NITROPHENYL BORONIC ACIDS AS DERIVATIZING AGENTS IN CHROMATOGRAPHY

by

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Dissertation Submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Chemistry

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May 1982

ACKNOWLEDGEMENTS

I would like to express my gratitude to the following persons for their help.

To Dr. Harold M. McNair for his encouragement to take a Ph.D, the financial support he gave me and the use of the instruments in his laboratories. More valuable has been his friendship and understanding throughout all these years.

To my friends Alex Apffel, Teng Man Chen, Dan Marsh and Mike Borgerding; their friendship has been invaluable during this long and hard struggle. A special place in my memory will be occupied by Alex and Teng Man who were particularly close to me.

No words can describe my gratitude to Esther Brann who was much more than just a mother figure, her understanding and patience as a friend will always be present in my life.

This work and my whole life would have been very different without the help and moral support of my wife Christine, she has been the most important influence during these long and trying years.

Finally this work is dedicated to my parents who in their own way, and perhaps without realizing it, have been my greatest source of inspiration.

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Introduction

Analytical chemistry has been defined as "the art of recognizing different substances and determining their constituents" (1). Taking into account modern concerns and technological advances, we could add to the previous definition"in very complex mixtures". As the demands on analytical chemists grow heavier, the ingenuity and resourcefulness of scientists seems to grow in a matching way through the development of ever more sophisticated and selective instruments and reagents, pushing the frontier of technology toward the ultimate goal in chemical analysis, "single atom or molecule determination".

Some of the paths followed by analytical chemists in their quest for results have been the improvement of the selectivity and sensitivity of their instruments and techniques. No method or instrumentation is absolutely accurate or precise when complex samples are analyzed, but through the use of extremely selective reagents or instruments very complex problems can be solved.

Chromatographic techniques have proven very helpful and effective in the resolution of complex mixtures. Gas chromatography (GC) is perhaps the most widely used analytical technique. It's sensitivity has reached attogram level (2) and efficiencies as high as 10^6 theoretical plates have been achieved (3) which is a remarkable fact considering it was obtained during the infancy of G.C. Comparable efficiences in very fast separations have also been recently reported (4).

Modern or high pressure liquid chromatography (HPLC) has grown very fast during the last 10 years, and it is now capable of good efficiencies (5) although somehow lagging in detection limits with respect to GC. Development of more advanced detection systems for HPLC is without doubt one of the more active areas of research. (6)

Modern Trends in Chromatography - Many improvements have occurred in GC. and HPLC over the years, most of them strongly related to the design and development of components for the chromatographic hardware. Capillary or microbore columns, element specific detectors, specialized sampling devices, multidimensional chromatography and hyphenated techniques are some of the most remarkable advances in chromatography.

Improvements in the chemistry aspects of the chromatographic process have also taken place. Developments like specific stationary phases, surface treatments, bonded phase chromatography and sample modification are perhaps the most notorious.

Efficiency, selectivity and sensitivity are undoubtedly the main concern of the chromatographer, and all major advances have been aimed at the improvement of these 3 aspects. It is difficult to appreciate where the limit is, but some opinions have been expressed concerning the theoretical limitations of chromatographic techniques (7). As theoretical limits are approached, chemists will turn to study factors which ultimately dictate the limits of chromatography: surface activities, enviromental effects, impurities, instrument stability, sample contamination to name a few.

Sample Modification in Chromatography - Several techniques like reaction chromatography, on column subtraction, pyrolysis and derivative formation are included under this title. By far the most popular is the formation of compounds derived from the original sample which are more suitable for chromatographic analysis.

Table I shows the most frequent reasons for taking this extra step in the analytical process along with the techniques where the related problems are frequently encountered. Somehow the need for derivative formation is a consequence of a GC's natural limitations such as low sample volatility which greatly limits its applicability. Other problem like poor peak shape due to sample polarity and active sites in the chromatographic column can seriously deteriorate efficiency, quantitation and sensitivity.

There are no sample volatility requirements in HPLC and poor peak shape although frequently encountered, can be easily solved in most cases by modifying the mobile phase, selecting the appropriate chromatographic mode and also by employing ion suppression or ion-pairing techniques.

Selectivity can be a serious problem in G.C. and HPLC. Capillary columns in G.C. have made this a secondary concern if no other sample problems are present. In HPLC, appropriate detectors and carefully selected conditions are usually enough to obtain the selectivity desired.

As mentioned before, detection limits are still a weak point in HPLC. In general G.C. detectors are more sensitive and of more

TABLE I

Derivatives in Chromatography

Increased Sample Volatility	Gas Chromatography
Improved Peak Shape	Gas Chromatography
Increased Selectivity	Gas and Liquid Chromatography
Enhanced Sensitivity	Gas and Liquid Chromatography

widespread use. Detector sensitivity usually grows along with detector selectivity, limiting the type of samples which can be analyzed at high sensitivities.

Sample inadequacy can be overcomed by derivative formation in both GC. and HPLC in order to use highly specific and sensitive detection systems. This can be done by the introduction of a suitable electrophore (for electron capture detector), chromophore (for ultraviolet visible detector) or fluorophore (for fluorescence detector), to the original sample. An electrochemically active group can also be attached expanding in this way the applicability of electrochemical detectors.

In conclusion, we can say that derivative formation in chromatography broadens and eases the applications of chromatography to otherwise very difficult or impossible problems.

Analytical chemistry has also been defined recently (8) as being "what analytical chemists can do" and we can readily see that through derivative formation it is possible to do "some more".

Scope and Goals - The present work will be devoted to the study of a special type of derivatization reagents: nitrophenyl boronic acids, and the following points are proposed as research goals:

a. The preparation and purification of the 3 possible isomers of nitrophenyl boronic acid.

b. The study of their derivatization performance employing a number of model compounds.

c. In depth study of the derivatives stability and molecular characteristics which may influence this aspect.

d. Evaluation of sensitivities and detection limits obtainable with different detectors for GC. and HPLC.

e. Exploration of the complete usefulness of these reagents.

A final objective will be the suggestion of improved boronic acid molecules and chromatographic conditions in order to expand the utility of boronic acids in chromatography.

General and Historical Information

Introduction

Derivative formation in chromatography can be regarded as a way to overcome some of the natural or technological limitations of chromatography as we practice it at the present state of the art.

The additional chemistry involved in this technique is obviously something most people would rather not get involved with, however, what can be seen as a complication is in many cases an additional refinement in the analytical process, which is likely to improve results without many problems and with no need for expensive or sophisticated instrumentation.

All chromatographic techniques or modes can benefit from this technique. Gas chromatography is however the field where it is more frequently applied. Historically, thin layer chromatography and paper chromatography were the first to use some kind of sample modification as a means to visualize the separation obtained (9). Nowadays, HPLC methodologies are using more often the formation of derivatives in order to increase the utility of some sensitive but rather selective detection systems.

Obviously, not all chemical reactions are adequate for the formation of derivatives. From a general point of view, Table II shows some of the desirable characteristics in a derivatization reaction applicable to both GC. and HPLC.

Derivatization in TLC constitutes a special case since in most

TABLE II

Desirable Characteristics of a Derivatization Reaction

- Fast and Quantitative
- Single Derivative or Product Obtained
- Mild Conditions Sufficient
- Requires no Corrosive Reagents
- Reagent and Derivative are Stable
- One Step Process
- Reagent(s) are Specific

situations it is done "in situ" after the separation has been completed and commonly used only for "visualization" or qualitative analysis purposes.

According with the reaction characteristics and the type of chromatography involved, the formation of derivatives can be done in several ways. Figure 1 shows a possible classification of those ways and the chromatographic modes where they are usually applied. In this figure, PRE or POST- column is understood as before or after the separation takes place and ON- or OFF-LINE as being either part of the chromatographic equipment or a separate procedure before the sample is introduced in the system.

Derivatization in Gas Chromatography -Traditionally GC has been the analytical technique which benefits the most from some type of chemical modification. As early as 1956, James and Martin (10,11) prepared methyl esters from fatty acids, just 4 years after GC was developed (12,13).

After the introduction of esterification as a derivatization reaction, the most significant development in this area took place in 1961 when Von Ruhlman and coworkers converted several aminoacids into N-trimethylsilyl trimethylsilyl esters by treating the amino acids salts with trimethylchlorosilane (14).

Chemical modification by the introduction of a trialkylsilyl group is perhaps the most popular and general derivatization reaction. Since its introduction, many reagents have been developed for this purpose;

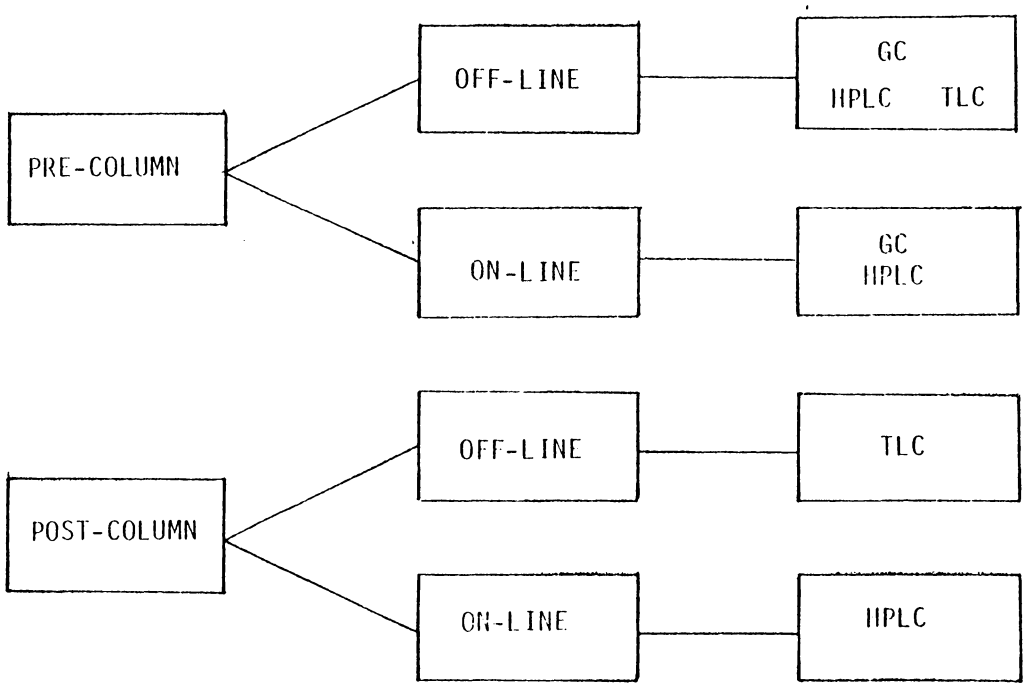


Figure 1. Derivatization Techniques

the silyl derivatives formed have excellent chromatographic properties; the reactions are usually fast and clean and in many cases the reagent itself serves as a solvent.

On the negative side, we find that silyl derivatives are sensitive to hydrolysis, the reaction may be difficult in the case of sterically hindered groups and detector contamination occurs in some cases. Two recent developments in the area of silanization reagents are the introduction of terbutyl dimethyl chlorosilane which forms derivatives more stable toward hydrolysis (15) and of pentafluorophenyl dimethyl-chlorosilane which greatly enhances electron capture detector response (16).

It would be very difficult and out of the scope of this work to present a complete review on silyl derivatives. Complementing the comments made so far, Table III shows the functional groups capable of forming this type of derivatives and Table IV gives a list of commonly used silanizing reagents. Excellent sources of information on this subject are readily available (16,17,18,19,20).

As mentioned before, one of the reasons to form derivatives is the possible enhancement of detector response. With relatively few exceptions, this approach has been applied in G.C. only in the case of the electron capture detector.

Gutenman and Lisk in 1963 were perhaps the first to increase detector response by esterifying some chlorophenoxy acetic acids with 2-chloroethanol. The introduction of a second halogen atom increased the sensitivity allowing detection at lower levels (21).

Table III

Functional Groups Which Form Silyl Derivatives

Group	Derivative
-OH	-O-TMS
-SH	-S-TMS
-COOH	-COO-TMS
-POH	-PO-TMS
-SOH	-SO-TMS
-NOH	-NO-TMS
$-NH_2$	-NH-TMS, $-N(TMS)_2$
-NH	= NTMS
$-CH_2-C=O$ |	-CH=C-O-TMS |

Table IV

Silylation Reagents

Reagent	Structure
Trimethyl Chlorosilane (TMCS)	$(CH_3)_3$-Si-Cl
Hexamethyl Disilazine (HMDS)	$(CH_3)_3$-Si-NH-Si-$(CH_3)_3$
t-Butyldimethyl Chlorosilane (BDMCS)	$(C_4H_9)Si(CH_3)_2Cl$
bis-trimethylsilyl Acetamide (BSA)	CH_3-C=N-Si$(CH_3)_3$ | O-Si$(CH_3)_3$
bis-trimethylsilyl Trifluoroacetamide (BSTFA)	CF_3-C-N(Si$(CH_3)_3$)(Si$(CH_3)_3$)
N-methyl N-(trimethylsilyl)trifluoroacetamide (MSTFA)	CF_3-C-N(CH_3)(Si$(CH_3)_3$)
Trimethyl Siyl Imidazole (TMSIM)	$C_3H_3N_2$-Si-$(CH_3)_3$
Pentafluorophenyldimethyl Silyl Chloride (Phlophemesyl Chloride)	C_6F_5-Si(CH_3)(CH_3)-Cl
N-Methyl N-t butyl dimethyl Silyl trifluoroacetamide (MTBSTFA)	CF_3-C(=O)-N(CH_3)-Si-(C_4H_9) | CH_3

Employing esterification or acylation reactions, a large number of electrophores can be attached to different samples. The most common type of electrophores used are perhalogenated hydrocarbon chains. It is interesting to point out that not all groups are equally useful, depending on the halogen atoms involved, the structure of the group, and the amount of halogen substitution present, very different response and chromatographic behavior is obtained (22).

In general, halogenated electrophores increase their relative response in the order I>BR>Cl>F. On the other hand, their volatility increases in the opposite order. These facts result in the need for a compromise between volatility and increment in response. Iodinated groups are too high in molecular weight to be of general use and fluorinated ones although very volatile and easy to chromatograph require extensive substitution to give good sensitivities (23).

Undesired column interaction with the sample can give poor peak shape due to chemisorption or partial sample decomposition. This situation which is a minor problem at high concentrations, can be a serious limitation in the system's capability to detect low sample concentrations.

Derivatives can greatly diminish or eliminate poor chromatographic behavior by eliminating sample activity. In general, this problem is associated with the samples ability to form hydrogen bonds, a phenomenum which is also responsible for limited sample volatility.

Symmetrical peak shape is a desirable requirement of all derivatives employed in chromatography, independently of any other

desired characteristics. Some multifunctional molecules cannot be totally derivatized in a single step and may require a multistep process with different reagents. A partially derivatized sample is usually not convenient from the volatility or peak shape point of view.

Many examples can be mentioned regarding the elimination of sample activity and undesirable column interaction. Several good and interesting cases are the analysis of steroids (24), alkaloids (25) and barbiturates (26).

Another way to circumvent this problem is to use highly inert columns with a minimum of active sites and essentially ideal behavior. The search for column materials which meet this requirement has been a long and laborious one, leading to the development of very inactive solid supports and tubing materials, nevertheless their performance is less than ideal.

One major development in column technology has been recently introduced (27). Capillary columns manufactured from fused silica which contain a chemically bonded stationary phases are very close to the "ideal column". Their surface is extremely inert and is possible to chromatogram very active samples in the underivatized form (28). It is possible that in the future the use of derivatization techniques to improve chromatographic behavior will be greatly reduced.

The performance of gas chromatography as an analytical technique is full of impressive achievements. One of the most remarkable examples of high efficiencies achieved in GC is the separation of optical isomers. Although some separations have been done without

resorting to derivatives (29), most cases require, besides derivative formation, the use of highly specific and/or very efficient capillary columns.

An excellent illustration of this kind of application is the resolution of amino acid enantiomers which can be derivatized with a chiral reagent and chromatographed in a capillary column coated with an enantiomeric stationary phase (30). This pioneering work by Gilav et. al. was the first practical application of G.C. to the field of optical isomer separation.

In addition to esterification and silanization reactions, many other types of derivatives have been formed and applied in G.C. A complete list of them would be exceedingly long and beyond the scope of the present work. To obtain further information, the following bibliographic references can be consulted (31,32,33,34).

Derivatization in Liquid Chromatography -We have previously mentioned that HPLC has sample requirements different from GC. High molecular weight samples are easily analyzed and ionic or strongly polar molecules can be handled with the use of appropriate columns and the help of ion suppression or ion pairing techniques.

The nature of the separation process in HPLC is such that mobile phase modification, along with careful column selection is usually enough to provide good peak symmetry, and the desired selectivity. Some exceptions to this situation are the resolution of racemic mixtures by derivatization with chiral reagents (35) and the improved

separation of acetylated chatecolamines (36) and methylated hydroxyanthones (37).

The need for derivatives in liquid chromatography comes as a consequence of the lack of general and sensitive detection systems. The refractive index detector which has a universal response is not sensitive enough for trace level determinations. The search for a good, universal and sensitive detector in HPLC has been an elusive quest (6).

Thin layer and paper chromatography visualization techniques have been employed for many years, many of them such as halogen vapor absorption and charring in the presence of strong oxidizers are hardly appropriate for modern HPLC. Some others like the ninhydrin visualization of amines (38) and fluorescent labeling (39) have found application in modern liquid chromatography.

The first reports of selective derivatization in HPLC appeared in the early 70's, mostly developed for ultraviolet and fluorescence detection.

The introduction of electrochemical detectors (40,41,42) opened a new dimension of selectivity and sensitivity in HPLC. Obviously, the possibility of derivatives with reducible or oxidizible groups were immediately explored (43).

Table V shows the most common derivatizing reagents used in HPLC along with the substrate to which they are applicable and the detection system employed. Excellent reviews and books are availabe on this subject (44,45,46,47).

Table V

Derivatization Reagents in HPLC

Reagents	Detector	Substrate(s)
Phenacycl Bromides	Ultraviolet	Carboxylic Acids
p.-Nitro NN'diisopropylurea	Ultraviolet	Carboxylic Acids
2,4 Dinitrophenylhydrazine	Ultraviolet Electrochemical	Carbonilic Compounds
p.-Nitro Benzoyl Chloride	Ultraviolet Electrochemical	Alcohols, Amines
1-Fluoro-2,4-Dinitrobenzene	Ultraviolet	Amines
Dansyl Chloride	Fluorescence	Amines (amino acids)
Fluorescamine	Fluorescence	Primary Acids
o.-Phthalaldehyde	Fluorescence	Amino Acids
4-Bromomethyl-7-Methoxy Cumarin	Fluorescence	Carboxylic Acids

The development of derivatives in HPLC is likely to be an active field for many years to come. This however may change if the long searched for "universal and sensitive detector" is ever developed. In the meantime, any new selective detection system is likely to generate the possibility of new reagents for specific types of derivatives. A particularly interesting case of this kind are the fluorinated derivatives for NMR detection (48).

Comparison of Derivatization in GC and HPLC - Several of the main differences between these two cases have already been mentioned. Perhaps the most relevant one is the need to increase the detector response in only a special situation in GC and at the present, almost the exclusive case in HPLC.

The possibility of post-column, on-line derivatization is a very attractive technique in HPLC. The nature of the mobile phases is particularly suited for many reactions. This same approach however, is rarely used in GC because of the high temperatures required and the possibility of detector interference.

On-line reactors in HPLC, capable of mixing one or more reagents with the column effluent, maintaining a desired temperature and a minimum of band dispersion have been studied and are now commercially available (49,50). This approach is generally more suitable in the post-column mode since in this way the separation conditions can be optimized independently.

Several types of on-line chemical modification have been

experimented with in GC, almost all of them in the pre-column configuration (51). Packed reactors and pyrolizers are among the devices used in those techniques, unfortunately they are not widely used.

A more popular technique in GC is the so called "on column derivatization". This approach has several small variations such as "Sandwich Technique" which consists of injecting the sample and reagents at the same time but slightly separated in the syringe; true "on column", injecting the reagent first and then the substrate; and finally the "microreactor" which consist of using the injection port of the column or the first few inches of it as a reaction bead coated with the reagent (52).

Typical applications of these "on column" techniques are the methylation of barbiturates and other drugs (53). Techniques of this type have not been applied to HPLC. The need for fast reactions, high temperatures or catalysts can be easily solved in GC, but in HPLC the process would impose severe restrictions on the separation conditions.

Excess derivatizing reagent is commonly employed in order to assure quantitative reactions. Both GC and HPLC can take care of this problem by strong or irreversible retention of the excess reagent or by eluting it well separated from the products of interest, avoiding in this way any strong interference with the detection system.

A special case in HPLC is on-line derivatization where the reagent is added to the column effluent stream. Usually the selective nature of the HPLC detectors is such that the reagents causes little

problem or in some other cases the reaction product is the only one generating a signal with the detection system.

Reverse phase chromatography is the most popular mode in HPLC and its range of applications has been expanded with the introduction of ion-pairing reagents. Ionizable samples are usually very water soluble and under normal conditions show little or no retention in reverse phase columns. These ionizable samples in the presence of ion-pairing reagents can form hydrophobic complexes which interact well with the long chain alkyl bonded phases of reverse phase packing materials (54,55). Ion-pair chromatography presents a good alternative to ion-exchange chromatography. Techniques of this nature do not exist in gas chromatography.

Bifunctional Molecules - Samples containing more than one reactive group present a special case for derivatization. Reaction or blocking of just one of several groups usually is not a desirable situation since the remaining ones can give undesirable column interaction. Another complication is sometimes the formation of more than one monoderivatized product.

Among the samples in this category we find: steroids, prostaglandins, lipids, catecolamines, nucleosides, aminoacids, carbohydrates etc. When the functionalities involved are such that one reagent or mixture of reagents can lead to a single product, the reactions usually require strong conditions or long periods of time to obtain full derivatization. If this approach is not suitable, or

multiple step process is usually required, two or more reactions are carried out in a predetermined sequence, maintaining the appropriate conditions in each step. Examples of this kind are the formation of n-butyl N-trifluoroacetyl amino acid derivatives, and acetylated alditol derivatives from sugars (56,57).

A third approach applicable only when the functional groups are located close to each other is the formation of cyclic derivatives. Specific reagents with this capability have been used successfully mostly in gas chromatography. Table VI mentions some of these reagents along with the substrates to which they have been applied to (58).

Boronic Acids - Among the reagents mentioned on Table VI which are capable of forming cyclic derivatives, only the boronic acids have a relatively wide range of applications. In general terms, they can react with 1-2, 1-3, 1-4 diols, hydroxy acids, amino alcohols, diamines, 1-2 enediols and aromatic molecules with ortho substituted phenol, amine and carboxylic acid group.

These acids have been known for a long time. Their usefulness in analytical chemistry covers several fields and different types of applications such as, complexing agents in liquid chromatography (59), ion exchange groups in resins (60) and derivative forming reagents in chromatography and mass spectrometry. This last application is particularly important and is possible because of the prominent molecular ion formed which is easily identified by the characteristic isotopic ratio of boron. Mass spectra of cyclic boronates are also

Table VI

Cyclic Derivatives

Reagent(s)	Substrate	Technique*
Acetyl Acetone	Biguanidines	GC-FID, NPD, EC
Hexafluoro Acetyl Acetone	Metals	
2,4 Pentanedione	Hydrazines	GC-FID
1,2 Diamino Benzene + TMCS	α Keto Acids	GC-FID
Dimethyl Dichloro Silane	1,2-1,3 Diols	GC-FID
Ethyl Phosphonothioic Dichloride	Diols, Aminoalcohols Diamines	GC-FID
Hexafluoroacetone-Diazomethane	Alcohols (esteroids) Cis Diols, α Amino Acids	GC-FID
Phenyl Isothiocyanate	α Amino Acids	GC-FID LC-UV
Boronic Acids	1,2-1,3 (Diols, Amino Alcohols Diamines, Hydroxy Acids)	GC-FID, ECD LC-UV

*NPD - Nitrogen Phosphorus Detector
FID - Flame Ionization Detector
EC - Electron Capture Detector
UV - Ultraviolet Detector

relatively simple and their fragmentation pattern reveals a good deal of structural information about the parent compound (61).

General Chemistry -Overall chemical aspects of boronic acids have been reviewed (62), and a list of all these acids prepared up to 1974 is available (63). In the next few paragraphs, the most relevant aspects of their chemistry will be mentioned as background for further discussion.

Organic boron acids of two kinds are known. Boronic acids which have the general structure R-B-$(OH)_2$ and a second type R_2-B-OH or borinic acids with no applications in analytical chemistry.

Most samples of boronic acids contain varying amounts of the trimeric anhydride, $(R\text{-}B\text{-}O)_3$. This fact complicates their characterization by simple physical constants such as melting point. In order to study the chemistry of the acids, and not of the anhydride, a recrystallization from water is recommended. Both forms can be distinguished by their infrared spectra.

Anhydride formation presumably occurs during the GC analysis of boronates when excess acid is present. This however, is no problem since the anhydrides are usually as reactive as the acids.

In order to have a more convenient way to characterize boronic acids S. Singhawangcha et. al. (64) proposed to use the corresponding pinacol boronates which have sharp melting points without decomposition and also good chromatographic and mass spectrometric characteristics. Another alternative for this purpose is to use the bis-trimethyl silyl

esters which have similarly good properties but unfortunately they are extremely sensitive to hydrolysis (55).

Boronic acid stability differs greatly depending on the R substituent. Acids containing an alkyl group are sensitive to oxidation with the slow formation of orthoboric acid. For this reason alkyl boronic acids are constantly maintained under water and in refrigeration (58). Aryl boronic acids are not sensitive to this type of oxidation.

Dissociation constants for these acids have been measured (66,67). The presence of aryl groups tends to increase the acidity (phenyl boronic acid is 10 times as strong as n-butyl boronic acid). Strong electron withdrawing groups substituted on the aromatic ring will result in increased dissociation constants as well, (phenyl boronic acid has a $k_a = 1.64 \times 10^{-11}$ compared with 4-fluorophenyl boronic acid which has $k_a = 3.66 \times 10^{-10}$).

An interesting observation has been reported concerning the acidity of nitrophenyl boronic acids (67). The ortho isomer is anomalously weak, $k_a = 0.56 \times 10^{-9}$ compared to the meta, $k_a = 6.9 \times 10^{-9}$ or the para $k_a = 9.8 \times 10^{-9}$. This appreciable difference has been attributed to internal ring formation or intramolecular hydrogen bond interaction.

The synthesis of boronic acids is usually achieved through the Grignard's reaction (68,69). Commonly, the yields range from 40% to 60% and final purification is almost invariably obtained by recrystallization from a suitable solvent.

Figure 2 shows the Grignard's reaction sequence and also the general derivatization reaction between boronic acids and bifunctional molecules. The product of this, a cyclic boronate is the derivative normally used in chromatography.

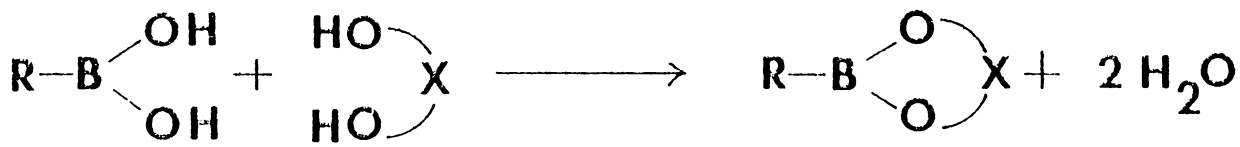
Interaction with Polyfunctional Molecules. Previous to the application of boronic acids in chromatography, their interaction with diols and carbohydrates was studied. J. Sugihara and C. Bowman reported on the formation of tribenzenboronates with some alditols and they also reported the fact that 5-, 6- and 7-member ring boronates are possible (70). J. C. Lockhart studied the ease of boronate formation from 1-2 and 1-3 diols concluding that there is no great difference between the two cases, he also suggested B-O bond fission as the mechanism of boronate formation (71).

The properties and structures of several phenylboronates obtained from polyols like mannitol, sorbitol etc, was reported by H. Kuivila et. al. (72) who also described a simplified method of boronate formation.

M. W. Wolfrom and J. Solms tried to crystallize benzenboronates of several aldoses and studied their stability in air. In all cases bis phenyl boronates were formed and many gave crystalline products with low yields. (73)

The thermal stability of n-butyl and phenylboronates was studied by P. B. Brendley et. al (74) concluding that alcoholysis was one of the possible decomposition paths.

a



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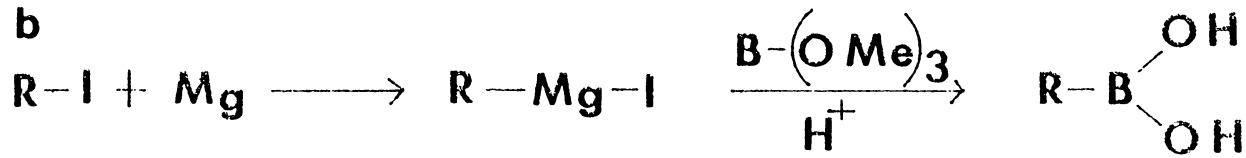


Figure 2. a) Boronic Acids General Derivatization Reaction
b) Boronic Acids Method of Preparation

The structure of several phenyl boronates obtained from acyclic polyhydroxy compounds was studied by E. J. Boorne et. al (75). Glycerol boronate was identified as being the 1,2 addition product. In the case of galactitol the 1,3, 4,6 bis boronate structure was proposed as the correct one. This report also discussed several conformational aspects of boronates, hydrogen bonding and boron oxygen coordination were some of the factors suggested to explain the influence of phenyl boronic acid on the chromatographic mobility of galactitol.

Measuring the weight gained by phenyl boronates upon standing in humid air, R. A. Bowie and O. C. Musgrave examined the stability of boronates of different ring size (76). In general 5 and 6 member rings were relatively stable; 7 member ring boronates were reported to hydrolize rapidly. A particularly interesting case of a stable boronate is the one formed from N,N-bis (2-hydroxy-1-naphthylmethyl) methyl amine which although forming a 10 member ring cycle was found to be stable toward hydrolysis. This unusual behavior was explained by the transanular N-B bond which makes the molecule particularly stable.

The interaction between several benzene boronic acids and different D-sugars has been studied by S. A. Baker et. al. (77) using optical rotation methods. Depending on the substituent attached to the aromatic ring, pH affects the complexation of sugars in different ways. It was found for example that 4-methoxybenzeneboronic acid complexes D-mannose at pH = 7; whereas 3-nitrobenzeneboronic acid begins to complex below pH = 6. These results are in agreement with the strength

of the acids.

Applications to Chromatography -As has been mentioned before, boronic acids were first used in chromatography in 1956 as complexing agents to modify the chromatographic behavior of some polyhydroxy compounds (59).

The examination of boronates by gas chromatography was first done by R. Koster et. al. in 1965 (78). They reported the analysis of phenyl boronates obtained from benzoin, mesohydrobenzoin and some diols. The reagent employed was 1,2-diphenyldiborane and the derivative structures were confirmed by mass spectrometry.

The application of alkyl boronic acids to the derivatization of more complex molecules was first reported in 1968 by C. J. Brooks et. al. (79) and later again by G. M. Anthony and C. J. Brooks in 1969 (80). These reports dealt with the use of n-butylboronic acid in the derivatization of corticosteroids, β-hydroxy amines and some other bifunctional molecules. Major emphasis was placed on the study of the GC characteristics of the boronates obtained. In some cases additional silanization was necessary in order to obtain complete derivatization of molecules containing isolated groups.

It is interesting to point out that these pioneering reports described some apparently successful attempts to analyze alkyl boronates by thin layer chromatography. In the case of corticosteroids, epimer separation was obtained and confirmed by mass-spectrometry.

The relative merits of methyl, n-butyl, phenyl and cyclohexyl

boronates for GC and MS analysis were studied in 1971 by C. J. Brooks and I. McLean (81). These authors found that while the MS spectra of methyl and phenyl boronates were relatively simple, those of n-butyl and cyclohexyl boronates were complicated by alkyl chain fragmentation. From the GC point of view, methyl boronates were somewhat thermally unstable and cyclohexyl ones had very long retention times.

In 1971 Frank Eisenberg reported the first GC analysis of carbohydrate boronates (82). n-butyl boronates of different sugars were prepared using pyridine as solvent and multiple peaks were observed in the cases of D-mannose and D-glucose.

As early as 1971, attempts were made to employ some kind of selective detection in the analysis of boronates. E. J. Sawenski et. al. (83) were perhaps the first in those attempts. Employing a flame photometric detector fitted with a 546 nanometer filter they were able to obtain detection limits of the order of a few nanograms for boron containing samples.

In 1973 R. Greenhalgh and P. J. Wood studied the response of an alkali flame ionization detector to boronates and boron containing molecules (84). Their results however, showed only a modest increment in response with respect to flame ionization detection. The detector's linear range on the other hand was found to be good, 10^3, and no detector contamination problems were encountered.

Regarding selective detection systems, it is convenient to mention at this point the recent report on the use of a dedicated inductively coupled plasma spectrophotometer for the selective analysis of

boronates (85). Systems of this type although complex and expensive, appear as a bright candidate for developing as the ultimate detector concerning selectivity, and applicability.

The electron capture detector since its introduction in 1958 by Lovelock (86) has proven to be one of the most sensitive devices ever produced and employed in analytical chemistry. Surprisingly the introduction of electrophores in the boronic acid structure took a long time to appear.

C. F. Poole and coworkers introduced 3,4-dichlorobenzene and 4-Iodo butan boronic acids as derivatizing reagents for electron capture detection in GC in 1978. Employing pinacol as a sample they studied the mechanisms of electron attachment and concluded that it was dissociative in both cases (87).

The same authors in 1979 (88), studied different "on column" derivatization methods. Their report mentions the effect of the amount of acid deposited in the column on the response obtained, no mention is made however of any loss of column efficiency and quantitative results are not clearly demonstrated.

Several other halogenated benzenboronic acids were later studied and compared by C. F. Poole et. al (89,90). Among the results mentioned in this paper were the quite different volatilities of the derivatives formed according with the halogen involved and the specific type of substitution. The reagents examined were the 2,6-dichloro, 2,4,6-trichloro, 3,5-(ditrifluoromethyl), 3,5-dichloro and 2,4-dichloro benzene boronic acids. In most cases good results were obtained with

detection limits of just a few picograms. Two reagents which did not give good results were the ones containing the pentafluorophenyl group which deboronates rather easily and the naphthalene one which gives extremely long retention times and high detection limits.

Nitrophenyl boronic acids have not been really studied. There is a brief mention of the meta isomer in which relatively long retention times for the corresponding boronates are reported and the extreme dependence of the EC. response on the detector temperature is also reported (91).

Besides their applications to gas chromatography, boronic acids have been applied to other chromatographic techniques. In liquid chromatography, meta amino phenyl boronic acid has been attached to polyacrylamide beads by a short aliphatic chain and the resulting material employed in the separation of plant polyphenolic substances (60). This same acid has also been coupled to an epoxy silane bonded phase on silica gel (92). Columns packed with this material have been used in the separation of carbohydrates, nucleosids and nucleotides. Slow equilibration between sample and bonded phase ligand was reported to limit the efficiency of the separation so obtained.

Applications of boronic acids as derivatizing reagent in HPLC have not been reported. There is only one report on the high performance thin layer separation of some insect hormones derivatized with naphthalenboronic acid which forms fluorescent derivatives (93). In this report nothing is mentioned about sensitivities or quantitative determinations.

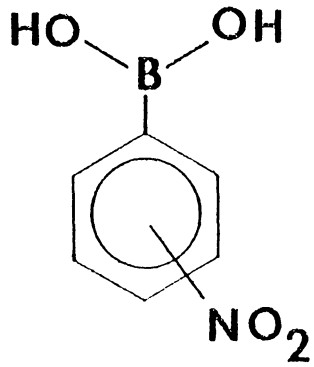
Nitrophenyl Boronic Acids - As mentioned before, this work will be devoted to an in depth study of the derivatization performance of nitrophenyl boronic acids. Figure 3 shows the structures of these reagents and some of the attractive features of these molecules as derivatizing reagents.

Nitro groups are well known to be excellent electrophores, a characteristic which is related to their strong electron withdrawing character. Good ultraviolet absorbance is also obtained when nitro groups are substituents in a aromatic ring. These two features alone are potentially beneficial for selective detection in GC and HPLC.

The possibility of electrochemical detection is also present if the derivatives formed are stable enough to be chromatographed under reverse phase conditions. This is a requisite as electrochemical detectors require highly conductive mobile phases.

The presence of the heteroatoms boron and nitrogen create another interesting alternative. The use of some kind of selective boron or nitrogen detector can lead to excellent specificity and sensitivity. We have already mentioned the use of an inductively coupled plasma spectrophotometer for this purpose but perhaps a more practical and economical approach is the use of a nitrogen selective detector from which at least two designs can be considered.

Thermoionic detectors are essentially the modern version of the old alkali flame ionization detector (94). These devices under optimum conditions and depending on the type of nitrogen containing sample, can give N/C specificity as good as 50,000. In this case however the



SUITABLE FOR FLAME IONIZATION DETECTION

GOOD ELECTRON CAPTURE RESPONSE

EXCELLENT ULTRAVIOLET ABSORPTION

CONTAINS AN ELECTROCHEMICALLY ACTIVE GROUP

POSSIBILITY OF NITROGEN OR BORON SELECTIVE DETECTION

Figure 3. Nitro Phenyl Boronic Acids Structures and Chromatographic Advantages of Boronates

selectivity obtained is in a way a measure of the sample's ability to form CN. radicals. It is possible that nitro groups would give a reduced response (95,96). This has been suggested but not studied in detail.

Another type of nitrogen detection system, is the Hall Electrolytic Conductivity detector which is less discriminating on the type of nitrogen functionality and perhaps a better approach in the case of derivatives of this kind. (97)

All of the previously mentioned benefits are in addition to the increased volatility and improved chromatographic behavior which the boronates are known to provide with respect to the original sample.

EXPERIMENTAL

In this section a complete description will be made of all instruments, accessories, reagents and techniques employed in the present study. Emphasis will be placed pointing out the important or critical details involved in the procedures. A full discussion of these details and their effects on results will be covered in the next section.

Reagents - A relatively large number of chemical reagents were employed in this work. In the next few paragraphs these reagents will be listed according to their use in specific procedures which will be described later in this section.

For the preparation of ortho, meta and para nitrophenyl boronic acids, phenyl boronic acid was used as a starting material which was obtained from Aldrich Chemical Co., Milwaukee, Wisconsin and Sigma Chemical Co., St. Louis, Missouri. No differences were noted between the two sources and in all cases the phenyl boronic acid was recrystallized from water and air dried before used.

Fuming colorless nitric acid (D=1.5), acetic anhydride and activated charcoal were obtained from Fisher Scientific Co., Fair Lawn, N.J., (ACS analytical reagent grade) and used without any additional treatment. Care was taken to maintain these reagents tightly closed when not in use. Nitric acid more than 6 months old usually develops a yellow color and it was not used after this point.

Anhydrous diethyl ether, sodium carbonate and concentrated hydrochloric acid obtained from J. T. Baker, Philipsburg, N.J., (ACS

analytical reagent grade) were used as received.

To study the derivatization efficiency of nitro phenyl boronic acids, a number of bifunctional model molecules were used as test samples.

Ethylene glycol, 1,3-propandiol, 2,4-pentandiol, 1,4-pentandiol, 1,4-butandiol, 1,3-propandiamine, 3-amino-1-propanol, all of no less than 98% purity were obtained from Aldrich Chemical Co., Milwaukee, Wisconsin. Lactic acid and 3-hydroxypropionic acid from the same supplier were of technical grade purity.

(-)Ephedrine, (+) phenyl propanol amine, (±) arterenol, dopamine, synefrine and epinefrine were purchased from Sigma Chemical Co., St. Louis, Missouri and used as free bases.

Pyrochatecol, salicylic acid and salicylamide of reagent grade purity were obtained from Eastman Kodak Co., Rochester, N.Y. Ortho-amino phenol and ortho phenylenediamine also from Eastman Kodak were practical grade.

Three different internal standards (analytical reagent grade) were employed in the stability studies. Triphenylmethane was obtained from Matheson Coleman & Bell, Cincinnati, Ohio, and di-n butyl phthalate was purchased from Fisher Scientific Co., Fairlawn, N.J.. The third internal standard was butyl hydroxy toluene (BHT), the preservative normally contained in tetrahydrofuran at 0.025% concentration level.

Stablized tetrahydrofuran containing 250 ppm BHT was the most commonly employed as reaction solvent. In addition, acetonitrile HPLC

grade from Fisher Scientific Co., Fairlawn, N.J. or Burdick & Jackson, Muskegon, Michigan was also employed. In the case of samples with limited solubility in THF or ACN, dimethylformamide from Fisher Scientific Co., was occasionally used.

To remove traces of water from the derivatization reactions, 2,2-dimethoxy propane was added as water scavenger. This was purchased from Aldrich Chemical Co. All solvents and moisture sensitive reagents were tightly capped when not in use.

In the case of samples to be analyzed by liquid chromatography, the solvent employed was tetrahydrofuran UV or HPLC grade purchased from Fisher Scientific Co., Fairlawn, N.J. Special precautions were taken by purging with nitrogen in the head space of the container, and also sparging the liquid with nitrogen purified through a moisture trap and an "oxysorb" oxygen scavenger filter.

Solvents used as mobile phases in HPLC were all HPLC grade and were purchased from different sources, mostly from Burdick & Jackson, Muskegon, Mi. and Ashland Chemical Co., Columbus, Ohio.

Chromatographic Instrumentation -A Varian gas chromatograph model 3700 equipped with flame ionization and electron capture detector was employed. The GC instrument was equipped with digital temperature controls and readout as well as digital linear temperature programming capabilities.

The radioactive source in the EC. detector was a Ni^{63} foil containing 8 millicurie of activity. The maximum recommended temperature of this source was 400°C. It is important to point out that

this detector is operated in the constant current, variable frequency mode. The base frequency selector switch inside the differential electrometer was set on the nitrogen position.

The gas chromatograph was attached to a Data Mark recorder model SR 6252 with variable chart speed and imput range. A 1 mv full scale range was used in all cases. To integrate the peak areas, a Varian CDS-111 electronic integrator was employed.

Prepurified nitrogen obtained from Airco, Montvale, N.J., was used as carrier gas for both FID and ECD detection. The nitrogen stream was further purified through a silica gel/molecular sieve combination filter (Foxboro, North Haven CT) and then through a "oxysorb" oxygen trap (Dow Chemical Co., Midland, MI.). In this way trace oxygen and water levels were reduced to an acceptable limit for the ECD detector.

All separations were performed using 6'x2mm I.D. 1/4" O.D. glass columns. These were packed with 3% OV-17 or 3% SP-2250 on Supelcoport 80/100 mesh obtained from Supelco Inc., Supelco Park, PA. The column carrier gas flow rate was set at 25 ml/min and measured weekly with the use of a soap bubble meter. Unless otherwise mentioned the conditions were as follows: Column temperature 180°, injector and detector temperature 260°C. In the case of phenyl boronates, the column temperature was 140°C and the injector and detector were set to 190°C.

Breathing air and hydrogen from Airco, Montvale, N.J., were used with the FID. The corresponding flow rates were set at 30 ml/min H_2 and 350 ml/min of air; these were also measured and reset if necessary at least once a week and used without additional purification.

The GC columns were packed "in house" with the aid of a water aspirator and a Dremel vibrator (Racine, Wis.). Approximately 2.5g of packing were necessary to completely fill each column. Both column ends were plugged with a small portion of silanized glass wool.

When the EC detector was employed, an additional stream of N_2 was added as a make-up gas. This was done using the hydrogen inlet line attached to the universal detector base. The combined carrier gas plus make-up streams were set to a total of 40 ml/min.

All the injections into GC columns were made with a Hamilton 701 10 microliter syringe. The septum was of the teflon faced type and replaced approximately every 50 or 60 injections.

The liquid chromatography studies were made with a Varian 5000 HPLC instrument. This chromatograph has a single piston, triple inlet valve pumping system and is capable of mixing any two of these solvents at a time. The liquid chromatograph was connected to a Varichrom variable wavelength detector with a spectral range of 190 to 800 nm. Depending on the sample type, 260 or 265 nm were selected as the working wavelengths.

Following the manufacturer's recommendations a spectral slit of 8 nm and a medium detector response time constant were routinely employed. All connections between column and injection valve or detector were kept to a minimum length using 1/16" O.D. stainless steel tubing with a 0.009" inside diameter.

The detector's output was fed to a Varian recorder model 9176 with variable chart speed and 1 mv full scale range. Peak area integrations

were obtained with a Varian CDS-111 electronic integrator.

The column employed in HPLC was a Lichrosorb 5 micron cyano bonded phase, 20 cm long x 4 mm I.D. provided by EM Sciences Laboratories. The mobile phase flow rate was normally set at 1 ml/min. All chromatograms shown in the results and discussion sections were obtained with mixture of 5% tetrahydrofuran UV grade in hexane as mobile phase.

All injections were made using a Valco 6 port high pressure sampling valve and a special 1 milliliter Glenco syringe adapted with a special needle to fit the valve. A 10 microliter sample loop was attached to the valve and the same one was used throughout all the LC studies.

In both gas and liquid chromatography, the integration conditions in the electronic integrator were adjusted to ensure optimum peak detectability during the chromatogram.

Spectroscopic Instrumentation - Nuclear magnetic resonance spectra were obtained in a model EM-390 Varian NMR Spectrometer which operates at a 90 MHz frequency and has a 14000 gauss magnet.

NMR samples were prepared in 5 mm O.D. precision glass tubes obtained from Norell Chemical Co., Landisville, N.J. No deuterated solvents were employed with the exception of D_2O for deuterium exchange experiments.

The tetrahydrofuran signals were used as internal references and no use was made of the lock-in operation mode. Every time the instrument was used the probe was carefully tuned and the "Y" and

curvature field homogeneity were also finely adjusted.

To determine UV spectra a scanning Hitachi UV-Vis Spectrometer model 100-60 was used. The spectra were recorded on a Hitachi model 50 potentiometric recorder synchronized with the spectrophotometer's scanning mechanism.

Fused silica sample cells with 10 mm path length were employed after careful chromic acid cleaning and final rinsing with UV grade THF.

All spectroscopic determinations were made at room temperature using THF stabilized with 0.025% BHT as a solvent in the case of NMR spectroscopy and UV grade THF in UV-Vis spectroscopy.

Miscellaneous Instrumentation - Melting point determinations were made on a Fisher-Jones hot plate melting point apparatus. The heating rate was maintained at approximately 2°C/min at the transition temperature.

Elemental analyses were performed by the Analytical Services of the Chemistry Department at Virginia Tech employing a Perkin Elmer model 240A CHN analyzer.

The analytical balances employed in this study were the Mettler balance models H-80 and H-23 with a maximum weighing capacity of 120 and 160g respectively. The readability was approximately 1 mg for the H-23 model and 0.1 mg for the H-80 one. Every time the balances were used the zero reading was adjusted after careful cleaning of the weighing pan.

To deliver small volumes, 50 and 100 microliter syringes were commonly used. When more accuracy and precision was desired, an adjustable volume micropipette was employed instead. Finnpipette (Finnpipette Ky, Finland) with a 50 to 200 microliter capacity was fitted with disposable polyethylene tips and calibrated to work with non aqueous solutions. The precision of the volumes delivered was estimated to be close to 1%.

Common laboratory glassware was employed after cleaning with detergent and then air dried. A final rinse with the solvent to be used was routinely made.

Procedures and Techniques - All laboratory techniques were followed as described in the next few pages. If modifications were introduced, these will be discussed and described in the next section.

Preparation of Meta Nitrophenyl Boronic Acid - The procedures described by W. Seaman and J. R. Johnson (98) were employed for this purpose with only minor modifications. These procedures are described as follows:

To a beaker containing 75 ml of colorless fuming nitric acid (D=1.5) placed in a dry ice bath at -15°C (40°F), 10g of phenyl boronic acid were slowly added during a period of approximately two hours. Good magnetic stirring was maintained and care was taken not to let the temperature rise above -9°C (15°F) during the addition period.

At all times the thermometer was placed inside the beaker with the mercury bulb well immersed in the liquid in order to have good

temperature readings.

When the addition was completed the reaction mixture was further stirred for 10 minutes and then poured over 100 ml of ice. At this point when the ice has melted, a solid in suspension was removed by filtration and the filtrate neutralized with concentrated KOH, after this the pH was adjusted to approximately 6 with dilute HNO_3.

Normally, after neutralization an abundant precipitate is obtained, this presumably consist of KNO_3. After removal of this salt by filtration, the solution is extracted twice with 100 ml of diethyl ether. After evaporation at room temperature a yellow crystalline residue is obtained.

The product obtained was mixed with the first solid and recrystallized from water with the addition of activated charcoal. The typical reaction yield was around 35% of the theoretical value.

The other 2 possible mononitrated isomers were also prepared by techniques described originally by Seaman and Johnson (98).

Preparation of Ortho- and Para-Nitrophenyl Boronic Acids - During a period of 45 min., 6g of colorless fuming nitric acid (D = 1.5) was added to a solution of acetic anhydride containing 10g of phenyl boronic acid. This solution was cooled at -15°C (4°F) in a dry ice bath regulating the rate of acid addition to avoid any drastic increase of temperature. The nitric acid was added with the aid of a 10 ml burette and vigorous magnetic stirring was maintained at all times.

The thermometer was immersed in the acetic anhydride solution for

accurate readings. After all the HNO_3 was added the solution was stirred for 90 minutes allowing the temperature to rise slowly to -7°C (19°F).

Magnetic stirring was continued until all the boronic acid had dissolved (usually 1 h.) allowing the temperature to rise but using occasional cooling to keep it below 20°C (64° F). When a clear solution was obtained, it was poured over 200 ml of ice and the mixture stirred until the ice was melted and the solution looked homogeneous.

Upon standing for a few hours or after concentrating by vacuum distillation (at approximately 25 mm Hg), a pale yellow solid was obtained, this product is essentially pure para nitrophenyl boronic acid. This solid was removed by filtration and further purified by recrystallization from water.

The filtrate was concentrated by reduced pressure distillation after the addition of 50 ml of water and the process repeated 2 or 3 times until the distillate has little odor of acetic acid. A final concentration was made to a very small volume until crystals separated from solution, the solid was then removed by filtration and saved for further purification (I).

The filtered solution was neutralized by adding Na_2CO_3 and then slightly acidified with HCl. After this it was extracted twice with 50 ml portions of diethyl ether. After evaporation of the ether at room temperature a crystalline solid was obtained (II). This product was mixed with the main fraction (I) and recrystallized from water with the addition of decolorizing charcoal.

The product obtained this way is a mixture which requires additional purification. Repeated washings with hot carbon tetrachloride and recrystallization from water are usually sufficient to obtain ortho nitro phenyl boronic acid of high purity.

Typically the reaction yields are, 35% of the ortho isomer and 5% of the para. The remaining products are mostly unreacted phenyl boronic acid, meta nitro phenyl boronic acid and nitrobenzene.

Derivatization Techniques - Derivatization in solution and also different "on-column" techniques were tried.

Solution or batch reactions were normally carried out by mixing appropriate volumes of equimolar solutions of the boronic acids and bifunctional samples. Normally a 1 molar excess of derivatizing reagent was added to the sample.

Tetrahydrofurane, acetonitrile and dimethyl formamide were the solvents employed and in all cases a small amount of 2,2-dimethoxy-propane was added to remove traces of water and ensure a complete reaction. Unless otherwise mentioned all derivatization reactions were carried out at room temperature which was in the range between 18-25°C.

In the case of samples prepared for calibration purposes, after the reaction was allowed to reach equilibrium, a final dilution was made to the calibration mark of a volumetric flask.

The same solution derivatization techniques were employed for both gas and liquid chromatographic analysis.

"On-column" derivatization was tried only with gas chromatography

by two slightly different techniques. The first one was the deposition by repeated injection of a relatively large amount of boronic acid onto the injection side of the column, followed by an injection of the sample. Another approach was the "reactor bed" which consists of coating a small portion of the column's packing material with a relatively large amount of the reagent. To accomplish this a concentrated solution of the acid was mixed with a weighted amount of packing and the solvent slowly evaporated at room temperature, after all the solvent was eliminated the coated packing was used to pack the first few inches of the GC column.

Column Packing and Conditioning - As mentioned before all GC columns employed in this study were packed in "house" following the technique described next.

The glass tubing was flushed with acetone and dried with a stream of nitrogen. The column packing was added slowly through the injection side while maintaining vigorous vibration and having the detector side of the column attached to a vacuum aspirator. The pressure obtained with the aspirator was close to 25 mm Hg. A small silanized glass wool plug was used to maintain the packing in place.

After the column was apparently full and no more packing could be added, the column was disconnected from the vacuum line and vibrated for 10 minutes more. The aspirator was then reconnected and while vibrating the packing level usually fell 5 inches. More packing was added and the process was repeated until no more packing could be added.

In total the whole packing procedure took about 60 minutes. A small plug of glass wool was placed on the injector's side and the column was conditioned.

In order to condition the column, the following procedure was observed; leaving the column disconnected from the detector and with the flow set at 25 ml/min with the temperature maintained isothermally at 120°C for 90 minutes; then it was temperature programmed at 1°/min up to 280°C and kept at this point for no less than 12 hours.

Sample Injection Techniques - Unless otherwise mentioned, the "solvent flush" technique was employed in all GC cases. This technique consists of the following steps:

- Enough solvent is taken to fill the syringe's needle dead volume (approximately 0.8 µl).
- The plunger is withdrawn until air appears visibly in the syringe's glass barrel.
- The desired volume of sample is taken.
- The plunger is further withdrawn until air appears again and the actual sample volumes is measured.
- Keeping the plunger withdrawn the injection is made.

All injections were performed on the "On-column" mode. The sample was actually deposited in the packing material to obtain maximum efficiency.

For liquid chromatography no special injection techniques were employed. With the use of a special 1 ml syringe adapted to fit the

Valco valve, the 10 µl sample loop was flushed with at least 5 times the loop's volume, after this the valve was switched to the inject position and maintained there for the analysis.

Stock Solution Preparation - Solutions containing single bifunctional molecules or boronic acids at 0.1 or 0.01 molar concentration level were employed throughout this study. These were prepared by careful weighing of the calculated amount of reagent in a volumetric flask and diluting to the calibration mark.

All solutions were tightly capped when not in use and maintained at room temperature. Although no evidence of decomposition was found, the solutions were used for no longer than three weeks.

The internal standard solutions were prepared in the same way and the same storing and use precautions were observed.

Micropipette Calibration -Small volumes of the stock solutions were handled with the use of a micropipette which was calibrated to work with non aqueous solvents. The calibration was done by weighing the amount of solvent delivered ten times with the volume set at the maximum capacity. Taking into account the solvent density and calculating the average volume delivered in 10 measurements the actual volume obtained was calculated.

Peak Integration Techniques - The following guidelines for peak area determination were observed:

- Maximum detector output was employed in order to obtain large integration counts.

- Solvent peaks and early eluting impurities were suppressed from the integration.
- In the cases where excessive overlap of the solvents tail with the peaks of interest was found, forced baselines were conveniently set in order to stablish the end of the integration.
- The initial peak width integration parameter was updated at least 2 or 3 times during the analysis.
- In order to simplify the integration report a minimum peak area count of 5000 counts was set.
- The end of the integration after the elution of the last peak was fixed to a time comparable with the integrator's own finding of the baseline.

Sample Preparation for NMR Spectroscopy - In order to obtain enough sample in a relatively small volume, the following procedure was employed: 2 ml of a 0.1 M solution of a model compound were evaporated to approximately 0.5 ml by blowing a gentle stream of dry nitrogen over a 5 ml sample vial containing the dilute solution.

Care was exercised to avoid condensation of water by warming to 35°C and evaporating very slowly. The concentrated solution was transferred to a sample NMR tube and the vial rinsed with a small volume of solvent.

The tube was manually shaken for a few seconds, tightly capped and after cleaning of the tube's outside wall installed in the sample

probe. It was always verified that enough sample was placed within the detector's zone.

The same evaporation procedure were followed when preparing solutions containing boronic acids.

Sample Preparation for UV Spectroscopy - Approximately 50 mg of sample were carefully weighed in a small vial and dissolved in UV-grade THF. This solution was quantitatively transferred to a 10 ml volumetric flask and the volume taken to the calibration mark.

After thorough mixing, 0.5 ml of the solution were transferred with the aid of a volumetric pipette to a 25 ml volumetric flask and the sample was diluted to the calibrated mark.

This final solution was employed to obtain the UV spectra which were scanned between 350 and 220 nanometers. Distilled water was placed in the reference cell. The absorbance readings were taken from the digital instrument's readout.

RESULTS AND DISCUSSION

In this section the results obtained will be presented. The discussion will cover 7 different areas which will be presented in the following order:

- Preparation and purification of ortho-, meta- and para-nitrophenyl boronic acids.
- Study of the derivatization reactions and chromatographic behavior of the derivatives obtained.
- Comparison of stabilities of different derivatives and discussion of the structural factors involved.
- Quantitative analyses, related problems and advantages and disadvantages of different detection systems.
- Chromatographic behavior in HPLC; discussion of the problems found and the potential use of boronates for this purpose.
- Scope of applications with different types of samples.
- Comments on the possible extension of the research done in this project.

In order to keep the discussion of results to a reasonable length, in some cases a generalization of the results obtained will be made. For practical reasons, only a limited number of examples will be fully discussed. In most situations however the conclusions suggested can be extended to all cases. When exceptions to these conclusions were found, those cases will be mentioned during the discussion.

It is convenient to keep in mind the purpose of the present work

which was analytical in nature. At some point during the discussion the emphasis will turn to not strictly analytical aspects. This was done to obtain more information on the molecules studied and its relevance to analytical chemistry may not be obvious at this point.

Preparation of Nitrophenyl Boronic Acids -The low temperature nitration technique described on page 44 and developed by Seaman and Johnson (98) was used and it is in general satisfactory. An interesting aspect of this technique is the change in the directing ability of the boronic acid group according with the solvent present in the reaction. When no solvent is present, such as in the preparation of the meta nitro isomer, the boronic acid group is electron withdrawing due to the electron deficient character of the boron atom.

The change in directing character is evident when a coordinating solvent like acetic anhydride is present in the reaction. The boronic acid group turns to electron donating and the reaction product in this case will be a mixture of ortho and para substituted isomers.

Figure 4 shows the contributing resonance forms in both cases. It is clear upon examining these forms what type of substitution will occur when the nitronium ion (NO_2^+) interacts with the forms in both reactions. Figure 5 shows the yields and reaction conditions required. A full discussion of these will be given in the pages ahead.

One of the first important practical details of these nitration reactions, is the type of nitric acid employed. Experiments with red fuming nitric acid or acid of less than 90% concentration were completely unsuccessful, resulting in extensive decomposition or

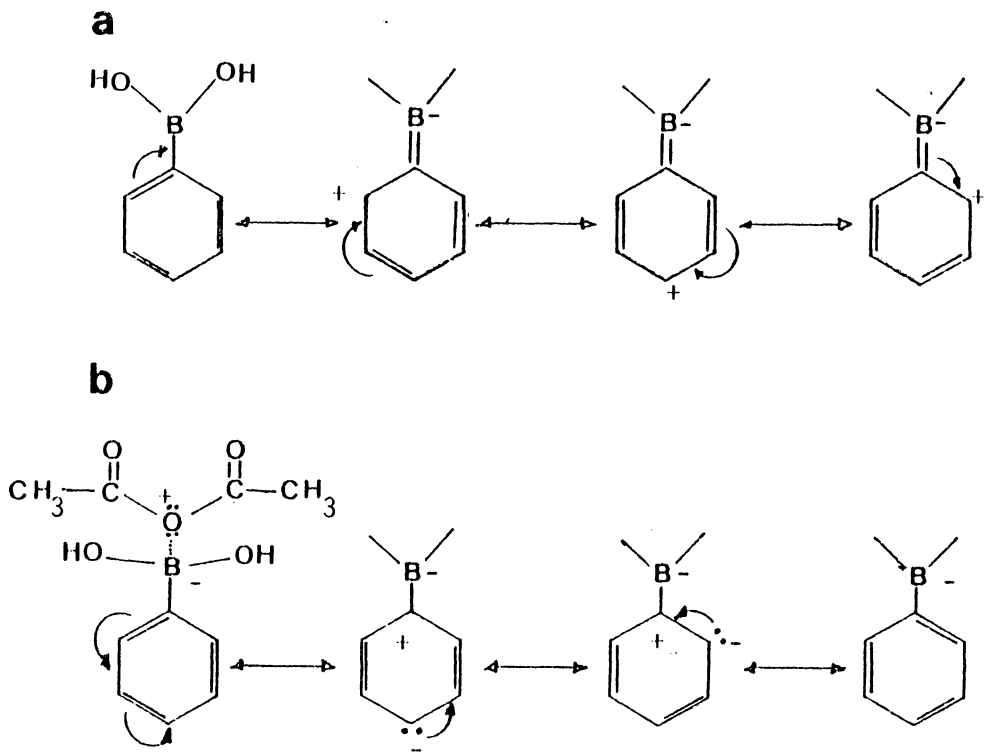


Figure 4. a) Resonance Structures of Phenyl Boronic Acid
b) Resonance Structures in the Presence of Acetic Anhydride

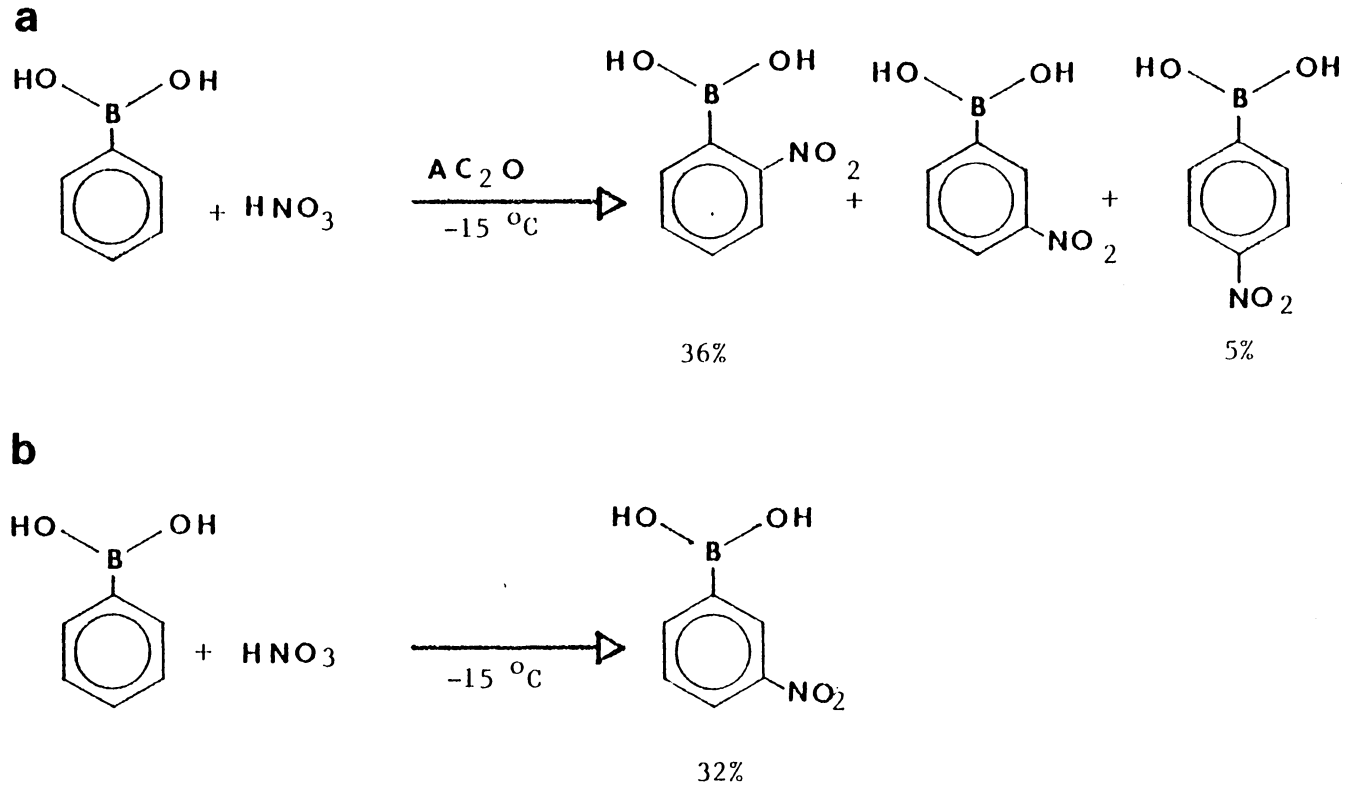


Figure 5. a) Preparation of Ortho and Para Nitro Phenyl Boronic Acids
b) Preparation of Meta Nitro Phenyl Boronic Acid

extremely low yields.

Temperature control during the reaction seems to be critical. In reactions where the temperature was higher than required less starting material remained unreacted, but the yield was generally lower.

Another important detail is to wash any product with cold water as soon as it is obtained, otherwise traces of nitric acid will oxidize the product, lowering the yield. An even better procedure is to recrystallize the products as soon as they come out of solution.

During the preparation of the ortho and para nitro isomers, the para isomer is obtained during a reduced pressure distillation step. In some cases no concentration was necessary to obtain this isomer since its solubility in water is relatively low. It is recommended, however to concentrate slightly and also to reduce the temperature a little after the concentration step in order to ensure complete removal of the para isomer. The ortho isomer is obtained after further distillation of the reaction solution.

Proceeding with the concentration at low pressure, the final distillation should be done carefully, just to the point when crystals separate from solution. Any further heating will char the product.

The preparation of meta nitro boronic acid is by far the easiest of all; the reaction is clean and the yield is good. There is a discrepancy however about the yield obtained in this study, around 35% and the one reported by Seaman and Johnson (98), around 65%. Possible explanations for this difference will be discussed later. The

reported yield of ortho nitro phenyl boronic acid is also twice the one obtained in this study.

Two practical recommendations which seem to increase the yield of ortho-para products are: (1) the addition of a gentle excess of nitric acid (around 20%),and (2) the fine grinding of phenyl boronic acid which increases the contact with acetic anhydride.

It is convenient to clarify that the yield figures in Figure 5 are only typical values. The actual ones will depend on many factors such as: the nitric acid employed, temperature, acetic anhydride purity, etc.

As it was mentioned earlier, the reaction in the presence of acetic anhydride will in theory give only a mixture of the ortho and para isomers. Unfortunately acetic anhydride is not a very good solvent for phenyl boronic acid and the reaction produces a significant amount of meta nitro isomer. This problem has been studied by D. R. Harvey and R. O. C. Norman (99). These authors reported essentially the same distribution of ortho, meta and para isomers as obtained in this work, but they did not suggest any practical way to resolve the mixture.

Purification of Products - The most difficult aspect in the preparation of the ortho and para isomers is the separation of the mixture obtained.

Gas chromatography has been used in the past to monitor the isomeric purity of these products, by a rather complicated method (99). This involves the transformation of the nitro acids to

chloronitrobenzenes by reaction with $CuCl_2$.

In this study the purity was analyzed by GC after derivatization with a diol. Figure 6 shows a chromatogram with the separation of the phenyl, ortho nitrophenyl and meta nitrophenyl boronates obtained with 2,4-pentandiol. Each pair of peaks corresponds to a particular type of substitution. The reason for two peaks is the diol used for this purpose which is a mixture of isomers (a d-l pair and a meso isomer) and the GC separation gives two distinctive peaks for each type of boronate.

It is a fortunate fact that the para nitro isomer is quite different from the others in its physical properties, in particular its solubility in water. This is particularly noticeable when recrystallizing from water, a relatively large volume of water is required and only at the boiling temperature it is possible to dissolve it. Upon cooling to room temperature crystallization occurs very rapidly. In essence the para isomer is self purifying, since it separates from the reaction mixture in a clean and efficient way due to its low solubility in water.

The remaining mixture contains mostly the ortho and meta isomers plus some unreacted phenyl boronic acid which typically can be as high as 20%. Purification of this mixture can be accomplished by repeated hot carbon tetrachloride extractions. Surprisingly this solvent preferentially removes the meta isomer and the phenyl boronic acid. When the ortho isomer fraction has reached the desired purity level, a final recrystallization from water is recommended.

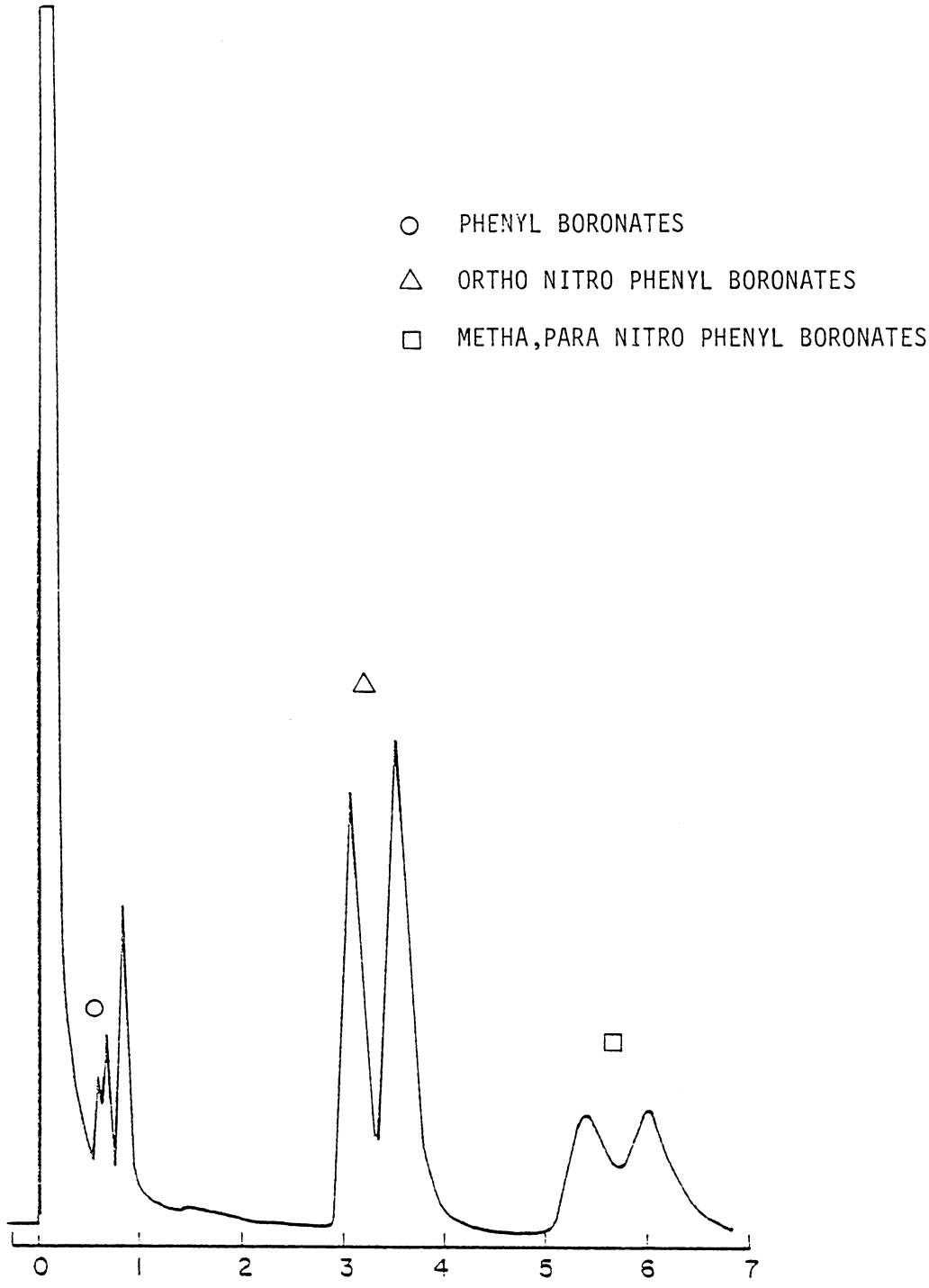


Figure 6. Separation of Phenyl, Ortho Nitro Phenyl, and Meta or Para Nitro Phenyl 2,4-Pentandiol Boronates

The purification process can be followed by GC according with the previously described procedure. Figures 7 and 8 show the 2,4-pentandiol derivatives formed from pure fractions of ortho and para nitro acids. Chromatographic conditions were as described on page 39.

A more tedious and less practical method is the repeated recrystallization from water at low temperatures. The first crystals coming out of solution consist of ortho isomer of relatively high purity. This method although possible, due to its practical limitations is not recommended.

As mentioned before, boronic acids do not have very sharp melting points, and therefore this is not a recommended process to check for purity. In this study elemental analysis and GC derivatization were used to determine the purity of the products obtained. Table VII shows the melting points and elemental analysis of the three nitro products obtained according to the procedures previously described.

Another interesting possiblity to determine the purity of these substances is NMR spectroscopy. This approach however requires a careful analysis of the signals obtained in the region 7-8 δ units. Of the three isomers, only the para has a relatively simple spectra; the ortho and meta have extremely complex patterns. These complicated signals arise from the natural boron isotopes and their respective nuclear spin quantum numbers. ^{10}B has a I equal to 3 and ^{11}B I equals 3/2, giving a heptuplet and a quartet when coupling with a proton. An additional problem is the lack of reference NMR and IR spectra for these substances.

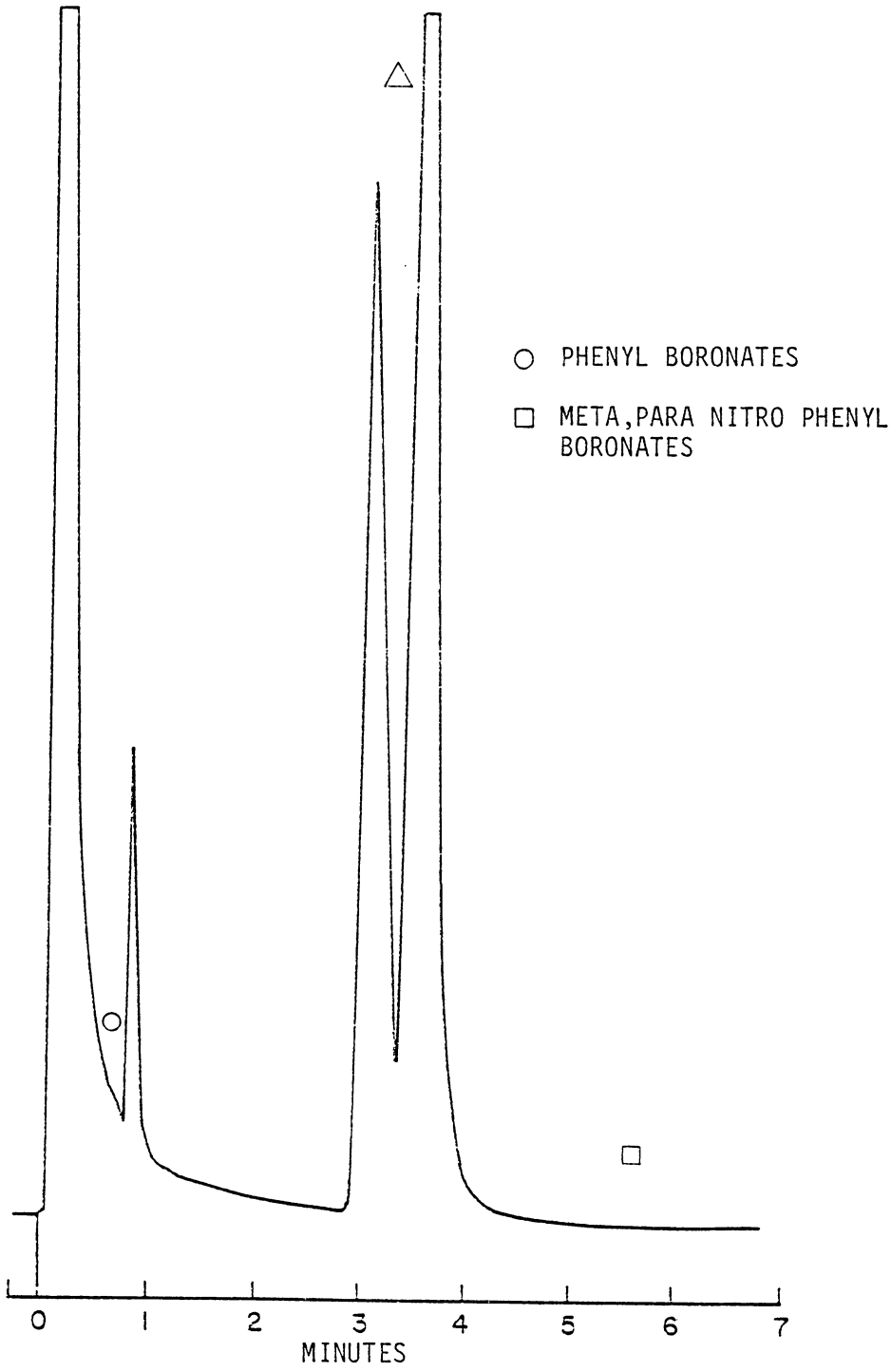


Figure 7. Pure Ortho Nitro Phenyl Boronic Acid Derivatized With 2,4-Pentandiol

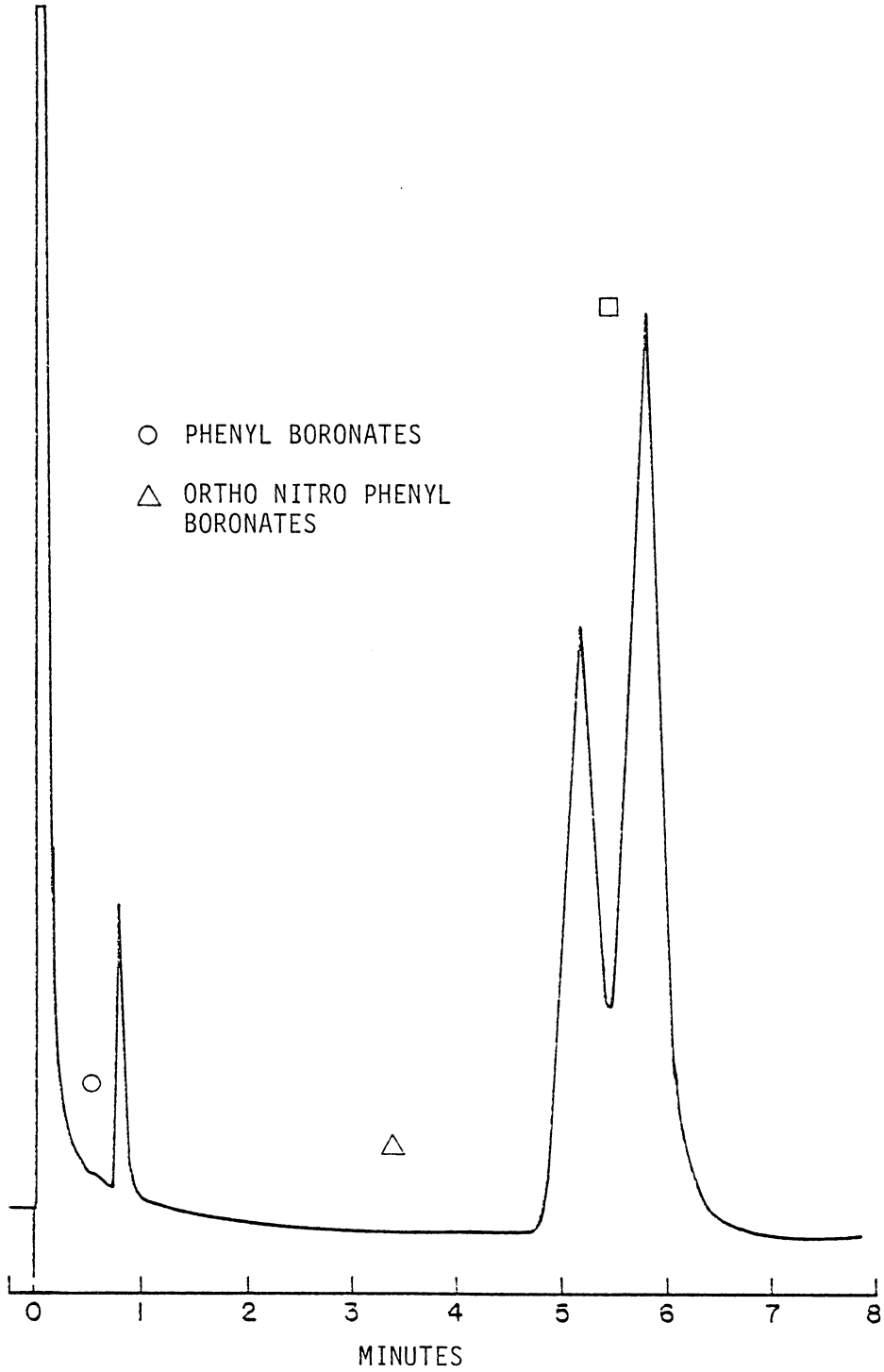


Figure 8. Pure Para Nitro Phenyl Boronic Acid Derivatized with 2,4-Pentandiol

Table VII

Boronic Acids Elemental Analysis and Melting Points

Product	Melting Point		Elemental Analysis*		
	Found	Reported (98)	% C	% H	% N
Ortho Nitro Phenyl Boronic Acid	135-145°C	138-139°C	41.98	2.88	8.09
Meta Nitro Phenyl Boronic Acid	280-295°C	285-286°C	42.60	3.44	8.18
Para Nitro Phenyl Boronic Acid	> 300°C	305°C	43.30	3.09	8.16

*Calculated % C = 43.19

% H = 3.59

% N = 8.38

The most obvious reaction byproduct is nitrobenzene. It is known that deboronation reactions of boronic acids are possible; phenyl boronic acid for example can be deboronated by heating with water at 140°C for 40-60 hours. In all the reactions carried out, a bright yellow color and a distinctive smell indicating the presence of nitrobenzene was always present.

An interesting observation reported by Seaman and Johnson (98) was the isolation of a small amount of a green oil, a possible nitroso compound obtained during the reduced pressure distillations. No similar product was ever observed with the reactions carried out in this project.

One difference between those previously published results and the present work was the isolation of a small amount of a extremely insoluble powder which coprecipitates with p.-nitrophenyl boronic acid. This fine powder is not recrystallizable from water and is of light brown color quite different in aspect from the para product. Somewhat surprising is the fact that this residue has the same elemental analysis as the three isomers. No other attempts were made to characterize this residue.

The discrepancy of reaction yields reported and the ones obtained in this work was mentioned earlier. Perhaps part of the explanation for this is the purity of the products obtained. Seaman and Johnson for example reported elemental analysis results which exceeded the calculated values by as much as 3% in carbon and 1% in nitrogen. It is obvious that Seaman and Johnson did not have in 1931 the benefits of

gas chromatography to characterize the purity of their products.

A final comment is that it is an unfortunate fact that ortho and para nitro phenyl boronic acids are the most difficult to prepare and purify, since they are the most recommended ones for use in chromatography as derivatizing reagents. An extended explanation of this point will be offered in the next section.

Derivatization Reactions - In order to study the performance of nitro phenyl boronic acids as derivatizing agents for bifunctional molecules, a series of model compounds were selected as test compounds. Table VIII shows the structure of these model compounds and their respective cyclic boronates.

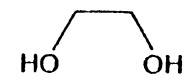
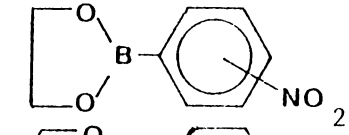
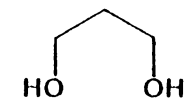
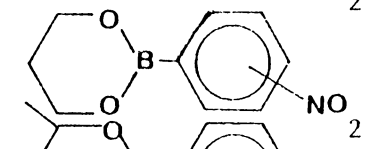
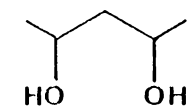
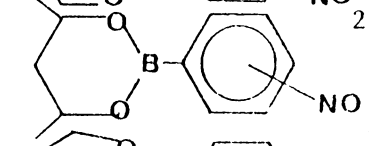
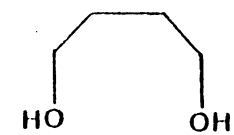
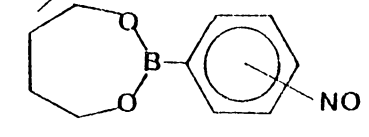
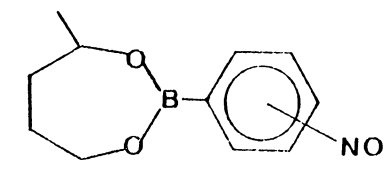
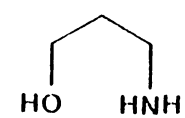
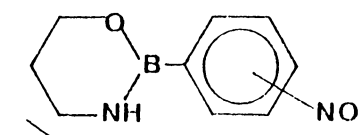
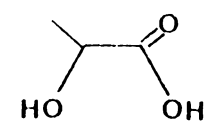
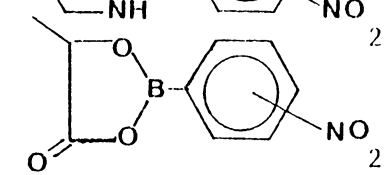
All of these molecules are difficult to chromatograph as free compounds due to their reduced volatility and high polarity. Another complicating factor is their lack of response toward selective detectors such as electron capture in GC or ultraviolet in HPLC.

Examining the boronate structures on Table VIII we see the different ring sizes which will be formed, some of which contain alkyl substituents. These differences make it possible to study different types of effects.

Both "On-Column" and solution derivatization techniques were studied, although most of this work was dedicated to the latter technique. During the next few paragraphs both techniques will be discussed along with some other aspects about the chromatographic behavior obtained in both cases.

TABLE VIII

MODEL COMPOUNDS AND BORONATES

Ethylene Glycol		
1,3-Propandiol		
2,4-Pentandiol		
1,4-Butandiol		
1,4-Pentandiol		
3-Amino-1-Propanol		
Lactic Acid		

Reactions in Solution - Convenient solvents for this type of reaction have been described in the past. In general polar non-reactive solvents such as tetrahydrofuran, acetonitrile, and dimethyl formamide are adequate for this purpose.

The characteristics of these reactions can be studied by different approaches. In this case NMR spectroscopy was chosen because it could easily give an indication of their speed and degree of completeness in a relatively easy way.

One of the important disadvantages of NMR spectroscopy is its relatively low sensitivity. In order to provide enough sample to the NMR spectrometer, concentrating procedures such as the one described on page 50 were necessary. Even under these conditions the instrument had to be operated at high amplification levels with the consequent problems of noise and satellite bands which become visible under these circumstances. Table IX lists the NMR spectrometer control settings employed to obtain the spectra in this study.

The spectra so obtained showed a remarkable abundance of signals, mostly from the solvent. A fortunate fact was that all the hydroxyl signals shown by the model molecules employed in this project were not seriously obscured by signals from the solvent. In the range between 2.3 and 3.2 delta units all of them are visible, although some are overlapped with satellite bands.

The first experiments adding a concentrated nitro phenyl boronic acid solution to a sample already in the NMR tube gave remarkable results. The speed of reaction was extremely fast; the hydroxyl bands

Table IX

NMR Spectrometer Conditions

End of Sweep	0
Sweep Width	10 ppm
Sweep Time	10 min.
Spectrum Amplification	Coarse - 1000, Fine-2
Filter	0.1 sec
Radio Frequency Power	0.02 mg
Sample Spinner	40 cps
Temperature	ambient

disappeared almost immediately providing no time to observe the reaction happening.

The solution added was concentrated in order to reduce the dilution effect which may affect the appreciation of the results.

In addition, the fact that the bands disappear completely indicate a rather extensive reaction with quite a favorable equilibrium for quantitative analysis. Figures 9, 10, 11 show three different reactions; in each case the spectrum on the left shows the solution before the addition of the boronic acid and the one on the right is the spectrum after the reaction has taken place.

From these figures we can also observe the complexity of the spectrum and the different positions of the hydroxyl bands. Although the cases presented refer only to reactions of p.-nitro phenyl boronic acid, the same results and discussion can be extended to the other isomers.

In order to see the reaction happening, a gradual addition of an equivalent of acid was made, recording the NMR spectra after each small addition. In this way the slow disappearance of the hydroxyl band was observed and it was also confirmed the reaction was complete after one equivalent of acid was added. Figures 12 through 15 show the reaction between ethylene glycol and 1,4-pentandiol and p.-nitrophenyl boronic acid at four different stages of completion. A word of caution is necessary when considering the NMR results. Due to the technique's lack of sensitivity, the instrumentation under normal circumstances can not detect small differences in concentration. In other words, the

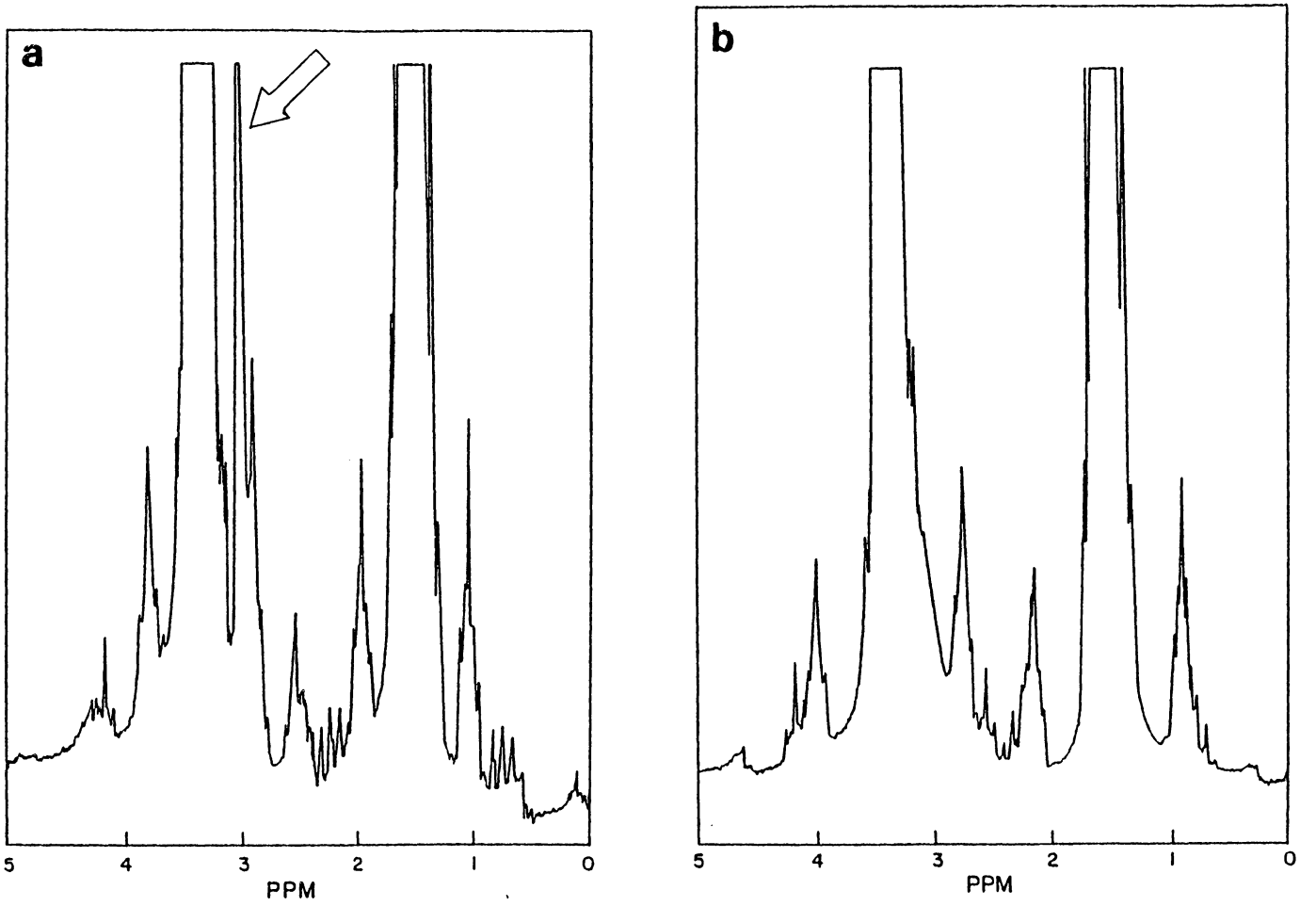


Figure 9. a) NMR Spectra of Ethylen Glycol in THF
b) Same After Addition of Para Nitro Phenyl Boronic Acid

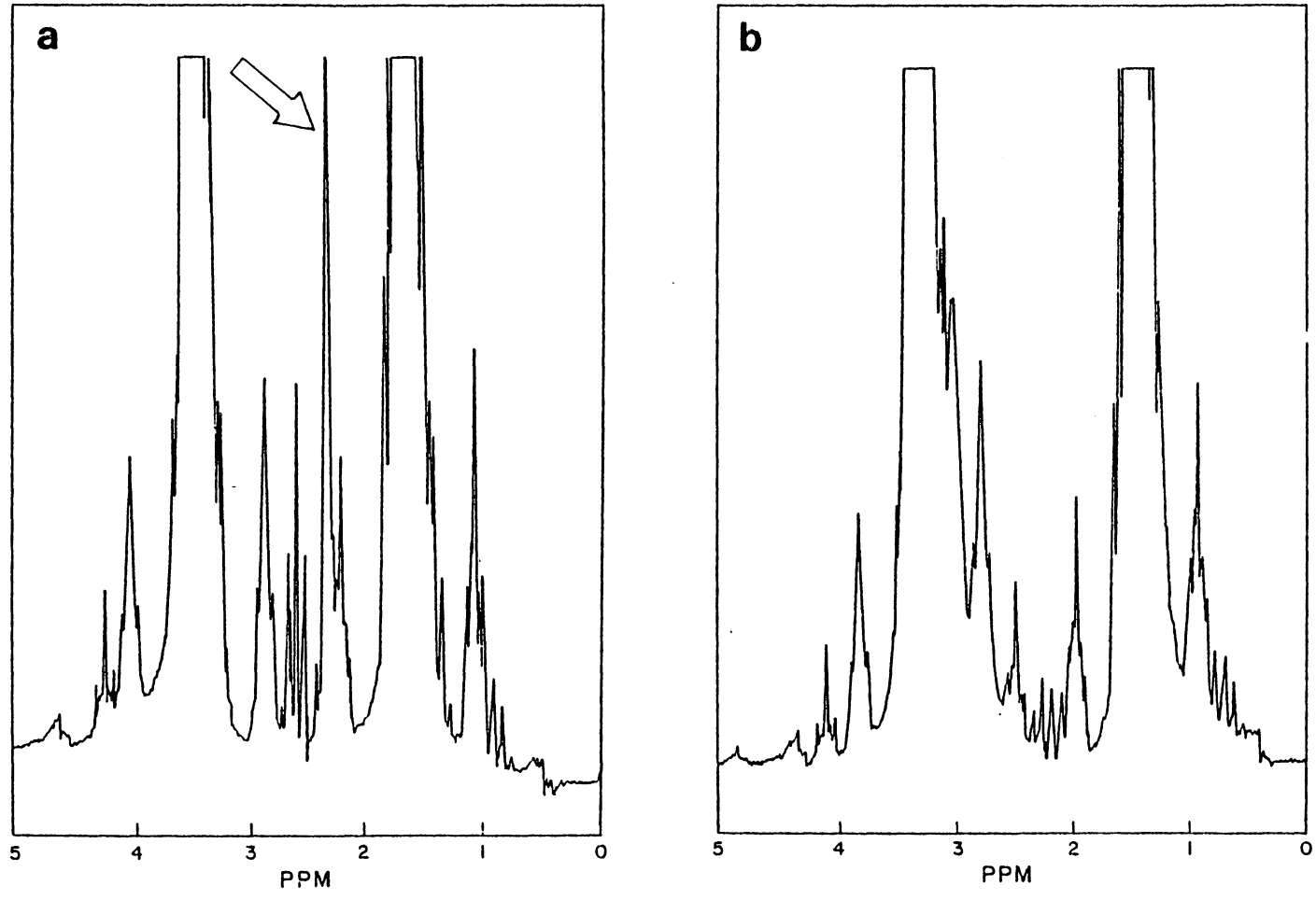


Figure 10. a) NMR Spectra of 3-Amino 1-Propanol in THF
b) Same After Addition of Para Nitro Phenyl Boronic Acid

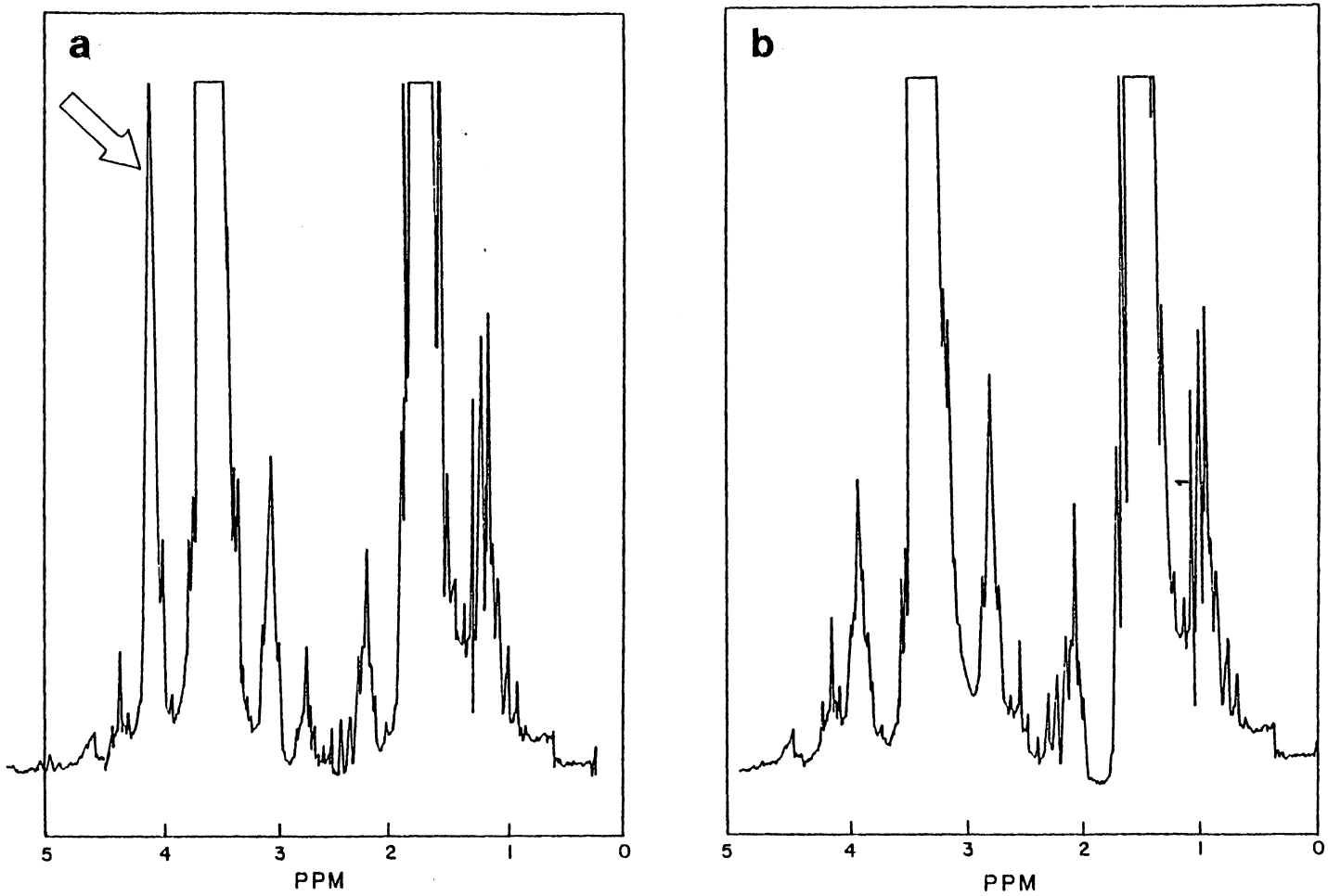


Figure 11. a) NMR Spectra of Lactic Acid in THF
b) Same After Addition of Para Nitro Phenyl Boronic Acid

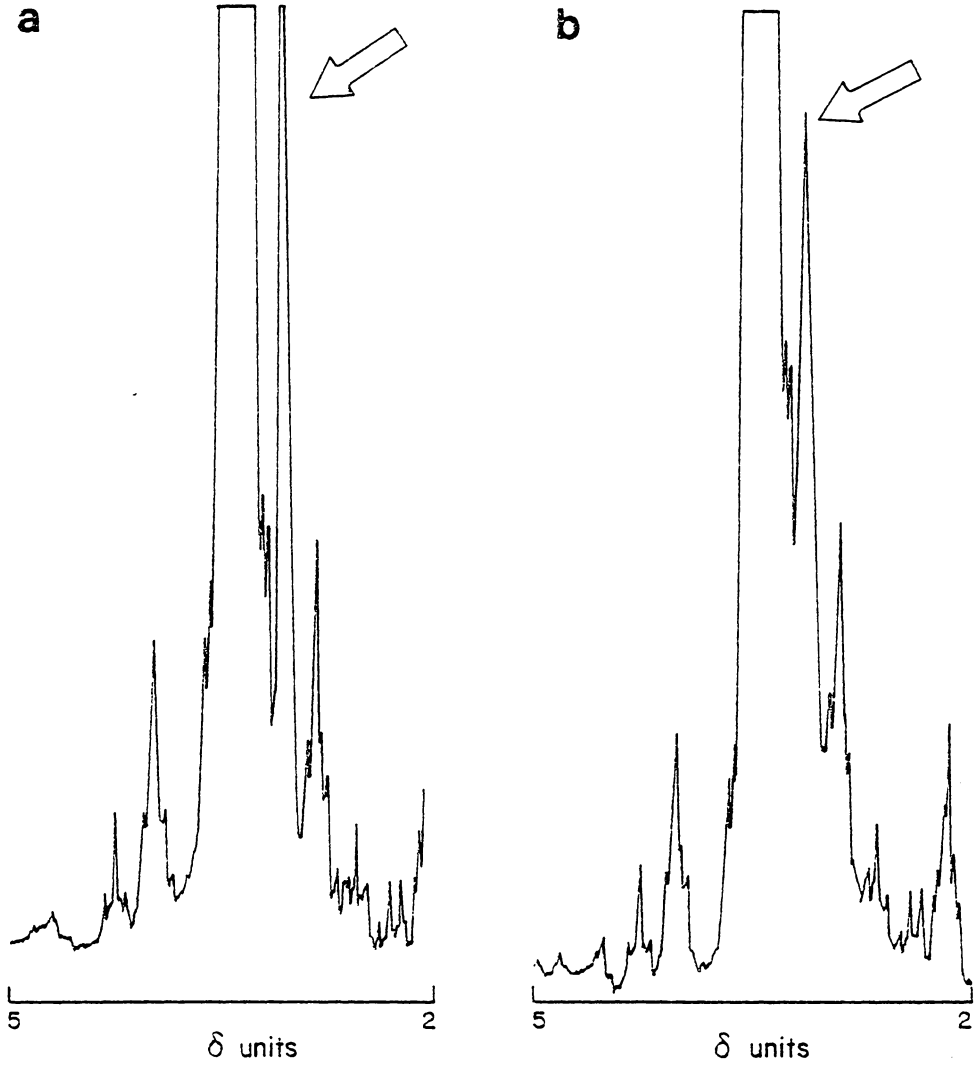


Figure 12. a) NMR Spectra of Ethylene Glycol in THF Before Reacting With Para Nitro Phenyl Boronic Acid

b) Same After Partial Reaction

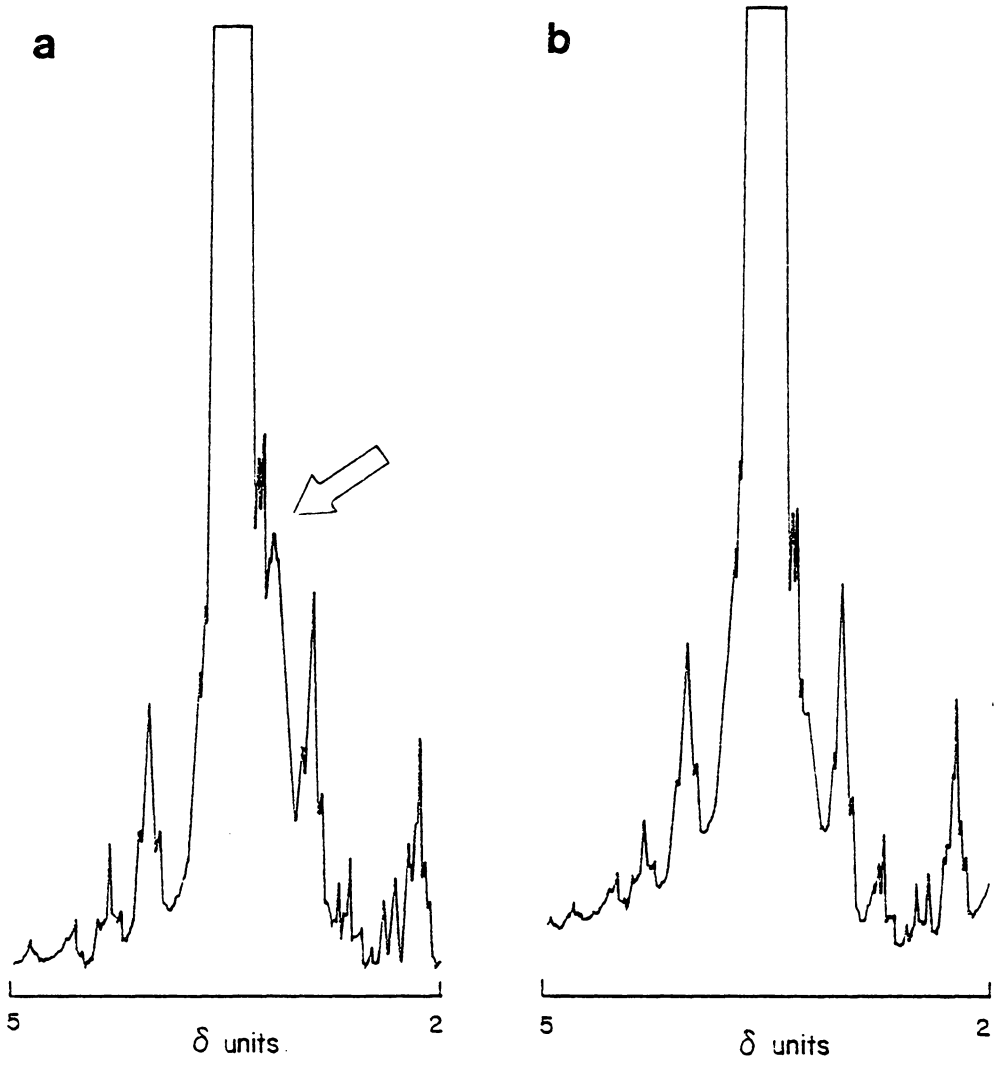


Figure 13. a) NMR Spectra of Ethylene Glycol Reacting With Para Nitro Phenyl Boronic Acid

b) Same When Reaction is Completed

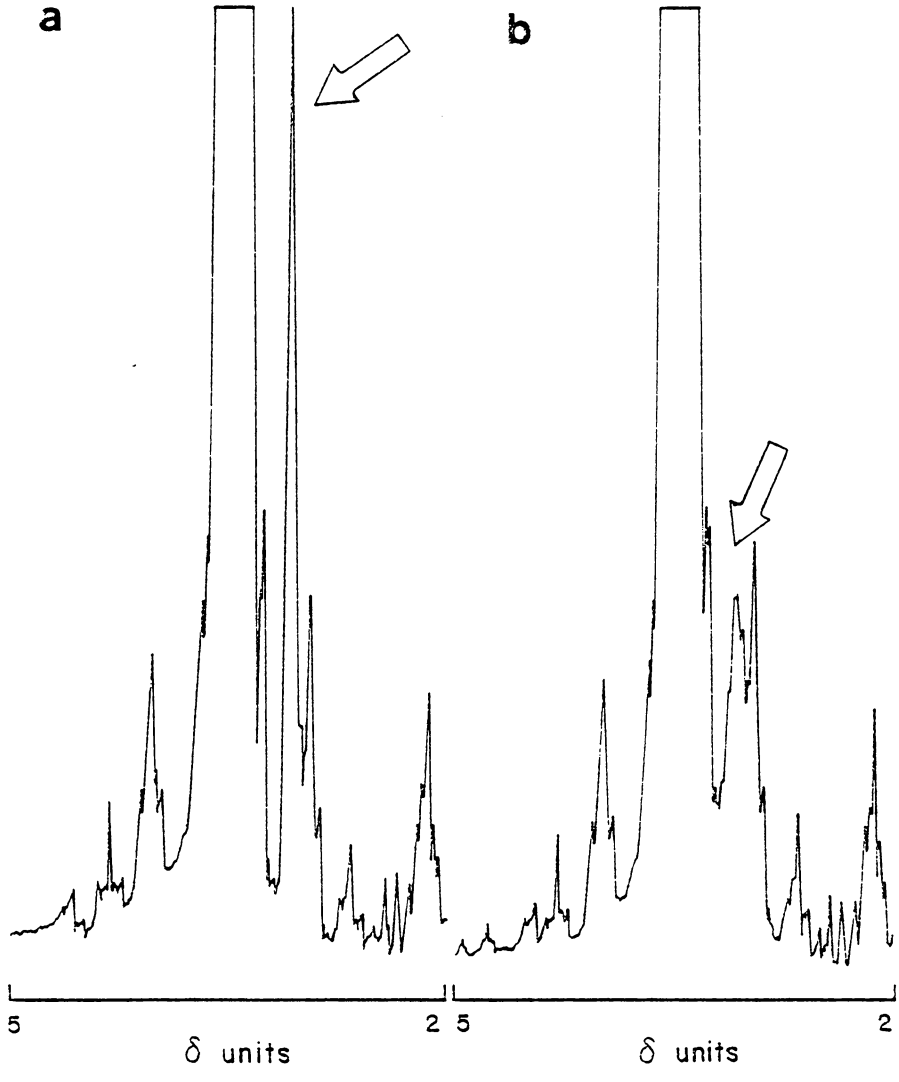


Figure 14. a) NMR Spectra of 1,4-Pentandiol in THF Before Reacting With Para Nitro Phenyl Boronic Acid

b) Same After Partial Reaction

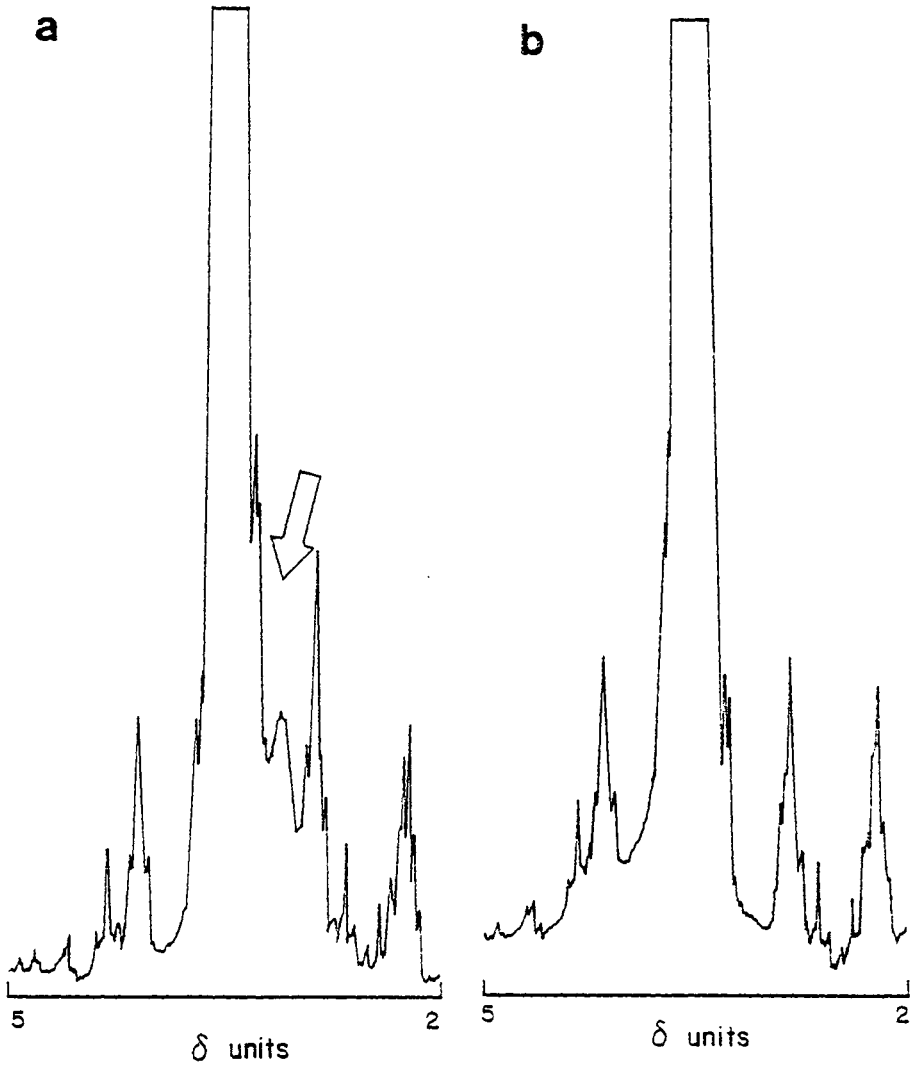


Figure 15. a) NMR Spectra of 1,4-Pentandiol Reacting With Para Nitro Phenyl Boronic Acid

b) Same When Reaction is Completed

reaction may appear to be at equilibrium, but this should be confirmed by some other means.

Looking at Figure 2 (page 27) we observe that one of the reaction products is water. One good way to displace the equilibrium is to remove this product formed or otherwise present in solution. This can be easily done by adding a water scavenger such as 2,2-dimethoxy-propane which will form methanol and acetone when removing traces of water.

An independent way to study the speed of reaction and the achievement of equilibrium, is to monitor the derivative's signal by GC. Employing an internal standard and measuring the peak height ratio of Boronate/I.S. at different times, it is possible to follow the reaction's progress. In this way it is possible, to determine that reactions with meta nitrophenyl boronic acid are essentially complete in 15 minutes, but the final equilibrium can take up to several hours.

Not all model compounds react at the same speed and although this method is too coarse to evaluate kinetic constants, it is possible to see some differences. Ethylene glycol for example, when reacting with meta nitro phenylboronic acid reaches 98% equilibrium after only 15 minutes, while 1,3-propandiol and 2,4-pentandiol reactions are only 88% complete. In the same time, 3-amino 1-propanol seems to react rather slowly, showing only 66% completion after the same period of time.

A different situation can be seen when 2,2-dimethoxy propane is present. All reactions are rapid achieving equilibrium in about 30 minutes. Some special cases are 2,4 pentandiol, 3-amino-1-propanol

and 1,3-propandiol which reach equilibrium only after 15 minutes. This fact is perhaps related to the stable structure of the boronates obtained, all of them are 6 membered cycles.

In view of the results obtained with the addition of 2,2-dimethoxy propane, it was considered convenient to routinely add a small amount of this additive to all derivatization reactions. The amount added was in proportion to the concentration of derivative formed, but it was in all cases at least three times the amount required to remove all the water formed. Although not completely studied, no abnormal results were observed when larger amounts were added.

Chromatographic Behavior - Nitro phenyl boronates have in most cases excellent peak shape. 3-amino 1-propanol boronates gave signals which are slightly tailed and unsymmetrical; this same observation is valid for 1,4-butandiol boronates.

Poor chromatographic characteristics were obtained only in the case of lactic acid. This factor and the absence of adequate boronate stability made it difficult to do any work with this sample and it was no longer considered.

All other boronates studied gave very symmetrical peaks with little distortion. A particularly good case was 2,4-pentandiol which seems to behave remarkably well.

Table X shows the relative retention values of boronates and the chromatographic conditions employed. Ethylene glycol was used as the reference compound to calculate the relative retention times. It is

TABLE X

RELATIVE RETENTION OF PHENYL BORONATES

	ORTHO NITRO	PARA NITRO	META NITRO
ETHYLENE GLYCOL	1.00	1.00	1.00
2,4-PENTANDIOL	1.36 1.51	1.66 1.71	1.57 1.70
1,3-PROPANDIOL	1.49	1.92	1.83
1,4-PENTANDIOL	2.05	2.80	2.73
3-AMINO-1-PROPANOL	2.09	3.18	2.71
1,4 BUTANDIOL	2.18	2.89	2.51
ETHYLENE GLYCOL	3.04 min.	3.84 min.	4.29 min.

Column 6' x 2 mm I.D. packed with 3% OV-17 onSupelcoport 80/100 mesh.
Temperature 180°C Flow 25 ml/min Nitrogen.
Injector and Detector Temperature 260°C H_2 30ml/min. Air 350 ml/min.

easy to see that as the molecular weight increases, it is more difficult to obtain good resolution. For example 1,4-butandiol and 1,4-pentandiol are not completely resolved, whereas to 1,3-propandiol and 1,4-pentandiol are.

Another conclusion from this table is the relative volatility exhibited by the 3 types of nitro phenyl boronates. The volatility increases in the order meta nitro < para nitro < ortho nitro. Considering volatility, the ortho nitro derivatives can be considered to be the most recommended ones for GC analysis. The benefit of increased volatility may extend the applicability of this reagent to higher molecular weights.

The derivatization of 2,4-pentandiol as it was mentioned before, gives two signals corresponding to isomers of this model compound. It was somewhat surprising that not all the derivatives gave the same separation for these isomers. The para nitro boronates are the least separated and the ortho nitro ones are the best separated. Perhaps, part of the explanation for this increment of efficiency, is the influence of the ortho nitro group which forces the boron group out of the plane of the aromatic ring, and this in turn may enhance the conformational differences between the isomers resulting in increased stationary phase selectivity.

It is commonly known in gas chromatography that adsorption and decomposition problems are due to active sites in the system, mostly in the column. A severe case of decomposition was found in the case of 1,4-butandiol boronates, when a new batch of column packing supposedly

identical to the other previously used, was tried. Figures 16, 17 illustrates the difference in performance between a good and a poorly coated packing material. The internal standard in this example was introduced for comparative purposes. Both batches of column packing were purchased from Supelco, Inc., Bellefonte, PA.

Seven member ring boronates are extremely sensitive to catalytic adsorption or decomposition. In particular 1,4-butandiol boronates turned out to be a specially difficult case. These boronates always gave slightly unsymmetrical peaks.

With these results in mind, it is recommended to test the suitability of GC columns for boronate analysis with a 1,4-butandiol sample. Specially deactivated columns may be necessary for trace level analysis since it is specially at low concentration levels when decomposition becomes more visible.

It is important to mention that 1,4-butandiol was the worst decomposition case studied in this project, other 7 membered ring boronates like the one formed with 1,4-pentandiol are much less sensitive to this problem. A fair statement is that although some decomposition problems are encountered in the GC analysis of boronates only a few cases are really difficult.

"On-Column" Derivatization - A technique of this kind has in general several distinctive advantages: simplicity, speed, lack of solvents, and ease of automation. In the case of boronic acid it has been reported before (88, 100, 101), but little has been said about its advantages or problems. The most important limitation of this

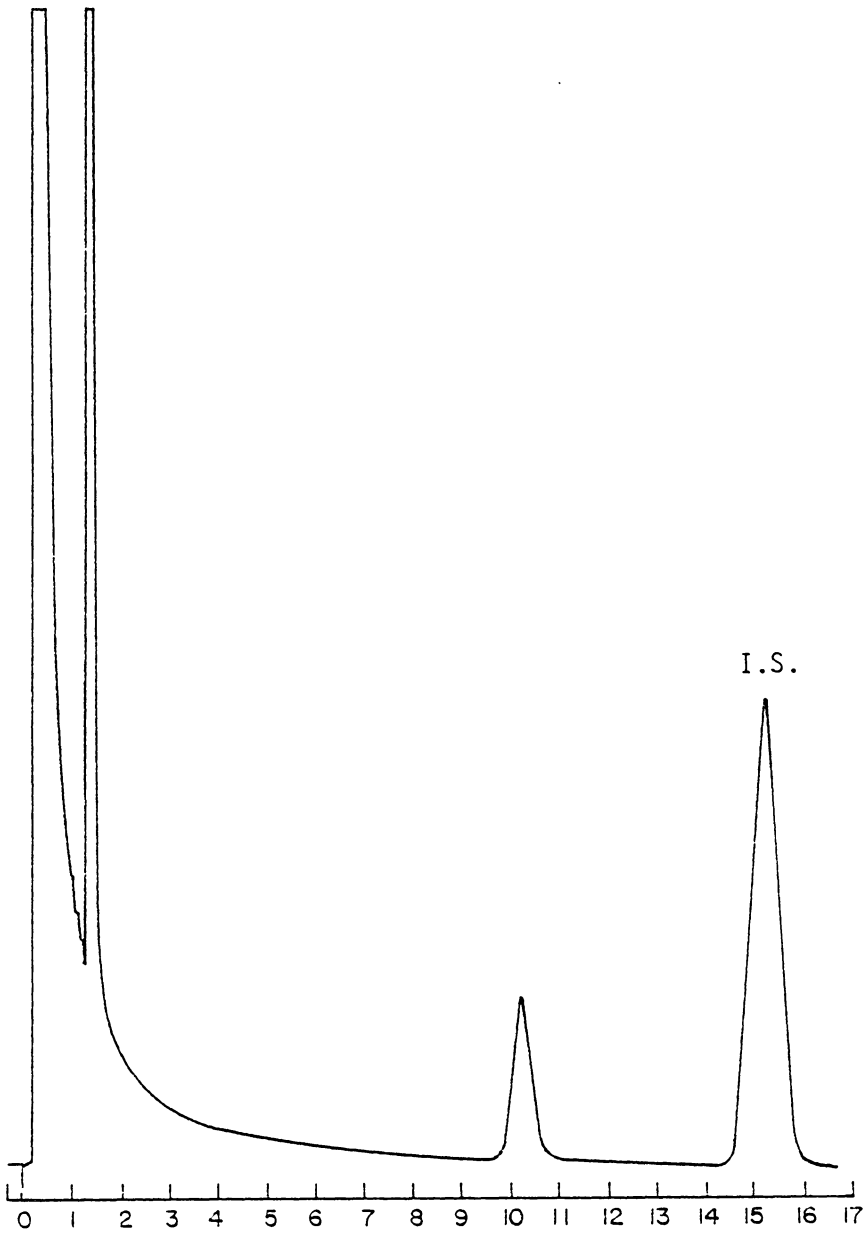


Figure 16. 1,4-Pentandiol Para Nitro Phenyl Boronate Chromatographed on a Good 3% OV-17 Packing

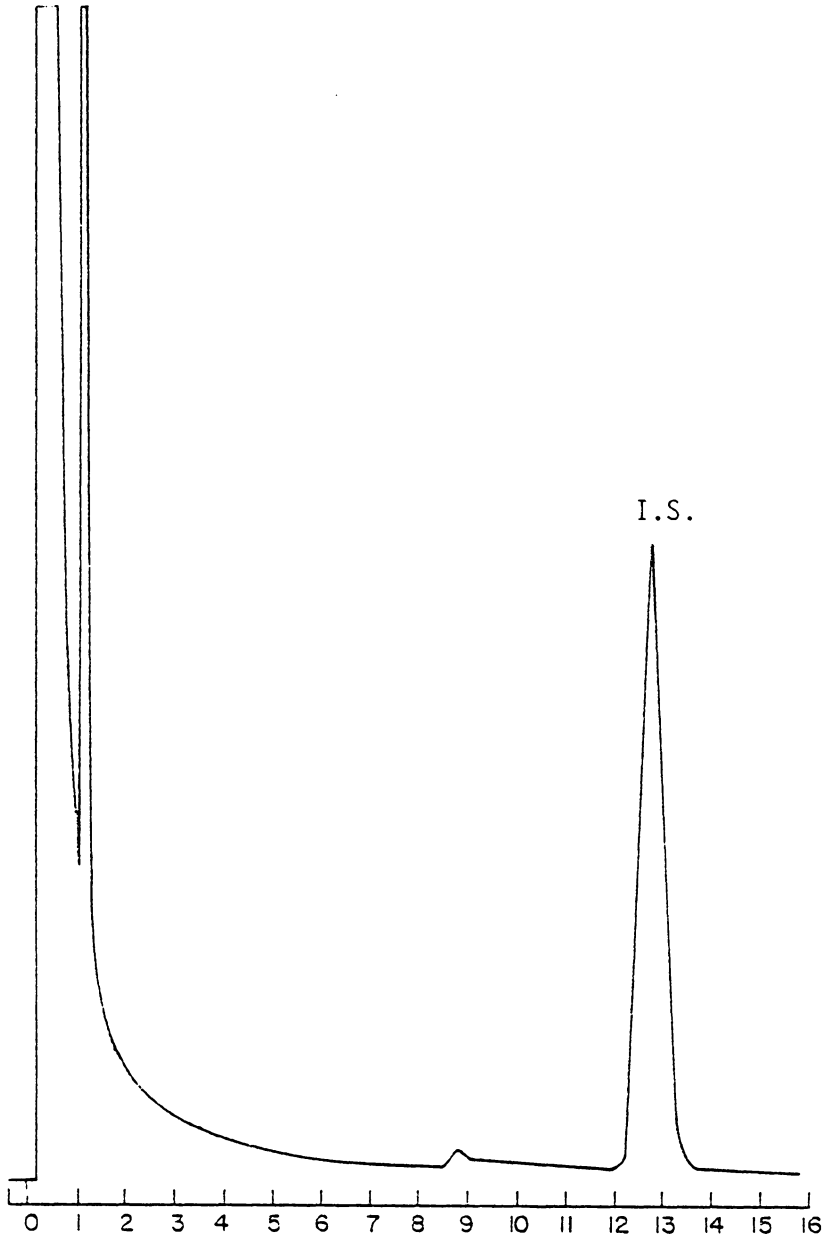


Figure 17. 1,4-Pentandiol Para Nitro Phenyl Boronate Chromatographed on a Poorly Prepared 3% OV-17 Packing

procedure is the response dependence on the amount of acid present in the column.

Employing para nitro phenyl boronic acid accumulated on top of the column and maintaining the injection zone at 230°C, it was found that injecting a constant amount of sample (10^{-7} moles), the response increases with the amount of acid present. When this amount of acid is about 200 times the required quantity for derivatization in solution, the response reaches a plateau. A main problem with this approach is the difficulty in maintaining a convenient amount of acid in the column as this is being constantly depleted by repeated sample injections.

The good reproducibility obtainable with this technique is surprising. The relative standard deviations usually are about 2-3%. This is however, dependent on the amount of acid. When the excess acid is relatively low (around 100 times excess) the reproducibility is poorer.

Although all the model compounds gave the same results, in the case of 1,4-pentandiol the reproducibilities are substantially lower, as much as 8% RSD. Surprisingly, a similar molecule, 1,4-butandiol gave much better results. A different approach to on-column derivatization is the packed reactor. This consist of a small length of column packed with a solid coated with the reagent. In this study the column packing (3% OV-17 Supelcoport 80/100) was coated with 12% by weight of p.-nitrophenyl boronic acid, and this material was packed into the first 8 cm of the column.

Maintaining the injection zone at 230°C and injecting one

microliter sample volumes (10^{-7} moles) of model compounds, good reproducibilities were obtained, in the order of 1-2% RSD.

Unfortunately there is an appreciable band broadening problem with the consequent loss of efficiency. Trying to increase the theoretical plates by using higher injection temperatures was tried but this in turn decreases the reproducibility.

The peak broadening was extremely bad in the case of 3-amino-1-propanol, where the peak shape was so poor that no reproducibility measurements were possible. Other model compounds such as lactic acid, do not react under on-column conditions.

The fact that 3-amino 1-propanol reacts relatively well with localized accumulated acid on the column suggests that this molecule requires a large amount of acid to react under on-column conditions.

In those cases where the on-column approach works acceptably well (ethylene glycol, 1,3-propandiol, 2,4-pentandiol), the retention times changed slightly as the reactor bed is depleted of reagent. This can be a serious disadvantage unless the compounds analyzed are well characterized or a constant replacement of the reactor bed is made.

There is one interesting point about on column reactions; considering the solvent front as a measure of the dead volume present in the system, the linear carrier gas velocity was calculated to be 10 cm/sec, with the reactor bed being 8 cm long, we see that all reactions essentially take place in 0.8 sec or less. This is a good appreciation of how fast the reaction can be at high temperatures.

The reactor bed approach gave increased responses compared to the

"point accumulation" previously described, but even in this case the overall performance is inferior to the case of derivatization reactions in solution.

On column derivatization was evaluated only with the p.-nitro phenyl boronic acid since this is the highest melting isomer (mp > 300°C). Other boronic acids do react in the same way but it is possible that some decomposition occurs.

In summary, these techniques can be reproducible if there is a careful control of the amount of reagent available for the reaction. Some loss of efficiency can be expected, and this can be severe depending on the sample. It is recommended that individual evaluations be carried out if the use of these techniques are contemplated.

Stability Studies -One important aspect related to the use of derivatives in chromatography is their stability. Ideally a perfectly inert and stable compound is desirable, but in practice only a few cases can be considered to meet these requirements. The best example of this is perhaps the fatty acids methyl esters which have relatively good long term stability.

From the analytical point of view a derivative should be stable at least for a few hours, giving enough time for the chromatographic analysis to be completed. Specialized situations may require more stability depending on particular analytical needs.

Boronates in general are known to be sensitive to hydrolysis and care should be taken to remove as much water as possible from the reaction. As was previously discussed, this also helps displace the

equilibrium to obtain a more favorable reaction for quantitative analysis.

Introducing an internal standard and measuring the peak height ratio Boronate/I.S. at different times, it was possible to study the stability of nitro phenyl boronates. All these determinations were made at the same conditions as described on Table X. Peak heights were measured manually with a stainless steel ruler calibrated in millimeters.

In order to plot the stability results, the first peak height ratio determined when the reaction had achieved equilibrium, was taken as 100%. All other ratios were calculated in reference to this first value.

Three internal standards were needed to study the stability of boronates. Meta and para nitro phenyl boronates are retained relatively long and the peaks are closely eluted, leaving no room for an internal standard of intermediate retention time. Triphenylmethane was chosen for this case since in the conditions described on Table X is eluted just after the last boronate peak.

Ortho nitro phenyl boronates are much less retained and although the same internal standard could be used, this would mean a waste of time. Because of its much shorter retention time, di-n butylphthalate was selected in this case.

Phenyl boronates required lower column temperatures than nitro phenyl boronates, the column was at 140°C and the injector and detector at 190°C. For this case BHT (t-butylhydroxytoluene) present in as

as preservative THF was the internal standard employed. Fortunately the BHT concentration normally present in THF was adequate to give a signal similar in intensity to the peaks of interest.

In all the stability studies a concentration level of $20x10^{-10}$ moles/microliter of each individual boronate was employed. The same concentration of internal standard was present along with a small amount of 2,2-dimethoxy propane (50 ul/10 ml of solution).

Figure 18 shows the relative stability of some ortho nitro phenyl boronates over a period of 5 days. This length of time was chosen for practical reasons assuming that after 120 hours no unusual behavior will be expected. In this and all the plots of this kind every point represents an average of 5 determinations. The reproducibility of these determinations was normally in the range of 2 to 4% RSD. During the first 24 hours of each experiment 5 points were determined at 6 hour intervals.

From Figure 18 we can see the stability of different boronate ring sizes: 1,3-propandiol which forms a 6 membered ring boronate is the most stable, followed by ethylene glycol and 1,4-butandiol which form 5 and 7 membered rings respectively. These results are not unexpected; this is the normal behavior of cyclic molecules. Perhaps what is surprising is the magnitude of the difference, while the 6-membered boronates has decayed only 6% after 5 days, the 7-membered boronate has decayed as much as 55%, being roughly ten times less stable.

Alkyl substituents increase the boronate's stability as we can see in Figure 19. In both cases shown, 1,4-butandiol and 1,4-pentandiol

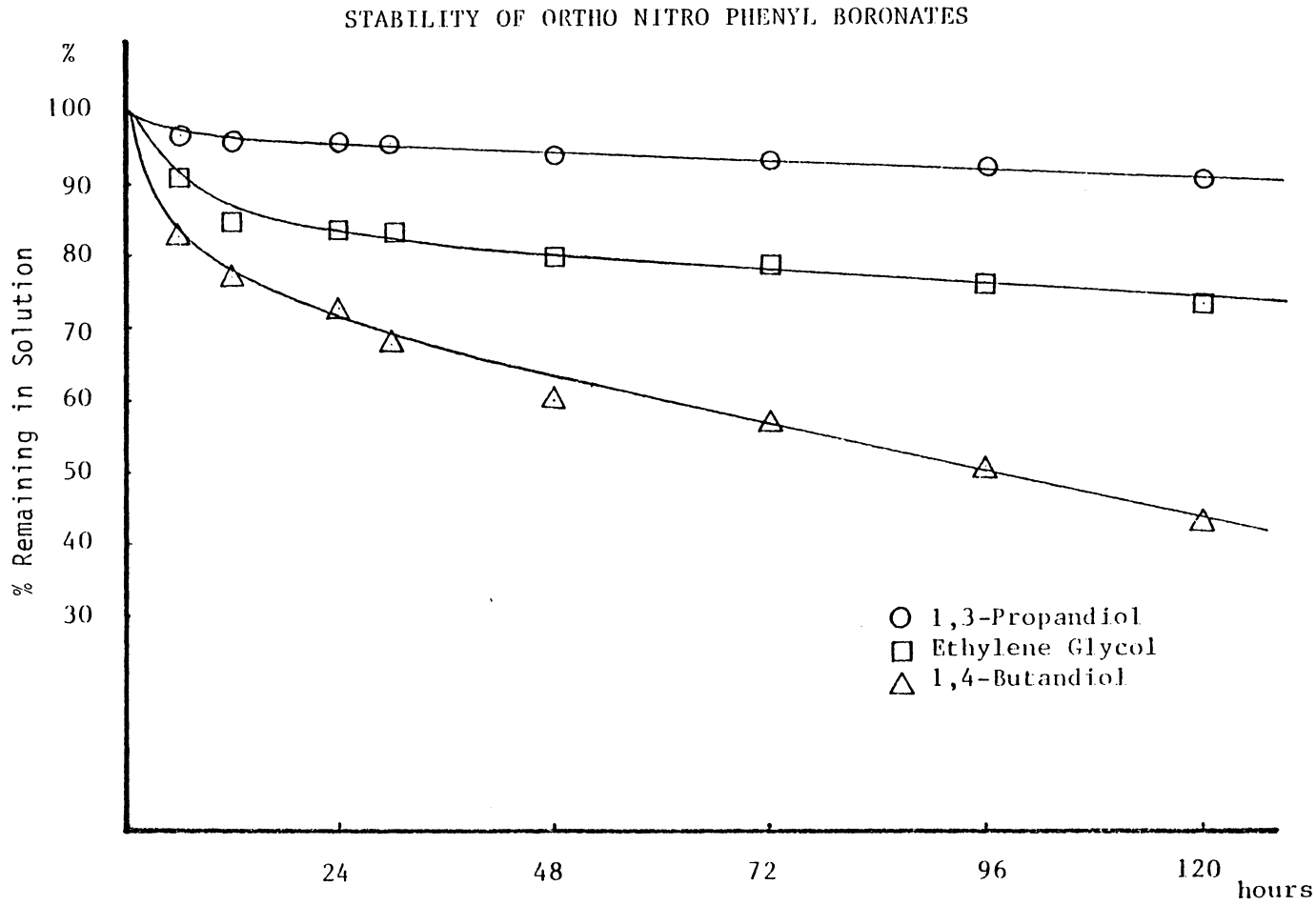


Figure 18. Comparative Stability of Ortho Nitro Phenyl Boronates

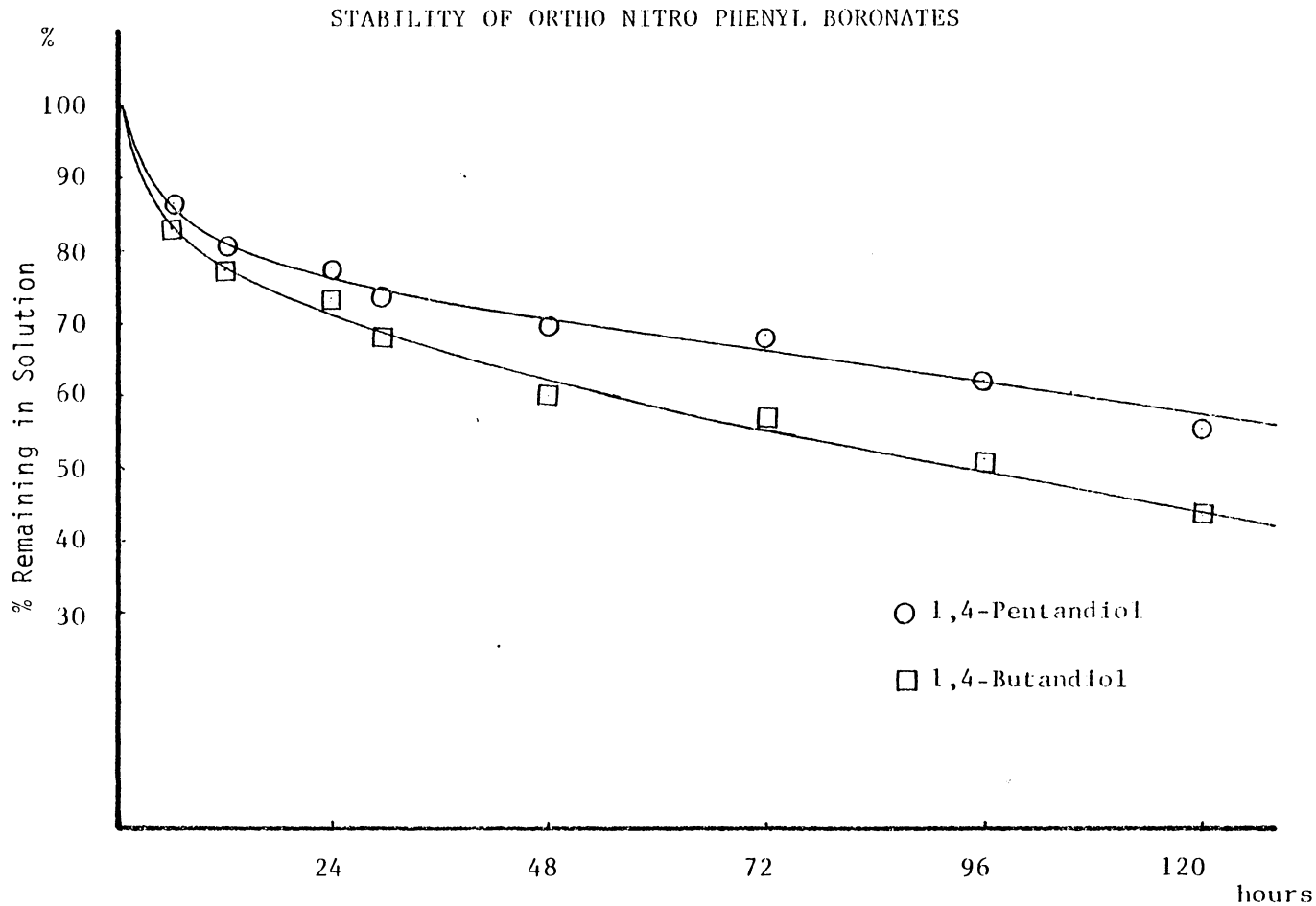


Figure 19. Stability of 7 Member Ring Ortho Nitro Phenyl Boronates

form 7-membered boronates and are unstable. However, the presence of a methyl group seems to enhance the stability. In any case, this type of boronate should be analyzed in no more than 2 hours after the reaction has achieved equilibrium in order to obtain good results.

A clearer effect of alkyl substitution can be seen in Figure 20 where the cases of 1,3-propandiol and 2,4-pentandiol are shown. The plot drawn for 2,4-pentandiol belongs to the longest retained isomer, but the behavior of the other isomer is identical to the one shown.

The difference between a 6 membered ring boronate with and without methyl substituents is not a drastic one; in order to see more clearly the difference in stability the % scale has been expanded. 2,4-pentandiol gives extremely stable boronates showing essentially no decay over the length of time studied, undoubtedly the methyl groups exert a stabilizing effect.

The nature of this effect was not studied, but it can be considered to be both an electronic and a steric effect. In this case the methyl substituent are located close to the boron atom and due to their relatively large volume is possible to think of a steric hindrance effect. The electron donating character of methyl groups is well known, in this case since the effect would have to be transmitted through a highly electronegative oxygen atom possibly this effect is not as important.

Another piece of information we can observe from Figure 20 is that 3-amino-1-propanol forms a boronate which decays essentially in same way as 1,3-propandiol. Both boronates are 6 membered ones without

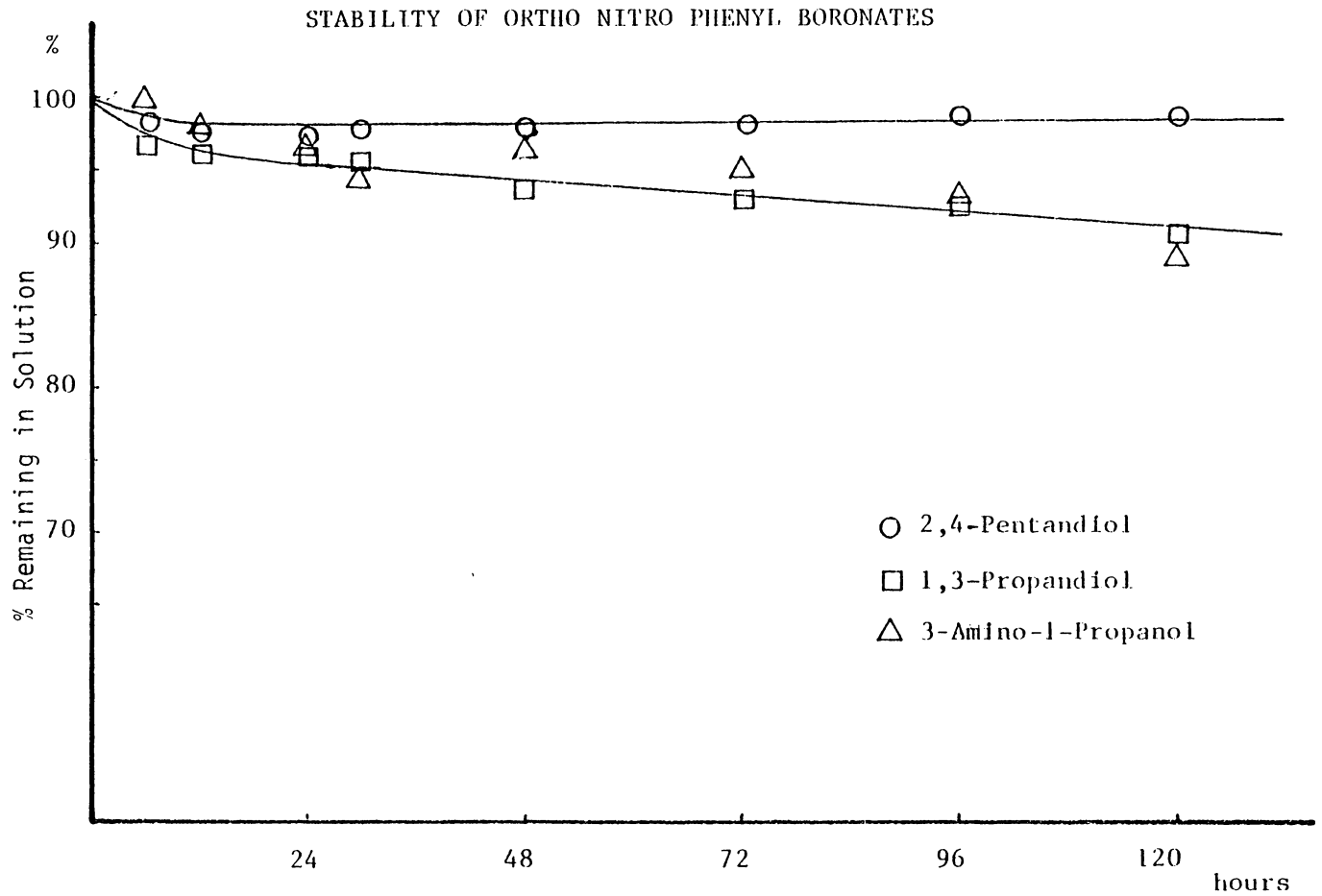


Figure 20. Stability of 6 Member Ring Ortho Nitro Phenyl Boronates

alkyl substitution.

The chromatographic behavior of 3-amino-1-propanol boronates is complicated by the residual basic character present in the amine group after derivatization. The peak shape is slightly asymmetrical and this may complicate its quantitative determination.

In the case of relatively unstable 7-membered boronates, the influence of the nitro group is clearly visible as is shown in Figure 21. Meta nitro phenyl boronates are relatively more stable than the ortho and para nitro ones.

A possible explanation for this observation are the contributing resonance forms possible in both types of substitution on the aromatic ring. Figure 22 shows these forms and it is easily observed that ortho and para nitro substituted aromatic rings contribute to the formation of a electron deficient carbon directly bonded to the boron atom. This in turn increases the electron deficient character of boron and propitiates the attack on that center. The meta nitro aromatic ring does not develop this character and although the electron deficiency continues, this is not as important as in the other cases.

Stable boronates, such as the ones formed with 1,3-propandiol, do not show drastic differences according to the nitro group position on the phenyl ring. In Figure 23 which has been greatly expanded in the % boronate axis in order to see the differences, it is obvious that meta nitro boronates are still the most stable. The differences however, with the other isomers is not as greatly marked, all the boronates shown in Figure 23 decay no more than 8% over a 5 day

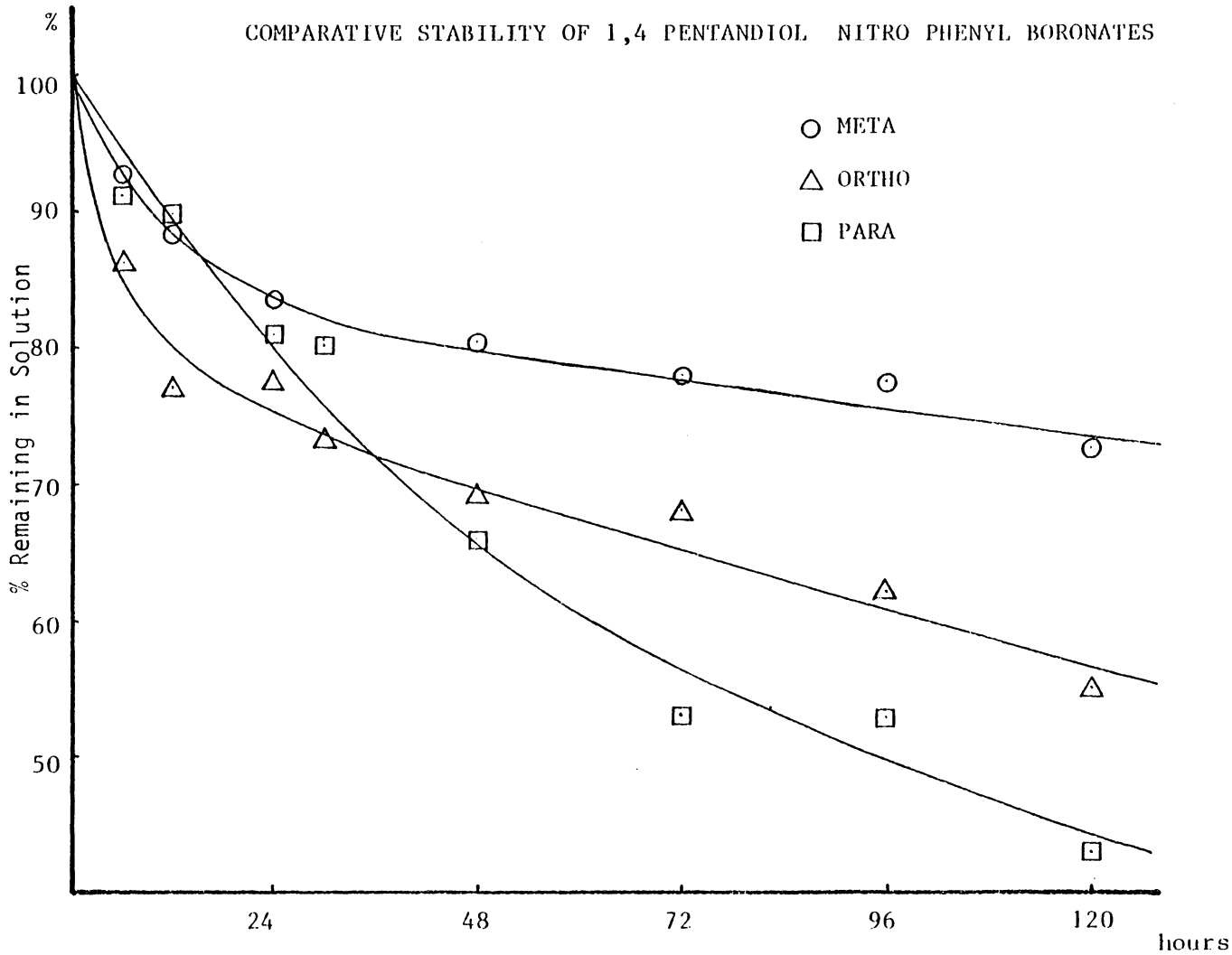


Figure 21. Comparative Stability of 7 Member Ring Nitro Phenyl Boronates

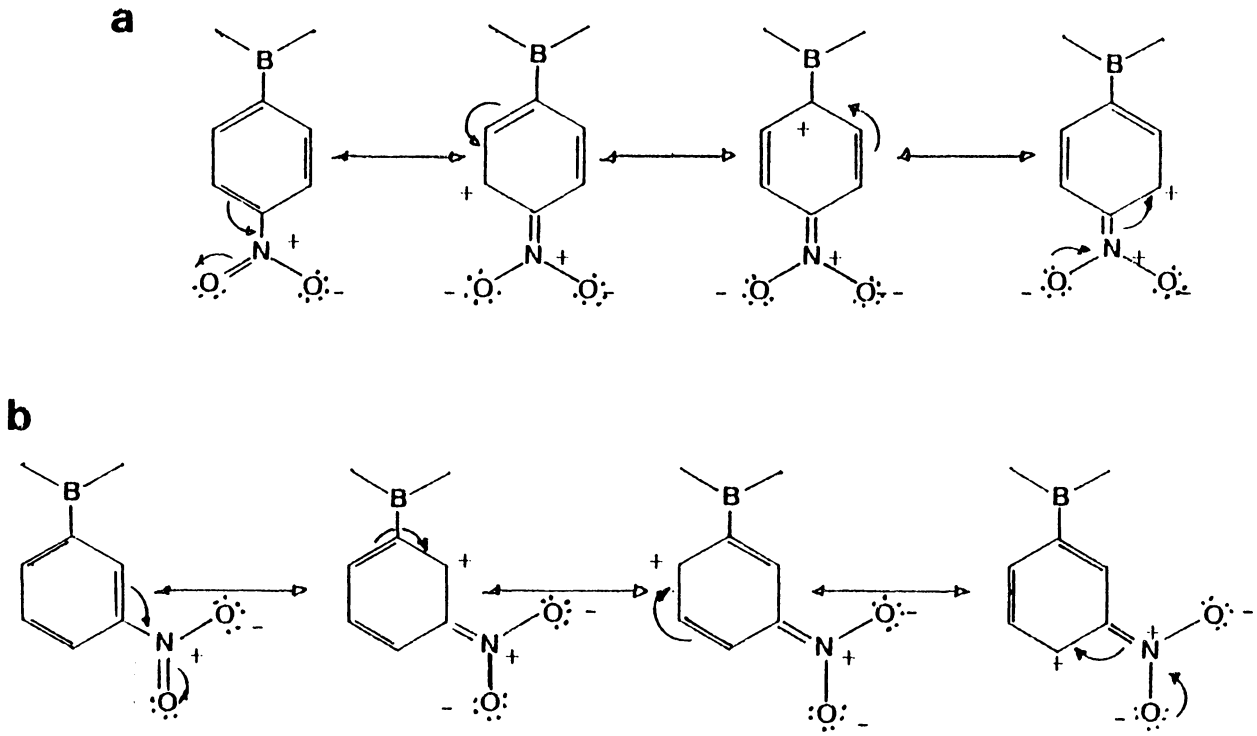


Figure 22. a) Resonance Structures of Ortho and Para Nitro Phenyl Boronates
b) Resonance Structure of Meta Nitro Phenyl Boronates

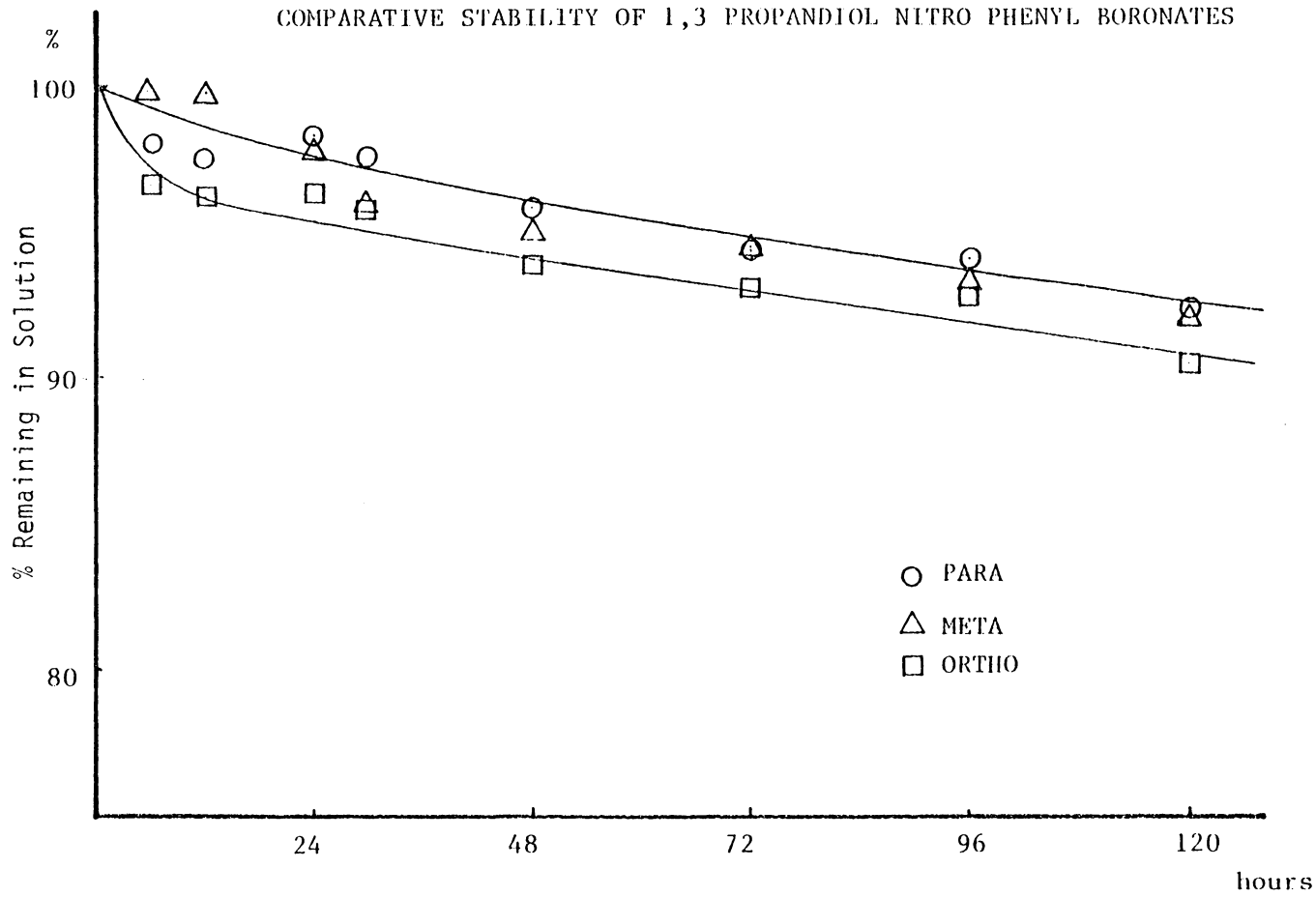


Figure 23. Stability of 1-3 Propandiol Nitro Phenyl Boronates

period. The method used in this project is not precise enough to see more clearly the small differences in stability among these derivatives.

Boronates obtained from 2,4-pentandiol are all equally stable regardless of the nitro group. They in general show little or no decay over the time period studied.

In summary, we see that the main structural factors determining the boronates stability are the ring size and the alkyl substitution present; the nitro group position has only minor influence.

Another stability aspect studied in this work was the overall influence of the nitro group on the stability of the boronates formed. Due to the strong electron withdrawing character of the nitro group, it is reasonable to assume the electron density in the aromatic ring is decreased. This in turn will increase the electron deficient character of the boron atom making it more liable to attack.

In order to determine the nitro group effect, the stability of phenyl boronates was studied. One of the unexpected findings in this study was the sensitivity of some phenyl boronates to thermal decomposition. Figure 24 shows the peak shape obtained for 1,4-butandiol when the injection temperature was 220°C and 190°C. A 30°C change was enough to appreciably decompose 7 member ring boronates of this type. 1,4-pentandiol phenyl boronate is much less sensitive to this decomposition.

All other phenyl boronates are temperature stable. No evidence of

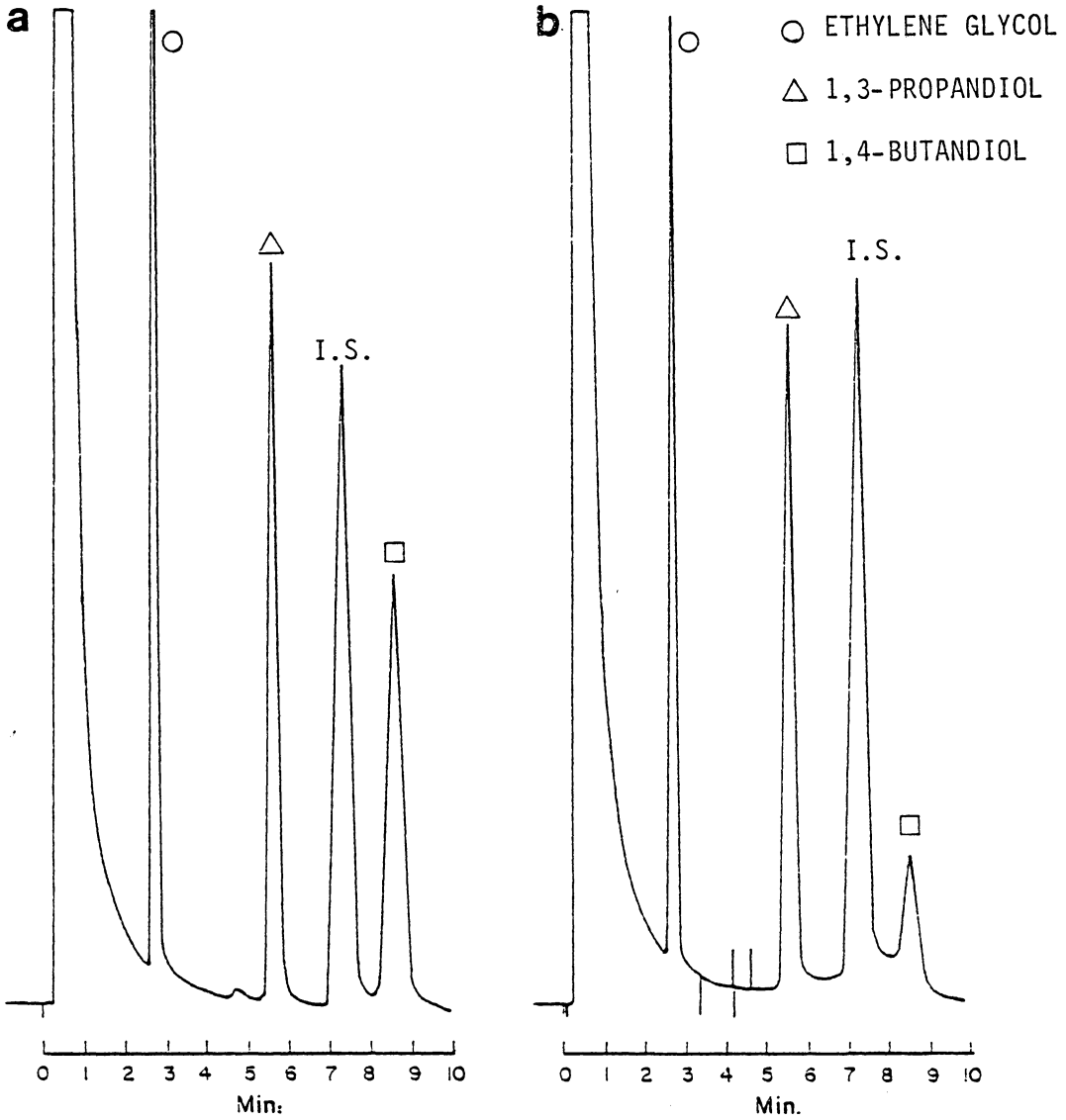


Figure 24. a) Chromatogram of Phenyl Boronates on a 3% OV-17 Supelcoport 80/100 Mesh Column Packing at 140°C. Injector and Detector Temperature 190°C.

b) Same, Except Injector and Detector Temperature 220°C

this kind of problem was detected in the case of nitro phenyl boronates in the range 230°-320°C. The conditions described on Figure 24 were taken as the standard ones for phenyl boronate analysis, all other conditions were as on Table X.

Phenyl boronates follow the same pattern of stability previously described. Their relative stability according to ring size and alkyl substitution is the same as with the nitro substituted ones.

Figure 25 displays stability curves for 7-membered ring boronates with and without nitro substitution. The meta nitro and the phenyl boronates are of comparable stability, a fact which once again points out the boronate ring size as the most important structural factor determining stability. For comparison the para nitro boronate is also shown, this being the least stable of all.

Figure 26 compares the stability of 6-membered ring boronates, here an obvious difference is seen. Although the stability is not very different, it is obvious the phenyl boronate is more stable. In conclusion, the nitro group has a destabilizing effect on the molecule. This effect however seems to be less important when the boronate is strained and intrinsically unstable.

Following the same general conclusions previously mentioned, 2,4-pentandiol phenyl boronates are extremely stable as in the case of nitro substitued ones.

Although the nitro group has this negative influence on the boronates stability, this effect is certainly counterbalanced by the chromophoric and electrophoric characters given to the molecule.

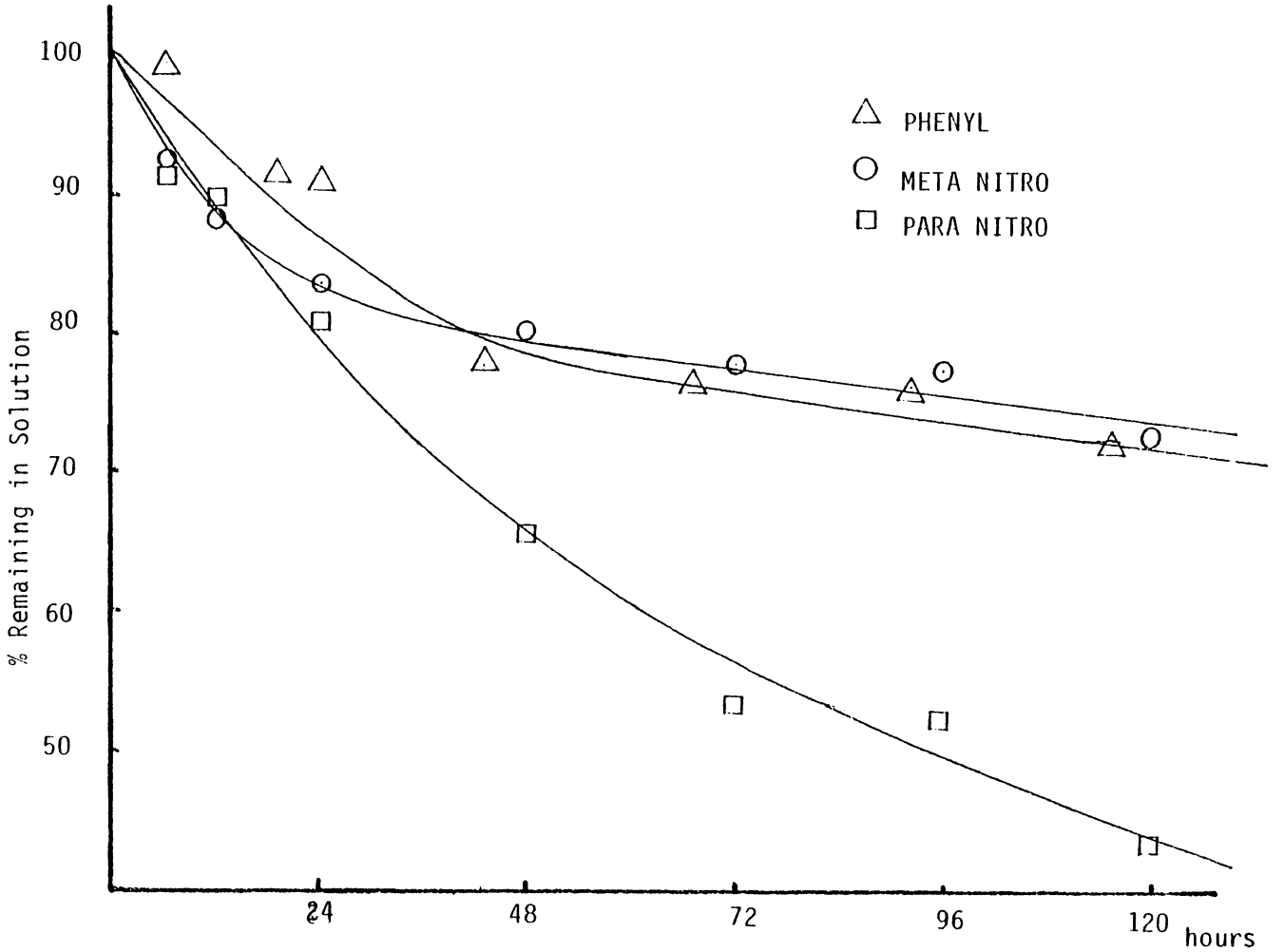


Figure 25. Stability of 1,4-Pentandiol Phenyl Boronates

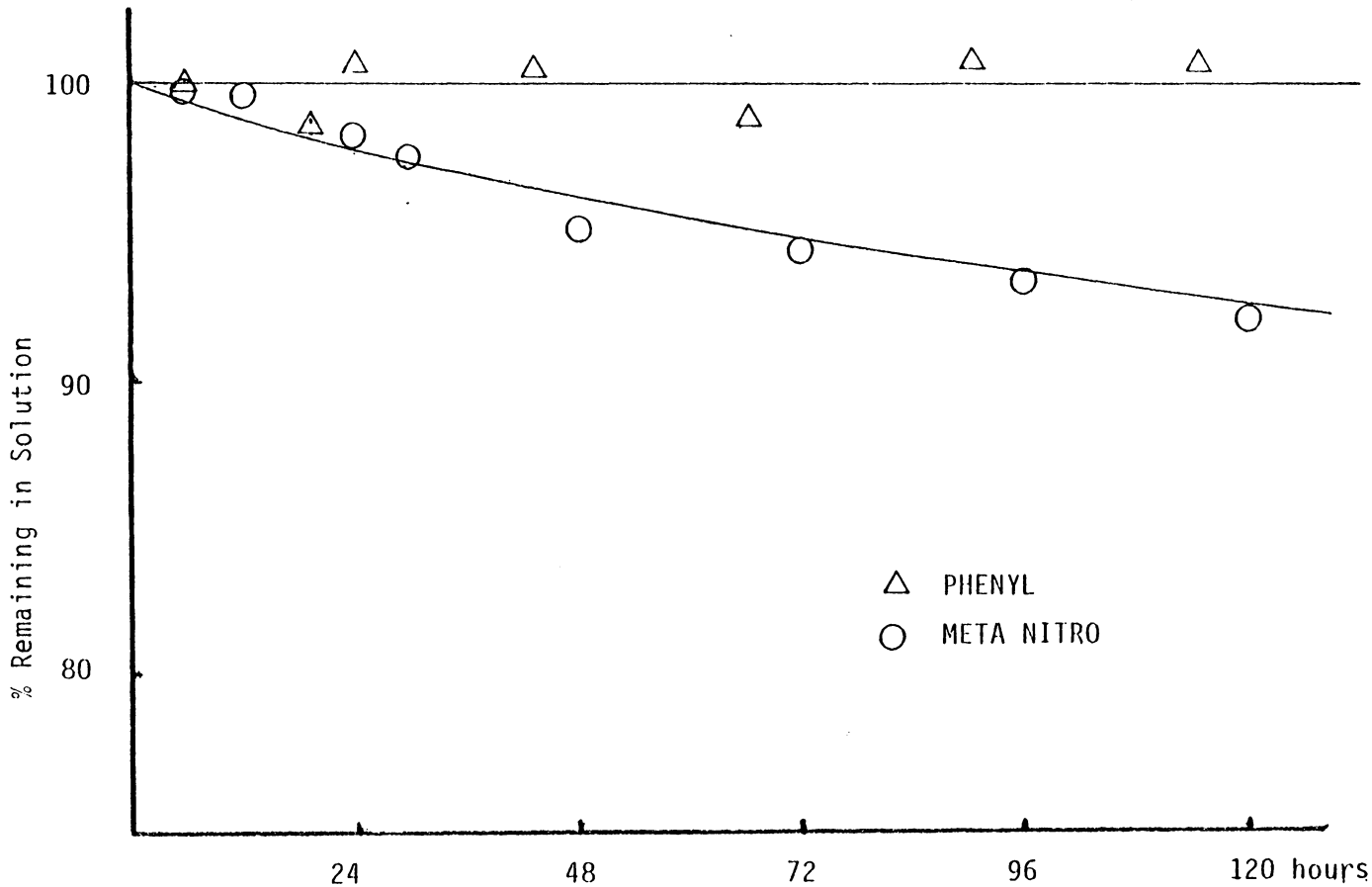


Figure 26. Stability of 1,3-Propandiol Phenyl Boronates

If high sensitivity is not needed, phenyl boronates can be adequate derivatives, since their electron capture and UV absorption properties are relatively good.

Decomposition Products - After studying the stability of nitro phenyl boronates and discussing the structural factors involved, the next logical question is what are the decomposition products?

One of the possible decomposition paths is the hydrolysis of the boronates formed in solution, however, dry solvents were employed and care was taken to remove the small amounts of water formed in the reactions by the addition of a water scavenger.

In addition to the above mentioned precautions, it is also necessary to remember that in all the reactions employed a one molar excess of boronic acid was always present. Another point is the on column reactivity of the boronic acids and the accumulation of these reagents in the column after repeated injections.

With this information it is difficult to think that hydrolysis would be an important pathway for the decay of the nitro phenyl boronates, at least in the experiments described in this study.

Another possible degradation path is the methanolysis reaction with the methanol produced by the 2,2-dimethoxypropane water scavenger. Acetone, the other product formed by DMP, can be considered to be inert.

The methanolysis reaction would produce dimethoxy nitro phenyl boronates, products which at least in theory should be detected by gas chromatography under the conditions employed in this work. Because of

molecular weight similarities, dimethoxy boronates would be expected to elute at similar retention times as ethylene glycol boronates.

However, it has been reported that derivatives of this kind are not stable enough to be analyzed by GC. This information combined with the fact that no additional signals were detected during the stability studies, indicate that if the product is formed, it is not stable enough to be analyzed by GC.

In order to evaluate the possibility of methanolysis being a decomposition path for nitro phenyl boronates, the effect of small amounts of methanol added to the boronates was studied.

When 200 microliters of methanol were added to a 10 ml solution containing 20×10^{-10} moles of meta nitro boronates, no appreciable decomposition was detected measuring the peak areas with respect to an internal standard. This amount of methanol is equivalent to roughly 400 times the quantity obtained by the removal of the water produced when obtaining that concentration of boronates. This is assuming 100% efficiency of removal by reaction with DMP.

The fact that no effect was visible with this relatively large amount of methanol even after 4 hours, shows the good stability of meta nitro phenyl boronates and also that methanolysis is perhaps a relatively slow decomposition reaction. A very dramatic effect however was found when the 1 ml of methanol was added; this is approximately 2000 times the amount formed under normal conditions. Figures 27, and 28 show the chromatograms before and after the addition of methanol. It is evident that methanolysis is a likely decomposition path although

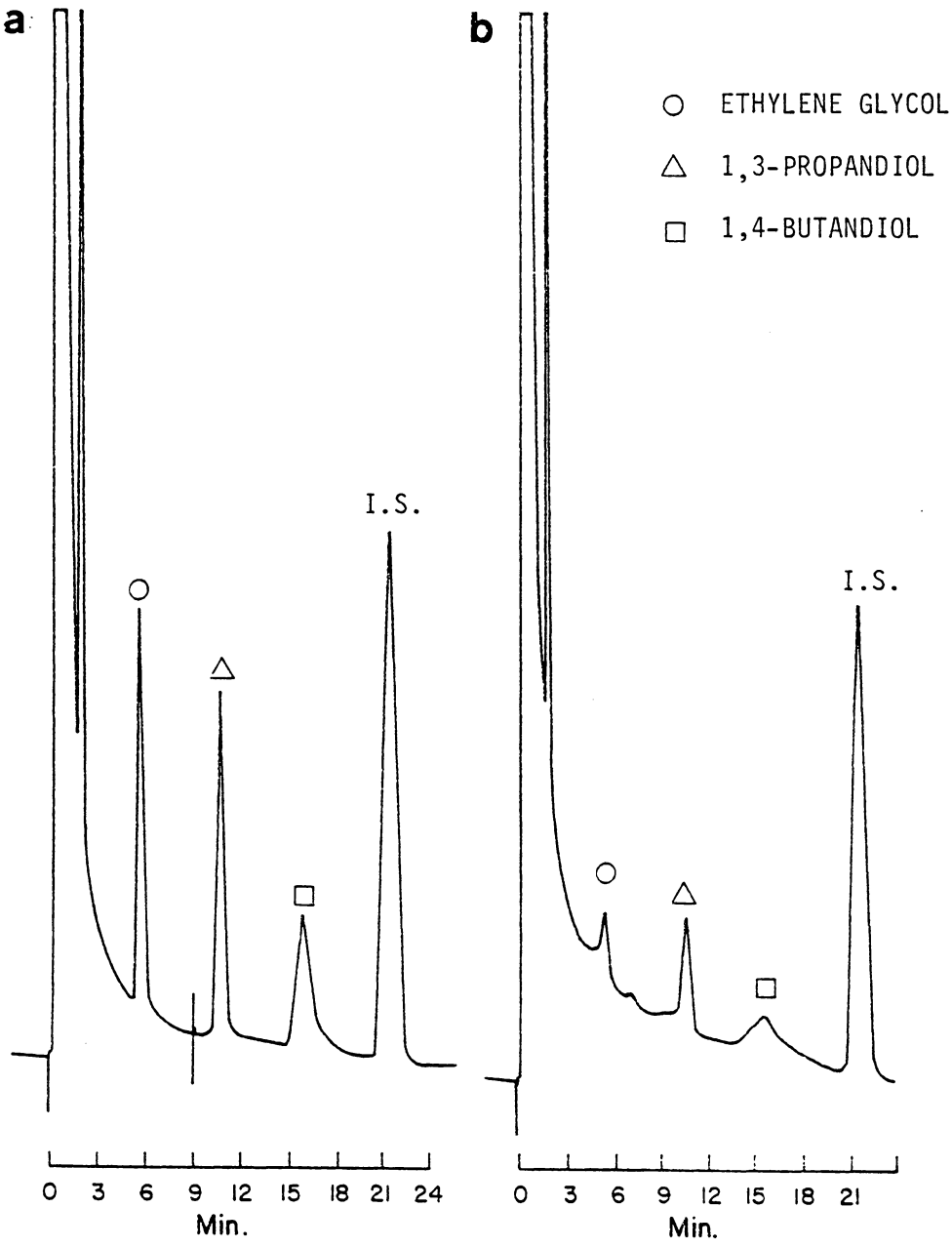


Figure 27. a) Meta Nitro Phenyl Boronates
b) Same After MeOH Addition

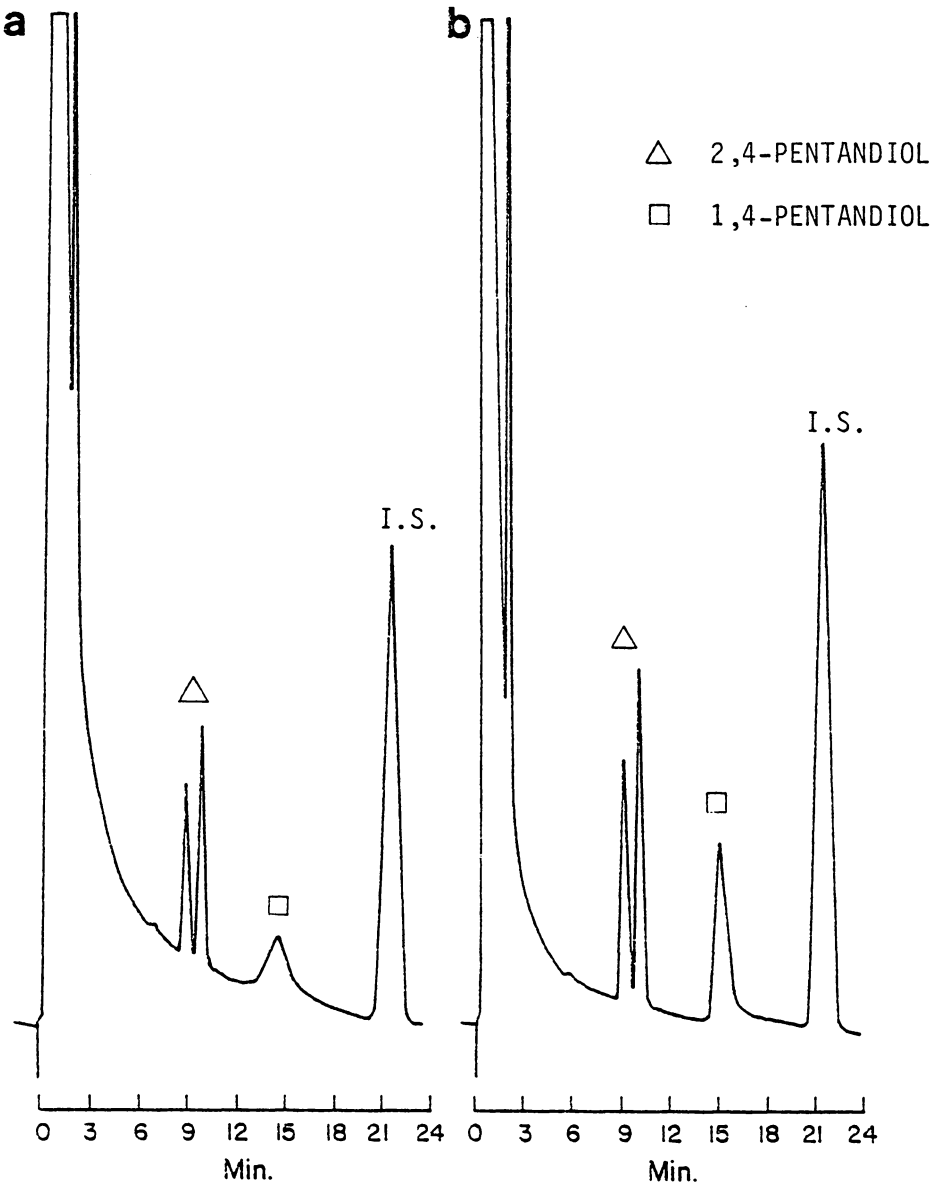


Figure 28. a) Meta Nitro Phenyl Boronates After MeOH Addition
b) Same Before Addition

a slow one.

With the methanolysis experiment additional information about boronates was found. As expected, the 7-membered ring boronates were the ones which show more extensive decomposition, 6-membered rings also show some decay, but it was relatively little in the case of 2-4 pentandiol.

In conclusion, methanolysis is a likely way for nitro phenyl boronates to decay, however, the decomposition seems to be slow. Little hydrolysis was likely to occur in these studies due to the precautions taken to displace the reactions equilibrium by water removal with DMP.

Quantitative Analysis - The extra sample manipulation involved in the formation of derivatives is always a concern for the analytical chemist. This is particularly true in the case of derivatives to be used for quantitative analysis, where control of reaction conditions and careful instrument calibration are special requirements.

Derivatives for selective detection systems are very frequently used for quantitative analysis, especially at trace levels.

As expressed in the introductory part of this dissertation, one of the objectives of this work, was to evaluate the quantitative analysis suitability of nitro phenyl boronates. Two GC detectors were employed for this purpose, flame ionization and electron capture.

By preparing reactions at different concentration levels and

injecting known volumes of them, calibration plots were obtained for each type of nitro boronate and all model molecules. These calibrating solutions were 10 ml containing a one molar excess of the boronic acid and 50 microliters of 2,2-dimethoxypropane (DMP). Other practical details of these procedures have been presented in the experimental section.

Figures 29 and 30 show the calibration plots obtained with flame ionization detector for para-nitrophenyl boronates. These plots are representative of the results obtained with other nitro boronates.

The plots range in concentration from 2000 to 50 picomoles and every point represents the average of 5 determinations. The reproducibility of those points was slightly different in every case but it was usually around 2 to 3% RSD. This was a function of the sample size; lower concentrations commonly gave lower reproducibilities. Another observation was that 3-amino 1-propanol and 7-membered ring boronates all tend to give higher % RSD values compared to the other cases.

The two signals obtained for 2,4-pentandiol, which corresponds to a meso isomer and a d-l pair, were treated as distinctive components and the values shown in the concentration axis are not really correct in this particular case. This was done in order to detect any difference in behavior between the isomers.

The straight lines on Figures 29 and 30 represent the best fit calculated for the experimental points, and the correlation coefficients were calculated with the linear experimental values, not

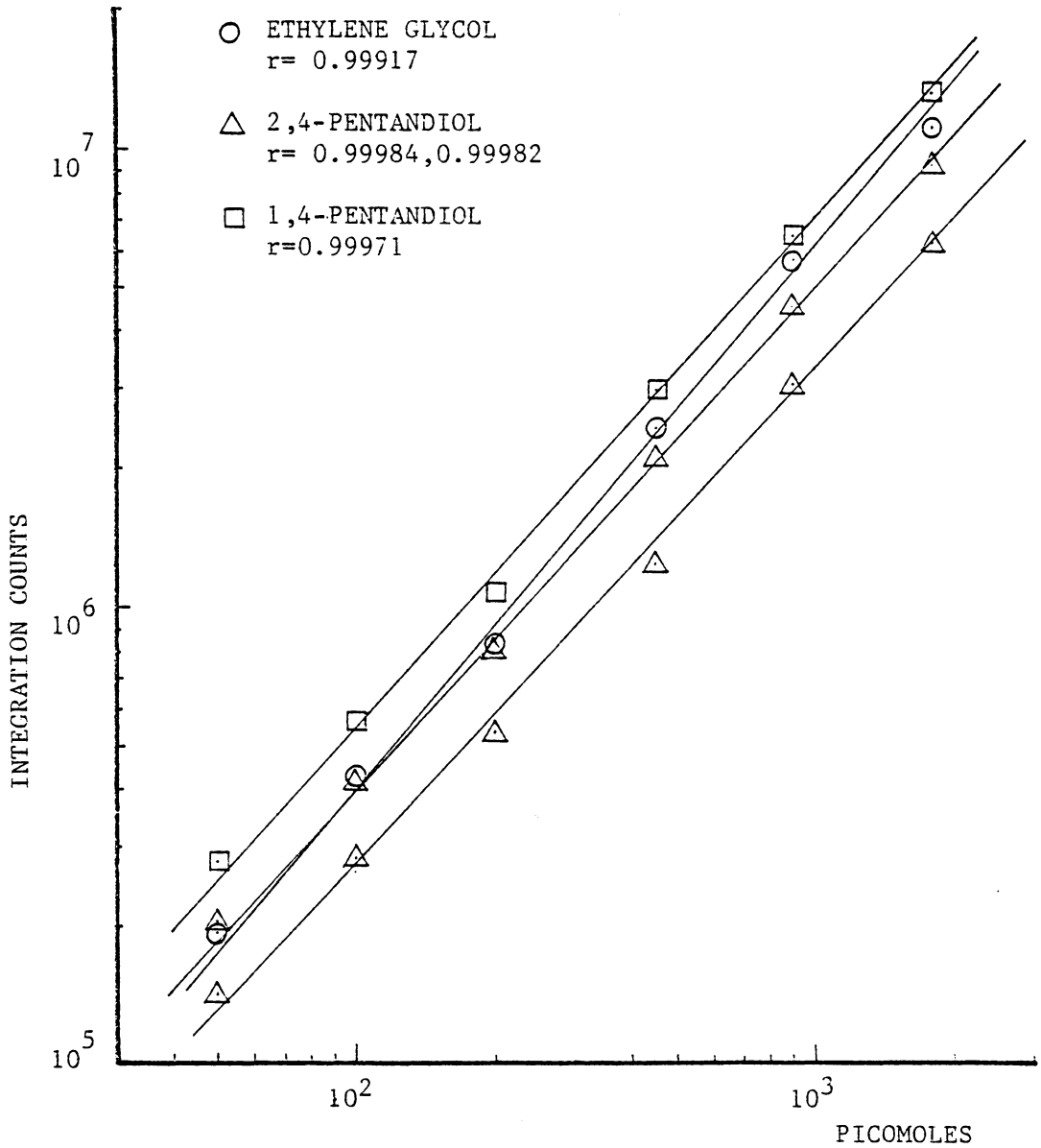


Figure 29. Calibration Plots for Para Nitro Phenyl Boromates With FID

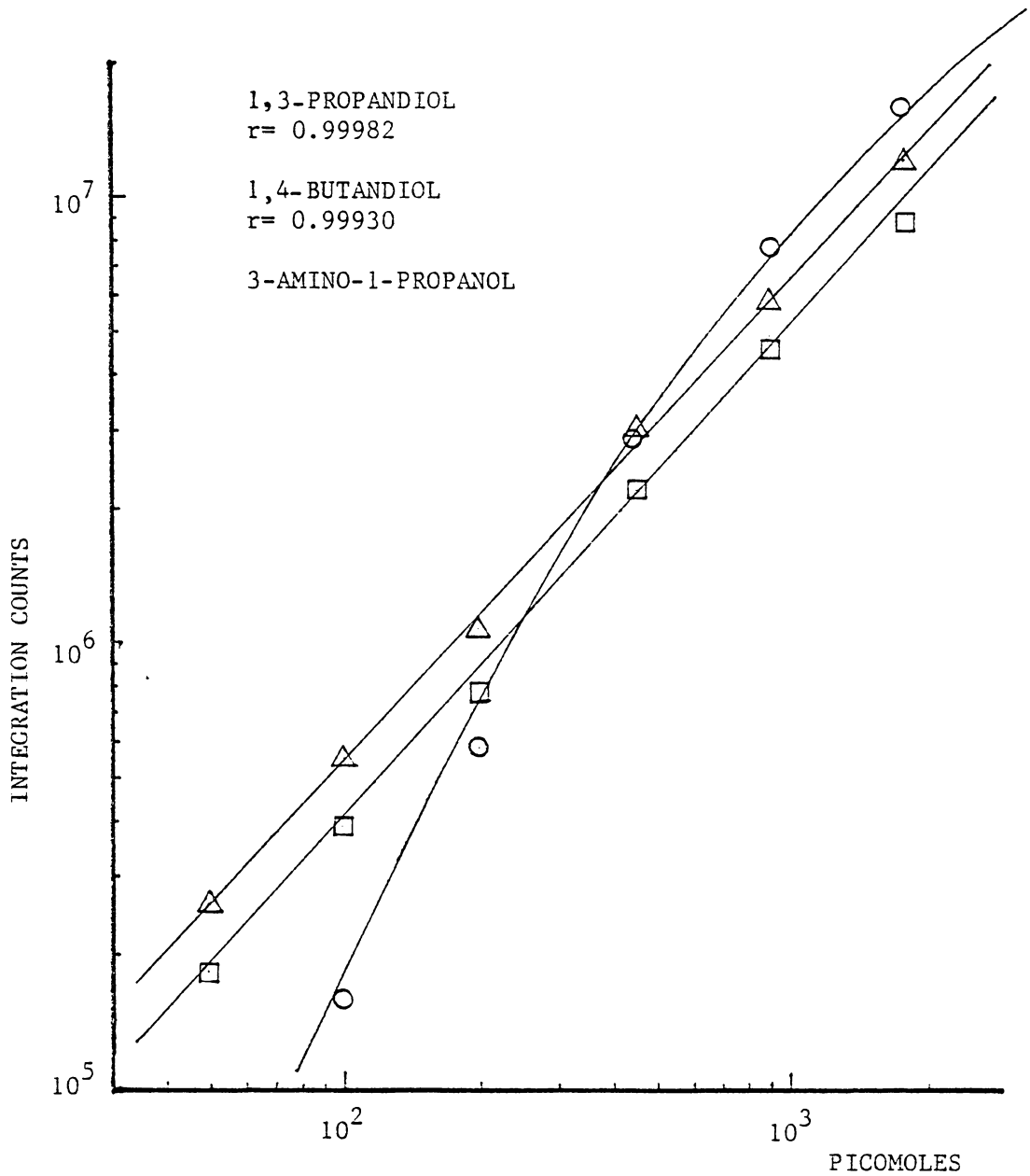


Figure 30. Calibration Plots for Para Nitro Phenyl Boronates With FID

by logarithmic conversion. These values indicate a very good fit to a straight line, and this in turn reflects the good reaction characteristics for quantitative analysis.

In the case of 3-amino-1-propanol, below the 1000 picomole level the response becomes non linear. In this plot, the last point was not drawn in order to keep the scale to a convenient size.

Non linear calibration plots are usually the results of adsorption problems. This is the sample's behavior when active sites give undesired column interaction and partial retention or decomposition of the sample. This kind of problem is particularly visible at low concentrations.

A possible explanation for this unusual or less than ideal behavior, is the residual basic character present in the boronate ring reacting with acidic sites on the column packing. Other boronates as seen on Figures 29 and 30 give excellent correlation coefficients.

It is convenient to clarify that this problem with 3-amino 1-propanol, although likely to be encountered, may not always be visible. Whether this will be present or not depends mostly on the column characteristics; the inertness of the packing, the conditioning process, previous history, etc. A possible solution to this inconvenience would be the use of specially deactivated columns, however, the use of basic additives such as KOH would not be recommended in this case.

The good performance obtained on Figures 29, 30 were representative of the other boronic acids, although non linear plots

were obtained in some cases with 7-membered ring boronates.

Below 50 picomole concentration levels the flame detector does not work well and the electron capture system becomes the detector of choice.

The high sensitivity of the EC detector creates some additional problems however. One of the most obvious problems found in this study was the solvent background.

Figures 31 and 32 show the background chromatogram obtained with two different qualities of THF, obviously the HPLC grade solvent is much more suitable for EC. analysis, but still it may be unacceptable in some cases.

Acetonitrile (HPLC grade) gives a much cleaner background as can be seen on Figure 33. The chromatograms, comparing FID and ECD responses are interesting because it shows the selective response obtained with the ECD. This solvent was chosen for EC analysis and as is obvious in Figure 33 it would also be a convenient one for FID work.

Calibration plots obtained with the EC detector look slightly different. Figures 34 and 35 show the plots for p. nitro boronates between 50 and 1 picomole concentration levels.

One of the first possible observations in the closeness of the two plots obtained for 2,4-pentandiol, if we compare these to the ones obtained with the flame detector, we can see they are further apart. Something else not shown on Figure 34 is that the plots are inverted with respect to each other. In other words, the isomer of higher response in FID is the one giving the lower signal in ECD. Since the

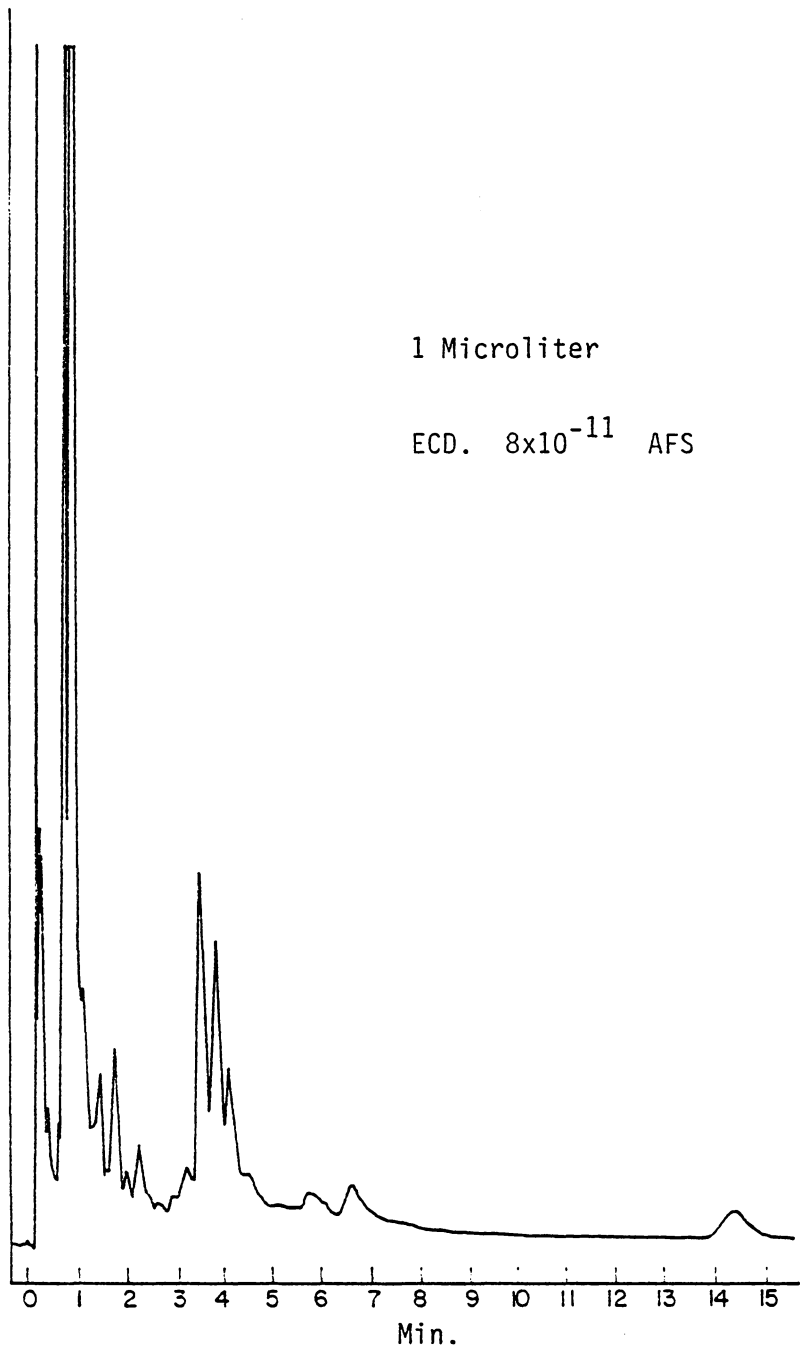


Figure 31. Solvent Background Obtained With THF ACS Reagent in ECD

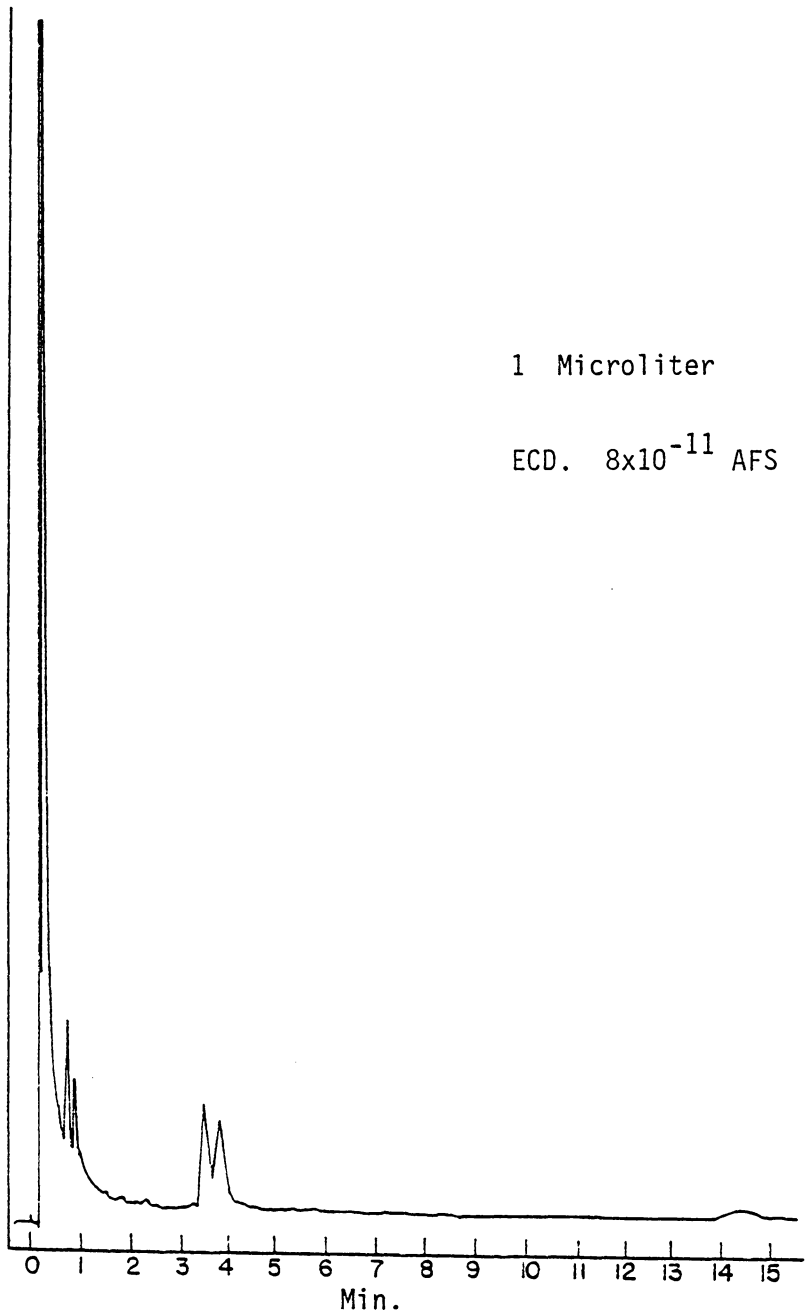


Figure 32. Solvent Background Obtained With THF HPLC Grade in ECD

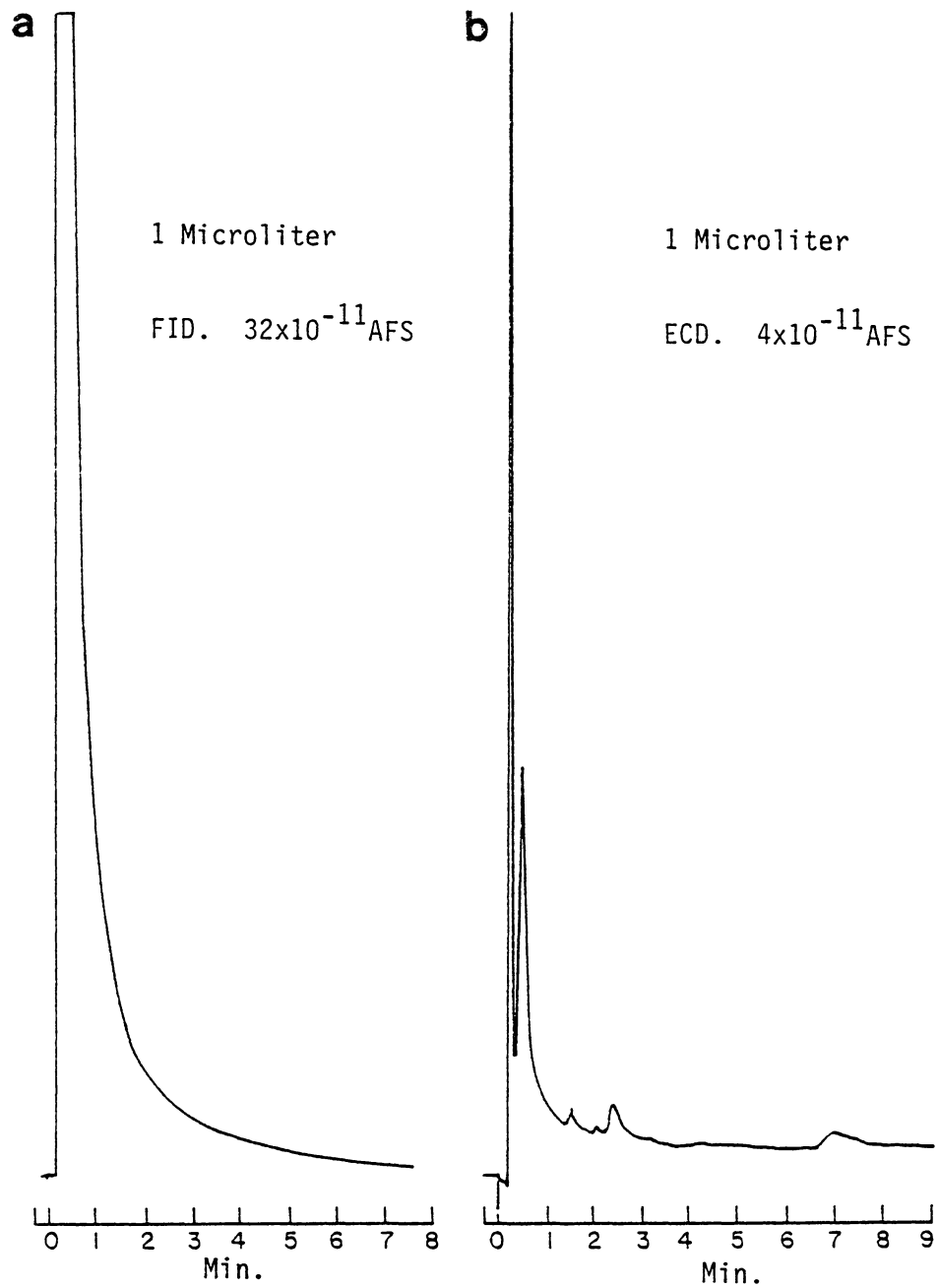


Figure 33. a) Solvent Background Obtained With Acetonitrile HPLC Grade in FID

b) Same in ECD

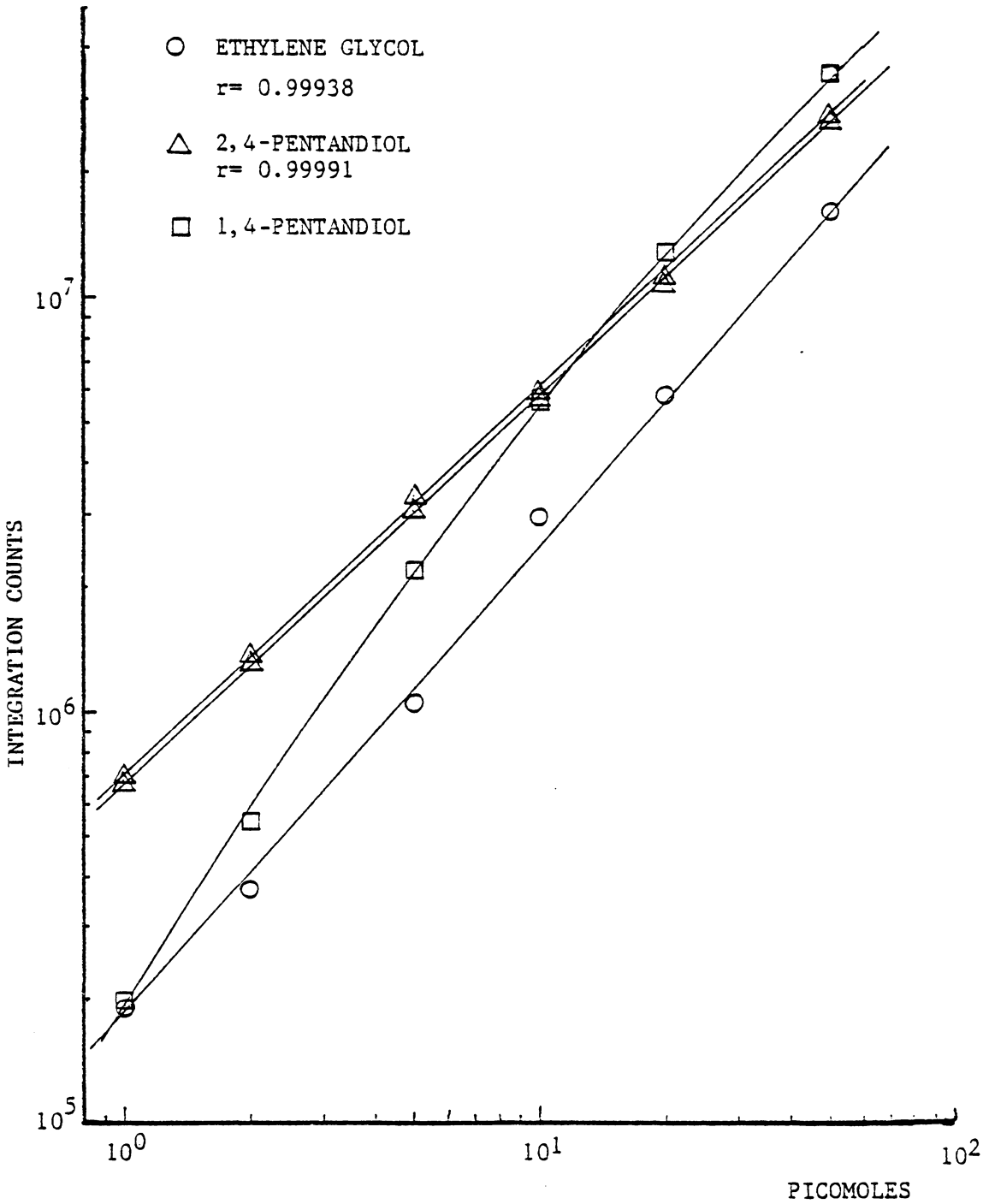


Figure 34. Calibration Plots for Para Nitro Phenyl Boronates With ECD

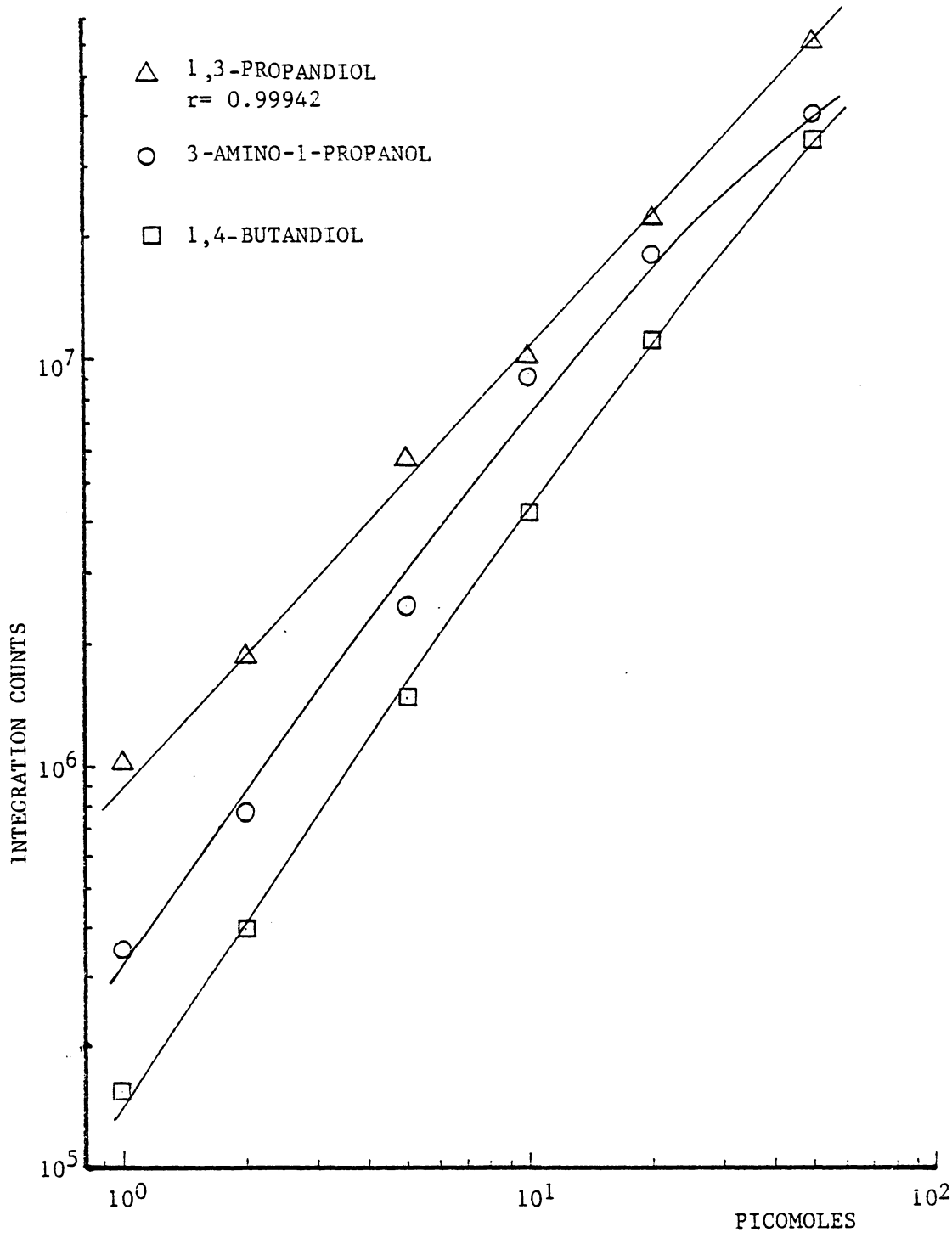


Figure 35, Calibration Plots for Para Nitro Phenyl Boronates With ECD

same sample was employed in both cases, it is obvious, the ECD is able to see a special property of these derivatives not visible with the more general FID. Why the EC detector sees this "configuration" of the molecules is not clear at this point, and it was not studied in this work.

From Figures 34 and 35 we also observe that 6- and 5-membered ring boronates give linear plots within the concentrations studied. As expected 3-amino 1-propanol also shows non linear behavior at these lower levels, although the curvature seems to be different.

Somewhat surprisingly, 7-membered ring boronates present non linear calibration plots. This is particularly visible with 1,4-pentandiol however not so clear with 1,4-butandiol. In fact the plot shown in Figure 35 looks very much like an straight line but in reality it is a smooth curve. This plot can be approximated to a straight line giving a correlation coefficient of 0.9963, however, the curve shown presents a better fit to the experimental points.

Small differences in the correlation coefficients can be taken as reflection of different stabilities or susceptibilities to decomposition. Ethylene glycol for example gave coefficients consistingly lower than 2,4-pentandiol.

It is interesting to compare the different sensitivities obtained with flame and electron capture detectors. Figures 36 and 37 display the overall difference in performance between these two cases. Both chromatograms represent the same sample size and were obtained under nearly identical conditions.

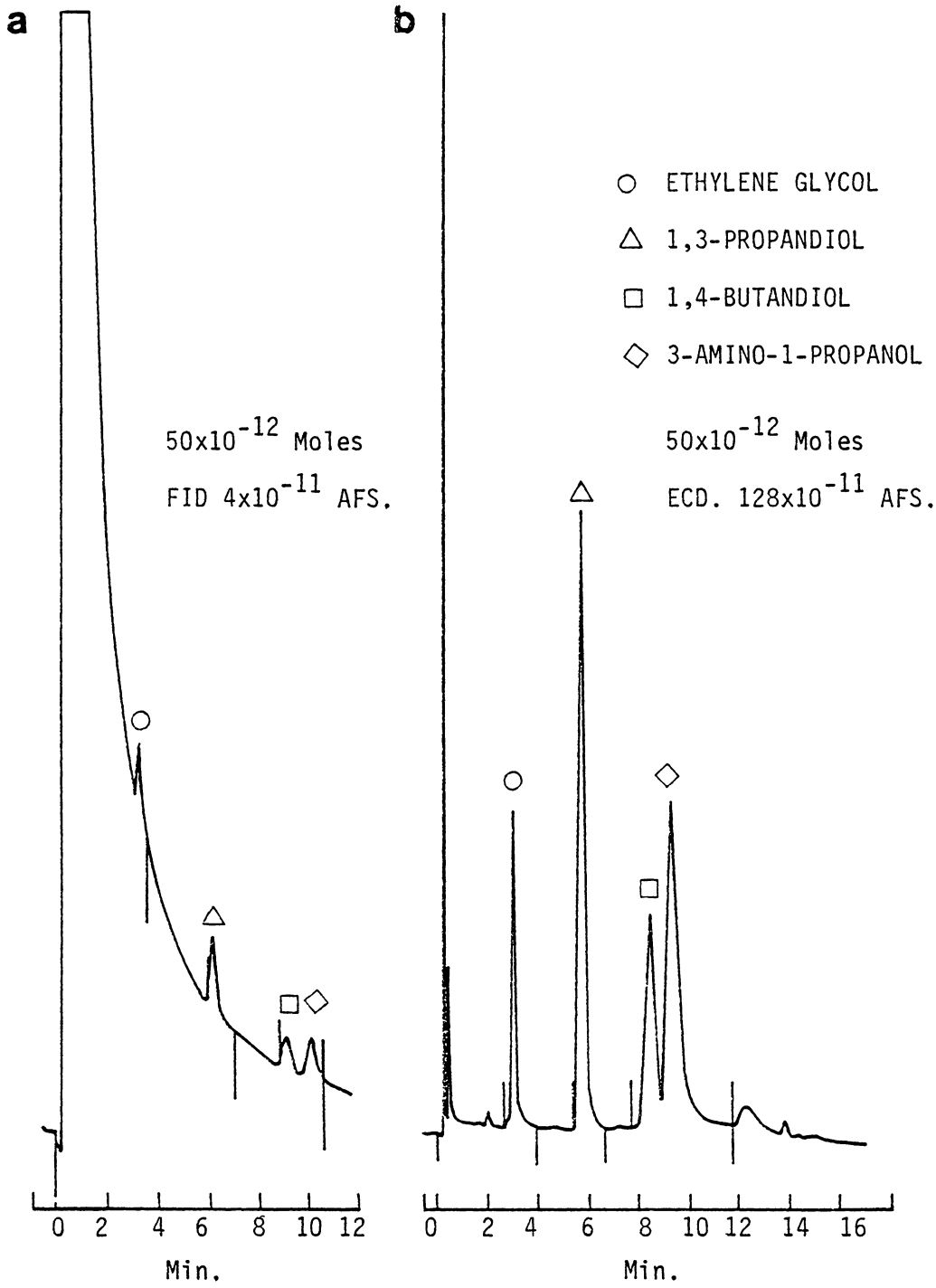


Figure 36. Sensitivity Comparison Between FID and ECD for Para Nitro Phenyl Boronates

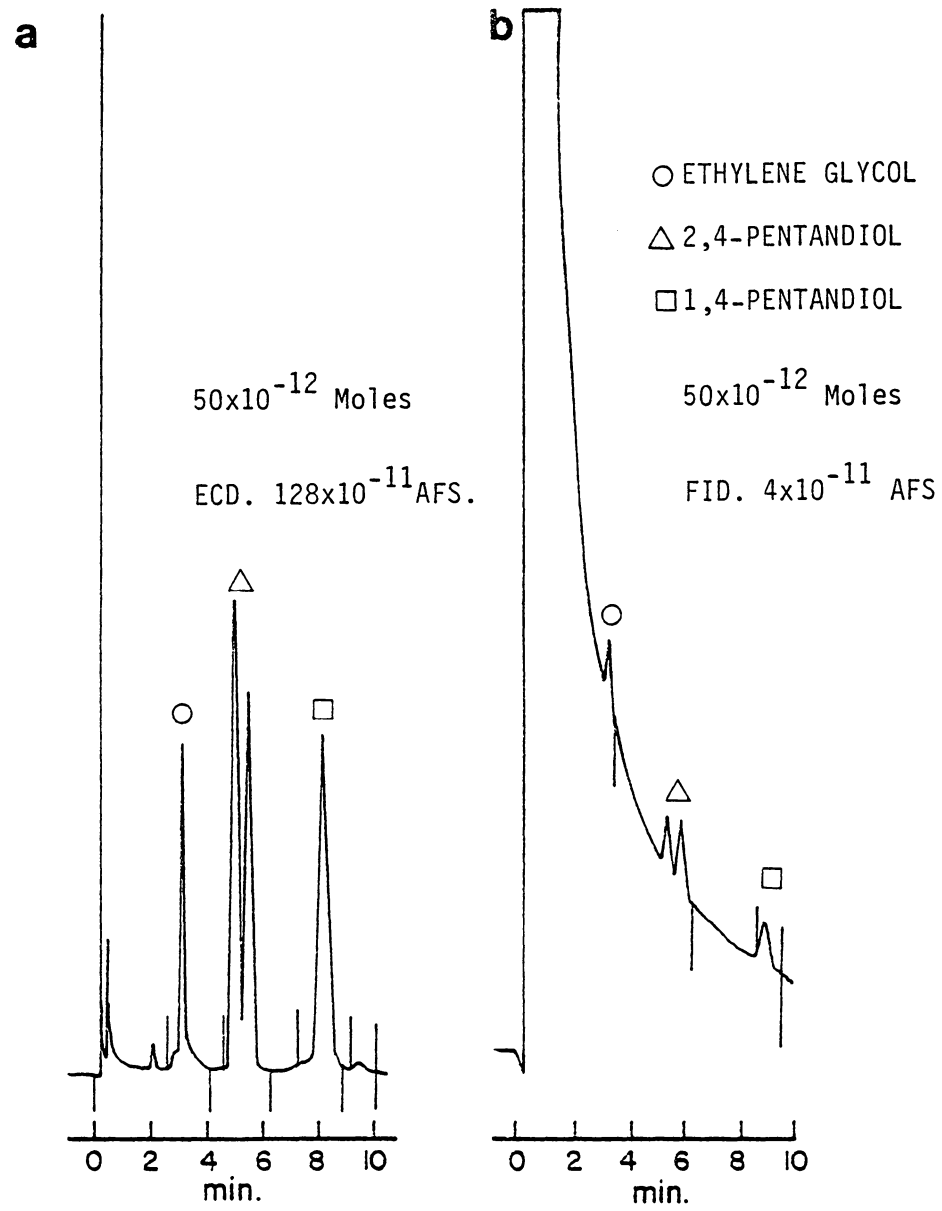


Figure 37. Sensitivity Comparison Between FID and ECD for Para Nitro Phenyl Boronates

Besides the obvious increased reponse obtained with the ECD, an additional advantage is the much lower solvent interference. This is particularly good at high sensitivity settings. A possible disadvantage is the detection of impurities not visible with FID.

Taking the ratio of integration values, it is possible to calculate the relative increment in response obtained with ECD. Ethylene glycol shows a relatively modest increment, a ratio of 165; 7-membered ring boronates show a much larger increment, ratios of 238 and 246 for 1,4-butandiol and 1,4-pentandiol respectively. 1,3-propandiol and 2,4-pentandiol show larger increments, ratios of 283 and 272 respectively. There is however an abnormal behavior of the longer retained isomer of 2,4-pentandiol with a ratio of only 188.

The case of 3-amino-1-propanol is the one that shows the largest increment found, 440 times. This value is a consequence of the good EC. sensitivity to heteroatom containing molecules. These values can not be expected to be very reproducible, due to the rather unusual behavior of the EC. detector. The values given should be taken as only an indication of possible increments of sensitivity.

Examining the plots on Figures 34 and 35 we see that even at the lowest point, the integration values are high, somewhere between 10^5 and 10^6 counts. Measuring the noise level with the integrator, and taking as the minimum detectable quantity a signal ten times the noise level, the detection limits calculated will fall well into the femtomole range. The noise counts with the EC. background signal were usually around 10^3 counts.

Figures 38, 39 and 40 compare chromatograms of p.-nitro boronates obtained with 50 picomoles and 300 femtomoles sample sizes. Here we can observe that although the EC. detector is extraordinarily sensitive, there are some additional factors which make the detection of these sample sizes extremely difficult.

Working in the femtomole range many impurities may obscure the peaks of interest. Such is the case of ethylene glycol, and due to sample decomposition 1,4-pentandiol and 1,4-butandiol give abnormally low responses. An additional problem is the solvent signal which at these low attenuations interferes with the signals of interest. In agreement with previous discussions, 3-amino-1-propanol, even at these extremely low levels gives a good response.

All these problems combined make it extremely difficult to be more specific about detection limits. These will depend on many different factors.

Relating the integration values obtained with the chromatograms shown on Figures 39 and 40 to the noise level measured at the same time, and taking as the minimum quantity a signal 10 times the noise level, the minimum detectable quantities shown on Table XI were calculated.

All the results discussed above were obtained under the conditions mentioned in the experimental section.

As mentioned before, other nitro boronates gave similar results without major differences among the isomers. Ortho nitrophenyl boronates suffered more from solvent interference due to their shorter

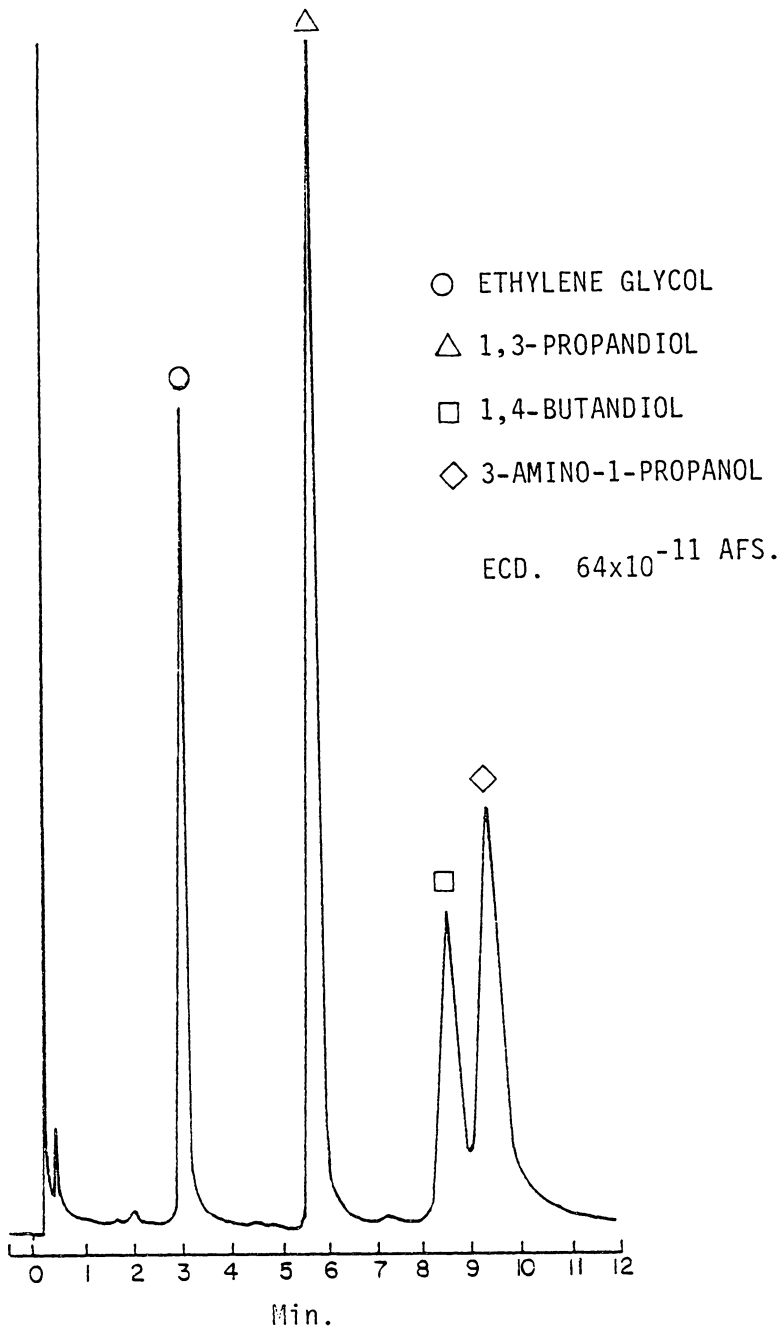


Figure 38. Para Nitro Phenyl Boronates 50 x 10^{-12} Moles Each

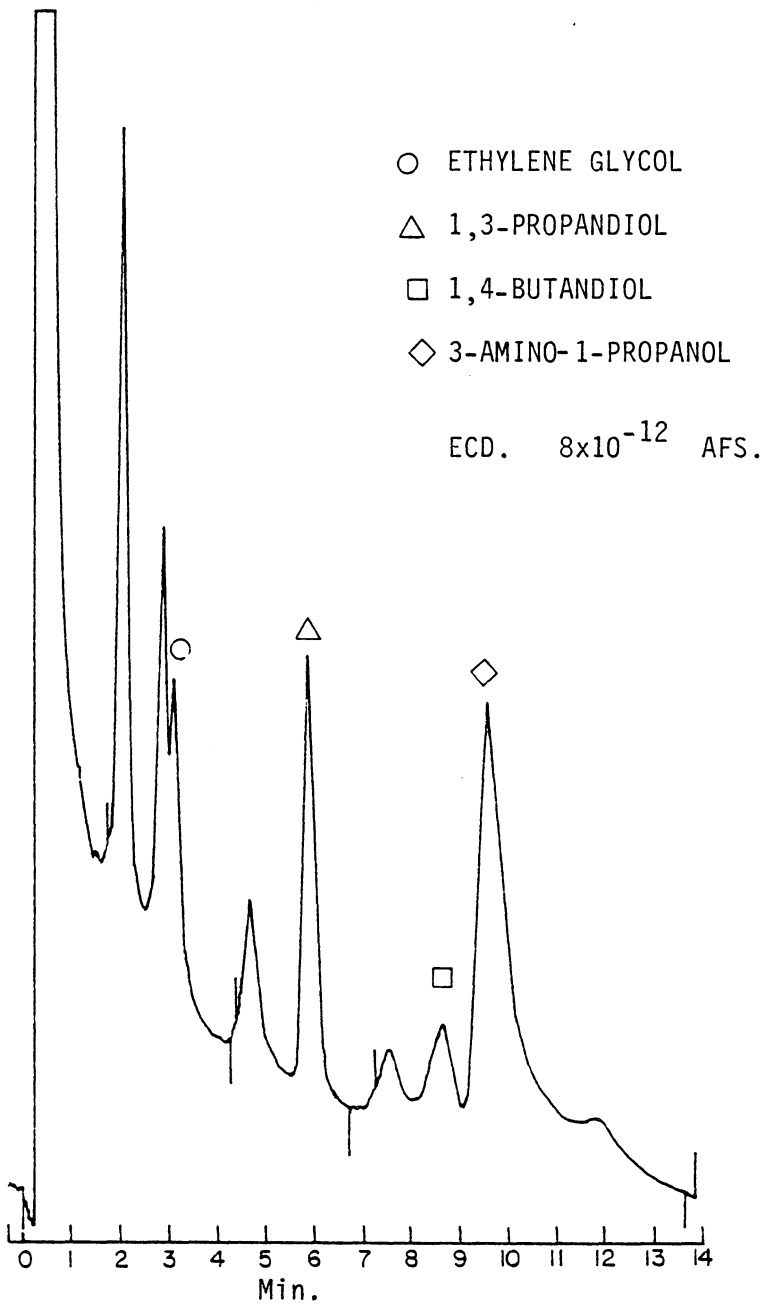


Figure 39. Para Nitro Phenyl Boronates 300 x 10^{-15} Moles Each

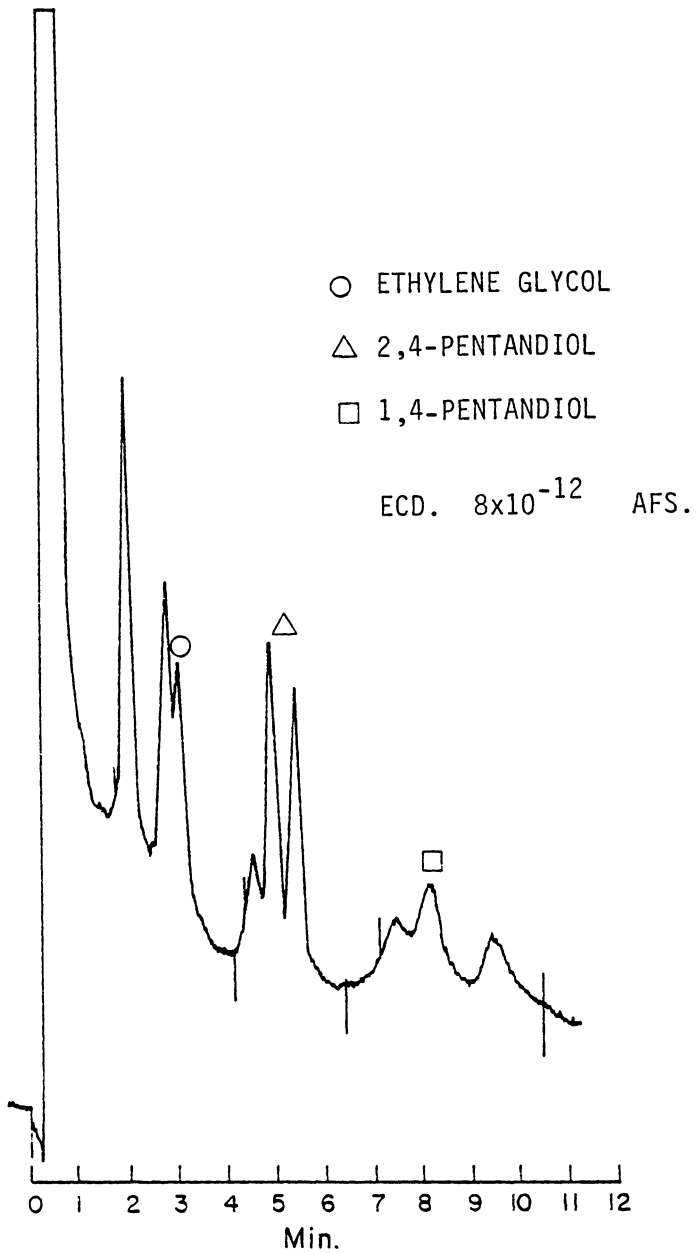


Figure 40. Para Nitro Phenyl Boronates 300 x 10^{-15} Moles Each

Table XI

Minimum Detectable Quantities
Para Nitro Phenyl Boronates - EC Detector

Ethylene glycol	1.9×10^{-15} moles
2-4 Pentandiol	1.11×10^{-15} moles
1-4 Pentandiol	2.54×10^{-15} moles
1-3 Propandiol	9.92×10^{-16} moles
1-4 Butandiol	2.62×10^{-14} moles
3-Amino-1-Propanol	4.5×10^{-16} moles

retention times. Meta nitro ones were very similar in behavior to the p. nitro boronates.

High Pressure Liquid Chromatography Analysis - As mentioned during the introduction to this dissertation, one of the attractive features of nitro phenyl boronates as derivatives in chromatography, is their good UV absorption, which could enhance the sensitivity in HPLC.

The magnitude of this good absorption can be appreciated in Figures 41, 42 and 43 which show the UV spectra in tetrahydrofuran of the three nitro phenyl boronic acids. The extinction coefficients measured from these spectra are very high at the wavelength of maximum absorption. These can be seen on the same figures.

From these extinction values, p.-nitrophenyl boronates seem to be the most recommended for HPLC analyses. As a comparison, phenyl boronic acid has an extinction coefficient of only 655 under similar conditions.

We can reasonably expect that the respective boronates will have similar spectroscopic properties since the chromophoric group will not be greatly affected with the formation of the derivative. However, solvent effects are likely to appear since according with the polarity of the media, the UV excited stated will be more or less stabilized.

Experimental evidence confirming this is the determination of the spectra in methanol. In this solvent the maximum were 265 nm for the para nitro acid, 274 nm for the meta and 265 nm for the ortho. The extinction values however were similar to the ones obtained in THF.

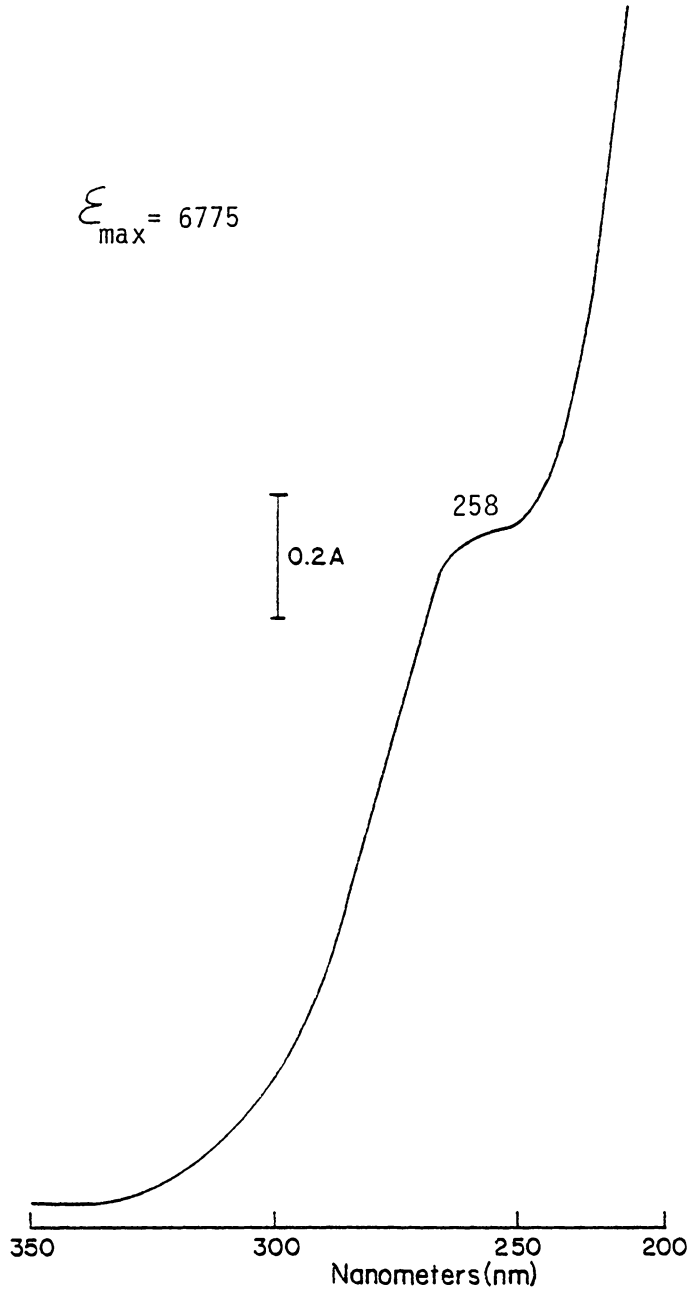


Figure 41. UV-Spectra of Ortho Nitro Phenyl Boronic Acid. Solvent THF UV Grade. Concentration 1.199×10^{-4} Molar.

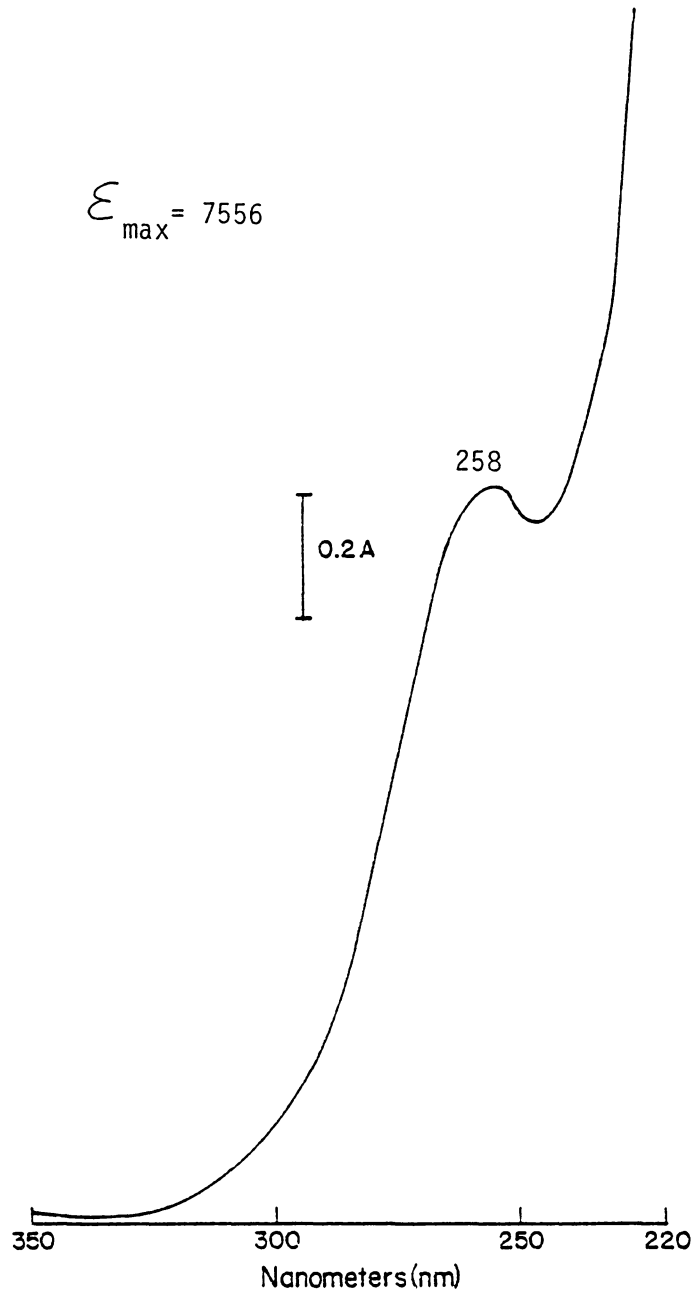


Figure 42. UV-Spectra of Meta Nitro Phenyl Boronic Acid. Solvent THF UV Grade. Concentration 1.199×10^{-4} Molar.

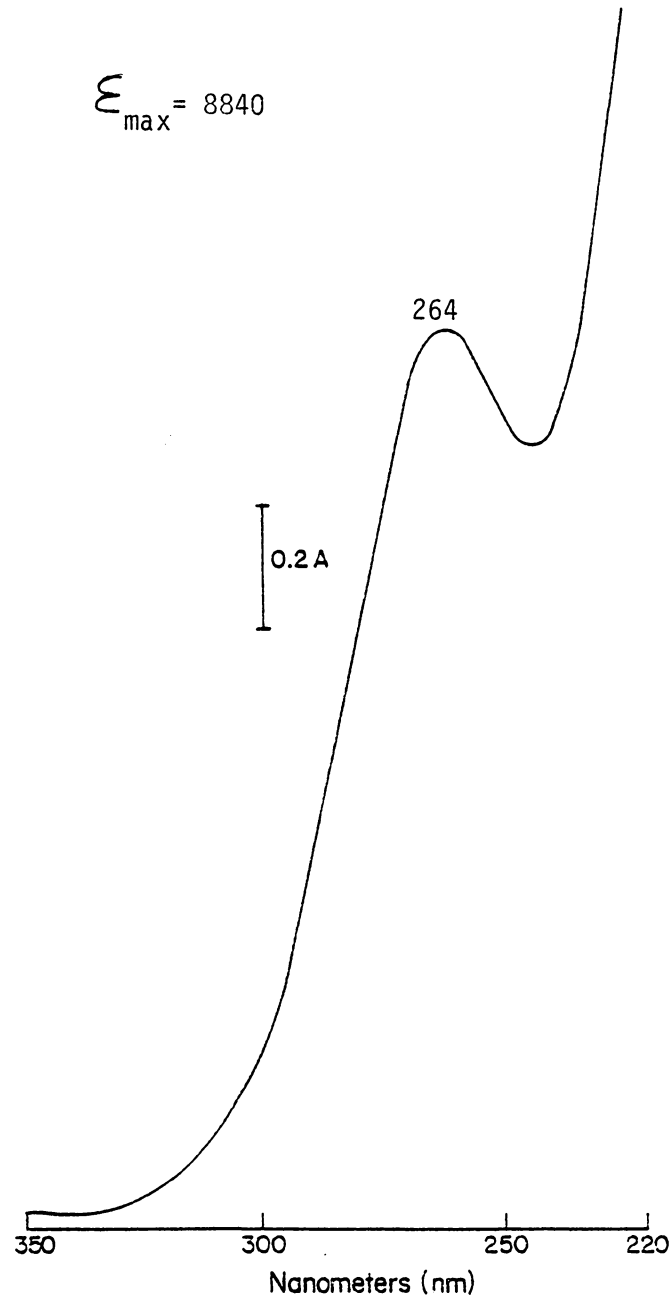


Figure 43. UV-Spectra of Para Nitro Phenyl Boronic Acid. Solvent THF Grade. Concentration 1.199 x 10^{-4} Molar.

The relative sensitivity of boronates toward hydrolysis became apparent during the attempts to use reverse phase liquid chromatography for their separations. In this chromatographic mode only the original boronic acid was detected. This is an unfortunate fact since the potential use of the electrochemical detector requires highly conductive mobile phases such as the ones normally employed in reverse phase chromatography.

Much better results were obtained with normal phase chromatography. Employing a cyano bonded phase column some of the nitro phenyl boronates gave a signal, however, even under these circumstances employing relatively non polar mobile phases, only the most stable of the nitro boronates survive the passage through the HPLC columns.

The best results were obtained with 2,4-pentandiol boronates which, are particularly stable. 1,3-propandiol and ethylene glycol boronates also gave good results, but according with their reduced response there is evidence of appreciable decomposition.

Normally HPLC is considered to be a better choice to analyze sensitive compounds. In this case however GC gave better results, since the thermal stability of boronates is good.

One possible explanation for the above described results, is the residual activity of the unreacted silanol groups on the surface of the silica base HPLC packings. It is an unfortunate fact that HPLC columns are so variable in their properties. Different procedures to chemically bond the stationary phase and other surface treatments

result in a wide range of materials. Some concern has been expressed recently about the lack of column uniformity (102).

A particularly important surface treatment which determines the residual column activity is the so called end-capping process. After the stationary phase has been chemically bonded to the silica surface, a certain amount of sterically hindered silanols remain present, these group are difficult to react with bulky reagents such as the ones used to manufacture stationary phases. In order to remove these groups an additional reaction can be made with a small methyl silane, the efficiency of both reaction will determine the column efficiency and activity.

Unfortunately end-capping is frequently done in the case of reverse phase columns, but only rarely with normal phase materials. With the presently available HPLC columns the decomposition problem of boronates seriously limit the applicability of these derivatives in HPLC.

On Figure 44 we see the results obtained with a cyano bonded phase column and 2,4-pentandiol p-nitro phenyl boronates. It was somewhat surprising that a separation was possible of the isomers clearly resolved in gas chromatography. The cyano group is more likely to interact with the polar nitro phenyl part of the derivative, being this part equal on both isomers, an expected result would be no separation in this column. The resolution is low but clear. This was possible because of the exceptionally high plate count of this particular cyano column, around 50,000 plates/meter.

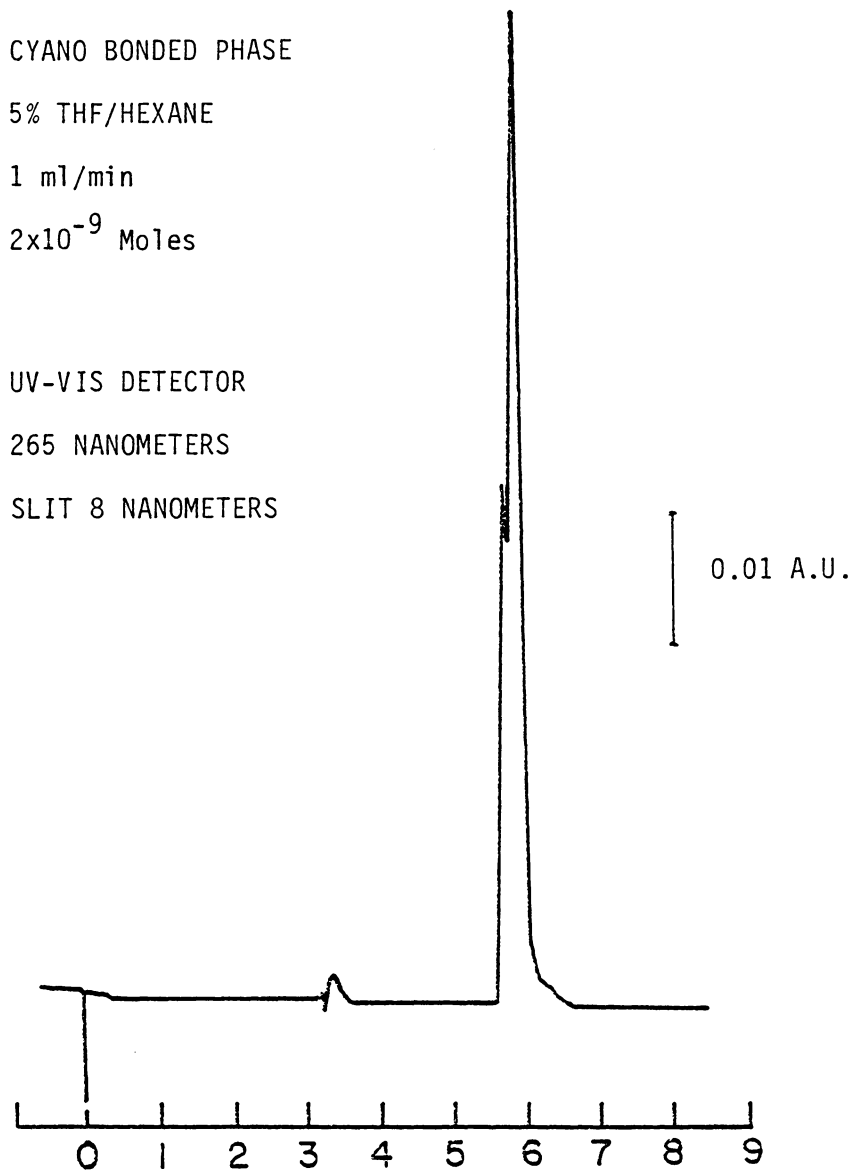


Figure 44. HPLC Separation of 2,4-Pentandiol Ortho Nitro Phenyl Boronate

Even with this particularly good case, there is some tailing of the peaks obtained, reflecting unwanted interaction with the silica support. This column was not end-capped and undoubtedly one that has been treated with this process and has been extensively reacted or covered with the stationary phase, would give much better results.

No signals were obtained with any of the 7-membered ring boronates or with amino alcohol boronates. The two other nitrophenyl boronates gave exactly the same results as the ones shown on Figure 44.

By careful tuning of the wavelength dial of the variable wavelength detector and injecting a constant amount of sample, the optimum frequencies for detection were determined. Ortho and meta nitrophenyl boronates show a maximum response at 260 nm and the para nitro ones at 265 nm. These results are slightly different from the ones shown on the UV spectra of the nitro phenyl boronic acids, but they are within reasonable instrumental error.

Good quantitative behavior was obtained with 2-4 pentandiol boronates, Figures 45, 46 and 47 show the calibration plots for the 3 nitro boronates.

At higher concentrations non linear behavior was displayed, this is a normal limitation of spectroscopic instrumentation. In the case of para nitro boronates the linear range for this detector was around 1200, a good value which compares with the ones commonly stated for detectors of this kind, about 2000.

It was relatively easy to approach the detection limits obtainable

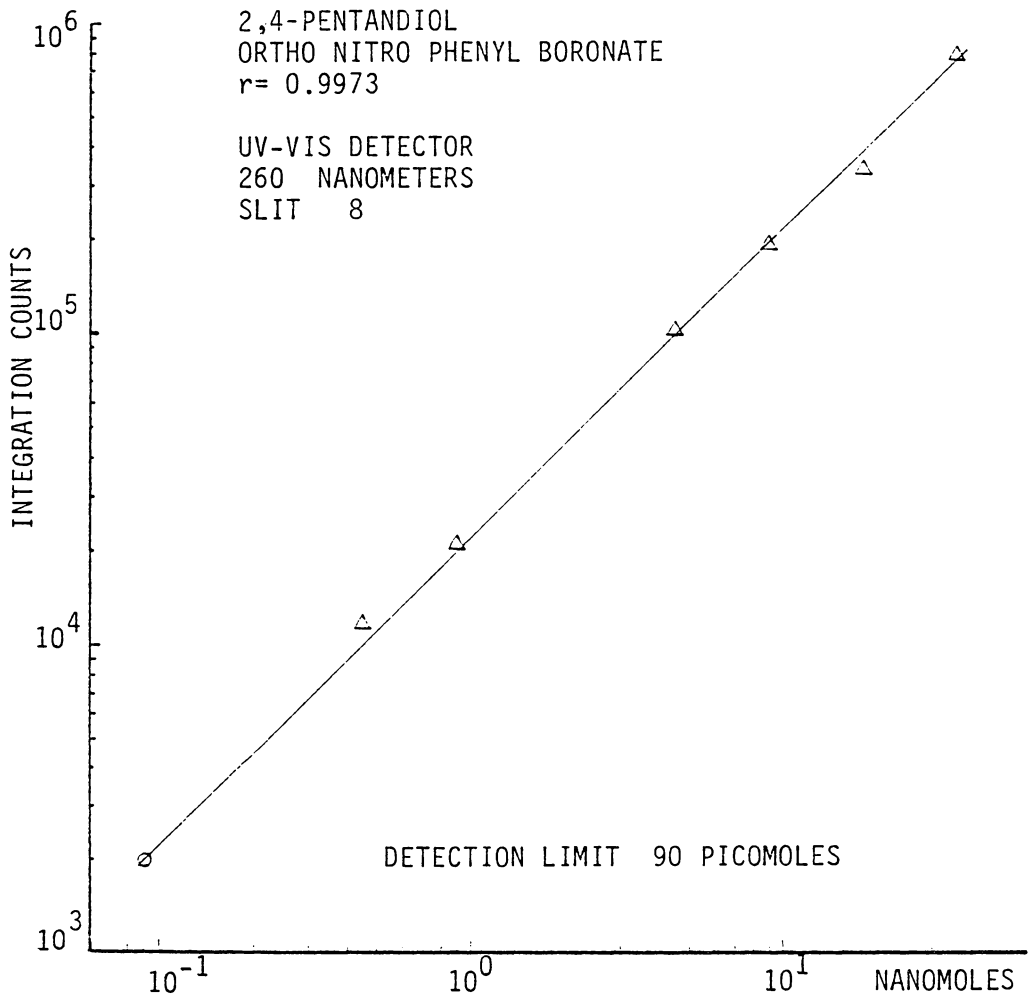


Figure 45. HPLC Calibration Plot for 2,4-Pentandiol Ortho Nitro Phenyl Boronates. Conditions as in Figure 44.

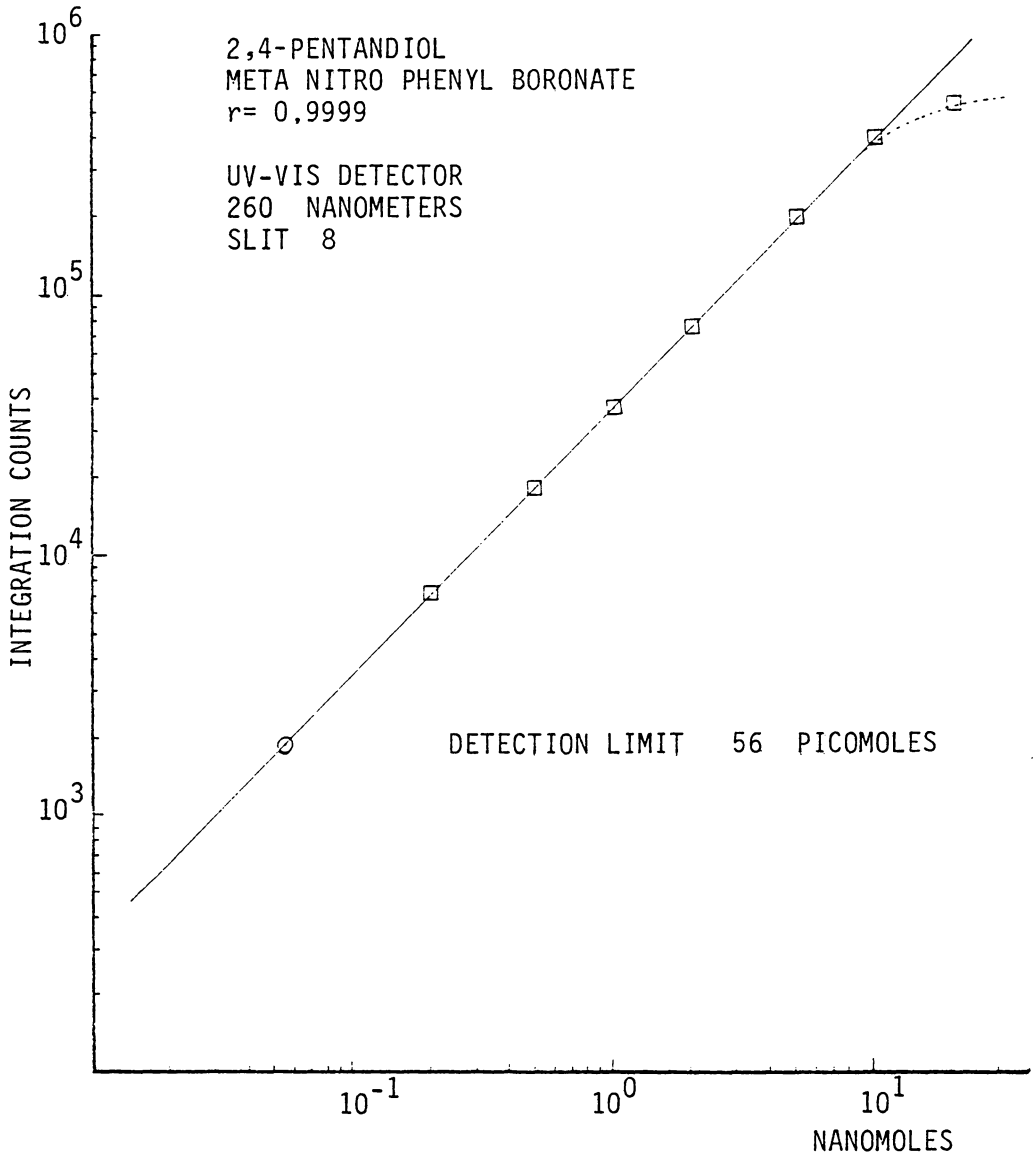


Figure 46. HPLC Calibration Plot for 2,4-Pentandiol Meta Nitro Phenyl Boronates. Conditions as in Figure 44.

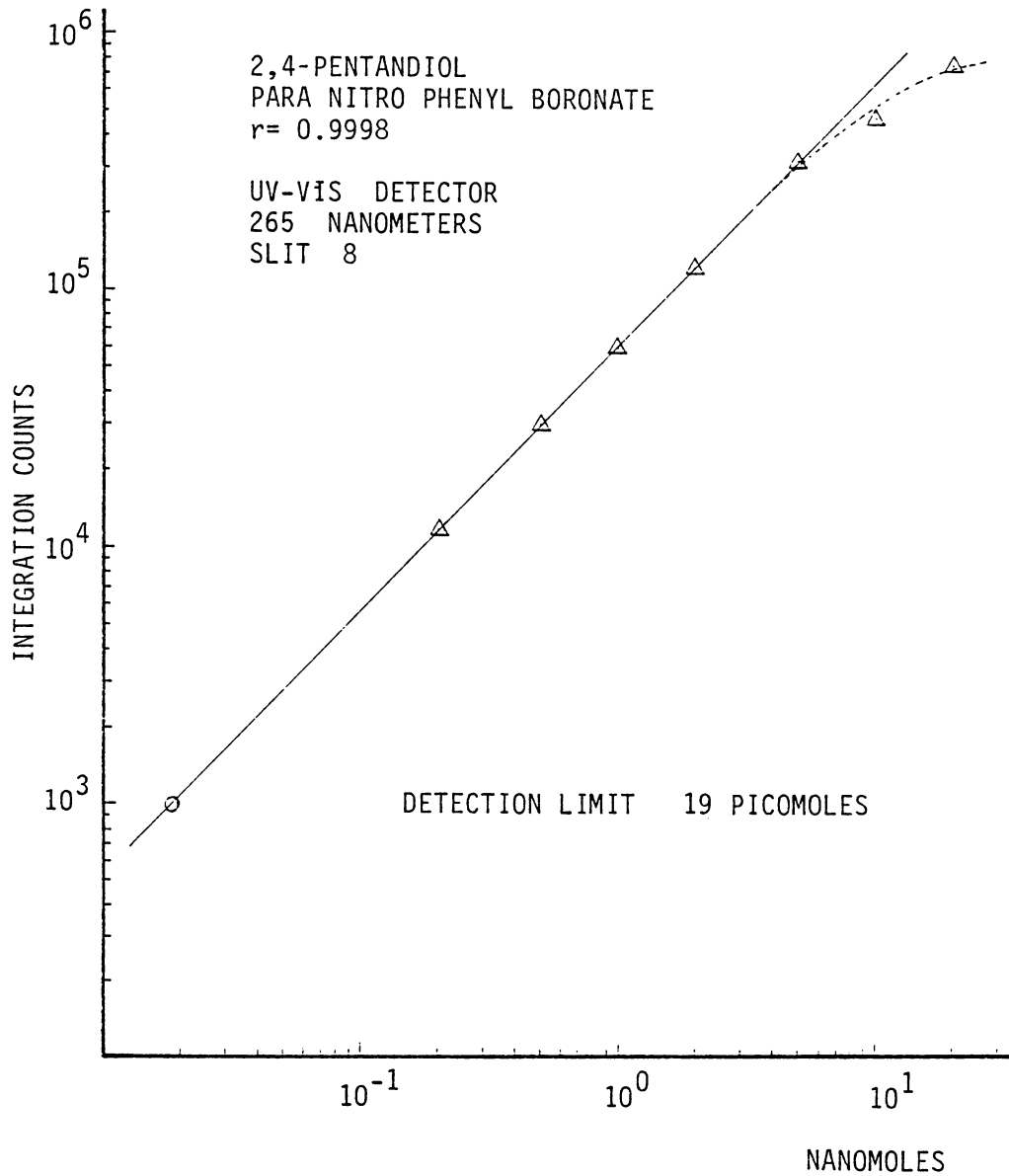


Figure 47. HPLC Calibration Plot for 2,4-Pentandiol Para Nitro Phenyl Boronates. Conditions as in Figure 44.

with these boronates. The p.-nitrophenyl ones because of their higher extinction coefficients show the lowest detection limits, these were commonly around 19 picomoles. This value transforms into 55 nanograms of sample, a good value for HPLC detectors.

The calibrating solutions employed in HPLC were prepared in the same way as the ones employed in GC. One difference was the solvent employed; UV grade THF was found to be very convenient for this case. As in the other calibration plots shown in this work, every point shown on Figures 45, 46 and 47 represent the average of 5 determinations.

Figure 48 shows the reproducibility obtained with the lowest experimental point of Figure 47. Even at this low concentration, the RSD value is quite acceptable, 2.42%.

Scope of Applications - Besides the model compounds mentioned so far, a large number of different types of compounds were tested as potential samples to be derivatized by nitro phenyl boronic acids.

As mentioned before, ortho nitro phenyl boronates are the most volatile of the boronates studied and this was the only type of nitro boronate used in studying the potential scope of applications.

Very good results were obtained with ortho disubstituted samples containing hydroxy and amino groups. Figure 49 shows the separation of several molecules of this type, as expected the relative retention occurs according to increasing molecular weight. Although not shown on Figure 49 good results were also obtained with o.-phenylenediamine, its ortho nitro boronate has a retention time very close to salicylic acid under the same conditions shown on Figure 49.

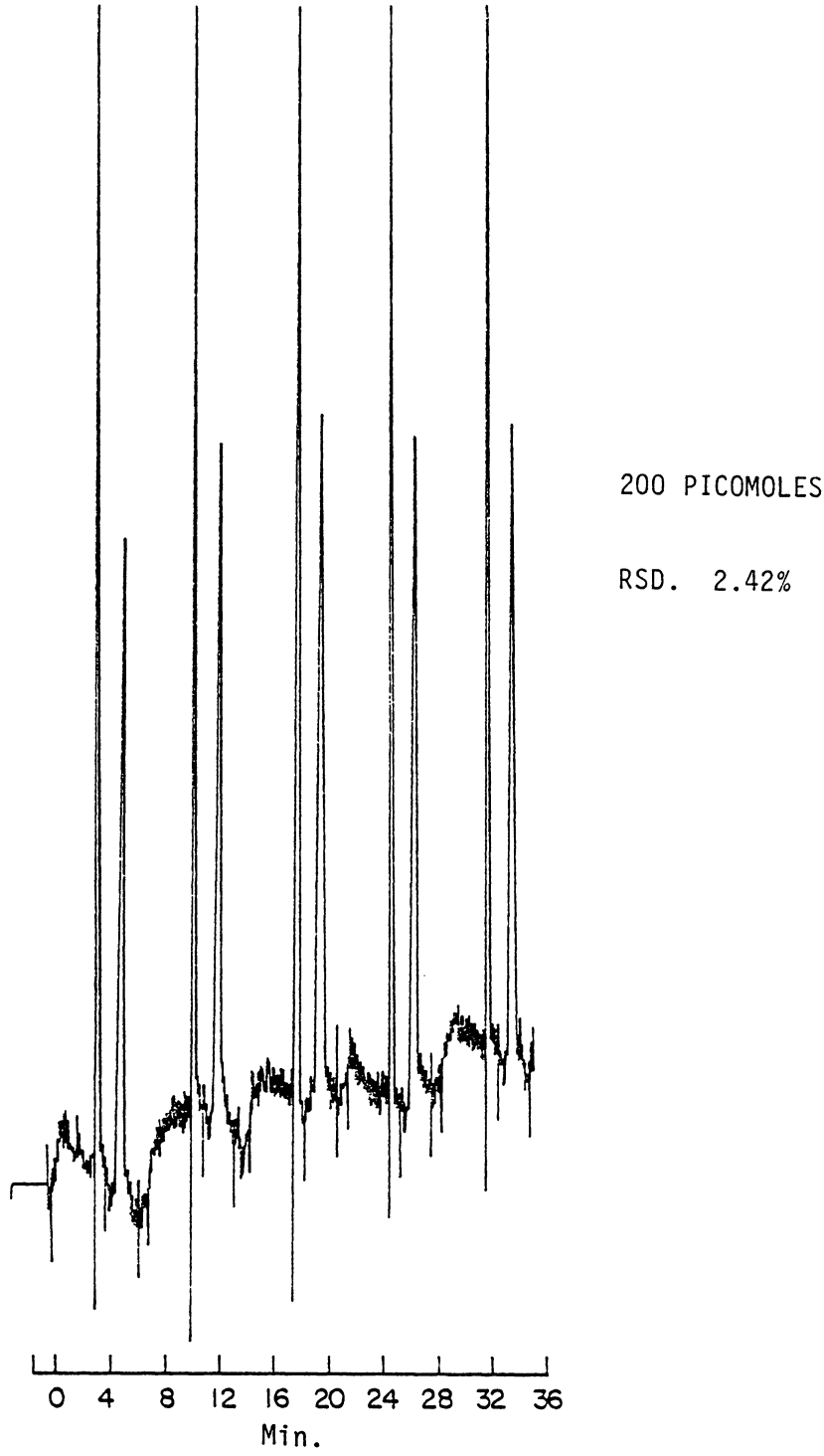


Figure 48. Injection Reproducibility in HPLC. 2,4-Pentandiol Para Nitro Phenyl Boronates. Conditions as in Figure 44.

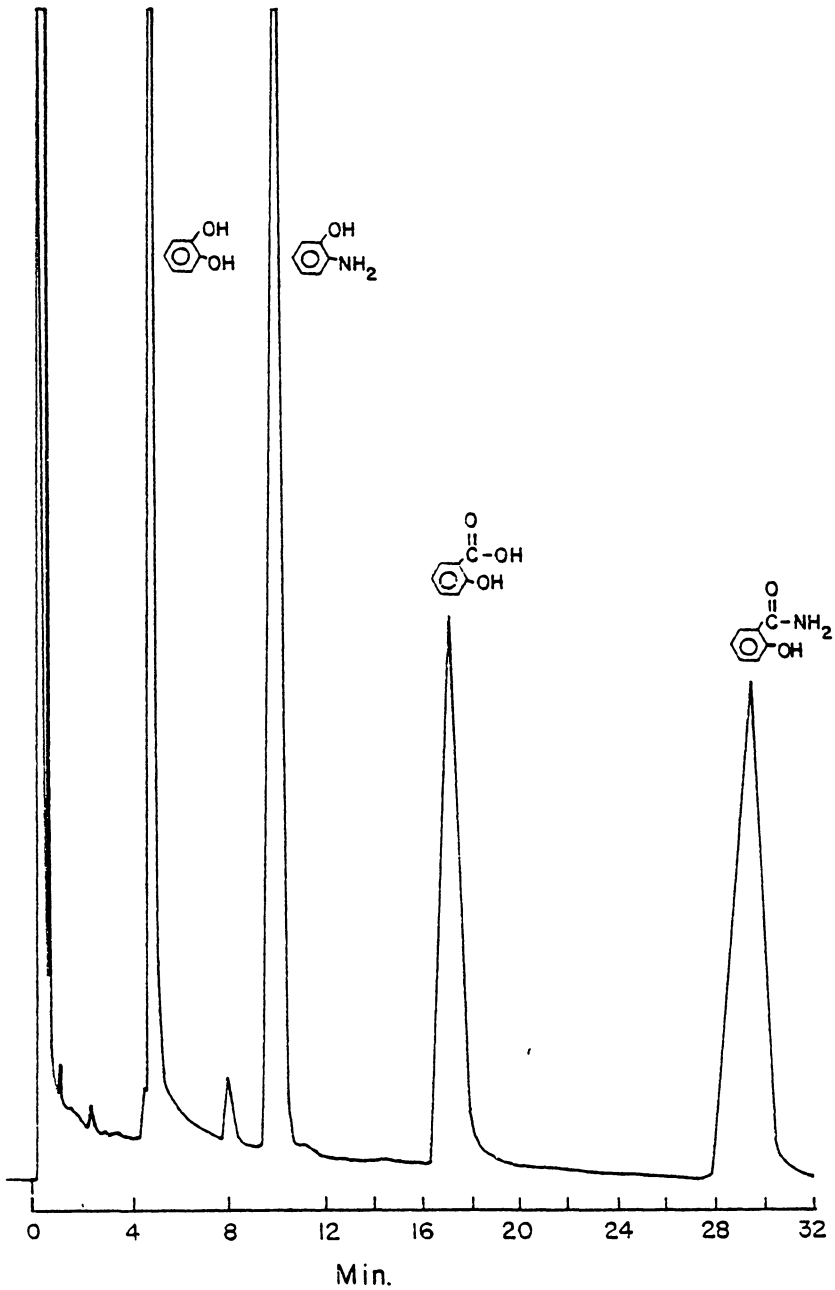


Figure 49. Separation Ortho Nitro Phenyl Boronates of Ortho Substituted Aromatic Molecules. Column Temperature 220°C. Other Conditions as in Table X.

Aromatic molecules containing an amino alcohol side chain also gave good results. The analysis of these derivatives however is slightly difficult due to the high molecular weight. Samples such as ephedrine and phenyl propanol amine gave very good results, others such as synephrine which contains an isolated hydroxyl group in the aromatic ring, seem to decompose at the relatively high temperatures required for convenient elution times, 230°C.

In the cases described so far, dimethyl formamide was a better solvent for the reaction, some samples such as phenyl propanol amine and salicylamide were not soluble in THF.

Hydroxy acids giving 5-membered ring boronates gave unstable boronates and asymmetrical peaks. 3-Hydroxybutyric acid gave very good results with nice symmetric signals and much better stability.

Mandelic acid and 1,3-propandithiol failed to react with ortho nitrophenyl boronic acid, they however react very well with the meta and para nitro ones. These boronic acids seem to be the first ones ever reported to form a stable derivative with 1,3-propandithiol.

Molecules which can be derivatized in more than two vicinal groups gave less than satisfactory results. The problem involved here was not the derivatization reaction itself but the product formed, in cases like this, the derivative is of very high molecular weight and difficult to chromatograph.

The increment in molecular weight obtained with nitro phenyl boronic acids is approximately 133 units, in the case of multiple addition. The increment may be 266 units or more. This added to the samples molecular mass may result in a extremely involatile boronate.

Multiple derivatization is possible in the case of samples like sugars and alditols. With some molecules of this kind it was possible to see the derivatives in GC, but these required high temperatures and a good peak shape was not normally found.

Some other unsuccessful cases were: citric acid, phthalic acid, and nadolo (cis 5-[3-(1,1-dimethyl ethyl) amino] - 2 hydroxy propoxy] - 1,2,3,4 tetrahydro-2,3-naphthalenediol.) This last case would form a derivative with a molecular weight of over 500 which is obviously too high for GC, but it is interesting to point out that the derivative seems to be formed. This is evident by the appearance of a high density yellow fluid which may be the derivative. Boronates in general are super cooled fluids and very few crystallize. Citric acid can also give multiple derivatives and phthalic acid which would form a 7-membered ring boronate is perhaps too unstable for GC analysis.

All the compounds which were successfully derivatized and gave a clear signal in GC were also tried by HPLC analysis, this however was largely unsuccessful. Although some compounds gave signals in HPLC when derivatized, the results were unclear and non conclusive.

It does not seem possible to use HPLC for boronate analysis until less reactive columns are available or better conditions are found.

Table XII lists the compounds tested indicating which were successful and unsuccessful forming a stable derivative(s).

Final Comments - From the information presented in this section, it is possible to appreciate that two main problems were encountered during the present study. Undesirable column interaction

Table XII

Compounds Tried as Samples

Successful Cases	Unsuccessful Cases
- Chatecol	Xylose
- o. Phenylenediamine	Arabinose
- Salicyclic Acid	Phthalic Acid
- Salicyl Amide	Ascorbic Acid
- o. Amino Phenol	Norepinefrine (arterenol)
- Ephedrine	Epinefrine
- Phenyl Propanol Amine	Dopamine
- 3 Hydroxybutyric Acid	Anthranilic Acid
- Fructose	
- Fucose	
- 1,3-Propandithiol	
- Mandelic Acid	

which leads to partial decomposition and non linear behavior in the case of GC, and total or excessive decomposition in the case of HPLC. The second problem was derivative instability, which makes the decomposition problems more evident in some cases.

There are essentially two approaches to follow in order to improve the overall derivatization and analysis process: better chromatographic equipment and improved derivatization reagents.

Along the approach of better equipment, in order to improve results, better column technology and different types of columns can be tried. It is possible that packed columns carefully or extensively deactivated, along with better coating procedures can improve the performance at the pico or femtomole level.

Another possibility is the use of fused silica capillary columns which are known to be extremely inert. This approach although expensive and difficult to use for quantitative analysis can extend the applicability of nitro phenyl boronates. A short fused silica capillary column is able to elute high molecular weight molecules in relatively short times. It is possible then, that a column like this can solve the problem of samples capable of derivatization in more than two closely positioned groups.

Better HPLC columns are likely to appear in the future. Totally organic columns have been recently discussed and will be available in the near future (103). It is not clear at this moment how successful these columns will be; as of now the column efficiency is limited and relatively few bonded stationary phases have been tried.

A better derivatizing reagent of this kind can be suggested in view of all the information obtained in this project. There are basically two modifications to the phenyl boronic acid molecule which can be tried. The first can be the introduction of an electron donating group, which is possible it will stabilize all the boronates and hopefully even the 7-membered ring ones.

A good candidate for this approach would be p.-methoxyphenyl boronic acid. Unfortunately a molecule like this, is not likely to have better electrophoric properties than phenyl boronic acid and most probably its properties will be worse. It is possible, however, that this reagent could be useful in HPLC due to its good UV absorption as long as the methoxyphenyl boronates are stable enough to be eluted without decomposition from HPLC columns. For GC derivatization, the ortho methoxyphenyl boronic acid would be a better reagent since the ortho substituted molecules are normally more volatile than the meta or para substituted ones.

Keeping the nitro group in the reagent's molecule, a better reagent would be one in which the boronic acid group is insulated or protected from attack. Following this approach, the benzyl nitrophenyl boronic acid is likely to be a better homolog than the phenyl one. A reagent like this, containing a methylene group as insulation from the strong electron withdrawing effects of the nitro group, is likely to give more stable derivatives and still have good EC and UV characteristics. The position of the nitro group would be less important in this case, but, still the ortho position is likely to give

shorter GC retention times and the para position most probably the strongest UV absorption, a desirable feature for HPLC analysis.

Another way to protect the boronic acid is by the presence of bulky alkyl groups on the reagent. With this in mind a suggested reagent will be 2,6-di-tert-butyl-4-nitrophenyl boronic acid. This molecule most probably will form derivatives totally unsuitable for GC analysis due to their high molecular weights, but these are likely to be more stable and less susceptible to attack by water and methanol.

If the stability of t-butyl nitro phenyl boronates is good, the use of reverse phase HPLC could greatly extend the applicability of boronic acids in chromatography. This case in particular would make possible the use of electrochemical detection and since no molecular weight restrictions are present in HPLC, the problem of multiple derivatization will be non existent.

In conclusion, these are some of the logical suggestions for extended research in this area. Undoubtedly more boronic acid studies and applications will be made in the future as part of the continuing effort of bringing chemistry into the separation science.

REFERENCES

1. I. M. Kolthoff., Anal. Chem. 1977, 49, 480A.

2. Corkill, J. A., et. al. Anal. Chem. 1982, 54, 481.

3. L. S. Ettre, A. Zlatkis, Eds., "75 Years of Chromatography" J. Chromatogr. Library Vol. 17 page 41, Elsevier Publs. Co. Amsterdam 1979.

4. J. Purcell, R. P. W. Scott, "Pittsburgh Conference 1982" paper 642, Atlantic City, N.J., 1982.

5. Scott, R. P. W., P. Kuceva, J. Chromatogr. 1976, 125, 251.

6. Stuart A. Borman, Anal. Chem. 1982, 54, 327A.

7. G. Guiochon, "High Performance Liquid Chromatography" Vol II Chapter i, C. Horvath Ed. Academic Press, N.Y. 1980.

8. T. Isenhour, Anal. Chem. 1982, 54, 462A.

9. N. Pellick, H. R. Bolliger, H. K. Mangold, "The History of Thin Layer Chromatography" Advances in Chromatography, Vol. 3, page 92, Marcel Dekker, N.Y. 1966.

10. A. T. James, J. Chromatogr. 1959, 2, 452

11. A. T. James, A. J. P. Martin, Biochem. J. 1956, 63, 144.

12. A. T. James, A. J. P. Martin, Biochem. J. 1952, 52, 242.

13. A. T. James, A. J. P. Martin, Biochem. J. 1952, 50, 679.

14. K. VonRuhlman, W. Giesecke, Angew Chem. 1961, 73, 113.

15. E. J. Corey, A. Venkateswarlu. J. Am. Chem. Soc. 1972, 94, 6190.

16. C. F. Poole, A. Zlatkis, J. Chromatog. Sci. 1979, 17, 115.

17. A. E. Pierce, "Silylation of Organic Compounds" Pierce Chemical Co., Rockford, IL, 1968.

18. "A Users Guide to Chromatography" Regis Chemical Co. Morton Grove Il, 1976.

19. Brittain, G. D., " A Bibliography of Silylation. Synthetic Methods and Analytical Uses" Pierce Chemical Co. Rockford IL.

20. Brittain G. D., "Handbook of Silylation and Other Special Techniques" Pierce Chemical Co. Rockford, IL, 1970.

21. W. H. Gutenman, D. J. Lisk, J. Assoc. Off. Agr. Chemists. 1964, 47, 353.

22. E. D. Pellizari, J. Chromatogr. 1974, 98, 343.

23. O. Gyllenhaal, P. Hartvig, J. Chromatogr. 1980, 184, 99.

24. H. H. Wotiz, S. J. Clark, "Gas Chromatography in the Analysis of Steroid Hormones" Chapter 8-9, Plenum Press, New York, 1966.

25. Mule, S. J., J. Chromatog. Sci. 1971, 9, 98.

26. Kanamen, G., J. Chromatog. Sci. 1973, 10, 283.

27. R. Dandeneau, et. al., Am. Lab. 1979, 11, 61.

28. P. Larson, L. Plotczyk, "Pittsburgh Conference 1982' paper no. 639 Atlantic City, N.J., 1982.

29. N. OI, M. Horiba, H. Kitahara, J. Chromatogr. 1980, 202, 299.

30. E. Gil-Av, B. Feibush, R. C. Sigler, Tetrahedron Lett. 1966, 1009.

31. K. Blau, G. King, "Handbook of Derivatives for Chromatography" Heyden, London 1977.

32. D. R. Knapp, "Handbook of Analytical Derivatization Reactions" John Wiley & Sons, New York, 1979.

33. J. Drozd., J. Chromatogr. 1975, 113, 303.

34. G. Zweig., J. Sherma, "Handbook of Chromatography". Volume II, page 191, CRC Press, Cleveland, Ohio 1972.

35. I. S. Krull, "Advances in Chromatography" Vol. 16, page 175, J. C. Giddings, Ed., Marcell Dekker, N.Y. 1978.

36. J. Merzhauser, et. al., Klin. Wochenschr. 1973, 51, 883.

37. K. Hostettman, H. M. McNair, J. Chromatogr. 1976, 116, 201.

38. R. Ertingshauser, H. J. Adler, A. S. Reichler, J. Chromatogr. 1968, 42, 355.

39. J. Rosmus, Z. Deyl, Chromatogr. Rev. 1971, 13, 83.

40. P. L. Joyner, R. J. Maggs, J. Chrom. Sci. 1970, 8, 427.

41. Y. Takata, G. Muto, Anal. Chem. 1973, 45, 1864.

42. P. T. Kissinger, et. al., Anal. Lett. 1973, 6, 465.

43. P. T. Kissinger, et. al. J. Chrom. Sci. 1979, 17, 137.

44. J. F. Lawrence, R. W. Frei, "Chemical Derivatization in Liquid Chromatography". J. Chromatogr. Library, Vol. 7, Elsevier Holland.

45. J. F. Lawrence, "Organic Trace Analysis by L.C." Chapter 7, Academic Press, London 1981.

46. R. W. Frei, J. F. Lawrence Eds., "Chemical Derivatization in Analytical Chemistry" Vol. I, Chapter 3-4, Plenum Press, N.Y. 1981.

47. J. Chrom. Sci., 1979, 17 (entire issue).

48. Spratt, M. P., Dorn, H. C., Unpublished work, Department of Chemistry, Virginia Tech, Blacksburg, VA, 1981.

49. R. W. Frei, J. F. Lawrence, "Chemical Derivatization in Analytical Chemistry" Vol. I, Chapter 4, Plenum Press, N.Y., 1981.

50. R. W. Frei, A. H. M. Scholten, J. Chrom. Sci. 1979, 17, 153.

51. L. S. Ettre, W. H. McFadden, Eds., "Ancillary Techniques of Gas Chromatography" Chapter 2, Wiley Interscience, N.Y., 1969.

52. L. S. Ettre, W. H. McFadden, Eds., "Ancillary Techniques of Gas Chromatography" Chapter 4, Wiley Interscience, N.Y., 1969.

53. Walter C. Kossa, et. al. J. Chrom. Sci. 1979, 17, 177.

54. T. Groen, J. C. Kraak, J. Chromatogr. 1977, 138, 245.

55. Tomlinson, E., T. M. Jeffries, C. M. Riley, J. Chromatogr. 1978, 159, 315.

56. C. Zomzely, et. al., Anal. Chem. 1962, 34, 1414.

57. D. H. Shaw, G. M. Mose, J. Chromatogr. 1967, 41, 350.

58. C. F. Poole, A. Zlatkis, J. Chromatogr. 1980, 184, 99.

59. E. J. Bourne, E. M. Lees, H. Weigel, J. Chromatogr. 1963, 11, 253.

60. C. A. Elliger, L. B. Rabin, J. Chromatogr. 1981, 216, 261.

61. R. W. Frei, J. F. Lawrence, "Chemical Derivatization in Analytical Chemistry" Vol. I, Chapter 2, Plenum Press, N.Y., 1981.

62. M. F. Lappert, Chem. Rev. 1956, 56, 959.

63. T. Onak., "Organoboron Chemistry" Academic Press, N.Y. 1975.

64. S. Singhawangcha et. al., J. High Res. Chrom. & Chrom. Comm. 1978, 1, 304.

65. W. J. A. VandenHeuvel et. al., Org. Mass. Spectrom. 1977, 12, 724.

66. D. L. Yabroff et. al., J. Am. Chem. Soc. 1934, 56, 1850.

67. B. Bettman et. al., J. Am. Chem. Soc. 1934, 56, 1865.

68. R. Bean, L. Johnson, J. Am. Chem. Soc. 1932, 54, 4415.

69. J. R. Johnson et. al., J. Am. Chem. Soc. 1938, 60, 111.

70. J. M. Sugihara, C. M. Bowman., J. Am. Chem. Soc. 1958, 80, 2443.

71. J. C. Lockhart, J. Chem. Soc. A, 1968, 869.

72. H. G. Kuivila et. al., J. Org. Chem. 1954, 19, 780.

73. M. I. Wolfrom, J. Solms, J. Org. Chem. 1956, 21, 815.

74. P. B. Brindley, W. Gerrard, M. F. Lappert, J. Chem. Soc. 1956, 1540.

75. E. J. Bourne, E. M. Lees, H. Weigel, J. Chem. Soc. 1965, 3798.

76. R. A. Bowie, O. C. Musgrave, J. Chem. Soc. 1963, 3945.

77. S. A. Barker et. al., Carbohydrate Res. 1973, 26, 33.

78. Koster, R., Rotermund, G. W., Ann. Chem. 1965, 689, 40.

79. Brooks, C. J. W., Watson, J., "Seventh Intl. Symposium on Gas Chromatography and its Exploitation" paper No. 8, Institute of Petroleum, London 1968.

80. G. M. Anthony, C. J. Brooks, et. al., J. Chrom. Sci. 1969, 7, 623.

81. C. J. Brooks, I. MacLean, J. Chrom. Sci. 1971, 9, 19.

82. Frank Eisenberg, Carbohydrate Res. 1971, 19, 135.

83. E. J. Sowinski, I. H. Suffet, J. Chrom. Sci. 1971, 9, 632.

84. R. Greenhalgh, P. J. Wood, J. Chromatogr. 1973, 82, 410.

85. S. W. Jordan, I. S. Krull, "Pittsburgh Conference 1981" paper No. 478, Atlantic City, N.J., 1982.

86. J. E. Lovelock, J. Chromatogr. 1958, 1, 35.

87. C. F. Poole, et. al., Analyst (London) 1978, 104, 82.

88. S. Singhawangcha et. al., J. High. Res. Chrom. & Chrom. Comm. 1979, 2, 77.

89. C. F. Poole, et. al., J. Chromatogr. 1978, 158, 33.

90. C. F. Poole, et. al., J. Chromatogr. 1979, 186, 307.

91. C. F. Poole, et. al., Chromatographic, 1978, 11, 347.

92. Magnus Glad, et. al., J. Chromatogr. 1980, 200, 254.

93. C. F. Poole, et. al., J. High. Res. Chrom. & Chrom. Comm. 1978, 1, 96.

94. Kolb, B., Aver, M., Pospisil, P., J. Chromatogr. 1977, 134, 65.

95. Kolb, B., Bishoff, J., J. Chrom. Sci. 1974, 12, 625.

96. Kolb, B., Aver, M., Pospisil, P., J. Chromatogr. Sci. 1977, 15, 53.

97. Randall C. Hall, Critical Reviews in Analytical Chemistry, page 323, December 1978.

98. W. Seaman, J. R. Johnson, J. Am. Chem. Soc. 1931, 53, 711.

99. D. R. Harvey, R. O. C. Norman, J. Chem. Soc. 1962, 3822.

100. G. Munro, et. al., J. Chromatogr. 1981, 204, 201.

101. E. Sherwood, et. al., J. Chromatogr. 1981, 205, 297.

102. A. P. Goldberg, Anal. Chem. 1982, 54, 342.

103. J. R. Benson, "Pittsburgh Conference 1982" paper 732, Atlantic City, N.J., 1982.

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NITROPHENYL BORONIC ACIDS AS DERIVATIZING AGENTS IN CHROMATOGRAPHY

by

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(ABSTRACT)

The suitability of nitrophenyl boronates as derivatives for GC and HPLC analysis has been studied.

Samples of ortho, meta and para nitrophenyl boronic acids were prepared and purified, the purity of these products was examined by GC. The derivatization performance of these acids was studied employing a number of bifunctional model compounds such as; diols, aminoalcohols, hydroxy acids etc.

The derivatives formed were studied to determine their stability, speed of formation, quantitative analysis and overall chromatographic behavior. The derivatization reactions were found to be very fast and essentially quantitative, reaching equilibrium in just a few minutes at room temperature. The boronates formed are of different stabilities depending on their structure, the single most important stability factor is the boronate ring size, this is followed by the alkyl substitution present and the position of the nitro group on the aromatic ring.

Excellent quantitative performance was obtained with flame ionization and electron capture detectors. Detection limits in the femtomole range were achieved with the EC detector. Solvent background and adsorption problems were found at the picomole level in some

cases.

The applicability of nitrophenyl boronates to HPLC analysis is seriously limited by the column activity due to residual silanol groups, this tends to decompose all but the most stable boronates. However, excellent results were obtained in the case of 2,4-pentandiol boronates, reaching detection limits in the picomole level with a variable wavelength ultraviolet detector.

Nitrophenyl boronic acids can also derivatize ortho disubstituted aromatic molecules containing hydroxyl, amino and carboxylic acid groups. Molecules which contain the reactive groups on the side chain attached to an aromatic ring can present a steric obstruction when reacting with the ortho nitrophenyl boronic acid. This same problem was found in the case of 1,3-propandithiol which reacts only with the meta and para nitro phenyl boronic acids.