# THE SEPARATION, IDENTIFICATION AND QUANTITATION OF TRICYCLIC ANTIDEPRESSANTS UTILIZING A CAPILLARY GAS CHROMATOGRAPH AND HASS SPECTROHETER

by

Robert M. Geidner

Submitted in Partial Fulfillnent of the Requirements

for the Degree of

Master of Science

in the

Chemistry

Program

Steven M. Schildcrout Aug. 13, 1986
Adviser Date

Dean of the Graduate School

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YOUNGSTOWN STATE UNIVERSITY

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#### ABSTRACT

THE SEPARATION, IDENTIFICATION AND QUANTITATION OF TRICYCLIC ANTIDEPRESSANTS UTILIZING A CAPILLARY GAS

CHROMATOGRAPH AND MASS SPECTROMETER

Robert M. Geidner

MASTER OF SCIENCE

YOUNGSTOWN STATE UNIVERSITY. 1986

Many procedures have been developed to separate, identify and quantitate various tricyclic antidepressants such as amitriptyline, protriptyline, doxepin, imipramine and nortriptyline using a gas chromatograph and various detectors. Huch of today's research work is done using fused silica capillary columns because of their increased resolving power due to the large number of theoretical plates. Still, much of the clinical work uses the somewhat outdated packed columns.

A recent study commented about inaccuracies in quantitation of various tricyclic antidepressants by many clinical laboratories (1). Many of these procedures Involve single or multi-step extractione, preparation of derivatives of these drugs and the use of various detectors in order to separate and identify them.

In the present study, state of the art equipment was ured to investigate the factors that might affect the reparation, identification and quantitation of three very

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ಲ್ಲಿ ಇತ್ತರ ಜನ್ನ ವಿಜಯದ ಕ್ಷೇತ್ರವನ್ನು ಪರೀಸ್ ಕ್ಷಾತ್ರವನ್ನು ಸಂತಿಕ್ಷಿತ್ರವನ್ನು ಸಂಕಟಕಾಗಿ ಬೆಂದುಕೊಳ್ಳಲ್ಲಿ ಬೆಂದುಕೊಳ್ಳಲ್ಲಿ ಪ್ರಶಸ್ತಿ ಪ್ರಶಸ್ತಿ ಪ್ರವಿಧಾನಕ್ಕೆ ಪ್ರತಿ ಪ್ರಶಸ್ತಿ ಪ್ರತಿ ಪ್ರಶಸ್ತಿ ಪ್ರಶಸ್ತಿ ಪ್ರತಿ ಪ್ರತಿ ಪ್ರಶಸ್ತಿ ಪ್ರಿಸಿ ಪ್ರಶಸ್ತಿ ಪ್ರತಿ ಪ್ರಶಸ್ತಿ ಪ್ರಶಸ್ತಿ ಪ್ರಶಸ್ತಿ ಪ್ರವಸ್ತಿ ಪ್ರಶಸ್ತಿ ಪ್ರಶಸ್ತಿ ಪ್ರವಸ್ತಿ ಪ್ರತಿ ಪ್ರಶಸ್ತಿ ಪ್ರವಸ್ತಿ ಪ್ರಶಸ್ತಿ ಪ್ರವಸ್ತಿ ಪ್ರವಿ ಪ್ರಶಸ್ತಿ ಪ್ರವಸ್ತಿ ಪ್ರವಸಿ ಪ್ರವಸ್ತಿ ಪ್ similar tricyclic antidepressents: amitriptyline,
nortriptyline and protriptyline. Thir was accomplished by
using the many advantages of capillary gar chromatography
(GC) combined with mass rpectrometry (MS) for increared
rerolution of complex mixtures and the detection of nanogram
levels of the individual drugs. In order to separate and
identify these three antidepressants, standards of varying
concentrations of each drug and mixtures of the three drugs
were run to obtain their optimal GC conditionr. In order to
further increase senritivity, samples were analyzed using
methane chemical ionization and selective ion monitoring.
Then many of the GC/MS parameters were varied in order to
see their effects on reparation and quantitation.

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#### **ACKNOWLEDGEMENTS**

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I would also like to thank my family and friends, especially my wife, Mary, and my children, Christopher, Nicholas and Megan for their constant unselfish support and patience.

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#### CHAPTER I

#### INTRODUCTION

#### Biochemical and Pharnacoloaical Background

most widely used clarser of psychotropic drugs for the treatment of endogenous depression. This type of depression is hypothesized to be the result of a genetic-biochemical abnormality affecting a person's ability to cope with stress. Depressive illness can be divided into two groups, depending on which neurotranrnitter is deficient:

- 1. Norepinephrine
- 2. Serotonin

Norepinephrine

Fig. 1. Chemical structure of Norepinephrine and Serotonin

#### CHVLLEE I

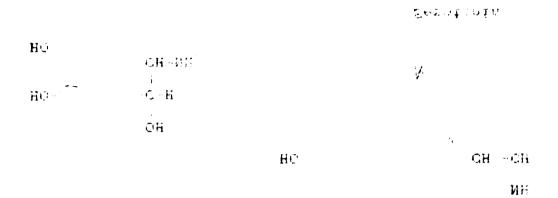
### INTECOURTEIN

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This finds antidepressants (TMA's) are in afthe mist stadely used places of payabotionic drugs for the treatment of antigenous depression. This type of depression is hypotheological to be the result of a squartiz biochemical abbitimulity officially affecting a person's ability to ence with sixeas. Everyonaive iliness can be divided into two proups, depending on which neurotransmitter is definition.

# 1. Menternaphric

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Each condition can be identified by decreases in the respective breakdown products of metabolism of the deficient neurotransmitter. In carer of norepinephrine-related depressiona, there is a decrease in usinary levels of normetanephrine (NMET), vanillylmandelic acid (VMA), and 3-methoxy-4-hydroxyphenylglycol (MMPG). For those patients suffering from serotonin-related depressione, the breakdown product of serotonin, 5-hydroxyindoleacetic acid (5-HIAA) is found in abnormally low levels in the spinal fluid (2).

Norepinephrine and rerotonin are both products of the body's metabolism of tyrosine; the latter goes through a longer pathway via tryptophan production. There neurotransmitter. carry nerve irpulrer across the synaptic cleft to be picked up by receptors on the neuron cell membrane, at which tire the synaptic transmission is terminated. The most effective action of antidepressants to relieve depression ir to block the uptake of there amine neurotranamitters at the rynaptic cleft, thereby allowing longer perairtence of neurotransmirrion.

It has been shown that amitriptyline giver better response in patients with rerotonin-related depression, while imipramine and its related tricyclic antideprerranta work better in patientr with norepinephrine defects. Since these drugs are usually given over a long period of time it is important to monitor their plasma lavele. This can help to

amperation of the transfer of the same of small of this case here and বুলিলের এইন একলের এইন হল কেন্তা কে কন্তার **হ**ল সেই হল সেই কা ស្គាន់ស្នង ខណៈ សិច្សាស្ត្រាស្ត្រ មន្ត្រី លោក ស្ត្រាស្ត្រីស្ត្រីប្រែក្រុមប្រជាព្រះ ។ ក្នុងសុខ នេះស្រែក 掌軸 表示 "我们也会们就是一个是一个本场的作品,这个实现,是一个人们的现在分词是最高的人们的企业人们的信息 in patients with services retates dopinates the child In the contract of the above that the final course for the contract of ក្រោស មុខ ស៊ី ១២. រួយ "យោក ១៣ ២២% ខេត្ត កា ភាពសេត្ neur therean the er the ernapte of thereby all early ಗಟ್ಟಿಕ್ ಇದ್ದು ಇದ್ದು ಕರ್ರದ್ದರು ಕರ್ಮಕ್ಷ ಕ್ಷಾತ್ರಕ್ಕೆ ಕ್ಷಾತ್ರಕ್ಷಕ್ಕೆ ಕ್ಷಾತ್ರಕ್ಕೆ ಕ್ಷಿತ್ರಕ್ಕೆ ಕ್ಷಿತ್ರಕ್ಷಕ್ಕೆ ಕ್ಷಿತ್ರಕ್ಕೆ ಕ್ಷಿತ್ರಕ್ಕೆ ಕ್ಷಿತ್ರಕ್ಕೆ ಕ್ಷಿತ್ರಕ್ಷಕ್ಕೆ ಕ್ಷಿತ್ರಕ್ಕೆ ಕ್ಷಿತ್ರಕ್ಕೆ ಕ್ಷಿತ್ರಕ್ಕೆ ಕ್ಷಿತ್ರಕ್ಕೆ ಕ್ಷಿತ್ರಕ್ಕೆ ಕ್ಷಿತ್ರಕ್ಕಿ ಕ್ಷಿತ್ರಕ್ಕೆ ಕ್ಷಿತ್ರಕ್ಕೆ ಕ್ಷಿತ್ರಕ್ಕೆ ಕ್ಷಿತ್ರಕ್ಕೆ ಕ್ಷಿತ್ರಕ್ಕಿತ್ರಕ್ಷಕ್ಕೆ ಕ್ಷಿತ್ರಕ್ಷಕ್ಕೆ ಕ್ಷಿತ್ರಕ್ಕೆ ಕ್ಷಿತ್ರಕ್ಕಿ ಕ್ಷಿತ್ರಕ್ಕಿ ಕ್ಷಿತ್ರಕ್ಕಿ ಕ್ಷಿತ್ರಕ್ಕಿ ಕ್ಷಿತ್ರಕ್ಕಿ ಕ್ಷಿತ್ರಕ್ಕಿ ಕ್ಷಿತ್ರಕ್ಷಕ್ಕಿ ಕ್ಷಿತ್ತಕ್ಕಿ ಕ್ಷಿತ್ತಕ್ಕಿ ಕ್ಷಿತ್ತಕ್ಕಿ ಕ್ಷಿತ್ತಕ್ಕಿ ಕ್ಷಿತ್ತಕ್ಕಿ ಕ್ಷಿತ್ tani in inated. Cha fuat Hifeotin - Lotable vi antiprinci in the c कर्मा कुछ कर के के किए प्राप्त हो के किए के अधिकार का कार्य के किए भारत के प्राप्त के किए किए किए के प्राप्त क ស្សាភាពស្តេច ១០ ដូច ១៩៣ ១៩៩៦ សមានសមាន **សមា**ស្តេចប្រើក្រុម ១០ ១៩៩០ ស្រុក ប្រឹក្សាសុសា powder corporation and applications or the checks of the things the loop is restained one of the character the detect of a 15% with Notable of the consultant presents and both to appear to

optimize therapeutic response and avoid toxic side- effects. The importance of monitoring can be realized by comparing therapeutic levels and toxic levels as seen in Table 1. It rhould also be noted that life-threatening cardiac toxicity or reizurer have been seen in cares of plasma concentrations over 1,000 ng/mL (3).

TABLE 1

THE ORAL DOSAGES, THE THERAPEUTIC PLASUA LEVELS AND THE TOXIC PLASUA LEVELS OF AUITRIPTYLINE (AMI), NORTRIPTYLINE (NOR), AND PROTRIPTYLINE (PRO) (4).

DRUG	DOSE (mg/day)	THERAPEUTIC LEVEL (ng/mL)	TOXIC LEVEL (ng/mL)
AMI	50-100	120-250	400
NOR	40-100	<b>50-</b> 150	200
PRO	15-40	50-150	200
	**************************************	<del></del>	

There are three tricyclic antideprerrants to be investigated in thir study:

- 1. Amitriptyline
- 2. Nortriptyline
- 3. Protriptyline

Amitriptyline is a potent antidepressant but maintains a low degree of toxicity. It also has mild side effects which include drowsiness, dizziness, nausea, headaches and hypotenrion. Nortriptyline is very similar to anitriptyline in both rtructure and function, although it does present

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THE OFAL DOLAGES, THE THEPAPEUTIO PLACENT VENALT AND THE TOLLED PLACEMA LEVELS OF ANITEDETALINE (ANI), NOTESIPTALINE (NOTE), AND PROTEIPTALINE (PEO) (4).

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<b>L</b> EO	15 -40	€D~TED	300
NOF	वंस्ट १३०)	<b>2</b> 0-120	Ţ <i>ār</i> )
VHI	21-700	TDO-TEO	400
DEAE	(wat qeb) (wat qeb)	(udyar) EHEEMBEGITT CEAEC	(Datar) LOXIO FEAET

There are throw tricyblic antidepresents to t including test in this polypy:

- 1. Amitripityline
- 2. Montaiptylone
- B. Protribitions

Amitriptyline is a potent antidepressent but meinten in a low degree of temporty. It else has mild side effects which include drowniness, dirminess, nauses, hesdaches and hypotenain. Meitriptyline is very similar to amitriptyline in bith structure and fountiin, although it does present

W.W. = 277

# NORTRIPTYLINE(Pamelor) C1=H21N

W.W. = 263

# PROTRIPTYLINE(VIVACTIL) C19H21N

M.W. = 263

Fig. 2. The chemical structure, chemical formula, molecular weight, and trade name of Amitriptyline, Nortriptyline, and Protriptyline.

rone more serious ride-reactions in some patients that would warrant discontinuing the drug. There would include tachycardia, hypotenmion, confusion, and hallucinations. Protriptyline is a rapid acting drug without the usual tranquilizing properties of other TCA's. It is similar to nortriptylina because of the serious consequences occuring in an overdose due to cardiac involvement (5).

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Cachain
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NORTPIPTYLINE(Pamelor) C. thath h.w. - ges CHOCK ON SHOWN

PROTRIPTYLINE(VIVACTIL) - CasHill N.W. = 163 CH -CH -CH - MH- OR

Fig. 1. The chamical structuri, chenical formuli, polecular wordshi, and trade name of Amitriptifians, Northly Militae, and Protriptyline.

some fmore statemental associations on abre refishing that would wastrant descentinging the drug. These would include tachycardia, hypotension, wastrant, and hallucinations. Protriptyline is a rapid detang drug without the upual tranquilizing properties of other TCA's. It is similar to northiptyline because of the serious concequences occuring northiptyline because of the serious concequences occuring in an everdese due to randiac invelvement (%).

# History of Analytical Methods

Since 1960 there have been nunerour methods developed for the measurement of TCA's. Most of there methods require extraction of the drug from an aqueous biological medium into an organic rolvent. This rxtract can then be analyzed by rpectrofluorometry, thin-layer chromatography (TLC), high performance liquid chromatography (HPLC), radioimmunoassay (RIA), enzyme inmunoasrayr such as enzyme multiplied innunoarsay (EMIT), and gas chromatography (GC). The main criteria for modern toxicological methods are rpecificity, sensitivity, accuracy, reproducibility, time, and cost of equipment and analysir. The following is a review of various modern methods for the measurement of TCA's uring the above noted criteria as quidelines.

An early opectrofluoronetric procedure developed by Moody and colleagues (6) involved a three-step extraction and acetylation before analymir. Thir method war not very rpecific because some of the parent drug's metabolites interfered with the analyrir. In 1978, Kaul and him colleagues (7) developed a method sensitive enough to measure 5 µg/L. This war a very time consuming procedure since it involved reacting amitriptyline with alpha-bromomethylacridine forming a quaternary product.

TLC procedurer have been developed by a number of researchers (8-9). It currently is a mtandard screening (qualitative) method with a sensitivity better than 1 ug/L,

## History of Anglyteese Maried

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An early observation of procedure developed by Mond: and colleagues (b) involved a three atap entraction and accordation before analysis. This method was not very specific because some of the parent drug's method was not very interfered vith the analysis. In 1979, Koul and hus colleannes (3) devaloped a method caronione govern to measure (4) devaloped a method caronione govern measure (4) vail. This was a very tamp containing procedure since it involved reaction interference outs.

The procedures tame been devisions for a rimber of reservables of a reservable of reservable of reservable of reservable of reservable of reservables o

and all resultr may be confirmed and quantitated by other methode.

The use of HPLC for the separation of TCA's was first described in 1975 by Knox and Jurand (10). The HPLC procedures are very similar in extraction method and sensitivity (approximately 1 µg/L) to GC procedures. The advantages of HPLC are its large carrying capacity and that the ranples are not destroyed as with GC. HPLC can also be used with a number of different detectorr such as mass spectrometer(MS) and visible and ultraviolet (UV) detectors.

RIA procedures for tricyclic antidepressants were introduced by Spector and colleagues in 1975 (11). Since then numerous other methods have been described with remarkable sensitivity and precision but with questionable specificity. This is due to crorr reactions with various other tricyclics and their netabolites. The RIA procedures have cone under fire in recent years because of inherent biohazard fectors and are currently being replaced by enzyme labelled methods such as EUIT.

The enzyme inmunoasray procedure. have recently found wide acceptance in the area of toxicology in clinical laboratories. This is because assays such as the EMIT procedures combine the specificity and sensitivity of immunoassay with the convenience, speed, and reproducibility of enzyme measurements.

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TABLE 2
SUMMARY OF METHODS USED TO MEASURE TRICYCLIC ANTIDEPRESSANT DRUGS IN SERUM (13).

METHODS S	SENSITIVITY(ug/L)	SPECIFICITYa	DERIVATIVE
Spec <b>trome</b> tric	100	1+	YES
Fluororetric	5-30	1+	YES
TLC	5	2+	NO
EMIT	3	3+	NO
RIA	0.1-2	3+	NO
GC **	10	2+	YES/NO
GC/MS	1	4+	YES/NO
HPLC /UV	2-10	2+	NO
HPLC/MS	0.5	4+	NO

specificity is graded on a scale of 1-4 or poor-excellent

The EMIT principle is as followrr (1) Drug-specific \_ antibodies are added to the patient specimen. (2)

Antibody-drug binding occurs. (3) The enzyme-labeled drug is added and will occupy any vacant antibody sites. (4) The free enzyme-labeled drug reacts with the substrate,

resulting in coenzyme conversion and an absorbance change which is measured rectrophotoretrically. As antibody-drug binding taker place, enzymatic activity is reduced.

Therefore, only the free enzyme-labeled drug can react with the substrate and coenzyme. Ar a rrrult, the rate of enzyme

<sup>\*</sup> denotes other detector. as noted on above.

SUMMARY OF METHODS USED TO MEASURE TRICYCLES ARRIDELLESDANT DRUGS IN SERVE (13).

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ec ws.	1	र्ग +	XETONO
<b>6</b> € * <i>E</i>	10	5+	VESVNO
RIA	$O\cup A=\mathbb{Z}$	<b>3</b> +	NO
EMIT	3	्री च	NO
TLC	ĉ'	**************************************	МО
Fluckimitrac	2 - 20	1 -	AEE.
Spentiomatri	100	1 -	or year or √or the sales
METHODS	SENSITIVITY (ug t)	SBECIFICIALS	DERIVATIVE

<sup>\*</sup> specificity to graded in a scale of 1-4 or poor-encellent \*\* denotes other detectors as noted in above

The EMIT principle is as follows: (1) Orașe ișceifii: antibodies are added to the patient specimen. (2)
Antibody-droe binding occurs. (5) The engyme-labeled drug is added ari will occupy any vacant antibody sites. (4) The free engyme-labeled drug results with the substrate, resulting in coemiyme conversion and an absorbance change which is measured spectrophifemetrically. As antibody drug binding takes place: engymetic activity in vedecad.
Therefore, only the free engyme-labeled drug can itset with

the substrate and coentyme. As a result, the sate of onzume

activity is directly proportional to the patient's drug concentration. In many of these procedures there ir still a lirited amount of crorr-reactivity to the drug'r own metabolite8 and other very similar drugs (13).

by A. T. James and A. J. Martin with their work in reparating volatile fatty acids (14). They used a packed column in which there is a stationary phase, usually a nonvolatile rolid or a nonvolatile liquid coating an inert rolid, and a mobile (gas) phase. This is a process in which volatile substances are reparated according to their vapor pressures. The higher the vapor pressure of a compound, the greater is its mobile-to-stationary phase ratio and therefore, the farter it will elute from the column (15). The solubility of the solute in the liquid phase is also a minor factor to be considered.

There are two typer of columns presently being used; packed columns and capillary columns. The packed columns contain a nonvolatile solid (stationary phare) and typically measure 6 ft. x 2 mm i.d. Whereas capillary columns will mearure 12-50 m x 100-500 µm i.d. There are three main typer of capillary columns: 1) rupport coated open tubular(SCOT)-a single coating of support material in a liquid matrix; 2) porous layer open tubular(PLOT)-porous layer of support material fixed to column wall; and 3) wall coated open tubular(WCOT)-liquid phare deposited directly upon column

solivity to directly proportional to the portional to the portional equal concentration. In many of the concentration is still a limited amount of cross-reactivity to the drug's byn metabolitas and other invry similar druge (19).

by A. T. James and A. J. Martin with their work in separating volatile fatty acids (14). They used a posked column in which there as a stationary phase, usually a nonvolatile solid or a nonvolatile liqued ecating on an unit, and a mobile (gas) phase. This is a princess in obtain volatile substances are caparated according to their vapor pressures. The mapper pressure at a compound, the greater to its mobile-to-stationary phase ratio and therefore, the faster it will elute from the limin (19). The colubility of the solute in the inquist phase is also a minor factor to be considered.

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The recent development of bonded phare capillary columnr has rerulted in ruch more durable, reliable, and reproducible columns. In there columns, the stationary pharer are polymer chains, ruch am polydinethyleiloxane, that give the column a particular polarity. These polymers are covalently bonded to the rilica surface of the column and are also cross-linked to each other. Thim bonding results in a stationary phase that will not deteriorate due to prolonged exposure to high temperatures or repeated injection of a wide variety of rolvents. There are also aone columns that can tolerate multiple injections of a polar rolvent ruch as water (16).

for the detection of TCA. It war first used for this in 1970 by Braithwaite and Whatley (17). There have been a number of different types of detectors utilized much as flama-ionization, electron-capture, and alkali flame-ionization (nitrogen-sensitive) detectors. There are numerour procedures using these detectorr and all show excellent precision and sensitivity, but the rpecificity is quertionable. This is because all of there detectors measure the total number of ion6 or reactive atomr reaching the detector and do not provide a means of identifying the drug being analyzed except for its retention time.

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Gas ships after as the sest widely used hethod for the dusaction of Mon. It was first used for this in 1970 by Essithasite sty Westiay (17). There have been a first of ordination types of ordinates and shall as first material natural and shall as first ordinates as a shall all three times as search these descriptions and senaltivity, for one apenitable. This is because all if these do so beauty the total numbers of the provide analysis the total numbers of the provide and analysis beauty on the style and to any provide and assert the total numbers of the provide and assert to a the day.

In 1976 Hammer and colleagues ured the combined gas chromatograph/ mass rpectrometer in the datection of nortriptyline (18). The result war a very rensitiva and precise procedure with a greatly improved rpecificity. In this care, the mass spectrometer detects the total number of ions and simultaneously measures the abundance of ion fragmentr according to their mass. Thir fragmentation pattern, which ir due to electron or chemical ionization, ir unique for each drug and can be used in combination with retention times to identify a rpecific drug. Sinca Hammer's work, there have been many improvements in procedures, mass spectrometry instrumentation, and the wide use of capillary columns to give greater reparation and identification of complex mixtures of drugo. Many of the tandem (GC/MS) instruments have been interfaced to computers for improved control of instrument parameterr and data handling.

There her been much research done on developing gas chromatography procedures for TCA's. These procedures have utilized both packed and capillary column, a number of sample preparation technique., and variour detectors. There arm many examples of techniques using packed columns and flame-ionization detectors (19), mass rectroneters (20) and nitrogen-sensitive detectors involving ringle and multi-step extractions (21-22). In recent years, there has bean an increased amount of work uring capillary columns and nitrogen/phosphorus sensitive detectors (23),

with warm investors was some standard that is the content to the con-पुष्ट परिचार के अधिकार के विषय के पूर्व अधिकार कराउने पूर्ण परिचार समाने के लिए

हा जिल्लामा १६८६ स्टब्स्ट स्थल क्रिकाला सङ्ख्या स्टब्स्ट स्टब्स्ट १६८६८ वर्ष និងប្រជាពីគេ ដែលប្រជាពីក្រាស់ថា សេខាស្ត្រី នា ផាគេបានប្រិស្សា អង្គិត សេខាណ្ឌ ១៩៩០ខ្លួលសង្គ្រាស់បន្ទាំ សម្រេចប ·斯尔克尔克克克·克尔克斯 人名意思多尔 克爾 医海绵 医皮肤 医多克 网络克雷斯 人名西格兰人姓氏克里特 经营业额

क्लिप्याच्या । त् १७३०८८७२मा विकासम्बद्धाः कृष्टाः वृष्टाः । इत्राह्मः वृष्टाः । व्यावराध्याम्य प्रवास्त मृत्रास्य मृत्राम् व्याप्तास्य स्वयाप्ता । स्वयास्य मृत्रा विश्वास्य स्वयाः । लक्**मार्**कित भए। शहरत्वेच एम् विरायक्षण **प्र**वेश । एक १८०० क्वारित्व (स्त्र प्रिये) or impart to gave greated appearancement and identification for ាស្មាធុនស្រាក់សុខស្នាស់ បាល់បានស្រែក្រុមសុខសុខសុខសុខ ១២១ ស្ទ្រឹង សុខផ្លែ ២៤២ ១៩ ១១៩៦១១៤៤ ಸಹಧಾಯವಾಗಲು ಧ್ಯಕ್ಷಣ ಎಂದು ಸಚ್ಚಿಕಲಿಥಿಸಲ್ಪಟ್ಟಿಗೆ ಹರಿಗಳುಸ್ಥಾರ ಪ್ರಸ್ತರ ಪ್ರಕ್ಷಣಗಳು (general supple nutition for exceptional sugments per pend to compression of the packars, sidh ta que to alegipon ca chimitat ionipach n' -ೂಗ್ರೌಗೀರ್ಚ್ಟ್ ನಾಪಾರ್ಗನ್ಯಾಗಡೆ ಭರ *ಭಲ್ಲಿಕು ಅನಕ್*ರು ಸೃಧ್ಯ ನರ ಅ**ಚಕ್ಕು**್ತುಗಳು ាល ពេលប្រជាព្រះស្រាស់ ស្រុក ស្រុ

मा देव देवेंच्या देवू (देविका १४ सम्भ १ देवता १६ देव्य देवे १० १० ५) र ರವರ್ಷ-ಪರ್ಮದ ಸಹವರಗಳ ವರ ಶ್ರಾಮ್ಯ ಸವರಾಧಿ ರಹಕ್ಕಿಗೆ ಕರ್ಮ ನಿರ್ದಿಸಿಗಳು ಅಂತಿ ಸಂಘಟನೆಗಳು មានស្វាទីពេល ស្យាត្តាប្រើប្រាប់ ស្ត្រីការកាប់៖ និងប្រាប់ ស្ថាមានការស្ថាទី និង ខ្លួន ស្គ errandaen tonationa en en estadena onmodation trocato in discontra ಕ್ರಾಣಾಯ ಕರ್ನಾಯದ ವಿಷ್ಣಾಗಿಯ ಪ್ರಾಥಾಯದ ನೀಡು ಮಾರ್ಯದ ಕ್ರಾಣಾಗಿಯ ಕ್ರಾಣಾಗಿಯ ಕ್ರಾಣಾಗಿಯ ಕ್ರಾಣಾಗಿಯ ಕ್ರಾಣಾಗಿಯ ಪ್ರಾಥಾಯ ಕ್ರಾಣಾಗಿಯ ಪ್ರಾಥಾಯ ಕ್ರಾಣಾಗಿಯ ಕ್ರ ಶರ ಕಾರಾಣ ಮಾತ್ರವಾಗಿ ಕಾರ್ಯಕ್ಷ ಕರ್ಮಕ್ಷಣಗಳ ಪ್ರಕ್ರೀಯ ಕ್ಷಾಣಗಳ ಮುಂದಿಗಳ ಮುಂದಿಗಳ ಬಿಡ್ಡಾಗಿ ಬಿಡ್ಡಾಗಿ ಕ್ಷಾಣಗಳ ಕ್ಷಾಣಗಳ ಪ್ರಕರಣಗಳ ಮುಂದಿಗಳ ಪ್ರಾಣಗಳ ಪ್ರಕರಣಗಳ ಪ್ರತಿಗಳ ಪ್ರಕರಣಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕರಣಗಳ ಪ್ರಕರಣಗಳ ಪ್ರಕರಣಗಳ ಪ್ರಕರಣಗಳ ಪ್ರಕರಣಗಳ ಪ್ರಕರಣಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕರಣಗಳ ಪ್ರಗಣಗಳ ಪ್ರಕರಣಗಳ ಪ್ರಕರಣಗಳ ಪ್ರಕರಣಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕರಣಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ತಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಣಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಣಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಗಳ ಪ್ರಕ್ಷಣ ರುಪ್ರತಿ ಮಾರ್ಕ್ಯಕ್ಷಾಗಿರುವ ದಿನವಿಗೆಗಳಲ್ಲಿ ವಿಶ್ವ ಗಳನ್ನುಲ್ ಅರ್ವಿಟ್ ತಿಗೆ कार्तर्राच्चा के प्रदेश के के का चार्च का विकास कर किए का का किए किए किए किए किए किए किए की का किए की का किए क विकास कर के किए की लक्षराव ....वेदल्केष्ट्रा एवं एवं इंडर वृद्धः पूर्णं का प्रकृतका वेद्दर १३०० वर्षः

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flame-ionization detectors (24) and mass spectrometers (25). The greatest improvement in renritivity and specificity are the result of the gar chromatograph / narr spectrometer combination.

### Gas Chromatograph and Mass Spectrometer

The potential analytical power of thir combination war first realized in 1957 when Holmes and Morrell crudely coupled the two instruments (26). Mass rpectrometera have three basic function. (1) to vaporize compound8 to be analyzed, (2) to ionize neutral molecular in the gar phase, and (3) to separate and detect ions according to their mass-to-charge ratios (m/z). In the GC/MS inrtrunentr vaporization is accomplished in the oven of the gas chromatograph and maintained throughout the ryrten. Ionization can be accomplished a number of ways; the ones of interest in this rtudy are electron impact ionization and chemical ionization. In electron impact ionization, the sample is introduced to the ion source as a vapor. This vapor ir then bombarded by high energy electron8 producing a positively charged nolecular ion (M\*). The electron energy can be varied but is typically 70-80 eV. The amount of energy required for ionization of most organic compoundr ir 7-13 eV. This ionization energy is the amount of energy needed to remove an electron from the highest occupied molecular orbital. This reaction is represented as follows:

#lementance of the control (24) and meso openiting the The openion in manufacture and specificate are the west, of the gas chrometograph of mass specificate combination.

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needed to remove an electrin from the highest cocupied 7-12 eV. This ithication energy is the amount of energy. energy required for abstration of seat organic contrands is can be marked but he typically 70:00 ov. The amount of positively obarged molecular ten (M+). The electron energy Adbor to their posterided by high coordinate transcriptions. cample to introduced to the ion scurce as a rapor. This chemical confidence. In electron impact forigation, the interest in this study are electron impact ionimation and Ionization man be accomplished a number of ways; the ones of chromatograph and maintained throughout the system vaporimation is accomplished in the oven of the gas made tomobards ratios (m/m). In the GC/MS instruments and (2) to separate and detect ions according to their analyzed. (2) to limize heutral molecules in the gas phase. three bosic functions (1) is veporable deepounds to be coupled the two instruments (26). Mass specifications tays with first realized in 1987 when Hollies and Morrell crudely The rotential analytical provar of this manhination

molecular orbiful. This resofich is represented as follower

Upon ionization, the excess energy may caure the decomposition of M\* to form fragmented ions (F1\*, F2\*, etc). This fragmentation pattern, or mars spectrum, can be used to identify the structure of the original molecule. The mass spectrum can be used for identification since it is like a reproducible fingerprint of the compound. It is these mass spectra and retention times that are used in identifying componento of a complex mixture.

Once the compound has been identified, the relative amount of the compound can be determined and its molecular mass confirmed by the use of chemical ionization. This has been used quite often for quantitation because of its increased sensitivity. In chemical ionization (CI), the sample is vaporized in the presence of a high pressure of reagent gas such as methane or ammonia which is bombarded by electronm. Since the reagent gas is found in excess compared to the mample, it is the reagent gas which is ionized by the electrons. The ionization of the sample is the result of ion-molecule collision reaction. between the ionized reagent gas and the rample molecules. Therefore, ionization is the result of a chemical reaction rather than direct bombardment of electrons. In this study methane (CH4) war used ar the reagent gas. The high concentration of reagent gar results in recombination reactions and further ionization.

Therefore, the following reactions occur during ionization:

April time! Item this bim sit is read which is assisted in the part MIN CONTRACTOR OF STANDARD THE SAME WILL BE CONTRACTOR ភភភភពពីពេល នៅក្រុម ប្រជាព្រះមានស្គាល់ ស្គ្រាល់ ស្គ្រាល់ ស្គ្រាល់ ស្គ្រាល់ ស្គ្រាល់ ស្គ្រាល់ ស្គ្រាល់ ស្គ្រាល់ ស ପର୍ ଜେନ୍ତ୍ରପ୍ରପର୍ଥ । ଏହି ହୁର୍ଗ୍ରେ ଅଧ୍ୟକ୍ତି ଓ କର୍ମ୍ୟର୍ଥ । ଅଧ୍ୟର ଜଣ । ୯୯୯ ଓ ଅଧ୍ୟ THE BOY OF THE CONTROL OF HER STORES OF THE SERVICE OF THE CONTROL OF THE PROPERTY OF THE ាល ខណៈស្ថាល ការាជ្រាធិស្សាយការ ប្រាស់ ១០១៩២ ស្រាល់ការបស់ ១៩ ក្រោយប្រាស់ day friq. ಕ್ಷಾರ್ಟ್-Minister (1911) ಇದಕ್ಕೆ ಸಂತಕ್ಷಣಗಳು ಕಟ್ಟಿಯಿಂದ ಕಟ್ಟಿಗಳು ಕಟ್ಟಿಗಳು ಕಟ್ಟಿಗಳು ಸಂಪತ್ರ ಹಾಡುತ್ತಿದ್ದರು. សត្ថិត្តិស្ថិតិសាស ស្ត្រីមួយ បានប្រជាពលរដ្ឋ ស្រាស់ សមាស្រាស់ សាស្ត្រី ស្ត្រី ស្ត្រី ស្ត្រី ស្ត្រី ស្ត្រី ស្ត្រ per appear on the first of a constant of the first of the first and the first of th ಸ್ವಾಪ್ತಿಗಳು, ಬರುವ ಗರ್ವಶ್ರವ ಸಂಗತ್ತಿಕೆ ಬರುವ ಅಂತರ ಕಾರ್ಯ ಕ್ರಾಪ್ತಿಕೆ ಕಾರ್ಯಕ್ಷಣೆಗಳು ಮಾಡುವುದು ಬರುವುದು ಮಾಡುವುದು ಮತ್ತು ಪ 建铁铁基丁烷 人名英格兰 医克尔氏试验 医二苯酚 化氯甲酚 电电子分离性电路 化二氯化二甲基甲基基酚 化电子电路电路电子 ತಿಕ್ಕೊಂಡಿ ಕೇಳಿದ್ದಾರೆ ಹೇಳಿದ್ದಾರು. ಇತ್ತಿಗಳ ಕೇಳಿ ಬರುಗಳು ಮುಂದಿ ಕೇಳಿದ್ದಾರೆ ಕೇಳಿದ್ದಾರೆ ಕೇಳಿದ್ದಾರೆ. ಅತ್ಯಕ್ಷಿಕ ಕೇಳಿ ಕೇ ಘರಣದ ಕಾರ್ಯಕ್ಷ ತಿರ್ವತನ ೧೯೯೬ ಆ ತಿರ್ಮ ೧೯೯೬ ಕಿಕ್ಕಳಲ್ಲಿ ಅಲ್ಲಾಲ್ ರಾಜಕ್ಕಿತ್ತು ಬರುಗಳು ស្គងល ខាលបា**ក្រស់**មិញ ភូមិ ខ្លាំង ខាងមាន គឺ ខាងម<mark>ែងស្តែការ</mark> ។ ប្រជាពីបាក់ប្រាប់ ប្រើបាន carde to a conformal fee been court to be soon as

$$CH_4 + e^- + CH_4^+ + 2e^-$$
 (2)

$$CH_{2}^{+} + CH_{4} \rightarrow C_{2}H_{8}^{+} + H_{2}$$
 (3)

$$CH_4^+ + CH_4 \rightarrow CH_8^+ + CH_8$$
 (4)

There is also a small amount of the C<sub>2</sub>H<sub>5</sub>+ ions formed during ionization. It is these ionr that react with the sample nolecules (M) to form the (M + H)+, (M + CH<sub>2</sub>)+, (M + C<sub>2</sub>H<sub>5</sub>)+, and (M + C<sub>2</sub>H<sub>5</sub>)+ ions. As a result of chemical ionization the fragmentation that is seen in electron impact ionization does not readily occur and a majority of sample ions are found in the MH+ form.

$$W + CH_{\bullet}^{+} \rightarrow WH + + CH_{\bullet}$$
 (5)

Therefore, with a properly tuned mass rpectoneter the MH+ ion concentration in the CI mode of operation may be ten times greater than the M+ ion in the EI node of operation. This in effect causes a ten-fold increase in sensitivity.

Once ionization is completed, the mass spectrometer must separate and detect the ions according to the mass to charge ratio (m/z). There are two principal methods for separating the ions to be discussed at this time. The first utilizes electrical and magnetic fields and the second user a quadrupole mass filter. The first magnetic-rector mass spectrometer, in which positive ions are deflected 180 degrees in a aagnetic field, was used by A. J. Denpster in 1918. The ions leaving the ion rource all have the same kinetic energy

$$KE = 1/2 \text{ av}^2 = \text{zeV} \tag{6}$$

$$CH^{*} + SU + CH^{*}U + TSU$$
 (3)

$$0.6\% \times 0.2\% \times 0.5\% \times 0.5\% \times 0.0\%$$

$$CH_4 \leftarrow CH_4 \rightarrow CH_3 \rightarrow CH_3 \qquad (4)$$

There is also a small amount of the  $C_3H_B$  liens formed during ionization. It is there ions that react with the sample milecules (H) to form the (H + H)·, (M +  $C_2H_B$ )·, (M +  $C_2H_B$ )· ions. As a result of chemical ionization the fragmentation that is seen in electron impact ionization does not rightly occur and a majority of sample ions are found in the MH· form.

$$\mathbf{N} + \mathbf{C}\mathbf{H}^{\mathbf{m}_{+}} \rightarrow \mathbf{N}\mathbf{H}_{+} + \mathbf{C}\mathbf{H}^{\mathbf{m}_{+}} \tag{2}$$

Therefore, with a properly tuned mass spectomet r the Mition concentration in the CI mode of operation may be tentimes arouter than the M+ ion in the EI mode of operation. This is stifust causes a ten-fold increase in sencitivity.

Must appoint and dotted the tone scronding to the mass to change taking forthand taking for the mass to change taking (n/m). There are two principal methods for separating the tens to be discussed at this time. The direct utilizes electrical and magnetic fields and the second used a quadrupole mass filter. The appet magnetic sector mass quadrupole mass filter. The appet magnetic postor mass discrimeter, in which resisting ions are differed lost digners and a magnetic field, was used by A. O. Dempster in 1910. The ions leaving the ion course all have the came winetic energy

where m is the mass of the ion, v is its velocity, z is tho number of charges on an ion, \(\begin{array}{c} \pm \section \\ \pm \end{array}\) the magnitude of the electronic charge, and V is the electrical potential. As ions enter the magnetic field they are separated according to their momentum and each m/z will have a unique radius of trajectory, R. The heavier ions have more momentum and are therefore influenced less by the magnetic field. This is seen by a flatter trajectory, whereas the lighter ions are nore strongly influenced and follow a nore curved trajectory. Finally, the ions must pass through a focusing slit to reach the detector. Thus, at a given magnetic field, B, only ions of a single m/z ( $m/z = B^2R^2/2v$ ) will have the proper trajectory to reach the datector. By varying the magnetic field strength, a wide range of m/z values can be scanned to be focused at the detector slit. This type of mass spectrometer is limited to a scan rate of 0.1 s/decade for the type of magnets available. A mass decade representsa ten-fold increase in mass, for example, 50-500 anu. A time of approximately 0.2 a is needed to reset the magnet between scans. Therefore, the total scan tine is 0.3 s.

The quadrupole **mass** filter consists of four rods carrying variable combinations of both radio frequency (RF) and direct current (DC) voltages. **Ions** interact with the electrostatic forces created by the rods after they are accelerated towards the detector. By controlling the DC-to-RF ratio, an electrical field can be established so

क्षेत्रक रिकार्यः अवद्यापन इतः विश्वप्रकृषावद्यारकारः । म् इतिदश्या वृद्यवृद्यः मृद्यवृद्ये वृद्या । रहितः । The quadrupole uses talter concists of fear solds sound. Therefore, the rotal good tupe have la a ರಕ್ಷ ಗಳಕ್ಕರುಗಡುತ್ತದಲ್ಲಿ 300 ರ. ಕನ್ನಡಗಾಗುತ್ತದೆ ಕಥ್ಮನಿಗಡಕ್ಕೆ ಕರ್ಮಿಸುತ್ತಿಗಳು a territia ... cesad in bira, for evappe, I.-TUN abu. A time To got the fire of magnoto avertants. A note donate representia mess appearant to the line for the second of agent mater of the adequade specimed to be focused on the detector ribble (give time as magnetic finid ctrangth, a widt range to red values can be the dreath trajectory to essent the determent by yerrang the ಕ್ಷಕ ಸಮ್ಮಣ. ಇದರ್ಶನ ರಕ್ಷ ತಂಡಕ್ಷಣಕ್ಕೆ ಹಂದ ಕಂಪ ದೇವಿ ಅವಿಕಲ್ಪಡೆದು ಗಳಿಸುತ್ತಿ №್ರಿಸಿ ally to reach the detector Thur, at a quien magnitud field, trendentary. Finally, the tent numb page through a feculaina more strengly influenced and desire a work recoved scen by a flatter trajectory, whenced the lighter cont and the store influenced less by the means'in built. This is traccontory. By The beginter lend have none mementum and and to their minerium and each was will have a unique radius of tunn enter the mounetim tield ther are separator errorating eladicining phange, and V is the electrical potential. Acthe best of charges on in tent is no the required of the where he is the mage of the ion, he is its velocity, in it the

manufilmy manusata nonthinations of hath reduc frugations of 68%; and darker manusant (23) mailingan. Land incarate right the clockreatering formes areased in the made arear they are areastered toward the despitation for a principle of the formes are area freely are formed to a contain the first particular and the formed areas are also are areas from the formed are areas are also are areas from the formed are areas and the formed are areas are also are are are areas and are areas are also are are areas and are areas are also are areas are also are areas are also are are also are are also are are also are are areas are also are are are also are are areas are also are are areas are also are areas are also are also are are also are are also ar

that ions of only one specific m/z value can pass through the mass analyzer, whereae almost all other m/z value ions collide with the rods and are neutralized. If the RF and DC amplitudes are simultaneously varied, a wide range of m/z value ions can be analyzed by the detector. The mass scanning range of the quadrupole mass filter is approximately 10-800 anu. A maximum scan rate of 780 amu/s can be reached before there is a significant decrease in resolution, peak shape, and intensity. This type of instrumentation is especially useful when a capillary column GC technique is performed. Capillary columnr with up to 10<sup>6</sup> theoretical plates can resolve on the order of ten peaks/s, especially early in the chronatogram. Therefore, in order to get reasonable reconstructed ion chromatograms. RIC, (graph of total ion current versus time) under these conditions, a scan rate of 100 scans/s is necessary (27-28). The conditions noted above are extremes and are beyond the capabilities of the instrumentation used in this study. The specifications of the Finnigan Model 1020B and capillary column used in this study are noted in the materialo-and apparatus section of this study.

column used in this study are not of in the materials and specifications of the Finnigan Model 100.5 and capillary capabilities in the inctrumentation used in this utudy. The The monditions noted above are intrames and are beyond the is than take of 100 shans's is necessary (27-28). condition. RIC. (graph of total ion current "ersus time) under these order ': get resconstle reconstructed ich chromatograms, pesting, especially e . In the chromatogram, Therefore, in 10° theoretical plates can resoure on the order of ton GC technique is performed. Capillary columns with up to instrumentation is especially useful when a dapillary dolumn respinition, pash shape, and intensity. This type of can be reached before there is a significant decrease in approximately ideaded amu. A maximum acan rate of 730 and/a scanning lange of the quadrupole mass filter is value into pair be analyzed by the detector. The maga  $\mathbf{S}^{-1}$  is the standing standard varied, a wide range of  $\pi/\pi$ a illiam with The rods and are neutralized. If the RF and DC the mass anathran, thereas almost all other miz value lane that tone of only one opecifie m m raise can pass through

apparatus section of this study.

### CHAPTER II

#### PURPOSE OF STUDY

It has been noted in a previous study that many clinical laboratories doing work in toxicology have great difficulty in the detection and quantitation of nunerous drugs (1). It is the purpose of this study to develop the optimum operating criteria for the separation, identification and quantitation of three very similar tricyclic antidepressants (AMI, NOR, and PRO) utilizing the Finnigan Model 1020 GC/MS with a capillary column. I have chosen to do this work using standards and not to deal with drug extraction procedures themselves because of the vast amount of work done in this area and due to the extra equipment and time necessary. It is also my intention to use available equipment such as a capillary column and an injector liner with as little pretreatment as possible to make clinical adaptation easier and less time consuming.

I will also attempt to thoroughly investigate as many variables or adjustable instrument parameters as possible to see their effects on the procedure. Therefore, the scope of this study is two-fold, to investigate the problem of developing an analytical procedure and to understand the capabilities of a state-of-art GC/MS instrument.

## CHVOLES II

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### CHAPTER III

### UATERIALS AND APPARATUS

All reagents were analytical grade including methanol supplied by Fisher Scientific Co. (Orangeburg, N. J. 10962) Methane, the reagent gas, and Helium, the carrier gas, were ultra-high purity grade supplied by Airco Products. The tricyclic antidepressants were supplied by Youngstown Osteopathic Hospital and purchased from the following companies: amitriptyline HCl (Elavil) and protriptyline HCl (Vivactil) from Merck Sharp & Dohme (West Point, PA 19486); and nortriptyline HCl (Pamelor) from Sandoz Pharnaceutical Div. (East Hanover, N. J. 07936).

All analyses were performed on a Finnigan Model

10208 Automated GC/MS which is computer interfaced for
instrument parameter control and data processing. In this
study the chemical ionization (CI) ion source war used, as
opposed to the electron ionization (EI) ion source, in order
to give optimum results during chemical ionization -procedures while allowing the flexibility to perform
electron ionization without a large loss in sensitivity. The
ion source is the actual enclosed compartment where
ionization occurs. The column is a fused silica capillary
column measuring 15 m X 0.25 mm I.D. with a polymer
stationary phase of polyvinyl- phenylnethylsiloxane (SE-54)

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with a film thickness of 0.25 µm. It was purchased from J & W Scientific, Inc. (Rancho Cordova, CA. 95670).

with a film thickness or 0.2% um. It was purchased from 0.6 W Ententific, Inc. (Fancto Orrdova, CA. 25670).

### CHAPTER IV

### **EXPERIXENTAL**

The drugs were ured in their hydrochloride salt forms and were dissolved in methanol to obtain stock rtandards of a concentration of 1 µg/µL for both anitriptyline (AMI) and nortriptyline (NOR) and 0.5 µg/µL for protriptyline (PRO). This was done so that approximately 1 µg of sample could be injected onto the column in a rrall solvent volume without overloading the column. The Finnigan Model 10208 GC/MS war zeroed and calibrated daily according to the manufacturer's specifications. A nanual tune was also carried out daily in order to adjust the instrument to obtain optimum resolution and intensity of the ions of interest in the range of 40-350 atomic mass units (amu). Anexample of the Finnigan computer syrtem's typical calibration report and the nanual tune settings are seen in Figures 3 and 4, respectively.

The standards and dilutions of the standards were ured to run analyrer in the electron ionization and chemical ionization modes to obtain reconstructed ion chromatogram and mass spectra of each drug standard. The mass spectrum of each drug was confirmed by comparing it to the electron ionization work by T. Mills et al (29)

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## CALIBRATION REPORT:

- 18 = LOWEST PEAK IN REFERENCE TABLE FOUND
- 614 = HIGHEST PEAK IN REFERENCE TABLE FOUND
  - 21 OF 21 REFERENCE PEAKS WERE FOUND
  - 1% OF PEAK WIDTH = RUS FIT ERROR
    ( RUS FIT ERROR <10% ACCEPTABLE)

Figure 3. An example of a calibration report

1.	ELECTRON	HULTIPLIER	SWITCH	ON
2.	ELECTRON	UULTIPLIER	VOLTAGE	-2200.00
з.	HIGH RESC	LUTION		133.00
4.	LOW RESOL	LUTION		125.00
5.	ION ENERG	}Y		2.51
6.	FILAMENT	SWITCH		ON
7.	ION PROGR	AM		3.22
8.	LENS VOLI	AGE		-166.00
9.	EXTRACTOR	1		2.00
10.	ELECTROHE	TER RANGE		7.00
11.	ELECTROHE	TER ZERO		2.05

Figure 4. Example of the typical manual tune settings

There mass rpectra are seen below in Figures 5-7. A mixture of the three drug etandards was then run in order to assure that separation was possible with the SE-54 capillary column. These standards were analyzed using the ieothermal

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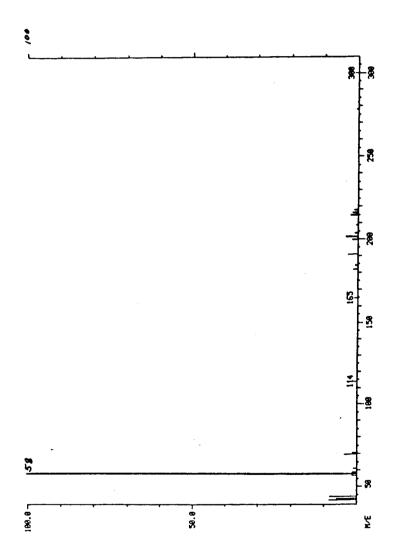


Figure 5. Reference EI Mass Spectrum of Amitriptyline from the work by T. Mills III.

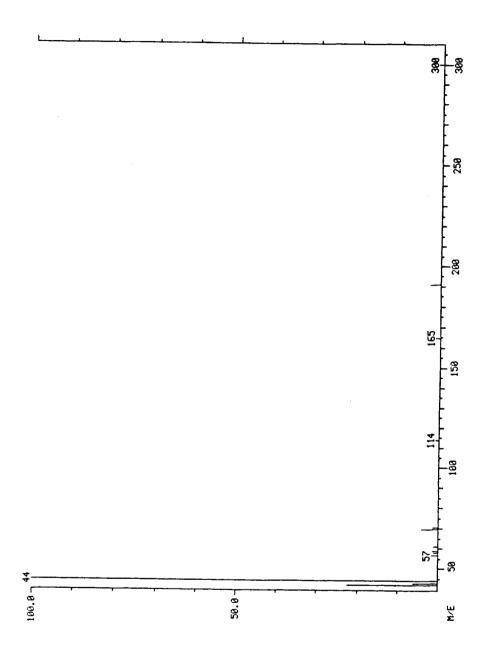


Figure 6. Reference EI Mass Spectrum of Mortriptyline from the work by  ${\tt T.}$  Mills III.

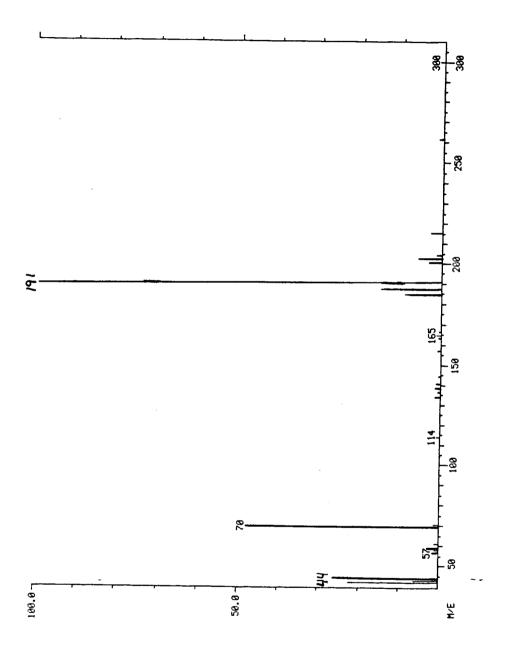


Figure 7. Reference EI Mass Spectrum of Protriptyline from  ${\mbox{\tiny -}}$  the work by T. Mills III.

## GC PARAMETERS:

```
250 deg. = Injector Temp. *

150 deg, = Initial Temp. *

220 deg. = Final Temp. *

1 min. = Initial Time *

5.0 D/H = Ramp Rate (deg/min) optional *

10 nin. = Final Time *

250 deg. = Separator Temp.

80 deg. = MS Manifold Temp.

INJECTION MODE: CAPILLARY

60 sec. = Split/Sweep Valve Time

90 sec. = Filament/Multiplier Off Time

SCAN FROU 40 AMU TO 350 AMU IN 1.0 sec. *
```

The separator is the area where the GC connects with the MS. A temperature higher than the final tamp. is needed to prevent condensation.

The manifold is the structure which houses the ion source, quadrupole analyzer, and ion detector.

Figure 8. An example of the typical GC/MS conditions

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### CHAPTER V

### RESULTS AND DISCUSSION

## Electron Ionization

The problem presented here is the separation of three tricyclic antidepressants, very similar in both structure and molecular weight. Uany procedures involve derivatization techniques to increase resolving power and sensitivity (30-31) but these problems should be eliminated by using capillary GC/MS. So, as in the work of Chinn et al (32) and Vinet (33) underivatized TCA's were used. First the etandards were analyzed in a programmed temperature node as seen in Figure 8. The data from these individual runs can be seen in Table 3. Thia phase of the analysis resulted in small but rather wide peaks in the reconstructed ion chromatograms (RIC) of all the drugs. The computer enhancement showed well-defined peaks with a slight amount of tailing resulting in excellent sensitivity for AMI and NOR and slightly decreased sensitivity for PRO. The sensitivity was determined by comparing peak height to baseline and background noise. The mass spectra for AMI, NOR and PRO can be seen in Figures 9-11 and all of these spectra correspond to the results seen by T. Mills III et al (30).

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# RESULTS AND DISCUSSION

## Electron fortration

The problem presented here to the separation of three tripyodite entidepressants, very similar in both structure and molecular hereby. Many promedures involve seasoning to increase restlying power and sensitivity (1973) but these problems chould be eliminated by using partillary 0.700, Set, as in the work of whith <u>st</u>

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Tomperators mode or seen in Figure 1. The data from these

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### TABLE 3

## DATA FOR EI OF INDIVIDUAL DRUG STANDARDS

DRUGS	Retention tine (min: sec)	Intensity* (units)	Sensitivity (ng)
IMA	2:34	8608	20
NOR	2:35	19680	30
PRO	2:45	7200 <b>°</b>	50

■ Intensity is measured by arbitrary units to compare the anounto of iono reaching the MS detector.

■ PRO's intensity value was doubled for comparison purposes because ita concentration was half of AMI's and NOR's.

Figures 9-11 also show how there fragmentation patterns in the mars spectra occur as a result of electron ionization.

Note that the molecular ion has a very low abundance in each case. It rhould also be noted that some of the primary ion fragment8 are further fragmented and are not seen in the mass rpectra.

## TABLE 3

DATA FOR EL OF INFIVIDARE DAUG ATARDAEL

F-E-1-23	Fatention time (min:sec)	Intensity <sup>a</sup> (unite)	Semnitivity (mg)
AMI	2:34	8808	54
NCE	7:38	19600	240
PR0	2:45	2.00 ps	<u>ē</u> 0

Intencity is accounted by arbitrary unity to compare the amounts of tone reading the MS detactor. PRO's intensity value was equal for compartion furposes because the compartion of hold of AMI's and NGF's.

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Figures 9-11 alor show how these firsprontation pattern in the mans sporting occur as a recult or electron ionization.

Note that the molecular ion has a roun law obundance in each case. It should sisp be noted that are of the ordering fragments are further first anented and six not seen in the mass sporting.

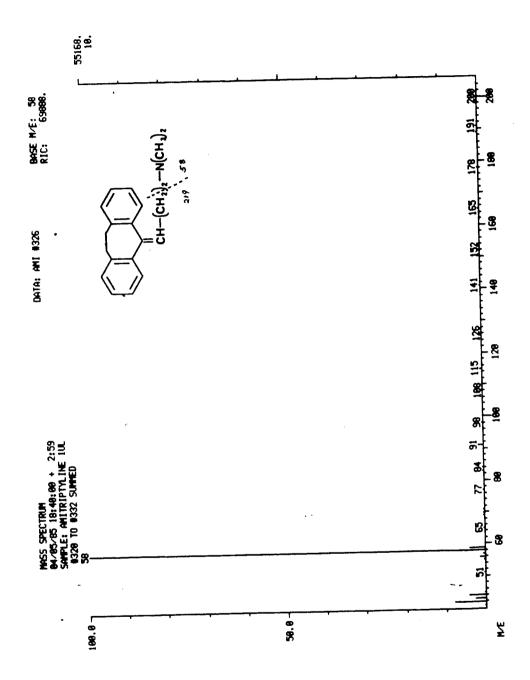


Figure 9. EI Mass Spectrum and Molecular Fragmentation of  ${\tt AMI.}$ 

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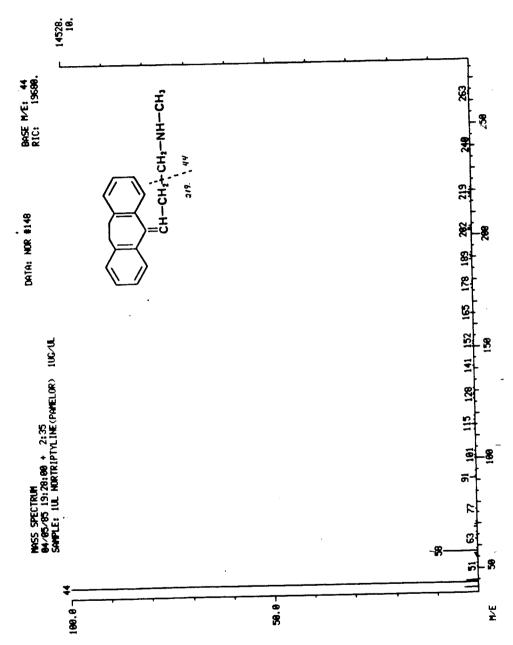


Figure 10. EI  ${\tt Mass}$   ${\tt Spectrum}$  and Holecular Fragmentation of NOR.

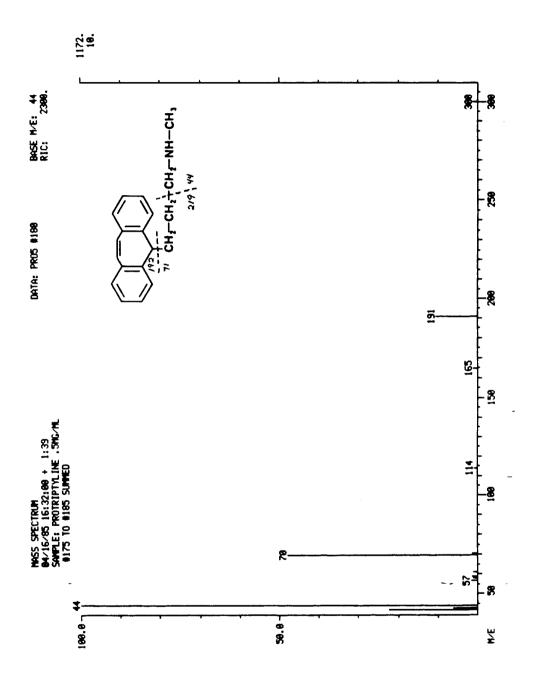


Figure 11. EI Mass Spectrum and Molecular Fragmentation of PRO.

Figure 11. El Mass Spectrus and Moscrus. Fragmentation in PSO.

### Chemical Ionization

In an attempt to improve the **sensitivity** of the individual **drugs, they** were analyzed by chemical ionization (CI) utilizing methane as the reagent gas as was done by Jenkins and **Friedel** (34). To determine the methane pressure to be used, mass spectra were obtained for the methane alone. This was done by varying the methane pressure by 0.1 torr increments from 0.0 torr to 1.0 torr (instrument manufacturer's recommended reagent gar range), and the results can be seen in Figure 12. It was by thie means that

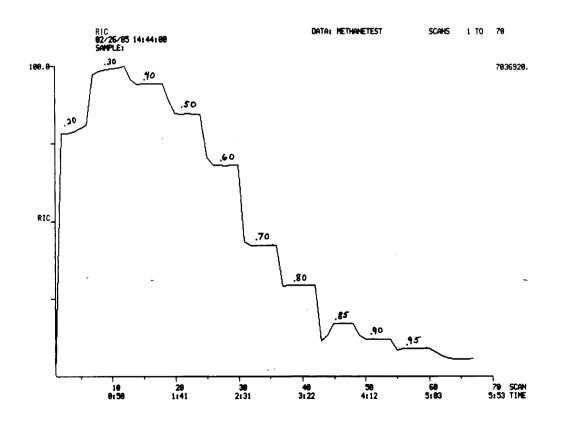


Figure 12. The total ion intensity of methane (the reagent gar). The pressure in torr 1s indicated at each level.

it war determined that the maximum concentration of the reactive CHs+ ions occurs at 0.4 torr and, therefore, this is the optimum methane operating pressure. The following changer in the GC parameter. were made in order to get the desired sensitivity and peak sharpness.

- 1. INITIAL TEMP. **150** deg.
- 2. RAMP RATE = 10.0 deg/min.
- 3. SPLIT/SWEEP VALVE = 60 sec.
- 4. FIL. /MULT. OFF TIME = 90 rec.
- 5. SCAN FROM 150 TO 350 AMU IN 1.0 sec.

The split/sweep valve tine is the amount of time that the valve is initially closed to allow all of the sample to get onto the column. The results are noted below in Table 4 and ar can be seen there war a vest improvement in the relative intensitier and sensitivity of all three drugr. The

TABLE 4

DATA FOR CI OF INDIVIDUAL DRUG STANDARDS

DRUGS	Retention time (min: sec)	Intenrity (units)	Sensitivity (ng)
IMA	5: 44	14944	10
NOR	5:53	11264	20
PRO	6:19	16480	30
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intensities for NOR and PRO, because of the broadness of their peaks, reflect the areas under the peaks. It must be remembered that the intensity referred to here is a relative term and will vary depending on the manual tune settings and other instrument conditions. The chemical ionizaton mass mpectra of AMI, NOR and PRO are seen below in Figuree 13-15. It should be noted that the ratio of MH+ to M+ is approximately 10 to 1. This agrees with statements made by the nanufacturer on CI results. The (M + C2H2)+ ions and (M + C<sub>2</sub>H<sub>2</sub>)\* ions are seen in the mass spectra, but (M + CH<sub>B</sub>)\* ions are not detected by this MS technique. This is because nearly all of the CHs+ that reacts with M forms MH+ and CH<sub>4</sub> as in reaction 5. It is this MH+ ion that remultr in the base peak in each of the cases. At this methane pressure some fragmentation of the drug molecule occure. This results in the detection of many characteristic mass peaks that can be umed to confirm the \_ identification of the analyzed drugs. So under these conditions, there are three criteria for identifying a ruspected drug (1) retention tine, (2) the rolecular weight of the compound am obtained from the mass of the MH+ ion, and (3) the nolecular fragmentation pattern.

A standard drug mixture of AMI, NOR and PRO, each with a concentration of 250 ng/µL, was analyzed using the same GC parameters am noted above. Since the split/sweep valve off-time is 60 sec the injection is splitlers and the vast majority of the sample reaches the column.

ក់ខ្លួនគ្រប់ប្រកស្លាប់ គ្នា ខ្លួម បានប្រជាជា ពិធីខណ្ឌគាល់ «ជាជាជាប្រកា នៅ សស្សស្រាល់ ឬ ស្ថាល់ការប្រជាពុធ្យាល់ សម្រាល់ ស្រែងការប្រជាព្ធិស្សាល់ ដែលប្រជាពុធ្យាការប្រជាពុធ្យាការប្រជាពិសាធា ក្នុង ២០ ក្រុម្ភាពសង្គមសង្គមសង្គម ប្រជាជនធ្វើ កន្លាំងនៅការប្រជាជនសាក្សា ប្រើប្រឹក្សា បានប្រឹក្សា បានប្រឹក្សា បានបានប ನಾರು, ಆ ಜನವಾರವಾಗಿಗಳು ಇತ್ತು ಪತ್ರೀಕಾಗಿಗೆ ಅತ್ಯಾಗಿಸುವ ಪ್ರತಾಣಕ ಕಣ್ಣ to contemporary graph programs of AME, MEE, wherefore the abli file tiv postivusas dragnami taon patitika to the rearrant to seather from the man in a line off the conor solve, the state  $m{x}$  ,  $m{x}$ ಪುರವುದ್ದರ್ವರ ಮುಂಡಲ ಕೃತ್ರಗಾಗಿ ಈ ಅವರ ಎಲ್ಲರ್ಗಳ ಅಪ್ರಭಾಷವಾಗಿ ಕ್ರಾಮ್ ಸ್ಥಾಮಿಕಿಗಳು ಕ್ರಾಮಿಕಿಗಳು ប្រធានពេលប្រជាពលរដ្ឋ ដែលប្រជាពលរដ្ឋ និង ប្រជាពលប្រធានក្នុង ប្រជាពលប្រជាពលប្រជាពលប្រជាពលប្រជាពលប្រជាពលប្រជាពលប្ ្សាមស្ថានពេទ្ធស្ថាននេះគេ ស្មាន។ សមន្ទាល់ ខេត្តពុល ព្រះស្វាស់ លេខស គ្គ សុស ពេល សេ ខណៈពេធិបានគេ នាប w into the resurred Than recoling an the detablish of hing ្សារ ២ ស្នះស្រុងមេខ ២០ខេស្ស ១០១០ ២០១២៩សុខ១០ ១៦ ខ្យុង ១០១៦ हर्म द्वारिक व्यक्तिहरू हरू द्वार प्रतिस्थिति विकास एक राज्या है, द्वार एक्टर अस ស្ពាររស់ស្ត្រាក្រុម ទី២៤ ស្ត្រី និង ១២ ១៩៥១៩៩២២ ភូមា ភូមា ១៩ ១៩១១ ភូមិ ១៩ រួម្នាក់ ការ គ្នាក្នុងក្រុម សមានរៀវ ការប្រជាជ្រួល ស្រុក ប្រជាជ្រួល ស្រុក ស្រុក ស្រុក ស្រុក ស្រុក សា (n. . )54%). ಇರಚಿತ ತನ್ನ ಚರ್ಧ ನಂಬರಗಳಾಗಿ ಕಟ್ಟಳಾಗ ಅಭಿಕ್ರಗಳನ್ನು ಗಳಿಗಳು ಕ್ರಾಂಡ (ಸ್ವ. ಕ. ೧೯೯೯), ಗಂಟಕ ಕ್ಷಾಂಡ ಸಂಪರ್ಚ ಸರ್ವೇಶಕ ೧೯೮೦ ಪರ್ವೀಸಿಸುತ್ತುವರು ស្សាល់ អភាម្រាញ់គ្នុន «សាក្សា នាម សូΣ ស្សាល់ពីនេះ «សាស្សា ស្គ្រាស់ ស្ត្រីពីនៅស្រុស សុសាល់ ಇಕ್ಕಳ ಸರ್ವಧಕಾರು ಸಿದ್ದಿ ಸರ್ವಿಸ್ತರ ಕ್ರಾರ್ಥಕ್ಷ ಅಭಿಕೃತ್ತಿಗಳ ಸರ್ವಿಸ್ತರ ಸಂಪ್ರವರ್ತಿಗಳ ಸರ್ವಿಸ್ತರ ಸರ್ವಿಸ್ತರ ಸರ್ವಿಸ್ತರ ಸ in teraph be proted that the cated as Marchall and sto and an high Man aut big dark som forell for all bilder in the ನಿಕೃತ್ವ ಗಟ್ಟಾತ್ರವು ಅರ್ವ ಸರ್ವಾಪ್ರವಾಗಿ ಕಾರಣ ಮತ್ತು ಕಾರ್ಯಕ್ಷಕ್ಕೆ ಕಾರ್ಯಕ್ಷಕ್ಕೆ ಮಾಡಿದ್ದಾರೆ. ಅರ್ಥನಿಗಳು term und mall mest depending on the Rendul mine pethings du the ស្រាស់ក្រោយពីស្រាឡា (2) ខណ្ឌ (ស្រាស់ សំពេករបស់ (រណៈ) កែកម្មាស់សេងជា (របស់ ស្រាស់មា សំគេ ១ ឧកខ្ពស់ (បស ស្នាមការ ជានារាស្រី បានអ្នកលេខ ស្ពាល បានគេរប់ គ្នាមានការ ខេត្ត ខេត្តប្រាប់ ភិជ្ជា មិនិទី១ ១៤

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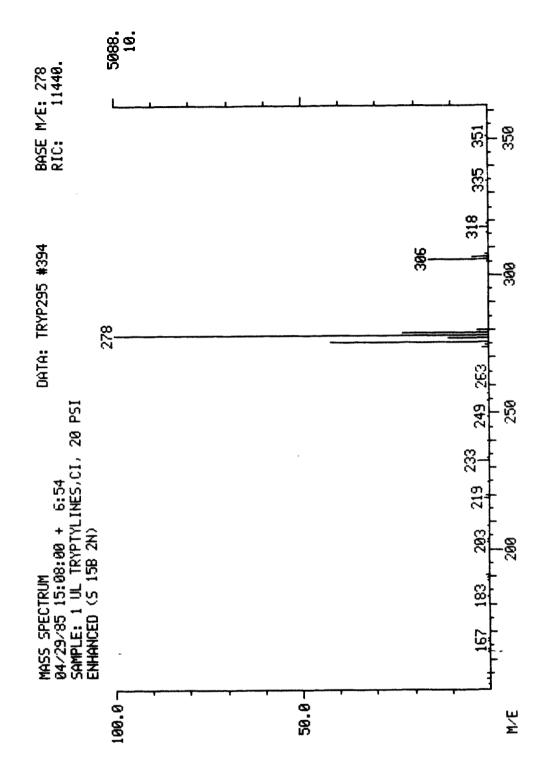


Figure 13. Chemical Ionization Mass Spectrum of AMI.

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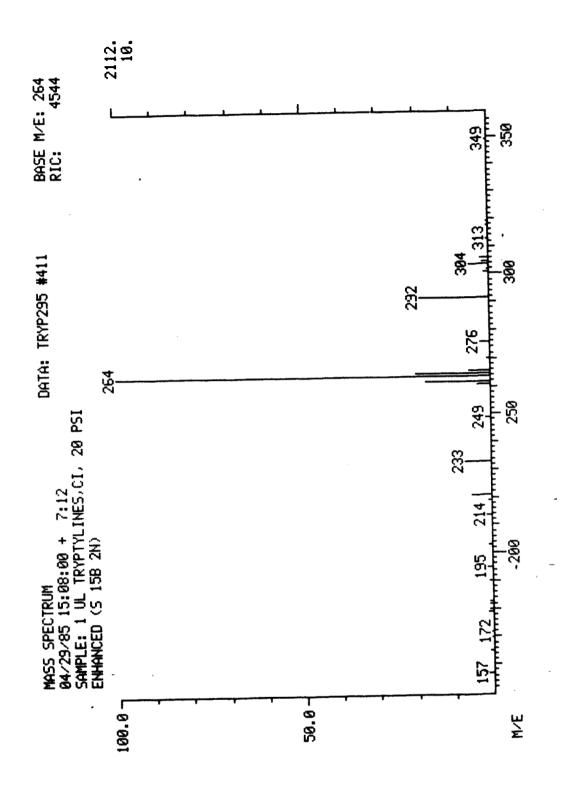


Figure 14. Chemical Ionization Mass Spectrum of NOR.

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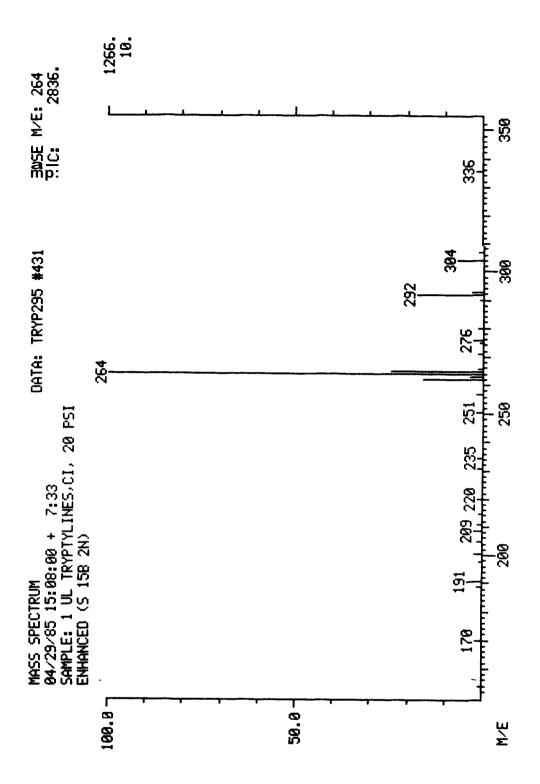


Figure 15. Chemical Ionization Mass Spectrum of PRO.

Figure 17. Chemista Isminstar: Mass Specimum et 77.

The RIC for one of the samplings is **seen** in Figure **16**, and it shows excellent **resolution**, **intensi**ty, and **sensitivi**ty. The statistical information for **this** analysis is seen in Table **5**. It should also be noted that the **intensities** in

TARI	F	5			

Data for Chemical Ionization of Drug Mixture

Drug	RT :	Intensity(Ht/A)	Rerponse Factor*
AMI	6: 56	44032/176496	0.85
NOR	7:13	23872/151524	1.00
PRO	7:36	18784/ <b>57925</b>	2.60

The peak aroa intensity value was urad to calculated the response factor

Table 5 are expressed in terms of peak height (Ht) and peak area (A). The response factors (Rf) seen in Table 5 are used in the quantitation of the drugs in an actual analysis. The response factor value is determined by comparing the instrument's ability to detect various substances of equal or known concentrations (as in this study). The response factor is calculated from the intensity readings of the various drugs compared to a reference. In this study, I used NOR as the drug of reference to calculate the response factors.

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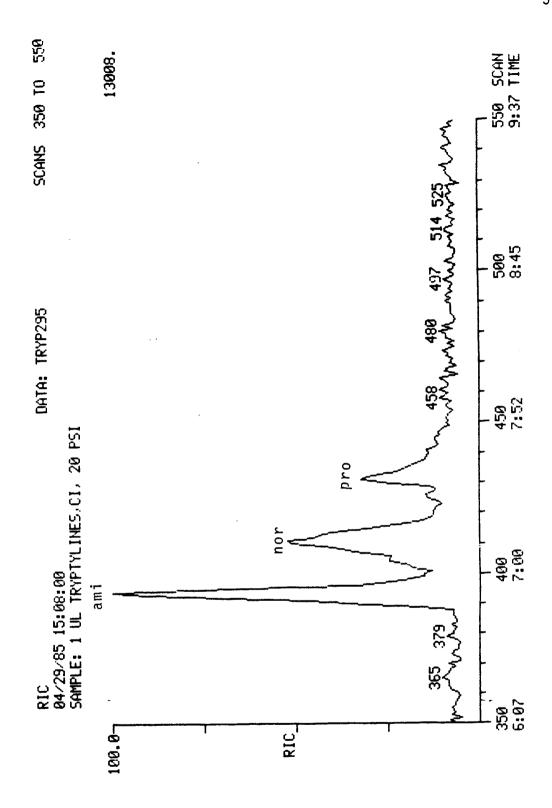


Figure 16. The RIC of 250 ng of each of the three drug standards analyzed in the programmed temperature mode.

(8)

(9)

#### **151524/** 176496 = 0.89

A response factor would have to be calculated for all rubstances to be analyzed. The concentration of unknown drugs would be determined using the following equation

(Iunh x Rf x Conc. Ista )/ Ista = Conc.unh As was noted above, intensity is a relative determination and can vary considerably, but this variation had little effect on calculating the response factors as seen in Table 5. Because of the broadness of the peaks and the large difference in peak height and peak area, I felt a more accurate response factor would be obtained by using peak area rather than peak height.

### Isothermal Conditions

If a procedure is to find clinical application, it should be as simple end easy to perform as possible. The programmed temperature mode is vary useful in analyzing complex mixtures and resulted in more than adequate separation of the drugs. But in clinical laboratorieg, a majority of their work involves therapeutic drug monitoring. In this case, we are not working with a complex mixture. Therefore, I have explored the possibilities of an isothermal procedure. The isothermal procedures are simpler and faster since the column does not need to reequilibrate its temperature (cool down to the initial temperature)

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before the next analysis. During this portion of the study only the initial and final temperature settings were varied but kept equal to each other. The same standard drug mixture war run through a series of CI analyses to find the optimum isothernal operating temperature. The RIC and the optimum temperature and other GC conditions are seen in Figures 17 and 18, respectively. The RIC shows good resolution and sensitivity for the relatively short retention times in comparison to the programmed mode. All three drugs ware injected onto a 200°C column and due to similarities in molecular weight and rtructure the rerult is three rather uniform peaks. This caused a substantial change in the response factors compared to earlier programmed temperature data. A tabular listing of the results of this isothermal analysis is seen in Table 6. The response factors in thie care were calculated using the peak height because of a difficulty in determining peak area due to peak overlap, although all peaks did appear uniform.

although all peaks did appear uniform. difficulty in determining peak area due to peak overlap. dade were daigulated uping the peak height because of c analysis is ocen in table 6. The rustones factors in this data. A takular teotang of the teoulity of them incithernal reaponse factions dumpared in darillor programmed tamparature unnious peaker This caused a substantial change in the molecular voight and ofructure thi result to three rither injected onto a 100m2 column and due to similarities in comparation to the programmed mode, all three drugs were eencaltityit: for the rejutifully abit; retent-in timen in and is, rappertively. The Plu chera good res lutton end tomperature and other 40 conditions are been in Figuric 17 isothermal remaring temperature. The fill and the optament was ron through a serial of OI analyses to tind the cottinum but keps thoughte each other. The toma standard drug miniture Only the initial and final temperations defings wire warings befuse the nucl snalpais forms thin portion of the brugy

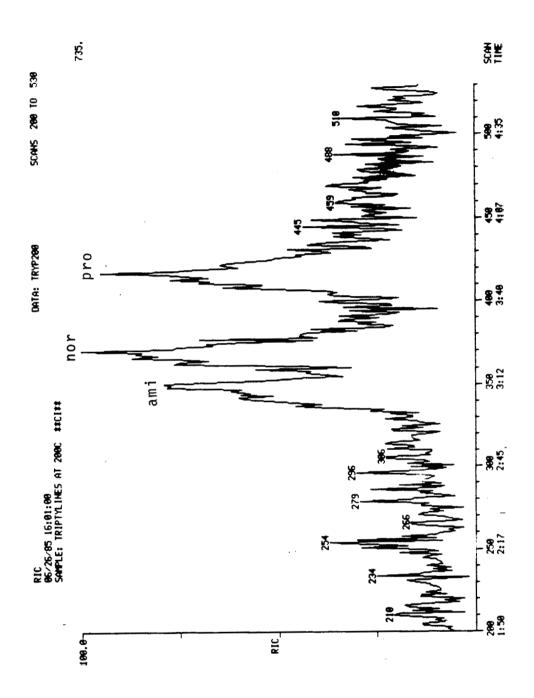


Figure 17. The RIC for the isothermal CI of the drug mixture analyzed at 200°C.

#### GC PARAMETERS:

250 deg. = Injector Temp.

200 deg. = Initial Tenp.

200 deg. = Final Tenp.

1 min. = Initial Time

0.0 D/H = Ramp Rate (deg/min)

10 nin. = Final time

250 deg. = Separator Tenp.

80 deg. = Hanifold Temp.

INJECTOR MODE: CAPILARY

60 sec. = Split/Sweep Valve Closed Time

90 sec. = Filament/Multiplier Off Time

SCAN FROM 150 AMU TO 350 AMU IN 0.5 sec.

UETHANE PRESSURE: 0.40 torr (used in CI)

Figure 18. Typical computer printout of GC conditions for isothernal analysis.

TABLE 6

Data for 200°C Ioothernal Analysis of the Drug Mixture\*

Drug	RΓ	In <b>tensi</b> ty	RF
AMI	4:29	771	1.13
NOR	4:43	870	1.00
PRO	5:23	631	1.38

\*The GC/MS conditions for isothermal analysis are eean in figure 18.

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#### GC/MS Variable Paraneters

Many researchers in reporting clinical procedureo merely state the conditions at which the analysis is performed. At this tire I will show the effect of varying many of the GC/MS parameters. This is done in order to give a better understanding of the overall GC/MS operation. This will show that adjusting many of the variables will result in a considerable change in the RIC. The first variable to be considered is the column temperature. It can be varied from 25°C to 300°C. The compromise is between the analysis tine, intensity, and resolution. As temperature is increased the analysis time and the rasolution will decrease. The sensitivity will increase with temperature and will plateau at the optimum temperature. As this temperature is exceeded, sensitivity and resolution will decrease as peaks begin to overlap. Table 7 shows the retention tire (RT) and the intensity (I) for the three drug rtandards at column temperatures from 190°C to 220°C while all other parameters were held constant. Table 8 shows the relative retention time (RRT) and the intensity (I) of the three rtandardr at varying column temperatures. This was accomplished by using the information from Table 7. The RRT is a means of standardizing retention times from numerous chronatograms. The RRT is determined by establishing one of the drugs as the standard or reference drug and its RRT is **1.00.** All of the other drugs RRT are determined by

李大大的 (1974) (1974) \$P\$ (1985) (1985) \$P\$ (1986) (1986) (1986) (1986) (1986) (1986) (1986) (1986) (1986) (1986) ាលស្ថា (Linguis និង Colon Logical State ) ។ បានជា ១០១៩ ស្រាប់ សាលា បាន<mark>មនុស្ស គ្រ</mark>ិត្រិតិ to construct the supplication of the security of the second sections. ರ ಉತ್ತರ ಆಗಿ ಕಟ್ಟಿ ಸ್ಥಾಪ್ರವಾಗಿ ಕೃತಿ ಕೃತಿ ಕಾರ್ಯಕ್ಷಣೆ ಮಾಡಿದ್ದಾರೆ. ಮಾಡಿದ ಕ್ಷಾಪ್ ಸ್ಟ್ರಿಸಿ ಕ್ಷಾಪ್ ಕ್ಷ್ಟಿ ಕ್ಷಾಪ್ ಕ್ಷಾಪ್ ಕ್ಷಾಪ್ ಕ್ಷಿಪ್ ಕ್ಷಾಪ್ ಕ್ಷಿಪ್ ಕ್ಷಾಪ್ ಕ್ಷಿಪ್ ಕ approved the first management of the first section which is the first of the self-**ಜ**ಳ ಭಾರತದಲ್ಲಿ ಇದು ಒಡುದು ದಿರುವ ಪರ್ಕಾರ ಇ ಪ್ರಕಾಣ ಒಟ್ಟಿಗಳಲ್ಲಿ ಇವರಿಗೆ ಸಿಲ್ಲಿಕ ស្នាក់ខណ្ឌល ស្រុក ស្ពេក្ស សក្សាកា ក្រុងក្នុង សុខសុខ សុខសុខ ១ ១៥៣ ខេឌ្ឌ សុខសុខ ខេ colored restrictions from The Color Thick Color Sit (4) Th A DECEMBER 1980 CONTRACTOR SERVICES AND A SERVICE SERVICE OF A SERVICE SERVICE OF A 我搬出就走,我看看我的话说,只要看到了这种大人,我们看到了,我们也没有一点,只要是这样的话,不是是 en y byspoed as ips observe constanted to serve product to ಇದರು. ಇದರ ಕೃಷ್ಣದ ಹಾಕಾಮರಾವರ್ಧವರ್ಷದ ಅವರ ಗಳಗಳು ಕರ್ಮ ಗರ್ವಹಗಳು ಸಾಮಾನಿಕಾಗಿ ಸಾಹಿ 👣 ರಂತಹಾತರು. ಭಾರತ ಗಾರ್ಯವರ್ಷ ನಿರ್ವಹಿಸಲಾಗಿ ನಿರ್ವಹಿಸಲಾಗಿ ನಿರ್ವಹಿಸಲಾಗಿ ನಿರ್ವಹಿಸಲಾಗಿ punt stores are en an american sur como contra como esta destablicada de la como establicada de la como establicad THE TOTAL SECTION OF THE SECTION OF ## - ೧೯೯೮ ಕರ್ಗಳಲ್ಲಿ ಎಂದು ವಿಕ್ಷೇತ್ರ ಕರ್ಮಕ್ಷ ಮತ್ತು ಮತ್ತು ಮತ್ತು ಕ್ಷಾಪ್ತಿಕ್ಕಾಗಿ ಮತ್ತು ಎಂದು ಎಂದು ಮತ್ತು ಮುಂದಿ ಇದೇ ಮತ್ತು grand ordination and the time of the control of the after described as MITTO DE SOUTE LA MILITURA COMO COMO CONTROLA CONTROLA MILITA DE CONTROLA CONTROLA DE CONTROLA CONTROL ·通·集·设理的 计对象 (对象性格 的 ) 化二氢二烷 ( ) 化二乙烷 ( ) 化二氢苯二氧甲基苯甲基苯甲基苯甲基  $m{p}$  (with the latter of the constant productions and the product of the constant  $m{x}$ where the second of the second  $\Sigma$  is the second of  $\Sigma$  in  $\Sigma$  $x\in \mathcal{K}$  . The constant of the interpretation of a difference of the state of the g(x) = g(x) + g(x) + g(x) = g(x) + g(x) +

### TABLE 7

The Retention Times (RT) and Intenritier (I) of the Drug Standards at Various Isothernal Temperature8

COLUMN TEMP.	AMI(RT/I)	NOR(RT/I)	PRO(RT/I)
190	4:55/1830	5:15/347	5:57/223
200	3:11/1898	3:23/2037	3:49/3561
210	2:17/1898	2:26/4244	2:44/1868
220	1:58/1180	2:02/1500	2:12/1650

TABLE 8

The Relative Retention Timer (RRT) and Intensities (I) of the Drug Standardr at Various Isothermal Temperatures

COLUMN TEHP.	AMI(RRT/I)	NOR(RRT/I)	PRO(RRT/I)
190	1.00/1830	1.13/347	1.22/223
200	1.00/1898	1.04/2037	1.12/3561
210	1.00/1898	1.04/4244	1.12/1868
220	1.00/1180	1.27/1500	1.34/1650

Analyres at temperatures less than 190°C reculted in extremely long RT and decreased intensity and sensitivty due to peak broadening. Analyses at temperatures greater than 220°C resulted in a complete loss of resolution because all three drugs appear as one large peak with an extremely

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ರ್ಷಕ್ಕೆ ಭಲ್ಲದಲ್ಲಿ ಇವರ ಇದೇ ಅದೇ ಮತ್ತು ಅವರ ಅವರ ಅಂದಿದ್ದಾರೆ. ಅವರ ಅವರ ಅಂತಿಯ ಕ್ರಾಮ್ ಅವರ ಮುಂದು ಮುಂದು ಮುಂದು ಮುಂದು ಮತ್ತು ಆರಂಭ್ಯ ಮುಂದು ಇವರ ಆರಂಭ್ಯ ಮುಂದು ಮು ng grading the protection of the state of th I CONTRACTOR OF THE SECOND CONTRACTOR OF THE PROPERTY OF THE P ម្នាល់ស្វាងសង្គ្រាប់ ប្រជាជាប្រើប្រាស់ស្គាល់ស្វាល់ ប្រជាជាប្រាជ្ធិស្វាស់ស្វាល់ ស្រែស្វាល់ ស្រែស្វាល់ ស្រែស្វាល់ ស្រែស្វាល់ ស្វាល់ March Dealer of Checks Control States and Control Control Control Control Control ត្រូវប្រជាព្រះស្រែក ខាន់ស្រែន និងស្រែន និងស្រែងស្រែ ស្រែសា ខាងស្គាស់ ស្រែក ១០ ខាងសុខជានេះ បានសុខភាព។

short retention **time.** The drugs could be distinguished only through conputer enhancement by scanning for individual characterietic masses.

This same effect is seen in the programmed temperature analyses when the column temperatures (initial and final) and the ramp rate are varied. It therefore appears that the degree of sensitivity and the resolution attained is directly related to the retention time which is controlled by the column temperature. Table 9 showe a comparison of the retention timer (RT) and the intensities (I) of the drug standards to the operating ramp rate in degrees per minute (D/M).

TABLE 9

The Retention tines and Intensities of the Drug Standards at Variour Ramp Ratesa

Ramp Rate(D/M)	AMI(RT/I)	NOR(RT/I)	PRO(RT/)
5	9:33/21180	9:49/9316	9: 57/7252
10	6:54/57228	7:12/49799	7:33/22466
15	5:36/43846	5:43/32458	6:00/10334
20	4:58/8768	/ <b>b</b>	/ <b>b</b>

All analyses were performed according to the conditions seen in Figure 8, except for ramp rate.

NOR and PRO appear as one peak at 5:02

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a Mil anstvæsæ ware performed sacardong to the conditions saer on Figure 4, ecospt for remp rate. Place NOP sau PFD appara og ode pesk at Tibl

The next parameter requiring investigation was the helium head pressure. Thir pressure can typically vary from 5 to 30 psi (pounds per square inch). The more common terminology for carrier gas (helium) neasurements is flow rate. Thie was mearured at preceures of 5 and 10 psi and rerulted in helium flow rates of 1.0 and 2.0 ml/min. Table 10 shows the effect of helium pressure on the drug's retention time while all other parameters remain constant. The RICs of this series of analyrer showed again that there is a direct correlation between intensity and retention time. The lower pressures resulted in long retention times and poor intensity due to very broad peaks that at times were nearly lost in the background noise, whereas the higher preesures resulted in rhorter retention times, greater intensities and excellent sensitivity. It must be

TABLE 10

The Retention Times of Drug Standards at Variour Helium Pressures (psi)\*

HELIUM -PRESSURE (psi)	RETENTION TIME (min:sec)		
•	AMI	NOR	PRO
10	11:02	11:30	12:05
20	9:33	9:49	9:57
30	8:33	8:45	9:25

\*All analyrar were at 5 deg/min ramp rate.

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remembered that varying this and other parameters that affect retention times nay reault in loss of resolution due to overlapping of RIC peaks.

As noted earlier the MS has the ability to scan for ions with a aasr-to-charge ratio from 1 to 800. In this study scanning war typically from 150 to 350 anu in 0.5 to 1.0 sec. The Finnigan model 1020B can also scan for a number of selected ion masses in a tine frame of 0.030 to 1.000 eec for each mass or mase range while the total scan nay take **0.50** to **4.00** rec. The instrument will then reset **itself** and begin scanning again. This concept is termed selected ion nonitoring (SIM). The advantage of this is two-fold; the background is decreased because fewer masses are being rcanned so fewer interfering ions are detected, and the selected masses are scanned for a longer period of tine than in previouely noted ranger resulting in more ions of a specific mass detected and higher intensity readings. These advantages result in an overall increase in detection sensitivity.

The effective use of CI and SIM are dependent upon the ability of the reagent gas to react with the parent molecules to be studied. This ability is reflected in the conversion rate of M+ to MH+ and the effectiveness is measured by the MH+ to M+ ratio. As noted earlier in Chapter V, methane is the reagent gar in this study and its optimum pressure was determined through independent methane

grabias in the secretar manager of castron decreases the second of the cast of the castron क्षित्रण, इ.स.च्या १९८० चुप्रकार एको १९८५ । आस्त्रण इत्याप्त्रण स्थापना विकास स्थापना स्थापना स्थापना स्थापना 他とは言葉をもてなば、もつから、これ、から、して、精神は、ラブで、ようが、原本中につるてある場合はで、それ。 កក់ក្រក់មហា ណូច ស្គា ៤០១០ ស្រង់។ នៅស្រីសាល់ ខណ្ឌនូលនៅ ១០០ ១០១០១០១៩៣០ ១៣០១៣ gro compress or got more see agon more as as as a company

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ក្រុមស្ត្រស្នាញ ស្រុកសុខ្មានស្រួល បាល ខណ្ឌស្តេចប្រូវ ក្រុមស្តែមានស ពីសា ឡាគ្នាសេសស្គាក់ស្ ಯಾರು 2001**,** ಗಳು ದಾರ್ಶಗಳ ಇಳಿಸಲಾಗಿದ್ದಾರೆ. ಆರೂ ಮುಖ್ಯಾಗಿ ಕರ್ಮದಿಸಿದರು ಸಂಚಿತ್ರಗಳಲ್ಲಿ ಕ್ರಾರ್ಥಿಕಿ कुछ एक रावार वर्ष । छ । ११३ वृद्ध का सामित्र विकास कर विवास । १५ वृद्ध विवास । १५ वृद्ध विवास । १५ वृद्ध विवास ಪ್ರಕೃತವಿಧಿಪ್ರ ವಿಶಾಸವಾಗ ಗಗರ ಜಪಕಾಸಿಕುವು ಕ್ಷಾಸ್ ಗ ಗಾರಚಿಕರ ಕಾರಾಗಗಳು ಗಳ ಕಾಗುಳ ಕಟ್ಟಿಗ ಹದಾಯದಾರವು ೨೯ ಇಗಳಾಗ ತಲ್ಲಿಕಾತಕಾಸಲಾಗಿತ್ತಿ ಕರಗಾಗಿ ಅಗಳ ಇಲ್ಲಾಗಿದ್ದಳಿ ನಟ್ಟು ಕೃತಿ ಧ್ಯವಶ್ವೀಸರ್ವಾಗವು ಕಂಗ ಪ್ರವರ್ಷದರ್ಶನ , ಕ್ಷಮು ಗಾಗಿ ಚಿತ್ರ ಸಾಧ್ಯವನ ಹಾಡುತ್ತಾರೆ ಹಾಗೂ ಪ್ರತಿಕಟ್ಟಿ is,  $\star$  three in  $\mathbb{I}_{p,h}$  . The constant of the constant of  $\mathbb{T}_p$ ರ್ವೀಸ್ಟ್ ಪ್ರಕ್ರಾಣಕ್ಕೆ ಪ್ರಕ್ಷಣಗಳ ಪ್ರಭಾವಿಗಳ ಪ್ರಭಾವಿಗಳ ಪ್ರಭಾವಿಗಳ ಪ್ರಭಾವಿಗಳ ಪ್ರಭಾವಿಗಳ ಪ್ರಭಾವಿಗಳ ಪ್ರಭಾವಿಗಳ ಪ್ರಭಾವಿಗ ್ರೀಕ್ಷಿಸ್ತ್ವರ ಕ್ಷಾರ್ ಕ್ಷಮ್ ಕ್ಷಾರ್ ೧೯೬೪ ನಿರ್ವಕ್ಷಿಸಲೀ ಅನ್ನು ೧೯೭೩ ಕಿರ್ಮು ಸರ್ವರ್ ಕ್ಷಮಾತ್ರವ ಮತ್ತ ng pagragas new basetro am e grea grama in historia (no 1996). 하는 사람이 이 교수를 입고하는 연습을 하는 사람이 물건들이 기계를 다 되었다. 보고를 보고 중 하는 기계를 하는 기계를 하는 것이다. ್ಕೆ ಸ್ವಾಪಿ ಹುದ್ದು ಚಳಕಾರಕ ಪ್ರತಿಕ ಕ್ರಮಿಕ ಕರ್ಮಕ್ಷಮ ನಿರಾಶಿ ಕೆಟ್ಟಿಕ್ಕು ನಿರ್ವದಿಕ್ಕು ಕೊಟ್ಟಿಗಳು ನಿರ್ವಹಿಸಿಕ

सके र गर्नाम चल्यार्मकर रहाते सन्धि होत्रा प्रमान प्रगार्थकर प्रमान Live segment and the EID broken ్ ఎక్కు ఇవ్వార్కువాడుని పాకార్యా ఎక్కువ కుర్యాట్స్కో నాయి. కార్యాల్లో కార్స్ కార్స్ కార్స్ కార్స్ కార్స్ కొట్ట 

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demonstrated through a series of analyses of the drug standards at various methane pressures. This was experimentally demonstrated through analyses of AMI in which the methane pressure was varied at 0.1 torr increments while monitoring the relative percentage of MH+ formed compared to the total amount of ions detected. Table 11 shows the results of this series of analyses.

TΑ	BLE	11	

Comparison of Methane pressures and the Relative amount of the UH+ formed during AMI analyses

Methane Pressure (torr)	Rel. * of MH*
0.20	11.93
0.30	18.10
0.40	12.00
0.50	9.71

This pressure ir measured in **the** ion source of the **MS** and it varies depending on the type of rolvent and the sample size, but mainly on the flow rate of reagent gas entering the ion source.

studies. The experiences proof on test option a protocole associated through a series of changes of the ire; standards at various methods prossures. This was experimentally described involute analyses of AMS in which the methods pressure was varied at the tire indicative bide membering the relative persenteds of the idea is reading the relative persenteds of the indicative of its dataphed. This is above the results of this series of analyses.

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Comparison of Methane pressures and the Felatize decomb of the MH+ formed during AMI preduced.

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Ø 30	18.10
0.20	11.53
Methane Fressure (tour)	₽#7° & # <b>₹ N</b> H+

This pressure is measured in the ich course of the MC and it varies depending on the type of solvent and the sample birst her mainly on the flow rate of respent gas entering the ion source.

## Clinical Comparison of Study

In thie study it was determined that sensitivity of the developed procedures ranged from 10 to 30 ng. It must be remembered that this is the actual amount of the drug injected onto the column. In many of the clinical procedures the following steps are followed

- 1. 1-3 mL of serum from patient is required.
- 2. 100-200 ng of internal standard is added.
- 3. A basic buffer is added to attain pH 9-11.
- 4. Extracting solution (such as hexane/alcohol) is added.
- 5. 70-99% of drug is extracted into organic phare.
- 6. Organic phase is drawn off and evaporated to dryness.
- 7. 5-100 µL of pure hexane is added to reconstitute residue.
- 8. 1-3 µL concentrated drug in hexane solution is injected.

The critical steps in concentrating the drug samples are steps 6 and 7. The above noted method is similar to the procedure developed by Rovei et al (30). Thus, the serum ANI concentration of 100 ng/mL would result in the injection of 30-40 ng of ANI being injected into the column. This is well within the sensitivity range of the above developed GC/MS procedure. The mathematical explanation of this is as follows:

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In this study at was determined that eencitivity is the developed procedures ranged from 10 to 30 mg. It must be remembered that this is the actual amount of the drug injected onto the solumn. In many of the clinical procedures the following steps are followed.

- 1.3 ML of serum from patient is required.
- 2. 100-200 ng of internal standard is added.
- 3. A basic buffer is edded to attain pH 9-11.
- 4. Entranting solution (such as hename clockel) is added.
- 5. 70-93% of drug is extracted into organic phase.
- E. Organic phase is drawn off and evaporated to dryness.
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- 8. 1.0 bl concentrated drug in hexane splution is

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100 ng/mL : 2#1 : 200 na

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Equation 10 shows that 2 mL of serum from this patient would contain 200 ng of AMI. The drug is completely extracted into an organic solvent and then this solvent is evaporated at 60°C under a stream of nitrogen gas. According to this procedure the residua is reconstituted with 20 uL of hexane. Therefore, as shown by equation 11 the 200 ng of AMI is now found in 20 uL or 10 ng/uL of hexane. Then 3-4 uL of this mixture or 30-40 ng of AMI is injected into the GC.

by Chinn et al (32) have eliminated the drying and concentrating steps. In this procedure the drug is extracted into 200 mL of a toluene/hexane/alcohol mixture and then 6 - mL is injected onto column. According to this extraction procedure a rerun AMI concentration of 120-250 ng/mL would - result in the injection of 7.2 to 15.0 ng of AMI onto the column.

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angration.

#### CHAPTER VI

#### CONCLUSION

As noted earlier, there have been numerous procedures developed for the separation, identification and quantitation of numerous tricyclic antidepressants including amitriptyline, nortriptyline and protriptylina. These procedures have included spectrometric methods, radioimmunoassays, thin-layer chromatography, enzyme-labelled immunoarsays, and most recently high prersure liquid chromatography (HPLC) and gas chromatography (GC). The latter method, GC, has become the most widely accepted methods for drug studies. Through the years many types of detectors have been used with gar chromatographs such as ultraviolet detectors, nitrogen-phosphorous sensitive detectors and mass spectrometers. It is the combination of gas chromatograph/ mass spectrometer (GC/MS) that has resulted in a highly senritive and highly specific instrument capable of detecting and identifying picogram quantities of drugs.

In spite of all the research accomplished, there was a limited amount of work done using fused silica capillary columns combined with GC/MS. This was especially true for capillary columns with the SE-54 stationary phase, since no research work was found that used this particular stationary

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phase with GC/MS. There is also a very small amount of work involved with studying the capabilitier and the limitations of capillary GC/MS. In this study, procedures and GC/MS parameters for both programmed or ramping temperature and isothermal nodes were investigated. The programmed temperature procedure could rupply excellent separation of a complex drug mixture and could be very useful in identifying an unknown drug. The isothermal procedure would provide more than adequate separation and more reliable response factors for the quantitation of drugs as in the case of therapeutic drug monitoring. In this case the use of electron ionization appeared less effective due to a lower sensitivity and specificity, whereas chemical ionization utilizing methane as the reagent gas resulted in significant increases in sensitivity and specificity. The specificity increased because the molecular weight of the parent molecule can easily be determined and the presence of additional fragmentation resulted in identifying many characteristic mass peaks.

It was the purpose of this study not only to develop a procedure of optimum conditions but also to report the effect of varying the conditions. The following is a list of the optimum GC/MS conditions for the programmed temperature node and the isothermal rode. The isothermal mode conditions are in parentheses if they are different than the programmed mode.

## GC PARAMETERS:

250 deg. \* Injector Temp.

150 deg. = Initial Temp. (200 deg.)

220 deg. = Final Temp. (200 deg.)

1 min. = Initial Time

10.0 D/M = Ramp Rate deg/min (0.0 D/M)

10 min. \* Final tine

260 deg. = Separator Temp.

80 deg. = Manifold Temp.

INJECTOR MODE: CAPILLARY

60 rec. = Split/Sweep Valve Closed Time

90 sec. = Filament/Multiplier Off Time

SCAN FROM 150 ANU TO 350 AMU IN 0.5 sec.

METHANE PRESSURE: 0.40 torr (used in CI)

HELIUM PRESSURE: 20 psi

Figure 19. The typical computer printout of the optimum GC/MS programmed temperature mode conditions. The optimum irothermal conditions, if different, are noted parenthetically.

In invertigating many of the adjustable parameters the key factor in reparating a drug mixture appears to be the retention time. This is affected by several variables such as column temperature (isothermal or programmed modes), carrier gas flow rate and column diameter and length. It must be remembered that an adjustment of any parameter may

result in a compromise in resolution, retention time, or sensitivity of a procedure's results.

One of the factors that was not investigated and could have a definite effect on the procedure is the stationary phase coating of the capillary column. The SE-54 coating used in this study is slightly polar and may tend to cause the tailing and broadened peaks evident in some of the RICs, whereas a less polar column coating, such as SE-30 or OV-101 that contain polydimathylsiloxane polymers, may be more effective due to the slightly polar characteristics of these drugs. There are also some columns available with slightly larger inside diameters or film thickness. These various column characteristice may result in sharper peaks without the tailing but some of the resolving power of the SE-54 coating would be lost.

Another factor that could be investigated is the reagent gas. Nethane was used in this study, but as suggested by Chinn et al (32), by using a methane-ammonia mixture as the reagent gas a larger percentage of the MH+ ions were produced. This is because a more stable MH+ ion is formed, less fragmentation of the parent molecule occure, and fewer secondary reactions occur. This could result in better sensitivity when analyses are performed by chemical-ionization selected-ion monitoring.

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#### REFERENCES

- 1. Van Brunt, N. Ther. Drug Monit., 1983, 5, 11-37.
- 2. Asperheir, M. K. <u>Pharmacologic Basis of Patient</u>
  <u>Care.</u> 5th ed. Philadelphia, PA.: W. B. Saunders Co., 1982.
- 3. Melnon, K. L.; Horrmlli, H. F. <u>Clinical</u>

  <u>Pharmacology</u>. 2nd ed. New York: MacMillan Publishing
  Co., 1978.
- 4. Baer, D. W. <u>Medical Laboratory Observer</u>, August 1985, 17, 12-13.
- 5. <u>Physicians' Desk Reference</u>, 39th ed., Edited by Edward R. Barnhart. **Oradell, N. J.: Medical** Economics Co., 1986.
- Woody, J. P.; Tait, A. C.; Todrick, A. <u>Br. J.</u>
   <u>Psychiat.</u>, 1967, 113, 183-193.
- Kaul, P. N.; Whitfield, L. R.; Clark, M. L. J. Pharr, Sci. 1978, 67, 60-62.
- Fenimore, D. C.; Meyer, C. J.; Davis, C. U.; Hsu, F.;
   Zlatkis, A. <u>J. Chroratoar</u>., 1977, 142, 399-489.
- Haefelfinger, P. <u>J. Chromatogr</u>., 1978, 145, 445-451.
- Knox, J. H.; Jurand, J. <u>J. Chroratoar</u>., 1975, 103, 311-326.
- 11. Spector, S.; Spector, N. L.; Alneida, M. P. Psychopharmacol. Commun. 1975, 1, 421.
- 12. Scoggins, B.; Haguire, K. P.; Norman, T. R.; Burrow\*, 6. B. Clin. Chem., 1980, 26, 13.
- 13. Emergency Toxicology Assays: Syva EMIT Handbooks, Syva Co., Palo Alto, CA., 1983.
- 14. James, A. T.; Martin, A. J. <u>J. Biochem.</u>, 1952, 50, 679.
- of Clinical Chemistry, Edited by Norbert Tietz,
  Philadelphia, PA.: W. B. Saunders Co., 1976.

## FILLETHIER

- 1. Van frant, N. Ther. Pres Menth., S. S. F. J. S. J.
- 4. Appendent (1. 1. 1. <u>Sharmary Login Bear A de Dalien)</u> Care, Sth ed. Philichtiph a. th. a (1. 1. Suchday) (で 1902)
- $2=\frac{He^{4\pi cn_{1}}}{12\pi^{1}}$  with Monthill, which was Militarian 1951 and  $61-45\pi^{1}$
- 4. Baer, D. M. Nedecal Laboratory A. errer. August 1945, 10, 12-29,
- 5. <u>Philodinings | Chapt Leferange</u>, sinh of . Edited by Editer by Editer's F. Berghirt, Object i, M. Jin Medical Editerial Const. (Const. 1996)
- Especially 1967, 113, 213 1931
- AR PROTUBLING WAITHERSON, D. R.; CLIFK, M. L. <u>Archarm. Sol</u>. 1974, 67, 98-51.
- As Seminores D. Cos Meyors C. Jos as des D. M.s. Heas, For-District As <u>de Chranalagr</u>o, 1987, 1987, 1989 489.
- न प्रदेश स्थापना क्रिया । विश्व क्रिया विश्व क्रिया विश्व क्षेत्र स्थापना स्थापना स्थापना स्थापना स्थापना स्थाप स्थित स्थित स्थापना स्
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- 15. Chatalogs, all the continuited plants in individual Care and C

## REFERENCES (Continued)

- 16. <u>Megabore Handbook</u>, J & W Scientific, Inc., Rancho Cordova, CA., 1985.
- Braithuaite, R. A.; Whatley, J. A. <u>J. Chromatogr.</u>, 1970, 49, 383-387.
- 18. Hammer, C. G. Anal. Chem., 1979, 48, 1708-1711.
- 19. Hucker, H. B.; Stauffer, S. C. <u>J. Pharm. Sci.</u>, 1974, 63, 296-297.
- 20. Biggs, J. T.; Holland, W. H.; Chang, S.; Hipps, P. P.; Shrrnan, W. R. J. Pharm. Sci., 1976, 65, 261-268.
- 21. Bredesen, J. E.; Ellingsen, O. F. <u>J. Chronatoar.</u>, 1981, 204, 361-367.
- 22. Corona, G. L.; Bonferoni, B.; Frattini, P.; Cucchi, M. L.; Santagortino, 6. J. Chronatoar., 1983, 277, 347-351.
- 23. Jones, D. R.; Lukey, B. J.; Hurrt, H. E.; 3. Chromatogr., 1983, 278, 291-299.
- 24. Plotczyk, L. L. <u>J. Chronatour</u>., 1982, 240, 349-360.
- 25. Bonderron, V.; Johansson, I. M. <u>J. Chromatogr.</u>, 1986, 377, 379-383.
- 26. Holmes, J. C.; Norrell, F. A. Appl. Spectrosc., 1957, 11, 86.
- 27. Andresen, B. D.; Wise, B. L.; Ng, K. J.; Wise, G. A. J. Med. Tech., 1985, 2, 245-250.
- 28. Holland, J. F.; Enkr, C. G.; Allison, J.; Stults, J. T.; Pinkston, J. D.; Newcome, B.; Watron, J. T. Anal. Chem. 1983, 55, 997A-1012A.
- 29. Mills III, T.; Price, W. N.; Price, P. T.;
  Roberson, J. C. <u>Instrumental Data for Drug Analysis</u>,
  Vol. I., Elsevier Co., New York, 1982.
- 30. Rovai, V.; Sanjuan, N.; Hrdina, P. D.
  J. Chromatogr., 1980, 182, 349-357.
- 31. Gupta, R. N.; Stefanic, M.; Eng, F. <u>Clin. Biochem.</u>, 1983, 16, 94-97.

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# REFERENCES (Continued)

- 32. Chinn, D. M.; Jennison, T. A.; Crouch, D. J.; Peat, M. A.; Thatcher, G. W. Clin. Chen., 1980, 26, 1201-1204.
- 33. Vinet, B. Clin. Chen., 1983, 29, 452-455.
- 34. Jenkins, R. G.; Friedel, R. O. <u>J. Pharn. Sci.</u>, 1978, 67, 17-23.

# REFERENCES (Continued)

- 00. Chinn, D. M.: Jennison, T. A.; Crouch, D. J.; Peat, M. A.; Thatcher, G. W. <u>Clin. Chem</u>., 1980, 26, 1201-1204.
- 33. Vinet, B. <u>Clin. Chem</u>., 1983, 23, 452-455.
- 34. Jenkins, R. G.; Friedel, P. O. J. Fharm. Sci., 1278, 67, 17 03.