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Identification and detection of conjugated quinones by direct LC/MS using NICI enhancement

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IDENTIFICATION AND DETECTION OF CONJUGATED QUINONES BY DIRECT LC/MS USING NICI ENHANCEMENT

by

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A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

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Abstract

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by

Kenneth K. Ng

Advisor: Professor David Locke

Sterically hindered phenols have been used effectively as antioxidants in commercial polymers, usually in the low tenths of a percent level. However, the additive itself can be oxidized to its quinone analog to provide new sources of undesirable discoloration. The detection of such colored species can be achieved by analyzing the colored polymer extract using HPLC with UV/Visible detection. The identification can be achieved only if standards are available, otherwise isolation or direct liquid chromatography/mass spectrometry (LC/MS) is needed. Due to the tremendous differences of the absorptivities between the UV and visible λ max, the presence of trace levels of undefined quinones can present problems of identification either

through isolation or direct LC/MS analysis.

Conjugated quinones give very strong molecular ions in the negative ion chemical ionization (NICI) mode. This is due to the fact that any quinone structure is a good candidate for electron capture. LC/MS equipped with a recently commercially available particle beam interface operating in the electron ionization and NICI modes with an on-line UV/Visible detector is a straightforward and powerful tool where structural information and compound characteristics can be obtained simultaneously. Increased sensitivity was achieved through some modifications of the commercially available system.

Acknowledgements

I want to express my sincere gratitude to my thesis advisor, Professor David Locke for his guidance and patience during this work.

I also acknowledge the support of Ciba-Geigy Corporation with whom I have been associated for the last seventeen years. I want to thank Dr. Jerrold Karliner for his encouragement and Dr. Kevin Volk for very stimulating discussions.

To my wife, Clardy, who makes it all possible and worthwhile. To my children, Verna and Daniel, for their love and understanding.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

In this dissertation, negative ion chemical ionization mass spectrometry in junction with high performance liquid chromatography with a particle beam interface is used to detect,identify and quantitate a class of sterically hindered conjugated quinones These compounds are present in low level quantities (usually sub ppm level) in degraded polymers which have been stabilized with common antioxidants, which are generally hindered phenol derivatives. The characterization of these compounds is important as part of the overall understanding of breakdown process of polymers.

Mass Spectrometry (MS)(1,2) has been proven over decades to be a very useful analytical technique because of its sensitivity and specificity, and the enormous amounts of structural data it is capable of generating. Use of different modes of ionization has also increased and has enhanced the sensitivity and specificity towards many different types of chemical compounds. One such mode, negative ion chemical

ionization (NICI) has high sensitivity and specificity based on the high electron affinity of certain compounds. These compounds are capable of capturing electrons to form anions during the chemical ionization process. In this research much attention has been given to the enhanced sensitivity of MS to steric hindered conjugated quinones NICI conditions.

High performance liquid chromatography (HPLC)(3,4) is not only a good complement to gas chromatography (GC) but has developed in recent years into a very powerful separation tool on its own merits. Not only has it decreased the analysis time but has also improved resolution dramatically through the availability of many different types of high efficiency columns. However, HPLC still suffers the general weakness of chromatography as a reliable tool for identification because of the lack of suitable detectors.

In a complex liquid chromatogram such as shown in Fig. I-1, components can be identified by matching retention times with those of known standards; otherwise the components have to be isolated and their

structures be elucidated by subsequent off-line spectral analysis. In most cases, this becomes a monumental undertaking and is impractical for trace level components. Such components are usually contaminated with background materials as well as unresolved, interfering components which can make the identification virtually impossible. The combination of two separate instruments, one providing high resolution separations and the other detailed structural information, has become not only a luxury but a necessity.

Direct Liquid Chromatography/Mass spectrometry (LC/MS) is an example of such a powerful hybrid instrument, combining a high power separation technique with a very sensitive and structurally informative tool. The obvious interfacing problems have been addressed in several recently commercially available systems. The particular system that has been evaluated in this research is the particle beam interface marketed by Hewlett-Packard.

The mechanisms leading to the formation of steric hindered conjugated quinones through oxidation are discussed in Chapter II. The development of many of

the commonly found quinone species during various stages of polymer degradation as represented by changing color intensities are illustrated.

The basic theory, development and utilization of NICIMS are discussed in Chapter III. in addition to its unique properties and applications. The selectivity and sensitivity of NICI are the essential features relevant to this research. Some of the earlier use of NICI to detect and identify different steric hindered conjugated quinones is reviewed. The general behavior of several reagent gases in the NICI mode was tested to try to enhance the sensitivity and the results are compared. Parameters that affect sensitivity of detection from the ionization process such as ion source pressure and temperature have been investigated.

The first part of Chapter IV is an overview of LC/MS while the latter part deals with the selection of one particular LC/MS system and the description of the initial configuration, the integral parts and various features of the entire system. Different conditions within the particle beam configuration that affect the sensitivity of detection of the quinones are discussed.

Modification of the configuration leading to the final system is described.

Chapter V is concerned with the overall results and presents a straightforward method to combine several powerful techniques to solve a complex chemical problem. Some future work is suggested and possibilities to branch out into other areas of analytical chemistry based on this concept are discussed.

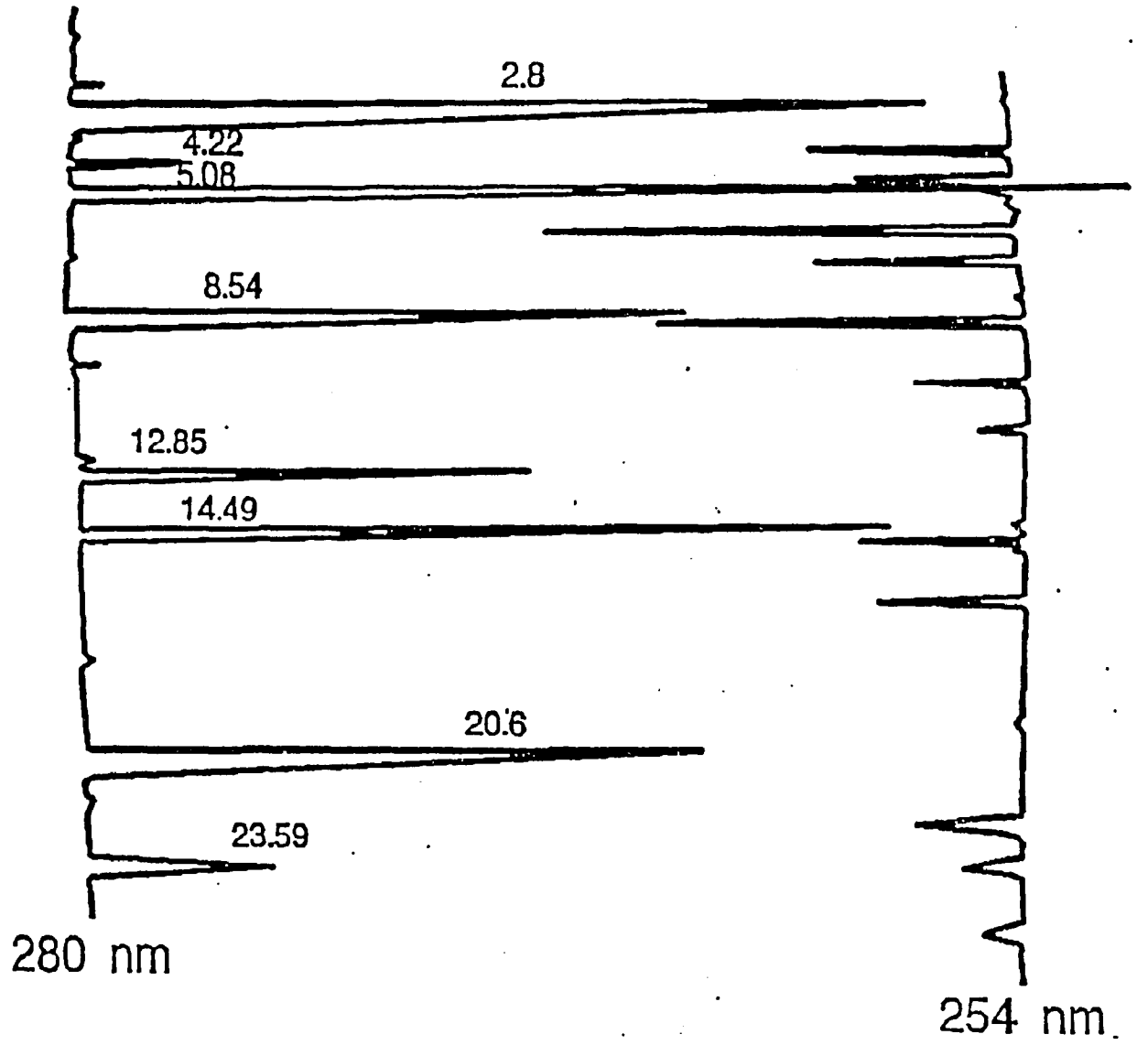


Fig. I-1 Liquid chromatogram of a synthetic mixture of a commercial antioxidant and its by-products using two UV wavelength detection

CHAPTER II

QUINONES AND CONJUGATED QUINONES FORMATION

The growth of the plastics industry leads directly to huge demands for plastic additives which are used to prolong the service life as well as to enhance the quality of the plastics goods. All polymeric materials undergo oxidation reactions with oxygen. Some oxidation reactions are purely thermal processes at usually elevated temperatures. Others need assistance of ultraviolet light. One of the manifestations of oxidation is discoloration of the polymer. One of the means to retard thermal oxidation is through the use of a particular class of additives called antioxidants. Section 1 deals with the basic concept of how antioxidants work.

1. Autoxidation and mechanisms of antioxidant action

a. Non-inhibited autoxidation

The reaction of organic compounds with molecular oxygen is called autoxidation, because such reactions take place "automatically", whenever organic

materials are exposed to the atmosphere. Autoxidation is characterized by two features: autocatalysis and inhibition by additives. Bolland and Gee (5) performed the first kinetic investigations of such reactions. Low molecular weight saturated hydrocarbons in the liquid phase were reacted with oxygen and the mechanism was depicted as:

INITIATION

$$RH \longrightarrow R^{\cdot} \qquad [1]$$

PROPAGATION

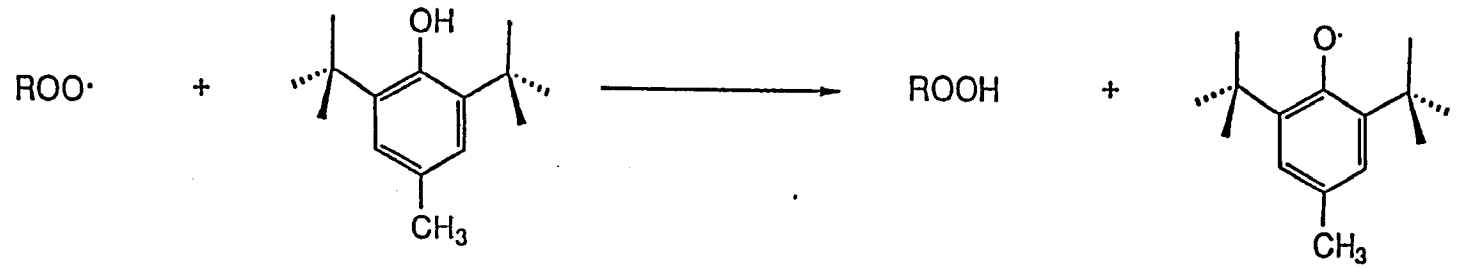
$$R^{\cdot} + O_2 \longrightarrow ROO^{\cdot} \qquad [2]$$

$$ROO^{\cdot} + RH \longrightarrow ROOH + R^{\cdot} \qquad [3]$$

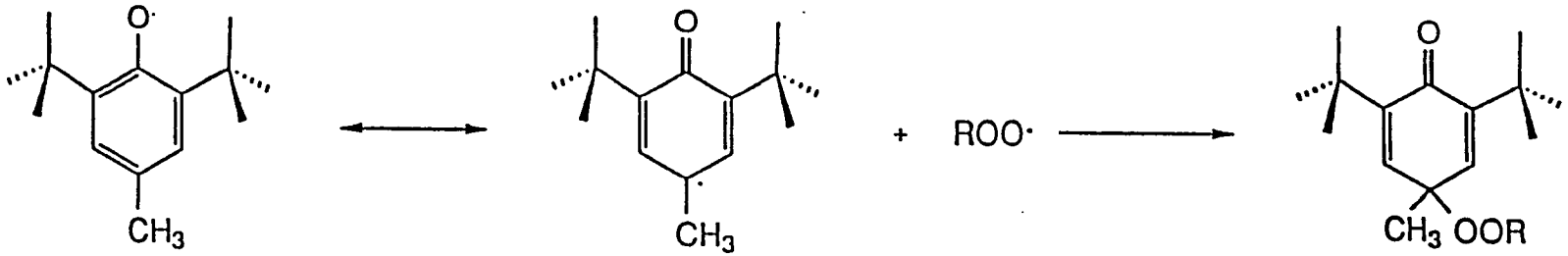
Up to now, the same sequence of reactions applies for the autoxidation of polymeric substances.

b. Inhibited autoxidation

The effectiveness of antioxidants is based on the fact that they are able to influence the autoxidation process (6). Normally they can be classified into two large basic groups of antioxidants: first, chain terminating or primary antioxidants and second, hydroperoxide decomposers or secondary antioxidants. Since in this research the primary interest lies in the primary antioxidants, the secondary antioxidants are beyond the scope of the discussion and shall be omitted. The majority of primary antioxidants are sterically hindered phenols or secondary aromatic amines. They are all capable of reacting quickly with peroxy radicals and are thus known as radical scavengers. As seen in SCHEME I, 2,6-di-t-butyl, 4-methylphenol (commonly known as BHT), a commonly used antioxidant, reacts according to [4] and [5].



[4]



[5]

SCHEME I

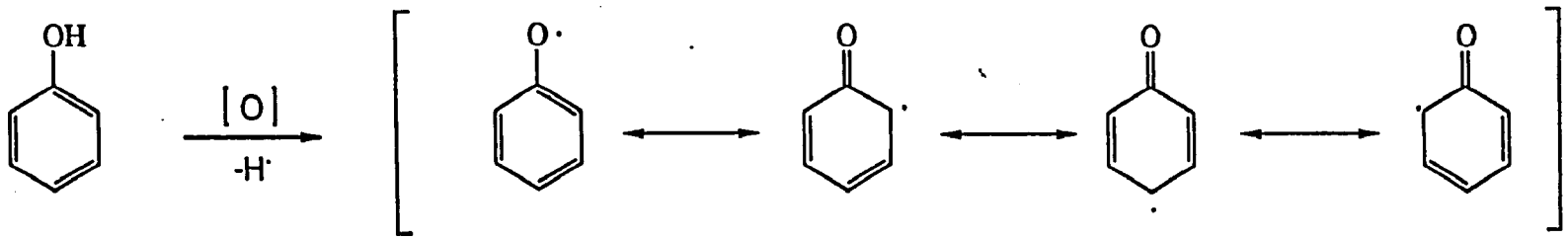
According to the above scheme, stabilization is achieved by the fact that reaction [4] competes with [3], transforming the reactive peroxy radical into the much less reactive phenoxy radical, which, in turn reacts with a second peroxy radical to complete reaction [5].

Unfortunately, these phenols can also undergo oxidation as shown in Section 2 which eventually lead to the formation of their quinones and conjugated quinone derivatives.

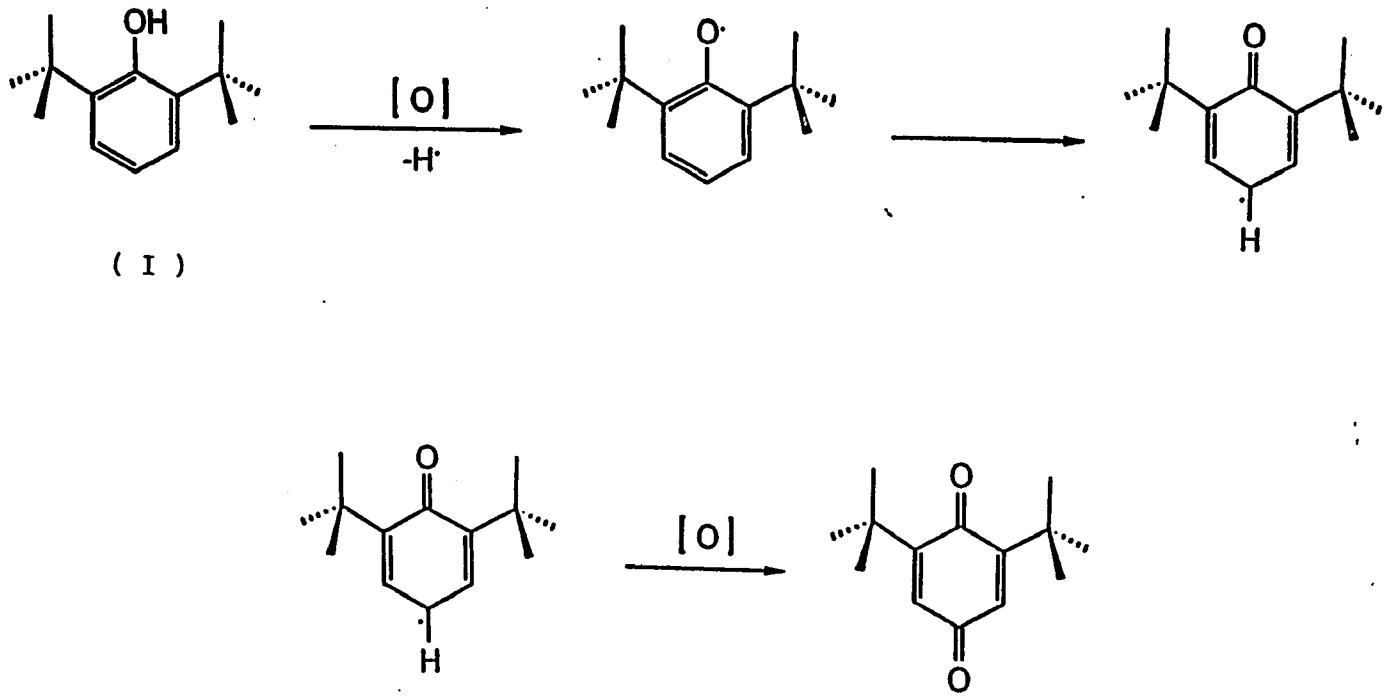
2. Oxidation of phenols

Oxidative attack seems to involve, as the first step, removal of the hydroxyl hydrogen to yield a phenoxy radical as shown in SCHEME II.

The subsequent course depends upon the substitution on the aromatic ring. In the case of 2,6-di-t-butylphenol (I), its quinone structure is formed as indicated in SCHEME III. The phenoxy radical can also form a dimerization product as in SCHEME IV. Dependent on the substituents, many different conjugated quinones may be formed following the same mechanism.

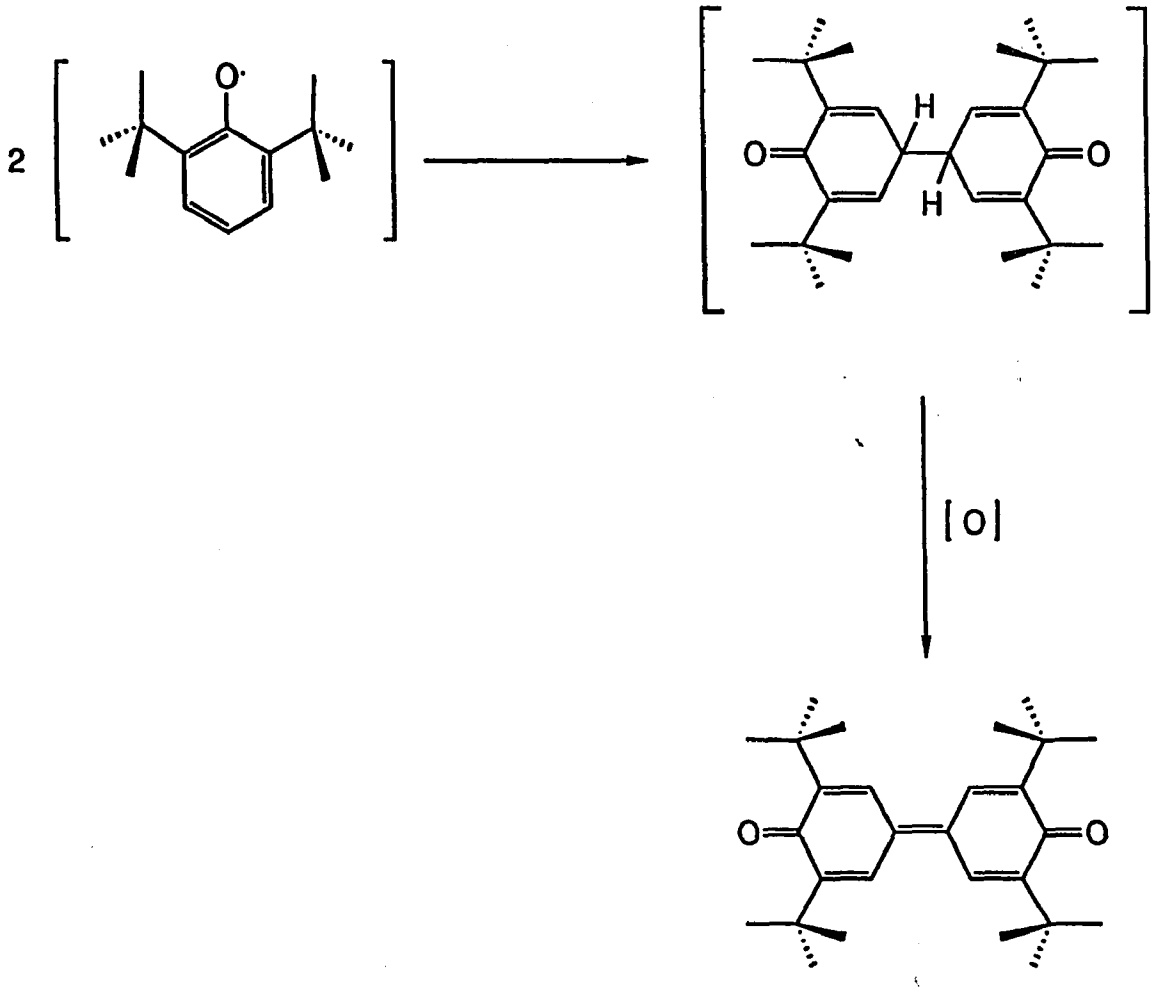


SCHEME II



(I)

SCHEME III



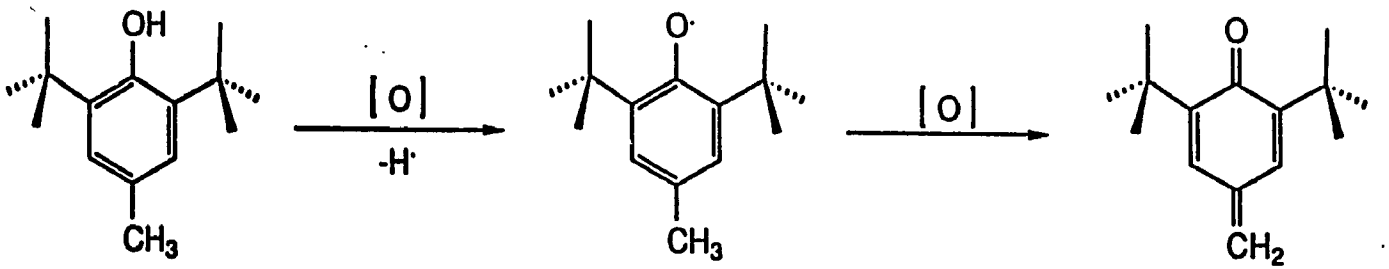
SCHEME IV

3. Discoloration of antioxidants

Ideally an antioxidant is colorless and should contribute as little as possible to discoloration of the substrate during long term use. However this is usually not the case. Depending on the conditions of aging, a yellowing effect may be caused by the substrate or by the additive. In some severe cases, the yellowing may be due solely to the specific antioxidant used. This usually depend on the processing conditions and the types of polymer involved. The hindered phenol BHT can undergo the same oxidative mechanism to form the so-called stilbenequinone (III) as seen in SCHEME V. This particular sterically hindered conjugated quinone is known to contribute significantly to the yellowing of polymers.

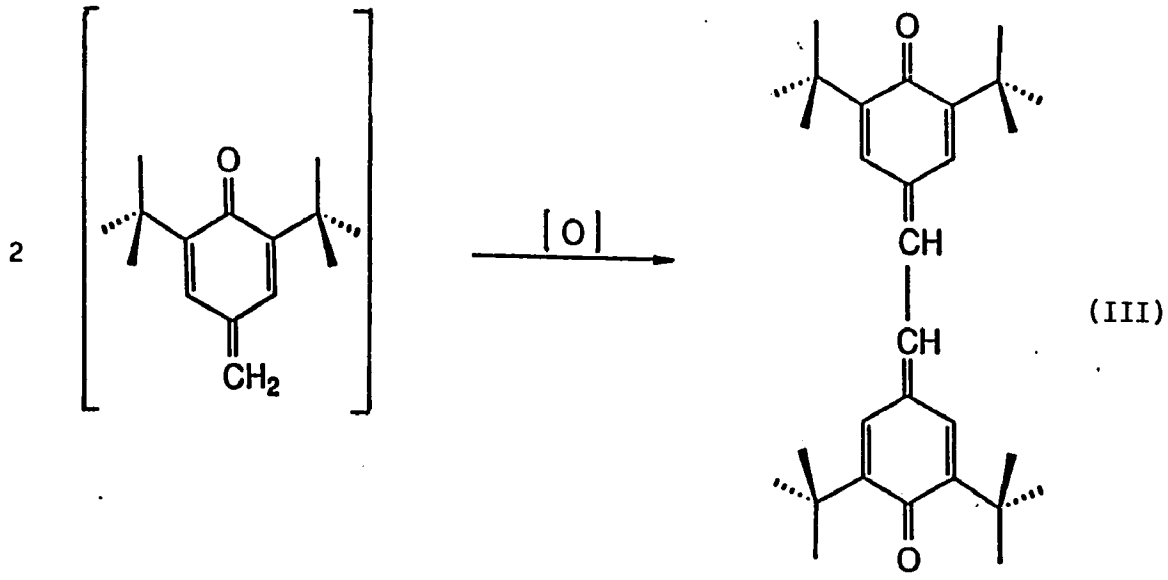
4. Common substituted conjugated quinones

The ultraviolet/visible absorption characteristics of several common substituted conjugated quinones derived from certain commercial antioxidants in Table II-1 which contribute to the discoloration of polymers are shown in Table II-2. The effect of



BHT

Methide Quinone



(III)

SCHEME V

different antioxidants on the development of color of a typical high activity catalyst polypropylene can been seen in Table II-3.

5. Detection of some antioxidants and their quinones by HPLC analysis

Since discoloration of polymer can be attributed partly to these quinones, their identity and level of presence are not only essential to the understanding of the degradation mechanism. This also become essential information when human and animal exposure are potentially involved since many of the polymers are eventually used in food handling utensils.

High performance liquid chromatography (HPLC) is generally used to detect and quantify these quinones that are present in a polymer of interest. The following experimental section is a description of a routine HPLC analysis to detect and quantitate these quiniones in some polymers as indicated.

Table II-1 Common hindered phenol commercial antioxidants

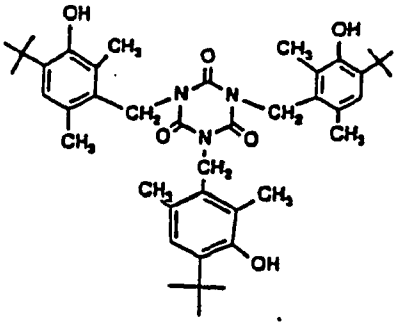
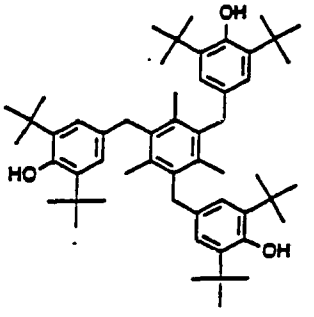
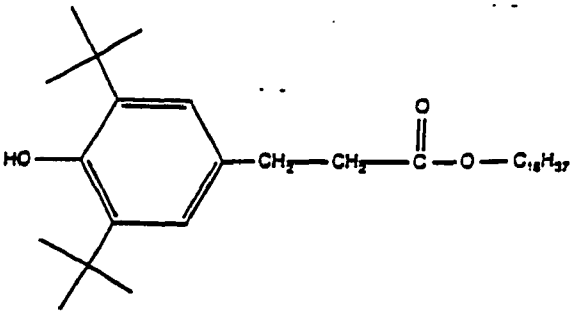
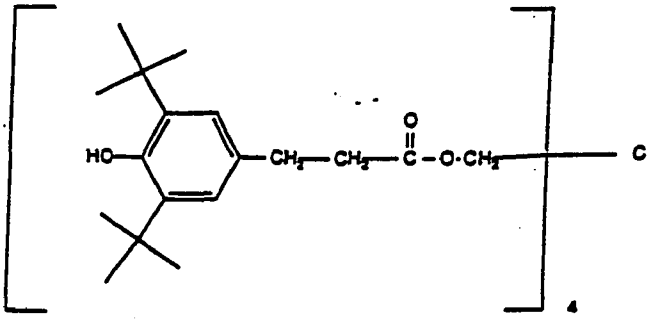
Tradename	Structure
CYANOX 1790	
ETHANOX 330	
IRGANOX 1076	
IRGANOX 1010	

Table II-2

UV and Visible Absorrption Characteristics of Antioxidants Oxidation Products

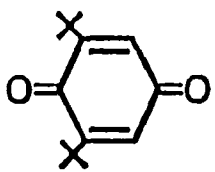
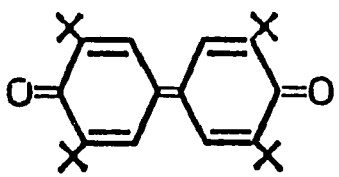
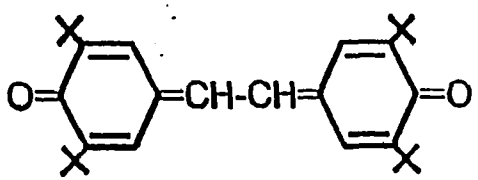
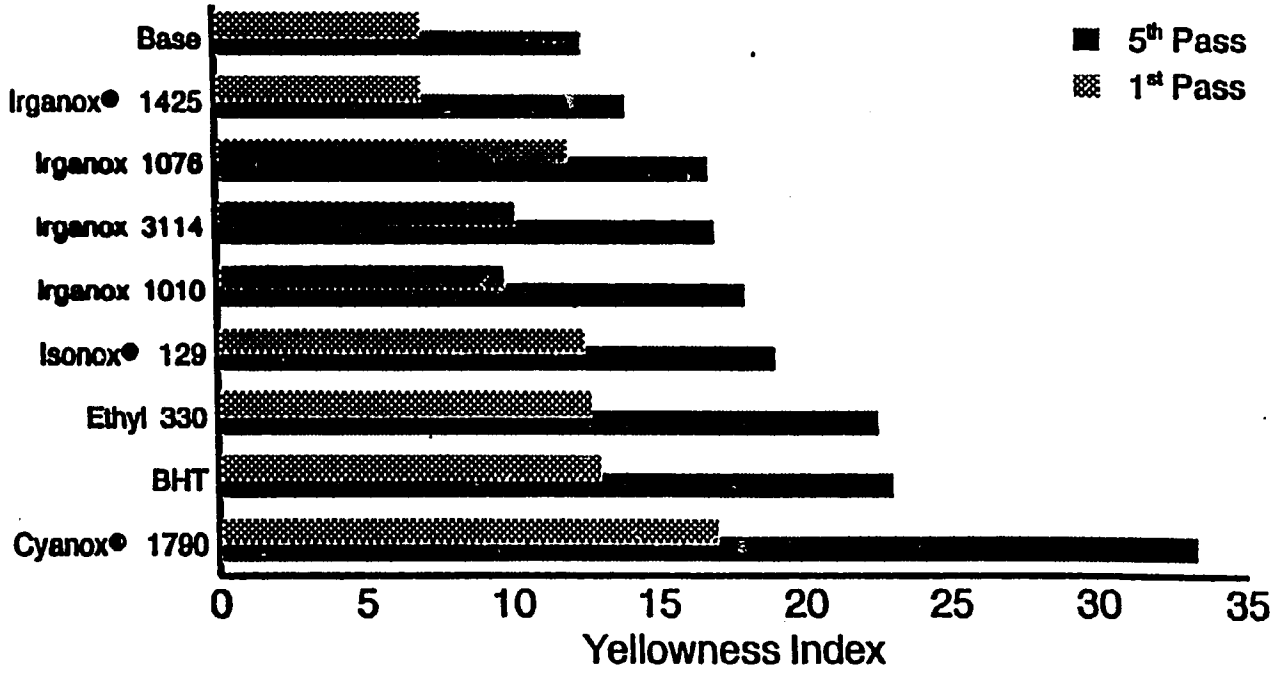
Compound	UV Absorption λMan (nm)	UV Absorption a* (cm-l/g)	Visible Absorption λMax (nm)	Visible Absorption a* (cm-l/g)
 Benzoquinone	254	41.5	443	0.14
 Diphenoquinone	260	10.4	423	178
 Stilbenequinone	240	14.2	452	244

Table II-3

High Activity Catalyst Polypropylene
Effect on Antioxidant on Color



Extrusion performed at 500°F - Base contained 750ppm CaSt

EXPERIMENTAL

Chemicals and reagents

The polymers were obtained from the research laboratory of the Additives Division of Ciba-Geigy Corporation. HPLC grade methanol was purchased from J.T.Baker (Phillipstown, N.J.) , spectroanalyzed ethyl acetate from Fisher Scientific (Fair Lawn, N.J.), toluene from EM Science (glass distilled) (Gibbstown, N.J.) and used without further purification.

Instrumentation

HPLC was carried out on a modular gradient liquid chromatograph made up of a Waters WISP 710 autosampler, two Waters 510 pumps, a Waters 840 system controller and a Waters model 481 variable ultraviolet/visible detector (Waters Chromatography Division, Milford, MA), using a 5 micron Zorbax ODS column (250mm x 4.6 mm .i.d.) from Mac-Mod Analytical, Chadds Ford, PA).

Procedures for sample preparation.

About one gram of the polymer was dissolved in 40 ml of toluene by refluxing the mixture for about one

hour. After cooling, the polymer was precipitated out by adding 65 ml. of methanol and removed by gravity filtration with a No.42 Whatman filter paper. The methanol solution was evaporated to dryness with a rotavap. The residue was redissolved in 1 ml of organic solvent and filtered. The filtrate was then analyzed by HPLC.

Two polymer materials were used. Sample A was a commercial high density polypropylene (HDPP) which was discolored from natural aging. Sample B was an ultra light polyethylene which was stabilized with a 0.3% of an experimental hindered phenol antioxidant and had been subjected to high temperature heating in 50% aqueous ethanol.

Chromatography

Sample A

The HPLC column was equilibrated for 10 minutes with the initial mobile phase, ethyl acetate/methanol/ water (20/60/20) prior to injection. After 50 ul was injected, the initial mobile phase was changed using a linear gradient to 84/12/4 mixture of the same solvents in 35 min. with 10 min. final hold. The flow rate was

1.0 ml/min. Solvent for injection was ethyl acetate/ methanol (1:1). Ultraviolet (UV) detection was carried out at 280 nm and visible detection was at 436 nm.

Sample B

The column was equilibrated with initial mobile phase for 10 min. prior to injection. The initial mobile phase was 70A/30B and changed linearly to 20A/80B in 25 min. A was methanol/water (3:1) and B was ethyl acetate. The flow rate was 1.0 ml/min. The volume of injection was 25 ul. The solvent for injection was same for sample A. The detection wavelengths were the same as sample A.

RESULTS AND DISCUSSION

As seen in Fig. II-1a, the liquid chromatogram for sample A showed the presence of many components in the extract by ultraviolet detection at 280 nm, while three major color components were detected using visible detection at 436 nm, as seen Fig. II-1b. One of the color components as indicated has been identified as a conjugated quinone derivative arising from one of the known additives present in the polymer. This was achieved by matching retention time with an available standard. In the case of sample B, as shown by Figs.

II-2a and 2b, two color components C and D were identified using retention time comparisons with standards. For the other two unknowns, we cannot rely on using standards. Off-line techniques are necessary to carry out the identification.

Preparative HPLC was performed on component A in sample B (Fig. II-2b). This was achieved by repeating the analysis using a semi-preparative column. The column was Zorbax ODS (250 cm. x 9.4 mm with a flow rate of 4.0 ml/min. The solvent systems remained the same. Each injection was 300 ul. Five runs were performed and the fractions were collected and combined. Contamination appears to be the biggest drawback. The high specific absorptivity of a conjugated quinone (Table II-2) causes what appears on the chromatogram to be a highly pure and significant amount of material. However it turn out to be a trace level of a plasticizer as indicated by direct-insertion probe mass spectrometric analysis. Incorporation of an on-line device for structural identification is necessary. GC/MS and LC/MS are the two most viable choices that can provide a fast and yet effective method with a high degree of success while requiring minimum sample preparation time. The merits

of LC/MS over GC/MS for this application will be discussed in Chapters III and IV.

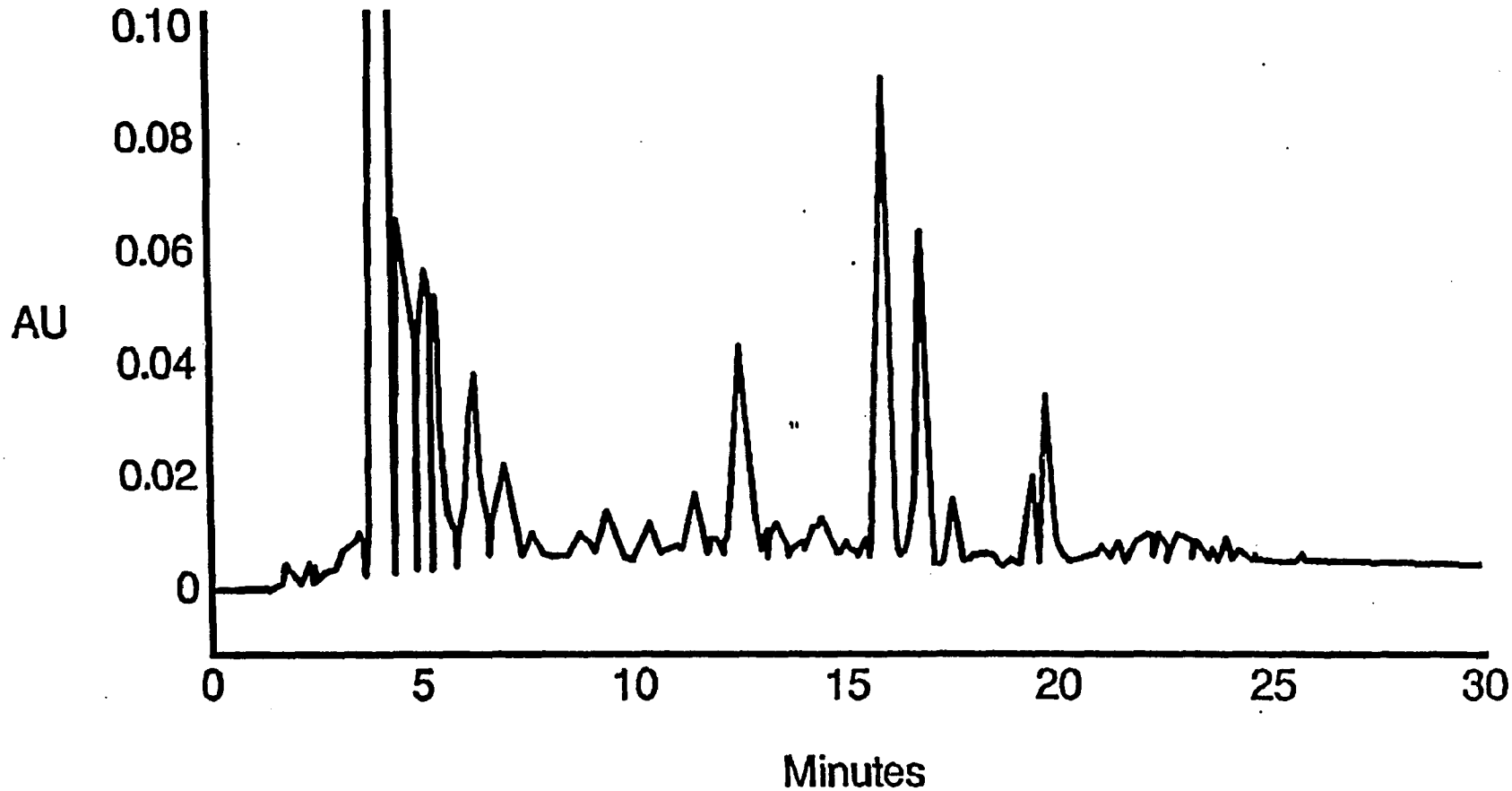


Fig. II-1a Liquid chromatogram of a commercial HDPP extract at 280 nm detection

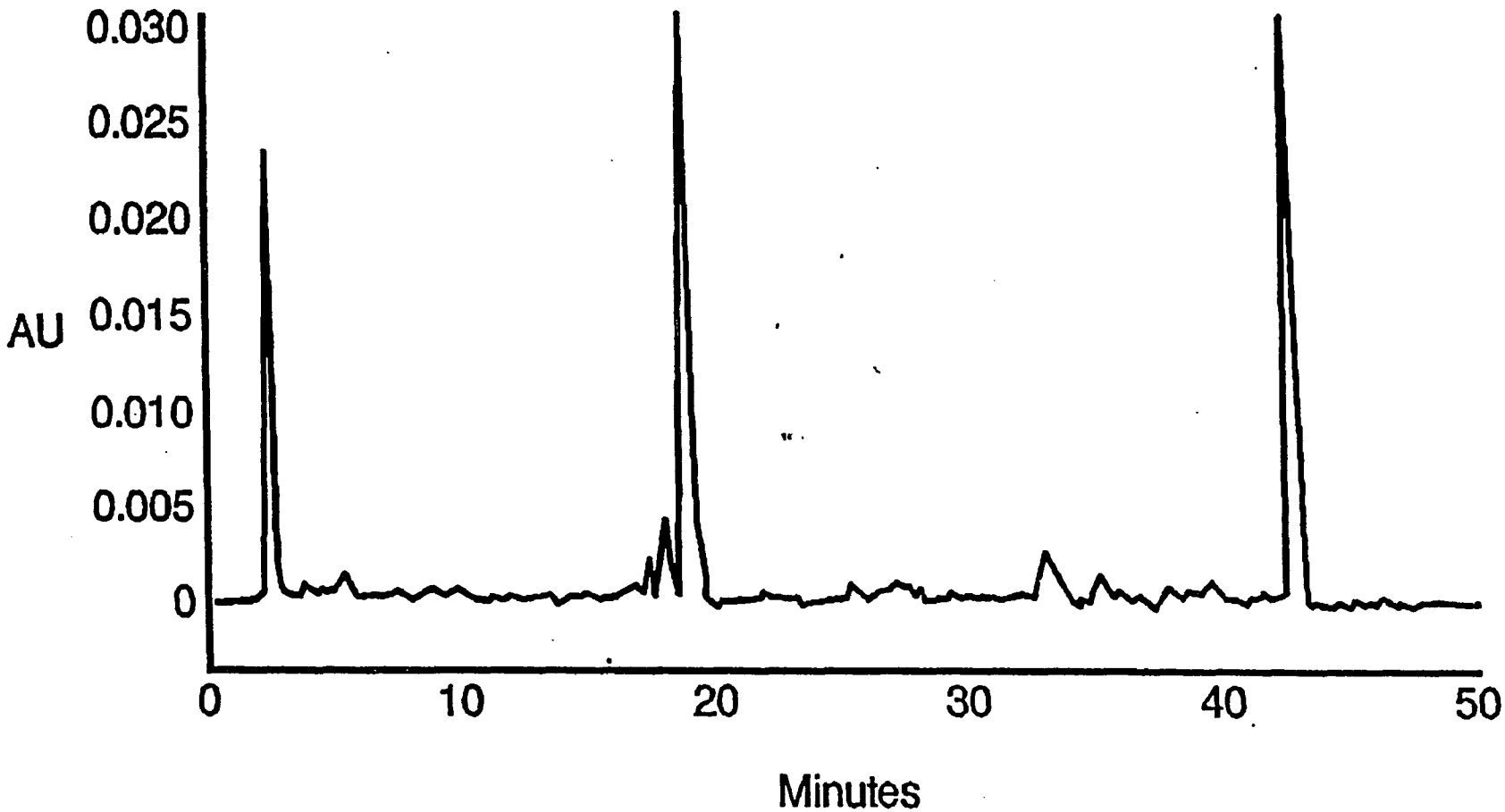


Fig. II-1b Liquid chromatogram of the same extract at 436 nm detection

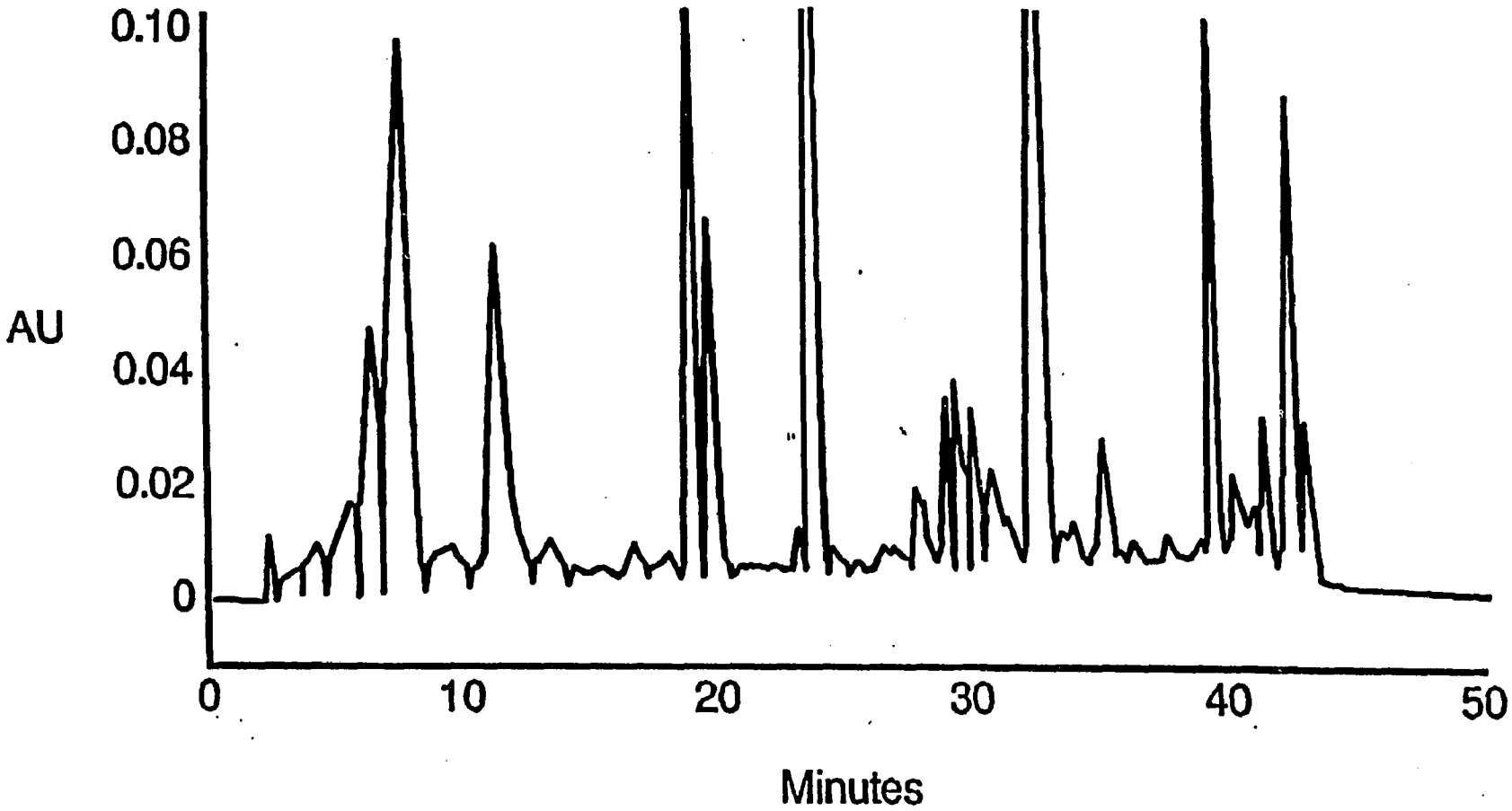


Fig. II-2a Liquid chromatogram of an extract of ULPE stabilized with an experimental antioxidant at 280 nm detection

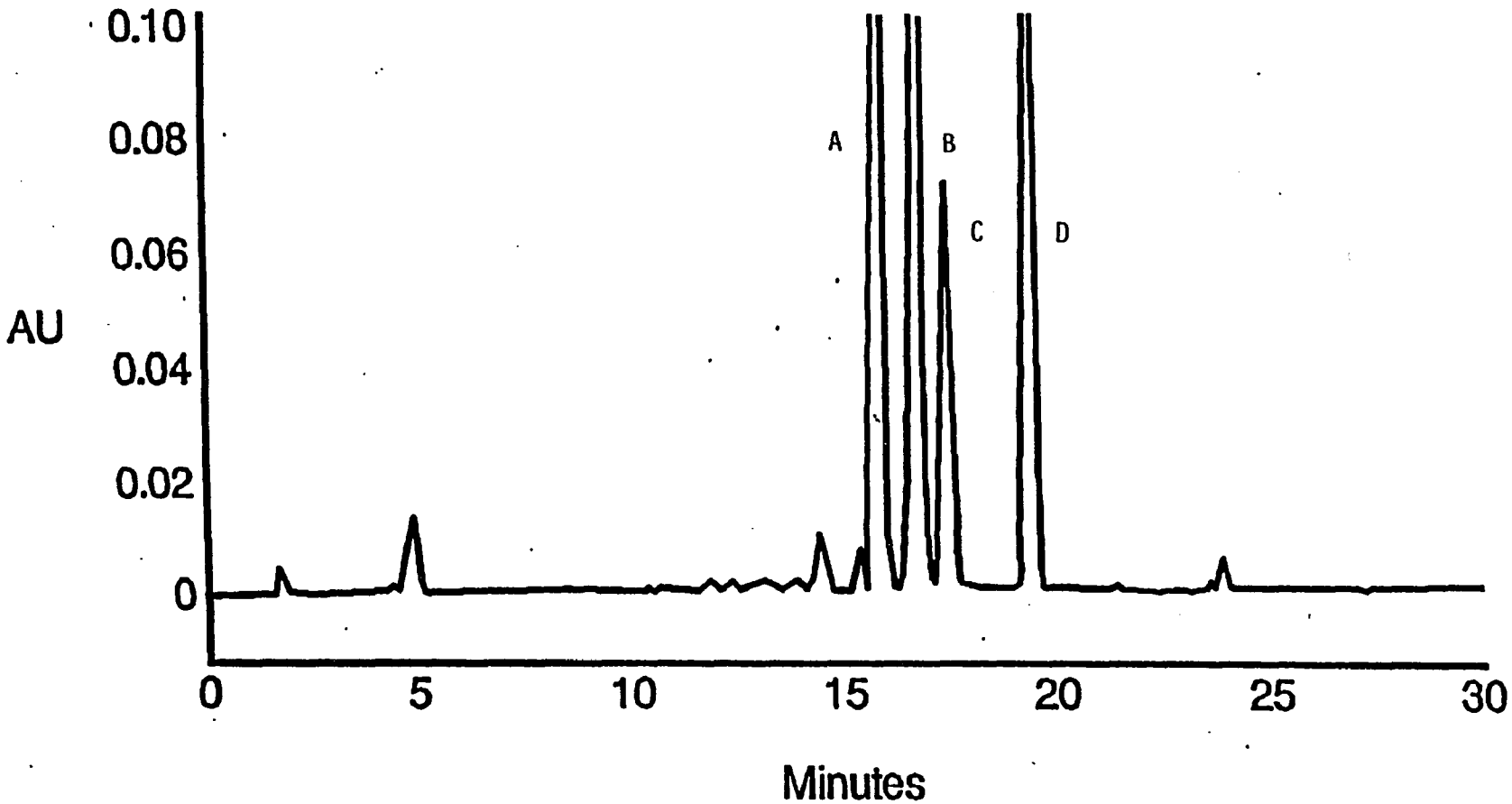


Fig. II-2b Liquid chromatogram of the same extract at 436 nm detection

CHAPTER III

NEGATIVE ION CHEMICAL IONIZATION MASS SPECTROMETRY

Traditionally negative ions have been overlooked in mass spectrometry. This is due partly to the lack of instrumental capabilities necessary for their detection, and fundamental differences in the nature of their production relative to positive ions under electron impact ionization conditions (7,8,9). One of the problems with negative ion mass spectrometry under the low pressure conditions of conventional EI mass spectrometry is the low electron capture cross section for high energy electrons (10).

When the ion source pressure is raised above ca. 10^{-5} torr by the addition of some nonreactive reagent gas, other kinds of reactions can begin to come into play. When Field and Munson (11) realized that an ion such as CH_5^+ could ionize sample molecules by transferring a proton to them in the gas-phase, they realized that the ionization process is totally different from ionization of a molecule by removal of an electron in the electron ionization mode. Chemical

Ionization Mass Spectrometry (CIMS) was born. CI is caused by a chemical reaction between the primary ion (reagent ion) and the sample molecule. In the case of negative ion chemical ionization (NICI) (12,13), two important types of reactions can occur as shown in Fig. III- 1. The formation of low-energy secondary electrons takes place as a part of the reagent gas ionization reactions encountered in conventional positive chemical ionization mass spectrometry. The resulting electrons are at thermal or near thermal energies and are available for capture by sample molecules. The excited anion resulting from this process can relax to a more stable vibrational energy level by collision with reagent gas molecule.

The other type of reaction which depend on the energy involved, is shown in Fig. III-2.

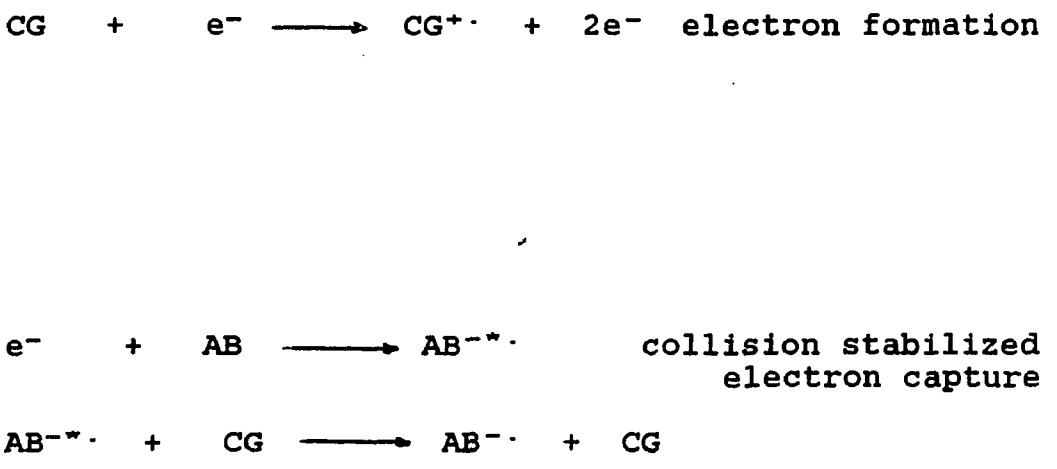


Fig. III-1 Mechanisms by which a non-reactive collision gas (CG) enhances the formation of negative ions from a sample molecule (AB)

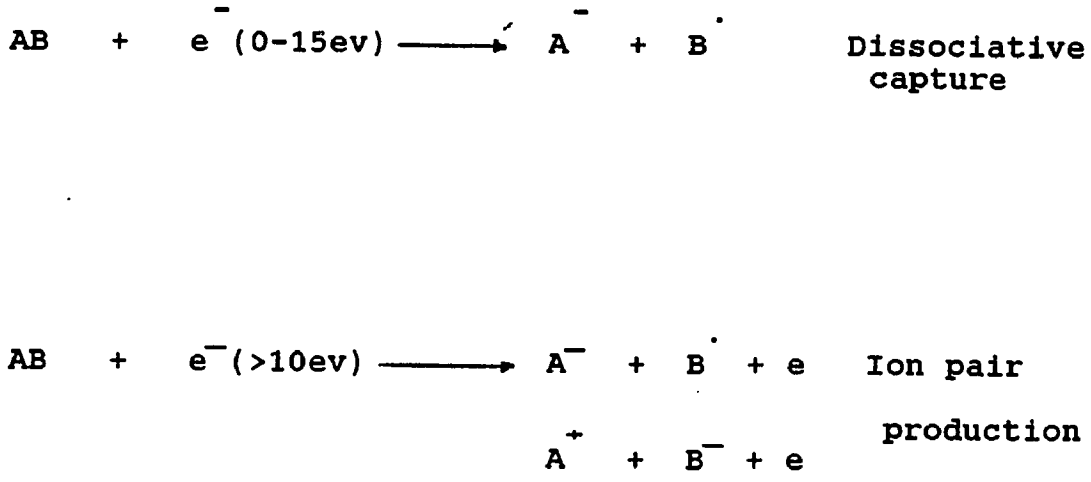


Fig. III-2 Negative ion formation dependent on energy involved

At even higher pressure, various kinds of ion-molecule reactions can also occur. The types of ion-molecule reactions observed under NICI conditions are CHARGE TRANSFER, DISSOCIATION, DISPLACEMENT, ABSTRACTION, ASSOCIATIVE DETACHMENT and TRANSFER.

In short, NICI differs from conventional positive ion chemical ionization (PICI) in several important aspects:-

1. Initial sample molecule ionization comes from interaction with electrons rather than cations. The energy of the ionization electron greatly affects the type of ion produced, which in turn affects the mass spectrum.

2. The sensitivity with which a sample can be detected depends upon the extent to which newly formed and excited anions can be stabilized by the reagent gas molecules present. An optimal source pressure has to be established to achieve maximum sensitivity so that collision processes are minimized to avoid fragmentation and/or electron detachment. Source temperature also can affect the internal energy of the reagent gas which in turn affects the relative importance of collisional

stabilization vs electron detachment processes.(14,15)

3. A wide range of ion-molecule reactions can occur, leading to chemically interesting products. Such reactions have rates that depend on choice of reagent gas and the energies of the ions and neutral species. Such variables cannot be overlooked.

With the development of chemical ionization mass spectrometry, NICI became increasingly more important, but it is still a relative new field of research. As discussed, two areas are of great and potentially interest:

(1) Ion-molecule reactions

(2) Sensitivity and selectivity relative to other methods of ionization.

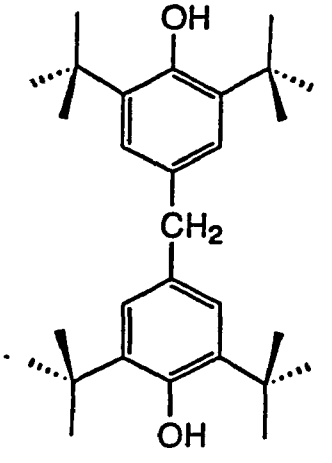
In this research, the sensitivity and selectivity that NICI can offer in the detection, identification and quantitation of substituted conjugated quinones are investigated. One of the most exciting features of NICI is the sensitivity associated with ion formation by electron capture in the ion source, which can be 100-1000 times greater than

that can be available using any positive ion methodology. This leads naturally to NICI's being the method of choice for analyzing certain organic compounds that are electron capturing species in complex mixtures which can be first separated by some kind of chromatography and then introduced into the ion source of the mass spectrometer. Many applications are now being handled by GC/MS.

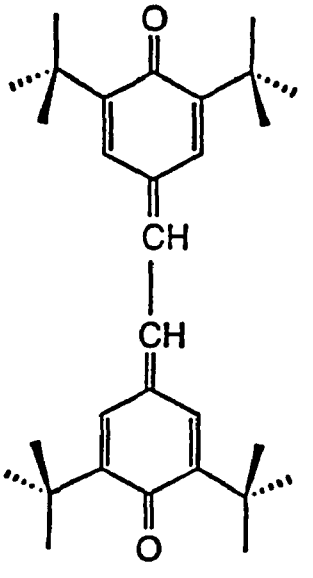
Because of their structures, many compounds are good electron capture candidates and are particularly useful in NICI, especially when molecular ion information is desired. Other compounds which are not electron capture candidates can be made into derivatives that are amenable to NICI. In this research, quinones and their conjugated derivatives are very good candidates for electron capture as seen in SCHEME VI. The product of a single electron capture leads to the formation of a semiquinone stabilized by resonance structures. (16)

Some initial work applying NICI to quinones was demonstrated by GC/MS. A test mixture of approximately equal concentrations of compound (II),

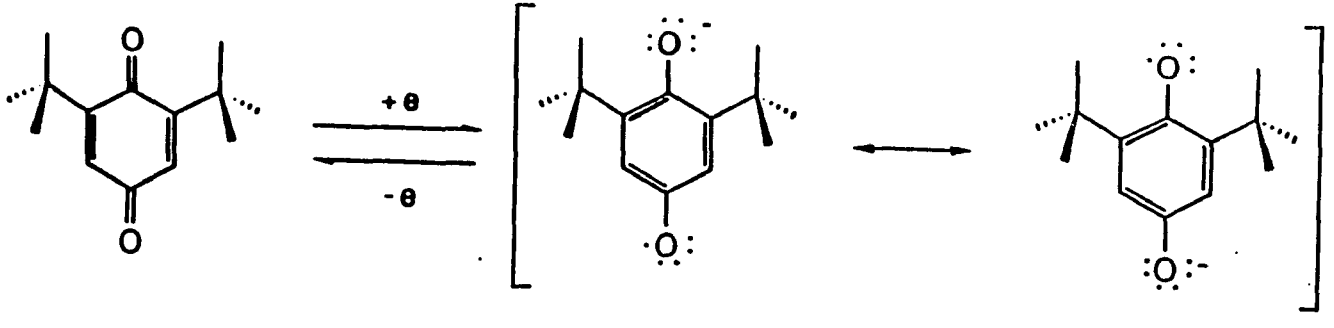
bis-3,5-di-t-butyl-4-hydroxyphenyl methane and 3,3',5,5'-tetra-t-butyl-4,4'stilbenequinone (III) was analyzed. The experimental conditions are described in the following section.



(II)



(III)



SCHEME VI

EXPERIMENTAL

Chemicals and Reagents

Compound (II) was obtained from the Additives Division of Ciba-Geigy Corporation and compound (III) was purchased from American Tokyo Kensel Inc.(Portland, Oregon) and used as is. The chemical reagent gas, isobutane, was from Matheson.

Instrumentation

The mass spectrometer used is a Finnigan Model 4600 single quadrupole instrument equipped with a SuperIncos data system with PPINICI (Pulsed Positive Ion Negative Ion Chemical Ionization)" accessory. The mass spectrometer was coupled directly to a Finnigan gas chromatograph with a 15m DB-1 fused silica capillary column with 0.25 micron film thickness (J & W, Folsom, CA).

" This accessory is an option from Finnigan MAT which enables the recording of positive and negative mass spectra alternately controlled by the Incos data system. The electronics module is called the ion control moduleICM and is described in the instrument's service manual.

Chromatography

The injection port was held at 250°C. The initial temperature was held at 40°C. After sample injection, the temperature was increased to 260°C at a rate of 18°C /min. The upper temperature was held for 10 min. Typically 1 uL of sample was injected.

Mass Spectrometry

(a) Ionization

Three ionization modes, electron ionization, positive chemical ionization, and negative chemical ionization. were performed on the same mixture. In addition, PPINICI was also performed. The reagent gas for chemical ionization, isobutane was present at a source pressure of 4 x 10^{-6} Torr. The filament current was set at 0.35 mA and electron energy at 70 ev.

(b) Reagent Gas

Again Compound (III) was chosen as the standard for testing the effect of different reagent gases on sensitivity under NICI conditions. It appears that reagent gases are more critical in negative ion molecule reactions (17,18) than the overall

sensitivity of the M^- under electron capture resonance conditions.

(c) Source Pressure and Temperature

As discussed previously, pressure and temperature play a very important role in the sensitivity of NICI. Since the two factors are very dependent on the instrument design, the optimum conditions will be described in Chapter IV.

RESULTS AND DISCUSSION

The compounds were chosen based on the applicability of GC/MS. Compound (II) was selected along with (III) to show the differences in the response under the three modes of ionization. Figs. III-3a, 3b and 3c represent the Total Ion Chromatograms (TIC) using of EI, PCI and NCI, respectively. Fig. III-3d is the TIC of the same mixture under the PPINICI mode. Figs. III-4a to 4f are individual mass spectra representative of the two compounds under different modes of ionization. Table III-1 is a summary of the responses of the two compounds under the different modes of ionization. As shown, it

can been easily seen that compound (III) in contrast to (II) not only selectively give better negative spectra but overall sensitivity is also greatly enhanced.

Four reagent gases, isobutane, methane, ammonia and nitrous oxide were used to maximize the sensitivity of compound (III) in the NICI mode. Under similar source pressure @ 4 x 10^{-5} Torr, isobutane was found to show the most enhancement. This is due to the fact that isobutane gives the most abundant thermal electrons under the conditions performed (19,20) as well as acting as a moderating gas for electron attachment.

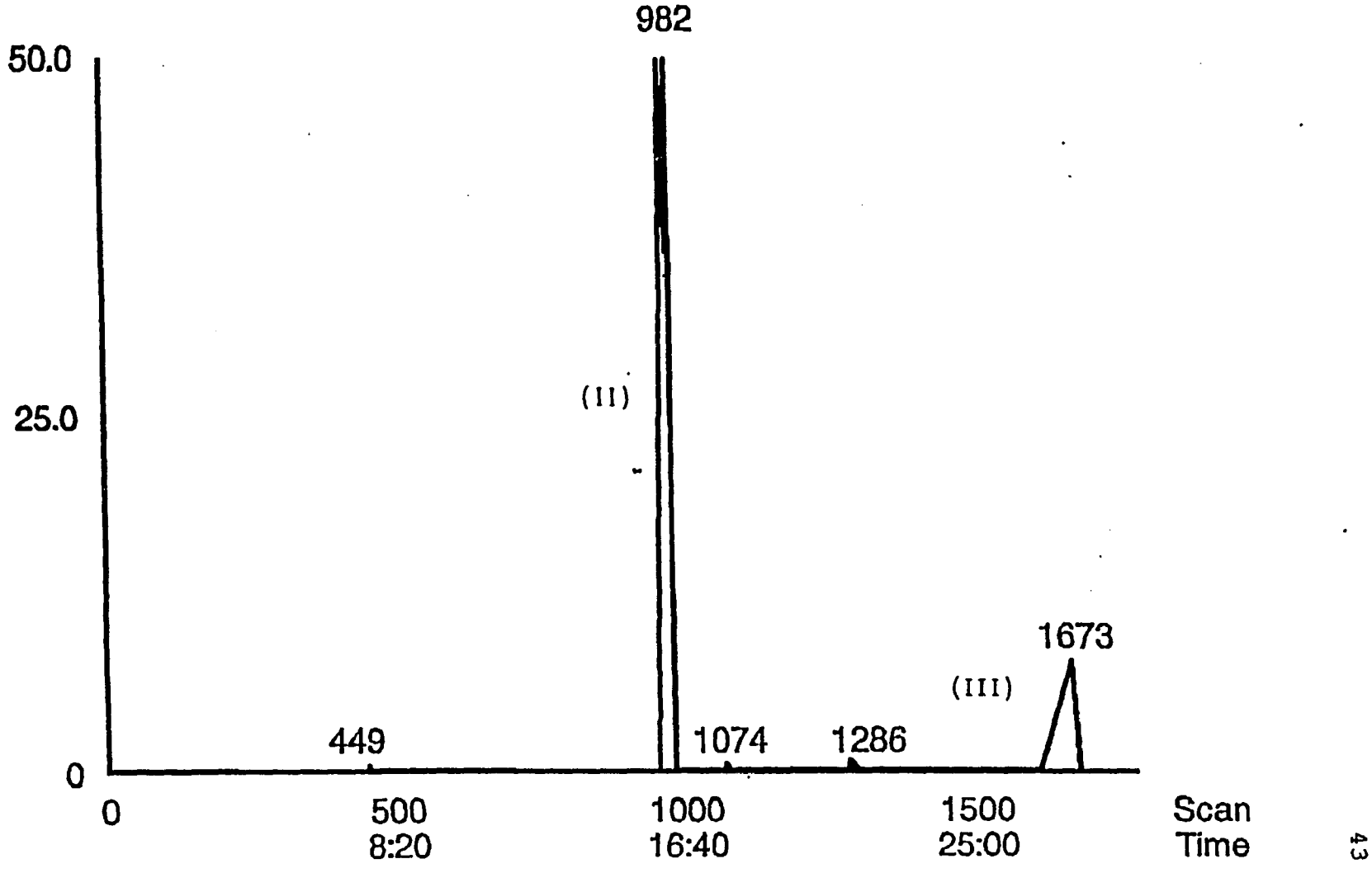


Fig. III-3a Total ion current of a mixture of Compounds (II) and (III) in EI mode

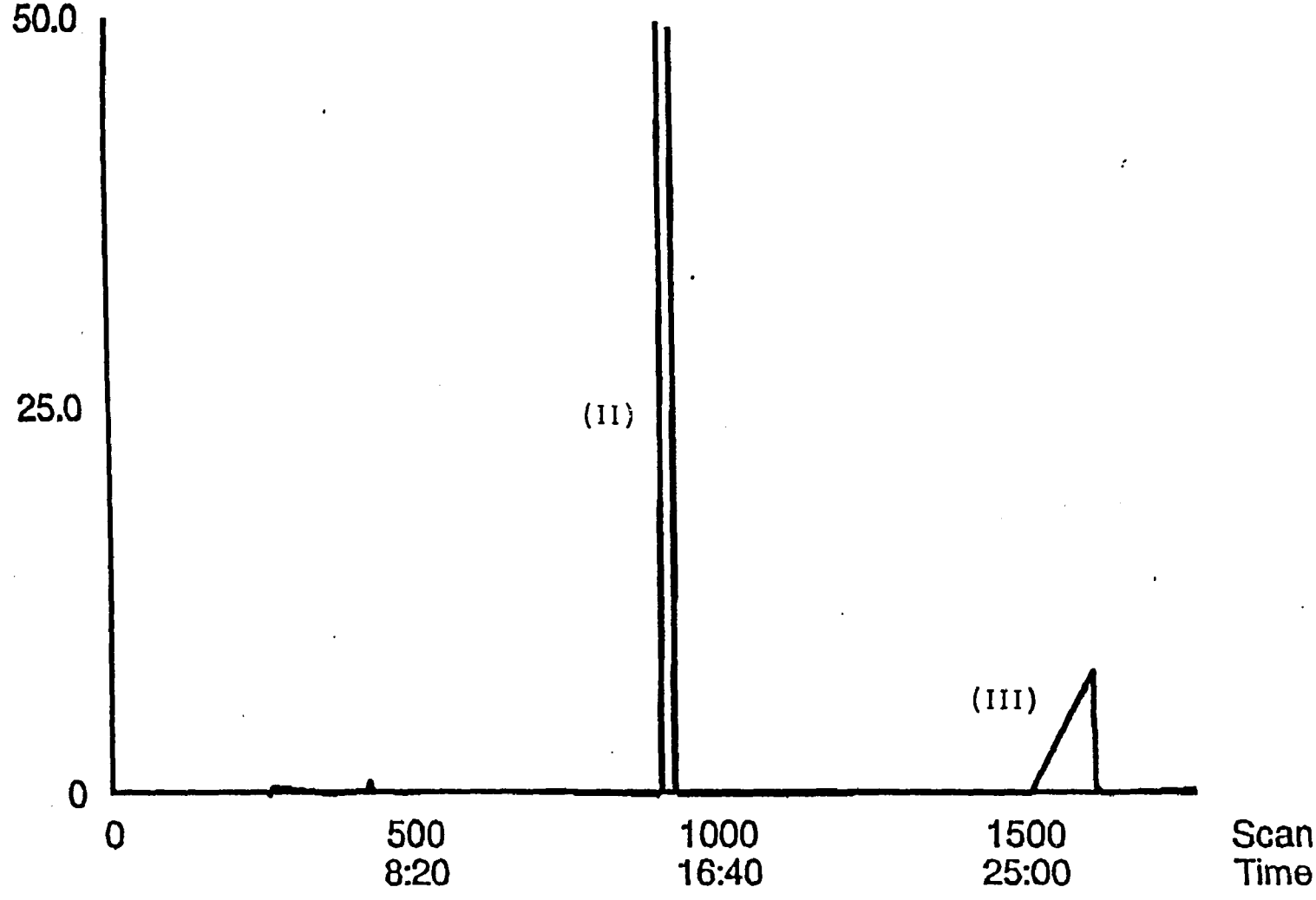


Fig. III-3b Total ion current of the same mixture in PCI mode

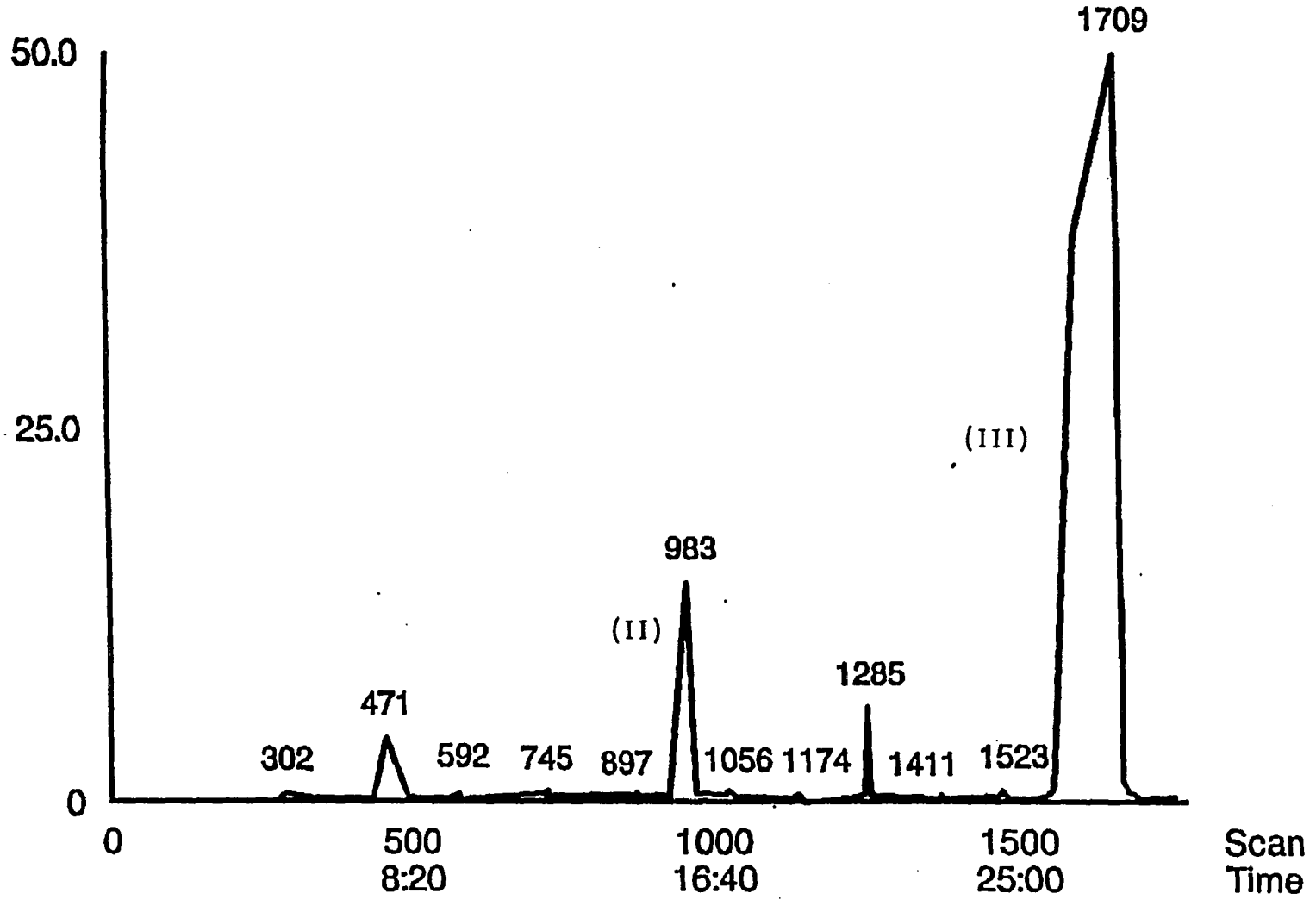


Fig. III-3c Total ion current of the same mixture in NICI mode

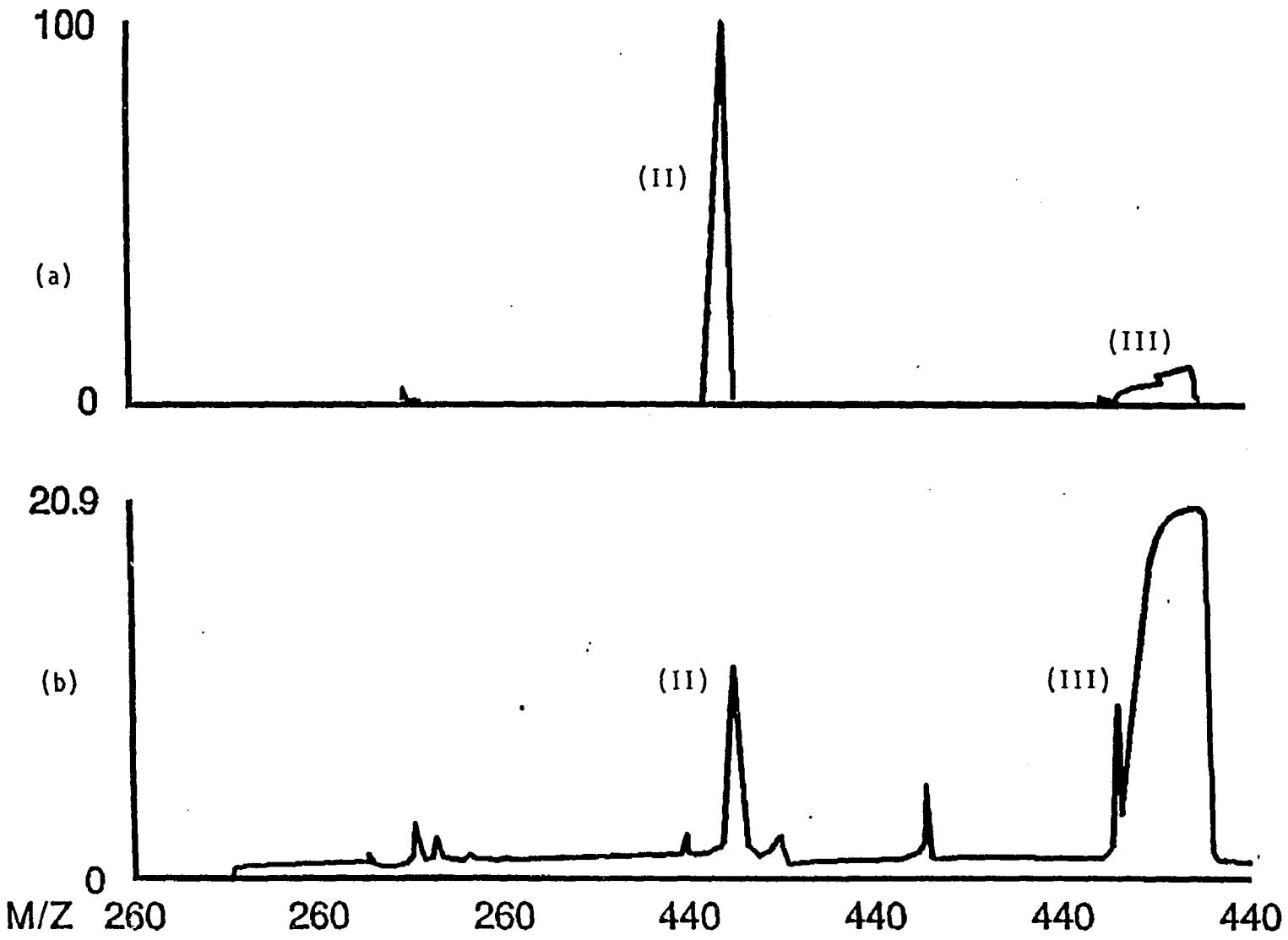


Fig. III-3d Total ion current of the same mixture in PPINICI mode, (a) PCI (b) NICI

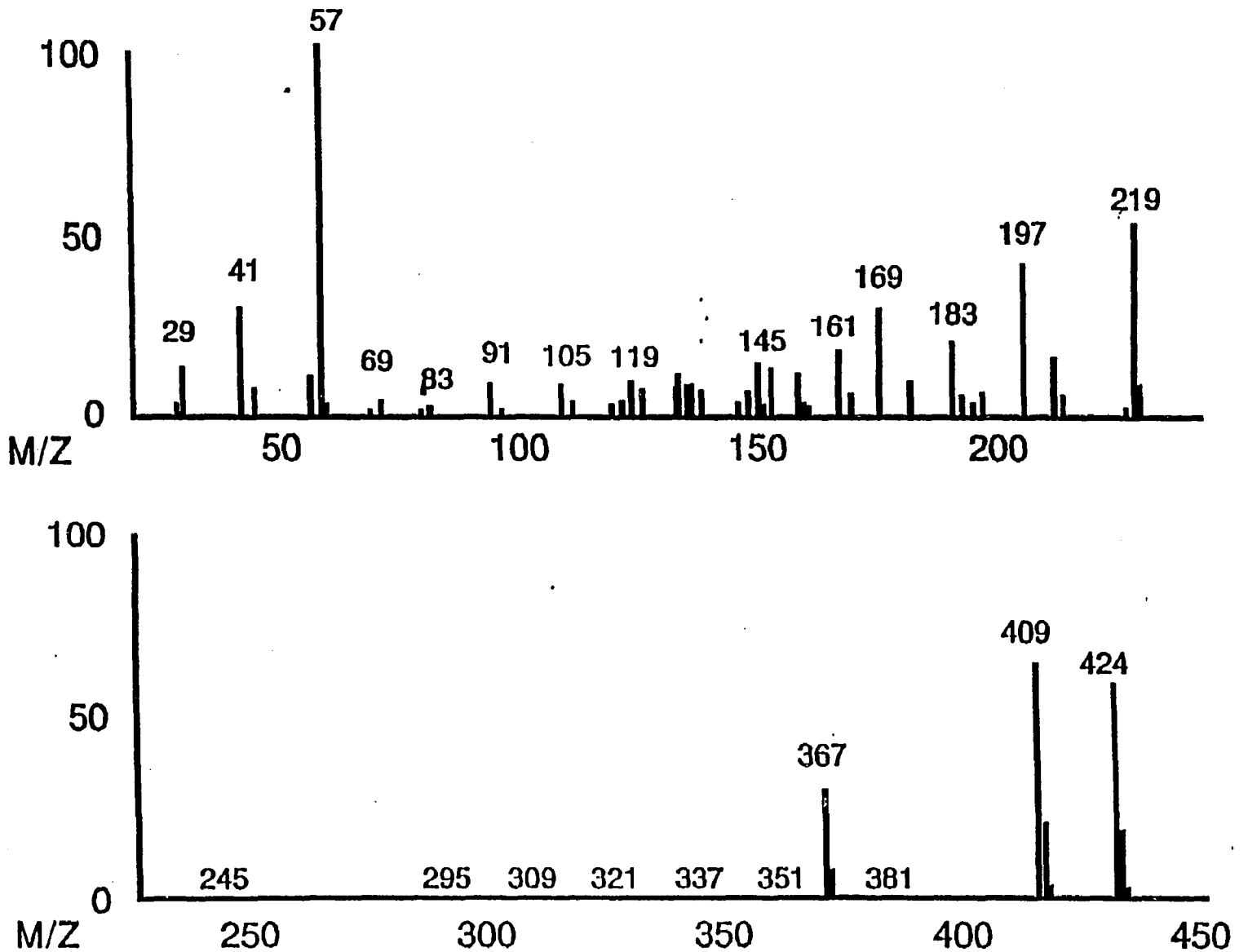


Fig. III-4a EI mass spectrum of Compound (II)

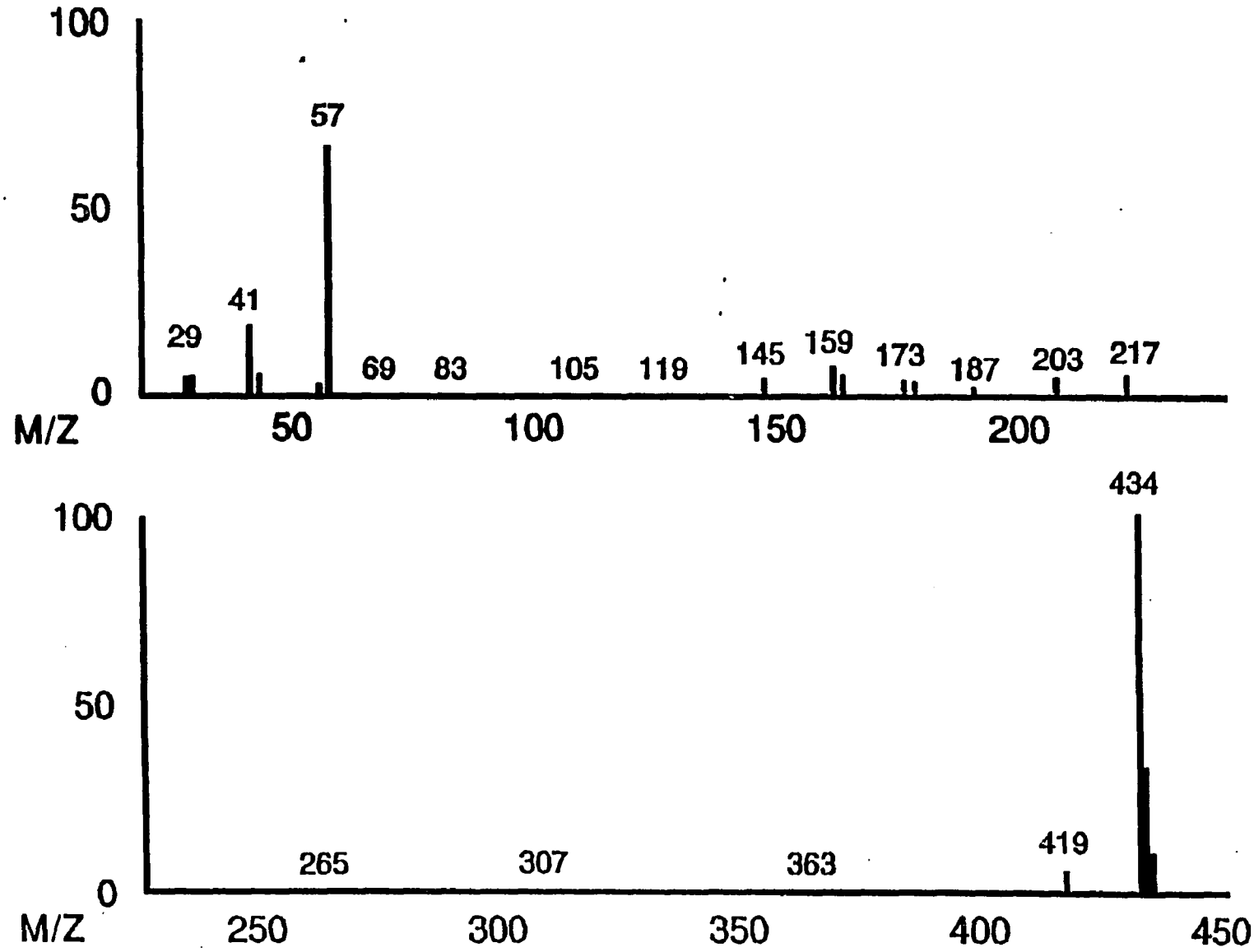


Fig. III-4b EI mass spectrum of Compound (III)

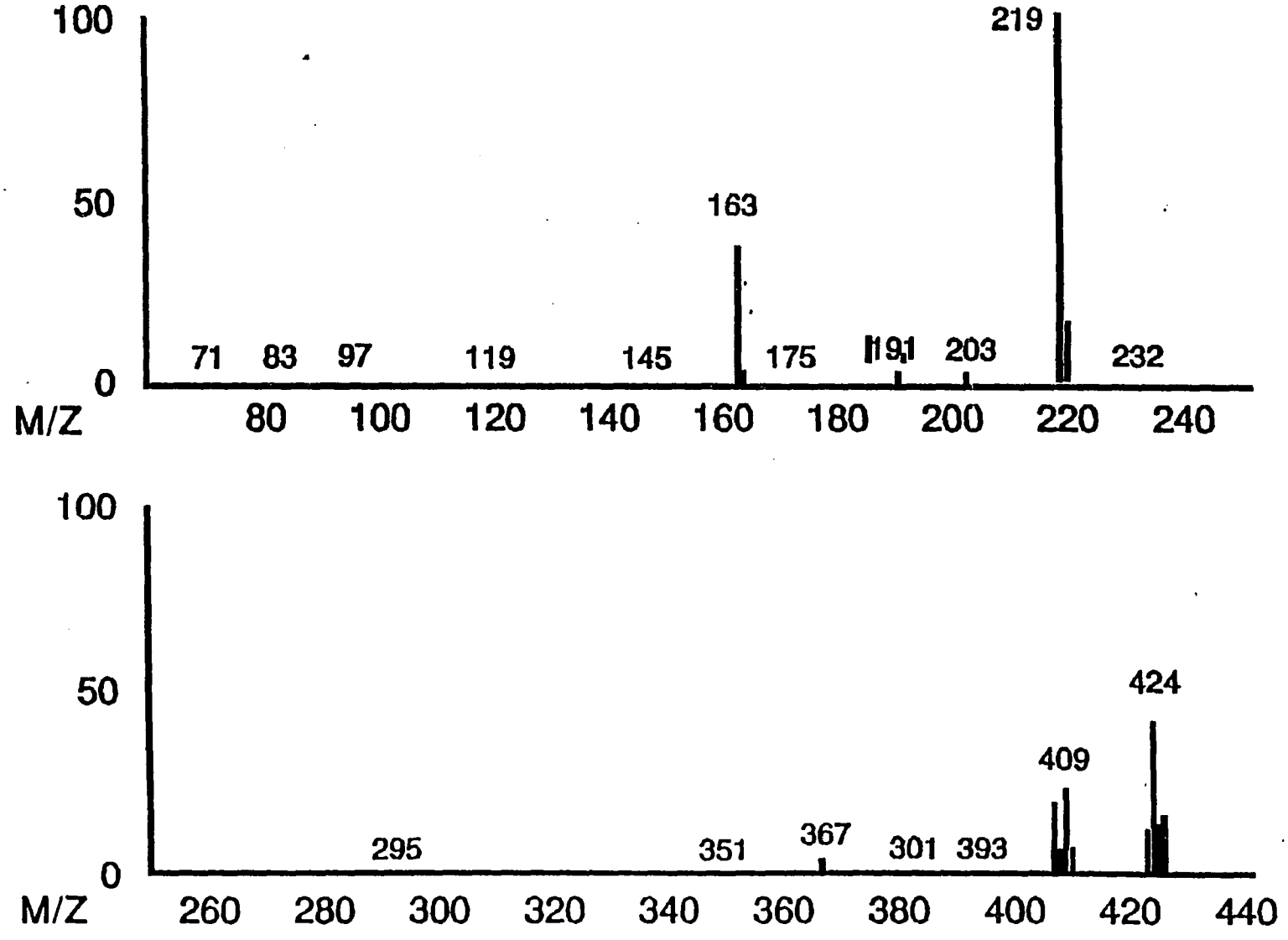


Fig. III-4c PCI mass spectrum of Compound (II)

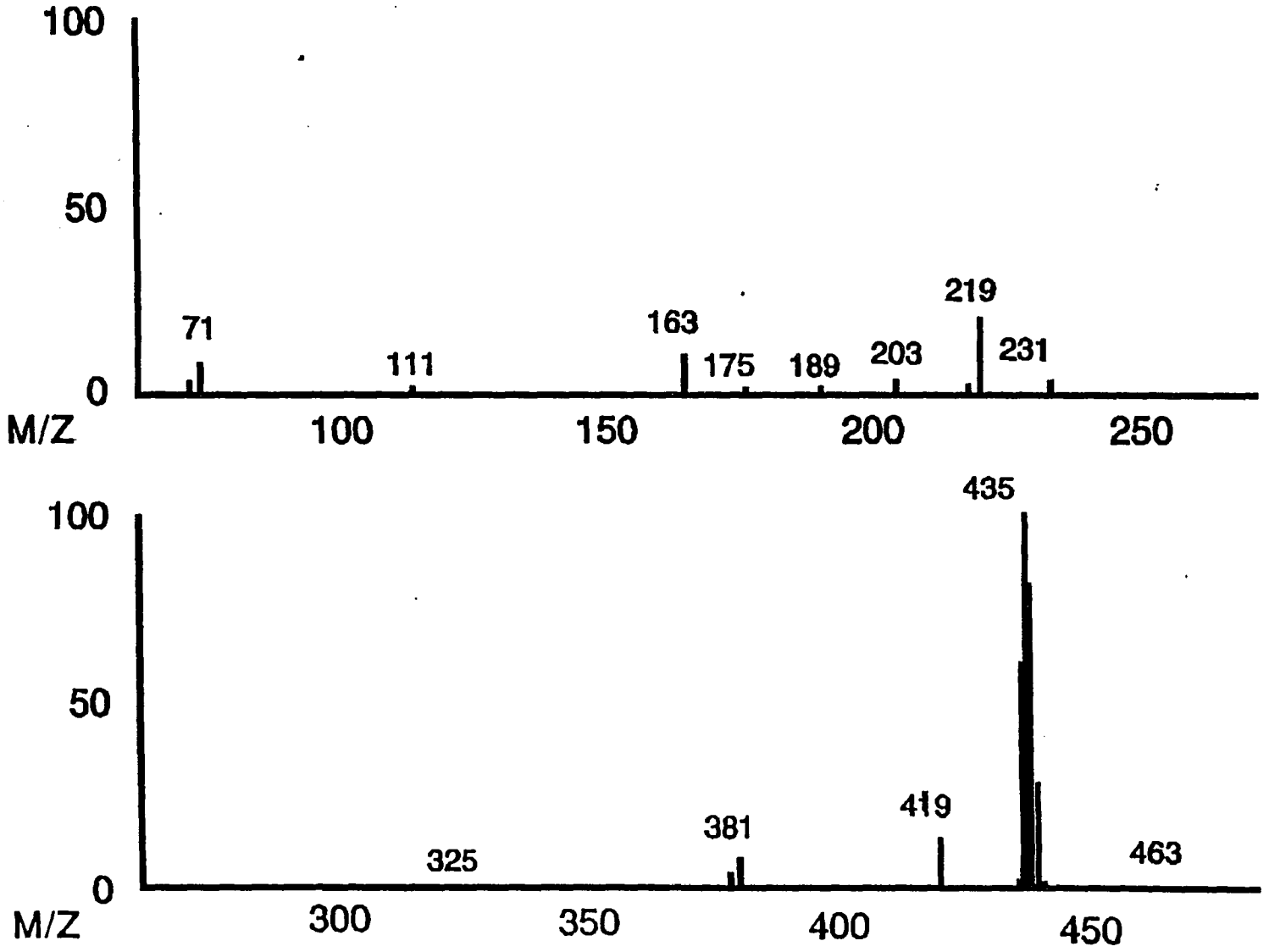


Fig. III-4d PCI mass spectrum of Compound (III)

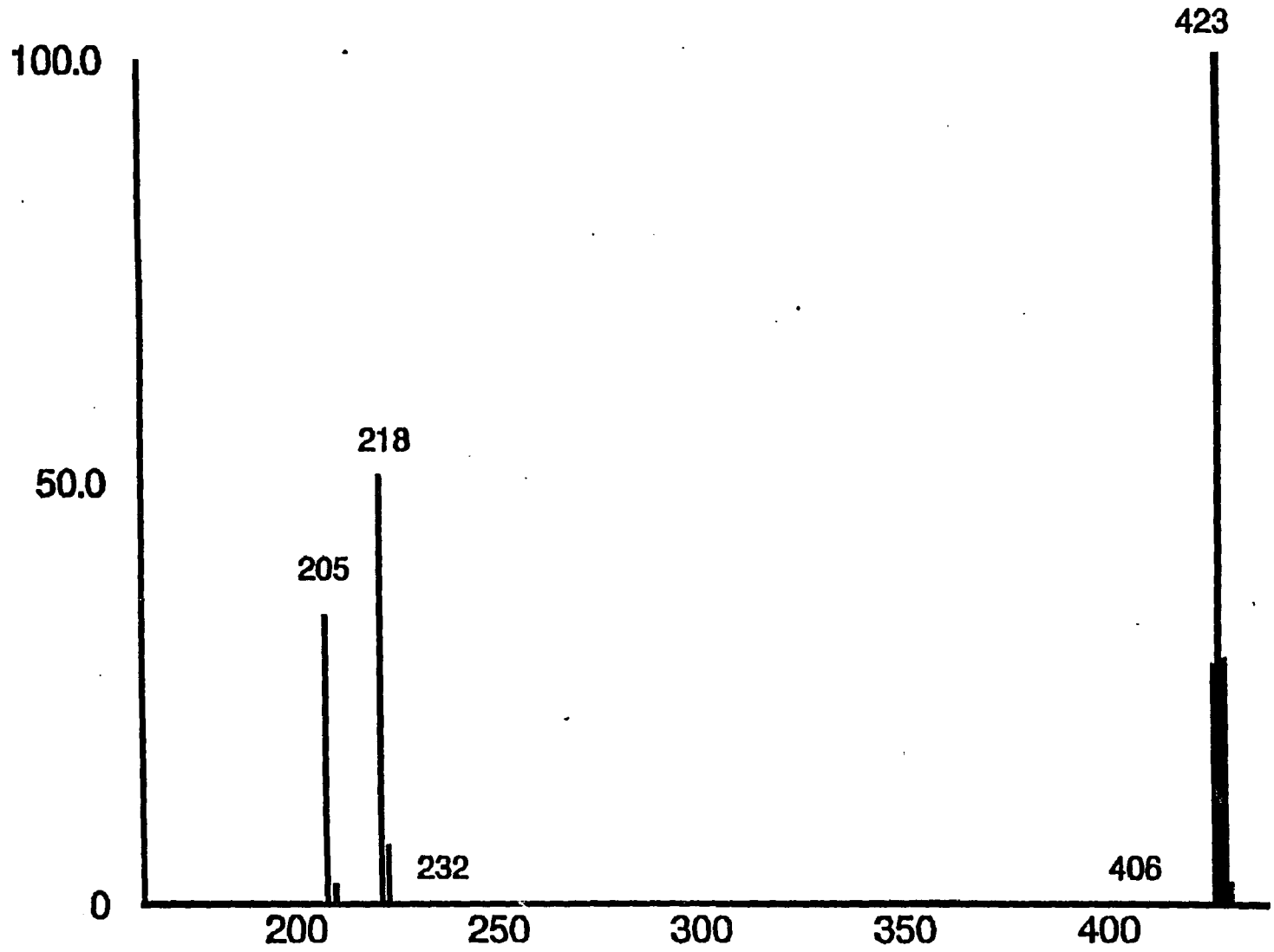


Fig. III-4e NICI mass spectrum of Compound (II)

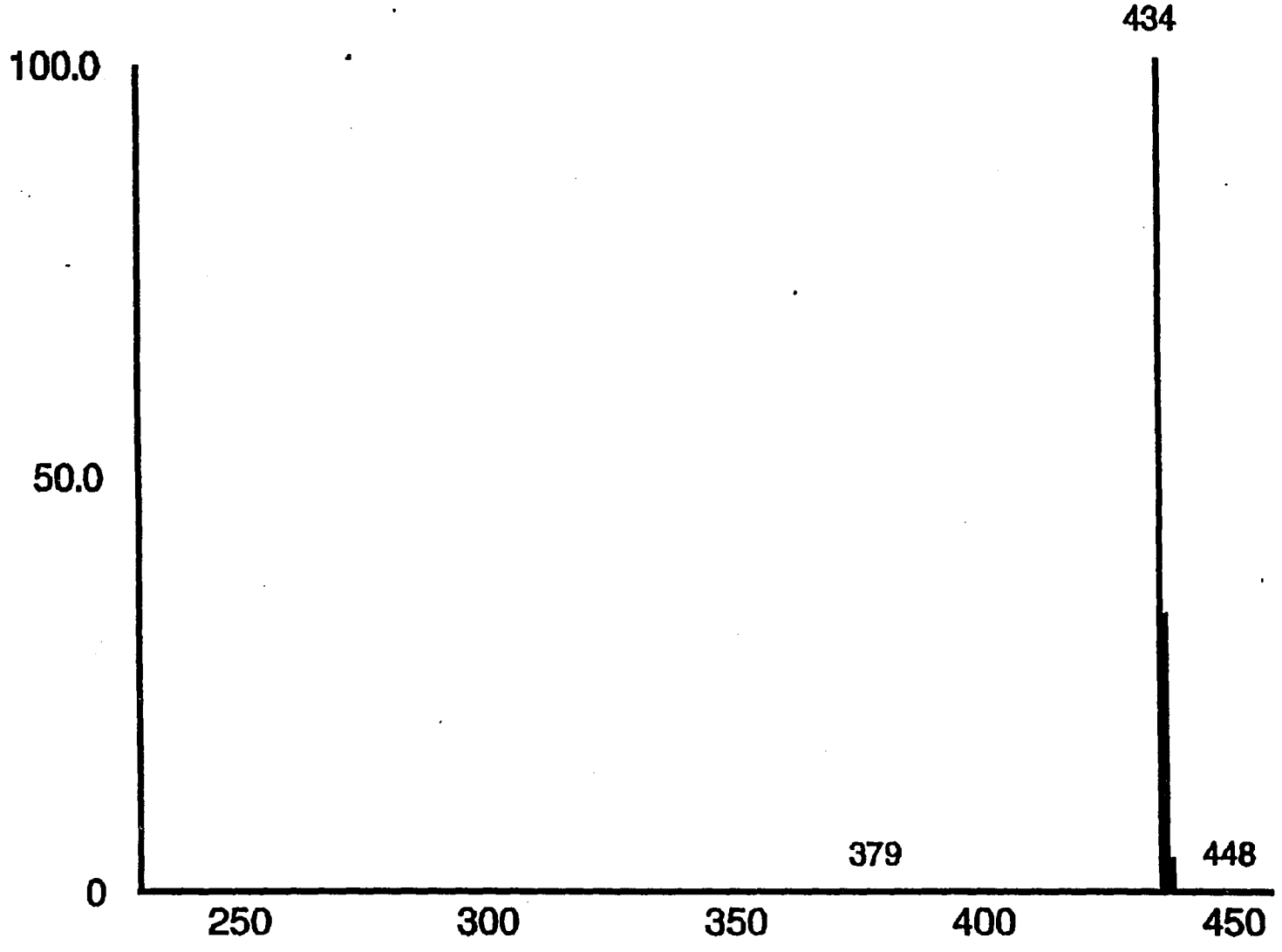


Fig. III-4f NICI mass spectrum of Compound (III)

TABLE III-1

Comparison of ion intensities of (II) and (III) by different modes of ionization

	Compound (II)		Compound (III)	
IONIZATION	IONS	INTENSITY	IONS	INTENSITY
EI	M^+ 424	8,008	M^+ 434	3,040
PCI	M^+ 424	4,960	MH 435	839
NICI	$M-H^-$ 423	2,324	M^- 434	14,800
PPINICI	M^+ 424	8,400	MH 435	1,732
	$M-H^-$ 423	3,792	M^- 434	13,536

INTENSITY measured by peak height

CHAPTER IV

LIQUID CHROMATOGRAPHY - MASS SPECTROMETRY

1. INTRODUCTION

Gas chromatography-Mass spectrometry (GC/MS) is a well established technique which combines two powerful instruments. It still has limitations for analyzing compounds that are either thermally labile or because of their polarities and molecular weights they cannot elute from the gas chromatographic columns. Figure IV.1 is a good approximation of such limits.(21)

Despite the tremendous advances made in the last ten to fifteen years (22), high performance liquid chromatography (HPLC) is still an essentially separation technique. Even with the various recently developed detectors listed in Table IV-1 (23), identification of HPLC peaks still relies on retention times of known standards. For on line identification of unknowns, it is necessary to interface the HPLC unit to a system that is capable of providing structural information about the analytes that elute from the column. Up to date, mass spectrometry (MS) is the most viable choice. To be able

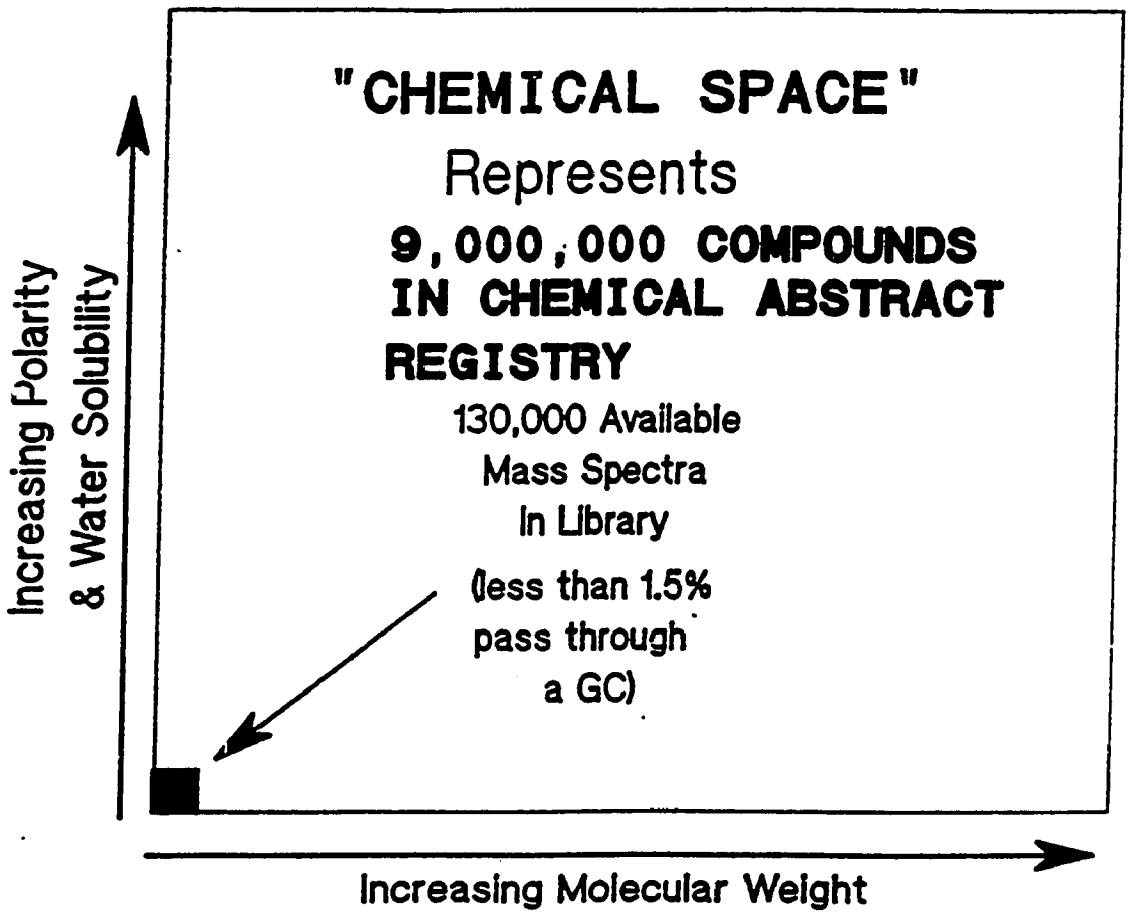


Fig. IV-1 Limitation of GC and GC/MS applications

Table IV-1 SOME COMMON HPLC DETECTORS

Criteria	UV/VIS	RI	Fluro.	Electr.
1. Universality	Medium	High	Low	Low
2. Selectivity	Medium	Low	Very Low	Very High
3. Sensitivity	High	Low	Very High	Very High
4. Gradient	Yes	No	Yes	No
5. Non-destructive	Yes	Yes	Yes	No
6. Structural Information	Low DAD-Medium	Low	Low	Low

DAD = Diode Array Detector

to interface HPLC to mass spectrometry would be ideal, not only to compliment the GC/MS technique but also to expand the analytical capability in all areas of chemistry as seen in Fig. IV-2.(24)

Several approaches have been successful in interfacing HPLC to mass spectrometry. The following is a list of the more accepted practices.

(a) Moving belt (25,26)
(b) Direct liquid introduction (DLI) (27-29)
(c) Thermospray (TSP) (30-32)
(d) Electrospray (ESP) (33,34)
(e) Ion spray (ISP) (33,34)
(f) Continuous flow/ fast atom bombardment (CF/FAB) (35,36)
(g) Particle beam (PB) (37)

To date, there is still not one widely accepted technique. The following is a discussion of the merits and limitations that each technique present.

(a) Moving belt involves the deposition of the HPLC effluent onto a chemically inert belt driven by a mechanical motor in a preliminary evaporation chamber.

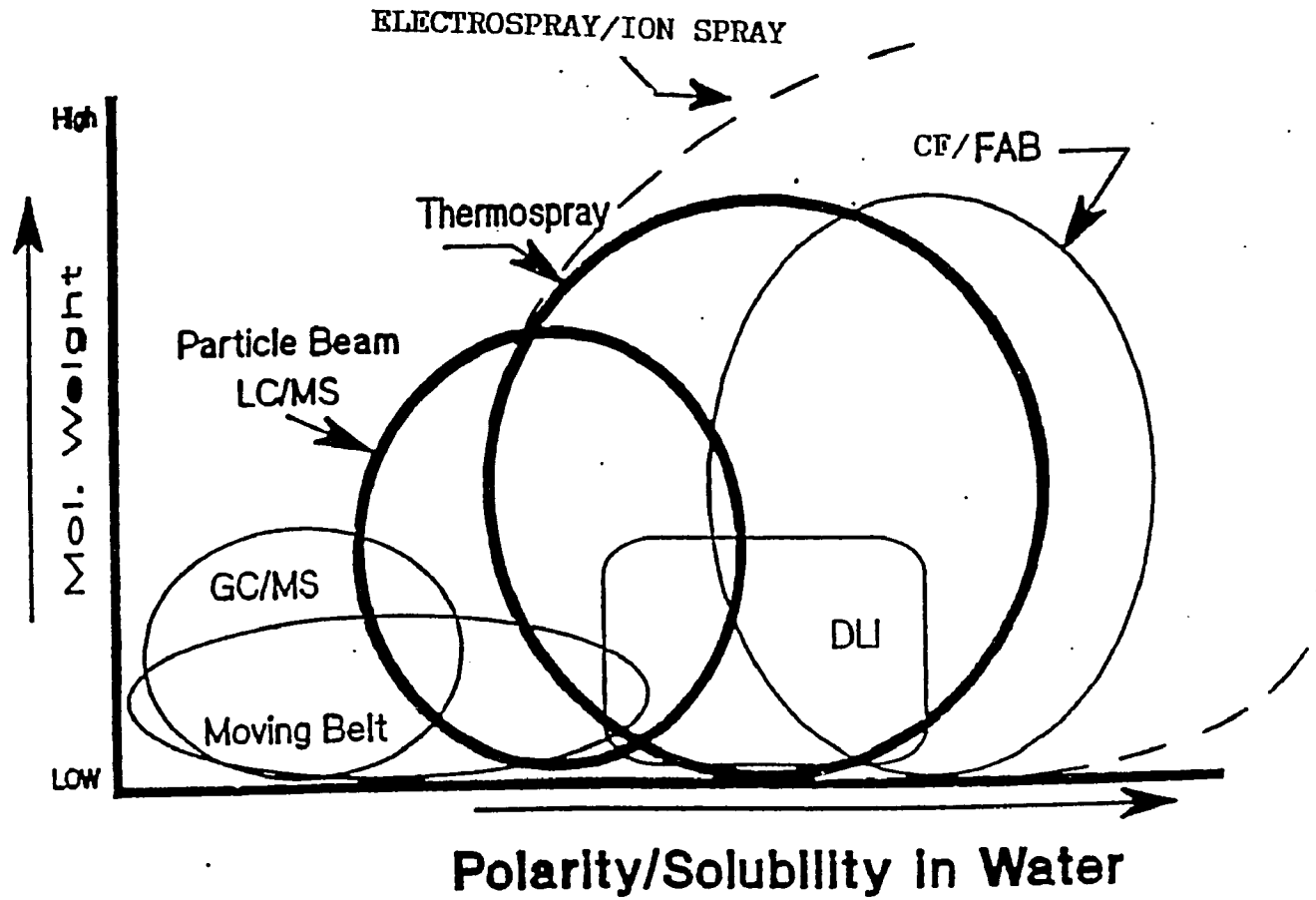


Fig. IV-2 Applicability of various LC/MS techniques

The belt then passes through two vacuum lock chambers and into the mass spectrometer where the sample is vaporized by the heated tip. Figure IV-3 is an illustration of the various parts of the moving belt LC/MS interface. It offers EI and CI spectra with reasonable sensitivity, and is solvent independent. However it suffers several disadvantages such as (1) memory effects, (2) cumbersome mechanical parts, (3) choice of buffers is limited and (4) high concentration of water is unacceptable. This particular technique, the first commercially available LC/MS interface, was quite exciting but is not much used to-day because of these limitations.

(b) The Direct Liquid Introduction (DLI) LC/MS interface is simple in design. The sample is split and only 1 % of the total effluent enters through a 5 micron pin hole into the ion source so that approximately 10 ul/min of the solution continuously enter the CI ion chamber. The bulk of the effluents was wasted and consequently the technique suffers from relative insensitivity. Fig. IV-4 demonstrates how the effluent is splitted and only a very minor of the actual sample gets into the source to be ionized. DLI only offers CI

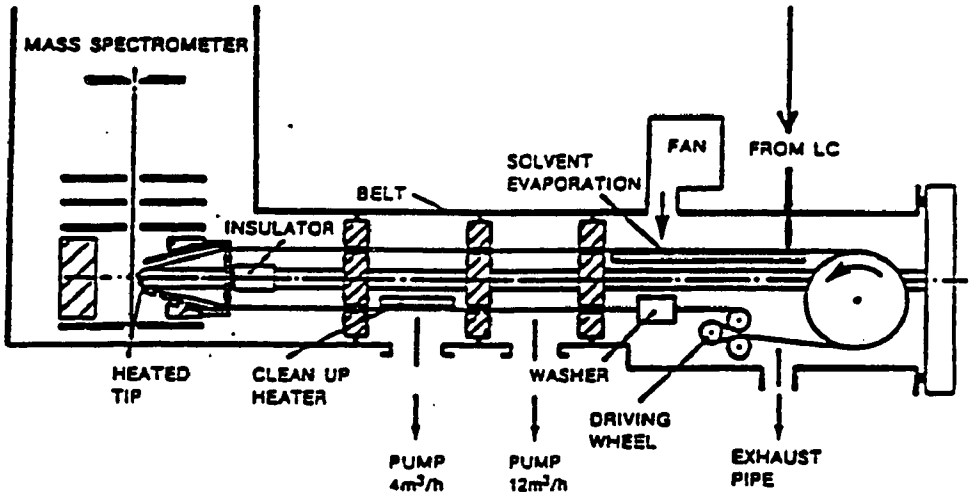


Fig. IV-3 A moving belt interface

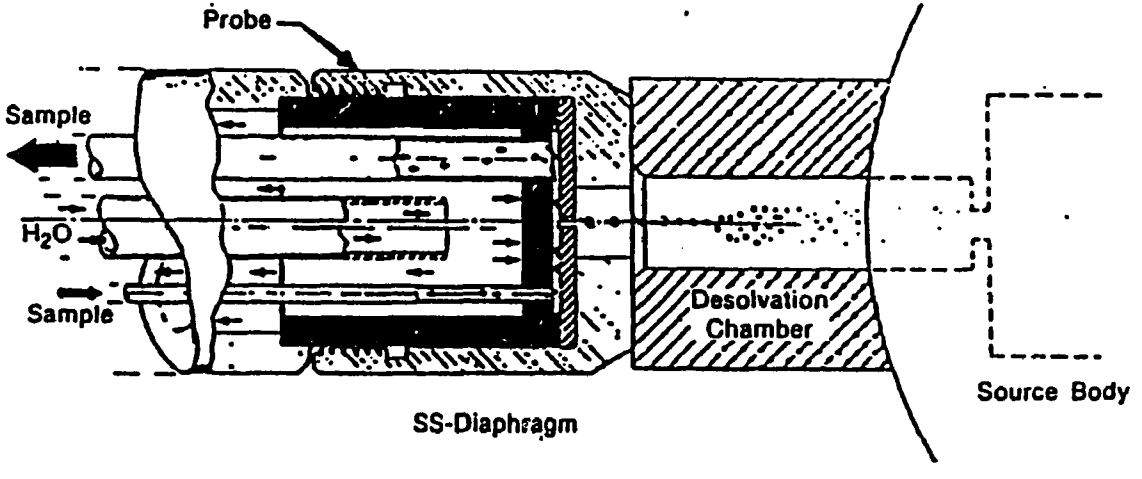


Fig. IV-4 Direct liquid introduction interface

spectra since the mobile phase functions as the chemical ionization plasma. Because of the additional potential problem of clogging of the interface, and the limited spectral information available, this particular technique has assumed very little importance in LC/MS laboratories.

(c) The Thermospray (TSP) interface developed by Vestal and his co-workers has become a very popular technique with a wide range of applications. It combines direct liquid introduction to the source and at the same time provides a soft ionization process. All major commercial mass spectrometer manufacturers have adopted this interface as one their standard options. Thermospray as defined by Vestal is the production of a jet of fine liquid particles by heating. Production of the visible thermospray jet is accomplished by partial vaporization of a liquid stream as it flows through a capillary tube merely by applying the proper amount of heat to the tube. A supersonic jet of vapor is produced which contains any unvaporized material as entrained liquid droplets or solid particles. Since samples of interest in HPLC are generally less volatile than the solvents, these tend to remain preferentially in the

droplets. It was then the thermospray ionization process takes place when the HPLC effluent is in a superheated, nebulized state upon leaving the vaporizer nozzle. Ion formation depends on the extent of desolvation and energy of the nebulized droplets. The direct ionization which occurs as the result of field assisted ion evaporation from charged liquid droplets requires ions in aqueous solution. For non-polar samples and mobile phases, external ionization means are required. These can be in the form of filament or discharge. Fig. IV-5 is the schematic of a Hewlett Packard TSP LC/MS interface with a dedicated ion source. The key to good results when using thermospray is maintaining a constant level of vaporization. Experiments show that peak mass spectrometer sensitivity is achieved when about 95% of the effluent is vaporized 95 % vaporization is commonly achieved when the thermospray probe exit temperature is 5-10°C lower than the temperature at which complete vaporization occurs. Fig. IV-6 shows a plot of control temperature vs. exit temperature. From this plot, the control temperatures which will result in complete vaporization and 95% vaporization can be determined. Such plots, called probe surveys, will have to performed according to the mobile phase, the flow rate, the rate

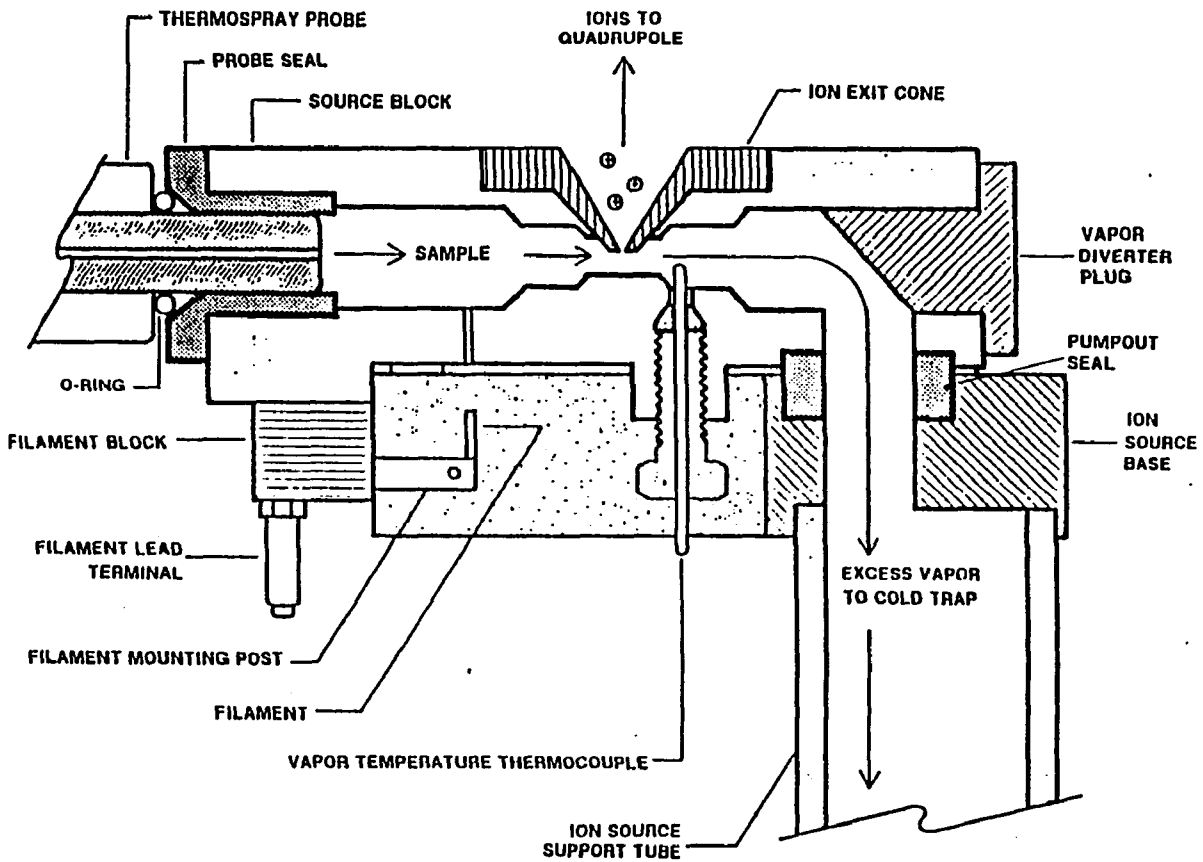


Fig. IV-5 A Hewlett-Packard TSP interface and dedicated ion source

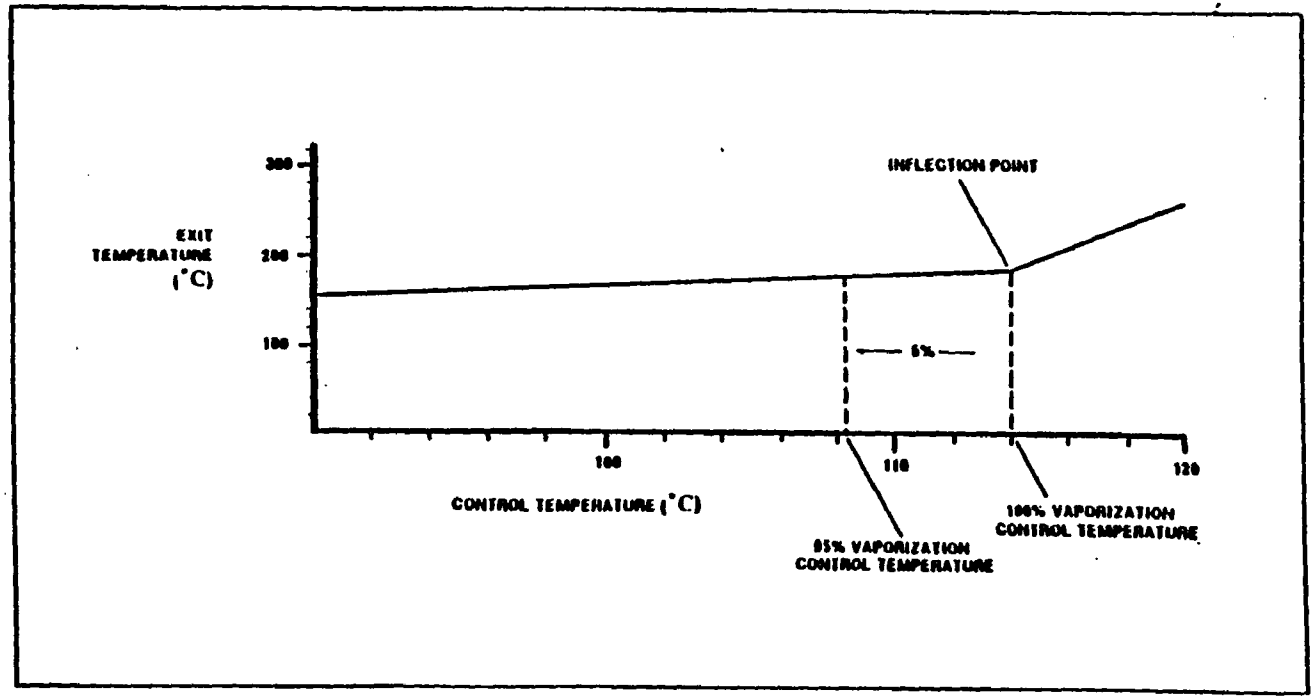


Fig. IV-6 Probe Survey. Exit temperature vs. Control temperature

of heating and the characteristic of individual probe. TSP has several advantages compared with other interfaces. There are no moving parts, it can analyze thermally labile and nonvolatile ionic organic compounds, and it can accept conventional HPLC flow rates. The technique does have limitations. Generally the results are strongly compound-dependent and usually only a pseudo-molecular ion and a few fragment ions that provide limited structural information are seen. Modification of the ion source such as addition of glow discharge or tandem mass spectrometry can help to generate fragmentation. However, TSP still suffers from not producing classical EI spectra. Hindered phenol antioxidants have been tested and found to ionize poorly by TSP.

(d) & (e) Electrospray (ESP) and ion spray (ISP) (pneumatically assisted electrospray) are gaining popularity since they offer alternate ionization efficiencies, especially on certain classes of compounds. In all LC/MS applications, uniform-sized droplets are formed from which analyte ions may be desorbed or ionized. This process involves the disruption of the bulk liquid surface to overcome the

surface energy of the solvent. Three common ways used are (a) heat of vaporization, (b) pneumatic nebulization incorporating a high-velocity jet of gas to shear droplets from the liquid stream, and (3) electrostatic potential to create an electric stress to generate droplets. These three approaches can be used alone or in combination to achieve the varying degrees of droplet sizes. Fig. IV-7 is a simple representation of how ion spray works. Both techniques generally suffers the same disadvantages as TSP in not producing conventional EI and CI spectra. These two new techniques are more applicable in biomedical polymers.

(f) Continuous flow /fast atom bombardment (CF/FAB) offers another LC/MS interface by transferring the HPLC effluent through a fused silica capillary directly to the continuous flow FAB probe. Usually a few percent of glycerol is added to each solvent of the mobile phase to assist ionization. The HPLC is normally operated at a flow of 5 ul/min. FAB ionization is a soft ionization process and applicable only to polar and ionic compounds. It is a "dirty" technique because the use of matrices such as glycerol for ionization causes tremendous source contamination.

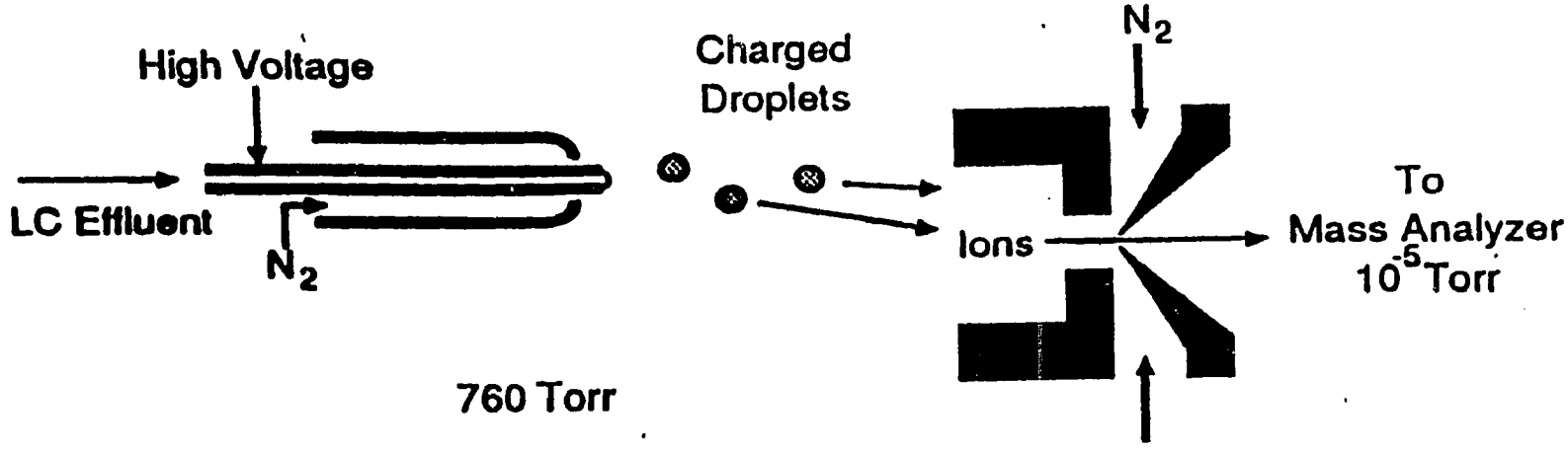


Fig. IV-7 An ion spray interface

(g) The Particle beam interface developed by Browner et al., based on the original " MAGIC" interface (Monodisperse Aerosol Generation Interface Combining LC with MS) (37) has been recently successfully commercialized and found to be a very versatile interface. A variety of chemical compounds have been shown (38) to be amenable to LC/MS analyses through the use of the PB interface. Since the analytes enter the source as dried particles, they can be ionized using the conventional EI/CI configuration, conventional, library searchable mass spectra are obtained.

Because of the availability of LC/MS instrumentation as well as the more conventional way of obtaining classical EI and CI spectra of this type of sterically hindered conjugated quinones, the Hewlett Packard LC/MS system equipped with the particle interface was chosen as the main tool to be used for this research.

A detailed description of its configuration and features and limitations will be discussed in the next section.

2. LIQUID CHROMATOGRAPHY/ PARTICLE BEAM/ MASS SPECTROMETRY (LC/PB/MS)

(a) OPERATING PRINCIPLE

The basic idea of the particle beam interface is as depicted in Fig. IV-8. The solvents in the mobile phase are efficiently removed while the solvent-free analytes in small diameter particles transverse in a tight beam to reach the mass spectrometer. In order to achieve this, the particle beam interface is made up of (i) nebulizer (ii) desolvation chamber (iii) momentum separator and (iv) transfer tube. The incoming LC effluent goes through a fused-silica capillary tube where helium as the dispersing gas is fed coaxially to form an aerosol. This is sprayed into the desolvation chamber at a pressure of about 200 torr. Upon entering the momentum separator, the flow rate is supersonic. When the analytes exit the second skimmer, they are essentially solvent-free and at a pressure of less than $2x10^{-2}$ torr. They then pass onto the ion source of the mass spectrometer through the transfer tube.

(b) PERFORMANCE

The three main process that affect the performance of the LC/PB/MS operation are (i) aerosol

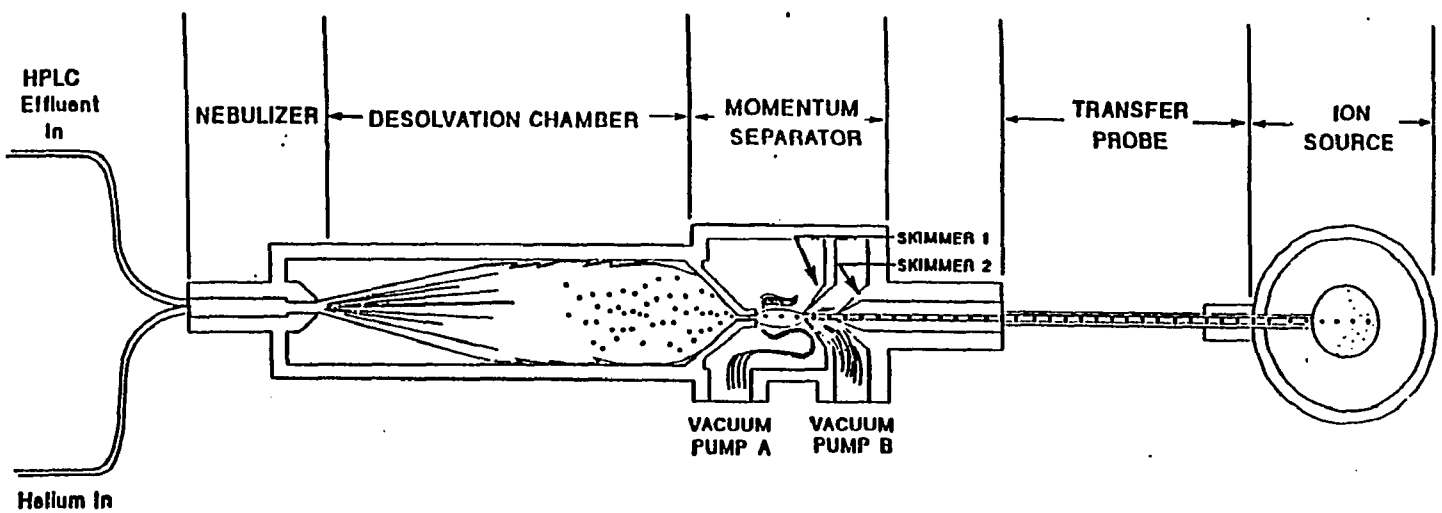


Fig. IV-8 A Hewlett-Packard particle beam interface

formation and (ii) analyte vaporization and ionization, and (iii) solute transport efficiency.

(i) Aerosol Formation

Droplet size is controlled by (i) nebulizer position, (ii) helium flow rate, and (iii) temperature of the desolvation chamber. A 10 um i.d. orifice of the nebulizer can produce uniform droplets of approximately 20 um diameter but they can vary with different composition of the mobile phase. The helium flow is a means to avoid coagulation of the droplets after aerosol generation and to provide dispersion to the desolvation chamber. Figs. IV-9 and 10 illustrate how these two factors independently affect the solute transport efficiency. (iii) has the least effect and usually is kept at 50°C. Only when high content of water is used in the mobile phase then the temperature is raised to avoid condensation in the desolvation chamber.

(ii) Analyte Vaporization and Ionization

Except for thermally labile compounds, it is advisable to maintain the source temperature of the mass spectrometer at about 300°C so that the high temperature

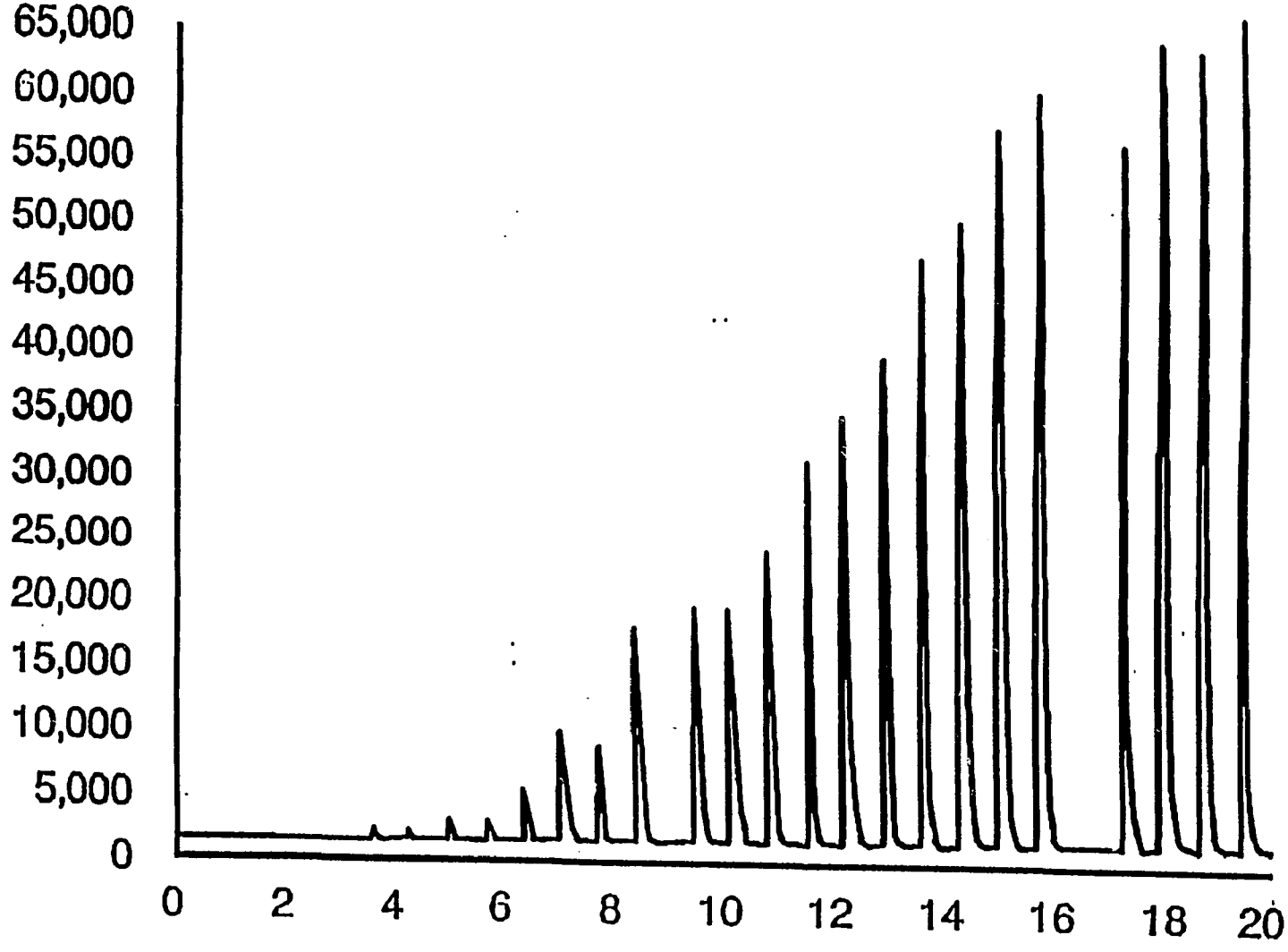


Fig. IV-9 Influence of nebulizer position on particle beam sensitivity. Each TIC peak represents a different setting of the nebulizer

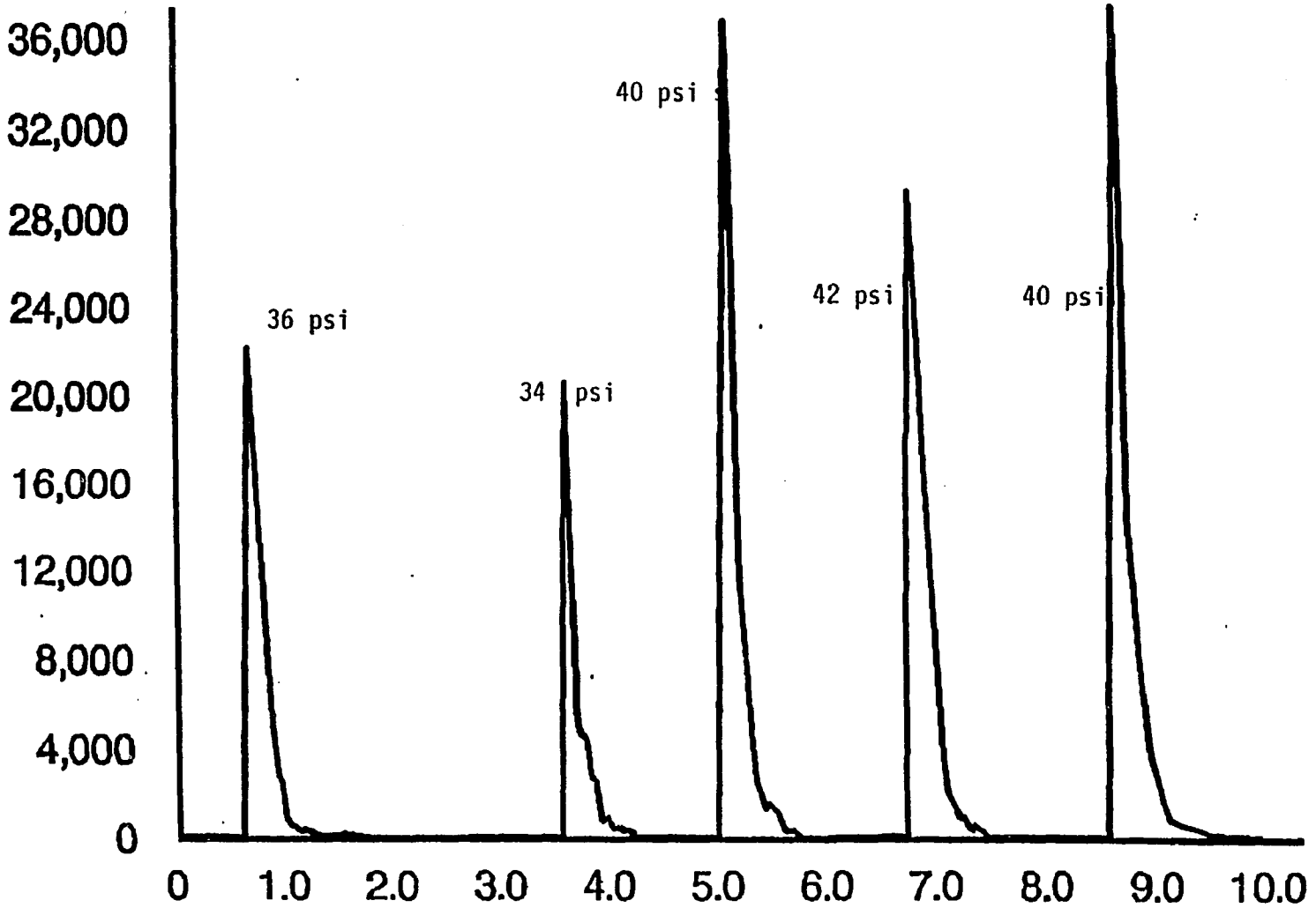


Fig. IV-10 Influence of helium flow on particle beam sensitivity. Each TIC peak represents a different pressure setting for Compound (III)

can assist the vaporization of the particles (39). Fig.IV-11 illustrates how the particle aggragates from the transfer probe get vaporized when they hit the hot source wall and then being ionized by the conventional electron ionization. As Seen in Fig. IV-12, Compound (III) shows no significant change in the sensitivity at the three temperatures, 240°, 270° and 300°C. in the NICI mode. The source temperature appears to have no significant effect on the vaporization and ionization for this type of compound and the source temperature will be kept at 300°C. for the entire study.

(iii) Solute Transport Efficiency

The mobile phase composition (36,40,41) plays a very significant role in the sensitivity of the system. For compound (V), Figs. IV-13a and 13b clearly demonstrate the significance of the mobile phase composition in the enhancement of the signal as the solutes enter the ion source of the mass spectrometer. The mechanism is not quite understood. Several suggestions have been made. These are:

(1) Molecular clusters formation. The analytes and the solvent form an effective mass through hydrogen

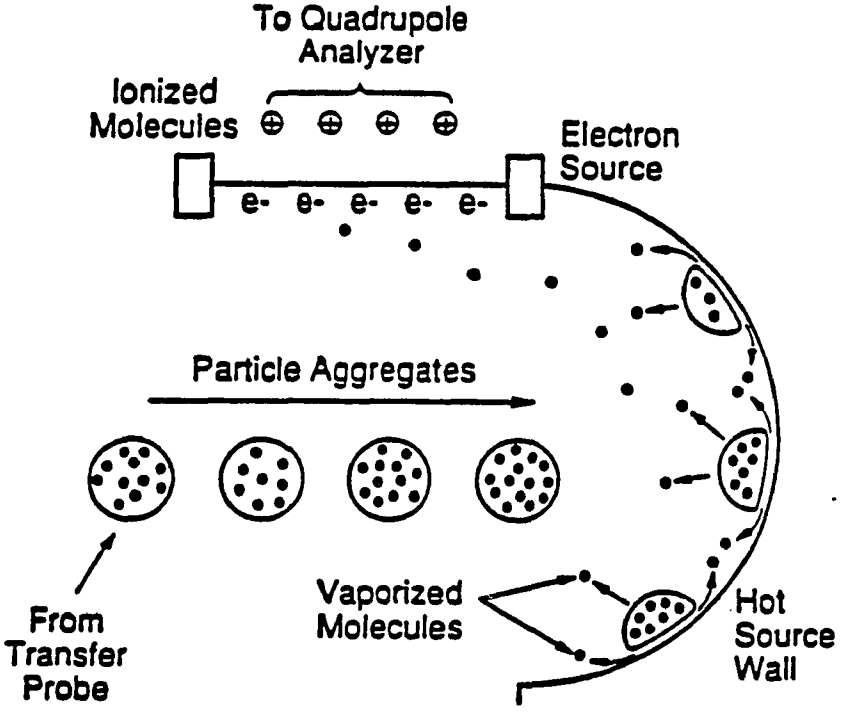


Fig. VI-11 Particle Beam EI ionization process

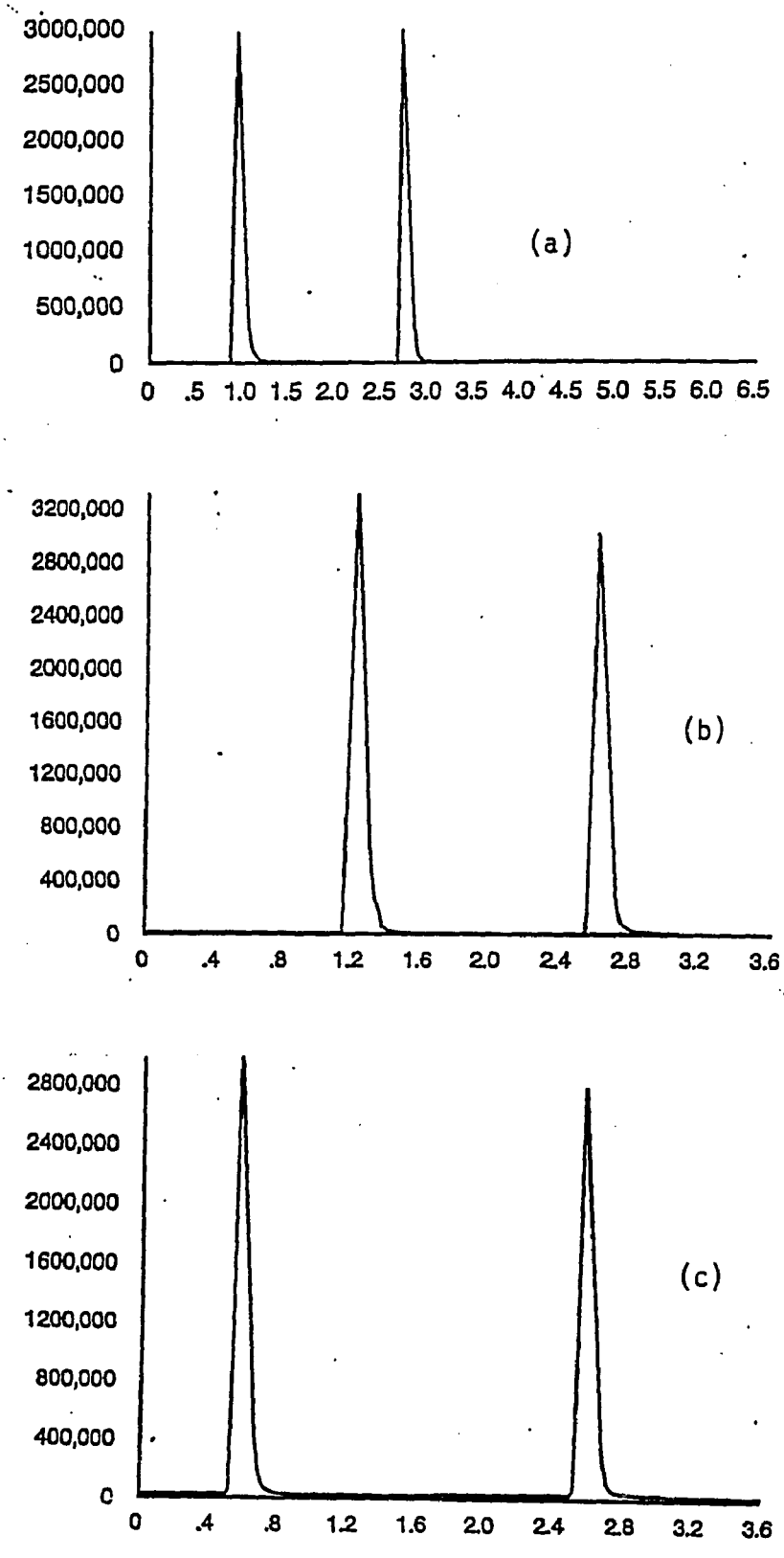


Fig. IV-12 Source temperature effect on Compound (III) in NICI mode, (a) 300°C (b) 270°C. (c). 240°C

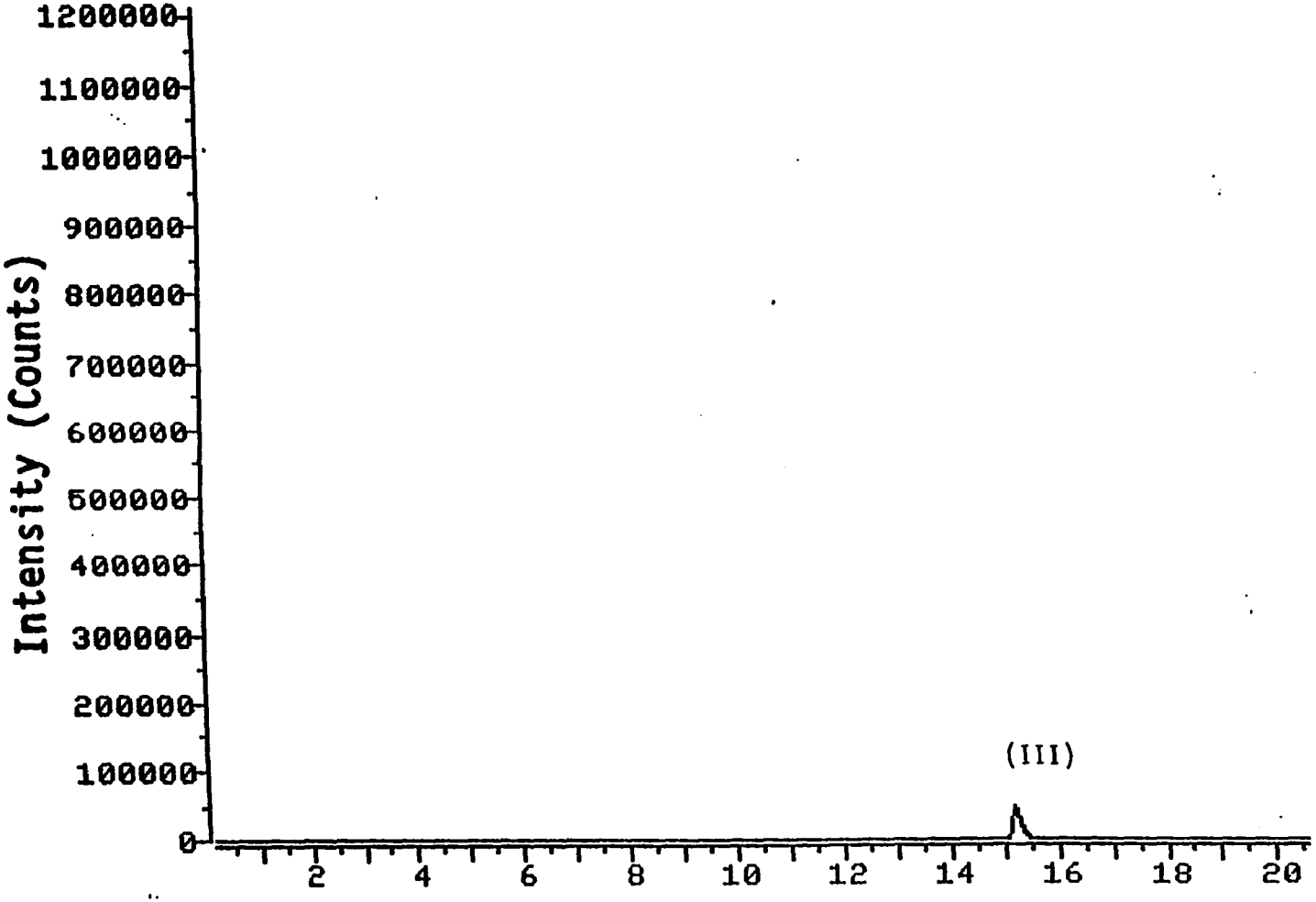


Fig. IV-13a Total ion current of Compound (III). HPLC conditions: mobile phase: CH3CN/H2O(50/50) to CH3CN linear gradient in 12 min. Flow rate: 0.2 ml/min. Post column addition: CH3CN at 0.3 ml/min.

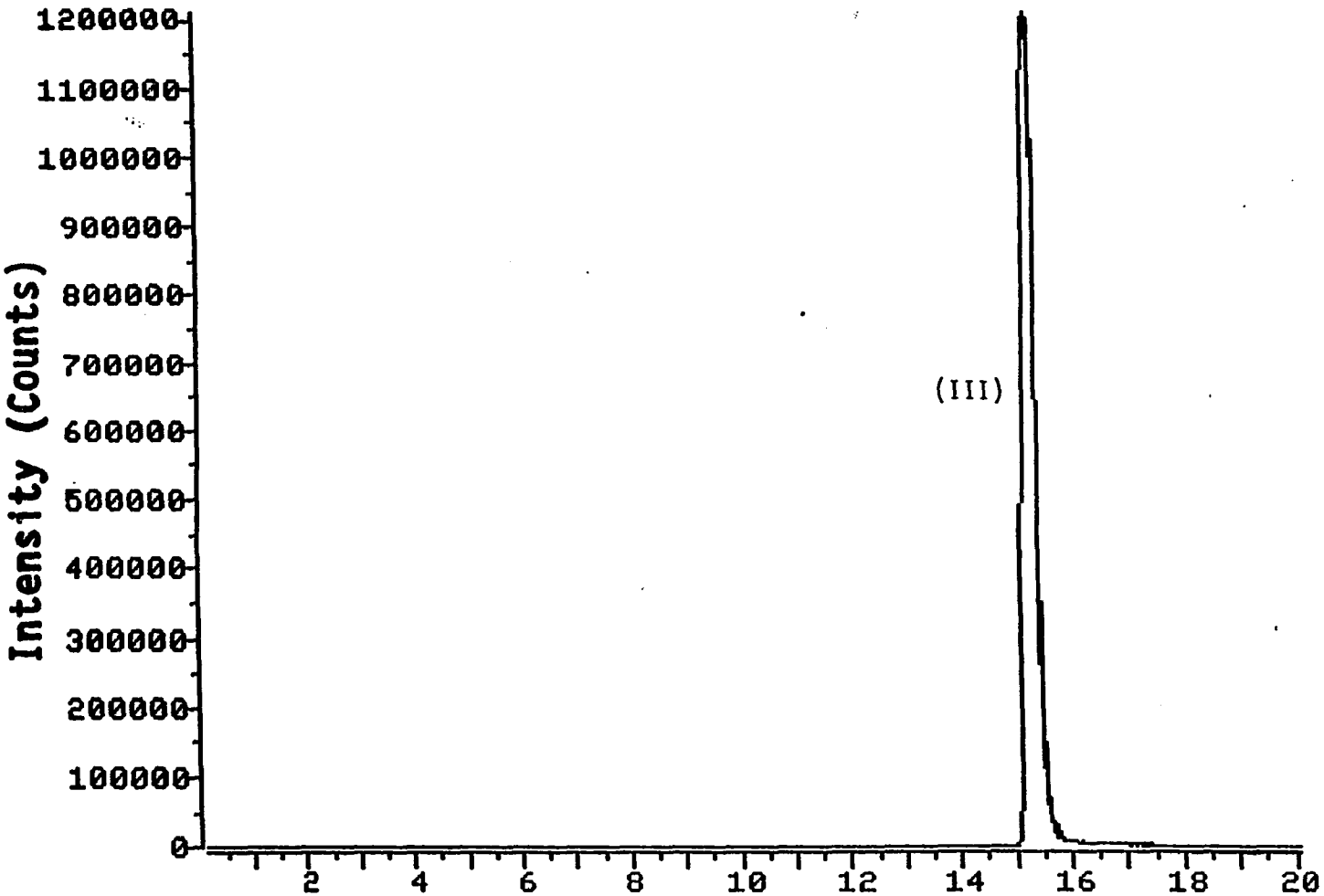


Fig. IV-13b Total ion current of Compound (III). HPLC conditions identical to Fig. IV-3a except for post column addition condition which is: methanol/ehtyl acetate(25/75) at 0.3 ml/min.

bonding or weak dipole-dipole interaction thus reducing the vapor pressure of the analytes and subsequently reduce the loss of the analytes in the momentum separator.

(2) Reduction in droplet size. The change in solvent from methanol to water increases the surface tension/density ratio resulting in the formation of larger droplets which in turn causes more difficulty to desolvate these larger droplets due to size and greater heat capacity of the water over methanol.

(3) Solvent volatility. More volatile solvent has higher cooling effects on the analytes. Rapid evaporation would result in a sudden temperature drop of the analyte, lowering its vapor pressure and, consequently, reducing solute evaporation in the desolvation chamber.

(c) REAGENT GAS PRESSURE

Based on the results discussed in Chapter III, because isobutane gives the most stable molecular ion and highest sensitivity, it is chosen as the reagent gas

for chemical ionization operation. The optimum source pressure is 2 x 10^{-4} Torr. The route of introduction of the reagent gas was modified as as depicted in Fig. IV-14. This allows the reagent gas to enter the ion source chamber with the analytes, maximizing the probability of collision of the analytes with the reactant gas.

(d) LIMITATIONS

The HPLC conditions have to be modified to be compatible with the particle beam interface, the operation which in turn is governed by the mass spectrometer.

(i) Flow Rate

The optimum flow rate is 0.5 ml/minute. At higher flow rates the particle beam interface cannot evaporate all the solvent, which then enters the ion source. At the recommended flow rate, the ion source pressure is usually at 2 x 10^{-5} Torr. which is about the limit for electron ionization.

(ii) Buffers

When the HPLC requires a buffered mobile phase,

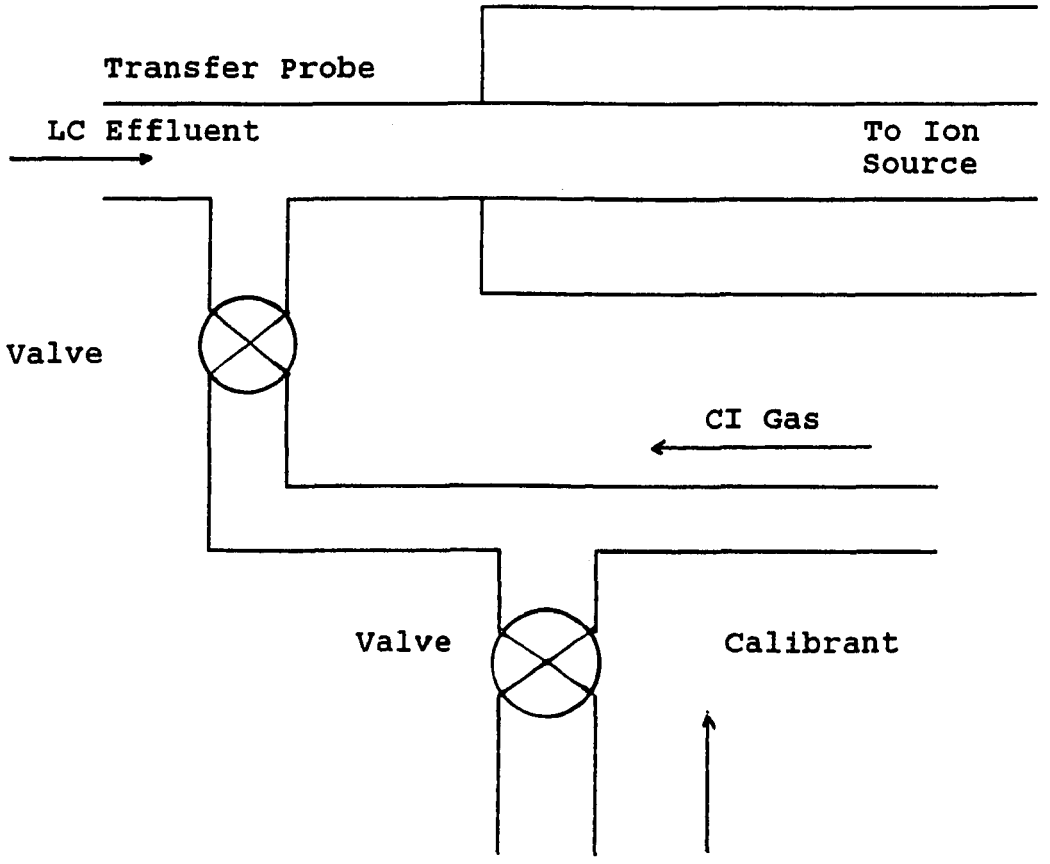


Fig. IV-14 Route of CI Reagent Gas

only volatile buffers can be used in order not to clog the skimmer orifice and interrupt the analysis. The common buffer of choice is ammonium acetate.

(iii) Columns

Short analytical columns are advisable because of the slower flow rate. This avoids peak broadening which affects the sensitivity of the mass spectrometry. Columns 8 cm. x 4mm i.d. have been found to be satisfactory.

(iv) Sensitivity

The sensitivity of LC/PB/MS is very compound-dependent. Linearity is often lacking over a short range in the low concentration level (< 2%). This is problematic for both quantitative and qualitative analyses. Enhancement of ion abundance of analytes during coelution again limits the applicability of this technique for quantitative analyses (40).

3. LIQUID CHROMATOGRAPHY/NICI MASS SPECTROMETRY COMPARED WITH GAS CHROMATOGRAPHY/MASS SPECTROMETRY FOR THE ANALYSIS OF ANTIOXIDANTS

Although GC/MS offers generally overall better sensitivity, especially using capillary columns, LC/NICI/MS offers two advantages that GC/MS cannot match for the application under study.

(a) LC/MS is applicable to a much wider range of compounds, such as those resulting from the degradation of hindered phenol antioxidants. Some conjugated quinones that were found as degradation products of many commercial or experimental antioxidant have very high molecular weights which exceed the volatility limit of gas chromatographic elution. Compound (IV) in Figure V.2 of Chapter V is such an example. Moreover, the use of NICI compensates for the poorer sensitivity of LC/MS.

(b) There is a correlation between the detection of a colored component by a visible light absorption detector and the response to the same component by the MS detection in the total ion current NICI mode. GC/MS lacks the possibility of such a direct

correlationship. This is important because not all compounds that give good NICI spectra are necessarily colored species. However components that both respond to the visible detector and give favorable NICI spectra are likely to be these conjugated quinones.

CHAPTER V

DESCRIPTION OF THE SYSTEM, OVERALL RESULTS AND CONCLUSION

The essential parts of the system are diagramed schematically in Fig. V-1.

(A) is a HP (Hewlett Packard) Model 1090 liquid chromatograph with a variable volume injector and a column switching valve that enables either flow injection (by-passing column) or on-column injection. In particle beam LC/MS analysis, preliminary optimization work has to be performed on all the variables critical to the performance of the interface as discussed in Chapter IV. The flow injection mode enables faster set up time than introducing samples through HPLC column. (B) is a HP Model 1050 micro flow cell variable wavelength ultraviolet/visible detector or a commercial diode array uv/vis detector. (C) is a HP Model 3392A on-line integrator. Solvent is added post column using a Waters 510 high pressure pump (D), through a swagelok tee in the 2' x 1/16" o.d. stainless steel tube leading to a 5988a HP particle beam interface (E). The HP MOdel 5988A mass spectrometer (F) is equipped with a single

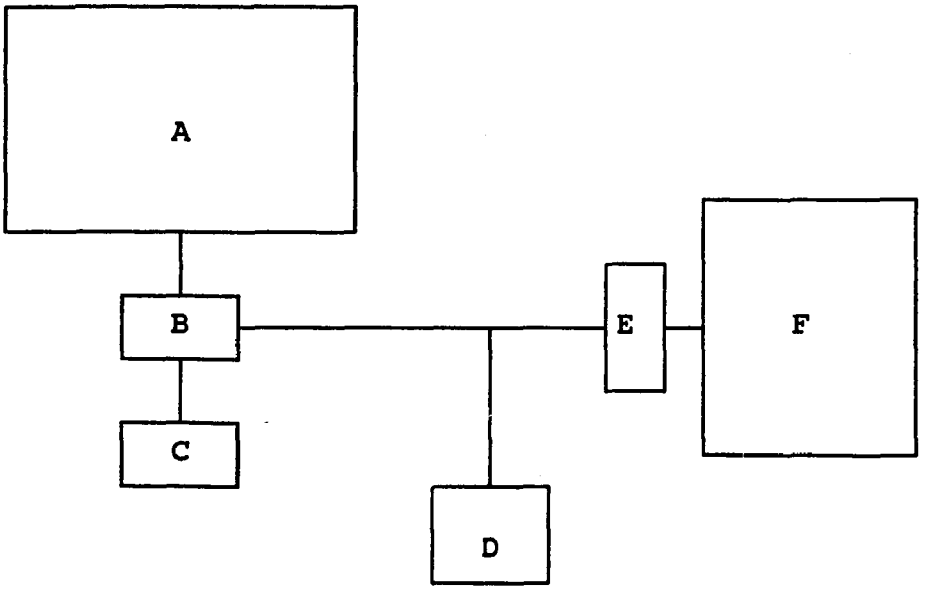
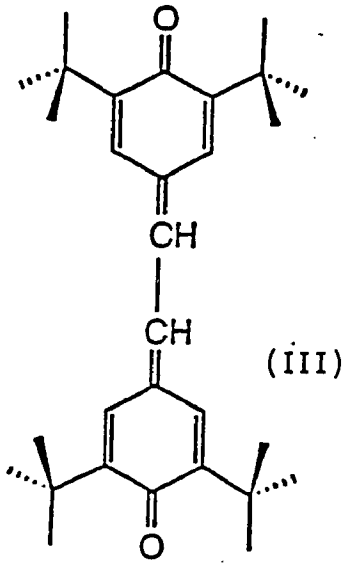


Fig. V-1 The complete LC/MS system

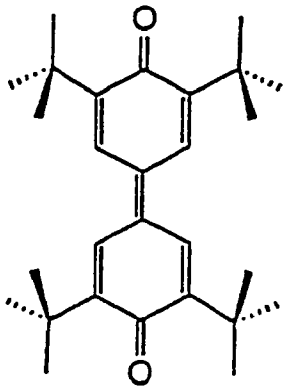
quadrupole; electron, positive, and negative ion chemical ionization capability; a mass range of 2000 daltons; and a RTE A data system. A triple quadrupole instrument could also be used to perform MS/MS experiments.

Application of this system was made to a synthetic mixture of conjugated quinones, hindered phenolic derivatives, and an internal standard (I.S.). The quinones, the phenolics and the I.S. are listed in Table V-1. Compound (III) had been discussed in previous chapters. Compound (IV) and (V) are conjugated quinones that derive from common commercial antioxidants. The I.S. is Araldite 6010, a commercial epoxy resin. Compounds (II) and (VI) are non-quinones but are hindered phenolic derivatives. Compound (II) was used in the NICI experiments in Chapter III. Compound (VI) is the dihydro derivative of (IV). Two LC/MS analyses were carried out sequentially. The first used the ultraviolet detector at 280 nm and EIMS mode. The second used visible light detection at 436 nm and NICIMS mode.

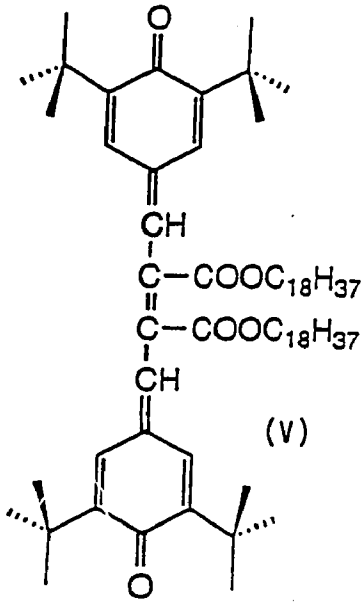
Table V-1 Structures of the six components in the synthetic mixture



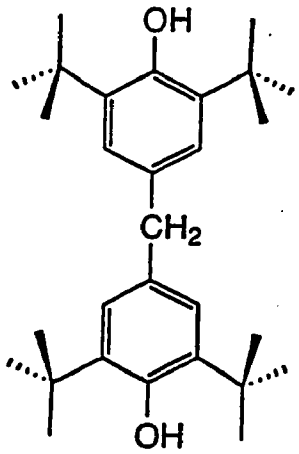
(III)



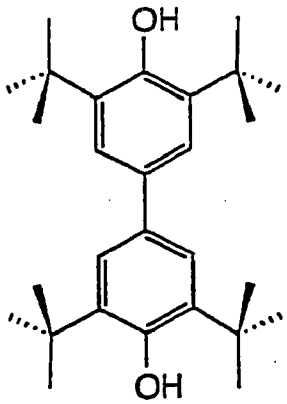
(IV)



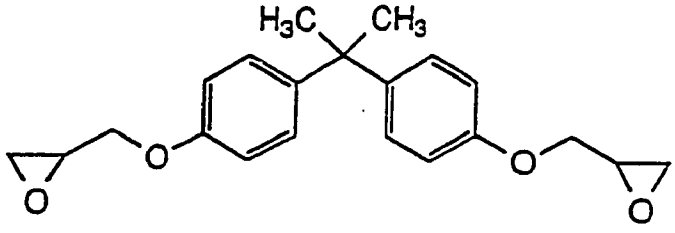
(V)



(II)



(VI)



(I.S.)

EXPERIMENTAL

Chemicals and reagents

Compounds (IV), (V) and (VI) were obtained from the Additives Division of Ciba-Geigy Corporation. The I.S. was obtained from the Plastics Division of Ciba-Geigy Corporation. The solvents and the reagent gas for chemical ionization were the same as those described in Chapters II,III and IV.

Sample Preparation

Approximately 3 mg each compound was accurately weighed out and dissolved in 3 ml of ethyl acetate to have a concentration of about 1 ug/ul. One half ml of each of the six solutions was combined in a vial to make the working solution in which the concentration of each component was approximately 0.18 ug/ul.

Chromatography

(1) On column Injection

A 8 cm x 4mm i.d. 5 um Zorbax ODS column (Mac-Mod Analytical, Chadds Ford, PA) was equilibrated

for 10 minutes with the initial mobile phase, ethyl acetate/methanol/water (20/60/20). After 5 ul injected, the initial mobile phase was changed using a linear gradient to 90/10/0 mixture of the same solvents in 20 min. with 10 min. final hold. The flow rate was 0.5 ml/min. Ultraviolet (UV) detection was carried out at 280 nm and visible detection at 436 nm.

(2) Flow Injection

Three mobile phases were used in the isocratic mode. They are (1) ethyl acetate, (2) methanol/ethyl acetate (30/70) and (3) water/methanol/ethyl acetate (20/60/20). The flow rate was 0.5 ml/min. Volume of injection was 5 ul.

Mass Spectrometry

(a) Particle Beam Interface

The nebulizer was set at the setting for the maximum response of Compound (V). Helium gas pressure was set at 40 psi as indicated by the pressure gauge in the particle beam interface module. The desolvation chamber temperature was set at 55°C.

(b) Ionization

Two ionization modes were used. In the EI mode, the filament current was set at 300 uamp emission and electron energy at 70 ev. In the NICI mode, the filament current was set at 400 uamp and electron energy at 180 ev. Reagent gas was isobutane. In the EI mode, the source pressure was 2×10^{-5} Torr as indicated by the source pressure gauge. In the NICI mode, the total ion source pressure was registered as 2×10^{-4} Torr.

RESULTS AND DISCUSSION

(a) Qualitative

As seen in the UV chromatogram, Fig. V-2a and the TIC in EI mode, Fig. V-2b, all the components were observed and resolved with the exception of Compounds (VI) and (II) which co-eluted as indicated by the EI mass spectra of the single peak at retention time of 8.53 minutes as shown in Figs. V-3 and 4. The mass spectra of Compound (III) and (V), Figs. V-5 and 6 showed evidence of reduction of the conjugated quinones to their corresponding reduced analogs (42,43) exhibiting M=2 peaks for their respective molecular

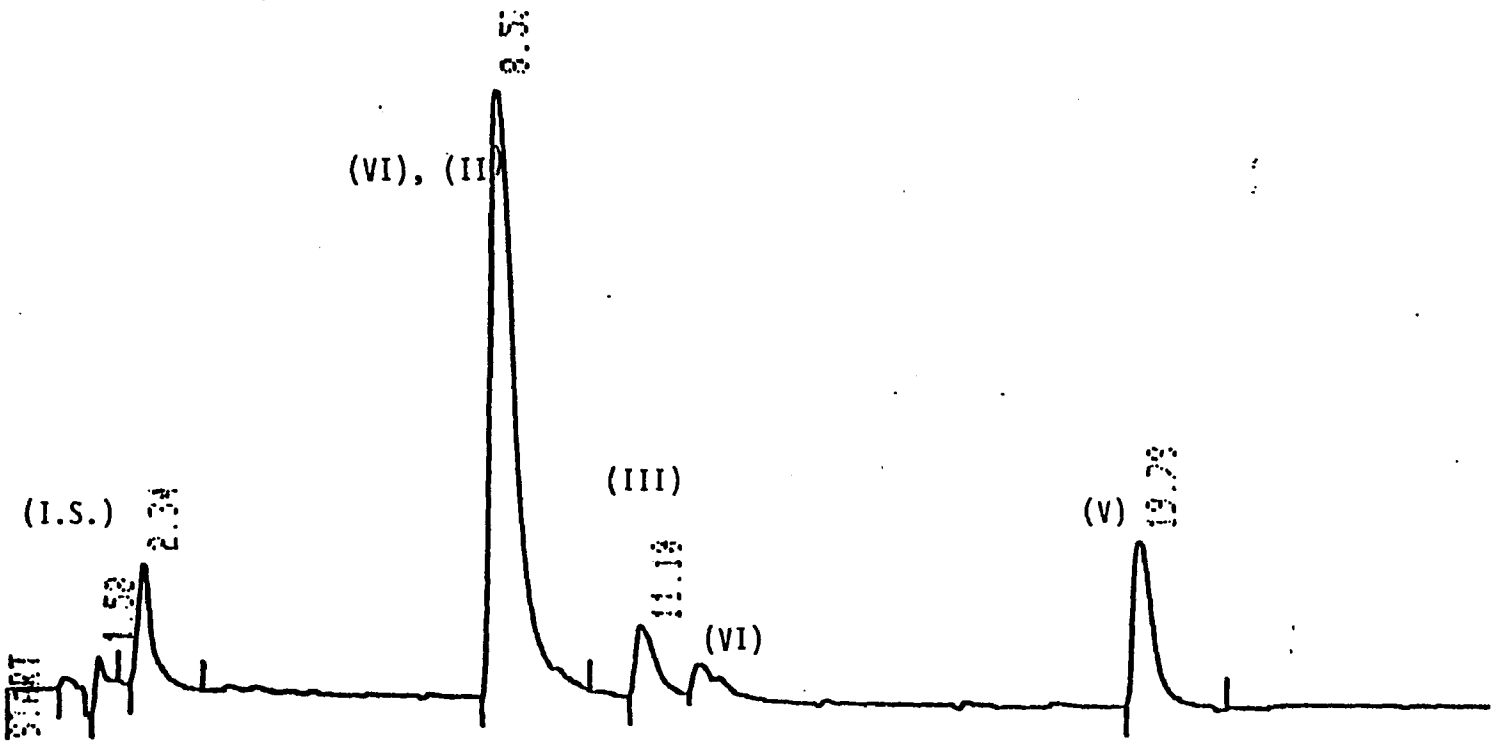


Fig. V-2a Liquid Chromatogram of the synthetic mixture at 280 nm detection

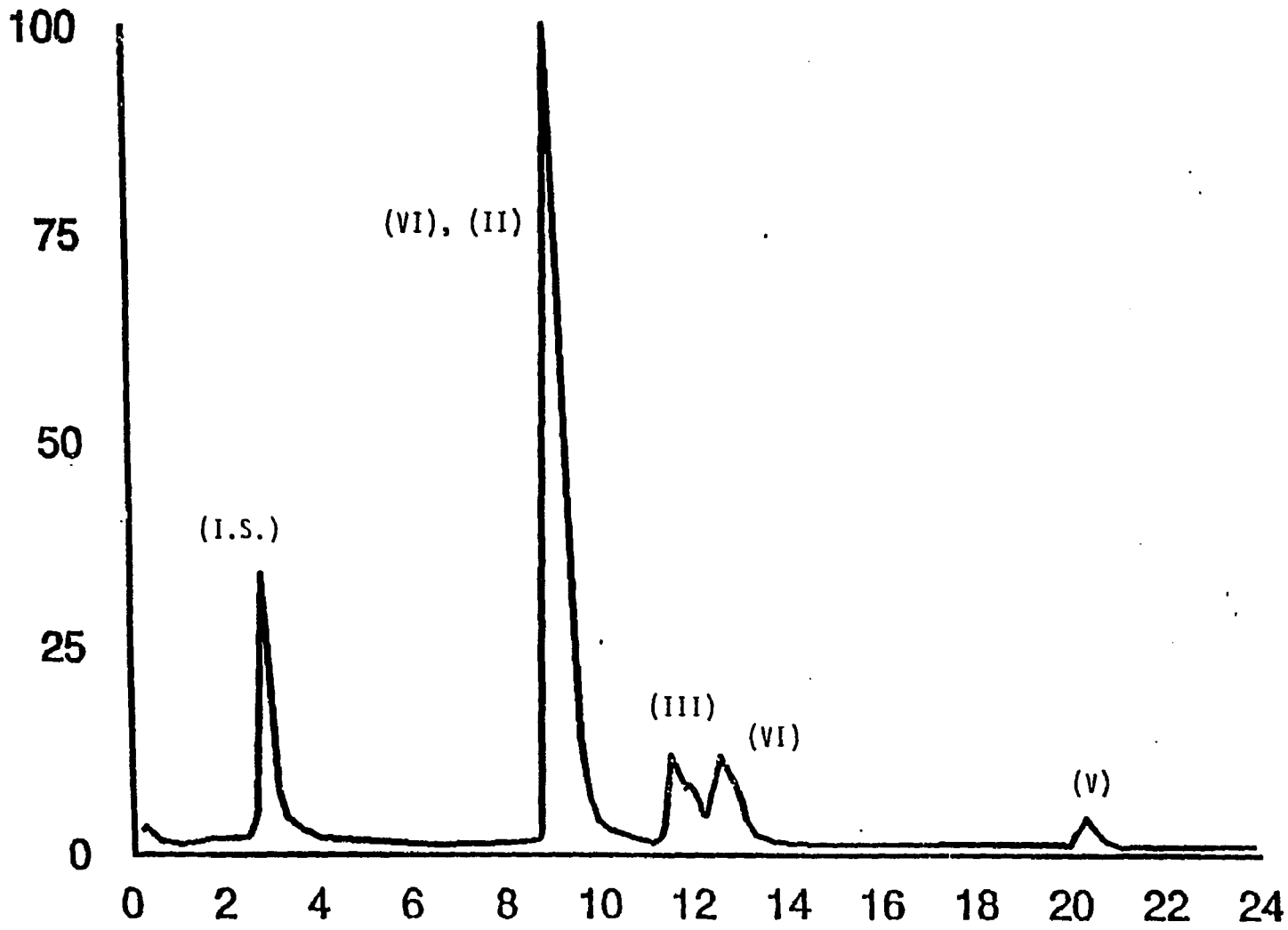


Fig. V-2b Total ion current of the synthetic mixture in EI mode. Compounds (II) and (IV) were identified by their mass spectra

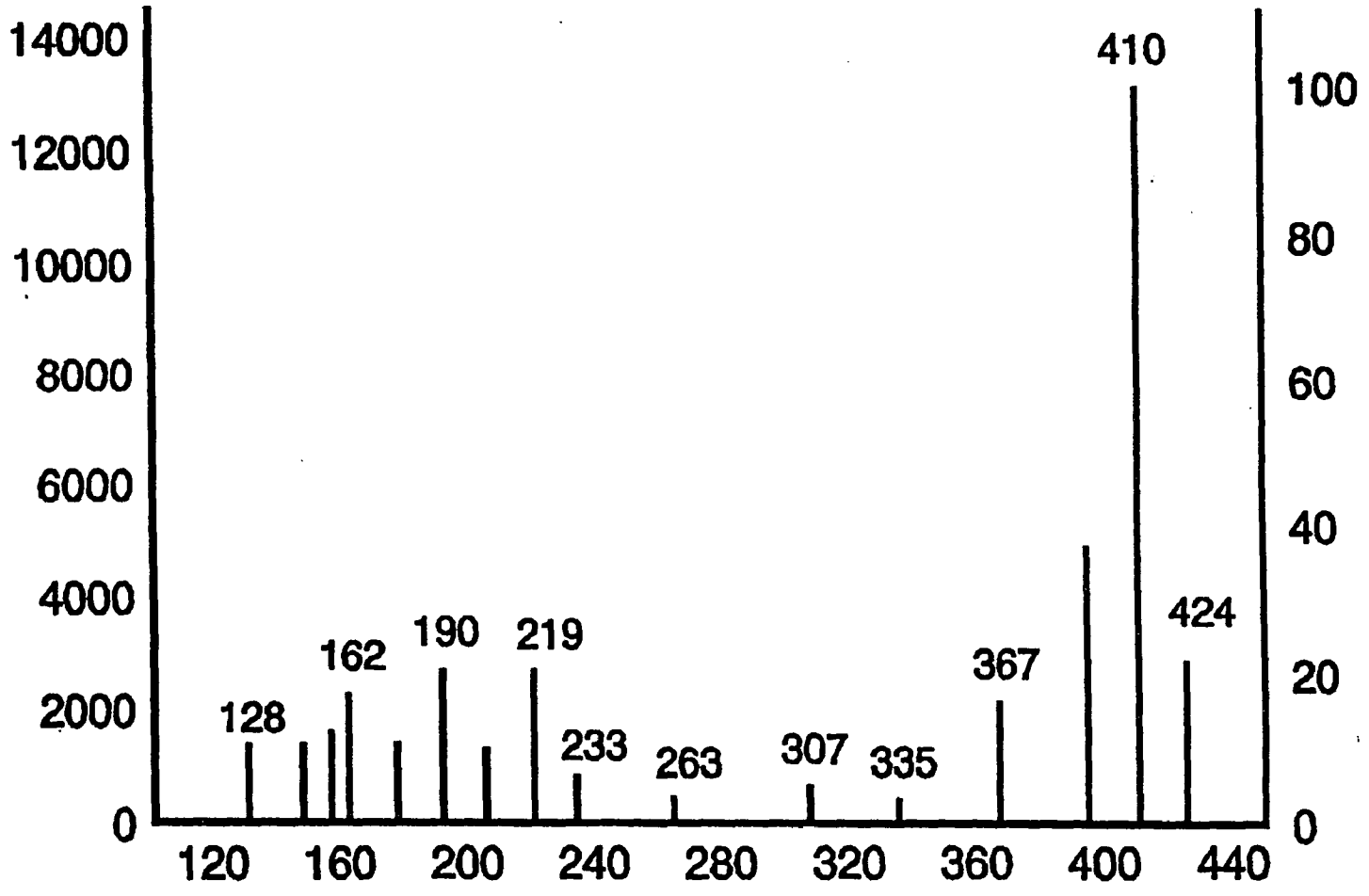


Fig. V-3 EI mass spectrum of Compound (VI)

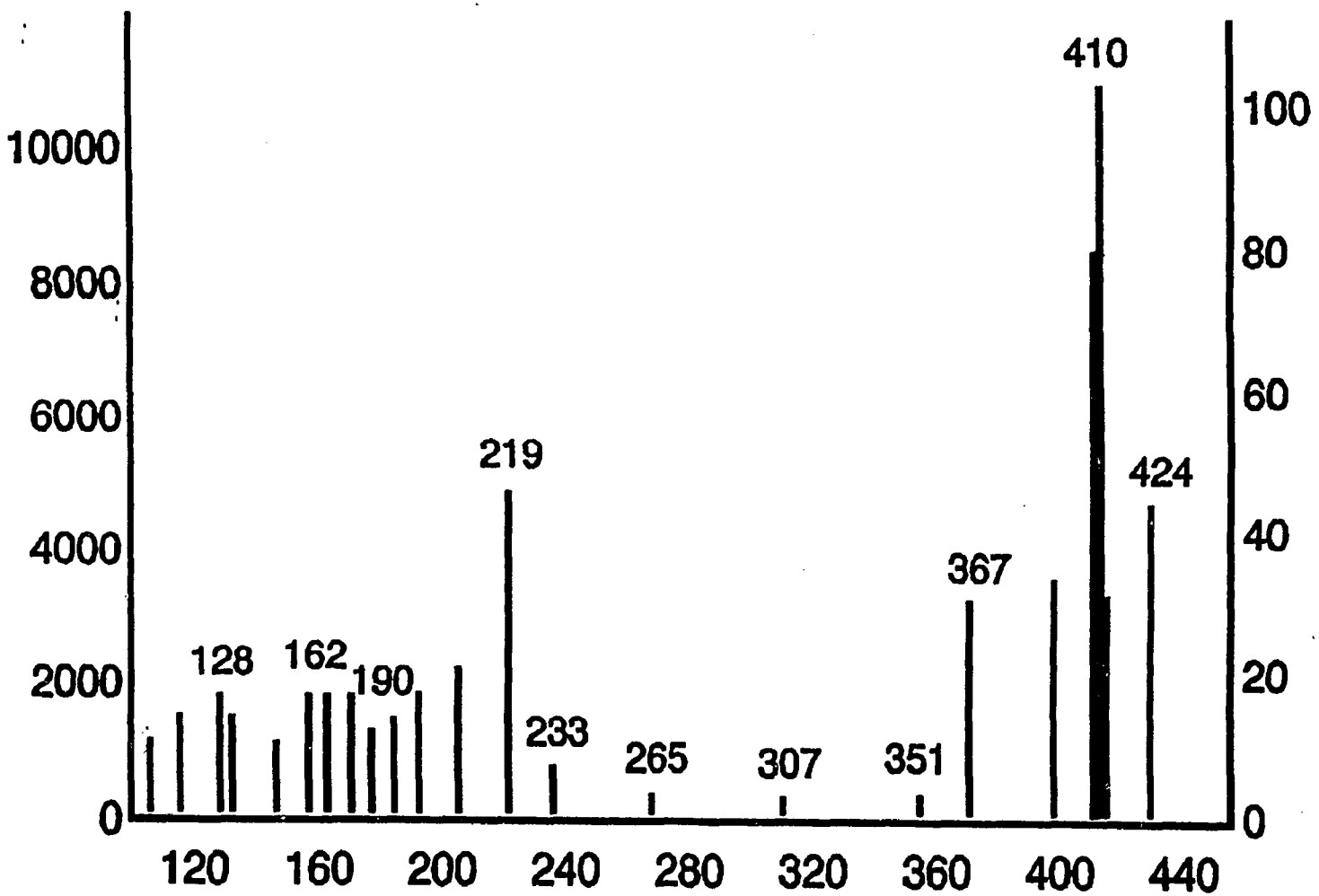


Fig. V-4 EI mass spectrum of Compound (II)

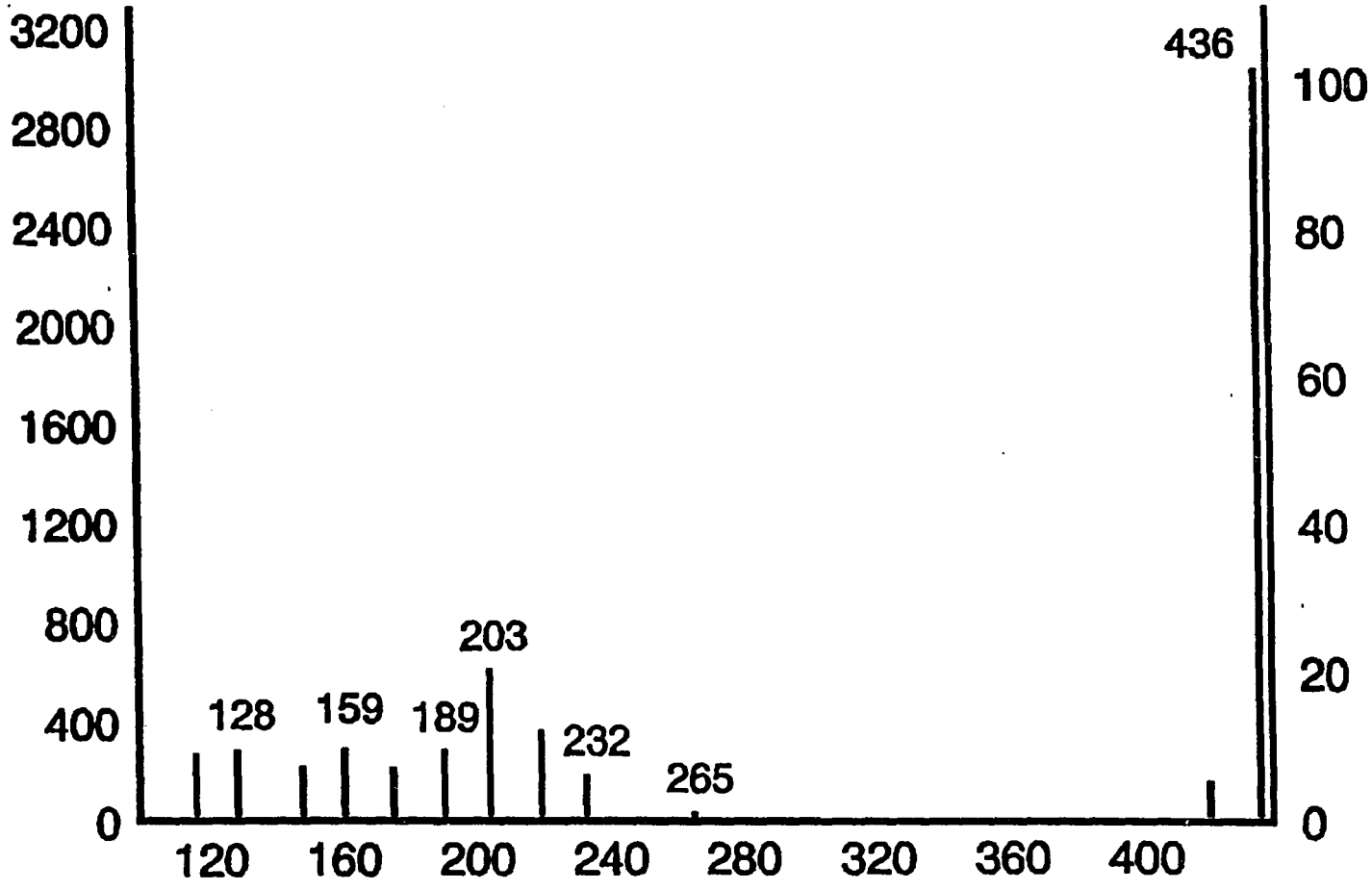


Fig. V-5 EI mass spectrum of Compound (III)

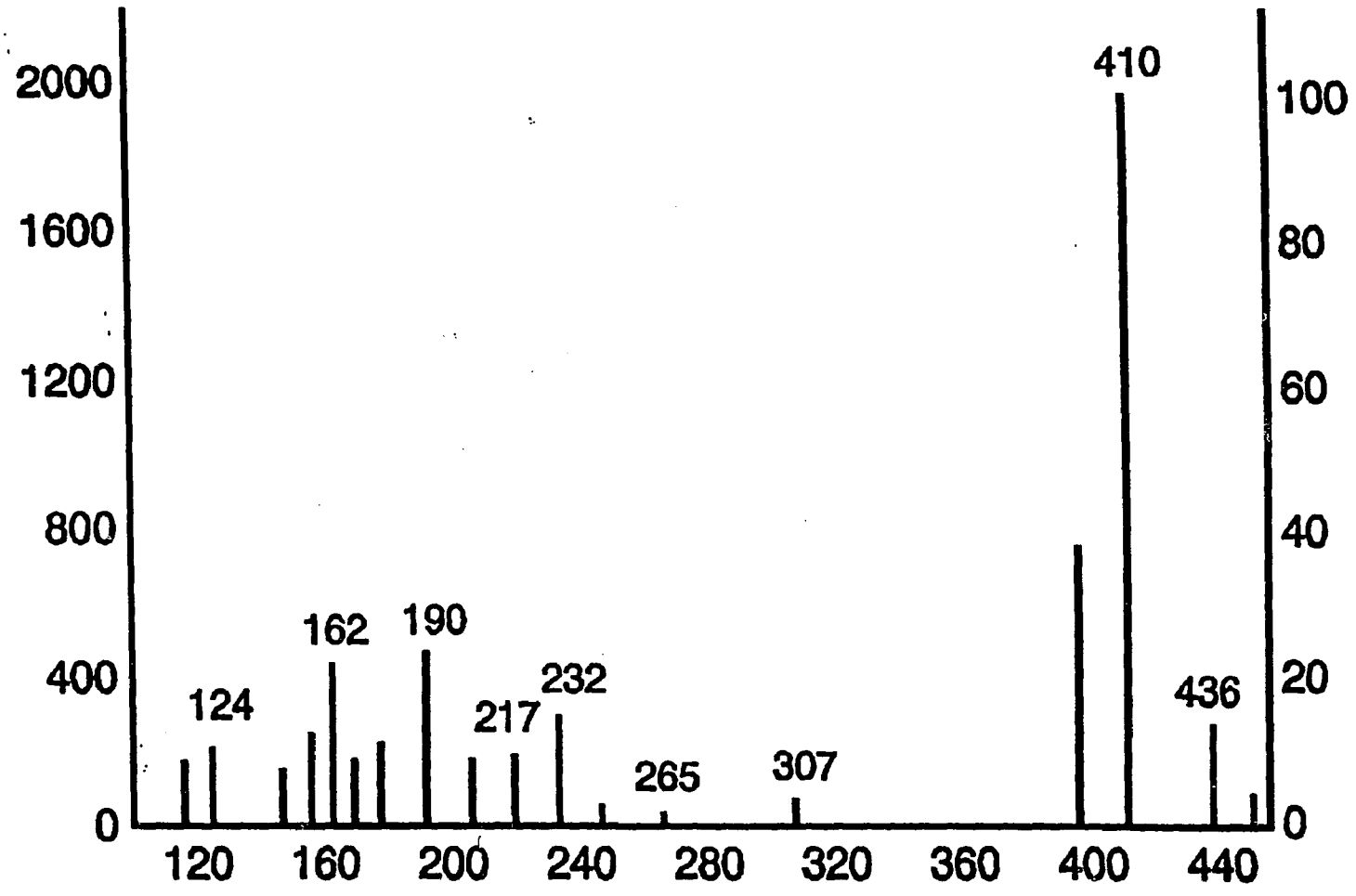


Fig. V-6 EI mass spectrum of Compound (IV)

ions. In fact, the spectrum of Compound (IV) in the EI mode, looked identical to that of Compound (VI). Because of the nature of the compound and the performance of the mass spectrometer, the molecular ion of Compound (V) was below detection level in the EI spectrum as shown in Fig. V-7.

When the analysis was switched to visible detection and NICI mode as seen in the Visible chromatogram, Fig. V-8a and the TIC of NICI, Fog. V-8b, the three compounds detected by both techniques were the three quinones, Compounds (III),(IV) and (V). The mass spectra gave unequivocal M^- ions corresponding to their respective molecular weights. Fig. V-9 is the NICI spectrum of Compound (V). Fig. V-10 are mass chromatograms of (III),(IV) and (V). A direct correlation can even be drawn based on the different absorptivities between the UV and visible λ_{max} of each component and their selective ionization between Ei and NICI modes. Close examination of the ion intensities of these three quinones clearly reveal that the quinones gave far superior ion intensities in the NICI than the EI mode.

As seen in Chapter IV, solvents have a strong

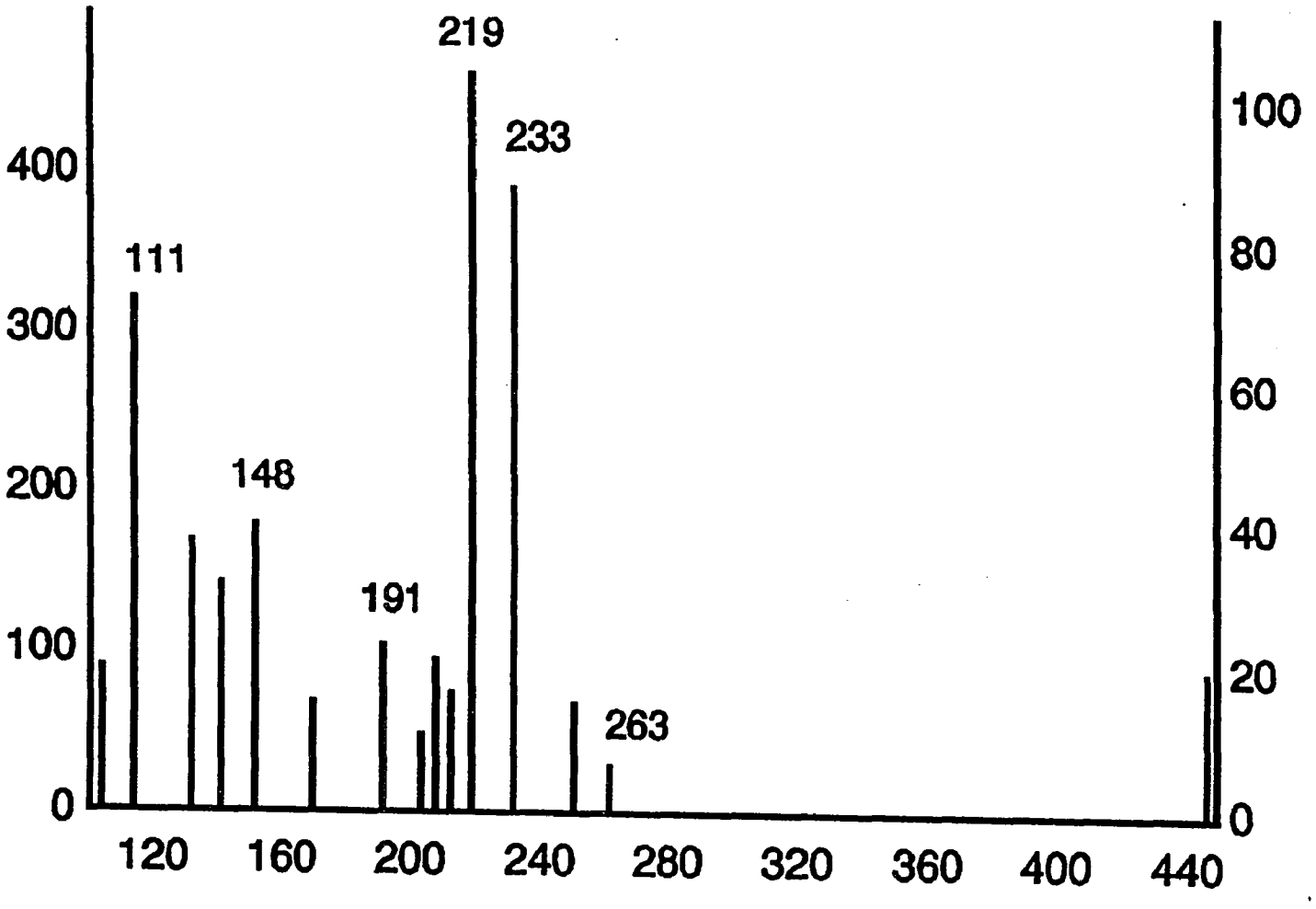


Fig. V-7 EI mass spectrum of Compound (V)

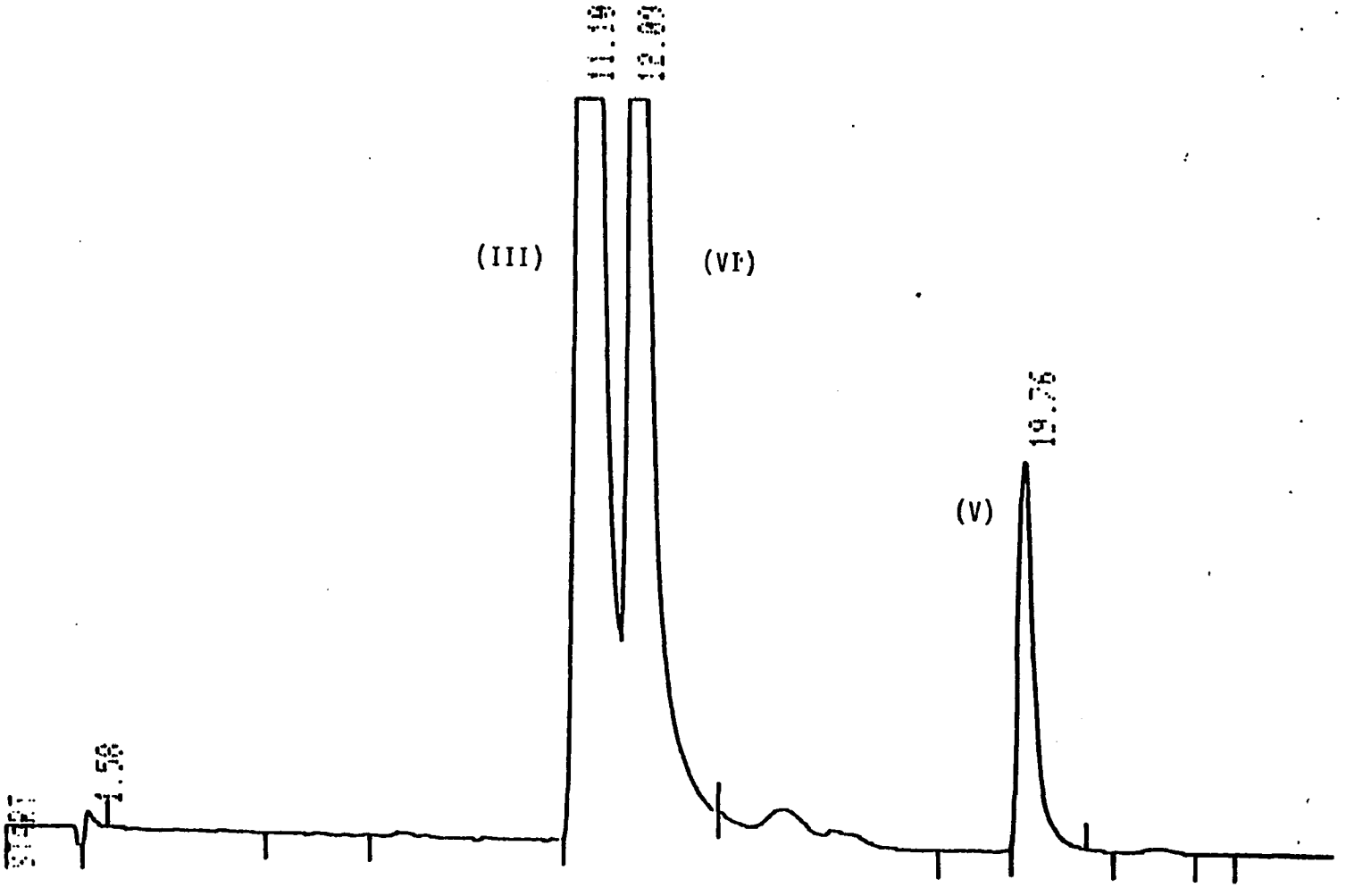


Fig. V-8a Liquid chromatogram of the synthetic mixture at 436 nm detection

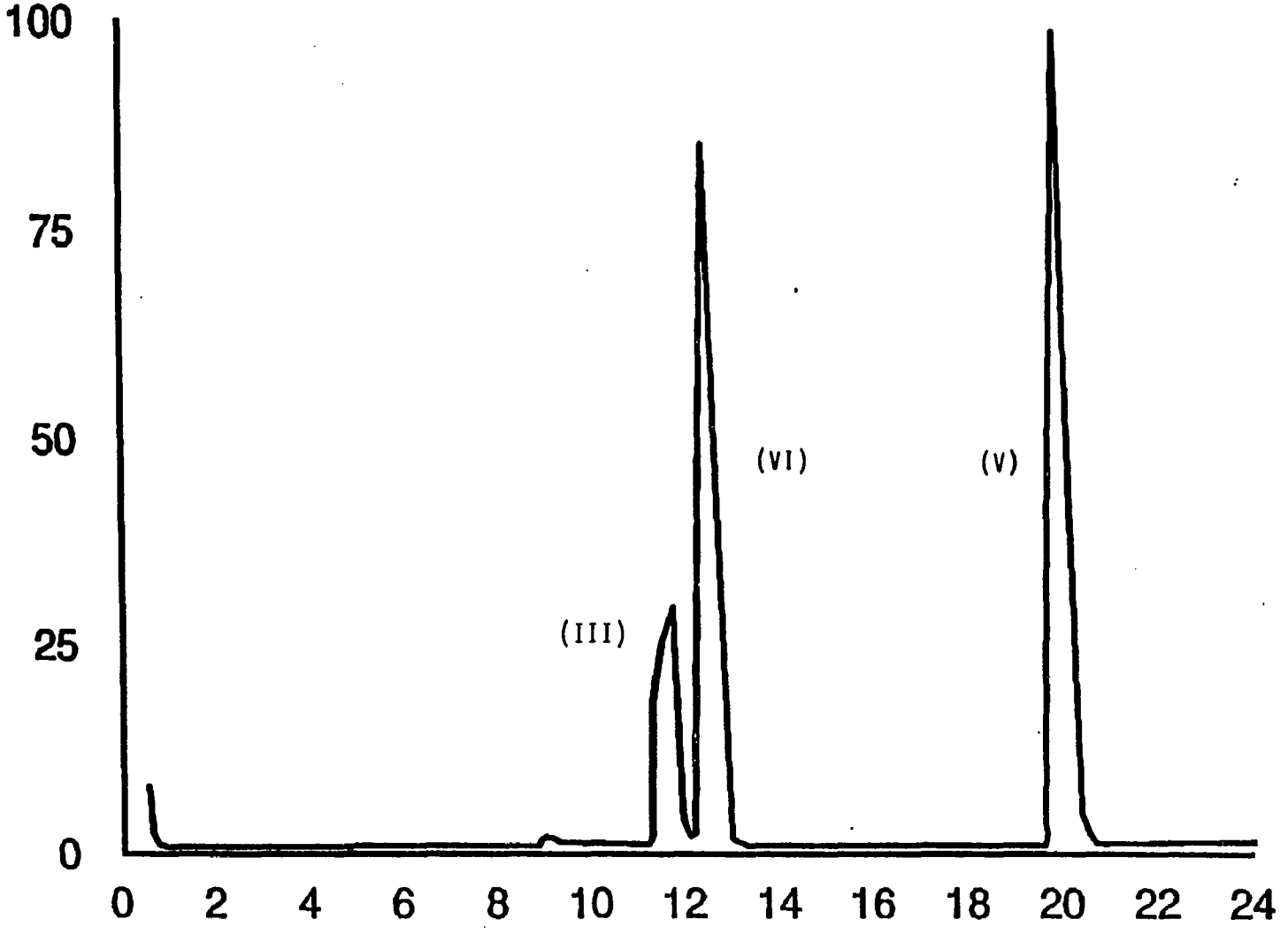


Fig. V-8b Total ion current of the synthetic mixture in NICI mode

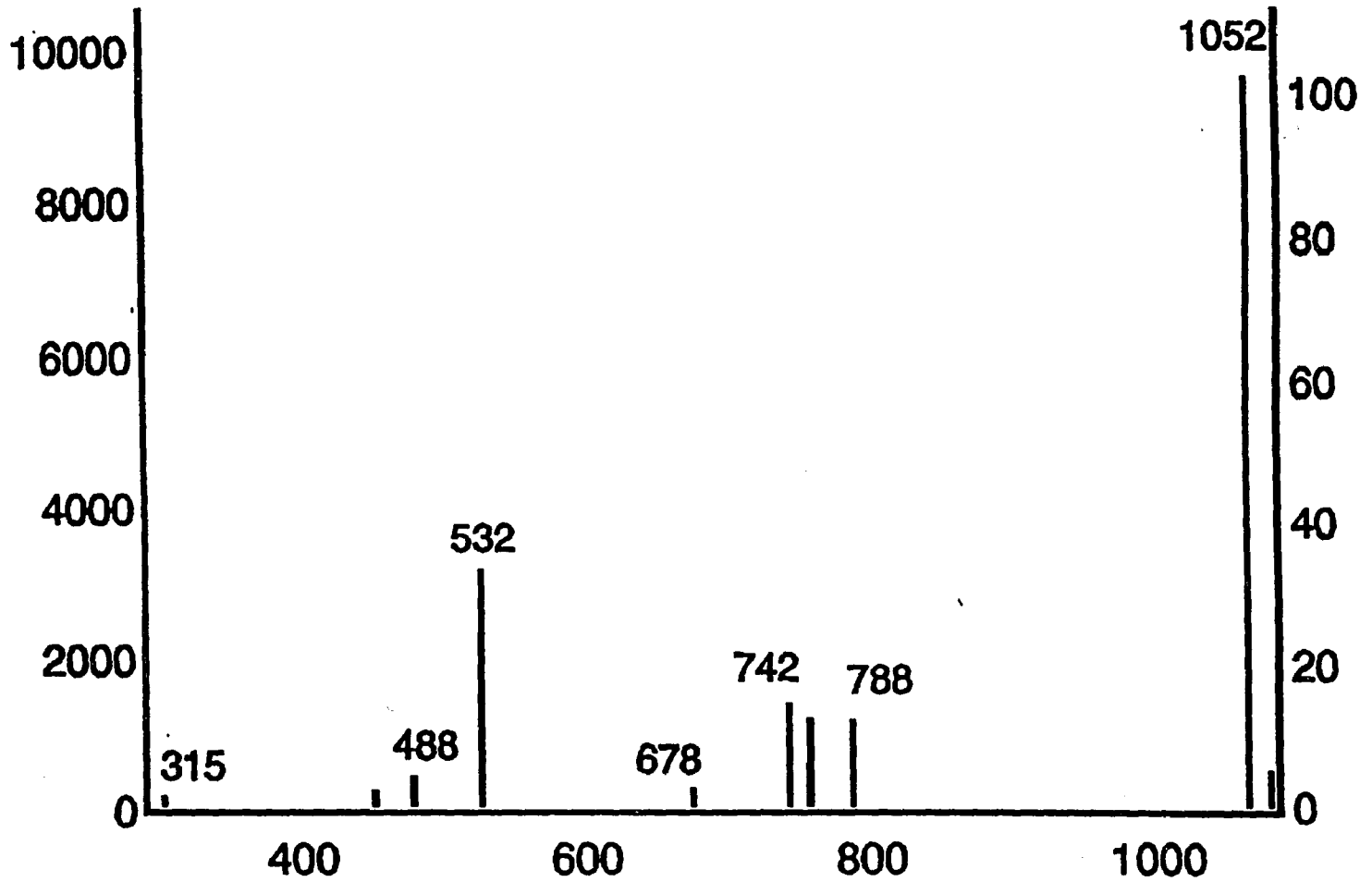


Fig. V-9 NICI mass spectrum of Compound (V)

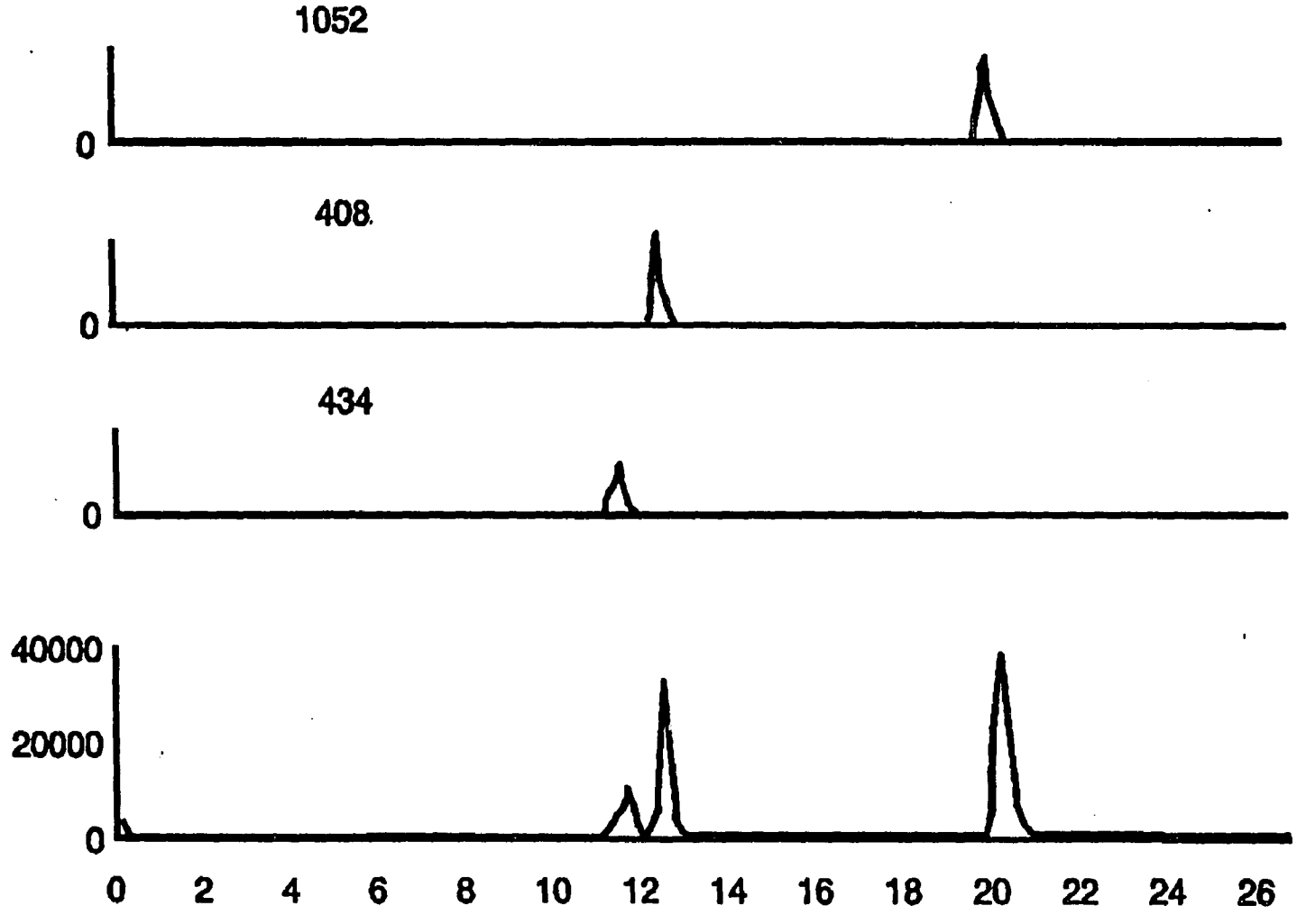


Fig. V-10 Total ion current and mass chromatograms of Compounds (III),(IV) and (V) in NICI mode

effect on the transport efficiency of analytes. In order to gain maximum sensitivity, the sample was subjected to the flow injection mode analysis, using three isocratic solvent conditions. Fig. V-11 shows that Compound (V) is most sensitive to the amount of ethyl acetate present. Ethyl acetate has less effect on the response to the other two quinones but, its presence is still critical.

Based on these findings as well as those in Chapter IV, post column solvent addition is necessary. This implementation has two advantages. Firstly, if a solvent is found to provide better transport, the mobile phase can be modified to allow the addition of that particular solvent. However, this is usually impractical for it will change significantly the characteristics of established chromatographic conditions. Secondly, there is still no theoretical approach to predict which solvent to use to increase the transport efficiency. Post column addition provides a fast screening process without disturbing the integrity of the liquid chromatogram.

(b) Quantitation

As part of the research, the detection and

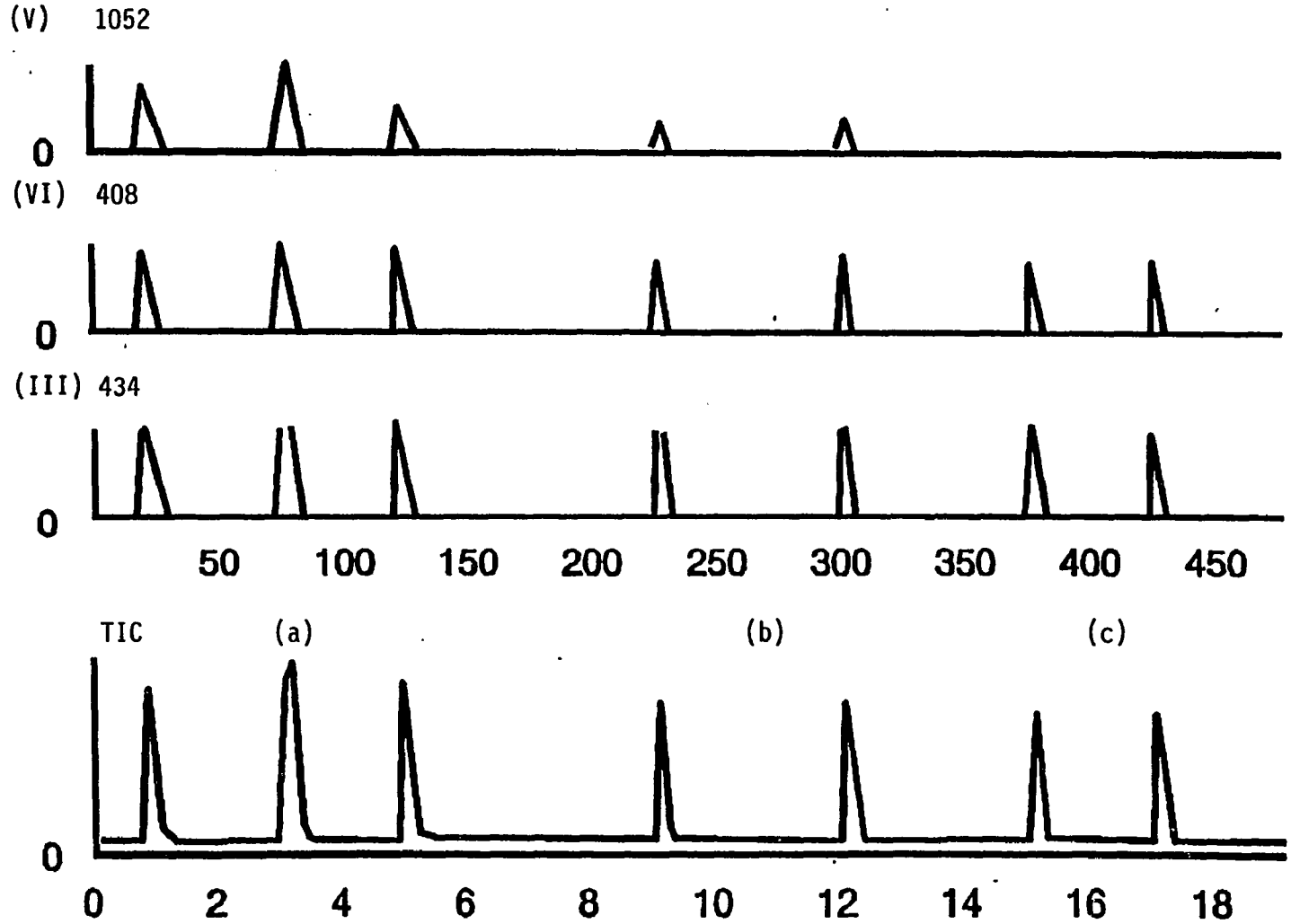


Fig. V-11 Total ion current and mass chromatograms of Compounds (V),(VI) and (III) by flow injection in three isocratic solvent conditions: (a) ethyl acetate (b) methanol/ethyl aetate(30/70) and (c) H2O/methanol/ethyl acetate(20/60/20). (a) is triplicate and (b)(c) duplicate injections

quantitation of the low level colored conjugated quinones are just as important as the identification of new unknown quinones. Quantitative analysis of trace organics using GC/MS in the selective Ion Detection (SID) or Selective ION Monitoring (SIM) mode is a well established technique. Not only is it sensitive, it can also provide specificity by using some structural information in terms of either a molecular or some characteristic ion or ions to be monitored. In this case, by combining LC/MS and NICI, these quinones can also be detected and quantitated. In the SID mode, which usually has an order of magnitude higher sensitivity than scanning over a die mass range, Compound (III) can easily be handled in the 20 ng range. This may not yet rival the visible detection, but certainly narrows the gap and at the same time provides better specificity.

(c) Limitations

There are certain limitations discovered during this research.

(i) Because of the particle beam design and because certain quinone structures undergo reduction in

the presence of water molecules in the ion source, sensitivity suffers when the chromatographic mobile phase is highly aqueous. Consequently, the use of water in the mobile phase should be avoided as much as possible, at least for this analysis.

(ii) It has been demonstrated that all type of quinones yield good negative ion spectra. However, not all components that yield good negative ion spectra are necessarily quinones. In the presence of such non-quinone species, interference could be problematic. The simultaneous response by the visible detection is therefore of the utmost importance.

(iii) NICI spectra show an intense M^- molecular ion but few structurally informative fragment ions. One remedy for such soft ionization techniques would be tandem mass spectrometry (45). In MS/MS experiments, the parent ion, in this case the M^- ion, is collisionally activated dissociation (CAD) through collisions with neutral gas molecules in the fragmentation region to yield various daughter ions, analogous to the fragmentation occurring during electron ionization. Subsequent mass analysis of the daughter ions by the

second mass analyzer permits identification of the compound.

CONCLUSION

The straightforwardness of combining an on-line ultraviolet/visible HPLC detector and the EI and NICI mass spectra using the particle beam interface to solve a very complicated chemical problem has been demonstrated. The following goals have been achieved

(1) Speed and Accuracy

Direct LC/PB/MS analysis is far more rapid and efficient than the tedious and time consuming alternative isolation procedure followed by off-line identification. It eliminates sample contamination, column and solvent background and avoids the poorer resolving power of either a preparative or semi-analytical column. The use of a subtraction routine of the modern mass spectral data system verifies the integrity of a pure component where the liquid chromatographic peaks are non-homogeneous.

(2) Selectivity

The simultaneous correlation of the UV detection and the EI mass spectral data followed by visible detection and NICI mass spectral data virtually guarantee the identification of this class of conjugated quinones and obviates second-guessing using an alternate technique.

(3) Sensitivity

The stability of the negative molecular ions of this type of quinone gives excellent sensitivity of the order of ten- or hundred-fold gain compared with EI spectra. There is also the parallel differential between the ultraviolet and visible absoprtivity of the same component.

Future work should include use of a diode array detector so that rapid maximum absorptivity can be obtained. The incorporation of a triple stage quadrupole mass spectrometer would enable tandem mass spectrometry to enhance structural identification capability. Some

very promising experimental results based on the same method have been obtained in the identification of some quinoidal structures resulting from oxidation of some N-phenyl-1 naphthylamine derivatives (46,47). These derivatives are used as additives in oil lubricants. Since the NICI technique is applicable to all electron-capturing species, p-hydroxyl nitro aromatic compounds are also good candidates. 2,6 Di-t-butyl-4-nitrophenol which forms a yellow solution, was used successfully to demonstrate the application of the technique to these systems.

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