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# A 'cold synthesis' of heroin and implications in heroin signature analysis Utility of trifluoroacetic/acetic anhydride in the acetylation of morphine

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## Abstract

Treatment of morphine, at room temperature, with a mixture of trifluoroacetic anhydride (TFAA) and acetic acid (20–30 min) affords good yields of heroin. GC–MS and HPLC examination shows that heroin produced by this route to be extremely clean, but the product contains slightly less heroin than observed via the more traditional acetic anhydride (AA) route (76.1% versus 83.55%); and greater quantities of 3-MAM and 6-MAM (6.9% versus 0.75% and 7.13% versus 0.63%). The concentration ratios of the major alkaloid impurities were found to be both production method (TFAA and AA) as well as morphine extraction methodology dependant. Data contained herein describe the impact of this new production method on current intelligence efforts, largely by-passing existing heroin signature programs and the UNDCP's efforts to restrict access to key synthetic precursors. Given the methodology dependency we find that examination of the major alkaloid ratios is unsuitable for the development of a new heroin signature program.

Further examination of the TFAA methodology allowed the identification of TFAA specific marker compounds, namely *bis*-trifluoroacetylmorphine (**30**), 3-trifluoroacetyl-6-acetylmorphine (**31**), 3-acetyl-6-trifluoroacetylmorphine (**32**) and trifluoroacetylcodeine (**33**). However, the hydrolytic lability of trifluoacetyl esters requires careful treatment of suspect samples, thus we propose a modification to existing HSP's in instances were the 6-MAM/WM ratio falls within the average minimum and maximum values of 6.17 and 17.32.  $\bigcirc$  2006 Elsevier Ireland Ltd. All rights reserved.

Keywords: Heroin; Signaure program; Acetylation of morphine; Cold synthesis

# 1. Introduction

Globally the heroin seized rises by about 5% per annum [1]. Within Australia these figures are even more disturbing as a result of record numbers of seizures, with the mid 1990s average of 250 kg per year increasing to between 450 and 730 kg per year since 2000 [2,3]. Indeed, despite education efforts within Australia a recent Australian National Drug Household survey indicates that domestic demand is on the increase and that the number of Australian heroin users has more than doubled since 1988 [4]. The semi-synthetic nature of heroin, being derived from morphine (Fig. 1) has facilitated the development and implementation of a series of heroin signature

programs (HSP). In a HSP seized samples are analysed by a number of different analytical procedures with the aim of providing crucial drug trafficking and distribution intelligence. HSP typically analyses and evaluates the major synthetic impurities and adulterants. These approaches have been extensively examined and reported previously [1,5-37]. Worldwide, the impurity profiling of illicit drugs is being increasingly utilised as an intelligence-gathering tool, to support and complement the work of law enforcement agencies allowing an objective, comparative analysis of the level of commonality between samples [9-13,32,33]. The chemical information provided from an impurity profile can be used to provide tactical intelligence, which involves establishing links between different samples [14,15] and strategic intelligence, which refers to the identification of the source of a sample [28-30], the tracing of distribution routes and identifying new production processes [31].

To date HSP analysis rapidly allows South-West Asian (SWA) heroin to be identified by significant quantities of both

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Fig. 1. Acetylation of morphine to yield diacetylmorphine (DAM, heroin).



Fig. 2. Route specific markers detected in the illicit production of heroin.

papaverine (PAP) and noscapine (NOS); South-East Asian (SEA) heroin by an absence of these alkaloids; Mexican heroin by its unique appearance; and South American heroin by its high purity and low acetyl codeine (AC) content. Further, quantitative major component data have been used successfully by a number of authors to determine the source of heroin [9,10,12,17,19].

Whilst a HSP typically concentrates on determination of geographic origin, it is also possible to elicit information relating to the routes of synthesis used in the actual manufacture of seized DAM. Standard HSP analysis is based on acetic anhydride (AA) as the principle acetylating agent. However, AA is not the sole acetylating agent available in the clandestine manufacture of heroin. There are currently two other acetylating agents described in the literature: acetyl chloride, which affords 1-chloroheroin (**3**) as a route specific marker; and ethylene diacetate, which

affords 3-[1-(1-carboxymethoxyethyl)]-6-acetylmorphine (4) as a route specific marker (Fig. 2) [5,38,39].

Over the past 30 years considerable work has been done developing parameters (Fig. 3, marker compounds 5-10) for the profiling of heroin. During this period only three acetylating agents have been reported AA, acetyl chloride and ethylene diacetate, with the former being employed almost exclusively. The recent discovery of traces of trifluoroacetic acid in a seized sample promoted an investigation into the use of this and related reagents in the synthesis of heroin [40].

# 2. General techniques and instrumentation

All solvents used herein were of HPLC grade.

#### 3. HPLC major impurity determination

The analyses were performed on a Perkin-Elmer ISS 200 Series HPLC equipped with an LC-235C diode array detector measuring at 240 and 225 nm. The chromatographic separation was achieved on an Alltec  $C_{18}$  column using a six-step methanol and phosphate buffer gradient (see Section 3.1) at a flow rate of 0.76 mL/min. The buffer (pH 2) comprised 1N sodium hydroxide (32 mL), phosphoric acid (11 mL) and hexylamine (3.5 mL) in millipore water (1000 mL).

## 3.1. Pump parameters

Step	Time (min)	Methanol (%)	Buffer (%)
1	10	5	95
2	20	30	70
3	6	30	70
4	10	80	20
5	4	80	20
6	10	5	95



Fig. 3. Chemical structures of the major alkaloids found in, and as a result of, the illicit synthesis of heroin.

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Comparison of chemical yields and major component analyses for the synthesis of heroin via the TFAA or the AA route	

Method	Target compounds								
	М	С	3-MAM	6-MAM	AC	DAM	NOS	PAP	Yield (%)
TFAA <sup>a</sup> AA <sup>b</sup>	$\begin{array}{c} 1.02 \pm 1.13 \\ 0.16 \pm 0.29 \end{array}$	$0.09 \pm 0.02 < 0.01$	$\begin{array}{c} 6.9 \pm 1.63 \\ 0.75 \pm 0.31 \end{array}$	$\begin{array}{c} 7.13 \pm 1.56 \\ 0.63 \pm 0.28 \end{array}$	$\begin{array}{c} 0.83 \pm 0.11 \\ 1.12 \pm 0.15 \end{array}$	$\begin{array}{c} 76.1 \pm 3.23 \\ 83.55 \pm 5.93 \end{array}$	<0.01 <0.1	<0.01 <0.1	$\begin{array}{c} 58\pm5\\ 64\pm12 \end{array}$

<sup>a</sup> Non-controlled precursor; reaction time 20-30 min; typically 3 equivalents TFAA; room temperature.

Listed on the International Narcotics Control Board's 'RED LIST': see http://www.incb.org/incb/en/red\_list.html; reaction time > 2 h; typically 10 equivalents AA; requires heating 80-90 °C.

Each sample under investigation was weighed (10 mg) into a 10 mL volumetric flask. Propiophenone (1 µL) was added as an internal standard and the mixture diluted to 10 mL with the addition of the injection solvent (5% methanol, 95% buffer).

## 3.2. GC-MS analysis

The analyses were performed on a Hewlett-Packard 6890 gas chromatograph equipped with a 5973 quadrupole massselective detector and an Agilent 7683 series autoinjector. The chromatographic separation was achieved on a HP-5MS (5% phenyl methyl siloxane) capillary column (length 30 m, i.d. 0.25 mm, film thickness 0.25  $\mu$ m) with helium carrier gas at a flow of 1.2 mL/min (pressure 88.9 kPa). The injection temperature was maintained at 260 °C, with 2 µL splitless injection. The column was heated at 100 °C for 1 min then increased at a rate 6 °C/min to 240 °C then 2 °C/min to 280 °C followed by 6 °C/min to 320 °C.

#### 3.3. Acidic and neutral trace impurity analysis

All reaction vials, vial inserts, pipettes and centrifuge tubes were silanised, to replace active hydrogen groups, prior to use.

Each sample under investigation was weighed (50 mg) into a glass centrifuge tube. Sulphuric acid (1N, 4 mL) and extraction mixture (60:40 ether-dichloromethane; 4 mL) were added to the sample. The solution was vortexed (15 min) and then centrifuged (15 min, 1000 rpm). An aliquot (3 mL) of the organic phase was pipetted into a reaction vial. The solution was dried under a stream of nitrogen at 70 °C. The sample was then treated with a derivatisation solution (250  $\mu$ L). The derivatising solution comprised BTFSA (1 mL), TMS (1%) and dichloromethane (1 mL). Next benzopinacolone (50  $\mu$ L) was added as the internal standard, at a concentration of benzopinacolone/dichloromethane of 2.5 mg/mL. The reaction vial was capped and heated at 70 °C for 30 min. The solution was allowed to cool and transferred to a GC vial for analysis.

GC-MS trace component analysis was used to determine the relative amounts of the target compounds in each sample.

## 3.4. Major component analysis

To 1 mg of each sample was added dichloromethane (250 µL) followed by 50 µL of internal standard (prepared as described above).

# 4. Results and discussion

There is literature precedent for the use of trifluoroacetic anhydride as an ester promoter, with two methods commonly described, differing only in the order of reagent addition [41,42]. Dissolution of morphine in TFAA followed by the addition of acetic acid gives rise to very low yields of DAM, but significant quantities trifluoroacetyl esters; conversely dissolution of acetic acid in TFAA forming the mixed anhydride followed by addition of morphine proved to be an extremely clean, efficient and elegant route for the production of high quality DAM.

The TFAA methodology provides for a four to six-fold decrease in reaction time in the absence of both heat and a large reagent excess (Table 1). Interestingly, neither acetic acid nor TFAA are restricted precursors, and can thus be readily obtained from chemical suppliers [43]. From a drug

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Alkaloid ratios for the AA and TFAA reactions using the Tas, SWA and SEA procedures

Method <sup>a</sup>	Ratio <sup>b</sup>						
	C/WM	NOS/WM	PAP/WM	6-MAM/WM			
Tas							
AA <sup>c</sup>	$1.48\pm0.22$	< 0.04	$0.01\pm0.02$	$0.80\pm0.36$			
TFAA <sup>d</sup>	$1.14\pm0.10$	< 0.01	$0.01\pm0.02$	$9.86 \pm 1.86$			
SWA							
AA <sup>e</sup>	$10.69\pm0.46$	$33.1\pm1.25$	$4.61\pm0.13$	$1.11\pm0.39$			
$\mathbf{TFAA}^{\mathrm{f}}$	$10.92 \pm 1.58$	$24.45\pm8.63$	$4.7\pm1.04$	$12.35\pm4.27$			
SEA							
AA <sup>e</sup>	$8.94\pm0.3$	$0.21\pm0.18$	$0.21\pm0.23$	$0.28\pm0.03$			
TFAA Base <sup>e</sup>	$9.07\pm0.09$	$0.25\pm0.22$	$0.06\pm0.2$	$9.54 \pm 2.7$			
TFAA HCl <sup>g</sup>	$9.78\pm0.07$	$2.12\pm0.34$	$0.26\pm0.1$	$9.03 \pm 1.31$			
Average							
$AA^{h}$	$5.56 \pm 4.35$	$6.68 \pm 13.93$	$0.99 \pm 1.91$	$0.71\pm0.42$			
TFAA <sup>i</sup>	$6.5\pm4.59$	$6.58 \pm 11.34$	$1.24\pm2.11$	$10.27\pm2.79$			

<sup>a</sup> Tas: Tasmanian procedure; SWA: South-West Asian procedure; SEA: South-East Asian procedure.

<sup>b</sup> C = total codeine (C + AC); $WM = (\%DAM \times 285.34/$ 369.42) + (%MAM × 285.34/327.38)(%MAM).

<sup>g</sup> n = 4.

- i n = 20.

<sup>&</sup>lt;sup>c</sup> n = 5.

<sup>&</sup>lt;sup>d</sup> n = 8.

<sup>&</sup>lt;sup>e</sup> n = 3.

 $<sup>^{</sup>f} n = 5.$ 

<sup>&</sup>lt;sup>h</sup> n = 11.

enforcement perspective the discovery of such a simple, rapid and facile method for DAM synthesis is significant. Not only does it offer the clandestine laboratories good yields with far greater throughput, but also the potential to circumvents the current UNDCP methods of tracking heroin production [43]. From Table 1, it is clear that the TFAA reaction gives rise to anomalous data outwith that anticipated in current HSP's. As can be seen from Table 1 the product produced via the TFAA route contains slightly less DAM (76.1%) than that observed via the more traditional AA route (83.55%); and greater quantities of 3-MAM (6.9% versus 0.75%) and 6-MAM (7.13% versus 0.63%). Furthermore all TFAA samples contained traces of morphine and codeine, whereas morphine was only detected in only two and codeine in none of the AA samples (data not shown). It is noteworthy to mention that trifluoroacetyl esters are notoriously unstable, readily hydrolysed under conditions required for analysis (see Section 6), meaning that their detection in clandestine laboratory samples is difficult [41,42], this is the most probable explanation for the increased concentrations of 3-MAM, 6-MAM, morphine and codeine detected.

Current HSP methodologies have evolved to deal with DAM synthesised via morphine acetylation by AA at elevated temperature, we thus felt it crucial that this new methodology be examined and key constituents analysed in an attempt to gather data as to how TFAA might impinge on existing HSP protocols. Accordingly we examined the amounts major illicit heroin components formed using the two methods (TFAA and AA) (Fig. 3 and Table 2) were compared by producing a number of replicate samples from three different morphine sources: Tasmanian poppy and typical South-West and South-East Asian morphine.

Table 2 highlights significant differences in the alkaloid ratios observed as a function of both the reaction method (TFAA or AA) and clandestine procedure employed. Variation in the noscapine, papaverine and codeine ratios between Tas, SWA and SEA series, can be explained by differences in the morphine extraction processes. The SWA procedure involves only a crude morphine extraction and consequently had the



Fig. 4. Trace level impurities targeted by the Australian National Heroin Signature Program.

highest NOS/WM (33.1 and 24.45 versus SEA 0.21, 0.25, 2.12 and TAS <0.01) and PAP/WM (4.61 and 4.7 versus SEA 0.21, 0.06, 0.26 and TAS 0.01) ratios. In contrast, the SEA employs steps to remove the majority of noscapine and papaverine, which resulted in the smaller NOS/WM (0.21, 0.25, 2.12 versus 33.1 and 24.45) and PAP/WM (0.21, 0.06, 0.26 versus 4.61 and 4.7) ratios observed in this series. The morphine used in Tas series had been refined using an unknown chemical process, which removed the majority of non-morphine alkaloids.

Our data clearly illustrate that the amounts of these nonmorphine alkaloids are related to the morphine extraction process and not the acetylating procedure. Consequently these ratios cannot be used as TFAA specific profiling parameters. In contrast, the 6-MAM/WM ratio is significantly higher for all TFAA produced samples (e.g. 9.86 versus 0.8 for Tas series) and remains constant irrespective of the morphine sample or clandestine procedure employed (TFAA reaction 9.86, 12.35 and 9.54, 9.03 for the Tas, SWA, SEA Base and HCl procedures, respectively). This indicates that the 6-MAM/WM ratio is both acetylating reagent and production process independent making it an ideal profiling parameter for the identification of the use of TFAA in the illicit synthesis of heroin.

Given that the major alkaloids are not suitable for determination of TFAA versus AA synthesis route we next turned our attention to analysis of the trace impurities (Fig. 4 and Table 3). Data presented in Table 3 clearly illustrate significant differences in the quantities of morphine related byproducts; **11–13** and **15** are produced when AA is employed as the acetylating agent, e.g. **13**: 38.74, 51.13 and 100 for the Tas, SWA and SEA Base methods, respectively, as compared with the TFAA method, e.g. **13**: 7.19, 9.92 and 50.33 for the Tas, SWA and SEA methods, respectively. These compounds are formed by the *N*-acetylation followed by demethylation of morphine and codeine N-oxide, which are transient intermediates formed during the acetylation process [22]. Not surprisingly our data confirm the TFAA routes as milder, resulting in lower initial concentrations of these intermediate species and hence lower amounts of **11–13** and **15**.

Further examination of Table 3 reveals a contrast between the relative proportions of thebaine related products formed during the two reactions. All three series show that the AA procedure resulted in the formation of substantially greater amounts of impurities (e.g. **19**: AA method: 6.16, 97.79 and 0.84; TFAA method 0.47, 4.75 and 0.02 for the Tas, SWA and SEA methods, respectively), with the exceptions of **17** in the SEA series (AA: 0.2; TFAA: 0.4) and impurities **20–23** which were formed in essentially equal amounts. Control experiments, treating thebaine according to the TFAA protocol showed that the observed differences (AA versus TFAA methods) are a result of thebaine being not reacting under the TFAA conditions. The application of heat to thebaine in the presence of TFAA results in the rapid decomposition of

Table 3 Analysis of major alkaloids and impurities as a result of the AA and TFAA methods of DAM synthesis

Target compounds	Average response and procedure used <sup>4</sup>							
	Tasmanian		SWA		SEA			
	AA <sup>b</sup>	TFAA <sup>c</sup>	AA <sup>d</sup>	TFAA <sup>d</sup>	AA <sup>d</sup>	TFAA <sup>d</sup>		
Morphine related comp	oounds							
11	$0.39\pm0.04$	$0.07\pm0.04$	$17.48 \pm 1.97$	$2.47\pm0.05$	$0.58\pm0.14$	$0.37\pm0.12$		
12	$14.91\pm0.98$	$4.46 \pm 1.5$	$20.58 \pm 2.01$	$10.95\pm2.54$	$35.96 \pm 6.46$	$20.25\pm 6.33$		
13	$38.74 \pm 1.22$	$7.19\pm3.25$	$51.13 \pm 0.85$	$9.92\pm2.0$	$100\pm5.30$	$50.33 \pm 18.1$		
14	< 0.05	< 0.05	$2.8\pm0.54$	$0.83\pm0.67$	$0.04\pm0.02$	$0.09\pm0.04$		
15	$2.56\pm0.37$	$0.3\pm0.16$	$12.85\pm1.16$	$1.82\pm0.11$	$18.17\pm4.70$	$6.52\pm2.13$		
16	< 0.05	< 0.05	$4.19\pm0.02$	$0.13\pm0.1$	$0.16\pm0.05$	$0.17\pm0.07$		
Thebaine related comp	ounds							
17	$2.13\pm0.13$	$0.16\pm0.06$	$61.36 \pm 4.14$	$1.76\pm0.69$	$0.2\pm0.06$	$0.4\pm0.05$		
18	$5.32\pm0.01$	$2.1\pm1.28$	$100\pm18.32$	$20.75\pm2.45$	$2.41\pm0.08$	$1.45\pm0.33$		
19	$6.16\pm0.55$	$0.47\pm0.34$	$97.79 \pm 10.56$	$4.75\pm0.35$	$0.84\pm0.09$	$0.02\pm0.01$		
20	$0.22\pm0.03$	$0.09\pm0.03$	$4.87\pm0.13$	$0.34\pm0.26$	$0.24\pm0.07$	$0.2\pm0.09$		
21	$0.12\pm0.02$	< 0.05	$9.11 \pm 1.69$	$0.92\pm0.31$	$0.03\pm0.08$	$0.02\pm0.02$		
22	< 0.05	< 0.05	$3.11\pm1.25$	$0.73\pm0.52$	$0.01\pm0.01$	< 0.05		
23	< 0.05	< 0.05	$6.22\pm0.85$	< 0.05	< 0.05	< 0.05		
Noscapine and N-norla	udanosine related comp	ounds						
24	< 0.05	< 0.05	$0.26\pm0.05$	< 0.05	< 0.05	< 0.05		
25	< 0.05	< 0.05	$0.18\pm0.01$	< 0.05	< 0.05	< 0.05		
26	< 0.05	< 0.05	$57.21 \pm 1.33$	$3.52\pm0.02$	$1.5\pm0.23$	< 0.05		
27	< 0.05	< 0.05	$1.57\pm0.01$	$0.14\pm0.03$	< 0.01	< 0.05		
28	< 0.05	< 0.05	$2.28\pm0.10$	$0.06\pm0.05$	< 0.01	< 0.05		
29	$0.22\pm0.15$	< 0.05	$8.73\pm3.8$	$5.93\pm0.38$	$100\pm9.9$	$7.74\pm0.25$		

<sup>a</sup> Response was calculated against an internal standard (i.e. compound response/internal standard × 100), data are then normalised against largest response.

<sup>b</sup> n = 3.

n = 6.n = 2. thebaine. Thus, although thebaine decomposes when heated in TFAA, formation of **17–23** (the major AA decomposition products) is not the preferred pathway for this reaction [21].

Significantly greater amounts of noscapine and *N*-norlaudanosine related compounds were produced when the AA method was employed (e.g. **26**: AA: 57.21 and 1.5; TFAA: 3.52 and <0.05 for SWA and SEA, respectively) (Table 3). The formation of **24** and **25** proceeds through the addition of acetic acid across the double bond in **27** and **28** [22]. The absence of these compounds in samples produced using TFAA can be explained by the absence of acetic acid and the smaller quantities of **27** and **28** present in the mixed anhydride system. The formation of **26** proceeds through the precursor, noscapine N-oxide [22]. The increased abundance of this compound in the AA samples is believed to be a result of smaller quantities of noscapine N-oxide being formed during the TFAA synthesis. Subsequent cleavage of the C1–N bond affords **27** and **28**.

The formation of **29** involves the acetylation of the secondary amine group in *N*-norlaudanosine [22], which is a function of nucleophile strength, with increased prevalence for attack at the trifluoroacetyl carbonyl, of the mixed anhydride formed in situ, increasing with the reactivity of the nucleophile [41]. Hence the TFAA procedure favours trifluoroacetylation, decreasing the amount of **29** observed.

Our data clearly establish that the amounts of these trace impurities were significantly different between the two reactions. However, when comparisons are made between the Tas, SWA and SEA series these differences were shown to be highly dependent upon the morphine extraction procedure (e.g. **19**: 6.16 versus 0.47, 97.79 versus 4.75 and 0.84 versus 0.02 for Tas, SWA and SEA, respectively). Consequently based on this data set, the trace organic component of a seized sample cannot be used as a TFAA specific profiling parameter.

With the currently identified trace organic impurities unable to distinguish between the two reactions, an attempt was made to identify TFAA reaction specific marker compounds. During this investigation morphine, thebaine, oripavine, codeine, noscapine and papaverine were treated with AA and TFAA, analysed by GC–MS and their respective products compared (Fig. 5).

The reaction of noscapine, papaverine, thebaine and oripavine with TFAA failed to produce any detectable products, which could be used as TFAA markers. However, when morphine and codeine were treated with TFAA and AA significant differences in the reaction products were observed. Fig. 6 shows the appearance of peaks in the TFAA samples at 23.5 min (**30** m/z = 477), 25 min (**31** m/z = 423) and 26 min (**32** m/z = 423) in morphine and 24 min (33 m/z = 395) in codeine, respectively. The molecular weights of these compounds suggest the addition of two CF<sub>3</sub>CO groups (m/z = 194), one CF<sub>3</sub>CO group and one CH<sub>3</sub>CO group (m/z = 140) to morphine (m/z = 285) and one CF<sub>3</sub>CO (m/z = 97) to codeine (m/z = 299), respectively, assuming the M-1 is detected in the GC-MS. We had anticipated that re-examination of our earlier preparations of heroin via the TFAA route that evidence for the formation of **30–33** would be forthcoming. This was not the case. However,



Fig. 5. (a) GC–MS analysis of a crude morphine sample after treatment with TFAA.  $R_t = 23.5 \text{ min} = bis$ -trifluoroacetylmorphine (**30**);  $R_t = 26 \text{ min} = mono-trifluoroacetylmorphine; <math>R_t = 27.7 \text{ min} = 3$ -MAM (**7**);  $R_t = 29 \text{ min} = \text{DAM}$  (**2**); and  $R_t = 31 \text{ min} = \text{IS}$  (benzopinacolone); (b) the same sample after typical pre-treatment for GC–MS analysis;  $R_t 27.2 \text{ min} = 6$ -MAM (**8**); 27.7 min = 3-MAM (**7**);  $R_t = 29 \text{ min} = \text{DAM}$  (**2**); and  $R_t = 31 \text{ min} = \text{IS}$  (benzopinacolone).

the current HSP methodology involves a  $1N H_2SO_4$  extraction and derivitisation step prior to analysis, given the extremely labile nature of trifluoroacetyl esters it was deemed probable that the current methodology precluded the detection of these TFAA specific markers. Consequently we re-examined the TFAA heroin synthesis in the absence of the extraction and derivatisation steps, in these instances both **32** and **33** were detected in all SEA and SWA samples produced using TFAA (Fig. 6).

Structural assignment of 30 and 31 was based on MS fragmentation pattern analysis. MS analysis shows 30 to have [M]<sup>+</sup> of 423 which suggests the addition of one acetyl and one trifluoroacetyl ester to morphine. This is supported by a significant  $[M-CH_2CO]^+$  (*m*/*z* = 381),  $[M-CF_3COOH]^+$  (*m*/ z = 310) and [M-CH<sub>2</sub>CO + CF<sub>3</sub>COOH] (m/z = 268) ions. Additionally, the  $[M-CH_2CHO]^+$  (*m*/*z* = 381) ion, which is characteristic of an aromatic acetate allowed the assignment of the acetate group to the 3-position [44]. The spectrum of 30 also showed significant peaks at m/z 215 and 174, which are characteristic of morphine skeleton fragmentation [45,46]. Similarly, **31** was determined to have  $[M]^+$  of 395, which is consistent with the addition of a trifluoroacetyl group to codeine. This was confirmed by the  $[M-CF_3COOH]^+$  (m/ z = 282) base ion. The spectrum of **31** also showed characteristic codeine skeleton fragmentation peaks at m/z229 and 188 supporting the assignment of 31 as trifluoroacetylcodeine [45,46].



Fig. 6. (a) Chemical structures of TFAA specific markers identified in this work; (b) GC/MS trace of the crude reaction mixture arising from the reaction of morphine and AA; (c) GC/MS trace of the crude reaction mixture arising from the reaction of morphine and TFAA; (d) GC/MS trace of the crude reaction of codeine and AA; (e) GC/MS trace of the crude mixture arising from the reaction of codeine and TFAA. (f) GC–MS trace typical of DAM synthesis using TFAA; expanded section shows the presence of 3-acetyl-6-trifluoroacetylmorphine (**32**) and trifluoroacetylcodeine (**33**).

#### 5. Conclusions

The use of TFAA provides a simple, quick and currently undetectable synthesis of heroin. To enable detection of this method a number of profiling parameters have been developed. In Australia, the routine profiling of heroin using major alkaloid analysis involves conversion of raw alkaloid concentration data into C/WM, NOS/WM, PAP/WM and 6MAM/WM ratios and its origin assigned in accord with the UNDCP data [38]. Herein we suggest that if the 6-MAM/WM ratio of a seized sample falls within the average minimum and maximum values (6.17–17.32) then a presumption would be raised that the sample was made using the TFAA route. Any samples, which raise this presumption, would then be subjected to an additional test to confirm the origin of the product. A DAM sample with a large 6-MAM/WM ratio may

also be explained by incomplete acylation or poor sample storage [25,47,48]. Additionally the presence of **32** or **33** may be due to the presence of an alternate trifluorinated reagent (e.g.  $CF_3COCI$ ).

## 6. Experimental

All solvents were HPLC grade or bulk solvents re-distilled from glass before use. Morphine used in the Tasmanian series was received from Tasmanian Alkaloids. In all other instances morphine utilised in synthesis was extracted (in a region specific manner) from samples seized by the Australian Federal Police using the methods described below. Thebaine, oripavine, morphine, codeine and papaverine reference standards were obtained from the National Analytical Reference Laboratory (NARL), Pymble, Australia.

## 6.1. Synthetic methods

#### 6.1.1. Morphine extraction

Opium (40 g) was dissolved in boiling water (30 mL), to which was added  $Ca(OH)_2$  (10 g) and the heating removed. The mixture was allowed to cool and stand overnight. The solution was filtered, the residue dissolved in boiling water (10 mL) and re-filtered. The combined filtrates were re-heated until the solution steamed, at this point the pH of the solution was adjusted to 8–9 with  $NH_4^+Cl$  and left to stand overnight. The solution was filtered to leave behind morphine base (2.61 g, 6.7%).

# 6.1.2. Morphine HCl

Morphine base (2.5 g) was dissolved in warm HCl (1 M, 25 mL) and 1 g of charcoal added. The solution was warmed for 10 min and then filtered. The filtrate was left to stand overnight and filtered to leave behind morphine hydrochloride (1.14 g, 41%).

## 6.2. Heroin synthesis

Trifluoroacetic anhydride synthesis.

## 6.2.1. Method 1

Glacial acetic acid (84  $\mu$ L, 1.75 mmol) was added slowly to trifluoroacetic anhydride (311  $\mu$ L, 2.1 mmol) in an ice bath and stirred for 10 min. Morphine (200 mg, 0.7 mmol) was added slowly and the mixture stirred for 20 min. The solution was then allowed to warm to room temperature and diluted with cold water (1 mL). The solution was put back in an ice bath and made basic (pH 8–10) with cold 10% sodium carbonate. The resulting aqueous solution was extracted with chloroform (3 × 10 ml), dried over MgSO<sub>4</sub> and evaporated to leave heroin base (157.6 mg, 78%).

## 6.2.2. Method 2

Trifluoroacetic anhydride (311  $\mu$ L, 2.1 mmol) was added slowly to morphine (200 mg, 0.7 mmol) in an ice bath and stirred for 10 min. Glacial acetic acid (84  $\mu$ L, 1.75 mmol) was added slowly and the mixture stirred for 20 min. The solution was then allowed to come to room temperature and evaporated under a stream of nitrogen. The residue dissolved in  $CH_2Cl_2$  and analysed by GC–MS (see Section 2).

## 6.3. Acetic anhydride synthesis

Acetic anhydride (600  $\mu$ L, 5.8 mmol) was added slowly to morphine (200 mg, 0.7 mmol). The resulting solution was heated and stirred at 85 °C for 2.25 h and allowed to cool to room temperature. The solution was diluted with cold water (1 mL). The solution was cooled on ice and made basic (pH 8– 10) with cold 10% sodium carbonate. The resulting aqueous solution was extracted with chloroform (3 × 10 mL), dried over MgSO<sub>4</sub> and evaporated to leave diacetylmorphine base.

## 6.4. South-East Asian procedure

Diacetylmorphine base (from morphine hydrochloride) (60 mg, 0.16 mmol) was dissolved in acetone (6 mL), and concentrated HCl (18 mL) was added and the solution evaporated under a stream of nitrogen to leave behind diacetylmorphine hydrochloride (57 mg, 86%).

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