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Quantitative Estimation of Diacetylmorphine by Preparative TLC and UV Spectroscopy

L Khan*, M T Siddiqui, N Ahmad and N Shafi

Medicinal Botanic Centre PCSIR Laboratories, Peshawar-25100, Pakistan

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A simple and efficient method for the quantitative estimation of diacetylmorphine in narcotic products has been described. Comparative TLC of narcotic specimens with standards showed presence of morphine, monoacetylmorphine, diacetylmorphine papaverine and noscapine, Resolution of the mixtures was achieved by preparative TLC. Bands corresponding to diacetylmorphine scraped, eluted UV absorbtion of extracts measured and contents quantified.

Key words: Heroin, Adulterated, Estimation, Diacetylmorphine.

Introduction

Diacetylmorphine commonly known as Heroin is a highly addictive morphine derivative, making up a large portion of the total illicit traffice in narcotics. It is powerful analgesic agent, but its undersirable side effects outweigh its value as a drug. It is also a labile product and transforms into monoacetylmorphine, morphine, and other metabolites during storage (Helen *et al* 1981). At the concern of the local drug abuse, law enforcing and antinarcotic authorities, the present studies were pursued to determine the contents of diacetylmorphine in the confiscated goods regarded as heroin. For the detection and estimation of diacetymorphine in illicit narcotic products, qualitative and quantitative procedures have been reported (Lurie and Guinness 1987; Helmet and Peter 1987; Billiet *et al* 1986). During the application of these methods when account is taken of the cost involved, availability of analytical instruments and technical know how, then some of them appear not practicalble. Among the various procedures, the commonly used volumetric method (Horwitz 1970) based on titration against standard sulphuric acid to quantify morphine content as heroin is suitable for assaying pure heroin products. Obviously applying this method for deriving conclusion regarding heroin content in sample adulterated with morphine, monoacetylmorphine papaverine and noscapine well yield erroneous results. For a similar reason assaying such products by extraction with methanol and measuring their UV absorptions (Horwitz 1975) will not yield reproducible findings due to solubilisation of the contaminants. For ascertaining diacetylmorphine in contaminated narcotic products, a modified procedure will be one that removes the undesired ingredients from the heroin matrix by chromatographic separation techniques. With the removal of interfering components, the heroin obtained will be reasonably pure for quantitative analysis. This work is part of an effort to apply the method for evaluation of illicit heroin.

*Author for correspondence

Materials and Methods

General solvents and reagents were obtained from BDH Ltd Pools U.K., Narcotics samples and reference standards were provided by Pakistan Customs, Anti Narcotics Force N.W.F.P. and the United Nation International Drug Control Programme UNDCP Islamabad. Silica gel 60 PF 254+366 for TLC was obtained from Merck Darmstadt. The UV absorptions, recorded on a Hitachi Model 1U-2000 instrument using chloroform solution.

Preparation of diacetylmorphine. Morphine 100 mg was dissolved in 2 ml glacial acetic acid and treated with 3 ml acetic anhydride containing few drops of periodic acid. The contents mixed and allowed to stand for 15 min at 5 °C. The mixture poured into 10 ml water and the solution treated with sodium carbonate to attain pH 7.8 and extracted with 50 ml ether. The ether solution washed with water, dried over anhydrous sodium sulphate. Ether removal furnished residue which was recrystallised from acetone to yield 58 mg product mp 170-172 °C (Budavari 1989).

Preparation of monoacetylmorphine. Diacetylmorphine 10 mg was dissolved in 2 ml ethanol with shaking and few drops of ammonia were added. The content was mixed and allowed to stand at 15 °C for 10 min. Solvent removal furnished residue which was extracted with ether and monoacetylmorphine recovered.

Comparative thin layer chromatography. TLC of 38 voucher specimens alongwith standards of morphine/ monoacetylmorphine, diacetylmorphine, papaverine and

noscapine was performed using pre-coated 0.25 mm thick silica gel G fluorescent plates. During examination 5 mg test and reference samples were withdrawn from their respective vials, dissolved in 0.5 ml chloroform, 10 µl applied on TLC plates and developed in a solvent system comprising of chloroform methanol: diethylamine (90:5:0.2). Spots on the chromatogram were detected by fluorescence quenching at 254, 366 nm, iodine vapour, spray reagents and Rf values (Khan *et al* 1986).

Purification by preparative thin layer chromatography. Voucher samples each weighing 10 mg were withdrawn and dissolved in 1 ml chloroform. Soluble extracts were carefully decanted and spotted without loss on 0.5 mm thick pre coated, fluorescent plates. Standard diacetylmorphine was also similarly treated. The plates were developed in the same solvent system used during the comparative TLC, Bands corresponding to diacetylmorphine were identified under UV light, scraped and eluted with methanol. These were extracted and total volume adjusted to 10 ml with chloroform.

UV spectroscopy. Chlorofrom solutions 1 ml each of test and standard diluted to 10 ml with chloroform and UV absorptions measured at 284 mm using a double beam spectrophotometer.

Results and Discussion

For acetylation of morphine, mild experimental conditions were chosen and as a results, TLC examination of the reaction mixture showed one major and several other minor components. In these experiments crystallisation from acetone succefully separated and purified the major component as diacetylmorphine from the reaction mixture.

The results of TLC evaluation of voucher samples against reference substances showed the samples as mixtures instead of pure heroin. Under the chromatographic conditions used, each sample resolved into five distinct constituents, such resolution was not possible with the reported TLC developing systems (Khan *et al* 1986, Mair *et al* 1986). The results of TLC finding are listed in Table-1. The samples were found adulterated with morphine, monoacetylmorphine, papaverine and noscapine. The results suggested presence of manufacturing impurities in the voucher samples.

For estimation of diacetylmorphine in the voucher samples by the reported multistage extraction, detection procedure (Horwitz 1970) using methanol for initial extractions, sodium hydroxide for partitioning and UV for measuring absorption of methanol sodium hydroxide extracts yielded heroin content ranging from 60-80%. These contents appeared invalid because TLC examination of the methanol extracts provided evidence for morphine, monoacetylmorphine, papaverine and noscapine.

Table 1
TLC profile of voucher sample

Constituents	Rf	Detection UV Fluorescence 254 nm	366 nm	$FeCl_3$	H_2SO_4 6%
Morpphine	0.04	Black	-	Blue	Brown
Monoacetyl-morphine	0.18	Black	-	Blue	Brown
Diacetyl-morphine	0.34	Black	-	-	Brown
Papaverine	0.78	Black	Light organe	Pink	Pale yellow
Noscapine	0.81	Black	Blue	Pink	Pink

Table 2
Contents of diacetylmorphine in the narcotic voucher sample

Code No.	Absorbance of the chloroform extract	*Diacetylmorphine%
1	0.14	4.5
2	0.13	4.19
3	0.36	11.61
4	0.37	11.93
5	0.28	9.03
6	0.52	16.77
7	0.39	12.58
8	0.44	14.19
9	0.41	13.22
10	0.51	16.61
11	0.58	18.96
12	0.42	13.54
13	0.28	9.03
14	0.33	10.64
15	0.86	27.74
16	1.29	41.61
17	0.87	28.06
18	1.25	40.32
19	0.41	13.22
20	0.43	13.87
21	0.54	17.41
22	0.62	20.0
23	0.63	20.32
24	0.47	15.16
25	0.84	27.09
26	0.62	20.00
27	0.45	14.51
28	1.05	33.87
29	0.79	25.48
30	2.2	70.96
31	1.16	37.41
32	0.88	28.38
33	0.57	18.38
34	0.27	8.70
35	0.24	4.74
36	0.38	12.25
37	0.26	8.88
38	1.10	35.48
Standard	3.1	100.00

*Absorbance of Test/Absorbance of standard x Concentration x '100.

For extraction and purification of diaceylmorphine from the voucher samples, preparative TLC was attempted due to the lack of sophisticated analytical instruments and reagents. Under the available facilities the chromatograhic method used resolved the ingredients of voucher samples with greater ease. After elution and extraction, the diacetylmorphine obtained appeared as completely diacetylmorphine in the voucher samples were calculated. The results are listed in Table-2. The voucher samples exhibited varying concentrations of diacetylmorphine which has not been obtained with the application of reported methods. One of the possible criticism on these determinations will be that the results obtained will not be reporducible due to adsorption of diacetylmorphine to silica gel layers. However in a separate resolution and evaluation work, using pure and adulterated heroin samples no major changes in the UV absorption of extracts were found. Undoubtedly preparative TLC is a routine technique undertaken for purification but its significance during evaluation of heroin product was not attempted on an earlier occasion. The work described has provided modified methodology for estimation of diacetylmorphine in the adulterated heroin products.

References

Billiet H A H, Wolters R, Galan De L, Huizer H 1986 Separation and identification of illicit heroin samples by liquid chromatography using lan alumina and C 18 coupled column system and photoiodide array detection. *J Chromatogr* **368** 2, 351-61.

Budavari S 1989 *The Merck Index* (Merck and Co Inc Raway NJ U.S.A) 11 ed p 468.

Helen C, Mehta A C, Calvert R T 1981 Stability of dimorphine in chloroform water mixture *Pharm J* **226** 6130, 682-83.

Helmut W, Peter M H 1987 Application of gas chromatographic capillary to capillary column switching system to the analysis of complex illicit drug samples. *J Chromatogr* **391** 2, 442-447.

Horwitz W 1970 *Official Methods of Analysis* of A.O.A.C. Benjamin Franklin station Washington DC, 11 ed pp 622-623.

Horwitz W 1970 *Official Methods of Analysis of A.O.A.C.* Benjamin Franklin Station Washington DC 12 ed pp 766.

Khan C, Haq I, Anis I M 1986 Developing systems for the identification of Opiates their acetyl derivatives and adultrants *J Chem Soc Pak* **8** (3), 417-19.

Lurie S I, Guinness K Mc 1987. The quantitation of heroin and selected basic impurities via reverse phase HPLC, II. The analysis of adulterated samples *J Liq Chromatogr* **10** (10) 2189-2204.

Mair N K, Navaratnam V, Rajahanda V 1986 Analysis of illicit heroin An effective TLC system for separating eight opiates and five adulterants *J Chromatogr* **366** 363-372.