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Methods for Total Drug and Related Substances in Pindolol

by

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ABSTRACT

A liquid chromatographic method for the assay of pindolol and related compounds in the bulk drug was developed. The method resolves six known and several unknown impurities from the drug and each other using a nitrile column, a mobile phase composed of acetonitrile-sodium acetate buffer (35:65), and a UV detector set at 219 nm. Minimum quantifiable amounts of impurities are 0.02% or less relative to the drug. Ten lots of pindolol raw material were evaluated for purity and drug content. Total levels of impurities in these samples, quantitated against pindolol, ranged from about 0.03% to 0.24%. Assay results were within the range of 98.5% to 101.5%.

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INTRODUCTION

Pharmaceutical Analysis

Factors to be considered in the choice of analytical technique for a pharmaceutical product include the chemical and physical nature of the drug, the matrix in which it exists, the purpose of the analysis and the analytical performance desired.

Most analytical techniques take advantage of a particular chemical or physical property of the drug. For example, it may contain a UV chromophore or a functional group with a characteristic infrared absorption which makes it amenable to spectroscopic techniques or it may be possible to devise a titrimetric method based on its acid-base properties. The solubility and stability of the drug in various media and under various conditions may also play a role in the choice of technique.

The sample presented to the analyst may be the bulk drug, a formulation or a biological sample. Each of these will have its own set of challenges. It may be crucial to be able to detect a potential toxic impurity at the parts per million level in the bulk drug or to be able to determine drug

content in several formulations such as coated or uncoated tablets, capsules, syrups, injectables, aerosols, oral solution, suppositories, creams or ointments, containing a wide variety of excipients which may cause interferences in the analysis. Biological samples present special problems which are not discussed in this thesis.

The method of analysis will be dictated to some extent by the performance criteria required. Performance is expressed by such analytical parameters as sensitivity, selectivity, accuracy, precision, limits of detection and quantitation, linearity, range and ruggedness.

The purpose of this work was to develop new methods for the determination of total drug and related compounds in pindolol raw materials. These methods would be used for regulatory purposes to analyze samples of pindolol prior to approval for sale in Canada. The methods would also be made available to the United States and British Pharmacopoeias.

Basis and Characteristics of Pharmaceutical Standards

1. Drug Regulations in Canada

To ensure the quality, safety and efficacy of pharmaceutical products, most nations have created laws and regulations governing the production, manufacturing, import and sale of drugs. In Canada, two acts form the basis of the drug laws: the Food and Drug Act and the Narcotic Control Act(1). The Health Protection Branch of the Department of National Health and Welfare is responsible for administering these Acts and the Regulations derived from them.

In this country, most single-ingredient drugs are manufactured to standards contained in the publications listed in Schedule B to the Food and Drug Act. These include the United States Pharmacopeia (USP)(2), the British Pharmacopoeia (BP)(3), and the International Pharmacopoeia (IP)(4) among others. In addition, certain drugs may have special requirements set out in the Regulations. Drugs listed in Schedule B publications or in the Regulations are known as official drugs. A manufacturer may use a "house" standard for a drug which is listed in a Schedule B publication so long as the most stringent criteria for purity and potency set out in these publications are met. For many new drugs, there are no

standards in the Schedule B publications or in the Regulations; in such a case the manufacturer must provide a standard, the professed standard, which must be acceptable to the Health Protection Branch.

2. Pharmacopeias

Many countries have established pharmacopeias which are volumes containing legal specifications for drugs and the methods of analysis which must be used to verify that these specifications are met.

The IP is somewhat of an exception, in that it does not have a legal status in any country, unless expressly introduced for that purpose by appropriate legislation. It is published by the World Health Organization (WHO) and is aimed primarily at developing countries. It contains simple methods which do not require expensive equipment or highly specialized personnel.

The European Pharmacopeia (EP)(5) is published under the direction of the Council of Europe and contains monographs for drug raw materials only. As of November 1988(6), eighteen countries were members of the Convention on the Elaboration of a European Pharmacopoeia: Austria, Belgium, Cyprus, Denmark, Finland, France, the Federal Republic of

Germany, Greece, Iceland, Ireland, Italy, Luxembourg, the Netherlands, Norway, Spain, Sweden, Switzerland, and the United Kingdom. Part I of the EP contains general texts such as a list of the members of the Convention, General Notices, methods of analysis, reagents, etc. Part II contains the monographs which are published in series twice yearly.

The BP has been the legal standard in the United Kingdom since 1864. The latest edition was published in 1988 and contains 2100 monographs in two volumes, I for drug raw materials and II for formulations, blood products, immunological products, radiopharmaceutical preparations and surgical materials.

The USP is recognized as the Official Compendia of the United States and is used by 40 other countries as a source of drug standards. It contains monographs on drug raw materials and dosage forms, medical devices and diagnostic tests. It is published by the United States Pharmacopeial Convention (USPC) which was established in 1820 and is composed of members from educational institutions, professional and scientific organizations and the federal government. The members of the USPC elect the General Committee of Revision which is responsible for reviewing additions and modifications to the USP. Several Canadians

are members of the Committee of Revision or carry out other USP functions.

Proposed revisions to the USP are subject to public scrutiny before they become official. Proposals for addition of a new monograph or modification of an existing one are reviewed by the appropriate committee or sub-committee which decides whether or not the proposed revision merits further consideration. If so, it is published in Pharmacopeial Forum (PF) a bimonthly USP publication of monograph revisions, new monographs and other compendial matters. The purpose of PF is to make proposed changes available for public comment before they become official. If there are no significant adverse comments, the method will become official in the USP. If the revision does elicit significant objections or suggestions for amendments, it will be revised and published once again in PF along with a summary of the comments received and perhaps a statement from the sub-committee regarding adoption into the USP.

The main volume of the USP is published every five years. Supplements are issued serially, as necessary. The main volume is composed of the following sections: People, Preamble, Admissions, General Notices, Monographs, General Chapters, Reagents, Tables and Index. The Preamble contains the articles of incorporation, constitution and bylaws,

rules and procedures, proceedings, and history of the USPC. The General Notices contain definitions of terms used in the USP and an explanation of the requirements stated in the monographs. The monographs are arranged in alphabetical order by drug name. Monographs for formulations immediately follow the monograph for the drug raw material. The General Chapters describe apparatus, chemical and physical tests, and general information such as US drug regulations and good manufacturing practice for finished pharmaceuticals.

3. Drug Monographs

Pharmacopeias are divided into monographs for individual drug articles. These contain specifications and methods of analysis. A monograph for a drug raw material would typically contain the common and chemical name of the drug, its chemical formula, structure, molecular weight, Chemical Abstracts Service (CAS) registry number and assay tolerance, one or more identity tests, an assay test, one or more purity tests, tests for heavy metals and loss on drying. The following tests might also be included: a test for optical purity, if the drug is a single optical isomer; a test for racemate composition, if it is a mixture of diastereomers; tests for specific ions, depending on the nature of the drug e.g. chloride; etc.

Monographs on drug raw materials usually contain a purity test. This may be a chromatographic purity test coupled with a nonspecific assay, a specific test and limit for a known impurity or a chromatographic purity test that also serves as the assay.

The USP defines five types of impurities: foreign substances, toxic impurities, concomitant components, signal impurities and ordinary impurities.

Foreign substances are adulterants or contaminants which do not result from the synthesis or manufacturing process. Depending on the nature and concentration of the foreign substance, it may or may not be detected using the pharmacopeial method. A drug found to contain a foreign substance would not meet monograph standards.

Toxic impurities are those which have a significant undesirable biological activity even at low concentrations and require identification and quantitation by specific tests. They may arise from the synthesis, preparation or degradation of the drug.

Concomitant components are not regarded as impurities but merely as a normal component of the drug. Examples include the inactive isomer of a drug which is administered as a

racemic mixture but for which only one optical isomer has biological activity.

Signal impurities are compounds which provide key information on the synthesis or degradation of the drug and require identification and quantitation by specific tests.

Ordinary impurities are considered innocuous in that they have no significant undesirable biological activity at or below the specified limits. These impurities arise from the synthesis, formulation or degradation of the drug.

In most cases, quantitation of impurities is done by comparison to an external standard, often the drug, rather than by comparison to individual impurity standards. The USP views 2.0% as the general limit for total ordinary impurities unless there is documentation to support a higher or lower level. Concomitant components, toxic or signal impurities are not included in the estimate of ordinary impurities and separate limits are set for these types of impurities, as necessary.

In addition to the types of impurities defined above, the terms process contaminants and related substances are also commonly used when referring to impurities. A related substance is a compound which is chemically related to the

drug and which arises from the manufacturing process or which forms upon storage. It may be a starting material, synthetic intermediate or degradation product. A process contaminant, according to the USP, is an identified or unidentified impurity which may be introduced during manufacturing or handling, but excluding related substances and water. Examples of process contaminants include inorganics (heavy metals, and ions such as chloride, sulfate), reagents, and solvents.

4. Characteristics of Purity and Assay Methods

Assay methods are used to determine drug content and purity methods are used for the determination of related substances. Purity and assay methods designed for regulatory purposes must be suitable for pharmaceuticals produced by several manufacturers, possibly using different routes of synthesis.

Pharmacopeial methods reflect the state of knowledge on a particular drug at the time of publication. As more knowledge is gained, the need to revise existing methods or develop new ones may arise. For example, the existence of new impurities formed as a result of a different route of synthesis may come to light or, alternatively, better analytical techniques may be developed.

Submissions to a pharmacopeia should contain a rationale identifying the need for the new or revised method, a clear and complete description of the method and sufficient documentation to show that the method has been properly validated. Validation is the process by which a method is tested and shown to be effective for its intended purpose. The type of method will determine which analytical parameters are to be evaluated and how these can be measured. Papers by Inman et al.(7), Debesis et al.(8) and the USP(9) provide excellent examples of guidelines for the validation of pharmaceutical methods. Parameters normally evaluated for related substances or assay methods are: selectivity, precision, accuracy, limit of detection, limit of quantitation, sensitivity, linearity and ruggedness.

Selectivity or specificity is the ability of the method to measure the analyte accurately in the presence of other components. For an HPLC assay method, it is usually necessary to show that the drug produces a suitable response, free from interferences from the sample matrix and that none of the impurities co-elute with the drug. For related substances methods, the individual impurities must all produce a response within a reasonable time frame and be resolved from the drug and the solvent front. For

impurities that are not known to be toxic, it is preferable, though not absolutely necessary for all impurities to be resolved from each other. In order to be able to demonstrate the selectivity of a method, the analyst will have to review various routes of synthesis, postulate which impurities may arise, and obtain standards of each of the possible related substances. Should a new impurity become available after a method is developed, it would be necessary to revalidate the method by showing that this impurity is resolved from the drug and can be suitably quantitated.

Precision is a measure of the reproducibility of the method, that is the degree of agreement between individual test results. To determine the precision of a method, a number of weighings of a particular sample are analyzed and the mean and relative standard deviation (RSD) of the assay results are determined. For chromatographic methods, it is also useful to determine the precision of the system. This is done by determining the mean response and RSD of replicate injections of a standard solution and gives an estimate of the error that is attributable to the operating system only and not to sample preparation. Debesis et al.(8) have suggested maximum allowable system and method RSD ranges for various assay acceptance ranges. For example, If the assay acceptance range is to be 98.5-101.5% and the method calls for duplicate determinations, then the

maximum method RSD for six replicates is 0.82% and the maximum allowable system RSD on six replicates is 0.58%. Generally, lower precision is required for related substances methods than for assay methods.

Accuracy is the closeness of a measured value to its true value. It is not possible to determine the accuracy of assay methods for raw materials. At best, results from different types of assay methods can be compared. However, when making these comparisons, it is important to keep in mind the limitations of the methods; for example, some assay methods are less specific than others and others involve the use of standards which are assumed to assay at 100.0%. Accuracy can be estimated to some extent in the case of assay methods for formulations: known amounts of the drug can be added to the formulation and the percentage of the drug recovered in the assay determined.

The limit of detection is the lowest amount of analyte that can be detected in a sample. For instrumental methods, different techniques can be used to determine the limit of detection. The investigator may compare test results from blanks and samples of increasingly low concentrations and establish at which concentration the analyte signal is distinguishable above the noise. Usually a signal to noise ratio of 2:1 or 3:1 is acceptable. Other investigators may

measure the background noise of several blank samples, determining the RSD of this response and multiply it by a factor, usually 2 or 3, to determine the limit of detection.

The limit of quantitation is the lowest amount of analyte that can be measured with a certain degree of precision or accuracy. For related substances methods, a high degree of precision is not normally required. With most HPLC integrators, it is not uncommon to observe RSD's of more than 50% at impurity levels approaching the limit of detection, then, as the concentration of the impurity increases, the RSD decreases. Many investigators define the limit of quantitation as 2X the limit of detection or a signal to noise ratio of 4:1. Others define the limit of quantitation as the concentration at which the RSD on a specified number of replicates is of a certain magnitude, for example 10% for five replicates. There should be, when possible, a factor of 5 to 10 between the limit of quantitation and the limit set in the specifications for a particular compound.

Linearity is the ability of the method to provide a measurable response to the analyte that is proportional to its concentration, over a given range. It is usually determined from regression analysis of a plot of response vs concentration. The variance of the slope of the regression

line calculated by the method of least squares provides a measure of linearity. Linearity is usually defined over a given range, e.g. 50-150% of the normal assay concentration in the case of an assay method. For a related substances method, it is usually necessary to demonstrate linearity from the minimum quantifiable amount to an amount exceeding the proposed limit on individual impurities.

Sensitivity is defined as the slope of the analytical response curve. It is the difference in response for a given difference in concentration. When several compounds are quantitated against a single standard, it is usually necessary to show whether the system is more or less sensitive to these compounds, relative to the standard.

Ruggedness or robustness is the degree of reproducibility of the method under a variety of normal test conditions, such as different equipment, different analysts or different days. Ruggedness can be demonstrated by showing that operational or environmental influences have no significant effect on test results.

Many factors can contribute to non-reproducibility of a method(10) and the analyst must try to build safeguards into the method to reduce their effect by such means as using well defined columns and designing a proper system

suitability test. System suitability tests are usually included in all HPLC methods submitted to pharmacopeias. They are a measure of the suitability of the entire system, hardware, electronics and solvents, to carry out the analysis. An analysis may be carried out only when the system suitability requirements can be met. It provides a means of ensuring good results without having to validate the method again.

Having the method evaluated by another analyst, preferably from another laboratory, provides a means to evaluate the ruggedness of the method. Such an external evaluation may reveal that the instructions are ambiguous or incomplete, or that differences in results can arise due to the use of different brands of equipment or chemicals.

Collaborative studies, such as those organized by the Association of Official Analytical Chemists (AOAC), in which a given set of samples are analyzed by a number of laboratories are probably the best way of demonstrating the ruggedness and precision of a method.

In addition to the method parameters listed above, other factors are simplicity and cost. The latter may be of less importance for methods used at the product development stage or for regulatory analysis but may be of considerable

importance for routine quality control methods(11).

High Pressure Liquid Chromatography (HPLC) in Pharmaceutical Analysis

1. General Principles of Chromatography

Chromatography is the process by which the separation of substances is achieved as the result of their differential distribution between two phases, a mobile phase and a stationary phase(12),(13). The mobile phase may be a gas or liquid and the stationary phase may be a supported liquid or solid.

The main types of chromatography used in pharmaceutical analysis are thin layer chromatography (TLC), gas chromatography (GC) and high pressure liquid chromatography (HPLC).

Below are some of the basic definitions and equations used in chromatography.

k: capacity factor is the ratio of the number of molecules in the stationary phase (n_S) to that in the mobile phase (n_M).

$$k = n_S/n_M$$

k depends upon the nature of the analyte, the stationary and mobile phases, and upon the temperature.

α: separation factor between two components, 1 and 2. α is the ratio of their capacity factors.

$$\alpha = k_2/k_1$$

N: the column efficiency or number of theoretical plates, by analogy to distillation. N is related to the height equivalent to a theoretical plate (h or HETP) and to the length of the column (L):

$$N = L/h$$

If the chromatographic peak is assumed to have a Gaussian shape, the number of theoretical plates, N, can be calculated from the retention time (t_R), and w_b, the width at the base or w_h, the width at half-height.

$$N = 16\ (t_R/w_b)^2$$

$$N = 5.54\ (t_R/w_h)^2$$

R_S: the resolution between two peaks, 1 and 2, is defined as the difference in retention times of the two peaks divided by the average peak width at the base:

$$R_S = (t_{R2} - t_{R1})\ /\ \tfrac{1}{2}\ (w_{b1} + w_{b2})$$

or, if widths at half height are used:

$$R_S = 1.18\ (t_{R2} - t_{R1})/(w_{h1} + w_{h2})$$

Resolution can also be expressed in terms of efficiency (N), separation factor (α) and the capacity factor (k):

$$R_S = 1/4\ N^{1/2}\ [(\alpha-1)/\alpha]\ [k/(1+k)]$$

This equation shows how changes in N, α or k will affect the resolution. Because resolution is proportional to the square root of efficiency, which in turn depends on the length of the column, to obtain a doubling in resolution between two components, it is necessary to increase the length of the column fourfold, which may not be possible in HPLC because of excessive back pressure. The efficiency of the column can also be increased by using a packing material of smaller diameter but here again, higher pressures may be

a limiting factor. An increase in k from 0 to 1 would increase the resolution from 0 to 0.5 and a further increase of k to 10 would increase the resolution to 0.91. Changes in k can be brought about by altering the mobile phase, the stationary phase or the temperature. Changes in α are more difficult to predict because the separation factor depends on the relative interactions of the components with the two phases.

Another way of improving the resolution of two components, in this case without changing the separation, would be to reduce band spreading. The van Deemter equation is a model used to explain the causes of band spreading.

$$h = A + B/u + Cu$$

The A term represents eddy diffusion. It arises because the analyte molecules do not all follow the same path around particles in the column; some will take longer paths, others shorter ones.

$$A = 2 \lambda d_p$$

where 2λ is a geometrical packing factor and d_p is the particle diameter.

The B term represents molecular diffusion. This is the normal diffusion in all directions of an analyte in a fluid.

$$B = 2 \gamma D_M$$

where γ is the labyrinth or tortuosity factor which depends on the nature of the packing, and D_M is the diffusivity of the analyte in the mobile phase.

C represents resistance to mass transfer. It is often divided into two components, C_s and C_M, the resistance to mass transfer in the stationary and mobile phases, respectively.

C_S is a function f_s of the capacity factor k.

$$C_S = f_s(k) \; d_f^2 \; / \; D_S$$

Thus C_S depends on the film thickness of the stationary phase (d_f^2), and the diffusion coefficient of the analyte in the stationary phase (D_S).

Similarly, C_M depends on the diffusion coefficient of the analyte in the mobile phase (D_M), the mean path length between particles in the column (d_p), and the affinity of

the analyte for the mobile phase.

$$C_M = f_m(k)\ d_p^2\ /\ D_M$$

The van Deemter equation shows the relationship between the plate height (h), particle size (d_p), linear velocity (u), solute diffusivity (D_M) and the degree of retention in a column. From this equation it is evident that h goes through a minimum as u is varied. Thus there is a single optimum u that gives minimum plate height and thus optimum efficiency.

The van Deemter equation can be derivatized to obtain the following expression of the minimum plate height

$$h_{min} = A + 2(BC)^{1/2}$$

h_{min} is directly proportional to particle size. Thus columns containing packing materials of a smaller particle size are more efficient. Unfortunately the increase in efficiency will be offset by an increase in operating pressure because the pressure is inversely proportional to the square of the particle size.

In reality, there are only a limited number of commercially available column lengths, diameters, packing types and

sizes. Commercial columns from manufacturers with strict quality assurance criteria are most suitable for regulatory methods because of the stringent reproducibility requirements.

2. High Performance Liquid Chromatography (HPLC)

According to Dennis(14), HPLC is the most widely used analytical procedure in pharmaceutical analysis. This technique provides good reproducibility and high quality results; the cost is reasonable for routine applications and it is fairly easy to operate the equipment. It can be used to determine the main product or impurities. It meets many of the analytical performance requirements that are normally required of pharmaceutical methods. It has several advantages over gas chromatography: separations are usually carried out at ambient temperature and therefore thermal degradation is less likely to occur; derivatization is not normally required and sample preparation is usually simpler, even for formulations. There are however some disadvantages when compared to GC: the resolving power of HPLC is generally lower, particularly when compared to capillary GC; and there is the problem of solvent waste disposal. The resolving power and sensitivity of HPLC are often greater

than TLC. However, quantitation is not possible in HPLC unless the compounds are eluted from the column whereas in TLC, all the components are on the plate and can be quantitated, if they are resolved, even if they do not move from the origin.

HPLC packing materials generally fall into one of four categories: normal phase, reverse phase, ion-exchange and size exclusion.

Columns packed with polar materials such as silica, alumina or polar bonded-phase silicas such as those containing diol or nitrile functionalities are used for normal phase chromatography. The packing is very polar and retains polar molecules. The polarity of the mobile phase determines the retention time of the compounds to be separated; the more polar the mobile phase, the faster polar compounds elute. Solvents such as hexane, methylene chloride and chloroform are most commonly used with normal phase columns.

There are two main theories to explain retention in normal phase systems. According to the Snyder-Soczewinski(15) theory, the analyte interacts strongly and specifically with sites on the stationary phase. Interaction with the mobile phase is not taken into account. The Scott-Kucera(16),(17) theory assumes that the strong

organic component in the mobile phase is attracted to the surface of the adsorbent in a Langmuir adsorption isotherm process and that the analyte molecules then interact with this adsorbed organic layer. Neither of these theories is considered to be universally applicable.

Reverse phase columns are those in which a functionality such as an alkyl of phenyl group has been bonded to the silica. This alters the surface of the silica particles and makes it non-polar and thus able to retain non-polar molecules. The rate of elution of non-polar molecules increases as the polarity of the mobile phase decreases. Typical mobile phases for reverse phase chromatography are mixtures of water or buffers and methanol, acetonitrile and tetrahydrofuran.

There is not yet a theory that accurately predicts retention behaviour in reverse-phase chromatography. Horvath et al.(18) have proposed the solvophobic theory which predicts that retention of a solute depends on the area of contact between the solute and an alkyl chain of the stationary phase, and the energy required to remove the mobile phase from this area of the alkyl chain.

Most chromatographic applications involve normal or reverse phase chromatography. Ionic solutes can be separated by ion

chromatography. Usually an aqueous mobile phase containing electrolytes and a stationary phase with weak ionic sites is used. The ionic solute displaces an eluent ion from the stationary phase then is subsequently displaced by another eluent ion; this process is repeated as the solute travels down the column.

In size exclusion chromatography a stationary phase consisting of particles of a suitable pore size is chosen so that components that are smaller than the pores are completely retained and those much larger than the pores are quickly eluted, while those of approximately the same size will be somewhat retained and separated. The retention times of the eluting compounds are compared to standards of known size and molecular weight thus providing an estimate of the molecular weight. Compounds with molecular weights greater than 2000 can be analyzed by size exclusion chromatography.

Samples are introduced into the liquid chromatograph manually by means of an injector or automatically using an autosampler and automatic injector. Ideally, samples are delivered as well-defined plugs of minimal thickness. The most commonly used type of injector is the valve injector. Among these, there are variable volume injectors whereby any volume within a certain range, for example 1-100 μL, may be

selected, and fixed loop injectors for which the volume injected depends on the size of the sample loop chosen. Autosamplers and autoinjectors increase lab productivity considerably because they can be set up to run outside of normal working hours and also because they allow the analyst to do other tasks such as analyzing data or writing reports while samples are being run. Typical autosamplers hold 21 to 121 vials. They may have such desirable features as cooling for biological samples or thermostating to regulate the temperature of samples requiring derivatization. Modern autosamplers can be programmed to add internal standard or derivatization solutions to vials, perform dilutions and extractions, mix the reagents within a vial, and do multiple injections per vial.

Pumping systems must be capable of developing pressures as high as several thousand psi and provide a steady, precise and accurate flow of mobile phase. There are two types of pumps; reciprocating pumps which are the most common, and syringe pumps which are use mainly for microbore systems. Solvent delivery may be accomplished using isocratic or gradient pumps. Many isocratic systems have time programmable flow rates. Ternary and quaternary solvent delivery systems allow eluants from up to three or four reservoirs respectively to be mixed in variable proportions isocratically or in a gradient. The eluants in these

reservoirs may be sparged with a suitable inert gas such as helium if the presence of oxygen causes interference with detection. Typical flow rates for analytical pumps, which include those used for microbore HPLC, are 0.001 to 10 mL/min. Semi-preparative and preparative HPLC pumps have flow rates of 0.1 to 150 mL/min.

The most common detectors for HPLC are: UV-visible, refractive index, fluorescence and electrochemical. Examples of more specialized detectors are polarimeters(19), Fourier Transform infrared spectrometers (FTIR)(20), mass spectrometers(21) and nuclear magnetic resonance spectrometers (NMR)(22). For certain applications, it may be useful to use two or more detectors in series.

UV-visible detectors are widely used for pharmaceutical methods. These detectors are based on the absorption of solutes in the ultraviolet (190-370 nm) or visible (370-555 nm) regions of the spectrum. Fixed wavelength detectors include single and dual wavelength detectors. These detectors contain a source which emits only certain discrete wavelengths, such as a low pressure mercury arc lamp. Variable wavelength detectors have a continuous source such as a high pressure xenon lamp and a monochromator or interference filter which allows selection of a specific

wavelength. An added advantage is the ability to program changes in wavelength at various times during an analysis. Diode array detectors permit on the fly scanning of eluting peaks over the UV-visible range, and possible identification of compounds with the aid of spectral libraries.

Refractive index detectors are used extensively for the analysis of carbohydrates and non-UV absorbing analytes. These detectors respond to the change in refractive index of the mobile phase as it passes through the detector cell. They are considered universal detectors because the presence of almost any solute in the mobile phase will cause a change in refractive index. However, they are not widely used for trace analysis because of their poor sensitivity. The sensitivity of good commercial refractive index detectors is of the order of 10^{-7} refractive index units which corresponds to analyte levels in the microgram range. The temperature of these detectors must be strictly controlled because refractive index varies with temperature.

Fluorescence detectors have many applications including amino acid and vitamin analysis. These detectors are equipped with filters to set the excitation and emission detection wavelengths. The limits of detection for fluorescence, typically parts per trillion (ppt), are generally much lower than for absorption, making this type

of detector ideally suited for trace-level analysis. Unfortunately not all compounds fluoresce.

The most widely used electrochemical detectors are those designed for amperometric measurements at constant potential. These detectors only sense compounds that are oxidized or reduced at the working electrode. This selectivity and the low limits of detection (ppt) are the main advantages of electrochemical detectors.

Column ovens and robots for sample preparation may also be integrated into HPLC systems.

Beta-blockers

1. General Characteristics of Beta-blockers

Beta-blockers are a class of drugs which reduce the effect of the sympathetic hormones noradrenaline and adrenaline on the heart by blocking the Beta receptors. There are two types(23): those that block β_1-receptors in the heart muscle, thus slowing the heart rate and reducing contractile force and those that block β_2-receptors in arteriolar smooth muscle, causing vasoconstriction in the extremities or bronchoconstriction.

The first commercially available beta-blocker was propranolol hydrochloride, discovered by Black et al.(24) in 1962. Black was awarded the Nobel Prize for Physiology and Medicine in 1988 for his work on propranolol and cimetidine, a histamine H_2-receptor antagonist(25). Since the early 1960's, many more beta-blockers have been developed and sales of these drugs in the United States are expected to surpass 1.2 billion dollars in 1992(26). They are used in the treatment of hypertension, cardiac arrhythmias, anxiety, migraine and glaucoma(27). Propranolol and pindolol have both β_1 and β_2 properties, but pindolol has a greater intrinsic sympathomimetic activity and less membrane stabilization (local anaesthetic) activity than propranolol.

Most beta-blockers have the following structural elements: an aromatic ring and a 2-hydroxy-3-(isopropylamino)-propoxy side chain. For some, the isopropyl group is replaced by a t-butyl group.

2. Pindolol

Sandoz was granted Swiss patents 469,002 and 472,404 for pindolol in 1969(28),(29).

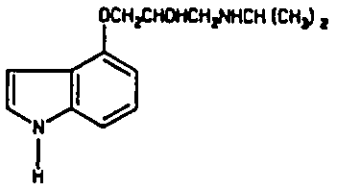
Pindolol is available in Canada from Sandoz Pharma as Visken tablets containing 5, 10 or 15 mg pindolol and as an injectable containing 0.2 mg/mL pindolol. Sandoz also markets pindolol/hydrochlorothiazide tablets(30). Apotex sells 5, 10 and 15 mg pindolol tablets under the trade name Apo-pindolol. Compulsory licences have been granted to Genpharm and Novopharm.

Chemical names and structures for pindolol related substances are given in Table 1 and Figure 1. The drug can be synthesized by reaction of II with epichlorohydrin and sodium hydroxide to produce III or VIII, which on further treatment with isopropylamine yields pindolol(31). II and VII are possible starting materials, III and VIII are intermediates, and IV, V and VI are potential side products.

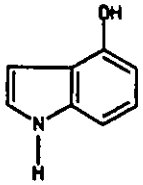
Table 1. Chemical Names of Pindolol Related Compounds

I	Pindolol: 4-[2-hydroxy-3-(isopropylamino)-propoxy]indole
II	4-hydroxy-indole
III	4-[2,3-(epoxy)-propoxy]indole
IV	1-[4-(2-hydroxy-3-isopropylamino-propyl)-indolyloxy]-3-isopropylamino-2-propanol
V	1-[7-(2-hydroxy-3-isopropylamino-propyl)-indolyloxy]-3-isopropylamino-2-propanol
VI	isopropylamino-N,N-bis-[3-(4-indolyloxy)-2-propanol] hemihydrate
VII	indole
VIII	4-(2-hydroxy-3-chloropropoxy)indole

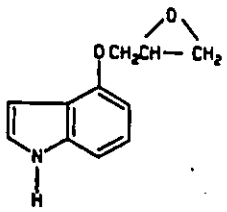
Figure 1. Structures of Pindolol Related Compounds



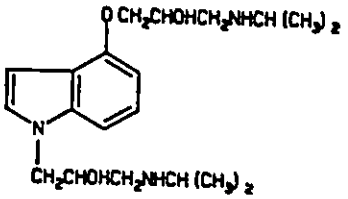
I



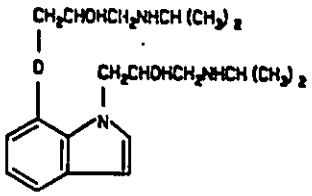
II



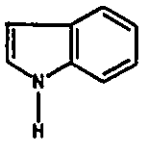
III



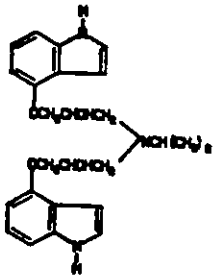
IV



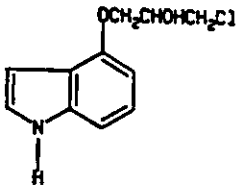
V



VII



VI



VIII

At the time work began to develop methods for pindolol, there was no official monograph for pindolol in the USP. Pindolol became official in the USP on May 15, 1987(32). The BP monograph for pindolol was added to the BP 1980 in the 1982 Addendum(33). The monograph for pindolol, including a chromatographic purity method by TLC and an assay by titration first appeared in Pharmacopeial Forum in the July-August issue of 1984(34).

The USP(2) monograph for pindolol contains a TLC method for chromatographic purity and assay by potentiometric titration with hydrochloric acid. The USP limits for impurities in raw materials are 0.5% for individual impurities and 2.0% total. The BP(3) test for related substances is similar to the USP method. The BP limits are 0.7% for compounds with an Rf of 0.1 and 0.3% for any other compounds. There is no limit on total impurities. The BP assay method is identical to the USP method; the limits for both are 98.5% to 101.0%.

There have been many papers describing the determination of pindolol in biological fluids by HPLC with ultraviolet(35),(36), fluorescence(37),(38) or amperometric detection(39), TLC(40), or GC with electron-capture detection(41),(42).

For many years, the Analytical Chemistry Section of the Bureau of Drug Research has been undertaking projects to evaluate pharmacopeial methods, and, if they are found not to meet requirements for sensitivity and selectivity, to develop new ones. The growing activity of generic companies which may produce a drug by processes completely different from that of the innovator and enhanced requirements of government regulations are the main reasons for these projects. This work was undertaken as part of a project to evaluate and develop methods for beta-blockers.

The initial step in the evaluation of the pindolol pharmacopeial methods was a review of the routes of synthesis used by various manufacturers to determine potential related compounds. After this review, the compounds were requested from the manufacturers.

The TLC method for chromatographic purity contained in the USP monograph for pindolol had been evaluated by F. Matsui of the Bureau of Drug Research in 1986(43). At that time it was shown to resolve five related compounds from the drug, although two (IV and V) were unresolved from each other. In October 1988, compound VI became available. This impurity was found to be well resolved from the drug and other related compounds. The limit of detection was about 0.05% for pindolol and each of the six related compounds.

However, the method was only semi-quantitative and lacked precision. The USP TLC method is a limit test. 5μL of two standard solutions corresponding to 0.05 and 0.025 mg/mL pindolol (0.5 and 0.25% level respectively) are spotted and used for comparison to impurity spots. It is generally accepted that such tests are not very precise, particularly when the estimation of impurity levels is done by visual inspection, as is the case in our laboratory. In addition, the color of the spot for 4-hydroxyindole (II) is different than for the other impurities and the drug. It was felt that an HPLC method might provide improvements in sensitivity and precision.

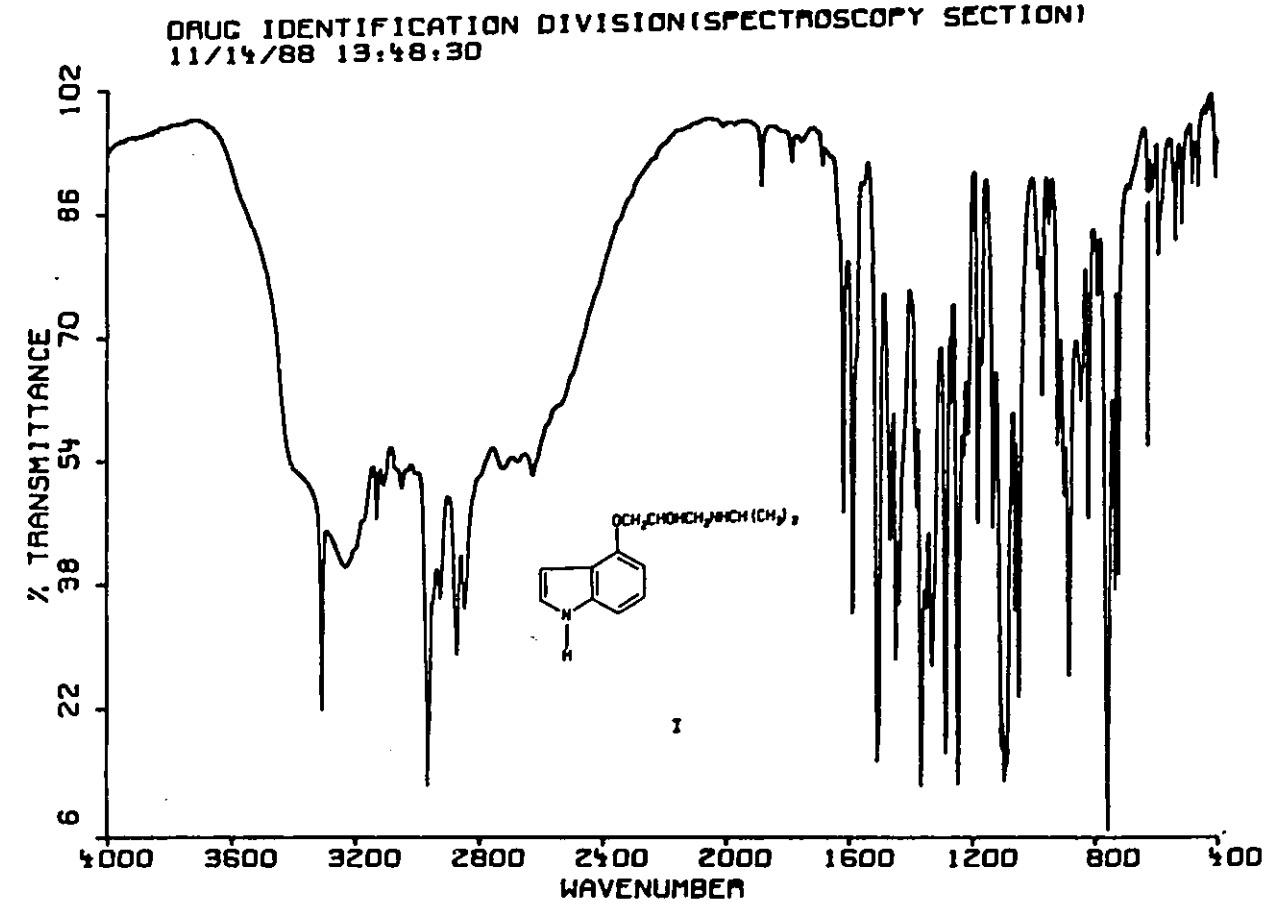
RESULTS AND DISCUSSION

Development of New Methods

1. Authentication of Pindolol and Related Compound Standards

The IR, PMR and mass spectra of the pindolol and related compound standards were consistent with their respective structures. (Figures 2 to 22)

Figure 2. IR Spectrum of Pindolol



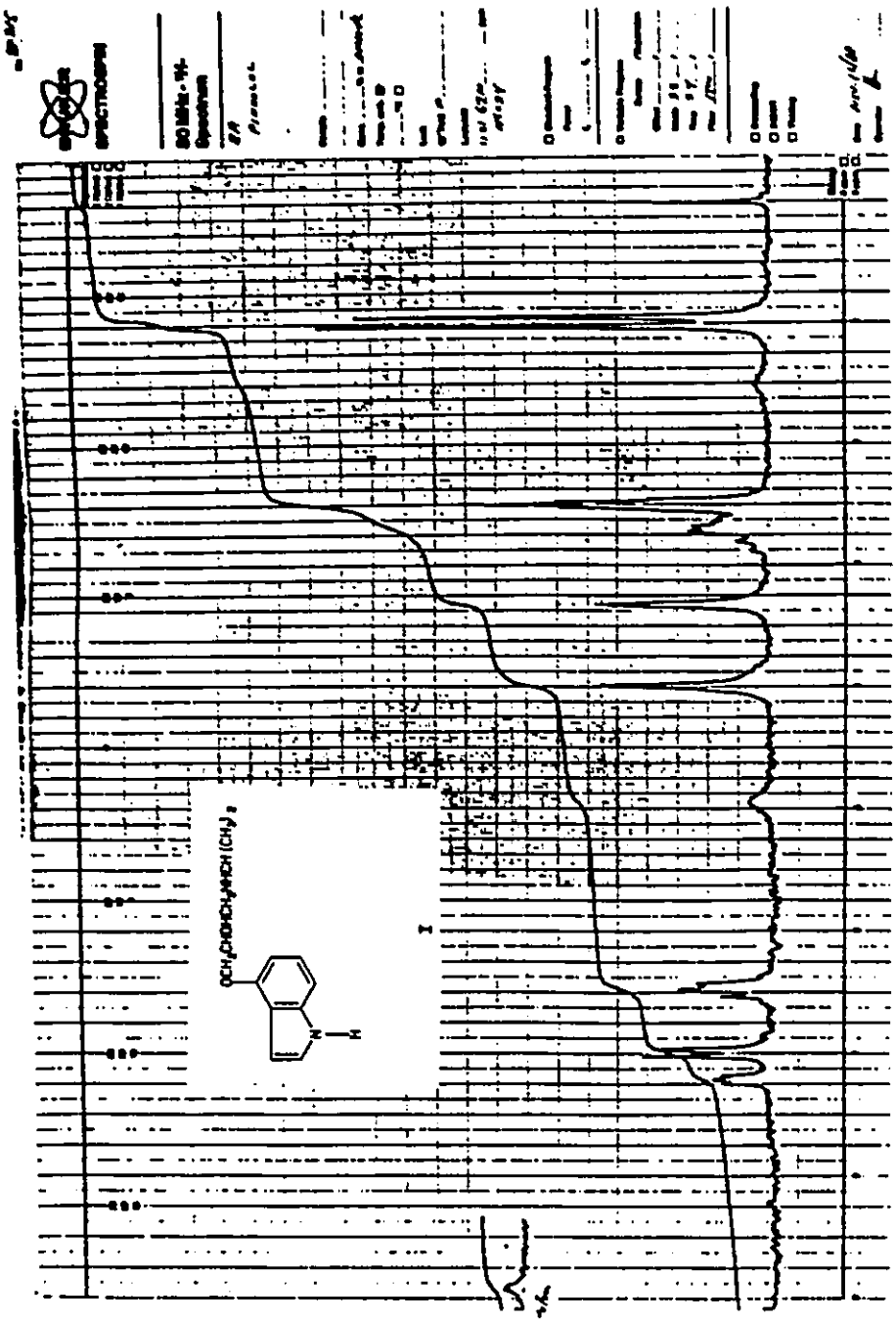


Figure 3. PMR Spectrum of Pindolol

Figure 4. Mass Spectrum of Pindolol

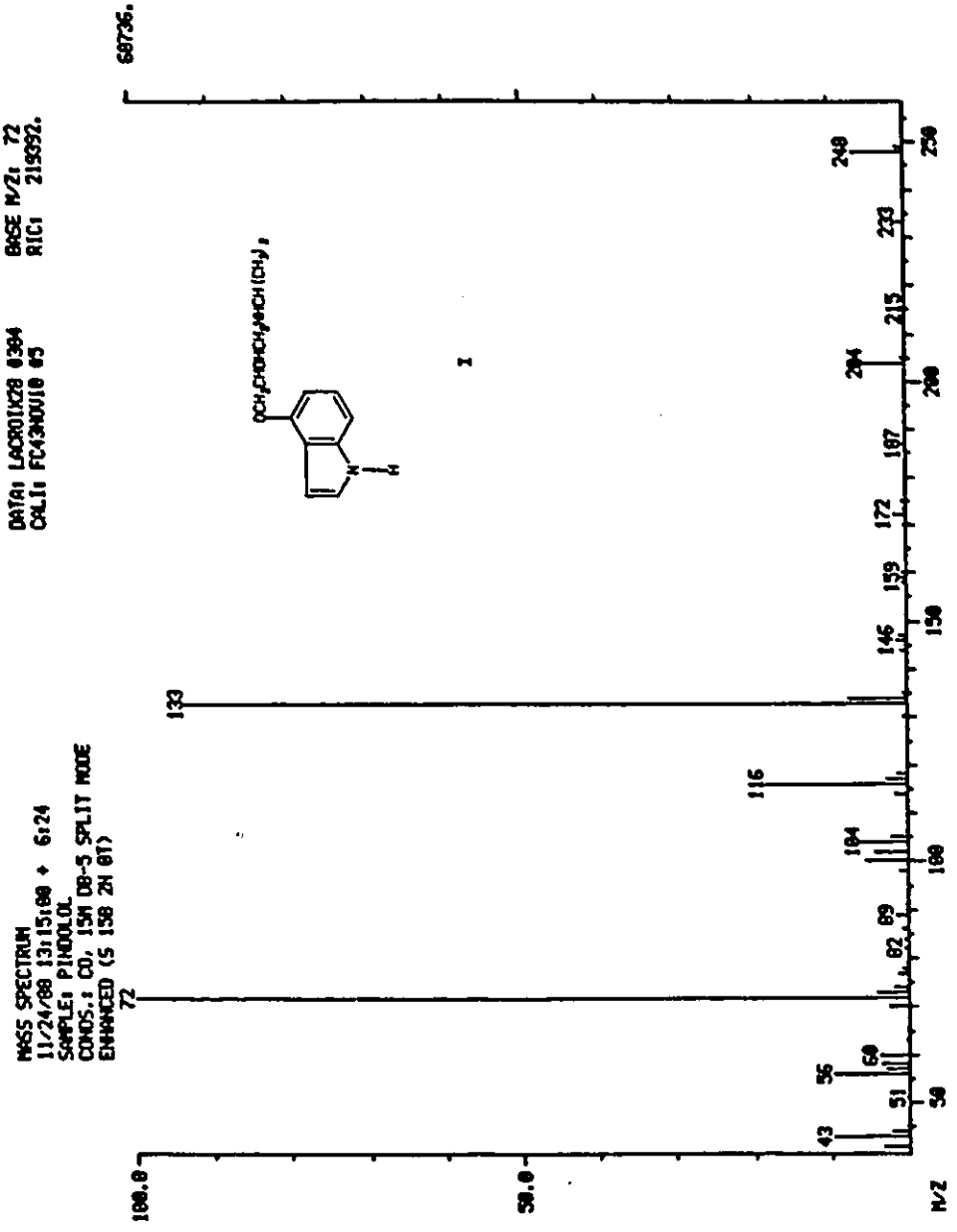
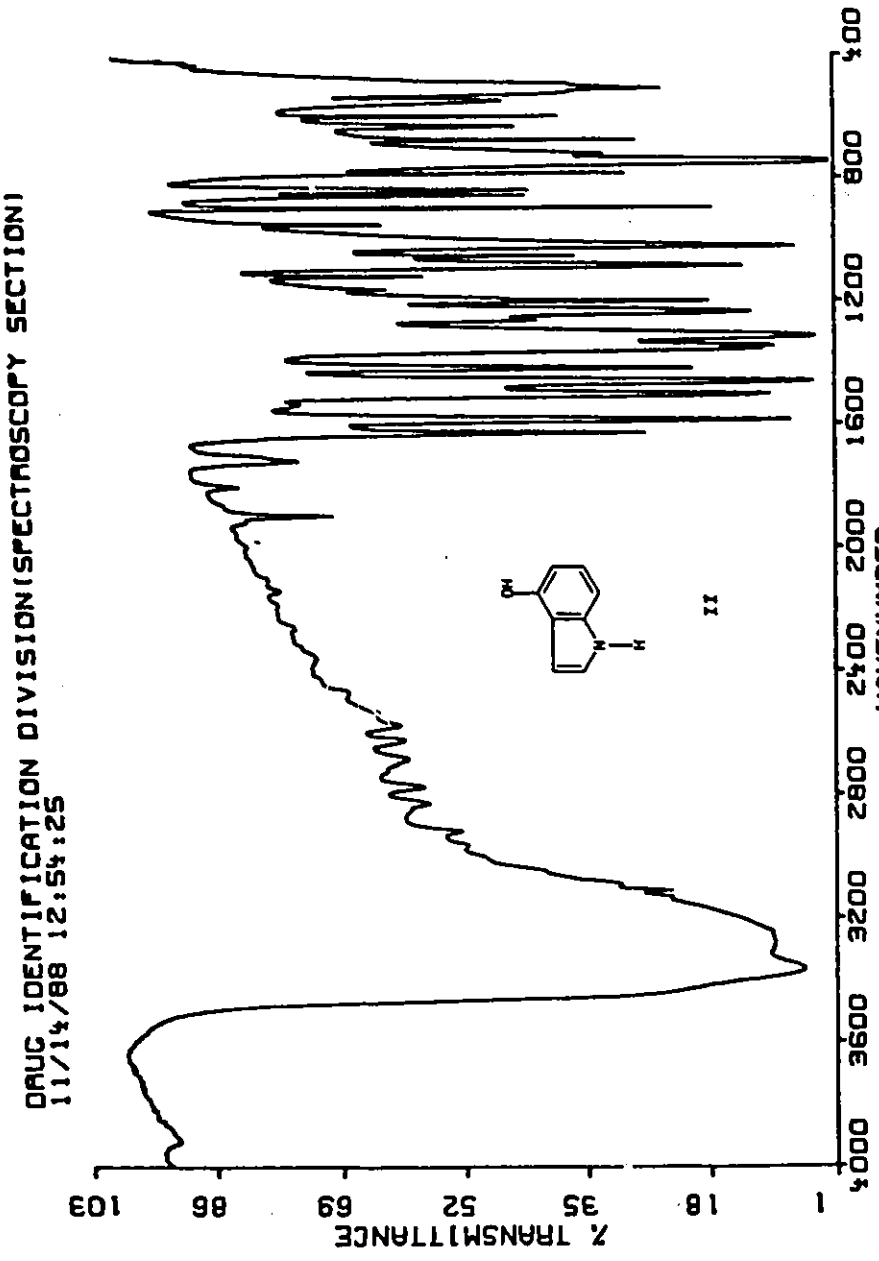


Figure 5. IR Spectrum of (II)



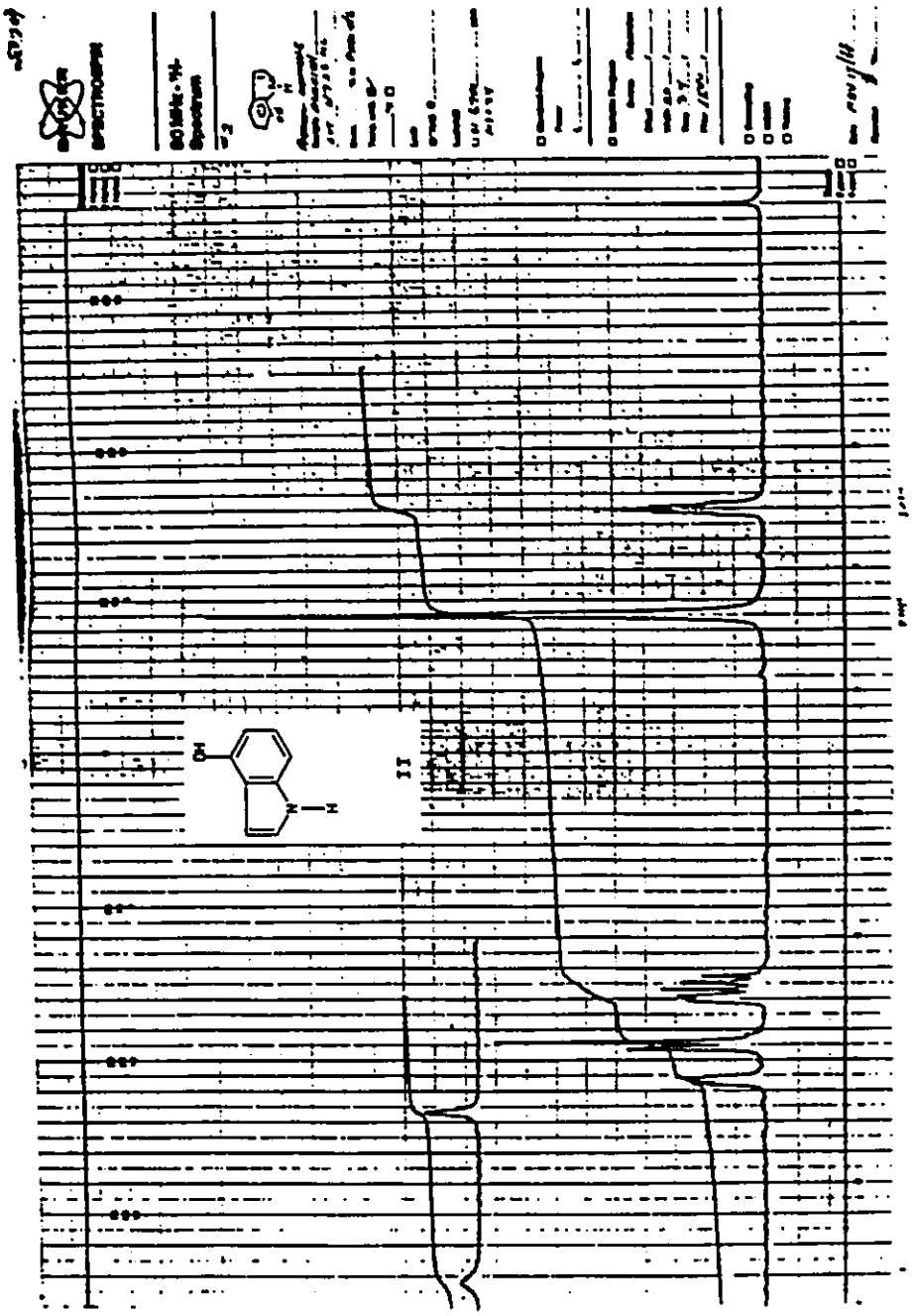


Figure 6. PMR Spectrum of (II)

Figure 7. Mass Spectrum of (II)

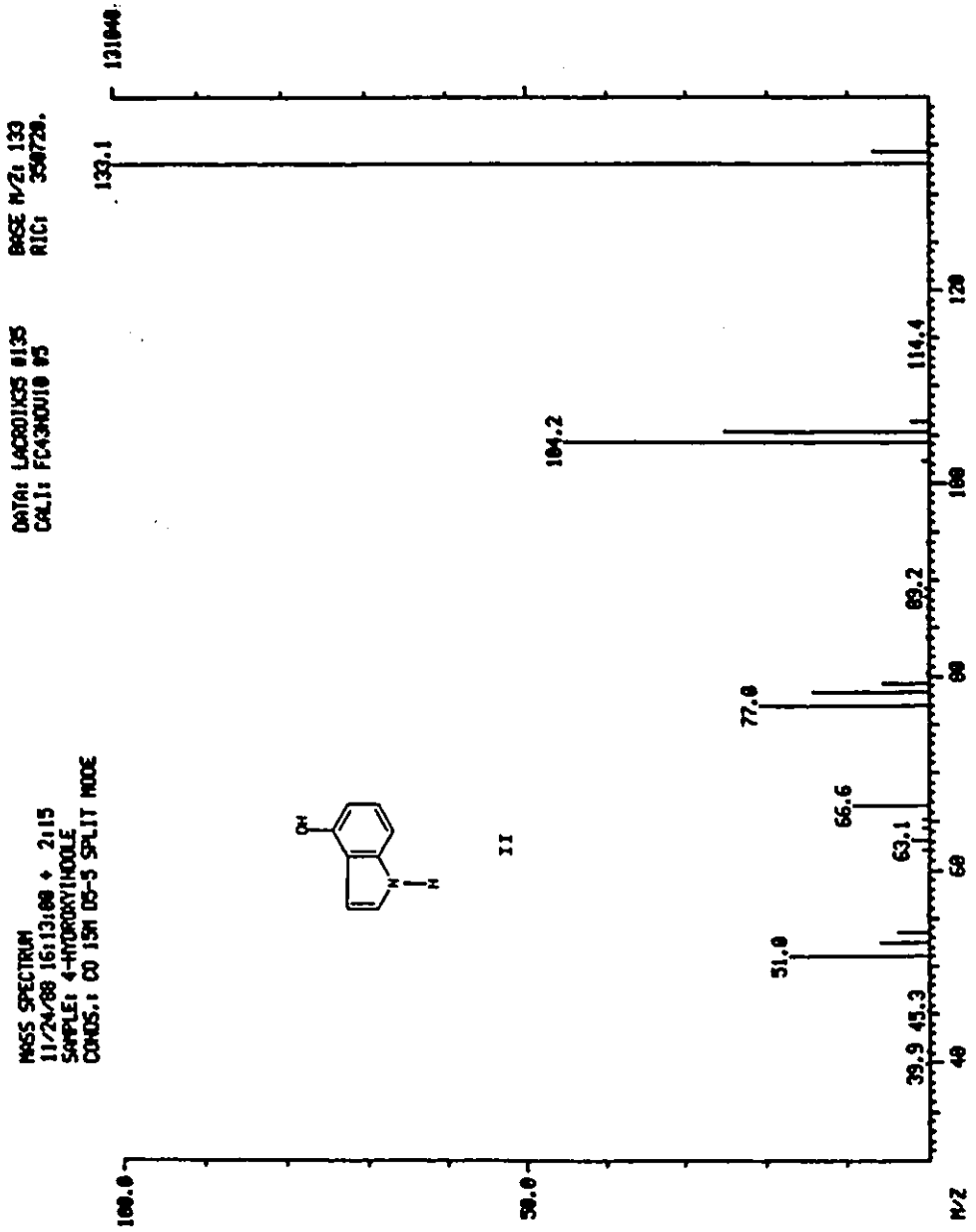
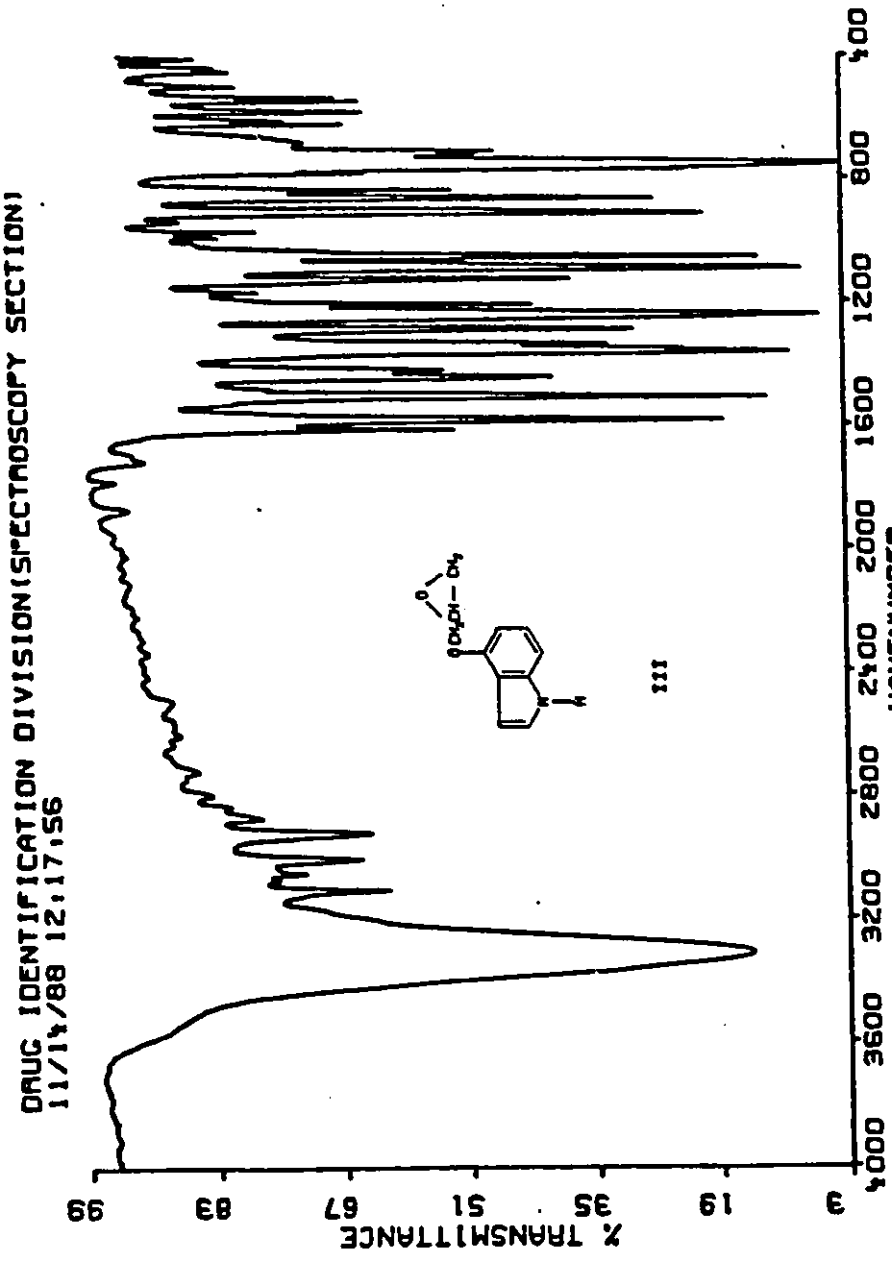


Figure 8. IR Spectrum of (III)



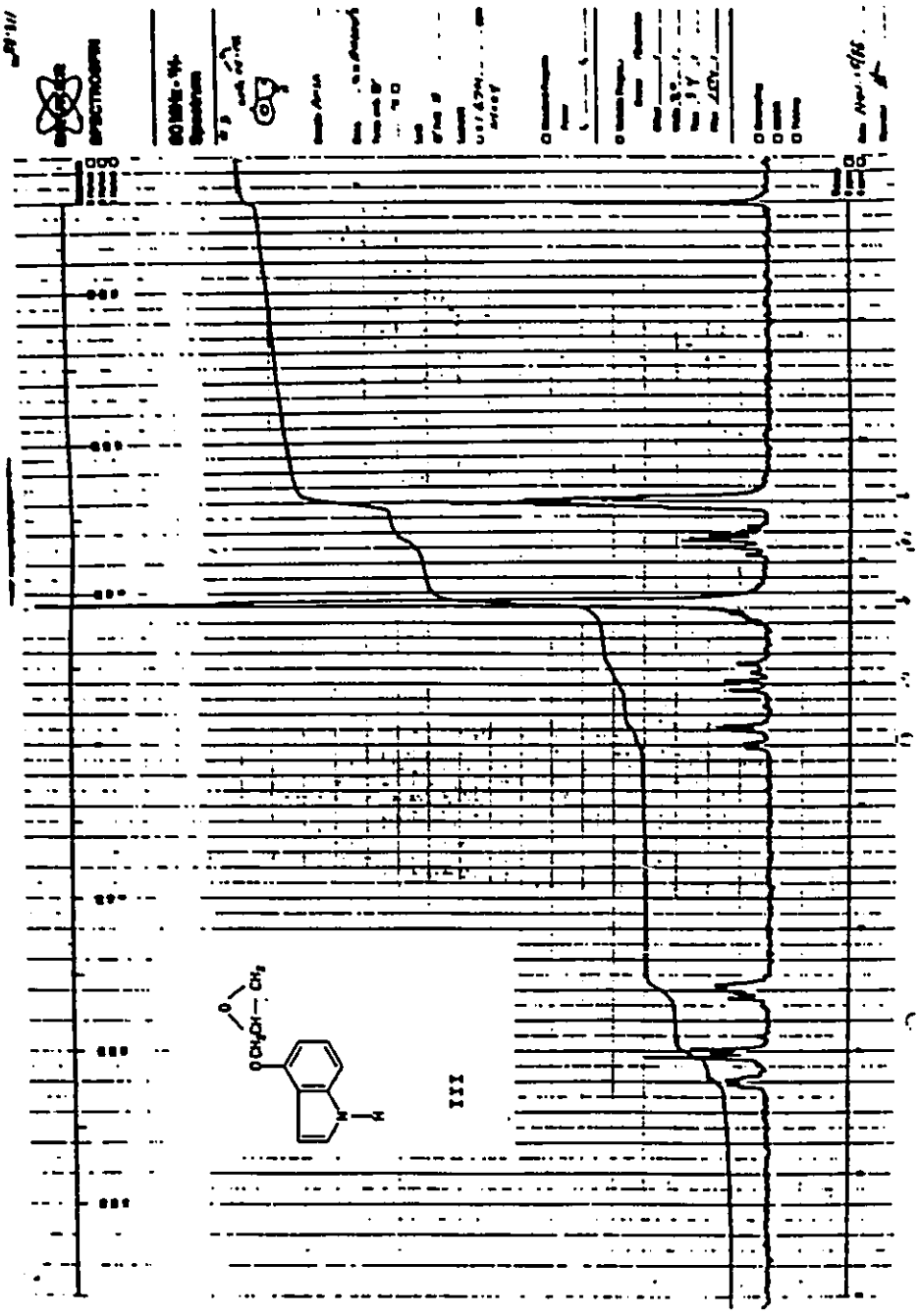


Figure 9. PMR Spectrum of (III)

Figure 10. Mass Spectrum of (III)

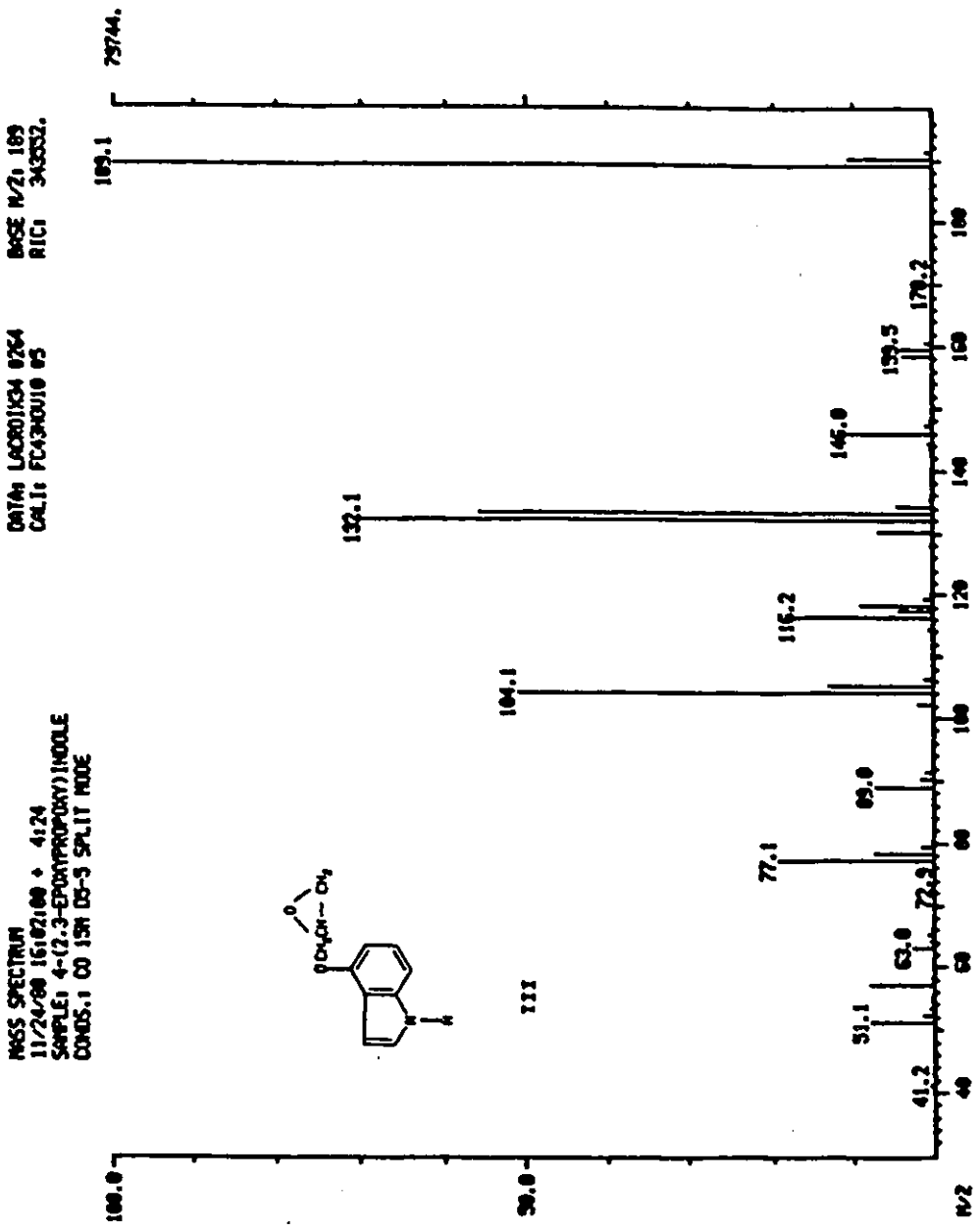
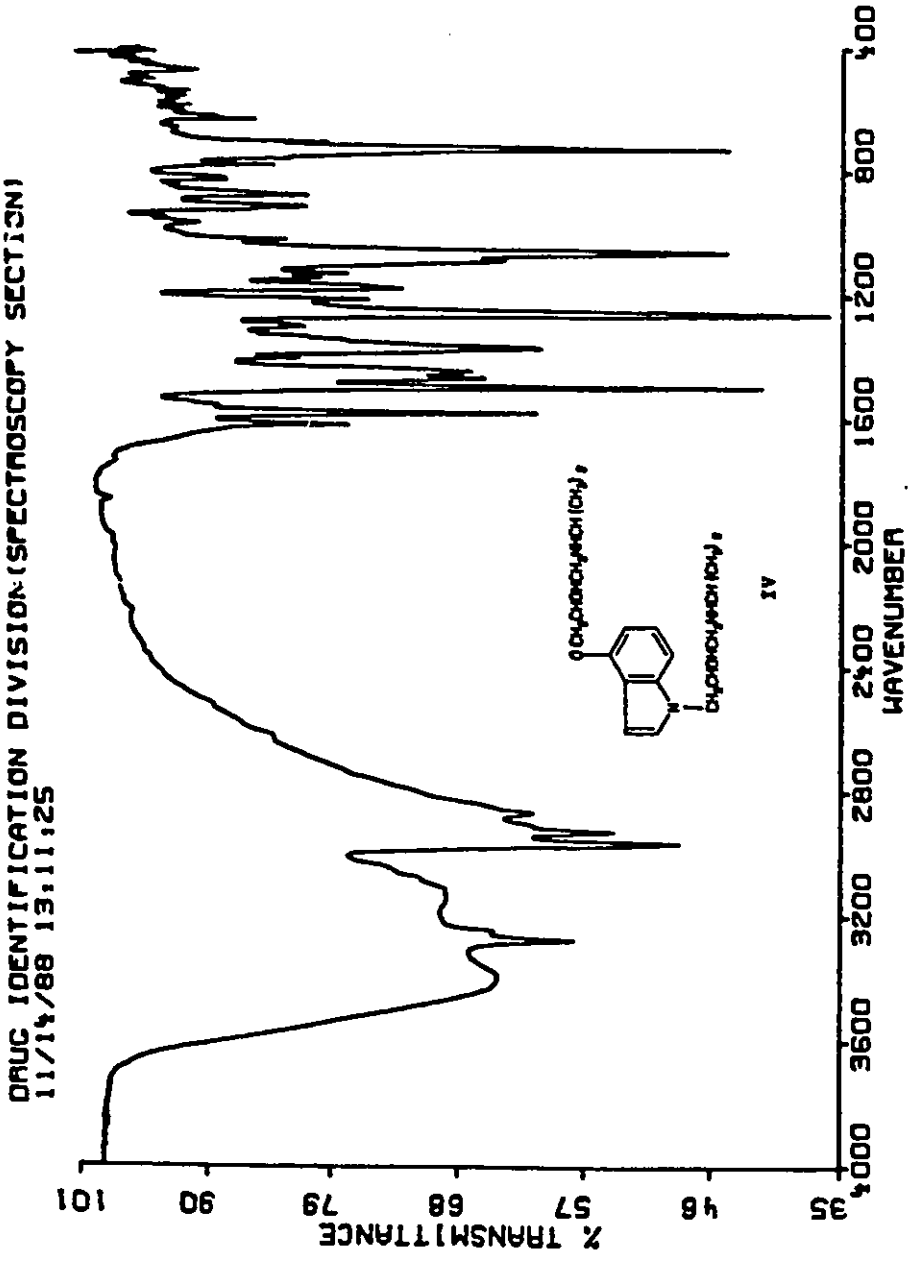


Figure 11. IR Spectrum of (IV)



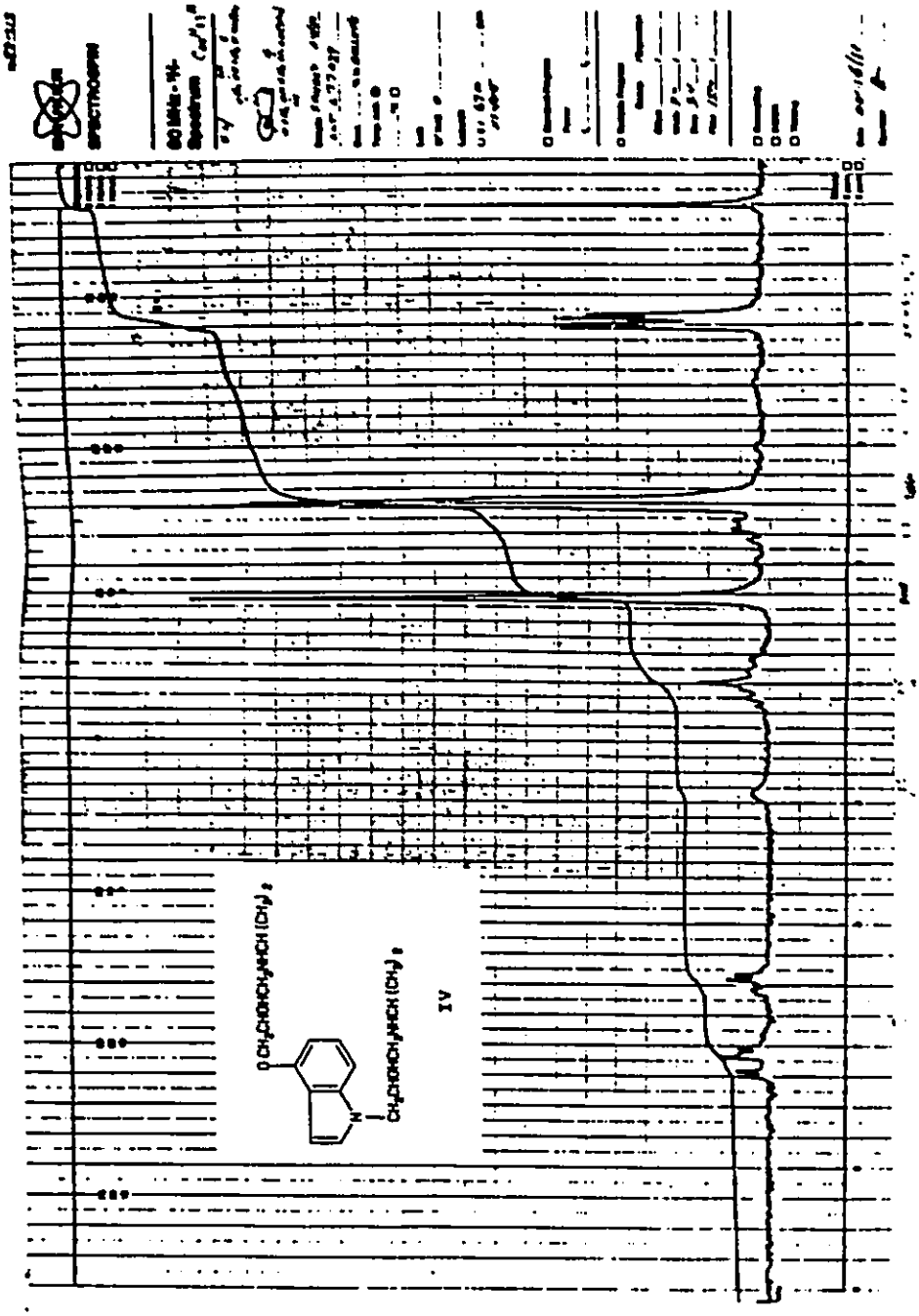


Figure 12. PMR Spectrum of (IV)

Figure 13. Mass Spectrum of (IV)

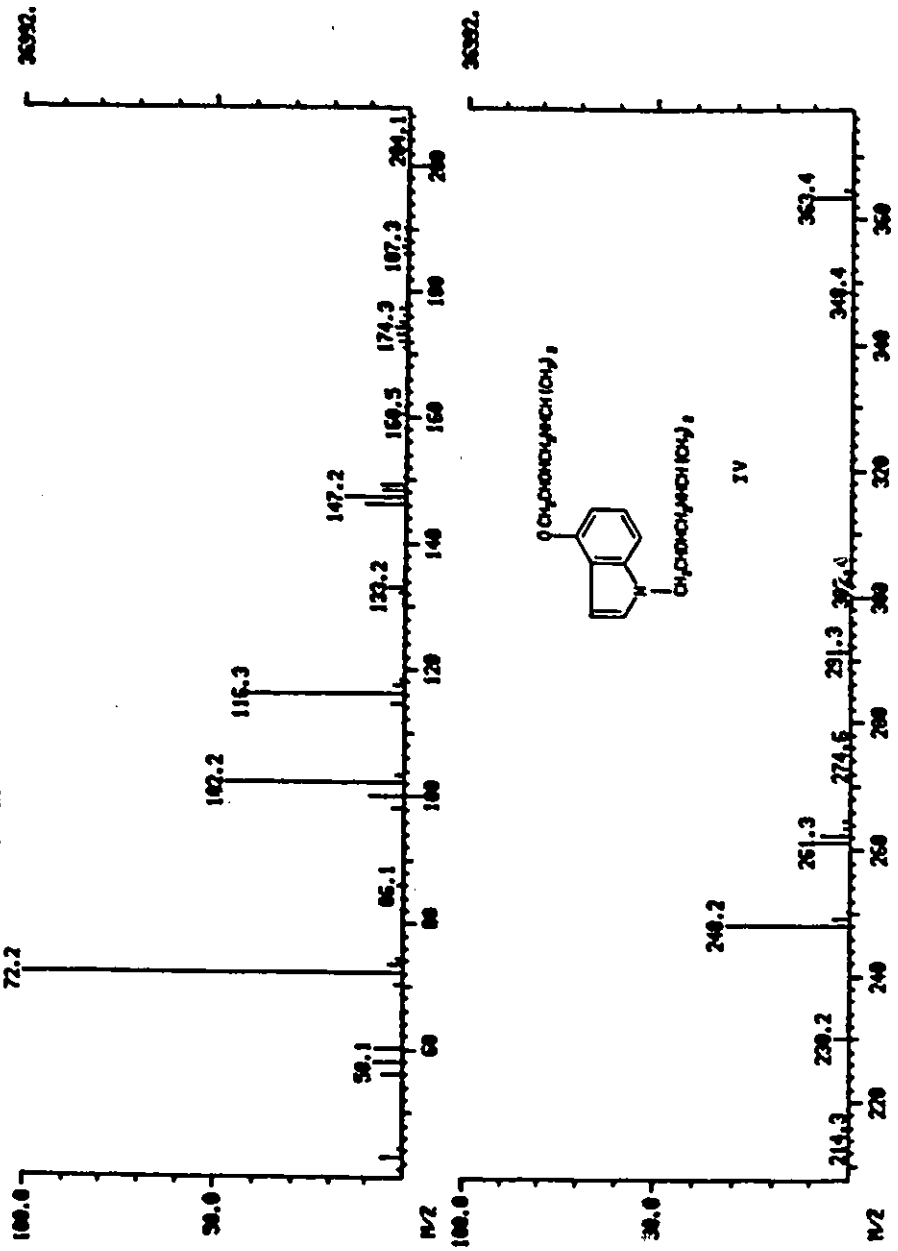
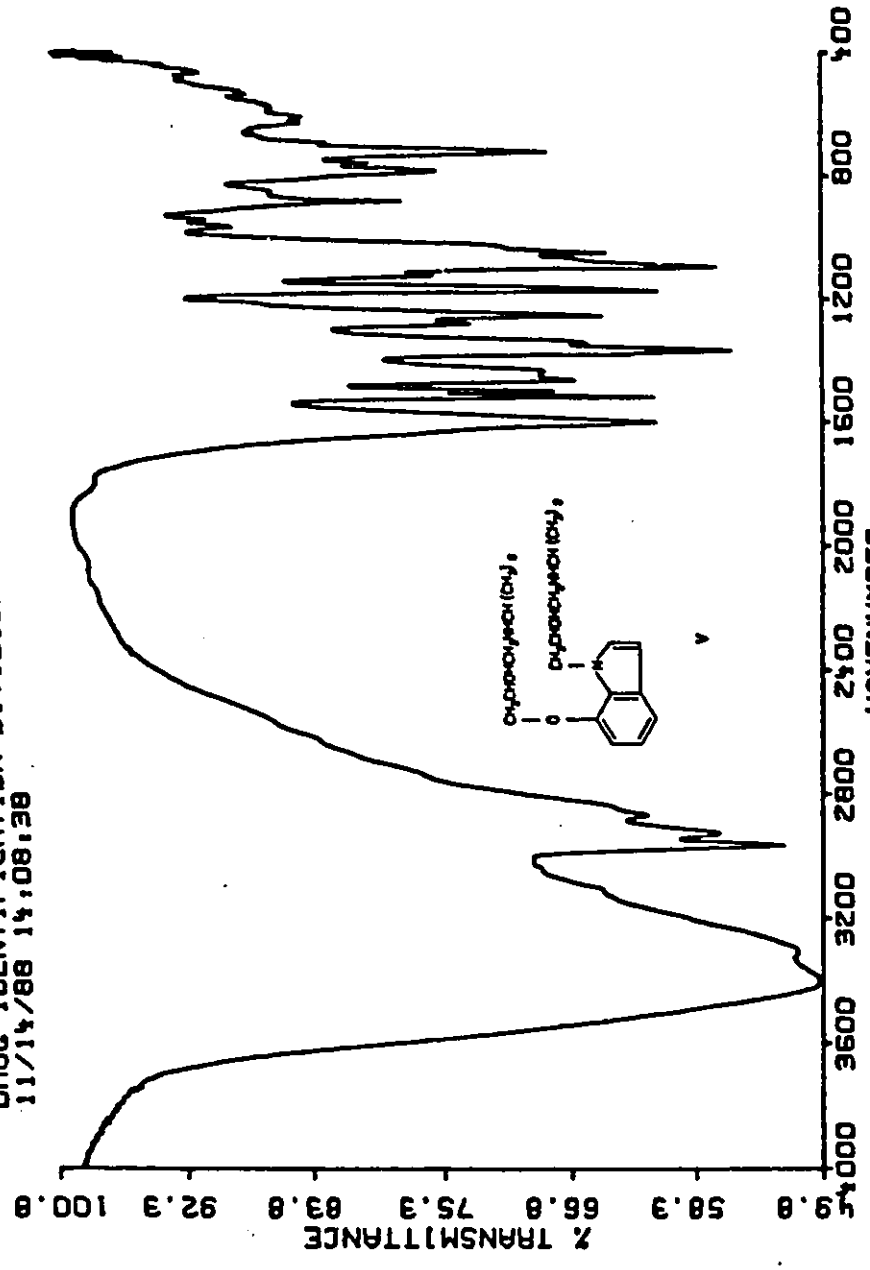


Figure 14. IR Spectrum of (V)



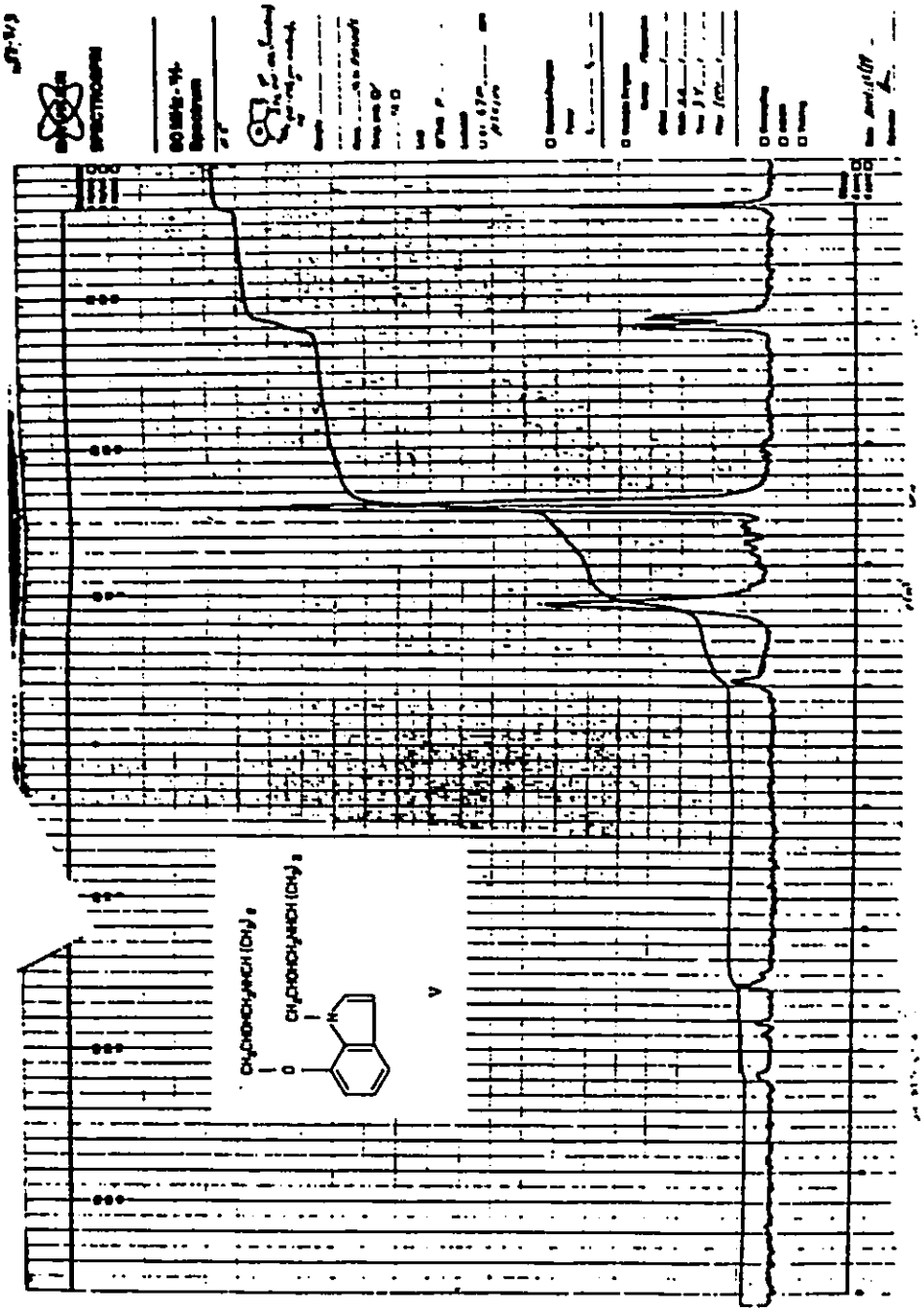
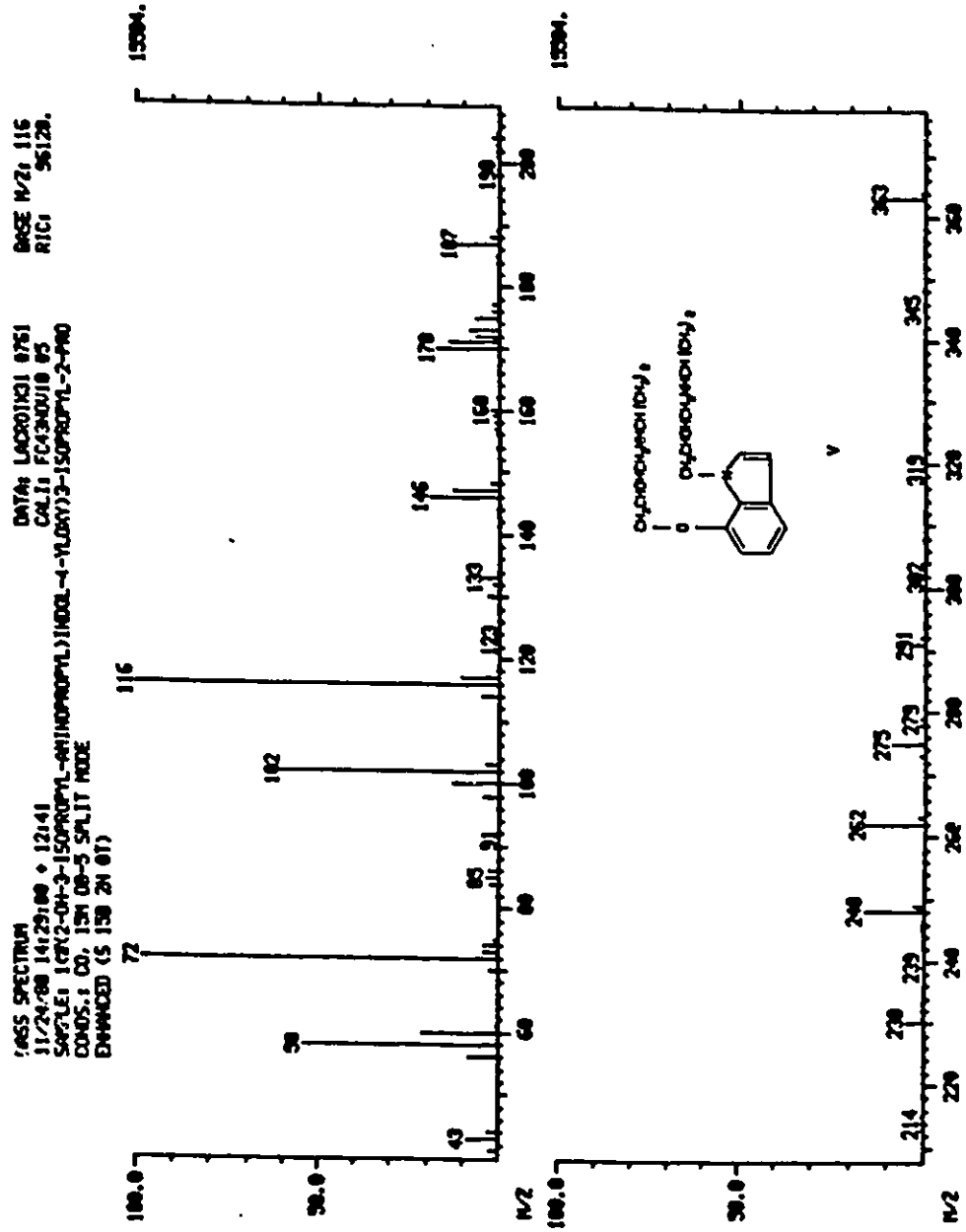


Figure 15. PMR Spectrum of (V)

Figure 16. Mass Spectrum of (V)



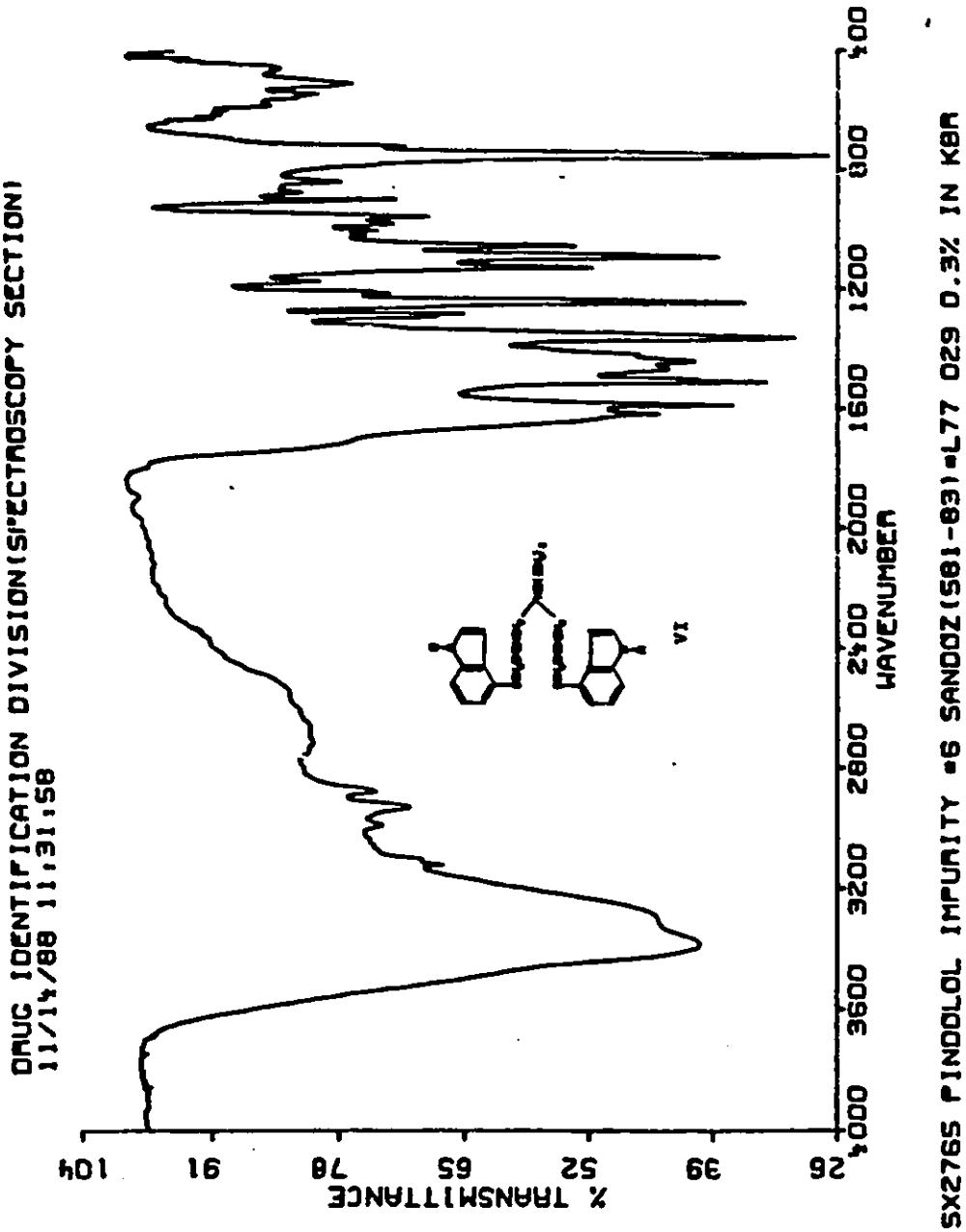


Figure 17. IR Spectrum of (VI)

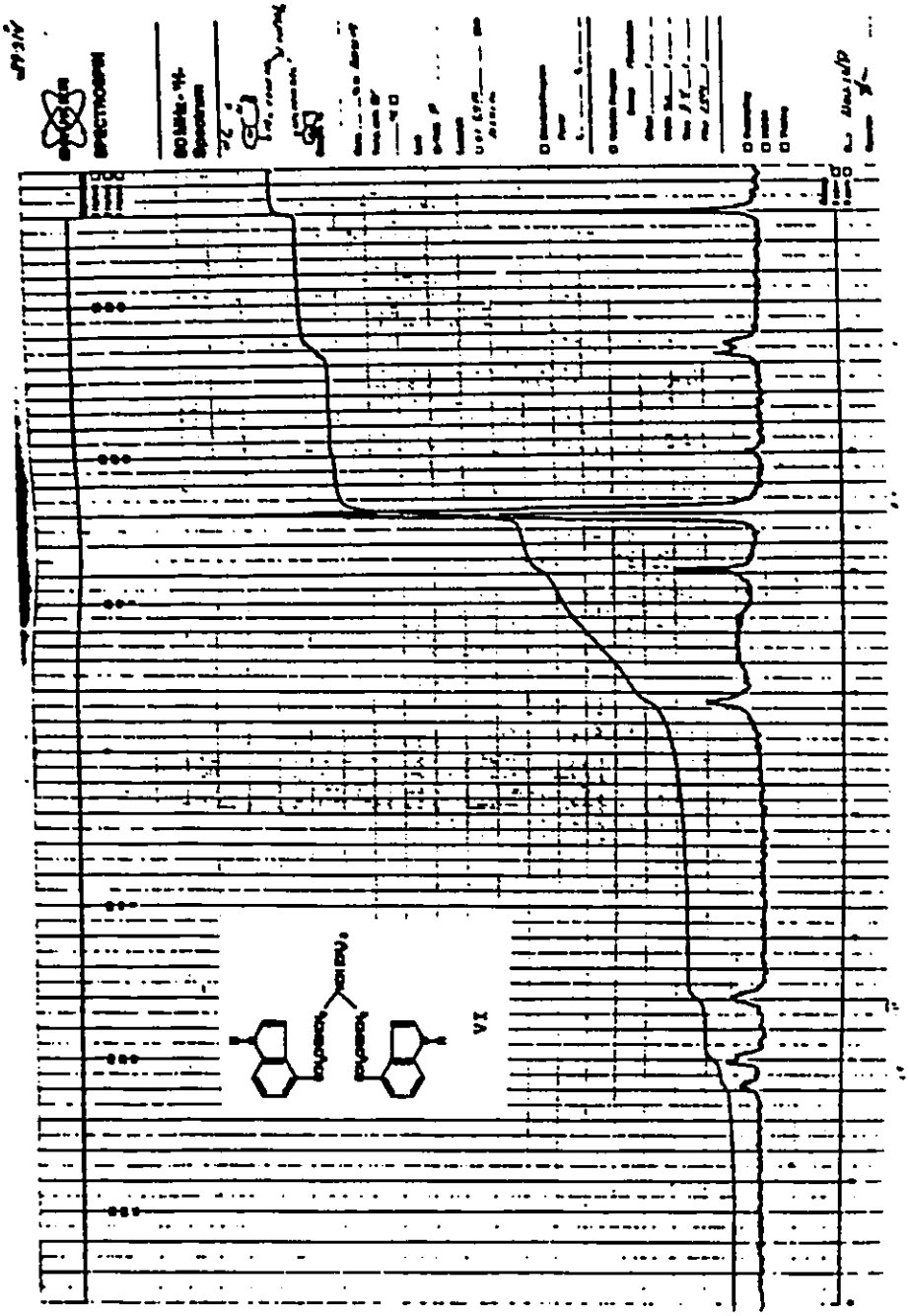


Figure 18. PMR Spectrum of (VI)

Figure 19. Mass Spectrum of (VI)

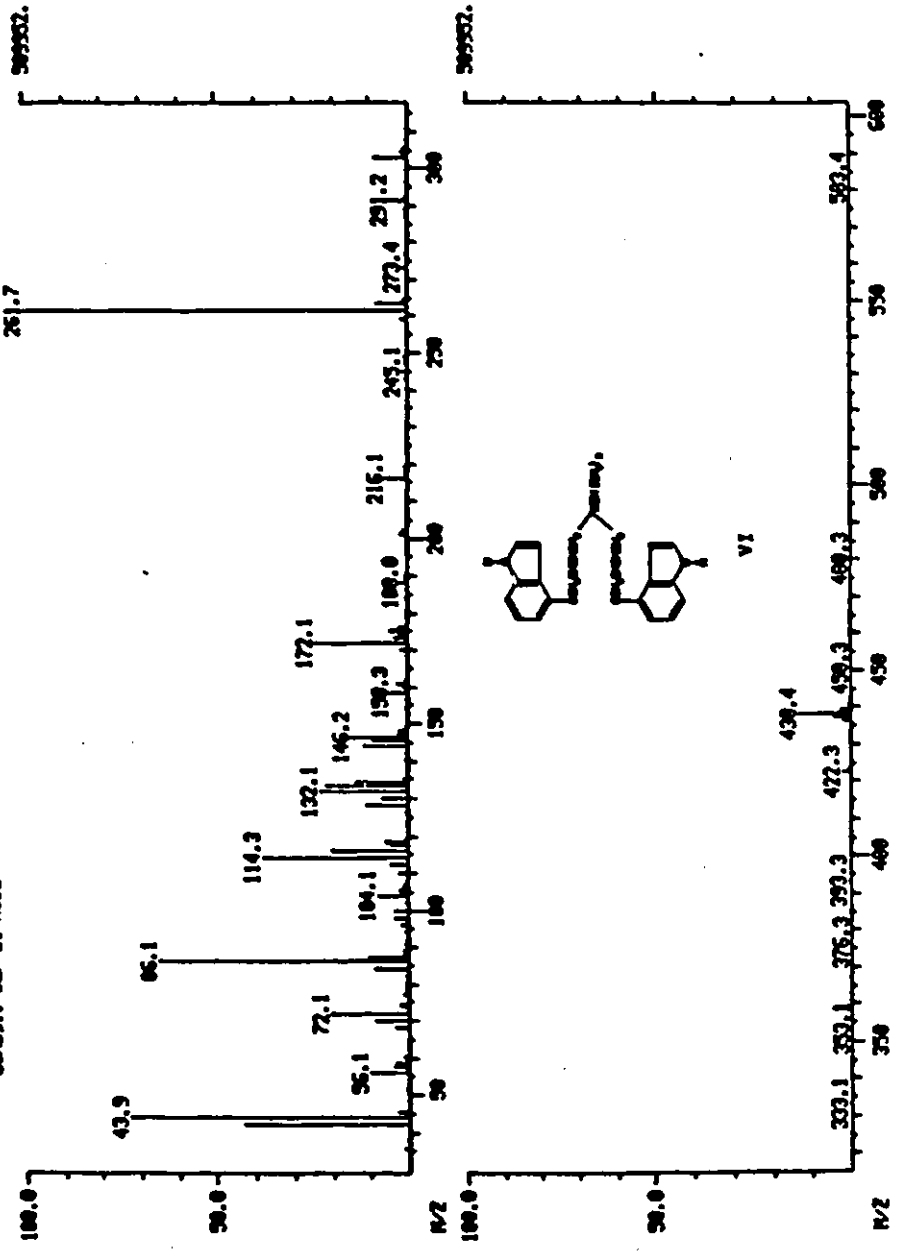
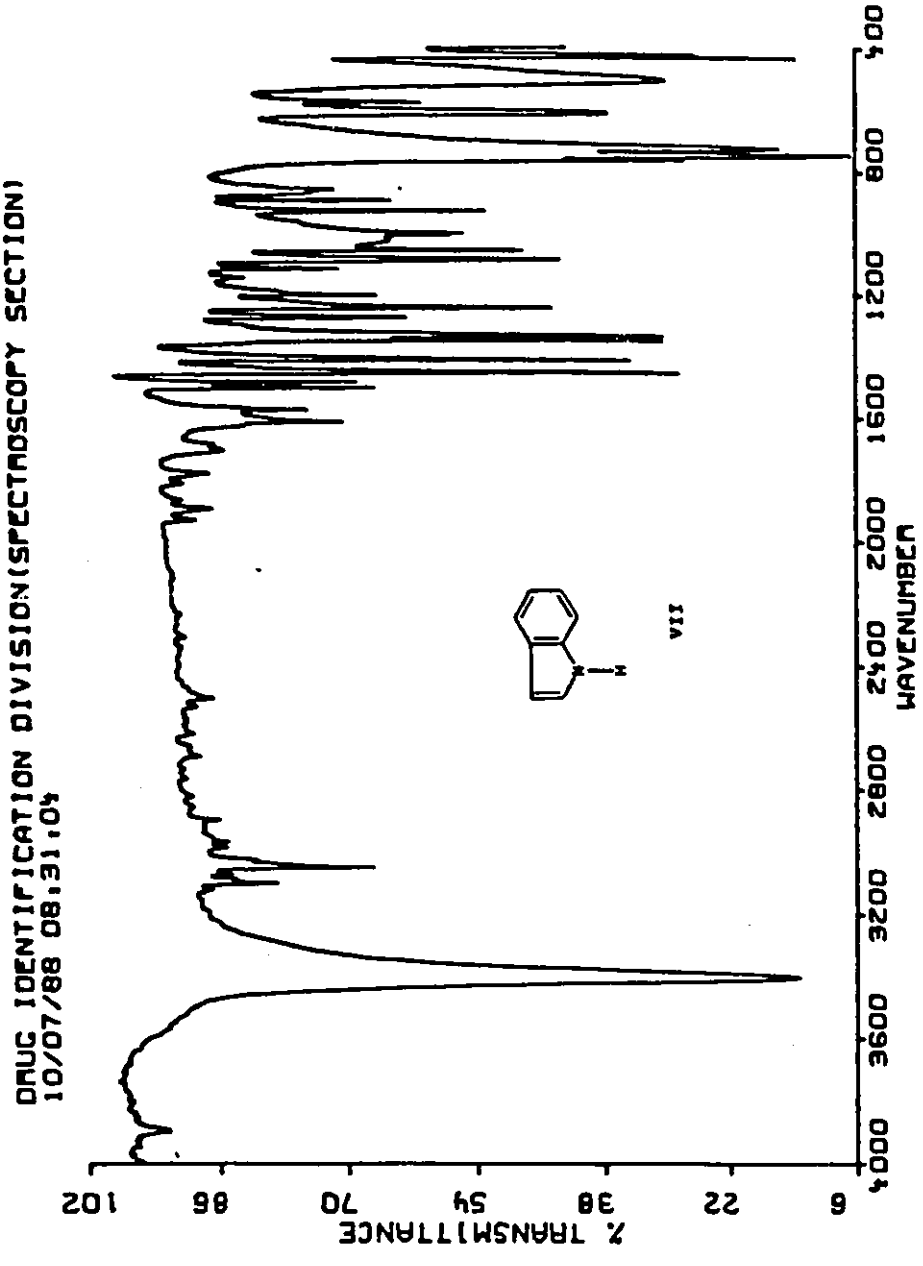


Figure 20. IR Spectrum of (VII)



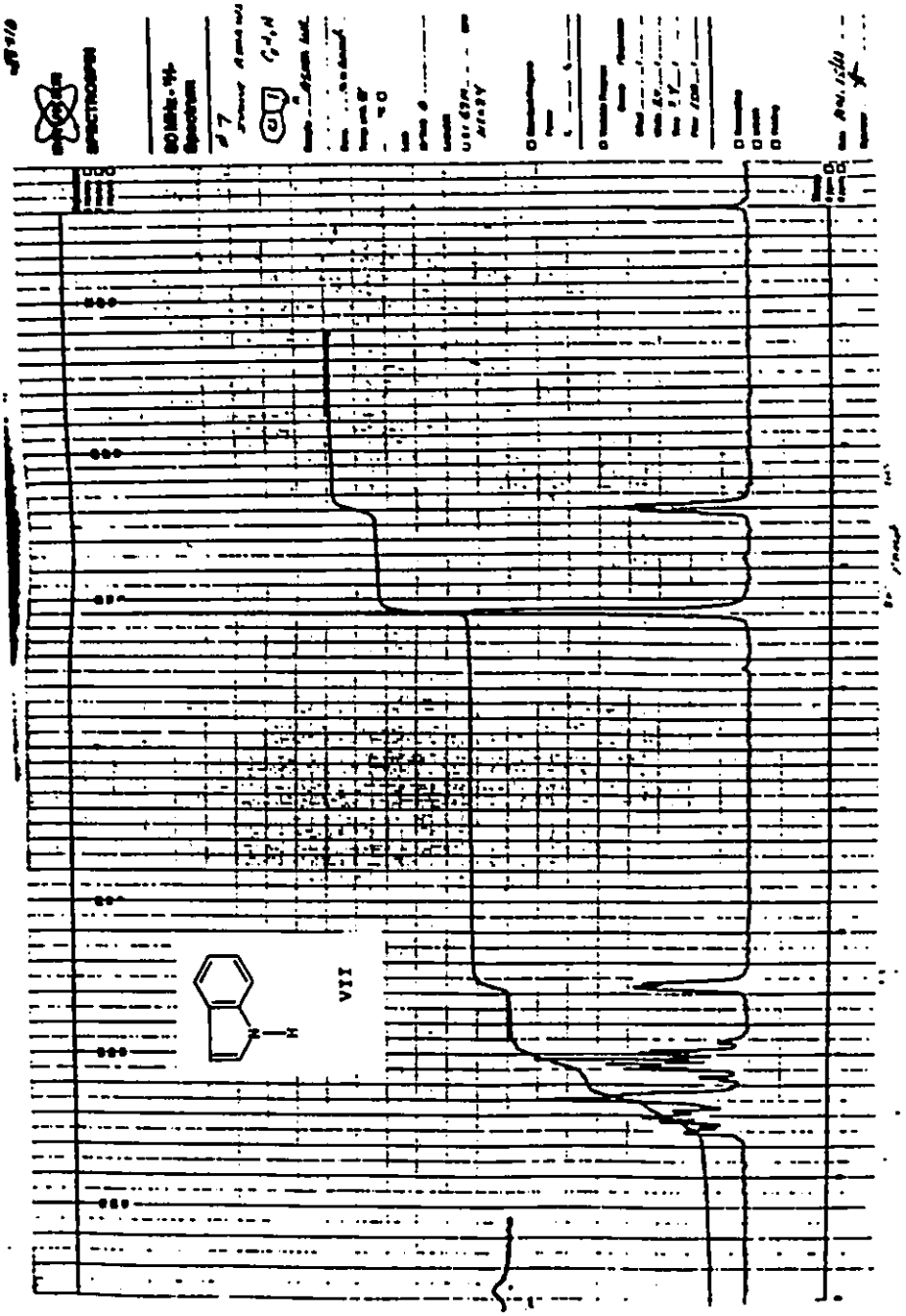
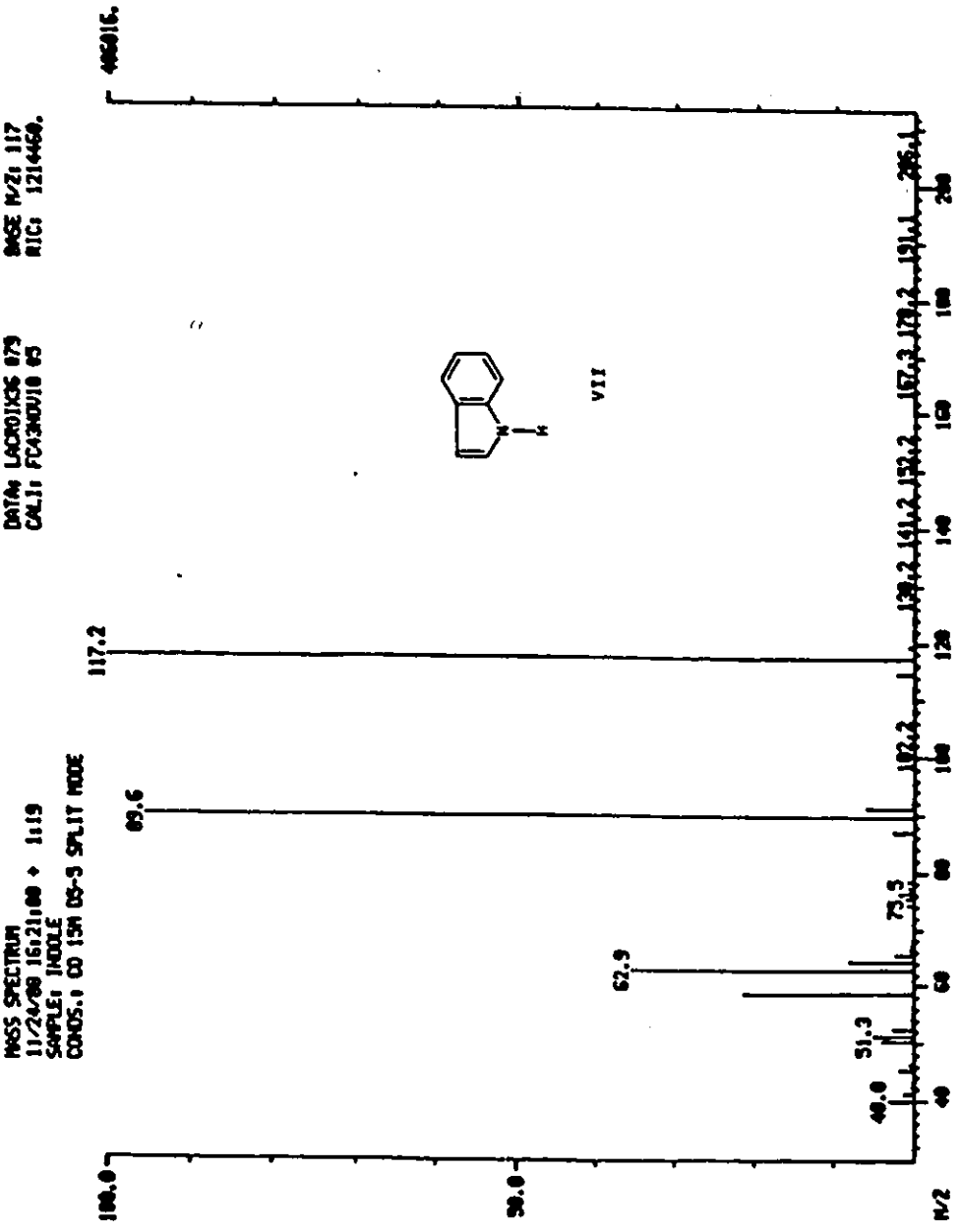


Figure 21. PMR Spectrum of (VII)

Figure 22. Mass Spectrum of (VII)



2. UV Absorbance

The UV spectra of pindolol and related compounds in methanol were obtained. The concentration of the drug and related substances, absorption maxima, absorbance at 219 nm and the absorbance relative to pindolol are presented in Table 2. All the related compounds and the drug exhibited a maximum between 217 and 220 nm, hence the choice of 219 nm as detector wavelength throughout method development and in the final HPLC method.

Table 2. UV Absorbance of Pindolol and Related Compounds

Compound	Conc. µg/mL[1]	Maxima nm	Absorbance at 219 nm	Relative Absorbance[2]
I	12.49	218, 265	1.936	1.00
II	11.71	217, 264	2.746	1.51
III	13.00	218, 264	2.407	1.19
IV	12.60	220, 266	1.142	0.58
V	15.50	218, 266	1.548	0.64
VI	12.50	218, 265	1.653	0.85
VII	12.20	218, 270	2.487	1.32

[1] In methanol

[2] Relative to pindolol

3. Chromatographic Systems Investigated

The syntheses of propranolol and pindolol follow a similar pathway and therefore the drugs have analogous intermediates and side-products. A HPLC method which had been developed for related substances in propranolol hydrochloride in 1986 appeared to be a good starting point for the resolution of pindolol related compounds(44). The column used was a 150 x 4.6 mm 3μm Spherisorb nitrile column. The mobile phase consisted of sodium acetate buffer 0.05 M adjusted to pH 5.0 with 0.05 M acetic acid/acetonitrile (ACN)/ tetrahydrofuran (THF) 70/15/15 (V/V) at a flow rate of 1 mL/min. The retention time of pindolol on this system was about 6 minutes whereas that of propranolol was 11-12 minutes (Figure 23).

Beginning with this mobile phase and flow rate, the system was modified and various mobile phase compositions were evaluated, using a test mixture composed of about 1 mg/mL drug and 0.05 mg/mL of each related compound in methanol. Mixing of the three components of the mobile phase was accomplished automatically by the ternary pumping system.

a) 70/15/15 buffer/ACN/THF (original propranolol system)

There was fairly good resolution of all impurities from the drug. II and III and VII eluted before the drug. V and IV eluted on the tail of the drug peak and VI was last as a doublet at about 24 minutes. (Figure 23)

b) 70/10/20 buffer/ACN/THF

Keeping the proportion of buffer constant but increasing the THF concentration and decreasing the ACN concentration resulted in a loss of resolution between pindolol and impurities VII, V and IV. VI eluted slightly later at about 28 minutes. (Figure 24)

c) 70/20/10 buffer/ACN/THF

Increasing the ACN concentration from 15 to 20% (v/v) and decreasing the THF concentration from 15 to 10% (v/v) had little effect on the resolution of most of the impurities, except IV and V which were less well resolved from the drug peak. (Figure 25)

Figure 23. Chromatogram of pindolol and related compounds using 70/15/15 buffer/ACN/THF (original propranolol system) as the mobile phase

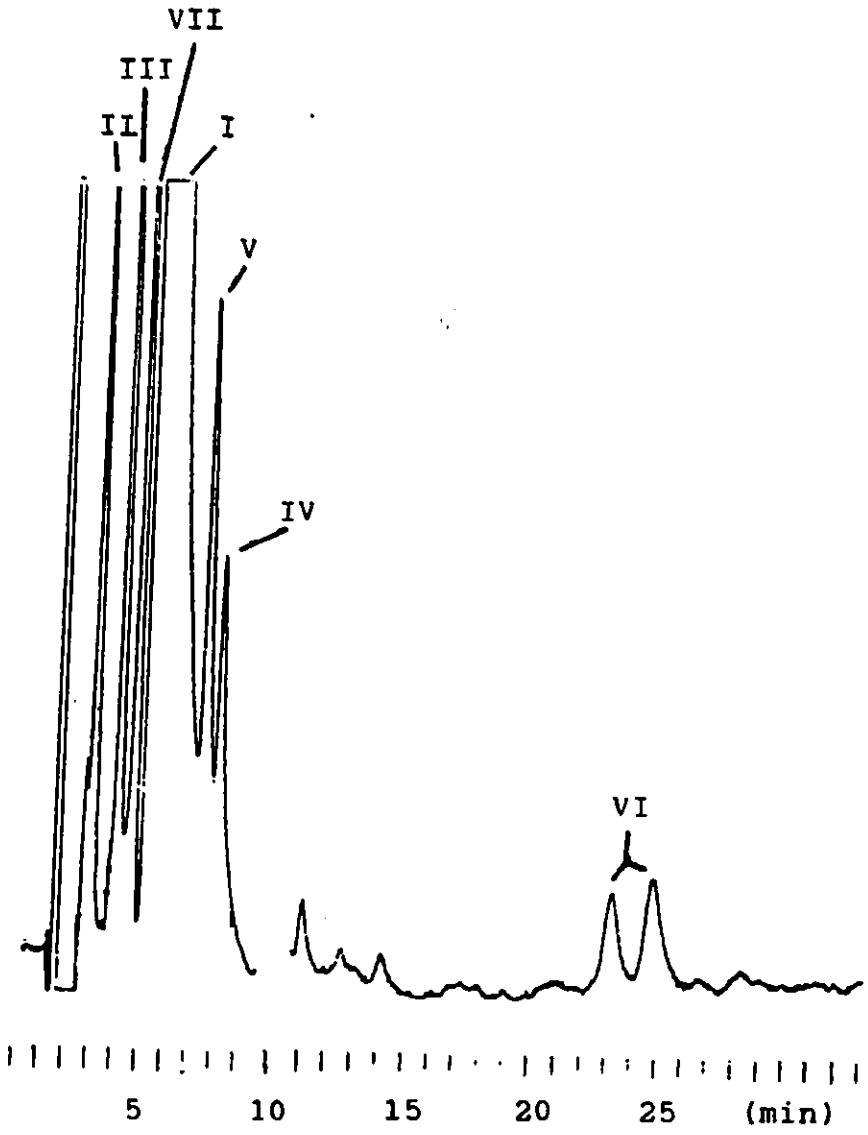


Figure 24. Chromatogram of pindolol and related compounds using 70/10/20 buffer/ACN/THF as the mobile phase

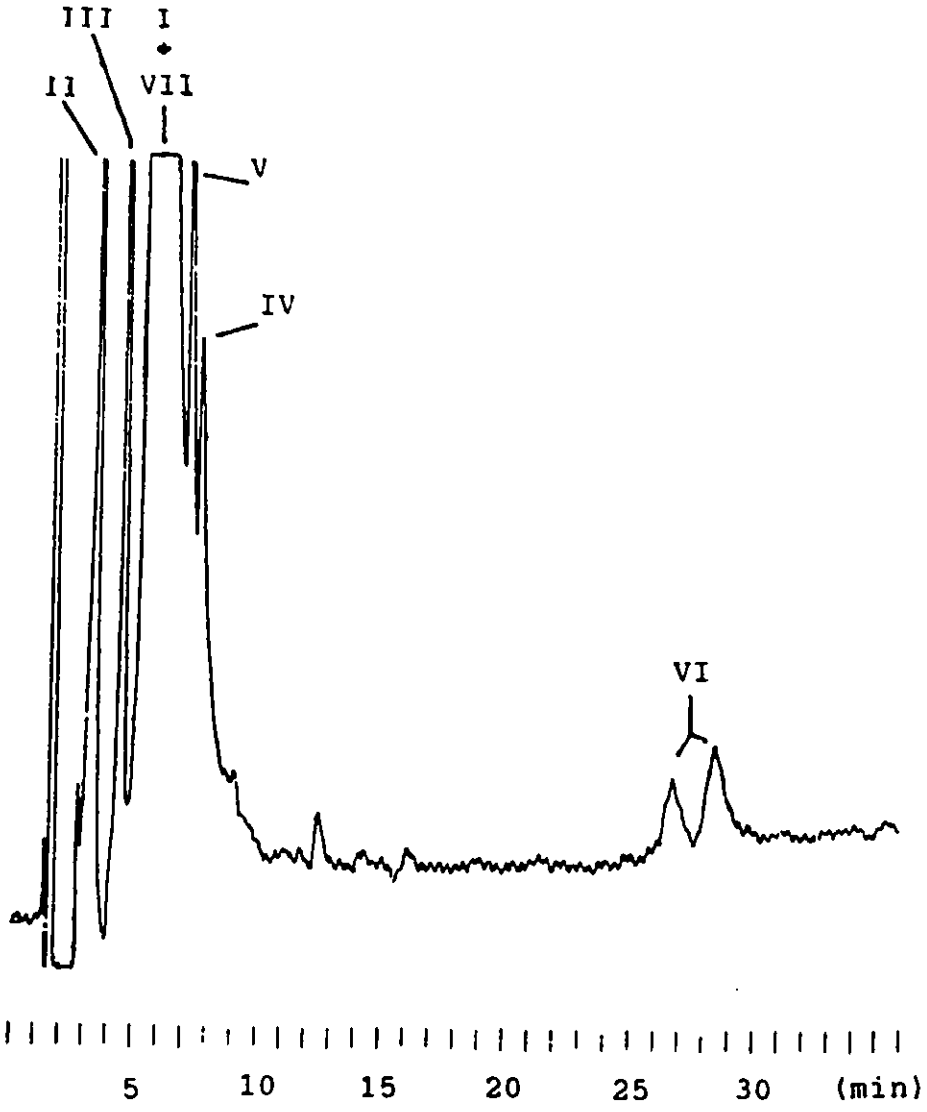
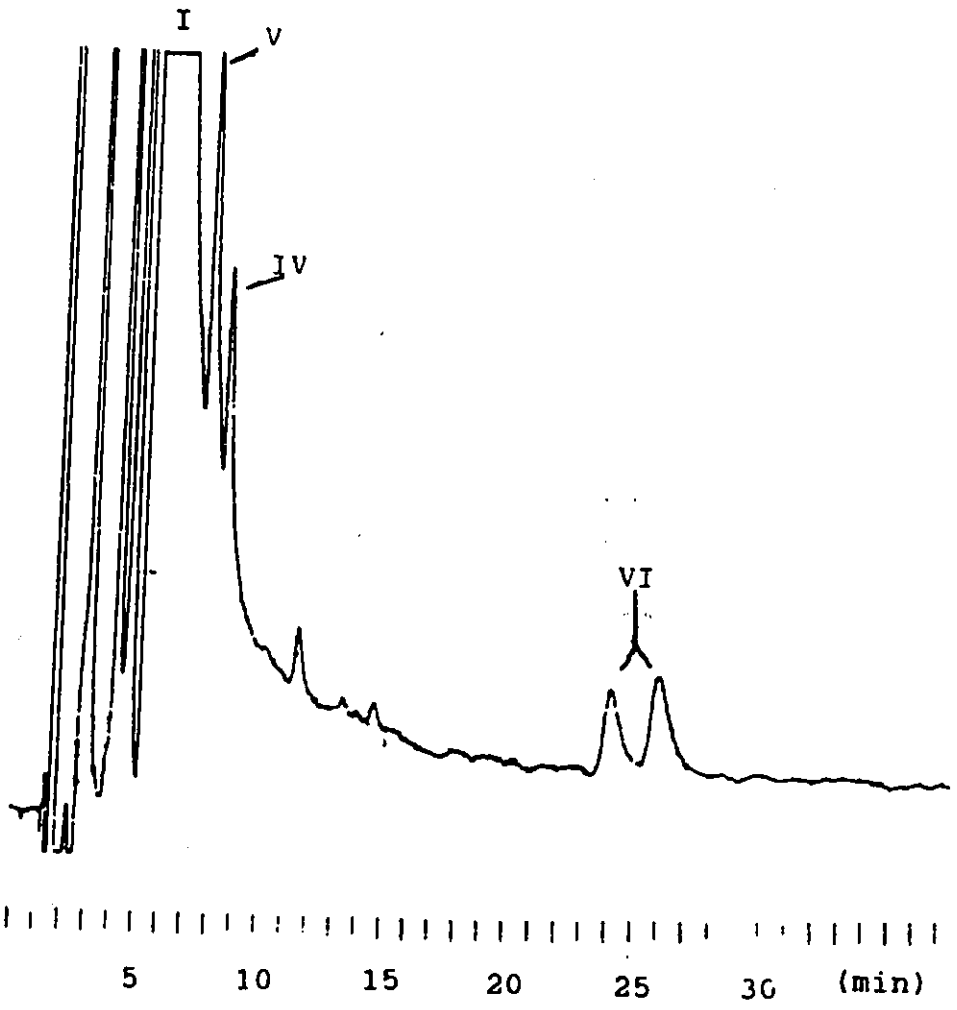


Figure 25. Chromatogram of pindolol and related compounds using 70/20/10 buffer/ACN/THF as the mobile phase



d) 70/30/0 buffer/ACN/THF

There was generally better resolution of the impurities from the drug. V and IV were lower on the tail of the drug, and shoulders on the drug peak from unknown compounds appeared. VI was eluted at about 26 minutes. (Figure 26)

e) 75/25/0 buffer/ACN/THF

Decreasing the acetonitrile concentration from 30% to 25% (v/v) resulted in a loss of resolution between the drug and impurities IV and V, and a slight increase in resolution between the drug and VI which eluted at about 32 minutes. (Figure 27)

f) 60/40/0 buffer/ACN/THF

Increasing the acetonitrile concentration to 40% (v/v) reduced the retention time of VI to about 15 minutes while increasing that of V and IV. There was almost baseline resolution between the drug and V. Three peaks from unknown compounds appeared on the tail of the drug, eluting before V. The early eluting compounds were still well resolved from the drug although two were not well resolved from each other. (Figure 28)

Figure 26. Chromatogram of pindolol and related compounds using 70/30/0 buffer/ACN/THF as the mobile phase

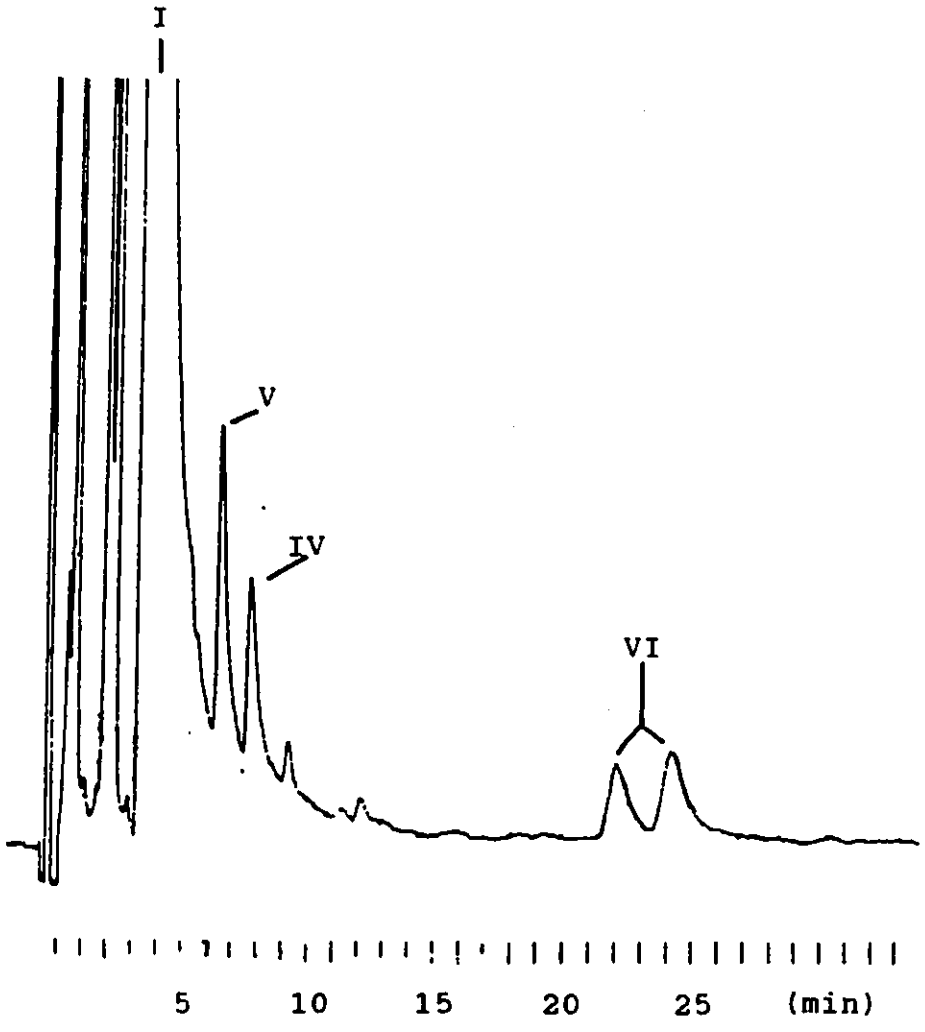


Figure 27. Chromatogram of pindolol and related compounds using 75/25/0 buffer/ACN/THF as the mobile phase

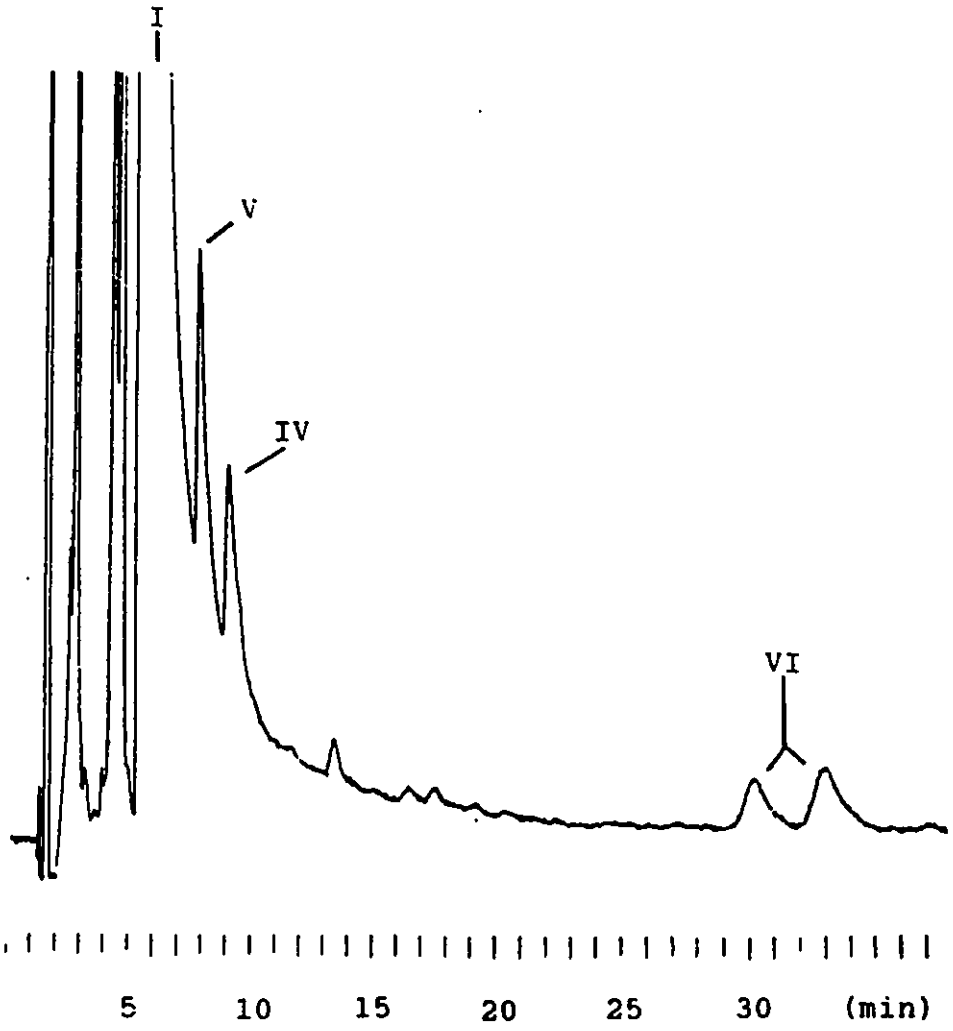
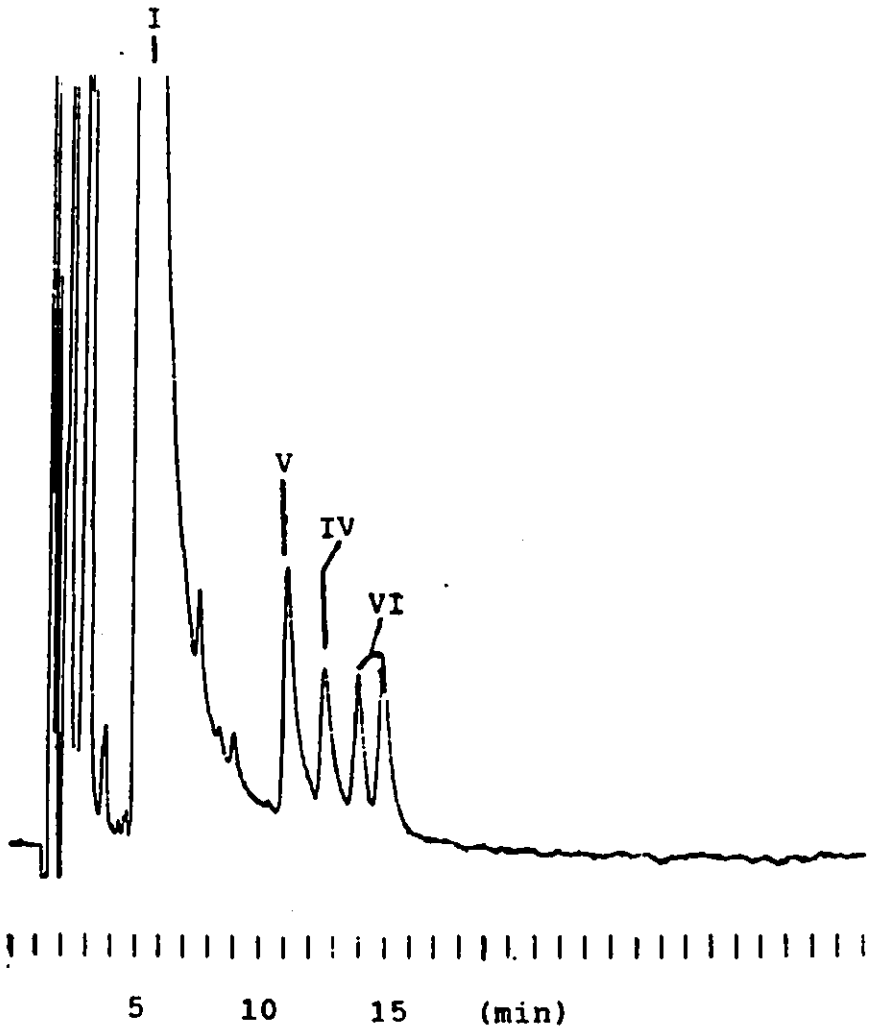


Figure 28. Chromatogram of pindolol and related compounds using 60/40/0 buffer/ACN/THF as the mobile phase



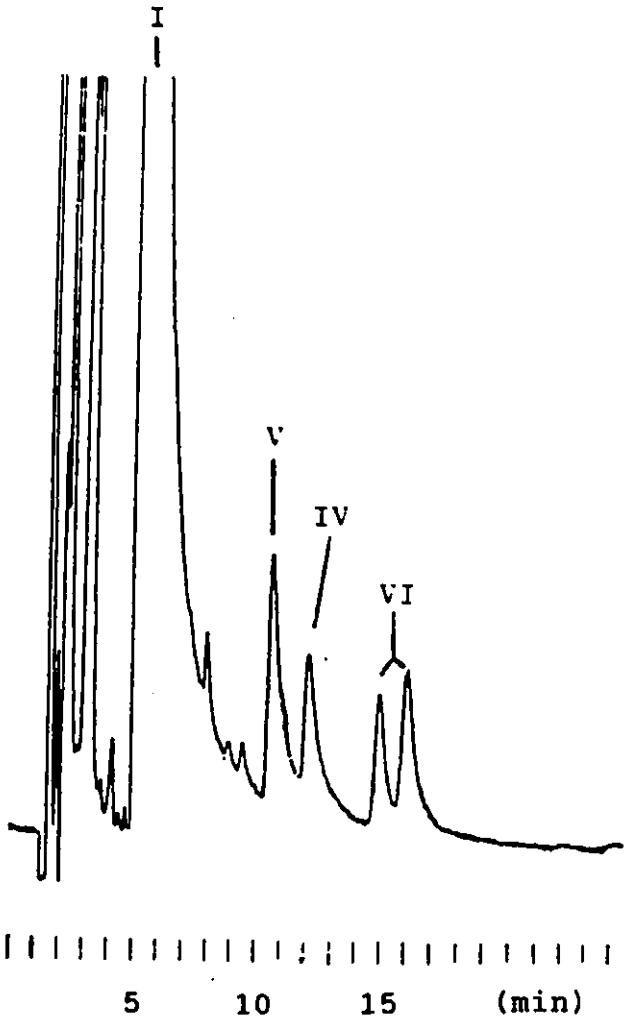
Differences in the configuration of ternary or quaternary pumping systems among various brands of liquid chromatographs can result in slight variations in the actual final concentration of the mobile phase. Therefore, in our laboratory, it is standard practice to check that a separation achieved by programming the ternary pump can also be obtained by mixing the same proportions of solvents manually. Once a suitable mobile phase is found for a separation, all subsequent validation work and sample analysis is done using a mobile phase that is mixed manually.

Up until this point, the mobile phase had been mixed by programming the ternary pumping system. Because a fairly good separation had been achieved with 60/40 buffer/ACN, the mobile phase was mixed by hand to see if any differences occurred.

g) 60/40 buffer/ACN (mixed by hand)

The retention time of VI was slightly increased but the system was still fairly good. (Figure 29)

Figure 29. Chromatogram of pindolol and related compounds using 60/40 buffer/ACN (mixed by hand) as the mobile phase



h) 58/42 buffer/ACN (mixed by hand)

A small increase in ACN concentration to 42% caused IV to co-elute with VI. (Figure 30)

i) 65/35 buffer/ACN (mixed by hand)

All the known impurities were well resolved from the drug, although two (III and VII) were not well resolved from each other. A shoulder and three peaks from unknowns were visible on the tail of the drug peak. (Figure 31)

j) 62/38 buffer/ACN (mixed by hand)

This system produced a loss of resolution between the unknown compounds and the drug. (Figure 32)

Figure 30. Chromatogram of pindolol and related compounds using 58/42 buffer/ACN (mixed by hand) as the mobile phase

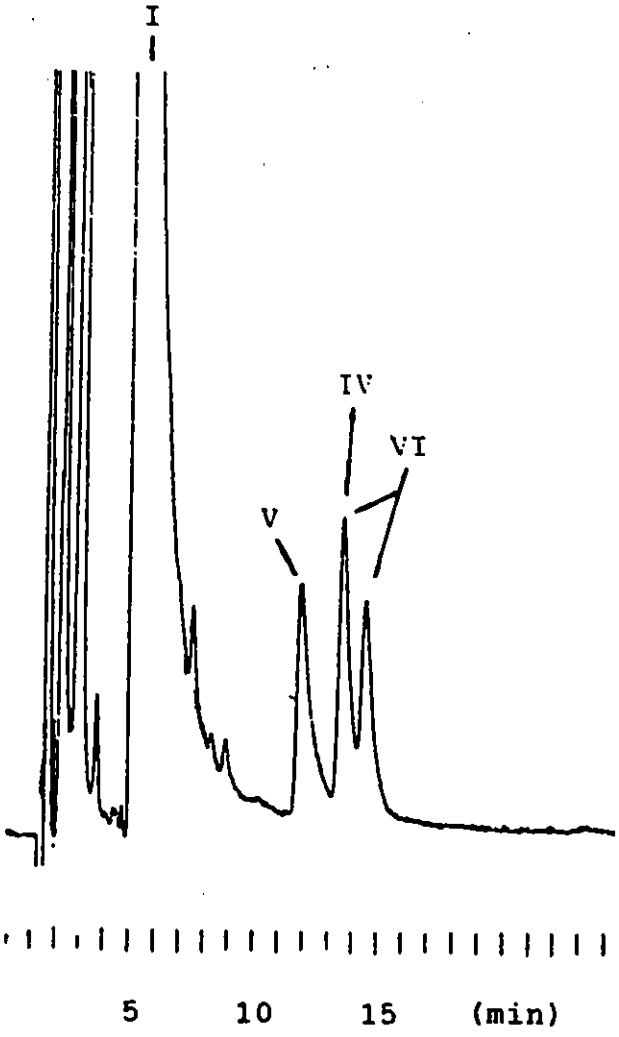


Figure 31. Chromatogram of pindolol and related compounds using 65/35 buffer/ACN (mixed by hand) as the mobile phase

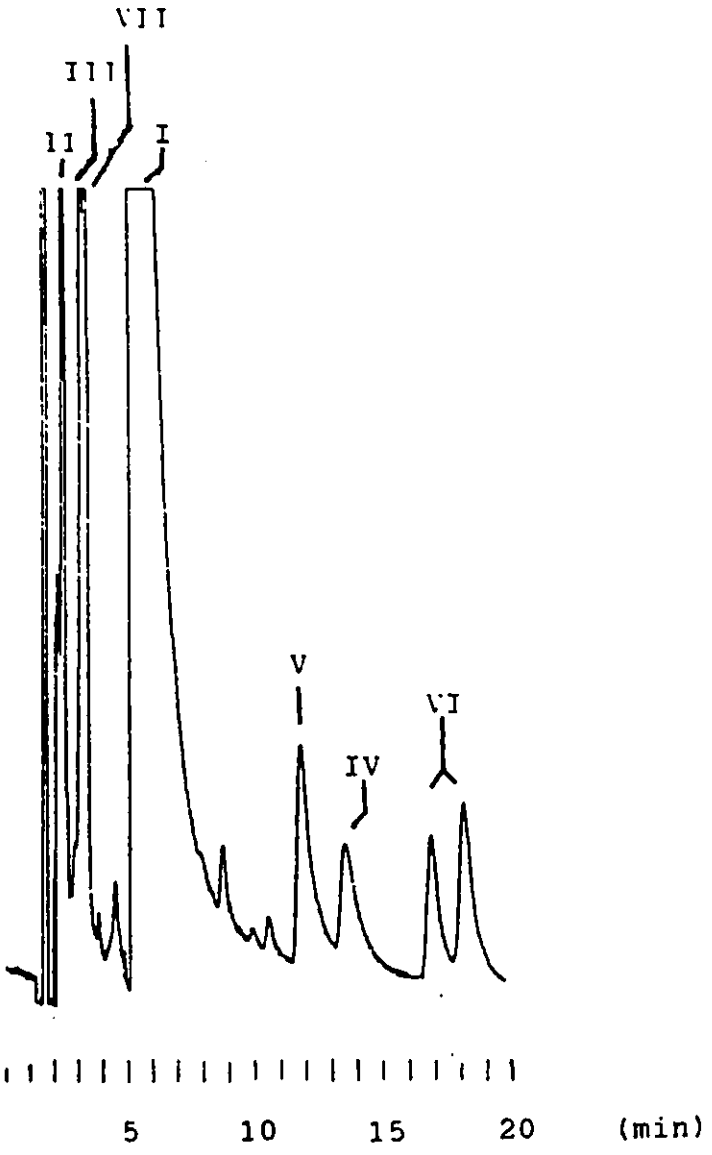
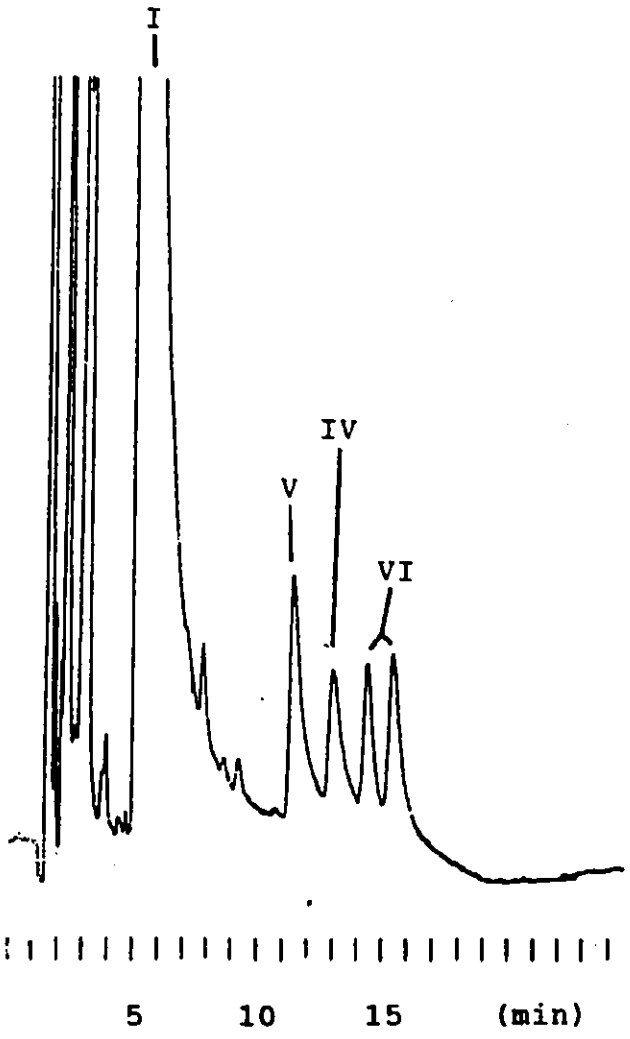


Figure 32. Chromatogram of pindolol and related compounds using 62/38 buffer/ACN (mixed by hand) as the mobile phase



Up to this point the 65/35 buffer/ACN combination was the best because it resolved the most compounds from the drug. Several systems with methanol in the mobile phase were evaluated to see if a better separation could be achieved.

k) 60/35/5 buffer/acetonitrile/methanol (mixed by hand)

This mobile phase produced poorer resolution between the unknown compounds and the drug than the 65/35 buffer/ACN mobile phase. There was also co-elution of IV and VI. (Figure 33)

l) 70/25/5 buffer/acetonitrile/methanol (mixed by hand)

There was coelution of IV and V and the unknowns on the tail of the drug peak. There was also an unresolved shoulder on the tail of the drug peak. (Figure 34)

Figure 33. Chromatogram of pindolol and related compounds using 60/35/5 buffer/acetonitrile/methanol (mixed by hand) as the mobile phase

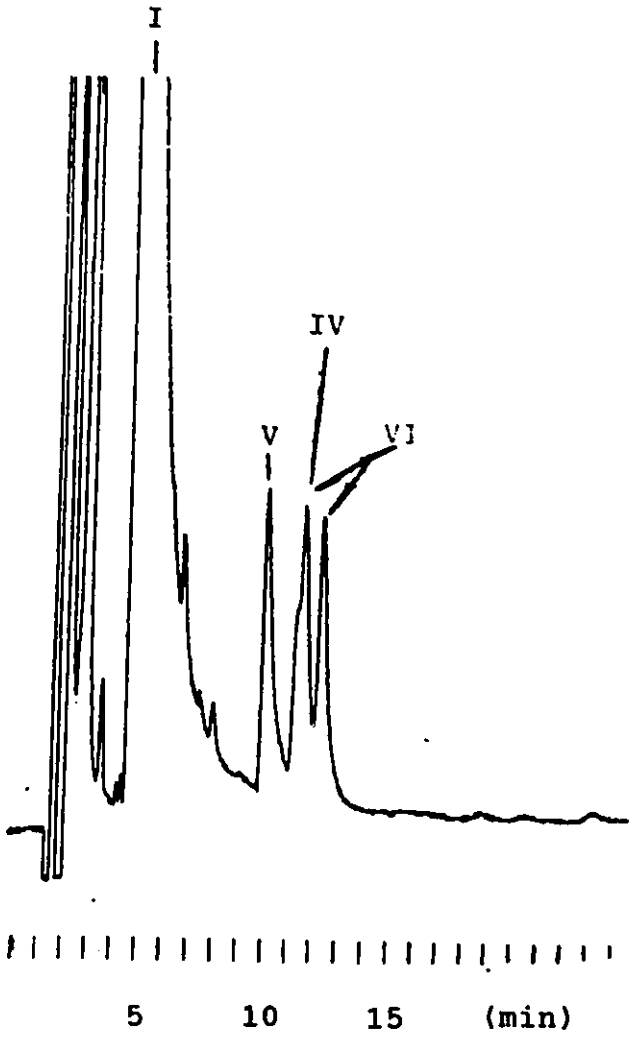
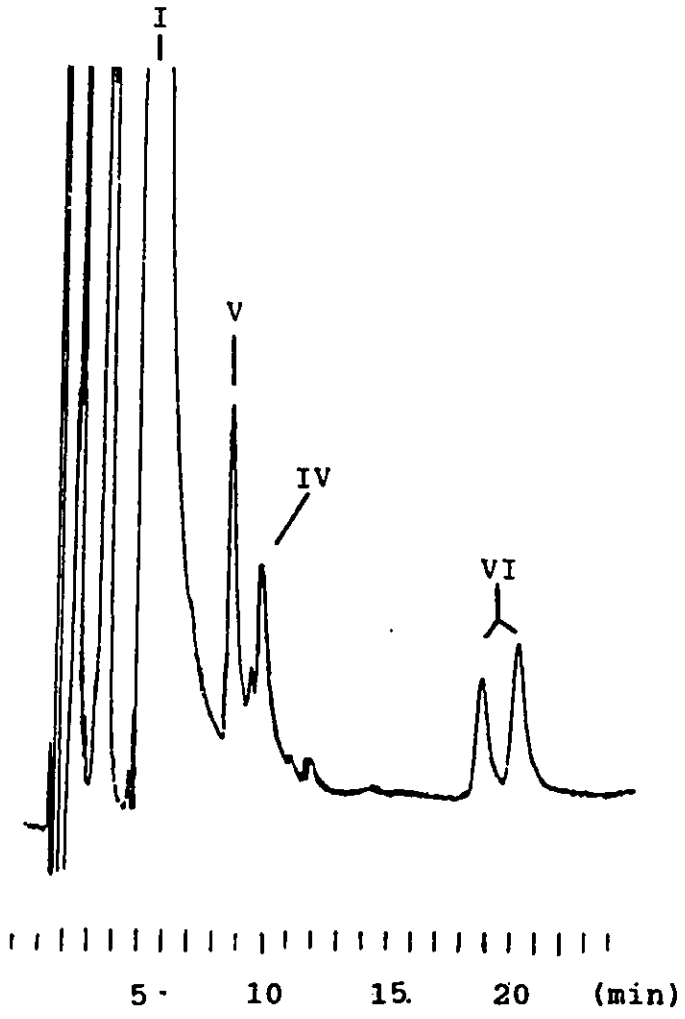


Figure 34. Chromatogram of pindolol and related compounds using 70/25/5 buffer/acetonitrile/methanol (mixed by hand) as the mobile phase



m) 70/20/10 buffer/acetonitrile/methanol (mixed by hand)

IV and V were closer to the drug peak than with the previous mobile phase. There was still a shoulder on the tail of the drug peak. (Figure 35)

Of those evaluated, the best overall was the 65/35 buffer/acetonitrile system and it was therefore chosen as the basis of the method. The retention times of the related compounds relative to pindolol on this system are given in Table 3.

Figure 35. Chromatogram of pindolol and related compounds using 70/20/10 buffer/acetonitrile/methanol (mixed by hand) as the mobile phase

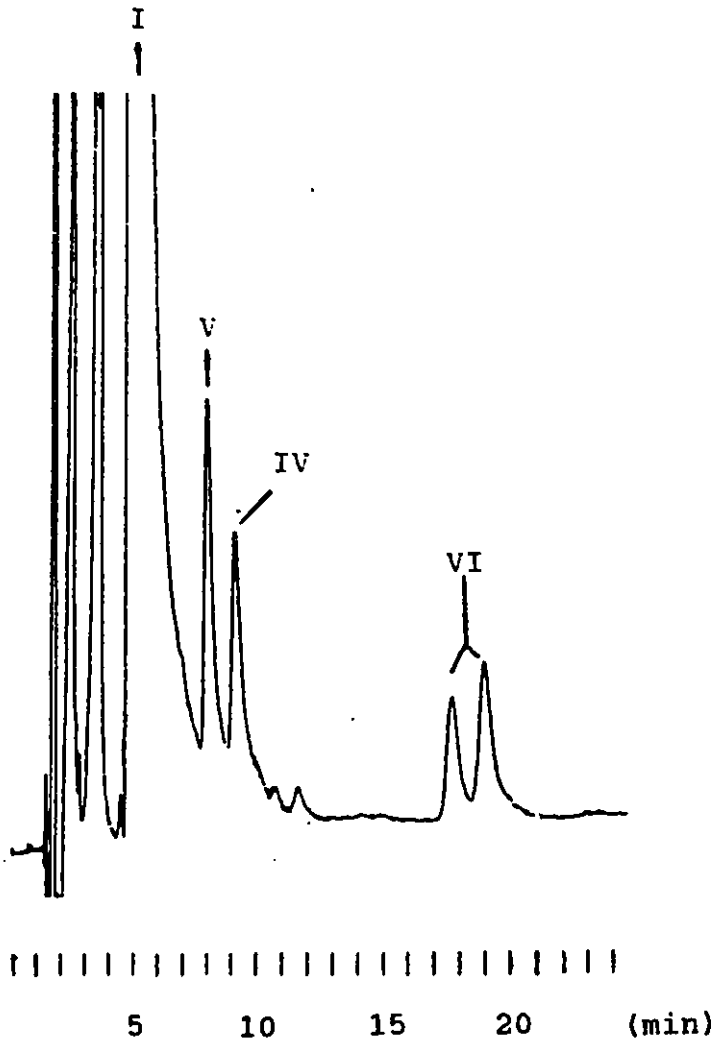


Table 3. Response of LC System to Pindolol and Related Compounds

Compound	RRT[1]	Min. Det.[2] %	Min. Quant[3] %	Slope[4] (Area/μg)	RSQ[5]	Rel. Resp.[6]
I	1.00	0.0025	0.005	1619000	0.947	1.00
II	0.46	0.001	0.002	4471000	0.977	2.76
III	0.60	0.001	0.002	2614000	1.000	1.61
IV	2.6	0.01	0.02	1564000	0.999	0.97
V	2.2	0.01	0.02	1529000	0.996	0.94
VI[7]	3.2	0.01	0.02	804000	1.000	0.50
	3.5			1211000	0.999	0.75
VII	0.64	0.001	0.002	3158000	0.998	1.95

[1] Retention time relative to pindolol

[2] Minimum detectable amount is 2X baseline noise, based on a 10 μg injection of pindolol

[3] Minimum quantifiable amount is 4X baseline noise, based on a 10 μg injection of pindolol

[4] Slope of the analytical response curve from the minimum quantifiable amount to the 5% level.

[5] Square of the correlation coefficient

[6] Response relative to pindolol

[7] This compound is a mixture of diastereomers

4. Linearity and Sensitivity

The response of the HPLC system to pindolol and the six related compounds was determined for levels ranging from the minimum quantifiable amount to 5% (0.5 μg on column). Duplicate weighings with dilutions were prepared for each compound. The results were analyzed by linear regression and are summarized in Table 3. The data and graphs are in Appendix I.

Table 3 also gives the minimum detectable and quantifiable amounts of pindolol and each related compound. The limits of detection of the HPLC method are lower than those of the USP TLC method by a factor of 5 to 50, depending on the impurity.

The response of the HPLC system to pindolol over the range of 50 to 150% of the assay concentration was also found to be linear with a slope of 1420000 area counts/μg on column (R^2 = 0.997).

Figure 36. Chromatogram showing the resolution of pindolol (I) at 1 mg/mL and the six known impurities at about 0.05 mg/mL. Column: 150 x 4.6 mm 3 µm Spherisorb nitrile (CSC No. 068808)

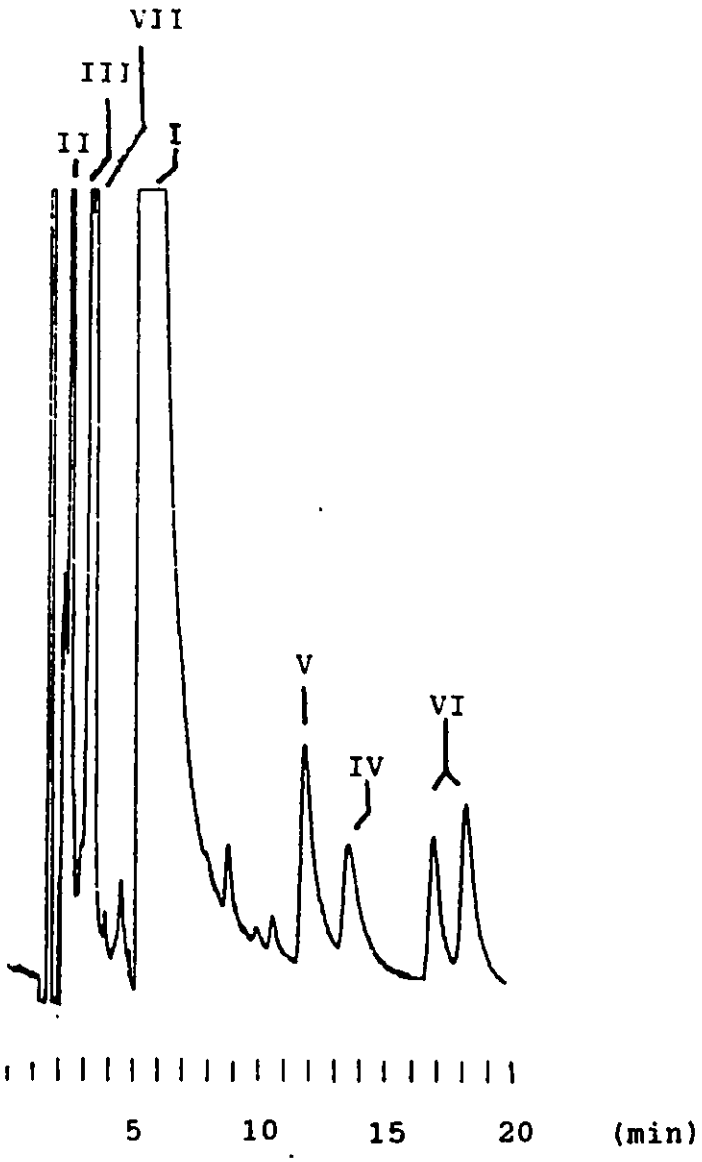
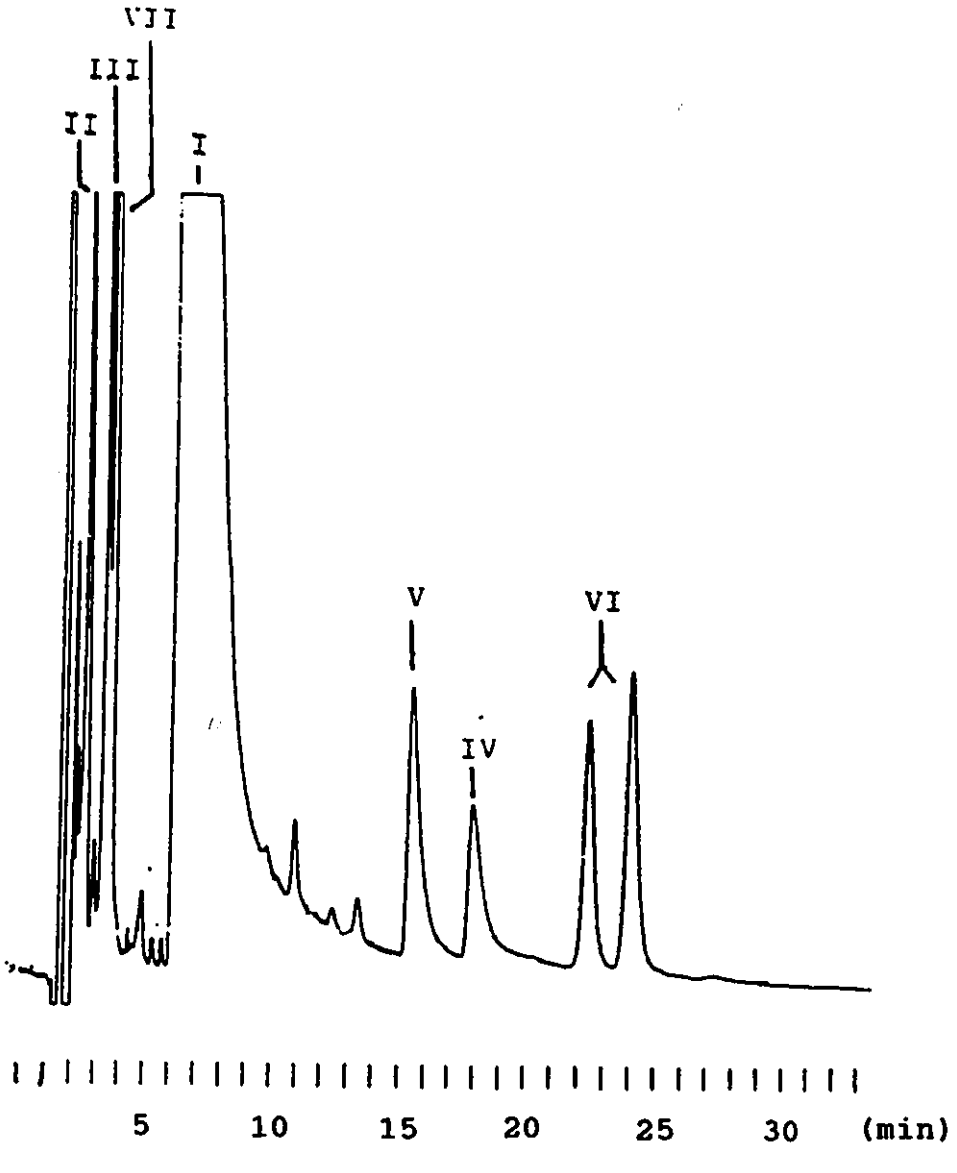


Figure 37. Chromatogram showing the resolution of pindolol (I) at 1 mg/mL and the six known impurities at about 0.05 mg/mL. Column: 150 x 4.6 mm 3 µm Spherisorb nitrile (Alltech No. 101088B-P)



5. Precision

The precision of the system for the assay method was determined by making six replicate injections of the standard solution (approximately 1.0 mg/mL, accurately known) and determining the relative standard deviation (RSD) of the peak responses. The relative standard deviations obtained on four different days were 0.27%, 0.35%, 0.12% and 0.10%. Debesis et al.(8) have proposed a maximum allowable RSD of 0.58% for an assay range of 98.5-101.5% when duplicate determinations are carried out.

6. Choice of System Suitability Parameters

The system suitability test requires the use of pindolol and indole (VII) which elutes before the drug. The resolution between indole and pindolol and the efficiency of the column were monitored during method development. The results obtained are listed in Table 4. When a new column from a different manufacturer (Alltech) was used, better resolution, higher efficiency and longer retention times were observed (Figure 37). This second column was used to validate the method, after the method had been developed and the samples analyzed on the first column. The retention time of pindolol was about 5.2 to 5.9 on the original column

and 6.5 to 7.6 on the second column. These ranges reflect changes over several weeks; for a given day, the variation was of the order of 0.1 minute. The choice of a maximum allowable RSD of 5% on the peak responses for the system suitability test for the related substances method was adequate for this type of method and for a 0.005 mg/mL solution, corresponding to only 0.5% of the concentration of the drug in the test solution. For the assay, however, where a greater precision is required, the RSD for 5 injections of the 1 mg/mL standard solution should be less than 0.6%.

Table 4. System Suitability Parameters[1]

Column #	Date	Retention Time (min)		Resolution[2]	Efficiency[3] (plates/m)	RSD[4] %
		I	VII			
068808	14-11-88	5.62	3.03	13.1	71,200	1.5
	13-12-88	5.45	3.04	7.8	22,900	2.7
	21-12-88	5.35	2.87	10.4	36,800	1.8
	04-01-89	5.56	2.98	10.8	41,200	1.6
	06-01-89	5.38	2.83	11.5	44,400	1.6
101088B-P	17-11-88	6.56	3.59	13.5	136,300	2.5

[1] Based on six injections of the system suitability solution

[2] Resolution between indole and pindolol

[3] Calculated using the pindolol peak

[4] Relative standard deviation of the response to pindolol

7. Stability of Solutions

Methanol was used throughout method development as solvent for the drug and related compound standards. It was necessary, however, to sonicate the test solutions to achieve complete dissolution. Chromatograms of pindolol test solutions injected over a 24 hour period showed no indication of degradation.

8. Ruggedness

As the column aged, the relative retention times of the compounds eluting after pindolol increased. The column could be regenerated by running several column volumes of acetonitrile through it.

New Methods

1. Method for Related Compounds in Pindolol Raw Materials

Equipment: Use a liquid chromatograph equipped with a detector set at 219 nm, a suitable integrator and an injector or autosampler capable of delivering a volume of 10 µL. Use a 15 cm x 4.6 mm column packed with 3 µm Spherisorb nitrile packing.

Solutions: Buffer - 0.05 M sodium acetate adjusted to pH 5.0 with 0.05 M acetic acid.
System suitability solution - 0.005 mg/mL pindolol (accurately known) and 0.005 mg/mL indole in methanol.
Test solution - Transfer quantitatively approximately 100 mg (accurately weighed) pindolol raw material to a 100 mL volumetric flask, add about 90 mL of methanol, dissolve the sample by placing in an ultrasonic bath for 5 minutes, cool, and add methanol to volume.

Mobile Phase: Buffer:acetonitrile : 65:35 parts by volume. Filter through a 0.45 µm filter. The flow rate is 1 mL/min.

System Suitability: Inject six 10 µL aliquots of the system suitability solution. The resolution between pindolol and indole is not less than 7 and the efficiency of the column calculated using the pindolol peak is not less than 20,000 plates/m. The relative standard deviation of the peak responses is not more than 5%. The retention times of indole and pindolol are typically 2.5 to 4.5 and 5.0 to 8.0 minutes, respectively.

Procedure: Separately inject 10 µL of the system suitability and test solutions into the chromatograph and run for 30 minutes. Calculate the amount of each impurity in the test solution as a percentage of the total amount of drug using the formula $100(A_u/A_s)(C_s/C_u)$ where A_u is the peak area due to the individual impurity, A_s is the area of the pindolol peak in the system suitability solution, and C_s and C_u are the concentrations of pindolol in the system suitability solution and the test solution, respectively.

2. Method for Assay of Pindolol Raw Materials

Dry reference standard and raw materials at 105°C for 4 hours.

Equipment: As for Method 1.

Solutions: Buffer and Test Solution - prepare as for Method 1.

Standard solution - Transfer quantitatively approximately 100 mg (accurately weighed) pindolol reference standard to a 100 mL volumetric flask, add about 90 mL of methanol, dissolve the sample by placing in an ultrasonic bath for 5 minutes, cool and add methanol to volume.

Mobile Phase: As for Method 1.

System Suitability: Inject two 10-μL aliquots of the system suitability solution. The resolution between pindolol and indole is not less than 7 and the efficiency of the column calculated using the pindolol peak is not less than 20,000 plates/m. The retention times of indole and pindolol are typically 2.5 to 4.5 and 5.0 to 8.0 minutes, respectively. In addition, inject five 10-μL aliquots of the standard solution. The relative standard deviation of the peak responses is not more than 0.6%.

Procedure: Separately inject 10 µL of the standard and test solutions into the chromatograph and run for 30 minutes. Calculate the percentage of pindolol using the formula $100(A_u/A_s)(C_s/C_u)$ where A_u and A_s are the areas of the pindolol peak in the test and standard solutions, respectively, and C_s and C_u are the concentrations of pindolol in the standard and test solutions, respectively.

Results of Sample Analysis

1. Related Compounds in Pindolol Raw Materials

Ten samples of pindolol raw material were analyzed in duplicate for related compounds according to Method 1. The results are given in Table 5. Quantitation was by comparison of peak areas to the area of a peak due to a pindolol in-house standard. All samples meet the purity requirements of the USP and BP.

Table 5. Related Compounds in Pindolol Raw Materials (%)

Lot	A	B	C	D	E	F	G	H	I	J[1]
Co.[2]	1	1	2	2	2	2	2	2	3	
RRT[3]										
0.54[4]			tr[5]	tr	tr			0.04		
0.66	0.01	0.01	0.01	0.01	0.01	0.01	tr	tr	0.01	
0.69							tr		tr	0.01
0.74		0.01				0.04	0.01	tr	0.02	0.04
0.79	0.02		0.04	0.04	0.04					
0.92	0.05	0.02	0.01	0.01	0.01	0.03	tr	tr	0.02	0.01
1.50				tr	tr				0.01	tr
1.6	tr	0.01	0.01	0.01	0.01	0.01	0.02	tr		
1.9		tr	tr	tr	tr	tr		0.16		
2.0									0.01	
2.2	tr		tr	tr	tr			0.04		
2.9		tr	tr	tr	0.04	tr				
3.1		tr				tr				
Total	0.08	0.08	0.07	0.07	0.11	0.09	0.03	0.24	0.07	0.06

[1] Raw material J is USP reference standard pindolol (Lot F)

[2] Designates the company that synthesized the raw material.

[3] Retention time relative to pindolol at about 5.2 minutes.

[4] May be compound III or VII, based on retention time.

[5] Trace (tr) is less than the minimum quantifiable amount.

2. Assay of Pindolol Raw Materials

The results of drug content determination using Method 2 are given in Table 6. The USP potentiometric titration assay method had been used to analyze several of these samples previously. These results are also reported in Table 6.

Table 6. Assay by HPLC and Potentiometric Titration (%)

Code	HPLC[1]	Potentiometric Titration[2]
A	100.2, 99.9	99.0, 99.1
B	100.5, 100.7	
C	100.4, 100.2	
D	99.8, 100.7	
E	100.1, 99.8	
F	100.3, 100.1	100.2, 100.1
G	99.6, 99.6	100.0, 100.2
H	100.1, 99.8	100.1, 100.1
I	100.4, 100.9	100.7, 100.3
J	100.8, 100.6	

[1] Samples were analyzed in December 1988 and January 1989. Sample A was used as in-house reference standard.

[2] Samples were analyzed in November, 1987.

Internal Evaluation of the New Methods

The methods were evaluated by an analyst who had not worked on their development. Results obtained by this analyst for two lots of raw materials are given in Table 7. The related substances determination was done on a similar chromatograph as the one that was used to develop the methods, whereas the assay results were obtained using the chromatograph that was used for method development.

His results were similar to those obtained during method development.

Table 7. Results of Pindolol Analysis by a Second Analyst

Related Substances		
RRT[1]	H	I
0.39		tr
0.64		tr
0.68	tr	tr
0.85		tr
0.91	tr	
1.9		0.02
2.5	0.13	0.01
3.0	0.04	
Total	0.17	0.03
Assay		
1st Weighing	100.0%	99.5%
2nd Weighing	101.1%	99.9%
Mean	100.6%	99.7%

[1] Retention time relative to pindolol.

The results this analyst obtained for the system suitability tests are in Table 8. This analyst had a lower system precision on his chromatograph, as can be seen from the RDS values for the pindolol peak in the system suitability solution (Table 8) and those he obtained for the standard solution: 1.04%, 0.92%. However, when he used the chromatograph on which the method was developed, he obtained an RSD 1.7% on the area of the pindolol peak in the system suitability solution and an RSD of 0.11% for six injections of the assay standard solution.

Table 8. System Suitability Results from 2nd Analyst[1]

Column #	Date	Retention Time (min)		Resolution[2]	Efficiency[3] (plates/m)	RSD[4] %
		I	VII			
101088B-P	20-02-89	7.54	4.47	10.9	71,100	4.3
	22-02-89	7.35	4.23	10.2	53,800	3.7
	07-03-89	7.55	4.10	10.7	52,192	4.5
068808	08-03-89	5.89	2.76	12.1	33,200	2.1
101088B-P[5]	14-03-89	7.59	4.03	12.1	51,900	1.7

[1] Based on six injections of the system suitability solution

[2] Resolution between indole and pindolol

[3] Calculated using the pindolol peak

[4] Relative standard deviation of the response to pindolol

[5] Analysis done on same chromatograph used for method development.

Discussion

The HPLC method gives only a partial resolution of impurities III and VII. However, this was not judged a serious shortcoming of the method because impurity VII occurs early in the synthetic route and is not very likely to be present in samples of raw materials. Impurity III is more likely to occur because it is an immediate precursor of the drug.

The system suitability solution is the same for both the related substances method and the assay method and the concentration of the test solutions for each method is 1 mg/mL, making it relatively easy to perform both analyses on the same day for a given set of samples.

Impurity levels in pindolol raw materials were determined by comparison to a pindolol standard. Because absorptivities are not equal and several compounds have a lower response relative to pindolol (Table 3), this could lead to an overestimation of compounds II, III and VII by about 180%, 60%, and 95%, respectively and a slight underestimation of IV and V. VI would probably also be underestimated by as much as 50% because each peak produced by this pair of diastereomers would be interpreted as a single compound unless a standard of this compound was available.

Pharmacopeial methods do not normally require quantitation of ordinary impurities against individual standards of related compounds. This would unnecessarily increase the time and costs of analyses. Instead, the sensitivity of the related compounds relative to the drug is taken into consideration when the limits on impurities are established.

EXPERIMENTAL

Equipment

The HPLC system (Varian 5560) was fitted with a 10 μL loop, an autosampler (Varian Model 8085), a variable wavelength detector set at 219 nm (Varian UV-200) and a data station (Varian 402). The second analyst used a different HPLC of the same make and model number. A Chromatographic Science Company (CSC) 3μm Spherisorb nitrile column 150 x 4.6 mm (No. 068808) was used for method development and an Alltech 3 μm Spherisorb nitrile column 150 x 4.6 mm (No. 101088B-P) was used for the internal evaluation of the method.

Other equipment used was a UV/VIS spectrophotometer (Varian DMS 90) connected to a computer (Hewlett-Packard HP-85) and a plotter (Hewlett-Packard HP 7470A), and an autotitrator (Mettler DL40RC Memotitrator) equipped with a 5 mL buret (DV 405) and a combination glass electrode (DG 112). Deionized water was obtained using a Sybron/Barnstead water purification system.

Chemicals and Supplies

Methanol, acetic acid, acetonitrile tetrahydrofuran and hexane: all HPLC grade (Baker, Phillipsburg, NJ). Sodium acetate, HPLC grade (Fisher Scientific, Fair Lawn, NJ). Hydrochloric acid (Anachemia Chemicals, Champlain, NY), methyl red (BDH, Toronto, Ontario), ethanol (Consolidated Alcohols, Toronto, Ontario), sodium carbonate (BDH, Poole, England). All mobile phase solvents were filtered though 0.2μ Nylon 66 filters (Pall Trinity Microcorp, Cortland, NY)

Pindolol related compounds were obtained as follows: II (Aldrich, Milwaukee, WI), II, III and VII (AMSA, Milan, Italy), IV, V and VI (Sandoz, Dorval, Quebec). Pindolol raw materials were obtained directly from manufacturers or were submitted to the Health Protection Branch in connection with New Drug Submissions. Information regarding the source of these materials has been removed in order to protect the confidentiality of this information.

UV Spectra

The spectrophotometer was operated with baseline correction in the double beam mode at a scan rate of 50 nm/min. The range scanned was 200 to 350 nm.

Solutions of pindolol and each related compound at a concentration of about 12.5 μg/mL were prepared in methanol. The UV spectrum and a printout of the absorbance were obtained for each solution.

Evaluation of the HPLC Column

A new column was used for method development: a 150 x 4.6 mm column (No. 068808) from Chromatographic Science Company (CSC) containing 3μm Spherisorb nitrile packing. This column was evaluated according to the manufacturer's specifications.

Mobile phase: 1% methanol in hexane filtered and degassed.
Flow rate: 1 mL/min
Detector wavelength: 254 nm
Test solution: toluene, nitrobenzene and di-n-butyl phthalate, 433, 24 and 834 μg/mL, respectively, in hexane.

The efficiency was determined, using the nitrobenzene peak and was found to be 79,900 plates per meter. The efficiency reported by the manufacturer was 104,700 plates per meter.

Investigation of Chromatographic Systems

A test mixture containing the drug at about 1 mg/mL and each of the impurities at 0.05 mg/mL was prepared using methanol as solvent. Beginning with the original propranolol mobile phase and flow rate, and a 150 x 4.6 mm column containing 3μm Spherisorb nitrile packing (No. 068808), the system was modified and the mobile phase compositions listed below were evaluated. Mixing of the components of the mobile phase was accomplished automatically with the aid of the ternary pumping system, except in the cases where hand mixing was used. The mobile phases were filtered and degassed prior to use. The flow rate in each case was 1 mL/min and the detector was set at 219 nm.

1) 70/15/15 buffer/ACN/THF (original propranolol system)
2) 70/10/20 buffer/ACN/THF
3) 70/20/10 buffer/ACN/THF
4) 70/30/0 buffer/ACN/THF
5) 75/25/0 buffer/ACN/THF
6) 60/40/0 buffer/ACN/THF
7) 60/40 buffer/ACN (mixed by hand)
8) 58/42 buffer/ACN (mixed by hand)
9) 65/35 buffer/ACN (mixed by hand)
10) 62/38 buffer/ACN (mixed by hand)
11) 60/35/5 buffer/acetonitrile/methanol (mixed by hand)

12) 70/25/5 buffer/acetonitrile/methanol (mixed by hand)

13) 70/20/10 buffer/acetonitrile/methanol (mixed by hand)

Chromatographic Conditions

Unless otherwise specified, the following system was used:

Column: 150 x 4.6 mm column (No. 068808) from Chromatographic Science Company (CSC) containing 3μm Spherisorb nitrile packing

Mobile phase: 65/35 (V/V) sodium acetate buffer 0.05 M adjusted to pH 5.0 with 0.05 M acetic acid/acetonitrile filtered and degassed.

Flow rate: 1 mL/min

Detector: 219 nm

Linearity, Sensitivity, Limits of Detection and Quantitation and Stability of Solutions

A) Linearity and Sensitivity at low concentrations, Limits of Detection and Quantitation and Stability of Solutions

Two stock solutions of the drug and each related substances were prepared as follows.

Stock A: About 50 mg (accurately known) was transferred to a 50 mL volumetric flask, dissolved in methanol and made up to volume. (Concentration: 1 mg/mL)

Stock B: About 50 mg (accurately known) was transferred to a 100 mL volumetric flask, dissolved in methanol and made up to volume. (Concentration: 0.5 mg/mL)

Dilutions of Stock A having the following concentrations were prepared: 0.1, 0.02, 0.01, 0.002, 0.001, 0.0002, 0.0001 mg/mL.

Dilutions of Stock B having the following concentrations were prepared: 0.05, 0.01, 0.005, 0.001, 0.0005, 0.0001, 0.00005 mg/mL.

The samples were run in the following order (2 injections/sample):

1) Test mix containing the drug at 1 mg/mL and each impurity at 0.05 mg/mL. This sample was run to see if the efficiency of the column was still adequate to resolve the unknown compounds on the tail of the peak.

2) Stock A

3) Dilutions of Stock A and B from the least to the most concentrated.

4) Stock A

Chromatograms of Stock A run near the beginning of the runs and at the end were examined to determine if degradation of the sample had occurred during analysis time (usually more than 8 hours).

The chromatograms were examined to determine the minimum detectable and minimum quantifiable amount.

The results were examined by linear regression. The slope of the analytical response curve was determined.

B) Linearity of pindolol in the assay concentration range

Solutions of pindolol in methanol, spanning the concentration range of 0.5 to 1.5 mg/mL were prepared and run on the system. The response to pindolol was measured in area counts. The results were examined by linear regression.

Precision of the System

Six injections of a 1 mg/mL solution of pindolol (accurately known) were run on the system. The mean area count and relative standard deviation were determined.

Monitoring of System Suitability Parameters

The system suitability solution was prepared as follows:

1) Stock indole solution: approximately 10.00 mg indole (accurately known) were transferred to a 10 mL volumetric flask, dissolved and diluted to volume with methanol.

2) Pindolol standard solution: approximately 50.00 mg pindolol standard (accurately known) were transferred to a 50 mL volumetric flask and about 40 mL methanol were added. The sample was sonicated for 5 minutes to dissolve the pindolol then cooled and diluted to volume with methanol.

3) Stock system suitability solution: 5 mL of the Stock indole solution and 5 mL of the pindolol standard solution were transferred to a 50 mL volumetric flask and diluted to volume with methanol.

4) System suitability solution: 5 mL of the stock system suitability solution were transferred to a 100 mL volumetric flask and diluted to volume with methanol.

Fresh solutions were prepared each day. System suitability solutions were run at various times during method development and before sample analysis, and on both columns.

Six injections of the system suitability solution were made. The retention times of pindolol and indole were monitored and the resolution between these two compounds was calculated. The efficiency of the column was calculated, using the pindolol peak. The relative standard deviation of the pindolol peak area was determined for six injections.

Evaluation of Second Column

The second column (Alltech 150 x 4.6 mm column containing 3 μm Spherisorb nitrile packing (No. 101088B-P)) was evaluated according to the manufacturer's specifications:

mobile phase: 5% ethanol in hexane
flow rate: 1 mL/min
injection size: 5 μL
detector wavelength: 254 nm

Test mixture: toluene, diethyl phthalate, dimethyl phthalate all at 1 mg/mL mobile phase (supplied with the column).

The efficiency was calculated from each of the peaks in the chromatogram. Efficiencies of 72900, 96100 and 98600 were obtained using the toluene, diethyl phthalate and dimethyl phthalate peaks, respectively. The manufacturer's results were as follows: 82500, 94900 and 94100, respectively.

The column was then evaluated using the system suitability test for pindolol.

Sample Analysis

Pindolol raw materials and standard were dried 4 hours at 105°C prior to use. Sample A was used as in-house reference standard.

Solutions prepared:

1) Stock indole solution: approximately 10.00 mg indole (accurately known) were transferred to a 10 mL volumetric flask, dissolved and diluted to volume with methanol.

2) Pindolol standard solution: approximately 50.00 mg pindolol standard (accurately known) were transferred to a 50 mL volumetric flask and about 40 mL methanol were added. The sample was sonicated for 5 minutes to dissolve the pindolol then cooled and diluted to volume with methanol.

3) Stock system suitability solution: 5 mL of the Stock indole solution and 5 mL of the pindolol standard solution were transferred to a 50 mL volumetric flask and diluted to volume with methanol.

4) System suitability solution: 5 mL of the stock system suitability solution were transferred to a 100 mL volumetric flask and diluted to volume with methanol.

5) Pindolol test solutions: approximately 50.00 mg pindolol (accurately known) were transferred to a 50 mL volumetric flask and about 40 mL methanol were added. The sample was sonicated for 5 minutes to dissolve the pindolol then cooled and diluted to volume with methanol. Duplicate weighings of each sample were analyzed.

The samples were usually run on the system in the following order (2 injections/vial): methanol blank, 3 vials of system suitability solution, 3 vials of standard solution, then alternating: sample, system suitability, sample, standard, etc.

Assay by Titration

The sodium carbonate standard was heated to 230°C for at least one hour prior to use. The pindolol samples were heated to 105°C for four hours prior to use.

The methyl red solution was prepared by transferring 9.83 mg to a 10 mL volumetric flask, dissolving with ethanol to volume.

The increment size on the titrator was set to 0.0025 mL.

1) Standardization of 0.1 N HCl

Approximately 10.00 mg sodium carbonate (accurately weighed) were transferred to a titration flask and dissolved in 50 mL of water. Two drops of methyl red TS were added. The solution was titrated with 0.1 N HCl and the end point was determined potentiometrically. The analysis was performed in duplicate. A blank was also titrated.

2) Assay of pindolol raw materials

Approximately 50.00 mg pindolol (accurately weighed) were transferred to a titration flask and dissolved in 50 mL of methanol. The solution was titrated with 0.1 N HCl and the end point was determined potentiometrically. The analysis was performed in duplicate. A blank was also titrated.

Intra-laboratory evaluation of the method

This work was done by N. Curran. Two HPLC systems were used:
the related substances determination was done on a system which was the same make and model as the one used for method development whereas the sample assays were carried out on the system used for method development. The column was an Alltech 3 μm Spherisorb nitrile column 150 x 4.6 mm (No. 101088B-P).

The chromatographic conditions were as described above. The samples and standard for the assay were dried under vacuum at 105°C for 4 hours prior to use.

Solutions were prepared as follows:

Stock indole and pindolol solutions: 25 mg (accurately known) of each compound were transferred to separate 50 mL volumetric flasks, dissolved and diluted to volume with methanol.

System suitability solution: 1 mL of each of the stock indole and pindolol solutions was transferred to a 100 mL volumetric flask and diluted to volume with methanol.

Test solutions: About 100 mg pindolol (accurately known) were transferred to a 100 ml volumetric flask. Approximately 90 mL of methanol were added and the sample was sonicated for 5 minutes. It was then diluted to volume with methanol.

Standard solution for assay: Approximately 100 mg pindolol standard (accurately weighed) was transferred to a 100 mL volumetric flask. About 90 mL of methanol were added and the sample was sonicated for 5 minutes. The sample was diluted to volume with methanol.

The related substances determination was carried out first. After several unsuccessful attempts to obtain a low enough RSD for six injections of the assay standard on his chromatograph, he then used the chromatograph on which the method was originally developed and, having met all the system suitability requirements, proceeded with the assay of the samples.

APPENDIX I

This appendix contains hard copies of the regression analysis of the raw data used to produce the values for the slope and square of the correlation coefficient given in Table 3, and the corresponding response curves for each of the available impurities. The computer program used for the regression analysis was Lotus 1-2-3, Release 2.01. Sigma-Plot, Version 3.1 was used to plot the response curves.

Figure 38. Regression Analysis for Pindolol (I)

Amt (ug)	Area	Pindolol Linearity		
0.0004920	2241	Regression Output:		
0.0004920	986	Constant		19079
0.0009840	3015	Std Err of Y Est		53132
0.0009840	2571	R Squared		0.947
0.0009856	3450	No. of Observations		26
0.0009856	3088	Degrees of Freedom		24
0.0019710	4208			
0.0019710	3901	X Coefficient(s)	1619214	
0.0049200	6334	Std Err of Coef.	78534	
0.0049200	7382			
0.0098400	17594			
0.0098400	20740			
0.0098560	23224			
0.0098560	24242			
0.0197100	49757			
0.0197100	51215			
0.0492000	64678			
0.0492000	62987			
0.0984000	184165			
0.0984000	189926			
0.0985600	246404			
0.0985600	254042			
0.1971000	478556			
0.1971000	484751			
0.4920000	748627			
0.4920000	744634			

Figure 39. Analytical Response Curve for Pindolol (I)

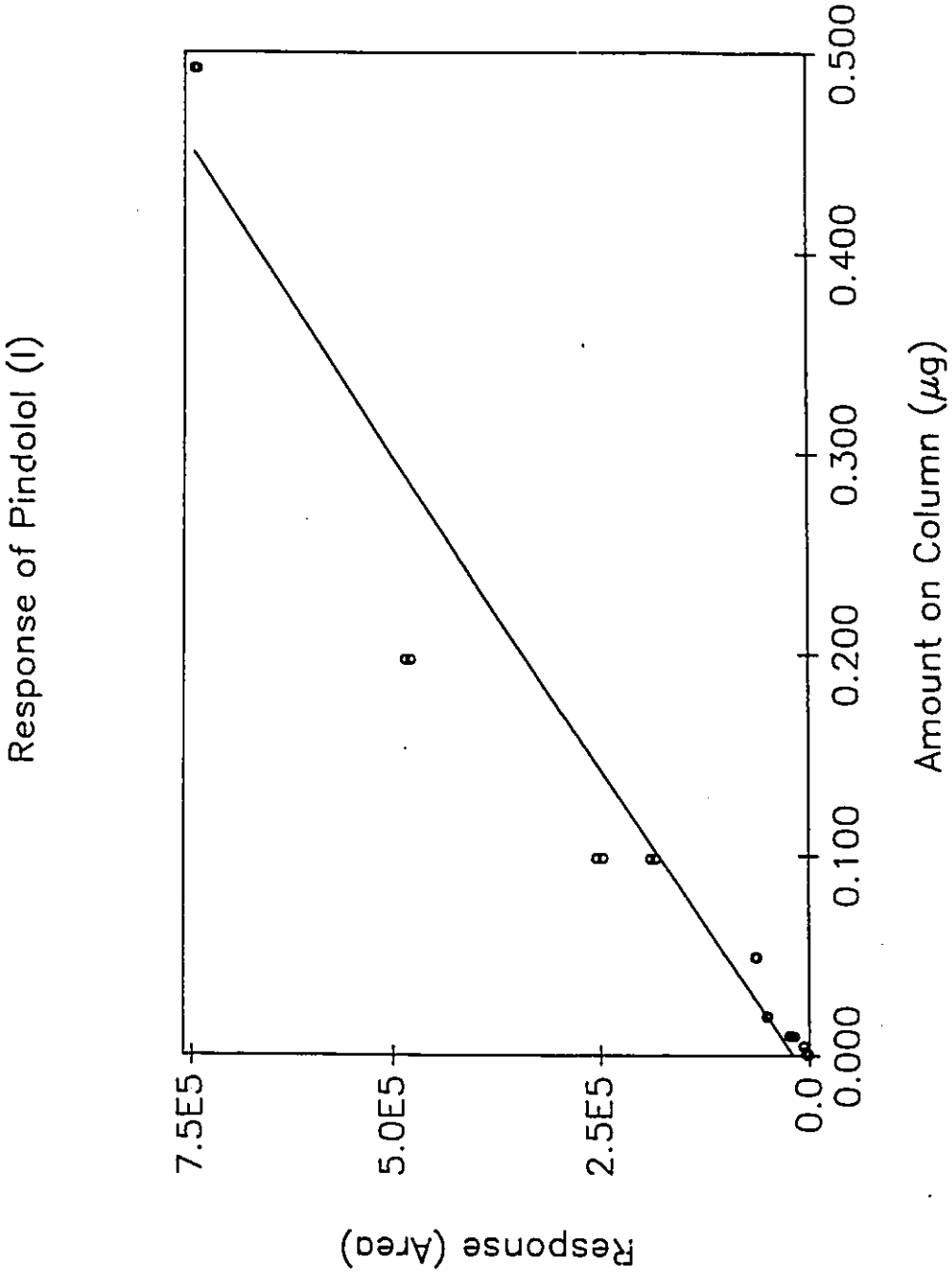


Figure 40. Regression Analysis for Impurity II

Amt (ug)	Area
0.0001962	76900
0.0001962	80621
0.0004810	66974
0.0004810	72545
0.0009620	91362
0.0009620	81358
0.0009810	65058
0.0009810	76350
0.0019620	86746
0.0019620	114417
0.0048100	90288
0.0048100	106901
0.0096200	143010
0.0096200	161616
0.0098100	161085
0.0098100	107376
0.0196200	202712
0.0196200	232508
0.0481000	331318
0.0481000	337337
0.0962000	497785
0.0962000	538050
0.0981000	575795
0.0981000	528145
0.1962000	1375410
0.1962000	1102700
0.4810000	2152860
0.4810000	2150770

Pindolol Imp II

Regression Output:

Constant	105394
Std Err of Y Est	89525
R Squared	0.977
No. of Observations	28
Degrees of Freedom	26
X Coefficient(s)	4471379
Std Err of Coef.	133541

Figure 41. Analytical Response Curve for Impurity II

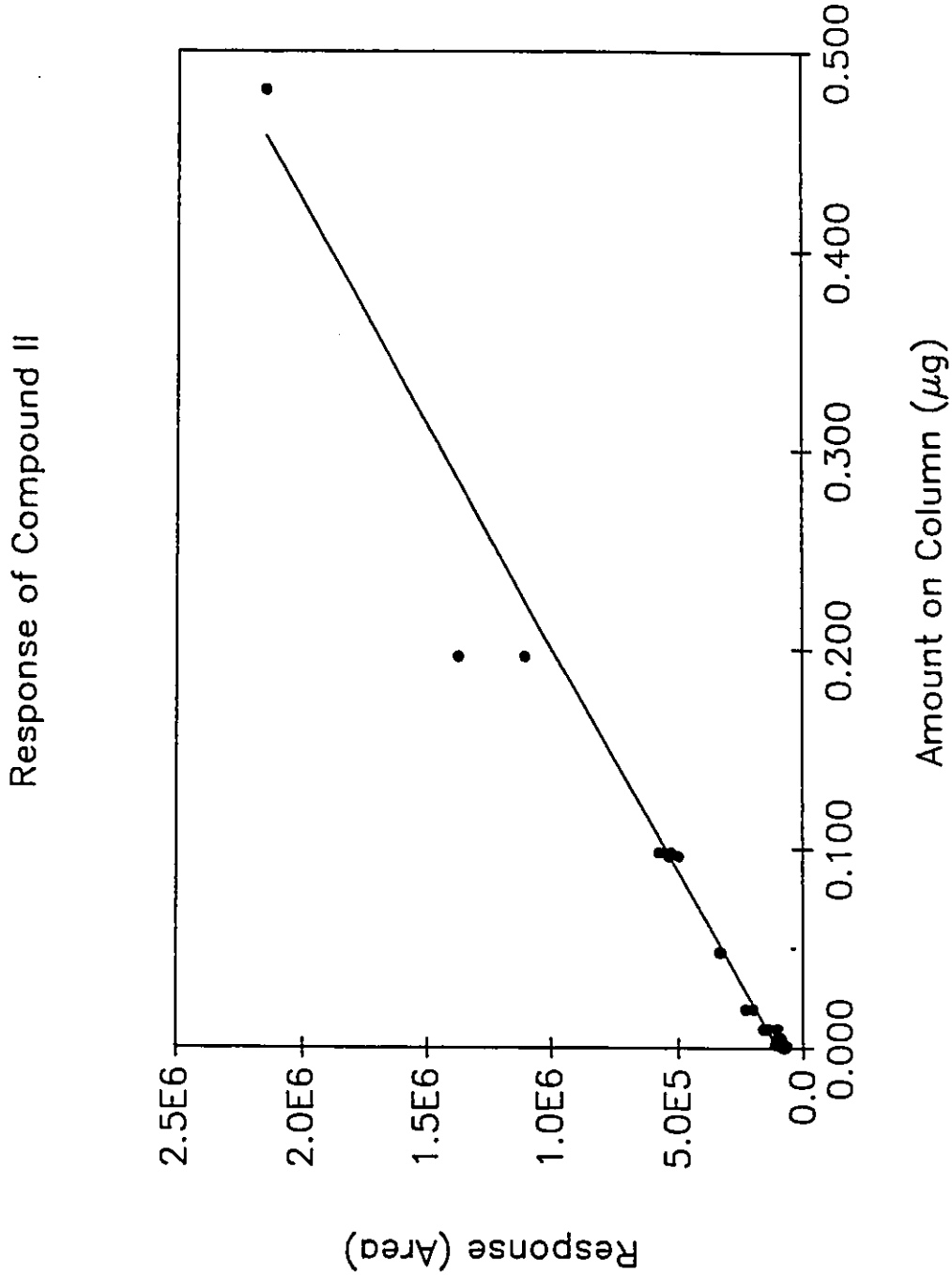


Figure 42. Regression Analysis for Impurity III

Amt (ug)	Area	Pindolol Imp III		
0.0001958	688	Regression Output:		
0.0001958	874	Constant		1396
0.0004720	1275	Std Err of Y Est		4776
0.0004720	1414	R Squared		1.000
0.0009440	2402	No. of Observations		28
0.0009440	2642	Degrees of Freedom		26
0.0009790	2890			
0.0009790	2731	X Coefficient(s)	2613515	
0.0019580	5202	Std Err of Coef.	7246	
0.0019580	5150			
0.0047200	12851			
0.0047200	11772			
0.0094400	23455			
0.0094400	26266			
0.0097900	25791			
0.0097900	26430			
0.0195800	50825			
0.0195800	50989			
0.0472000	123328			
0.0472000	124938			
0.0944000	243713			
0.0944000	248921			
0.0979000	262480			
0.0979000	265975			
0.1958000	526130			
0.1958000	526528			
0.4720000	1227290			
0.4720000	1229930			

Figure 43. Analytical Response Curve for Impurity III

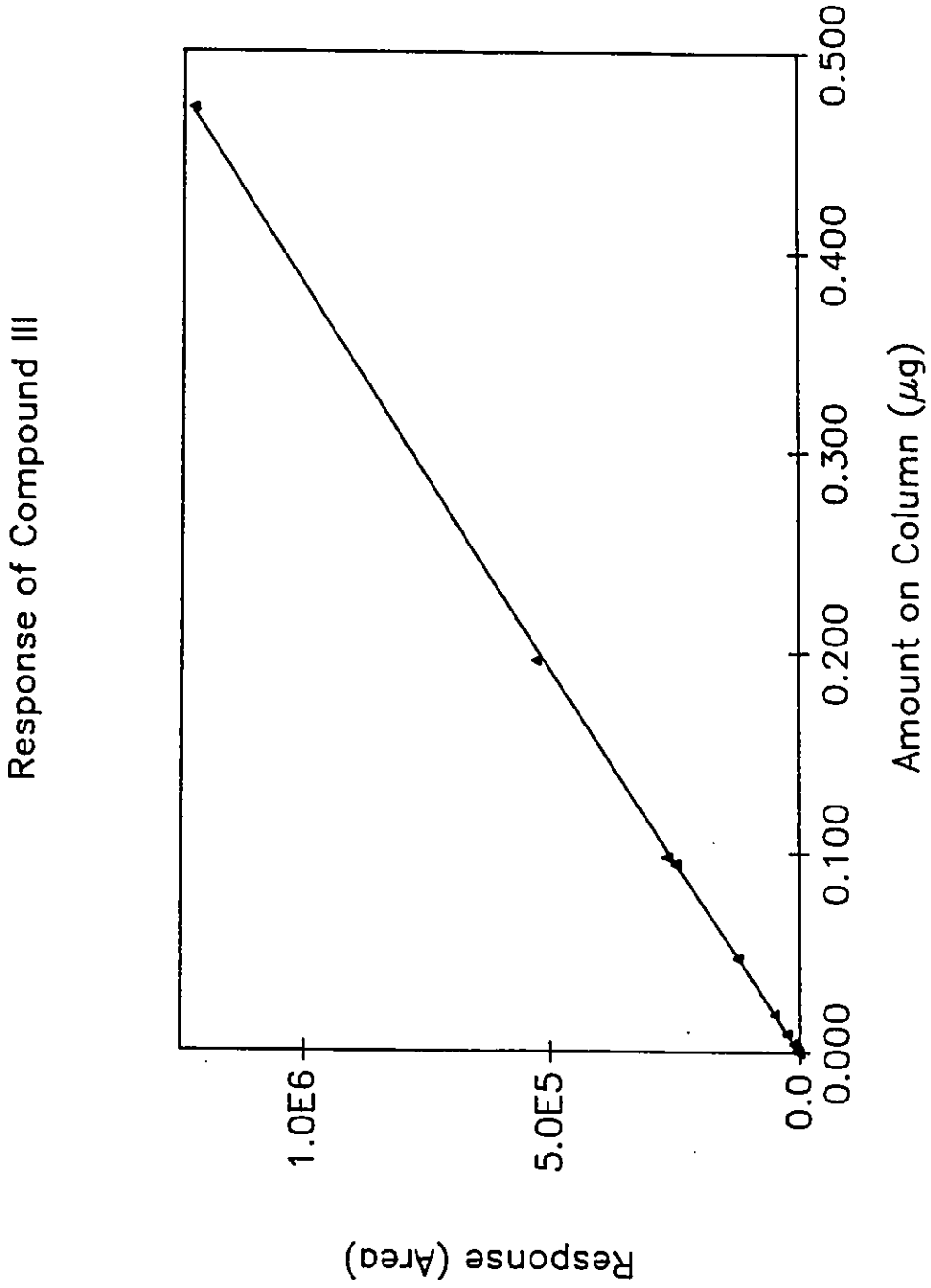


Figure 44. Regression Analysis for Impurity IV

Amt (ug)	Area
0.0020700	5633
0.0020700	6844
0.0047100	14623
0.0047100	12593
0.0094200	23491
0.0094200	22987
0.0103500	26164
0.0103500	25745
0.0207000	48834
0.0207000	46544
0.0471000	89830
0.0471000	89927
0.0942000	165623
0.0942000	173879
0.1035000	174878
0.1035000	175848
0.2070000	341771
0.2070000	333818
0.4710000	746070
0.4710000	742957

Pindolol Imp IV

Regression Output:

Constant		11645
Std Err of Y Est		6051
R Squared		0.999
No. of Observations		20
Degrees of Freedom		18
X Coefficient(s)	1564429	
Std Err of Coef.	9738	

Figure 45. Analytical Response Curve for Impurity IV

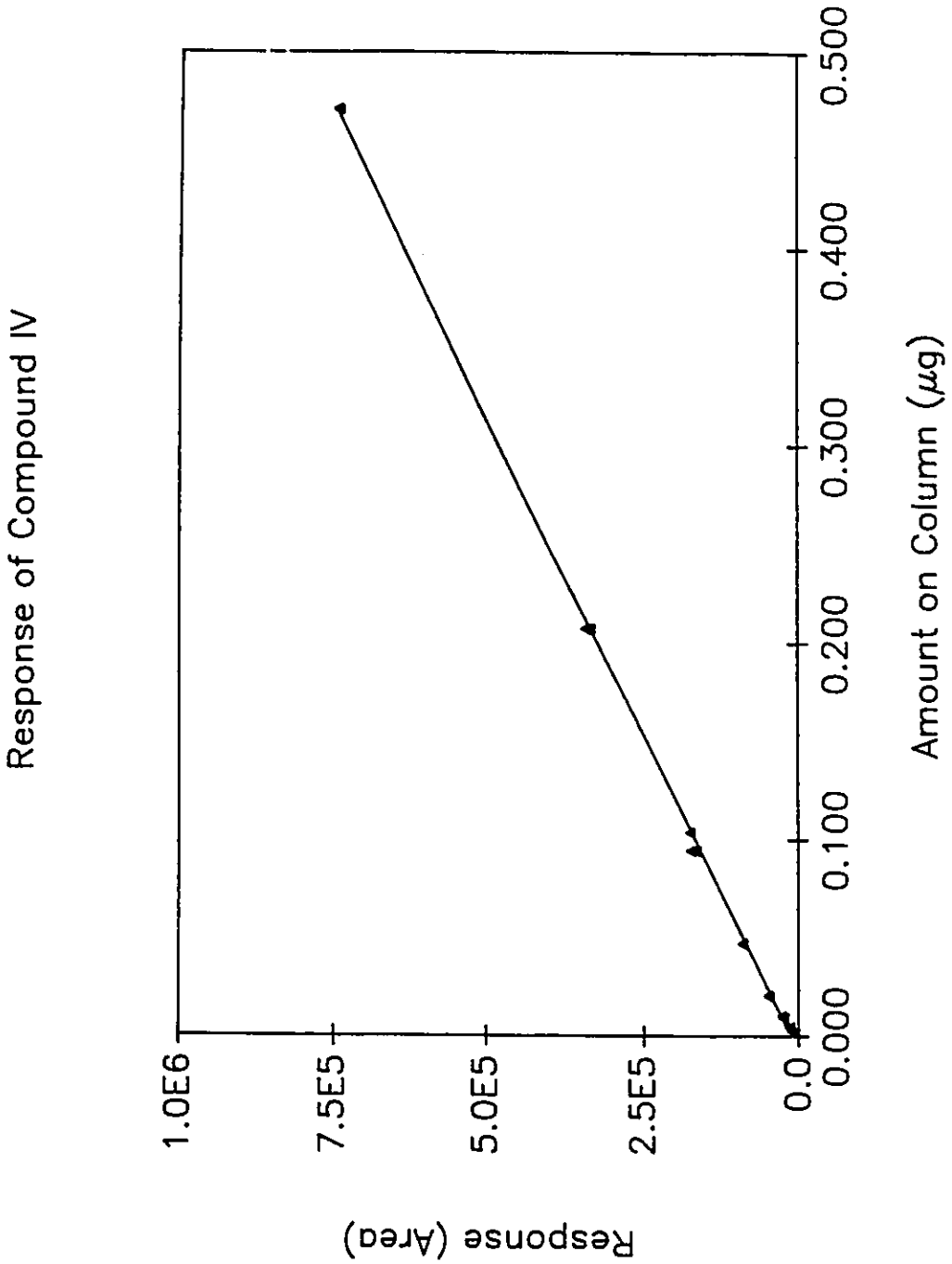


Figure 46. Regression Analysis for Impurity V

Amt (ug)	Area	Pindolol Imp V		
0.0010080	6602	Regression Output:		
0.0010080	6318	Constant		9947
0.0010260	8794	Std Err of Y Est		14054
0.0010260	6091	R Squared		0.996
0.0020520	10852	No. of Observations		24
0.0020520	8722	Degrees of Freedom		22
0.0050400	19249			
0.0050400	22060	X Coefficient(s)	1529002	
0.0100800	26663	Std Err of Coef.	20522	
0.0100800	27673			
0.0102600	25569			
0.0102600	30236			
0.0205200	39994			
0.0205200	49605			
0.0504000	97134			
0.0504000	111320			
0.1008000	183849			
0.1008000	181343			
0.1026000	155326			
0.1026000	157883			
0.2052000	291484			
0.2052000	288056			
0.5040000	790958			
0.5040000	790659			

Figure 47. Analytical Response Curve for Impurity V

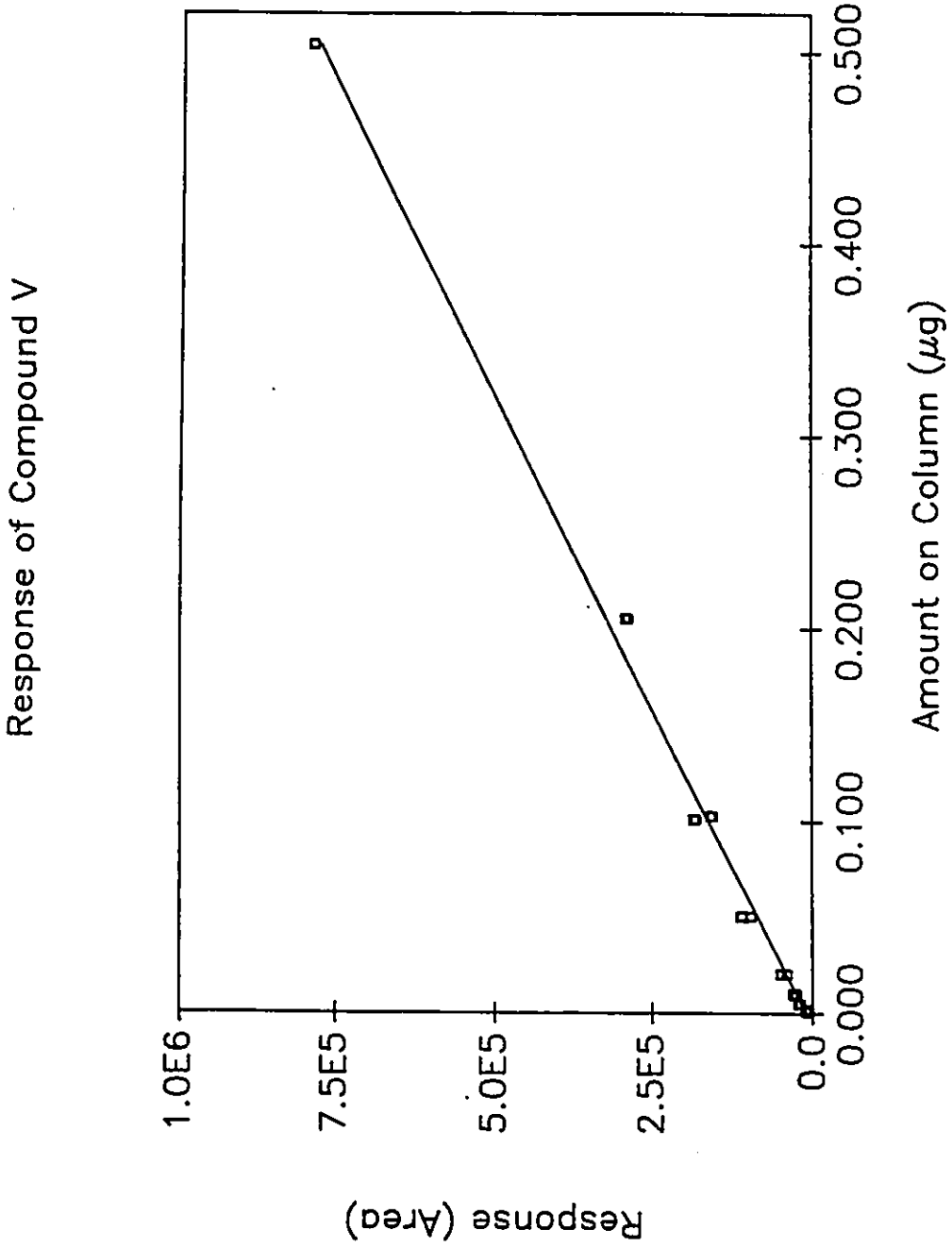


Figure 48. Regression Analysis for Impurity VIa

Amt (ug)	Area	Pindolol Imp VI-A		
0.0021760	1756	Regression Output:		
0.0021760	1694	Constant		523
0.0046400	3787	Std Err of Y Est		2161
0.0046400	3993	R Squared		1.000
0.0092800	7073	No. of Observations		20
0.0092800	7371	Degrees of Freedom		18
0.0108800	9466			
0.0108800	11391	X Coefficient(s)	803792	
0.0217600	17960	Std Err of Coef.	3503	
0.0217600	17564			
0.0464000	36355			
0.0464000	36572			
0.0928000	71565			
0.0928000	74019			
0.1088000	89182			
0.1088000	90613			
0.2176000	178653			
0.2176000	181109			
0.4640000	370787			
0.4640000	372314			

Figure 49. Analytical Response Curve for Impurity VIa

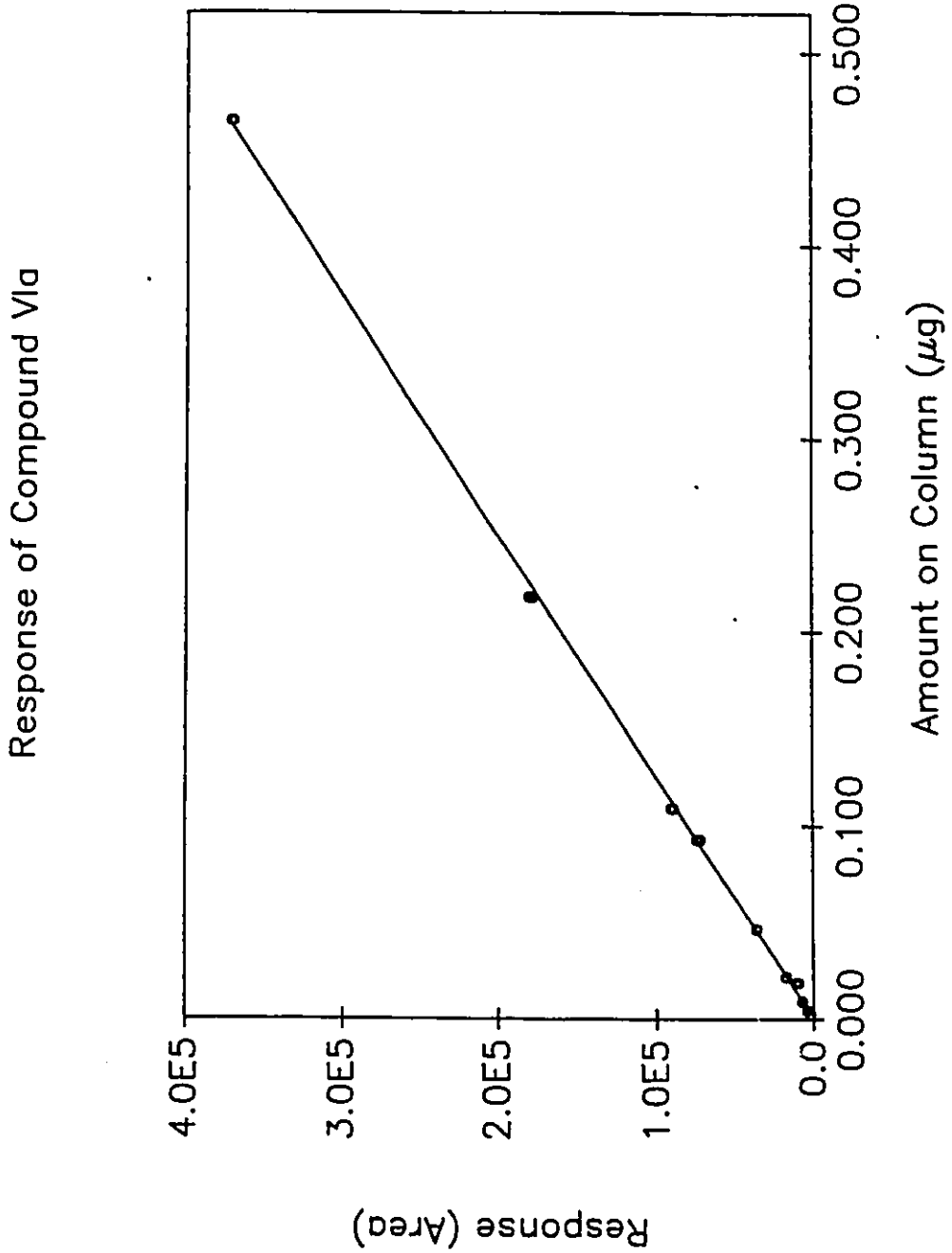


Figure 50. Regression Analysis for Impurity VIb

Amt (ug)	Area
0.0021760	1997
0.0021760	1782
0.0046400	5547
0.0046400	5260
0.0092800	10635
0.0092800	10574
0.0108800	13632
0.0108800	16136
0.0217600	22311
0.0217600	23537
0.0464000	50893
0.0464000	51362
0.0928000	107750
0.0928000	107800
0.1088000	132407
0.1088000	142118
0.2176000	273556
0.2176000	271581
0.4640000	557698
0.4640000	556840

Pindolol VI-B

Regression Output:

Constant		-301
Std Err of Y Est		4968
R Squared		0.999
No. of Observations		20
Degrees of Freedom		18
X Coefficient(s)	1210952	
Std Err of Coef.	8054	

Figure 51. Analytical Response Curve for Impurity VIb

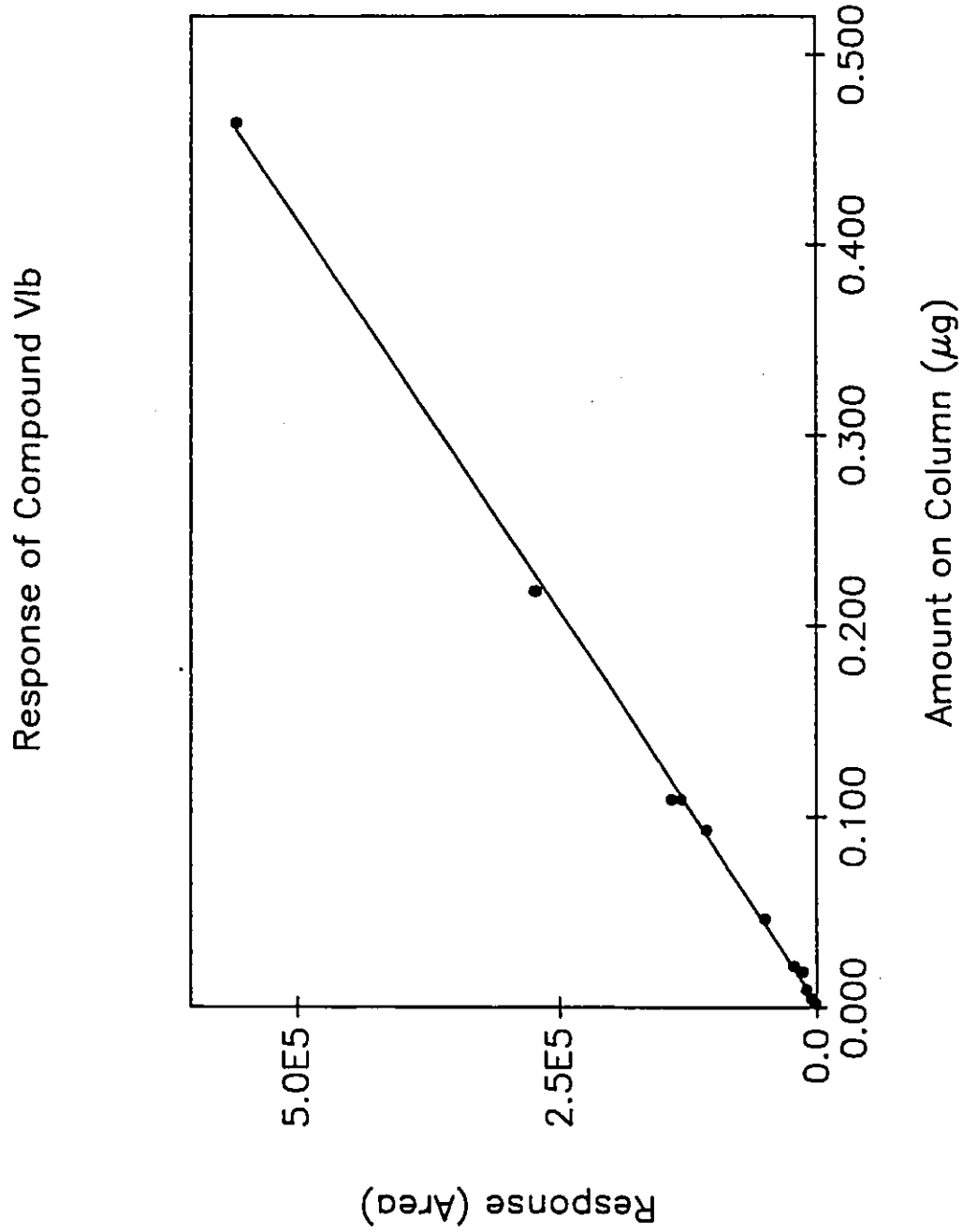
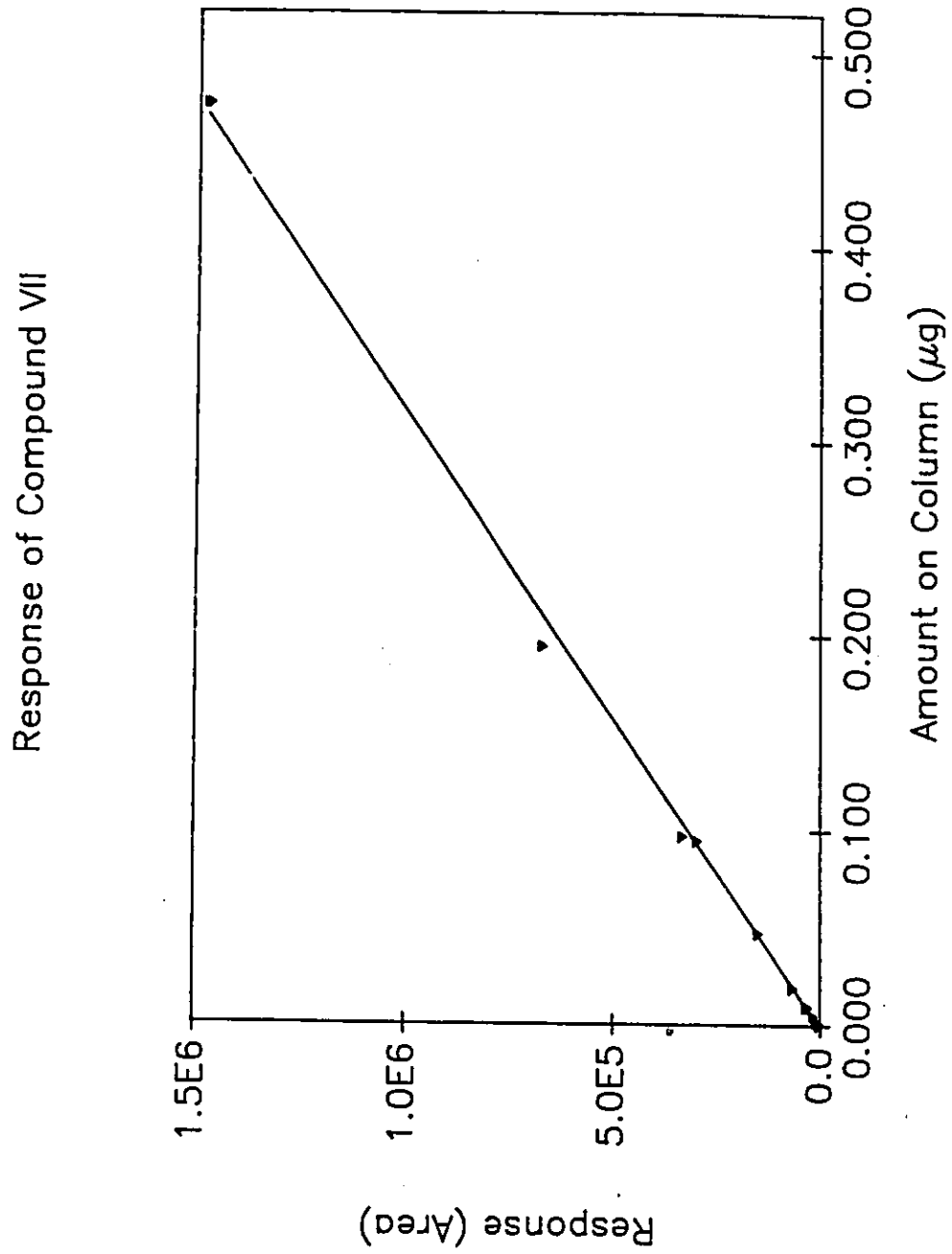


Figure 52. Regression Analysis for Impurity VII

Amt (ug)	Area	Pindolol VII		
0.0000946	1169	Regression Output:		
0.0000946	1150	Constant		6167
0.0001940	2274	Std Err of Y Est		16233
0.0001940	2334	R Squared		0.998
0.0004730	2344	No. of Observations		30
0.0004730	2321	Degrees of Freedom		28
0.0009460	3973			
0.0009460	3525	X Coefficient(s)	3157940	
0.0009700	4842	Std Err of Coef.	24367	
0.0009700	4906			
0.0019400	8525			
0.0019400	9211			
0.0047300	16505			
0.0047300	16548			
0.0094600	30711			
0.0094600	30263			
0.0097000	33840			
0.0097000	35435			
0.0194000	68930			
0.0194000	68602			
0.0473000	152370			
0.0473000	153408			
0.0946000	298636			
0.0946000	298670			
0.0970000	334274			
0.0970000	334432			
0.1940000	668417			
0.1940000	667756			
0.4730000	1473440			
0.4730000	1480320			

Figure 53. Analytical Response Curve for Impurity VII



SUMMARY

A liquid chromatographic method was developed for the quantitative determination of total drug and related substances in pindolol raw material. The method resolves six related compounds and several unknown impurities from the drug and from each other. The assay method is more specific than the existing USP tritrimetric method. The limits of detection for the related substances method are lower than the existing USP TLC method by factors of 5 to 50, depending on the impurity.

The method was presented as a poster at the 103rd Association of Official Analytical Chemists (AOAC) Annual International Meeting in September, 1989 and has been accepted for publication in the Journal of the AOAC. The method has also been proffered to the United States Pharmacopeia.

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CLAIMS TO ORIGINAL RESEARCH

1) Liquid chromatographic conditions were investigated to optimize the separation of pindolol related compounds from the drug and each other.

2) The HPLC methods based on this separation were suitable for drug assay within a range of 50 to 150% and for the quantitation of related compounds at levels as low as 0.002%.

3) The assay method was validated for use as a pharmacopeial standard. The following analytical parameters were evaluated: selectivity, sensitivity, linearity, precision, and ruggedness, including two columns of the same type from different manufacturers.

4) The related substances method was validated for use as a pharmacopeial standard. The following analytical parameters were evaluated: selectivity, sensitivity, linearity, precision, limits of detection and quantitation, and ruggedness, including two columns of the same type from different manufacturers.

5) The viability of the methods was demonstrated by evaluation of ten lots of pindolol from various manufacturers for purity and drug content.