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# CHROMATOGRAPHIC IDENTIFICATION OF ORGANIC COMPOUNDS; USE OF DERIVATIVES*

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Chromatography, particularly paper and gas chromatography, have rapidly come into general use in criminalistics and toxicological laboratories, hence, a discussion of its application for identification of organic chemical compounds, particularly narcotics, poisons, and drugs is timely. By chromatography, unless a specified method is designated, all types of chromatography paper, gas, column, and electrophoresis are included. This discussion will be limited to an analysis of the information which can be derived from the position parameters measured in the process for the purpose of identification, and nothing will be said about the conditions of chromatography or the advantages of chromatographic separation and purification.

Chromatography generally is a flow process and causes a separation of certain components being chromatographed, depending on conditions. This result of a chromatographic process does not measure a unique property of a substance; such as does infrared spectroscopy, nuclear paramagnetic resonance spectroscopy, or x-ray diffraction spectroscopy. Hence in a chromatographic system, more than one substance may produce the same qualitative response. This likelihood of identical qualitative responses increases as the molecular weights and chemical complexity of the materials to be identified increase.

A given chromatographic identification process, when established with a limited number of reference compounds, may appear to be quite specific; this is particularly true when reference compounds include only closely related simple compounds of one homologous chemical series. However, as an increasing number of reference compounds are included in the analytical system, and as the sensitivity of detection increases, or the detection method is made less specific, more and more apparent identities appear.

The reliability of identification can be improved by use of a battery of chromatographic systems; i.e., by multiple runs at changed conditions, substrates, detectors, or all of these. Also, one may use preliminary steps to reduce the probability of misidentification; that is, separate the substance into various chemical or solubility classes and test each class separately. Even so, reliable identifications are dificult to achieve.

*Examples*

Paper Chromatography: A great many things have same Rf. A number of things of the same class e.g. *Amines*, have the same Rf.

Gas Chromatography: e.g. the Cadman (5) alcohol system carbon bisulfide and ethyl alcohol have same elution time.

Extreme Example: In the Mannering (7) method of testing urine for opiates, urine is treated to extract chloroform soluble substances into the following classes: Acidic, neutral, basic and amphoteric bases. Morphine is an amphoteric base; it gives an unusual blue color when treated with iodoplatinate, and is at Rf .39 in a particular system; yet there is in about 1% of the cases, a substance separating with the amphoteric bases, giving a blue color with the reagent, at Rf .39, appears and is not morphine.

In cases involving analyses of biological samples, particularly post mortem samples where complex factors of random chemical ingestion, metabolism, putrefaction, and artifact formation are involved, the identification problem can be most acute.

An elegant approach to this problem was suggested in a urine analysis similar to the above except the material tested for was codeine. In this biological system, ingested codeine is excreted principally in 3 forms, codeine (and conjugate) as norcodeine conjugate and as morphine conjugate (1). Paper chromatography of urine extracts revealed 3 substances distributed in 2 fractions, each of in-

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dividual Rf value and response to locator reagent, corresponding to codeine, norcodeine, and morphine.

A solution to this problem, then, is to chemically prepare and identify a derivative or derivatives of the product tentatively recognized to authenticate the identification.

As a starting basis one can use the classical organic chemical techniques; however the inherent advantage of the tremendous resolving power of the chromatographic process adds a whole new dimension to the technique. Derivatives need no longer be the purified solid products of a nearly quantitative chemical reaction with few side products or without competing reactions. In fact reactions utilizing chain reactions, carbonium and carbinium ions, pyrolysis, reduction, photolysis, complex oxidation, and other mechanisms, even those which give a multiplicity of products can now be used. The production of a number of similar products will not now preclude their use as identification information but may now even make the information much more reliable, however, with a reduction in sensitivity. Also reactions which produce gaseous or liquid derivatives can be of value.

Further attention is called to the fact that the requirement that large quantities (centigrams) of material, needed to produce derivatives in order that they may be successfully separated and purified, is now negated by use of the relatively sensitive chromatographic techniques.

*Examples*

Classical derivative process.

Ketone + semicarbazide in buffer yields a precipitate of semicarbazone; the product is separated, recrystallized, and identified by melting point.

Paper chromatographic derivative process.

Ketonic opiate narcotic salt applied to paper; 10 L of semicarbazide in buffer added to spot; allowed to dry at R. T.; Chromatographed.

Resultant semicarbazone has lower Rf than original ketonic narcotic, but has same response to locator reagent (4).

Pyrolysis

Compound is thermally dissociated in appropriate apparatus. After dissociation, recombination usually is statistical and the products are chromatographically separated and measured. Besides a loss of sensitivity, this method also has the disadvantage that in mixtures the amount and identity of products may be a function of composition of mixture.

Oxidative degradation (Paper chromatography)

A narcotic salt is applied to paper, 10 L of potassium of permanganate in phosphoric acid is added. After drying, chromatography. Some narcotics are not affected; others are destroyed and products are not readily revealed. However codeine, dihydrocodeinone, dihydrohydroxycodeinone, and ethyl morphine yield from 1–12 new products which are readily demonstrated by ultraviolet light and permit individualization of the original material. (4) This process of oxidation has marked advantages over the pyrolytic process described above.

One can easily control the reaction over a tremendous range of chemical potential; from mild oxidation which will attack particular chemical bonds, (e.g. tertiary amine + hydrogen peroxide gives amine oxide) or a generalized attack such as described using permanganate in acid which may yield many products; to complete oxidation to $CO_2$, water, nitrogen, sulfate etc.

Also, such a process should be less affected by the relative amounts of material present in a mixture, provided excess of oxidant is present.

It is proposed to utilize chemical changes generally for the following purpose.

1. To produce additional evidence of identification by chemical reaction to produce recognizable derivatives.
2. To demonstrate chemical properties of substance.
3. To improve the results of chromatography
   a. by making the tests more sensitive
   b. by making chromatographic methods feasible.

It is proposed to produce the derivatives directly on or within the chromatographic system without use of accessory chemical apparatus, and in one operation, if feasible.

*Examples*

*Amphetamine* has low sensitivity to detector reagent Iodoplatinate. Reliable limit—50 ug./cm$^2$. When spotted on paper chromatogram and alkylated by allowing 10 L dimethyl sulfate in 20% alkali to dry on spot, the sensitivity to the reagent is increased ten fold and the Rf is changed (4).

*Morphine* during paper chromatography is not

visible until process is completed and locator reagent is added. It may be chemically changed before chromatography by adding 10 L nitrous acid in acetic acid to sample spot on chromatogram; the resulting nitroso morphine (?) (6) is colored yellow, has nearly the same Rf, is markedly fluorescent and is an indicator. Morphine in urine extracts behaves the same as the standards. Urine blanks are negative. This increases reliability of identification, and increases sensitivity. Similar behavior is shown by other amphoteric opiates, but the non phenolic opiates do not react thus with nitrous acid (4).

*Morphine and dilaudid* have nearly same Rf. To differentiate, react with semicarbazide on paper. Morphine is unaffected. Dilaudid forms semicarbazone of different Rf (4).

*Heroin*, in some chromatographic systems, has Rf value near that of commonly used excipients and diluents in illicit narcotics mixtures, particularly procaine and other local anesthetics. Advantage may be taken of the easy hydrolysis of the heroin to morphine, which may be done on the spot applied to the paper before chromatography, under conditions mild enough to be without effect on other substances which may be present. The morphine is of very different Rf, acidity, and response to locator reagents. In practice, 10 L of N sodium hydroxide is added to spot before chromatography, and allowed to dry at room temperature, followed by chromatographic process (3).

*Morphine* may be converted to recognizable derivatives in a gas chromatograph by a later addition of a reactive acid chloride, which in overtaking the morphine, forms esters which have different, reproducible retention times. Even mixed esters may be formed in this manner and identified, increasing the certainty of the identification (2).

The possibilities are many. In fact with almost any general purpose chromatographic system and existing detectors a complete system of qualitative organic chemical analysis is possible in a microgram scale with a minimum of customary chemical procedures. Additional possibilities feasible now include:

a. Resolution of optical isomers

b. Microscale Liebig & Dumas combustion processes

c. "Tailoring" of substances which will not chromatograph successfully in a given system to make process feasible, e.g.,

1. Volatile substances converted into non volatile derivatives for application of paper chromatography.
2. Colorless substances into colored for application of column chromatography or continuous electrophoresis.
3. Convert non fluorescent substances into fluorescent.
4. Convert neutral substances into charged substances which will migrate in electric field.
5. Convert substances into halogenated or nitrated derivatives which will respond to electron capture detector for gas chromatography.
6. Convert substance into a radioactive derivative for ultimate in sensitivity.
7. Labile substances converted into more stable derivatives for processing and identification.

In addition, with a general purpose chromatographic system and a suitable battery of derivatives, the inconvenience and expense involved in maintaining multiple chromatographic systems, columns, substrates etc., will be minimized, and much time now lost in changing over from one system to another, equilibrating temperatures, standardizing, etc., could be saved.

That a great many of the above objectives have already been established may be seen by perusing any recent authoritative text on chromatography. It is believed that further application of these principles will make the chromatographic processes more useful, more applicable, and more reliable.

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