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IDENTIFICATION AND QUANTITATIVE DETERMINATION OF MORPHINE IN THE URINE OF HEROIN DEPENDENTS

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ABSTRACT

Morphine in the urine of heroin addicts was identified by TLC using methanol, water, benzene and glacial acetic acid (80:15:15:5) and ethyl acetate, methanol and ammonium hydroxide (25:10:5) as solvent systems and potassium idoplatinate and iodine as the developing reagents. Potassium iodate in alkaline medium gives a yellowish golden colour having maximum absorbance at 450 nm. By this reaction the minimum limit of identification and quantitative determination of morphine is 2 µg/ml. It provides a basis for the spectrophotometric determination of morphine M the urine of heroin addicts.

Introduction

Morphine and its diacetyl derivative diamorphine are frequently used in aqueous oral and parenteral medicines. Studies in laboratory animals *in Woo* and in *vivo* indicate that heroin (diacetylmorphine) was rapidly diacetylated, first to 6-acetylmorphine and then to morphine. The major portion of a given dose of heroin in man could be accounted for as free morphine and as morphine conjugate (Yeh et *al*, 1976). Diamorphine is known to undergo hydrolysis in aqueous solution to the monoacetyl derivative and morphine itself (Beaumont and Decks, 1982). Morphine is more stable in aqueous solution than heroin (diamorphine), but does not decompose on beating in strong base or on storage (Yeh and Mcquinn, 1975).

Many analytical techniques have been used for the identification and quantitative determination of heroin and morphine. In chromatographic methods such as TLC (Davey *et al*, 1968; Rao, 1977), GC (Moore and Elena, 1972), LC (Rasmussen et at, 1978) and HPLC (Twitchett, 1975; Wu and Wttick, 1973), long procedures are involved. Specially in TLC methods, there are errors due to loss of morphine and heroin during extraction. In the voltametric (Proksa *el al*, 1978) determination of morphine with graphite and platinum electrodes, sensitive control of pH and temperature is necessary. In the polarographic method (Popescus and Baiulescu, 1977) thebaine and codeine show similar behavior as morphine, while in the phase-resolved phosphorimetry (Yen and Winefordner, 1977) attempts to determine morphine were unsuccessful. Prior to the spectrophotometric determination of morphine in urine samples, tedious column extraction with a number of organic solvents and stringent control of experimental conditions were required (Breiter *et al.*, 1978). A color test was reported by Sarwar and Tahir (1976) for the determination of morphine using potassium iodide in acidic medium, employing an organic solvent, chloroform, to produce a pink colour.

The first phase of the present investigation deals with the extraction of morphine from the urine of heroin addicts and its identification by thin layer chromatography (TLC). The second phase involves the application of a spectrophotometric method (Sax-war and Aman, 19M) for the determination of morphine in the urine of heroin addicts, because heroin after smoking/inhaling is almost completely converted to morphine (Way *et al*, 1960) and excreted as such in the urine.

Materials and Methods

Chemicals and reagents

Morphine sulphate, ammonium chloride, dipotassium hydrogen phosphate, hydrochloric acid, sodium carbonate and sodium hydroxide were obtained from Merck. Potassium iodate was obtained from Judex (England). All other chemicals and reagents used were of analytical grade from BDH and Merck.

Solvent systems for TLC

1. Methanol, water, benzene, glacial acetic acid (80:15:5:2, v/v).
2. Ethyl acetate, methanol, ammonium hydroxide (35% ammonia) (85:10:5, v/v).
3. Benzene, chloroform, acetone, methanol, diethylamine, (60:15:15:5:5, v/v).
4. Iso-propyl alcohol, ammonia (90:10, v/v).
5. Chloroform, ethyl acetate, n-propanol (96:2:2, v/v).
6. Chloroform, 95% ethanol (80:20, v/v).
7. Cyclohexane, chloroform, diethylamine (50:40:10, v/v).

Spraying reagents

1. (a) 1 g hexa-chloroplatinic acid hexa hydrate (Merck) dissolved in 10 ml distilled water.
   (b) 30 g potassium iodide dissolved in 200 ml distilled water.
   (a) and (b) were mixed, the volume was made upto 250 ml and the solution stored in an amber glass bottle.
2. Ninhydrin solution (10% w/v) in ethanol.
3. Iodine vapours in iodine chamber.

Morphine identification by TLC

Urine samples were collected within 6-8 hours of heroin smoking/inhalation from healthy male heroin addicts with an average age of 35 (24-45) years and weight of 58 (51-65) kg, under closely controlled conditions after admission to the Addiction Treatment Center, Psychiatry Department, Mayo Hospital, Lahore (Pakistan). All subjects were judged to be in good health form from recent history and physical and laboratory examination includ'ng tests of heamatological, renal, hepatic and cardiac functions.

10 ml urine was mixed with 1 ml of 50% HCI in a 20 ml test tube plugged with cotton wool and autoclaved (Autoclave, Hiryamo, Japan) at 120°C for 15 minutes at 15 psi. After the test tube was cooled, 0.5 ml sodium carbonate (70% w/v solution) was added and the contents shaken till effervescence stopped. Then 2 ml concentrated ammonia solution containing 35% ammonia and ammonium chloride and 60 ml of the extraction solvent consisting of 1- butanol and chloroform (1:9) were added. The contents of the flask were shaken on an Gyrotory shaker Model G2 for 15 minutes at 190 rpm. The contents were poured in a separating funnel. The upper aqueous layer was discarded and the contents of the lower organic layer were washed twice with 5-7 ml K2HPO4 buffer (pH 9.5). The organic layer was filtered through Whatman filter paper No .1 in a beaker and evaporated at room temperature to dryness. The dried residue was dissolved in 0.4 ml methanol and 20 p1 of the solution along with the standard morphine solution were spotted on *TLC* plates (silica gel 60 GF 254 and Kieselgel PF 254, 366, Merck) and developed in different solvent systems to achieve the best separation. After development the *TLC* plates were dried. Potassium iodoplatinate and ninhydrin solutions were sprayed on two separate plates and the th'rd plate was placed in the iodine chamber for few minutes. The colour of the spots was noted and the Rf values calculated. The spots were also observed under UV light at 254 and 366 nm to find any change in the colour. Navy blue and yellow colour of morphine appeared on 250 pm TLC plates which could be confirmed by comparing with that of the standard. The TLC plates were heated at 100°C and 160°C in a Mommert air oven for 30 minutes and the colour of the spots was noted again under UV light to observe any change as shown in Table 1.

Preparation of standard morphine calibration graph

Different dilutions of morpine solution (1 mg/ml) containing 2 to 200 *pg/ml* of the morphine were prepared. One ml of each dilution was mixed with I ml of 2% potassium iodate, a bright yellow golden colour developed after shaking. IN sodium hydroxide (0.05 to 1 ml) was used to adjust the pH between 11 and 12 preferably 115. The solution was heated at 90-95°C for 5 minutes, cooled to room temperature and the volume made upto 10 ml with distilled water. The absorbance of the solution was measured at 450 nm on the Spectronic 20 spectrophotometer. The experiment was repeated with different concentrations of standard morphine solution and a calibration graph was prepared as shown in Figure 1. The colour reaction was found to obey Beer's Law relation in the concentration range 2 to 200 pg/ml.

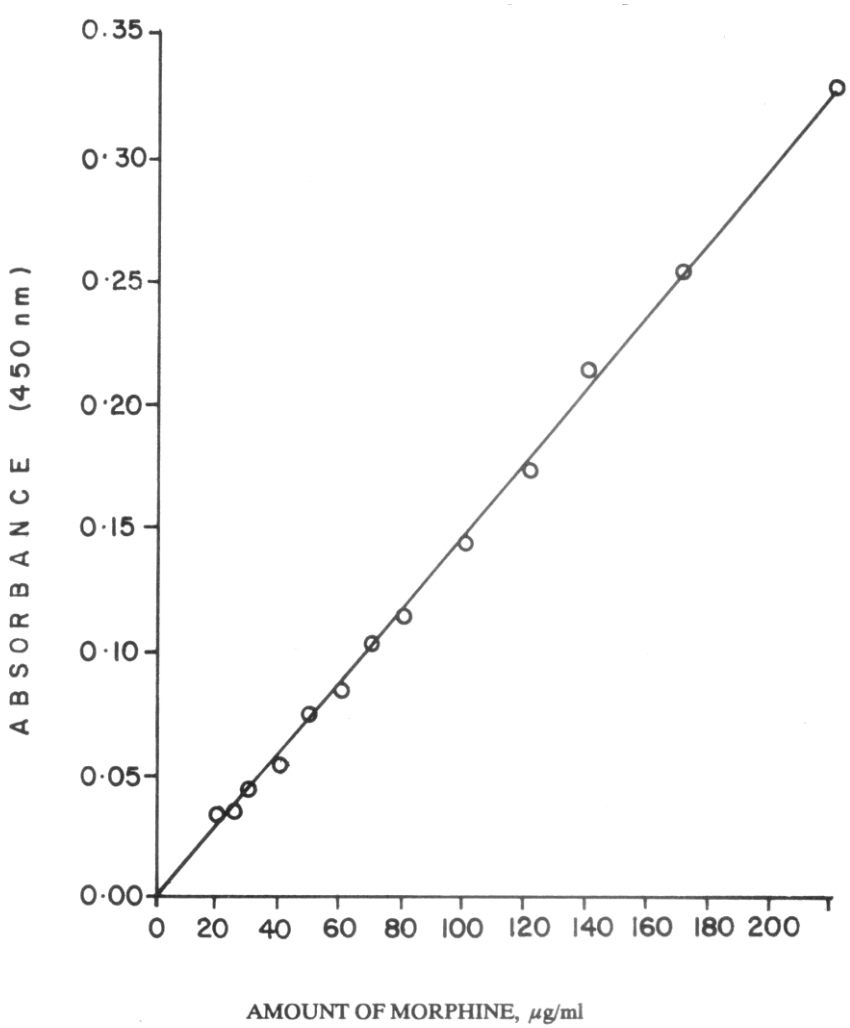

Figure 1. Calibration curve for morphine

Morphine extraction from addict urine for quantitative determination

50 ml addict urine was made alkaline with 4 ml of 0.1N ammonia in a separating funnel. 20 ml of chloroform and isopropyl alcohol mixture (3:1) was added and the solution shaken for few minutes. The organic layer was filtered through a filter paper into a beaker. The extraction was repeated five times. The extract of light yellow colour due to morphine was then evaporated to dryness on a water bath at 60 to 90°C. The dried yellowish crystalline residue was dissolved in 0.5 ml of 0.1N HC1, the solution was transferred into 25 ml measuring flask and the volume was made up to the mark with distilled water. An aliquot was taken and the spectrophotometric measurements were carried out as described above.

Results and Discussion

On developing the TLC plates coated with fluorescent Kieselgel PF 254, 366 and non-fluorescent silica gel 60 F 254 a highly characteristic pattern ranging from a single spot to a remarkable combination of spots, bands and patches with brown to brownish black colour were observed under UV light at 254 and 366 nm. When the TLC plates were sprayed with ninhydrin, morphine showed navy blue and yellowish brown spots while bright yellow spot was observed when placed in iodine chamber. When observed under UV light at 254nm, morphine spots were bluish black and dark brown and at 366 am the spots were invisible or black on both the fluorescent and non-fluorescent TLC plates. On heating the plates at 100 °C the morphine spot became more visible and dark in colour but at 160°C the spots became faint and with ninhydrin spray the morphine spots were invisible (Table 1).

There was a significant (P c O.001) change in the Rt values of morphine spot on the fluorescent and non-fluorescent TLC plates after development with the same solvent. The time taken by the TLC plates to develop differs with different solvents. Methanol, water, benzene, glacial acetic acid system (80:15:5:2) was observed to be a good solvent as it also separated other opium alkaloids, benzodiazipines, and chlorpromazine. Ethyl acetate, methanol, ammonium hydroxide system (85:10:5) showed best separation of morphine only.

The Rt values recorded in Table 1 are the average of five determinations and in most instances are fairly reproducible. These values may vary for a particular colour appearing for a particular compound at 100° and 160°C. This could be due to changes in the colour properties when heated at 100° and 160°C to different admixtures of compounds formed *at* the two temperatures and having different effects on the mobility of the spots. Studies were restricted to 100° and 160°C as significant differences in colour were not observed at intermediate temperatures and the colour faded at temperatures above 160°C. Good results were obtained when the heating time was about 15 minutes.

Table 1
Colour* profile of morphine after extraction from heroin addict urine on fluorescent silica TLC plates developed with different solvents and spraying reagents.

Solvent System	Spraying Reagent	Colour of spot after spraying	Visibility in UV light 254 nm	366 nm	Colour of spot after heating at 100°C	Visibility in UV light 254 nm	366 nm	Rf value of sample/standard
1	Pot. Iodo-platinate	Nb	B1	-	Db	Db	-	0.84/0.87
	ninhydrin	Yb	LYb	Yb	Yb	Db	-	
	Iodine	Db	Db	Yb	Yb	Db	B1	
2	Pot. Iodo-Platinate	Nb	Bu	B1	Db	B1	-	0.18/0.17
	Ninhydrin	Db	B1	B1	V	-	-	
	Iodine	Yb	Db	B1	Y	B1	B1	
3	Pot. Iodo-Platinate	Nb	Bu	-	Bu	LY	-	0.43/0.42
	Ninhydrin	-	-	-	-	-	-	
	Iodine	Y	Yb	Yb	LY	LY	G	
4	Pot. Iodo-Platinate	Nb	G	-	Bu	-	-	0.45/0.48
	Ninhydrin	-	-	-	-	-	-	
	Iodine	Y	Br	Yb	Y	-	-	
5	Pot. Iodo-Platinate	Nb	Bu	Bb	Bu	G	-	0.18/0.22
	Ninhydrin	-	-	-	LY	LG	G	
	Iodine	Y	Db	Db	LG	G	G	

*Nb = Navy Blue; Bu = Blue; Db = Dark Blue; Y = Yellow; LY = Light Yellow; Yb = Yellowish Brown; LG = Light Gray; LYb = Light Yellowsih Brown; Br = Brown; Db = Dark Brown; Bb = Brownish Black; Bl = Black; G = Gray; V = Violet; - = Invisible.

The coloured product of morphine has an absorption maximum at 450 um, hence all measurements were carried at this wave length. The colour intensity was maximum at pH 11-12, which was obtained by the addition of IN sodium hydroxide, therefore, all the determinations were carried out at this pH. The intensity of the bright-yellow golden colour was maximum when heated on a water bath at 90-95°C for the completion of reaction. The colour was stable upto 30 minutes and measurements during this period showed no change in absorbance values.

The quantity of morphine detected in urine varies (Table 2) in different groups, depending upon the purity and quantity of heroin inhaled or smoked and the total amount of water and fluid intake daily by the addict. Since it is known that heroin is deacetylated to 6-acetyl morphine and then to morphine (Way and Adler, 1962), it seems likely that morphine is excreted in large amounts as conjugated morphine in the bile (Robinson and Williams, 1971) and the rest of it as total or free morphine. When morphine is given intravenously, it produces high initial plasma levels of free morphine, rapidly distributes in the body tissue and is excreted as *free* morphine and conjugated with glucuronide.

Table 2
Urinary excretion of morphine in heroin addicts after 8 hours of heroin inhalation/smoking.

Group	Amount of heroin inhaled/smoked daily (g)	Amount of morphine detected in urine µg/ml
I N˙ =15	0.5-2	20-10
II N˙ =15	2-4	80-150
III N˙ =15	4-6	150-320

˙N = No. of addict patients
Total urinary output = 13 to 1.8 litres daily in all groups.

The urinary excretion of free and conjugated morphine within 4 hours is the same as that after I/M or S/C injection. The rapid distribution, metabolism and excretion of I/V morphine results in lowering blood levels of free morphine from 15 minutes to 3 hours compared with those after IIM injection. Morphine is a basic amine, it rapidly leaves the plasma and concentrates in the tissue. Free morphine is handled in the kidney by the organic cation system and morphine-3-glucuronide by the organic anion system (Brunk and Delle, 1974). Heroin is rapidly converted to morphine within 8 hours and excreted in the urine. With this method total (free and conjugates) morphine was detectable in the first 8 hour urine sample as reported by *Yeh et al.* (1976). The method is satisfactory for the determination of morphine in the urine of heroin addicts.

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