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TRACE ANALYSIS OF ORGANIC COMPOUNDS

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Abstract - The interest in organic trace analysis and its importance is demonstrated by a brief statistical review of the literature. The difficulties of organic trace analysis are compared to the complications found in inorganic trace analysis. There are numerous additional problems. Their solution calls for so much knowledge from neighbouring fields that organic trace analysis can be considered as an independent constantly expanding branch of analytical chemistry with many interdisciplinary connections.

INTRODUCTION

In organic trace analysis various organic constituents are identified qualitatively or determined quantitatively at very low concentrations. In many cases the sample is of natural origin. The determination of traces in samples from industry is of less importance.

Less attention is given to the trace analysis of organic compounds than to inorganic trace analysis. For example, about one third of all papers given at this symposium deal with the determination of organic substances. Only one article in ten is devoted to organic trace analysis. This is in sharp contrast to the fact that there are at least 1000 times more organic substances than inorganic.

There are two possible reasons for the discrepancy. One is that the micro and trace analysis of organic compounds is of no interest. The other is that the difficulties are so great that results are more tedious to obtain than in inorganic analysis.

It is the aim of this paper to demonstrate

(a) that there is a great interest in organic trace analysis and

(b) that there are more difficulties in this field than in inorganic trace analysis.

INTEREST IN ORGANIC TRACE ANALYSIS

To demonstrate this it may be useful to look at the number of analytical publications. About 10,000 articles were abstracted in Analytical Abstracts in 1976.

TABLE 1. Distribution of publications in analytical chemistry (calculated from Analytical Abstracts, 1976)

Field	Percentage of publications within field	Percentage of publications dealing with trace analysis
Organic chemistry	57	9
Inorganic chemistry	31	9
Analytical techniques	12	1
Sum	100	19

Table 1 indicates that there are about twice as many articles published on the analysis of organic compounds as on inorganic compounds or ions. About one fifth of all analytical publications deal with problems of trace analysis.

About the same number of papers is published on trace analysis of organic compounds as on inorganic trace analysis.

The 57% of publications on organic chemistry can be sub-divided into groups as shown in Table 2, depending on the materials to be analysed.

TABLE 2. Percentage of publications in organic analytical chemistry referring to different samples

Type of material analysed	Percentage of publications	Percentage of publications dealing with traces
biochemical samples	20	4
industrial organic chemicals	13	1
air,water	8	1
pharmaceuticals	7	1
food	6	1
agricultural samples	3	1
organic substances	57	9

From Table 2 it can be seen that organic trace analysis plays an important role in the life sciences and in ecology. The analysis of food samples, specimens from agriculture, air and water is the subject of about one fifth of all publications in analytical chemistry. The increasing importance of the analysis of these samples with direct relevance to each individual member of mankind is demonstrated by the fact that the literature on this branch of analytical chemistry is continually increasing.

The trace analysis of organic compounds obviously plays a fairly important part in those fields that are currently at the focus of public interest, such as "protection of the environment" and "purity of food", and also in biochemistry.

THE DIFFICULTIES OF ORGANIC TRACE ANALYSIS

If organic trace analysis is of importance but there is only a comparatively small number of publications, a possible reason for this might be that organic trace analysis is even more complicated and difficult than inorganic trace analysis, which itself is difficult enough already. It is the purpose of this article to examine this question and to give some explanations - mainly by comparison with inorganic trace analysis.

The first difficulty met in attempting an organic trace analysis is that there is practically no scheme available, comparable to the separation schemes used in inorganic trace analysis which make it possible to analyse for all ions.

This lack of a systematic approach also becomes apparent in a search for textbooks. There are practically no handbooks or extended treatments. On the other hand the field is constantly expanding. New compounds are produced in profusion, in contrast to inorganic chemistry. Interest may suddenly focus on substances which previously had little significance, such as polychlorinated biphenyls. The toxicity of these compounds has only lately been recognized and air must be tested for their presence. This changing character of the topics of organic trace chemistry is one of the reasons for the lack of handbooks.

Another contrast to inorganic chemistry should also be mentioned at the beginning. Sometimes - mostly in biochemistry - the chemical nature of a compound which gives a certain effect is not known. This compound is to be isolated and determined by procedures which are to be developed. The number of substances imaginable in organic chemistry is practically unlimited, in contrast to the case with inorganic compounds.

Difficulties in sampling and storage of samples

The difficulties can be illustrated by the determination of the insecticide content of air. It is well known that after insecticides have been sprayed, they disappear in time either through chemical transformation or through physical processes, in most cases evaporation. Insecticides that can evaporate in this way have a relatively high vapour pressure. A

Other problems that are not found in trace analysis of inorganic compounds arise because of the ease of decomposition, oxidation, and enzymatic or bacterial transformation of the samples to be stored. Such interferences lead to serious problems in the storage of blood samples, for example.

Though the literature data(8-11) are unfortunately somewhat contradictory, they show, for example, that the concentrations of bilirubin and cholesterol change within two days even when the serum samples are stored at 3°C. The phosphate and ammonia contents of blood samples increase rapidly, since organic compounds are decomposed with formation of these products and an accompanying decrease in the concentration of the parent organic compounds.

Enzyme activities in blood or serum samples are also influenced by the manner in which the samples are stored. The activities of lactate dehydrogenases and phosphatases decrease within two days when the sample is kept at 0°C. For the trace analyst who has been concerned with inorganic compounds an entirely new aspect is presented by the behaviour of some enzymes, suspensions of which in aqueous ammonium sulphate solution lose their activity on freezing and thawing(12).

This instability leads to problems of organization in clinical laboratories where a very large number of samples must be examined. Efforts are made to avoid bacterial decomposition by storage at -23°C or by freeze-drying, as well as by the addition of chemical stabilizers. However, this procedure is not entirely free from problems, since these additives can interfere with the analyses. The safest method is to work up the samples as soon as possible.

The problem of instability of samples is particularly serious where constant concentrations or activities of calibrated standards must be maintained for long periods.

Digestion methods

Digestion methods play a much greater role in the trace analysis of inorganic compounds than of organic trace constituents. In particular, destructive digestion methods are rarely of any interest in the latter field, with the exception of the determination or organic molecules by means of some representative "inorganic" atom. A survey of the insecticides which are used most frequently today(13) shows that about 40% of them contain chlorine or other halogens and 35% are organophosphates. It is reasonable, therefore, to analyse for such insecticides by determination of phosphorus or halogen after separation and wet-ashing of the insecticide(14).

Sample preparation is very important in the disintegration of cellular tissues to liberate special components or to obtain selected cell fractions or particle fractions. The methods used range from immunological methods to centrifugation in solutions of specified density, ultracentrifugation, and electrophoresis of whole cells.

The removal of the main component, protein, to obtain traces of low molecular weight substances is also a step full of problems. It can lead to low values, e.g. by occlusion of the trace components or as a result of their adsorption on the precipitated protein, but it can also lead to high values through removal of amino-acids from the proteins (in amino-acid analyses).

Separation methods

The most critical step in the trace analysis of organic compounds lies in the choice of a suitable separation method. All the test materials in which great interest is shown (blood, tissue, food, urine, and apparently even systems as "simple" as air or water) are complicated and consist of a large number of principal and minor components. Separations are unavoidable in the great majority of cases.

A "preliminary" publication (more than 250 pages long) on the influence of 9000 drugs on the results of clinical analyses(15) shows that the "blood system" cannot by any means be regarded as a constant matrix. For precision, therefore, the trace component of interest should preferably be separated from this variable system by methods which are not influenced by the composition of the sample. Such methods are sometimes difficult to find and often enough the yield from separation procedures in organic trace analysis is low.

As a consequence a trend has lately developed to apply "direct" procedures which do not require any separations. The advantages are obvious. But since with samples of biological origin or from environmental sources constancy of composition of the matrix cannot be expected - as it can in the analysis of alloys or minerals - it is difficult to calibrate these procedures. Separation procedures seem to be more reliable. They are certainly more often applied - as is indicated by the literature (see Table 4).

certain insecticide content can therefore be expected in air. It is likely that some of the insecticide will be held on dust particles, but because of its high vapour pressure it will also be present to an appreciable extent as a free component in the gas phase. Difficulties arise in the determination of this fraction in the ng/m^3 range, since condensation to reduce the vapour pressure results in the formation of aerosols, which cannot be quantitatively determined. The lower the temperature to which the gas sample is cooled for "quantitative" condensation, the smaller are the aerosol particles, with the result that the yield diminishes.

One method that gives high yields even for nanogram quantities involves the passage of the air containing insecticides through gauze coated with polyethylene glycol(1). Non-polar substances dissolve in the polyethylene glycol with high efficiency, as can be shown by the use of ^{14}C-labelled insecticides. They can then be determined by gas chromatography. Results are given in Table 3.

TABLE 3. Differentiation between gaseous and aerosol fraction of insecticides in air samples

Sample taken at	content of p,p'-DDT found (ng/m^3)		content of lindane found (ng/m^3)	
	aerosol fraction	gaseous fraction	aerosol fraction	gaseous fraction
University of Mainz	2	191	0	7
" "	13	550	0	26
Neustadt	0	0	22	17
Schauinsland	1	61	0.3	2

The total DDT content of certain samples has been found to be as high as 1.8 µg/m^3. This value corresponds to the findings of Stanley(2) for air samples from the United States. The lindane content reported by Stanley is of the same order of magnitude as we found. The American authors used a sampling system which guaranteed quantitative collection of all insecticides. The usual data found in the literature for the insecticide content of "air" are measured by passing the air through filters, so that only the aerosol fraction is collected. This fraction is generally only a small part of the entire insecticide content of the air. These examples, already 4 years old, may serve to demonstrate some of the difficulties encountered in sampling.

In the analysis of blood samples in clinical work, differences are often found according to the point from which the sample was taken, since the concentrations of many substances in venous blood are different from those in arterial blood(3). The sampling conditions, including the point from which the sample was taken, must therefore be indicated very precisely in analyses of this type.

The problems arising in the storage of samples containing organic trace components are the same as those in the trace analysis of inorganic compounds, i.e. adsorption on the walls of the storage vessels. Insecticides in water samples, for example, are adsorbed on the walls of polyethylene vessels after a very short time(4). Losses of cholesterol to polypropylene pipette tips have been reported(5). Such tips are widely used in clinical laboratories. Quinine, which has to be determined at the ng/ml level by fluorescence spectroscopy, is practically all adsorbed on the walls of the glass cells within seconds, even when the alkaloid solution is 0.1N in sulphuric acid(6). To avoid losses of this nature, it is recommended that the walls of the apparatus should be saturated with a suitably dilute solution of the trace component to be determined. It is advisable to keep the surface area of the vessel as small as possible in order to minimize the adsorption effect. A change of solvent or of the composition of the test solution is also helpful in many cases.

In one respect organic trace analysis is better off than inorganic trace analysis: there are only a few contamination problems since the compound to be determined is usually not a permanent constituent of the laboratory environment, reagents, glasswase etc. Nevertheless, blank values must still be determined. There may be very unusual effects due to the non-specific determination procedures which must be applied in organic trace analysis. In the determination of theophylline in blood by gas chromatography, erroneously high values have been found owing to some volatile material given off by the rubber stopper used to close the sample tubes(7).

TABLE 4. Percentage of publications in organic trace analysis (from Analytical Abstracts 1976) in which separation steps are mentioned

Number of separation steps	Percentage of publications
no separation necessary	4
one separation step necessary	32
two separations combined	49
more than 2 separation methods required	15

Except for a few cases of highly specific enzymatic procedures which did not require any separation of the component to be determined (and which make up the 4% in Table 4) it has always been necessary to suggest at least one separation step during organic trace analysis. About half of all publications describe the combination of two separation methods - generally a preseparation or "clean-up" step, during which the compound of interest is separated together with others to form a "group" - and a final separation during which the compound of interest is isolated from other interfering members of the group.

According to Bock's scheme(16) separation methods can be divided into two groups. Separation is achieved either by differences in distribution of substances between two non-miscible phases or by differences in mobility in one phase. Data from the literature (see Table 5) indicate that the first type of separation procedure is applied only in organic trace analysis.

TABLE 5. Percentage of publications on organic trace analysis in which a particular separation procedure is mentioned (calculated from Analytical Abstracts 1976)

Separation procedure	Percentage of publications
liquid-liquid distribution	33
gas chromatography	29
thin-layer chromatography	18
other types of chromatography (including 6% high-pressure liquid chromatography and 3% ion-exchange chromatography)	16
miscellaneous	4

Liquid-liquid distribution is obviously of greatest significance. The procedure is simple and often rapid, and the physicochemical principles of the separation step hold valid over many orders of magnitude of concentration of the extracted species. There are two main group separations which can be achieved rather simply:

(a) separation of polar substances from non-polar and
(b) separation of polar substances into acidic, basic and neutral substances, depending on the pH.

This separation scheme is rather old and has been used successfully in drug analysis. It is the basis of many modern procedures.

Next to liquid-liquid distribution the different types of chromatography are of importance. They are often combinations of separation procedures and methods of determination. They are consequently difficult to classify.

A serious problem in the separation of traces from biochemical sources is that many of these substances are hydrophilic. The only separation methods that can really be considered for them are consequently methods in which water is used as an essential component of the system.

Although there are many separation procedures suggested, improvements are often required. Emulsion formation, for instance, poses a severe problem in the otherwise very successful liquid-liquid distribution method. Consequently it is difficult to automate separation methods of this type.

One improvement is gained by a combination of the two separation principles given by Bock. As long as 40 years ago, a procedure was invented, called "diasolysis", which is a combination of liquid-liquid distribution and dialysis which is obtained by separating two non-miscible liquid phases by a membrane which is semipermeable to one solute(17).

We suggest using silicone rubber membranes(18). They have the interesting feature of swelling when treated with suitable organic solvents. Such a swollen membrane forms a barrier to compounds of high molecular weight. If the swelling agent is a non-polar liquid such as toluene or hexene, polar substances are not dissolved within the membrane, which thus becomes inpenetrable to these compounds. It is also possible to apply these liquid-liquid distribution separations to systems which otherwise form a foam or an emulsion, since no shaking is necessary. The automation of the extraction procedure is no problem. Remote control of toxic or radioactive solutions is possible. Deproteination is not necessary as long as there is no protein-binding of the substance to be diasolysed. Protein-binding, on the other hand, can be detected and measured in this way. Furthermore, trace constituents can be easily concentrated from a dilute solution into a solvent in which the species to be extracted shows good solubility. Since two liquid phases are separated by the swollen membrane barrier it is even possible to use two solvents which mix with each other. The diffusion process within the swollen membrane increases the specificity of the separation procedure, as is demonstrated in Table 6.

TABLE 6. Yield of diasolysis of barbiturates which are transferred from an aqueous solution (pH 8) into solvent 3 through a silicone rubber membrane swollen with solvent 2

Solvent 2	Solvent 3	Transferred %		
		barbitone	phenobarbitone	amylobarbitone
$CHCl_3$	methanol	47	54	87
$CHCl_3$	propan-2-ol	37	43	80
diethyl ether	acetone	57	63	87
CCl_4	methanol	0	6	41
n-heptane	methanol	0	0	17
CH_2Cl_2	methanol	5	18	89

These experiments show the influence of tiny differences in the molecular structure of the three barbiturates. They have the same structure except for one group at position 5.

Analogously, 50 ng of DDT can be extracted from 1 litre of water into a few ml of toluene with a yield of 85%. Other insecticides were extracted directly from a slurry of spinach in water to which the radioactively labelled compound had been added. It was even possible to handle the two sample materials which pose the biggest problems in liquid-liquid distribution - beer and milk.

The data in Table 6 are just one example of a very common experience in the trace analysis of organic compounds - the yields are usually smaller than in inorganic trace analysis. However, losses can often be very effectively recognized and taken into account by the isotope dilution method.

Here again, additional difficulties are encountered in comparison to inorganic trace analysis. Very often it is possible to prepare an inorganic radioactive isotope by neutron activation. In organic trace analysis intact molecules are required as tracers. The radioactivation by direct irradiation in a nuclear reactor almost always leads to Szilard-Chalmers reactions and destruction of the irradiated compound. Labelled compounds must be prepared by micro variants of typical organic synthesis. Fortunately, many organic compounds can be bought

already labelled with ^{14}C or tritium. It is necessary, though, to control the chemical purity of these substances, as radiolysis and decomposition of the radioactive compounds occurs.

To improve the efficiency of separation, it is sometimes suggested that derivatives of the trace components should be prepared, e.g. in the field of ultramicro preparative gas chromatography. However, problems are also often encountered here, since microgram or submicrogram quantities of organic substances react differently with the very large excess of reagent than with the roughly stoichiometric amounts usually used in preparative organic chemistry. The disturbing influence of water is particularly drastic in some derivative formation reactions if the quantity of the trace component is small in comparison with the water films that are adsorbed even on small micro vessels (in microgram quantities). The result may be a severe limitation of the separation methods.

Methods of determination

In the methods for the determination of organic trace components serious problems are again found in addition to those pertinent to the trace analysis of inorganic constituents.

In the analysis of organic substances meaningful results are nearly always obtainable only by use of methods of determination that are specific. Elementary analysis at best gives only hints in the trace and micro ranges. For this reason, an extremely effective tool for the trace analysis of inorganic compounds, radiochemical activation analysis, is almost useless for the trace analysis of organic compounds.

The detection of specific isomers is another problem that is fairly unusual in the trace analysis of inorganic compounds.

The methods for the determination of an organic compound can be separated into two groups:

(a) molecular properties are measured directly by physical (e.g. infrared, ultraviolet, nmr or mass spectrometry), chemical or biological methods;

(b) the presence of a particular species is indicated indirectly (e.g. by its mobility in a chromatographic system and detection by a non-specific method such as flame ionization).

A look at the literature shows a picture similar to that for separation methods. Only a few procedures are extensively used though many methods have been suggested (see Table 7).

TABLE 7. Percentage of publications (calculated from Analytical Abstracts 1976) which describe certain determination methods of organic trace analysis

Procedure	Percentage of publications	Sensitivity given in literature
gas chromatography	30	pg
ultraviolet spectrometry	20	ng
fluorescence spectrometry	19	ng
gas chromatography + mass spectrometry	14	ng
thin-layer scanning methods	9	ng
radioimmunoassay	5	pg
electrochemical methods	3	ng

Gas chromatographic methods, with their great sensitivity, are widely used. Unfortunately, they must be classified as less specific, indirect procedures. To compensate for these disadvantages the combination of gas chromatography with other, often molecule-specific, procedures - such as mass spectrometry - are becoming more and more important and widely used. Many other very useful combinations are described in two textbooks(19,20).

Unfortunately, most modern procedures of instrumental analysis(21) have detection limits in the microgram region, the notable exception being mass spectrometry. These methods are useless for high sensitivity trace analysis (only 0.1% of all publications in 1976 suggested nmr spectrometry as a tool in organic trace analysis).

With the aid of highly refined special procedures, however, the molecule-specific methods of determination (ultraviolet, infrared and nmr spectrometry) can be used for the investigation of submicrogram quantities. An increase in sensitivity of 1-2 powers of ten can often be achieved here by electronic averaging(22).

Mention should be made of the fundamental difficulty of most molecule-specific instrumental methods of analysis, that they require more or less pure substances. Preliminary separations thus acquire corresponding significance within the analytical plan as a whole. After the trace component has been isolated (with the least possible loss), it must be quantitatively transferred to the instrument with the aid of micro techniques.

Extremely high sensitivity, coupled in most cases with excellent specificity, is offered by enzymatic methods and immunological techniques. Among the latter, radioimmunoassay is at present the only practicable possibility for the fast, specific determination of organic trace components in a large number of samples(23). The method is based on the principle that the substance to be determined, which must act as an antigen, is allowed to react with a specific antibody. This antibody is added as the "reagent" in a substoichiometric quantity for the analysis. A small quantity of the substance to be determined, but in a radioactively labelled form, is also added to the sample. The antigen originally present in the sample itself and the added antigen compete to react with the substoichiometric quantity of antibody. If there is a large quantity of antigen that originally came from the sample, correspondingly less of the labelled antigen will be bonded to the antibody, and the activity found after isolation of the antigen-antibody complex is then a measure of the antigen concentration in the sample.

The method depends on the availability of the radioactively labelled antigen and of the antibody, and it is necessary to verify that the latter specifically and stoichiometrically reacts with the antigen. Interference by other plasma components, which may vary in concentration, must be ruled out.

The effectiveness of the method may be demonstrated by the determination of digoxin and digitoxin in blood. About 10 - 20% of people over 60 years of age require cardiotonic agents of this type. Because of their very narrow therapeutic range, it is desirable to know their concentration in the patient's blood. Good therapeutic results are obtained, for example, with digoxin in a concentration of 1 ng/ml in serum, but toxic effects appear at a concentration of 2 - 3 ng/ml. The objective determination of such low concentrations has until now been possible only by very complicated analytical methods; however, an elegant alternative is now offered by radioimmunoassay(24).

CONCLUSION

The trace analysis of organic compounds is a fascinating field, and is of great general important. In addition to the difficulties found in trace analysis of inorganic compounds, the investigator in this field is very often faced with numerous other problems. Their solution calls for so much knowledge from neighbouring fields that it seems justifiable to consider their analytical chemistry as an independent scientific field with interdisciplinary connections.

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