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Isolation and purification of heroin from heroin street samples by preparative high performance liquid chromatography

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ABSTRACT

The present study established a novel method using preparative high performance liquid chromatography to isolate and purify heroin·HCl from heroin street samples to be used as a reference standard. Different kinds of mobile phases and columns were used, ultimately the mobile phase consisting of hexane–isopropanol–methanol (65:28:7, v/v) and the SIL preparative column prepared in laboratory were selected as the final condition. Heroin was further purified by the drowning-out crystallization method using isopropanol–methanol (50:1, v/v) and hexane as drowning-out anti-solvents and salting-out agents, respectively. The purity was assessed by analytical high performance liquid chromatography and the confirmation of the chemical structure was performed by IR and NMR. About 110.7 mg of heroin·HCl at a purity of over 99.52% was obtained from 180 mg of heroin street samples which contained 156.15 mg of heroin·HCl component by preparative high performance liquid chromatography. This method is suitable for preparing heroin standards in forensic science area.

1. Introduction

In recent years, there has been a tremendous increase in illicit drug consumption, overdoses and deaths related to drugs, especially heroin, in China. Illegal heroin abuse still represents a main problem in our society. Therefore, the analysis of heroin samples confiscated from the illicit market remains one of the main roles for forensic specialists.

Heroin, mainly found on the illicit market, is usually produced by acetylation of morphine originating from opium (Scheme 1). In clandestine laboratories, the purification of morphine and heroin is seldom efficient. Therefore, other alkaloids from opium and their acetylated derivatives can be found in what is sold in the illicit market as clandestine heroin (Fig. 1). In addition, most illicit heroin street samples are adulterated and/or diluted several times. Adulterants such as analgesics, local anaesthetics and caffeine are pharmacologically active substances that mimic the bitter taste of heroin, whereas carbohydrates such as lactose, mannitol and sucrose are inactive and are often used for dilution purposes [1].

The Chinese legal system requires that content determination of the confiscated drugs should be included in the expertise report to provide reliable basis for conviction and sentencing to the criminals involved in drug trafficking. Furthermore, it is important to determine active components and their content in drug detection process, because it also can be used to distinguish the source and manufacturing process of illegal drugs (e.g. heroin) [2–7].

In quantitative determination process for illegal drugs, related reference materials of high performance usually play an important role in accurate quantification. However, domestic reference materials cannot meet the requirement of laboratory accreditation at present. It will lead to uncertainty in drug detection and have an effect on accurate determination. On the other hand, reference materials which are purchased from foreign countries are all in liquid state (e.g. heroin·HCl 1.0 mg base/mL solution in acetonitrile), which is inconvenient to use, especially in pharmacodynamics study. Therefore the research for preparation of illegal drug reference material is extremely urgent.

Preparative high performance liquid chromatography (PHPLC) is an effective method for isolation and purification of active components from natural products [8–11]. It is also reported to be used for isolation of impurities of bulk drugs [12–15]. However, preparative HPLC has seldom been used in forensic science area, only Peters et al. [16] used semi-preparative HPLC to isolate and purify the designer drug metabolite after it was synthesized by a biotechnological method.

The drowning-out method, which was also used in our research, is widely used as an enhancement to the crystallization processes for the isolation and separation of compounds, such as pharmaceuticals, due to its low cost, high energy efficiency and good sensitivity to operational conditions. It is considered as one of the most important separation techniques, especially when separation

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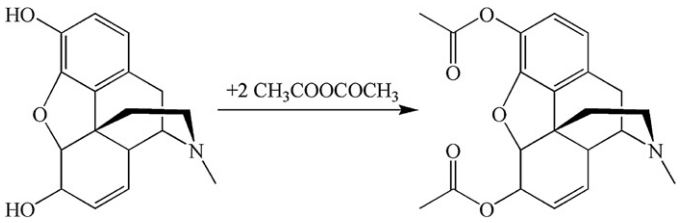


Scheme 1. Acetylation of morphine to yield diacetylmorphine (DAM, heroin).

of solutes from multi-component solutions is required [17]. Drowning-out is a reactive crystallization technique based upon three stages, i.e. dissolution in drowning-out anti-solvents, addition of salting-out agents and precipitation [18].

On this basis, the present study aims to establish a method for isolation and purification of heroin·HCl from heroin street samples. Preparative HPLC combined with the drowning-out method was chosen as the most efficient method to obtain heroin standard.

2. Materials and methods

2.1. Chemicals and reagents

All organic solvents used for preparative and analytical HPLC analysis were of HPLC grade. Methanol, tetrahydrofunan, isopropanol and n-hexane were all purchased from Fisher Scientific (Fair Lawn, NJ, USA). Diethylamine was purchased from ACROS Organics (New Jersey, USA). Trifluoroacetic acid (HPLC grade) used for HPLC analysis was obtained from J&K Chemical Ltd. (Beijing, China). Ultrapure water purified via the Synergy Purification System (Millipore, Molsheim, France) was used during both preparative and analytical HPLC analysis.

Heroin·HCl, 3-monoacetylmorphine·NH_2HSO_3, 6-monoacetylmorphine·HCl, morphine·HCl, acetylcodeine·HCl and codeine·H_3PO_4 were provided by Institute of Forensic Science, Ministry of Public Security (Beijing, China).

Heroin street samples as off-white powders were confiscated from the illicit market by the Guangdong Police (China) and were submitted for research in our laboratories.

2.2. Sample preparation

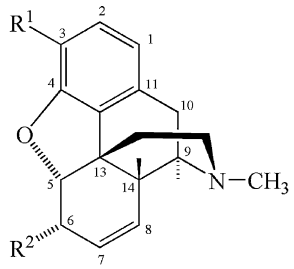
One mg of illicit heroin street samples was dissolved in 1 mL of mobile phase for analytical HPLC analysis. For preparative HPLC analysis, 500 mg of heroin street samples were firstly dissolved in 1 mL of methanol and then 4 mL of isopropanol was added. Both sample solutions were in an ultrasonic bath for 5 min and then filtered.

2.3. Analytical methods

The isolation procedure was monitored with one of the following sets of conditions.

2.3.1. RP-HPLC

The analysis was performed on a Shimadzu LC-20AD HPLC system equipped with a SIL-20A auto-injector and a SPD-M20A diode array detector (DAD) set at 210 nm



	R^1	R^2
Morphine	OH	OH
Heroin	$OCOCH_3$	$OCOCH_3$
3-Monoacetylmorphine	$OCOCH_3$	OH
6-Monoacetylmorphine	OH	$OCOCH_3$
Codeine	OCH_3	OH
Acetylcodeine	OCH_3	$OCOCH_3$

Fig. 1. Structures of heroin and related compounds.

(Shimadzu, Japan) using a Shim-pack VP-ODS column (250 mm × 4.6 mm I.D., 5 μm) fitted with a C18 guard column. A mobile phase consisting of 23% methanol/water (0.05% trifluoroacetic acid in water, v/v) was used at a flow rate of 1.0 mL/min. The column temperature was 35 °C.

2.3.2. NP-HPLC

The analysis was performed on the HPLC system described in Section 2.3.1 using an Inertsil SIL 100A column (250 mm × 4.6 mm I.D., 5 μm). Hexane–tetrahydrofunan/isopropanol–0.75% diethylamine in methanol (75:20:5, v/v) was used as the mobile phase. Flow rate was 1.0 mL/min and the column temperature was 35 °C.

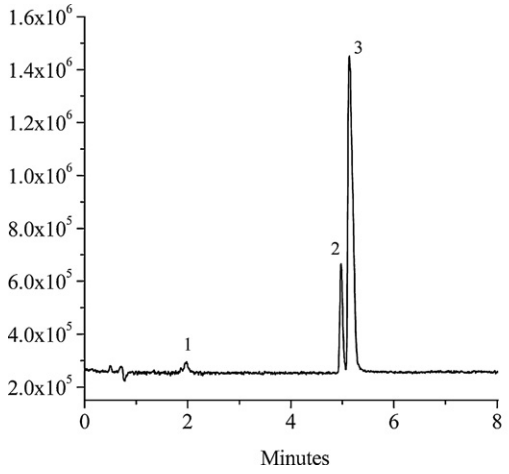
2.3.3. LC–MS

The LC–MS system consisted of LC-20AD binary pumps, a SIL-20A autoinjector, a CTO-20A column oven and a LCMS-2020 instrument (Shimadzu, Japan). An electrospray source was used in positive mode and ion spray voltage was set to 1500 V. Nitrogen was used as nebulizer gas (1.5 L/min) and drying gas (10 L/min). A Shim-pack XR-ODS column (100 mm × 2.0 mm I.D., 5 μm) was used for the analysis. Mobile phase A was 0.5% formic acid in acetonitrile and mobile phase B was 0.5% formic acid in water. The system was run in a linear gradient from 10% A to 30% A in 10 min. The total flow rate was 0.4 mL/min and the column temperature was maintained at 40 °C.

2.4. Preparative HPLC separation

2.4.1. Preparative RP-HPLC separation

The chromatographic separation was performed with a Shimadzu LC-8A preparative HPLC system equipped with a SPD-M20A diode array detector, a



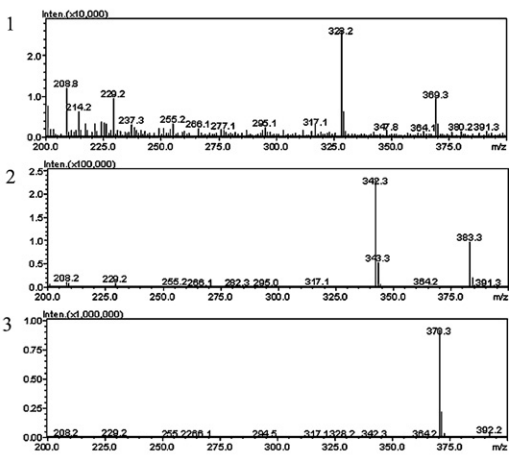


Fig. 2. Total ion chromatogram (TIC) and its corresponding mass spectra of heroin samples. Peaks: 1 = 6-monoacetylmorphine, 2 = acetylcodeine, 3 = heroin.

SIL-10AP automatic injector and a FRC-10A automatic fraction collector (Shimadzu, Japan). The preparative HPLC was performed with a Shim-pack VP-ODS preparative column (250 mm × 20 mm I.D., 15 μm). A mobile phase consisted of 20% methanol–0.05% trifluoroacetic acid in water. The flow rate was 20 mL/min while the detected wavelength was 210 nm. The injection volume was 200 μL. The preparative HPLC equipment was controlled by Shimadzu LC-solution Chromatography Data Software (Shimadzu, Japan).

2.4.2. Preparative NP-HPLC separation

The chromatographic separation was performed with the preparative HPLC system described in Section 2.4.1. Preparative HPLC was performed with a NP SIL-100A stainless steel preparative column (250 mm × 10 mm I.D., 5 μm) that was packed in laboratory. Hexane–tetrahydrofunan–methanol (60:32:8, v/v) or hexane–isopropanol–methanol (65:28:7, v/v) was used as the mobile phase respectively. The flow rate was 8 mL/min or 10 mL/min respectively while the detected wavelength was 210 nm. The injection volume was 300 μL.

2.5. Drowning-out crystallization

The fraction, which included heroin collected from the eluent of preparative HPLC, was concentrated and dried by rotatory evaporator. Then, the residue was re-dissolved with isopropanol–methanol (50:1, v/v) and transferred into a centrifuge tube. An equal volume of hexane was added into the centrifuge tube, which was then placed overnight at 4 °C. The analytes were largely precipitated via the drowning-out crystallization and then centrifuged (3000 rpm/min, 10 min) with an Anke TDL-40B centrifuge (Shanghai, China). The supernatant was carefully removed and the final product was obtained after being washed and dried.

2.6. Identification of heroin

The preparative heroin was identified by IR, ^{1}H NMR and ^{13}C NMR spectrometry. IR analysis was performed on a Thermo Electron Nexus 8700 Flourier Transform Infrared Spectrometer (Thermo Nicolet, USA). ^{1}H NMR and ^{13}C NMR spectrometry was recorded on an Avance DRX-500 (500 M Hz) NMR spectrometer (Bruker Biospin GmbH, Germany).

3. Results and discussion

3.1. LC–MS analysis of heroin samples

Illicit heroin street sample was firstly determined by the LC–MS method described in Section 2.3.3. Seen from the total ion chromatogram (TIC) and its corresponding mass spectra (Fig. 2), heroin street samples comprised heroin $[M+H]^+$ (m/z 370.3), acetylcodeine $[M+H]^+$ (m/z 342.3) and a small quantity of 6-monoacetylmorphine $[M+H]^+$ (m/z 328.2).

3.2. Preparative HPLC

In the beginning, the preparative RP-HPLC method described in Section 2.4.1 was used to isolate pure heroin from street heroin samples. Though heroin has been well separated from other components by analytical HPLC (Fig. 3a), using the similar mobile phase, it was not resolved well from other components by preparative HPLC (Fig. 4a). What is more, after the fraction including heroin was collected from the eluent of preparative HPLC, it was concentrated and dried by evaporator. Then a good deal of monoacetylmorphine was found because heroin was likely to hydrolyze when it was in water especially heated. It suggested that the preparative RP-HPLC was not the suitable method to isolate heroin from heroin samples.

Illicit heroin street samples could also be analyzed by the NP-HPLC method as described in literature [19]. It also suggested it was possible to transform analytical scale to preparative scale to obtain heroin standard. Hexane–dichloromethane–0.75% diethylamine in methanol (75:20:5, v/v) was used as the mobile phase in this method. Because the maximum absorption wavelength of heroin is 205 nm, tetrahydrofunan and isopropanol which have cut-off wavelengths at 210 nm were selected as substitutes of dichloromethane whereas dichloromethane has a cut-off wavelength at 245 nm. Furthermore, as the boiling points of tetrahydrofunan and isopropanol are 66 °C and 82 °C respectively, they could be evaporated with hexane and methanol easily when the fraction was concentrated and dried by evaporator.

The hexane–tetrahydrofunan–methanol system was firstly used as described in Section 2.3.2, and heroin was well separated from other components (Fig. 3b). Then this system (diethylamine

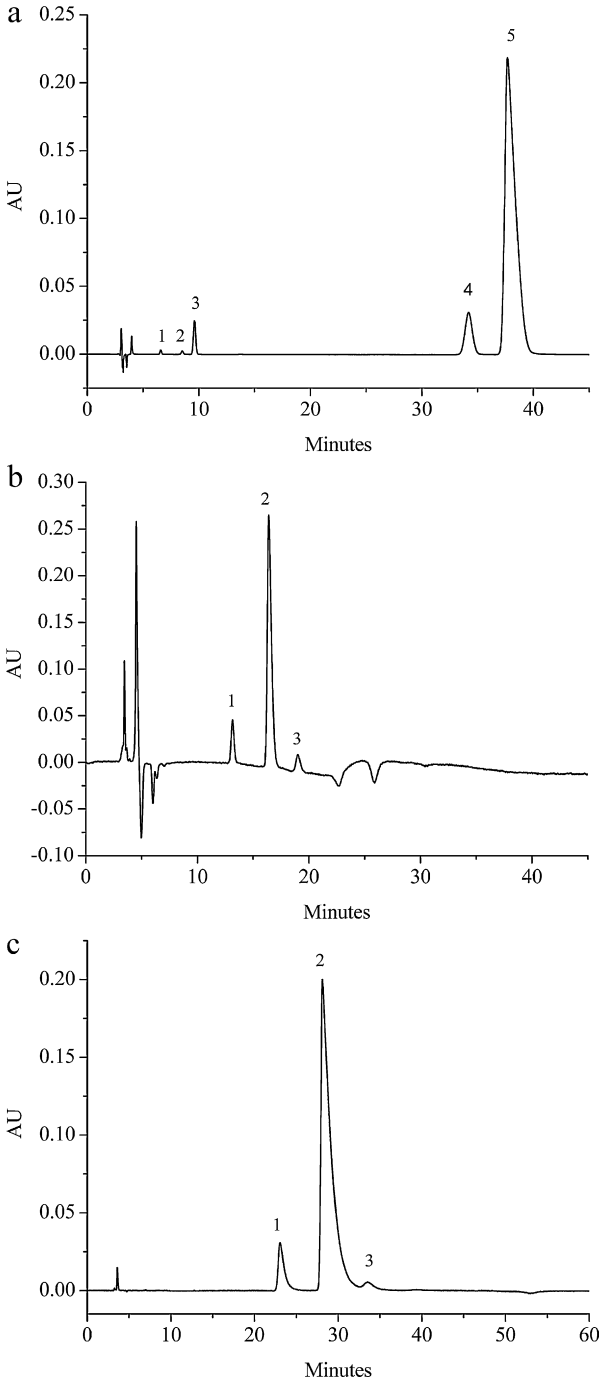


Fig. 3. Analytical HPLC chromatograms of heroin samples: (a) analytical RP-HPLC chromatogram of heroin samples. Peaks: 1 = codeine, 2 = 3-monoacetylmorphine, 3 = 6-monoacetylmorphine, 4 = acetylcodeine, 5 = heroin. (b) Analytical NP-HPLC chromatogram of heroin samples. Analytical conditions: hexane–tetrahydrofunan–methanol (0.75% v/v diethylamine in methanol) (75:20:5, v/v). Peaks: 1 = acetylcodeine, 2 = heroin, 3 = 6-monoacetylmorphine. (c) Analytical NP-HPLC chromatogram of heroin samples. Analytical conditions: hexane–isopropanol–methanol (0.75% v/v diethylamine in methanol) (75:20:5, v/v). Peaks: 1 = acetylcodeine, 2 = heroin, 3 = 6-monoacetylmorphine.

was removed in case of hydrolization) was applied in preparative HPLC as described in Section 2.4.2. Tetrahydrofunan was used as the mobile phase, and heroin could be separated from acetylcodeine (Fig. 4b). Though butyrolactone which was identified as oxidation product of tetrahydrofunan was detected by GC–MS, it could be removed by rotatory evaporator. Initially, during evaporation, the heroin began in its crystal form as heroin·HCl, then, as the evaporation continued, more oxidation products of tetrahydrofuran were generated, changing the heroin's characteristics. Thus, this system was suitable to isolate pure heroin from street heroin samples; however, not in its crystal form.

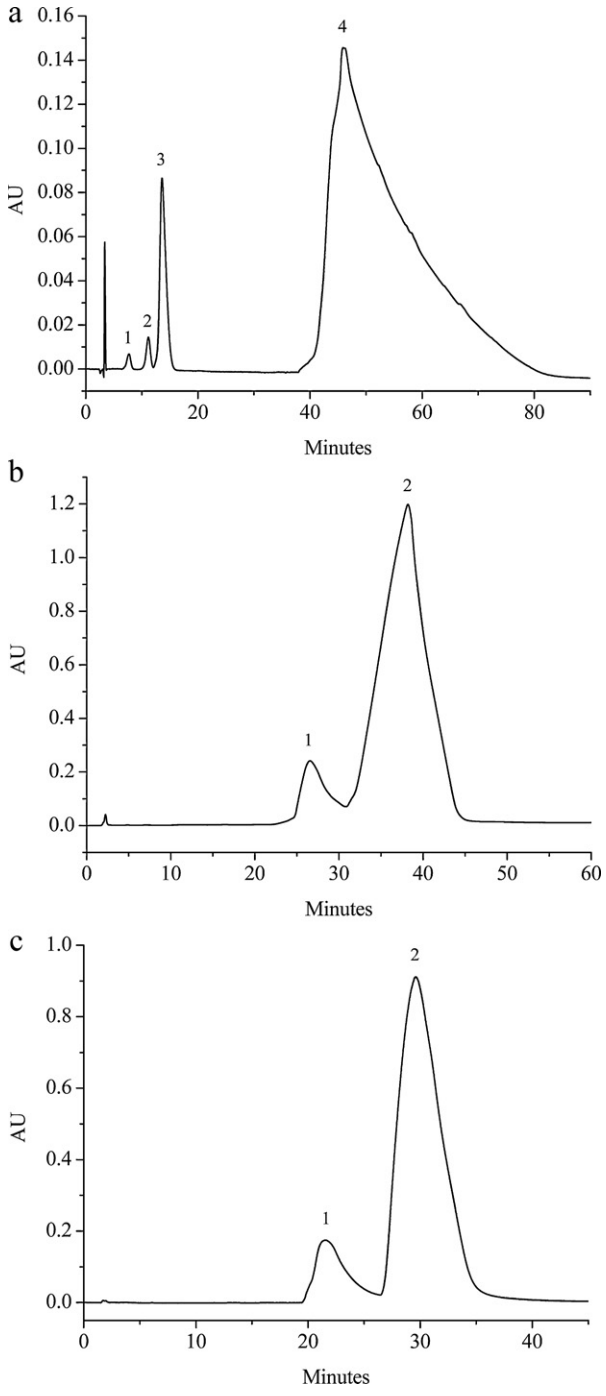


Fig. 4. Preparative HPLC chromatograms of heroin samples: (a) preparative RP-HPLC chromatogram of heroin samples. Peaks: 1 = codeine, 2 = 3-monoacetylmorphine, 3 = 6-monoacetylmorphine, 4 = mixture of acetylcodeine and heroin. (b) Preparative NP-HPLC chromatogram of heroin samples. Preparative conditions: hexane–tetrahydrofunan–methanol (60:32:8, v/v). Peaks: 1 = acetylcodeine, 2 = heroin. (c) Preparative NP-HPLC chromatogram of heroin samples. Preparative conditions: hexane–isopropanol–methanol (65:28:7, v/v). Peaks: 1 = acetylcodeine, 2 = heroin.

The hexane–isopropanol–methanol system as described in Section 2.3.2 was used. The analytical HPLC chromatography (Fig. 3c) demonstrated that heroin has not been separated well from monoacetylmorphine. But the content of monoacetylmorphine was less than 1% in heroin samples, which could be removed by drowning-out crystallization process subsequently. Firstly the hexane–isopropanol–methanol (60:32:8, v/v) solvent system and the flow rate of 8 mL/min were used. However, heroin could not separate well from acetylcodeine. Then the condition was improved. The isopropanol–methanol proportion was reduced, and flow rate was increased. Finally, hexane–isopropanol–methanol (65:28:7, v/v) and flow rate of 10 mL/min were used, heroin was resolved well from acetylcodeine (Fig. 4c). Eventually, 140.7 mg off white heroin powder with a small quantity of monoacetylmorphine was obtained from 180 mg of heroin street samples which contained 156.15 mg of heroin·HCl. The purity was determined as 99.13% by HPLC area percent with the method described in Section 2.3.1.

3.3. Further purification of heroin

In order to obtain white heroin crystal with a high purity and remove minor impurities co-eluted from the preparative HPLC, the heroin fraction was further purified by the drowning-out crystallization method as described in Section 2.5. Isopropanol–methanol (50:1, v/v) and hexane were selected as the drowning-out anti-solvent and salting-out agent, respectively. There was 110.7 mg of heroin·HCl obtained from 140.7 mg of heroin fraction above with the recovery of 78.68%. Fig. 5 showed the analytical HPLC chromatogram of heroin fraction which was concentrated and purified by the drowning-out crystallization method with the purity from 99.13% to 99.52%.

3.4. The structural identification

Heroin fraction after the drowning-out crystallization treatment was submitted to the IR, ^{1}H NMR and ^{13}C NMR analysis. IR spectrum (Fig. 6a) with the ν_{max} (KBr) 3439, 2954, 2630, 1762, 1737, 1446, 1370, 1245, 1217, 1178, 1157, 1037 cm^{-1} indicated that the product was heroin·HCl (Fig. 6c) rather than heroin base (Fig. 6b) compared with their infrared spectral data from the Bio-Rad/Sadtler IR Data Collection. ^{1}H NMR (500 MHz, methanol-d$_4$)

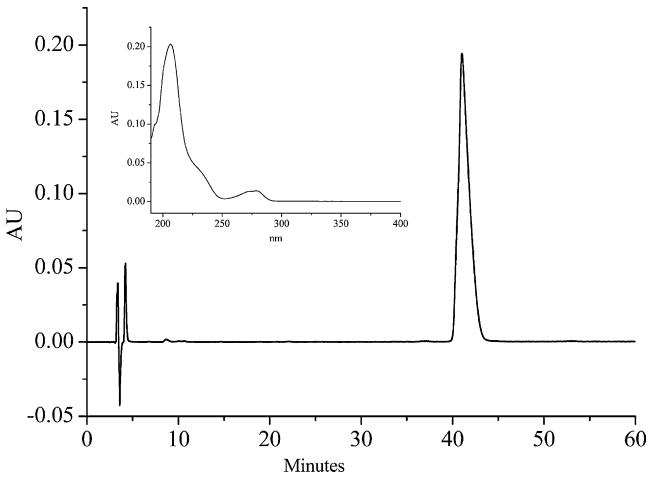


Fig. 5. Analytical RP-HPLC chromatogram of heroin fraction with corresponding UV spectrum.

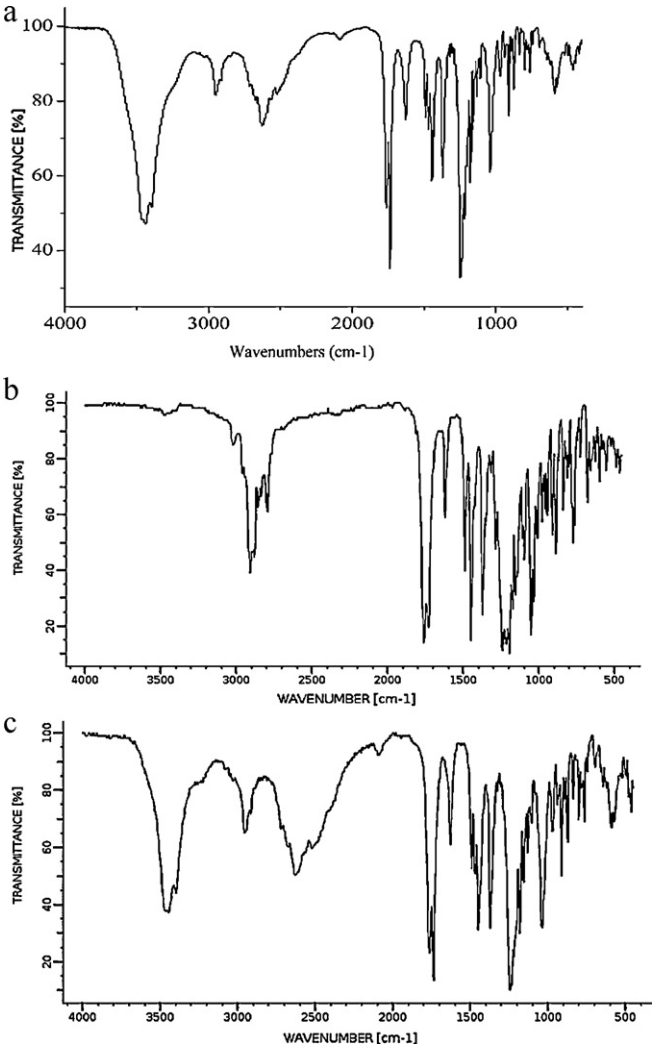


Fig. 6. IR absorption spectra of heroin: (a) IR spectrum of heroin fraction. (b) IR spectrum of heroin base from the Bio-Rad/Sadtler IR Data Collection. (c) IR spectrum of heroin·HCl from the Bio-Rad/Sadtler IR Data Collection.

δ ppm: 6.87 (d, J = 8 Hz, 1H, H-2), 6.75 (d, J = 8 Hz, 1H, H-1), 5.77–5.74 (m, 1H, H-8), 5.52 (d, J = 10 Hz, 1H, H-7), 5.22 (s, 1H, H-5), 5.22 (s, 1H, H-6), 4.21 (s, 1H, H-9), 3.37–3.03 (m, 2H, $-CH_2-$), 3.35–2.95 (m, 2H, $-CH_2-$), 3.16 (s, 1H, H-14), 3.03 (s, 3H, N–CH_3), 2.38–2.12 (m, 2H, $-CH_2-$), 2.25 (s, 3H, $-CH_3$), 2.10 (s, 3H, $-CH_3$). ^{13}C NMR (125 MHz, methanol-d_4) δ ppm: 172.43 (–CO–), 170.58 (–CO–), 151.45 (C-4), 134.48 (C-3), 132.27 (C-8), 131.20 (C-11), 130.63 (C-12), 127.19 (C-7), 124.96 (C-2), 121.67 (C-1), 89.54 (C-5), 69.18 (C-6), 62.80 (C-14), 49.53 ($-CH_2-$), 43.04 (C-13), 42.15 (N–CH_3), 40.26 (C-9), 34.23 ($-CH_2-$), 23.09 (C-10), 20.99 ($-CH_3$), 20.87 ($-CH_3$). The NMR data also indicated this compound was heroin·HCl.

4. Conclusions

The present work developed a novel method for isolation and purification of heroin·HCl from heroin street samples to be used as a reference standard. Pure heroin·HCl was obtained by preparative HPLC using a mobile phase consisting of hexane–isopropanol–methanol (65:28:7, v/v). Then it was further purified by the drowning-out crystallization method. There was 110.7 mg of heroin·HCl obtained from 140.7 mg of heroin fraction that was separated by preparative HPLC from 180 mg of heroin street samples which contained 156.15 mg of heroin·HCl, the yield 70.89%. The purity was 99.52% by analytical HPLC and the confirmation of the chemical structure was performed by IR and NMR spectroscopy. The described method is suitable for preparing useable quantities of pure heroin·HCl as reference standard in forensic science area.

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