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CONTENTS

A review of laboratory methods for the analysis of opiates and diluents in illicit drug traffic

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ABSTRACT

The paper summarizes the utility of currently available analytical techniques for the detection of heroin and other opiates in non-biological samples. It focuses on the following techniques: thin-layer chromatography/high-performance thin-layer chromatography, gas-liquid chromatography, high-performance liquid chromatography, gas chromatography/mass spectrometry. Recent innovations for the identification of compounds using high-performance liquid chromatographic techniques are also discussed. The applications of each methodology based on the experience of the authors are outlined.

Introduction

The rapid and accurate identification of substances found in illicit drug traffic is important in the implementation of drug-suppression activities. Further, the physical and chemical characteristics of the various substances found in the illicit market and those in illicit traffic provide valuable "clues" and support the development of a sound drug intelligence information system. It must be fully realized that the laboratory identification and characterization forms only one facet or source of information to the overall drug intelligence information system. When this information is collated with other sources of information, analysed and exchanged, a distinct picture begins to emerge. Hence, it is essential that any laboratory surveillance programme developed must be an integral part of the overall supply reduction activity.

Laboratory analytical technology has developed rapidly over the past two to three years. New methods have been developed to identify as well as to quantify substances and their diluents found in illicit traffic. These techniques have facilitated the determination of these substances in several forms. For example, samples for analysis may be biological fluids (e. g. urine, blood, secretions etc.) or body tissues. Non-biological samples (e. g. mainly those found in illicit drug traffic) may be in the raw unprocessed form or as the final product, available either in bulk quantities or in small "street" amounts.

This paper focuses on reviewing the laboratory analytical methods for non-biological opiate samples detected by enforcement agencies. It is common knowledge that samples intercepted by such agencies are diverse mixtures containing various substances, which include opiate compounds originally present in the opium plant, of which the major substances are the alkaloids, e. g. morphine, codeine, noscapine (narcotine), papaverine and thebaine. Other opiate compounds present which arise from the chemical manipulation of the raw materials include diacetylmorphine (heroin), monoacetylmorphine and acetylcodeine. In "street" samples, non-opiate substances which are commonly encountered include adulterants such as atropine, quinine, caffeine, strychnine and barbitone, as well as diluents such as sugars; various colouring agents are also found.

The compounds listed above, although not exhaustive, are diverse. It is apparent that while sample treatment can separate the diverse chemical classes of diluent substances found in illicit samples, individual substance separation is not possible. The use of methods which are capable of distinguishing between individual compounds and quantifying each one of them is necessary.

Numerous techniques have been developed to detect and measure opiate substances and their diluents. These include chromatographic and other techniques:

Chromatographic techniques

Thin-layer chromatography (TLC)
High-performance thin-layer chromatography (HPTLC)
Gas-liquid chromatography (GLC)
High-performance liquid chromatography (HPLC)
Gas chromatography/mass spectrometry (GC/MS)
Liquid chromatography/mass spectrometry (LC/MS)

Other techniques

Spectrophotometry
Fluorometry
Colorimetry
Circular dichroism
Crystallography
Microcrystal tests
Microdiffusion analysis

Other procedures based on the immunoassay principle are available but have only proved to be useful for analytical work in biological fluids.

Chromatographic techniques

The various types of chromatographic techniques are based on differential distributions of sample components between two phases, one stationary and the other mobile. It is through such a mechanism that the separation of components in a complex sample is achieved. This is accomplished by optimizing the factors which control resolution or separation. In this way, individual components can be eluted and monitored separately, free from possible interference by other components and contaminants. This mechanism, which separates sample components into individual "peaks" or "spots", is basically responsible for the specificity of chromatographic systems. The mode of detection in most chromatographic systems is non-specific and cannot distinguish between different compounds, except when mass spectrometry is used for detection. Differences between the various types of chromatography are because of differences in the nature of the mobile and stationary phases. As a result of such differences, different types of chromatography vary in their applications and potential usefulness.

Thin-layer chromatography and high-performance thin layer chromatography

High-performance thin-layer chromatography (HPTLC) is a recent development and can be thought of in simple terms as a new, improved version of conventional thin-layer chromatography (TLC). Some of the advantages offered by HPTLC over TLC are:

(a) Separations of compounds and their analyses are faster and more distinct;

(b) HPTLC chromographic plates are easier to handle;

(c) HPTLC is more suitable for further analysis, including quantification, because the plates can be cut into strips or special shapes and prepared for subsequent analysis. Conventional glass plates cannot be used in this manner.

TLC and HPTLC techniques have long been used in drug-abuse screening programmes. They are often regarded as the most suitable techniques for the rapid detection of drugs of abuse both in biological and non-biological material because they meet the criteria of:

Minimal instrumentation
Low cost
Simplicity
Minimum laboratory space and maintenance
Rapidity of analysis
Specificity and resolution that is superior to non-chromatographic techniques
Ease of interpretation of results

TLC and HPTLC techniques have been applied to the chemical analysis of illicit opiate samples. They are mainly used as a basic or primary screening technique to detect opiate and non-opiate compounds in illicit samples. This information can then be used as a guide for further analytical work that will confirm the initial findings.

With non-biological specimens, TLC/HPTLC can be performed directly, for example, using methanolic solutions of illicit drug samples except crude, unprocessed preparations of vegetable origin, without the lengthy extraction steps required for biological samples.

Numerous TLC/HPTLC methods have been published, including those capable of separating heroin from morphine (Steele, 1965), heroin or morphine from monoacetylmorphine (Mule, 1964; Comer and Comer, 1967), and heroin from acetylcodeine (Kaistha, 1972). Data on the detection of the lesser-abused opiate alkaloids, namely thebaine, papaverine and noscapine, are scanty. Common contaminants such as barbital, caffeine and strychnine can, however, be distinguished from the compounds of interest, namely codeine, heroin and morphine.

TLC/HPTLC techniques can also be used for quantitative determinations. Various quantification methods have been described which include:

Elution, followed by either spectrophotometry or fluorometry
Spectrodensitometry
Radioautography
Bioautography

Elution, followed by spectrophotometry, or fluorometry and spectrodensitometry, are commonly used, while radioautography and bioautography are less frequently utilized. As quantitative procedures, TLC/HPTLC are inferior to GLC or HPLC in terms of rapidity, ease of operation, convenience, precision and sensitivity.

However, TLC/HPTLC techniques play an invaluable role when used as preceding steps to more detailed analysis, and can be carried out simply, cheaply and rapidly. The selectivity of the TLC/HPTLC system will facilitate the rational use of other analytical techniques. The TLC technique is currently used for routine screening at the National Drug Research Centre, and work is being carried out to regularize the HPTLC method.

Gas-liquid chromatography and high-performance liquid chromatography

Gas-liquid chromatography (GLC) and high-performance liquid chromatography (HPLC) techniques are superior to TLC/HPTLC in the quantification of heroin and other opiate drugs. In addition, GLC and HPLC offer greater selectivity for the separation of the various compounds. Until recently, gas chromatography was the best and most widely used

method for the quantification of heroin and other opiate drugs in illicit traffic on a routine basis. Numerous GLC methods have been published (Kaistha, 1972; Gudzinowicz and Gudzinowicz, 1978). The introduction of HPLC techniques into illicit drug analysis and in particular the methodologies based on reverse-phase systems have seen a change, however. Criticisms directed at either method have often distorted the value of GLC and HPLC, but have recognized that both methodologies are important in the analysis of heroin, other opiate agents, and their diluents.

Gas chromatography can only analyse compounds in the gaseous phase; the compounds to be analysed have to be volatile in nature or derivatized into volatile agents. For example, trimethylsilyl (TMSi) derivatives of heroin (Grooms, 1968) and other opiates are commonly used. The thermal stability of heroin does not appear to pose any problems. Generally, GLC techniques are more sensitive than HPLC methods because of the more sensitive electrochemical detectors used. For non-biological samples, however, this is not an important factor since HPLC methods possess sufficient sensitivity to detect the amounts present in such specimens. HPLC on the other hand can analyse non-derivatized species without the problems of tailing encountered in GLC. In addition, HPLC techniques are easier to run than GLC techniques; the selectivity available in HPLC is broader. The instrument and running costs of HPLC are, however, higher.

It appears that at this stage, on a routine basis, both GLC and HPLC techniques will continue to be the main methodologies for the quantitative analysis of heroin in illicit samples.

The recent introduction of capillary columns in GLC further enhances the selectivity that can be achieved using gas chromatographic techniques. This can be regarded as an important step in the development of GLC technology. Capillary column techniques have been developed for the analysis of opiates. For example (Edlund, 1981), described a method for the simultaneous determination of morphine, 6-acetylmorphine and codeine.

Several modes of HPLC have been described for the detection and quantification of heroin and other opiate agents, namely:

(a) Normal phase (Verporte and Svendsen, 1974);

(b) Ion-exchange chromatography (Knox and Jurand, 1973);

(c) Paired-ion reverse-phase chromatography (Soni and Dugar, 1979);

(d) Isocratic reverse-phase chromatography (Love and Pannell, 1980; Yoshifumi Nobuhara and others, 1980; Poochikian and Cradock, 1980).

Reverse-phase chromatography is the most popular mode of liquid chromatography at present. Briefly, the major disadvantages of normal phase HPLC lie in the highly non-polar nature of the mobile phase, the

possibility of column inactivation by water, contamination by polar compounds and lower potential in terms of selectivity. Ion-exchange chromatography generally gives poor reproductibility and caused problems of reduced column life. The use of paired-ion chromatographic reagents with reverse-phase systems is a recent innovation. The drugs of interest can, however, be satisfactorily eluted by adjusting the pH of mobile phase using appropriate buffers (Love and Pannell, 1980).

Hence, the development of paired-ion chromatography reagents in the analysis of heroin and other opiate drugs does not represent an indispensable innovation since these drugs can be satisfactorily eluted within the pH range considered optimal for reverse phase columns (pH 2—8). Clearly then, paired-ion chromatography reagents, with their added cost, are only indispensable when the compounds of interest are very strong acids or bases.

The weakness of detection specificity in HPLC is generally well-known since, like all other chromatographic techniques, HPLC is primarily a separating technique. The quality of detection therefore depends on the capabilities of the detection system used. Conventionally, spectrophotometry (Love and Pannell, 1980) and fluorometry (Nelson, Nolan and Bedford, 1982) are most often used as detectors in HPLC systems used for the analysis of heroin and opiates. However, since UV absorption and fluorescence are properties exhibited by so many compounds of diverse chemical structures the specificity of HPLC systems are only as good as the separation achieved. Consequently, attempts have been made to provide more specific detection while using conventional HPLC detector systems for quantification.

Baker, Skelton and Ma (1979), devised an identification system using three solvent-column systems (both normal and reverse-phase). The relative retentions and the ratio of absorptions at 254 mm and 280 mm using these three chromatographic systems were used to identify compounds. Among the compounds studied were morphine, codeine, heroin, papaverine and noscapine (narcotine). It was reported that 95 per cent of the 101 drugs of forensic interest were characterized using these procedures.

Another approach put forward by Janes (1981) used a double-beam, diode-array detector incorporating a fast, integrated microprocessor. The effectiveness of this approach, however, requires the use of computer technology, thus incurring initially higher costs and obviously a more skilled technologist. The usefulness of this innovation in the analysis of heroin and other opiate agents has not been investigated although the potentials are enormous.

Gas chromatography/mass spectrometry

An integrated gas chromatography/mass spectrometry (GCMS) system combines the unexcelled identification of mass spectrometry with gas chromatography's powerful separation capabilities. While detection of

other GC systems tends to be non-specific, detection by mass spectrometry offers a high degree of specificity in the identification of compounds not normally attained with more common techniques such as a flame-ionization detector or an electron capture detector. This specificity is due to the ability of the mass spectrometer to provide a unique pattern of mass fragment intensities for each compound. Different compounds, even those which are closely related, will produce differing responses unique in themselves to the specific compound.

GC/MS is often regarded as the most sophisticated or powerful analytical tool currently in use. Connecting a computer to a GC/MS system considerably expands the potential of the system.

Owing to its inherent specificity and sensitivity, GC/MS is regarded as a definitive method. The running costs, however, are high. In addition, highly skilled operators are required to operate the instruments. In view of the high running and instrument costs, as well as the complexity of operations, it is difficult to envisage GC/MS as a routine method for opiate identification in the near future. It will, nevertheless, play an essential role in confirmative and definitive analyses of drugs. At the National Drug Research Centre, only "new" compositions of illicit opiates detected are analysed using the computer-based GC/MS, and the spectrums are stored for reference.

Other techniques

The National Drug Research Centre has been involved in developing and modifying analytical methodologies for the identification and quantification of drugs found in illicit traffic, particularly the opiates. Based on these experiences it would appear that only the chromatographic techniques among methods currently available have the potential to distinguish between different opiate compounds. On their own, spectrometric and fluorometric techniques lack the specificity to distinguish between different opiate compounds. These techniques can, however, be utilized as detection methods when interfaced with chromatographic systems.

Conclusion

Based on their experience, the authors are of the opinion that:

(a) In countries where mass spectrometry is not available, reliable identification and quantification can be achieved by the proper application of an appropriate GLC method and an HPLC method;

(b) TLC has an important role to play in the screening and analysis of opiate compounds, particularly in less-developed countries. For many

applications, this approach is more cost-effective than the analysis of samples directly on multi-column GLC or HPLC;

(c) There are several HPLC systems which will separate most of the opiate compounds, but there is no single system which will resolve all the opiates and all the common "cutting" agents.

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Bibliography

Baker, J. K., R. E. Skelton *and* C. Y. Ma. Identification of drugs by high pressure liquid chromatography with dual wavelength ultraviolet detection. *Journal of chromatography* (Amsterdam), 168:417:27, 1979.

Comer, J. P. *and* I. Comer. Applications of thin-layer chromatography in pharmaceutical analyses. *Journal of pharmaceutical sciences* (Washington, D.C.), 56:413–416, 1967.

Edlund, P. O. Determination of opiates in biological samples by glass capillary gas chromatography with electron-capture detection. *Journal of chromatography* (Amsterdam), 206:109–116, 1981.

Grooms, J. O. Quantitatives gas chromatographic determination of heroin in illicit samples. *Journal of the Association of Official Analytical Chemists* (Washington, D.C.), 51:1010–1013, 1968.

Gudzinowicz, B. J. *and* M. J. Gudzinowicz. Analysis of drugs and metabolites by gas chromatography, mass spectrometry. New York, Marcel Dekker, 1978. Vol. 5, pp. 1–184.

James, G. E. Wavelength chromatography: simultaneous multi-wavelength L. C. monitoring using the HP 8450A spectrophotometer. *Cancer research* (Chicago), 13:39–43, 1981.

Kaistha, K. K. Drug abuse screening programs: detection procedures, development costs, street-sample analysis and field tests. *Journal of pharmaceutical sciences* (Washington, D.C.), 61:655–678, 1972.

Knox, J. H. *and* J. Jurand. Separation of morphine alkaloids, heroin, methadone and other drugs by ion exchange chromatography. *Journal of chromatography* (Amsterdam), 82:398–401, 1973.

Love, J. L. *and* L. K. Pannell. A high performance liquid chromatographic method for the analysis of illicit heroin. *Journal of forensic sciences* (Chicago), 25:320–326, 1980.

Mule, S. J. Determination of narcotic analgesics in human biological materials: application of ultraviolet spectrophotometry, thin-layer and gas liquid chromatography. *Analytical chemistry* (Washington, D.C.), 36:1907–1914, 1964.

Nelson, P. E., S. L. Nolan *and* K. R. Bedford. High-performance liquid chromatography detection of morphine by fluorescence after post-column derivatization. *Journal of chromatography* (Amsterdam), 234:407–414, 1982.

Poochikian, G. K. *and* J. C. Cradock. Stability of Brompton mixtures: determination of heroin (diacetylmorphine) and cocaine in presence of their hydrolysis products. *Journal of pharmaceutical sciences* (Washington, D.C.), 69:637–639, 1980.

Separation and determination of opium alkaloids by high-performance liquid chromatography. *By* Yoshifumi Nobuhara *and others. Journal of chromatography* (Amsterdam), 190:251–255, 1980.

Soni, S. K. *and* S. M. Dugar. Separation of standard opiates and their analysis in pharmaceutical and illicit preparations by paired ion reverse phase high pressure liquid chromatography. *Journal of forensic sciences* (Chicago), 24:437–447, 1979.

Steele, J. A. Solvent systems for the identification of opiates in narcotic seizures by thin-layer chromatography. *Journal of chromatography* (Amsterdam), 19:300–303, 1965.

Verpoorte, R. *and* A. B. Svendsen. High speed liquid chromatography of alkaloids. *Journal of chromatography* (Amsterdam), 100:227–230, 1974.