it was reasonable to consider they were by-products from the same reaction that occurs between codeine, iodine and red phosphorus.

In order to clarify the chemical composition of "krokodil", the obtained extract was analyzed with HPLC-DAD (Fig. 2S). The wide polarity range of the mobile phase highlighted the large chemical diversity of the sample, which can be divided into three categories according to its polarity. The apolar constituents were eluted within the first minutes. Since the final products were not submitted to any purification step, we hypothesized that these contaminants were derived from plasticizers family. The second

category was composed by compounds with intermediate polarity, which were eluted almost at the end of one hour. Desomorphine was identified here with a retention time of 50.13. It is expected that the other morphinans may be present within this category. Finally, the third category, composed by the polar constituents of the mixture, were eluted almost at the end of two hours. Due to the harsh reaction conditions at elevated temperatures, it is expected that polar sub-products are obtained during the synthesis procedure.

As phosphorus is a key element in "krokodil" preparation, we analyzed the presence of phosphorus-containing molecules present on it by ^{31}P-NMR (Fig. S3). Four phosphorous species are involved in the "krokodil" manufacture namely, H_3PO_3, H_3PO_4, PI_3 and PH_3. PH_3 is a highly reactive and toxic gas that promptly reacts with water and oxygen. PI_3 is the most unstable phosphorous trihalide and reacts easily with water [24]. Considering the reduction mechanism using HI as catalyst, H_3PO_3 is formed as a by-product of the SN_2 reaction that leads to 6-iodocodeine and by PI_3 hydrolysis. As refereed above, the formation of H_3PO_4 is explained by the thermal decomposition (T> 200ºC) of H_3PO_3. Apparently, H_3PO_3 and H_3PO_4 are not present in "krokodil", since their typical signals at δ 3.5 ppm and δ 0 ppm [25], respectively, were not observed. ^{31}P NMR spectra usually cover the region between -500 ppm and 1400 ppm [25]. However, all signals in the ^{31}P NMR spectrum of "krokodil" are present in a narrow range (-11.74 ppm to 5.86 ppm). These absorptions are compatible with the presence of phosphanes or phosphorous/phosphoric acid derivatives, such as phosphate esters, phosphonate esters or phosphates salts [26]. Since H_3PO_3 and H_3PO_4 were not evident in the ^{31}P NMR "krokodil" spectrum we could assume that they were converted into its derivatives during the extremely harsh reaction.

Quantitative Analysis

Several chromatographic conditions, such as column oven temperatures and gas flow rate, were tested in order to achieve the best peak separation of the analytes of interest. These tests led to the optimized conditions presented above and the analytes of interest were detected in 28 min. The retention time of desomorphine was 19.99 minutes, the retention time of codeine was 21.45 minutes and the retention time of the IS was 12.78 minutes (Figure 3A). Despite Srimurugan and colleagues [27] had

synthesized a deutered analogue for desomorphine, it was not available to purchase and phenacetin was chosen as IS since it proved to be effective for opioids analysis [28]. The integration of the chromatographic peaks for quantitative analysis was performed by Selective Ion Monitoring (SIM) mode, increasing selectivity and allowing more precise peak integration, especially relevant when we led with small concentrations [16].

To evaluate the efficacy of the synthesis, codeine and desomorphine concentrations were analyzed in 10 synthesized "krokodil" samples. The concentration of codeine in "krokodil" samples was residual and lower than the LLOQ of the method. On the other hand, the medium desomorphine concentration was approximately to 0.56 (±0.35) mg/mL (yield of 5.5%±3.5). Due to the homemade character of synthesis, different amounts of desomorphine were produced. This variability is also common in homemade synthesis. Indeed, according to users' skills, different "krokodil" batches may be produced. These results proved that the synthesis procedure allows the consumption of almost all the codeine present in the original tablets, but other compounds besides desomorphine are formed (Figure 3B).

Stability of "krokodil"

Regarding "krokodil" stability, the best storage temperature was shown to be 4ºC since higher concentrations of desomorphine were observed (Table 1S). Stock solutions are usually stored in freezer and, to make a working solution it is necessary to bring the stock solution to room temperature [29]. Karinen, Oiestad [29] described the stability of different substances in stock solution at room temperature, in the freezer and refrigerator. Opioids were shown to be stable at different temperatures for at least one year, except tramadol. There are no data describing the stability of desomorphine. The short stability of desomorphine in "krokodil" samples might be explained by extremely acidic pH of the final sample. Moreover we hypothesized that the lower stability at -20ºC when compared to 4ºC may be justified by the freezing-thawing phenomenon that occurs when a solution is stored at low temperatures.

CONCLUSIONS

"Krokodil" was produced mimicking street synthesis. The laboratory route for desomorphine production from codeine is totally different since the reduction is catalyzed by thionyl chloride. Despite both reaction methods are reductions, the by-products are very different due to the starting materials used. A sensitive, reproducible and simple GC-EI/MS method was developed and validated to screen and quantify desomorphine and codeine in "krokodil" samples. The qualitative analysis of the samples also showed the presence of other two morphinans (*i.e.* dihydromorphine-3,6-dideoxy and morphinan-4,5-epoxy-3-ol) due to the highly reductive environment. It is believe that the proposed analytical methodology will be a powerful tool for forensic laboratories in cases where street samples require laboratorial analysis. Finally, a more systematic investigation of the reaction conditions is needed in order to obtain additional information about the chemistry behind "krokodil" synthesis. Indeed, despite the fact that GC-EI/MS is a highly sensitive technique, the identification of other morphinans is difficult namely due to non-volatile and unusual fragmentation patterns. Further elucidation and identification of side-products might be possible by liquid chromatography-high-resolution mass spectrometry and nuclear magnetic resonance.

There is no doubt that "krokodil" is an extremely dangerous mixture of compounds, which contain desomorphine as its main psychoactive ingredient. The use of harmful substances in the synthesis and the absence of proper purification methods before the drug consumption results in the formation of a very damaging mixture. Chemical content analysis of "krokodil" should provide the needed information about its active ingredients and contaminants and about the chemical process undergoing its homemade production. It has been reported by the media that the life expectancy of people who inject the drug is reduced to about 2-3 years. Both consumers and service providers suggest that skilled "cooks" can prepare a cleaner intravenous "krokodil" solution, which causes less toxic effects.

Our group is dedicated to understand the complete toxicology of "krokodil". We initiated this work trying to obtain reliable samples of "krokodil". The authors contacted users to understand the synthesis process and to know all the social and legal issues that lead a person to abuse "krokodil". All this information was compiled and can be found in a previous work [1]. To further understand "krokodil"-related toxic effects, a "clean"

(not homemade) synthesis using quality laboratorial starting materials would be interesting. Moreover, biochemical and histological analysis aiming to compare to the toxic alterations of blank samples ("krokodil" without codeine), "krokodil" samples and "clean" krokodil need to be done. Indeed, we are in progress with *in vivo* experimental studies using "krokodil" samples, aiming to understand its mechanism of toxicity and the main target organs. Certainly, conjugating the chemical and *in vivo* toxicological data it will be possible to understand which compounds are actually being responsible for signs and symptoms of intoxication. Moreover, these findings should contribute to preventive measures for reducing the harmful toxic effects of this drug. Ultimately, specific therapeutic approaches for "krokodil" abusers can be proposed and developed.

CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest.

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FIGURE LEGENDS

Figure 1 – "Krokodil" synthesis procedure. A – Sample dilution. B – "Krokodil" extraction. C – Derivatization procedure.

Figure 2 – Proposed mechanism for desomorphine synthesis using the hydriodic acid/red phosphorous reduction method.

Figure 3 – A - GC-EI/MS SIM mode chromatogram for desomorphine (*m/z* =148, 286 and 271), codeine (*m/z*= 178, 229 and 280), phenacetin (IS, *m/z* = 162, 236 and 251) and a blank sample (red ink). Chromatograms were obtained using a standard containing desomorphine and codeine at 3 µg/mL and phenacetin (IS) at 2 µg/mL. **B -** GC-EI/MS Fullscan mode chromatogram of a synthesized "krokodil" sample.

Figure 1S – Mass spectra of desomorphine and the fragmentation pattern of the morphinan derivatives found on "krokodil" samples.

Figure 2S – Representative HPLC-UV chromatogram of a "krokodil" sample.

Figure 3S – Representative ^{31}P-NMR (121 MHz, D2O) spectrum of a "krokodil" sample. δ = 5.86 ppm (s; 0.14P); 0.49 ppm (s; 0.16P); -0.76 ppm (s; 1P); -3.73 ppm (s; 0.17P); -11.74 ppm (s; 0.54P).

TABLE LEGENDS

Table 1 – A - Parameters of the analytical curves of desomorphine and codeine standard solutions (0.6 – 10 µg/mL) obtained by the least squares method in three different days. B - Precision, accuracy and recovery (%) for desomorphine and codeine evaluation at 3 different spiked concentrations. LOD, limit of detection; LLOQ, limit of quantification.

Table 1S - Desomorphine concentrations (mg/mL) in "krokodil" samples storage at 3 different temperatures (*i.e.* room temperature, 4ºC and -20ºC). RT = room temperature; NA = not applied.

Figure 1

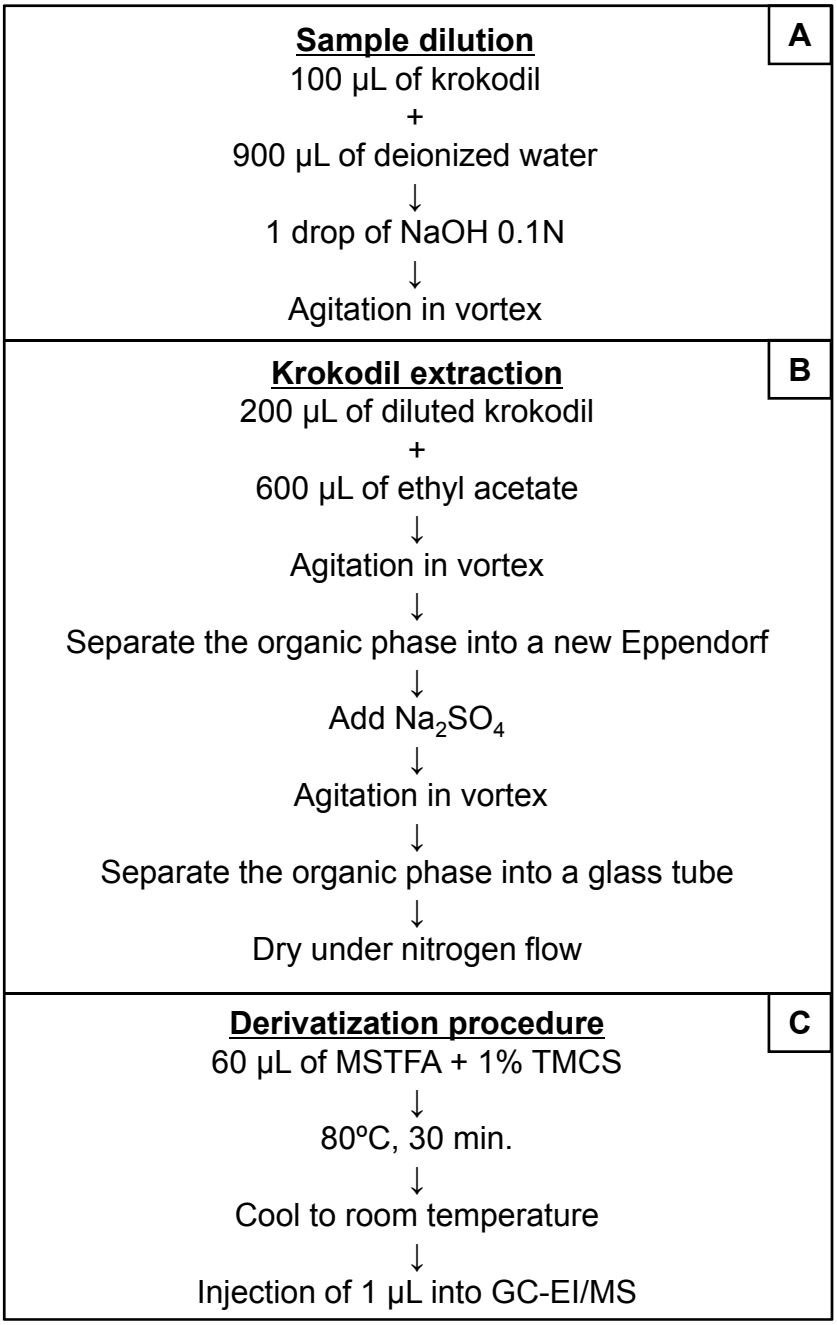

Figure 2

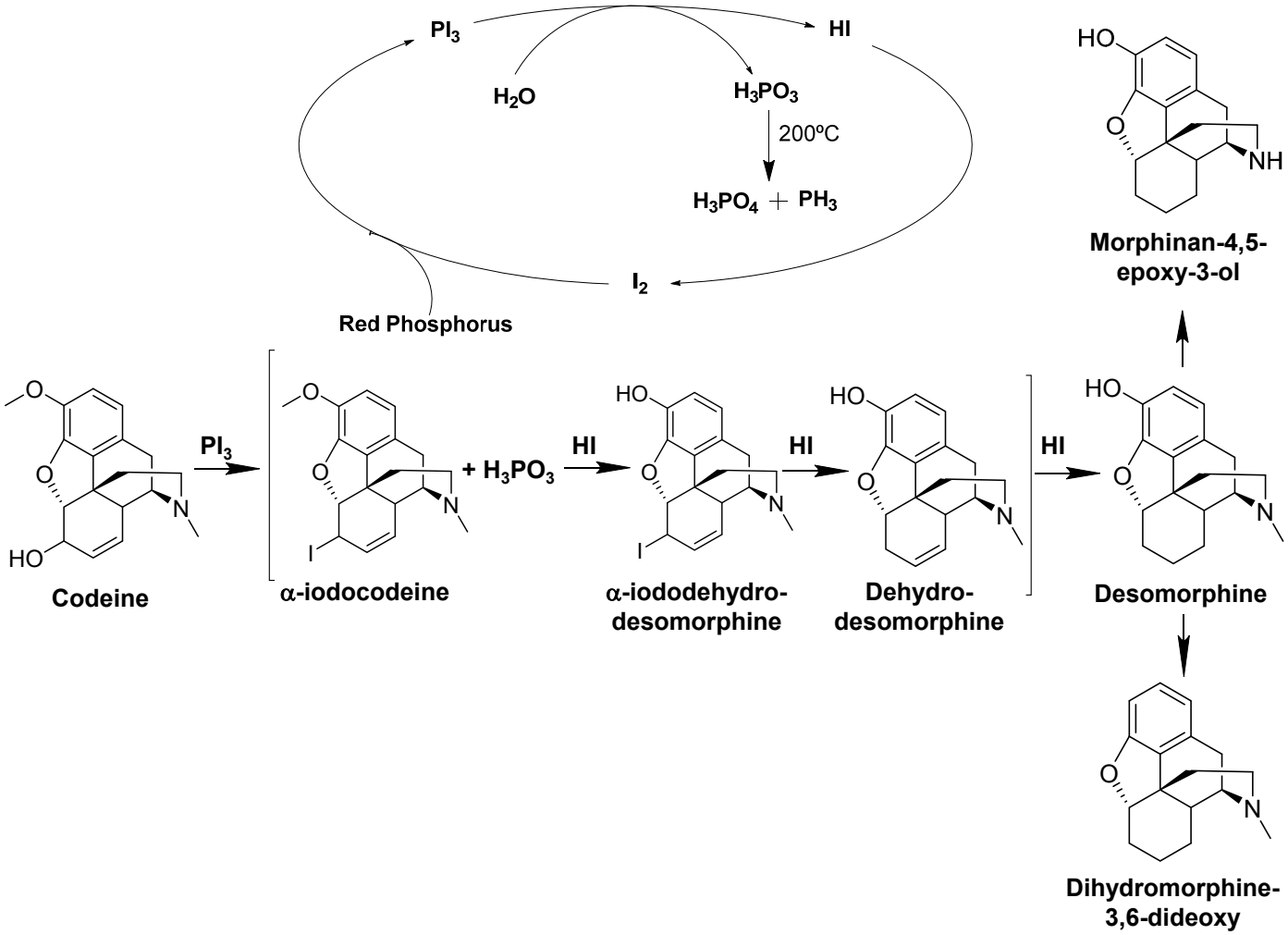

Figure 3

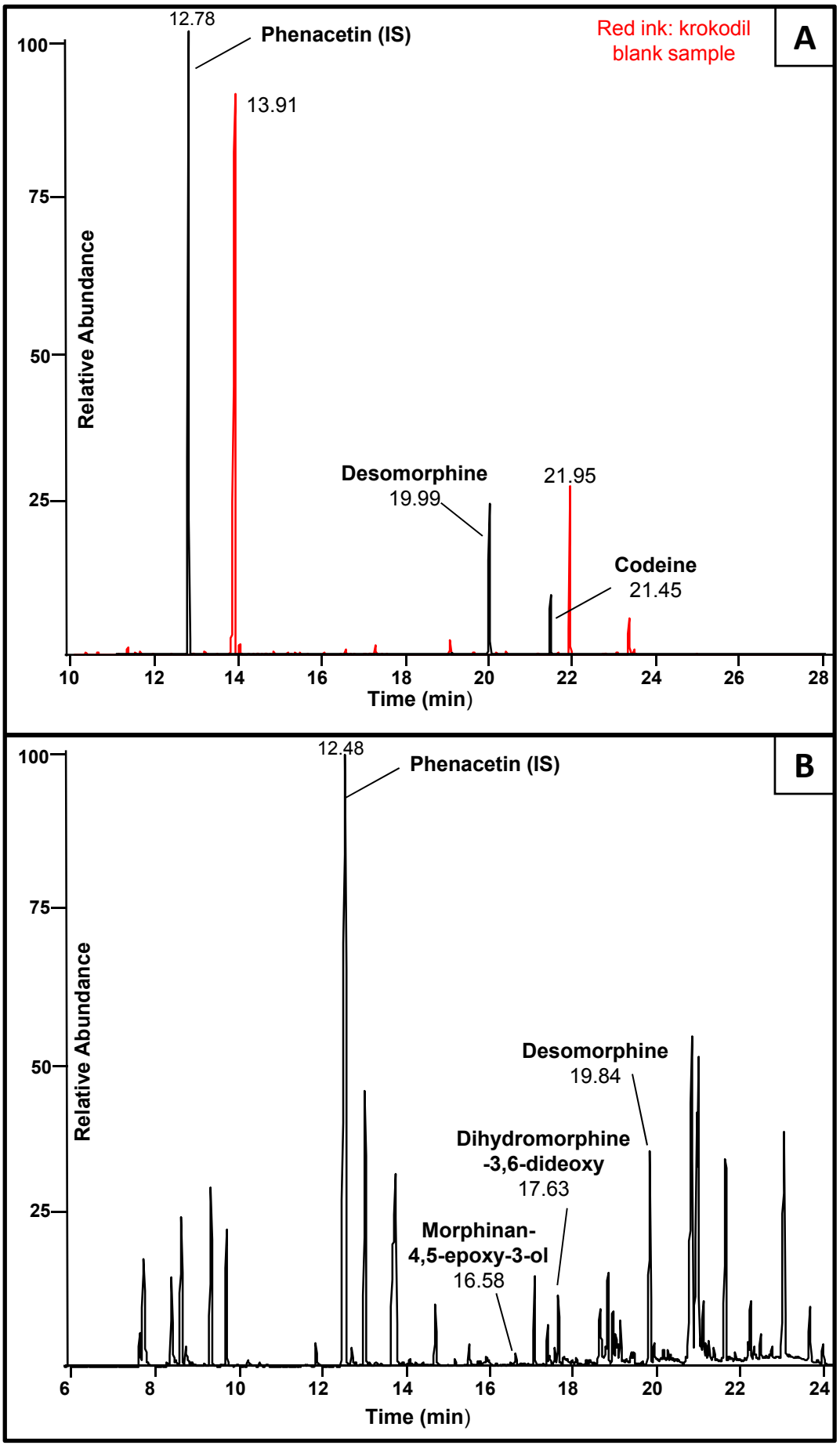

TABLE 1

	A						B				
Xenobiotic	**n=3**	**y = mx + b**	**Concentration range (µg/mL)**	***r*²**	**LOD (µg/mL)**	**LLOQ (µg/mL)**	**Concentration (µg/mL)**	**Intra-day precision (%, n=3)**	**Inter-day precision (%, n=3)**	**Accuracy (%, n=3)**	**Recovery (%)**
Desomorphine	day1	y=0.071x-0.022	0.625-10.0	0.9919	0.150 ± 0.002	0.490 ± 0.002	0.625	9.84	11.5	101.3	97.55
	day2	y=0.013x-0.001	0.625-10.0	0.9982			2.50	11.6	0.49	108.8	92.41
	day3	y=0.033x-0.012	0.625-10.0	0.9904			10.0	10.4	3.91	110.2	78.32
Codeine	day1	y=0.040x-0.011	0.625-10.0	0.9934	0.170 ± 0.002	0.570 ± 0.002	0.625	8.03	3.60	106.6	93.76
	day2	y=0.045x-0.000	0.625-10.0	0.9985			2.50	6.84	5.35	101.3	90.94
	day3	y=0.012x-0.001	0.625-10.0	0.9995			10.0	8.89	3.58	104.9	93.94