Some applications of Py-GC-MS to the identification of drugs and microorganisms.

By

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A thesis presented for the degree of

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in the

University of Aston in Birmingham.

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SUMMARY

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The development of pyrolysis in combination with other analytical techniques is briefly discussed.

The use of pyrolysis-gas chromatography(Py-GC) and pyrolysis-mass spectrometry (Py-MS) as a tool for the identification of microorganisms is discussed. Results are presented on the optimisation of the Py-GC system for the analysis of microorganisms and data on the reproducibility of the pyrograms given.

A brief review of the use of pyrolysis in the analysis of drugs is given. The behaviour of medicinal sulphonamides on pyrolysis is shown to be characterised by fission around the sulphonamido group to produce aniline and a characteristic heterocyclic amine although this is complicated in some instances by secondary reactions. The sulphonamides are identified by the retention time data or by direct determination of the heterocyclic amine by pyrolysis-gas chromatography-mass spectrometry(Py-GC-MS). The technique is also used for formulated mixtures with the excipients having no apparent effect on the pyrogram. The pyrolytic fragmentation of sulphonamides is shown to be dissimilar to that occurring under electron-impact conditions. Py-GC is also investigated as a quantitative analytical technique for sulphonamides.

Propionic acid derivatives are analysed by Py-GC-MS and all of these drugs undergo decarboxylation and elimination to yield the characteristic ethyl and vinyl derivatives.

The use of Py-GC-MS in the analysis of drugs and their metabolites in urine is reported. The pyrogram produced by a control urine sample is shown to have no large interfering peaks in the areas of interest and the excreted drugs and their metabolites can clearly be seen in the urine pyrograms.

Seventeen components of the urine pyrogram are identified and the pyrolytic products of several components of urine are investigated. The pyrolytic products of hippuric acid are identified and it is shown that the relative intensities of these products are altered if hippuric acid is pyrolysed with urea. Various amino acids are pyrolysed and their respective products are shown to be affected if pyrolysed with urea.

KEY WORDS.

Pyrolysis, Drugs, Urine, Microorganisms,
Pyrolysis-gas chromatography-mass spectrometry.

CONTENTS

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" The greater our knowledge increases the greater our ignorance unfolds. "

J.F.Kennedy, 1962

INTRODUCTION

Pyrolysis has been defined [1] as the transformation of a compound into another substance or substances through the agency of heat alone. One of the first applications of pyrolysis in direct combination with another analytical technique was described by Zemany [2] in 1952. He reported the use of pyrolysis-mass spectrometry (Py-MS) of natural and synthetic polymers and concluded that it was possible to use pyrolysis in conjunction with other analytical techniques for identification of the original material. The use of pyrolysis-gas chromatography (Py-GC) was communicated by Davison et.al. [3] who reported that the elution pattern of the peaks (pyrogram) could be used as a fingerprinting method for identification purposes. The first use of Py-GC in biomedical analysis was by Janak [4] who described the pyrolysis of the sodium salts of a series of barbiturates and was able to show that unique fragments were produced. He also described the pyrograms of some natural oils and fats. The use of Py-GC in the analysis of microorganisms was reported by Oyama in 1963 [5]. This application has since been developed by the use of pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS)by Simmonds [6]. Probably the most elegant use of the technique , to date, was that reported by Biemann [7] who described the Py-GC-MS apparatus to be used as a life-detection system on the Viking expedition[8] to Mars. There havebeen numerous applications of the technique of pyrolysis and the area has been extensively reviewed [9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23].

Pyrolysers can be divided into two main groups, i.e. (a) Continuous mode and (b) pulse mode pyrolysers.

(a) Continuous mode pyrolysers consist of a heated tube which is surrounded by a heating source, normally an electric coil, and also a means of introducing the sample into the hot zone. Pyrolysis occurs within this zone and the products are removed by flushing with carrier gas (in Py-GC). The heated injection port of a normal GC is probably the most simple of these pyrolysers and has in fact been used for the pyrolytic study of antibiotics [24]. The advantages and disadvantages of these pyrolysers have been thoroughly discussed elsewhere [25].

(b) Pulse mode pyrolysers are more commonly used than the continuous mode type and may be broadly classified into two groups i.e. (i) Hot filament and (ii) Curie point.

(i) With the hot filament the material is kept in very close proximity to the heated filament if not actually on the filament. These pyrolysers have been used extensively in the qualitative and quantitative analysis of polymers [26]. The system is normally designed to be as close to the analyser as possible and in the case of Py-GC is normally situated directly on top of the column. The pyrolysis fragments are immediatly diluted in the carrier gas and are flushed directly onto the GC column. Much detailed work has been undertaken to increase the reproducibility of these pyrolysers with special emphasis on the temperature rise time (TRT). The advent of boosted filament pyrolysers has enabled TRTs of 12ms from ambient to 700°C. A detailed discussion [27] concerning the use of filament and Curie

Composition	Curie Point (°C)
Fe/Co/V	980
Co/Ni (60/40)	860
Co/Ni (45/55)	770
Fe	700
Co/Ni (33/67)	650
Ni/Fe (60/40)	520
Ni/Cr/Fe (51/1/48)	430
Ni/Fe (45/55)	330
Ni	300

Table 1. Composition and temperatures of some Curie point wires

point pyrolysers emphasised the need for fast TRT's combined with low sample weights.

(ii) Curie point pyrolysis, the apparatus used in this study, was first described for the analysis of polymers by Py-GC in 1960 [28]. The technique was developed as a routine analytical tool by Simon and his co-workers [29, 30, 31]. The Curie point is the temperature at which there is a transition from ferro- to para-magnetism. The pyrolysis wire is placed inside a quartz tube whch in turn is surrounded by a coil. This coil is fed with a high frequency current and an alternating magnetic flux is set up in the wire. Hysteresis losses cause the wire to heat up to the Curie point, at which temperature it does not absorb any further energy. Heat is transfered to cooler parts of the wire and also by losses to the carrier gas and hence the temperature drops below the Curie point and enables more energy to be absorbed. This process enables stabilisation of the temperature around the Curie point within a few degrees. The temperature of the Curie point is dependant on numerous factors [30] such as the frequency of the current, composition of the wire and carrier gas flow rate. The wires are normally produced of different alloys and a typical range of compositions is given in Table 1.with the temperatures obtained at a fixed frequency. The Curie point pyrolyser used in this study is shown in Fig. 1. with the pyrolysis wire in position at the centre of the quartz tube. The coupling body joins the pyrolyser directly to the top of the GC column which ensures that the dead volume of the pyrolyser is kept to a minimum.

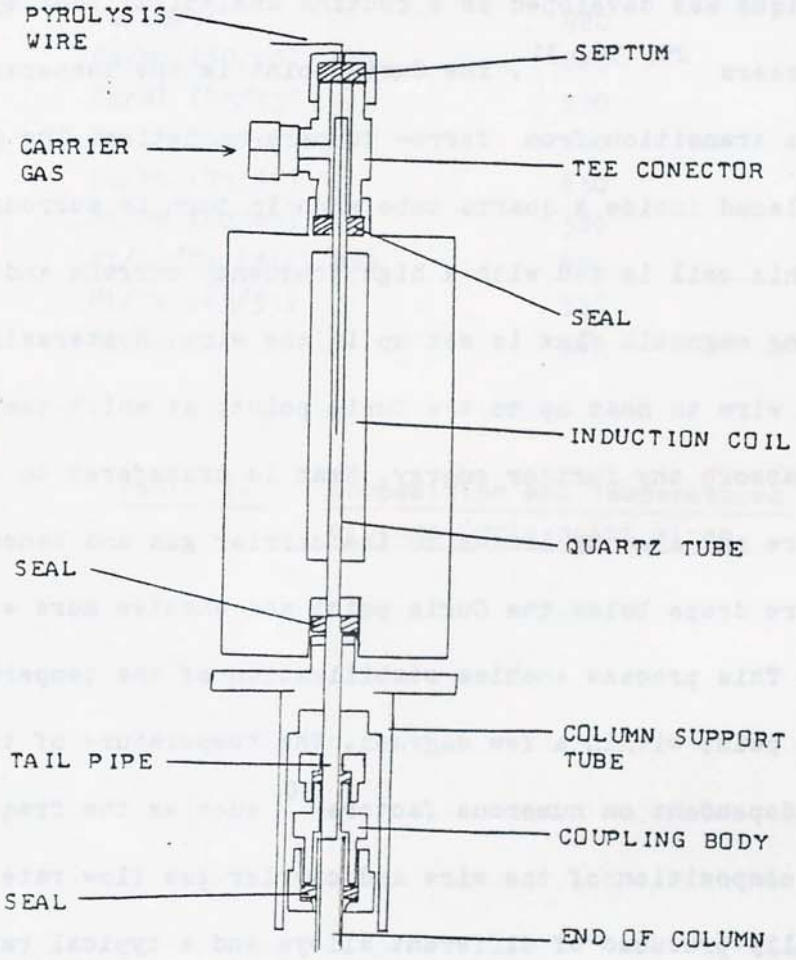


Fig 1. Diagram of Pye Curie point pyrolyser

Various systems of detection have been used for pyrolysis but the majority of the published work has involved either Py-GC or Py-MS. Py-GC is the most common method and the pyrolysis fragments are separated on the GC column and the information obtained can be used for qualitative or quantitative purposes. As a qualitative tool Py-GC provides either a characteristic fingerprint or unique fragments which are diagnostic for particular molecule(s). When fingerprints are used great care must be taken to ensure that the reproducibility of the pyrogram is determined as small differences in peak ratios are used to determine whether an unknown pyrogram matches that of a known sample [11]. When unique fragments are formed identification may be made more easily [32]. Most quantitative work has been reported on the analysis of polymers. The pyrograms produced are relatively simple and usually consist of the monomers or dimers of the polymer. Much work has been reported on the standardisation of this application [25]. The use of mass spectrometry(MS) for the identification of the eluates (Py-GC-MS) adds a further refinement [6]. As well as the normal flame ionisation detector (FID), electron capture detectors (ECD) can also be used and there have been reports of a nitrogen detector being used for specific Py-GC analyses [33].

Py-MS has recently been developed for a number of applications [34] with the advantage that the analysis times are kept to a minimum (sometimes less than 1 min.). The data obtained is usually in the form acceptable for direct computer analysis which is essential if elaborate statistical methods are to be used [35]. As well as the conventional

electron impact (EI) method of ionisation, chemical ionisation (CI) has been used in conjunction with pyrolysis [36]. These milder ionisation techniques enable more information concerning the molecular fragments to be gained and towards this end field ionisation (FI) and field desorption (FD) have been used in studies on biopolymers and microorganisms [37, 38].

Chapter 1 of this study is concerned with the optimisation of the pyrolytic and chromatographic parameters with respect to the analysis of microorganisms. Chapter 2 describes the use of Py-GC-MS in the analysis of drugs and their metabolites.

CHAPTER 1 MICROORGANISMS

A INTRODUCTION

Oyama [5] first described the Py-GC of bacteria when discussing experiments for extra-terrestrial life detection. The pyrograms of three microorganisms isolated from soil samples , although the amount pyrolysed seemed excessive (10 mg), exhibited good chromatographic resolution particularly when the results are compared to some more recent work. This work was extended [39] when the effect of the growth media on the pyrograms was discussed. In addition , comments on the handling of normalised pyrograms when using computer matching of unknown samples were made. The origin of certain peaks was discussed with respect to the pyrolysis of albumin and the constituent amino acids of albumin.

The pioneering work in the use of Py-GC as a taxonomic tool was undertaken by Reiner and his co-workers. The successful analysis of ninety five coded samples of mycobacteria by Py-GC was reported [40] and the advantages over current techniques discussed. No statistical data on the significance of the differences in peak ratios was given. This information would appear to be essential when only quantitative differences in the pyrograms were observed. However, the need for stringent control to determine reproducibility was discussed later [41] . It was felt that the possibility of unique fragments being present in the pyrograms was slight and that the data from which classifications were made should be available. The possibility of using computer matching methods was also mentioned. The use of Py-GC for the rapid characterisation of salmonella organisms was discussed together

with the comparative serology and chemistry of the organisms. All fifty-four coded samples were identified correctly and the possibility of utilising Py-GC-MS to associate the differences in the pyrograms to a particular chemotype were discussed. This work was extended when results obtained from the Py-GC of some Gram-negative organisms were given [42]. Attempts were made to differentiate between bacteria which differed only in flagellar antigens using E.coli cultures of known serotype. Very small differences, described as consistent, were observed but no statistical information regarding the significance of these differences was given. Comments were made on the fact that it is very difficult to place any clinical emphasis on differences in the the pyrograms per se as bacteria with great serological differences can exhibit very similar pyrograms. The Py-GC of fifty samples of mycobacteria were reported [43] and the reproducibility of the retention times of two significant peaks in the pyrograms from five organisms were discussed. An interesting comparison was also made of the results obtained from two different gas chromatographs with the conclusion that the organisms pyrolysed were identified correctly using both instruments. Bar graphs were used for interspecies variation comparisons and suggestions made that these bar graphs could well be computerised for more objective comparisons. The work on the mycobacteria was expanded [44] when the effects of culture age and media on the pyrograms were discussed. These changes were apparently dependant on the individual organism and were difficult to predict, but changing the media had more of an

effect on the pyrogram than the culture age. An interesting comment was made on the differences in the pyrogams from drug-resistant and drug-susceptible organisms. Menger et.al.[45] extended the work reported earlier by Reiner by using computer matching of pyrograms from pathogenic organisms. Although the program gave the correct result in 90% of the cases it was rather too simplistic in nature (as recognised by the authors). Only peaks at the end of the pyrogram were utilised and these were split into two groups :- Those with amplitudes of 3 or 4 mm and those greater than 4 mm. (Peaks of 2 mm or smaller were rejected). The program then compared the test pyrogram altering the error limits on retention times if no peaks were found. The advantage of this technique would be that subjective judgements as to a match or no-match should be eliminated. A general overview of this work has recently been given [46] in which the proposed applications of Py-GC be extended to material such as cancer cells. The use of Py-GC for the differentiation of Clostridium botulinum Types A,B and E has been reported [47] . Interesting comparisons were made on the effect of the growth media on the pyrograms and the effect of sporulation was profound with a small shoulder in the vegetative cell pyrogram becoming the most intense peak in the pyrogram produced from the spores. It was possible, using Py-GC, to differentiate these organisms at the strain level but not always possible at the type level. An attempt was made to detect toxin production by using a modification of the dialysis sac technique and pyrograms were given for the dialysate supernatant fluid (DSF),

DSF plus partially purified toxin and partially purified toxin. However, the peaks in the pyrogram due to the toxin were obscured by other fragments.

The use of Curie point Py-GC of bacteria on open tubular columns was reported in 1972 [48] and comments on interlaboratory reproducibility were made with reference to the wide range of techniques used and the effect of sample preparation. It was refreshing to see a large section devoted to the experimental technique and the advantages of using low loadings (5-15µg) were discussed. The pyrograms produced from different strains of Neisseria menigitidies were given, but only small differences were observed. This work was continued by reporting the differentiation of strains of streptococci which differed by only one antigen, using the Py-GC of cell wall fractions [49]. This study of a well defined bacteria was used in an attempt to understand the biochemical basis for the observed differences in bacterial pyrograms. Polysaccharides were extracted from the cell wall and their respective pyrograms compared, it was concluded from these results that the differences in cell wall pyrograms were due solely to the differences in the polysaccharide present. The automation of Curie point Py-GC was described [9] which enabled unattended operation for 24-30 hours. Attempts were also made to automate the data handling but this was unsuccessful due to unacceptable errors, by the digital integrator, when calculations were made involving fused or tailing peaks. The limitations of

this technique were recognised by the authors and ideas proposed concerning the utilisation of sophisticated computer programs incorporating multidimensional correlation functions.

Py-GC was utilised to differentiate between Types of Vibrio cholerae [50] . The classification was accomplished using the differences in relative peak heights, it was also shown that Vibrio cholerae could be distinguished from other aerobic Gram-negative organisms. These classifications corresponded to those obtained by more conventional methods. The development of a high resolution Py-GC system, for the identification of microorgansms, was described by Quinn [51] . Great care was given to the optimisation of the pyrolysis and of the chromatographic conditions. This approach is to be welcomed as many of the earlier workers did not devote enough space to this type of discussion. The pyrograms from various bacteria were reproduced and it was recognised that some of the peaks may be due to secondary reactions within the pyrolyser. A brief report comparing Py-GC and the GC of extracted, derivatised fatty acids as an aid to the identification of streptococci has appeared [52] . The conclusion was drawn that Py-GC gave more satisfactory results. The use of Py-GC for the identification of oral streptococci has briefly been discussed [53] and expanded [54] . This later paper [54] used Py-GC to compare streptococci from deposits on teeth and in blood with thirty named strains. The magnitude of a selection of peaks in the pyrogram were used to obtain similarity coefficients which in turn

were used for the differentiation of samples. These results were also compared to those obtained in different laboratories and various elaborate statistical techniques used, the results from which were discussed fully. Investigation of the chromatographic conditions were studied with respect to the Py-GC of Gram-negative bacteria [55] . Both polar and non-polar packed columns were investigated but due to improved resolution support coated open tubular (SCOT) columns were used. It was found possible to trap the pyrolysate at the top of the column and then chromatograph normally when the correct flow rate was restored. Although correct identification was achieved when six organisms were classified by a visual matching routine this was not achieved when computer matching was attempted. Problems, similar to those noted by Quinn [51] , were encountered due to the deposition of high-boiling material at the injection end of the column. The use of a disposable precolumn was unsuccessful but a modified backflush method was suggested.

Several Bacillus species, which were difficult to identify morphologically were studied using Py-GC [56] . Difficulty was encountered when the cells were taken straight from the media as peaks due to contaminents e.g. Nutrient agar, produced inconsistencies in the pyrograms. A technique was therefore developed in which the bacteria were grown on a membrane filter placed on the nutrient enabling the cells to be harvested rapidly and cleanly from the filter. This work was extended [57] when it was stated that the effect of culture age on the pyrogram becomes most pronounced

after 48 hours and that sporulation would appear to have a stabilising influence on the cellular constituents.

In 1970 Simmonds [6] described the use of Py-GC-MS to identify most of the fragments produced by the pyrolysis of Micrococcus luteus and Bacillus subtilis var. niger. These experiments were a natural extension of those earlier described for the detection of extra-terrestrial life [5, 39]. Forty eight fragments were identified and tabulated according to their possible biochemical origin e.g. Proteins, carbohydrates, nucleic acids, lipids, and porphyrins. All the major peaks were identified but some (e.g. Acetamide) were not assigned to a particular class. Certain fragments were unique to one organism e.g. Methyl styrene was only seen in M.luteus. Comparisons were made with those results obtained from the previous pyrolysis of geochemical samples and 91% of the fragments seen on the pyrolysis of desert soil were also observed in the pyrolysate of M.luteus.

Meuzelaar et.al. [58, 59,60] described the development of Curie point Py-MS using low voltage electron-impact ionisation. Examples of the pyrograms from various materials were given with an emphasis on short (<1 min) analysis times. The use of pyrolysis followed by FIMS of organisms was described [61]. The molecular ion was used to characterise the pyrolysis fragments. Fragments from m/e 14-142 were tabulated as their high resolution masses together with their probable identity. Many of these structures were consistent with those proposed by Simmonds [6]. These similarities

are perhaps surprising since there is no close taxonomic relationship between the organisms studied in the two reports. This emphasises that the differences on pyrolysis are mainly quantitative and therefore care should be taken when interpreting the results.

The application of Py-MS in the differentiation of strains of streptococci was described [62] and the samples were taken directly from the culture plate. The pyrograms were obtained via a signal averaging unit which permitted study of a single time-averaged pyrogram. This was a useful development as small deviations due to changes in the diffusion rates of fragments from the pyrolysis zone were removed. A brief description of the algorithm for the matching program was given but the need for refined pattern recognition techniques was expressed.

Other attempts to characterise bacteria have been made using degradation followed by MS analysis. The direct insertion of lyophilised bacteria into the ion source of a double-focussing MS to produce characteristic mass spectra has been described [63]. These spectra were shown to originate from the pyrolysis products of phospholipids and ubiquinones. The temperature of the ion-source was held at 300°-350°C and direct probe heating was not used. Reproducibility tests were carried out on different preparations from a single strain. The use of linear-programmed thermal degradation MS as a tool for the identification of bacteria has recently been described [36]. This approach utilises the production of certain fragments at a specific temperature and use of chemical

ionisation enables information concerning the molecular species, of the fragments produced, to be obtained. Ion profiles were reproduced which were used to classify the ten bacteria studied and and the algorithms for making the identification given. Correlation coefficients were supplied for replicate analyses of Citrobacter fruendii.

Meuzelaar et.al.[34, 35,64] reported the extension of their Py-MS work with descriptions of fully automated sample-handling and data-reduction techniques. The instrument was similar to that previously described [58] and utilised an automatic sample introduction turntable which had a capacity of thirty-six samples. The data obtained is treated on a large computer using sophisticated pattern recognition techniques involving multivariate analysis. This is probably the most sophisticated technique in use to date and if the results could be processed on a 'mini' computer the whole apparatus could operate as an independant system which would have great promise as a routine analytical method. It should be interesting to note futura publications concerning the long term reproducibility which is essential if large data banks , necessary for these kinds of comparisons, are to be kept efficiently.

The use of Py-GC as an aid to the rapid diagnosis of viral and fungal diseases in plants was reported in 1969[65] . This work involved pyrolysing leaf slices and characterising normal and infected leaves. Pyrograms were given for various diseasd leaves and also of a spore population of Puccinia striiformis. The same

laboratory also investigated the use of a selective detector, i.e. a nitrogen detector, in order to determine the origins of various peaks in the pyrograms of biological materials [33]. Various dermatophytes were studied by Py-GC [66] and the effects of colony age, sample size and culture media on the pyrograms investigated. However, the pyrograms were poorly resolved and in an extension of this work [67] the chromatography was substantially improved. When considering the problems associated with data evaluation it was found that insufficient information was available from the twenty most intense peaks in any one pyrogram but better results, for taxonomic purposes, were obtained if all one hundred and fourteen peaks were included. A Taxometric map was produced to indicate the relations between the twenty-one strains based on the information derived from the pyrograms.

Py-GC was used as an aid to the identification of Penicillium species [68]. Data was given on the reproducibility of the retention times for twenty-nine peaks over six replicate runs, but the column resolution was not sufficient to separate many groups of fused peaks. This work was extended [69] on the Py-GC of Aspergillus species and the effect of hyphal age and sporulation were investigated. The chromatographic resolution obtained had deterioated from the previous study [68] and no useful conclusions were drawn.

The use of Py-GC-MS in the study of Streptomyces longisporoflavus has been recently described [70]. Most of the fragments were identified and assigned to various biochemical classes as in a previous study, on bacteria, by Simmonds [6]. Comments were made on the appearance of two unique peaks in the pyrogram of the

actinomycete these were isobutane and methylnaphthalene, and the biochemical information obtained regarding the cellular structure was also discussed.

Py-MS was used in an attempt to chemically characterise soil organic polymers[71] . The pyrogram from the Py-MS of melanin, isolated from Eurotium echinulatum, was compared to that obtained from a model polymer. Melanins from other fungi were pyrolysed and the different spectra discussed. The same Py-MS instrument was used as an aid to fungal taxonomy[72] . Various cell fractions, soluble lipid and cell walls, were pyrolysed separately in order to determine the origin of various peaks in the spectrum.

B RESULTS AND DISCUSSION

There have been previous reports on the optimisation of the parameters associated with the Py-GC analysis of microorganisms[48,51], but these were not applicable to the apparatus available. Therefore the initial part of this study was concerned with the optimisation of the various parameters involved with both pyrolysis and the ensuing gas chromatography. As the pyrolysate is flushed directly onto the top of the column (see Fig 1.) the technique should be considered as an integrated system but standard pyrolysis conditions were utilised while the gas chromatography was optimised. A pyrolysis temperature of 980°C and a pyrolysis time of 10seconds were used in these preliminary studies.

(1) Sample preparation.

The bacteria used for this work was Pseudomonas aeruginosa NCTC 6750 and the samples were kindly provided by colleagues in the microbiology laboratory, using the following method:-

The cells were grown in chemically defined media[73] and harvested during the log. phase of growth. They were centrifuged and the resulting pellet was washed with distilled water and again spun down. The pellet was then dried on a watch glass, over P_2O_5, under vacuum for 48 hours.

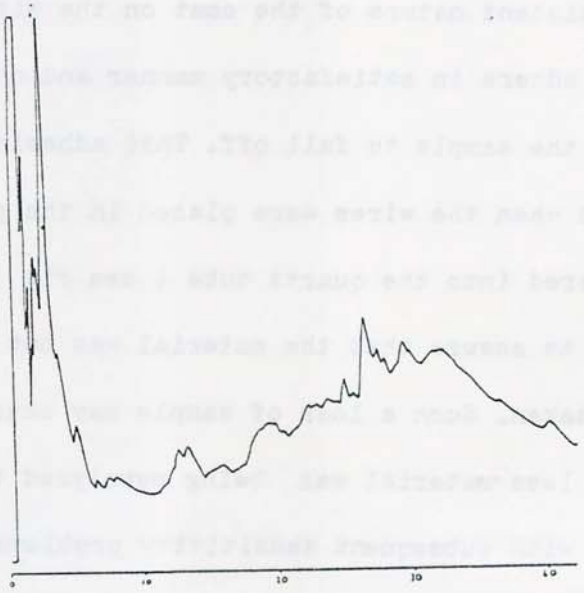
The resulting material was of a crystalline nature and adhered very strongly to the surface of the watch glass. After scraping the material from the glass, the cells were then weighed and ground down to a powder. N.B.This grinding process proved difficult and an uneven powder was obtained due to the extreme crystalline nature of

the sample. The ground material was placed in a small glass tube (40mm x 3mm ID) and carbon disulphide was used as a suspending agent [48]. A Pasteur pipette was then used to transfer the suspension to the heated zone of the pyrolysis wire (25 mm) and the solvent was removed by placing in a stream of warm air from a hairdryer. Due to the inconsistent nature of the coat on the wire the material did not appear to adhere in satisfactory manner and only required a slight knock for the sample to fall off. This adhesion problem was further aggravated when the wires were placed in the pyrolyser, as they had to be lowered into the quartz tube (see Fig 1) and it proved very difficult to ensure that the material was not dislodged when this was undertaken. Such a loss of sample may cause two problems :

(i) less material was being pyrolysed than was desired, with subsequent sensitivity problems and

(ii) the material would lodge at the bottom of the quartz tube and may restrict the carrier gas flow or act as an adsorbing site for polar materials produced on pyrolysis.

In order to improve adhesion various solvents were used in place of carbon disulphide e.g. methanol, ethanol, acetone and ethyl acetate, but no improvement was seen with these and in some cases coagulation of the material ensued. The use of desiccated material was obviously unsatisfactory and therefore the use of lyophilised cells was investigated. The cells were grown and harvested as previously described and a home-made Freeze-Dryer was utilised. The dried cells were very different to those obtained by the desiccation process, with the material having a pubescent appearance,



Retention Time (mins)

Fig 2. Py-GC of P.aeruginosa using 2% OV17 column

and were easily ground down to a homogeneous mass. Due to the low density of the material care had to be taken when handling these potentially pathogenic cells and all manipulations were carried out in a fume cupboard which was kept almost closed. The operator wore a lint mask, to prevent inhalation of any cells, and all surfaces and equipment were regularly washed down with a phenol solution. These cells produced an even suspension with carbon disulphide whose homogeneity was enhanced when the tubes were left (50% submerged) in a low-powered ultrasonic cleaning bath. Prior to coating the end of the wire was flamed.(N.B. The effect of flaming on the reproducibility of the pyrogram was later investigated and no effect was discernible, in contrast to previous work [48] using Curie point pyrolysis.) The wire was placed in the chuck of a mixer motor and rotated in the horizontal plane, along its cylindrical axis, at 600R.P.M. A drop of suspension was placed on the rotating wire, and further drops added until the required coating was obtained. This procedure resulted in a tightly bound coat and was used throughout these experiments unless otherwise specified.

(2) GC Conditions

A semi-polar column was initially investigated and a 1.5m x 4mm I.D. 2% OV17 column was prepared and conditioned. After pyrolysis the column was held at 70°C for 8 mins and then programmed at 5 C/min to 190°C at which temperature it was maintained for ten mins. The resulting pyrogram is displayed in Fig 2 and it is evident that there are several peaks in the initial eluate but after 5 mins

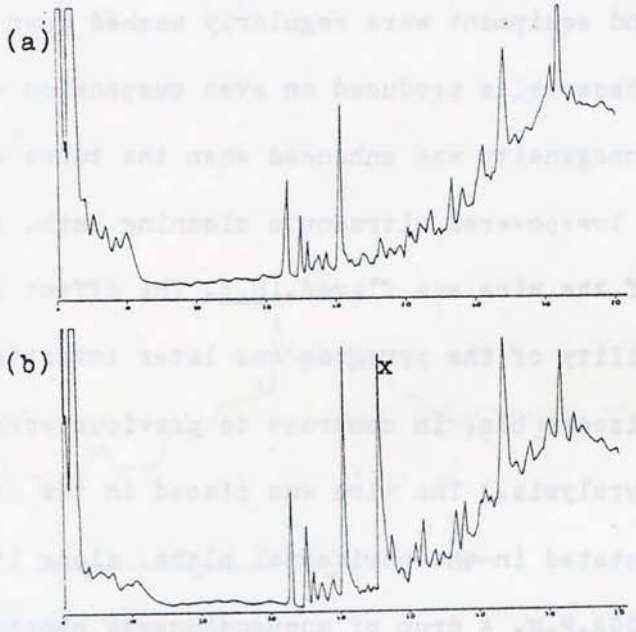


Fig 3. (a) Py-GC of P.aeruginosa
(b) Py-GC of S.aureus

no resolved peaks were apparent. This was obviously unsatisfactory and in an attempt to improve the resolution, the effect of changing the carrier gas flow rate was assessed. The rate was altered over the range 25mls/min to 70 mls/min (measured at an oven temperature of 100°C) but no significant increase in column resolution was seen. After each pyrolysis it was discovered that the inside of the quartz tube, adjacent to the pyrolysis zone, became contaminated with a tarry brown deposit. There was the possibility that this deposit could cause adsorption and a lack of reproducibility and therefore a fresh tube was used for each pyrolysis. The used tubes were cleaned by the following procedure :

(i) Rinsed with water followed by acetone.

(ii) Inside wall of tube scraped with wire followed by thorough wash with acetone and water.

(iii) Tubes placed in Aqua regia (80%HCl, 20% HNO_3) for two weeks.

(iv) Rinsed thoroughly with distilled water followed by acetone.

(v) Dried using a hairdryer.

(vi) Clean tubes were stored in a sealed polythene bag prior to use.

Following the poor results obtained from the semi-polar column the use of a polar column was investigated. A 1.5m x 4 mm I.D. 2% KOH+ 8% CARBOWAX 20M column was prepared and conditioned. After a number of pyrolyses the resolution was greatly improved. The resulting pyrogram from the pyrolysis of P.aeruginosa is shown in Fig 3 . The peaks were well resolved although there were areas in the pyrogram

in which there was a distinct lack of components. After obtaining these initial results on the polar column another bacterial sample was pyrolysed to determine the differences in the pyrograms. Staphlococcus aureus was grown, harvested and prepared as previously described. The pyrogram is shown in Fig 3 and the appearance of the large peak X in the S.aureus pyrogram enabled the two pyrograms to be differentiated by a cursory visual inspection.

(3) Pyrolysis Conditions

Following these initial results the optimisation of the pyrolysis and chromatographic conditions was attempted and the following parameters were individually assessed in order to obtain a well resolved pyrogram in the shortest possible analysis time :

(i) Pyrolysis temperature.

(ii) Pyrolysis time.

(iii) Loading of wire (weight of sample pyrolysed).

(iv) Carrier gas flow rate.

(v) Temperature program rate.

(vi) Initial and final oven temperatures.

(vii) Duration of isothermal periods.

(viii)Length of column.

(i) Pyrolysis temperature

Curie point pyrolysis only enabled variation of the temperature over discrete values and the temperatures chosen for this study were 410°, 510°, 610°, 770°, and 980°C. At the lower two temperatures (410° and 510°C) very poor pyrograms were obtained with no large or well resolved peaks apparent. The three higher temperatures (610°,

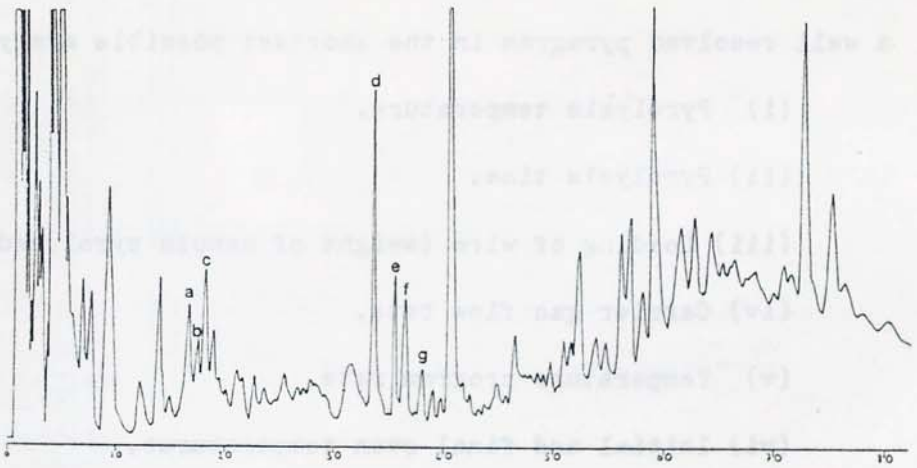


Fig 4. Py-GC of P.aeruginosa for statistical analysis of pyrolysis temperature and time effects

770° and 980°C) all produced similar pyrograms to each other. Seven peaks were measured and normalised to the most intense peak. Fig 4 gives the pyrogram with the seven peaks marked a→g. Peak d was used as the internal standard and the results obtained from replicate runs at the three higher temperatures are given in Appendix 1. The coefficients of variation (CV's) are given and from the comparison of these figures it can be seen that the reproducibility of the ratios obtained at 610°C are worse than those obtained at 770° and 980°C. For example, the CV for peak a at610°C was 21.4% which compared to the 770° and 980°C values of 7.7% and 8.4% respectively. From these latter two results it would appear that the results obtained at 770°C were slightly more reproducible than those obtained at 980°C, but if the CV of the respective peak heights were compared it can be seen that the 980°C value (for peak a) is 58.9% and at 770°C it is 39.2%. The large variation in peak heights at 980°C,due to variation in loading, did not cause a correspondingly high variation in the peak ratios and as this was not the case 980°C was chosen as the pyrolysis temperature.

(ii) Pyrolysis time

The duration of pyrolysis was varied through the values 1.0 sec, 2.0 sec, 5.0 sec and 7.5 sec. Three replicate pyrograms were obtained for each time, except for the 7.5 sec period for which only two were recorded. The peaks chosen for the statistical analysis are the same as in (i) and can be seen in Fig 4 , with the data on the peaks in Appendix 2 . It was expected that the shorter times (1.0 and 2.0 secs) would yield unreproducible results as the cycling

time for the Pye Curie point pyrolyser at 980°C is 1.4 sec. However, this did not appear to be the case with no marked difference between all four times. A previous study [27] had demonstrated that the Pye Curie point pyrolyser was probably underpowered (nominal output 30 watts) for the fast TRT's that are normally required. For this reason the 5 sec period was chosen although it was not certain that this time was short enough to minimise the risk of secondary reactions. It was thought possible that if the pyrolysis time was excessive then the material impacted on the inside of the quartz tube could be pyrolysed again and so cause secondary fragments to be flushed onto the column.

(iii) Loading of the wire

The amount on the wire was varied between 200µg and 2mg. There were problems experienced with weighing the amount of material on the wire as the coated wires would not fit into the weighing compartment of the most sensitive torsion balance available. No obvious differences in the pyrograms were observed, except for intensities increasing with loading. It was concluded that a loading of about 800µg would be aimed at for the bacteria pyrograms, as this would give reproducible pyrograms with a medium amplifier attenuation (10×10^2).

N.B. At this time the Pye 104 could only be used with a single column and therefore the lower the amplifier attenuation the more the baseline rose, during temperature programming, due to column bleed. This rising baseline made the accurate assessment of peak heights or areas more difficult.

(iv) Carrier gas flow rate.

The carrier gas flow was varied from 25mls/min, of nitrogen, through to 70mls/min. There are two opposing problems associated with the flow rate :- If the flow rate was too fast then the components had short residence times and although resolution was not significantly effected the peaks eluted very close to each other and therefore retention time data was more prone to error. Alternatively, if the flow rate was too slow the analysis time was lengthened unduly with the possibility of unacceptably broad peaks.

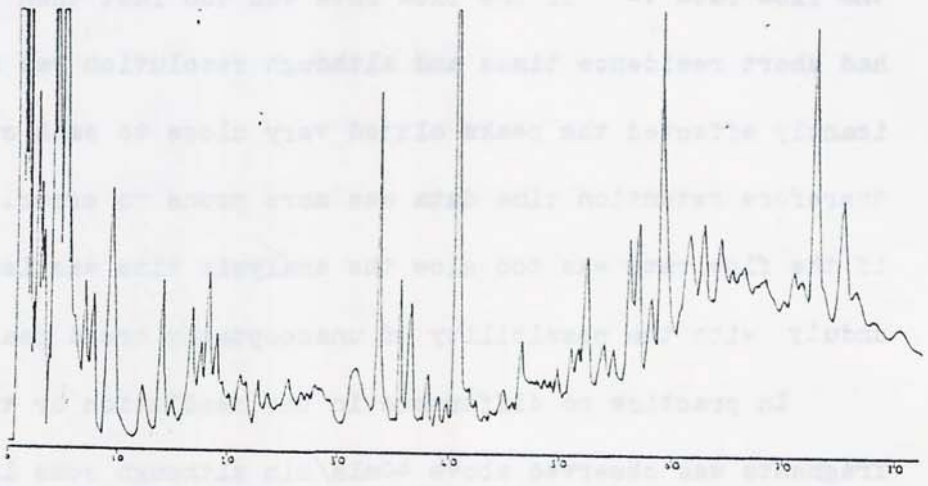
In practice no difference in the resolution or the number of fragments was observed above 40mls/min although some loss of resolution was observed below this. It was decided to use a nitrogen flow rate of 50mls/min (measured at 100°C).

(v) Temperature program rate

The rate of temperature programming was varied between 1.5°/min and 10°C/min. Up to the value of 5°C/min no significant differences were observed but at higher rates some of the smaller peaks were not completely resolved. A rate of 2.5°C/min was decided upon which gave a total analysis time of 80 mins although in later runs this was changed to 5°C/min in order to decrease the analysis time to 45 mins.

(vi) Initial and final temperatures

The initial temperature was set at 50°C, although this would appear to be too low for a CARBOWAX 20M column as the physical state of the polymer changes at about 64°C from a liquid to a solid[74] and therefore retention time data for peaks eluting below this temperature may not be reproducible. However, since a significant



Retention Time (mins)

Pyrolysis Temperature 980°C

Pyrolysis Period 5 seconds

Amount of sample on wire 800µg

Carrier gas (nitrogen) flow rate 50 mls/min

Temperature program rate 50°C/ 4 mins/ → 2.5°C/min /200°C /10 mins

Column 2.1m x 4mm (ID) 2%KOH + 8% CARBOWAX 20M

Fig 5. Optimised pyrogram of P.aeruginosa with the pyrolysis and chromatographic variables.

percentage of the fragments produced by pyrolysis of complex biological material were very volatile and elute below this value , as much information as is available should be obtained from this volatile section. The upper temperature limit for CARBOWAX 20M was about 250°C but when routinely used to this temperature the initial 5-10 cms of the column rapidly darkened and the performance was adversely effected. With temperatures in excess of 200°C no well resolved peaks were seen and therefore a final temperature of 200°C was used.

(vii) Duration of isothermal peroids

These were determined by the amount of significant information obtained at these temperatures. The initial eluent contained many fragments but the following period was relatively bereft of information and therefore a compromise of an initial period of 4mins was used. The later period was determined by the time required for all the significant peaks to be eluted and this was set at 10 mins.

(viii) Length of column

The column length was varied through 1.5m, 2.1m and 2.7m (all 4mm ID) giving analysis times of 60,80 and 110 mins respectively. A compromise was reached between increased resolution and a short analysis time utilising the column of 2.1m.

A final optimised pyrogram for Pseudomonas aeruginosa is shown in Fig 5 with the pyrolysis and chromatographic parameters tabulated below. These conditions were used for all the pyrograms in the following discussion unless stated otherwise.

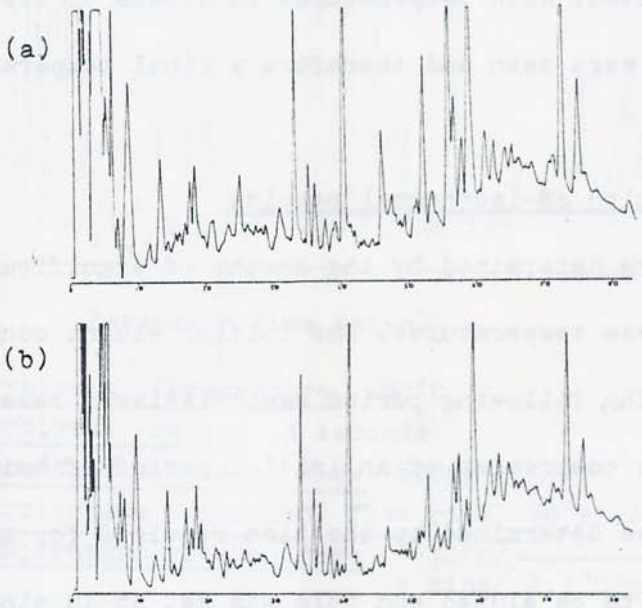


Fig 6. Py-GC of (a) E.coli
(b) P.aeruginosa

An objective method was then sought in order to differentiate between two different bacteria using the pyrograms alone. Esherischia coli and Pseudomonas aeruginosa were chosen as examples and their pyrograms can be compared in Fig 6 . From these two pyrograms it was not possible to assess whether any of the peaks were unique to one organism and it was assumed that none were [41] . In order to differentiate between the two pyrograms on the basis of differences in peak ratios it was essential to determine the deviations in replicate runs of the same sample and also determine the deviations in runs from replicate preparations from the same strain. For these tests twelve replicate samples of P.aeruginosa were pyrolysed and twenty-two peaks were measured manually and normalised to the largest peak. The pyrogram is displayed in Fig 7 with the peaks used labelled from a → v. The peak heights for the eleven replicates can be found in Appendix 3 and the statistical analysis, on the ratios of the individual peaks to peak k, are displayed in Appendix 4 . From the coefficients of variation (CV) given in Appendix 4 it can be seen that this normalisation technique did not provide a satisfactory representation of the pyrogram. It was considered that perhaps better reproducibility would be obtained if the pyrogram was divided into three sections and this was achieved using the retention time boundries of ⟨30 mins, ⟩30 min and ⟨45 mins, and ⟩45 mins. The largest peak within each of the three areas was used as the internal standard (peaks g,k and u in Fig 7) the ratios and statistical analysis are given in Appendix 5 . The CV's are improved over those

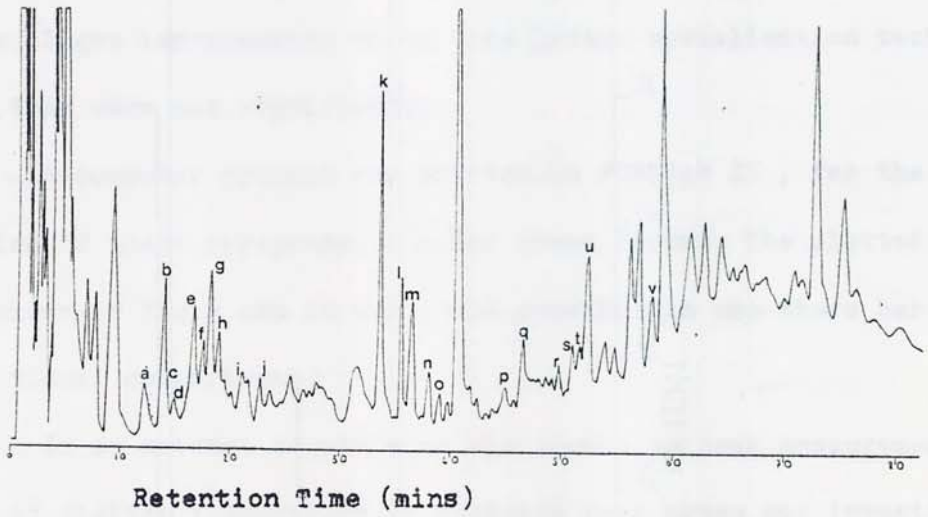


Fig 7. Py-GC of Paeruginosa with the peaks (a-u) utilised for statistical analysis

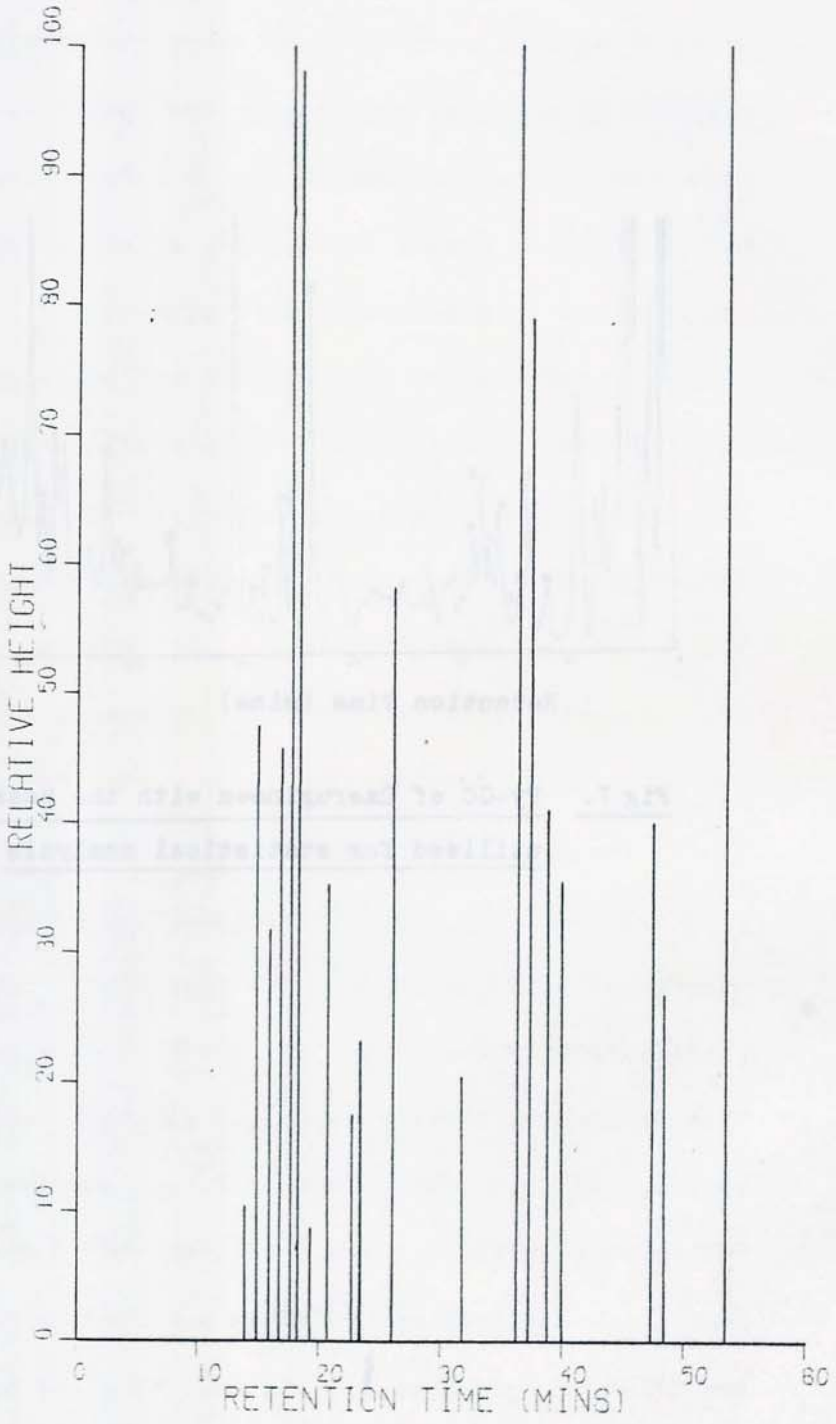


Fig 8 Computer plot of normalised pyrogram

obtained by the previous normalisation technique but are still not satisfactory and an alternative three peaks were chosen, for comparative purposes. Peaks b,l and v were used (see Fig 7)and the ratios and statistical analysis can be seen in Appendix 6 . There were some slight improvements using this latter normalisation technique but they were not significant.

A computer program was written,in FORTRAN IV , for the representation of these pyrograms in a bar graph format. The plotted product is shown in Fig 8 and it was found possible to use these bar graphs for visual comparisons.

In an attempt to minimise the errors in peak measurement the use of digital integrators to estimate peak areas was investigated and three commercially produced instruments were assessed (i.e. Pye DP 88, Infotronics CRS 204 and a Varian CDS101). These did not provide satisfactory results due to their method of estimation of the baseline which was not consistent and errors were found in the evaluation of the areas of fused peaks and unresolved shoulders. This problem is essentially one of correct estimation of the true baseline which is never obvious as the pyrogram consists entirely of overlapping peaks. It was concluded that for correct curve fitting, in this application, the solution would appear to be to obtain the raw data, via some data-logging system, and treat this using a sophisticated curve fitting approach on a main frame computer.

C CONCLUSION

From these results it has been shown that the reproducibility of bacterial pyrograms is dependent on numerous factors which include both the pyrolysis and the chromatographic conditions. If the technique is to be used as a routine method the overall intralaboratory reproducibility must be improved with extensive trials on the interlaboratory reproducibility. Much work has been done to investigate the variables associated with this technique [48,51]. It is indicative of the problems associated with the interpretation of the results obtained that although Oyama [5] first mentioned the possibility of using pyrolysis as a taxonomic tool in 1963 no single method has yet emerged although several techniques are in use in separate laboratories. The identification of over forty pyrolysis fragments by Py-GC-MS by Simmonds [6] could have led to a better understanding of the pyrolysis process but this work has not been expanded.

The most hopeful note would appear to be in the area of Py-MS of microorganisms [34] in which developments are underway to have an independent computer to directly analyse the data using sophisticated statistical and pattern recognition techniques [75].

CHAPTER 2 DRUGS

A INTRODUCTION

(1) General background

Since 1960 there have been numerous reports on the use of pyrolysis in the analysis of pharmacologically active molecules. Many of the early applications of pyrolysis were of great interest at the time of publication but due to the rapid development of techniques in analytical chemistry many of these would not now be the methods of choice for the drugs concerned. This is particularly true for the pyrolysis studies of those high molecular weight antibiotics whose complete structures have only recently been elucidated. Numerous reports have also been made of on-column degradations and rearrangements but in most cases it is not clear whether these reactions are induced by heat alone or are dependent on some active site on the support material itself. For example the decomposition of tolazamide [76] and biguanides [77], the on-column pyrolysis of menadione bisulphite addition compounds, a synthetic analogue of Vitamin K [78], and also the on-column breakdown of some prostaglandins [79]. A thermally-induced on-column Lossen rearrangement of the drug 4-butoxyphenylacetohydroxamic acid was described by Dell et.al. [80]. A brief mention of other thermal conversions of drugs has been made in a review on the use of GC in drug analysis [16]. Reaction gas chromatography has also been used extensively, for example by Rodecka and Nigram [81] in the analysis of tropane alkaloids and by Pella and Galombo [82] who used pyrolysis over various carbon surfaces in order to determine the amount of oxygen in the molecule.

Brodasky[83] used the conventional injection-port heater for low temperature (200-400°C) pyrolysis studies of various antibiotics and

Fig 9. Structural analogues of lincomycin used in Py-GC study (Brodasky [83])

also a conventional pyrolyser for high temperature work (600-1300°C). Lincomycin was examined by low temperature pyrolysis and it was possible to characterise the alkyl substituents at the three major positions of substitution of lincomycin (see Fig 9). Some of these pyrolysis products were identified which enabled determination of these substituents. High temperature pyrolysis (usually at 1000°C) was used to distinguish between pairs of antibiotics whose structures were uncertain. High (900°C) and low (380°C) temperatures were also used in Py-GC studies of polyene antifungal antibiotics candicidin, levorin and trichomycin . No statistical data was given on the reproducibility and no quantification was possible. The analysis of lasalocid, an antibiotic isolated from cultures of Streptomyces lasalensis [84], involved a thermally-induced retroaldol rearrangement to yield a ketone, which occured quantitatively in the injection-port at 310°C.

A quantitative and qualitative study of penicillins and cephalosporins by Py-GC and Py-GC-MS was described in 1976 by Roy and Szinai [32]. On pyrolysis it was observed that fragmentation occured around the amido group to yield, in most cases, a characteristic fragment. This is demonstrated in Table 2 , in which the major fragments are shown. The cracking severity, i.e. the percentage of the material available for pyrolysis that was actually pyrolysed, was given for four antibiotics and this varied from 97.9% for methicillin to 100% for penicillin G. Quantitative studies were carried out using the most intense (and symetrical) peak in the pyrogram and standard curves were drawn in which the log. of the amount on the filament was plotted against the log. response over the range 10ng to 100µg, the regression

Table 2. Major pyrolysis products from pyrolysis of penicillins (Roy & Szinai[32])

coefficients were given for the plots.

An interesting study was reported on the pyrolysis of polymeric sulphonamides [85]. These polymers, produced as potential antibacterials and carriers for anti-tumour agents, were analysed by Py-MS and Py-FDMS and information on the structures of the monomer and dimer obtained. The pyrimidine sulphonamides have been pyrolysed and the products compared with those obtained on hydrolysis [86].

One of the first descriptions of the use of Py-GC in the biomedical field was given by Janak [4] in 1960 who reported the analysis of a series of sodium salts of the barbiturates. He demonstrated that this technique yielded volatile hydrocarbons from which characterisation of the parent molecule was possible, he also noted that the fragments produced were not solely due to the simple fission of the parent molecule but also that thermally-induced rearrangements occurred, e.g. o-xylene was produced from the pyrolysis of phenobarbitone. This work was extended when the Py-GC of twenty-seven substituted barbituric acids was described [87]. Comparisons were made of the pyrograms produced by pyrolysing the free acid, the sodium salt and of mixing the free acid and anhydrous potassium carbonate. The peaks were digitised and represented on a log, time scale, this treatment produced results which were easily compared with each other. The barbiturate work was continued with the identification of the various peaks [88]. The major pyrolysis products of a number of free acids and their salts were given with the various nitriles being the predominant peaks. These nitriles were not as noted earlier by Janak [4] but the authors argue

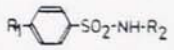
that the chromatography conditions used by Janak probably led to the nitriles being retained on the column. The pyrograms of sodium seconal and sodium phenobarbitone were reported as an application of a new pyrolyser [89]. In a study on the quantitative aspects of Py-GC the analysis of two barbiturates was reported [90]. The drugs were separated from the four inactive substances in the tablet and were pyrolysed after conversion to their sodium salts. Data on the reproducibility was given and this was better than $\pm$ 3% for both of the drugs.

The quantitative analysis of atropine by Py-GC was described [91] and eleven major peaks were obtained when the alkaloid was pyrolysed inside a glass capillary and chromatographed on APIEZON L at 80°C. Two of the larger peaks were used for quantification and an accuracy of $\pm$5% was obtained when 30µg of atropine was pyrolysed.

A comprehensive account was given of the Py-GC of indole alkaloids [92], and the fragments produced were identified by comparison of the retention times with standards. Different classes of alkaloids were differentiated by this means and it was shown that methoxy substituents on the indole ring yielded characteristic fragments. The analysis of twenty-one alkaloids by Py-GC was described [93] and it was shown that they can be identified using the lower hydrocarbon (C_1-C_4) pyrolysis products and trimethylamine with the exception of morphine and heroin which were differentiated due to the production of acetic acid from heroin but not from morphine. The data obtained was treated by various statistical techniques and by using multivariate methods it was possible to differentiate between all the drugs studied.

Many investigations have attempted to identify and suggest the origins of the various constituents of tobacco and tobacco smoke [94]. Pyrolysis provides a good model for the study of this problem and has been used by several workers in the investigation of the process. The breakdown of DDT in tobacco smoke has been studied [95] by pyrolysing DDT in a nitrogen atmosphere and separating the various products by GC and LC. In another experiment to examine the non-volatile products from the breakdown of DDT in tobacco smokes [96] the condensate, after pyrolysis, was analysed by fractionation on florosil and then by GC. The pyrolytic degradation of nornicotine and mysomine was reported [97] and the products identified included quinoline, isoquinoline and 3-cyanopyridine. The pyrolysis of polyenes was studied [98] in an attempt to understand the formation of aromatic hydrocarbons in cigarette smoke.

The volatile products from the pyrolysis of nineteen phenothiazines [99] were studied, and a tentative identification of three of the gaseous hydrocarbons was made. The pyrograms were digitised and normalised, relative to sodium pentobarbital, in the same manner described for barbiturates [87] and therefore the pyrograms were easily compared. However, no mention was made of the results obtained from pyrolysing mixtures of these drugs, as would be found in the clinical or forensic situation. The analysis of saccharin in various soft drinks and multivitamin products was carried out by Szinai and Roy [100] using Py-GC. It was also found possible to assay sodium saccharide by prior conversion to the free imide. The identification of the volatile constituents produced by pyrolysis of cannabidiol was reported [101] using both air and hydrogen as the carrier gas. The volatile components



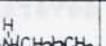
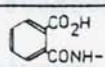
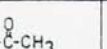
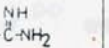
No.	SULPHONAMIDE	R_1	R_2	PROPRIETARY PRODUCT
1	BENZENE SULPHONAMIDE	H-	-H	
2	BENZENE SULPHANILIDE	H-		
3	CHLORPROPAMIDE	Cl-		DIABINESE (PFIZER)
4	p-ETHYL BENZENE SULPHANILIDE	CH_3CH_2-		
5	PHTHALYLSULPHATHIAZOLE			THALAZOLE (MAY & BAKER)
6	SUCCINYLSULPHATHIAZOLE			SULPHASUXIMIDE (MSD)
7	SULPHACETAMIDE	H_2N-		EYE DROPS
8	SULPHANILAMIDE	H_2N-	-H	
9	SULPHADIAZINE	H_2N-		SULPHATRIAD (MAY & BAKER)
10	SULPHADIMETHOXINE	H_2N-		MADRIBON (ROCHE)
11	SULPHADIMIDINE	H_2N-		SULPHAMEZATHINE (ICI)
12	SULPHAFURAZOLE	H_2N-		GANTRISIN (ROCHE)
13	SULPHAGUANIDINE	H_2N-		
14	SULPHAMERAZINE	H_2N-		SULPHATRIAD (MAY & BAKER)
15	SULPHAMETHOXAZOLE	H_2N-		GANTANOL, BACTRIM (ROCHE) SEPTRIN (WELLCOME)
16	SULPHAMETHOXYDIAZINE	H_2N-		DURENATE (BAYER)
17	SULPHAMETHOXYPYRIDAZINE	H_2N-		MIDICEL (PARKE DAVIS) LEDERKYN (LEDERLE)
18	SULPHAMETOPYRAZINE	H_2N-		DALYSEP (SYNTEX) KELFAZINE (MONTEDISON)
19	SULPHAPHENAZOLE	H_2N-		ORISULF (CIBA)
20	SULPHAPYRIDINE	H_2N-		M & B 693 (MAY & BAKER)
21	SULPHATHIAZOLE	H_2N-		SULPHATRIAD (MAY & BAKER)
22	TOLBUTAMIDE	CH_3-		RASTINON (HOECHST)
23	p-TOLUENE SULPHONAMIDE	CH_3-	-H	

Table 3. Structures of medicinal sulphonamides

were identified directly by GC-MS and the less volatile ones were derivatised and by using MS the number of hydroxyl groups available for silylation were estimated. A brief mention has also been made of the possibility of using Py-GC in the estimation of various cardenolides and the replicate pyrograms of digitoxigenin reproduced [46].

The drugs used in this study were sulphonamides and propionic acid derivatives and brief comments only will be made on their uses and methods used for analysis.

(2) Sulphonamides

The sulphonamide antibiotics are characterised by the sulphanilamide molecule with each individual drug differing in the substituent (normally an aromatic heterocyclic moeity)on the amine of the sulphonamido group. These drugs ,and their structures are tabulated in Table 3 .

The sulphonamides have a very broad spectrum of antibacterial activity being active against both Gram-positive and Gram-negative organisms. The action of the sulphonamides is largely bacteriostatic although in high concentrations, in the urine, they can be bacteriocidal. They act by inhibiting the uptake of p-aminobenzoic acid (PABA) which is essential, in bacteria, for the production of folic acid. The most frequently used sulphonamides are active in the treatment of urinary tract infections and are also used in the treatment of eye infections, bacillary dysentry and meningitis. They are normally metabolised by conjugation and acetylation of the free para-amino group on the sulphanilamide part of the molecule. The toxic effects of the sulphon-

amides are usually associated with urinary complications but the use of mixed sulphonamide preparations and lower dose sulphonamides have helped to reduce the rate of crystalluria in patients undergoing sulphonamide therapy. The drugs can broadly be split into four main groups according to their activities. i.e.Short-acting, intermediate acting, long acting and poorly absorbed. These classifications have been discussed elsewhere in detail [102].

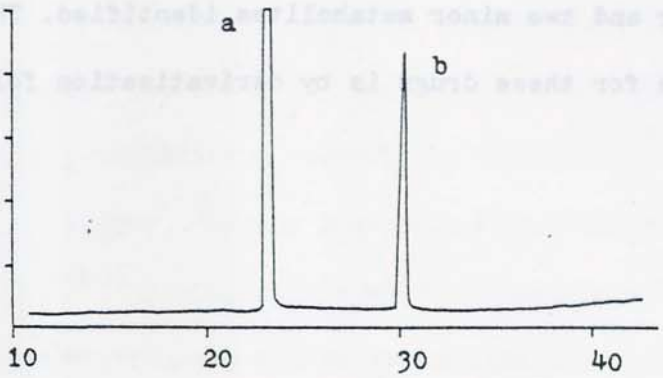
The sulphonamides are generally too polar to be analysed directly by GC. There have been previous reports of methylation[103,104], permethylation and perethylation [105] for GC-MS studies and the use of perfluoroacyl and pentafluorobenzyl compounds for ECD studies[106]. Methylated acetal derivatives have recently been used for this purpose[107]. Degradation techniques have been limited to hydrolysis and GC of the liberated amines[108], and to preparative pyrolysis and thin-layer chromatography (TLC) for pyrimidine sulphonamides[86]. The TAS [109] (thermomicro and transfer-application substance) procedure has been applied to some sulphonamides[110]. Paper and thin-layer chromatography [111] have been used in addition to high-pressure liquid[112] and ion-pair partition chromatography[113].

(3) Propionic acid derivatives

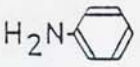
The drugs in this section are characterised by the substituent in position 2 of the propionic acid molecule. These compounds were developed for their analgesic, antiinflammatory and antipyretic effects and the first one, ibuprofen, was released for clinical use in 1969.

It has been suggested that these drugs exhibit their antiin-

flammatory action by the inhibition of prostaglandin synthetase [114]. The metabolites of ibuprofen has been studied in depth by GC [115] and two major and two minor metabolites identified. The most common assay procedure for these drugs is by derivatisation followed by GC.

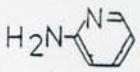


MS Peak a



m/e	93	66	65	92	39	94	67	40
RI(%)	100	35	13	10	8	8	5	5

MS Peak b



94	67	93	66	41	40	39	68
100	81	31	23	23	11	11	8

Fig 10. Py-GC-MS of sulphapyridine

B RESULTS AND DISCUSSION

(1) Sulphonamides

(a) Qualitative analysis

The sulphonamides used in this study are listed in Table 3 with the characteristic substituents given in conjunction with the manufacturer's name (as marketed in the UK). All of these marketed sulphonamides were obtained, as the pure and formulated products, from the named manufacturers.The Py-GC conditions used initially were similar to those utilised in the Py-GC of bacteria with the exception of the initial and final isothermal temperatures which were set at 100°C and 250°C respectively. Most of the medicinal sulphonamides differ only in the heterocyclic substituent on the sulphonamido group and the first one that was used clinically (sulphapyridine) was chosen for the initial Py-GC-MS investigation.

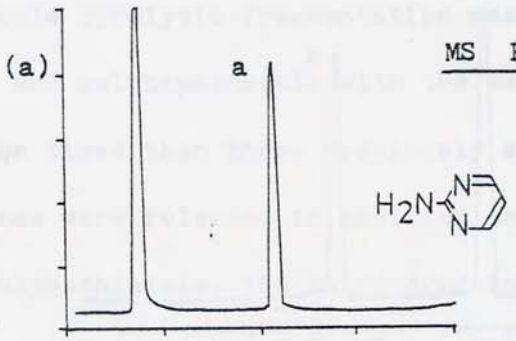
The pyrogram produced by sulphapyridine, as shown in Fig 10, was interesting for its simplicity. There were only two peaks of any magnitude and the structures of these fragments are given with the MS data obtained. The MS data is tabulated in a similar form to that used in the Aldermaston Eight Peak Index [116], i.e. the eight most intense peaks were tabulated on the top line, in order of decreasing intensity, with the relative intensities (expressed as a percentage of the base peak) on the bottom row. It should be noted that the eight peaks were not always in complete agreement with those quoted in the Aldermaston Index and the corresponding relative intensities also differed. These dissimilarities were probably due to the different ionisation

Sulphapyridine Aniline 2-aminopyridine

Scheme 1. Pyrolytic fragmentation of sulphapyridine

energies used. Most mass spectra in the Index were recorded at an ionisation energy of 70eV, however, in this study the GC eluent was followed by a total-ion monitor and the carrier gas (helium) was also ionised if an ionisation energy of 70eV was used, and therefore small fluctuations in the carrier gas flow resulted in an apparently drifting baseline. It was found that by operating with an ionisation energy of 20-22eV (helium appearance potential 24eV) a much improved total-ion current trace was obtained. However, with this lower energy differences would be expected in the relative intensities of some MS fragments when compared to the values given in the Aldermaston Index. In the case of sulphapyridine the two pyrolysis fragments were identified as anilne and 2-aminopyridine by MS data and comparison of retention times with standards. This fragmentation process is summarised in Scheme 1 . The mass spectra of both aniline and 2-aminopyridine are characterised by the loss of HCN from the base peak (also the molecular ion) which is the typical fragmentation seen with aromatic and hetrocyclic amines. The data from the Py-GC-MS analysis of three pyrimidine sulphonamides (i.e.sulphadiazine, sulphamerazine and sulphadimidine) is summarised in Fig 11 . All three drugs underwent similar fragmentation to sulphapyridine with aniline being produced in all three cases along with the characteristic heterocyclic amine. It was possible to identify the heterocyclic amine from the retention data alone, as aniline provided a useful internal standard, but the information obtained from the MS data made identification unequivocal.

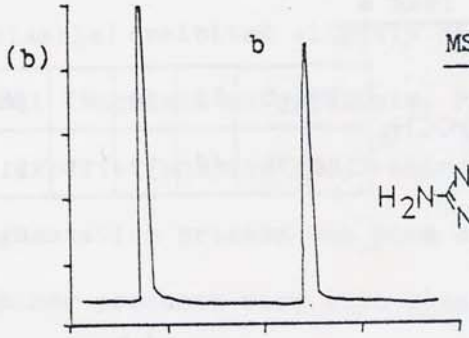
The MS fragmentation of the amine residues was dominated by the



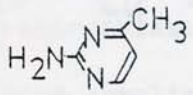
MS Peak a

95	68	42	67
100	40	18	13

96	94	43	39
8	8	8	5

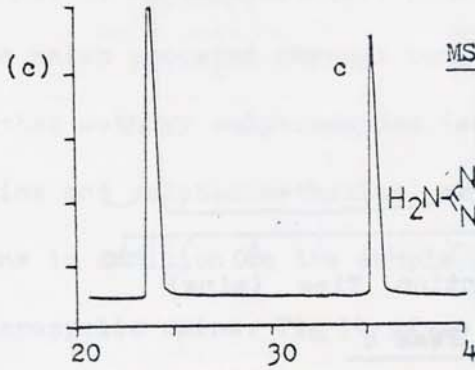


MS Peak b

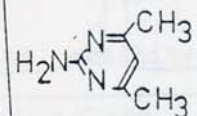


109	82	67	43
100	26	14	13

94	93	41	68
11	10	10	9



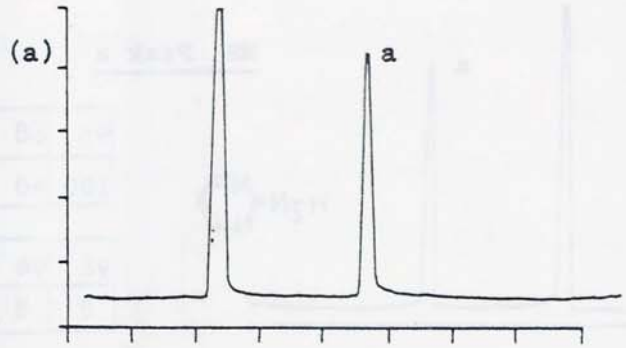
MS Peak c



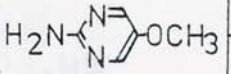
123	96	95	43
100	29	19	19

67	82	124	83
15	10	8	8

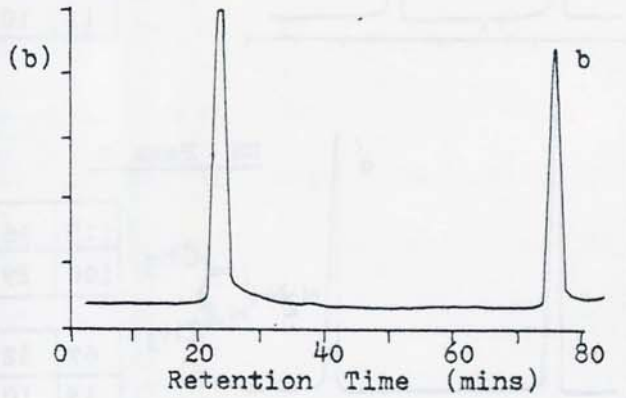
Fig 11. Py-GC-MS of pyrimidine sulphonamides (a) Sulphadiazine (b) Sulphamerazine (c) Sulphadimidine



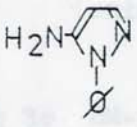
MS Peak a



125	110	82	55	83	56	40	126
100	74	68	37	21	11	11	10



MS Peak b



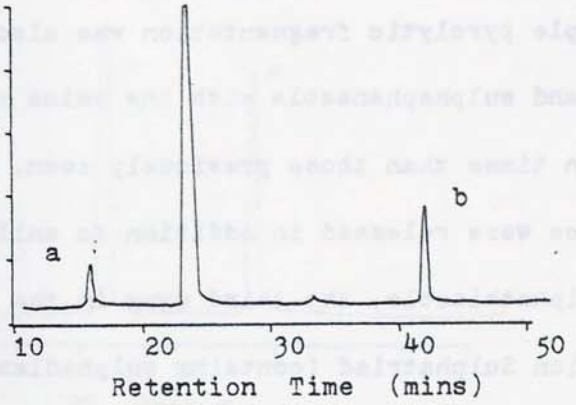
159	158	77	104	92	93	65	160
100	38	31	25	25	19	13	11

Fig 12. Py-GC-MS of (a) sulphamethoxydiazine (b) sulphaphenazole

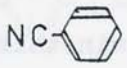
loss of HCN from the molecular ion and the molecular ions (each separated from each other by 14 mass units) were also the base peaks. This simple pyrolytic fragmentation was also exhibited by sulphamethoxydiazine and sulphaphenazole with the amine residues having longer retention times than those previously seen. The MS data confirmed that the amines were released in addition to aniline , see Fig 12 .

Sulphathiazole, the third drug in the triple sulphonamide preparation Sulphatriad (containg sulphadiazine, sulphamerazine and sulphathiazole) exhibited slightly different behaviour by producing two additional fragments on pyrolysis. Fig 13gives the pyrogram obtained, and the expected aniline and 2-aminothiazole were clearly seen. However, the fragmentation process was more complicated than in previous cases as two minor products were also obtained and the larger of these (peak a) was identified as benzonitrile, possibly formed from a secondary reaction which proceded through benzothiazole as an intermediary.

Other methoxy sulphonamides (sulphametopyrazine, sulphamethoxypyridazine and sulphadimethoxine) exhibited evidence of secondary reactions in addition to the simple fragmentation to yield aniline and the heterocyclic amine. Fig 14 gives the results obtained from the Py-GC-MS analyses of sulphametopyrazine and sulphamethoxypyridazine. Sulphametopyrazine yields the expected products of aniline and 2-amino-3-methoxypyrazine but in addition there was also a peak (peak y) of slightly shorter retention time than aniline. This additional peak was identified, by MS and retention time data, as N-methylaniline. The MS fragmentation of these substituted amines is dominated by the $(M-1)^{+\cdot}$ and the $M^{+\cdot}$ doublet. N-methylaniline was also produced by the pyrolysis

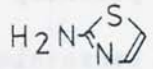


MS Peak a



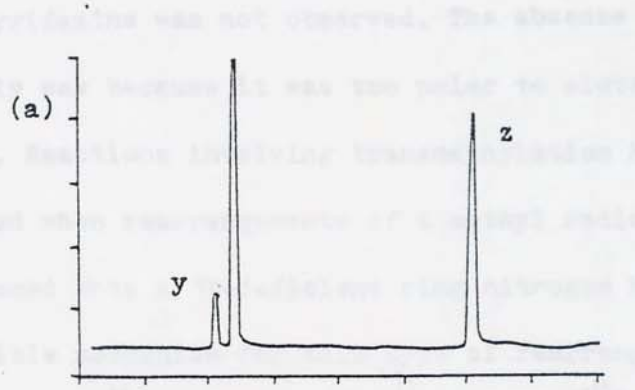
103	76	50	51	102	104	39	78
100	30	15	12	9	7	7	6

MS Peak b

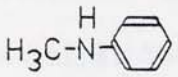


100	58	73	101	46	60	102	43
100	59	25	7	7	7	6	6

Fig 13. Py-GC-MS of sulphathiazole

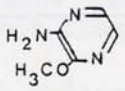


MS Peak y



106	107	77	79	78	65	39	92
100	83	30	24	15	14	10	7

MS Peak z



125	96	82	124	95	68	97	126
100	45	27	20	15	10	9	7

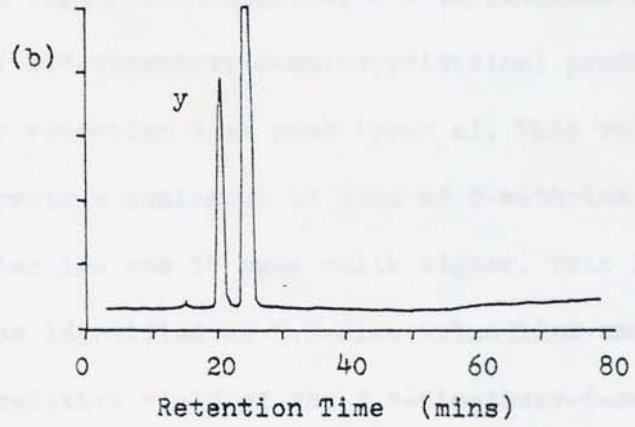
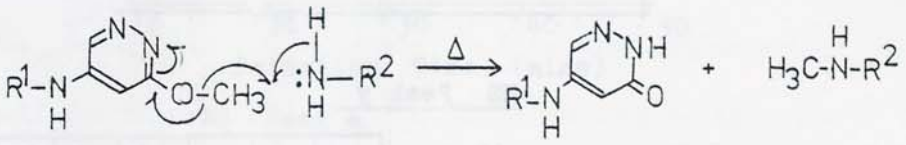


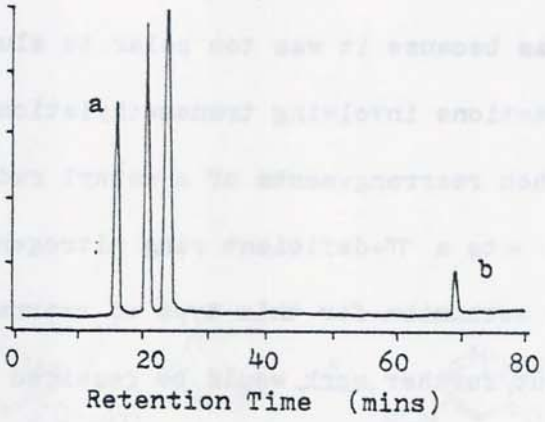
Fig 14. Py-GC-MS of (a) Sulphametopyrazine
(b) Sulphamethoxypyridazine



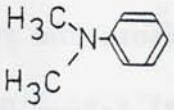
Scheme 2. Possible mechanism for methylation of methoxy sulphonamides

of sulphamethoxypyridazine but in this case the expected 3-methoxy-6-aminopyridazine was not observed. The absence of this amine residue probably was because it was too polar to elute from the CARBOWAX 20M column. Reactions involving transmethylation have previously been reported when rearrangements of a methyl radical from methoxy groups positioned α to a π-deficient ring nitrogen have occured [117,118]. A possible mechanism for this type of rearrangement is given in Scheme 2 but further work would be required to determine at which stage of the fragmentation the transmethylation occured, i.e. before or after the release of aniline. The study of sulphadimethoxine by Py-GC-MS was undertaken as that molecule had two methoxy groups, both of which were α to π-deficient ring nitrogens. The resulting pyrogram can be seen in Fig 15 with aniline, N-methylaniline and the characteristic amine (2,4-dimethoxy-6-aminopyrimidine) produced in addition to a shorter retention time peak (peak a). This volatile component had a mass spectrum analogous to that of N-methylamine except that the molecular ion was 14 mass units higher. This lower retention time peak was identified as N,N-dimethylaniline and indicated with the lower relative yield of the 2,4-dimethoxy-6-aminopyrimidine, that under these conditions sulphadimethoxine was more prone to undergo transmethylation on pyrolysis.

Sulphacetamide was the only sulphonamide studied which had an aliphatic, instead of an aromatic, substituent. On pyrolysis of the sodium salt the pyrogram appeared more complex than those previously seen and six major components were visible. Fig 16 displays this pyrogram but it should be noted that the GC variables were altered

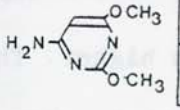


MS Peak a



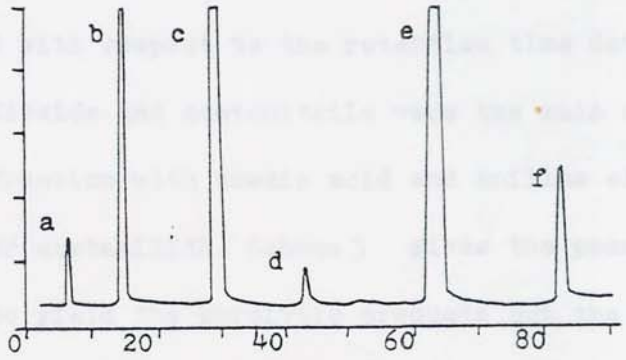
120	121	77	105	104	122	51	79
100	80	29	17	16	9	8	7

MS Peak b



154	155	125	110	67	68	126	156
100	81	36	31	26	20	19	11

Fig 15 Py-GC-MS of sulphadimethoxine



MS Peak a

CO_2

44	28
100	

MS Peak b

SO_2

64	48	66
100	14	2

MS Peak c

$H_3C\text{-}CN$

41	40	39	42
100	43	8	2

MS Peak d

$H_3C\,CO_2H$

43	60	45	42	40
100	99	79	9	9

MS Peak e

H_2N-

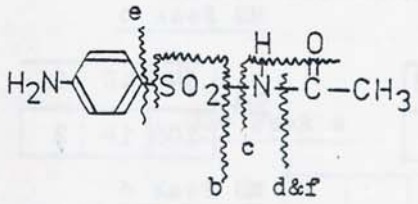
93	66	65	92	94	67	40	39
100	27	7	6	5	3	2	2

MS Peak f

$H_3C\text{-}\overset{O}{\overset{\|}{C}}\text{-}\overset{H}{N}$-

93	135	66	43	94	45	59	44
100	22	13	11	5	5	5	4

Fig 16. **Py-GC-MS of sulphacetamide**

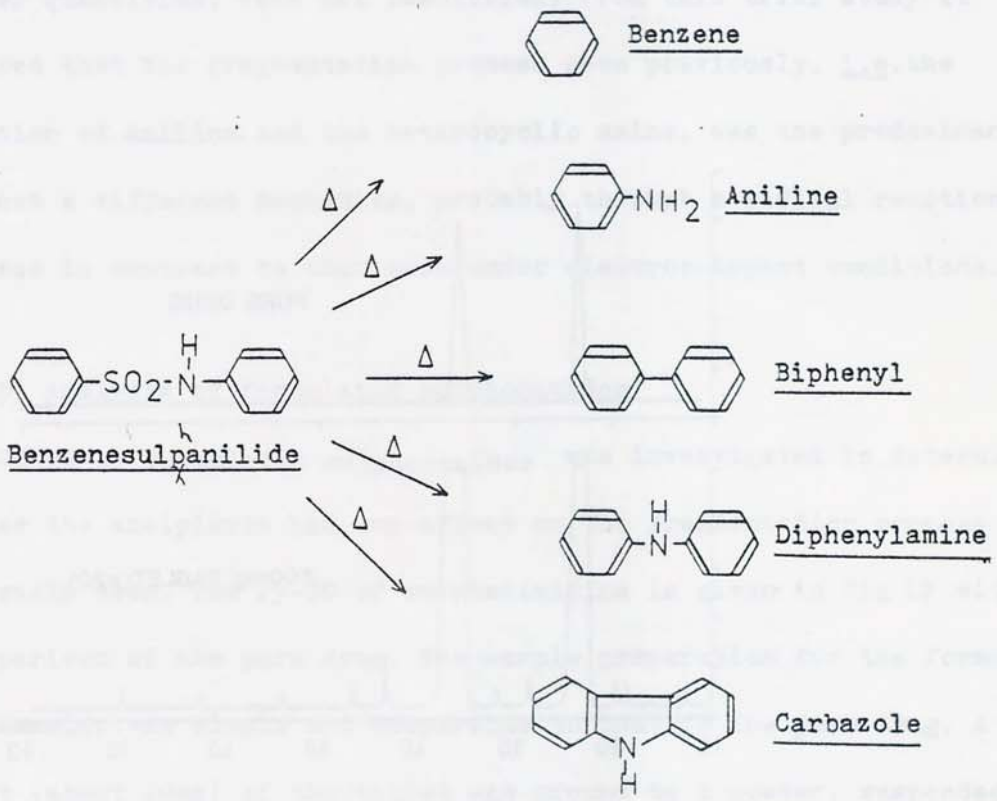


Scheme 3. Probable fragmentation pathway of sulphacetamide (letters refer to Fig16.)

to facilitate an improved separation of the gaseous fraction and therefore this pyrogram should not be compared to those previously displayed with respect to the retention time data. Carbon dioxide, sulphur dioxide and acetonitrile were the main components of the gaseous fraction with acetic acid and aniline eluting later with the more polar acetanilide. Scheme 3 gives the possible fragmentation pathway to yield the pyrolytic products but the formation of acetanilide probably involves an intermolecular rearrangement. Although more complex the unique production of acetanilide sufficed for qualitative analysis.

In a further attempt to understand the pyrolytic fragmentation of sulphonamides, model compounds were prepared (see experimental) in order to compare the products obtained from the electron-impact induced fragmentation to those observed on pyrolysis. The predominant pathway seen with EIMS of sulphonamides was the extrusion of SO_2 to yield the disubstituted amine. If this same process occured on pyrolysis of the medicinal sulphonamides then the resulting diamine would be too polar to be eluted using the GC conditions previously described.

Benzene sulphanilide was prepared and the suggested pyrolytic products are given in Scheme 4 . In an initial Py-GC study the retention time data was obtained for these possible products and compared to the pyrogram obtained from benzene sulphanilide. Only benzene and aniline were evident and it was concluded that the other products were formed, if at all, in very low yields. The quartz pyrolysis tubes were washed with methanol, after pyrolysis of benzene sulphanilide, and the concentrate analysed using TLC. Eight components were resolved and, by comparison with the RF values obtained from the



Scheme 4. Possible pyrolysis products of benzenesulphanilide

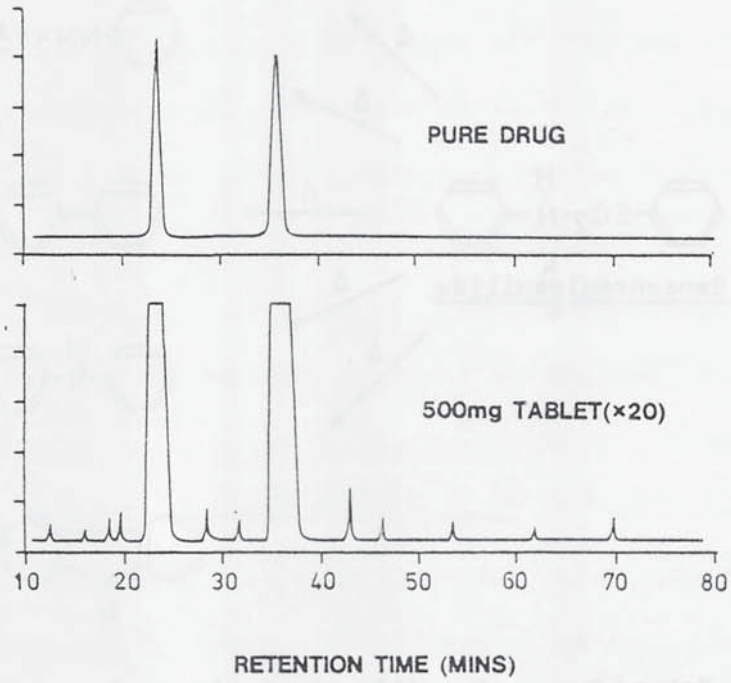


Fig 17. Py-GC of pure and formulated sulphadimidine

concurrent use of standards, the major products were identified as biphenyl(RF 0.97) and carbazole(0.58) with a much smaller amount of diphenylamine(0.79) but the other five products, present in much smaller quantities, were not identified. From this brief study it appeared that the fragmentation process seen previously, i.e. the formation of aniline and the heterocyclic amine, was the predominant pathway and that a different mechanism, probably through a radical reaction, was used in contrast to that seen under electron-impact conditions.

(b) Analysis of formulated sulphonamides

The Py-GC-MS of formulated sulphonamides was investigated to determine whether the excipients had any effect on the fragmentation process previously seen. The Py-GC of sulphadimidine is given in Fig 17 with a comparison of the pure drug. The sample preparation for the formulated sulphonamide was simple and comparable to that of the pure drug. A small amount (about 20mg) of the tablet was ground to a powder, suspended in carbon disulphide and coated onto the wire, as previously described. The excipients present (probably starch, magnesium stearate, gelatin etc.) can be seen to have no discernable effect on the pyrolysis products and that if the peaks due to aniline and 2-amino-4,6-dimethyl-pyrimidine were brought onto scale then the small peaks would be negligible.

A frequently prescribed sulphonamide preparation is the triple sulphonamide tablet Sulphatriad with the constituent drugs being sulphadiazine, sulphamerazine and sulphathiazole. Fig 18 gives the pyrograms of these drugs and also the pyrogram obtained from a

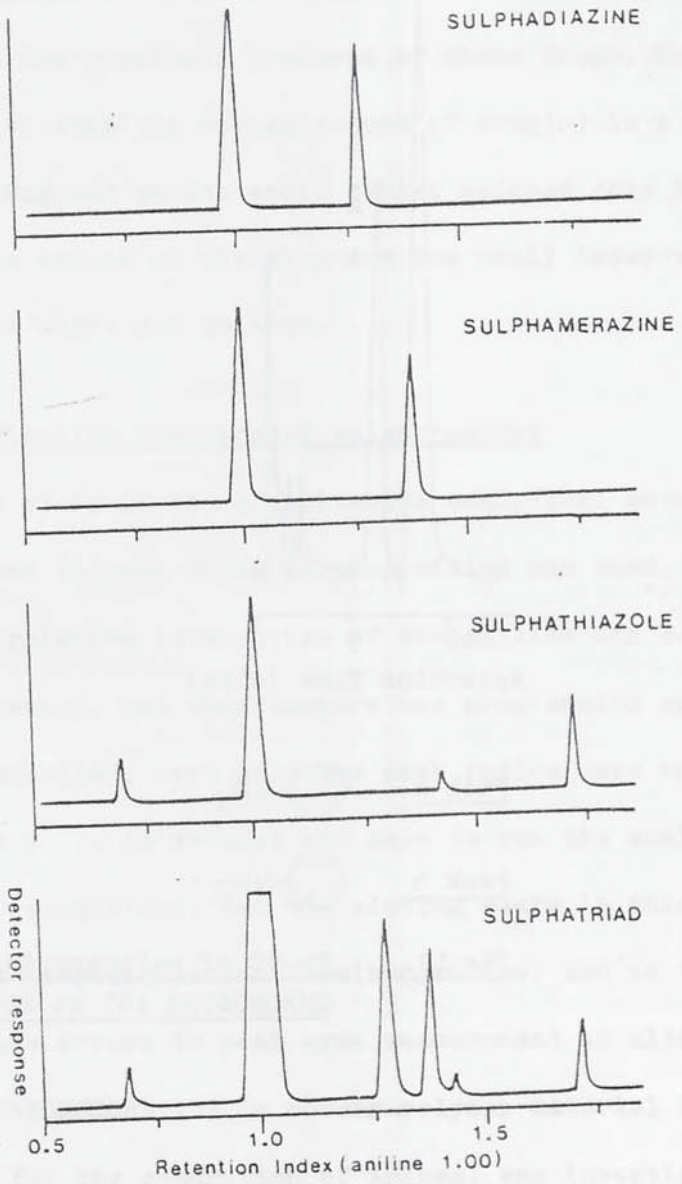
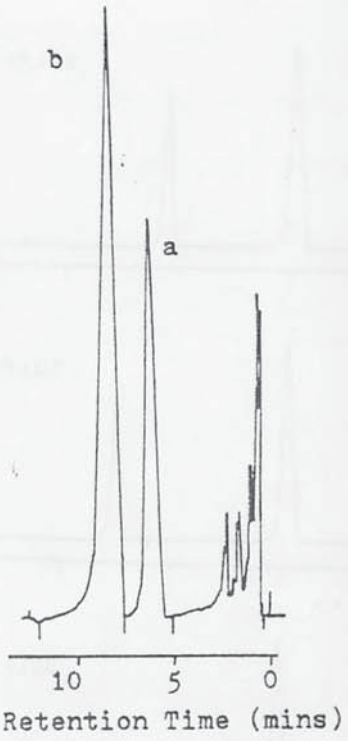
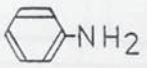


FIGURE 18 PYROGRAM OF A FORMULATED MIXTURE



Peak a 

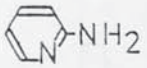
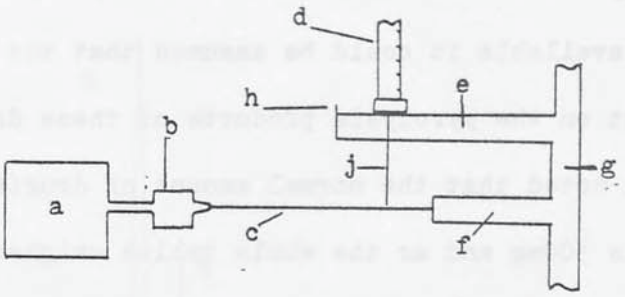
Peak b 

Fig 19. Py-GC of sulphapyridine on CHROMOSORB 103 at 220°c

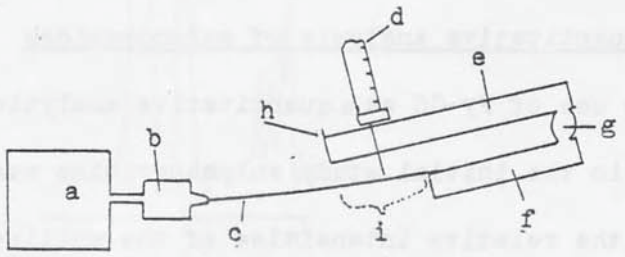
Sulphatriad tablet. The characteristic heterocyclic amines from each of the constituent drugs were evident with no apparent interference in the pyrolytic mechanism when these were pyrolysed together. From the evidence available it could be assumed that the excipients do not have any effect on the pyrolysis products of these drugs. However, it should be noted that the normal amount of drug(s) in a sulphonamide tablet was 500mg and as the whole tablet weighed only 600mg there was a considerable excess of the drug and any small interfering effects of the excipients might not be seen.

(c) Quantitative analysis of sulphonamides

The use of Py-GC as a quantitative analytical method was investigated and in the initial study sulphapyridine was used. The reproducibility of the relative intensities of the aniline and 2-aminopyridine peaks was assessed, but when temperature programming conditions, as previously described, were used the peak ratios were not reproducible (see Appendix 7). An attempt was made to run the analysis using isothermal GC conditions, but the eluting peaks in this case exhibited undue tailing (especially the 2-aminopyridine) and as tailing was likely to cause errors in peak area measurement an alternative column was sought. CHROMOSORB 103 (a porous polymer material designed specifically for the separation of amines) was investigated and found to be satisfactory. After optimising the various parameters (eg. flow rate, oven temperature etc) the pyrogram of sulphapyridine appeared as shown in Fig 19 . A pyrolysis temperature of 770°C was used in an investigation of how the amount of drug on the wire effected the



Front Projection



Plan

KEY

a	Motor	f	Wire support
b	Chuck	g	Stand
c	Pyrolysis wire	h	Syringe stop
d	Syringe	i	Zone of loading
e	Syringe support	j	Needle

Fig 20. Projection and plan of loading equipment for quantitative pyrolysis studies

reproducibility. It seemed probable that the less material on the wire the greater would be the reproducibility, but it was also essential for this study that a method be used for coating the wire which enabled an exact amount of the material to be loaded.

The previous method of using a suspension was suspect because variable amounts could be taken up and deposited on the wire even if the volume used could be carefully controlled. It was desirable therefore to use a solvent in which sulphapyridine was soluble and dilute solutions of the drug in methanol (1.0-3.0mg/ml) were found to be satisfactory. A microsyringe (10µl SGE glass syringe with plunger guide) was adapted by use of a shortened needle(23mm). A known weight of material was coated onto the wire by carefully controlled application of a set volume, normally in the range 5-20µl. The wire had to rotate evenly when being loaded and the previously described equipment was adapted so that the free end of the wire was restricted. N.B. If the wire was slightly bent, on rotating the free end would exhibit undue movement and when a drop of solution was placed on it the centrifugal force would exceed that due to the surface tension and the drop would be forced off the wire before the solvent had evaporated. A syringe guide was used to ensure that the same surface area was coated in each case. The equipment used for the coating procedure is shown, in diagrammatic form, in Fig 20 .

The amount of sulphapyridine on the wire was varied between 50ng and 200µg. Although the two peaks were clearly seen at the lower levels (50ng-1µg) problems were encountered with the baseline taking an excessive time to stabilise after the insertion of a new wire into the

pyrolyser. This was due to the effect of contamination of the carrier gas by air, and this caused baseline fluctuations as it was flushed from the column. This baseline instability proved only a serious problem at the low amplifier attenuation required for these low loadings. In an attempt to combat this problem a different method of interfacing the pyrolyser with the GC column was attempted. Instead of being permanently attached to the GC the pyrolyser was fitted with a long needle through which the pyrolysate was flushed. This needle was then inserted through the injection septum, the pyrolyser was fired and after the products had been flushed onto the column the needle was withdrawn. Although this technique prevented the contamination of the column with air, problems were encountered with adsorption of the pyrolysis products by the metal needle and although various attempts were made to resolve this adsorption problem a satisfactory solution was not found.

Using the conventional Py-GC interface, a loading of approximately 50µg of drug on the wire was considered to be the optimum level. Using these conditions eleven replicate runs were recorded in order to assess the reproducibility of the aniline/2-aminopyridine peak ratio. The data was obtained and the results from the peak area measurements (CRS 304-30) were compared with those obtained from using manual peak height measurements. The results are given in Appendix 8 . The coefficients of variation were 7.0% for the area ratios and 7.3% for the height ratios, and although these figures were not considered satisfactory for quantitative analysis, further work was attempted on the concurrent pyrolysis of two sulphonamides.

In this experiment one sulphonamide was used as an internal

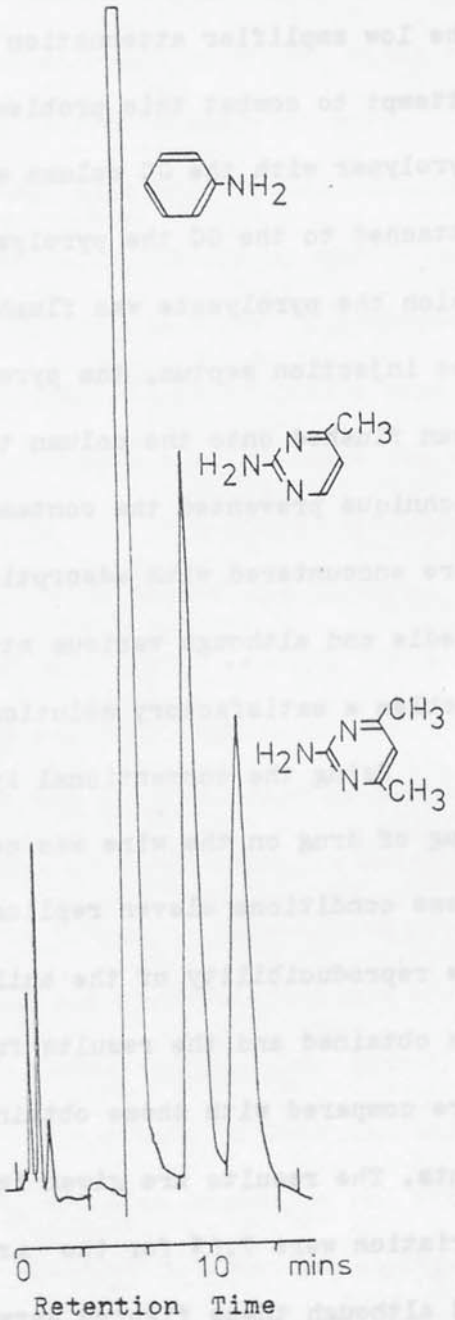


Fig 21 Py-GC of sulphamerazine and sulphadimidine

standard and the amount of the other one was varied. Sulphamerazine and sulphadimidine were used with the latter as the internal standard. The pyrogram obtained from these two drugs can be seen in Fig 21 with the wires loaded using the same technique as described for sulphapyridine. In the preliminary investigation the amount of sulphadimidine on the wire was kept constant and the amount of sulphamerazine was varied. Using this method a calibration curve was drawn and the results, for both the area measurements and the height measurements, are given in Appendix 9 . The area measurements proved more accurate than the height measurements with correlation coefficients of 0.997 and 0.967 respectively. An attempt was made to reduce this error by keeping the total amount of drug on the wire constant for each concentration. The results from this analysis are given in Appendix 10 with the correlation coefficients, of 0.987 and 0.982, having worsened from the previous analysis. This worsening was not readily explicable and the figures obtained did not give an accuracy of better than $\pm$ 5% which is that obtained with other methods (GLC) [108] used for the quantitative analysis of mixed sulphonamide preparations. These variations in results are probably due to inaccuracies in making up the standard solutions and also to variations in the TRT of the Curie point pyrolyser used in this study.

(d) Analysis of sulphonamide metabolites

After the assessment of Py-GC-MS in the analysis of both pure and formulated sulphonamides the use of Py-GC to follow the excretion of these drugs and their metabolites was investigated.

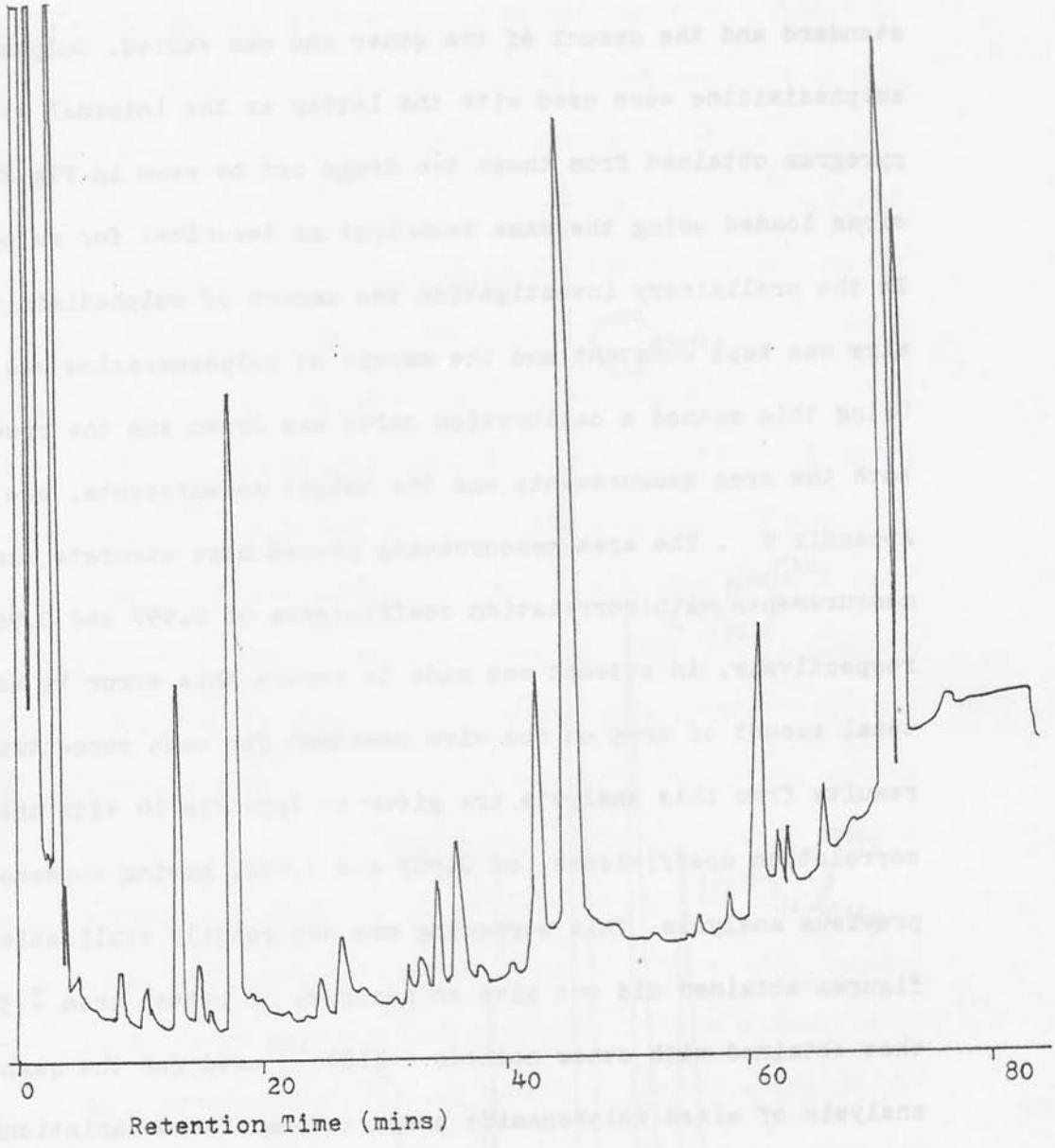


Fig 22. Py-GC of control urine sample

The sample preparation was again simple, i.e. a small aliquot of urine (usually 25mls) was taken , freeze-dried and a small portion of the total solids remaining were coated onto a 770°C pyrolysis wire. A pyrogram obtained from the pyrolysis of a control urine specimen is shown in Fig 22 . There are a few (about sixteen) significant peaks but the pyrogram is surprisingly simple when the total number of components in urine[119] is considered.

The diurnal changes in the urine constituents might have effected the pyrogram and this was studied using samples taken at 4 hourly intervals throughout a normal day (0700 hrs to 2300hrs) but no variation in the overall appearance was observed. Another variable was the source of the urine . Samples were taken from subjects of different races, i.e.caucasian and negroid, and also samples from smokers and non-smokers. Once again no significant fluctuations were observed in any of the peak ratios and no new fragments appeared that were unique to any one subject or group. Fig23(b)gives the pyrogram obtained from pyrolysing a urine sample taken from a patient who had commenced sulphadimidine therapy four hours earlier. The control urine sample, Fig 23(a), can be seen above. The superimposition of the aniline and 2-amino-4,6-dimethylpyrimidine peaks on the control pyrogram was clearly seen. A longer retention time peak is also observed and this later peak was identified as acetanilide, formed from the pyrolysis of the N-acetyl metabolite of sulphadmidine. Sulphadimidine is excreted in the urine as 50% of the unchanged drug and 50% of the N-acetyl metabolite[102]. For comparative purposes the N-acetyl analogue of sulphadimidine was prepared (see experimental) and the pyrogram shown in Fig 23(c). This metabolite

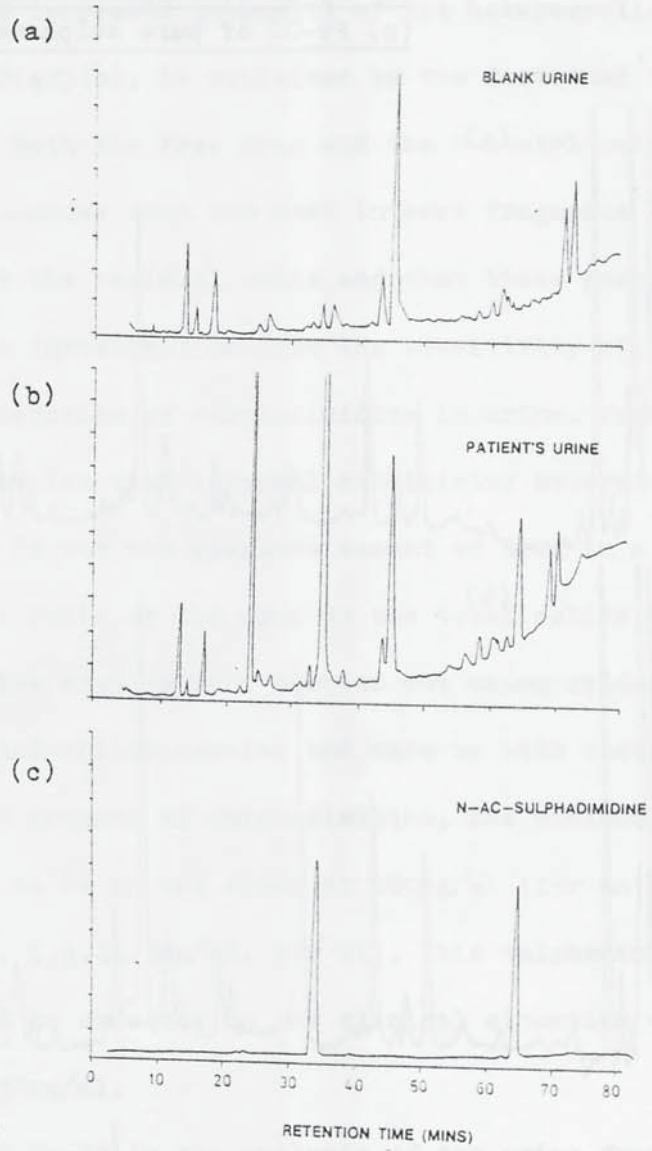
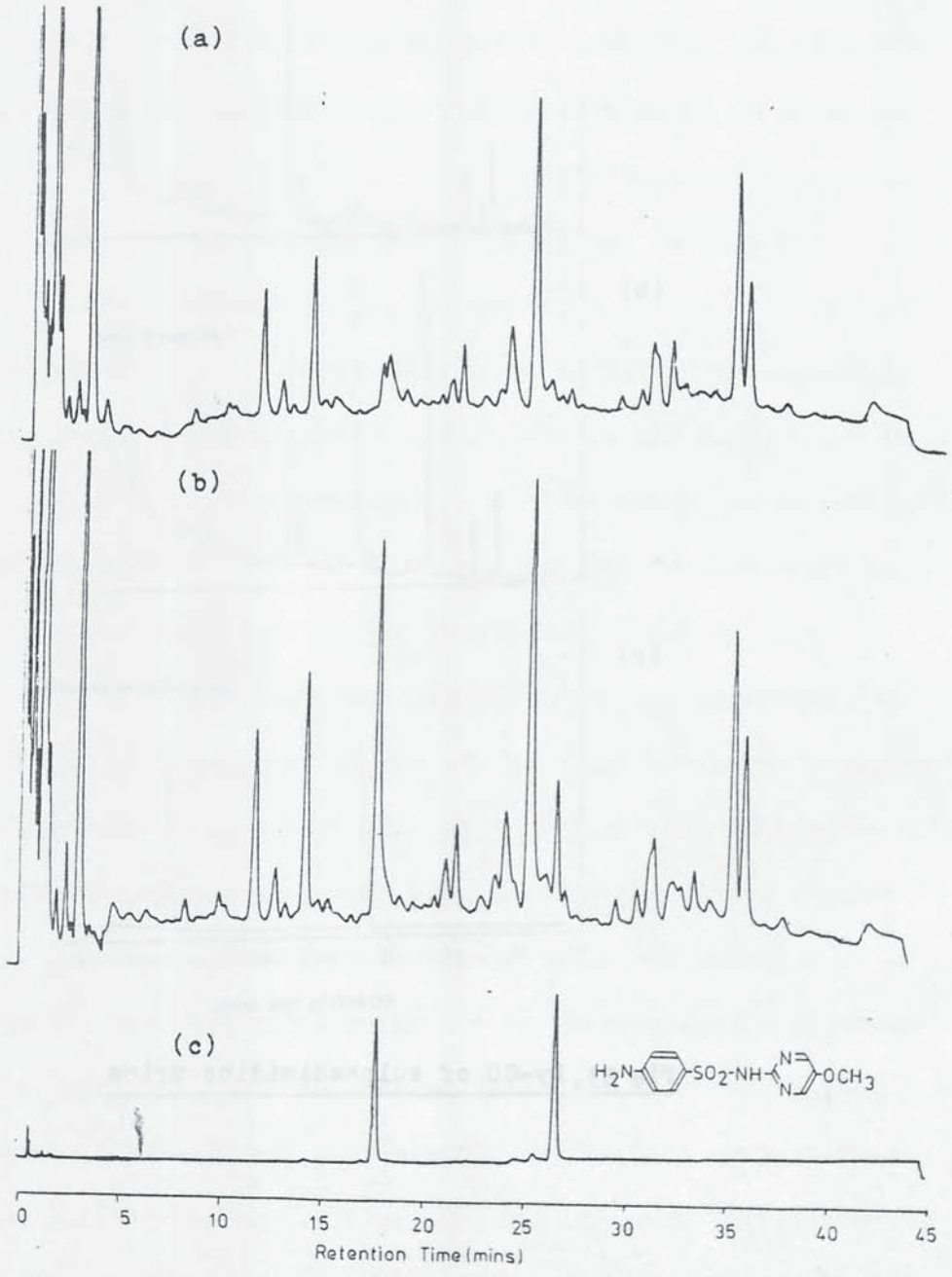


Fig 23. Py-GC of sulphadimidine urine

Fig 24. (a) Py-GC control urine sample
(b) Py-GC of sulphamethoxydiazine urine
(c) Py-GC of pure sulphamethoxydiazine



underwent simple fragmentation on pyrolysis with only two major peaks apparent which were identified as 2-amino-4,6-dimethyl pyrimidine and acetanilide. The increased intensity of the heterocyclic amine in the test pyrogram, Fig23(b), is explained by the fact that this is produced on pyrolysis of both the free drug and the N-acetyl metabolite.

Fig 23 (b) shows that the most intense fragments in the pyrogram were aniline and the residual amine and that these peaks were offscale. This prompted an investigation into the sensitivity of the method with respect to the detction of sulphadimidine in urine. However, this was slightly more complex than a normal sensitivity determination as the limiting factor is not the absolute amount of drug in a given volume of urine but the ratio of the drug to the total solids in that volume of urine. Sensitivity studies were carried out using reconstituted urine (from pooled total-solids samples and made up with distilled water) and adding known amounts of sulphadimidine. The minimum detectable level was found to be in the order of 200µg/ml (for an average quantity of total solids, i.e.21.5mg/ml, per ml). This sulphonamide level was far lower than would be expected in the clinical situation which would be in the order of 1.52mg/ml.

The use of Py-GC in the analysis of the urine froma patient bein treated with a low dose sulphonamide[102] was undertaken with sulphamethoxydiazine used as an example. The normal daily dose of sulphamethoxydiazine is 500mg (orally) with an initially higher loading dose, compared to a daily dose of 4g for sulphapyridine.
The pyrogram produced is shown in Fig 24(b) with a control sample (a).

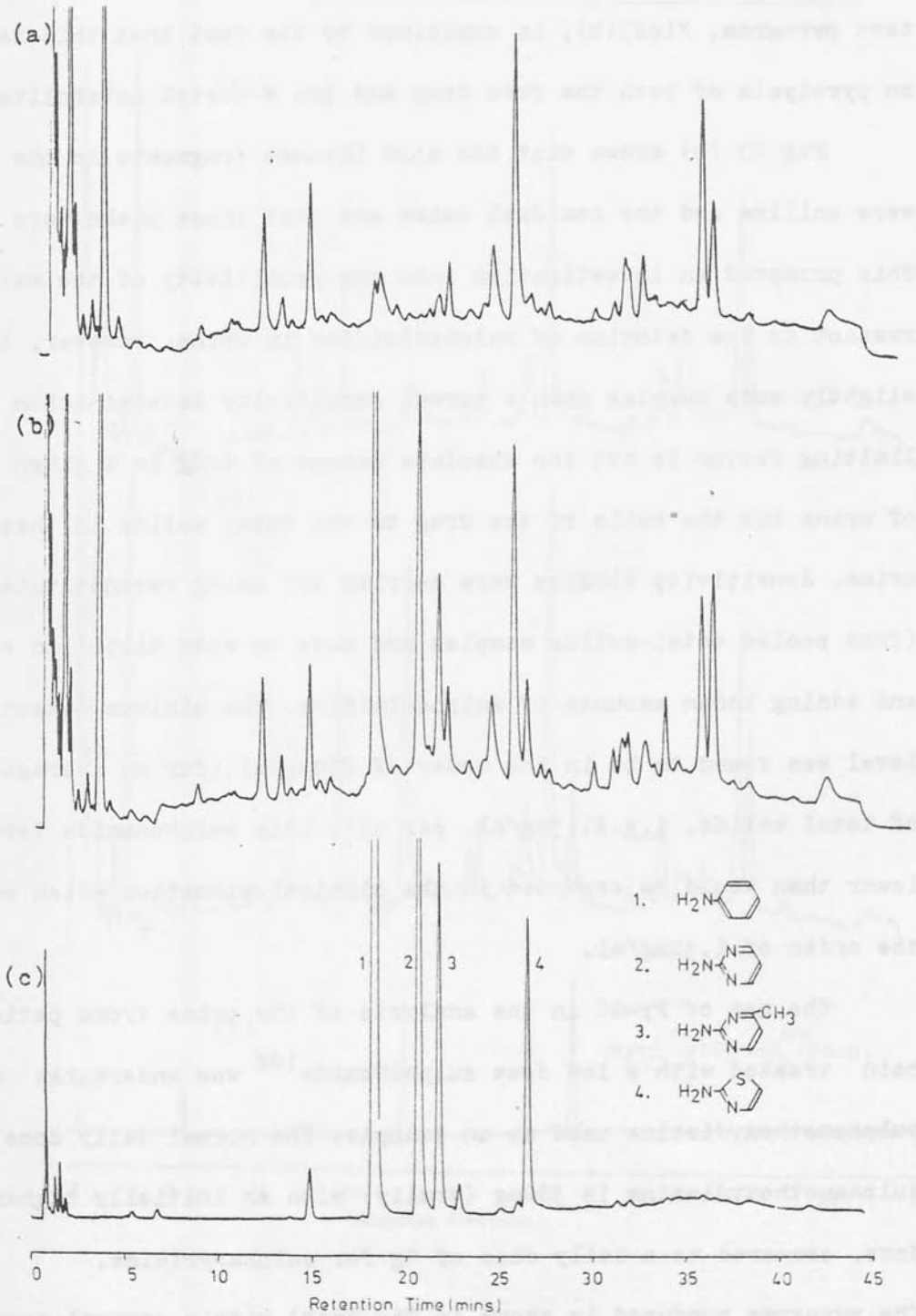
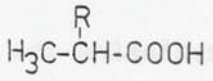


Fig 25. (a) Pyrogram of control urine
(b) Pyrogram of Sulphatriad urine
(c) Pyrogram of Sulphatrid tablet

and the pyrogram obtained from the pure drug (c). The chromatography has been improved with the use of a faster temperature programming rate (5°C/min instead of 2.5°C/min) resulting in an analysis time of 45 minutes. With sulphamethoxydiazine the amine residue was not the largest peak in the pyrogram, as was the case with sulphadimidine, but it was still clearly evident.

The final study of sulphonamide excretion by Py-GC can be seen with the triple sulphonamide Sulphatriad. The analysis of a patient's urine can be seen in Fig 25 with the three components clearly superimposed on the control urine with the exception of the benzonitrile peak from sulphathiazole, which was exactly coincident with a peak seen in the control urine (Fig 25 (a)). Acetanilide could also be identified but in this case all three drugs undergo N-acetylation and therefore an excretion and metabolism study could not be made on one individual component of a mixed formulation when using this technique.



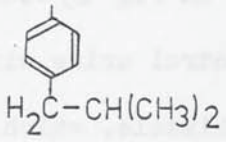
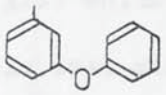
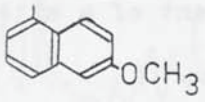
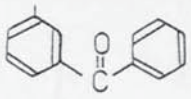
Drug	Radical R
Ibuprofen	
Fenoprofen	
Naproxen	
Ketoprofen	

Table4. Structures of propionic acid derivatives

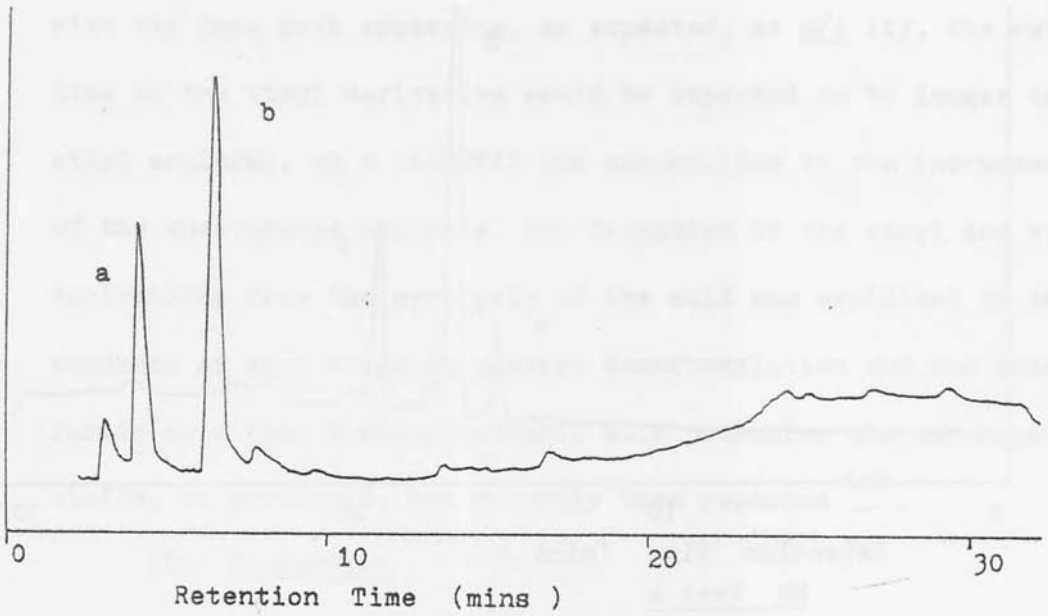
(2) Propionic acid derivatives

(a) Formulated drugs

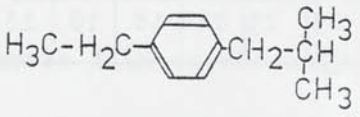
In this Py-GC-MS investigation the pyrolytic fragmentation of four propionic acid derivatives was studied, i.e.ibuprofen, fenoprofen, naproxen and ketoprofen. The characteristic substituents of these four drugs are given in Table 4 . All four drugs were analysed directly as the formulated product utilising the same technique as described earlier, i.e.a small quantity of the tablet was ground,suspended in carbon disulphide and coated onto the pyrolysis wire. The chromatographic conditions were identical to those described for the sulphonamides except that the initial GC oven temperature was 120°C and no initial isothermal period was used. Using this new initial temperature the first significant peak eluted after four minutes (from ibuprofen) and although the resolution of the fragments was not effected the analysis time was shortened by twelve minutes.

(i) Ibuprofen

On pyrolysis ibuprofen was seen to yield two major fragments. Fig26 displays the pyrogram and the MS data obtained from peak a (retention time 4.3 mins) and peak b(retention time 6.6 mins). Peak a was identified as 4-ethyl-isobutylbenzene, the mass spectrum of which exhibited the characteristic aromatic hydrocarbon fragmentation with fission occurringaround the β carbon of the iso-butyl fraction to yield a base peak at m/e 119. Further fragmentation resulted in characteristic fragments at m/e's 105, 91 and 65. Peak b could be seen to have an analagous electron-impact fragmentation except that the major peaks

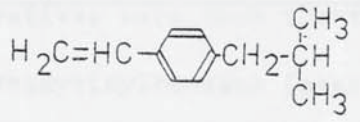


MS Peak a



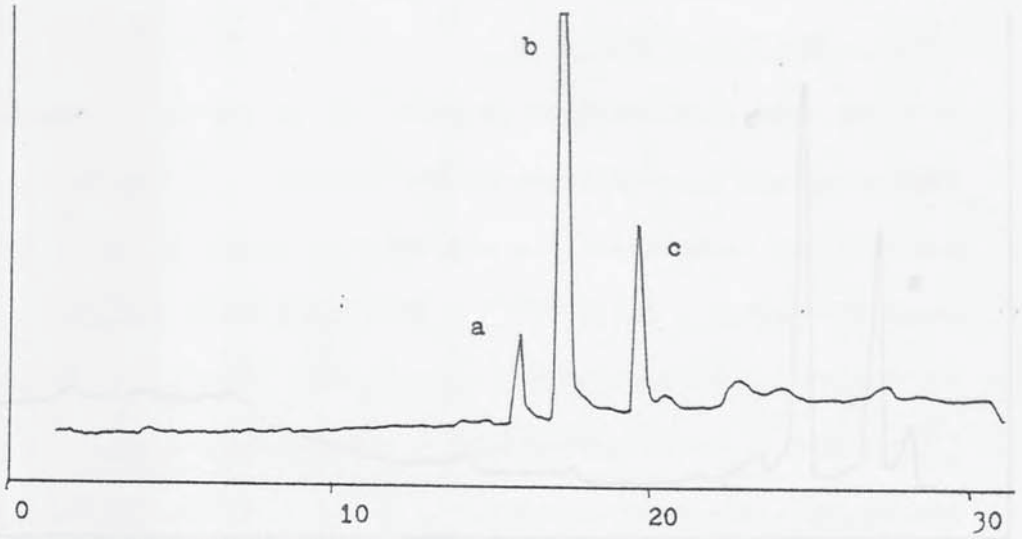
119	162	120	91	105	117	163	65
100	30	22	22	10	8	4	4

MS Peak b

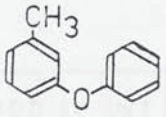


117	160	118	115	91	119	65	161
100	32	21	11	9	8	5	4

Fig 26. Py-GC-MS of a formulated preparation of ibuprofen

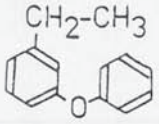


MS Peak a



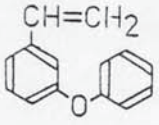
184	141	159	91	169	155	185	65
100	25	23	22	18	18	16	11

MS Peak b



198	183	105	153	77	199	184	170
100	58	36	30	28	18	16	14

MS Peak c



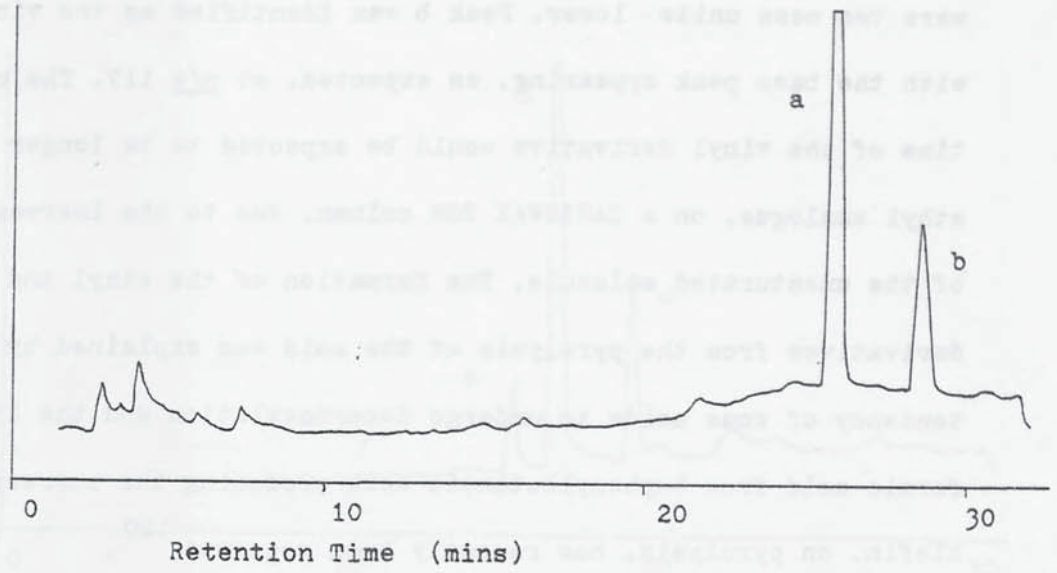
196	167	77	153	197	168	152	165
100	42	30	24	20	17	15	12

Fig 27. Py-GC-MS of a formulated preparation of fenoprofen

were two mass units lower. Peak b was identified as the vinyl analogue with the base peak appearing, as expected, at m/e 117. The retention time of the vinyl derivative would be expected to be longer than the ethyl analogue, on a CARBOWAX 20M column, due to the increased polarity of the unsaturated molecule. The formation of the ethyl and vinyl derivatives from the pyrolysis of the acid was explained by the tendency of some acids to undergo decarboxylation and the loss of formic acid from 4-phenylbutanoic acid producing the corresponding olefin, on pyrolysis, has recently been reported [120].

(ii) Fenoprofen

The fenoprofen sample was prepared as previously described except that the tablet was film-coated and it was necessary to peel back the polymeric film to use only the tablet core for the Py-GC-MS anlysis. The results obtained from the pyrolysis of fenoprofen are summarised in Fig 27. A similar pyrolytic degradation is evident to that previously witnessed with ibuprofen, in that the ethyl and vinyl derivatives were both identified. 3-Phenoxyethylbenzene (peak b) and 3-phenoxyvinylbenzene (peak c) both underwent the expected MS fragmentation and lost a methyl radical from the molecular ion (m/e 198 & 196 which were also the base peaks). A shorter retention time fragment (peak a) was observed and identified as 3-phenoxytoluene. In the study of the pyrolysis of fenoprofen the methyl analogue was readily visible but although the pyrograms from the other three drugs (ibuprofen, naproxen and ketoprofen) had baseline deviations at the retention times of the expected methyl analogues these were never intense enough to be positively identified by MS.



MS Peak a

133	210	105	181	134	77	211	182
100	80	54	24	17	14	13	6

MS Peak b

208	131	105	77	103	209	207	181
100	80	68	32	30	12	10	8

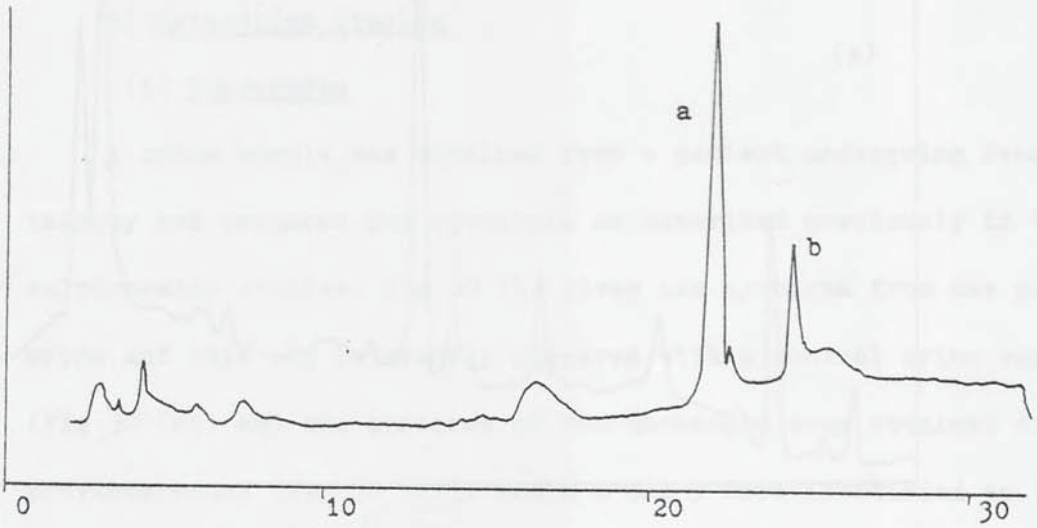
Fig 28. Py-GC-MS of a formulated preparation of ketoprofen

(iii) Ketoprofen

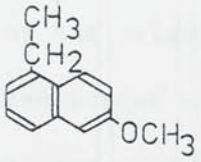
From the pyrogram of ketoprofen, Fig 28 , it was evident that the same pyrolytic fragmentation was occuring to yield the characteristic doublet comprising of the ethyl and vinyl analogues. The MS fragmentation was of some interest when comparing the base peak of the vinyl and ethyl residues. The base peak in the MS of the vinyl compound was also the molecular ion but in the corresponding ethyl derivative the base peak appeared to be due to the $(m-77)^{+\cdot}$ fragment.

(iv) Naproxen

The pyrolytic fragmentation of naproxen appeared to yield the expected products and the results are summarised in Fig 29 . As in the ketoprofen MS studies the vinyl group appeared to confer an increased electronic stability on the molecule resulting in the molecular ion being the base peak in the vinyl residue but the $(M-15)^{+\cdot}$ peak being the most intense in the ethyl analogue.

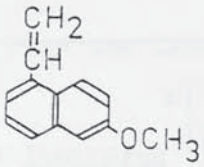


MS Peak a



171	186	127	172	187	155	143	154
100	53	16	11	7	5	4	3

MS Peak b



184	141	169	115	134	185	127	142
100	77	35	33	21	19	15	13

Fig 29. Py-GC-MS of a formulated preparation of naproxen

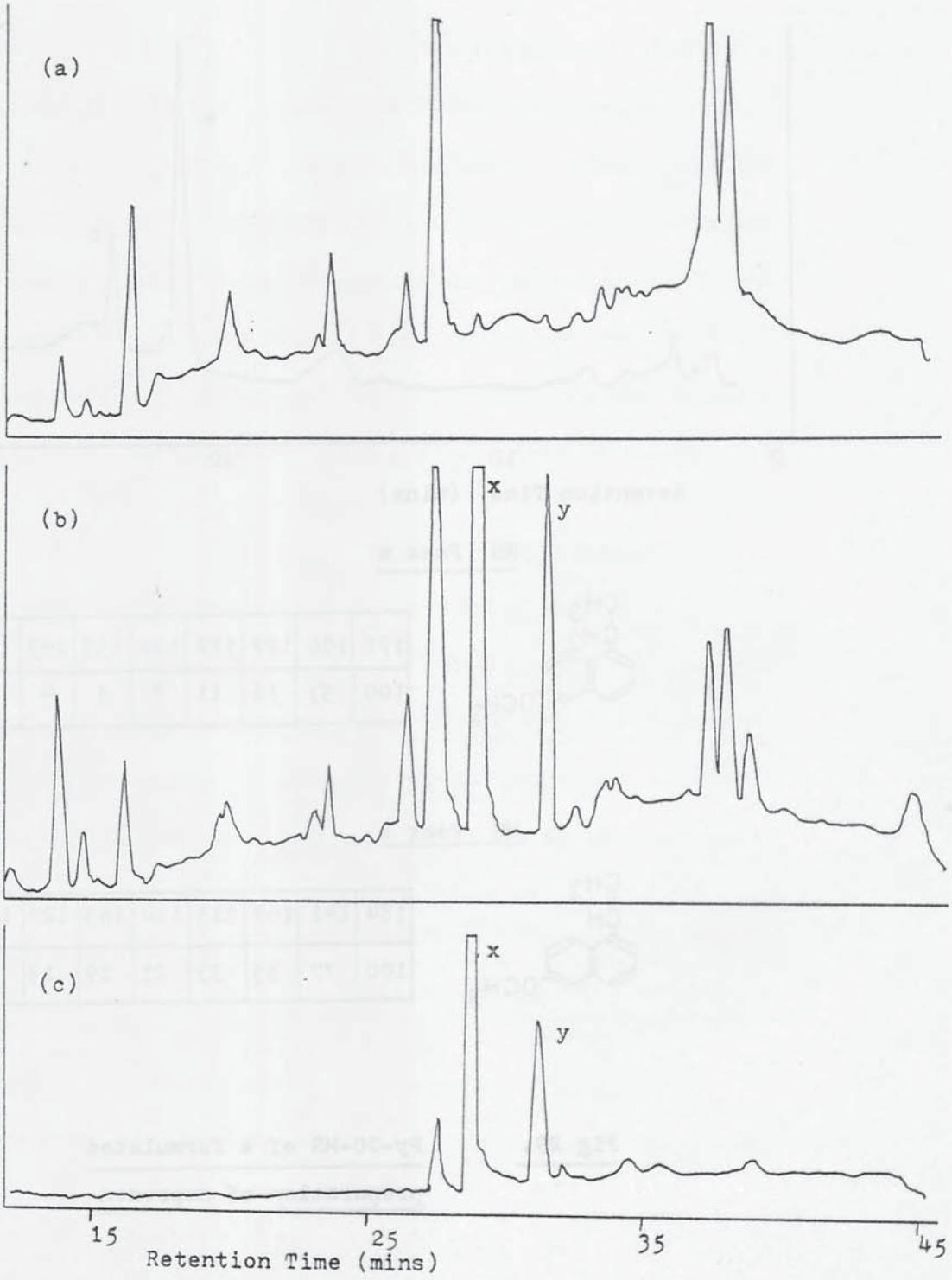


Fig 30. Py-GC-MS of (a) Control urine sample
(b) Fenoprofen urine sample
(c) Formulated fenoprofen sample

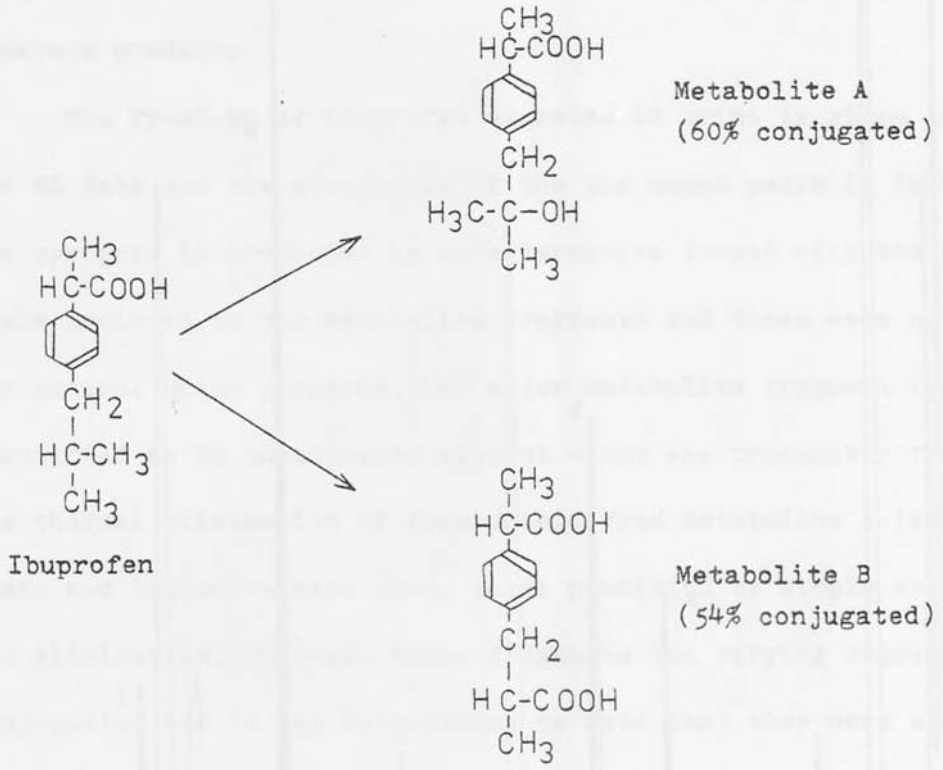
(b) Metabolism studies

(i) Fenoprofen

A urine sample was obtained from a patient undergoing fenoprofen therapy and prepared for pyrolysis as described previously in the sulphonamide studies. Fig 30 (b) gives the pyrogram from the patients urine and this can be readily compared with a control urine sample (Fig 30 (a)) and the pyrogram of the unchanged drug obtained from the previous study (Fig 30 (c)). Peaks x and y were identified as the ethyl and vinyl residues respectively. In a previous report on the metabolism of fenoprofen [121] 45% of the drug was excreted as the glucuronide conjugate of the original drug, 45% excreted as the glucuronide of the 4'-hydroxyfenoprofen metabolite and 4-10% excreted as the unconjugated metabolites. The glucuronide of the unmodified drug would exhibit the same fragmentation as the pure drug but although the vinyl and ethyl derivatives of the 4'-hydroxy metabolite would still be formed they would not elute from the CARBOWAX 20M column.

(ii) Ibuprofen

The use of Py-GC-MS in the analysis of ibuprofen metabolites posed many interesting problems. The metabolism of ibuprofen has been studied in depth[115], and it was shown that there were two major metabolites and the metabolic pathway is summarised in Scheme 5. In the studies on the pyrolytic fragmentation of the pure drug, the carboxylic acid formed an active site for rearrangements and eliminations. After metabolism, Metabolite B (see Scheme 5) had two carboxylic acid groups in the molecule and Metabolite A had one alcohol and one



Scheme 5. Major metabolic products of ibuprofen

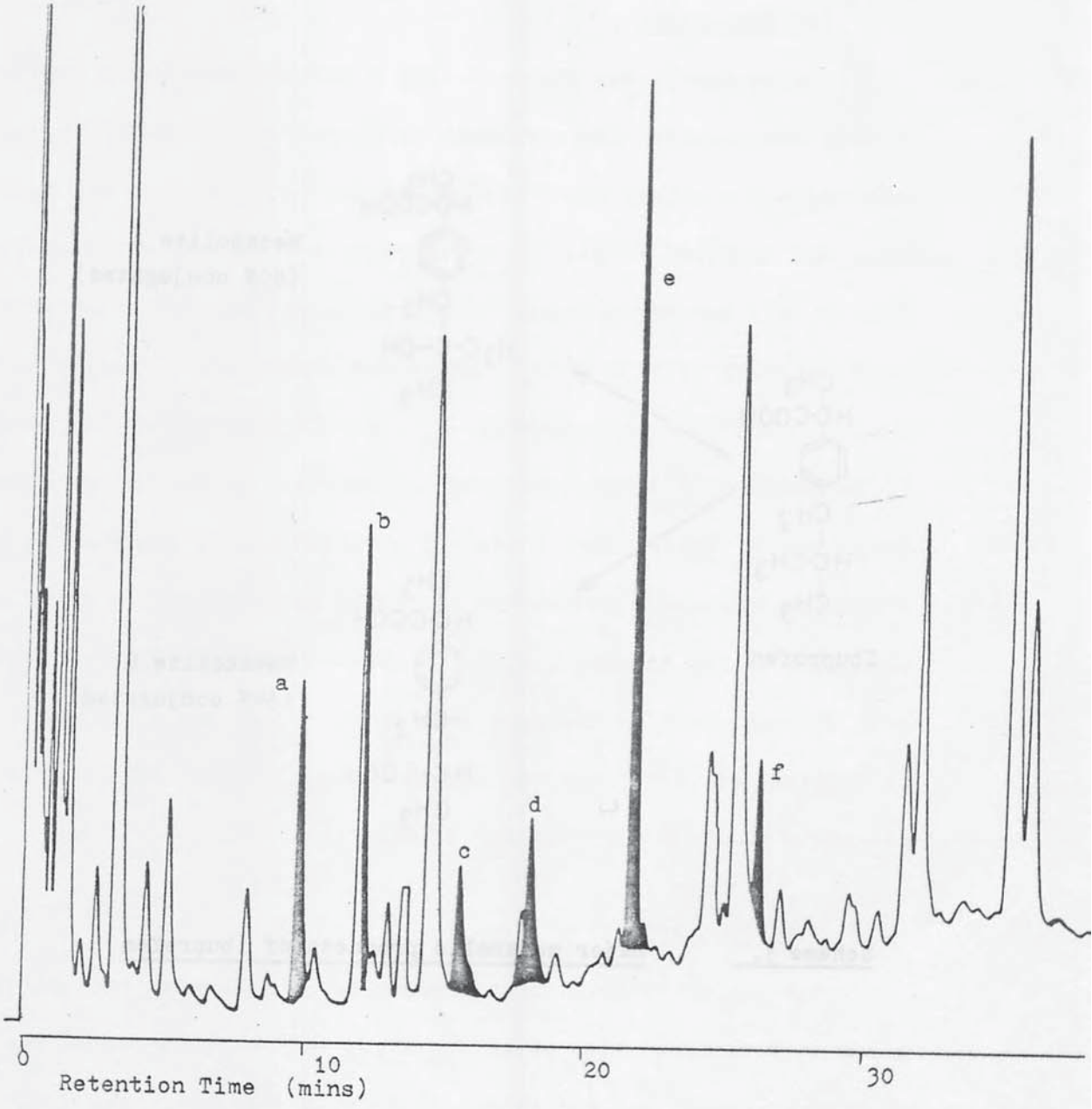


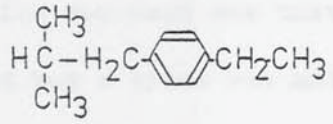
Fig 31. Py-GC-MS of urine from ibuprofen patient (shaded peaks do not appear in control urine pyrogram)

carboxylic acid group. With the acid metabolite there are a number of possibilities with respect to the pyrolytic fragmentation and likewise the alcohol could well have dehydrated an pyrolysis to yield various fragments. It was possible therefore, to envisage at least twenty separate products.

The Py-GC-MS of ibuprofen excreted in urine is given in Fig 31 with the MS data and the structures of the six named peaks in Table 5 . The pyrogram is presented in an alternative format with the shaded peaks assigned to the metabolite fragments and these were not seen in the control urine pyrogram. The major metabolite fragment (peak e) was identified as an unsaturated alcohol which was presumably formed from the thermal elimination of formic acid from Metabolite A (see Scheme 5). Peaks a-d inclusive were among those predicted by simple decarboxylation and elimination. However, these fragments had varying degrees of conjugation and it was interesting to note that they were eluted in order of increasing polarity and apparently irrespective of molecular weight. Peak f was identified as the corresponding nitrile(4-(2-cyano)propyl-ethylbenzene) from Metabolite B. The formation of a nitrile was initially perplexing but much information was gained from the behaviour of urine components on pyrolysis (see next chapter).

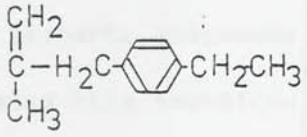
Although six peaks were identified there were certainly more than six fragments produced on pyrolysis of the ibuprofen urine. In a further investigation, time was obtained on a VG 16F GC-MS linked to a VG 2035 data system. This equipment enabled continuous scanning (every 3sec) throughout the pyrogram and then sophisticated treatment of the data

MS Peak a



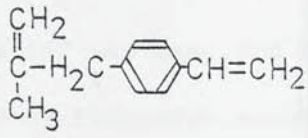
119	162	91	120	105	117	163	65
100	22	21	18	11	9	3	3

MS Peak b



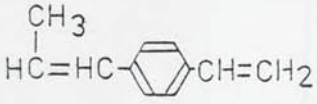
131	39	145	117	160	91	129	115
100	37	36	31	29	27	11	11

MS Peak c



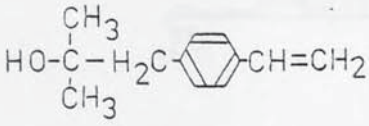
105	143	158	77	117	115	91	159
100	88	53	53	38	32	21	9

MS Peak d



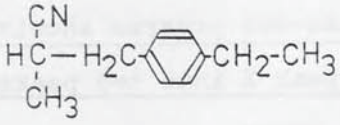
104	77	144	129	128	117	115	65
100	56	42	38	35	19	15	15

MS Peak e



117	105	120	116	90	133	91	118	163
100	38	37	37	35	27	23	7	5

MS Peak f



119	91	104	173	120	118	117	115	144
100	12	9	8	7	6	6	5	4

Table 5. MS data from Fig 31.

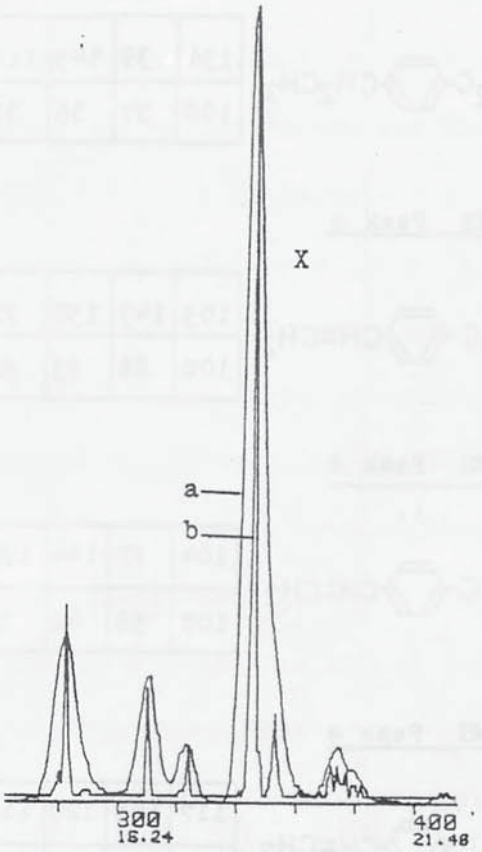


Fig 32. Output from Mass-max program showing resolution of peak X into two peaks

(a)- Total ion trace

(b)- Mass max trace

using the Mass-max[122] program. The advantage of the Mass-max data reduction approach was that eluting peaks were not plotted by a total-ion current but a trace was made by assessment of the fluctuation of individual m/e values and if one peak contained two different components then they would be resolved by this method. (N.B. The program would not resolve isomeric compounds which had identical mass spectra). Fig 32 illustrates this technique on a portion of the pyrogram with the total-ion trace shown above the Mass-max trace. Peak x can be seen to consist of two separate compounds. Unfortunately there were software difficulties when the 16F was used and no additional information concerning the Py-GC-MS of ibuprofen metabolites was forthcoming.

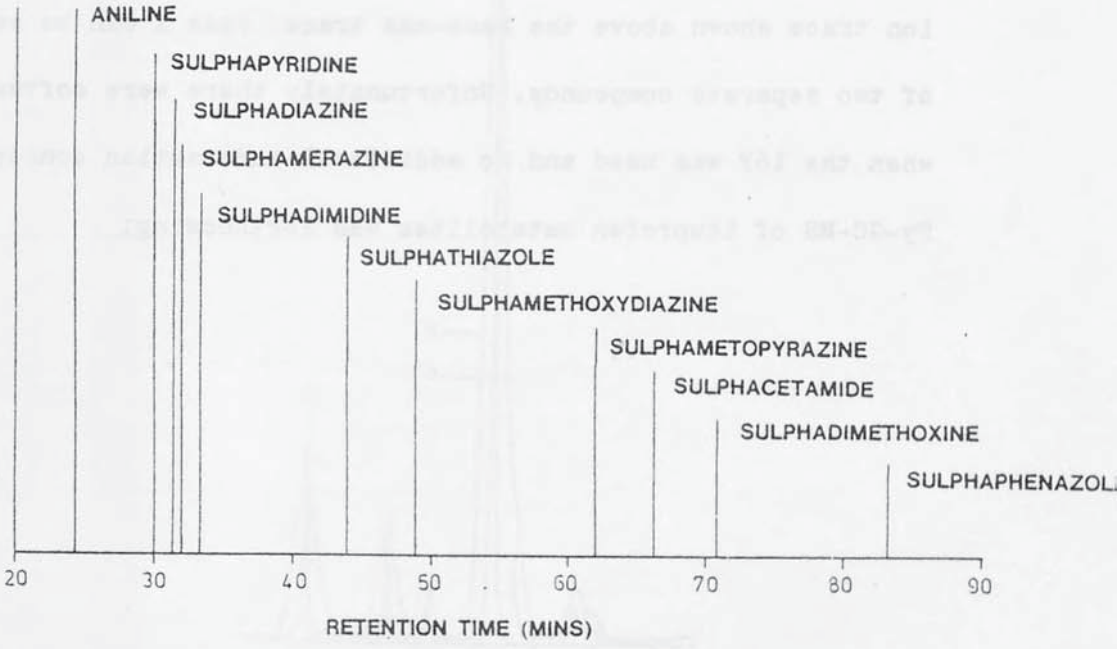


Fig 33. Summary of characteristic fragments produced by pyrolysis of medicinal sulphonamides

C CONCLUSION

The advantages of Py-GC-MS in the analysis of formulated drugs are that no elaborate sample preparation is required and that the analysis system was virtually identical for the sulphonamides and the propionic acid derivatives. This simple sample preparation is of obvious benefit with the sulphonamides when some GC techniques involve elaborate separation procedures[108]. The method can be used for the qualitative analysis of sulphonamides and this may be achieved by comparison of retention times alone (as summarised in Fig 33). The mechanism of fragmentation is not clear although it would appear that it is dissimilar from that observed in the mass spectrometer. The use of Py-GC as a quantitative method required that the chromatographic conditions be changed but when analysis of an unknown sample was undertaken an accuracy of better than ±5% was not achieved. This inaccuracy was probably due to the low-powered pyrolysis unit which permitted fluctuations in the TRT. The effect of the thickness and uniformity of the coat on the wire might also have contributed to this lack of reproducibility.

The analysis of some propionic acid derivatives with analgesic and antipyretic properties was also demonstrated, with unique fragments being formed in each case. The analysis of the metabolites, by Py-GC-MS of the total solids from urine,with no prior separation required, was demonstrated to be a method that could be used with both high and low dose sulphonamides and also mixed sulphonamide preparations. However, it was not possible to follow the excretion and metabolism of an individual drug in the formulated mixture as the other components, which also

took the same metabolic route, yielded identical products on pyrolysis.

CHAPTER 3 URINE AND URINE COMPONENTS

A INTRODUCTION

Urine is an aqueous solution of water soluble compounds whose components have been quoted in detail [119]. The main components are urea(20-35g,excreted in 24 hrs), creatinine(2.15g), hippuric acid(700mg) and various amino acids. There have been attempts to run urine profiles by GC and GC-MS for normal urine constituents [123,124], abnormal metabolites [125] and also for drugs [126]. Py-GC has previously been used as a profiling technique in order to characterise different cell types [46] and also to distinguish between normal haemoglobin and that obtained from a patient with sickle cell anaemia [127]. There have been no previous reports on the Py-GC-MS analysis of urine.

Some of the individual components of urine have been studied in detail but the results were often contradictory and ambiguous. There have been numerous studies on the pyrolysis of amino acids but the different products obtained in each case make it essential that the work be repeated using standardised conditions. An example is that of the various reports on the products obtained from the pyrolysis of phenylalanine. The volatile amines produced on pyrolysis of phenylalanine were tabulated by Winter and Albro [128]. They used a filament pyrolyser but wrapped the amino acid in a glass fibre filter paper, which could encourage secondary reactions. The amines determined, by comparison of retention volumes with standards, were NH_3, $MeNH_2$, Me_2NH, $EtNH_2$, Pr_3N, Pr_2NH and Bu_3N. The higher amines were probably formed by secondary reactions in the cooler parts of the pyrolysis chamber. The GC conditions used in this study only permitted the elution of the volatile fraction.

The Curie point pyrolysis of phenylalanine [29] yielded a simple pyrogram which consisted of benzene(5%), toluene(78%), ethylbenzene(2%) and styrene(15%) [129]. In this study capillary column GC was used and an excellent resolution of the components was obtained. A further study, using Py-GC-MS, claimed that on pyrolysis of seventeen amino acids unique products were formed in every case. Benzene was stated to be unique for phenylalanine although in the Curie point analysis this accounted for only 5% of the pyrolysate. A further Py-GC-MS study of aromatic amino acids [130], using a furnace pyrolyser, reported the use of three different GC columns and three different pyrolysis temperatures (400°C, 500°C and 600°C). The relative percentages of the pyrolysis products from phenylalanine were given in each case. Toluene was the major product and seven other fragments were well resolved but phenylethylamine appeared as a badly tailing peak.

It was concluded that the overall processes were comparable but the discrepancies were probably caused by the great variation in the chromatographic conditions, pyrolysis methods(i.e. furnace, filament and Curie point) and the method of peak identification. It would appear that if the pyrolysis of amino acids was to be used as a guide to the structure of proteins [10, 131, 132, 133, 5, 128] and urine pyrograms then further standardisation is essential.

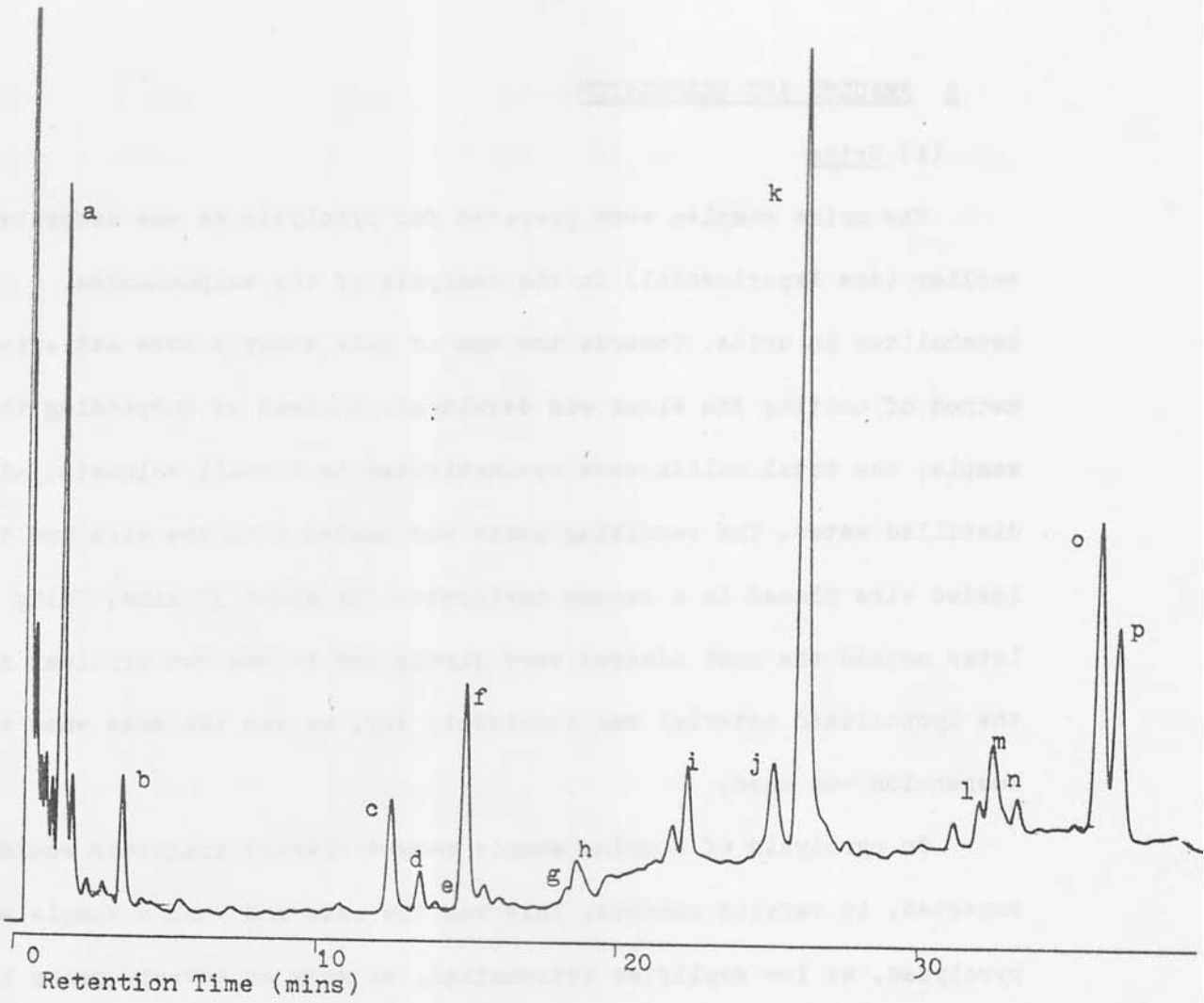
B RESULTS AND DISCUSSION

(1) Urine

The urine samples were prepared for pyrolysis as was described earlier (see Experimental) in the analysis of the sulphonamide metabolites in urine. Towards the end of this study a more satisfactory method of coating the wires was developed. Instead of suspending the sample, the total solids were reconstituted in a small volume(0.5ml) of distilled water. The resulting paste was coated onto the wire and the loaded wire placed in a vacuum desiccator for about 30 mins. Using this later method the coat adhered very firmly and it was not critical if the lyophilised material was absolutely dry, as was the case when a suspension was used.

On pyrolysis of a urine sample many different fragments would be expected, in varying amounts. This was the case and when a sample was pyrolysed, at low amplifier attenuation, as many as seventy peaks have been resolved, most of which were of low intensity. However, using the attenuation required for the metabolism studies (see previous chapter) about twenty peaks were seen in a pyrogram from a control urine sample.

Identification of seventeen components was achieved and the pyrogram is displayed in Fig 34 with the corresponding MS data in Table 6. The most intense peak, peak k, was identified as p-cresol,the isomer being assigned by retention time comparisons. The mass spectrum of the cresol exhibited the characteristic intense doublet of the $M^{+\cdot}$ and $(M-1)^{+\cdot}$ ions. It was perhaps surprising that the acidic phenol and p-cresol(peaks j and k) did not display tailing when eluting from the basic CARBOWAX 20M + KOH column but the chromatographic data obtained



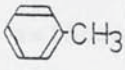
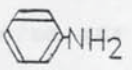
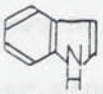
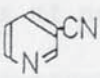
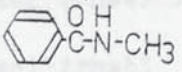
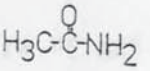
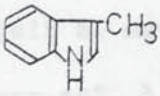
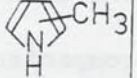
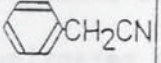
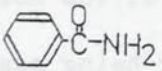
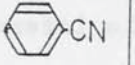
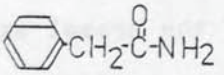
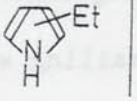
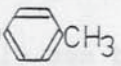
Peak	Structure	Peak	Structure	Peak	Structure
a		g(1)		l	
b		g(2)		m	
c		h		n	
d&e		i		o	
f(1)		j		p	
f(2)		k			

Fig 34. Py-GC-MS of urine

MS Peak a								
	91	92	65	39	63	51		
	100	75	25	13	8	4		

MS Peak b								
	79	52	51	80	78	53	50	39
	100	56	12	6	6	5	5	5

MS Peak c								
	67	41	39	40	38	37		
	100	22	16	3	3	2		

MS Peak d&e								
	81	80	53	27	82	52	39	51
	100	91	30	21	9	6	6	3

MS Peak f(1)								
	103	76	50	104	75	51	39	52
	100	45	9	8	7	4	4	3

MS Peak f(2)								
	80	95	39	67	53	94	41	
	100	78	56	33	33	28	22	

MS Peak g(1)								
	104	77	50	105	76	51	52	
	100	60	16	11	11	7	5	

MS Peak g(2)								
	93	66	40	92	39	94	65	67
	100	48	26	17	17	13	13	9

MS Peak h								
	44	59	43	42	41	60		
	100	62	34	14	5	3		

MS Peak i								
	117	90	116	89	91	118	63	51
	100	76	41	32	16	11	8	5

MS Peak j								
	94	66	65	55	95	40	39	38
	100	30	17	10	9	8	7	7

MS Peak k								
	107	108	77	79	91	39	51	80
	100	70	30	22	14	13	11	10

MS Peak l								
	117	90	59	118	91	63	39	116
	100	45	28	13	13	6	6	4

MS Peak m								
	105	134	77	135	106	78	51	136
	100	78	67	33	13	11	9	7

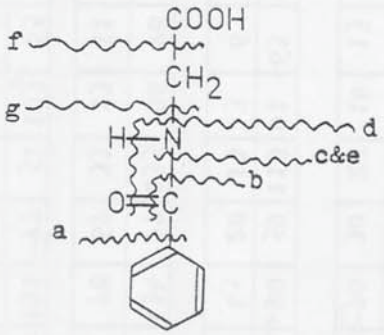
MS Peak n								
	130	131	77	51	103*	65	655*	132*
	100	93	19	15	33	11	4	15

MS Peak o								
	105	121	77	78	122	51	50	76
	100	74	60	10	9	8	3	5

MS Peak p								
	92	91	59	135	65	77	121	136
	100	75	53	18	15	10	8	5

*N.B. Bleed peaks also

Table 6. MS data from Fig 34.



Scheme 6. Probable fragmentation pathway of hippuric acid on pyrolysis

N.B. Letters refer to Fig 35.

by injecting standards was identical to that seen in the pyrogram. The mass spectra of the aromatic amides (peaks o and p) and N-methylbenzamide (peak m) made identification straightforward with the peak at m/e 105 in the benzamide(peak o) mass spectrum characteristic of the benzoyl fragment and the base peak of m/e 92 confirming the structure of the phenylacetamide fragment (peak p). It was tempting to assign some fragments to common amino acids, e.g. indole and 3-methylindole to tryptophan, but further work was undertaken to determine the products from the pyrolysis of some common components of urine.

(2) Urine components

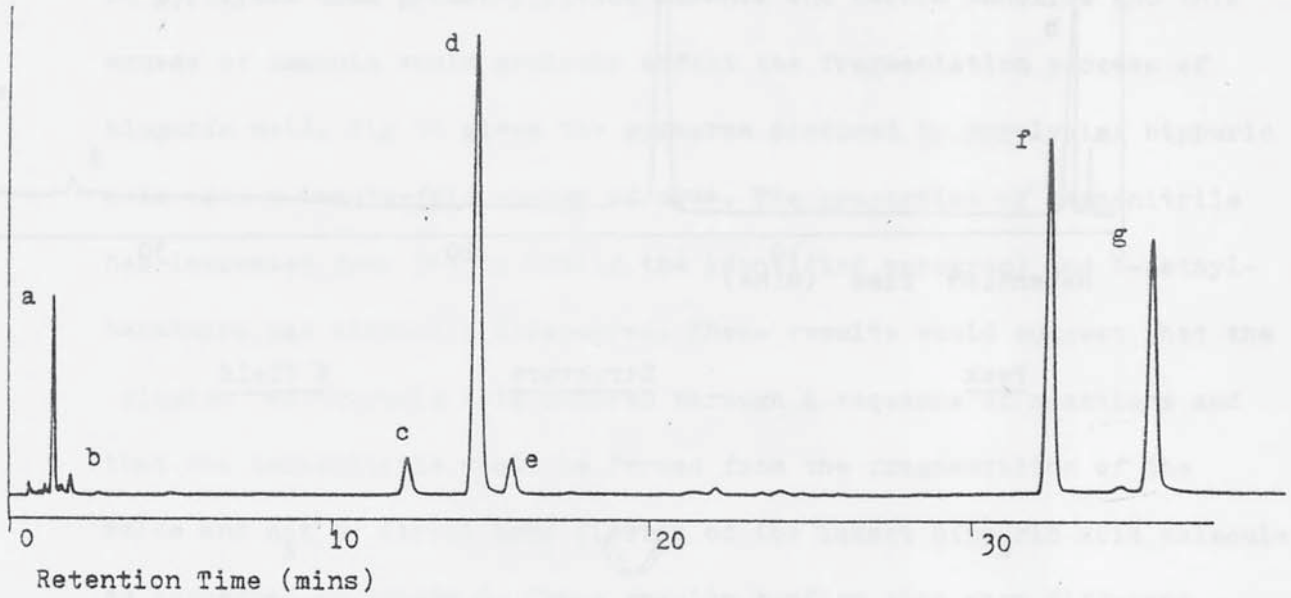
(a) Hippuric acid

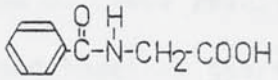
Hippuric acid (700mg excreted every 24 hrs) was pyrolysed using the same conditions as utilised for the urine analysis and the resulting pyrogram can be seen in Fig 35 with the corresponding MS data given in Table 6. The pyrolysis products are readily explicable by direct bond fission, as summarised in Scheme 6, with the exceptions of toluene and acetophenone whose formation was dependent on secondary rearrangements. From the relative amounts of the products (expressed as a percentage of the total area of the identified pyrogram) given in Fig 35, it was evident that benzonitrile (peak d), N-methylbenzamide (peak f) and benzamide (peak g) were the major fragments. These products were identified by their respective mass spectra with the characteristic benzoyl ion at m/e 105 in the products (peaks d and e).

Although these results, concerning the pyrolytic fragmentation of hippuric acid, were interesting they were not significant in elucidating the components of the urine pyrogram as hippuric acid, in urine samples,

MS Peak a	78	77	52	79	51	39	50	76
	100	20	17	12	8	6	5	5
MS Peak b	91	92	65	39	51	93		
	100	74	21	11	5	5		
MS Peak c	105	77	106	51	50	78	52	
	100	84	79	26	16	16	11	
MS Peak d	103	76	104	77	50	52	75	51
	100	33	11	6	6	3	3	2
MS Peak e	105	77	120	51	76	43	106	78
	100	67	19	19	15	11	11	7
MS Peak f	105	134	77	135	106	78	51	136
	100	91	61	30	12	8	7	5
MS Peak g	105	77	121	51	78	122	50	76
	100	64	63	13	10	6	4	4

Table 7. MS data from Fig 35.





Hippuric acid

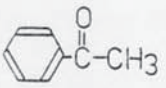
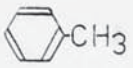
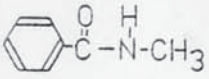
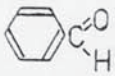
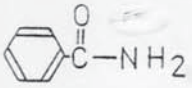
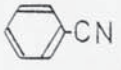
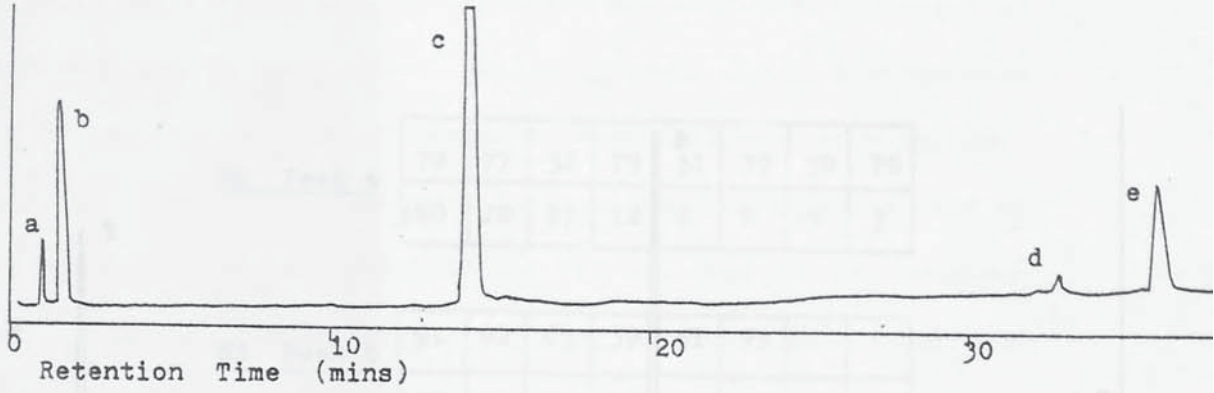
Peak	Structure	% Yield	Peak	Structure	% Yield
a		6	e		3
b		1	f		28
c		3	g		23
d		36			

Fig 35. Py-GC-MS of hippuric acid



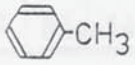
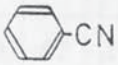
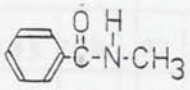
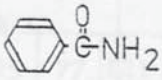
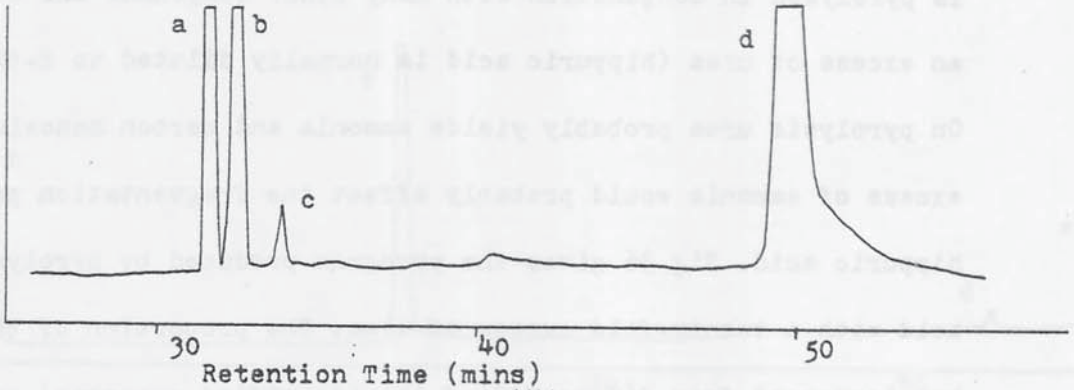
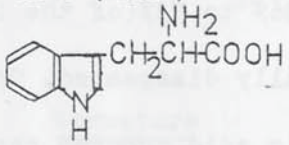
Peak	Structure	% Yield
a		2
b		9
c		73
d		1
e		15

Fig 36. Py-GC-MS of hippuric acid with urea

is pyrolysed in conjunction with many other compounds and especially in an excess of urea (hippuric acid is normally diluted to 2-5% in urea). On pyrolysis urea probably yields ammonia and carbon monoxide and this excess of ammonia would probably affect the fragmentation process of hippuric acid. Fig 36 gives the pyrogram produced by pyrolysing hippuric acid with a twenty-fold excess of urea. The proportion of benzonitrile has increased from 36% to 73%(of the identified pyrogram) and N-methyl-benzamide has virtually disapeared. These results would suggest that the fission of hippuric acid occured through a sequence of reactions and that the benzonitrile would be formed from the fragmentation of the amide and not by direct bond fission of the intact hippuric acid molecule as suggested in Scheme 6. These results confirm that when different compounds are pyrolysed together there is a possibility that the resulting pyrogram is not simply produced by superimposing the pyrograms of the individual components.





Tryptophan

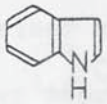
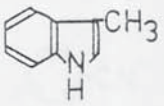
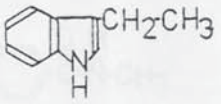
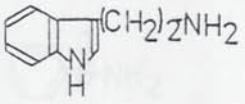
Peak	Structure	% Yield
a		31
b		26
c		2
d		41

Fig 37. Py-GC-MS of tryptophan

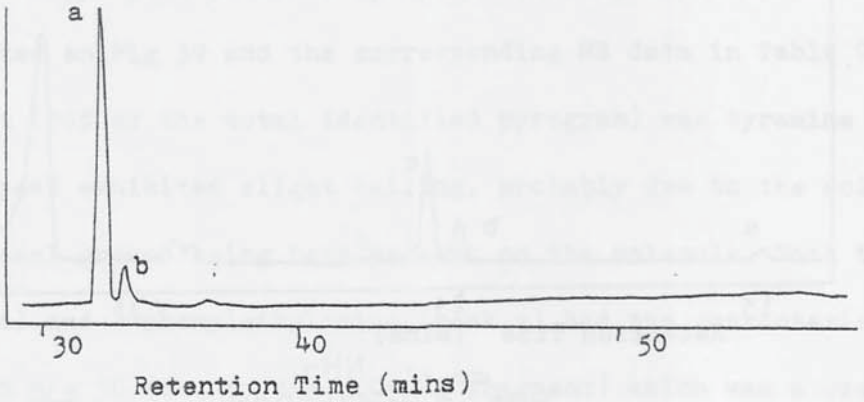
(b) Tryptophan

Tryptophan (30mg excreted every 24hrs) was pyrolysed and the resulting Py-GC-MS trace, together with the structures of the fragments produced, is given in Fig 37. The MS data of the four major fragments is presented in Table 8. The products were indole (peak a) and 3-methylindole (peak b) as seen in a previous study [130, 134], and also a smaller quantity of 3-ethylindole (peak c) was produced. Formation of tryptamine (peak d) in such a high yield (41% of the total identified pyrogram) was unexpected and the molecular ion of tryptamine was also high for an amine of this type.

Tryptophan was also pyrolysed with an excess of urea and the resulting pyrogram presented in Fig 38. The pyrogram exhibited considerable differences to that seen on pyrolysis of the pure molecule and most noticable was the complete absence of the amine tryptamine. The percentage yield of the indole had increased from 31% to 89% with 3-methylindole making up the other 11%. This simpler pyrogram (Fig 38) was probably due again to the complex nature of the pyrolytic environment.

MS Peak a	117	90	89	118	116	63	91	39
	100	50	38	12	12	11	8	6
MS Peak b	130	131	77	51	103	66.5	65	102
	100	58	17	11	11	8	6	6
MS Peak c	130	145	144	59	117	131	77	103
	100	62	58	38	35	31	27	23
MS Peak d	130	131	30	77	103	160	117	102
	100	60	43	37	34	18	16	15

Table 8. MS data from Fig 37.



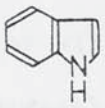
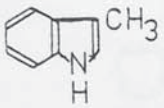
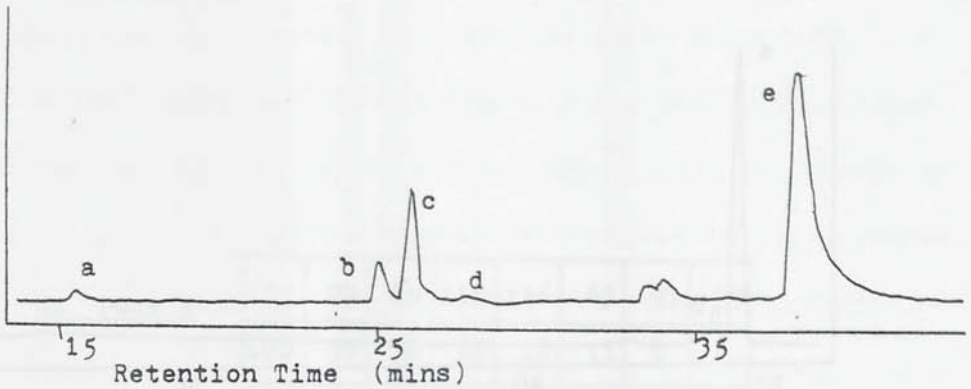
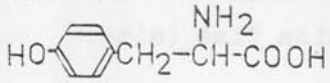
Peak	Structure	% Yield
a		89
b		11

Fig 38. Py-GC-MS of tryptophan with urea





Tyrosine

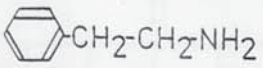
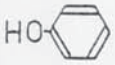
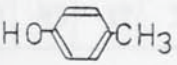
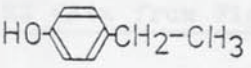
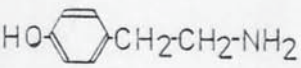
Peak	Structure	% Yield
a		1
b		6
c		16
d		1
e		76

Fig 39. Py-GC-MS of tyrosine

(c) Tyrosine

The pyrogram produced by tyrosine (56mg excreted in 24hrs) is presented in Fig 39 and the corresponding MS data in Table 9. The main product (76% of the total identified pyrogram) was tyramine (peak e) whose peak exhibited slight tailing, probably due to the acidic and basic functional groups being both present on the molecule. Both tyramine (peak e) and 2-phenylethylamine (peak a) had the characteristic base peak at m/e 30 (due to the $H_2C=\overset{+\cdot}{N}H_2$ fragment) which was a useful guide as the molecular ions of amines were not always evident.

When pyrolysed with an excess of urea, Fig 40, the analagous fragmentation was observed to that seen with tryptophan in that the pyrogram was much simpler with equal amounts of phenol(peak a) and p-cresol (peak b) and a total dissappearance of the tyramine fragment.

MS Peak a

30	91	65	39	92	51	121	
100	15	15	8	8	8	5	

MS Peak b

94	66	65	39	40	55	95	38
100	30	21	19	12	9	8	6

MS Peak c

107	108	77	79	91	39	80	51
100	71	29	24	12	12	11	11

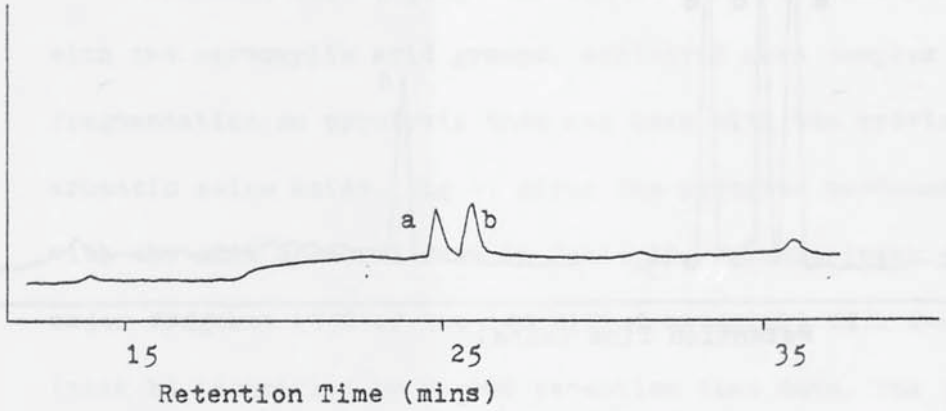
MS Peak d

107	122	77	91	27	108	39	51
100	31	31	23	19	16	16	12

MS Peak e

30	108	107	76	91	109	137	65
100	40	27	12	6	5	4	3

Table 9. MS data from pyrogram in Fig 39.



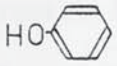
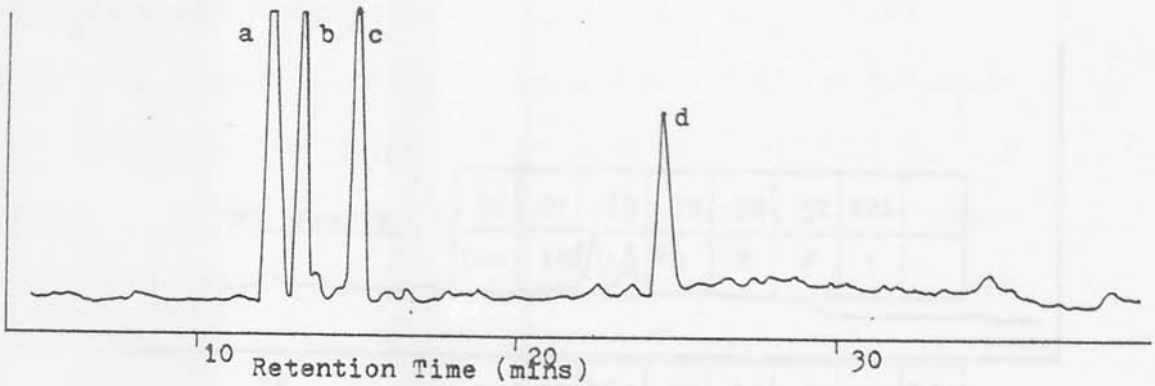
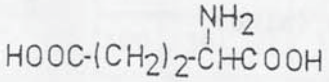
Peak	Structure	% Yield
a		51
b		49

Fig 40. Py-GC-MS of tyrosine with urea





Glutamic acid

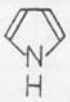
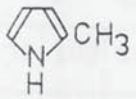
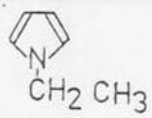
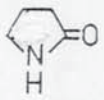
Peak	Structure	% Yield
a		45
b		20
c		19
d		16

Fig 41. Py-GC-MS of glutamic acid

(d) Glutamic acid

Glutamic acid (250mg excreted in 24hrs), an aliphatic amino acid with two carboxylic acid groups, exhibited more complex pyrolytic fragmentation on pyrolysis than was seen with the previously studied aromatic amino acids. Fig 41 gives the pyrogram produced by glutamic acid with the mass spectral data in Table 10. Pyrrole (peak a) was the major fragment (45% of the identified material) with 2-methylpyrrole (peak b) identified by MS and retention time data. The later two peaks (c and d) were tentatively assigned to N-ethylpyrrole and 2-pyrrolidone respectively. The formation of pyrrole, from pyrolysis of glutamic acid, may be explained by decarboxylation and ring closure but the mechanism of the formation of 2-methylpyrrole and N-ethylpyrrole is less than certain.

When glutamic acid was pyrolysed with urea no well resolved peaks were observed and even pyrrole was not evident. This result might indicate that the initial intermolecular reactions are significant even in the formation of the simplest pyrolytic fragments, as the dilution factor, due to urea, will inhibit intermolecular reactions but should not affect intramolecular ones.

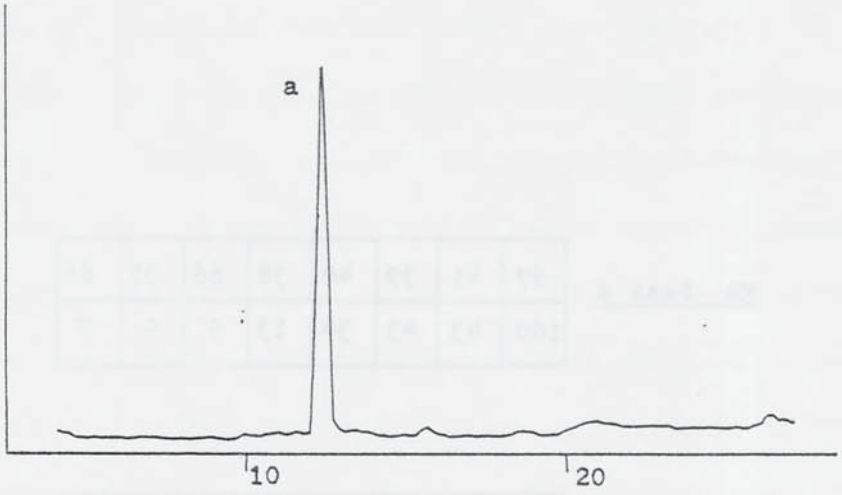
MS Peak a								
	67	41	39	40	38	66	37	68
	100	43	43	34	13	9	9	7

MS Peak b								
	80	81	53	27	39	51	52	82
	100	73	37	25	15	10	8	6

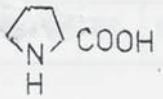
MS Peak c								
	80	95	53	94	39	67	41	27
	100	52	26	27	25	12	12	8

MS Peak d								
	85	41	42	30	84	27	39	56
	100	76	69	66	35	26	23	16

Table 10. MS data from Fig 41.



Retention Time (mins)



Proline

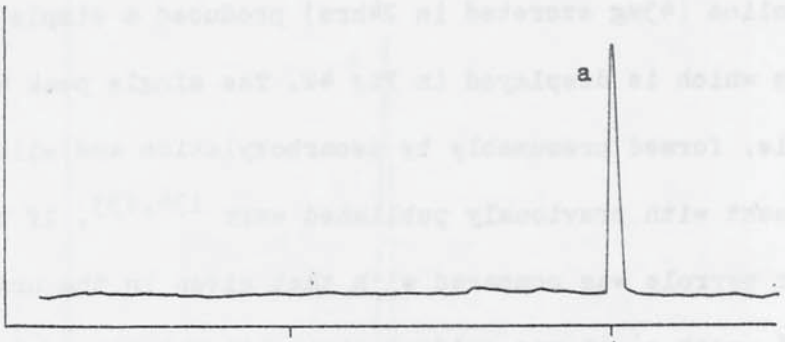
MS Peak a

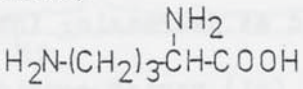
67	41	39	40	38	37
100	84	51	39	16	9

Fig 42. Py-GC-MS of proline

(e) Proline

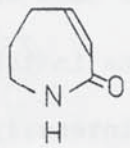
Proline (43mg excreted in 24hrs) produced a simple pyrogram on pyrolysis which is displayed in Fig 42. The single peak was identified as pyrrole, formed presumably by decarboxylation and elimination, and is in agreement with previously published work [134,135]. If the MS data given for pyrrole was compared with that given in the urine pyrogram (Table 6, peak c) it was evident that although the m/e's were in the same order (presented as decreasing intensities) the corresponding relative intensities (RI) varied considerably. e.g.m/e 41 had a RI of 22 in Table 6 but in Fig 42 the RI was 84. These differences indicate that care must be taken when identification of unknown compounds is based on relative intensities, and that an alternative comparison (e.g.retention time data) should always be used when available. The RI differences were probably due to variations in the initiation of the MS scan on the eluting peak and also to the intensity of the peak itself (variation in source pressure, due to eluate, with respect to time).





Lysine

MS Peak a

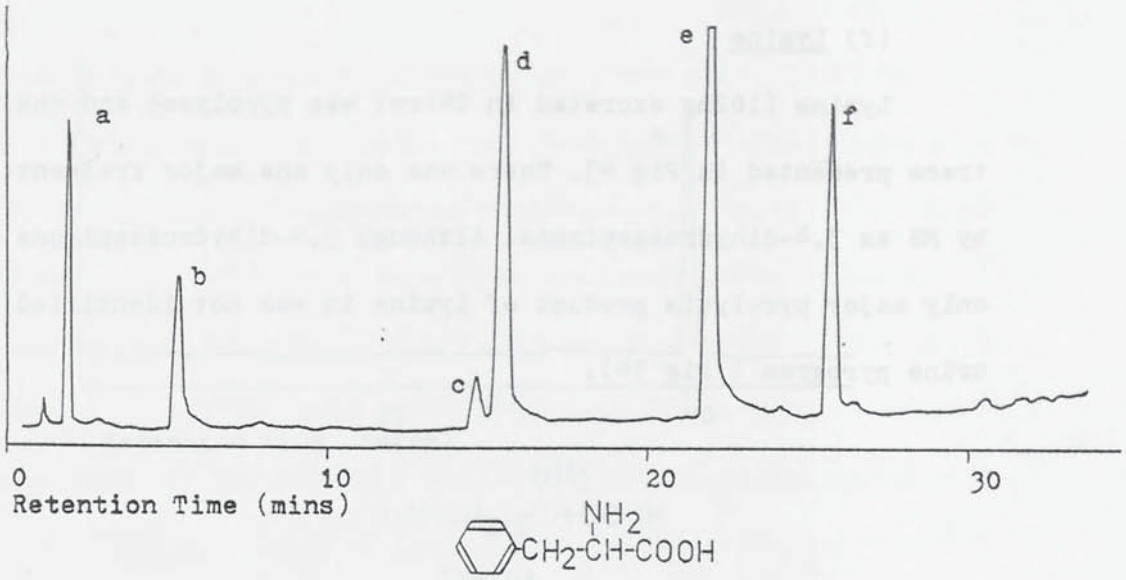


42	56	83	30	29	27	111	110
100	98	83	73	28	23	22	12

Fig 43. Py-GC-MS of lysine

(f) Lysine

Lysine (102mg excreted in 24hrs) was pyrolysed and the resulting trace presented in Fig 43. There was only one major fragment, identified by MS as 3,4-dihydroazepinone. Although 3,4-dihydroazepinone was the only major pyrolysis product of lysine it was not identified in the urine pyrogram (Fig 34).



Phenylalanine

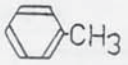
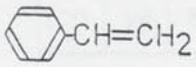
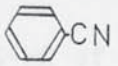
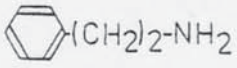
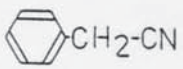
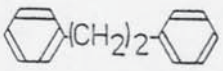
Peak	Structure	% Yield
a		8
b		10
c		3
d		22
e		41
f		16

Fig 44. Py-GC-MS of phenylalanine

(g) Phenylalanine

Phenylalanine (29mg excreted in 24hrs) had previously been analysed by Py-GC-MS [130] using a furnace pyrolyser. The pyrogram obtained in this work is shown in Fig 44 with one minor and five major products, these were identical to those previously recorded [130]. An interesting product was 1,2-diphenylethane (peak f) which must have been formed by a secondary reaction and was also present in the previous study. The chromatography utilised [130] did not permit good resolution of the phenylethylamine but as can be seen in Fig 44 (peak d) the KOH + CARBOWAX 20M column enabled good resolution to be achieved.

MS Peak a	91	92	65	39	51	63	93	
	100	59	18	14	10	10	7	

MS Peak b	104	103	78	77	51	105	50	39
	100	59	59	27	23	14	14	14

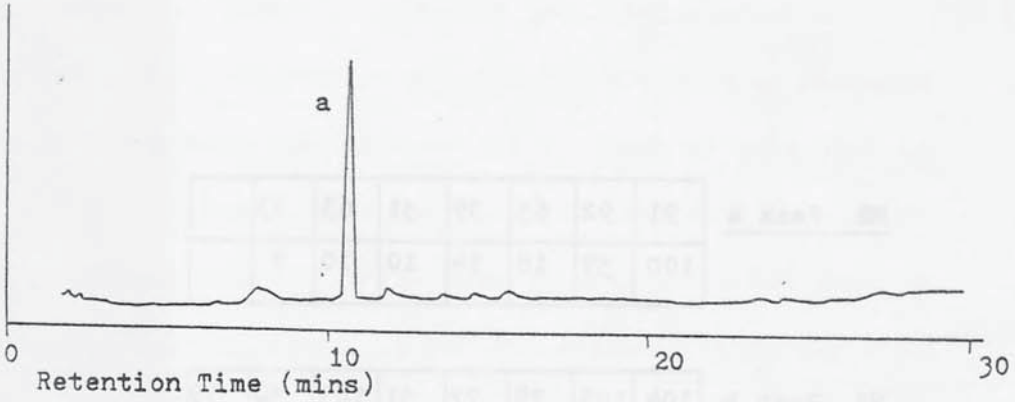
MS Peak c	103	76	39	50	104	51	75	
	100	53	27	13	10	7	6	

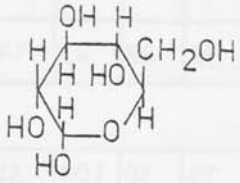
MS Peak d	30	91	65	92	39	51	121	
	100	27	10	8	8	6	4	

MS Peak e	117	90	116	89	91	51	118	63
	100	70	51	41	19	16	14	14

MS Peak f	91	182	65	92	104	39	51	183
	100	17	17	12	6	5	4	3

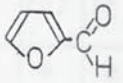
Table 11. MS data from Fig 44.





Glucose

MS Peak a



96	95	39	29	67	40	38	37
100	99	33	14	13	13	11	5

Fig 45. Py-GC-MS of glucose

(h) Glucose

There have been previous reports of the pyrolysis of carbohydrates [136]. Glucose (72mg excreted in 24hrs) was pyrolysed and the resulting pyrogram is given in Fig 45 with furfural the major product. The pyrolysis of glucose with urea was attempted in anticipation of the appearance of pyrroles or pyridines produced by exchange of the heterocyclic oxygen with nitrogen. This exchange would have explained the lack of any substituted furans in the urine pyrogram. However, the resulting pyrogram did not exhibit any resolved peaks.

C CONCLUSIONS

Seventeen components from the urine pyrogram were identified and from the ensuing study of urine components the origins of some of the fragments became apparent (e.g. indole and 3-methylindole from tryptophan). However, it is evident that the urine pyrogram is not simply the summation of the individual components as the presence of other components, e.g. urea, profoundly affect the fragmentation of the constituent molecules. There would appear to be a further degree of complexity, as the results of the pyrolysis of hippuric acid with urea would not explain why the benzamide peak is often more intense than the benzonitrile peak. The data from Fig 36 (pyrolysis of hippuric acid with urea) would suggest that the benzonitrile should predominate. The presence of pyridine(Fig 34, peak b) has not been satisfactorily explained as no pyridine was seen in any of the component pyrograms.

The behaviour of the amino acids with urea helped to explain the formation of nitriles by ibuprofen metabolites (Chapter 2), as the carboxylic acid groups could readily be converted to the amide and hence dehydrate to the corresponding nitrile.

Further study should be initiated to determine if urine with abnormal levels of some components could be screened by this method.

APPENDIX 1.

Data for Variation of peak heights and ratios with pyrolysis temperatures.

For pyrogram see Fig 4.

	1	2	3	Mean	S.Dev	CV (%)
a	35	96	17	49.33	41.40	83.93
a/d	.333	.331	.472	.379	.081	21.35
b	35	96	15	48.67	42.19	86.70
b/d	.333	.331	.417	.360	.049	13.62
c	79		31	55.00	33.94	61.71
c/d	.752		.661	.707	.064	9.11
d	105	290	36	143.67	131.34	91.42
d/d	1.000	1.000	1.000			
e	74	215	27	105.33	97.84	92.88
e/d	.705	.741	.75	.732	.024	3.25
f	33	93	13	46.33	41.63	89.86
f/d	.314	.321	.361	.332	.025	7.64
g	19	52	8	26.33	22.90	86.96
g/d	.181	.179	.222	.194	.024	12.51

Data for 3 replicate runs at 610°C for 5 seconds

	1	2	3	Mean	S.Dev	CV (%)
a	32	62	75	56.33	22.05	39.15
a/d	.372	.408	.434	.405	.031	7.69
b	28	45	53	42.00	12.77	30.40
b/d	.326	.296	.306	.309	.015	4.94
c	62	100	117	93.00	28.16	30.28
c/d	.721	.658	.676	.685	.032	4.74
d	86	152	173	137.00	45.40	33.14
d/d	1.000	1.000	1.000			
e	61	106	122	96.33	31.63	32.83
e/d	.709	.697	.705	.704	.006	0.86
f	31	49	53	44.33	11.72	26.43
f/d	.360	.322	.306	.329	.028	8.42
g	18	28	30	25.33	6.43	25.38
g/d	.209	.184	.173	.189	.018	9.78

Data for 3 replicate runs at 770°C for 5 seconds

	1	2	3	Mean	S.Dev	CV (%)
a	81	24	71	58.66	30.44	51.88
a/d	.422	.358	.380	.387	.033	8.41
b	34	16	38	29.33	11.72	39.95
b/d	.177	.239	.203	.206	.031	15.09
c	83	39	96	72.67	29.87	41.11
c/d	.432	.582	.513	.510	.075	14.74
d	192	67	187	148.67	70.77	47.60
d/d	1.000	1.000	1.000			
e	102	56	109	89.00	28.79	32.35
e/d	.531	.836	.583	.650	.163	25.10
f	55	22	55	44.00	19.05	43.30
f/d	.286	.328	.294	.303	.022	7.36
g	37	17	39	31.00	12.17	39.24
g/d	.193	.254	.209	.219	.032	14.46

Data for 3replicate runs at 980°C for 5 seconds

APPENDIX 2.

Data for variation of peak heights and ratios with pyrolysis times.

For pyrogram see Fig 4.

	1	2	3	Mean	S.Dev	CV (%)
a	22	7	17	15.33	7.637	49.81
a/d	.440	.583	.654	.559	.109	19.50
b	20	5	11	12.00	7.56	62.91
b/d	.400	.417	.423	.413	.012	2.89
c	42	9	20	23.67	16.80	71.00
c/d	.840	.750	.769	.786	.047	6.03
d	50	12	26	29.33	19.21	65.52
d/d	1.000	1.000	1.000			
e	53	8	25	28.67	22.72	79.27
e/d	1.060	.667	.962	.896	.205	22.82
f	17	5	10	10.67	6.03	56.51
f/d	.340	.417	.385	.381	.039	10.16
g	11	3	7	7.00	4.00	57.14
g/d	.220	.250	.269	.246	.025	10.03

Data for 3 replicate runs at 980°C for 1 second

	1	2	3	Mean	S.Dev	cv (%)
a	24	17	28	23.00	5.57	24.21
a/d	.453	.515	.491	.486	.031	6.43
b	17	13	19	16.33	3.06	18.70
b/d	.321	.394	.333	.349	.039	11.21
c	37	25	40	34.00	7.94	23.34
c/d	.698	.758	.702	.719	.034	4.66
d	53	33	57	47.67	12.86	26.98
d/d	1.000	1.000	1.000			
e	50	35	55	46.67	6.41	22.30
e/d	.943	1.061	.965	.990	.061	6.34
f	17	11	18	15.33	3.79	24.69
f/d	.321	.333	.316	.323	.009	2.70
g	11	7	13	10.33	3.06	29.57
g/d	.209	.212	.228	.216	.011	4.90

Data for 3 replicate runs at 980°C for 2 seconds

	1	2	3	Mean	S.Dev	CV (%)
a	81	24	71	58.66	30.44	51.88
a/d	.442	.358	.380	.387	.033	8.41
b	34	16	38	29.33	11.72	39.95
b/d	.177	.239	.203	.206	.031	15.09
c	83	39	96	72.67	29.87	41.11
c/d	.432	.582	.513	.509	.075	14.75
d	192	67	187	148.67	70.77	47.60
d/d	1.000	1.000	1.000			
e	102	56	109	89.00	28.79	32.35
e/d	.531	.836	.538	.650	.163	25.10
f	55	22	55	44.00	19.05	43.30
f/d	.286	.328	.294	.303	.022	7.37
g	37	17	39	31.00	12.17	39.24
g/d	.193	.254	.209	.219	.032	14.46

Data for 3 replicate runs at 980°C for 5 seconds

	1	2	Mean	S.Dev	CV (%)
a	51	26	38.50	17.68	45.92
a/d	.372	.377	.375	.004	.094
b	40	19	29.50	14.85	50.34
b/d	.292	.130	.211	.115	54.29
c	93	27	60.00	46.67	77.78
c/d	.679	.391	.535	.204	38.06
d	137	69	103	48.08	46.68
d/d	1.000	1.000			
e	84	32	58.00	36.77	63.40
e/d	.613	.462	.538	.107	19.86
f	46	21	33.50	17.68	52.77
f/d	.336	.304	.320	.022	7.07
g	34	21	27.50	9.19	33.43
g/d	.248	.304	.276	.040	14.35

Data for 2 duplicate runs at 980°C for 7.5 seconds

APPENDIX 3.

Data from peak heights from eleven replicate runs from pyrolysis of *P. aeruginosa*.

For pyrogram see Fig 7.

	1	2	3	4	5	6	7	8	9	10	11
A	22	40	17	26	32	14	48	26	28	17	54
B	56	83	44	79	81	47	107	63	68	60	114
C	10	12	7	15	14	8	18	10	12	10	19
D	22	33	16	31	34	20	41	25	27	25	47
E	47	74	43	78	74	39	102	59	62	52	104
F	27	38	24	41	40	24	53	33	36	31	56
G	60	87	52	88	88	54	113	73	78	68	122
H	17	31	14	28	30	17	31	18	21	21	38
I	27	41	22	39	40	23	52	32	34	29	56
J	24	38	21	37	36	20	48	29	31	26	50
K	86	151	83	148	136	78	171	104	110	102	197
L	58	89	46	79	79	51	99	67	70	61	113
M	35	55	27	49	49	30	61	40	41	36	73
N	21	34	14	26	30	18	36	23	24	21	39
O	17	21	13	18	19	15	23	17	18	16	26
P	16	19	12	11	14	7	16	10	11	8	17
Q	21	26	19	20	12	14	26	18	20	15	28
R	26	34	18	11	23	17	29	26	25	16	27
S	17	30	21	31	28	12	36	22	23	21	42
T	16	20	12	11	19	11	25	14	16	12	22
U	49	74	52	45	57	34	80	57	58	42	76
V	42	32	36	31	37	23	34	26	19	40	45

APPENDIX 4.

Peak ratios and statistical analysis of peak ratios after normalisation to Peak K.

For pyrogram see Fig 7.

	1	2	3	4	5	6	7	8	9	10	11
A	.256	.265	.205	.176	.235	.179	.281	.250	.255	.167	.274
B	.651	.550	.530	.534	.596	.603	.626	.606	.618	.588	.579
C	.116	.079	.084	.101	.103	.103	.105	.096	.109	.098	.096
D	.256	.219	.193	.209	.250	.256	.240	.240	.245	.245	.239
E	.547	.490	.518	.527	.544	.500	.596	.567	.564	.510	.528
F	.344	.252	.289	.277	.294	.308	.310	.317	.327	.304	.284
G	.698	.576	.627	.595	.647	.692	.661	.702	.709	.667	.619
H	.198	.205	.169	.189	.221	.218	.181	.173	.191	.206	.193
I	.314	.272	.265	.264	.294	.295	.304	.308	.309	.284	.284
J	.279	.252	.253	.250	.265	.256	.281	.279	.282	.255	.254
K	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
L	.674	.589	.550	.574	.581	.654	.579	.644	.636	.598	.574
M	.407	.364	.330	.331	.360	.385	.357	.385	.373	.353	.371
N	.244	.255	.170	.176	.221	.231	.211	.221	.218	.206	.198
O	.198	.139	.160	.122	.140	.192	.135	.221	.164	.157	.132
P	.186	.126	.145	.074	.103	.090	.094	.096	.100	.078	.086
Q	.244	.172	.229	.135	.088	.179	.152	.173	.182	.147	.142
R	.302	.225	.217	.074	.169	.218	.170	.250	.227	.157	.137
S	.198	.199	.253	.209	.206	.154	.211	.212	.209	.206	.213
T	.186	.132	.145	.074	.140	.141	.146	.135	.145	.118	.112
U	.570	.490	.627	.304	.419	.436	.468	.548	.527	.412	.386
V	.488	.212	.434	.209	.272	.295	.199	.250	.173	.392	.228

	Mean	S.Dev	CV
A	.231	.042	18.170
B	.589	.038	6.513
C	.099	.011	10.618
D	.236	.020	8.475
E	.536	.032	5.970
F	.298	.021	7.051
G	.654	.045	6.882
H	.195	.017	8.722
I	.290	.018	6.201
J	.264	.013	4.921
K	1.000	----	-----
L	.601	.045	7.490
M	.365	.023	6.300
N	.211	.022	10.430
O	.160	.031	19.380
P	.107	.033	30.815
Q	.168	.043	25.820
R	.195	.062	31.664
S	.206	.023	11.165
T	.134	.027	20.465
U	.472	.093	19.677
V	.287	.105	36.705

APPENDIX 5.

Peak ratios and statistical analysis of peak ratios after normalisation to Peaks G,K and U.

For pyrogram see Fig 7.

	1	2	3	4	5	6	7	8	9	10	11
A	.367	.460	.330	.295	.364	.259	.425	.356	.359	.250	.443
B	.600	.954	.850	.898	.920	.870	.947	.863	.872	.882	.984
C	.167	.138	.130	.170	.159	.148	.159	.137	.154	.147	.156
D	.367	.379	.310	.352	.386	.370	.363	.342	.346	.368	.385
E	.783	.851	.830	.886	.841	.722	.903	.808	.759	.765	.852
F	.450	.437	.460	.466	.455	.444	.469	.452	.462	.456	.459
G	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
H	.283	.356	.270	.318	.341	.315	.274	.247	.269	.309	.311
I	.450	.471	.420	.443	.455	.420	.460	.438	.436	.426	.459
J	.400	.437	.400	.420	.409	.370	.425	.397	.397	.382	.410
K	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
L	.674	.589	.550	.534	.581	.654	.579	.644	.636	.598	.574
M	.407	.364	.330	.331	.360	.385	.357	.385	.373	.353	.371
N	.244	.225	.170	.176	.221	.231	.211	.221	.218	.206	.198
O	.198	.139	.160	.122	.140	.192	.135	.221	.164	.157	.132
P	.327	.257	.230	.244	.246	.206	.200	.175	.190	.190	.224
Q	.429	.351	.370	.444	.211	.412	.325	.316	.345	.357	.368
R	.531	.459	.350	.244	.404	.500	.368	.456	.431	.381	.365
S	.347	.405	.400	.689	.491	.352	.450	.386	.397	.500	.553
T	.327	.270	.230	.244	.333	.324	.313	.246	.397	.286	.289
U	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
V	.857	.432	.690	.689	.649	.676	.425	.456	.328	.952	.592

	Mean	S.Dev	CV
A	.361	.069	19.12
B	.880	.097	11.02
C	.153	.011	7.19
D	.364	.024	6.59
E	.817	.054	6.61
F	.455	.009	1.98
G	1.000	----	-----
H	.302	.033	10.93
I	.445	.017	3.82
J	.405	.018	4.44
K	1.000	----	-----
L	.601	.045	7.49
M	.365	.023	6.30
N	.211	.022	10.43
O	.160	.031	19.38
P	.229	.042	18.34
Q	.362	.063	17.40
R	.407	.076	18.67
S	.459	.100	21.79
T	.295	.047	15.93
U	1.000	----	-----
V	.631	.193	30.59

APPENDIX 6.

Peak ratios and statistical analysis of peak ratios after normalisation to Peaks B3, L and V.

For pyrogram see Fig 7.

	1	2	3	4	5	6	7	8	9	10	11
A	.393	.482	.390	.329	.395	.298	.449	.413	.412	.283	.474
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
C	.179	.142	.160	.190	.173	.170	.168	.159	.176	.167	.167
D	.393	.398	.364	.392	.420	.426	.383	.397	.397	.417	.412
E	.839	.892	.977	.987	.914	.830	.953	.937	.912	.867	.912
F	.482	.458	.545	.519	.494	.511	.495	.524	.529	.517	.491
G	1.071	1.048	1.182	1.114	1.086	1.149	1.056	1.159	1.147	1.133	1.070
H	.304	.373	.318	.354	.370	.362	.290	.286	.309	.350	.330
I	.482	.494	.500	.494	.494	.489	.486	.508	.500	.483	.491
J	.429	.458	.477	.468	.444	.426	.449	.460	.456	.433	.439
K	1.483	1.697	1.804	1.873	1.722	1.529	1.727	1.552	1.571	1.672	1.743
L	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
M	.603	.618	.587	.620	.620	.588	.616	.597	.586	.590	.646
N	.362	.382	.304	.329	.380	.353	.364	.343	.343	.344	.345
O	.293	.236	.283	.228	.241	.294	.232	.254	.257	.262	.230
P	.381	.594	.333	.355	.378	.304	.471	.385	.579	.200	.378
Q	.500	.813	.528	.645	.324	.609	.765	.692	1.053	.375	.622
R	.619	1.036	.500	.355	.622	.739	.853	1.000	1.316	.400	.600
S	.405	.938	.583	1.000	.757	.522	1.029	.846	1.211	.525	.933
T	.327	.652	.333	.355	.514	.478	.735	.538	.842	.300	.489
U	1.167	2.313	1.444	1.452	1.514	1.478	2.353	2.192	3.053	1.050	1.689
V	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

	Mean	S.Dev	CV
A	.397	.065	16.37
B	1.000	----	-----
C	.168	.012	7.14
D	.402	.019	4.73
E	.906	.052	5.74
F	.503	.025	4.97
G	1.106	.046	4.16
H	.334	.031	9.28
I	.492	.008	1.63
J	.447	.016	3.58
K	1.670	.122	7.31
L	1.000	----	----
M	.608	.019	3.13
N	.349	.022	6.30
O	.255	.024	9.41
P	.389	.122	28.79
Q	.619	.199	32.15
R	.799	.291	36.42
S	.783	.249	31.80
T	.447	.148	33.11
U	1.780	.571	32.08
V	1.000	----	-----

APPENDIX 7.

Data from replicate runs of pyrolysis of sulphapyridine using temperature programmed GC.

Run No	Area of peak a	Area of peak b	a/b
1	19830	21039	0.94
2	19072	21046	0.91
3	16570	19046	0.87
4	18187	18402	0.99
5	23714	21664	1.09

Quantitative data from sulphapyridine temperature programmed runs

Peak a = aniline Peak b = 2-aminopyridine

Mean (a/b)	0.96
S.Dev	0.08
CV.	9.0%

APPENDIX 8.

Data from eleven replicate runs from the pyrolysis of sulphapyridine using CHROMOSORB 103 isothermally. For pyrogram see Fig 19.

	Ratio of peak b/a	
Run No	Heights	Areas
1	1.5211	2.2840
2	1.5846	2.2810
3	1.3763	2.0810
4	1.5455	2.3700
5	1.4583	2.1440
6	1.6842	2.5960
7	1.4167	2.0380
8	1.3166	2.2700
9	1.5385	2.3840
10	1.5303	2.3990
11	1.3729	2.2140
Mean	1.4859	2.2783
Stan. Dev	0.1084	0.1594
CV	7.2978	6.9958

APPENDIX 9.

Calibration data (peak heights and peak areas) for pyrolysis of sulphamerazine/sulphadimidine mixture with different amounts on the wire.

For pyrogram see Fig 21.

Sulphamerazine/sulphadimidine peak height ratios.

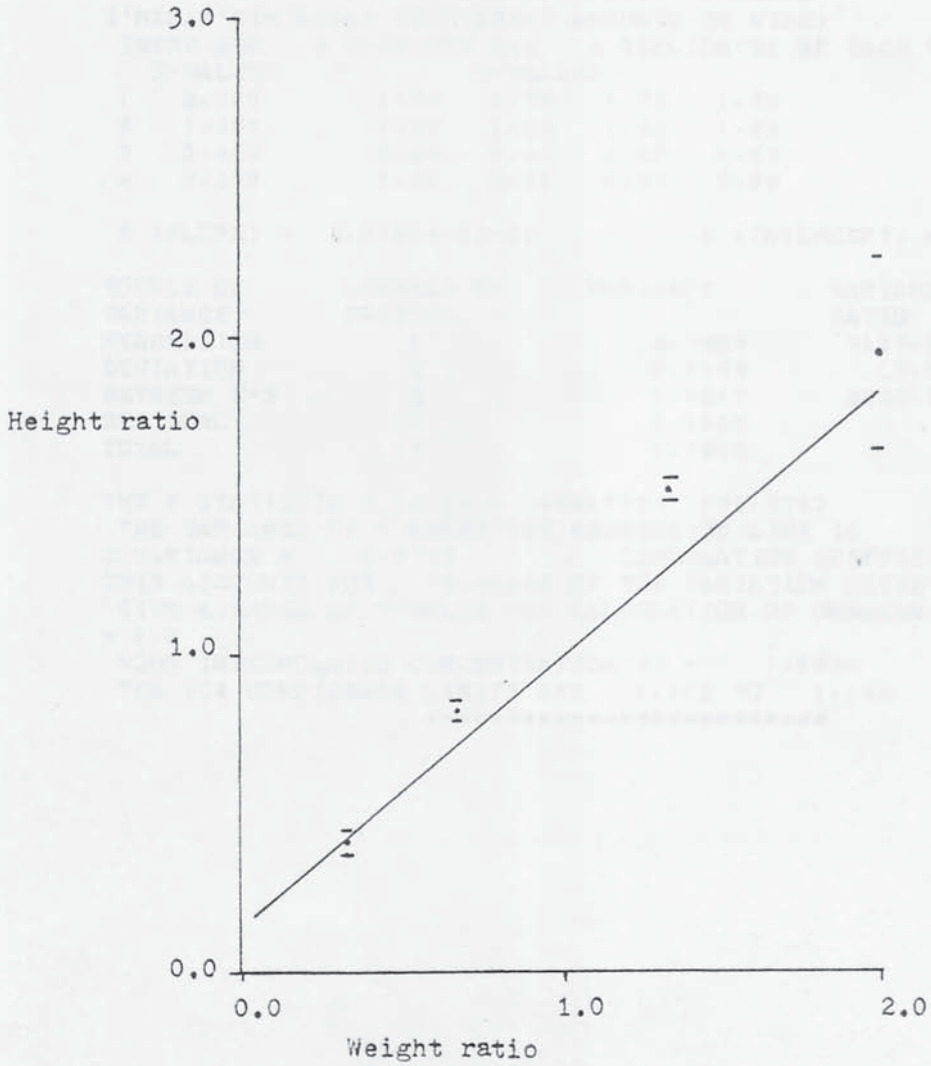
```
                    **************************
S'MER/S'DIM HGTS (DIFFERENT AMOUNTS ON WIRE)
 THERE ARE   4 X-VALUES AND   4 REPLICATES OF EACH Y-VALUE
   X-VALUES              Y-VALUES
1    2.000        1.93    2.15    2.17    1.53
2    1.330        1.55    1.51    1.52    1.52
3    0.660        0.87    0.80    0.85    0.85
4    0.330        0.38    0.43    0.41    0.43

 B (SLOPE) =  9.1481066E-01          A (INTERCEPT) =   1.9325449E-01

SOURCE OF       DEGREES OF      VARIANCE        VARIANCE
VARIANCE        FREEDOM                         RATIO
REGRESSION          1             5.5160        244.8397
DEVIATION           2             0.0564          2.5032
BETWEEN X-S         3             1.8763         83.2820
RESIDUAL           12             0.0225
TOTAL              15             0.3933

THE F STATISTIC ( 1, 2) =   97.8097  [99.27%]
 THE VARIANCE OF Y ABOUT THE REGRESSION LINE IS     0.0274
COVARIANCE =    0.4020          CORRELATION COEFFICIENT =    0.9670
THIS ACCOUNTS FOR   93.5052% OF THE VARIATION OBSERVED
 GIVE AVERAGE OF Y-VALUE FOR CALCULATION OF UNKNOWN CONCENTRATION
- 1.0
 YOUR INTERPOLATED CONCENTRATION IS =    0.8819
 THE 95% CONFIDENCE LIMITS ARE   0.607 TO   1.157
                    **************************
```

Sulphamerazine/sulphadimidine peak height plot (different amounts on wire).



Sulphamerazine/sulphadimidine peak area ratios.

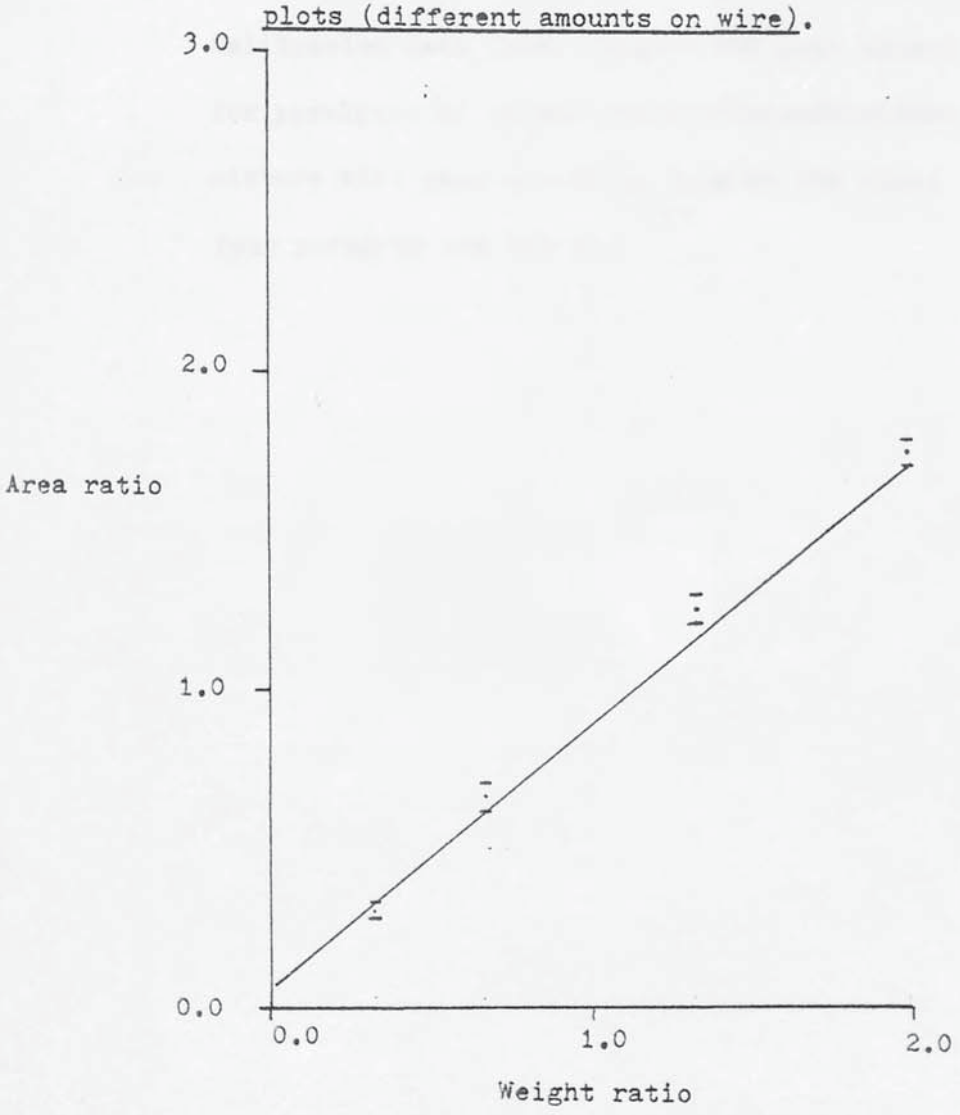
```
                    ************************
S'MER/S'DIM AREAS (DIFFERENT AMOUNTS ON WIRE)
 THERE ARE    4 X-VALUES AND    4 REPLICATES OF EACH Y-VALUE
   X-VALUES              Y-VALUES
 1    2.000        1.74    1.76    1.73    1.76
 2    1.330        1.27    1.22    1.25    1.23
 3    0.660        0.69    0.67    0.62    0.69
 4    0.330        0.32    0.31    0.33    0.29

 B (SLOPE) =  8.5180240E-01          A (INTERCEPT) =   7.2553405E-02

SOURCE OF       DEGREES OF      VARIANCE        VARIANCE
VARIANCE        FREEDOM                         RATIO
REGRESSION          1              4.7824        9109.2560
DEVIATION           2              0.0099          18.8958
BETWEEN X-S         3              1.6007        3049.0159
RESIDUAL           12              0.0005
TOTAL              15              0.3206

THE F STATISTIC ( 1, 2) =  482.0784  [99.87%]
 THE VARIANCE OF Y ABOUT THE REGRESSION LINE IS     0.0019
COVARIANCE =    0.3743          CORRELATION COEFFICIENT =    0.9973
THIS ACCOUNTS FOR   99.4564% OF THE VARIATION OBSERVED
 GIVE AVERAGE OF Y-VALUE FOR CALCULATION OF UNKNOWN CONCENTRATION
- 1.0
 YOUR INTERPOLATED CONCENTRATION IS =    1.0888
 THE 95% CONFIDENCE LIMITS ARE   1.012 TO   1.166
                    ************************
```

Sulphamerazine/sulphadimidine peak area plots (different amounts on wire).



APPENDIX 10.

Calibration data (peak heights and peak areas) for pyrolysis of sulphamerazine/sulphadimidine mixture with same amount of drug on the wire.

Foor pyrogram see Fig 21.

Sulphamerazine/sulphadimidine peak height ratios.

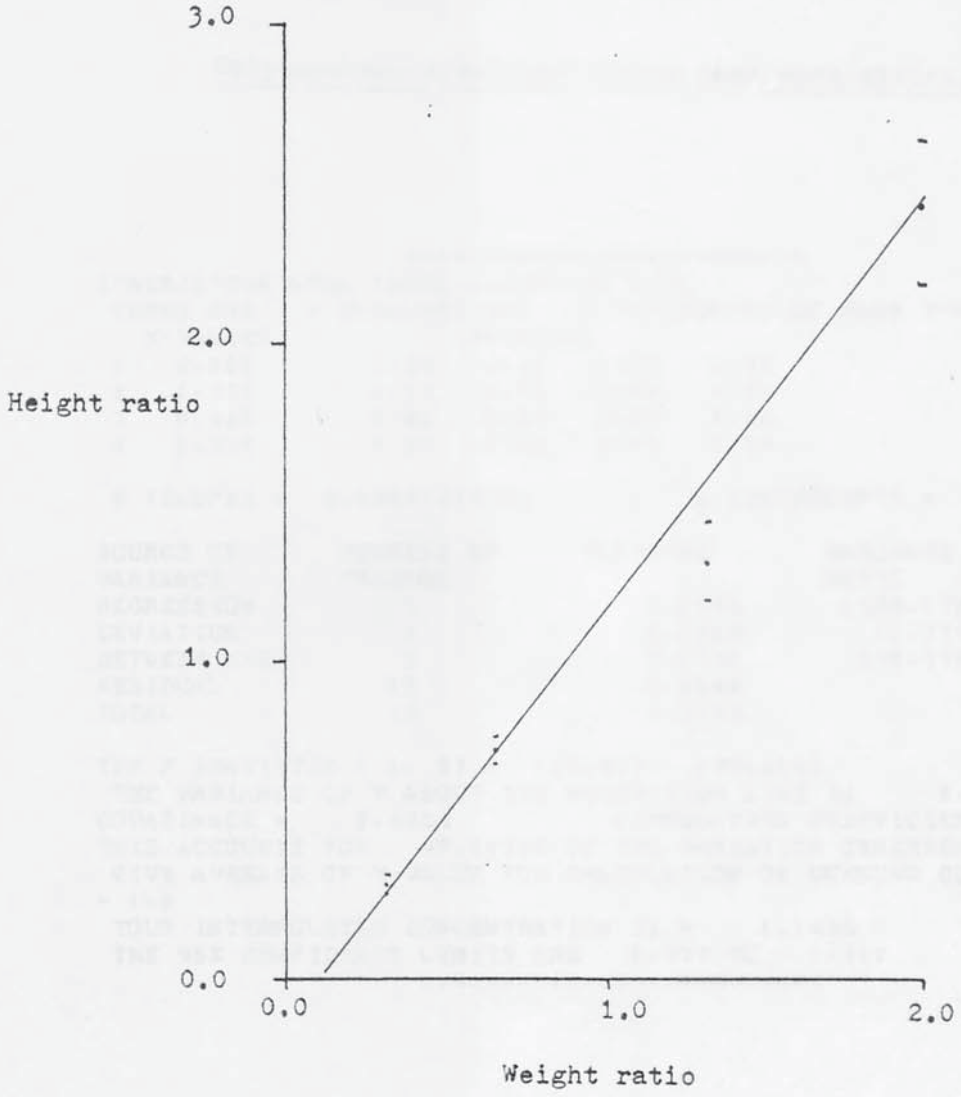
```
                    **************************
S'MER/S'DIM HGTS  (SAME AMOUNT ON WIRE  )
 THERE ARE    4 X-VALUES AND    4 REPLICATES OF EACH Y-VALUE
   X-VALUES                Y-VALUES
1    2.000          2.27    2.23    2.64    2.60
2    1.330          1.43    1.37    1.15    1.33
3    0.660          0.76    0.70    0.68    0.77
4    0.330          0.28    0.31    0.31    0.31

 B (SLOPE) =  1.2366640E 00          A (INTERCEPT) =  -1.3934716E-01

SOURCE OF       DEGREES OF      VARIANCE        VARIANCE
VARIANCE        FREEDOM                         RATIO
REGRESSION          1             10.0802        641.1983
DEVIATION           2              0.0966          6.1432
BETWEEN X-S         3              3.4244        217.8283
RESIDUAL           12              0.0157
TOTAL              15              0.6975

THE F STATISTIC ( 1, 2) =  104.3751  [99.32%]
 THE VARIANCE OF Y ABOUT THE REGRESSION LINE IS     0.0273
COVARIANCE =    0.5434          CORRELATION COEFFICIENT =    0.9816
THIS ACCOUNTS FOR   96.3506% OF THE VARIATION OBSERVED
 GIVE AVERAGE OF Y-VALUE FOR CALCULATION OF UNKNOWN CONCENTRATION
- 1.0
 YOUR INTERPOLATED CONCENTRATION IS =    0.9213
 THE 95% CONFIDENCE LIMITS ARE   0.718 TO   1.124
```

Sulphamerazine/sulphadimidine peak height plot (same amount on wire).



Sulphamerazine/sulphadimidine peak area ratios.

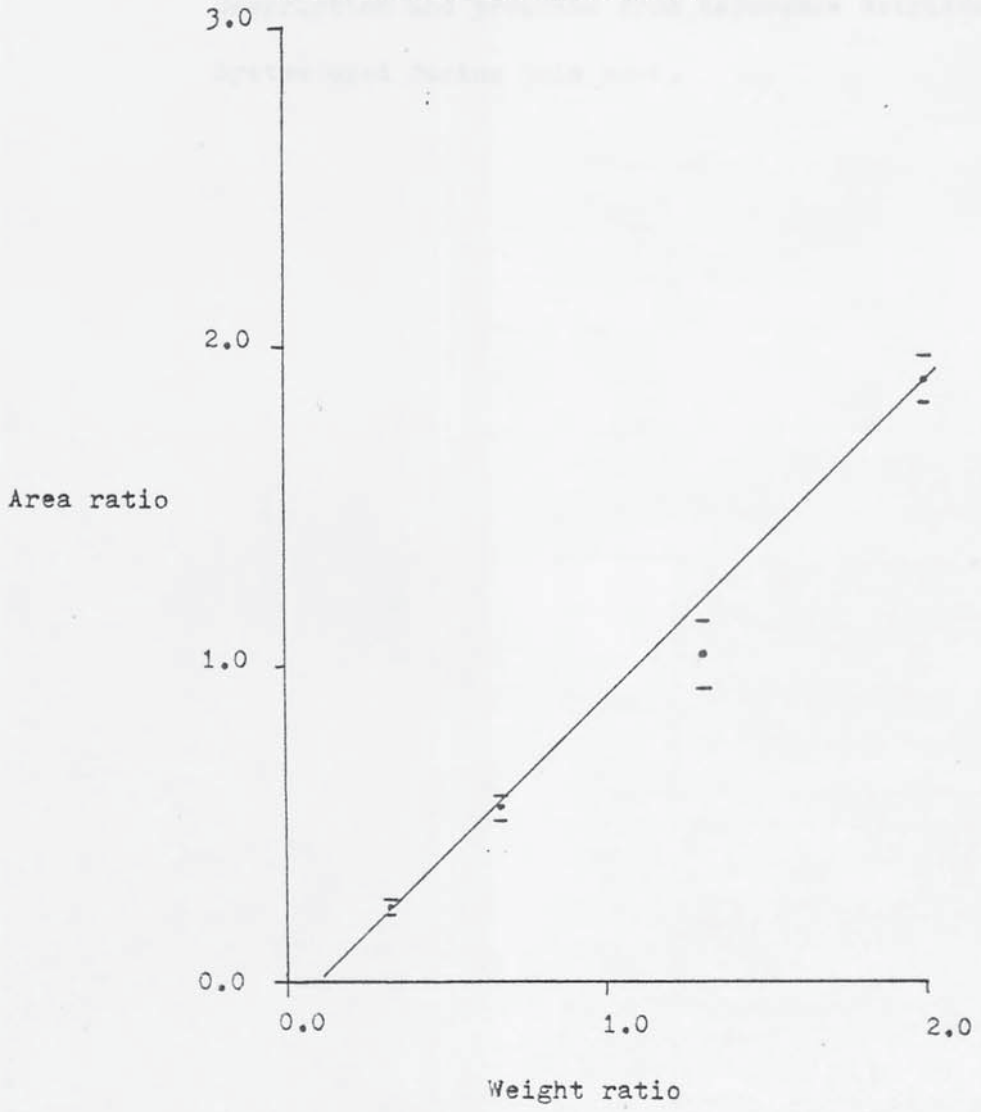
```
                    **************************
S'MER/S'DIM AREA (SAME AMOUNT ON WIRE )
 THERE ARE    4 X-VALUES AND    4 REPLICATES OF EACH Y-VALUE
   X-VALUES                Y-VALUES
 1    2.000          1.84    1.83    1.99    1.93
 2    1.330          1.17    1.06    0.92    1.02
 3    0.660          0.60    0.55    0.54    0.60
 4    0.330          0.23    0.25    0.23    0.24

 B (SLOPE) =  9.6355747E-01            A (INTERCEPT) =  -1.0314207E-01

SOURCE OF       DEGREES OF      VARIANCE        VARIANCE
VARIANCE        FREEDOM                         RATIO
REGRESSION          1              6.1196        1388.1785
DEVIATION           2              0.0519          11.7784
BETWEEN X-S         3              2.0745         470.5784
RESIDUAL           12              0.0044
TOTAL              15              0.4184

THE F STATISTIC ( 1, 2) =  117.8577  [99.41%]
 THE VARIANCE OF Y ABOUT THE REGRESSION LINE IS      0.0112
COVARIANCE =     0.4234          CORRELATION COEFFICIENT =     0.9874
THIS ACCOUNTS FOR    97.5026% OF THE VARIATION OBSERVED
 GIVE AVERAGE OF Y-VALUE FOR CALCULATION OF UNKNOWN CONCENTRATION
- 1.0
 YOUR INTERPOLATED CONCENTRATION IS =     1.1449
 THE 95% CONFIDENCE LIMITS ARE    0.979 TO    1.311
                    **************************
```

Sulphamerazine/sulphadimidine peak area plot (same amount on wire).



APPENDIX 11.

Description and programs from Reference Retrieval System used during this work.

```
0           RFINFO
1           ******
2
3
4  INTRODUCTION
5  ************
6
7  THIS PACKAGE IS DEVISED FOR THE INPUT AND OUTPUT OF A
8  PERSONAL REFERENCE RETRIEVAL SYSTEM.
9  SOME DEFINITIONS OF COMPUTING TERMS ARE
10 GIVEN AT THE END OF THIS LISTING
11
12 REFERENCE  UNIT
13 *********  ****
14
15 EACH REFERENCE MUST BE PUT INTO A STANDARD FORMAT , AS
16 REFERENCES ARE RETRIEVED BY CHARACTER COMPARISONS AND ONLY
17 EXACT MATCHES ARE RECOGNISED, IT IS THEREFORE ESSENTIAL THAT
18 THE FORMAT BE STRICTLY ADHERED TO.
19
20 THE LAYOUT OF A REFERENCE UNIT IS AS DISPLAYED BELOW
21
22 COLUMNS  1         2         3         4         5         6
23 1234567890123456789012345678901234567890123456789012345678901234567890
24 AUTHOR.1.        AUTHOR.2.        AUTHOR.3.
25 AUTHOR.4.        AUTHOR.5.        AUTHOR.6.
26 KEY.W.1.KEY.W.2.KEY.W.3.KEY.W.4.
27 KEY.W.5.KEY.W.6.KEY.W.7.KEY.W.8.
28 TITLE
29 TITLE
30 JOURNAL OR BOOK              VOL PAGES   YEAR
31 COMMENTS
32 COMMENTS
33 COMMENTS
34 COMMENTS
35
36 A BRIEF DESCRIPTION OF EACH TYPE IS GIVEN BELOW :
37
38 AUTHORS    THERE ARE SIX AUTHOR BLOCKS, EACH BLOCK CONSISTING
39           OF SIXTEEN CHARACTERS . THE RECOMMENDED FORM OF
40           LISTING IS SURNAME FOLLOWED BY INITIALS WITH A
41           FULL STOP AFTER THE SURNAME AND FOLLOWING EACH
42           INITIAL .
43
44 KEYWORDS   THERE ARE EIGHT KEYWORD BLOCKS WITH EACH BLOCK
45           CONSISTING OF EIGHT CHARACTERS .
46
47 TITLE      THERE ARE TWO BLOCKS FOR THE TITLE AND EACH BLOCK
48           CONSISTS OF SIXTY CHARACTERS . ANY CHARACTER
49           STRING CAN BE PLACED IN THIS SPACE AS NO SEARCHING
50           IS DONE ON THE TITLE .
51
52 JOURNAL    ONE BLOCK OF TWENTY EIGHT CHARACTERS ,NO SEARCHING
53           ON JOURNAL NAME .
54
55 VOLUME     ONE BLOCK OF FOUR CHARACTERS , NO SEARCHING ON
56           VOLUME
57
58 PAGE       ONE BLOCK OF EIGHT CHARACTERS NO SEARCHING ON PAGE
59
60 YEAR       ONE BLOCK OF FOUR CHARACTERS , IMPORTANT THAT YEAR
61           IS IN FULL FORM  EG. 1974 . IF WHEN SEARCHING TWO
62           REFERENCES HAVE EQUAL SCORES THEN THE MOST RECENT
63           (BY YEAR) IS OUTPUT FIRST .
64
65 COMMENTS   FOUR BLOCKS EACH OF SIXTY CHARACTERS , NO
66           SEACHING ON COMMENTS
67
68 FILES IN THE PACKAGE
69 ***** ** *** *******
70
71 THESE CAN BE DIVIDED INTO THREE SECTIONS :
72
73    (1) INPUT AND OUTPUT PROGRAMS
74    (2) DATA FILES
75    (3) FILE MAINTENANCE MACROS
76
77 ALL FILE NAMES ARE PREFIXED WITH RF AND FILE NAMES ARE
78 DESIGNED SO THAT THEIR FUNCTION SHOULD BE SELF EVIDENT .
79
80      ABBREVIATIONS AND DEFINITIONS ARE TABULATED BELOW :
81
82     AUTH   AUTHOR              BN    BINARY PROGRAM
83     COPY   COPY MACRO          CRD   CARD (BACKGROUND)
84     DATA   DATA                FI    FILE
85     IN     INPUT               INFO  INFORMATION FILE
86     INT    INTERACTIVE         KEY   KEYWORD
87     NUM    INDEX NUMBER        OUT   OUTPUT
88     RF     REFERENCE           RV    RETRIEVAL MACRO
89     SRC    SOURCE PROGRAM      ST    DUPLICATE FILE
```

```
 90
 91 THE PROGRAMS AND DATA FILES ARE TABULATED BELOW FOLLOWING
 92 THE NAME IS AN INDEX NUMBER WHICH IS THE ADDRESS THAT A LISTING
 93 OF THIS FILE CAN BE FOUND. IN THE COLUMN CONTAINING THE BINARY
 94 NAMES THE LISTINGS UNDER THESE INDEX NUMBERS ARE THOSE
 95 OBTAINED WHEN THE PROGRAMS ARE RUN , AND NOT A LISTING OF
 96 THE BINARY PROGRAM . IN SECTION 2 CONTAINING THE DATA FILES AN
 97 EXACT DUPLICATE OF THE MAIN DATA FILE IS ALSO KEPT FOR
 98 SECURITY PURPOSES .
 99
100 (1) PROGRAMS FOR INPUT AND OUTPUT
101     ******** *** ***** *** ******
102
103 PROGRAM FUNCTION    MACRO      SOURCE          BINARY
104 ******* ********    *****      PROGRAM         PROGRAM
105                                *******         *******
106
107 INPUT
108 -----
109 REFERENCES :
110    CARDS          RFCRDIN(2)  RFCRDINSRC(3)   RFCRDINBN(4)
111    INTERACTIVE    RFINTIN(5)  RFINTINSRC(6)   RFINTINBN(7)
112
113 KEYWORDS :        RFKEYIN(8)  RFKEYINSRC(9)   RFKEYINBN(10)
114
115 OUTPUT
116 ------
117 REFERENCES :
118    CARDS          RFCRDOUT(11) RFCRDOUTSRC(12) RFCRDOUTBN(13)
119    INTERACTIVE    RFINTOUT(14) RFINTOUTSRC(15) RFINTOUTBN(16)
120
121 AUTHORS :         RFAUTHOUT(17)RFAUTHOUTSRC(18)RFAUTHOUTBN(19)
122
123 DATA FILE :       RFFIOUT(20)  RFFIOUTSRC(21)  RFFIOUTBN(22)
124
125
126 (2) DATA FILES
127     **** *****
128
129    FUNCTION          FILENAME          DUPLICATE FILENAME
130    ********          ********          ********* *********
131
132    REFERENCES :      RFDATAFI(23)        RFDATAFIST
133
134    KEYWORDS :        RFKEYFI(24)         RFKEYFIST
135
136    INDEX NUMBER :    RFNUMFI(25)         RFNUMFIST
137
138
139 (3) FILE MAINTENANCE MACROS
140     **** *********** ******
141
142    FUNCTION          MACRO NAME
143    ********          ***** ****
144
145    HARDWARE OR       RFCOPY(26)
146    SOFTWARE FAULT
147    DURING INPUT RUN
148
149    RETRIEVES ALL     RFRV(27)
150    PROGRAMS IN THE
151    PACKAGE
152
153
154  BRIEF DESCRIPTIONS OF THE ABOVE MACROS AND PROGRAMS ARE
155  GIVEN BELOW . FULL DETAILS CANNOT BE GIVEN FOR RUNNING
156  THE PROGRAMS SO IT IS SUGGESTED THAT LISTINGS OBTAINED
157  FROM PREVIOUS RUNS ARE STUDIED
158
159     RFCRDIN
160     *******
161
162 TO RUN THIS PROGRAM THE DATA HAS TO BE IN A TEMPORARY INPUT
163 FILE (NAMED INFILE) . A CONTROL CARD IS PLACED AFTER EACH
164 REFERENCE UNIT , AND THIS CARD MUST HAVE THE LETTER
165  ' A ' IN ONE OF THE FIRST FOUR COLUMNS , IF THIS CARD IS
166 BLANK THEN IT IS TAKEN AS A TERMINATOR . THE MAIN DATA FILE
167 IS AMMENDED BY THE REFERENCES IN THIS TEMPORARY FILE AND THE
168 INDEX FILE IS INCREMENTED BY THE NUMBER OF REFERENCES IN THE
169 TEMPORARY INPUT FILE . A LISTING IS PRODUCED OF THE
170 TEMPORARY INPUT FILE AND ALSO OF THE AMMENDED SECTION OF THE
171 MAIN DATA FILE . THESE LISTINGS ENABLE CHECKING OF THE INPUT
172 DATA , IF ERRORS ARE FOUND IN THIS INPUT DATA THEN THE MAIN
173 DATA FILE MUST BE EDITED IN THE CONVENTIONAL WAY (DEPENDANT
174 ON THE OPERATING SYSTEM ) .
175
176
```

```
177      RFINTIN
178      *******
179
180  THIS PROGRAM FACILITATES THE INTERACTIVE INPUT OF REFERENCES
181  INTO THE MAIN DATA FILE . FULL INSTRUCTIONS ON THE INPUT OF
182  DATA CAN BE OBTAINED AT THE START OF THE PROGRAM ,
183  ALTERNATIVELY AN ABBREVIATED FORM IS USED . THE AUTHORS AND
184  KEYWORDS ARE INPUT ONE PER LINE AND A BLANK IS RECOGNISED
185  AS A TERMINATOR FOR EACH SET , THE COMMENTS ARE ALSO
186  TERMINATED BY A BLANK . AFTER INPUT OF A COMPLETE REFERENCE
187  THIS IS THEN DISPLAYED FOR CHECKING AND IF ERRORS ARE
188  PRESENT THEN AN EDITING OPTION IS OFFERED . EACH REFERENCE
189  IS THEN TRANSFERED TO THE MAIN DATA FILE .
190
191
192      RFKEYIN
193      *******
194
195  THIS PROGRAM EDITS THE KEYWORD FILE , IT HAS BEEN DESIGNED
196  TO BE USED INTERACTIVELY BUT CAN ALSO BE RUN IN A BACKGROUND
197  MODE . THE KEYWORD IS INPUT (UP TO EIGHT CHARACTERS )
198  FOLLOWED BY , ON A SEPARATE LINE , THE DEFINITION (UP TO
199  SIXTY CHARACTERS ) . THE PROGRAM THEN INSERTS THE NEW
200  KEYWORD INTO THE MAIN FILE ALPHABETICALLY . IF THE NEW
201  KEYWORD IS ALREADY ON THE FILE THEN THIS INFORMATION IS
202  DISPLAYED AT THE END OF THE RUN AND THE FILE IS NOT AMMENDED
203  BY THAT KEYWORD . THE PROGRAM IS TERMINATED BY A BLANK
204  KEYWORD .
205
206
207      RFCRDOUT
208      ********
209
210  THIS PROGRAM RETRIEVES REFERENCES FROM THE MAIN DATA FILE
211  BY MATCHING EITHER AUTHOR OR KEYWORDS (BUT NOT A MIXTURE ) .
212  A FILE IS CREATED WITH THE INPUT DATA CALLED INFILE , FOR
213  ONE SEARCH UP TO SIX AUTHOR OR EIGHT KEYWORDS ARE PERMITTED
214  (ONE PER LINE ), EACH INPUT DATA SET IS TERMINATED BY A
215  BLANK LINE . THE PROGRAM GIVES A WEIGHTING TO EACH WORD
216  SEARCHED FOR (SIX FOR THE FIRST AUTHOR , FIVE FOR THE SECOND
217  ETC. AND EIGHT FOR THE FIRST KEYWORD ETC. ). AFTER THE BLANK
218  TERMINATOR THERE IS A CARD CONTAINING THE MINIMUM SCORE
219  REQUIRED , THIS ENSURES THAT REFERENCES WHOSE SCORES ARE
220  BELOW THIS MINIMUM ARE NOT OUTPUT . THE NEXT CARD IS A
221  CONTROL CARD  1 TO FINISH , 2 FOR ANOTHER SEARCH , IF A
222  FURTHER SEARCH IS REQUIRED THEN THE DATA IS INPUT AS BEFORE
223
224
225      RFINTOUT
226      ********
227
228  THIS IS SIMILAR TO RFCRDOUT EXCEPT THAT IT IS RUN
229  INTERACTIVELY . THE SAME INPUT IS REQUIRED EXCEPT THAT NO
230  MINIMUM SCORE IS REQUIRED AS THE MATCHES ARE OUTPUT IN
231  BLOCKS OF FIVE REFERENCES AND AFTER EACH BLOCK AN OPTION IS
232  GIVEN TO FINISH . NO INPUT FILE IS REQUIRED AND THE FULL
233  INSTRUCTIONS , CONCERNING DATA INPUT , ARE GIVEN WHEN THE
234  PROGRAM IS RUN . NB. IT IS NOT PRACTICAL TO RUN SEARCHES
235  INTERACTIVELY WHEN LARGE DATA FILES ARE IN USE (IE. GREATER
236  THAN 1000 ENTRIES ) AS THE TAPE SPEED MAKES THE WAITING TIME
237  EXCESSIVE .
238
239
240      RFAUTHOUT
241      *********
242
243  THIS PROGRAM PRODUCES AN ALPHABETIC AUTHOR LIST WITH THE
244  INDEX NUMBERS OF OF THE REFERENCES OUTPUT NEXT TO THE
245  AUTHOR'S NAME .  THIS PROGRAM IS OPERABLE ONLY IN A
246  BACKGROUND MODE WITH LINEPRINTER OUTPUT .
247
248
249      RFFIOUT
250      *******
251
252  THIS PROGRAM OPERATES IN A BACKGROUND MODE ONLY AND PRODUCES
253  THE MAIN REFERENCE DATA FILE IN LINEPRINTER FORM .
254
255
256    DATA FILES
257    **** *****
258
259  EACH OF THE THREE MAIN DATA FILES , AS DESCRIBED BELOW HAVE
260  AN EXACT DUPLICATE FILE .  THIS DUPLICATE FILE IS MAINTAINED
261  FOR SECURITY REASONS AND IF THERE IS A BREAKDOWN WHEN INPUT
262  PROGRAMS ARE BEING RUN THEN THE DUPLICATE FILES ARE COPIED
263  INTO THE MAIN DATA FILES USING THE MACO RFCOPY .
264
```

```
265     RFDATAFI
266     ********
267
268 THIS IS THE MAIN DATA FILE WHICH CONTAINS ALL THE REFERENCES
269 EACH REFERENCE IS INDEXED AND THIS NUMBER APPEARS ON THE
270 FIRST LINE OF THE REFERENCE BLOCK . THE FILE IS APPENDED
271 WHEN EACH NEW REFERENCE IS ENTERED . EACH BLOCK CONSISTS OF
272 THIRTEEN LINES - 1ST LINE INDEX NUMBER , LINES 2-12 CONTAIN
273 THE REFERENCE AND THE 13TH LINE IS BLANK . THE FILE IS
274 TERMINATED BY **** STARTING IN COLUMN NINE ,FOLLOWED BY SIX
275 BLANK LINES AND THEN **** STARTING IN COLUMN ONE .
276
277
278     RFKEYFI
279     ********
280
281 THIS FILE CONTAINS AN ALPHABETIC LIST OF KEYWORDS AND THEIR
282 CORRESPONDING DEFINITIONS . EACH ENTRY CONSISTS OF EIGHT
283 COLUMNS FOR THE KEYWORD , ONE BLANK COLUMN AND SIXTY COLUMNS
284 FOR THE DEFINTION . THE FILE IS TERMINATED BY TWO BLANK
285 LINES AND A FURTHER LINE WITH **** BEGINING IN COLUMN ONE .
286
287
288     RFNUMFI
289     ********
290
291 THIS FILE CONTAINS THE INDEX NUMBER OF THE LAST ENTRY IN
292 THE MAIN DATA FILE . THIS IS THEN READ AND INCREMENTED
293 WHEN NEW REFERENCES ARE ENTERED .
294
295
296   FILE MAINTENANCE MACROS
297   **** ************ ******
298
299     RFCOPY
300     ******
301 THIS FILE IS RUN WHEN THERE HAS BEEN A SYSTEMS BREAKDOWN
302 ( EITHER HARDWARE OR SOFTWARE ) WHEN INPUTING REFERENCES OR
303 KEYWORDS . IF A BREAKDOWN DOES OCCUR THE FILES DO NOT
304 CONTAIN THE CORRECT TERMINATORS AND A SUBSEQUENT ATTEMPT TO
305 RUN A SEARCH PROGRAM OR INPUT FURTHER DATA WOULD BE
306 UNSUCCESSFUL . THE DUPLICATE DATA FILES ARE COPIED BACK INTO
307 THE MAIN DATA FILES .
308
309
310     RFRV
311     ****
312
313 THIS MACRO BRINGS ALL FILES INTO AN IMMEDIATE ACCESS
314 SITUATION , IE. RETRIEVING ALL THE FILES IN THE PACKAGE
315 (EXCEPT ITSELF ! ) .
316
317
318
319 THE FOLLOWING TERMS ARE DEFINED TO AVOID ANY MISUNDERSTANDING
320
321 MACRO -  A COMMAND STATEMENT WHICH IS EXPANDED BY THE
322       OPERATING SYSTEM INTO A SERIES OF BASIC COMMANDS . BY
323       THIS MEANS A COMPEX OPERATION WHICH REQUIRES MANY
324       COMMANDS CAN BE INITIATED BY A SINGLE STATEMENT.
325
326 PROGRAM- A SET OF INSTRUCTIONS IN A COMPUTER LANGUAGE FOR
327     .  THE PERFORMANCE OF SOME SYMBOL MANIPULATION
328       (E.G. COMPUTATION) ON A DIGITAL COMPUTER.
329
330 BINARY
331 PROGRAM- A PROGRAM WHICH HAS BEEN CONVERTED INTO A MACHINE
332         CODE.
333
334
335
```

```
RFCRDIN
*******
 0 CE OUTFILEA
 1 CE !
 2 IN !,T4444
 3 TC/****/
 4 PE,E
 5 4444
 6 ED RFDATAFI,RFDATAFI(+1),1
 7 ER !
 8 MZ 5000
 9 LO RFCRDINBN
10 AS *CR0,CARDFILE
11 AS *CR2,RFNUMFI
12 AS *LP2,OUTFILEA
13 AS *LP1,RFNUMFI(+1)
14 AS *CP0,RFDATAFI(APPEND)
15 EN 0
16 CE !
17 IN !,T4444
18 T/****/
19 IE          ****
20
21
22
23
24
25
26 E,E
27 4444
28 ED RFDATAFI,RFDATAFI(+1),1
29 ER RFDATAFI(-1)
30 ER RFDATAFI(-2)
31 ER RFNUMFI(-1)
32 ER !
33 CY RFDATAFI,RFDATAFIST(+1)
34 ER RFDATAFIST(-1)
35 CY RFNUMFI,RFNUMFIST(+1)
36 ER RFNUMFIST(-1)
37 ER RFNUMFI(-1)
38 LF CARDFILE,NU,*LP
39 ER CARDFILE
40 LF OUTFILEA,NU,*LP
41 ER OUTFILEA
42 ****
43
```

```
RFCRDINSRC
**********

 0        SHORTLIST
 1        PROGRAM (FXXX)
 2        INPUT 1 =CR0
 3        INPUT 3 =CR2
 4        OUTPUT 2 =LP0
 5        OUTPUT 4 =LP1
 6        OUTPUT 6=LP2
 7        OUTPUT 7 =CP0
 8        COMPRESS INTEGER AND LOGICAL
 9        EXTENDED DATA
10        TRACE 0
11        END
12        MASTER CARD
13 C      THIS PROGRAM READS INPUT FROM A FILE(CR0)
14 C      AND APPENDS A DATAFILE(CP0) WITH THE INPUT DATA
15 C      THEN INCREMENTS THE INDEX FILE(LP1) BY THE
16 C      NUMBER OF REFERENCES ENTERED
17 C      DATAFILE (AMMENDED PART) IS OUTPUT TO FILE(LP2)
18        DIMENSION TREAD(11,15)
19        DATA BLANK/4H    /
20        READ(3,100)JN
21   100  FORMAT(I5)
22  1000  DO 10 I=1,11
23        DO 11 J=1,15
24    11  TREAD(I,J)=BLANK
25    10  CONTINUE
26        DO 12 I=1,11
27    12  READ(1,101)(TREAD(I,J),J=1,15)
28   101  FORMAT(15A4)
29        JN=JN+1
30        WRITE(7,102)JN
31        WRITE(6,1020)JN
32  1020  FORMAT(1H ,I4)
```

```
      102 FORMAT(I4)
          DO 14 I=1,11
          WRITE(6,1010)(TREAD(I,J),J=1,15)
 1010 FORMAT(1H ,15A4))
   14 WRITE(7,101)(TREAD(I,J),J=1,15)
          WRITE(7,103)BLANK
          WRITE(6,1030)BLANK
 1030 FORMAT(1H ,A4)
          READ(1,103)LAB
  103 FORMAT(A4)
          M=4
          CALL COMP(M,LAB,1,BLANK,1)
          IF(M.EQ.4)GO TO 1001
          GO TO 1000
 1001 WRITE(4,104)JN
  104 FORMAT(1H ,I5)
          STOP
          END
          FINISH
****
```

```
RFINTIN
*******

CE !
IN !,T4444
TC/****/
PE,E
4444
ED RFDATAFI,RFDATAFI(+1),1
ER !
MZ 20000
LO RFINTINBN
RP AB,CM,LG
OL *CR0
AS *CR2,RFNUMFI
OL *CR1
OL *LPT
AS *LP1,RFNUMFI(+1)
AS *CP0,RFDATAFI(APPEND)
EN 0
CE !
IN !,T4444
T/****/
IZ          ****


E,E
4444
ED RFDATAFI,RFDATAFI(+1),1
ER RFDATAFI(-1)
ER RFDATAFI(-2)
CY RFDATAFI,RFDATAFIST(+1)
CY RFNUMFI,RFNUMFIST(+1)
ER RFDATAFIST(-1)
ER RFNUMFIST(-1)
ER !
****
```

```
      RFINTINSRC
      **********

      0        SHORTLIST (LP)
      1        PROGRAM  (FXXX)
      2        INPUT 1 = CR0
      3        INPUT 3 = CR2
      4        INPUT 5 = CR1
      5        OUTPUT 2= LP0
      6        OUTPUT 4= LP1
      7        OUTPUT 7=CP0
      8        COMPRESS INTEGER AND LOGICAL
      9        EXTENDED DATA
     10        TRACE 0
     11        END
     12        MASTER T
     13 C      THIS PROGRAM INTERACTIVELY ACCEPTS REFERENCES(CR1),
     14 C      LISTS THE INPUT REFERENCE FOR CHECKING AND IF REQUIRED
     15 C      PROVIDES EDITING OPTION.FULL INSTRUCTIONS FOR INPUT CAN BE
     16 C      OBTAINED BY TYPING 'I' AT START OF PROGRAM,OTHERVISE CONCI
SE
     17 C      INSTRUCTIONS ARE GIVEN.PROGRAM RECOGNISES BLANK LINE AS
     18 C      TERMINATOR FOR EACH DATA SET.DATAFILE IS APPENDED(CP0)
     19 C      WITH SATISFACTORY REFERENCES,AND INDEX FILE IS INCREMENTED
(CR2,LP1)
     20        DIMENSION TREAD(11,15),AI(6,4),RK(8,2),RCOM(4,15),
     21       1RTIT(2,15)
     22        DATA BLANK/4H    /
     23        IQX=0
     24        WRITE(2,260)
     25    260 FORMAT(1H0,' TYPE 1 FOR FULL INSTRUCTIONS ')
     26        READ(1,261)IQX
     27    261 FORMAT(I0)
     28        IDB=0
     29   1001 IE=0
     30   1000 IAU=1
     31        IKW=1
     32        ITI=1
     33        IJO=1
     34        ITO=1
     35        IVP=1
     36        ICO=1
     37        DO 300 I=1,15
     38        DO 301 J=1,11
     39    301 TREAD(I,J)=BLANK
     40    300 CONTINUE
     41    200 IF(IAU.EQ.0)GO TO 201
     42        DO 302 I=1,6
     43        DO 303 J=1,4
     44    303 AI(I,J)=BLANK
     45    302 CONTINUE
     46        IF(IQX.NE.1)GO TO 2600
     47        WRITE(2,100)
     48    100 FORMAT(1H0,' TYPE AUTHORS AS SLACK.J.A.  ',
     49       1' UP TO SIX AUTHORS, ONE PER LINE '//,' NOT MORE ',
     50       2' THAN 16 CHARACS PER AUTHOR ')
     51        GO TO 2601
     52   2600 WRITE(2,2620)
     53   2620 FORMAT(1H0,' AUTHORS ')
     54   2601 N=0
     55        II=0
     56        DO 1 I=1,6
     57        READ(1,101)(AI(I,J),J=1,4)
     58    101 FORMAT(4A4)
     59        M=4
     60        CALL COMP(M,AI(I,1),1,BLANK,1)
     61        IF(M.EQ.4)GO TO 2
     62      1 CONTINUE
     63      2 IL=1
     64        K=1
     65        DO 4 I=1,6
     66        DO 5 J=1,4
     67        TREAD(IL,K)=AI(I,J)
     68      5 K=K+1
     69        IF(I.NE.3)GO TO 4
     70        IL=2
     71        K=1
     72      4 CONTINUE
     73    201 IF(IKW.EQ.0)GO TO 202
     74        DO 304 I=1,2
     75        DO 305 J=1,8
     76    305 RK(J,I)=BLANK
     77    304 CONTINUE
     78        IF(IQX.NE.1)GO TO 2602
     79        WRITE(2,103)
     80    103 FORMAT(1H0,'TYPE KEY WORDS,UP TO 8 CHARACS PER WORD',
     81       1' ONE WORD PER LINE,'//,' UP TO 8 KEY WORDS')
     82        GO TO 2603
     83   2602 WRITE(2,2621)
     84   2621 FORMAT(1H0,' KEY WORDS ')
     85   2603 DO 6 I=1,8
     86        READ(1,104)(RK(I,J),J=1,2)
     87    104 FORMAT(2A4)
```

```
      M=4
      CALL COMP(M,RK(I,1),1,BLANK,1)
      IF(M.EQ.4)GO TO 7
    6 CONTINUE
    7 IL=3
      K=1
      DO 8 I=1,8
      DO 9 J=1,2
      TREAD(IL,K)=RK(I,J)
    9 K=K+1
      IF(I.NE.4)GO TO 8
      IL=4
      K=1
    8 CONTINUE
  202 IF(ITI.EQ.0)GO TO 203
      DO 306 I=1,2
      DO 307 J=1,15
  307 RTIT(I,J)=BLANK
  306 CONTINUE
      IF(IQX.NE.1)GO TO 2604
      WRITE(2,105)
  105 FORMAT(1H0,' TYPE TITLE ,NOT MORE THAN 2 LINES ,',
     1'60 CHARACS PER LINE')
      GO TO 2605
 2604 WRITE(2,2622)
 2622 FORMAT(1H0,' TITLE ')
 2605 DO 10 I=1,2
   10 READ(1,106)(RTIT(I,J),J=1,15)
  106 FORMAT(15A4)
      IL=5
      DO 11 I=1,2
      DO 12 J=1,15
   12 TREAD(IL,J)=RTIT(I,J)
   11 IL=IL+1
  203 IF(IJO.EQ.0)GO TO 204
      IF(IQX.NE.1)GO TO 2606
      WRITE(2,107)
  107 FORMAT(1H0,'TYPE JOURNAL OR BOOK,NOT MORE THAN 28',
     1' CHARACS')
      GO TO 2607
 2606 WRITE(2,2623)
 2623 FORMAT(1H0,' JOURNAL ')
 2607 READ(1,108)(TREAD(7,I),I=1,7)
  108 FORMAT(7A4)
  204 IF(IVP.EQ.0)GO TO 205
      IF(IQX.NE.1)GO TO 2608
      WRITE(2,109)
  109 FORMAT(1H0,' TYPE VOL(4 CHARACS),PAGE(8 CHARACS),',
     1' YEAR(4 CHARACS) EG 1976',/,' ON SEPERATE LINES')
      GO TO 2609
 2608 WRITE(2,2624)
 2624 FORMAT(1H0,' VPY ')
 2609 READ(1,110)TREAD(7,8)
  110 FORMAT(A4)
      READ(1,111)TREAD(7,9),TREAD(7,10)
  111 FORMAT(2A4)
      READ(1,112)TREAD(7,11)
  112 FORMAT(A4)
  205 IF(ICO.EQ.0)GO TO 207
      DO 308 I=1,15
      DO 309 J=1,4
  309 RCOM(J,I)=BLANK
  308 CONTINUE
      IF(IQX.NE.1)GO TO 2610
      WRITE(2,1113)
 1113 FORMAT(1H0,'TYPE COMMENTS,60 CHARACS PER LINE,',
     1' UP TO 4 LINES')
      GO TO 2611
 2610 WRITE(2,2625)
 2625 FORMAT(1H0,' COMMENTS ')
 2611 DO 13 I=1,4
      READ(1,113)(RCOM(I,J),J=1,15)
  113 FORMAT(15A4)
      M=4
      CALL COMP(M,RCOM(I,1),1,BLANK,1)
      IF(M.EQ.4)GO TO 140
   13 CONTINUE
  140 IL=8
      DO 14 I=1,4
      DO 15 J=1,15
   15 TREAD(IL,J)=RCOM(I,J)
   14 IL=IL+1
  115 FORMAT(1H ,I5)
  207 WRITE(2,217)
  217 FORMAT(1H0,'REF FILED AS BELOW:-')
      DO 16 I=1,2
   16 WRITE(2,117)(TREAD(I,J),J=1,12)
  117 FORMAT(1H ,12A4)
      DO 17 I=3,4
   17 WRITE(2,118)(TREAD(I,J),J=1,8)
  118 FORMAT(1H ,8A4)
```

```
179         DO 18 I=5,6
180      18 WRITE(2,119)(TREAD(I,J),J=1,15)
181     119 FORMAT(1H ,15A4)
182         WRITE(2,120)(TREAD(7,J),J=1,11)
183     120 FORMAT(1H ,10A4,A4)
184         DO 19 I=8,11
185      19 WRITE(2,119)(TREAD(I,J),J=1,15)
186         WRITE(2,124)
187     124 FORMAT(1H0,'TYPE 1 TO TRANSFER,0 TO EDIT,-1 TO CANCEL')
188         READ(1,125)IE
189     125 FORMAT(I0)
190         IF(IE)2001,20,21
191    2001 IE=1
192         GO TO 1000
193      20 WRITE(2,126)
194     126 FORMAT(1H0,' TYPE 1 TO EDIT ,0 OK-,AUTH,KW,TITLE,JOUR,'
195        1'VOL PAGE YEAR,COMMENTS; 6I0')
196         READ(1,127)IAU,IKW,ITI,IJO,IVP,ICO
197     127 FORMAT(6I0)
198         GO TO 200
199      21 IF(IDB-EQ-1)GO TO 310
200         IDB=1
201         READ(3,114)JN
202     114 FORMAT(I5)
203     310 JN=JN+1
204         WRITE(7,128)JN
205     128 FORMAT(I4)
206         DO 22 I=1,2
207      22 WRITE(7,1117)(TREAD(I,J),J=1,12)
208    1117 FORMAT(12A4)
209         DO 23 I=3,4
210      23 WRITE(7,1118)(TREAD(I,J),J=1,8)
211    1118 FORMAT(8A4)
212         DO 24 I=5,6
213      24 WRITE(7,1119)(TREAD(I,J),J=1,15)
214    1119 FORMAT(15A4)
215         WRITE(7,1120)(TREAD(7,J),J=1,11)
216    1120 FORMAT(11A4)
217         DO 25 I=8,11
218      25 WRITE(7,1119)(TREAD(I,J),J=1,15)
219         WRITE(2,123)
220     123 FORMAT(1H0,' TYPE 1 TO FINISH')
221         READ(1,125)IQ
222         IF(IQ-EQ-1)GO TO 1006
223         WRITE(7,135)BLANK
224     135 FORMAT(A4)
225         GO TO 1001
226    1006 WRITE(4,115)JN
227         WRITE(7,136)BLANK
228     136 FORMAT(A4)
229         STOP
230         END
231         FINISH
232 ****
```

```
RFKEYIN
*******

0 MZ 8000
1 LO RFKEYINBN
2 AS *CP0,RFKEYFIST(+1)
3 OL *CR0
4 AS *CR1,RFKEYFI
5 OL *LP0
6 OL *LP1
7 EN 0
8 CY RFKEYFIST,RFKEYFI(+1)
9 ER RFKEYFI(-1)
10 ER RFKEYFIST(-1)
11 ****
```

RFKEYINSRC
****

```
      SHORTLI (LP)
      PROGRAM  (FXXX)
      INPUT 1=CR0
      INPUT 3=CR1
      OUTPUT 4=CP0
      OUTPUT 2=LP0
      OUTPUT 5=LP1
      COMPRESS INTEGER AND LOGICAL
      EXTENDED DATA
      TRACE 0
      END
      MASTER KEYX
C     THIS PROGRAM EDITS A KEYWORD FILE (CR1)
C     ALPHABETICALLY AND INSERTS NEW KEYWORDS AND DEFINITIONS
C     RUNS CHECK TO ENSURE THAT KEYWORD IS NOT ALREADY
C     ON FILE. RECOGNISES BLANK AS TERMINATOR FOR DATA SET.
C     FULL INSTRUCTIONS FOR INPUT OF DATA ARE GIVEN IF USED
C     INTERACTIVELY.WRITES TO NEW FILE(CP0) AT END OF RUN
      COMMON KEYIN(30,2),IDEF(30,15),KEY(150,2),KEYDEF(150,15),
     1ISCO(150),IBUK(2),IBUD(15)
      DIMENSION LETTR(30)
      DATA LETTR/1H-,1HZ,1HY,1HX,1HW,1HV,1HU,1HT,1HS,
     11HR,1HQ,1HP,1HO,1HN,1HM,1HL,1HK,1HJ,1HI,1HH,1HG,
     21HF,1HE,1HD,1HC,1HB,1HA,1H/,1H.,1H /
      DATA IBLANK/4H    /
      DO 10 I=1,150
      ISCO(I)=0
      DO 11 J=1,2
   11 KEY(I,J)=IBLANK
      DO 12 J=1,15
   12 KEYDEF(I,J)=IBLANK
   10 CONTINUE
      DO 13 I=1,30
      DO 14 J=1,2
   14 KEYIN(I,J)=IBLANK
      DO 15 J=1,15
   15 IDEF(I,J)=IBLANK
   13 CONTINUE
      DO 16 I=1,15
   16 IBUD(I)=IBLANK
      IBUK(1)=IBLANK
      IBUK(2)=IBLANK
      DO 150 I=1,150
      READ(3,170)(KEY(I,K),K=1,2),(KEYDEF(I,K),K=1,15)
  170 FORMAT(2A4,1X,15A4)
      M=4
      CALL COMP(M,KEY(I,1),1,IBLANK,1)
      IF(M.NE.4)GO TO 149
      INE=1
      GO TO 151
  149 DO 148 J=1,2
      DO 147 NA=1,30
      M=1
      CALL COMP(M,KEY(I,1),J,LETTR(NA),1)
      IF(M.NE.1)GO TO 147
      ISCO(I)=ISCO(I)+NA
      IF(J.EQ.1)ISCO(I)=ISCO(I)*100
      GO TO 148
  147 CONTINUE
  148 CONTINUE
  150 CONTINUE
  151 DO 20 I=1,30
      WRITE(2,110)
  110 FORMAT(1H0,' TYPE KEYWORD ')
      READ(1,100)(KEYIN(I,K),K=1,2)
  100 FORMAT(2A4)
      M=4
      CALL COMP(M,KEYIN(I,1),1,IBLANK,1)
      IF(M.NE.4)GO TO 30
      II=I-1
      GO TO 21
   30 WRITE(2,111)
  111 FORMAT(1H0,' TYPE DEF 15A4 ')
      READ(1,101)(IDEF(I,K),K=1,15)
  101 FORMAT(15A4)
   20 CONTINUE
   21 DO 330 I=1,II
      IBS=0
      DO 32 M=1,2
      DO 33 K=1,30
      N=1
      CALL COMP(N,KEYIN(I,1),M,LETTR(K),1)
      IF(N.NE.1)GO TO 33
      IBS=IBS+K
      IF(M.EQ.1)IBS=IBS*100
      GO TO 32
   33 CONTINUE
   32 CONTINUE
```

```
      39 DO 40 IJ=1,INE
         IF(ISCO(IJ)-IBS)41,50,40
      41 CALL ISORT(INE,IJ,IBS,I)
         GO TO 330
      50 IBT=0
         IBQ=0
         DO 54 M=3,4
         DO 55 NA=1,29
         N=1
         CALL COMP(N,KEYIN(1,1),M,LETTR(NA),1)
         IF(N.NE.1)GO TO 55
         IBT=IBT+NA
         IF(M.EQ.3)IBT=IBT*100
         GO TO 54
      55 CONTINUE
      54 CONTINUE
         DO 56 M=3,4
         DO 550 NA=1,29
         N=1
         CALL COMP(N,KEY(IJ,1),M,LETTR(NA),1)
         IF(N.NE.1)GO TO 550
         IBQ=IBQ+NA
         IF(M.EQ.3)IBQ=IBQ*100
         GO TO 56
     550 CONTINUE
      56 CONTINUE
         IF(IBQ-IBT)41,58,40
      58 DO 71 IK=1,4
         ICO=0
         ICI=0
         DO 73 IL=1,29
         M=1
         CALL COMP(M,KEYIN(1,2),IK,LETTR(IL),1)
         IF(M.NE.1)GO TO 73
         ICO=IL
         GO TO 80
      73 CONTINUE
      80 DO 74 IL=1,29
         M=1
         CALL COMP(M,KEY(IJ,2),IK,LETTR(IL),1)
         IF(M.NE.1)GO TO 74
         ICI=IL
         GO TO 81
      74 CONTINUE
      81 IF(ICI-ICO)41,71,40
      71 CONTINUE
         GO TO 119
      40 CONTINUE
     119 WRITE(2,120)(KEYIN(1,K),K=1,2)
     120 FORMAT(1H0,' KEYWORD ',2A4,' IS ALREADY ON FILE ')
     330 CONTINUE
         DO 90 I=1,INE
      90 WRITE(4,130)(KEY(I,K),K=1,2),(KEYDEF(I,K),K=1,15)
     130 FORMAT(2A4,1X,15A4)
         WRITE(4,391)IBLANK
     391 FORMAT(A4)
         STOP
         END
         SUBROUTINE ISORT(INE,IJ,IBS,I)
         COMMON KEYIN(30,2),IDEF(30,15),KEY(150,2),KEYDEF(150,15),
        1ISCO(150),IBUK(2),IBUD(15)
         INE=INE+1
         DO 90 KA=IJ,INE
         IBF=ISCO(KA)
         ISCO(KA)=IBS
         IBS=IBF
         DO 91 KB=1,2
         IBUK(KB)=KEY(KA,KB)
         KEY(KA,KB)=KEYIN(I,KB)
      91 KEYIN(I,KB)=IBUK(KB)
         DO 92 KC=1,15
         IBUD(KC)=KEYDEF(KA,KC)
         KEYDEF(KA,KC)=IDEF(I,KC)
      92 IDEF(I,KC)=IBUD(KC)
      90 CONTINUE
         RETURN
         END
         FINISH
****
```

RFCRDOUT
****

```
 0 MZ 35000
 1 CE !
 2 LO RFCRDOUTBN
 3 AS *CR0,INFILE
 4 AS *LP1,OUTFILE
 5 AS *CR1,RFDATAFI
 6 AS *MT0,!(WRITE)
 7 EN 0
 8 ER !
 9 LF OUTFILE,*LP
10 ER OUTFILE
11 ER INFILE
12 ****
```

RFCRDOUTSRC
****

```
 0        SHORTLIST (LP)
 1        PROGRAM  (FXXX)
 2        INPUT 1 =CR0
 3        INPUT 3 = CR1
 4        OUTPUT 2 =LP1
 5        OUTPUT 6 =LP0
 6        USE 7 =MT0
 7        COMPRESS INTEGER AND LOGICAL
 8        EXTENDED DATA
 9        TRACE 0
10        END
11        MASTER SEARCH
12 C      THIS PROGRAM RETRIEVES REFERENCESFROM A FILE(CR1)
13 C      AND WRITES THEM ONTO A WORKFILE(MT0).THEY ARE THEN
14 C      READ BACK IN AGAIN AND MATCHED AGAINST THE TEST
15 C      FILE(CR1),EACH REFERENCE IS GIVEN A SCORE,DEPENDENT
16 C      ON THE NUMBER OF MATCHES,AND THEY ARE PUT IN ORDER,WHEN A
17 C      SEARCH IS COMPLETED A FILE IS WRITTEN TO(LP1)WHICH
18 C      CONTAINS ALL THE MATCHES UP TO A MINMUM SCORE WHICH IS
19 C      READ IN WITH THE TEST FILE,SEARCHES AREUNDERTAKEN
20 C      ON AKEYWORD OR AUTHOR BASIS AND CANNOT BE MIXED
21 C      WITHIN ONE RUN.PROGRAM RECOGNISES BLANK CARD AS TERMINATOR
22 C      FOR DATA SET.
23        COMMON IREFIN(12,16),IREFMO(150,12,16),IRKW(8,2),
24       1IRAUT(6,4),IRETF(12,16),ISCOA(150),IREF(12,16)
25        DATA IBLANK/4H    /
26        DO 800 I=1,12
27        DO 801 J=1,16
28   801  IREF(I,J)=IBLANK
29   800  CONTINUE
30        IREF(8,11)=0
31        REWIND 7
32        DO 803 I=1,5000
33        READ(3,804)IBO
34   804  FORMAT(A4)
35        READ(3,804)IREF(1,1)
36        M=4
37        CALL COMP(M,IREF(1,1),1,IBLANK,1)
38        IF(M.EQ.4)GO TO 860
39        WRITE(7)IREF(1,1)
40        DO 806 J=2,7
41        READ(3,805)(IREF(J,K),K=1,16)
42   806  WRITE(7)(IREF(J,K),K=1,16)
43   805  FORMAT(16A4)
44        READ(3,807)(IREF(8,K),K=1,11)
45   807  FORMAT(10A4,I4)
46        WRITE(7)(IREF(8,K),K=1,11)
47        DO 808 J=9,12
48        READ(3,805)(IREF(J,K),K=1,16)
49   808  WRITE(7)(IREF(J,K),K=1,16)
50        GO TO 803
51   860  ITT=I-1
52        END FILE 7
53        WRITE(2,1110)ITT
54  1110  FORMAT(1H0,' NUMBER OF REFS ON FILE = ',I4)
55        GO TO 2001
56   803  CONTINUE
57  2001  REWIND 7
58        DO 2 J=1,12
59        DO 3 K=1,16
60        IRETF(J,K)=IBLANK
61        IREFIN(J,K)=IBLANK
```

```
      DO 1 I=1,150
      IREFHO(I,J,K)=IBLANK
      ISCOA(I)=0
    1 IREFHO(I,8,11)=0
    3 CONTINUE
    2 CONTINUE
      IRETF(8,11)=0
      IREFIN(8,11)=0
      ILL=0
      DO 4 I=1,2
      DO 5 J=1,8
    5 IRKW(J,I)=IBLANK
    4 CONTINUE
      DO 6 I=1,4
      DO 7 J=1,6
    7 IRAUT(J,I)=IBLANK
    6 CONTINUE
      READ(1,101)IAS
  101 FORMAT(I0)
      IF(IAS.NE.1)GO TO 200
      CALL AUTH(IBLANK,ITT,ILL)
      GO TO 900
  200 CALL KEYW(IBLANK,ITT,ILL)
  900 READ(1,208)IM
  208 FORMAT(I0)
      IF(IM.NE.2)GO TO 2000
      GO TO 2001
 2000 STOP
 2001 END
      SUBROUTINE AUTH(IBLANK,ITT,ILL)
      COMMON IREFIN(12,16),IREFHO(150,12,16),IRKW(8,2),
     1IRAUT(6,4),IRETF(12,16),ISCOA(150),IREF(12,16)
      ILL=0
      IHO=0
      DO 8 I=1,6
      READ(1,103)(IRAUT(I,J),J=1,4)
  103 FORMAT(4A4)
      M=4
      CALL COMP(M,IRAUT(I,1),1,IBLANK,1)
      IF(M.EQ.4)GO TO 910
      ITA=I
    8 CONTINUE
  910 READ(1,104)ISCMIN
  104 FORMAT(I0)
    9 IIE=0
      CALL READIN(IIE,ILL,IBLANK,ITT)
      IF(IIE.EQ.1)GO TO 616
      ISCORE=0
      DO 14 I=1,ITA
      DO 15 K=2,3
      DO 16 KI=1,13,4
      IM=0
      DO 17 KJ=1,4
      KK=KJ-1
      M=4
      CALL COMP(M,IRAUT(I,KJ),1,IREFIN(K,KI+KK),1)
      IM=IM+M
   17 CONTINUE
      IF(IM.NE.16)GO TO 16
      ISCORE=ISCORE+(7-I)
      GO TO 14
   16 CONTINUE
   15 CONTINUE
   14 CONTINUE
      IF(ISCORE.LT.ISCMIN.OR.ISCORE.EQ.0)GO TO 9
      CALL ISORT(IHO,ISCORE)
      GO TO 9
  616 WRITE(2,207)
  207 FORMAT(1H0,'AUTHORS SEARCHED ',2X,'WEIGHTING',
     1/,17(1H=),3X,9(1H=))
      DO 20 JJ=1,ITA
      IWGTA=7-JJ
   20 WRITE(2,210)(IRAUT(JJ,JC),JC=1,4),IWGTA
  210 FORMAT(1H ,4A4,5X,I1)
      WRITE(2,216)ISCMIN
  216 FORMAT(1H0,'MINIMUM SCORE = ',I2,/)
      CALL IOUT(IHO)
      RETURN
      END
      SUBROUTINE KEYW(IBLANK,ITT,ILL)
      COMMON IREFIN(12,16),IREFHO(150,12,16),IRKW(8,2),
     1IRAUT(6,4),IRETF(12,16),ISCOA(150),IREF(12,16)
      IHO=0
      ILL=0
      DO 30 I=1,8
      READ(1,110)IRKW(I,1),IRKW(I,2)
  110 FORMAT(2A4)
      M=4
      CALL COMP(M,IRKW(I,1),1,IBLANK,1)
```

```
      IF (M.EQ.4)GO TO 310
      ITA=I
   30 CONTINUE
  310 READ(1,104)ISCMIN
  104 FORMAT(I0)
   31 IIE=0
      CALL READIN(IIE,ILL,IBLANK,ITT)
      IF(IIE.EQ.1)GO TO 618
      ISCORE=0
      DO 36 I=1,ITA
      DO 37 K=4,5
      DO 38 KI=1,7,2
      IM=0
      DO 39 KJ=1,2
      KK=KJ-1
      M=4
      CALL COMP(M,IRKW(L,KJ),1,IREFIN(K,KI+KK),1)
      IM=IM+M
   39 CONTINUE
      IF(IM.NE.8)GO TO 38
      ISCORE=ISCORE+(9-I)
      GO TO 36
   38 CONTINUE
   37 CONTINUE
   36 CONTINUE
      IF(ISCORE.EQ.0.OR.ISCORE.LT.ISCMIN)GO TO 31
      CALL ISORT(IHO,ISCORE)
      GO TO 31
  618 WRITE(2,211)
  211 FORMAT(1HO,'KEYWORDS SEARCHED  WEIGHTING',
     1/18(1H*),2X,9(1H*),/)
      DO 21 JJ=1,ITA
      IWGTA=9-JJ
   21 WRITE(2,212)(IRKW(JJ,JC),JC=1,2),IWGTA
  212 FORMAT(1H ,2A4,15X,I1)
      WRITE(2,217)ISCMIN
  217 FORMAT(1HO,'MINIMUM SCORE = ',I2,/)
      CALL IOUT(IHO)
      RETURN
      END
      SUBROUTINE READIN(IIE,ILL,IBLANK,ITT)
      COMMON IREFIN(12,16),IREFHO(150,12,16),IRKW(8,2),
     1IRAUT(6,4),IRETF(12,16),ISCOA(150),IREF(12,16)
      ILL=ILL+1
      IF(ILL.GT.ITT)GO TO 6131
      READ(7)IREFIN(1,1)
      DO 10 I=2,7
   10 READ(7)(IREFIN(I,J),J=1,16)
      READ(7)(IREFIN(8,J),J=1,11)
      DO 11 I=9,12
   11 READ(7)(IREFIN(I,J),J=1,16)
      GO TO 613
 6131 IIE=1
  613 RETURN
      END
      SUBROUTINE ISORT(IHO,ISCORE)
      COMMON IREFIN(12,16),IREFHO(150,12,16),IRKW(8,2),
     1IRAUT(6,4),IRETF(12,16),ISCOA(150),IREF(12,16)
      IHO=IHO+1
      IF(IHO.GT.150)IHO=150
      DO 18 I=1,IHO
      II=I
      IF(ISCORE-ISCOA(I))18,19,20
   19 IF(IREFIN(8,11)-IREFHO(I,8,11))18,20,20
   18 CONTINUE
   20 DO 21 J=II,IHO
      ISCOAT=ISCOA(J)
      ISCOA(J)=ISCORE
      ISCORE=ISCOAT
      DO 22 JI=1,12
      DO 23 JN=1,16
      IRETF(JI,JN)=IREFHO(J,JI,JN)
      IREFHO(J,JI,JN)=IREFIN(JI,JN)
   23 IREFIN(JI,JN)=IRETF(JI,JN)
   22 CONTINUE
   21 CONTINUE
      RETURN
      END
      SUBROUTINE IOUT(IHO)
      COMMON IREFIN(12,16),IREFHO(150,12,16),IRKW(8,2),
     1IRAUT(6,4),IRETF(12,16),ISCOA(150),IREF(12,16)
      IF(IHO.EQ.0)GO TO 251
      DO 25 I=1,IHO,2
      IF(ISCOA(I+1).EQ.0)GO TO 260
      WRITE(2,1007)ISCOA(I),ISCOA(I+1)
 1007 FORMAT(1H ,'SCORE = ',I2,51X,'SCORE = ',I2)
      DO 27 J=1,7
      WRITE(2,106)(IREFHO(I,J,JI),JI=1,15),
     1(IREFHO(I+1,J,JI),JI=1,15)
```

```
240    106 FORMAT(1H ,30A4)
241     27 CONTINUE
242        WRITE(2,115)(IREFHO(I,8,JI),JI=1,11),
243       1(IREFHO(I+1,8,JI),JI=1,11)
244    115 FORMAT(1H ,10A4,I4,16X,10A4,I4)
245        DO 116 J=9,12
246    116 WRITE(2,106)(IREFHO(I,J,JI),JI=1,15),
247       1(IREFHO(I+1,J,JI),JI=1,15)
248        GO TO 25
249    260 WRITE(2,1008)ISCOA(I)
250   1008 FORMAT(1H ,'SCORE = ',I2)
251        DO 261 J=1,7
252    261 WRITE(2,1009)(IREFHO(I,J,JI),JI=1,15)
253   1009 FORMAT(1H ,15A4)
254        WRITE(2,1010)(IREFHO(I,8,JI),JI=1,11)
255   1010 FORMAT(1H ,10A4,I4)
256        DO 262 J=9,12
257    262 WRITE(2,1009)(IREFHO(I,J,JI),JI=1,15)
258        GO TO 29
259     25 CONTINUE
260        GO TO 29
261    251 WRITE(2,118)
262    118 FORMAT(1H0,' NO MATCHES ')
263     29 RETURN
264        END
265        FINISH
266 ****
267
```

RFINTOUT
****

```
0 MZ 35000
1 CE !
2 LQ RFINTOUTBN
3 AS =CR1,RFDATAF1
4 AS =MT0,!(WRITE)
5 OL =LP1
6 OL =CR0
7 RP AB,CM,LG
8 EN 0
9 ER !
10 ****
11
```

RFINTOUTSRC
****

```
0       SHORTLIST (LP)
1       PROGRAM  (FXXX)
2       INPUT 1 =CR0
3       INPUT 3 = CR1
4       OUTPUT 2 =LP1
5       OUTPUT 6 =LP0
6       USE 7 =MT0
7       COMPRESS INTEGER AND LOGICAL
8       EXTENDED DATA
9       TRACE 0
10      END
11      MASTER SEARCH
12 C    THIS PROGRAM RETRIEVES REFERENCESFROM A FILE(CR1)
13 C    AND WRITES THEM ONTO A WORKFILE(MT0).THEY ARE THEN
14 C    READ BACK IN AGAIN AND MATCHED AGAINST THE TEST
15 C    FILE(CR0),EACH REFERENCE IS GIVEN A SCORE,DEPENDENT
16 C    ON THE NUMBER OF MATCHES,AND THEY ARE PUT IN ORDER,WHEN A
17 C    SEARCH IS COMPLETED A FILE IS WRITTEN TO(LP1)WHICH
18 C    CONTAINS ALL THE MATCHES UP TO A MINMUM SCORE WHICH IS
19 C    READ IN WITH THE TEST FILE,SEARCHES AREUNDERTAKEN
20 C    ON AKEYWORD OR AUTHOR BASIS AND CANNOT BE MIXED
21 C    WITHIN ONE RUN.PROGRAM RECOGNISES BLANK CARD AS TERMINATOR
22 C    FOR DATA SET.
23      COMMON IREFIN(12,16),IREFHO(150,12,16),IRKW(8,2),
24     1IRAUT(6,4),IRETF(12,16),ISCOA(150),IREF(12,16)
25      DATA IBLANK/4H    /
26      DO 800 I=1,12
27      DO 801 J=1,16
28  801 IREF(I,J)=IBLANK
29  800 CONTINUE
30      IREF(3,11)=0
31      REWIND 7
32      DO 803 I=1,5000
33      READ(3,804)IBO
34  804 FORMAT(A4)
35      READ(3,804)IREF(1,1)
36      M=4
37      CALL COMP(M,IREF(1,1),1,IBLANK,1)
38      IF(M.EQ.4)GO TO 860
```

```
      WRITE(7)(IREF(1,I)
      DO 806 J=2,7
      READ(3,805)(IREF(J,K),K=1,16)
  806 WRITE(7)(IREF(J,K),K=1,16)
  805 FORMAT(16A4)
      READ(3,807)(IREF(8,K),K=1,11)
  807 FORMAT(10A4,I4)
      WRITE(7)(IREF(8,K),K=1,11)
      DO 808 J=9,12
      READ(3,805)(IREF(J,K),K=1,16)
  808 WRITE(7)(IREF(J,K),K=1,16)
      GO TO 803
  860 ITT=I-1
      END FILE 7
      WRITE(2,1110)ITT
 1110 FORMAT(1H0,' NUMBER OF REFS ON FILE = ',I4)
      GO TO 2001
  803 CONTINUE
 2001 REWIND 7
      DO 2 J=1,12
      DO 3 K=1,16
      IRETF(J,K)=IBLANK
      IREFIN(J,K)=IBLANK
      DO 1 I=1,150
      IREFHO(I,J,K)=IBLANK
      ISCOA(I)=0
    1 IREFHO(I,8,11)=0
    3 CONTINUE
    2 CONTINUE
      IRETF(8,11)=0
      IREFIN(8,11)=0
      ILL=0
      DO 4 I=1,2
      DO 5 J=1,8
    5 IRKW(J,I)=IBLANK
    4 CONTINUE
      DO 6 I=1,4
      DO 7 J=1,6
    7 IRAUT(J,I)=IBLANK
    6 CONTINUE
      READ(1,101)IAS
  101 FORMAT(I0)
      IF(IAS.NE.1)GO TO 200
      CALL AUTH(IBLANK,ITT,ILL)
      GO TO 900
  200 CALL KEYW(IBLANK,ITT,ILL)
  900 READ(1,208)IM
  208 FORMAT(I0)
      IF(IM.NE.2)GO TO 2000
      GO TO 2001
 2000 STOP
      END
      SUBROUTINE AUTH(IBLANK,ITT,ILL)
      COMMON IREFIN(12,16),IREFHO(150,12,16),IRKW(8,2),
     1IRAUT(6,4),IRETF(12,16),ISCOA(150),IREF(12,16)
      ILL=0
      IHO=0
      DO 8 I=1,6
      READ(1,103)(IRAUT(I,J),J=1,4)
  103 FORMAT(4A4)
      M=4
      CALL COMP(M,IRAUT(I,1),1,IBLANK,1)
      IF(M.EQ.4)GO TO 910
      ITA=I
    8 CONTINUE
  910 READ(1,104)ISCMIN
  104 FORMAT(I0)
    9 IIE=0
      CALL READIN(IIE,ILL,IBLANK,ITT)
      IF(IIE.EQ.1)GO TO 616
      ISCORE=0
      DO 14 I=1,ITA
      DO 15 K=2,3
      DO 16 KI=1,10,4
      IM=0
      DO 17 KJ=1,4
      KK=KJ-1
      M=4
      CALL COMP(M,IRAUT(I,KJ),1,IREFIN(K,KI+KK),1)
      IM=IM+M
   17 CONTINUE
      IF(IM.NE.16)GO TO 16
      ISCORE=ISCORE+(7-I)
      GO TO 14
   16 CONTINUE
   15 CONTINUE
   14 CONTINUE
      IF(ISCORE.LT.ISCMIN.OR.ISCORE.EQ.0)GO TO 9
      CALL ISORT(IHO,ISCORE)
      GO TO 9
```

```
      616 WRITE(2,207)
      207 FORMAT(1H0,'AUTHORS SEARCHED ',2X,'WEIGHTING',
         1/,17(1H=),3X,9(1H=))
          DO 20 JJ=1,ITA
          IWGTA=7-JJ
       20 WRITE(2,210)(IRAUT(JJ,JC),JC=1,4),IWGTA
      210 FORMAT(1H ,4A4,5X,I1)
          WRITE(2,216)ISCMIN
      216 FORMAT(1H0,'MINIMUM SCORE = ',I2,/)
          CALL IOUT(IHO)
          RETURN
          END
          SUBROUTINE KEYW(IBLANK,ITT,ILL)
          COMMON IREFIN(12,16),IREFHO(150,12,16),IRKW(8,2),
         1IRAUT(6,4),IRETF(12,16),ISCOA(150),IREF(12,16)
          IHO=0
          ILL=0
          DO 30 I=1,8
          READ(1,110)IRKW(I,1),IRKW(I,2)
      110 FORMAT(2A4)
          M=4
          CALL COMP(M,IRKW(I,1),1,IBLANK,1)
          IF (M.EQ.4)GO TO 310
          ITA=I
       30 CONTINUE
      310 READ(1,104)ISCMIN
      104 FORMAT(I0)
       31 IIE=0
          CALL READIN(IIE,ILL,IBLANK,ITT)
          IF(IIE.EQ.1)GO TO 618
          ISCORE=0
          DO 36 I=1,ITA
          DO 37 K=4,5
          DO 38 KI=1,7,2
          IM=0
          DO 39 KJ=1,2
          KK=KJ-1
          M=4
          CALL COMP(M,IRKW(L,KJ),1,IREFIN(K,KI+KK),1)
          IM=IM+M
       39 CONTINUE
          IF(IM.NE.8)GO TO 38
          ISCORE=ISCORE+(9-I)
          GO TO 36
       38 CONTINUE
       37 CONTINUE
       36 CONTINUE
          IF(ISCORE.EQ.0.OR.ISCORE.LT.ISCMIN)GO TO 31
          CALL ISORT(IHO,ISCORE)
          GO TO 31
      618 WRITE(2,211)
      211 FORMAT(1H0,'KEYWORDS SEARCHED  WEIGHTING',
         1/18(1H=),2X,9(1H=),/)
          DO 21 JJ=1,ITA
          IWGTA=9-JJ
       21 WRITE(2,212)(IRKW(JJ,JC),JC=1,2),IWGTA
      212 FORMAT(1H ,2A4,15X,I1)
          WRITE(2,217)ISCMIN
      217 FORMAT(1H0,'MINIMUM SCORE = ',I2,/)
          CALL IOUT(IHO)
          RETURN
          END
          SUBROUTINE READIN(IIE,ILL,IBLANK,ITT)
          COMMON IREFIN(12,16),IREFHO(150,12,16),IRKW(8,2),
         1IRAUT(6,4),IRETF(12,16),ISCOA(150),IREF(12,16)
          ILL=ILL+1
          IF(ILL.GT.ITT)GO TO 6131
          READ(7)IREFIN(1,1)
          DO 10 I=2,7
       10 READ(7)(IREFIN(I,J),J=1,16)
          READ(7)(IREFIN(8,J),J=1,11)
          DO 11 I=9,12
       11 READ(7)(IREFIN(I,J),J=1,16)
          GO TO 613
     6131 IIE=1
      613 RETURN
          END
          SUBROUTINE ISORT(IHO,ISCORE)
          COMMON IREFIN(12,16),IREFHO(150,12,16),IRKW(8,2),
         1IRAUT(6,4),IRETF(12,16),ISCOA(150),IREF(12,16)
          IHO=IHO+1
          IF(IHO.GT.150)IHO=150
          DO 18 I=1,IHO
          II=I
          IF(ISCORE-ISCOA(I))18,19,20
       19 IF(IREFIN(8,11)-IREFHO(I,8,11))18,20,20
       18 CONTINUE
       20 DO 21 J=II,IHO
          ISCOAT=ISCOA(J)
```

```
      ISCOA(J)=ISCORE
      ISCORE=ISCOAT
      DO 22 JI=1,12
      DO 23 JN=1,16
      IRETF(JI,JN)=IREFHO(J,JI,JN)
      IREFHO(J,JI,JN)=IREFIN(JI,JN)
   23 IREFIN(JI,JN)=IRETF(JI,JN)
   22 CONTINUE
   21 CONTINUE
      RETURN
      END
      SUBROUTINE IOUT(IHO)
      COMMON IREFIN(12,16),IREFHO(150,12,16),IRKV(8,2),
     1IRAUT(6,4),IRETF(12,16),ISCOA(150),IREF(12,16)
      IF(IHO.EQ.0)GO TO 251
      DO 25 I=1,IHO,2
      IF(ISCOA(I+1).EQ.0)GO TO 260
      WRITE(2,1007)ISCOA(I),ISCOA(I+1)
 1007 FORMAT(1H ,'SCORE = ',I2,51X,'SCORE = ',I2)
      DO 27 J=1,7
      WRITE(2,106)(IREFHO(I,J,JI),JI=1,15),
     1(IREFHO(I+1,J,JI),JI=1,15)
  106 FORMAT(1H ,30A4)
   27 CONTINUE
      WRITE(2,115)(IREFHO(I,8,JI),JI=1,11),
     1(IREFHO(I+1,8,JI),JI=1,11)
  115 FORMAT(1H ,10A4,I4,16X,10A4,I4)
      DO 116 J=9,12
  116 WRITE(2,106)(IREFHO(I,J,JI),JI=1,15),
     1(IREFHO(I+1,J,JI),JI=1,15)
      GO TO 25
  260 WRITE(2,1008)ISCOA(I)
 1008 FORMAT(1H ,'SCORE = ',I2)
      DO 261 J=1,7
  261 WRITE(2,1009)(IREFHO(I,J,JI),JI=1,15)
 1009 FORMAT(1H ,15A4)
      WRITE(2,1010)(IREFHO(I,8,JI),JI=1,11)
 1010 FORMAT(1H ,10A4,I4)
      DO 262 J=9,12
  262 WRITE(2,1009)(IREFHO(I,J,JI),JI=1,15)
      GO TO 29
   25 CONTINUE
      GO TO 29
  251 WRITE(2,118)
  118 FORMAT(1HO,' NO MATCHES ')
   29 RETURN
      END
      FINISH
****
```

RFAUTHOUT
****

```
0 MZ 47000
1 LO RFAUTHOUTBN
2 AS *CR0,RFDATAFI
3 AS *LP0,OUTFILEC
4 EN 0
5 LF OUTFILEC,*LP
6 ER OUTFILEC
7 ****
```

RFAUTHOUTSRC
****

```
0         SHORTLIST (LP)
1        PROGRAM  (FXXX)
2        INPUT 3=CR0
3        OUTPUT 2=LP0
4        COMPRESS INTEGER AND LOGICAL
5        EXTENDED DATA
6        TRACE 0
7        END
8        MASTER ORDER
9 C      THIS PROGRAM READSREFERENCE FILE(CR0)
10 C     AND OUTPUTS (LP0) ALPHABETICAL AUTHOR LISTING WITH
11 C     INDEX NUMBERS OF REFERENCES.
12       COMMON ISCO(800),INAME(800,4),IREF(800,48),
13      1IAUT(6,4),IBUNAM(4),IBUREF(48),KBUREF(48),INAUT(2,12)
14       DIMENSION LETTR(29)
15       DATA LETTR/1H-,1HZ,1HY,1HX,1HW,1HV,1HU,1HT,1HS,
16      1 1HR,1HQ,1HP,1HO,1HN,1HM,1HL,1HK,1HJ,1HI,1HH,1HG,
17      2 1HF,1HE,1HD,1HC,1HB,1HA,1H.,1H /
18       DATA IBLANK/4H    /
19       IOE=0
20       IDD=IBLANK
```

```
      DO 10 I=1,800
      ISCO(I)=0
      DO 11 J=1,4
   11 INAME(I,J)=IBLANK
      DO 12 K=1,48
   12 IREF(I,K)=IBLANK
   10 CONTINUE
 1000 DO 4  I=1,4
      IBUNAM(I)=IBLANK
      DO 5 J=1,6
    5 IAUT(J,I)=IBLANK
    4 CONTINUE
      DO 6  I=1,48
      KBUREF(I)=IBLANK
    6 IBUREF(I)=IBLANK
      DO 13 I=1,2
      DO 14 J=1,12
   14 INAUT(I,J)=IBLANK
   13 CONTINUE
      READ(3,100)IBO
  100 FORMAT(A4)
      READ(3,100)IDD
      M=4
      CALL COMP(M,IDD,1,IBLANK,1)
      IF(M.EQ.4)GO TO 150
      INE=0
      READ(3,101)(INAUT(1,I),I=1,12)
  101 FORMAT(12A4)
       KXI=9
      DO 15 J=1,9,4
      M=4
      CALL COMP(M,INAUT(1,J),1,IBLANK,1)
      IF(M.NE.4) GO TO 15
       KXI=10
       GO TO 20
   15 INE=INE+1
      READ(3,101)(INAUT(2,J),J=1,12)
      DO 16 J=1,9,4
      M=4
      CALL COMP(M,INAUT(2,J),1,IBLANK,1)
      IF(M.EQ.4)GO TO 20
   16 INE=INE+1
   20 DO 201 KS=1,KXI
  201 READ(3,100)IAX
      KK=-4
      JJ=1
      DO 21 I=1,INE
      KK=KK+4
      IF(KK.LT.9)GO TO 25
      KK=0
      JJ=2
   25 DO 22 J=1,4
   22 IAUT(I,J)=INAUT(JJ,KK+J)
   21 CONTINUE
      DO 30 I=1,INE
      IOE=IOE+1
      IBS=0
      DO 32 M=1,2
      DO 33 K=1,29
      N=1
      CALL COMP(N,IAUT(I,1),M,LETTR(K),1)
      IF(N.NE.1)GO TO 33
      IBS=IBS+K
      IF(M.EQ.1)IBS=IBS*100
      GO TO 32
   33 CONTINUE
   32 CONTINUE
   39 DO 40 II=1,IOE
      IF(ISCO(II)-IBS)41,50,40
   41 CALL ISORT(IOE,II,IBS,I,IDD)
      GO TO 30
   50 IBT=0
      IBQ=0
      DO 54 M=3,4
      DO 55 NA=1,29
      N=1
      CALL COMP(N,IAUT(I,1),M,LETTR(NA),1)
      IF(N.NE.1)GO TO 55
      IBT=IBT+NA
      IF(M.EQ.3)IBT=IBT*100
      GO TO 54
   55 CONTINUE
   54 CONTINUE
      DO 56 M=3,4
      DO 57 NA=1,29
      N=1
      CALL COMP(N,INAME(II,1),M,LETTR(NA),1)
      IF(N.NE.1)GO TO 57
      IBQ=IBQ+NA
```

```
110         IF(M.EQ.3)IBQ=IBQ+100
111         GO TO 56
112      57 CONTINUE
113      56 CONTINUE
114         IF(IBQ-IBT)41,58,40
115      58 DO 70 IJ=2,4
116         DO 71 IK=1,4
117         ICO=0
118         ICI=0
119         DO 73 IL=1,29
120         M=1
121         CALL COMP(M,IAUT(I,IJ),IK,LETTR(IL),1)
122         IF(M.NE.1)GO TO 73
123         ICO=IL
124         GO TO 80
125      73 CONTINUE
126      80 DO 74 IL=1,29
127         M=1
128         CALL COMP(M,INAME(II,IJ),IK,LETTR(IL),1)
129         IF(M.NE.1)GO TO 74
130         ICI=IL
131         GO TO 81
132      74 CONTINUE
133      81 IF(ICI-ICO)41,71,40
134      71 CONTINUE
135      70 CONTINUE
136         DO 85 JA=1,48
137          M=4
138         CALL COMP(M,IREF(II,JA),1,IBLANK,1)
139         IF(M.NE.4)GO TO 85
140         IREF(II,JA)=IDD
141         IOE=IOE-1
142         GO TO 30
143      85 CONTINUE
144      40 CONTINUE
145      30 CONTINUE
146         GO TO 1000
147     150 DO 110 IK=1,IOE
148         WRITE(2,120)IK,(INAME(IK,J),J=1,4),(IREF(IK,J),J=1,24)
149     120 FORMAT(1H ,I4,1X,4A4,1X,24A4)
150         M=4
151         CALL COMP(M,IREF(IK,25),1,IBLANK,1)
152         IF(M.EQ.4)GO TO 110
153         WRITE(2,120)IK,(INAME(IK,J),J=1,4),(IREF(IK,J),J=25,48)
154     110 CONTINUE
155         STOP
156         END
157         SUBROUTINE ISORT(IOE,II,IBS,I,IDD)
158         COMMON ISCO(800),INAME(800,4),IREF(800,48),
159        1IAUT(6,4),IBUNAM(4),IBUREF(48),KBUREF(48),INAUT(2,12)
160         KBUREF(1)=IDD
161         DO 90 KA=II,IOE
162         IBF=ISCO(KA)
163         ISCO(KA)=IBS
164         IBS=IBF
165         DO 91 KB=1,4
166         IBUNAM(KB)=INAME(KA,KB)
167         INAME(KA,KB)=IAUT(I,KB)
168      91 IAUT(I,KB)=IBUNAM(KB)
169         DO 92 KC=1,48
170         IBUREF(KC)=IREF(KA,KC)
171         IREF(KA,KC)=KBUREF(KC)
172      92 KBUREF(KC)=IBUREF(KC)
173      90 CONTINUE
174       - RETURN
175         END
176         FINISH
177 ****
178
```

```
RFFIOUT
*******

0 MZ 10000
1 LO RFFIOUTBN
2 AS *CR0,RFDATAFI
3 AS *LP0,OUTFILE
4 EN 0
5 LF OUTFILE
6 LF OUTFILE
7 ER OUTFILE
8 ****
9
```

RFFIOUTSRC
****

```
  0       SHORTLIST (LP)
  1       PROGRAM (FXXX)
  2       INPUT 1 =CR0
  3       OUTPUT 2 =LP0
  4       COMPRESS INTEGER AND LOGICAL
  5       EXTENDED DATA
  6       TRACE 0
  7       END
  8       MASTER LIST
  9 C     THIS PROGRAM READS FROM DATA FILE(CR0) AND
 10 C     OUTPUTS(LP0) THE FILE IN 120 COLUMNS .
 11       DIMENSION IREFIN(11,30),INT(2)
 12       DATA IBLANK/4H    /
 13       DO 1 I=1,5000
 14       ITA=1
 15       IT=1
 16       IJ=15
 17     4 READ(1,100)INT(ITA)
 18   100 FORMAT(/A4)
 19       M=4
 20       CALL COMP(M,INT(ITA),1,IBLANK,1)
 21       IF(M.EQ.4)GO TO 99
 22       DO 2 JA=1,11
 23     2 READ(1,101)(IREFIN(JA,JA),JA=IT,IJ)
 24   101 FORMAT(15A4)
 25       IF(ITA.EQ.2)GO TO 7
 26       ITA=2
 27       IJ=30
 28       IT=16
 29       GO TO 4
 30     7 WRITE(2,102)INT(1),INT(2)
 31   102 FORMAT(1H0,A4,56X,A4)
 32       DO 3 IA=1,11
 33     3 WRITE(2,103)(IREFIN(IA,IB),IB=1,30)
 34   103 FORMAT(1H ,30A4)
 35     1 CONTINUE
 36    99 IF(ITA.EQ.1)GO TO 999
 37       WRITE(2,104)INT(1)
 38   104 FORMAT(1H0,A4)
 39       DO 5 JA=1,11
 40     5 WRITE(2,105)(IREFIN(JA,JC),JC=1,15)
 41   105 FORMAT(1H ,15A4)
 42   999 STOP
 43       END
 44       FINISH
 45 ****
 46
```

RFDATAFI (LAST 60 LINES)
****

```
4850  374
4851 FOLMER.O.F.      AZARRAGA.L.V.
4852
4853 PYRO     PY/GC    POLY     GC
4854 LAS/PY
4855 A LASER PYROLYSIS APPARATUS FOR GAS CHROMATOGRAPHY
4856
4857 J CHROMATOGR SCI              7   665-70   1969
4858 MAINLY POLYMERS - COMPARISON OF FILAMENT TO LASER .
4859
4860
4861
4862
4863  375
4864 PERRY.S.G.
4865
4866 PYRO     PY/GC    POLY     GC
4867
4868 PYROLYSIS SUB-GROUP OF THE GAS CHROMATOGRAPHY
4869 DISCUSSION GROUP
4870 J CHROMATOGR SCI              7   193-4    1969
4871 INAUGRAL MEETING - COMMENTS ON APPLICATIONS AND TECHNIQUES
4872 USED . SET UP INITIAL TEST .
4873
4874
4875
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4876  376
4877 CAUWENBERGHE.K.VVANDEWALL.M.     VERZELE.M.
4878
4879 PYRO     PY/GC    ST/DET  GC
4880 MS
4881 DETERMINATION OF THE BRANCHING DEGREE IN ALKYL BENZENES
4882 BY PYROLYSIS GAS CHROMATOGRAPHY .
4883 J CHROMATOGR SCI            7   696-700 1969
4884 COMPARISON OF PYRO AND MS . EMPIRICAL METHOD .
4885
4886
4887
4888
4889  377
4890 WILLMOTT.F.W.
4891
4892 PYRO     PY/GC    GC       POLY
4893 CU.PT
4894 PYROLYSIS - GAS CHROMATOGRAPHY OF POLYOLEFINES
4895
4896 J CHROMATOGR SCI            7   101-8   1969
4897 POLY ONLY
4898
4899
4900
4901
4902          ****
4903
4904
4905
4906
4907
4908
4909 ****
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RFKEYFI
=======

0	AA	ATOMIC ABSORPTION
1	ALKAL	ALKALOIDS
2	ALK/AG	ALKYLATING AGENTS
3	AM/MET	AMINE METABOLISM
4	AMINO.A	AMINO ACIDS
5	ANT/INF	ANTI INFLAMATORY DRUGS
6	ANTIBIO	ANTIBIOTICS
7	APA	ANALYTICAL PYROLYSIS AMSTERDAM (76)
8	API	ATMOSPHERIC IONISATION (MS)
9	AUTO	AUTOMATION
10	BACT	BACTERIA
11	BIO/PRO	BIOLOGICAL PROFILES
12	BIOCHEM	BIOCHEMICAL
13	BIOPOL	BIOPOLYMERS
14	BIOTRAN	BIOTRANSFORMATION
15	BLOOD	COMPONENTS IN BLOOD
16	BOTAN	BOTANICAL
17	CAMS	COLLISIONAL ACTIVATION MS
18	CAN/C	CANCER CELLS
19	CAP/GC	CAPILLARY GC
20	CIMS	CHEMICAL IONISATION MASS SPECTROMETRY
21	CLIN	CLINICAL STUDIES
22	COL	COLORIMETRIC
23	COMP	COMPUTERS
24	CU.PT	CURIE POINT
25	DATA/P	DATA PROCESSING (USUALLY MASS SPEC)
26	DERIV	DERIVATIVES (GC)
27	DETECT	DETECTORS (GC)
28	DEUT	DEUTERIUM LABELLING
29	DRUGS	DRUGS
30	ECD	ELECTRON CAPTURE DETECTOR (GC)
31	EIMS	ELECTRON IMPACT MS (USUALLY COMPARISON O OTHER ION SRCE
32	ENVIR	ENVIROMENTAL
33	ENZYMES	ENZYMES
34	FDMS	FIELD DESORPTION MS
35	FIMS	FIELD IONISATION MS
36	FLUOR	FLUORESENCE
37	FOOD/A	FOOD ANALYSIS
38	FOR	FORENSIC
39	FUNGI	FUNGI
40	GC	GAS CHROMATOGRAPHY
41	GC/MS	GAS CHROMATOGRAPHY/MASS SPECTROMETRY
42	HORM	HORMONES
43	HPLC	HIGH PRESSURE(PERFORMANCE) LIQUID CHROMATOGRAPHY
44	INFO/RET	INFORMATION RETRIEVAL
45	INFO/TH	INFOMATION THEORY
46	INORG	INORGANIC CHEMISTRY
47	IR	INFRA-RED
48	LAS/PY	LASER PYROLYSIS
49	LIBSEAR	LIBRARY SEARCH (COMPUTER)
50	MECH	MECANISMS(CHEMICAL)

```
51 METAB     METABOLISM
52 METABDIS METABOLIC DISORDERS
53 MS        MASS SPECTROMETRY
54 MYCOBACT MYCOBACTERIA
55 NATPROD   NATURAL PRODUCTS
56 NMR       NUCLEAR MAGNETIC RESONANCE
57 NUC-AC    NUCLEIC ACIDS
58 PCA       PRINCIPLE COMPONENT ANALYSIS
59 PEPTIDES PEPTIDES
60 PIMS      PULSED ION MS
61 POLY      POLYMERS
62 PYRO      PYROLYSIS
63 PY/GC     PYROLYSIS/GC
64 PY/GC/MS PYROLYSIS/GC/MS
65 PY/MS     PYROLYSIS/MS
66 QUAT      QUATERNARY AMMONIUM COMPDS
67 RA/ASS    RADIOIMMUNOASSAY
68 REV       REVIEW
69 SOIL      SOIL CHEMISTRY ( GEOCHEMISTRY )
70 SPEC/INT SPECTRAL INTERPRETATION
71 SSMS      SPARK SOURCE MS
72 STAT/AN   STATISTICAL ANALYSIS
73 STEROID   STEROIDS
74 ST/DET    STRUCTURAL DETERMINATION
75 ST/PH     STATIONARY PHASES(GC)
76 SUGARS    SUGARS
77 SULPHON   SULPHONAMIDES
78 URINE     URINE
79 UV        ULTRA VIOLET
80 VIRUS     VIRUS
81 ZOOL      ZOOLOGICAL
82
83
84 ****


RFNUMFI
*******

0   377
1


RFCOPY
******

0 CY RFDATAFIST,RFDATAFI(+1)
1 CY RFNUMFIST,RFNUMFI(+1)
2 CY RFKEYFIST,RFKEYFI(+1)


RFRV
****
0 RV RFINFO
1 RV RFCRDIN
2 RV RFCRDINSRC
3 RV RFCRDINBN
4 RV RFINTIN
5 RV RFINTINSRC
6 RV RFINTINBN
7 RV RFKEYIN
8 RV RFKEYINSRC
9 RV RFKEYINBN
10 RV RFCRDOUT
11 RV RFCRDOUTSRC
12 RV RFCRDOUTBN
13 RV RFINTOUT
14 RV RFINTOUTSRC
15 RV RFINTOUTBN
16 RV RFAUTHOUT
17 RV RFAUTHOUTSRC
18 RV RFAUTHOUTBN
19 RV RFFIOUT
20 RV RFFIOUTSRC
21 RV RFFIOUTBN
22 RV RFDATAFI
23 RV RFDATAFIST
24 RV RFKEYFI
25 RV RFKEYFIST
26 RV RFNUMFI
27 RV RFNUMFIST
28 RV RFCOPY
29 ****
30
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EXPERIMENTAL

Instrumentation

Pyrolyser- Pye Curie point pyrolyser, nominal output 30 watts.

Gas Chromatographs- Pye 104 (model 4) and Pye GCV.

Integrator- Infotronics CRS 304-30.

Recorders- Pye AR25, Phillips PM 8222, W+W 1100.

GC-MS- VG Micromass 12B

Operating conditions -

Magnet current scan - 1-5 amps.

Electron multiplier voltage - 3.5 kV.

Trap current - 100μamps.

Ionisirg potential - 22eV.

Scan time - Drugs - 10 sec 1-5 amps

Urine - 10 sec 0.9 - 3.4 amps

Accelerating voltage 4 kV.

Chopper amplifier - 1×10^{-7} response- 0.003.

Total-ion monitor - 1×10^{-9} amps.

UV recorder range scale 1 x 3 x 10.

GC interface - Single glass jet separator.

GC outlet temperature 220°C.

GC-MS jets temperature 220°C.

MS inlet temperature 230°C.

MS source temperature 280°C.

Optimised procedures

(a) Bacteria

The cells were grown in chemically defined media[73] and harvested during the log phase of growth. They were centrifuged and the resulting pellet was washed with distilled water and again spun down. The cells were then lyophilised in a home-made unit.

The dried material was placed in a small glass tube (40mm x 3mm) and CS_2 was used as the suspending agent. The tube was kept in an ultra-sonic cleaning bath and the cells were transfered to the rotating pyrolysis wire(600 RPM) *via* a Pasteur pipette. The CS_2 was evaporated by placing the wire in a heated airstream.

Conditions used-

Pyrolysis Temperature 980°C.

Pyrolysis Time 5 secs.

Amount of sample on wire 800 μg.

N_2 (carrier gas flow rate) 50 mls/min.

Temperature program rate 50°/4min/ ——→ / 200°C
2.5°C.min^{-1}/10 mins

Column 2.1m x 4 mm(ID) 2% KOH + 8% CARBOWAX 20M.

(b) Drugs

(i) Qualitative

The drug was placed in a glass tube and suspended in CS_2 (see bacteria) and coated onto a 770°C pyrolysis wire. When temperature programmed from 100°C to 250°C the baseline drifted, due to column bleed, and it was therefore necessary to use the integrator baseline adjustment facility and a program used for the Py-GC study of sulphametopyrazine is shown below.

Threshold level	2000
Minimum area	5000
Skim ratio	100
Noise	30
Baseline 1	390
Baseline 2	480
Baseline 3	670
Baseline 4	720
Baseline 5	750
Baseline 6	820
Peak width	10
Peak width doubles at	100

N.B. All the time units are in 0.1 of a minute(i.e. 6 secs.)

(ii) Quantitative

For quantitative analysis the sulphonamides were dissolved in methanol and a known amount transfered to the pyrolysis wire(770°C) using a microsyringe. The porous polymer packing 'CHROMOSORB 103 was used at an oven temperature of 235°C. The other GC parameters were as follows:

Amplifier attenuation	200
Injection-port temperature	280°C.
Detector oven temperature	350°C.
Nitrogen flow rate	50 mls/min.
Air pressure (FID)	6 lb/in^2.
Hydrogen pressure (FID)	20lb/in^2 .

The integrator program was as follows:

	100	L
	9999	M
	10	S
	200	N
	160	R
15		W
	1	Sx
50	8	Sx
51	1	Rx
145	8	Rx

N.B. The external commands were used for :

(1) Integrate inhibit.

(8) Valley to valley integration.

(c) Urine

The urine samples were taken and the total volume recorded. An aliquot was lyophilised and the resulting total solids stored in glass containers prior to analysis.

The wires were loaded by reconstituting the total-solids by adding a small (0.5 mls) volume of distilled water and the resulting paste was coated onto the wires. The wires were then placed in a vacuum desiccator for 30 mins prior to pyrolysis. The Py-GC conditions for the urine and urine components are given below:

Pyrolysis Temperature 770°C. Pyrolysis Time 5 sec.

Column 1.5m x 4mm (ID) 2% KOH + 8% CARBOWAX 20M.

Temperature program 100°C / ——→ / 245°C
5 mins / 5°C. min^{-1} / 8 mins

Amplifier attenuation 16×10^2

Integrator program :

100	L
9999	M
10	S
50	N
430	R
15	W
8	Sx
429 8	Rx

Synthetic Procedures.

(1) Preparation of benzenesulphanilide.

Preparation as previously recorded [137].

24 mls of aniline were added to 12mls of benzene sulphonyl chloride and the temperature of the mixture allowed to rise to 70°C. The mixture was then cooled and an excess of H_2O added. The colourless crystals were filtered off and recrystallised from acetic acid/water (50/50). An 80% yield was obtained with the above procedure.

(2) Preparation of N-acetylsulphadimidine.

5g of sulphadimidine was refluxed with 20mls of acetic anhydride for 30mins. The mixture was cooled and after filtration the crystals were recrystallised from 95% ethanol. The N-acetylsulphadimidine was obtained in a 67% yield.

TLC of benzenesulphanilide pyrolysate

The quartz pyrolysis tubes were washed with methanol and the extract spotted onto a plate. Alumina GF_{254} (Type 60/E), (Merck) was used and the solvent was Toluene/n-Hexane (90/10). The following data was obtained by the concurrent use of standards.

Rf.	Identification	Rf	Identification
0.97	Biphenyl	0.79	Diphenylamine
0.58	Carbazole	0.43	Unknown
0.35	Unknown	0.30	Unknown
0.23	Unknown	0.00	Unknown

Further experimental details are given in the results and discussion sections.

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" The more we have read, the more we have learned,
the more we have meditated, the better conditioned
we are to affirm that we know nothing."

Voltaire, 1764.