Federal Office of Road Safety

Analysis for Drugs in Saliva

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Abstract

The results are reported of research investigating the use of saliva analysis as a research tool in determining drug usage by drivers who have not been involved in crashes. The research shows that saliva analysis is a valid research method for screening for a wide variety of drugs with the potential to impair driving skills, in a non-invasive and more easily obtainable manner than would be the case for blood and/or urine samples. In addition, details are provided of drug findings from the first 618 saliva samples from a related survey of truck drivers, bus drivers and general population drivers

Keywords

DRUGS, DRIVING, SALIVA, SURVEYS

CONTENTS

SUMMARY

1. A rationale is given for the use of saliva analysis to validate surveys which address the extent of drug usage by non-compromised drivers.

2. The methodology for the collection of samples, their processing and subsequent analysis by gas chromatography and tandem mass spectrometry (GC-MS-MS) is detailed.

3. Results for preliminary experiments in which the ability to detect known drugs in spiked (drug added to saliva) saliva samples and in saliva samples from subjects who had taken known drugs are presented.

4. Details of drug findings from the first 618 saliva samples in the survey are shown. These comprise 318 truck drivers, 90 bus drivers and 210 general drivers.

5. A total of 417 drugs were detected in the 318 truck driver samples. This number was reduced to 106 when caffeine and cotinine (nicotine metabolite) were excluded. Stimulant drugs (other than caffeine) were found in 91 samples (28.61%).

6. A total of 100 drugs were detected in the 90 bus driver samples. This number was reduced to 13 when caffeine and cotinine were excluded. Stimulant drugs (other than caffeine) were found in 6 samples (6.66%).

7. A total of 211 drugs were detected in the 210 general driver samples. This number was reduced to 23 when caffeine and cotinine were excluded. Stimulant drugs (other than caffeine) were found in 6 samples (2.86%).

8. The prevalence of stimulant drugs in truck drivers thus appears, at this stage, to be very different from that in the other driver populations sampled.

9. The authors consider that saliva analysis, using the method described in the body of the report, has proved to be a viable non-invasive way of screening for a wide variety of drugs which are considered to pose problems for traffic safety. It should be noted that the same limitations apply to saliva analysis as applies to the detection of drugs in blood by such a broad screening procedure. That is, we will not be able to detect drugs which are highly water soluble, such as chloral hydrate and sodium valproate and drugs which are taken in very small doses, such as lysergic acid diethylamide (LSD).

10. The relevance of Δ^9-tetrahydrocannabinol (THC) detection in saliva requires more research in view of the New Zealand finding (Cairns *et al*, 1990) that there was a moderate correlation between subjective perception of intoxication and THC levels in stimulated saliva. There was only correlation between THC levels and impaired vigilance early in the course of intoxication. The authors concede that the relationship was probably incidental because THC is sequestered in the mouth after smoking. It is much less certain whether THC can ever diffuse into saliva from the blood. Thus, although detection of THC in saliva probably indicates recent cannabis usage, and is therefore of some value in enforcement, it is probably of little relevance as an efficient predictor of the likely extent of THC-induced impairment.

1. GENERAL INTRODUCTION

Driving a motor vehicle is a multifunctional task involving visual search and recognition, vigilance, information processing under variable demand, decision-making, risk-taking and enough sensorimotor control to carry out all these activities simultaneously (Starmer *et al.*, 1988). It is also an overlearned task (that is, practice has obviated the need for conscious recall), where critical high level demands are very infrequent.

Impairment of driving performance has been defined (Consensus Report, 1985), in a general sense, as the failure to exercise the expected degree of prudence or control to ensure safe operation of the vehicle under the traffic conditions pertaining at the time. This is often expressed as traffic violations and traffic crashes.

Such alterations of behaviour are not specific to drugs, however, and can be associated with distraction, emotional lability, aggression, fatigue, physical illness, psychiatric illness and many other factors which can show complex interactions. Della-Glustina (1977) also pointed to the complex interactions of age, chronic disease and prescription drug use as well as non-medical factors in traffic accidents.

The medical and social costs of traffic accidents to the community are immense. Transport accidents in 1988 were conservatively estimated to have cost Australian society $6.6 billion. Road accidents contributed $6.1 billion (94%) to this total. Aviation accidents cost $64 million (1%), rail $94 million(1.4%) and maritime $264 million (4%) (Bureau of Transport and Communications Economics, 1992). Although road crashes are responsible for just over 2% of total deaths in Australia each year, they account for almost 7% of years of life lost through all causes of death. This is more than the years lost through cerebrovascular disease and cancer. When only years of life lost during the working age span are considered, road accidents account for more years lost than through all forms of heart disease and about 75% of those lost through all cancers (Federal Office of Road Safety, 1991). The Federal Office of Road Safety Road Fatality Statistics for 1990 indicate that there were 2328 fatalities in Australia in that year and about ten times that number of serious injuries. Each fatality costs the Australian community $300,000 (in 1985 terms), and each serious injury $52,000.

The relationships between alcohol consumption and increased traffic violation rates and increased crash risk is now well established. The first recommendation for mandatory alcohol testing for crash-involved drivers was by Widmark in 1914. Because of this, many alcohol countermeasures have been put in place and the extent of enforcement is now the main criterion of success. With the introduction of *per se* drink-drive legislation, where the perceived risk of apprehension has been greatly increased, and of community awareness and education programs, alcohol-related accidents have begun to decline.

Given that many drugs other than alcohol have central nervous system effects which are remarkably similar to those of alcohol (and may even exhibit cross-tolerance), it would be surprising if these drugs did not have a similar potential to impair driving ability. There are a number of fragmented lines of evidence which support the hypothesis that drugs (other than alcohol) can induce impairment which may constitute a traffic safety problem. These are:

1. Many drugs (prescribed, bought over the counter and recreational) are intrinsically impairing and/or can exacerbate alcohol-induced performance deficits.

2. Many drugs which are capable of causing psychomotor impairment are widely used in the community (eg Reynolds *et al.*, 1977; Hendtlass, 1983).

3. There is an apparent over-representation of the same drugs in drivers who are killed on the roads, who present at hospital as the result of a crash or who are apprehended by police for driving in an aberrant manner.

4. Evidence from prospective surveys, such as that carried out by Skegg *et al.* (1979), has indicated a highly significant association between the prescription of minor tranquillisers and the risk of a serious road accident.

Nevertheless, concern that drugs other than alcohol might be important in traffic safety has only recently been expressed. Even more recently, epidemiological studies have begun to identify many prescription and over-the-counter drugs as being over-represented in drivers who are killed or injured in traffic accidents. Information on the behavioural toxicology (which, in this context, includes the adverse effects of the drug on human skills performance, such as the ability to drive a motor vehicle, operate machinery or work in a hazardous environment) of these drugs, is scant, however. Most of the drugs so identified have the ability to impair driving-related skills. Thus, although massive campaigns have been organised to counter the detrimental effects of alcohol on driving, the results of drugs driving research have been claimed to be mostly of academic interest and to have little significance in licensing and regulatory terms (Irving, 1986). This may be because therapeutic drugs taken for legitimate therapeutic purposes are tacitly assumed to restore driver ability towards normal. This may or may not be the case (de Gier et al., 1986; Gerhard & Hobi, 1986).

Alcohol is a simple but quite inappropriate model for the study of the effects of other drugs on driving ability and traffic safety. Most drugs which impair driving performance require much smaller doses to produce a measurable effect. Most drugs have considerably more complex pharmacodynamic (how the drug works and the effects it produces) and pharmacokinetic (the movement of drugs within the body as a function of time) profiles than

alcohol, often with asynchrony between blood concentration and pharmacological effect. There is no equivalent of the "Breathalyser" for drugs other than alcohol and body fluid sampling is a major logistical problem in cases which do not involve injury or death.

Most of the epidemiological studies in which body fluid samples were analysed have found a 5-15% incidence of drug involvement in road crash victims, the variation appearing to depend largely on whether cannabis was included in the screen (Cairns et al., 1984). Both analytical and survey approaches have been adopted in studies where there has been an attempt to define the involvement of drugs other than alcohol in traffic violations and traffic crashes which have resulted in injury or death (reviewed Starmer *et al.*, 1988). These approaches are in many ways complementary and when used together should provide a more accurate picture than either method alone (Vine & Watson, 1982). For example, a severe disadvantage of the blood analysis approach is that such work is costly and very time-consuming and is, therefore, applicable to only relatively small populations. The questionnaire approach can be applied to much larger populations but suffers from the disadvantage that it relies on truthful and accurate answers from drivers. Attempts to combine the two approaches (Finkle *et al.*, 1968; Sterling-Smith, 1975) have required considerable resources of manpower and equipment and have required access to police, medical and social records.

It has been stated (McLean *et al.*, 1985) that drugs-driving studies are very much the "art of the possible" as regards permitted sampling procedures and acquisition of data and crash variables. There are, in consequence, problems in the interpretation of the results. A logical approach would be to determine whether prescription, over-the-counter and illicit drug usage in the driving population (and its sub-populations) reflects that in the population at large after an appropriate adjustment for age and gender. Then it would be possible to determine whether modifications of these patterns occur in those who commit traffic offences or who are involved in traffic crashes. No such studies have yet been published.

There is an obvious problem with body fluid sampling which is compounded by difficulties in selecting suitable and affordable analytical methodology. Although blood samples can usually be obtained from drivers who are admitted to hospital as the result of a crash, it is unrealistic to expect to get permission to take blood from drivers surveyed at the roadside, although this has been done in Finland (Honkanen *et al.*, 1980). Urine sampling is unsatisfactory, largely because urinary drug levels seldom, if ever, reflect the activity of a drug and can only confirm usage. There are also problems which pertain to the fluid balance of the individual. Measurement of drug concentrations in saliva appears to be the only viable approach.

Again, it should be emphasised that an important priority is to establish the extent and nature of drug use and abuse among non-compromised drivers. Then the findings for drugs in compromised populations of drivers can be

findings for drugs in compromised populations of drivers can be put into context and the design of any new studies which might be envisaged can be optimised.

This is the objective of the current Federal Office of Road Safety Research Program on Drugs and Driving. Attitudinal/ behavioural information has been obtained on an anonymous basis from groups (Table 1) of non-compromised bus, truck and general drivers in all mainland states of Australia and saliva samples have been collected from all 1700 respondents in the study. These are currently undergoing analysis for a wide range of drugs.

In this report, the methodology for drug analysis in saliva is discussed and preliminary results are presented.

Table 1 Driver populations from whom attitudinal/behavioural information in relation to drug-taking was obtained (numbers of respondents)

Location	Driver Group	Total
New South Wales / ACT (793)	General (S) General (L) Truck Bus	143 256 294 100
Victoria (354)	General (S) General (L) Truck Bus	142 97 80 35
Queensland (211)	General (S) General (L) Truck Bus	81 64 36 30
South Australia / Northern Territory (206)	General (S) General (L) Truck Bus	67 53 61 25
Western Australia (211)	General (S) General (L) Truck Bus	42 56 30 10
TOTAL (1702)	General (S) General (L) Truck Bus	475 526 501 200

Note: (S) = short journey; (L) = long journey

1.1 THE SALIVARY GLANDS AND SALIVA SECRETION

In any consideration of the use of saliva sampling for drug identification and monitoring, it is important to understand the way in which saliva is elaborated and the factors which influence its secretion.

The principal function of the salivary glands is the synthesis of macromolecules and their secretion, along with water and electrolytes, to form saliva (Hand, 1987). At the cellular level, it is apparent that the synthetic functions are paramount. The parotid and submandibular salivary glands collectively contribute about 88% of the total salivary flow in man (Dawes, 1978). The secretory cells in the major human salivary glands are of two types, serous and mucous. Serous secretions are watery and protein-rich whereas mucous secretions are viscous and carbohydrate-rich (Tandler, 1987). The human parotid gland is a pure serous gland, with all its secretory cells of the serous variety, whereas the submandibular gland is a mixed gland containing both serous and mucous cells, with the serous cells predominating by 12 to 1 (Tandler, 1987).

The serous cells of the parotid and submandibular glands are arranged into spherical clusters, termed acini, which are one cell thick and enclose a central lumen. Up to 90% of the glandular volume is made up of acinar cells (Williams *et al.*, 1973).

The cytoplasm of unstimulated serous cells in both parotid and submandibular salivary glands contain numerous membrane-bound secretory granules (1 μm diameter) with a moderately dense matrix and which contain spherules. The spherules consist, in part, of proline-rich protein (Kousvelari *et al.*, 1982). Other secretory products, such as amylase, lysozyme, lactoferrin and lactoperoxidase (Hatakeyama *et al.*, 1985; Tandler & Riva, 1986) have been localised in human serous cells and are probably confined to the secretory granules. On receipt of the appropriate stimulus, the granules are released to the exterior of the cell (exocytosis).

Mucous cells of the submandibular glands are crammed with structureless mucous droplets. There are also myoepithelial cells (basket cells) which have marked ATPase activity (cause breakdown of adenosine triphosphate, liberating energy) and have a contractile function (Garrett & Emmelin, 1979). These cells are joined to the acinar cells and can work in a coordinated manner (Taugner & Schiller, 1980). The simultaneous contraction of many of myoepithelial cells can result in a gush of saliva into the mouth.

The first ducts from the secretory elements are the small diameter intercalated ducts. They are considerably longer in the parotid than in the

submandibular gland and the cells may contain secretory granules and probably add protein and some lactoferrin and amylase to the saliva. The intercalated ducts coalesce to form striated ducts which are more extensive in the submandibular gland than in the parotid. The striated ducts of the major salivary glands resorb electrolytes (principally sodium), which results in saliva becoming hypotonic (Young & Shogel,1966).

At the periphery of each lobule, striated ducts join to form excretory or interlobular ducts which are embedded in septal connective tissue. The secretory ducts probably also participate in the resorption of electrolytes from the saliva (Schneyer *et al.*, 1972).

Excretory ducts coalesce to form the main excretory ducts, which extend from the hilus of the gland to the mouth. In the parotid gland, the main duct (Stenson's duct) opens into the mouth opposite the upper second molar. In the submandibular gland, the main duct (Wharton's duct) opens at the apex of the sublingual papilla, lateral to the lingual frenulum. The ducts are lined with pseudostratified epithelium which contains occasional ciliated and goblet cells. It is likely that the main excretory ducts have only a minor role in electrolyte resorption (Schneyer *et al.*, 1972). Deep to the epithelium there is a muscular layer, consisting of longitudinally oriented smooth muscle without any circular fibres.

The autonomic nerve routes to the salivary glands are not always as precise anatomically as is commonly believed (Garrett, 1987). No universal statement can be made about the overall arrangements of sympathetic or parasympathetic nerves in different glands nor about their neuroeffector arrangements. Neurotransmitters provide the first message to the cell signalling it to secrete. Binding of the neurotransmitter to the appropriate surface receptor activates the receptor initiating the cascade of events resulting in secretion. Several types of neurotransmitter are considered to have a role in salivary gland secretion and include noradrenaline, acetylcholine, vasoactive intestinal peptide, substance P, 5-hydroxytryptamine and adenosine triphosphate. Non-conventional neurotransmitter substances, mostly neuropeptides, are known to exist in both sympathetic and parasympathetic nerves to the salivary glands.

In general terms, the final common stimulus for salivary glands to secrete is *via* their nerve supply (Garrett, 1987) and not, as in many other parts of the gastrointestinal tract, by hormones as well, except in the sense that hormonal balance maintains a correct milieu for secretion to occur. Also in general terms, the main stimulus for inducing the flow of saliva comes from parasympathetic impulses. Parasympathetic stimulation can occur in isolation, causes some exocytosis and induces myoepithelial contraction. Vasodilation is part of the secretory process. Parasympathetic stimulation thus produces a copious continuing secretion that has a relatively low

concentration of protein. Sympathetic stimulation, on the other hand, depending on the gland, has only a moderate, low or absent ability to mobilise fluid and fluid secretion will cease altogether if the vasoconstriction is severe. However, any fluid which is secreted tends to contain a relatively high concentration of protein (Garrett, 1987). Sympathetic impulses tend to modulate the composition of the saliva to satisfy special needs, which include digestive purposes. In life, it is thought that there is a continuous background of reflex parasympathetic stimulation, even at rest, and thus any sympathetic impulses normally occur on top of a background of parasympathetic secretion.

There is a vast literature on reflex salivary secretion in man, especially concerning flow rates and ionic composition (Garrett, 1987).

The amount of amylase in human parotid saliva is increased under standardised conditions of oral stimulation, when the subjects were immersed in cold water, which was taken to indicate that sympathetic activity increases the amylase output (Spiers *et al.*, 1974) but it was not established whether this was due to increased secretory nerve activity, increased catecholamine output or both.

Chewing has long been used as a reflex stimulus for obtaining parotid secretion. This has repeatedly been shown to have the most dominant secretory effect on the gland on the same side as the chewing. It has usually been considered that the reflex stimulation comes *via* muscle afferents but recent studies give a strong indication that the most important input in people with natural teeth arises from periodontal mechanoreceptors (Hector, 1985).

The most widely accepted model for saliva production hypothesis is a two-step process (Izutsu, 1987). In the first step, an isotonic primary system is thought to be produced by the acini and in the second step, the fluid is rendered hypotonic as it flows through the ducts. The latter process involves a ductal reabsorption of sodium chloride and the secretion of potassium and bicarbonate (Thayson, 1960). At low flow rates, ductal reabsorption will be extensive and the sodium content of the saliva will be low but at high flow rates, the primary secretion will transit the ducts too quickly to allow much reabsorption and the sodium concentration will be high. It is now considered that the process is more complex and must take into account non-isotonic primary secretion and variable rates of ductal sodium chloride reabsorption (Young & van Lenep, 1979; Baum *et al.*, 1984).

Salivary electrolyte levels in man are usually dependent on flow rate. Parotid sodium concentration increases with increasing flow rate but, in contrast, parotid potassium and inorganic phosphate concentrations both

decrease with increasing flow rate (Dawes, 1984). These concentrations do not depend on the duration of stimulation. Parotid chloride and bicarbonate concentrations both increase with increasing flow rate but their subsequent concentrations depend on the duration of stimulation. The increase in bicarbonate concentration is responsible for an increase in parotid salivary pH with increasing flow rate (Izutso, 1981).

In both submandibular and sublingual saliva, the flow rate-dependent changes in submandibular salivary sodium, chloride, bicarbonate and inorganic phosphate concentrations and pH are generally the same as in parotid saliva (Dawes, 1984). Submandibular potassium concentration is not strongly affected by flow rate while calcium concentration decreases with increasing flow rate. Only chloride, bicarbonate and pH changes are dependent on duration of stimulation.

Salivary glands are thus typical exocrine glands and the general mechanisms by which salivary glands secrete are comparable to other exocrine tissues (Baum, 1987). Exocrine secretion involves two functional events; water and salt transport and protein release.

1.2 Secretion of drugs into saliva

Most drugs diffuse passively from plasma into saliva, the rate and extent to which this occurs being governed by a number of physicochemical actors, such as plasma protein-binding and the pKa (which reflects the acidity of alkalinity) of the drug. Only unbound drug is available for diffusion and, generally, only the un-ionised (uncharged molecule) form of the drug is the diffusible molecular species. Acidic drugs with pKa values greater that 8.5 and basic drugs with pKa values less than 5.5 are almost totally un-ionised in plasma. It can be shown that at the normal plasma pH (7.4), the saliva and plasma concentrations of unbound drugs are equal.

For example with the minor tranquilliser, diazepam, which is about 95% bound to protein in plasma, the saliva:plasma concentration ratio is about 0.03. At normal therapeutic blood concentrations (500-2000ng/ml), therefore, the concentration of diazepam in saliva might be expected to be of the order of 1-6 ng/ml. Thus, although the concept of saliva assay for drugs is well understood (Idowu & Caddy, 1982; Peel *et al.*, 1984), until the advent of MS-MS instruments (tandem mass spectrometer - see below), the measurement of many drugs in saliva was impossible or unreliable because the drug or metabolite concentrations attained in saliva were beyond the sensitivity threshold of conventional assay equipment. The triple stage quadrupole (TSQ) mass spectrometer, which has been used in the present study, is up to two orders of magnitude more sensitive than conventional instruments and can, therefore, detect a therapeutic concentration of diazepam.

2. METHODOLOGY

2.1 Analytical - Rationale for the use of Tandem Mass Spectrometry

The rapid detection of a wide range of drugs at normal therapeutic levels in small samples of body fluids presents a most difficult analytical challenge. If many hundreds or thousands of samples are to be screened, a very rapid and, ideally, highly automated method must be used. However, this would not normally be consistent with highly selective detection at low levels. High sensitivity analysis is usually achieved by optimising detection of a specific compound or group of compounds at the expense of the other components of the mixture.

Drugs have greatly differing chemical structure and therefore screening for a sufficiently wide range of compounds in a reasonable time has been impractical. The studies carried out by Vine & Watson (1982) and Warren *et al.* (1981) were limited by this problem which placed restrictions on the number of drugs included and the minimum levels at which they could be detected.

In epidemiological studies, it is important that there be no false positives. Each drug identification must be authentic to avoid unduly influencing the results due to the relatively small numbers (usually < 500) of samples which are screened. At the same time it is equally important to ensure that there are no false negatives because many of the drugs which are particularly liable to impair driving ability are taken in low doses and are present at very low levels in body fluids.

The detection of drugs in body fluids can be achieved by physico-chemical analytical techniques, such as chromatography or mass spectrometry, or by biological methods, such as immunoassay. The latter, while providing high sensitivity for specific compounds are of only limited use in screening because only one drug at a time can be detected and the number of drugs for which reagents have been developed is quite small. Also, this technique does not always provide unambiguous identification of a drug.

Undoubtedly, the methods best suited to drug screening are those based on mass spectrometry or a combination of mass spectrometry with chromatography. Mass spectrometers rely on the electrical properties of substances to identify minute quantities of drugs in complex mixtures, such as blood or saliva.

The smallest particle of an element is an atom, which has an atomic weight or mass which is specific to that element. An element can be identified by determining the mass of its atom. The smallest particle of a chemical compound is a molecule. Each molecule is made up of a number of atoms which are electrically bonded together in a specific pattern. A chemical compound, such as a drug, has a characteristic molecular weight or molecular mass, which is the sum of the masses of its constituent atoms. It should be

noted, however, that different compounds can have the same nominal molecular weight (made up of different atomic masses) and it follows that it is not always possible to uniquely identify a compound by its molecular mass alone. For example, the tranquilliser diazepam, has the same molecular mass (284) as stearic acid, which is a constituent of human and animal fats. A distinction can be made between molecules of the same mass by using energy to break them up into smaller fragments, which form a characteristic spectrum for that compound. This can be carried out by a mass spectrometer which identifies a compound by fragmenting it into its unique spectrum and then measuring the mass of each fragment which can be printed or displayed on screen.

Normally the mass spectrometer is coupled to a gas chromatograph (GC) to enable an initial separation of the components of the mixture which are to be analysed by the mass spectrometer. This technique, gas chromatography-mass spectrometry (GCMS), was, until recently, the most sensitive and selective method for the detection of drugs. The current situation is that although purely chromatographic techniques, especially those utilising different columns and detectors, are useful in screening, ultimately mass spectrometry is required for confirmation of drug identity. However, in common with other analytical methods, GCMS becomes less effective as the range of drugs to be detected increases and samples become more and more complex.

Although conventional mass spectrometers are capable of detecting organic compounds at picogram (10^{-12} gm) levels, such sensitivity is rarely achieved in practice except for very pure reference standards. In the analysis of "real" samples, such as extracts from plants, from body fluids and tissues and from the environment, detection of such compounds is generally possible only at the nanogram (10^{-9} gm) level. Despite the use of sophisticated "clean-up" procedures and gas or liquid chromatographic separation methods, interfering compounds produce a level of chemical noise which, in effect, determines the limit of detection of a particular compound.

The development in recent years of a new generation of mass spectrometer, the so-called tandem or MS-MS instrument, has now provided a way of removing this chemical noise and allowing detection limits closer to those of the pure reference standards to be attained. Although there are several types of MS-MS instrument, it is the triple stage quadrupole mass spectrometer (TSQ) which has demonstrated effectiveness in rapid screening procedures (Brotherton & Yost, 1983; Hunt *et al.*, 1985).

Mass analysis in a quadrupole mass spectrometer is achieved by accelerating the ions into the region within the quadrupole rods (Figure 1), where they come under the influence of two electric fields, one an rf frequency applied to the rods and the other a +/- d.c. voltage gradient applied to opposite pairs of rods. These fields cause the ions to move through the rods in a spiral path and only those for which the electric fields produce the correct momentum (mass x velocity) will pass through and be detected. The others, because the field is incorrect for their mass, will be discharged on the rods and will not be detected.

This is also referred to as a quadrupole mass filter because masses which are not of interest can be filtered out.

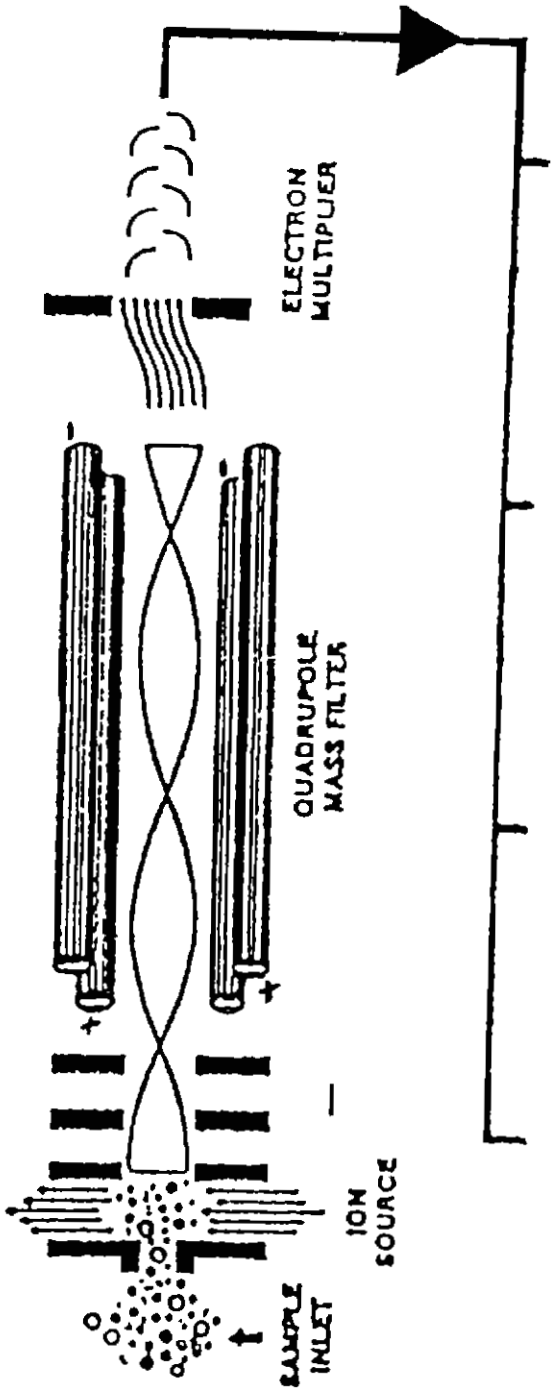


Figure.1 Quadrupole mass filter

The TSQ (Figure 2) consists of a linear arrangement of three quadrupoles (Q1, Q2, Q3) in which the first (Q1) and last (Q3) operate as mass analysers and the second (Q2) is used as a collision chamber in which ions (charged molecules) produced in the source and selected by Q1 are brought into collision with neutral gas molecules causing fragmentation of these ions. This process is referred to as collision-activated decomposition (CAD). Separation of the components of a mixture may therefore be achieved in the gas phase, after ionisation, by using Q1 to allow only a characteristic ion or group of ions to enter Q2 and to undergo fragmentation and subsequent mass analysis in Q3. Choice of ions for collision and analysis is computer-controlled and a large number of components may be analysed simultaneously.

Thus, the sample as it elutes from a gas chromatograph, is introduced into the ion source (1) and is ionised. In most cases, a gas such as methane is used to ionise the sample molecules (chemical ionisation), mainly to protonated molecular ions (ions formed by the addition of a proton). In the first quadrupole (2), the computer can be programmed to scan for the parent ions (protonated molecular ions) of drugs of interest. As the parent ions leave Q1, they are focused into Q2 (3) where they are brought into collision with energised atoms of an inert gas, usually argon. This breaks up the parent ions into characteristic daughter ions. The daughter ions pass into Q3 (4) and are mass analysed to produce a daughter ion spectrum . The spectra of parent and the corresponding daughter ions are scanned simultaneously. Finally, the ions pass to the electron multiplier (5) which functions as an amplifier.

Chemical noise can be reduced to such an extent by the TSQ that samples may even be analysed directly without prior chromatography and with little or no pre-treatment. This makes it possible, for example to detect drugs directly in blood (Brotherton & Yost, 1983).

Even greater selectivity may be attained by combining capillary column gas chromatography with a TSQ mass spectrometer. This combination extends the number of compounds which can be detected in a single sample to several hundreds. The capillary column is used to provide a preliminary separation of sample components so that the TSQ need only monitor a relatively small number of compounds at any given time.

The sophisticated software of the TSQ data system allows interactive real-time control of all operating modes. Alternatively, it is possible to sequence a particular analysis so that operating conditions are optimised for each component which is to be detected. As each component or group of components is eluted from the column, the computer automatically chooses the ionisation method, collision energy and daughter ions appropriate for optimum detection. This is the mode of operation which is used to detect drugs in blood and saliva samples from drivers. The net result is the production of a unique "finger print" for each drug of interest which provides positive identification. A data base has been established for approximately 200 common drugs which are likely to influence the ability to drive a motor vehicle. Most of these drugs may be readily detected at normal therapeutic levels in small (1-2 ml) samples of blood or saliva. Identification is based on computer matching with a reference

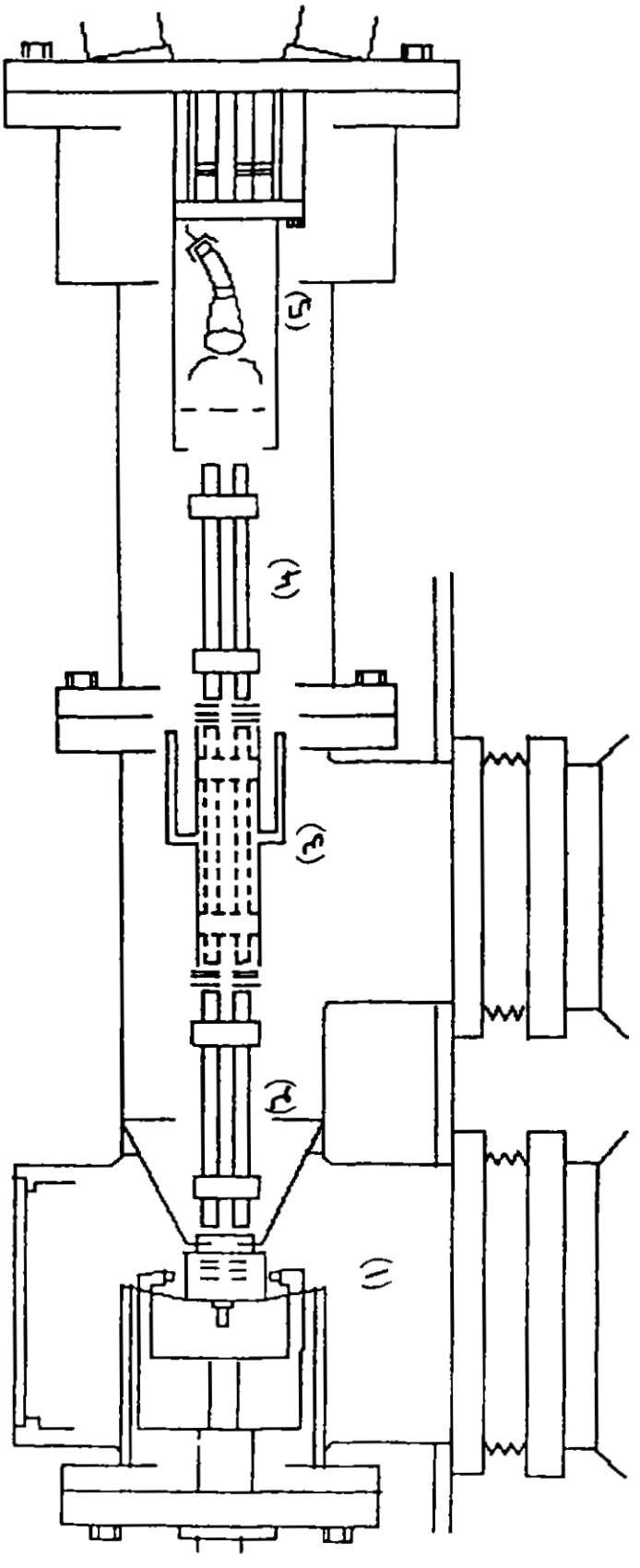


Figure 2. The Finnegan Triple Stage Quadrupole mass spectrometer

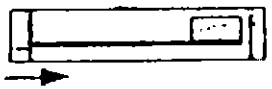
library of the daughter ion spectra of the 200 or so target drugs. It has now proved possible to automate the analytical procedure.

2.2 Processing of the samples

The processing of saliva samples parallels that already developed for the detection of drugs in venous blood. Blood samples (~ 7 ml) for drug analysis are collected into silanized glass McCartney bottles (10 ml) with teflon-lined screw caps and containing an aqueous solution (500 mL) of potassium oxalate (5% w/v) and sodium fluoride (1% w/v). After addition of the blood, the contents of the bottle are shaken vigorously and then frozen and stored at -20°C until analysed.

Whole saliva is a complex mixture of parotid, submandibular, sublingual and minor salivary gland secretions. There are additional problems which relate to the small size of the sample and the presence of food particles, enzymes, bacteria, leucocytes and sloughed epithelial cells. To overcome these difficulties we have used the saliva collection device (Epitope, Beaverton, Or. USA). This is basically an absorbent swab surrounded by a porous plastic membrane. The respondent places the swab in his/her mouth in the area between the back teeth and the side of the mouth (near the opening of Stenson's duct). The swab tastes slightly salty and will stimulate parotid saliva flow. The swab needs to be in the respondent's mouth for 2-3 minutes. When this time has elapsed, the subject places the swab in a plastic vial and breaks off the stick, which is discarded. The vial is then capped. The vial contains 0.2 ml of a blue buffer solution which also contains a preservative which confers sample stability, and this is also taken up into the swab. The procedure is shown in Figure 3. After collection, the vials are kept cool and stored and transported to Sydney under refrigeration where they are held at -15°C until analysed. In the laboratory, the vials are inverted, the nipple is broken off (revealing a small hole) and the whole vial is centrifuged to remove the saliva sample. Using this method, about 100 ml of a sample, which is essentially an ultrafiltrate of parotid saliva, can be obtained.

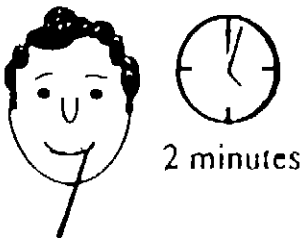
SPECIMEN COLLECTION PROCEDURE

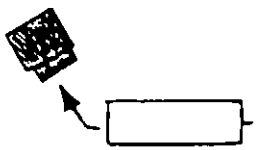


1. Open collection pad pouch at the stick end and remove the collection pad.

2. Place the pad into the mouth between the lower cheek and gums. Stroke the pad back and forth 20 times to moisten, then hold the pad in place for 2 minutes with the mouth closed.

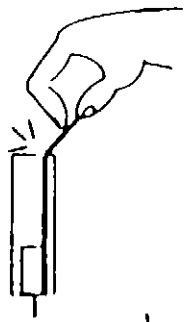
3. While the pad is held in the patient's mouth, remove the specimen vial from the pouch. Be careful <u>not</u> to break off the tip at the bottom of the vial.





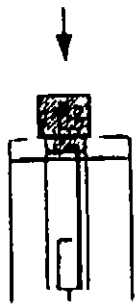
4. Holding the vial upright, remove the cap by bending ti over and away from the vial. This motion facilitates cap removal and prevents spillage of the preservative solution contained in the vial.

5. After the 2 minute time period has elapsed, remove the pad from the mouth. Insert the pad to the bottom of the vial.



6. Bend the pad handle until the upper half of the stick snaps off.

7. Replace the cap into the vial. Be sure to press the cap <u>completely</u> into the vial until it is fully closed. You will feel and hear a final "click" when the cap is all the way in. This indicates that the cap has been correctly seated into the vial.



8. Return the vial to the laboratory for specimen processing.

Figure 3. Saliva sample collection procedure

2.2.1 Extraction of samples (Figure 4)

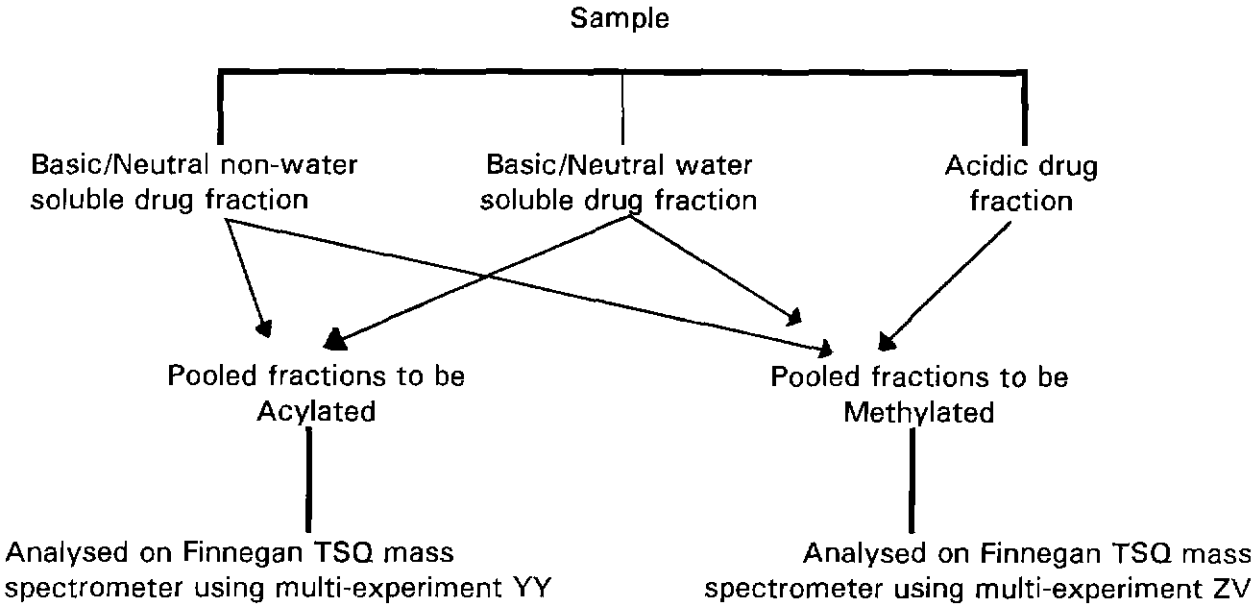


Figure 4. Processing of body fluid samples for GC-MS-MS analysis

2.2.1.1 Basic and neutral drugs

Saliva (~0.2 ml) and internal standard (50 mL), deuterated drug mixture (ibuprofen-d2, 44ng/mL; phenytoin, 22 ng/ml and nordiazepam-d4, 4.4ng/ml in ethanol) are placed in a culture tube (100 x 12.5 mm) with a teflon-lined screw cap and mixed gently on a rotorack for 5 min. An aliquot of saliva (0.1 ml) is transferred to a culture tube (100 x 12.5 mm) for extraction as described under acidic drugs. To the remainder is added borate buffer pH 9.5 (2 ml). Chlorobutane (5 ml) is then added and the tube contents are mixed on a rotorack for 15 min and then centrifuged for 10 min. The organic layer is transferred to a clean culture tube (100 x 15 mm), triethylamine (40 ml) is added and the mixture evaporated to dryness under a gentle flow of nitrogen at room temperature (Extract A). To the aqueous layer is added 10 M sodium hydroxide solution (400 mL) and dichloromethane (8 ml) and the tube is shaken vigorously by hand. Further mixing is carried out on a rotorack for 15 min and the tube is centrifuged for 10 min. The aqueous layer is discarded and the organic layer is transferred to the tube containing Extract A. The contents of this tube are evaporated to dryness (Extract A + B).

2.2.1.3 Acidic drugs

To the aliquot of saliva (0.1 ml) reserved above is added sodium acetate buffer pH 4.5 (0.5 ml) and Chlorobutane (3 ml) and the contents of the tube are mixed (10 min) and centrifuged as described previously. The organic layer is transferred to a clean culture tube (100 x 12.5 mm) and evaporated to dryness under nitrogen at room temperature (Extract C).

transferred to a clean culture tube (100 x 12.5 mm) and evaporated to dryness under nitrogen at room temperature (Extract C).

The complete extraction of 20 saliva samples by these methods can be carried out by one person in approximately 7-8 hours, the last two hours of which are taken up by slow evaporation of extracts at room temperature.

2.2.2 Derivatisation of extracts for GC-MS-MS analysis

Because of the instability of certain drugs, some must be acylated and others methylated in order to be detected by the instrument. Extracts A + B are dissolved in chloroform (1 ml) and an aliquot (500 ml) is transferred to the tube containing Extract C and evaporated to dryness. The residue is taken up in ether/methanol (9:1, 200 ml) and a solution of diazomethane (200 ml) is added. The mixture is allowed to stand at room temperature for 10 min and is then evaporated to dryness. The residue is reconstituted in chloroform (20 ml) and 2ml is injected into the GC-MS-MS for analysis of basic, acidic and neutral drugs using the multiple experiment descriptor ZV.

The remainder of the Extract A + B is reconstituted in chloroform (100 ml), and is transferred to a reactivial to which trifluoroacetic anhydride (100 ml) is added. The vial is tightly capped using a teflon-faced septum and is heated at 70°C for 10 min. The vial is cooled to room temperature and the contents are evaporated very gently to dryness under nitrogen. The residue is analysed on the same day after reconstitution in chloroform (20 ml). An aliquot (2 ml) is injected into the GC-MS-MS and acylated drugs are analysed using multiple experiment descriptor YY.

2.2.3 GC-MS-MS analysis

Analyses are carried out on a Finnigan TSQ-46 triple stage quadrupole gas chromatograph/mass spectrometer (GC-MS-MS). A fused silica bonded phase Ultra 1 capillary column (12.5 m x 0.2 mm i.d. x 0.3 mm film thickness, Hewlett Packard) is inserted directly into the ion source of the TSQ. The column is held at 80°C for 1 min after the splitless injection and then programmed at 25°C/min to 300°C. Helium (2 ml/min) is used as the carrier gas and methane is added through the makeup valve to give a source pressure of 133 Pa. Positive and negative ions are generated at an ion source temperature of 140°C with an electron beam energy of 100 eV.

The TSQ is operated in the daughter ion mode, using argon as the collision gas at a pressure of 0.2 Pa and the collision energies of -25 eV for positive ions and + 30 eV for negative ions.

The methylated basic and acidic drug extracts (Extract B + C) are analysed using multiple experiment descriptor ZV. The trifluoroacylated extracts are analysed using multiple experiment descriptor YY. A summary of the drugs which can be scanned for in multi-experiments is shown in Appendix 1.

Data processing is carried out using custom-written Incos data selection procedures. These procedures detect peaks in the selected reaction monitoring chromatograms produced by the multiple experiment descriptors and produce a hard copy of any chromatogram which contains a peak whose abundance exceeds 10 standard deviations above average noise level.

Analytical sensitivity and retention time reproducibility are monitored by the inclusion of deuterium-labelled standards for several key drugs. Any marked variation in sensitivity or retention time of these standards in a particular batch of samples results in those samples being re-extracted and re-analysed.

2.2.4 Selection of the target drugs

These have been selected on the basis of the frequency of their prescription under the Australian Pharmaceutical Benefits Scheme, the extent of their perceived illicit use in Australia and their potential to disrupt human skills performance. In addition, two frequently used drugs, aspirin and paracetamol, with no proven effects on performance, have been included, as well as caffeine and cotinine, a metabolite of nicotine.

Drugs which cannot be detected, either because of their very small molecular size, water solubility or dose, include chloral hydrate, sodium valproate and lysergic acid diethylamide.

It should also be noted that the presence of D^9-tetrahydrocannabinol (THC) in saliva can only be used to infer that the individual has used cannabis recently. THC is so lipophilic that once it has gained access to the blood, it cannot be transferred back to saliva. Nevertheless, THC often persists longer in saliva than in blood (Cairns et al., 1990).

3. RESULTS

3.1 Preliminary experiments

These were undertaken to establish whether the sample processing and analytical techniques which have been developed for blood were suitable for the detection of drugs in saliva. As indicated in the Methodology section, different extraction procedures must be used for basic/neutral non-water soluble , basic/neutral water soluble and acidic drugs. These are summarised in Figure 5.

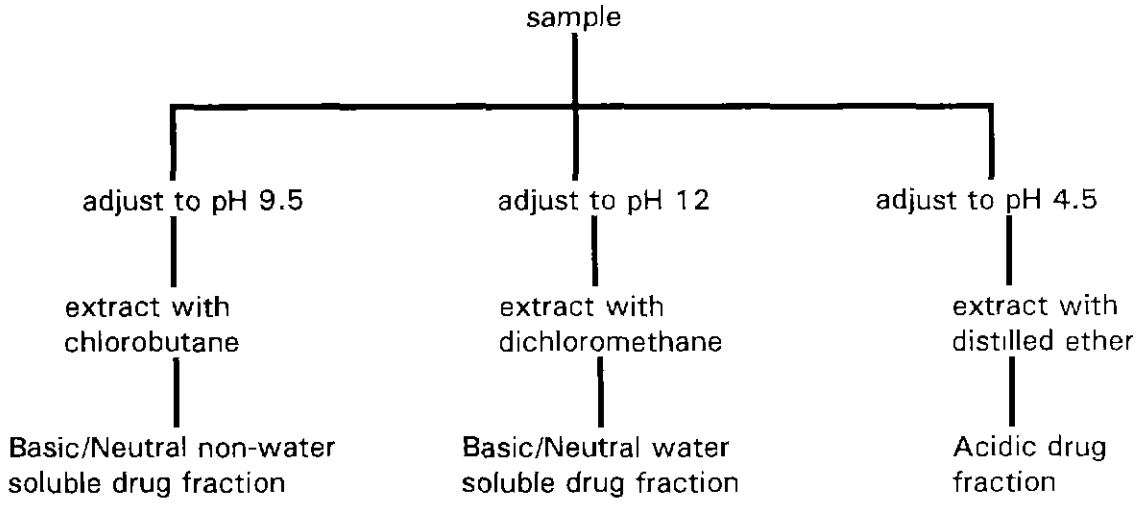


Figure 5. Extraction of the sample

3.1.1 The ability to detect diazepam, morphine and pentobarbitone in saliva

Examples of drugs which fall into the above categories are diazepam, morphine and pentobarbitone. These drugs were all detectable. The result for diazepam was considered to be encouraging because this drug has a very low plasma : saliva ratio (about 5%). At this stage of the investigation it was also established that the following drugs could be detected in saliva:

Stimulants:	amphetamine, caffeine
Benzodiazepines:	diazepam, nordiazepam, temazepam, oxazepam
Anticonvulsants:	carbamazepine,
Hypnotics:	pentobarbitone, tetrahydrocannabinol
Opioids:	morphine
α-Adrenoceptor agonists:	methoxyphenamine

3.1.2 Modified preliminary processing of the saliva samples

Because of the very small size of the saliva samples (~ 100 µl), a modified method for the preliminary processing of the samples was investigated. This is shown in Figure 6. The sample extracts are then processed as indicated in Figure 4.

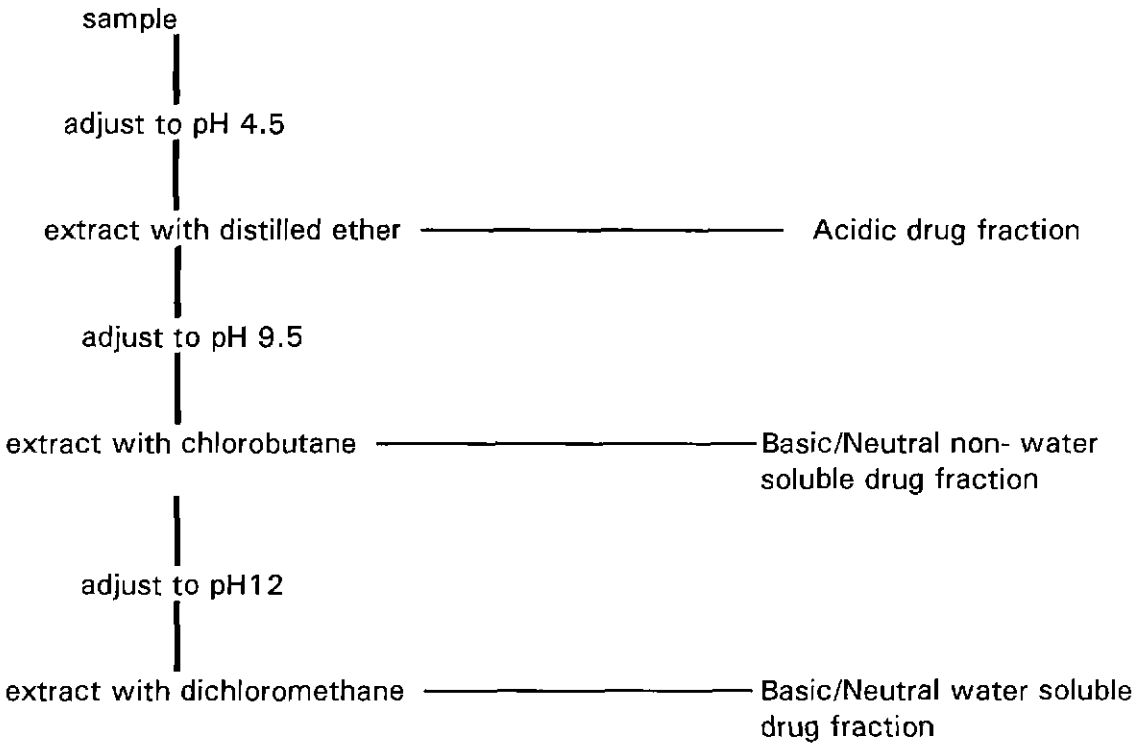


Figure 6. Modified preliminary processing of the sample.

3.1.3 Detection of pentobarbitone in saliva over time after pentobarbitone administration to a subject.

Saliva samples (approximately 1 ml) were obtained from a subject before and at 60 and 90 minutes after an oral pentobarbitone (200 mg) dose. These samples were processed as indicated above and analysed on the TSQ. The mass chromatograms are shown in Figures 7 - 9. From these it can be seen that a pentobarbitone peak was detected at 60 min with a smaller peak at 90 min. This is in accord with the expected blood levels at 60 and 90 minutes.

Figure 7 **Mass chromatogram of the saliva of a subject before he took an oral dose of pentobarbitone (200mg). The expected position of the pentobarbitone peak is indicated.**

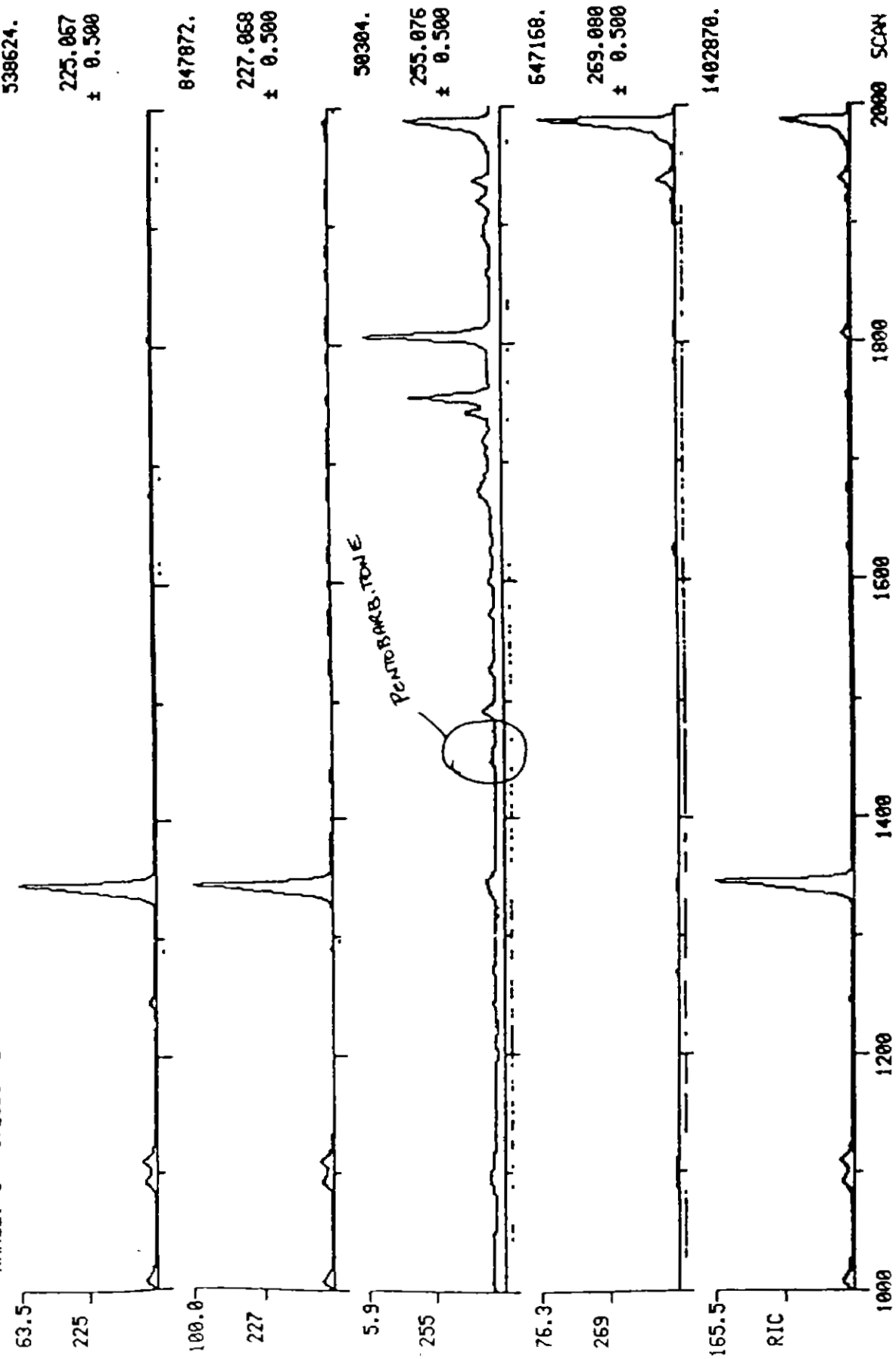


Figure 8 **Mass chromatogram of a saliva extract from a subject taken 60 min after an oral dose of pentobarbitone (200mg). The position of the pentobarbitone peak is indicated.**

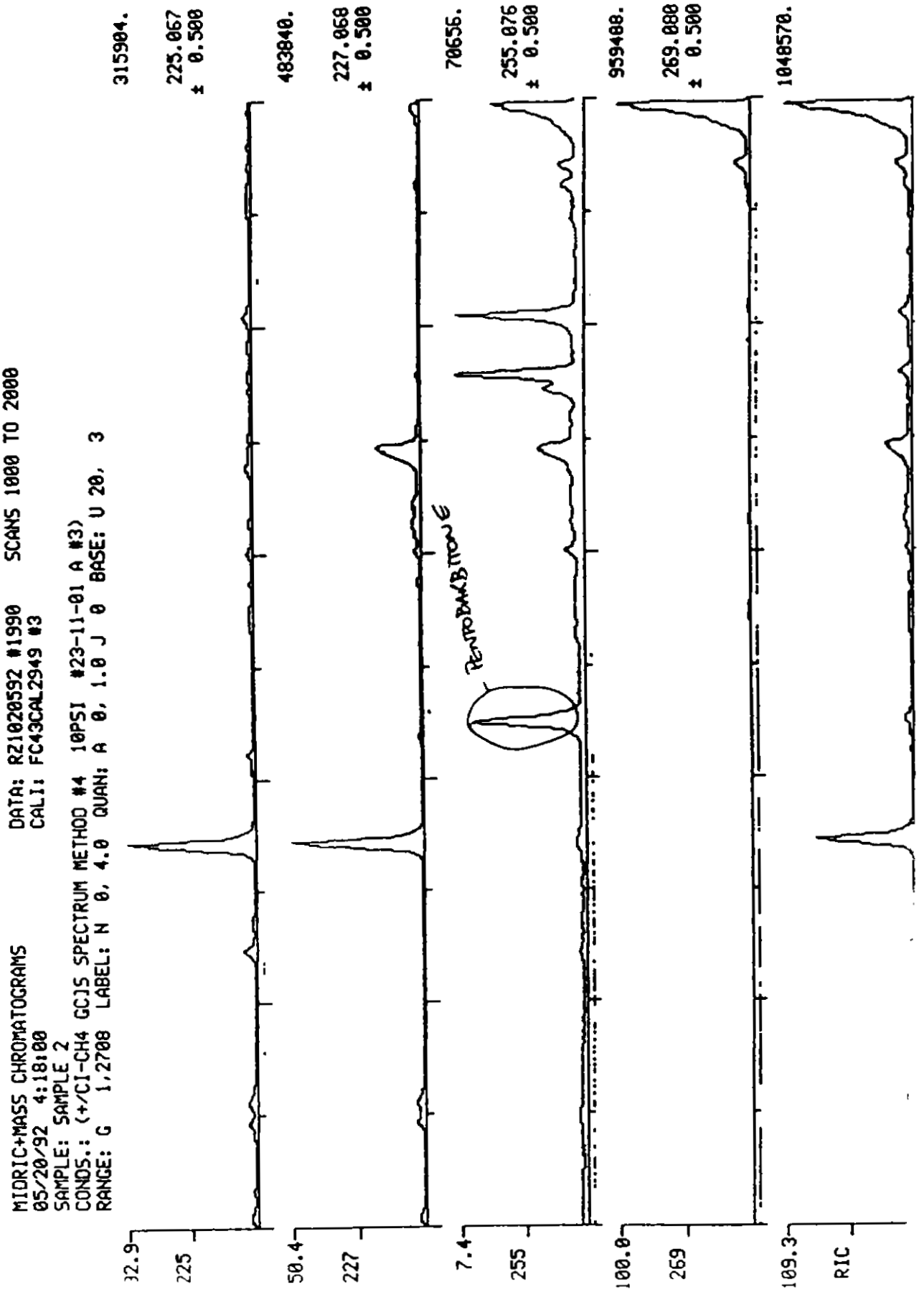
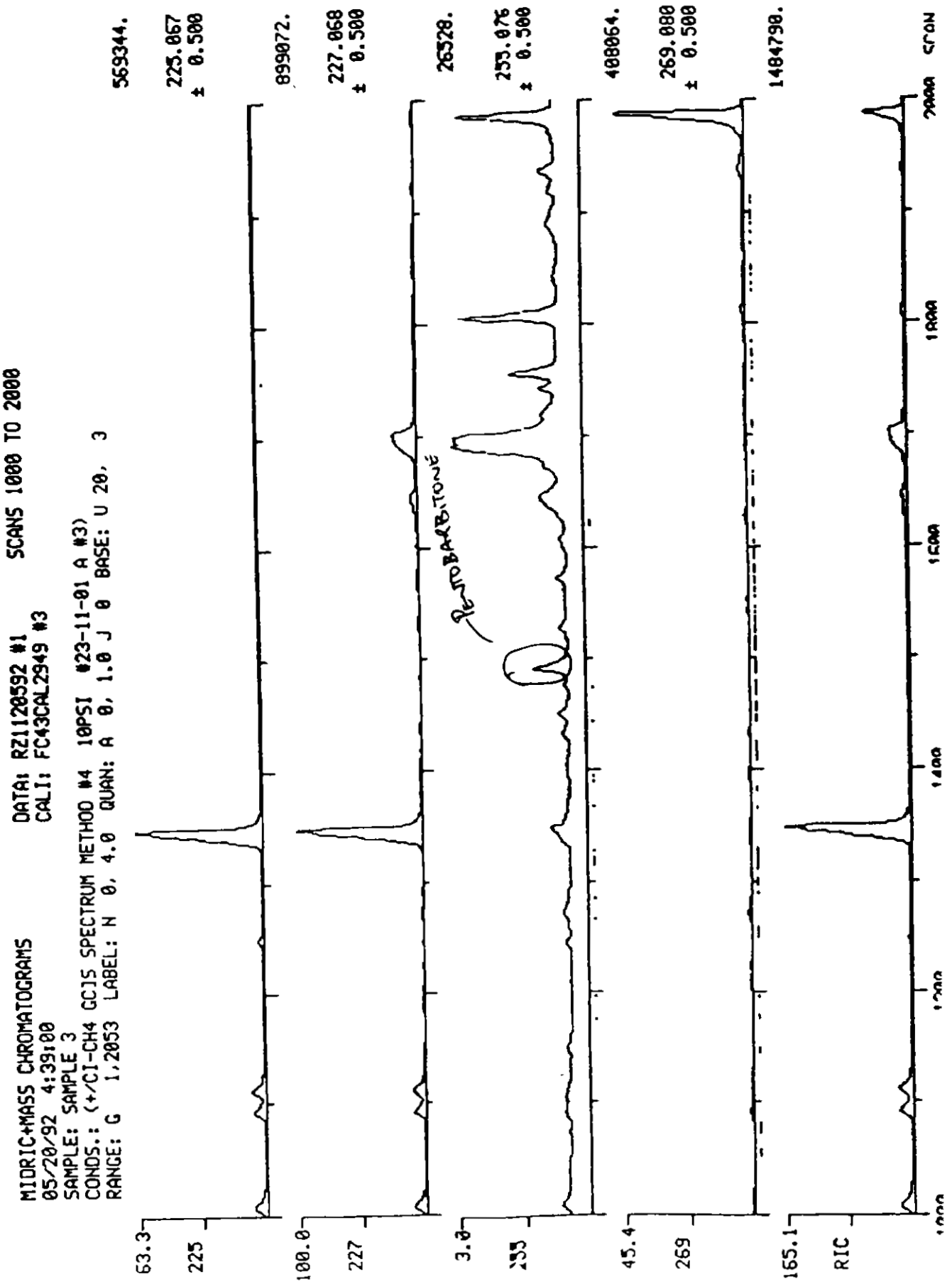


Figure 9 **Mass chromatogram of a saliva extract from a subject taken 90 min after an oral dose of pentobarbitone (200mg). The position of the pentobarbitone peak is indicated.**



3.1.4 Detection of benzodiazepines in saliva.

Saliva samples (approximately 1 ml) were obtained from subjects who had received oral doses of diazepam (20 mg) 60 min earlier. These were processed by the basic/neutral non-water soluble extraction procedure (Figure 5) with a deuterated nordiazepam internal standard. The samples were analysed by GC-MS-MS and quantified against the deuterated internal standard. The results are shown in Figures 10-12 and summarised below:

Subject	Nordiazepam (ng/ml)	Diazepam (ng/ml)	% Recovery
1	2.4	4.2	58.5
2	16.0	8.1	78.5
Blank standard			58.1

3.1.5 Recovery of drugs from spiked saliva samples

In this series of experiments, drugs of interest were added to saliva. The samples were then processed and the extracts were injected into the GC-MS-MS. The results are shown in the Figures 13 - 37. All the drugs tested were readily detectable in saliva.

Figure 10 **Detection of diazepam and nordiazepam in saliva. Mass chromatogram showing the deuterated internal standard (D4-nordiazepam) peak.**

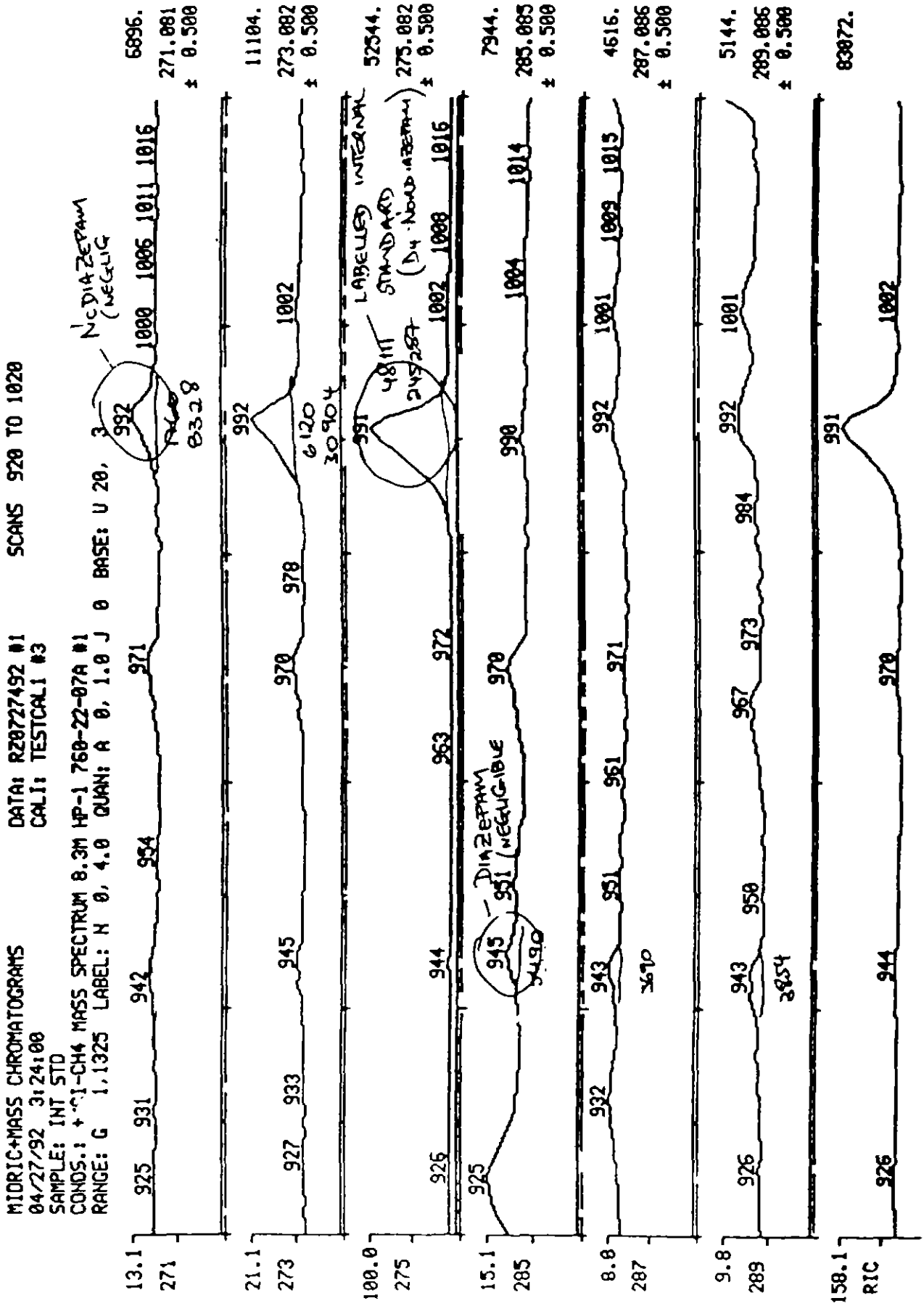


Figure 11 **Mass chromatogram of a saliva extract from a subject (No: 1) who had taken an oral dose of diazepam (20mg) one hour previously. The positions of the diazepam and nordiazepam peaks are indicated, together with that of the internal standard.**

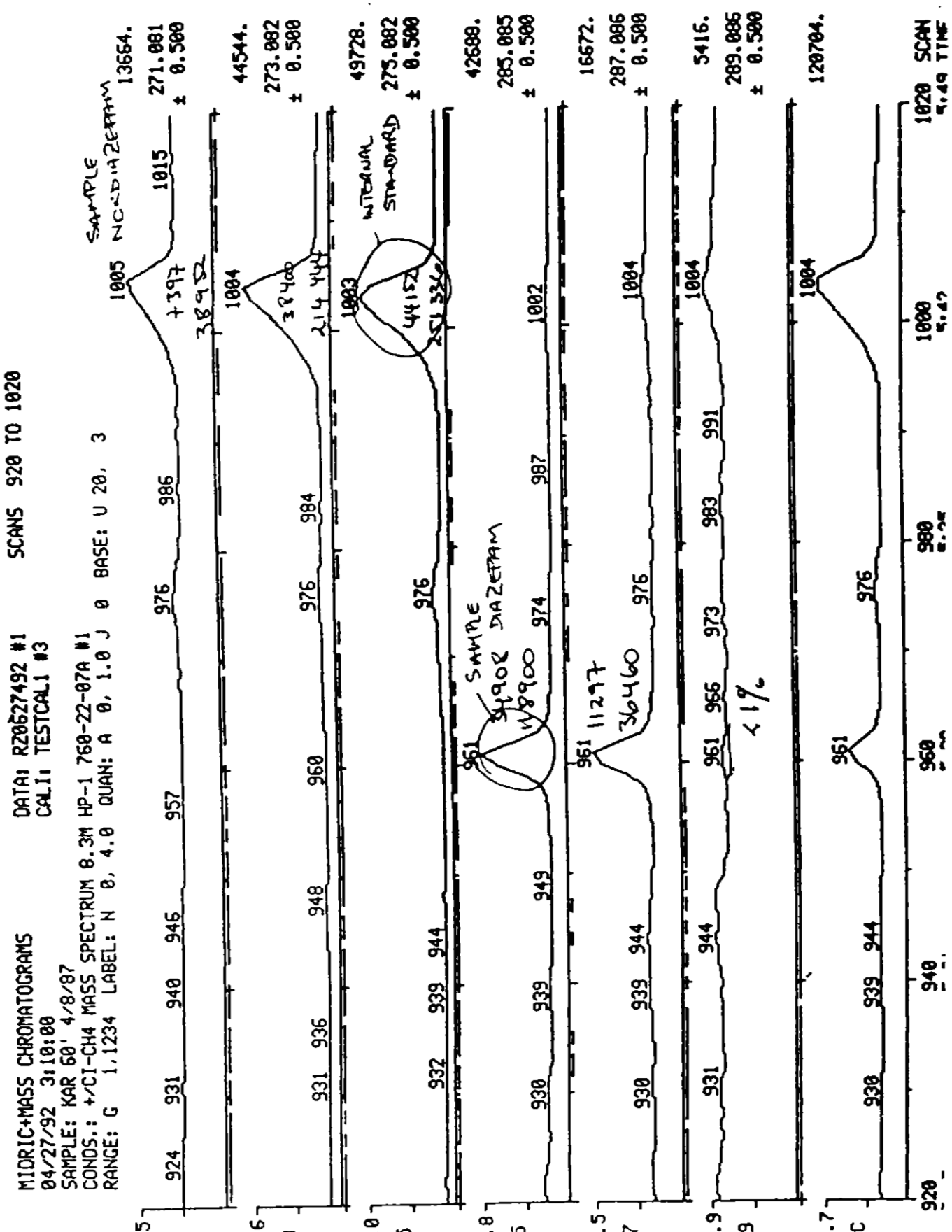


Figure 12 **Mass chromatogram of a saliva extract from a subject (No: 2) who had taken an oral dose of diazepam (20mg) one hour previously. The positions of the diazepam and nordiazepam peaks are indicated, together with that of the internal standard.**

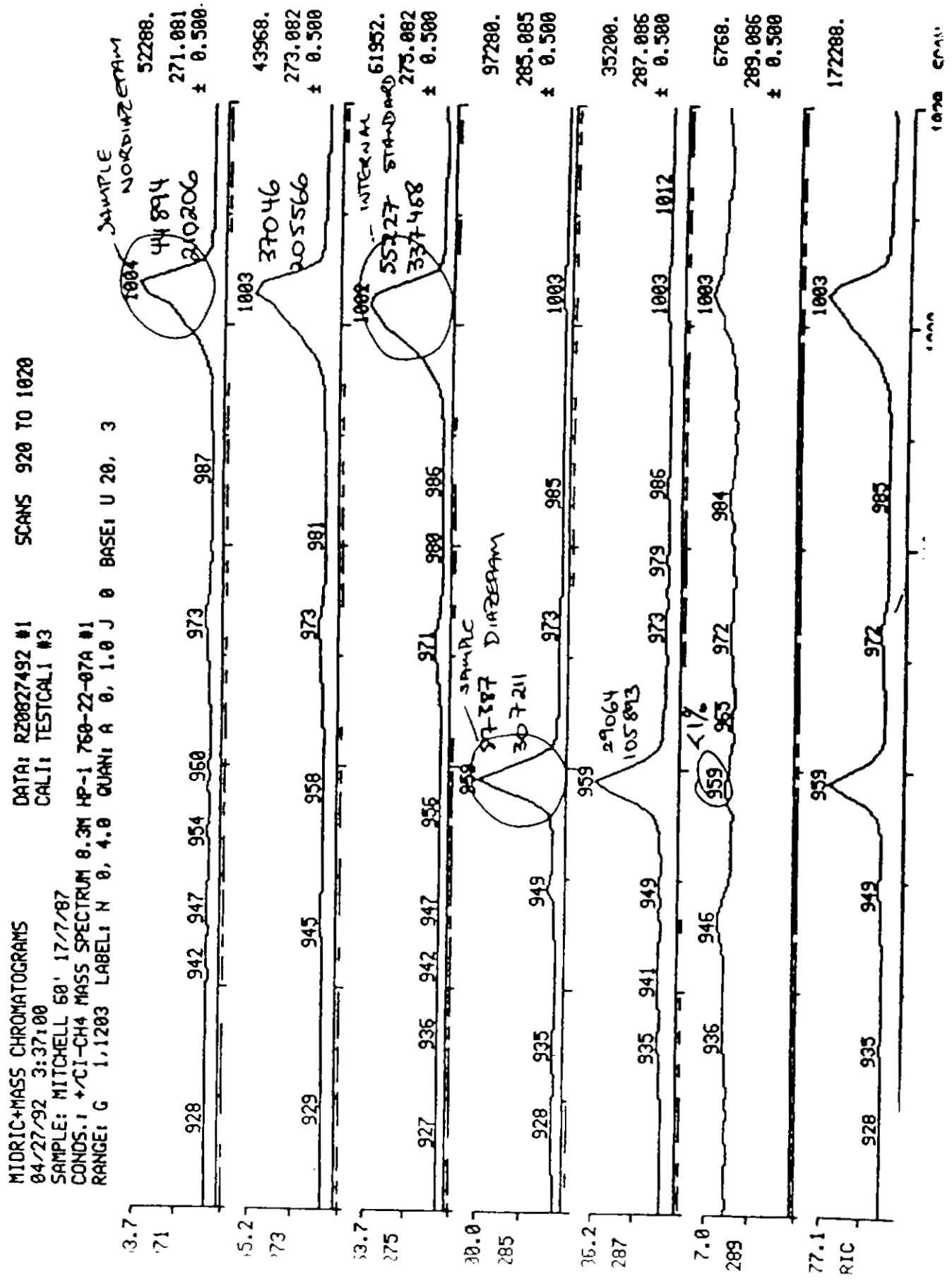


Figure 13 **Total ion chromatogram of the drug mixture standard in multi-experiment YY. These drugs were added to saliva & the samples were processed before introduction into the GC-MS-S. Drug peaks are indicated.**

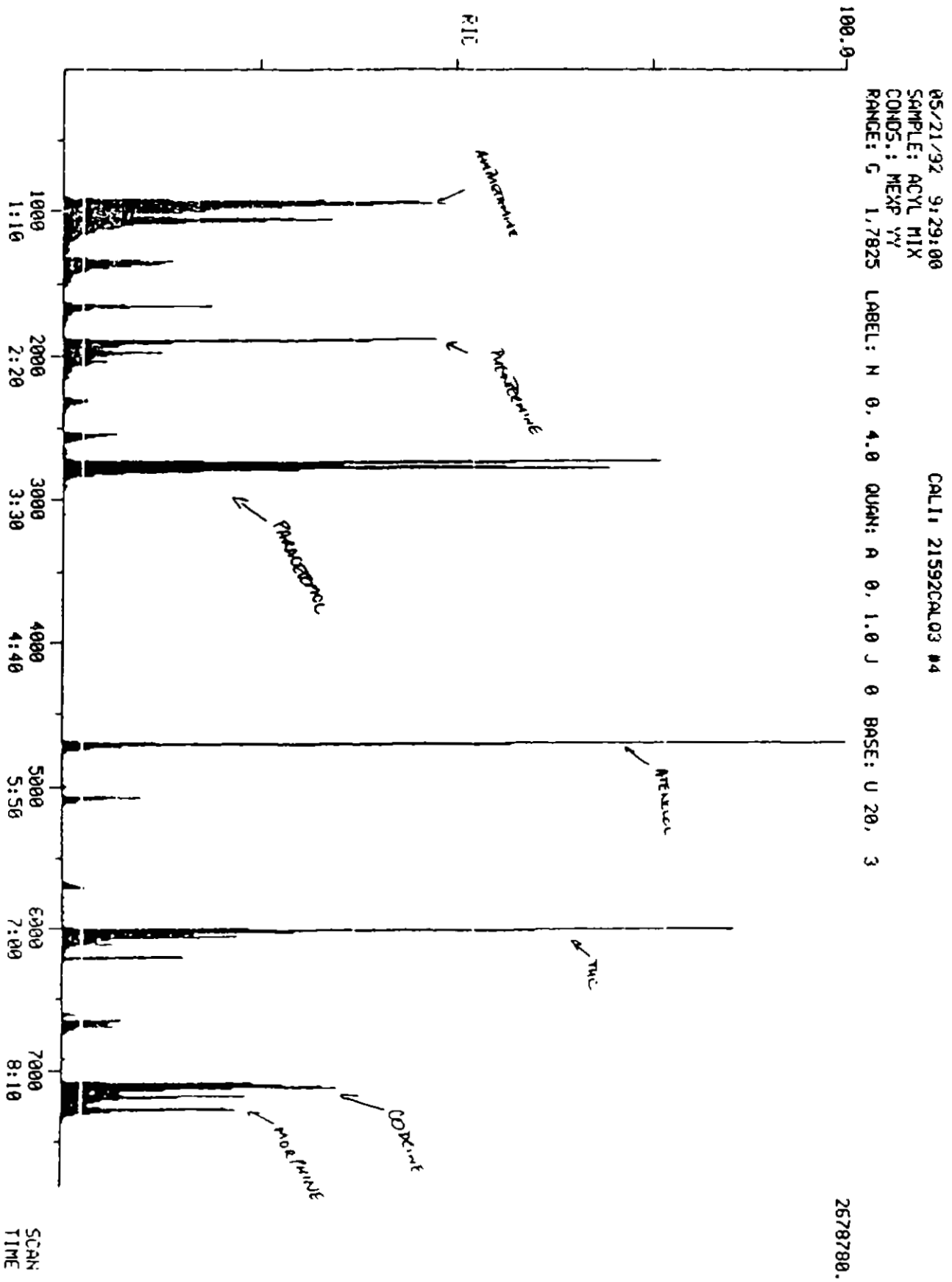


Figure 14 **Mass chromatogram of amphetamine which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.**

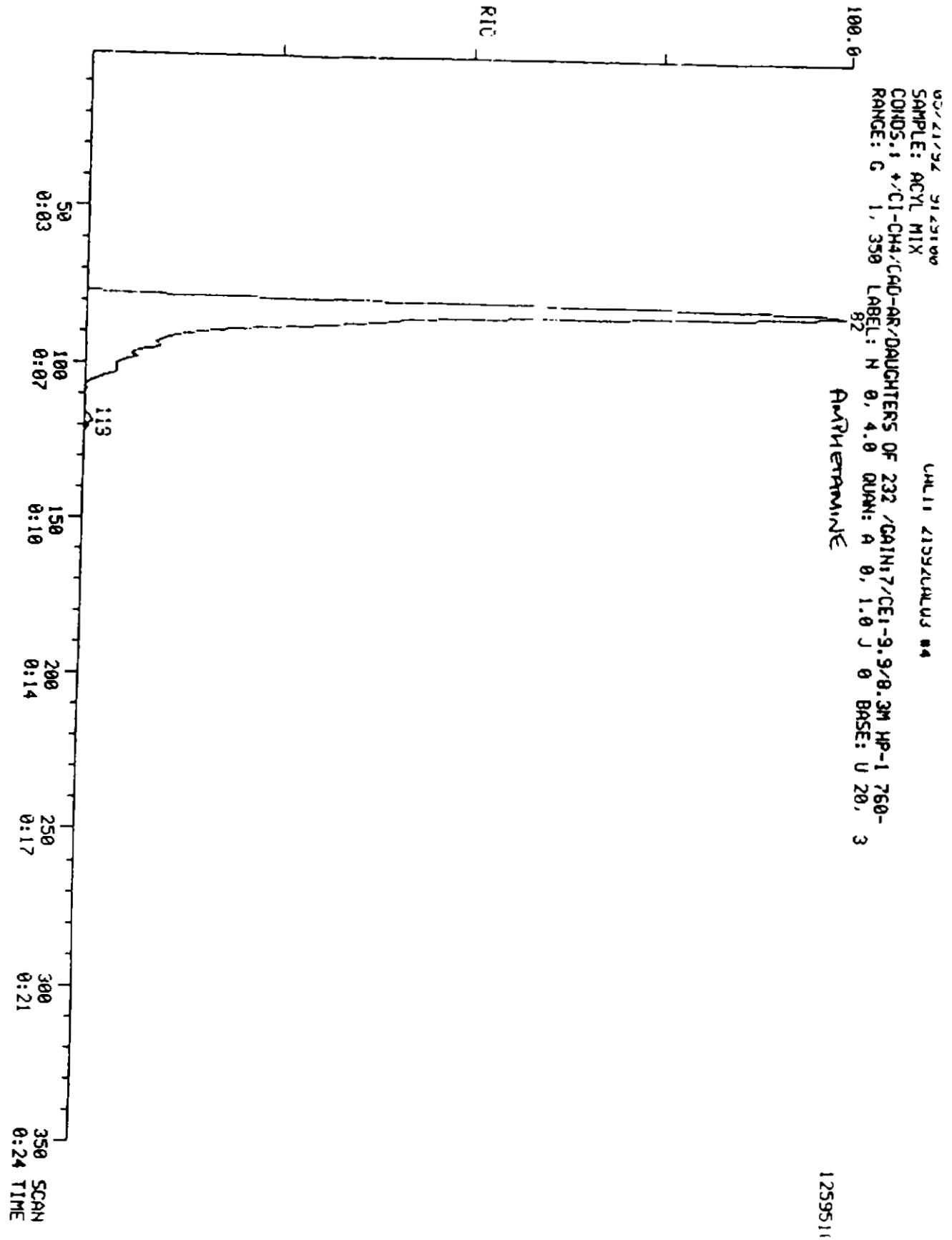


Figure 15 **Mass chromatogram of the antihistamine, tranylcypromine, which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.**

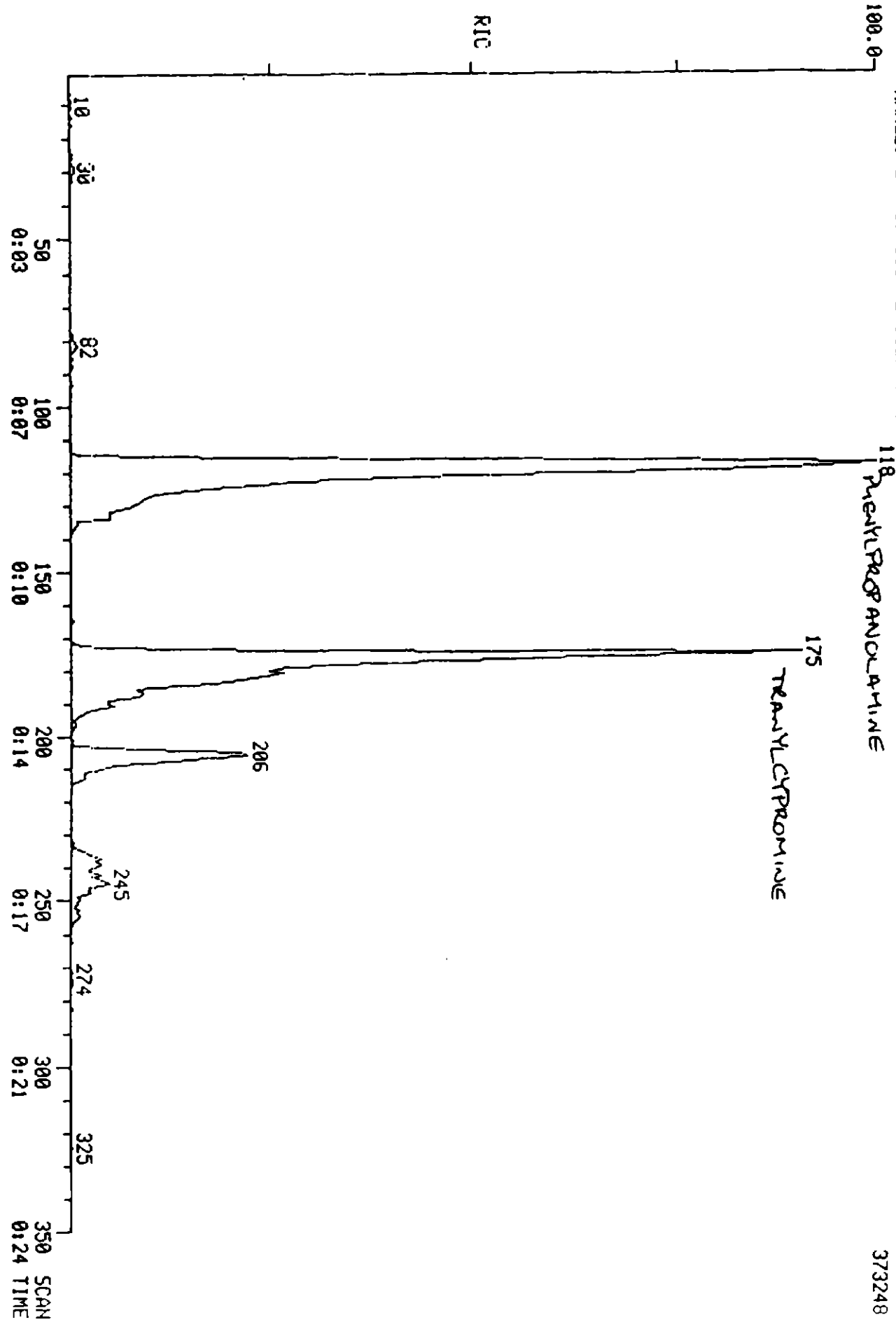
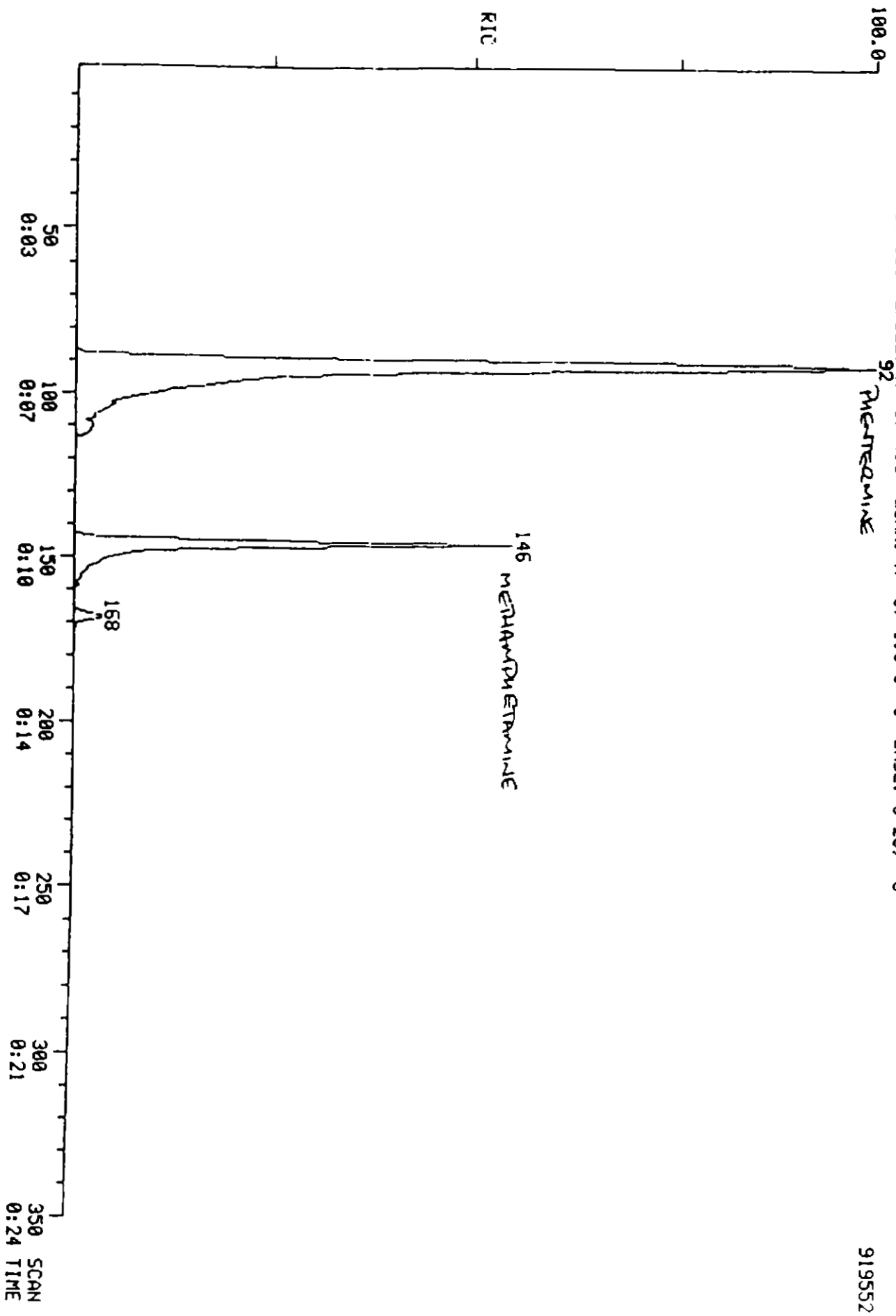


Figure 16 Mass chromatogram of the stimulant methylamphetamine, which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.



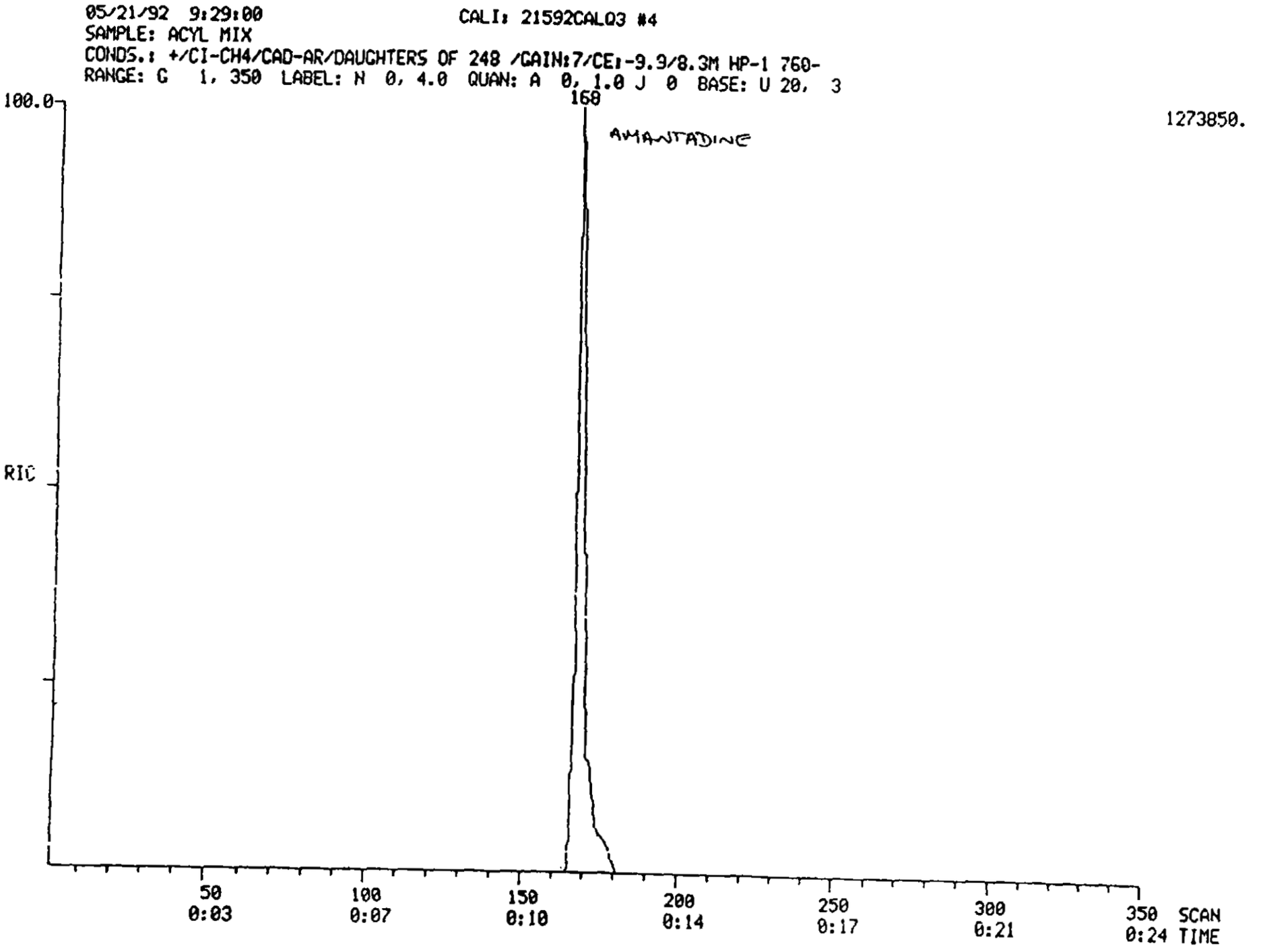


Figure 17 Mass chromatogram of amantadine which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.

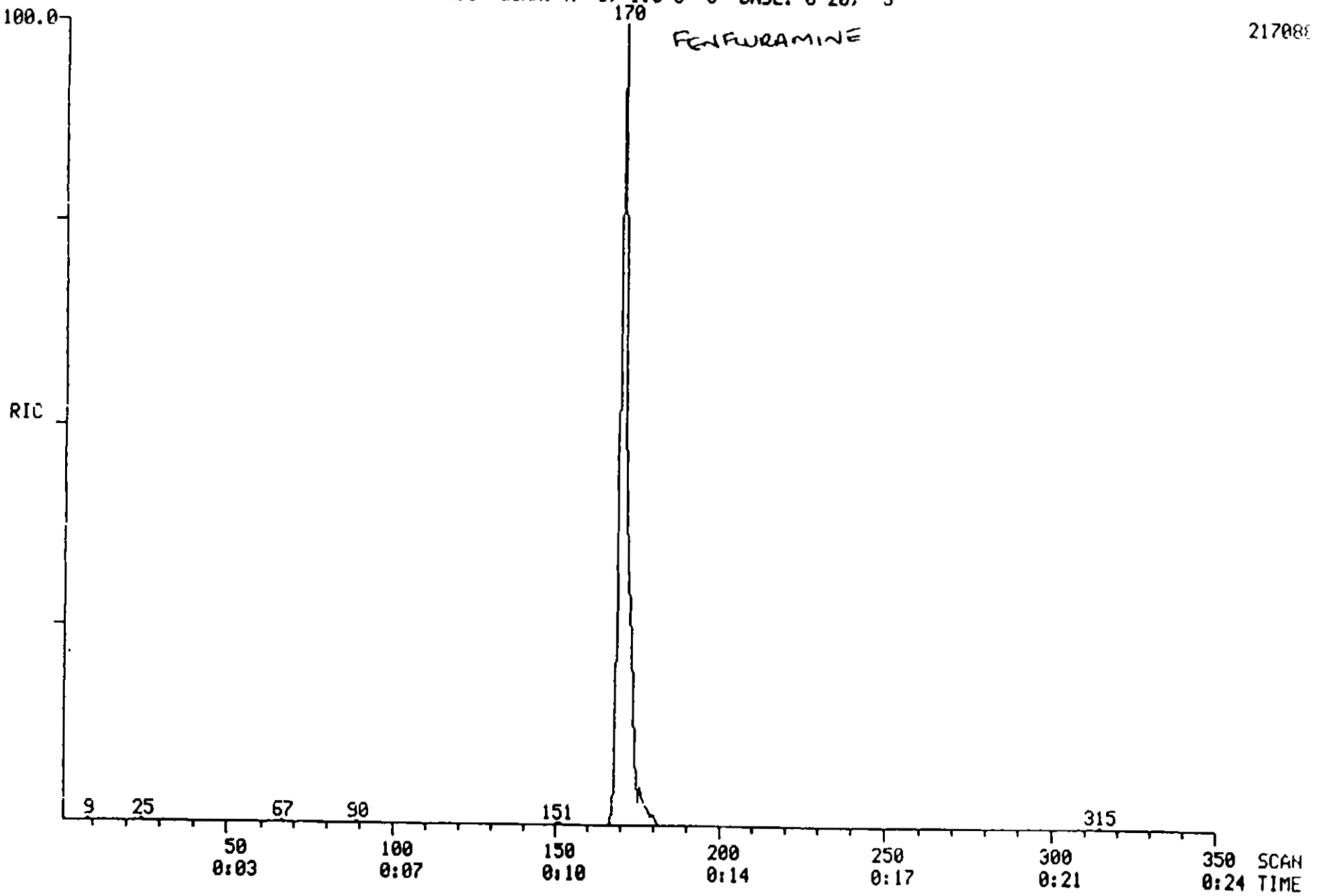


Figure 18 Mass chromatogram of the anorexiant drug, fenfluramine, which was added to a saliva sample which was then processed and introduced into the GC-MS-MS.

Figure 19 **Mass chromatogram of the stimulant drug, ephedrine, which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.**

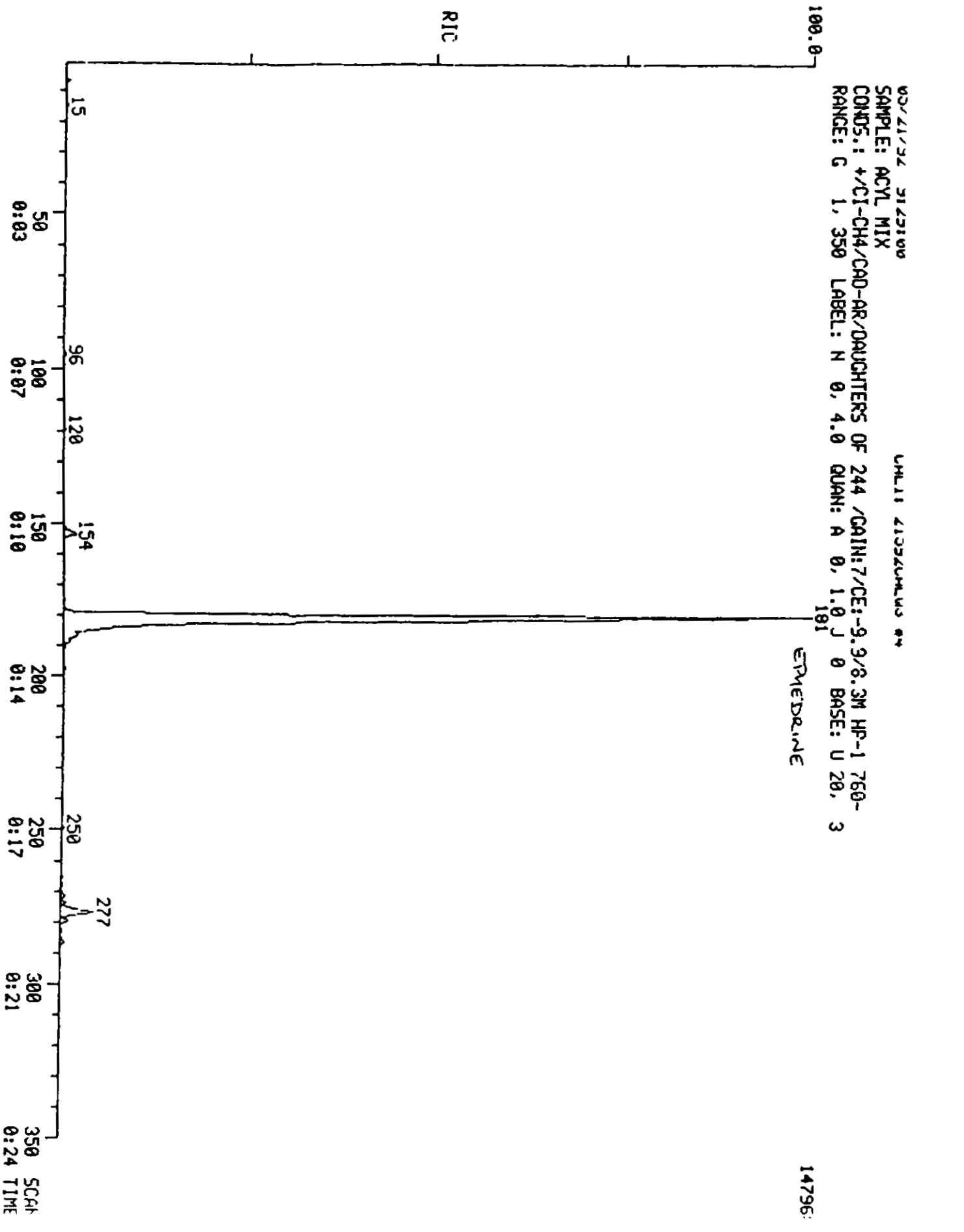


Figure 20 **Mass chromatogram of the expectorant drug, guiaphenesin, which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.**

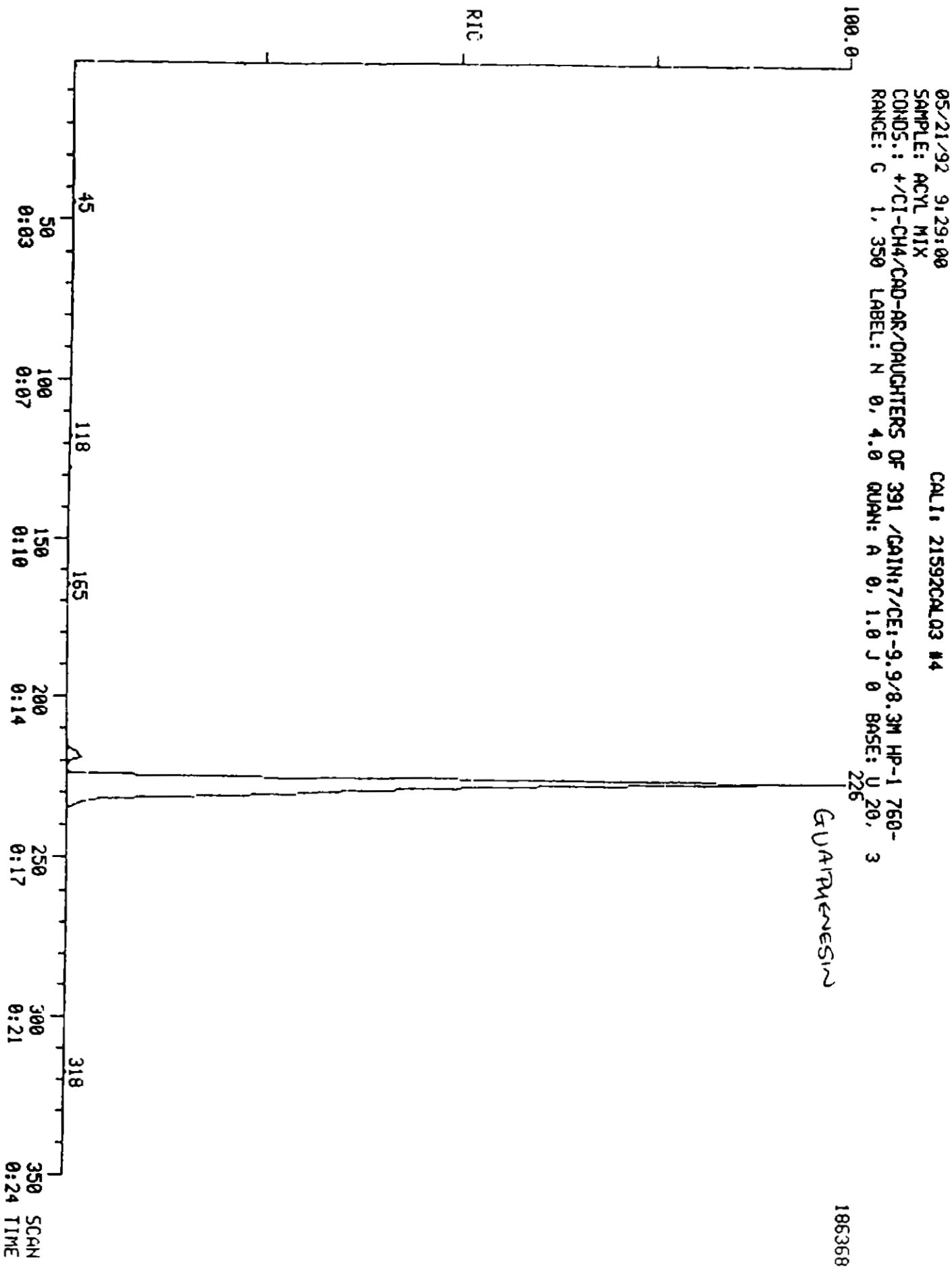


Figure 21 **Mass chromatogram of the antipyretic analgesic, paracetamol, which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.**

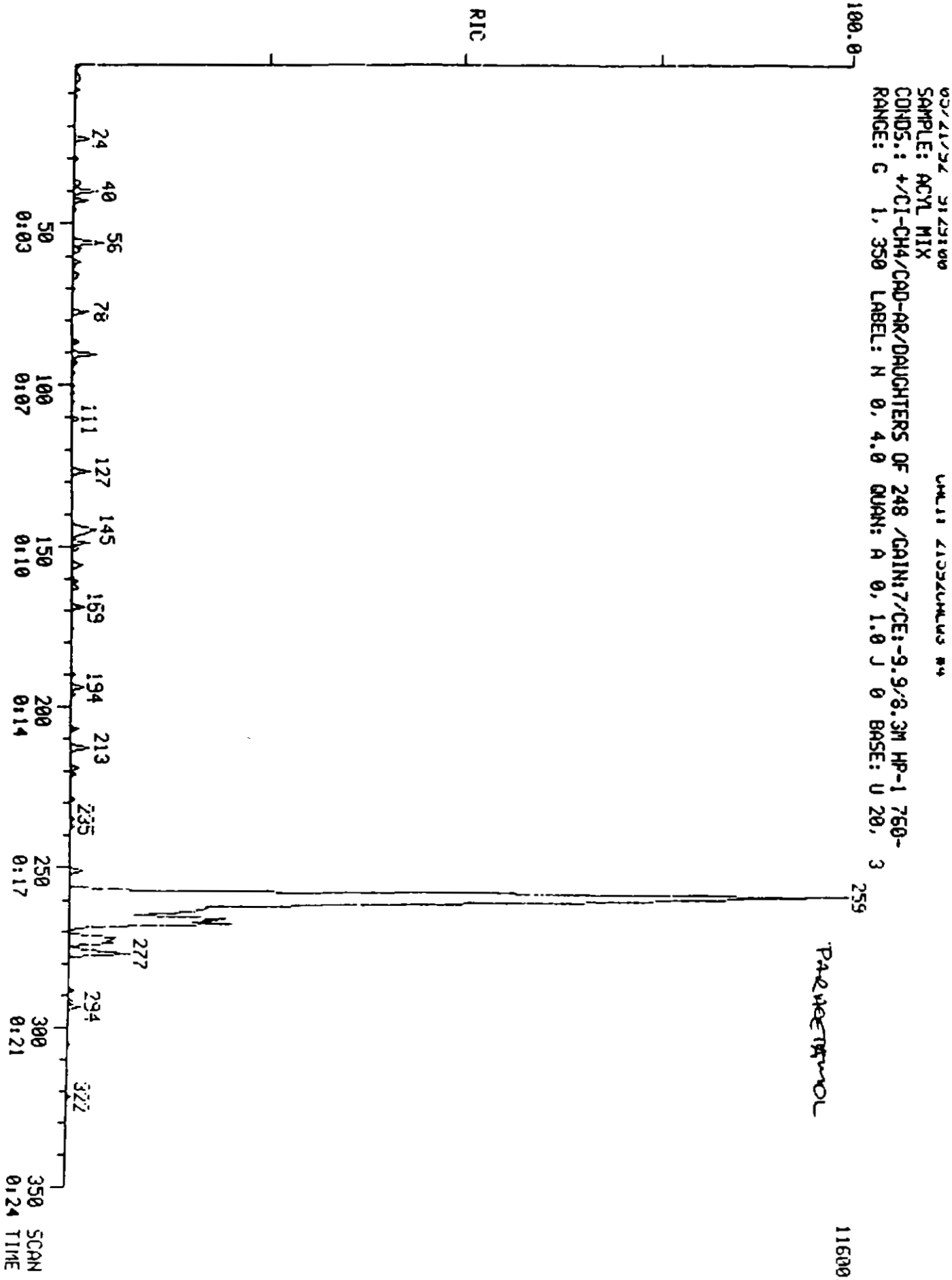


Figure 22 **Mass chromatogram of the anti-arrythmic drug, mexiletine, which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.**

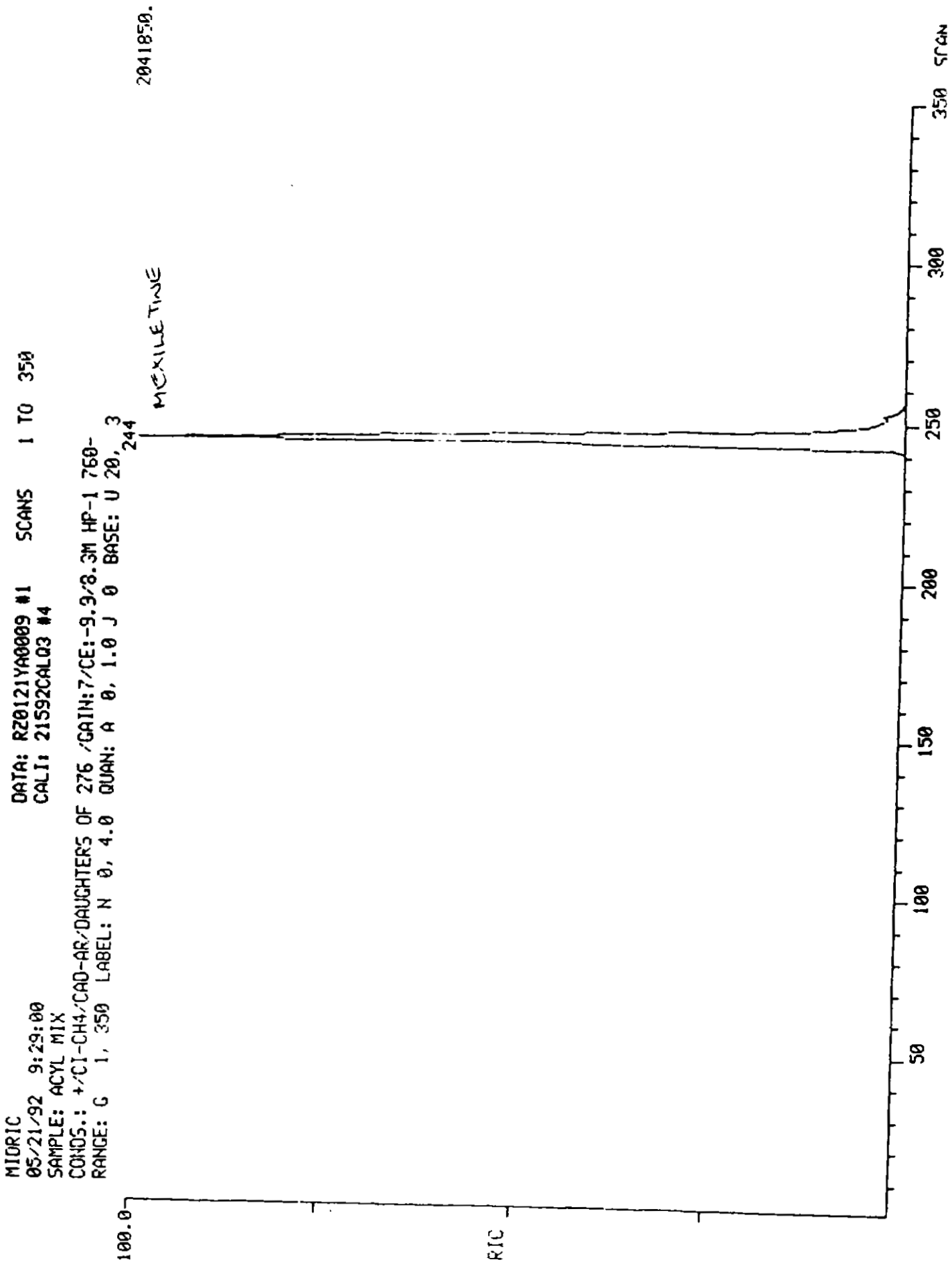


Figure 23 **Mass chromatogram of tetrahydrocannabinol which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS. the peak for the internal standard of deuterated THC can also be seen.**

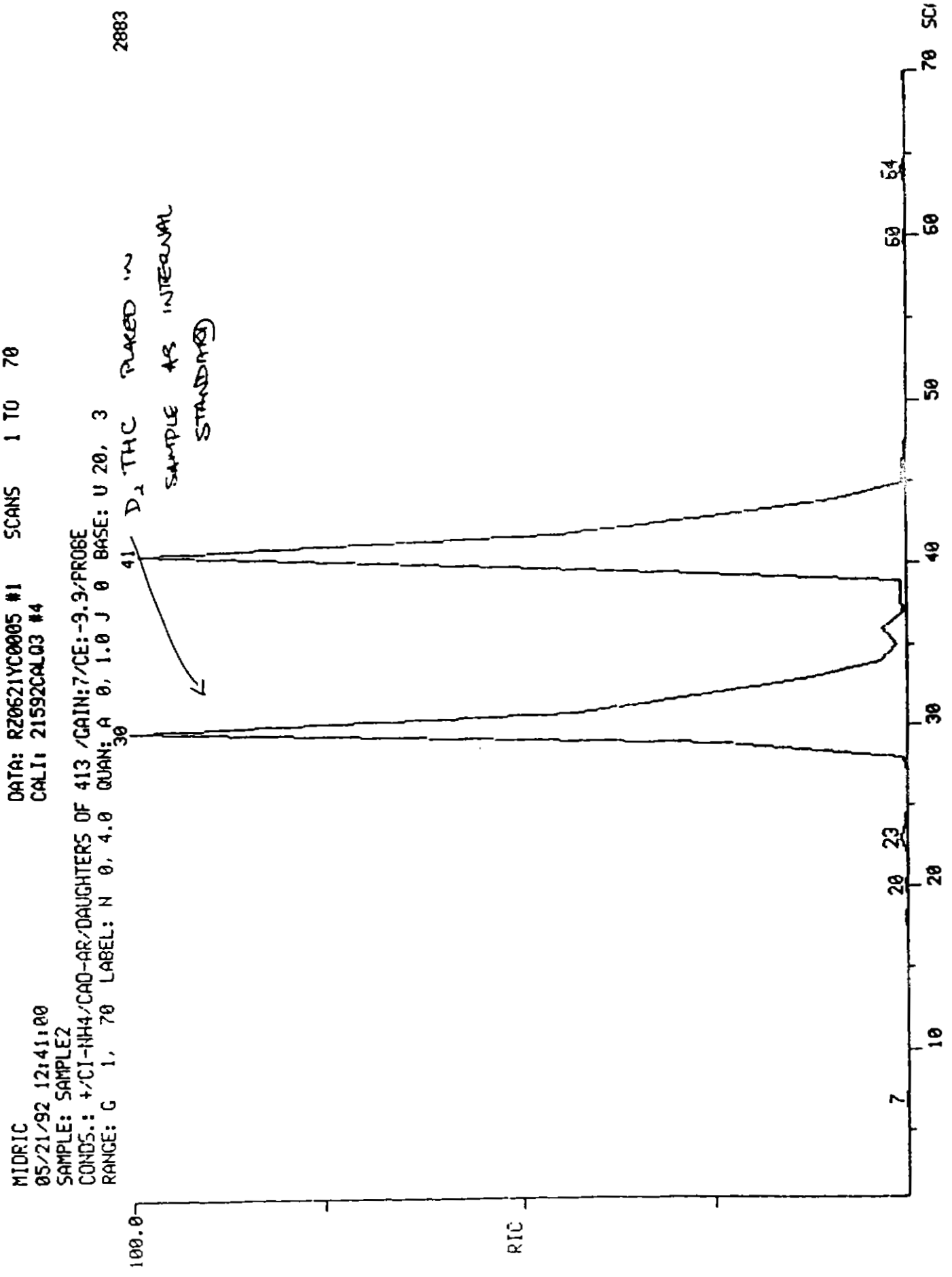


Figure 24 **Mass chromatogram of the opioid, morphine, which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.**

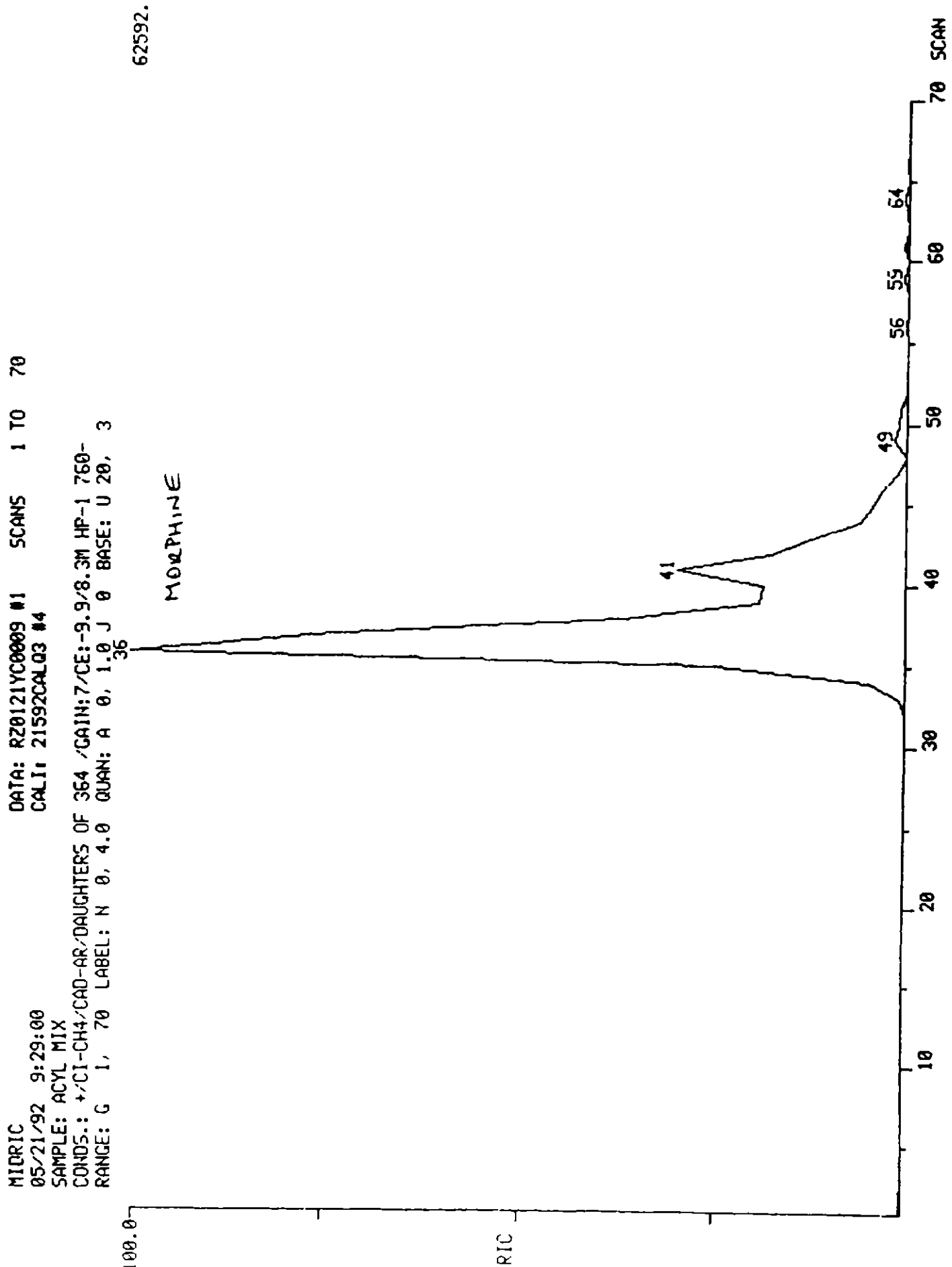


Figure 25 **Mass chromatogram of the vasodilator drug, perhexilene, which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.**

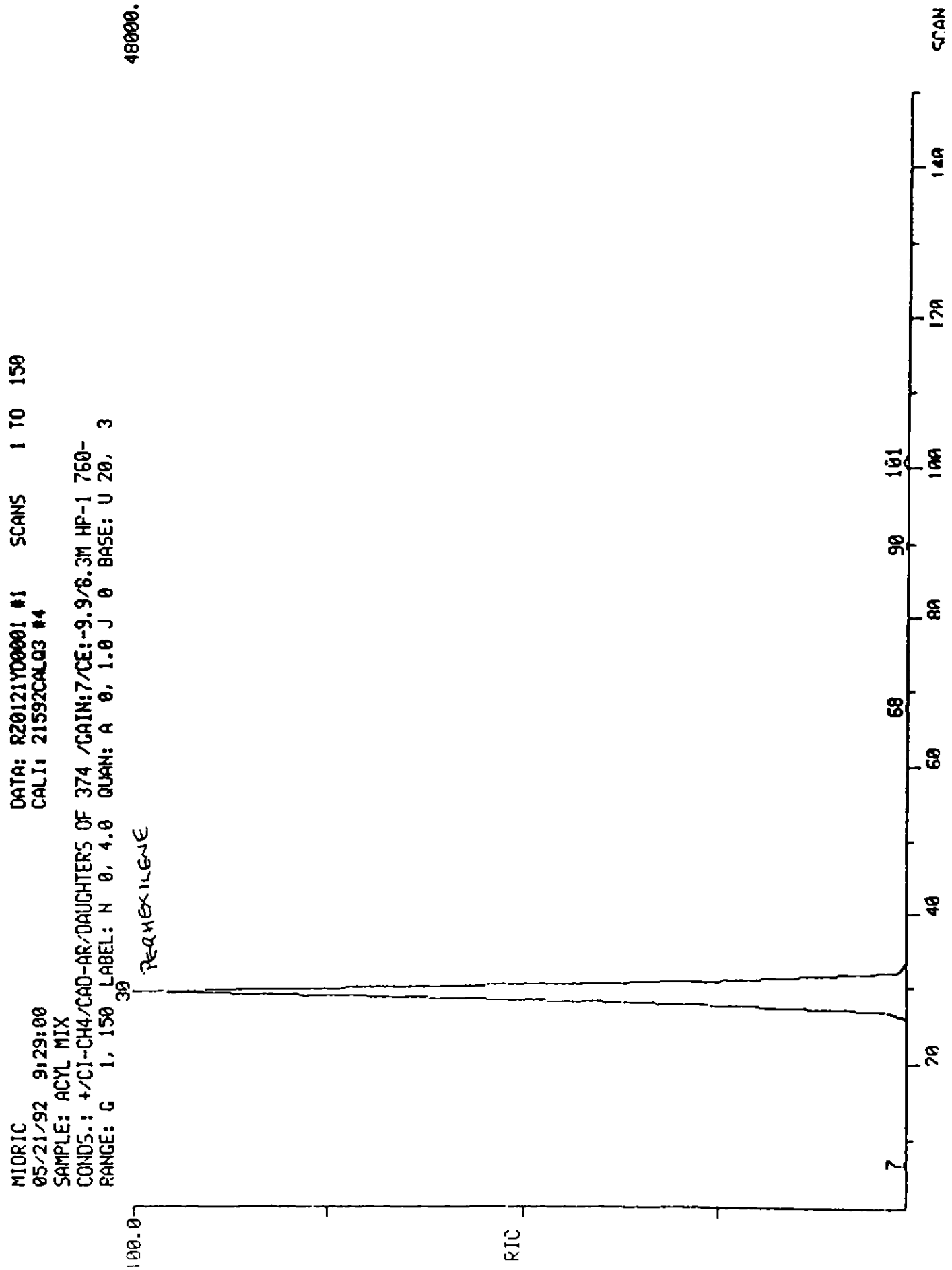


Figure 26 **Mass chromatogram of the analgesic drug, dihydrocodeine, which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.**

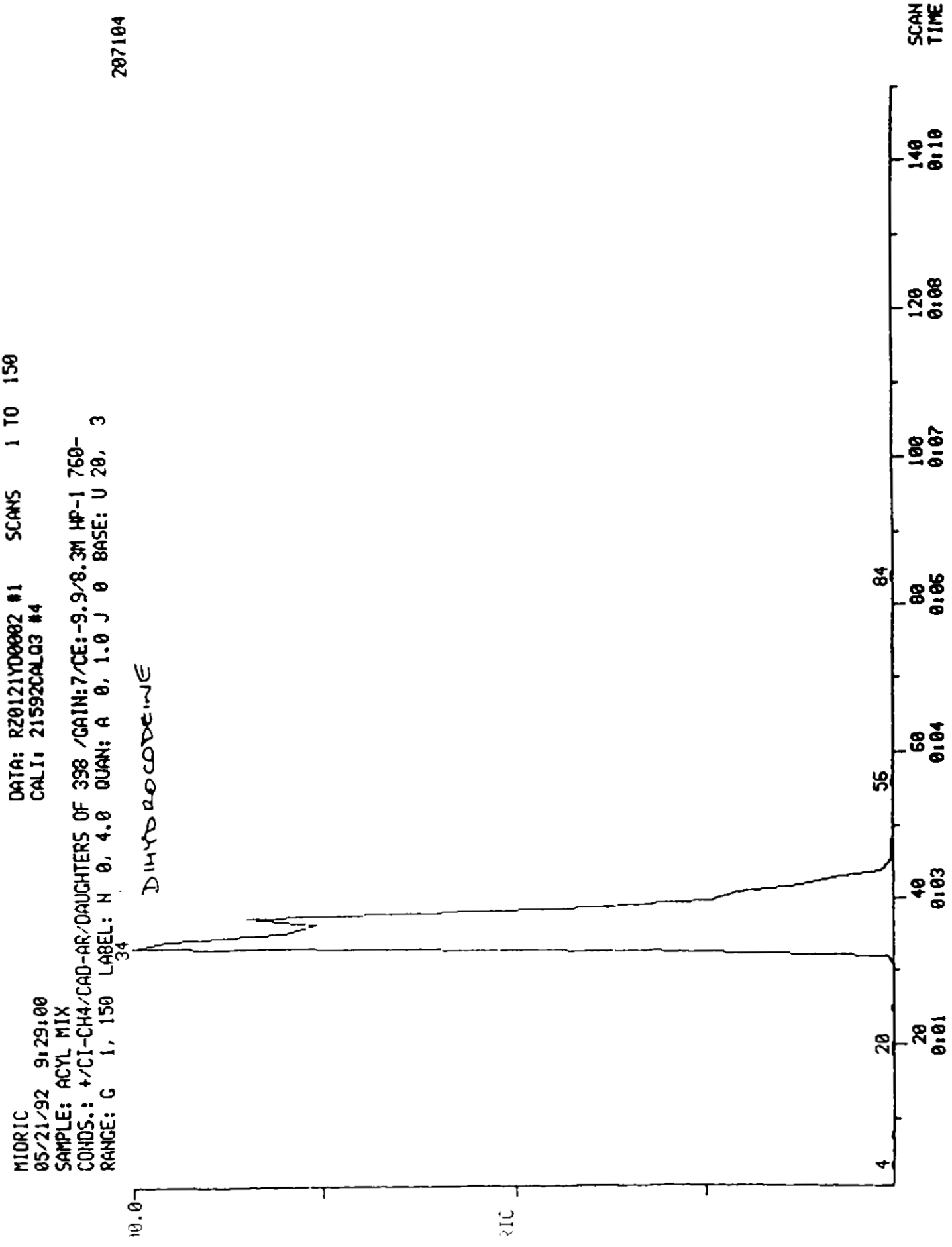


Figure 27 **Mass chromatogram of the opioid, codeine, which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.**

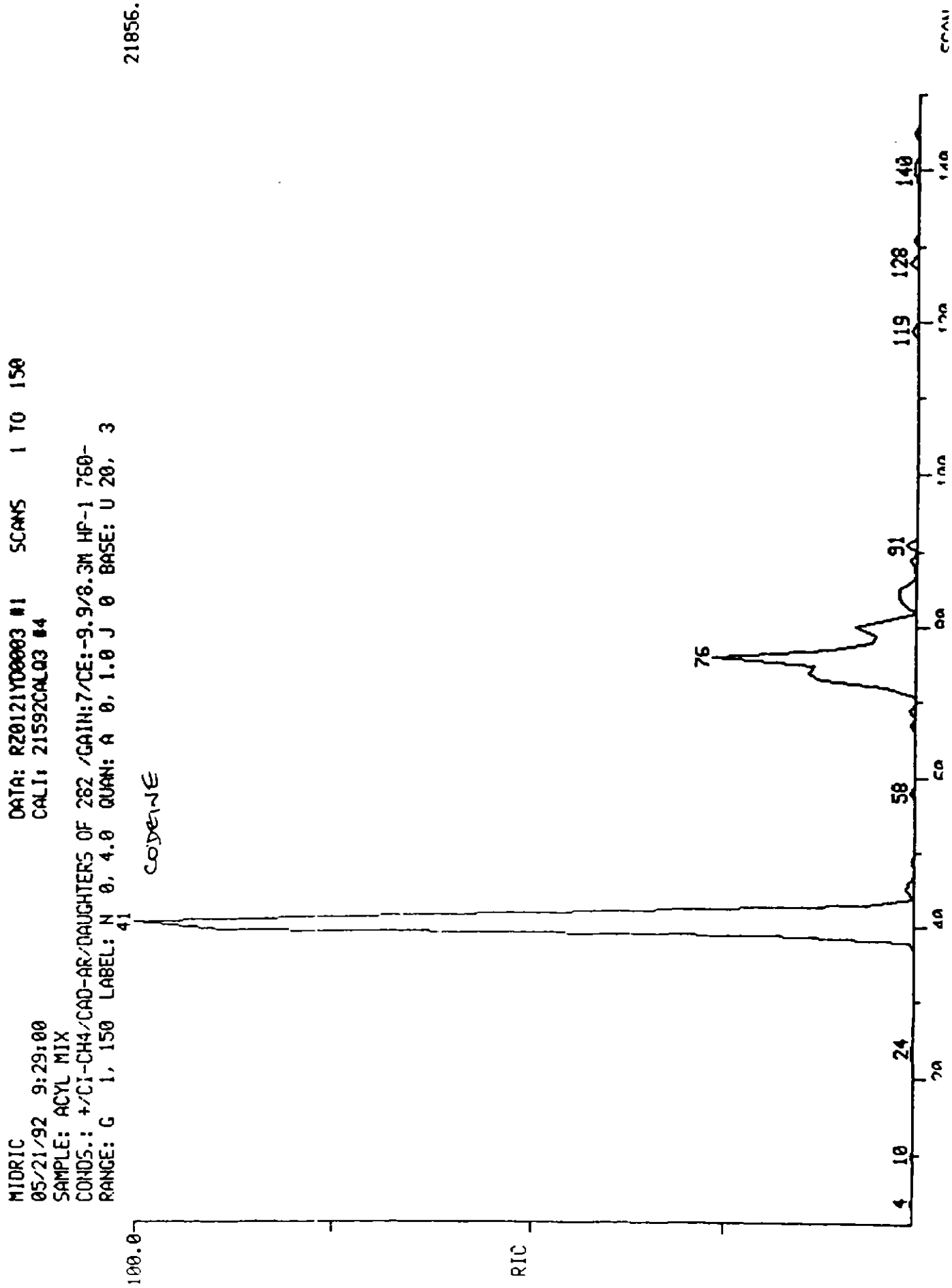


Figure 28 **Mass chromatogram of the opioid, ethylmorphine, which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.**

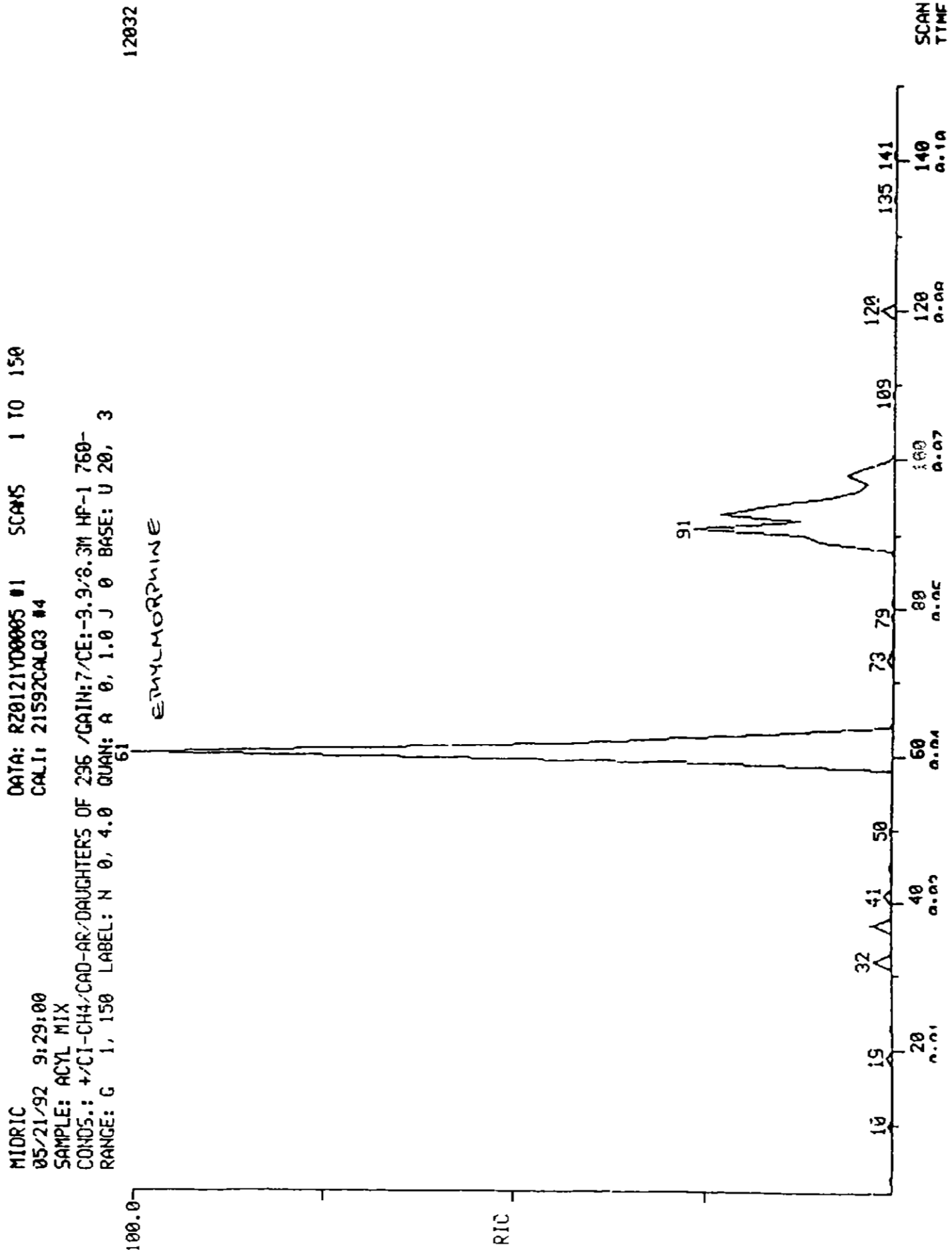


Figure 29 **Mass chromatogram of the beta-adrenoceptor blocking drug, practolol, which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.**

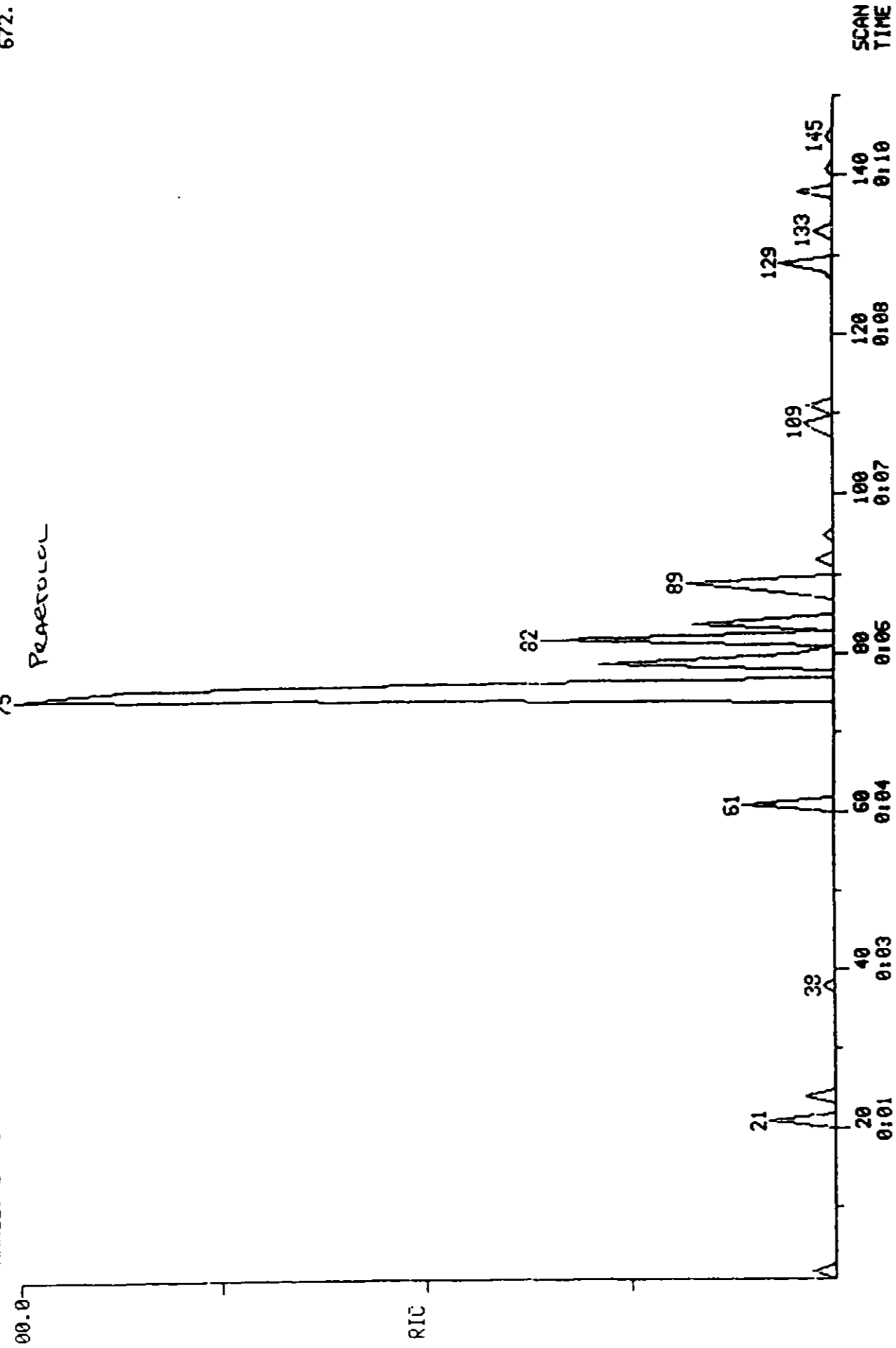


Figure 30 **Mass chromatogram of the tricyclic antidepressant drugs nortriptyline and protriptyline which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.**

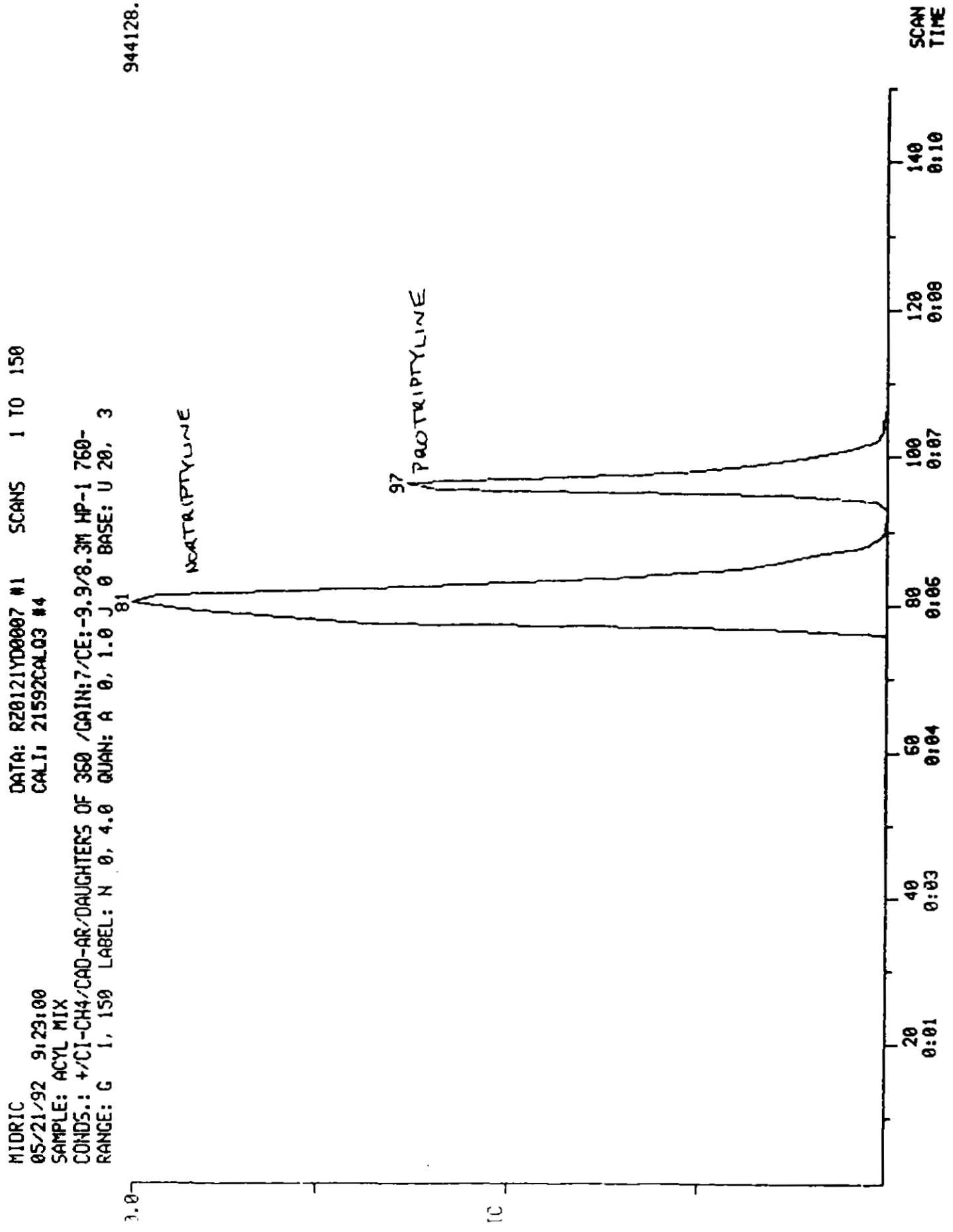


Figure 31 **Mass chromatogram of the antidepressant drug, desipramine, which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.**

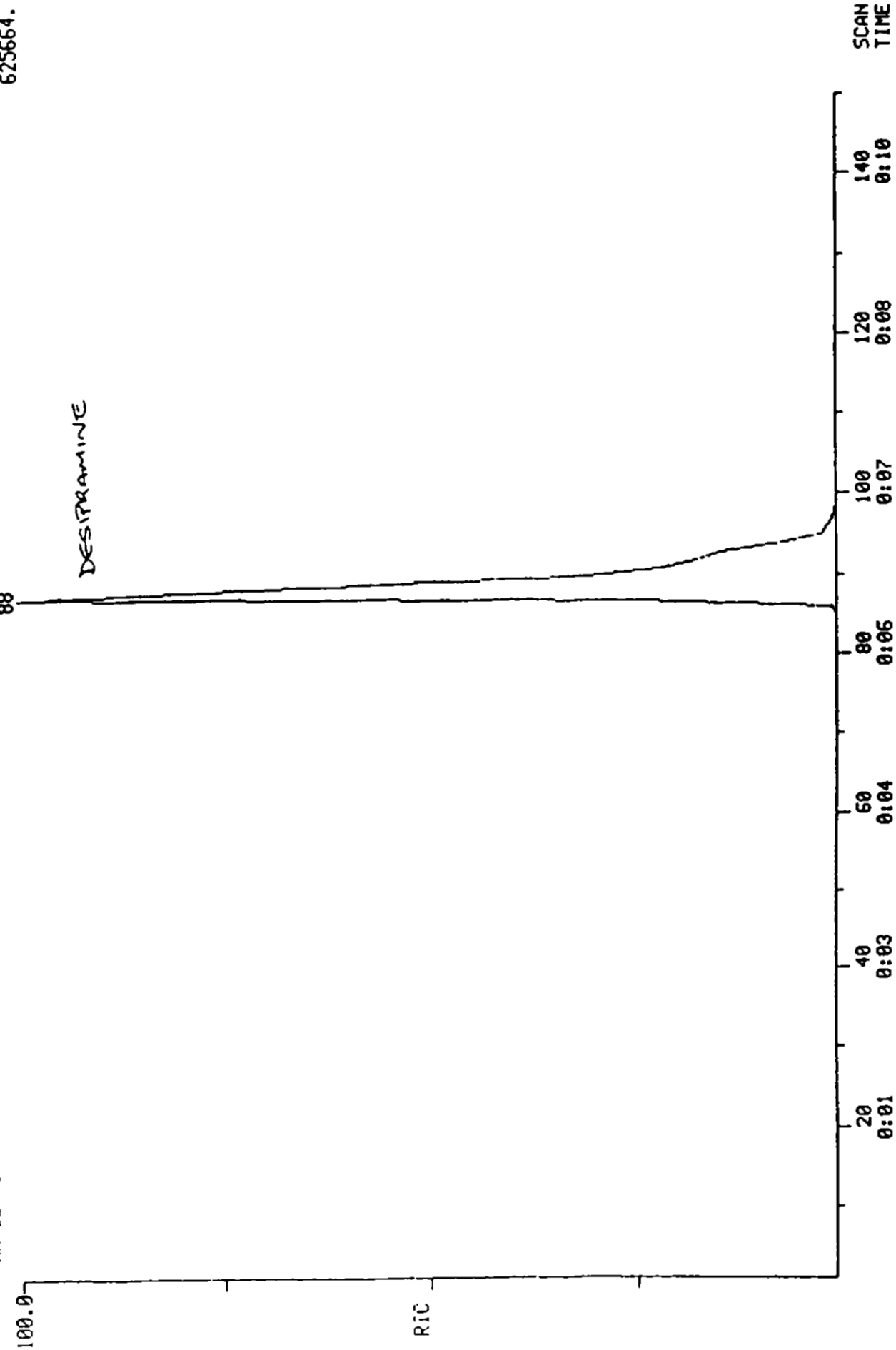


Figure 32 **Mass chromatogram of the decongestant drug, methoxyphenamine which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.**

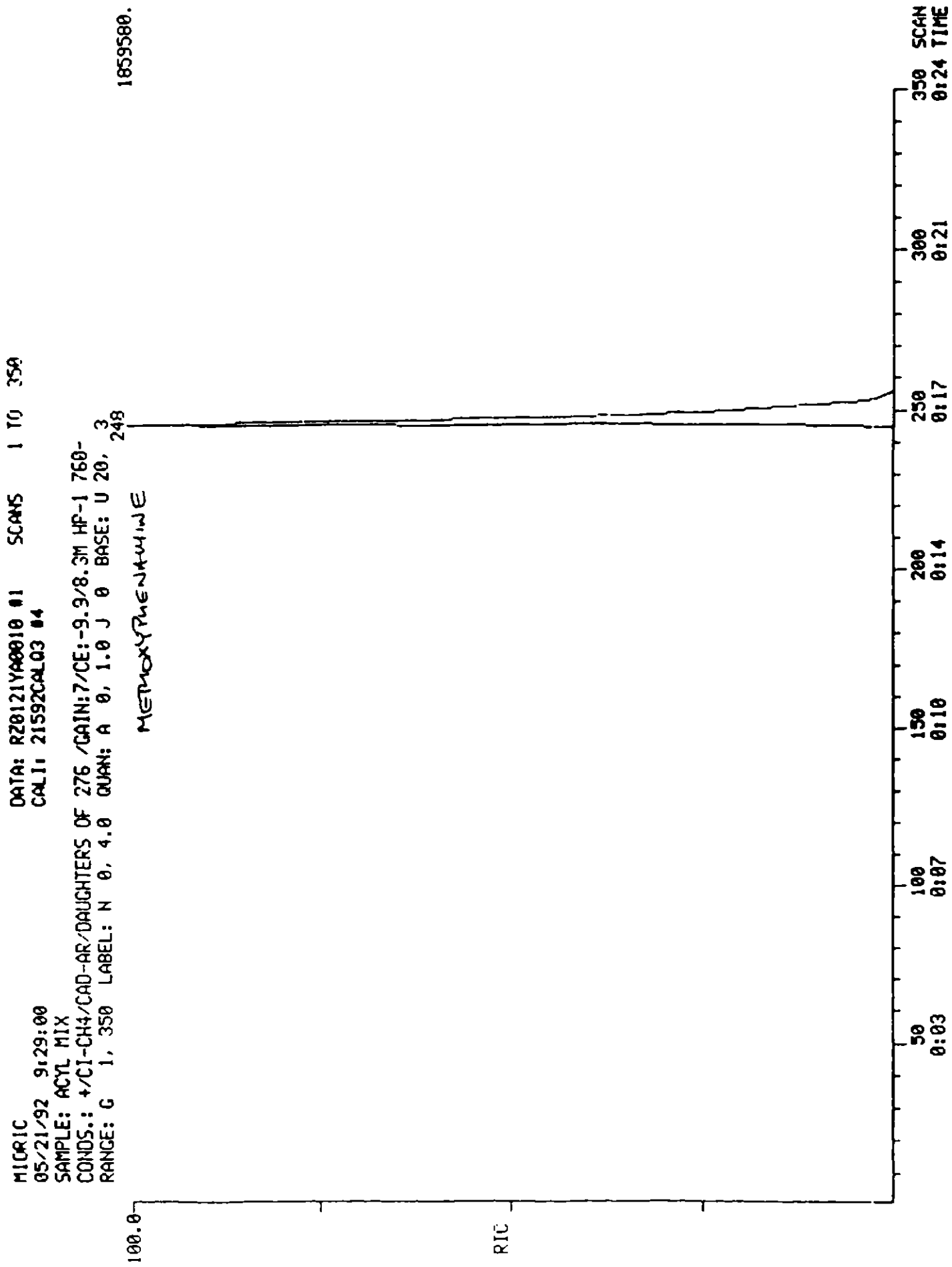
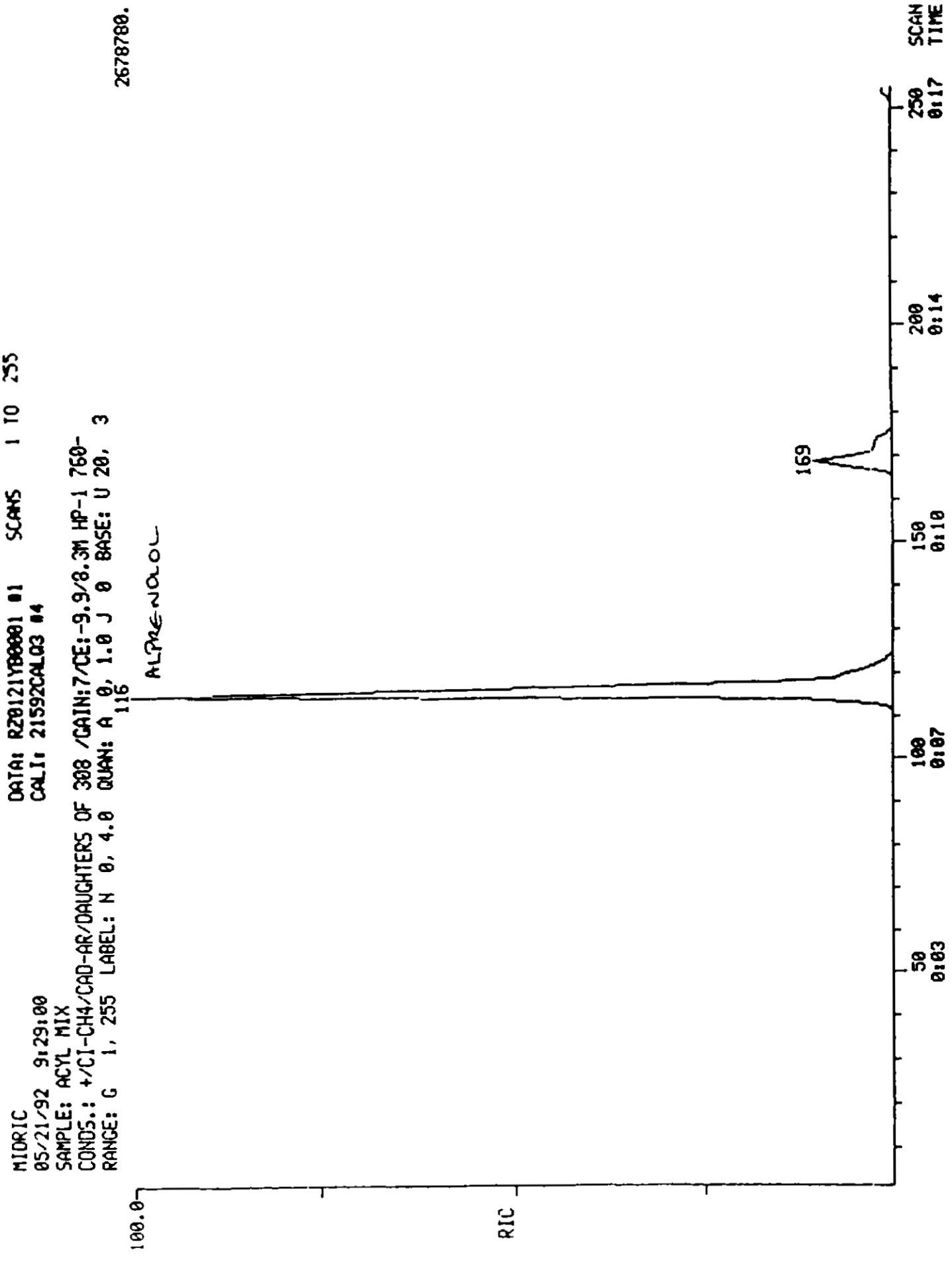


Figure 33 **Mass chromatogram of the beta-adrenoceptor blocking drug alprenolol, which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.**



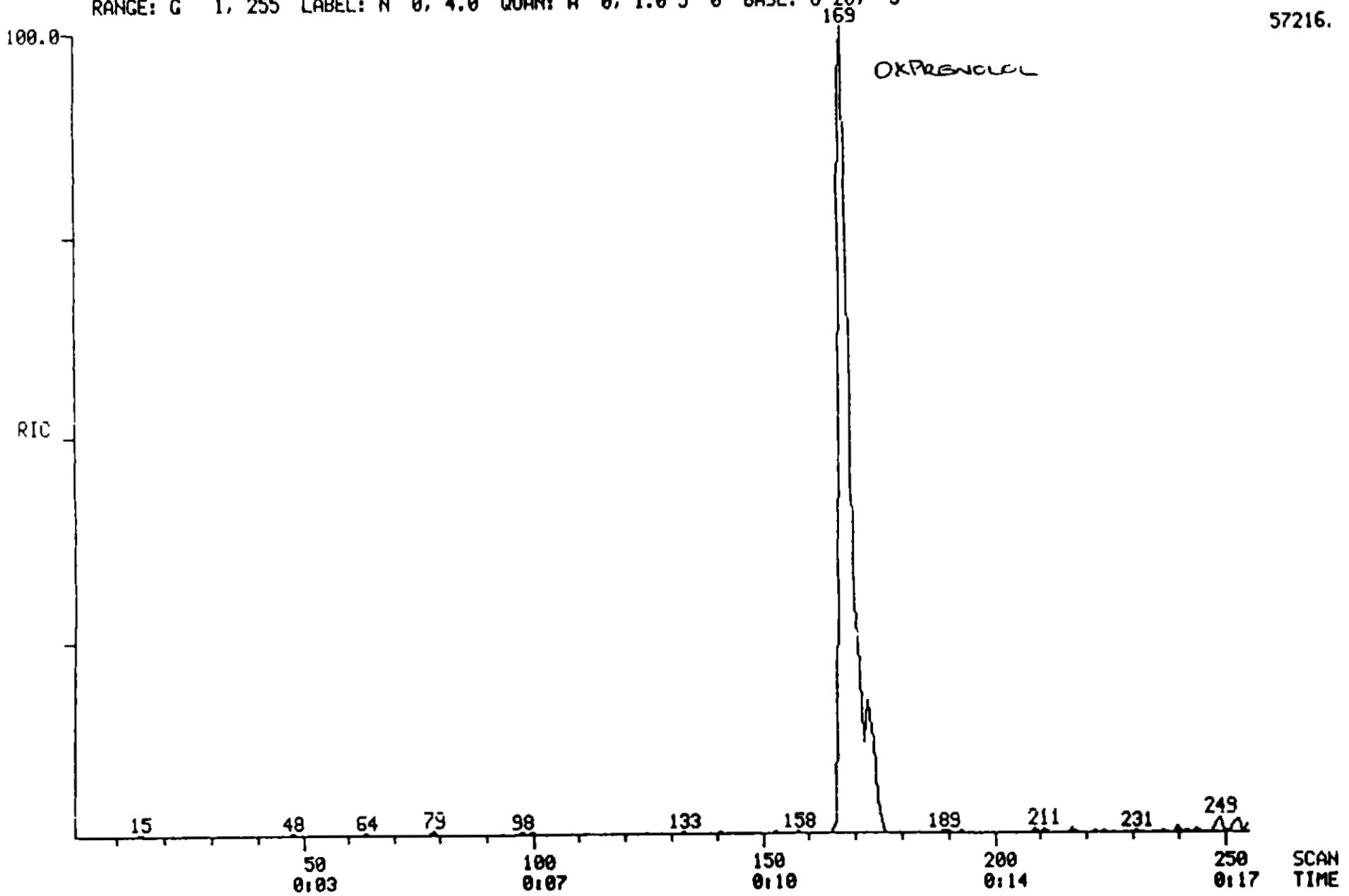


Figure 34 Mass chromatogram of the beta-adrenoceptor blocking drug, oxprenolol, which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.

Figure 35 **Mass chromatogram of the antidepressant drug, nomifensine, which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.**

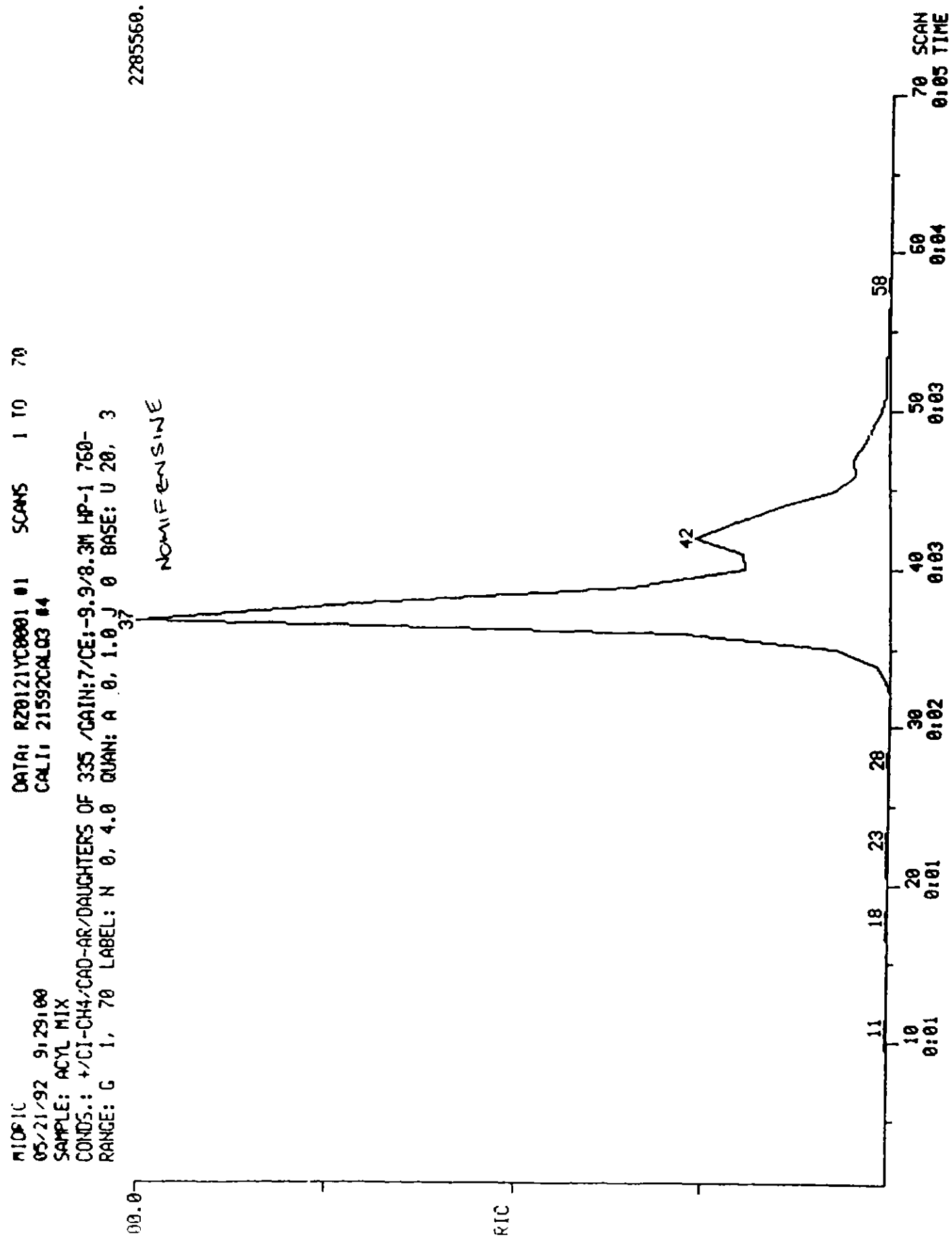


Figure 36 Mass chromatogram of the beta-adrenoceptor blocking drug, propranolol, which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.

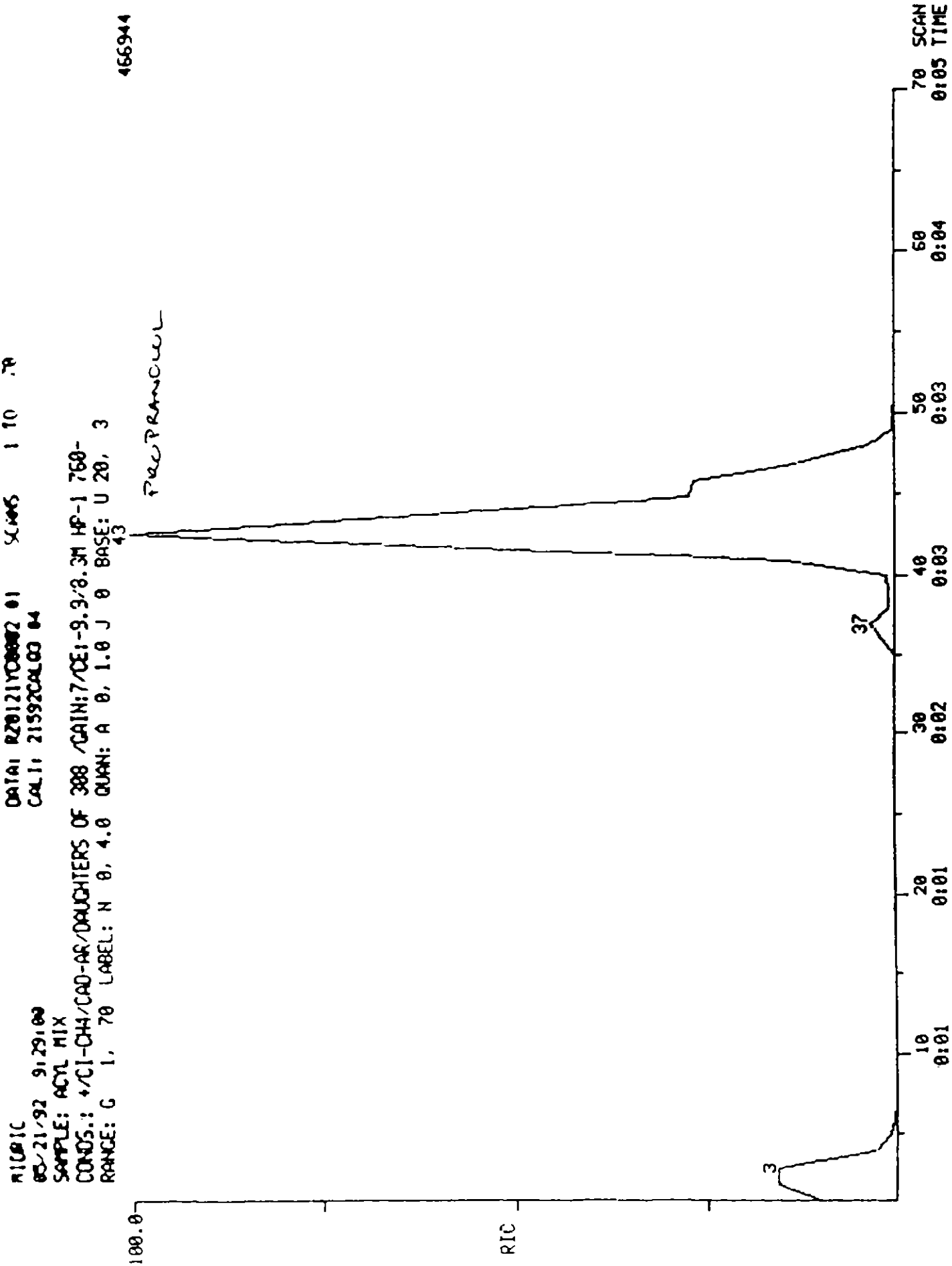
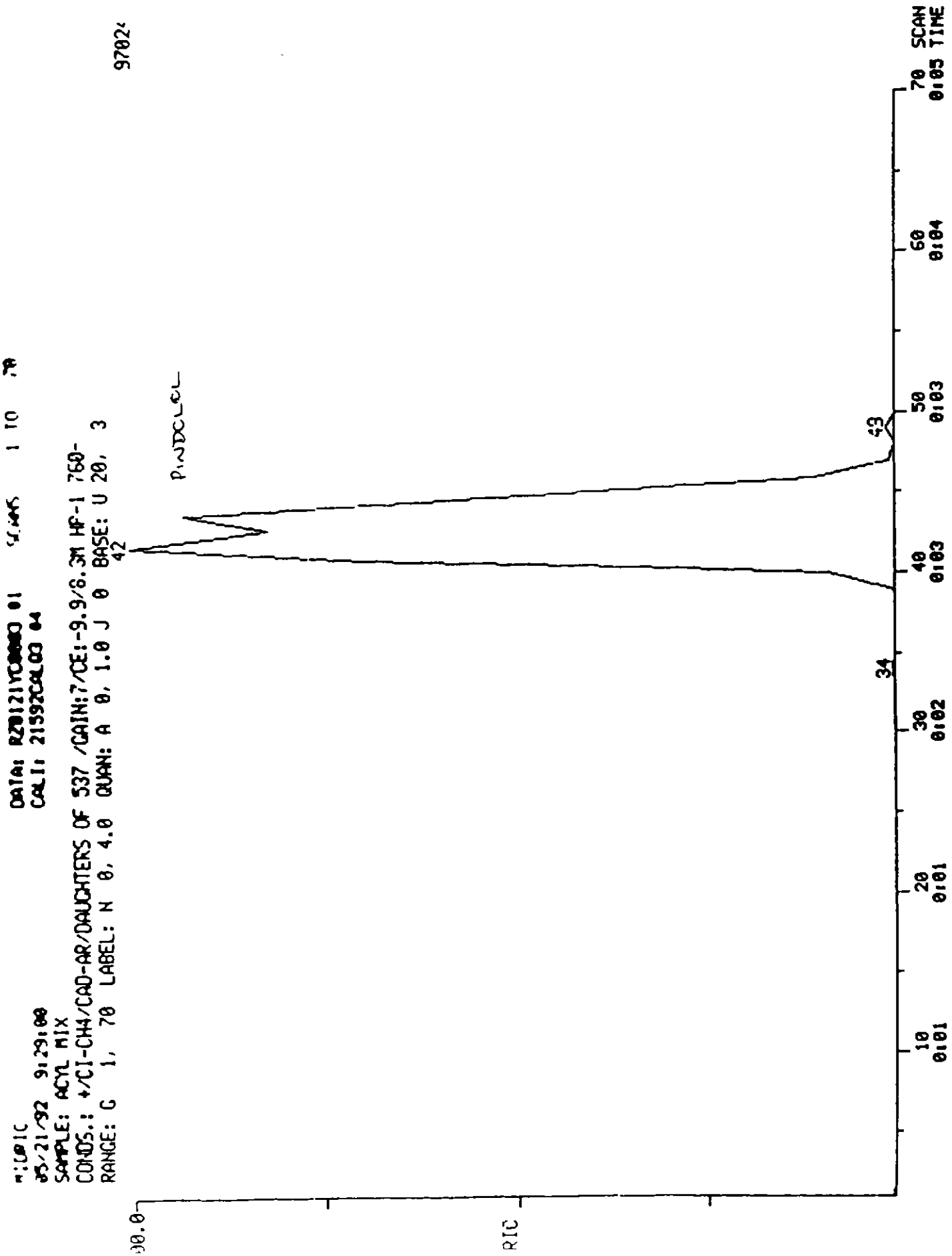


Figure 37 **Mass chromatogram of beta-adrenoceptor blocking drug, pindolol, which had been added to a saliva sample which was then processed and introduced into the GC-MS-MS.**



3.2. Drugs found in saliva samples from drivers.

A total of 618 saliva samples have been analysed at the time of compilation of this report. The results are summarised in Tables 2 - 4.

3.2.1 Drugs found in truck drivers and comparison survey data

Table 2. TRUCK DRIVER SAMPLE (n = 318)

DRUG	FREQUENCY	%
AMPHETAMINE*	16	5.03
ASPIRIN	1	0.31
CAFFEINE	251	78.9
CODEINE	1	0.31
COTININE	60	18.9
DIAZEPAM	3	0.94
EPHEDRINE*	25	7.86
FENFLURAMINE*	1	0.31
GUAIPHENESIN	1	0.31
LIGNOCAINE	1	0.31
METHAMPHETAMINE*	16	5.03
METHOXYPHENAMINE*	1	0.31
NORDIAZEPAM	3	0.94
PARACETAMOL	2	0.63
PHENTERMINE*	24	7.55
PHENYLPROPANOLAMINE*	8	2.52
PHENYTOIN	2	0.63
TRIFLUOPERAZINE	1	0.31

*Stimulants other than caffeine and cotinine = 91 (29.6%)

Notes:

1. A total of 417 drugs were found in the 318 truck driver samples

2. A total of 106 drugs were found in the 318 truck driver samples after excluding caffeine and cotinine

3. Excluding caffeine and cotinine, the numbers of drugs which were found in individual truck drivers were:

Numbers	Occurrences
1	48
2	14
3	4
4	3
6	1

Relevant survey data for comparison (n = 500)

Q6: If you are tired, you can either stop of keep driving. Which of the following would you do, to help cope with the tiredness?

Have a drink of coffee, tea	182	(36%)
Have a drink of coke, pepsi	164	(33%)
Smoke a cigarette	158	(32%)
Take stay awake pills	126	(25%)
Take other medication	22	(4%)
Smoke cannabis	7	(1%)

Q 10: Truck drivers' perceived use of stay awake pills by other truck drivers

1-10%	434	(87%)
90-100%	55	(11%)
Don't know	12	(2%)

Q 11: Do you agree that it is more responsible to take some form of pill or medication to help you stay awake than to drive while you are tired?

Yes	355	(71%)
No	127	(25%)
Don't know	19	(4%)

Q 12a: How often would you say you take stay awake pills or medicine on:

	Long Runs		**Short Runs**	
Several times per run	50	(10%)	2	(<1%)
Once per run	40	(8%)	6	(1%)
Occasionally	150	(30%)	43	(9%)
Never	258	(51%)	449	(90%)
Used to	3	(1%)		

Q 13: Suppose you've been driving 8 hours and you've got 4 to go. Is this the sort of situation where you might take something to help you stay awake?

Yes	148	(30%)
No	91	(18%)
Don't know	262	(52%)

Q 14: Under what (other) circumstances would you take stay awake pills or medication?

Feeling tired/drowsy	42	(8%)
In a hurry/no time to sleep	23	(5%)
Meet a deadline	89	(18%)
Long trip	42	(8%)
Stress/pressure	25	(5%)
Other reasons		All under 5%

Q 15: What do you think are the best stay awake pills or medication for drivers to use?

Ephedrine	104	(21%)
Shakers	59	(12%)
Tenuate Dospan	37	(7%)
Brickettes	40	(8%)
Duromine	116	(23%)
Fedrine	28	(6%)
Speed	33	(7%)
Don't know	209	(42%)
Others (Sudafed, methedrine, anorectics, cough & cold preparations, vitamins, No-Doze, caffeine pills, licit drugs, illicit drugs, coffee/tea, coke/pepsi)		All under 5%

Q 17: Do you have any ongoing health problems that need medication?

Yes	76	(15%)
No	424	(85%)

Q18: What medication do you use?

Anti gout preparations	8	(11%)
Analgesics	11	(14%)
Anti-asthmatics	13	(17%)
Cough & cold preparations	4	(5%)
Antacids, antiflatuents	9	(12%)
Anti-diabetic drugs	4	(5%)
Anti-peptic ulcerants	6	(8%)
Beta blockers & antihypertensives	14	(18%)
Antihistamines	7	(9%)
Other drugs		(5%)

Note: In all, 76 truck drivers stated that they used medicines for ongoing medical problems

3.2.2 Drugs found in bus drivers

Table 3. BUS DRIVER SAMPLE (n = 90)

DRUG	FREQUENCY	%
AMANTADINE	1	1.11
AMPHETAMINE*	1	1.11
CAFFEINE	72	80.0
COTININE	15	16.7
EPHEDRINE*	2	2.22
ETHOSUXIMIDE	1	1.11
MEDAZEPAM	1	1.11
METHAMPHETAMINE*	1	1.11
METHOXYPHENAMINE*	1	1.11
NORDIAZEPAM	1	1.11
NORTRIPTYLINE	1	1.11
PARACETAMOL	1	1.11
PHENTERMINE*	1	1.11
PHENYTOIN	1	1.11

*Stimulants other than caffeine and cotinine = 6 (6.66%)

Notes:

1. A total of 100 drugs were found in the 90 bus driver samples
2. A total of 13 drugs were found in the 90 bus driver samples after excluding caffeine and cotinine
3. Excluding caffeine and cotinine, the numbers of drugs which were found in individual bus drivers were:

Numbers	Occurrences
1	5
3	1
5	1

3.2.3 Drugs found in general driver population

Table 4. GENERAL DRIVER SAMPLE (n = 210)

DRUG	FREQUENCY	%
AMPHETAMINE*	2	0.95
ASPIRIN	1	0.48
CAFFEINE	167	79.5
COTININE	21	10.0
CARBAMAZEPINE	1	0.48
Δ^9-TETRAHYDROCANNABINOL	3	1.43
DIAZEPAM	3	1.43
EPHEDRINE*	3	1.43
METHAMPHETAMINE*	1	0.48
NORDIAZEPAM	4	1.90
PARACETAMOL	2	0.95
PHENYTOIN	2	0.95
TEMAZEPAM	1	0.48

*Stimulants other than caffeine and cotinine = 6 (2.86%)

Notes:

1. A total of 211 drugs were found in the 210 general driver samples
2. A total of 23 drugs were found in the 210 general driver samples after excluding caffeine and cotinine
3. Excluding caffeine and cotinine, the numbers of drugs which were found in individual general drivers were:

Numbers	Occurrences
1	14
2	3
3	1

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Appendix 1 Summary of Drugs Scanned for in Multi-Experiments

Multi-Experiment Editor - Input File: YY - Number of Experiments: 5
1 60 cycles of Experiment of (1.89 s/cycle) Total time: 1.53

1: (MID OF - 1.89 Sec) +/Q3 MASS SPECTRUM/GAIN: 7

2 350 cycles of Experiment YA (0.77 s/cycles) Total time: 4.31

Amphetamine	1:	(MID XA - 0.07sec)	+/Daughters of 232	/Gain: 7/CE -9.9
Phenylpropanolamine[1]	2:	(MID XD - 0.07sec)	+/Daughters of 230	/Gain: 7/CE -9.9
Phentermine[2]	3:	(MID XB - 0.07sec)	+/Daughters of 246	/Gain: 7/CE -9.9
Amantadine	4:	(MID XG - 0.07sec)	+/Daughters of 248	/Gain: 7/CE -9.9
Fenfluramine	5:	(MID XH - 0.07sec)	+/Daughters of 328	/Gain: 7/CE- 9.9
Ephedrine	6:	(MID XI - 0.07sec)	+/Daughters of 244	/Gain: 7/CE -9.9
Guaiphenesin	7:	(MID XC - 0.07sec)	+/Daughters of 391	/Gain: 7/CE -9.9
Paracetamol	8:	(MID X6 - 0.07sec)	+/Daughters of 248	/Gain: 7/CE -9.9
Mexiletine	9:	(MID XJ - 0.07sec)	+/Daughters of 276	/Gain: 7/CE -9.9
Methoxyphenamine	0:	(MID XK - 0.07sec)	+/Daughters of 276	/Gain: 7/CE -9.9
Iproniazid	1:	(MID XL - 0.07sec)	+/Daughters of 276	/Gain: 7/CE -9.9

3 255 cycles of Experiment YB (0.42 s/cycle) Total time: 1.47

Atenolol[3]	1:	(MID XM - 0.07sec)	+/Daughters of 308	/Gain: 7/CE -9.9
Methocarbamol	2:	(MID XN - 0.07sec)	+/Daughters of 338	/Gain: 7/CE -9.9
Oxprenolol	3:	(MID XO - 0.07sec)	+/Daughters of 344	/Gain: 7/CE -9.9
Atropine	4:	(MID XQ - 0.07sec)	+/Daughters of 272	/Gain: 7/CE -9.9
Metoprolol	5:	(MID XM - 0.07sec)	+/Daughters of 428	/Gain: 7/CE- 9.9
Nomifensine	6:	(MID X9 - 0.07sec)	+/Daughters of 335	/Gain: 7/CE -9.9
Pindolol	7:	(MID XS - 0.07sec)	+/Daughters of 537	/Gain: 7/CE -9.9

4 70 cycles of Experiment YC (0.75 s/cycle) Total time: .52

Nomifensine	1:	(MID X9 - 0.07sec)	+/Daughters of 335	/Gain: 7/CE -9.9
Atenolol[4]	2:	(MID XM - 0.07sec)	+/Daughters of 308	/Gain: 7/CE -9.9
Pindolol	3:	(MID XS - 0.07sec)	+/Daughters of 537	/Gain: 7/CE -9.9
THC	4:	(MID XV - 0.13sec)	+/Daughters of 411	/Gain: 7/CE -9.9
$d_{2\text{-}THC}$	5:	(MID XY - 0.13sec)	+/Daughters of 413	/Gain: 7/CE -9.9
Perhexiline	6:	(MID X7 - 0.07sec)	+/Daughters of 374	/Gain: 7/CE -9.9
Dihydrocodeine	7:	(MID XZ - 0.07sec)	+/Daughters of 398	/Gain: 7/CE -9.9
Codeine	8:	(MID XE - 0.07sec)	+/Daughters of 282	/Gain: 7/CE -9.9
Morphine	9:	(MID XX - 0.07sec)	+/Daughters of 364	/Gain: 7/CE -9.9

5 150 cycles of Experiment YD (0.71 s/cycle) Total time: 1.47

Perhexiline	1:	(MID X7 - 0.07sec)	+/Daughters of 374	/Gain: 7/CE -9.9
Dihdrocodeine	2:	(MID XZ - 0.07sec)	+/Daughters of 398	/Gain: 7/CE -9.9
Codeine	3:	(MID XE - 0.07sec)	+/Daughters of 282	/Gain: 7/CE -9.9
Morphine	4:	(MID XX - 0.07sec)	+/Daughters of 364	/Gain: 7/CE -9.9
Ethylmorphine	5:	(MID XW - 0.07sec)	+/Daughters of 296	/Gain: 7/CE -9.9
Practolol	6:	(MID XF - 0.07sec)	+/Daughters of 459	/Gain: 7/CE -9.9
Nortriptyline[5]	7:	(MID XP - 0.07sec)	+/Daughters of 360	/Gain: 7/CE -9.9
Metoclopramide	8:	(MID X4 - 0.07sec)	+/Daughters of 396	/Gain: 7/CE -9.9
Desipramine	9:	(MID XT - 0.07sec)	+/Daughters of 457	/Gain: 7/CE -9.9
Quinine	10:	(MID X3 - 0.07sec)	+/Daughters of 421	/Gain: 7/CE -9.9

1. *Also Tranylcypromine*
2. *Also Methamphetamine*
3. *Also Alprenolol, Propranolol*
4. *Also Protriptyline*

Multi-Experiment Editor - Input File: ZV - Number of Experiments: 10

1 40 cycles of Experiment of (1.89 s/cycle) Total time: 1.15

1: (MID OF - 1.89 Sec) +/Q3 MASS SPECTRUM/GAIN: 7

2 130 cycles of Experiment Z1 (0.55 s/cycles) Total time: 1.11

Ethosuximide	1:	(MID SZ - 0.55sec)	+/Daughters of 156	/Gain: 7/CE -24.8
Aspirin	2:	(MID SZ - 0.55sec)	+/Daughters of 153	/Gain: 7/CE -24.8

3 310 cycles of Experiment ZA (0.44 s/cycles) Total time: 2.15

Cotinine	1:	(MID QA - 0.02sec)	+/Daughters of 177	/Gain: 7/CE -24.8
Phensuximide	2:	(MID QA - 0.02sec)	+/Daughters of 190	/Gain: 7/CE -24.8
Methsuximide	3:	(MID QA - 0.02sec)	+/Daughters of 204	/Gain: 7/CE -24.8
Barbitone	4:	(MID QA - 0.02sec)	+/Daughters of 213	/Gain: 7/CE -24.8
Butobarbitone	5:	(MID QA - 0.02sec)	+/Daughters of 241	/Gain: 7/CE -24.8
Methyl Phenidate	6:	(MID QG - 0.03sec)	+/Daughters of 234	/Gain: 7/CE -24.8
Beclamide	7:	(MID QI - 0.03sec)	+/Daughters of 198	/Gain: 7/CE -24.8
Prolintane	8:	(MID QI - 0.03sec)	+/Daughters of 218	/Gain: 7/CE -24.8
Carbromal	9:	(MID QE - 0.03sec)	+/Daughters of 237	/Gain: 7/CE -24.8
Amylobarbitone	10:	(MID QE - 0.03sec)	+/Daughters of 255	/Gain: 7/CE -24.8
Methohexitone	11:	(MID RN - 0.02sec)	+/Daughters of 267	/Gain: 7/CE -24.8
Quinalbarbitone	12:	(MID RN - 0.02sec)	+/Daughters of 277	/Gain: 7/CE -24.8
Phendimetrazine	13:	(MID RA - 0.03sec)	+/Daughters of 192	/Gain: 7/CE -24.8
Diethylcarbamazine	14:	(MID QF - 0.03sec)	+/Daughters of 200	/Gain: 7/CE -24.8
Diethylpropion	15:	(MID QM - 0.03sec)	+/Daughters of 206	/Gain: 7/CE -24.8
Ibuprofen	16:	(MID QQ - 0.02sec)	+/Daughters of 221	/Gain: 7/CE -24.8
d_4 - Ibuprofen	17:	(MID SO - 0.03sec)	+/Daughters of 225	/Gain: 7/CE -24.8
Clofibrate	18:	(MID QZ - 0.03sec)	+/Daughters of 229	/Gain: 7/CE -24.8
Pethidine	19:	(MID RE - 0.03sec)	+/Daughters of 248	/Gain: 7/CE -24.8
Pentobarbitone	20:	(MID RI - 0.03sec)	+/Daughters of 255	/Gain: 7/CE -24.8
Metronidazole	21:	(MID QC - 0.03sec)	-/Daughters of 171	/Gain: 7/CE -24.8

125 cycles of Experiment ZD (0.60 s/cycle) Total time: 1.15

Glutethimide	1:	(MID QI - 0.03sec)	+/Daughters of 218	/Gain: 7/CE -24.8
Isocarboxazid	2:	(MID QI - 0.03sec)	+/Daughters of 232	/Gain: 7/CE -24.8
Prilocaine	3:	(MID QH - 0.02sec)	+/Daughters of 221	/Gain: 7/CE -24.8
Lignocaine	4:	(MID QH - 0.02sec)	+/Daughters of 235	/Gain: 7/CE -24.8
Phencyclidine	5:	(MID QP - 0.02sec)	+/Daughters of 243	/Gain: 7/CE -24.8
Phenobarbitone	6:	(MID QP - 0.02sec)	+/Daughters of 261	/Gain: 7/CE -24.8
Ethotoin	7:	(MID QF - 0.03sec)	+/Daughters of 205	/Gain: 7/CE -24.8
Mephenytoin	8:	(MID QW - 0.02sec)	+/Daughters of 219	/Gain: 7/CE -24.8
Meprobamate	9:	(MID QD - 0.03sec)	+/Daughters of 219	/Gain: 7/CE -24.8
Dimenhydrinate	10:	(MID RD - 0.03sec)	+/Daughters of 229	/Gain: 7/CE -24.8
Ketamine	11:	(MID QU - 0.03sec)	+/Daughters of 238	/Gain: 7/CE -24.8
Pheniramine	12:	(MID RM - 0.04sec)	+/Daughters of 241	/Gain: 7/CE -24.8
Naproxen	13:	(MID RI - 0.03sec)	+/Daughters of 245	/Gain: 7/CE -24.8
Pethidine	14:	(MID RE - 0.03sec)	+/Daughters of 248	/Gain: 7/CE -24.8
Diphenydramine	15:	(MID RC - 0.03sec)	+/Daughters of 256	/Gain: 7/CE -24.8
Fenoprofen	16:	(MID RN - 0.02sec)	+/Daughters of 257	/Gain: 7/CE -24.8
Flurbiprofen	17:	(MID RO - 0.02sec)	+/Daughters of 259	/Gain: 7/CE -24.8
Diflunisal	18:	(MID RV - 0.03sec)	+/Daughters of 265	/Gain: 7/CE -24.8
Orphenadrine	19:	(MID RG - 0.03sec)	+/Daughters of 270	/Gain: 7/CE -24.8
Doxylamine	20:	(MID RH - 0.03sec)	+/Daughters of 271	/Gain: 7/CE -24.8
Clioquinol	21:	(MID SI - 0.02sec)	+/Daughters of 320	/Gain: 7/CE -24.8
Caffeine	22:	(MID QO - 0.03sec)	+/Daughters of 195	/Gain: 7/CE -24.8
Ornidazole	23:	(MID QO - 0.03sec)	-/Daughters of 153	/Gain: 7/CE -24.8

5 95 cycles of Experiment ZE (0.50 s/cycle) Total time: .47

Methaqualone	1:	(MID QI - 0.03sec)	+/Daughters of 251	/Gain: 7/CE -24.8
Tolbitamide	2:	(MID QI - 0.03sec)	+/Daughters of 285	/Gain: 7/CE -24.8
Cyclizine	3:	(MID RC - 0.03sec)	+/Daughters of 267	/Gain: 7/CE -24.8
Diphenpyraline	4:	(MID RC - 0.03sec)	+/Daughters of 282	/Gain: 7/CE -24.8
Ketoprofen	5:	(MID QM - 0.03sec)	+/Daughters of 269	/Gain: 7/CE -24.8
Normethadone	6:	(MID QM - 0.03sec)	+/Daughters of 296	/Gain: 7/CE -24.8
Methadone	7:	(MID QM - 0.03sec)	+/Daughters of 310	/Gain: 7/CE -24.8
Naproxen	8:	(MID RI - 0.03sec)	+/Daughters of 245	/Gain: 7/CE -24.8
Mepivacaine	9:	(MID QL - 0.03sec)	+/Daughters of 247	/Gain: 7/CE -24.8
Pyrimethamine	10:	(MID RV - 0.03sec)	+/Daughters of 249	/Gain: 7/CE -24.8
Thenyldiamine	11:	(MID QK - 0.03sec)	+/Daughters of 262	/Gain: 7/CE -24.8
Dextromethorphan	12:	(MID RR - 0.03sec)	+/Daughters of 272	/Gain: 7/CE -24.8
Chlorpheniramine	13:	(MID RT - 0.03sec)	+/Daughters of 275	/Gain: 7/CE -24.8
Etidocaine	14:	(MID QV - 0.02sec)	+/Daughters of 277	/Gain: 7/CE -24.8
Chlophedianol	15:	(MID QA - 0.02sec)	+/Daughters of 290	/Gain: 7/CE -24.8
Chlorpropamide	16:	(MID RF - 0.02sec)	+/Daughters of 291	/Gain: 7/CE -24.8
Probenecid	17:	(MID ST - 0.03sec)	+/Daughters of 300	/Gain: 7/CE -24.8
Dicyclomine	18:	(MID QN - 0.03sec)	+/Daughters of 310	/Gain: 7/CE -24.8
Brompheniramine	19:	(MID SE - 0.03sec)	+/Daughters of 319	/Gain: 7/CE -24.8
Tinidazole	20:	(MID QO - 0.03sec)	-/Daughters of 247	/Gain: 7/CE -30.0

6 55 cycles of Experiment ZF (0.37 s/cycle) Total time: .20

Propoxyphene	1:	(MID QI - 0.03sec)	+/Daughters of 131	/Gain: 7/CE -24.8
Primidone	2:	(MID QI - 0.03sec)	+/Daughters of 219	/Gain: 7/CE -24.8
Medazepam	3:	(MID QI - 0.03sec)	+/Daughters of 271	/Gain: 7/CE -24.8
Amitriptyline	4:	(MID QI - 0.03sec)	+/Daughters of 278	/Gain: 7/CE -24.8
Phenytoin	5:	(MID RH - 0.03sec)	+/Daughters of 267	/Gain: 7/CE -24.8
Cocaine	6:	(MID RH - 0.03sec)	+/Daughters of 304	/Gain: 7/CE -24.8
Mianserin	7:	(MID QA - 0.02sec)	+/Daughters of 265	/Gain: 7/CE -24.8
Doxepin	8:	(MID QA - 0.02sec)	+/Daughters of 280	/Gain: 7/CE -24.8
Trimipramine	9:	(MID QA - 0.02sec)	+/Daughters of 295	/Gain: 7/CE -24.8
Nalidixic Acid	10:	(MID RR - 0.03sec)	+/Daughters of 247	/Gain: 7/CE -24.8
Diclofenac	11:	(MID RR - 0.03sec)	+/Daughters of 310	/Gain: 7/CE -24.8
d^3 - Phenytoin	12:	(MID SR - 0.03sec)	+/Daughters of 269	/Gain: 7/CE -24.8
Tolmetin	13:	(MID QQ - 0.02sec)	+/Daughters of 272	/Gain: 7/CE -24.8
Triprolidine	14:	(MID RQ - 0.03sec)	+/Daughters of 279	/Gain: 7/CE -24.8
Imipramine	15:	(MID QH - 0.02sec)	+/Daughters of 281	/Gain: 7/CE -24.8
Mepyramine	16:	(MID QS - 0.03sec)	+/Daughters of 286	/Gain: 7/CE -24.8
Probenecid	17:	(MID ST - 0.03sec)	+/Daughters of 300	/Gain: 7/CE -24.8
Benzhexol	18:	(MID QL - 0.03sec)	+/Daughters of 302	/Gain: 7/CE -24.8
Methadone	19:	(MID QM - 0.03sec)	+/Daughters of 310	/Gain: 7/CE -24.8

7 cycles of Experiment ZK (0.41 s/cycle) Total time: .32

Dothiepin	1:	(MID QA - 0.02sec)	+/Daughters of 296	/Gain: 7/CE -24.8
Trimeprazine	2:	(MID QA - 0.02sec)	+/Daughters of 299	/Gain: 7/CE -24.8
Cyproheptadine	3:	(MID QJ - 0.03sec)	+/Daughters of 288	/Gain: 7/CE -24.8
Pizotifen	4:	(MID QJ - 0.03sec)	+/Daughters of 296	/Gain: 7/CE -24.8
Carbamazepine	5:	(MID RL - 0.02sec)	+/Daughters of 237	/Gain: 7/CE -24.8
Nalidixic Acid	6:	(MID RR - 0.03sec)	+/Daughters of 247	/Gain: 7/CE -24.8
Mazindol	7:	(MID RU - 0.03sec)	+/Daughters of 267	/Gain: 7/CE -24.8
Tolmetin	8:	(MID QQ - 0.02sec)	+/Daughters of 272	/Gain: 7/CE -24.8
Triprolidine	9:	(MID RQ - 0.03sec)	+/Daughters of 279	/Gain: 7/CE -24.8
Promazine	10:	(MID QH - 0.02sec)	+/Daughters of 285	/Gain: 7/CE -24.8
Pentazocine	11:	(MID GE - 0.03sec)	+/Daughters of 386	/Gain: 7/CE -24.8
Bupivacine	12:	(MID GY - 0.02sec)	+/Daughters of 289/Gain: 7/CE -24.8	
Azatadine	13:	(MID RV - 0.03sec)	+/Daughters of 291/Gain: 7/CE -24.8	
d_7 - Azatadine	14:	(MID SU - 0.03sec)	+/Daughters of 298/Gain: 7/CE -24.8	
Benzhexol	15:	(MID QL - 0.03sec)	+/Daughters of 302/Gain: 7/CE -24.8	
Oxazepam	16:	(MID QB - 0.03sec)	-/Daughters of 268/Gain: 7/CE -24.8	
Lorazepam	17:	(MID QB - 0.03sec)	-/Daughters of 302/Gain: 7/CE -24.8	

1. Also Promethazine

8 110 cycles of Experiment ZL (0.59 s/cycles) Total time: 1.05

Clomipramine	1:	(MID QH - 0.02sec)	+/Daughters of 315	/Gain: 7/CE -24.8
Chlorpromazine	2:	(MID QH - 0.02sec)	+/Daughters of 319	/Gain: 7/CE -24.8
Clemastine	3:	(MID QX - 0.03sec)	+/Daughters of 215	/Gain: 7/CE -24.8
Mebhydrolin	4:	(MID QI - 0.03sec)	+/Daughters of 277	/Gain: 7/CE -24.8
Chlordiazepoxide	5:	(MID RA - 0.03sec)	+/Daughters of 284	/Gain: 7/CE -24.8
Azatadine	6:	(MID RV - 0.03sec)	+/Daughters of 291	/Gain: 7/CE -24.8
Dothiepin	7:	(MID QA - 0.02sec)	+/Daughters of 296	/Gain: 7/CE -24.8
Methdilazine	8:	(MID QL - 0.03sec)	+/Daughters of 297	/Gain: 7/CE -24.8
Clobazam	9:	(MID SD - 0.02sec)	+/Daughters of 301	/Gain: 7/CE -24.8
Oxycodone	10:	(MID SH - 0.04sec)	+/Daughters of 316	/Gain: 7/CE -24.8
Tetrabenazine	11:	(MID RK - 0.04sec)	+/Daughters of 318	/Gain: 7/CE -24.8
Phenylbutazone	12:	(MID QR - 0.02sec)	+/Daughters of 323	/Gain: 7/CE -24.8
Disopyramide	13:	(MID RX - 0.02sec)	+/Daughters of 340	/Gain: 7/CE -24.8
Flurazepam	14:	(MID RX - 0.02sec)	-/Daughters of 287	/Gain: 7/CE 30.0
Temazepam	15:	(MID QB - 0.03sec)	-/Daughters of 298	/Gain: 7/CE 30.0
Lorazepam	16:	(MID QB - 0.03sec)	-/Daughters of 302	/Gain: 7/CE 30.0
Nordiazepam	17:	(MID RY - 0.02sec)	-/Daughters of 269	/Gain: 7/CE 30.0
d_4 - Nordiazepam	18:	(MID SV - 0.03sec)	-/Daughters of 273	/Gain: 7/CE 30.0
Diazepam	19:	(MID RS - 0.02sec)	-/Daughters of 283	/Gain: 7/CE 30.0
Warfarin	20:	(MID SB - 0.02sec)	-/Daughters of 307	/Gain: 7/CE 30.0
Nitrazepam	21:	(MID QC - 0.03sec)	-/Daughters of 295	/Gain: 7/CE 30.0
Flunitrazepam	22:	(MID QC - 0.03sec)	-/Daughters of 313	/Gain: 7/CE 30.0
Bromazepam	23:	(MID SQ - 0.02sec)	-/Daughters of 314	/Gain: 7/CE 30.0
Midazolam	24:	(MID SP - 0.03sec)	-/Daughters of 324	/Gain: 7/CE 30.0

9 125 cycles of Experiment ZM (0.58 s/cycles) Total time: 1.12

Prochlorperazine	1:	(MID QZ - 0.03sec)	+ Daughters of 374	/Gain: 7/CE -24.8
Trifluoperazine	2:	(MID QH - 0.03sec)	+ Daughters of 408	/Gain: 7/CE -24.8
Indoprofen	3:	(MID RW - 0.02sec)	+ Daughters of 296	/Gain: 7/CE -24.8
Fentanyl	4:	(MID QM - 0.03sec)	+ Daughters of 337	/Gain: 7/CE -24.8
Griseofalvin	5:	(MID RR - 0.03sec)	+ Daughters of 353	/Gain: 7/CE -24.8
Carboxy-THC	6:	(MID SQ - 0.04sec)	+ Daughters of 359	/Gain: 7/CE -24.8
Phenoperidine	7:	(MID RZ - 0.03sec)	+ Daughters of 368	/Gain: 7/CE -24.8
Haloperidol	8:	(MID RB - 0.07sec)	+ Daughters of 376	/Gain: 7/CE -24.8
Doxapram	9:	(MID QK - 0.03sec)	+ Daughters of 379	/Gain: 7/CE -24.8
Dextromoramide	10:	(MID SJ - 0.03sec)	+ Daughters of 393	/Gain: 7/CE -24.8
C_{28}	11:	(MID SM - 0.02sec)	+ Daughters of 393	/Gain 7/CE -24.8
Nitrazepam	12:	(MID QC - 0.03sec)	+ Daughters of 295	/Gain 7/CE 30.0
Flunitrazepam	13:	(MID QC - 0.03sec)	+ Daughters of 313	/Gain: 7/CE 30.0
Temazepam	14:	(MID QB - 0.03sec)	+ Daughters of 298	/Gain: 7/CE 30.0
Alprazolam	15:	(MID QB - 0.03sec)	+ Daughters of 308	/Gain: 7/CE 30.0
Econazole	16:	(MID QB - 0.03sec)	+ Daughters of 381	/Gain: 7/CE 30.0
Miconazole[2]	17:	(MID QN - 0.03sec)	+ Daughters of 255	/Gain: 7/CE 30.0
Clonazepam	18:	(MID SN - 0.03sec)	+ Daughters of 329	/Gain: 7/CE 30.0
Indomethacin	19:	(MID RU - 0.03sec)	+ Daughters of 371	/Gain: 7/CE 30.0
Bromazepam	20:	(MID SQ - 0.02sec)	+ Daughters of 324	/Gain: 7/CE 30.0
Midazolam	21:	(MID SP - 0.03sec)	+ Daughters of 324	/Gain: 7/CE 30.0

10 380 cycles of Experiment ZJ (0.49 s/cycles) Total time: 3.06

C_{32}	1:	(MID SM - 0.02sec)	+/Daughters of 449	/Gain: 7/CE -24.8
Thioridazine	2:	(MID QL - 0.03sec)	+/Daughters of 371	/Gain: 7/CE -24.8
Meclozine	3:	(MID RP - 0.06sec)	+/Daughters of 391	/Gain: 7/CE -24.8
Pholcodine	4:	(MID SL - 0.06sec)	+/Daughters of 399	/Gain: 7/CE -24.8
Thiethylperazine	5:	(MID QZ - 0.03sec)	+/Daughters of 400	/Gain: 7/CE -24.8
Thiopropazate	6:	(MID RI - 0.03sec)	+/Daughters of 446	/Gain: 7/CE -24.8
Diphenoxylate	7:	(MID RJ - 0.06sec)	+/Daughters of 453	/Gain: 7/CE -24.8
Verapamil	8:	(MID RB - 0.07sec)	+/Daughters of 455	/Gain: 7/CE -24.8
Sulindac	9:	(MID SK - 0.07sec)	-/Daughters of 370	/Gain: 7/CE 30.0
Thiothixene	10:	(MID RM - 0.04sec)	-/Daughters of 399	/Gain: 7/CE 30.0

2. Also Isoconazole