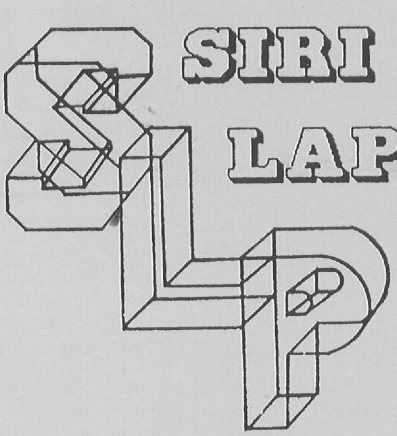

An Evaluation And Development Of Chromatographic Methods For The Study of Chemical Profiles Of Illicit Heroin Samples

Pusat Penyelidikan Dadah dan Ubat-Ubatan
(Centre for Drug Research)
U.N./W.H.O. Research and Training Centre
Universiti Sains Malaysia
11800 USM Penang
MALAYSIA

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TERHAD

AN EVALUATION AND DEVELOPMENT OF CHROMATOGRAPHIC METHODS FOR THE STUDY OF CHEMICAL PROFILES OF ILLICIT HEROIN SAMPLES

Shobha Kumari d/o Chhunnu Lal
N.K. Nair
V. Navaratnam

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LIST OF ABBREVIATIONS

AAN	Acetylanhydronornarceine
Acc	Acetylcodeine
AL	Acetylnorlaudanosine
AN	Acetylnornarcotine
ANM	Acetylnormorphine
AT	Acetylthebaol
C	Codeine
CRY	Cryptopine
DAM	Diacetylmorphine
DANC	Diacetylnorcodeine
DANM	Diacetylnormorphine
Lev	Levallorphan
M	Morphine
3-MAM	O^3-Monoacetylmorphine
6-MAM	O^6-Monoacetylmorphine
Me	Meconin
Na	Narceine
Nal	Nalorphine
NC	Norcodeine
NM	Normorphine
No	Noscapine
P	Papaverine
T	Thebaine
TANM	Triacetylnormorphine
TL	Thebaol
Acet	Acetone

Acet	Acetone
$nBuNH_2$	n-Butylamine
nBu_2O	n-Butyl ether
Bz	Benzene
C.C.	Capillary column
CDMS	Cyclohexanedimethanol succinate
cycHex	Cyclohexane
DEA	Diethylamine
Diox	Dioxane
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
EtOH	Ethanol
HBFA	Heptafluorobutyric anhydride
nHex	n-Hexane
HOAc	Acetic acid
iOct	Isooctane
iPrOH	Isopropanol
MeCN	Acetonitrile
MeOH	Methanol
MSTFA	N-methyl-N-trimethylsilyl-trifluoroacetamide
NH_4OAc	Ammonium acetate
P.C.	Packed column
nPrOH	n-Propanol
TBAP	Tetrabutylammonium phosphate
TMA	Tetramethylammonium hydroxide
Tol	Toluene
TEA	Triethylamine

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ABSTRAK

Sistem-sistem kromatografi lapisan nipis (TLC), kromatografi cecair keupayaan tinggi (HPLC) dan kromatografi gas (GC) telah diperkembangkan untuk memisah serta mengkuantitasi opiat-opiat utama dan juga opiat-opiat surih yang hadir dalam sampel-sampel heroin haram.

Sistem TLC dengan n-heksana - kloroform - trietilamina (9:9:4) sebagai eluen dapat memisahkan lapan opiat utama iaitu morfina, kodeina, O^6-monoasetilmorfina (6-MAM), diasetilmorfina (DAM), papaverina, asetilkodeina, noskapina, tebaina dan empat opiat surih atau hasil-hasil sampingan bersekutu iaitu narseina, nalorfina, levallorfan dan asetiltebaol. Semua opiat pada mulanya diidentifikasikan di bawah cahaya UV dan seterusnya dikenalpastikan berdasarkan kepada nilai R_f masing-masing dan warna yang dihasilkan dengan reagen semburan iodoplatinat berasid. Had kuantitasi pada jarakgelombang 254 nm adalah 0.7 μg bagi levallorfan, 5 μg bagi tebaina, papaverina dan asetiltebaol dan 0.2 μg bagi opiat-opiat yang lain.

Sistem HPLC dengan 1% trietilamina di dalam air [diselaraskan kepada pH 6 dengan asid ortofosforik] dan metanol di dalam nisbah 40:60 sebagai fasa bergerak telah memberi resolusi garis dasar yang baik bagi asetiltebaol dan lapan opiat utama yang telah disebutkan tadi. Kolum jenis oktadesilsiloksana fasa terikat (C_{18}) digunakan sebagai fasa pegun bersama dengan pengesan UV pada jarakgelombang 280 nm. Kadar pengaliran fasa bergerak adalah 1.00 ml min^{-1}. Had pengesanan dalam satu campuran adalah 11 ng bagi asetiltebaol, asetilkodeina dan 12 ng bagi 7 opiat utama yang lain.

Kolum-kolum GC berpadat OV-1, OV-3, OV-17 dan OV-210 telah dinilai bagi pemisahan opiat-opiat dengan pengesan pengionan nyala. Walau bagaimanapun, hanya fasa pegun secara relatif bukan polar, OV-3 dapat memisahkan 9 opiat. Bagi analisis kuantitatif, kolum rerambut SE-54 [fasa pegun setara dengan OV-3] yang telah memberi resolusi 11 opiat, digunakan dengan pengesan perangkap ion pada kedua-dua mod imbasan penuh dan pengesanan ion berganda. Had pengesanan di dalam mod pengesanan ion berganda adalah 400 pg bagi DAM, asetilkodeina dan papaverina, 600 pg bagi kodeina dan 6-MAM, 800 pg bagi levallorfan dan tebaina, 2 ng bagi morfina dan nalorfina dan 5 ng bagi noskapina dan narseina.

Sistem-sistem TLC, HPLC dan GC telah diguna bagi menganalisa 50 sampel rampasan heroin haram tempatan yang tidak diketahui asal geografikalnya. Keputusan-keputusan menunjukkan kehadiran hanya DAM, 6-MAM, asetilkodeina, kodeina dan morfina dalam sampel-sampel rampasan tersebut. Tempat asal sampel-sampel ditentukan oleh dua kaedah iaitu pertama dengan membandingkan data analisis kualitatif dan kuantitatif sampel-sampel dengan yang dilaporkan oleh O'Neil [22] dan kedua, dengan kaedah Narayanaswami [18]. Kaedah kedua menunjukkan bahawa hanya 17% daripada sampel-sampel berasal dari Asia Tenggara manakala dengan kaedah pertama 60% daripada sampel-sampel mempunyai profil kimia sama seperti sampel-sampel Asia Tenggara yang dilaporkan oleh O'Neil. Perbezaan ini dapat dijelaskan jika terjadinya hidrolisis DAM ke 6-MAM bagi sampel-sampel heroin haram semasa penyimpanan yang lama. Maka nisbah eksperimental DAM ke asetilkodeina yang telah digunakan bagi menentukan tempat asal sampel-sampel heroin haram oleh kaedah Narayanaswami tidak akan mencerminkan nisbah tepat yang hadir semasa penyediaan sampel-sampel.

ABSTRACT

Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) dan Gas Chromatography (GC) systems were developed for separation and quantitation of the major and some of the trace opiates present in illicit heroin samples.

The TLC system with n-hexane - chloroform - triethylamine (9:9:4) as eluent was capable of separating eight major opiates namely morphine, codeine, O^6-monoacetylmorphine (6-MAM), diacetylmorphine (DAM), papaverine, acetylcodeine, noscapine, thebaine and four trace opiates or associated byproducts namely narceine, nalorphine, levallorphan and acetylthebaol. All the opiates were first located under UV light and subsequently identified on the basis of their R_f values and the colour produced with acidified iodoplatinate as the spray reagent. The limits of quantitation at 254 nm was 0.7 µg for levallorphan, 5 µg for thebaine, papaverine and acetylthebaol and 0.2 µg for the other opiates.

The HPLC system with 1% triethylamine in water [adjusted to pH 6 with orthophosphoric acid] and methanol in the ratio 40:60 as the mobile phase gave good baseline resolution for acetylthebaol and the eight major opiates mentioned previously. An octadecylsiloxane bonded phase [C_{18}] column was used as the stationary phase together with a UV detector set at 280 nm. The flow rate of the mobile phase was 1.00 ml min^{-1}. The limits of detection in a mixture was 11 ng for acetylthebaol, acetylcodeine and 12 ng for the other seven opiates.

The OV-1, OV-3, OV-17 and OV-210 packed GC columns were evaluated with a flame ionization detector for the separation of the opiates. However, only the relatively non-polar stationary phase, OV-3 was able to separate 9 opiates. For quantitative analysis, the SE-54 capillary column [equivalent stationary phase to OV-3] which was able to resolve 11 opiates was used with an ion trap detector on both the full scan and multiple ion detection (MID) mode. The limits of detection in the MID mode was 400 pg for DAM, acetylcodeine and papaverine, 600 pg for codeine and 6-MAM, 800 pg for levallorphan and thebaine, 2 ng for morphine and nalorphine and 5 ng for noscapine and narceine.

The TLC, HPLC and GC systems were used to analyse 50 locally siezed illicit heroin samples of unknown geographical origin. The results showed the presence of only DAM, 6-MAM, acetylcodeine, codeine and morphine in the seized samples. The origin of the samples were deduced firstly by comparing the qualitative and quantitative data with those reported by O'Neil [22] and secondly, by Narayanaswami's method [18]. The latter method suggested that only 17% of the samples were from South East Asia whereas 60% of the samples had a chemical profile similar to South East Asian samples reported by O'Neil. The differences would be accounted for if hydrolysis of DAM to 6-MAM occurred during prolonged storage of illicit heroin samples. The experimental ratios of DAM to acetylcodeine which were utilized for determining the origin of the illicit heroin samples by Narayanaswami's method thus would not reflect the ratios which were present during the preparation of the samples.

CONTENTS

Chapter 1

INTRODUCTION

1.1 General Introduction

Opium is derived from the unripe seed capsules of the opium poppy, PAPAVER SOMNIFERUM L. The latex obtained by incision of the unripe seed capsules is dried or partly dried by heat or spontaneous evaporation to yield opium which contains about 25 alkaloids [1-4].

Morphine, the most important alkaloid in opium, is present to an extent varying from 10-16% by weight of opium [5,6]. The other alkaloids present are codeine (0.8-2.5%), noscapine or narcotine (4-8%), papaverine (0.5-2.5%) and thebaine (0.5-2%) [7]. These major alkaloids and other trace alkaloids such as narceine, codamine, cryptopine, oripavine, norlaudanosine constitute about 25% by weight of opium [8,9].

Opium is the major source of morphine needed for the preparation of heroin. Heroin (diacetylmorphine, DAM) is prepared by the diacetylation of morphine [7].

1.2 Opiates

The opiates present in illicit heroin include natural and synthetic opium alkaloids. The names and structures of commonly occuring opiates in illicit heroin are listed in Fig. 1.1. Table 1.1 lists the formula, relative molecular weight, pKa values and melting point of the opiates [7, 10-12].

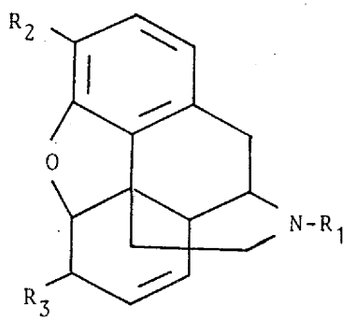
1.3 Clandestine Production of Illicit Heroin

Various methods have been reported for the clandestine production of illicit heroin [13-18]. Basically, all the methods involve the extraction of morphine from opium followed by acetylation of the morphine with acetic anhydride to obtain heroin [Fig. 1.2].

The normal method employed involves treatment of opium with lime and water, followed by filtratation. Ammonium chloride is added to the filtrate to precipitate morphine which is then redissolved in sulphuric acid containing charcoal. After filtration, ammonium hydroxide is added to reprecipitate morphine which is filtered and air dried. Acetic anhydride is added to the dry morphine and refluxed. Neutralization with sodium carbonate precipitates crude heroin base which is then filtered and washed with water.

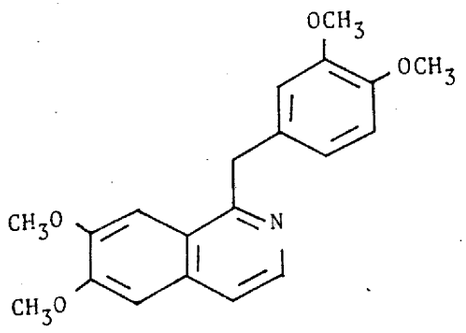
1.4 Chemical Composition of Illicit Heroin

The production of illicit heroin in South East Asia has recently been given wide publicity. Depending on the purity of the starting material (morphine base vs raw opium) and the manufacturing process involved, the chemical composition of illicit heroin can usually be classified into five groups:

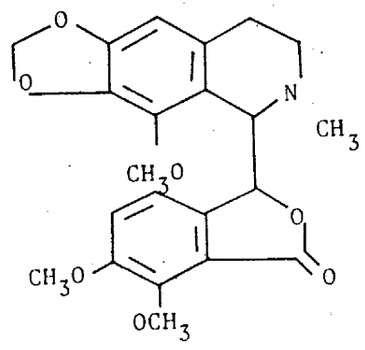


Compound	R_1	R_2	R_3
1. Morphine	CH_3	OH	OH
2. O^6-monoacetyl-morphine (6-MAM)	CH_3	OH	$OCOCH_3$
3. Diacetylmorphine (DAM)	CH_3	$OCOCH_3$	$OCOCH_3$
4. Codeine	CH_3	OCH_3	OH
5. Acetylcodeine	CH_3	OCH_3	$OCOCH_3$
6. Thebaine	CH_3	OCH_3	OCH_3
7. Nalorphine	C_3H_5	OH	OH

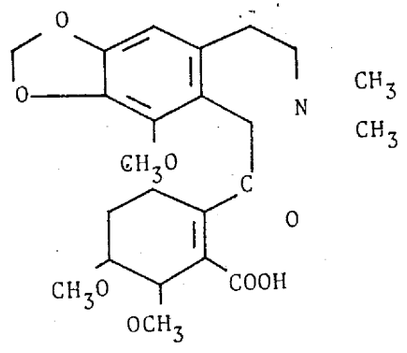
Fig. 1.1: Opiates



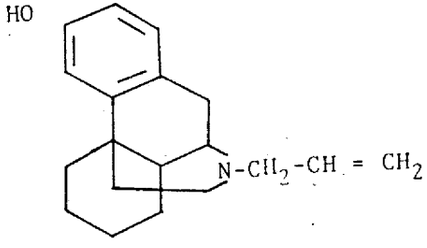
8. PAPAVERINE



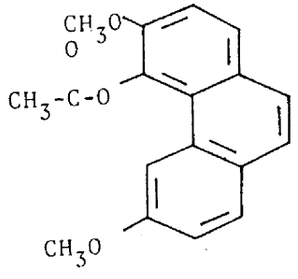
9. NOSCAPINE



10. NARCEINE



11. LEVALLORPHAN



12. ACETYLTHEBAOL

Table 1.1

Name, Formula, Relative Molecular Weight, pKa Values and Melting Points of Opiates

Name	Formula	Relative Molecular Weight	pKa	Melting Point
Morphine	$C_{17}H_{19}NO_3$	285	9.9	250°C
6-MAM	$C_{19}H_{21}NO_4$	327	-	265°C
DAM	$C_{21}H_{23}NO_5$	369	7.6	230°C
Codeine	$C_{18}H_{21}NO_3$	299	8.2	155°C
Acetylcodeine	$C_{20}H_{23}NO_4$	341	-	142°C
Thebaine	$C_{19}H_{21}NO_3$	311	8.2	193°C
Papaverine	$C_{20}H_{21}NO_4$	339	6.4	215°C
Noscapine	$C_{20}H_{23}NO_4$	413	6.2	175°C
Narceine	$C_{23}H_{27}NO_8$	445	9.3	192°C
Nalorphine	$C_{19}H_{21}NO_3$	311	7.8	260°C
Levallorphan	$C_{19}H_{25}NO$	283	6.9	176°C
Acetylthebaol	$C_{18}H_{15}O_4$	296	-	122°C

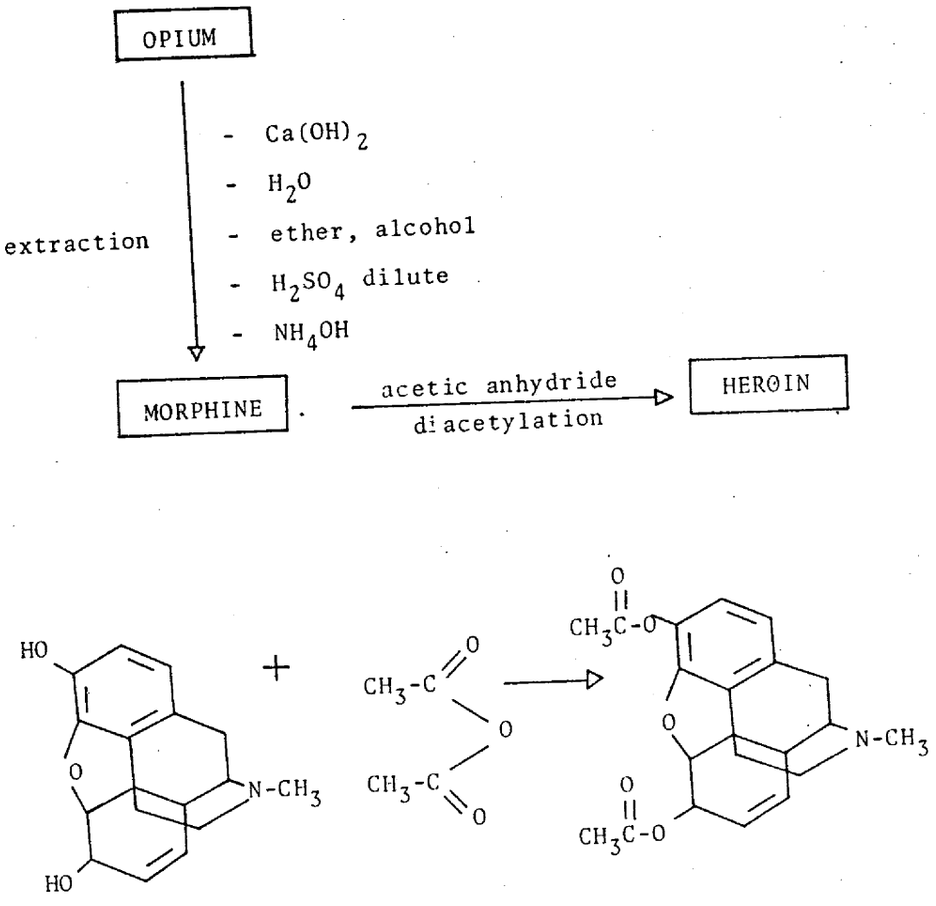


Figure 1.2: Preparation of Illicit Heroin

i. presence of naturally occurring opium alkaloids such as codeine, morphine, noscapine, papaverine, thebaine, which may have passed unchanged through the extraction, acetylation and purification procedure [18-25];

ii. presence of synthetic opium alkaloids such as 6-MAM, acetylcodeine, nalorphine which could result from the acetylation step, or hydrolysis [26-30];

iii. low level of trace impurities and associated by-products such as acetylthebaol [20-21, 31-33], norcodeine [34], normorphine [34], acetyl norlaudanosine [31, 35], acetyl nornarcotine [31, 35], diacetylnorcodeine [36], resulting from rearrangement reactions during the manufacture of illicit heroin;

iv. presence of diluents such as caffeine, quinine, sugar, talc which are added for bulk after preparation [37-40];

v. presence of adulterants such as methadone, cocaine, ephederine which are added after preparation, for extra pharmacological effects [41-42].

1.5 Characterisation and Comparison of Illicit Heroin Samples

Illicit heroin often contains trace impurities which are associated with the manufacturing process (Section 1.4). Attempts have been made to characterize these impurities by qualitative and quantitative analysis in order to determine typical chemical features of illicit heroin samples. Recently, several workers have reported characterisation and comparison of illicit heroin samples by employing different types of criteria [18-19, 21-22, 33].

Law et al. [21] suggested that comparisons of seized illicit heroin can yield information regarding its origin and distribution pattern. Law proposed that analysis of the major components and associated impurities in a seized batch of illicit heroin would give a unique chemical profile or "chemical signature" for that particular batch of illicit heroin. Although the illicit heroin initially might be relatively pure, it was often progressively diluted or adulterated as it passed down a distribution chain. Thus two samples from the same batch of heroin could have completely different chemical compositions when seized at "street level". Law suggested that in order to compare such samples, it was only necessary to quantitate those components present in, or derived from, the original opium. Although the amount of various alkaloids would vary with the degree of dilution, the overall ratios would remain the same. Utilizing this criterion, Law identified and quantitated trace impurities of acetylthebaol and thebaol as well as the major opiates, morphine, codeine, 6-MAM, DAM, acetylcodiene and papaverine in illicit heroin samples by GC and HPLC methods. He concluded that a given heroin sample could be chemically characterized. The quantitative analysis

of the same impurities in illicit heroin was also carried out by Huizer [33] who utilized a HPLC method with fluorimetric detection. The method also permitted discrimination between different heroin samples.

O'Neil et al. [22] reported some physical and chemical features of illicit heroin indicative of their origin. Samples taken from seizures of illicit heroin preparations of known geographical origin were examined by GC and HPLC. The chromatographic characteristics were considered in relation to the physical appearance of the materials and it was found possible in many instances to discriminate between samples of different origin.

Nair et al. [19] stated that the chemical composition of heroin intercepted in the illicit drug traffic and at consumer level varies substantially, depending on factors such as the geographical source, the manufacturing process used (extraction, acetylation and purification) and the distribution pattern. They suggested that a detailed chemical analysis of a sufficient number of samples would contribute towards providing data relating to their origin and distribution pattern. A number of illicit heroin samples were analysed by GC and subsequently grouped on the basis of their relative opiate compositions.

Another method for determining the source of supply or origin of illicit heroin samples was proposed by Narayanaswami [18]. The ratios of morphine to codeine obtained from opium samples of known origin as well as the ratios of DAM to acetylcodeine in illicit heroin samples that were found to belong to the same source of supply as the known opium samples, were used as the basic criteria for a comparison to determine the origin of illicit heroin samples. According to Narayanaswami, the criteria that were useful in determining the origin of samples of seized heroin should be based on the compounds carried over from the extraction of morphine from opium raw material and from the acetylation process. Quantitation by GC, showed that the theoretical ratio of DAM to acetylcodeine increased two-fold at each stage of the chemical conversion in the sequence opium-morphine- heroin. The ratios of DAM to acetylcodeine obtained from opium samples of known origin showed significant variation which enabled the author to make distinct composition profiles of the alkaloids for each geographical area studied. Such profiles made it possible to compare heroin samples of known origin with illicit heroin samples of unknown origin and to determine the geographical area from which the latter originated.

1.6 Analytical Methods for Heroin Analysis

For many years, heroin analysis depended on a series of presumptive tests such as colour, microcrystalline, solubility and precipitation tests [44, 45]. However in recent years, more sophisticated analytical methods have been developed. These techniques include thin layer chromatography (TLC), liquid chromatography (LC), gas chromatography (GC), spectroscopic methods

such as ultraviolet (UV), infrared (IR), fluorescence or luminescence and also radioimmunoassay (RIA). Mass spectrometry (MS) has been used for identification and confirmation, often on-line with gas/liquid chromatography [46].

The most extensively used colour tests for the identification of heroin are with Marquis reagent, Froehde reagent and nitric acid [47], whereas mercuric iodide in hydrochloric acid and platinum chloride are used for microcrystalline tests. In some cases, a combination of tests are performed for a specific analytical scheme to prove the identity of heroin. A good review of such methods currently being used by U.S. crime laboratories is available from the Law Enforcement Assistance Administration in conjunction with its laboratory proficiency testing program [48].

In the past few years, there have been a number of papers in which a particular chromatographic technique has been used for the separation and quantitation of heroin samples. Splies et al. [49] published data that question the validity of colour and microcrystalline tests for heroin analysis. Their studies showed that several common colour tests, along with platinum chloride and gold bromide microcrystalline tests, were incapable of distinguishing heroin from structurally related morphine compounds.

Hider [50] proposed an analytical scheme consisting of the Marquis test and the mercuric chloride microcrystalline test to identify heroin. Fulton [51] suggested that the Marquis, Froehde and Mecke colour tests in combination with mercuric iodide and gold bromide microcrystalline tests were sufficient for positive identification of heroin.

A combination of TLC and colour tests have been extensively used for the identification of heroin and other opium alkaloids [52-56]. Curry and Patterson [30] on the other hand, suggested a preliminary examination of crude heroin by IR. Schaler and Jerpe [57] advocated combining GC with IR for identification of heroin; while Nakamura et al. [58] demonstrated the utility of gas chromatography-mass spectrometry (GC-MS) for heroin identification.

In conclusion, in recent times the trend has been towards the utilization of chromatographic methods for the analysis of illicit heroin [59-61].

1.7 Chromatographic Techniques for Illicit Heroin Analysis

Chromatography is a technique that involves separation of solutes based on selective interactions between a stationary phase and a mobile phase. The chromatographic techniques used in illicit heroin analysis are thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and gas chromatography (GC).

1.7.1 Thin Layer Chromatography (TLC)

TLC has been extensively applied for the detection and identification of opiates in illicit heroin samples [10,19, 30, 45-46, 52, 59-60, 62-98]. It is the most popular screening method for opiates. Table 1.2 summarises some of the published TLC systems for screening opiates commonly encountered in illicit heroin samples.

Several TLC solvent systems were examined by Clark [45] for use with silica gel plates. The eluent consisting of n-butyl ether - diethyl ether - diethylamine (45:45:10) separated morphine, DAM, noscapine but 6-MAM was not separated from papaverine and acetylcodeine overlapped with thebaine.

Three workers [46, 77, 97] separated the same five opiates namely morphine, 6-MAM, DAM, codeine and acetylcodeine using different solvent systems. Manura et al. [46] investigated various systems and concluded that benzene - dioxane - ethanol - ammonium hydroxide [50:40:5:5) as eluent gave the best separation for the above mentioned five opiates. Van Welsum [77] used a system with chloroform - diethyl ether - methanol - 25% ammonium hydroxide [75:25:5:1] as the mobile phase for separation of five opiates, whereas Marumo et al. [97] employed diethyl ether saturated with water - acetone - diethylamine (85:8:7). Quantitation was done by the latter workers by UV absorption at 280 nm and 360 nm. The detection limit reported for the five opiates were 1 ug.

Three solvent systems were reported by Gough et al. [59] for opiate screening. The TLC system with chloroform - cyclohexane - diethylamine (8:10:3) as eluent separated 6-MAM, DAM, acetylcodeine, papaverine but morphine was not separated from codeine. This system was the best among the three in terms of resolution of the opiates.

Kaistha et al. [63] examined four TLC systems (Table 1.2) for opiate screening and system B with ethyl acetate-cyclohexane - methanol - ammonia solution in the ratio 70:15:10:5 gave separation of seven opiates namely morphine, 6-MAM, DAM, codeine, acetylcodeine, papaverine and thebaine. These seven opiates were also separated by the TLC system proposed by Steele [64] with a mobile phase consisting of dioxane - chloroform - ethyl acetate - ammonia solution [60:25:10:5]. Another system reported by Steele [64] with ethyl acetate - benzene - acetonitrile - ammonia (25:30:40:5) as eluent was capable of separating six opiates namely morphine, 6-MAM, DAM, codeine, papaverine and noscapine. Acetylcodeine was not separated from thebaine and all the opiates were detected by spraying with potassium iodoplatinate reagent.

Table 1.2

Summary of the published TLC systems on illicit heroin analysis

Reference	OPIATE separated	Solvent system	Adsorbent	Sensitivity
STEELE 1965	M, 6-MAM, DAM, C, ACC, P, T	Diox - $CHCl_3$ - EtoAC - NH_3 (60 : 25 : 10 : 5)	Silica	
KAISTHA 1972	M, 6-MAM, C, No, T	A. EtOAC - cycHex - Diox - MeOH - H_2O - NH_3 (50 : 50 : 10 : 10 : 1.5 : 0.5)	"	-
	M, 6-MAM, DAM, C, ACC, P, T	B. EtOAC - cycHex - MeOH - NH_3 (70 : 15 : 10 : 5)	"	-
	M, No, P	C. EtOAC - cycHex - Diox - MeOH - H_2O - NH_3 (50 : 50 : 10 : 10 : 0.5 : 1.5)	"	-
	M, 6-MAM, C, ACC, No, P	D. EtOAC - cycHex - NH_3 - MeOH - H_2O (70 : 15 : 2 : 8 : 0.5)	"	-
		Two dimension		
VIALA 1973	C, M, DAM, Na, No, 6-MAM	i) MeOH - $CHCl_3$ - 23% NH_4OH (85 : 15 : 0.7)	"	-
		ii) Et_2O - Acet - DEA (85 : 8 : 7)		
VAN WELSUM 1973	M, 6-MAM, DAM, C, ACC	$CHCl_3$ - Et_2O - MeOH - NH_3 (75 : 25 : 5 : 1)	"	-
CLARK C.C. 1976	M, DAM, No	nBu_2O - Et_2O - DEA (45 : 45 : 10)	"	-
MACHOVICOUA 1977	C, M, P, T, Na, No	Xylene - CH_2Cl_2 - Acet - EtOH - NH_4OH (40 : 20 : 20 : 6 : 2.5)	"	-
MASOUD 1977	C, M, P, DAM	$CHCl_3$ - Et_2O - MeOH - NH_4OH (75 : 25 : 5 : 1)	"	-

Reference	OPIATE SEPARATED	Solvent system	Adsorbent	Sensitivity
CLARK E.G. 1978	M, C, P, T	A. Bz - Diox - EtOH - NH_3 (50: 40 : 5 : 5)	Silica	-
	M, 6-MAM, C, ACC	B. EtOH - HOAC - H_2O (6 : 3 : 1)	"	-
	M, 6-MAM, No	C. MeOH - NH_3 (100 : 1.5)	"	-
MANURA 1978	C, M, DAM, 6-MAM, ACC	Bz - Diox - EtOH - NH_4OH (50: 40 : 5 : 5)	"	-
STAHL 1978	C, M, DAM, P, T, ACC, Na, No, 6-MAM	Tol - Acet - EtOH - NH_4OH (45 : 45 : 7 : 3)	"	-
		$CHCl_3$ - MeOH (9 : 1)		
ENGELKE 1979	M,C, No, P	Tol - Acet - EtOH - NH_3 (20 : 20 : 3 : 1)	"	-
BUDD 1980	M, DAM, C, ACC, No, P, T	nHex - $CHCl_3$ - DEA (50 : 30 : 7)	"	-
MARI 1982	M, 6-MAM, DAM, C, P, No	A. Tol - Acet - EtOH - DEA (45 : 45 : 7 : 3)	"	-
	M, P, No	B. $CHCl_3$ - Acet (9 : 1)	"	-
GOUGH 1982	6-MAM, DAM, ACC, P	A. $CHCl_3$ - cycHex - DEA (8 : 10 : 3)	"	-
	M, 6-MAM, C	B. $CHCl_3$ - EtOH - Acet - NH_3 (20 : 20 : 5 : 1)	"	-
	M	C. $CHCl_3$ - MeOH - DEA (16 : 3 : 1)	"	-

Reference	OPIATE SEPARATED	Solvent system	Adsorbent	Sensitivity
HUIZER 1983	M, 6-MAM, DAM, C, P	Tol - Acet - EtOH - DEA (30 : 60 : 7 : 3)	Silica	-
WAGNER 1984	M, C, P, 6-MAM, DAM, No	Tol - Acet - EtOH - NH_3 (40 : 40 : 6 : 2)	"	-
NAIR 1986	C, M, 6-MAM, DAM, P, T, ACC, No	$CHCl_3$ - nHex - TEA (9 : 9 : 4)	"	0.1 g

Abbreviations:

M = morphine; C = codeine; DAM_6 = diacetylmorphine;
ACC = acetylcodeine; 6-MAM = O^6-monoacetylmorphine;
P = papaverine; T = thebaine; No = noscapine; Na = narceine;
Lev = levallorphan; Nal = nalorphine

Acet = acetone; Bz = benzene; cycHex = cyclohexane;
Diox = dioxane; DEA = diethylamine; EtOAC = ethyl
acetate; Et_2O = diethylether; EtOH = ethanol;
iPrOH = isopropanol; MeOH = methanol; MeCn = acetonitrile;
nBu_2O = n-butylether; n-Hex = n-hexane; Tol = toluene.

Budd [65] developed a TLC system with n-hexane-chloroform-diethylamine (50:30:7) as mobile phase which separated seven opiates namely morphine, DAM, codeine, acetylcodeine, papaverine, noscapine and thebaine. However, 6-MAM and caffeine which is a common diluent found in illicit heroin were unresolved.

A two-dimensional thin layer chromatographic method for opiates described by Viala et al. [66] separated morphine, 6-MAM, DAM, codeine, noscapine and narceine. The chromatograms were developed in the first direction with methanol - chloroform - ammonia solution (85:15:0.7) and in the second direction with diethyl ether - acetone - diethylamine (85:8:7).

Nair and co-workers [19] reported a TLC system with chloroform - n-hexane - triethylamine (9:9:4) as a eluent capable of separating eight opiates namely morphine, 6-MAM, DAM, codeine, acetylcodeine, papaverine, thebaine, noscapine and five adulterants. The limit of detection of the individual opiates in the mixture was 0.1 µg. The opiates were first located under UV and subsequently identified on the basis of their R_f values and the colour produced with spray reagents.

In conclusion, the literature review on TLC systems showed that the maximum number of opiates were separated by the system reported by Nair and co-workers [19].

1.7.2 High Performance Liquid Chromatography (HPLC)

HPLC is gaining importance as a technique for the routine analysis of illicit heroin [99-100]. Various modes of HPLC systems have been reported for illicit heroin analysis including ion-exchange [41, 101-105], adsorption [33, 37, 71, 106-115], reversed phase ion-pair [116-121] and reversed-phase chromatography [20, 24, 122-129]. Table 1.3 summarises some of the published HPLC systems for illicit heroin analysis.

Cashman and Thorton [104] were among the earliest workers to use HPLC for the analysis of illicit heroin. A 37- to 50- nm silica (Porasil T) column was used with a mobile phase of chloroform-methanol (4:1) for separating morphine, 6-MAM and DAM. Procaine, an adulterant sometimes found in illicit heroin samples, coeluted with DAM.

Twitchett [102] utilized ion-exchange chromatography with gradient elution to examine the components of illicit heroin. Morphine, 6-MAM, DAM were separated together with five adulterants and 20 illicit heroin samples were quantitated for these compounds. The chromatographic conditions employed were boric acid (0.2M aqueous) adjusted to pH 9.3 with 40% sodium hydroxide as eluent, together with a Zipax SCX strong cation-exchange resin column and UV absorbance detector.

Table 1.3

Summary of published HPLC systems on illicit heroin analysis

REFERENCE	HPLC MODE	MOBILE PHASE	Buffer	pH	Column	OPIATES SEPARATED	DETECTOR	Analysis time	Sensitivity
KNOX 1973	Ion exchange	MeCN - nPrOH - buffer (12 : 2 : 86)	0.04 M NaOH aqueous	9.3	Zipax SCX resins	M, 6-MAM, DAM	uv (280 nm)	30 min	-
WITTWER 1973	"	boric buffer - phosphate buffer (85 : 15)	0.01 M KH_2PO_4, 0.01M H_3BO_3	6.0 9.5	SAX Zipax resin	M, C, P, T	uv (254 nm)	30 min	-
TWITCHETT 1975	"	NaOH - buffer (40 : 60)	0.2 M aq H_3BO_3	9.3	Zipax SCX resin	M, 6-MAM, DAM	uv (270 nm)	10 min	-
LAURENT 1984	"	MeOH - MeCN - buffer (12.5: 12.5 : 7.5)	0.01 M aq TMA	6.5	Alumina	M, DAM, ACC	uv (254 nm)	10 min	-
ZIEGLER 1975	Adsorption	$CHCl_3$ - MeOH - DEA (100^3 + 300 + 1)	-	-	Si (Corasil)	M, C, P, T, No, CRY	uv (254 nm)	26 min	-
HUIZER 1977	"	Et_2O - iOct - MeOH - DEA - (52^8 + 35 + 12 + 0.2)	-	-	Lichrosorb Si60	C, 6-MAM, DAM, ACC	uv (227 nm)	-	-
WITTWER 1981	"	cyc Hex - mixture (750 ml : 250 ml) mixture: NH_4OH - MeOH - $CHCl_3$ ($1 + {}^3200 + 800$)	-	-	10 m-Si (Porasil)	6-MAM, DAM, ACC	uv (254 nm)	15 min	-
HUIZER 1983 (p.32)	"	n-Hex - CH_2Cl_2 - MeOH (0.75%DEA) (75: 20: 5)	-	-	Lichrosorb Si60	M, 3-MAM, DAM, ACC	uv (227 nm)	42 min	-

REFERENCE	HPLC MODE	MOBILE PHASE	BUFFER	pH	COLUMN	OPIATES SEPARATED	DETECTOR	Analysis Time	Sensitivity
HUIZER 1983 (p.40)	Adsorption	n-Hex - CH_2Cl_2 MeOH (0.75% DEA) (75: 20: 5)	-	-	Lichrosorb Si60	AT, AL	Fluorimetric Detection ex = 260 nm em = 400 nm	10 min	-
WHITE 1983	"	iOct - Et_2O - MeOH - DEA - H_2O (400 + 300 + 225 + 0.5 + 15)	-	-	Si	M, C, 6-MAM, DAM, ACC	uv (279 nm)	12 min	-
OLIEMAN 1977	Reversed phase ion pair	MeOH - buffer (40 : 60)	0.005M n - $C_7H_{15}SO_3H$ in H_2O	-	µBondapak C_{18}	M, C, DAM, T	uv (254 nm)	20 min	-
LURIE 1977	"	H_2O - CH_3COOH buffer (59: 1: 40)	0.005m n-$C_7H_{15}SO_3H$ in MeOH	3.5	µBondapak C_{18}	M, C, P, T, No	uv (254 nm)	20 min	-
SONI 1979	"	MeOH - buffer (47 : 53)	0.01 M TBAP	7.5	µBondapak C_{18}	M, C, 6-MAM, DAM, ACC	uv (254 nm)	20 min	-
LURIE 1982	"	MeCN - H_3PO_4 - buffer (12: 1: 87)	0.02 M CH_3SO_3H in H_2O	2.2	µBondapak C_{18}	M, C, 6-MAM, DAM, P, No	uv (220 nm, 254 nm)	60 min	-
GALESWSKY 1984	"	MeCN - buffer (10 : 90)	10 mM $KClO_4$ + 5 mM $nBuNH_2$	3.0	C_{18}	M, C, P, T	uv (280 nm)	7 min	-
TRINLER 1975	Reversed phase	MeCN - aq. buffer (65 : 35)	0.1% by weight $(NH_4)_2CO_3$ in H_2O	-	µBondapak C_{18}	M, DAM	uv (254 nm)	10 min	-

REFERENCE	HPLC MODE	MOBILE PHASE	BUFFER	pH	COLUMN	OPIATES SEPARATED	Detector	Analysis Time	Sensitivity
WU 1977	Reversed phase	MeCn - Buffer (25 : 75)	0.1 M NaH_2PO_4	4.8	µBondapak C_{18}	M, C, P, T, No	uv (254 nm)	40 min	-
LOVE 1980	"	MeCN - Buffer (65 : 35)	0.75 g NH_4OAc per 100 ml H_2O	7.0	"	C, 6-MAM, DAM, ACC	uv (280 nm)	12 min	-
NOBUHARA 1980	"	MeCN - diox - Buffer (10: 10: 80)	1% NH_4OAc	5.8	10 CN nucleosil	M, C, P, T, No, CRY	uv (254 nm)	25 min	-
BAKER 1981	"	MeCN - Buffer (85 : 15)	0.005 M TBAP	-	amino-propyl bonded Si	M, C, 6-MAM, DAM, ACC	uv (284 nm)	16 min	-
LURIE 1984	"	MeCN - MeOH - Buffer (25.2: 13.4: 61.4)	phosphate buffer	2.2	C_{18}	AT, AN, AL, Me	uv (260 nm)	25 min	-
BILLIET 1986	"	MeCN - MeOH - Buffer (17: 28: 55)	0.01 M citrate/TMA	6.0	C_{18} and Alumina coupled	M, C, DAM, P, No, ACC, AT	uv (254 nm)	16 min	-

Abbreviations:

iOct = isooctane; NH_4OAc = ammonium acetate; TBAP = tetrabutyl ammonium phosphate; TMA = tetramethylammonium hydroxide;

AT = acetylthebaol; AN = acetylnornarcotine; AL = acetylnorlaudanosine; CRY = cryptopine; Me = meconin. TL = thebaol; nPrOH = n-propanol; nBuNH$_2$ = n-butyl amine

Ion-pair reversed phase chromatography was used by Soni dan Dugar [116] with a mobile phase consisting of 47% methanol with 0.01M tetrabutylammonium phosphate solution (pH 7.5). The stationary phase was a prepacked μ-Bondapak C_{18} column and detection was at 254 nm. This system gave separation of morphine, 6-MAM, DAM, codeine and acetylcodeine.

Another HPLC method for the analysis of illicit heroin was described by Love and Pannell [125]. A μ-Bondapak C_{18} column was utilized and the mobile phase at pH 7 consisted of 65% acetonitrile, and 35% aqueous buffer containing 0.75 g of ammonium acetate per 100 ml water. Six opiates were separated namely morphine, 6-MAM, DAM, codeine, acetylcodeine, papaverine but morphine and papaverine were not resolved to baseline. In addition, tailing was observed for DAM. Samples were assayed directly in methanol using a fixed UV detector at 280 nm.

In a recent report, Wittwer [113] described a method for the analysis of "brown" heroin samples of the "Mexican process" variety. A 10 μm Porasil column was used. The mobile phase was composed of 750 ml of cyclohexane and 250 ml of a mixture of ammonium hydroxide - methanol - chloroform (1:200:800) and detection was at 254 nm. 6-MAM, DAM, acetylcodeine, procaine and acetylprocaine were separated and quantitated in the "brown" heroin samples.

Separation and quantitation of the components of illicit heroin using reversed phase HPLC, was also described by Baker and Gough [24]. Using an amino-propyl bonded silica packing and a mixture of 85% acetonitrile and 15% 0.005 M tetrabutylammonium phosphate as the mobile phase, seven opiates and two adulterants were separated. The opiates morphine, 6-MAM, DAM, codeine, acetylcodeine were well separated but papaverine and noscapine were not resolved to baseline. In addition, tailing was observed in the morphine peak.

White et al. [37] reported a dual-column HPLC system for analysis of the opiate and sugar content of illicit heroin preparations. The analysis for morphine, 6-MAM, DAM, codeine and acetylcodeine was performed on a 25 cm x 4.9 mm internal diameter (ID) stainless steel column packed with irregular shaped silica with isooctane - diethyl ether - methanol - diethylamine - water (400:325:225:0.5:15) as eluent. However, morphine displayed a broad peak and tailing. The sugar analysis utilized a similar type of column (12.5 cm x 4.9 mm ID) with 75% acetonitrile, 25% water containing 0.01% (v/v) pentaethylenehexamine as eluent. Glucose, sucrose and lactose were separated and quantitated by this system.

Huizer et al. [111] described the analysis of heroin seizures in the Netherlands. A 5 µm-Lichrosorb Si 60 column was used with a mobile phase composed of diethyl ether - isooctane - methanol - diethylamine (52.8:35:12:0.2). Good baseline separation of 6-MAM, DAM, codeine, acetylcodeine and caffeine was obtained. Using the same column with n-hexane - dichloromethane - methanol with 0.75% v/v diethylamine (75:20:5) as eluent, Huizer (71) was also able to separate O^3-monoacetylmorphine (3-MAM), which is also found in uncut heroin. However, 6-MAM and codeine were unresolved by this system. More recently, Huizer [33] again utilized the same column and mobile phase and separated 6-MAM, DAM, acetylcodeine, papaverine and noscapine. Quantitation of these additional opiates gave better discrimination between illicit heroin samples than in the previous study [111]. Subsequently, studies were extended with flourimetric detection whereby acetylthebaol and thebaol were separated [33]. The excitation and emission wavelengths were experimentally found to be optimum at 260 nm and 400 nm respectively.

Recently, an improved reversed phase ion-pairing chromatographic method for the analysis of illicit heroin was developed by Lurie et al. [118]. Retention data relative to heroin and 220:254 nm absorbance ratios were reported for 46 adulterants or synthetic by-products in heroin samples. A Partisil 10 ODS-3 column was employed with a mobile phase consisting of 12% acetonitrile, 87% water, 1% phosphoric acid and 0.02 M methane sulfonic acid, adjusted to pH 2.2 with 2N sodium hydroxide. Separation of 6-MAM, DAM, acetylcodeine, noscapine was reported. Another system reported by the same worker [114] utilized a µBondapak C_{18} column and a mobile phase consisting of 40% heptane sulfonic acid in methanol (pH 3.5), 1% glacial acetic acid, 59% water. The system was able to separate morphine, codeine, thebaine, noscapine and papaverine. However, codeine and 6-MAM as well as DAM and acetylcodeine were unresolved.

Another reversed phase system was developed for acetylated thebaine, noscapine, morphine, codeine and norlaudanosine products by Lurie [127]. A C_{18} column was utilized with acetonitrile - methanol - phosphate buffer (pH 2.2) [25.2:13.4:61.4] as eluent. Acetylthebaol, acetylnornarcotine, meconin, acetylnorlaudanosine, diacetylnorcodeine and other by-products were separated by this system.

A HPLC system for heroin analysis on alumina by ion-exchange utilizing a mixed aqueous-organic mobile phase was described by Laurent et al. [41]. The mobile phase utilized was citric acid and tetramethylammonium hydroxide (0.01 M) at pH 6.5, in methanol - acetonitrile - water [12.5:12.5:7.5]. This system was able to separate morphine, DAM, acetylcodeine, but 6-MAM and codeine were unresolved.

Billiet et al. [20] reported separation and identification of illicit heroin samples by using an alumina and C_{18} coupled column system with UV detection at 254 nm. The mobile phase consisting of 28% methanol, 17% acetonitrile, 55% tetramethylammonium hydroxide (0.01 M) buffer at pH 6 was utilized. The alumina and C_{18} columns were used either in series or as individual columns by a switching system. The opiates separated by the switching system were morphine, DAM, codeine, acetylcodeine, papaverine and noscapine. By using the two columns in series, 6-MAM, DAM, acetylcodeine, papaverine, noscapine, acetylthebaol were separated.

In conclusion, the literature review on reported HPLC systems showed that the system reported by Billiet et al. [20] could separate to baseline the maximum number of opiates.

1.7.3 Gas Chromatography (GC)

Gas chromatography (GC) is one of the most frequently used techniques in illicit heroin analysis because it offers minimal sample preparation, high resolution and good sensitivity. Several recent papers on illicit heroin analysis by GC have been reported [18-19, 21-22, 25-28, 30-32, 34-36, 38, 42-43, 45-46, 57, 72, 130-138] and some are summarised in Table 1.4.

Moore and Bena [130] developed a rapid and accurate method for heroin in illicit preparations. A 3% OV-1 packed column with nitrogen as carrier gas, and a flame ionisation detector (FID) was used. An isothermal run at 255°C separated 6-MAM, DAM, papaverine. Another method was later developed by the same workers to determine and quantitate morphine, codeine and 6-MAM in illicit heroin with an electron capture detector (ECD) [27]. These three opiates were reacted with heptafluorobutyric anhydride (HFBA) rendering them suitable for ECD and quantitation. The fluorinated derivatives were extracted from acetonitrile - sodium bicarbonate solution into light petroleum in a rapid one-step extraction procedure. The derivatives were chromatographed on a 3% OV-17 packed column and morphine, codeine, 6-MAM were quantitated in heroin at levels as low as 0.001%, 0.01% and 0.01% respectively. Minimum detectable quantity reported was 20 pg for morphine, 80 pg for codeine and 100 pg for 6-MAM. The same worker also identified and quantified 3-MAM in illicit heroin samples in addition to these three opiates [28]. The same chromatographic conditions were used as reported earlier [27] and in addition, mass spectral characterization was done by GC-MS.

The content of morphine and 6-MAM in illicit heroin samples were determined by Machata et al. [43] by extractive propionylation in aqueous solution (disodium hydrogen phosphate buffer) with ethyl acetate as extractive solvent.

Table 1.4

Summary of the published GC systems on illicit heroin analysis

REFERENCE	DETECTOR	COLUMN	CARRIER GAS	DERIVATIZATION	OPIATES SEPARATED	SENSITIVITY	Analysis Time
CURRY 1970	GC-FID	3% CDMS P.C.	N_2	-	C, 6-MAM, DAM, ACC	-	15 min
DEZAN 1972	GC-FID	3% OV-1 P.C.	N_2	-	6-MAM, DAM	-	15 min
MOORE 1972	GC-FID	3% OV-1 P.C.	N_2	-	6-MAM, DAM, P	-	12 min
LIM 1978	GC-FID	3% OV-17 P.C.	N_2	-	C, DAM	-	16 min
MOORE 1978 (V147)	GC-ECD	3% OV-17 P.C.	N_2	+ (HFBA)	M, C, 6-MAM	M = 20 pg C = 80 pg 6-MAM = 100 pg	10 min
MOORE 1978 (V154)	GC-ECD	3% OV-17 P.C.	N_2	+ (HFBA)	M, C, 6-MAM, 3-DAM,	-	12 min
MACHATA 1980	GC-NPD	2.5% OV-1 P.C.	N_2	- (propionyla-tion)	6-MAM, DAM, ACC	-	-
GOUGH 1981	GC-FID	3% OV-210 P.C.	N_2	+ (Silyl 8)	M, C, 6-MAM, DAM, ACC	-	25 min
CHOW 1982	GC-MS	3% OV-1 P.C.	He	-	DAM, ACC	-	-
MARI 1982	GC-FID	2% OV-17 P.C.	N_2	-	6-MAM, DAM, ACC	-	14 min

REFERENCE	DETECTOR	COLUMN	CARRIER GAS	DERIVATIZATION	OPIATES SEPARATED	SENSITIVITY	Analysis Time
DEMEDTS 1982	GC-NPD	CP-Sil 5 C.C.	He	-	M, C, 6-MAM, DAM, ACC	Each opiate = 20 ng	20 min
NEUMANN 1982	GC-FID	SE-54 C.C.	H_2	+ (MSTFA)	AT, DANC, TANM, Me	-	30 min
LAW 1983	GC-FID	2.5% SE-30 C.C.	N_2	-	M, C, DAM, P	-	-
CHIAROTTI 1983	GC-FID	SE-54 C.C.	H_2	-	M, C, DAM, P, NAL	DAM = 0.2 NG	20 min
MOORE 1983	GC-ECD	OV-17 C.C.	He	+ (HFBA)	M, C, 6-MAM, 3-MAM, NC, NM, ANM, DANM	-	25 min
O'NEIL 1984	GC-FID	3% OV-210 P.C.	N_2	+ (Silyl 8)	M, C, 6-MAM, DAM, ACC, P	-	35 min
ALLEN 1984	GC-FID	DB-1 C.C.	H_2	+ (MSTFA)	AT, AL, AN, AAN	-	25 min
NEUMANN 1984	GC-FID	SE-54 C.C.	H_2	+ (MSTFA)	M, C, P, T, No, He, TL	-	30 min
NARAYANASWAMI 1985	GC-FID	3.8% UCW-98 P.C.	N_2	-	C, 6-MAM, DAM, ACC	-	-
NAIR 1986	GC-FID	3% OV-210 P.C.	N_2	-	C, 6-MAM, DAM, ACC, P, No	-	30 min
MOORE 1986	GC-ECD	DB-1 C.C.	H_2	+ (HFBA)	DANC, TANM, Me	-	30 min
NEUMANN 1987	GC-FID	OV-17 & SE-54	He	+ (MSTFA)	AT, AL, AN, AAN	-	25 min

Abbreviations

MSTFA = N-methyl-N-trimethylsilyl-trifluoroacetamide; HBFA = heptafluorobutyric anhydride; CDMS = cyclohexane-dimethanol succinate;

3-MAM = O^3-monoacetylmorphine; DANC = diacetylnorcodeine; TANM = triacetylnormorphine; NC = norcodeine; NM = normorphine; ANM = acetyl-normorhpine; DANM = diacetylnormorphine; AAN = acetylanhydronornarceine;

P.C. = packed column; C.C. = capillary column

Only the phenolic 3-OH-group of morphine reacts to 3-O-propionylmorphine, whereas 6-O-propionylmorphine cannot be prepared by this method. Using 2.5% OV-1 or SE-52 packed column and nitrogen-phosphorus detector (NPD), DAM, 3-propionylmorphine and 6-acetyl-3-propionylmorphine were separated and quantitated.

Gough and Baker [25] studied the suitability of several stationary phases for use in the separation and quantitation of morphine, codeine, 6-MAM, DAM, acetylcodeine with a FID. They reported that the best stationary phase was silanised OV-210 (trifluoropropyl-methyl silicone) in terms of ability to separate the five opiates, the reproducibility of retention times and losses of the compounds, particularly morphine which suffered adsorption losses. However the losses were reproducible and satisfactory quantitative data were obtained. Recently, O'Neil et al. [22] utilized these chromatographic conditions and quantitated the five opiates and papaverine in illicit heroin with a view to discriminate samples of different origin. Papaverine was also separated and quantitated without interference from the five opiates.

A mass spectrometry method in selected ion monitoring (SIM) mode was developed for the quantitative analysis of illicit heroin by Chow [137]. Ions m/z 369 and 327 of DAM and m/z 375 and 331 of deuterated DAM were used for monitoring and quantitation. Analysis was carried out with a 3% OV-17 packed column which separated DAM and acetylcodeine. Mari et al. [72] utilized the same column and FID, to separate 6-MAM, DAM, acetylcodeine in illicit heroin samples. In addition to these three opiates, Demedts et al. [42] separated morphine, codeine and adulterants in illicit heroin with the use of a fused silica capillary column CP-Sil 5 and NPD. However, 6-MAM and acetylcodeine were unresolved. The minimum detectable limit reported was 20 ng for the five opiates.

Law et al. [21] identified and quantified morphine, codeine, DAM, papaverine on a 2.5% SE-30 capillary column at 250°C with FID, and also 6-MAM and acetylcodeine after derivatization. Chiarotti et al. [26] reported the analysis of illicit heroin samples with a SE-54 capillary column and FID. The opiates separated and quantified were morphine, codeine, DAM, papaverine and nalorphine. However, 6-MAM and acetylcodeine were unresolved and the minimum detectable limit reported for DAM was 0.2 ng.

The same column and detector was utilized by Neumann et al. [36] to develop a method for analysis of trace impurities in illicit heroin samples. The trace impurities identified and separated were acetylthebaol, diacetylnorcodeine, triacetylnormorphine and meconin which were derivatized with N-methyl-N-trimethylsilyl-trifluoroacetaminde (MSTFA) prior to injection. The procedure was used to obtain chemical profiles

of illicit heroin samples. Using the same chromatographic conditions, Neumann et al. [138] investigated impurity profiles of opium and crude morphine samples. Morphine, codeine, thebaine, papaverine, noscapine, thebaol, meconin were identified and separated. Recently Neumann et al. [31] also reported a further study on trace impurities of illicit heroin samples. Two capillary columns SE-54 and OV-17 of different selectivity, were coupled by a switching device. The dual-oven GC was equipped with two FID. Trace impurities acetylthebaol, acetylnorlaudanosine, acetylnornarcotine, acetylanhydronornarceine were separated by this method. These trace impurities were also identified and separated in illicit heroin by Allen et al. [35] utilizing a FID and a fused silica capillary column, DB-1. After extraction from the bulk matrix, trace quantities of compounds such as narcotine and norlaudanosine and related impurities of illicit heroin were subjected to derivatization using MSTFA.

Moore [34] recently reported a preliminary study which utilized a fused silica capillary column, OV-17, with ECD that separated and quantitated the trace impurities found in illicit heroin, namely codeine, morphine, 6-MAM, 3-MAM, norcodeine, normorphine, 6-acetylnormorphine, 6-diacetylnormorphine. These heroin impurities were subjected to derivatization with HFBA and this was followed by a one-step extraction into an isooctane - ethyl ether mixed solvent. Determination of other neutral impurities such as diacetylnorcodeine, triacetylnormorphine, meconin, were also studied by Moore et al. [32] with an ECD and a fused capillary column, DB-1. The acetylated impurities were isolated from the bulk heroin matrix after which they were subjected to reduction with lithium aluminium hydride followed by derivatization with HFBA in the presence of pyridine. The resultant HFB electrophiles were detected on-column.

A 3.8% UCW-98 packed column with FID was utilized by Narayanaswami [18] to separate and quantitate codeine, 6-MAM, DAM, acetylcodeine in illicit heroin samples. In addition to these opiates, Nair et al. [19] separated and quantitated papaverine and noscapine using a 3% OV-210 packed column with FID in illicit heroin samples.

Among the GC systems reported, the system reported by Moore [34] gave the separation of maximum number of opiates.

It is clear from literature review that the published chromatographic methods were only able to separate a limited number of opiates. Therefore, in this study, three new chromatographic systems namely TLC, HPLC and GC systems were developed to separate and quantitate not only the major opiates, morphine, codeine, 6-MAM, DAM, papaverine, acetylcodeine, noscapine, thebaine but also the trace opiates and associated by-products namely narceine, nalorphine,

levallorphan, acetylthebaol which were reported to be present in some illicit heroin samples. These methods were then used to examine the chemical profile of 50 locally seized illicit heroin samples. The samples were subsequently grouped on the basis of their relative opiate compositions in order to determine whether such data would provide information relating to their origin.

Chapter 2

EXPERIMENTAL

2.1 Instruments

A. TLC System

A Shimadzu CS-920 High Speed TLC Scanner was used to scan developed TLC plates. The opiate standards were prepared in chloroform - methanol (9:1) solution and 5 µl samples were spotted manually on silica gel plates and developed in the solvent system, n-hexane - chloroform - triethylamine (9:9:4). The developed plates were scanned from 200 nm to 600 nm in order to determine the wavelengths of maximum signal response, λ_{max} for the opiates. However, for quantitation a wavelength of 254 nm was used for all of the opiates for ease of analysis.

B. HPLC System

A Waters (Milford, Mass., U.S.A.) HPLC equipped with Waters Model 510 HPLC pumps and U6K Universal Liquid Chromatograph Injector was used in combination with a Lambda-Max Model 481 UV detector. A Waters Automated Gradient Controller attached to the HPLC system was used for gradient elution and results were integrated by a Hewlett-Packard 3390 A Integrator.

A Lichrosorb RP-18 Column (250 x 4.0 mm I.D.) with packing material of particle size 7 µm was utilized [E. Merck, Darmstadt, F.G. Germany]. The opiate samples were dissolved in methanol and 10 µl of samples were injected manually. The mobile phase used was aqueous 1% triethylamine (adjusted to pH 6.0 with orthophosphoric acid) and methanol at a ratio 40:60 in an isocratic run. The flow rate of the mobile phase was set at 1.00 ml min^{-1} and the column was maintained at room temperature. The mobile phase was filtered three times prior to use through a 0.45 µm filter (D-3354 Dassel, West Germany). After use, the column was flushed with distilled water at a flow rate of 1.00 ml min^{-1} for two hours and stored in methanol - water (70:30) mixture, to maintain the efficiency of the column.

C. GC System

The GC analysis was carried out utilizing the following three systems:

1. a Hewlett-Packard HP 5880A Gas Chromatograph equipped with a flame ionization detector. The glass columns (6 ft x 0.22 mm I.D.) were packed with 3% OV-210, OV-101, OV-17, OV-3 on 100-120 mesh Chromosorb W. The carrier gas was nitrogen and results were integrated by a control keyboard HP 5880 A Level Four Integrator. The opiate samples were prepared in chloroform-methanol (9:1) solution and 1 µl samples were injected manually. Prior to GC analysis, the columns were conditioned overnight at 15-20°C below the maximum temperature of the stationary phase at a flow rate of 45 ml min^{-1} of carrier gas. Conditioning of GC columns is necessary in order to remove absorbed air, stationary phase impurities, and residual solvents from the coating operation. Using the columns prior to conditioning can result in excessive "bleed" of the stationary phase (or other volatile substances) into the detector which in turn can create high noise levels and reduced sensitivity.

2. a Hewlett-Packard HP 5890 Gas Chromatograph equipped with a Ion Trap Detector (ITD) [Series 700 Finnigan Mat] which is a universal and specific detector for capillary-column gas chromatography.

   The data acquiring system attached to the GC-ITD consisted of an IBM Computer XT with keyboard, video display monitor, fixed disc and printer m-1109 (Brother). The capillary column used was a crosslinked 5% phenylmethyl silicone gum phase, SE-54 (25 m x 0.22 mm ID with a 0.33 µm film thickness). Helium was used as the carrier gas at a flow of 0.50 ml min^{-1}. The opiate samples were dissolved in chloroform-methanol (9:1) and 1 µl samples were injected manually with a split ratio of 1:10.

3. a Hewlett-Packard HP 5890 Gas Chromatograph equipped with a Mass Selective Detector [5970 Series, HP]. A capillary column, crosslinked 5% phenyl- methylsilicone gum phase, SE-54 (25 m x 0.2 mm ID with a 0.33 µm film thickness) was used with helium as the carrier gas at a flow of 0.70 ml min^{-1}.

D. Infra Red Spectrophotometer [Acculeb I, Beckmann]

2.2 Chemicals and Materials

All chemicals and solvents were of analytical reagent grade and only methanol was purified further by distillation.

Acidified potassium iodoplatinate reagent was prepared by dissolving 0.25 g of platinic chloride and 5.0 g of potassium iodide in water and making the volume to 100 ml. 2 ml of concentrated hydrochloric acid was added to acidify the solution [44].

Pre-coated TLC plates were obtained from Merck (20 x 20 cm, aluminium backed; silica gel 60 GF_{254}, 0.2 mm thickness).

Local seized illicit heroin samples were provided by the Anti Drug Division of the Royal Malaysian Police.

2.3 Standards

Morphine, codeine, O^6-monoacetylmorphine, diacetylmorphine, acetylcodeine, thebaine, papaverine, noscapine, narceine, nalorphine,

levallorphan were obtained from the United Nations Division on Narcotic Drugs, Vienna. Acetylthebaol was prepared using the procedures of Allen et al. [139].

Preparation of Acetylthebaol

120 mg of thebaine was dissolved in 2 ml of acetic anhydride containing 80 mg of lithium chloride in a round bottomed flask. The solution was refluxed for 6 hours at 100°C in a water-bath. The solvent was evaporated in a rotary evaporator (10 mm Hg) at 70°C. The residue was dissolved in diethyl ether-dichloromethane (60:40) and washed successively with 1 M sodium carbonate, 0.25 M sulphuric acid and 1 M sodium carbonate. The organic layer was dried by using sodium sulphate and evaporated to an oil. The oil was dissolved in diethylether and chromatographed on a column packed with neutral alumina using diethyl ether as the eluent. Solvent removal from the eluent yielded the compound, acetylthebaol [Fig. 1.1]. Melting point, TLC, IR, GC-MSD, were performed to confirm the product and to determine its purity.

Results

Product: 40.5 mg (33.5%)

Melting point: 120-121°C [literature (140) : 118-122°C]

TLC: was performed on silica gel plates with benzene-acetone-methanol (7:2:1) as solvent system. Detection was accomplished by UV light. R_f value was 0.80 for acetylthebaol [literature (141) : 0.86].

IR (cm^{-1}): 1745, 1600, 1500, 1050 [Figure A1 in Appendix]

GC-MSD: m/z (relative abundance)

296 (31.8), 255 (17.2), 254 (10.0), 240 (10.6), 239 (2.9), 211 (13.3), 210 (8.9), 152 (11.3), 139 (13.7), 91 (2.6) [Figure A2 in Appendix]

The values were in accordance with reported values [141].

Chapter 3

RESULTS AND DISCUSSION

3.1 Thin Layer Chromatography (TLC)

A number of TLC systems have been reported for the screening of opiates in illicit heroin samples [section 1.7.1].

Nair and co-workers [19] evaluated 35 TLC systems for their ability to resolve the major opiates in illicit heroin without interference from five frequently used adulterants. Of the 35 systems evaluated, only three systems [63-65] could separate seven major opiates. The TLC system proposed by Kaistha et al. [63] and Steele [64] could resolve seven opiates, namely morphine, 6-MAM, DAM, codeine, acetylcodeine, papaverine and thebaine. The TLC system proposed by Budd [65] could also resolve seven opiates namely morphine, DAM, codeine, acetylcodeine, papaverine, thebaine and noscapine. Nair et al. [19] developed a TLC system which was capable of separating eight opiates, namely the above mentioned seven opiates and 6-MAM. Based on the ability to resolve the maximum number of opiates, these four potential systems [19, 63-65] and an additional system which was a modification of the system proposed by Budd [65], were selected and evaluated for the separation of the 12 opiate standards.

3.1.1 Solvent Systems

Table 3.1 lists the solvent systems of the five selected TLC systems.

Table 3.1: TLC Systems Evaluated for Opiate Analysis

TLC System No.	Solvent System	Reference
1	n-hexane-chloroform-triethyl-amine (9:9:4)	19
2	ethyl acetate-cyclohexane-methanol-ammonia solution (70:15:10:5)	63
3	dioxane-chloroform-ethyl acetate-ammonia solution (60:25:10:5)	64
4	n-hexane-chloroform-diethylamine (50:30:7)	65
5	n-hexane-chloroform-diethylamine (50:30:3.5)	

System 5 which was a modification of system 4 was constituted by reducing the volume of diethylamine in system 4, so as to obtain the same solvent strength as in system 1.

The solvent system parameter ε^o is defined by Snyder [142] as the adsorption energy per unit area of the solvent. The value ε^o for a ternary system [ε_{ABC}] can be obtained from the following equation which relates the ε^o values of pure components A, B and C of the ternary solvent system [ε_A, ε_B, ε_C] and the average value, n_b, of the areas of the molecules of A, B and C on the adsorbent surface:

$$\varepsilon_{ABC} = \varepsilon_A + \frac{\log N_A / \theta_A}{\alpha' n_b} \qquad [3\text{-}1]$$

α' is a constant that varies with adsorbent activity or water content ($0.6 < \alpha' < 1.0$), and the solvent strength is assumed to increase in the order A, B, C. N_A is the mole fraction of solvent A in the mobile phase and θ_A is the mole fraction of solvent A in the adsorbed phase. θ_A for a ternary solvent system is obtained by the following equation:

$$\theta_A = \frac{1}{1 + r_{AB}K_{AB}(1 + r_{BC}K_{BC})} \qquad [3\text{-}2]$$

where the equilibrium constants, K_{AB} and K_{BC} are defined by $K_{AB} = 10^{\alpha' n_b(\varepsilon_B - \varepsilon_A)}$, $K_{BC} = 10^{\alpha' n_b(\varepsilon_C - \varepsilon_B)}$

and the mole fraction ratios, r_{AB} and r_{BC} for a ternary system are given by $\frac{N_B}{N_A}$ and $\frac{N_C}{N_B}$ respectively.

In deriving equations [3-1] and [3-2], Snyder [143, 144] assumed that a Langmuir type equilibrium existed for the adsorption of the various pure solvent components A, B, C onto the adsorbent surface with formation of an adsorbed monolayer.

Table A1 in the appendix lists the necessary solvent properties required to calculate the solvent strength of systems 1, 4 and 5. The values given are for alumina but these values are generally similar to those for silica being about 0.8 fold smaller on the average [142]. According to Snyder [143], α' is generally between 0.6 - 0.7 for water deactivated adsorbents. Thus an average value of α' = 0.65 was selected. Table 3.2 shows the solvent strength for the three ternary systems which were calculated using equations [3-1] and [3-2].

Table 3.2

Average n_b, N_A, θ_A and ε_{ABC} Values for Ternary System 1, 4 and 5

TLC System No.	Average n_b	N_A	θ_A	ε_{ABC}
1	5.40	0.330	0.0190	0.374
4	5.97	0.464	0.0148	0.408
5	5.97	0.484	0.0206	0.375

The retention factor (R_f) values obtained for all the 12 opiates by the five solvent systems are tabulated in Table 3.3.

Solvent system 1 was able to resolve all the 12 opiates (Table 3.3). System 2 and 3 were unable to separate levallorphan, noscapine and acetylthebaol. System 4 could not separate narceine, morphine and nalorphine in addition to codeine and 6-MAM. Although system 5 has nearly the same solvent strength as system 1, it was unable to separate narceine, morphine and nalorphine, codeine from 6-MAM and thebaine from papaverine (Table 3.2). Therefore, the addition of triethylamine provided the selectivity for the separation of the 12 opiates although both triethylamine and diethylamine belong to the same selectivity group (Group I) in Snyder's solvent classification [142].

Figure 3.1 shows a thin layer chromatogram of the 12 opiates in a mixture using system 1.

All the opiates were first located under UV light and subsequently identified on the basis of their Rf values and the colour produced with acidified iodoplatinate as the spray reagent (Table 3.4).

Table 3.3

R_f Values of the Opiates

Opiates	R_f x 100				
	System 1	System 2	System 3	System 4	System 5
1. Narceine	0.0	0.0	0.0	0.0	0.0
2. Morphine	2.9	12.6	2.1	0.0	0.0
3. Nalorphine	5.1	32.1	7.1	0.0	0.0
4. Codeine	13.2	25.2	44.7	14.1	7.7
5. 6-MAM	23.6	39.6	54.6	14.1	7.7
6. DAM	29.3	42.7	57.8	40.1	24.6
7. Acetylcodeine	35.9	50.9	64.1	45.8	29.6
8. Thebaine	39.9	47.8	61.7	52.8	35.9
9. Papaverine	42.5	62.9	67.4	48.9	35.9
10. Levallorphan	51.4	71.0	71.6	31.0	14.8
11. Noscapine	54.1	71.0	71.6	54.0	42.3
12. Acetylthebaol	57.2	71.0	71.6	60.6	52.8

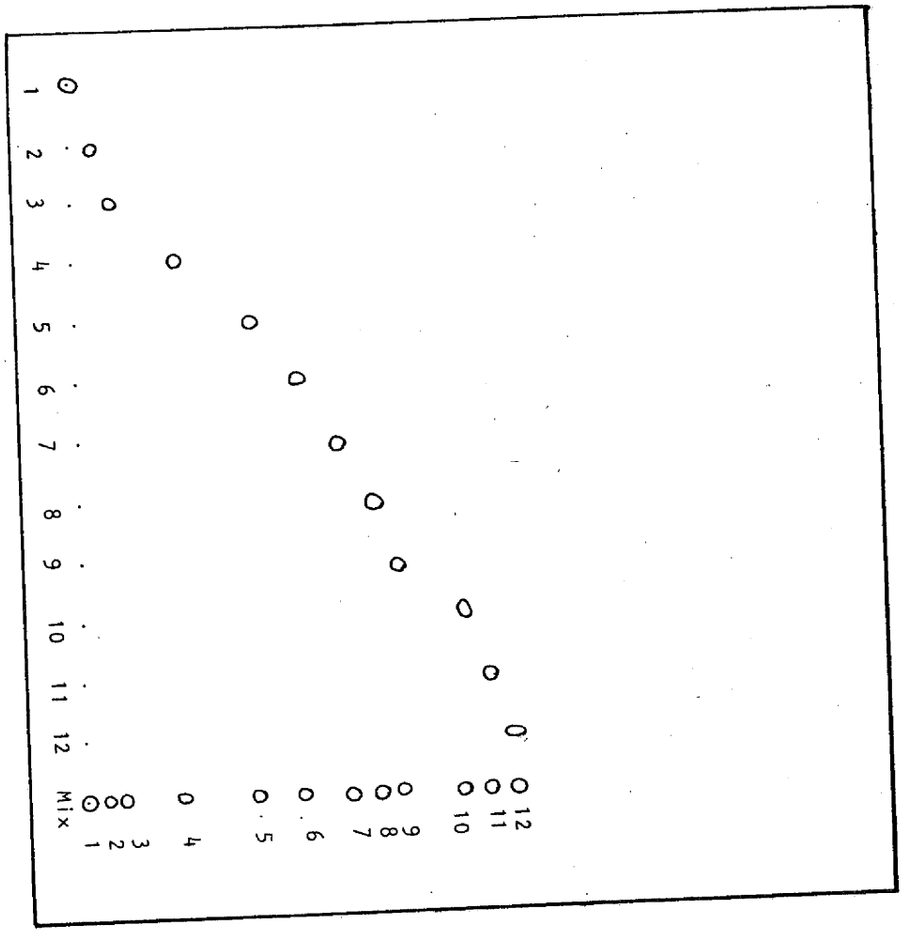


Fig. 3.1: Thin-layer chromatogram developed using system 1 for a mixture of 12 opiates
1 = narceine, 2 = morphine, 3 = nalorphine, 4 = codeine, 5 = 6-MAM, 6 = DAM, 7 = acetylcodeine, 8 = thebaine, 9 = papaverine, 10 = levallorphan, 11 = noscapine, 12 = acetylthebaol

Table 3.4

Colour Reactions of Opiates With Acidified Iodoplatinate Spray Reagent

Compounds	Colour
Narceine	Blue
Morphine	Blue
Nalorphine	Blue
Codeine	Blue
6-MAM	Dark blue
DAM	Dark blue
Acetylcodiene	Purple
Thebaine	Red brown
Papaverine	Purple brown
Levallorphan	Brown
Noscapine	Purple
Acetylthebaol	Brown

The order of separation of the 12 opiates by system 1 together with the pK_b values [7, 146-147] of most of the opiates are given in Table 3.5.

Table 3.5

Order of Separation of the 12 Opiates by TLC System 1

Compounds		pK_b
Narceine		4.7
Morphine		4.1
Nalorphine		6.2
Codeine		5.8
6-MAM	increasing	6.0
DAM	R_f	6.4
Acetylcodeine	Value	-
Thebaine		5.8
Papaverine		7.6
Levallorphan		7.1
Noscapine		7.8
Acetyltheboal		-

The mobile phase in system 1 consisted of n-hexane-chloroform-triethylamine (9:9:4). Triethylamine (pK_b = 3.36) being a basic solvent competes with the basic solutes for the active silanol sites on the silica surface. The basic compounds will tend to form hydrogen bonds with the surface silanol groups which functions as a Lewis acid. Thus if secondary effects are neglected, the stronger the basicity the stronger will be the bonding and the lower the Rf value.

The molecular structures of the 12 opiates (Figure 1.1) suggest that they can be classified into two groups based on their frame structure. Morphine, nalorphine, codeine, 6-MAM, DAM, acetylcodeine, thebaine, levallorphan and acetylthebaol all possess the phenanthrene frame structure, whereas narceine, papaverine and noscapine belong to the benzylisoquinoline alkaloid group.

In the phenanthrene group, acetylthebaol is the only compound without a tertiary amine group and therefore would be expected to yield the highest Rf value. The order of separation in terms of increasing Rf values which is to be expected on the basis of the pK_b values of the compounds in the phenantherene group (Table 3.5) is morphine, codeine and thebaine (unresolved), 6-MAM, nalorphine, DAM, levallorphan and acetylthebaol. The order of separation observed was similar to the predicted order except thebaine and nalorphine showed an inverted order of separation. Subsequently, codeine and thebaine which were predicted to be unresolved, however were resolved in the observed order. Although the pK_b value for acetylcodeine was unavailable in literature, it would be a weaker base in comparison to codeine and consequently yield a higher Rf value.

In the benzylisoquinoline group, the predicted order of separation in terms of increasing Rf values based on the pK_b values is narceine, papaverine and noscapine and this was also the observed order.

3.1.2 Quantitative Analysis

A high speed TLC scanner was used for quantitation. In-situ reflectance measure units yielded area readings proportional to the concentrations of the opiates between the range of 30 to 1000 $\mu g\ ml^{-1}$.

A volume of 5 μl of each mixture of 12 opiates with concentrations 1000, 500, 250, 125, 62, 30 $\mu g\ ml^{-1}$ were spotted on silica gel plates and developed using system 1 (Section 3.1.1). The spot area counts at wavelength 254 nm at various concentrations is given in Table A2 in appendix. The average of four readings of spot area counts were calculated for each concentrations and standard calibration curves were obtained for each opiate [Figure 3.2a - 3.2c]. A linear

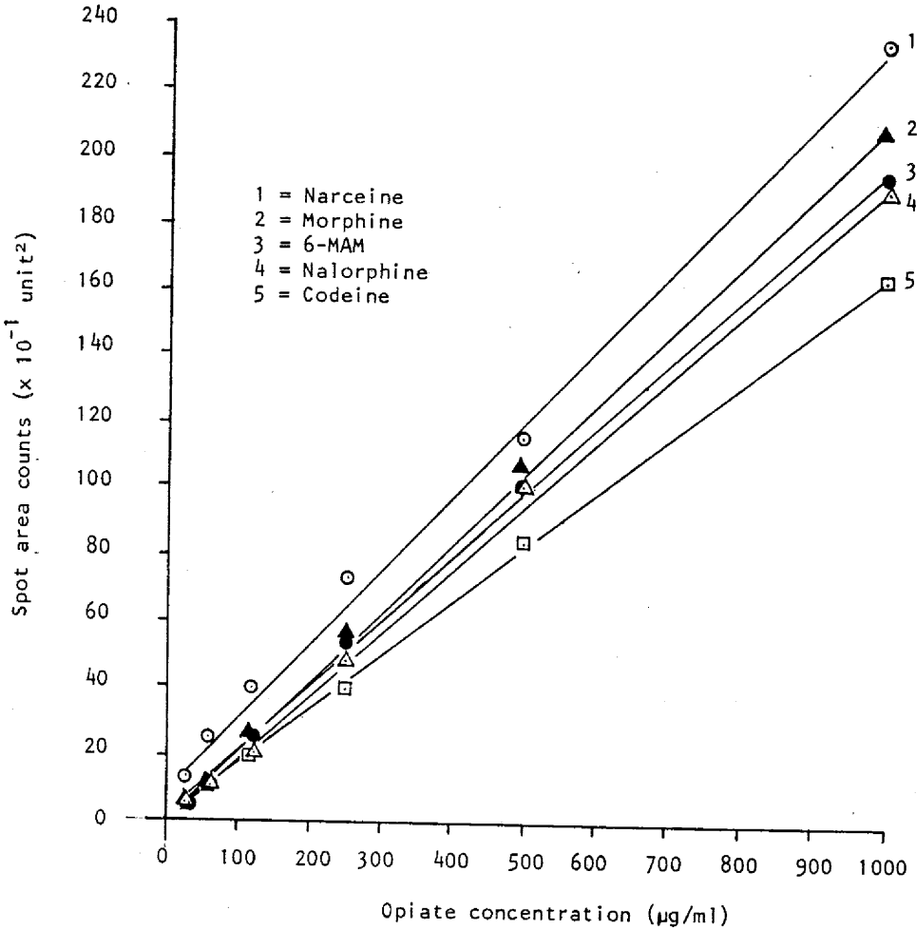


Fig. 3.2a: Calibration graph of narceine, morphine, 6-MAM, nalorphine and codeine using the TLC system

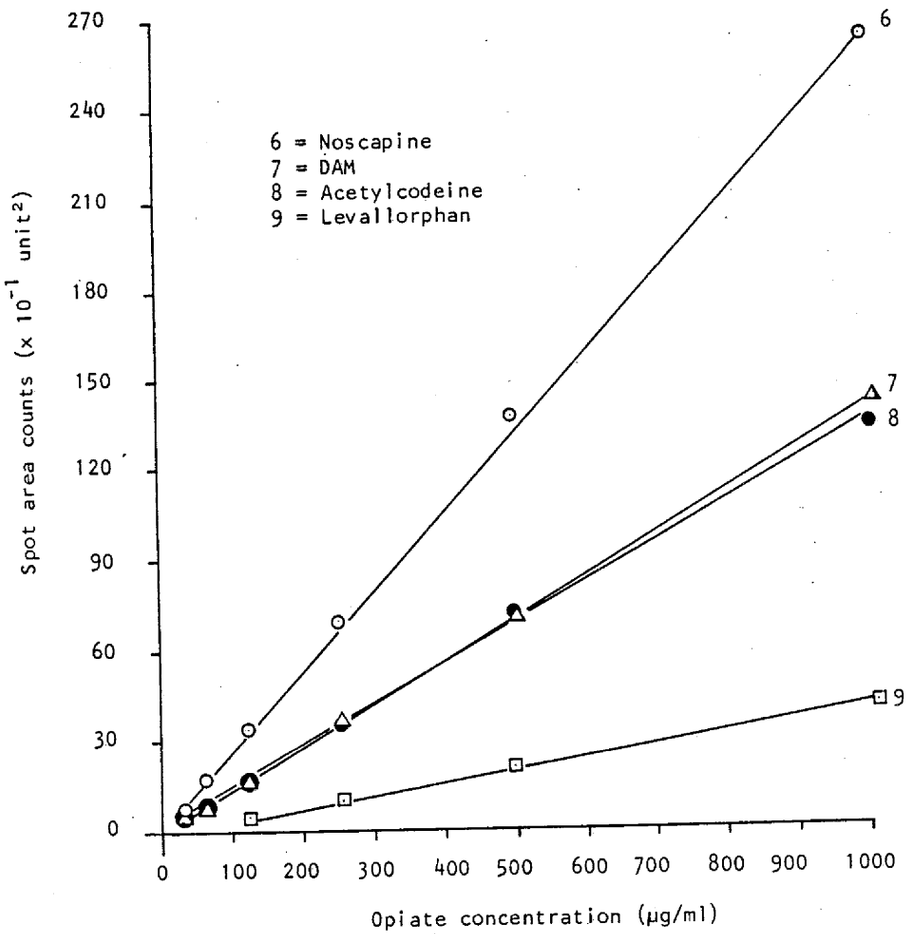


Fig. 3.2b: Calibration graph of DAM, acetylcodeine, levallorphan and noscapine using the TLC system

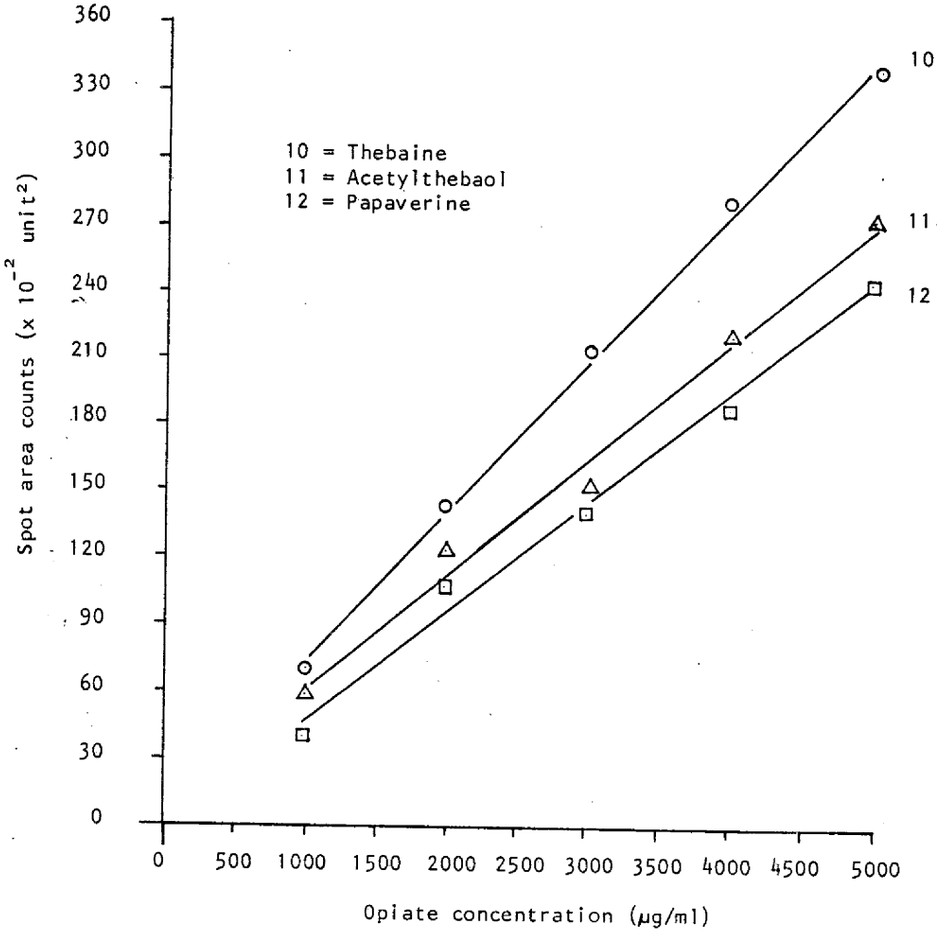


Fig. 3.2c: Calibration graph of thebaine, papaverine and acetylthebaol using the TLC system

correlation existed within the range of 30 to 1000 µg ml^{-1} for all the 12 opiates with an average coefficient of regression of 0.997. All the samples were chromatographed on the same TLC plate in order to minimize the variation in chromatographic conditions.

The limit of quantitation by the TLC Scanner for each opiate standard at 254 nm is shown in Table 3.6.

However, earlier workers [19] reported the limit of detection by UV light (254 nm) and spray reagents of the eight opiates namely morphine, codeine, 6-MAM, DAM, acetylcodeine, thebaine, papaverine and noscapine in a mixture as 0.1 µg with TLC system 1. Marumo et al. [97] reported the limit of quantitation for morphine, 6-MAM, DAM, codeine and acetylcodeine as 1 µg. Limits of detection of opiates for other TLC systems in the literature (Section 1.7.1) have not been reported.

Table 3.6

Densitometer Limits of Quantitation for 12 Opiates with TLC System 1

Opiates	λ_{max}(nm)	Minimum Amount Quantitated (μg)	
		λ_{max}	λ = 254 nm
Narceine	290	0.005	0.2
Morphine	250	0.16	0.2
Nalorphine	230	0.02	0.2
Codeine	250	0.16	0.2
6-MAM	230	0.16	0.2
DAM	290	0.16	0.2
Acetylcodeine	250	0.08	0.2
Thebaine	300	0.02	5.0
Papaverine	260	0.002	5.0
Levallorphan	290	0.16	0.7
Noscapine	320	0.08	0.2
Acetylthebaol	260	0.003	5.0

3.2 HPLC

A number of HPLC systems have been reported for the analysis of opiates in illicit heroin samples in the literature [Section 1.7.2]. Of the HPLC systems reported, reverse-phase (RP) mode has been most widely used [20, 24, 122-129]. Billiet et al. [20] separated 7 opiates namely morphine, codeine, DAM, papaverine, noscapine, acetylcodeine and acetylthebaol by using an alumina and C_{18} coupled column system. Baker and Gough [24] also used RP mode and reported separation of 7 opiates namely morphine, codeine, 6-MAM, DAM, acetylcodeine, papaverine and noscapine. However, poor baseline resolution was obtained for papaverine and noscapine. Since RP mode has given the separation of maximum number of opiates in literature, this mode was selected for the separation of the 12 opiate standards. Furthermore, RP offers several advantages. For example, the selectivity is mainly controlled by eluent effects. Another advantage is that the stationary phase is quite stable since it is chemically bonded to the support.

The resolution, R of a compound in a mixture by HPLC is given by the following equation:

$$R = \frac{1}{4}(\alpha - 1) \cdot \left[\frac{k'}{(1 + k')}\right] \sqrt{N} \qquad [3\text{-}3]$$

where α is the selectivity factor, k' is the capacity factor and N is the column efficiency. The selectivity factor, α, is defined by equation [3-4]:

$$\alpha = \frac{k'_2}{k'_1} \qquad [3\text{-}4]$$

for peaks 1 and 2. [It is assumed that the two peaks elute close together and that $k'_2 > k'_1$].

The capacity factor, k', is the ratio of the average time of a compound spent in the stationary phase to the average time in the mobile phase. The k' value of a compound in a chromatogram is determined by the following equation:

$$k' = \frac{t_R - t_o}{t_o} \qquad [3\text{-}5]$$

k' is thus equal to the ratio of the difference between the dead time, t_o, and the peak retention time, t_R, to the dead time. The dead time, t_o is related to the column length, L and the linear flow velocity, u, by

$$t_o = L/u \qquad [3\text{-}6]$$

The factor k', which is mainly controlled by the mobile phase solvent strength produces the largest effect on the resolution. For small k' values, the resolution increases rapidly, while $k' > 10$ results in long analysis time and broadening of peaks, which makes detection more difficult. The accepted values for k' in terms of resolution, analysis time and peak detection are in the range $1 \leqslant k \leqslant 10$.

Water, methanol and triethylamine (TEA) was used as the mobile phase with an octadecylsiloxane bonded phase (C_{18}) column as the stationary phase in the developed RP-HPLC system. A UV detector was used at 280 nm since and this was the most frequently used wavelength in the reported HPLC systems (section 1.7.2).

3.2.1 Optimization of the Mobile Phase System

The optimization of the mobile phase system for the separation of 8 opiates was achieved by systematically varying in sequence, the mobile phase composition, the pH of the aqueous composition and the percentage of the basic modifier, TEA, in the aqueous composition of the mobile phase.

For the optimization of the mobile phase composition, the percentage of methanol [B] in the mobile phase was varied in the range 45% to 62% B. The aqueous phase contained 1% TEA and was adjusted to pH 6 with orthophosphoric acid [A]. The flow rate of the mobile phase was set at 1.00 ml min^{-1} and maintained at room temperature.

Table 3.7 gives the capacity factor, k′, and selectivity factor, α, values obtained for the 8 opiates using equation [3-4] and [3-5]. With reference to the table, k′ values of the opiates obtained were in the optimum range $1 < k' < 10$ at B greater than 58%. At 58% B, thebaine and noscapine were unresolved ($\alpha = 1$). At 60% B, all the 8 opiates were separated with good baseline resolution with α values greater than 1.193. At 61 and 62% B, DAM and papaverine were partially resolved ($\alpha = 1.144$ at 62% B). Figure 3.3 shows the plots of k′ values versus %B. The plot for all the 8 opiates showed a similar trend in that k′ values decreased as %B increased from 45 to 62%. As the %B increased in the mobile phase, the polarity of the mobile phase decreased. Therefore the compounds were less strongly retained by the hydrocarbonaceous stationary phase and hence they eluted much faster with consequent decrease in k′ values.

For the optimization of pH, the pH of the aqueous component of the mobile phase which consisted of 1% TEA in water was varied from pH 4.5 to 6.0 using orthophosphoric acid. The percentage of methanol was fixed at 60% and the flow-rate of the mobile phase was set at 1.00 ml min^{-1}. Table 3.8 gives the k′ and values obtained for the range of pH 4.5 to 6.0. The k′ values for all values of pH fall within the range $0.13 < k' < 7.55$. All the opiates were separated with good baseline resolution only at pH 6.0 ($0.67 < k' 6.71$). Figure 3.4 shows the plots of k′ values versus pH. All the plots showed the same trend in that k′ values increased as pH increased. This effect was to be expected for basic compounds such as the opiates since the dissociation of the compounds decreased as the pH increased. The ionized molecules were distributed preferentially into the aqueous or more polar phase whereas the undissociated or unionized form of these compounds was less water soluble and consequently more strongly retained

Table 3.7

Capacity factor (k′) and selectivity factor (α) values with increasing percentage of methanol

PERCENTAGE METHANOL	45		50		55		58		60		61		62	
OPIATE	k′	α	k′	α	k′	α	k′	α	k′	α	k′	α	k′	α
1. MORPHINE	0.84	-	1.15	-	0.98	-	0.83	-	0.67	-	0.65	-	0.77	-
2. 6-MAM	1.91	2.274	2.10	1.826	1.68	1.714	1.33	1.602	1.06	1.582	1.02	1.569	1.15	1.494
3. CODEINE	2.51	1.314	2.78	1.324	2.27	1.351	1.88	1.414	1.56	1.472	1.49	1.461	1.65	1.435
4. DAM	7.32	2.916	6.52	2.345	4.45	1.960	3.33	1.771	2.61	1.673	2.44	1.638	2.57	1.558
5. PAPAVERINE	9.66	1.320	9.34	1.433	6.24	1.402	4.44	1.333	3.17	1.215	2.85	1.168	2.94	1.144
6. ACETYLCODEINE	9.66	1.00	9.34	1.00	6.90	1.106	5.57	1.255	3.89	1.277	3.67	1.288	3.86	1.313
7. THEBAINE	14.50	1.501	13.85	1.483	14.46	2.096	9.40	1.688	4.64	1.193	4.44	1.210	4.72	1.223
8. NOSCAPINE	14.50	1.00	13.85	1.00	14.46	1.00	9.40	1.00	6.71	1.446	6.00	1.351	6.02	1.275

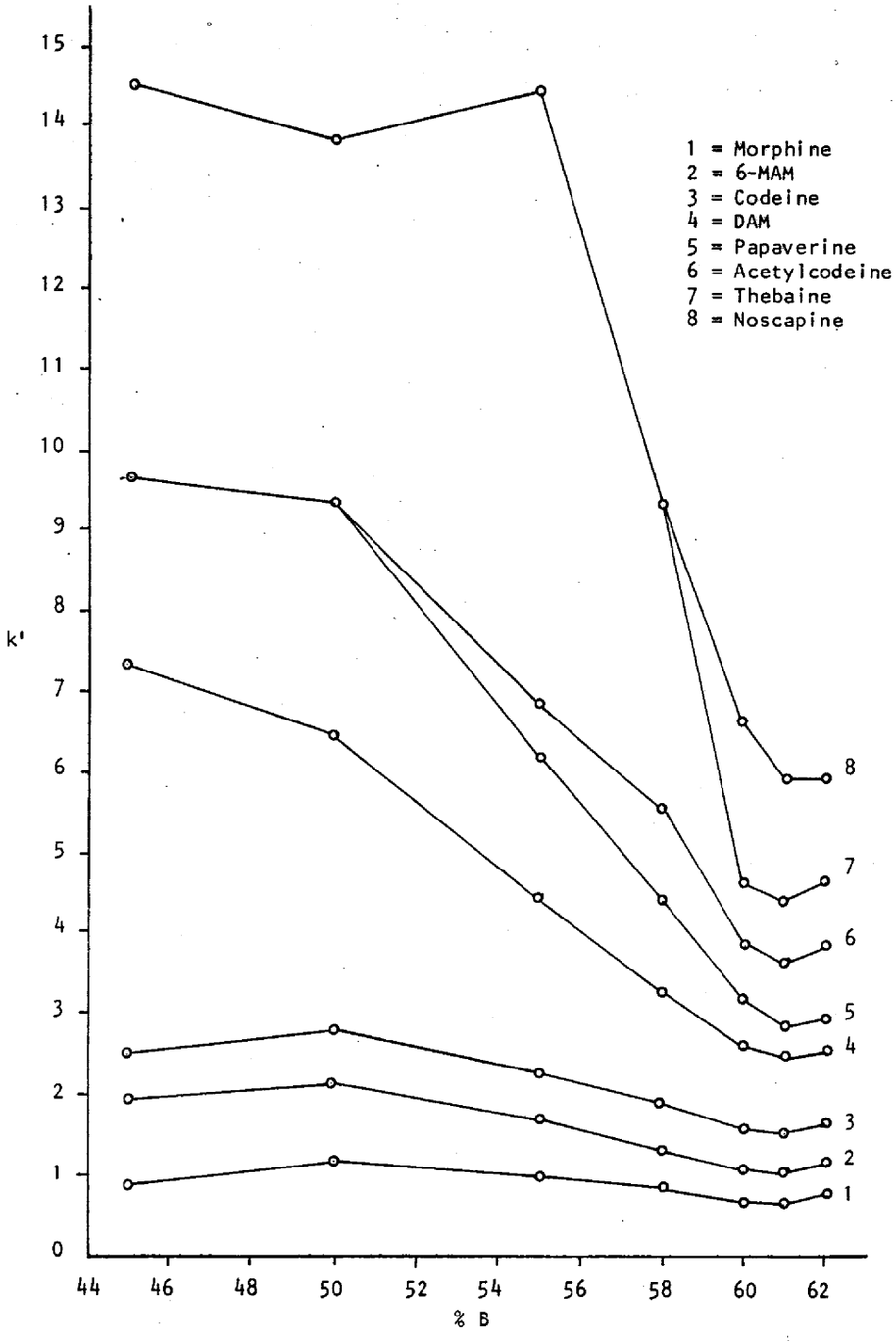


Fig. 3.3: Plots of capacity factor (k') versus percentage methanol (%B) in the mobile phase; at pH 6

Table 3.8

Capacity factor (k') and selectivity factor (α) values with increasing pH of the aqueous mobile phase

	pH	4.5		4.8		5.5		5.7		5.8		6.0	
	OPIATE	k'	α	k'	α	k'	α	k'	α	k'	α	k'	α
1.	MORPHINE	0.13	-	0.46	-	0.49	-	0.54	-	0.74	-	0.67	-
2.	6-MAM	0.26	2.00	0.54	1.174	0.80	1.633	0.88	1.630	1.16	1.568	1.06	1.582
3.	CODEINE	0.41	1.577	0.68	1.259	1.08	1.35	1.27	1.433	1.64	1.414	1.56	1.472
4.	DAM	0.89	2.171	1.08	1.588	2.04	1.889	2.35	1.850	2.89	1.762	2.61	1.673
5.	PAPAVERINE	0.89	1.00	1.20	1.111	3.11	1.525	3.34	1.421	3.68	1.273	3.17	1.215
6.	ACETYLCODEINE	2.75	3.090	3.03	2.525	3.11	1.00	3.34	1.00	3.68	1.00	3.89	1.277
7.	THEBAINE	2.75	1.00	3.03	1.00	3.72	1.196	3.64	1.090	4.57	1.242	4.64	1.193
8.	NOSCAPINE	4.77	1.735	5.63	1.858	7.41	1.992	6.99	1.920	7.55	1.652	6.71	1.446

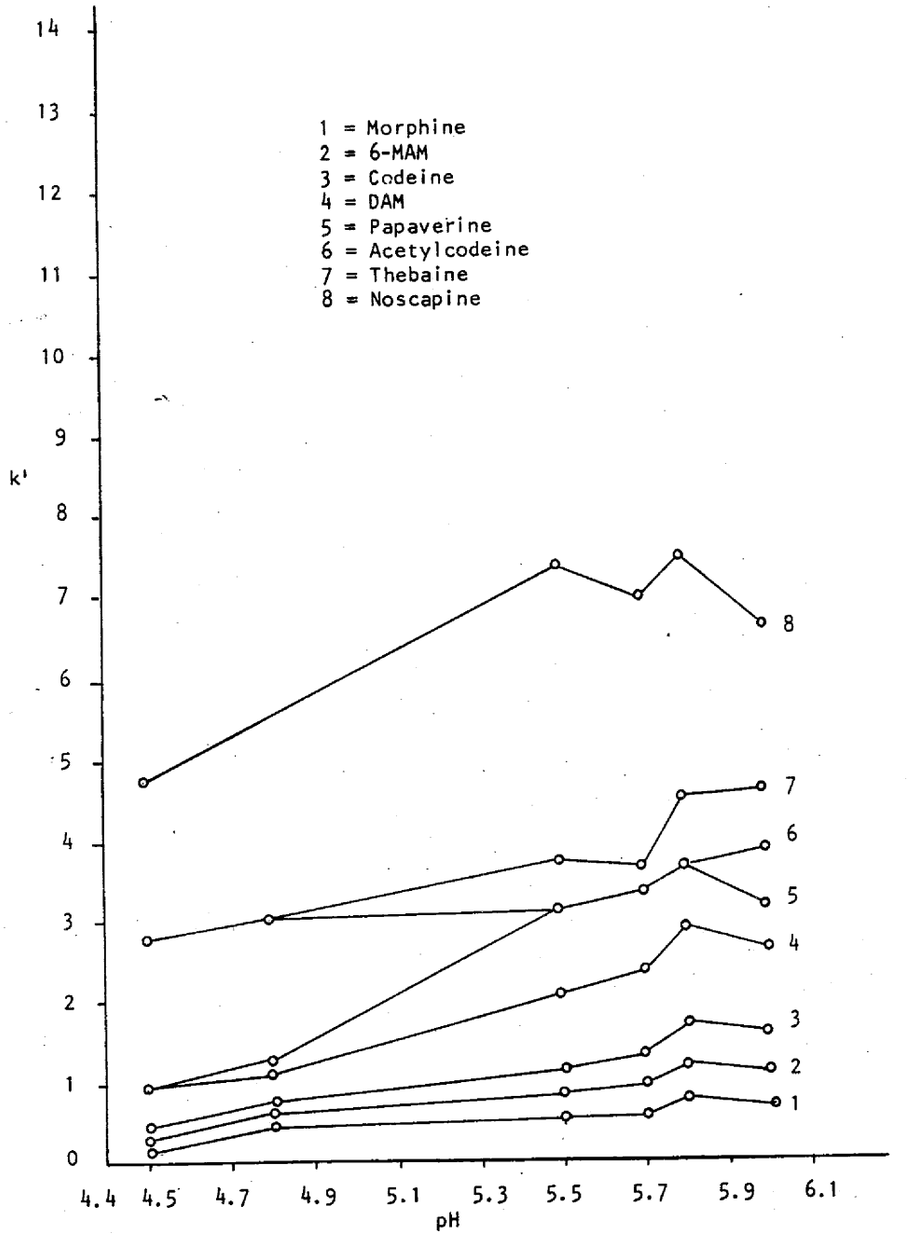


Fig. 3.4: Plots of capacity factor (k') versus pH of aqueous component of mobile phase; at 60% B (methanol)

by the stationary phase. As the pH increased, more unionized form of the compounds were formed which caused the compounds to elute more slowly and hence k' values increased.

For the optimization of TEA in the aqueous component of the mobile phase, the percentage was varied from 0.5 to 2.0% and adjusted to pH 6 with orthophosphoric acid. The percentage of methanol was fixed at 60% and the flow rate of the mobile phase was set at 1.00 ml min^{-1}. Table 3.9 gives the k' values and α values obtained for all the 8 opiates for the range 0.5 to 2.0% TEA. The k' values obtained were in the range $0.56 < k' < 7.32$. At 1% TEA, all the 8 opiates were separated with good baseline resolution.

Figure 3.5 shows the plots of k' values versus % TEA in water. The general trend shown by all the plots is that as the % TEA increased from 0.5 to 1.0%, the k' values decreased. However, as the % TEA increased from 1.0 to 2.0%, there was no appreciable change in the k' values. Furthermore, in the absence of TEA in the mobile phase, none of the compounds were eluted. These observations can be explained if it is assumed that a high proportion of unreacted silanol groups were still present in the stationary phase [142, 148-149]. These residual unreacted silonal groups were therefore available for interaction with solutes in a manner similar to that in normal-phase chromatography (NPC). Hence, a dual retention mechanism could occur on such stationary phases. Residual unreacted silanol groups are of particular importance when designing chromatographic separations involving basic compounds such as opiates since such compounds can also interact with the silanol groups and become retained on the surface [150-151]. Such heteroenergetic retention gives rise to peak tailing and poor reproducibility. Thus, TEA was added as a basic modifier to the mobile phase in order to reduce silanophilic interactions between the solutes and the stationary phase. This reduction in silanophilic interactions could account for the decrease in k' values as the percentage of TEA increased from 0 (total retention) to 1%. As the percentage of TEA increased from 1% to 2%, there was no appreciable change in the k' values. This could suggest that masking of the available surface residual silanol groups were complete with 1% TEA.

The optimized mobile phase system which consisted of 1% TEA in water (adjusted to pH 6 with orthophosphoric acid) and methanol in the ratio 40:60 at flow rate of 1.00 ml min^{-1}, gave good baseline resolution of the major 8 opiates namely morphine, 6-MAM, codeine, DAM, papaverine, acetylcodeine, thebaine and noscapine. Acetylthebaol was also resolved without interference from the other opiates with this optimized mobile phase. However, narceine, nalorphine and levallorphan were not separated by this sytem as they coeluted with morphine, codeine and DAM respectively. Figure 3.6 shows a HPLC chromatogram for a mixture of the 9 opiates with the optimized HPLC system.

Table 3.9

Capacity factor (k′) and selectivity factor (α) values with increasing percentage of triethylamine

PERCENTAGE TRIETHYLAMINE	0.5		1.0		2.0	
OPIATE	k′	α	k′	α	k′	α
1. MORPHINE	0.86	-	0.67	-	0.56	-
2. 6-MAM	1.38	1.605	1.06	1.582	0.92	1.643
3. CODEINE	2.02	1.464	1.56	1.472	1.30	1.413
4. DAM	3.50	1.733	2.61	1.673	2.34	1.800
5. PAPAVERINE	3.50	1.00	3.17	1.215	3.39	1.499
6. ACETYLCODEINE	5.29	1.511	3.89	1.277	3.65	1.077
7. THEBAINE	6.52	1.233	4.64	1.193	4.55	1.247
8. NOSCAPINE	7.32	1.123	6.71	1.446	7.02	1.543

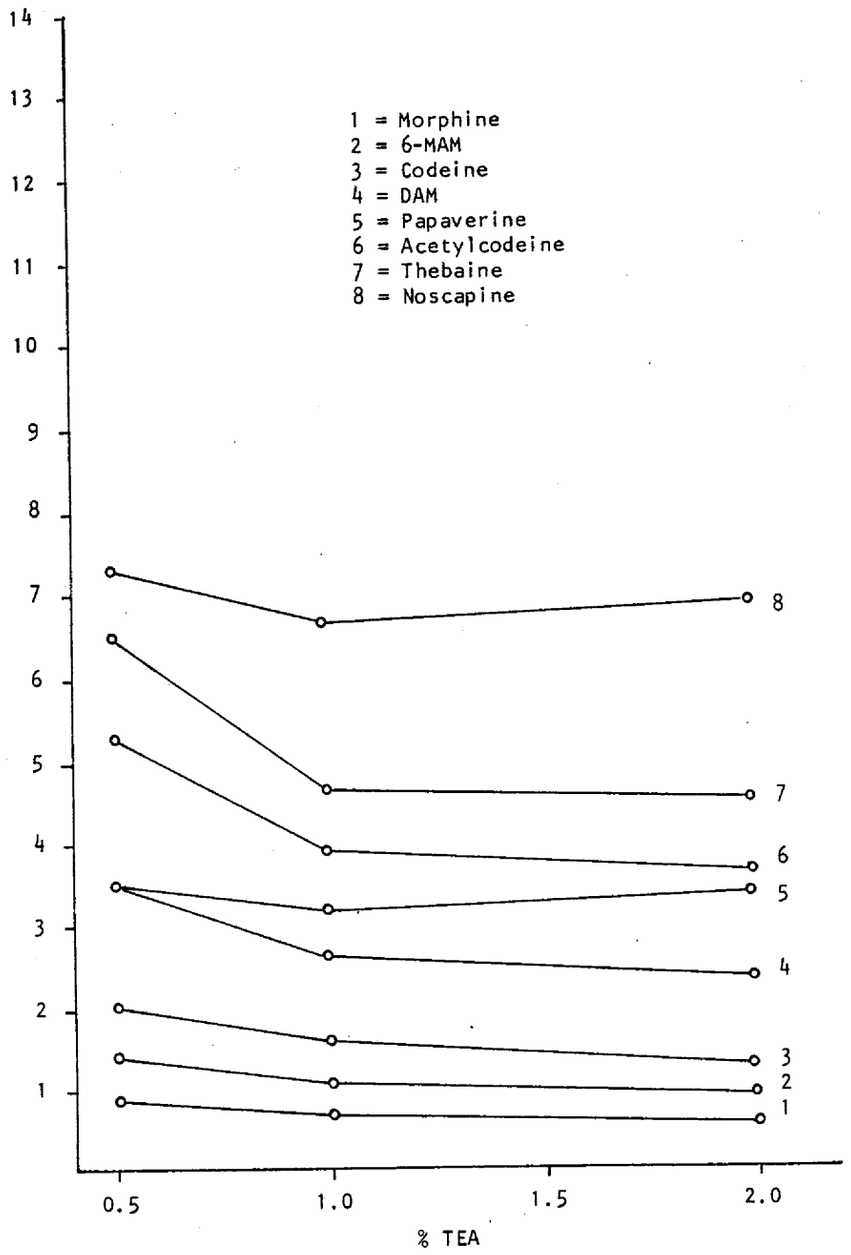


Fig. 3.5: Plots of capacity factor (k') versus percentage TEA (% TEA) in aqueous component of mobile phase; at pH 6 and 60% B

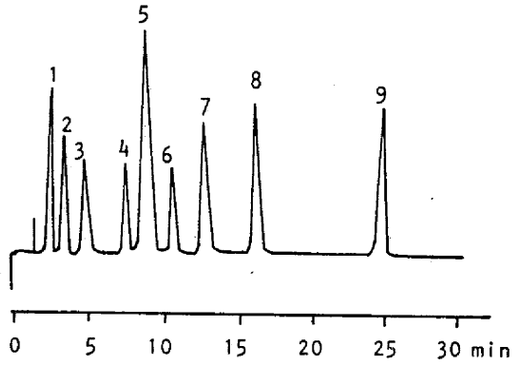


Fig. 3.6: HPLC chromatogram of a mixture of 9 opiates

(1 = morphine, 3.80 min; 2 = 6-MAM, 4.70 min; 3 = codeine, 5.83 min; 4 = DAM, 8.23 min; 5 = papaverine, 9.50 min; 6 = acetylcodeine, 11.16 min; 7 = thebaine, 12.87 min; 8 = noscapine, 17.57 min; 9 = acetylthebaol, 25.10 min)

Chromatographic Conditions:

Column:	250 x 4.00 mm I.D., 7 um RP-18 reverse phase material (Lichrosorb)
Mobile phase:	[A] 1% TEA in water (adjusted to pH 6 by orthophosphoric acid
	[B] 60% methanol
Flow rate:	1.00 ml min^{-1}
Column temperature:	ambient
Detector:	UV (280 nm)

When 1% TEA in the optimized mobile phase was substituted with 1% diethylamine, baseline resolution was not obtained for DAM and papaverine and peak tailing was also observed. This indicated that the function of TEA in the mobile phase could not be solely attributed to the masking of the silanol groups since both DEA and TEA should have similar capabilities to penetrate and interact with the silanol groups both in terms of size and nature of interaction. Thus, it is reasonable to infer that interactions of the amine modifier in the mobile phase also contribute to resolution and selectivity.

3.2.2 Order of Elution

The separation mechanism in reverse phase chromatography (RPC) with bonded hydrocarbon phases is still not fully understood. Three major separation mechanisms have been proposed [152].

The first mechanism postulates that partitioning of the solutes occur between the two liquid phases (the mobile and stationary phase) in which the solutes have different distribution coefficients. Kirkland and DeStefano [153] favoured this mechanism because they observed that the capacity factor, k' increased with increasing amount of bonded material while the specific surface area remained constant. Locke [154] considered the stationary phase as an inert sponge and as a consequence suggested that the thermodynamic activity of the eluent inside the gel was different from that of the bulk liquid in the interstitial volume.

The second type of mechanism was proposed by Knox and Pryde [155]. The organic modifier in the aqueous mobile phase is assumed to be preferentially adsorbed on the stationary phase. Partition of the solute molecules takes place between the mobile phase and the new "mixed" stationary phase. Thus, the role of the modified silica gel is simply to provide a surface on which the stationary phase is coated.

The third mechanism has been investigated by a number of workers [156-161]. These workers proposed that solute retention was primarily due to the formation of reversible complexes by solvophobic interactions between the solute molecules and chemically bonded moieties at the surface of silica. The interaction of solute molecules (S) with the hydrocarbonaceous ligands (L), at the surface of the stationary phase in solvophobic chromatography is considered a reversible association and the solute retention is governed by the equilibrium:

$$S + L \rightleftharpoons SL$$

where the complex, SL is assumed to be formed by solvophobic interactions and the process is characterized by the equilibrium constant, K, which is defined by $K = \frac{[SL]}{[S][L]}$

They suggested that two factors governed the chromatographic process in RPC. Firstly, the greater the lipophilic character of a compound, the longer the retention and secondly the smaller the lipophilic character of the mobile phase, the longer the retention.

The order of elution of the 9 opiates is given in Table 3.10.

Table 3.10

Order of Elution of 9 Opiates by the HPLC System

No.	Opiates	
1.	Morphine	increasing retention time ↓
2.	6-MAM	
3.	Codeine	
4.	DAM	
5.	Papaverine	
6.	Acetylcodeine	
7.	Thebaine	
8.	Noscapine	
9.	Acetylthebaol	

The order of elution can be explained by the third mechanism. The polarity of the functional groups of the opiates were used to obtain an estimate of the lipophilic character of the compounds. The order predicted in terms of increasing retention time for the 9 compounds in the phenantherene group (Section 3.1) on the basis of increasing lipophilic character was morphine, 6-MAM, nalorphine, codeine, levallorphan, DAM, acetylcodeine, thebaine and acetylthebaol. This was also the order observed except that nalorphine and codeine as well as levallorphan and DAM coeluted. For the 3 compounds in the benzylisoquinoline group, the order predicted in terms of increasing retention time was narceine, papaverine and noscapine and this was also the order observed.

3.2.3 Quantitative Analysis

For quantitative analysis, 9 different concentrations of the mixture of the 8 opiates were prepared. 3 µl of each mixture with concentrations of 1000, 500, 250, 125, 62, 31, 16, 8, 4 µg ml^{-1} were analysed with the optimized HPLC system. For acetylthebaol, the concentrations used were 100, 80, 60, 40, 20, 10 µg ml^{-1}.

Table A3 in Appendix gives the average peak height for each opiate standard at the various concentrations. Standard calibration curves were obtained for the lower concentration range of 4 to 62 µg ml^{-1} and for the higher concentration range of 125 to 1000 µg ml^{-1} for each opiate (Figure 3.7a - 3.7f). For acetylthebaol, only one curve was obtained for the range of 10 to 100 µg ml^{-1} (Figure 3.7g).

The within day precision of the method was assessed by repeated analysis of a mixture of standard opiates with the following concentrations: 750, 500, 250, 125, 50, 25 µg ml^{-1} (Table 3.11). Five replicates were analysed for each concentration. The percentage coefficient of variation (% CV) were calculated and the overall % CV were within the range of 1.03 to 5.69. The day-to-day precision was determined by conducting repeated analysis over three consecutive days for the following standard concentrations: 500, 125, 50 µg ml^{-1} (Table 3.12). The % CV calculated were within the range of 0.57 to 4.83 indicating good reproducibility of the system.

Table 3.13 shows the lowest detectable limit for the 9 opiates by the HPLC system.

The limits of detection for the system were better than those reported by Jane [111] who obtained the limit as 50 ng for morphine and DAM by UV detection. However, Peterson et al. [143] reported the limit of detection of morphine as 100 pg but the system utilized both a UV and an electrochemical detector in parallel. Other HPLC systems in the literature did not report the detection limits for the opiates.

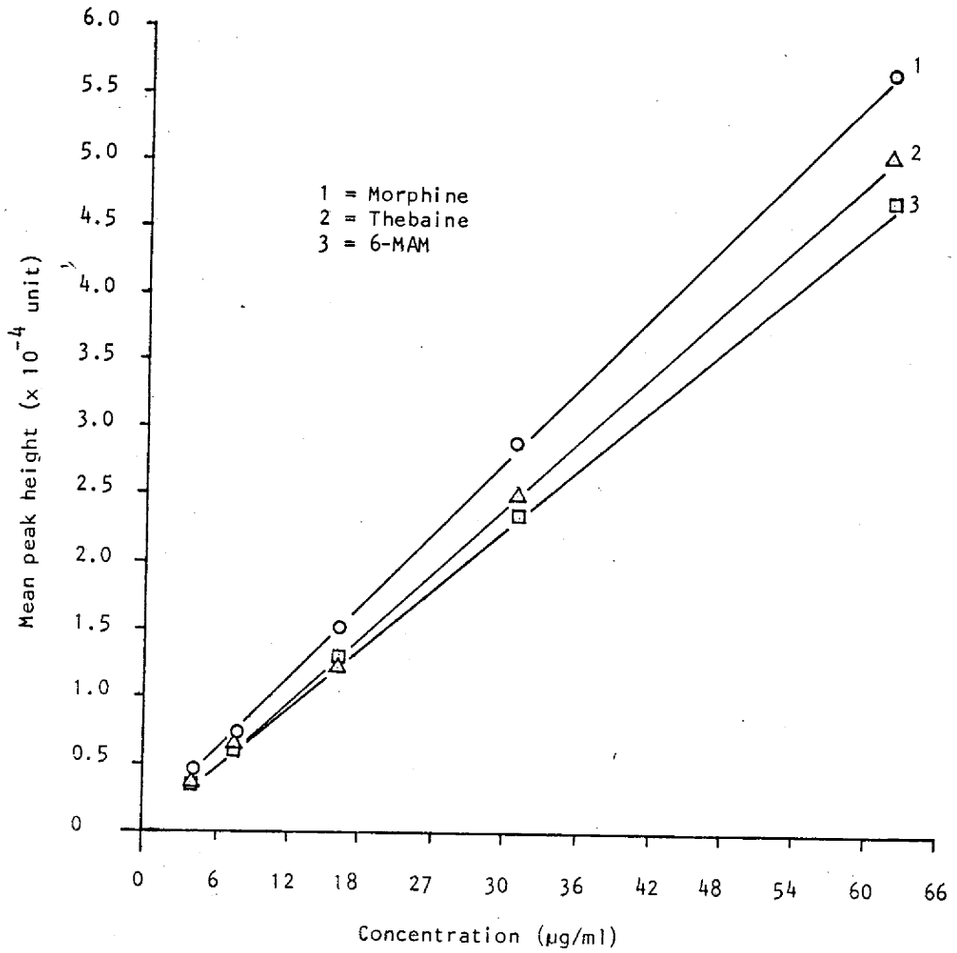


Fig. 3.7a: Standard curves for morphine, thebaine and 6-MAM for the concentration range of 0 - 62 µg/ml with the HPLC system

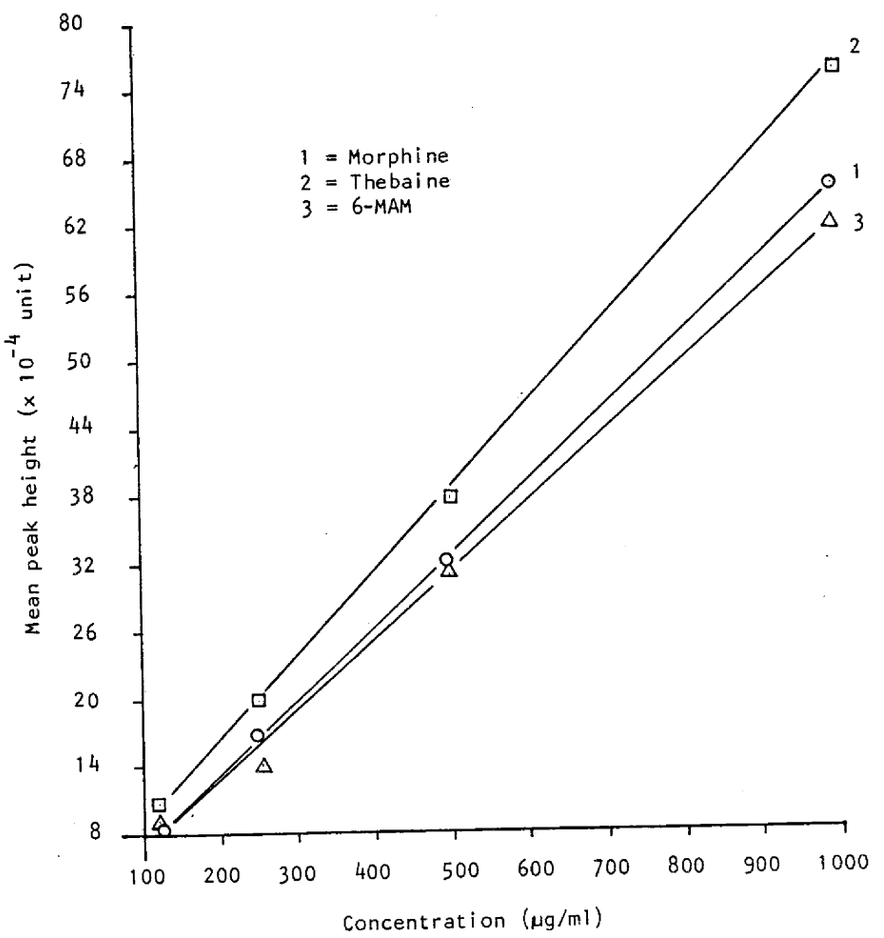


Fig. 3.7b: Standard curves for morphine, thebaine and 6-MAM for the concentration range of 125 - 1000 µg/ml with the HPLC system

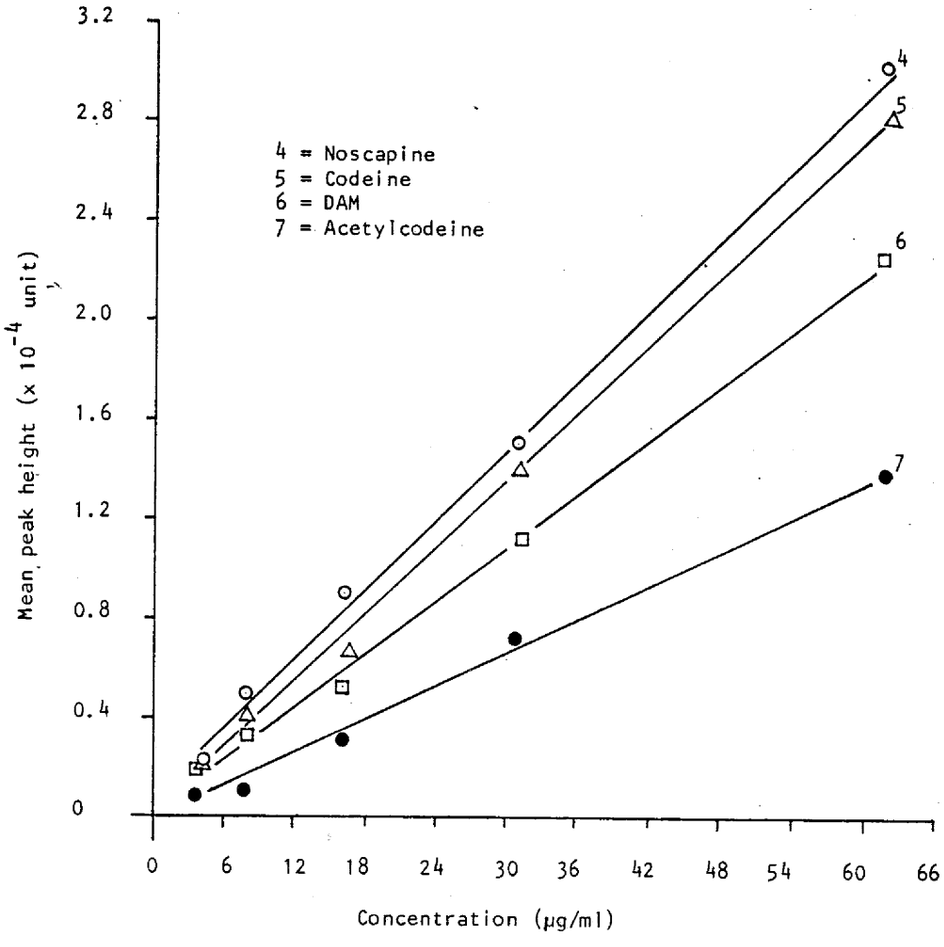


Fig. 3.7c: Standard curves for noscapine, codeine, DAM and acetylcodeine for the concentration range of 0 - 62 µg/ml with the HPLC system

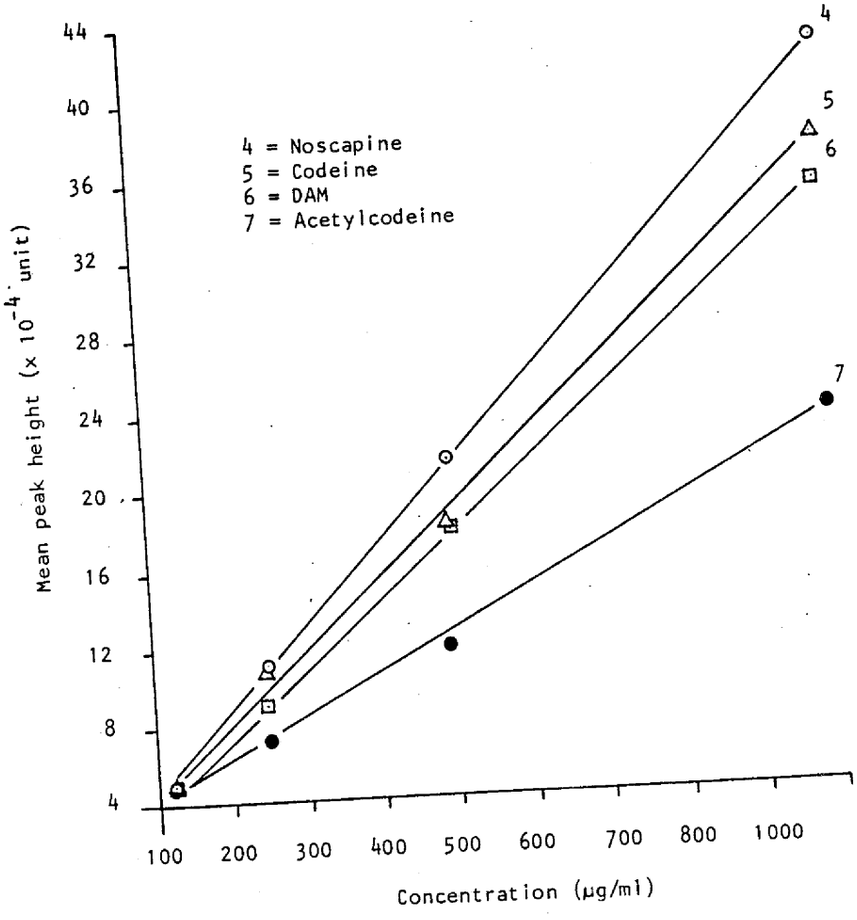


Fig. 3.7d: Standard curves for noscapine, codeine, DAM and acetylcodeine for the concentration range of 125 - 1000 µg/ml with the HPLC system

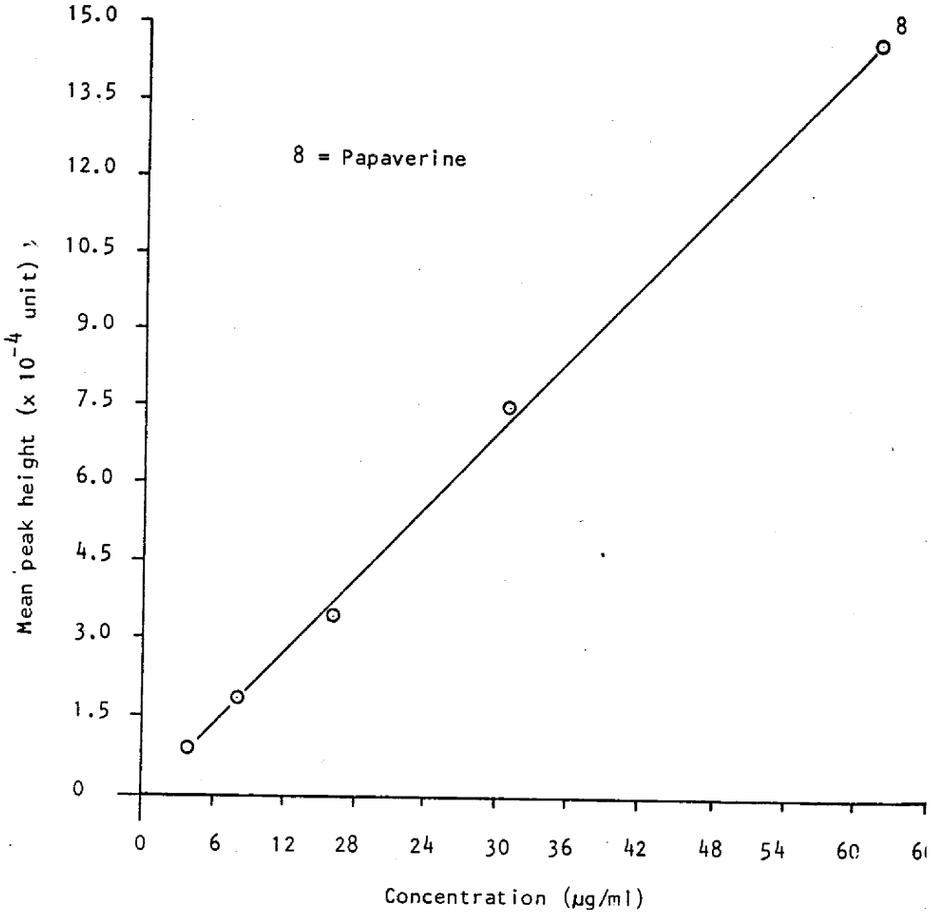


Fig. 3.7e: Standard curve for papaverine for the concentration range of 0 - 62 µg/ml with the HPLC system

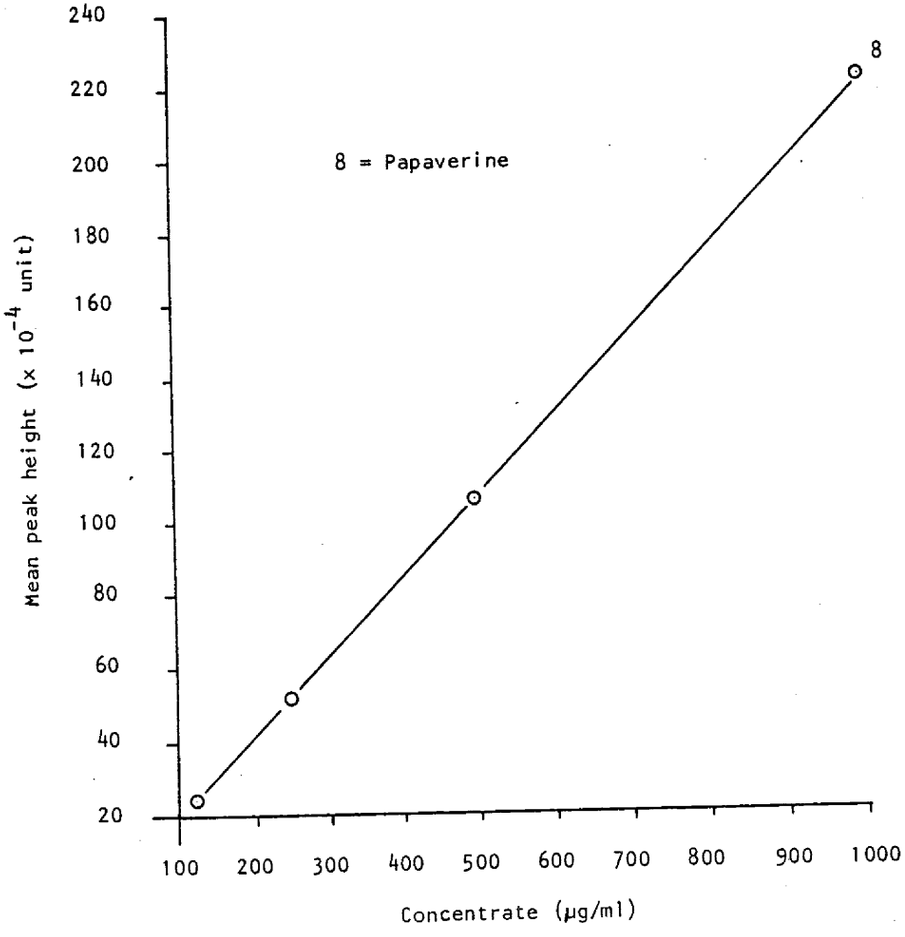


Fig. 3.7f: Standard curve for papaverine for the concentration range of 125 - 1000 µg/ml with the HPLC system

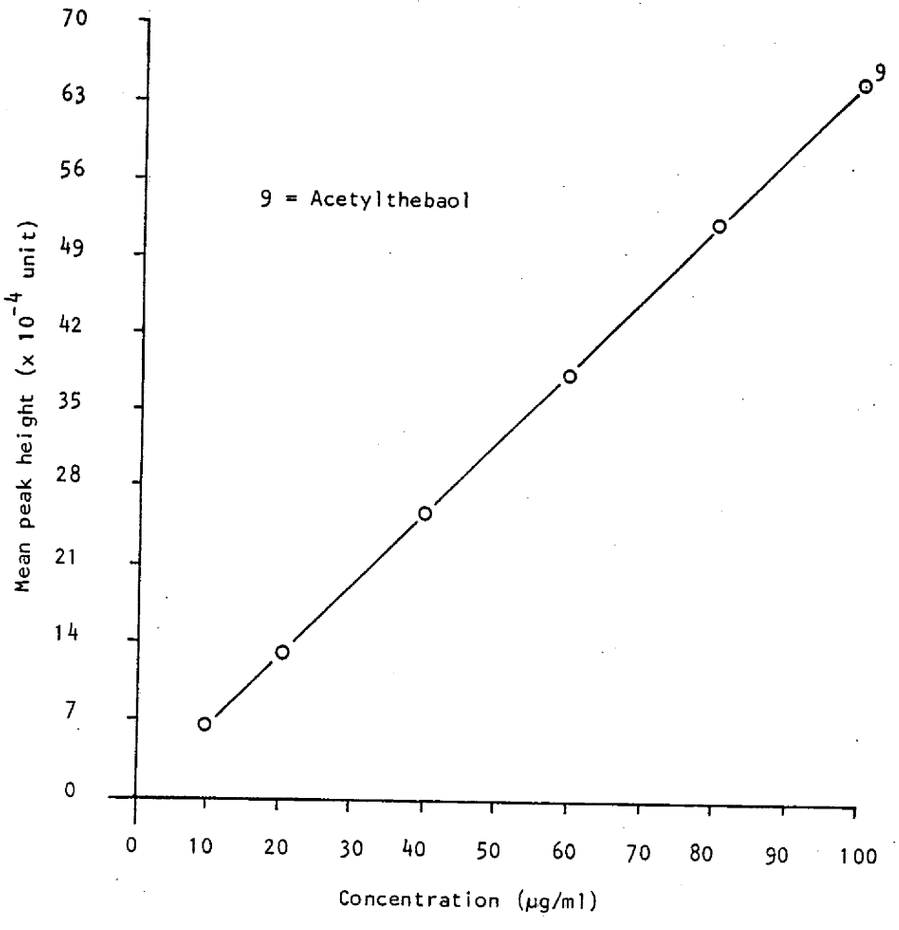


Fig. 3.7g: Standard curve for acetylthebaol for the concentration range of 0 - 100 µg/ml with the HPLC system

Table 3.11

Within-day Percentage Coefficient of Variation (%CV) Values for the Opiates by the HPLC System

		OPIATES								
CONCENTRATION (µg/ml)	REPLICATE	MORPHINE	6-MAM	CODEINE	DAM	PAPAVERINE	ACETYLCODEINE	THEBAINE	NOSCAPINE	ACETYL-THEBAOL
25	5	4.01	3.94	4.05	5.69	2.70	2.23	5.54	1.55	3.85
50	5	1.73	2.11	1.51	2.11	2.15	2.20	3.15	1.03	2.16
125	5	4.48	4.83	4.96	4.36	4.25	3.66	3.78	4.73	4.05
250	5	4.06	3.70	4.09	4.03	3.14	4.10	3.50	3.41	3.70
500	5	2.82	1.73	1.18	1.55	2.91	1.41	1.63	2.88	2.18
750	5	3.88	4.21	3.54	3.73	3.14	3.14	2.72	3.20	3.22

Table 3.12

Day-to-day Percentage of Variation (%CV) Values for the Opiates by the HPLC System

CONCENTRATION (µg/ml)	REPLICATE	OPIATES								
		MORPHINE	6-MAM	CODEINE	DAM	PAPAVERINE	ACETYLCODEINE	THEBAINE	NOSCAPINE	ACETYL-THEBAOL
50	15	2.20	2.66	1.37	0.57	1.54	1.89	2.36	1.95	2.80
125	15	3.79	2.85	3.94	2.34	2.97	3.19	1.69	1.05	3.64
500	15	1.26	1.14	0.95	3.27	1.33	1.67	4.83	1.11	4.15

Table 3.13

Limit of Detection of 9 Opiates by the HPLC System

Opiates	Detection Limit (ng)
Morphine	12.0
6-MAM	12.0
Codeine	12.0
DAM	12.0
Papaverine	12.0
Acetylcodeine	11.0
Thebaine	12.0
Noscapine	12.0
Acetylthebaol	11.0

3.3 Gas Chromatography (GC)

Several workers have reported analysis of opiates in illicit heroin samples by GC methods with various stationary phases in packed or capillary columns (Section 1.7.3). Opiates are generally chromatographed on either relatively non-polar or intermediate polarity stationary phases. In view of this, two relatively non-polar stationary phases, OV-1 and OV-3 and two intermediate polarity stationary phase, OV-17 and OV-210 were selected for the separation phase of the 12 opiate standards. The composition of these stationary phases are given in Table 3.14.

Isothermal runs were carried out over the temperature range 170°C to 240°C with OV-1, OV-3, OV-17 and OV-210 glass packed columns and FID. The optimized conditions which were obtained by gradient programming are listed in Table 3.15. The chromatograms of each column with the optimized conditions are shown in Figure 3.8a to 3.8e.

The intermediate polarity stationary phase OV-17 gave separation of only 5 opiates namely codeine, 6-MAM, thebaine, DAM and papaverine (Figure 3.8a). Levallorphan coeluted with codeine, acetylcodeine with 6-MAM, acetylthebaol with thebaine, noscapine and narceine with papaverine. Morphine and nalorphine did not elute and were adsorbed to the column. Moore et al. [28, 34] resolved 4 opiates namely morphine, codeine, 6-MAM and 3-MAM with a OV-17 packed column and 8 opiates namely morphine, codeine, 6-MAM and 3-MAM, normorphine, norcodeine, acetylnormorphine and diacetylnormorphine on a DB-17 capillary column. In these reported systems, morphine was not adsorbed because it was derivatized with HFBA. The GC parameters used by the developed system and the reported OV-17 system were different.

When compared to OV-17, the OV-210 packed column, which is also of intermediate polarity, gave separation of 7 opiates namely levallorphan, codeine, acetylcodeine, 6-MAM, DAM, papaverine and noscapine (Figure 3.8b). However, thebaine and acetylthebaol coeluted with 6-MAM whereas with OV-17 thebaine was resolved. Since the maximum operating temperature of OV-210 was 275°C, narceine were unable to elute as it required much higher temperatures for elution. As in the case of OV-17, nalorphine did not elute and was most probably adsorbed to the column. Nair and co-workers [19] using different operating conditions but the same column reported resolution for 6 opiates namely codeine, acetylcodeine, 6-MAM, DAM, papaverine and noscapine.

In contrast to the OV-210, the relatively non-polar stationary phase OV-1 was able to resolve only 6 opiates, namely codeine, 6-MAM, DAM, papaverine, noscapine and narceine (Figure 3.8c). The opiates which coeluted were levallorphan and morphine with codeine, acetylcodeine, thebaine, nalorphine and acetylthebaol with 6-MAM. However, Machata et al. [43] reported resolution of codeine, morphine and 6-MAM on an OV-1 packed column. A capillary column SE-30 which had a stationary phase equivalent to OV-1 was used by Law et al. [21] to resolve codeine, morphine, DAM and papaverine. In both systems, resolution was obtained as a result of derivatization.

Table 3.14

Composition of Commonly Used Stationary Phases in Opiate Analysis by GC Systems

Trade Names	Composition	Polarity	Solid Supports	Equivalent Phases	Temp. Limit Min/Max °C	References
OV-1	100% Methyl Silicone Gum	Non-polar	Chromosorb W-HP	DB-1, SE-30	100/350	21, 32, 35, 38, 43, 130 137
OV-3	5% Phenyl Methyl Silicone	Non-polar	Chromosorb W-HP	SE-54	50/320	26, 36, 138
OV-17	50% Phenyl Methyl Silicone	Intermediate	Chromosorb W-HP	DB-17	0/375	27, 28, 31, 34, 72, 136
OV-210	50% 3,3,3-Trifluoro-propyl	Intermediate	Chromosorb W-HP	OV-202	0/275	19, 22, 25

Table 3.15

Optimized Parameters Obtained for the Separation of 12 Opiates on 5 Columns by the GC Systems

Parameter \ Column	OV-17 Packed	OV-210 Packed	OV-1 Packed	OV-3 Packed	SE-54 Capillary
Detector	FID	FID	FID	FID	ITD
Initial temp (^{o}C)	200	175	220	205	240
Initial time (min)	3	15	10	15	21
Rate (^{o}C min^{-1})	5	10	10	10	25
Final temp (^{o}C)	220	245	280	285	295
Final time (min)	1	10	6	7	22
Rate A (^{o}C min^{-1})	1	.	.	.	.
Final temp A (^{o}C)	240	.	.	.	.
Final time A (min)	5	.	.	.	.
Rate B (^{o}C min^{-1})	20	.	.	.	.
Final temp B (^{o}C)	290	.	.	.	.
Final time B (min)	10	.	.	.	.
Injector temp (^{o}C)	250	250	250	250	250
Detector temp (^{o}C)	300	300	300	300	300
Column flow (ml min^{-1})	40	45	30	45	0.5
Run time	45.5	32	22	30	45.2
Split ratio	.	.	.	.	1:10
No. of Opiates Separated	5	7	6	9	11

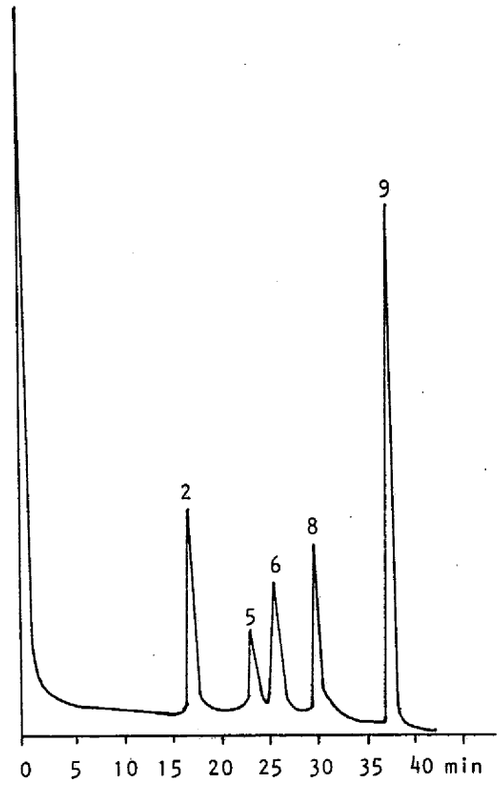


Fig. 3.8a: GC chromatogram showing separation of 5 opiates with a OV-17 packed column

(2 = codeine, 17.64 min; 5 = 6-MAM, 23.94 min; 6 = thebaine, 26.40 min; 8 = DAM, 30.71 min; 9 = papaverine, 38.23 min)

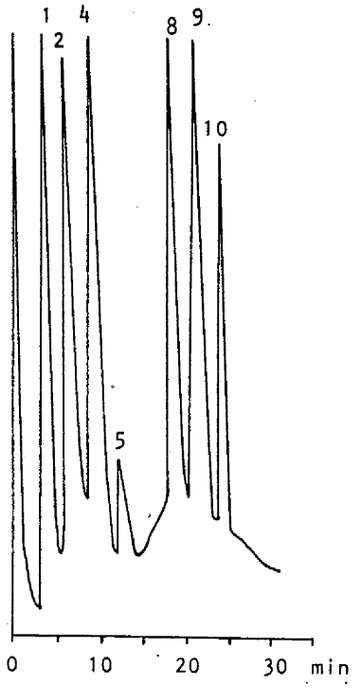


Fig. 3.8b: GC chromatogram showing separation of 7 opiates with a OV-210 packed column

(1 = levallorphan, 3.60 min; 2 = codeine, 6.22 min; 4 = acetylcodeine, 9.25 min; 5 = 6-MAM, 12.63 min; 8 = DAM, 18.58 min; 9 = papaverine, 21.32 min; 10 = noscapine, 24.90 min)

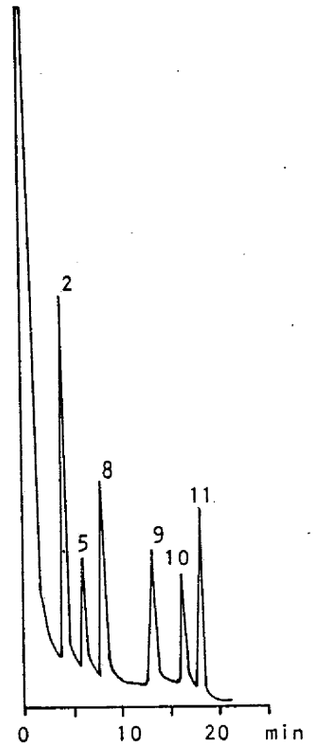


Fig. 3.8c: GC chromatogram showing separation of 6 opiates with a OV-1 packed column

(2 = codeine, 4.54 min; 5 = 6-MAM, 6.58 min; 8 = DAM, 8.56 min; 9 = papaverine, 13.39 min; 10 = noscapine, 17.07 min; 11 = narceine, 18.77 min)

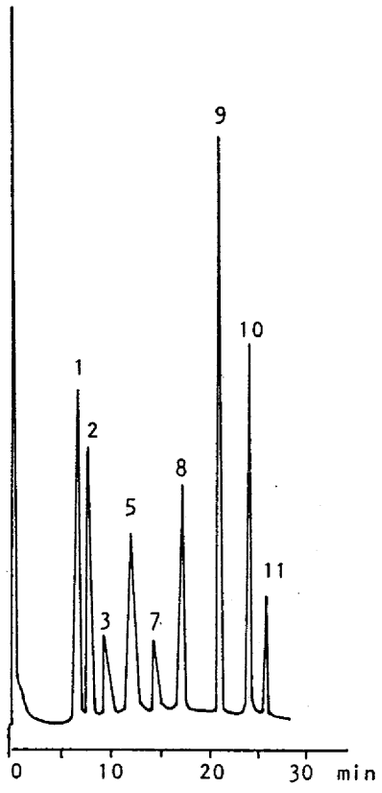


Fig. 3.8d: GC chromatogram showing separation of 9 opiates with a OV-3 packed column

(1 = levallorphan, 6.73 min; 2 = codeine, 7.79 min; 3 = morphine, 9.45 min; 5 = 6-MAM, 12.22 min; 7 = nalorphine, 14.56 min; 6 = DAM, 17.34 min; 9 = papaverine, 21.15 min; 10 = noscapine, 24.19 min; 11 = narceine, 25.93 min)

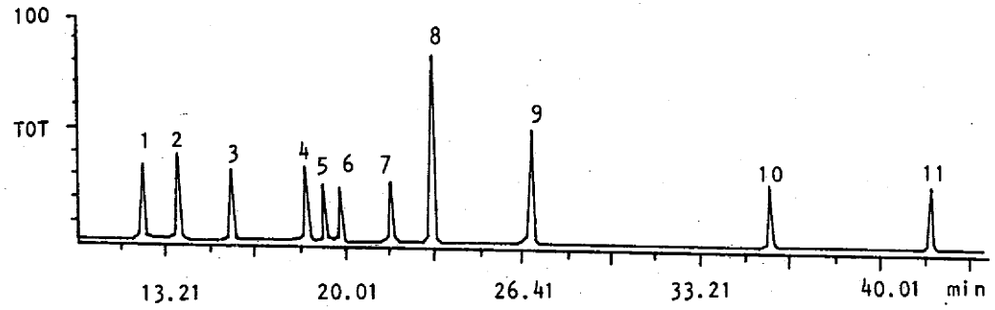


Fig. 3.8e: GC chromatogram showing separation of 11 opiates with a SE 54 capillary column

(1 = levallorphan, 12.35 min; 2 = codeine = 13.53 min; 3 = morphine, 15.56 min; 4 = acetylcodeine, 18.47 min; 5 = 6-MAM, 19.29 min; 6 = thebaine, 20.05 min; 7 = nalorphine, 21.50 min; 8 = DAM, 23.17 min; 9 = papaverine, 27.02 min; 10 = noscapine, 36.06 min; 11 = narceine, 42.06 min)

The OV-3 which is also a relatively non-polar stationary phase, was however able to resolve levallorphan, codeine, morphine, 6-MAM, nalorphine, DAM, papaverine, noscapine and narceine (Figure 3.8d). As in the case of OV-1, acetylcodeine, thebaine and acetylthebaol coeluted with 6-MAM. No studies have been reported in literature on the use of OV-3 column for the separation of opiates.

In view of the resolution of 9 opiates, a capillary column with equivalent stationary phase to OV-3 was selected for the separation of the 12 opiates. Capillary columns being much longer than packed columns can generate a larger number of theoretical plates thereby improving separation by increasing resolution. This means the component peaks are sharper and that more components can be separated per unit time. The SE-54 capillary column, was able to resolve not only the 9 opiates separated by OV-3 but in addition acetylcodeine and thebaine. Neumann et al. [138] reported separation of thebaol, codeine, thebaine, morphine, papaverine and noscapine with a SE-54 capillary column. However, derivatization was necessary for the resolution of the 6 opiates.

The results showed that of the 4 stationary phases, SE-54 or OV-3 were able to resolve more opiates in the mixture. The suitability of a stationary phase for resolving the components of the mixture of opiates depends upon the selectivity of the phase. This is a measure of the degree to which polar compounds are retarded relative to their elution on a non-polar phase. Stationary phases have been classified on the basis of their polarity by Rohrschneider [163] and by McReynolds [164]. Based on the Rohrschneider constants, the stationary phases can be arranged in order of increasing polarity in the following sequences: OV-1, OV-3, OV-17 and OV-210. The slight degree of polarity in OV-3 or SE-54 due to the substitution of a small amount of phenyl groups (5%) into the methyl silicones provided the best selectivity for the separation of the opiates.

The order of elution of the opiates in terms of increasing retention time with the SE-54 capillary column was in the following sequence: levallorphan, codeine, morphine, acetylcodeine, 6-MAM, thebaine, nalorphine, DAM, papaverine, noscapine and narceine.

In GC, separations depend upon differences in boiling points, molecular weights and structural variations of the compounds. The relative volatility or separation factor of a compound depends upon the interactions of the solute and the liquid phase of the stationary phase. These cohesive forces are of three types namely London dispersion forces, Debye induction forces and Keesom orientation forces [165, 166].

However, in this case, the order of elution of the opiates could not be predicted by utilizing any one of the factors mentioned above indicating that the retention index is a composite function of many effects.

3.3.1 Quantitative Analysis

For quantitative analysis SE-54 capillary column which could separate 11 opiates was used with an ITD. The ITD which is a mass spectrometer enables identification of the opiates both by retention time and mass spectra.

Quantitative analysis on the ITD was done on both the scan modes namely full scan and multiple ion detection (MID).

On the full scan mode, the mass range 60 to 450 u was scanned at 1 scan per second. 8 different concentrations of the mixture of 11 opiates were prepared. A volume of 1 µl of each mixture with concentrations of 1000, 750, 600, 500, 400, 300, 200, 100 µg ml^{-1} were analysed with the optimized conditions given in Table 3.15. Since acetylthebaol coeluted with thebaine, solutions containing 1000, 900, 800, 500, 300, 200, 100 µg ml^{-1} of acetylthebaol were prepared and analysed separately. Table A4 in the appendix gives the average peak area for each opiate standard at the various concentrations. Standard calibration curve were obtained for the concentration range 100 to 1000 µg ml^{-1} for each opiate (Figure 3.9a - 3.9d). A liner correlation existed within the range of 100 to 1000 µg ml^{-1} with a correlation coefficient of 0.991 on average for the 12 opiates.

The within day precision of the method was assessed by repeated analysis of a mixture of 11 standard opiates with the following concentrations: 1000, 750, 600, 400, 200 µg ml^{-1} (Table 3.16). Five replicates were analysed for each concentration level. The percentage coefficient of variations (% CV) were calculated and the overall % CV were within the range of 0.12 to 3.79.

The day-to-day precision was determined by conducting repeated analysis over three consecutive days for the following standard concentrations: 1000, 500, 200 µg ml^{-1} (Table 3.17). The % CV were within the range of 0.47 to 4.33 indicating good reproducibility of the system.

3 opiates namely codeine, DAM, papaverine were used to standardize relative abundances in electron ionization mass spectra. These opiates were used to determine the precision of relative abundances (RA) measurements in full mass range scans. The mass range scanned for codeine, papaverine and DAM was 60 to 300 u, 60 to 340 u and 60 to 370 u respectively with a 1 second per scan time. Five consecutive GC injections of 5 ng of each compound was carried out using the experimental conditions described in Section 3.3. 10 key ions for each compound were selected. The mean RA for the five replicates, standard deviation (SD) and relative standard deviation (RSD or % CV) of the RA for the 10 key ion are given in Table 3.18. The statistics in Table 3.18 were computed with abundance data from the scan at the apex of each GC peak. Table 3.18 shows that the precision of the RA measurements of 10 key codeine

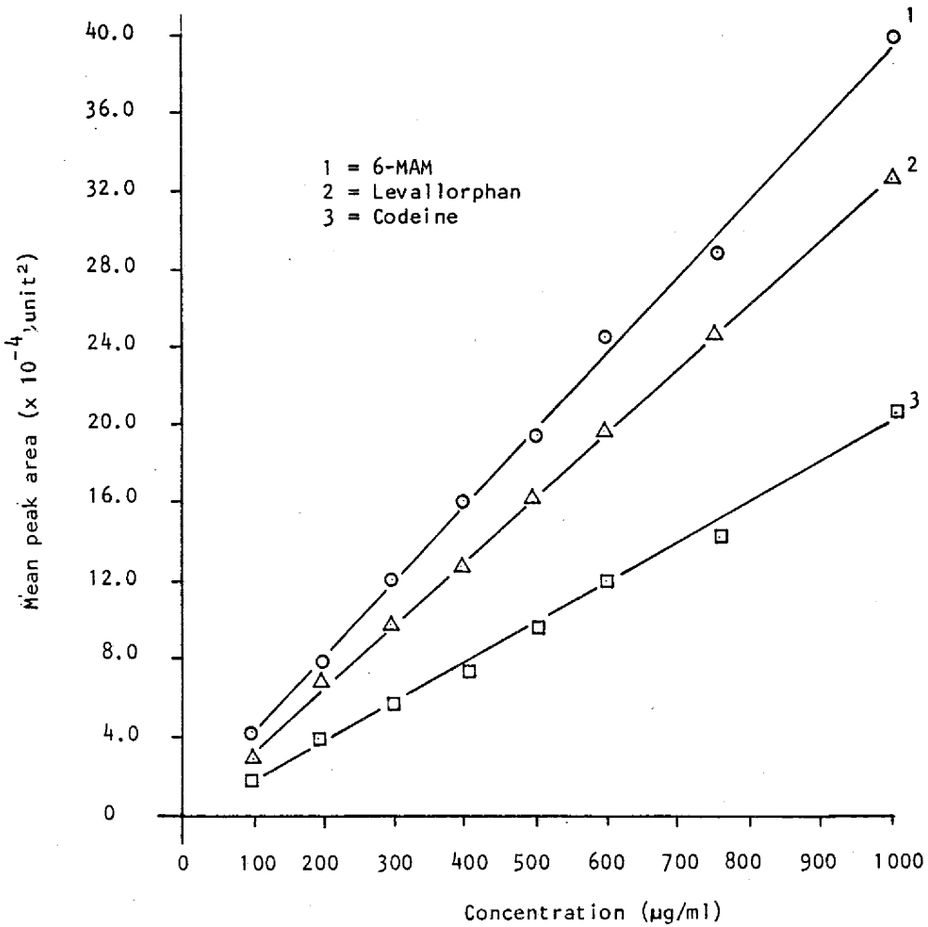


Fig. 3.9a: Standard curves for 6-MAM, levallorphan and codeine for the concentration range of 0 - 1000 µg/ml in the scan mode on GC-ITD

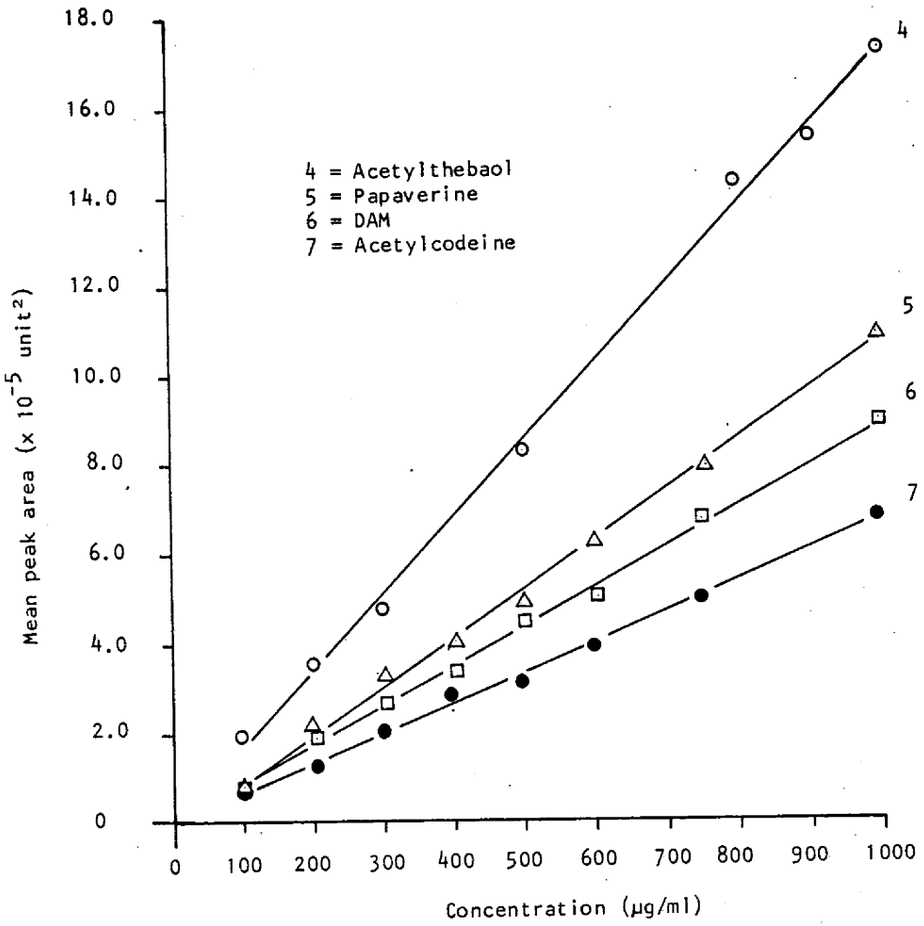


Fig. 3.9b: Standard curves for acetylthebaol, papaverine, DAM and acetylcodeine for the concentration range of 0 - 1000 µg/ml in the scan mode on GC-ITD

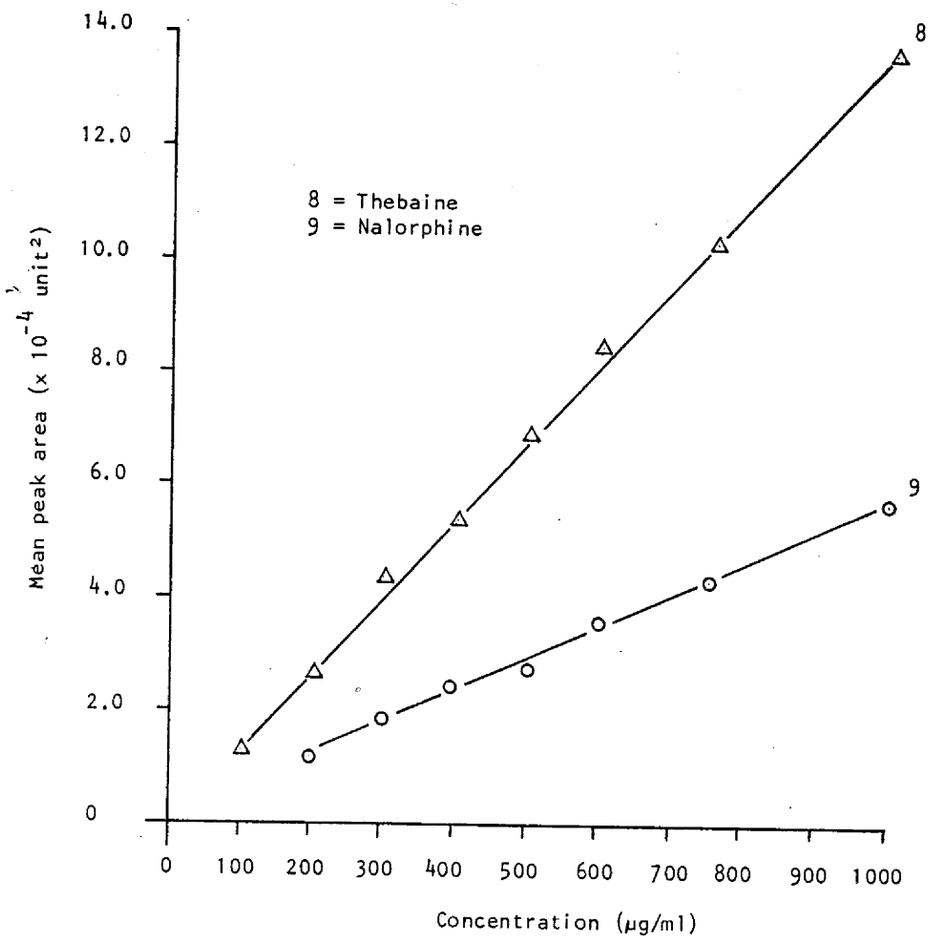


Fig. 3.9c: Standard curves for thebaine and nalorphine for the concentration range of 0 - 1000 µg/ml in the scan mode on GC-ITD

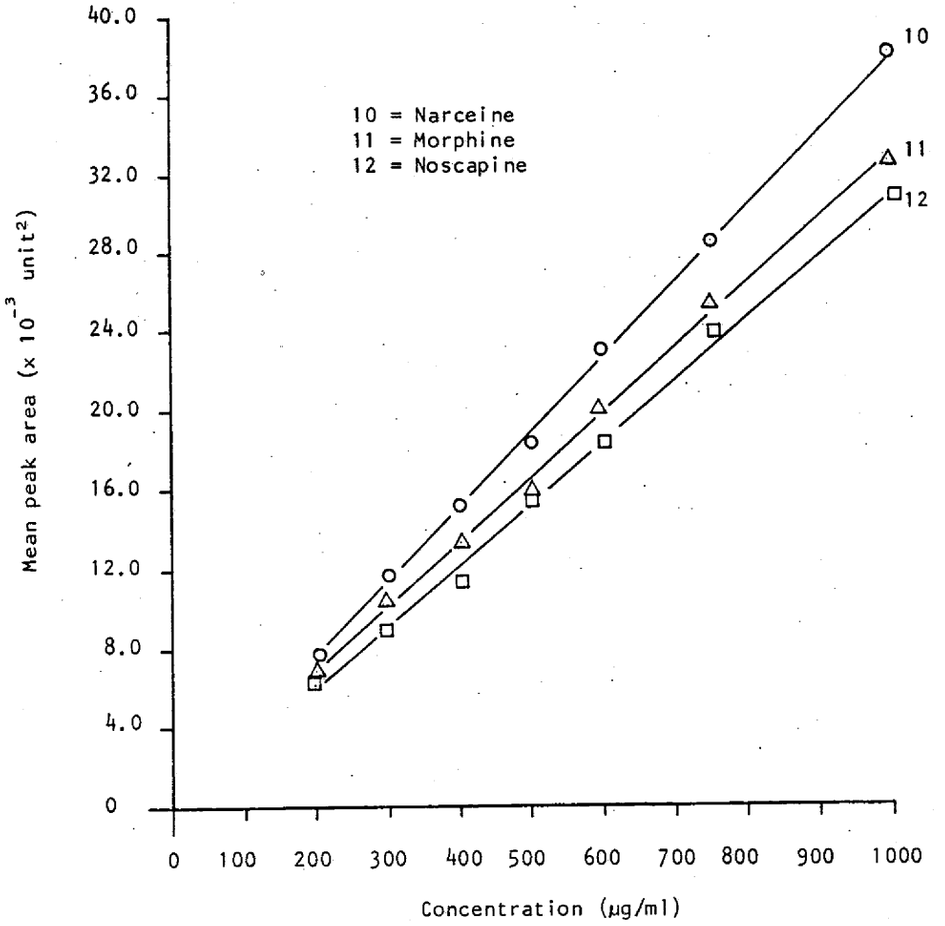


Fig. 3.9d: Standard curves for narceine, morphine and noscapine for the concentration range of 0 - 1000 µg/ml in the scan mode on GC-ITD

Table 3.16

Within-day Percentage Coefficient of Variation (% CV) Values of the Opiates by the GC System

Opiates	Conc. / Replicates	% CV 200 µg ml^{-1}	400 µg ml^{-1}	600 µg ml^{-1}	750 µg ml^{-1}	1000 µg ml^{-1}
Levallorphan	5	1.37	0.79	0.58	0.34	0.12
Codeine	5	1.54	0.95	0.72	0.36	0.16
Morphine	5	3.49	2.85	2.34	1.69	1.15
Acetylcodeine	5	1.69	1.23	0.85	0.41	0.20
6-MAM	5	1.26	0.89	0.66	0.25	0.18
Thebaine	5	2.31	1.14	0.92	0.32	0.21
Nalorphine	5	3.28	2.66	2.15	2.03	1.02
DAM	5	1.14	0.88	0.56	0.21	0.13
Papaverine	5	1.20	0.94	0.67	0.25	0.12
Noscapine	5	3.79	2.70	2.12	2.01	1.13
Narceine	5	3.44	2.35	2.18	2.00	1.08

Table 3.17

Day-to-Day (for 3 days) Percentage Coefficient of Variation (% CV) Values of the Opiates by the GC System

Opiates	Conc. / Replicates	% CV 200 µg ml^{-1}	500 µg ml^{-1}	1000 µg ml^{-1}
Levallorphan	15	1.88	1.06	0.48
Codeine	15	1.79	1.08	0.47
Morphine	15	4.12	3.05	2.16
Acetylcodeine	15	1.92	1.21	0.54
6-MAM	15	1.65	1.13	0.49
Thebaine	15	2.74	1.56	0.63
Nalorphine	15	3.15	2.89	1.65
DAM	15	1.48	1.03	0.44
Papaverine	15	1.60	1.11	0.51
Noscapine	15	4.24	3.14	2.06
Narceine	15	4.33	3.08	2.17

Table 3.18

Mean, Standard Deviation (SD) and Relative Standard Deviation (RSD) of the Relative Abundances (RA) of Key Ions in the Electron Ionization (EI) Mass Spectra of Compounds Used to Standardize EI Mass Spectra

Opiates	Mass	Mean RA	SD	RSD (% CV)
Codeine	77	15.5	0.33	2.1
	81	14.1	0.14	1.0
	91	8.5	0.20	2.3
	115	19.7	0.67	3.4
	162	23.0	1.40	6.1
	214	9.9	0.81	8.2
	229	16.0	0.88	5.5
	282	10.8	0.22	2.0
	298	15.0	0.54	3.6
	299	100.0	-	-
DAM	63	6.1	0.32	5.2
	81	19.1	1.18	6.2
	146	11.7	1.17	10.0
	162	7.4	0.67	9.1
	204	23.0	2.69	11.7
	268	42.2	3.17	7.5
	310	39.6	2.53	6.4
	327	100.0	-	-
	368	14.8	0.64	4.3
	369	68.3	3.69	5.4
Papaverine	63	9.6	0.32	3.3
	77	5.6	0.24	4.2
	154	3.4	0.16	4.7
	191	3.7	0.23	6.1
	266	6.5	0.34	5.2
	293	11.8	0.30	2.5
	308	9.9	0.36	3.6
	324	26.1	1.49	5.7
	338	28.9	1.39	4.8
	339	100.0	-	-

ions were better than the precision of RA measurements of 10 key DAM ions. All the 3 sets were good however, considering the small quantity injected and the potential for variation during the experiment.

On the MID mode, the most abundant ions of the 11 opiates were scanned at 1 scan per second. The data were acquired using a standard 12 ion range MID protocol shown in Table 3.19. The mass ranges selected for each opiate were chosen to include the most abundant mass peaks for that particular opiate.

Table 3.19

MID Ranges Used for 12 Opiates

	MID Table	
	Mass Range	Opiates
1.	220 to 222	Noscapine
2.	256 to 257	Levallorphan
3.	282 to 286	Levallorphan, Morphine
4.	294 to 300	Nalorphine, Thebaine, Codeine
5.	311 to 312	Thebaine, Nalorphine
6.	324 to 328	6-MAM, DAM
7.	339 to 340	Papaverine
8.	341 to 342	Acetylcodeine
9.	369 to 370	DAM
10.	413 to 414	Noscapine
11.	427 to 428	Narceine
12.	445 to 446	Narceine

A volume of 1 µl of each mixture of 11 opiates with concentrations in the range 0.4 µg ml^{-1} to 10 µg ml^{-1} were analysed under the MID mode using the optimized conditions described in section 3.3. Table A5 in the appendix gives the average peak area for each opiate standard at various concentrations. Standard calibration curves were obtained for each opiate and a linear range extends from 0.4 µg ml^{-1} to 10 µg ml^{-1} with a correlation coefficient of 0.990 on average for the 11 opiates (Figure 3.10a - 3.10b).

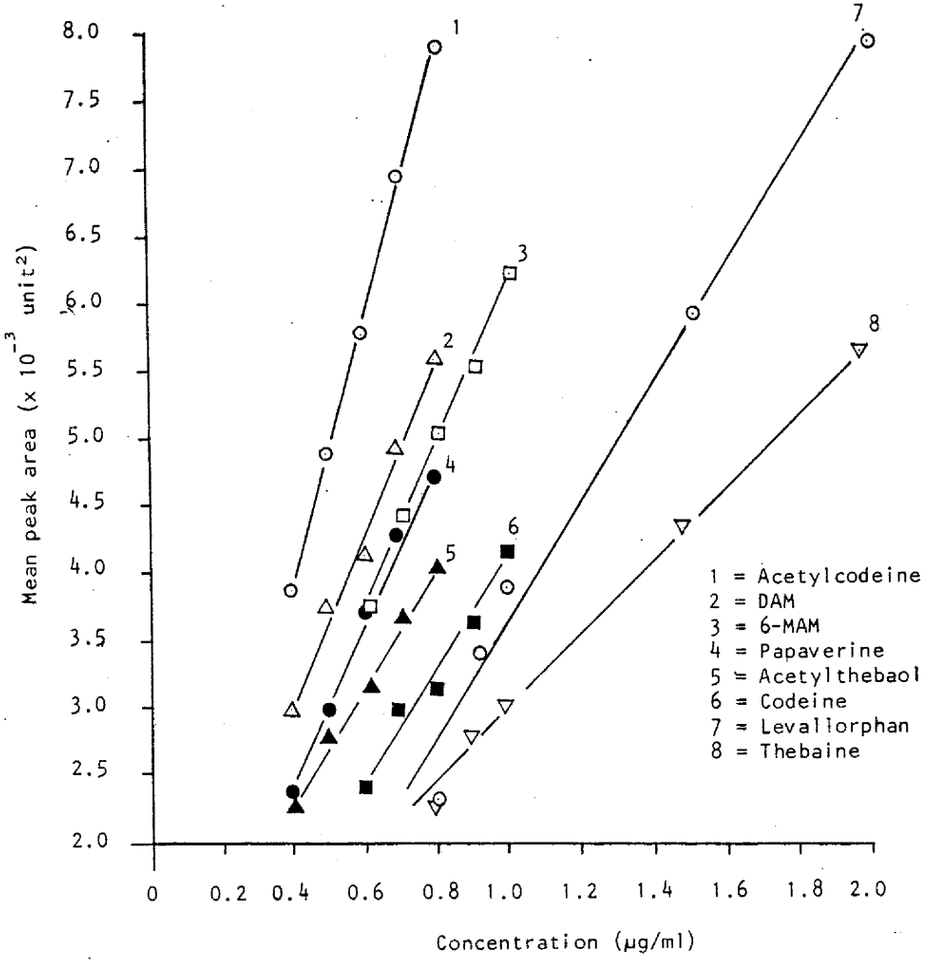


Fig. 3.10a: Standard curves for acetylcodeine, DAM, 6-MAM, papaverine, acetylthebaol, codeine, levallorphan and thebaine for the concentration range 0.4 - 2.0 µg/ml in the MID mode on GC-ITD

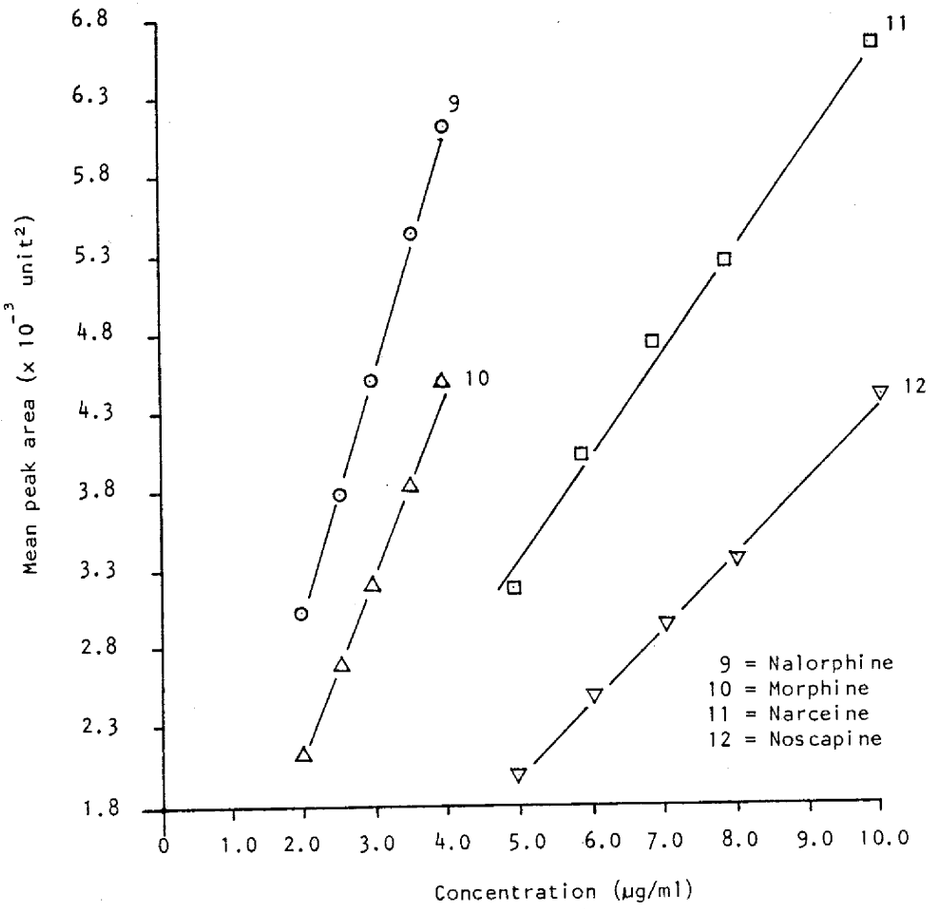


Fig. 3.10b: Standard curves for nalorphine, morphine, narceine and noscapine for the concentration range 2 - 10 µg/ml in the MID mode on GC-ITD

The limit of detection of all the opiates which could separate on the 5 columns is given in Table 3.20. It is clear from Table 3.20 that the SE-54 capillary column with an ITD on the MID mode gave the best limit of detection. The limit reported for this system is better than the reported limit of 20 ng by Demedts [42] for morphine, codeine, 6-MAM, DAM and acetylcodeine using a CP-Sil5 capillary column with a NPD. The limit reported by Chiarotti [26] for DAM was 0.2 ng on SE-54 capillary column with a FID. Other systems in the literature did not report the limit of detection for the opiates separated.

Table 3.20

Limits of Detection for the 12 Opiates on 5 Columns by the GC Systems

Opiates	Packed Column				Capillary Column	
	OV-17	OV-1	OV-210	OV-3	SE 54	
					Full Scan Mode	MID Mode
1. Levallorphan	-	-	12 ng	15 ng	3 ng	800 pg
2. Codeine	20 ng	20 ng	40 ng	15 ng	2 ng	600 pg
3. Morphine	-	-	-	120 ng	10 ng	2 ng
4. Acetylcodeine	-	-	12 ng	-	1 ng	400 pg
5. 6-MAM	20 ng	30 ng	100 ng	20 ng	2 ng	600 pg
6. Thebaine	20 ng	-	-	-	3 ng	800 pg
7. Nalorphine	-	-	-	250 ng	10 ng	2 ng
8. DAM	20 ng	20 ng	4 ng	10 ng	1 ng	400 pg
9. Papaverine	20 ng	20 ng	10 ng	15 ng	1 ng	400 pg
10. Noscapine	-	40 ng	40 ng	30 ng	20 ng	5 ng
11. Narceine	-	40 ng	-	30 ng	20 ng	5 ng
12. Acetylthebaol	-	-	-	-	1 ng	-

3.4 Analysis of Illicit Heroin Samples

50 locally siezed illicit heroin samples of unknown geographical origin were analysed by using the TLC, HPLC and the GC systems described in Section 3.1, 3.2 and 3.3.

A volume of 5 µl of each seized illicit heroin sample with a concentration of 1000 µg ml^{-1} was analysed by the TLC system for the presence of 12 opiates namely morphine, codeine, 6-MAM, DAM, acetylcodeine, thebaine, papaverine, noscapine, narceine, nalorphine, levallorphan and acetylthebaol. The analysis results (Table A6 in the appendix) showed that only 4 opiates were present in the samples namely DAM, 6-MAM, acetylcodeine and morphine. DAM was present in 44 samples in the range 63.3 to 100%, 6-MAM in 32 samples in the range 4.5 to 100%, acetylcodeine in 15 samples in the range 6.2 to 21.4% and morphine in only 4 samples in the range 4.2 to 30.9%.

On the HPLC system, a volume of 3 µl of each seized illicit heroin with a concentration of 1000 µg ml^{-1} was analysed for the presence of 9 opiates namely DAM, 6-MAM, acetylcodeine, codeine, morphine, thebaine, papaverine, noscapine and acetylthebaol. The analysis results (Table A7 in the appendix) showed that in addition to the 4 opiates obtained with TLC, codeine was also present in some of the samples. This was because the concentration of codeine in the samples was below 40 µg ml^{-1} which was the detection limit of the TLC method. DAM was present in 45 samples in the range 59.2 to 94.4%, 6-MAM in 48 samples in the range 1.2 to 100%, acetylcodeine in 46 samples in the range 1.3 to 21.9% (with the exception of sample number 6 which had 100% acetylcodeine), morphine in 8 samples in the range 2.6 to 28.7% and codeine in 12 samples in the range 1.7 to 9.2%.

A volume of 1 µl of each seized illicit heroin sample with a concentration of 100 µg ml^{-1} was analysed by the GC ststem on both the full scan and MID mode for the presence of 11 opiates namely DAM, 6-MAM, acetylcodeine, codeine, morphine, thebaine, papaverine, noscapine, narceine, nalorphine and levallorphan. Unlike the HPLC method which showed the presence of morphine in some samples, the GC analysis results (Table A8 in the appendix) showed that only 4 opiates were present namely DAM, 6-MAM, acetylcodeine and codeine because the concentration of morphine was below the detection limit. DAM was present in 45 samples in the range 59.2 to 100%, 6-MAM in 39 samples in the range 1.1 to 100%, acetylcodeine in 45 samples in the range 1.3 to 21.3% (with the exception of sample number 6 which had 100% acetylcodeine) and codeine in 7 samples in the range 2.2 to 14.6%.

In conclusion, the analysis results of the 50 locally seized illicit heroin samples by the TLC, HPLC and GC systems showed the presence of only 5 opiates namely DAM, 6-MAM, acetylcodeine, codeine and morphine. The remaining 7 opiates namely thebaine, papaverine, noscapine, narceine, nalorphine, levallorphan and acetylthebaol were not detected in any of the samples.

Figures 3.11a, 3.11b and 3.11c show TLC, HPLC and GC chromatograms respectively of a representative selection of the seized illicit heroin samples. The analysis results obtained with the TLC system for DAM, 6-MAM, acetylcodeine and morphine in the seized samples were compared with the HPLC system (Figure 3.12a - 3.12d). The correlation was conventionally expressed with the assumption that HPLC data were exact and all the errors were in TLC data. The correlation coefficient was 0.960 for DAM, 0.968 for 6-MAM, 0.974 for acetylcodeine and 0.999 for morphine. In the same manner, the analysis results obtained with the HPLC system for DAM, 6-MAM, acetylcodeine and codeine in the seized samples were compared with the GC system (Figure 3.12e - 3.12h). The correlation were conventionally expressed with the assumption that HPLC data were exact and all the errors were in GC data. The correlation coefficient was 0.937 for DAM, 0.912 for 6-MAM, 0.963 for acetylcodeine and 0.907 for codeine.

For characterization and comparison purposes, the HPLC quantitative analysis data was used for the 50 seized illicit heroin samples since 5 opiates were detected by this method. Table 3.21 shows the 10 possible groupings of the 50 illicit heroin samples based on the qualitative analysis data.

The presence of 6-MAM in the illicit samples has been reported to be due to the breakdown or hydrolysis of DAM to 6-MAM and to partial acetylation of morphine to 6-MAM [71]. If 6-MAM in the samples is assumed to be due to either hydrolysis of DAM or to a combination of hydrolysis of DAM and partial acetylation of morphine to 6-MAM then group 9 samples would fall into group 7, those in group 4 into group 1, those in group 6 into group 3 and those in group 8 into group 5.

It is also clear that the procedure utilized for the preparation of samples in group 2 and 3 differed substantially since all the codeine in the former group underwent complete acetylation with incomplete acetylation of the morphine whereas in the latter group the reverse situation occured. In contrast, the samples in group 7 and 9 showed total absence of codeine as well as other opiates indicating the extraction procedure was extremely efficient. In the case of samples in group 5 and 8 the acetylation procedure was efficient since morphine and codeine were absent which is in contrast to those samples in group 1 where both were present. The one sample in group 10 could not be considered as an illicit heroin sample since it only contained acetylcodeine. On this basis, it is reasonable to conclude that the samples were probably prepared in at least 5 different clandestine operations.

O'Neil et al. [22] analysed over 200 illicit heroin samples of known geographical origin for the presence of 7 opiates namely DAM, 6-MAM, acetylcodeine, morphine, codeine, papaverine and noscapine by GC and HPLC. A qualitative and quantitative analysis of these opiates in the samples was used by these workers to determine the geographical origin of illicit heroin and to discriminate between samples of different origin.

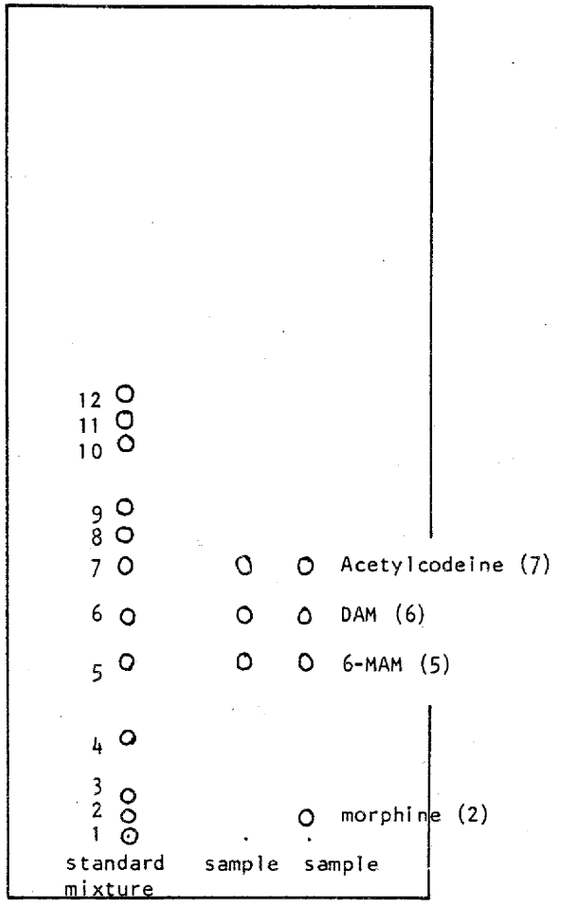


Fig. 3.11a: TLC chromatogram of two illicit heroin samples

(1 = narceine; 2 = morphine; 3 = nalorphine;
4 = codeine; 5 = 6-MAM; 6 = DAM;
7 = acetylcodeine; 8 = thebaine; 9 = papaverine;
10 = levallorphan; 11 = noscapine; 12 = acetylthebaol)

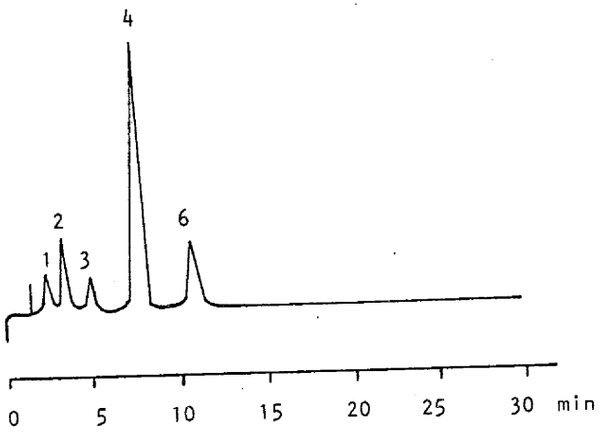


Fig. 3.11b: HPLC chromatogram of an illicit heroin sample

(1 = morphine; 2 = 6-MAM; 3 = codeine; 4 = DAM;
6 = acetylcodeine)

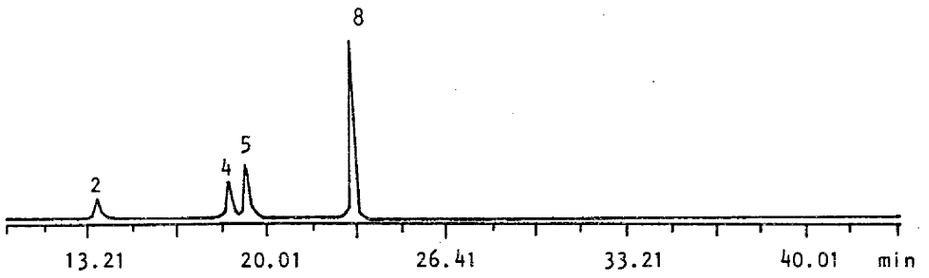


Fig. 3.11c: GC-ITD chromatogram of an illicit heroin sample

(2 = codeine; 4 = acetylcodeine; 5 = 6-MAM; 8 = DAM)

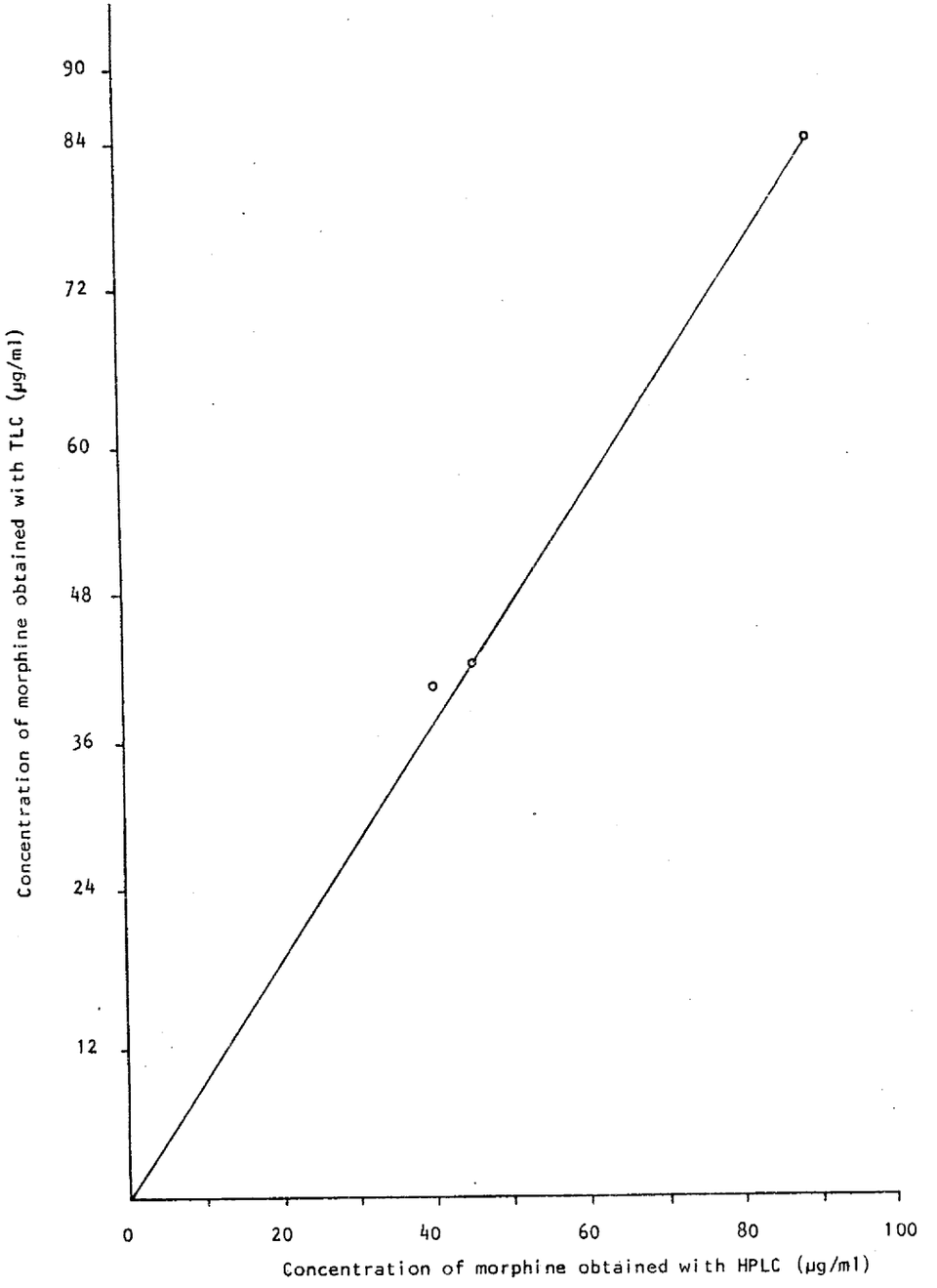


Fig. 3.12a: **Correlation plot of data obtained from TLC versus HPLC for morphine concentrations in the illicit heroin samples**

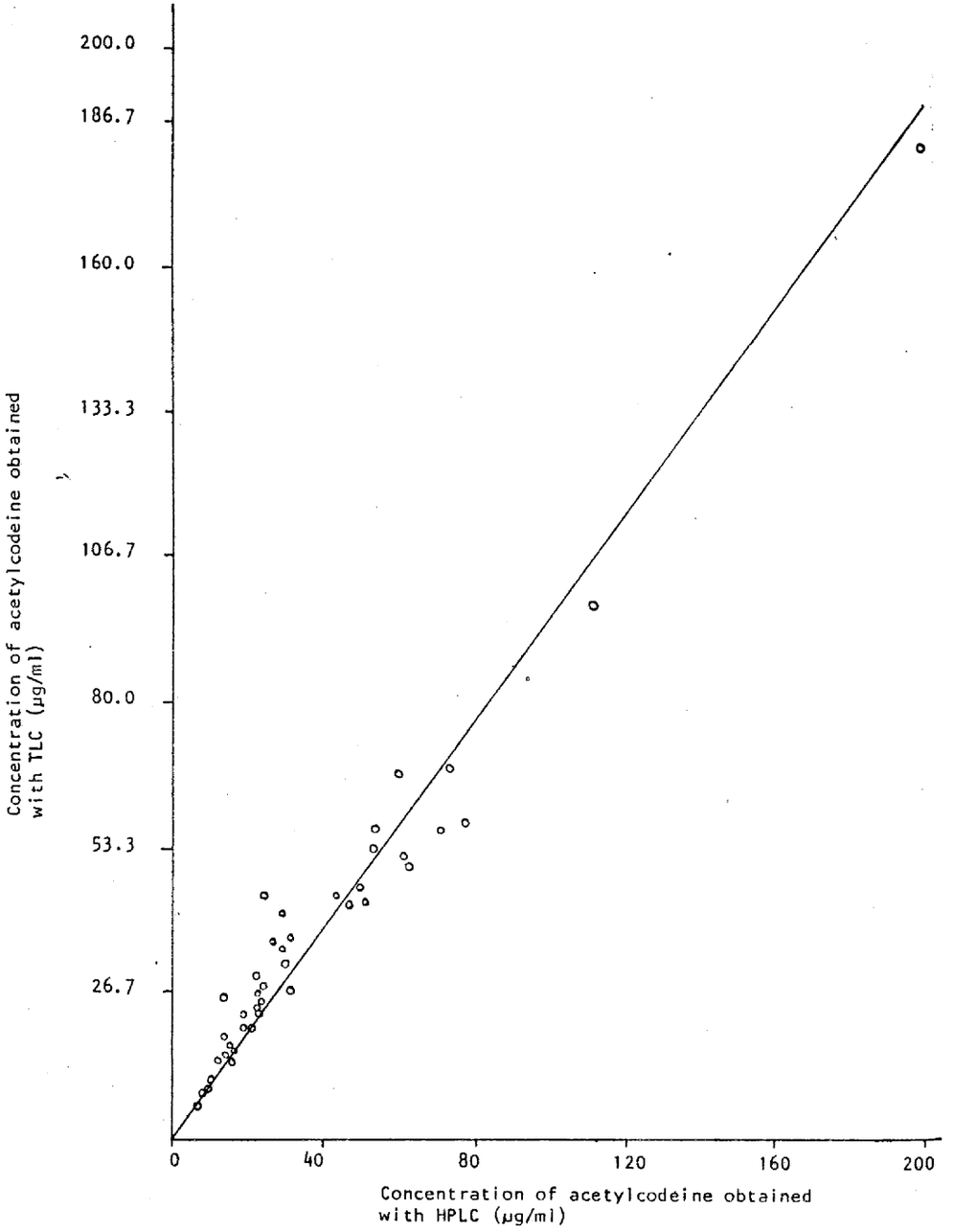


Fig. 3.12b: Correlation plot of data obtained from TLC versus HPLC for acetylcodeine concentrations in the illicit heroin samples

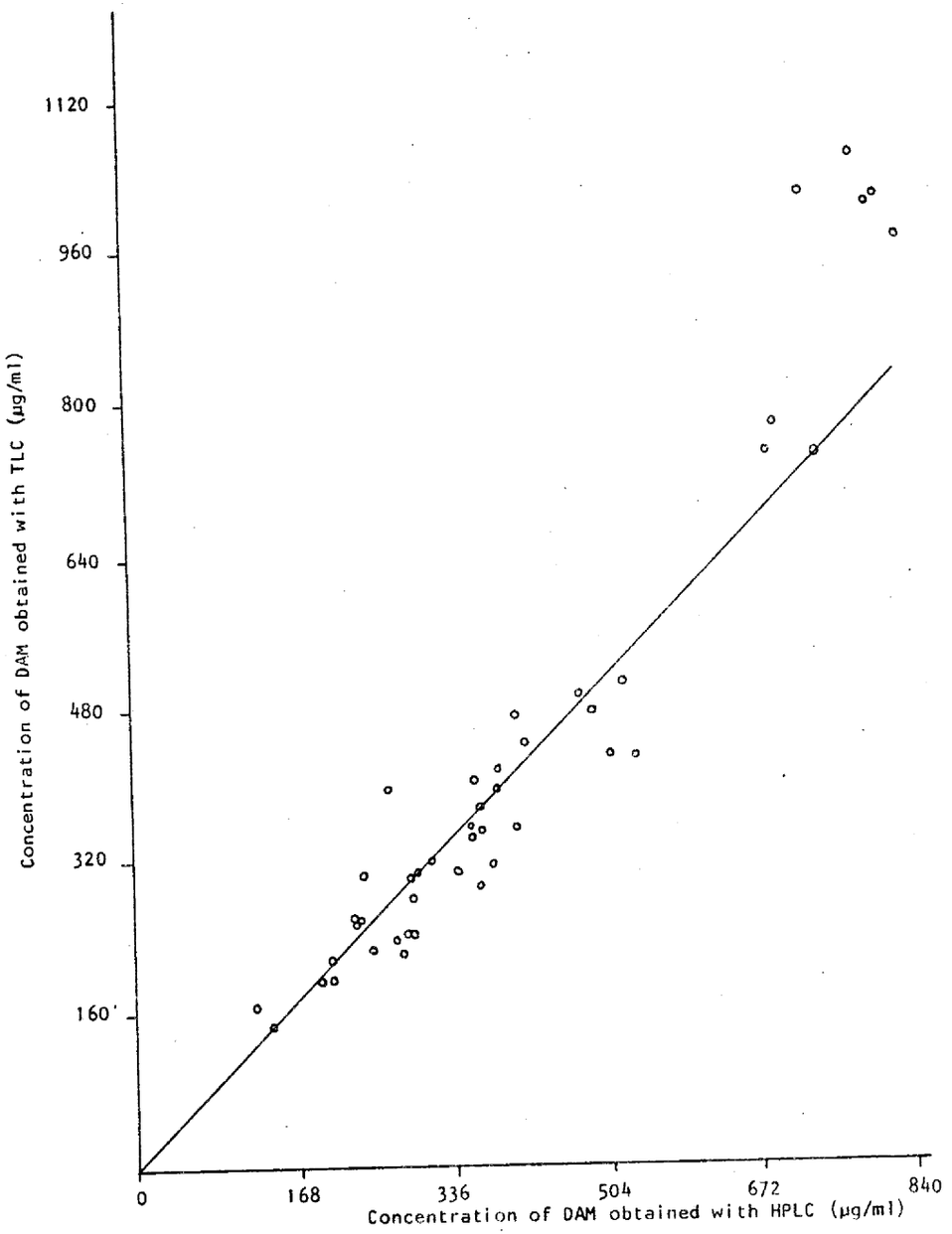


Fig. 3.12c Correlation plot of data obtained from TLC versus HPLC for DAM concentrations in the illicit heroin samples

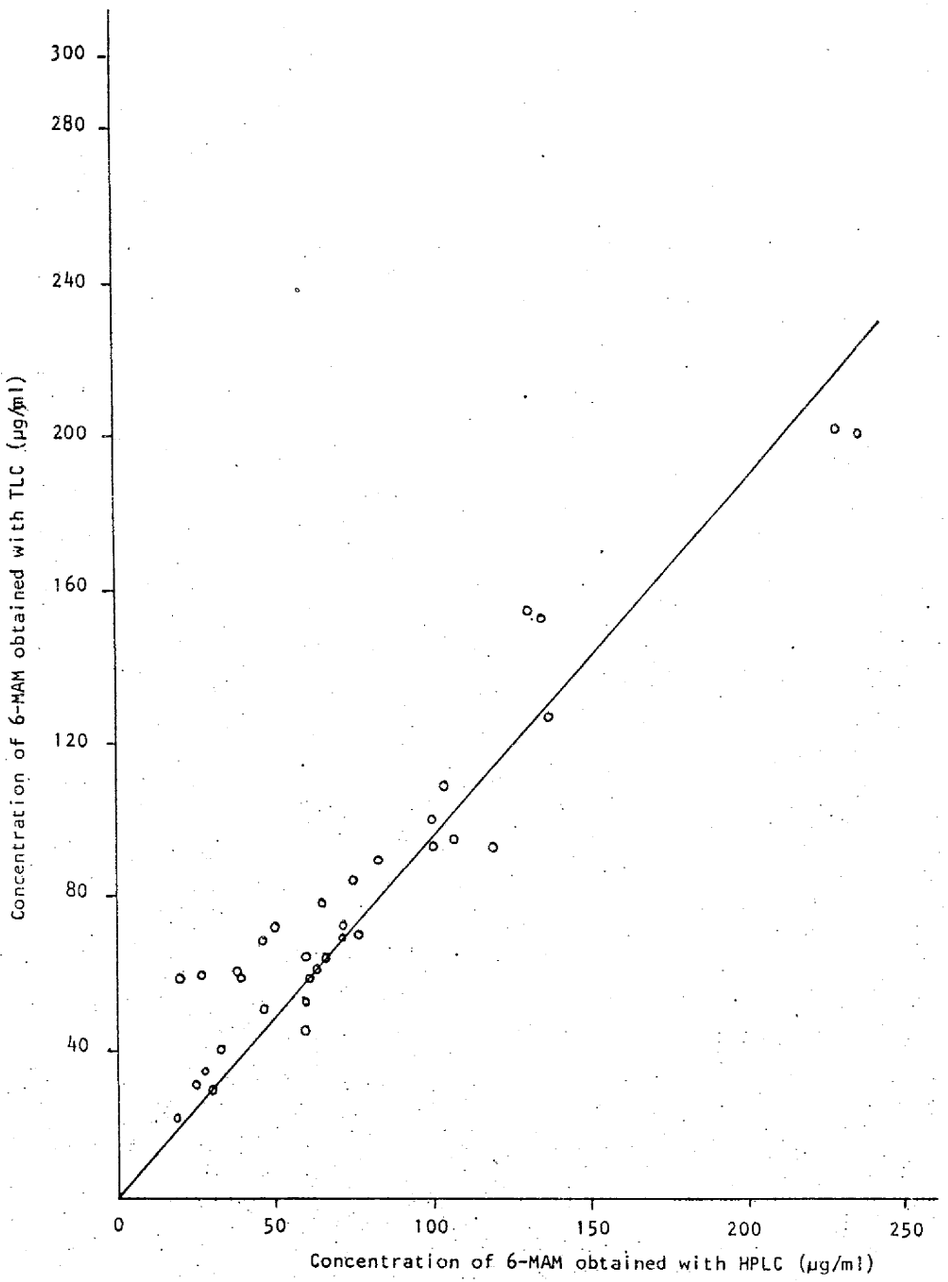


Fig. 3.12d: Correlation plot of data obtained from TLC versus HPLC for 6-MAM concentrations in the illicit heroin samples

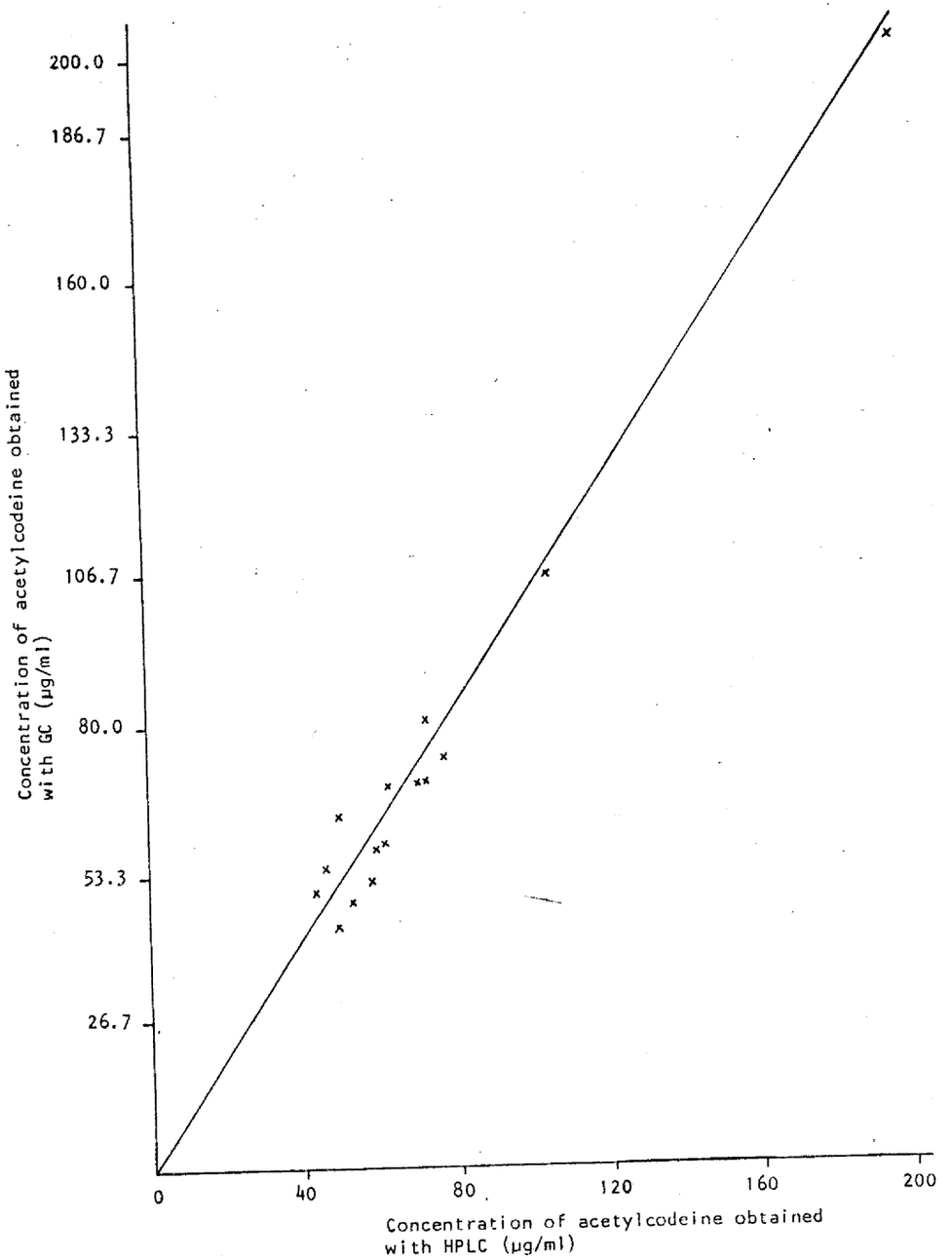


Fig. 3.12e: Correlation plot of data obtained from GC versus HPLC for Acetylcodeine concentrations in the illiict heroin samples

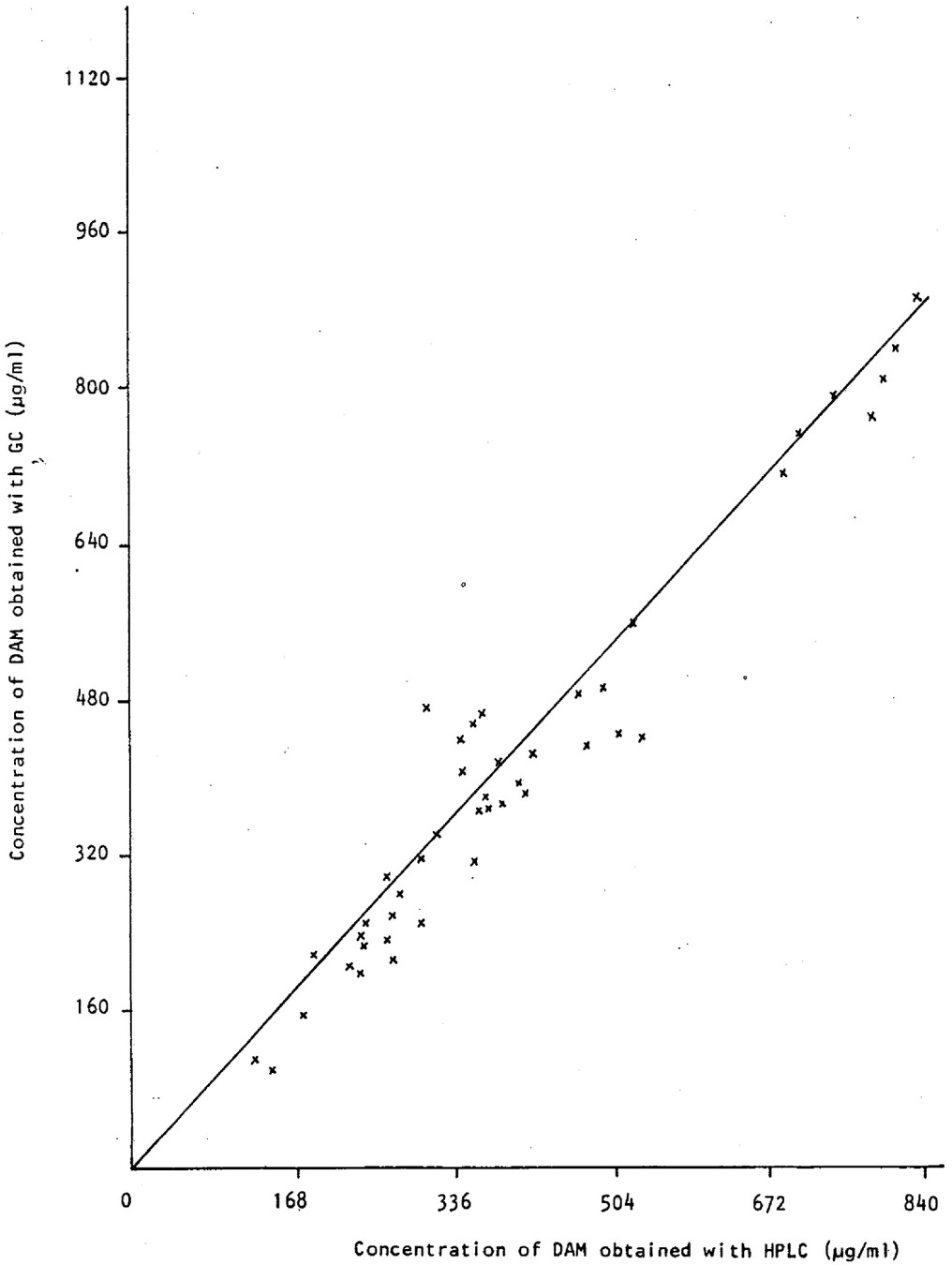


Fig. 3.12f: **Correlation plot of data obtained from GC versus HPLC for DAM concentrations in the illicit heroin samples**

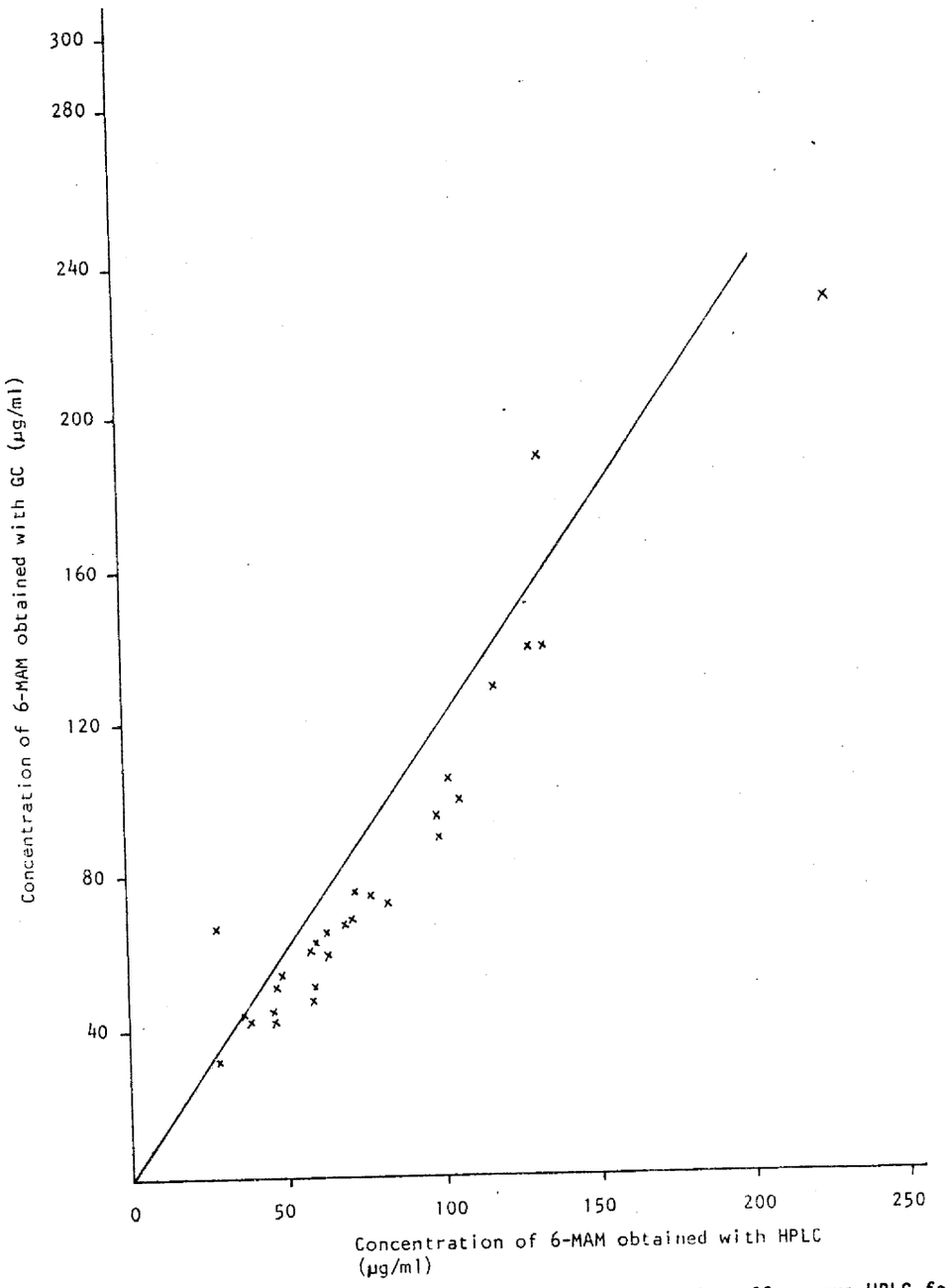


Fig. 3.12g: Correlation plot of data obtained from GC versus HPLC for 6-MAM concentrations in the illicit heroin samples

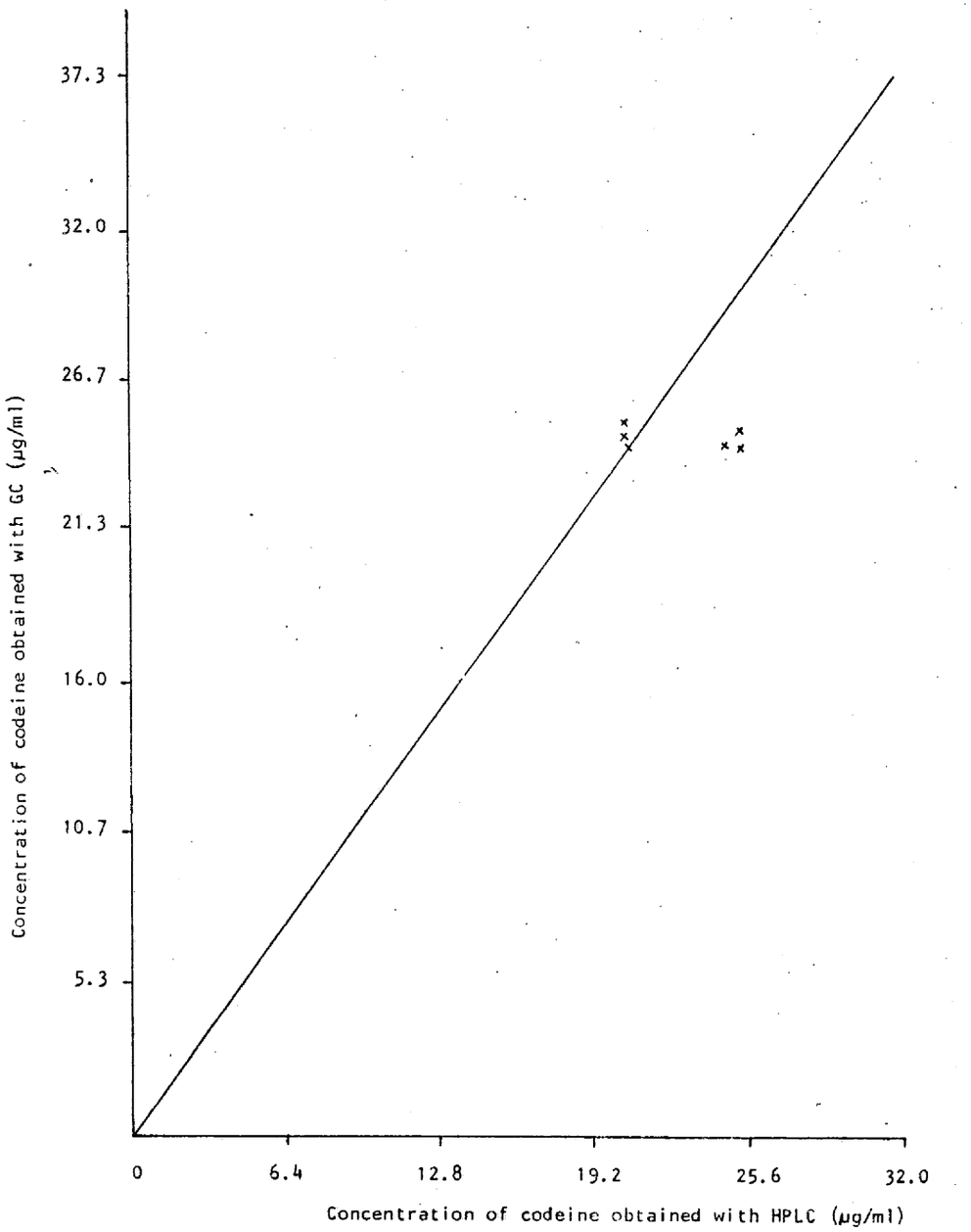


Fig. 3.12h: **Correlation plot of data obtained from GC versus HPLC for Codeine concentrations in the illicit heroin samples**

Table 3.21

Grouping of the 50 Illicit Heroin Samples Based on HPLC Analysis

(Values given are relative peak-height percentages)

Group	No. of Samples	DAM	6-MAM	Acetylcodeine	Morphine	Codeine
1	2	82.8 - 87.9	1.3 - 3.9	6.4 - 7.7	1.4 - 4.3	1.7 - 2.6
2	4	82.2 - 94.4	1.2 - 8.2	1.3 - 8.9	2.6 - 5.1	-
3	7	76.3 - 93.4	1.5 - 11.8	2.9 - 13.8	-	2.1 - 6.4
4	2	-	58.9 - 70.5	3.8 - 6.5	13.7 - 28.6	8.6 - 9.2
5	29	59.2 - 93.2	1.4 - 32.1	1.6 - 21.6	-	-
6	1	-	76.8	16.8	-	6.5
7	2	75.5 - 81.8	18.8 - 24.5	-	-	-
8	1	78.1	-	21.9	-	-
9	1	-	100.0	-	-	-
10	1	-	-	100.0	-	-

Based on the characterization of O'Neil, it was possible to deduce the origin of some of the samples. The 29 samples in group 5 had only DAM (59.2 to 93.2%), 6-MAM (1.4 to 32.1%) and acetylcodeine (1.6 to 21.6%). The 2 samples in group 7 had only DAM (75.5 to 81.8%) and 6-MAM (18.8 to 24.5%). The analysis of 14 samples of Indian origin by O'Neil showed presence of DAM (46.5 to 91.8%) and 6-MAM (0.3 to 9.8%) in all the 14 samples, and acetylcodeine (0.9 to 8.9%) in 12 samples. Morphine, codeine, noscapine and papaverine were not detected in any of the samples. The analysis of 20 samples of South East Asian origin by O'Neil also showed presence of only DAM (55.8 to 95.0%), 6-MAM (0.8 to 29.1%) and acetylcodeine (2.4 to 15.2%) in all the samples with exception of only 1 sample which had 1% papaverine and noscapine. Out of 94 samples from Pakistan analysed by O'Neil, 33 samples showed presence of only DAM (40.4 to 97.0%), 6-MAM (0.5 to 21.6%) and acetylcodeine (1.0 to 10.9%). Therefore, the 31 samples in groups 5 and 7 could be from India, South East Asia or Pakistan.

The 2 samples in group 1 showed similar qualitative analysis results as 1 sample from Pakistan. In addition, the 4 samples in group 2 also showed similar qualitative analysis results as 5 samples from Pakistan. Thus the qualitative and quantitative analysis results showed that the chemical profile of 29 samples in group 5 were typical of those from South East Asia, 6 samples of group 1 and 2 showed profiles unique to some of the Pakistan samples and 2 samples in group 7 showed profiles unique to some of the Indian samples. However, none of the samples analysed by O'Neil yielded a chemical profile similar to the 8 samples in groups 3 and 6.

Narayanaswami [18] also proposed a method for determining the source of supply or origin of illicit heroin samples. In his analysis, Narayanaswami observed that the ratio of DAM to acetylcodeine increased approximately two-fold at each stage of conversion in the series opium - morphine - heroin. He found that a correspondence of 1:3.8 existed between the morphine to codeine ratio in opium and their respective acetylated products in clandestine heroin samples. With this observation, Narayanaswami obtained values for the ratio of DAM to acetylcodeine for illicit heroin samples using the conversion factor 3.8 for opium samples of known geographical origin. A correlation between the theoretical ratios of DAM to acetylcodeine for opium samples of known origin and the experimental ratios of DAM to acetylcodeine for illicit heroin samples from various countries and areas was then established. The results showed significant variations in DAM to acetylcodeine ratios of samples from different geographical areas.

In order to verify Narayanaswami's observation, the experimental ratios of DAM to acetylcodeine were determined for the 43 illicit heroin samples which contained both these compounds. Based on the correlation data proposed by Narayanaswami, the origins of the illicit heroin samples were deduced [Table 3.22a].

The results showed that of the 43 samples, only 24 samples originated from Asian countries namely India (4 samples), Hong Kong (3 samples), China (4 samples), Afghanistan (3 samples), Pakistan (3

Table 3.22a

Classification of the 50 Illicit Heroin Samples Based On Narayanaswami's and O'Neil's Methods

Group	Total samples	Sample No.	DAM/ acetylcodeine ratio	Country (Narayanaswami's method)	Country (O'Neil's method)
1	2	14	12.9	India	Pakistan
		23	11.4	Hong Kong	
2	4	16	25.6	Laos	Pakistan
		17	9.2	-	
		19	18.6	Singapore	
		25	75.5	-	
3	7	1	5.5	-	
		4	12.7	India	
		5	10.8	Hong Kong	
		10	23.2	Mexico	
		15	12.8	India	
		21	31.8	Turkey	
		30	35.4	-	
4	2	2	-	-	
		3	-		
5	29	7	7.4	-	South East Asia
		8	7.8	China (Manchuria)	
		9	4.7	-	

Group	Total Samples	Sample No.	DAM/ acetylcodeine ratio	Country (Narayanaswami's method)	Country (O'Neil's method)
		13	15.9	Singapore	
		20	14.5	Pakistan	
		22	8.7	China (Manchuria)	
		24	22.9	Mexico	
		26	14.7	Pakistan	
		27	3.6	-	
		28	41.5	-	
		29	22.5	Afghanistan	
		31	23.9	Mexico	
		33	5.8	-	
		34	7.5	-	
		35	5.4	-	South East Asia
		36	21.7	Afghanistan	
		37	17.2	Singapore	
		38	8.6	China (Manchuria)	
		39	29.7	Turkey	
		40	16.9	Singapore	
		41	15.9	Singapore	
		42	12.4	India	
		43	7.8	China (Manchuria)	
		44	33.7	Vietnam	
		46	14.7	Pakistan	
		47	6.8	-	
		48	11.5	Hong Kong	
		49	20.1	Afghanistan	
		50	55.3	-	
6	1	12	-		-

Group	Total Samples	Sample No.	DAM/ acetylcodeine ratio	Country (Narayanaswami's method)	Country (O'Neil's method)
7	2	32	-		India
		45	-		
8	1	18	3.6	-	
9	1	11	4.7	-	
10	1	6	-		

samples), Singapore (5 samples), Laos (1 sample) and Vietnam (1 sample). 3 samples originated from Mexico and 2 samples from Turkey. The remaining 14 samples yielded ratios which did not agree with any of the samples from the countries examined by Narayanaswami.

The 29 samples in group 5 which yielded a chemical profile typical of South East Asia samples showed that only 4 samples were of South East Asian origin (Singapore) by Narayanaswami's method. The other samples in group 5 showed they originated from China (4 samples), Pakistan (3 samples), Afghanistan (3 samples), India (1 sample), Vietnam (1 sample), Hong Kong (1 sample), Turkey (1 sample) and Mexico (2 samples). The origin of the remaining 9 samples in group 5 could not be deduced. The origin of the 2 samples in group 7 which showed a chemical profile similar to Indian samples could not be deduced by Narayanaswami's method. Similarly, although the 6 samples in groups 1 and 2 yielded a chemical profile similar to samples from Pakistan Narayanaswami's method indicated they had originated from other countries.

The 20 illicit heroin samples of South East Asian origin analysed by O'Neil was also subjected to Naranayanaswami's method (Table 3.22b). The results showed that only 3 samples were from Singapore and 1 sample from Vietnam. The remaining samples originated from India (3 samples), Hong Kong (4 samples), China (4 samples), Pakistan (1 sample), Afghanistan (1 sample) and Japan (1 sample). The origin of the remaining 2 samples could not be deduced.

It is thus clear that although all 50 illicit heroin samples were seized in Malaysia, Narayanaswami's method indicated that only 17% of the samples were of South East Asian origin. Similarly of the 20 samples from South East Asia analysed by O'Neil, only 17% of the samples indicated South East origin by Narayanaswami's method. However, prior history of the 59 illicit heroin samples such as information relating to the nature and period of storage of the seized samples were not provided by the police. Some investigators have reported that prolonged storage of some samples resulted in hydrolysis of DAM to 6-MAM. There is however a difference in opinion as to the origin of the monoacetylmorphines present in heroin samples. Some workers have attributed the occurence of 6-MAM to hydrolysis of heroin during salt formation and to storage conditions of heroin [74, 104, 125, 167-169]. Davey and Murray [170] reported that the decomposition of heroin proceeded mainly through the formation of 6-MAM, whereas other workers were of opinion that 6-MAM originated from incomplete acetylation of morphine [74, 135]. However, Moore and Klein [28] found that incomplete acetylation did not lead to 6-MAM but to 3-MAM and that 6-MAM was mainly a product formed by hydrolysis of heroin. In view of this breakdown process, the experimental ratio of DAM to acetylcodeine determined for the illicit heroin samples may not be a true reflection of the ratio which occurred during the preparation of the illicit heroin samples. Furthermore, Narayanaswami had obtained a ratio of DAM to acetylcodeine from opium samples seized in Singapore which is a non-cultivating opium country.

Table 3.22b

Classification of the 20 of O'Neil's Illicit Heroin Samples of South East Asian Origin by Narayanaswami's Method

Group	Total samples	Sample no.	Dam/ acetylcodeine ratio	Country (Narayanaswami's method)
Illicit heroin sample analysed by O'Neil - South East Asian origin	20	196	15.2	Singapore
		197	4.6	-
		198	5.1	-
		199	14.5	Pakistan
		200	12.3	India
		201	22.7	Afghanistan
		202	34.5	Vietnam
		203	18.0	Singapore
		204	9.4	Hong Kong
		205	9.3	Hong Kong
		206	9.8	Hong Kong
		207	6.2	China
		208	7.3	China
		209	12.1	India
		210	6.6	China
		211	10.9	Hong Kong
		212	13.8	India
		213	19.7	Japan
		214	6.3	China
		215	18.1	Singapore

3.4.1 Conclusion

It can be concluded from Table 3.23 that majority of the illicit heroin samples analysed had a ratio of DAM/acetylcodeine in the range 1 to 20 and this was also observed for the majority of South East Asian samples analysed by O'Neil. However when the qualitative and quantitative analysis results of the 50 illicit heroin samples were compared with the samples analysed by O'Neil, it was found that 30 samples in groups 5 and 8 had a typical chemical profile as for samples that originated from South East Asia. Eight samples in groups 1, 2 and 4 showed a profile similar to a few samples from Pakistan and 3 samples in groups 7 and 9 showed profile similar to samples from India. However, none of the samples analysed by O'Neil showed a chemical profile as similar to the 8 samples in groups 3 and 6. The sample in group 10 could not be considered as an illicit heroin sample as it only contained acetylcodeine.

Table 3.23

Number of Samples in Specific Ranges of DAM/Acetylcodeine Ratio

Range of DAM/ acetylcodeine ratio	50 Seized Samples	29 Samples of Group 5	20 O'Neil's South East Asian Samples
1 - 10	15	11	9
11 - 20	16	10	9
21 - 30	7	5	1
31 - 40	3	1	1
41 - 50	1	1	0
51 - 60	1	1	0
61 - 70	0	0	0
71 - 80	1	0	0

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Table A1

ε^o, n_b and density values for the solvents used in the TLC systems 1, 4 and 5 [142, 145]

Solvent	ε^o	n_b (nm^2)	Density (gcm^{-3})
n-Hexane	0.01	6.0	0.659
Chloroform	0.40	4.4	1.492
Triethylamine	0.48	5.8	0.726
Diethylamine	0.63	7.5	0.707

Table A2

Average spot area values for each opiate at various concentrations by the TLC system

Opiate	Concentration ($\mu g\ ml^{-1}$)	Average Spot Area ($unit^2$)
1. Narceine	30	141
	62	253
	125	395
	250	728
	500	1155
	1000	2336
2. Morphine	30	88
	62	130
	125	256
	250	572
	500	1062
	1000	2064
3. Nalorphine	30	46
	62	98
	125	206
	250	482
	500	994
	1000	1893
4. Codeine	30	45
	62	97
	125	207
	250	405
	500	834
	1000	1638
5. 6-MAM	30	46
	62	127
	125	251
	250	527
	500	997
	1000	1933

Opiate	Concentration ($\mu g\ ml^{-1}$)	Average Spot Area ($unit^2$)
6. DAM	30	37
	62	83
	125	165
	250	362
	500	711
	1000	1424
7. Acetylcodeine	30	45
	62	80
	125	160
	250	362
	500	722
	1000	1323
8. Thebaine	1000	7079
	2000	14208
	3000	21243
	4000	28101
	5000	33884
9. Papaverine	1000	3955
	2000	10830
	3000	14100
	4000	18710
	5000	24477
10. Levallorphan	125	46
	250	96
	500	202
	1000	414
11. Noscapine	30	75
	62	162
	125	341
	250	700
	500	1362
	1000	2620
12. Acetylthebaol	1000	5950
	2000	12246
	3000	15302
	4000	22105
	5000	27200

Table A3

Average Peak Height Values for Each Opiate at Various Concentrations by the HPLC System

Opiate	Concentration (µg/ml)	Average Peak Height (unit)
Morphine	4.0	4,643
	8.0	5,397
	16.0	10,797
	31.0	20,932
	62.0	46,299
	125.0	81,589
	250.0	163,934
	500.0	371,881
	1000.0	599,876
6-MAM	4.0	3,427
	8.0	5,857
	16.0	9,814
	31.0	18,844
	62.0	38,498
	125.0	69,696
	250.0	142,145
	500.0	310,809
	1000.0	512,720
Codeine	4.0	2,086
	8.0	3,889
	16.0	6,410
	31.0	14,109
	62.0	31,059
	125.0	50,415
	250.0	106,476
	500.0	200,218
	1000.0	379,982

Opiate	Concentration (µg/ml)	Average Peak Height (unit)
DAM	4.0	1,801
	8.0	3,110
	16.0	5,167
	31.0	11,159
	62.0	22,538
	125.0	47,875
	250.0	87,782
	500.0	198,019
	1000.0	325,594
Papaverine	4.0	8,774
	8.0	17,836
	16.0	34,421
	31.0	65,448
	62.0	146,039
	125.0	303,651
	250.0	560,848
	500.0	1,059,097
	1000.0	1,989,250
Acetylcodeine	4.0	892
	8.0	1,201
	16.0	3,200
	31.0	13,756
	125.0	28,848
	250.0	52,539
	500.0	117,503
	1000.0	192,176
Thebaine	4.0	3,474
	8.0	6,229
	16.0	12,059
	31.0	25,056
	62.0	50,279
	125.0	108,351
	250.0	200,318
	500.0	452,505
	1000.0	753,840

Opiate	Concentration (μg/ml)	Average Peak Height (unit)
Noscapine	4.0	1,211
	8.0	3,843
	16.0	6,877
	31.0	14,777
	62.0	30,048
	125.0	61,896
	250.0	110,600
	500.0	272,651
	1000.0	409,610
Acetylthebaol	10.0	64,400
	20.0	129,580
	40.0	255,020
	60.0	383,500
	80.0	511,880
	100.0	643,100

Table A4

Average Peak Area Values for Each Opiate at Various Concentration on Full Scan Mode by the GC System

	Concentration (μg/ml)	Average Peak Area (unit^2)
Levallorphan	100.0	29,730
	200.0	89,719
	300.0	98,290
	400.0	128,330
	500.0	134,102
	600.0	166,726
	750.0	225,200
	1000.0	327,628
Codeine	100.0	17,292
	200.0	39,735
	300.0	57,431
	400.0	73,212
	500.0	108,247
	600.0	139,232
	750.0	143,727
	1000.0	204,677
Morphine	200.0	6,804
	300.0	10,213
	400.0	12,026
	500.0	16,143
	600.0	18,090
	750.0	24,079
	1000.0	32,269
Acetylcodeine	100.0	72,842
	200.0	166,771
	300.0	248,954
	400.0	302,512
	500.0	312,927
	600.0	385,602
	750.0	454,298
	1000.0	669,966

	Concentration (µg/ml)	Average Peak Area (unit^2)
6-MAM	100.0	43,971
	200.0	80,738
	300.0	130,157
	400.0	158,575
	500.0	243,285
	600.0	264,726
	750.0	289,335
	1000.0	396,442
Thebaine	100.0	13,105
	200.0	26,010
	300.0	42,724
	400.0	54,037
	500.0	69,091
	600.0	84,031
	750.0	102,159
	1000.0	136,075
Nalorphine	200.0	11,690
	300.0	18,036
	400.0	23,691
	500.0	32,054
	600.0	36,086
	750.0	43,057
	1000.0	58,477
DAM	100.0	84,627
	200.0	219,281
	300.0	265,337
	400.0	332,260
	500.0	384,820
	600.0	503,170
	750.0	663,324
	1000.0	884,342

	Concentration (µg/ml)	Average Peak Area (unit^2)
Papaverine	100.0	75,696
	200.0	217,996
	300.0	326,703
	400.0	394,508
	500.0	490,196
	600.0	620,989
	750.0	791,054
	1000.0	1,083,391
Noscapine	200.0	6,323
	300.0	8,862
	400.0	11,301
	500.0	15,394
	600.0	18,300
	750.0	23,717
	1000.0	30,490
Narceine	200.0	6,714
	300.0	10,851
	400.0	16,262
	500.0	18,303
	600.0	22,823
	750.0	26,267
	1000.0	37,582
Acetylthebaol	100.0	151,866
	200.0	218,438
	300.0	471,745
	500.0	824,523
	800.0	1,421,386
	900.0	1,528,184
	1000.0	1,717,628

Table A5

Average Peak Area Values for Each Opiate at Various Concentration on MID Mode by the GC System

Opiate	Concentration (μg/ml)	Average Peak Area ($unit^2$)
Levallorphan	0.8	2995
	0.9	3371
	1.0	3857
	1.5	5285
	2.0	7781
Codeine	0.6	2383
	0.7	2936
	0.8	3122
	0.9	3610
	1.0	4108
Morphine	2.0	2114
	2.5	2701
	3.0	3102
	3.5	3718
	4.0	4473
Acetylcodeine	0.4	3882
	0.5	4826
	0.6	5747
	0.7	6910
	0.8	7882
6-MAM	0.6	3744
	0.7	4419
	0.8	4980
	0.9	5478
	1.0	6199
Thebaine	0.8	2256
	0.9	2763
	1.0	2945
	1.5	4547
	2.0	5580

Opiate	Concentration (μg/ml)	Average Peak Area ($unit^2$)
Nalorphine	2.0	3015
	2.5	3765
	3.0	4482
	3.5	5660
	4.0	6114
DAM	0.4	2969
	0.5	3692
	0.6	4104
	0.7	4882
	0.8	5530
Papaverine	0.4	2346
	0.5	2960
	0.6	3700
	0.7	4234
	0.8	4715
Noscapine	5.0	2029
	6.0	2516
	7.0	2940
	8.0	3357
	10.0	4185
Narceine	5.0	3210
	6.0	3816
	7.0	4741
	8.0	5265
	10.0	6319

Table A6

TLC Analysis Results of 50 Illicit Heroin Samples

SAMPLE NO.	CONCENTRATION (µg/ml)				RELATIVE OPIATE COMPOSITION (%)			
	MORFINE	6-MAM	DAM	ACETYLCODEINE	MORFINE	6-MAM	DAM	ACETYLCODEINE
1	-	-	421.0	70.0	-	-	85.7	14.3
2	85.0	190.0	-	-	30.9	69.1	-	-
3	43.0	299.0	-	-	12.6	87.4	-	-
4	-	41.0	463.0	-	-	8.1	91.9	-
5	-	-	490.0	50.0	-	-	90.7	9.3
6	-	-	-	-	-	-	-	-
7	-	62.0	383.0	64.0	-	12.2	75.2	12.6
8	-	-	208.0	-	-	-	100.0	-
9	-	54.0	263.0	58.0	-	14.4	70.1	15.5
10	-	44.0	318.0	-	-	12.2	87.9	-

SAMPLE NO.	CONCENTRATION (µg/ml)				RELATIVE OPIATE COMPOSITION (%)			
	MORFINE	6-MAM	DAM	ACETYLCODEINE	MORFINE	6-MAM	DAM	ACETYLCODEINE
11	-	232.0	-	-	-	100.0	-	-
12	-	140.0	-	-	-	100.0	-	-
13	-	42.0	474.0	-	-	8.1	91.7	-
14	41.0	43.0	813.0	59.0	4.2	4.5	85.1	6.2
15	-	-	850.0	70.0	-	-	92.4	7.6
16	43.0	70.0	800.0	-	4.7	7.7	87.6	-
17	-	42.0	790.0	75.0	-	4.6	87.1	8.3
18	-	-	385.0	105.0	-	-	78.6	21.4
19	-	-	319.0	-	-	-	100.0	-
20	-	130.0	347.0	-	-	27.3	72.8	-

SAMPLE NO.	CONCENTRATION (µg/ml)				RELATIVE OPIATE COMPOSITION (%)			
	MORFINE	6-MAM	DAM	ACETYLCODEINE	MORFINE	6-MAM	DAM	ACETYLCODEINE
21	-	-	820.0	-	-	-	100.0	-
22	-	-	440.0	52.0	-	-	89.4	10.6
23	-	-	900.0	82.0	-	-	91.7	8.4
24	-	74.0	567.0	-	-	11.5	88.5	-
25	-	-	720.0	-	-	-	100.0	-
26	-	75.0	255.0	-	-	22.7	77.3	-
27	-	-	760.0	205.0	-	-	78.8	21.2
28	-	-	476.0	-	-	-	100.0	-
29	-	100.0	443.0	-	-	18.4	81.6	-
30	-	-	100.0	-	-	-	100.0	-

SAMPLE NO.	CONCENTRATION (µg/ml)				RELATIVE OPIATE COMPOSITION (%)			
	MORFINE	6-MAM	DAM	ACETYLCODEINE	MORFINE	6-MAM	DAM	ACETYLCODEINE
31	-	65.0	112.0	-	-	36.7	63.3	-
32	-	-	-	-	-	-	-	-
33	-	67.0	432.0	70.0	-	11.8	75.9	12.3
34	-	68.0	411.0	54.0	-	12.8	77.1	10.1
35	-	110.0	300.0	44.0	-	24.2	66.1	9.7
36	-	-	449.0	-	-	-	100.0	-
37	-	68.0	247.0	-	-	21.6	78.4	-
38	-	51.0	220.0	-	-	18.8	81.2	-
39	-	51.0	399.0	-	-	11.3	88.7	-
40	-	-	499.0	-	-	-	100.0	-

SAMPLE NO.	CONCENTRATION (µg/ml)				RELATIVE OPIATE COMPOSITION (%)			
	MORFINE	6-MAM	DAM	ACETYLCODEINE	MORFINE	6-MAM	DAM	ACETYLCODEINE
41	-	96.0	374.0	-	-	20.4	79.6	-
42	-	66.0	239.0	-	-	21.6	78.4	-
43	-	45.0	383.0	48.0	-	9.45	80.5	10.1
44	-	60.0	251.0	-	-	19.3	80.7	-
45	-	73.0	227.0	-	-	24.3	75.7	-
46	-	140.0	250.0	-	-	35.9	64.1	-
47	-	90.0	156.0	-	-	36.6	63.4	-
48	-	-	232.0	-	-	-	100.0	-
49	-	47.0	214.0	-	-	18.0	82.0	-
50	-	-	203.0	-	-	-	100.0	-

Table A7

HPLC Analysis Results of 50 Illicit Heroin Samples

SAMPLE NO.	CONCENTRATION (µg/ml)					RELATIVE OPIATE COMPOSITION (%)				
	MORFINE	6-MAM	CODEINE	DAM	ACETYLCODEINE	MORFINE	6-MAM	CODEINE	DAM	ACETYLCODEINE
1	-	30.4	20.6	392.3	71.1	-	5.9	4.0	76.3	13.8
2	89.6	184.4	27.0	-	11.8	28.7	58.9	8.6	-	3.8
3	45.6	235.2	30.8	-	21.8	13.7	70.5	9.2	-	6.5
4	-	34.8	11.6	395.6	31.1	-	7.4	2.5	83.6	6.6
5	-	27.9	20.7	478.7	44.5	-	4.9	3.6	83.7	7.8
6	-	-	-	-	16.9	-	-	-	-	100.0
7	-	65.9	-	378.9	50.9	-	13.3	-	76.4	10.3
8	-	28.8	-	214.6	27.5	-	10.6	-	79.21	10.2
9	-	49.4	-	282.9	60.7	-	12.6	-	72.0	15.5
10	-	46.6	25.3	309.1	13.3	-	11.8	6.4	78.4	3.4

SAMPLE NO.	CONCENTRATION (µg/ml)					RELATIVE OPIATE COMPOSITION (%)				
	MORFINE	6-MAM	CODEINE	DAM	ACETYLCODEINE	MORFINE	6-MAM	CODEINE	DAM	ACETYLCODEINE
11	-	223.2	-	-	-	-	100.0	-	-	-
12	-	135.8	11.4	-	29.7	-	76.8	6.5	-	16.8
13	-	40.0	-	379.6	23.9	-	9.0	-	85.6	5.4
14	41.8	38.3	24.8	803.3	62.3	4.3	3.9	2.6	82.8	6.4
15	-	20.4	20.7	812.9	63.3	-	2.2	2.3	88.6	6.9
16	45.6	73.6	-	749.6	29.3	5.1	8.2	-	83.5	3.3
17	30.0	47.5	-	716.7	78.0	3.4	5.4	-	82.2	8.9
18	-	-	-	397.7	111.7	-	-	-	78.1	21.9
19	10.6	20.0	-	365.7	19.5	2.6	4.8	-	88.0	4.7
20	-	120.6	-	324.2	22.4	-	25.8	-	69.4	4.8

SAMPLE NO	CONCENTRATION (µg/ml)					RELATIVE OPIATE COMPOSITION (%)				
	MORFINE	6-MAM	CODEINE	DAM	ACETYLCODEINE	MORFINE	6-MAM	CODEINE	DAM	ACETYLCODEINE
21	-	12.8	18.1	790.6	24.9	-	1.5	2.1	93.4	2.9
22	-	15.9	-	486.0	55.8	-	2.9	-	87.1	10.0
23	12.9	12.7	16.4	839.6	73.6	1.4	1.3	1.7	87.9	7.7
24	-	78.1	-	534.1	23.3	-	12.3	-	84.1	3.7
25	23.3	8.7	-	693.2	9.2	3.2	1.2	-	94.4	1.3
26	-	72.0	-	277.4	18.8	-	19.5	-	75.3	5.1
27	-	12.5	-	710.9	199.1	-	1.4	-	77.1	21.6
28	-	19.8	-	353.3	8.5	-	5.2	-	92.6	2.2
29	-	106.8	-	352.2	15.6	-	22.5	-	74.2	3.3
30	-	14.2	8.2	149.8	4.2	-	8.0	4.6	84.9	2.4

SAMPLE NO	CONCENTRATION (µg/ml)					RELATIVE OPIATE COMPOSITION (%)				
	MORFINE	6-MAM	CODEINE	DAM	ACETYLCODEINE	MORFINE	6-MAM	CODEINE	DAM	ACETYLCODEINE
31	-	62.4	-	132.1	5.5	-	31.2	-	66.0	2.8
32	-	6.8	-	30.8	-	-	18.2	-	81.8	-
33	-	70.8	-	428.3	74.1	-	12.3	-	74.7	12.9
34	-	62.0	-	354.9	47.3	-	13.4	-	76.5	10.2
35	-	103.2	-	271.7	50.4	-	24.3	-	63.9	11.9
36	-	13.4	-	499.8	23.1	-	2.5	-	93.2	4.3
37	-	71.7	-	257.6	15.0	-	20.8	-	74.8	4.4
38	-	60.1	-	195.5	22.8	-	21.6	-	70.2	8.2
39	-	49.0	-	415.0	14.0	-	10.2	-	86.8	2.9
40	-	15.6	-	525.8	31.2	-	2.7	-	91.8	5.4

SAMPLE NO.	CONCENTRATION (µg/ml)					RELATIVE OPIATE COMPOSITION (%)				
	MORFINE	6-MAM	CODEINE	DAM	ACETYLCODEINE	MORFINE	6-MAM	CODEINE	DAM	ACETYLCODEINE
41	-	100.1	-	372.8	23.5	-	20.2	-	75.1	4.7
42	-	79.3	-	244.8	19.7	-	23.1	-	71.2	5.7
43	-	60.7	-	419.9	53.8	-	11.4	-	78.6	10.1
44	-	64.1	-	247.2	7.3	-	20.1	-	77.6	2.3
45	-	81.6	-	251.0	-	-	24.5	-	75.5	-
46	-	130.8	-	307.5	20.9	-	28.5	-	67.0	4.6
47	-	99.3	-	182.9	27.0	-	32.1	-	59.2	8.8
48	-	31.0	-	278.3	24.1	-	9.3	-	83.5	7.2
49	-	59.6	-	282.2	14.0	-	16.8	-	79.3	3.9
50	-	27.1	-	243.3	4.4	-	9.9	-	88.5	1.6

Table A8

GC Analysis Results of 50 Illicit Heroin Samples

SAMPLE NO.	CONCENTRATION (µg/ml)				RELATIVE OPIATE COMPOSITION (%)			
	6-MAM	CODEINE	DAM	ACETYLCODEINE	6-MAM	CODEINE	DAM	ACETYLCODEINE
1	6.2	2.5	32.0	5.7	13.4	5.4	68.9	12.3
2	15.3	2.5	-	1.4	79.4	13.2	-	17.4
3	20.2	3.9	-	2.4	76.3	14.6	-	9.1
4	4.0	-	42.3	3.7	8.1	-	84.6	7.3
5	5.8	2.6	53.9	4.4	8.7	3.8	80.8	6.6
6	-	-	-	1.5	-	-	-	100.0
7	7.8	-	29.3	4.5	18.8	-	70.5	10.8
8	3.5	-	21.9	3.2	12.1	-	76.7	11.2
9	7.2	-	23.8	6.8	19.1	-	62.9	18.0
10	6.8	2.5	28.2	2.6	17.0	6.2	70.3	6.5

SAMPLE NO.	CONCENTRATION (µg/ml)				RELATIVE OPIATE COMPOSITION (%)			
	6-MAM	CODEINE	DAM	ACETYLCODEINE	6-MAM	CODEINE	DAM	ACETYLCODEINE
11	20.3	-	-	-	100.0	-	-	-
12	12.8	-	-	3.4	78.7	-	-	21.3
13	5.9	-	35.6	2.5	13.5	-	80.8	5.7
14	6.0	2.5	101.8	5.2	5.2	2.2	88.2	4.5
15	5.8	2.5	102.2	5.0	5.0	2.2	88.5	4.3
16	8.3	-	75.8	4.1	1.1	-	98.4	0.5
17	5.0	-	102.8	5.9	4.4	-	90.4	5.1
18	-	-	40.3	9.9	-	-	80.3	19.7
19	2.1	-	39.6	2.0	4.8	-	90.5	4.7
20	9.3	-	32.6	2.9	20.8	-	72.7	6.5

SAMPLE NO.	CONCENTRATION (µg/ml)				RELATIVE OPIATE COMPOSITION (%)			
	6-MAM	CODEINE	DAM	ACETYLCODEINE	6-MAM	CODEINE	DAM	ACETYLCODEINE
21	-	-	107.0	4.5	-	-	95.9	4.1
22	-	-	50.1	5.6	-	-	89.9	10.1
23	-	-	94.8	6.9	-	-	93.2	6.8
24	7.1	-	51.6	2.5	11.6	-	84.4	4.0
25	-	-	73.9	1.1	-	-	98.5	1.5
26	7.0	-	30.7	2.3	17.6	-	76.7	5.7
27	-	-	78.7	18.4	-	-	81.1	18.9
28	-	-	31.1	1.0	-	-	96.9	3.1
29	9.5	-	36.0	1.4	20.2	-	76.8	3.0
30	-	-	15.1	-	-	-	100.0	-

SAMPLE NO.	CONCENTRATION (µg/ml)				RELATIVE OPIATE COMPOSITION (%)			
	6-MAM	CODEINE	DAM	ACETYLCODEINE	6-MAM	CODEINE	DAM	ACETYLCODEINE
31	6.4	-	16.9	0.6	26.7	-	70.7	2.6
32	0.5	-	2.6	-	16.6	-	83.4	-
33	5.8	-	46.9	7.1	9.6	-	78.5	11.9
34	5.3	-	41.0	4.3	12.3	-	79.4	8.4
35	10.9	-	22.5	4.6	28.6	-	59.2	12.2
36	-	-	48.5	2.6	-	-	94.8	5.2
37	7.2	-	22.8	1.7	22.7	-	71.8	5.5
38	5.3	-	19.7	2.5	19.3	-	71.7	9.0
39	5.3	-	36.3	1.6	12.2	-	84.2	3.7
40	-	-	46.4	3.2	-	-	93.6	6.4

SAMPLE NO.	CONCENTRATION (µg/ml)				RELATIVE OPIATE COMPOSITION (%)			
	6-MAM	CODEINE	DAM	ACETYLCODEINE	6-MAM	CODEINE	DAM	ACETYLCODEINE
41	9.9	-	35.1	2.2	21.0	-	74.3	4.7
42	8.0	-	26.1	2.1	22.2	-	72.0	5.8
43	6.0	-	48.1	5.3	10.2	-	80.9	9.0
44	6.1	-	25.6	0.8	18.9	-	78.7	2.5
45	8.9	-	30.5	-	22.7	-	77.3	-
46	15.7	-	31.1	2.1	32.1	-	63.7	14.2
47	9.3	-	19.9	3.6	28.4	-	60.6	11.0
48	3.0	-	24.4	2.8	9.8	-	80.9	9.3
49	5.8	-	24.5	1.9	18.2	-	76.1	5.8
50	3.1	-	26.6	-	10.6	-	89.5	-

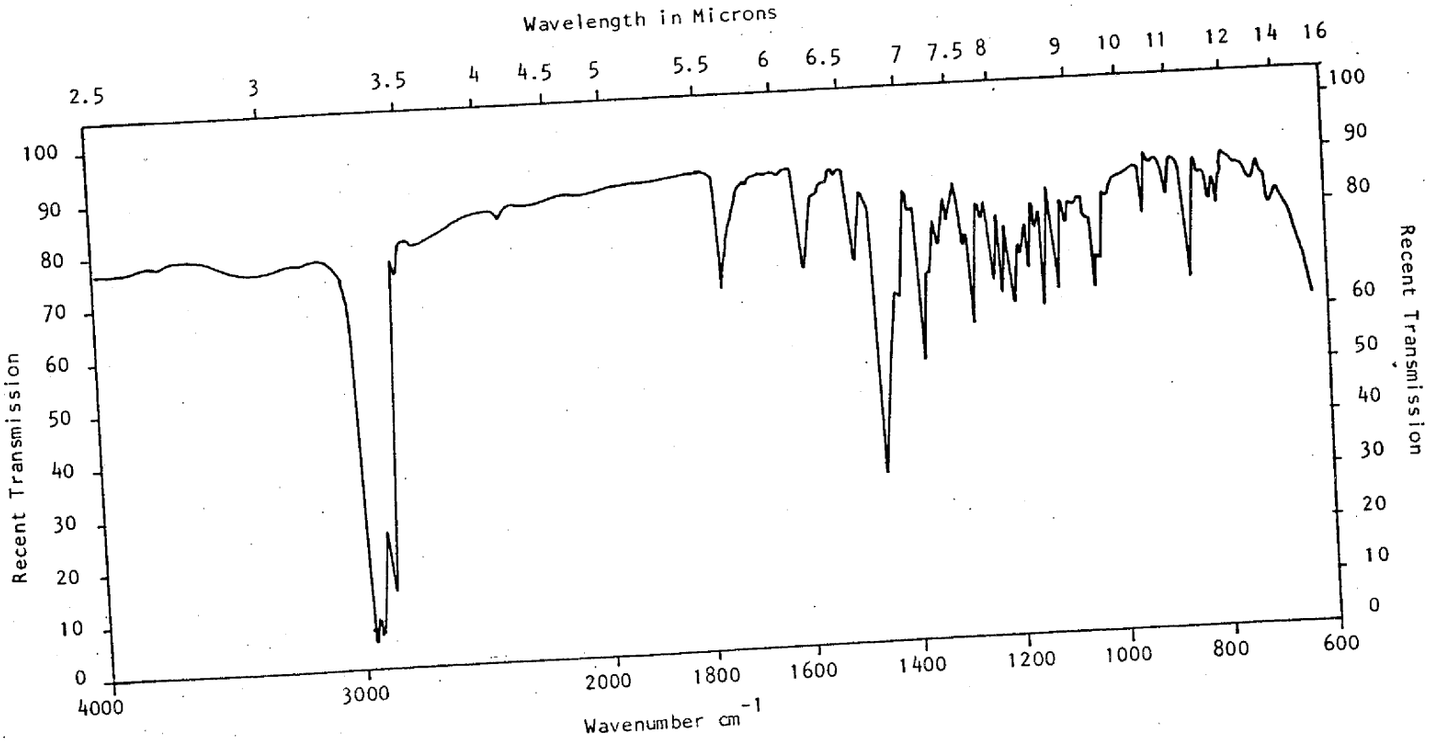

Figure A1: Infrared spectrum of Acetylthebaol

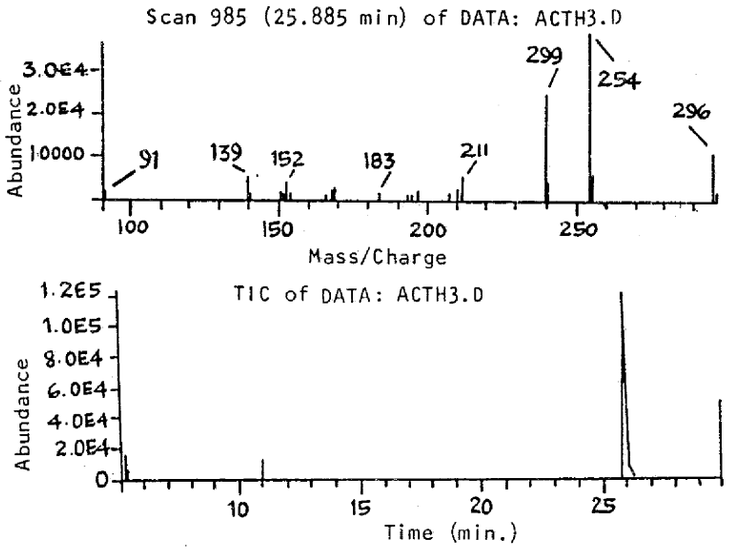

Figure A2: Mass spectrum and gas chromatogram of Acetylthebaol

Figure A2 (contd.)

Scan 986 (25.885 min) of DATA: ACTH3.D

ACETYLTHEBAOL

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
91.00	946	153.15	1707	194.05	839	240.10	3917
139.15	5082	165.10	1491	196.05	2026	253.20	1279
140.05	1220	167.10	2361	207.15	1416	254.20	36992
150.15	1290	168.10	2477	210.15	3278	255.20	6370
151.05	1466	183.20	1224	211.15	4916	296.25	11765
152.15	4175	193.20	1069	239.10	24744	297.25	2169