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ANALYSIS OF BIOLOGIC SAMPLES FOR MORPHINE AND MORPHINE-RELATED COMPOUNDS BY GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC METHODS

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20. Abstract

Methods were investigated for the analysis of biologic samples containing morphine and morphine-related compounds through use of bioanalytical systems involving gas chromatograph-mass spectrometer-computer combined instruments. Most of the work was carried out with a quadrupole mass spectrometer designed for chemical ionization work. Methane was used as the reagent gas.

The studies included the synthesis of stable isotope labeled compounds and derivatives of morphine and morphine-related compounds, and the development of analytical procedures for the determination of free and total morphine and morphine-related compounds in biologic samples. Mass spectral studies were carried out by electron impact ionization, chemical ionization (0.5-1 Torr) and atmospheric pressure ionization mass spectrometry. The procedures were applied in the analysis of a large number of urinary and blood (serum, plasma) samples.

Methods based upon gas chromatograph-mass spectrometer-computer bioanalytical systems show high specificity and high sensitivity in detection, and are generally regarded as reference methods of analysis. The general procedures developed in the course of this work can be used in other applications.

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I. INTRODUCTION

At the present time most analytical methods used for the study of organic compounds in complex mixtures of biologic origin fall into one of three categories. Gas phase analytical methods based upon the use of gas chromatograph-mass spectrometer-computer (GC-MS-COM) analytical systems are coming into wide use; they provide the most reliable and effective methods now known for the analysis of drugs and drug metabolites in biologic samples, and they are also used in environmental research studies for the detection and estimation of toxic organic compounds. Procedures based on saturation analysis are also widely used. Protein binding methods have largely been replaced by radioimmunoassay procedures, and a series of special methods have been developed for drug assays. The EMIT (enzyme mediated immunoassay technique) system is now being evaluated in clinical chemistry laboratories for use in estimating blood concentrations of anticonvulsant drugs, and it may prove to be useful in specific applications of this kind. RIA (radioimmunoassay) and FRAT (free radical assay technique) procedures have been used widely. All of these methods are based upon an essentially biological phenomenon, and their chief weakness is that related compounds may interfere with the determination. Liquid chromatographic methods are used for preliminary purification of the sample when this effect is present. A second difficulty when drug studies are involved is that multiple determinations for several drugs or drug metabolites may be required, and the necessary reagents may not be available. A third group of procedures, many of which are still in use, are based upon spectrophotometric or related techniques developed before the introduction of more precise analytical methods. A current example is the use of high performance liquid chromatography, with an ultraviolet absorption detector, for the analysis of drugs in blood. These methods are not usually highly specific or highly sensitive, but they can be used in some applications.

The specific problem under study, described in this report, was to develop and apply analytical procedures for the determination of morphine and morphine-related compounds in blood and in urine, using a gas chromatograph-mass spectrometer-computer analytical system. This approach was taken because methods based upon these systems provide both a high degree of reliability and high sensitivity of detection; wide ranges of concentrations of drugs in biologic fluids can be determined with a degree of specificity not associated with other methods. Procedures based upon these analytical systems are now generally regarded as reference methods.

This report contains a discussion of gas phase analytical procedures for the study of morphine and morphine-related compounds. Other types of methods are not discussed. The experimental procedures which were developed and used are described in detail, and results are given.

II. BACKGROUND

The most important development in analytical chemistry in many years has been the introduction, development and use of gas phase analytical methodology. The initial step in this direction was the

establishment of the basic concepts and technology for the separation of organic compounds by gas chromatography. This was followed by combining gas chromatography with mass spectrometry, leading to a combined instrument in which the mass spectrometer acted as a detector with unparalleled capabilities for identification and quantification. When small computers (4-8K core) were developed for laboratory use, it became possible to design a new kind of instrument: an analytical system based on a combination of a gas chromatograph, a mass spectrometer and a computer. These systems are the most powerful tools now available for the analysis of complex mixtures of biologic origin for compounds other than macromolecules.

The function of the gas chromatograph in an analytical system is to separate components of the mixtures under study. Many investigations have been carried out dealing with both the theory and practice of gas chromatography, but the most important early advances of importance for biologic studies were the development of thin-film columns, the introduction of derivatization procedures for reducing bonding forces between molecules, and the invention of ionization detectors. The first of these developments occurred in 1959-1960. At that time VandenHeuvel, Sweeley and Horning (1) described the preparation of thin-film GC columns prepared with a thermostable liquid film (a methylsiloxane polymer known as SE-30) which were suitable for the separation of steroids. This work provided the first demonstration of the applicability of GC methods to the separation of an important class of biologic compounds previously thought to be essentially non-volatile and therefore not separable by gas phase methods. (Most organic chemists were aware of the fact that some steroids could be purified by sublimation, but since this was generally carried out under reduced pressure it was thought that a separation which employed atmospheric pressure conditions would not be suitable). Methods for preparing columns of this type, published a few years later (2), are still in use, and the thermostable siloxane polymer SE-30 is still the most widely used of all liquid phases for separations carried out at moderately high temperatures (200-320^{o}). Most of the columns used in this work were SE-30 columns.

The most significant and still most widely used derivatives are trimethylsilyl ethers. These were introduced for steroid separations by Luukkainen, VandenHeuvel, Haahti and Horning (3). At the time that this work was carried out, it was recognized that the most serious difficulty which prevented the wider use of GC methods was the fact that many compounds of biologic significance contained hydroxyl groups, and the resulting hydrogen bonding between molecules made it impossible to volatilize these substances without decomposition. Attempts had been made to use acetyl derivatives in some instances, but when trimethylsilyl derivatives were investigated it was found that they were generally superior to other types of derivatives because of their great thermal stability (hydrolysis usually occurs readily, but thermal elimination of trimethylsilanol does not) and volatility. Several new reagents for preparing trimethylsilyl ether derivatives have been introduced since the initial studies, but the principles and practices developed at that time are still valid and silylation is still the most widely used reaction for derivative formation. It was used in this work.

The introduction of the argon ionization detector and the flame ionization detector were important successive steps in the development of gas chromatography, since they permitted relatively small samples to be used. The argon ionization detector is no longer employed, but flame ionization detection is used almost universally as a general, non-specific and sensitive method of GC detection.

The concepts involved in the design of the combined gas chromatograph-mass spectrometer were familiar to a number of scientists during the period 1958-1960. The principal problem was that of developing a "molecule separator" which would lead to exclusion of most of the carrier gas in the effluent stream from a gas chromatograph, while allowing the sample to proceed into the ion source of a mass spectrometer. This problem was solved by Ryhage through the development of a jet-orifice separator (4); other types of separators were introduced later. The Ryhage separator, together with fast scanning, made it possible to design the first commercial combined GC-MS instrument (LKB 9000).

The next significant development was the introduction of the technique of selective ion monitoring by Holmstedt (5). One or more ions characteristic of the substance under study were monitored during the course of a GC separation in a combined GC-MS instrument. By monitoring both an ion or ions from a reference compound (the internal standard) and the compound of interest it was possible to carry out highly sensitive and highly specific quantification procedures. Holmstedt called the procedure "mass fragmentography". This method is widely used, and it was employed in this work.

The development of small laboratory computers made it possible to design analytical systems of the GC-MS-COM type. These are now used with a disk to provide additional storage. Many operating parameters can be controlled by a computer (or by modern circuitry with microprocessors), and the computer is also used for the acquisition and analysis of data. When a computer is employed, it is also possible to use a repetitive scan technique with computer-based analysis of data at the end of the run in order to identify specific compounds. This procedure, developed by Biemann (6), is called "mass chromatography".

All early GC-MS instruments and GC-MS-COM systems were designed with electron impact (EI) sources. This is the best arrangement when problems of identification are involved. For purposes of quantification, however, chemical ionization (CI) conditions are now generally preferred. The usual reagent gas is methane, although other reagents may be preferred in specific applications.

The current state of development of GC-MS-COM systems may be summarized in the following way. The mass spectrometer may be an electrical field (quadrupole) instrument, or a magnetic field mass spectrometer. Older magnetic field instruments use magnetic field changes for scanning, and accelerating voltage changes for selective ion detection. Design changes are in process for magnetic field instruments in order to avoid the requirement for relaxation time. Quadrupole instruments are well suited to computer-based operation and control. Both CI and EI sources are used; CI conditions are usually employed in quantitative work, while

EI techniques are preferred for identification purposes. The computer is usually a small (4-8K core, 12- or 16-bit) laboratory computer. A disk is often added; provision for visual display of spectra is generally included. Microprocessor control of operation is not yet a fully established technology. The gas chromatograph is usually a packed column instrument of conventional design, although open tubular columns will probably come into wider use in the next few years. Hardware devices for multiple ion detection or selective ion detection (mass fragmentography) are available, but computer-based operation is often preferred.

The system employed in this study was based upon a quadrupole mass spectrometer, with mass range to 800 amu, arranged for chemical ionization. The gas chromatograph was of conventional design, and packed columns (glass) were employed. The computer was a small laboratory computer with a disk, and visual display of spectra was possible. Selective ion detection was employed in quantitative studies.

A prototype atmospheric pressure ionization mass spectrometer was used in some studies. This instrument shows very high sensitivity of detection. Samples were introduced by platinum wire probe. Details are included in a later section.

Investigations were carried out of hydrolysis conditions, extraction and purification methods, procedures for derivative formation, mass spectral characterization and quantitative GC-MS-COM methods for use with morphine and morphine-related compounds. These methods were used for the analysis of urine and blood samples.

III. SURVEY OF METHODS

A. Metabolism and distribution

The literature dealing with morphine and morphine-related compounds is extensive and is distributed through many disciplines. Comparatively little quantitative data relating to morphine metabolism and distribution in humans are available, however, because of the earlier lack of analytical methods with sufficient sensitivity of detection and specificity for use in human studies. The recent review of Boerner, Abbott and Roe (7) summarizes current knowledge of the metabolism of morphine and heroin in humans.

Diacetylmorphine (heroin) is rapidly metabolized to both 6-acetylmorphine and 3-acetylmorphine, but the rate of enzymic hydrolysis of the ester group at the 3-position is considerably faster than that of the corresponding group at the 6-position. As a consequence, the apparent route of metabolism is: 3,6-diacetylmorphine → 6-acetylmorphine → morphine.

Since hydrolytic enzymes are widely distributed in the body, the removal of the 3- and 6-acetyl groups probably begins immediately and occurs at many sites after heroin ingestion. The distribution of these three compounds, however, is likely to be somewhat different for each

compound. The rate of entry of diacetylmorphine into the central nervous system is believed to be faster than that of morphine. After a very short period, however, the metabolic processes which occur are those of morphine itself. The concentration of morphine in blood falls relatively rapidly after a single dose of either heroin or morphine, but low concentrations of morphine persist for a long time. The pattern of distribution is not known in detail, but the primary site of metabolism is the liver and excretion occurs both through urinary and biliary pathways.

From a mass transfer point of view, the principal metabolite of morphine is the 3-glucuronide, and urinary excretion is the principal pathway of excretion. The 6-glucuronide is also a human (and animal) metabolite, but the rate of formation of the 6-glucuronide is very much slower than that of the 3-isomer. For example, in the rabbit the 3-glucuronide accounted for 45% of administered morphine, while the 6-isomer accounted for 0.3%. The formation of sulfate conjugates from phenols is a common reaction, and in the case of morphine the 3-sulfate would be an expected product. Yeh (8) found that the ratio of 3-glucuronide to 3-sulfate in the human (in a pooled urine experiment) was 4:1. The 3-sulfate accounts for 5-10% of administered morphine. The 6-sulfate has not been found as a metabolic product, although it is probably formed in small amount. The rate of reaction of the 6-hydroxyl group is very much slower than that of the 3-hydroxyl group in both types of conjugation reactions. The proportion of glucuronide to sulfate is likely to vary with individuals, and species differences may be large. For example, the sulfate is the major urinary conjugate of morphine in the cat and chicken (9).

The metabolic problems of interest, particularly in terms of physiologically active compounds, involve reactions other than conjugation. Two routes of considerable interest are N-demethylation to form normorphine and O-methylation to form codeine. Since both types of reactions will occur, another metabolite to be expected is norcodeine (N-demethylation of codeine and O-methylation of normorphine). Compounds in the codeine series can form only 6-conjugates, but normorphine can form both a 3-glucuronide and a 3-sulfate. Since 6-conjugation is a relatively slow reaction, codeine would be an expected urinary product, with little conjugation, while normorphine would presumably be excreted in both free and conjugated form. It has been established in a variety of studies that codeine and normorphine are authentic morphine metabolites, but there is also some disagreement about the extent to which these metabolites (particularly codeine) are formed in the human, and the nature of the conjugated products. One study (10) indicated that about 6% of a total morphine dose was converted to urinary codeine, largely in conjugated form. Since codeine is known to undergo N-demethylation, norcodeine would be an expected metabolite as well under these circumstances. The determination of normorphine is more difficult than that of morphine, but recent studies (8,11,12) indicate that both free (about 1-5%) and conjugated (about 1-3%) normorphine will be present as urinary metabolites of morphine. Yeh (13) found 1% free normorphine and 4% total normorphine.

These metabolites account for about 75-85% of an administered dose of morphine. The fate of the remaining material is not known. Some biliary excretion will occur, but conjugates are generally hydrolyzed in the gut and the biliary products are often reabsorbed. It seems likely that additional morphine metabolites remain to be detected, and these may be formed by known types of reaction. The conversion of a tert-amine to an N-oxide is a known metabolic pathway, and morphine N-oxide has been detected as a urinary component after morphine administration (14). A second drug (amiphenizole) was also given at the same time, however, and it is not certain if the N-oxide was a result of enzymic oxidation. A potientially important observation was made in a study (15) of morphine metabolites in rat brain. Two metabolites were detected; these were considered to have catechol or quinoid structures, and they reacted with sulfhydryl groups of proteins. These observations parallel the results of Bolt, Kappus and Remmer (16) with respect to the protein binding of ethynylestradiol after metabolic activation. The P450 oxidation of ethynylestradiol was believed to yield 2-hydroxy-ethynylestradiol. In the case of morphine, the corresponding compound would be 2-hydroxymorphine.

During the past few years, numerous investigations have established the fact that drugs and other exogenous compounds containing olefinic bonds or aromatic rings are metabolized in part through the epoxide-diol pathway. In some instances epoxides have been found as relatively stable metabolites, and in others the evidence rests upon the isolation of dihydrodiols (from aromatic compounds) or other diols of appropriate structure. The formation of a catechol from a phenolic substance may also depend upon epoxidation, although this view is based upon chemical analogies rather than upon direct evidence. Interest in the epoxide pathway of metabolism has increased in recent years because of demonstrations that epoxides can react with cellular components including cofactors, proteins containing SH groups, and DNA. Specific types of epoxides may be required for reaction with DNA, according to the Hulbert (17) hypothesis, but many epoxides will react with sulfhydryl containing proteins. This may be the basis of the cytotoxicity of epoxides.

In the case of morphine, the expected products of epoxidation would be the 7,8-epoxide, 2-hydroxymorphine, and possibly an 11,12-epoxide. It is probable that the 7,8-epoxide would be formed more rapidly from diacetylmorphine and from 6-acetylmorphine than from morphine. None of these substances has been identified with certainty, but the metabolites of Misra, Mitchell and Woods (15) may be related to 2-hydroxymorphine. The suggestion (15) that altered cellular function, caused by reaction of a metabolite with receptor protein, may be the basis of the development of tolerance is difficult to prove, but there is increasing evidence that epoxides can react with cellular protein. It seems likely that the 7,8-epoxide from morphine, diacetylmorphine and 6-acetylmorphine would be formed by microsomal P450 oxidation, and these compounds may be involved in the development of tolerance.

Recognized major and minor pathways, accounting for 75-85% of administered drug, are shown in Chart 1. Compounds which have not been identified as morphine metabolites but which are probably also formed as human products, include the 3-glucuronide of 6-acetylmorphine,

the 6-sulfate of morphine, the 3-glucuronide and 3-sulfate of normorphine (the expected conjugates), norcodeine and unidentified conjugates of codeine. Since the deacetylation reaction occurs very rapidly, studies of the metabolism of diacetylmorphine after an initial period become equivalent to a study of morphine metabolism. The determinations of interest are those of free and conjugated morphine, free and conjugated normorphine and free and conjugated codeine. Low concentrations of morphine last for a long time after the initial period of metabolism.

The chief analytical problems arise from the relatively low concentrations of drug and drug metabolites to be expected in urine and in blood; these problems are discussed in later sections. The isolation of epoxide metabolites, and the detection of 2-hydroxymorphine, were not attempted in this study. These may be important compounds since they may be involved in the development of tolerance.

B. Hydrolysis of conjugates and isolation of samples

The principal urinary compounds arising from diacetylmorphine or morphine ingestion are free morphine, morphine 3-glucuronide and morphine 3-sulfate. Small amounts of normorphine, normorphine 3-glucuronide and normorphine 3-sulfate should also be present, along with a little codeine and norcodeine. In one recent study (18) with several modes of administration of morphine, 65-70% of the dose was excreted in urine as conjugates and 3-9% as free morphine. In another study (13), morphine conjugates amounted to 64% of the dose, while 10% of free morphine was found, along with 4% of normorphine conjugates and 1% of free normorphine (accounting for 83% of the dose).

Although the urinary excretion of morphine occurs relatively rapidly, small amounts continue to be excreted for a long time after an initial dose. The reasons for the retention of morphine in the body are not known; protein binding has been suggested as a possible explanation, and fat solubility may also be involved. As a consequence of this property, however, it is desirable to employ analytical methods capable of detecting and estimating both relatively high concentrations of morphine in urine during the initial period of excretion, and low concentrations for the ensuing hours or days. Since the concentration in blood falls rapidly after the initial dose of diacetylmorphine or morphine, the methods should also be capable of measuring low concentrations in blood of both free and conjugated morphine.

The method chosen for hydrolysis of urinary and blood conjugates of morphine was adapted from techniques used in the study of human urinary steroids. The enzyme was Glusulase; this contains both glucuronidases and sulfatases. The hydrolysis rates of glucuronides and sulfates with this enzyme mixture are influenced by steric effects, but both 3- and 6-conjugates of morphine are hydrolyzed relatively easily. Urinary rates may be slowed by inhibitors (13).

The method used for the extraction of morphine was based upon studies of salt-solvent pair extraction of drugs (19). The fluid (urine or diluted 3:2 plasma or serum) was saturated with ammonium carbonate

and extracted with ethyl acetate. Free morphine is extracted under these conditions; the initial extraction process, however, yields a mixture that requires additional treatment. Morphine and its basic metabolites (normorphine, codeine) were returned to an aqueous phase by extraction of the organic phase with dilute hydrochloric acid. The reextraction of the aqueous solution was carried out with 3:1 chloroform:isopropanol after saturation with ammonium carbonate. This procedure provides a sample suitable for derivatization and instrumental analysis.

When enzymic hydrolysis of urine was employed, morphine and its basic metabolites were removed from aqueous solution by ion exchange chromatography (AG50W). After elution with hydrochloric acid (4N), the desired compounds were extracted with ethyl acetate/ammonium carbonate.

In previous studies, the extraction of normorphine has been recognized as being more difficult than that of morphine. It is necessary to employ alkaline conditions (a pH of 9.3-9.4 has been recommended), and chloroform:isopropanol 3:1 is usually employed for solvent extraction (20). Sodium chloride is often added to depress the solubility of morphine and normorphine in the aqueous phase. The organic bases are returned to an aqueous phase by extraction with dilute hydrochloric acid, and reextracted in the same fashion as in the original extraction step.

The initial solvent extraction provides a sample containing both neutral and basic substances. Neutral materials are largely eliminated when a reextraction step is employed. The effectiveness of the extraction depends upon the pH of the aqueous solution and the extracting solvents, and upon a salting-out affect. The recommended pH varies form 9.4 to 10.3; chloroform:isopropanol 3:1 was the preferred extractant mixture in earlier studies.

Ion exchange column chromatographic procedures have been widely used for the selective removal of bases from biologic samples. In this study, the chief problem proved to be that of separating urinary neutral and basic components, and an ion exchange procedure proved to be satisfactory.

Acidic, alkaline and enzymic procedures have all been used for the hydrolysis of morphine conjugates; alkaline conditions result in very low recovery of free drug, possibly because of air oxidation. The recovery after acidic or enzymic hydrolysis is about the same (95-100%). The use of Glusulase is necessary in order to hydrolyze sulfate as well as glucuronide conjugates.

The direct study of glucuronides by gas phase procedures is possible; the most satisfactory derivatives are the methyl ester-trimethylsilyl ethers. Conjugates of morphine have not been studied in this way, however, since the analytical information that is usually needed is that resulting from an estimation of free and conjugated morphine. In this work, free and conjugated values were determined.

C. Gas chromatography

The most satisfactory derivative of morphine for analytical purposes is the ditrimethylsilyl ether. The phenolic group, and the allylic hydroxyl group, are readily converted to trimethylsilyl ethers by the usual silylating reagents. Bis-trimethylsilylacetamide, bis-trimethylsilyltrifluoracetamide or N-trimethylsilylimidazole may be used; the reaction may be catalyzed by the addition of trimethylchlorosilane, but this is not required. The trimethylsilyl (TMS) derivative has good gas chromatographic properties.

This derivative was employed by Wilkinson and Way (21) in an early quantitative study of morphine metabolism, and it has been used many times in later investigations. Although trimethylsilyl ethers undergo hydrolysis relatively easily, they are thermally stable and show little adsorption on GC columns. Column loss may occur if acidic conditions develop on the column packing; the best way of avoiding this circumstance is to employ an initial 1-2 cm zone of 10% SE-30 packing, according to the practice described by Thenot and Horning (22). The TMS derivative was used in the present study and in the recent method described by Clarke and Foltz (23). Other studies (24-30) have also been based upon the use of TMS derivatives.

Codeine forms a 6-trimethylsilyl ether; this derivative is suitable for analytical studies. Diacetylmorphine does not require derivative formation. 6-Acetylmorphine forms a 3-trimethylsilyl ether. Normorphine forms a ditrimethylsilyl ether, in the same fashion as morphine. The secondary amino group will also react with most silylating reagents, but not with N-trimethylsilylimidazole, to yield an N-trimethylsilyl derivative. Compounds of this type are active silylating agents, and when they are employed as derivatives it is not unusual to find both the free amine and the N-trimethylsilyl derivative present during the GC separation.

Acetyl derivatives of amines have frequently been used in GC identification studies, but the perfluoracyl derivatives usually have better GC properties. Ebbinghausen, Mowat, Vestergaard and Kline (31) recently developed an analytical procedure for the study of morphine and codeine based upon the use of the trifluoracetyl derivative (morphine) and the heptafluorobutyryl derivative (codeine). Smith and Cole (32) used the 3-trifluoracetyl derivative of 6-acetylmorphine in a study of diacetylmorphine metabolism. Ebbinghausen, Mowat and Vestergaard (33) used trifluoracetyl derivatives in a study of codeine metabolism. Diacetylmorphine has been detected and quantified in illicit preparations (34-36).

An internal standard is usually employed when quantitative studies are carried out by GC techniques. Tetraphenylethylene was used in the early work of Wilkinson and Way (21); this is suitable when a flame ionization detector is employed. Smith and Cole (32) used a nitrogen detector; the internal standard was ethylmorphine acetate.

A number of screening procedures in which the identification step is based upon GC data have been described. Derivative formation is not necessary if the purpose is to detect diacetylmorphine or methadone but most screening procedures have as their purpose the detection of a

number of drugs. The problems associated with the development and use of screening methods are manifold. The method of "on-column" silylation (37) is useful in screening work, but in research studies involving quantitative work it is desirable to complete the derivatization step before the sample is analyzed.

D. Mass spectrometry and GC-MS-COM methods

Analytical systems based upon a combination of a gas chromatograph, a mass spectrometer and a computer, and operated as a single instrumental system, provide the most powerful and most reliable method of analysis now known for the study of complex mixtures of biologic origin. They are particularly valuable in studies of drugs and drug metabolism. The function of the gas chromatograph is to separate components of the mixtures under investigation. For example, most drugs yield multiple metabolites; some metabolites may have a physiological action related to that of the original drug, some may have toxic properties due to their specific structure, and some may be inactive. The structural differences introduced through metabolic transformations are usually such that separation of the parent drug and individual metabolites is possible with ordinary GC columns. It is usually necessary to prepare derivatives prior to the instrumental analysis step, since many metabolites contain polar groups which would lead to undue adsorption if derivatives were not prepared. The mass spectrometer provides an intermittent or continuous record of mass spectral data. If the primary purpose of the analysis is to obtain qualitative data, the system may be operated manually so that mass spectra are obtained for each peak detected in the GC effluent stream. In this mode of operation, the "total ion current" is usually used as a guide to determine when spectra should be obtained. A second mode of operation, given the descriptive name of mass chromatography, involves the continuous cycling of the mass spectrometer to provide a series of mass spectra obtained throughout the separation process. Each scan may require about 2 to 6 seconds, depending upon the mass range selected for the scan. Some relaxation time is required between scans when the scan is accomplished by magnetic field changes; if the scan involves electrical field or accelerating voltage changes, the cycling is essentially continuous. An analysis may require 5-10 min if only one or two compounds are under study; if multicomponent analyses are needed, the analysis time may be 30-60 min or more. The mass spectral data are subjected to computer-based analysis. The programs may be relatively simple, but generally a sophisticated program is required in order to deal with problems of incomplete separation. The greatest value of this approach lies in its unparalled capabilities for the detection, often in small amount, of specific compounds of interest because of their beneficial or toxic physiological action. For this reason, electron impact spectra are almost always obtained for analysis. It is also possible in some instances to employ a charge transfer mode of operation with nitrogen as a carrier gas, but this form of operation has never been investigated in detail. The advantage of using fragmentation spectra lies in the fact that it is usually possible to arrive at a unique identification when EI mass spectral data are combined with GC data. Retention behavior is a physical property which is based upon the free energy of solution of the solute under the conditions of the separation, while the fragmentation spectrum is based upon the chemical structure of the compound.

Some types of isomers give virtually identical EI mass spectra, but the retention times will be different. Structurally unrelated compounds may show virtually identical retention behavior with a specific column, but the EI spectra will be different. Identifications based upon criteria involving both physical properties and chemical structure have high validity.

Analytical systems are also used for quantitative purposes. The usual mode of operation is to monitor two or more ions during the course of the separation. The technique was originally called mass fragmentography; other terms that have been used are multiple ion detection, selective ion detection and selected ion detection. In early applications, EI conditions were used with magnetic field instruments, and the usual procedure was to monitor at least two ions derived from the compound under study, and one or two ions derived from an internal reference compound. Response factors were needed to relate observed ion intensities to mass relationships, and ratio measurements were carried out to compare ion intensities for the compound under study and the internal standard. Two fragment ions, or the molecular ion (M^+) and a fragment ion, were used to decrease the possibility of interference from other compounds. Two recent developments in quantitative work have been the use of chemical ionization techniques and the use of stable isotope labeled compounds. The advantage of CI over EI techniques is that it is usually possible to choose an ion found in high yield as the ion whose intensity is to be used (this is frequently the protonated molecule, MH^+) and it is usually possible to conserve the stable isotope label in the ion used for quantification. The preferred stable isotope label is ^{13}C, since there is no discernable isotope effect in the separation or ionization processes for ^{13}C compounds. It is customary to introduce three or more ^{13}C atoms. The adsorption losses will be the same for both labeled and unlabeled compounds, and the retention behavior will be the same. Deuterium labeled compounds are also used. These are usually satisfactory, although there may be a recognizable difference in retention behavior (and perhaps in adsorption losses). For compounds with NCH_3 groups, the usual label is NCD_3. Homologs and analogs have also been used as internal standards. They are less satisfactory than stable isotope labeled compounds, but they have been used in some applications. The usual practice is to monitor two or four ions (one or two each for the compound under investigation and one or two each for the internal standard), and most programs allow for the monitoring of eight ions if necessary (for multicomponent analyses).

The technical problems associated with quantitative work are not simple. From an instrumental point of view, it is necessary to employ power supplies of high stability in quadrupole instruments, and to have a means of detecting or correcting drift for both magnetic field and electrical field instruments. The peak setting is usually made to the nearest 0.1 amu, and adjustments for mass defects may be required. Derivatives should be selected to minimize adsorption losses, and the sample size should be large enough to avoid errors in ion intensity measurements.

In this work, an electrical field (quadrupole) instrument was used in the CI mode with methane as the carrier gas. A conventional 4 mm glass GC column was employed for the separation processes. The internal standard was morphine-d_3 (NCD_3 morphine) prepared from ordinary morphine by N-demethylation followed by conversion to the NCD_3 compound. Two ions were monitored for morphine and for the internal standard. For morphine, these were at 340.2 (corresponding to $(MH-90)^+$) and 414.2 (corresponding to $(MH-15)^+$) for the plasma analyses, and 430.2 (corresponding to MH^+) and 414.2 for the urinary analyses. The corresponding ions were higher by 3 amu for the internal standard. The formation of an ion at $(MH-15)^+$ is normally observed for trimethylsilyl derivatives of all kinds; derivatives of alcohols also usually show strong $(MH-90)^+$ ions under methane CI conditions.

This approach was also used by Clarke and Foltz (23). The same internal standard was used, and morphine ions at 340 amu $(MH-90)^+$ and at 414 amu $(MH-15)^+$ were employed; the di-TMS derivative was prepared with bistrimethylsilylacetamide.

A related approach based upon trifluoracetyl and heptafluorobutyryl derivatives was used by Ebbighausen, Mowat, Vestergaard and Kline (31) and by Ebbighausen, Mowat and Vestergaard (33).

A number of reference substances were prepared and studied by mass spectrometry during the course of this work, and analytical procedures were also employed for the detection of diacetylmorphine, 6-acetylmorphine, codeine and normorphine. Both diacetylmorphine and 6-acetylmorphine are short-lived in the human, but normorphine and codeine should be present in low amount in parallel with morphine concentrations.

Since it was expected that low concentrations in plasma would be encountered, a study was carried out of ionization reactions in an atmospheric pressure ionization (API) mass spectrometer. This is a new instrument (38-43) showing subpicogram sensitivity of detection, in which the ionization process is carried out at atmospheric pressure in a small reaction chamber external to the mass analyzer region of a quadrupole mass spectrometer. Conditions were examined for the formation of MH^+ and M^+ ions. For diacetylmorphine, morphine and codeine, one of the problems in analysis is that the group attached at the 6-position (hydroxyl, acetyl, trimethylsilyloxy) is readily lost under both EI and CI conditions, with the result that MH^+ or M^+ ions are present in low intensity. When M^+ ions are formed from these substances by charge transfer from nitric oxide ions (NO^+), however, the M^+ ions are the base peak. This observation by Jardine and Fenselau (44) was confirmed in API studies. The predominant reaction observed was M^+ ion formation, through charge transfer.

IV. EXPERIMENTAL

A. Synthesis of reference compounds

1. Acyl derivatives

Acetyl derivatives of alcohols or phenols are best prepared by reaction with acetic anhydride, usually in pyridine solution. The preparation of acetylcodeine is a typical procedure. Thirty mg (0.01 mM) of codeine were dissolved in 5 ml of pyridine. One ml of acetic anhydride was added and the mixture was allowed to stand for 24 hours. Ice and water were added, and the product was extracted with 5 portions of 10 ml of chloroform. The combined extracts were dried over anhydrous sodium sulfate, and the solvents were evaporated. The yield was 31 mg (77%) of 6-acetylcodeine as the acetate salt.

The preparation of perfluoroacyl derivatives was described by Ebbinghausen, Mowat, Vestergaard and Kline (31).

2. Alkyl derivatives

The procedure used in this work was first described by Corey (45,46) and later employed by Hakomori (47) for the permethylation of sugars and by Haegele et al (48) for the peralkylation of peptides and amino acids. The preparation of diethylmorphine was carried out in the following way. Morphine hydrochloride (10.7 mg, 0.1 mM) was dissolved in 600 µl of dimethylsulfoxide (distilled over calcium hydride). To this solution, 150 µl of a 1 M solution of methylsulfinylmethide carbanion was added. The reaction mixture was sonicated for 10 minutes to break gel particles which were formed. This was followed by the addition of 10.5 µl of ethyl iodide (equimolar excess) and the reaction mixture was sonicated for 50 minutes. Ice and water were added (approximately 1 ml) and the diethylmorphine was extracted with 2 ml of chloroform. The chloroform solution was washed 3 times with 1 ml portions of water, and the solvent was removed with a stream of nitrogen. The reaction is conveniently carried out in a 3.5 ml screw cap vial, which is flushed with nitrogen when reagents are added, since the carbanion solution is extremely sensitive to moisture and to oxygen. The yield was 10.9 mg (96%).

Dimethylmorphine and ethylcodeine were prepared in the same fashion. Deuterated derivatives were also prepared.

3. Trimethylsilyl (TMS) derivatives

The procedure described by Thenot and Horning (49) was used with slight modification. In a typical procedure, 100-200 µg of compound was reacted with 100 µl of silylating reagent (bistrimethylsilylacetamide or bistrimethylsilyltrifluoracetamide) at 60-100°C for 60 minutes. Aliquots of these solutions were injected.

The expected derivatives were obtained from morphine, codeine and 6-acetylmorphine. Normorphine formed a tri-TMS derivative. Deuterated derivatives were also prepared.

carbamate ester which does not require the preparation of normorphine as the starting compound for the introduction of the N-CD_3 group.

The most satisfactory method involved the use of ethyl chloroformate to effect N-demethylation of morphine, leading to formation of the corresponding normorphine carbamate as described by Elison et al. (51). The reduction of N-carbophenoxynormorphine, according to Abdel-Monem and Portoghese (52), and reduction of N-trichlorocarbethoxynormorphine, as described by Montzka et al. (53), were not as satisfactory.

c. Synthesis of O^3,O^6,N-tricarbethoxynormorphine

The procedure described by Elison et al.(51) was followed without major change, but the final product was identified as O^3,O^6,N-tricarbethoxynormorphine, and not O^3,N-dicarbethoxynormorphine as indicated by the authors.

Normorphine as the free base (28 mg, 0.01 mM), 0.4 ml (4 mM) of ethyl chloroformate, and 1 g (20 mM) of potassium hydroxide in 6 ml of water and 10 ml of chloroform were shaken in a separatory funnel for 15 minutes. The chloroform layer was collected, and the aqueous phase was extracted with 2 portions of 10 ml of chloroform. The combined chloroform extracts were washed with 1N hydrochloric acid and with water. The chloroform solution was evaporated. The yield was 44.2 mg (88%) of a slightly yellow, resin-like material identified by its mass spectrum as a diester carbamate.

d. Preparation of N-CD_3-morphine (morphine-d_3)

O^3,O^6,N-tricarbethoxynormorphine (79 mg, 0.162 mM) was dissolved in 5 ml of tetrahydrofuran. (The tetrahydrofuran was freshly distilled from lithium aluminum hydride.) To this solution, a suspension of 42 mg (1 mM) of lithium aluminum deuteride in 2 ml of tetrahydrofuran was added dropwise and with stirring. After the addition was completed, the reaction mixture was heated under reflux for 2 hours. Ethyl acetate was added to destroy excess reagent. This was followed by the addition of 25 ml of 2N hydrochloric acid and 4 g of potassium tartrate, and the mixture was heated under reflux for 2 hours. After adjusting the pH to 8.3 with aqueous potassium hydroxide, the mixture was extracted with methylene chloride for 24 hours by using a continuous extractor. After evaporation of the solvent, 22 mg (47%) of N-CD_3-morphine (morphine-d_3) was obtained.

B. Mass spectral data

1. Electron impact mass spectra

Electron impact mass spectra were obtained with an LKB 9000 GC-MS combined instrument. The conditions were: ionizing voltage, 20 eV; current, 60 μA; accelerating voltage, 3.5 kV. The column was a 9 ft x 4 mm id glass coil containing 1% SE-30 liquid phase on 100-120 mesh Gas Chrom Q. Helium was used as the carrier gas. Both temperature programmed and isothermal conditions were used.

2. Chemical ionization mass spectra

Chemical ionization mass spectra were obtained with a Finnigan 3200 quadrupole mass spectrometer designed for chemical ionization work. Methane was used as the carrier and reagent (0.5-1 Torr) gas. The ionizing voltage was 100 eV. The glass column (U-tube) was 6 ft x 4 mm id containing 1% SE-30 liquid phase on 100-120 mesh Gas Chrom Q. Both temperature programmed and isothermal conditions were used. The mass range extended to 800 amu.

3. Atmospheric pressure ionization mass spectra

The atmospheric pressure ionization mass spectrometer was a prototype instrument. The mass analyzer was a quadrupole mass spectrometer equipped with pulse counting circuitry. The ionization chambers utilized a ^{63}Ni source or a corona discharge source. Details of the design and operation of this instrument have been published (38-43). Samples were introduced with a platinum wire probe. A liquid chromatograph-mass spectrometer-computer system was also used.

C. Analysis of urine

1. Free morphine and other bases in urine

a. Extraction and derivatization

The extraction step was carried out by the salt-solvent pair extraction procedure of M. G. Horning et al. (19). Ammonium carbonate (solid) was added to saturate 5.0 ml of urine, to which 1.5 μg of morphine-d_3 (NCD_3-morphine) had been added, and the aqueous solution was extracted twice with 5 ml portions of ethyl acetate. The combined organic extracts were dried with anhydrous sodium sulfate, and the solvent was evaporated with the aid of a nitrogen stream.

For the determination of morphine, the sample was converted to derivative form by treatment with bistrimethylsilylacetamide (25-50 μl; 25 μl was used when morphine concentrations were low) at 60° for 20 min. Under these conditions morphine forms a ditrimethylsilyl derivative, while codeine forms a monotrimethylsilyl derivative. 6-Acetylmorphine forms a monotrimethylsilyl derivative, but diacetylmorphine remains unchanged. For the determination of normorphine, 25 μl of N-trimethyl-

4. Preparation of internal reference compounds labeled with deuterium

Internal reference compounds labeled with stable isotopes are the most suitable standards for quantitative analysis by gas chromatograph-mass spectrometer-computer techniques. Deuterium labeled standards possess physical properties nearly identical with their corresponding unlabelled analogues but they are distinguishable by mass spectrometry. Due to the ease and low cost of synthesis, deuterium labeled internal standards are commonly used for the purpose of quantitative analysis of drugs and drug metabolites. Since many drugs contain an N-methyl function, the most accessible site for the introduction of the deuterium label is by forming the N-demethylated compound (normorphine in this work) which in turn is then alkylated using $\underline{d}_3$-labeled methyl iodide to form the N-$\underline{d}_3$-labelled drug (N-$\underline{d}_3$-morphine); the reduction of the carbamate with lithium aluminum deuteride is another method.

a. Preparation of normorphine

Cyanogen bromide method of von Braun (50)

Diacetylmorphine acetate (215 mg, 0.5 mM) was dissolved in 4 ml of anhydrous chloroform. A solution of 96 mg of cyanogen bromide (0.9 mM) in 1 ml of chloroform was added to the solution, and the reaction mixture was heated under reflux for 2.5 hours. The chloroform was evaporated, and the residue was treated with 5 ml of boiling water. The solution was allowed to cool and the precipitate was removed by filtration. After recrystallization from ethanol/water, colorless needles of diacetyl-N-cyanonormorphine were obtained. The yield was 164 mg (86%).

Upon refluxing 121 mg (0.33 mMol) of diacetyl-N-cyanonormorphine for 5 minutes with concentrated hydrochloric acid, the two ester functions were saponified and N-cyanonormorphine crystallized from the cooled mixture. To complete the crystallization process, the mixture was refrigerated overnight. The product was removed by filtration. The yield was 94 mg (95%).

N-Cyanonormorphine was converted to normorphine by refluxing 94 mg (0.317 mM) with 60 ml of 6% hydrochloric acid for 6 hours. The solvent was removed *in vacuo* and the residue was dissolved in ethanol. Normorphine hydrochloride was precipitated upon addition of *n*-pentane. Storage of the mixture (freezer) completed the precipitation process. The product was removed by filtration, washed with cold *n*-pentane, and dried. The yield was 79 mg (81%) of normorphine hydrochloride.

b. Preparation of morphine-N-CD_3 (morphine-$\underline{d}_3$)

The synthesis of morphine labeled with deuterium in the N-methyl group may be accomplished either through direct methylation of normorphine with $\underline{d}_3$-methyl iodide or by the reduction of a carbamate ester of normorphine with lithium aluminum deuteride. The direct route was used by Ebbighausen *et al.*, but as expected the yield was low and other products were obtained as well (codeine-$\underline{d}_6$, unreacted normorphine and norcodeine-$\underline{d}_3$). The method of choice is therefore the utilization of a

silylimidazole was used as the derivatizing reagent under the same conditions; a ditrimethylsilyl derivative was formed. (Conversion to a tritrimethylsilyl derivative occurs with bistrimethylsilylacetamide).

b. GC-MS-COM procedure

A Finnigan Model 3200 GC-MS combined instrument, with a Model 6000 data system, was employed. Methane was used as the carrier and reagent (0.5-1 Torr) gas. A 6 ft x 4 mm id glass U-tube column with 1% SE-30 on 100-120 mesh Gas Chrom Q column packing was used for separation and sample introduction. The column was programmed at 4°/minute from 180°. The ionizing voltage was 100 μA. A solvent/reagent bypass was used.

The system was calibrated with perfluorotributylamine, and then with an authentic sample of the ditrimethylsilyl derivative of morphine.

The ions of interest for the quantification of morphine are at 430.2, 414.2 and 340.2 amu. These correspond to the ions MH^+, $(MH-16)^+$ and $(MH-90)^+$. The related ions for morphine-d_3 are 3.0 amu greater. A study of possible interferences indicated that measurements of each pair of these ions from morphine and morphine-d_3 would be satisfactory; the ions at 414.2/417.2 and 430.2/433.2 amu were chosen. After calibration with an authentic sample of the TMS derivative of morphine, the values 414/417 and 430/433 amu were used.

Ratios of ion intensity values were used to calculate the morphine concentration in the sample. No examples of interference from other substances were encountered, but two pairs of ions were always monitored in order to decrease the possibility of error due to unrecognized interference by other urinary components.

Derivatized samples were subjected to analysis for codeine and normorphine. The codeine analysis was based upon a comparison of ion intensities at 372 amu (MH^+) for codeine and at 433 amu (MH^+ for morphine-d_3), after determination of the response factor under the conditions of operation. Normorphine was not detected in any sample.

Urinary samples which were relatively high in morphine concentration were examined for the presence of diacetylmorphine (370 and 328 amu) and 6-acetylmorphine (as the TMS derivative with ions at 400 and 358 amu). These compounds were not detected in any sample.

2. Total morphine and other bases in urine

a. Hydrolysis, extraction, purification and derivatization

A 5.0 ml sample of urine, to which 1.5 μg of morphine-d_3 had been added, was adjusted to pH 4.5 with 0.5 g of sodium acetate trihydrate and a few drops of acetic acid, and 0.2 ml of Glusulase (Endo Laboratories Inc., Garden City, New York) was added (this corresponds to 30,000 units of β-glucuronidase and 8,000 units of sulfatase). The mixture was kept at 37° for 18 hours. This condition results in the

hydrolysis of morphine glucuronide and morphine sulfate, but it also liberates free steroids from urinary conjugated steroids. Direct extraction results in a mixture containing both morphine and urinary steroids; a fractionation step is necessary before analysis.

Ion exchange fractionation. A small ion exchange column containing 0.30 g of the sulfonic acid ion exchange resin AG 50W-X8 (200-400 mesh) (Bio Rad Laboratories, Richmond, California) in the acid form was prepared in a disposable Pasteur pipette. The flow rate was controlled (at the exit) at 1.5 ml/minute through use of a four-channel peristaltic pump. The hydrolyzed urine sample was passed through the column, and the column was washed with 10 ml of 0.2 N hydrochloric acid. Morphine was eluted with 25 ml of 4N hydrochloric acid. The eluate was evaporated at 40-50° under reduced pressure.

This procedure was developed with the aid of ^{14}C-labeled morphine, as indicated later.

Fractionation by back extraction. The hydrolyzed urine was extracted as described for the isolation of free morphine samples by extraction with ethyl acetate after saturation with ammonium carbonate. Hexane (1 ml) was added to the ethyl acetate solution from the initial extraction. Morphine was extracted into an aqueous solution by washing the organic solution with 2 x 1 ml of 0.1 N hydrochloric acid. The aqueous solution was extracted with ethyl acetate (5 ml) after saturation with solid ammonium carbonate. The ethyl acetate solution was dried with anhydrous sodium sulfate, and the solvent was evaporated with the aid of a nitrogen stream.

Direct extraction. The hydrolyzed urine was extracted with ethyl acetate after saturation with solid ammonium carbonate, as described for the isolation of free morphine samples. The resulting mixture contained the urinary steroids androsterone, etiocholanolone and dehydroepiandrosterone, as well as morphine.

Recovery experiments. Radioactive morphine (N-$^{14}CH_3$), was obtained from Amersham Searle Corp., Arlington Heights, Illinois. This material was used in recovery studies of three procedures: direct extraction, extraction followed by back extraction into aqueous solution, and isolation through use of an ion exchange column. In each instance, the final sample was prepared in scintillation vials; the residue obtained after evaporation of the solvent was dissolved in 0.5 ml of methanol, and 10 ml of toluene/POPOP solution was added for counting.

Preparation of derivatives. These were prepared in the same way as described for free morphine samples.

Trimethylsilyl derivatives of androsterone, etiocholanolone and dehydroepiandrosterone were prepared employing the conditions used for morphine samples. The methoxime-trimethylsilyl derivatives of these steroids were prepared in the usual way (54).

b. GC analyses

Comparisons of retention behavior for the trimethylsilyl derivatives of morphine, codeine and normorphine, and for diacetyl morphine, with the properties of the trimethylsilyl derivatives of androsterone, etiocholanolone and dehydroepiandrosterone, indicated that interference would be expected under the usual conditions, based on use of SE-30 columns. Methylene unit (MU) values were compared for a 1% SE-30 column.

c. GC-MS-COM analyses.

Instrumental analyses of samples were carried out in the same way as for free morphine determinations.

Studies were made by selective ion detection to determine the degree of overlap of urinary steroids and morphine and related substances in the separation process.

Comparisons of results indicated that a fractionation or purification step would be required in order to prevent interference by steroids in the morphine determination. The procedure selected for use was the ion exchange method of sample isolation. This method was used in subsequent urinary analyses.

D. Analysis of serum

1. Free morphine and other bases in serum

a. Extraction, purification and derivatization

Serum samples were extracted by the salt-solvent pair procedure of M. G. Horning et al. (19) as described for urine analyses. One ml of serum, to which 1.5 µg of morphine-d_3 had been added, was extracted twice with 5 ml of ethyl acetate. Hexane (1 ml) was added to the combined ethyl acetate extracts and the organic phase was washed twice with 1 ml of 0.1N hydrochloric acid. The aqueous phase containing morphine and other bases was then extracted with ethyl acetate or with a mixture of chloroform-isopropanol (3:1) after neutralization and saturation with solid ammonium carbonate. The organic solution, dried with anhydrous sodium sulfate, was evaporated with the aid of a nitrogen stream. The residue was treated with 25-50 µl of bistrimethylsilylacetamide at 60° for 20 minutes. Trimethylsilyl derivatives of morphine, codeine and normorphine were formed under these conditions.

In some experiments, proteins were precipitated with tungstic or trichloroacetic acid prior to the extraction step. Lower recoveries were obtained, due to protein binding. The most satisfactory extraction method was that described here.

b. GC-MS-COM analyses

Analyses of serum samples were carried out in the same way as for urine samples. The ions which were monitored were at 414/417 and 340/343 amu.

When the back extraction procedure was used, there was no interference with morphine determinations by endogenous substances.

2. Total morphine and other bases in serum

a. Hydrolysis, extraction, purification and derivatization

The internal standard (1.0 µg) was added to the serum sample (1 ml) and the solution was adjusted to pH 4.5 with acetate buffer after 3:2 dilution with water. The conjugated morphine was hydrolyzed, employing the same conditions as described for total morphine determinations in urine. After hydrolysis, the sample was extracted, purified and derivatized by procedures identical to those described for free morphine determinations in serum.

In some experiments, samples of 0.1 or 0.2 ml were analyzed; these were diluted to 0.5 ml before hydrolysis, and a correspondingly lower amount of morphine-d_3 was added.

b. GC-MS-COM analyses

Analyses of serum samples were carried out in the same way as for free morphine measurements.

After enzymic hydrolysis and purification by back extraction, serum samples gave cleaner mass fragmentograms than urine samples.

V. RESULTS AND DISCUSSION

A. Mass spectral data

1. Electron impact mass spectra

The chief value of electron impact (EI) mass spectral data lies in uses in detection and identification studies, although quantitative work is carried out in some laboratories by EI techniques. In this work, where the objectives involved quantitative measurements, a study of EI spectra in the morphine series was carried out to determine if there were any advantages to be gained by using EI methods with a variety of morphine derivatives. The structures of all compounds used in this work were also validated by EI procedures.

The behavior of organic bases under EI conditions is not always predictable, but M^+ ions are often formed; $(M-H)^+$ ions may also be present. Fragmentation pathways may lead to cleavage products with or without a nitrogen-containing group. For morphine, and morphine-related compounds, elimination of the substituent at the 6-position (Chart 2) and elimination of most of the ring structure containing the 6-position, occurs relatively easily. The base peak is usually M^+, but for diacetylmorphine the most prominent ion corresponds to $(M-CH_2CO)^+$; this is not unexpected for acetates. The major ions for morphine itself are at 285, 215 and 162 amu. The molecular ion (M^+) at 285 amu is the base peak; the ion at 215 amu corresponds to $(M-70)^+$, indicating loss of most of the ring containing the 6-position. O^3,O^6-Dimethylmorphine and O^3,O^6-diethylmorphine had good gas chromatographic properties, and the mass spectra were very similar, allowing for the difference in substituent groups. The major ions corresponded to M^+. Groups that were eliminated from dimethylmorphine led to ions at $(M-15)^+$, $(M-31)^+$ and $(M-84)^+$; the $(M-84)^+$ ion for dimethylmorphine corresponds to $(M-70)^+$ for morphine and $(M-98)^+$ or 243 amu in the diethylmorphine mass spectrum. The ions at 176 and 178 amu for dimethylmorphine correspond to the ions at 190 and 192 amu for diethylmorphine, suggesting that these fragments contain one of the O^3 or O^6 substituent groups. The spectra for the deuterated derivatives dimethylmorphine-d_6 and diethylmorphine-d_{10} showed ions at 179 and 181 amu, and at 195 and 197 amu respectively, indicating that these ions contain only one substituent group of the O^3 and O^6 pair.

Diacetylmorphine has been used as a derivative in gas chromatographic studies, and 6-acetylmorphine is a metabolite of diacetylmorphine. The most suitable derivative of 6-acetylmorphine is O^3-trimethylsilyl-O^6-acetylmorphine. Mass spectra of these compounds are in the Figures.

O^3,O^6-Ditrimethylsilylmorphine is a good derivative of morphine for quantitative studies. The major EI peak is at 429 amu, corresponding to M^+; the ions at 234 and 236 amu correspond to ions found at 176 and 178 amu for dimethylmorphine. The d_9 derivative shows the expected shifts in amu values. A prominent peak at $(M-90)^+$ was not present (elimination

of trimethylsilanol) for the derivative, but the elimination evidently occurred to give an ion at $(M-90-15)^+$ or 324 amu.

The mass spectrum of morphine-$\underline{d}_3$ (NCD_3-morphine) showed a shift of the major peaks of morphine to 288 (M^+), 218 and 165 amu; these were all shifted by 3 amu, indicating that the NCD_3 group was present in these ions. In the spectrum of the ditrimethylsilyl derivative, ions at 432 amu, or M^+, 417, 404, 290, 239, 237, 199 and 149 amu all showed a shift of 3 amu, indicating that all of these ions contained the NCD_3 group.

The TMS derivative of 6-acetylmorphine has good gas chromatographic properties. A characteristic mass spectrum showing ions at 399, 357, 234 and 196 amu was obtained. These ions correspond to M^+, $(M-42)^+$ corresponding to $(M-CH_2CO)^+$, and to the ions at 234 and 196 amu found in the mass spectrum of O^3,O^6-ditrimethylsilylmorphine. This indicates that the ions at 234 and 196 amu contained the aromatic ring with its 3-substituent group, and as shown earlier, the NCH_3 group.

Mass spectra were also obtained for a group of compounds in the normorphine series. When the NH group was present, the base peak was a cleavage product; when the $NSi(CH_3)_3$ group was present, the molecular ion was the base peak. The peak at 222 amu for the ditrimethylsilyl derivative of normorphine probably corresponds to the peak at 237 observed for the related derivative of morphine.

Intermediates in syntheses leading to morphine-$\underline{d}_3$ were also characterized by their mass spectra. These spectra are included in the Figures.

2. Chemical ionization mass spectra

All methane chemical ionization mass spectra obtained for morphine and morphine derivatives, and for related compounds, showed well defined fragmentation patterns. Ions due to MH^+ and M^+ were present, along with ions resulting from the elimination of the substituent group at the 6-position. Small peaks corresponding to $(M+29)^+$ and $(M+41)^+$ were also present.

Morphine showed ions at 286, 285, 284 and 268 amu, corresponding to MH^+, M^+, $(M-H)^+$ and $(MH-18)^+$. Ions from the ditrimethylsilyl derivative of morphine were found at 430, 429, 428, 414 and 340 amu. These correspond to MH^+, M^+, $(M-H)^+$, $(MH-16)^+$ and $(MH-90)^+$. The methyl group elimination leading to the $(MH-CH_4)^+$ ion involves a trimethylsilyl group, and not the NCH_3 group.

The same effects are shown in the methane chemical ionization mass spectrum of the O^3-trimethylsilyl derivative of morphine. The base peak corresponds to $(MH-18)^+$, arising from the elimination of the 6-hydroxyl group as water. Other peaks were found to correspond to MH^+, M^+ and $(M-H)^+$, and to $(MH-CH_4)^+$.

Diacetylmorphine showed ions at 370, 369, 368 and 310 amu, corresponding to MH^+, M^+, $(M-H)^+$ and $(MH-CH_3COOH)^+$. The base peak resulted from the elimination of the substituent group in the 6-position.

Codeine gave the expected ions at 300, 299, 298 and 382 amu, corresponding to MH^+, M^+, $(M-H)^+$ and $(MH-18)^+$, with the latter ion as the base peak. 6-Acetylcodeine showed ions at 342, 341, 340 and 282 amu, corresponding to MH^+, M^+, $(M-H)^+$ and $(MH-CH_3COOH)^+$.

Normorphine showed ions at 272, 271, 270 and 254 amu, corresponding to MH^+, M^+, $(M-H)^+$ and $(MH-18)^+$, with the MH^+ ion as the base peak. The tritrimethylsilyl derivative of normorphine gave ions at 488, 487, 472 and 398 amu, corresponding to MH^+, M^+, $(MH-16)^+$ and $(MH-90)^+$.

Isobutane CI spectra were similar to methane CI spectra, but the mass spectrum of the ditrimethylsilyl ether showed only two (rather than three) major peaks, corresponding to MH^+ and $(MH-90)^+$. The same effect was found in the mass spectrum of the trimethylsilyl derivative of normorphine. This difference is characteristic of the reagents.

The condition chosen for quantitative work was based on chemical ionization with methane as the reagent gas, and with derivatives obtained by silylation.

3. Atmospheric pressure ionization mass spectra

The observation that chemical ionization mass spectra of morphine and related compounds, obtained with methane or isobutane as reagent gases, usually led to fragment ions as the base peaks (due to elimination of the 6-position substituent group) led Jardine and Fenselau (44) to investigate the use of nitric oxide as a reagent gas. It was found that M^+ ions were formed by charge transfer, and that fragmentation did not occur. Similar studies were carried out by atmospheric pressure ionization techniques. It is not difficult to use 0.1% nitric oxide in helium as the ionizing gas, since a heated filament is not present. Both morphine, codeine and diacetylmorphine formed M^+ ions by charge transfer, as indicated in the Figures. This is a satisfactory method for the ionization of morphine and morphine-related compounds, and it may be used to detect impurities in morphine-related preparations. For example, a sample of acetylcodeine (O^3-methyl-O^6-acetylmorphine) was analyzed by atmospheric pressure ionization mass spectrometry with nitric oxide as the reagent gas. The original sample evidently contained free morphine, since the minor components were found to be diacetylmorphine, a monoacetylmorphine, and morphine, as well as codeine.

A sample of the trimethylsilyl derivative of codeine gave an unusual mass spectrum indicating the presence of a codeine-related impurity; this result requires further study.

In a separate study of the use of a liquid chromatograph-mass spectrometer-computer analytical system based on API mass spectrometry, the sensitivity of detection of diethylmorphine was investigated. The

d_{10} labeled substance was employed, since this would be required as an internal reference compound for the diethyl derivative. About 1 ng could be detected (Figure 40); the limiting sensitivity of detection of the LC-MS(API)-COM system is about 0.5 ng. On a concentration basis, this is about the same as the subpicogram sensitivity of detection demonstrated for the API mass spectrometer alone.

B. Analysis of urine

1. Free morphine and other bases in urine

Exploratory studies with the procedure described in the Experimental Section indicated that interfering substances were not likely to be encountered in urine except after hydrolysis. The free morphine method, with the ditrimethylsilyl derivative, was then applied to a number of urine samples. The internal reference compound was morphine-d_3 (NCD_3-morphine). To avoid the possibility of error, two ions were monitored, as indicated in the procedure.

The results of a series of urinary analyses are in the Appendix. Since morphine is excreted largely in conjugated form, the concentrations of morphine as free morphine are much less than those expressed as total morphine for all samples containing an appreciable concentration of morphine. For samples containing only trace amounts of morphine, the concentrations may be nearly the same. Free codeine was found in samples obtained soon after drug ingestion in amounts corresponding to 1-5% of the morphine concentration.

Diacetylmorphine and free 6-acetylmorphine were not detected in urine samples, for those samples containing relatively large amounts of morphine.

2. Total morphine and other bases in urine

The determination of total morphine concentration in urine presents a number of difficulties. Yeh (13) found that the hydrolysis of the 3-glucuronide of morphine was slow and dependent upon the volume of urine. This effect may be due to unrecognized inhibitors, but a more likely source of difficulty lies in the fact that steroid glucuronides are present and these may act as competitive substrates. When a large excess of enzyme was used, the hydrolysis of conjugates was complete. Direct extraction, however, yielded a sample that was not suitable for analysis. Continued study of the problem indicated that steroids were responsible for the observed interferences. This may also have been the source of the interferences described by Ikekawa et al. (55) for samples obtained by acid hydrolysis of urine.

Figures 41 and 42 show the nature of the problem, and two solutions. Ion monitoring at 430.2, 414.2 and 340.2 amu for the ditrimethylsilyl ether of morphine, and at the corresponding masses for the derivative of morphine-d_3 (3 amu greater) showed a considerable amount of interference due to other compounds. Back extraction into an aqueous solution, followed by reextraction, gave suitable samples (Figure 41). The use of an ion exchange column also gave good results. The analytical samples prepared in this way were free of interference from steroids (Figure 42).

Chart 3 shows the origin of the interference due to urinary steroids. Trimethylsilyl derivatives of androsterone, etiocholanolone and dehydroepiandrosterone are eluted from non-polar columns with nearly the same retention time as the trimethylsilyl derivative of morphine. These three steroids, under the conditions used for the derivatization of morphine, will form both the expected O^3-trimethylsilyl derivatives and the derivatives of the enol form of the steroids (O^3,O^{17}-ditrimethylsilyl derivatives), as indicated in Chart 2. The GC separation with an SE-30 column of the O^3-trimethylsilyl derivatives of androsterone and dehydroepiandrosterone, and of the ditrimethylsilyl derivative of morphine, is shown in Figure 43. This was obtained by selective ion monitoring under CI conditions. The O^3,O^{17}-ditrimethylsilyl derivatives of androsterone and etiocholanolone are also not well separated from the morphine derivative, so that interference may be expected from three steroid derivatives: the two derivatives of the enol forms of androsterone and etiocholanolone, and the O^3-derivative of dehydroepiandrosterone. Introduction of the ion exchange procedure for sample treatment resulted in analytical samples which were free of interference from steroids. Figure 42 shows the change in a typical sample; the ion exchange procedure decreases greatly the degree of interference for morphine-$\underline{d}_3$ ions which would otherwise be present.

The conditions for the elution of morphine from the ion exchange column were established by use of radioactive morphine. Figure 44 shows the elution step as accomplished with 4N hydrochloric acid.

Urinary samples were analyzed for total morphine content after hydrolysis and with use of the ion exchange method of sample purification. The results are in the Tables in the Appendix. The excretion of morphine occurs relatively rapidly, but small amounts are present in urine for a number of days after the major period of excretion has ended. This effect has been noted previously. The urinary excretion of morphine occurs primarily through conjugation.

Normorphine was not detected as a urinary metabolite.

In a few instances a slight increase in urinary morphine was noted well after establishment of trace excretion concentrations. The effect is not believed to be due to analytical artifacts or interference from unknown sources (for example, a human metabolite), but the increases are also so small that direct drug ingestion is not likely. Indirect transfer through smoke may be a possibility; this is known to occur for nicotine.

C. Analysis of serum

The determination of morphine in plasma, serum or cerebrospinal fluid, for samples containing relatively low concentrations of morphine, is difficult because of the small sample size available for analysis, and because of sample loss during the process of isolation and transfer into a small volume of derivatization reagent(s). These problems were discussed by Wilkinson and Way (21) for a gas chromatographic method based upon the use of the ditrimethylsilyl ether of morphine as the derivative of choice for the determination. Some sample loss was en-

countered due to the adsorption of morphine hydrochloride on glass during the concentration of a 1N hydrochloric acid extract. The internal reference compound was tetraphenylethylene.

The method developed in the course of this work involved extraction by the salt/solvent procedure of M. G. Horning et al. (19), followed by back extraction with 0.1N hydrochloric acid (after the addition of hexane to depress the solubility of morphine hydrochloride in the organic solution), and reextraction with chloroform:isopropanol (3:1) after saturation with ammonium carbonate. The final evaporation of the transfer solvent (methanol) and the derivatization step were carried out in conical tubes (Reactivials). In this sequence of steps, the back extraction showed considerable losses until hexane was added to the ethyl acetate solution containing the sample.

The internal reference compound was morphine-d_3, and the instrumental analysis was carried out by GC-MS-COM techniques using methane chemical ionization. The ion pairs which were monitored were at 414/417 and 340/343 amu; the derivatives were the ditrimethylsilyl ethers of morphine and morphine-d_3. The instrumental analysis procedures were similar to those used in urinary analyses.

Morphine concentrations were determined both as free morphine and as total morphine, after enzymic hydrolysis with Glusulase of diluted samples.

Blood samples (serum or plasma) from humans and from animal experiments with baboons were analyzed both for free and total morphine. The results are in the Appendix.

Figure 45 shows a comparison of morphine analyses both before and after enzymic hydrolysis. Most of the morphine in blood, after morphine or diacetylmorphine ingestion, is present in conjugated form. From urinary studies, the major conjugate is known to be the 3-glucuronide. The transformation of diacetylmorphine into 6-acetylmorphine and morphine is extremely rapid; in the dog (32) the half-lives of the acetylated compounds are a few minutes.

VI. CONCLUSIONS

The most reliable and satisfactory methods for the analysis of biologic samples containing morphine and morphine-related compounds are based upon the use of gas chromatograph-mass spectrometer-computer analytical systems. In this work, a system based upon a quadrupole (electrical field) mass spectrometer was employed; the instrument was designed for chemical ionization work, and methane was used as the reagent gas.

A series of studies were carried out which included the synthesis of stable isotope labeled compounds and of a variety of derivatives of morphine and morphine-related compounds, and the development of analytical procedures for the determination of free and total morphine and morphine-related compounds in biologic samples. Mass spectral studies

were carried out by electron impact ionization, chemical ionization (0.5-1 Torr) and atmospheric pressure ionization mass spectrometry. The methods were applied in the analysis of a large number of urinary and some blood (serum,plasma) samples.

The procedures developed and applied in the course of this work can be used in other applications. Methods based upon GC-MS-COM systems show high specificity and high sensitivity in detection, and are generally regarded as reference methods of analysis.

METABOLIC PATHWAY OF DIACETYLMORPHINE

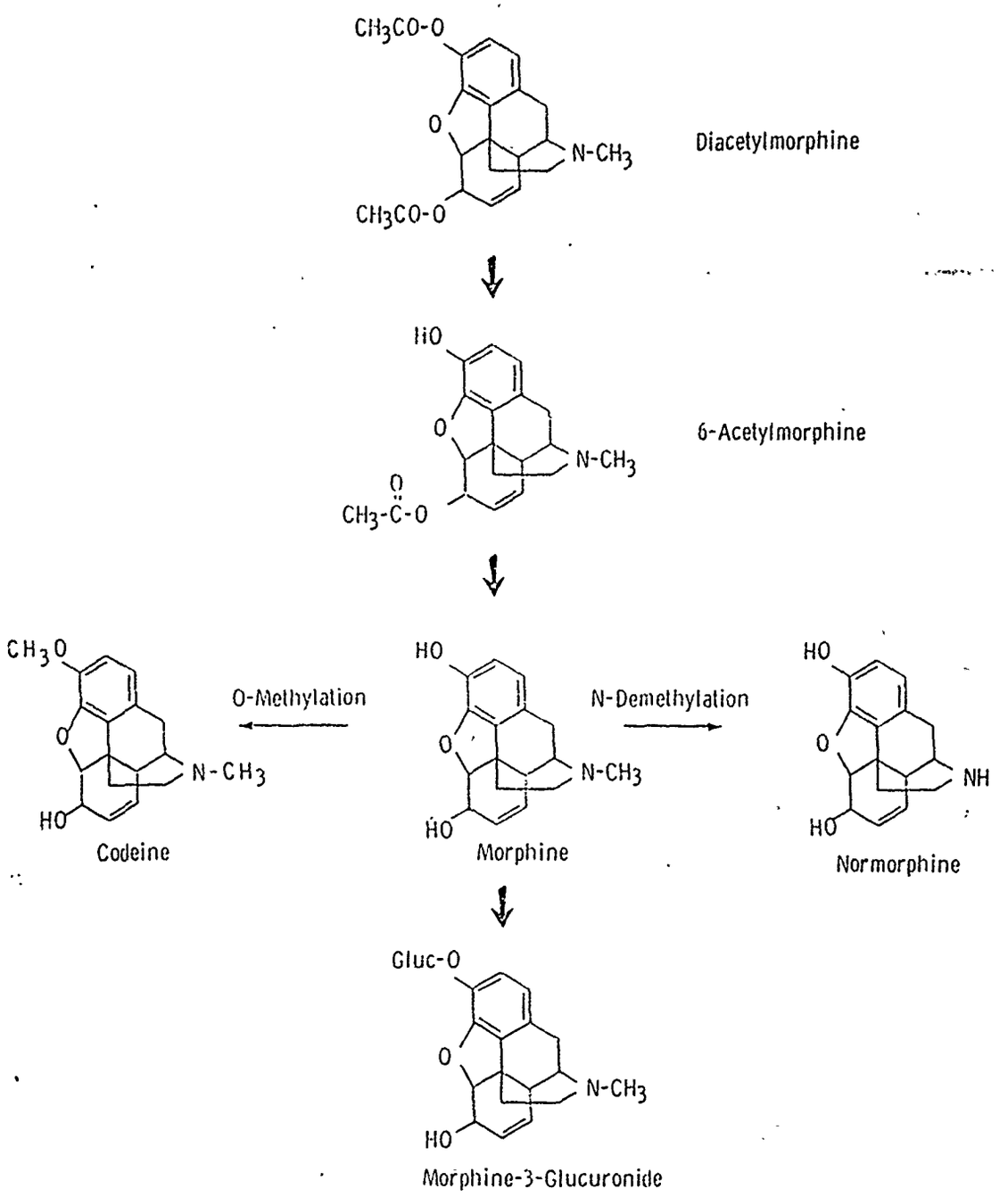
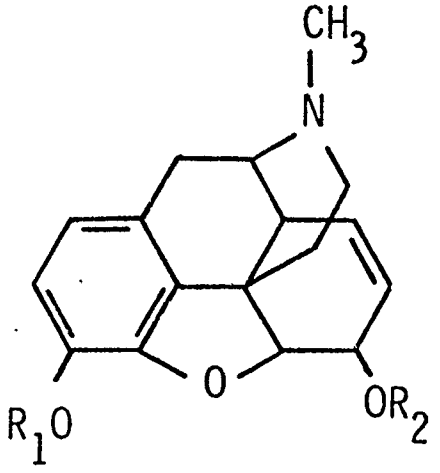
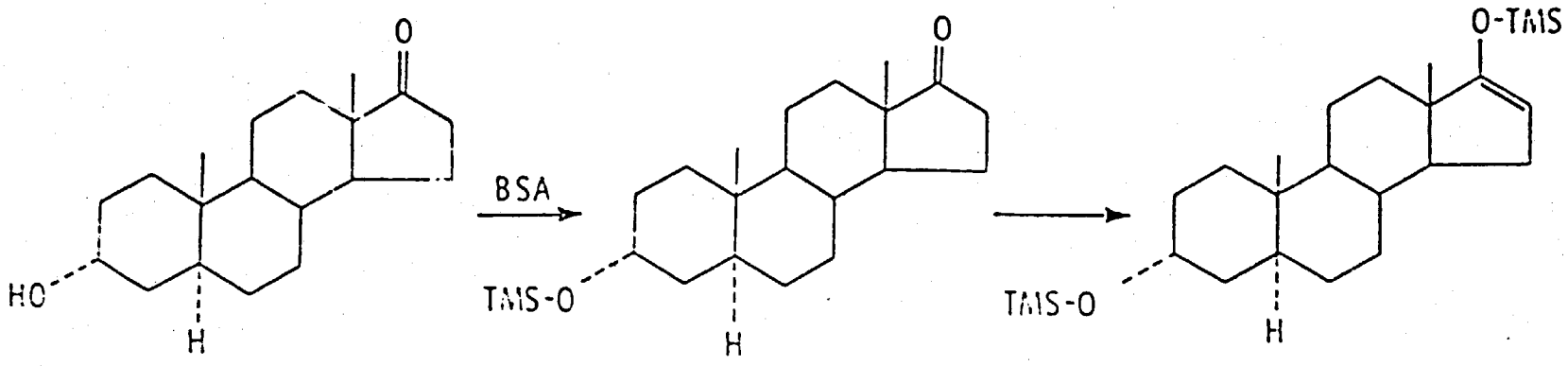


Chart 1



$R_1 = H$	$R_2 = H$	MORPHINE
$R_1 = CH_3$	$R_2 = H$	CODEINE
$R_1 = CH_3CO$	$R_2 = CH_3CO$	O^3, O^6-DIACETYLMORPHINE
$R_1 = H$	$R_2 = CH_3CO$	O^6-ACETYLMORPHINE
$R_1 = CH_3$	$R_2 = CH_3$	O^3, O^6-DIMETHYLMORPHINE
$R_1 = C_2H_5$	$R_2 = C_2H_5$	O^3, O^6-DIETHYLMORPHINE
$R_1 = Si(CH_3)_3$	$R_2 = Si(CH_3)_3$	O^3, O^6-DITRIMETHYLSILYLMORPHINE
$R_1 = CH_3$	$R_2 = C_2H_5$	O^3-METHYL-O^6-ETHYLMORPHINE

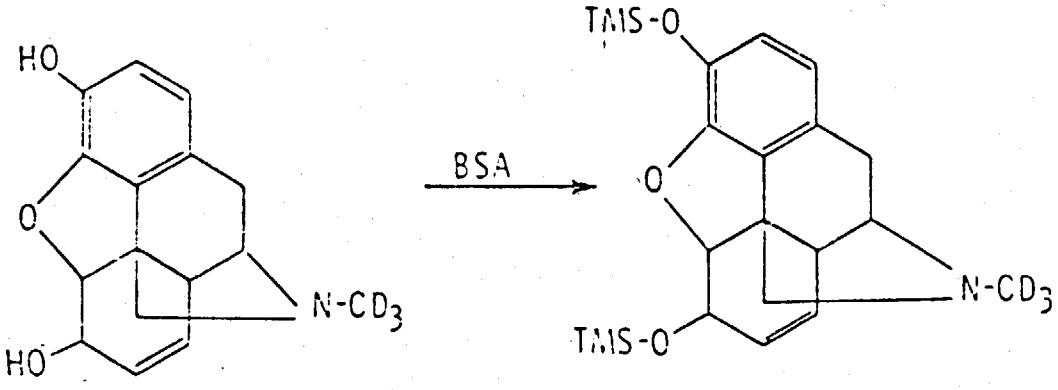
NORMORPHINE SERIES : NH IN PLACE OF NCH_3



ANDROSTERONE
MW = 290

MW = 362
MU = 24.57

MW = 434
MU = 25.22



MORPHINE N-CD$_3$
MW = 288

MW = 432
MU = 25.05

1% SE 30

Chart 3

Titles to Figures

Figure 1. Mass spectrum of morphine; EI, 20 eV.

Figure 2. Mass spectrum of O^3,O^6-dimethylmorphine; EI, 20 eV.

Figure 3. Mass spectrum of deuterium labeled O^3,O^6-dimethylmorphine, with deuterium (d_6) labels in the methyl groups; EI, 20 eV.

Figure 4. Mass spectrum of O^3,O^6-diethylmorphine; EI, 20 eV.

Figure 5. Mass spectrum of deuterium labeled O^3,O^6-diethylmorphine, with deuterium ($\underline{d}_{10}$) labels in the ethyl groups; EI, 20 eV.

Figure 6. Mass spectrum of diacetylmorphine; EI, 20 eV.

Figure 7. Mass spectrum of O^3,O^6-ditrimethylsilylmorphine; EI, 20 eV.

Figure 8. Mass spectrum of deuterium labeled O^3,O^6-ditrimethylsilylmorphine, with deuterium ($\underline{d}_{18}$) labels in the trimethylsilyl groups; EI, 20 eV.

Figure 9. Mass spectrum of deuterium labeled morphine, with deuterium ($\underline{d}_3$) labels in the N-methyl group; EI, 20 eV.

Figure 10. Mass spectrum of deuterium labeled O^3,O^6-ditrimethylsilylmorphine, with deuterium ($\underline{d}_3$) labels in the N-methyl group; EI, 20 eV.

Figure 11. Mass spectrum of O^6-acetylmorphine; EI, 20 eV.

Figure 12. Mass spectrum of deuterium labeled O^6-acetylmorphine, with deuterium ($\underline{d}_3$) labels in the N-methyl group; EI, 20 eV.

Figure 13. Mass spectrum of O^3-trimethylsilyl-O^6-acetylmorphine; EI, 20 eV.

Figure 14. Mass spectrum of O^3,O^6-ditrimethylsilylnormorphine; EI, 20 eV.

Figure 15. Mass spectrum of O^3,O^6,N-tritrimethylsilylnormorphine; EI, 20 eV.

Figure 16. Mass spectrum of O^3-methyl-O^6-trimethylsilylnormorphine; EI, 20 eV.

Figure 17. Mass spectrum of O^3-methyl-O^6,N-ditrimethylsilylnormorphine; EI, 20 eV.

Figure 18. Mass spectrum of O^3,O^6-diacetyl-N-cyanonormorphine; EI, 20 eV.

Figure 19. Mass spectrum of N-cyanonormorphine; EI, 20 eV.

Figure 20. Mass spectrum of O^3,O^6,N-tricarbethoxynormorphine.

Figure 21. Mass spectrum of morphine; CI, methane.

Figure 22. Mass spectrum of O^3,O^6-ditrimethylsilylmorphine; CI, methane.

Figure 23. Mass spectrum of O^3-trimethylsilylmorphine; CI, methane.

Figure 24. Mass spectrum of O^3,O^6-diacetylmorphine; CI, methane.

Figure 25. Mass spectrum of O^3-methylmorphine (codeine); CI, methane.

Figure 26. Mass spectrum of O^3-methyl-O^6-trimethylsilylmorphine (O^6-trimethylsilyl ether of codeine); CI, methane.

Figure 27. Mass spectrum of O^3-methyl-O^6-acetylmorphine (O^6-acetyl derivative of codeine); CI, methnae.

Figure 28. Mass spectrum of normorphine; CI, methane.

Figure 29. Mass spectrum of O^3,O^6,N-tritrimethylsilylnormorphine; CI, methane.

Figure 30. Mass spectrum of morphine; CI, isobutane.

Figure 31. Mass spectrum of O^3,O^6-ditrimethylsilylmorphine; CI, isobutane.

Figure 32. Mass spectrum of O^3,O^6-diacetylmorphine; CI, isobutane.

Figure 33. Mass spectrum of O^3-methylmorphine (codeine); CI, isobutane.

Figure 34. Mass spectrum of O^3-methyl-O^6-acetylmorphine (O^6-acetyl derivative of codeine); CI, isobutane.

Figure 35. Mass spectrum of normorphine; CI, isobutane.

Figure 36. Mass spectrum of O^3,O^6,N-tritrimethylsilylnormorphine; CI, isobutane.

Figure 37. Mass spectrum of a sample of codeine derivatized by formation of the O^6-trimethylsilyl ether; API, nitric oxide.

Figure 38. Upper panel: mass spectrum of O^3,O^6-diacetylmorphine; API, nitric oxide. Lower panel: mass spectrum of a sample of codeine derivatized by acetylation; API, nitric oxide. Some unreacted codeine was present. Morphine was also present as an impurity, leading to te presence of O^3,O^6-diacetylmorphine, a monoacetylmorphine and morphine in the analytical sample.

Figure 39. Upper panel: mass spectrum of morphine; API, nitric oxide. Lower panel: mass spectrum of codeine; API, nitric oxide.

Figure 40. Detection of O^3,O^6-diethylmorphine-$\underline{d}_{10}$ (labeled with deuterium in the ethyl groups) with a LC-MS-COM system based on an API mass spectrometer.

Figure 41. Selective ion detection charts for the analysis of morphine in urine, employing the O^3,O^6-ditrimethylsilyl ether of morphine as the derivative, and with CI (methane) mode of operation. Left panel: analysis of a sample extracted directly from urine. Right panel: analysis of a sample partially purified by back extraction. In both instances the internal standard was morphine-$\underline{d}_3$ (NCD_3-morphine).

Figure 42. Selective ion detection charts for the analysis of morphine in urine, employing the O^3,O^6-ditrimethylsilyl ether of morphine as the derivative, and with CI (methane) mode of operation. Left panel: analysis of a sample extracted directly from urine. Right panel: analysis of a sample partially purified by an ion exchange procedure. In both instances the internal standard was morphine-$\underline{d}_3$ (NCD_3-morphine).

Figure 43. Selective ion detection chart showing the interference of the trimethylsilyl ether derivative of dehydroepiandrosterone with the O^3,O^6-ditrimethylsilyl ether derivative of morphine. The trimethylsilyl ether derivative of androsterone is eluted before the derivative of morphine; the corresponding derivative of etiocholanolene is also eluted before the derivative of morphine.

Figure 44. Elution of morphine from an ion exchange column (AG 50Wx8) with 4N hydrochloric acid.

Figure 45. Selective ion detection charts showing the analysis of a plasma sample for free (left panel) and total (right panel) morphine. Morphine-$\underline{d}_3$ (NCD_3-morphine) was used as the internal standard; the derivatives were the O^3,O^6-ditrimethylsilyl ethers.

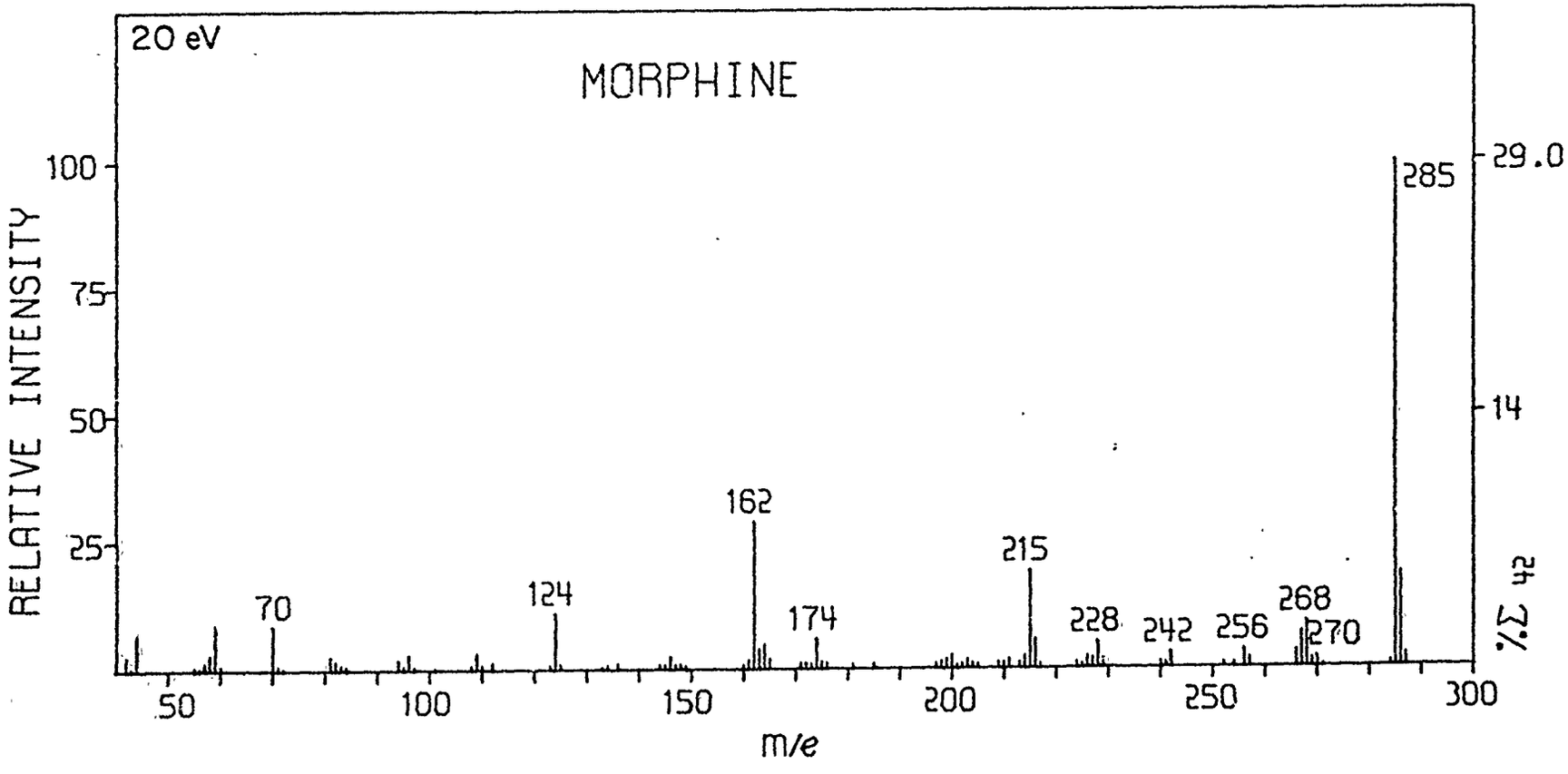

20 eV
MORPHINE
RELATIVE INTENSITY
100
75
50
25
29.0
14
24.7%
285
162
215
124
70
174
228
242
256
268
270
50
100
150
200
250
300
m/e

Figure 1

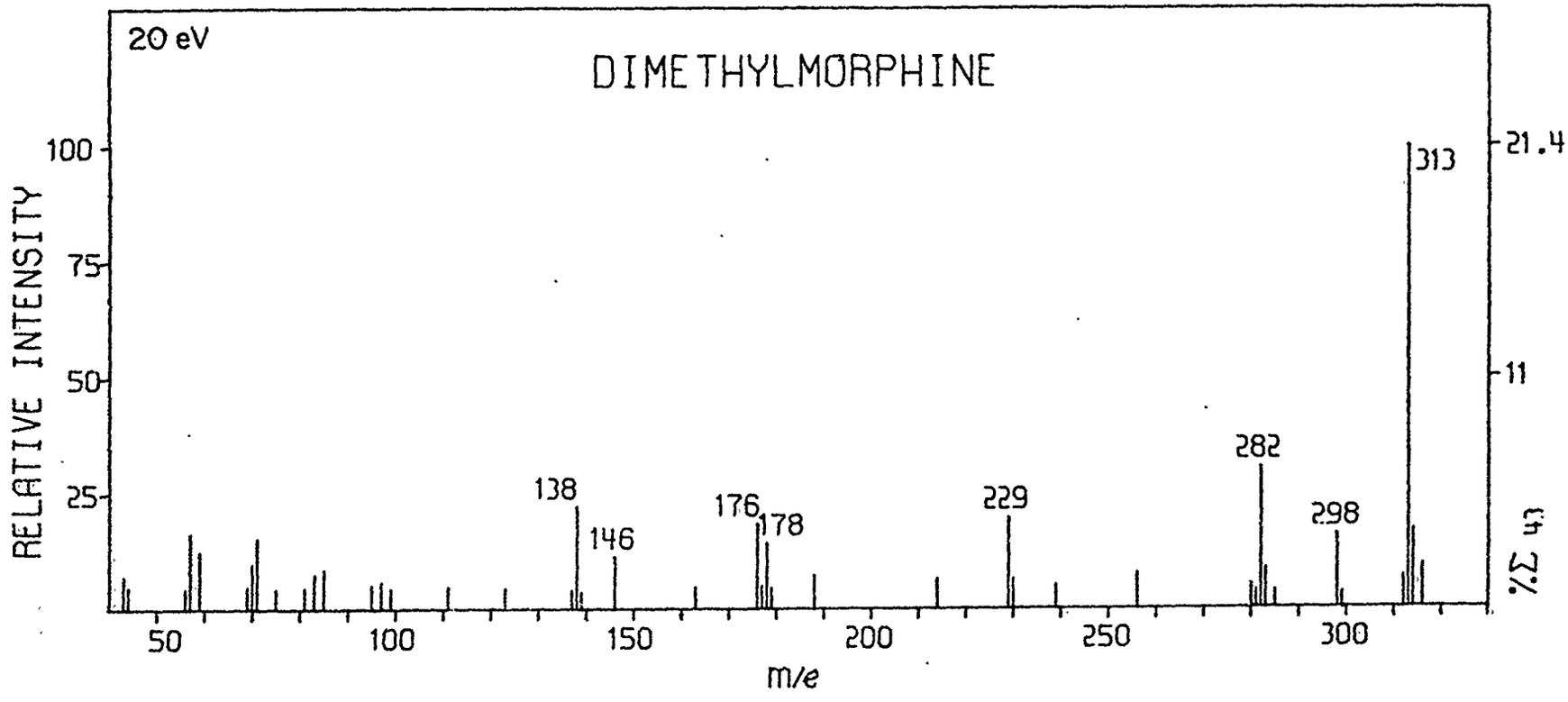

20 eV
DIMETHYLMORPHINE
RELATIVE INTENSITY
100
75
50
25
21.4
11
4.7%
313
282
298
229
176
178
138
146
50
100
150
200
250
300
m/e

Figure 2

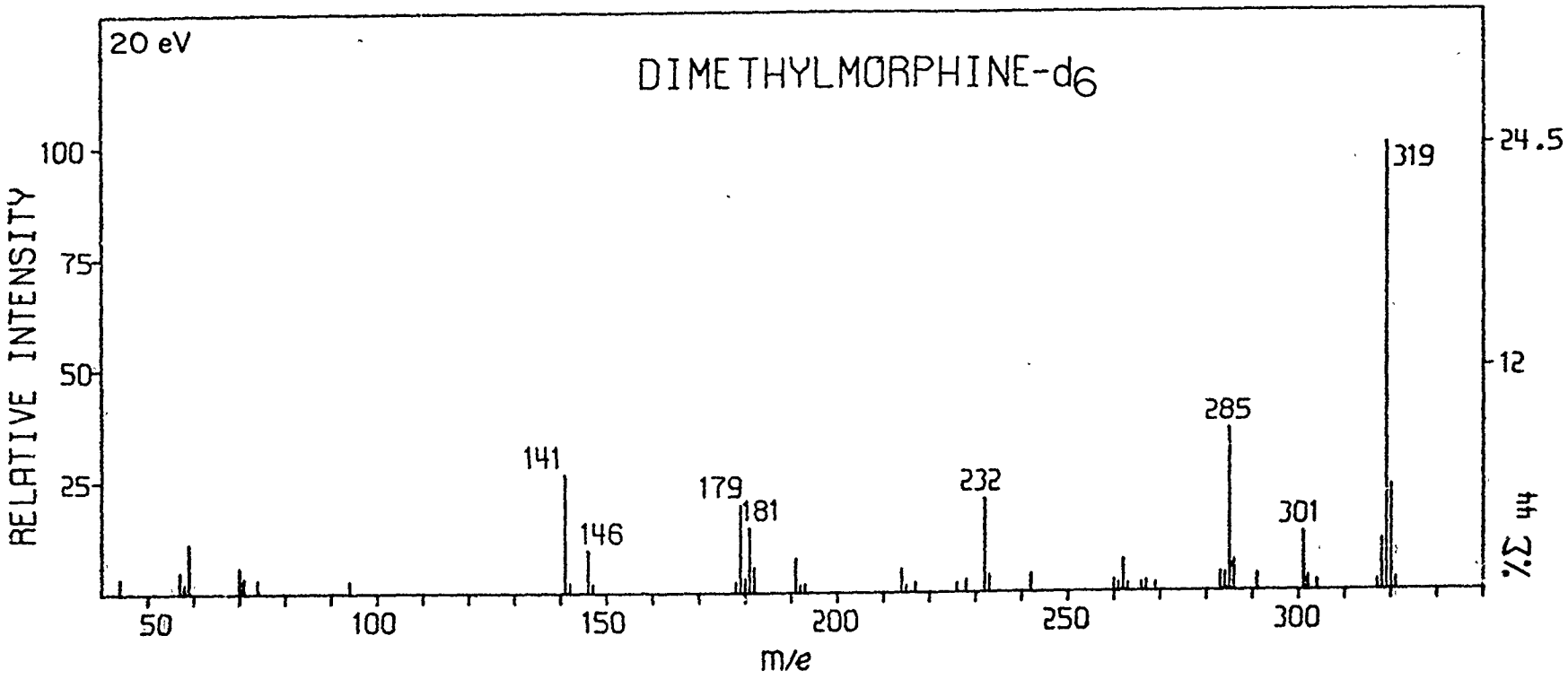

20 eV
DIMETHYLMORPHINE-d6
RELATIVE INTENSITY
100
75
50
25
24.5
12
Σ% 44
319
285
232
301
141
146
179
181
50
100
150
200
250
300
m/e

Figure 3

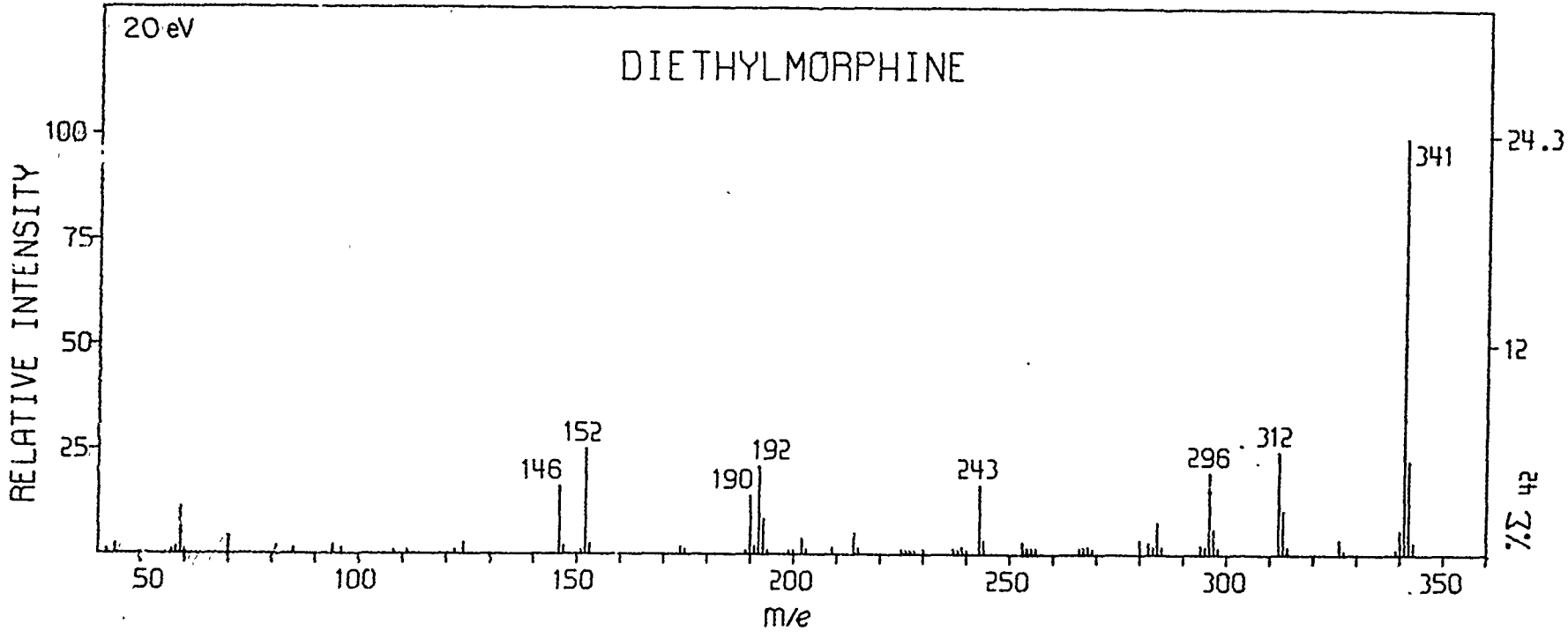

20 eV
DIETHYLMORPHINE
RELATIVE INTENSITY
100
75
50
25
146
152
190
192
243
296
312
341
24.3
12
42 Σ%
50
100
150
200
250
300
350
m/e

Figure 4

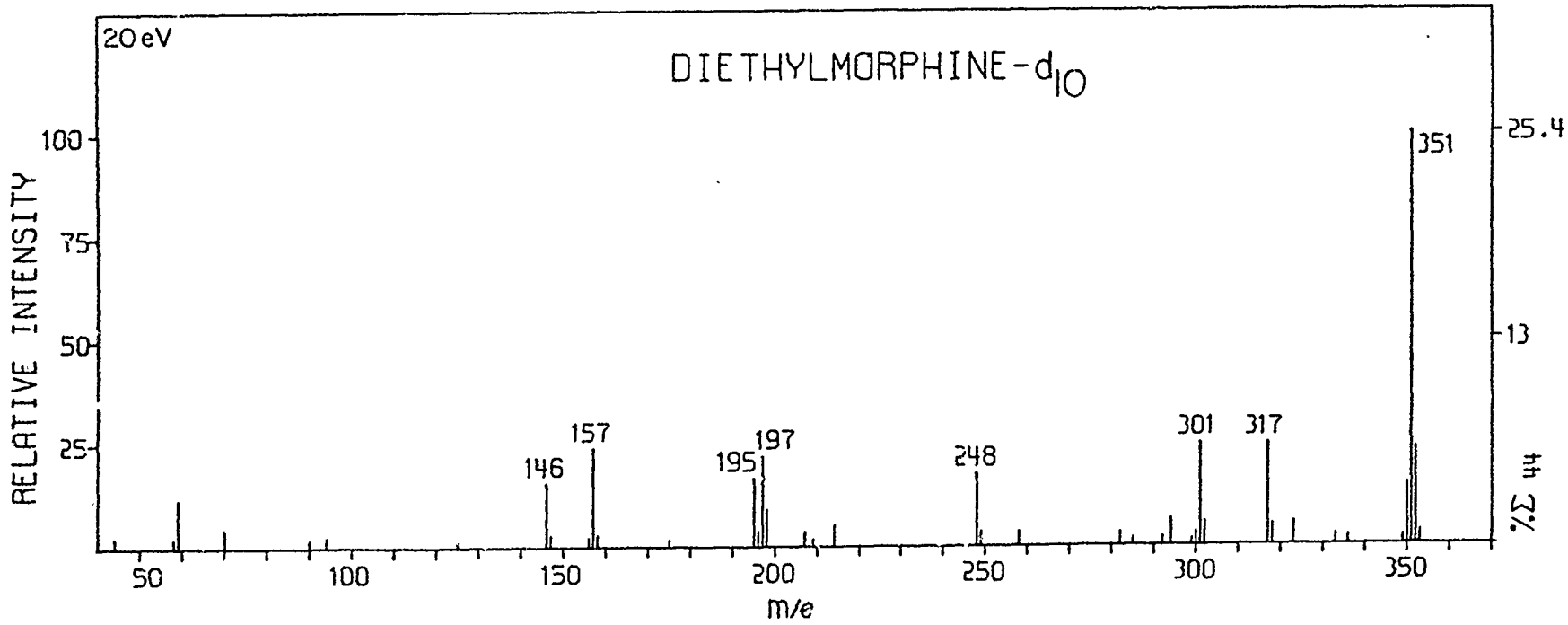

20 eV
DIETHYLMORPHINE-d10
RELATIVE INTENSITY
100
75
50
25
25.4
13
44 Σ%
351
301
317
157
197
146
195
248
50
100
150
200
250
300
350
m/e

Figure 5

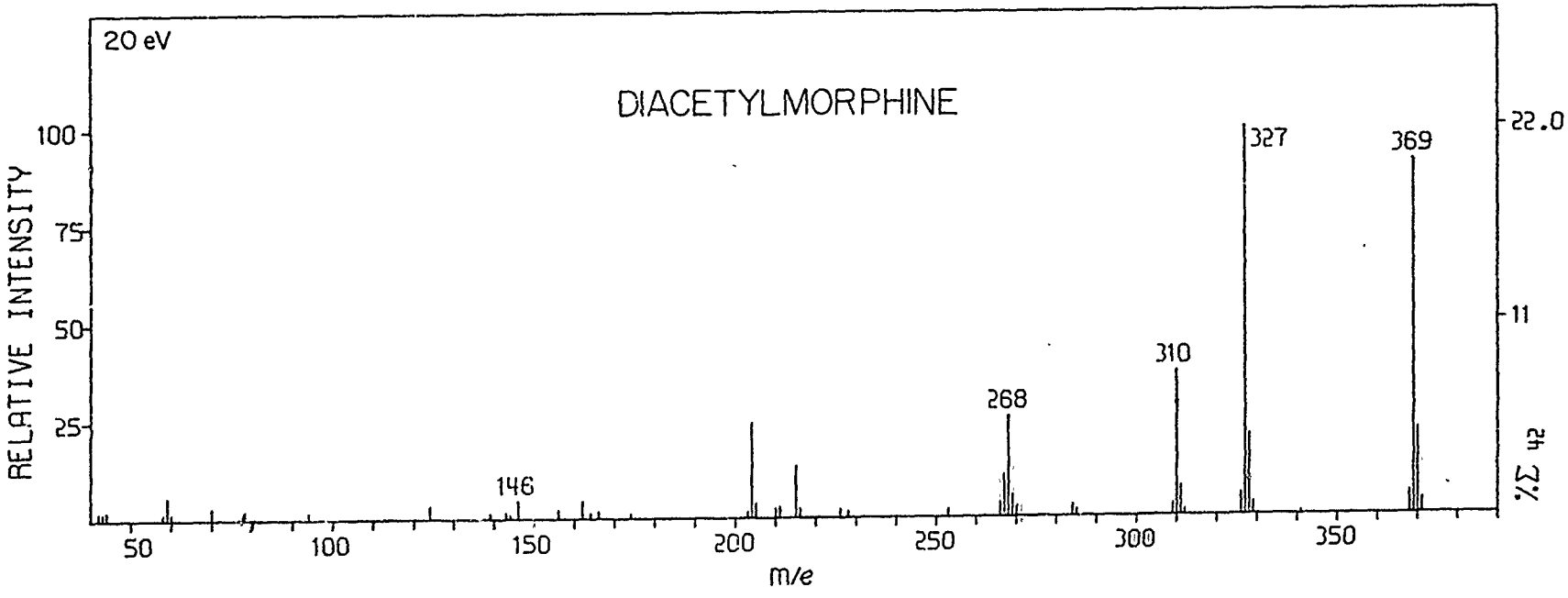

20 eV
DIACETYLMORPHINE
RELATIVE INTENSITY
100
75
50
25
22.0
11
0
%
327
369
310
268
146
50
100
150
200
250
300
350
m/e

Figure 6

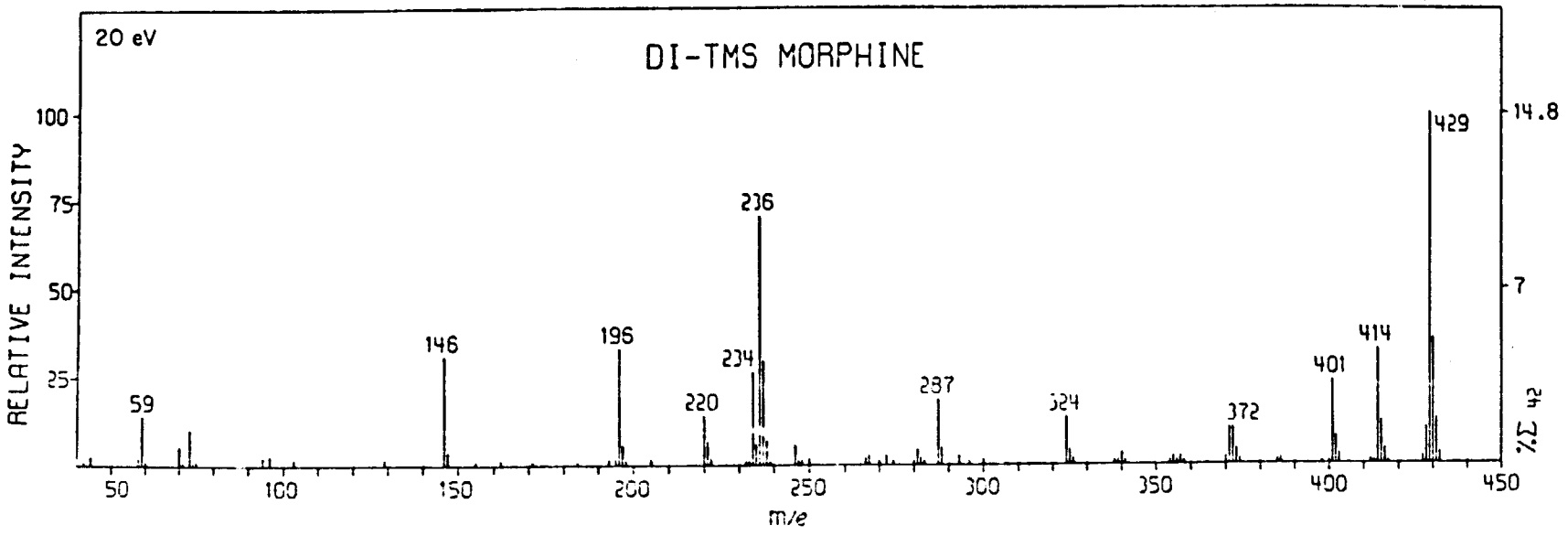

20 eV
DI-TMS MORPHINE
RELATIVE INTENSITY
100
75
50
25
59
146
196
220
234
236
287
324
372
401
414
429
14.8
7
% Σ
50
100
150
200
250
300
350
400
450
m/e

Figure 7

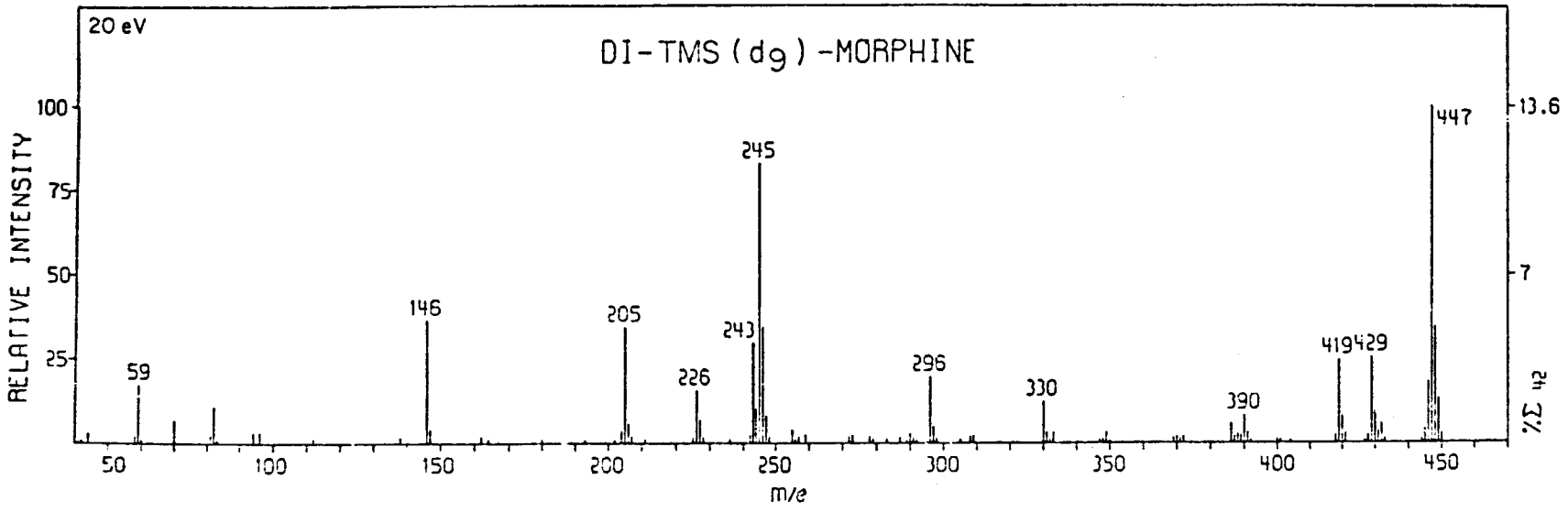

20 eV
DI-TMS (d9) -MORPHINE
RELATIVE INTENSITY
100
75
50
25
59
146
205
226
243
245
296
330
390
419
429
447
13.6
7
%
50
100
150
200
250
300
350
400
450
m/e

Figure 8

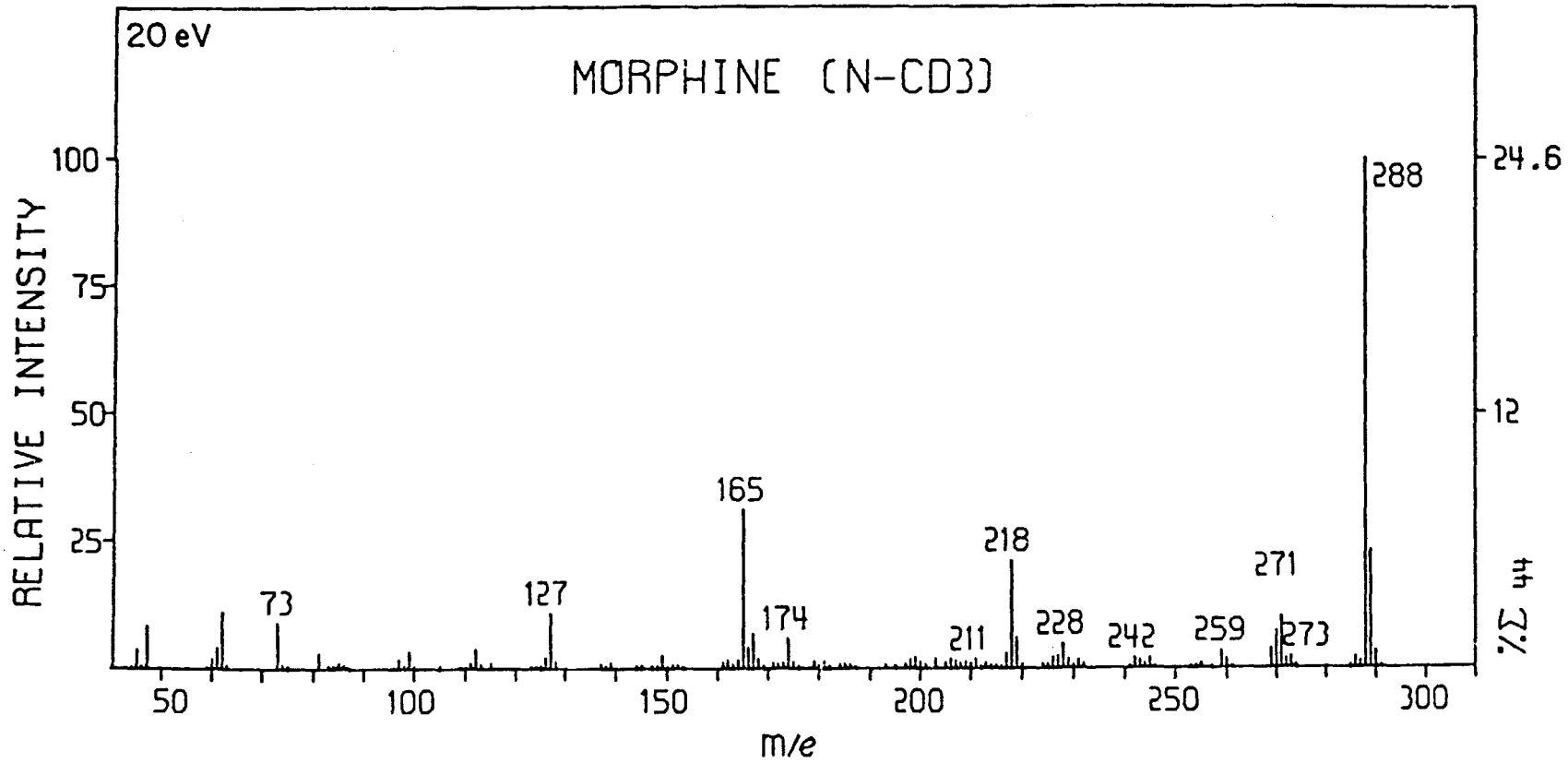

20 eV
MORPHINE (N-CD3)
RELATIVE INTENSITY
100
75
50
25
24.6
12
0
288
165
218
271
73
127
174
211
228
242
259
273
50
100
150
200
250
300
m/e

Figure 9

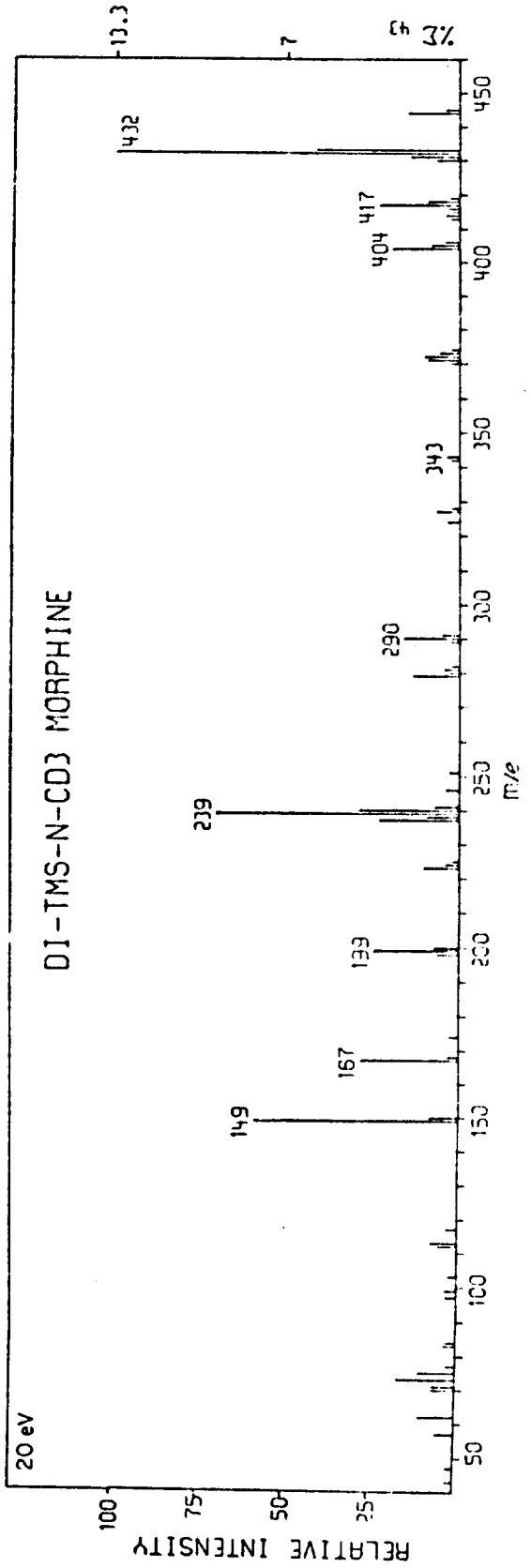

DI-TMS-N-CD3 MORPHINE
20 eV
RELATIVE INTENSITY
100
75
50
25
50
100
150
200
250
300
350
400
450
m/e
149
167
139
239
290
343
404
417
432
13.3
7
5
%

Figure 10

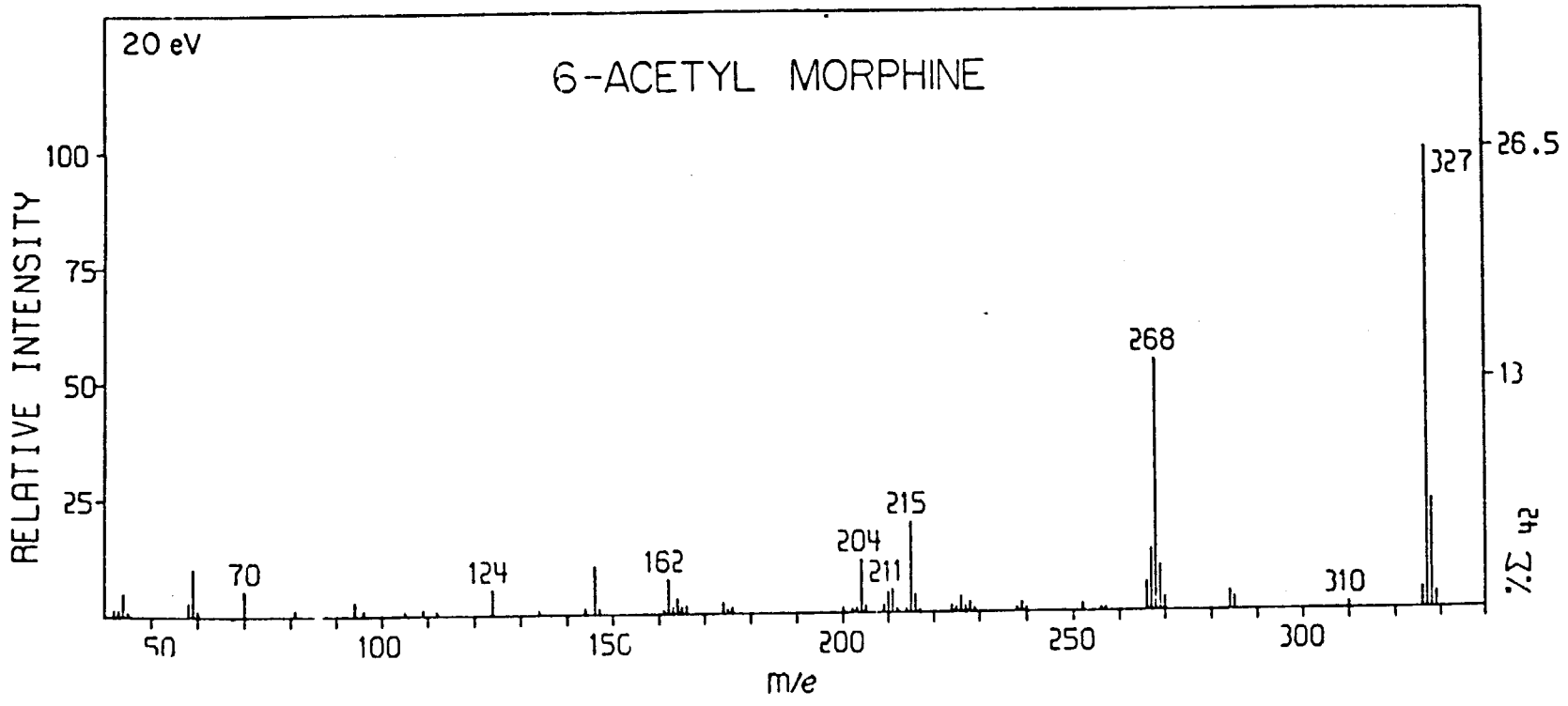

20 eV
6-ACETYL MORPHINE
RELATIVE INTENSITY
100
75
50
25
26.5
13
24.2%
327
268
215
204
211
162
124
70
310
50
100
150
200
250
300
m/e

Figure 11

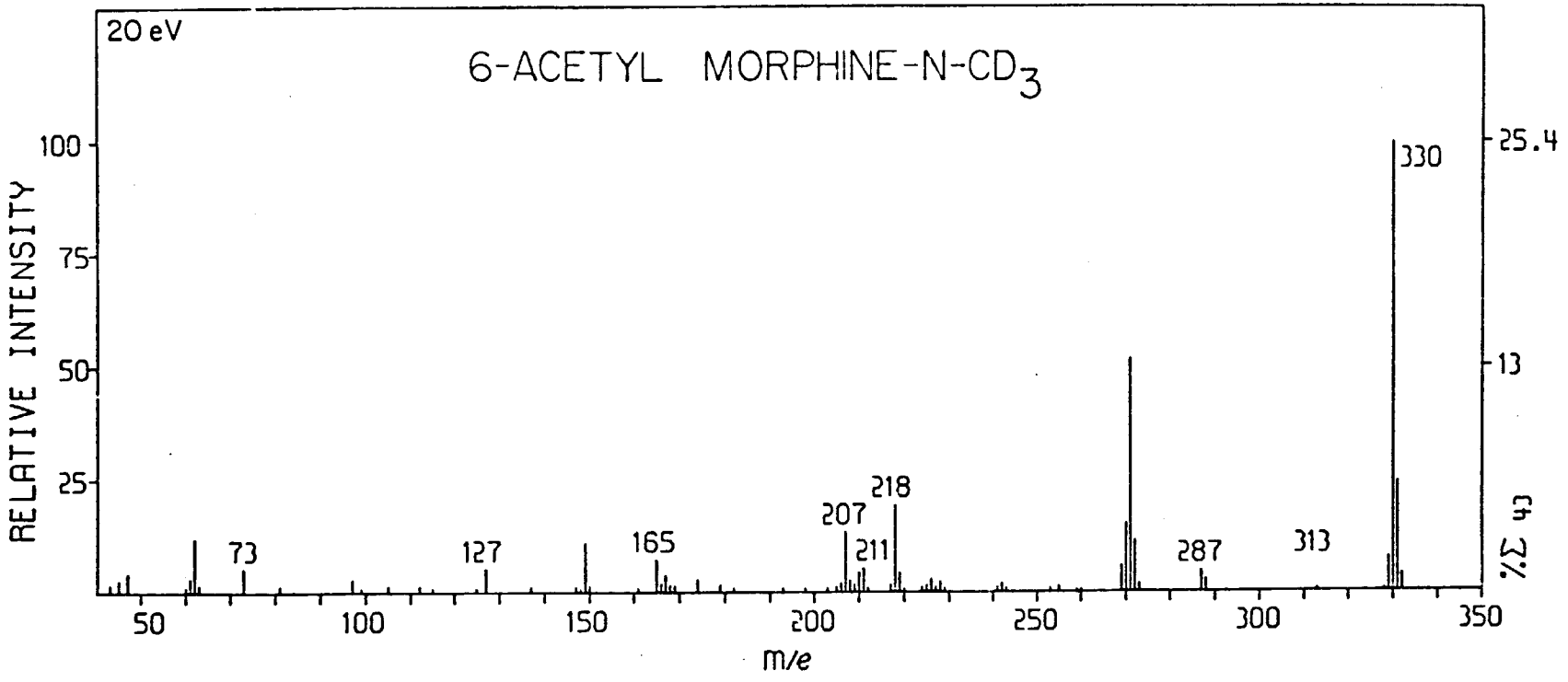

20 eV
6-ACETYL MORPHINE-N-CD3
RELATIVE INTENSITY
100
75
50
25
25.4
13
% Σ
330
218
207
211
73
127
165
287
313
50
100
150
200
250
300
350
m/e

Figure 12

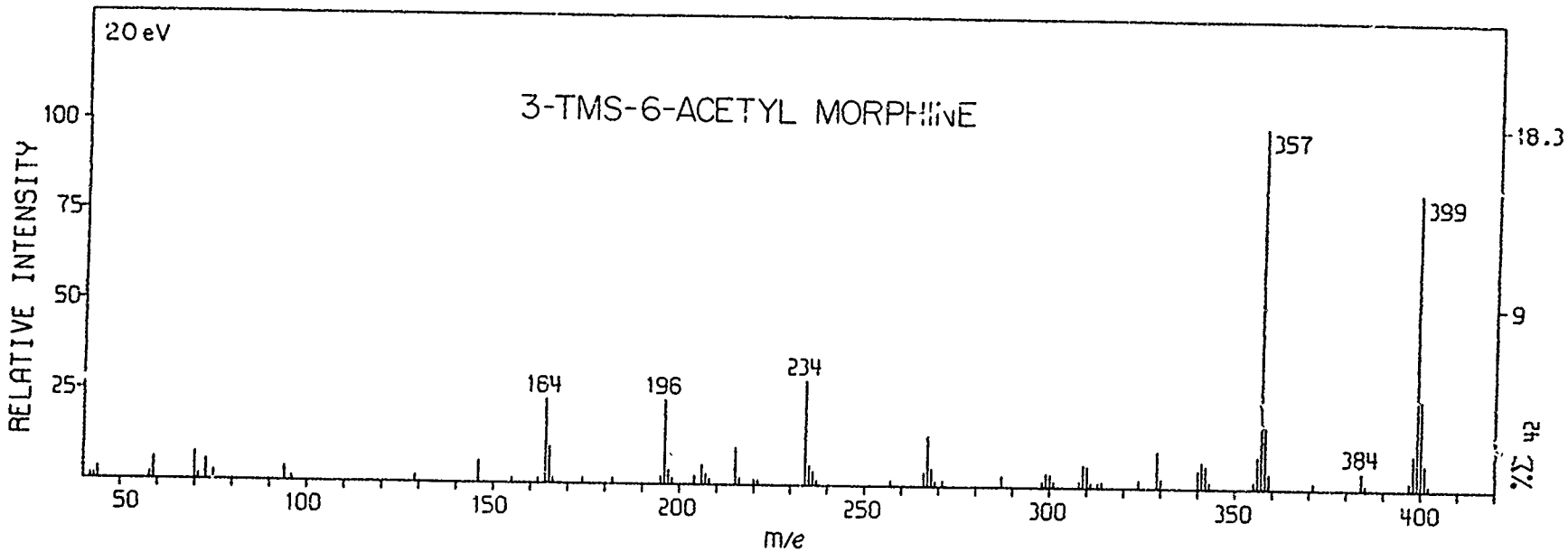

20 eV
3-TMS-6-ACETYL MORPHINE
RELATIVE INTENSITY
100
75
50
25
357
399
234
196
164
384
18.3
9
24
%
50
100
150
200
250
300
350
400
m/e

Figure 13

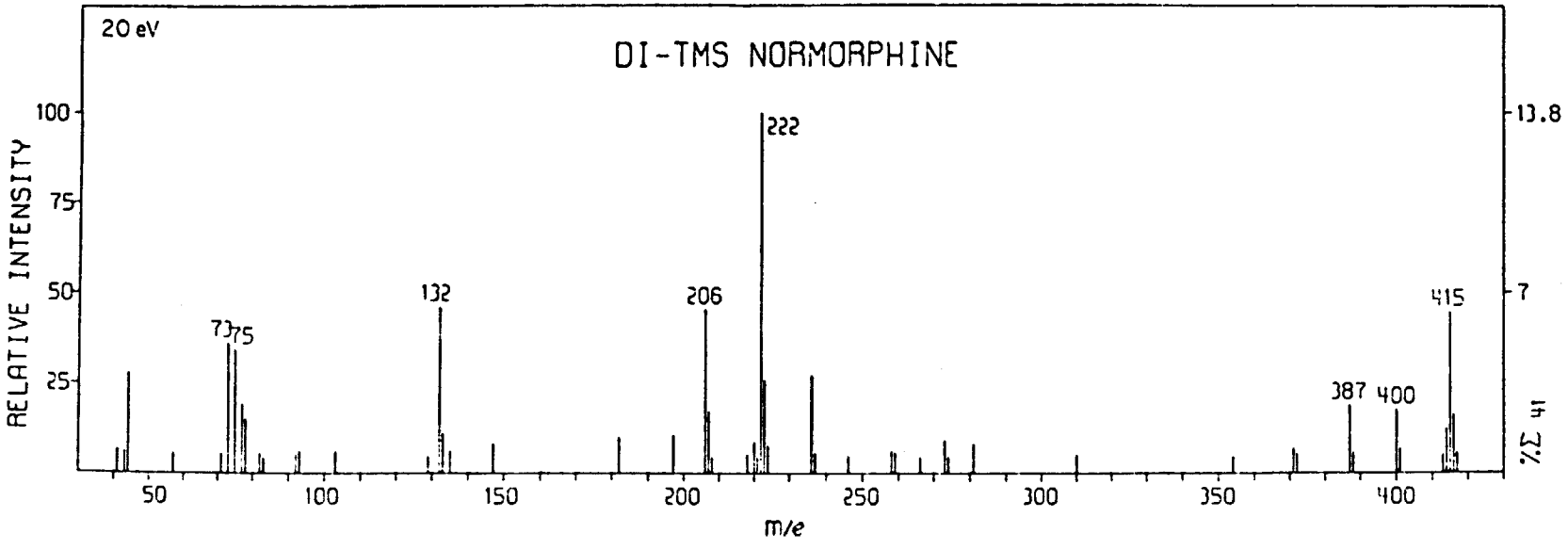

20 eV
DI-TMS NORMORPHINE
RELATIVE INTENSITY
100
75
50
25
73
75
132
206
222
387
400
415
13.8
7
1.41 %
50
100
150
200
250
300
350
400
m/e

Figure 14

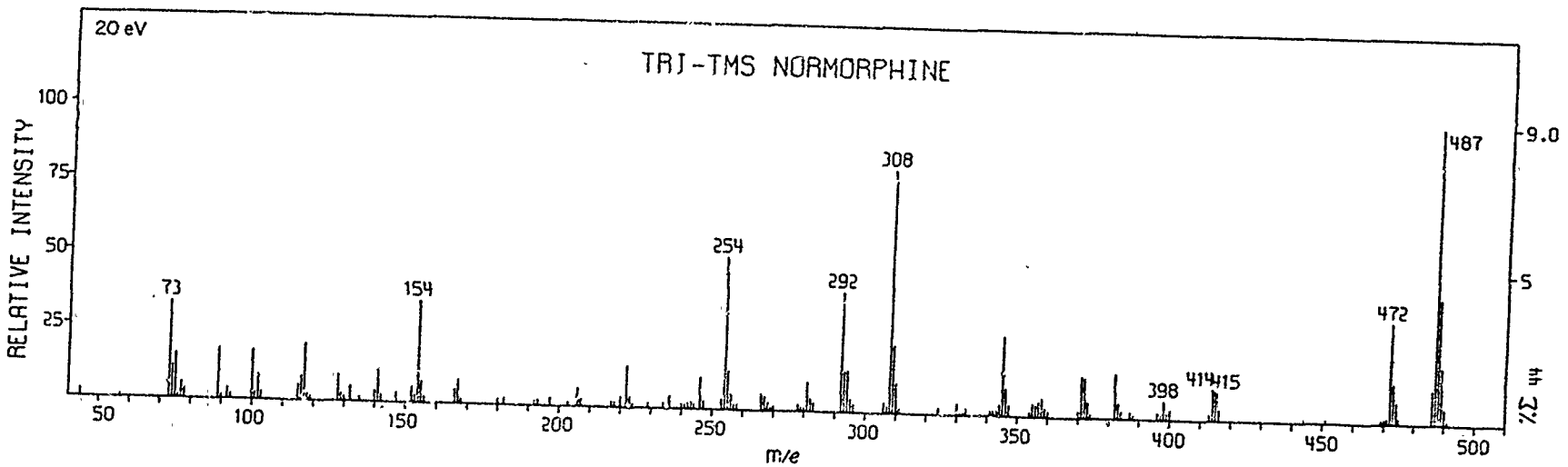

20 eV
TRI-TMS NORMORPHINE
RELATIVE INTENSITY
100
75
50
25
73
154
254
292
308
398
414
415
472
487
9.0
5
Σ%
50
100
150
200
250
300
350
400
450
500
m/e

Figure 15

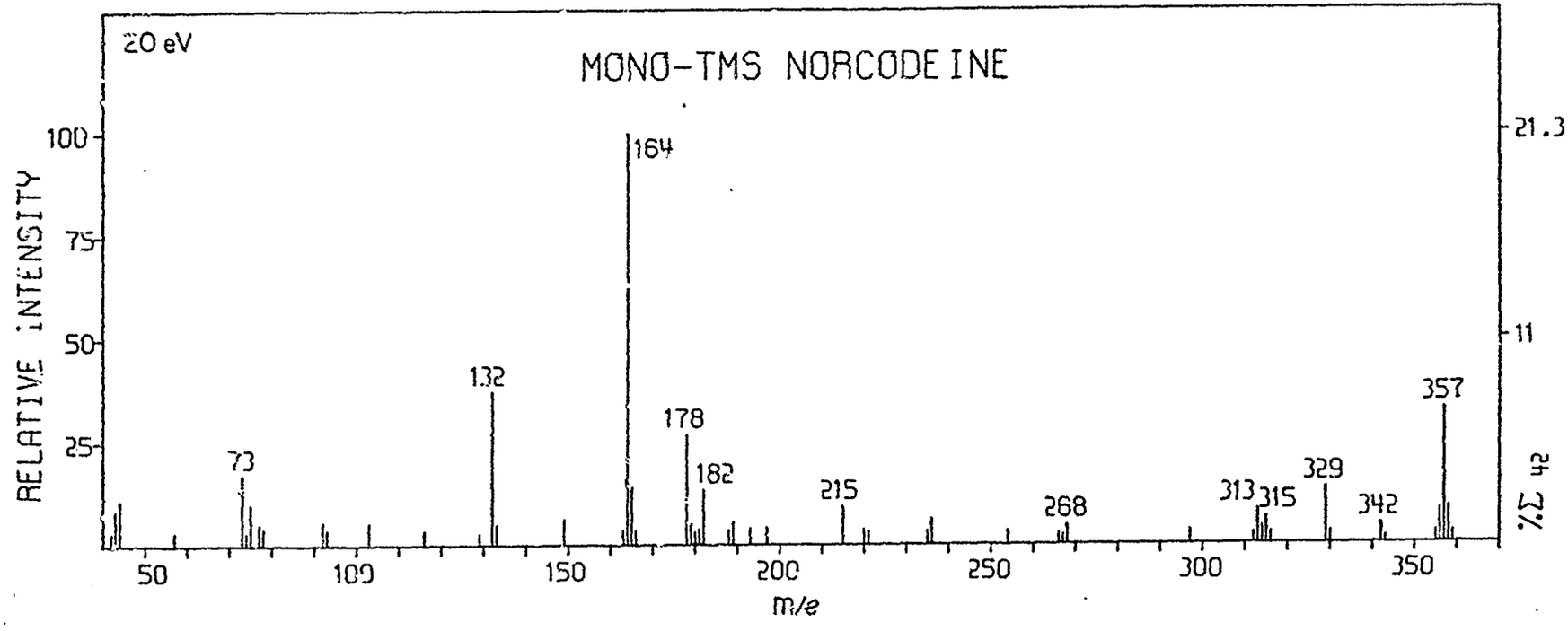

20 eV
MONO-TMS NORCODEINE
RELATIVE INTENSITY
100
75
50
25
21.3
11
0
Σ%
164
132
73
178
182
215
268
313
315
329
342
357
50
100
150
200
250
300
350
m/e

Figure 16

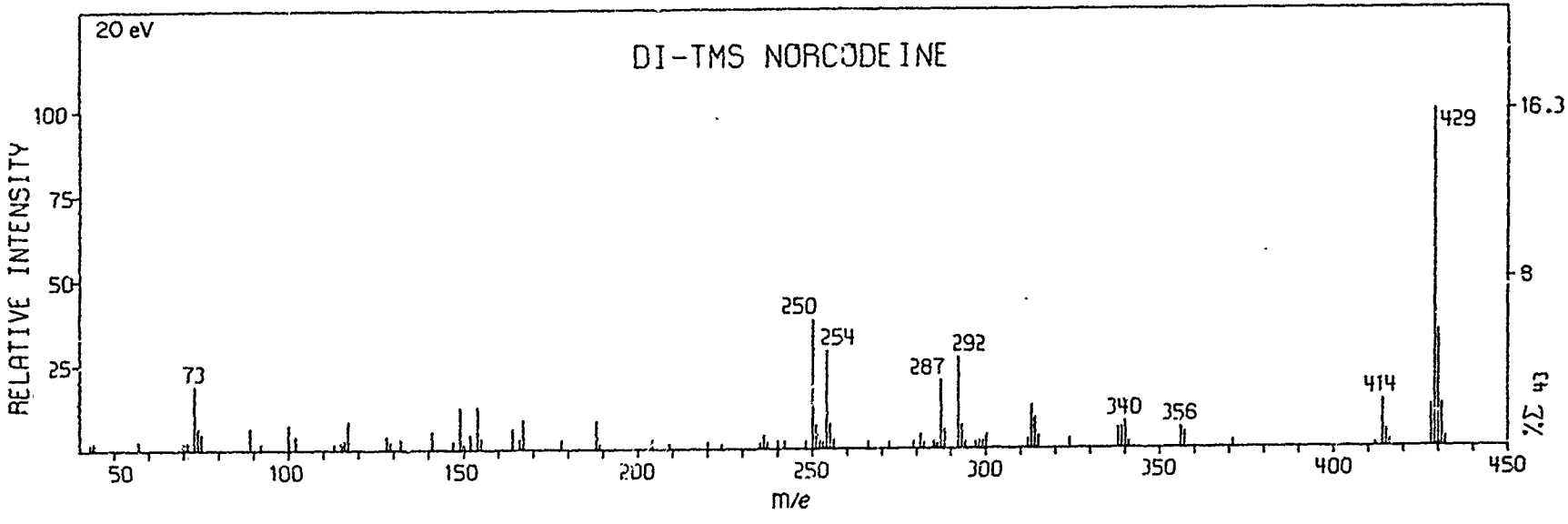

20 eV
DI-TMS NORCODEINE
RELATIVE INTENSITY
100
75
50
25
16.3
8
0
Σ%
73
250
254
287
292
340
356
414
429
50
100
150
200
250
300
350
400
450
m/e

Figure 17

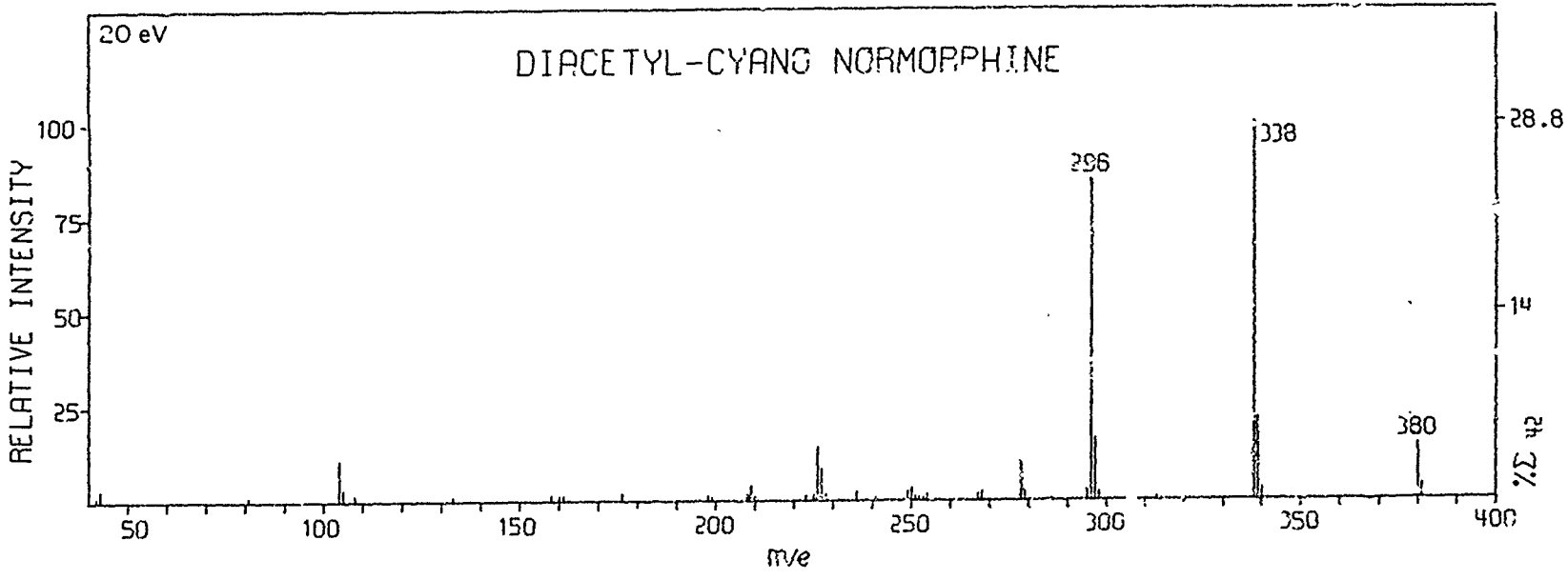

20 eV
DIACETYL-CYANO NORMORPHINE
RELATIVE INTENSITY
100
75
50
25
296
338
380
28.8
14
2h 3%
50
100
150
200
250
300
350
400
m/e

Figure 18

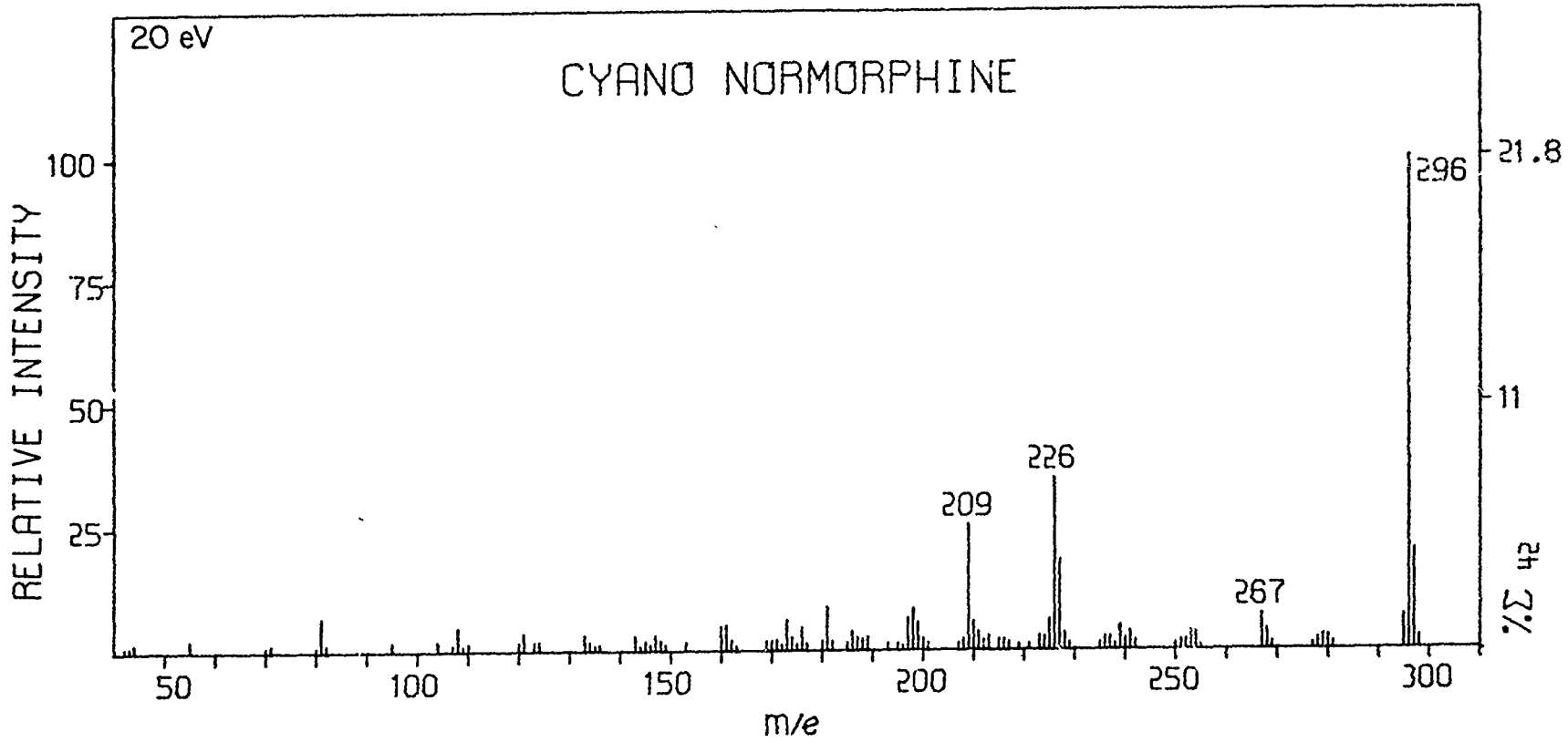

20 eV
CYANO NORMORPHINE
RELATIVE INTENSITY
100
75
50
25
296
21.8
11
226
209
267
24 Σ%
50
100
150
200
250
300
m/e

Figure 19

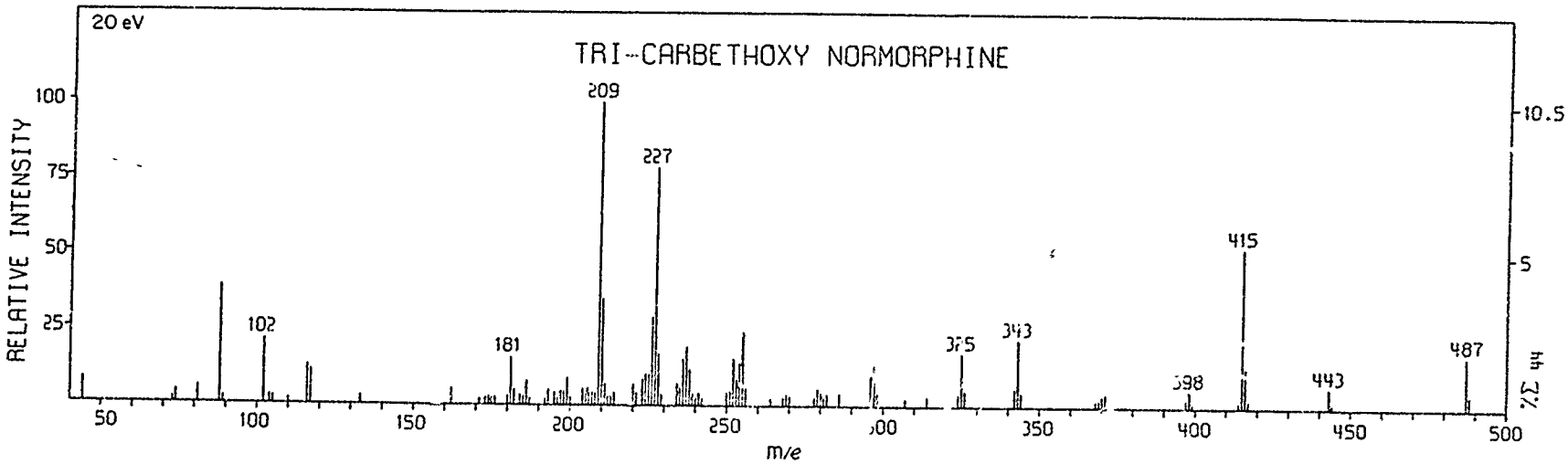

20 eV
TRI-CARBETHOXY NORMORPHINE
RELATIVE INTENSITY
100
75
50
25
209
227
102
181
415
343
487
598
443
10.5
5
m/e
50
100
150
200
250
350
400
450
500

Figure 20

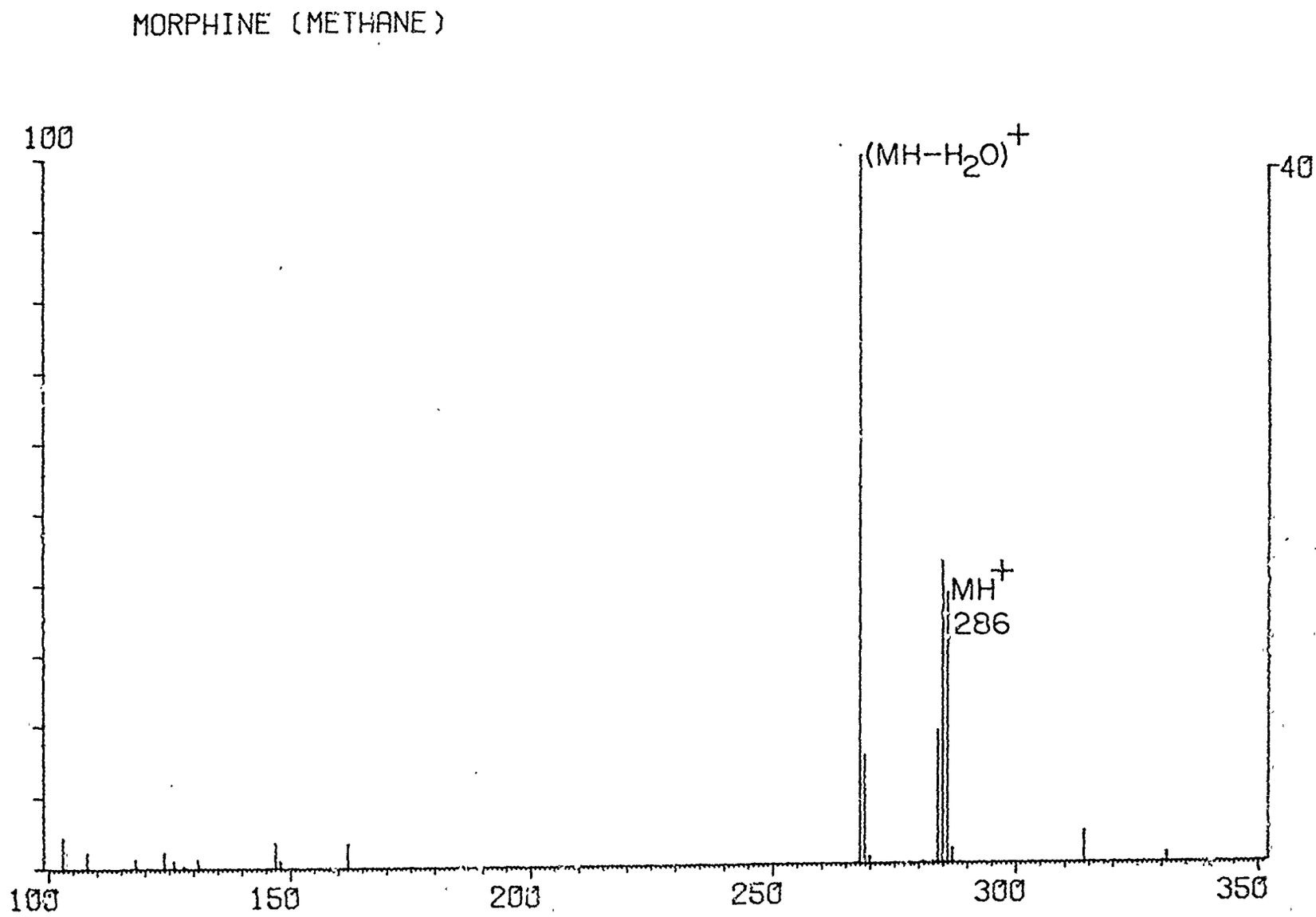


Figure 21

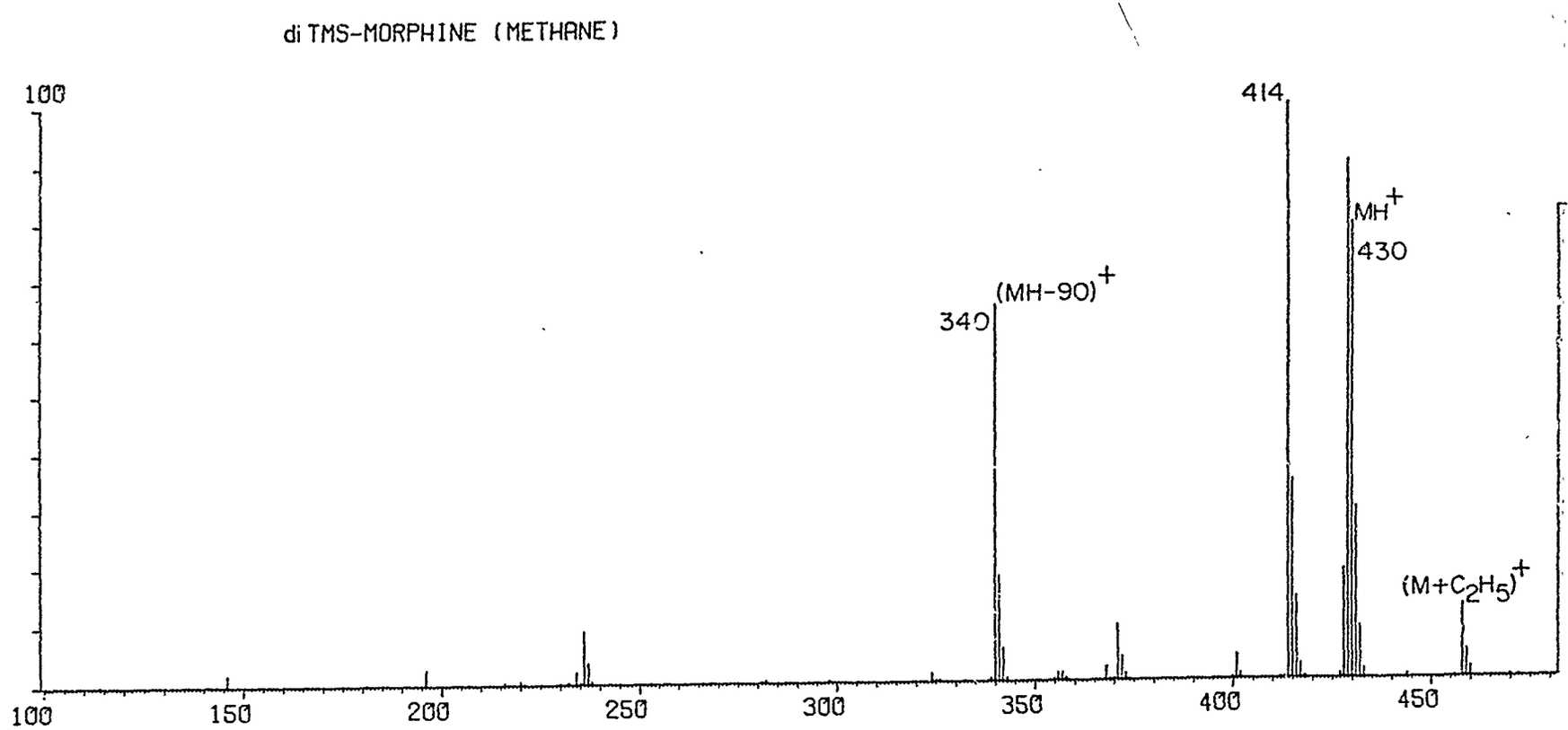

di TMS-MORPHINE (METHANE)
100
414
MH+
430
(MH-90)+
340
(M+C2H5)+
100
150
200
250
300
350
400
450

Figure 22

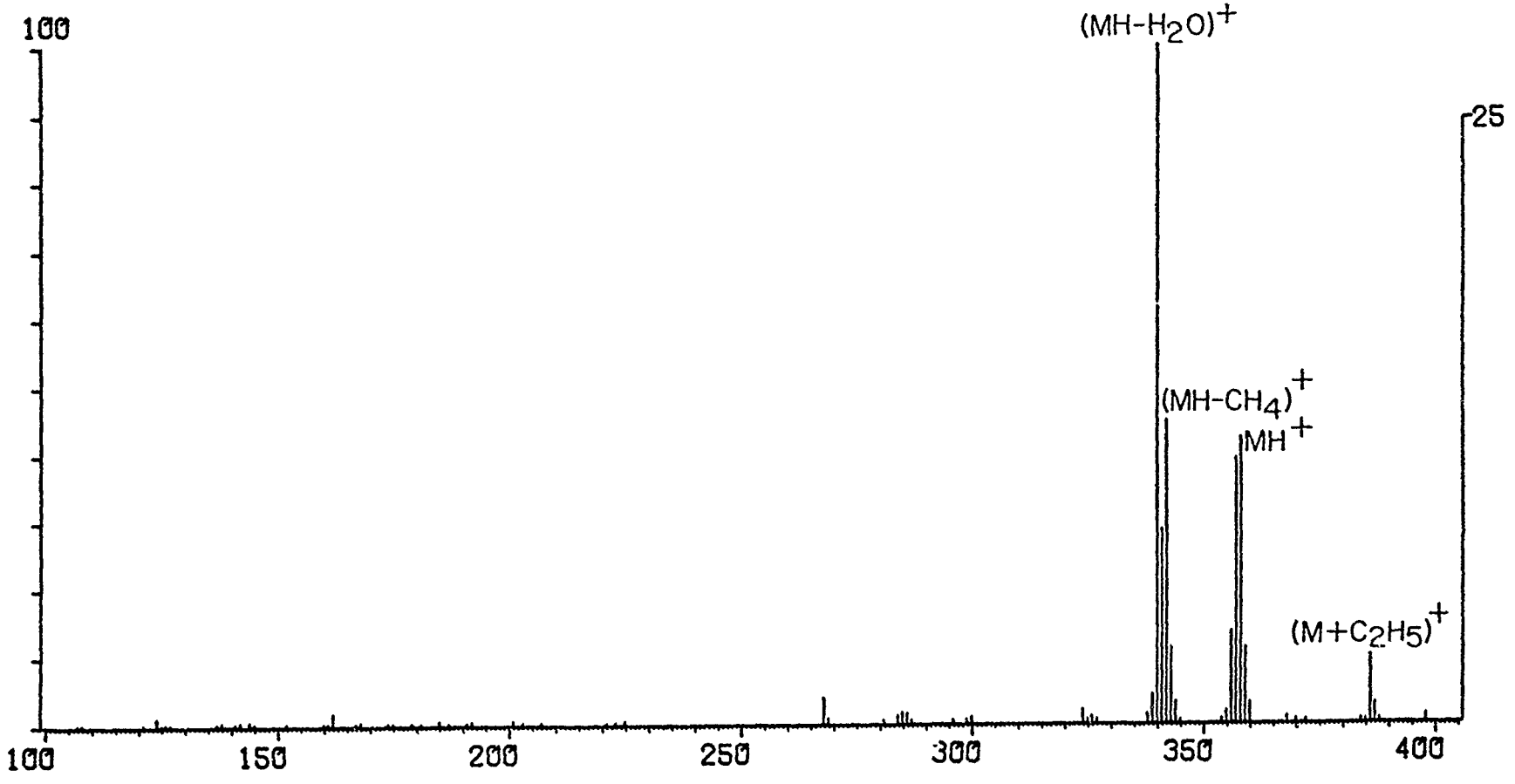

MORPHINE-3-TMS CH4
100
(MH-H2O)+
25
(MH-CH4)+
MH+
(M+C2H5)+
100
150
200
250
300
350
400

Figure 23

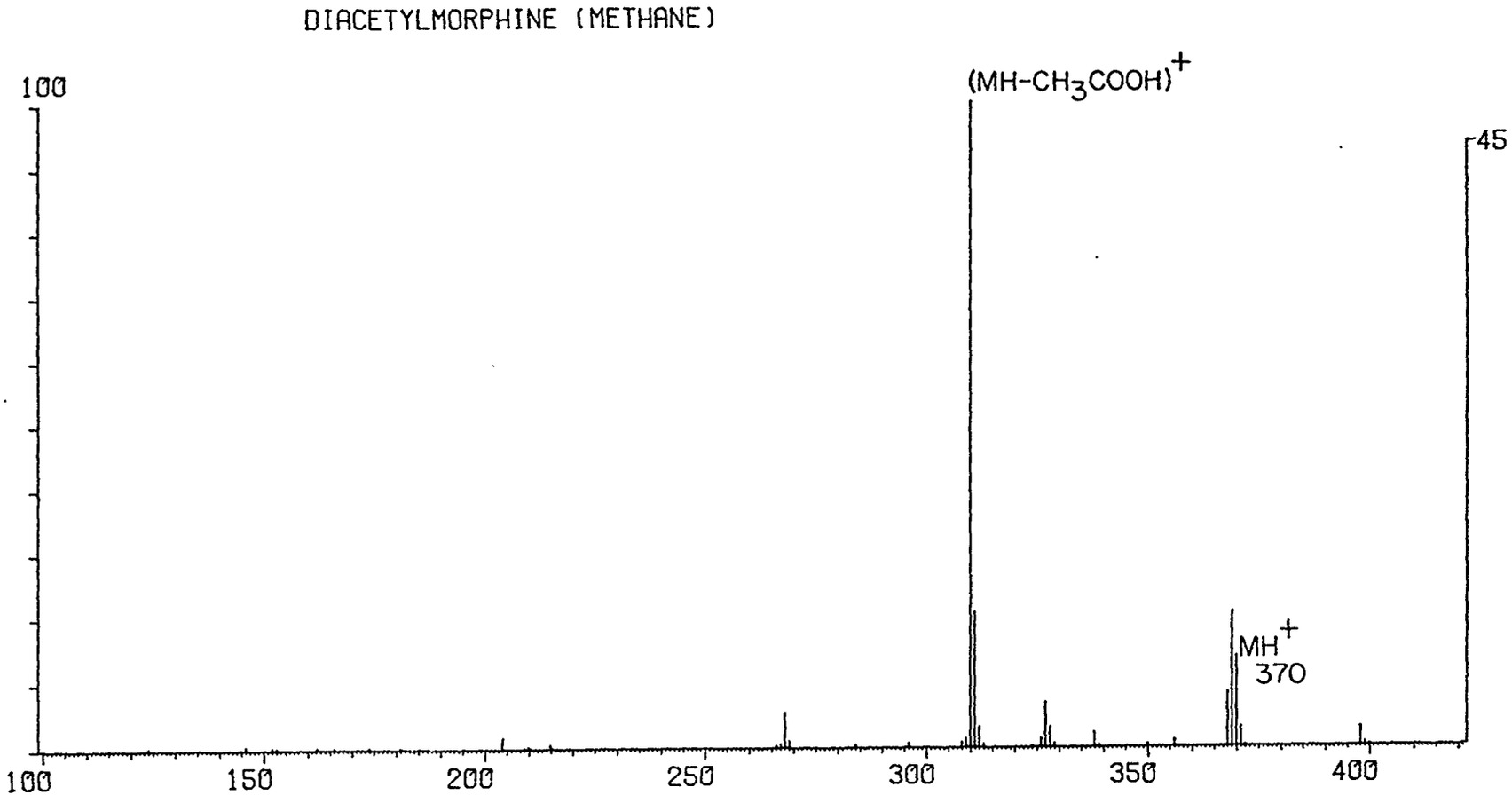

DIACETYLMORPHINE (METHANE)
100
(MH-CH3COOH)+
45
MH+
370
100
150
200
250
300
350
400

Figure 24

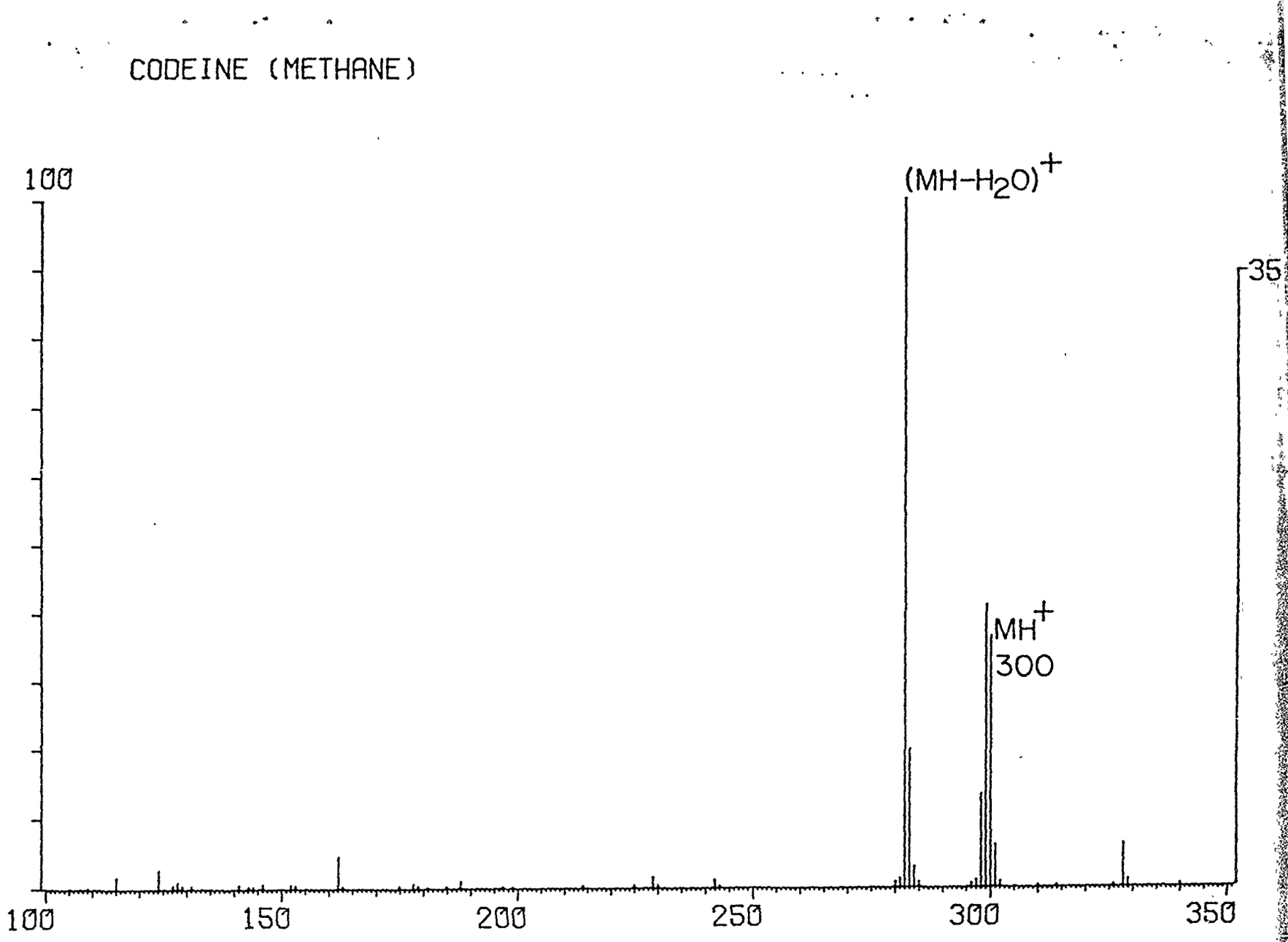


Figure 25

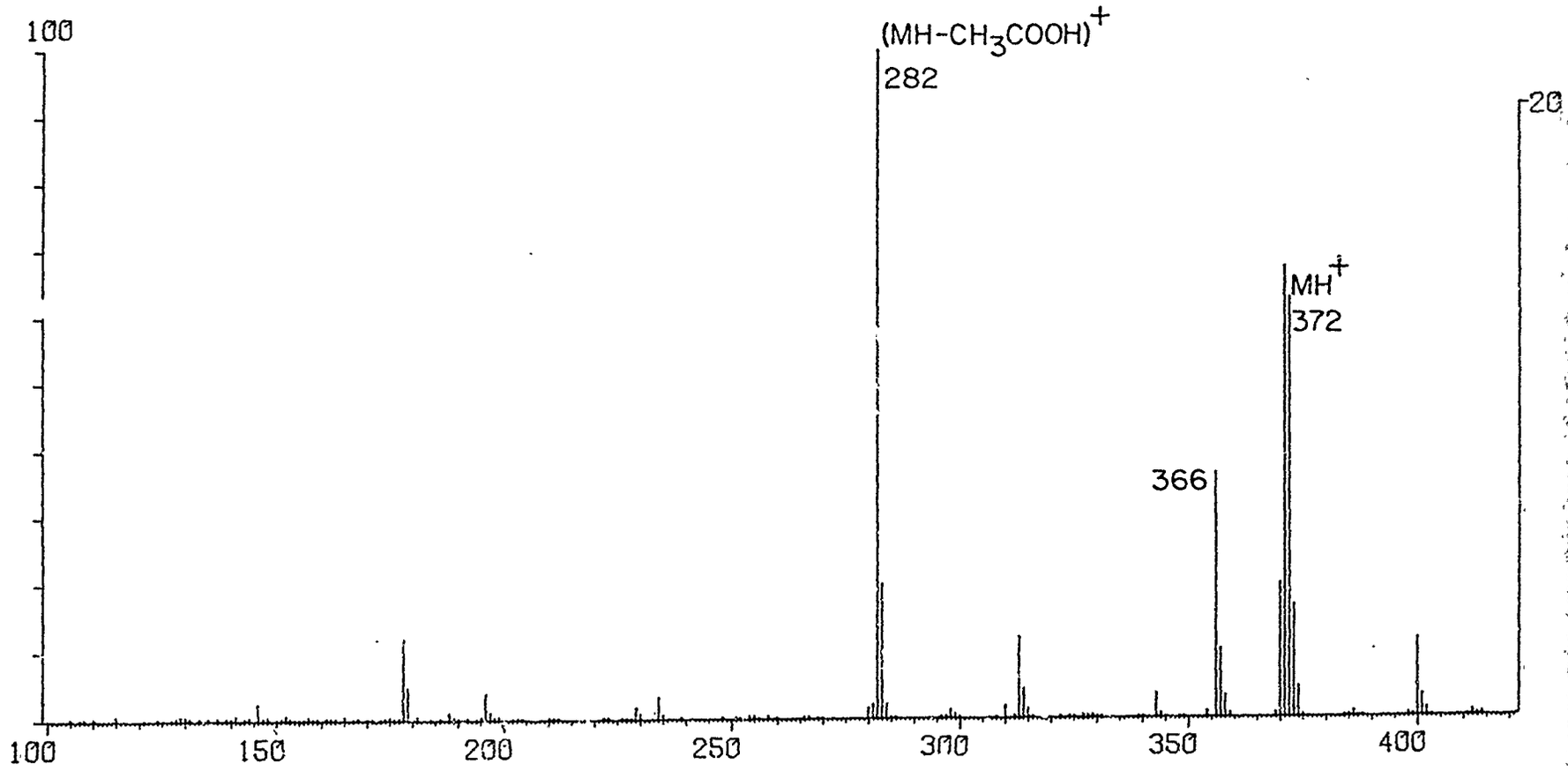

CODEINE TMS (METHANE)
100
(MH-CH3COOH)+
282
20
MH+
372
366
100
150
200
250
300
350
400

Figure 26

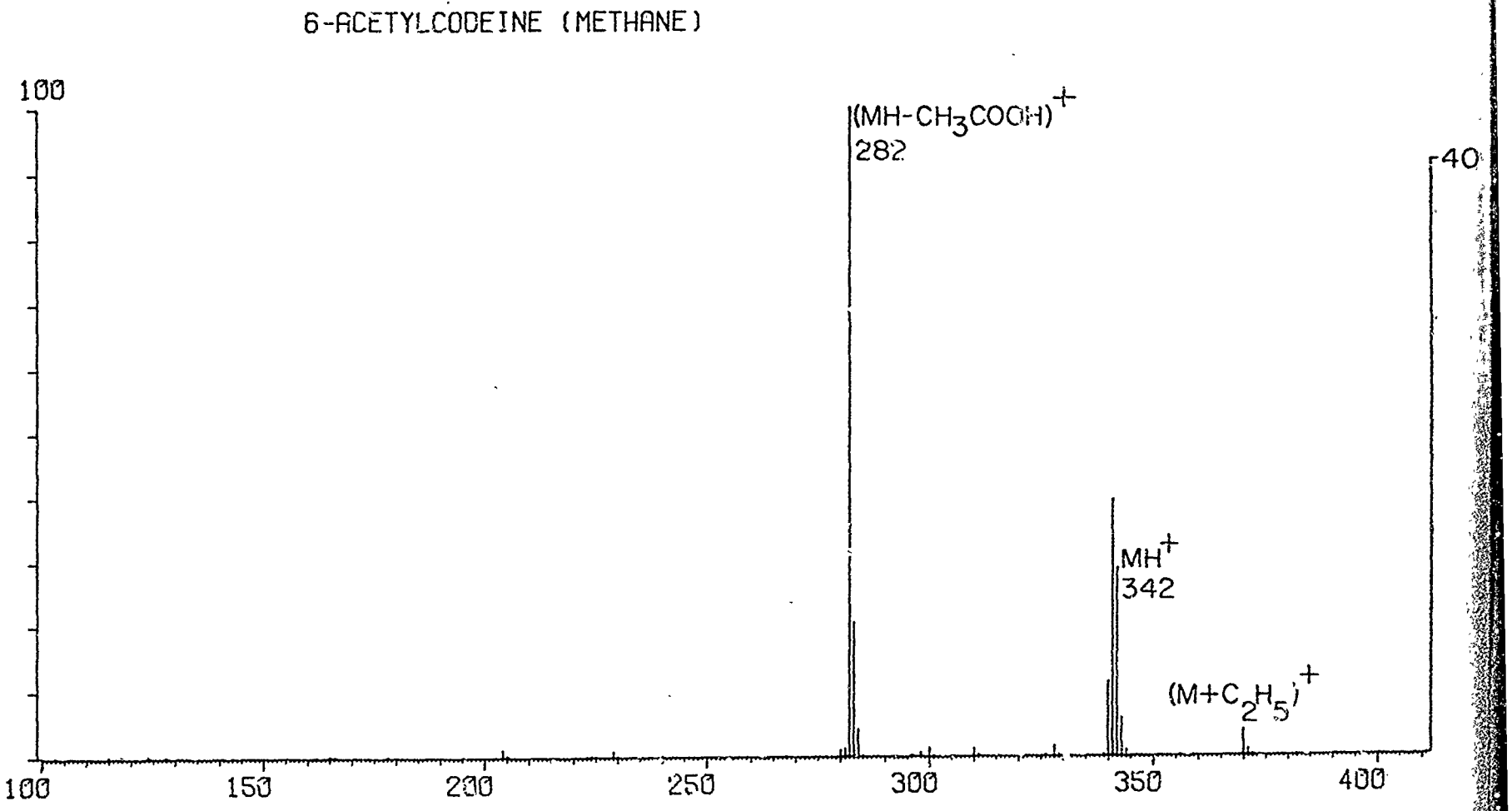

6-ACETYLCODEINE (METHANE)
100
(MH-CH3COOH)+
282
40
MH+
342
(M+C2H5)+
100
150
200
250
300
350
400

Figure 27

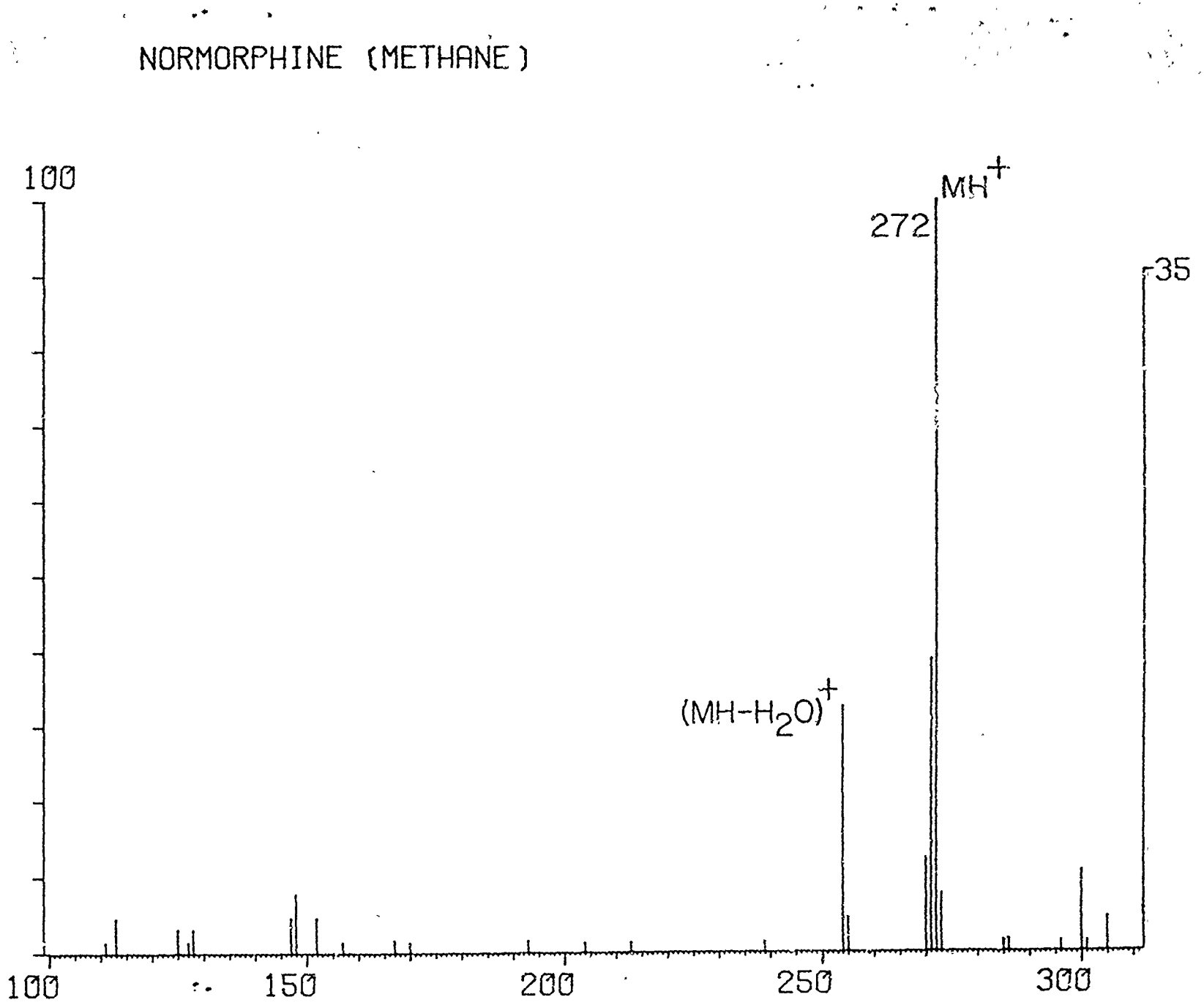


Figure 28

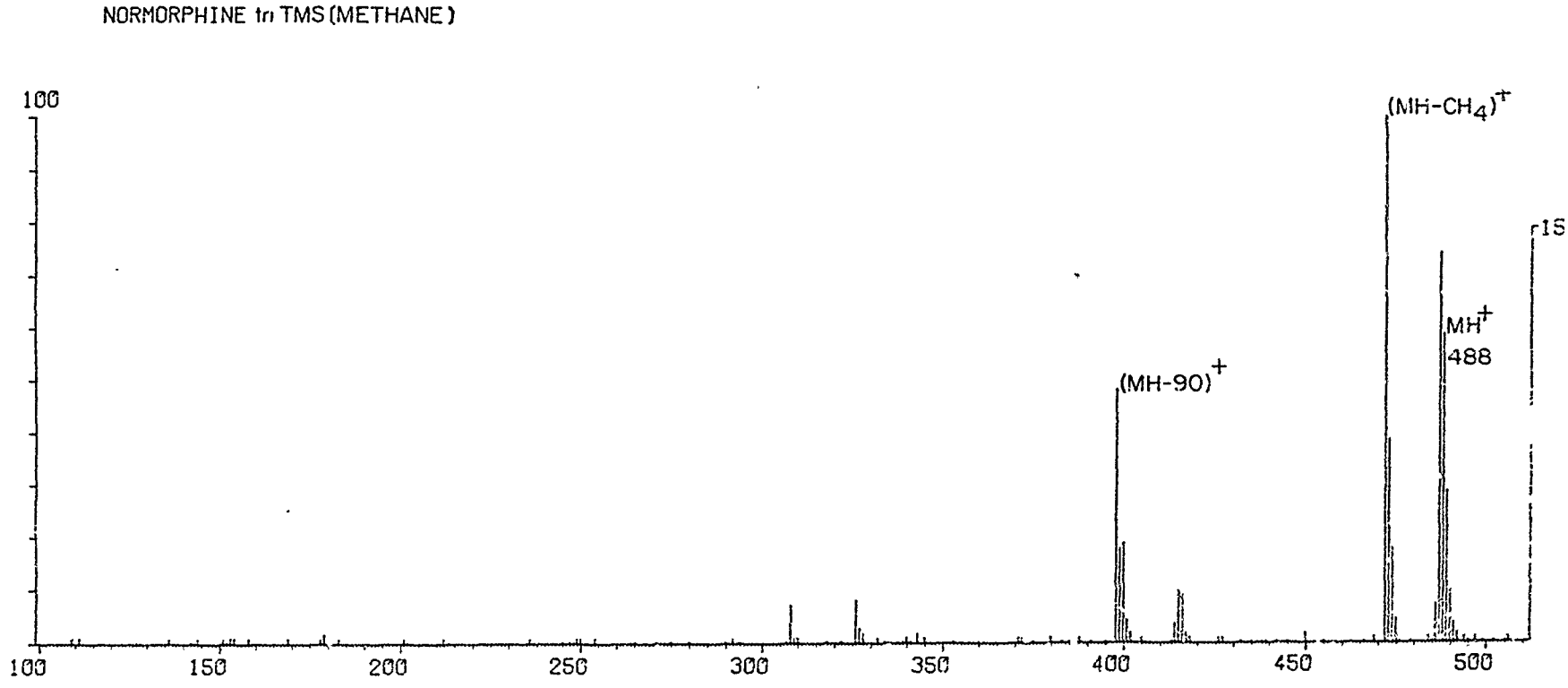

NORMORPHINE tri TMS (METHANE)
100
(MH-CH4)+
IS
MH+
488
(MH-90)+
100
150
200
250
300
350
400
450
500

Figure 29

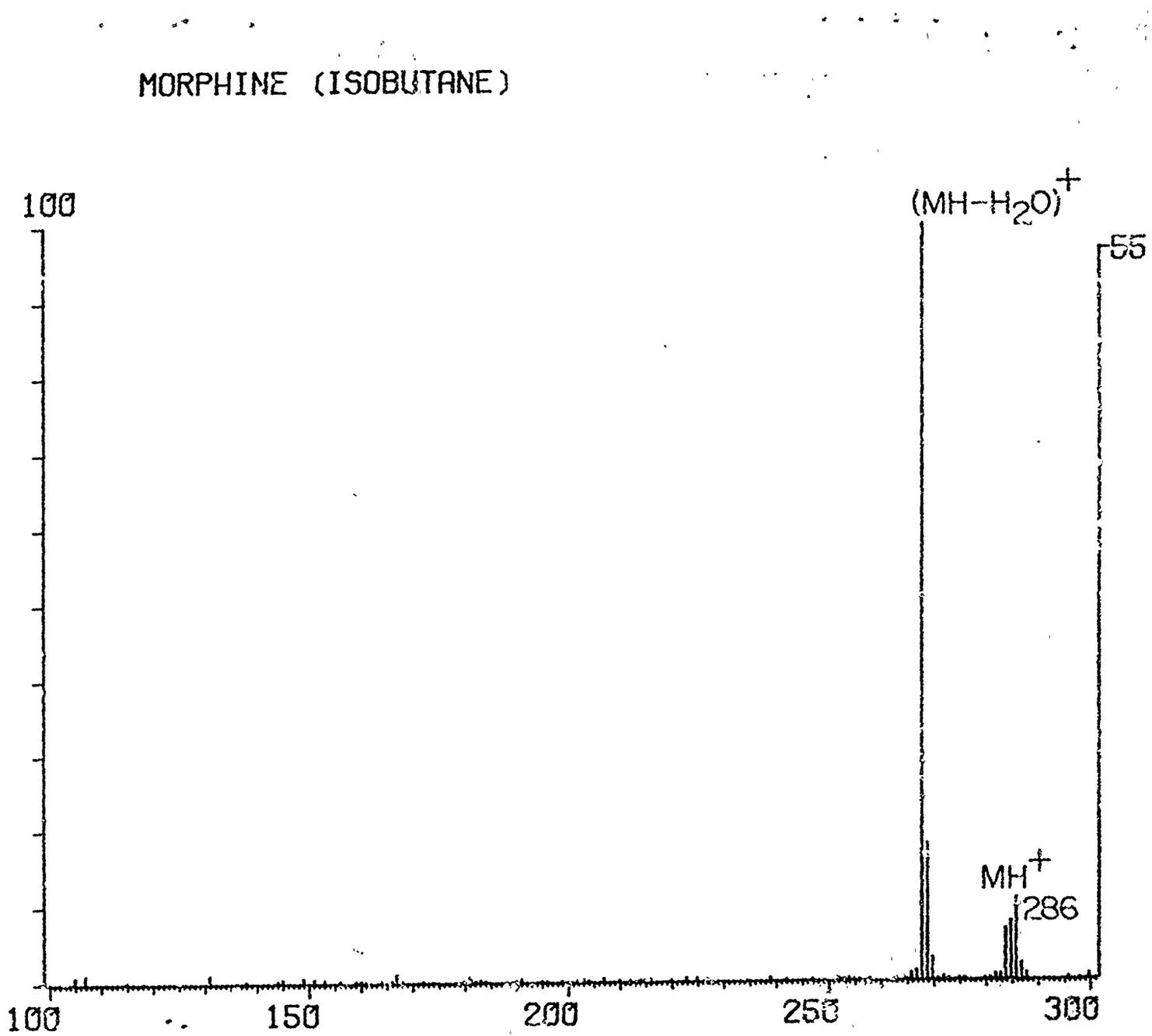


Figure 30

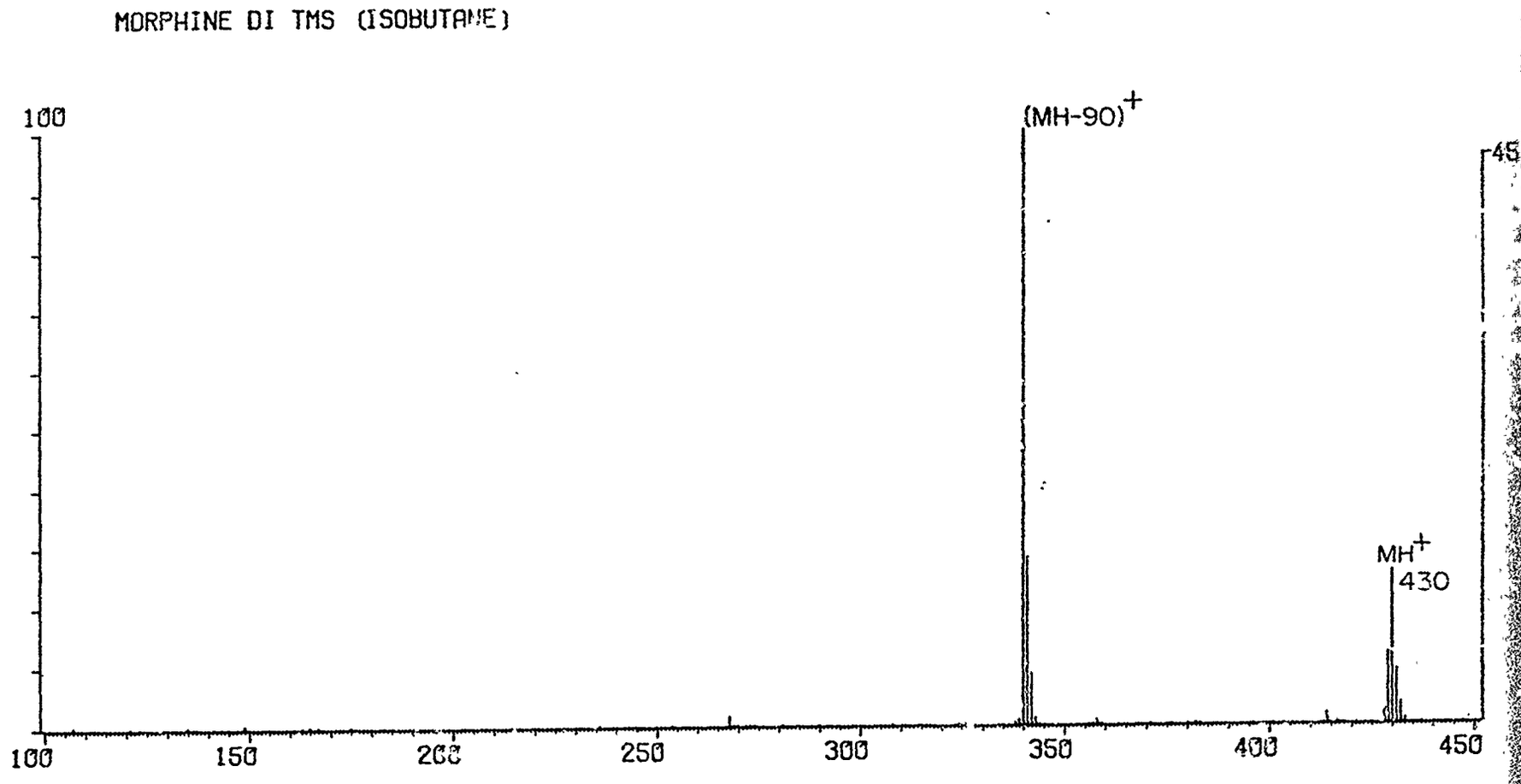

MORPHINE DI TMS (ISOBUTANE)
100
(MH-90)+
45
MH+
430
100
150
200
250
300
350
400
450

Figure 31

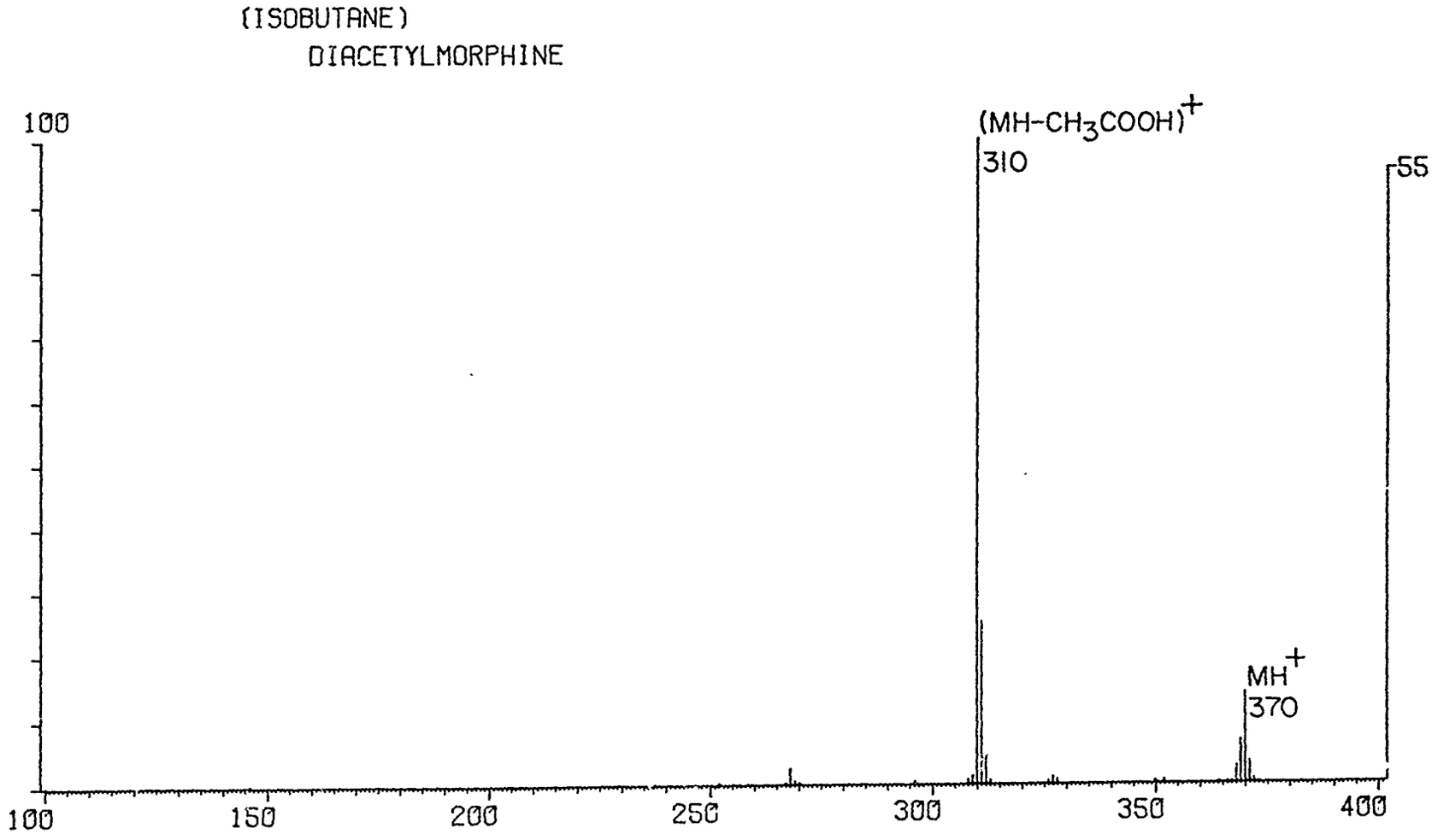

(ISOBUTANE)
DIACETYLMORPHINE
100
(MH-CH3COOH)+
310
55
MH+
370
100
150
200
250
300
350
400

Figure 32

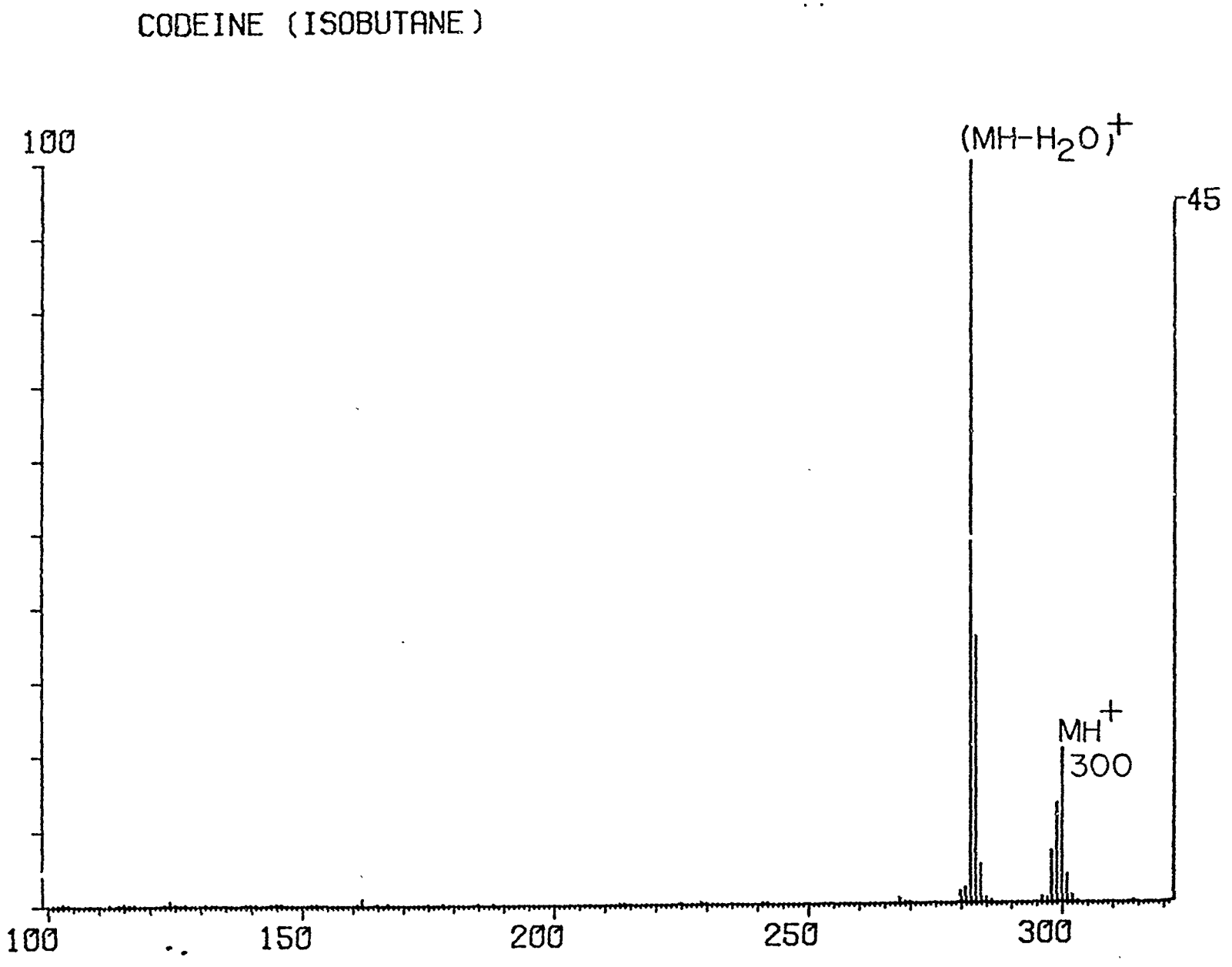


Figure 33

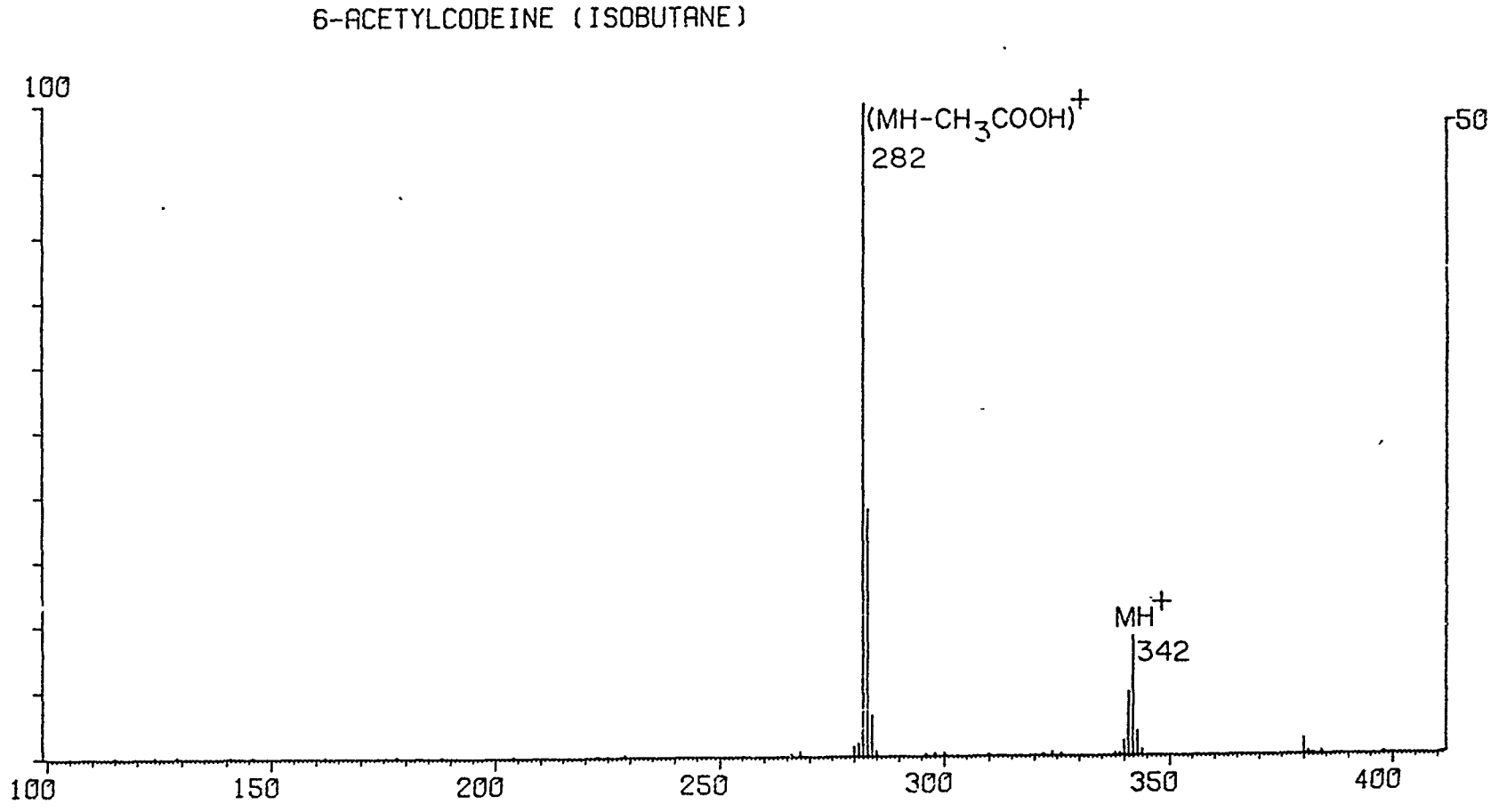

6-ACETYLCODEINE (ISOBUTANE)
100
(MH-CH3COOH)+
282
50
MH+
342
100
150
200
250
300
350
400

Figure 34

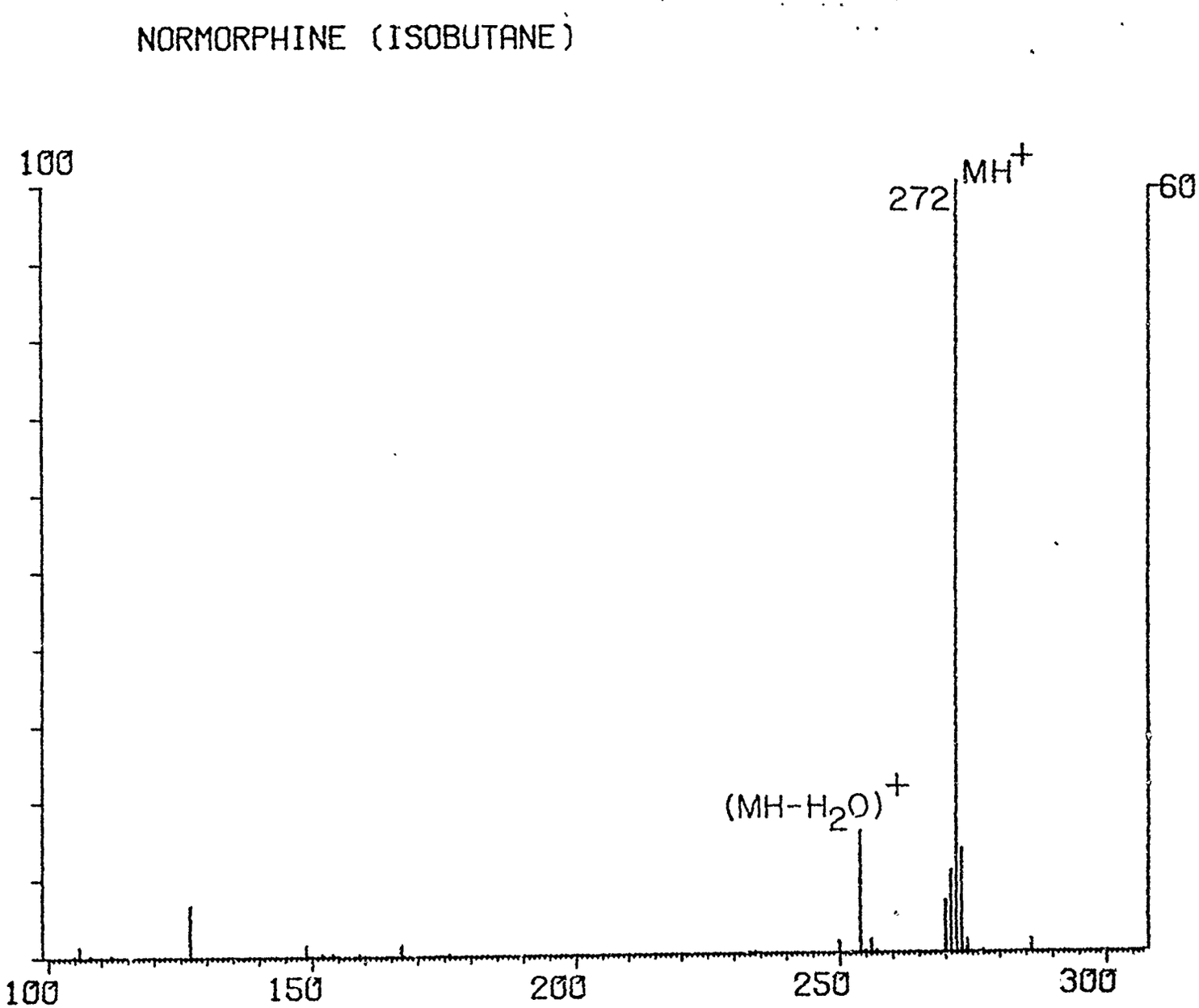

NORMORPHINE (ISOBUTANE)
100
272
MH+
60
(MH-H2O)+
100
150
200
250
300

Figure 35

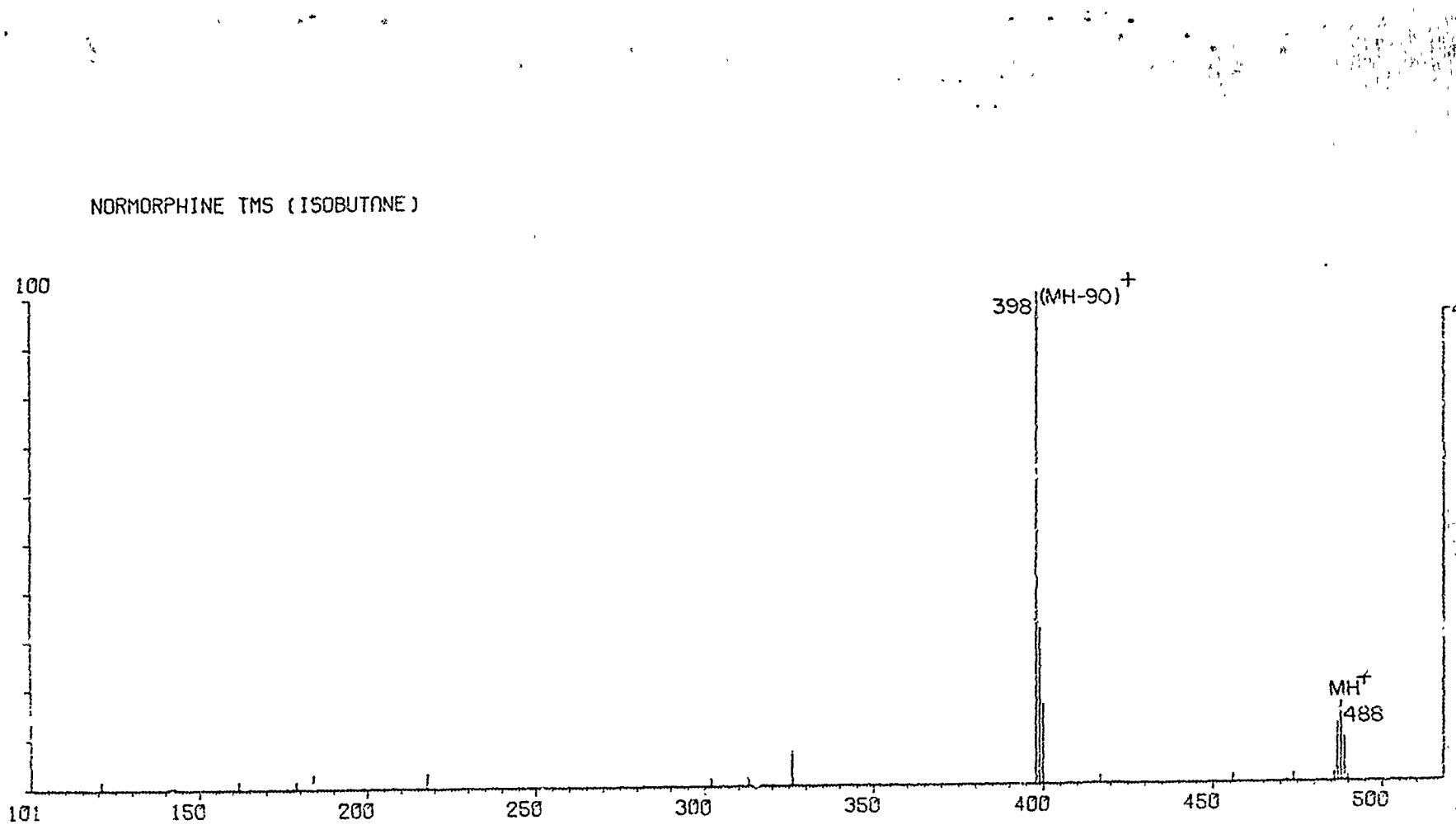

NORMORPHINE TMS (ISOBUTANE)
100
398
(MH-90)+
45
MH+
488
101
150
200
250
300
350
400
450
500

Figure 36

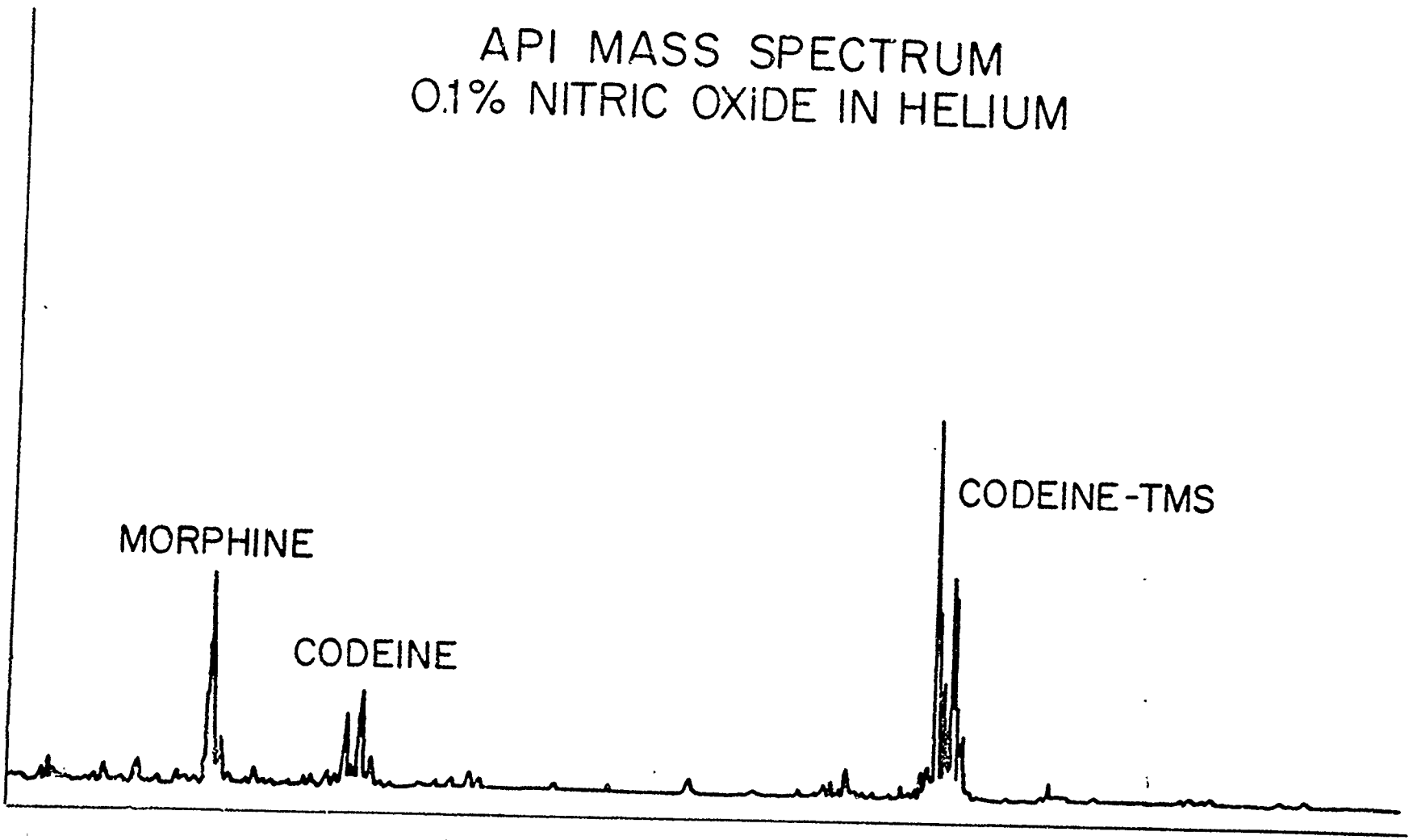
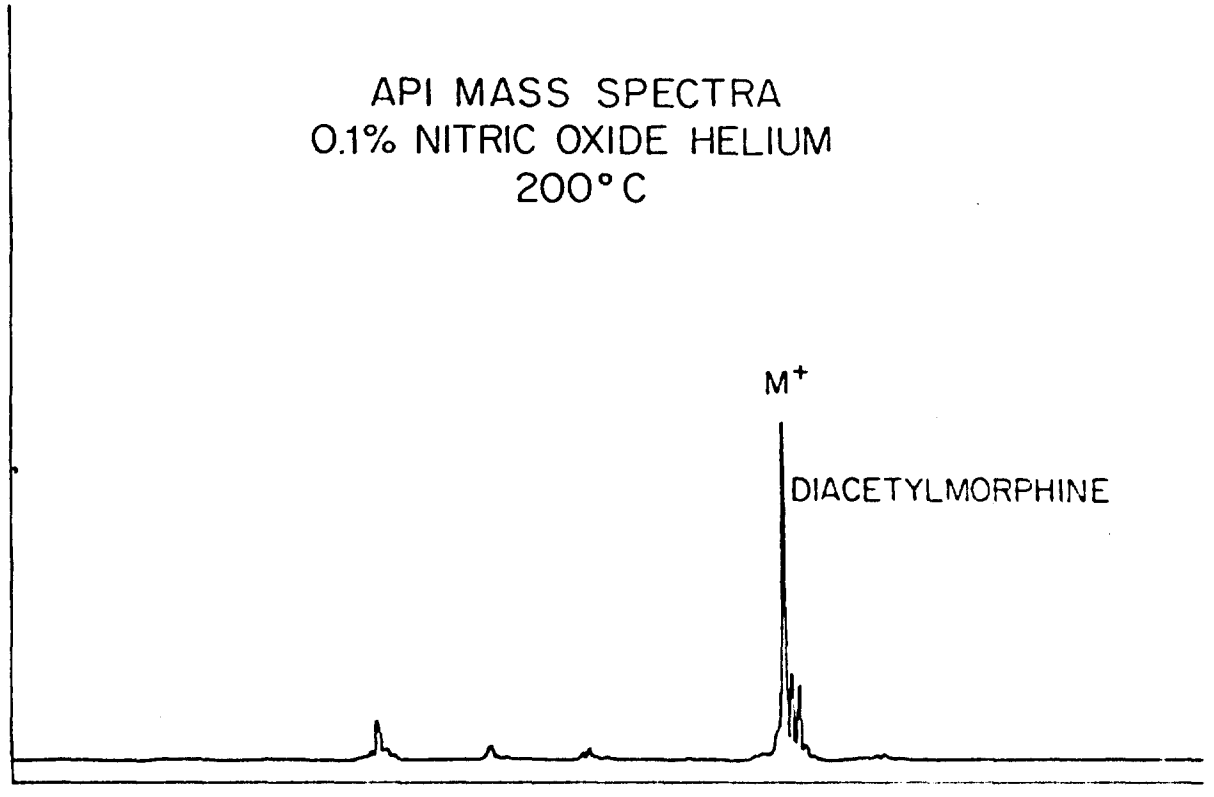

API MASS SPECTRUM
0.1% NITRIC OXIDE IN HELIUM
MORPHINE
CODEINE
CODEINE-TMS

Figure 37


API MASS SPECTRA
0.1% NITRIC OXIDE HELIUM
200° C
M+
DIACETYLMORPHINE

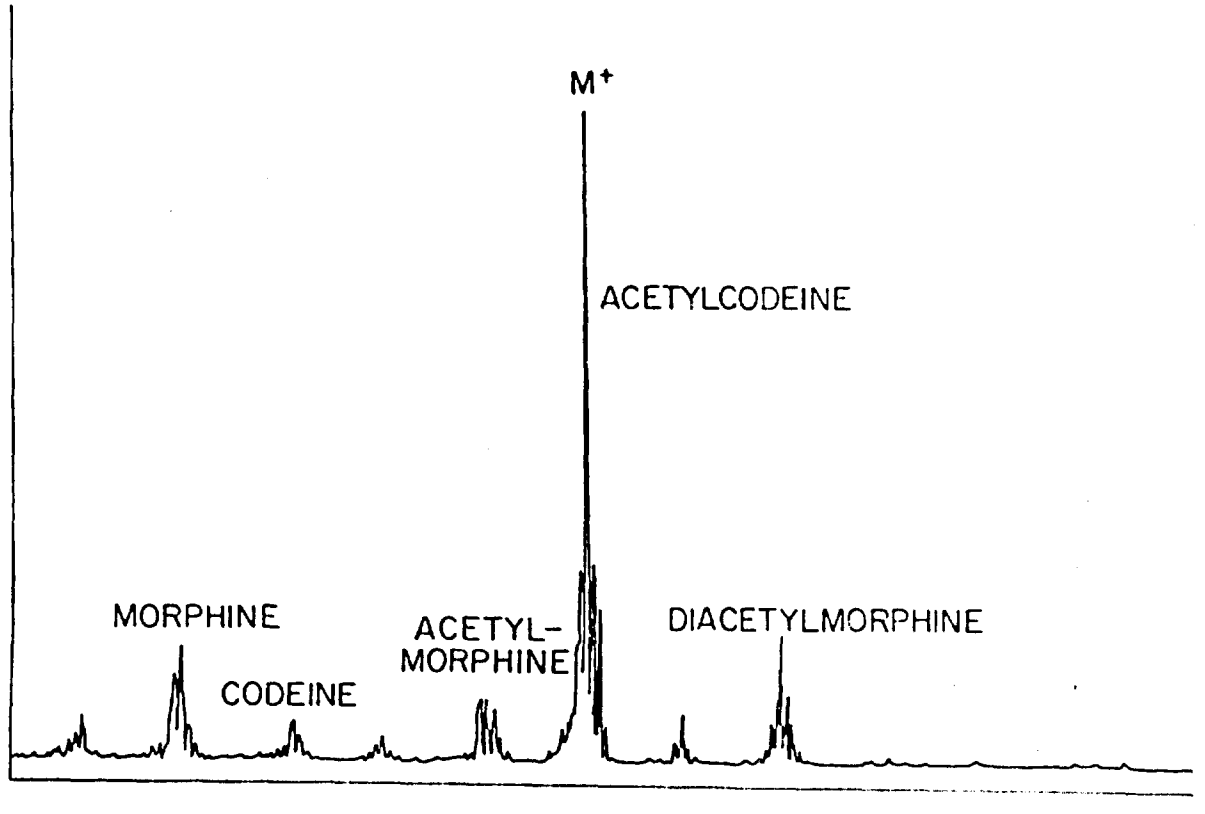

M+
ACETYLCODEINE
MORPHINE
CODEINE
ACETYL-
MORPHINE
DIACETYLMORPHINE

Figure 38

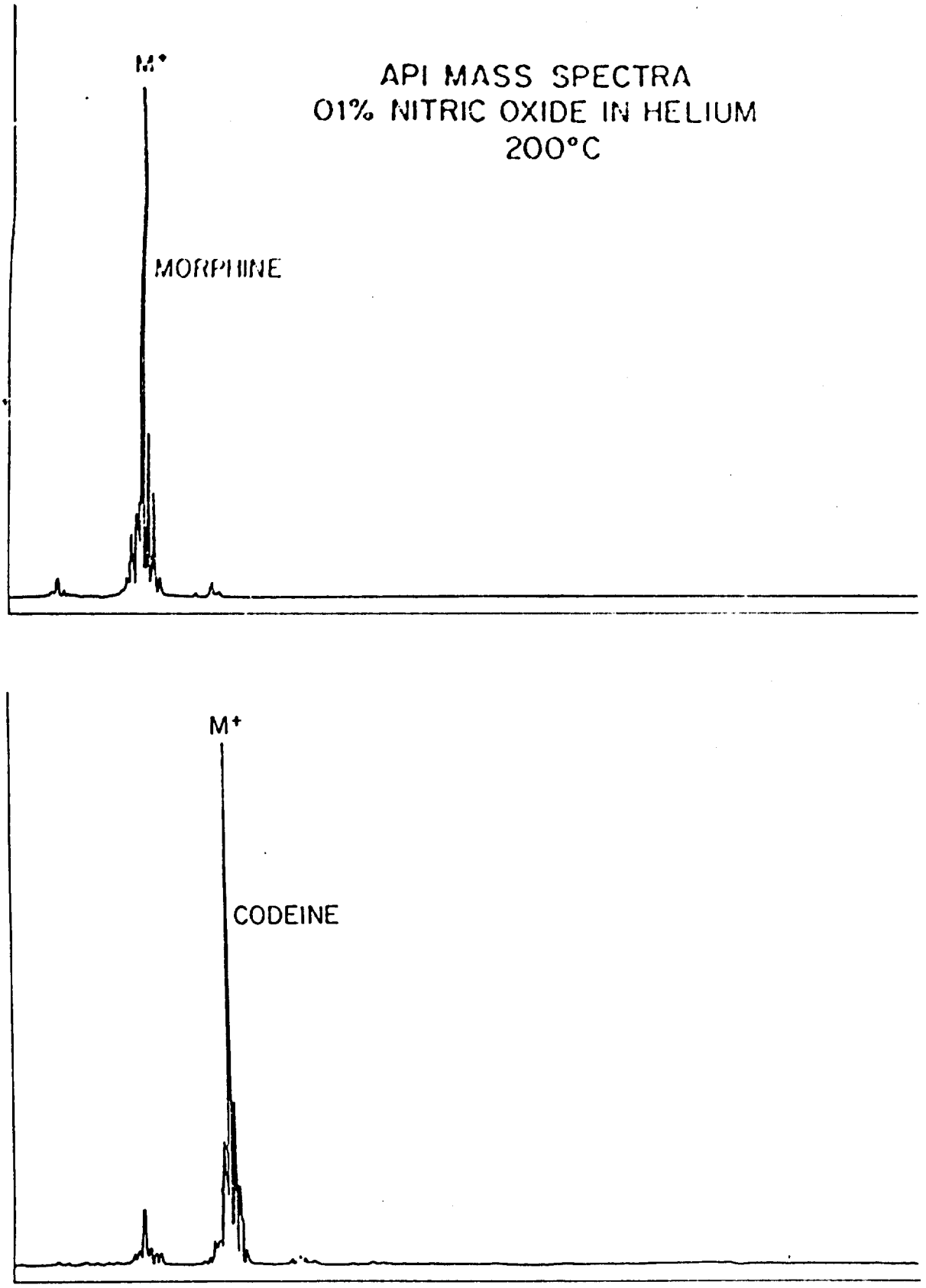

API MASS SPECTRA
01% NITRIC OXIDE IN HELIUM
200°C
M+
MORPHINE
M+
CODEINE

Figure 39

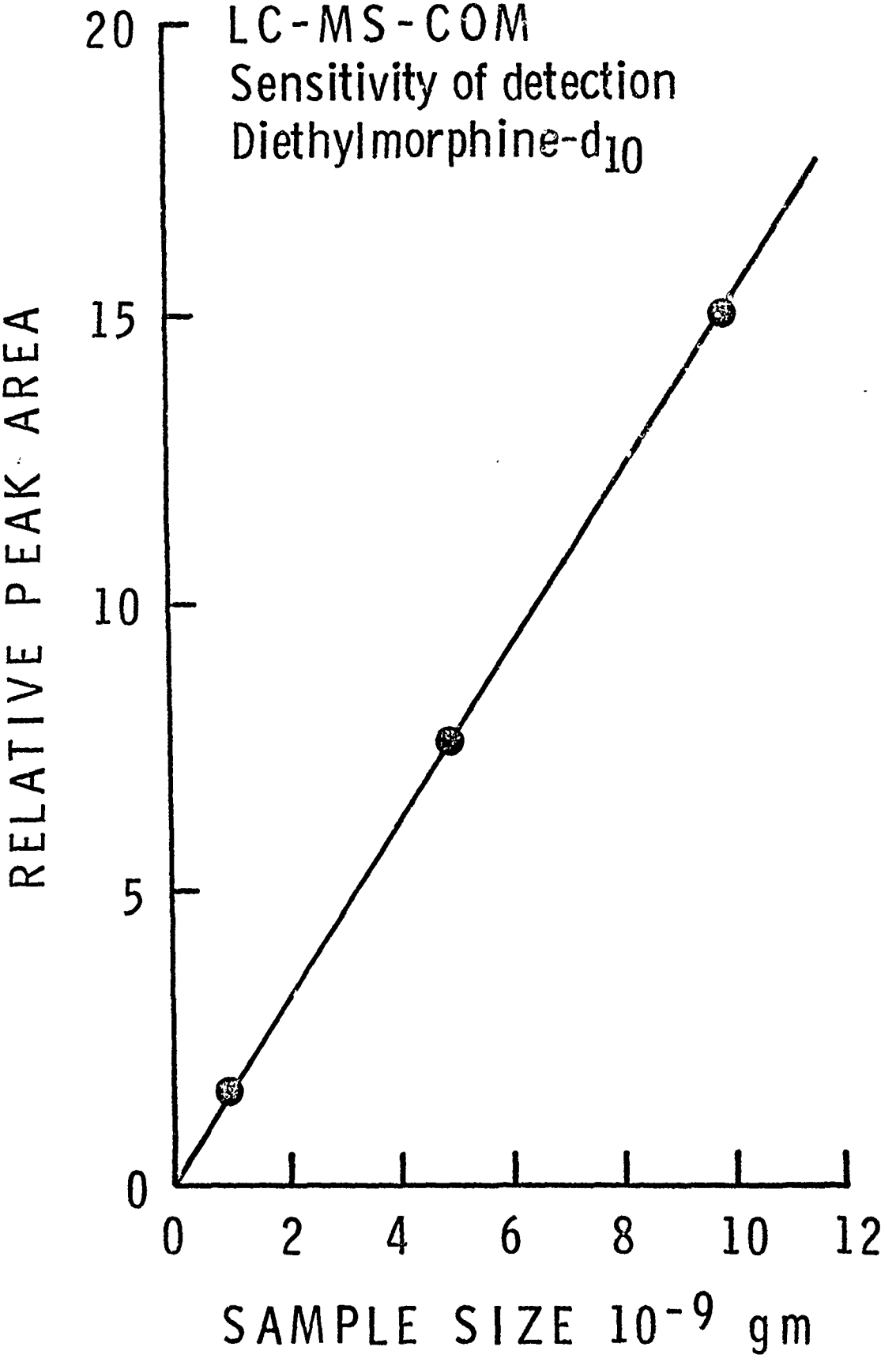

LC-MS-COM
Sensitivity of detection
Diethylmorphine-d10
RELATIVE PEAK AREA
20
15
10
5
0
0
2
4
6
8
10
12
SAMPLE SIZE 10-9 gm

Figure 40

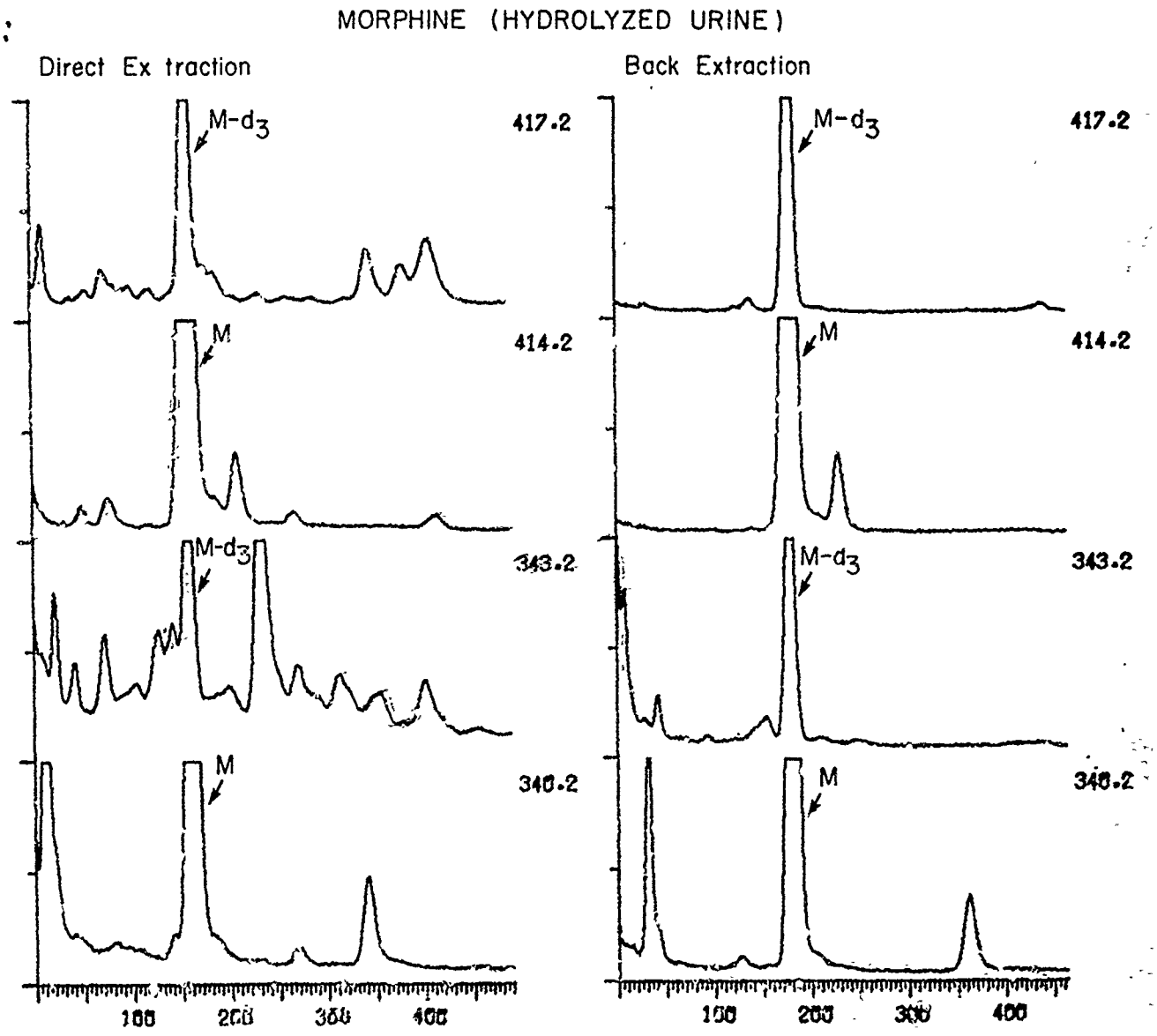

MORPHINE (HYDROLYZED URINE)
Direct Ex traction
Back Extraction
M-d3
M
417.2
414.2
343.2
340.2
100
200
300
400

Figure 41

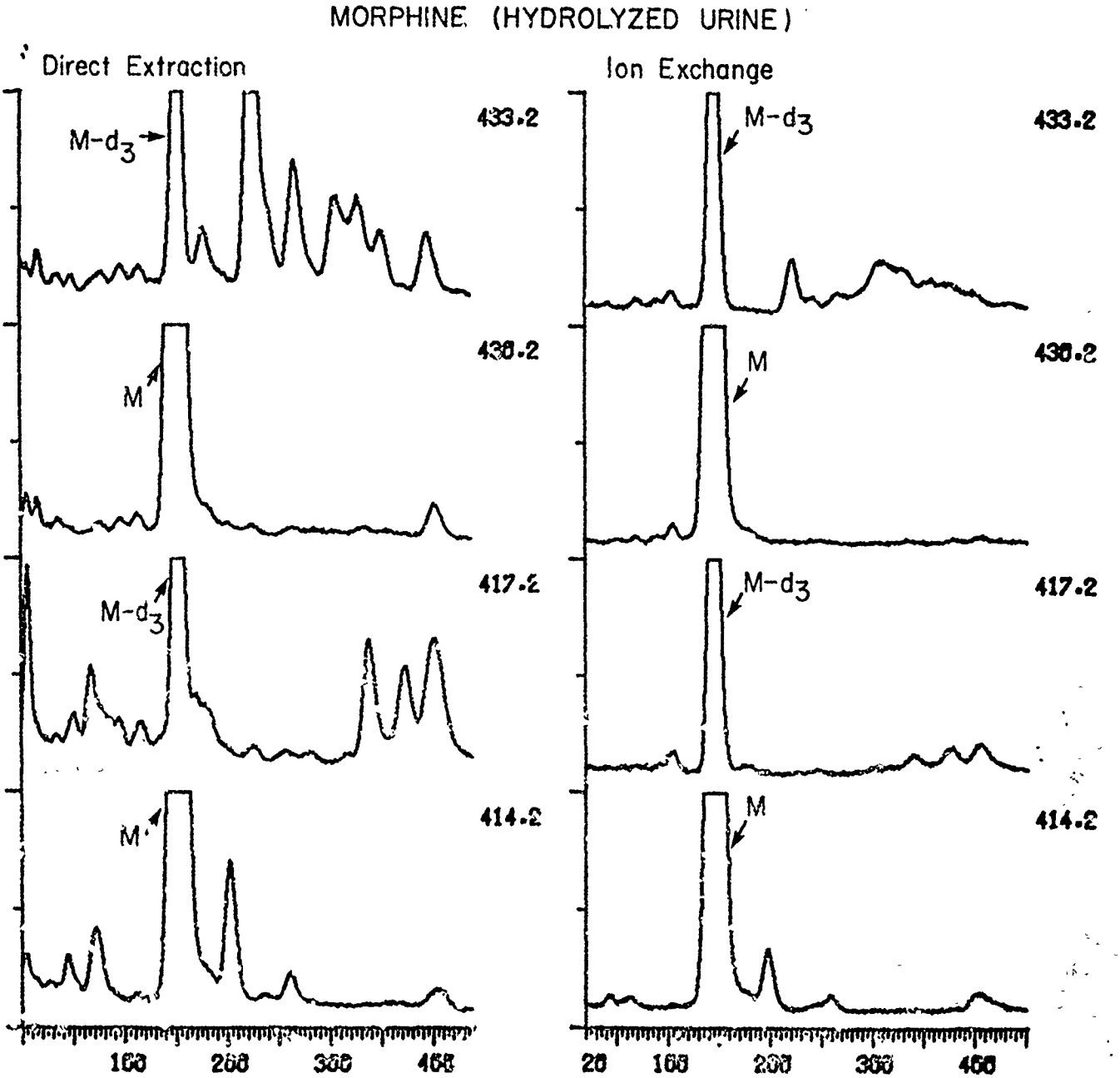

MORPHINE (HYDROLYZED URINE)
Direct Extraction
Ion Exchange
M-d3
433.2
M
436.2
M-d3
417.2
M
414.2
100
200
300
400
20
100
200
300
400

Figure 42

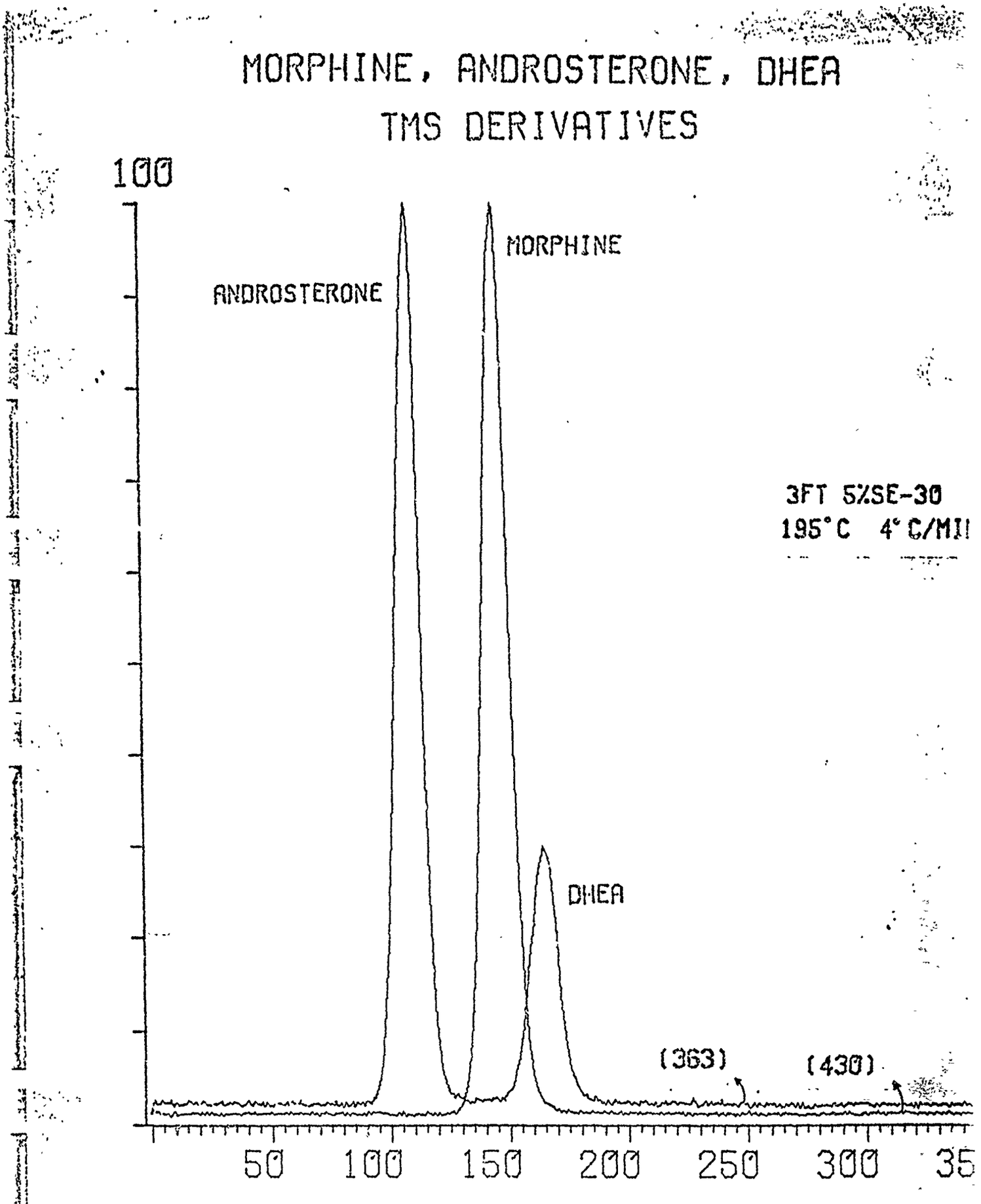

MORPHINE, ANDROSTERONE, DHEA
TMS DERIVATIVES
100
ANDROSTERONE
MORPHINE
3FT 5%SE-30
195°C 4°C/MI
DHEA
(363)
(430)
50
100
150
200
250
300
35

Figure 43

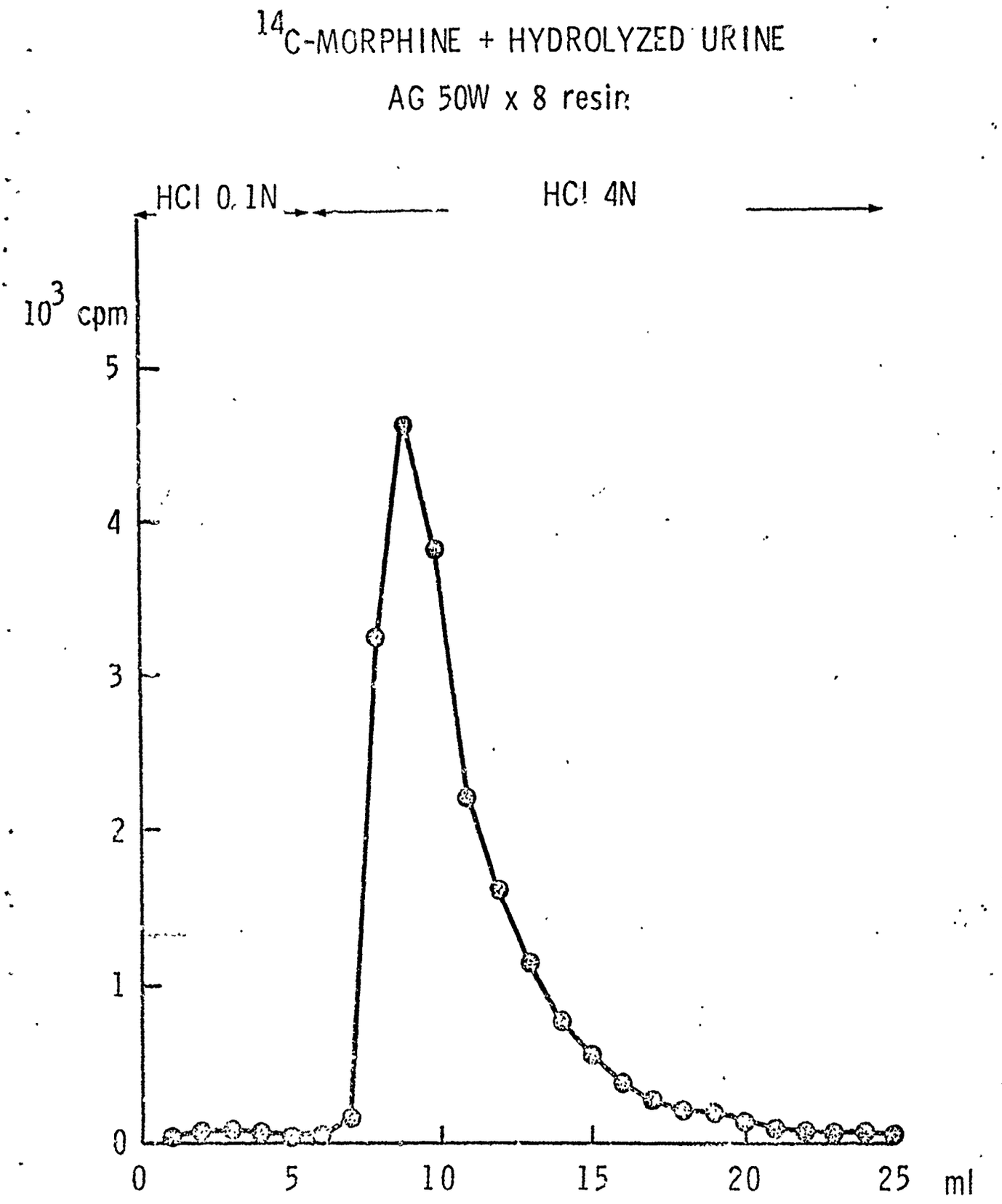

^{14}C-MORPHINE + HYDROLYZED URINE
AG 50W x 8 resin
HCl 0.1N
HCl 4N
10^3 cpm
5
4
3
2
1
0
0
5
10
15
20
25
ml

Figure 44

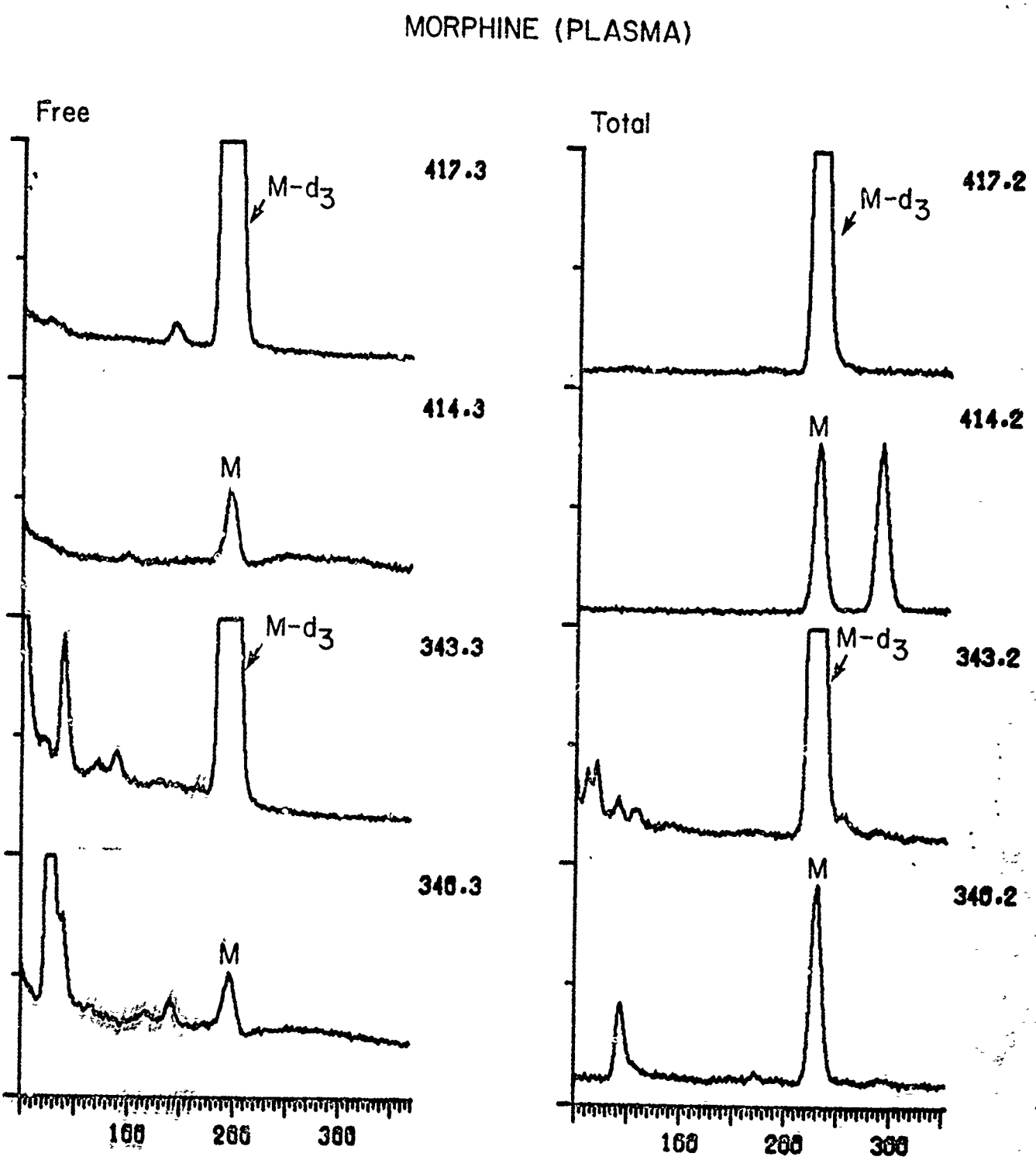

MORPHINE (PLASMA)
Free
Total
M-d3
M
417.3
414.3
343.3
340.3
417.2
414.2
343.2
340.2
100
200
300

Figure 45

VII. LITERATURE CITED

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VIII. APPENDIX

PHASE I

SYMBOLS:

ND -- Never detected

\+ -- Trace detected (amount too small to measure)

R -- Repeat analysis (value changed from 1st results sent)

* -- Corrected result, error on first results sent

o -- Results not sent previously

Patient/Day	Specimen No.	GC-MS Total Morphine µg/ml	GC-MS Free Morphine µg/ml	Frat Value	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
1-0	6-12205	8.57	0.35	5.850	+	ND
1-5	6-12435	0.08	0.03	0.040	ND	ND
1-7	6-12504	0.04	0.00	0.042	ND	ND
1-12	6-13034	0.02	0.00	0.011	ND	ND
2-0	6-12936	0.01	----	0.020	ND	ND
2-1	6-12708	0.00	----	0.020	ND	ND
2-3	6-12567	0.00	----	0.040	ND	ND
2-4	6-12462	0.01	0.00	0.010	ND	ND
2-5A	6-12935	0.01	0.00	0.025	ND	ND
2-5B	6-12702	0.01	0.00	0.035	ND	ND
3-1	6-12332	3.79	0.32	2.840	ND	ND
3-3	6-12770	0.28	0.03	0.190	ND	ND

Patient/Day	Specimen No.	GC-MS Total Morphine µg/ml	GC-MS Free Morphine µg/ml	Frat Value	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
3-5	6-12236	0.00	0.00	0.037	ND	ND
3-6	6-12570	0.00	0.00	0.170	ND	ND
3-7	6-12505	0.00	0.00	0.008	ND	ND
3-8	6-12366	0.00	0.00	0.006	ND	ND
3-9	6-12433	0.00	0.00	0.022	ND	ND
3-10A	6-12535	0.00	0.00	0.017	ND	ND
3-10B	6-13033	0.00	0.00	0.017	ND	ND
4-3	6-12764	0.35	0.02	0.470	ND	ND
4-5	6-12832	0.11	0.00	0.165	ND	ND
4-6	6-12510	0.03	0.00	0.028	ND	ND
4-7	6-12501	0.04	0.00	0.122	ND	ND
4-8	6-12904	0.01	0.00	0.170	ND	ND
4-9	6-12531	0.02	0.00	0.012	ND	ND

Patient/Day	Specimen No.	GC-MS Total Morphine µg/ml	GC-MS Free Morphine µg/ml	Frat Value	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
4-10	6-12963	0.01	0.00	0.041	ND	ND
4-11	6-12139	0.02	0.00	0.062	ND	ND
4-12	6-13009	0.00	0.00	0.004	ND	ND
5-2	6-12432	3.87	0.17	2.500	ND	ND
5-3	6-12837	0.69	0.05	0.910	ND	ND
5-6	6-12539	0.03	0.00	0.001	ND	ND
5-8	6-12709	0.01	0.00	0.037	ND	ND
5-9	6-12169	0.00	----	0.004	ND	ND
5-10	6-12831	0.00	----	0.073	ND	ND
6-0	6-12040	0.12	0.01	0.130	ND	ND
6-1	6-12110	1.06	0.12	0.940	ND	ND
6-4A	6-12234	0.01	0.00	0.010	ND	ND

Patient/Day	Specimen No.	GC-MS Total Morphine µg/ml	GC-MS Free Morphine µg/ml	Frat Value	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
6-4B	6-12903	0.01	0.00	0.020	ND	ND
6-5	6-12509	0.01	0.00	0.000	ND	ND
7-2	6-12335	0.97	0.04	2.310	ND	ND
8-0	6-12166	723	72.6	21.263	1.59	+
8-1	6-12934	52.1	4.71	18.800	0.96	ND
9-0	6-12962	9.57	0.48	5.140	1.05	ND
9-1	6-12336	3.57	0.11	2.300	+	ND
10-0	6-13107	3.20	0.07	3.760	1.37	ND
10-1	6-12561	83.9	1.57	14.300	ND	ND
11-0	6-12931	0.00	----	0.025	ND	ND

Patient/Day	Specimen No.	GC-MS Total Morphine µg/ml	GC-MS Free Morphine µg/ml	Frat Value	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
12-0	6-12767	9.73	0.31	12.600	ND	ND
13-0A	6-12161	14.8	0.32	3.900	0.18	ND
13-0B	6-12334	3.54	0.32	3.200	2.05	ND
13-1	6-12736	2.85	0.11	2.280	ND	ND
13-2	6-12207	0.12	0.00	0.182	ND	ND
13-4	6-12340	0.02	0.00	0.030	ND	ND
13-5	6-12738	0.01	0.00	0.082	ND	ND
13-7	6-12438	0.03	0.00	0.030	ND	ND
13-8	6-12370	0.01	0.00	0.045	ND	ND
13-9	6-12068	0.00	----	0.008	ND	ND
13-10	6-12706	0.01	0.00	0.023	ND	ND
13-11	6-12165	0.03	0.00	0.012	ND	ND

Patient/Day	Specimen No.	GC-MS Total Morphine µg/ml	GC-MS Free Morphine µg/ml	Frat Value	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
13-12	6-12201	0.02	0.00	0.062	ND	ND
13-12B	6-12362	0.02	0.01	0.060	ND	ND
14-1	6-12303	18.2	0.44	6.530	0.90	ND
14-2	6-12464	3.46	0.18	4.010	0.32	ND
14-3	6-12803	0.10	0.03	0.140	ND	ND
14-6	6-12361	0.04	----	0.085	ND	ND
14-7	6-12502	0.02	0.02	0.152	ND	ND
14-8	6-12568	0.03	0.01	0.116	ND	ND
14-9A	6-12268	0.00	----	0.082	ND	ND
14-9B	6-12508	0.02	0.00	0.065	ND	ND
14-10	6-12106	0.01	0.00	0.030	ND	ND
14-12	6-12436	2.52	0.97	2.600	0.85	ND

Patient/Day	Specimen No.	GC-MS Total Morphine µg/ml	GC-MS Free Morphine µg/ml	Frat Value	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
15-2	6-12304	1.36	0.04	1.500	ND	ND
15-7	6-12108	0.02	0.00	0.027	ND	ND
15-9	6-12307	0.01	0.00	0.054	ND	ND
15-10	6-12563	0.00	-----	0.028	ND	ND
15-14	6-12835	0.00	----	0.006	ND	ND
16-0	6-12565	24.6	0.79	6.980	0.779	ND
16-1	6-12209	3.49	0.24	2.40	--	--
17-0	6-12132	8.26	0.48	8.400	ND	ND
17-8A	6-12133	0.00	----	0.041	ND	ND
17-8B	6-12410	0.02	0.01	0.035	ND	ND
17-9	6-12733	0.02	0.00	0.017	ND	ND
17-10	6-12434	0.00	-----	0.008	ND	ND
17-11	6-12910	0.01	----	0.009	ND	ND

Patient/Day	Specimen No.	GC-MS Total Morphine µg/ml	GC-MS Free Morphine µg/ml	Frat Value	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
17-12	6-12240	0.01	----	0.011	ND	ND
17-13	6-12067	0.01	0.00	0.000	ND	ND
19-1A	6-12261	0.02	----	0.016	ND	ND
19-1B	6-12306	0.00	----	0.010	ND	ND
19-3	6-12104	0.00	----	0.009	ND	ND
19-4	6-12468	0.00	----	0.001	ND	ND
19-5	6-12802	0.00	----	0.000	ND	ND
20-2	6-12066	3.64	0.08	2.870	ND	ND
20-4	6-12331	1.58	0.19	1.860	ND	ND
20-5	6-12237	0.55	0.10	1.300	ND	ND
20-6	6-12305	0.16	0.10	0.220	ND	ND
20-7	6-12905	0.04	0.02	0.170	ND	ND

Patient/Day	Specimen No.	GC-MS Total Morphine µg/ml	GC-MS Free Morphine µg/ml	Frat Value	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
20-9	6-12208	0.01	0.00	0.050	ND	ND
20-9B	6-12365	0.01	----	0.023	ND	ND
20-10A	6-12235	0.00	0.00	0.049	ND	ND
20-10B	6-12363	0.00	----	0.000	ND	ND
20-11	6-12901	0.04	0.00	0.004	ND	ND
20-12	6-13103	0.00	----	0.007	ND	ND
20-13	6-12532	0.00	----	0.035	ND	ND
20-14	6-12206	2.62	0.42	3.500	5.21	ND
21-1	6-12063	31.7	2.63	6.520	1.04	ND
21-2	6-12102	3.36	0.41	2.720	+	ND
21-3	6-12467	1.14	0.25	1.480	ND	ND
21-8	6-12406	0.07	0.03	0.450	ND	ND
21-10	6-12808	0.08	0.02	0.251	ND	ND

Patient/Day	Specimen No.	GC-MS Total Morphine µg/ml	GC-MS Free Morphine µg/ml	Frat Value	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
21-11	6-12867	0.08	----	0.299	ND	ND
21-13	6-12131	0.02	0.01	0.114	ND	ND
22-1	6-12266	3.70	0.15	2.900	+	ND
22-2	6-12836	0.96	0.08	1.160	ND	ND
22-4	6-12470	0.01	----	0.035	ND	ND
22-6	6-12431	0.00	----	0.054	ND	ND
22-8	6-12810	0.01	----	0.089	ND	ND
22-9	6-12701	0.01	----	0.042	ND	ND
22-10A	6-12863	0.00	----	0.020	ND	ND
22-10B	6-12202	0.00	0.00	0.035	ND	ND
22-11	6-12069	0.03	----	0.035	ND	ND
22-12	6-12466	0.01	----	0.057	ND	ND

Patient/Day	Specimen No.	GC-MS Total Morphine µg/ml	GC-MS Free Morphine µg/ml	Frat Value	GC-MS Metabolites	
					Codeine %	Nor-morphine %
23-1	6-12405	4.41	0.51	3.900	--	--
23-2	6-12908	6.59	0.65	4.520	+	ND
23-3	6-12339	1.86	0.11	1.200	ND	ND
23-4	6-12134	0.09	0.00	0.041	ND	ND
23-5	6-12704	0.04	0.02	0.006	ND	ND
23-9	6-12308	0.00	0.00	0.017	ND	ND
23-10	6-12801	0.00	----	0.010	ND	ND
23-13	6-12137	0.00	----	0.020	ND	ND
23-14	6-13010	0.00	----	0.004	ND	ND
24-1	6-12806	2.67	0.14	2.200	ND	ND
24-2	6-12210	0.61	0.05	0.840	ND	ND
24-4	6-1256	0.01	0.00	0.022	ND	ND
24-6	6-12533	0.00	0.00	0.017	ND	ND

Patient/Day	Specimen No.	GC-MS Total Morphine µg/ml	GC-MS Free Morphine µg/ml	Frat Value	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
24-8	6-12062	0.00	----	0.030	ND	ND
24-12	6-12705	0.04	0.04	0.072	ND	ND
24-13	6-12834	0.00	----	0.006	ND	ND
25-0	6-12036	0.00	----	0.016	ND	ND
25-2	6-12907	0.00	----	0.016	ND	ND
25-3	6-12710	0.00	----	0.006	ND	ND
25-6	6-12809	0.01	----	0.002	ND	ND
27-2	6-12964	0.48	0.03	0.540	ND	ND
27-4	6-12805	0.07	0.01	0.230	ND	ND
27-5	6-12238	0.01	0.00	0.063	ND	ND
27-6	6-12707	0.00	----	0.002	ND	ND
27-7	6-12961	0.00	----	0.020	ND	ND

Patient/Day	Specimen No.	GC-MS Total Morphine µg/ml	GC-MS Free Morphine µg/ml	Frat Value	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
27-8	6-12569	0.00	----	0.022	ND	+
27-10	6-12103	0.00	----	0.062	ND	ND
27-11A	6-12070	0.00	----	0.009	ND	ND
27-11B	6-12469	0.00	----	0.001	ND	ND
27-12	6-12140	0.01	----	0.004	ND	ND
27-13	6-12168	0.01	----	0.002	ND	ND
28-2	6-12838	1.79	0.15	1.700	ND	ND
28-4	6-13036	0.35	0.02	0.490	ND	ND
28-5	6-13038	0.14	0.01	0.301	ND	ND
28-13	6-13105	0.06	0.00	0.065	ND	ND
30-3	6-13035	12.7	0.74	8.720	1.00	ND
30-6	6-12965	1.87	0.09	2.040	+	ND
30-10	6-13039	0.03	0.00	0.083	ND	ND

Patient/Day	Specimen No.	GC-MS Total Morphine µg/ml	GC-MS Free Morphine µg/ml	Frat Value	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
31-5	6-13004	0.08	0.00	0.174	ND	ND
31-6	6-13007	0.01	0.00	0.000	ND	ND
31-8	6-13005	0.01	0.00	0.022	ND	ND
32-6	6-13006	0.01	----	0.001	ND	ND
32-8	6-12970	0.01	----	0.017	ND	ND
33-9	6-13003	0.01	----	0.008	ND	ND
34-7	6-12968	0.02	0.01	0.012	ND	ND
35-4	6-13102	0.01	----	0.035	ND	ND
36-3	6-12966	0.02	0.00	0.012	ND	ND
36-4	6-13040	0.01	----	0.057	ND	ND
37-2	6-12439	0.05	0.00	0.140	ND	ND

Patient/Day	Specimen No.	GC-MS Total Morphine µg/ml	GC-MS Free Morphine µg/ml	Frat Value	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
38-0	6-12938	9.38	4.18	14.400	ND	ND
99-1	6-12906	0.01	0.00	0.012	ND	ND
99-2	6-12302	0.00	----	0.030	ND	ND
99-3	6-12865	0.00	----	0.006	ND	ND
99-14	6-12262	0.00	----	0.045	ND	ND

REPEATS FROM PHASE I AS REQUESTED BY ARMY

Patient/Day	Specimen No.	GC-MS Total Morphine µg/ml	GC-MS Free Morphine µg/ml
4-3	6-12764	0.27	0.01
4-7	6-12501	0.06	0.01
4-9	6-12531	0.02	0.00
4-12	6-13009	0.01	0.00

PHASE II

SYMBOLS:

ND -- Never detected

\+ -- Trace detected (amount too small to measure)

R -- Repeat analysis (value changed from 1st results sent)

* -- Corrected result, error on first results sent

o -- Results not sent previously

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
1-0	6-00708	925	4.28	3.96	0.34	0.31	5.85	ND	ND
1-1	6-00714	475	6.31	3.00	0.43	0.20	5.70	ND	ND
1-2	No Specimen	---	---	---	---	---	1.71	--	--
1-3	6-00736	445	0.41	0.18	0.01	0.00	0.67	ND	ND
1-4	6-00741	525	0.02	0.01	0.00	0.00	0.017	ND	ND
1-5	6-00761	1065	0.01	0.01	0.00	0.00	0.004	ND	ND
1-6	6-00769	880	0.01	0.01	0.01	0.01	0.063	ND	ND
1-7	6-00794	360	0.01	0.01	0.01	0.00	0.042	ND	ND
1-8	6-00795	615	0.01	0.00	0.00	0.00	0.028	ND	ND
1-9	6-00796	585	0.01	0.00	0.00	0.00	0.023	ND	ND
1-10	6-00800	440	0.00	0.00	0.00	0.00	0.023	ND	ND
1-11	6-00608	555	0.00	0.00	0.00[R]	0.00[R]	0.00	ND	ND
1-12	6-00619	2325	0.01	0.01	0.00	0.00	0.017	ND	ND

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
2-0	6-00707	705*	0.00	0.00	0.00	0.00	0.00	ND	ND
2-1	6-00713	1915	0.00	0.00	0.00	0.00	0.002	ND	ND
2-2	6-00725	1675	0.00	0.00	0.00	0.00	0.016	ND	ND
2-3	6-00735	1120	0.00	0.00	0.00	0.00	0.004	ND	ND
2-4	6-00743	1005	0.00	0.00	0.00	0.00	0.001	ND	ND
2-5	6-00760	820	0.01	0.01	0.00	0.00	0.035	ND	ND
2-6	6-00770	1270	0.00	0.00	0.00	0.00	0.025	ND	ND
2-7	6-00793	515	0.01	0.00	0.00	0.00	0.00	ND	ND
3-0	6-00724	535	18.4	9.87	2.60	1.39	12.600	+	ND
3-1	6-00737	1720	1.91	3.28	0.30	0.51	2.840	ND	ND
3-2	6-00742	540	1.77	0.96	0.23	0.13	2.800	ND	ND
3-3	6-00759	607	0.20	0.12	0.00*	0.00	0.190	ND	ND
3-4	6-00768	485	0.04	0.02	0.00	0.00	0.037	ND	ND

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
3-5	6-00797	535	0.01	0.01	0.00^{R}	0.00^{R}	0.037	ND	ND
3-6	6-00798	845	0.33	0.28	0.00	0.00	0.017	ND	ND
3-7	6-00799	885	0.00	0.00	0.00^{R}	0.00^{R}	0.00	ND	ND
3-8	6-00601	615	0.01	0.00	0.00	0.00	0.006	ND	ND
3-9	6-00609	715	0.00	0.00	0.00	0.00	0.022	ND	ND
3-10	6-00618	750	0.00	0.00	0.00	0.00	0.017	ND	ND
4-0	6-00616		40.8		6.56		35.4	.61	ND
4-1	6-00633	1885	11.6	21.9	0.70	1.33	6.521	.10	ND
4-2	No Specimen	---	---	---	---	---	5.4	--	--
4-3	No Specimen	---	---	---	---	---	0.47	--	--
4-4	No Specimen	---	---	---	---	---	0.260	--	--
4-5	6-00407	695	0.05	0.04	0.01	0.01	0.165	ND	ND
4-6	6-00412	750	0.01	0.01	0.00	0.00	0.030	ND	ND

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
4-7	No Specimen	---	---	---	---	---	0.122	--	--
4-8	6-00445	690	0.07	0.05	0.00	0.00	0.170	+	ND
4-9	No Specimen	---	---	---	---	---	0.012	--	--
4-10	6-00469	760	0.03	0.03	0.00	0.00	0.041	ND	ND
4-11	6-00497	855	0.01	0.01	0.00	0.00	0.062	ND	ND
4-12	No Specimen	---	---	---	---	---	0.004	--	--
5-0	No Specimen	---	---	---	---	---	---	--	--
5-1	6-00634	1100	4.15	4.57	0.30	0.33	4.40	ND	ND
5-2	6-00650	900	2.99	2.69	0.00	0.00	2.50	ND	ND
5-3	6-00665	685	0.47*	0.32*	0.01	0.01	0.910	ND	ND
5-4	6-00693	1270	0.00	0.00	0.00	0.00	0.023	ND	ND
5-5	6-00403	1495	0.00	0.00	0.00	0.00	0.002	ND	ND
5-6	6-00413	1620	0.00	0.00	0.00	0.00	0.001	ND	ND

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value ug/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
5-7	6-00429	940	0.00	0.00	0.00	0.00	0.020	ND	ND
5-8	6-00446	935	0.01	0.01	0.00	0.00	0.037	ND	ND
5-9	6-00458	1215	0.01	0.01	0.00	0.00	0.004	ND	ND
5-10	6-00470	910	0.00	0.00	0.00	0.00	0.073	ND	ND
5-11	6-00498	905	0.00	0.00	0.00	0.00	0.050	ND	ND
6-0	6-00617	385	0.61	0.24	0.01	0.00	0.130	ND	ND
6-1	6-00635	1375	0.80	1.10	0.04	0.05	0.940	ND	ND
6-2	6-00651	1250	0.01	0.01	0.01	0.01	0.054	ND	ND
6-3	6-00666	1090	0.00	0.00	0.00	0.00	0.012	ND	ND
6-4	6-00694	1560	0.00	0.00	0.00	0.00	0.010	ND	ND
6-5	6-00409	1950	0.00	0.00	0.00	0.00	0.025	ND	ND
11-0	6-00433	870	0.01	0.01	0.00	0.00	0.025	ND	ND
11-1	6-00454	795	0.00	0.00	0.00	0.00	0.035	ND	ND

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
11-2	No Specimen	---	---	---	---	---	---	--	--
11-3	No Specimen	---	---	---	---	---	---	--	--
11-4	6-00496	1740	0.01	0.02	0.00	0.00	---	ND	ND
11-5	6-01319	1510	0.00	0.00	0.00	0.00	---	ND	ND
11-6	6-01339	920	0.00	0.00	0.00[R]	0.00[R]	---	ND	ND
11-7	6-01344	1240	0.00	0.00	0.00	0.00	---	ND	ND
11-8	6-01367	530	0.01	0.00	0.00	0.00	---	ND	ND
11-17	12-10384	390	0.00	0.00	0.00	0.00	---	ND	ND
13-0	6-00468		3.47		0.45		3.90	2.82	ND
13-1	6-00493	675	1.43	0.97	0.13	0.09	2.28	0.91	ND
13-2	6-01320	1440	0.11	0.16	0.01	0.01	0.1820	ND	ND
13-3	6-01340	510	0.03	0.01	0.00	0.00	0.054	ND	ND
13-4	6-01346	860	0.01	0.01	0.00	0.00	0.030	ND	ND

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
13-5	6-01365	1610	0.01	0.01	0.00	0.00	0.082	ND	ND
13-6	No Specimen	---	---	---	---	---	---	--	--
13-7	6-01388	880	0.01	0.00	0.00	0.00	0.030	ND	ND
13-8	6-01399	675	0.01	0.01	0.00	0.00	0.045	ND	ND
13-9	12-10246	1650	0.00	0.00	0.00	0.00	0.008	ND	ND
13-10	12-10262	1080	0.01	0.01	0.00	0.00	0.023	ND	ND
13-11	12-10291	750	0.00	0.00	0.00	0.00	0.012	ND	ND
13-12	12-10335	2120	0.01	0.02	0.00	0.00	0.062	ND	ND
13-13	12-10371	2390	0.00	0.00	0.00	0.00	0.012	ND	ND
13-14	12-10382	1120	0.00	0.00	0.00	0.00	0.007	ND	ND
14-0	No Specimen	---	---	---	---	---	---	--	--
14-1	6-00494	880	11.8	10.4	0.31	0.27	6.53	ND	ND
14-2	No Specimen	---	---	---	---	---	4.01	--	--

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
14-3	6-01341	510	0.06	0.03	0.01	0.00	0.140	ND	ND
14-4	6-01347	870	0.02	0.02	0.00	0.00	0.082	ND	ND
14-5	6-01366	905	0.02	0.02	0.00	0.00	0.082	ND	ND
14-6	6-01370	470	0.03	0.02	0.00	0.00	0.085	ND	ND
14-7	6-01387	470	0.06	0.03	0.00	0.00	0.152	ND	ND
14-8	6-01400	495	0.01	0.01	0.00	0.00	0.116	ND	ND
14-9	12-10247	490	0.02	0.01	0.00	0.00	0.082	ND	ND
14-10	12-10261	850	0.01	0.00	0.00	0.00	0.030	ND	ND
14-11	12-10292	630	0.00	0.00	0.00	0.00	---	ND°	ND°
14-12	12-10336	455	0.01	0.00	0.00	0.00	2.60	ND	ND
14-13	12-10370	685	0.00	0.00	0.00	0.00	0.030	ND	ND
14-14	No Specimen	---	---	---	---	---	0.057	--	--
15-0	No Specimen	---	---	---	---	---	---	--	--

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frac Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
15-1	6-00495	3258	1.01	3.29	0.06	0.20	6.12	ND	ND
15-2	6-01321	1310	1.03	1.35	0.03	0.04	1.50	ND	ND
15-3	No Specimen	---	---	---	---	---	---	--	--
15-4	6-01345	1130	0.02	0.02	0.00	0.00	0.016	ND	ND
15-5	6-01364	1100	0.00	0.00	0.00	0.00	0.016	ND	ND
15-6	6-01369	1000	0.00	0.00	0.00	0.00	0.052	ND	ND
15-7	6-01386	640	0.00	0.00	0.00	0.00	0.025	ND	ND
15-8	6-01397	480	0.00	0.00	0.00	0.00	0.030	ND	ND
15-9	12-10248	430	0.00	0.00	0.00	0.00	0.054	ND	ND
15-10	12-10259	1240	0.00	0.00	0.00	0.00	0.016	ND	ND
15-11	12-10293	890	0.00	0.00	0.00	0.00	0.662	ND	ND
15-12	12-10334	1270	0.00	0.00	0.00[R]	0.00[R]	0.017	ND	ND
15-13	12-10372	1490	0.00[o]	0.00[o]	0.00[o]	0.00[o]	---	ND[o]	ND[o]
15-14	12-10383	1100	0.00	0.00	0.00[o]	0.00[o]	0.006	ND	ND

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
19-0	12-10217	580	0.00	0.00	0.00	0.00	0.020	ND	ND
19-1	12-10242	1505	0.00	0.00	0.00	0.00	0.016	ND	ND
19-2	12-10264	1570	0.00	0.00	0.00	0.00	0.00	ND	ND
19-3	12-10295	1395	0.00	0.00	0.00	0.00	0.009	ND	ND
19-4	12-10339	1500	0.00	0.00	0.00	0.00	0.001	ND	ND
19-5	12-10365	910	0.00	0.00	0.00	0.00	0.00	ND	ND
22-0	No Specimen	---	---	---	---	---	18.6	--	--
22-1	12-10546	1014	3.42	3.46	0.15	0.15	2.9	1%	ND
22-2°	12-10574	715	5.06	3.62	0.04	0.03	1.16	ND	ND
22-3°	12-10589	795	0.05	0.04	0.01	0.01	0.110	ND	ND
22-4	12-10810	1185	0.01	0.01	0.00	0.00	0.035	ND	ND
22-5	12-10829	1530	0.00	0.00	0.00	0.00	0.017	ND	ND

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
22-6	12-10841	900	0.00	0.00	0.00	0.00	0.054	ND	ND
22-7	12-10857	740	0.00	0.00	0.00	0.00	0.020	ND	ND
22-8	12-10868	610	0.00	0.00	0.00	0.00	0.089	ND	ND
22-9	No Specimen	---	---	---	---	---	0.042	--	--
22-10	12-10892	1120	0.00	0.00	0.00	0.00	0.035	ND	ND
22-11	12-10910	1110	0.00	0.00	0.00	0.00	0.035	ND	ND
22-12	12-10925	605	0.04	0.03	0.00	0.00	0.057	ND	ND
22-13	12-10947	1000	0.00	0.00	0.00	0.00	0.045	ND	ND
22-14	12-10966	630	0.00	0.00	0.00	0.00	0 013	ND	ND
23-0	12-10524	850	37.7	31.5	3.18	2.70	13.63	5%*	ND
23-1	12-10543	1875	7.61	14.2 *	0.67	1.26	3.90	0.45%*	ND
23-2	12-10571	590	3.87	2.28	0.33	0.20	4.52	ND	ND
23-3	12-10588	650	0.75	0.49	0.08	0.05	1.20	ND	ND

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-Morphine %
23-4	12-10807	1400	0.02	0.03	0.00	0.00	0.041	ND	ND
23-5	12-10826	1800	0.01	0.01	0.00	0.00	0.006	ND	ND
23-6	12-10843	760	0.06	0.05	0.00	0.00	0.035	ND	ND
23-7	12-10859	2030	0.01*	0.02	0.01	0.01	0.015	ND	ND
23-8	12-10870	1100	0.01	0.01	0.00	0.00	.037	ND	ND
23-9	12-10884	1270	0.01	0.01	0.01	0.01	.017	+	ND
23-10	12-10893	1600	0.01	0.01	0.01	0.02	.010	ND	ND
23-11	12-10911	1845	0.67	1.23	0.01	0.02	.062	ND	ND
23-12	No Specimen	---	---	---	---	---	.094	--	--
23-13	12-10948	1185	0.01	0.01	0.01	0.01	.020	ND	ND
23-14	12-10967	1470	0.01	0.01	0.00	0.00	.004	ND	ND
24-0	12-10525	440	11.1	4.91	0.17	0.08	7.47	ND	ND
24-1	12-10544	3880	3.36	13.0	0.14	0.55	2.20	ND	ND

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-Morphine %
24-2	12-10572	4240	0.34	1.45	0.05	0.20	.840	ND	ND
24-3	12-10590	1930	0.01	0.02	0.00	0.00	.023	ND	ND
24-4	12-10808	1580	0.01	0.01	0.01	0.01	.022	ND	ND
24-5	12-10827	2510	0.00	0.00	0.00	0.00	.028	ND	ND
24-6	12-10842	1915	0.00	0.00	0.00	0.00	.017	ND	ND
24-7	12-10858	1690	0.00	0.00	0.00	0.00	.049	ND	ND
24-8	12-10869	1000	0.01	0.01	0.01	0.01	.030	ND	ND
24-9	12-10882	2385	0.00	0.00	0.00	0.00	.004	ND	ND
24-10	12-10894	1640	0.01	0.01	0.01	0.01	---	ND	ND
24-11	12-10912	2025	0.01	0.03	0.00	0.00	.028	ND	ND
24-12	12-10920	1420	0.03	0.04	0.02	0.03	.072	ND	ND
24-13	12-10949	1580	0.01	0.01	0.00	0.00	.006	ND	ND
24-14	12-10968	1350	0.01	0.01	0.01	0.01	.028	ND	ND

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-Morphine %
25-0	12-10526	300	0.01	0.00	0.01	0.00	.016	ND	ND
25-1	12-10545	1310	0.00	0.00*	0.00R	0.00R	.016	ND	ND
25-2	12-10573	1245	0.02	0.02	0.00R	0.00R	.016	ND	ND
25-3	12-10587	860	0.01	0.01	0.01	0.01	.006	ND	ND
25-4	12-10809	1185	0.00	0.00	0.00R	0.00R	.011	ND	ND
25-5	12-10828	1045	0.01	0.01	0.00	0.00	.000	ND	ND
25-6	12-10844	220	0.00	0.00	0.00	0.00	.002	ND	ND

PHASE III

SYMBOLS:

ND -- Never detected

\+ -- Trace detected (amount too small to measure)

R -- Repeat analysis (value changed from 1st results sent)

* -- Corrected result, error on first results sent

o -- Results not sent previously

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
16-0	12-10218	610	6.68	4.07	0.83	0.50	6.98	0.54	ND
16-1	12-10244	725	2.63	1.91	0.24	0.17	2.40	0.65	ND
17-0	12-10216	690	8.65	5.97	0.80	0.55	8.4	0.36	ND
17-1	12-10243	1430	16.6	23.8	0.74	1.06	5.4	ND	ND
17-2	12-10263	2130	3.77	8.03	0.35	0.73	3.2	ND	ND
17-3	12-10294	510	0.72	0.37	0.17	0.09	0.94	ND	ND
17-4	12-10338	820	0.12	0.10	0.09	0.07	0.17	ND	ND
17-5	12-10368	665	0.05	0.03	0.05	0.03	0.24	ND	ND
17-6	12-10385	820	0.05	0.04	0.05	0.05	0.07	ND	ND
17-7	12-10523	650	0.08	0.06	0.07	0.04	0.08	ND	ND
17-8	12-10541	1700	0.01	0.02	0.0	0.0	0.41	ND	ND
17-9	12-10569	1785	1.49	2.66	0.06	0.11	0.02	ND	ND
17-10	12-10593	925	0.01	0.01	0.0	0.0	0.01	ND	ND

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
17-11	12-10805	1560	0.01	0.02	0.0	0.0	0.01	ND	ND
17-12	12-10824	1245	0.29	0.35	0.10	0.13	0.01	ND	ND
17-13	12-10845	1195	0.26	0.31	0.11	0.13	0.0	ND	ND
20-0	No Specimen	---	---	---	---	---	16.4	--	--
20-1	12-10296	600	7.44	4.46	1.50	0.90		1.56	ND
20-2	12-10337	1040	1.26	1.31	0.14	0.15	2.87	0.48	ND
20-3	12-10367	860	0.84	0.72	0.05	0.05	1.40	0.48	ND
20-4	12-10386	670	0.92	0.62	0.10	0.07	1.86	0.87	ND
20-5	12-10522	797	0.47	0.37	0.06	0.05	1.30	0.43	ND
20-6	12-10542	1120	0.12	0.13	0.01	0.01	0.22	ND	ND
20-7	12-10570	1330	0.10	0.13	0.0	0.0	0.17	ND	ND
20-8	12-10592	700	0.03	0.02	0.0	0.0	0.07	ND	ND
20-9	12-10806	990	0.05	0.05	0.0	0.0	0.05	ND	ND

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
20-10	12-10825	1320	0.06	0.07	0.0	0.0	0.05	ND	ND
20-11	12-10839	720	0.04	0.03	0.0	0.0	0.0	ND	ND
20-12	12-10855	1770	0.02	0.03	0.0	0.0	0.01	ND	ND
20-13	12-10866	375	0.04	0.01	0.0	0.0	0.04	ND	ND
20-14	12-10886	620	1.62	1.01	0.17	0.11	3.50	2.59	ND
21-0	No Specimen	---	---	---	---	---	---	--	--
21-1	12-10297	665	75.7	50.3	1.02	0.68	6.52	0.13	ND
21-2	12-10340	430	2.58	1.11	0.12	0.05	2.72	ND	ND
21-3	12-10366	1125	1.13	1.27	0.13	0.15	1.48	1.41	ND
21-4	No Specimen	---	---	---	---	---	1.04	--	--
21-5	No Specimen	---	---	---	---	---		--	--
21-6	12-10540	335	0.31	0.10	0.06	0.02	0.58	3.87	ND
21-7	No Specimen	---	---	---	---	---	0.46	--	--

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
21-8	12-10591	---	0.23	---	0.03	---	0.45	4.00	ND
21-9	12-10804	610	0.18	0.11	0.03	0.02	0.36	ND	ND
21-10	12-10823	530	0.16	0.08	0.02	0.01	0.251	2.56	ND
21-11	12-10840	470	0.13	0.06	0.12	0.01	0.299	ND	ND
21-12	12-10856	910	0.06	0.05	0.01	0.01	0.093	ND	ND
21-13	12-10867	565	0.07	0.04	0.01	0.0	0.114	ND	ND
21-14	12-10888	660	0.05	0.03	0.01	0.01	0.130	ND	ND
27-0	No Specimen	---	---	---	---	---	20.5	--	--
27-1	6-00499	1495	2.21	3.30	0.11	0.17	2.64	2.81	ND
27-2	6-01323	1585	0.44	0.70	0.02	0.02	.54	ND	ND
27-3	6-10343	1435	0.13	0.19	0.01	0.01	.15	ND	ND
27-4	6-10348	1120	0.07	0.08	0.00	0.00	.13	ND	ND
27-5	6-10368	1800	0.02	0.03	0.02	0.03	.063	ND	ND

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
27-6	6-01372	1260	0.00	0.00	0.00	0.00	.00	ND	ND
27-7	6-01385	1214	0.01	0.02	0.00	0.00	.02	ND	ND
27-8	6-01398	1470	0.01	0.01	0.00	0.00	.022	ND	ND
27-9	12-10245	1540	0.01	0.01	0.00	0.00	.000	ND	ND
27-10	12-10260	1210	0.00	0.00	0.00	0.00	.062	ND	ND
27-11	12-10290	1520	0.00	0.00	0.00	0.00	.009	ND	ND
27-12	12-10333	1000	0.00	0.00	0.00	0.00	.004	ND	ND
27-13	12-10369	2100	0.00	0.00	0.00	0.00	.002	ND	ND
28-0	12-10913	1500	9.23	13.85	0.95	1.42	4.70	19.71	ND
28-1	12-10950	1285	2.11	2.71	0.15	0.19	---	ND	ND
28-2	12-10977	940	0.39	0.36	0.02	0.02	1.70	ND	ND
28-3	No Specimen	---	---	---	---	---	---	--	--
28-4	12-10402	460	0.22	0.10	0.01	0.01	.49	1.36	ND

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
28-5	12-10418	980	0.10	0.10	0.01	0.01	.301	.97	ND
28-6	12-10450	1645	0.02	0.03	0.00	0.00	.190	trace	ND
28-7	12-10458	610	0.02	0.01	0.01	0.01	.012	ND	ND
28-8	12-10486	1150	0.14	0.16	0.00	0.00	.035	2.96	ND
28-9	12-10717	740	0.00	0.00	0.00	0.00	.019		ND
28-10	12-10746	820	0.00	0.00	0.00	0.00	.043	ND	ND
28-11	12-10763	1355	0.01	0.01	0.00	0.00	.004	trace	ND
28-12	12-10794	1330	0.00	0.00	0.00	0.00	.017	ND	ND
28-13	12-11916	1520	0.00	0.00	0.00	0.00	.065	ND	ND
28-14	No Specimen	---	---	---	---	---	.084	--	--
29-0	12-10915	1000	527	527	27.4	27.4	13.2	0.65	ND
29-1	No Specimen	---	---	---	---	---	---	---	--
29-2	12-10946	740	63.10	46.69	6.08	4.50	6.7	0.45	ND

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
29-3	12-10964	565	54.3	30.7	5.23	2.95	10.54	1.56	ND
29-4	No Specimen	---	---	---	---	---	4.60	---	--
29-5	12-10401	400	17.3	6.94	1.36	0.54	6.60	0.33	ND
29-6	12-10416	855	51.9	44.3	8.24	7.04	8.50	3.24	ND
29-7	12-10448	1070	5.82	6.23	0.39	0.42	4.13	0.33	ND
30-0	No Specimen	---	---	---	---	---	15.40	--	--
30-1	No Specimen	---	---	---	---	---	---	--	--
30-2	12-10954	930	23.2	21.5	1.38	1.29	5.20	ND	ND
30-3	12-10965	320	19.3	6.20	1.45	0.47	8.72	ND	ND
30-4	12-10978	735	5.15	3.78	0.29	0.21	3.05	ND	ND
30-5	No Specimen	---	---	---	---	---	---	--	--
30-6	12-10420	1000	2.45	2.45	0.12	0.12	2.04	ND	ND
30-7	12-10451	790	1.74	1.38	0.11	0.09	1.40	ND	ND

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
30-8	12-10459	890	0.40	0.35	0.02	0.02	0.38	ND	ND
30-9	12-10487	720	0.11	0.08	0.01	0.00	0.17	ND	ND
30-10	12-10718	980	0.04	0.04	0.01	0.01	0.083	ND	ND
30-11	12-10747	1070	0.02	0.02	0.00	0.00	0.016	ND	ND
30-12	12-10765	1205	0.01	0.02	0.00	0.00	0.043	ND	ND
30-13	No Specimen	---	---	---	---	---	---	--	--
30-14	12-11917	1080	0.02	0.03	0.00	0.00	0.027	ND	ND
31-0	12-10980	1455	8.71	12.68	0.55	0.79	4.60	ND	ND
31-1	12-10405	1805	7.13	12.87	0.40	0.73	3.80	ND	ND
31-2	12-10424	600	4.08	2.45	0.19	0.12	3.12	ND	ND
31-3	12-10453	505	0.19	0.09	0.01	0.00	0.27	ND	ND
31-4	12-10461	835	0.04	0.03	0.00	0.00	.072	ND	ND
31-5	12-10489	920	0.09	0.08	0.01	0.01	0.174	ND	ND

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
31-6	12-10720	1160	0.00	0.00	0.00	0.00	0.000	ND	ND
31-7	12-10749	980	0.00	0.00	0.00	0.00	0.004	ND	ND
31-8	12-10769	1770	0.00	0.00	0.00	0.00	0.022	ND	ND
31-9	12-10797	1335	0.00	0.00	0.00	0.00	0.035	ND	ND
31-10	12-11911	830	0.00	0.00	0.00	0.00	0.050	ND	ND
31-11	12-11930	940	0.00	0.00	0.00	0.00	0.076	ND	ND
31-12	12-11945	1320	0.00	0.00	0.00	0.00	---	ND	ND
31-13	12-11963	1800	0.00	0.00	0.00	0.00	---	ND	ND
32-0	12-10979	700	3.59	2.51	0.28	0.20	3.76	5.24	ND
32-1	12-10404	1460	3.23	4.71	0.19	0.28	2.98	2.07	ND
32-2	12-10422	1630	0.96	1.56	0.06	0.09	1.20	0.42	ND
32-3	12-10452	1100	0.07	0.08	0.01	0.01	0.074	+	ND
32-4	12-10460	985	0.01	0.01	0.01	0.01	0.027	+	ND

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
32-5	No Specimen	---	---	---	---	---	---	--	--
32-6	12-10719	1770	0.01	0.02	0.00	0.00	0.001	33.33	ND
32-7	12-10748	1420	0.00	0.00	0.00	0.00	0.000	ND	ND
32-8	12-10767	1575	0.00	0.00	0.00	0.00	0.017	ND	ND
32-9	No Specimen	---	---	---	---	---	---	--	--
32-10	12-11910	1320	0.00	0.00	0.00	0.00	0.006	ND	ND
32-11	12-11929	730	0.01	0.00	0.00	0.00	0.035	ND	ND
32-12	12-11944	1640	0.00	0.00	0.00	0.00	0.00	+	ND
32-13	12-11962	1620	0.00	0.00	0.00	0.00	0.00	+	ND
33-0	12-10981	620	4.81	2.98	0.26	0.16	3.90	5.05	ND
33-1	No Specimen	---	---	---	---	---	2.44	--	--
33-2	12-10426	545	0.58	0.32	0.06	0.03	1.24	ND	ND
33-3	12-10454	710	0.14	0.10	0.01	0.01	0.29	ND	ND

Patient/Day	Specimen No.	Total Volume (ml)	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-morphine %
33-4	12-10462	960	0.02	0.02	0.01	0.01	0.028	ND	ND
33-5	12-10490	405	0.01	0.00	0.00	0.00	0.028	ND	ND
33-6	12-10721	705	0.01	0.01	0.00	0.00	0.035	ND	ND
33-7	12-10750	960	0.01	0.01	0.00	0.00	0.028	ND	ND
33-8	12-10771	980	0.01	0.00	0.00	0.00	0.000	ND	ND
33-9	12-10798	835	0.01	0.00	0.00	0.00	0.008	ND	ND
33-10	12-11912	1160	0.00	0.00	0.00	0.00	0.042	ND	ND
33-11	12-11931	960	0.00	0.00	0.00	0.00	0.065	ND	ND
33-12	12-11946	1040	0.00	0.00	0.00	0.00	---	ND	ND
33-13	12-11964	1300	0.00	0.00	0.00	0.00	---	ND	ND

PHASE IV

Patient/day	Specimen No.	Total Volume ml	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µl/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-Morphine %
1-0	6-00708	925	4.28	3.96	0.340	0.310	5.85	ND	ND
1-1	6-00714	475	6.31	3.00	0.430	0.200	5.70	ND	ND
1-2	no specimen						1.71		
1-3	6-00736	445	0.410	0.182	0.010	0.004	0.67	ND	ND
1-4	6-00741	525	0.020	0.011	0.003	0.001	0.017	ND	ND
1-5	6-00761	1065	0.013	0.014	0.002	0.004	0.004	ND	ND
1-6	6-00769	880	0.011	0.010	0.012	0.011	0.063	ND	ND
1-7	6-00794	360	0.013	0.005	0.006	0.002	0.042	ND	ND
1-8	6-00795	615	0.005	0.003	0.003	0.002	0.028	ND	ND
1-9	6-00796	585	0.005	0.003	0.002	0.001	0.023	ND	ND
1-10	6-00800	440	0.003	0.001	0.004	0.002	0.023	ND	ND
1-11	6-00608	555	0.001	0.001	0.025	0.014	0	ND	ND
1-12	6-00619	2325	0.005	0.012	0	0	0.017	ND	ND
2-0	6-00707	405	0.004	0.003	0.004	0.003	0	ND	ND
2-1	6-00713	1915	0.003	0.006	0	0	0.002	ND	ND

Patient/day	Specimen No.	Total Volume ml	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-Morphine %
2-2	6-00725	1675	0.002	0.004	0	0	0.016	ND	ND
2-3	6-00735	1120	0.002	0.002	0	0	0.004	ND	ND
2-4	6-00743	1005	0.002	0.002	0	0	0.001	ND	ND
2-5	6-00760	820	0.007	0.005	0	0	0.035	ND	ND
2-6	6-00770	1270	0.002	0.003	0	0	0.025	ND	ND
2-7	6-00793	515	0.008	0.004	0	0	0	ND	ND
3-0	6-00724	535	18.4	9.87	2.60	1.39	12.6	+	ND
3-1	6-00737	1720	1.90	3.27	0.299	0.514	2.84	ND	ND
3-2	6-00742	540	1.77	0.958	0.233	0.126	2.80	ND	ND
3-3	6-00759	607	0.201	0.122	0.033	0.002	0.190	ND	ND
3-4	6-00768	485	0.040	0.020	0	0	0.037	ND	ND
3-5	6-00797	535	0.010	0.006	0.053	0.029	0.037	ND	ND
3-6	6-00798	845	0.334	0.283	0	0	0.017	ND	ND
3-7	6-00799	885	0.003	0.003	0.017	0.015	0	ND	ND
3-8	6-00601	615	0.005	0.003	0.004	0.003	0.006	ND	ND

Patient/day	Specimen No.	Total Volume ml	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-Morphine %
3-9	6-00609	715	0.003	0.002	0	0	0.022	ND	ND
3-10	6-00618	750	0.004	0.003	0.002	0.001	0.017	ND	ND
4-0	6-00616		40.8		6.56		35.4	0.61	ND
4-1	6-00633	1885	11.6	21.9	0.703	1.32	6.52	0.10	ND
4-2	no specimen						5.4		
4-3	no specimen						0.47		
4-4							0.260		
4-5	6-00407	695	0.052	0.036	0.010	0.007	0.165	ND	ND
4-6	6-00412	750	0.014	0.010	0.004	0.004	0.030	ND	ND
4-7	no specimen						0.122		
4-8	6-00445	690	0.073	0.050	0	0	0.170	+	ND
4-9	no specimen						0.012		
4-10	6-00469	760	0.034	0.026	0.003	0.002	0.041	ND	ND
4-11	6-00497	355	0.010	0.009	0.004	0.003	0.062	ND	ND
4-12	no specimen						0.004		

Patient/day	Specimen No.	Total Volume ml	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-Morphine %
5-0	no specimen								
5-1	6-00634	1100	4.15	4.56	.302	.332	4.40	ND	ND
5-2	6-00650	900	2.99	2.69	0	0	2.50	ND	ND
5-3	6-00665	685	.047	.032	.012	.008	0.910	ND	ND
5-4	6-00693	1270	0	0	0	0	0.023	ND	ND
5-5	6-00408	1495	0	0	0	0	0.002	ND	ND
5-6	6-00413	1620	0	0	0	0	0.001	ND	ND
5-7	6-00429	940	0	0	0	0	0.020	ND	ND
5-8	6-00446	935	.008	.008	.001	.001	0.037	ND	ND
5-9	6-00458	1215	.006	.007	.002	.002	0.004	ND	ND
5-10	6-00470	910	.004	.003	.002	.002	0.073	ND	ND
5-11	6-00498	905	.003	.003	.009	.007	0.050	ND	ND
6-0	6-00617	385	.609	.235	.005	.002	0.130	ND	ND
6-1	6-00635	1375	.800	1.100	.035	.048	0.940	ND	ND
6-2	6-00651	1250	.011	.014	.007	.008	0.054	ND	ND

Patient/day	Specimen No.	Total Volume ml	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-Morphine %
6-3	6-00666	1090	.002	.003	.003	.003	0.012	ND	ND
6-4	6-00694	1560	.002	.004	.002	.004	0.010	ND	ND
6-5	6-00409	1950	.002	.004	.003	.005	0.025	ND	ND
11-0	6-00433	870	.006	.006	0	0	0.025	ND	ND
11-1	6-00454	795	.003	.002	0	0	0.035	ND	ND
11-2	no specimen								
11-3	no specimen								
11-4	6-00496	1740	.009	.015	.002	.003		ND	ND
11-5	6-01319	1510	0	0	.003	.005		ND	ND
11-6	6-01339	920	.002	.002	.014	.013		ND	ND
11-7	6-01344	1240	.002	.002	0	0		ND	ND
11-8	6-01367	530	.006	.003	.002	.001		ND	ND
11-17	12-10384	390	0	0	.004	.001		ND	ND
13-0	6-00468		3.47		.447		3.90	2.82	ND

Patient/day	Specimen No.	Total Volume ml	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-Morphine %
13-1	6-00493	675	1.42	.965	.134	.090	2.28	0.91	ND
13-2	6-01320	1440	.114	.164	.005	.007	0.182	ND	ND
13-3	6-01340	510	.028	.014	.004	.002	0.054	ND	ND
13-4	6-01346	860	.011	.009	.003	.003	0.030	ND	ND
13-5	6-01365	1610	.005	.009	.003	.006	0.082	ND	ND
13-6	no specimen								
13-7	6-01388	880	.005	.004	0	0	0.030	ND	ND
13-8	6-01399	675	.014	.010	.003	.002	0.045	ND	ND
13-9	12-10246	1650	.004	.006	0	0	0.008	ND	ND
13-10	12-10262	1080	.006	.007	.001	.001	0.023	ND	ND
13-11	12-10291	750	.004	.003	0	0	0.012	ND	ND
13-12	12-10335	2120	.011	.023	.002	.004	0.062	ND	ND
13-13	12-10371	2390	.001	.003	.001	.002	0.012	ND	ND
13-14	12-10382	1120	.001	.002	.001	.001	0.007	ND	ND
14-0	no specimen						*		
14-1	6-00494	880	11.8	10.4	0.311	0.274	6.53	ND	ND
14-2	no specimen						4.01		

Patient/day	Specimen No.	Total Volume ml	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-Morphine %
14-3	6-01341	510	0.057	0.029	0.005	0.002	0.140	ND	ND
14-4	6-01347	870	0.023	0.020	0.003	0.002	0.082	ND	ND
14-5	6-01366	905	0.018	0.017	0.003	0.003	0.082	ND	ND
14-6	6-01370	470	0.031	0.015	0	0	0.085	ND	ND
14-7	6-01387	470	0.061	0.028	0	0	0.152	ND	ND
14-8	6-01400	495	0.013	0.006	0.004	0.002	0.116	ND	ND
14-9	12-10247	490	0.016	0.008	0.003	0.001	0.082	ND	ND
14-10	12-10261	850	0.005	0.004	0.002	0.002	0.030	ND	ND
14-11	12-10292	630	0.003	0.002	0.001	0.001	*		
14-12	12-10336	455	0.005	0.002	0	0	2.60	ND	ND
14-13	12-10370	685	0.002	0.001	0	0	0.030	ND	ND
14-14	no specimen						0.057		
15-0	no specimen						*		
15-1	6-00495	3258	1.01	3.29	0.061	0.199	6.12	ND	ND
15-2	6-01321	1310	1.03	1.34	0.031	0.041	1.50	ND	ND
15-3	no specimen						*		

Patient/day	Specimen No.	Total Volume ml	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-Morphine %
15-4	6-01345	1130	0.015	0.017	0.001	0.001	0.016	ND	ND
15-5	6-01364	1100	0.003	0.003	0	0	0.016	ND	ND
15-6	6-01369	1000	0.004	0.004	0	0	0.052	ND	ND
15-7	6-01386	640	0.001	0.001	0.002	0.001	0.025	ND	ND
15-8	6-01397	480	0.003	0.002	0.001	0	0.030	ND	ND
15-9	12-10248	430	0.001	0	0.002	0.001	0.054	ND	ND
15-10	12-10259	1240	0	0	0.002	0.003	0.016	ND	ND
15-11	12-10293	890	0	0	0	0	0.062	ND	ND
15-12	12-10334	1270	0	0	0.016	0.020	0.017	ND	ND
15-13	12-10372	1490					*		
15-14	12-10383	1100	0.001	0.002			0.006	ND	ND
19-0	12-10217	580	.002	.001	0	0	.020	ND	ND
19-1	12-10242	1505	.003	.004	.003	.004	.016	ND	ND
19-2	12-10264	1570	.002	.003	.003	.004	0	ND	ND
19-3	12-10295	1395	.003	.004	.002	.002	.009	ND	ND
19-4	12-10339	1500	.001	.001	.001	.002	.001	ND	ND

Patient/day	Specimen No.	Total Volume ml	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-Morphine %
19-5	12-10365	910	.001	.001	.002	.002	.0	ND	ND
22-0	no specimen						18.6		
22-1	12-10546	1014	3.45	3.46	.149	.151	2.9	1	ND
22-4	12-10810	1185	.008	.010	.004	.005	0.035	ND	ND
22-5	12-10829	1530	0	0	.003	.004	0.017	ND	ND
22-6	12-10841	900	.002	.001	0	0	0.054	ND	ND
22-7	12-10857	740	0	0	0	0	0.020	ND	ND
22-8	12-10868	610	0	0	0	0	0.089	ND	ND
22-9	no specimen						0.042	ND	ND
22-10	12-10892	1120	0	0	0	0	0.035	ND	ND
22-11	12-10910	1110	0	0	0	0	0.035	ND	ND
22-12	12-10925	605	.044	.027	0	0	0.057	ND	ND
22-13	12-10947	1000	.001	.001	0	0	0.045	ND	ND
22-14	12-10966	630	0	0	0	0	0.013	ND	ND
23-0	12-10524	850	37.7	31.5	3.17	2.70	13.6	.05	ND

Patient/day	Specimen No.	Total Volume ml	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-Morphine %
23-1	12-10543	1875	7.60	1.42	.671	1.25	3.90	.0045	ND
23-2	12-10571	590	3.87	2.28	.333	.197	4.52	ND	ND
23-3	12-10588	650	.752	.489	.080	.052	1.20	ND	ND
23-4	12-10807	1400	.020	.027	.001	.002	.041	ND	ND
23-5	12-10829	1800	.006	.010	.004	.007	.006	ND	ND
23-6	12-10843	760	.063	.048	.003	.002	.035	ND	ND
23-7	12-10859	2030	.002	.016	.005	.010	.015	ND	ND
23-8	12-10870	1100	.006	.007	.004	.004	.037	ND	ND
23-9	12-10884	1270	.010	.012	.009	.011	.017	+	ND
23-10	12-10893	1600	.005	.008	.011	.017	.010	ND	ND
23-11	12-10911	1845	.668	1.23	.008	.015	.062	ND	ND
23-12	no specimen						.094		ND
23-13	12-10948	1185	.005	.006	.005	.006	.020	ND	ND
23-14	12-10967	1470	.007	.010	.002	.003	.004	ND	ND
24-0	12-10525	440	11.1	4.91	.169	.075	7.47	ND	ND
24-1	12-10544	3880	3.35	13.0	.141	.547	2.26	ND	ND

Patient/day	Specimen No.	Total Volume ml	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-Morphine %
24-2	12-10572	4240	.342	1.44	.046	.197	.840	ND	ND
24-3	12-10590	1930	.009	.017	0	0	.023	ND	ND
24-4	12-10808	1580	.006	.010	.005	.008	.022	ND	ND
24-5	12-10827	2510	.001	.004	.005	.013	.028	ND	ND
24-6	12-10842	1915	0	0	0	0	.017	ND	ND
24-7	12-10858	1690	.004	.007	0	0	.049	ND	ND
24-8	12-10869	1000	.006	.006	.010	.010	.030	ND	ND
24-9	12-10882	2385	0	0	.002	.006	.004	ND	ND
24-10	12-10894	1640	.005	.008	.008	.014	xxx	ND	ND
24-11	12-10912	2025	.014	.027	0	0	.028	ND	ND
24-12	12-10927	1420	.031	.043	.018	.026	.072	ND	ND
24-13	12-10949	1580	.007	.011	0	0	.006	ND	ND
24-14	12-10968	1350	.009	.013	.008	.011	.028	ND	ND
25-0	12-10526	300	.006	.002	.013	.002	.016	ND	ND
25-1	12-10545	1310	.002	0	.022	.028	.016	ND	ND
25-2	12-10573	1245	.018	.023	.202	.251	.016	ND	ND

Patient/day	Specimen No.	Total Volume ml	GC-MS Total Morphine µg/ml	GC-MS Total Morphine mg/24 hr	GC-MS Free Morphine µg/ml	GC-MS Free Morphine mg/24 hr	Frat Value µg/ml	GC-MS Metabolites Codeine %	GC-MS Metabolites Nor-Morphine %
25-3	12-10587	860	.006	.005	.010	.008	.006	ND	ND
25-4	12-10809	1185	.003	.003	.023	.027	.011	ND	ND
25-5	12-10828	1045	.006	.006	0	0	.000	ND	ND
25-6	12-10844	220	.001	0	0	0	.002	ND	ND

Baboon serum samples (all have the prefix FJSBP520)

Sample	Free Morphine ug/ml	Sample	Free Morphine ug/ml	Sample	Free Morphine ug/ml
1.	0.06	31.	0.00	61.	0.00
2.	0.07	32.	0.00	62.	0.01
3.	0.05	33.	0.09	63.	0.13
4.	0.03	34.	0.00	64.	0.02
5.	0.49	35.	0.00	65.	0.00
6.	0.03	36.	0.00	66.	0.00
7.	0.12	37.	0.00	67.	QNS
8.	0.10	38.	0.00	68.	QNS
9.	0.02	39.	0.00	69.	0.00
10.	0.02	40.	0.02	70.	0.00
11.	0.00	41.	0.00	71.	0.00
12.	0.09	42.	0.00	72.	0.00
13.	0.00	43.	0.00	73.	0.00
14.	0.00	44.	0.00	74.	0.00
15.	0.00	45.	0.00	75.	0.00
16.	0.00	46.	0.00	76.	0.00

Sample	Free Morphine µg/ml	Sample	Free Morphine µg/ml	Sample	Free Morphine µg/ml
17.	0.00	47.	0.00	77.	0.00
18.	0.00	48.	0.00	78.	0.00
19.	0.00	49.	0.00	79.	QNS
20.	0.00	50.	QNS	80.	0.00
21.	0.09	51.	QNS	81.	0.00
22.	QNS	52.	0.17	82.	0.00
23.	0.01	53.	0.00	83.	0.00
24.	0.16	54.	QNS	84.	0.00
25.	0.05	55.	0.00	85.	0.02
26.	0.00	56.	0.00	86.	0.01
27.	0.00	57.	0.00		
28.	0.16	58.	0.00		
29.	0.00	59.	0.00		
30.	0.12	60.	0.00		

QNS means quantity not sufficient to do the analysis.

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