

Opioids: Analytical and Toxicological Aspects

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Abstract

A sensitive Gas chromatography- mass spectrometry (GC) method was developed and validated for determination of Morphine, Codeine, 6MAM, Normorphine, from Heroin in deceased human plasma.

Gas Chromatography was accomplished with Capillary column: MS5 (0.25 x 30 m) (5% phenylmethyl siloxane), Temperature column: 150°C for 7 min- rate 15 °C min, 280°C for 12 min).

Sample: 1ml of blood extracted with 4ml of diethyl ether, centrifugation and elimination of the supernatant, suspension of the biological matrix in physiologic solution.

Internal standard: Nalorphine (200ng). Injection; 1 µl for stock solutions

Retention time Codeine – 15.9 – 16.1, monitored ions Codeine 371- 414- 440.

Heroin (diacetylmorphine) semisynthetic derivative of morphine marketed by Bayer in 1898. Fig. 1

Usually taken intravenously, currently also nasal and inhaled (smoked).

In the body is converted rapidly (10-15 min.), by deacetylation in 3-MAM and 6-MAM and then more slowly (4-6 hours) to morphine.

Morphine derivative heroin has a very large volume of distribution (2-5 L / Kg); Undergoes conjugation with acid glucuronic transformed into morphine-3-glucuronide and morphine-6-glucuronide.

Metabolic pathway of opiates in man: methylation (I= N-methylation, II= N-dimethylation, III=o-methylation, IV= o-demethylation). Fig. 2

Criteria of subject selection and sampling documented history of drug abuse, detectable by criterion circumstantial - medical history. Blood sampling during the course of autopsy;

Autopsy performed in cases, within 20-60 hours from the discovery of the corpse.

Keywords: Overdose, Morphine, Heroin, GC- mass.

1. Introduction

Opium (from the greek OPOS = juice) is the Papaver somniferum album latex, which is obtained by incising the unripe capsules.

Morphine took its name after Morpheus, the divinity of sleep, because of the opium derivative's depressive action on the central nervous system and the capacity to induce somnolence.

Heroin [1,2] (diacetylmorphine) semisynthetic derivative of morphine was marketed by Bayer in 1898.

1.1 Heroin; Name, formula, molecular weight, colour, odour, synonyms

3,6 – diacetossi – 7,8 – deidro 4,5 – epossi – N – metilmorphinan. $C_{21} H_{23} N O_5$: M.W. 369,4.

Colour: white crystals, changes to pink and emits vinegar odour after prolonged exposure to air.

Synonyms; Acetomorphine, Diacetylmorphine, Diamorphine,

7,8 – diidro - 4,5 α epossi – 17 - metilmorphinan – 3,6 - α - diol diacetate.

Melting point: 170 °C – 172 °C, 171 - 174 °C.

1.2 Heroin hydrochloride; name, formula, molecular weight, colour, odour, synonyms

3,6 – diacetossi – 7,8 – deidro 4,5 – epossi – N – metilmorphinan. $C_{21} H_{23} N O_5 \cdot HCl \cdot H_2 O$: M.W. 423,9.

Heroin hydrochloride has a white colour, in the form of crystalline powder. Inodorous when freshly prepared which, however emits a characteristic odour of acetic acid when stored.

Synonyms; 3,6-di-O-acetylmorphine HCl, H₂O. Diacetyl morphine HCl, Diamorphine. HCl

7,8 – diidro - 4,5 α epossi – 17 - metilmorphinan – 3,6 - α - diol diacetate. HCl. H₂O.

Melting point: 229 – 233 °C, 243 – 244 °C.

1.3 Identification of Drug of Abuse by High Performance Liquid Chromatography, Thin- layer Chromatography, Paper Chromatography [3,4].

1.4 Sensitive Gas Chromatographic quantitation, GC- Mass [5,6,7,8,9].

1.5 Syntheses of Heroin: it is a derivative of morphine, it is obtained semi-synthetically treating morphine with acetic anhydride or acetyl chloride. Fig. 1

1.6 Stability and Degradation: heroin in alkaline solutions is rapidly hydrolyzed even in vivo where in contact with the blood it turns into monoacetylmorphine and then in a slower way it turns into morphine [10].

Heroin is also hydrolyzed in monoacetylmorphine in aqueous solution buffered at pH 7,4 a 23 °C.

Hydrolysis is much faster at higher pH values (6,4), while there is no further conversion to morphine at pH 7,4 during 24 hours.

In a solution 0.5M of sodium carbonate, heroin is hydrolyzed to amonoacetylmorphine with a half-life of only 4.2 minutes. Further hydrolysis to morphine occurs after 55.5 minutes.

Hydrolysis in human blood has a half-life of 12.6 minutes, in serum 19.8 minutes.

At pH 4 with phosphate buffer the half-life was 415 minutes [11].

2. Method

2.1 Opium Alkaloids Extraction. Natural opium alkaloids produce latex (dried for one day) of incarnation of the *Papaver somniferum* immature capsule. The extraction process can be schematized as follows:

Opium sheets (trituated): extraction with water at 30 ° (1 kg of opium: 3 l of water), filtration:

a) aqueous solution with morphine, codeine, thebaine, papaverine and narcotine present as sulphates and methacrylates.

b) Solid (narcotics) most of it does not melt, it has no therapeutic interest

Water solution of calcium chloride at 60 ° C for 2 days at rest

Solution: Alkaloids Chlorides,

Vacuum evaporation at 30 ° -40 °, in a refrigerator at 0 ° (crystallization)

Mostly crystals of morphine and codeine are formed (in smaller amounts of papaverine and thebaine).

2.2 Criteria of subject selection and sampling.

Extraction method

Organic Matrices: Blood 1ml, Extraction with 4ml diethylether

Centrifugation and elimination of the supernatant

Suspension of the biological matrix in physiological solution.

Enzyme hydrolysis: β -glucuronidase (1 mL) at T ° of 55 ° C For 1h and ½.

Extraction: Basic to PH (9) and controlled ionic force, using a patented extractive system (Toxi- Tubes™ TYPE "A")

Derivatization: BSTFA + 1% TMS (50 μ L)

Internal Standard: Nalorphine (200 ng)

3. Instrumentation

Instrumentation: GC (Agilent 5890) / MS (Agilent 5973 N), How to Acquire in SIM [12,13].

Capillary column: MS5 (0.25 x 30 m) (5% phenylmethylsiloxane)

T column: 150 ° C for 7 min - rate 15 ° C / min, 280 ° C for 12 min. Injection: 1 μ l

Retention time: Tr Codeine: 15.9-16.1 Tr Morphine: 16.3-16.4 Tr 6MAM: 16.7- 16.9 Tr Nalorphine: 17.1- 17.4

Monitored Ions: Codeine: 371/414/440 Morphine: 429/414/440 6 MAM: 399/340/287 Nalorphine: 455/414/440

Calibration curves: Codeine / Morphine / 6 MAM. Range: 0.2 to 500 ng / ml.

4. Results and discussions

Usually taken intravenously, currently also nasal and inhaled (smoked).

In the body is converted rapidly (10-15 min.) by deacetylation in 3-MAM and 6-MAM and then more slowly (4-6 hours) to morphine.

Morphine derivative heroin, has a very large volume of distribution (2-5 L / Kg); Undergoes conjugation with acid glucuronic transformed into morphine-3-glucuronide and morphine-6-glucuronide. Fig. 3

Criteria of subject selection and sampling documented history of drug abuse, detectable by criterion circumstantial - medical history. Blood Sampling during the course of autopsy;

Autopsy performed in cases, within 20-60 hours from the discovery of the corpse.

Acute opioid intoxication (defined as overdose) is characterized by euphoria, rash, itching, myosis, drowsiness, decreased frequency and amplitude of respiration, hypotension, bradycardia and decreased temperature.

In severe intoxication severe respiratory depression can evolve to the exitus.

Factors determining acute narcotism: intake of doses characterized by high purity, taking doses higher than the individual tolerance level (or after a period of abstinence) polydrug; "overdose" in body-packers (involuntary lethal dose-taking dosers).

5. Conclusions

Although death diagnosis from opioid acute opiate narcotism provides the primary reference to the polydistrict distribution of morphine, the utility of extending the dosage to other heroin metabolites (6- MAM Codeine).

Indeed, the integrated evaluation of the three metabolites can provide useful information about "the age of death"

Regarding 6MAM concentrations: - if death occurs within 3 hours of heroin intake, a significant blood concentration and a low cerebral concentration should be observed, in deaths occurring more than 3 hours after intake, a low blood concentration of 6MAM is usually detectable compared to higher cerebral concentration.

With regard to the concentrations of Codeine (on blood), it has emerged that this analyte may be helpful as "evidence of chronicity of abuse" as it is significantly distributed in the habit of ordinary consumers.

Finally, the relationship between blood and blood concentrations of heroin metabolites should always be integrated with other clinical and / or medical-legal evaluation criteria.

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FIG.1

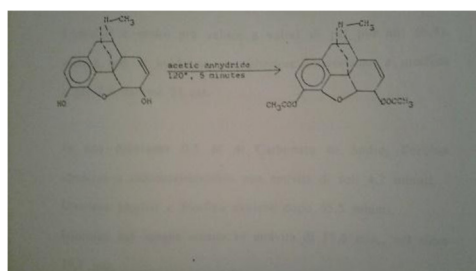


FIG.2

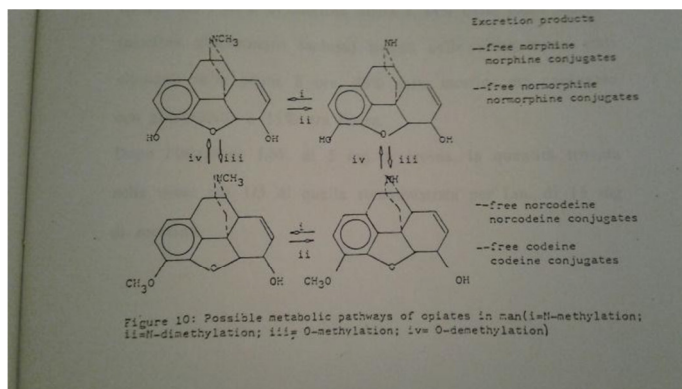


FIG.3

