

Analysis of Diacetylmorphine, Caffeine, and Degradation Products after Volatilization of Pharmaceutical Heroin for Inhalation

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Abstract

Pharmaceutical smokable heroin was developed for a clinical trial on medical co-prescription of heroin and methadone. This product, consisting of 75% w/w diacetylmorphine base and 25% w/w caffeine anhydrate, was intended for use via "chasing the dragon", that is, inhalation after volatilization. This procedure involves heating the powder mixture, which may lead to formation of degradation products that could subsequently be inhaled. We developed a method that used a high-performance liquid chromatography system that was compatible with photodiode-array detection and mass spectrometric detection to separate diacetylmorphine- and caffeine-related compounds in a wide polarity range for analysis of the vapor. This method was used to analyze the contents of the plastic drinking straws that were used by patients to inhale the vapors from pharmaceutical heroin used via chasing the dragon, which were considered to be representative of the vapors the patients inhaled. They contained primarily unchanged diacetylmorphine, its main metabolite 6-acetylmorphine, caffeine, and some morphine. Several unidentified peaks were observed in the straw chromatograms. Chemical structures were proposed for nine degradation products: morphine derivatives with different substitution patterns of the C₃, C₆, and/or N₁₇ positions, which comprised 0.4–9.7% of the straw sample residue weight. Activity and toxicity of most of these compounds are unknown and require further investigation.

Introduction

A clinical trial was conducted in the Netherlands to evaluate the effect of medical co-prescription of heroin and methadone on mental and physical health and social functioning of chronic

treatment-resistant heroin-dependent patients (1). In the Netherlands, 75–85% of heroin addicts use heroin by chasing the dragon (2). In this procedure, drug users heat heroin powder on aluminum foil with a cigarette lighter until it melts and evaporates. The vapors are subsequently inhaled through a straw in the mouth. The popularity of this route of administration was the reason that two separate study protocols were developed for the clinical trial: one trial testing the efficacy of the prescription of an inhalable form of heroin and another trial testing the efficacy of the prescription of injectable heroin. In preparation for the first trial, pharmaceutical heroin for inhalation after volatilization was developed: a powder mixture of 75% (w/w) diacetylmorphine base and 25% (w/w) caffeine anhydrate (3,4). Caffeine anhydrate was added to increase the recovery of diacetylmorphine base after volatilization and to reduce degradation upon heating (5).

In vitro experiments with this product have shown that the vapors that developed on heating consisted mainly of unchanged diacetylmorphine and caffeine and some 6-acetylmorphine. However, as several authors have reported degradation of heroin and formation of pyrolysis products on heating heroin samples (5–7), it was decided to develop a method of analysis suitable for separation and identification of the constituents of the volatilized pharmaceutical smokable heroin. A high-performance liquid chromatography (HPLC) method for quantification of heroin, caffeine, 6-acetylmorphine, and morphine and separation of known and likely degradation products of diacetylmorphine and caffeine in a wide polarity range was devised. Furthermore, the LC system was compatible with both photodiode-array detection (DAD) and mass spectrometric (MS) detection, to enable structural identification and confirmation of the analytes. This system was used to analyze paraphernalia (plastic straws and aluminum foils) that were used by heroin addicts in a pharmacokinetic study comparing smoked heroin with injected heroin (8). These samples could provide reliable information on the exposure of

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patients to any degradation products after inhaling volatilized heroin, especially because they were obtained from a controlled clinical trial using pharmaceutical heroin, eliminating the possible influence of impurities, diluents, and adulterants present in street heroin.

Experimental

We analyzed the plastic straws (cut to ± 11 cm) and aluminum foils (10×20 cm) that were used by heroin addicts in a pharmacokinetic study (8). In this study, 10 male patients, participating in the Dutch Heroin trial (1), were admitted to a closed clinical research facility to study the pharmacokinetics and pharmacodynamics of smoked heroin compared to injected heroin. All patients received pharmaceutical heroin twice a day for four consecutive days. Pharmaceutical heroin for inhalation after volatilization consisted of a powder mixture of 75% w/w diacetylmorphine base and 25% (w/w) caffeine anhydrate. Maintenance doses (200–300 mg) were used, but the total morning dose was varied double-blindly: each patient used 66%, 100%, or 150% of his maintenance dose (overall dose range 133–450 mg), and the morning dose was dispensed in two unequal portions (40% and 60%, w/w). New paraphernalia (plastic straws and aluminum foil) were dispensed with each new (portion of a) dose. Patients volatilized the pharmaceutical heroin by placing it on a piece of aluminum foil and heating it with a cigarette lighter until it melted and evaporated. The resulting fumes were inhaled through a plastic straw. The patients were allowed a maximum of 30 min to complete the inhalation procedure.

Apparatus

The HPLC system with DAD consisted of an 1100 series binary HPLC pump (model G1312A, Agilent Technologies, Amstelveen, The Netherlands), a SpectraSERIES model AS3000 automatic sample injection device, equipped with a 100- μ L sample loop (Thermo Separation Products, Breda, The Netherlands) and a photodiode-array detector (WatersTM 996, Waters Chromatography B.V., Etten-Leur, The Netherlands). Chromatograms were processed using Chromeleon[®] software (Dionex Corporation, Sunnyvale, CA).

The LC system used for MS detection was an HP1100 LC (Agilent Technologies, Palo Alto, CA), consisting of a binary pump, autosampler, degasser, and column oven. The LC flow was split 1/20 before entering an API3000 triple quadrupole MS equipped with an electrospray ion source (Sciex, Thornhill, ON, Canada). The quadrupoles were operated in the positive ion mode with unit resolution. The ion spray voltage was 5500 V, and the source temperature was set at 400°C. A range of m/z 250–500 amu was scanned for the identification of the unknown degradation products. A step size of 0.1 amu was used with dwell times of 2 and 5 s (product ion scans and Q1 scans, respectively).

In both LC systems, separation was achieved using a Zorbax Bonus RP analytical column (15 cm \times 4.6-mm i.d., 5- μ m particle size, Rockland Technologies, Inc., Newport, DE) that was

protected by a reversed-phase guard column (10 \times 3-mm i.d., Varian) and kept at 32°C during analysis. The mobile phase consisted of a 5mM ammonium acetate buffer (pH 5.7), mixed with acetonitrile according to a programmed gradient: 0–2 min, 3% acetonitrile; 2–2.6 min a linear rise from 3 to 13% acetonitrile; 2.6–8 min, 13–15.5%; 8–15 min, 15.5–80%; and 15.1–24 min, 3% acetonitrile. Flow was 1 mL/min, and the injection volume was 20 μ L.

Diacetylmorphine base was manufactured specifically for the clinical trial and obtained through the Central Committee on the Treatment of Heroin Addicts (Utrecht, The Netherlands). Caffeine anhydrate and morphine hydrochloride were purchased from Bufa (Uitgeest, The Netherlands), and 6-acetylmorphine hydrochloride was from Sigma Aldrich (Zwijndrecht, The Netherlands). Normorphine and 6-acetylcodeine were obtained from Radian International (via Schmidt, Amsterdam, The Netherlands), and N,3,6-triacetylnormorphine was a gift from Diosynth (Oss, The Netherlands). Other chemicals used were analytical grade or HPLC grade.

Methods

In preparation for analysis, the plastic straws were placed in 20 mL of a 1:1 (v/v) mixture of 5mM ammonium acetate solution (pH 4) and acetonitrile and were subsequently sonicated for 15 min. The resulting solutions were diluted to 25.0 mL with the same solvent mixture and either stored at -20°C or diluted further for HPLC analysis. The aluminum foil samples were cut in smaller pieces before placing them in 20 mL of a 1:1 (v/v) mixture of 5mM ammonium acetate solution (pH 4) and acetonitrile. Sonication of the samples was replaced by mechanical shaking for 30 min because the aluminum foil disintegrated when sonicated. The resulting solutions were analyzed after dilution to 25.0 mL with the same solvent or stored at -20°C .

Six calibration standards were used to construct a calibration line for quantification of four analytes. The concentration ranges were 1–10 $\mu\text{g/mL}$ for morphine and 6-acetylmorphine, 1–50 $\mu\text{g/mL}$ for caffeine, and 1–100 $\mu\text{g/mL}$ for diacetylmorphine. Quality control solutions containing all four analytes, with concentrations in the lower, middle, and high ranges of the calibration lines, were used. For optimization experiments, a mixture of seven reference standards as well as separate standard solutions in 5mM ammonium acetate buffer (pH 4) were prepared (concentrations: 5–10 $\mu\text{g/mL}$ for normorphine, morphine, 6-acetylmorphine, 6-acetylcodeine, and N,3,6-triacetylnormorphine and ± 28 $\mu\text{g/mL}$ for diacetylmorphine and caffeine).

Results and Discussion

HPLC–DAD method

The analysis of heroin for bio-analytical, forensic, or pharmaceutical purposes has been the subject of research for decades, and many papers have been published on the subject. However, analytical methods that can be used in the study of the volatilization process of heroin (when it is smoked or 'chased') have received relatively little attention. The earliest

study on this subject used an aspecific determination of 'total phenol content' (9), but different chromatographic methods have been developed more recently. Several authors mention the use of HPLC with UV detection (5,6,10) or gas chromatography (GC)-MS (7,11,12) for quantification and identification of compounds. GC was not preferred because using elevated temperatures (200–300°C) in the analysis (injector and/or transfer line) could result in on-line degradation of the analyte(s), which could in turn lead to problems identifying the true pyrolysis products.

Therefore, we decided to develop an HPLC method to be used specifically in the study of the volatilization process of heroin. Easy quantification of heroin, caffeine, 6-acetylmorphine, and morphine was required, and the chromatographic system should be able to separate the known and/or likely pyrolysis products from the components mentioned. Furthermore, the LC system had to be suitable for both photodiode-array detection and MS, to enable identification of unknown components.

Most degradation products that were reported in literature to have formed on heating pure diacetylmorphine base or its hydrochloride salt were found to be structurally related to mor-

phine (Table I). The three active sites on the molecule (C₃, C₆ and N₁₇, see Figure 1) appear to be able to either receive or lose methyl (ether) and acetyl groups during the volatilization process, creating differently substituted morphine analogues. Therefore, we selected reference standards that represented many substitution types (mono-, di-, and triacetylation and/or methylation of the morphinan structure), so a large polarity range would be covered and the chance of missing compounds in the analysis would be minimized. Seven reference substances were available: diacetylmorphine, 6-acetylmorphine, morphine, normorphine, normorphine, 6-acetylcodeine, and N,3,6-triacetylnormorphine.

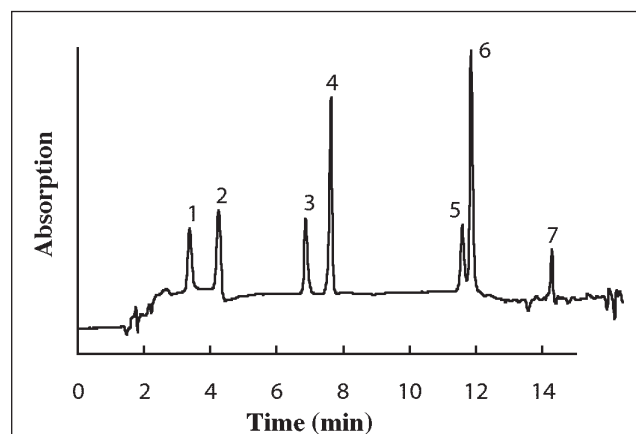
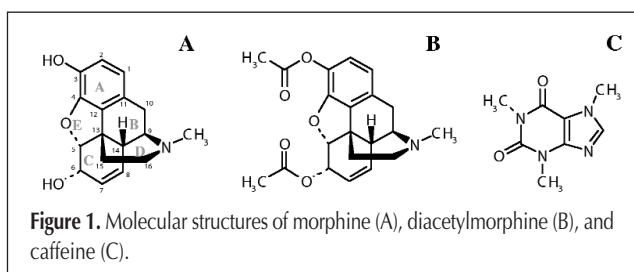
A 5mM acetate buffer was selected as the polar component of the mobile phase and as the solvent because it is compatible with MS and has a suitable buffering range (pK_a 4.76) for optimal stability of diacetylmorphine [pH 4–4.5, (13,14)]. The mobile phase gradient with acetonitrile was based on the gradient that was used in the bioanalysis of heroin and that was developed in our institution to analyze 16 components in a large polarity range (15). To achieve optimal separation and peak shape for all of the seven reference standards, optimization experiments were carried out. The mobile phase gradient was modified and flow, pH, and column temperature were subsequently optimized. The resulting LC system effectively separated the seven reference standards (Figure 2) and calibration lines with R² > 0.999 were obtained for diacetylmorphine, 6-acetylmorphine, morphine, and caffeine. The highest and lowest calibration solutions showed coefficients of variation < 1.5% (n = 6), indicating that quantification was sufficiently precise.

Because the LC system was optimized for diacetylmorphine-related compounds, it was considered necessary to ensure that caffeine-related compounds were also separated. Eight reference standards, structurally related to caffeine, were shown to be sufficiently separated in this system: 1-methyl uric acid had a retention time of 5.53 min; 1-methylxanthine, 5.73 min; theobromine, 5.85 min; 1,7-dimethylxanthine, 6.38 min; 1,7-dimethyl uric acid, 6.53 min; theophylline, 6.60 min; and 5-acetylamino-6-amino-3-methyluracil, 3.2 min.

Table I. List of Pyrolysis Products Mentioned in the Literature*

Sample	Pyrolysis Products	References
Diacetylmorphine base	6-Acetylmorphine	5–7,10–12
	3-Acetylmorphine	7
	N,6-Diacetylnormorphine	5–7
	N,3,6-Triacetylnormorphine	5–7
	Morphine	5,7
Diacetylmorphine HCl	3-Acetylmorphine	7
	6-Acetylmorphine	5,6
	N-Acetylnormorphine	7
	N,6-Diacetylnormorphine	5,6
	N,3,6-Triacetylnormorphine	5,6
	N,3-Diacetyl-6-O-methylnormorphine	7
	6-Acetylcodeine	7
	3,4-Diacetoxypheanthrene	6
	1,10-Diacetoxypheanthrene	7
	9-Hydroxypheanthrene	7
	N-(2-Phenylethyl)-acetamide	7
	Isoquinoline	7

* Pyrolysis products were found after heating diacetylmorphine base or salt at temperatures ranging from 250 to 400°C, using different heating methods [a quartz boat in a furnace (6), aluminum foil and a lighter (5,7), or a smoking device (11)].



Quantitative analysis of paraphernalia samples

The weight of the residue on the aluminum foil and in the straw samples was calculated from their weight before and after use in the process of chasing the dragon. The mean residue weight in the straw samples was 11.9 ± 9.4 mg (range 0–62 mg, $n = 119$) and on the aluminum foil samples a mean residue of 4.8 ± 6.2 mg (range –2–61 mg, $n = 120$) was found. All foil samples showed thick black residues on the side that was in close contact with the cigarette lighter during the volatilization of heroin. This is because of the natural inclination of addicts to make optimal use of the dispensed drug: they heated the powder mixture with great care for as long as it took for all signs of vaporization to cease, creating thick layers of soot on the under side of the foil and very small, carbonized residues on the top. A simple experiment showed that heating a 'blank' piece of aluminum foil for 2 min with a cigarette lighter resulted in a weight increase of 0.6 mg. This indicates that it is likely that most of the weight increase of the foil after use was due to the deposition of soot from the cigarette lighter because the chasing-the-dragon process took 16 min on average, creating 8 times as much soot residue as in the 2-min experiment. This is probably also the explanation for the significant correlation of the weight of the foil residues with the dose of heroin used (Pearson correlation 0.369, $p < 0.001$): more time is needed to volatilize a larger dose, leading to more deposited soot on the aluminum foil. Straw sample residue weight was also significantly correlated with the dose of pharmaceutical heroin used (Pearson correlation = 0.447, $p < 0.001$), which could be explained by increasing amounts of vapor being released from larger doses, depositing more residue on passing through the straw. Therefore, recoveries will be reported corrected for dose: as milligram recovered per 100 mg heroin (100 mg heroin = 100 mg diacetylmorphine base + 33 mg caffeine anhydrate). The corrected mean residue weights were 6.2 ± 4.2 mg/100 mg heroin (range 0.0–25.0) in straw samples and 2.4 ± 2.3 mg/100 mg heroin (range –1.7–20.3) on aluminum foil samples. Apparently, during chasing the dragon, only about 6% of a heroin dose is lost to absorption through deposition in the straw, indicating that inhalation through a straw during chasing the dragon allows effective inhalation after volatilization. Efficient inhalation (maximizing the amount of vapor reaching the airways) will probably depend mostly on the chasing technique of

the user and on the circumstances (e.g., air turbulence disturbing the vapors).

Straw sample chromatograms showed that all residues contained 6-acetylmorphine (2.2 ± 1.5 mg/100 mg heroin, $n = 119$), caffeine (1.2 ± 0.7 mg/100 mg heroin, $n = 119$), and diacetylmorphine (1.2 ± 1.3 mg/100 mg heroin, $n = 119$). Only 10 samples showed traces of morphine (0.2 ± 0.1 mg/100 mg heroin). In total, 74% of the residue weight was recovered as diacetylmorphine, caffeine, or one of the hydrolysis products of diacetylmorphine, which implies that 26% of the residue weight consists of unknown substances and (probably) soot. Large variations in recovery of known and unknown compounds were observed (Figure 3) between and within patients. We should, however, bear in mind that the quantitative composition of the straw residue is not necessarily similar to the quantitative composition of the inhaled vapors, as the tendency to deposit inside the straw could differ between the constituents of the vapors.

As expected, even the 10 foil residues with the largest residue weights (10–61 mg, 3.3–20.3 mg/100 mg heroin) contained only very small amounts of diacetylmorphine, caffeine, or degradation products. Only 0.08–10% (w/w) of these residues was recovered as one of the four quantified analytes: 6-acetylmorphine (mean 0.12 mg/100 mg heroin, range 0.004–0.48) and diacetylmorphine (0.08 mg/100 mg heroin, range 0.01–0.25) were present in all 10 foil samples, whereas caffeine was found in five samples (0.01 mg/100 mg heroin, 0.004–0.02) and morphine in only one sample (0.02 mg/100 mg heroin).

Identification of unknown peaks

The HPLC–DAD chromatograms of some of the straw samples contained several small, unidentified peaks: two examples are shown in Figure 4. Even though baseline separation was not achieved for all unknown compounds, resolution was sufficient to select eight peaks for identification. The peaks that were selected for identification (A–H) varied in detection frequency: compound G and C were observed in only 6 and 20 of the 120 straw samples, respectively, whereas A, B, E, F, and D occurred in 94, 108, 111, 112, and 118 of the samples, respectively. Foil sample chromatograms contained very few unidentified peaks: five of the samples (analyzed without prior dilution) contained peak D, and peak E was detected in six samples.

Retention times and relative peak areas of the unknown compounds are listed in Table II. A UV-spectrum could be obtained

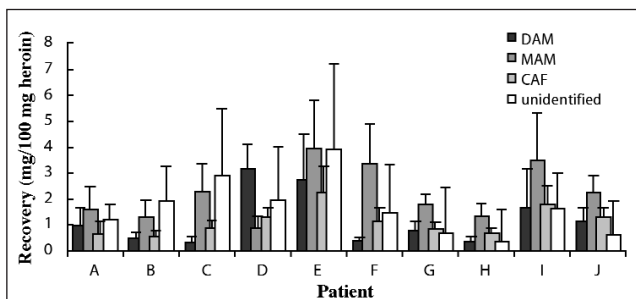


Figure 3. Results of the quantitative analysis of straw samples. For each patient (11–12 samples per patient), the mean recoveries of diacetylmorphine (DAM), 6-acetylmorphine (MAM), and caffeine (CAF) are given, as well as the size of the unidentified part of the residue. All values are corrected for dose (100 mg heroin = 100 mg diacetylmorphine + 33 mg caffeine); error bars indicate standard deviations.

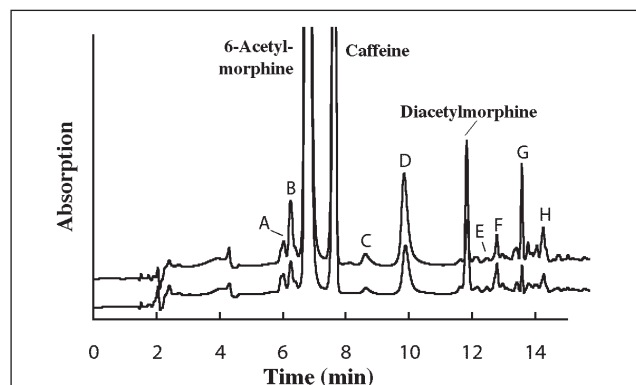


Figure 4. Chromatograms of two straw samples showing degradation peaks A–H (blank subtracted).

for six of the eight unknown peaks, all of which were similar in shape and absorption maximum (λ_{\max}) to morphine and morphine-like reference standards (Table II). Only compound H showed a λ_{\max} (279 nm) that resembled the slightly shifted maximum found in diacetylmorphine and N,3,6-triacetylnormorphine. None of the peaks showed UV-spectra comparable to that of caffeine ($\lambda_{\max} = 272$ nm) or caffeine-related compounds, which generally have a lower λ_{\max} (234–271 nm) and higher specific absorbances than morphine-related compounds. Potential mass-to-charge ratio values for molecular ions ($[M+H]^+$) in peaks of compounds A–H were obtained from Q1 mass

spectra of straw samples, and product ion spectra were obtained for each of these parent masses (Figure 5, Table II).

Compound A. The peak shape of unknown peak A suggests that it is not quite pure (Figure 4), and slightly different absorption maxima could be distinguished for the first (A1) and last (A2) part of the peak: 280 and 286 nm, respectively (Table II), in some straw samples. Both UV-spectra, however, were morphine-like in shape, and the retention time of 5.7 min was suggestive of slightly less polar morphine derivatives. The Q1 scan of the signal at 5.7 min showed a dominant mass at m/z 286 and a smaller signal at 302. The product ion spectrum of

the dominant mass resembled that of the morphine reference standard (Figure 6), which could be explained by the presence of hydromorphine (m/z 286 = $[M+H]^+$) (Figure 7A). A hydromorphine reference standard showed the same principal peaks as the morphine reference standard, and it showed the same retention time and absorption maximum as compound A1, confirming the proposed structure (Table II).

The second observed Q1 mass, m/z 302, might then have been associated with the second part of unknown peak A, with the absorption maximum of 286 nm. This parent mass suggests the addition of oxygen to morphine, which could indicate the formation of, for example, morphine-N-oxide (Figure 7C), 10-hydroxymorphine (Figure 7D), or 14-hydroxymorphine. Hydroxylation of the phenyl ring of morphine is also possible, but unlikely in this case, as such a structural change would cause a bathochromic shift in the UV spectrum compared to that of morphine. Furthermore, hydroxylation of C_{14} in a morphine-like structure was reported to be possible only when C_{14} was unsaturated (like in thebaine) (16), which would suggest C_{10} as the preferred site for hydroxylation. Oxidation is a major mechanism of degradation of morphine in aqueous solutions, and degradation is known to be catalyzed by oxygen of air, sunlight, UV irradiation, and organic impurities (17). Morphine-N-oxide is a well-known oxidation product of morphine in aqueous solutions (17), and it would be expected to have a longer retention time than morphine, as opposed to 10-hydroxymorphine, which is more polar than morphine. Therefore, we propose morphine-N-oxide as a possible structure of compound A2 (Figure 7C).

Compound B. The UV-spectrum of the peak at 6.2 min (compound B) shows a small shift of λ_{\max} , compared to morphine, similar to that of the 6-acetylmorphine reference standard (Table II). The parent mass found at this retention time was also the same as for 6-acetylmorphine (m/z 328, $[M+H]^+$), but the product ion mass spectrum differed slightly: m/z 268

Table II. Properties of Reference Standards and Unidentified Peaks*

Name	T_R (min)	λ_{\max} (nm)	Relative Peak Area (%)	Parent m/z	Principal Peaks (m/z)
Normorphine	3.4	285		272	165, 181, 153, 209, 121-201
Morphine	4.2	285		286	152, 128, 165, 115, 189
A1	5.7	280		286	152, 165, 128, 115, 127, 151
A2	5.7	286	0.05 (0.01–0.28)	302	
Hydromorphine	5.7	280		286	128, 152, 115, 157, 165
B	6.2	282	0.29 (0.01–2.64)	328	268, 193-219, 211, 191, 237, 165, 286
6-Acetylmorphine	6.7	283		328	211, 193, 165, 183-191-209, 201
Caffeine	7.3	272		195	138, 195, 110
C	8.4	285	0.19 (0.01–0.31)	344	268, 215, 162, 165-191-266
D	9.7	284	1.56 (0.01–4.58)	344	268, 162, 215-266, 145
6-Acetylcodeine	11.6	284		342	225, 197, 282
Diacetylmorphine	11.8	279		370	268, 328, 211, 193-237
E	12.3		0.21 (0.02–0.80)	384	225, 283, 207-251, 342
F	12.7	285	0.35 (0.06–0.55)	386	268, 162-215, 327-310
G	13.5		0.71	356	254, 219, 191, 237, 211
Triacetylnormorphine	14.2	279		398	237, 254, 219, 296, 356
H	14.2	279		398	237, 296, 254, 219, 356, 211

* Median values for relative peak area (%) of the unknown peaks in straw sample chromatograms are given with the range within parentheses; principal peaks are given in order of decreasing intensity with equally large peaks separated by a hyphen.

was the base peak instead of 211, and higher relative intensities were observed for the peaks at m/z 193, 219, and 237 (Table II). Because m/z 268 indicates the loss of acetic acid from the parent mass, it was likely that the main difference between compound B and 6-acetylmorphine was due to the position of the acetyl group, suggesting the presence of 3-acetylmorphine (Figure 7B). 3-Acetylmorphine was expected to have a very similar UV-spectrum and retention time to 6-acetylmorphine, but no reference standard was available for definite confirmation by direct comparison of 3-acetylmorphine as the proposed structure for compound B.

Compounds C and D. Although the retention times of compounds C and D were very different, both had morphine-like UV-spectra and a parent mass at m/z 344 in their Q1 scan. The resulting product ion mass spectra were also very similar, they differed only in the relative intensities of the fragments with m/z 165, 181, and 191 (Figure 5). The parent mass of 344 suggests addition of oxygen to 6-acetylmorphine, which was confirmed by the presence of intense peaks at m/z 266/268 and 284 and suggest the loss of acetic acid from 6-acetylmorphine ($[M+H]^+ = 328$) as well as from $[M+O+H]^+ = 344$. Furthermore, both spectra contain a peak at m/z 326 that could indicate the loss of water from the parent, which was not observed in any of the reference standards (Figure 6), indicating that it might be due to added oxygen. 6-Acetylmorphine is the main degradation product of diacetylmorphine found after volatilization and unidentified compounds could therefore be the result of degradation of 6-acetylmorphine. Oxidation of 6-acetylmorphine during heating and subsequent volatilization was considered a potential degradation mechanism because oxidation is known

to be important in degradation of morphine in aqueous solutions (17). Similar to oxidation of morphine, 6-acetylmorphine could be oxidized to 6-acetylmorphine-N-oxide (Figure 7C). Furthermore, following the same reasoning as under Compound A, the parent mass of 344 could also have been the result of hydroxylation of another position of the 6-acetylmorphine molecule, for example, C₁₀ (Figure 7D). 6-Acetyl-10-hydroxymorphine is expected to elute before 6-acetylmorphine-N-oxide. Therefore, 6-acetyl-10-hydroxymorphine is proposed as the structure of compound C, and compound D is proposed to be 6-acetylmorphine-N-oxide.

Compound E. The peak of compound E in the HPLC-DAD chromatograms was too small to obtain an UV-spectrum, but it was present in quantities large enough to obtain a parent mass (m/z 384) in a Q1 scan. The product ion mass spectrum (Figure 5) contained fragments suggesting the loss of two acetyl groups from the parent ion (m/z 342 and 300), as well as loss of acetic acid (324 and 264), indicating that compound E could be a 3,6-diacetyl-substituted morphine. The parent mass suggests the addition of CH₂, which is most likely to occur at the tertiary amine, possibly combined with opening of the piperidine ring. This mechanism was proposed by Cook et al. (18) to explain a pyrolysis product that they found after volatilization of diacetylmorphine hydrochloride. However, the product ion mass spectrum also shows a base peak with m/z 225 that was also observed in codeine (data not shown) and 6-acetylcodeine, but not in spectra of other morphine derivatives (Figure 6). However, the spectra of 6-acetylmorphine, diacetylmorphine, and N,3,6-triacetylmorphine did show a fragment with m/z 211, corresponding to 225 after loss of CH₂. These fragments could be the result of elimination of the piperidine (D-)ring and elimination of the group at C₆, leading to a structure with intact A, B, and E rings (see Figure 1), a conjugated C ring and either -OH (m/z 211) or -OCH₃ (m/z 225) on C₃. The fragment with m/z 283 in the product ion mass spectrum of compound E could then correspond to the 225 fragment, with an acetyl

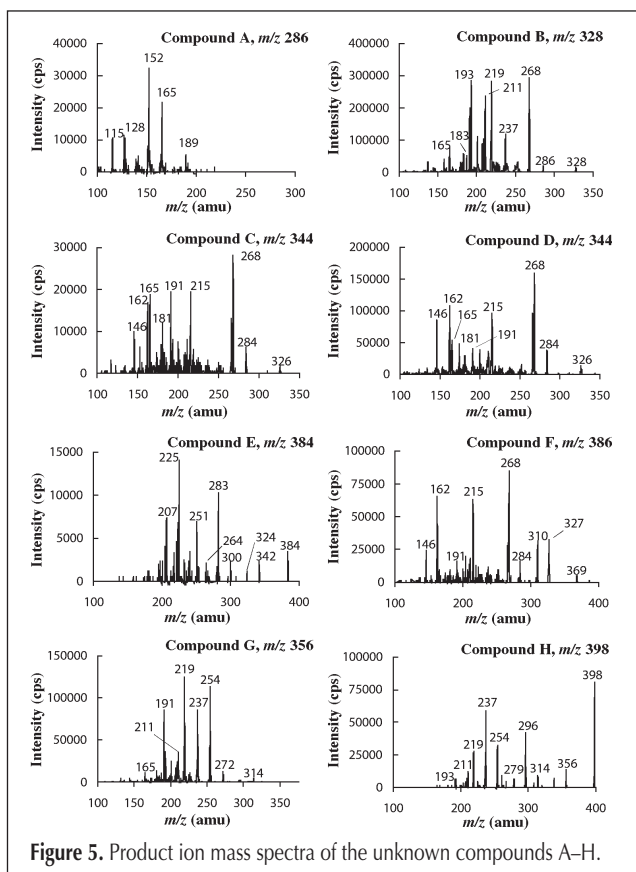


Figure 5. Product ion mass spectra of the unknown compounds A-H.

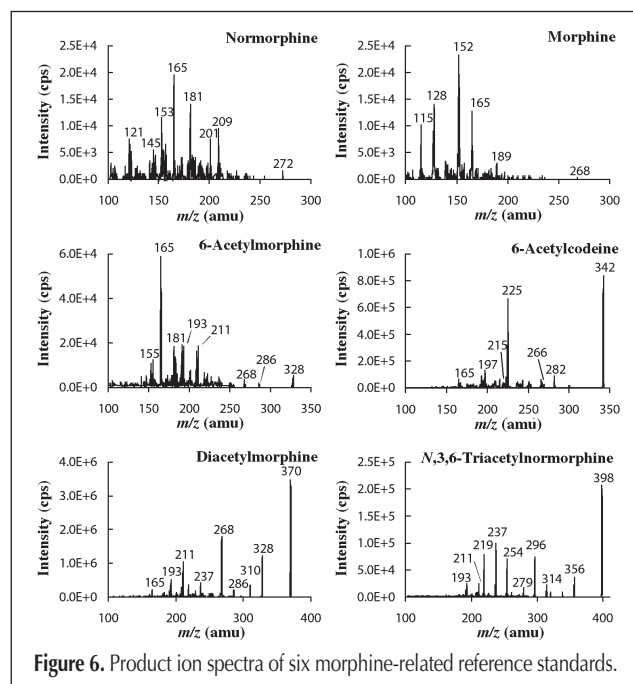


Figure 6. Product ion spectra of six morphine-related reference standards.

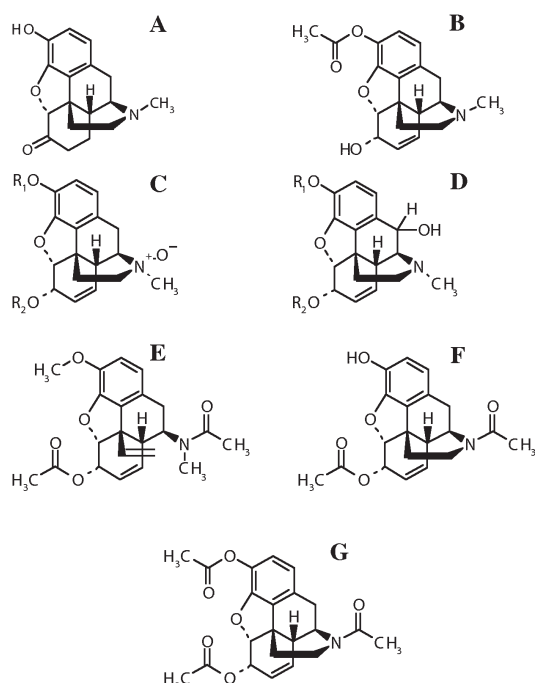


Figure 7. Structure proposals for compounds A–H. Compound A1, hydromorphone, m/z 286 (A); compound B, 3-acetylmorphine, m/z 328 (B); compound A2, *N*-oxide formation (C) or 10-hydroxy-formation (D), m/z 302 ($R_1=R_2=H$); compound C/D, *N*-oxide formation (C) or 10-hydroxy-formation (D), m/z 344 ($R_1=H$, $R_2=COCH_3$); compound F, *N*-oxide formation (C) or 10-hydroxy-formation (D), m/z 386 ($R_1=R_2=COCH_3$); compound E, *N*-methylation with opening piperidine ring, m/z 384 (E); compound G *N*,6-diacetylnormorphine, m/z 356 (F); and compound H, *N*,3,6-triacetylnormorphine, m/z 398 (G).

group at C_6 . Considering this, the structure in Figure 7E was proposed for compound E. It could have formed from diacetylmorphine during volatilization by elimination of C–O from the C_3 -acetyl group and subsequent opening of the piperidine ring according to the mechanism by Cook et al. (18).

Compound F. Compound F combined a morphine-like spectrum with a retention time of 12.7 min. At this retention time, the most abundant mass in the Q1 spectrum was found to be m/z 386, corresponding to addition of oxygen to diacetylmorphine. The product ion mass spectrum was very similar to that of compound D (Figure 5), and the same line of reasoning as in the Compound A and Compounds C and D sections might be followed to derive the most likely position of the added oxygen in the morphinan structure. Formation of an *N*-oxide is more likely than 10-hydroxylation because of the similarity of the product ion spectra of compounds D and F, and because compound D elutes later than diacetylmorphine, indicating that it is less polar. Therefore, the proposed structure for compound D is 3,6-diacetylmorphine-*N*-oxide (Figure 7C).

Compound G. The chromatographic peak for compound G was superimposed on a blank peak, which caused interference in its UV-spectrum. However, the Q1 scan clearly showed a single most abundant mass at 13.5 min: m/z 356. The product ion mass spectrum of this parent ion showed peaks indicative of the loss of one (m/z 314) or two acetyl (m/z 272) groups as well as one acetyl group and acetic acid (m/z 254). This information

suggests that the structure contains two acetyl groups, one of which is positioned on N_{17} in the morphinan structure. Furthermore, because the parent ion mass suggests addition of a carbonyl (C=O) to acetylmorphine, and 6-acetylmorphine is abundantly present as a degradation product during volatilization, we propose *N*,6-diacetylnormorphine as the structure of compound G (Figure 7F). This proposal was supported further by the similarity between the product ion spectra of compounds G and H because the latter was also identified as an *N*-acetylated morphine derivative. *N*,6-Diacetylnormorphine has been reported before as a degradation product occurring after heating diacetylmorphine base or hydrochloride (Table I).

Compound H. The most likely candidate for the identity of compound H is easily derived from Table II: *N*,3,6-triacetylnormorphine. Both compounds showed the same retention time, a similar shift in UV-spectrum, the same parent mass, and similar principal peaks in the product ion scan. We therefore conclude that compound H is *N*,3,6-triacetyl-normorphine (Figure 7G).

Overall recovery

Morphine-related compounds with different substitution patterns for C_3 , C_6 , and N_{17} (i.e., codeine, 6-acetylmorphine, normorphine, dihydromorphine, and diacetylmorphine) have similar specific absorbances ($A^{1\%}_{1\text{cm}} = \pm 50$) (19). Therefore, the relative peak area of the unidentified peaks in the LC–UV chromatograms could be used to calculate the relative amounts of the unknown compounds present in the straw samples (Table II). The total peak area of the unidentified compounds in the straw chromatograms was 0.4–9.7%, and diacetylmorphine, 6-acetylmorphine, morphine, and caffeine accounted for a mean of 74% of the residue weight in straws, which suggests that not all constituents of the residue were identified. Similar to the foil samples, part of the straw residue might also have consisted of soot from the cigarette lighter flame, as over 80% of the samples showed a light-brown to black-colored residue. Moreover, many showed signs of melting, indicating that the flame had come close enough to the straws to deposit soot.

The identities of the constituents of the straw sample residues that were analyzed in this study can be considered representative of the composition of the vapors that patients inhale when they use pharmaceutical smokable heroin by inhalation after volatilization. These samples were found to contain mainly unchanged constituents of the volatilized pharmaceutical product, as well as the well-known hydrolysis products of diacetylmorphine, that are considered to be its active metabolites (6-acetylmorphine and morphine). The degradation products that were observed in the straw samples were in agreement with those found in *in vitro* experiments involving heated diacetylmorphine base in literature (Table I), with the exception of the proposed *N*-oxide and 10-hydroxyl derivatives, which were not reported before. The majority of the degradation products were morphine derivatives with different substitution patterns on the C_3 , C_6 , and N_{17} positions (Figure 1). Only one proposed structure (compound E) showed ring cleavage that could lead to the formation of phenanthrene derivatives (Table I), which are known to be potentially toxic. Some of the compounds that were detected could bind to μ -receptors and could have analgesic and euphoric effects. Hydromorphone (compound A1) is

a well-known active analgesic (trade name Dilaudid) that is considered to be twice as potent as heroin (19–21). Opiate binding studies have shown that the lack of a free 3-hydroxyl group leads to a low binding affinity to μ -receptors (20,22,23). Moreover, substitution at N₁₇ also influences receptor binding affinity (20). This means that 3-acetylmorphine (compound A1), N,6-diacetylnormorphine (compound G), and N,3,6-triacetylnormorphine (compound H) are probably not active, even though they could be metabolized to active compounds after systemic absorption. Deacetylation of 6-acetyl-10-hydroxymorphine (compound C) would yield 10-hydroxymorphine, which has been reported to be active (24). Morphine N-oxide (compound A2) was reported to have weak analgesic effects, weaker subcutaneous and intravenous toxicity than morphine, and no teratogenic effects in mice (17). In summary, some of the degradation products could have opioid activity, but further research is required to assess their activity and toxicity after chronic administration via inhalation after volatilization.

Conclusions

We successfully used an HPLC–DAD–MS method to analyze the contents of plastic straws and aluminum foil samples used by addicts chasing the dragon in a clinical study. The residue in the straw samples, that was considered representative for the vapor reaching the patient's lungs, was found to consist mainly (75%) of unchanged diacetylmorphine, 6-acetylmorphine, caffeine, and morphine. Chemical structures were proposed for nine degradation products that were found to be morphine derivatives with different substitution patterns of the C₃, C₆, and/or N₁₇ positions and that were estimated to comprise 0.4–9.7% of the straw sample residue weight. Activity and toxicity of most of these compounds is unknown and requires further investigation.

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