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Chemical characterization and quantitative estimation of narcotic drugs in the seized illicit samples by GC-MS and GC-FTIR, identification of source and possibility of isotopic substitution

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Abstract : Qualitative and quantitative forensic investigation of about thirty selected street drugs samples seized in between 1998-2003 from different regions of India were made using standard procedures followed by the use of sophisticated methods like GC-MS, GC-FTIR as per forensic protocol. The analysis of samples showed the presence of heroin, codeine, acetylmorphine, acetylcodeine, papaverine, noscapine, phenobarbital, methaqualone, paracetamol, strychnine, caffeine, nitrazepam with other variants and diluents. Adulterants and diluents formed 80% of the illicit samples. Heroin percentage were found to be low in most cases.

Attempts were made to gather information regarding geographical origin and the distribution network of illicit samples.

Methods suggested for the identification of source were analyzed. Isotopic signature of the elements in the samples using IRMS and biomarker identification appeared to be the best methods for source determination but could not be utilized due to lack of IRMS instrument and absence of data on biomarkers.

However, no isotopic substitution was observed in the samples from GC-MS experiments but idea of isotopic abundance of elements collectively was obtained. FTIR measurements, used to identify the compounds and group frequencies of the compounds in the illicit samples could be utilized to determine the ratios of $^{13}C/^{12}C$ of the compounds.

However, determination of source or route of drug trafficking appeared to be illusive unless there are vast collection of authentic illicit drugs from different countries are available.

Keywords : Biomarker, GC-FTIR, GC-MS, heroin, illicit drugs, isotopic signature, morphine, source.

Introduction

Morphine, cannabis and related 'scheduled' narcotic addictive drugs have a history of their use for euphoric pleasures from time immemorial. Morphine, an addictive analgesic and exceedingly valued sedative is probably the last resort for painful sufferings of the dying cancer patients.

However, the abuse of these narcotic drugs due to addiction is on the increase and the use of morphine and its acetylated product heroin have become a menace to the society.

These drugs have psychological, physiological and physical dependence on human behavior[1]. Naturally, there is a vast international market for the drugs. Due to their high clandestine demand, the prohibited drugs have become revenue earners for many countries and for the terrorists, extremists all over the world.

Increase in demand of the drugs with potentially high revenue earning capability is responsible for wide scale drug trafficking by smugglers and terrorists to extract and earn money. The demands are the reasons for the unofficial or clandestine preparation of heroin from morphine and morphine from the milky brownish juice of unripe pod of the poppy (morphine content is about 4–14%) with adulterants to satisfy the needs of habituated drug addicts.

The seizure of scheduled illicit drugs is a routine ac-

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tivity for the law enforcing authorities from different regions in India and the seized materials were sent to the Central Forensic Science Laboratory (CFSL), Kolkata for detailed forensic investigations.

The objectives were

(i) To purify the samples and make detailed qualitative and particularly quantitative estimations of the important drugs.

(ii) To find out the compositions of the seized illicit drugs along with adulterants to enable the authorities to impose duties, where applicable.

(iii) To establish the probable origin or source of the drugs like morphine, heroin and unfolding the distribution network of the illicit drugs[2]. The flow of such drugs to CFSL has been continuing till now. Generally, it is the usual practice to send few selected samples from each seizure for examinations.

The present paper deals with detailed forensic investigation of about thirty typical samples collected in between 1989 to 2003. The investigation included the purification and preliminary analysis of the samples followed by the use of sophisticated methods like GC-MS, GC-FTIR[2–6] as per forensic protocols and find out the presence of adulterants present in the samples. It is rational to make attempts to find out the sources of the samples and establish though difficult drug trafficking routes. The results are presented in this communication.

Materials and methods

Reagents and samples :

The standard samples of codeine and morphine were obtained from Government Opium and Alkaloid Works, Gajipur(UP), India. Methanol (HPLC grade, E. Merck, India), ethylacetate (AR, E. Merck, India), ammonia (AR, E. Merck, India), and acetic anhydride (AR, BDH, India) were utilized for the experimental purpose. Standard samples of acetanilide, papaverine, noscapine, phenobarbital were used for the estimation of compounds in the mixtures. However, standard samples of methaqualone, paracetamol, strychnine, nitrazepam, caffeine, sorbital acetate were not available. The most important lacuna was the non-availability of standard samples of monoacetyl morphine, monoacetyl codeine and heroin. These were prepared in the laboratory, purified and standardized using standard procedures.

The illicit drug samples obtained from different investigating agencies were dissolved in methanol and subjected to preliminary analysis, particularly TLC, to have an idea of the constituents present in the samples. Among the opiates the order of elution in GC was codeine, morphine, acetyl codeine, acetyl morphine, heroin and papaverine. The GC-MS of a typical sample is shown in Fig. 1.

Confirmatory analysis :

Further qualitative and quantitative analysis of the samples were carried out by dissolving the samples in methanol and passing through GC-MS. GC separates the constituents of the mixtures depending on the mass, polarities of the solvents used, flow rate of the inert gas and temperature. The instrument (GC-MS) was standardized taking anthracene as an internal standard. From GC-MS, constituents of the narcotic drugs were identified to be heroin, monoacetyl morphine and acetyl codeine (Fig. 1) together with other adulterants in sugar base or along with the raw components associated with crude preparation of morphine or substituted morphine. The main adulterants identified from GC-MS varied from sample to sample. The results are given in Table 1. The results were the averages of at least 3 readings. The results were reproducible and accurate (within 0.1%). The concentrations of some of the adulterants whose standard samples were not available in the laboratory are also presented in the Table 1. These were methaqualone, acetanilide, paracetamol, phenobarbital, strychnine, caffeine, nitrazepam, sorbital acetate. Total Ion Chromatograms (TIC) of each of the components in the mixtures were determined and compared with GC-MS data of the appropriate standards (by library matching). The results were confirmed by GC-FTIR (Fig. 2) and IR library matching (Figs. 3-7).

Preparation of acetyl codeine :

Codeine base was refluxed with requisite amount of liquid acetic anhydride at 100 °C for 2 h. The solution was neutralized with Na_2CO_3 when acetyl codeine was precipitated. The precipitate was repeatedly washed with water, dried and recrystallized from chloroform solution. The purity of the sample was checked by GC-MS. The R_t

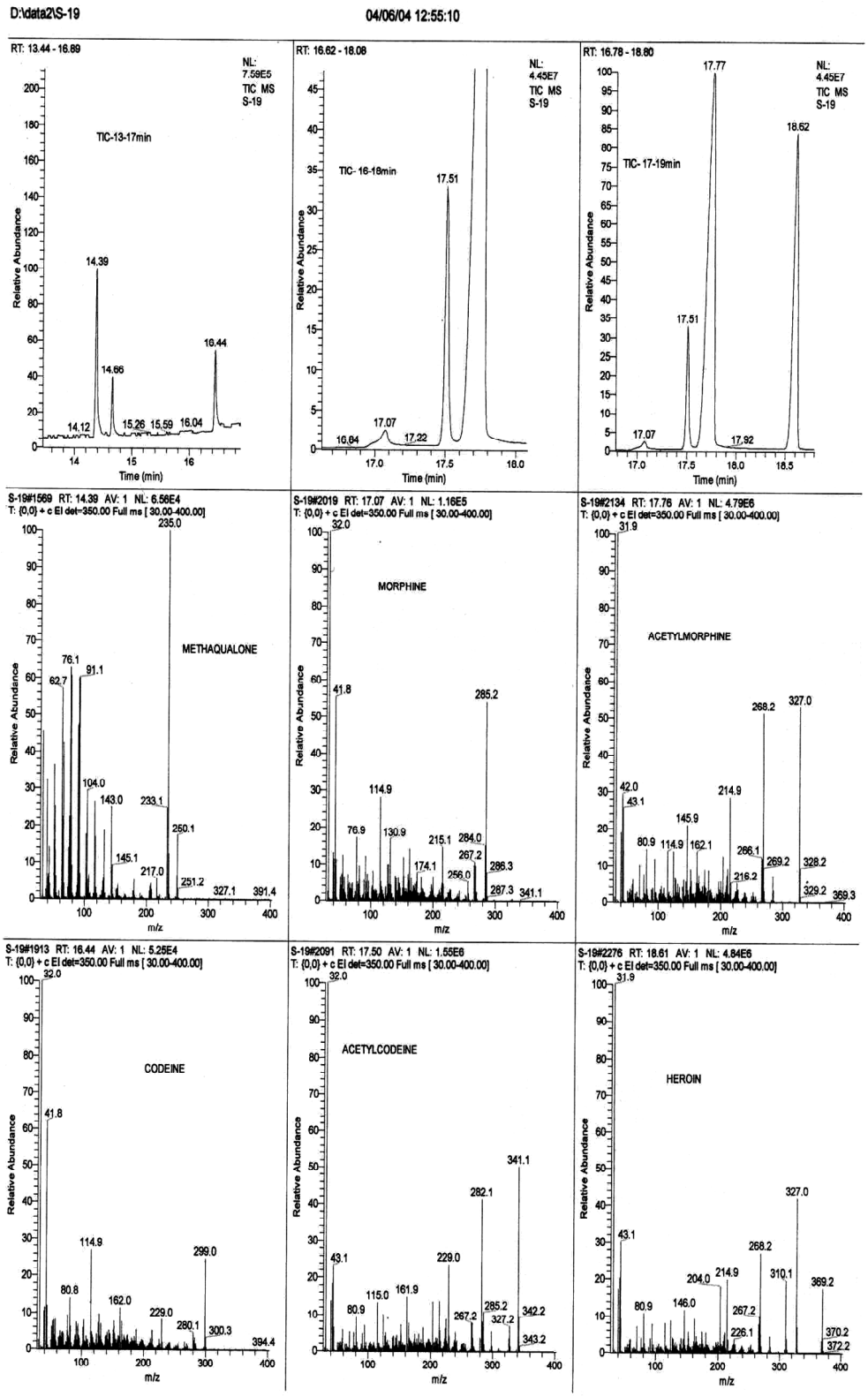

Fig. 1. The total ion chromatogram (TIC) of a street sample of illicit drug and Mass spectra of the individual components present in the sample.

value of acetyl codeine is 14.8 min and the *m/z* values of major fragments are shown in Fig. 1.

Preparation of heroin :

The known amount of dried morphine base was dissolved in slightly more than the requisite amount of acetic anhydride and refluxed for 4 h at constant temperature 100 °C. The solution was neutralized by Na_2CO_3 when heroin (diacetylmorphine) was precipitated. The precipitate (collected by filtration) was washed repeatedly with water, dried and recrystallized from chloroform solution. The purity of the sample was verified by GC-MS.

Table 1. The physical appearance, location, year, % of the constituents present in different street samples, ratio of herion/acetyl codeine and probable source

Sl. no.	Physical appearance	Location	Year	% of heroin	% of acetyl morphine	% of acetyl codeine	% of morphine	Heroin/acetyl codeine ratio and possible source	Other ingredients with percentage and effects
1.	Off white globules	Nagaland	1989	4.7	57.8	6.2			
2.	Off white globules	Monipur	1989	22.3	23.6	2.2		10.1 Hong kong	
3.	Off white globules	Mizoram	1989	2.5	3.6	0.33		7.8 China (Manchuria)	
4.	Off white globules	Tripura	1990						
5.	Light brown powder	Kolkata	1990		0.6				Acetanilide (23.28) (antipyretic and analgesic), methaqualone (oral hyonotic), Codeine (0.28) (narcotic analgesic)
6.	Light brown globules	Mizoram	1991	1.8	10.6	2.0			
7.	Light brown globules	Mizoram	1991	41.19	10.7	2.48		16.6 Iran	
8.	Light brown powder	Kolkata	1993	9.12	4.06	1.02		8.9 China	
9.	Light brown powder	Kolkata	1994	3.19	1.46	0.16	6.28	19.93 Afganisthan	Codeine (1.37) (narcotic analgesic)
10.	Light brown powder	Kolkata	1994	43.2	15.0	3.8	1.34	11.4 Hong kong	Codeine (0.89) (narcotic analgesic)
11.	Light brown powder	Kolkata	1995	7.2	0.43	0.34	1.33	21.2 Afganisthan	Papaverine (0.82) (narcotic analgesic)
12.	Light brown powder	Kolkata	1995		0.12	0.24	0.39		Phenobarbital (6.8) (sedative and anticonvulsant), methaqualone (oral hypnotic)
13.	Brown powder	Kolkata	1995		0.13	0.22	0.76		Noscapine (0.42) (narcotic analgesic)
14.	Brown powder	Kolkata	1995	5.26	5.9	1.43	3.65		
15.	Brown powder	Kolkata	1995	4.82	3.4	1.54	3.12		
16.	Brown powder	Kolkata	1995	4.82	1.5	0.56	1.51	7.71 China	

Table-1 (contd.)

17.	Brown powder	Kolkata	1996	4.65	5.36	2.18	2.83	2.1	Papaverine (1.31) (narcotic analgesic), caffeine (CNS stimulant)
18.	Brown powder	Kolkata	1996	Tr	2.4	0.26	1.58		Papaverine (0.84), Codeine (0.26), Noscapine (0.47) caffeine, strychnine (bitter and analeptic) paracetamol (antipyretic and analgesic)
19.	Dark brown crystalline	Kolkata	1996	23.1	1.5	3.8	0.72	6.08 China	Codeine (1.52), methaqualone
20.	Dark brown crystalline	Kolkata	1996		0.78	0.54	2.8		Codeine (1.07), paracetamol
21.	Dark brown crystalline	Kolkata	1996	3.77	12.7	4.7	0.83		Sorbitol acetate (irritant)
22.	Dark brown crystalline	Kolkata	1997	9.87	4.4	3.2	0.65	3.08	
23.	Dark brown crystalline	Kolkata	1998	0.67	18.2	1.18	2.36		Codeine (1.23)
24.	Dark brown crystalline	Orissa	2000	1.03	0.59	0.5	2.78	2.06	Papaverine (1.37) (narcotic analgesic)
25.	Brown powder	Orissa	2001						
26.	Brown powder	Orissa	2001		1.56	0.41			Codeine (0.25) (narcotic analgesic), paracetamol
27.	Brown powder	Delhi	2003	11.18	10.50	4.2			
28.	Brown powder	Delhi	2003	7.07	13.5	3.0	0.31	2.66	
29.	Brown powder	Kolkata	2003	0.79	0.46	0.25		2.36	Caffeine
30.	Brown powder	Kolkata	2003	1.2	3.5	0.81		3.04	
31.	Brown powder	Kolkata	2003	1.4	0.42	0.96			Nitrazepam (tranquilizer)

Preparation of monoacetyl morphine :

A weighed amount of morphine was acetylated with liquid acetic anhydride for 15 min at room temperature. The morphine was totally converted to monoacetyl morphine and diacetylmorphine. Absence of morphine was confirmed by GC-MS and the compounds were separately identified by GC-MS. The ratio of the peak area of monoacetylmorphine and diacetylmorphine was 5 : 1. The area of monoacetylmorphine and corresponding amount was considered for calculation of the monoacetylmorphine present in the samples.

Quantitative analysis of the illicit samples by GC-MS :

The analysis and quantitative estimation of heroin, monoacetylmorphine, acetyl codeine, morphine, codeine, papaverine, acetanilide, phenobarbital and noscapine present in the samples were performed in a Finnigan Trace GC and Trace MS by injecting variable volumes of standard solutions of the above samples and the experimental solutions in methanol by auto sampler using the following parameters.

GC parameters : Column - capillary, RTX - 5MS, length 15 m, 0.25 ID, 0.25 micron film thickness coating.

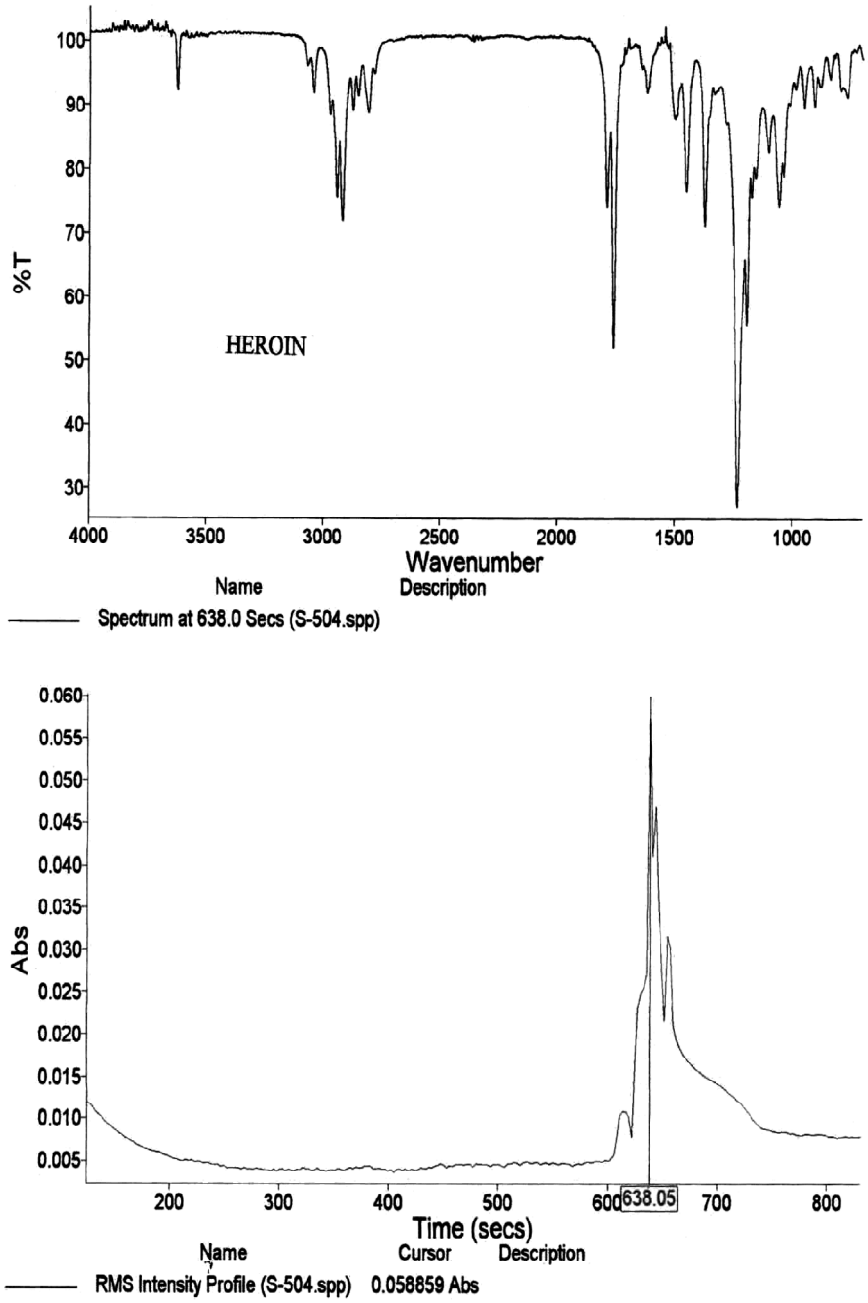

Fig. 2. GC-FTIR chromatogram of a street sample of illicit drug and IR spectra of the individual component present in the sample.

Oven temperature was programmed between 80–280 °C with 10 °C/min increments and held 2 min at 280 °C. The injector and interface temperatures were 260 °C and 280 °C, respectively. Helium was the carrier gas with a flow rate 1.2 ml/min at constant flow mode.

MS parameters : Source temperature 250 °C, energy 70 eV, source current 150 mA, lens 1 = 5 volts, lens 2 = 100 volts, repeller 1.2 volts, mass range scanned 30–400 amu, detector voltage 350 V.

GC-FTIR :

GC-FTIR analysis was carried out by Perkin-Elmer, Clarus 500 GC and Spectrum GX FTIR system with Perkin-Elmer Spectrum GC/FTIR interface having MCT detector.

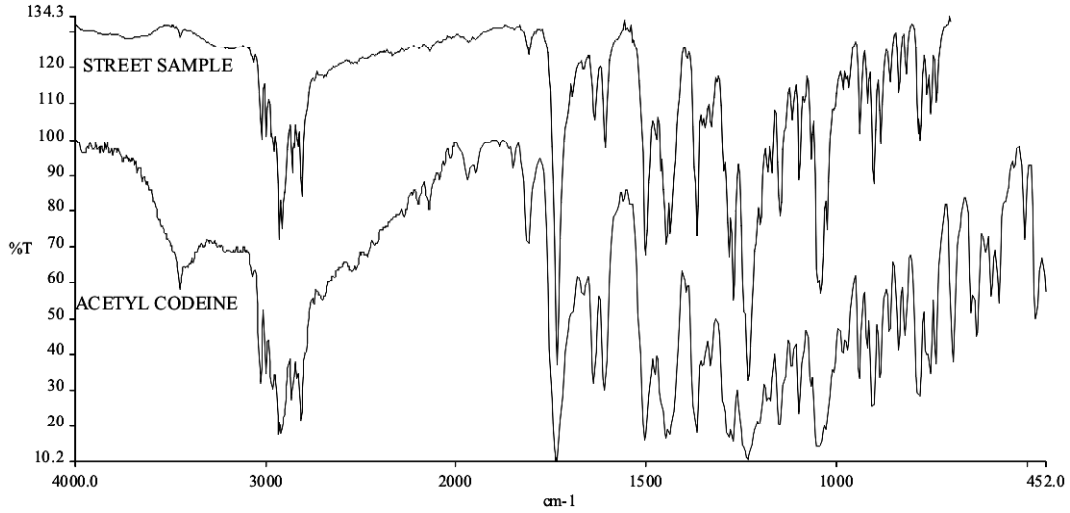

Fig. 3. The sample spectrum was taken by using Perkin-Elmer spectrum GX FTIR auto microscope system. Parameters are : Detector MCT, J-stop size 7.92 mm, B-stop size 21.20 mm, IR HeNe wave number 15789.01 cm^{-1}, resolution 4.00 cm^{-1}, J-stop resolution 4.00 cm^{-1}, Apodization strong, Gain 1, OPD velocity 0.2 cm/s, Interferogram Bidirectional-double sided, Phase correction self-256. The sample spectrum is matched with acetyl codeine.

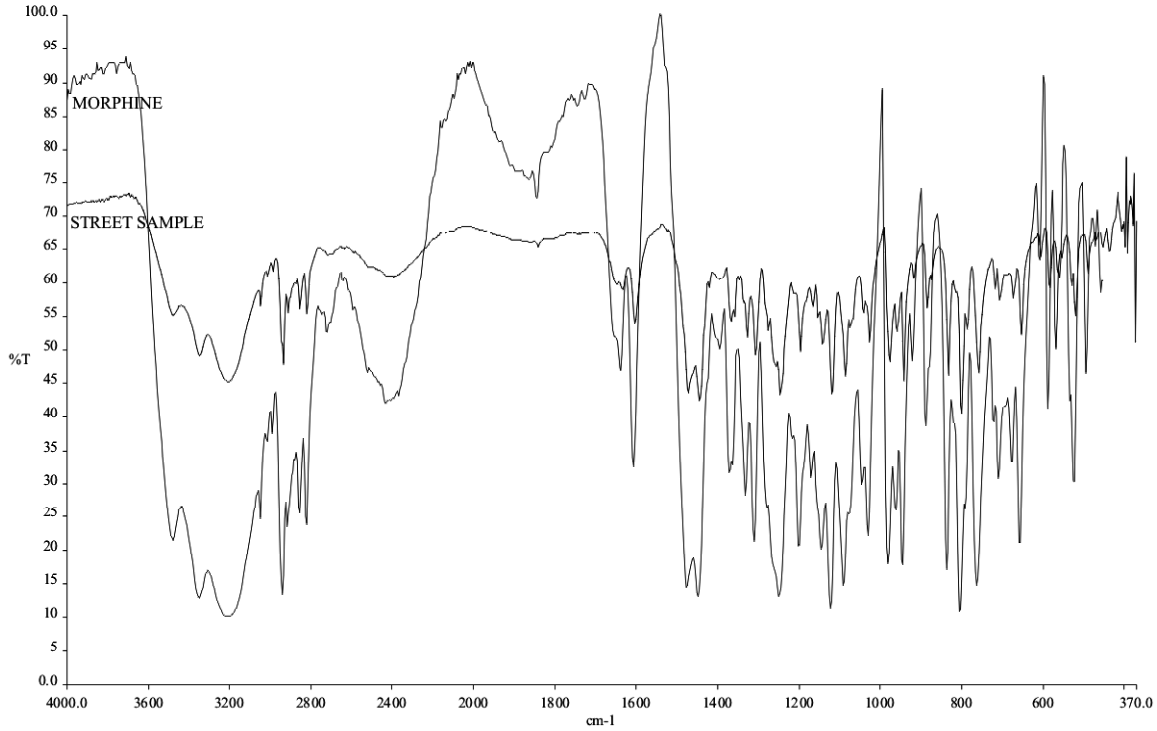

Fig. 4. Instrument parameters are same as IR spectrum 1. The sample spectrum is matched with morphine.

GC parameters - Column - DB 5, 15 m length, ID 0.32 mm, film thickness 0.25 micron. The injector temperature was 270 ºC. The system was run at constant pressure mode of 12 psi. Helium was the carrier gas. The oven temperature was programmed between 90 ºC to 280 ºC with 10 ºC/min increment and 6 min hold at 280 ºC. The transfer line temperature was 270 ºC. MCT detector with liquid N_2 temperature was used.

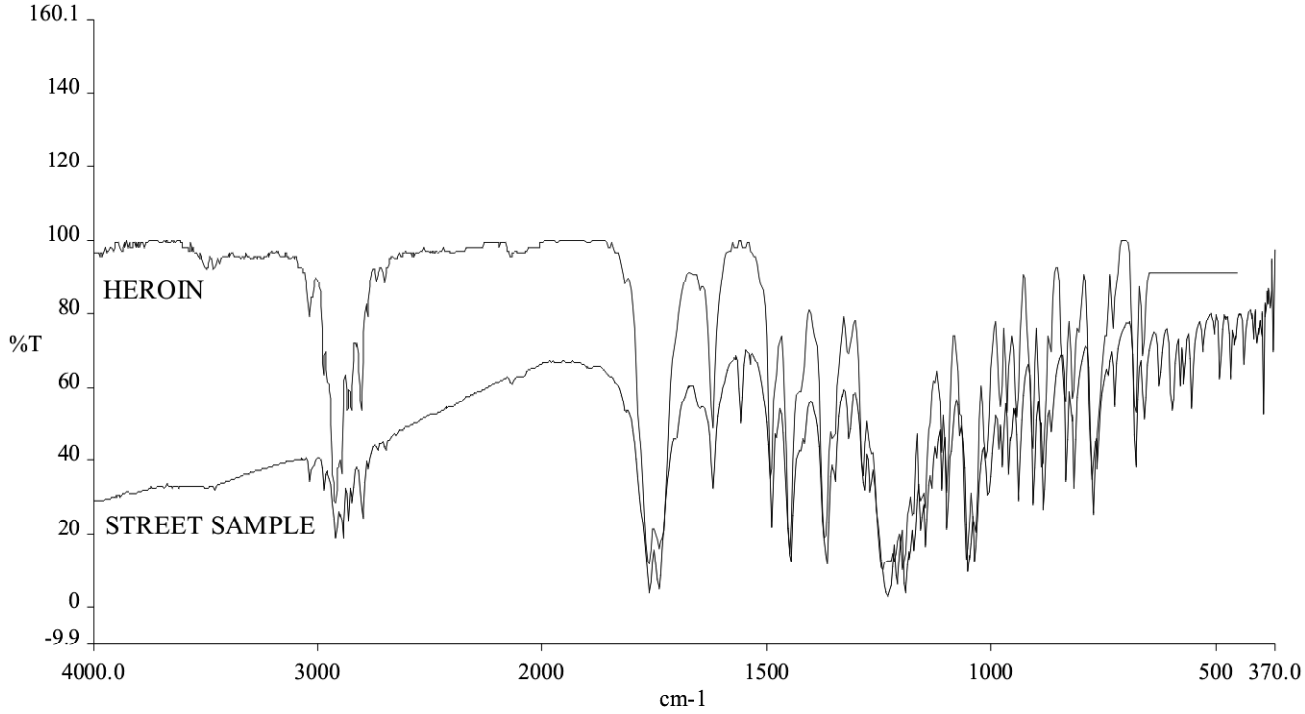

Fig. 5. Instrument parameters are same as IR spectrum 1. The sample spectrum is matched with heroin.

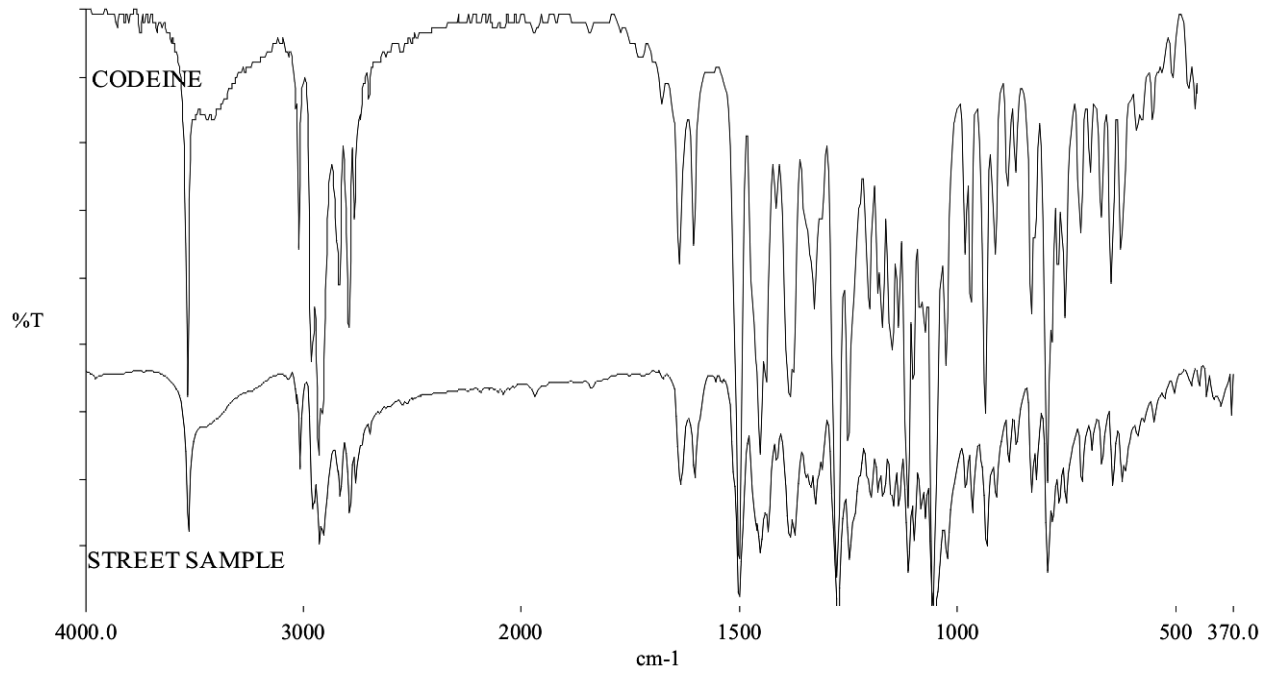

Fig. 6. Instrument parameters are same as IR spectrum 1. The sample spectrum is matched with codeine.

Results and discussion

A typical mass spectra of the illicit drug sample given in Fig. 1 showed the presence of heroin, codeine, acetyl morphine, acetyl codeine, papaverine, noscapine with other variants. Comparison of the TIC of the experimental samples (Fig. 1) with those of the standard samples mentioned before gives the percentage of the constituents in the samples. IR spectrum of a molecule not only identifies functional groups but due to its fingerprint value can identify the whole molecule. Comparison the group frequencies of the experimental samples (Fig. 2) with those of the standard samples corroborates the presence of heroin, morphine, codeine in the experimental samples. Though FTIR was not much used for quantitation of organic samples in forensic investigations, but comparison of the peak heights or rather area under the absorption peaks preferably of the strongest group of the experimental and standard samples are the concentration or % of the components of the constituents present in the illicit samples. The peak area of the C=O group was utilized for FTIR analysis. The values were reproducible and could be determined within accuracy[7–10] (observed from at least 16 scans for each of the sample). The results show the percentage concentrations of the main constituents like heroin, acetyl codeine, acetyl morphine etc. present in the samples and are given in Table 1.

The seizures of the illicit samples were from 1989-2003. The color of the samples varied with time. The samples collected in the early periods were light brown cubes or powders indicating the possible presence of trace impurity and were sugar based. The samples collected in the latter periods were colored. The color suggests the presence of impurities and is probably due to the origin of the illicit drugs where clandestine preparation of heroin was made from the milky brownish juice of unripe pod of the poppy which contained 4–14% morphine[1]. The crude preparations were used as such and diluted with other ingredients as the concentration of heroin in the samples were low (except sample numbers 1, 2, 7, 10, 19, 28, 29). Adulterants and diluents formed 80% of the illicit samples and proper acetylations were not performed. But they were purchased by the addicts. There was no attempt for sampling of the drugs based on heterogeneity of the powders or particle size of the components for statistical sampling for quantitative purpose as done by Dujourdy *et al.*[9] as the collected samples were few and

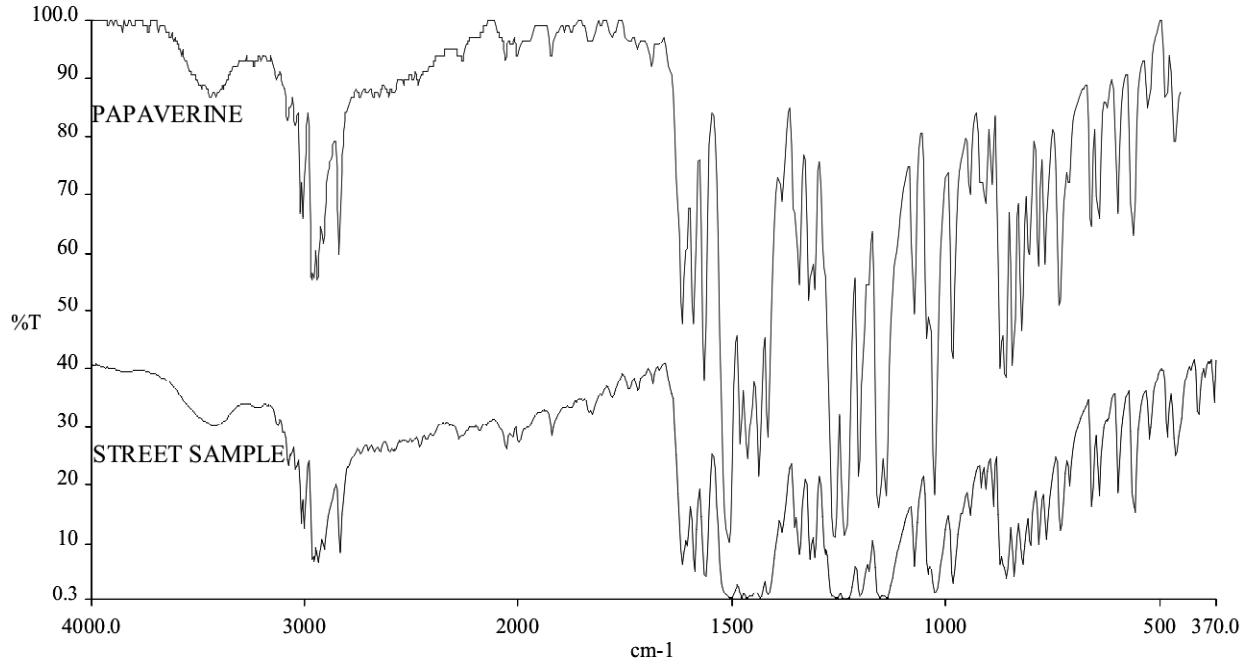

Fig. 7. Instrument parameters are same as IR spectrum 1. The sample spectrum is matched with papaverine.

spread over 1989-2003. But enormous variable factors or impurities limited the effectiveness of the procedure.

The quantification of the major narcotic drugs and level of adulterants (Table 1) are however, unable to provide any information regarding the geographical origin and the distribution network of the illicit samples. For investigative purposes, it is necessary to establish relationships between seizures or to provide evidence of links between a dealer and user.

Origin or source determinations from seized illicit narcotic drugs (or any drugs or compound) can be obtained using the following methods :

(i) Comparison of physical and chemical properties of drugs using composition profile of the samples, color ingredients, spectral properties using UV-Visible spectrophotometer and the quantities of the major narcotic constituents (using GC-MS, GC-FTIR, HPLC, HPTLC)[3–6,11-13] in the authentic samples of illicit drugs from different origin and the illicit samples seized.

White and colleagues[14] proposed a comparison of illicit heroin samples based on its diluent sugar content. However, the differences in the sugar contents may be due to processing of the samples by the different dealers and give no positive evidence regarding the origin[15].

Comparison of the endogenous trace impurities in the samples by chromatographic analysis may provide evidence regarding the origin.

(ii) Isolation and identification of unique marker compound or its derivatives[16].

A unique alkaloid oripavine (or its products derived from oripavine) was utilized as marker compounds for heroin from poppy seeds of Tasmanian origin (found in Australia), the marker compounds were not available in illicit heroin samples from Turkey, Pakistan, Columbia and Myanmar. In this connection it can be mentioned the petroleum fuels or products from different origin contain different biomarkers. Mineral oil biomarkers were utilized to suggest the source of fuels or culprits in forensic arson cases or in the preparation and identification of the source of the explosives[17–19].

(iii) Variations in the isotopic forms of elements like ^{13}C, ^{2}H, ^{18}O, ^{15}N in the elemental constituents of natural organic compounds or isotopic signature of the elements present[19–21]. The isotopic compositions of the natural products are determined by the biosynthetic pathway that has produced the compound and depend on the geographical origin. Isotopic labeling in the illicit samples may be made during acetylation to act as taggant or marker, but it is rarely done.

The use of GC-MS may be utilized to identify qualitatively the existence and variations of isotopes and estimate quantitatively the isotopic contents in the samples. The method is reproducible and accurate.

GC-MS[16,17] :

MS (EI+) was used to identify quantitatively the probable existence of isotopes in the sample[14] by determining the signals X, X+1, X+2, say of heroin (X = 369) using selective ion monitoring mode. The relative intensities of X+1, X+2, were calculated assuming the intensities of the first peak to be 100% (i.e. X = 100%). The results are compared with the theoretical factors calculated and are given in Table 2. The factors due to isotope variation are calculated by multiplying the natural abundance of the particular isotope with number (*n*) atoms present in the molecular ion to determine X+1, X+2. From the comparison of the results the following conclusions may be derived.

(a) Considerable increase in the intensity of the signals suggested the possibility of isotopic substitutions.

(b) The agreement between the observed intensities of signals X+1, X+2 with the calculated results indicate that the intensities are due to natural abundance of isotopes.

(c) Moderate changes in intensities usually suggest isotopic abundance of the elements. The isotopic abundance of elements are dependent on the geographical origin, climate, altitude, the amount of available moisture, the fertility of the soil, the age of the plant, the time of lancing and the variety of *Papaver somniferum* from which the natural drugs are obtained. Obviously, the isotopic composition of the drug are very much dependent on the biosynthetic pathway of the origin of the drugs. From the results given in Table 2, it is apparent that there was no possibility of isotopic substitution in the seized samples but the natural origin of the samples was different. The results also indicated no isotopic labeling in the illicit

Table 2. Abundances of X, X+1, X+2, X+3 of heroin in the samples

Sl. No.	*m/z* = 369	*m/z* = 370	*m/z* = 371	*m/z* = 372
1.	100	22.94	4.253	0.531
2.	100	19.334	2.997	0.374
3.	100	24.262	4.859	0.4129
6.	100	24.443	3.815	0.451
7.	100	21.115	3.74	0.430
8.	100	24.294	3.004	0.4126
9.	100	23.194	3.445	0.302
10.	100	22.471	2.954	0.417
11.	100	23.356	4.225	0.431
14.	100	23.884	3.726	0.477
15.	100	23.390	3.602	0.462
16.	100	23.294	3.431	0.436
17.	100	24.183	3.757	0.452
18.	100	23.674	3.997	0.423
19.	100	21.844	3.188	0.496
20.				
21.	100	22.179	3.197	0.402
22.	100	22.035	3.238	0.368
23.	100	23.869	3.865	0.429
24.	100	24.752	4.231	0.485
27.	100	21.343	3.445	0.402
28.	100	22.563	3.402	0.440
29.	100	22.789	4.856	0.427
30.	100	21.762	3.878	0.504
31.	100	23.595	4.366	0.442

samples to act as taggant or marker.

Determination of isotopic signature of the elements[19–21]:

The determination of 'isotopic signature' of the elements of the drugs (as well as of explosive materials not of importance here) are of great importance in determining the origin or source of the illicit samples.

The most important method of determining the isotope signature of elements in forensic samples is to use isotopic ratio mass spectrometry (IRMS). Little use of this method has been made and the method is not great success in many cases.

Use of ^{13}C NMR :

NMR can be used to determine site specific isotope ratios on individual site of a molecule. The use of ^{13}C NMR was utilized as a tool by Besacier *et al.*[22] for comparison and origin assignment of seized heroin samples. However, ^{13}C NMR shows the presence and abundance of ^{13}C isotope in the sample. IRMS or MS (SIM) gives the overall isotopic ratios but gives no idea regarding the natural isotopic abundance of the elements H, C, O, N in the illicit drug samples such as heroin. Two chemically identical substances may have different stable isotopic composition depending on the biosynthetic pathway of the production of original compound (e.g. morphine). Because H, C, N and O are the basic compounds and major elements in both natural and manufactured compounds of forensic interest, it is desirable to determine the natural abundance of isotopes of the elements in compounds from different sources and countries. Comparison of the natural abundance of the isotopes of C, O, N etc. between the standard compounds and the experimental samples may give an idea of the source.

FTIR method :

Inspite of low absorption of different groups containing low isotopic abundance of H, N, O etc., it is possible to use FTIR to have an idea of isotopic substitution of different atoms.

The frequency $\bar{\nu}$ of a group or bond can be determined from the spectra. $\bar{\nu}$ is group or bond dependent

$$\text{and } \bar{\nu} = \frac{1}{2\pi c}\sqrt{\frac{k}{\mu}} \qquad (1)$$

($\bar{\nu}$ frequency in wave number),

where k and μ are the force constant and reduced mass for the particular group. k is independent of the isotope of the atoms in the group or bond but μ is dependent on mass of the atoms as

$$\mu = \frac{m_1 m_2}{m_1 + m_2}$$

where m_1 and m_2 are the atomic masses of the atoms forming the bond. The frequency shift and calculated frequency of any group or bond due to presence of isotope should agree well with the observed experimental frequency. The ratio of areas under two peaks (considering peaks without and with isotopes) should give the ratio of isotopes present. The intensity of the frequency of the peak with increased area may be due to isotopic abundance or due to isotopic substitution.

However, the low natural isotopic abundances of H,

N, O gives peaks with low intensities of absorption peaks with possibility of appreciable error.

However, ^{13}C was utilized due to its relatively high natural abundance. The $^{12}C=^{16}O$ peak of heroin was observed at 1759.79 cm^{-1} and the value for $^{13}C=^{16}O$ peak was 1720.8 cm^{-1} in agreement with the observed peak (Table 3). The ratio of areas of the absorption curves under the peaks 1720.8 cm^{-1} and 1759.79 cm^{-1} gives the ratio $^{13}C/^{12}C$ to be near 1.11 corroborated from the results (Table 3). It has been observed that the natural abundance of ^{13}C was 1.1 except in few cases [sample no 8 (1.25); 9 (1.375); 10 (1.256); 11 (1.365); 14 (1.314); 19 (1.47); 28 (1.323); 30 (1.225)] suggesting that the compounds were of different origin. The samples might be clubbed into three groups (8, 10, 30), (9, 11, 14, 28) and (19). It is apparent that isotopic abundance or isotopic substitution of different atoms can be estimated using FTIR measurements.

FTIR measurement was utilized by Sharma and Lahiri[10] for the determination of isotopic abundance in case of nitro explosives.

The method is relatively little used in forensic analy-

Table 3. $\bar{\nu}$ of $^{12}C=^{16}O$, $^{13}C=^{16}O$ at 250 °C and $^{12}C/^{13}C$ ratios in the samples

Sl. No.	$\bar{\nu}$ of $^{12}C=^{16}O$ at 250 °C observed value in cm^{-1}	$\bar{\nu}$ of $^{13}C=^{16}O$ at 250 °C observed (calculated value 1720.8 cm^{-1})	Observed abundance of $^{12}C/^{13}C$ (natural abundance = 100/1.1)
1.	1759.79	1723.3	100/1.162
2.	1759.74	1720.1	100/1.294
3.	1759.79	1719.3	100/1.090
4.			
5.			
6.	1759.71	1722.7	100/1.068
7.	1760.1	1719.6	100/1.064
8.	1759.79	1719.10	100/1.250
9.	1759.65	1719.23	100/1.375
10.	1760.12	1720.47	100/1.256
11.	1759.62	1722.43	100/1.365
12.			
13.			
14.	1760.43	1722.31	100/1.314
15.	1759.79	1722.11	100/1.089
16.	1759.63	1719.13	100/1.169
17.	1759.59	1719.22	100/1.129
18.	1759.79	1720.70	100/1.132
19.	1759.68	1720.10	100/1.370
20.			
21.	1759.23	1720.60	100/1.067
22.	1760.62	1720.80	100/1.006
23.	1759.77	1722.40	100/1.076
24.	1759.77	1721.10	100/1.064
25.			
26.			
27.	1759.79	1720.8	100/1.323
28.	1760.20	1720.21	100/1.026
29.	1760.62	1721.30	100/1.225
30.	1760.00	1720.74	100/1.127
31.	1760.01	1721.11	100/1.101

sis of samples.

Use of ICP-MS analysis of inorganic elements[23] :

Recently, Liu *et al*. utilized ICP-MS to determine inorganic elements (Ag, As, Ba, Cd, Co, Cr, Cu, Mn, Ni, P, Pb, Se, Sb, Th, Tl, U, V and Zn) in heroin samples. They selected 10 elements (P, V, Cr, Ni, Cu, Zn, As, Se, Pb and V) and 7 elements ratios (U/Ba, Ba/Pb, Cd/Mn, Co/Ni, V/Cr, P/V, Cd/V) for classification of origin of heroin samples from heroin samples of 'Golden Crescent' (countries between Pakistan, Afghanistan and Iran) and Golden Triangle (countries between Mayanmar, Laos, Thailand) utilizing the data set of these 17 variables in 150 authentic heroin samples, classification of origin was achieved utilizing principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA). The authors claimed the method to be a reliable tool for screening illicit samples.

The method is new and requires further studies with large number of samples collected from authentic sources as well as illicit samples.

Empirical method :

A simple and indigenous method of assigning the source or origin of heroin samples used by Narayanswami[24,25] was utilized in the present study.

The origin of illicit samples were assigned from heroin/acetyl codeine ratio (in the illicit samples) as the absolute contents of the major alkaloids and the ratio of heroin/acetyl codeine depend on the origin of the opium (Table 4). The contents may vary due to dilution or adulteration of other factors enumerated before but heroin/acetyl codeine in the clandestine manufacture of heroin remains unaltered in the end product. It is to be noted that acetyl codeine, which is an impurity of illicit heroin synthesis, was suggested as a marker of heroin in human hair by Girod and Staub[26].

Table 4. (Narayanaswami) Ratio of heroin/acetyl codeine and possible origin

Ratio of heroin/acetyl codeine	Possible origin
11.05, 9.8, 11.2, 10.5, 10.7, 11.3, 11.8	Hong kong
21.9, 20.1, 21.3	Afganisthan
5.95, 5.99, 6.18, 6.02, 6.12, 7.06, 9.1, 5.5	China
8.1, 8.2, 7.76, 8.4, 8.6, 8.2	China (Manchuria)
18.6, 16.8	Iran

A comparative table of heroin/acetyl codeine of representative samples of different countries is given by Narayanswami[24,25]. The heroin/acetyl codeine ratio of the illicit samples were determined and compared with the values given in the table to identify the possible source of the samples. The possible origin of the illicit samples (7, 8, 9, 10, 11, 16, 19) is given in Table 1. Samples (14, 15, 21, 30) or (17, 24, 28, 29) are probably made by mixing two or more original samples and arbitrary dilution with other diluents.

Recently morphine (M) to codeine (C) concentration ratio in blood and urine was used as a marker of illicit heroin in forensic autopsy samples by Konstantinova *et al.*[27]. According to them, both blood and urine (M/C) > 1 can be used to separate heroin users from other cases, positive for morphine and codeine. However, the method cannot be utilized for the determination of origin or source in illicit samples.

But whatever may be the analytical method utilized to ascertain the source of illicit drugs but in most cases determination of the source of illicit drugs are impossible except from solid intelligence supports.

However a well-equipped library with a vast collection of authentic samples of illicit drugs like morphine, codeine etc. from large number of countries with known history of poppy cultivation and other sources and proper inventory are needed for such studies. Unfortunately no forensic laboratory in India is equipped with such an artifact.

Conclusions

(1) The illicit samples contained heroin, acetyl morphine, acetyl codeine, morphine, codeine together with other adulterants like papaverine, noscapine, phenobarbital, methaqualone, paracetamol, strychnine, caffeine, nitrazepam. Obviously, samples were of varied origin and obtained mostly from tertiary sources.

(2) The origin of the samples could be best determined from the mode of transport, nature of packaging and questioning the carrier.

(3) True knowledge of the source require the authentic samples of illicit drugs from different origin but it is generally not obtainable. Comparison of

heroin/acetyl codeine ratios of illicit samples with standard references as utilized by Narayanswami may be helpful. Impurities or drug adulterants may provide some evidence. However, heroin/ acetyl codeine ratio may not be foolproof as the ratio will depend on the acetylation process and conditions from the preparation of the heroin from crude or refined sources.

(4) No isotopic substitution was observed.

(5) Presence of isotopes may be inferred from MS peaks with proper selective ion monitoring mode. However, since IR peaks are group specific or bond specific, IR peaks particularly for C=O bond may provide evidence for the natural isotopic abundance or isotopic substitution. Isotopic substitutions of atoms like H, O, N can also be determined from FTIR.

(6) In view of lack of authentic illicit samples, a data base containing isotopic abundance of C of different agricultural products including poppy seed from poppy cultivating regions may be made. The data may give valuable evidences regarding the origin of illicit drugs.

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