GAS–LIQUID CHROMATOGRAPHY OF SUBMICROGRAM AMOUNTS OF DRUGS

I. PREPARATION, SCOPE AND LIMITATION OF COLUMNS

COLIN McMARTIN AND HAROLD V. STREET
Department of Forensic Medicine, University of Edinburgh (Great Britain)
(Received October 25th, 1965)

For certain aspects of toxicological work, it is essential to be able to carry out analyses *rapidly*. If gas–liquid chromatography is to be used in such analyses, the retention times of the drugs involved must, therefore, be made quite short. Retention times may be reduced by (a) using a high flow-rate of carrier gas, (b) shortening the column, (c) raising the column temperature or (d) reducing the percentage of liquid phase on the column. However, (a) and (b) are limited because if the flow-rate is too high or the column too short the resolving power of the column is lost, and there is an obvious limitation under (c) depending on the thermal stability of the stationary phase and of the sample. This means that support material coated with a small amount of a non-polar liquid phase must be used if short retention times are to be obtained for polar compounds of high molecular weight. Unfortunately, the use of column packings prepared from diatomaceous earth coated lightly with non-polar liquids for gas chromatography of drugs and other polar compounds results in an unsatisfactory peak shape, manifested by a sharp leading edge and a flat trailing edge which returns slowly to the baseline. When smaller samples are used, the time between injection and peak maximum increases and the peak shape is more distorted. Use-preconditioning may also occur. These effects can be explained in terms of adsorption of the sample by the surface of the support material. Drugs with hydrogen atoms capable of forming hydrogen bonds seem to be adsorbed strongly and this would suggest that adsorption may involve hydrogen bond formation. Several attempts to reduce this adsorption have been described.

Various support materials have been investigated by other workers. Of these, supports prepared from diatomaceous earth seem to have been used most widely and a number of ways of treating calcined diatomaceous earth have been reported. Using glass microbeads PARKER, FONTAN AND KIRK[1] found that the resolution of the column was poor and peak tailing occurred. A support material prepared from "Tide" was described by DECORA AND DINNEEN[2] and shown to give better results than an acid-washed support prepared from diatomaceous earth. SMITH AND RADFORD[3] compared a number of diatomaceous supports and showed that the adsorption by firebrick was greater than that of several flux-calcined products.

Acid-washing of support material was described by JAMES AND MARTIN[4] in an early paper on gas chromatography. Celite was washed with concentrated hydrochloric acid and then with water until neutral. The function of acid-washing

was stated to be the removal of metallic oxides. Acid-washing has often been used in conjunction with other procedures for improving support material and this makes it difficult to assess the importance of acid-washing. PARKER AND KIRK[5] described the behaviour of barbituric acid derivatives on acid-washed firebrick coated with SE-30. 1 to 10 μg of derivative were used but the method of acid-washing was not stated.

Treatment of supports with dimethyl-dichlorosilane (DDS) was described by HORNING, MOSCATELLI AND SWEELEY[6] who exposed their support material, which had been washed with concentrated hydrochloric acid, to DDS vapour. HOLMES AND STACK[7] found the conditions for adequate treatment with DDS to be critical. They recommend a detailed procedure in which acid-washed support is treated with a very dilute solution of DDS. ANDERS AND MANNERING[8,9] have reported results for a column packed with acid-washed, DDS-treated support used for phenothiazine derivatives and other drugs.

Hexamethyldisilazane (HMDS)-treatment of support materials was first described by BOHEMEN, LANGER, PERRETT AND PURNELL[10] who believed that acid-washing was unimportant and that their treatment, which involved refluxing support material with HMDS in petroleum ether, was superior to that involving DDS. BROCHMANN-HANSSEN AND SVENDSEN[11,12] used a column packed with acid-washed, HMDS-treated, support for barbiturates and sympathomimetic amines. Improvement of columns by *injection* of HMDS has been described by ATKINSON AND TUEY[13]. HMDS and DDS are believed to react with hydroxyl groups on the surface of the support material and thus remove adsorbing sites.

PARKER, FONTAN AND KIRK[14] described the application of a column containing support material coated with potassium hydroxide to the analysis of sympathomimetic amines. They found, not surprisingly, that barbiturates could not be run on this column. The use of polar additives to suppress tailing sometimes makes a column unsuitable for a particular class of drugs. Furthermore, the additives may be thermally unstable and thus limit the temperature range of the column.

The large number of variations involved make it virtually impossible to draw satisfactory comparisons of the various treatments of support material described by other workers. Because of this, it was decided to investigate some of these treatments. Using Chromosorb W, the effects of acid-washing and treatment with HMDS or DDS were tested by packing treated, but uncoated, powder into a column. The uncoated powder was used because it was thought that this would provide a more critical test of adsorption. These experiments indicated that acid-washing followed by treatment with DDS gave the best results. Further study indicated that poor results were obtained when very *dry* support material was treated with DDS. This discovery led to the treatment of *damp* support material with DDS. It was found that under these conditions remarkably good results could be obtained.

During these investigations we found that support material treated with dimethyldichlorosilane improves on heating. This prompted us to investigate the effect of heat on acid-washed Chromosorb W which had been coated with the silicone polymer SE-30. An improvement in results was obtained with temperatures above 300° in the absence of oxygen. In this connection, it is interesting to note that PARKER AND KIRK[5] describe the heating of a column, packed with acid-washed firebrick coated with SE-30, in argon (30 ml/min) at 280° for 24 h, and that GOLDBAUM,

SCHLOEGEL AND DOMINGUEZ[15] recommend that columns packed with SE-30 or QF-1 coated Chromosorb W should be heated at 300° for 8 h. These workers have not reported any development of this procedure and it is not clear from their articles whether they appreciated the effect that heating could have on column performance. The heating may have been intended to remove, from the column, material which caused a high recorder base-line.

It is relevant to note here that a procedure has been described for the production of water-repellent glass surfaces involving coating the surface with a silicone and then heating in air. JOHANNSON AND TOROK[16] stress that the surface must be degreased before coating, either by heating at 400° in air for 1 h, or by cleaning with a solvent. After coating, heating at 300° for 30 min or at lower temperatures for longer periods of time gives the best results. In an article on the thermal and oxidation stabilities of polymethylsiloxanes, ATKINS, MURPHY AND SAUNDERS[17] report that changes occur when these compounds are heated in an oxidising atmosphere at 200° and that cracking occurs in an inert atmosphere at 250°. It is likely that the treatment of surfaces by heating with silicone polymers will be more effective in an *inert* atmosphere, where the effect of increasing the temperature and duration of the treatment may be studied without fear of oxidation occurring. HUNTER *et al.*[18] have reported experiments on the treatment of glass surfaces with a number of silane derivatives, including polymers and lauryl and stearyl derivatives. Their experiments on the effect of heating were rather surprisingly carried out in air.

In a further investigation of the effect of heating support materials with SE-30, we varied the conditions of heating. Good results were obtained when the diatomaceous earth, coated with SE-30, was placed in a slow stream of nitrogen and heated in a furnace at 350° for 1 h. It was, however, not possible to obtain good results when the experiment was repeated. This led to a study of the factors which govern the effectiveness of the treatment. It was observed that a number of factors influence the behaviour of the columns prepared from support material which has been treated in the above manner with SE-30. The method of coating the support material before baking would seem to be critical. After much trial and error, a satisfactory coating procedure was developed in which the diatomaceous earth, which had not been thoroughly dried, was boiled with a solution of SE-30. After excess solution had been removed by filtration, the remaining solvent was removed from the powder by evaporation whilst stirring.

Another important factor is the position of the powder in the tube during the heating at 350°. In an experiment in which the flow rate of nitrogen during heating was much faster than usual, support material with very poor characteristics was obtained. It seemed possible that this was due to the removal of SE-30 or its products from the diatomaceous earth and it occurred to us that this might apply, to a lesser extent, to the powder into which nitrogen at the slower flow rate passed first. To test this, an experiment was performed in which an excess of powder was heated in a slow stream of nitrogen and only powder which had been well down-stream was used for packing the column. On some occasions very good results were obtained by this method.

On one occasion a remarkable improvement in a column which had formerly given poor results, occurred after a liver extract had been injected into the column. This led us to try the effect of tristearin on the performance of the column. When

tristearin was injected and the injector temperature was raised for a short time and then lowered, the results were similar to those obtained with the liver extract. Unfortunately, in some cases, this improvement was only temporary but the results were sufficiently encouraging to warrant further investigation involving tristearin. This was done by heating support material with SE-30, removing excess SE-30 by rinsing with petroleum or toluene and recoating with a mixture of tristearin and SE-30. This procedure yielded very good results. One of the features of tristearin treatment is that the peak height increases even when the tailing is not reduced very much.

Anomalous behaviour of certain metal columns led us to compare a number of stainless steel columns which were packed with portions of the same packing material. Some of the columns, including a new column which had not been used before, gave very much worse results than others. It was subsequently discovered that such columns could be rendered satisfactory by heating them in air to about 550° and then heating them packed with SE-30 coated support material with carrier gas passing.

The elucidation of many of these effects requires further investigation but we feel that it is important that some of the results we have obtained so far should be available for those interested in developing methods for the preparation of columns suitable for gas–liquid chromatography of small quantities of certain compounds.

Although the application of gas chromatography to drugs has been described by a number of workers, little attention seems to have been paid to the *minimum* amounts of drugs which could be run satisfactorily on the columns used. In general, the minimum amounts stated to have been used have been of the order of 1 or 2 μg. This paper includes a description of the gas-chromatographic behaviour of a number of different types of drugs on columns prepared by our new technique (see below). The limitations of these columns have been investigated and it has been found that, in general, the minimum amounts of drugs required for satisfactory analysis are about 25 times smaller than those described by most previous workers.

LLOYD *et al.*[19] give results for the gas chromatography of a number of high molecular weight alkaloids. The size of sample they used was between 5 and 30 μg and the column was packed with unwashed Chromosorb W coated with SE-30. The actual size of samples with which the reported results were obtained was not given.

PARKER, FONTAN AND KIRK[1,14] state their findings for 1 to 8 μg of tranquillizers on a column containing glass microbeads, and for 1 to 10 μg of a large range of drugs on columns packed with acid-washed Chromosorb W coated with SE-30. These drugs include barbiturates, tranquillizers and alkaloids such as morphine, quinine, emetine and strychnine. ANDERS AND MANNERING[8,9] have applied a column packed with dimethyldichlorosilane-treated Gas-Chrom S, coated with SE-30, to some phenothiazine derivatives and a number of other compounds. Results are given for 5 to 10 μg of phenothiazines, for 2.5 μg amphetamine and 30 μg of morphine.

KAZYAK AND KNOBLOCK[20] used 10 to 30 μg of sample in their analysis of a wide range of drugs including morphine, quinine and strychnine. These workers used a column, conditioned at 300° for 8 h, packed with Anakrom ABS coated with SE-30. They show a chromatogram obtained with 25 μg of morphine after extraction from urine. BROCHMANN-HANSSEN AND SVENDSEN[11,12] report results for 5 to 10 μg of a large number of barbituric acid derivatives and sympathomimetic amines. Barbiturates were analysed on a column containing SE-30 at 137° and amphetamine was

run on SE-30 at 82°. In both columns, the support material was Chromosorb W, acid-washed and treated with hexamethyldisilane. CIEPLINSKI[21] describes the preparation of a column for barbituric acid derivatives and shows results for 10 μg per μl of solution but he does not state what volume of this solution was injected.

JAIN, FONTAN AND KIRK[22] describe a column and procedure by which 0.08 μg of a barbituric acid derivative can be analysed after a simple extraction from blood. The column temperature they used was 230°, which would make their column unsuitable for high molecular weight compounds. VANDENHEUVEL, HAAHTI AND HORNING[23] describe the application of a column to a number of compounds (including barbiturates) but they do not state the amount of samples used in the recordings which they reproduce. They do, however, imply that they could "recognize" down to about 0.1 μg but, again, the meaning of their statement is not clear.

Our experimental investigations of some of the factors limiting the sensitivity of columns used for the gas chromatography of drugs resulted in the preparation of columns which gave satisfactory results with 0.04 μg of some barbituric acid derivatives at 160°. Some of these drugs could be detected at the 0.01 μg level. Also, a number of drugs either containing groups which we thought might cause the drug to run badly (*e.g.* with severe tailing) or else of high molecular weight were tested. The temperature of the column was adjusted to give a retention time of 2 to 8 min and the response for different quantities of sample was obtained.

EXPERIMENTAL

A Perkin-Elmer model 800 Gas Chromatograph equipped with a flame ionisation detector was used. The signal was recorded on a Honeywell —0.25 to +2.5 mV recorder. The carrier gas was nitrogen (oxygen free) 30 ml/min.

Preparation of column packing

About 200 ml of Chromosorb W were washed several times with concentrated hydrochloric acid and the powder was then boiled in the acid in a conical flask for 10 min. The powder was rinsed several times with concentrated hydrochloric acid and then with water until the supernatant liquid was neutral to a pH paper. The suspension of the powder in water was then boiled for 10 min, rinsed several times with water, the "fines" decanted after each rinsing and excess water removed by vacuum filtration. The powder was placed in flat glass dishes and dried on a boiling water bath. 50 ml of this washed powder were then boiled for 10 min with an excess of toluene. A volume of a 10 % (w/v) solution of SE-30 in toluene, equal to the volume of toluene present was added to the mixture which was then thoroughly stirred and boiled briefly. The powder was then drained in three portions by vacuum filtration and each portion was dried with stirring on a hot-plate. The three portions were placed in a Pyrex glass tube measuring 2.5 cm in diameter and 40 cm long and fitted at one end with a sintered glass disc. The tube and contents were then heated in a stream of nitrogen (about 30 ml/min) in a furnace at 350° for 1 h. That portion which had lain between the other two portions during the heating was placed in a sintered glass filter funnel and rinsed 4 times with toluene and 3 times with a solution containing 2 % (w/v) SE-30 and 0.1 % (w/v) tristearin in toluene. Each rinse involved stirring followed by vacuum filtration. The final rinse was followed by thorough

filtration and the powder was then dried with stirring on a hot-plate. The powder was packed into a metal column prepared as described below.

Preparation of metal column

The column consisted of a 6 ft. length of stainless steel tube $^1/_8$ in. O.D. and 0.085 in. I.D., coiled into a helix about 3 in. in diameter and 18 in. long. A steel sintered plug was pressed into one end of the tube. The column was then heated in air in a furnace at 550° for 1 h. After cooling, the column was packed (as described below) with one of the end-portions of powder which had been coated with SE-30 and heated as described above. This column was then heated at 300° in the gas chromatograph with carrier gas flowing at 30 ml/min for 2 h. The column was then removed, emptied, and repacked with the middle fraction prepared as describe above. After heating in the gas chromatograph at 250° for 2 h, the column was ready for use.

Procedure for packing the column

The sintered steel disc end of the column was connected to a water pump. The open end of the column was held uppermost and powder was poured into it through a small filter funnel attached by rubber tubing. The column was tapped gently until no more powder entered. This procedure took less than 5 min. The open end of the column was then plugged with glass wool which had been silanised by wetting it with a 1 % (v/v) solution of DDS in petroleum ether, rinsing with methanol and then with petroleum ether, and drying.

Drug solutions

These were prepared in ethanolic solution and were injected into the gas-chromatograph with a 10 μl Hamilton graduated syringe. For nomenclature of the drugs, we have used that given in the Merck Index 1960.

RESULTS AND DISCUSSION

The results with Chromosorb W which was *rapidly* washed with concentrated hydrochloric acid and not allowed to stand with the acid were as good as those obtained with Chromosorb W which had *prolonged* contact with acid. Washing with aqua regia and concentrated nitric acid did not give better results than washing with concentrated hydrochloric acid.

Injection of HMDS or DDS into columns containing these acid-washed Chromosorb W preparations caused a large improvement. Silanisation of the acid-washed powder gave much better results than those of the silanised non-acid-washed powder. Treatment with HMDS similar to that described by BOHEMEN *et al.*[10] gave good results which deteriorated when the column was heated to 260°.

A commonly given explanation of the effects of silanising agents on support material is the formation of silyl ethers of reactive groups on the surface of the support. BOHEMEN *et al.*[10] suggest that HMDS reacts with $-\overset{|}{\underset{|}{Si}}-OH$ groups to produce the inert group $-\overset{|}{\underset{|}{Si}}-O-Si(CH_3)_3$. If this explanation is correct it would appear that this ether is not stable at 260°. In the same way reaction with DDS might be supposed to involve reaction of a DDS molecule with two neighbouring surface

—Si—OH groups or with one surface group and with a molecule of methanol. If methanol rinsing were omitted, this scheme suggests that free —Si—Cl groups would hydrolyse to form harmful —Si—OH groups. In fact, under these circumstances, DDS-treatment was found to be ineffective.

An adequate explanation of the effect of DDS must also take into account the importance of water and the improvement of treated support material on heating. A possible explanation of the importance of water is that adsorbing surface groups (which might be metallic oxides or derived from silica) are converted to a reactive form in the presence of water, *i.e.* metallic oxides might hydrate to give a hydroxide. In this connection, it is of interest that treatment of *wet* Chromosorb W (which had not been acid-washed) with DDS was successful. It is possible, however, that in this case the hydrochloric acid, released in the reaction of DDS with water, fulfilled the same function as acid-washing.

A rather different explanation of the results is also possible. If only a few of the adsorbing sites on the surface of the powder were reactive with DDS, DDS molecules would only be attached to a few sites along the surface. In the presence of water the formation of polymers on these sites would occur and this might lead to a situation in which adsorbing sites were screened by layers of silicone polymers on the surface.

A certain amount of information has been collected about the changes which occur when support material is heated with SE-30. When nitrogen was passed through coated support material and then through uncoated support material, both portions of support material being heated at 350°, the uncoated support was found to improve, but not to the same extent as occurred when *coated* support was heated under the same conditions. The improvement obtained by heating support material with SE-30 was still considerable even after heating in nitrogen at 500° for 1 h, by which time the stationary phase had been removed, (presumably by thermal decomposition and vaporisation). When powder which had been heated with SE-30 was rinsed thoroughly with toluene or with petroleum ether and recoated with SE-30 the results were still very good. These observations suggest a reaction in which parts of silicone polymers are chemically bound to the surface of the support material. It would appear that a reaction can occur between the support material and volatile products formed by heating SE-30.

The importance of a number of other factors must also be taken into account in an explanation of the effect of heating with SE-30. The presence of water on the support material and the coating procedure are important. Also when powder is heated in a high flow-rate of nitrogen poor results are obtained. Furthermore, that portion of the powder (in the glass heating tube) through which the nitrogen passed first yielded inferior results. Possibly the contact of SE-30 with the surface is essential for obtaining good results, and hence uniform coating of the surface is important. The role of water might then be connected with the spreading of the SE-30, or the water might be involved in the reaction. The adverse effect of a high flowrate of nitrogen might be due to traces of oxygen in the nitrogen or it might be connected with removal of SE-30 from the powder through which the nitrogen first passes. These suggestions are only tentative and further investigation of the effect of heating with SE-30 on support materials may yield useful results.

The improvement that occurs when tristearin is injected has some unexpected features. The quantity of tristearin required is very low (500 μg) and, in some cases, there is a large change in peak height with little change in tailing. The mode of action of the tristearin is not known but the results indicate that a process other than simple adsorption can influence the results obtained in gas–liquid chromatography.

Adsorption which affects the results must lie somewhere between the extremes of weak adsorption by a large number of sites and strong adsorption by a few sites. These types of adsorption will exert different effects on the results. With weak adsorption, distortion of the peak shape will occur but it should be possible to run small quantities through the column although the retention time may increase slightly. Strong adsorption will be characterised by the removal of a fixed amount of sample and by the fact that good results can be obtained for a sufficiently large sample (which saturates the adsorbing sites). A column with strongly adsorbing sites may show marked use-conditioning.

It might, therefore, be thought that tristearin occupies a few strongly adsorbing sites. Tristearin, however, does not behave as a polar phase because a 0.1 % coating of tristearin does not alter the retention times of a column. In view of this, if the removal of adsorbing sites occurs after the injection of tristearin it must be caused either by polar decomposition products or by reaction.

The difficulty with this explanation is that tristearin affects the peak heights over a wide range of sample sizes. If, for instance, 0.1 μg of a sample were strongly adsorbed the results with 0.1 μg would be poor and with 1 μg they would be good. Suppose injection of tristearin reduced the adsorption to 0.01 μg. The results with 0.1 μg would improve enormously and the peak height with 1 μg by a factor of 10/9. In practice, on some occasions, the peak heights from 0.04 to 2 μg of barbituric acid derivatives approximately doubled following injection of tristearin.

It would appear then that although adsorption may play an important part in the non-ideal behaviour of packed columns, another effect operates which prevents a certain fraction of sample from emerging in the main sample peak and this effect is controlled by tristearin.

Two suggestions of possible causes of this phenomenon may clarify the situation. The carrier gas used in this work was oxygen-free nitrogen. This gas may contain up to 5 p.p.m. of oxygen and a calculation shows that, if a sample is in the column for 4 min, enough oxygen will have passed through to oxidise about a μg of sample or, alternatively, to produce sites which would capture a similar quantity of sample. Suppose this reaction were slow and the rate depended on the concentration of sample in the gas phase, an amount of sample proportional to sample size would be eliminated. Tristearin might remove oxygen by reaction or it might react with sites as they were produced. However, an attempt to eliminate oxygen from the carrier gas did not produce better results.

An alternative explanation is that after reaction with SE-30 a layer of molecules attached to the surface forms a screen through which sample can diffuse slowly to adsorbing sites which have not reacted. The control of the rate of adsorption can be seen in terms of closely spaced polymer molecules which will occasionally adopt a configuration allowing access to the surface. The tristearin or its products might slowly diffuse through the polymer layer and thus modify this effect. The rate of sample diffusion might depend on concentration and hence similar fractions of sample

would be removed. The importance of the coating procedure before baking might, therefore, be due to the production of a dense layer of silicone polymers chemically bound to the surface.

The condition of stainless steel columns and methods for improving such columns are of great importance. The inferior results obtained with some untreated columns may be due to contamination but, if this is the case, the contamination must be such that it could not be removed by toluene, petroleum ether or methanol. Metal columns have some obvious advantages over glass columns; they are strong, can be bent without heating into almost any shape desired and are not fragile. This means that packing can be done rapidly and firmly because the column can be tapped without fear of breakage whilst it is being packed.

The following approximate quantities, calculated for the materials and apparatus used in this work may be useful in a consideration of factors which limit the column performance.

1. The surface area of the powder in one of our packed columns is about 10 square metres.

2. The surface area of the inside of one of our columns is about 0.01 square metres.

3. A 2 % (w/w) loading of the powder with SE-30 should give a layer, on average, 3 molecules deep.

4. 250 μg of tristearin could cover an area of 0.2 square metres.

5. 2 μg of sample could cover an area of 0.002 square metres.

As regards the scope of the column prepared as described above, the results show that there is a wide variation in the performance of the column for different compounds. Table I shows the retention times for a given column temperature and the minimum detectable amounts of various drugs using our column. The drugs were selected because they contained a group or groupings which might be expected to prevent the compound from running satisfactorily on the column. These drugs were,

TABLE I

MINIMUM DETECTABLE AMOUNT OF DRUGS CHOSEN TO TEST THE SCOPE OF THE COLUMN

Drug	*Column temperature (°C)*	*Retention time (min)*	*Minimum amount of drug detectable (μg)*
Amphetamine	120	2.5	0.2
Phenelzine	140	3.1	1
Salicylic acid	160	1.5	2
Secobarbital	160	9.2	0.01
4-Hydroxyacetanilide	180	3.5	2
Caffeine	180	4.9	0.04
Cyclizine	215	2.7	0.01
Phenylbutazone	215	7.2	0.01
Scopolamine	225	4.6	0.2
Morphine	245	3.2	0.2
Librium*	245	3.6 and 7.3	0.04
Trifluoperazine	245	6.9	0.04

* Librium shows two peaks. The first peak to emerge may be a decomposition product of librium.

therefore, used as a test of the ability of the column to handle small amounts of compounds of a range of chemical types encountered in toxicological analysis. It is difficult to identify the features of a drug which cause it to run badly from the small selection of drugs considered here but the following observations can be made.

The highly conjugated drugs phenylbutazone, caffeine and librium can be detected in amounts less than 0.04 μg. Drugs containing the piperazine ring, substituted so as to give two tertiary amine groups, such as cyclizine and trifluoperazine can be detected in small quantities whereas the same amount of scopolamine, which contains a tertiary amine group and an alcohol group, does not give as satisfactory a result. 0.2 μg of morphine (a tertiary amine, a phenol and an alcohol) and amphetamine (a primary amine) is the minimum quantity to give satisfactory results. More than 1 μg of phenelzine (a derivative of hydrazine), salicylic acid (a carboxylic acid and a phenol) and 4-hydroxy-acetanilide are required for satisfactory results. It is interesting to note that *p*-aminophenol gives better results than its N-acetylated derivative 4-hydroxyacetanilide. The high molecular weight drugs quinine, brucine and emetine were not detected when up to 10 μg were injected at 245°. Retention times for these compounds have been reported by other workers but in some cases the reported retention time is so short that it seems likely that the observed peak was due to a decomposition product.

One of the most important facts which emerges from our results is that, with most of the drugs we have used, the retention time does not increase as the concentration of the injected drug is reduced. For example, identical retention times were obtained for 0.2 μg and 0.01 μg of secobarbital; 0.2 μg and 0.01 μg of phenylbutazone; for 0.2 μg and 0.04 μg of librium; and for 0.2 μg and 0.04 μg of trifluoperazine. In all these examples, the volume of solution injected was 1 μl. This point is discussed further in our article dealing specifically with the analysis of barbiturates and some related compounds in biological media (see McMARTIN AND STREET[24]).

Our results suggest that our column could be used to develop a procedure to screen biological samples for the presence of an unknown drug in poisoning cases, where the quantity of drug present would be sufficiently large for the technique to be used to cover rapidly a large number of drugs. Work along these lines is now being carried out.

The column we used was designed to have short retention times so that high molecular weight compounds could be analysed at reasonable temperatures and, hence, so that the column would be applicable, with temperature-programming, to a large range of drugs. For work on a single drug, it would be possible to use a column containing more phase or a more polar phase which, whilst more limited in the range of drugs to which it were applicable, would give results with smaller quantities of certain drugs than those described in this paper.

A technique capable of estimating 0.01 μg or less of a drug would be very useful in studies connected with the correlation of the "free" drug level in the plasma and therapeutic effectiveness in the human.

ACKNOWLEDGEMENT

This research programme has been supported by a grant from the Scottish Hospital Endowments Research Trust.

SUMMARY

Experiments on Chromosorb W show that if hexamethyldisilazane (HMDS) or dimethyldichlorosilane (DDS) is to be used to reduce adsorption then acid-washing is also necessary in addition to the silanisation to obtain satisfactory results with drugs. Treatment with HMDS is not thermostable. Acid-washed Chromosorb W which has been treated with DDS improves after heating at 260°. The presence of water on the acid-washed support is necessary for satisfactory DDS treatment. A column packing which gives satisfactory results with 0.04 μg of some barbiturates at 200° has been prepared by treatment of *wet* Chromosorb W with DDS.

Some of the factors limiting the quantities of certain drugs which can be analysed by GLC are described. Support material suitable for small quantities of certain barbituric acid derivatives was prepared by heating the support material, coated with SE-30 at 350° in a stream of nitrogen. Consistent results required special procedures for coating and heating the powder. A remarkable improvement in poor columns sometimes occurred following the injection of tristearin. The detailed preparation of SE-30–tristearin columns is described. The effect of tristearin has been exploited in the preparation of good columns. The state of the metal column is important if good results are to be obtained and even new columns may be unsatisfactory. A satisfactory procedure for improving poor metal columns is described. Good results were obtained with 0.04 μg of certain barbituric acid derivatives at 160° using an SE-30–tristearin column.

The scope of this type of column was tested with drugs chosen to represent a wide range of chemical types. For each drug the temperature was adjusted to give a retention time of about 4 min and the minimum quantity of drug to give satisfactory results under these conditions was determined. This quantity varied from 5 μg for 4-hydroxyacetanilide to 0.01 μg for cyclizine and phenylbutazone. 0.04 μg of a number of drugs can be analysed successfully using an all-metal system.

REFERENCES

1 K. D. PARKER, C. R. FONTAN AND P. L. KIRK, *Anal. Chem.*, 34 (1962) 757.
2 A. W. DECORA AND G. U. DINNEEN, *Anal. Chem.*, 32 (1960) 164.
3 E. D. SMITH AND R. D. RADFORD, *Anal. Chem.*, 33 (1961) 1160.
4 A. T. JAMES AND A. J. P. MARTIN, *Biochem. J.*, 50 (1952) 679.
5 K. D. PARKER AND P. L. KIRK, *Anal. Chem.*, 33 (1961) 1378.
6 E. C. HORNING, E. A. MOSCATELLI AND C. C. SWEELEY, *Chem. Ind. (London)*, (1959) 751.
7 W. L. HOLMES AND E. STACK, *Biochim. Biophys. Acta*, 56 (1962) 163.
8 M. W. ANDERS AND G. J. MANNERING, *J. Chromatog.*, 7 (1962) 258.
9 M. W. ANDERS AND G. J. MANNERING, *Anal. Chem.*, 34 (1962) 730.
10 J. BOHEMEN, S. H. LANGER, R. H. PERRETT AND J. H. PURNELL, *J. Chem. Soc.*, (1960) 2444.
11 A. BROCHMANN-HANSSEN AND A. B. SVENDSEN, *J. Pharm. Sci.*, 51 (1962) 318.
12 A. BROCHMANN-HANSSEN AND A. B. SVENDSEN, *J. Pharm. Sci.*, 51 (1962) 938.
13 A. P. ATKINSON AND G. A. P. TUEY, *Nature*, 199 (1963) 482.
14 K. D. PARKER, C. R. FONTAN AND P. L. KIRK, *Anal. Chem.*, 35 (1963) 356.
15 L. R. GOLDBAUM, E. L. SCHLOEGEL AND A. M. DOMINGUEZ, in A. STOLMAN (Editor), *Progress in Chemical Toxicology*, Academic Press, London, New York, 1963, p. 41.
16 O. K. JOHANNSON AND J. J. TOROK, *Proc. Inst. Radio Engrs.*, 34 (1946) 296.
17 D. C. ATKINS, C. M. MURPHY AND C. E. SAUNDERS, *Ind. Eng. Chem.*, 39 (1947) 1395.
18 M. J. HUNTER, M. S. GORDON, A. J. BARRY, J. F. HYDE AND R. D. HEIDENREICH, *Ind. Eng. Chem.*, 39 (1947) 1389.
19 H. A. LLOYD, H. M. FALES, P. F. HIGHET, W. J. A. VANDENHEUVEL AND W. C. WILDMAN, *J. Am. Chem. Soc.*, 82 (1960) 3791.

20 L. KAZYAK AND E. C. KNOBLOCK, *Anal. Chem.*, 35 (1963) 1448.
21 E. W. CIEPLINSKI, *Anal. Chem.*, 35 (1963) 256.
22 N. C. JAIN, C. R. FONTAN AND P. L. KIRK, *Microchem. J.*, 8 (1964) 28.
23 W. J. A. VANDENHEUVEL, E. O. A. HAAHTI AND E. C. HORNING, *Clin. Chem.*, 8 (1962) 351.
24 C. MCMARTIN AND H. V. STREET, *J. Chromatog.*, 1966, in press.