ANALYSIS FOR HEROIN BY THIN LAYER CHROMATOGRAPHIC (TLC) METHOD

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SUMMARY

Heroin containing market (street) samples and urine samples of addicts were analysed (for active heroin component) qualitatively by thin layer chromatographic (T.L.C) method. Patients admitted in Drug Abuse Treatment Center (DATC) can get heroin by different secret channels. It was, therefore, necessary to check the heroin levels in urine of admitted patients.

INTRODUCTION

The success of any treatment of the addicts using street sample of heroin lies very much on the complete compositional knowledge of the samples and monitoring the level of its metabolites i.e. 6-monoacetyl morphine and morphine in biological samples (urine) during treatment. Since heroin has long history of abuse and is one of the more commonly encountered illicit drugs, it must often be identified and quantified in illegal drug proceedings and forensic analysis.[1,2,3] Such street samples contain a wide range of heroin concentration. The urine samples of addicts were pre-extracted with chloroform at pH 9, while the street samples [1,2,3] were directly dissolved in chloroform and spotted on the T.L.C. plate. Three solvent systems were used during the research.

MATERIAL AND METHODS

Material

Standard morphine (B.D.H)
Standard Heroin (from P.C.S.I.R. Labs)
Market samples (from local Hospitals)
Urine samples (from local Hospitals)

Sample Collections

Market samples were collected by various sources, including DATC.

Biological samples (Urine) were also collected from the patients admitted for detoxification in the DATC unit.

Preparation of Solutions

a) Preparation of Control

10 mg of standard morphine was dissolved in 1 ml methanol (100%). 10 mg of market sample was dissolved in 1 ml methanol. The extract of biological sample was dissolved in methanol.

b) Preparation of Phosphate Buffer

350 gm of dibasic potassium phosphate (K_2HPO_3) in 1 litre H_2O, was adjusted to the pH 9 by further addition of dihydrogen phosphate (NaH_2PO_4) and stored at room temperature.

c) Preparation of Extract Solvent

Extraction solvent was prepared by mixing 18 ml of chloroform with 2 ml of n-butanol.

d) Preparation of Coating Plate

20 x 20 mm glass plates were coated with silica gel and plaster of paris in the following range.

30 gm plaster of paris
70 gm silica gel powder
60 gm water

Slurry was prepared and coating done by a spreader. The plates were activated.

TLC Procedure

e) Extraction Procedure

10 ml urine sample was mixed with 1 ml 50% HCL. The solution was heated in a water bath for one hour; cooled and 0.5 ml 70% K_2CO_3 was added. The tube was shaken till effervescence stopped. 2 ml alkaline buffer was added to it followed by 20 ml extraction solution. The tube was stoppered and agitated 5 minutes in mechanical shaker and centrifuged for 3 minutes at 1000 r.p.m. Top aqueous layer was discarded. The organic phase was shacked twice with 10 ml phosphate buffer and upper layer was discarded each time. It was then filtered through Watman No. 1 filter paper and evaporated at 75 C by a stream of compressed air. The extract was dissolved in 50 ul of methanol. Spotting of the extract was done on TLC plates and developed through three different solvent systems. They were finally dried and sprayed with iodoplatinate solution. R_f of 10 positive urine were calculated (see Table 1,2,3). Control was spotted along with the sample and comparative study was carried out. Market samples of heroin were also spotted and developed through the same solvent system and impurities were detected.

Confirmatory test for the presence of morphine

The plate was sprayed with ammonical silver nitrate and. heated in the oven at 100 C for 5 minutes. Morphine spot turned black.

Table - 1

R_f 100 VALUES OF 10 MARKETING SAMPLES OF HEROIN AND 10 BIOLOGICAL SAMPLES IN GLACIAL ACETIC ACID, ETHANOL AND WATER (3:6:1)

S.No	Marketing Samples	Impurities(R_f)	Biological Samples
1.	43.7	28.6 19.2 15.0 14.3	44.2 34.0
2.	43.9	26.0	42.9 33.0
3.	43.6	27.9 15.0	36.0
4.	42.7	26.4 21.0 19.2	40.7 29.8
5.	42.7	19.2	36.7 40.7
6.	43.7	26.2 16.0	41.4 38.0
7.	42.8	37.5 23.7 19.2 15.0	40.7 33.0
8.	44.3	25.0 20.0 15.0	43.9 34.0
9.	44.2	19.2 15.0	32.6 19.2
10.	44.2	27.9 15.0	37.5 15.8

Rf of Ref. = 0.44 = 44.0 %
Rf of Std. = 0.437 = 43.7 %

RESULTS

TLC

R_f values in 3 different solvent systems were determined for 10 market samples and 10 urine samples. Results are given in Tables 2 and 3. The mean value of 10 samples was highly significant ($P<.001$. two tail). Results for the impurities present in the market samples are also given.

DISCUSSION

The purpose of the present work has been to illustrate the capability and suitability of TLC as analytical tool for the analysis of market and urine samples

Table - 2

R_F * 100 VALUES OF 10 MARKETING SAMPLES OF HEROIN AND 10 BIOLOGICAL SAMPLES IN METHANOL (25%) AND AMMONIUM, HYDROXIDE (200:3)

S.No	Marketing Samples	Impurities(R_f)	Biological Samples
1.	45.5	27.0 18.0 14.0 12.0	44.5 35.0
2.	46.2	28.2	43.5 34.2
3.	45.9	29.3 16.4	44.0
4.	44.9	28.4 23.5	64.1 33.3
5.	46.0	20.4	45.6 35.1
6.	46.4	28.2 17.0	44.9 39.2
7.	47.9	39.2 24.5 20.3 16.2	44.2 33.6
8.	45.7	27.3 21.0 17.0	43.2 33.2
9.	45.4	21.0 17.0	35.8 21.5
10.	46.3	29.0 17.0	44.9 20.6

Rf of Ref. = 0.46 = 46%
Rf of Std. = 0.458 = 45.8%

Table - 3

R_F * 100 VALUES OF 10 MARKETING SAMPLES OF HEROIN AND 10 BIOLOGICAL SAMPLES IN METHANOL.

S.No	Marketing Samples	Impurities(R_f)	Biological Samples
1.	38.0	32.2 27.0 18.0 14.0	40.0 33.0
2.	35.8	27.0	39.0 34.0
3.	36.6	28.4	14.5
4.	38.0	30.0 20.0	34.0 15.0
5.	38.0	19.0 15.0	34.0 35.0
6.	35.8	32.2	39.0 33.0
7.	37.0	26.9 22.0 16.0 13.0	35.0 32.0
8.	38.0	26.0 14.0 12.0	37.0 36.0
9.	34.6	18.0 14.0	34.0 30.0
10.	36.9	22.0 12.0	36.0 31.0

Ref. value = 0.38% = 38%
Ref. value = 0.37% = 37%

containing heroin. TLC determination of the heroin powder samples and the extracted samples of urine provided a rapid and accurate method with the major advantage of allowing individual quantitation.

R_f values for active heroin were determined in three solvent system. The solvent systems employed for TLC determination on silica gel plates were:

1. Glacial acetic acid: ethanol and water (3:6:1)
R_f for heroin is 0.437 (43.7%)
2. Methanol: 25% ammonium hydroxide (200:3)
R_f is 0.46 (46%)
3. Methanol
R_f is 0.38 (38%)

The values compare very well with the reference values given in Tables 1,2,3. No interference of the impurities with the heroin takes place under experimental conditions.

However, the variation of impurities Rf values used for various samples indicates the adulteration of the market samples by different diluents.

REFERENCES

1. Manura JJ, Chao JM and Seferatan RJ. Forensic Ser. 1978; 23: 44.
2. Love JL and Pannell LK. J Forensic Sci. 1980; 23: 320.
3. Clark CC. J Forensic Sci. 1977; 22: 418.