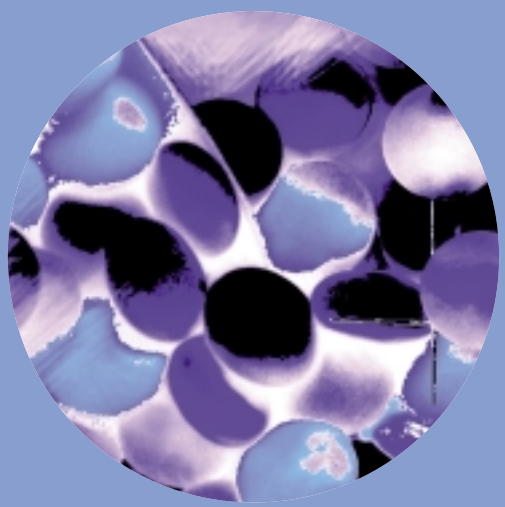
Analysis of Pharmacologically Relevant Compounds using GC/MSD – EI/PCI/NCI

Compendium of Applications



Agilent Technologies

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Preface

The combination of gas chromatography with mass spectrometry has been successfully used for decades. The now standard and universal technique of electron impact ionization (EI) has provided, with respect to sensitivity and compound identification, extraordinary performance and the volume of spectral reference data has continued to increase over the years. Commercially available mass spectral libraries now contain more than 350,000 entries. Instrument sensitivity has improved such that sufficient sensitivity is available in full scan mode for determinations at analyte concentrations in the range of 10pg/µl to 1pg/µl. However the EI technique still leaves some analytical demands unfulfilled. For example, if the EI spectrum shows little or no definitive information about the analyte molecule, such as molecular weight, or, in samples where matrix interferences convolute the spectral information due to insufficient selectivity, then successful detection can decrease drastically.

For such cases, chemical ionization techniques (CI) are available which enhance and extend the use of mass spectrometry. The positive chemical ionization (PCI) mode can usually lead to molecular weight information since protonated and adduct species of sufficient intensity can provide distinctive information about the correct molecular mass. For compounds with high electron affinity, the electron capture negative ion chemical ionization (ECNI or NCI) mode offers selectivity and the utmost sensitivity even in difficult matrices. Detection limits in the femtogram range can often be easily achieved. Stable instrumental parameters can insure high reproducibility over a wide concentration range. Importantly, the new instrumental platforms provide user-friendly CI operational features, such as autotuning, that make CI similar to EI in convenience.

With CI techniques, mass spectrometry becomes a powerful, unique, problem-solving tool. CI is the most creative MS technique. After becoming familiar with some criteria and the effects of certain instrumental parameters, the user will rapidly achieve positive results, and the usefulness and fascination of CI will become readily apparent. To assist and convey such an experience is the aim of this compendium.

I would like to thank Dr. Harry Prest, Senior Application Chemist, Agilent Technologies, Chemical Solution Business Division, Palo Alto, USA, for his helpful comments and advice in preparing this applications compendium.

H.-Jürgen Schulz
February, 2002

1. Introduction

The technique of chemical ionization mass spectrometry (CIMS) was pioneered by Munson and Field in 1966. CIMS can be considered an alternative ionization approach to electron impact (EI) and offers the opportunity to determine the molecular weight of the analytes and in selected cases, CIMS measurements show very high selectivity and outstanding sensitivity.

1.1 Distinctions between EI & CI

In EI mode, relatively high energy electrons (70 eV) collide with analyte molecules producing positive ions and other species. The fragmentation process, executed under constant conditions, is well understood and the positive ion fragmentation pattern, which is the EI mass spectrum of the analyte, is used for compound identification. Whereas EI is a direct energy transfer process with electron kinetic energy deposited directly in an analyte molecule, CI is an indirect process involving an intermediate chemical agent. This is particularly true in positive chemical ionization (PCI). In PCI, the ion source is filled with a reagent gas which is ionized to create reagent ions which react with the analyte. The "interesting" reaction products are positive ions which are collected and measured in PCI. Negative ions are also formed by this chemical process and this is a form of NCI. The gas filling the source can also be used to "buffer" or "thermalize" electrons. These "slow" electrons can be captured very efficiently by analyte molecules to form negative ions which is called electron capture negative ionization (ECNI). In casual usage, when the resulting ions being measured are positive, it is referred to as PCI and when negative, NCI.

2. Positive Chemical Ionization (PCI)

PCI mode is preferred in cases that the EI mass spectrum contains mostly low mass-to charge ratio fragments and therefore little or no information about the molecular weight of the analyte. The success of the technique is strongly dependent on the choice of the applied reagent gas. The literature documents a multitude of examples for structure elucidations with PCI techniques by using different reagent gases, in addition to the elementary use of PCI for molecular weight. PCI sensitivity is comparable to EI sensitivity but can offer significant improvements in actual samples depending on the choice of CI reagent gas and nature of the interferences. In terms of the signal-to-noise of the base peaks, PCI often provides a better result than EI and therefore is well suited to selected ion monitoring (SIM) acquisitions.

2.1 Ion Source Configuration

The CI ion source resembles the EI source but is designed to have an ionization chamber (< 1ml volume) where the reactions take place that is much more enclosed. The filament generating the electrons is positioned just outside the chamber. The electrons emitted from the filament into the ion chamber are accelerated to between 100 and 200 eV for optimal penetration of the reagent gas. The electron entrance orifice is small to keep the chamber tight and reagent gas pressure high. The reagent gas enters the ionization chamber via the GC/MSD interface. Because the amount of reagent gas in the source completely overwhelms the analyte, the reaction of the analyte is very efficient. The shape of the source is designed that the partial pressure in the outer chamber is about 10^{-5} to 10^{-6} torr in order to maintain a collision free path for the ions to reach the analyser. Mass spectrometry using such a source design is named as High Pressure Mass Spectrometry (HPMS).

2.2 PCI reactions of different Reagent Gases

The most frequently used reagent gases are methane, iso-butane and ammonia. The reagent gas is ionized by electrons entering the ionization source and, because the pressure of the reagent gas is high, a number of reactions occur. The principal methane reactions are:

$$CH_4 + e^- \rightarrow CH_4^+, CH_3^+, CH_2^{+\cdot}$$
$$CH_4 + CH_4^+ \rightarrow CH_5^+ + CH_3^{\cdot} \quad mz\ 17$$
$$CH_2^{+\cdot} + CH_4 \rightarrow C_2H_4^{+\cdot} + H_2 \quad mz\ 28$$
$$CH_2^{+\cdot} + CH_4 \rightarrow C_2H_3^+ + H_2^{\cdot}H \quad mz\ 27$$
$$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2 \quad mz\ 29$$
$$C_2H_3^+ + CH_4 \rightarrow C_3H_5^+ + H_2 \quad mz\ 41$$

This stepwise description of the gas phase reactions is for clarity only and in fact all the processes are proceeding simultaneously. Of special interest is the CH_5^+ ion which is a strong proton donator and can form a protonated molecule with an analyte. Additionally methane forms characteristic molecular adduct ions :

$$CH_5^+ + M \rightarrow [MH]^+ + CH_4 \quad mz\ M+1$$
$$C_2H_5^+ + M \rightarrow [M+C_2H_5]^+ \quad mz\ M+29$$
$$C_3H_5^+ + M \rightarrow [M+C_3H_5]^+ \quad mz\ M+41$$

Such processes are described by the term "Proton Affinity" (PA). PA is defined as the thermochemical

ability of the reaction partners to transfer protons. In order to generate a protonated molecule, the PA of the analyte must be greater than that of the reagent gas ion. As the difference between the PA of the analyte and the PA of the reagent gas ion increases the amount of fragmentation of the analyte increases. Referring to literature, the PA of organic compounds is between 180kcal/mol to 240kcal/mol. In most cases, the PA of the compound of interest is unknown and successful PCI requires experimentation with reagent gases in each case.

Reagent Gas Ions and their Proton Affinities (kcal/mol):

Hydrogen	H_3^+	101
Methane	CH_5^+	132
iso-Butane	$C_4H_9^+$	196
Ammonia	NH_4^+	204

Ammonia has a high PA value which is closest of the common gases to the PA of most organic molecules so it often provides better differentiation (more selectively) between the sample matrix and the analyte. Non-polar compounds or matrices consisting mostly of C and H, are less readily ionized in comparison to more polar compounds. For example, carboxylic esters, such as the phthalates (Agilent Technologies Application Note 5988-2244EN) show a high affinity for ammonia because of the polar ester-linkages (COO groups).

Formation of Ammonia Reagent Gas Ions:

$NH_3 + e^- \rightarrow NH_3^+, NH_2^+$
$NH_3^+ + NH_3 \rightarrow NH_4^+ + NH_2^+$ *mz* 18
$NH_2^+ + NH_3 \rightarrow NH_3^+ + NH_2$ *mz* 17
$\rightarrow (NH_4NH_3)^+$ *mz* 35
$\rightarrow (NH_4(NH_3)_2)^+$ *mz* 52

Typical ammonia reactions with an analyte (M)

$M + NH_4^+ \rightarrow (M+NH_4)^+$ M + 18
$M + NH_4^+ \rightarrow (M+H)^+ + NH_3$ M + 1

Analytes suited for ammonia reaction generate the characteristic $[M+NH_4]^+$ adduct ion, some times the protonated molecule and most often both.

The choice of the applied reagent gas determines the fragmentation behavior of the analyte and consequently the result of the PCI measurement. PCI processes generating little fragmentation are referred to as "soft ionization" and the corresponding the reagent gases are called "soft reagent gas". Conversely, reactions creating a great deal of fragmentation are named "hard ionization" and the gas, a "hard reagent gas" .

In some cases the Hydride Abstraction Reaction is of interest, and often occurs with long chain alkanes or compounds containing long chain alkyl groups:

$CH_5^+ + M \rightarrow (M-H)^+ \ldots$

In these cases the PA of the reagent gas ion is greater than the PA of the analyte.

2.3 Examples of EI/PCI

Figure 1 shows an example of the differences in response for the different ionization processes and PCI reagent gases for methyl palmitate. In full scan mode the total ion response follows EI > PCI/CH_4 > PCI/NH_3.
The spectra reflects the characteristics of each reaction, Figure 2

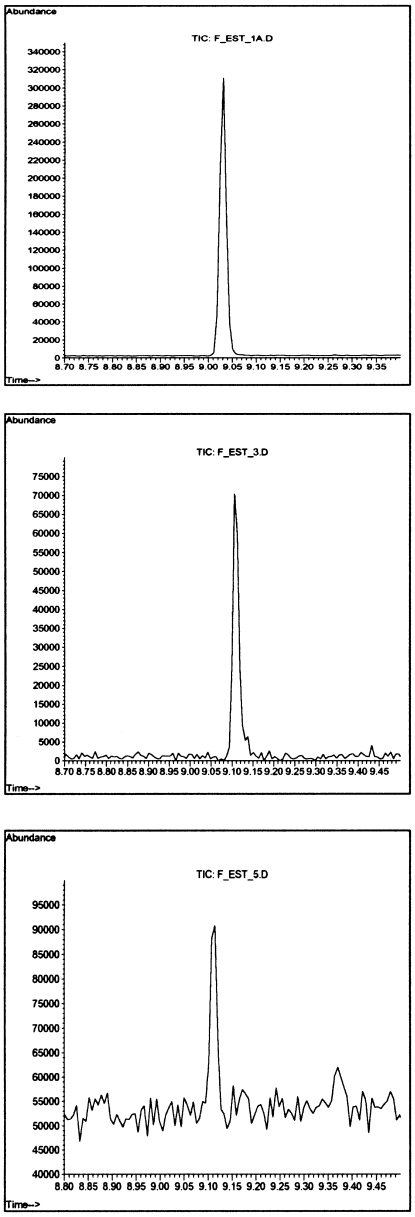


Figure 1. Total ion chromatograms of methyl palmitate at 600pg injected, in EI (top), PCI-CH_4 (middle) and PCI-NH_3 (bottom)

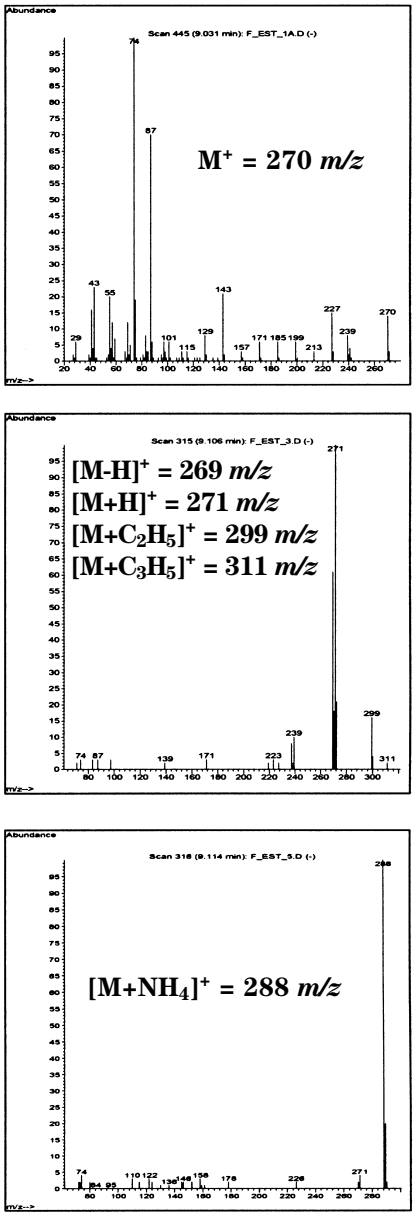


Figure 2. Full Scan Spectra referring to Figure 1

3. Electron Capture Negative Ionization (ECNI)

The importance of the ECNI technique becomes readily evident in view of the selectivity of the detection of suitable analytes at very low concentration levels (ppt). In addition, the typical sample matrix interferences are generally suppressed in ECNI MS mode resulting in very high signal-to-noise values. Suitable analytes for NCI, have a high electron capture capacity or high electron affinity (EA). The expression "chemical ionization" is not applicable because the important part of the reaction is achieved by capture of low energy electrons – "thermal" electrons – by the analyte so there are no ion molecular reactions involved in the initial ion creation process. Thermal electrons are generated by collision of electrons emitted from the filament with buffer gas (e.g. methane) molecules located at high pressure in the ionization chamber of the source:

$\mathbf{e^{**-}}$ (70 eV) **+** $\mathbf{CH_4}$ (buffer gas) → $\mathbf{e^{*-}}$ **thermal Electrons** (2 eV)
$\mathbf{e^{*-} + M \rightarrow M^-}$

Analytes with high EA form stable molecular anions, M^-, and show a simple spectrum. (See Figure 3). This process is resonant electron capture and commonly called NCI. Electrons with an energy potential of about 15 eV lead to dissociative reactions:

$\mathbf{e^*}$ (15eV) **+ MX → M + X**$^-$

Mass spectra resulting from these reactions exhibit more fragmentation and less sensitivity compared with the ECNI process. Though it is hard to predict which analytes are suitable for NCI some rules of thumb are used. Good results are often obtained with compounds already successfully analyzed by GC/ECD. ECNI candidates contain multiple halogen groups, or nitro groups, or double bond and/or conjugated structures, and/or hetero atoms. Polar compounds which are suitable for derivatization can be modified with perfluoro reagents in order to integrate a group with a high EA into the molecular structure and improve chromatography.

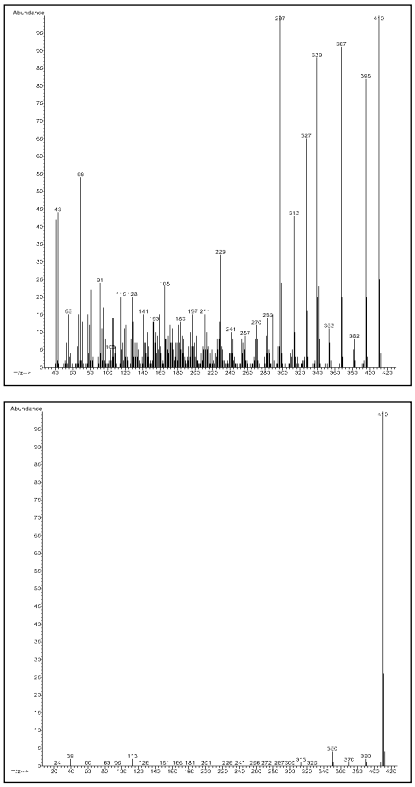


Figure 3. Mass spectra of tetrahydrocannabinol as the trifluoroacetic acid anhydride derivative (nominal mol. wt. 410 g/mol) in EI (top) and ECNI-CH4 (bottom)

3.1 Optimizing ECNI measurements

In ECNI mode some ion source operating parameters can be modified to improve sensitivity.

Buffer Gas

Raising the flow amount of the buffer gas, which influences the collision process, will sometimes lead to an increase in response. Such optimization refers to the term "High Pressure Electron Capture Mass Spectrometry" (HPECMS). The quality and purity of the buffer gas are of importance. The gas purity should be in the range of 99.95% (3.5) to 99.995% (4.5). In addition to methane, ammonia and carbon dioxide are frequently applied. Oxygen and water are very efficient at collecting electrons and can suppress analyte response and great care must be taken that they are be excluded.

Ion Source Temperature

The ionization chamber temperature dramatically affects the reaction yield and the fragmentation behavior and consequently, the sensitivity. Low ion source temperature favors the electron capture process. As a consequence of the operation of the source (hot filament) and the GC (hot carrier gas), the lowest practical source temperature is about 150°C. Considering the analyte elution temperature, the ion source body sometimes is a "cold spot" and might be a reason for tailing peak shape and matrix effects.

Tuning Parameters

The tuning program controls the parameters of electron energy (eV) and emission current (µA). Electron energy (EE) has some influence on the mobility of the electrons and their penetration efficiency in the ionization chamber. A high EE value is usually advantageous. Emission current (EC) is related to the amount of electrons emitting from the filament. Increasing the EC value leads to response additional but filament lifetime has to be considered. Higher ECs lower filament life and the accuracy of the filament position above the electron entrance slit may become degraded due to deformation.

4. Practical Hints

The following hints are based on the experience of the author and are to be considered as advice and do not represent a guarantee in all circumstances or applications.

4.1 Gas Chromatography

Chemical Ionization requires the same GC criteria as applied in EI mode. Carrier gas purifiers are highly recommended. Also an air-water check should be performed prior to switching to CI. Before developing a CI method, all GC operating parameters such as injection technique and the capillary column should be tested in EI mode in order to avoid any kind of sample discrimination. Thermally labile compounds are analysed using on-column or PTV injection systems which permit cryo-focussing and sample enrichment. Splitless injection executed in pulsed-pressure mode

has the advantages of almost quantitative sample transfer from the injection liner into the column and the reduced residence time of the sample in the injector inlet. The liner type has to be appropriate to the injection technique. In this compendium, for splitless injection a deactivated double-taper liner (Agilent Part Nr. 5181-3315) was used. The choice of the column is always related to the analytical problem, however, for the majority of the analytes documented here, the MSD standard column (HP-5ms, 30m x 0.25mm x 0.25µm, Agilent Part Nr. 19091S-433) was adequate. Sample matrix interferences may suggest use of another phase and /or GC oven program.

4.2 PCI and NCI Conditions

For both PCI and NCI techniques, the MSD software includes sophisticated autotuning programs. These autotune programs assist adjusting the flow of reagent gas and the other parameters necessary for successful CI operation. These autotune values are a good starting point. The value for Electron Multiplier Voltage (EM Voltage) normally needs to be increased in over autotune values by about 400 V. In PCI mode, the results are primarily dictated by choice and pressure of the reagent gas, altering autotune parameter values has almost no benefits. In ammonia PCI, increasing the pressure typically increases the formation of adduct ions. In NCI mode the previously discussed tune parameters are worth adjusting in order to improve sensitivity. In any case frequent tuning should avoided, especially in ECNI mode where the residual tuning gas can increase the background for several hours due to the extreme sensitivity in ECNI. The analyte amount (absolute amount onto the column) recommended for method development in scan mode are 10ng for PCI and 1ng or less for NCI.

4.3 Derivatization

Polar, chromatographically difficult compounds frequently need derivatization before analysis. In NCI mode, derivatized analytes show increased sensitivity. The following recommendations refer to the literature references and to the author's lab experiences. Derivatization reagents and reaction (incubation) criteria are matter of choice and may be varied in order to improve reaction yield and sensitivity. For a reaction vial, the "High Recovery Vial", 1.5ml volume, conical bottom autosampler vial (Agilent Part Nr. 5182-3454) is recommended.

Care must be taken to insure that all solvents used for derivatization are free of water. The solution containing the sample that is to be derivatized is evaporated (blown-down) with purified dry nitrogen introduced into the vial by means of a capillary steel tube (1/16" o.d.) or glass pipet. The tube's opening is positioned some millimeters above the liquid surface and a gentle gas flow (checked prior to placing over the reaction vial) which forms only a small depression on the surface, is applied until the solvent has completely evaporated. The derivatization reagent is added to the dry residue, the vial sealed, and allowed to react for a specific length of time, at a regulated and usually elevated temperature. Depending on the chemical nature of the derivatizing reagent (i.e., capable of degrading the capillary column phase), it may have to be removed by nitrogen blow-down as described above and reconstituted in an appropriate solvent before the sample is injected. Sometimes an adequate dilution must be prepared. According to experience, most derivatives are unstable, even if they are stored at low temperature. In most cases, when analyzing a sample instead of a standard, derivatization happens to analytes and to any reactive matrix compounds present. Such byproducts can complicate the chromatogram and spectral analysis and should be minimized or eliminated by further sample preparation steps or improved chromatography.

Chemically aggressive reagents will deteriorate the stationary phase of the column, especially when applying splitless or on-column injection. With split injection, care should be taken to avoid corrosion of the gas tubing connected to the injector and that the the gas regulation module (EPC module) is not damaged.

5. Instrumentation

The documented applications were executed with the Agilent Technologies GC/MSD System:

- Gas Chromatograph 6890plus, split/splitless and On Column Injector, Autosampler 7673
- Mass Spectrometer MSD 5973N, CI Option
- HP Kayak XA, ChemStation Software Vers. G1701CA

As reagent gas or buffer gas methane (4.5) and ammonia (3.5 or 4.0), Linde Gas AG, were used. All gas supply tubes were of stainless-steel material and a gas purifier (Agilent Part Nr. 1999-80410 used for **only** methane) were installed between the MSD and the gas bottles. For ammonia, an appropriate, corrosion resistant gas regulator was used. The ammonia gas stainless steel supply tube was coiled and the pressure was adjusted to approximately 7 psi (0,5 bar) in order to avoid generating droplets. (For more information on operating in ammonia, refer to Agilent Technolgies Technique Brief Nr. 5968-7844E).

6. Literatur

"Chemical Ionization Mass Spectrometry", 2nd Edition, Alex G. Harrison, CRC Press, ISBN 08493-4254-6

"Introduction to Mass Spectrometry", Chapter Six, 2, J. Throck Watson, Raven Press, New York

"High Pressure Electron Capture Mass Spectrometry", W. B. Knighton, L. J. Sears and E. P. Grimsrud, Mass Spectrometry Reviews, 1996, 14, 327-343

"Handbook of Analytical Derivatization Reactions", D. R. Knapp, John Wiley & Sons, ISBN 0-471-03469-X

"Handbook of Derivatives for Chromatography", 2nd Edition, K. Blau and J. Halket, John Wiley & Sons, ISBN 0-471-92699-X

"Silylating Agents", Fluka, ISBN 3-905617-08-0

Contents

Component

Summary

Acepromazine

CAS-Nr. 61-00-7
Molecular formula: $C_{19}H_{22}N_2OS$

GC-Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 0.7ml/min, 30cm/sec
Mode: Constant Flow
Injection: Split, 250°C
Oven Temp. Program
120°C (0.3 min) - 20°C/min to 300°C (4 min)

MS-Parameter

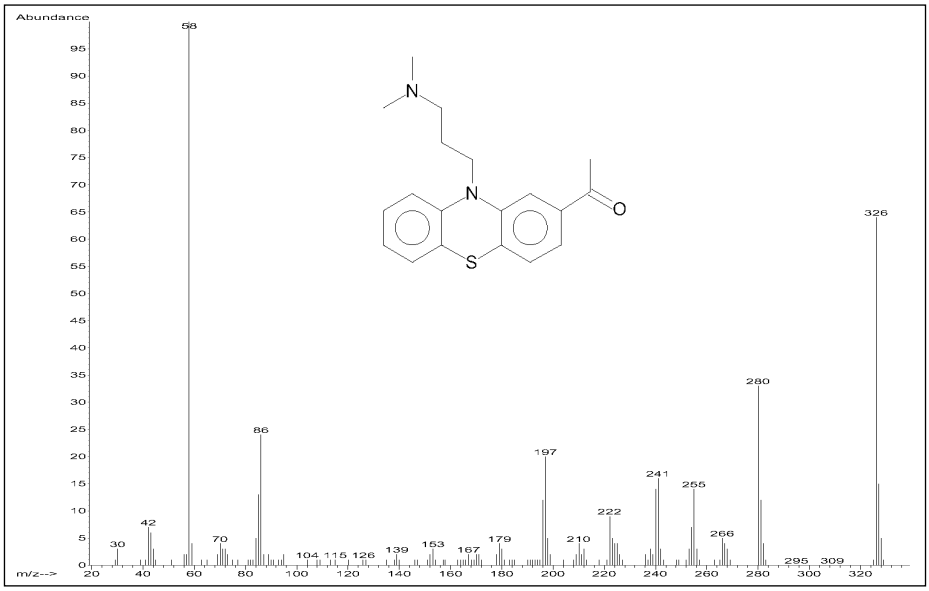
Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C

Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1 ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V

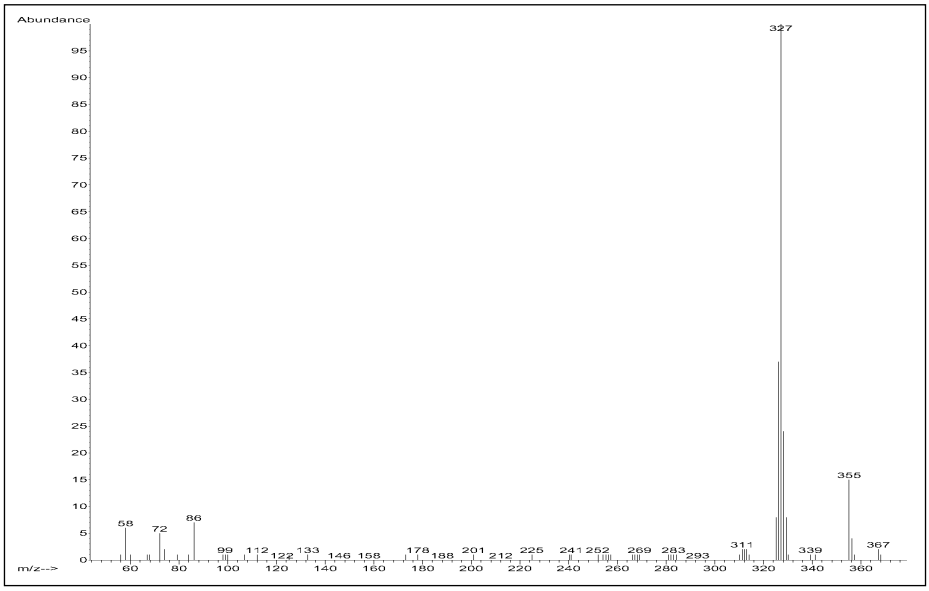
Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1 ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V

Results

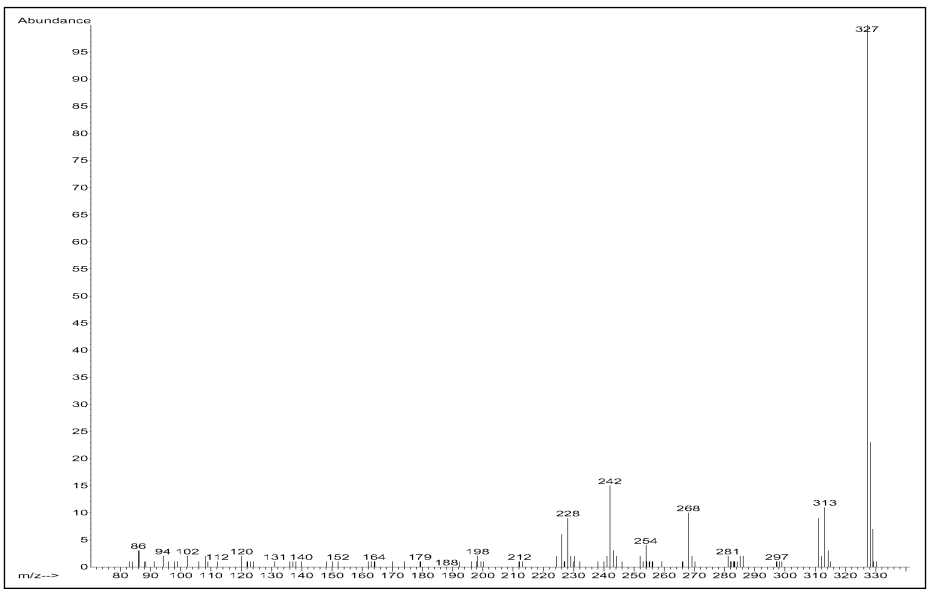
Analyte Retention Time: 11.60min
Analyte Concentration: 4ng/µl
Signal/Noise Ratios for TIC
PCI/CH_4 Scan: > 18/1
PCI/NH_3 Scan: > 25/1



EI Spectrum, Acepromazine: *m/z* 326; M^+



PCI/CH_4 Spectrum, Acepromazine: *m/z* 327, 355, 367; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



PCI/NH_3 Spectrum, Acepromazine: *m/z* 327; $[M+H]^+$

Barbiturates

Amobarbital CAS-Nr. 57-43-2
Molecular formula: $C_{11}H_{18}N_2O_3$
Barbital CAS-Nr. 57-44-3
Molecular formula: $C_8H_{12}N_2O_3$
Butethal CAS-Nr. 77-28-1
Molecular formula: $C_{10}H_{16}N_2O_3$
Pentobarbital CAS-Nr. 76-74-4
Molecular formula: $C_{11}H_{18}N_2O_3$
Secobarbital CAS-Nr. 76-73-3
Molecular formula: $C_{12}H_{18}N_2O_3$

GC-Parameter

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 0.7ml/min, 30cm/sec
Mode: Constant Flow
Injection: Pulsed splitless, 250°C
Oven Temperature Program
60°C (1min) – 20°C/min to 180°C
10°C/min to 300°C

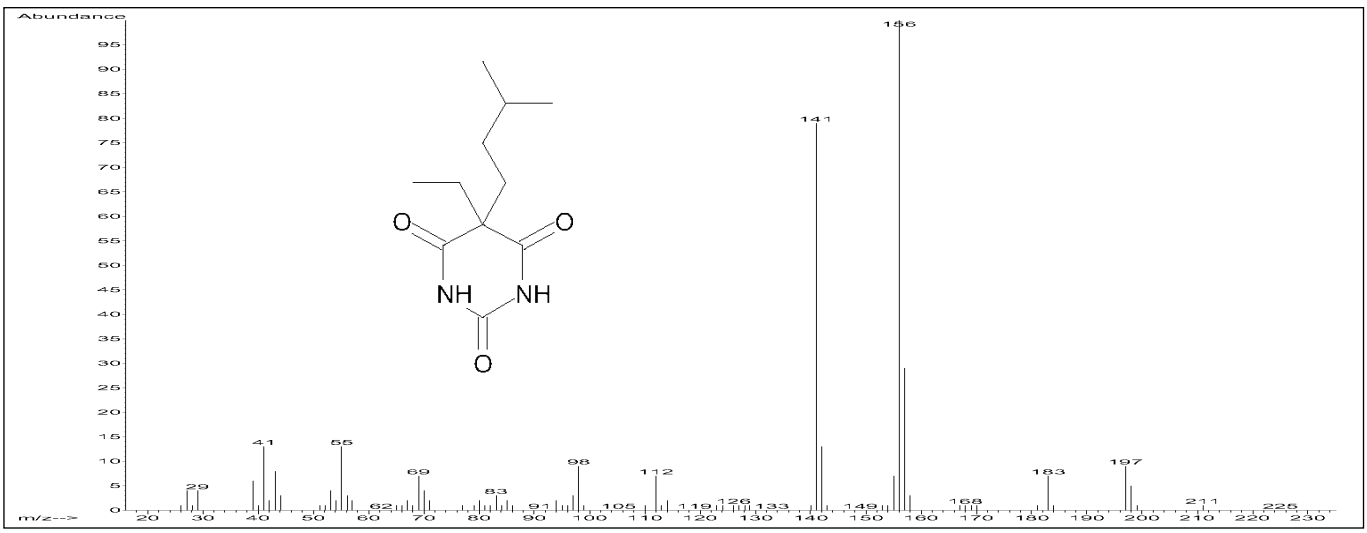
MS-Parameter

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C
Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V
Mode: ECNI/CH_4 – SCAN/SIM
Buffer Gas: Methane
Flow (Setting): 2ml/min (40)
Tune: ECNI-Methane Tune File
Temperatures:
Source 150°C, Quad 106°C

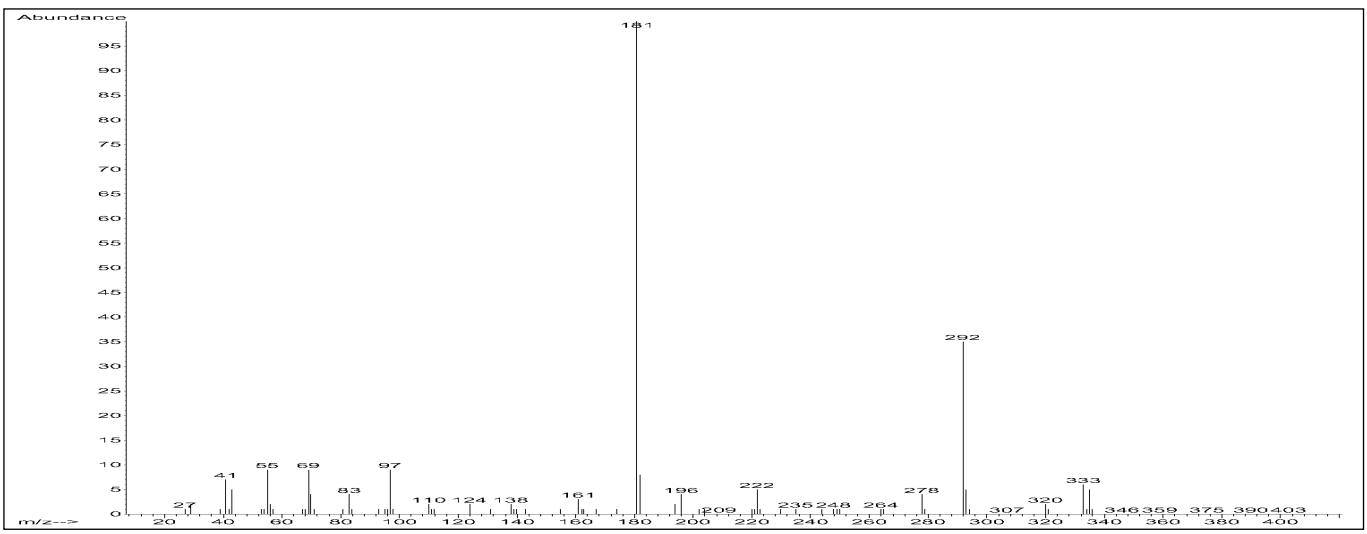
Remarks

Derivatization with Penta-fluorobenzylbromide (PFBB)
To 100µl of the Barbiturate Standard (SIGMA D 3155), concentration 20ng/µl each, diluted in ethylacetate, 10µl of the derivatization reagent and 10µl of triethylamine are added and the mixtures is incubated for 60 min at 60°C. The reaction leads to the mono-derivatives. An aliquot of the derivatized solution is used for GC/MSD measurement.
Caution: Only diluted samples are injected in order to avoid column stationary phase deterioration.

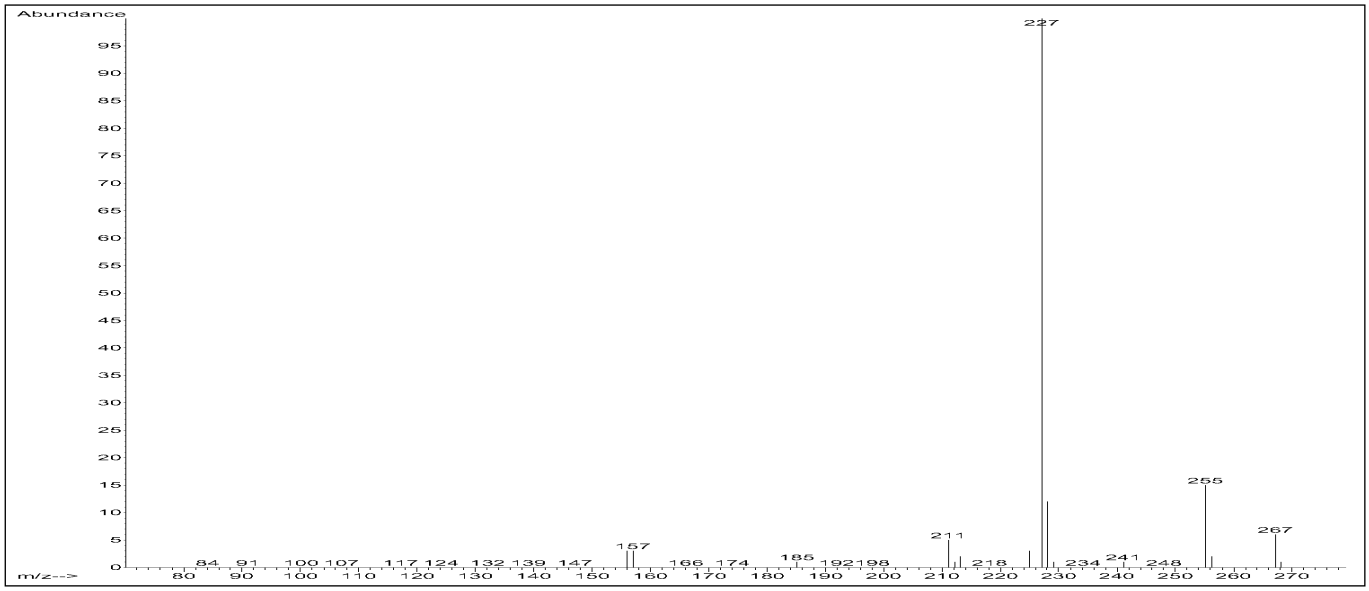
Results
Underivatized Barbiturates can be measured without problems. The derivatization improves sensitivity and also increases molecular mass (+181amu) which is advantageous especially in SIM mode. A drastic improvement is noticed in ECNI mode. The signal/noise ratio for 1pg/µl analyte concentration in ECNI mode exceeds 200:1. Sensitivity for the derivatives in PCI with NH_3 is very low.



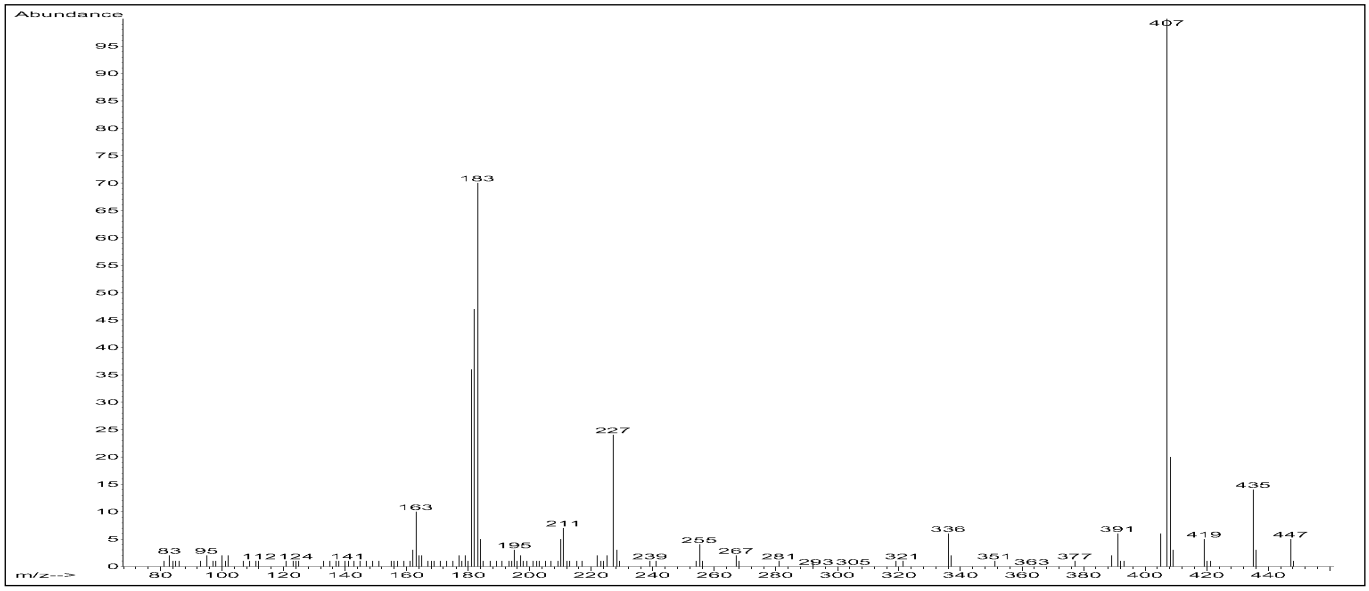
EI-Spectrum, Amobarbital, underivatised, *m/z* 226; M^+



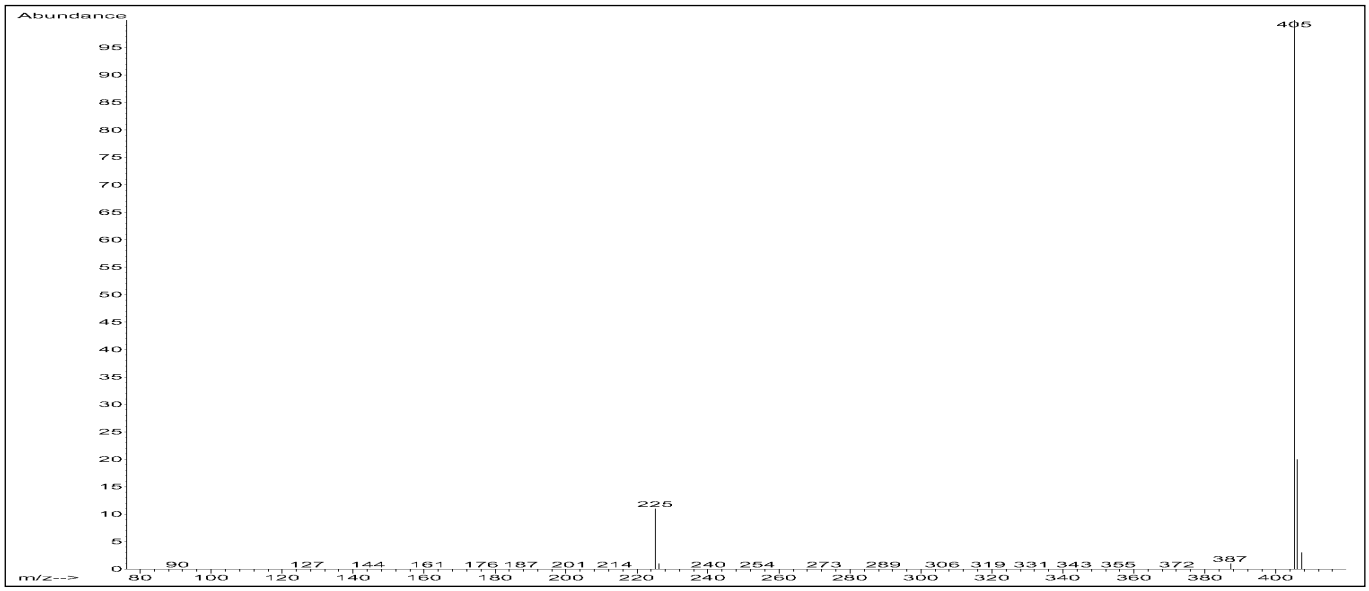
EI-Spectrum, Amobarbital, PFBB-derivative, *m/z* 406; M^+



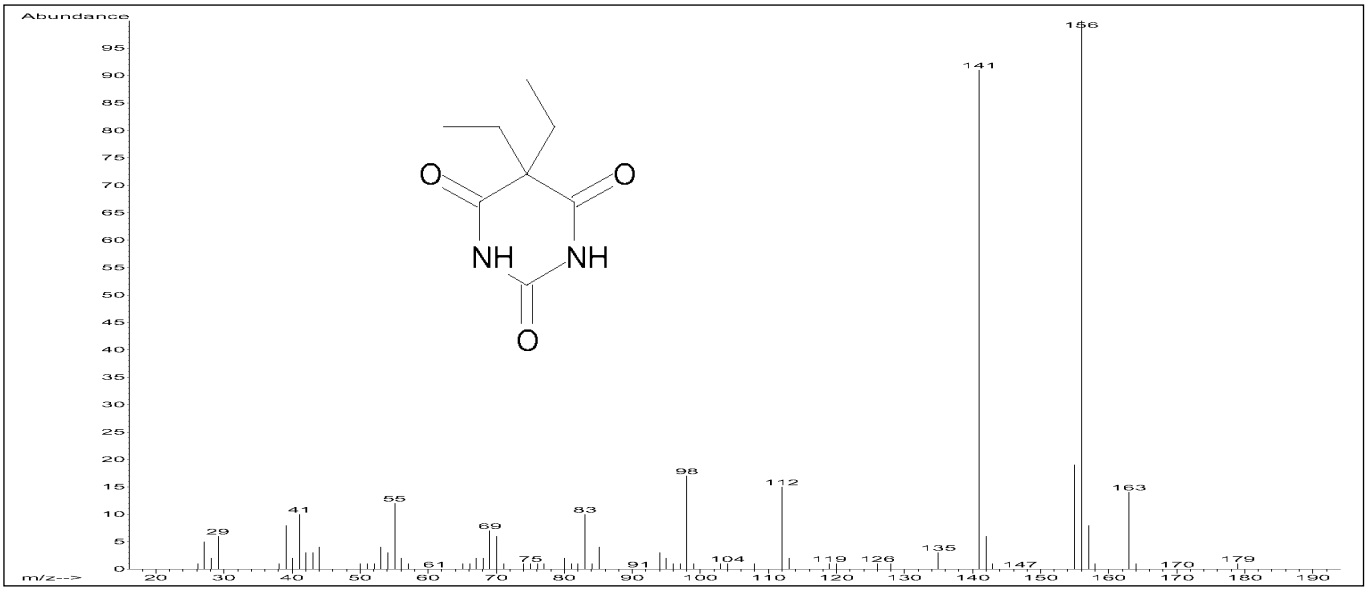
PCI/CH_4-Spectrum, Amobarbital, underivatised: *m/z* 227, 255, 267; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



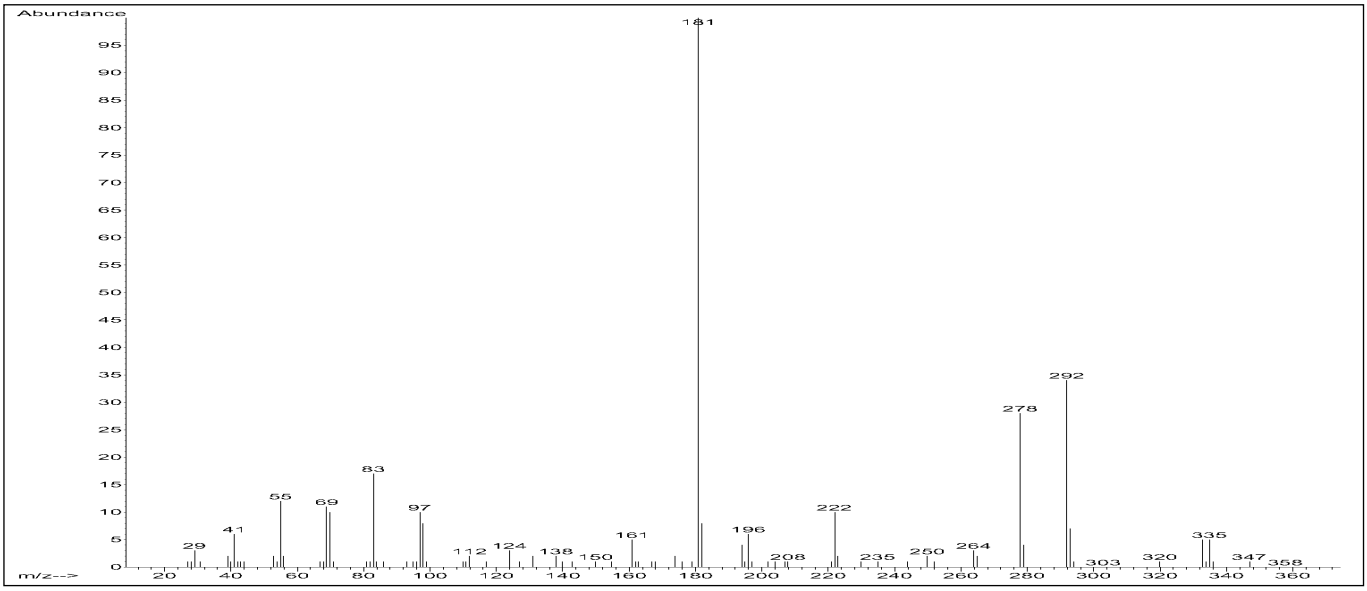
PCI/CH_4-Spectrum, Amobarbital, PFBB-derivative: *m/z* 407, 435, 447; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



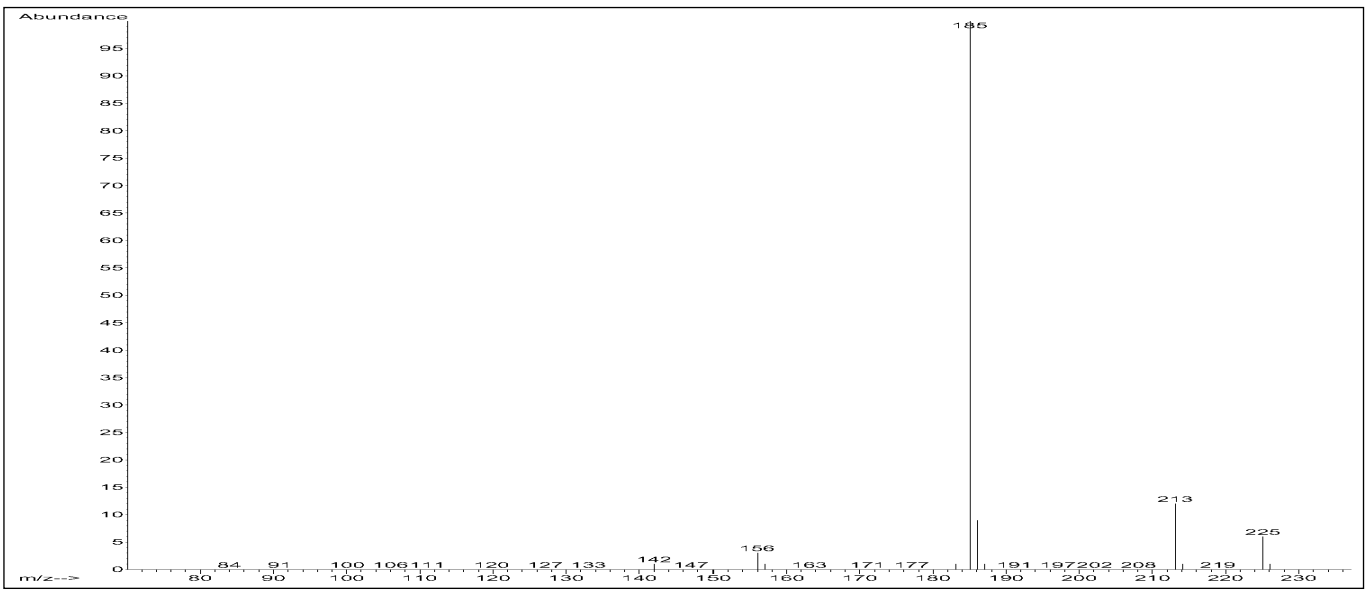
ECNI/CH_4-Spectrum, Amobarbital, PFBB-derivative: *m/z* 405; $[M-H]^-$



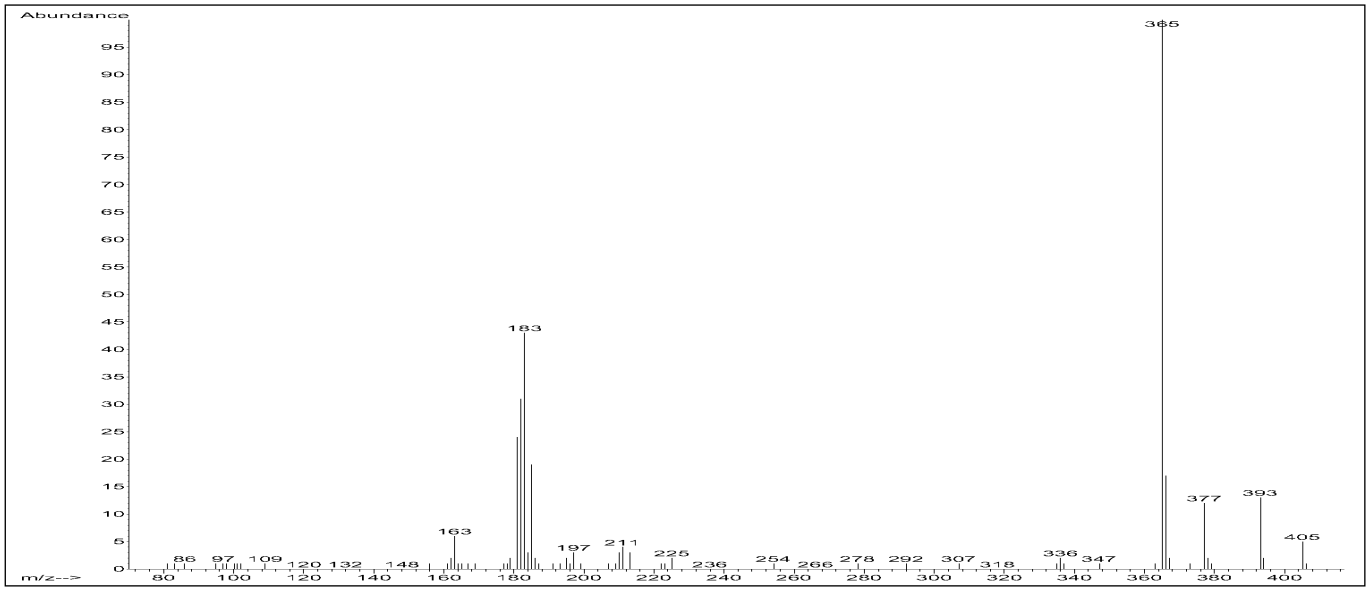
EI-Spectrum, Barbital, underivatised, *m/z* 184; M^+



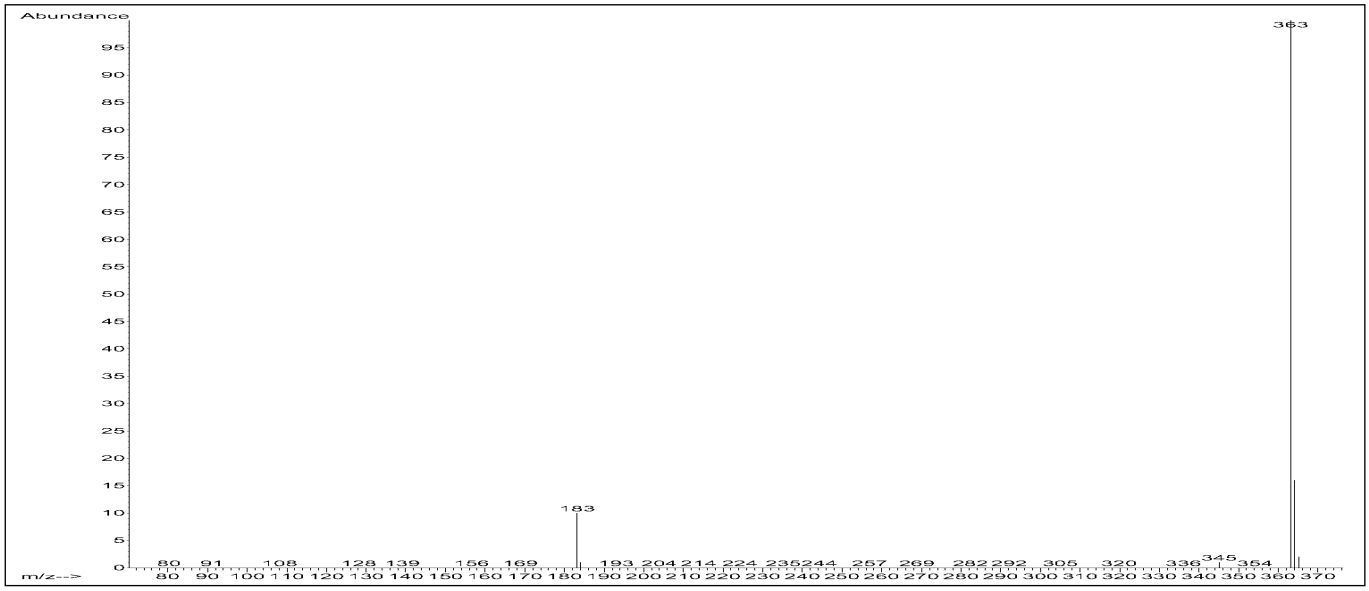
EI-Spectrum, Barbital, PFBB-derivative, *m/z* 364; M^+



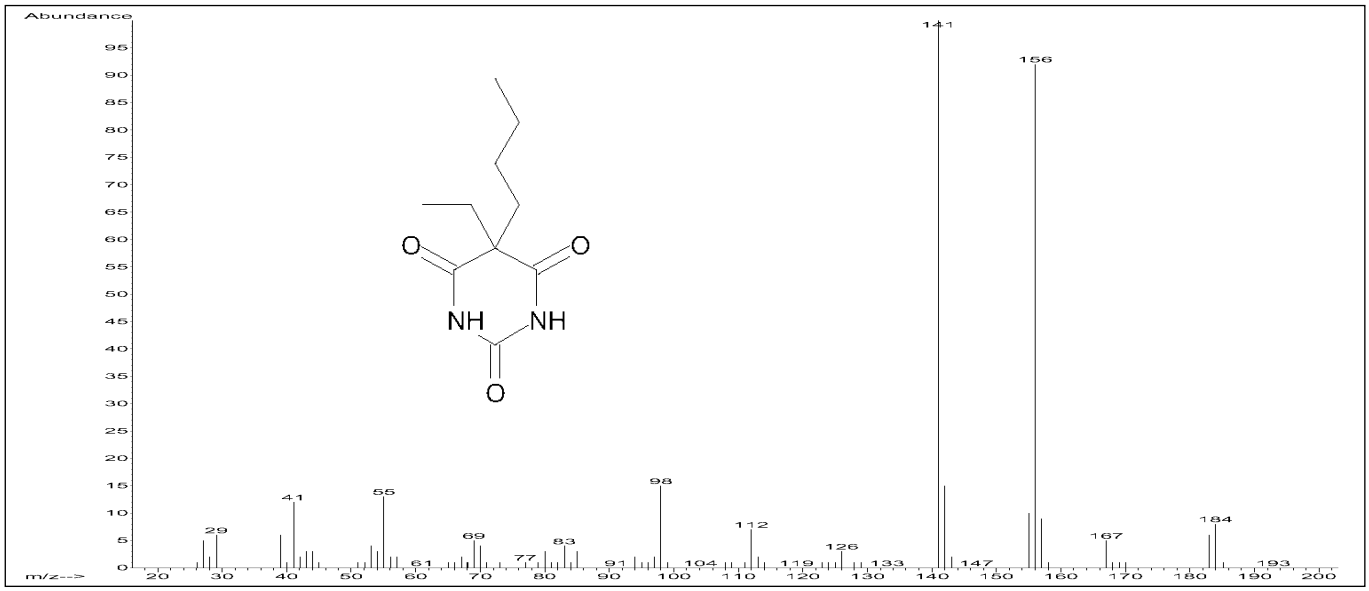
PCI/CH_4-Spectrum, Barbital, underivatised: *m/z* 185, 213, 225; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



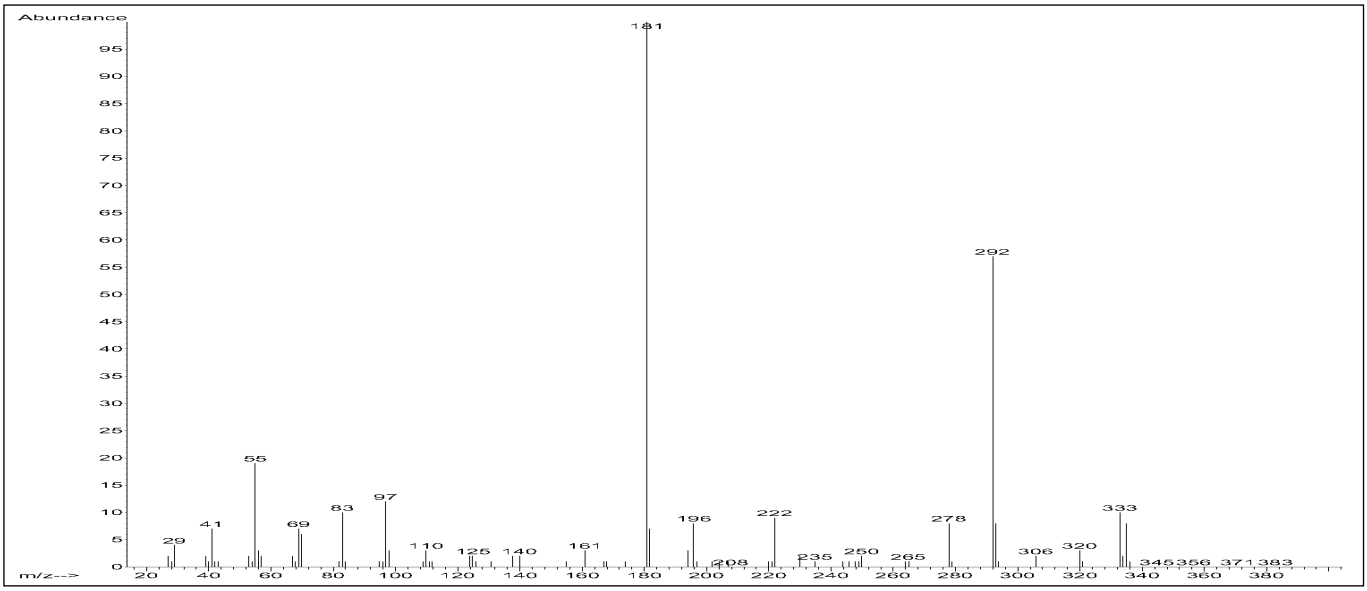
PCI/CH_4-Spectrum, Barbital, PFBB-derivative: *m/z* 365, 393, 405; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



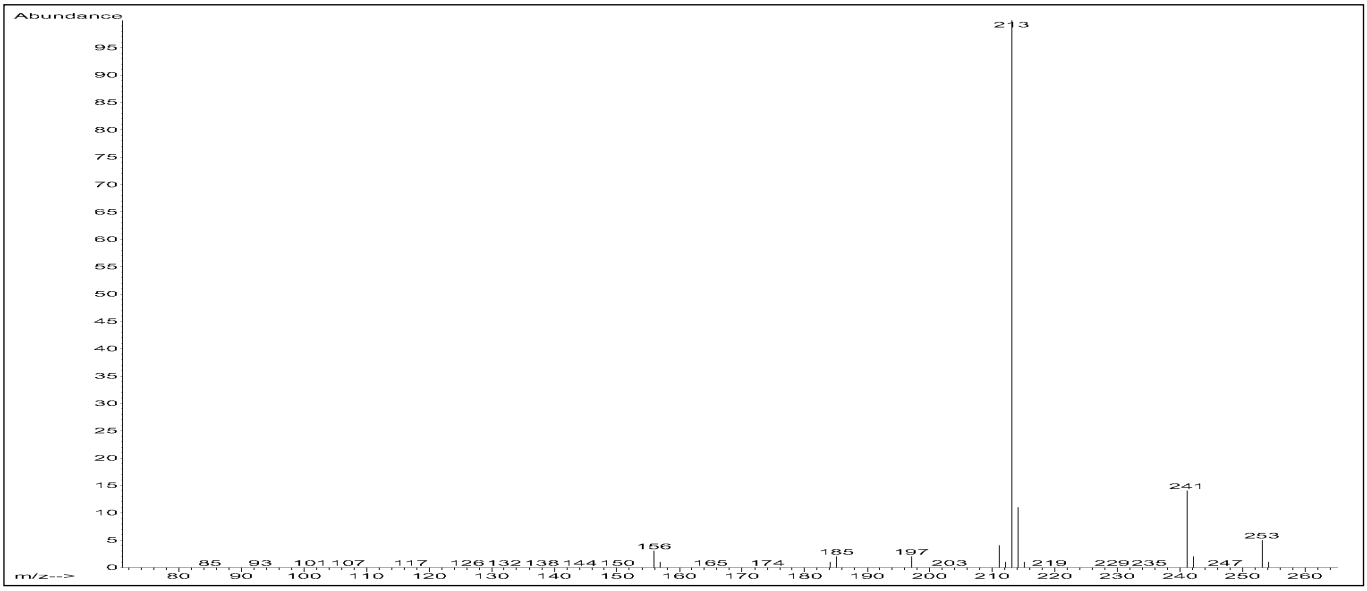
ECNI/CH_4-Spectrum, Barbital, PFBB-derivative: *m/z* 363; $[M-H]^-$



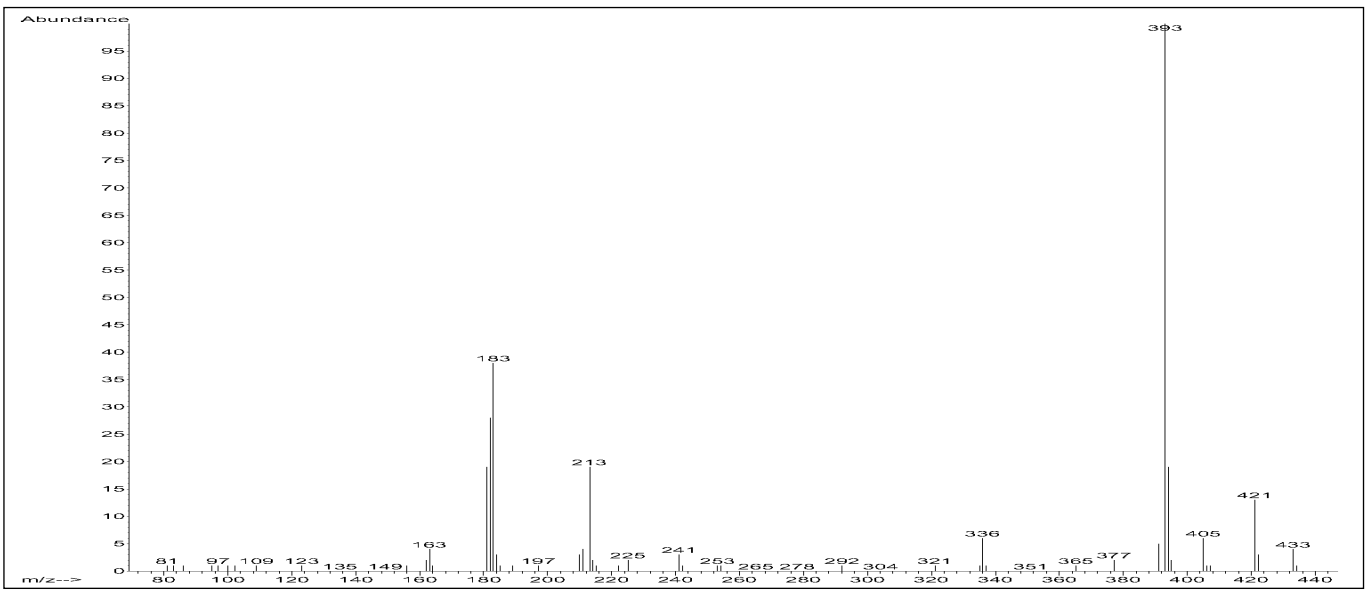
EI-Spectrum, Butethal, underivatised, *m/z* 212; M^+



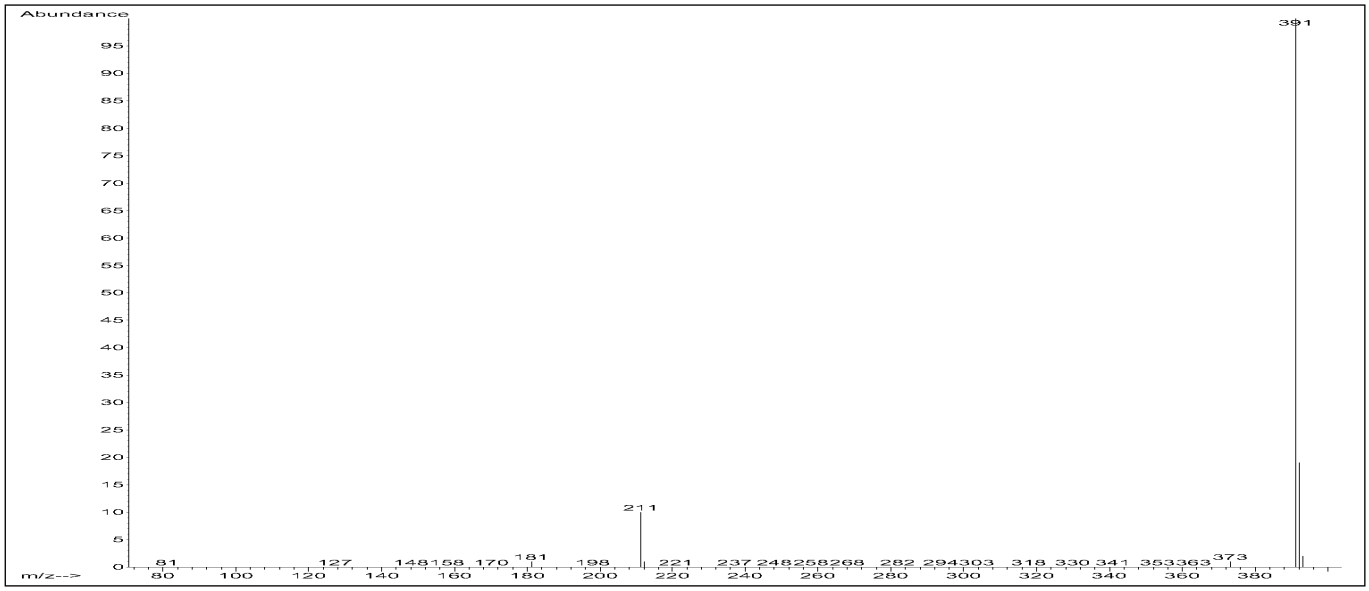
EI-Spectrum, Butethal, PFBB-derivative, *m/z* 392; M^+



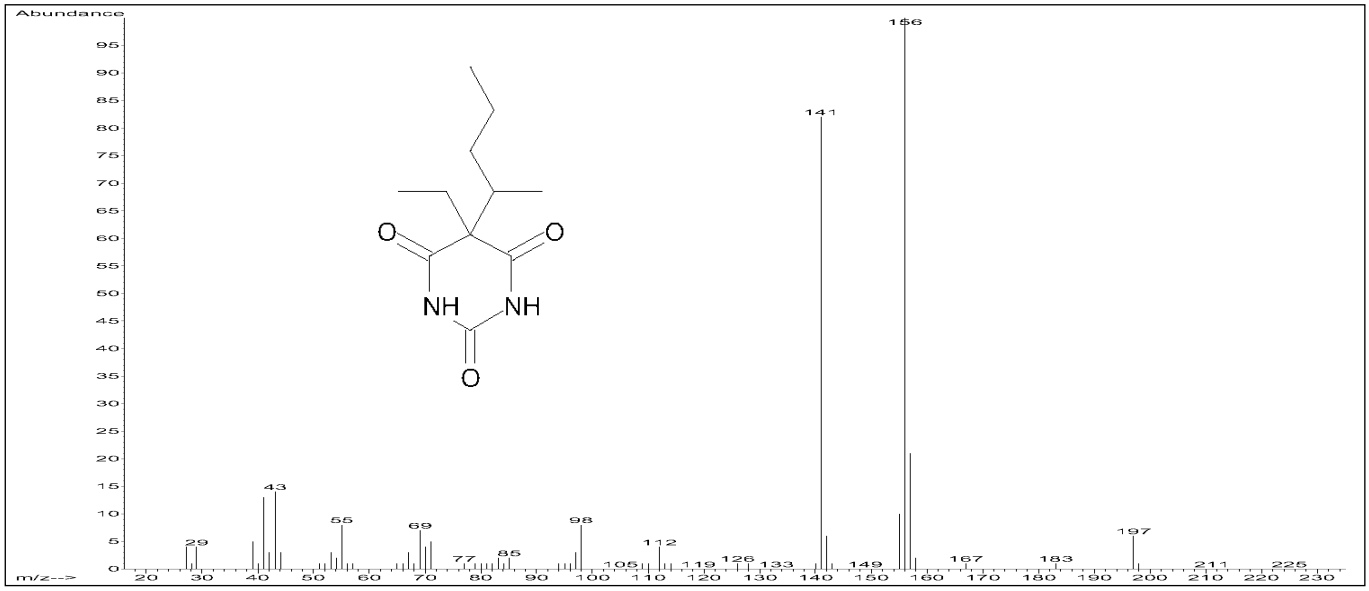
PCI/CH_4-Spectrum, Butethal, underivatised: *m/z* 213, 241, 253; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



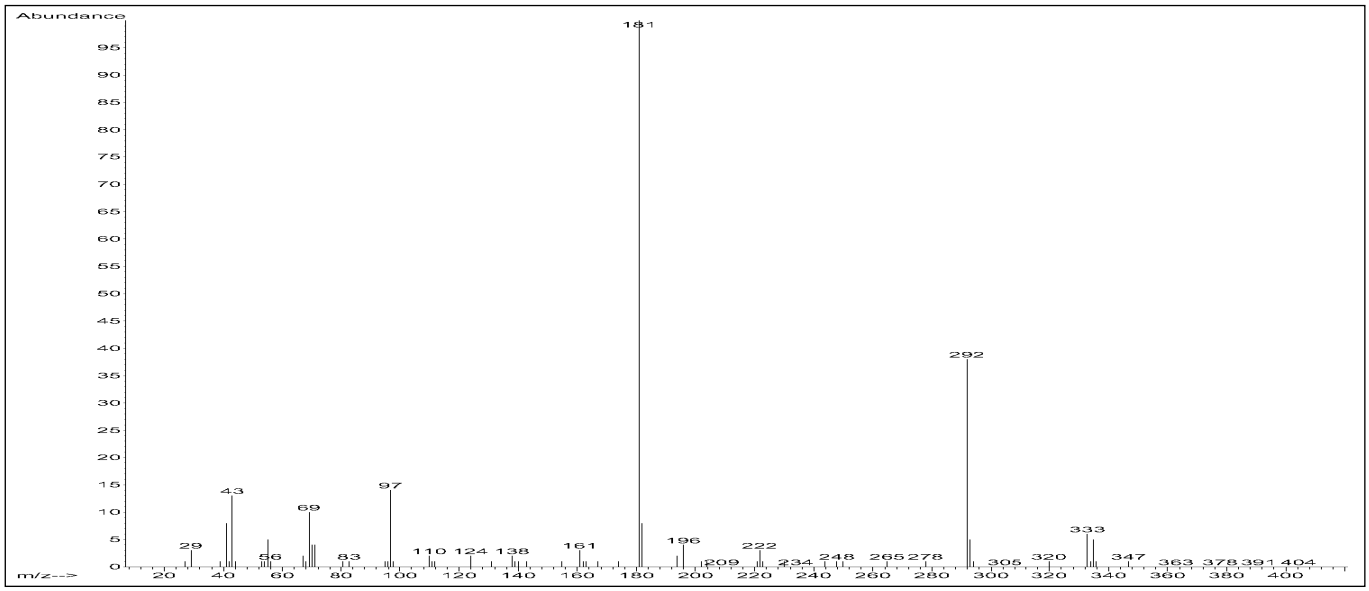
PCI/CH_4-Spectrum, Butethal, PFBB-derivative: *m/z* 393, 421, 433; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



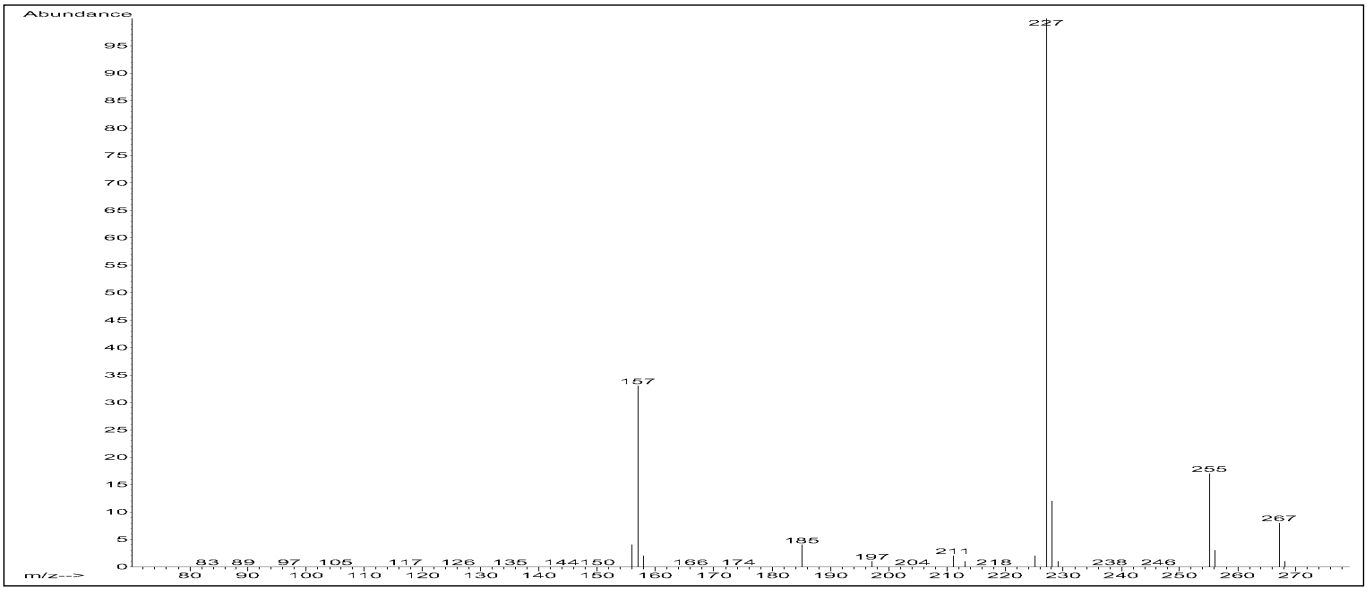
ECNI/CH_4-Spectrum, Butethal, PFBB-derivative: *m/z* 391; $[M-H]^-$



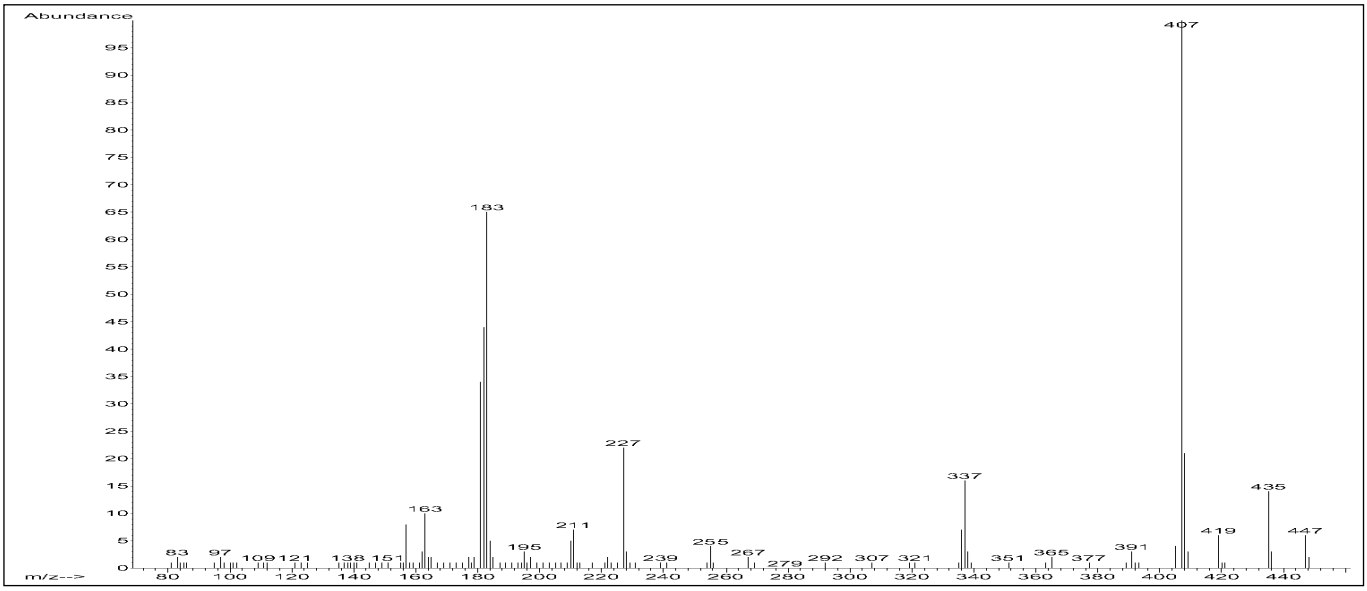
EI-Spectrum, Pentobarbital, underivatised, *m/z* 226; M^+



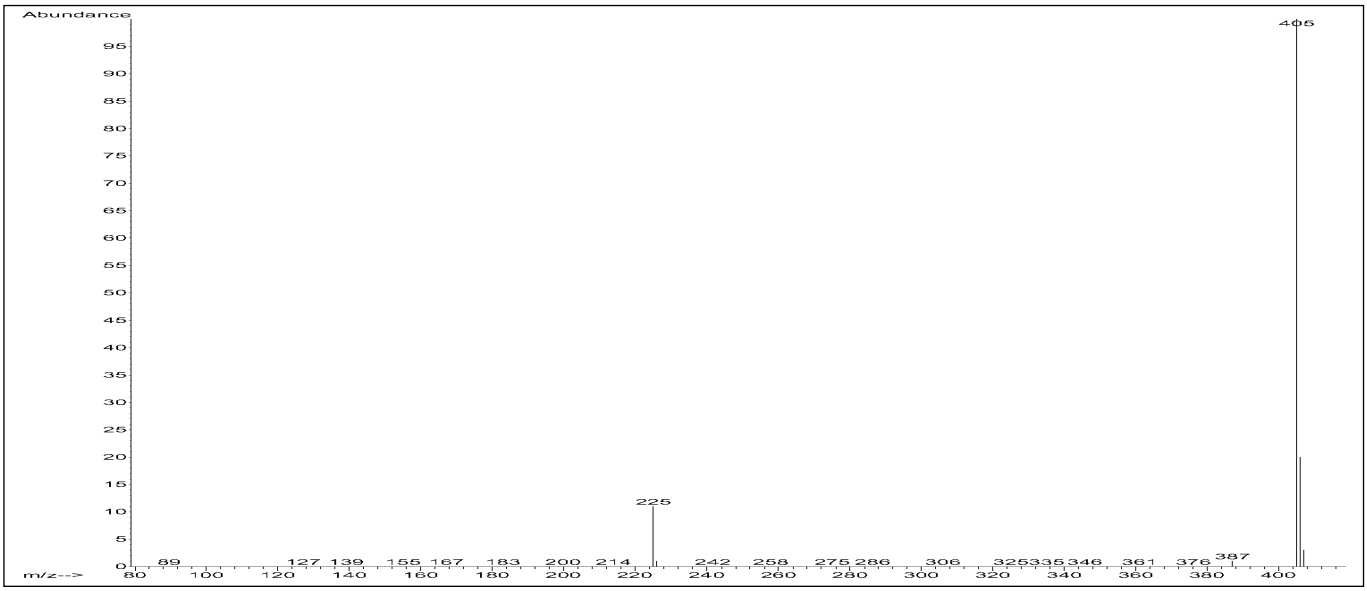
EI-Spectrum, Pentobarbital, PFBB-derivative, *m/z* 406; M^+



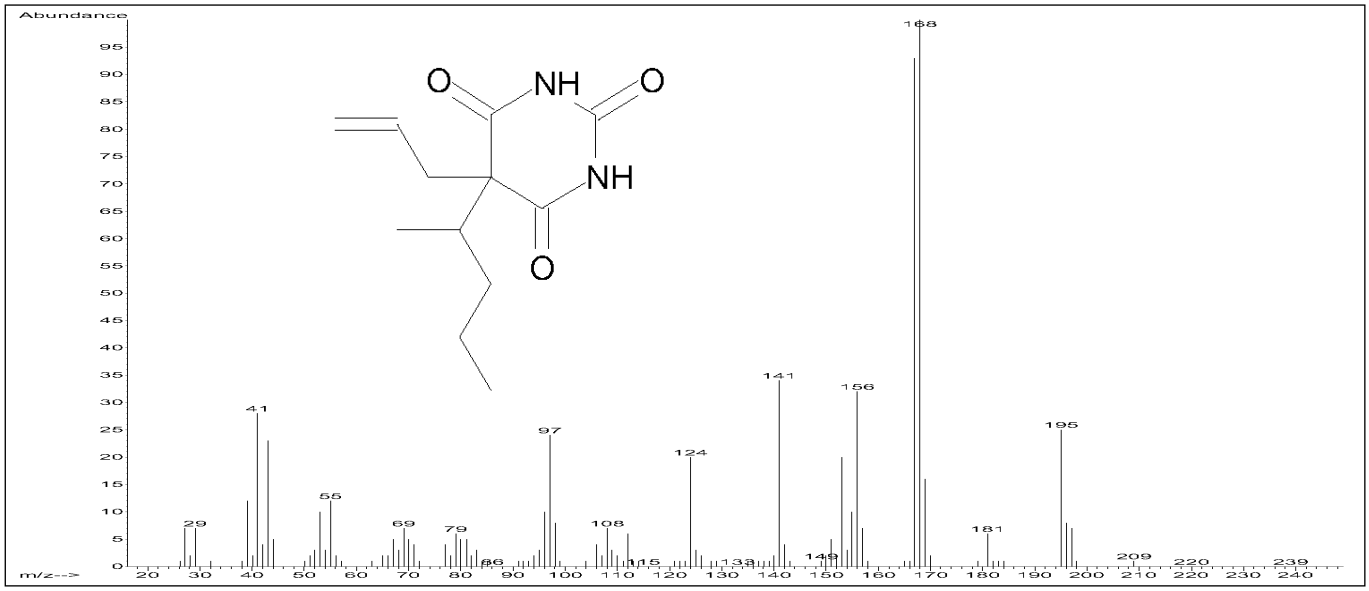
PCI/CH_4-Spectrum, Pentobarbital, underivatised: *m/z* 227, 255, 267; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



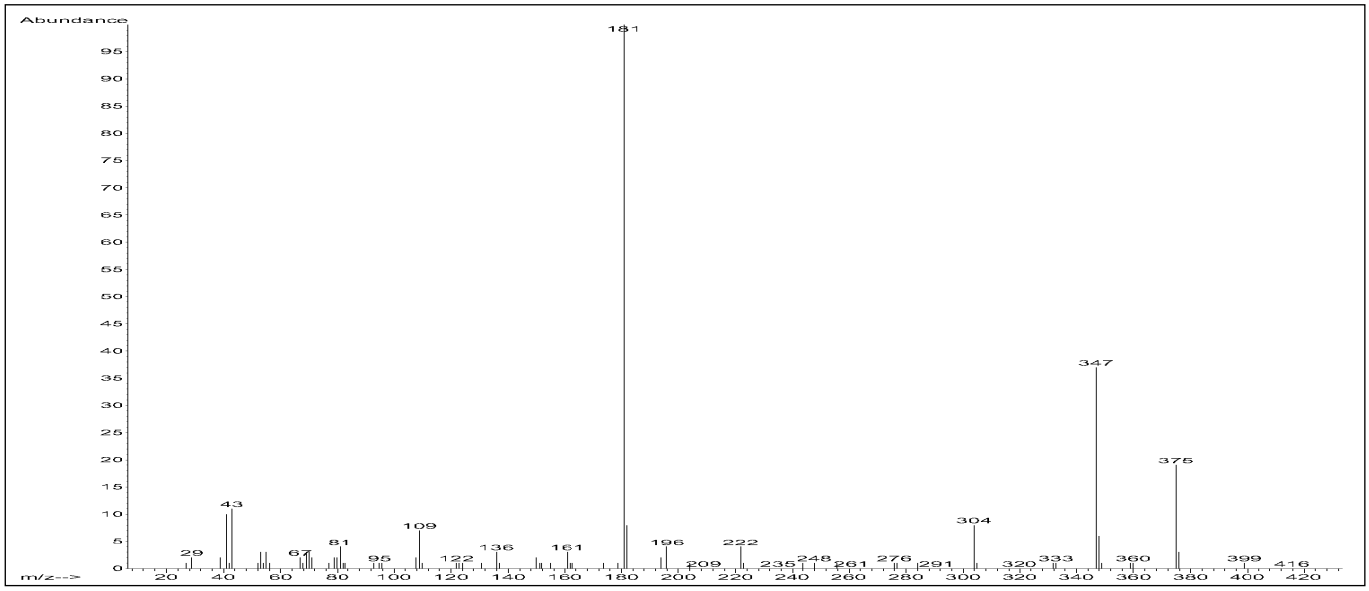
PCI/CH_4-Spectrum, Pentobarbital, PFBB-derivative, *m/z* 407, 435, 447; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H^5]^+$



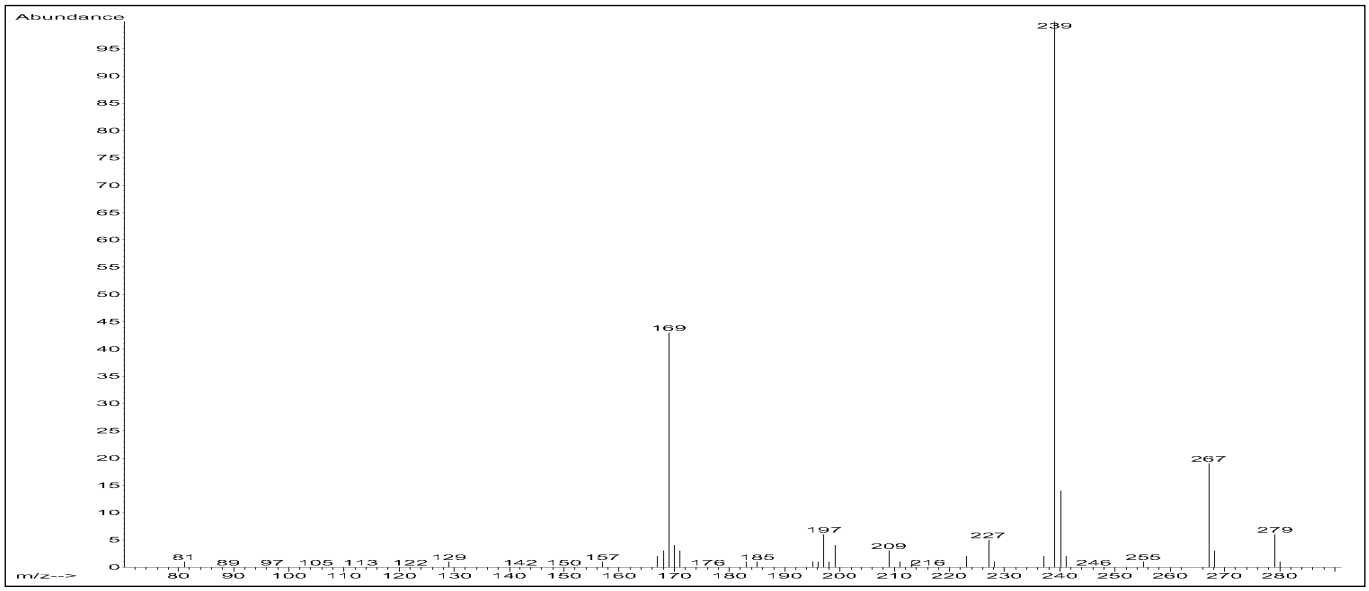
ECNI/CH_4-Spectrum, Pentobarbital, PFBB-derivative: *m/z* 405; $[M-H]^-$



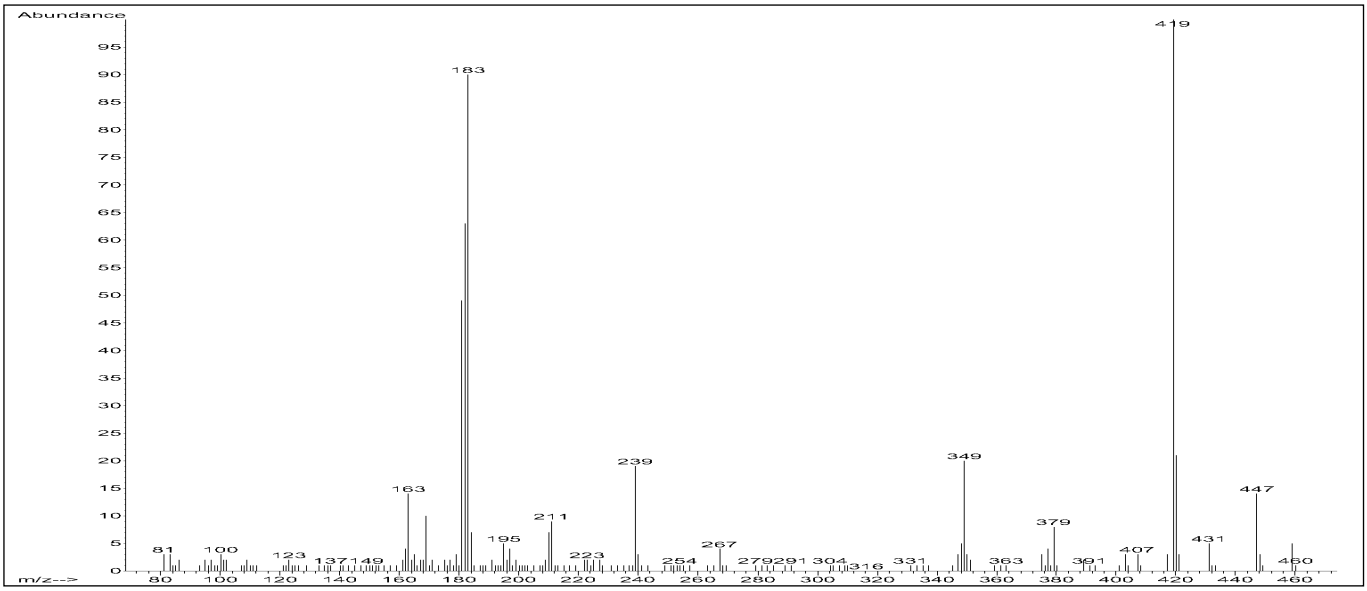
EI-Spectrum, Secobarbital, underivatised, *m/z* 238; M^+



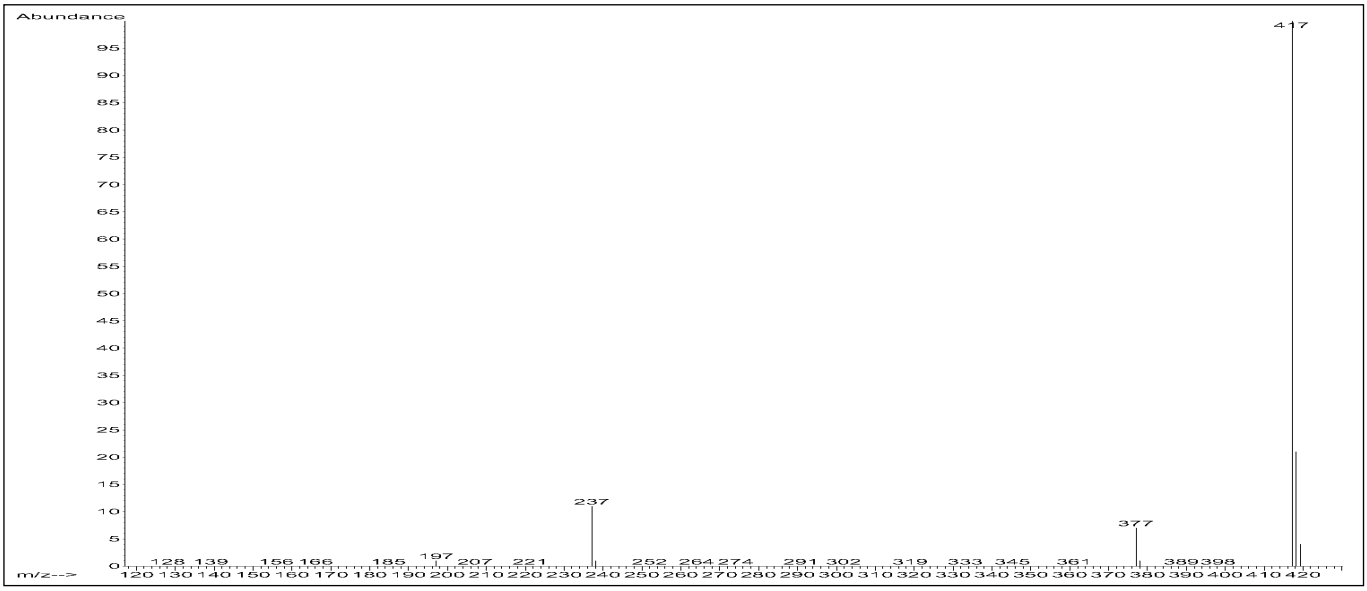
EI-Spectrum, Secobarbital, PFBB-derivative, *m/z* 418; M^+



PCI/CH_4-Spectrum, Secobarbital, underivatised: *m/z* 239, 267, 279; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



PCI/CH_4-Spectrum, Secobarbital, PFBB-derivative: *m/z* 419, 447, 459; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$

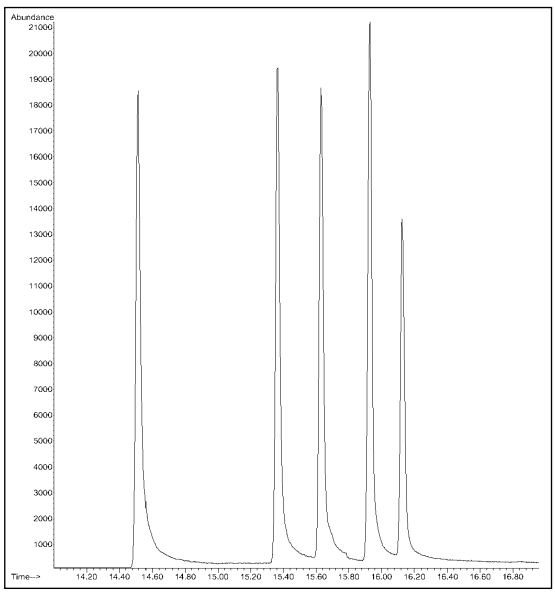


ECNI/CH_4-Spectrum, Secobarbital, PFBB-derivative: *m/z* 417; $[M-H]^-$

ECNI SIM, 5 Barbiturates

Analytes in elution order	RT (min)	Nominal ions (*m/z*)	Relative Signal/Noise Ratio
Barbital	14.52	363	1
Butethal	15.37	391	1.03
Amobarbital	15.63	405	1.00
Pentobarbital	15.93	405	1.15
Secobarbital	16.13	417	0.72

Table 1



ECNI SIM, 5 Barbiturates, 1 pg/µl each, see Table 1

Benzodiazepines

Alprazolam CAS-Nr. 28981-97-7
Molecular formula: $C_{17}H_{13}ClN_4$
Bromazepam CAS-Nr. 1812-30-2
Molecular formula: $C_{14}H_{10}BrN_3O$
Diazepam CAS-Nr. 439-14-5
Molecular formula: $C_{16}H_{13}ClN_2O$
Flunitrazepam CAS-Nr. 1622-62-4
Molecular formula: $C_{16}H_{12}FN_3O_3$
Triazolam CAS-Nr. 28911-01-5
Molecular formula: $C_{17}H_{12}C_{l2}N_4$

GC-Parameters

Column: HP-5ms
Agilent Part Nr.19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 0.7ml/min, 30cm/sec
Mode: Constant Flow
Injection:
On Column: 100°C/40°C
Oven Temp.Program
Scan: 100°C (0.3min)
SIM: 40°C (0.3min) -
25°C/min to 300°C (6min)
Flunitrazepam
Scan/SIM: 100°C (0.3min)

MS-Parameter

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C
EM Voltage: Tune + 400V
Mode: ECNI/CH_4 – SCAN/SIM
Buffer Gas: Methane
Flow (Setting): 2ml/min (40)
Tune: ECNI-Methane Tune File
Temperatures:
Source 150°C, Quad 106°C
EM Voltage: Tune + 400V

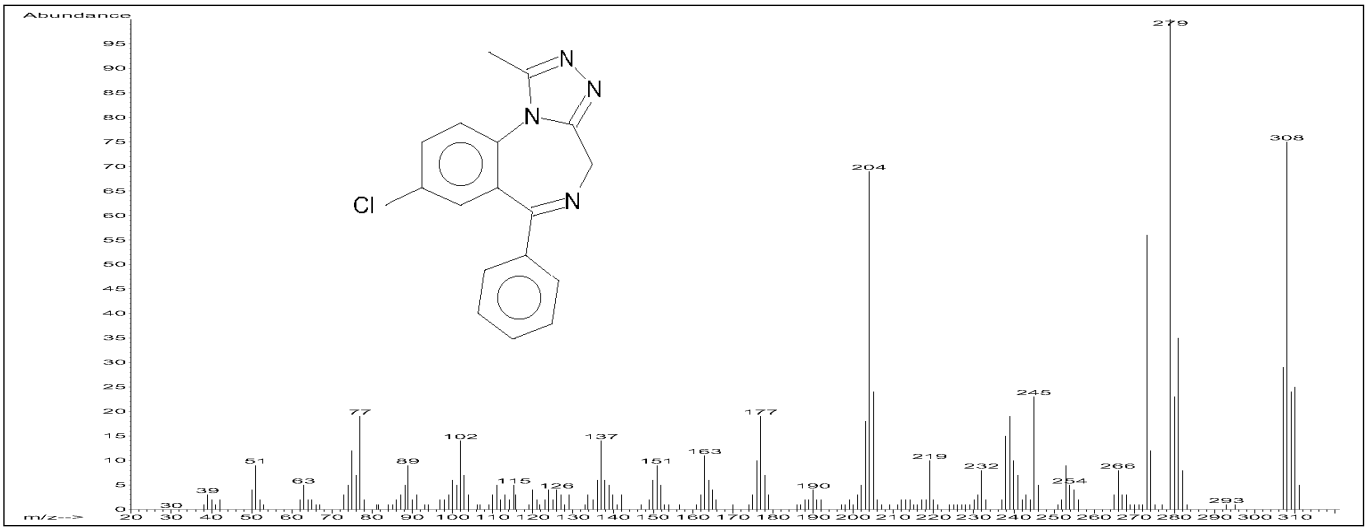
Remarks

Injection
"Active" surfaces in the inlet system may causes discrimination. For these measurements, on-column injection technique with a fused silica needle syringe was applied to allow a better comparison.

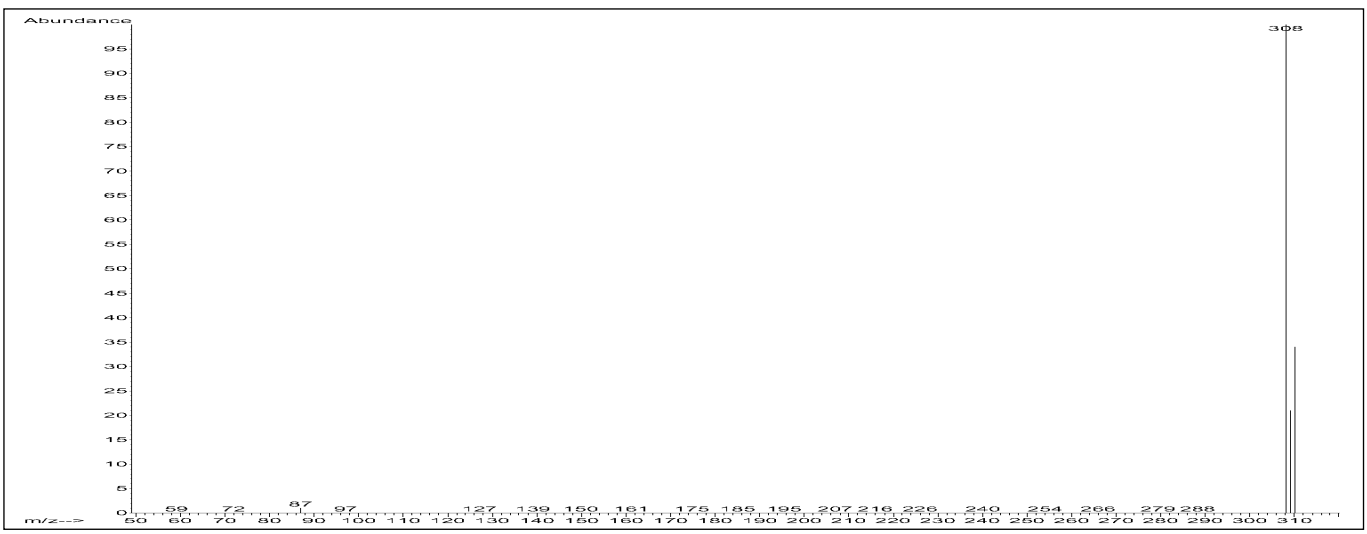
ECNI Parameters
The commonly applied parameters for improving ECNI measurements (like Emission Current, Flow, NH_3 Buffer Gas) showed no positive effects.

Results
Sensitivity in ECNI Scan mode was measured using concentrations in the range of 0.3ng/µl to 1ng/µl and is in that range. SIM mode measurements were made at about 1000 times lower; 3pg/µl to 10pg/µl. Flunitrazepam showed the highest relative response (at < 200fg/µl).

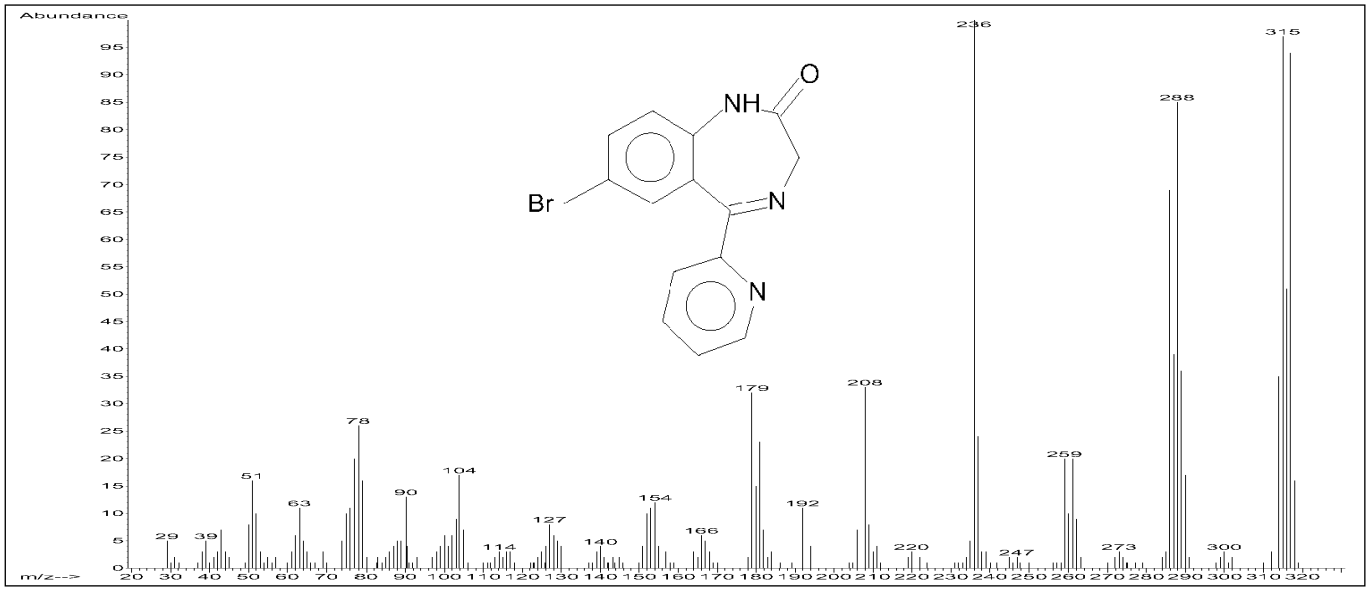
References
"Application of Electron Capture Negative Chemical Ionization for the detection of a Date Rape Drug"
A. Negrusz, Ch. Moore, H. Prest
Agilent Pub. Nr. 5968-4364E



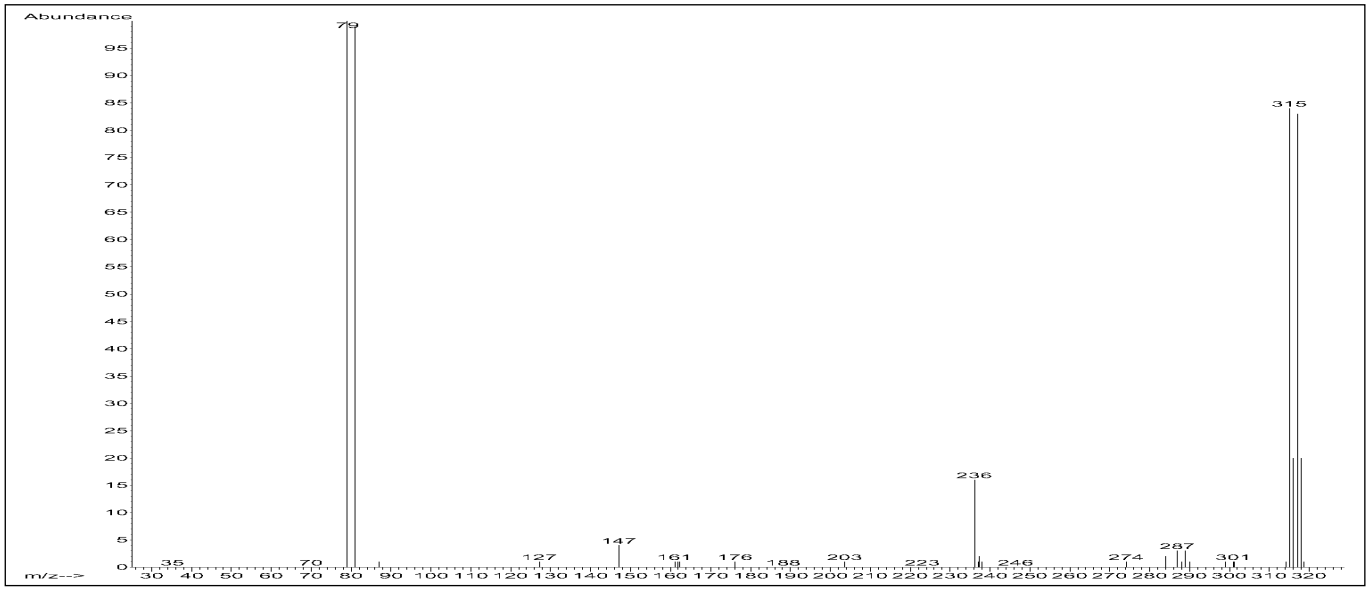
EI-Spectrum, Alprazolam: *m/z* 308; M^+



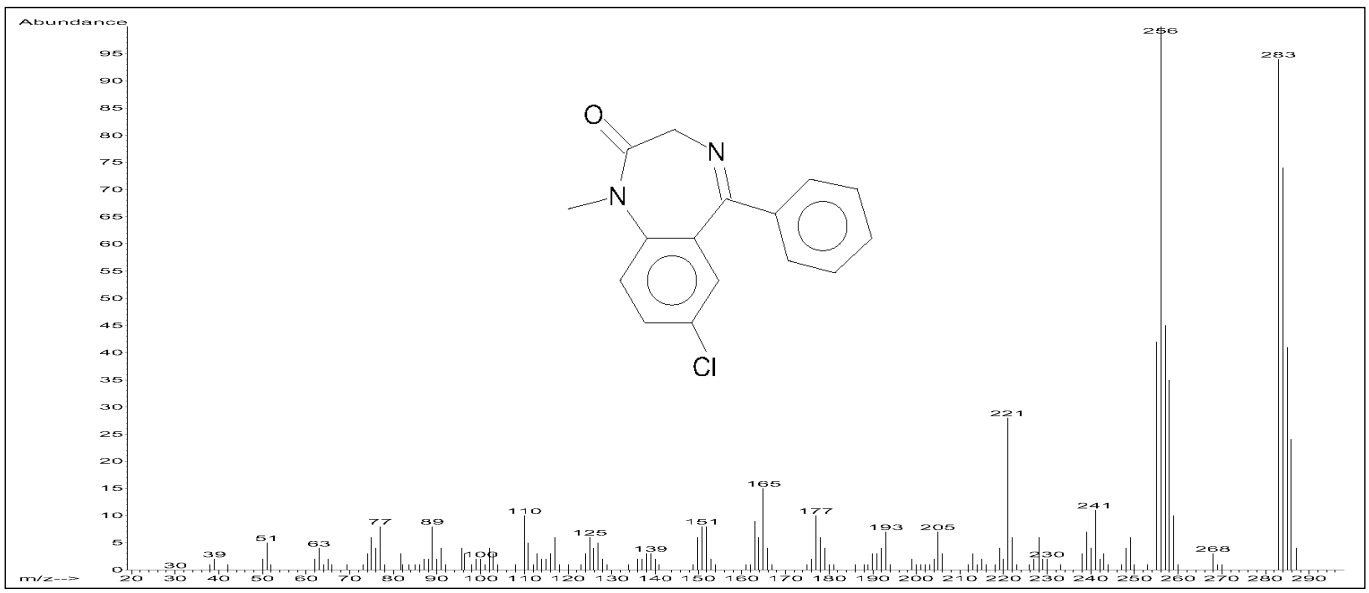
ECNI-Spectrum, Alprazolam: *m/z* 308; M^-



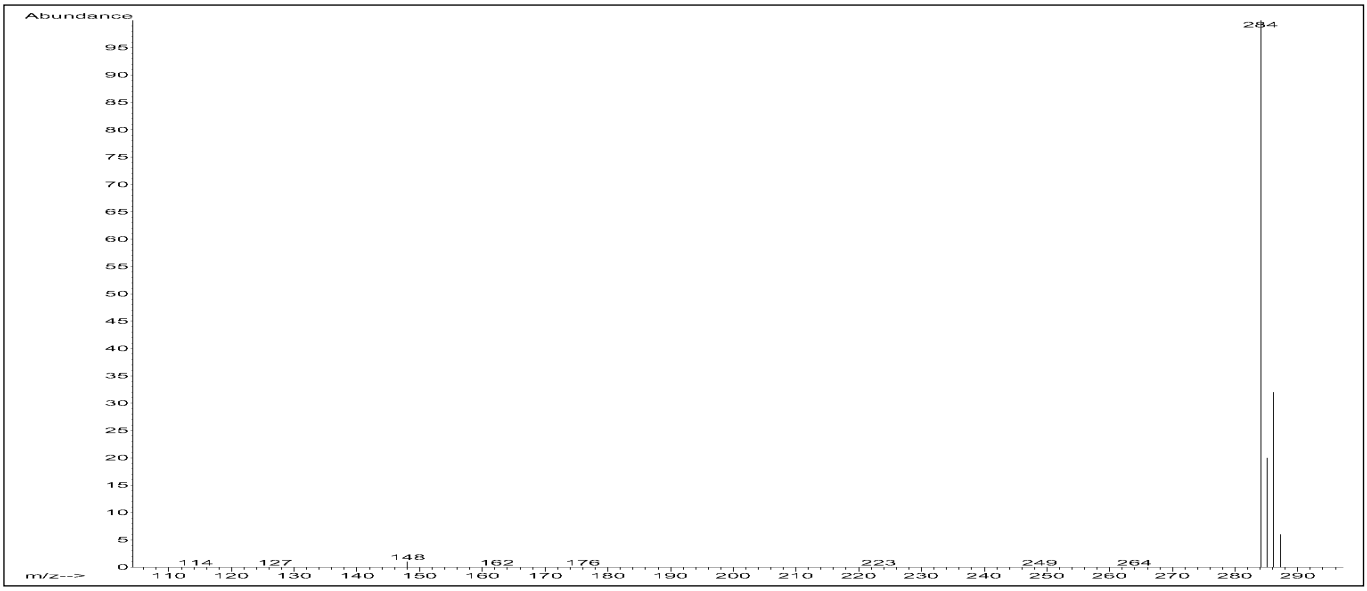
EI-Spectrum, Bromazepam: *m/z* 315; M^+



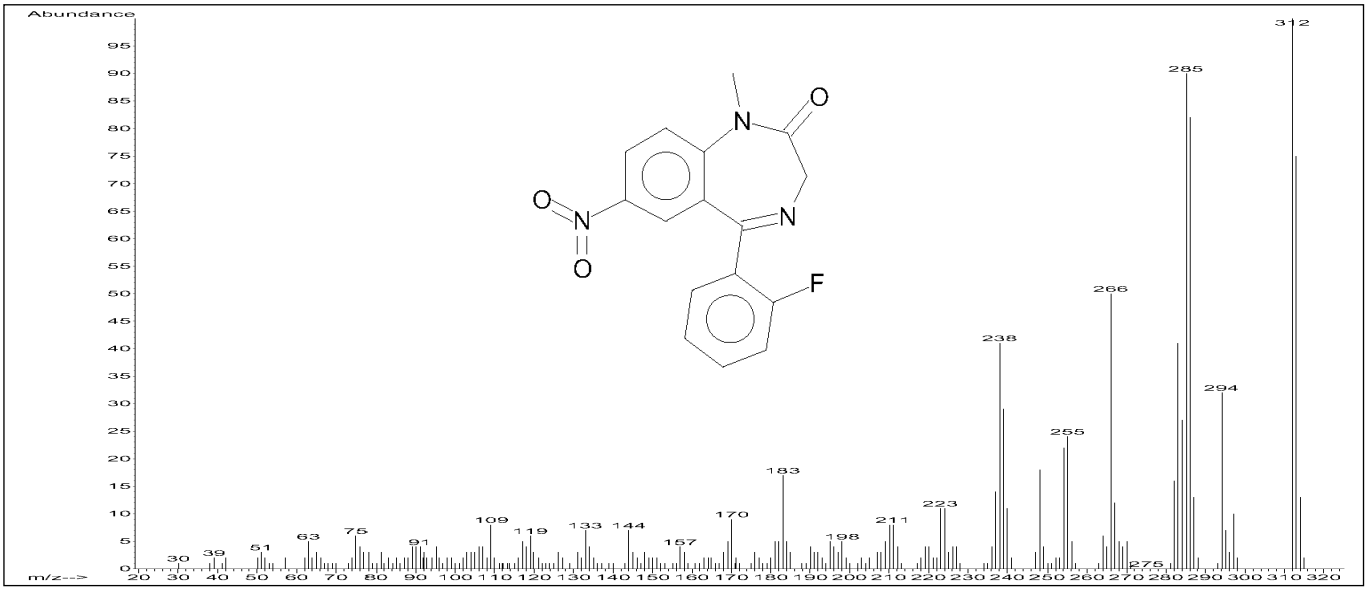
ECNI-Spectrum, Bromazepam: *m/z* 315; M^-



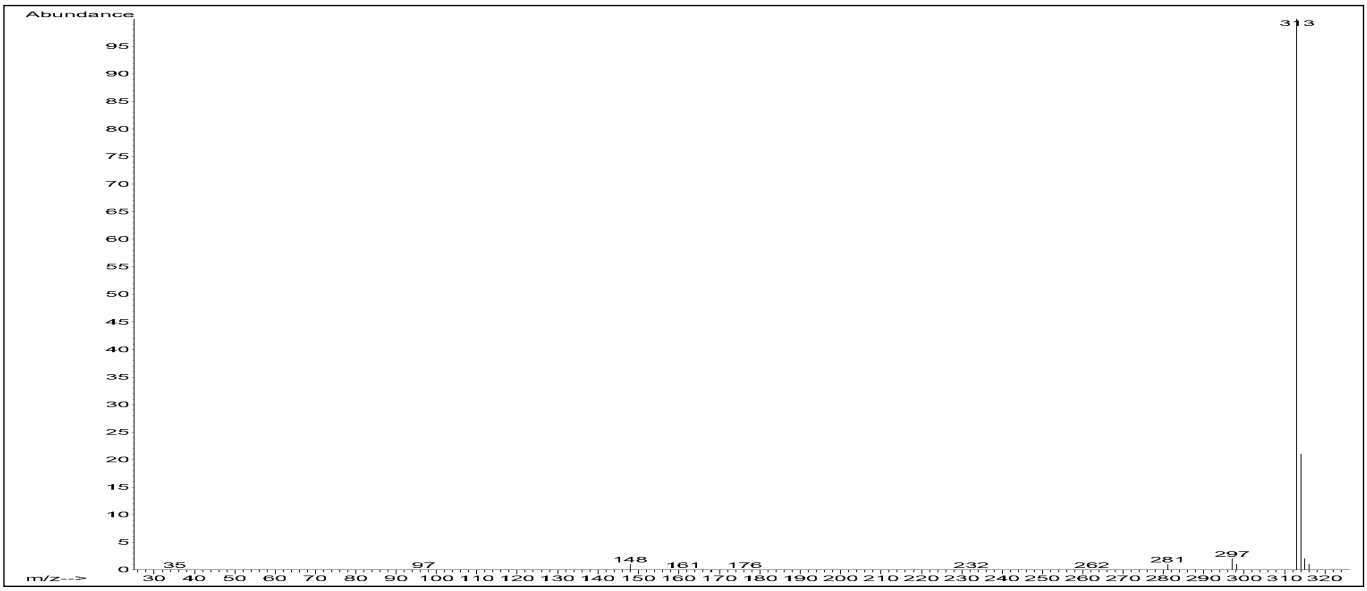
EI-Spectrum, Diazepam: *m/z* 284; M^+



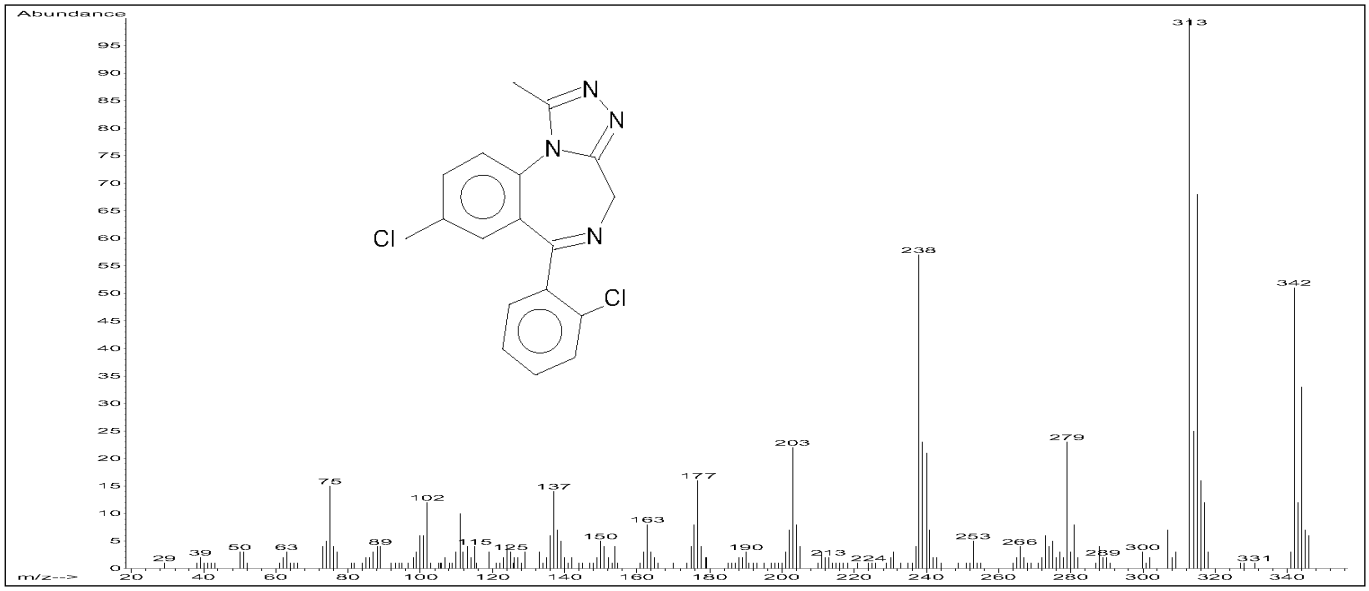
ECNI-Spectrum, Diazepam: *m/z* 284; M^-



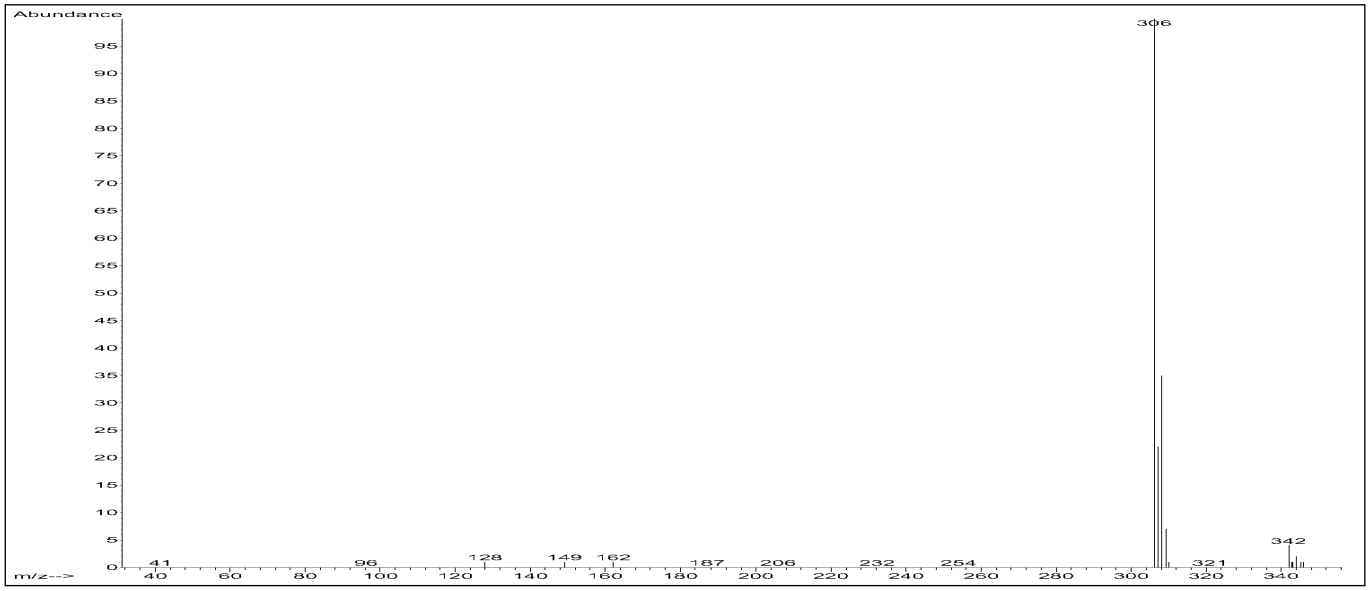
EI-Spectrum, Flunitrazepam: *m/z* 313; M^+



ECNI-Spectrum, Flunitrazepam: *m/z* 313; M^-

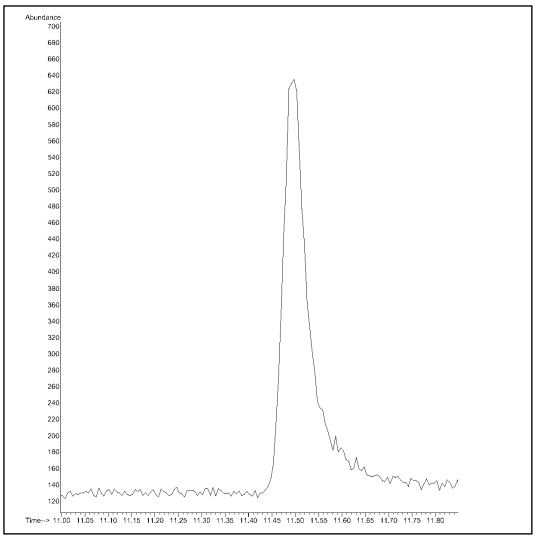


EI-Spectrum, Triazolam: *m/z* 342; M^+



ECNI-Spectrum, Triazolam: *m/z* 342; M^-

ECNI SIM

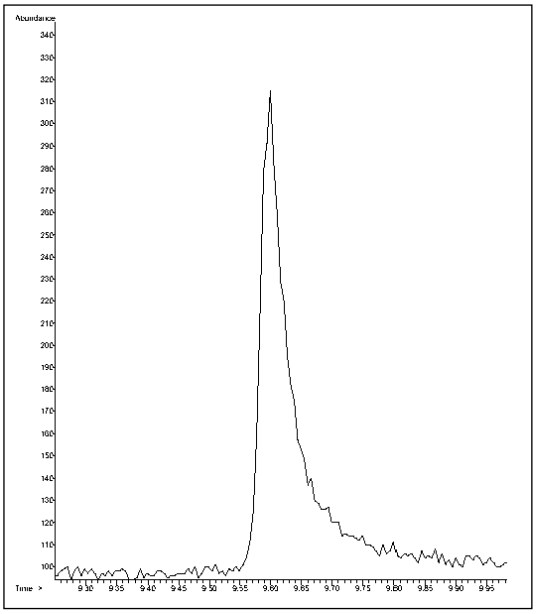


ECNI SIM, Alprazolam

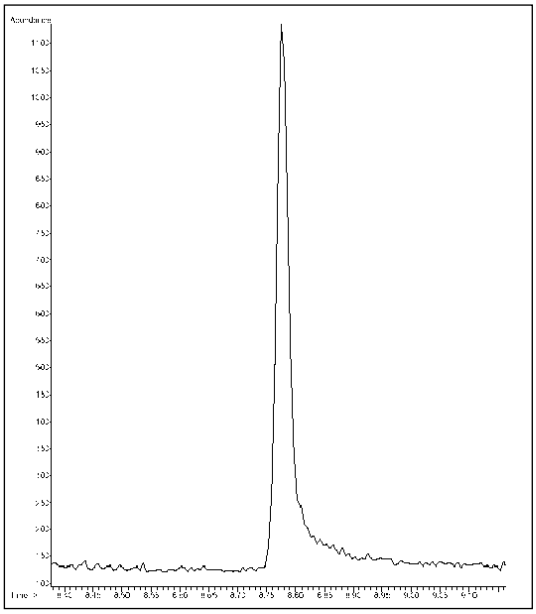
ECNI SIM

Analytes	Retention Time (min)	Ions (*m/z*)	Signal/Noise Relative to Diazepam (TIC)
Alprazolam	11.49	308, 310	0.6
Bromazepam	9.60	315, 317	0.08
Diazepam	8.77	284, 286	1
Flunitrazepam	9.49	313	16
Triazolam	12.32	306, 308	0.7

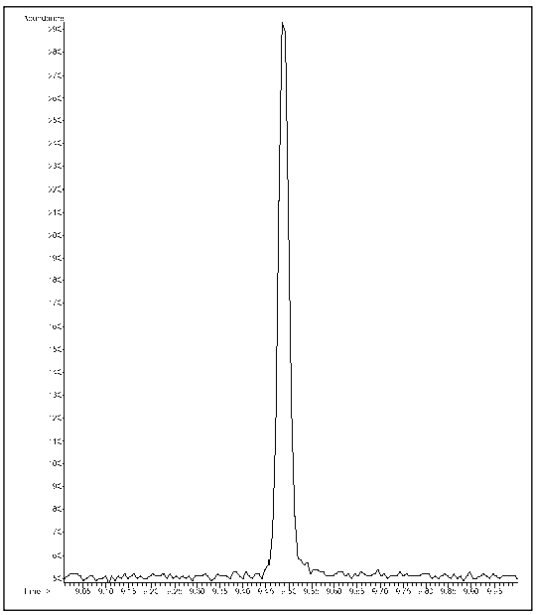
Table: Sensitivity Benzodiazepines



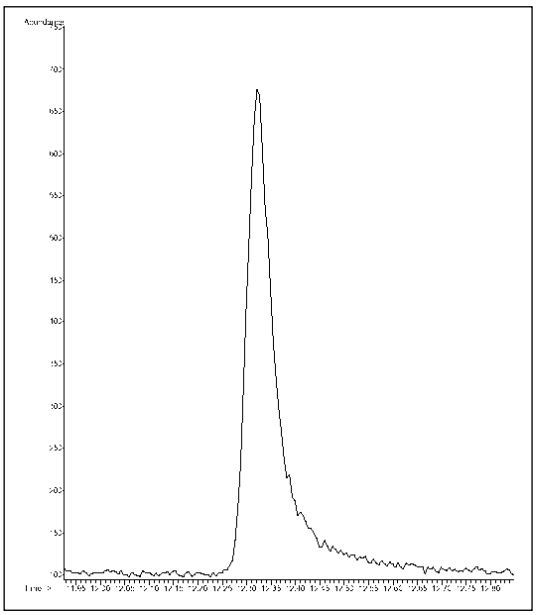
Bromazepam



Diazepam



Flunitrazepam



Triazolam

Benzoylecgonine

CAS-Nr. 519-09-5
Molecular formula: $C_{16}H_{19}NO_4$

GC-Parameters

Column: HP-5ms
Agilent Part Nr.19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 1.2ml/min, 40cm/sec
Mode: Constant Flow
Injection: Pulsed splitless, 250°C
Oven Temp. Program
70°C (1min) – 25°C/min to
300°C (5min)

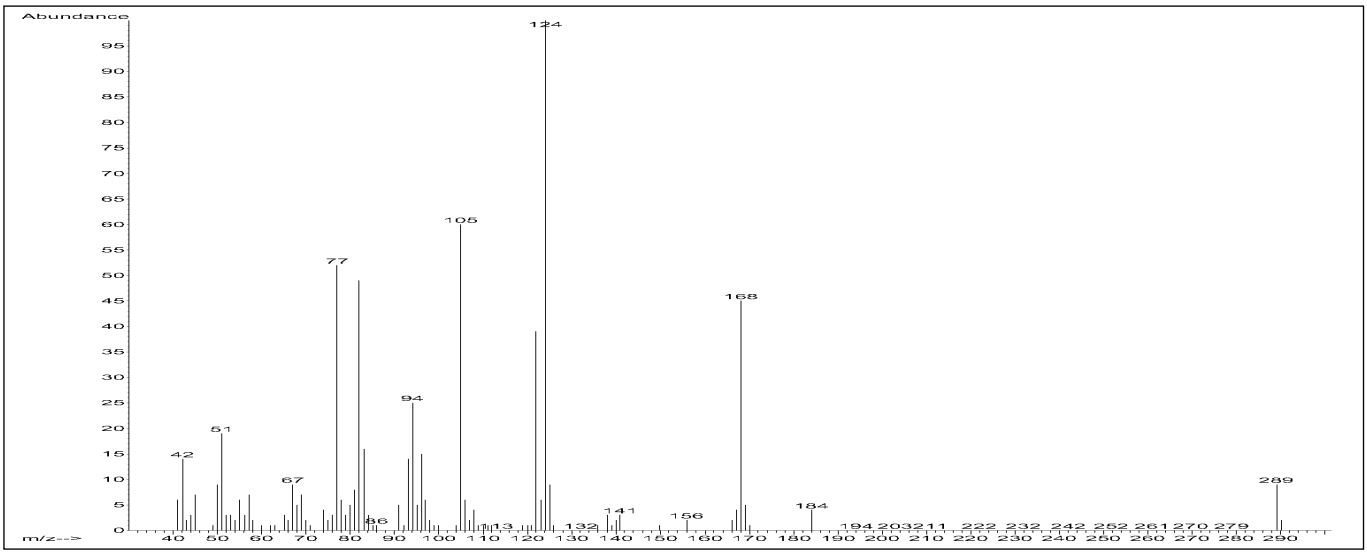
MS-Parameter

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C
Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V
Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V

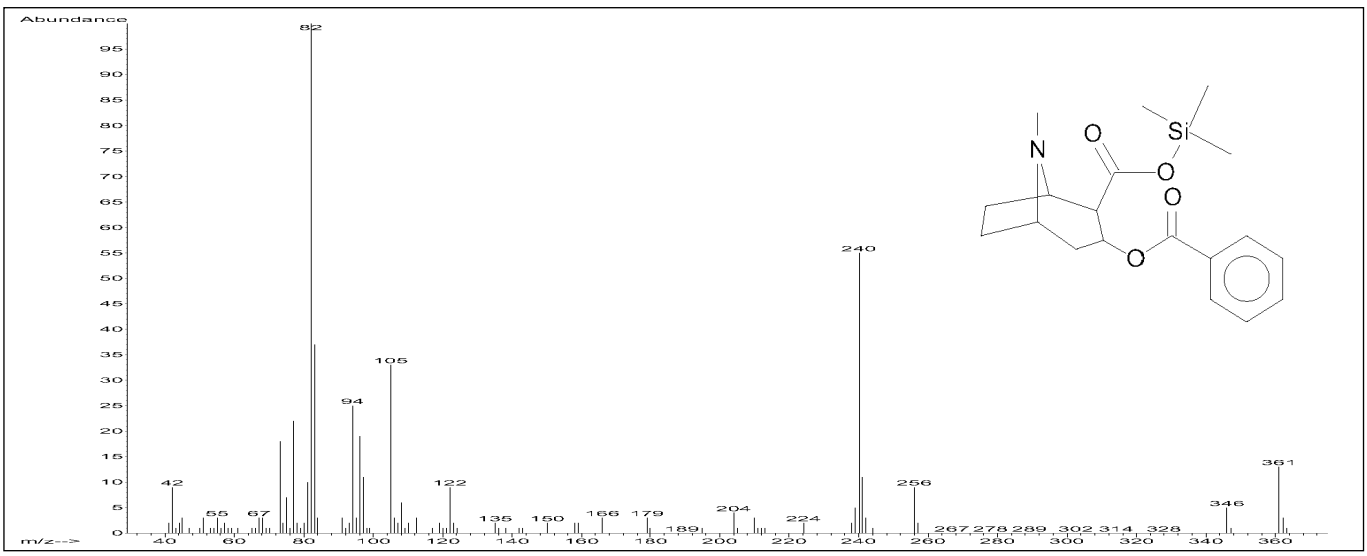
Remarks

Derivatization
a) Trimethyl silylation –TMS– with MSTFA (Reagent: Fluka 69479)
b) Reaction with Pentafluoro-propionic Acid Anhydride (PFPA) (Reagent: Fluka 77292)
a) The standard solution (SIGMA B 8900), concentration 100ng/µl, diluted in ethyl acetate, was evaporated with a gentle nitrogen flow. To the residue, 50µl reagent is added and the reaction mixture is incubated for 20min at 60°C. Gentle evaporation with nitrogen is repeated and the residue redissolved in ethyl acetate.
b) Procedure as described above. After the first evaporation the residue, 80µl of the PFPA reagent and 20µl of hexafluoro-isopropanol (FLUKA 52517) are added and the reaction mixture is incubated for 30 min at 70°C. Then evaporation, dilution and GC/MSD analysis.

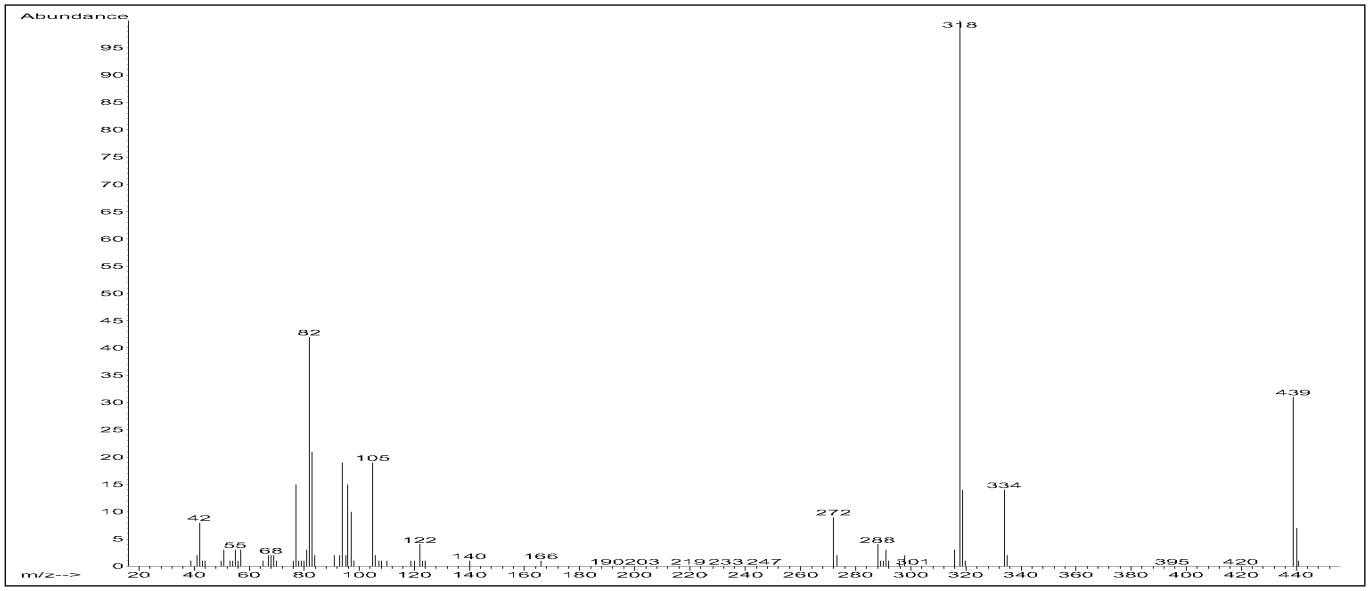
Results
Derivatization is recommended. In PCI/NH_3 Mode, TMS Derivative, the degree of fragmentation is related to sample concentration for this analyte.
Sensitivity is directly related to the derivatization and to the applied reagent gas, see table. In SIM mode, analyte concentration of 1pg/µl is measured with signal/noise ratio of approximately 10/1.



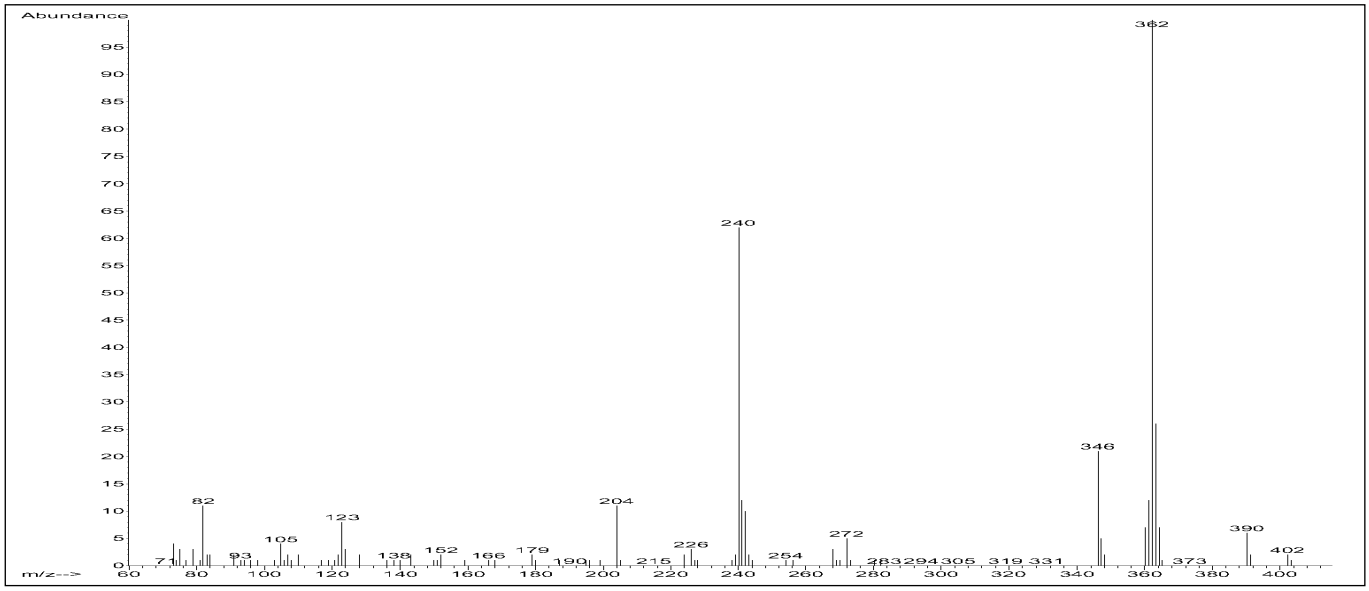
EI-Spectrum, Benzoylecgonine, underivatised: *m/z* 289; M^+



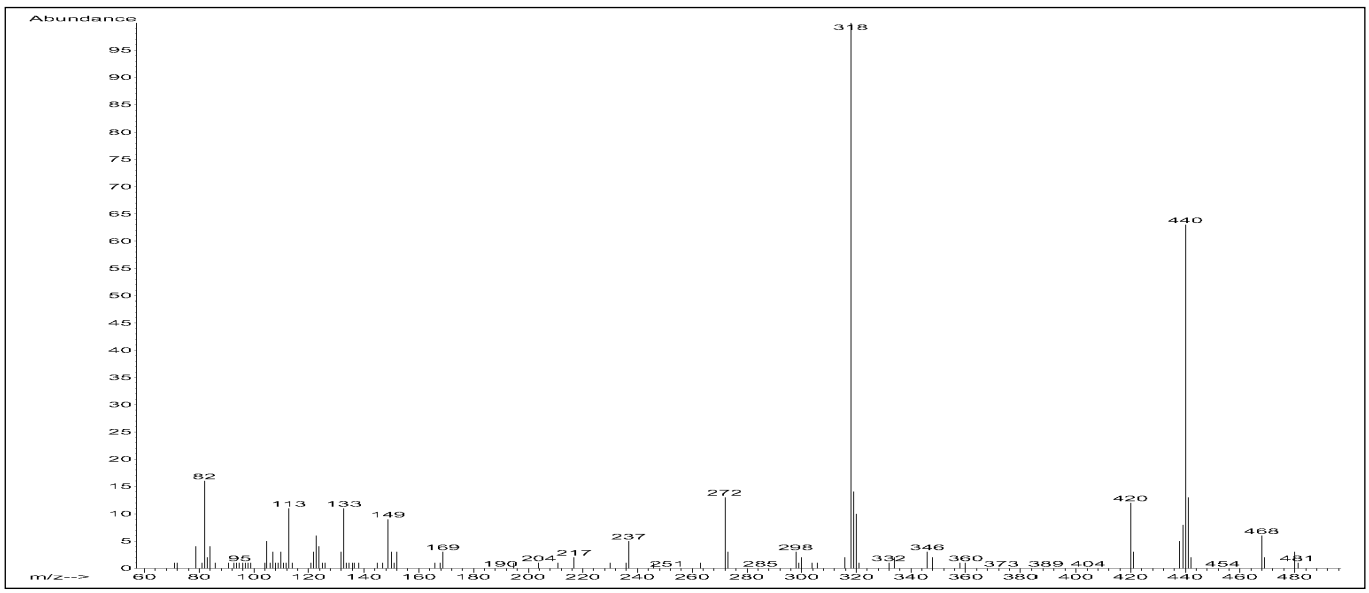
EI-Spectrum, Benzoylecgonine, TMS Derivative: *m/z* 361; M^+



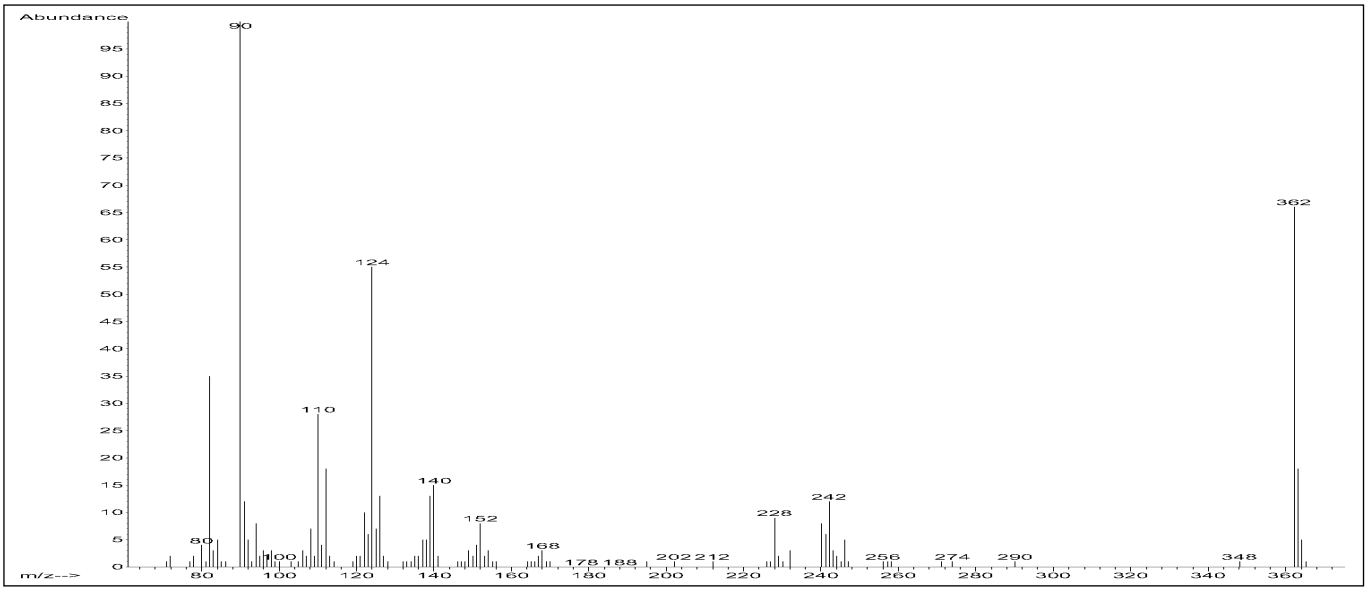
EI-Spectrum, Benzoylecgonine, PFPA Derivative, -O-CH-$(CF_3)_2$: *m/z* 439; M^+



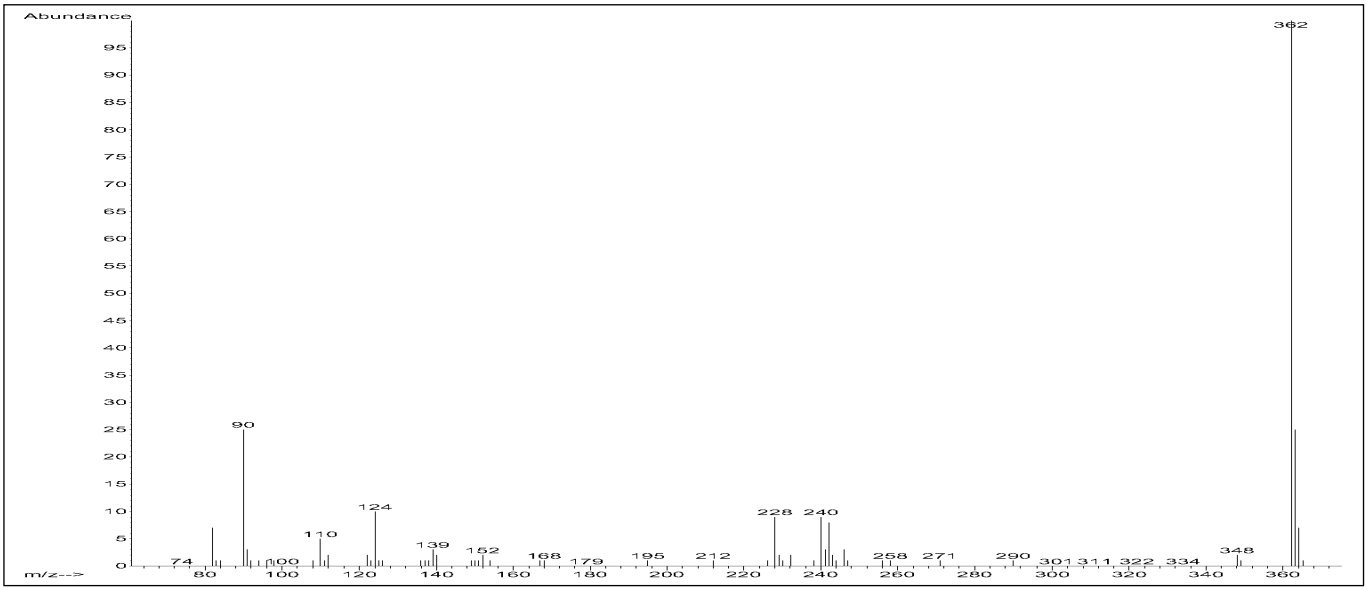
PCI/CH_4-Spectrum, Benzoylecgonine, 50ng/µl, TMS Derivative: *m/z* 362, 390, 402; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



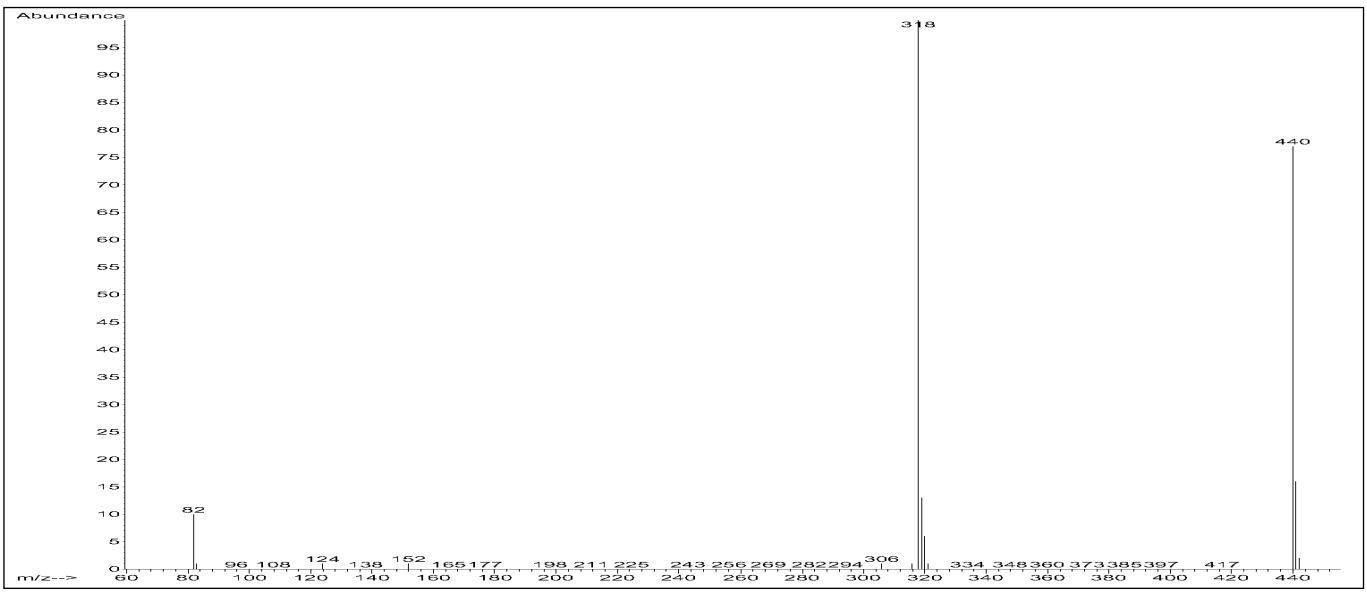
PCI/CH_4-Spectrum, Benzoylecgonine, 10ng/µl, PFPA Derivative, -O-CH-$(CF_3)_2$: *m/z* 440, 468, 480; $[M+H]^+$, $[M+C_2H_5]^+$, $[M + C_3H_5]^+$



PCI/NH_3-Spectrum, Benzoylecgonine, 10ng/µl, TMS Derivative: *m/z* 362; $[M + H]^+$

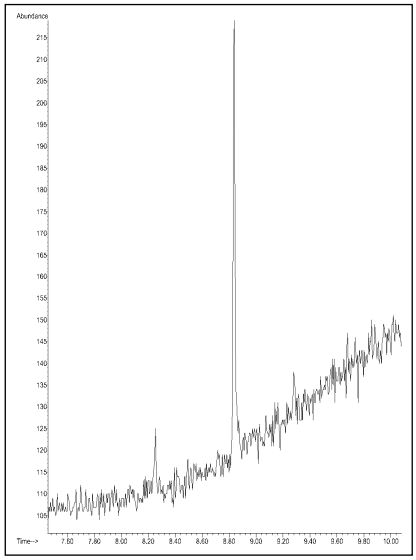


PCI/NH_3-Spectrum, Benzoylecgonine, 50ng/µl, TMS Derivative: *m/z* 362; $[M+H]^+$



PCI/NH_3-Spectrum, Benzoylecgonine, 10ng/µl, PFPA Derivative, -O-CH-$(CF_3)_2$: *m/z* 440; $[M+H]^+$

PCI/NH_3 – SIM Mode



Benzoylecgonine, PFPA Derivative,
Retention Time: 8.84min
1pg/µl, SIM Ions: 318, 440 *m/z*

Derivative	Ion - Mode	Peak to Peak Signal:Noise Ratio
TMS	PCI/CH_4	> 10 : 1
TMS	PCI/NH_3	> 35 :1
PFPA	PCI/CH_4	> 100 : 1
PFPA	PCI/NH_3	> 200 : 1

Table : Benzoylecgonine, Sensitivity (S/N), Scan Acquisition, 10ng/µl each

Chloramphenicol

CAS-Nr. 56-75-7
Molecular Formula: $C_{11}H_{12}C_{l2}N_2O_5$
CAS-Nr. O,O-TMS Derivative:
21196-84-9

GC-Parameter

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 0.7ml/min, 30cm/sec
Mode: Constant Flow
Injection: Pulsed splitless
Oven Temp. Program
70°C (1min) - 30°C/min to 150°C
15°C/min to 300°C

MS-Parameter

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C
Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V
Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1.75ml/min (35)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V
Mode: ECNI/CH_4 – SCAN/SIM
Buffer Gas: Methane
Flow (Setting): 2ml/min (40)
Tune: ECNI-Methane Tune File
Temperatures:
Source 150°C, Quad 106°C
EM Voltage: Tune + 400V
SIM Tune+ 600V

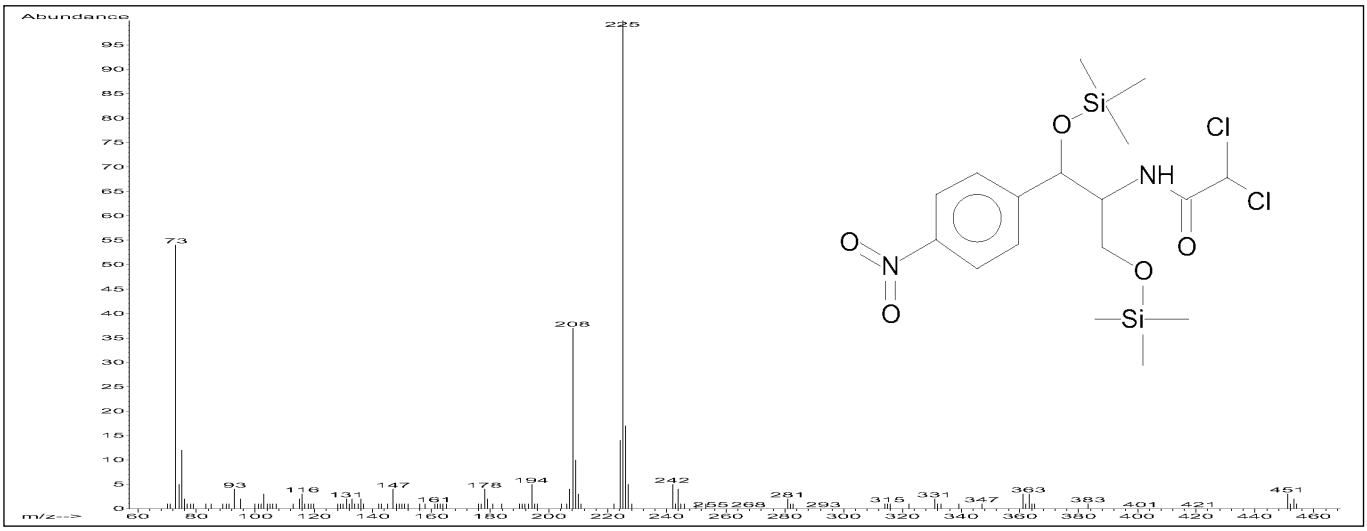
Remarks

Derivatization
Trimethyl silylation with HMDS/TMCS at 2/1 in Pyridine (Reagent: Fluka 85431)
The standard solution (SIGMA C 0378), concentration 20ng/µl, diluted in ethyl acetate, is gently evaporated under dry nitrogen. To the residue, 50µl reagent is added and the reaction mixture is incubated for 2 min at 50°C. Evaporation to dryness is repeated and the residue diluted in hexane. The solution is ready for injection.

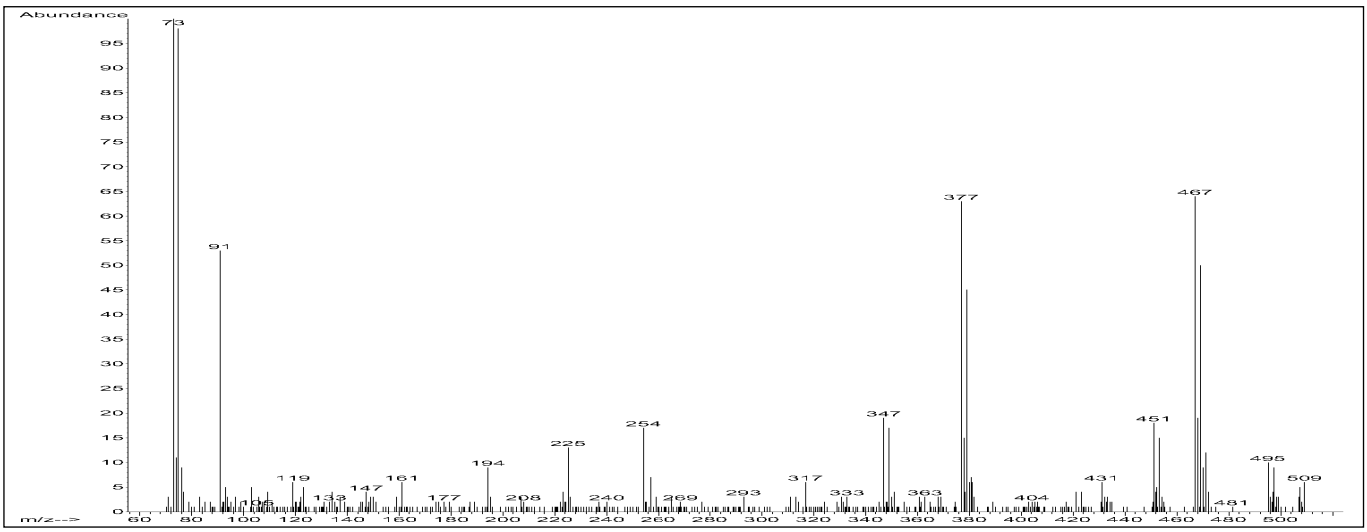
Results
Analyzing this compound as O,O TMS derivative is preferred over the parent.

PCI Scan sensitivity is on the order of 20ng/µl with methane however ammonia produces 5 times greater sensitivity.

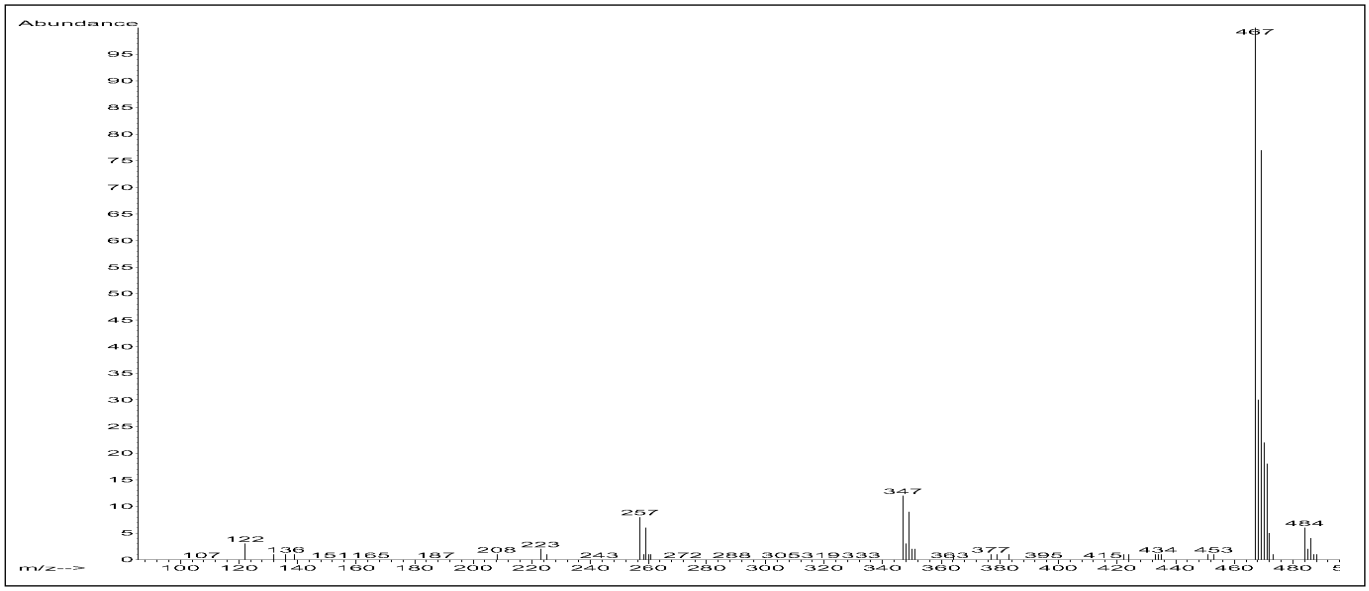
The greatest sensitivity is achieved in ECNI SIM mode which allows detection at 0.1 pg/µl. Calibration curves show good linearity over the concentration range of 0.1pg/µl to 100pg/µl.



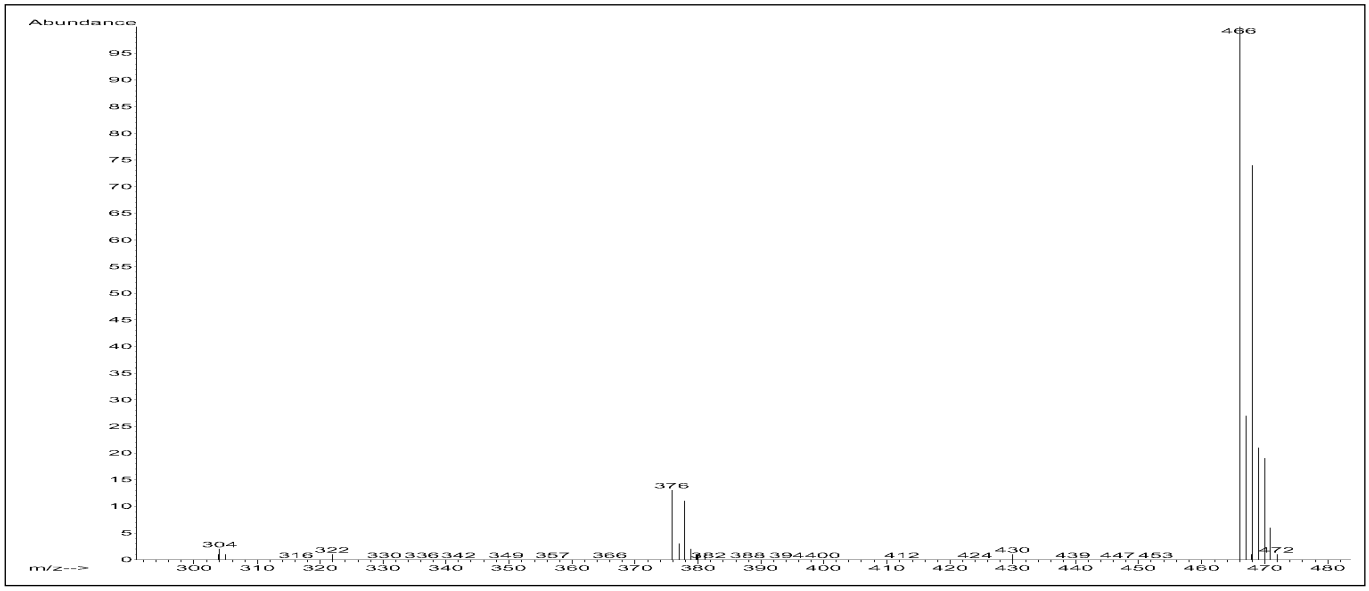
EI Spectrum, Chloroamphenicol, O,O-TMS Derivative: *m/z* 466; M^+



PCI/CH_4 Spectrum, Chloroamphenicol, O,O-TMS Derivative: *m/z* 467, 495, 507; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$

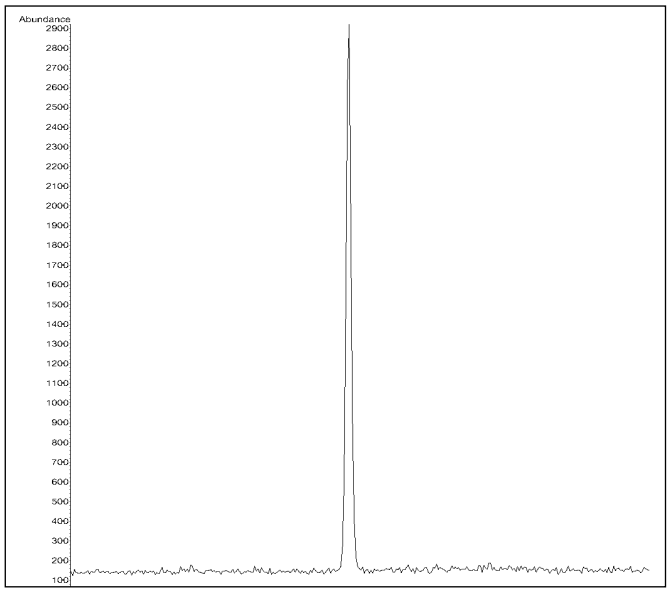


PCI/NH_3 Spectrum, Chloroamphenicol, O,O-TMS Derivative: *m/z* 467, 484; $[M+H]^+$, $[M+NH_4]^+$

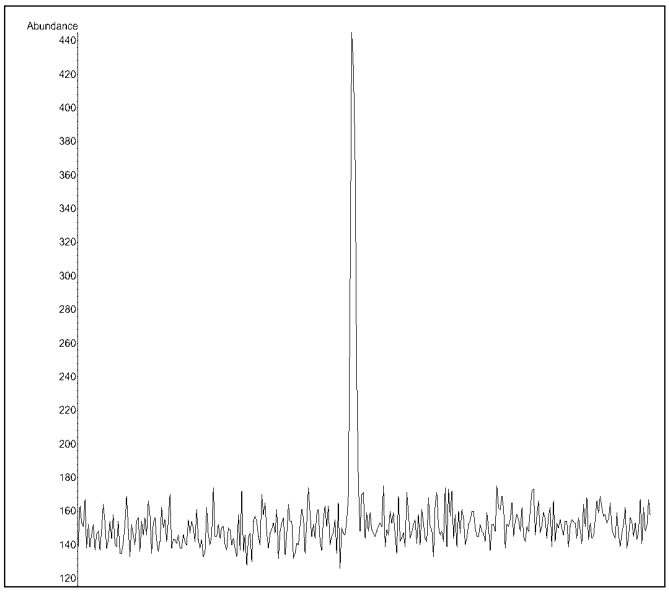


ECNI/CH_4 Spectrum, Chloroamphenicol, O,O-TMS Derivative: *m/z* 466; M^-

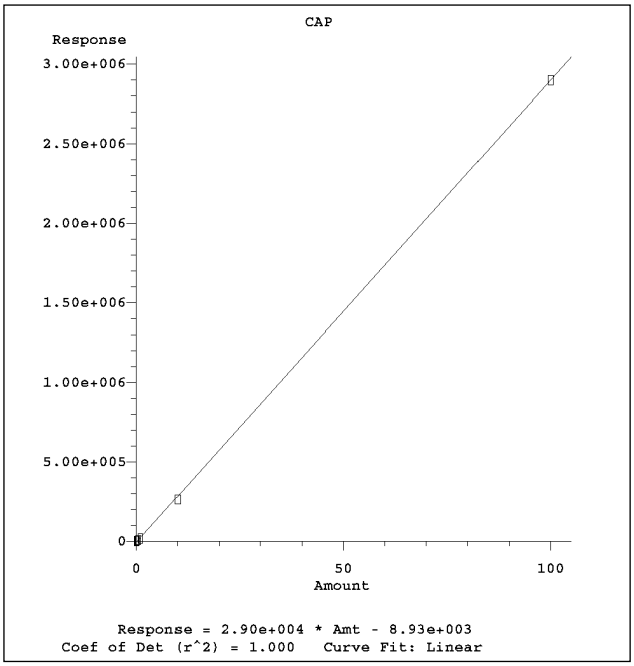
SIM Mode (Ions: 465.9, 467.9, 469.9 *m/z*)

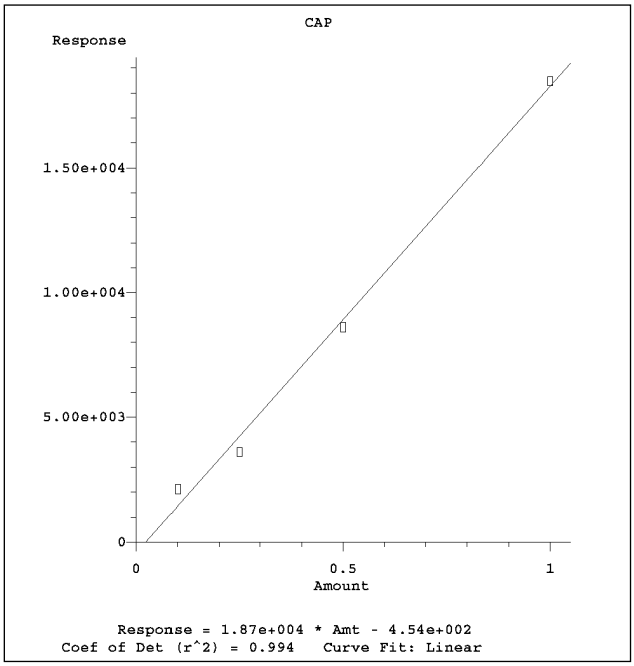


ECNI/CH_4 Chloroamphenicol, O,O-TMS Derivative, 1pg/µl



ECNI/CH_4 Chloroamphenicol, O,O-TMS Derivative, 0.1pg/µl





Calibration Curves: ECNI/CH_4 Chloroamphenicol, O,O-TMS Derivative
left: 0.1pg/µl to 100pg/µl, right: 0.1pg/µl to 1pg/µl

Chlorphenoxamine

CAS-Nr. 77-38-3
Molecular Formula: $C_{18}H_{22}ClNO$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 0.7ml/min, 30cm/sec
Mode: Constant Flow
Injection: Split, 250°C
Oven Temp Program
120°C (0.3min) – 20°C/min to 300°C (4min)

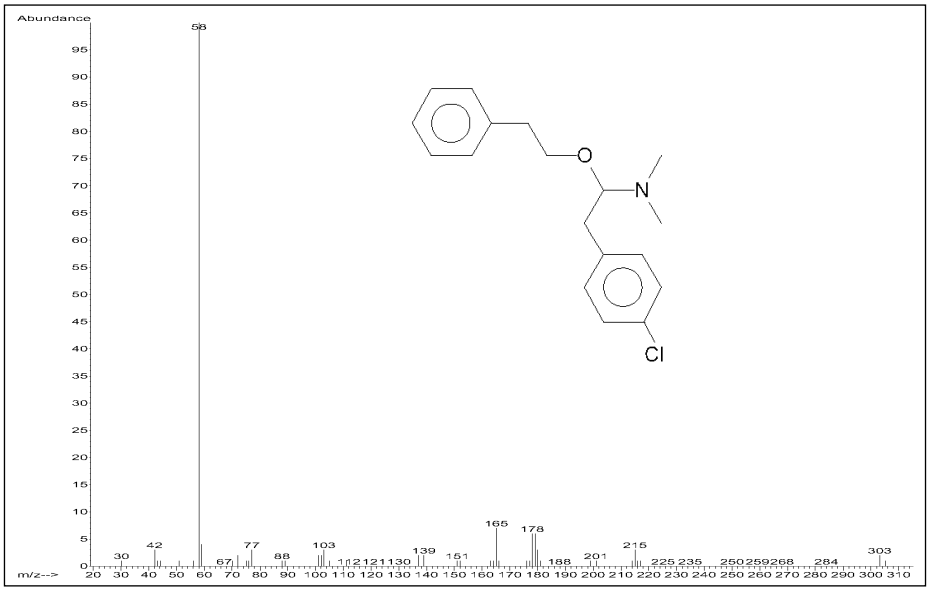
MS Parameters

Mode: EI - SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C

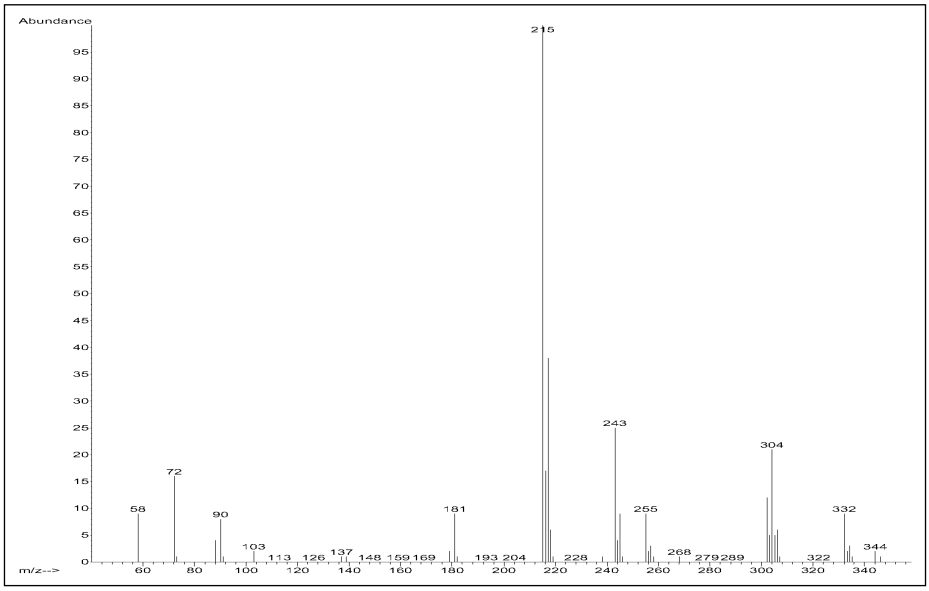
Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V

Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V

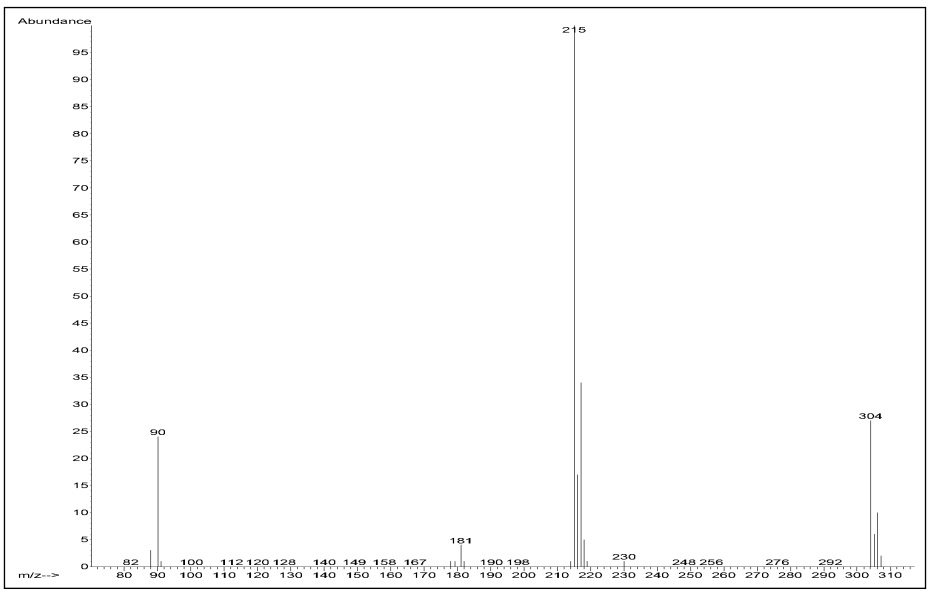
Results
Analyte Retention Time: 15.04min
Analyte Concentration: 4ng/µl
Signal/Noise Ratios for TIC
PCI/CH_4 Scan: > 500/1
PCI/NH_3 Scan: >100/1



EI-Spectrum, Chlorphenoxamine: *m/z* 303; M^+



PCI/CH_4-Spectrum, Chlorphenoxamine: *m/z* 304, 332, 344; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



PCI/NH_3-Spectrum, Chlorphenoxamine: *m/z* 304; $[M+H]^+$

Chlorprothixene

CAS-Nr. 113-59-7
Molecular Formula: $C_{18}H_{18}ClNS$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 0.7ml/min, 30cm/sec
Mode: Constant Flow
Injection: Split, 250°C
Oven Temp Program
120°C (0.3min) – 20°C/min to 300°C (4min)

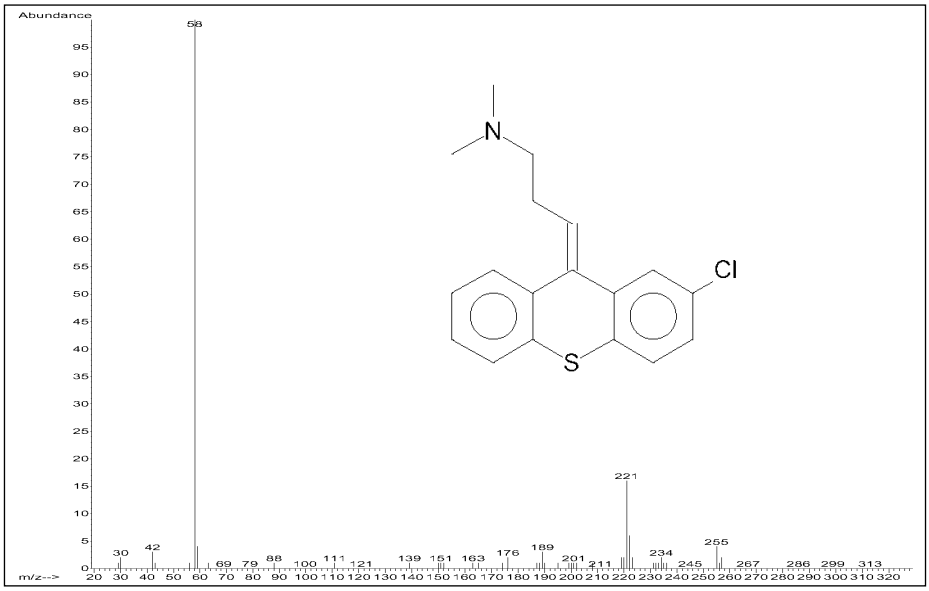
MS Parameters

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C

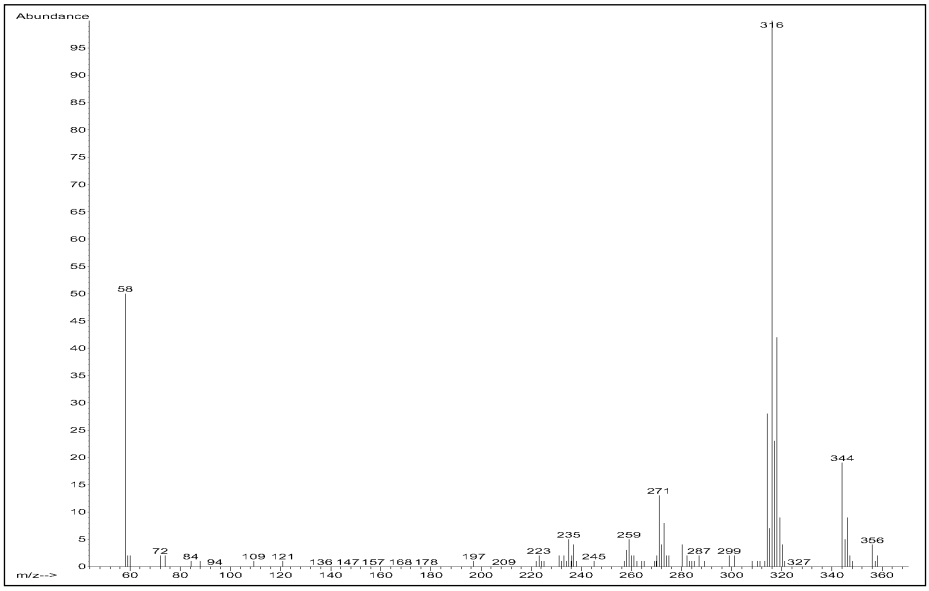
Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V

Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V

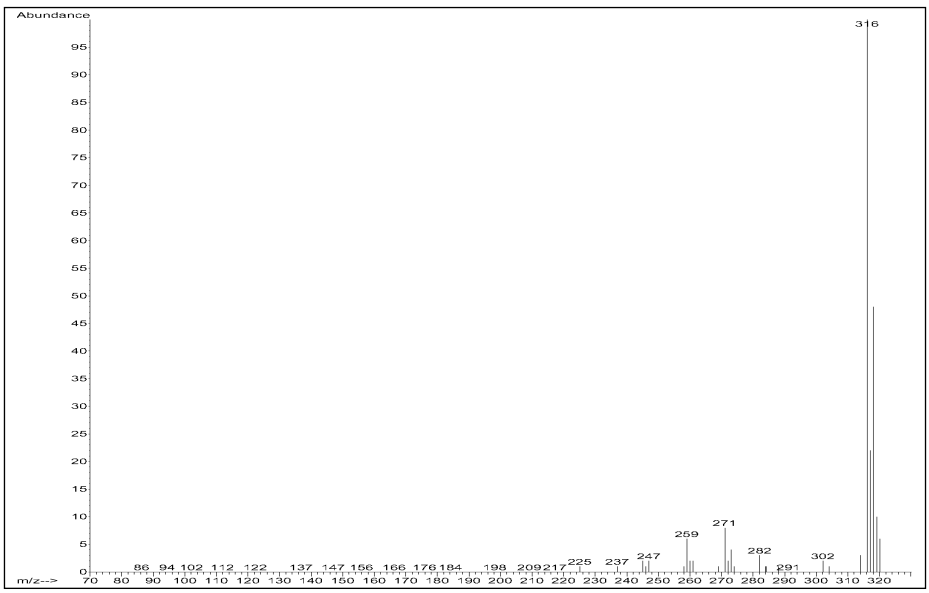
Results
Analyte Retention Time: 10.56min
Analyte Concentration: 4ng/µl
Signal/Noise Ratios for TIC
PCI/CH_4 Scan: > 25/1
PCI/NH_3 Scan: > 60/1



EI-Spectrum, Chlorprothixene: *m/z* 315; M^+



PCI/CH_4-Spectrum, Chlorprothixene: *m/z* 316, 344, 356; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



PCI/NH_3-Spectrum, Chlorprothixene: *m/z* 316 : $[M+H]^+$

Cimaterol

CAS-Nr. 54239-37-1
Molecular Formula: $C_{12}H_{17}N_3O$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 1.2ml/min, 40cm/sec
Mode: Constant Flow
Injection: Pulsed splitless, 250°C
Oven Temp. Program
70°C (1min) – 25°C/min to
280°C (5min)

MS Parameters

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C

Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V

Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V

Mode: ECNI/CH_4 – SCAN/SIM
Buffer Gas: Methane
Flow (Setting): 2ml/min (40)
Tune: ECNI-Methane Tune File
Temperatures:
Source 150°C, Quad 106°C
EM Voltages: Tune + 400V

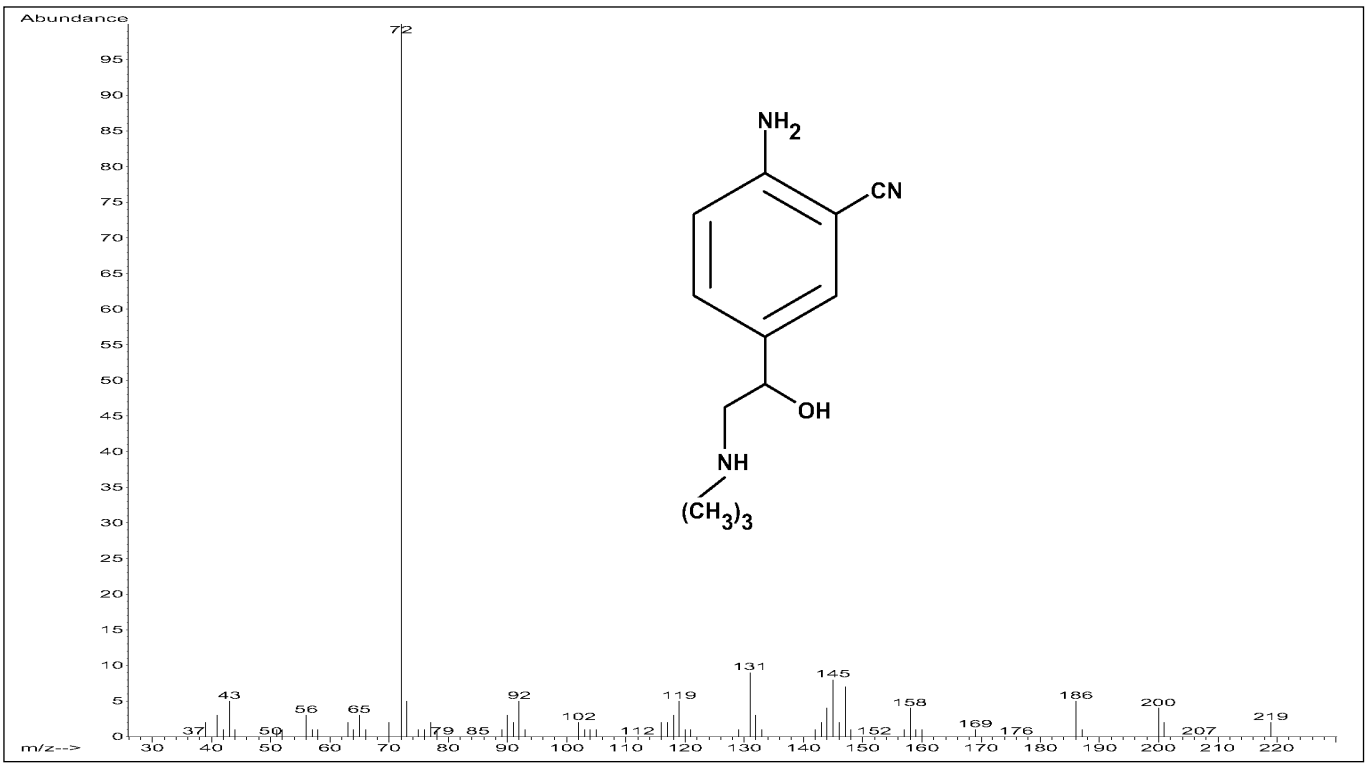
Remarks

Derivatizations: TMS and PPFA
a) Trimethyl silylation (TMS) with BSTFA/TMCS
(Reagent: Fluka 15238)

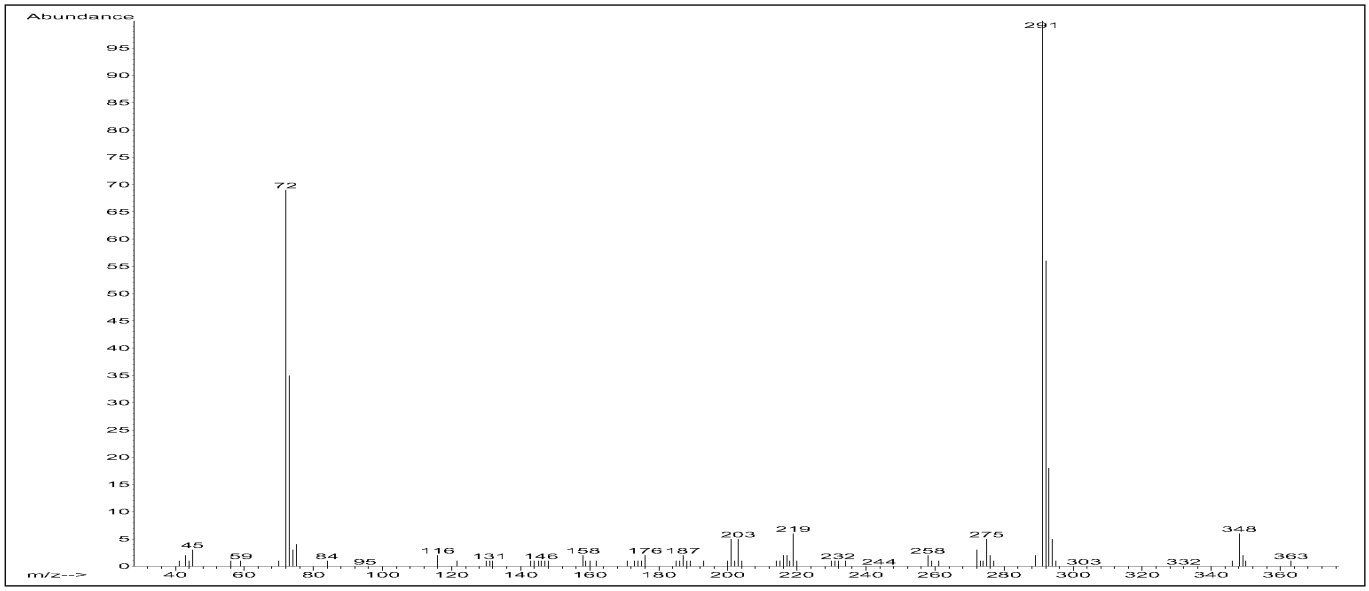
The standard solution (Boehringer Ingelheim), concentration 1mg/ml, diluted in methanol, is evaporated to dryness with a gentle nitrogen flow. To the residue, 50µl reagent and 125 µl pyridine is added and the reaction mixture is incubated for 30min at 60°C. Evaporation is repeated and the residue is redissolved in chloroform. The solution is ready for injection.

b) Reaction with Pentafluoropropionic Acid Anhydride (PFPA) (Reagent: Fluka 77292)
The standard is treated as in a) above. After the first evporation the residue is treated with 80µl of the PFPA reagent, 20µl of Hexafluoroisopropanol (Fluka 52517) is added and the reaction mixture is incubated for 30min at 70°C, followed by evaporation, dilution and GC/MSD analysis.

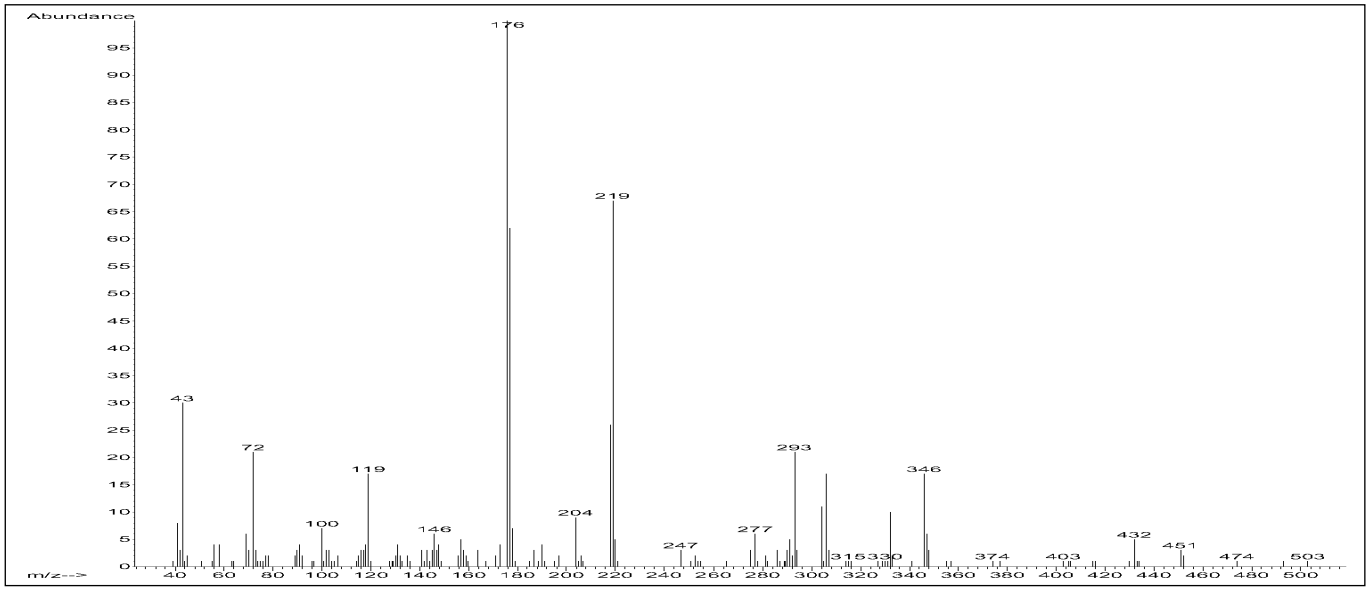
Results
The EI spectra of underivatised and derivatized analytes show low intensity for the molecular ion. Both the TMS and PFPA derivatization reactions form the di-derivatives. The TMS derivative response is higher comparing to the PFPA derivative. PCI/NH_3 response shows increased sensitivity by factor 2.6. Both modes present the characteristic PCI adduct ion information. Sensitivity for the TMS derivative in PCI/NH_3 SIM mode results in a signal-to-noise ratio of >20:1 at an analyte concentration of 50pg/µl.



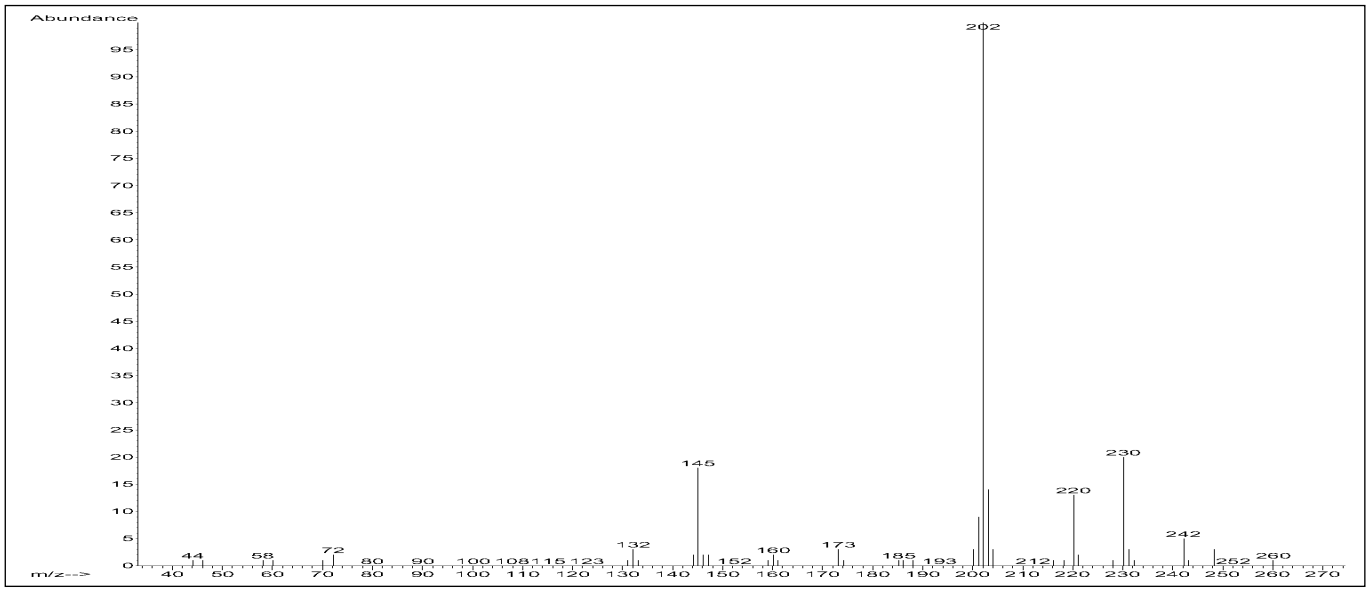
EI-Spectrum, Cimaterol, underivatised: *m/z* 219; M^+



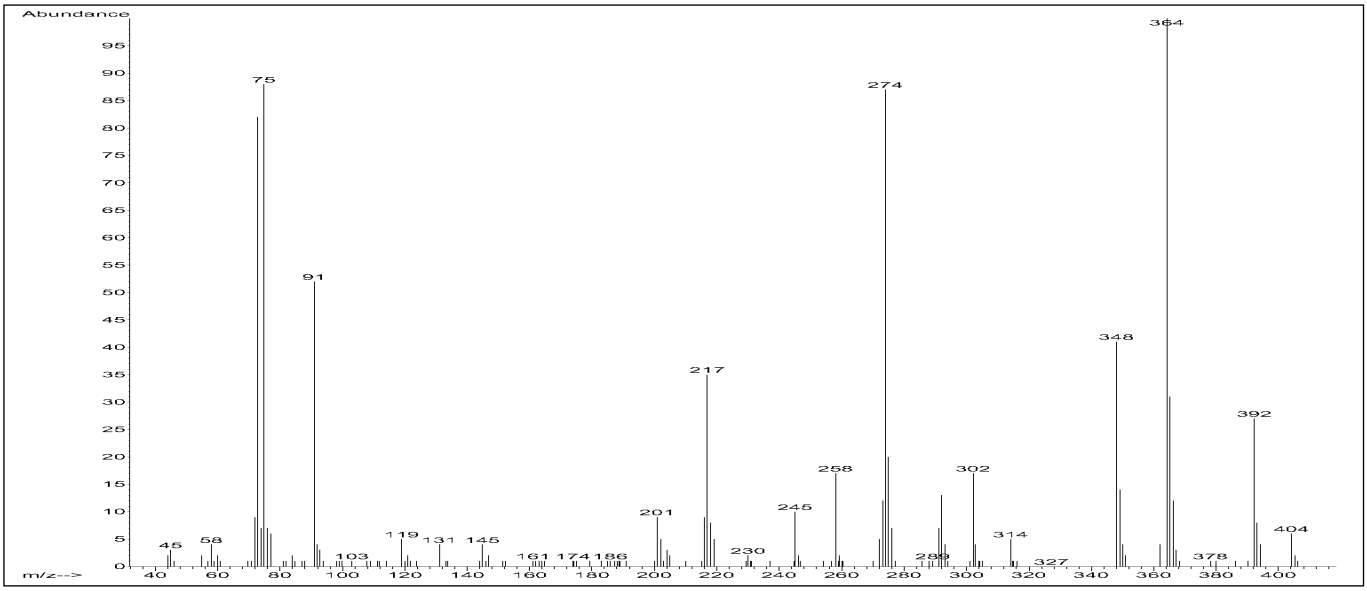
EI-Spectrum, Cimaterol, TMS Derivative: *m/z* 363; M^+



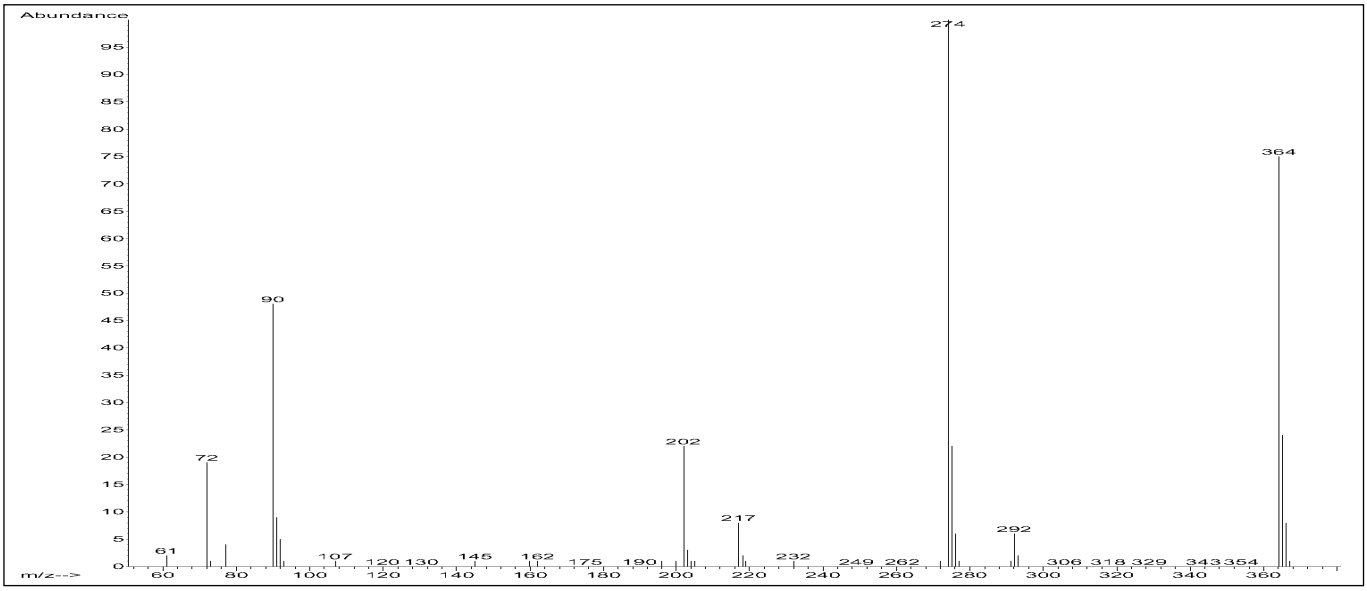
EI-Spectrum, Cimaterol, PFPA Derivative: *m/z* 511; M^+



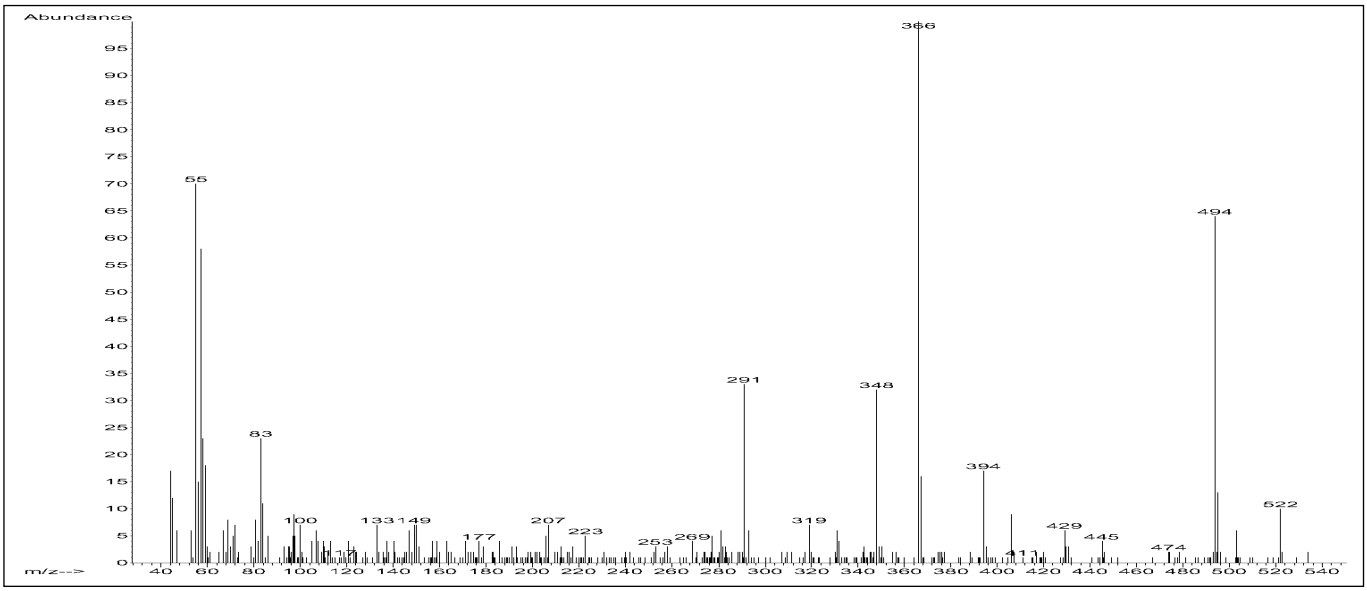
PCI/CH_4-Spectrum, Cimaterol, underivatised: *m/z* 220, 248, 260; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$; *m/z* 202, 230, 242; $[M-OH]^+$, $[M+C_2H_5-OH_2]^+$, $[M+C_3H_5-OH_2]^+$



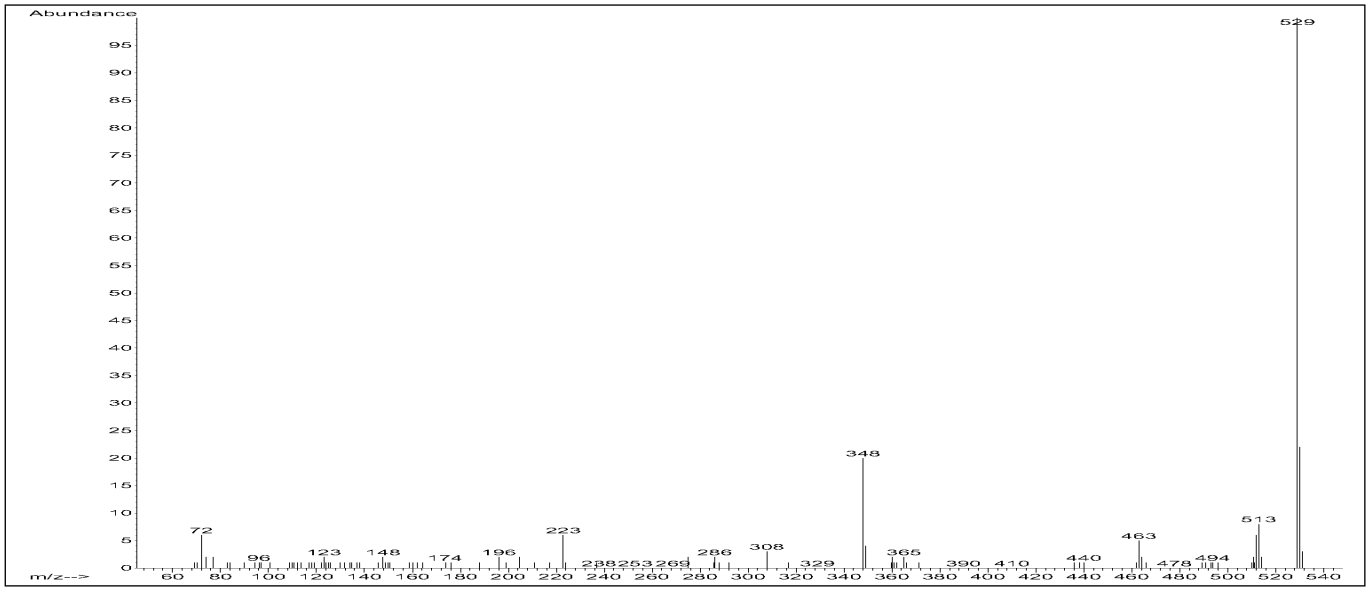
PCI/CH_4-Spectrum, Cimaterol, TMS Derivative: *m/z* 364, 392, 404; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



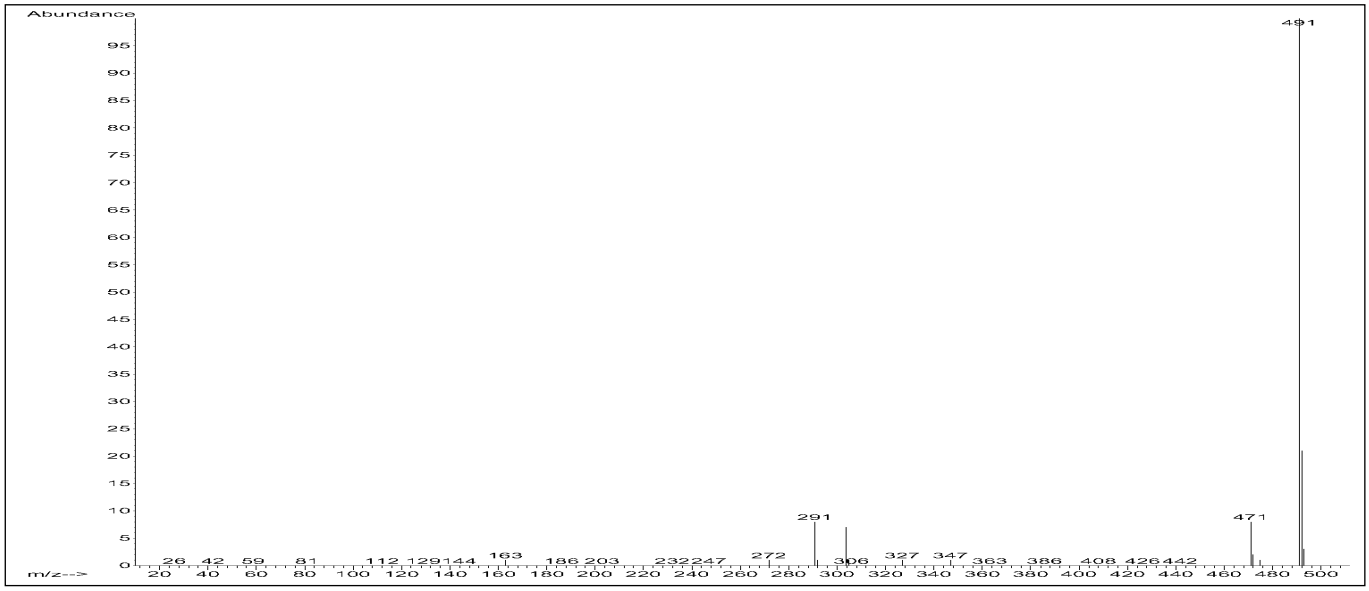
PCI/NH_3-Spectrum, Cimaterol, TMS Derivative: *m/z* 364 $[M+H]^+$



PCI/CH_4-Spectrum, Cimaterol, PFPA Derivative: *m/z* 494, 522, 534; $[M-OH]^+$, $[M+C_2H_5-OH_2]^+$, $[M+C_3H_5-OH_2]^+$

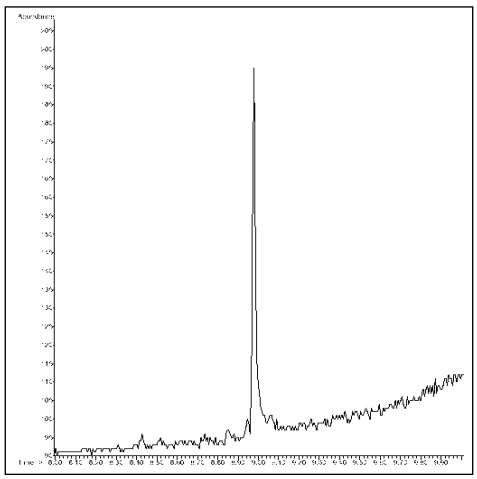


PCI/NH_3-Spectrum, Cimaterol, PFPA Derivative: *m/z* 529; $[M+NH_4]^+$



ECNI/CH_4-Spectrum, Cimaterol, PFPA Derivative: *m/z* 491; $[M-HF]^-$

PCI/NH_3 – SIM Mode



Cimaterol, TMS Derivative, 50pg, Retention Time: 8.98min
Ions: 274, 364 *m/z* ; Signal/Noise: > 20/1

Clenbuterol

CAS-Nr. 37148-27-9
Molecular Formula: $C_{12}H_{18}C_{l2}N_2O$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 1.2ml/min, 40cm/sec
Mode: Constant Flow
Injection: Split & Pulsed splitless, 250°C
Oven Temp. Program
Split: 100°C (0.3min) – 25°C/min to 280°C
Pulsed splitless: 80°C (1min) – 25°C/min to 265°C (1min)

MS Parameters

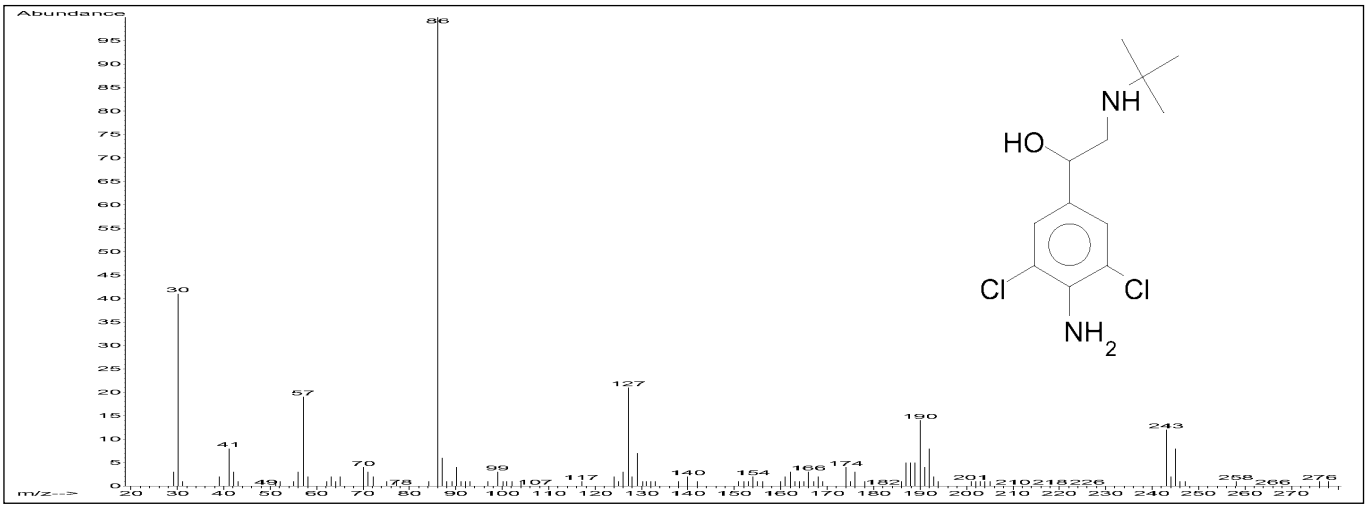
Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C
Mode: PCI/CH_4 – SCAN/SIM
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V
Mode: PCI/NH_3 – SCAN/SIM
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V
Mode: ECNI/CH_4 – SCAN/SIM
Buffer Gas: Methane
Flow (Setting): 2ml/min (40)
Tune: ECNI-Methane Tune File
Temperatures:
Source 150°C, Quad 106°C
EM Voltage: Tune + 400V

Remarks

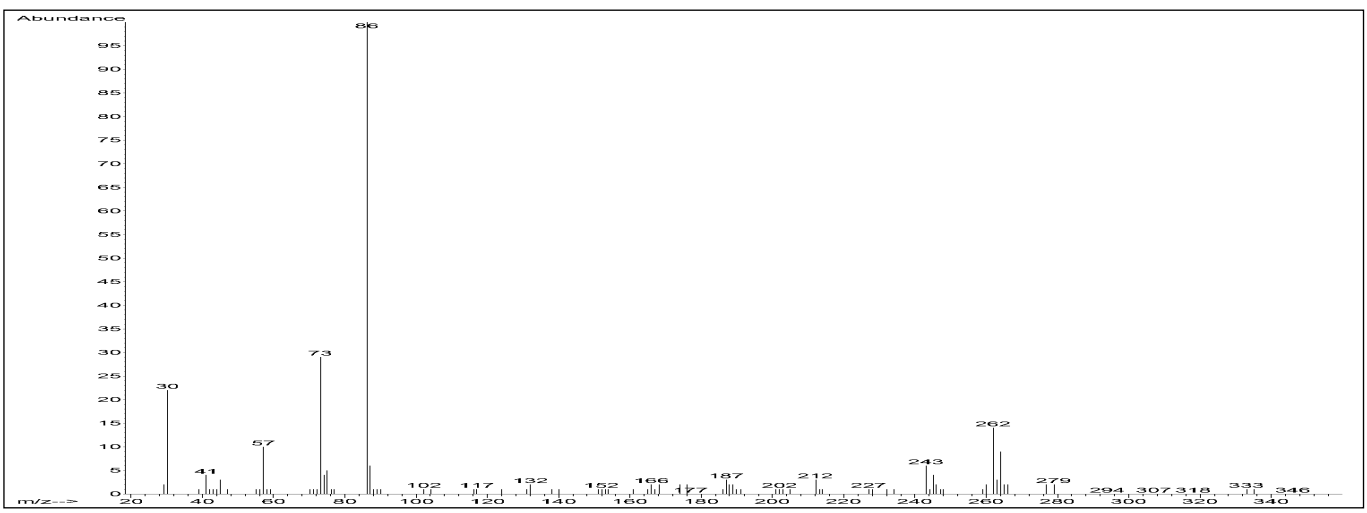
Derivatization
Trimethyl silylation with BSTFA/TMCS (Reagent: Fluka 15238)
Derivatization conditions (e.g, incubation temperature and time) have some impact on the product formation. Applying 60°C for 30min generates both mono- and di-derivatives at a ratio of 1:0.75.
The standard solution (SIGMA C 5423), concentration 1mg/ml, in methanol, is evaporated with a gentle nitrogen flow. To the residue a mixture of Pyridine/TMS-Reagent (2.5/1) is added and incubation is done for 30min. The excess reagent is evaporated and the residue redissolved in chloroform. The solution is ready for injection.

Results
The degree of fragmentation is related to the sample concentration for this analyte. The response in PCI/NH_3 mode is higher comparing to PCI/CH_4 mode. Weak response is observed in ECNI/CH_4 mode.

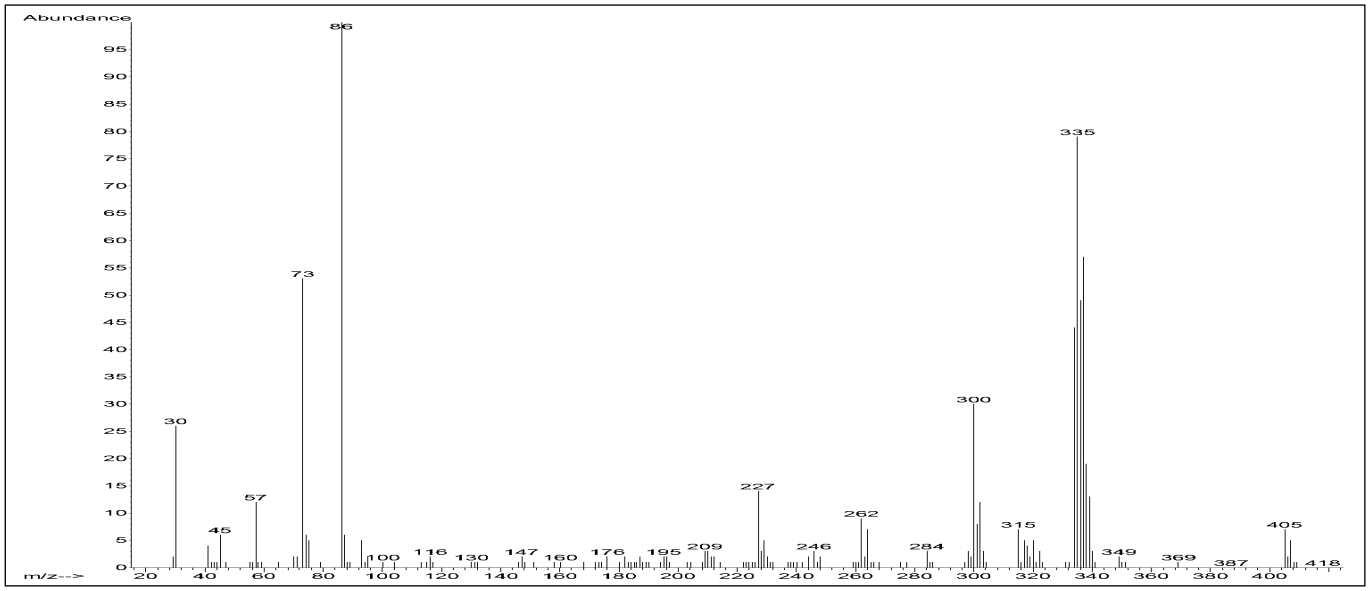
References
"Analysis of Clenbuterol by GC-MS…", F. David, RIC, Agilent Pub. Nr. 5962-9427E
"Clenbuterol and Norandrosterone…", B. Wüst, Agilent Pub. Nr. 5980-0908E



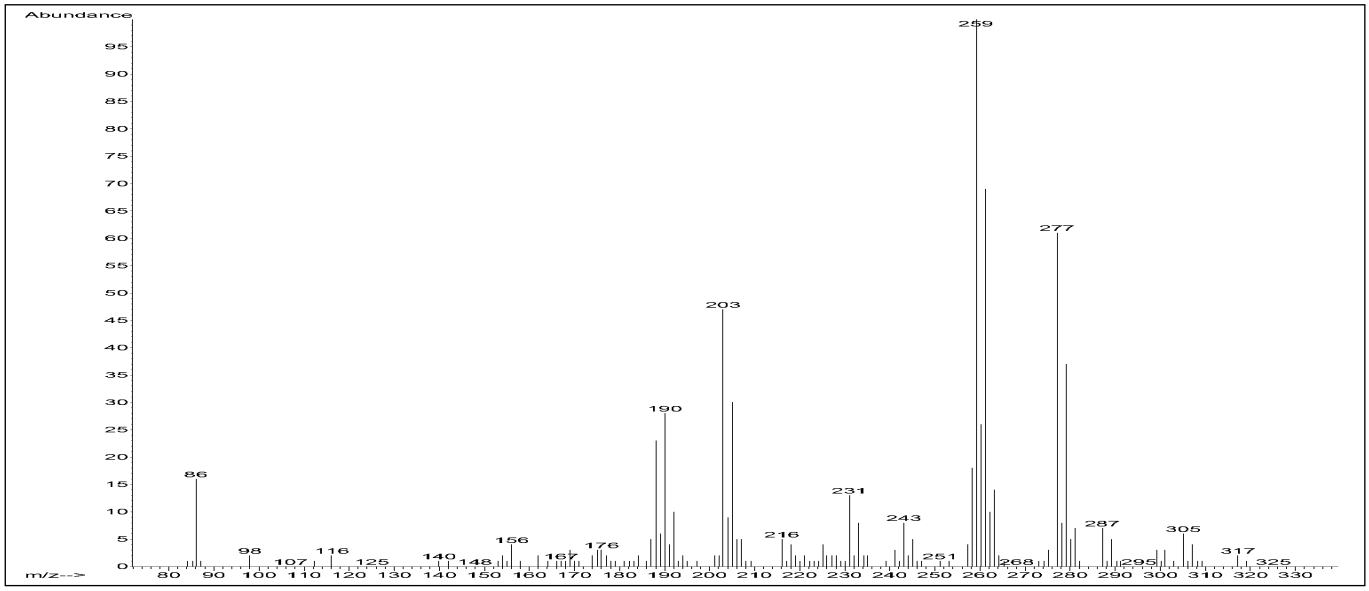
EI-Spectrum, Clenbuterol, underivatised: *m/z* 276; M^+



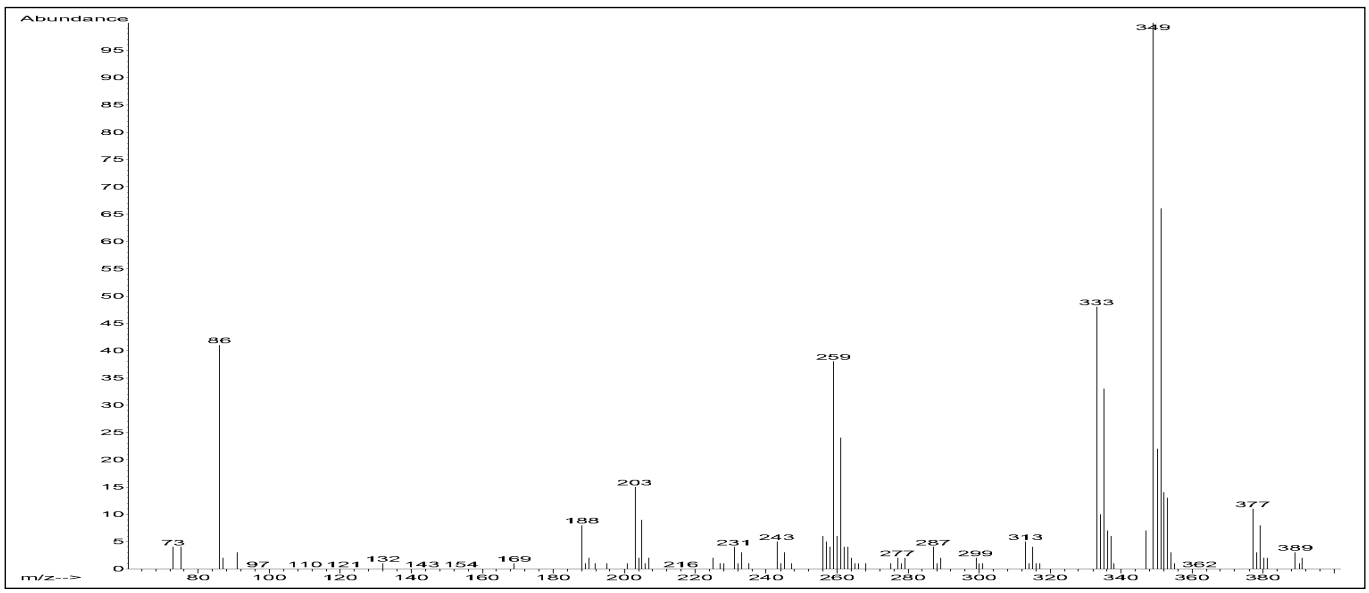
EI-Spectrum, Clenbuterol, BSTFA-mono-derivative: *m/z* 348; M^+



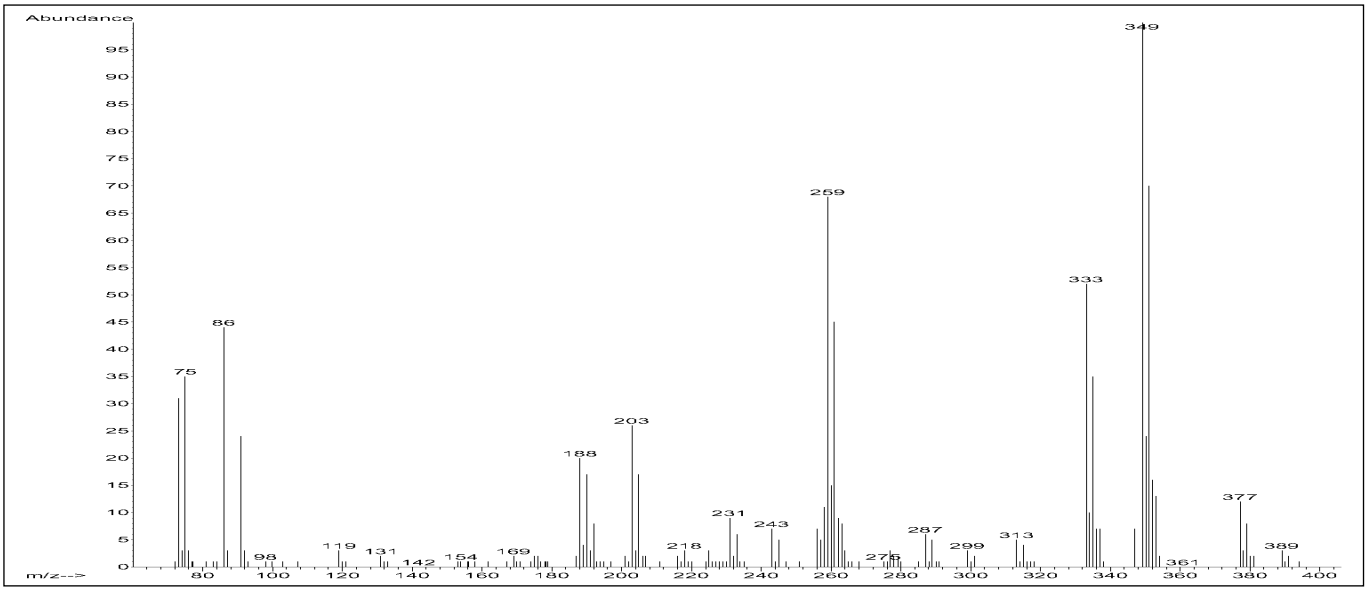
EI-Spectrum, Clenbuterol, BSTFA-di-derivative:, *m/z* 420; M^+



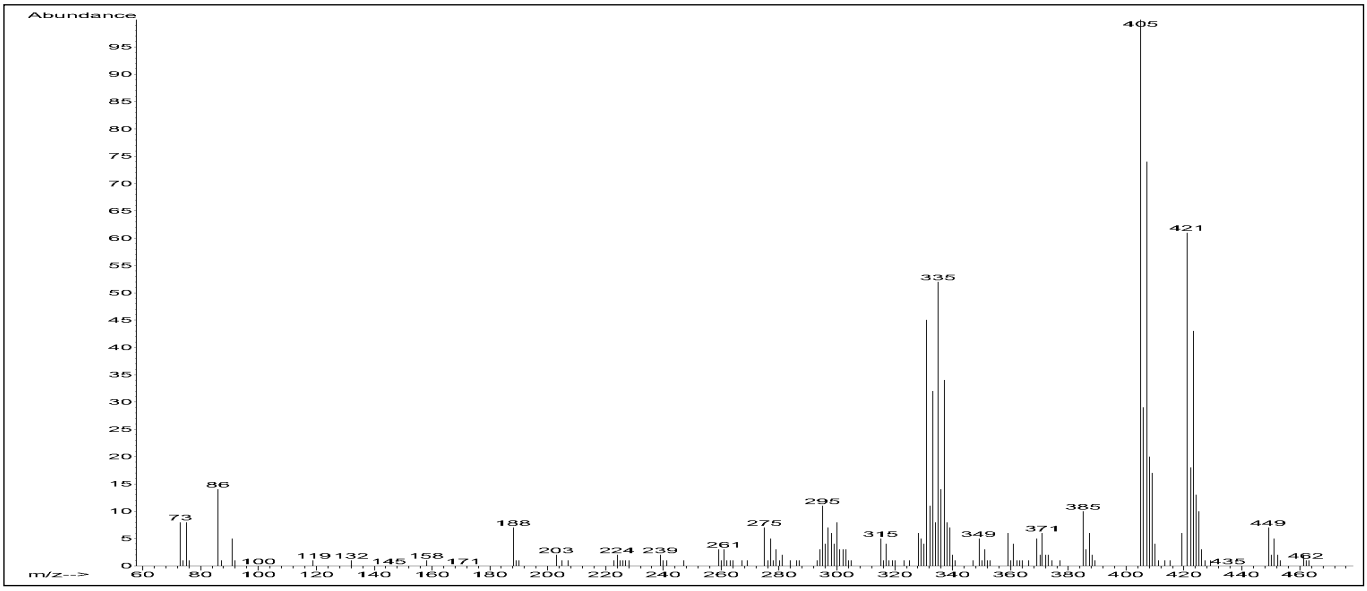
PCI/CH_4-Spectrum, Clenbuterol, underivatised: *m/z* 277, 305, 317; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



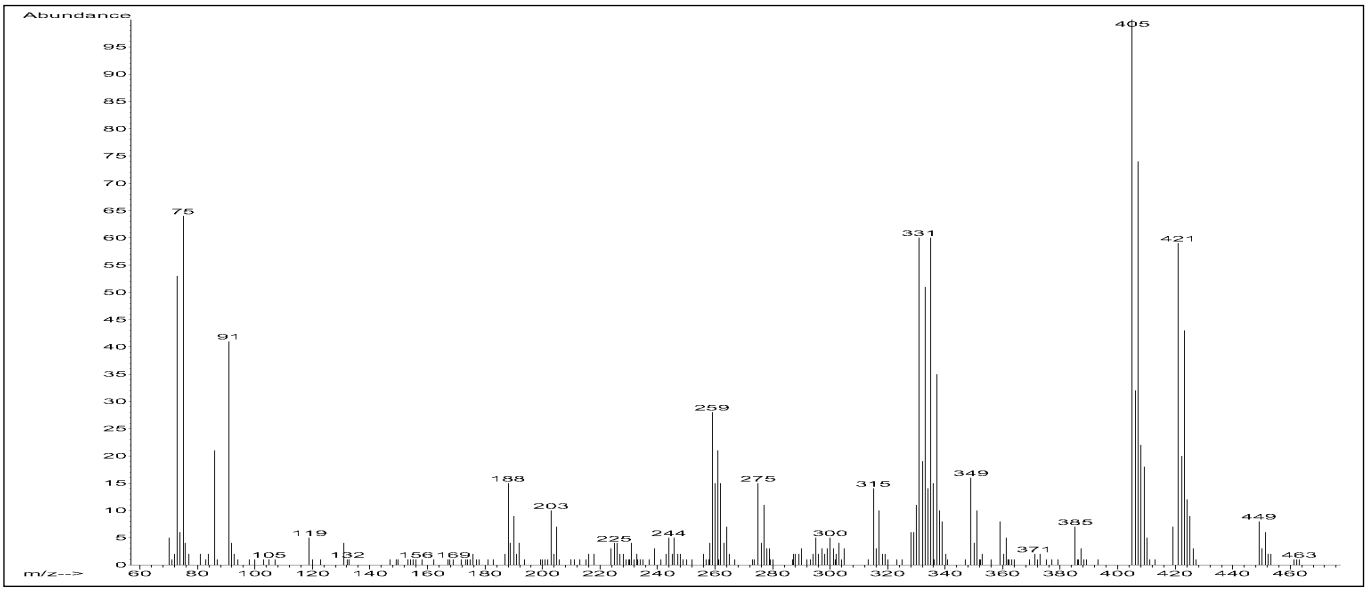
PCI/CH_4-Spectrum, Clenbuterol, BSTFA-mono-derivative, approx. 30ng: *m/z* 349 , 377 , 389 ; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



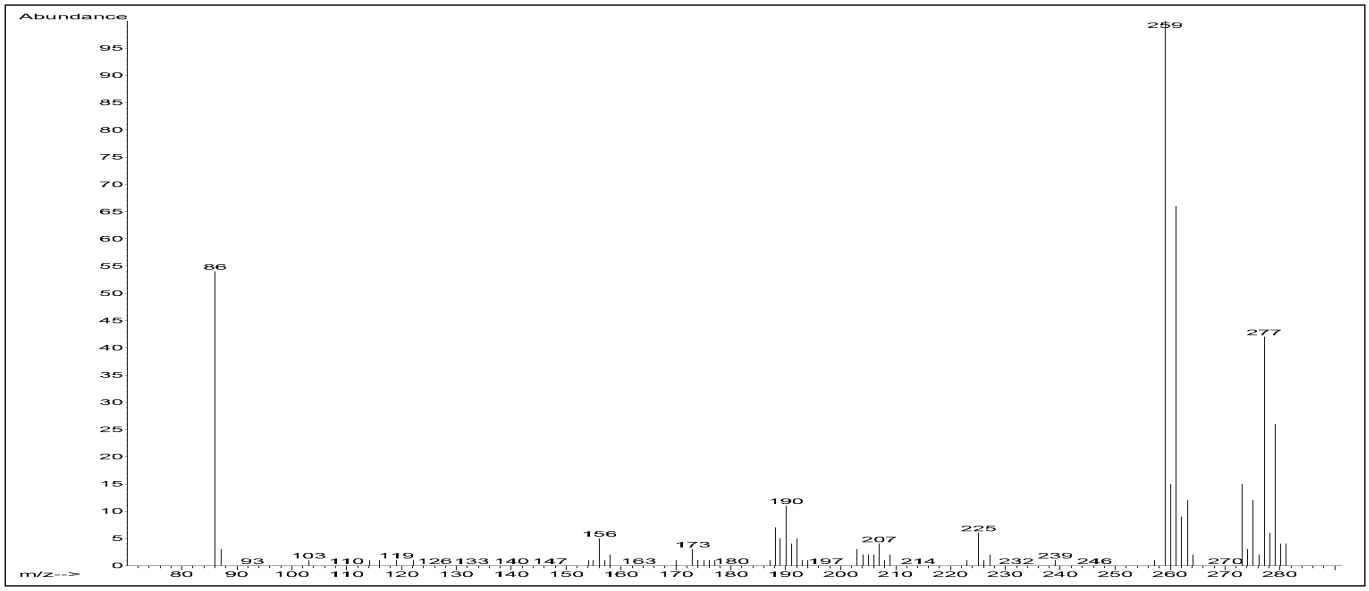
PCI/CH_4-Spectrum, Clenbuterol, BSTFA-mono-derivative, approx. 1ng, *m/z* 349, 377, 389; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



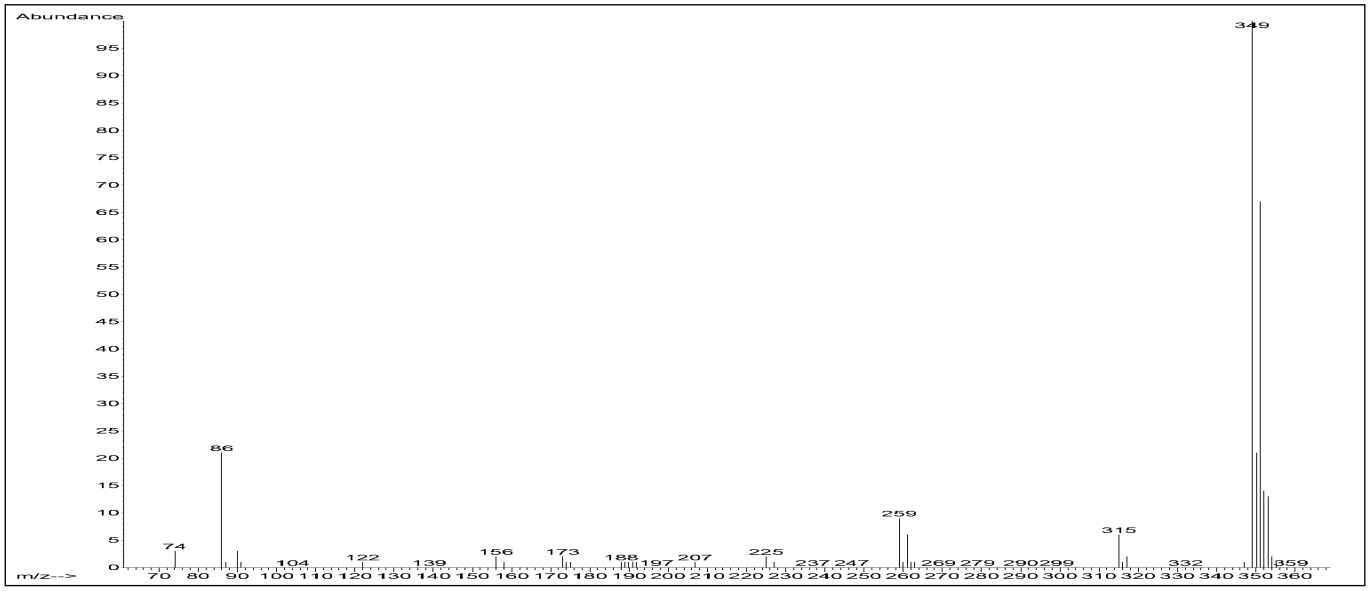
PCI/CH_4-Spectrum, Clenbuterol, BSTFA-di-derivative, approx. 40ng: *m/z* 421, 449, 461; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



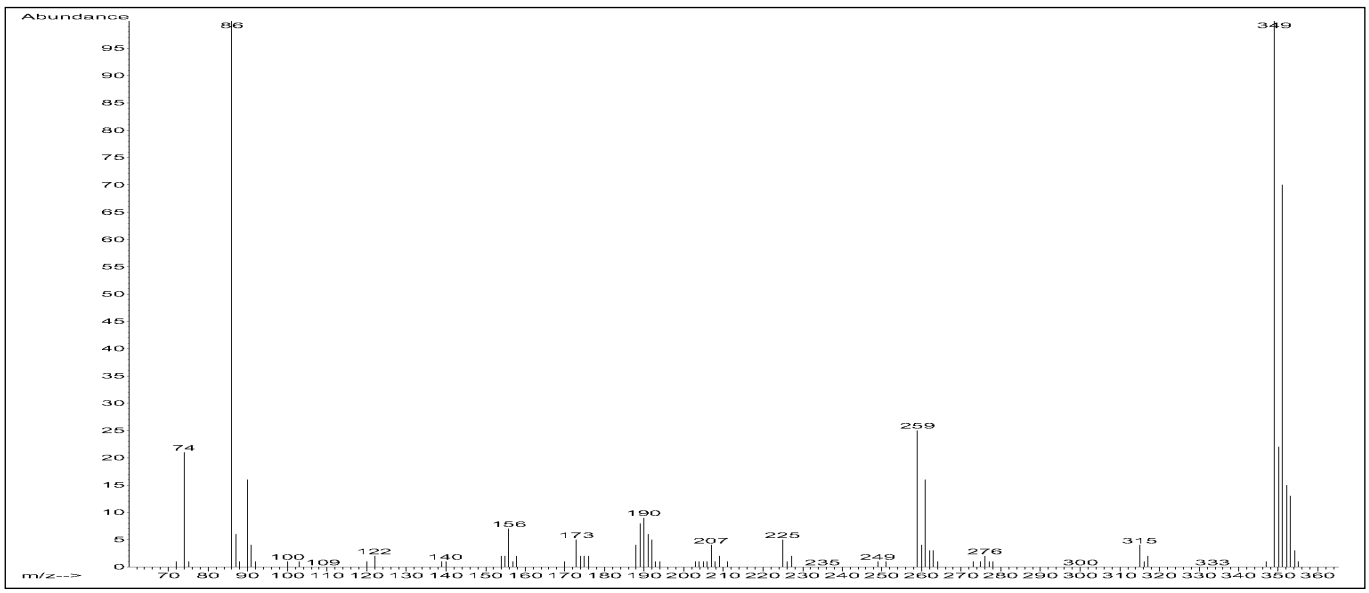
PCI/CH_4-Spectrum, Clenbuterol, BSTFA-di-derivative, approx. 1ng: *m/z* 421, 449, 461; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



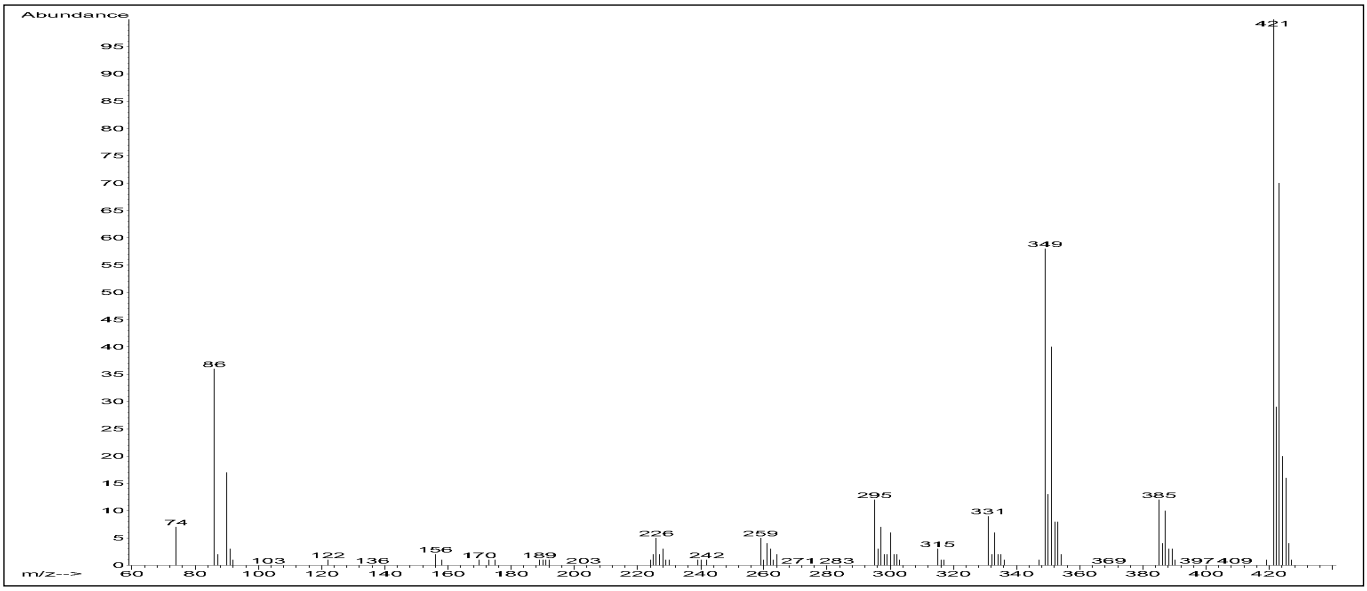
PCI/NH_3-Spectrum, Clenbuterol, underivatised: *m/z* 277; $[M+H]^+$



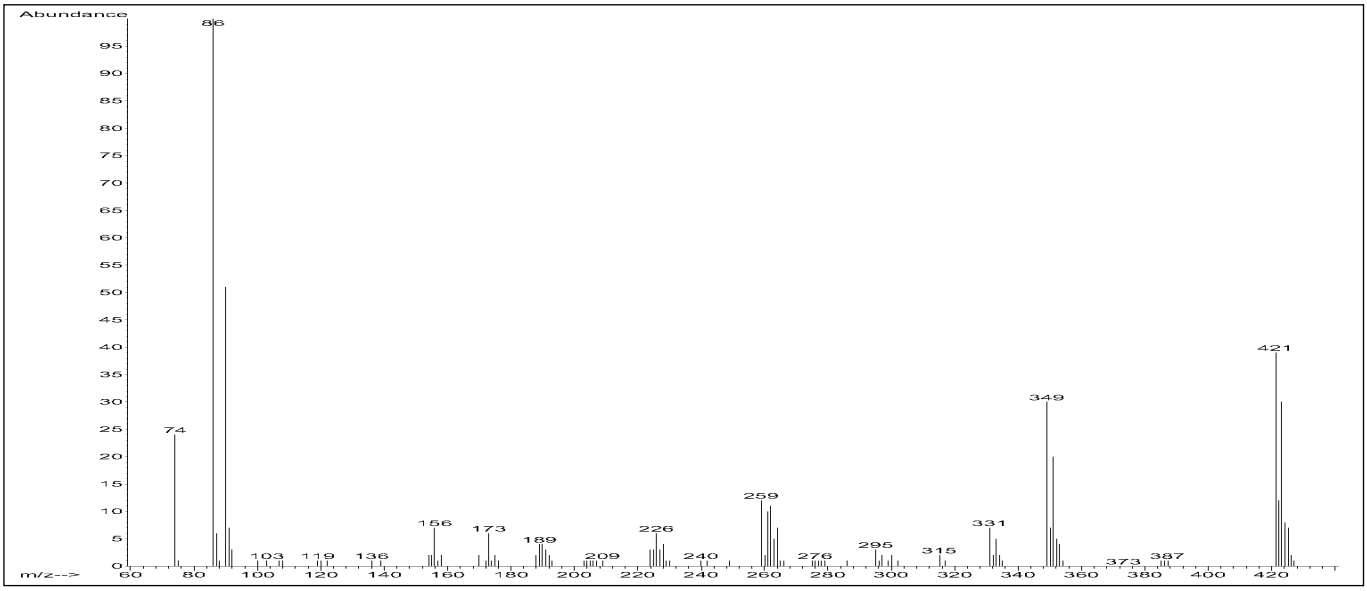
PCI/NH_3-Spectrum, Clenbuterol, mono-derivative, approx. 30ng: *m/z* 349; $[M+H]^+$



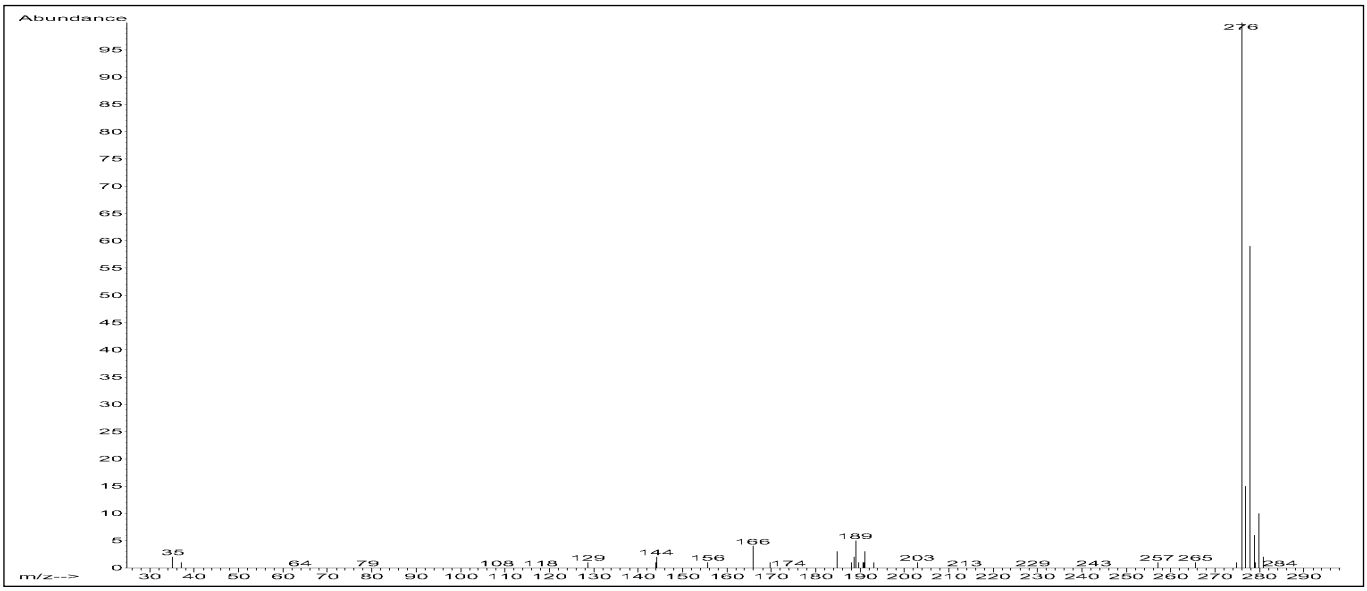
PCI/NH_3-Spectrum, Clenbuterol, mono-derivative, approx. 1ng: *m/z* 349; $[M+H]^+$



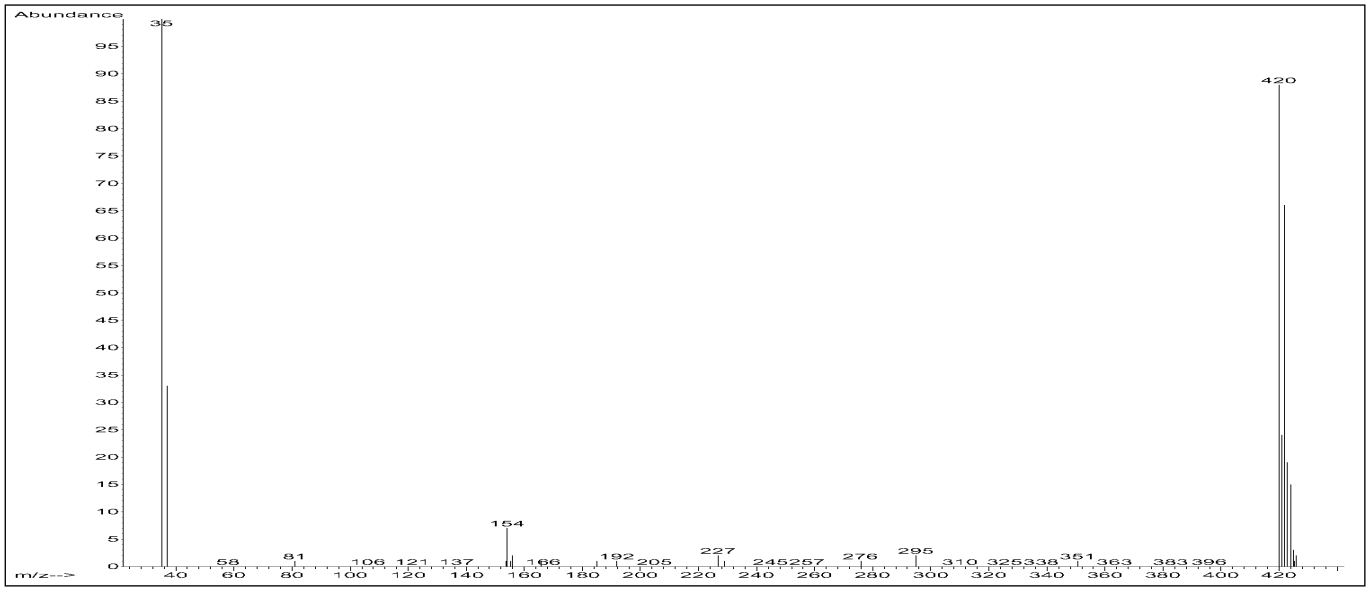
PCI/NH_3-Spectrum, Clenbuterol, approx. 40ng, di-derivative: *m/z* 421; $[M+H]^+$



PCI/NH_3-Spectrum, Clenbuterol, di-derivative, approx. 1ng: *m/z* 421; $[M+H]^+$

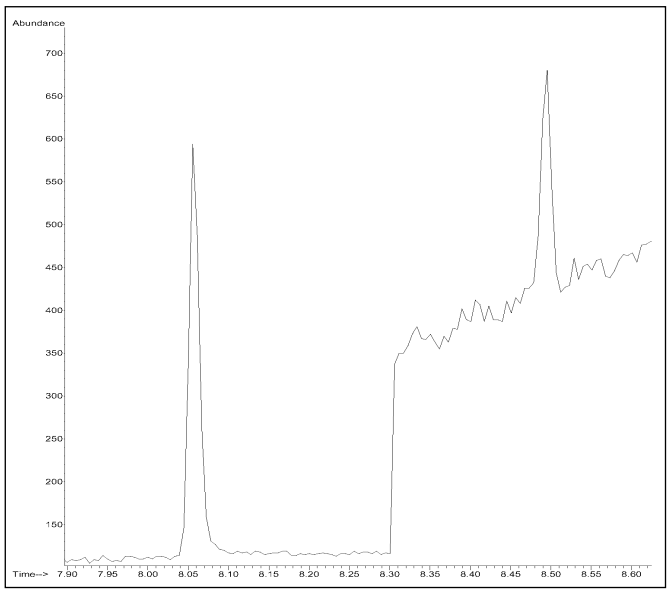


ECNI/CH_4-Spectrum, Clenbuterol, approx. 1ng, underivatised: *m/z* 276; M^-

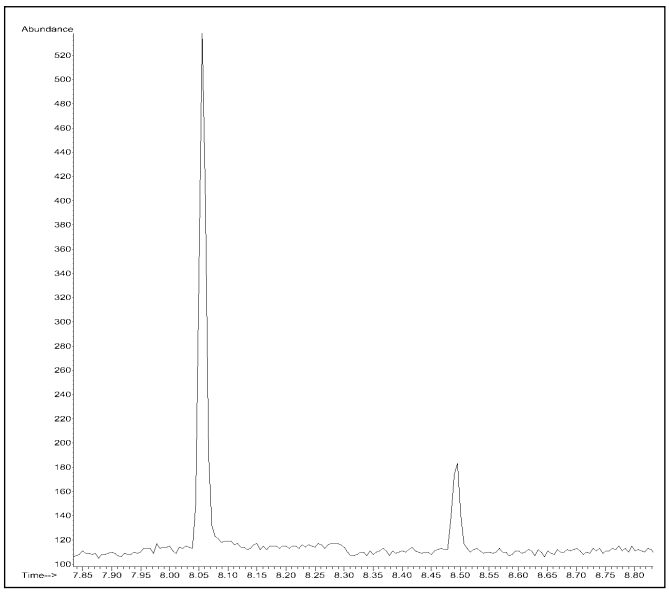


ECNI/CH_4-Spectrum, Clenbuterol, approx. 1ng, di-derivative: *m/z* 420; M^-

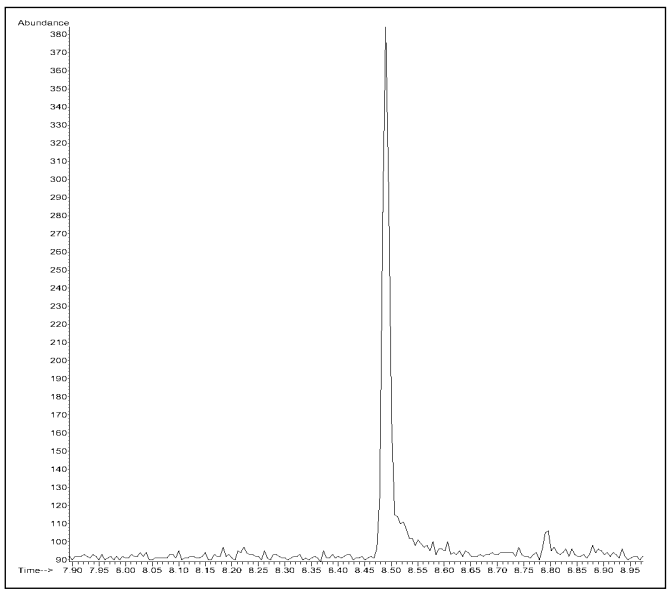
SIM Mode



PCI/CH_4, Clenbuterol, approx. 15pg, mono & di-derivative: S/N ≈ 70/1 & ≈ 5/1



PCI/NH_3, Clenbuterol, approx. 1.5pg, mono &di-derivative: S/N ≈ 55/1 & ≈ 8/1



ECNI/CH_4, Clenbuterol, approx. 15 pg, di-derivative: S/N ≈ 30/1

Cocaine

CAS-Nr. 50-36-2
Molecular Formula: $C_{17}H_{21}NO_4$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 1.2ml/min, 40cm/sec
Mode: Constant Flow
Injection: Pulsed splitless, 250°C
Oven Temp.Program
70°C (1min) – 25°C/min to
300°C (5min)

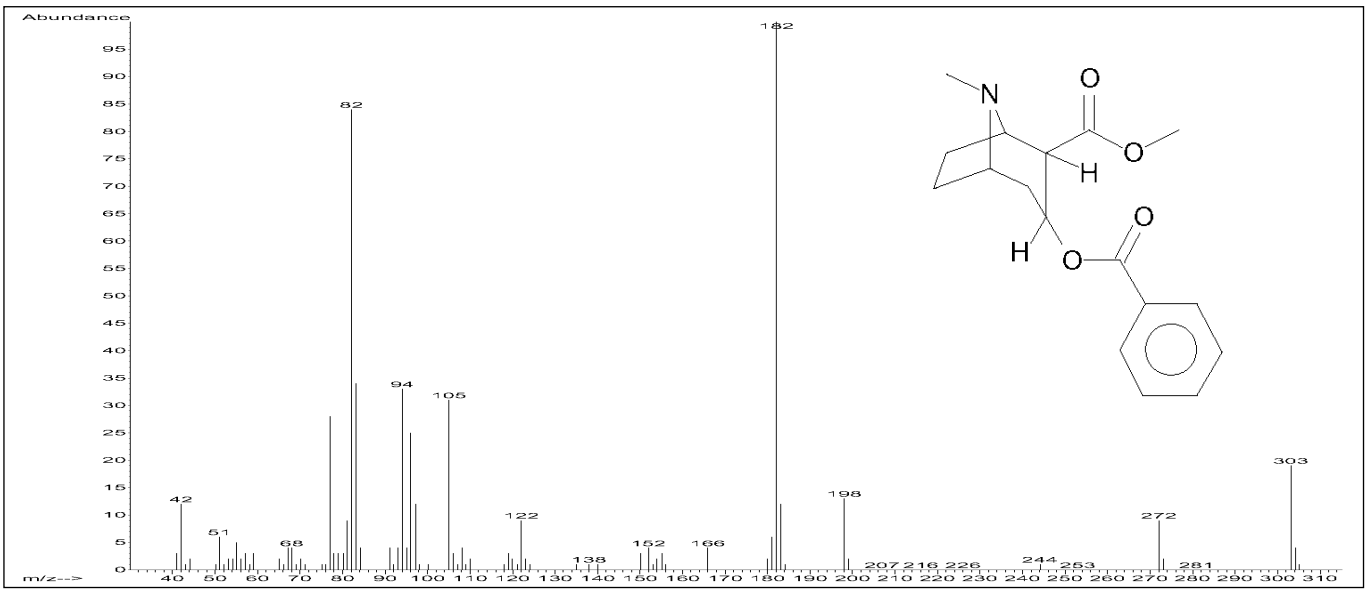
MS Parameters

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C

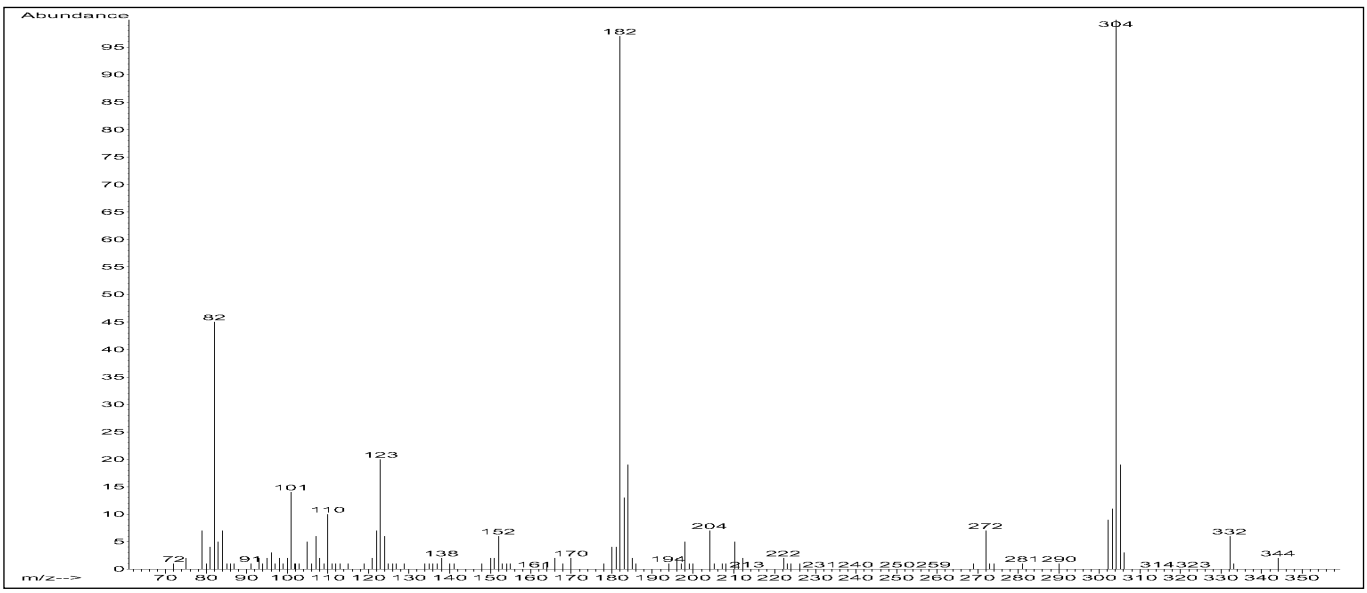
Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V

Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V

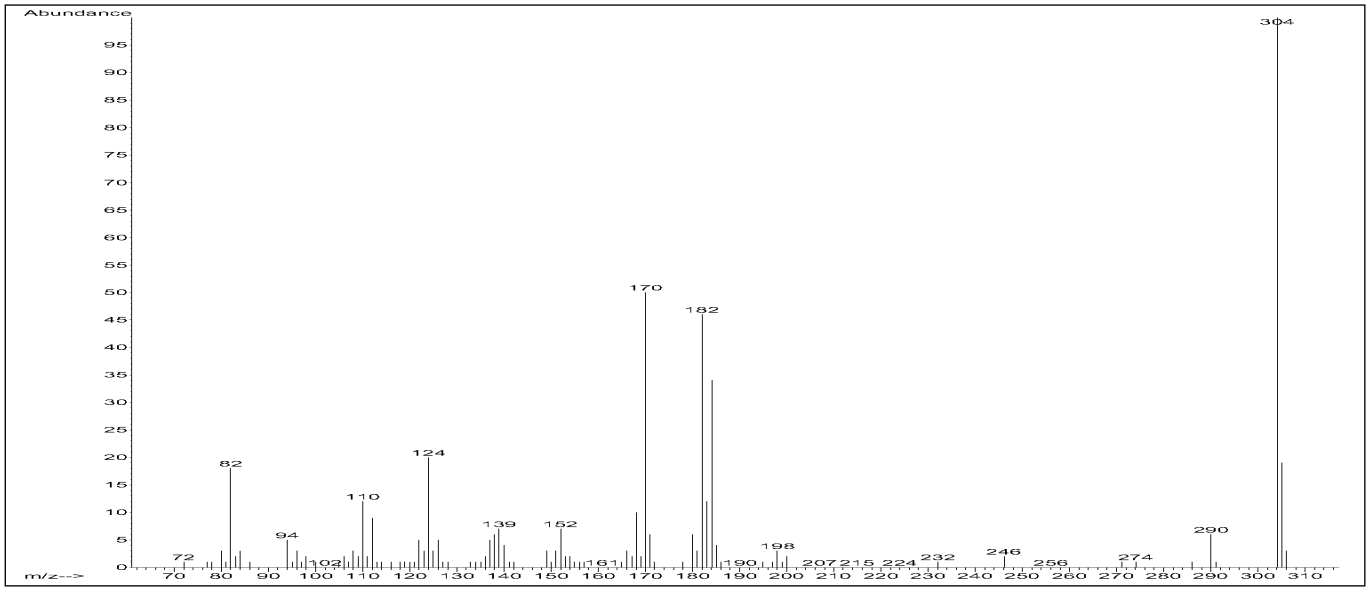
Results
In PCI mode, SCAN acquisition, ammonia is the preferred reagent gas due to higher response compared to methane. In SIM mode there were no significant differences in responses observed. In PCI/CH_4 SIM mode signal/noise ration of >20/1 was measured for 10pg/µl. ECNI showed no relevant spectrum.



EI-Spectrum, Cocaine: *m/z* 303: M^+

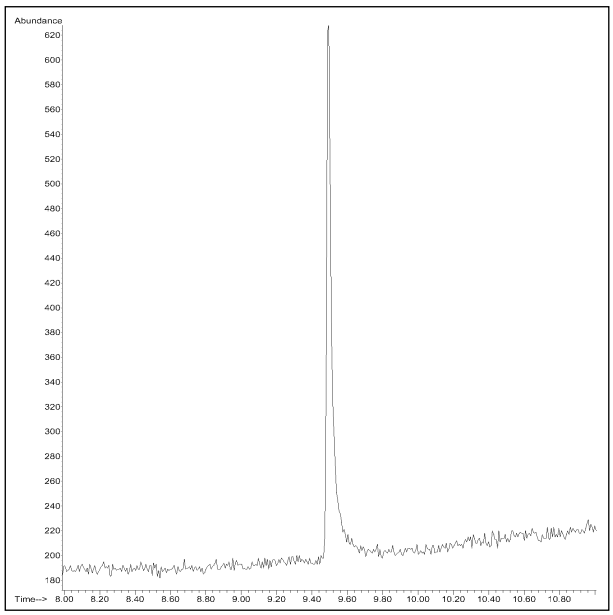


PCI/CH_4-Spectrum, Cocaine: *m/z* 304, 332, 344; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



PCI/NH_3-Spectrum, Cocaine: *m/z* 304; $[M+H]^+$

PCI/CH_4 – SIM Mode



Cocaine, Retention Time: 9.50min, 10pg/µl: Ions 182, 304 *m/z* ; S/N > 25/1

Codeine

CAS-Nr. 76-57-3
Molecular Formula: $C_{18}H_{21}NO_3$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 1.2ml/min, 40cm/sec
Mode: Constant Flow
Injection: Pulsed splitless, 250°C
Oven Temp. Program
70°C (1min) – 25°C/min to 300°C (5min)

MS Parameters

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C

Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V

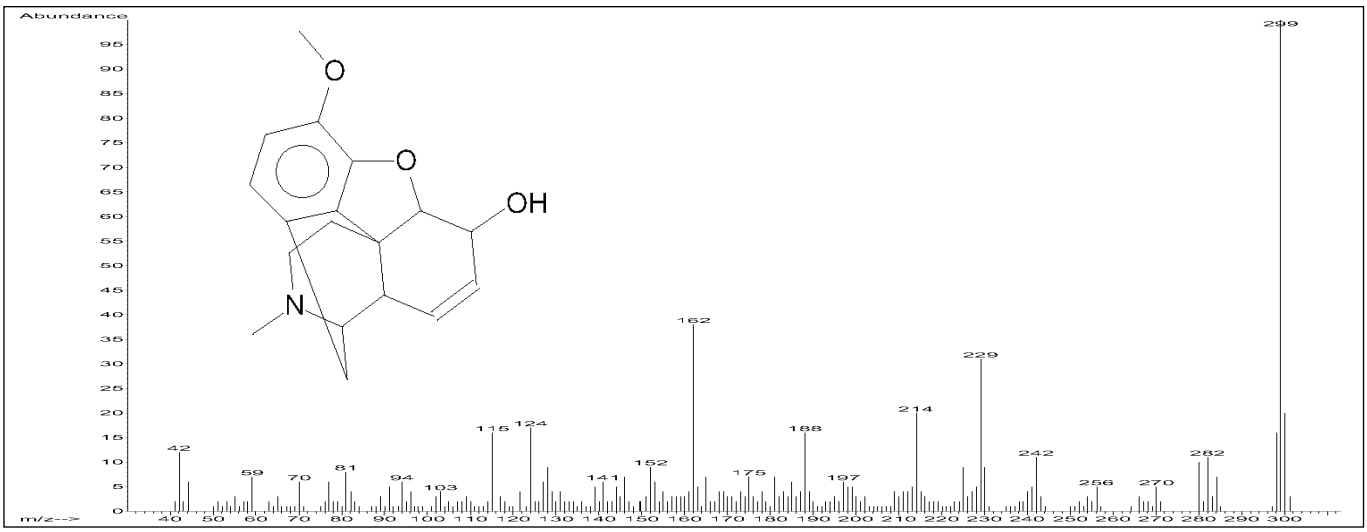
Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V

Mode: ECNI/CH_4 – SCAN/SIM
Buffer Gas: Methane
Flow (Setting): 2ml/min (40)
Tune: ECNI-Methane Tune File
Temperatures:
Source 150°C, Quad 106°C
EM Voltage: Tune + 400V

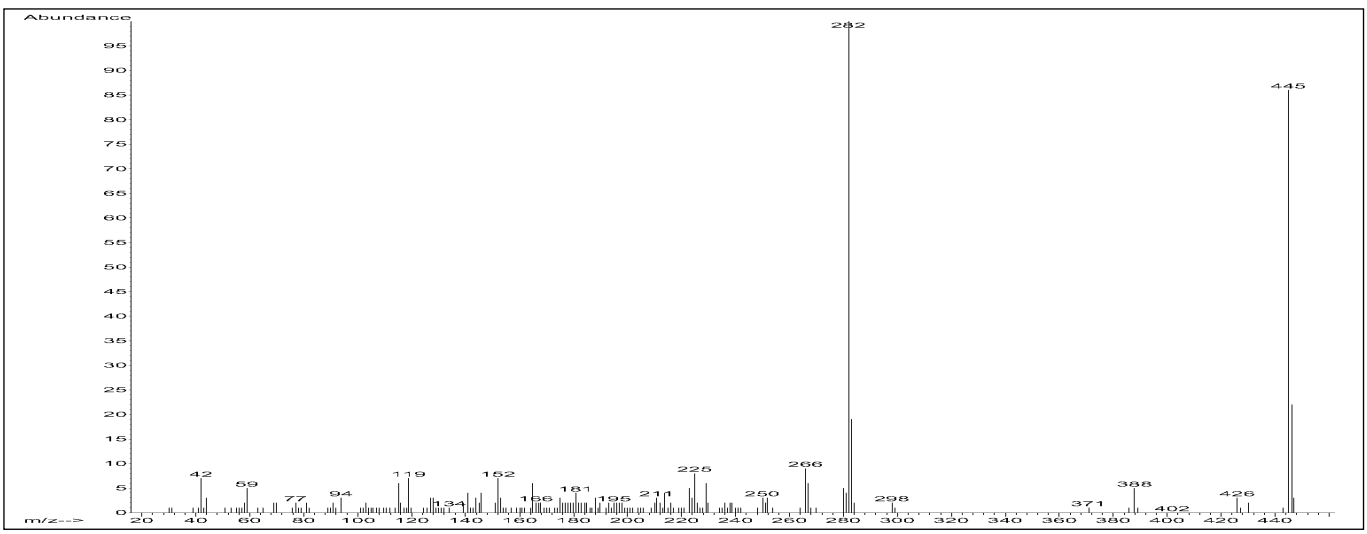
Remarks

Derivatization
Reaction with Pentafluoro-propionic Acid Anhydride (PFPA) (Reagent: Fluka 77292)
The standard solution (SIGMA C 1653), concentration 100ng/µl in ethyl acetate, is evaporated with a gentle flow of nitrogen. To the residue 80µl of the PFPA reagent and 20µl of Hexafluoroisopropanol (Fluka 52517) is added and the reaction mixture is incubated for 30min at 70°C. Evaporation is repeated and the residue redissolved in ethyl acetate. The solution is ready for injection and analysis.

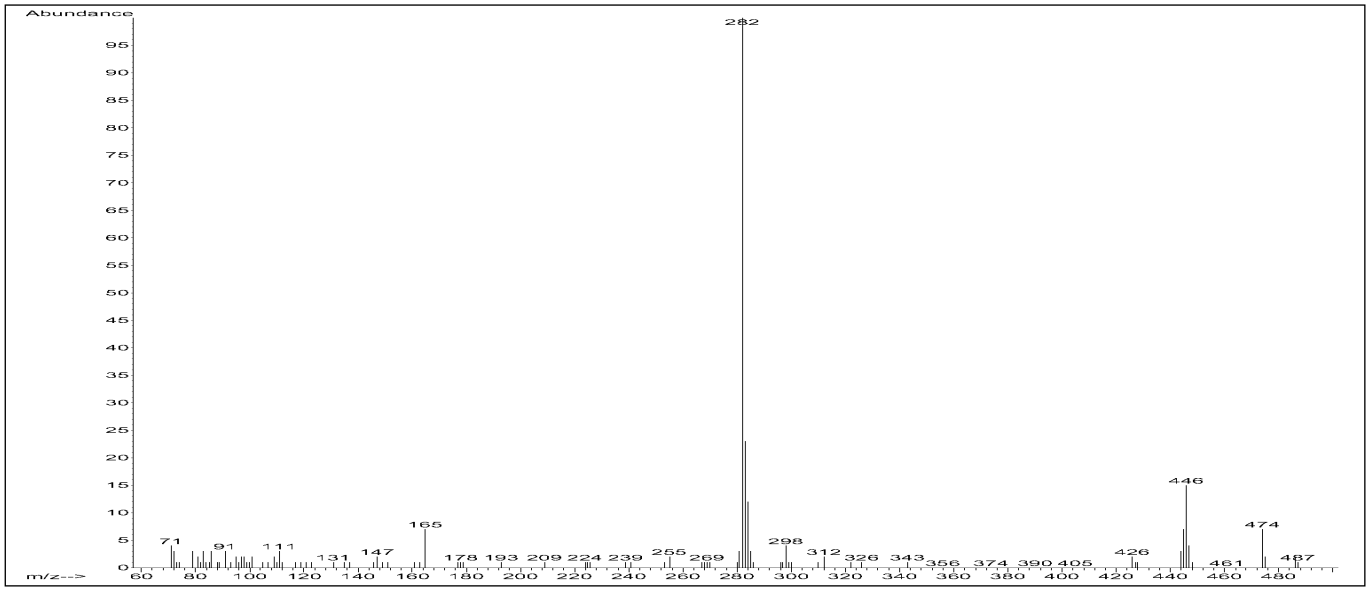
Results
Even the underivatized analyte is measured without chromatographic discrimination. In PCI mode, the response of the derivatized analyte was 2-times higher using ammonia reagent gas than methane.
ECNI/CH_4 SIM measurements were highly sensitive for the derivatized analyte; 5pg/µl resulted in signal/noise ratio of >125/1.
The acetylated analyte showed no significant ECNI spectrum.



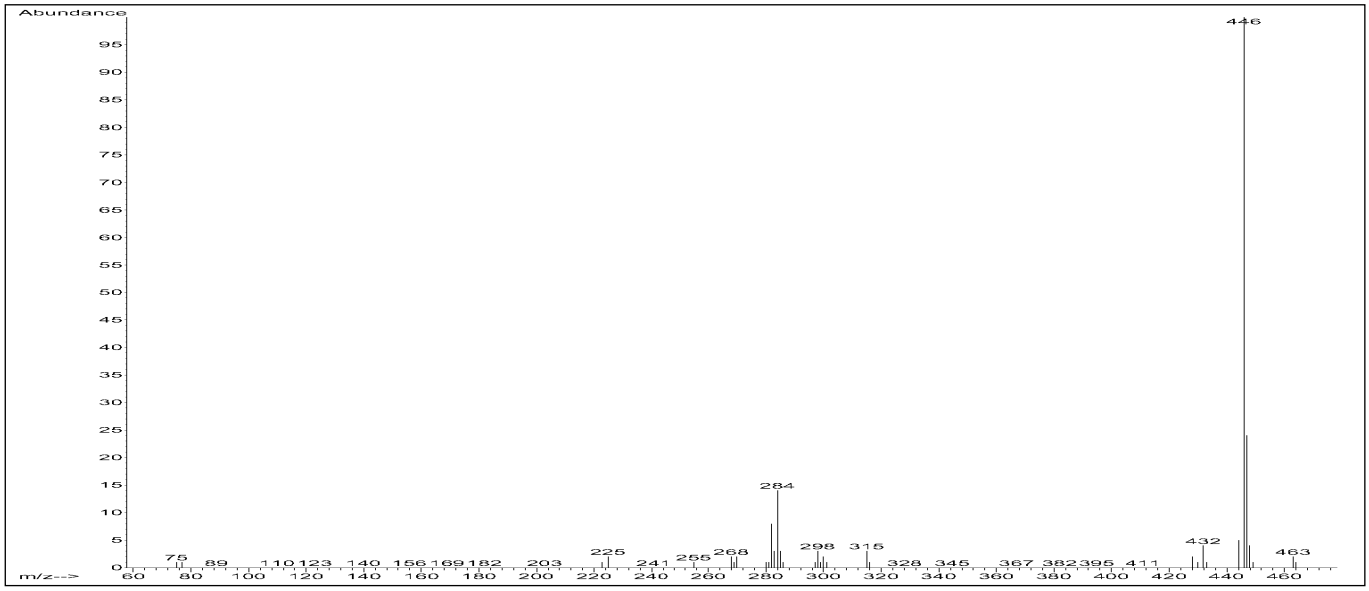
EI-Spectrum, Codeine, underivatised: *m/z* 299 ; M^+



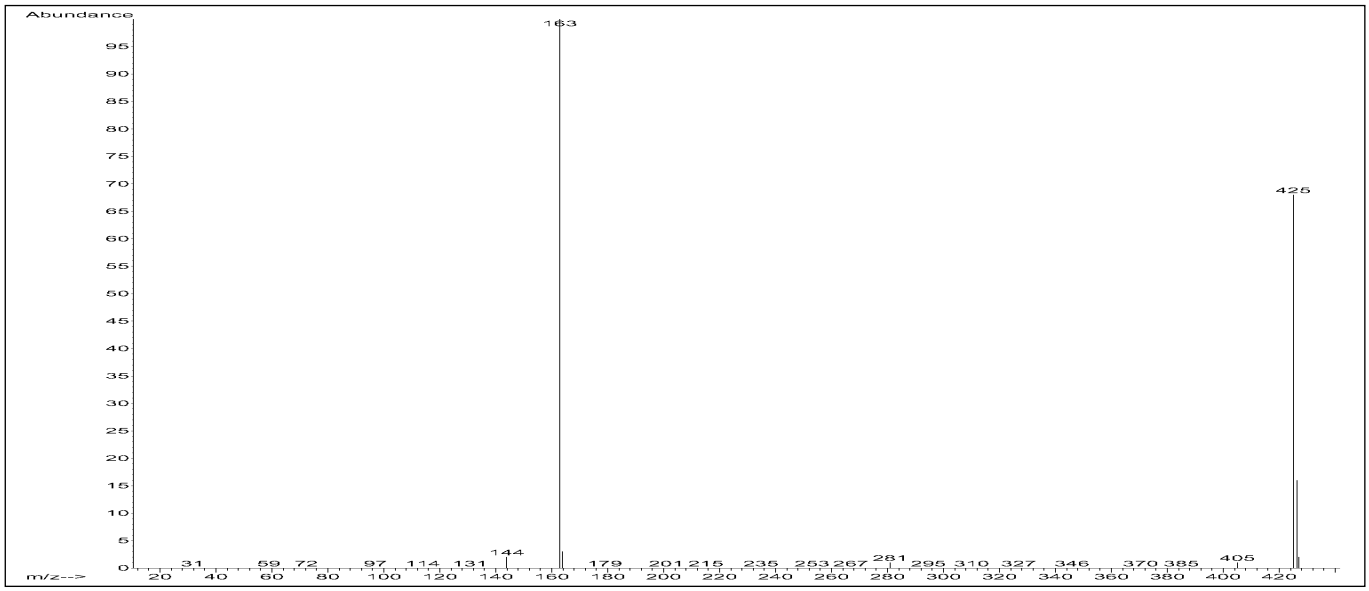
EI-Spectrum, Codeine, PFPA Derivative: *m/z* 445 ; M^+



PCI/CH_4-Spectrum, Codeine, PFPA Derivative: *m/z* 446, 474, 486; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$

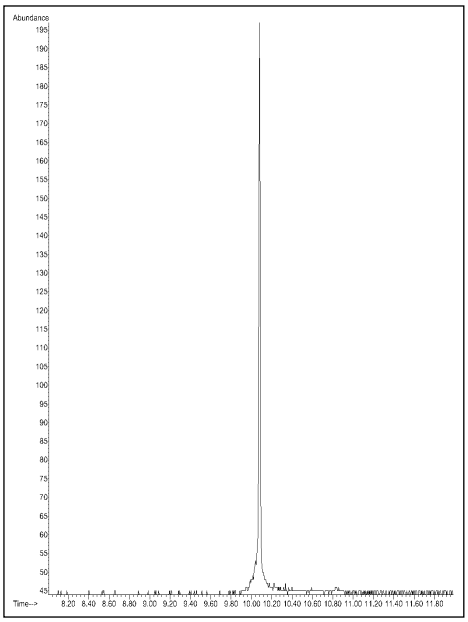


PCI/NH_3-Spectrum, Codeine, PFPA Derivative: *m/z* 446, 463; $[M+H]^+$, $[M+NH_4]^+$



ECNI/CH_4-Spectrum, Codeine, PFPA Derivative: *m/z* 425; $[M-HF]^-$

ECNI/CH_4 SIM



Codeine, PFPA Derivative, 5pg, Retention Time: 10.08min
Ion: 425 *m/z*, Signal/Noise ≈ 150/1

Dimethindene

CAS-Nr. 5636-83-9
Molecular Formula: $C_{20}H_{24}N_2$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 0.7ml/min, 30cm/sec
Mode: Constant Flow
Injection: Split, 250°C
Oven Temp. Program
120°C (0.3min) – 20°C/min to 300°C (4min)

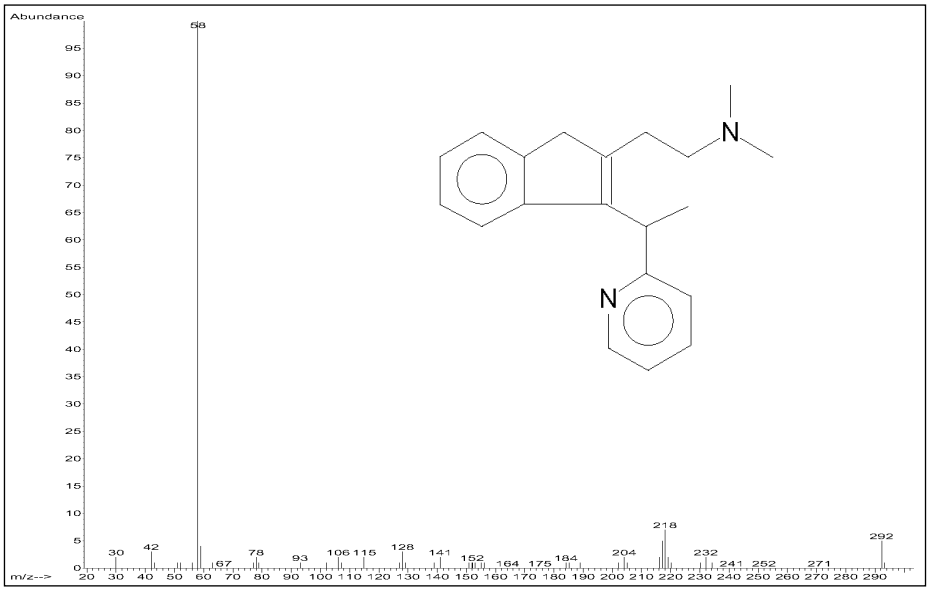
MS Parameters

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C

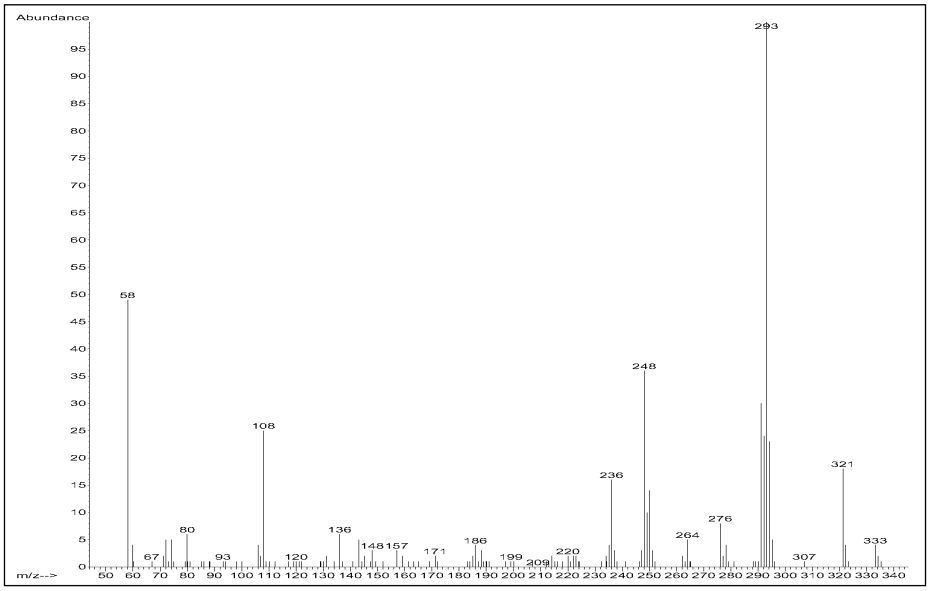
Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V

Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V

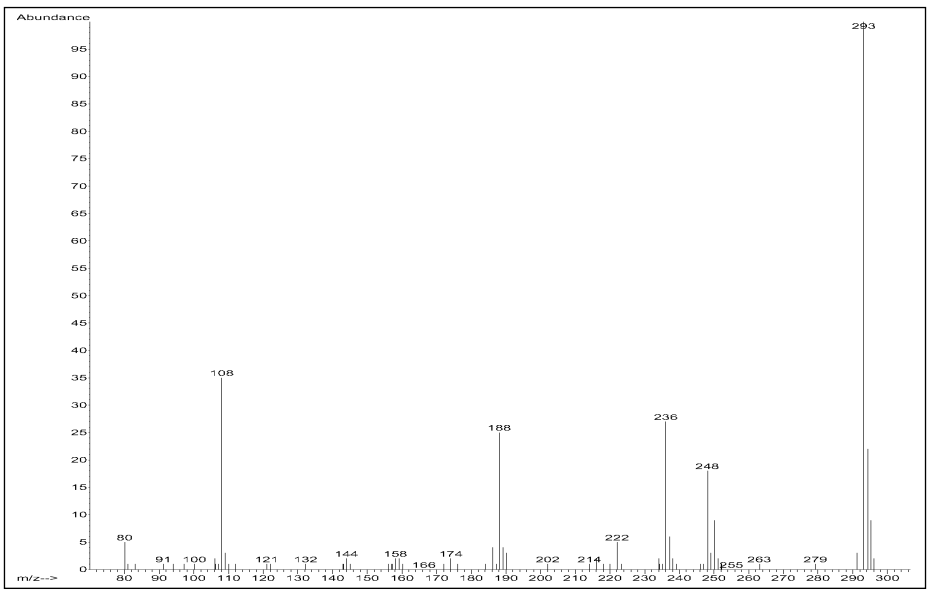
Results
Analyte Retention Time: 9.59min
Analyte Concentration: 4ng/µl
Signal/Noise Ratios for TIC
PCI/CH_4 Scan: > 5/1
PCI/NH_3 Scan: > 10/1



EI-Spectrum, Dimethindene: *m/z* 292; M^+



PCI/CH_4-Spectrum, Dimethindene: *m/z* 293, 321, 333; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



PCI/NH_3-Spectrum, Dimethindene: *m/z* 293; $[M+H]^+$

Diphenhydramine

CAS-Nr. 58-73-1
Molecular Formula: $C_{17}H_{21}NO$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 0.7ml/min, 30cm/sec
Mode: Constant Flow
Injection: Split, 250°C
Oven Temp. Program
120°C (0.3min) – 20°C/min to 300°C (4min)

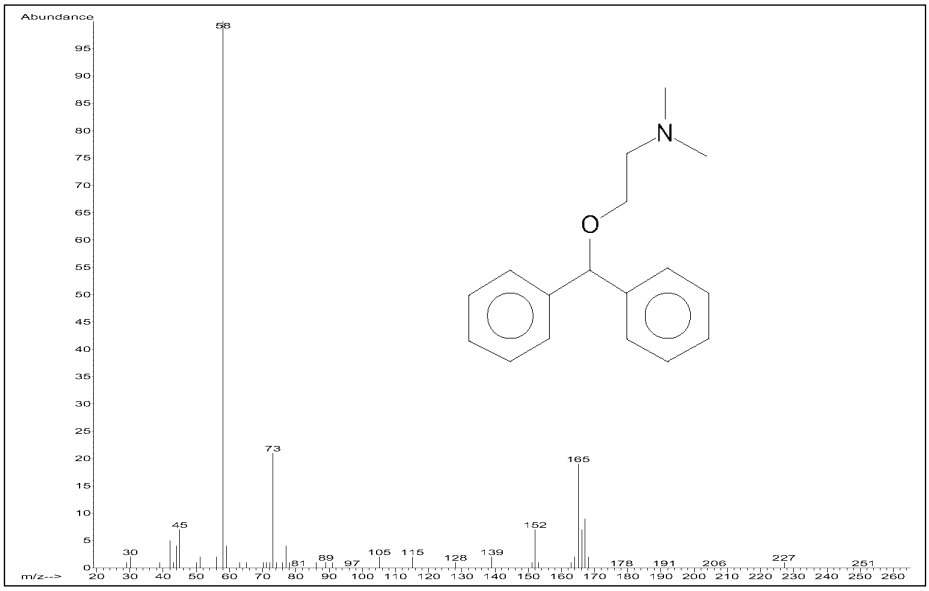
MS Parameters

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C

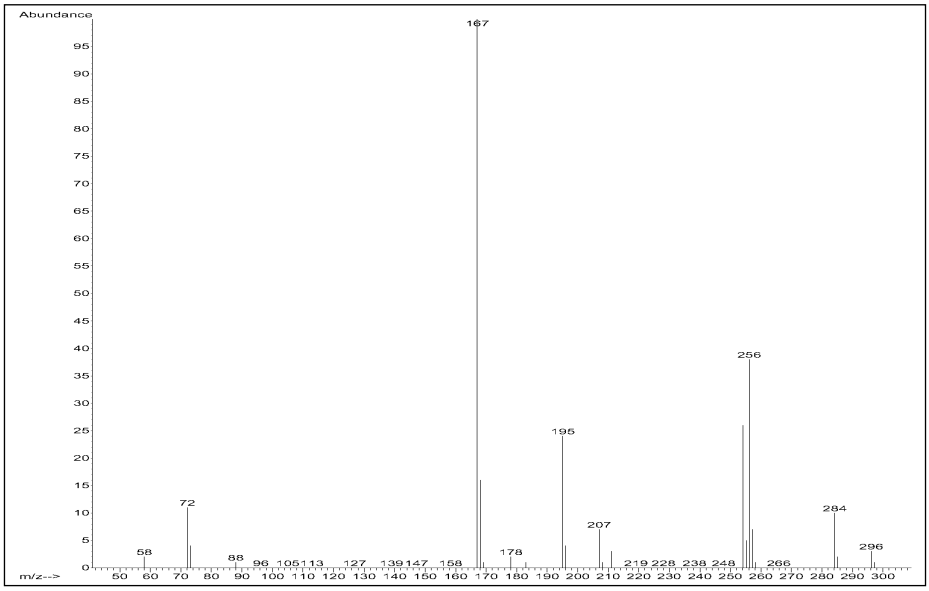
Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V

Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V

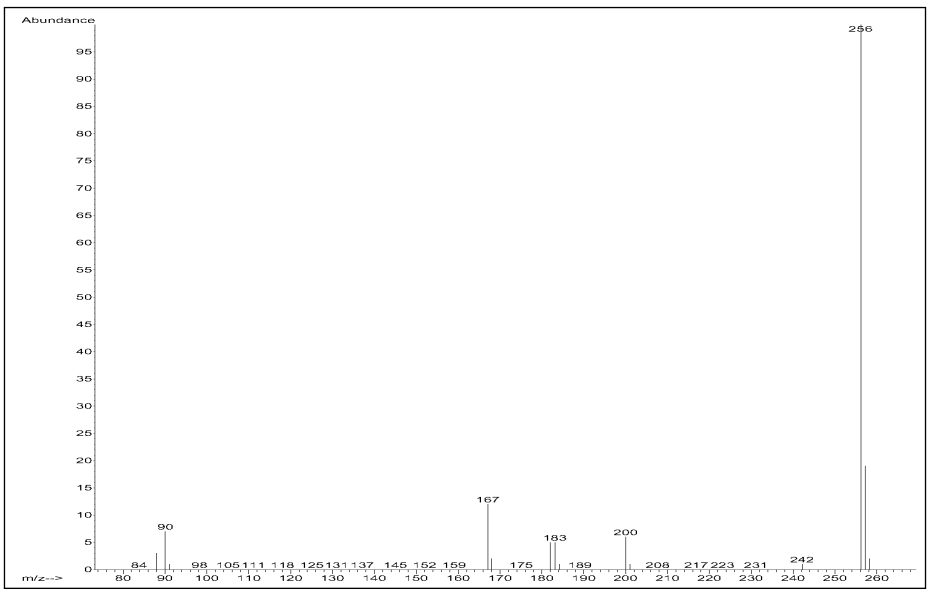
Results
Analyte Retention Time: 12.81min
Analyte Concentration: 4ng/µl
Signal/Noise Ratios for TIC
PCI/CH_4 Scan: >500/1
PCI/NH_3 Scan: >80/1



EI-Spectrum, Diphenhydramine: *m/z* 255; M^+



PCI/CH_4-Spectrum, Diphenhydramine: *m/z* 256, 284, 296; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



PCI/NH_3-Spectrum, Diphenhydramine: *m/z* 256; $[M+H]^+$

Lidocaine

CAS-Nr. 137-58-6
Molecular Formula: $C_{14}H_{22}N_2O$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 0.7ml/min, 30cm/sec
Mode: Constant Flow
Injection: Split, 250°C
Oven Temp. Program
120°C (0.3min) – 20°C/min to 300°C (4min)

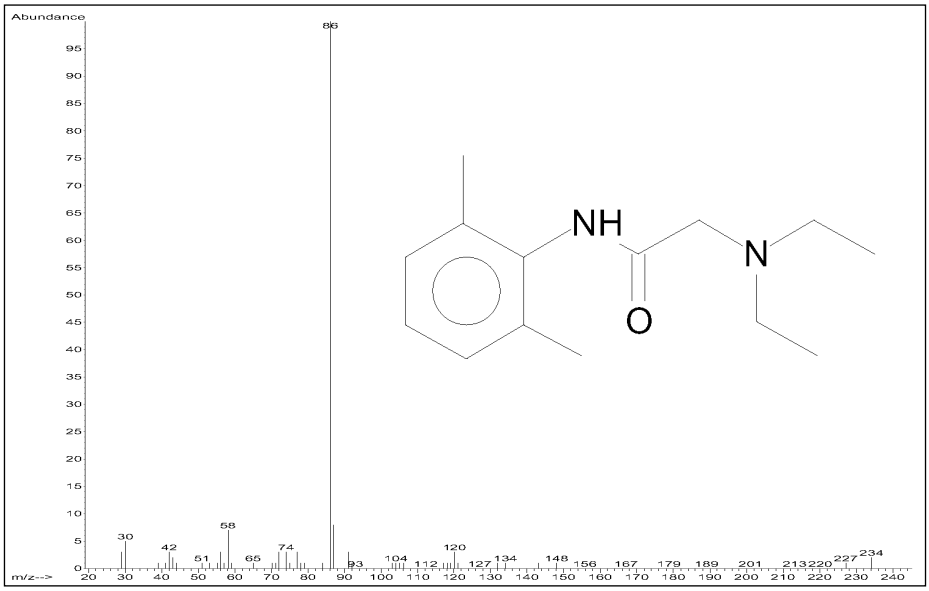
MS Parameters

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C

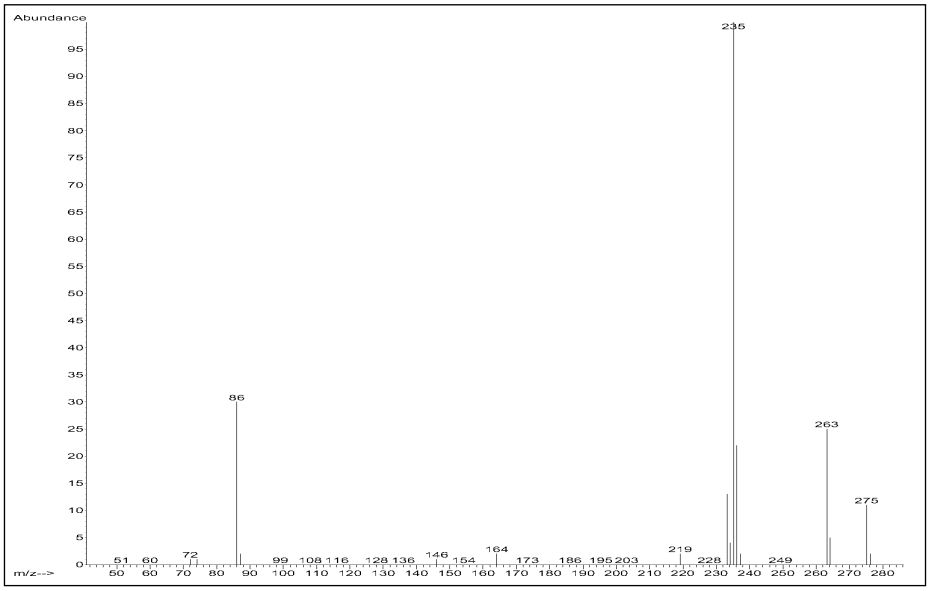
Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperature:
Source 250°C , Quad 106°C
EM Voltage: Tune + 400V

Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V

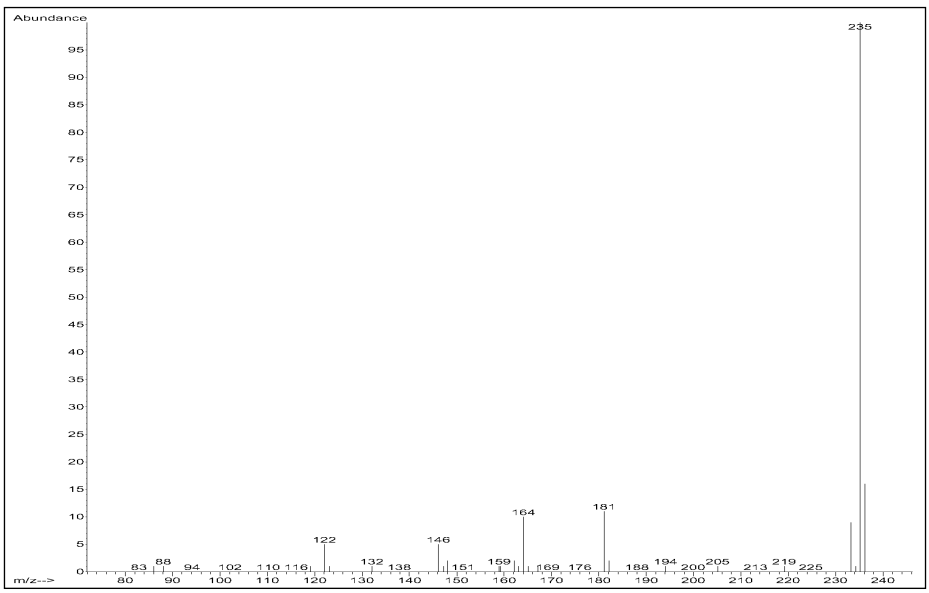
Results
Analyte Retention Time: 12.95min
Analyte Concentration: 4ng/µl
Signal/Noise Ratios for TIC
PCI/CH_4 Scan: >350/1
PCI/NH_3 Scan: >80/1



EI-Spectrum, Lidocaine: *m/z* 234; M^+



PCI/CH_4-Spectrum, Lidocaine: *m/z* 235, 263, 275; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



PCI/NH_3-Spectrum, Lidocaine: *m/z* 235; $[M+H]^+$

Mabuterol

CAS-Nr. 56341-08-3
Molecular Formula: $C_{13}H_{18}ClF_3N_2O$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 1.2ml/min, 40cm/sec
Mode: Constant Flow
Injection: Pulsed splitless, 250°C
Oven Temp. Program
70°C (1min) – 25°C/min
to 280°C (5min)

MS Parameters

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C

Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V

Mode: PCI/NH_3 – SCAN/SIM
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V

Mode: ECNI/CH_4 – SCAN/SIM
Buffer Gas: Methane
Flow (Setting): 2ml/min (40)
Tune: ECNI-Methane Tune File
Temperatures:
Source 150°C, Quad 106°C
EM Voltage: Tune + 400V

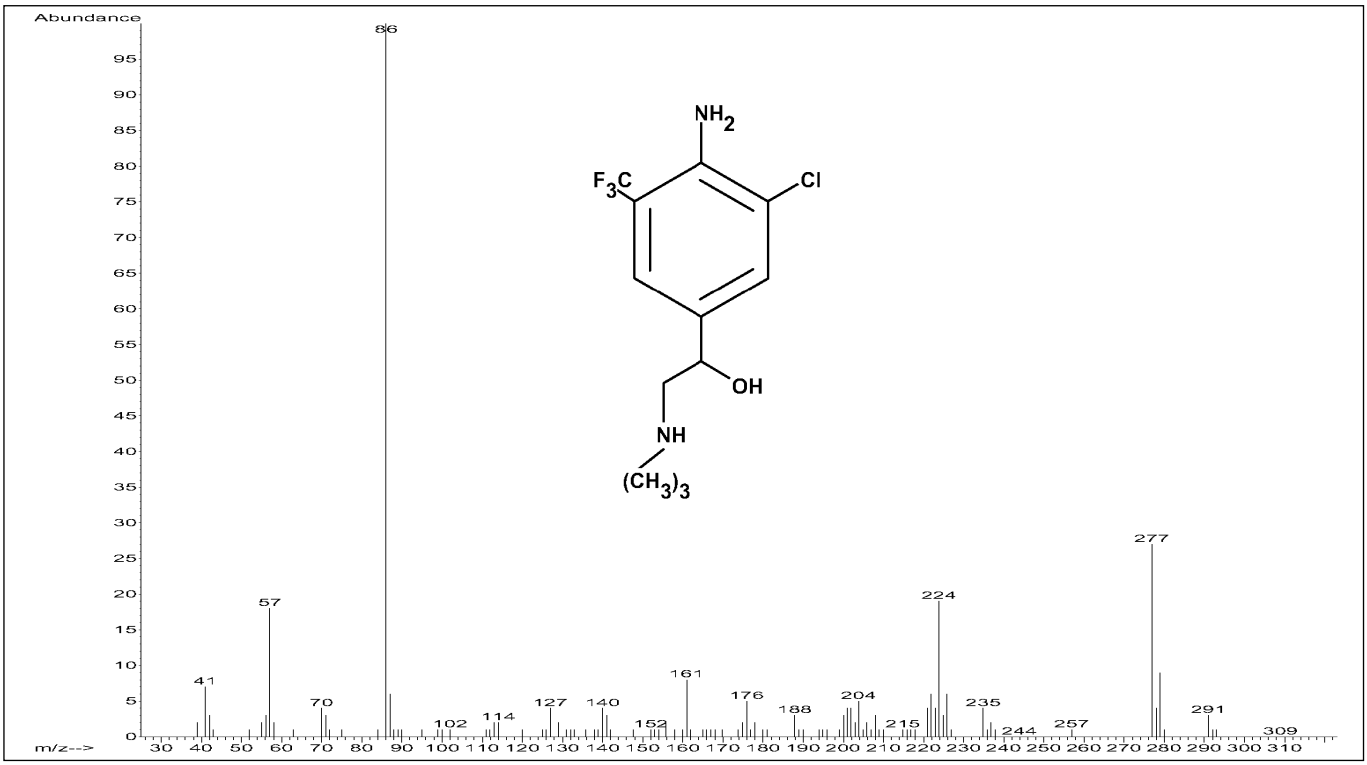
Remarks

Derivatization
a) Trimethyl silylation with BSTFA/TMCS
(Reagent: Fluka 15238)
b) Reaction with Pentafluoropropionic Acid Anhydride – PFPA –
(Reagent: Fluka 77292)

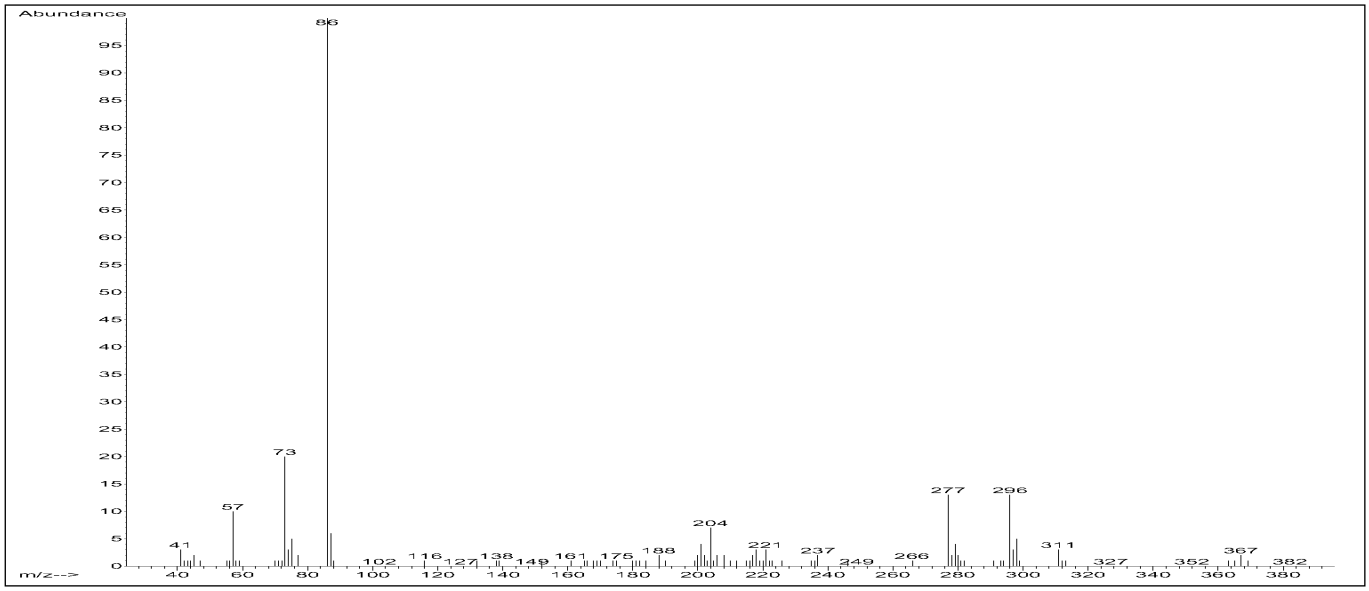
a) 100µl of the hydrochloride standard (Boehringer Ingelheim), concentration 1.6mg/ml, is dissolved in methanol and evaporated with a gentle flow of nitrogen. To the residue, 50µl of derivatization reagent and 125µl of dry pyridine are added and the reaction mixture is incubated for 30min at 60°C. Gentle evaporation with nitrogen is repeated and the residue dissolved in chloroform.

b) Procedure as described above. After the first evaporation to the residue, 80µl of the derivatization reagent and 20µl of hexafluoro isopropanol (Fluka 52517) are added and the reaction mixture is incubated for 30min at 70°C. Then evaporation, dilution and GC/MSD analysis.

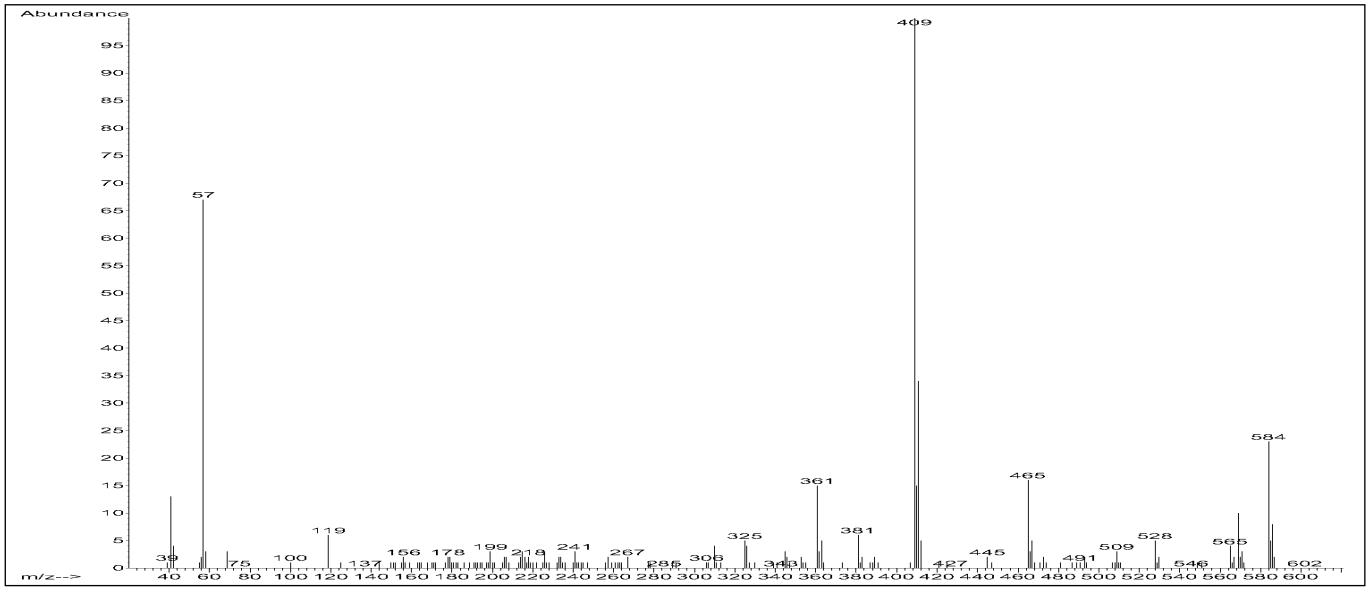
Results
The EI spectra of the underivatized and derivatized analytes show low intensity for the molecular ion. The TMS derivatization react to form a mono-derivative and the PFPA derivatization forms a di-derivative. The PFPA spectra show M-18 fragmentation. In PCI/CH_4 mode the derivatives generate the molecular ions and show the characteristic adduct ions. The measurements of the TMS derivatives in PCI/NH_3 Scan mode are by factor 6 more sensitive than the PCI/CH4 measurements. The PFPA derivative shows less response than the TMS derivative in PCI mode.
An improvement is noticed in ECNI mode for the PFPA derivative.
In SIM mode, the signal/noise ratio is approximately 40:1 for an analyte concentration of 200fg/µl.



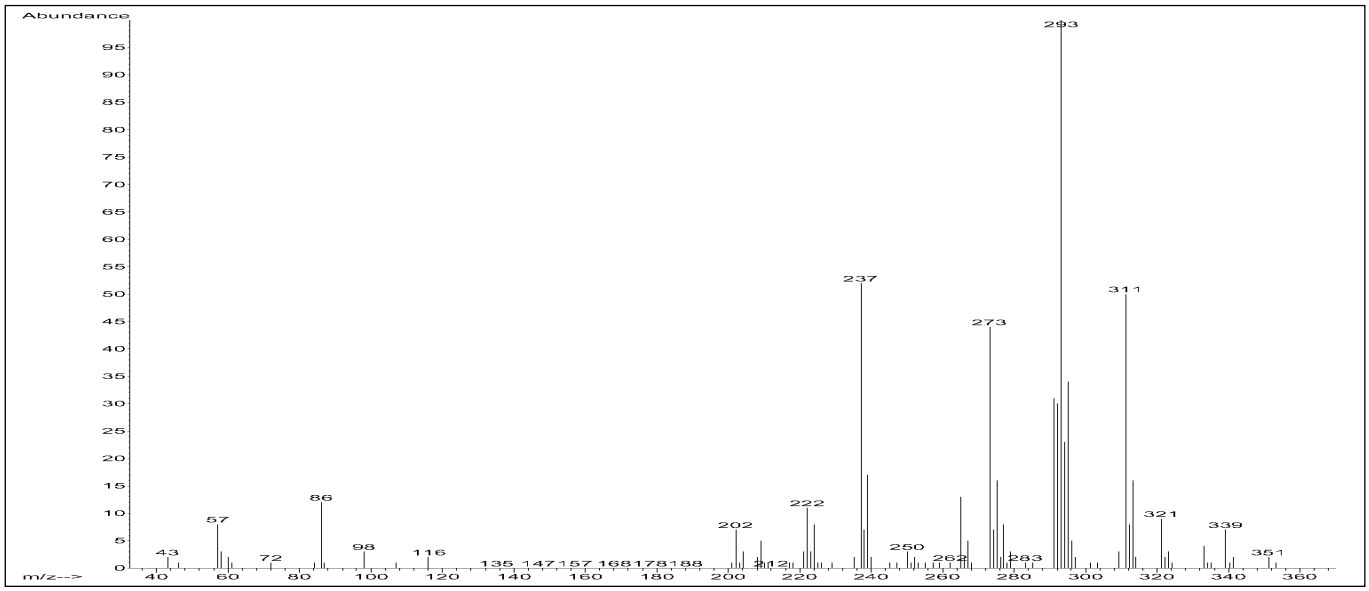
EI-Spectrum, Mabuterol, underivatised, *m/z* 310; M^+



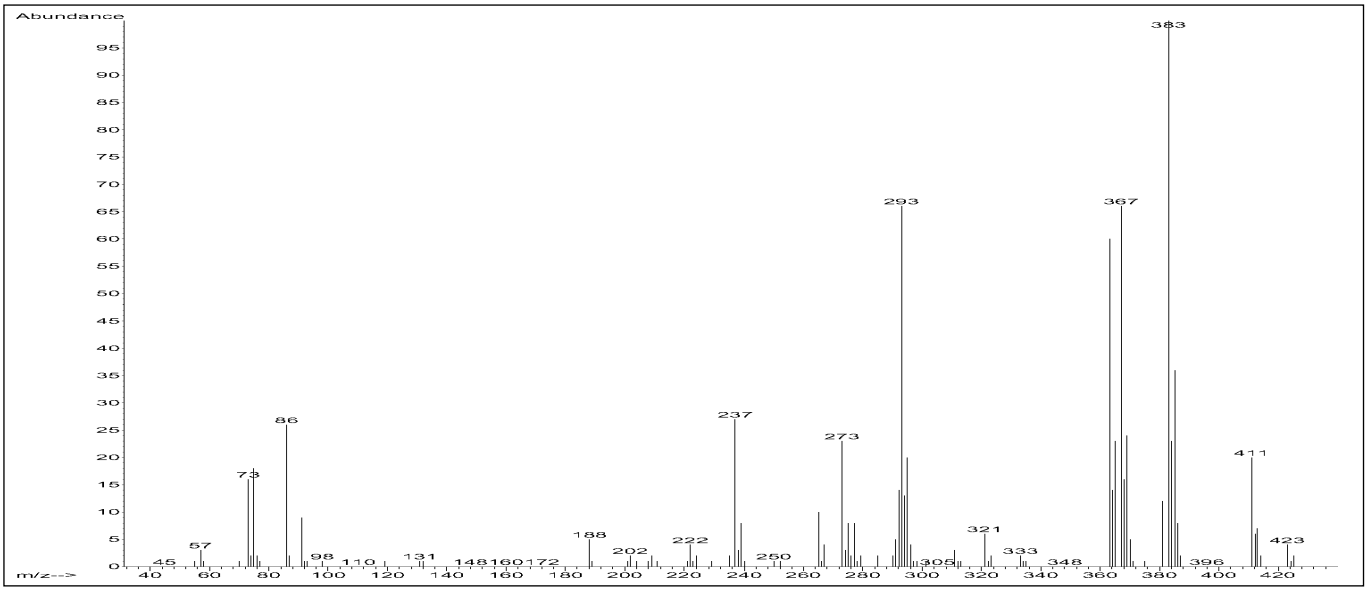
EI-Spectrum, Mabuterol, TMS mono-derivative, *m/z* 382; M^+



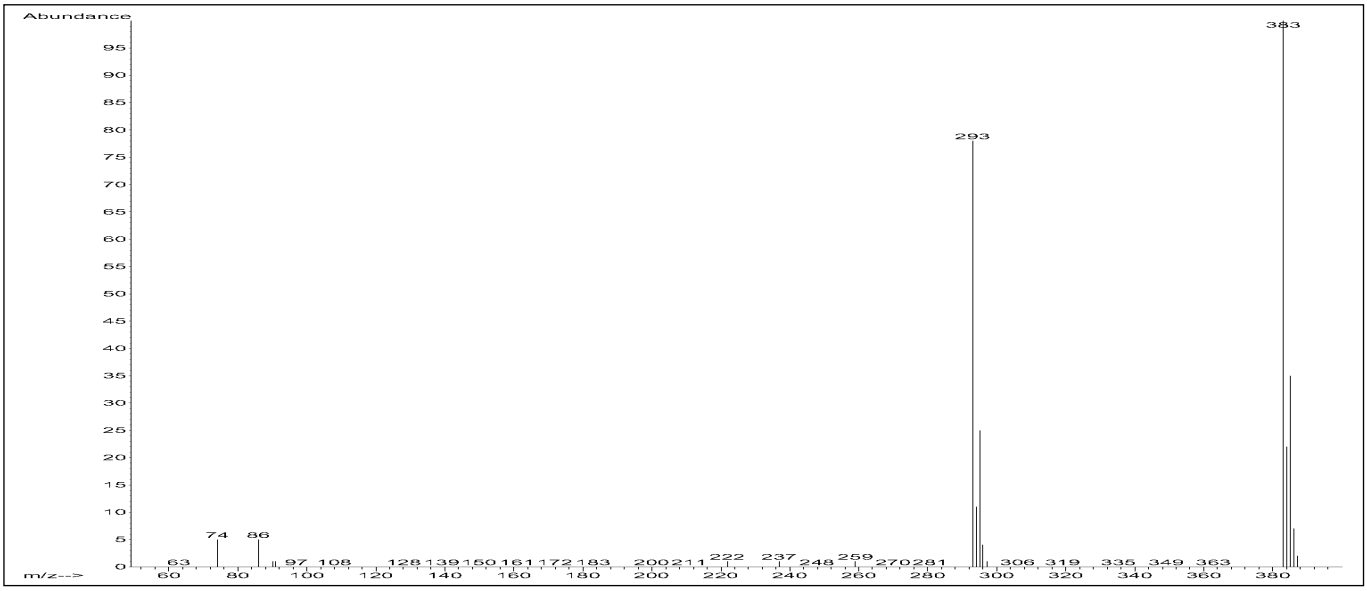
EI-Spectrum, Mabuterol, PFPA di-derivative, *m/z* 602; M^+



PCI/CH_4-Spectrum, Mabuterol, underivatised, *m/z* 311, 339, 351; $[M + H]^+$, $[M + C_2H_5]^+$, $[M + C_3H_5]^+$

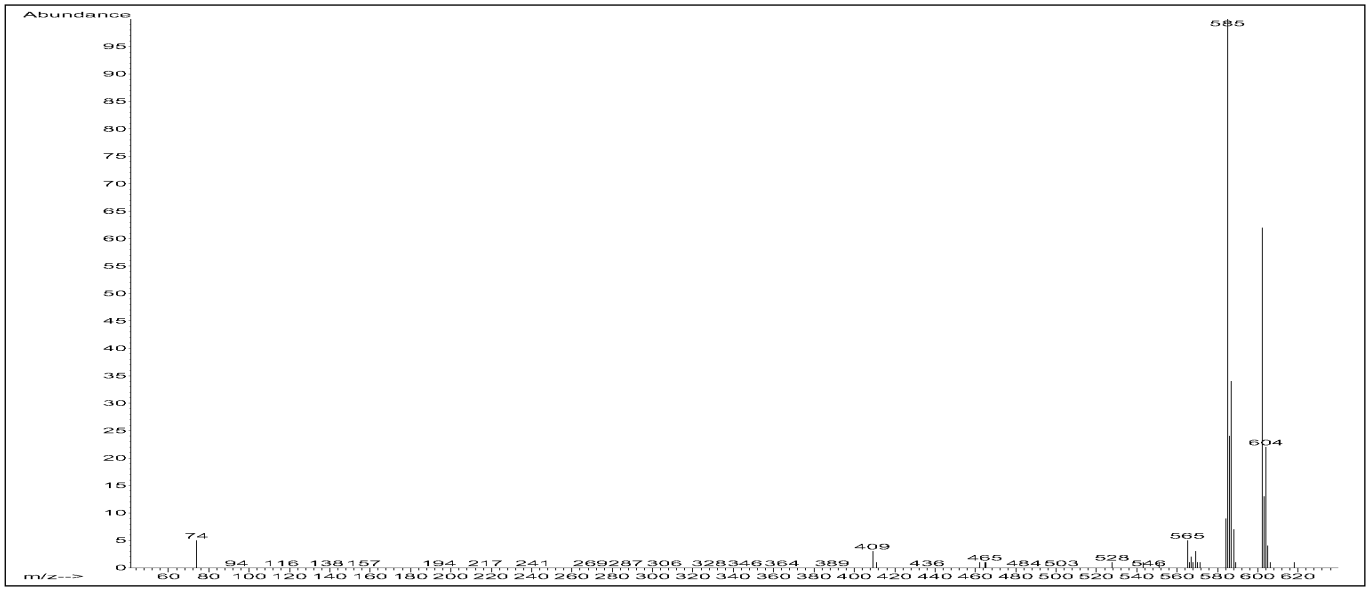


PCI/CH_4-Spectrum, Mabuterol, TMS mono-derivative, *m/z* 383, 411, 423; $[M + H]^+$, $[M + C_2H_5]^+$, $[M + C_3H_5]^+$

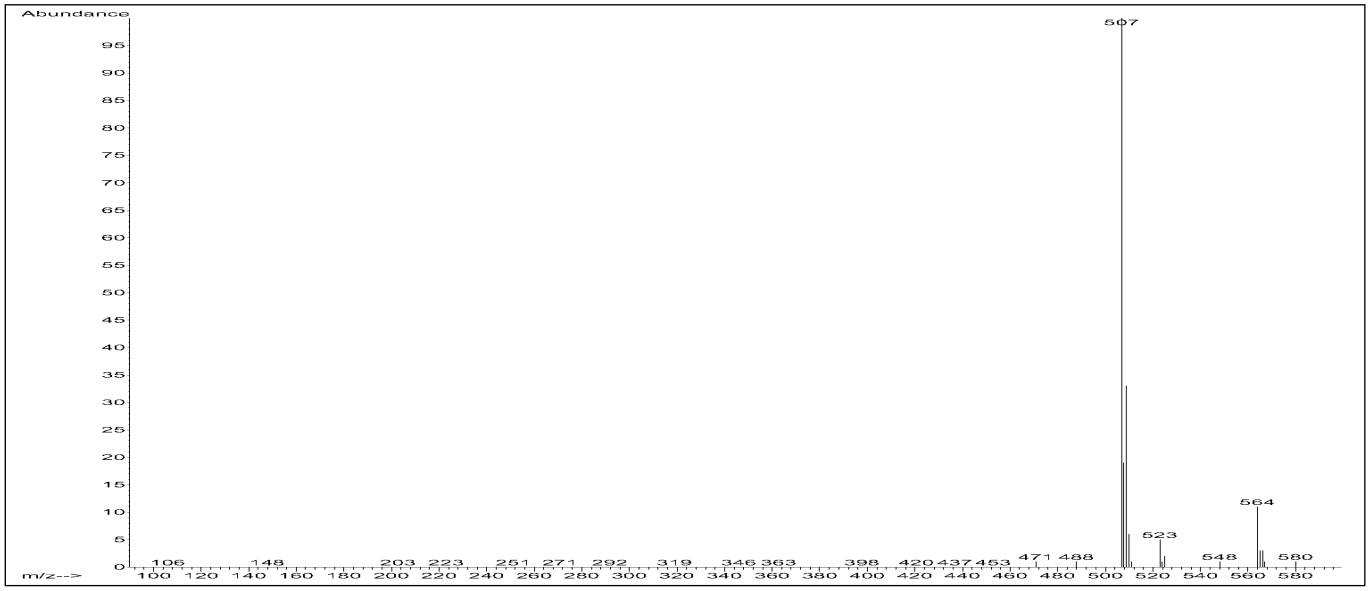


PCI/NH_3-Spectrum, Mabuterol, TMS mono-derivative, *m/z* 383; $[M + H]^+$

PCI/CH_4-Spectrum, Mabuterol, PFPA di-derivative, *m/z* 585, 613, 625; $[M-18 + H]^+$, $[M-18 + C_2H_5]^+$, $[M-18 + C_3H_5]^+$

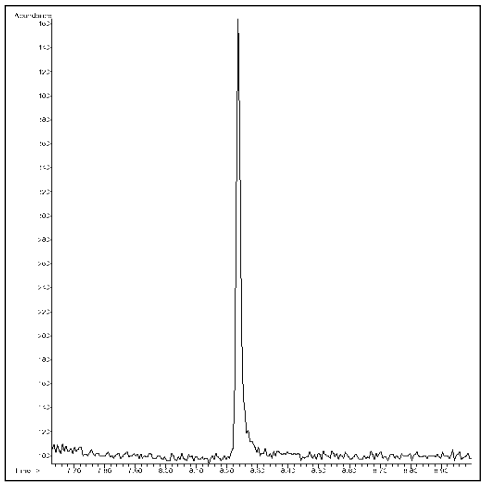


PCI/NH_3-Spectrum, Mabuterol, PFPA di-derivative, *m/z* 602; M^+



ECNI/CH_4-Spectrum, Mabuterol, PFPA di-derivative, molecular mass = 602 u

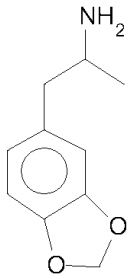
ECNI/CH_4 – SIM Mode



Mabuterol, PFPA derivative, 200fg,
Retention Time: 8.24min
Ions: m/z 507/509, Signal/Noise: 40/1

MDA

3,4 Methylendioxyamphetamine
CAS-Nr. 4764-17-4
Molecular Formula: $C_{10}H_{13}NO_2$



GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 1.2ml/min, 40cm/sec
Mode: Constant Flow
Injection: Pulsed splitless, 250°C
Oven Temp. Program
70°C (1min) – 25°C/min to 300°C (5min)

MS Parameters

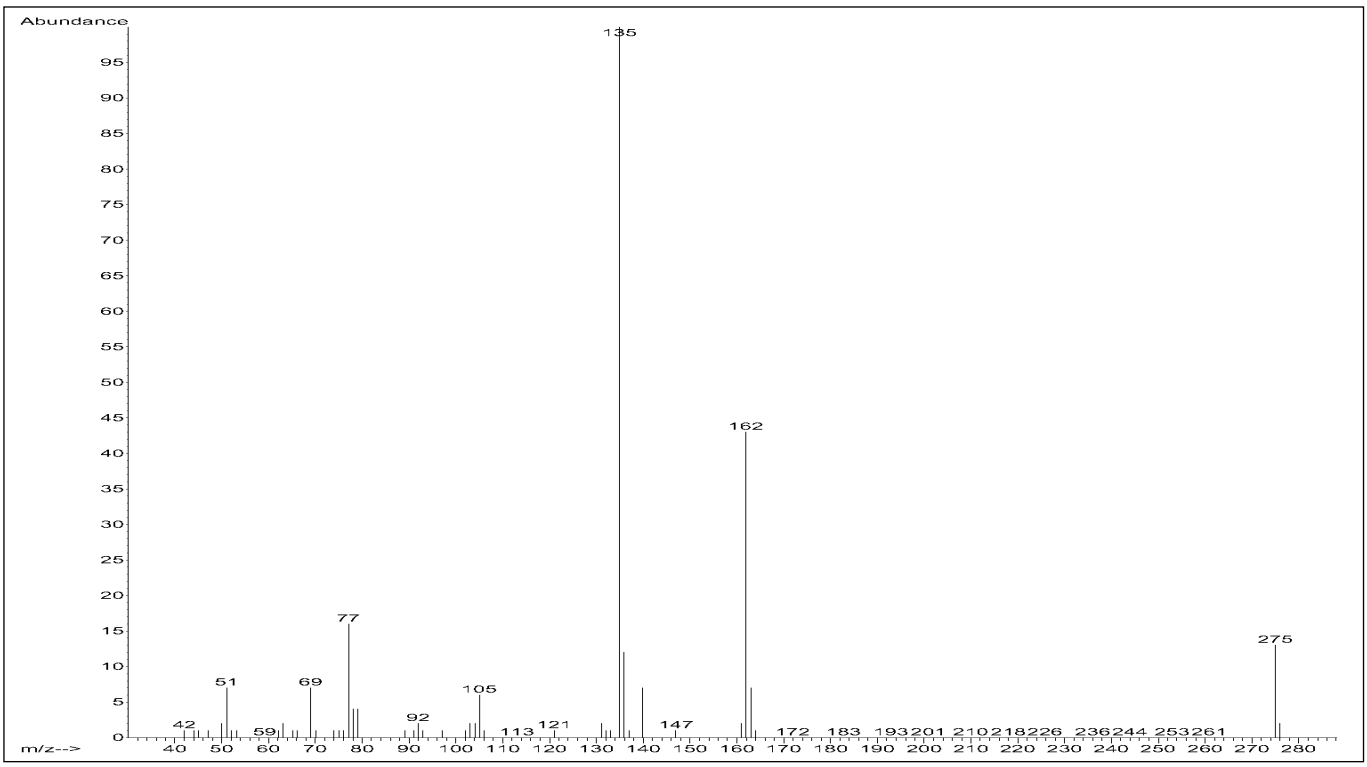
Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C
Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V
Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V
Mode:
ECNI/CH_4/NH_3 – SCAN/SIM
Buffer Gas: Methane
Flow (Setting): 2ml/min (40)
Buffer Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: ECNI-Methane Tune File
Temperatures:
Source 150°C, Quad 106°C
EM Voltage: Tune + 400V

Remarks

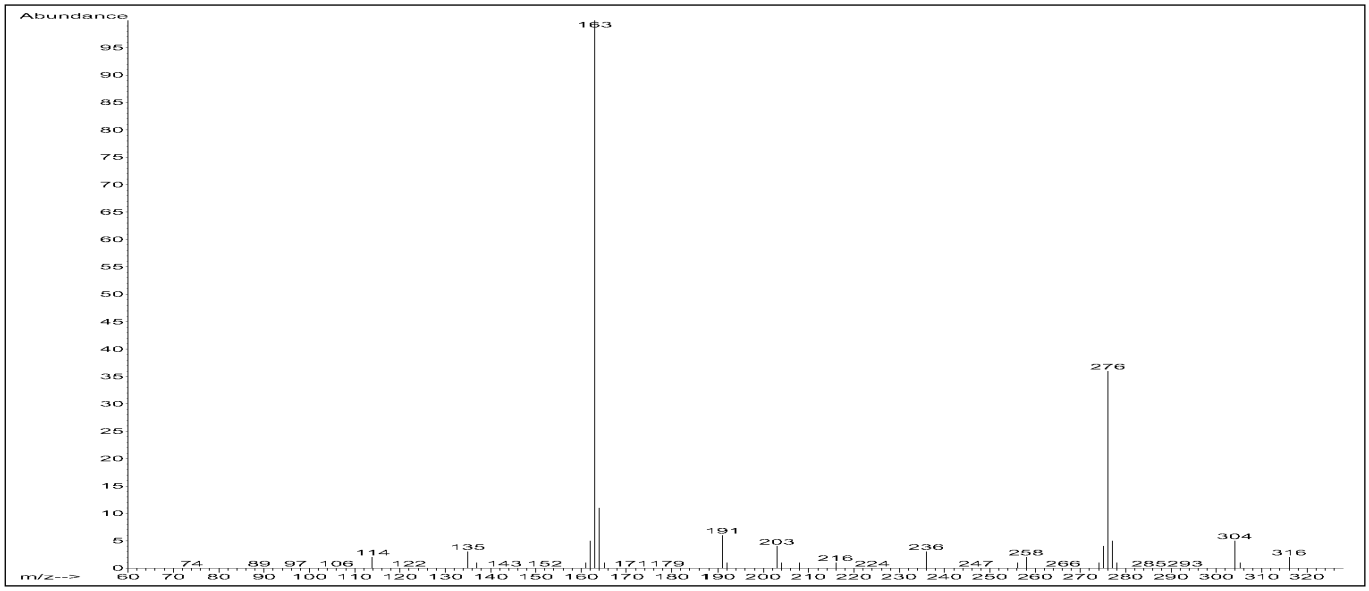
Derivatization
a) Trifluoroacetylation (TFA) with MBTFA (Reagent: Fluka 65943)
b) Reaction with Pentafluoropropionic Acid Anhydride – PFPA – (Reagent: Fluka 77292)

a) 100µl of the standard (SIGMA M 3272), 100ng/µl MDA concentration, is dissolved in ethyl acetate and evaporated with a gentle flow of nitrogen. To the residue 50µl derivatization reagent is added and the solution is incubated for 30min at 80°C. Gentle evaporation with nitrogen is repeated and the residue dissolved in ethyl acetate.
b) Procedure as described above. After the first evaporation to the residue, 80µl of the derivatization reagent and 20µl of hexafluoroisopropanol (Fluka 52517) are added and the reaction mixture is incubated for 30min at 70°C. Then evaporation, dilution and GC/MSD analysis.

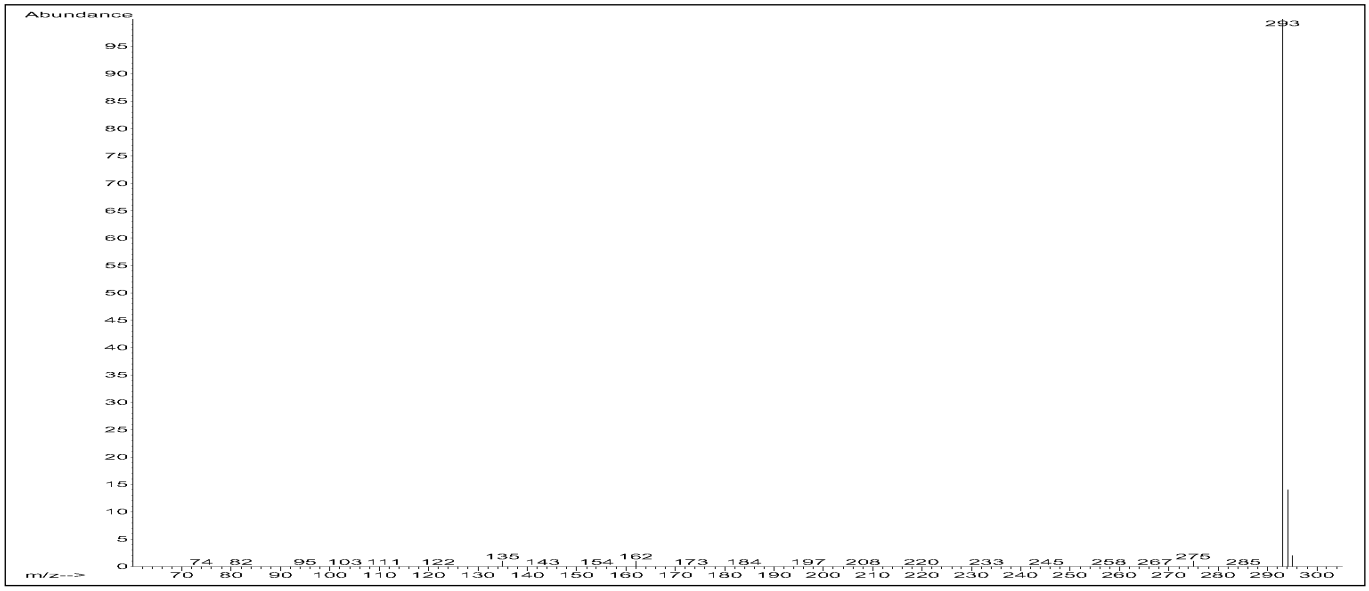
Results
Derivatization is recommended for this analyte. In PCI/NH_3 mode the analyte spectrum shows the NH_4 adduct ion as base peak. Signal is a factor 1.5 more sensitive compared to PCI with CH_4. In ECNI mode the TFA derivative response is relatively low. PFPA derivatization is the reaction of choice for both PCI and ECNI modes. Fragmentation and sensitivity are related to the choice of derivatizing reagent and buffer gas. In ECNI/NH_3 SIM mode the signal:noise ratio is approximately 65:1 for the 5pg PFPA derivative.



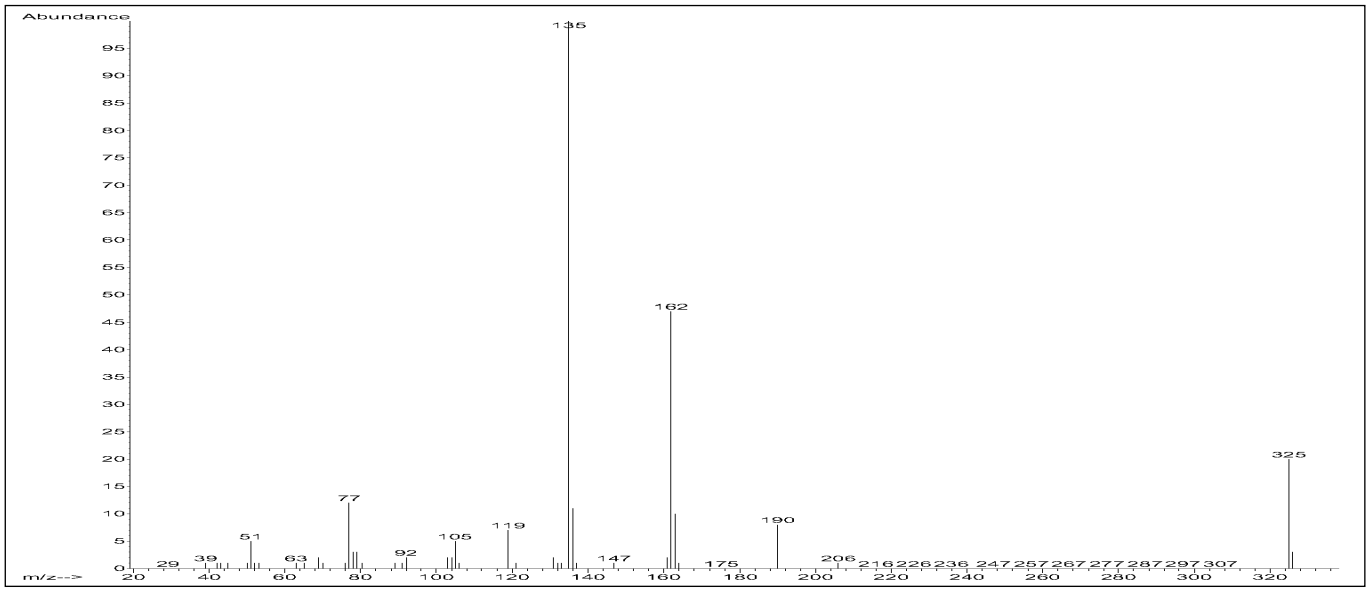
EI-Spectrum, MDA, TFA derivative, *m/z* 275; M^+



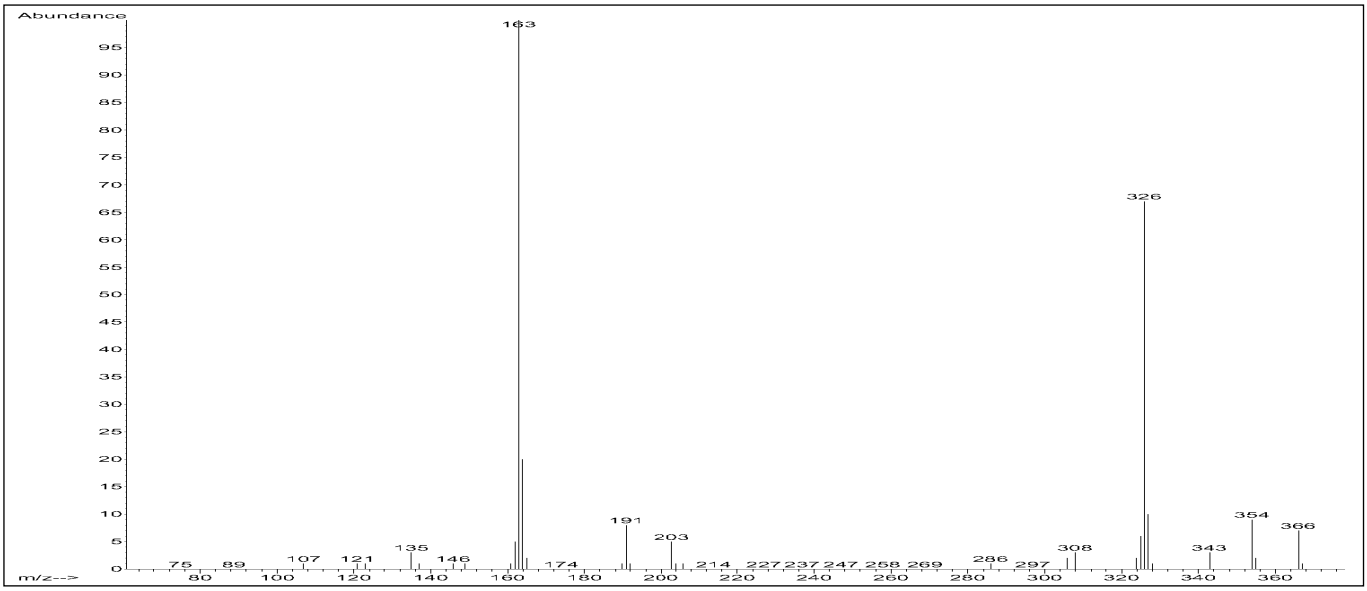
PCI/CH_4-Spectrum, MDA, TFA derivative, *m/z* 276, 304, 316; $[M+H]^+$, $[M + C_2H_5]^+$, $[M + C_3H_5]^+$



PCI/NH_3-Spectrum, MDA, TFA derivative, *m/z* 293; $[M + NH_4]^+$

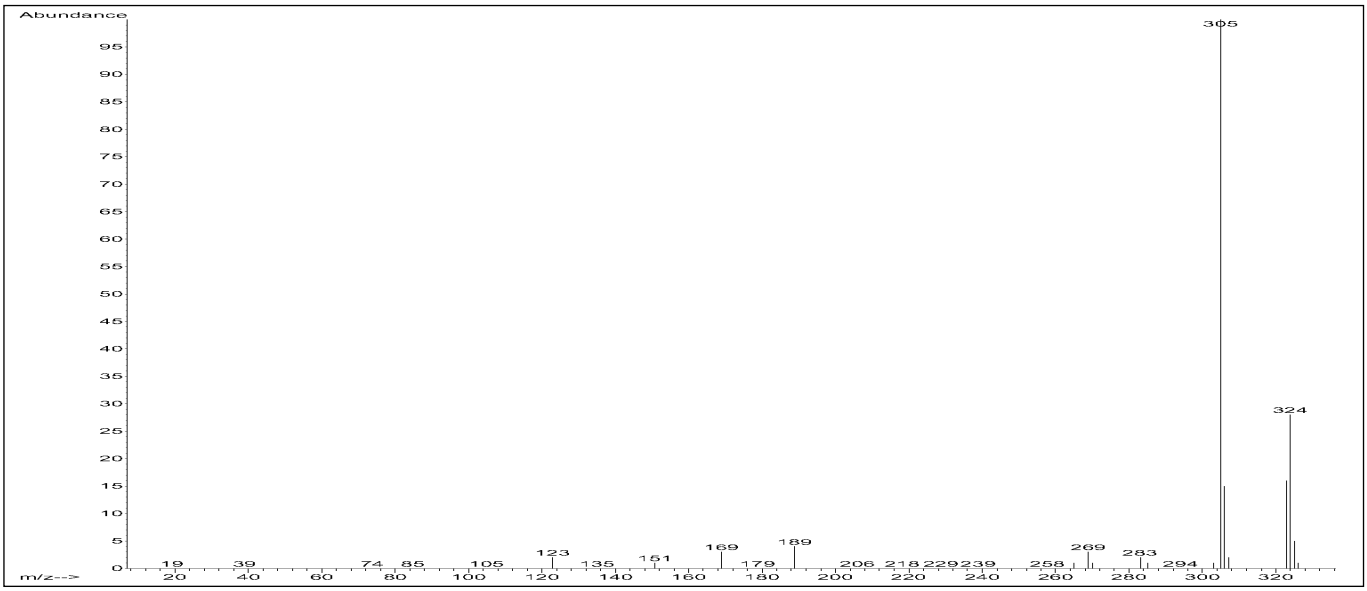


EI-Spectrum, MDA, PFPA derivative, *m/z* 325; M^+

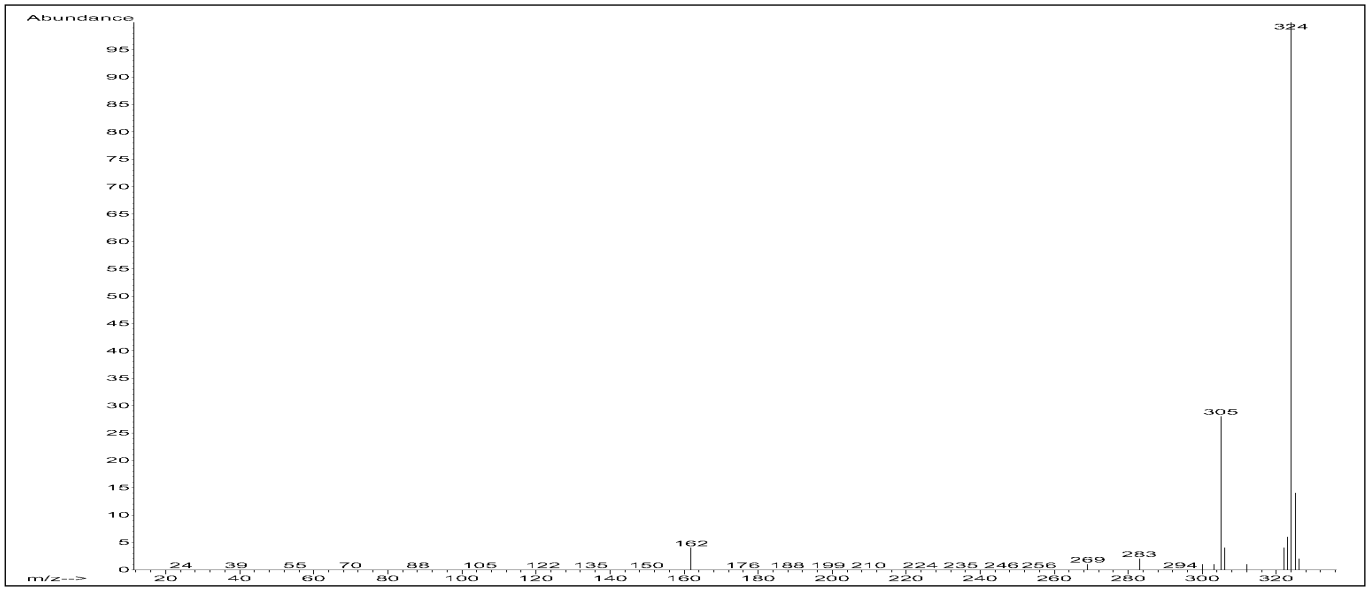


PCI/CH_4-Spectrum, MDA, PFPA derivative, *m/z* 326, 354, 366; $[M+H]^+$, $[M + C_2H_5]^+$, $[M + C_3H_5]^+$

PCI/NH_3-Spectrum, MDA, PFPA derivative, *m/z* 343; $[M + NH_4]^+$

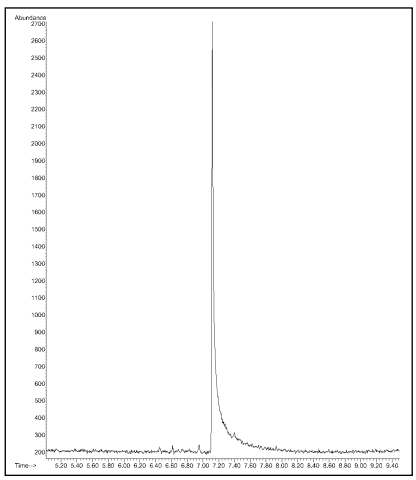


ECNI/CH_4-Spectrum, MDA, PFPA derivative, *m/z* 305, 324; $[M-HF]^-$, $[M-H]^-$



ECNI/NH_3-Spectrum, MDA, PFPA derivative, *m/z* 305, 324; $[M\text{-}HF]^-$, $[M\text{-}H]^-$

ECNI/NH_3 – SIM Mode



MDA, PFPA derivative, 5pg,
Retention Time: 7.12min
Ions: m/z 305, 324; Signal: Noise ≈ 65/1

Mepivacaine

(Carbocaine)
CAS-Nr. 96-88-8
Molecular Formula: $C_{15}H_{22}N_2O$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 0.7ml/min, 30cm/sec
Mode: Constant Flow
Injection: Split, 250°C
Oven Temp. Program
120°C (0.3min) – 20°C/min to 300°C (4min)

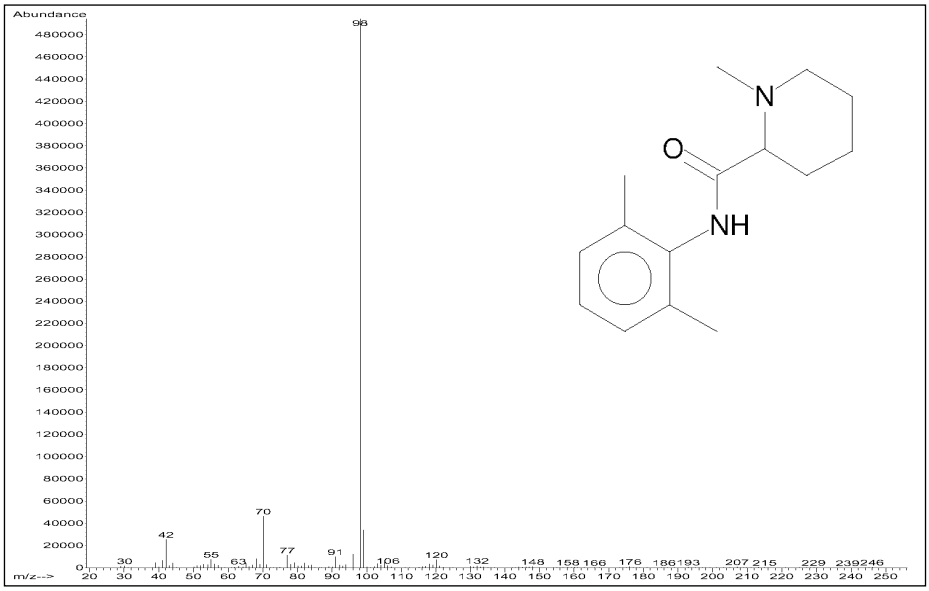
MS Parameters

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C

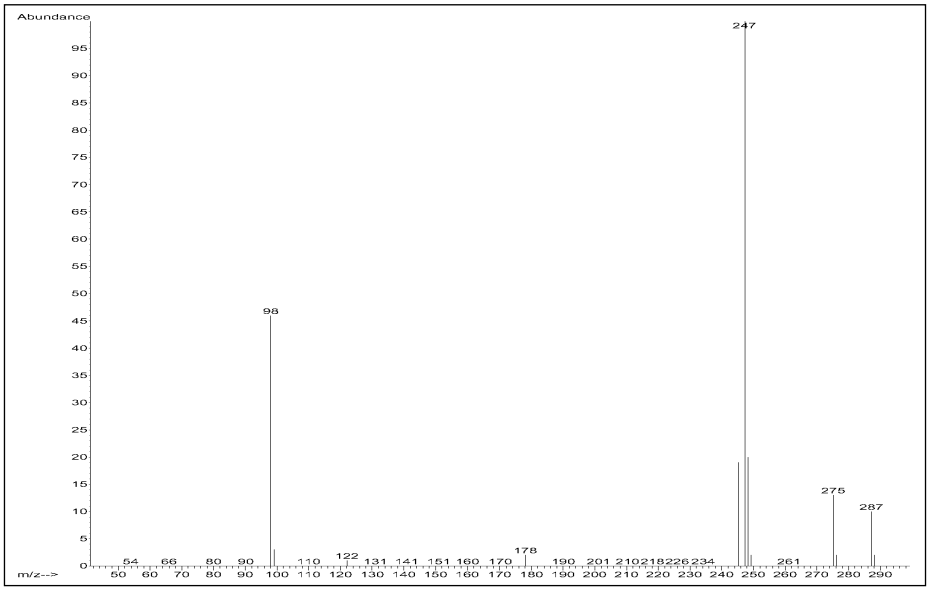
Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperature:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V

Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V

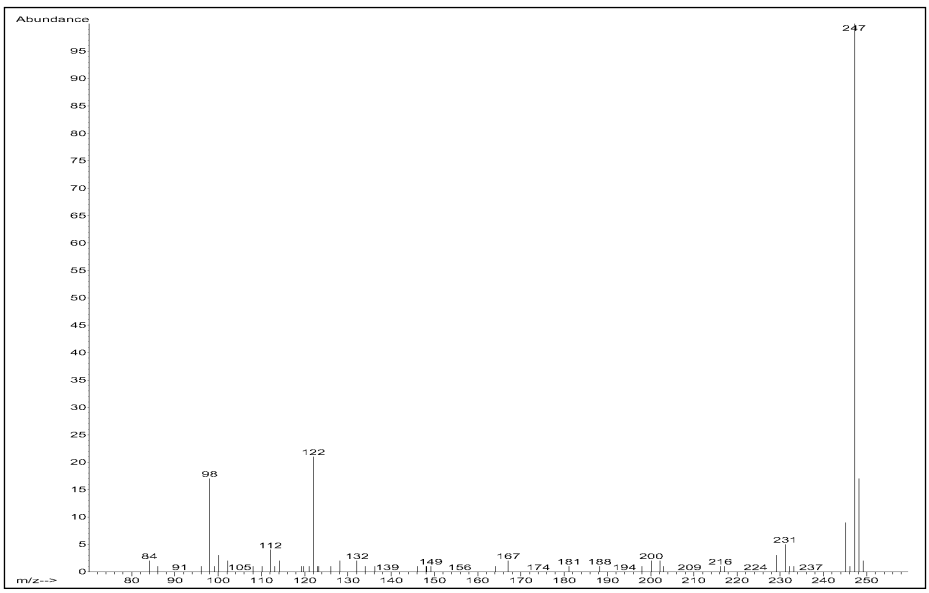
Results
Analyte Retention Time: 14.89min
Analyte Concentration: 4ng/µl
Signal/Noise Ratios for TIC
PCI/CH_4 Scan: > 680/1
PCI/NH_3 Scan: > 70/1



EI-Spectrum, Mepivacaine: *m/z* 246; M^+



PCI/CH_4-Spectrum, Mepivacaine: *m/z* 247, 275, 287; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



PCI/NH_3-Spectrum, Mepivacaine: *m/z* 247; $[M+H]^+$

Methadone

CAS-Nr. 76-99-3
Molecular Formula: $C_{21}H_{27}NO$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 0.7ml/min, 30cm/sec
Mode: Constant Flow
Injection: Split, 250°C
Oven Temp. Program
120°C (0.3min) – 20°C/min to 300°C (4min)

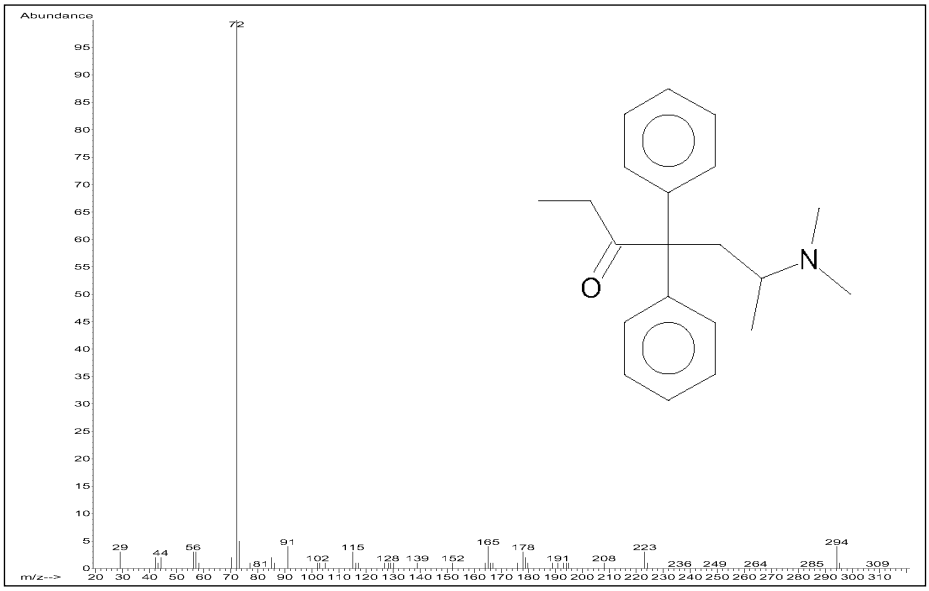
MS Parameters

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C

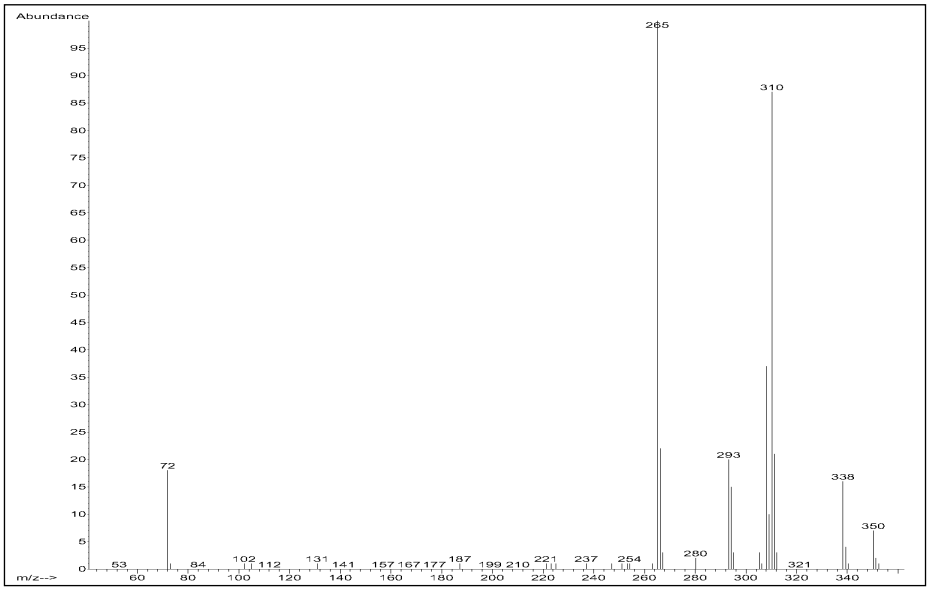
Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V

Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V

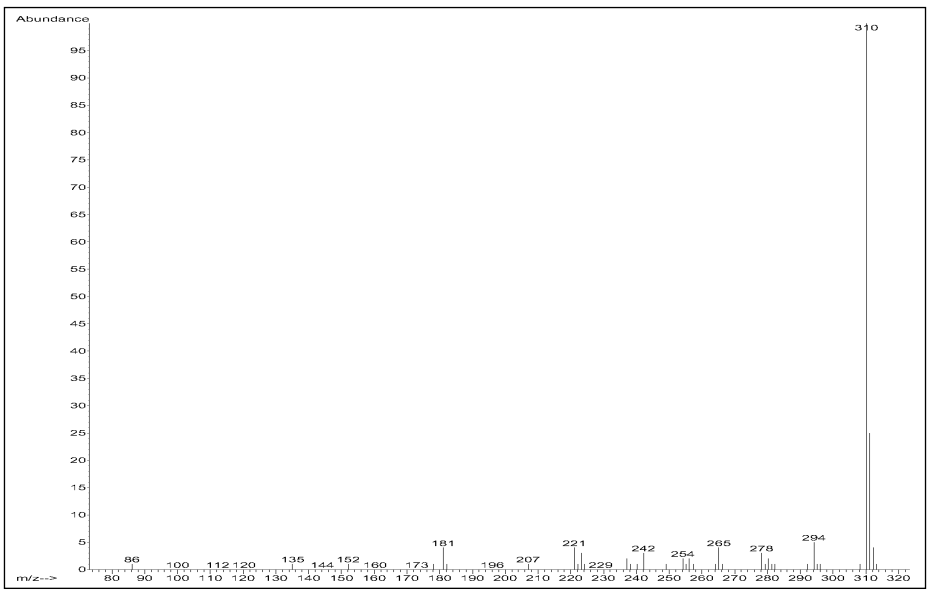
Results
Analyte Retention Time: 15.54min
Analyte Concentration: 4ng/µl
Signal/Noise Ratios for TIC
PCI/CH_4 Scan: > 300/1
PCI/NH_3 Scan: > 30/1



EI-Spectrum, Methadone: *m/z* 309; M^+



PCI/CH_4-Spectrum, Methadone: *m/z* 310, 338, 350; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



PCI/NH_3-Spectrum, Methadone: *m/z* 310; $[M+H]^+$

Morphine

CAS-Nr. 57-27-2
Molecular Formula: $C_{17}H_{19}NO_3$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 1.2ml/min, 40cm/sec
Mode: Constant Flow
Injection: Pulsed splitless, 250°C
Oven Temp. Program
70°C (1min) – 25°C/min to 300°C (5min)

MS Parameters

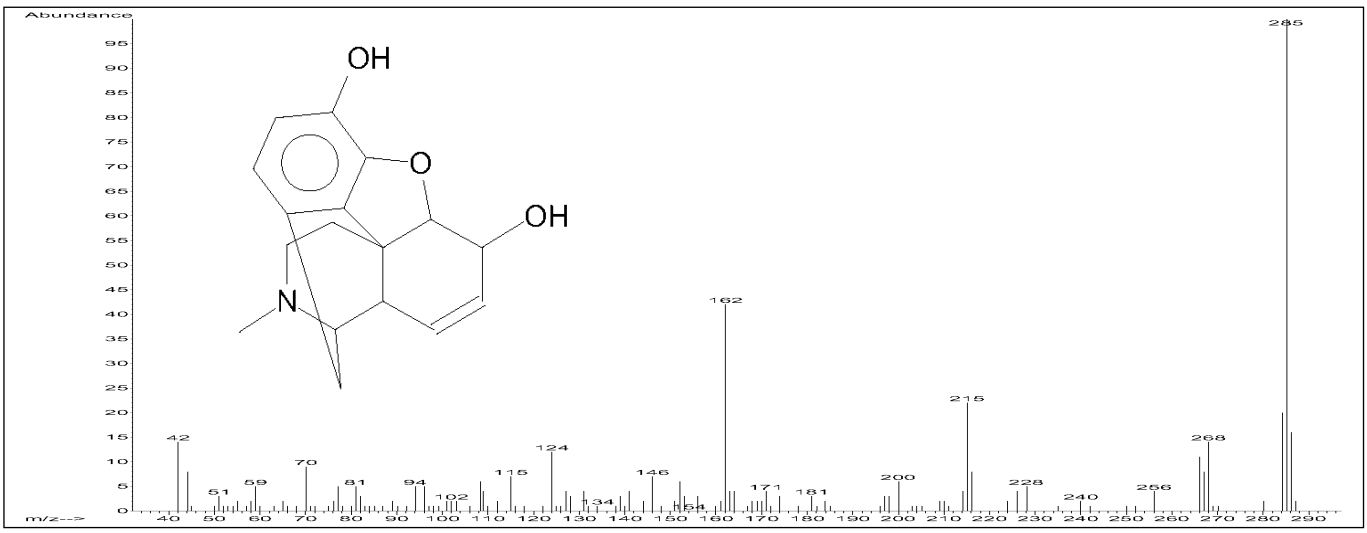
Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C
Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V
Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V
Mode: ECNI/CH_4 – SCAN/SIM
Buffer Gas: Methane
Flow (Setting): 2ml/min (40)
Tune: ECNI-Methane Tune File
Temperatures:
Source 150°C, Quad 106°C
EM Voltage: Tune + 400V

Remarks

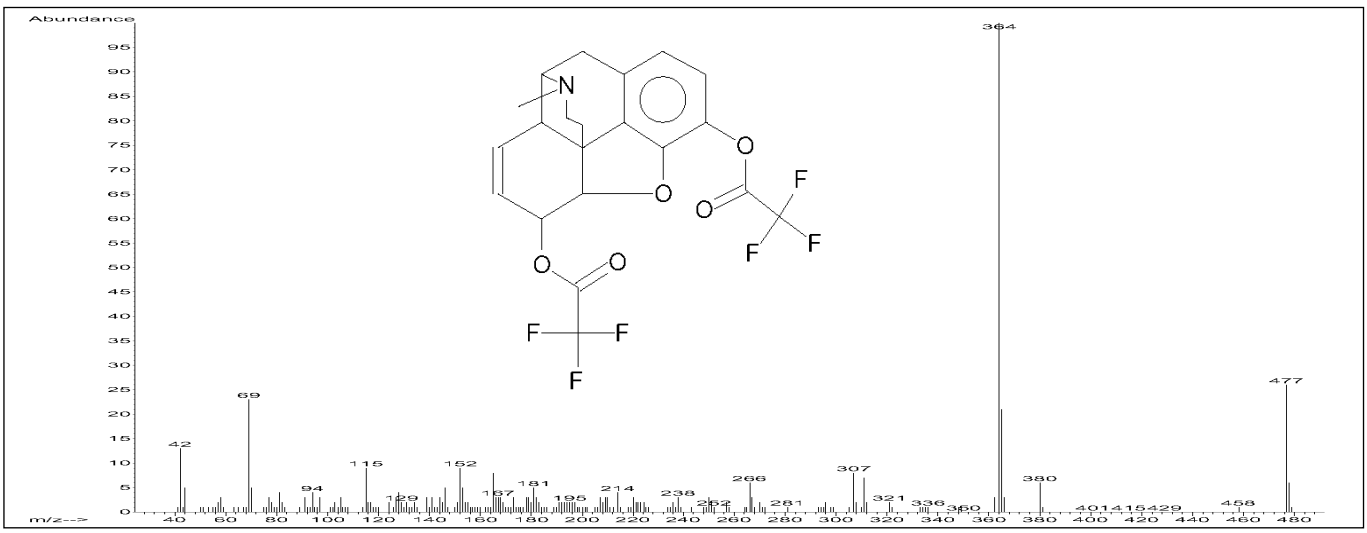
Derivatization
Reaction with Pentafluoro-propionic Acid Anhydride (PFPA) (Reagent: Fluka 77292)

100µl of the standard (SIGMA M 9524), concentration 100ng/µl, dissolved in ethyl acetate is evaporated with a gentle flow of nitrogen. To the residue 80µl derivatization reagent and 20µl hexafluoroisopropanol are added and the mixture is incubated for 30min at 70°C. Gentle evaporation with nitrogen is repeated and the residue redissolved in ethyl acetate. The solution is ready GC/MSD analysis.

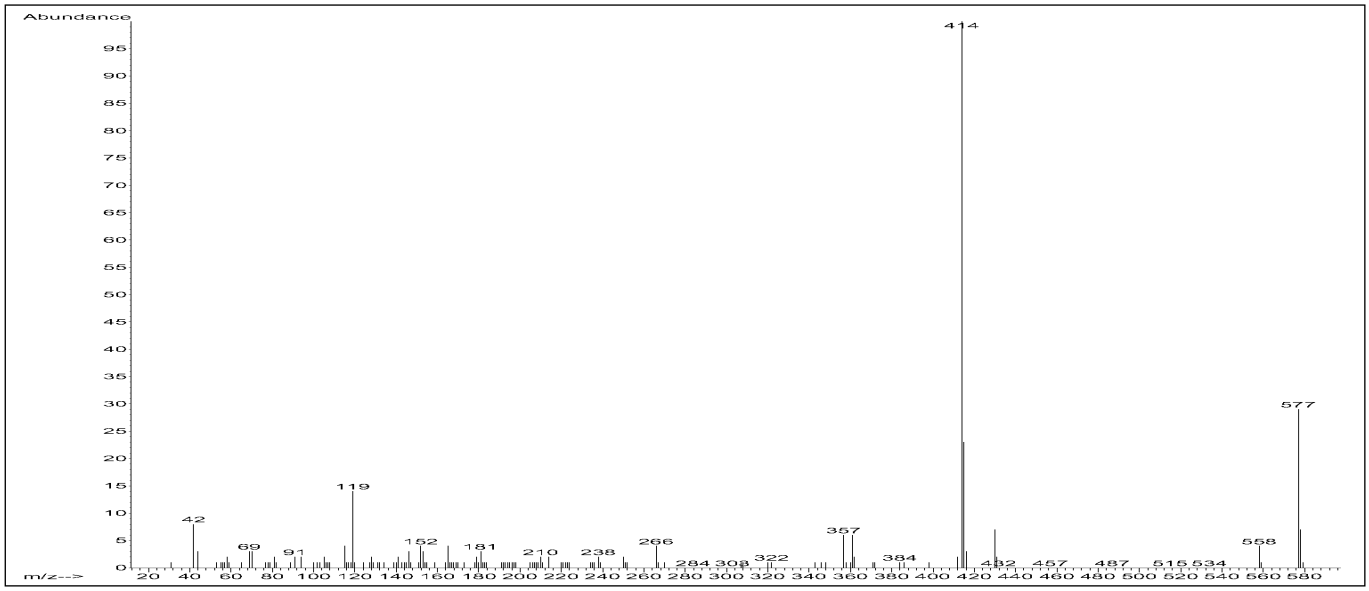
Results
Derivatization is recommended. The trifluoroacetylation (TFA) with MBTFA leads in EI mode to a moderately intense molecular ion. In ECNI mode the TFA analyte spectrum shows no distinctive results for the derivative.
The PFPA derivatization is suitable for both PCI/NH_3 and for ECNI/CH_4 measurements. The signal-to-noise ratio for 1pg analyte PFPA derivative in ECNI/CH4 mode is approximately 30:1.



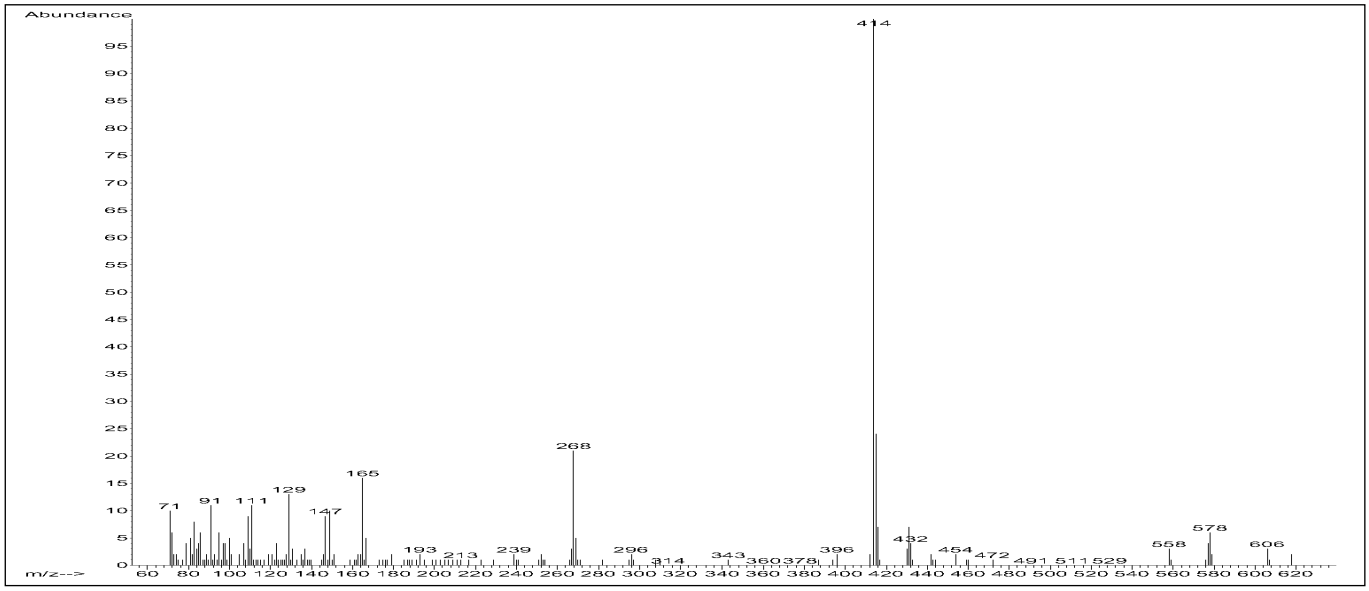
EI-Spectrum, Morphine, underivatized, *m/z* 285; M^+



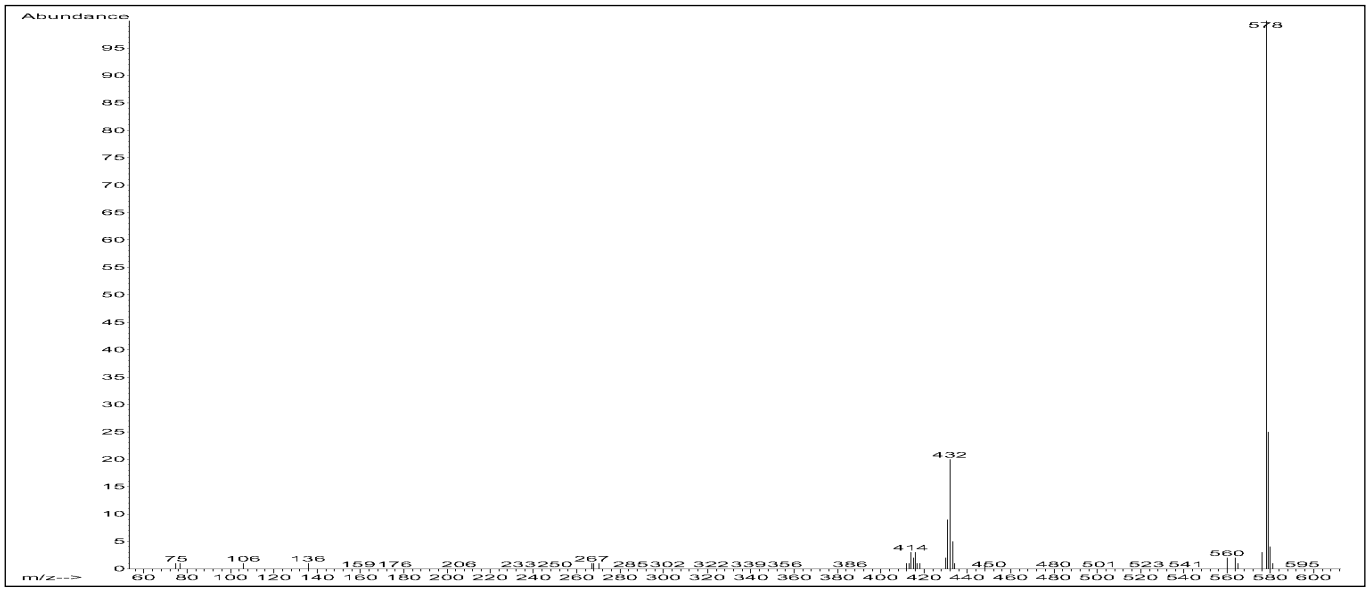
EI-Spectrum, Morphine, Trifluoroacetyl derivative, *m/z* 477; M^+



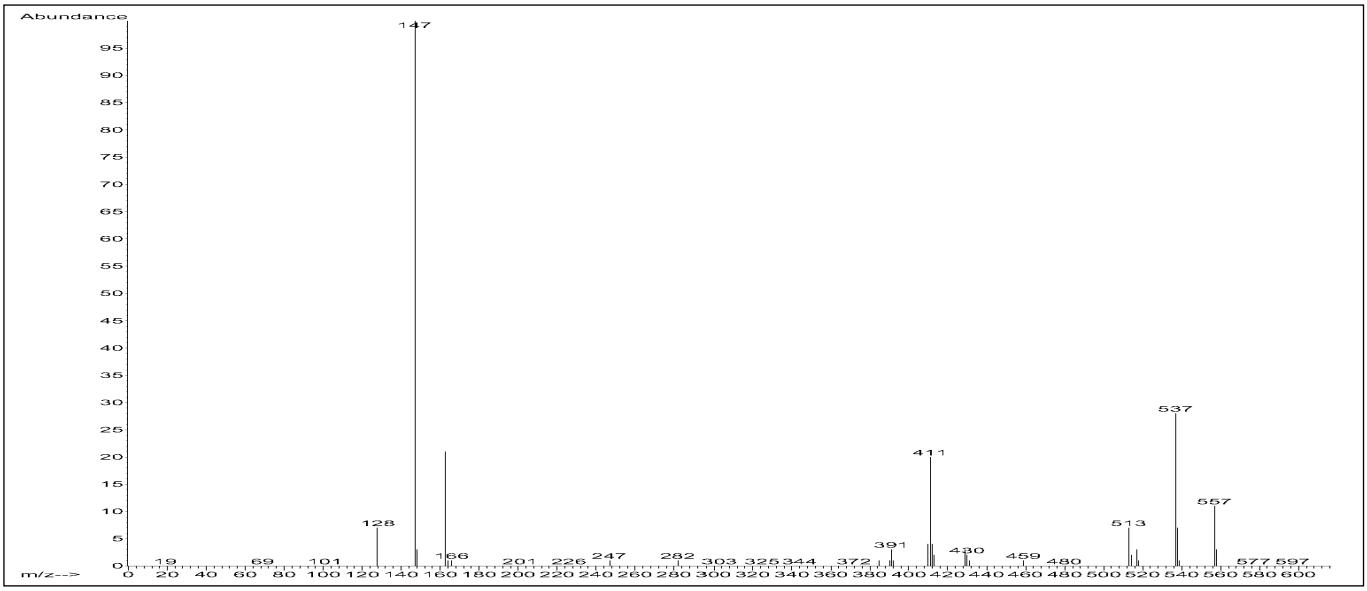
EI-Spectrum, Morphine, PFPA derivative, *m/z* 577; M^+



PCI/CH_4-Spectrum, Morphine, PFPA derivative, *m/z* 578, 606, 618; $[M + H]^+$, $[M + C_2H_5]^+$, $[M + C_3H_5]^+$

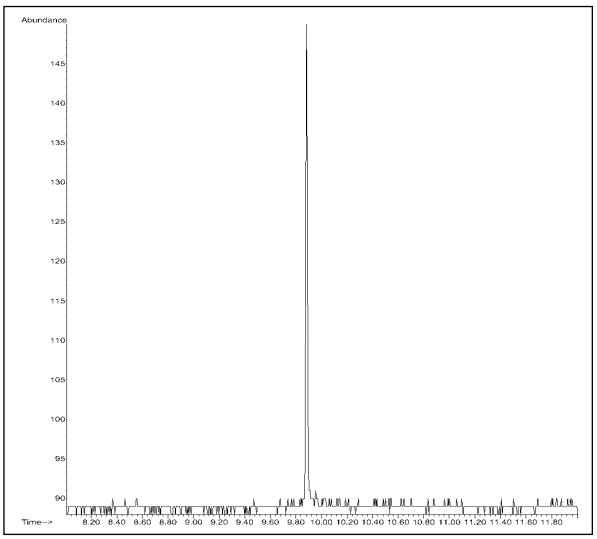


PCI/NH_3-Spectrum, Morphine, PFPA derivative, *m/z* 578; $[M + H]^+$



ECNI/CH_4-Spectrum, Morphine, PFPA derivative, *m/z* 537, 557, 577; [M – 2(HF)]$^-$, [M-HF]$^-$, [M]$^-$

ECNI/CH_4 – SIM Mode



Morphine, PFPA derivative, Retention Time: 9.89min
1pg/µl, Ions: *m/z* 537, 557; S/N ≈ 30/1

Acquisition Mode	Analyte conc.	Approximate Signal/Noise Ratio
EI-Scan	10ng/µl	250/1
PCI/CH_4-Scan	10ng/µl	70/1
PCI/NH_3-Scan	10ng/µl	150/1
ECNI/CH_4-Scan	10ng/µl	110/1
ECNI/CH_4-SIM	1pg/µl	30/1

Table: Morphine, PFPA derivative, Sensitivity (S/N), EI/PCI/ECNI, Scan/SIM

Nalorphine

CAS-Nr. 62-67-9
Molecular Formula: $C_{19}H_{21}NO_3$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 1.2ml/min, 40cm/sec
Mode: Constant Flow
Injection: Pulsed splitless, 250°C
Oven Temp. Program
70°C (1min) – 25°C/min to 300°C (5min)

MS Parameters

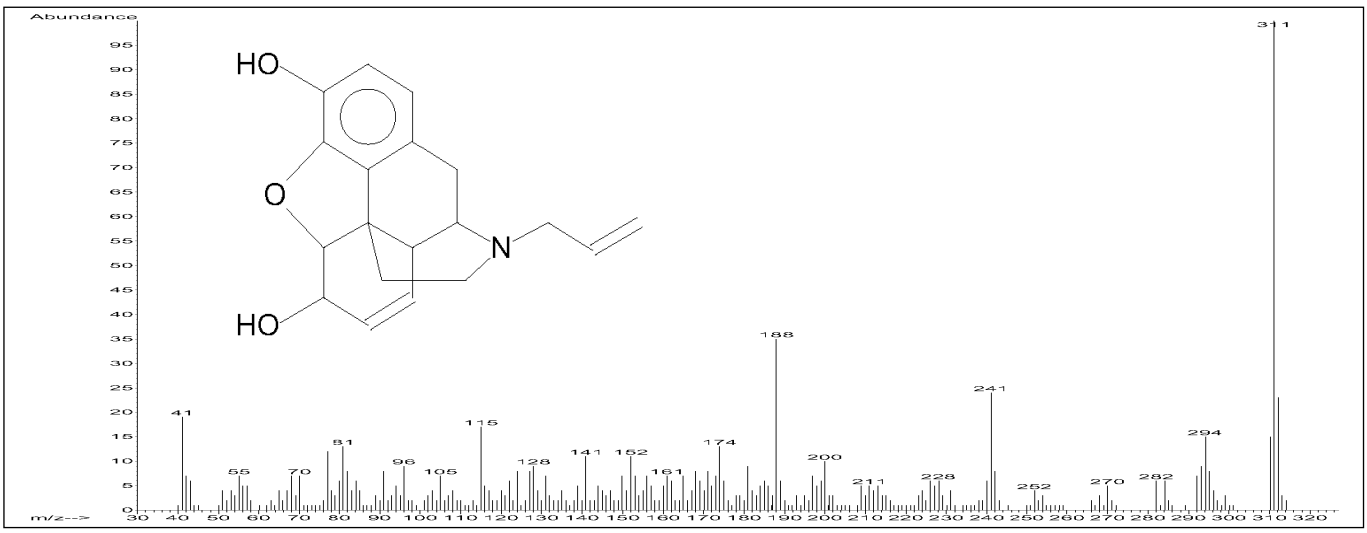
Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C
Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V
Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V
Mode: ECNI/CH_4 – SCAN/SIM
Buffer Gas: Methane
Flow (Setting): 2ml/min (40)
Tune: ECNI-Methane Tune File
Temperatures:
Source 150°C, Quad 106°C
EM Voltage: Tune + 400V

Remarks

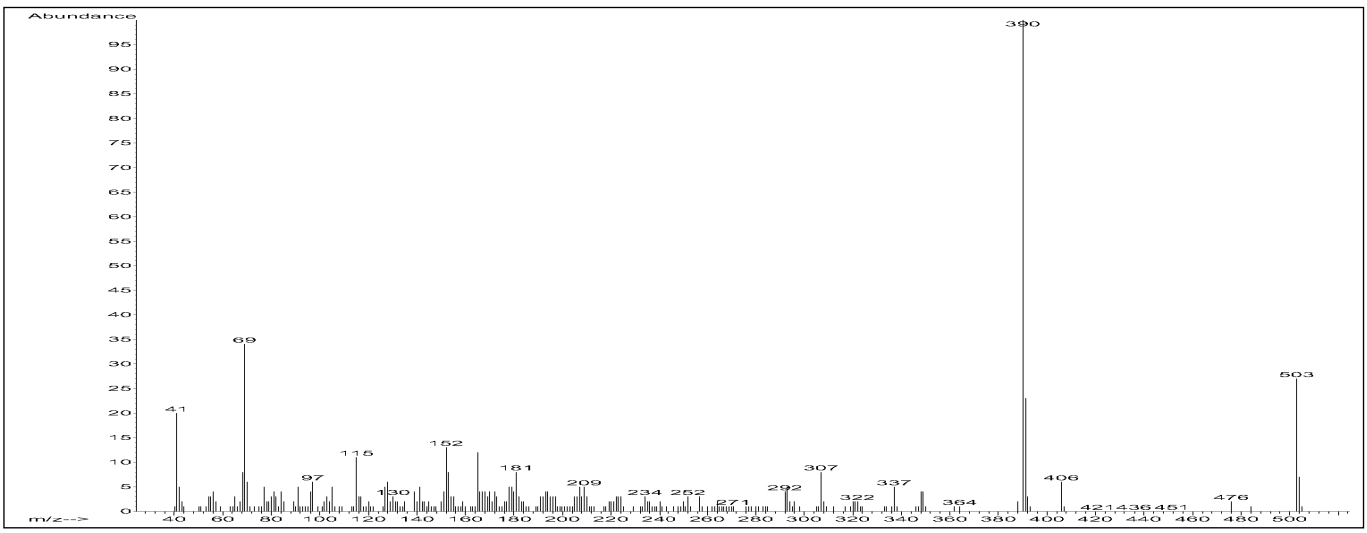
Derivatization
Reaction with Pentafluoropropionic Acid Anhydride (PFPA)
(Reagent: Fluka 77292)

100µl of the standard (SIGMA N 0762), concentration 100ng/µl, dissolved in ethyl acetate is evaporated with a gentle flow of nitrogen. To the residue, 80µl of derivatization reagent and 20µl hexafluoroisopropanol are added and the mixture is incubated for 30min at 70°C. Gentle evaporation with nitrogen is repeated and the residue redissolved in ethyl acetate. The solution is ready GC/MSD analysis.

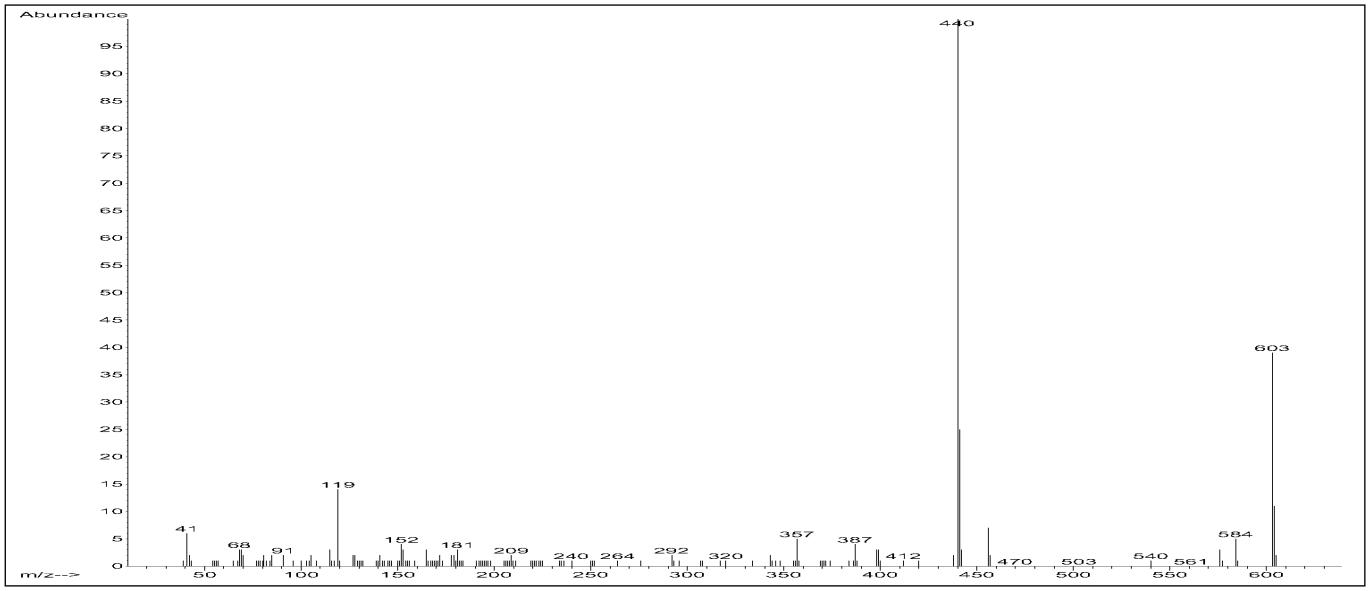
Results
Derivatization is recommended. The trifluoroacetylation (TFA) with MBTFA leads in EI mode to a moderately intense molecular ion. In ECNI mode the TFA analyte spectrum exhibits no distinctive features.
The PFPA derivatization is suitable for PCI/NH_3 and for ECNI/CH_4 measurements. The signal/noise ratio for 1pg analyte PFPA derivative in ECNI/CH_4 mode is approximately 15:1.



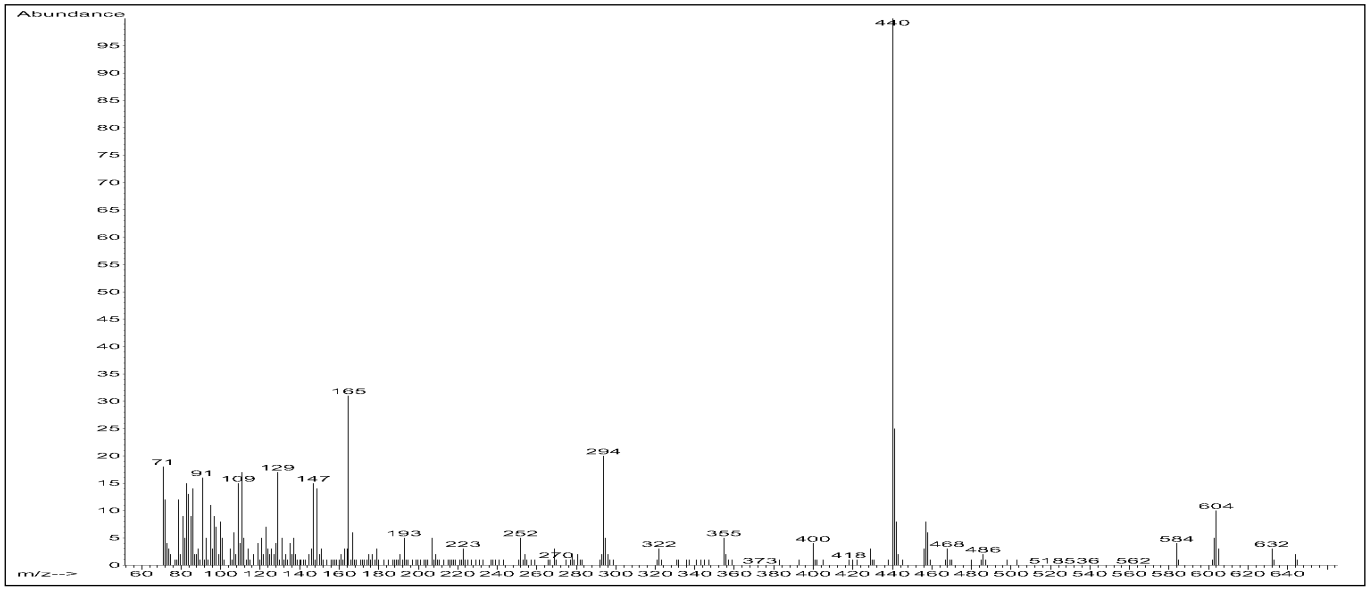
EI-Spectrum, Nalorphine, underivatized, *m/z* 311; M^+



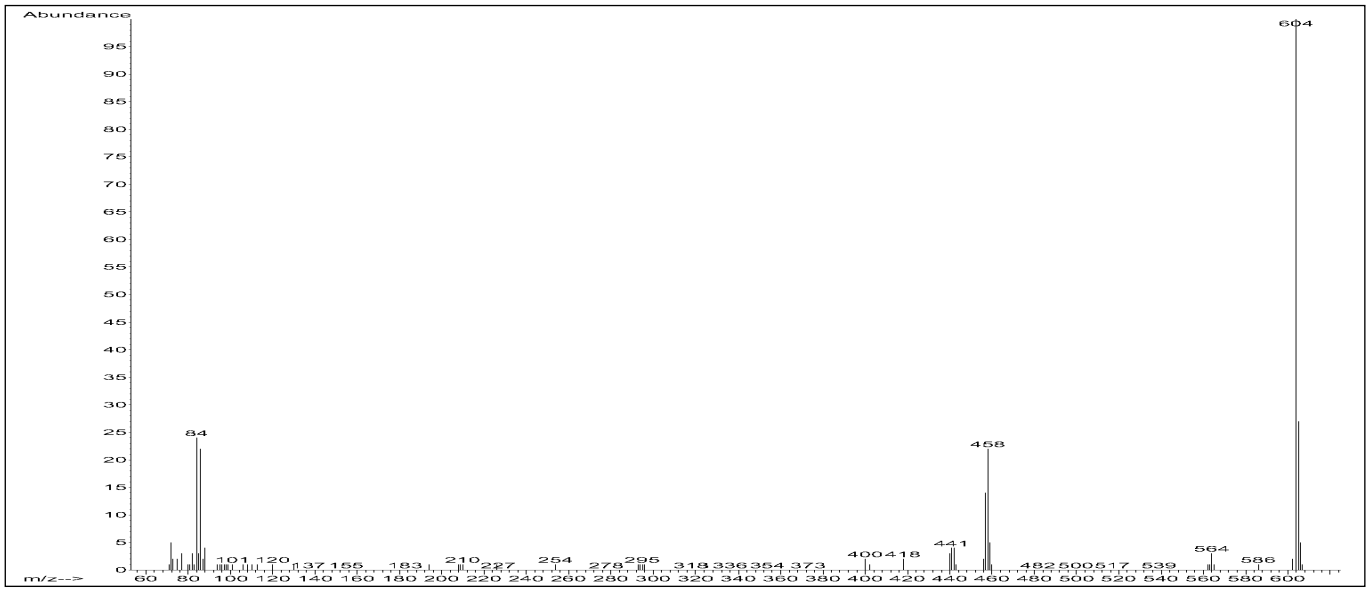
EI-Spectrum, Nalorphine, Trifluoroacetyl derivative, *m/z* 503; M^+



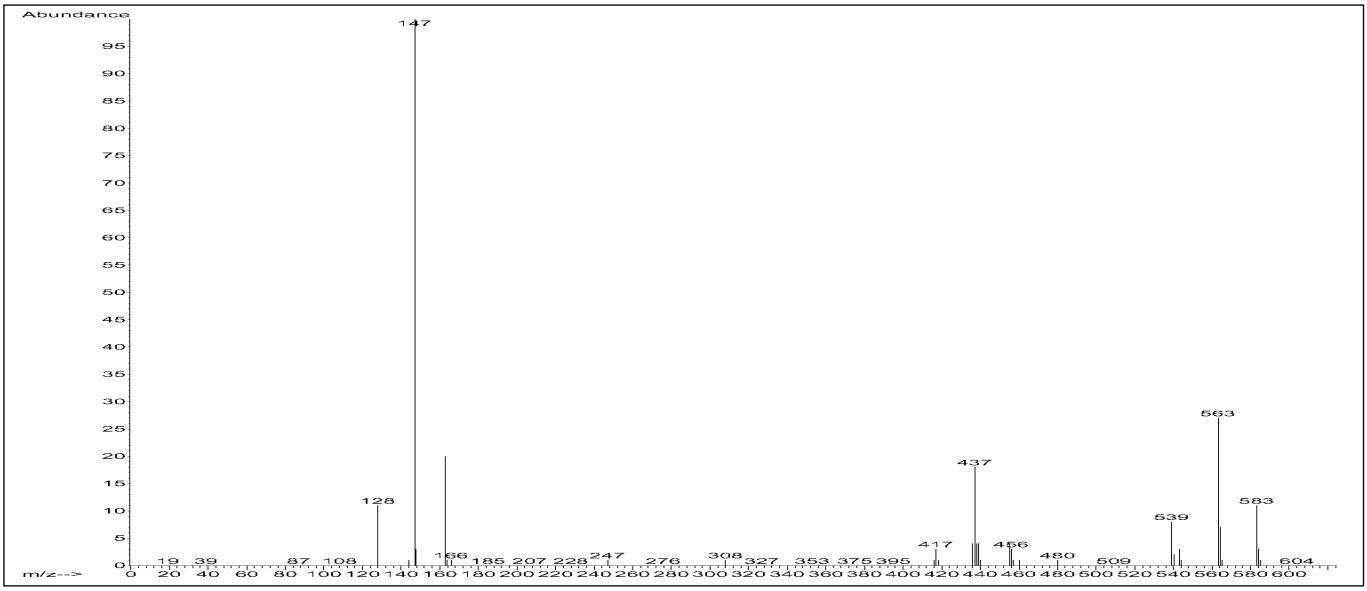
EI-Spectrum, Nalorphine, PFPA derivative, *m/z* 603; M^+



PCI/CH_4-Spectrum, Nalorphine, PFPA derivative, *m/z* 604, 632, 644; $[M + H]^+$, $[M + C_2H_5]^+$, $[M + C_3H_5]^+$

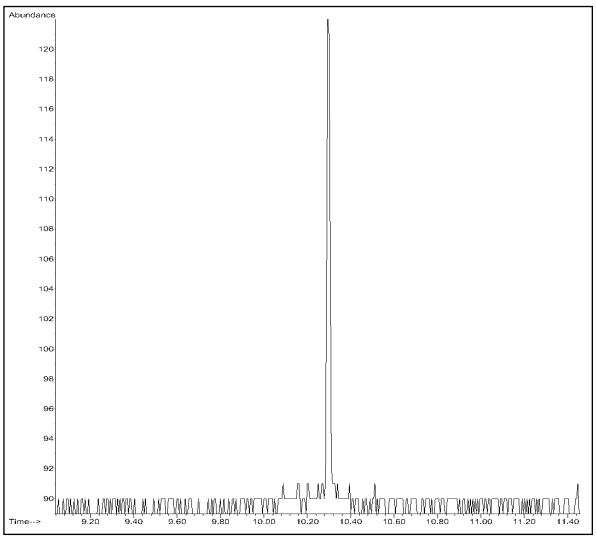


PCI/NH_3-Spectrum, Nalorphine, PFPA derivative, *m/z* 604; $[M + H]^+$



ECNI/CH_4-Spectrum, Nalorphine, PFPA derivative, *m/z* 563, 583, 603; $[M - 2(HF)]^-$, $[M - HF]^-$, M^-

ECNI/CH_4 – SIM Mode



Nalorphine, PFPA derivative, Retention Time: 10.30min
1pg/µL, Ions: *m/z* 563, 583; S/N: 16/1

Acquisition Mode	Analyte conc.	Approximate Signal/Noise Ratio
EI-Scan	10ng/µl	240/1
PCI/CH_4-Scan	10ng/µl	75/1
PCI/NH_3-Scan	10ng/µl	110/1
ECNI/CH_4-Scan	10ng/µl	200/1
ECNI/CH_4-SIM	1pg/µl	15/1

Table: Nalorphine, PFPA derivative, Sensitivity (S/N), EI/PCI/ECNI, Scan/SIM

Nitroimidazoles

Dimetridazole
CAS-Nr. 551-92-8
Molecular Formula: $C_5H_7N_3O_2$
Ronidazole
CAS-Nr. 7681-76-7
Molecular Formula: $C_6H_8N_4O_4$
Metronidazole
CAS-Nr. 443-48-1
Molecular Formula: $C_6H_9N_3O_3$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 0.7ml/min, 30cm/sec
Mode: Constant Flow
Injection: Pulsed splitless, 250°C
Oven Temp. Program
60°C (1min) – 25°C/min to 270°C

MS Parameters

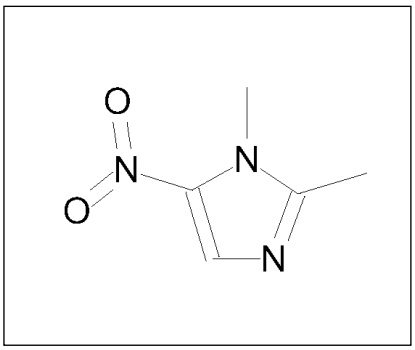
Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C

Mode: ECNI/CH_4 – SCAN/SIM
Buffer Gas: Methane
Flow (Setting): 2ml/min (40)
Buffer Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: ECNI-Methane Tune File
Temperatures:
Source 150°C, Quad 106°C
EM Voltage Scan: Tune + 300V
EM Voltage SIM: Tune + 400V

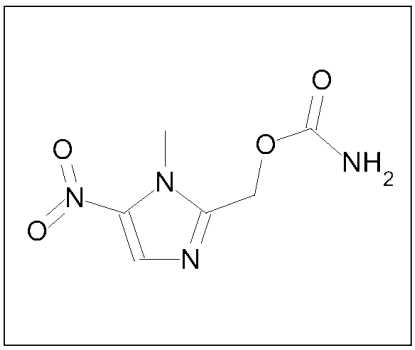
Remarks

Derivatization
Ronidazole (Reagent: SIGMA R 7635) and Metronidazole (Reagent: SIGMA M 1547) can be silylated with MSTFA (Reagent: Fluka 69479)
Each standard solution, concentration 1ng/µl in ethyl acetate, is evaporated with a gentle nitrogen flow. To the residue, 50µl of the reagent is added and incubated at 15min at 60°C.
The derivatized solutions are used for GC/MSD measurements.

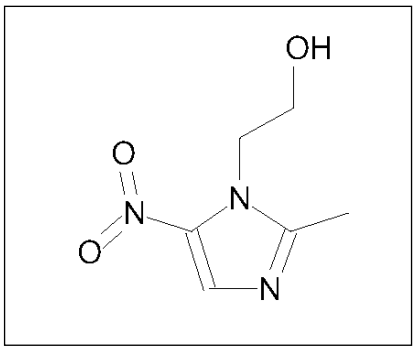
Results
The spectrum of Ronidazole shows no molecular ions either in EI or in ECNI mode. Spectral base peaks of the analytes are related to the fragmentation at the carbamide ester positions. The degree of fragmentation is affected by choice of the buffer gas. Both methane and ammonia show different responses. Considering absolute signal intensitiy, ammonia is the preferred buffer gas. However the signal/noise ratios indicate that both gases perform comparably. The relative S/N ratios in SIM mode for these nitroimidazoles are approximately: Dimetridazole : Metronidazol : Ronidazole = 100 : 10 : 1.



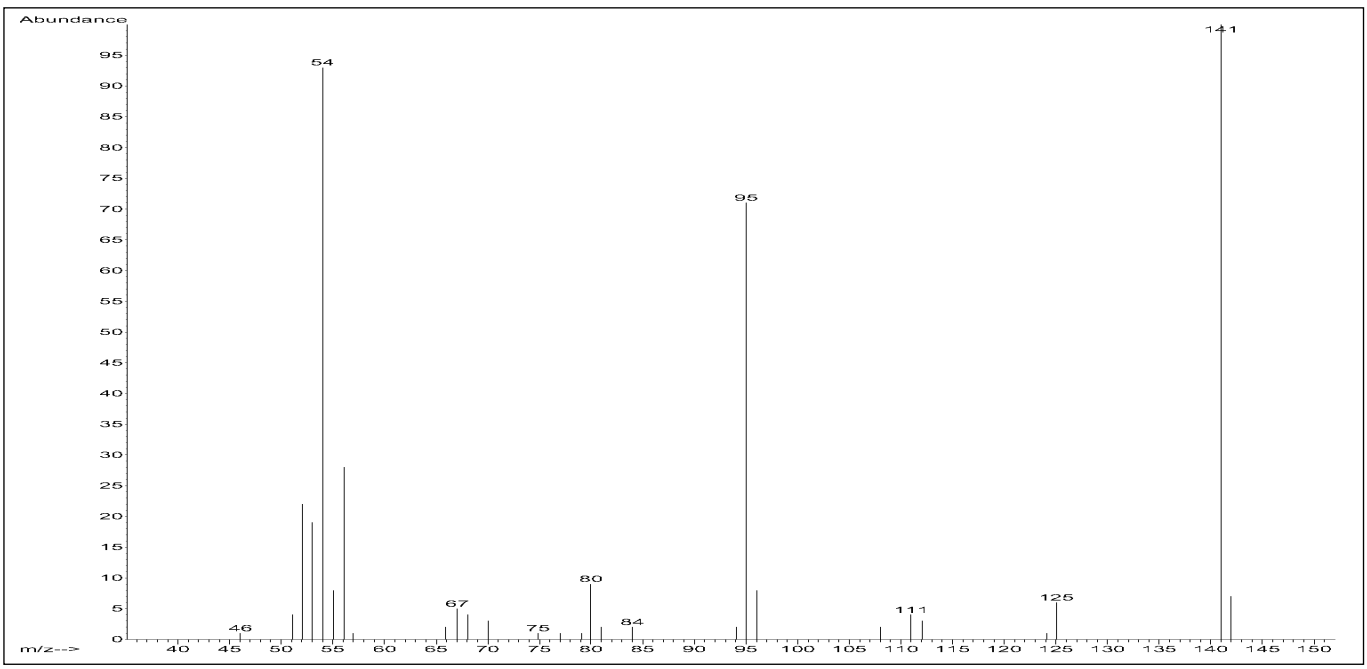
Dimetridazole



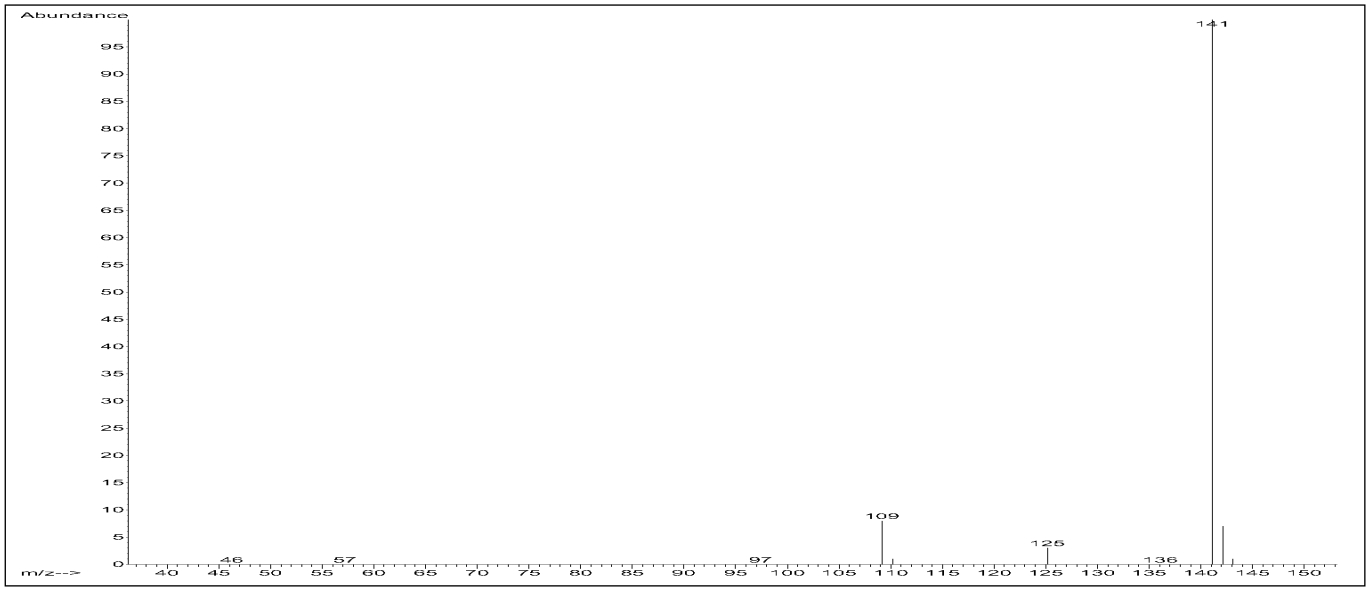
Ronidazole



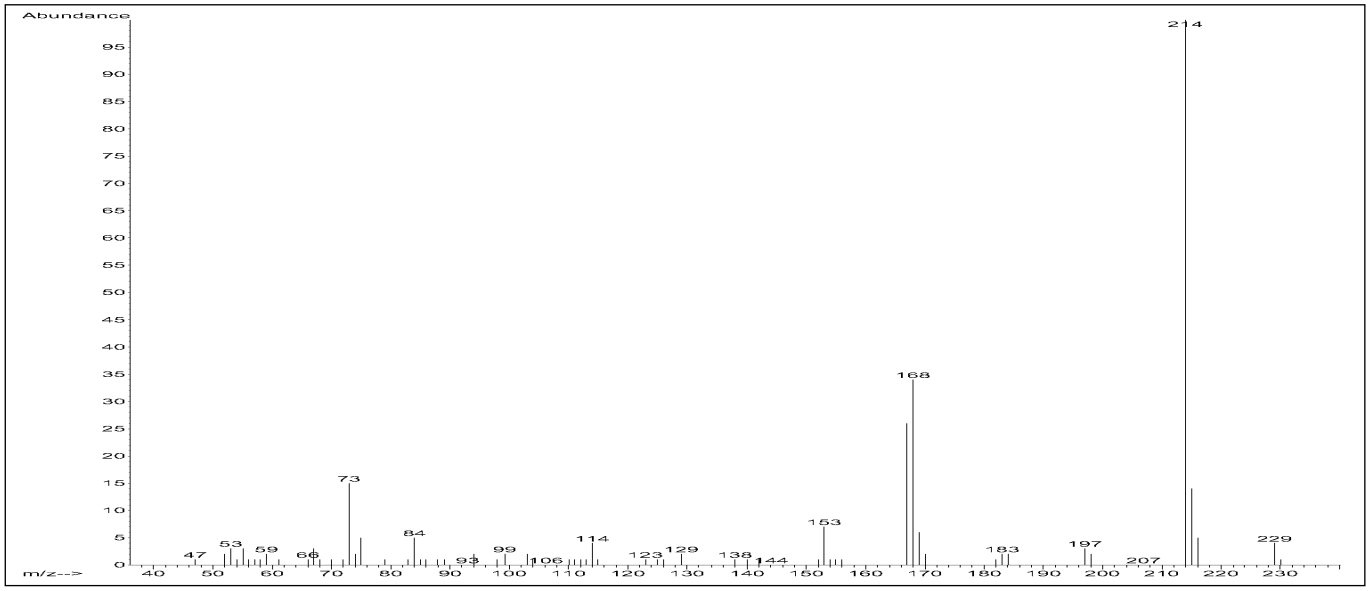
Metronidazole



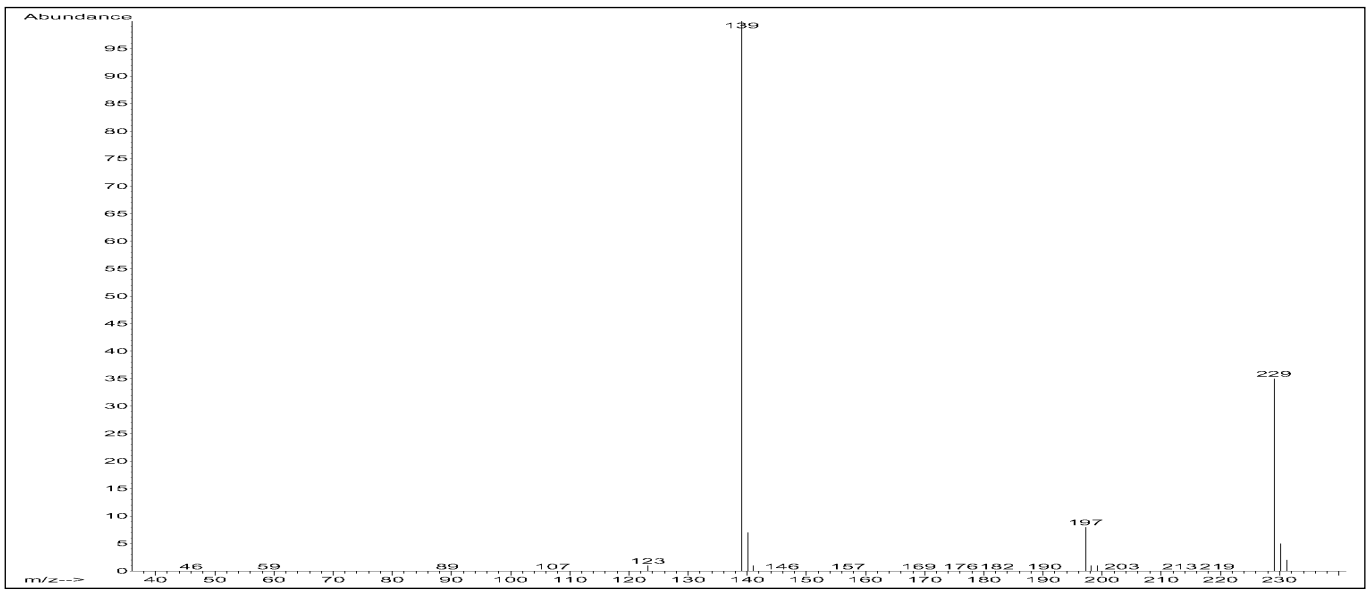
EI-Spectrum, Dimetridazole, *m/z* 141; M^+



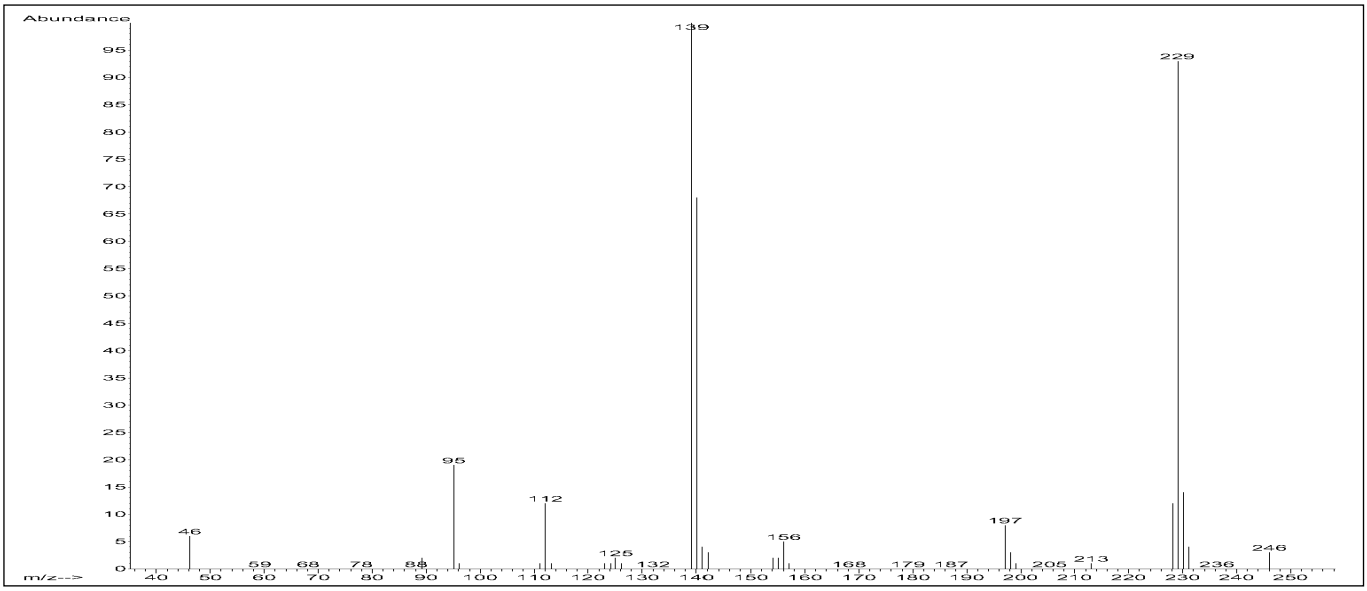
ECNI/CH_4-Spectrum, Dimetridazole, *m/z* 141; M^-



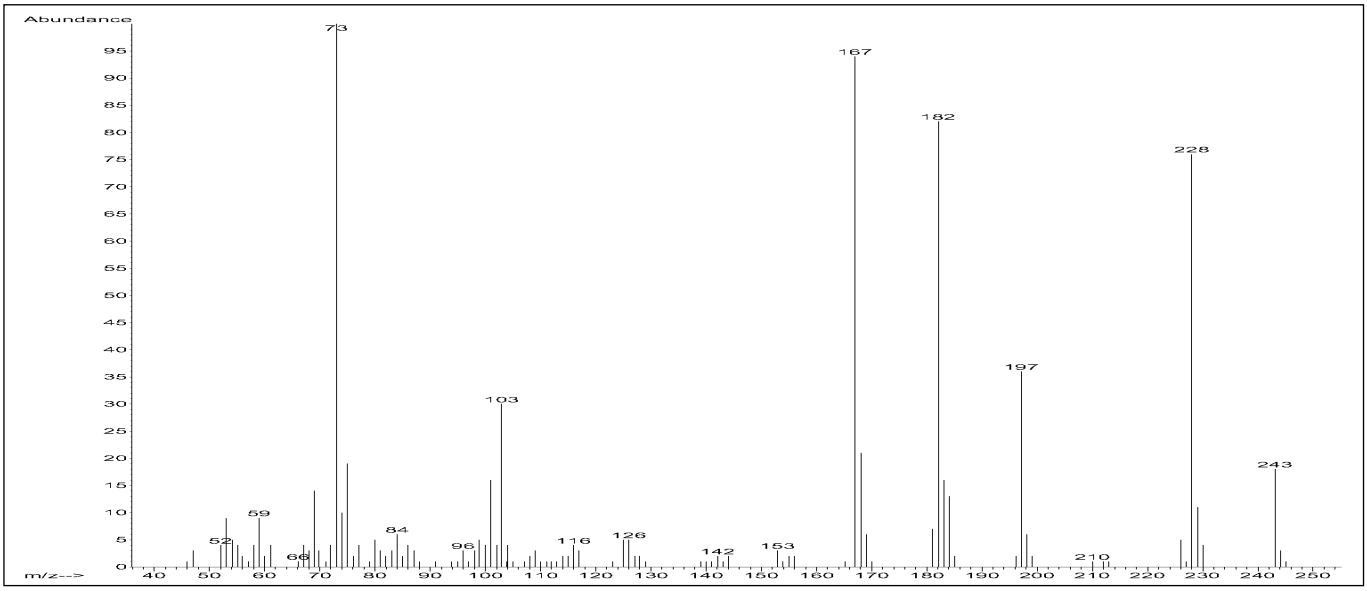
EI-Spectrum, Ronidazole, TMS derivative, molecular mass = 272 u



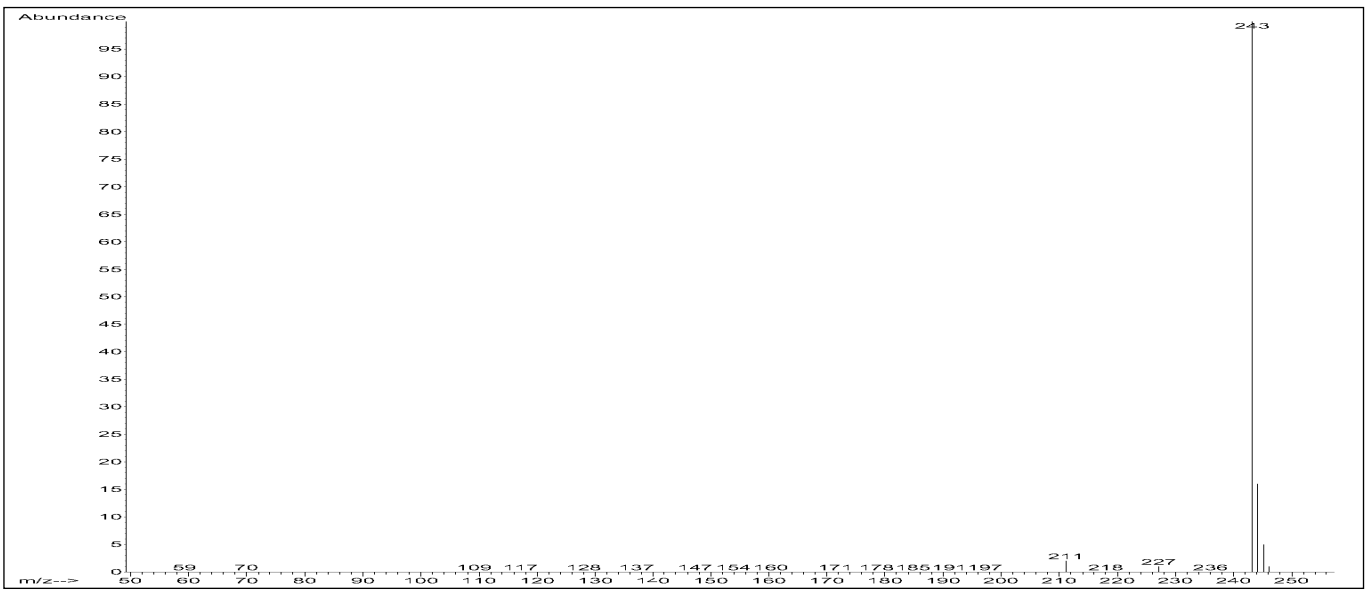
ECNI/CH_4-Spectrum, Ronidazole, TMS derivative, molecular mass = 272 u



ECNI/NH_3-Spectrum, Ronidazole, TMS derivative, molecular mass = 272 u

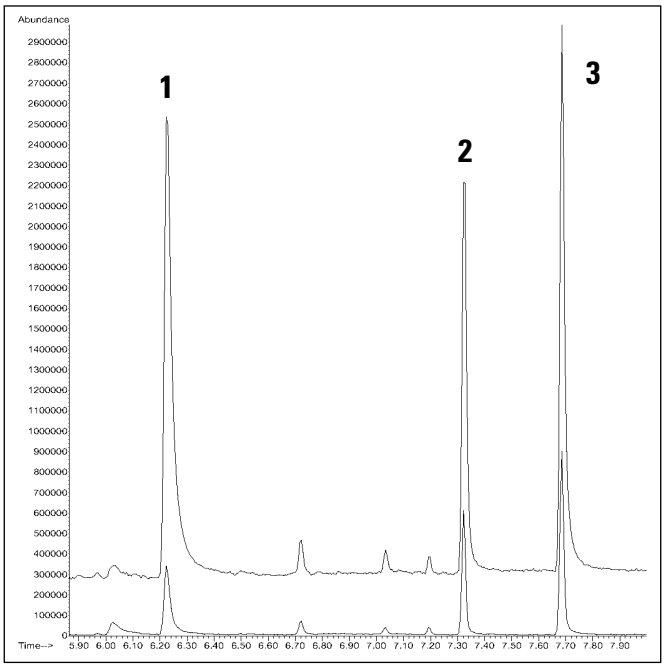


EI-Spectrum, Metronidazole, TMS derivative, *m/z* 243; M^+



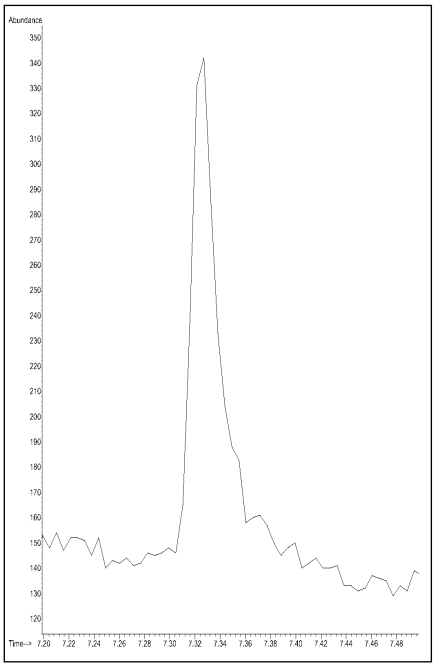
ECNI/CH_4-Spectrum, Metronidazole, TMS derivative, *m/z* 243; M^-

Scan Mode, ECNI, 1ng each

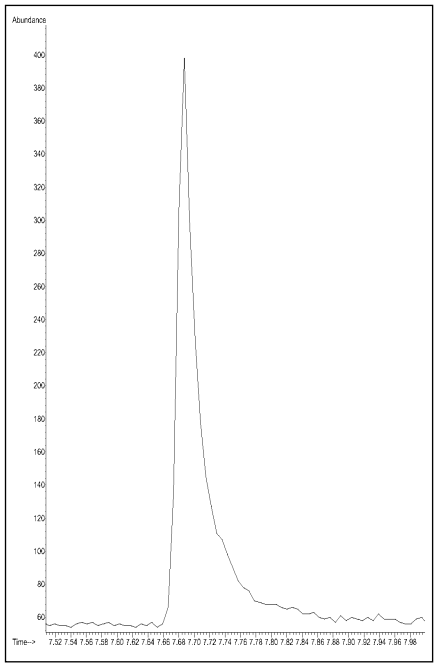


TIC of Dimetridazole(1), Ronidazole(2), Metronidazole(3)
Buffer Gas: Methane, bottom; Ammonia, top

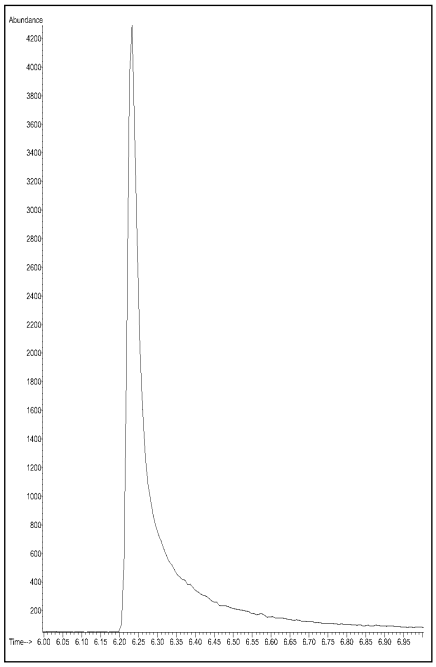
SIM Mode, ECNI/CH_4, 1 pg each



Ronidazole, S/N ≈ 6/1
Ions *m/z* 139/229



Metronidazole, S/N ≈ 60/1
Ion *m/z* 243



Dimetridazole, S/N ≈ 600/1
Ion *m/z* 141

Orphenadrine

CAS-Nr. 83-98-7
Molecular Formula: $C_{18}H_{23}NO$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 0.7ml/min, 30cm/sec
Mode: Constant Flow
Injection: Split, 250°C
Oven Temp. Program
120°C (0.3min) – 20°C/min to 300°C (4min)

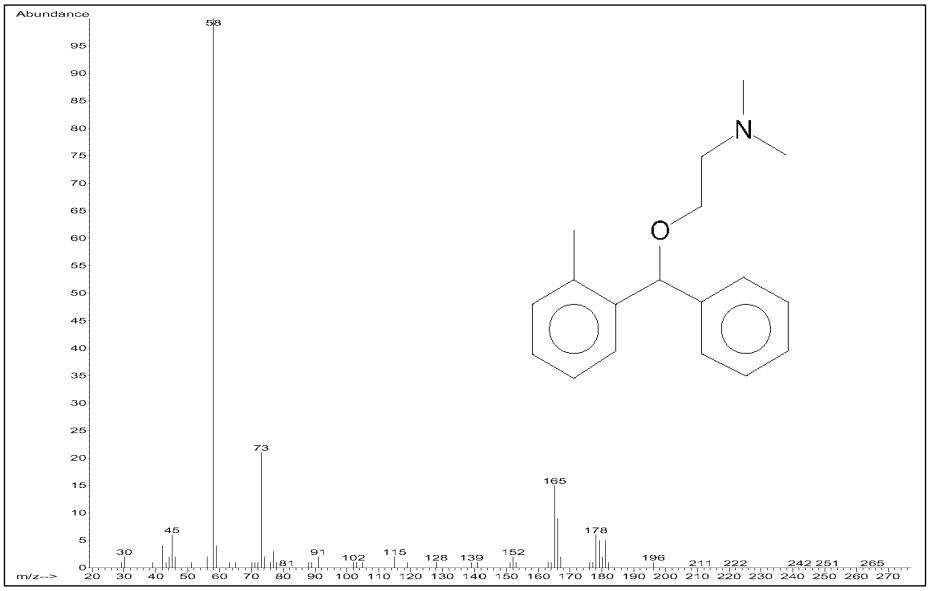
MS Parameters

Mode: EI – SCAN
Tune: Atune
Temperature:
Source 230°C, Quad 150°C

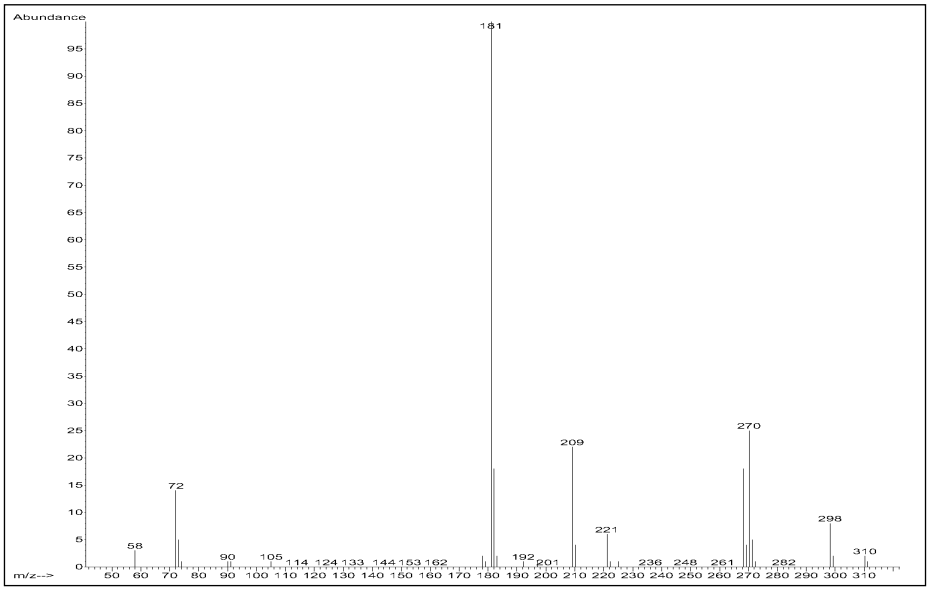
Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V

Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V

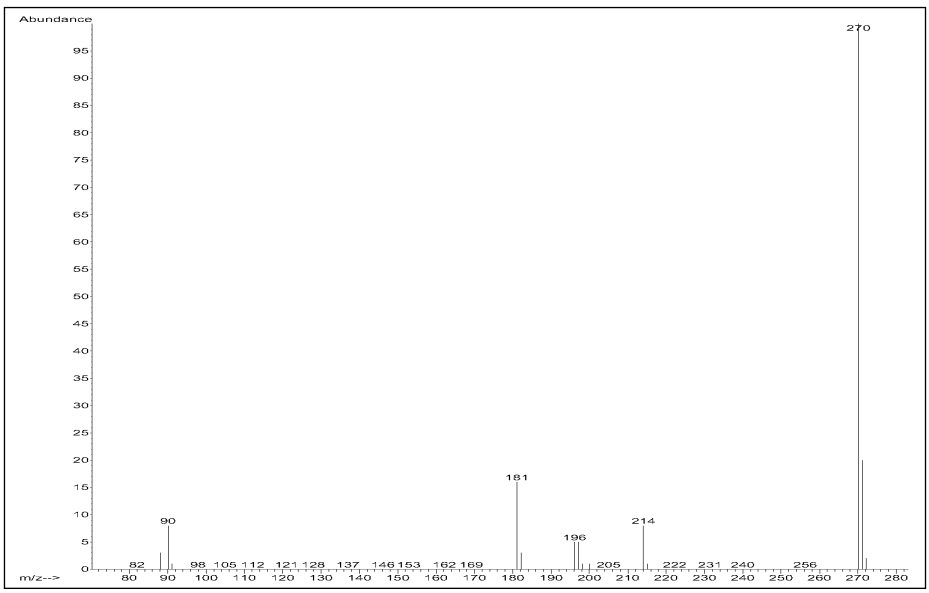
Results
Analyte Retention Time: 13.54min
Analyte Concentration: 4ng/µl
Signal/Noise Ratios for TIC
PCI/CH_4 Scan: > 300/1
PCI/NH_3 Scan: > 50/1



EI-Spectrum, Orphenadrine: *m/z* 269; M^+



PCI/CH_4-Spectrum, Orphenadrine: *m/z* 270, 298, 310; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



PCI/NH_3-Spectrum, Orphenadrine: *m/z* 270; $[M+H]^+$

Phenylbutazone

CAS-Nr. 50-33-9
Molecular Formula: $C_{19}H_{20}N_2O_2$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 1.2ml/min, 40cm/sec
Mode: Constant Flow
Injection: Pulsed splitless, 250°C
Oven Temp. Program
70°C (1min) – 25°C/min to 300°C (2min)

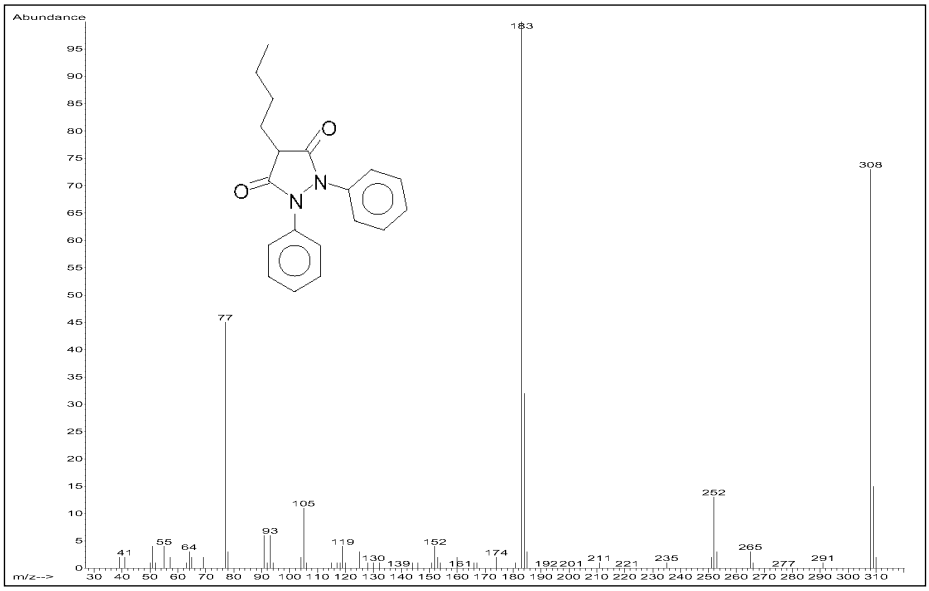
MS Parameters

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C

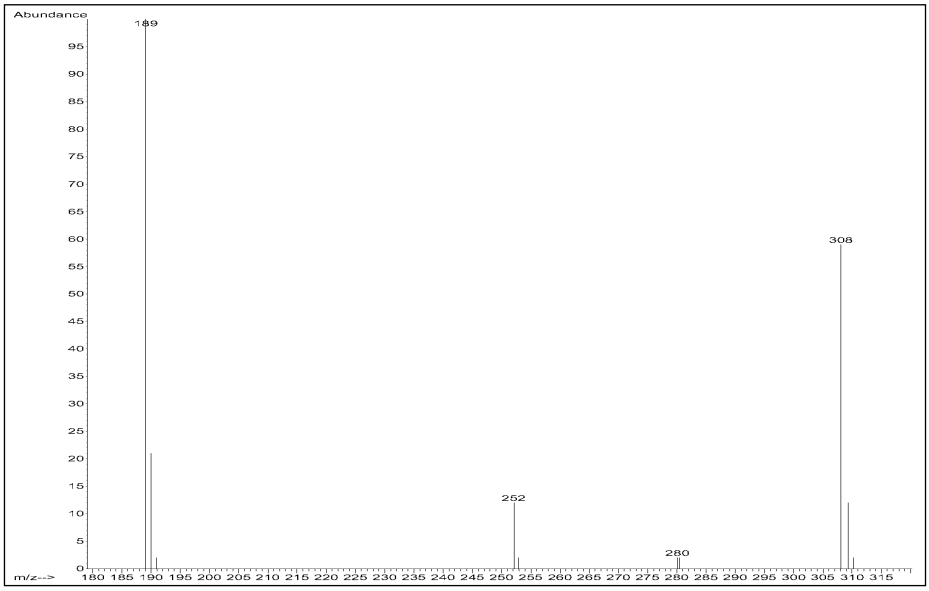
Mode: ECNI/CH_4 – SCAN/SIM
Buffer Gas: Methane
Flow (Setting): 2ml/min (40)
Tune: ECNI-Methane Tune File
Temperatures:
Source 150°C, Quad 106°C
EM Voltage: Tune + 400V

Remarks

Results
In EI mode, the analyte shows a moderately intense molecular ion. ECNI/CH_4 mode generates the molecular anion and SIM measurements in this mode result in an approximate signal/noise ratio of 15:1 for 1pg of analyte.

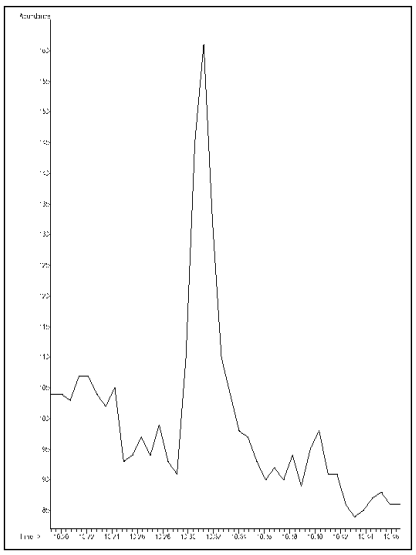


EI-Spectrum, Phenylbutazone, *m/z* 308; M^+



ECNI/CH_4-Spectrum, Phenylbutazone, *m/z* 308; M^-

ECNI/CH_4 – SIM Mode



Phenylbutazone, 1pg, Retention Time: 10.32min
Ion: *m/z* 308, Signal/Noise ≈ 7/1

Promethazine

CAS-Nr. 60-87-7
Molecular Formula: $C_{17}H_{20}N_2S$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 0.7ml/min, 30cm/sec
Mode: Constant Flow
Injection: Split, 250°C
Oven Temp. Program
120°C (0.3min) – 20°C/min to 300°C (4min)

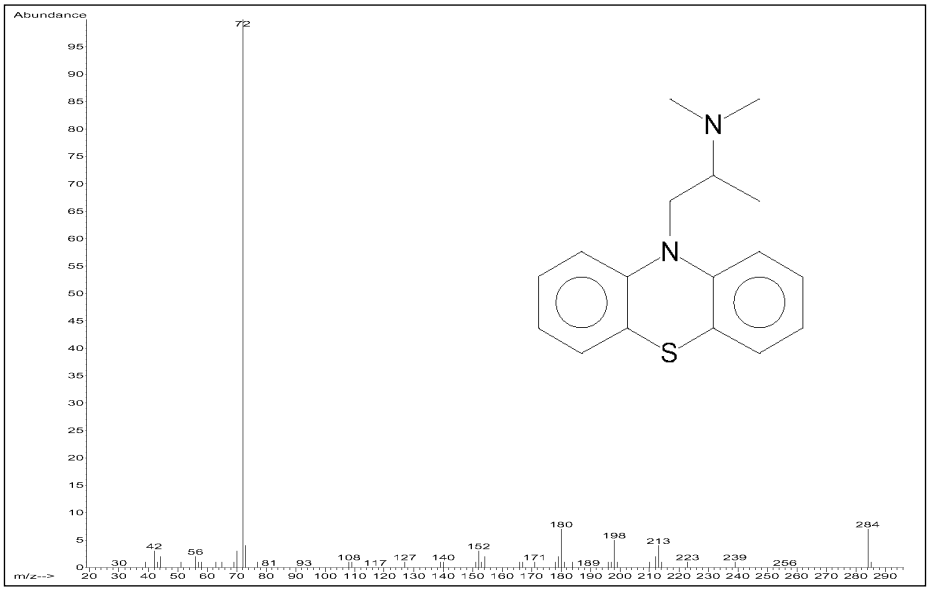
MS Parameters

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C

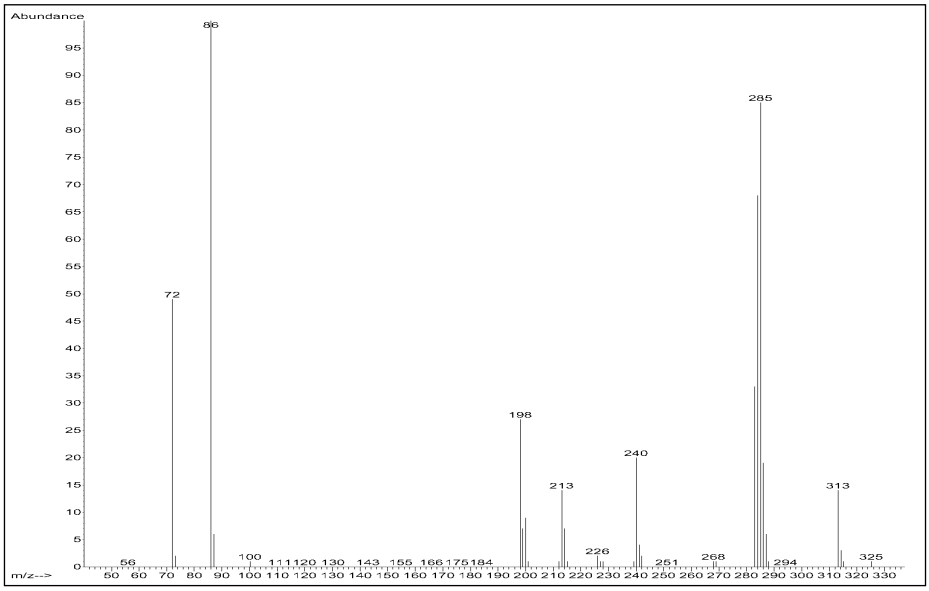
Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V

Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V

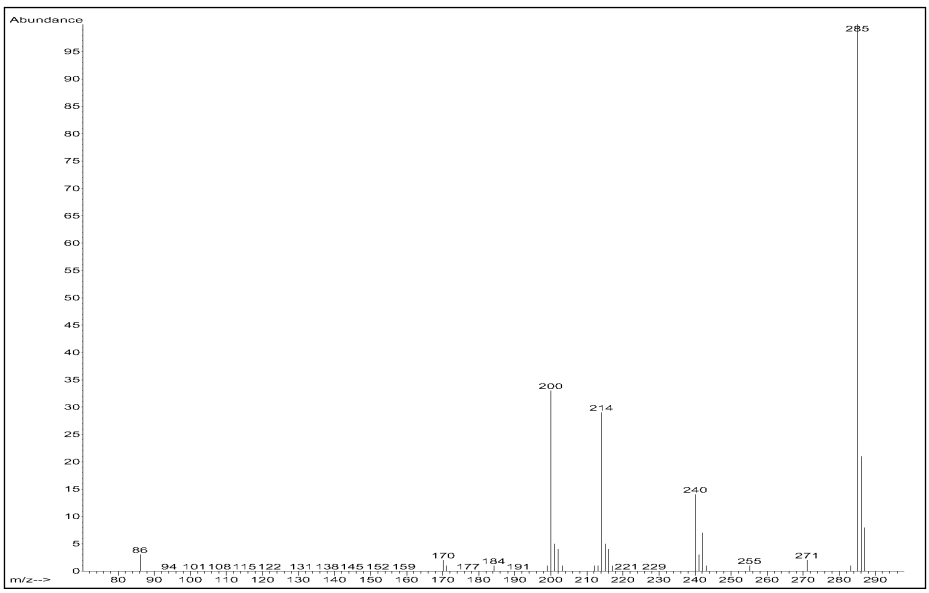
Results
Analyte Retention Time: 16.79min
Analyte Concentration: 4ng/µl
Signal/Noise Ratios for TIC
PCI/CH_4 Scan: > 200/1
PCI/NH_3 Scan: > 90/1



EI-Spectrum, Promethazine: *m/z* 284 ; M^+



PCI/CH_4-Spectrum, Promethazine: *m/z* 285, 313, 325; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



PCI/NH_3-Spectrum, Promethazine: *m/z* 285; $[M+H]^+$

Propionylpromazine

Combelen
CAS-Nr. 3568-24-9
Molecular Formula: $C_{20}H_{24}ON_2S$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 0.7ml/min, 30cm/sec
Mode: Constant Flow
Injection: Split, 250°C
Oven Temp. Program
120°C (0.3min) – 20°C/min to 300°C (4min)

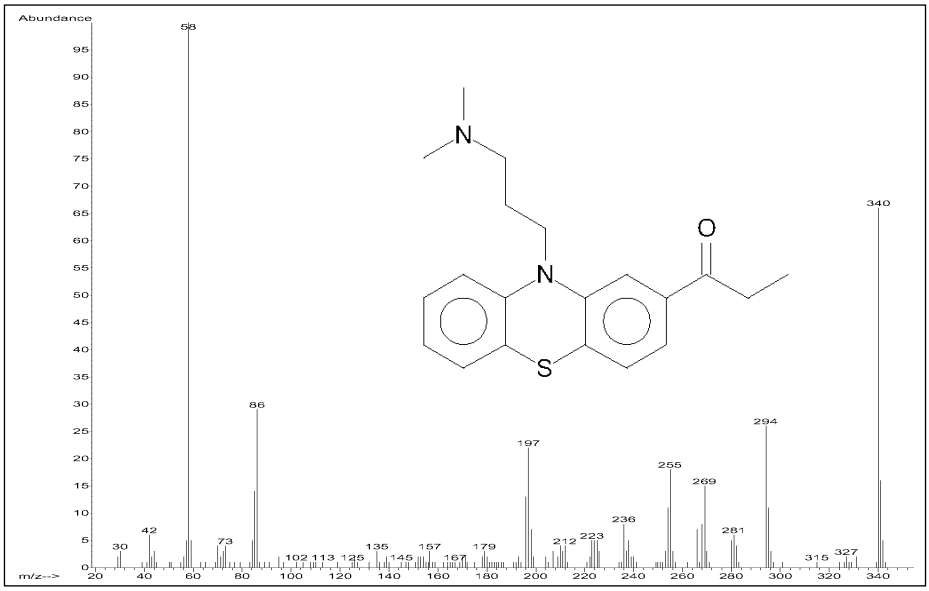
MS Parameters

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C

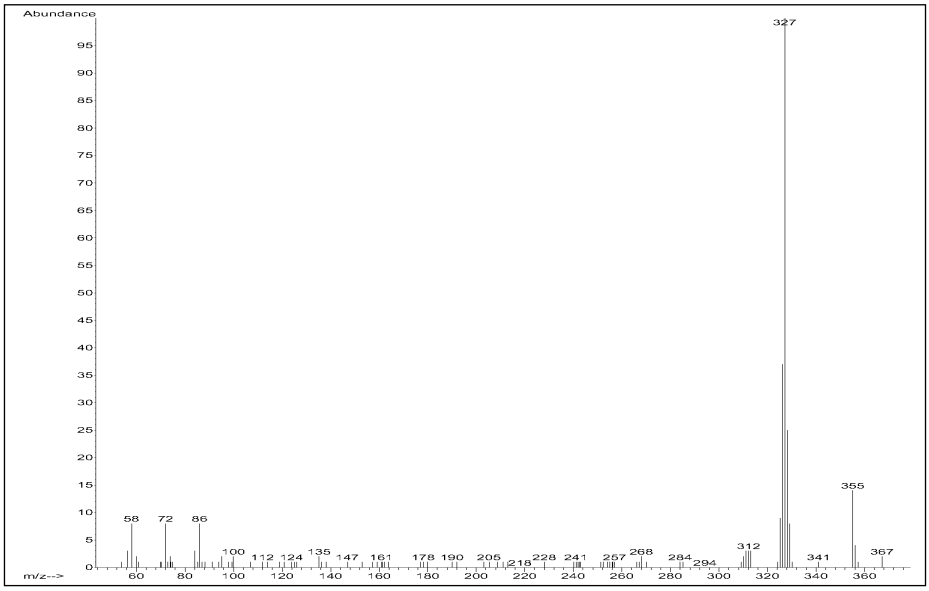
Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V

Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V

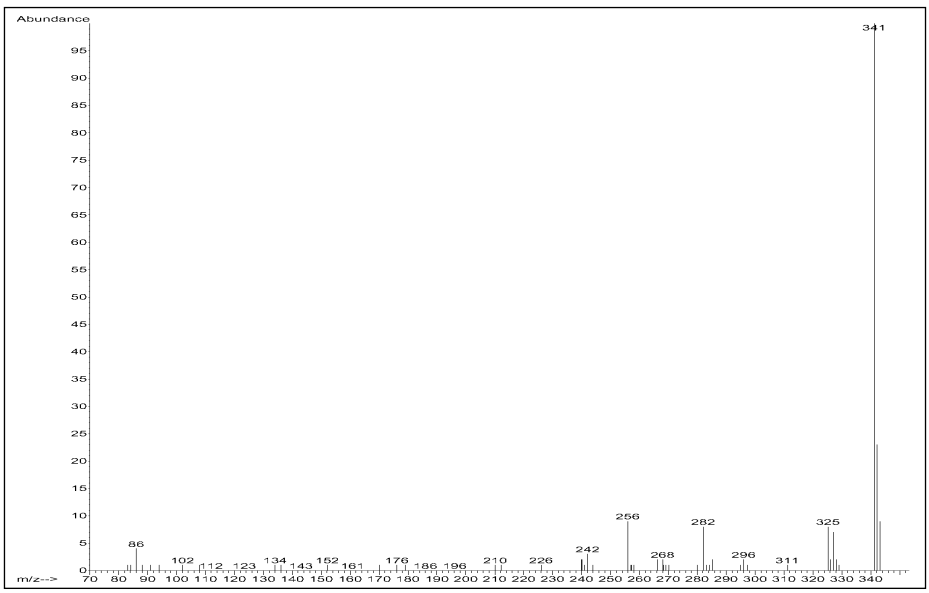
Results
Analyte Retention Time: 9.25min
Analyte Concentration: 4ng/µl
Signal/Noise Ratios for TIC
PCI/CH_4 Scan: > 8/1
PCI/NH_3 Scan: > 16/1



EI-Spectrum, Propionylpromazine: *m/z* 340; M+



PCI/CH_4-Spectrum, Propionylpromazine: *m/z* 327, 355, 367; $[M+H-CH_2]^+$, $[M+C_2H_5-CH_2]^+$, $[M+C_3H_5-CH_2]^+$



PCI/NH_3-Spectrum, Propionylpromazine: *m/z* 341; $[M+H]^+$

Ractopamine

CAS-Nr. 99095-19-9 (HCl)
Molecular Formula: $C_{18}H_{23}NO_3$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 1.2ml/min, 40cm/sec
Mode: Constant Flow
Injection: Pulsed splitless, 250°C
Oven Temp. Program
70°C (1min) – 25°C/min to 300°C (5min)

MS Parameters

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C

Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V

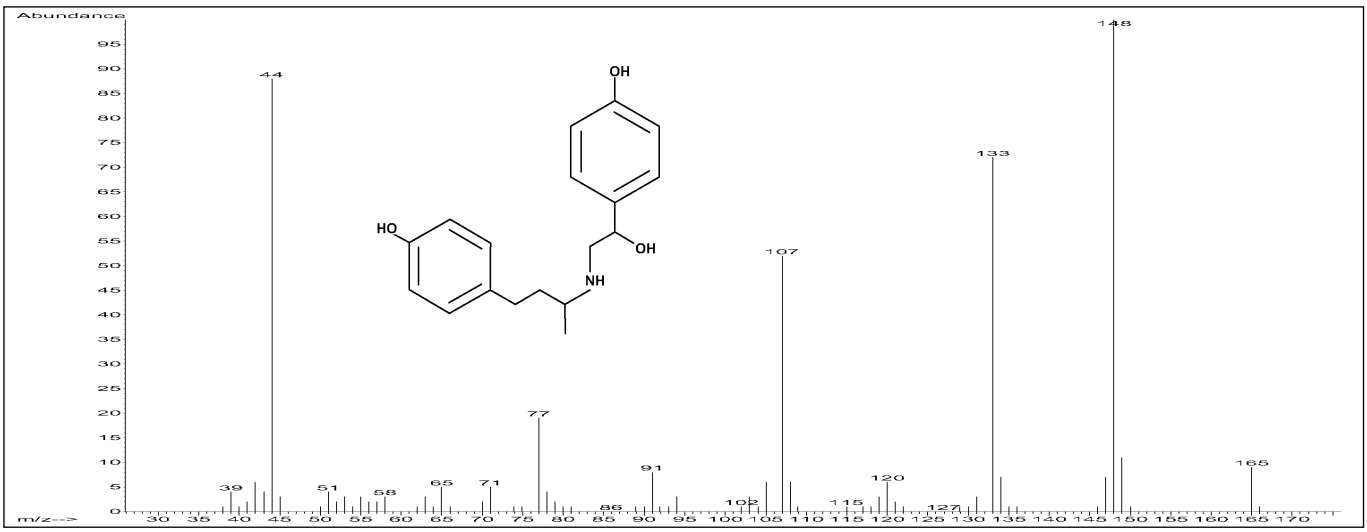
Mode: PCI/NH_3 – SCAN/SIM
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V

Remarks

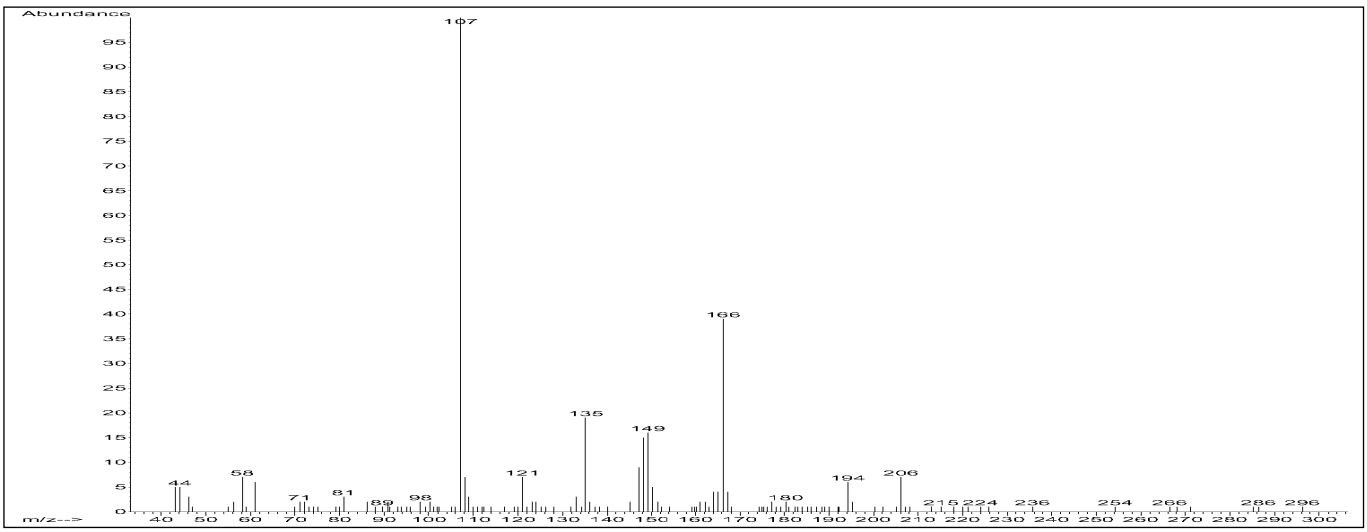
Derivatization
Silylation (TMS) with BSTFA/TMCS (Reagent: Fluka 15238)

100µl of the hydrochloride standard (Lilly Research Laboratories), concentration 1.6mg/ml in methanol, is evaporated with a gentle flow of nitrogen. To the residue, 50µl derivatization reagent and 125µl pyridine are added and the mixture incubated for 30min at 60°C. Gentle evaporation with nitrogen is repeated and the residue redissolved in chloroform. The solution is ready GC/MSD analysis.

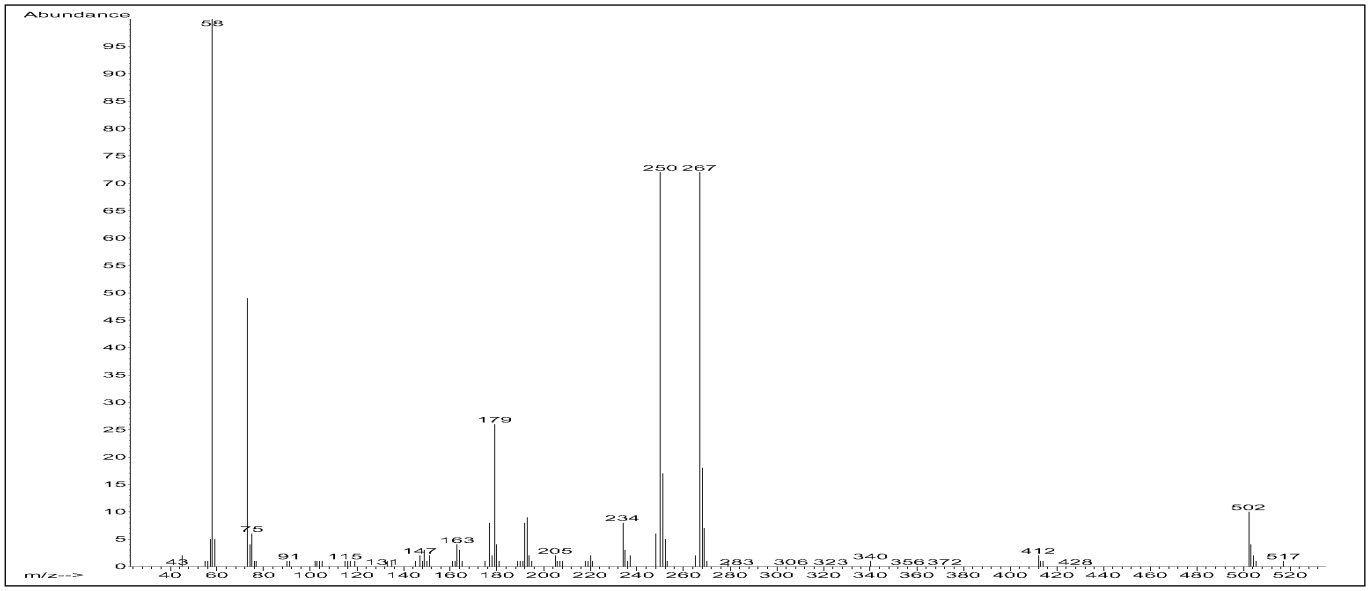
Results
Derivatization is recommended due to the polar nature of the analyte. The TMS reaction leads to the tri-derivative. The underivatised analyte shows no protonated molecule or molecular adducts in PCI/CH_4. The PCI/NH_3 reaction of the silylated analyte is the method of choice. In PCI/NH_3 SIM the signal/noise ratio for 100pg of analyte is calculated as approximately 30/1.



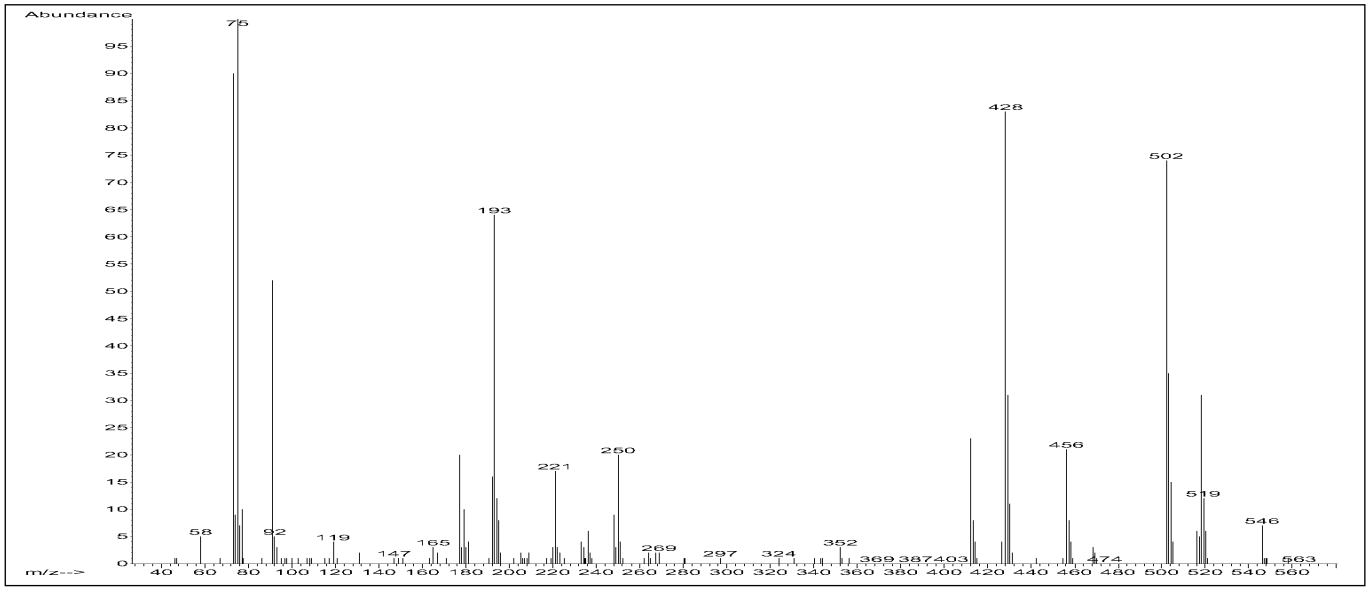
EI-Spectrum, Ractopamine, underivatized, molecular mass = 301 u



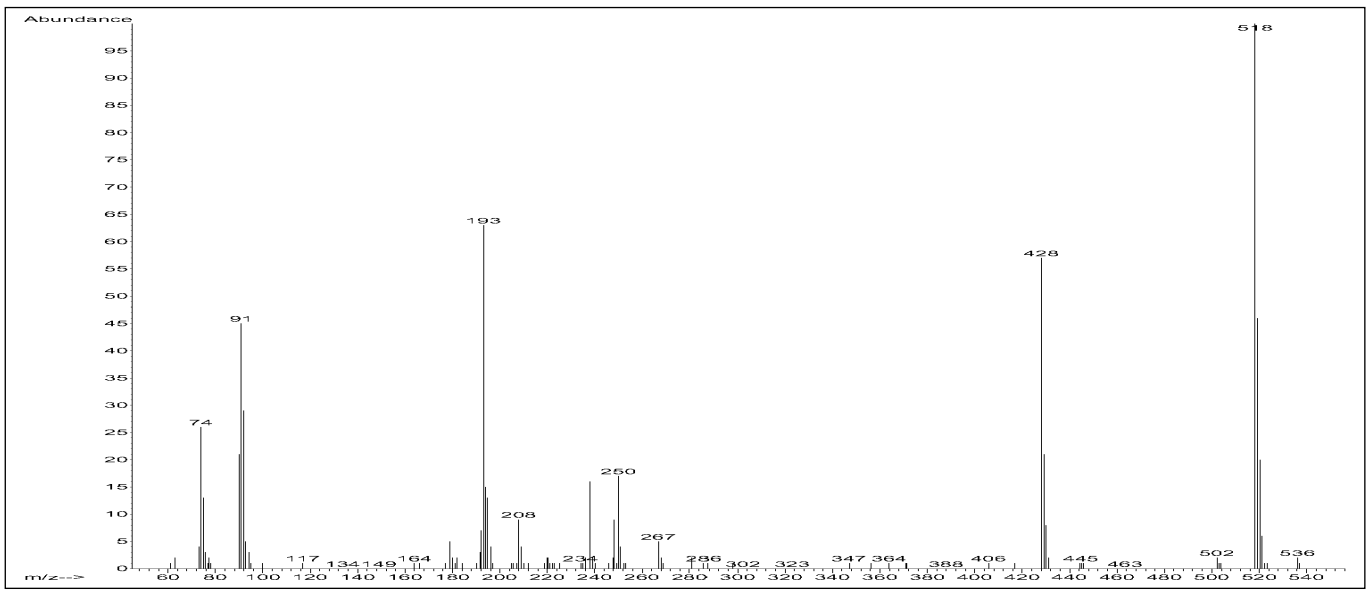
PCI/CH_4-Spectrum, Ractopamine, underivatized, molecular mass = 301 u



EI-Spectrum, Ractopamine, TMS derivative, *m/z* 517; M^+

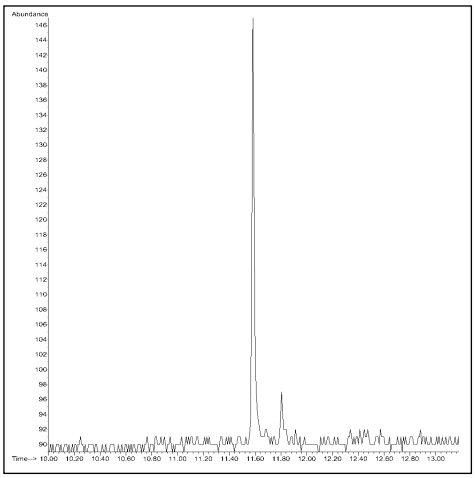


PCI/CH_4-Spectrum, Ractopamine, TMS derivative, *m/z* 518, 546, 558; $[M + H]^+$, $[M + C_2H_5]^+$, $[M + C_3H_5]^+$



PCI/NH_3-Spectrum, Ractopamine, TMS derivative, *m/z* 518, 535; $[M + H]^+$, $[M + NH_4]^+$

PCI/NH_3 – SIM Mode



Ractopamine, TMS derivative, 100pg,
Retention Time: 11.58min
Ions: *m/z* 428, 518; Signal/Noise ≈ 30/1

Steroids

Cholesterol CAS-Nr. 57-88-5
Molecular Formula: $C_{27}H_{46}O$
Estradiol CAS-Nr. 7681-76-7
Molecular Formula: $C_{18}H_{24}O_2$
Estrone CAS-Nr. 443-48-1
Molecular Formula: $C_{18}H_{22}O_2$
Testosterone CAS-Nr. 58-22-0
Molecular Formula: $C_{19}H_{28}O_2$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 0.7ml/min, 30cm/sec
Mode: Constant Flow
Injection: Pulsed splitless, 250°C
Oven Temp. Program
60°C (1min) – 30°C/min to 270°C (5.5min)

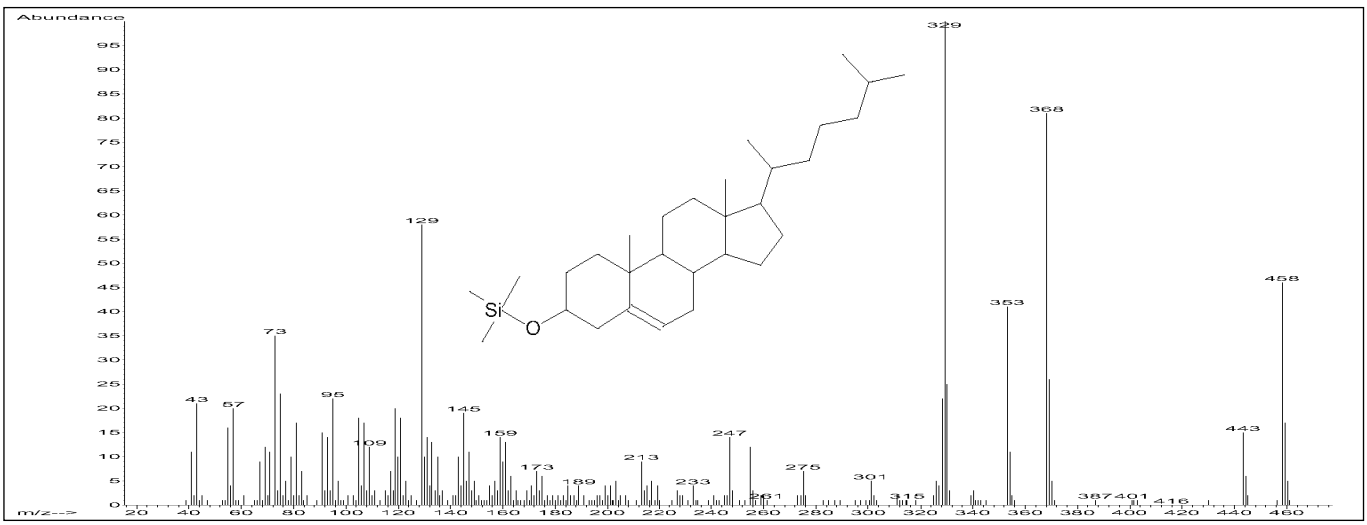
MS Parameters

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C

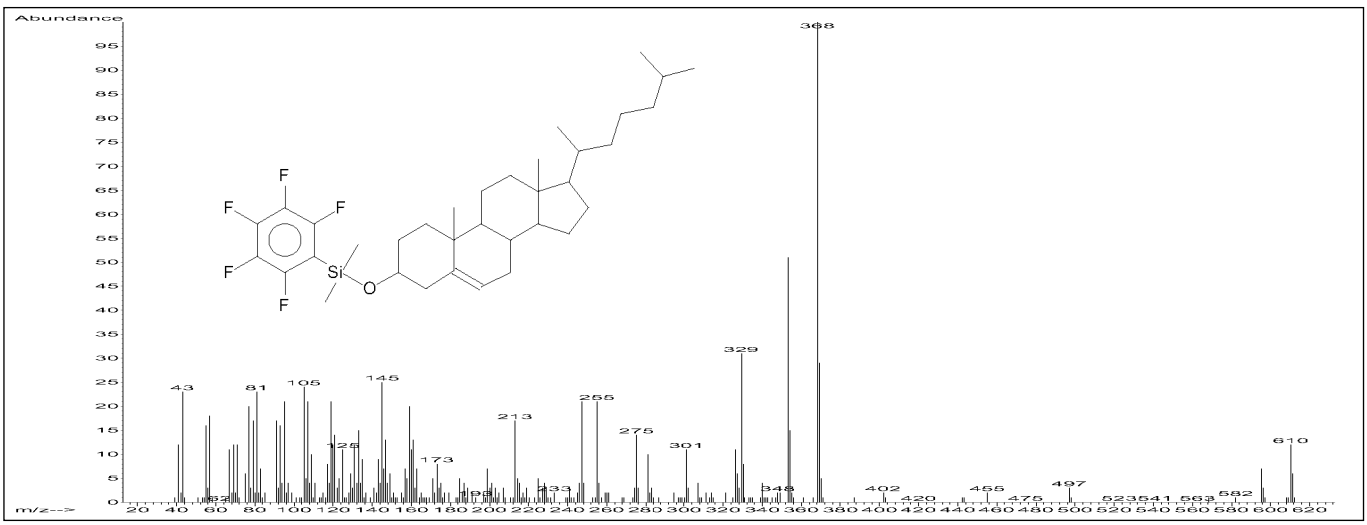
Mode: ECNI/CH_4 – SCAN/SIM
Buffer Gas: Methane
Flow (Setting): 2ml/min (40)
Tune: ECNI-Methane Tune File
Temperatures:
Source 150°C, Quad 106°C
EM Voltage Scan: Tune + 400V
EM Voltage SIM: Tune + 800V
Optimization of Tune Parameters
Emission Current: Tune + 150µA
Flow (Setting): 3ml/min (60)

Remarks

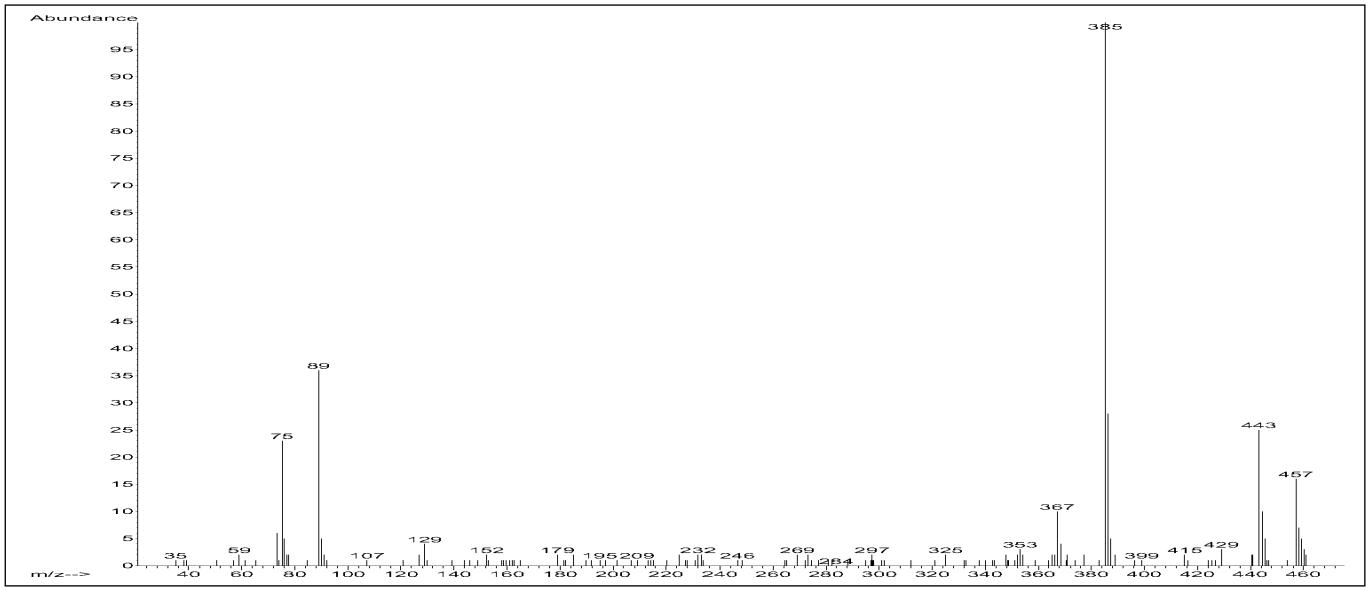
Derivatization
The analytes (Cholesterol/Estradiol/ Estrone/Testosterone SIGMA C 8667/E 8750/ E 9750/ T 1500) can be derivatized with BSTFA and with Pentafluorophenyl/DMCS as well as other approaches.
Derivatization with TMS
To 200µl of the analytes at a concentration of 1ng/µl each in chloroform, 100µl of the derivatization reagent (BSTFA, Fluka 15238) is added and the mixture is incubated for 30min at 60°C. After evaporation under a gentle flow of nitrogen, the residue is redissolved in ethyl acetate; the solution is ready for injection.
Derivatization with PFPh
The analytes, concentration 1-2mg/ml each, are dissolved in 100µl acetonitrile and 100µl pyridine is added. The mixture is shaken (Vortex-Minishaker) for 2min and to the clear solution 50µl of derivatization reagent (Pentafluorophenyl/ DMCS, Fluka 76750) is added. Be unconcerned with the precipitate. The reaction takes 20min at room temperature. After addition of 800µl chloroform the precipitate dissolves and an aliquot is diluted prior to injection.
Caution: PFPh derivatives are unstable even at low temperatures.
Results
The TMS derivatives show weak response in ECNI Scan mode, e.g. for Cholesterol, concentration 18ng/µl, signal/noise ratio ≈ 10/1. Favourable responses are obtained with the PFPh derivatives of Estrone and Estradiole: low concentrations (≈ 200fg/µl) can be detected in SIM mode.



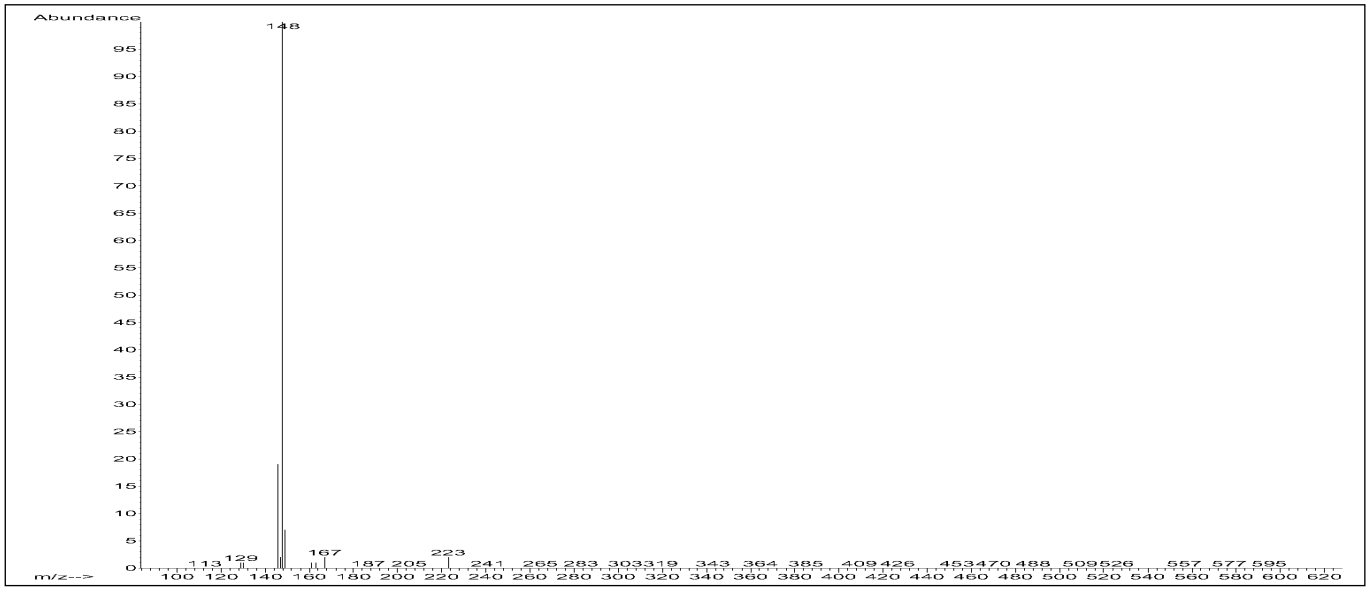
EI-Spectrum, Cholesterol TMS derivative, *m/z* 458; M^+



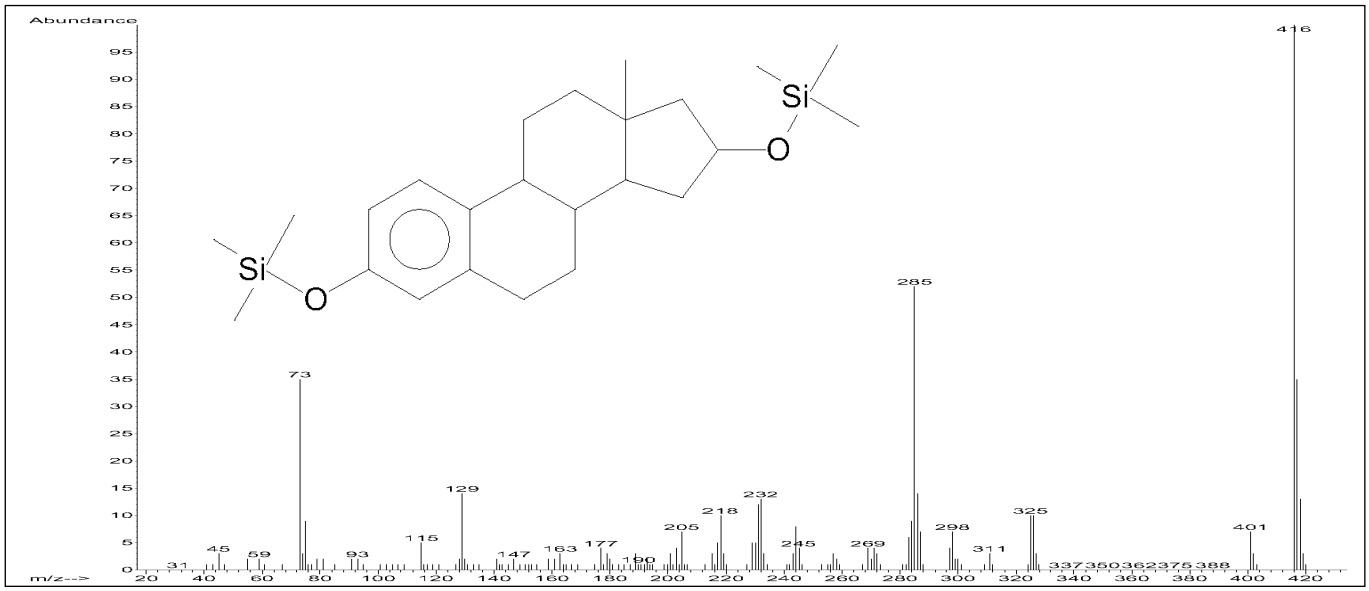
EI-Spectrum, Cholesterol PFPh derivative, *m/z* 610; M^+



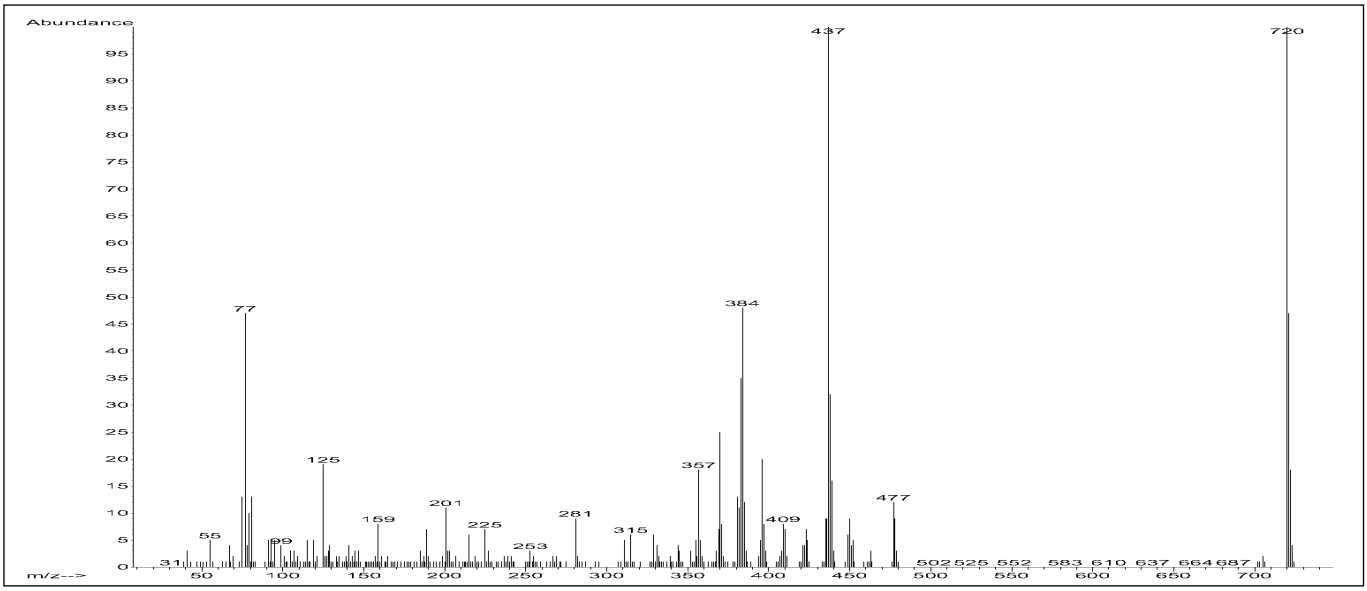
ECNI/CH_4-Spectrum, Cholesterol TMS derivative, *m/z* 458; M^-



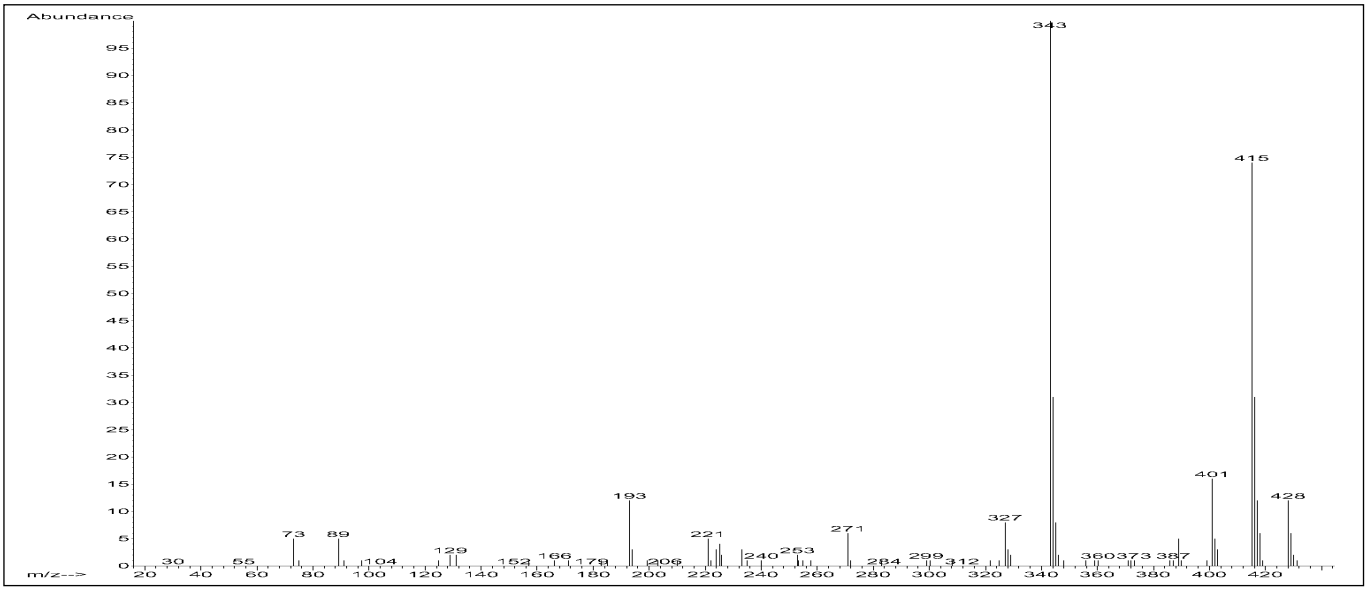
ECNI/CH_4-Spectrum, Cholesterol, PFPh derivative, *m/z* 610; M^-



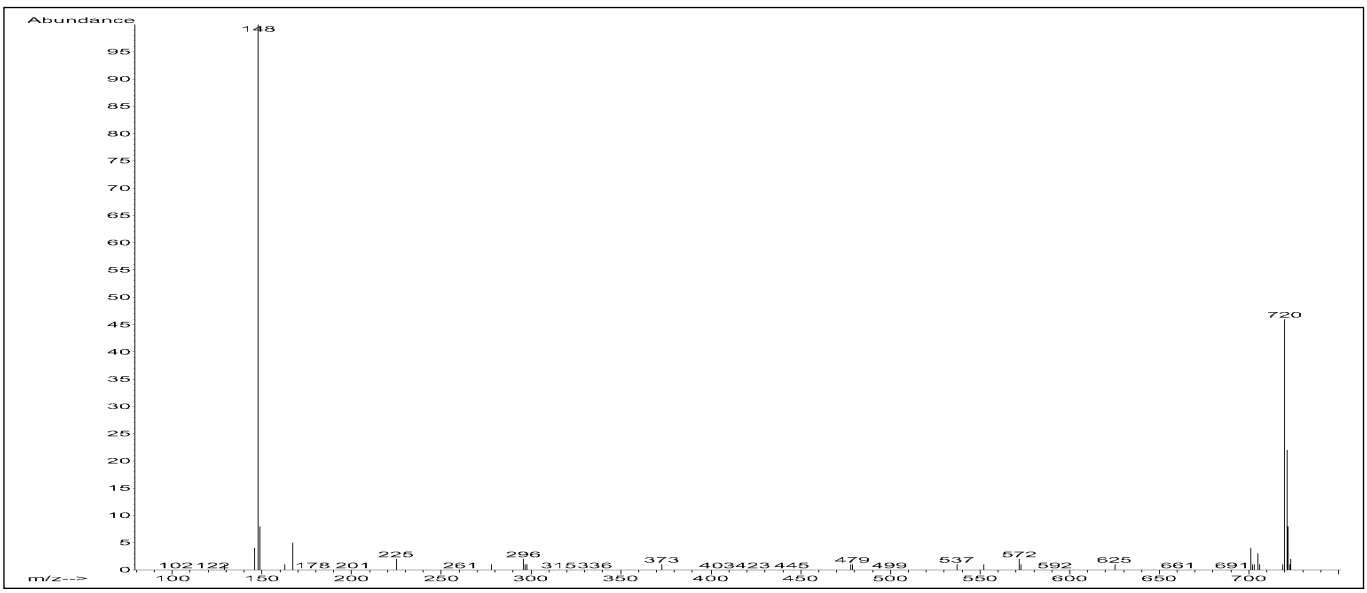
EI-Spectrum, Estradiol TMS derivative, *m/z* 416; M^+



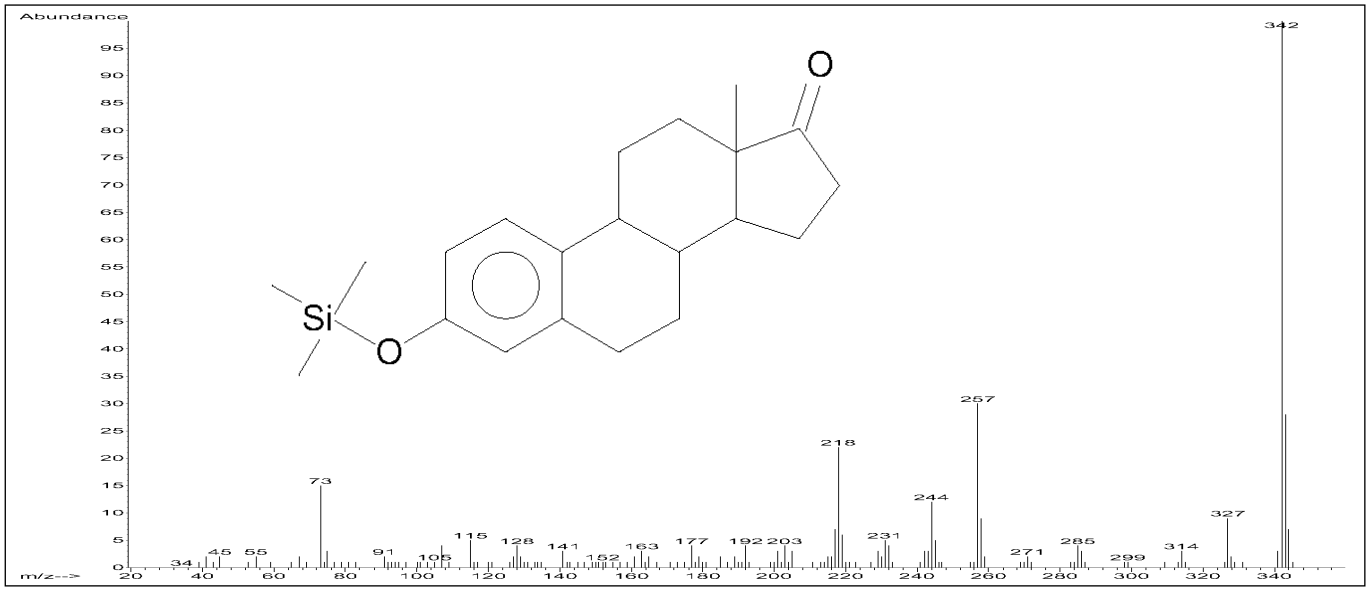
EI-Spectrum, Estradiol PFPh derivative, *m/z* 720; M^+



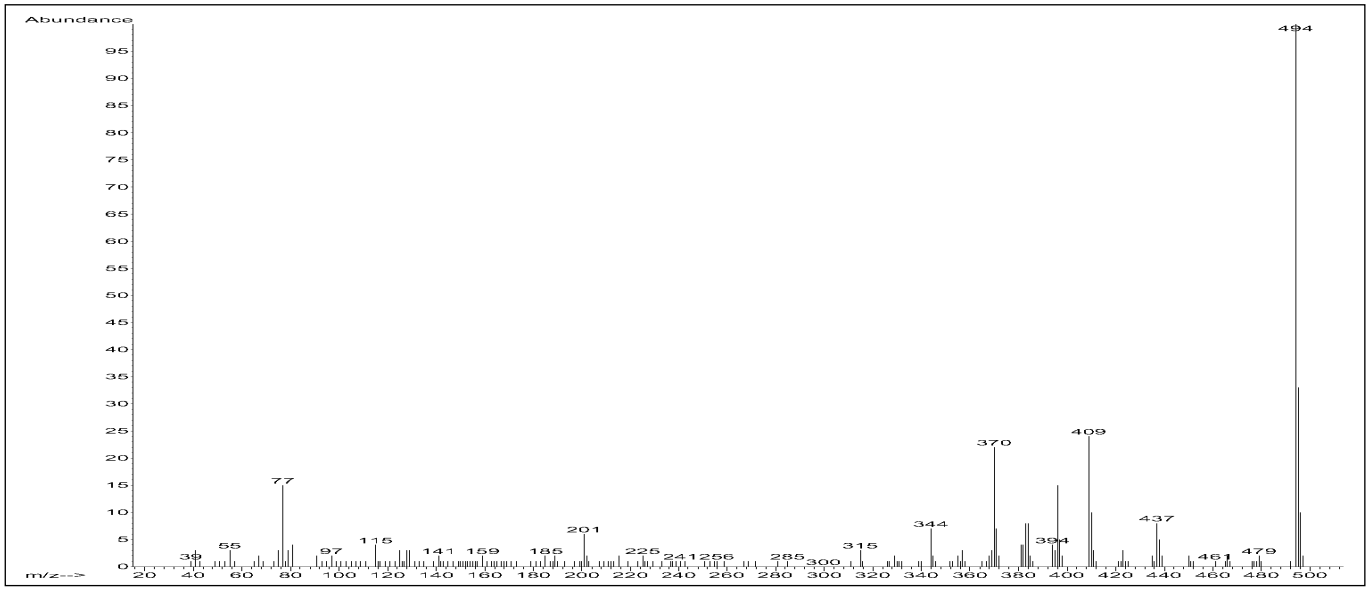
ECNI/CH_4-Spectrum, Estradiol TMS derivative, *m/z* 416; M^-



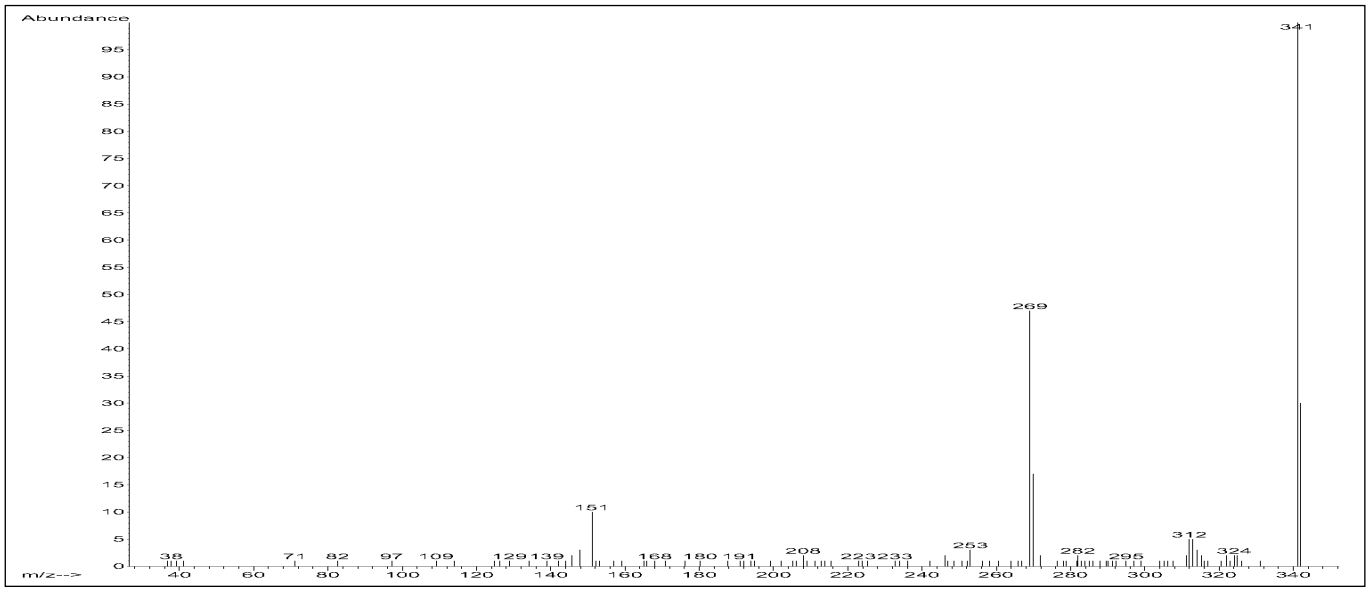
ECNI/CH_4-Spectrum, Estradiol PFPh-derivative, *m/z* 720; M^-



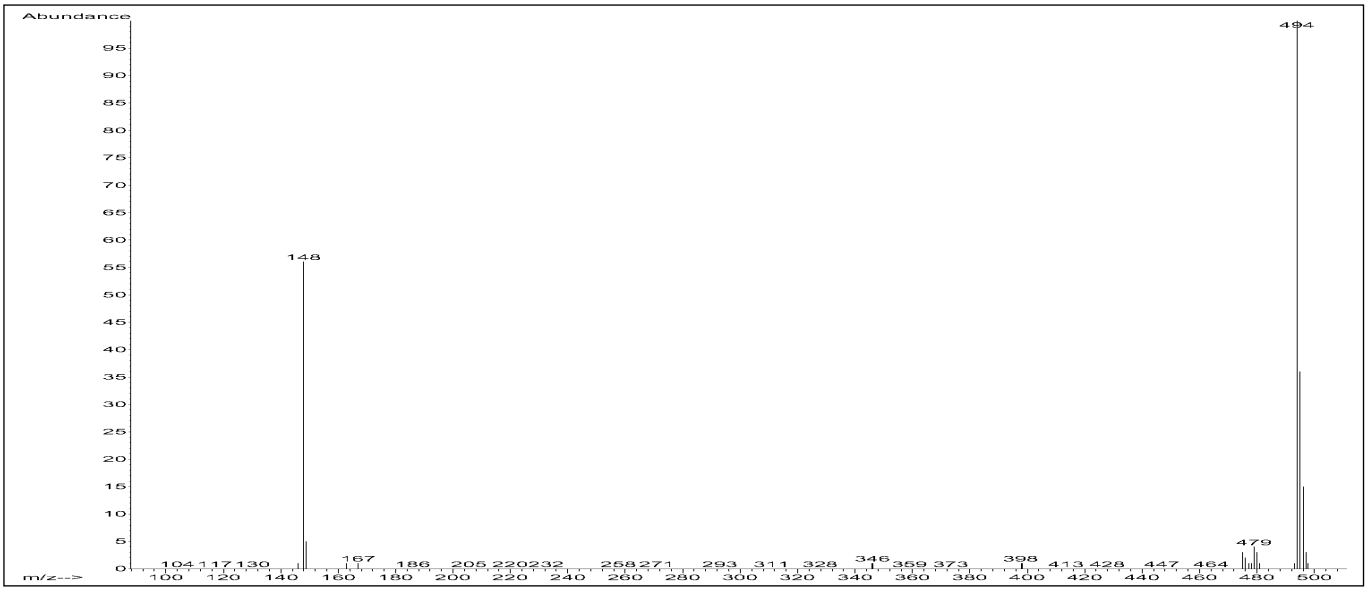
EI-Spectrum, Estrone TMS derivative, *m/z* 342; M^+



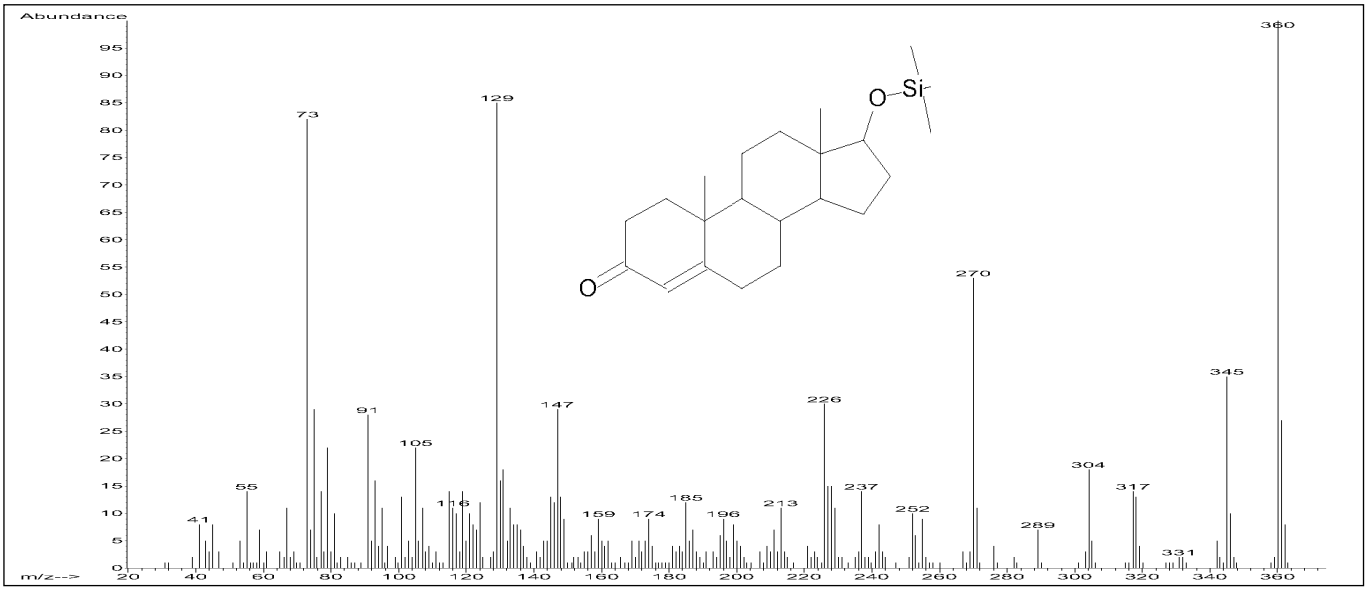
EI-Spectrum, Estrone PFPh-derivative, *m/z* 494; M^+



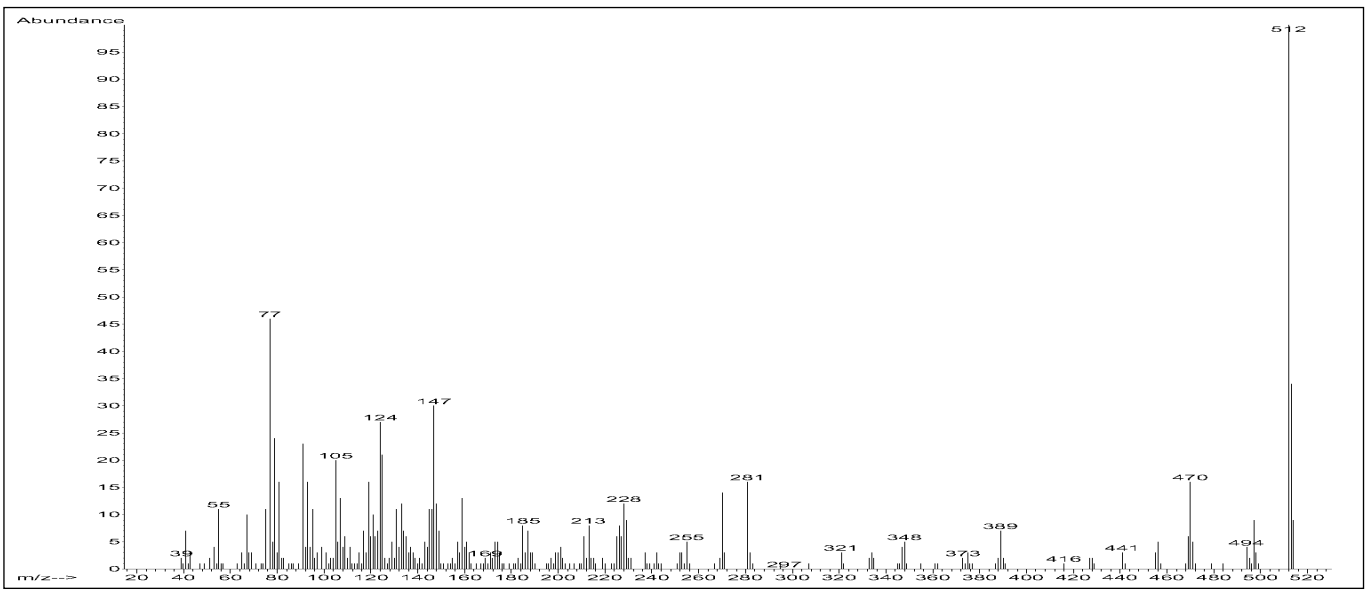
ECNI/CH_4-Spectrum, Estrone TMS-derivative, M^-: 342*m/z*



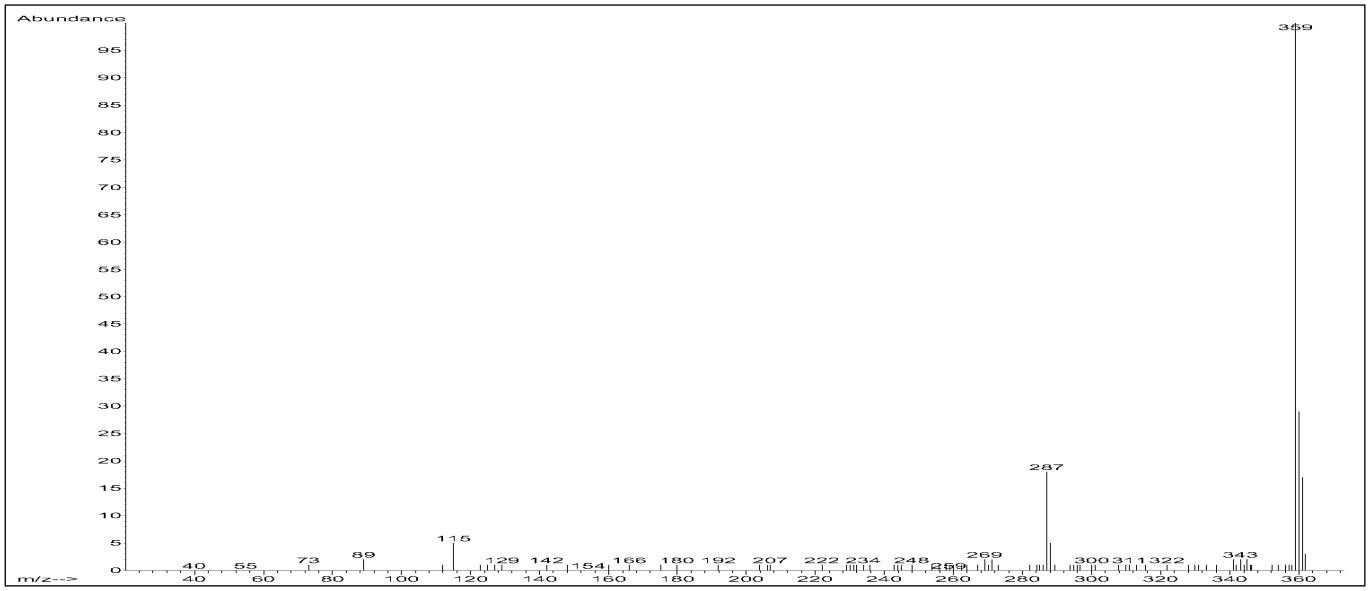
ECNI/CH_4-Spectrum, Estrone PFPh derivative, *m/z* 494; M^-



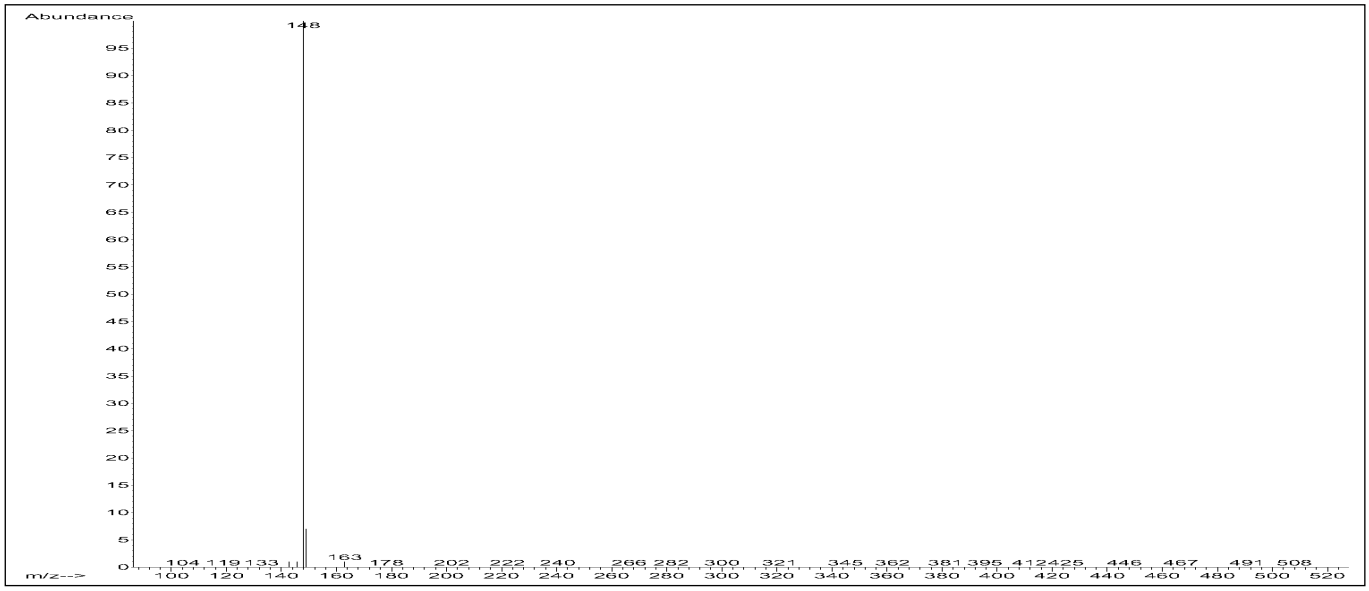
EI-Spectrum, Testosterone, TMS derivative, *m/z* 360; M^+



EI-Spectrum, Testosterone, PFPh derivative, *m/z* 512; M^+

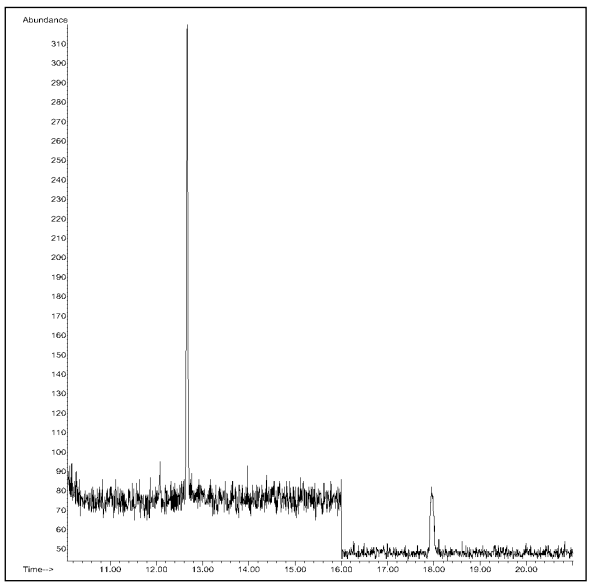


ECNI/CH_4-Spectrum, Testosterone, TMS derivative, *m/z* 360; M^-



ECNI/CH_4-Spectrum, Testosterone, PFPh derivative, molecular mass = 512 u

ECNI/CH_4 – SIM



Estrone (left); Estradiol (right);
SIM Ions: *m/z* 494; *m/z* 720; 0.2pg/µl each

Tetrahydrocannabinol

(d9-THC) CAS-Nr. 1972-08-3
Molecular Formula: $C_{21}H_{30}O_2$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 1.2ml/min, 40cm/sec
Mode: Constant Flow
Injection: Pulsed splitless, 250°C
Oven Temp. Program
70°C (1min) – 25°C/min to 300°C (5min)

MS Parameters

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C

Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V

Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V

Mode: ECNI/CH_4 – SCAN
Buffer Gas: Methane
Flow (Setting): 2ml/min (40)
Tune: ECNI-Methane Tune File
Temperatures:
Source 150°C, Quad 106°C
EM Voltage: Tune + 400V
Emission Current: 150µA

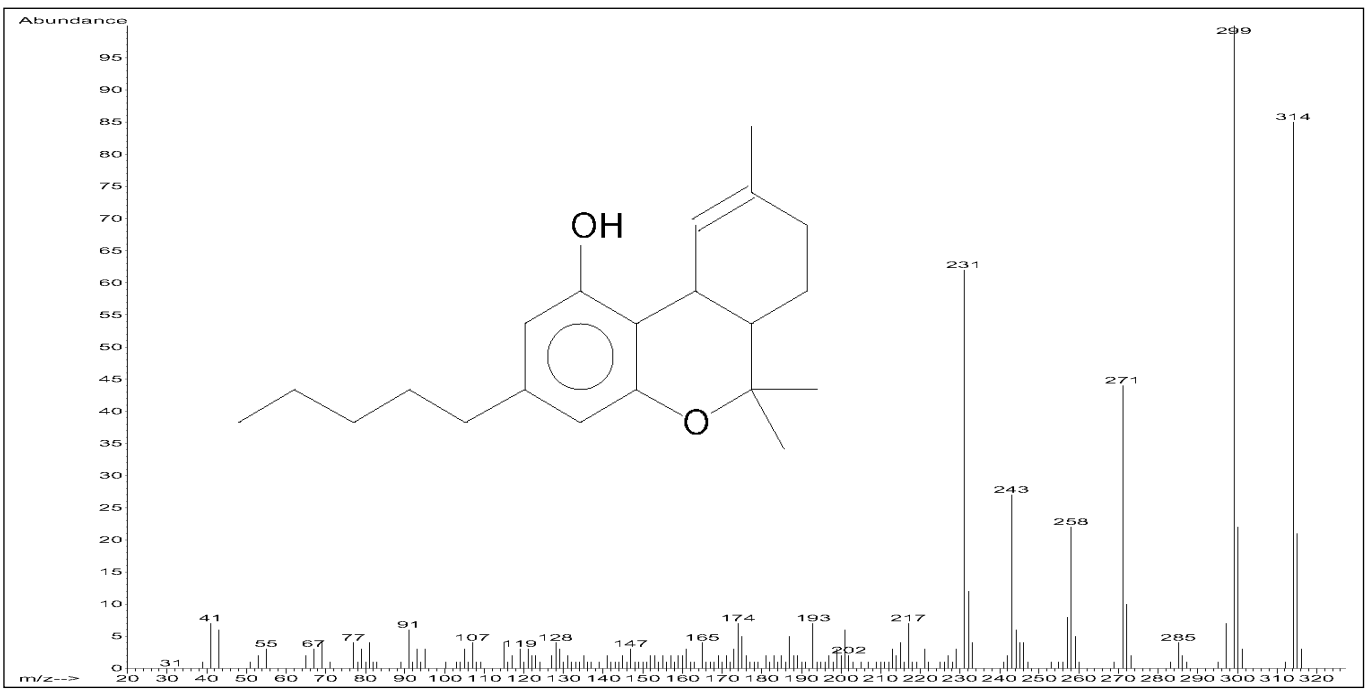
Remarks

Derivatization
a) Trifluoroacetylation (TFA) with MBTFA (Reagent: Fluka 65943)
b) Reaction with Pentafluoropropionic Acid Anhydride (PFPA) (Reagent: Fluka 77292)

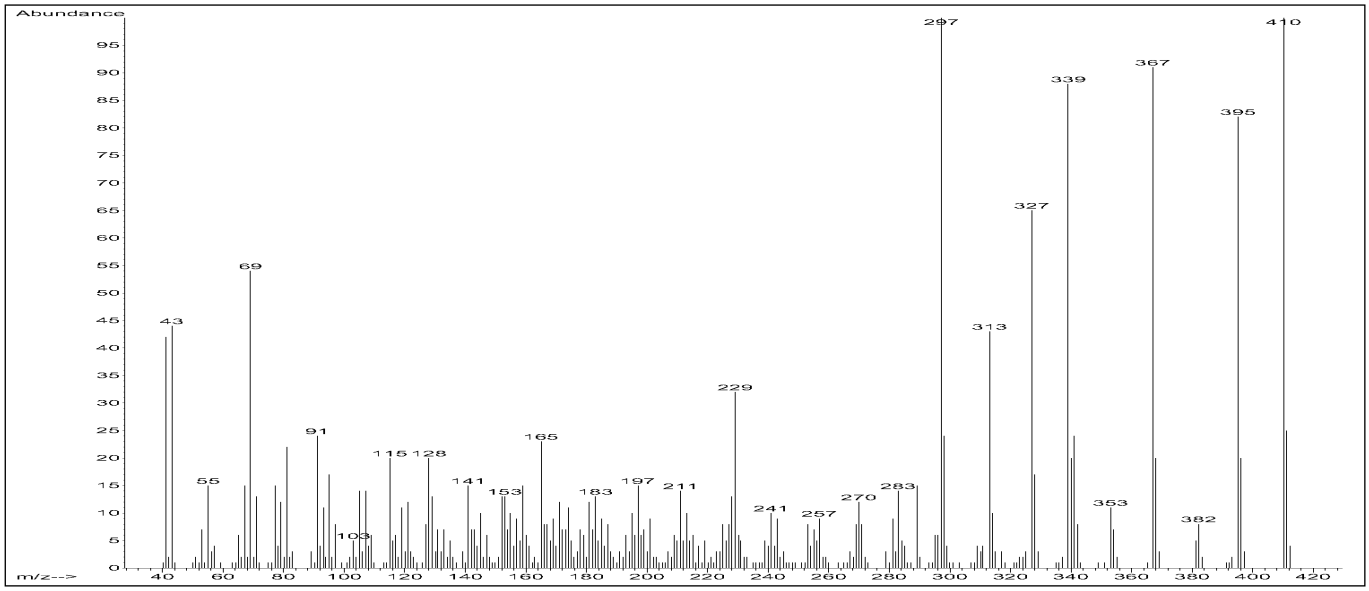
a) 100µl of the standard (SIGMA T 4764) at a concentration of 100ng/µl is dissolved in ethyl acetate and evaporated with a gentle flow of nitrogen. To the residue 50µl derivatization reagent is added and the solution is incubated for 30min at 80°C. Gentle evaporation with nitrogen is repeated and the residue redissolved in ethyl acetate.
b) Procedure as described above. After the first evaporation of the residue, 80µl of the derivatization reagent and 20µl of hexafluoroisopropanol (Fluka 52517) are added and the reaction mixture is incubated for 30min at 70°C. Then evaporation, dilution and GC/MSD analysis.

Results
Even the underivatized analyte at a concentration 10ng/µl can be measured without chromatographic discrimination. The described derivatization reactions lead to THC by-products, not considered here. The trifluoroacetylated analyte can be measured in ECNI mode, however the PFPA derivative shows no significant ECNI spectrum. The cited literature give additional information about THC determinations in PCI and ECNI modes.

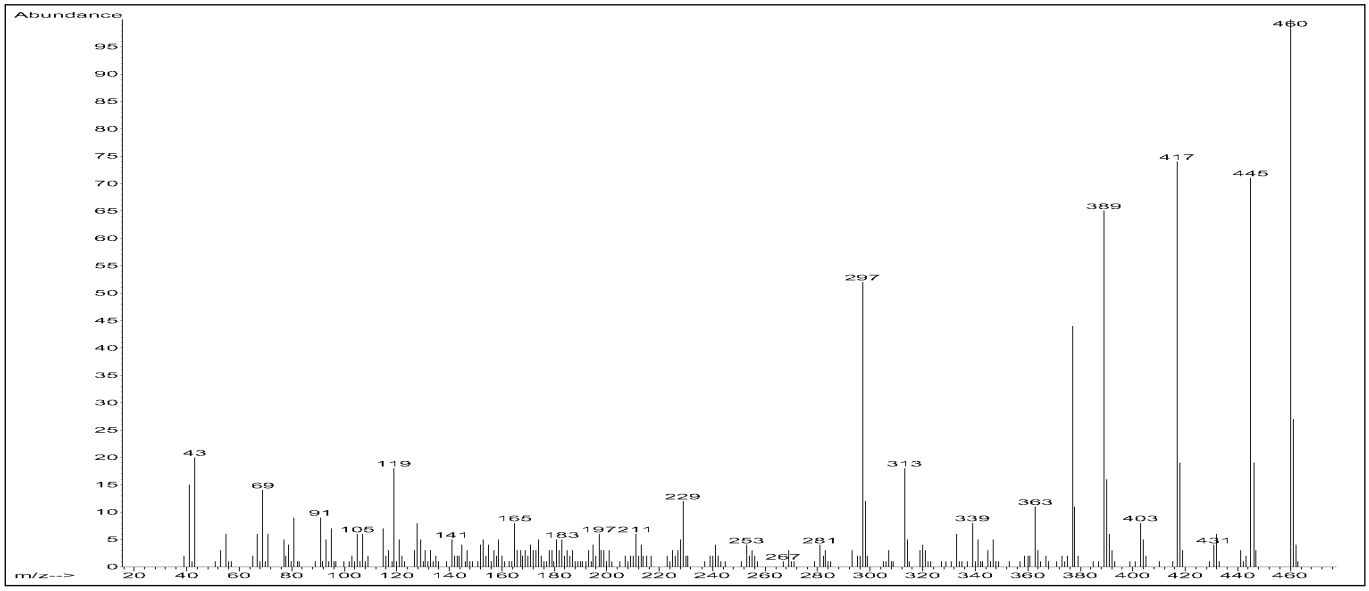
Literature
"Cannabinoids in Blood: Advantage of Positive Chemical Ionization in Mass Spectroscopy"
Harry Prest,
Agilent Pub. Nr. 5967-6103
"Determining Cannabinoids in Blood Using Electron Capture Negative Chemical Ionization …"
Harry Prest,
Agilent Pub. Nr. 5967-6331



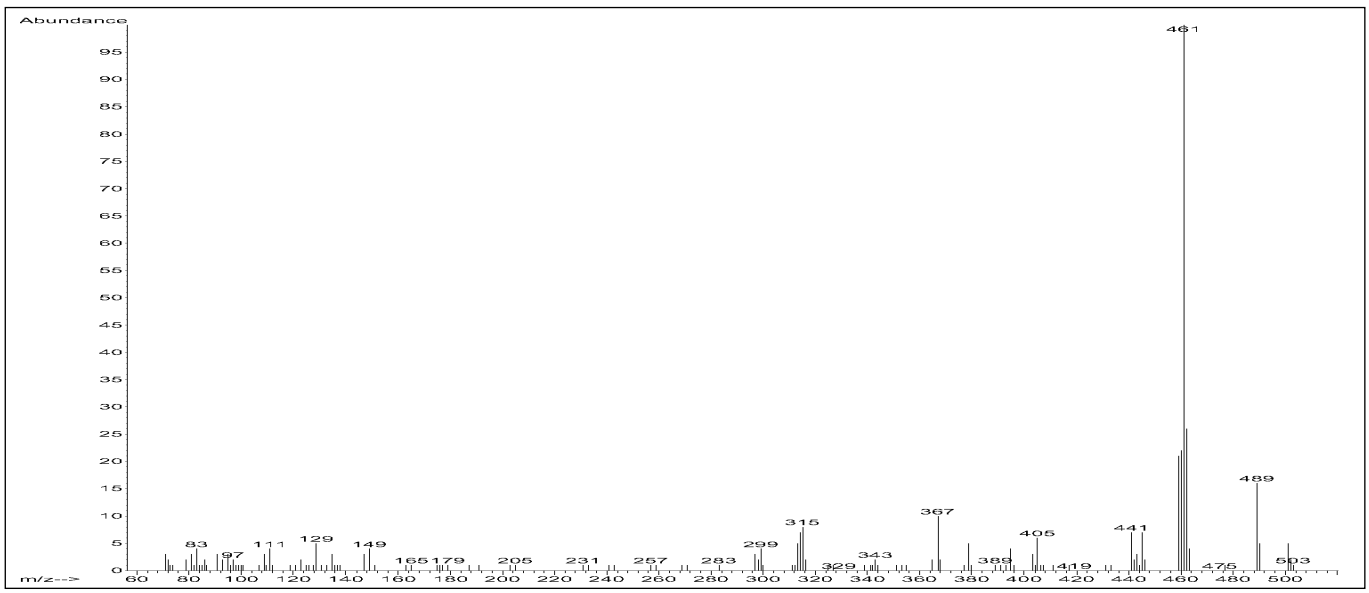
EI-Spectrum, Tetrahydrocannabinol, underivatized, *m/z* 314; M^+



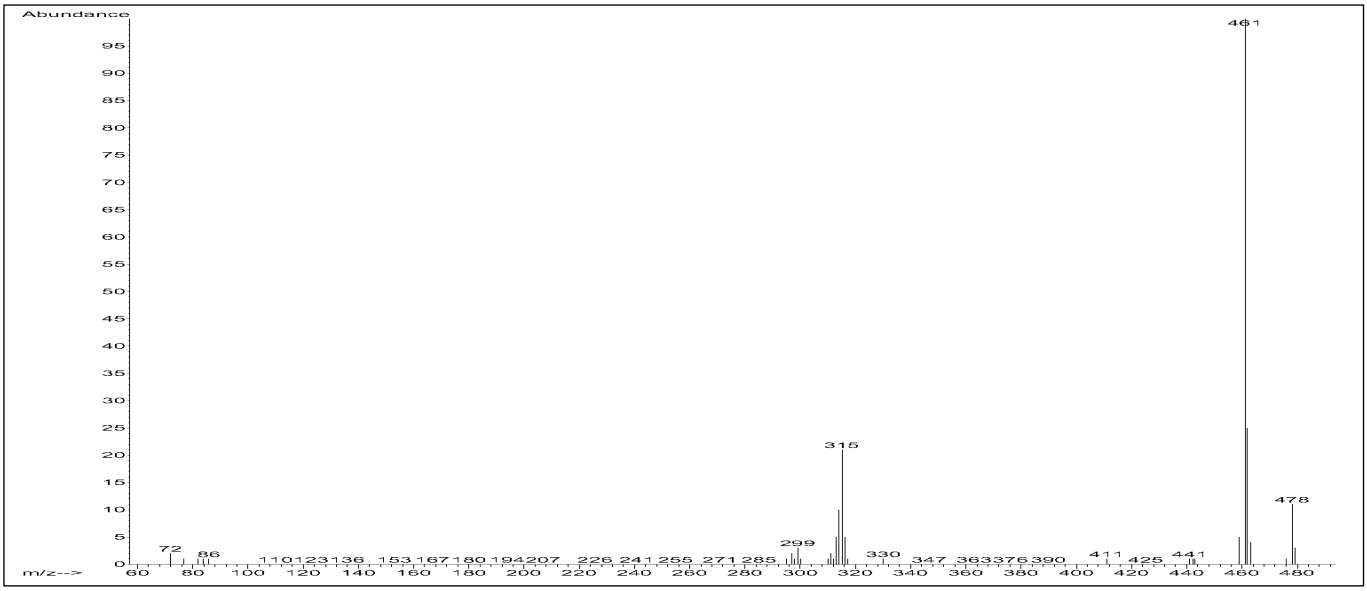
EI-Spectrum, Tetrahydrocannabinol, TFA derivative, *m/z* 410; M^+



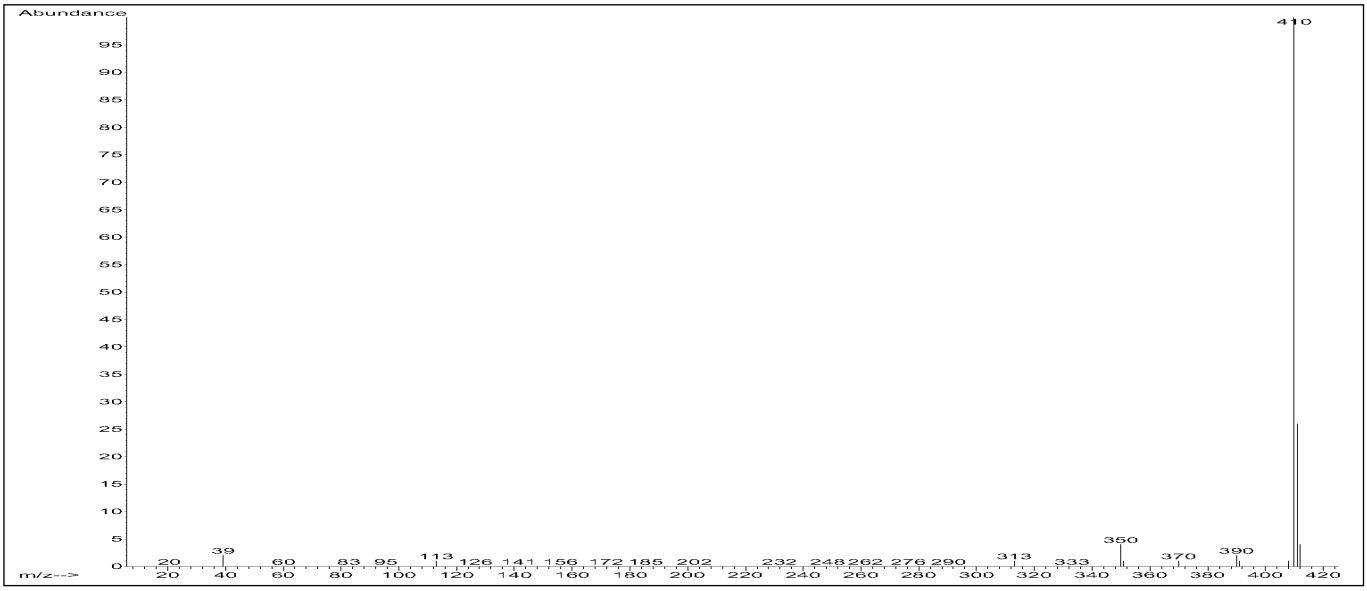
EI-Spectrum, Tetrahydrocannabinol, PFPA derivative, *m/z* 460; M^+



PCI/CH_4-Spectrum, Tetrahydrocannabinol, PFPA derivative, *m/z* 461, 489, 501; $[M + H]^+$, $[M + C_2H_5]^+$, $[M + C_3H_5]^+$



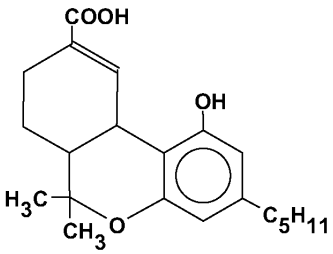
PCI/NH_3-Spectrum, Tetrahydrocannabinol, PFPA derivative, *m/z* 461, 478; $[M + H]^+$, $[M + NH_4]^+$



ECNI/CH_4-Spectrum, Tetrahydrocannabinol, TFA derivative, *m/z* 410; M^-

THC COOH

11-Nor-d9-Tetrahydrocannabinol
-9-Carboxylic Acid
CAS-Nr. 56354-06-4
Molecular Formula: $C_{21}H_{28}O_4$



GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 1.2ml/min, 40cm/sec
Mode: Constant Flow
Injection: Pulsed splitless, 250°C
Oven Temp. Program
80°C (1min) – 30°C/min to 300°C (3min)

MS Parameters

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C

Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V

Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V

Mode: ECNI/CH_4/NH_3 – SCAN/SIM
Buffer Gas: Methane
Flow (Setting): 2ml/min (40)
Buffer Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: ECNI-Methane Tune File
Temperatures:
Source 150°C, Quad 106°C
EM Voltage: Tune + 400V
Emission Current: 150µA

Remarks

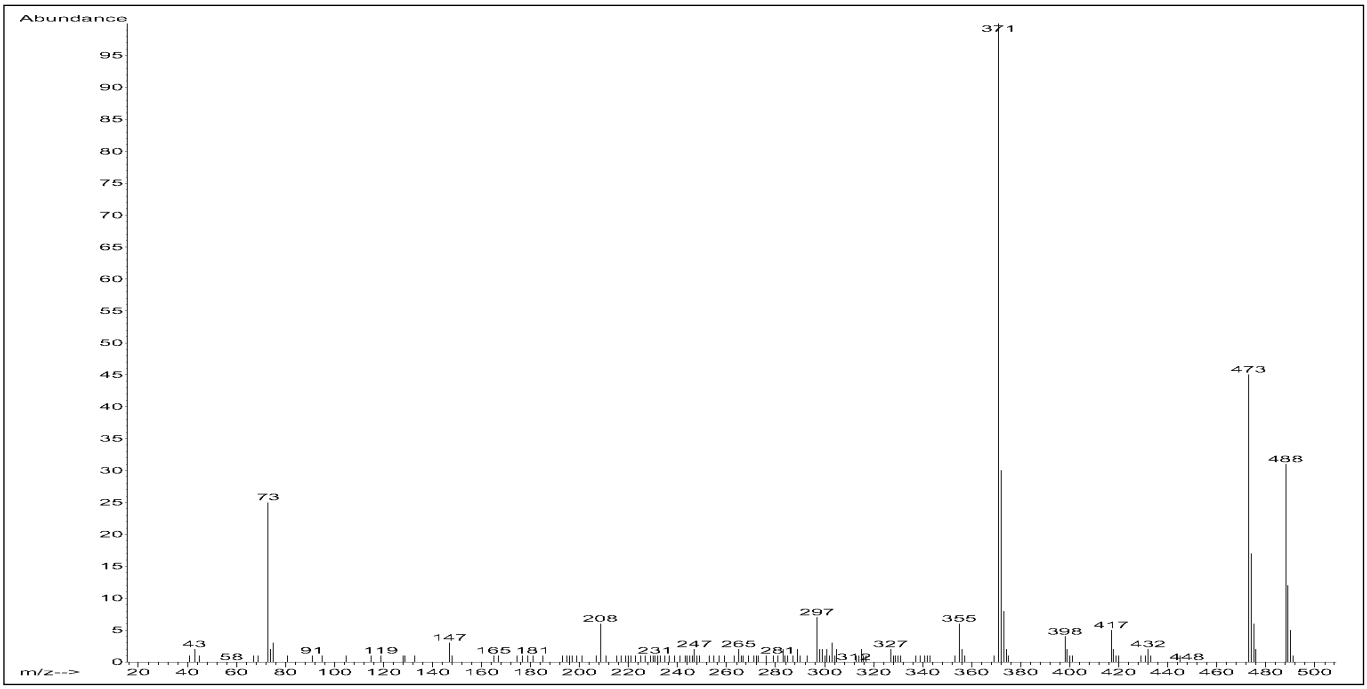
Derivatization
a) Silylation (TMS) with BSTFA/ TMCS (Reagent: Fluka 15238)
b) Reaction with Pentafluoropropionic Acid Anhydride (PFPA) (Reagent: Fluka 77292)

a) 100µl of the standard (SIGMA N 3142) at a concentration of 100ng/µl is dissolved in ethyl acetate and evaporated with a gentle flow of nitrogen. To the residue, 50µl of derivatization reagent is added and the solution is incubated for 30min at 80°C. Gentle evaporation with nitrogen is repeated and the residue redissolved in ethyl acetate.
b) Procedure as described above. After the first evaporation, 80µl of the derivatization reagent and 20µl of hexafluoroisopropanol (Fluka 52517) are added to the residue and the reaction mixture is incubated for 30min at 70°C. Then evaporation, dilution and GC/MSD analysis.

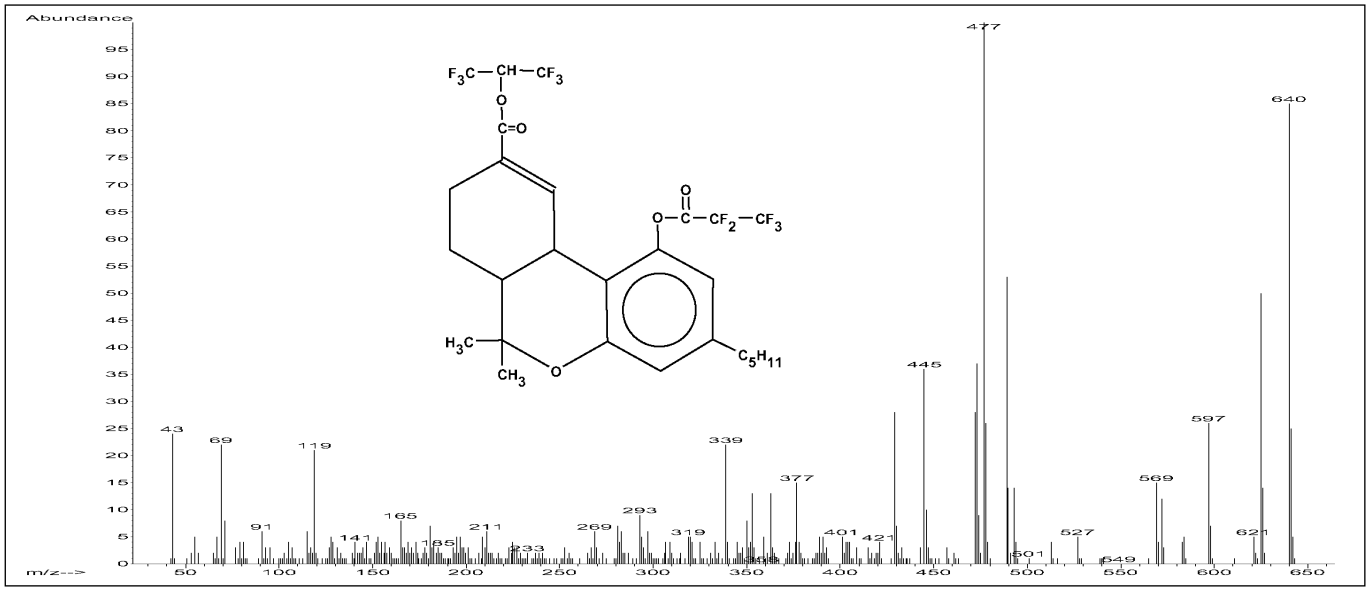
Results
Derivatization is recommended for this polar analyte. The analyte response is related to the choice of reagent/buffer gases applied. The PFPA derivative is more suitable for ECNI measurements and the highest sensitivity is achieved in ECNI/NH_3 SIM mode.

Literature
"Zum Nachweis von 11-nor-d9-Tetrahydrocannabinolcarbonsäure in menschlichen Haaren mittels GC/MS"
U. Dressler, Dissertation 2000, Med. Fakultät der Universität München

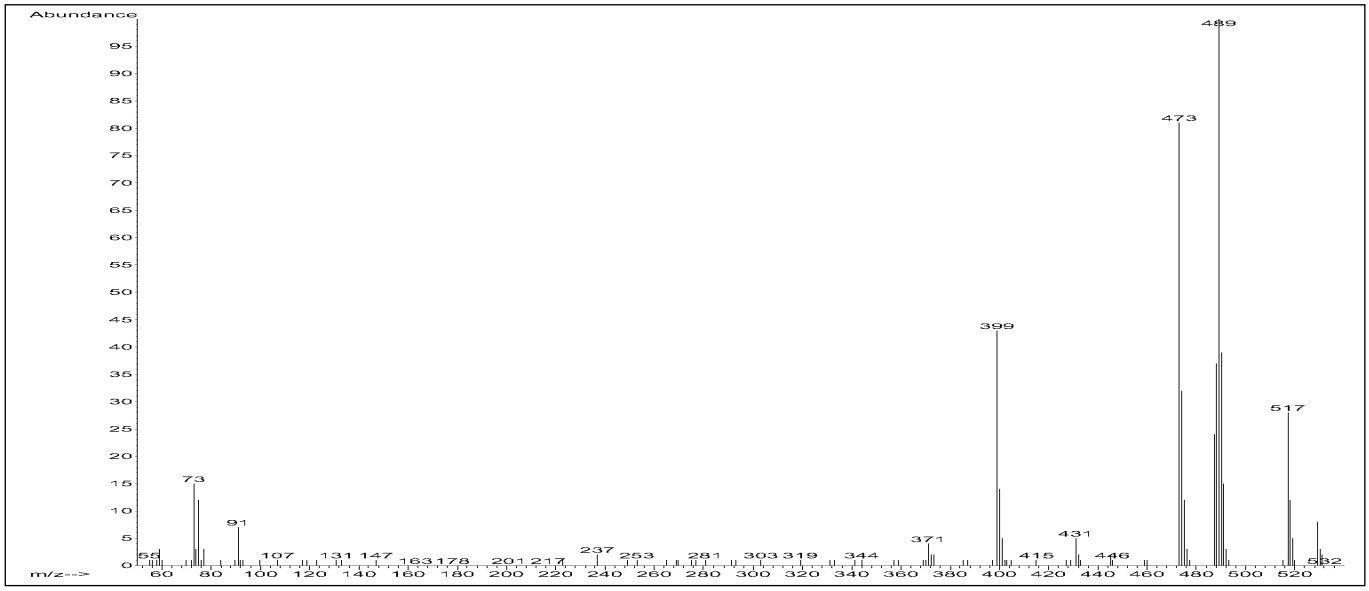
"Determining Cannabinoids in Blood Using ECNCI with HP 5973 CI MSD"
Harry Prest,
Agilent Publication Nr. 5967-6331



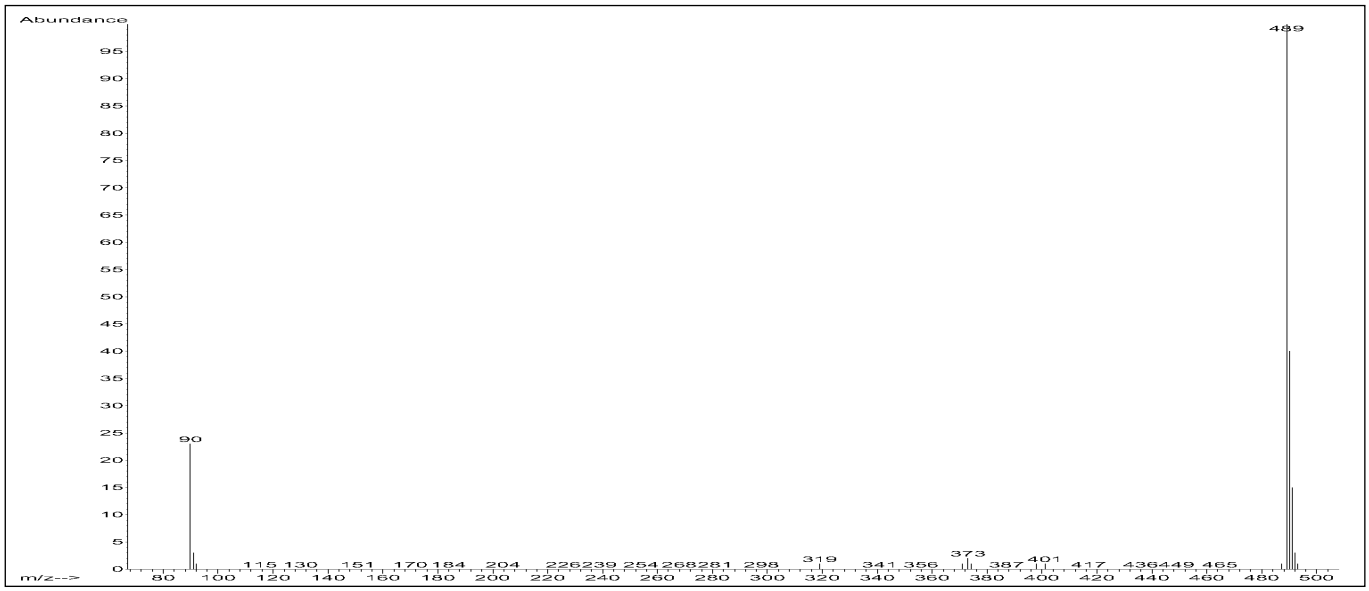
EI-Spectrum, Tetrahydrocannabinol Carboxcylic Acid, TMS derivative, *m/z* 488; M^+



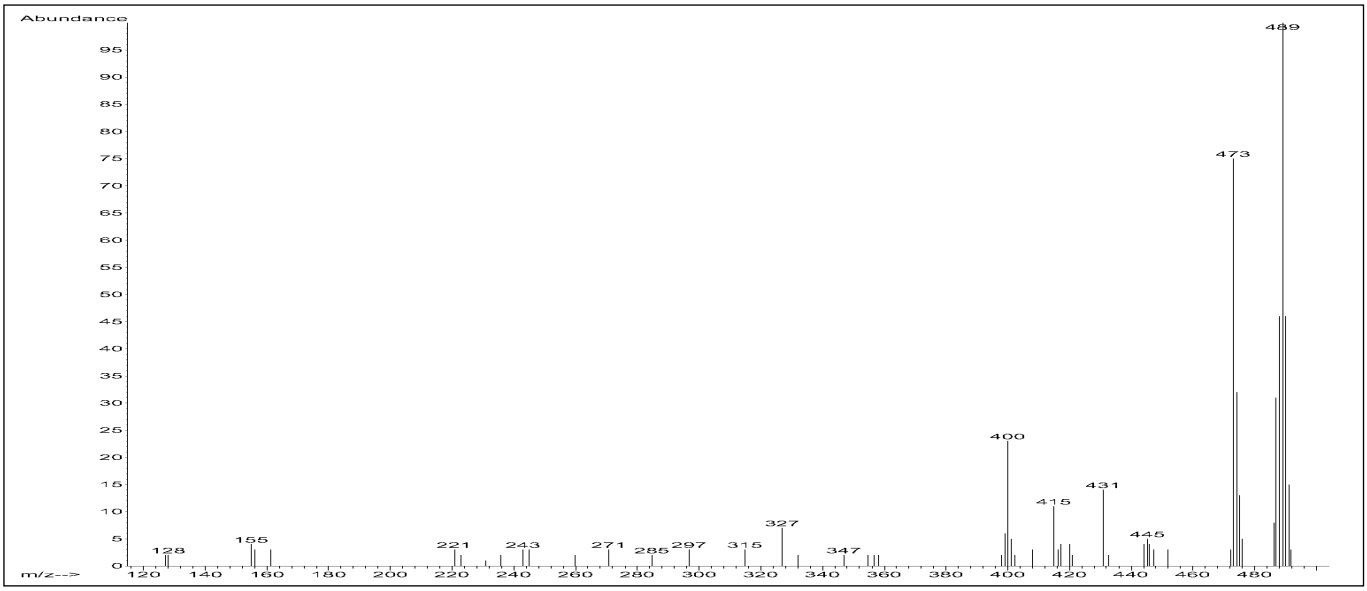
EI-Spectrum, Tetrahydrocannabinol Carboxcylic Acid, PFPA derivative, *m/z* 640; M^+



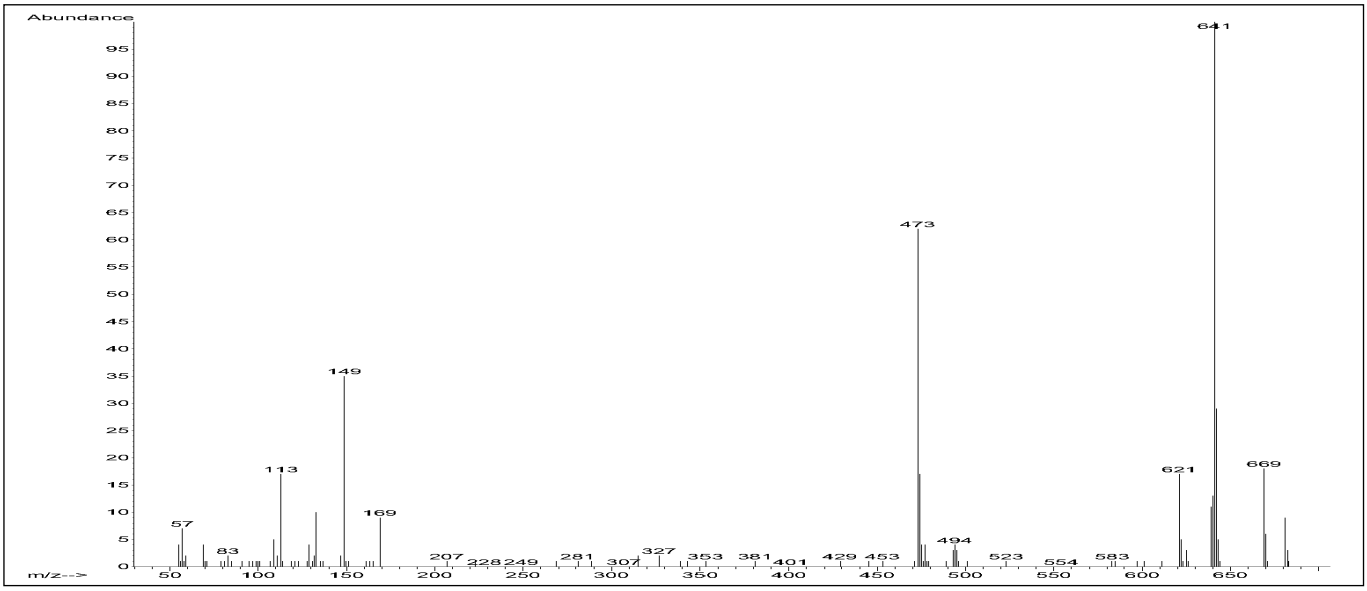
PCI/CH_4-Spectrum, Tetrahydrocannabinol Carboxcylic Acid, TMS derivative, *m/z* 489, 517, 529; $[M + H]^+$, $[M + C_2H_5]^+$, $[M + C_3H_5]^+$



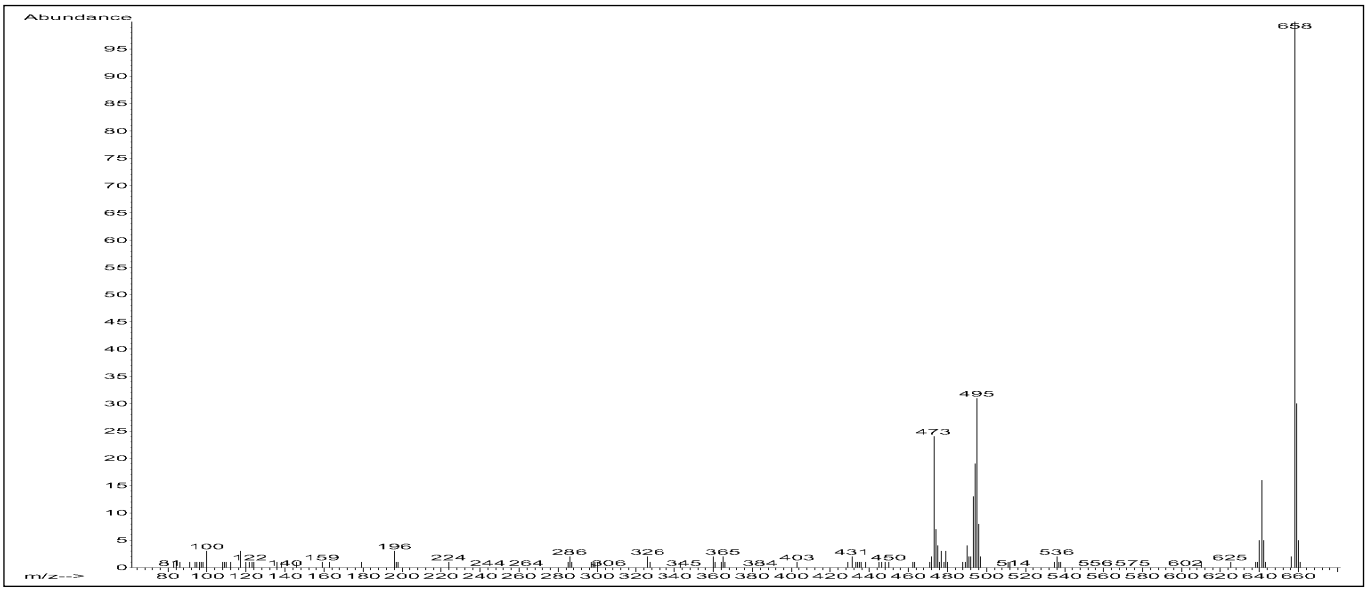
PCI/NH_3-Spectrum, Tetrahydrocannabinol Carboxcylic Acid, TMS derivative, *m/z* 489; $[M + H]^+$



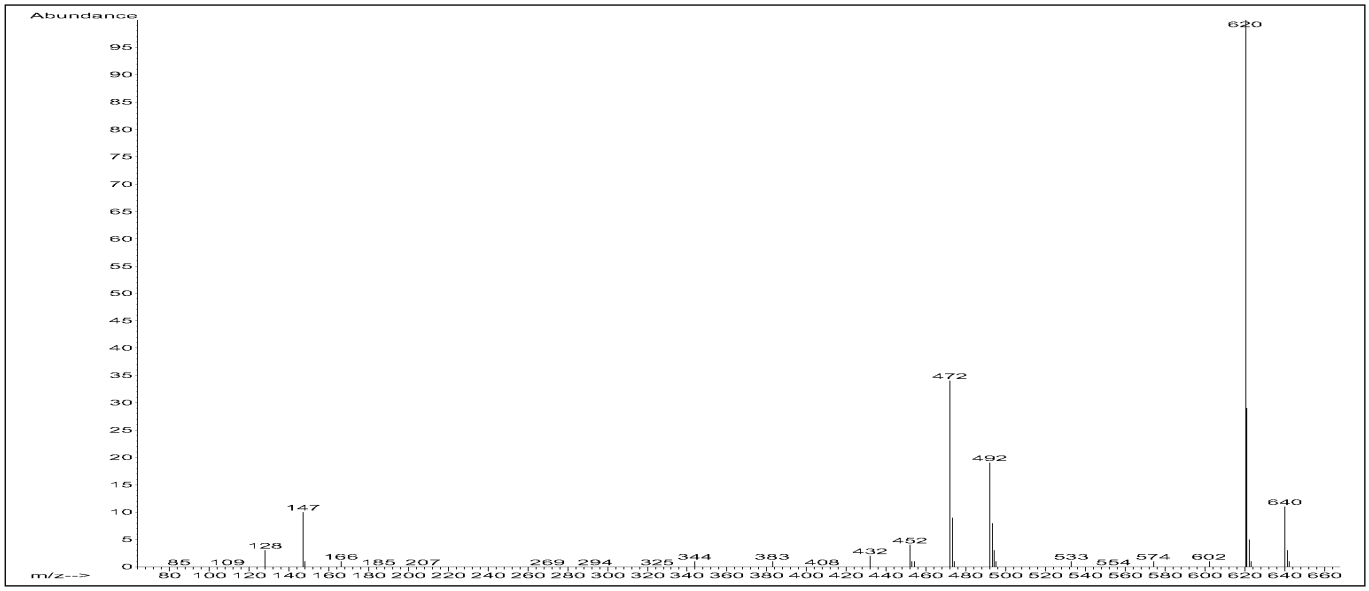
ECNI/CH_4-Spectrum, Tetrahydrocannabinol Carboxcylic Acid, TMS derivative, *m/z* 489; $[M+H]^-$



PCI/CH_4-Spectrum, Tetrahydrocannabinol Carboxcylic Acid, PFPA derivative, *m/z* 641, 669, 681; $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$



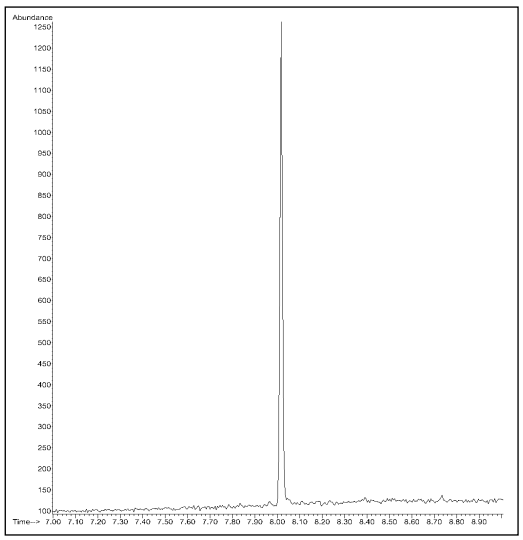
PCI/NH_3 -Spectrum, Tetrahydrocannabinol Carboxcylic Acid, PFPA derivative, *m/z* 641, 658; $[M+H]^+$, $[M+NH_4]^+$



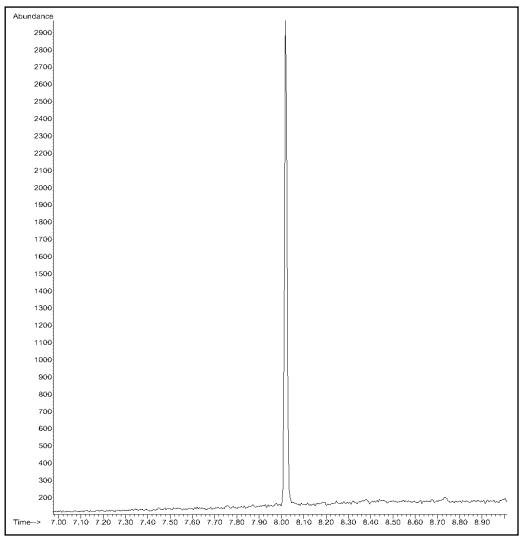
ECNI/CH_4-Spectrum, Tetrahydrocannabinol Carboxcylic Acid, PFPA derivative, *m/z* 620, 640; $[M-HF]^-$, $[M]^-$

SIM Mode, PFPA derivative

0.5pg/µl each, Ions: *m/z* 620, 640



ECNI/CH_4, Tetrahydrocannabinol Carboxcylic Acid



ECNI/NH_3, Tetrahydrocannabinol Carboxcylic Acid

Ion Mode	Derivative	Concent.	Acquis. Mode	Approximate Signal/Noise Ratio
EI	TMS	10ng/µl	Scan	650/1
EI	PFPA	10ng/µl	Scan	620/1
PCI/CH_4	TMS	10ng/µl	Scan	40/1
PCI/NH_3	TMS	10ng/µl	Scan	80/1
NCI/CH_4	TMS	10ng/µl	Scan	10/1
PCI/CH_4	PFPA	10ng/µl	Scan	140/1
PCI/NH_3	PFPA	10ng/µl	Scan	75/1
NCI/CH_4	PFPA	10pg/µl	Scan	15/1
NCI/CH_4	PFPA	0.5pg/µl	SIM	40/1
NCI/NH_3	PFPA	0.5pg/µl	SIM	60/1

Table: Tetrahydrocannabinol Carboxcylic Acid, Responses

trans Zearalenone

CAS-Nr. 17924-92-4
Molecular Formula: $C_{18}H_{22}O_5$

GC Parameters

Column: HP-5ms
Agilent Part Nr. 19091S-433
30m x 0.25mm x 0.25µm
Carrier Gas: Helium
Flow: 1.2ml/min, 40cm/sec
Mode: Constant Flow
Injection: Pulsed splitless, 250°C
Oven Temp. Program
70°C (1min) – 25°C/min to 300°C (4min)

MS Parameters

Mode: EI – SCAN
Tune: Atune
Temperatures:
Source 230°C, Quad 150°C

Mode: PCI/CH_4 – SCAN
Reagent Gas: Methane
Flow (Setting): 1ml/min (20)
Tune: PCI-Methane Autotune
Temperatures:
Source 250°C, Quad 106°C
EM Voltage: Tune + 400V

Mode: PCI/NH_3 – SCAN
Reagent Gas: Ammonia
Flow (Setting): 1ml/min (20)
Tune: PCI-Ammonia Tune File
EM Voltage: Tune + 400V

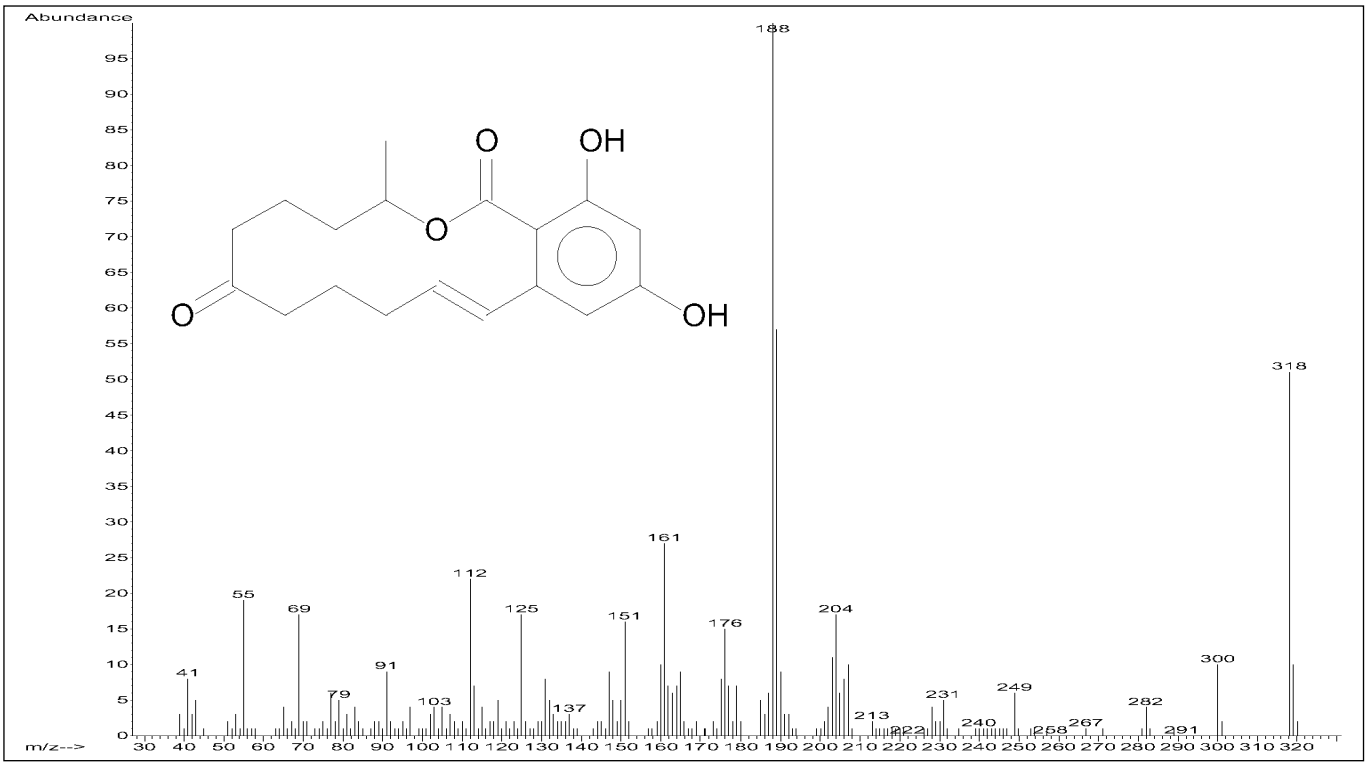
Mode: ECNI/CH_4– SCAN/SIM
Buffer Gas: Methane
Flow (Setting): 2ml/min (40)
Tune: ECNI-Methane Tune File
Temperatures:
Source 150°C, Quad 106°C
EM Voltage: Tune + 400V

Remarks

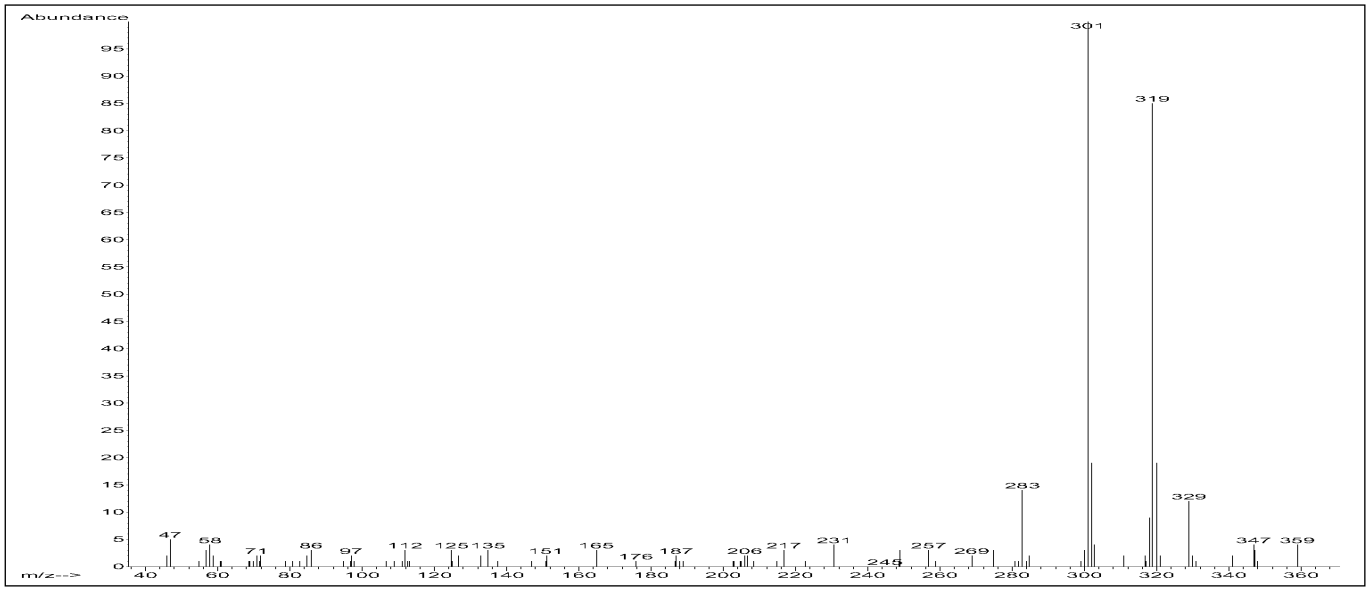
Derivatization
a) Silylation (TMS) with BSTFA/TMCS (Reagent: Fluka 15238)
b) Reaction with Pentafluoropropionic Acid Anhydride (PFPA) (Reagent: Fluka 77292)

a) 50µl of the standard (SIGMA Z 2125) at a concentration of 10mg/ml in methanol is evaporated with a gentle flow of nitrogen. To the residue, 50µl of derivatization reagent is added and the solution is incubated for 30min at 60°C. Gentle evaporation with nitrogen is repeated and the residue dissolved in ethyl acetate.
b) Procedure as described above. After the first evaporation to the residue, 40µl of the derivatization reagent and 20µl of hexafluoroisopropanol (Fluka 52517) are added and the reaction mixture is incubated for 30min at 70°C. Then evaporation, dilution and GC/MSD analysis.

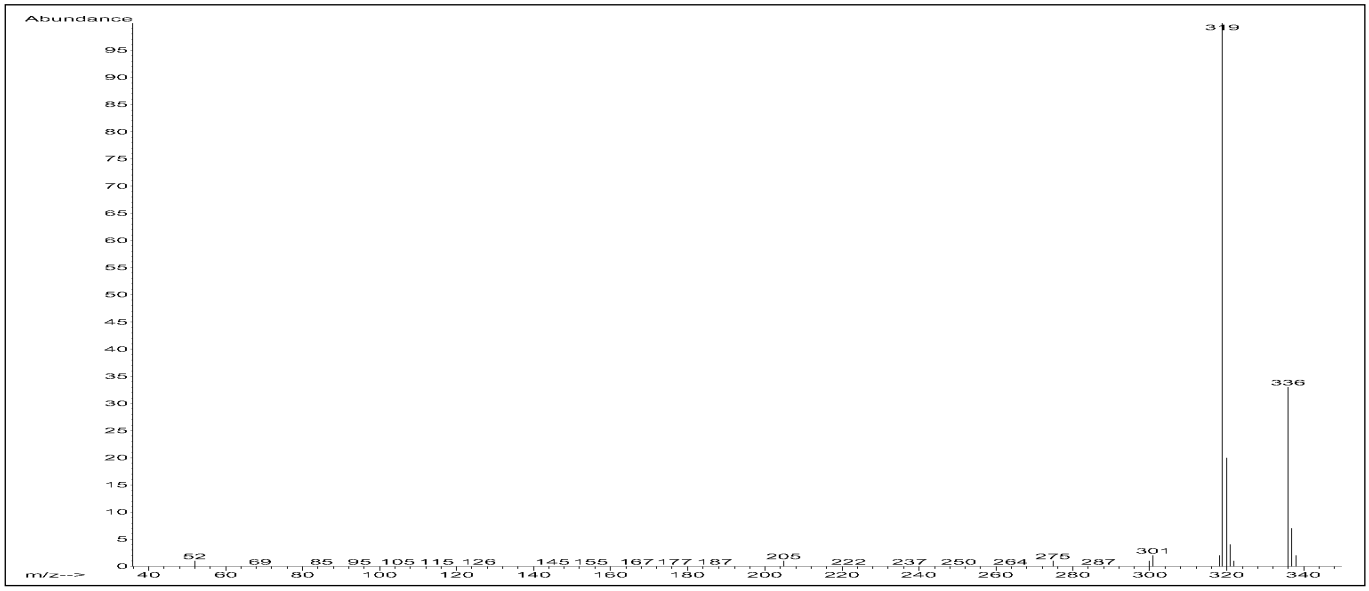
Results
Underivatized analyte:
In EI scan mode measurements in the concentrations range of 1ng to 10ng are achievable. In PCI mode, ammonia is the preferred reagent gas.
TMS derivative:
The analyte reacts to make the mono and di-derivative with a response ratio of ≈ 0.4 : 1. PCI/NH_3 responses are a factor of three more sensitive compared to the PCI/CH_4.
PFPA derivative:
The di-derivative is preferentially produced (90%) and elutes prior to the mono-derivative. PCI/CH_4 is the preferred mode compared to ammonia reagent gas.
In ECNI/CH_4 mode the mono-derivative dominates by a factor of more than 10 to one. In SIM a concentration of 0.5pg/µl produces approximately 30:1 signal:noise.
In addition to the mentioned derivatization reactions, pentafluorophenyl reaction was tested and resulted in less sensitive response when compared to the PFPA data.



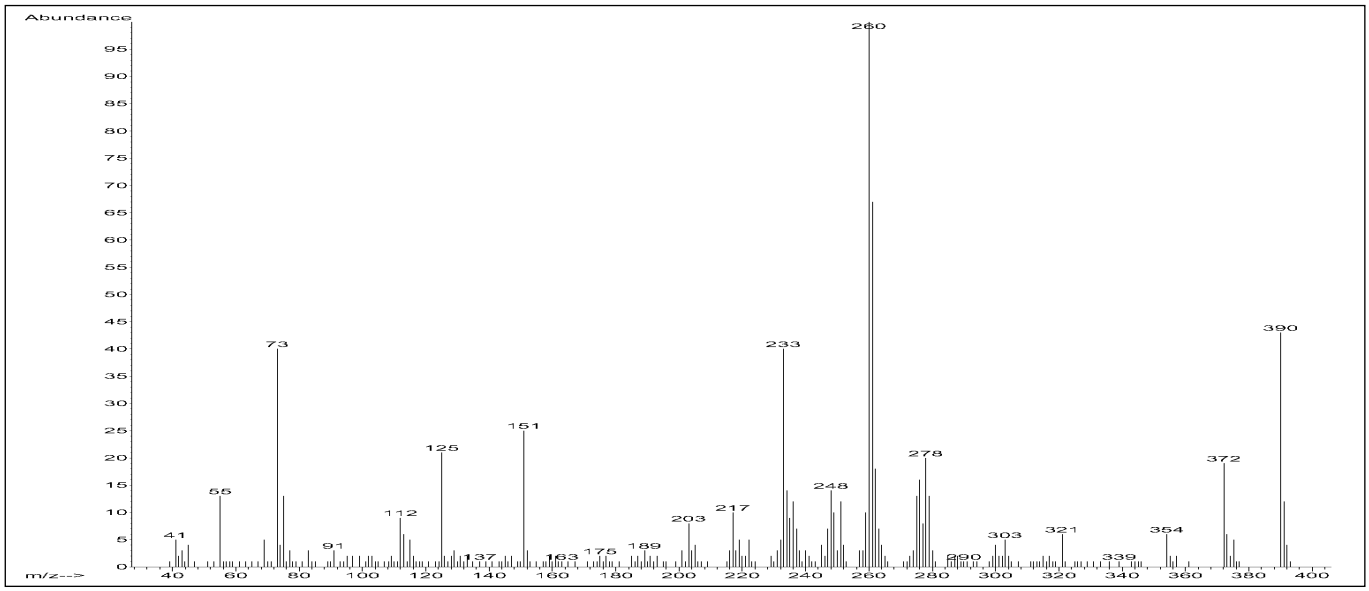
EI-Spectrum, Zearalenone, underivatized, *m/z* 318; M^+



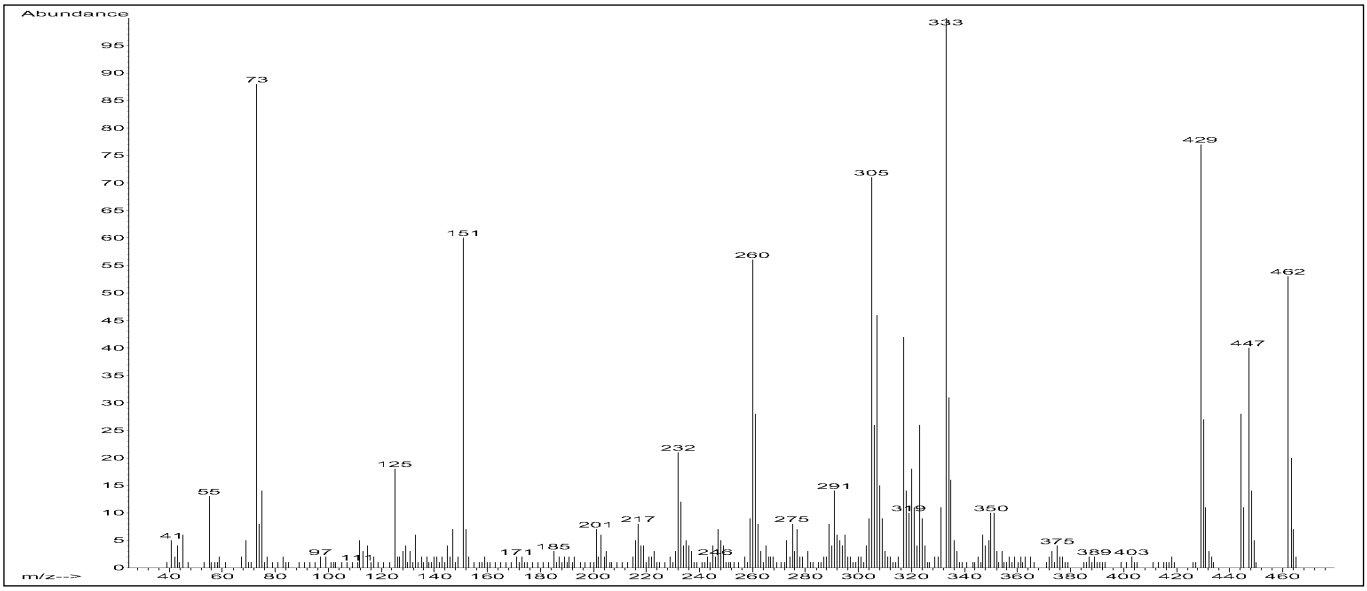
PCI/CH_4-Spectrum, Zearalenone, underivatized, *m/z* 319, 347, 359; $[M + H]^+$, $[M + C_2H_5]^+$, $[M + C_3H_5]^+$



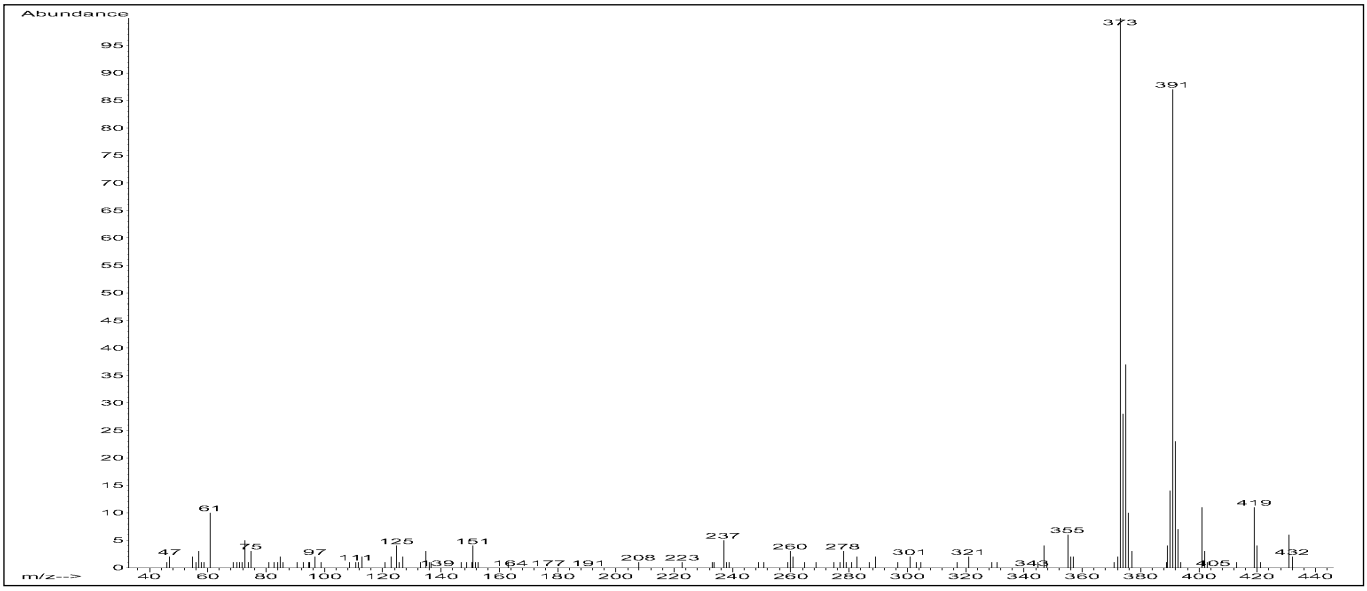
PCI/NH_3-Spectrum, Zearalenone, underivatized, *m/z* 319, 336; $[M + H]^+$, $[M+NH_4]^+$



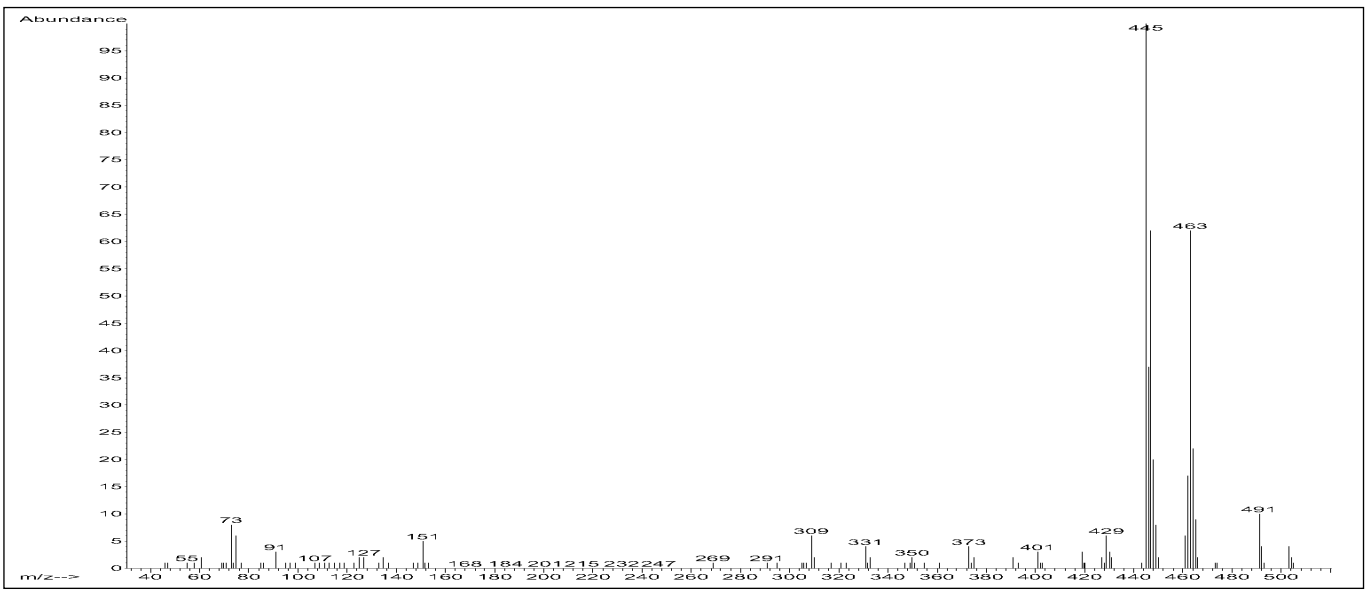
EI-Spectrum, Zearalenone, TMS mono-derivative, *m/z* 390; M^+



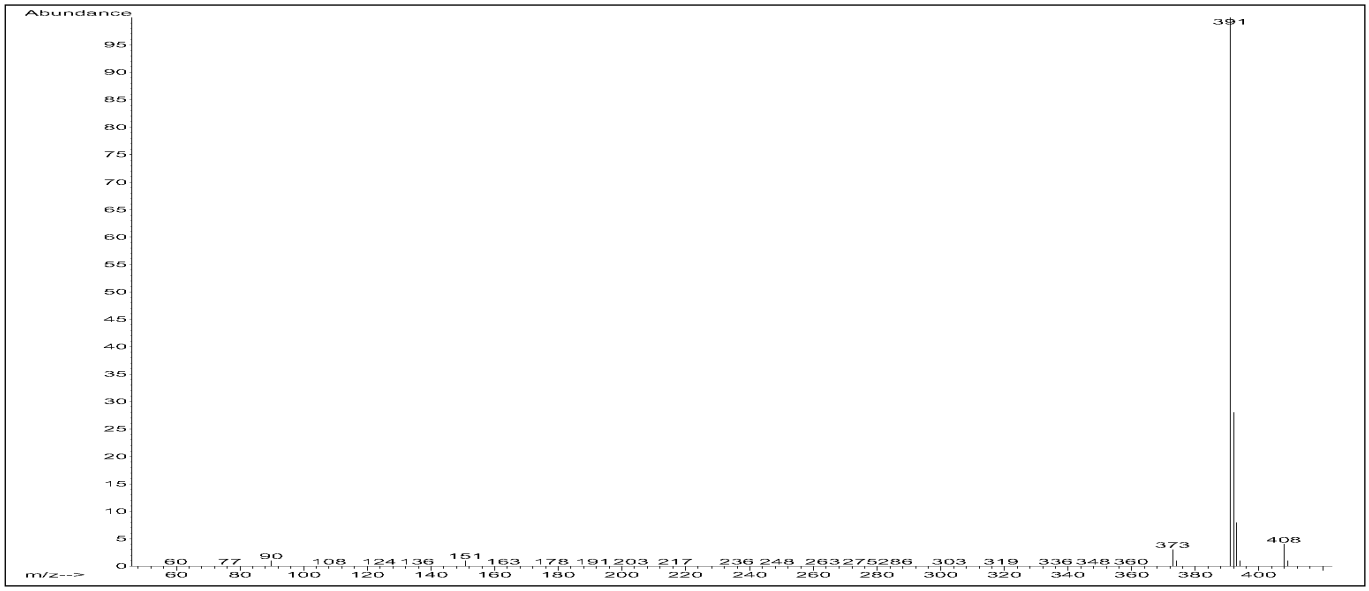
EI-Spectrum, Zearalenone, TMS di-derivative, *m/z* 462; M^+



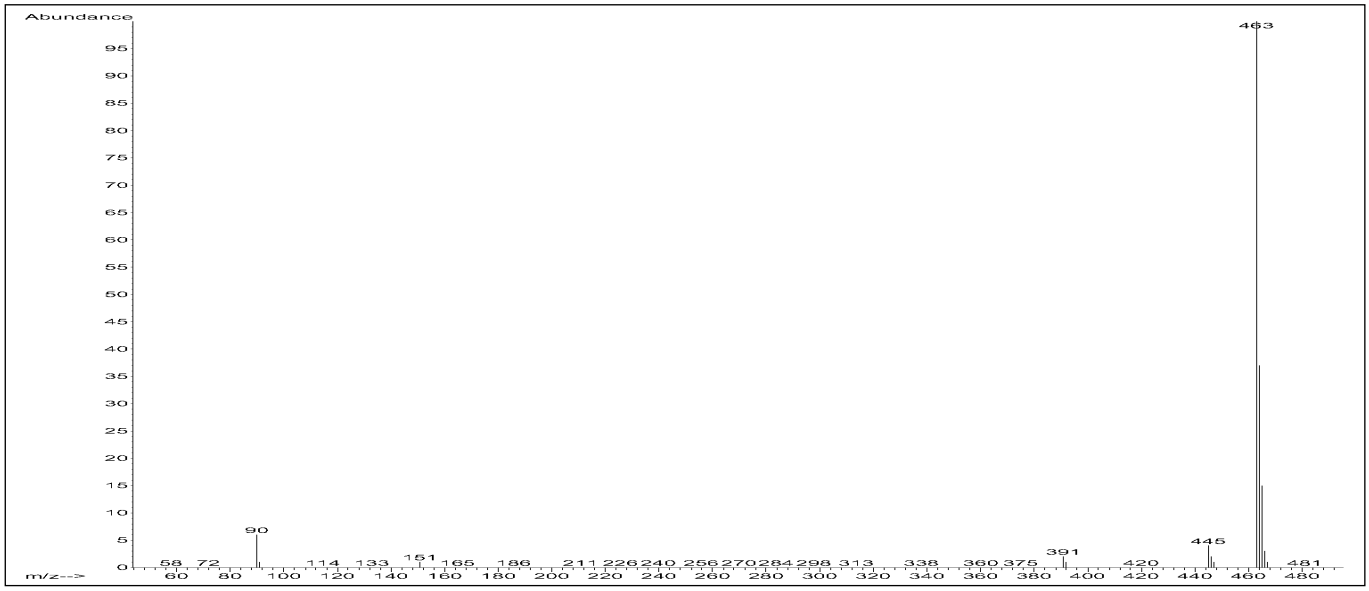
PCI/CH_4-Spectrum, Zearalenone, TMS mono-derivative, *m/z* 391, 419, 431; $[M + H]^+$, $[M + C_2H_5]^+$, $[M + C_3H_5]^+$



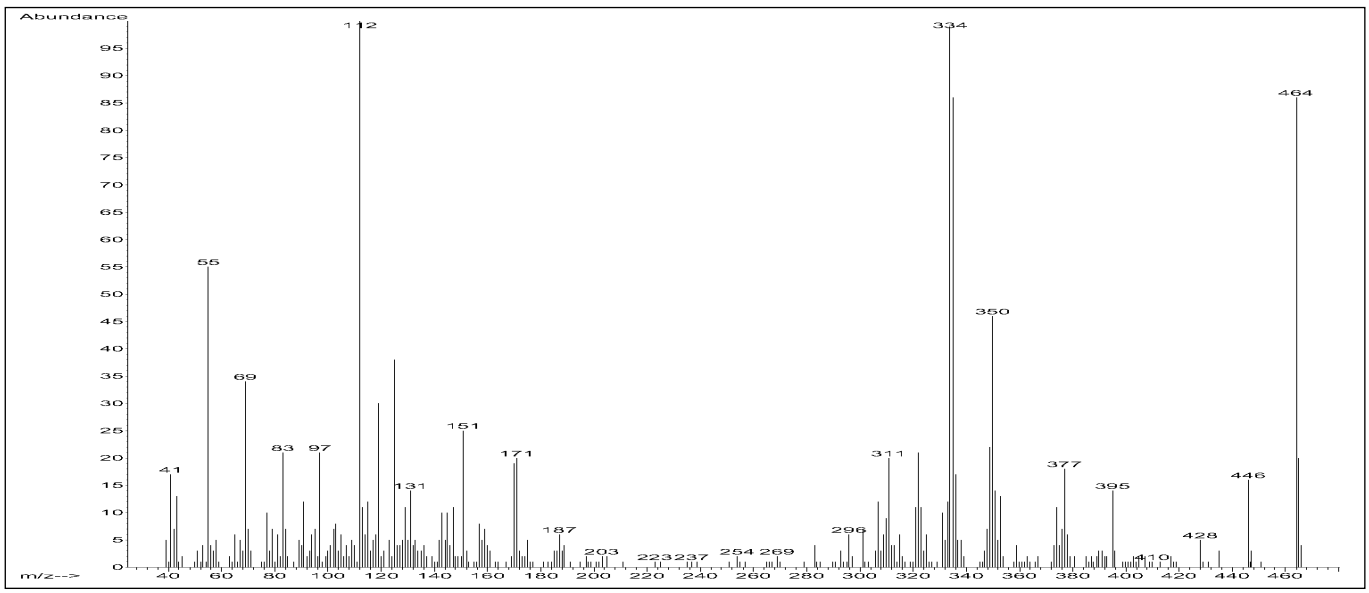
PCI/CH_4-Spectrum, Zearalenone, TMS di-derivative, *m/z* 463, 491, 503; $[M + H]^+$, $[M + C_2H_5]^+$, $[M + C_3H_5]^+$



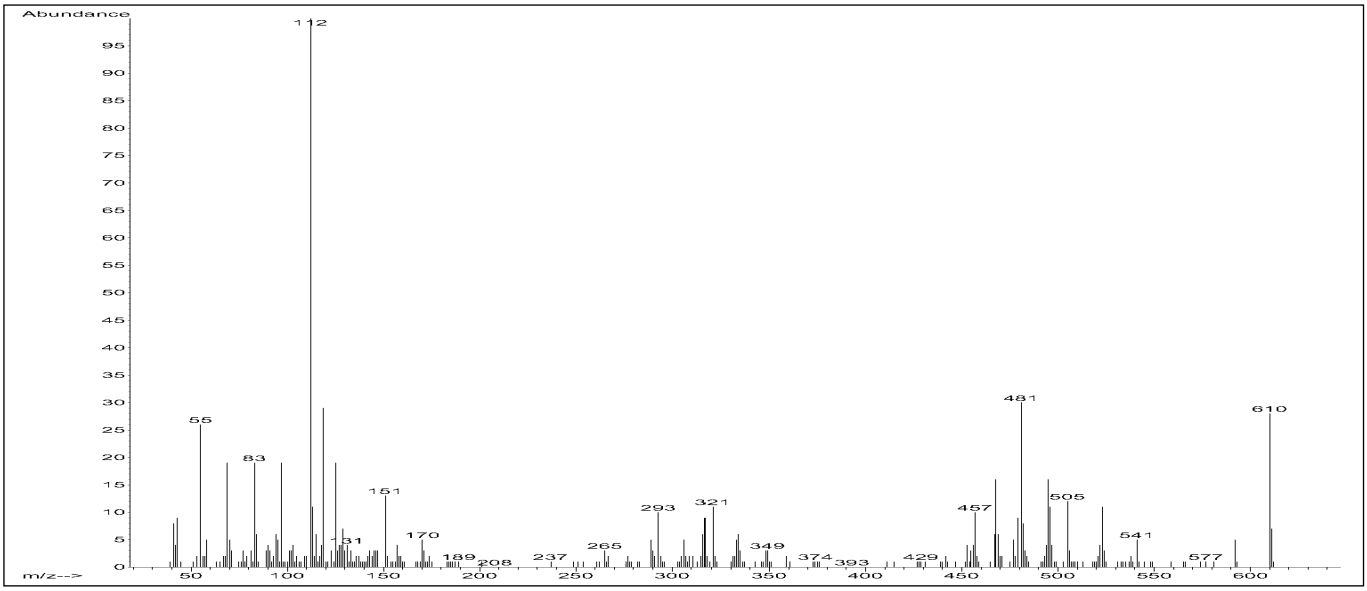
PCI/NH_3-Spectrum, Zearalenone, TMS mono-derivative, *m/z* 391, 408; $[M + H]^+$, $[M+NH_4]^+$



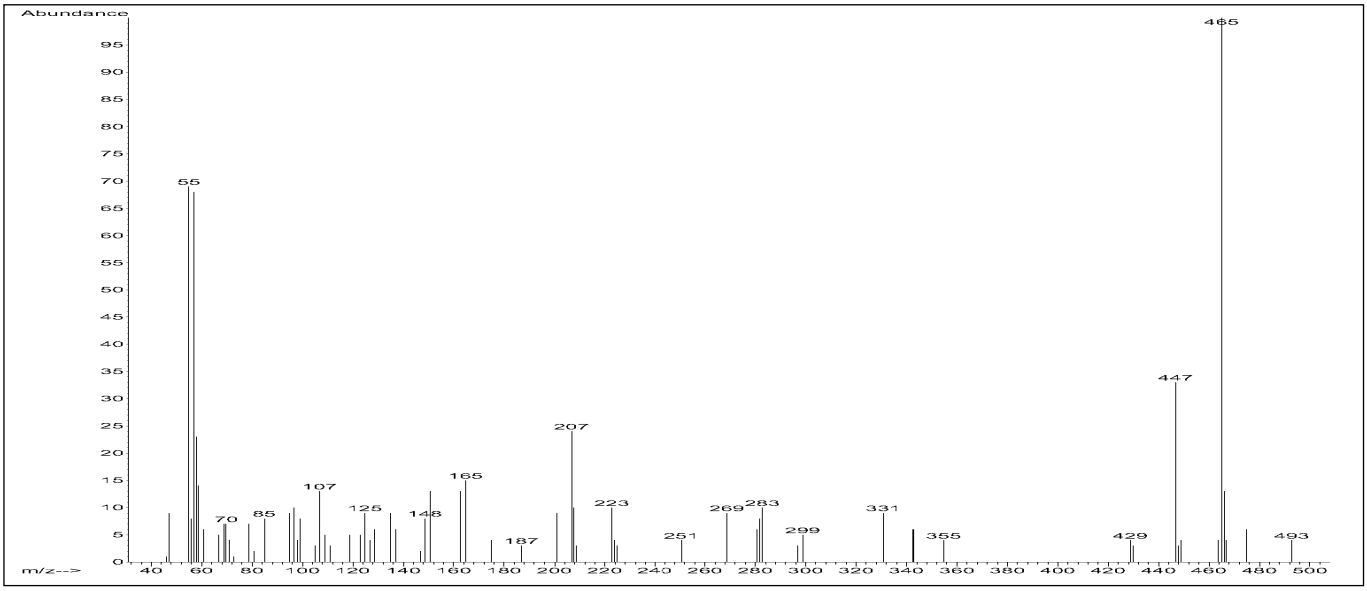
PCI/NH_3-Spectrum, Zearalenone, TMS di-derivative, *m/z* 463 ; $[M + H]^+$



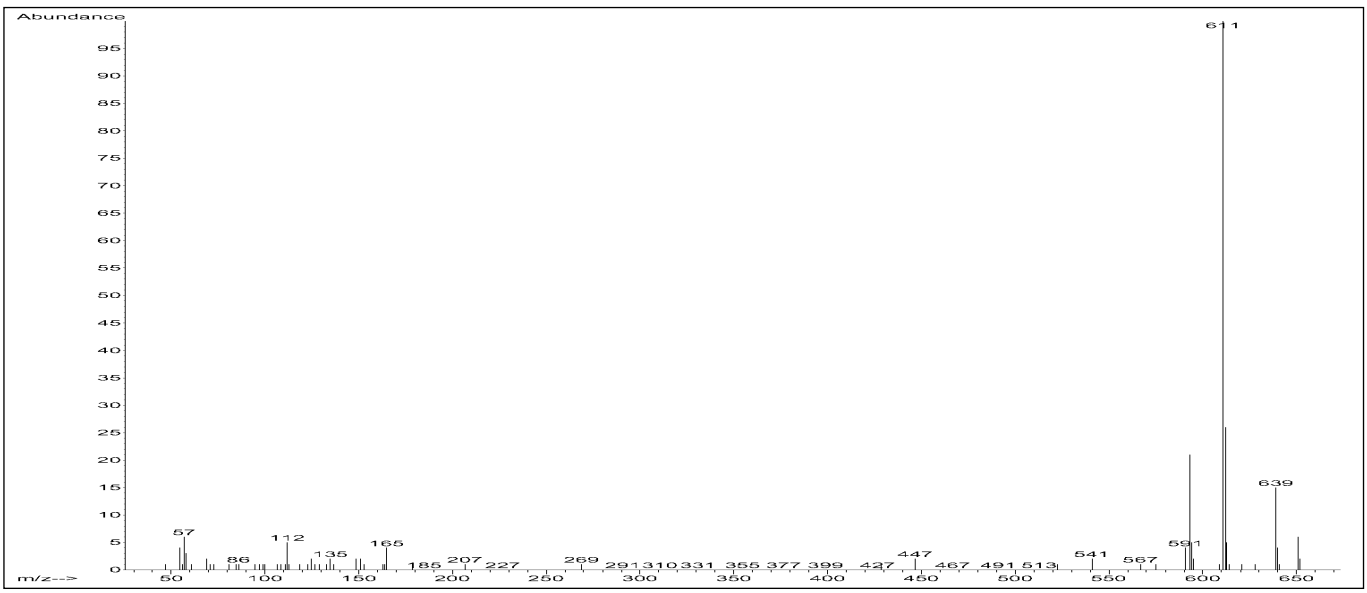
EI-Spectrum, Zearalenone, PFPA mono-derivative, *m/z* 464; M^+



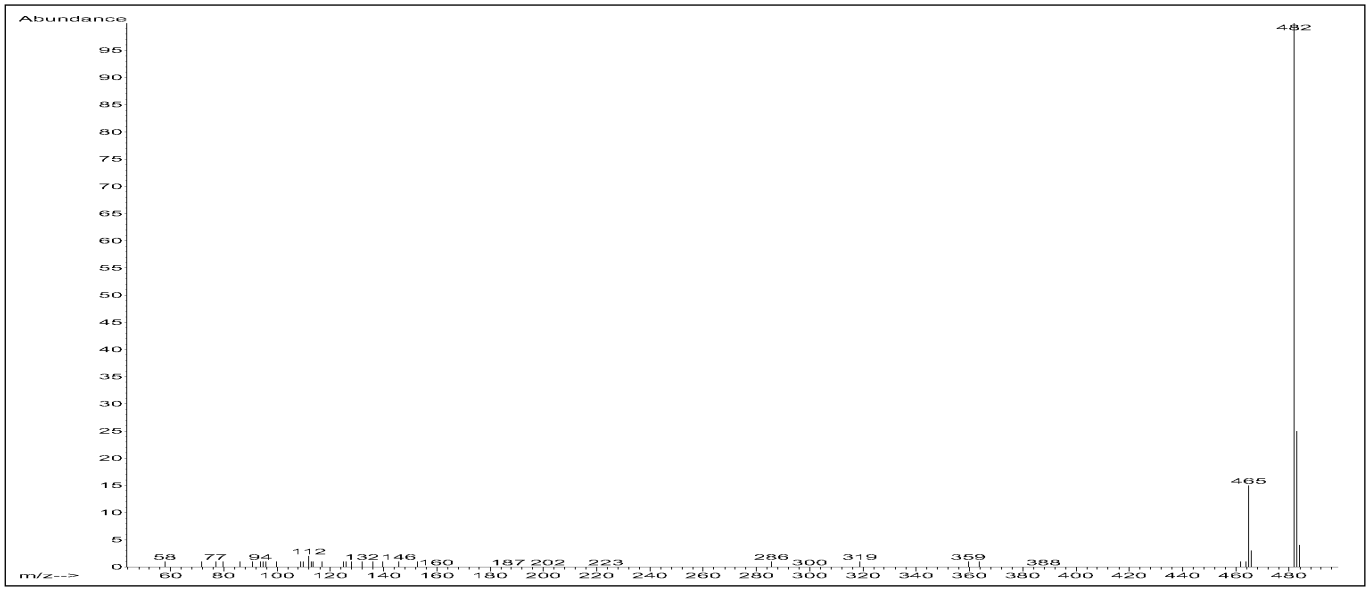
EI-Spectrum, Zearalenone, PFPA di-derivative, *m/z* 610; M^+



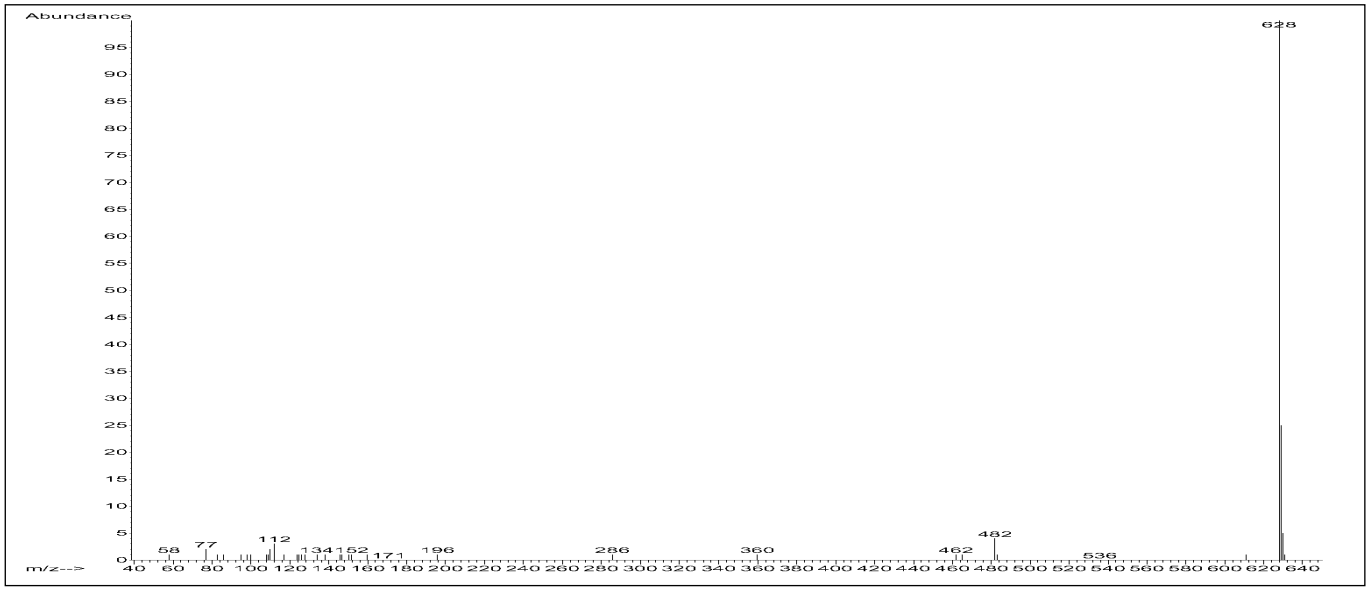
PCI/CH_4-Spectrum, Zearalenone, PFPA mono-derivative, *m/z* 465, 493; $[M + H]^+$, $[M + C_2H_5]^+$



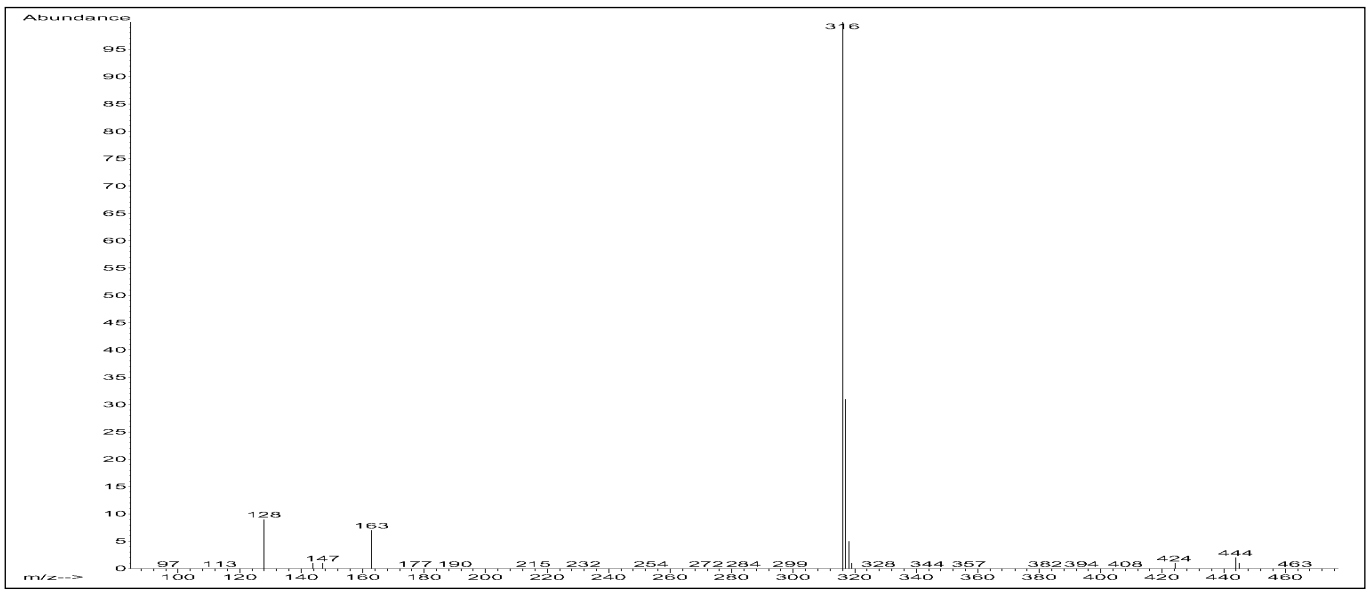
PCI/CH_4-Spectrum, Zearalenone, PFPA di-derivative, *m/z* 611, 639, 651; $[M + H]^+$, $[M + C_2H_5]^+$, $[M + C_3H_5]^+$



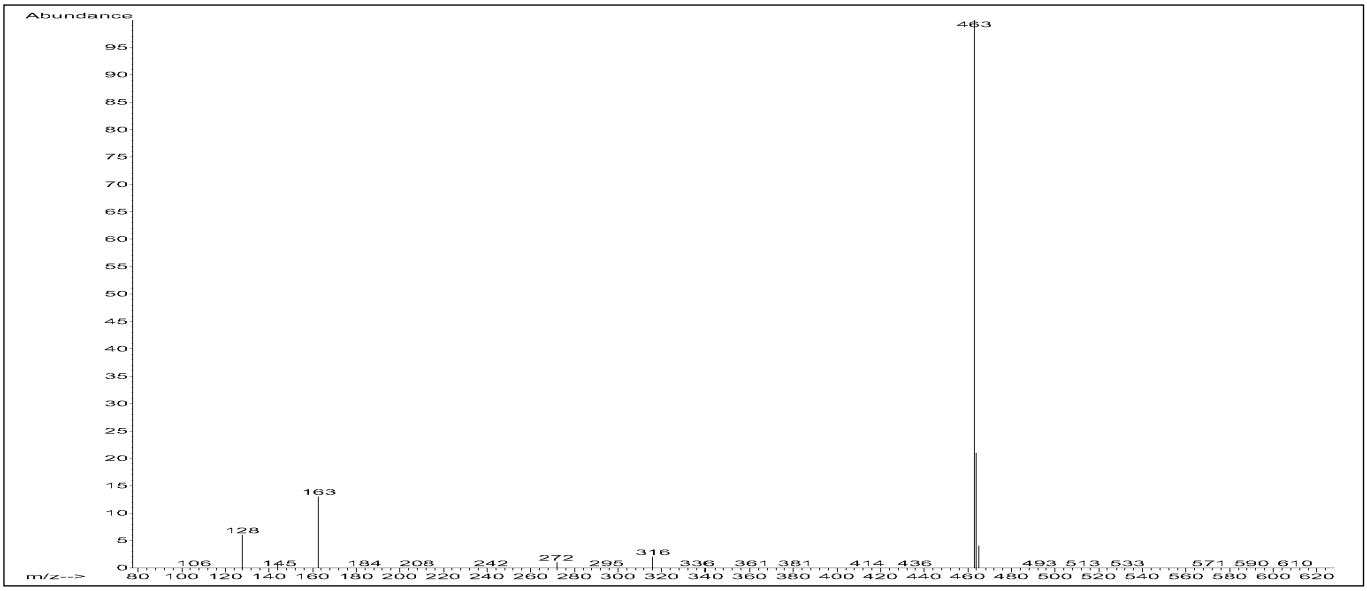
PCI/NH_3-Spectrum, Zearalenone, PFPA mono-derivative, *m/z* 465, 482; $[M + H]^+$, $[M + NH_4]^+$



PCI/NH_3-Spectrum, Zearalenone, PFPA di-derivative, *m/z* 628; $[M + NH_4]^+$

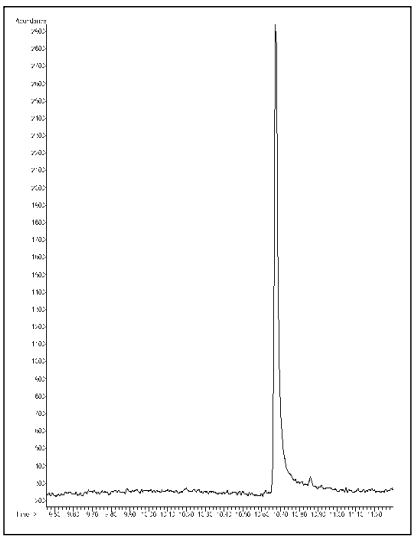


ECNI/CH_4-Spectrum, Zearalenone, PFPA mono-derivative, *m/z* 316, 444, 464; $[M - 148(PFPA+H)]^-$, $[M-HF]^-$, $[M]^-$



ECNI/CH_4-Spectrum, Zearalenone, PFPA di-derivative, molecular mass = 610 u *m/z* 463 ; $[M-147]^-$ (PFP = 147 u)

ECNI/CH_4 – SIM Mode



**Zearalenone, PFPA mono-derivative, 200fg,
Retention Time: 10.68min, Ion: *m/z* 316;
Signal/Noise ≈ 30/1**

The analysis of pharmacologically relevant compounds is carried out using different analytical techniques. The combination of gas chromatography (GC) with mass spectrometry (MS) is one of the most frequently applied because it offers high separating power, which is advantageous in analyzing complex mixtures, and reliable identification of unknown compounds. In the field of human and veterinarian medicine, drugs of abuse can be unambiguously determined and accurately quantitated.

This brochure emphasizes the standard mass spectral technique of electron impact ionization (EI) and both chemical ionization (CI) techniques; positive chemical ionization (PCI) and electron capture negative ionization (ECNI or NCI). The data presented unmistakably indicates CI is not merely a supplement to EI, but for most of the documented examples, improves analytical results related to compound selectivity and detection sensitivity. To assist and encourage the user in exploring CI, this brochure also presents an elementary understanding of CI-theory and useful practical operating advice for CI on the Agilent MSD.

Data for 43 compounds are presented from a wide variety of drug classes; barbiturates, benzodiazepines, ß-agonists, narcotics and steroids and others. All GC and MS method parameters, derivatisation techniques, and the resulting EI/PCI/ECNI mass spectra are documented. The results are briefly discussed for the different operating modes and reagent gases applied. Attention is given to the quantitative data obtained especially in terms of signal-to noise. The intention being to assist the user in choosing successful conditions and modes to analyze samples for particular drugs at trace concentrations.

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