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Approaches to Drug Sample Differentiation. I: A Conceptual Review

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ABSTRACT: Differentiation of drug samples can be based on at least three different approaches. Conventional methods use qualitative and quantitative determinations of impurities or minor components. Samples may also be differentiated by variations in a naturally occurring isotope such as carbon-13, whose content varies because of differences of carbon fixation conditions for cultivated plant drugs or from differences in reaction conditions for synthesized drugs. A third approach involves the determination of various diastereoisomeric or enantiomeric compositions or both. This last approach also serves an essential purpose in cases where one isomer is under regulation while other(s) may not be. Representative works are reviewed.

KEYWORDS: toxicology, drug identification, chemical analysis, sample differentiation, isotope ratio, diastereoisomer, enantiomer, chiral lanthanide shift reagent, chiral stationary phase

A major and overriding characteristic that sets criminalistics apart from other scientific disciplines is its unique concern with the process of individualization. Other sciences are satisfied when an object is classified into a unit place in the discipline's taxonomy. Criminalistics strives to relate the object to a particularized source [*1*].

Perhaps the oldest and still the most successful and familiar illustration of this statement is the characterization and comparison of fingerprints.

Recent advances in all fields of forensic science have also achieved limited differentiation on various samples. For example, by grouping various genetic markers, forensic serologists are now in a much better position to discriminate [*2*] among various body fluids and secretions, such as human semen [*3*] and bloodstains [*4*].

The purpose of this paper is to review the current state of the characterization, "fingerprinting," or individualization of drug samples of forensic science interest. It is intended to describe recent approaches involving various instrumental methods of analysis that either have resulted in some degree of success or possess high potential for sample individualization. Some of these approaches were developed in various crime and general-purpose analytical laboratories; some of them were developed in the author's laboratory. Approaches presented here include rather untested methods in the hope of contributing to the further development of this field.

Basically, three different approaches can be used for sample source differentiation. The traditional and still the most common method involves the qualitative and quantitative de-

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termination of impurities or minor components. A second method of differentiation involves the measurement of isotope ratios of naturally occurring isotopes. The third approach involves the determination of diastereoisomeric or enantiomeric compositions of certain drug samples such as the widely abused cocaine and amphetamine.

Determination of Minor Constituents

It is a well-known and widely utilized fact that determinations of impurities in illegal preparations can be of great help in the characterization and comparison of these drug samples. The types of impurities detected and the quantities present reflect precursor chemicals used, synthetic paths, and reaction conditions.

Because of the frequent amphetamine-manufacturing activities in clandestine laboratories [*5*], many studies have been conducted on the determination of impurities observed in these types of samples. For instance, the analysis of methamphetamine preparations, presumably prepared by the Leuckart reaction, reveals contamination by dibenzylketone [*6*], α-benzyl-*N*-methylphenethylamine [*6, 7*], *N*,α,α'-trimethyldiphenethylamine [*6–8*], *N*-formylmethamphetamine [*7*], and phenyl-2-propanone [*8*]. It has also been emphasized that the identification of these impurities "can aid in identifying methamphetamine samples which are of common origin and in distinguishing between samples of legitimate and illicit manufacture" [*7*].

Similarly, but in a quantitative fashion, the composition of illicit heroin samples was determined and used to draw conclusions about the common origin or common trade route of different samples [*9*].

The same approach was also used in the differentiation of cultivated plant drug samples. A simple direct inlet probe mass spectrometric procedure was used to identify [*10*] cannabinoids and further used to differentiate various samples by the measurement of the relative concentrations of the major cannabinoids present [*11*]. Novotny and his co-workers' investigation [*12*] on the use of combined high-resolution gas-liquid chromatography/mass spectrometry highlighted the analysis of marijuana samples from different origins. Thirty-eight constituents were identified by this approach. With more sample pretreatment and analysis, the high resolution used offered higher specificity.

Similarly, alkaloidal constituents in opium samples from different countries were analyzed by gas-liquid chromatography and were used in the determination of the origin of seized samples [*13*].

Determination of Naturally Occurring Isotopes

Since the ^{13}C composition in a plant is controlled by the adopted carbon fixation pathway and the environmental conditions [*14*], drugs from different sources of cultivated plants may be differentiated based on their $^{13}C/^{12}C$ ratio. Since the same carbon fixation pathway is adopted for plants of the same species, the difference observed in the $^{13}C/^{12}C$ ratio reflects the environmental conditions under which the plant was cultivated. These variations will undoubtedly be small, but they can be detected by specially designed double-inlet/double-collector mass spectrometers. It was reported [*14*] that *Cannabis sativa* L. samples grown indoors and in a metropolitan area contain lower ^{12}C content. If these comparisons are carried out on individual chemical fractions or compounds rather than on the gross sample, high resolution can be achieved.

It is highly possible that sources of synthesized drugs could be differentiated based on their ^{13}C content, which varies through reaction isotope effects in accordance with the synthesis conditions.

A similar approach was used for the differentiation of trinitrotoluene (TNT) samples [*15*].

Parallel to the tagging of explosives [*16*], commercially manufactured drugs with high

potential for being diverted for illegal trade may, in principle, be tagged with various contents of ^{13}C. Labeling drugs with ^{13}C or other naturally occurring stable isotopes is widely done in biochemical research for establishing drug metabolism and human organ functions [*17*]. A study is currently under way labeling methamphetamine with various amounts of ^{13}C methylamine in the preparation process.[2] It is predicted that, by using 0 to 0.1% of ^{13}C methylamine, the product can be easily differentiated.

Determination of Diastereoisomers and Enantiomers

The differentiation of diastereoisomers and enantiomers has two significant implications in forensic science analysis. These differentiations are essential in cases where one isomer is under regulation while others may not be. On the other hand, determination of isomeric composition can provide valuable information about the common origin of samples.

Certain drugs derived from natural sources occur only in one form of isomer, whereas synthesized counterparts usually result in a mixture of diastereoisomers or enantiomers or both [*18*]. In this sense, enantiomeric determination could classify a sample as to either a synthetic or naturally occurring origin. Further analysis on isomeric composition (if present) may differentiate samples prepared under different synthetic conditions.

Because of differences in their physical properties, diastereoisomers can often be determined by conventional methods. On the other hand, the differentiation of enantiomers requires special method development.

Several approaches have been explored in enantiomeric analyses. Earlier procedures used microcrystal tests [*19*] and optical methods [*20,21*]. Recent advances in gas chromatography/mass spectrometry/data system greatly improve the chromatographic method of analysis because the mass spectrometric component provides valuable chemical information. Therefore, the use of chiral derivatizing reagents, such as N-trifluoroacetyl-*l*-prolyl chloride [*22,23*] and (s)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride [*24*] to form diastereoisomeric derivatives from *d*- and *l*-amphetamine prior to chromatographic analysis, provided a valuable method of enantiomeric determination. Alternatively, the use of a chiral stationary phase[3] should provide a direct chromatographic procedure for enantiomer analysis.

Recently, nuclear magnetic resonance (NMR) spectrometry, which provides definite chemical information, has been applied to the resolution of enantiomers. Four different approaches are being used in the NMR determination of enantiomer composition. Before the use of chiral lanthanide shift reagents, enantiomers were derivatized with a chiral reagent [*25*] or combined with a chiral solvent [*26*] to induce a chemical shift difference ($\Delta\Delta\delta$) between corresponding enantiomeric groups. The third approach involves the use of a chiral solvent and an achiral lanthanide shift reagent [*27*]. The fourth approach employs optically active shift reagents to induce nonequivalent NMR spectra of enantiomers [*28–30*]. The applications of these approaches to the analysis of samples of forensic science interest are demonstrated by the use of a chiral solvent [*31*], (−)α-phenethyl alcohol, and a chiral shift reagent [*32*], tris(3-trifluoroacetyl-α-camphorato)europium(III), in the determination of cocaine enantiomers. A more effective chiral shift reagent, tris(*d,d*-dicampholylmethanato) europium(III), has been used in the determination of *d*-and *l*-amphetamine [*33*].

Discussion

Undoubtedly, each method surveyed in the previous sections has its unique merit and is suitable for a specific kind of sample or specific analytical goal. However, none of these methods can be considered as truly universal. For example, the identification and quantita-

[2]C. T. Tsay, unpublished data.
[3]J. H. Liu and W. Ku, unpublished data.

tion of minor components or impurities serves the purposes of qualitative identification and possibly source identification. However, this approach is limited by the fact that different sample storage conditions may influence the rate of decomposition and in this way affect the final composition [*9*]. Composition may also be changed as the drug is diluted and adulterated by successive dealers. On the other hand, precise determination of isotope ratio involves extensive sample preparation and instrumentation, which are not normally available. The idea of isomer determinations is excellent because of the need to identify a specific isomer [*34*]. Unfortunately, the logical choice of NMR method may not be practical because of the limited resolving window of NMR spectrometric method.

It is this author's view that the best approach should include the high resolving power of capillary gas chromatography and mass spectrometry. The gas chromatograph component should have the capacity to resolve enantiomers by using either a chiral column or a chiral derivatizing agent. The mass spectrometer component should be able to monitor single ions to provide adequate isotope ratio information if needed. In essence, this single analytical procedure will provide information on minor components or impurities and on isomers and isotope ratio for samples of interest. A report on this approach will appear shortly.

Conclusion

In principle, differences exist in all samples. Differentiations may be intrinsic, partially resulting from variations in sample cultivation or synthetic conditions, or extrinsic, attributable to variation in sample history or simply inhomogeneity. Each kind of difference requires a unique approach. As more approaches are explored and further advanced, more success will be expected in the pursuit of sample differentiations.

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