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# Characterization of a heroin manufacturing process based on acidic extracts by combining complementary information from two-dimensional gas chromatography and high resolution mass spectrometry

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ABSTRACT

A comprehensive two-dimensional gas chromatography mass spectrometric approach (GC×GC-TOFMS) supported by one-dimensional gas chromatography high resolution mass spectrometers (GC-HRTOFMS, GC-FTICRMS) were used to track and confirm characteristic chemical impurities through an authentic illicit heroin production process. Minor and trace compounds present in illicit heroin that result from the used opium and the manufacturing process generate a specific impurity profile of each batch. Based on that, comparative analysis of heroin samples is possible, which can contribute amongst others to the uncovering of links between seized samples. The illicit manufacturing processes are thereby only vaguely described in the literature. Neutral and acidic compounds of the unadulterated impurity profiles at different stages during an authentic manufacturing process of heroin were analyzed by comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC×GC-TOFMS). The focus was set on 44 compounds, mostly acetylation products, found in the heroin base including four unaltered opium alkaloids and 18 target compounds used within the German Heroin Analysis Program (HAP) at the Bundeskriminalamt (Federal Criminal Police Office; BKA). 12 compounds were found to be preserved unaltered through the manufacturing process. In case of 21 acetylated compounds the original alkaloid is known or could be assigned, i.a. by analysis of separately acetylated standards.

Using GC coupled to high resolution time-of-flight mass spectrometry (GC-HRTOFMS), major fragments caused by electron impact ionization (EI) were determined. The corresponding molecular weight was obtained by GC hyphenated to Fourier transform mass spectrometry mass spectrometer (FTICRMS) via atmospheric pressure chemical ionization (APCI) as soft ionization interface.



## 1. Introduction

The illicit manufacturing processes for heroin are only vaguely described in the literature. Most of them rely on the Thiboumery and Mohr process, also known as the lime method which involves the extraction of morphine using calcium oxide and ammonium chloride and subsequent acetylation with acetic anhydride [1–3]. During the isolation of morphine, other main and minor alkaloids present in opium poppy are extracted, and the subsequent chemical conversion of morphine to the corresponding diacetylmorphine (heroin) leads to the formation of various by-products. Several publications about the formation mechanism of by-products occurring during illicit heroin manufacturing were released in the past years. They are mainly focused on acetylated products related to the major alkaloids present in opium poppy [4–6]. These minor and trace compounds present in illicit heroin generate a specific impurity profile of each batch. Comparison of seized heroin samples based on these impurities provides an important contribution to the uncovering of links amongst seized samples and thus is of substantial value for drug intelligence. Profiling methods routinely used by forensic institutes are commonly based on gas chromatographic analysis and (ultra) high performance liquid chromatography followed by quantitation of mainly acetylated

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alkaloids frequently found in illicit heroin samples [7–12]. The German Heroin Analysis Program (HAP) of the Bundeskriminalamt (Federal Criminal Police Office; BKA) comprises a determination of exterior attributes, the quantitative determination of main compounds (major opium alkaloids), adulterants, diluents, solvents and the analysis of selected organic trace impurities based on acidic extraction of the samples.

Opium, the starting material for illicit heroin manufacturing, is the dried latex of opium poppy (*Papaver somniferum*), that is obtained by scratching its crude seed vessel. According to the United Nations Office on Drugs and Crime (UNODC) Afghanistan continued to be the largest global opium producer. Estimates suggest that up to 90% of heroin seized in Western Europe originates from Afghanistan [2,13]. In 2004 the opportunity to observe an authentic heroin manufacturing process in Afghanistan was offered to collaborators of the BKA in Germany. Besides detailed documentation of the process itself, it provided the occasion to collect authentic samples during the complete manufacturing. During the presented study the impurity profiles of these samples obtained by acidic extraction were analyzed in detail using comprehensive two-dimensional gas chromatography and high resolution mass spectrometry to provide basic information on the origin and composition of the detected impurity compounds based on which further conclusion for the heroin analysis program may be drawn.

In a proof of concept study we already demonstrated the applicability of two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOFMS) for the analysis of illicit heroin samples [14]. In the following this technique could be successfully applied in further studies on the profiling of forensically relevant samples [15–17]. GC×GC-TOFMS benefits from its very high chromatographic resolution combined with the high sensitivity and the selectivity of the mass spectrometric detection. If only unit resolution is provided by the instrument, compound identification is mainly possible by comparison of the fragmentation pattern generated by electron ionization, to mass spectral libraries. One possible solution for the characterization of unknown compounds is the implementation of high resolution spectrometry to allow for the elucidation of the elemental composition based on the accurate mass and the isotopic pattern.

During this study the samples were therefore analyzed on a gas chromatograph coupled to high resolution time-of-flight mass spectrometer system (GC-HRTOF MS). An example for the capability of the new technique in the field of forensic science was already shown [18]. The absence of an observable molecular ion for some compounds is the major drawback of electron impact ionization (EI). In order to circumvent the strong fragmentation of molecules, soft ionization techniques, such as photo ionization [19] or chemical ionization [20] have to be applied. One opportunity is atmospheric pressure chemical ionization (APCI), which was introduced in 1973 by Horning et al. and is based on pressurized nebulization into a corona discharge at atmospheric pressure [21]. The generated ionized nitrogen and water clusters cause protonation of the analytes. For the presented work, a gas chromatograph was coupled to a Fourier transform ion cyclotron resonance mass spectrometer (FTICRMS) using APCI as interface. The ultrahigh mass resolution provided by FTICRMS system combined with the chromatographic separation (GC) and the soft ionization (APCI) is an efficient tool for the characterization of a sample and the elucidation of the elemental composition/molecular formula based on the observed molecular ion. The combination of GC×GC and GC hyphenated to high-resolution mass spectrometry was used in this study to characterize the acidic and neutral impurities in samples taken from the different steps during an authentic heroin manufacturing process to obtain basic information about the composition and origin of the impurities resulting from such a controlled synthesis.

## 2. Experimental

Samples from a documented authentic heroin manufacturing in Afghanistan were kindly provided by the Bundeskriminalamt. Samples were collected at all stages of the manufacturing process, starting from raw opium. The described process did not involve the processing of opium itself. As intermediates and final products the morphine base, the heroin base (brown), the heroin base (white) and the heroin hydrochloride were collected.

### 2.1. Manufacturing of the samples

The raw opium used was seized in Nangarhar but due to frequent distribution of opium between different provinces its actual origin is not evident. The opium was crushed, poured into barrels and dissolved in hot water while stirring. Calcium oxide (anhydrous lime) was added and left overnight. The resulting morphine solution was filtered and the residue was re-dissolved several times to increase the yield. Ammonium chloride was added and stirred until the morphine base precipitated. The morphine base was filtered the next day, subsequently air-dried and weighed for calculation of the required amount of acetic anhydride. The appropriate amount of acetic anhydride was added to the morphine base, stirred and heated for 30 min. The mixture was then poured into hot water and subsequently filtered. Sodium carbonate solution was added to the filtered solution until the brown heroin base precipitated. The heroin base was filtered out and washed twice with hot water. To convert the brown heroin base to the white heroin base, the brown base was dissolved in diluted hydrochloric acid and precipitated using diluted ammonia solution. By filtration the white heroin base was obtained. For conversion to the corresponding hydrochloride it was dissolved in a mixture of hydrochloric acid and acetone followed by filtration and evaporation on a water bath until the heroin hydrochloride precipitated. A detailed documentation is given in [22].

The described manufacturing process was based on 70 kg of four visually distinguishable batches of raw opium with a total morphine content of 8.5% (6.1–11.1%). The extracted morphine base revealed a purity of 53.1%. Finally, the brown and the white heroin base and the heroin hydrochloride revealed a purity of 68.1%, 78.5% and 74.0%, respectively. During data evaluation, particular interest was set on the brown heroin base with its acetylated alkaloids as this is the predominantly seized type of heroin in Europe.

### 2.2. Sample extraction

Sulphuric acid, toluene and eicosane were obtained from Sigma Aldrich (Steinheim, Germany). Extraction was done according to the guidelines of the heroin analysis program (HAP) [8]. 250 mg of each sample was dissolved in 5 mL sulphuric acid (0.25 mol/L) and 5 mL of toluene were added. The toluene contained 10 mg/L of tetracontane as internal standard. Samples were placed in an overhead shaker for 5 min was applied followed by centrifugation for 5 min. 3.2 mL of the organic phase were evaporated to dryness under nitrogen and finally resolved in 200 μL of pure toluene. The extraction protocol was applied to the raw materials, intermediates and products of the manufacturing process.

The extracts of raw opium were additionally treated with trimethylsilyldiazomethane (TMSD) (Sigma Aldrich, Schnelldorf, Germany) prior to analysis for methylation of the fatty acids. 2.5 μL of methanol (Merck, Darmstadt, Germany) and 2.5 μL of trimethylsilyldiazomethane were added to 25 μL of opium extract. The solution was left for 20 min at room temperature and subsequently injected into the GC.

### 2.3. Acetylation of alkaloids

In addition laboratory syntheses were carried out by acetylation of individual alkaloids to study the resulting acetylation products. Comparison of the retention times and mass spectra of the acetylated products and the impurities of the heroin extract was used to determine the origin of unknown impurities and to reveal the basic molecular structure of these compounds.

Alkaloid standards were purchased from different suppliers: Reticuline (Toronto Research Chemicals Inc., Toronto, Canada), Thebaine (Lipomed, Weil am Rhein, Germany), Codeine and Papaverine (Merck, Darmstadt, Germany), Papaveraldine (Serva, Heidelberg, Germany), Laudanosine, Narcotin and Narceine (Ferak Berlin GmbH, Berlin, Germany). For acetylation acetic anhydride (Fluka, Steinheim, Germany) was added to the individual alkaloids and heated up for 6 h at 115 °C. Afterwards the solution was evaporated to dryness and dissolved in methanol and toluene.

### 2.4. Instrumentation

#### 2.4.1. Comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC×GC-TOFMS)

Two-dimensional gas chromatography unit resolution mass spectrometry was done on a GC×GC-TOFMS instrument (Leco, St Joseph, MI, USA) which consists of a standard gas chromatograph (6890N, Agilent Technologies, Palo Alto, CA, USA) equipped with a dual-stage, four-jet cryogenic (liquid $N_2$) modulator and a