THE DETERMINATION AND PHARMACOKINETICS OF METHIMAZOLE IN BIOLOGICAL FLUIDS.

THESIS

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ABSTRACT

The Determination and Pharmacokinetics of Methimazole in Biological Fluids.

The development of methimazole therapy, its mechanism of action and pharmacokinetics in man have been briefly reviewed.

Specific, sensitive analytical methods were developed for methimazole and 3-methyl-2-thiohydantoin using gas chromatography- mass spectrometry. Trideutromethylimidazole was successfully synthesised and used as the internal standard. Extraction methods were developed for the separation of methimazole from various biological media. Using these specific methods, various clinical investigations were undertaken.

Dosage linearity was shown when the plasma concentrations and kinetics of patients on a low dosage regimen where compared to those on a high dosage regimen. A lack of accumulation was also indicated as the plasma concentrations were shown to be at steady-state. Plasma concentrations of methimazole could be correlated with its effect on the inhibition of organification. Thus, the great divergence in the therapeutic response to the drug in thyrotoxicosis is obviously not due to differences in plasma pharmacokinetics or the extent of inhibition of organification of iodine.

No significant differences between pharmacokinetic parameters after oral administration to euthyroid and hyperthyroid patients were observed. Thus, it appears that thyroid hormones do not depress or

increase the metabolism of methimazole and therefore there are no pharmacokinetic reasons to adjust the dose of methimazole during treatment of thyrotoxicosis.

Results for intrathyroidal concentrations supported the concept that methimazole is actively concentrated in the thyroid gland by showing no linearity with dose, an increase in thyroid/plasma concentration ratios with time and a decrease in thyroid/plasma concentration ratio with increasing dose thus indicating a saturated system. However, results for the percentage inhibition of iodide organification appears to be in direct conflict with the intrathyroidal levels. It is likely that the perchlorate discharge test underestimates the duration of action of methimazole as it does not gauge the extent of inhibition of coupling by methimazole.

The analysis of infant plasma samples in conjunction with maternal plasma and milk samples showed that the maternal milk/plasma concentration ratio approached unity and that infant plasma levels of methimazole were significant. However, the clinical indices showed no abnormalities in their thyroid function. Therefore, with low doses and careful thyroid monitoring, methimazole is suitable for the treatment of hyperthyroidism in breast-feeding mothers.

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CHAPTER 1

INTRODUCTION

1.1 REVIEW OF HYPERTHYROIDISM AND THE ACTION OF METHIMAZOLE

1.1.1 Introduction

The thyroid gland carries out a specific biochemical process in the formation and secretion of the thyroid hormones, two physiologically potent amino-acids, L-thyroxine (T_4) and L-triiodotyronine (T_3). These hormones exert stimulatory effects on cell metabolism and are essential for the normal growth and development of the body.

A common disorder of the thyroid gland is hyperthyroidism in which the thyroid gland secretes excessive amounts of the thyroid hormones. There are two forms of thyroid hyperfunction which are recognised. 'Diffuse toxic goiter' (Graves disease or Basedow's disease) is overactivity of the whole gland and occurs most commonly in young adults. The second condition is the overactivity of one or more thyroid nodules, 'toxic nodular goiter' , which occurs primarily in older patients and usually arises from long-standing non-toxic goiter. Both disorders can be successfully treated with antithyroid drugs which interfere directly with the synthesis of the thyroid hormones.

Although it has been previously observed that cabbage plants contain a goitrogenic substance (Chesney et al.,1928; Hercus and Purves,1936), the modern era of antithyroid drug therapy began in 1941 with two independent chance observations. MacKenzie et al. studying the antibiotic effects of sulfaguanidine on intestinal bacteria noted thyroid

hyperplasia in the rats exposed to this drug. At the same time, while studying the taste sensation in rats, Richter and Clisby (1942) observed goiters in those fed with phenylthiourea.

It was concluded that this goitrogenic activity was due to the inhibition of thyroid synthesis (Astwood et al., 1943a; MacKenzie and MacKenzie, 1943) and the active moiety was thiourea. Astwood et al. (1943b,1945) screened over two hundred compounds containing the thiocarbamide group for antithyroid activityand reported the first successful clinical treatment of hyperthyroidism with thiouracil. However, thiouracil was found to cause side effects especially agranulocytosis and was abandoned with the introduction, in 1946, of 6-propylthiouracil (Astwood et al.) which displayed increased anti-thyroid activity with decreased toxicity. Methimazole, which displayed even greater potency, was introduced several years later (Stanley and Astwood, 1949) followed by carbimazole (Lawson et al., 1951a,b). Carbimazole is a carbethoxy derivative of methimazole, originally developed in the hope of obtaining a longer acting drug than methimazole. However, carbimazole is now known to be rapidly and totally bioactivated to methimazole after oral intake and its antithyroid action is attributed to methimazole itself.

At present carbimazole is primarily used in Europe whereas propylthiouracil and methimazole are administered almost exclusively in North America.

The most important class of antithyroid compounds are the thiocarbamides (Figure 1.1), of which thiourea is the simplest member.

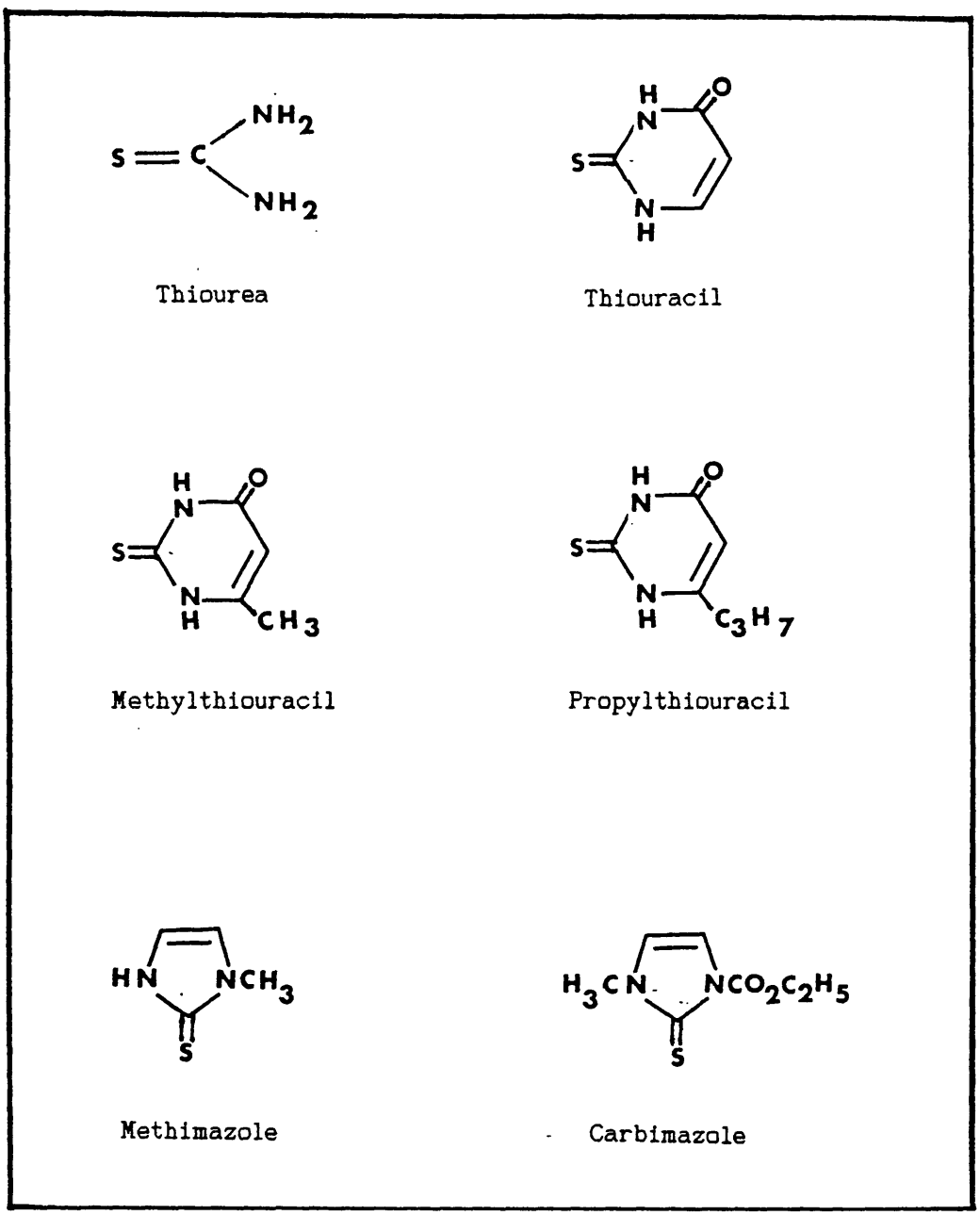


Figure 1.1 The structures of the thioureylene antithyroid drugs formerly and currently used in the treatment of hyperthyroidism.

Although most of them incorporate the entire thioureylene group, in some, one of the nitrogen atoms is replaced by oxygen or another sulphur so that only the thiocarbamide group is common to all.

1.1.2 The Formation of the Thyroid Hormones

The formation of the thyroid hormones involves:-

1)The active transport of extracellular iodide into the gland,

2)the oxidation of iodide and the iodination of tyrosyl groups of thyroglobulin forming monoiodotyrosine (MIT) and diiodotyrosine (DIT),

3)the coupling of the iodotyrosines, MIT and DIT, to form the iodothyronines, triiodothyronine (T_3) and tetraiodothyronine (thyroxine, T_4) and

4)proteolysis of thyroglobulin and the release of T_3 and T_4 into the bloodstream

The pathways of iodide metabolism are shown in Figure 1.2.

Iodine ingested in the diet reaches the circulation in the form of iodide. This plasma iodide is largely extracellular and is removed mainly by the kidneys and the thyroid. The thyroid cells extract iodide from plasma and concentrate it in the interior of the cell and in the colloid. This iodide transport mechanism is an energy dependent process requiring oxygen.

Iodide is oxidised by hydrogen peroxide (H_2O_2) in the microvilli of the apical cell membrane. There seems conclusive proof that the NADPH-cytochrome c reductase is one source of H_2O_2 for the peroxidation of

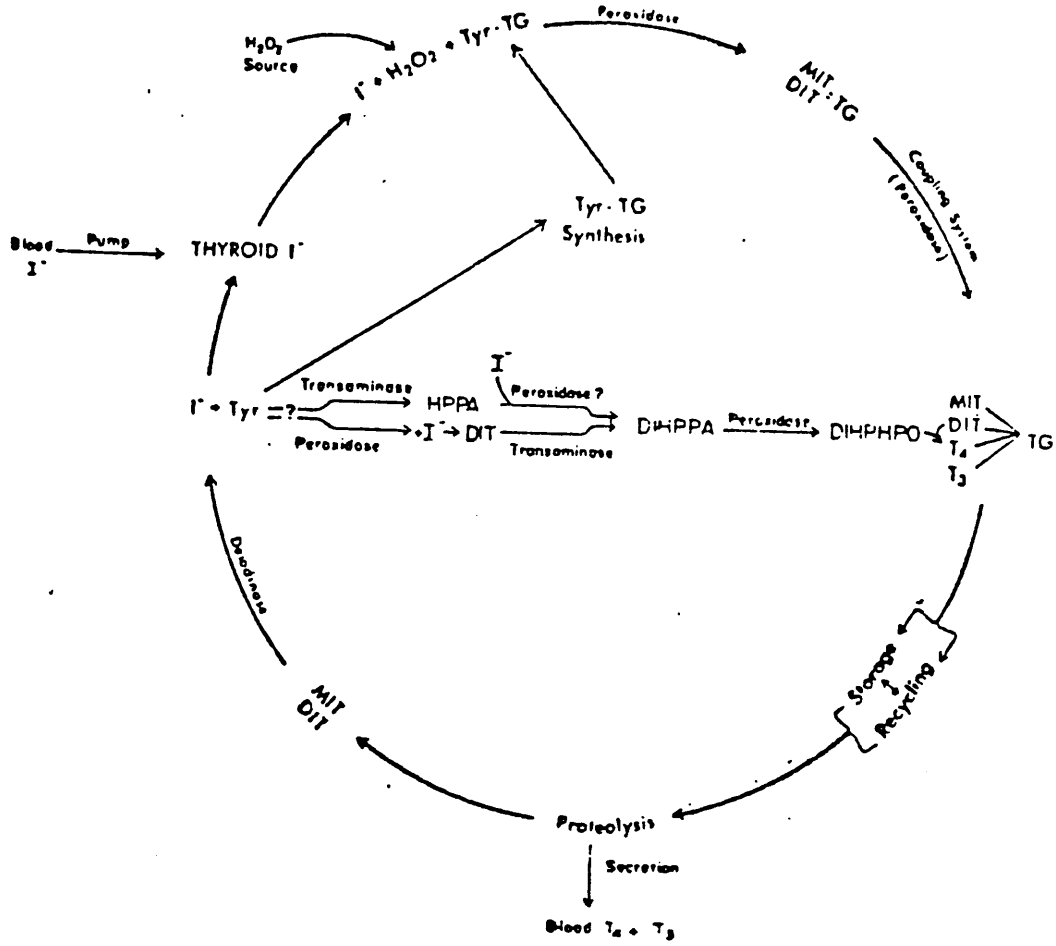


Figure 1.2 Pathways of iodide metabolism by the thyroid gland.

DeGroot and Niepomniszcze, (1977)

iodide(DeGroot et al., 1972). The transfer of reducing equivalents from NADH to NADH-cytochrome B reductase and cytochrome B is another source of H_2O_2 in the thyroid (Ohtaki et al., 1973). Thus, two and possibly more enzymes may be involved in the H_2O_2 generation, since other studies suggest H_2O_2 production by monoamine oxidase (Fischer et al., 1966, 1968). The oxidised form of iodine immediately becomes covalently bound to peptide-linked tyrosyl groups in the thyroglobulin molecule adjacent to it, to form monoiodotyrosine (MIT) and diiodotyrosine (DIT).

The iodothyronine molecules are formed by the attachment of one iodotyrosine (MIT or DIT) to the phenolic group of a nearby DIT molecule. It has been suggested (DeGroot and Niepomnizcze, 1977) that the initial iodination of tyrosyl residues occurs on an immature TG in an uncoiled form with its tyrosyl groups readily open to the iodination enzymes. During the next phase of maturation, the molecule may develop a coiled secondary structure as disulphide bonds, and ionic or hydrogen bonds are formed, thus placing the iodinating tyrosyls close to one another in neighouring coils and facilitating transfer of an iodophenyl group (Figure 1.3).

The normal secretory process begins with the formation of an intracellular 'colloid droplet' by the action of the microvilli of the cell apical membrane. Once inside the cell, the droplets fuse with a lysosome, which has been mobilized from the cell base toward the cell apex, forming a phagosome. Here, the TG is probably completely degraded to its component amino-acids, and the released T_3 and T_4 make their way to the bloodstream. The iodotyrosine are deiodinated by a microsomal

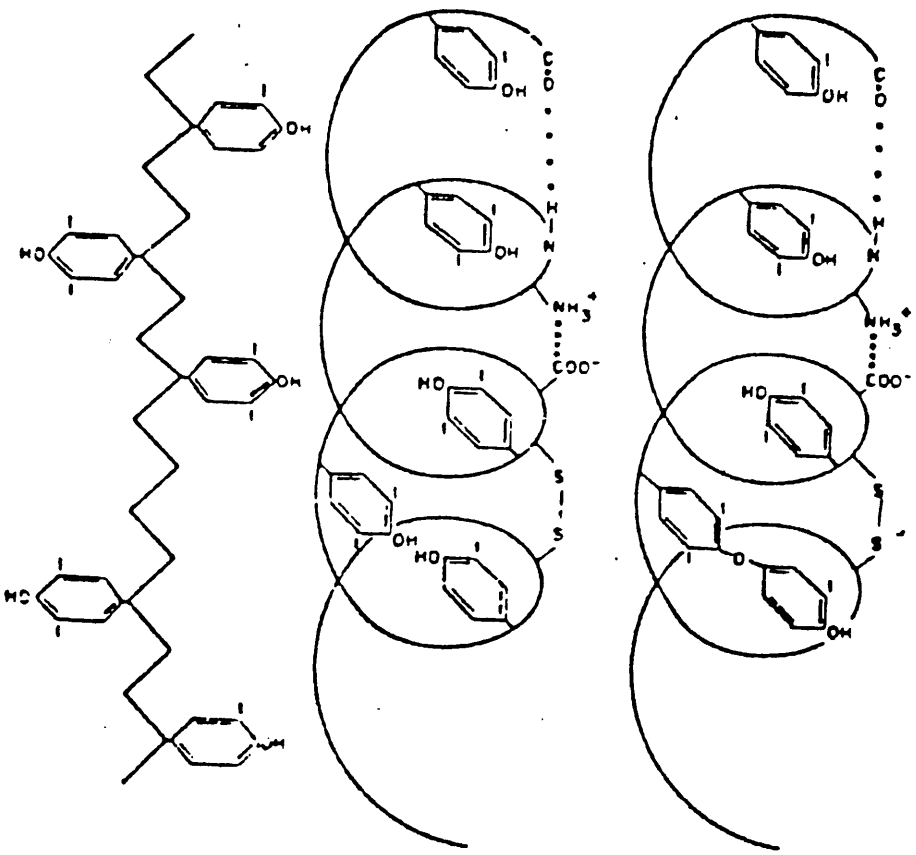


Figure 1.3 A hypothetical model of the intramolecular iodothyrosine coupling process. De Groot and Niepomniszcze, (1977)

flavoprotein deiodinase and the liberated iodide is partially reutilized and partially lost from the cell. This internal iodide cycle is important, for three to five times as much iodide is formed inside the gland each day by deiodinase activity as enters the cell from the serum.

Synthesis and secretion of thyroid hormone is regulated predominately by Thyroid Stimulating Hormone (TSH) from the pituitary gland (Tong, 1971). The secretion rate of TSH is determined by the balance between the negative influence of plasma T_3 and T_4 (Sterling and Lazurus, 1977) and the positive hypothalmic stimulus, Thyroid Releasing Hormone (TRH). TRH stimulates both the release and the synthesis of TSH. TSH concentrations in the serum are markedly reduced or absent in patients with hyperthyroidism due to Graves' disease or toxic nodular goiter.

Normal thyroid function obviously requires an adequate intake of iodine. Without it, normal amounts of hormone cannot be made, thyrotropin is secreted in excess, and the thyroid hypertrophies in response to the need for greater efficiency in extraction of residual traces of iodide from the blood

Prolonged iodine deficiency leads to preferential synthesis of T_3 relative to T_4 , which may be an important iodine-sparing mechanism. High concentrations of iodide appear to influence all important aspects of iodine metabolism by the thyroid gland. Acute effects of iodide to inhibit organification of iodide in the thyroid gland are well-known i.e. the Wolff-Chaikoff Effect (Wolff and Chaikoff, 1948). The intracellular rather than the extracellular concentration of the anion appears to be the major determinant and, with time, the effect was found to be

temporary. This is due to an autoregulatory decrease in iodine transport and a lowered intracellular iodide concentration, allowing organic binding and hormone synthesis to resume (Braverman and Ingbar, 1963).

Iodide also antagonizes the ability of both thyrotropin and cyclic AMP to stimulate endocytosis of colloid, proteolysis and hormone secretion (Piserav et al., 1971).

1.1.3 Site of Action of Methimazole

The main site of action of methimazole is beyond the step of iodide concentration, directly on the synthesis of thyroid hormones from accumulated iodide. Each step in hormone synthesis is affected, however, the main site of action is the initial iodination of tyrosine (Burgi and Haberli, 1977)

All these reaction steps leading to thyroid hormone production are known to be catalysed by a thyroid peroxidase (TPO) enzyme and methimazole has been shown to be an inhibitor of TPO in vitro (Alexander et al., 1959; DeGroot and Davies, 1962; Hosoya, 1963; Mahoney and Igo, 1966; Morris and Hager, 1966; Yip, 1966; Coval and Taurog, 1967). Thus, there is the possibility that methimazole exerts its inhibitory effects by blocking a TPO enzyme in vivo.

There have been a variety of proposed mechanisms for the action of methimazole in relation to TPO (Morris and Hager, 1966; Maloof et al., 1969 ; Taurog, 1976; Michot et al., 1977 and Davidson et al., 1978) two

of which are given in figures 1.4 and 1.5. All of these proposed mechanisms contain some common points. In vivo, an irreversible complex with TPO can be formed in the absence of iodide. There appears to be competition of methimazole with iodide for TPO or TPO-H_2O_2 under certain conditions. The presence of iodide appears to make the inhibition of TPO by methimazole reversible and studies in vivo suggest that methimazole causes reversible inhibition of TPO.

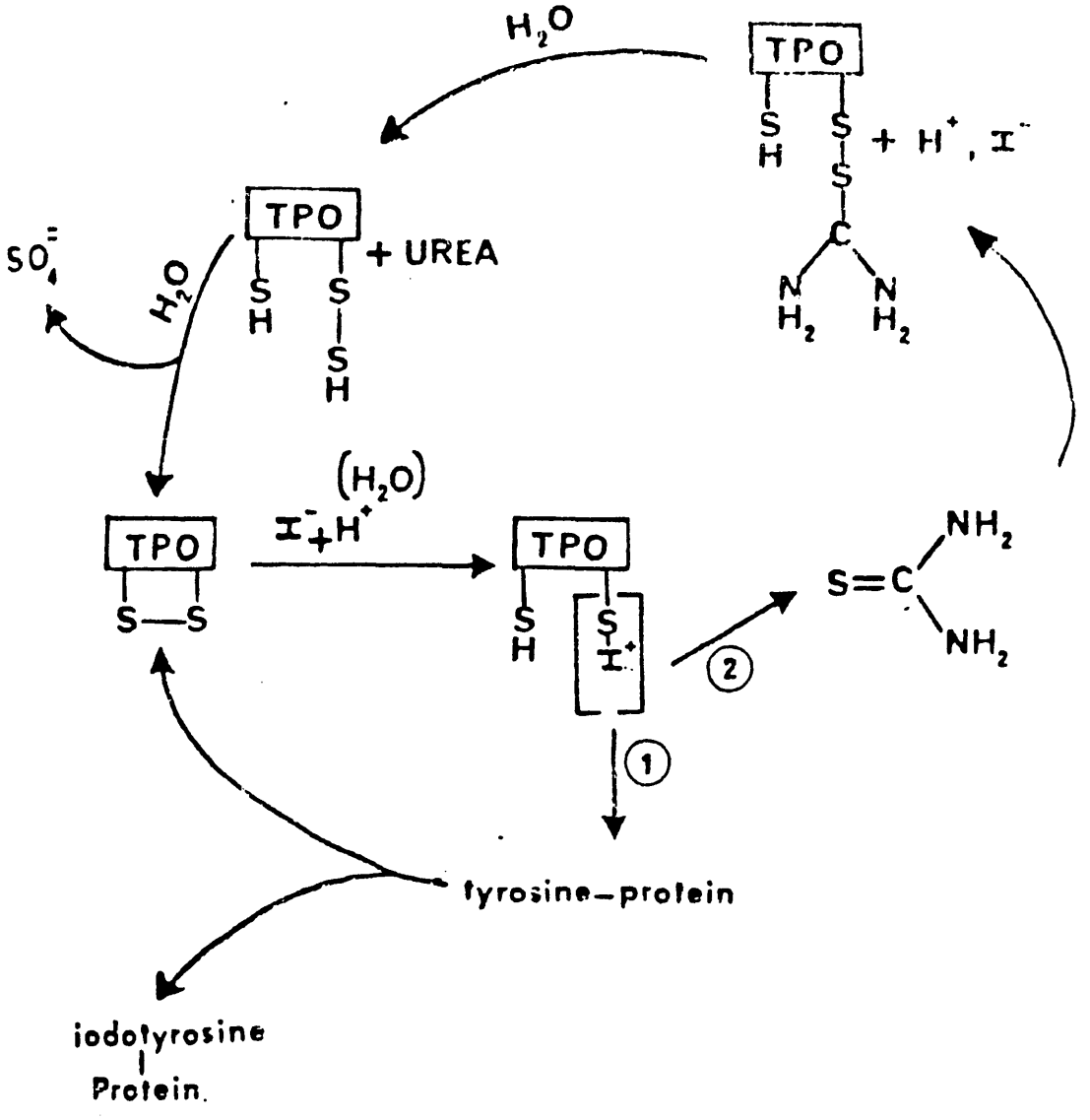


Figure 1.4 Mechanism of action of thioureylene antithyroid drugs on TPO and their resulting oxidation based on proposals by Maloof et al. (1969 a,b).

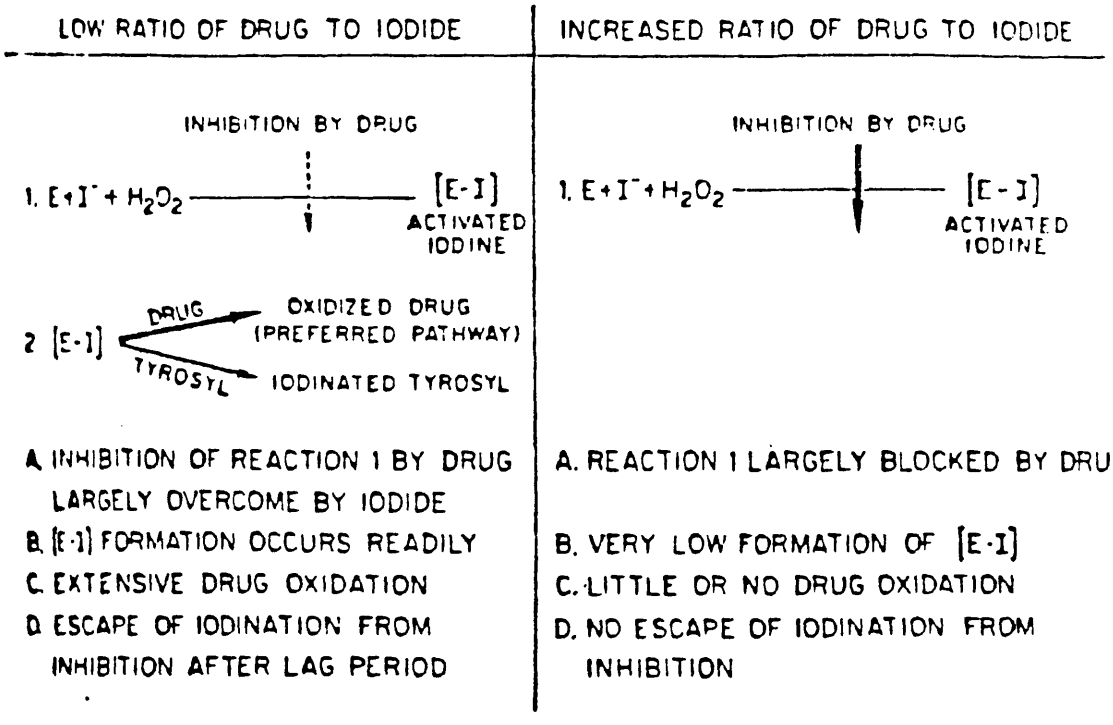


Figure 1.5 Scheme proposed by Taurog (1976) for the mechanism of inhibition by thioureylene drugs of TPO-catalysed iodination.

1.2 REVIEW OF ANALYTICAL METHODS FOR METHIMAZOLE MEASUREMENT

A variety of chemical analytical methods have been used to study the fate of methimazole in biological systems. The techniques which have been used are colorimetry, high performance liquid chromatography (H.P.L.C) , gas-liquid chromatography (G.L.C.) and radioimmunoassay (RIA). An outline of each method is presented in this literature survey.

The first method of measurement of methimazole was colorimetric. This was based on a colour reaction for thiouracils (McAllister, 1951 a,b). It entailed reaction of the methimazole thiol moiety with a 2:6-dichloroquinone-chlorimide reagent. No studies in humans, however, were reported before Pittman et al (1971) improved the method by changing the colour reaction to one with N-2,6-trichloro-p-benzoquinoneimine, and estimated the plasma concentration and urinary excretion of methimazole in healthy euthyroid subjects. Vesell et al (1975) also used this method for further human kinetic studies but the lack of specificity of this method meant that sulphur-containing metabolites of methimazole would contribute to the colour. This would explain the long half-life value obtained by Vesell compared with those of later authors using more specific methods. This problem of specificity was alleviated with the development of H.P.L.C. assays. Meulemans et al.,(1980) combined the 2:6-dichloroquinone-chloroimide procedure and H.P.L.C. to obtain a sensitive method for the determination of methimazole in plasma. The drug complex was detected with a U.V. detector at a wavelength of 405nm. The sensitivity of the method allowed detection to concentrations of 5ng/ml. The method did not employ an internal standard and both antithyroid

Table 1.1 Studies of analytical methods of measuring methimazole concentrations.

Method first published	Method	Use in Human Kinetic Studies
McAllister (1951)	Colorimetric	Vessell et al (1975)
Pittman et al (1971)	Colorimetric	Hallengren et al (1982)
Skellern et al (1974)	HPLC	Skellern et al (1977)
Skellern et al(1976)	HPLC	Melander et al (1980)
		Johansen et al (1982)
Bending and Stevenson (1978)	GLC	
Skellern et al (1980a)	HPLC	Skellern et al (1980a,b)
		Low et al (1981)
Floberg et al (1980)	GC-MS	Tegler and Lindstrom (1980)
		Dahlberg et al (1981)
		Jansson et al (1983a,b)
		Jansson et al (1985)
Meulemans et al (1980)	HPLC	
Cooper et al (1984)	RIA	Cooper et al (1984)
Tatsuhara et al (1985)	HPLC	Okamura et al (1986)
Hengstmann and Hohn (1985)	HPLC	Hengstmann and Hohn (1985)

drugs, propylthiouracil and carbimazole were found to elute from the column with the same retention time as methimazole.

Skellern et al (1974, 1976, 1980a) also developed H.P.L.C. assays capable of measuring quantities of methimazole in plasma. The initial method (1974) used a Porasil C column(2ft X 2.3mm) with n-hexane:tetrahydrofuran 50:50 mobile phase. This method had a limit of detection of only 100ng/ml due to low column efficiency and methimazole not being completly resolved from the solvent peak. Chromatography was improved by the use of a 10um alumina column (100 X 4.6mm) with a mobile phase which consisted of 2% methanol in chloroform. However, this procedure (1976) was still only suitable for the determination of methimazole in plasma above a concentration of 0.1ug/ml. Finally, the replacement of benzamide, the internal standard with p-toluamide (1980), resulted in a slightly shorter analysis time, but had the problem of interference caused by theophylline.

H.P.L.C. with electrochemical detection has also been used to study methimazole in plasma and urine samples (Tatsuhara et al. 1985). This method was capable of detecting 10ng/ml of methimazole.

To date, the most sensitive H.P.L.C. method is that devised by Hengstmann and Hohn(1985) which claims detection of picogram amounts of methimazole. H.P.L.C. separation was performed on a 30 x 0.4cm stainless steel column containing 10um Bondapack C-18, reverse phase material. The solvent system consisted of nine parts of 0.05M potassium phosphate buffer and 1 part of methanol.1,5-dimethyl-2-mercaptoimidazole was employed as the internal standard and methimazole was back extracted

from dichloromethane into 0.1N NaOH thus increasing recovery and reducing interference from primary and secondary phenolic amines.

Stenlake et al. (1970) used G.L.C. to measure methimazole recovered from rat urine. Extracted methimazole was treated with methyl iodide to form the S-methyl methimazole derivative. Reaction time was critical because the instability of the derivative could lead to the retention of free methimazole on the column. The sensitvity of this assay was 250ng/ml. Bending and Stevenson (1978) developed a method using G.L.C. with thermionic nitrogen-phosphorous detection which could measure methimazole down to levels of 30ng/ml. Methimazole was extracted into chloroform and was added to tetramethylammonium hydroxide. Flash methylation by tetramethyl ammonium hydroxide occurs at 300°C in the injection port. 6-hydroxypyridazin-3(2H)-one, the internal standard, was added after the extraction procedure because of poor recovery from the plasma into chloroform.

The most sensitive and specific method yet published for the determination of methimazole was presented by Floberg et al. (1980) using a G.C.-M.S system. The drug was transferred from the sample and derivatised in one step by extractive alkylation. This reaction was with either benzyl chloride or pentafluorobenzyl bromide. A deuterium-labelled analogue, 1-trideuteromethylimidazole-2-thiol was used as the internal standard. The sensitivity of this method was 2ng/ml with a coefficient of variation of about 6%. However, in this technique, the choice of derivative depends on prior knowledge of the expected drug level.

The final development was presented by Cooper et al. (1984) who quantified methimazole by a simple, rapid and precise radioimmunoassay. A methimazole derivative, 1-methyl-2-mercapto-5-carboxyimidazole, was conjugated to porcine thyroglobulin and antibodies to the conjugate were raised in rabbits. The assay has a sensitivity (25ng/ml) which is comparable to those of the chromatographic techniques. The putative methimazole metabolites 3-methyl-2-thiohydantion and 1-methylimidazole had minor cross-reactivities of 2.1% and .5% respectively.

1.3 CLINICAL PHARMACOKINETICS OF METHIMAZOLE

1.3.1 Absorption

The majority of drugs are given extravascularly but are intended to act systemically. Before the drug can exert a pharmacological effect, the drug must be absorbed, prior to distribution to the receptor site, where it will elicit its effect.

The absorption step may, therefore, be responsible for non-ideal drug therapy due to poor absorption or delays in the absorption process.

There are many extravascular routes but oral administration is the most frequently used mode, largely because of its convenience. The extent of material in the systemic circulation from an extravascular formulation compared with an intravenous formulation is given as its percentage absolute bioavailabilty

There are various steps through which a dosage form has to pass before reaching the systemic circulation i.e. disintegration, dissolution and passage through membranes. The dissolution of the solid is often the rate limiting step to drug absorption i.e. the rate of absorption is controlled by how fast the drug dissolves in fluids at the absorption site.

Once in solution the drug has to pass to the membrane for absorption where in most cases it is presumed to be transferred across the membrane by passive diffusion. This is determined by the lipophilic nature of the drug and the membrane. The majority of drugs cross the

lipid membrane of the gastrointestinal tract in their unionised form. Thus, it can be seen that a drug which remains ionised will have poor membrane permeability and this will become the rate determining step in the drug absorption process. The absorption rate is related not only to the permeability of the compound through the membrane but also to the surface area available for transport. Hence, it is immediately obvious that the small intestine is conducive to rapid transfer of drugs. The removal of material from the absorption site increases the effective concentration.

There are also several other physiological factors which may affect bioavailability. As already mentioned, blood flow to the gastro-intestinal tract can greatly affect absorption of drugs. Logically the less the blood flow, the slower the drug is taken away which could possibly compromise absorption.

Any factor which prevents the transfer of drugs to the small intestine could delay absorption. Some drugs decrease (propantheline), whereas others (metoclopramide) increase gastric emptying and, therefore, may influence their own absorption or that of co-administered drugs. The presence of food is a very complex factor with regard to gastric emptying. Blood flow to all parts of the stomach and small intestine are increased 2 to 3 fold after feeding. Consequently the presence of food could in fact increase the absorption rate and maybe the extent, by virtue of increased blood flow, of drugs which are taken both with and just before food. However, large volumes of food tend to slow stomach emptying causing delays in absorption and increased

exposure to stomach acidity. Cold foods tend to delay stomach emptying; low viscosity liquids are emptied more rapidly than high viscosity liquids which also retard drug movement to sites of absorption. Large quantities of electrolytes, fats and some proteins tend to slow the rate of emptying whereas carbohydrates have no effect. Finally the more bulky the food, the slower the emptying rate. Thus, one cannot generalise about the effect of food on the rate and extent of drug absorption.

Preliminary studies in two subjects administered oral and intravenous doses of ^{35}S-methimazole indicated almost complete absorption of the drug with bioavailabilities of 81 and 99% respectively (Alexander et al., 1969; Shimmins et al., 1969) These results have been corroborated by measurements of total excretion of radioactive material in urine and faeces after oral administeration of either ^{35}S-methimazole or ^{35}S-carbimazole (Alexander et al.,1969; Marchant, 1979) The use of total radiolabel data however gives no indication as to whether the drug was metabolized.

After oral dosing any loss of the administered material by microfloral metabolism in the lumen prior to absorption, transmucosal metabolism or hepatic metabolism is termed 'first pass metabolism' since any absorbed material must first pass these potential sites of elimination before reaching the systemic circulation. Thus, radiolabelled studies can only really be used for information on absorption of the total drug related material. To gain an exact measure of the bioavailability of methimazole it is necessary to conduct a

cross-over study measuring unchanged drug in plasma after oral and intravenous administrations.

Studies in hyperthyroid and euthyroid patients administered both oral and intravenous methimazole (Hengstmann and Hohn, 1985), gave values of 0.49 and 1.5 respectively. The value for hyperthyroid patients seems to indicate first pass metabolism. A value greater than one only occurs when drug disposition differs depending on the route of administration. The explanation given by the authors for these different results between the groups is the existence of an unknown metabolite originating from gut wall metabolism. This metabolite interferes with the determination of distribution procedure only in euthyroid subjects. However this explanation seems highly unlikely.

Further studies however seem to rule out first pass metabolism. A bioavailability of 0.93 was achieved for a study of healthy subjects (Jansson et al., 1985). A further study of both normal and hyperthyroid patients found the AUC to be very similiar between intravenous and oral administration even though the different modes of administration were not to the same individuals (Okamura et al., 1986). Therefore, the general, conclusion appears to be that the bioavailability of unchanged methimazole is high, if not complete.

Many investigators have calculated the time taken to reach the peak plasma concentration tmax and the peak plasma concentration values Cmax during pharmacokinetic studies following the oral administration of methimazole. These have been listed in Table 1.2. Generally these studies indicate fairly rapid absorption, with tmax values ranging from

Table 1.2 Studies on the peak plasma concentrations of methimazole following oral administration.

Authors	MMI	tmax (h)	Cmax(ug/ml)	Status
Nelander et al. 1980	60mg 12x5mg	2.04+-0.66	1.325+-0.58	EU
Hallengren et al. 1982	40mg 1x40mg	1.03+-0.58 1.02+-0.79	0.824+-0.14 0.830+-0.20	EU HU
Cooper et al. 1984	30 and 60mg	2.0 1.0	.65+/-.09;1.54+/-.2 .78+/-.1;1.35+/-.01	EU HU
Skellern et al. 1980	60mg 6x10mg	0.5-1.0	1.54-0.45	HU
Pittman et al. 1971	60mg 6x10	1.0	0.92+/- 0.85	EU
Okamura et al. 1986	10mg	1.8+/- 1.4 2.3+/-0.8	0.213+/- 0.84 0.299+/-0.92	EU HU
Jansson et al. 1985	10mg	0.48+/-0.18	0.248+/-0.03	EU
Hengstmann and Hohn 1985	40mg	0.83+/-0.24	0.584+/-0.05	HU
Jansson et al. 1983	10mg	0.9+/-0.5	0.149+/-0.015	EU

0.5 to 3 hours but that there is considerable interindividual variation in both the tmax and Cmax values after oral administration.

1.3.1.1 Conversion of Carbimazole to Methimazole

Carbimazole is a carbethoxy derivative of methimazole, and was introduced by Lawson et al (1951a, 1951b) with a view to obtaining a longer acting antithyroid drug. According to their report, carbimazole is stable in acid solution but is readily hydrolysed in neutral or alkaline solution. Later studies confirm this rapid hydrolysis 'in vivo' in human plasma samples (Stenlake et al., 1970; Skellern et al., 1974)

A marked accumulation of ^{35}S-radioactivity was found in the rat thyroid following the administration of ^{35}S-carbimazole (Marchant et al., 1972), however chromatographic analysis of the ^{35}S-activity in the thyroid showed that all the carbimazole had been metabolised to methimazole, sulphate and protein bound ^{35}S

The conversion of carbimazole to methimazole presumably occurs rapidly either in the gastrointestinal tract or immediately after entry into the circulation (Nakashima et al., 1979, Skellern et al., 1980a) Even three minutes after intravenous injection of ^{35}S-carbimazole to rats, no unchanged ^{35}S-carbimazole, only ^{35}S methimazole could be detected and thus Nakashima et al.,(1979) have concluded that the conversion of carbimazole to methimazole is enzymatic.

In vitro studies have differed over the antithyroid potency of carbimazole. One study showed only a slight inhibitory effect on the

peroxidase activity in human thyroid tissue (Melander et al., 1980) while another showed its potency between that of propylthiouracil and methimazole (Nakashima et al., 1979). However, with its rapid conversion in vivo to methimazole, this inherent antithyroid potency is unimportant.

No major differences in the kinetics of methimazole after dosing carbimazole or methimazole have been reported (Melander et al, 1980; Skellern et al, 1974, 1980a; Jansson et al, 1983) Thus, carbimazole acts in vivo as a 'prodrug' which is rapidly and totally bioactivated to the active methimazole and as such offers no kinetic or dynamic advantage over methimazole.

1.3.2 Distribution

The concentration in the plasma with time following the administration of a single dose depends on the rate and extent of distribution to the tissues and on how rapidly the drug is eliminated. The concentration achieved after distribution is complete is a result, not only of the dose, but also the extent of distribution into the tissue

The rate of distribution of a drug between blood and tissue can be limited either by perfusion or by diffusion. A perfusion rate-limtation prevails when the tissue membranes are poorly perfused with blood i.e. skin, muscle, fat. A diffusion-rate limitation occurs if passage of a drug across the tissue membrane is hampered by the degree of ionisation of that drug in plasma. Due to the lipoid nature of membranes,

distribution equilibrium will be attained more quickly when ionisation is suppressed.

The extent of distribution is determined from the relationship between the concentration of drug in plasma and the known amount of drug in the body. The apparent volume into which a drug distributes in the body at equilibruim is called the ' apparent volume of distribution', (V) .

A few studies have been carried out concerning the volume of distribution of methimazole. In the study of Alexander et al. (1969) using radioactive ^{35}S-methimazole, values of 34.2 and 35.7 L were found in two patients after intravenous administration. Skellern and co-workers (1980a) calculated the total volume of distribution to be 41.9 L after oral administration of either 60mg carbimazole or methimazole to fifteen hyperthyroid patients. To gain a true estimate of the volume of distribution however, studies must be performed after intravenous injection with drug levels measured by a specific analytical technique. In recent years such studies have been undertaken by various investigators. Using the GC-MS assay developed by Floberg et al., 1980; Jansson et al. (1985) estimated the apparent volume of distribution for seven normal patients following a single intravenous bolus injection of 10mg of methimazole. The values (26.5-63L), roughly equalled the total body water as did a similar study by Hengstmann and Hohn (1985). Using H.P.L.C. with electrochemical detection, a value of 56+/-6L was determined following an intravenous injection of 40mg methimazole to eight hyperthyroid patients. However, in the following year, Okamura et al

Table 1.3 Studies on the apparent volume of distribution of methimazole

Study	No. of Subjects	V(L) +/- S.D.	Method
Alexander et al. (1969)	2HT	35 +/- 1	r/a
Skellern et al. (1980a)	15HT	42 +/- 38	HPLC
Jansson et al. (1985)	7EU	45 +/- 11	GC-MS
Hengstmann and Hohm (1985)	8HT	56 +/- 6	HPLC
Okamura et al. (1986)	[1]5EU	*2.1 +/- 0.6	HPLC
	15HT	*1.9 +/- 1.1	

1 EU = normal

* Volume given in (L/kg)

Weight for normal patients = 61.8 +/- 4.5

Weight for hyperthyroid patients = 49.2 +/- 6.2

calculated the volume of distribution after intravenous administration of 10mg methimazole to five normal (86-179L) and fifteen hyperthyroid (34-166L) patients. These values, which are much higher than previous studies, suggest that methimazole is more widely distributed.

The extent of distribution throughout the body is dependent on that particular drug's physico-chemical properties. Studying the chemistry of methimazole, it can be seen that in plasma (pH7.4) it will be unionised and therefore free to move across the lipid membrane into tissues. Also, methimazole displays neglible protein binding and thus the percentage of the dose available for distribution will be high. Thus, methimazole would be expected to distribute quite widely. However, methimazole is not considered to be highly bound to tissue proteins, a condition which produces large apparent volumes of distribution. Also, for small lipid soluble compounds like methimazole, the time to equilibrate tends to be perfusion rate limited. Highly perfused tissues tend to equilibrate rapidly; poorly perfused tissues slowly. As methimazole is rapidly eliminated, distribution equilibrium may not have been achieved in more poorly perfused tissues such as muscle and fat.

1.3.2.1 Placental Transfer of Methimazole

Again, because of its lipid solubility, low protein binding and suppressed ionization, methimazole can leave the maternal circulation and cross the placenta becoming a risk to foetal thyroid function. These assumption were confirmed by Marchant et al.,(1977). The placental

transfer of ^{35}S-methimazole and carbimazole was measured in euthyroid women undergoing therapeutic abortion after 8 to 18 weeks of pregancy. By comparing maternal and foetal serum concentrations at delivery two hours after oral administration it was found that the foetal:maternal ratio was 0.72 to 1.09

Unfortunately, addition of thyroxine or tri-iodothyronine seems of little value as the placenta is relatively impermeable to thyroid hormones and the supplement may increase the dose of methimazole needed to control the maternal hyperthyroidism.

1.3.2.2 Concentration in the Thyroid

The distribution of methimazole in the human thyroid has been studied following single oral doses of ^{35}S-methimazole and carbimazole in patients requiring thyroidectomy (Marchant et al., 1972; Lazarus et al., 1975). These studies have shown that methimazole is actively concentrated by the thyroid gland. Thyroid/serum ratios were greater than 1 in thyrotoxic and normal thyroid tissue. In normal thyroid tissue eight to twelve hours after administration, greater than 90% of iodine organification was inhibited. In thyrotoxic tissue the mean inhibition was 80% at eight hours and a considerable interindividual variation was seen in antithyroid effect at the same drug dose.

A much more marked thyroidal accumulation of methimazole was found by Jansson et al.(1983b). Using a GC-MS method, they studied intrathyroidal concentrations in twenty euthyroid patients with Grave's

disease. On treatment with carbimazole and thyroxine they found thyroid to serum ratios of 5, three to six hours and 61, seventeen to twenty hours after the last oral dose. These studies indicate that only a small proportion of a dose is taken up in the thyroid gland of a patient receiving continuous therapy. Jansson et al. postulated that this could be due to self-inhibition of thyroidal uptake by methimazole and might explain why they could not demonstrate a statistical difference in intrathyroidal methimazole concentrations between patients receiving 30-45mg of carbimazole and those receiving 15-20mg. Also, the mean intrathyroidal drug concentrations did not differ between the group receiving the final dose three to six hours preoperatively and the group taking the final dose seventeen to twenty hours before excision. This indicates that the elimination of methimazole from the thyroid gland is much longer than the elimination from the peripheral circulation.

1.3.3 Elimination

Elimination is defined as the irreversible loss of substances from the site of measurement within the body. The rate of elimination is often expressed as the elimination half-life ($t_{½}$) i.e. the time taken for the concentration to decrease by one half. This parameter is measureable only when elimination is the major disposition entity i.e. the terminal phase of the concentration versus time profile. The $t_{½}$ values published for methimazole are given in Table 1.4. Drugs may be eliminated by one of many routes e.g. metabolism, excretion, biliary

secretion, into saliva, into sweat, into milk; the first two usually constitute the major routes of elimination.

1.3.3.1 Renal Excretion

The renal route is the ultimate in elimination of some drugs and most metabolites. In fact all low molecular weight compounds which are not bound to high molecular weight proteins are glomerular filtrated. Some compounds are also actively secreted from plasma into the tubular lumen, mainly along the proximal tubule. The extent of protein binding has no effect on secretion.

Water is reabsorbed along the length of the kidney tubule. This water reabsorption leads to drugs which were filtered and perhaps secreted, being hundred times more concentrated by the distal-collecting tubules. Thus, because of the possible concentration gradient there is a tendency for the drugs to be reabsorbed. The degree of reabsorption is proportional to the degree of ionization of the compound.

The amount of methimazole excreted unchanged in the urine of euthyroid and hyperthyroid subjects has varied from 5 and 12% of the administered dose (Marchant, 1979; Pittman et al., 1971; Okamura et al., 1986). Also the elimination of methimazole remained unaltered in patients with renal impairment (Jansson et al., 1985). These results exclude renal excretion as a major pathway of elimination of unchanged drug. However, chromatographic analysis of the radioactivity in urine 48 hours after ^{35}S-methimazole administration showed four ^{35}S-

Table 1.4 Studies on the elimination half-life of methimazole

Study	Subjects	t½(h)	Method
Alexander et al. (1969)	1HT	13.8	Total r/a
	1EU	20.7	
Pittman et al. (1971)	11EU	6.4	Colorimetric
Crooks et al. (1973)	?HT	7.9	Total r/a
	?EU	11.2	
Skellern et al. (1974)	3EU	3.5-4.4	HPLC
Vesell et al. (1975)	4HT	6.9+/-0.6	Colorimetric
	15EU	9.3+/-1.4	
Balzer et al. (1975)	5HT	28	Total r/a
	7EU	20	
Bending and Stevenson (1978)	1HT	3.7	GLC
Melander et al. (1980)	11EU	3.7+/-1.6	HPLC
Skellern et al. (1980)	15HT	3.1+/-0.8	HPLC
Johansen et al. (1982)	5EU	4.9+/-2.0	HPLC
Jansson et al. (1983)			
Cooper et al. (1984)	5HT	6.8	RIA
	6EU	6.0	
Jansson et al. (1985)	14EU	5.3+/-0.5	GC-MS
Hengstmann and Hohn (1985)	3HT	6.1+/-0.3	HPLC
	2EU	5.1+/-0.1	
Okamura et al. (1986)	[1]5EU	20.7+/-9.6	HPLC
	15HT	18.5+/-12.9	

1 EU = normal

compounds: unchanged methimazole (7%); an unknown major polar metabolite (about 50%) not bound to either glucuronic or sulphuric acid; inorganic sulphate (6%); and a minor ^{35}S-metabolite (1.5%)
[Marchant, 1979]. Another minor metabolite was also identified, this time by thin layer chromatography and H.P.L.C. (Skellern et al., 1977). Skellern et al. showed that small amounts of 3-methyl-2-thiohydantoin could be demonstrated in urine and also plasma and thyroid tissue after administration of either carbimazole or methimazole. Thus, it is fair to say that the metabolites are renally cleared with the main metabolite being a strongly polar non-glucuronide compound. As the readsorption is proportional to the ionisation of the compound, this non-lipid soluble, polar metabolite would show very little readsorption along the distal tubule.

1.3.3.2 Metabolism

As already mentioned, metabolism is considered to be the major route of elimination. Generally metabolism is considered to occur in the liver. However it can also take place in the gut wall, plasma, kidneys and other tissues. Metabolism occurs because the body recognises the molecule as 'foreign' and, if it is unable to eliminate it unchanged, can alter it into a form which is more soluble in physiological fluids and hence more readily excreted. Generally, this means increasing its water solubility. The acidity of the molecule may be increased and this will aid solubility because most physiological fluids are slightly basic.

Two studies in patients with hepatic impairment both showed a prolonged elimination half-life of methimazole (Jansson et al., 1985; Cooper et al., 1984). These observations indicate that the major elimination route is hepatic metabolism.

The biliary excretion of equimolar doses of ^{35}S-methimazole and ^{35}S-carbimazole (Papapetrou et al.,1972) and a dose of ^{14}C-methimazole (Sitar and Thornhill, 1973) has been studied in rats. The total amount of ^{35}S radioactivity excreted in the bile 5 hours after ^{35}S-methimazole and carbimazole was 21.1 +/-2.8 and 31.71+-8.16% of the dose, respectively. In contrast, the total amount of ^{14}C-radioactivity excreted in 10 hours was only 9% of the dose which probably reflects the biliary excretion of a greater number of methimazole metabolites containing sulphur than carbon. It is also interesting to note that hepatic cytochrome oxygenases were capable of oxidising methimazole to 3-methyl-2-thiohydantoin and N-methylimidazole (Neal and Lee,1978). Conversion of methimazole to 3-methyl-2-thiohydantoin involves the production of a new chemical group on the molecule. This kind of metabolism is usually consistent between different species, varying only in quantity. The oxidation at the olefinic carbon atom gives increased water solubility and therefore increased elimination. The metabolism probably occurs in the endoplasmic reticulum of liver cells by enzymes known collectively as Cytochrome P-450.

In studies of ^{35}S-methimazole much more drug is excreted in the bile than in the faeces (Alexander et al.,1969). Thus it would seem likely that an enterohepatic circulation occurs for methimazole and/or its

metabolites and indicates that the majority of the radioactivity excreted in the bile may be reabsorbed and finally excreted by the kidney. Indeed the half-life of 3-methyl-2-thiohydantoin was calculated to be 13.5 hours (Skellern et al.,1980a); i.e. at least three times longer than methimazole which certainly points to the possibility of enterohepatic cycling. This could be verified by animal experiments involving cannulation of the common bile duct. An animal is dosed with radiolabelled methimazole and the bile collected. This is reinfused into the intestine of another animal from which bile is also collected. If radioactivity is recovered in the bile from the second animal, enterohepatic circulation is said to occur.

Agreement exists as to the main metabolite of methimazole being a methimazole glucuronide in the bile (Papapetrou et al.,1972; Sitar et al.,1973). There is considered to be a molecular weight requirement for the biliary excretion of drugs. For many, attainment of such a molecular weight is usually achieved by metabolism; e.g. conjugation with glucuronic acid. Such metabolites can the appear in the faeces. However conjugation of methimazole with glucuronic acid gives a molecular weight of 290 which is not large enough to allow such a process. Instead this conjugate would be hydrolysed in the gut by the gut bacteria and enter into enterohepatic recycling. Conjugation reactions are usually mediated by high energy intermediates (PAPS,UDPGA) and often occur in the cytoplasm. Qualitative differences can occur from species to species, however MMI-S-glucuronide has been shown to occur both in rats

(Marchant and Alexander,1972; Skellern et al.,1973) and man (Alexander et al.,1969).

1.3.3.3 Excretion of Methimazole in Breast Milk

Excretion of xenobiotics in breast milk is a relatively minor pathway but important in terms of the effect of ingestion by the suckling infant. Milk is a complex fluid with high fat and protein levels with significant changes in composition during the course of lactation. A selective blood/milk barrier exists for the mammary ducts of most species studied. The duct membrane is permeable to water but milk remains isosmotic to plasma.

Since human breast milk is an aqueous fluid of heterogenous and varying composition, the amount of drug excreted in milk will vary with both the composition and the yield. Fundamental processes for xenobiotic elimination determine which drugs will be excreted. Physiochemical properties of the drug influence both its passage and 'trapping' into milk components. A schematic representation of drug transfer between milk and plasma is given in Figure (1.6).

The main factors which affect drug transport into milk are: milk and plasma pH; milk and plasma protein binding; milk fat partitioning. Of these factors, the ones affected by changes in milk composition are milk fat partitioning and protein binding.

The extent and affinity of drug binding to both plasma and milk proteins are a determinant of drug concentration in whole milk.

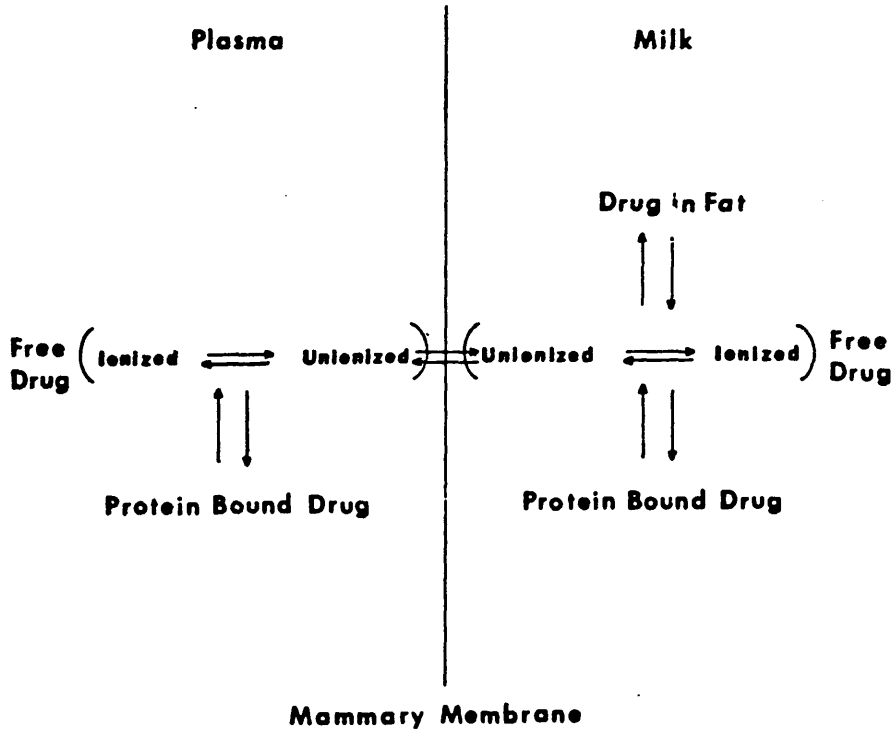


Figure 1.6 Schematic representation of a model for drug transfer between milk and plasma.

Johansen et al. (1982) showed that there was only a negilible amount of methimazole bound to plasma proteins. Also, Cooper et al.,(1984) showed from studies with ^{35}S-methimazole that greater than 99% of the radioactivity was recoverable from the aqueous phase of milk after centrifugation and thus concluded that milk protein binding of methimazole was also negligible.

Drug transfer into milk is generally considered to be characterized by the following assumptions: only the unbound, unionized form of the drug which is located in the aqueous phase of the plasma and milk can diffuse across mammary membranes, and no carrier mediated transfer occurs. Methimazole, with a pKa value of 11.5 (Skellern et al.,1981) remains unionised in both plasma (pH6.6-6.8) and milk (pH 7.4-7.7)

The coefficient of lipid solubility for an unionised drug determines both its penetration of the biological membrane to gain entrance to milk and also its concentration in fat.

Methimazole has a high lipid solubility and thus, considering this factor and also that it is a non-protein bound drug showing no ionisation in a hydrophilic enviroment, indicates that it would tend to equilibrate across the lipid barrier of the mammary gland. Despite these simple physiological considerations, it was the general belief that methimazole was concentrated in breast milk. This was based on the findings of Williams et al. (1944) who demonstrated that the level of thiouracil in breast milk was three times higher than the level in whole blood.These results were later found to lack sensitivity and specificity, Schuppan et al.(1973), but the findings had already been extended to

methimazole. However, five recent studies have challenged this assumption (Low et al., 1979; Tegler etal., 1980: Johansen et al., 1982; Cooper et al., 1984 and Notarianni et al., 1986). These studies show a milk : plasma ratio of methimazole from 0.58 to 1.19 and that the drug was never concentrated in the milk.

1.3.4 Design and Optimization of Dosage Regimen

Dosage describes both the route and the rate of administration of drug. Some drugs e.g. analgesics, hypnotics, neuromuscular blocking agents, bronchodilators and antiemetics are used effectively as a single dose. More frequently drugs are used on a continuous basis. Also most drugs are used in a dosage regimen that results in measureable and often pharmacologically active levels of drug persist in the body when the next dose is administered.

For drugs administered in a fixed dosage interval, the peak plasma level following the second and succeeding doses is higher than the peak level after the first dose therefore the drug accumulates in the body relative to the first dose. Under such conditions drug accumulation proceeds at a decreasing rate with increasing number of doses until a steady state plasma level of drug is achieved. At steady state the plasma concentration of drug at any point in time during any dosing interval will be identical. For the continuous maintenance of

therapeutic amount of drug in the body, the initial and maintenance doses must be given at dosing intervals that keep the amount above a minimum effective level and below a level producing excessive side effects and toxicity. The rate and extent of accumulation of a drug are dependent on the relative magnitudes of the dosing interval and the half-life of the drug.

In the treatment of hyperthyroidism with methimazole various dosages are recommended. Some authors favour a fixed dosage in all thyrotoxic patients (Havard, 1974; Irvine and Toft, 1976), while others recommend individualized doses based on an assessment of the severity of the disease (Braverman, 1978; Solomon, 1978). In the latter case it can sometimes be difficult in a given patient to determine a suitable methimazole dosage regimen. However there is also the problem of adverse reaction with methimazole usually occuring within four months from the start of treatment. The most common side effects are nausea, headaches, rashes and arthralgia. Rarer reactions include alopecia, agranulocytosis and jaundice. Complete spontaneous reversibility is the rule after withdrawal of the drug. Goitre and hypothyroidism are usually a result of overtreatment. Occurence of adverse reactions, especially near the beginning of treatment where high doses are used, seems to indicate a dosage relationship.

This problem of amount of dose needed for efficacy versus side effect was studied by Romaldini et al. (1983). He compared the remission rate in patients receiving high dose therapy (60mg daily) with those in patients receiving a maintenance dose (13.6mg daily). The group

receiving high-dose therapy had an almost twofold higher remission rate after a mean follow-up period of forty-two months. However the rate of side effects was also higher.

The dosage regimen currently recommended for methimazole (British National Formulary 1987) is 30-60mg daily until the patient becomes euthyroid (four to eight weeks) when it is reduced to a maintenance dose of between 5 and 15mg daily.

The dosage regimen employed is assessed using appropriate chemical indices (Alexander et al.,1973; Wise et al.,1973; Low et al.,1979; Dahlberg et al., 1981; Romaldini et al.,1983). There are many laboratory techniques available for assessing the level of thyroid function. These fall into three catagories:-

(1) total circulating levels of thyroid hormone

(2) circulating levels of free thyroid hormone e.g. Free Thyroxine Index (FTI) and

(3) dynamic tests of thyroid function e.g. ^{132}I Uptake, ^{99m}Tc Uptake and the Potassium Perchlorate test.

Some of these clinical indices are not reliable indications of thyroid overactivity especially basal metabolic rates or serum thyroxine (T_4) levels (Wayne, 1960; Larsen, 1975; Nusynowitz and Young, 1979)

Pharmacokinetics can serve as a useful means of evaluating existing dosage regimens. Plasma concentrations, however, can only be used for any correlation if they are at steady state and the literature is very sparse for multiple dosage kinetic studies. One study by Dahlberg et al.(1981) found that although mean concentrations of methimazole showed

a linear dose response (80ng/ml to 35ng/ml after dose reduction of from 30 to 15mg daily) they did not correlate significantly with the actual T_3 levels.

One of the major questions relating to methimazole therapy is whether patients require single or multiple daily doses of medication. Pharmacokinetic studies suggest that methimazole with a half-life of three to six hours cannot be effective when administered in single daily doses. Yet a study by Wise et al.(1973) showed that after divided dose therapy had blocked thyroidal hormonogenesis, this block could be maintained with an equivalent single daily dose.

Therfore, clearly plasma concentrations cannot be related to therapeutic efficacy. There are two possible reasons for the apparent lack of correlation. Firstly, the ratio of plasma methimazole concentration to methimazole at the site of action does not remain constant over time. As discussed earlier (section 1.3.2.2), methimazole is actively concentrated by the thyroid gland and therfore the intrathyroidal methimazole concentrations are more clearly related to clinical effect. Secondly, no account as been made of metabolite activity. As discussed earlier (section 1.3.3.2), 3-methyl-2-thiohydantoin has a plasma half-life three times longer than methimazole and prolonged duration of action may be partially attributable to the thyroid activity of this metabolite. 3-methyl-2-thiohydantion has been shown to have antithyroid activity in the rat (Searle et al., 1951), but this remains to be demonstrated in humans.

1.3.5 Pharmacokinetics in Hyperthyroidism

Many pharmacokinetic studies are performed in normal human subjects but little information has appeared on the effects which disease states may have on drug pharmacokinetics. Disease states by definition change the normal functions of the body, so it is not suprising that drugs administered under these conditions could have altered pharmacokinetics. In particular, usual dosage regimens may need to be substantially modified in patients with renal function impairment and liver disorders (altered metabolism and elimination), congestive heart failure (protracted and erratic drug absorption, reduced liver and renal blood flow giving the risk of toxicity), gastrointestinal disorders (altered absorption) and thyroid disorders.

Hyperthyroidism increases the general metabolic rate. Excess thyroid hormones result in increased protein synthesis and enzymatic activity. The heart, diaphragm, liver and kidneys are all markedly stimulated by thyroxine. Thus, it would seem logical to suspect that the biotransformation of drugs would be enhanced in patients with this disorder.

The comparison of certain pharmacokinetic parameters between hyperthyroid and euthyroid patients should give a reflection of the physiological variables of that disease state and the dosage regimen can be adjusted accordingly.

The problem of altered metabolism of methimazole in hyperthyroidism has attracted many investigators. Early studies using non-specific

methods suggested a shortened half-life in hyperthyroidism and a prolonged one in hypothyroidism (Balzer et al., 1975; Crooks et al., 1973; Vesell et al., 1975). However, with the development of the GC-MS method (Floberg et al., 1980), the kinetic profiles of oral methimazole were compared in hyperthyroid patients both during the hyperthyroid and euthyroid states and no significant differences in Cmax, tmax, t½ or AUC were found. These results were confirmed in similiar studies involving oral administration of methimazole undertaken by Cooper et al. (1984) using RIA and Jansson et al. (1985) again using GC-MS. Thus it appeared that hyperthyroidism did not affect the kinetics of methimazole. However, conflicting data was then published by Hengstmann and Hohn (1985). Using both oral and intravenous data they found a prolonged half-life, lower clearance and bioavailability in hyperthyroid patients. These results were the complete reverse that would be expected if the increased metabolic rate was to affect methimazole at all.

The following year the most extensive study to date was undertaken by Okamura et al (1986). Their results were compatible with those of Hallengren, Cooper and Jansson and showed that hyperthyroidism does not affect the kinetics of methimazole.

The final solution to this question must come from studies using both intravenous drug administration and the same patients before and after treatment to correct the altered thyroid state. In this way any fluctuations caused by variable gastrointestinal absorption and interindividual variation will be minimized.

1.4 AIMS AND OBJECTIVES OF THE STUDY

This review has outlined the development of methimazole therapy, discussed in detail the theories of its mechanism of action and described what is known about its metabolism and pharmacokinetics in man. There is a substantial amount of information concerning the absorption, distribution and excretion of methimazole but there are still some areas which require further investigation in order to obtain a truly comprehensive view of the disposition of methimazole. With a greater understanding of the kinetics involved more accurate predictions for suitable dosage regimens will be made resulting, ultimately, in improved drug efficiacy.

The main areas for investigation are the frequency and size of dose, variation in drug handling with thyroid status, and a detailed examination of the levels of methimazole achieved in maternal blood, umbilical cord and in milk during pregnancy and lactation.

The proposed investigation will aim to develop a simple but selective extraction and gas chromatography-mass spectrometry assay for the measurement of methimazole from a variety of biological matrices. This technique will then be used for some of the clinical investigations mentioned above. Dose response relationships will be studied in patients at two doses and the concentration time curves will be correlated with the effect of organification. The variation of pharmacokinetics will be compared in hyperthyroid and euthyroid patients. Thyroidal methimazole levels will be measured and correlated

to the drug action. A study of maternal blood and milk and infant blood will be undertaken to assess the safety of methimazole treatment in lactating mothers with regard to the suckling infant. Finally, an additional study will be made of methimazole levels in plasma and urine of the racing greyhound. This will be used to compare plasma and urinary levels and to investigate the possibility of measuring 3-methyl-2-thiohydantion by a specific gas chromatographic-mass spectrometry method.

CHAPTER 2

GAS CHROMATOGRAPHY-MASS SPECTROMETRY

2.1 INTRODUCTION

Gas-liquid chromatography (GLC) is a process in which a mixture of organic compounds in the vapour state are separated into their constituent parts by partition between a mobile gaseous phase and a stationary liquid phase.This technique was first introduced to the field of analytical chemistry by James and Martin (1952).

Mass spectrometry is a technique for separating charged particles derived from the molecule of interest. The development of the mass spectrometer dates historically from 1897 when Wien showed that a beam of positive ions could be deflected using electric and magnetic fields. The first mass spectrometers were available for commercial use in 1940 (Washburn and Hoover). The coupling of the mass spectrometer to the gas chromatograph for use as a detector and analyser of GLC effluents was achieved by Ryhage (1964). The dynamic combination of a gas chromatograph and a mass spectrometer permits direct identification of compounds. The mass spectrometer gives additional selective information about the compound, other than that of retention time alone. Figures 2.1 and 2.2 show a pictorial and schematic diagram of a gas chromatograph - mass spectrometer, respectively.

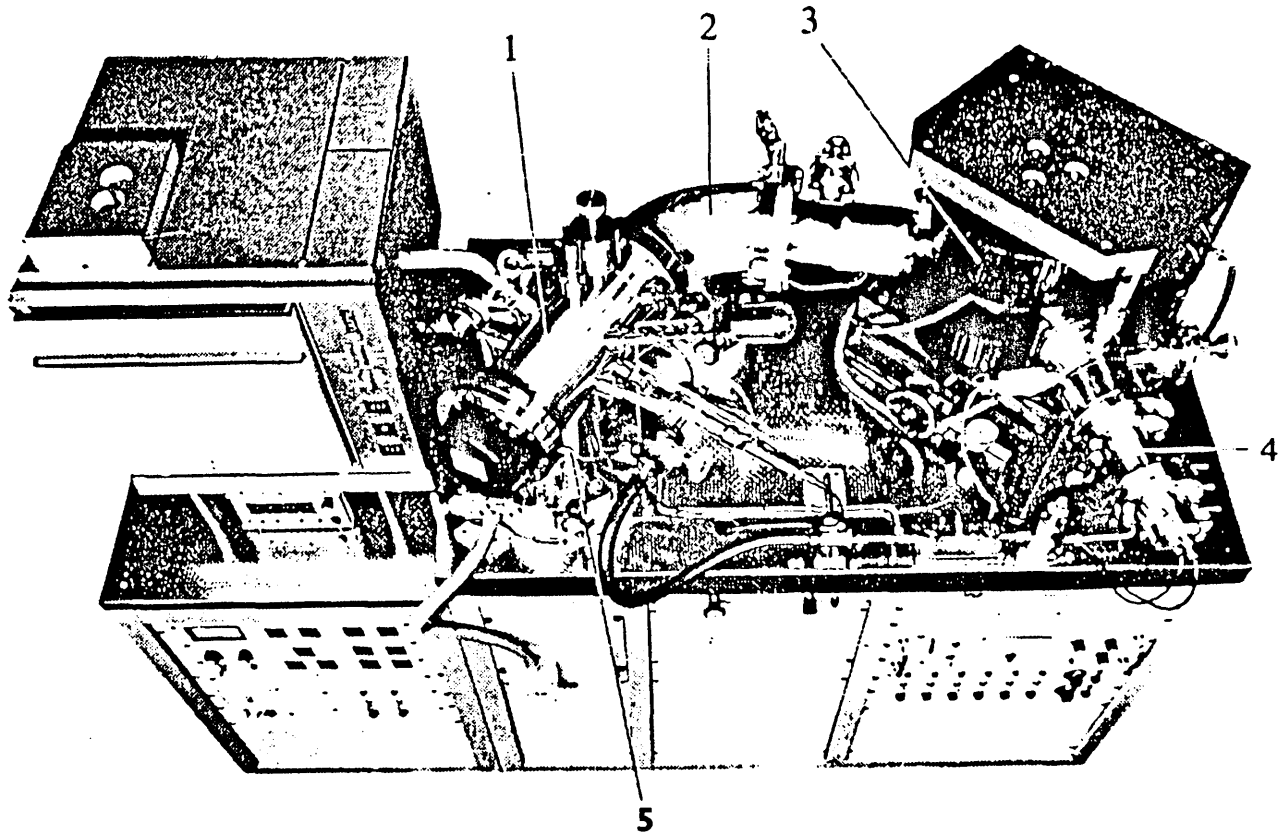


Figure 2.1 Angled view of a modern, double-focussing magnetic sector mass spectrometer (the Micromass 7070) to show the ion source, analyser region and detector. 1, ion source; 2, electric sector; 3, magnetic sector; 4, detector (an electron multiplier). The inlet line for chemical ionization reactant gas is also labelled (5). Photograph by kind permission of VG Analytical Ltd, Altrincham, England.

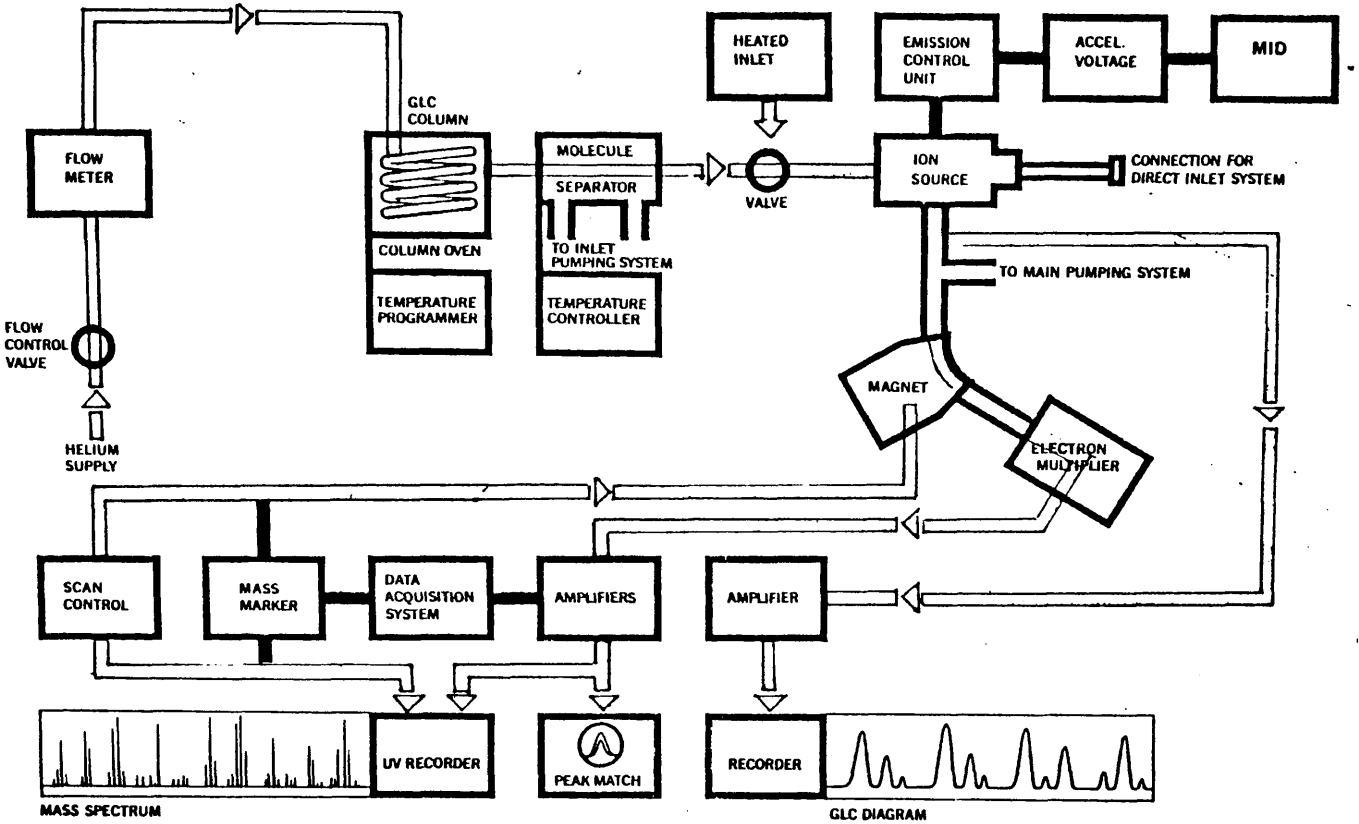


Figure 2.2. Block diagram of integrated LKB 9000 gas chromatograph-mass spectrometer. Courtesy of LKB.

2.2 GAS CHROMATOGRAPHY

2.2.1 Columns

The GC column is the heart of a GC/MS system. Without its power of separation the mass spectral data would be impossible to interpret.

2.2.1.1 Packed Columns

Simple mixtures are best suited to packed columns which can give relatively short analysis times.

The column tubing, either glass or stainless steel, is filled with small solid particles (solid support) each coated with a liquid phase. The ideal features of a solid support are that it should consist of inert uniformly spherical particles having a large surface area per unit volume and that it should be mechanically strong over a wide temperature range. The most commonly used supports are diatomaceous earths, either kieselguhr or crushed firebrick.

A wide variety of stationary phases are available commercially. They range from non-polar to very polar materials. The stationary phase is a liquid which is non-volatile at the operating column temperature. Separation of the sample is achieved by the difference in the solubilities of its individual components in the liquid phase. The liquid phase is only loosely bound to the support and slowly elutes as "column bleed" as the temperature increases,thus limiting the maximum operating temperature (MAOT) and the lifetime of the column.

2.2.1.2 Capillary Columns

This type of column, which is made from either glass or fused silica, has no packing material. The liquid phase is bonded either directly to the column walls (WCOT) or to a support material coating the inner wall surface (SCOT). There is, consequently, no peak broadening caused by the paths of the gas through the packing and so efficiency is improved. However , for there to be effective interaction between the two phases; the internal diameter needs to be small (0.1 - 0.3mm) and gas flow rates are therefore low (0.1 to 2ml/min).

Unfortunately, capillary columns are limited in their sample and solvent carrying capacity. It is often necessary to split the sample at the injector to prevent column damage by a relatively large injection of hot solvent. To overcome the problem of sample loss due to split injections, a number of injection techniques have been developed. The Grob Injector (Figure 2.3) can operate in both split and splitless mode. This is a technique carried out with a split injector in which the sample is introduced with the split vent closed, and with the column adjusted to a low temperature in the region of the boiling point of the sample solvent. In this way the evaporated sample from the injector liner tends to stay in the top of the column. After an initial waiting time, usually in the region of 20 to 30 seconds, the split vent is opened to flush out the liner and the column oven is temperature programmed to the sample requirements. This technique gives a narrow injection band with the advantage of low sample loss and no solvent tail. However, to prevent the loss of sample almost entirely, on-column injectors have been

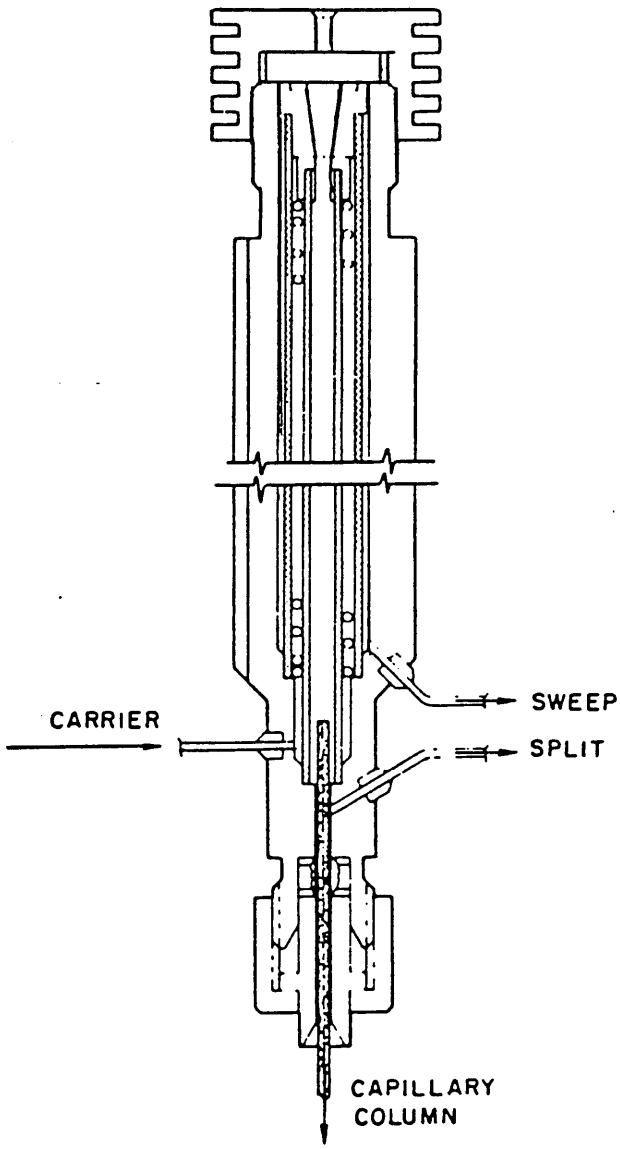


Figure 2.3 Grob type injector for capillary column systems. (Copyright © 1982, Finnigan Corporation. All rights reserved.)

developed. In this case, the liquid sample is introduced through a precision guide and placed directly into the precooled column.

2.2.2 Derivatives

As the sample moves through the column, absorption problems can be met with compounds of a more polar nature containing functional groups such as hydroxyls, carboxylic acids and amines leading to poor peak shape. Problems may also be experienced with compounds of low volatility. In many cases it proves possible to improve or confer suitable GLC properties on a molecule by derivatisation.

On derivatisation, the character of the molecule is changed from polar and active to non-polar and inert. This is generally coupled with an increase in volatility and thermal stability which are the properties most desired for gas chromatography. Interactions between the column support and the derivatised form of the molecule are reduced to a minimum and peak shape is sharper. Also the derivative must suit the requirements of the detector system. The most important requirement of a molecule for mass spectrometry is that it should give abundant and distinctive ions in its mass spectrum. Reagents commonly used include diazomethane for methylating carboxylic acids, the perfluorinated anhydrides for acylating amines and the various silylating reagents which have universal applications.

2.3 MASS-SPECTROMETRY

2.3.1 MS Interfaces

Between the GLC outlet and the ion source of the mass spectrometer a pump or 'molecular separator' is inserted. It is necessary to remove the carrier gas, helium, from the GLC eluate since the operating pressure at the column outlet is incompatible with the operating pressure of the mass spectrometer. As the sample moves through the separator it is preferentially enriched in the organic components.

Wherever possible, direct connection to the ion source is preferred for capillary columns. This is acceptable because of the low gas flow rates. With the ultraflexible fused silica columns it is not uncommon for the column to be taken right into the source without any connections and associated dead volumes. This is not only a simple and practical arrangement but also one that conveys the maximum amounts of sample to the mass spectrometer ion source.

2.3.2 Ionisation

There are several methods for producing ions in the source of a mass spectrometer. The most common mode of ionisation is by electron bombardment of the gaseous sample. The resultant positively charged molecular ion (M+) fragments into ions of lower mass, as determined by the structural features of the parent molecule.

Electrons are produced in the ion source by thermal emission from a metal filament. The effectiveness in ionising the sample molecule increases with the electron energy (5-100eV). Most reference spectra are reported at 70eV because at this level, changes in electron energy have negligible effect on ion production and fragmentation patterns

Many polar molecules do not yield molecular ions under the conditions of electron impact ionisation. If molecules could be ionised without a significant addition of energy, fragmentation would be reduced and molecular ions would be much more abundant. In recent years a number of soft ionisation techniques such as chemical ionisation, field desorption and fast atom bombardment have been developed which yield more abundant molecular ions without loss of sensitivity.

2.3.3 Mass Analysers

2.3.3.1 Single Focussing Magnetic Instrument

The ion beam is accelerated through an electric field prior to separation. The most common mode of separation is by magnetic deflection. The flight of a moving particle in a magnetic field is given by the following equation

$$m/e = H R / 2E \qquad (1)$$

H=magnetic field, E=accelerating field, R=radius

Keeping the accelerating potential constant and altering the magnetic field, ions of different m/e values are brought sequentially to focus on the detector plate, giving the full mass spectrum.

2.3.3.2 Double Focussing Mass Spectrometers

Ions of the same m/e value can have different kinetic energies. If the ion beam is passed through an electrostatic field prior to the magnetic sector, the field will act as a direction and velocity focussing device. Thus, the ions will have a narrower band of kinetic energy and therefore improved resolution.

2.3.3.3 Quadrupole Mass Spectrometers

The separation of ions by their m/e can also be achieved by electric fields alone. The quadrupole separator consists of four parallel cylindrical rods which are diagonally electrically connected. Between each pair of opposite and electrically connected rods is applied a d.c. voltage and a superimposed radio-frequency (rf) potential. With the rf/dc voltage constant, the voltages are varied to effect separation of ions according to mass.

2.3.4 Collecting and Recording of Ions

As the separated ion beam impinges on the collector plate of the detector, the plate emits an electrical signal which is amplified prior to display on photosensitive paper or, more commonly nowadays, is computer processed.

Since Hites and Biemann (1970) demonstrated the utility of a computer in processing and storing mass spectral data when the GLC

effluent is analysed by automatic scanning, new developments have given scope for data display, manipulation and output in real time, i.e., during or shortly after data acquisition. Thus GC/MS with an integral data system has evolved into a powerful analytical tool.

Computer-generated digital signals are converted into analog levels by a digital-to-analog convertor (DAC) and are fed to the mass spectrometer electronics through a buffer circuit. The output analog signal can then be used to control the magnetic current, magnetic field and accelerating voltage. The main software elements of all GC/MS data systems will include functions such as; mass spectrometer tuning and calibration, control of scanning, data acquisition, display presentation, library searching, survey searching and quantitation routines.

The work space store or memory must be accessible on demand and capable of transferring data at high rates. To cope with this problem, the computer has an integral memory unit, which is often referred to as the core. The computer acquires data in a foreground or priority mode and allows processing and display of the data in a background, second priority mode. In this way data can be viewed and manipulated in real time immediately after acquisition. Disk-based storage provides necessary extension to the computer memory. After initial processing, data are written into a data file on a magnetic disk.

Having acquired the data, a VDU and keyboard are necessary for examination of the results and a printer or plotter is essential for permanent records.

2.3.5 Tuning and Calibration

The purpose of tuning is to achieve the best mass spectrometer sensitivity across the mass range consistent with peak shapes that enable mass measurement and possibly quantitation to the required degree of precision.

Symmetrical peak shape is important if accurate mass assignments are made. Under normal operating conditions there are nearly always some background or bleed ions that serve for basic tuning purposes.

The assignment of mass values to acquired mass spectrometer data requires that the instrument be calibrated. The degree of accuracy required will determine both the frequency with which calibrations must be made and the way in which they are achieved. In all cases a calibration compound must be introduced into the mass spectrometer, which should give spectra with ions evenly spaced across the mass range of interest. This is usually a fluorinated hydrocarbon, perfluoronated kerosene (PFK) being the most common choice. The fragmentation pattern for PFK is given in figure 2.4. The compound should be easy to introduce into the ion source and cause no major contamination problems.

2.3.6 Qualitative Mass Spectrometry

Once the compound under study has been ionised and its spectra recorded, the data obtained can be used for structure elucidation. The most abundant peak in a spectrum is called the base peak and is usually set to 100% when the raw spectrum is normalised. The molecular ion (often

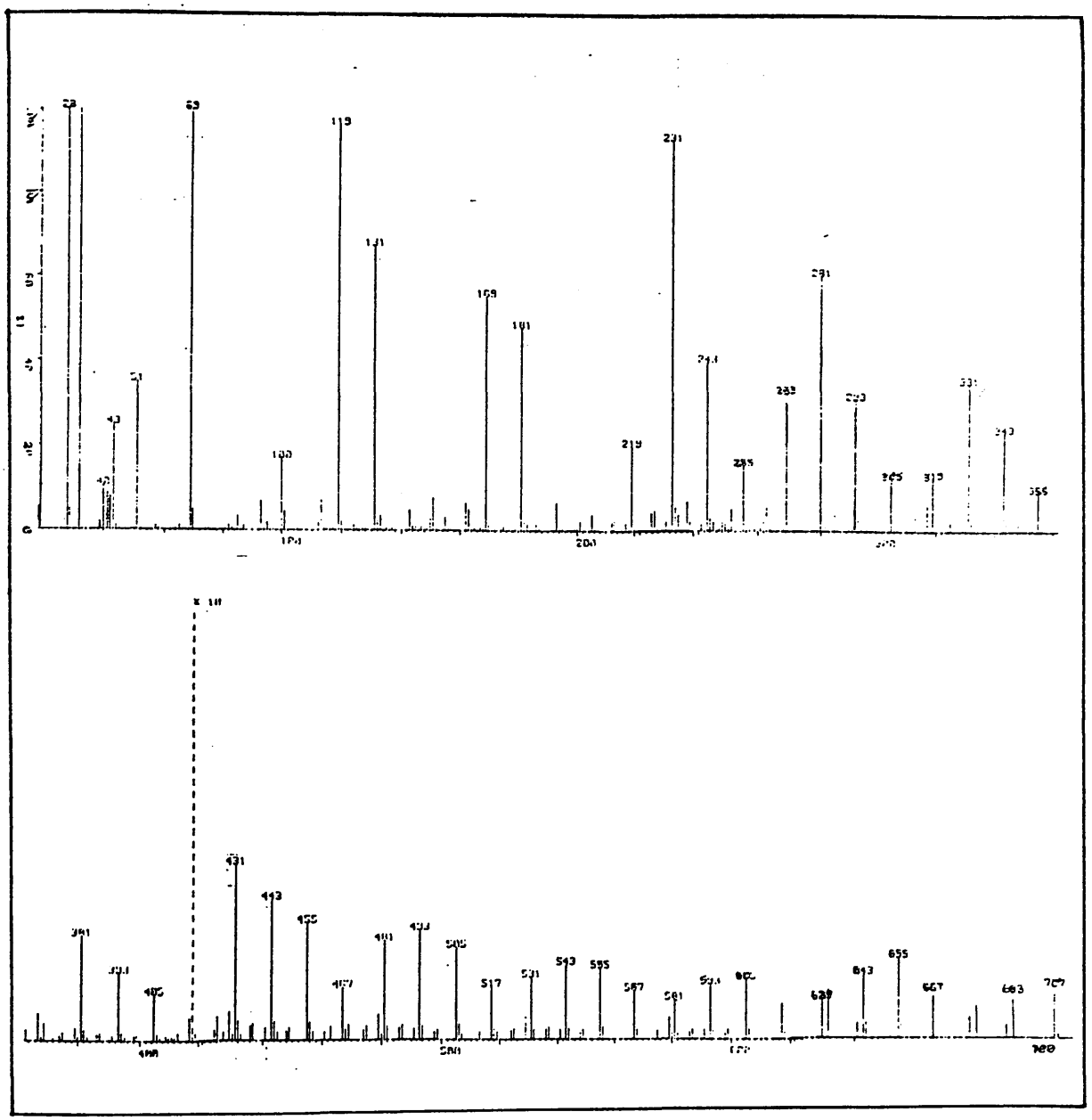


Figure 2.4 Mass fragmentation pattern of perfluoronated kerosene (PFK).

the base peak) is called the parent ion. Integration of all ion intensities in a spectrum gives the total ion current, which is sometimes used as a normalizing value when plotting the relative intensities.

The physico-chemical properties of the compound and the conditions under which it is ionized determine the shape of the spectrum. Thus, the spectrum gives structural information and, depending on the ionization conditions, the normal molecular weight. The mass spectrum of a compound constitutes conclusive evidence of its identity and accordingly serves as such for positive identification; e.g., by comparison with library spectra.

2.3.7 Quantitative Mass Spectrometry

Two methods are used to quantify compounds introduced into the GCMS. One is to monitor the intensity of the compound being determined, by switching the mass spectrometer analyser rapidly between two reference ions which are different only by a few molecular weights and in whose range the chosen ion of the compound lies. This method, known as 'selected ion monitoring', is one of the fastest growing areas of MS. The second method is to scan the spectrum repetitively, with the data from these scans being acquired by a computer. The varying intensity of selected ions can be retrieved from the scans, and the output presented in a similar fashion to the real time output of the first method.

2.3.7.1 Selected Ion Monitoring

Selected ion monitoring (SIM) is the simultaneous detection of one or more fragment ions, as opposed to the scanning of the whole spectrum in conventional mass spectrometry. The detection of subnanogram quantities of naturally occurring substances and drugs in biological materials is the major application of this technique.

Initally developed by Sweeley et al (1966) the importance of SIM in GCMS analyses, as being a highly specific gas chromatographic detector was described by Brooks and Middleditch (1971). The mass spectrometer is adjusted to detect only those selected ions characteristic of the compound of interest.

As can be seen from equation (1), the mass of the ion focussed on the collector is inversely proportional to the applied accelerating voltage. Therefore by the rapid and automatic adjustment of the accelerating voltage (at constant magnetic field) the ion current at 2 or 3 selected ions is focussed alternatively on to the detector for short periods of time (50-250 msecs).

The selected ion current is recorded and has the appearance of a conventional gas chromatograph except that the tracings are in duplicate or triplicate depending on whether 2 or 3 ions are being monitored. Peaks in the characteristic ion current profile at the expected retention is good evidence for the presence of the compound of interest.

The three most important aspects of the method to consider are: sensitivity, specificity and the choice of internal standard.

Since the sensitivity decreases as the number of ions monitored increases, because less time is spent monitoring each ion, maximum sensitivity in detecting a minimum of 50 pg (Brooks and Middleditch, 1971) is achieved by monitoring a single ion. However the more ions monitored, the more confidence can be placed in the results.

2.3.8 Internal Standard

With an internal standard, GCMS assay reliability benefits through correction for losses which may occur during extraction, purification and derivatization. Furthermore, variations in sample injection into the GCMS system, together with unnoticed fluctuations in instrumental sensitivity are automatically compensated for. The ideal internal standard should be chemically and physically very similar to the compound of interest and yet be readily distinguishable by GCMS. These needs are met either by the compound itself labelled with a stable isotope or by a structural analogue.

Generally, isotopically labelled analogues are the most effective internal standards for GCMS because they are practically identical in chemical properties to the respective unlabelled compounds whilst being readily distinguishable by mass spectrometry because of their difference in mass. Since partition coefficients can be taken as identical, the ratio of compound to internal standard will remain constant even though the extraction conditions may change inadvertently from one run to the next. Likewise, the rate of derivatisation will be similar except for those cases where the cleavage of a bond to a labelled atom is involved, or

where a secondary isotope effect is rather large. Finally, the retention times on the gas chromatograph column are similar. Internal standards labelled with ^{13}C, ^{15}N, ^{18}O, and ^{37}Cl are even more similar chemically to the corresponding unlabelled compounds than those labelled with ^{2}H (D), but they are not as popular because they are expensive and generally less easy to synthesise. The isotopic label(s), especially if deuterium, must be incorporated at a 'stable' position in the molecule, obviating loss of heavy isotope during the analytical procedure. The internal standard should be labelled to a high degree of isotopic purity, with less than 1% of residual unlabelled molecules if acceptable 'blanks' are to be achieved in the assay. One other disadvantage apart from the cost, is poorer precision. Besides the disadvantage of using two channels rather than one, there is also the point that drift on each channel may occur at a different rate and direction. Together with the normal magnetic drift of the instrument , this would mean a change in the ratio of the two responses over a course of several hours for the same solutions injected. However the mass spectrometer precision is of less importance than the poor precision in other stages of the analysis.

2.3.9 Errors

With a data acquisition system, the accuracy of mass measurement is vastly more dependent upon the setting up of the mass spectrometer than on the data system itself.

Factors which affect the reproducibility of a spectrum include the temperature of the source and inlet system, the state of cleanliness of

the source, the condition of the filament, the electron beam, the trap current and the repeller voltage.

Finally, when using selected ion monitoring, magnetic drift can be a problem and this must be compensated for by frequent checking of the magnet tuning throughout the course of an analysis.

CHAPTER 3

DEVELOPMENT OF AN ANALYTICAL ASSAY FOR METHIMAZOLE

SECTION 1 : GC-MS METHOD

3.1 SYNTHESIS OF INTERNAL STANDARD

3.1.1 Introduction

With an internal standard, GC-MS assay reliability benefits through correction for losses which may occur during extraction, purification and derivatization. Furthermore, variations in sample injection into the GC-MS system, together with unnoticed fluctuations in instrumental sensitivity are automatically compensated for. As discussed in Chapter 2 (section 2.3.8) these needs are met by the compound itself labelled with a stable isotope. In the case of methimazole, this philosophy was proven to work satisfactorily (Floberg et al.,1980). These workers who synthesised deuterium labelled methimazole, and after identical extractive alkylation as methimazole, was introduced to the GC-MS as the internal standard. Deuterium-labelled methimazole is a good candidate for use as an internal standard because it is relatively cheap and easy to synthesise with the label in a stable position. This was the obvious choice for further work.

The synthesis was carried out in two stages. Firstly, the synthesis of acetalylthiocarbimide (Easson and Pyman, 1932) which was further reacted with trideuteromethylamine.HCl using a modification of a previous method (Floberg et al., 1980) to give 1-trideuteromethylimidazole-2-thiol

3.1.2 Materials

Ethanol, Ethyl Acetate and Chloroform were obtained from Rathburn Chemicals Ltd. Walkerburn. Carbon Disulphide and Lead Acetate were products of British Drug House (BDH), Dorset. Aminoacetal, Sodium Hydroxide and Trideuteromethylamine.HCl were obtained from Sigma Chemicals Ltd, Poole, Dorset. All other chemicals were obtained as follows: Anhydrous Potassium Chloride from Hopkins and Williams Ltd., Essex; and Sulphuric Acid from May and Baker Ltd., Dagenham, Essex.

Chromatography (PTLC) was performed on 20x 20cm glass plates coated with silica gel (Kiesel Gel 60(2mm)). The suppliers were E.Merck Ltd., Darmstadt, G.F.R..

3.1.3. Experimental

3.1.3.1.The Preparation of Acetalylthiocarbimide

A mixture of aqueous sodium hydroxide (45ml of 5N), carbon disulphide (20g) and a solution of aminoacetal (27g) in water (100ml) was warmed and gently shaken until the carbon disulphide had dissolved. The solution was treated at 0°C with an ice-cold solution of basic lead acetate (30g) in water (100ml),a reddish-yellow precipitate forming. An ice-cold concentrated solution of normal lead acetate (60g) was then added gradually and with shaking. The mixture was kept cold for 30 minutes and then gradually warmed on a steam bath with continual shaking. The coloured precipitate blackened. Acetalylthiocarbimide was removed by steam distillation,isolated and

dried (anhydrous potassium carbonate) in ether and distilled under diminished pressure.

3.1.3.2. Preparation of 1-trideuteromethylimidazole-2-thiol

Acetalylthiocarbimide (5.45g) was mixed with trideutero-methylanmine.HCl (2.86g) and 5ml ethanol in a screw-capped tube. The mixture was cooled in ice water and sodium hydroxide (1.64g) was added. The tube was then slowly shaken until it reached room temperature, where it was allowed to stand for two hours. The sodium chloride which formed was filtered off and the solvent was evaporated. The residue was hydrolysed by refluxing with 20ml of 30% sulphuric acid for 30 minutes. After cooling it was neutralised with 4M sodium hydroxide solution and extracted with ethyl acetate (4 x 50ml). Evaporation of the solvent gave a crude residue which was purified by preparative thin layer chromatography (solvent system 96:4 chloroform:ethanol)

3.1.4 Results

3.1.4.1 Yield and Combustion Analysis

The yields for acetalylthiocarbimide and deuterium labelled methimazole were 6.96g (19.4%) and 310mg (8.8%), respectively. The combustion analysis results for both compounds are given in Table 3.1

Table 3.1 Combustion analysis data

Acetalylthiocarbimide ($C_7H_{13}NSO_2$)				
	Carbon	Hydrogen	Nitrogen	Sulphur
Found	46.7	7.25	7.6	17 0
Requires	48.0	7.5	8.0	18.3

1-trideuteromethylimidazole-2-thiol ($C_4H_3D_3N_2S$)				
	Carbon	Hydrogen	Nitrogen	Deuterium
Found	40.25	2.5	23.2	5.0
Requires	41.0	2.6	23.9	5.1

3.1.4.2 Infra-Red Spectroscopy

The infra red spectrum for acetalylthiocarbimide was obtained using the pure liquid and is shown in Figure 3.1.

Deuterated and pure methimazole infra-red spectra were obtained using potassium bromide discs. The infra-red examination showed similiar spectra for the two compounds (Figures 3.2 and 3.3). The principal functional group peaks for both compounds are $-CH_3$ (1466cm) -NH (1570cm) and -CSNH- (1271cm).

3.1.4.3 Mass Spectrometry

The direct inlet mass spectrum of synthesised acetalylthiocarbimide with its m/e ion intensities is shown in Figure 3.4. The molecular ion (m/e 175) fragments at the carbon to carbon single bond to give the base ion m/e 103 [$\dot{C}(OC_2H_5)_2$]. Other important fragments are m/e 74 (HCSNH$\dot{C}H_2$) and m/e 130 (HCSNHC$\dot{H}$COC$_2$H$_5$)

The mass spectra of the two compounds (deuterated and pure methimazole) showed the same pattern of ion peaks. The expected increase of three mass units for the molecular ion due to the deuterium atoms was clearly visible; i.e. m/e 117 instead of 114 (Figures 3.5 and 3.6).

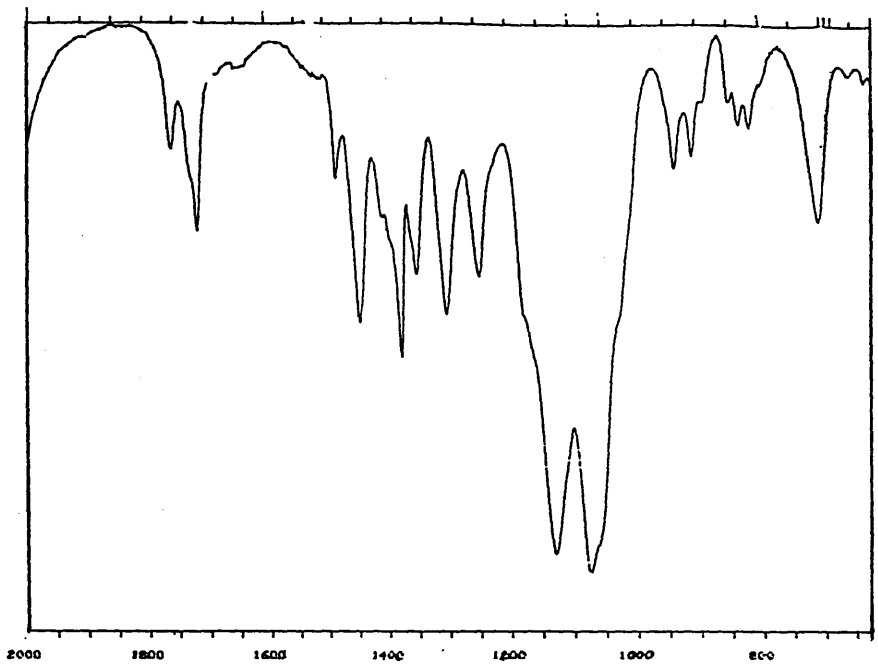


Figure 3.1 Infra-red spectrum for acetalylthiocarbimide.

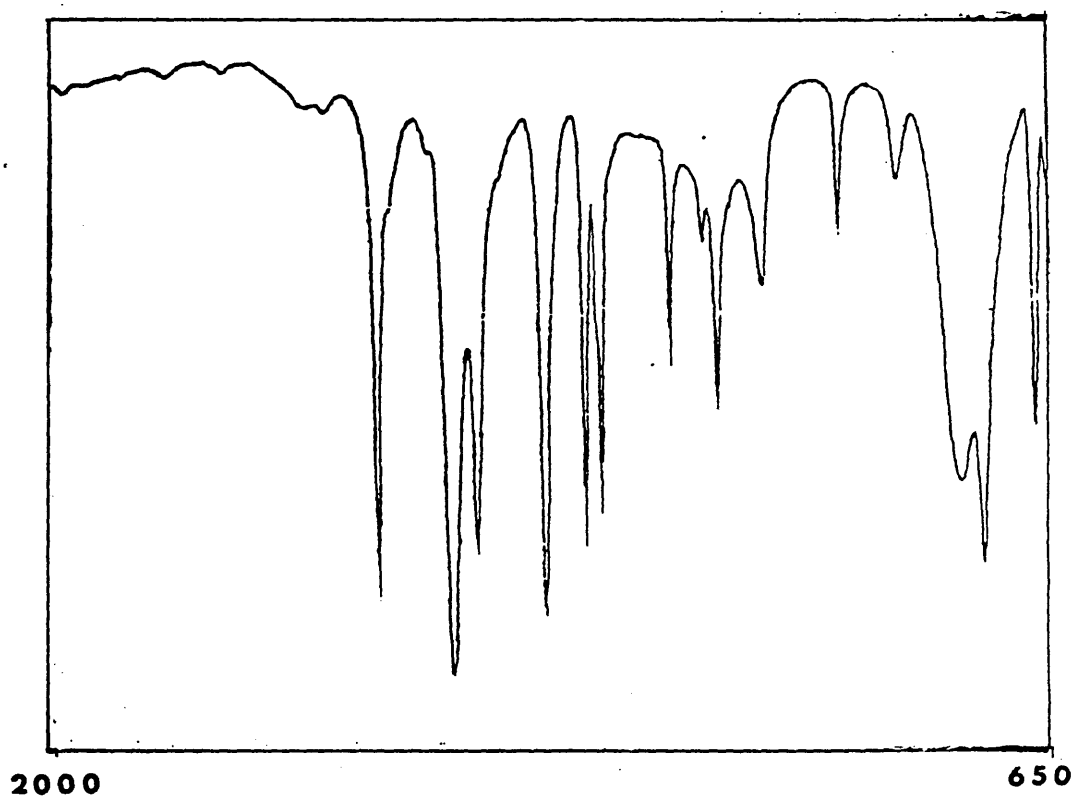


Figure 3.2 Infra-red spectrum for deuterium labelled methimazole.

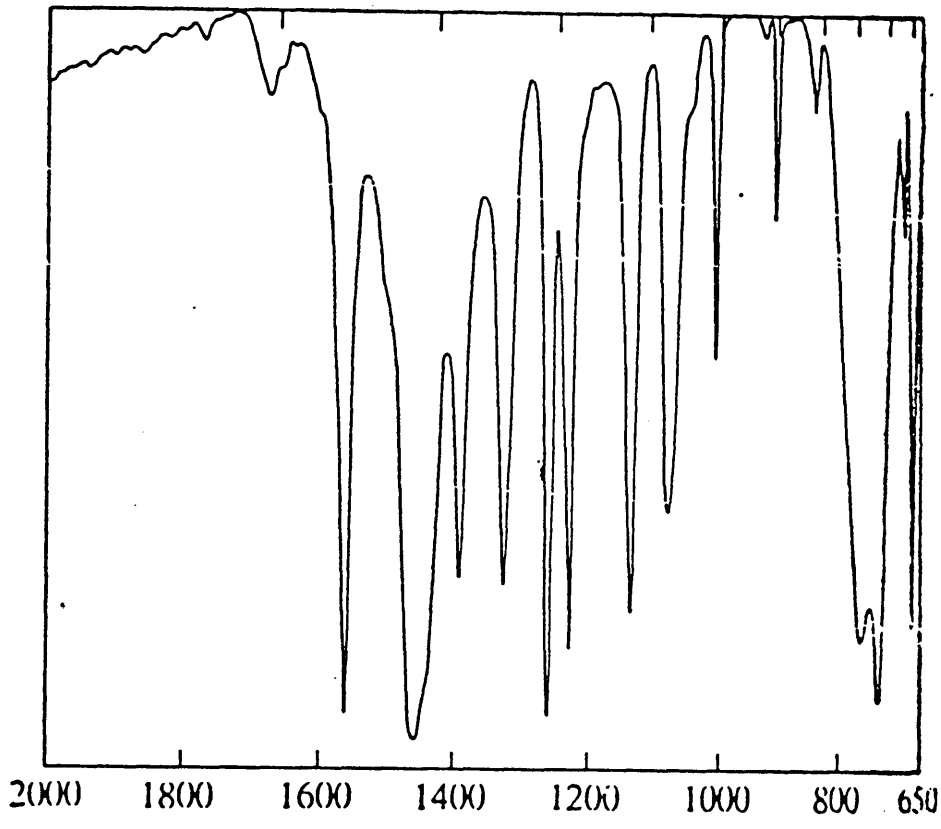


Figure 3.3 Infra-red spectrum for pure methimazole.

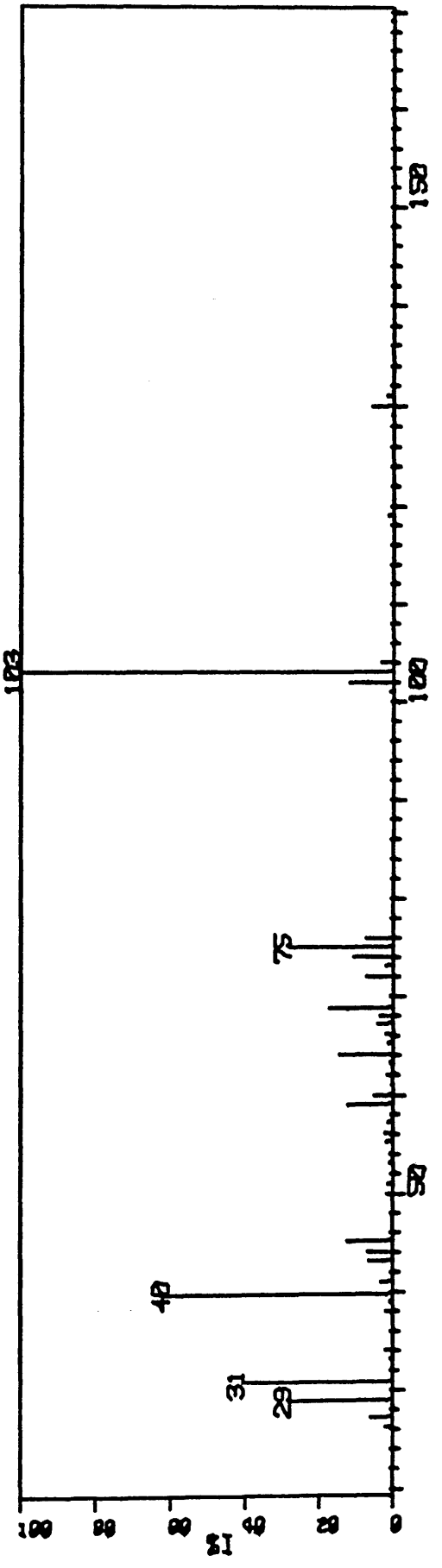


Figure 3.4 Mass fragmentation pattern of acetalylthiocarbimide

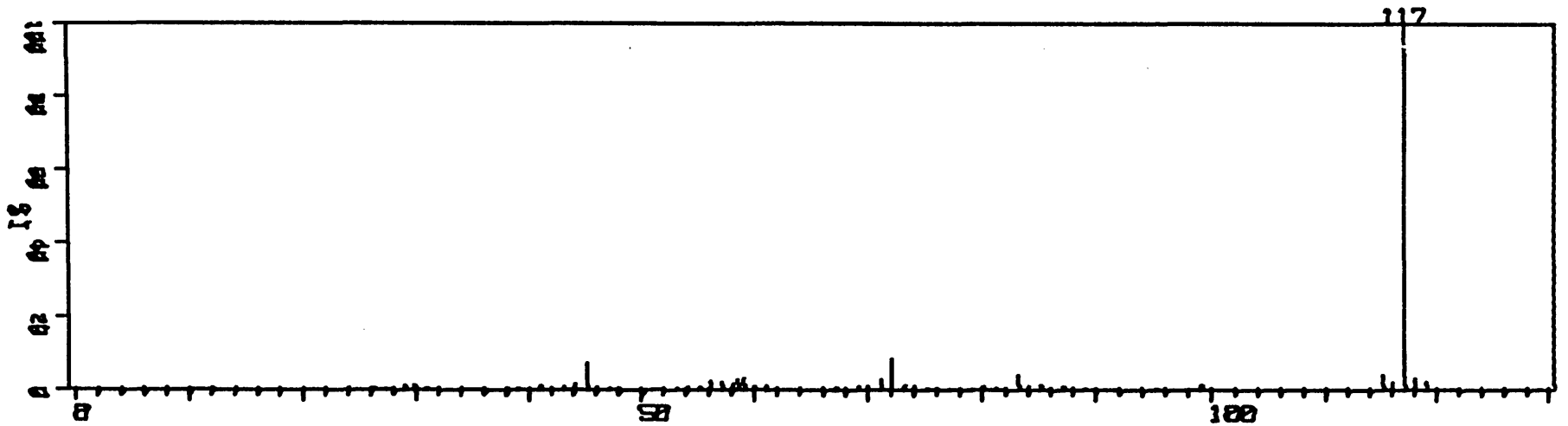


Figure 3.5 Mass fragmentation pattern of deuterium labelled methimazole

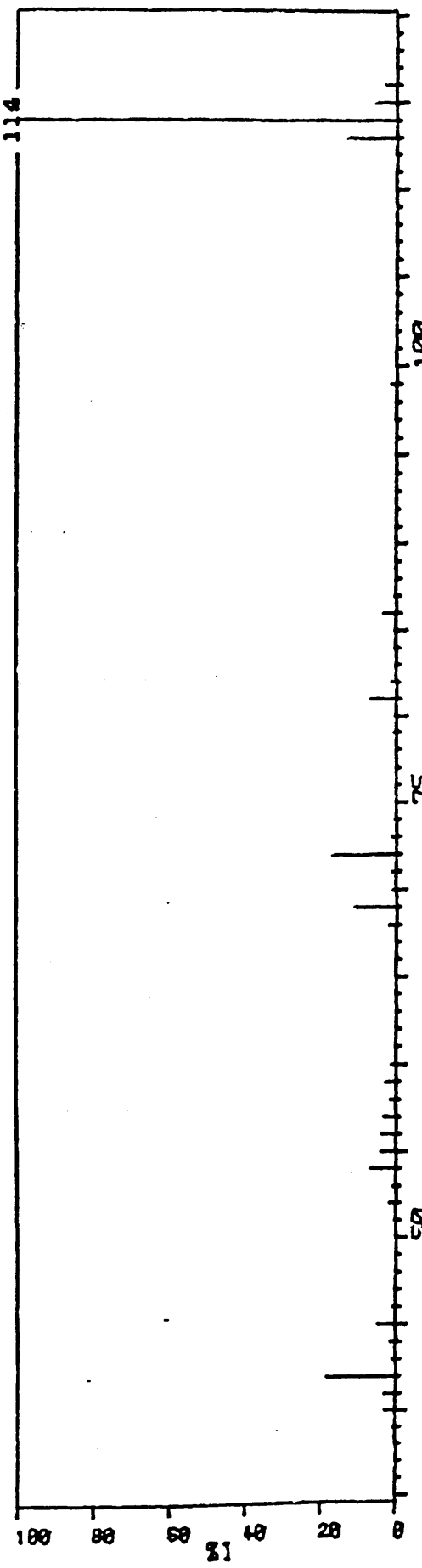


Figure 3.6 Mass fragmentation pattern of pure methimazole.

3.1.5. Discussion

Synthesis of trideuterated methimazole via acetalylthiocarbimide, was successfully achieved with the appropriate spectroscopic analysis used for verification.

The final yields, quoted above, were much lower than those achieved by the respective authors (60% for acetalylthiocarbimide and 21% for trideuteromethimazole). However, although acetalylthiocarbimide contained some impurities, the final crystals of trideuteromethimazole were of a purity acceptable for a GC-MS (SIM) internal standard. Adequate purification was achieved by the use of PTLC as opposed to column chromatography which was advocated in the orignal article.

3.2 DERIVATISATION

3.2.1. Introduction

The underivatised thiol, methimazole does not chromatograph well because of non-specific absorption effects. However, there are a wide variety of different derivatisation procedures suitable for reaction with the thiol functional group, the most commonly used being acylation, alkylation and silylation.

Acylation is popular for amino acids, giving very stable derivatives. Thus, it becomes a less suitable derivatisation method for methimazole in which the thiol group is in resonance with an amino group. This could give rise to a mixture of products and hence a complex mass spectrum.

Alkylation has previously been used to derivatise methimazole for GC (Stenlake et al.,1970; Bending and Stevenson,1978) and GC-MS (Floberg et al.,1980).

Methyl iodide was used to form the S-methyl methimazole derivative (Stenlake et al., 1970). However, reaction time was critical and the instability of the derivative could lead to the retention of free methimazole on the column. Methylation with diazomethane was also attempted but was found to cause cleavage of the imidazole ring.

The best alkylation for GC to date has been flash methylation in the injection port with tetramethylammonium hydroxide (Bending and Stevenson, 1978). However, methylation only increases the molecular weight of methimazole by 14 mass units. For SIM, the derivative needs to have a significant increase in weight from 114 to give good resolution.

For GC-MS, alkylation has been performed using two derivatives, benzyl chloride and pentafluorobenzyl bromide (Floberg et al., 1980). These gave good chromatography however benzyl chloride produced a derivative with a prominent mass of 207. This is a prominent peak in the mass spectrum of the common silicone derivative gas chromatographic phases. These vapours 'column bleed', are a considerable source of interference in GC-MS analysis. Their fragmentation pattern will be present in each spectrum and may occlude the presence of methimazole in blank samples. Pentafluorobenzyl bromide is a strong lachrymator and should be handled with extreme caution and as such is an undesirable option.

As yet no investigators have attempted the formation of a silyl derivative of methimazole for GC or GC-MS analysis. However, this

technique is applicable to all compounds containing active hydrogen functions.

Silylation ,therefore, was investigated as a potential method for the derivatisation of methimazole and the resultant fragmentation pattern from the total ion scan studied to choose the characteristic ions most suitable for SIM. It was decided to convert methimazole to the t-butyldimethylsilyl derivative using the silylation reagent N-methyl N-tertbutyldimethylsilyltrifluoroacetamide (MTBSTFA).

3.2.2 Materials and Methods

MTBSTFA was supplied by Pierce Chemicals Ltd., Luton, Bedfordshire. Samples of methimazole and 1-trideuteromethylimidazole-2-thiol (1ug) were derivatised using MTBSTFA reagent (10ul). Then the derivatised compounds were introduced into the mass spectrometer (set on 'scan mode') through the gas chromatograph (1ul injection of each compound was used). The mass spectrum of each of the above compounds was thus obtained.

3.2.3 Results

The mass spectra of the TBDMS-derivatives of methimazole and trideuterimethimazole are shown in Figures 3.7 and 3.8, respectively. Table 3.2 shows the major ion peaks and their relative abundancies.

3.2.4 Discussion

The silylation reaction is considered to involve the formation of a transition state (Figure 3.9).

The properties most desired of X, the leaving group are low basicity, the ability to stabilize a negative charge in the transition state and little or no (p-->d) back-bonding between X and silicon. The basicity of the leaving group should be less than that of Y as the formation of the transition state is a reversible process which is favoured by X, the weak base.

The reaction mechanism for the derivatisation of methimazole by MTBSTFA is given in Figure 3.10.

In this case the leaving group, trifluoroacetamide, can stabilize a partial negative charge through resonance, and the presence of its carbonyl group ensures that its basicity is less than the deprotonated methimazole. This derivative is more stable to hydrolysis than corresponding TMS derivatives and consequently samples do not need to be meticulously dried before silylation.

In general,the reaction time is dependent on the silylating conditions and on the structural features of the drug. This rate of reaction can be increased by :

the addition of an acid or base catalyst

the correct choice of solvent (polar and able to dissolve both reagent and sample without reacting with either).

an increase in temperature

However, in the case of MTBSTFA with methimazole, the reaction between such a powerful silyl donor and unhindered drug site meant the

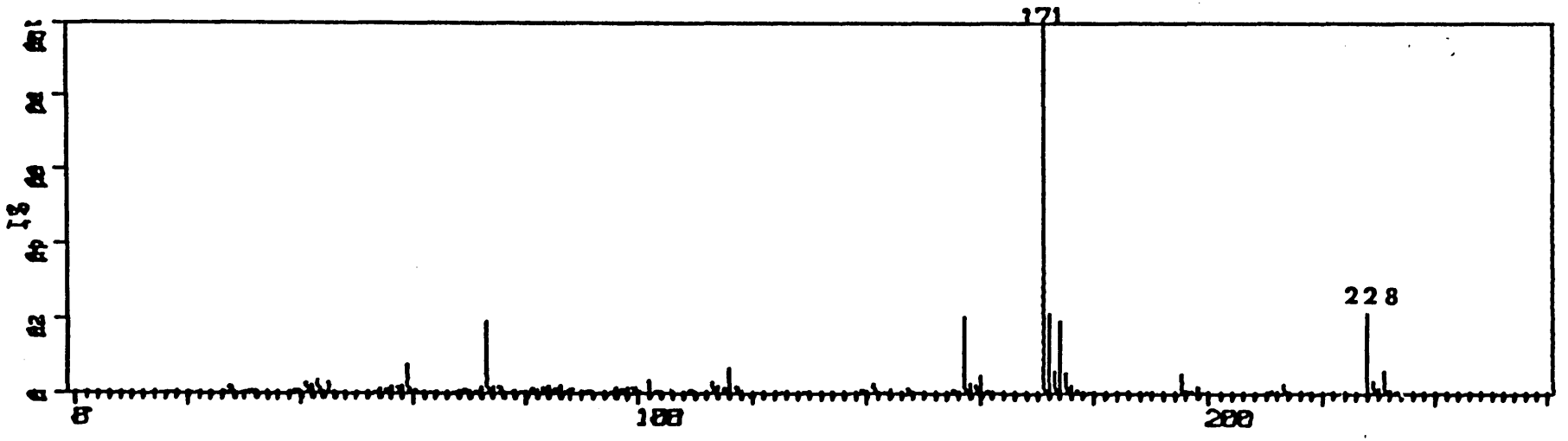


Figure 3.7 Mass fragmentation pattern of TBDMS-methimazole.

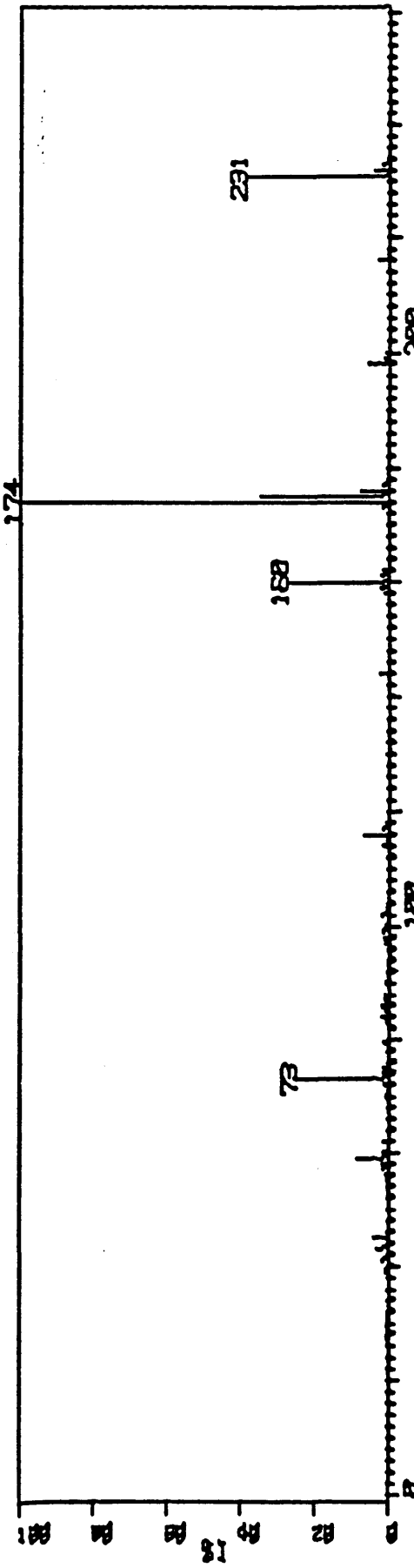


Figure 3.8 Mass fragmentation pattern of TBDMS-deuterium labelled methimazole

Table 3.2 The major ions and their relative intensities of the TBDMS derivatives of methimazole and trideuteromethimazole.

TBDMS-Methimazole		TBDMS-Trideuteromethimazole	
Mass	Intensity%	Mass	Intensity%
59.0	7.6	59.0	8.5
73.2	18.9	73.1	25.1
157.1	20.5	160.0	27.8
171.1	100.0	174.1	100.0
172.1	21.5	175.1	34.8
173.1	6.1	176.1	7.6
228.0M	21.3	231.0M	38.9

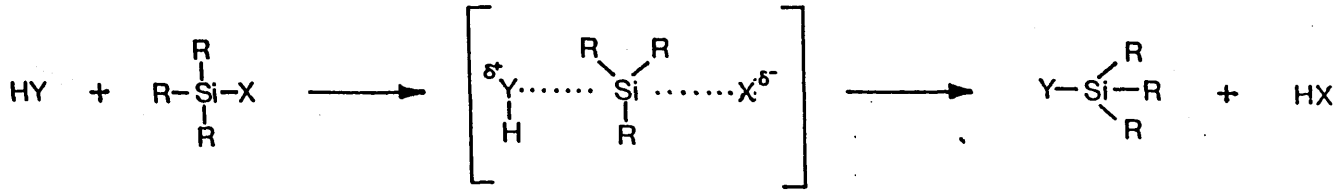


Figure 3.9 Mechanistic diagram of a silylation reaction.

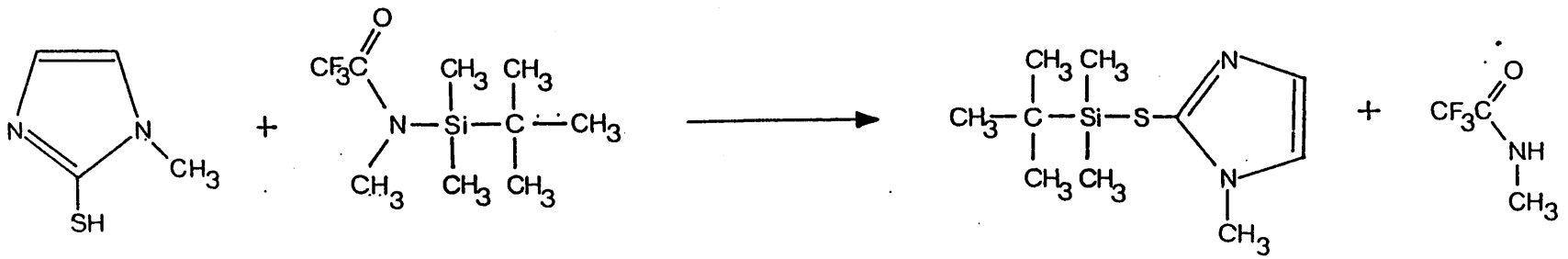


Figure 3.10 Derivatisation reaction of MTBSTFA with methimazole.

reaction was virtually instantaneous at room temperature without the help of a catalyst or the need for a solvent

In addition, compounds possessing a TBDMS function produce simpler, more easily interpretable mass spectra. Characteristically, the loss of a tert-butyl function produces a prominent mass spectrum fragment of 57 (molecular weight) and M-57. The major ion fragments of the TBDMS derivatives of methimazole and trideuteromethimazole appeared at m/e 171 and m/e 174 respectively. This corresponds to cleavage of the tert-butyl group. The molecular ions of their TBDMS derivatives appeared at m/e 228 and m/e 231 for the drug and the labelled drug respectively. Thus m/e 171 and 174 were used to monitor the compounds in the application of mass fragmentography.

3.3 GAS CHROMATOGRAPHY AND MASS SPECTROMETRY CONDITIONS

3.3.1 Introduction

In this study, the instrument used was a 3B Perkin-Elmer Sigma gas chromatograph and VG 16F magnetic sector mass spectrometer. This was connected to a VG2050 datasystem.

Capillary GC was chosen for sample analysis. The gas chromatograph was directly interfaced to the mass spectrometer source. The gaseous sample was ionized by electron impact and the spectra recorded at 70eV.

The Foreground/Background selective ion recording datasystem (F/BSIR) allows the user to quantitatively monitor and store the intensities of ions of selective mass throughout a gas chromatography

run. The datasystem can monitor the intensities of up to ten ions simultaneously and the stored data can be processed to provide accurate peak height, area concentration and a retention time measurement.

Using this, instrumental optimum parameters were adopted for studying TBDMS-Methimazole by selective ion monitoring.

3.3.2. Methods

(i) GC Conditions for TBDMS-Methimazole

Column type	: WCOT Fused Silica CP Sil5 CB MAOT=350°C
Length	: 25 m
Diameter	: 0.22 mm
Film thickness	: 0.12 mm

The temperature program used was:

1st stage : 140°C - 180°C at 5°/min

2nd stage : 180°C - 250°C at 15°/min

Injector temperature : 225°C

Detector temperature : 225°C (ion source)

(ii) MS Conditions for SIM of m/e 171 and 174

Source temperature : 225°C

Amperes	: 0.1 uA
Gain	: 3
Response	: 0.003 s
Electron energy	: 70 eV
Multiplier supply	: 2.5 kV

Perfluorokerosene (PFK) fragmentation ions; C_3F_7 (m/e 168.9888) and C_4F_7 (m/e 180.9888) were used as reference ions.

Channel 1 was set to monitor m/e 171.041

Channel 2 was set to monitor m/e 174.064

3.3.3 Results and Discussion

Capillary columns may be made either of glass or fused silica. It was decided that a fused silica column would be the best choice as they have the advantage of flexibility and strength. Thus silica columns may be threaded through complex pipework to emerge at the mass spectrometer ion source.

The stationary liquid phase may be chemically bonded to the capillary walls. This liquid should have low volatility, high thermal stability and must not react with the components of the sample.

It is a general rule to select phases which are chemically similar to the compound of interest. This means that the solubility of the components will be high hence giving favourable **k** values. The formation of the TBDMS derivative of methimazole has resulted in decreased polarity therefore an apolar phase was selected for analysis.

A medium column length was chosen with a narrow bore diameter measurement to give better resolution and to complement the injector system. A thin film was used so that the column bleed would be minimised. The column type has a maximum operating temperature (MAOT) of 350°C. This temperature is quoted assuming isothermal operation with a flame ionisation detector. A mass spectrometer will impose greater limitations because of its susceptibility to column bleeding. Therefore the column temperature was kept well below this limit.

The injector, the column and the detector are heated separately, the injector and detector usually to a temperature of 50°C above the column temperature. The choice of column temperature will be a compromise. It should be high enough to give rapid analysis and yet low enough to allow the desired separation. As high sensitivity is required, a fairly short retention time is favourable. Thus the column was temperature programmed to rise from 140°C to 180°C at 5°/min to separate the components and then from 180°C to 250°C at 15°/min to remove any sample contamination.

Methimazole and 1-trideuteromethylimidazole-2-thiol tertiary butyl dimethyl silyl derivatives were previously scanned in order to find the major ion of each compound. Once determined, the necessary adjustments to the accelerating voltage can be made which will allow the focussing of these particular ions on the collector plate; i.e. the conditions can be set for selective ion monitoring.

Two perfluorokerosene (PFK) fragmentation ions are used as the reference ions to define the mass window of ions allowed to reach the collector plate. Obviously the two ions selected for the methimazole analysis are within this mass range.

The mass spectrometer is equipped with a 10 channel multiple ion detection unit which selects ions by programming the accelerating voltage. Channel 1 was set to monitor the major ion of m/e 171.041 for TBDMS-methimazole and channel 2 was set on m/e 174.064 for the internal standard TBDMS-trideuteromethimazole.

3.4 SUMMARY

A good analytical method for the quantitation of methimazole has been developed using GC-MS. An internal standard, trideuteromethylimidazole, has been successfully synthesised and chromatographs in an identical manner to methimazole whilst being clearly distinguishable by mass spectrometry. Finally, a derivative has been chosen which not only chromatographs well on the chosen column, but also gives a lucid fragmentation pattern containing distinct major ions for methimazole and the internal standard which are appropriate for SIM GC-MS.

SECTION 2 : EXTRACTION FROM BIOLOGICAL SAMPLES

3.5 EXTRACTION

3.5.1 Introduction

In general, biological fluids are not used for direct injection onto the analytical system, but rather an extract is used. This is because the drug is usually present in very low concentrations and therefore a concentration step is needed. Also the sample will contain numerous endogenous compounds that are often present in much higher concentration than the drug and these may co-elute preventing accurate quantitation or simply contaminate the column, shortening its effective lifetime.

Extraction can be based on liquid-liquid or liquid-solid distribution. In liquid-solid extraction the solid phase has a greater attraction for the isolate than the solvent in which the isolate is dissolved. As the sample solution passes through the sorbent bed, the isolate concentrates on this surface while other samples pass through the bed. The sample is then eluted with a solvent that will displace it from the absorbent.

Some examples of absorbents are XAD-2 (Amberlite), a polystyrene divinyl co-polymer, Alumina(Al_2O_3), Extrelut (diatomaceous earth) and ion exchangers. In recent years there has been the development of bonded silica absorbents. The specific properties of a given bonded silica sorbent are a result of the functional group covalently bonded to the silica substrate. A variety of different bonded silicas are

commercially available, offering a wide range of selective properties for extraction. However liquid-liquid extraction is still usually preferred when results of high accuracy and precision are required. Normally pure solvents or mixtures of solvents are used. Commonly used solvents include diethyl ether, chloroform, dichloromethane, dichloroethane, ethyl acetate and butanol. Preconditions for the choice of a suitable solvent are that the two phases shouldbe immiscible, inert towards the solute, easily separated by low speed centrifugation and alow boiling point for sample concentration.

Methimazole is fairly soluble in a number of organic solvents. These include chloroform, ethyl acetate, acetonitrile and diethyl ether. Compared with the solvents mentioned, chloroform has the greatest solubility for methimazole and has the weakest polarity.

In order for the drug to preferentially dissolve in the organic phase, it must have no net charge; i.e. its ionisation must be suppressed. The pH of the environment will therefore play an important role in determining the level of un-ionised drug available to partition into the organic solvent.

The reversible binding of drugs to normal body macromolecule is one of the most important matrix effects of the biological material in drug analysis. Complex formation between a drug and a macromolecule in the biological material will, in principle, decrease the degree of extraction. This problem is more pronounced in cell-rich materials and especially in tissues with a high fat content. Procedures for denaturation of proteins can be of advantage. However, every form of precipitation of proteins can give serious disturbances in low concentration analysis.

Methimazole was reported to be 40% protein bound in plasma (Skellern et al., 1974) but these findings were later revoked (Skellern et al;1980(a)). Recent studies showed that no serum protein binding of methimazole could be demonstrated by the use of ultrafiltration (Balzer et al., 1975; Johansen et al., 1982) , therefore a protein denaturation step should not be required for the plasma assay.

In urine analysis the high concentration of inorganic salts and urinary pigments can present problems. Also urine is highly influenced by intake of food and other substances. It is therefore a good idea to first dilute the urine with at least an equal volume of water or appropriate buffer before extracting the sample.

Tissue samples are usually homogenized as the first step of treatment. Thyroid tissue is strictly more proteinaceous than fatty, therefore it would be better to homogenize under polar conditions. After homogenization the remaining solids can be separated by centrifugation. Although methimazole is not protein bound in plasma, it could be attached to endogenous macromolecules in thyroid tissue. Therefore, recovery could possibly be increased by the addition of a protein degradation agent.

The analysis of breast milk can be readily accomplished using standard analytical procedures. The primary difference between breast milk and other body fluids normally analysed is that breast milk contains a relatively high concentration of fatty acids and related lipids. These lipid materials can reduce extraction efficiency and also interfere with analysis, particularly gas-liquid chromatography. Accordingly, multiple solvent extractions are necessary for the

complete extraction of compounds with high lipid solubility. Techniques for the removal of lipids include washing with a low polarity solvent such as hexane, column chromatography, protein precipitation prior to extraction, steam distillation and lyophilisation.

However, studies with [^{35}S] MMI indicated that greater than 99% of the radioactivity was recoverable from the aqueous phase of milk after centrifugation at 3000xg (Cooper et al., 1984). Therefore the top lipid layer obtained by centrifugation can be discarded.

3.5.2 Materials

Chloroform was obtained from Rathburn Chemicals Ltd.,Peebleshire. Methimazole was kindly donated by Nicholas Laboratories, Slough. Extrelut was obtained from E. Merck Ltd., Darmstadt, G.F.R. (Extrelut columns were prepared by packing 0.7g of chloroform washed extrelut into a pasteur pipette containing a glass wool frit) Buffer solution was chloride-borate/NaOH 0.05M pH 9 (Meulemans et al.,1980)

3.5.3 Methods

3.5.3.1.Plasma Extraction

Plasma (1 ml) containing methimazole(aq) (1 ug/ml), pH9 chloride-borate buffer (0.05M,1ml) and internal standard (trideuteromethimazole,20ul of 10ug/ml aqueous solution giving

0.2ug/ml) were mixed on a vortex for 30 seconds. Chloroform (5ml) was added and the sample was rollermixed for 15 min then centrifuged at 3000 r.p.m. for 10 min. The organic phase was removed using a pasteur pipette and evaporated undr oxygen-free nitrogen. The extract was derivatised as described in section 2.3.

3.5.3.2. Urine extraction

Aliquots of urine (0.5 ml) were placed in screw-capped test-tubes (10 ml). Internal standard (trideuteromethimazole 40 ul of 10ug/ml aqueous solution giving 0.4ug/ml) was added and the tubes vortex mixed for ten seconds. Chloride-borate/NaOH buffer (pH9 0.05M, 2ml) and chloroform (8ml) was added to the spiked urine. The test tubes were capped, rollermixed for 15 minutes and then centrifuged at 3000 r.p.m. for 10 minutes. After removal of the upper aqueous layer the organic extracts were transferred to tapered test-tubes and evaporated under a stream of oxygen-free nitrogen at 30°C. The samples were reconstituted in pH9 chloride-borate/NaOH buffer (0.05M,200ul) and sonicated for five minutes. The extracts were then applied to extrelut columns and after ten minutes were eluted with chloroform (2ml) into ½ dram vials and completely evaporated. The extract was derivatised as described in section 2.3.

3.5.3.3. Thyroid Tissue Extraction

Thyroid tissue (1g) was cut into small pieces and pH9 chloride-borate/NaOH buffer (0.05m,10ml) was added together with internal

standard (10ug/ml trideuteromethimazole (100ul)). The mixture was homogenized and then centrifuged at 3000 rpm for 10 minutes. The supernatant was decanted to a separate test-tube where it was vortexed for 10 seconds and a 1ml aliquot removed to a 10ml screw-capped tube. Chloroform (5ml) was added to the spiked supernatant. The test tube was capped, rollermixed for 15 minutes and then centrifuged at 3000 r.p.m. for 10 minutes. After removal of the upper aqueous layer, the organic extract was transferred to a tapered test-tube and evaporated under a stream of oxygen-free nitrogen at 30°C. The sample was then manipulated as for urine.

3.5.3.4. Milk Extraction

Aliquots of milk (1ml) were centrifuged for 10 minutes at 3000 r.p.m. After removal of the upper layer, the samples were placed in a 10ml screw-capped test-tube. Internal standard (trideuteromethimazole 40ul of 10ug/ml aqueous solution giving 0.4 ug/ml) and pH9 chloride-borate/NaOH buffer (0.05M, 1ml) was added and the tubes vortex mixed for 10 seconds. The samples were then extracted as for urine.

3.5.4 Results and Discussion

Methimazole is a very weak acid with a pKa value of 11.5 (Skellern et al., 1981). Therefore any decrease in pH from 11.5 will increase the level of drug which is un-ionized. Table 3.3 shows the percentage recovery of methimazole in plasma extracts over the pH range 5 - 10 in comparison with that of a standard chloroform solution of methimazole

Table 3.3 Percentage recovery of methimazole following extraction over the pH range 5-10.

pH	Peak Height	% Recovery
control	4983	100
5	2988	59.8
6	3518	70.4
7	3262	65.2
8	3838	76.8
9	3512	70.2
10	2399	48.0

at the same concentration. Over this pH range there was little difference in recovery. However the chromatogram at pH9 showed the least interference and therefore this was the buffer system chosen.

A problem with chloroform extractions is the formation of emulsions. To prevent this, the solvent volume was much larger than the sample volume. Also centrifugation will break up any emulsions which may still occur.

After liquid-liquid extraction alone, urine, thyroid and milk extracts all still contained endogenous compounds which co-eluted off the GC column with methimazole. Therefore an extrelut column was used as a clean-up step. It would have been much simpler if this could have been used as a single extraction step; i.e. if the sample was loaded directly onto the column and the eluant evaporated and derivatised. However, as extrelut is an absorbent medium, the sample volumes at this stage would be such as to overload the column.

As was mentioned in the introduction, methimazole could be bound to endogenous macromolecules in thyroid tissue. Table 3.4 shows the recoveries of methimazole in spiked and patient samples, three with and three without Subtilisin A (0.1 mg/ml), a proteolytic enzyme.

All the samples were put into a water bath with a shaking device and were incubated for 1hr at 50^{o}C before extraction. A spiked control sample was extracted in the normal way to check that the temperature did not have any adverse effect on methimazole recovery. The results from Table 3.4 show that there was no improvement in recovery by employing Subtilisin A, and therefore it can be assumed that methimazole is not significantly bound to proteins in the thyroid gland.

Table 3.4 Peak height recovery of methimazole from thyroid tissue both with and without pretreatment with subtilisin A.

Sample		Sutilisin A	Peak Height
Spiked	1	Yes	16160
	2	Yes	16212
	3	Yes	16194
	4	No	16102
	5	No	16172
	6	No	16182
Patient	1	Yes	10148
	2	No	15589
Control			16365

Thus, sufficient extraction methods have been developed for all of the matrixes to be analysed. Typical chromatograms for methimazole extracted from plasma, urine, milk and thyroid tissue are shown in Figures 3.11 to 3.14

3.6 METHOD VALIDATION

3.6.1 Introduction

Before a new assay is used to determine the quantity of a drug in patient samples, it must be shown to be precise, accurate, sensitive and specific. The presence of an internal standard is used to compensate for losses during sample preparation and should help to increase accuracy and precision. However, there must be some kind of validation by the repeated analysis of spiked samples to determine exactly the accuracy and precision of the method.

The mean recoveries with their standard deviations give a measure of the accuracy of the method, while the coefficient of variation gives a measure of the precision of the method. The calibration curve gives an idea of the linear range of the method.

Biological materials for analysis of drugs are almost exclusively stored at -20°C. This is usually sufficient to prevent significant changes of the biological material including microbiological degradation. At this temperature most drugs are not decomposed to any great extent even during prolonged storage; e.g. for several months. However, repeated freezing and thawing of a biological material may

give rise to decomposition and therefore the stability of a drug in a certain biological material must always be studied

3.6.2 Methods

3.6.2.1a Preparation of Calibration Standards

Aliquots of control plasma and urine were spiked with appropriate amounts of an aqueous standard solution of methimazole (constituting < 10% of the total mixture) to produce at least six standards over the required concentration range. The samples were analysed according to the described methods. The ratio of the peak areas (Y) resulting from the molecular ions 171 and 174 of the silyl derivatives were plotted against the concentration of methimazole (X) expressed in units of ug/ml.

3.6.2.1b Fitting of the calibration data

The standard deviation of experimentally determined ratios from the fitted line is expressed in concentration units as

$\lambda = s/b$ where:

s = standard deviation in peak area ratio units.

$$s = \sqrt{\left(\frac{\Sigma d_i^2}{n-2}\right)}$$

the 'd_i' being the deviation of experimental peak area ratios from the line and 'n' the number of points.

b = slope of the line in units of peak area ratio/concentrations.

$$= \frac{\Sigma(X-\bar{x}).(Y-\bar{y})}{\Sigma(X-\bar{x})^2}$$

$$= \frac{\Sigma XY - \frac{(\Sigma X).(\Sigma Y)}{n}}{\Sigma X^2 - \frac{(\Sigma X)^2}{n}}$$

where the X and Y are the concentrations and their associated area ratios.

A limit of reliable determination (LRD) for each calibration line is estimated as follows:

$$LRD = 2\lambda \text{ for intercept } a > 0,$$

$$= -a/b + 2\lambda \text{ for intercept } a < 0.$$

The relative standard deviation (RSD) of each calibration is estimated by expressing the concentration standard deviation, λ, as a percentage of the mean spiked concentration, x, that is:

$$RSD = 100\lambda/x$$

$$\text{where } x = \frac{\Sigma X_i}{n}$$

the X_i being the spiked concentrations assayed to construct the calibration line.

3.6.2.2.Reproducibility

The reproducibility of the method was evaluated by performing three replicate assays on spiked plasma, urine, milk and thyroid tissue at three different concentrations of methimazole. These aliquots were then analysed as previously described. The variability of the peak area ratios at each concentration were expressed as coefficients of variation (cv).

3.6.2.3.Extraction Efficiency

The extraction efficiency of methimazole and the internal standard from plasma, urine, milk and thyroid tissue was calculated by comparing peak heights from the extracts with those of standard chloroform solutions.

3.6.2.4.Stability Study

6 ml aliquots of each plasma (0.5 and 1.0ug/ml) and urine samples (1.0 and 2.0ug/ml) were stored at -20°C for periods of 1 day, 3days, 7 days, 14 days, 1 month, 3 months and 6 months

The samples were assayed for methimazole against freshly prepared calibration standards in the appropriate biological fluid. Stability of methimazole was assessed by comparing the results of successive assays with the concentration determined initially.

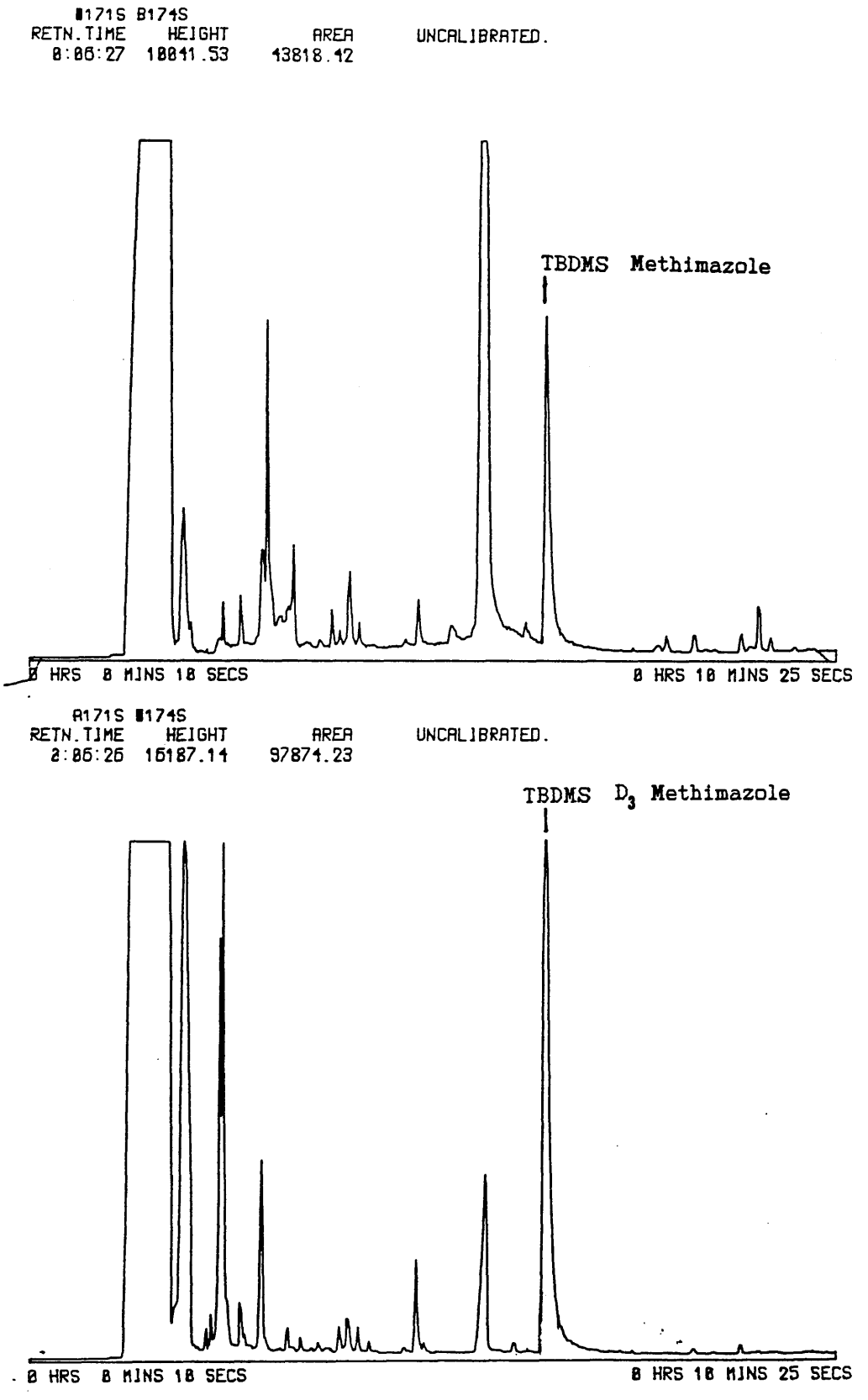


Figure 3.11 Typical chromatogram of methimazole in plasma

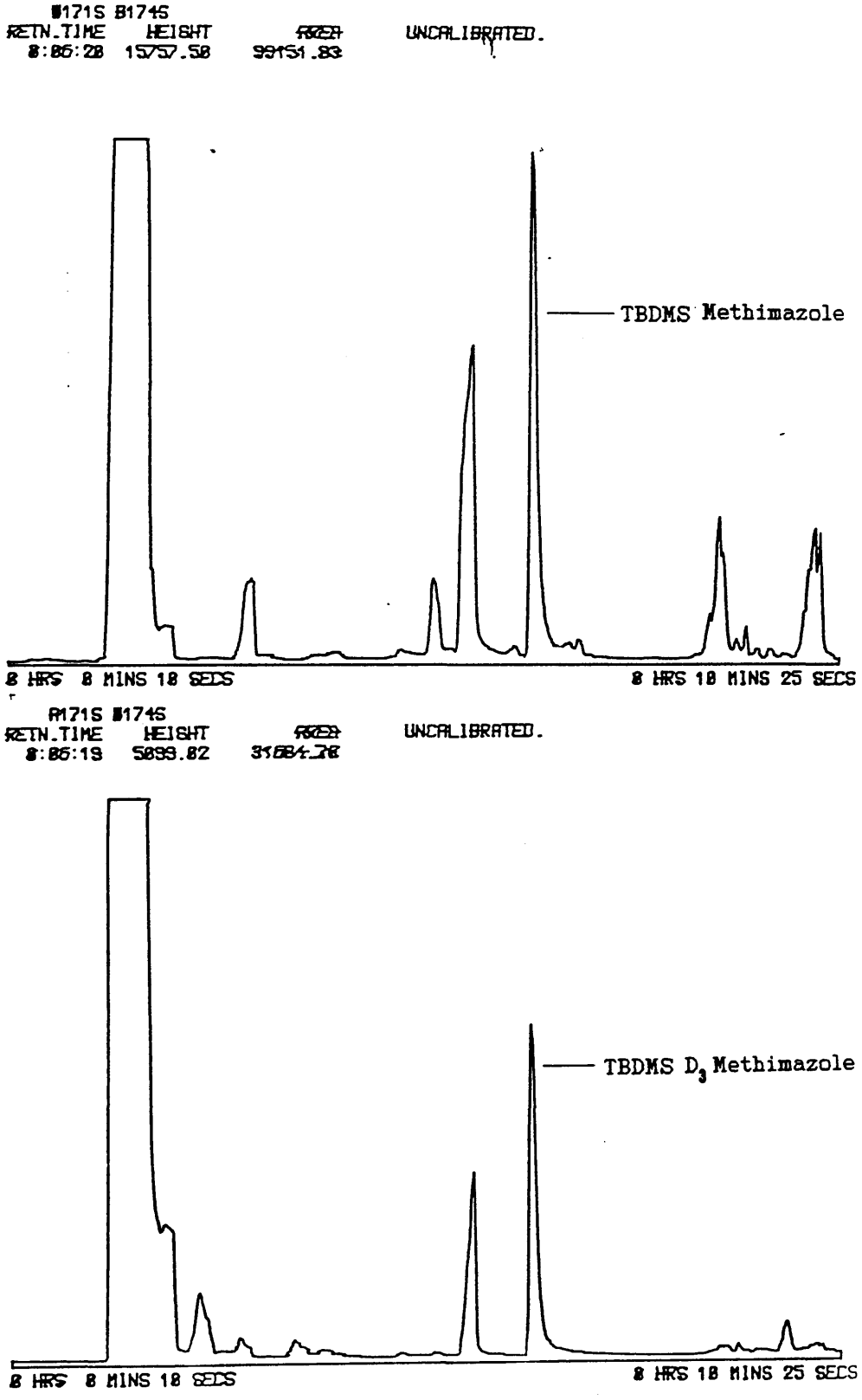


Figure 3.12 Typical chromatogram of methimazole in urine

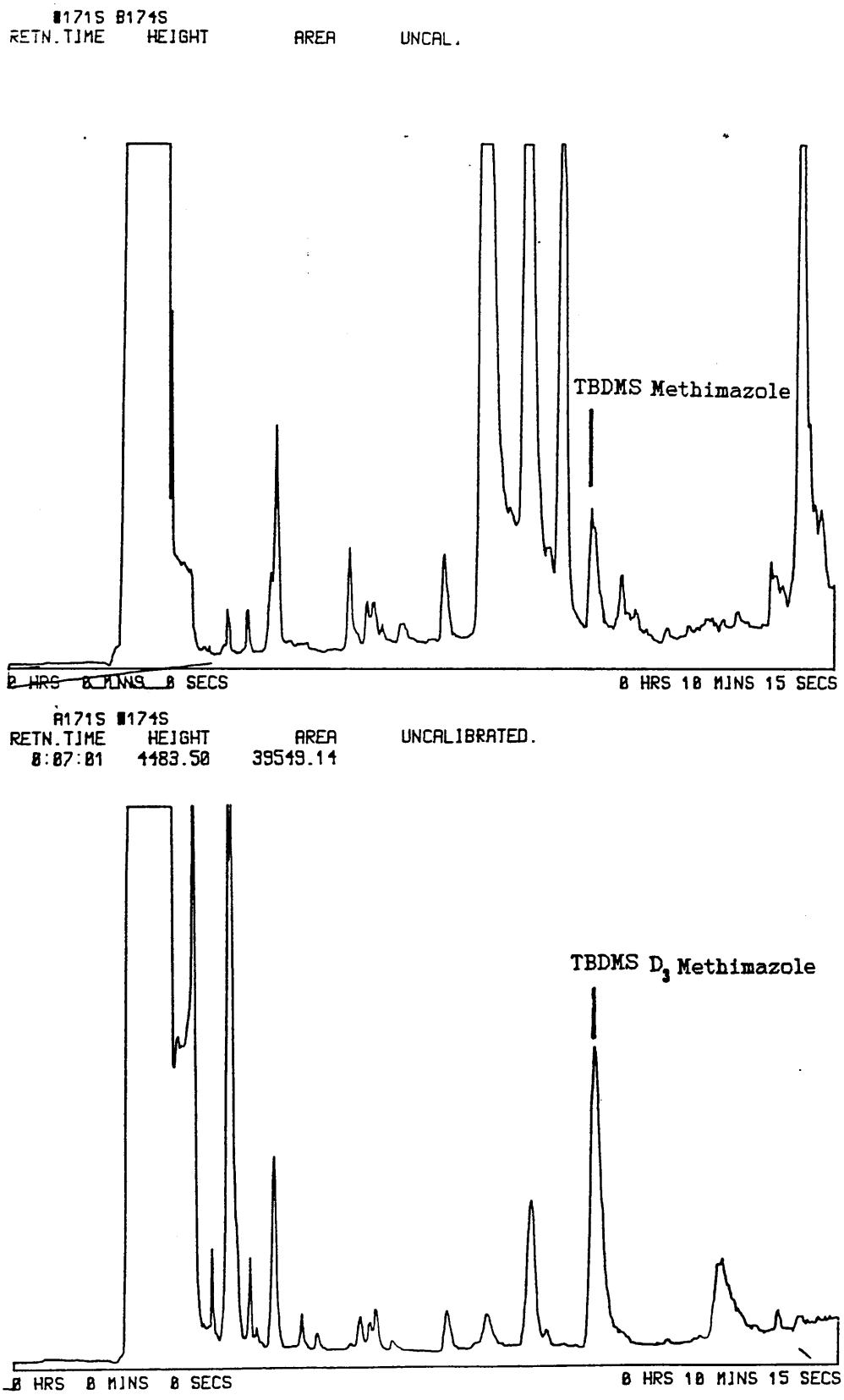


Figure 3.13 Typical chromatogram of methimazole in milk

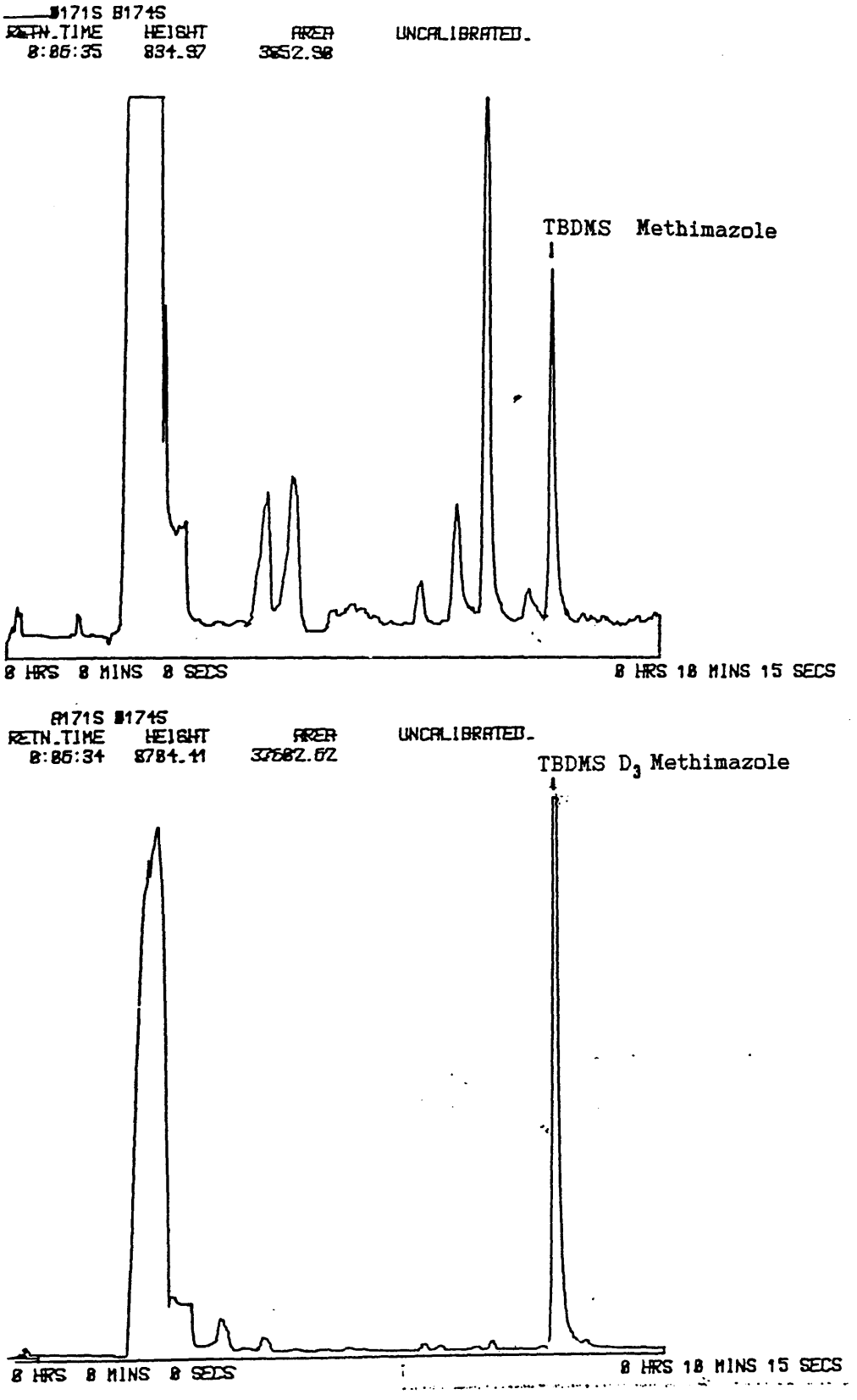


Figure 3.14 Typical chromatogram of methimazole in thyroid tissue.

Table 3.5 Linear regression analysis of calibration curves for methimazole in human plasma, milk and thyroid tissue and from greyhound urine.

Calibration Curve Range (ug/ml)	Relative Standard Deviation (%)	Limit of Reliable Determination (ug/ml)
Human Plasma		
0-.05	4.47	0.003
.05-.5	5.31	0.027
Human Milk		
.025-1.0	3.75	0.020
Human Thyroid		
.10-5.0	6.19	0.094
Greyhound Urine		
.05-5.0	7.70	0.103

Table 3.6 Reproducibility of replicate extractions of methimazole from human plasma.

Concentration (ug/ml)	Methimazole Area	Trideuterated Methimazole Area	Ratio	CV%
	6396	279390	0.229	
0.05	10716	48154	0.223	3.51
	8408	35223	0.239	
	10677	21068	0.507	
0.1	26710	52697	0.507	0.11
	10737	21397	0.508	
	129581	124221	1.043	
0.5	138085	134877	1.024	6.65
	79877	86845	0.920	

Table 3.7 Reproducibility of replicate extractions of methimazole from human milk

Concentration (ug/ml)	Methimazole Area	Trideuterated Methimazole Area	Ratio	CV%
	20124	52118	0.386	
0.1	17996	52135	0.345	4.95
	18733	46484	0.403	
	23530	64466	0.365	
	39133	77185	0.507	
0.5	40500	70927	0.571	6.05
	59812	112851	0.530	
	62910	69056	0.911	
1.0	78359	89247	0.878	1.84
	54796	61293	0.894	

Table 3.8 Reproducibility of replicate extractions of methimazole from human thyroid tissue.

Concentration (ug/ml)	Methimazole Area	Trideuterated Methimazole Area	Ratio	CV%
	9178	24071	0.381	
0.1	20506	62422	0.328	7.84
	20496	9962	0.342	
	21941	104304	1.169	
1.0	102769	87619	1.173	3.73
	177943	162212	1.097	
	367716	157796	2.330	
2.0	411349	188450	2.183	3.50
	255184	110520	2.309	

Table 3.9 Reproducibility of replicate extractions of methimazole from greyhound urine.

Concentration (ug/ml)	Methimazole Area	Trideuterated Methimazole Area	Ratio	CV%
	124466	115064	1.08	
0.1	117148	109926	1.07	7.58
	25279	26871	0.94	
	181690	101498	1.79	
0.5	135258	71188	1.90	4.77
	151433	87533	1.73	
	194645	75884	2.56	
1.0	172620	69048	2.50	3.47
	143432	60013	2.39	

Table 3.10 Extraction recoveries of methimazole from human plasma, milk and thyroid tissue and from greyhound urine.

	Methimazole Concentration (ug/ml)	Extraction Efficiency (%)
a)Human Plasma	(1) 0.05 (2) 0.1 (3) 0.5	80 71 86.5 Mean= 75.8 +/- 4.5
b)Human Milk	(1) 0.1 (2) 0.5 (3) 1.0	59 67 69 Mean = 65 +/- 4.3
c)Greyhound Urine	(1) 0.1 (2) 0.5 (3) 1.0	65 72 60 Mean =65.7 +/- 4.9
d)Human Thyroid	(1) 0.1 (2) 1.0 (3) 2.0	60.0 81.7 74.4 Mean = 72 +/- 9

Table 3.11 Concentration of methimazole in spiked human plasma and urine samples after storage at -20 C for up to six months

Sample	Plasma		Urine	
	1ug/ml	0.5ug/ml	1.0ug/ml	2.0ug/ml
Initial	0.903	0.489	0.940	1.956
Day 1	0.447	0.624	-	2.390
Day 3	0.664	0.506	0.617	1.940
Day 7	-	0.423	1.280	2.030
Day 14	1.110	0.519	1.120	2.000
1 month	1.080	0.538	1.020	2.220
3 month	1.360	0.590	1.400	2.350
6 month	1.670	0.522	1.560	2.040
Mean	1.03	0.526	1.13	2.120
(CV%)	(40)	(12)	(28)	(8.5)

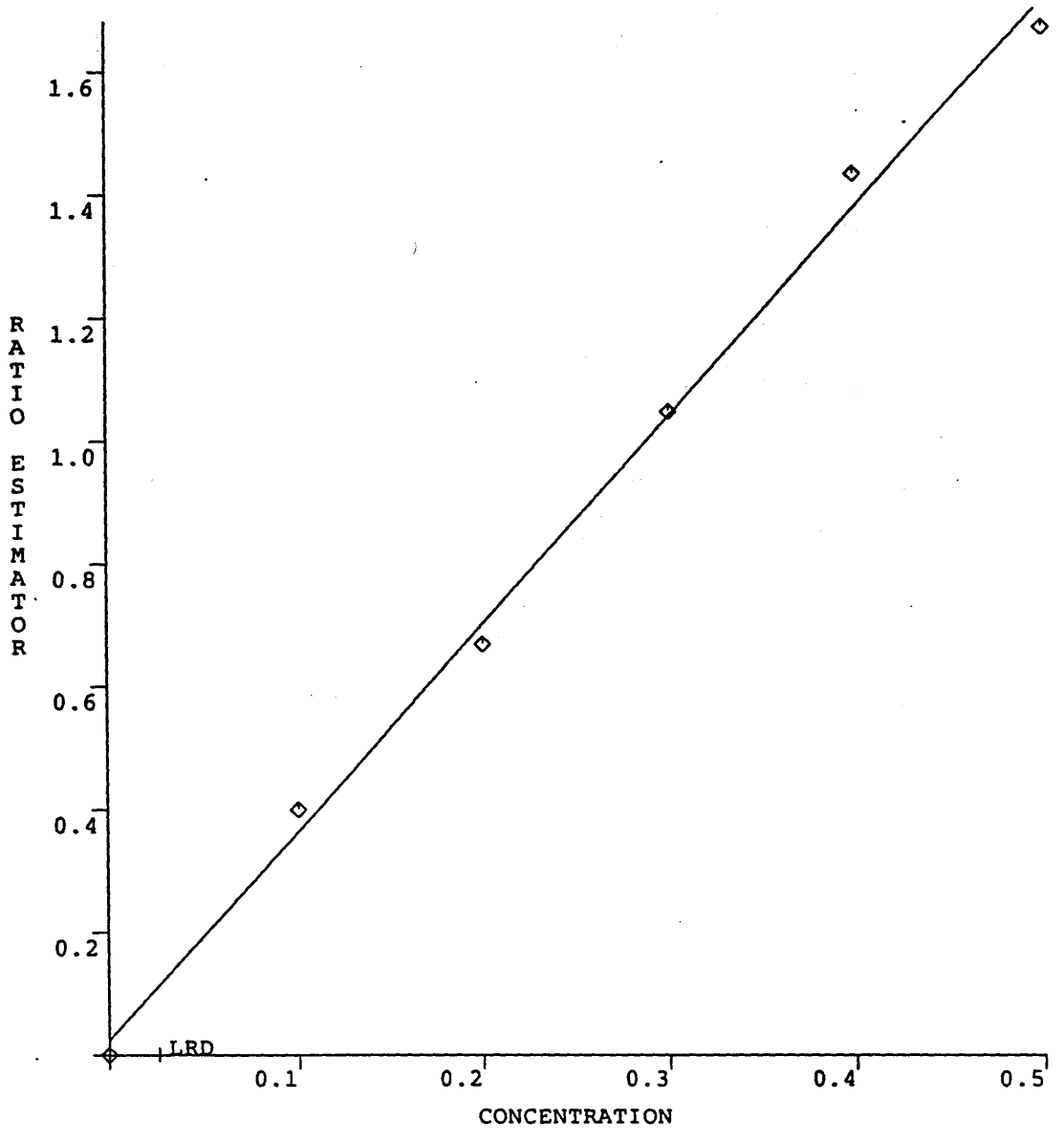


Figure 3.15 Calibration curve for methimazole in plasma (range 0 - 0.5ug/ml)

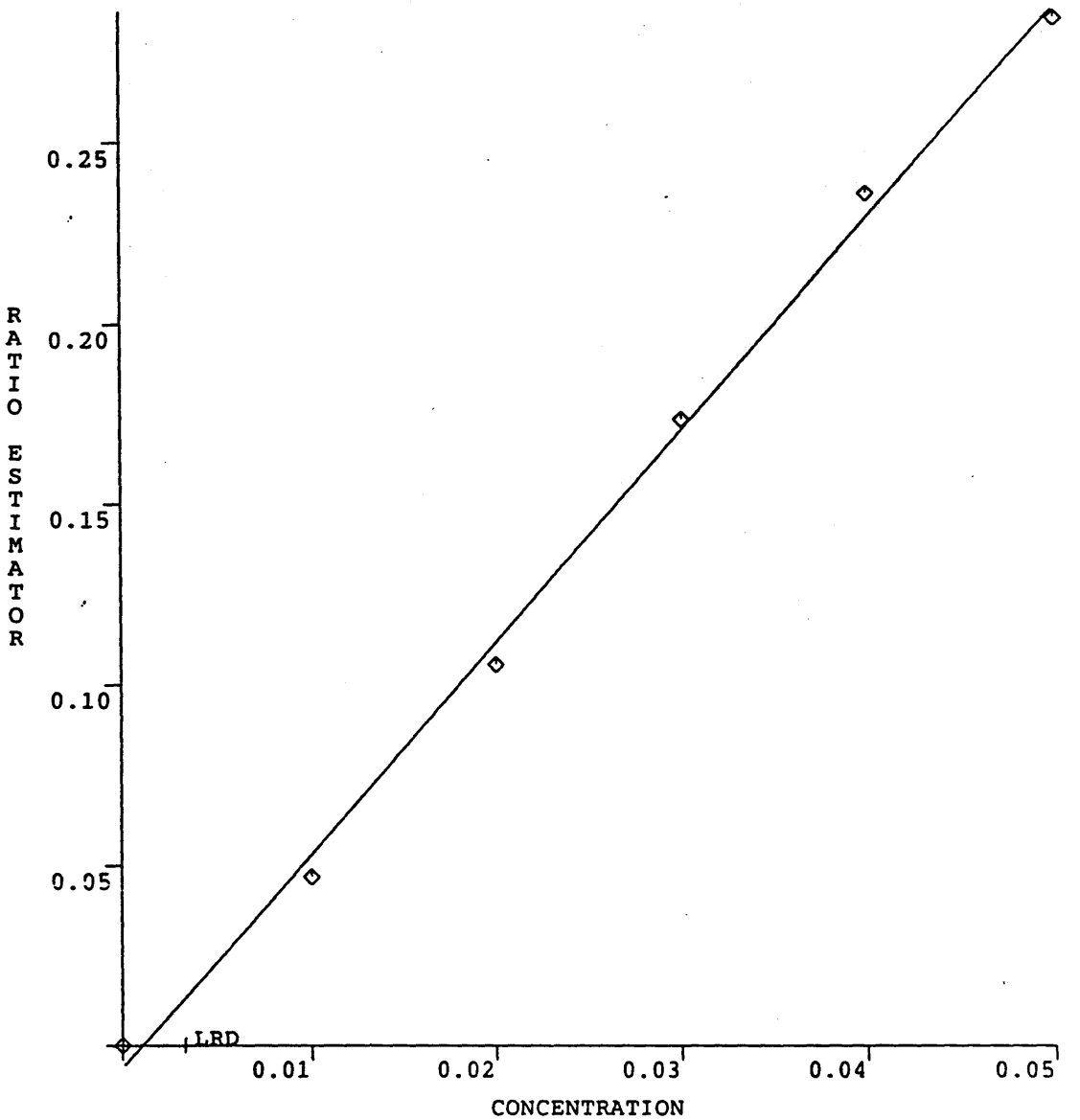


Figure 3.16 Calibration curve for methimazole in plasma (range 0 - 0.05ug/ml)

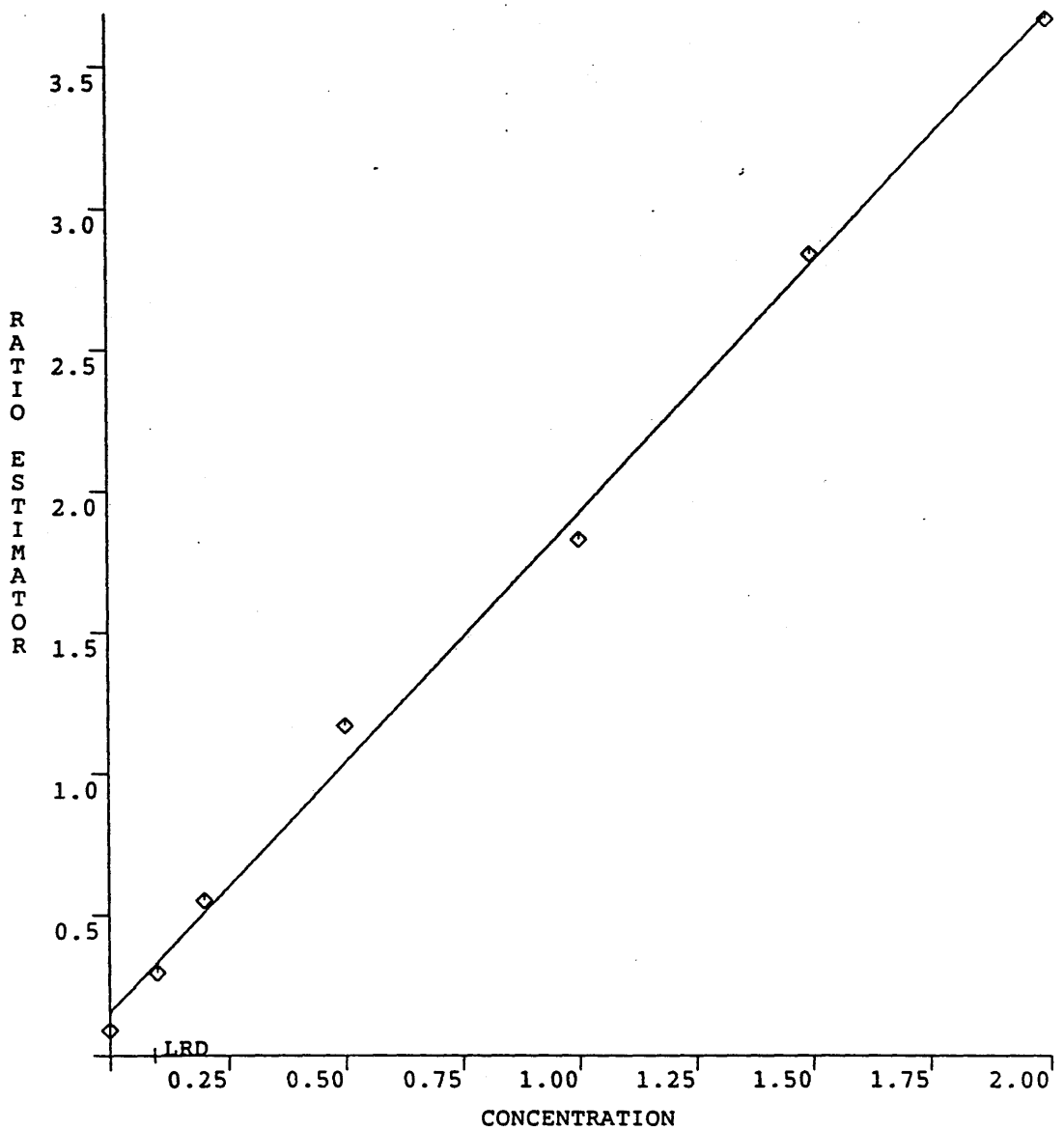


Figure 3.19 Calibration curve for methimazole in thyroid tissue.

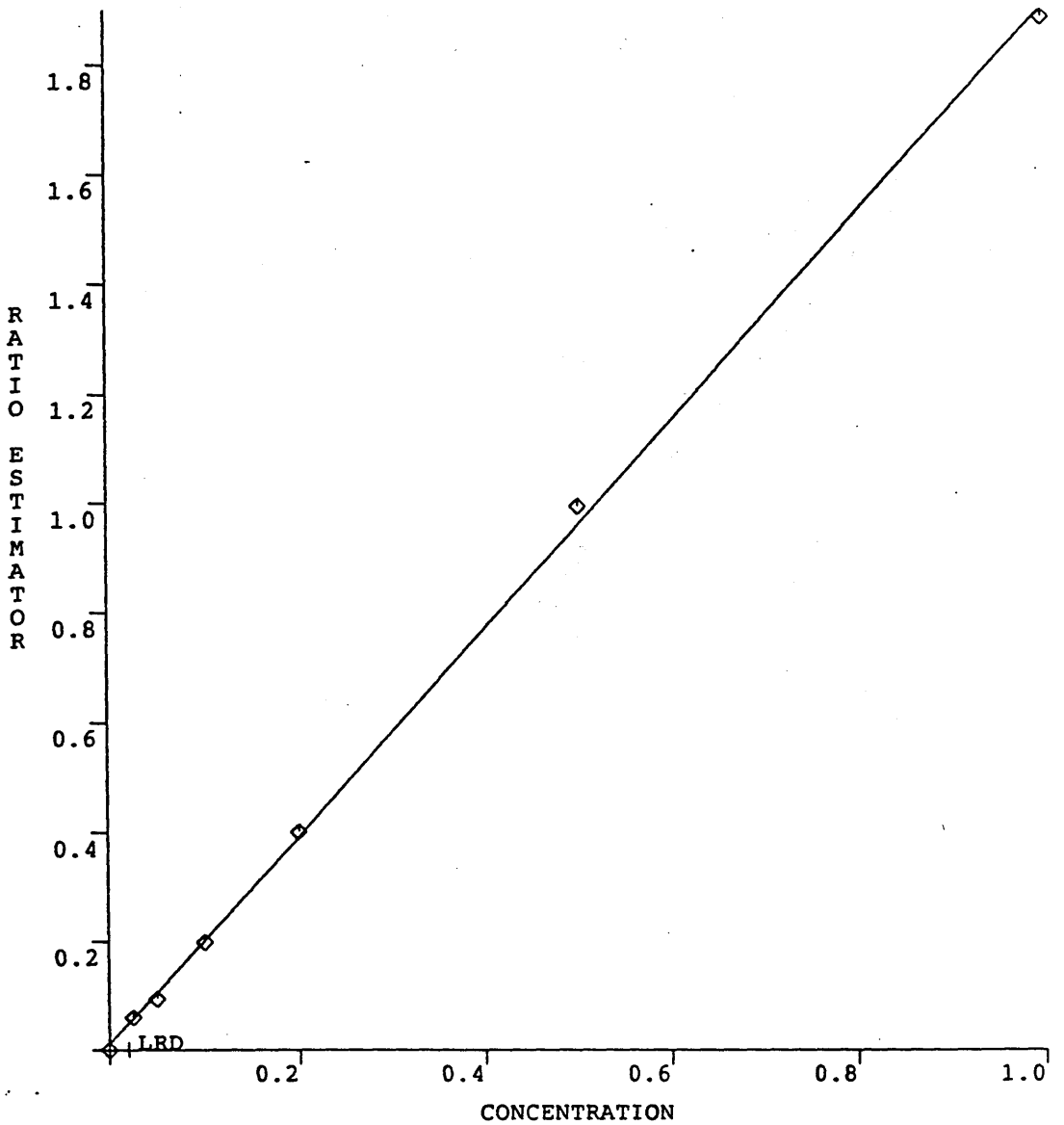


Figure 3.17 Calibration curve for methimazole in milk

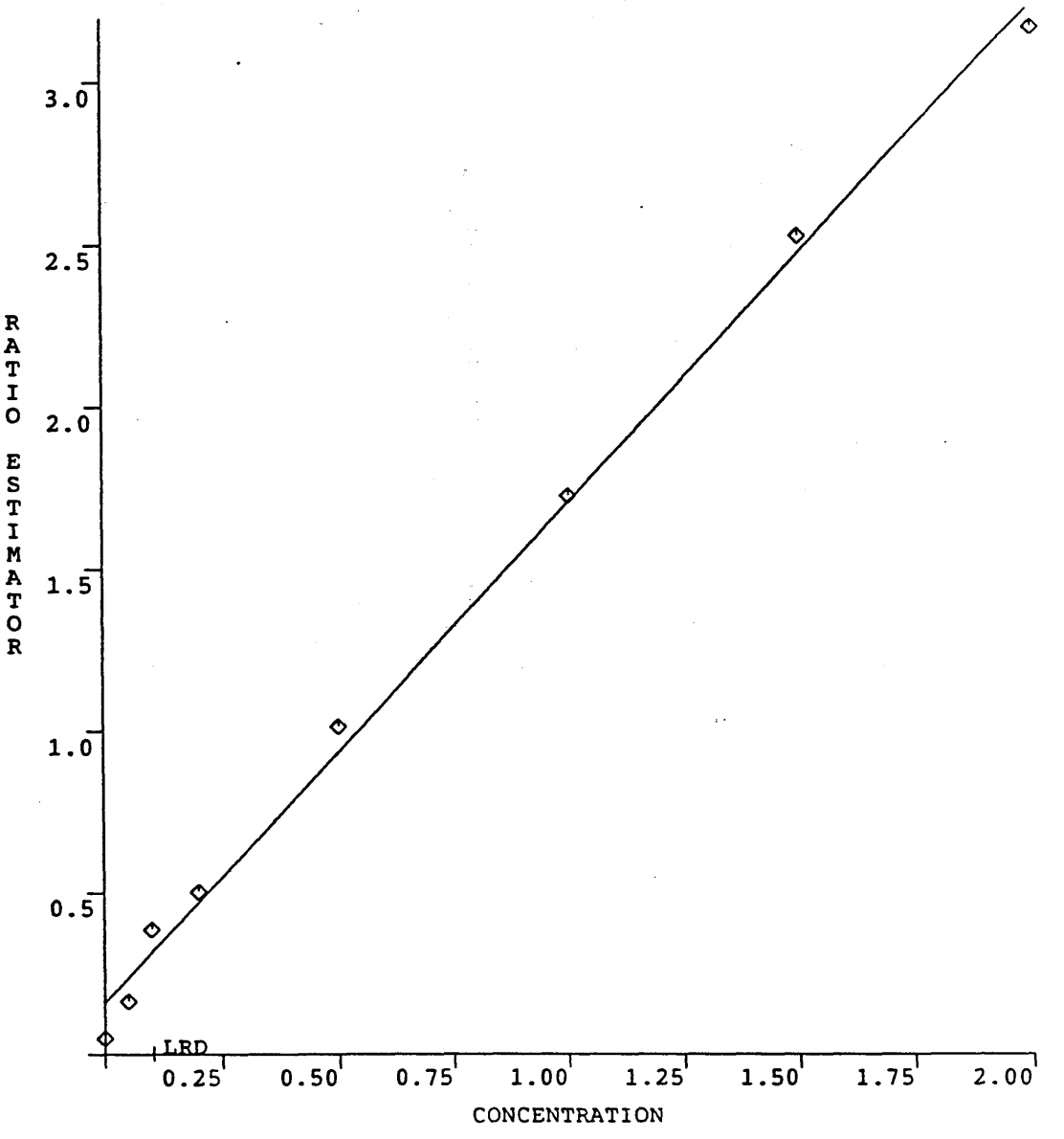


Figure 3.18 Calibration curve for methimazole in urine.

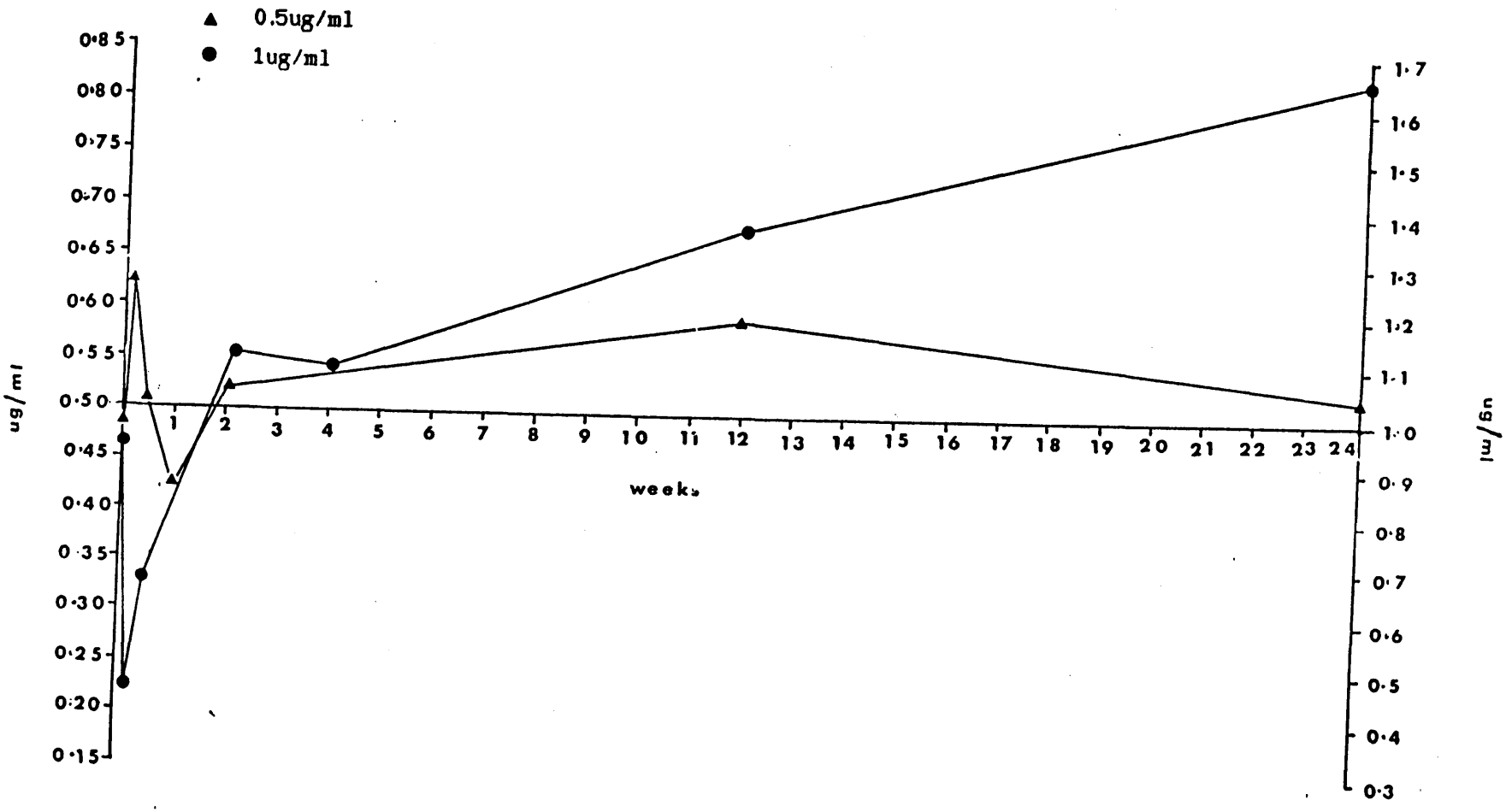


Figure 3.20 Plot of concentration versus time for plasma stability standards over six months

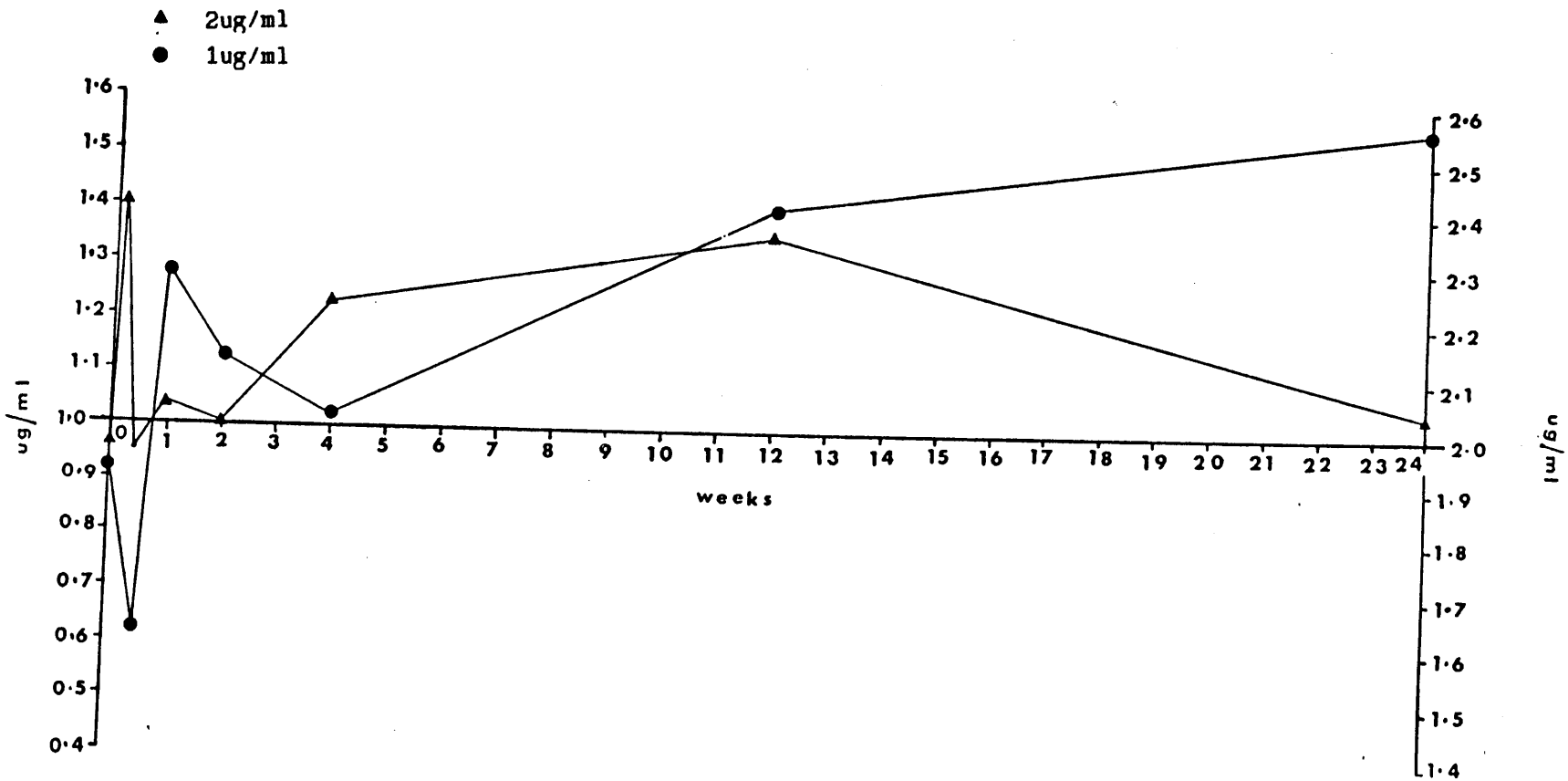


Figure 3.21 Plot of concentration versus time for urine stability standards over six months

3.6.3 Results and Discussion

Factors leading to compound losses during sample preparation are critical at low concentrations and can adversely affect the reliability of the assay. In this section, from the study of the overall recovery (Table 3.10), the precision (Table 3.6 to 3.9) and accuracy (Table 3.5), it can be seen that the sample preparation has been optimized to enable the development of a reliable and validated method for methimazole which is suitable for use in clinical therapeutic monitoring.

The overall extraction efficiencies ranged from 65 to 76%. Plasma extraction gave the best recovery probably because it involved less stages. Extraction from milk had the lowest recovery perhaps suggesting a loss of compound at the separation of the aqueous and lipid layers.

The reproducibility of the assay is high as shown by the coefficients of variation which were all below 10%.

The calibration curves were shown to be linear over the ranges studied (figures 3.15 to 3.18) with relative standard deviations which were all below 8% (Table 3.5). The samples showed good stability after storage at -20°C for up to six months. The stability sample results are given in Table 3.11 and are graphically presented in Figure 3.20&21.Despite a wide variation, the mean value was very close to the actual value. Thus, it was concluded that there was no significant sample loss and the stability of methimazole over six months was satisfactory. However, from figures 3.20 and 3.21, the graphs appear to show a positive trend which may reflect a change in the sample matrix with time (Appendix I, p.286).

CHAPTER 4

URINARY ELIMINATION OF METHIMAZOLE AND 3-METHYL-2-THIOHYDANTOIN IN THE RACING GREYHOUND

4.1 INTRODUCTION

4.1.1. Doping in Greyhound Racing

The racing of greyhounds is an international industry worth £3000 million, with its popularity in Britain second only to football. The huge sums of money associated with the sport, make it important to have a doping control programme. At present race-tracks registered by the National Greyhound Racing Club (NGRC) have a pre-race testing laboratory. Here, each dog's urine is tested by thin layer chromatography following solvent/solvent extraction for the presence of barbiturates and common neutral drugs and alkaloids (Bogan and Smith,1968). The Fujiwara test is used to detect chloral drugs. If any of these procedures prove positive or if there is any other reason for suspicion (e.g. altered performance), track officials send a sample to the Department of Forensic Medicine and Science at Glasgow University where a range of analytical techniques are carried out.

Drugs which block pain can make an injured dog run faster and depressants can also sometimes improve the performance of nervous or stressed greyhounds. However, as the dose-response relationship of drugs in the racing greyhound are not widely known, it is likely that the administration of any drug could be counterproductive to a good performance. With this aim, an opponent may deliberately dope the greyhound. Alternatively, because dogs are usually run in graded races, on the basis of their past performance, the owner can use this ploy in

order to mask the dog's potential. Then, its odds will be improved in subsequent races where a dope test will reveal nothing. In reality, this sophisticated plan is very difficult to achieve with the limited body of knowledge available on greyhound drug reactions.

Drugs which are most commonly administered are oxyphenylbutazone, phenylbutazone, caffeine and procaine (Association of Official Racing Chemists, 1985) but often, especially when attempting to slow a dog, trainers use all sorts of drugs which could result in any manner of effects.

4.1.2. Possible Physiological Effects of Methimazole in the Racing Greyhound.

Hormones have a central modulatory effect on the very fundamental molecular processes which govern various metabolic activities at both basic cellular and organ level. An excess or deficiency of a specific hormone may therefore produce profound and widespread physiological effects.

As discussed in the introduction (section 1.1.2), thyroid function is regulated by the specific hormone, thyrotropin (TSH). The rate of secretion of thyrotropin is delicately controlled by the quantity of thyroid hormone in the circulation. If extra hormone is given, the secretion of thyrotropin is suppressed and the thyroid becomes inactive and regresses, whereas any decrease in the normal rate of secretion of

the thyroid evokes an enhanced secretion of thyrotropin and the thyroid is stimulated to increased growth and function.

Thus, it can be seen that the administration of methimazole will have two effects with time. Initially, as it inhibits production of thyroid hormones it will produce the symptoms of hypothyroidism. However, negative-feedback action of thyroid hormone will reverse the situation i.e. there will be an increase in thyroid hormone production resulting in hyperthyroidism.

In the hypothyroid state, the appetite is poor, gastrointestinal activity is diminished and abdominal distention and constipation are common (Bell et al., 1977; Forfar et al., 1980). The voluntary muscles are weak and flabby and deep-tendon reflexes are slowed. The heart is often dilated and cardiac output is diminished.

In the hyperthyroid state, the muscles are weak and tremulous; the heart rate is rapid and the heart beat is forceful, the arterial pulses are prominent and bounding but the cardiac output is decreased on exercise (Forfar et al., 1982; Sobel and Braunwald, 1971).

Obviously, the extent of either of these conditions will depend very much on the size of the dose and how often and regularly it is administered.

4.1.3 Comparison of Urinary Excretion of Methimazole and Metabolites in Different Species.

4.1.3.1 Metabolism of Methimazole in Rats

Metabolic studies using ^{35}S methimazole (Marchant and Alexander,1972) and 2- ^{14}C methimazole (Skellern et al.,1973) in rats have shown the presence of inorganic sulphate and at least six other metabolites in urine. There is also evidence for the presence of a glucuronide of methimaozle in the urine of rats receiving methimazole (Sitar et al.,1973). The nature of the compounds excreted in rat urine after methimazole administration is summarised in Table 4.1.

4.1.3.2. Metabolism of Methimazole in Man

The metabolism of methimazole in man has been discussed earlier (section 1.3.3.2). Comparing human metabolism studies with those in the rat there are some differences in results. A high percentage of methimazole-glucuronide was found to be excreted in rat urine (Sitar et al., 1973) but, although a methimazole -glucuronide has been shown in human bile, none was detected in urine (Marchant et al., 1979). The urinary metabolites found in the radioactive studies (Alexander et al., 1969; Marchant et al., 1979) have not been further characterised, but a minor metabolite, 3-methyl-2-thiohydantoin, was later identified (Skellern et al., 1977). 3-methyl-2-thiohydantoin is formed by a

Table 4.1 Nature of compounds excreted in urine after administration of ^{35}S and/or ^{14}C-methimazole to the rat.

Reference	Dose (mg/kg) and label	Cumulative Excretion Time (hrs)	Nature and Amount (% administered dose) of Compounds Excreted			
			MMI	MMI-G	Sulphate	Total unknowns
Marchant and Alexander (1972)	0.5 ^{35}S	24	11 +/- 1	-	3	a) 36 +/- 2% b) 4 +/- 1 c) 1 d) 1
Skellern et al (1973)	3.0-3.5 ^{14}C	12	11 +/- 2	-	-	a) 38 +/- 2% b) 6 +/- 1 c) 6 +/- 1 d) 4 +/- 1 e) 2 +/- 1
Sitar et al (1973)	20 ^{14}C	24	14-21	36-46	-	a)10-20%

Phase I metabolism reaction i.e. introduction of a new chemical group on a molecule, in this case by the hydrolysis of methimazole. This type of reaction is probably common to all species, varying only in degree, as hydrolytic enzymes are widely distributed to deal with endogenous compounds present in the diet.

However, a Phase II metabolism i.e. addition of an endogenous molecule to a compound such as glucuronidation, may not be common to all species. It is, for example, already known that the cat is virtually incapable of metabolic conjugation with glucuronic acid.

4.1.3.3. Metabolism of Methimazole in Greyhound

We cannot predict what a drug, well studied in man, will do in another species or how it will be metabolised. Even the physiologic differences between the racing greyhound and the domestic dog are quite large.

The racing greyhound has a very large aerobic capacity partly because of the quality of it's blood which has a very high concentration of the oxygen-carrying red blood cells. The increased viscosity of their blood means that in order to pump efficiently, their heart is huge compared with the rest of their body. Most of the greyhound mass, some 60%, is devoted to muscle which has been found to have only low stores of glycogen.

From this limited physiological information, it seems that the greyhound has quite unique energy stores and it is reasonable to assume

that the enzymes intermediating in these processes might be quantitatively or qualitatively different from other species.

Therefore, it is impossible to say much about the metabolism of methimazole, except that by the nature of its common reaction pathway there is a possibilty that 3-methyl-2-thiohydantoin could be formed.

4.1.4. Aim of Study

The aim of this study is to determine the plasma concentration versus time profile and urinary excretion of methimazole in the greyhound following a single oral dose. Also an attempt will be made to identify methimazole metabolites in greyhound urine.

4.2 MATERIALS AND METHODS

MTH-glycine was obtained from Sigma Chemicals Ltd. Methanol was obtained from Rathburn Chemicals Ltd., Walkerburn. Ammonium Hydroxide was a product of British Drug House (BDH), Dorset. Thin layer chromatography (TLC) was performed on 5 x 20 cm glass plates coated with silica gel (Kieselgel 60, (0.25mm)). The supplier was E.Merck, Darmstadt, G.F.R. Chromogenic sprays were supplied by Shandon, Cheshire, England. All other reagents are as listed in 3.5.2.

A single oral dose study in greyhounds was conducted by The Wellcome Surgical Institute, Glasgow University, Garscube Estate, Glasgow. Two healthy greyhounds were administered methimazole. The male dog was given 5mg while the female dog was given 10mg of methimazole. The dose was given in a cellulose capsule without excipients. The dogs were housed individually in metabolism cages for 48 hours under conditions of controlled temperature and humidity. They were fed twice a day at 10.30am and 4.00pm with meal and tinned dog food.

Blood samples (10ml) were taken from the jugular vein predose and at frequent time intervals up to 6 hours following drug administration. The samples were collected into heparinised tubes and after centrifugation, the resultant plasma was stored at -20°C. Urine was also collected at regular time intervals up to 48 hours following drug administration and was stored at -20°C.

At the end of the study, the samples were immediately taken to The Department of Forensic Medicine and Science. Urine samples were thawed

for volume measurement then, like the plasma samples, stored at -20°C until assayed.

Eight samples of urine from Walthamstow Stadium were sent for analysis over a period of nine weeks. The initial sample was sent because of improved performance; with the pre-race routine tests proving negative. After detection of methimazole ,further samples where sent each time the dog competed at this stadium. The samples were sent by post and on arrival immediately frozen at -20°C until assayed.

The samples were assayed for methimazole as described in 3.5.3.2.

4.3. RESULTS

4.3.1. Methimazole Plasma and Urine Concentrations

The plasma concentration-time data of methimazole for the two greyhounds are listed in Table 4.2 and depicted in Figure 4.1. A typical chromatogram of methimazole from greyhound urine is shown in figure 4.2.

Following dosing, maximum plasma concentrations (Cmax) were 306 and 546ng/ml for the 5 and 10mg doses, respectively. They occurred between 1 and 2 hours post-dosing. The areas under the curve to the last data point were 973 and 2002 for the 5 and 10mg doses, respectively. Doubling the dose resulted in approximate two-fold increases in AUC (2.06) and Cmax values (1.8) indicating a linear dose response.

Urinary excretion of methimazole (Tables 4.3 and 4.4) was low, with 5.5 and 8.2% of the dose being recovered in 48 hours after administration of the 5 and 10mg doses, respectively. The excretion was rapid since the majority of the eliminated material (47.3 and 57.3%) appeared during the first 6 hours after administration.

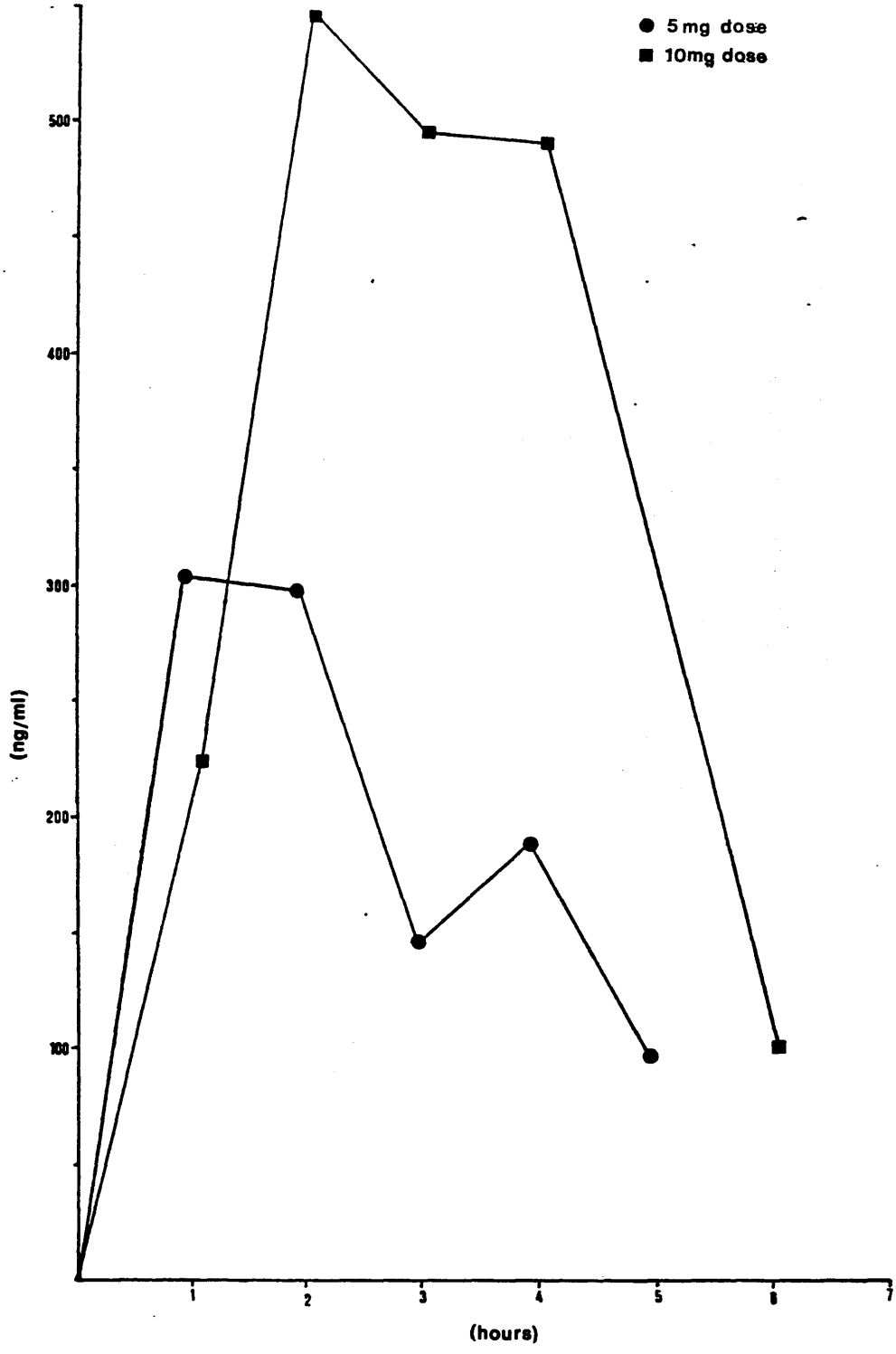


Figure 4.1 Plasma concentration versus time curve for the two greyhounds following oral administration of 5mg or 10mg of methimazole.

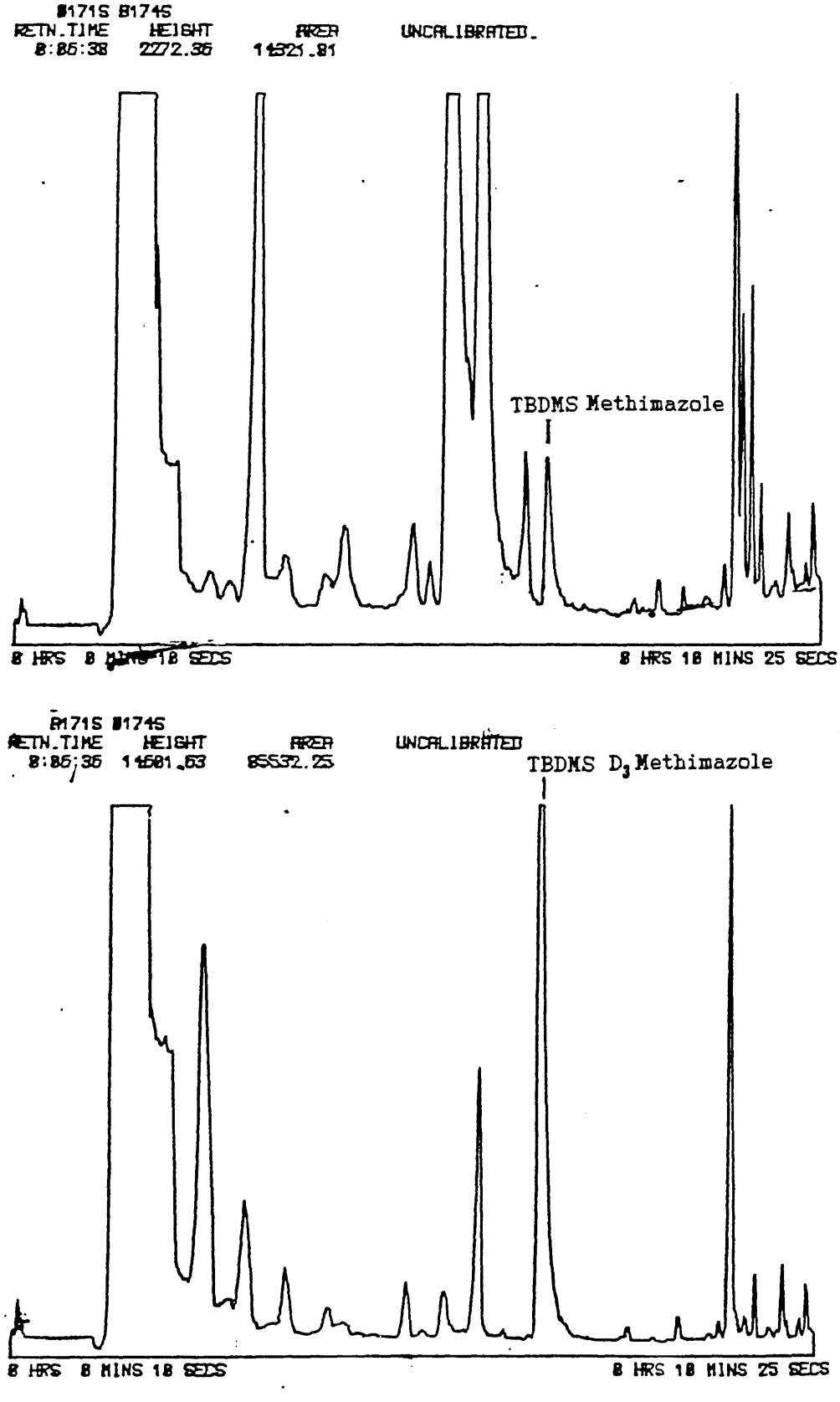


Figure 4.2 Typical chromatogram of methimazole in greyhound urine.

Table 4.2 Plasma concentrations of methimazole (ng/ml) in two greyhounds after the oral administration of 5 and 10mg doses

Time(hour)	5mg dose	10mg dose
0	0	0
1	306	223
2	298	546
3	145	495
4	190	492
5	98	-
6	-	100

Table 4.3 Urinary excretion of methimazole following oral administration of 10mg to a greyhound.

Urine Collection (hour)	Volume (ml)	Urine Concentration (ng/ml)	Amount Excreted (mg)	Percentage Dose
0		0	-	-
0-6	350	1343	0.470	4.7
6-24	505	620	0.313	3.1
0-24	855	1963	0.783	7.8
24-30	75	373	0.028	0.28
30-48	116	138	0.016	0.16
24-48	191	511	0.044	0.44

Table 4.4 Urinary excretion of methimazole following oral administration of 5mg to a greyhound.

Urine Collection (hour)	Volume (ml)	Urine Concentration (ng/ml)	Amount Excreted (mg)	Percentage Dose
0		0	-	-
0-6	112	1161	130	2.6
6-24	366	396	145	2.9
0-24	478	1557	275	5.5
24-30	754	-	-	-
30-48	196	-	-	-
24-48	950	-	-	-

4.3.2. 3-Methyl-2-Thiohydantoin

4.3.2.1. Total Ion Monitoring

A total ion mass spectrum of authentic 3-methyl-2-thiohydantoin was obtained using a stock solution (0.1mg/ml). It's mass spectrum shows an intense molecular ion at m/e 130 and a prominent ion at 102 corresponding to the expulsion of carbon monoxide (figure 4.3). The mass spectrum of 3-methyl-2-thiohydantoin is shown in figure 4.4. Table 4.5 shows the major peaks and their relative abundancies.

Greyhound urine (20ml) was extracted by the methimazole urine assay (section 3.5.3.2) with the same ratio of sample to solvent. If the metabolite 3-methyl-2-thiohydantoin was present it was hoped that by comparing the intensities of the molecular ions of methimazole (m/e 114) and 3-methyl-2-thiohydantion (m/e 130), a ratio versus time curve could be obtained. However, 3-methyl-2-thiohydantoin was only identified from the total ion scan of one sample, the 6-24hour collection after the 10mg dose administration. It's spectrum is shown in figure 4.5.

4.3.2.2 Selected Ion Monitoring

A stock solution of 3-methyl-2-thiohydantoin was derivatised using MTBSTFA reagent following the same procedure as given for methimazole (section 3.2.2). The mass spectrum of the TBDMS-derivative of 3-methyl-2-thiohydantoin is shown in figure (4.6). Table (4.5) shows the

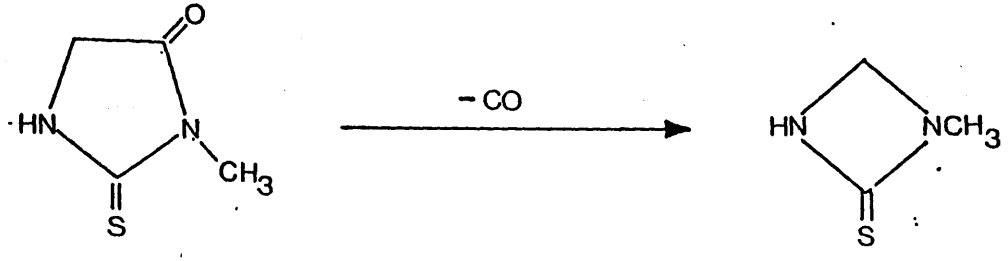


Figure 4.3 Expulsion of carbon monoxide from 3-methyl-2-thiohydantoin on electron impact in the ion source of the mass spectrometer.

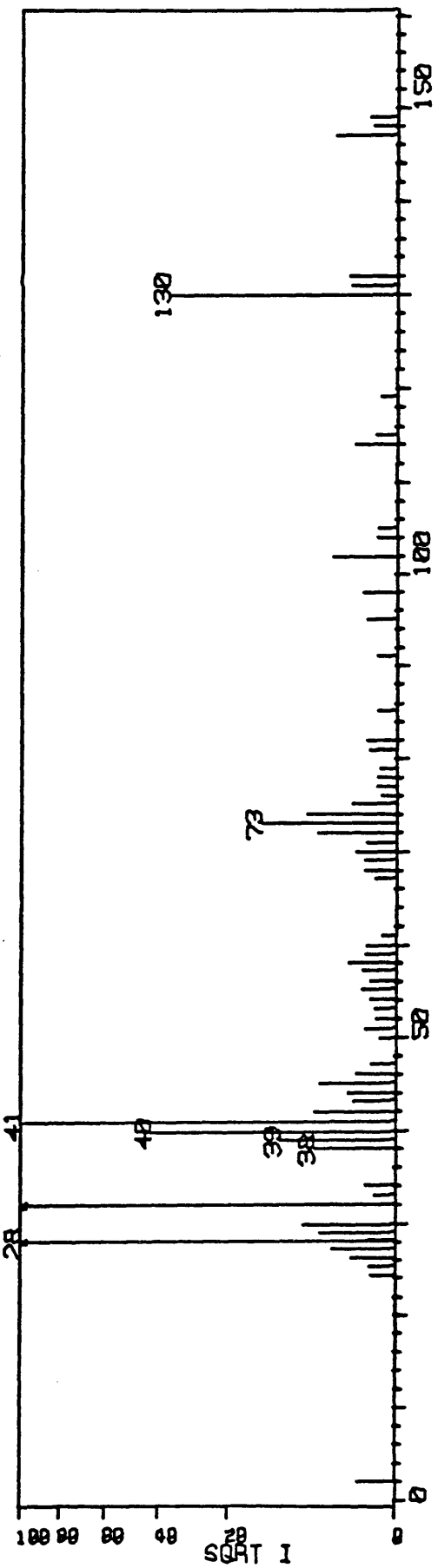
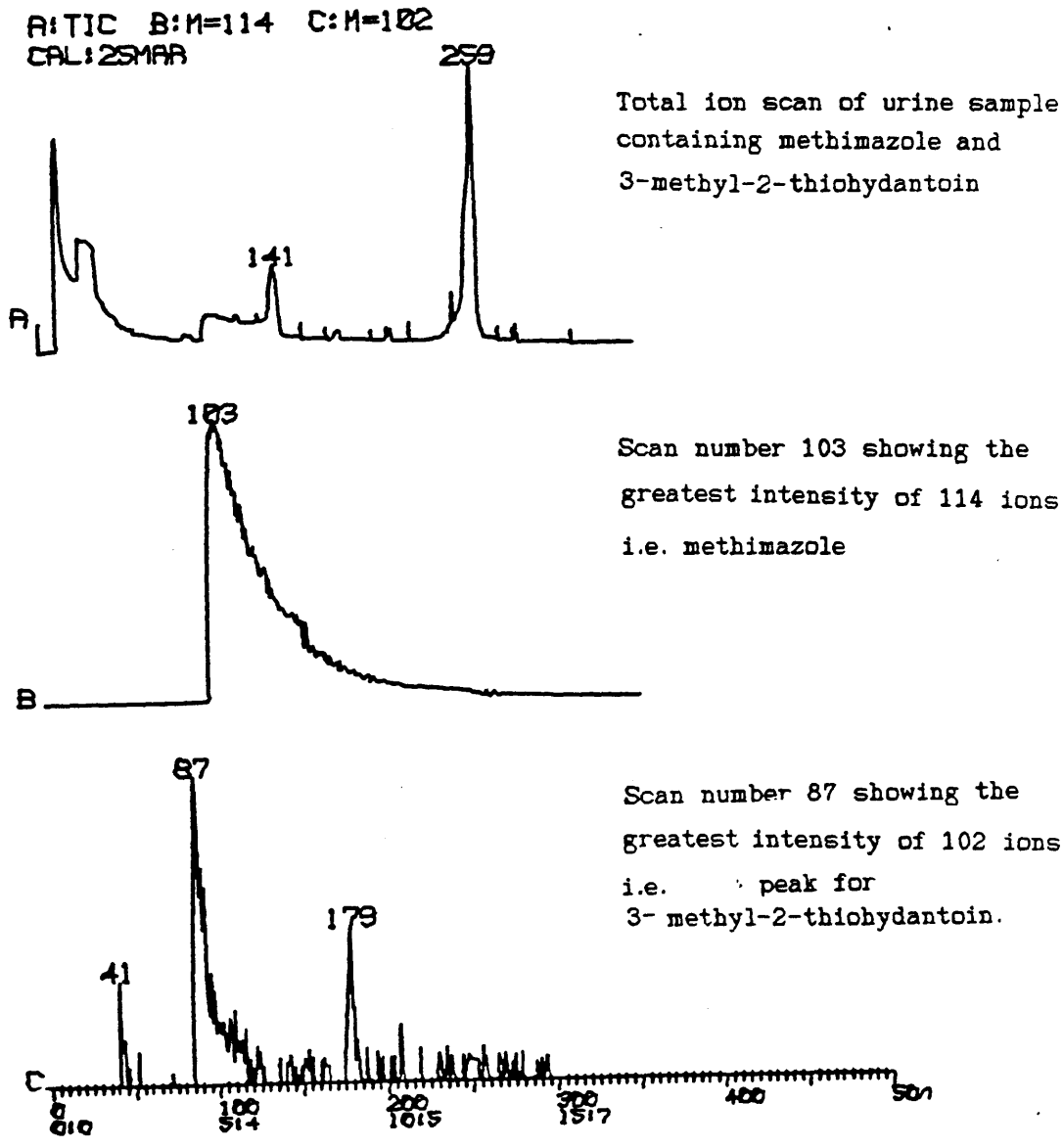


Figure 4.4 Mass fragmentation pattern of 3-methyl-2-thiohydantoin.



Total ion scan of urine sample containing methimazole and 3-methyl-2-thiohydantoin

Scan number 103 showing the greatest intensity of 114 ions i.e. methimazole

Scan number 87 showing the greatest intensity of 102 ions i.e. peak for 3- methyl-2-thiohydantoin.

Figure 4.5 Total ion scan of the 6-24 hour urine collection after oral administration of methimazole.

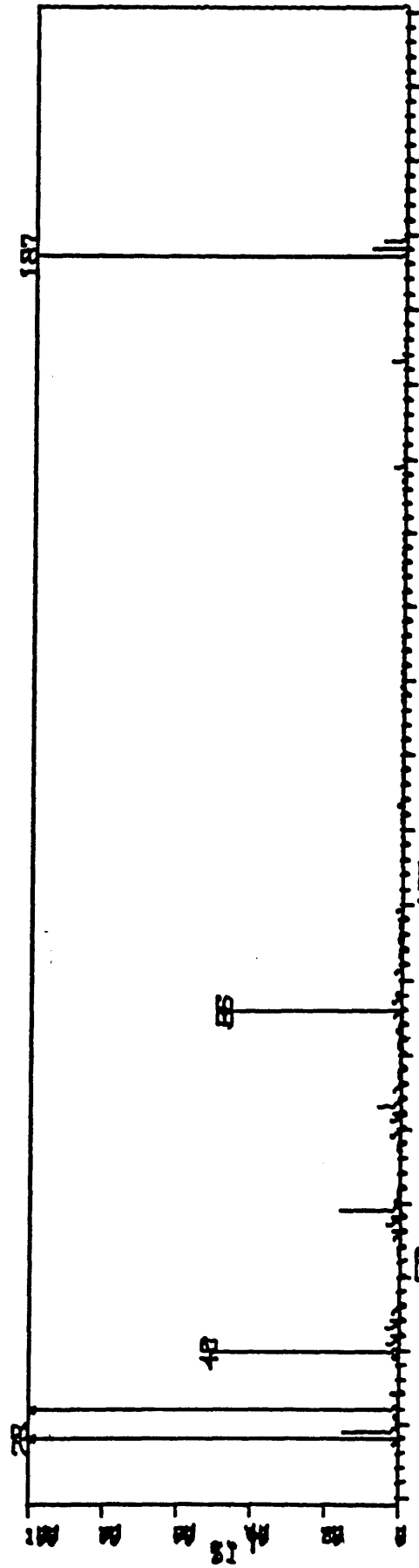


Figure 4.6 Mass fragmentation pattern of TBDMS-3-methyl-2-thiohydantoin.

Table 4.5 The major ions and their relative intensities of 3-methyl-2-thiohydantoin and it s TBDMS derivative.

3-Methyl-2-Thiohydantoin		TBDMS-Methylthiohydantoin	
Mass	Intensity	Mass	Intensity
131.2	0.4	229.1	2.8
130.1	3.7	189.1	5.9
102.1	0.4	188.1	9.1
75.2	0.5	187.1	100.0
74.1	0.8	86.0	46.0
73.1	1.8	59.0	16.0
69.1	0.5	57.2	3.7

major ion peaks and their relative adundancies. The major ion fragment appears at m/e 187. This corresponds to cleavage of the tert-butyl group and can be used in the application of selected ion monitoring (figure 4.7).

In order to have the ability of monitoring the TBDMS derivatives of methimazole, trideuterated methimazole and 3-methyl-2-thiohydantoin simultaneously; Perfluorokerosene (PFK) fragmentation ions C_3F_7 (m/e 168.9888 and C_5F_7 (m/e 192.9888) were used as reference ions

Channel 1 was set to monitor m/e 171.041

Channel 2 was set to monitor m/e 174.064

Channel 3 was set to monitor m/e 187.036

The rest of the GC-MS conditions were as described for TBDMS-methimazole (section 3.2.2).

A chromatogram for 3-methyl-2-thiohydantoin in urine is shown in Figure 4.8.

The limit of detection for stock solutions derivatised to TBDMS-3-methyl-2-thiohydantoin was 50ng/ml.

However, when extraction of 3-methyl-2-thiohydantoin was attempted from urine samples following the same procedure as for methimazole, recovery was very poor with a limit of detection of 0.5ug/ml. 3-methyl-2-thiohydantoin has a lower pKa value than methimazole because of the presence of a carboxyl group. Thus, at pH9, its ionisation may not be completely suppressed resulting in apoor extraction into organic solvent. Consequently, although chromatographic conditions suitable for the analyses of 3-methyl-2-thiohydantoin had been developed, the lack of

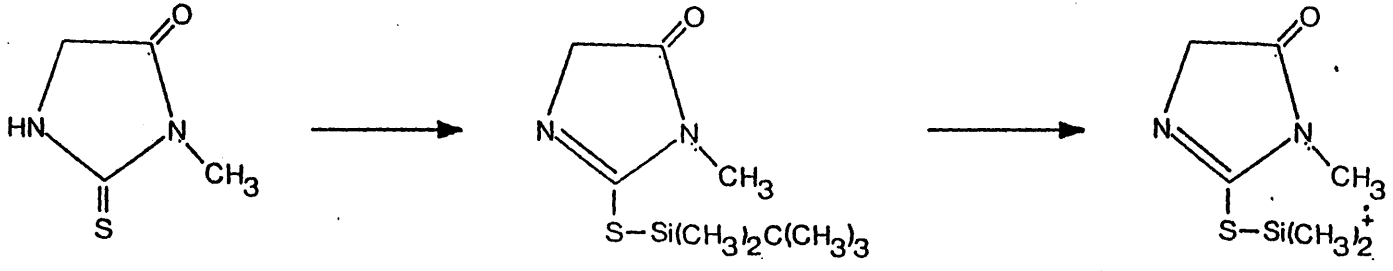


Figure 4.7 Derivatisation reaction of MTBSTFA with 3-methyl-2-thiohydantoin

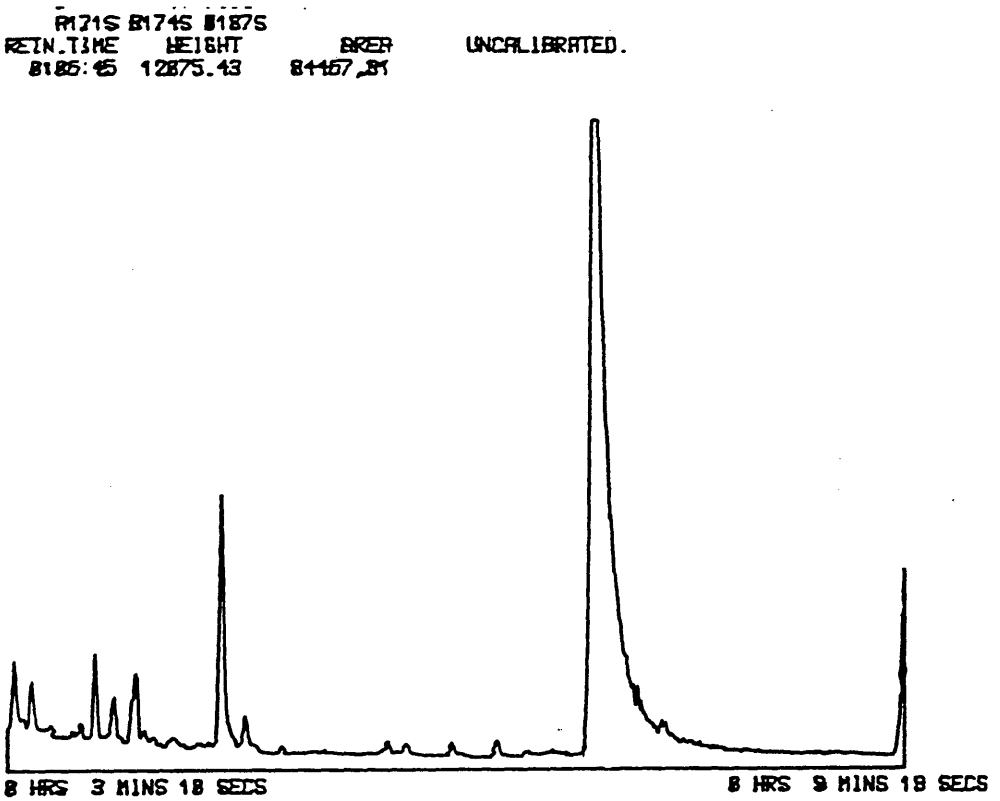


Figure 4.8 Typical chromatogram of 3-methyl-2-thiohydantoin in greyhound urine.

sufficient extraction recovery meant that the method was not of sufficient sensitivity to detect the levels present in one millilitre of urine. Therefore, the extraction was carried out using 20ml of urine, as was done for total ion monitoring. However, it was found in this case that 3-methyl-2-thiohydantoin was not fully resolved from endogenous urinary components and could not be accurately determined.

4.3.3. Methimazole Doping in the Racing Greyhound

4.3.3.1.Routine Extraction Procedure

Dilute ammonium hydroxide was added to urine (20ml) until the sample became alkaline. Ethyl acetate (40mls) was added to the sample. After shaking, the ethyl acetate layer was removed and dried with sodium sulphate. After filtration into an evaporating basin, the sample was placed on a steam bath and evaporated to dryness. The residue was reconstituted in a few drops of chloroform and spotted on two thin layer chromatography (TLC) plates. The plates were developed using the ascending technique in a glass chamber (Shandon TLC Chromotank) saturated with the solvent system ,chloroform:methanol (3:1). The solvent was run to a height of 15cm. The plates were allowed to air dry. Various chromogenic reagents were used to visualise the TLC plates. Both plates were exposed to hydrochloric acid fumes and then the first plate was sprayed with iodoplatinate solution to locate the

alkaloids and the second plate was sprayed with furfuraldehyde to locate the carbamates.

A brownish spot on was detected the first plate with an Rf x 100 value of 74. As the TLC plate showed a positive result, the urine residue was taken for further identification by total ion monitoring where it showed enhanced ion intensity at m/e 114, the molecular ion of methimazole.

In order to determine if this positive spot was methimazole the TLC system was repeated but this time the plate was spotted with the extracted urine sample and a sample of methimazole in chloroform. After spraying, the spot corresponding to methimazole had the same Rfx100 value as the unknown spot from the urine sample.

4.3.3.2. Selected Ion Monitoring

The initial urine sample was extracted and derivatised as described in section 3.5.3.2. The sample gave a value of 2ug/ml.

Any subsequent samples from the same dog were then analysed by this method for methimazole. The analysis results for the samples are given in Table 4.6.

Table 4.6 Methimazole concentrations found in urine samples of a racing greyhound.

Sample code	Methimazole concentration (ug/ml)
G84312	2.00
G84328	0.91
G85008 (pre-race 18.10)	1.06
G85009 (pre-race 21.50)	0.73
G85010 (post-race 22.50)	0.11
G85031	0.34
G85037	Negative
G85052	Negative

4.4 DISCUSSION

The amount of methimazole excreted unchanged in the urine of greyhounds confirmed that renal excretion is a minor pathway for the elimination of unchanged drug. In fact, the percentage levels are very similiar to those already found in man (Marchant 1979; Pittman et al., 1971; Okamura et al., 1986). Metabolism is the major route of elimination and one of the metabolites of methimazole already identified in rat and man, 3-methyl-2-thiohydantoin, was identified in greyhound urine in this study.

3-methyl-2-thiohydantoin is more polar and less lipophilic than methimazole and therefore would be expected to show very little reabsorption along the distal tubule of the kidney. However, the quantities of this metabolite were so small that it was only identifiable in one sample. Thus, it would appear that 3-methyl-2-thiohydantoin is, as with rat and man, only a minor metabolite perhaps because of further metabolism within the liver. The urine fraction in which 3-methyl-2-thiohydantoin was identified was 6-24 hours after a 10mg dose suggesting a longer half life than methimazole. In one human study, the half-life of 3-methyl-2-thiohydantoin was calculated to be 13.5 hours (Skellern et al., 1980a) i.e. about 2 to 3 times longer than methimazole. 3-methyl-2-thiohydantoin has been shown to have antithyroid activity in the rat (Searle et al., 1951), but this remains to be demonstrated in other species. However, this potentiality makes the metabolite worthy of kinetic studies. A plasma concentration time

curve for 3-methyl-2-thiohydantoin has been measured over 24 hours by HPLC (Skellern et al., 1980a). However, this method suffered interference from caffeine and theophylline and endogenous plasma material. The selected ion monitoring method described in this chapter allows chromatographic analysis of methimazole and 3-methyl-2-thiohydantoin simultaneously without interference. Thus, following refinement of the extraction procedure, this method could be used for kinetic studies on 3-methyl-2-thiohydantoin.

Obviously, there are further metabolites of methimazole present in the greyhound which have not been identified. Conjugation at the thiol functional group of methimazole is a strong possibility either by glucuronidation catalysed by UDP-glucuronyltransferase or by glycosidation involving UDP-glycosyltransferase.

The mass spectral characteristics of glucuronide conjugates have been studied (Feug et al., 1983; Billets et al., 1973; and Feuselau and Johnson (1980). The glucuronides usually have to be derivatised if molecular ions are to be detected in their mass spectra, and these are usually found in low abundance. Consequently the identity of the molecular ion in an unknown spectrum may not be immediately apparent. Thus, identification of glucuronides would be more easily undertaken by HPLC which offers many advantages when dealing with such polar compounds.

The amount of methimazole excreted in the urine samples sent from the racetrack was considerably higher than the levels found in the study conducted at The Wellcome Surgical Institute and therefore suggest that

a dose greater than 10mg had been administered to the greyhound before racing.

CHAPTER 5

METHIMAZOLE PHARMACOKINETICS AT DIFFERENT DOSE LEVELS AND DISEASE STATES

5.1 INTRODUCTION

5.1.1 Methimazole Pharmacokinetics in Different Dosage Regimens

The clinical use of antithyroid drugs has largely been governed by uncontrolled clinical observation. Doses at the higher end of the range produce an unacceptable incidence of hypothyroidism when used without thyroid hormone supplementation. Doses at the lower end of the range in use are effective in a majority of, but by no means all, patients.

One clinical test which has been used to estimate the duration of antithyroid effect of methimazole in patients throughout a dose is the perchlorate discharge test (McCruden et al., 1987).

Potassium perchlorate interferes with the uptake of iodide. The administration of this substance to a normal subject, therefore, interferes with the incorporation of further iodide into the thyroid but does not lead to significant discharge of iodide from the gland, since trapped iodide is organified very rapidly. Methimazole inhibits iodide organification but not uptake, therefore, patients undergoing methimazole treatment will show a significant discharge of iodide after the administration of potassium perchlorate and the extent of discharge is related to the degree of inhibition of organification .

Oral doses of methimazole above 20mg daily reduce the organification of thyroidal iodide to very small levels five hours after one of two divided daily doses (McCruden et al., 1981). The response below this dose is more variable. It is not clear whether this variability is due

to differences between patients in the levels of plasma methimazole attained. In animals the relationship between dose of antithyroid drug and plasma levels is non-linear (Marchant et al., 1978).

The basic goal of therapy with many drugs , is to achieve steady state blood and tissue levels of the drug that are both efficacious and non-toxic

Steady-state levels are characterised by the concentration-time profile reproducing itself between any given two doses. When equal maintenance doses are given at uniform time intervals, then steady-state levels would be characterised by the concentration-time profile for the entire 0 to 24 hour period reproducing itself each day. Thus, for a one compartment model total area under curve is given by

$$AUC = F.D/V.k \quad \text{(p.o. dose)}$$

i.e. AUC is directly proportional to dose, provided V and k do not change. Hence, fraction of AUC not seen in the interval is directly proportional to the fraction of dose carried over, f; i.e. AUC during a dose interval at steady state is the same as the total area under curve extrapolated to infinity for a single dose.

Generally, increasing the dose of a drug produces a proportional increase in the plasma concentration at all times. Hence, the time for the peak concentration remains unchanged but its magnitude increases proportionally with dose. The explanation is readily apparent. If the dose is doubled ; then, at any given time the amount absorbed is doubled

and with twice as much entering the body, twice as much is eliminated. Being the difference between the amount absorbed and that eliminated, the amount of drug in the body at any time is, therefore, also doubled and so too is the area under the curve.

This dosage linearity has been illustrated for methimazole in two studies (Jansson et al.,1983a and Okamura et al., 1986). Jansson showed that a 1.5 fold increase in dose from 10mg to 15mg produced a 1.5 fold increase in the mean area under the curve (AUC) from 1611 +/- 322ng.h/ml to 2454 +/- 495 ng.h/ml in healthy subjects. The later study by Okamura examined three dose levels 10, 15 and 30mg in hyperthyroid patients. Similarly to Jansson, the dose change from 10mg to 15mg gave a 1.48 fold increase in the mean AUC values (3228 +/- 1064ng.h/ml to 4773 +/- 1299ng.h/ml). However, although the 30mg dose gave a further increase in mean AUC (7136 +/- 718ng.h/ml) this only corresponded to a 1.5 and a 2.2 fold increase from the 15mg and 10mg mean AUC values, respectively. In fact, the peak plasma concentration values (Cmax) which would normally be more prone to deviations showed a better correlation to dose increases. The mean Cmax values for the 10, 15 and 30mg doses were 299 +/- 92 ng/ml, 380 +/- 64 ng/ml and 803 +/- 147 ng/ml respectively. These values correspond to a 1.3, 2.1 and 2.69 fold increase from the 10mg to the 15mg dose, and the 15mg and 10mg doses to the 30mg dose respectively.

Frequency of dosing is as important as level of dosing. For a drug of short to intermediate half-life (20 min - 8 hours), the major considerations are therapeutic index and convenience of dosing. A drug

with a high therapeutic index need only be administered once every one to three half-lives. A drug with a low therapeutic index must be given approximately every half-life. Again, by studying the steady-state concentration-time profile one can optimise the frequency of dosing.

Before pharmacokinetically interpreting a concentration-time profile, the data is often first fitted to a 'compartment model'

Pharmacokinetic models assume that results are decided by prior causes which may be inherited or environmental. Deviations and fluctuations are usually attributed to experimental error. Parameters such as volumes and rate constants, are assumed to remain constant over the entire observation period.

The schematic diagrams of linear pharmacokinetic models all have either first order or zero order input rate constants, and first order distribution and elimination rate constants. The solution diagrams of the differential equations of such linear compartmental systems can be described by poly-exponential equations, the number of terms of which are identical to the number of phases, thus

$$C = \sum_{i=1}^{n} Ci\ e^{\lambda_i t}$$

C = concentration at time t

C_i = the ith coefficient

λ_i= exponent of the ith exponential term

The 'method of residuals' is used to determine whether the data may be adequately described by a polyexponential equation. The difference between an observed and a calculated value is called a residual. This

difference when divided by an estimate of its error is termed a standardised residual, r

i.e. $r = y-f/s$

A digital computer may be programmed to perform non-linear least squares analysis of the data. A good criterion of how well the technique has been applied to a given set of data is to consider the sum of squared deviations and the errors on the fitted parameters (should be approximately 30% or less based on a Students t-value at the 5% probability level.)

Previous investigators of the pharmacokinetics of methimazole after oral administration have found that the data is adequately described by a one compartment model (Skellern et al.,1980a; Jansson et al., 1985)

A one-compartment model depicts the body as a single, kinetically homogenous unit. It is assumed that the rate of change in drug concentration in the plasma reflects quantitatively the change of drug concentration throughout the body.

5.1.2 Methimazole Pharmacokinetics in the Euthyroid and Hyperthyroid State

The disposition of many drugs may be altered in the hyperthyroid patient. Such alterations are of greatest importance for drugs with a

low therapeutic ratio, or in cases where a drug is being used to control the hyperthyroid condition itself or one of its manifestations.

Hormones have a central modulatory effect on the very fundamental molecular processes which govern various metabolic activities at both basic cellular and organ level. An excess of thyroid hormones may therefore produce profound and widespread physiological effects (Table 5.1).

The influence of hyperthyroidism on drug kinetics has been reviewed (Shenfield et al., 1981) and is summarised in Table 5.2.

Investigations into the effect of hyperthyroidism on methimazole pharmacokinetics was discussed earlier (section 1.3.5) and in general appear to indicate that there is no alteration in methimazole disposition in this disease state although one recent study has indicated otherwise (Hengstmann and Hohn, 1985).

By analysing the known physiological effects of hyperthyroidism presented in Table 5.1, we can identify the pharmacokinetic parameters which would reflect the changes, if any, in methimazole disposition.

Increased gastrointestinal function may result in an increased rate of absorption which would be reflected by a decreased tmax and increased Cmax value.

The increase in blood flow, renal and, especially in the case of methimazole, liver function could all have an effect of an increased rate of elimination. This would be shown by a lowering in the peak plasma concentration, Cmax; increased total body clearance, Cl; a larger

Table 5.1 The physiological effect of hyperthyroidism

Pharmacokinetic variable	Pathophysiological effect	Reference
Absorption	Stomach emptying and gastrointestinal motility increased, occasionally leading to malabsorption	Forfar et al. (1980); Miller et al. (1978); Thomas et al. (1973)
Liver function	Increased liver blood flow; increased microsomal enzyme activity; abnormal liver function, centrilobular necrosis occasionally	Eichelbaum (1976); Miller et al. (1978); Myers et al. (1950); Wells et al. (1983)
Renal function	Increased renal blood flow and glomerular filtration in some patients	Bradley et al. (1974)
Blood flow	Cardiac output increased; decreased output on exercise	Forfar et al. (1982); Sobel & Braunwald (1971)
Binding proteins	Albumin and plasma α_1-acid glycoprotein reduced	Kimberg (1971); Scott et al. (1984)

O'Connor and Feely, 1987

Table 5.2 The influence of hyperthyroidism on drug pharmacokinetics.

Parameter	Change	Drug[a]	Reference
Absorption			
Rate	↑	Paracetamol	Forfar et al. (1980)
	↑	Propranolol	Bell et al. (1977)
	↓	Riboflavin	Levy et al. (1972)
Amount	↓	? Digoxin	Lawrence et al. (1977)
			Watters & Tomkin (1975)
Distribution	↑	Propranolol (25%)	Wells et al. (1983)
	↑	Digoxin (variable results)	Shenfield (1977, 1981)
	↔	Phenytoin	Hansen et al. (1978)
Metabolism			
Oxidative	↑	Antipyrine (33%)	Shenfield (1981)
	↑	Propranolol (50%)	Feely et al. (1981b)
	↑	Metoprolol (50%)	Hallengren et al. (1982)
	↑	Tolbutamide	Kampman & Skovsted (1975)
	↑	Theophylline (20%)	Vozeh et al. (1984)
	↑	Warfarin ($t_{1/2}$ - single dose)[b]	McIntosh et al. (1970)
	↔	Warfarin ($t_{1/2}$ - long term therapy)[b]	Schrogie & Solomon (1967)
	↔	Phenytoin	Hansen et al. (1978)
	↔	Propylthiouracil	Kampman & Skovsted (1975)
	↔	Carbamazole	Cooper (1984)
	↔	Diazepam	Ochs et al. (1981)
Glucuronidation	↑	Paracetamol (24%)	Forfar et al. (1980)
	↑	Oxazepam (65%)	Scott et al. (1984)
Hormonal	↑	Cortisol ($t_{1/2}$ - 55%)[b]	Gallagher et al. (1972)
	↑	Thyroxine ($t_{1/2}$ - 34%)[b]	Ingbar & Frunhel (1958)
	↑	Triiodothyronine (118%)	Bianchi et al. (1978)
	↑	Insulin (45%)	Demetriadis et al. (1985)
Renal clearance	↑	Digoxin	Lawrence et al. (1977)
	↔	Digoxin	Bonsnes et al. (1978)
	↔	Atenolol	Doherty & Perrone (1986)
	↔	Sotalol	Shenfield et al. (1977)
	↔	Nadolol	Hallengren et al. (1982)
			Aro et al. (1982)
			Wilkinson & Burr (1984)
Binding	↓	Propranolol	Feely et al. (1981c)
	↓	Warfarin	Feely et al. (1981c)

a Percentage change relates to clearance unless specified

b Assessed by measurement of half-life ($t_{1/2}$)

Key ↑ = increase ↔ = no change ↓ = decrease

Shenfield et al., 1981

elimination rate constant, ke and consequently a smaller area under the plasma concentration curve from zero to infinity ($AUC_{0\rightarrow\infty}$)

Alterations in the concentration of the major binding proteins are often associated with alterations in the volume of distribution of a number of drugs. However, methimazole is not highly protein bound and thus an increase in the volume of distribution would not be expected in this particular case.

5.1.3 Aim Of Study

The aim of this study is to examine the dose-response relationships for methimazole and any variation in drug handling with thyroid status.

Twelve patients were divided into four groups designated as group 1 (patients 1,2,3 and 4), group 2 (patients 5 and 6), group 3 (patients 7, 8 and 9) and group 4 (patients 10,11 and 12). Groups 1 and 2 were administered 5mg d.b. of methimazole orally and groups 3 and 4 were administered 20mg d.b. of methimazole orally. Patients in groups 1 and 3 had reached chemical euthyroidism whereas patients in groups 2 and 4 had remained hyperthyroid. Organification of thyroidal iodide was estimated at various timepoints and plasma samples were taken regularly after oral administration. From a study of the results it is hoped to answer the following questions:-

i) Is there a correlation between plasma concentration and extent of organification of iodine?

ii) Can methimazole be given as a single daily dose which would

encourage greater patient compliance?

iii) Does methimazole disposition vary with thyroid status which might call for the dosage to be reassessed as the patient reaches chemically induced euthyroidism?

5.2. EXPERIMENTAL

5.2.1. Methods

Twelve female patients, all within the first three months of treatment, gave written informed consent to the study which had been approved by the ethical committee of the Western Infirmary, Glasgow.

Methimazole (Tapazole) was obtained from Eli Lilly, Indianapolis, USA through the hospital pharmacy and was given in one of two oral regimens: 5mg or 20mg twice daily. The dietary regime was fasting at the time of the plasma profiles until after the three hour sample. Samples for methimazole and perchlorate discharge measurements were taken after one of the twice daily doses. These doses were not changed in individual patients. Pre sample collection clinical and biochemical characteristics of the patients are shown in Table 5.3. T4 supplementation was added as required to avoid symptomatic hypothyroidism.

Serum Free T3, Free T4 and TSH were estimated using Amerlex RIA kits (Amersham U.K.). Some TSH levels, shown in brackets, were estimated using an immunoradiometric assay (IRMA).

A gamma-camera/data analysis system was used to monitor the thyroid uptake of ^{123}I-iodide for 30 minutes following intravenous adminstration of the tracer. Sodium perchlorate (300mg) was then administered intravenously. The discharge of tracer was followed for a further 30 minutes and the percentage discharge was calculated.

Table 5.3 The pre-study clinical indices for the twelve patients

Subject no.	Age	Thyroid status	Dose (mg) b.d.	T_3 (nmol/L)	T_4 (nmol/L)	TSH (mu/L)
1	30	EU	5	1.29	51	
2	32	EU	5			
3	31	EU	5	1.59	60	(10.7)
4	21	EU	5	1.30	150	(0.83)
5	52	HT	5	3.15	106	1.1
6	33	HT	5	2.58	162	1.0
7	34	EU	20	2.80	80	(0.17)
8		EU	20	1.47	153	(0.28)
9	24	EU	20	2.20	95	(0.08)
10	29	HT	20	2.75	121	1.0
11	19	HT	20	2.89	197	0.5
12	50	HT	20	3.59	162	0.9
Normal range				0.89-2.46	54-142	

Blood samples were collected into EDTA tubes pre-dose and at frequent time intervals up to 12 hours following drug administration. After centrifugation, the resultant plasma was stored in the Gardiner Institute, Department of Medicine, The Western Infirmary at -20°C. After transportation to the Department of Forensic Science and Medicine, Glasgow University; the samples remained stored at -20°C until assayed. The plasma samples were assayed for methimazole using the method described in section 3.5.3.1.

5.2.2 Assessment of Pharmacokinetic Parameters

Terminal log-linear points from four hours after administration were used to calculate the elimination rate constant, λz and the elimination half-life, t½; using the * MODFIT program. The area under the plasma concentration-time curves to the last data point (AUC) and the area under the first statistical moment curve (AUMC), were determined by a combined linear-logarithmic trapezoidal method using the *AUCDAT program. The area under the first moment curve (AUMC) is the area under the curve of a plot of the product of concentration and time versus time from zero to the last data point. The mean residence time (MRT) and the apparent oral clearances (Cl/F) were calculated by model independent methods as follows:-

$$MRT = \frac{AUMC^{SS}_{0\rightarrow\tau} + \tau\left(\frac{C_{\tau_{ss}}}{\lambda_z}\right)}{AUC^{ss}_{0\rightarrow\tau}} \quad ; \quad Cl/F = Dose/AUC$$

where τ = dosing interval

C_τ = concentration at the end of the dosing interval

The mean residence time in the body based on plasma concentrations is defined as the expected interval of time that a drug introduced into the plasma or central compartment spends in the central and peripheral compartments before irreversibly leaving the central compartment.

Peak concentration (Cmax) was defined as the highest value actually recorded, and the time to reach peak concentration (tmax) was obtained accordingly.

5.2.3. Statistical Analysis

The statistical significance of difference was assessed by the analysis of variance using the *ANOVA digital computer program.

* MODFIT, AUCDAT and ANOVA are pharmacokinetic programs developed at Beechams Pharmaceuticals, Harlow and are not available for use outside the company.

5.3 RESULTS

The plasma concentration-time profiles of methimazole at the two dose levels (5mg and 20mg b.d.) and between the two patient catagories (euthyroid and hyperthyroid) are presented in Tables 5.4 to 5.7 and in linear and log-linear graphic form in Figures 5.1 to 5.4, respectively.

Typical chromatograms for the two dose levels are given in Figures 5.5 and 5.6, respectively.

The individual pharmacokinetic parameters of methimazole for the twelve patients are given in Tables 5.8 to 5.11, respectively.

The Cmax values for methimazole increased with increasing dose levels in both thyroid status groups. The mean Cmax values of methimazole following the 5mg dose were 198 and 194.5ng/ml in euthyroid and hyperthyroid patients respectively. The mean Cmax values of methimazole following the 20mg dose were 627 and 311ng/ml in euthyroid and hyperthyroid patients respectively.

The area under the plasma concentration-time curve to infinty ($AUC_{0\rightarrow\infty}$) for methimazole increased with increasing dose levels in both thyroid status groups. The mean AUC values of methimazole following the 5mg dose were 501 and 564ng.h/ml in euthyroid and hyperthyroid patients respectively. The mean AUC values of methimazole following the 20mg dose were 3395 and 1283ng.h/ml in euthyroid and hyperthyroid patients respectively. After dose normalisation, there was no significant

Table 5.4 The plasma concentrations following oral administration of methimazole for group 1.

Time (hr)	Subject 1 (ng/ml)	Subject 2 (ng/ml)	Subject 3 (ng/ml)	Subject 4 (ng/ml)
0.0	29			
0.5	119	225	17	46
1.0	184	363	200	N.S.
1.5	79	284	50	38
2.0	84	79	44	26
3.0	90	110	38	22
4.0	79	82	30	20
5.0	60	78	37	15
6.0	34	100	26	22
7.0	33	69	20	3
8.0	26	50	60	

Table 5.5 The plasma concentrations following oral administration of methimazole for group 2.

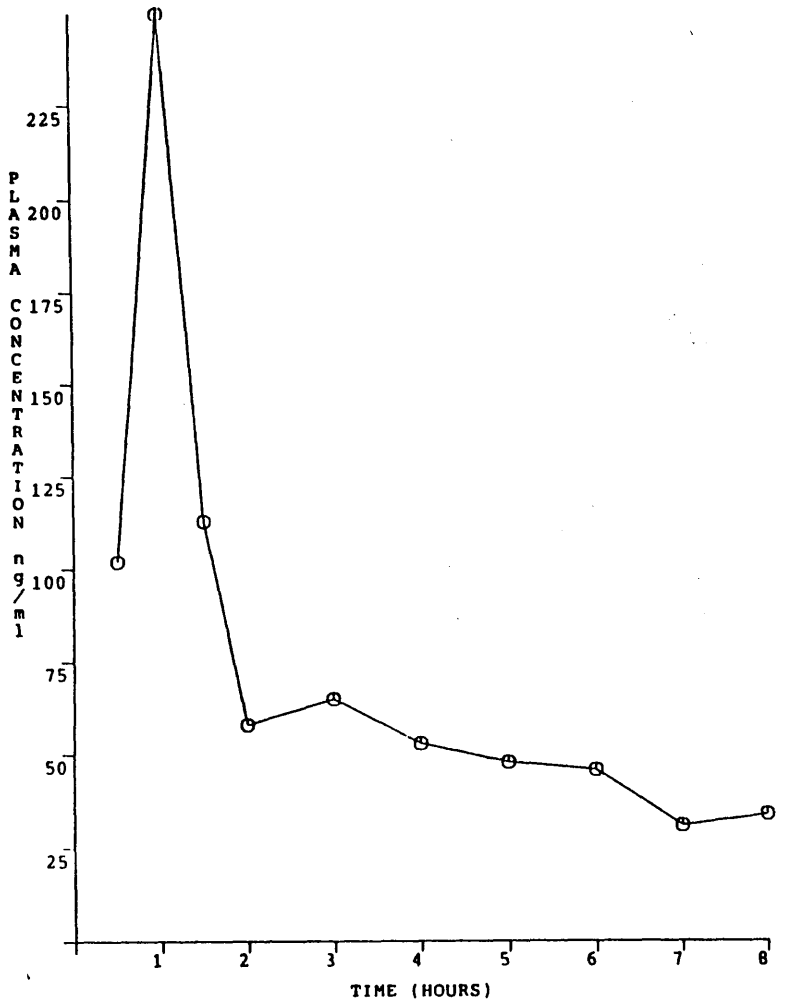
Time (hr)	Subject 5 (ng/ml)	Subject 6 (ng/ml)
0.0	24	5
0.5	92	56
1.0	123	158
1.5	121	266
2.0	82	56
3.0	76	93
4.0	44	67
5.0	54	64
6.0	43	42
7.0	39	42
8.0	87	45

Table 5.6 The plasma concentrations following oral administration of methimazole for group 3

Time (hr)	Subject 7 (ng/ml)	Subject 8 (ng/ml)	Subject 9 (ng/ml)
0.0	33	97	112
0.5	438	583	580
1.0	672	617	332
1.5	575	629	300
2.0	545	587	164
3.0	377	495	305
4.0	414	458	278
5.0	338	409	184
6.0	243	265	171
7.0	209	261	128
8.0	92	245	115
13.5	N.S.	79	N.S.
24.0	N.S.	N.S.	52

Table 5.7 The plasma concentrations following oral administration of methimazole for group 4.

Time (hr)	Subject 10 (ng/ml)	Subject 11 (ng/ml)	Subject 12 (ng/ml)
0.0	18	110	22
0.5	N.S.	N.S.	102
1.0	190	N.S.	157
1.5	289	488	136
2.0	187	442	N.S.
2.5	N.S.	366	N.S.
3.0	N.S.	257	N.S.
3.5	N.S.	361	N.S.
4.0	113	262	N.S.
4.5	N.S.	254	N.S.
5.0	58	N.S.	66
5.5	N.S.	243	N.S.
6.0	36	N.S.	72
6.5	N.S.	226	N.S.
7.0	24	N.S.	56
7.5	N.S.	176	N.S.
8.0	20	148	47
9.0	N.S.	N.S.	30
12.0	15	N.S.	N.S.



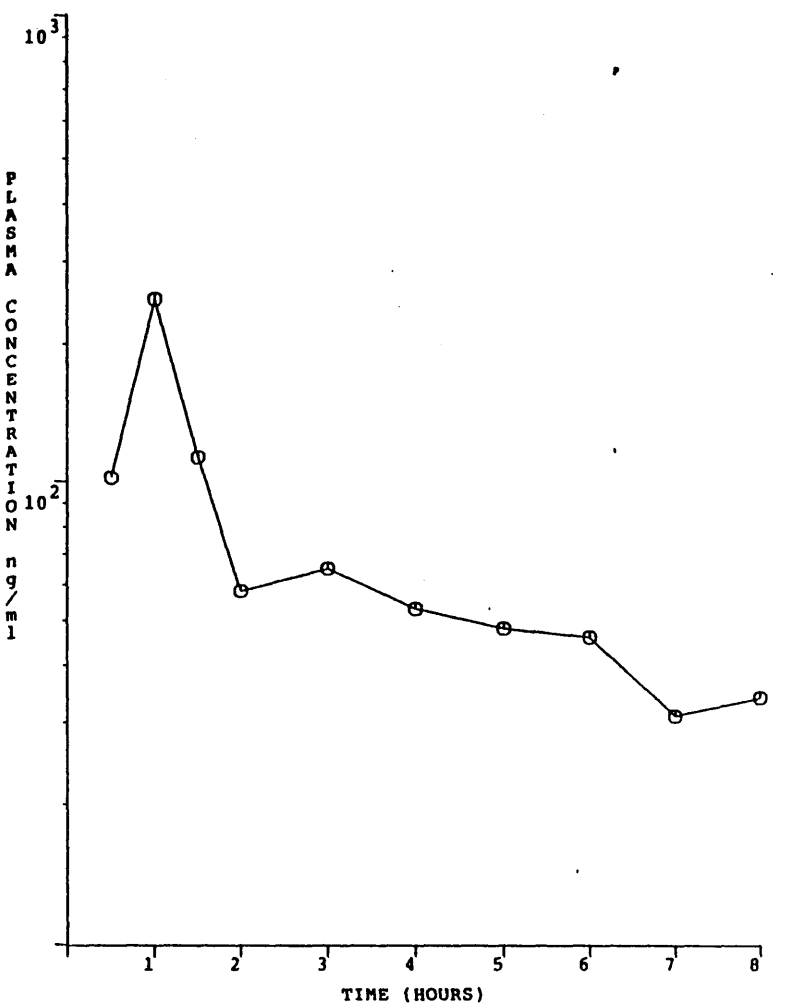


Figure 5.1 Mean linear and log-linear plasma concentration versus time curves following oral administration of 5mg of methimazole to four euthyroid patients

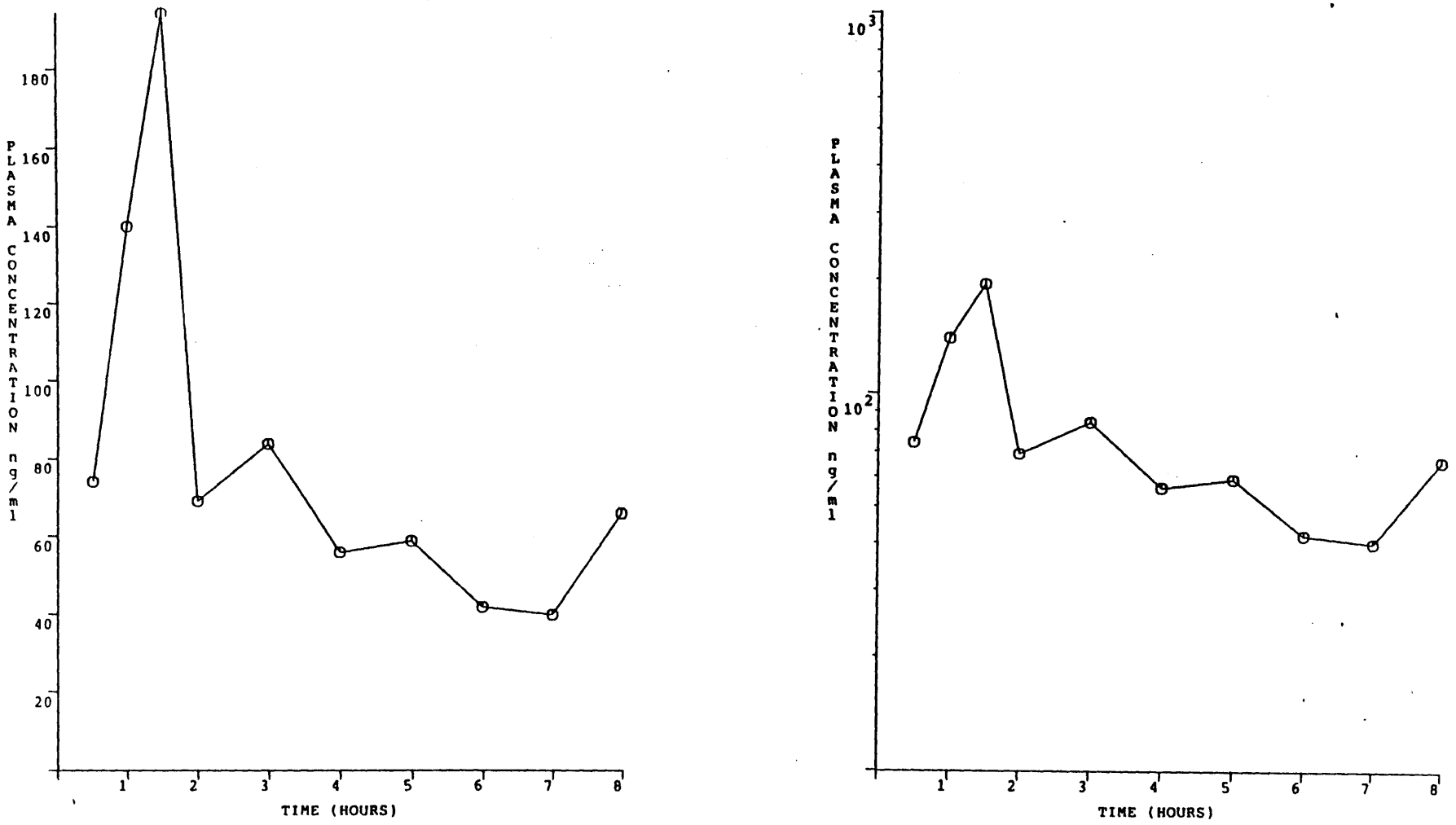


Figure 5.2 Mean linear and log-linear plasma concentration versus time curves following oral administration of 5mg of methimazole to two hyperthyroid patients

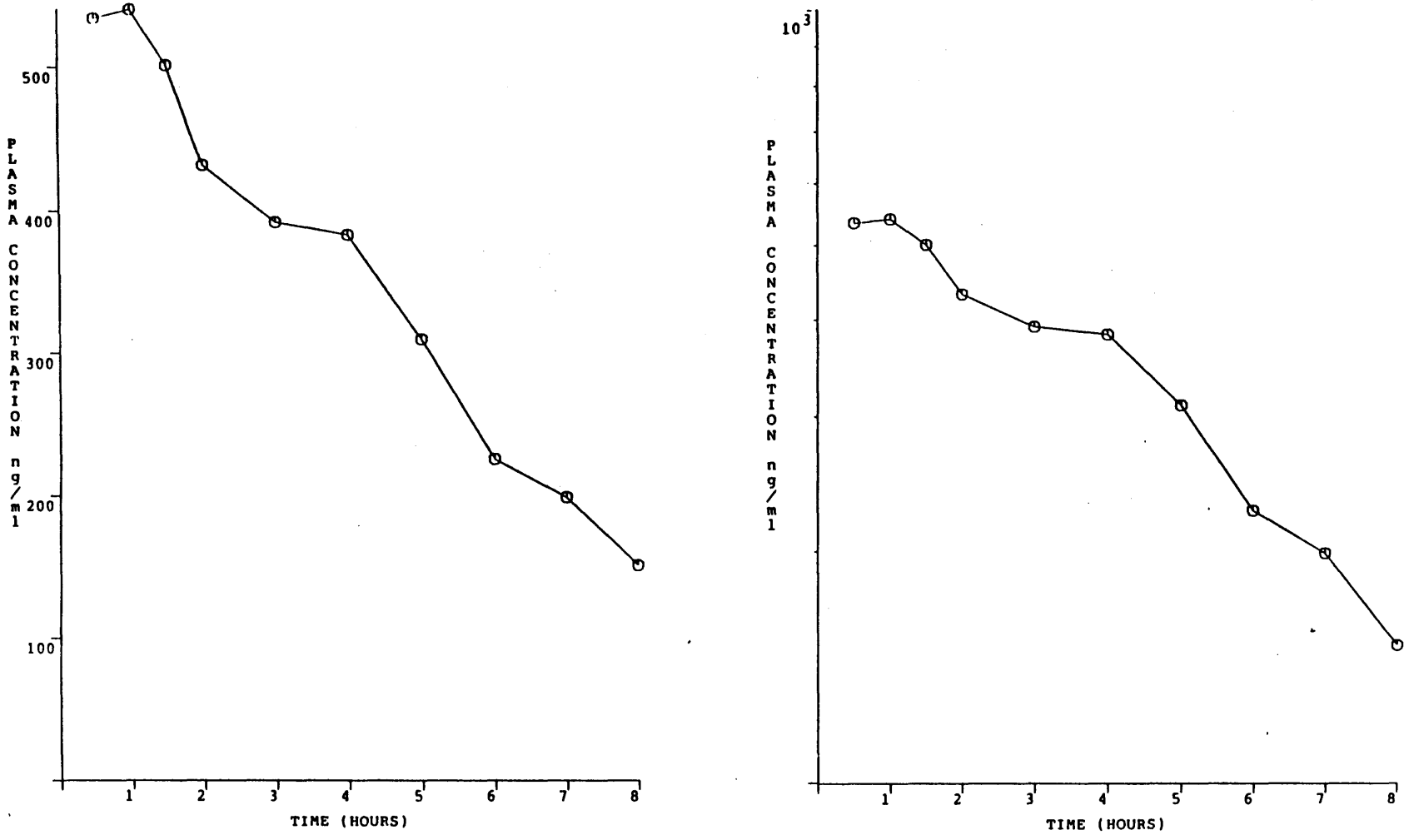


Figure 5.3 Mean linear and log-linear plasma concentration versus time curves following oral administration of 20mg of methimazole to three euthyroid patients

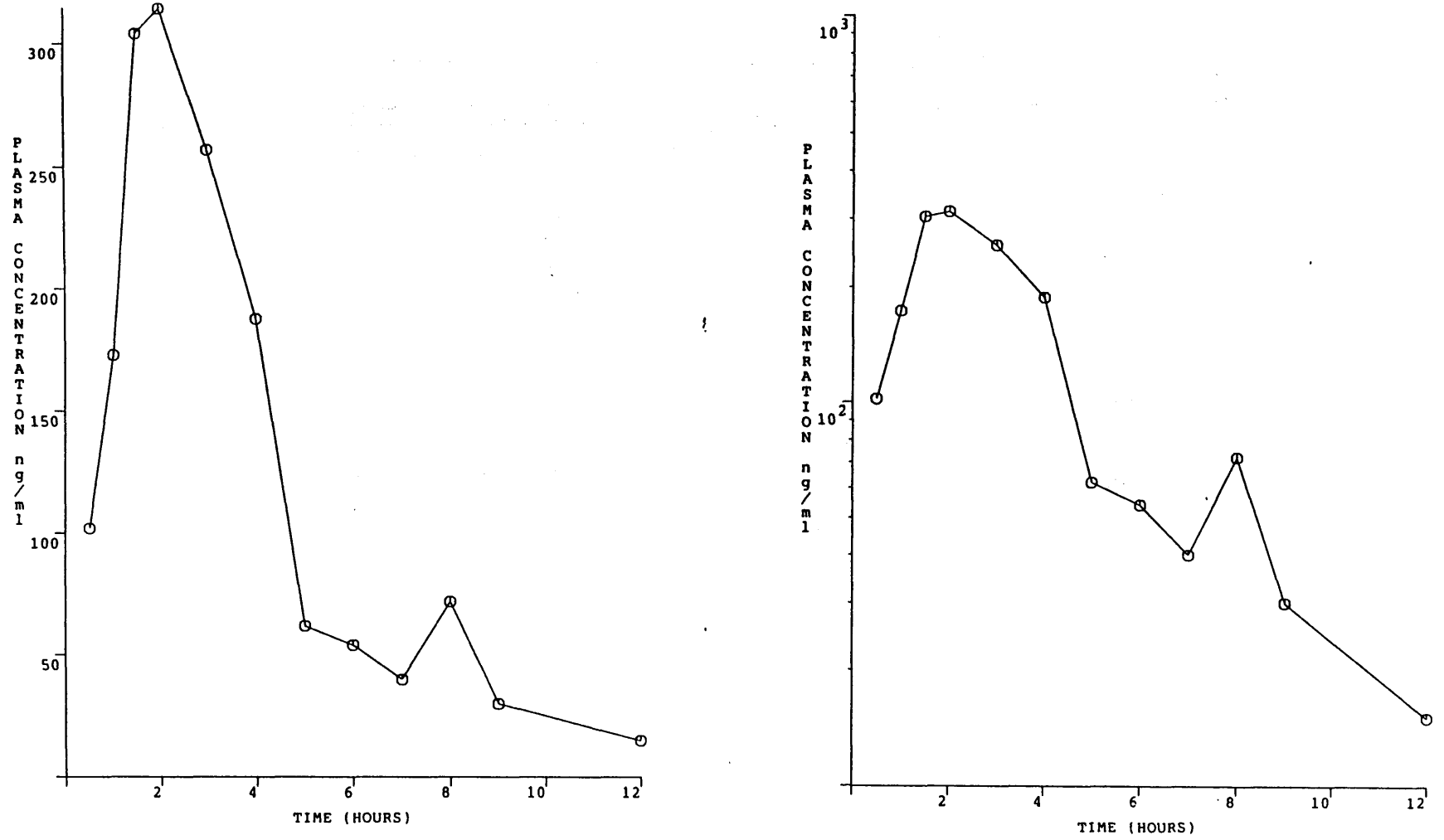


Figure 5.4 Mean linear and log-linear plasma concentration versus time curves following oral administration of 20mg of methimazole to three hyperthyroid patients.

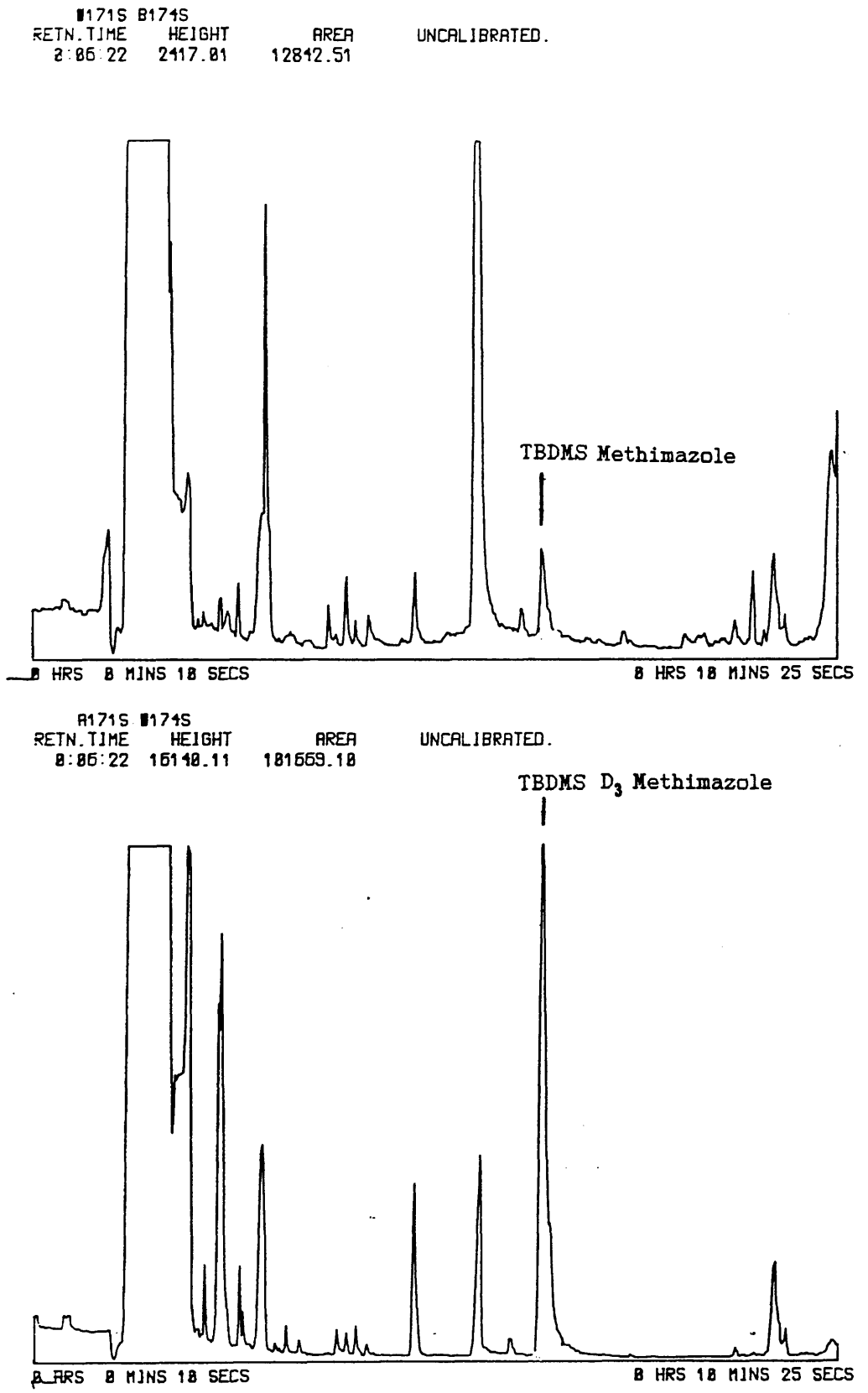


Figure 5.5 Typical chromatogram of methimazole in human plasma following oral administration of 5mg of methimazole.

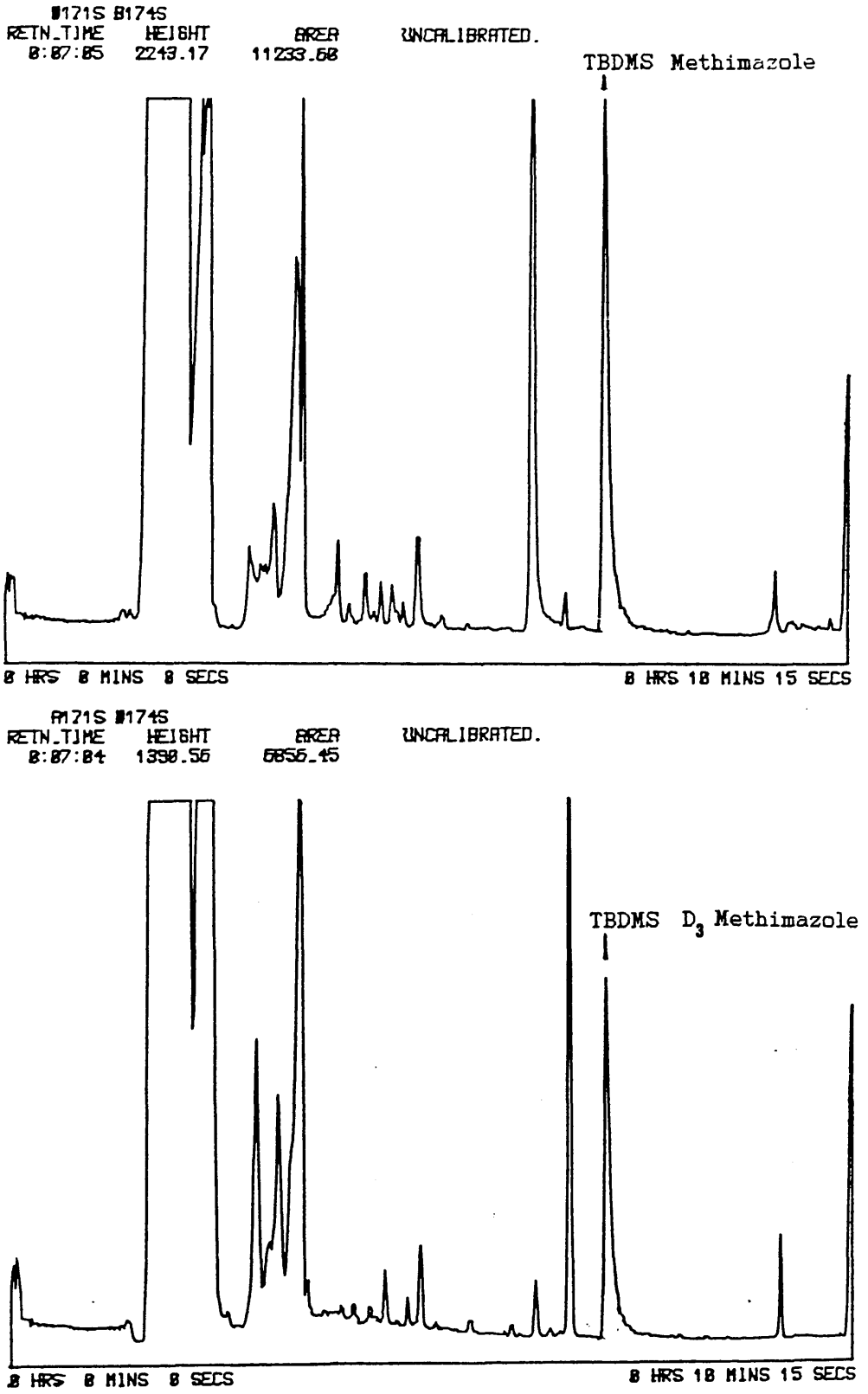


Figure 5.6 Typical chromatogram of methimazole in human plasma following oral administration of 20mg of methimazole.

Table 5.8 The non-compartmental pharmacokinetic parameters for group 1 following oral administration of methimazole

Subject no.	Cmax (ng/ml)	tmax (hr)	AUC (ng.h/ml)	AUMC ($ng.h^2/ml$)	MRT (hr)	λ_z (h^{-1})	$t_{1/2}$ (hr)	CL/F (ml/min)
1	184	1.0	565	1731	5.10	0.3025	2.29	147
2	363	1.0	944	2899	3.07	0.1084	6.39	88
3	200	1.0	335	1129	3.37	0.1406	4.93	249
4	46	0.5	159	446	2.80	0.4837	1.43	524
Mean	198	0.875	501	1551	3.58	0.2588	3.76	252
+/- S.D.	130	0.25	339	1041	1.04	0.1723	2.3	193
CV%	65.5	28.6	68	67	29	44.3	61.2	77

Table 5.9 The non-compartmental pharmacokinetic parameters for group 2 following oral administration of methimazole.

Subject no.	Cmax (ng/ml)	tmax (hr)	AUC (ng.h/ml)	AUMC ($ng.h^2/ml$)	MRT (hr)	λz (h^{-1})	t½ (hr)	CL/F (ml/min)
5	123	1.0	529	1819	9.77	0.0860	8.06	158
6	266	1.5	598	1947	3.63	0.2660	2.61	139
Mean	194.5	1.25	564	1883	6.70	0.176	5.34	148
+/- S.D.	101	0.35	49	91	4.34	0.127	3.85	13
CV%	52.0	28.3	9	5	65	72.3	72.2	9

Table 5.10 The non-compartmental pharmacokinetic parameters for group 3 following oral administration of methimazole.

Subject no.	Cmax (ng/ml)	tmax (hr)	AUC (ng.h/ml)	AUMC ($ng.h^2/ml$)	MRT (hr)	λz (h^{-1})	t½ (hr)	CL/F (ml/min)
7	672	1.0	2868	9425	3.71	0.3249	2.13	116
8	629	1.5	4189	19826	6.22	0.1868	3.71	80
9	580	0.5	3129	25101	13.25	0.0821	8.45	107
Mean	627	1.0	3395	18117	7.73	0.1979	4.76	101
+/- S.D.	46	0.5	700	7976	4.95	0.1218	3.29	19
CV%	7.3	50	21	44	64	61.5	69.2	19

Table 5.11 The non-compartmental pharmacokinetic parameters for group 4 following oral administration of methimazole.

Subject no	Cmax (ng/ml)	tmax (hr)	AUC (ng.h/ml)	AUMC ($ng.h^2/ml$)	MRT (hr)	λz (h^{-1})	t½ (hr)	CL/F (ml/min)
10	289	1.5	884	2941	3.88	0.4399	1.58	377
11	488	1.5	2236	8122	8.61	0.1185	5.85	149
12	157	1.0	730	2588	5.58	0.1773	3.91	457
Mean	311	1.33	1283	4550	6.02	0.2452	3.78	328
+/- S.D.	167	0.29	829	3098	2.40	0.1711	2.14	160
CV%	53.5	21.7	65	68	40	69.8	56.6	49

difference between dose levels (0.958, P= 0.05) or between euthyroid and hyperthyroid patients (0.184, P = 0.05).

There were no consistent changes in the terminal half-lives (t½) of methimazole for either group at either dose level. The mean values of t½ of methimazole following the 5mg dose were 3.76h (range 1.43 - 6.39h) and 5.34h (range 2.61 - 8.06h) in euthyroid and hyperthyroid patients respectively. The mean values of t½ of methimazole following the 20mg dose were 4.76h (range 2.12 - 8.45) and 3.78h (range 1.58 - 5.85h) in euthyroid and hyperthyroid patients respectively.

There were no consistent changes in the oral clearance (Cl/F) of methimazole for either patient group at either dose level. The mean Cl/F values of methimazole following the 5mg dose were 252 ml/min (range 88 - 524 ml/min) and 148 ml/min (range 139 - 158 ml/min) for euthyroid and hyperthyroid patients, respectively. The mean Cl/F values of methimazole following the 20mg dose were 101 ml/min (range 80 - 116 ml/min) and 328 ml/min (range 149 - 377 ml/min) for euthyroid and hyperthyroid patients respectively.

The tmax values of methimazole remain very similar between all categories. The mean tmax values of methimazole following the oral administration of 5mg were 0.875h (range 0.5 - 1.0h) and 1.25h (range 1.0 - 1.5 h) for euthyroid and hyperthyroid patients respectively. The mean tmax values of methimazole following the oral administration of 20mg were 1h (range 0.5 - 1.5h) and 1.33h (range 0.5 - 1.5h) for euthyroid and hyperthyroid patients respectively.

The sodium perchlorate discharge results along with methimazole concentration found at the time of discharge are presented in Table 5.12. and Figure 5.7 and represent the change in percentage perchlorate discharge with time and the relationship between percentage perchlorate discharge and plasma concentration respectively.

Table 5.12 Comparison of the perchlorate discharge results and the plasma concentration at the nearest equivalent timepoint following oral administration of methimazole

Patient	Dose (mg)	Thyroid status	ClO_4 Discharge (%)	Interval from MMI dose (h)	Cp at the nearest timepoint (ng/ml)
1	5	EU	75.5	5.0	60
2	5	EU	27.0	24.5	0 (12h)
3	5	EU	0.0	25.0	0 (12h)
4	5	EU	79.7	13.0	0 (12h)
5	5	HT	80.1	3.1	76
6	5	HT	81.8	2.5	64
7	20	EU	7.5	24.0	N.S.
8	20	EU	85.4	23.0	79
9	20	EU	30.9	25.0	52 (24h)
10	20	HT	69.2	5.0	58
11	20	HT	76.8	13.0	110 (12h)
12	20	HT	78.2	5.5	66 (5h)
					72 (6h)

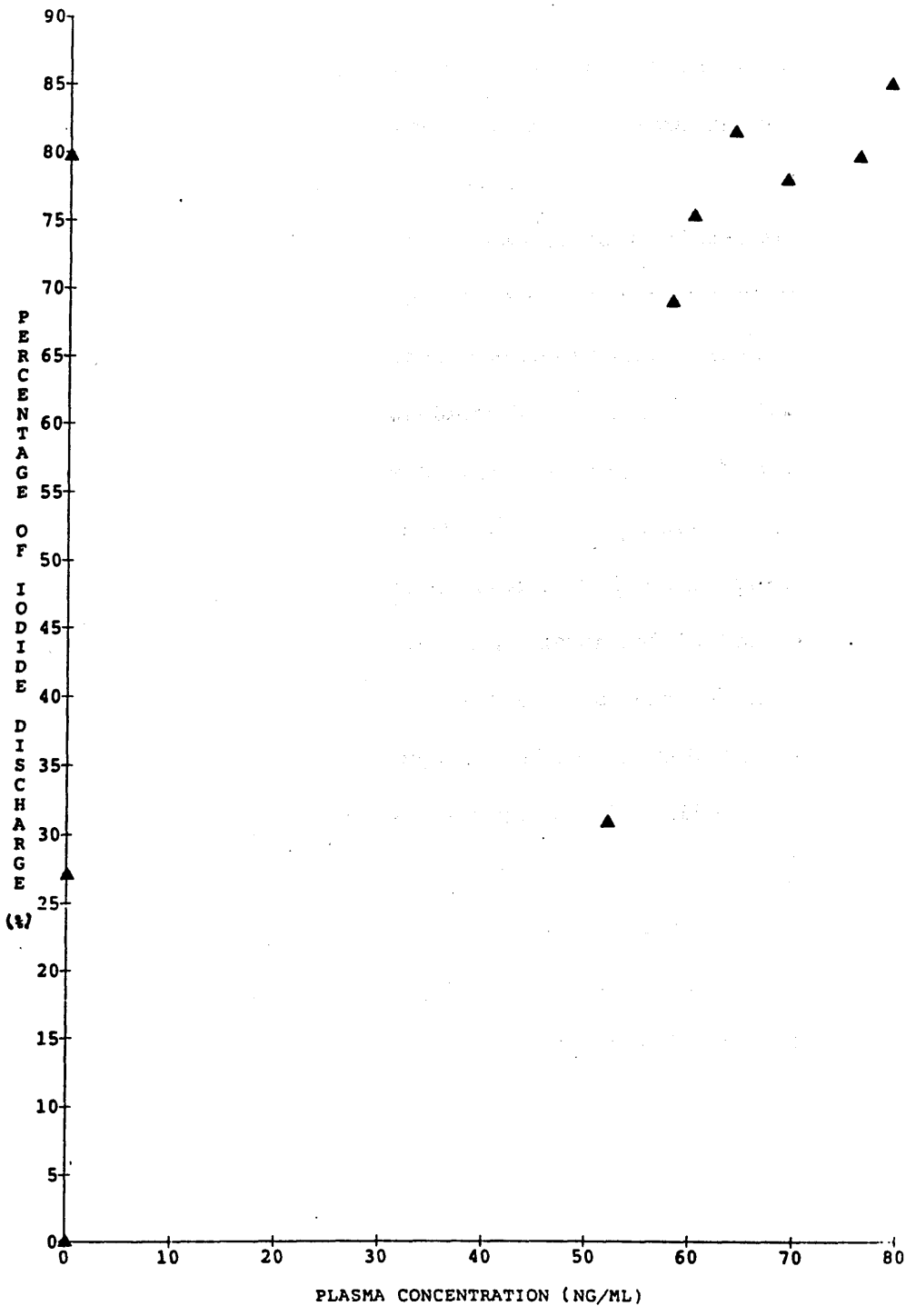


Figure 5.7 Plasma concentration versus percentage iodide discharge.

5.4 DISCUSSION

All patients had been receiving the methimazole treatment for more than one month when the samples were collected. Thus, at this stage in the treatment, with such a dosage regimen, all patients were expected to be achieving steady-state plasma levels.

As mentioned in section 5.1. the plasma concentration-time curve of methimazole after oral administration is normally adequately described by a one compartment model. Thus, the methimazole concentrations after the oral doses were analysed by least-square non-linear regression. However, the one compartment model gave unacceptably high sum of squared deviations (>30%) for the generated parameters , therefore, the pharmacokinetic parameters were derived from the post-peak log-linear points and the appropriate equations as described in section 5.2.2. From studying the plots of log C vs time, the terminal elimination phase was measured from four hours and this timepoint selection is in agreement with previous studies (Jansson et al., 1985). To gain an adequate estimate of the slope of the terminal phase of a plasma concentration-time curve, it is necessary to follow its course for at least three half-lives. As the patients are being dosed twice daily, the sampling for each dose ends at eight hours. However the zero hour time point is equivalent to the twelve hour timepoint from the previous dose. At steady state, for every dose, the concentration at each timepoint should remain the same. This theory can be illustrated by subject ten who was sampled at two consecutive twelve hour timepoints and gave

levels of 18 and 15ng/ml respectively (the difference in levels being equivalent to the standard deviation of the assay).

As no intravenous administration data was obtained for this study, the bioavailability of methimazole could not be calculated. Therefore, calculation of absorption and distribution kinetics was not justified.

Pharmacokinetic parameters of methimazole following oral administration have been described in several papers and the results for terminal half-life (t½), oral clearance (Cl) and area under the curve (AUC) are summarised in Table 5.13. In the present study, there is a high degree of interindividual variation within each group for all the measured parameters except tmax which would be unlikely to change as methimazole is known to be rapidly absorbed. These variations could be caused by several factors e.g. the wide age range within the patients and perhaps differences in liver function (diagnostic enzymolgy results such as serum bilirubin and transaminases levels were not detailed but none of the patients were reported to have abnormal liver conditions). Such patient variations would particularly affect plasma half-life and systemic clearance. However, the mean values show a good agreement with the studies summarised in table 5.13.

Peak plasma methimazole levels occurred after about 0.5-1.5h, with a second smaller peak occurring in 50 % of the subjects after 3 h. Similiar observations have been made with carbimazole (Skellern et al., 1976) where it was postulated that the second peak was due to slow carbimazole hydrolysis to methimazole in the small intestine, with subsequent absorption of methimazole. However, this is unlikely as the

Table 5.13 Pharmacokinetic parameters following methimazole administration.

Study	Subject no.	Dose	CL (ml/min)	AUC (ng.h/ml)	Method
Skellern et al (1980)	15HT	60mg CBZ	171.7 +/-188		HPLC
Jansson et al (1985)	7EU	10mg MMI		1661 +/- 322	GC-MS
	8HT	15mg MMI	106.7	2422 +/- 509	
	EU		105.2	2454 +/- 295	
Hengstmann and Hohn (1985)	3HT	40mg MMI	140 +/-9	4763 +/- 300	HPLC
	2EU				
Okamura et al (1986)	[1]5EU	10mg MMI	63.8 +/- 11.6	2657 +/- 544	HPLC
	15HT		56.2 +/- 18.7	3228 +/- 1064	
	7HT	15mg MMI	56.8 +/- 10.3	4773 +/- 1299	
	6HT	30mg MMI	76.7 +/- 16.4	7136 +/- 718	

[1] EU = normal

conversion of carbimazole to methimazole occurs very rapidly (Nakashima et al., 1979; Skellern et al.,1980a and Jansson et al., 1983a). Also, this phenomenon was noted after methimazole administration (Cooper et al., 1984) whch cast doubt on this hypothesis although no alternative suggestion was offered. A possible explanation for the second peak is that it is being caused by enterohepatic recirculation of methimazole. Once excreted into the bile, a drug may be reabsorbed from the intestinal tract. As discussed in section 1.3.3.2., it seems likely from radioactive studies that enterohepatic circulation occurs for methimazole and/or its metabolites. Normally the levels reabsorbed by this cycle would not significantly affect the plasma concentration-time profile. However, the gall-bladder can store up to 60mls of bile. After the intake of food, this bile is released under the influence of vagus nerve stimulation and the compound cholecystokinin released by the duodenum. Thus, the plasma concentration could be raised shortly after meals and the second peak could be a result of this phenomenon. As discussed in section 1.3.3.2., enterohepatic recirculation can be tested by bile cannulation, but obviously this technique is only suitable for animal studies. Various methods have been suggested for human studies. The bile can be removed from the body by a t-tube set-up and any alteration in the plasma concentration profile noted. However this technique introduces a new compartment and thus can itself alter the pharmacokinetics. Antibiotics such as neomicin and oxytetracycline can be co-administered with the drug under investigation. They kill the gut flora thus the conjugated drug is not broken down to the drug and

reabsorbed and therefore the second peak is no longer present. However, an unpleasant side-effect of killing gut flora is to cause diarrhoea in the subject. Activated charcoal can be co-adminstered with the drug. It acts like a sponge for the drug which is consequently not available for biliary excretion. Finally, it has been suggested that the natural bile secretion stimulator, cholecystokinin, be administered at certain intervals during the plasma concentration time profile. If a secondary peak corresponds to the cholecystokinin administration then enterohepatic recirculation can be said to be occurring. This test would be the most definitive, however, it is difficult to judge at what dosage level the cholecystokinin should be given and, as it has shown some toxicity this method is not favoured.

In order to study the linearity of concentration between a low and high dose, mean Cmax and AUC for groups 1 and 2 were compared with those for groups 3 and 4. For euthyroid patients there was a 3.2 fold increase in Cmax and a 5.0 fold increase in AUC between doses and for hyperthyroid patients, the same parameters showed a 1.6 fold and a 1.5 fold increase , respectively.

Although all comparisons gave a definitive increase, none gave the 4 fold increase which should be expected. However, as shown in the results section, there was no significant difference after dose normalisation suggesting linearity and as mentioned in the section 5.1.1, the postulated and calculated values have failed to agree in a similar study (Okamura et al., 1986). Also, a more accurate insight would have been gained in this study if the same patients had received the two

doses thereby eliminating interindividual variations. However, the subjects were patients undergoing necessary medical treatment defined by their condition and not the dictates of this study.

No significant differences between pharmacokinetic parameters after oral administration to euthyroid and hyperthyroid patients were observed. The results can be seen in tables 5.8 to 5.11. Cmax decreased in hyperthyroid patients as would be expected if there was an increased rate of elimination, however the decrease was not significant. Contrary to prediction, tmax was increased in hyperthyroid patients but, again, the increase was not substantial. If there was an increase in the rate of absorption, tmax would be expected to decrease. However, the rate of absorption of methimazole is not likely to show a marked increase as, in normal patients, it has been shown to be rapid (Jansson et al., 1985). The values for clearance, elmination rate constants and areas under the curve all had a large degree of variation and showed no particular trend when all the groups were compared. Also clearance values rely on the bioavailabilty of methimazole which varies from individual to individual ragardless of disease state. This is illustrated by the range of F values quoted in the literature and previously discussed in section 1.3.1. As mentioned already in this dicussion bioavailibility values were not within the scope of this study therefore the mean value derived from the most detailed study in the literature was used (Jansson et al., 1985). However this mean values does not take into account the fluctuations in bioavailability between individuals. Obviously, as was the case of the dosage comparison, a more representative picture of the

changes in disposition between thyroid states would have been available if the same patients had been compared first in the hyperthyroid state and then again when they had reached chemical euthyroidism. However, this was not possible within the confines of this study. This study does however suggests that thyroid hormones do not depress or increase the metabolism of methimazole.

The question thus remains as to why thyroid hormones affect the diposition of several drugs but not methimazole. A possible explanation (Hallengren et al., 1982) could be that the influence of hyperthyroidism on drug oxidation becomes apparent mainly for drugs that are subject to extensive presystemic clearance e.g. propranolol and metoprolol (John and Regardh, 1976). Methimazole oxidation is unaffected because it has a high bioavailability and therefore undergoes little or no first-pass metabolism also Hallengren postulated that methimazole could be metabolized by different oxidative systems.

The data on peak plasma methimazole concentrations and methimazole plasma clearance in euthyroid and hyperthyroid patients are consistent with those in previously published reports. (see table 5.14). Thus, in agreement with most investigators, there are no pharmacokinetic reasons to adjust the dose of methimazole during treatment of thyrotoxicosis.

The perchlorate discharge test was used to estimate the duration of antithyroid effect of the two doses of methimazole. Although there was some deviation in response around thirteen hours, in general, discharge of radioiodine from the thyroid by perchlorate diminished in both groups with time after methimazole administration.

The plot of the percentage discharge of radioiodine from the thyroid by perchlorate against the plasma concentration at the time of discharge test (figure 5.7) gave an interesting result. Apart from a wide variation in percentage discharge at zero plasma concentration, in general, at the selected timepoints, the comparison showed a linear response. Thus, it appears from these results that the plasma concentrations of methimazole can be correlated with its effect on the inhibition of the organification of iodine. Yet, as discussed in section 1.3.4, the plasma concentrations of methimazole have shown no correlations with clinical indices. Consequently, the great divergence in the therapeutic response to the drug in thyrotoxicosis is obviously not due to differences in plasma pharmacokinetics or the extent of inhibition of organification of iodine.

In conclusion, plasma pharmacokinetics of methimazole have shown quite large interindividual variation which could be expected as the study involved patients rather than healthy volunteers. The concentration/time profile often had a second, smaller peak which may possibly be due to enterohepatic recycling. This study gave results which indicate dosage linearity and the methimazole plasma concentrations seem to correlate with its effect on the inhibition of the organification of iodine. The pharmacokinetic parameters generated for hyperthyroid and euthyroid patients gave no evidence of any variation of drug handling with thyroid status.

CHAPTER 6

INTRATHYROIDAL METHIMAZOLE LEVELS

6.1 INTRODUCTION

An important aspect of the pharmacology of antithyroid drugs is their concentration by the thyroid gland. The accumulation of methimazole by its target organ enables maintenance of an effective concentration at the site of action with a minimum extrathyroidal effect.

Some endogenous substances are transported across membranes against their concentration gradients or against a gradient of electrical potential. Metabolic energy is necessary to drive such movements and the process is known as active transport. Active transport mechanisms also make use of carrier molecules. Examples of active transport mechanisms include those involved in the secretion of H^+ into gastric juice and urine of the kidney tubules, and the sequestration of iodine by cells of the thyroid gland.

A few drugs that are chemically related to nutrients are absorbed by active transport mechanisms e.g. methyldopa and α-aminopenicillins are actively absorbed from the gastrointestinal tract, chlorpromazine is concentrated in the brain and some penicillins are actively secreted across the proximal tubule of the kidney.

The thyroidal accumulation of radioactive methimazole has been studied following oral doses of ^{35}S-methimazole and carbimazole (Marchant et al., 1972; Lazarus et al., 1975) and also by a gas chromatographic-mass spectrometric method (Jansson et al., 1983) following carbimazole administration. The results of these studies in

terms of the thyroid/plasma concentration ratios, are summarised in Table 6.1.

The two radioactivity studies showed considerable agreement with, in most cases, a low thyroid/plasma ratio being found. However the GC-MS method, which should give a more accurate assessment of methimazole levels, showed more pronounced intrathyroidal accumulation. Also, the ratio increased with increasing time interval from the last dose. The GC-MS method gives the first data on direct measurement of intrathyroidal concentrations of methimazole and these, along with the corresponding plasma concentrations, are given in Table 6.2. The mean intrathyroidal drug concentrations did not differ between the group receiving the final dose 3-6 hours preoperatively and the group taking the final dose 17-20 hours before excision. This indicates that the elimination time for methimazole in the thyroid gland is much longer than in blood.

The estimation of thyroid binding of iodide from the uptake of radioiodine and its discharge by perchlorate or other ions provides a measure of the thyroidal effect of methimazole. It has been shown (Wartosky and Ingbar, 1971; Barnes and Bledsoe, 1972) that, depending on dose, methimazole is effective for 12 hours or more as an inhibitor of iodide organification within the human thyroid gland. However, the results of more recent inhibition studies following oral doses of ^{35}S-methimazole and carbimazole are contradictory. These results are summarised in Table 6.3 . The first study (Lazarus et al., 1975) is in agreement with previous ideas, showing that for most patients receiving

Table 6.1 Review of the thyroid/serum ratio of methimazole following oral administration of carbimazole or methimazole.

Reference	Subject no.	Dose (mg)	Time (hr)	Normal Gland	
				Mean	Range
Marchant et al (1972)	1	5	3.5	1.7	
	2	10	8-9	6.9	1.4 -12.4
Lazarus et al (1975)	3		4	1.4	0.9 - 2.2
	4		8	3.6	0.5 - 10.0
	1		12	2.0	
Jansson et al (1975)	10	10 (CBZ)	3 - 6	5.3	2 - 12
	10		17 - 20	61	15 - 195

Table 6.2 Plasma and intrathyroidal concentrations of methimazole following administration of 10mg of carbimazole.

Patient no.	Time after MMI Dose (h)	Intrathyroidal concentration of MMI (ng/g)	Plasma concentration of MMI (ng/ml)
1	3 - 6 h	270	90
2		1135	94
3		765	110
4		230	108
5		230	120
6		350	96
7		495	128
8		550	83
9		460	82
10		695	110
Mean +/- S.D.		518 +/- 90	102 +/- 5
11	17 -20 h	370	10
12		380	16
13		370	9
14		300	20
15		1110	16
16		600	8
17		980	25
18		1895	40
19		780	4
20		480	7
Mean +/- S.D.		727 +/- 157	16 +/- 3

Jansson et al., 1983

Table 6.3 Percentage intrathyroidal inhibition of organic binding of iodine after oral administration of carbimazole or methimazole.

Reference	Dose (mg)	Time after MMI dose (h)	Subject no.	Normal Tissue		Method
				Mean	+/- S.D.	
Lazarus et al (1975)		8	2	94.4	1.1	eTCA ppt
		12	2	90.8	0.8	
McCruden et al (1985)	6	5 - 7	20	37.7	17.3	ClO_4^- discharge
	10		18	61.2	15.4	
	20		13	74.8	11.4	
	24		18	85.5	7.0	
	30		11	87.9	7.6	
	10	12 - 14	8	19.6	14.4	
McCruden et al (1987)	5	2	4	20.2	8.1	ClO_4^- discharge
		5	6	21.8	7.1	
		13	6	26.5	7.5	
		25	5	37.6	17.0	
	20	2	2	15.9	2.7	
		6	6	19.9	6.7	
		13	6	21.6	6.4	
		25	6	32.3	9.9	

carbimazole or methimazole, inhibition of iodide organification was greater than 90% up to 8 hours after administration. However, in a study using the perchlorate discharge test (McCruden et al., 1985), inhibition of iodide organification by methimazole and carbimazole was shown to have diminished between 5 and 12 hours after administration. Further investigations by the same authors over an extended period of up to 25 hours (McCruden et al., 1987) served to confirm the previous results. Thus, there remains a disparity between intrathyroidal methimazole concentrations and its duration of effect as measured by perchlorate discharge.

The aim of this present study is to investigate intrathyroidal methimazole concentrations in euthyroid patients with Graves disease who are undergoing subtotal thyroidectomy. Thyroid/plasma concentrations at two different doses and at different time intervals will be compared. Examination of the differences in intrathyroidal concentrations between the two doses will be compared to give insight into the existence of an active transport process. Finally, perchlorate discharge data from these patients will be compared with the intrathyroidal concentrations to investigate further this apparent discrepancy.

6.2 EXPERIMENTAL

Six female patients previously studied in Chapter 5 (Subject numbers 1, 3, 4, 7, 9 and 11) with hyperthyroidism due to diffuse toxic goiter, underwent subtotal thyroidectomy. All patients were treated with methimazole in one of two oral regimens : 5mg b.d. (subjects 1,3 and 4) or 20mg b.d. (subjects 7,9 and 11) for at least two months before surgery. All patients were euthyroid, based on the clinical investigations given in Table 6.4, at the time of subtotal thyroidectomy. Three patients were given the last dose of methimazole at a nominal time of 25 hours before surgery; two at 13 hours before surgery and one patient at 5 hours before surgery. All patients were hospitalised for at least 24 hours before surgery. The last doses of methimazole were given under supervision.

At least 2g of thyroid gland tissue were obtained from each sampling site of the gland at operation. In each patient, a tissue sample was obtained from each lobe of the gland. Blood samples were taken at the time of gland excision and collected into EDTA tubes. After centrifugation, the resultant plasma was stored, with the thyroid gland tissue, at $-20^{o}C$, in the Gardiner Institute, Department of Medicine, The Western Infirmary. After transportation to the Department of Forensic Science and Medicine, Glasgow University; the samples remained stored at $-20^{o}C$ until assayed. The plasma and thyroid tissue samples were assayed for methimazole using the methods described in sections 3.5.3.1 and 3.5.3.3., respectively.

Table 6.4 Clinical indices and actual sampling times for patients undergoing subtotal thyroidectomy following oral administration of methimazole.

Subject no.	Dosage (mg)	Nominal interval from methimazole dose (h)	Actual interval from methimazole dose (h)	T_3 (nmol/L)	T_4 (nmol/L)	TSH (mu/L)
1	5	5	5	0.8	122	N.S.
3	5	24	22.5	1.01	99	2.92
4	5	13	13	1.3	150	0.83
7	20	24	24.5	2.8	80	0.17
9	20	24	25	2.01	152	N.S.
11	20	13	13	1.8	172	1.2

Normal ranges T_3 = 0.8 - 2.46

T_4 = 54 - 142

N.S.= no sample

The tablets and clinical diagnostic tests were supplied by the manufacturers listed in section 5.2.1.

Less than two weeks before surgery, and after the patients had reached euthyroidism, a perchlorate discharge test was carried out. The test was carried out as described in section 5.2.1. Each patient underwent the test at the same time interval from the last methimazole dose that the thyroid excision would occur.

6.3. RESULTS

Typical chromatograms for methimazole extracted from thyroid tissue from patients on the 5mg and 20mg dosage regimen are given in Figures 6.1 and 6.2, respectively. Both chromatograms were from samples taken 13 hours after the last methimazole dose. The plasma concentration time values along with the thyroid tissue concentration time values (for both lobes) are presented in Table 6.5. The plasma to mean thyroid concentration ratios, which increased with time, are also given in this table. A graphical description of ratio versus time is given in Figure 6.3.

In the 5mg dosage group, the patient who was operated on 5h after the last dose of methimazole did not differ in mean intrathyroidal methimazole concentrations from the patient operated on 13h after the last dose of methimazole. The patient undergoing thyroidectomy 25h after the last dose of methimazole showed a significant increase in mean intrathyroidal methimazole concentrations when compared with the 5h and 13h patients. In contrast , the plasma concentrations showed a definite decrease in levels with increasing time interval.

In the 20mg dosage group the patient, operated on 13h after the last dose of methimazole did not differ significantly in mean intrathyroidal methimazole concentration from the two patients who were nominally operated on 24h after the last dose of methimazole. In this case the plasma concentrations did not decrease as the time interval increased and in fact the mean concentration at 24h was greater than the value

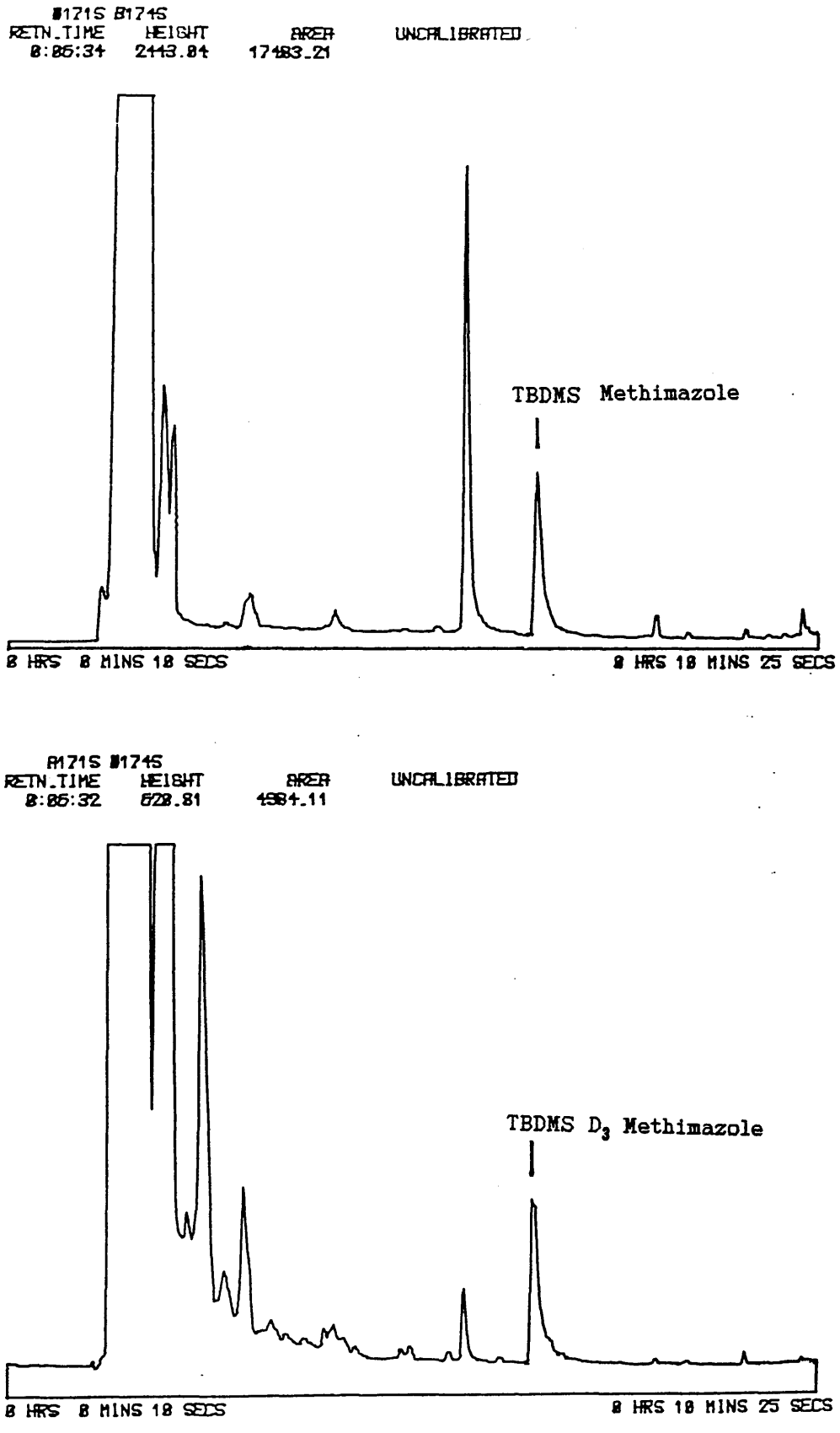


Figure 6.1 Typical chromatogram of methimazole in thyroid tissue following oral administration of 5mg of methimazole.

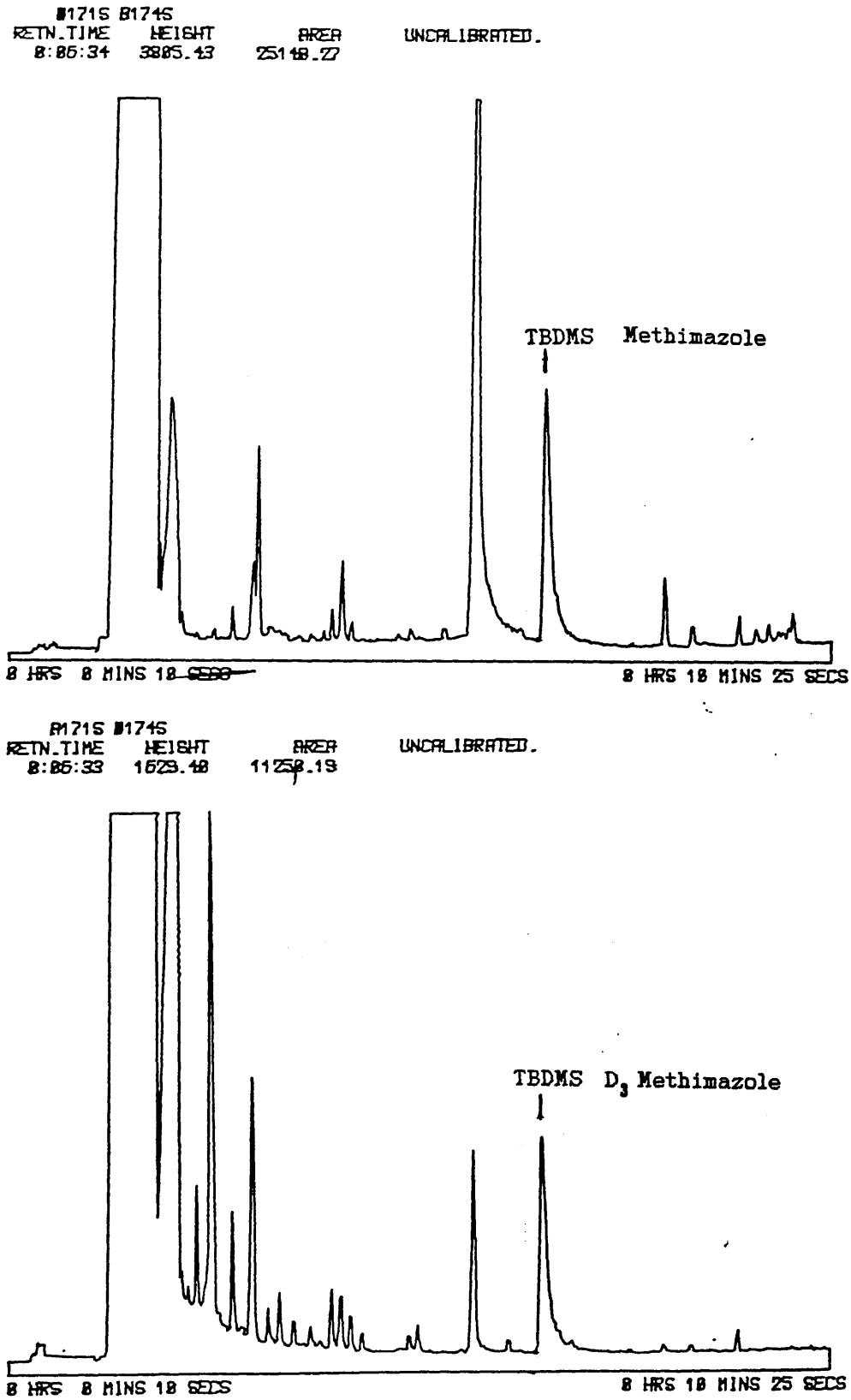


Figure 6.2 Typical chromatogram of methimazole in thyroid tissue following oral administration of 20mg of methimazole.

Table 6.5 Plasma and thyroid gland tissue concentrations of methimazole for patients undergoing subtotal thyroidectomy following oral administration of methimazole.

Subject no.	Dose (mg)	Interval from MMI dose (h)	Plasma concentration (ng/g)	Thyroid concentration (ng/g)			T/P
				Left Lobe	Right Lobe	Mean	
1	5	5	180	467	483	475	2.6
3	5	24	18	692	658	675	37.5
4	5	13	21	492	442	467	22.2
7	20	24	18	233	142	188	10.4
9	20	24	39	242	192	217	5.6
11	20	13	12	305	292	298	24.8

T/P = mean thyroid to plasma concentration ratio

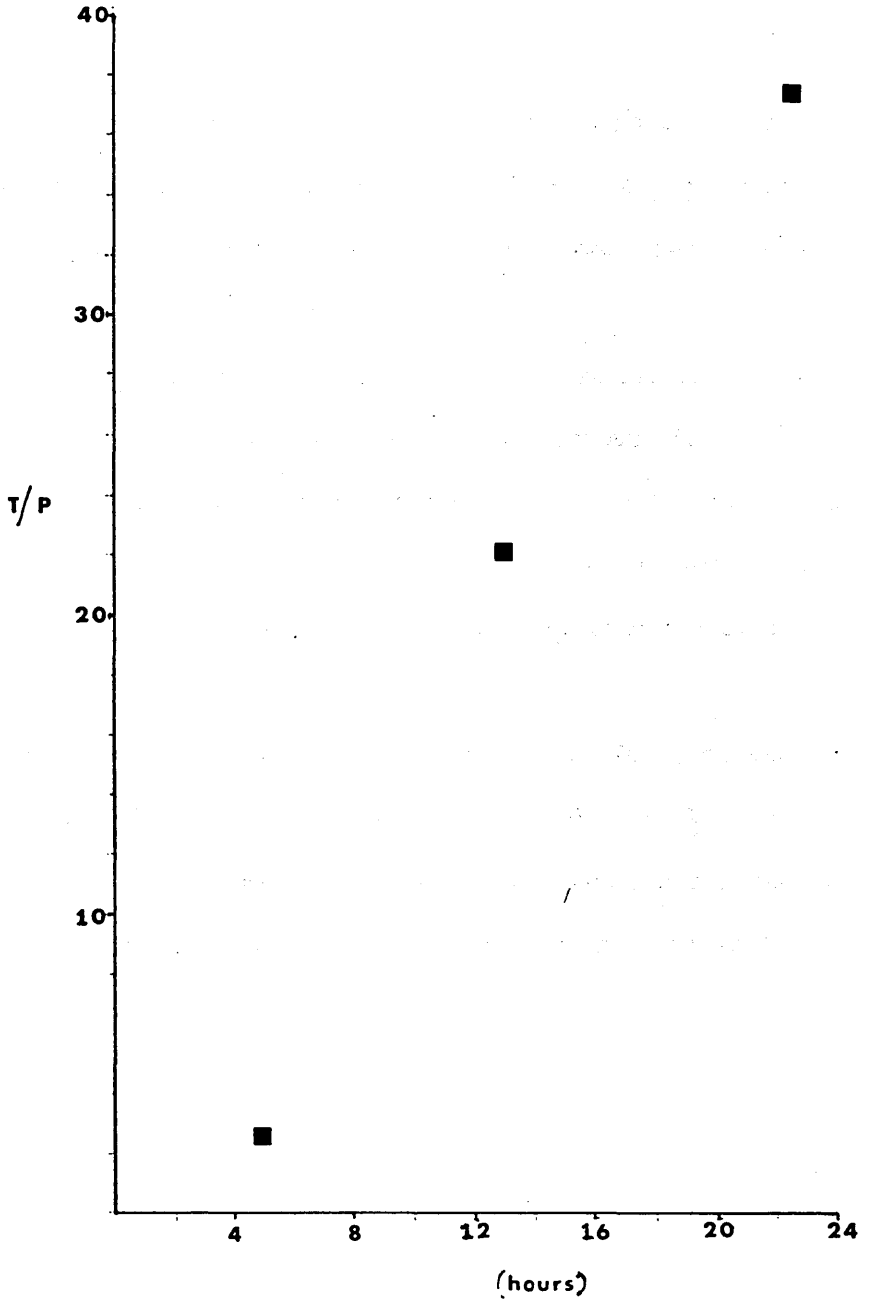


Figure 6.3 Thyroid/plasma concentration ratio versus time following the 5mg dose

calculated for the 13h sample. However there was a wide individual variation between the two 24h samples (cv=52%).

A comparison of the mean intrathyroidal concentrations at the same dose interval showed that the 5mg patients gave significantly higher values than the 20mg patients i.e. 675 +/- 24ng/g compared to 202+/- 46ng/g after 24 hours and 467 +/- 35ng/g compared to 298 +/- 9ng/g after 13 hours, respectively.

The variation between two specimens from separate lobes in the patients was 11%. This indicated a homogenous tissue concentration of methimazole. No correlation between intrathyroidal and plasma concentrations of methimazole was found. No correlation was found between pretreatment plasma T3 values and intrathyroidal methimazole concentrations.

The sodium perchlorate discharge results for each patient (except patient no. 11) are presented in Table 6.6 . Following the 20mg oral administration, the perchlorate discharge results for the two 24 hour patients , showed a wide individual variation (c.v.=86%). This interindividual variation was in agreement with the plasma concentrations already discussed for these patients.

Table 6.6 The percentage of the 30 minute ^{123}I-iodide discharge by perchlorate following intravenous administration of the tracer at selected time intervals after oral administration of methimazole.

Subject no	Dose (mg)	Interval from MMI dose (h)	Discharge (% 30-minute uptake)
1	5	5	75.5
3	5	24	0
4	5	20	79.9
7	20	24	7.5
9	20	24	30.9
11	20	13	-

6.4 DISCUSSION

This study provides the first combined data on direct measurement of intrathyroidal concentrations of methimazole together with percentage inhibition of iodide organification in the same individuals.

The interindividual differences in intrathyroidal concentrations in the present study showed considerable variation which was possibly related to the intrathyroidal iodine content, as this has been shown to markedly affect methimazole accumulation in the rat (Marchant et al., 1972) and in a model system (Engler et al., 1983).

As discussed in section 6.1, methimazole is known to be actively concentrated in the thyroid gland and the results of this study support this concept, in several ways. Firstly, the intrathyroidal concentrations do not show any linearity with dose, giving instead higher concentrations for the lower dosage group. Also, the results are within the same range as those quoted in Table 6.2. (Jansson et al., 1983b) which were measured after oral administration of 10mg of methimazole. This lends weight to the notion of a saturable thyroidal uptake mechanism. The intrathyroidal concentrations between dose groups at the 24 hour timepoint were compared with the corresponding plasma concentrations, in the form of thyroid/plasma concentration ratios. At the low dose, the thyroid/plasma ratio is high, reflecting active transport, but at the higher dose, this ratio falls when the transport system is saturated by high plasma concentrations.

Examples of other drugs which are involved in an active transport mechanism are given in section 6.1. In the case of methimazole, it is particularly fortuitous that the site of active transport and of action are one and the same. The blood flow to the thyroid gland is higher than that to most other tissues of the body thus methimazole is concentrated by the thyroid very shortly after drug administration.

The transport system used by methimazole has not been identified. The follicle cells of the thyroid gland take up iodide through the activity of an energy-dependent pump within the membrane. However, methimazole blocks the incorporation of iodide into protein but not the iodide pump and therefore is not in competition, unlike perchlorate ions, for this particular transport system.

Various methods which have been used for identifying transport systems might be employed to investigate the mechanism of methimazole concentration. In some systems, transport has been shown to be inhibited by compounds that react with proteins, such as phenylisothiocyanate. Some drugs act as inhibitors of transport systems by competing for carrier sites e.g. some penicillins are rapidly eliminated from the body by an active transport process for weak acids in the kidney tubules. A synthetic weak acid, probenecid, combines with the same carrier and so competes with penicillin and as a result, blood levels of penicillin are maintained longer. Finally, metabolic energy is necessary to drive any movement across concentration gradients, thus drugs that block production of ATP (e.g. cardiac glycosides) will inhibit active transport. However, this is not a good choice for the

investigation of methimazole uptake as this would also inhibit the iodide pump and consequently affect thyroid hormone production.

The mean intrathyroidal concentrations did not vary (regardless of dose) between the group receiving the final dose 5 hours (or 13 hours) preoperatively and the group taking the final dose 24 hours before excision. These results are in agreement with the previous study on intrathyroidal levels (Jansson et al.,1983b) and strongly indicate that the elimination time for methimazole in the thyroid gland is much longer than in blood. Thus, the data from the direct measurement of intrathyroidal levels of methimazole are in agreement with previous studies (Barnes and Bledsoe, 1972; Bouma and Kammer, 1980; Jansson et al.,1983) and indicate that a much longer antithyroid effect could be expected than that suggested by the decline in blood concentrations.

However, the results for the percentage inhibition of iodide organification appears to be in direct conflict with the intrathyroidal levels giving virtually no inhibition after 24 hours on the high dose and none at all following the low dose. Various reasons for this apparent discrepancy between the two studies (McCruden et., 1987; Jansson et al., 1983)have been suggested by one of the authors (McCruden et al., 1987). Intrathyroidal iodine content is thought to have an affect on the extent of methimazole metabolism (Taurog et al., 1976; Engler et al., 1983). If the patients undergoing the perchlorate discharge test had a higher thyroidal concentration of inorganic iodide than those in the other study, they would not oxidise methimazole and thus shorten its duration time. However, this theory has been disproven

by the present results which were measured in the same individuals. Another theory (McCruden et al., 1987) is that methimazole might have a greater duration of effect on the coupling of iodotyrosines than the inhibition of organification in which case, the perchlorate discharge test would not give a true estimate of duration of action. Methimazole does inhibit the formation of thyroxine in concentrations lower than those which decrease the iodination of tyrosine; it therefore seems likely that small doses of methimazole have a selective effect on the coupling reaction. However, this action has been taken as evidence that thyroid peroxidase may be involved in the coupling reaction as well as in the formation of iodotyrosines. It is thought that the coupling reaction is facilitated by the stucture of thyroglobulin, and autoradiographic evidence suggests that iodination of thyroglobulin takes place at the apical cell membrane, in the region where biochemical analyses have suggested that the thyroid peroxidase is bound. These facts, taken together make it difficult to imagine that a change in distribution of methimazole would not affect both stages of hormone production. Autoradiographic techniques following radiolabelled methimazole need to be undertaken to clarify this particular area of study.

This study has confirmed the apparent disparity between intrathyroidal methimazole concentrations and their duration of effect as measured by the perchlorate discharge test. It is likely that the perchlorate discharge test underestimates the duration of action of methimazole as it does not gauge the extent of inhibition of coupling of

iodotyrosines. However, until some method is developed which can measure the coupling effect or autoradiography can give evidence of the distribution of methimazole to different sites within the thyroid gland, the intrathyroidal concentrations of methimazole at 24 hours are not sufficient evidence for the instigation of a once daily regimen.

In conclusion, the intrathyroidal concentrations have shown a large interindividual variation which is possibly related to individual variation in intrathyroidal iodine content. The results i.e. non-linearity with dose, an increase in thyroid/plasma concentration ratio with time and its subsequent decrease with increasing dose, support the concept of active transport of methimaozle in the thyroid gland. The methimazole thyroidal concentration levels do not correlate with its effect on the inhibition of the organification of iodine and it is postulated that a truer correlation might be found with its effect on the inhibition of the coupling process.

CHAPTER 7

METHIMAZOLE IN BREAST MILK

7.1 INTRODUCTION

As the benefits of breast-feeding have become more widely recognised, more and more women have chosen to breast-feed their newborn infants. Therefore, studies concerning the pharmacokinetics of drug excretion into breast milk and predictive models estimating drug concentration in milk are of growing interest and importance to the clinician.

Several studies have been undertaken to investigate whether the treatment of lactating mother with methimazole could have an adverse effect on the infant's thyroid function. The results of these studies are listed in Table 7.1.

As was dicussed in Chapter one (section 1.3.3.3), since methimazole is a non-protein-bound drug with a high lipid solubility and is unionised in a hydrophilic enviroment, an almost equal concentration of methimazole in plasma and milk could be anticipated.

All studies are in reasonable agreement that the serum/milk ratio is close to unity as predicted from pharmacokinetic considerations.

The dose of methimazole which would be given to a hyperthyroid infant is not different from the adult dose if weight relations are considered (Howard and Hayles, 1978). Therefore, conversion of the dose received through milk to its equivalent amount in an adult gives a more representative picture of the possible effect of that dose in the infant. For this reason, Table 7.1 gives a comparison of the studies' findings in terms of weight related percentage dose and equivalent dose for a 70kg man.

Table 7.1 Studies of the distribution of methimazole after administration to lactating mothers.

Author	Maternal Thyroid Status	Maternal Dose	Equivalent Dose (mg)	Percentage Dose	Serum/Milk Ratio
Low et al 1979	-	10mg (1)	0.94	9.4	0.88 to 0.58
Tegler 1980	HT	2.5mg (2)	0.18-0.4	7-16	1.16+-0.12
Johanson 1982	EU	*40mg (1)	0.88-1.52	2.2-3.8	0.98+-0.13
Cooper 1984	EU	40mg	1.2	2.6-3.5	1.03+-0.16
Notarianni 1986	HT	10-20mg (3)	0.26-0.52	2.6	1.22+-0.16

(1) single oral dose

(2) b.d. oral dose

(3) daily dose

* 40mg carbimazole= 24.5mg methimazole

Both early studies (Low et al., 1979; Tegler et al., 1980) show a relatively high percentage dose in the milk. The higher percentage obtained by Low et al. could be due to the analytical method employed. By using ^{35}S-labelled carbimazole, any metabolites of methimazole which contained sulphur would also be included in the final measurement. In fact, radioactivity measurements are not normally suitable for pharmacokinetic analysis unless no metabolism is demonstrated.

High percentage values, however, were also obtained (Tegler et al., 1980) in a later study. From these values, Tegler postulated that, assuming similar pharmacokinetic relations, the normal therapeutic dose of 5mg, four times a day, would give the child up to 3mg of methimazole daily in the milk, which he viewed as a risk to the neonate's thyroid function.

Although Cooper et al. (1984) found a much reduced percentage excretion similar to those of Johansen et al. (1982) and Notarianni et al. (1986), he felt that at this dosage level (40mg) the amount excreted in the milk was a significant risk for the suckling infant.

However such calculations are fraught with difficulties. It is impossible to measure accurately how much milk the baby consumes. Also the amount of drug available will vary with the feeding time in relation to dosing time and the milk composition at that particular stage. The complex nature of drug dosing via breast milk is summarised by a list of factors described in Table 7.2. For a conclusive answer to this problem clinical studies on the suckling infant are necessary.

Table 7.2 Factors affecting excretion of a drug in breast milk and dose consumed by infant.

1.Maternal Pharmacology

a)Drug dose,frequency and route
b)Clearance rate
c)Plasma protein binding
d)Metabolite profile

2.Breast

a)Blood flow and pH
b)Yield capacity
c)Ion and other transport mechanisms
d)Drug metabolism (and reabsorption)

3.Milk

a)Composition (fat,protein,water)
b)pH

4.Infant

a)Suckling behaviour,including equal time on each breast
b)Amount consumed per feeding
c)Feeding intervals (regular or irregular)
d)Time of feeding in relation to maternal dosing

5.Drug

a)pKa (ionisation at plasma and milk pH)
b)Solubility characteristics in fat and water
c)Protein binding characteristics
d)Molecular weight

Wilson et al.,1980

Of the studies already mentioned, only Notorianni et al (1986) used any of the common clinical indices of the thyroid function to assess the effect of the methimazole levels on the child. In the four infants studied all had normal thyroid function except one whose TSH levels remained high for the first 10 days.

Therefore, it is clear that before there is a revision of the dogma of restricting breast-feeding mothers to propylthiouracil treatment in preference to methimazole, further pharmacokinetic studies must be undertaken.

In particular, there is a need to examine both the immediate and long term effects of maternal methimazole treatment in the suckling infants. This must include the analysis of several plasma or urine samples from the infant in order to substantiate projected exposure from the breast milk dosing.

The aim of this present study was to provide documentation of milk/plasma ratios of methimazole over a dose interval, both at the beginning and at the end of feeds. Plasma concentrations of methimazole in the infant should record their level of exposure which can be correlated to the methimazole milk concentrations.

Maternal thyroid status and maintenance of the euthyroid state in the babies over their period of exposure to methimazole will be monitored using standard chemical indices.

7.2 EXPERIMENTAL

7.2.1. Description

A three and a half month study into the effect of treatment of a lactating mother with carbimazole and its effect on her babies was undertaken at The Childrens Hospital, Birmingham.

The mother developed thyrotoxicosis two months after giving birth to healthy female twins. In the absence of any specific evidence of harmful effects of carbimazole in this situation, and after detailed discussion, she was advised to continue breast feeding. She was started on carbimazole when the twins were four and a half months old. The dose was initially 30mg/day but was later reduced when she became euthyroid.

7.2.2. Sample Collection

Tables 7.3 and 7.4. give the timing of maternal and infant samples respectively. Plasma samples were obtained as soon as practicable after blood collection by centrifugation (3000 rpm for 15 minutes) in a refrigerated centrifuge. Each plasma and milk sample was placed in a labelled tube and frozen immediately at -20°C.

Table 7.3 Maternal sample collection data

DATE	DOSE (24hr)	FREQUENCY	BLOOD	MILK
25/10/85	30mg	15mg b.d. 10.00, 22.00	10.30 12.00 13.00 14.30 16.30 22.30	10.30 (A) 13.30 (B)
28/10/85	30mg	10mg 07.30,15.30,23.00		10.00 (B) 10.45 (A)
29/10/85	30mg	As previous		10.00 (B)
30/10/85	30mg	As previous		9.00 (B)
31/10/85	30mg	As previous		10.00 (B)
1/11/85	30mg	As previous	12.00 15.00 16.00	14.00 (A)
8/11/85	30mg	15mg b.d. 10.00, 22.00	10.00	
15/11/85	20mg	10mg b.d. 10.00, 22.00	10.30	
22/11/85	15mg	5mg 10.00 10mg 22.00	11.00	
9/12/85	7.5mg	22.00	11.20	
13/1/86	7.5mg	As previous		10.00 (B)
14/2/86	10mg	As previous		12.00 (A)

(B)= Before Feed
(A)= After Feed

Table 7.4 Infant sample collection data

DATE	FEEDING FREQUENCY 24hrs	BLOOD SAMPLE TIMES TWIN 1	TWIN 2
25/10/85	4-5/24hrs 10.00 14.00 18.00 22.00	10.00 13.15 16.35	10.00 13.10
1/11/85	4-5 + cereal As above	10.00	10.00
8/11/85	As above	10.00	10.00
15/11/85	4-5 + cereal lunch dinner	10.30	10.30
22/11/85	As above	10.45	10.50
9/12/85	As above	11.25	11.15
14/2/86	As above	11.30	11.30

7.2.3. Transport of Samples

The samples were kept frozen by transportation in an ice-box from The Childrens Hospital, Birmingham to the Department of Forensic Medicine and Science, Glasgow; where they were stored at -20°C until assayed.

7.2.4. Assay of samples

Plasma and milk samples were assayed for methimazole as described in section 3.5.3.1 and section 3.5.3.3. Milk samples for the calibration standards were obtained from The Queen Mother's Hospital, Glasgow from healthy lactating mothers who were not on any prescribed drug regimen.

7.3 Results

The clinical indices for the mother and the two twins were monitored throughout the study. The results are summarised in Tables 7.5 to 7.7. Changes in thyroid hormonal levels in the mother were used as a reflection of a need for dosage regimen alteration. In the two infants, thyroid stimulating hormone, thyroxine and triiodothyronine remained normal throughout the study. Free T4 and free T3 were measured on three days between weeks 8 and 16. The free T3 values in twin 1 were 9.5, 10.0 and 10.5 pmol/l, a small increase from the normal.

Methimazole was assayed in the mother's and her babies plasma and in breast milk. Table 7.8 shows the maternal plasma concentrations (ng/ml) throughout the study and it is apparent that there is a wide intraindividual variation of concentration values at the same time point. At 30 minutes after the administration of the first dose the plasma concentration was 61ng/ml whereas, at the same time point for the second dose the value was 27ng/ml. The plasma concentration at the same time point at different dosages also does not show any consistent pattern. At 30 minutes, after a dose of 10mg t.d., the plasma concentration gives 72ng/ml and, at a lower dose of 10mg b.d., the same time interval gives a value of 173ng/ml. However, it is unrealistic to attempt comparisons in this way when the regimen varies so much, resulting in a variation in the amount of methimazole still present at the next administration. The concentrations remain well within the therapeutic range with the highest values being recorded at the highest

Table 7.5 Maternal clinical indices throughout the study

Date	Status	T_4 (nmol/L)	T_3 (nmol/L)	TSH (mu/L)	Free T_3 (pmol/L)	Free T_4 (pmol/L)
21/10/85	HT	161	11.2	<0.3	-	-
25/10/85	HT	141	5.2	<0.3	-	-
1/11/85	EU	85	1.0	<0.3	-	-
8/11/85	EU	53	1.0	<0.3	-	-
15/11/85	EU	41	1.4	<0.3	-	-
22/11/85	EU	47	1.2	0.8	-	-
9/12/85	EU	94	7.1	<0.3	7.1	-
6/1/86	EU	144	-	<0.3	13.0	32.8
14/2/86	EU	143	-	0.3	6.5	24.8

Table 7.6 Clinical indices for twin 1 throughout the study.

Date	T4 (nmol/l)	T3 (nmol/l)	TSH (mu/l)	Free T3 (pmol/l)	Free T4 (pmol/l)
21/10/85	113	3.4	2.8	–	–
25/10/85	116	4.1	5.1	–	–
1/11/85	140	2.1	4.0	–	–
8/11/85	148	2.1	4.0	–	–
15/11/85	139	3.0	4.7	–	–
22/11/85	129	3.3	6.5	–	–
9/12/85	125	10.5	2.5	10.5	–
6/1/86	130	–	5.5	9.5	15.5
14/2/86	124	–	4.4	10.0	18.0

Table 7.7 Clinical indices for twin 2 throughout the study

Date	T4 (nmol/l)	T3 (nmol/l)	TSH (mu/l)	Free T3 (pmol/l)	Free T4 (pmol/l)
21/10/85	92	–	1.5	–	–
25/10/85	116	5.2	4.1	–	–
1/11/85	105	–	3.5	–	–
8/11/85	125	1.9	3.5	–	–
15/11/85	123	2.4	3.9	–	–
22/11/85	133	2.6	3.5	–	–
9/12/85	124	9.1	1.9	9.1	–
6/1/86	103	12.9	4.9	8.8	–
14/2/86	122	14.1	2.5	7.4	–

Table 7.8 Maternal plasma concentrations of methimazole

Date	Dose	Time	Interval From Dose (hr)	Concentration (ng/ml)
25/10/85	15mg	10.30	0.5	61
	b.d.	12.00	2.0	114
		13.00	3.0	196
		14.30	4.5	174
		18.30	8.5	86
		22.30	0.5	27
1/11/85	10mg	12.00	4.5	51
	t.b.d.	15.00	7.5	56
		16.00	0.5	72
8/11/85	15mg	10.00	12.0	59
	b.d.			
15/11/85	10mg	10.30	0.5	173
	b.d.			
22/11/85	5mg 10.00	11.00	1.0	55
	10mg 22.00			
9/12/85	7.5mg	11.20	13.5	53

dosage. Figure 7.1 shows the concentration versus time curve for maternal plasma over 8 hours at a dosage of 15mg b.d. The values for tmax and Cmax are 3 hours and 196ng/ml, respectively.

Methimazole was measured in breast milk on 10 occasions on 8 study days. Table 7.9 shows the milk concentration levels (ng/ml). The mean concentration was 43ng/ml (range 0-92ng/ml). On three occasions a milk collection was taken at the same timepoint following the same dosage regimen and these gave values of 54, 92, 53ng/ml. Figure 7.2 shows a typical chromatographic trace of a breast milk sample.

Plasma methimazole was measured on 9 occasions in twin 1 and on 7 occasions in twin 2. Table 7.10 gives the plasma concentrations found in the twins. The mean plasma concentration of methimazole in twin 1 was 45ng/ml (range 0-105ng/ml) and that in twin 2 was 52ng/ml (range 0-156ng/ml). The highest plasma concentration were recorded when the mother was on the largest dose of carbimazole (30mg/day) at the high dose level. On the first sample day, plasma concentrations in each infant were comparable. However, in later weeks these showed greater deviation. This can be explained by the change in diet as the infants were introduced to solid feeding, which would cause variation in their individual milk consumption.

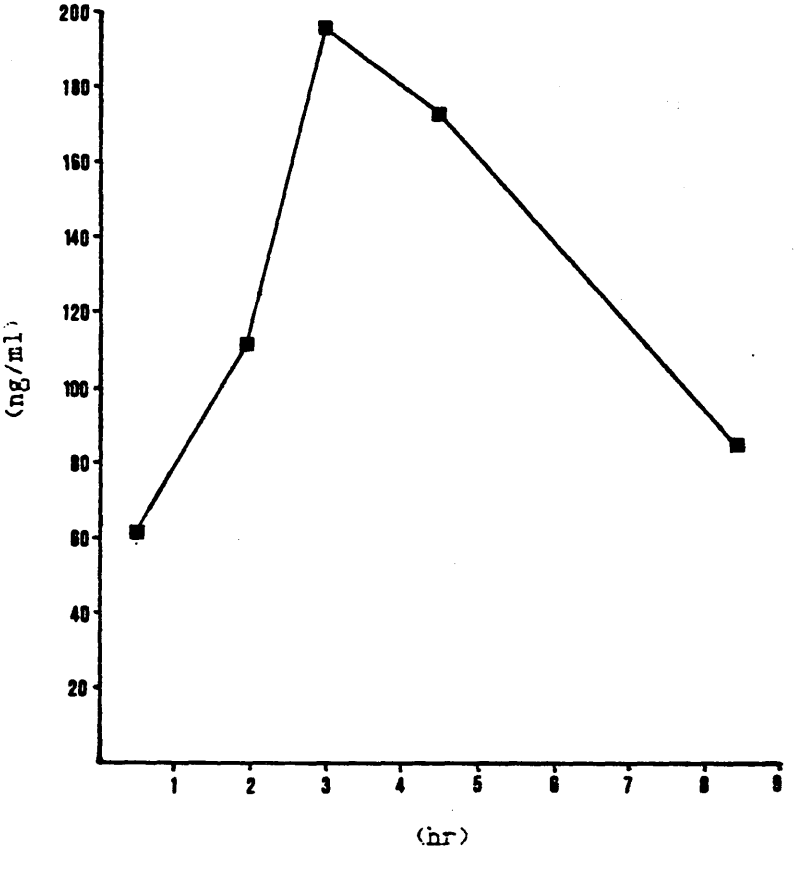


Figure 7.1 Maternal plasma versus time profile.

Table 7.9 Maternal milk concentrations of methimazole

Date	Dose	Sample Time	Interval from Dose(hr)	Concentration
25/10/85	15mg	10.30	0.5	18
	b.d.	13.30	3.5	38
28/10/85	10mg	10.00	2.5	54
	t.b.d	10.45	3.25	60
29/10/85	10mg	10.00	2.5	92
	t.b.d			
30/10/85	10mg	9.30	2.0	45
	t.b.d			
31/10/85	10mg	10.00	2.5	53
	t.b.d			
1/11/85	10mg	14.00	6.5	38
	t.b.d			
13/1/86	7.5mg	10.00	12.0	34
14/2/86	10mg	12.00	14.0	0

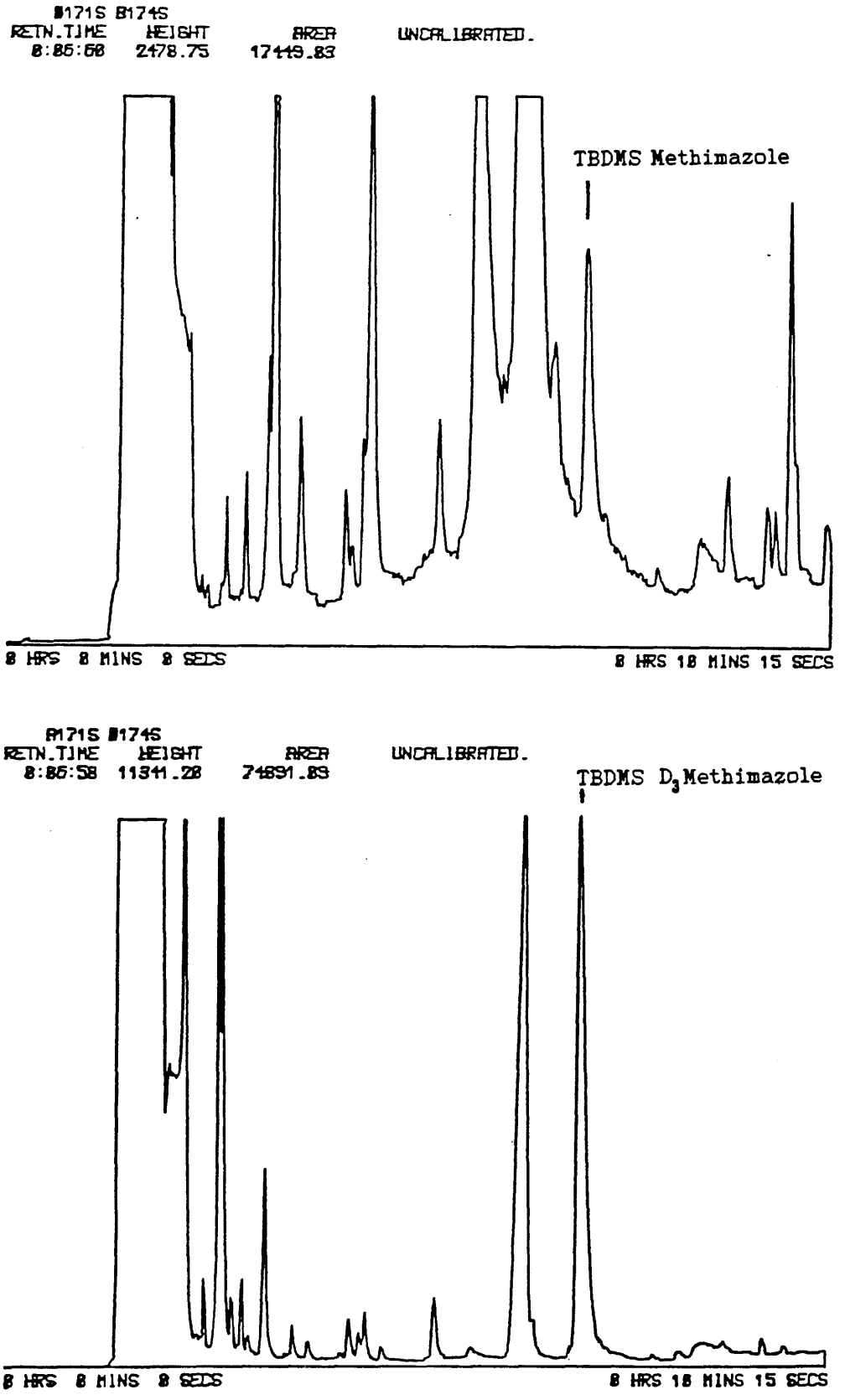


Figure 7.2 Typical chromatogram of methimazole in human breast milk.

Table 7.10 Infant plasma concentrations of methimazole

Date	Dose	Time	Interval From Dose(hr) (feeding)	Concentration(ng/ml) 1	2
25/10/85	15mg b.d	10.00	0/12	105	156
		13.30	3.5	81	83
		16.30	6.5	79	
1/11/85	10mg t.b.d	10.30	3.0	52	
8/11/85	15mg b.d	10.00	0/12	3	0
15/11/85	10mg b.d	10.45	0.75	9	82
22/11/85	5mg 10.00 10mg 22.00	10.50	0.75	55	33
9/12/85	7.5mg	11.20	13.33	0	0
14/2/86	10mg	11.30	13.5	18	12

7.4 DISCUSSION

Excretion of methimazole (or any free unionised drug) in breast milk is expected to follow the disposition model shown in Figure 7.2. Pharmacokinetics operative for methimazole need to be developed so that the paediatric consequences of dosing via breast milk can be minimised.

By analysing infant plasma samples, in conjunction with maternal plasma and milk samples, this study is an attempt to verify the pharmacokinetic predictions of previous studies.

However, by it's very nature, such a study involving suckling infants limits it's own calculation of pharmacokinetic parameters. Firstly, the total milk volume cannot be measured and secondly, because of the ethics involved in frequent blood collection from infants.

However parameters derived from milk volumes and concentrations have been calculated in previous studies. Also, although the infant plasma sampling is incomplete and cannot be used to generate any pharmacokinetic data, the results can be used to look at trends in the context of present literature.

The dosage regimen is constantly being reassessed throughout the study, using thyroid function tests, to make sure that the dosage is as low as possible to firstly achieve and then maintain euthyroidism in the mother. In this way, as little methimazole as possible will be present in the milk and consequently the twins.

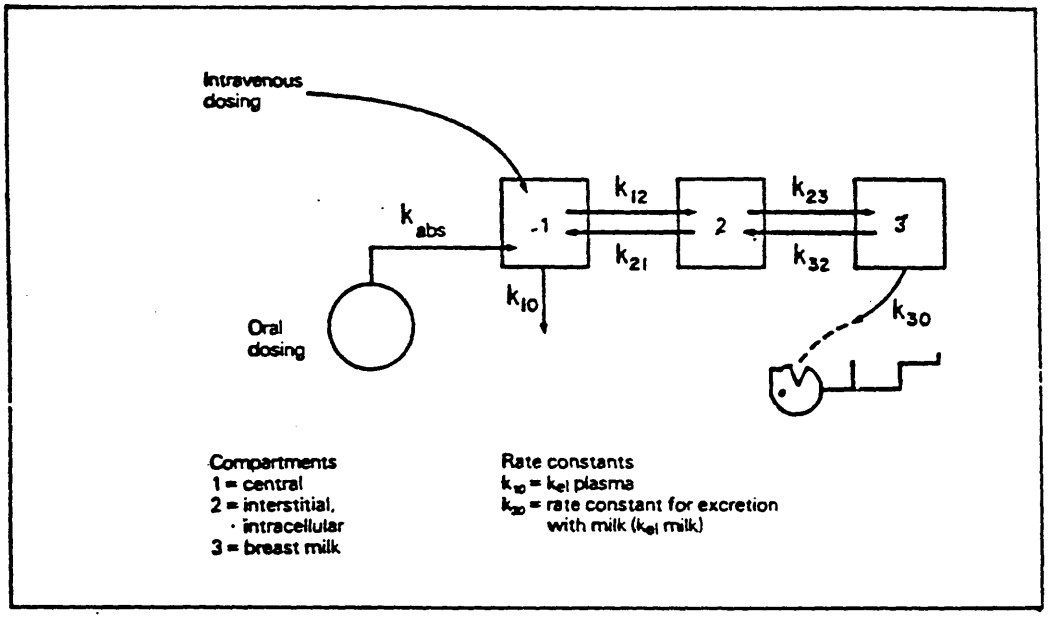


Figure 7.3 A three-compartment model for drug excretion in breast milk.

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Although maternal blood sample collection was stopped on the last day of breast feeding, elimination of the drug could be affected at this time in the treatment. The lack of nursing allows accumulation of milk and drug in the breast. This drug is available for movement back into the interstitial compartment (Figure 7.3). As this movement from the interstitial compartment to the blood is presumed higher than from the milk into the interstitial compartment; k_{32} would become the rate-limiting step for drug elimination from plasma.

Frequent measurements of drug concentration in milk and plasma can be made during breast feedings which occur at different times in relation to a dosing interval. These measurements provide meaningful data for calculation of the milk/plasma ratio and for assessment of average concentrations during a feeding.

M/P = AUCmilk/AUCplasma Average concentration = AUCmilk/t

Data to calculate the AUC for milk was not available in this study. Firstly, milk could not be collected frequently throughout a dosage interval as it was needed for the infants. Secondly, its collection could not be evenly spread throughout the study because of the variations in dosage regimen. However, from Tables 7.8 and 7.9, some single points can be compared. For 25/10, paired values of 61ng/ml for plasma and 18ng/ml for milk which gives a milk/plasma concentration ratio of only 0.3, however this time point is only 30 minutes after administration of the oral dose and therefore is perhaps not sufficient

time to have equilibrated across the membrane. Another day, 1/11, has samples, (14.00 and 15.00) although not paired, close enough in time for reasonable comparison. These give a substantially higher value of 0.68. This value is still lower than the general trend to unity shown previously in Table 7.1 . However, during multiple dosing, the ratio refers to average steady-state concentrations and milk/plasma ratio at any one time during a dosing interval may vary.

Milk to plasma concentration ratios (M/P), fraction not bound to milk proteins (fm) and skim to whole milk concentration ratios (S/M) of a drug can be affected by compositional change in the milk. Milk compositon may be affected by factors such as the stage of lactation, the time of day and the time during a feeding. Fat content increases during the course of a feeding while protein composition remains fairly constant. From Table 7.9, a comparison of concentration levels both before and at the end of feeding can be made for the 28/10. There is little difference between the values. Thus an increase in fat content in milk has no effect on the methimazole concentration i.e. most of the methimazole remains in the aqueous phase as was found by Cooper et al (1984).

The milk concentration values cannot be used for calculation of the amount of drug delivered to the babies again because there are not enough sample points over one dosing interval and also milk volumes were not recordable because of breast-feeding. Estimates of yield cannot be made as for a given individual the volume varies each day and

a further influence on yield include twins as compared with single infant nursing.

However, the concentration values themselves appear to be in a similiar range to those found in previous studies and thus suggest similiar excretion levels.

Clinical studies on the suckling infant showed the highest plasma concentrations when the mother was on 30mg of carbimazole daily dose. The level subsequently decreased with decreasing maternal dose and a move onto more solid foods. Calculation of infant daily dose is not possible because of the limited number of blood samples which can be reasonably taken from an infant. However, the single values observed were at the lower end of the range of concentrations (50-100ng/ml) purported to cause thyroid suppression in adults with thyrotoxicosis (Benker and Reinwein, 1982). These figures alone seem to confirm the projected levels expected in infant plasma from previous studies (Table 7.1) which were considered to represent a significant risk to the suckling infants thyroid function.

However, clinical examination and thyroid function tests (Tables 7.6 and 7.7) done on each twin throughout the study showed no evidence of thyroid suppression.

The normal range quoted for total serum T4 is 60-150nM/L and 1-2.6nM/l for serum T3. It is generally accepted that the thyroid status is determined by the concentration of free (i.e. non-protein bound) thyroid hormones. The normal range for free T4 is 10-22pM/L and the T3

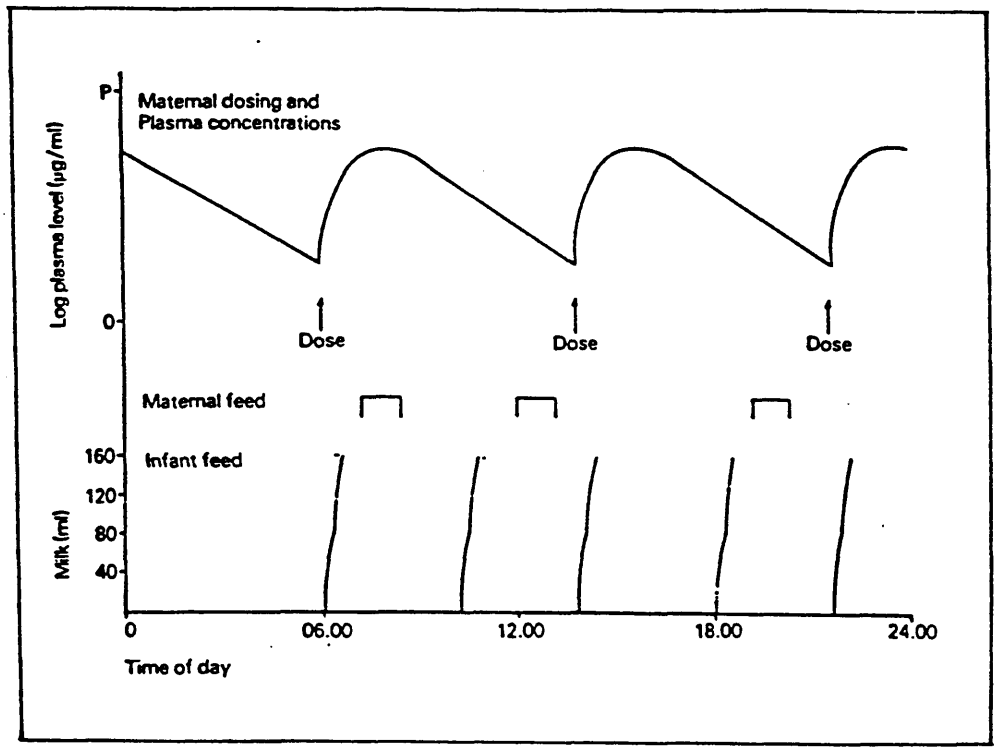

Figure 7.4 An idealised profile for maternal dosing and infant breast feeding.

The milk volume plot represents the known rapid secretion of fluid during the early period of breast feeding. The 'notch' in the plot depicts a change from one breast to the other. Plasma concentrations are shown for steady-state conditions.

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is 2-8pM/L. The total T4:T3 is about 65:1 and the free T4:T3 is about 2:1 in normal man.

An increased ratio of T3:T4 is seen in hypothyroidism as a iodine-sparing mechanism but the slight increase in free T3 values did not cause a substantial change in the ratio. Also, all other values remained within the normal range.

Breast feeding occurs at different times in relation to dose throughout the day and hence methimazole concentration in milk will vary according to some relationship with plasma concentrations (figure 7.4). In this study, the highest plasma methimazole levels in each twin were recorded 2-4 hours post-maternal dosing; the lowest occuring 6 or more post dosing. Discarding breast milk produced 2-4 hours following a carbimazole dose would therefore be expected to reduce the infants daily methimazole load considerably.

Thus, this study is in agreement with similiar results from a long term study of seven children (Notorianni et al., 1986). It suggests that carbimazole in doses of 30mg or less per day can be used to treat thyrotoxicosis in breast-feeding mothers and that current advice that propylthiouracil is the preferred drug in this instance should be revised.

This is the first clinical study of methimazole to the suckling infant and the results of the concentrations of methimazole found in infant blood have confirmed the predictions made by other authors.

CHAPTER 8

GENERAL DISCUSSION.

The present investigation was undertaken to assist the clinician with the treatment of hyperthyroidism using the antithyroid drug, methimazole. This was done in order to determine and correlate plasma pharmacokinetics with pharmacodynamics in the thyroid gland, the therapeutic effect and to investigate any changes in pharmacokinetics with variation in thyroid status. Also, a study was undertaken to assess the suitability of methimazole treatment for hyperthyroidism in breast-feeding mothers. In order to examine these questions in detail, a suitably sensitive analytical method for the measurement of methimazole needed to be developed.

An analytical method for the quantification of methimazole was developed using GC-MS. An internal standard, trideuteromethylimidazole, was successfully synthesised and chromatographs in an identical manner to methimazole whilst being clearly distinguishable by mass spectrometry. A derivative was selected which, not only chromatographs well on the chosen column, but also gives a lucid fragmentation pattern containing distinct major ions for methimazole and the internal standard which are appropriate for SIM GC-MS. Sample preparation in various biological fluids was optimised to enable the development of a sensitive, selective and robust method for methimazole which is suitable for use in clinical therapeutic monitoring. In fact, the limit of detection of plasma samples was 3ng/ml which is comparable to the GC-MS method developed by Floberg et al. (1980). They could detect down to levels of 2ng/ml of methimazole in plasma. However, that method had inherent problems with the internal standard and with column bleed. Only an

HPLC method developed by Hengstmann and Hohn, (1985), which claims to detect picogram levels of methimazole, is more sensitive.

Further analytical work in greyhound urine identified a minor metabolite 3-methyl-2-thiohydantoin, which may play a part in the antithyroid effect of the compound. The same derivatisation reaction that was employed for methimazole, allowed selected ion monitoring of methimazole and 3-methyl-2-thiohydantoin simultaneously without interference and this method could form the basis of kinetic studies of 3-methyl-2-thiohydantoin.

Plasma pharmacokinetics can only be used for correlation with effect if they have been examined at steady-state. In this study patients on long term treatment were investigated. Previous studies had concentrated mainly on single dose pharmacokinetics or the kinetics following only a short-term study and the literature is very sparse for multiple dosage kinetic studies.

Dosage linearity was shown when patients on a low dosage regimen where compared to those on a high dosage regimen. The results were in agreement with two other similiar studies (Jansson et al.,1983a; Okamura et al., 1986)The lack of accumulation of methimazole was also indicated as the plasma concentrations returned to approximately the same levels at the end of the dosing interval following either dosage (i.e. steady-state conditions). Thus, as far as plasma concentrations are predictive, a dosage regimen of twice daily, at either 5mg or 20mg, achieve steady-state plasma levels in the range that should be both efficacious and non-toxic.

No significant differences between pharmacokinetic parameters after oral administration to euthyroid and hyperthyroid patients were observed which is consistent with results from two previously published reports (Cooper et al.,1984; Jansson et al., 1985), but contradictory to one previous study (Hengstmann and Hohn, 1985). However it would appear that thyroid hormones do not depress or increase the metabolism of methimazole and therefore there are no pharmacokinetic reasons to adjust the dose of methimazole during treatment of thyrotoxicosis. Thus, the strategy of individualised doses based on the assessment of the severity of the disease suggested by some authors (Braverman, 1978 and Solomon, 1978) is not justified. Further radioactive and metabolic studies need to be undertaken to understand why variation in thyroid status does not appear to affect the kinetics of methimazole but does affect other drugs. However, before this work can be undertaken, all the metabolites of methimazole must be identified and their pathways elucidated.

The perchlorate discharge test was used to estimate the duration of antithyroid effect of the two doses of methimazole. In general, discharge of radioiodine from the thyroid by perchlorate diminished in both groups with time after administration of methimazole. Thus, plasma concentrations of methimazole could be correlated with its effect on the inhibition of organification. Yet, plasma concentrations have shown no correlation with clinical indices. Consequently, the great divergence in the therapeutic response to the drug in thyrotoxicosis is obviously not due to differences in plasma pharmacokinetics or the extent of inhibition of organification of iodine.

However, the most important aspect of the pharmacology of antithyroid drugs is their concentration level at the site of action, the thyroid gland. This study provides the first combined data on direct measurement of intrathyroidal concentrations of methimazole together with percentage inhibition of iodide organification in the same individuals. Methimazole is known to be actively concentrated in the thyroid gland and the results of this study support this concept in several ways. The intrathyroidal concentrations do not show any linearity with dose, instead, giving higher concentrations for the lower dosage group. Also, the intrathyroidal concentrations between dose groups at the same timepoint were compared with the corresponding plasma concentrations, in the form of thyroid/plasma concentration ratios. At the low dose, the thyroid/plasma ratio is high, reflecting active transport, but at the higher dose, this ratio fell when the transport system is saturated by high plasma concentrations. The transport system used by methimazole has not yet been identified and further studies using various chemical inhibitors could be undertaken to identify this transport system.

The intrathyroidal concentrations remained high, regardless of dose, 24 hours after administration of methimazole. This result is in agreement with the only other similiar study (Jansson et al., 1983b) i.e. that the elimination time for methimazole in the thyroid gland is much longer than in blood. This indicates that a much longer antithyroid effect could be expected than that suggested by the decline in blood concentrations. However, results for the percentage inhibition of iodide

organification appear to be in direct conflict with the intrathyroidal levels, giving virtually no inhibition after 24 hours on the high dose and none at all following the low dose. The fact that the perchlorate discharge results and intrathyroidal concentrations are from the same patients disproves the proposed theory (McCruden et al., 1987) that the vast difference between the results was due to variation in intrathyroidal iodine content between the two study groups (Jansson et al., 1983b and McCruden et al., 1987). It is more likely that the perchlorate discharge test underestimates the duration of action of methimazole as it does not gauge the extent of inhibition of coupling of iodotyrosines, which is the main site of action of methimazole, (McCruden et al., 1987). This theory can be examined if a method is developed which can measure the coupling effect in a way that the perchlorate discharge test measures iodination, or if autoradiographic studies can give evidence of the distribution of methimazole to different sites within the thyroid gland.

One of the major questions relating to methimazole therapy is whether patients require single or multiple daily doses of medication. In terms of the results of this study, although the plasma and thyroid data conflicts, intrathyroidal drug concentrations are more clearly related to antithyroid effect. Also, it seems that the perchlorate discharge test tends to underestimate the duration of antithyroid action. Therefore, it would appear that low single daily doses are sufficient to achieve maintenance of therapeutic levels of methimazole in the body and that higher doses at a shorter interval may only be necessary in terms of

achieving steady state quickly. However, a long-term follow-up study of forty two months(Romaldini et al., 1983) found the remission rate for a group receiving high-dose therapy was almost twofold higher than patients receiving low-dose therapy. However, the rate of side effects was also higher. Thus the question of a single daily dose regimen remains a complex issue and will only be answered satisfactorily when a more direct method of measuring the antithyroid effect is developed. Only then will it be possible to select the optimum dosage regimen of long-term remission with no untoward side-effects. If this is achieved, measurement of plasma concentrations would only be recommended in patients responding poorly for no apparent reason.

The study of drug excretion in breast milk is important with regard to the safety of the suckling infant. Several studies have been undertaken to investigate whether the treatment of lactating mothers with methimazole could have an adverse effect on the infant's thyroid function (Low et al., 1979; Tegler and Lindstrom, 1980; Johansen et al., 1982; Cooper et al., 1984 and Notarianni et al., 1986). This present study provides documentation of milk/plasma ratios of methimazole over a dose interval both at the beginning and at the end of feeds after the oral administration of carbimazole. This is also the first clinical study of methimazole to the suckling infant and is of special interest as the study has been carried out in twins.

A comparison of maternal milk to plasma concentration ratios, although slightly lower, tended towards unity as was shown in previous studies. In addition, a comparison of concentration levels both before

and at the end of feeding showed that an increase in fat content has no effect on the methimazole milk concentration.

Plasma concentrations of methimazole in the infant should record their level of exposure which can be correlated to the methimazole milk concentrations. The values obtained were at the lower end of the range of concentrations purported to cause thyroid suppression in adults (Benker and Reinwein, 1982) and seemed to confirm the projected levels expected in infant plasma from the previous studies. However clinical examination and thyroid function tests done on each twin throughout the study showed no evidence of thyroid suppression. In this study, the highest plasma methimazole levels in each twin were recorded 2-4 hours post-maternal dosing; the lowest occuring 6 or more post dose. Discarding breast milk produced 2-4 hours following a dose would therefore be expected to reduce the infants daily methimazole load considerably. Finally, this study suggests that carbimazole in doses of 30mg or less per day can be used to treat hyperthyroidism in breast feeding mothers and that the current advice recommending propylthiouracil as the preferred drug in this instance should be revised.

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SAFETY OF INTRAVENOUS IMMUNOGLOBULIN

SIR,—Dr Thomson and colleagues (March 7, p 539) describe the use of alpha interferon to treat non-A, non-B hepatitis transmitted by an intravenous immunoglobulin preparation. Readers may conclude that these patients were infected via 'Sandoglobulin', this being the only intravenous immunoglobulin referred to in the paper. This was not so; they were infected via another preparation (as stated in ref 5 in the March 7 paper). Despite extensive use worldwide, no case of post-transfusion hepatitis fulfilling the normal criteria for non-A, non-B hepatitis[1] has been ascribed to the use of sandoglobulin or any other intravenous immunoglobulin prepared by the pH4/pepsin method.[2]

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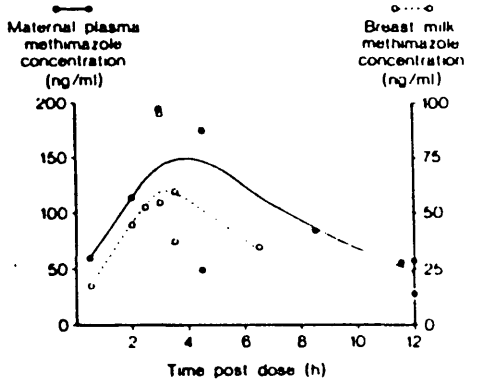
CARBIMAZOLE AND BREASTFEEDING

SIR,—Most prescribing information states that thyrotoxic mothers treated with carbimazole should not breastfeed.[1] 0·47–16% of a maternal carbimazole dose is estimated to reach the child through breast milk.[2,3] These results span a wide range and there is little information on the pharmacology of carbimazole in the young infant. Because breastfeeding and carbimazole therapy for thyrotoxicosis are in their separate ways advisable, we report on breastfed twins whose mother was taking carbimazole.

The mother had thyrotoxicosis 2 months after giving birth to healthy twins. In the absence of any specific evidence of harmful effects of carbimazole in this situation,[1] and after detailed discussion, she was advised to continue breastfeeding. She was started on carbimazole, initially 30 mg daily, reducing when she became euthyroid.

Clinical examinations and thyroid function tests were done on each twin over the next 4 months. Methimazole (the active metabolite) was assayed in the mother's and her babies' plasma and in breastmilk. Thyroid stimulating hormone, thyroxine (T4), and triiodothyronine (T3) remained normal throughout weeks 1–16. Free T4 and free T3 were measured on three days between weeks 8 and 16. The free T3 values in twin 1 were 9·5, 10·0, and 10·5 pmol/l, a small increase (normal range unavailable in the paediatric age group, adult range 2–8). All other values remained normal.

Free methimazole was measured in breastmilk on 10 occasions between weeks 2 and 16 and the mean concentration was 43 ng/ml (range 0–92 ng/ml). Milk volumes were not recordable because of breastfeeding and breastmilk excretion of methimazole could not be calculated. However, three paired breastmilk/plasma samples were analysed, giving ratios of 30–70%. Plasma methimazole was measured in twin 1 between weeks 1 and 16 and in twin 2 between weeks 2 and 16. The concentration of methimazole in twin 1 was 45 ng/ml (range 0–105 ng/ml) and that in twin 2 was 52 ng/ml (range 0–156 ng/ml). The highest values were recorded when the mother was on 30 mg carbimazole daily. Plasma methimazole concentrations in the twins were at the lower end of the range of concentrations purported to cause thyroid suppression in adults with thyrotoxicosis (50–100 ng/ml).[4] However, thyroid function tests and clinical examination showed no evidence of thyroid suppression.



Maternal plasma and milk methimazole concentrations after carbimazole doses of 30 mg daily.

The figure shows the maternal plasma and milk methimazole concentrations, related to time after the dose of carbimazole. The highest plasma methimazole levels in each twin were recorded 2–4 h post dose; the lowest occurred 6 h or more post dose. Discarding breastmilk produced 2–4 h after a carbimazole dose would therefore be expected to reduce the infants' daily load of methimazole considerably.

These data, together with similar results in a long-term study of 7 children,[4] suggest that carbimazole in doses of 30 mg or less per day can be used to treat thyrotoxicosis in breastfeeding mothers.

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RELEASE OF ATRIAL NATRIURETIC PEPTIDE DURING PREGNANCY AND IMMEDIATE PUERPERIUM

SIR,—Atrial natriuretic peptide (ANP) is a serious candidate for the natriuretic hormone whose existence has been postulated for 25 years. However, it is not yet clear how important ANP is for the regulation of sodium and water balance under physiological conditions.

During pregnancy some 900 mmol sodium progressively accumulates and total body water increases by 6–8 litres, distributed amongst the fetus, placenta, and increased maternal blood and interstitial fluid volumes. After delivery this excess maternal sodium and water load is no longer required and is excreted, significant diuresis being evident from the third to fourth day post partum.[1] If ANP has relevance as a natriuretic and diuretic hormone one would expect the redistribution of body fluids post partum to be a major stimulus to release of the hormone.

We have measured plasma ANP concentrations serially in seven healthy women throughout pregnancy and the early puerperium. The women gave informed consent to blood sampling. The women, aged between 26 to 36 years (mean 31), received no proprietary medications during pregnancy other than iron and vitamin supplements. All patients had uneventful pregnancies: blood pressures remained below 140/90 mm Hg, maternal weight gain was normal (mean 10·4 [SE 1·3] kg); and delivery was in the third trimester (at 38·4 [SE 0·9] weeks). Six patients were delivered vaginally and one had a caesarean section for cervical dystocia. Although four patients received intravenous sodium chloride in crystalloid solution during labour, none was given more than 250 mmol, and ANP concentrations at least 72 h later should not have been affected. Blood samples were taken during routine antenatal clinic visits at the beginning, middle, and end of the second trimester, in the last month of pregnancy, and one in the postnatal ward 3–5 days post partum. All venesections were done between 0800 and 0900 hours after the women had fasted overnight and

Appendix I

Stability Study

Each data set was subjected to linear regression analysis and the subsequent lines of best fit gave positive slopes. However, there was a wide variation in the degree of slope between concentrations for both fluids. In the plasma study, the 0.5ug/ml data had a slope approximately a factor of 10 less than the 1.0ug/ml data. In the urine study, the 2ug/ml data had a slope approximately a factor of 10 less than the 1.0ug/ml data. This wide variation between the slopes of the concentrations was further studied in the plasma samples by a t-test which gave a value of 1.133. A value of 2.37 is needed for significance at the 5% level. Thus, there is no evidence of correlation between the two concentration data sets. This positive trend could be due to endogenous material interfering with the methimazole chromatographic peak. However, the lack of correlation between the two sets of concentration data which make this explanation less likely. However, as a precaution against errors due to interference from the sample matrix, spiked samples (quality controls) should be stored with the patient samples. Any deviation of the quality controls from the actual spiked concentration will give a reflection of the accuracy of the values generated for the patient samples.