REACTION MONITORING USING VIBRATIONAL SPECTROSCOPY

University of Bergen
Department of Chemistry
Bergen

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Reaction monitoring using vibrational spectroscopy

Thesis for the degree of European Master in Quality in Analytical Laboratories

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Contents

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List of Abbreviations

CCD	Charge-coupled device
CLS	Classical least squares
DCM	Dichloromethane
DMF	Dimethyl formamid
DOE	Design of experimental
FDA	Food and drug administration
FT-NIR	Fourier transform near infrared
GC	Gas chromatography
GC-MS	Gas chromatography and mass spectrometry
I	Intensity
ILS	Inverse least squares
IR	Infrared
K	Kelvin
MLR	Multiple linear regression
MSDS	Material safety data sheet
NIPALS	Non-linear iterative partial least squares
NIR	Near infrared
NMR	Nuclear magnetic resonance
PAL	Process analytical technology
PCA	Principle component analysis
PCR	Principle component regression
PLS	Partial least squares
PMT	Photomultiplier tubes
PPH_3	Triphenylphosphine
R^2	Coefficient of determination
T	Transmittance
TBAB	Tetrabutylammonium bromide
THF	Tetrahydrofuran
USEPA	United State Environmental Protection Agency
VS	Vibrational spectroscopy
WLS	Weighted least squares

Abstract

Reaction monitoring by vibrational spectroscopy (VS), especially NIR spectroscopy, is being increasingly used during process development. Analysts usually use spectroscopy to qualitatively monitor the progress of reactions.

Cross-coupling reactions of organic electrophiles and organometallic reagents have increased popularity as a powerful synthetic tool and innumerable improvements have been done from the first protocol that allows a wide range of coupling partners to be combined efficiently[1]. The aim of cross-coupling reaction is carbon-carbon bond formation. The Suzuki coupling reaction is one of the most efficient methods for the construction of C-C bonds.

The objective of the thesis is to monitor a Suzuki coupling reaction by vibrational spectroscopy and to use chemometrics to build the model that can serve to anticipate how far the reaction has proceeded. Our plan is to investigate whether NIR or Raman spectroscopy is better suited for monitoring the reaction.

An efficient, versatile and non-destructive method to monitor the reaction by using VS is described. By using this method it is possible to monitor the reaction without interrupting it. In this way time and money are saved.

A Suzuki cross-coupling reaction was chosen to be monitored. In this reaction 1-iodo-2-nitrobenzene was used as an organometallic reagent. This reacted with an organic electrophile phenylboronic acid to form 2-nitrobiphenyl as a product.

For the occurrence of this reaction the catalyst palladium (II) acetate must be added. A base should be present to promote the transmethyilation process. Potassium carbonate was used as a base.

This reaction was monitored by VS under standard conditions which are disclosed elsewhere[2]. The method allows us to know when the product of interest was formed and to stop the reaction to avoid the formation of contaminations. The recorded data was treated with multivariate data analysis such as PLS (Partial Least Squares) and PCA (Principle Component Analysis). Once a protocol for proper reaction monitoring was developed an experimental design was set up. The purpose of this was to build the model that could serve to predict how far the reaction had proceeded. Temperature, concentrations and reaction time were the variables investigated.

The designed experiments were monitored using VS enabling continuous prediction of reaction progress. GC-MS was used to validate the VS prediction results. This necessitated sampling the reaction mixture at predefined intervals for off-line analysis.

1 Introduction

Synthetic chemistry continues to amaze with its power to construct diverse molecular structures of complex architecture. In this field it is important to discover new strategies to develop reactions that can serve to construct new complex molecules as they might serve the protocol of Suzuki coupling reactions[3].

The carbon-carbon bond construction method[4,5,6] has allowed chemists to assemble complex molecular frameworks which has been used to prepare numerous natural products[7], drug precursors[8], biological compounds[9], organic products[10] and herbicides[11]. Lately, the conditions developed for the cross-coupling reaction have many desirable features for large scale syntheses and are persuasively used in the industrial synthesis of pharmaceuticals and chemicals products.

In daily laboratory work, time together with accuracy plays a crucial role in monitoring techniques particularly in in-situ reactions.

Nowadays in-situ reaction monitoring techniques coupled with multivariate analysis can serve as the best approach in order to face the challenges like interruption of reaction and the long reaction times needed in the pharmaceutical and chemical industries.

NIR and Raman spectroscopy are excellent instrumental techniques to monitor the forming of a desired product in real time. In comparison to GC-MS analysis which requires a considerable period of time to provide a response the NIR and Raman spectroscopies provide an immediate result that considerably shortens the overall process time. By these techniques it can be possible to obtain a huge amount of information about the reaction with minimum amount of effort. Another advantage is that NIR and Raman provide information on the accumulation of reaction intermediates when we are monitoring the reaction. They also give information about whether the reaction is going in the right direction. These advantages play a key role in choosing the technique to be used in pharmaceutical and industrial production.

A literature screening revealed a vast amount of material concerning the Suzuki coupling reaction. Surprisingly very few reports[12,13] described usage of FT-NIR and Raman spectroscopy in cross-coupling reaction. To the best of my knowledge, no reports on using NIR spectroscopy and multivariate analysis in monitoring the Suzuki cross-coupling reaction have been published.

Except for the tremendous good thing that Suzuki coupling reaction possesses it has a drawback which is to define the end time of reaction, thus avoiding the formation of byproducts. Moreover, some Suzuki coupling reactions give very low yield, as is the case of 2-chloro phenylboronic acid with 1-iodo-2nitrobenzene which gives 2% yield[2]. The

present thesis aims to find which spectroscopic techniques are better (either NIR or Raman) and tries to optimize the reaction using multivariate analysis and experimental design to predict how far the reaction has proceed and stop it in due time. During the reaction the samples were withdrawn and analyzed by GC-MS. In order to be sure that we have our product of interest the sample after work up it was analyzed by NMR as well.

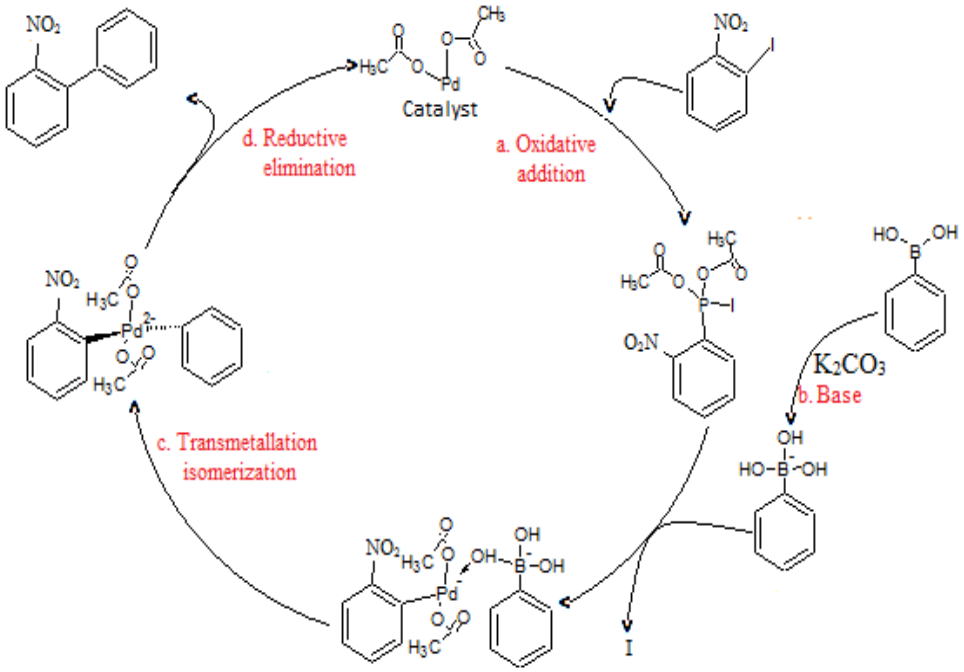
2 Literature review

2.1 Suzuki Coupling Reaction

In the past few decades, there has been an immense use of metal-catalyzed cross-coupling reactions, called Suzuki coupling. The Suzuki coupling scheme[3] has found numerous applications in modern synthesis. The popularity of this method arises from both the variety of organometallic reagents utilized in these reactions, and the broad range of functional groups that can be included into these reagents.[14] This reaction plays a key role in organic synthesis, and the importance of palladium-catalyzed cross coupling in organic synthesis was recognized by the awarding of the Nobel Prize in chemistry 2010 to Richard Heck, Ei-ichi Negishi, and Akira Suzuki[15].

The first step of the Boscalid synthesis was chosen as a model for our Suzuki coupling reaction. Boscalid is a modern fungicide that can be used as broad-spectrum fungicide for the protection of crops such as fruits, vegetables and horticultural plants. Boscalid can be used in combinations with other fungicides allowing many different diseases to be combated in a wide variety of crops. In comparison to other fungicides, Boscalid has a high level of environmental compatibility according to the USEPA (United States Environmental Protection Agency)[16]. This makes Boscalid the most useful fungicide in the world.

Instead of using 1-chloro-2-nitrobenzene as substrate as well as the protocol of Boscalid synthesis we have used the 1-iodo-2-nitrobenzene. This is due to the higher activity of ArI compared with ArCl in oxidative addition (ArI>>ArBr>>ArCl[17,18]). Phenylboronic acid was used as organoboron compound. This organohalide and this organoboron lead to the formation of a new carbon-carbon single bond product called 2-nitrobiphenyl in the presence of palladium acetate as base.
The mechanism of the reaction is given in **Scheme 1**.



Scheme 1. Cross-coupling reaction between 1-iodo-2-nitrobenzene and phenylboronic acid, in the presence of palladium acetate as catalyst and K_2CO_3 as base.

The first step (a) is an oxidative addition of 1-iodo-2-nitrobenzene to palladium acetate to give an organopalladium compound. The phenylboronic acid in the presence of base (b) was used as a coupling partner in palladium-catalyzed cross coupling with substrate. The base activation of phenylboronic acid as an intermediate facilitated the transfer of the organic group from boron to palladium. This is called transmetallation (c). In this step, the two organic groups are assembled on the same palladium atom via palladium-carbon bonds. In the final step (d) the nitrobenzene and benzene compounds couple with one another to give a new carbon-carbon single bond and 2-nitrobiphenyl is released from palladium acetate. In this process Pd^{2+} is reduced to Pd^0 and therefore the final step is called a reductive elimination.

As for most reaction, temperature plays an important role. In addition, Suzuki coupling reactions are affected by the choice of: catalyst, base, and solvent.

2.1.1 Catalyst

A catalyst is a substance that increases the rate of a process. It is of tremendous importance to oganometallic chemistry. The catalyst is not consumed in the reaction so it can participate in multiple transformations. Originally palladium was used as the catalyst in Suzuki coupling reactions[3]. During the time of developing the reaction significant progresses were made trying to replace Pd with a Ni-catalyst[19]. Lately a

huge number of new catalysts have been reported for the Suzuki coupling reaction[20,21,22]. Sometimes in addition to the catalyst in the reaction a ligand is needed as well. The role of ligands is to stabilize the catalyst in the mixture. The most frequently used ligands are tetrabutylammonium bromide (TBAB)[23] and triphenylphosphine (PPh_3)[24]. As the most used palladium catalysts are $Pd(PPh_3)_4$, $Pd(OAc)_2$ and $PdCl_2$ when the halobenzene is bromide or iodide. Since in the protocol 1-iodo-2-nitrobenzene is as substrate we have used $Pd(OAc)_2$ as a catalyst. Also the ligand is not needed because the palladium (II) acetate used is stable.

2.1.2 Base

The choice of base is crucial in the Suzuki coupling reaction protocols in organic synthesis. The uses of various bases in dependence with solvents have improved the Suzuki coupling reaction so selecting a suitable base is important to obtain high yields. K_2CO_3 and K_3PO_4 perform well in dimethyl formamid (DMF) whereas a stronger base such as NaOH is more efficient in tetrahydrofuran and water (THF/H_2O). Some protocols of products synthesis via cross-coupling reaction which do not require base for the reagent activation has been reported[25,26]. In the present thesis, K_2CO_3 was used as base.

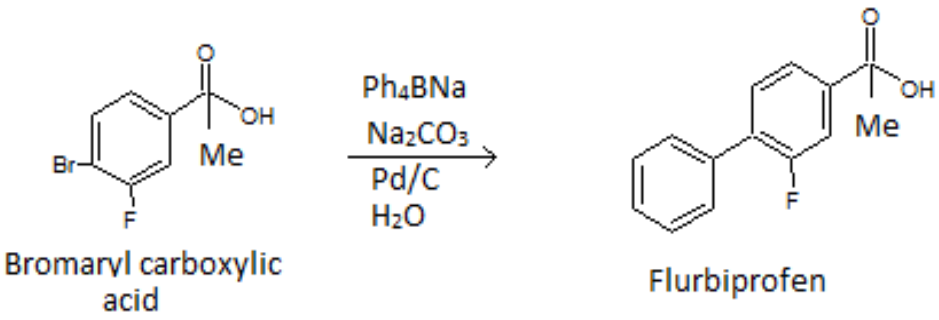
2.1.3 Solvent

Solvent as well is one of components that affects the yield of Suzuki-coupling reactions. Based on the patent literature[27] for protocol of Boscalid synthesis THF:H_2O and t-BuOH:H_2O were found to be the best solvents. In comparison to the monophasic solvents like methanol and DMF a biphasic solvent system such as methanol/H_2O is better suited. It has been reported that for the same conditions[2] biphasic solvent methanol/H_2O provided higher yield compared to methanol as a reaction media. This is the reason why we have chosen the biphasic solvent in our protocol synthesis. Noteworthy to mention as an important parameter in Suzuki-cross coupling reaction is the temperature. It has been disclosed in the protocol of synthesis of 2,2'-dinitrobiphenyls[2] that by increasing the temperature the yield will substantially increase as well.

We have said before that Suzuki cross coupling reaction has had a large impact on synthetic organic chemistry. This reaction has revolutionized the modality of how organic molecules are assembled. The reason of widespread use is due to the mild conditions associated with the reactions and also the tolerance of broad range of functional groups. This reaction was applied to the synthesis of numerous natural products and biologically active compounds which have found application in the

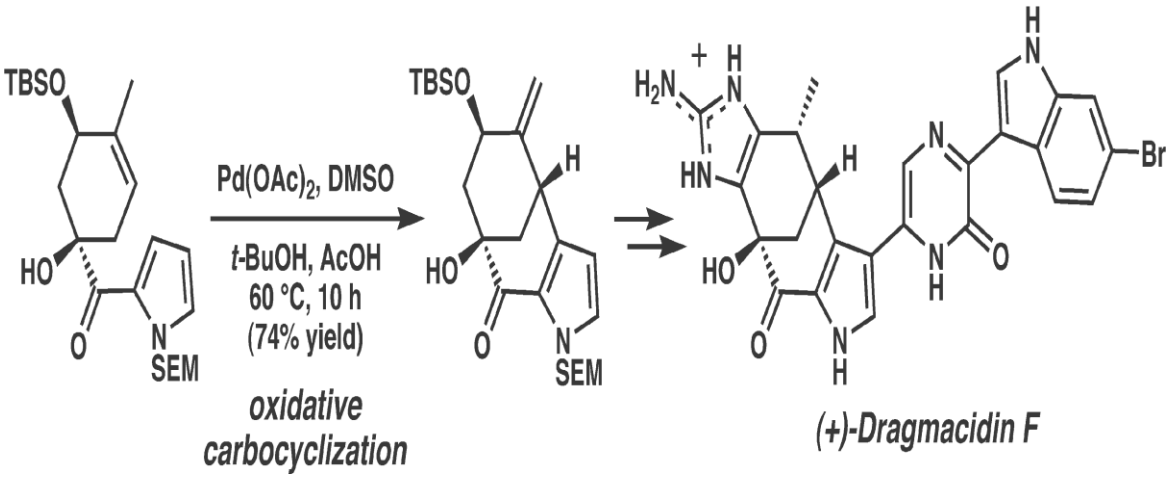
pharmaceutical and chemical industries. Some examples of the use of the Suzuki cross-coupling reactions in industrial application are given below.

Nonsteroidal anti-inflammatory and analgesic drugs, such as flurbiprofen, felbinac and fenbufen which are used to treat pain and inflammation were a synthesized via Pd-catalyzed Suzuki coupling reactions[28]. The key step of forming the biaryl in the synthesis of flurbiprofen was performed via Pd/C-catalyzed Suzuki coupling reaction. This step is given in **Scheme 2**.



Scheme 2. Construction of the biaryl fragment in synthesis of flurbiprofen[28].

Dragmacidin F-bromoindole alkaloid, a well-known antiviral compound which was extracted from the Mediterranean sponge Halicortex sp. was a synthesis via a halogen-selective Suzuki coupling[29] as can be seen in the following **Scheme 3.**



Scheme 3. Total synthesis of dragmacidin F[29].

These are just two examples among the others[28,30] that emphasize the great importance that the Suzuki cross coupling has in synthesis of the pharmaceutical compound as well as biologically active compound.

2.2 Vibrational Spectroscopy

During the last 15 years, online vibrational spectroscopy has become an increasingly useful tool for research and process development[31,32,33,34]. Vibrational spectroscopy is the interacting of light with matter, and in particular the energy of the light. This energy is sufficient to excite vibrations of the molecule which absorb it. When molecules are excited, they achieve a state of higher energy, where the vibrational amplitude is increased as it is given in **Figure 1**.

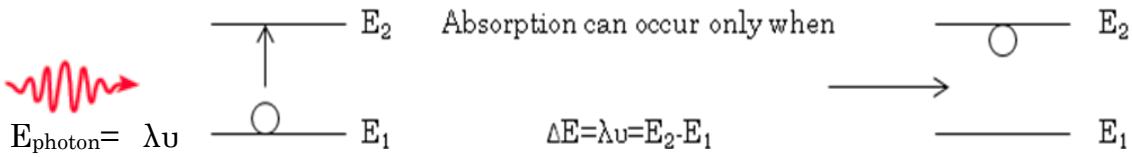


Figure 1. Excited state of molecule by absorption the energy.

Vibrational spectra are commonly measured by two very different techniques, IR spectroscopy and Raman spectroscopy. Basically, NIR spectroscopy work in such a way that light in the NIR region is passed through a sample and the intensity of the transmitted light is measured at each frequency. In Raman spectroscopy we do not observe transmitted light but light scattered from the sample.

Generally infrared spectroscopy instrumentation is more sensitive than Raman spectroscopy due to the high amount of signal that we can get from a given amount of sample.[35] However these two techniques are complementary. In order to get a complete picture of the vibrational state of a compound it is good to use two techniques if it is possible. In general the strong bands in the IR spectrum of a compound correspond to weak bands in the Raman and vice versa. This complimentary nature is due to the electrical characteristic of the vibration. Vibrational spectroscopy has widespread applications in process development. It is possible by this technique to obtain a spectrum from samples in many different forms such as solids, liquids, as well as gas. As drawback of these techniques is the difficulty of interpreting spectra records, sometimes we have difficulties in extracting chemical information from the spectra.

2.2.1 Near-Infrared Spectroscopy

Near-infrared spectroscopy is a powerful technique utilized in process development[36,37] which provides unique information that is hardly accessible by any other technique. This technique has an early historical background. Near infrared region was discovered in 1800.[38] Nevertheless,

NIR has been used extensively only in the second half of the 20th century, especially after 1985 as it can be seen in **Figure 2**.

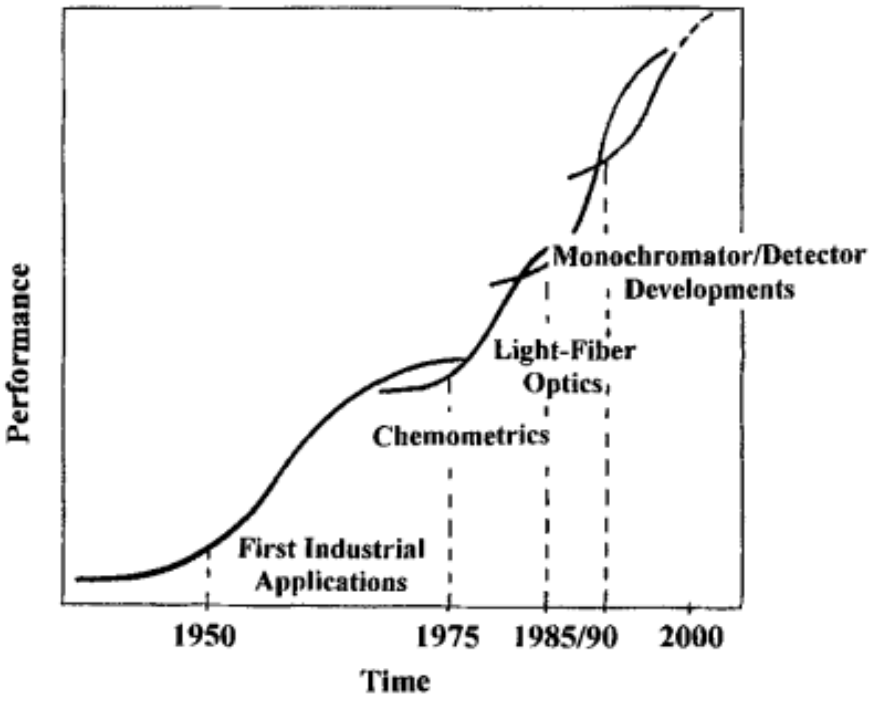


Figure 2. The development of near-infrared spectroscopy in industry.[39]

At temperatures above zero Kelvin (K) all the atoms in molecules are in continuous vibration. For molecules with N atoms the degrees of freedom in the coordinate axes (x, y, z) are six if they are non-linear, respectively five if they are linear. In the following **Figure 3**, the three different movements are shown**.**

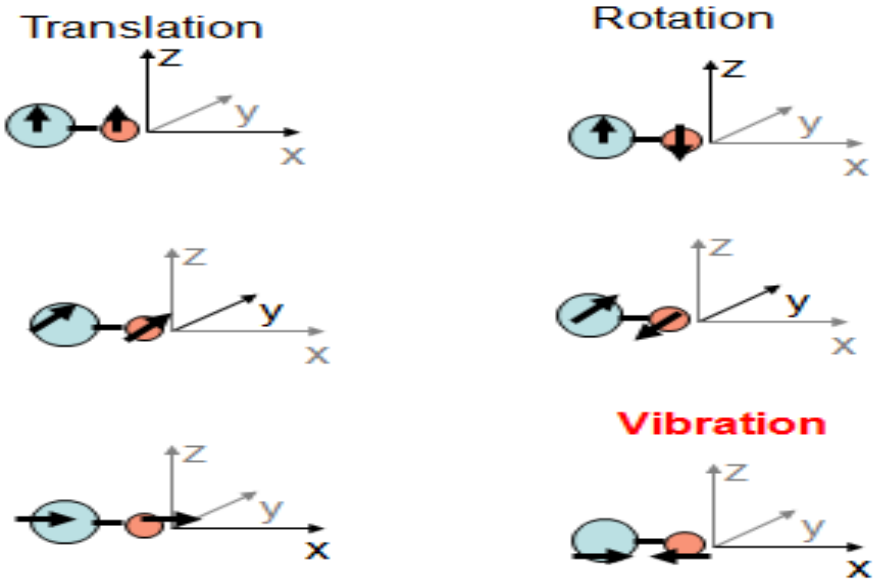


Figure 3. The degrees of freedom for molecule with N atoms. 3 translations and 2 rotations if it is linear and 3 translations, 2 rotations and 1 vibration if it is non-linear.

An NIR-spectrophotometer is an instrument which measures the absorption of near infrared radiation by a sample as a function of wavelength (780-2500mm). NIR spectra are recorded by detecting changes in transmittance (absorption) intensity as a function of frequency. Transmittance (T) is the ratio of intensity transmitted by the sample (I) to the intensity of the incident radiation (I_0) whereas absorbance (A) is the logarithm of the reciprocal of the T.

$$T = I/I_0 \quad => \quad A = \log_{10}(1/T) = -\log_{10}(I/I_0)$$

NIR-spectrophotometer consists of three basic components:

- emitting source;
- wavelength discriminator (monochromator); and
- sensitive detector.

Mainly there are three types of emitting sources which produce continuous radiations but with different energy profiles. Nernst glower a filament of mixed Zircon-Yttrium and Ecbiumoxides with a short lifetime. The other two are: Globar (silicon carbide) and Nichrome coil.

A monochromator is a device used to disperse the radiation of the near infrared source. Monochromator components are: a). an echelette grating which separates the light into different wavelengths; b). an optical filter; c). an entrance slit which creates a point shaped light source; d). exit slit and f). mirrors, used for focusing the beam.

The detector is used to transform the near infrared radiation into an electrical signal. There are two classes of detectors: thermal and photon detectors. Thermal detectors attend the changes in temperature; they specifically measure the heating effect which is produced by near infrared radiation. Thermocouples, thermistors, and pneumatic devices are included in thermal detectors. Photon detectors recorded the interaction of IR radiation and a semiconductor material. They are more sensitive than thermal detectors.

The near infrared technique can be considered as a versatile technique. Its advantages are: a). it is a non-destructive technique; b). no need for sample preparation; c). less time-consuming, ability to perform in field measurements (no collection of samples); d). more than one analyte can be determined simultaneously and f). a robust technique for online analysis of processes.

Due to the above advantages NIR can be considered as a powerful technique for pharmaceutical and industrial applications. The following **Table 1** shows the major fields of application.

Table 1. Usage of NIR spectroscopy

Applications of NIR spectroscopy	
Agriculture	Fruit quality[40] Identification of fish species[41] Predictions of nematofauna abundance and composition[42]
Pharmaceutical	Plastic packaged products[43] Tablets dissolution[44] Determining active ingredients[45]
Industrial	Analysis of fuels[46] Biodiesel quality[47] Polymer properties[48] Analysis of textiles[49]

2.2.2 Raman Spectroscopy

When the incident radiation hits the sample the frequency of photons changes due to the interaction with a sample. The incident radiation will be scattered by the sample in all directions. The scattered radiation will have a different frequency from the incident radiation. These changes in frequencies represent characteristics of samples by giving information about vibrational and rotational movement and constitute the so-called Raman spectrum.

Incident radiation (laser light) with frequency υ_0 excites molecules and then these molecules reemit light with three different frequencies[50]:

- when the excited molecules reemit light with the same frequency (υ_0) as that of the incident beam they constitute the so-called Rayleigh scattering;

- when the excited molecules reemit the light with the frequencies (υ_m) shifted from the initial frequency (υ_0) and if these frequencies are in the lower side of the Rayleigh line they form the Raman frequency called Stokes frequency, $\upsilon_S=\upsilon_0-\upsilon_m$;

- if these frequencies are on the higher side of the Rayleigh line they constitute the Raman frequency called Anti-Stokes frequency, $\upsilon_{AS}=\upsilon_0+\upsilon_m$.

This phenomenon can be seen in the following **Figure 4**.

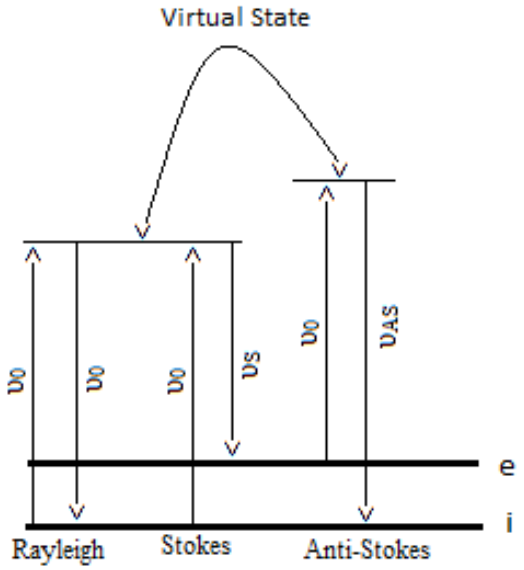


Figure 4. Raman transitional state. i-basic vibrational state; e-excited vibrational state. $\upsilon_m = E_e - E_i$

A Raman spectrometer consists of four major units:

- excitation source;
- sample optics;
- wavelength selector (monochromator); and
- detector.

In Raman spectrometers usually a laser beam is used as an excitation source, whereas the scattered light is collected with a lens and is sent through the spectrophotometer to obtain the Raman spectrum. Before the discovery of lasers Raman spectroscopy suffered from the disadvantage that relatively more concentrated solutions were needed due to the low intensity of Raman spectral lines.

The sample optics serve as the light collector, and its role is to orientate the light in the spectrophotometer. When the scattered light is collected it is transmitted into a monochromator.

The function of monochromator is to characterize the frequency component of the collected light. Wavelength separation is usually done by using a grating monochromator, especially a holographic grating or a Michelson interferometer.

Usually a photodiode array, such as charge-coupled devices (CCD) and photomultiplier tubes (PMT), is used as a detector. Nowadays the charge-coupled devices are more often used due to the sensitivity of these detectors.

Raman spectroscopy is a very important method for investigating molecular and physical properties of molecules due to the molecular vibrations. Advantages of Raman spectroscopy are: a). it is a non-

destructive technique; b). no need for sample preparation prior to analysis; c). the analysis can be done through a transparent container; d). it has a high spatial resolution (=1 μm).

The main drawback of Raman spectroscopy is the interference from the fluorescence. The fluorescence appears when the incident light is completely absorbed and the molecule is transferred to an excited state from which it can go to various lower states but only after a certain resonance lifetime. In contrast, the Raman effect is therefore not a resonant effect. A fluorescence process typically requires more than 10^{-9} seconds from its vibrationally excited level of the electronic state to return at the lowest vibrational level. In contrary, a Raman transition is completed for 10^{-12} seconds or less.

Depending on the laser wavelength, fluorescence may or may not exist. If the photon does not provide sufficient energy to the molecule, the required transition to generate fluorescence will not take place.

Based on the above advantages Raman spectroscopy is widely used in pharmaceutical and industrial applications. Some examples are given in following **Table 2**.

Table 2. Examples of Raman Spectroscopy use.

Applications of Raman spectroscopy	
Pharmaceutical	Quantitatively determination of active ingredients[51]
	Analysis of solid form drugs[52]
Industrial	Fruit characterization[53]
	Meat quality[54]
	Characterization of different pigments[55]
	Polymer properties[56]

2.3 Chemometrics techniques

2.3.1 Multivariate analysis

Multivariate analysis is a collection of methods used to describe the analysis of data where several measurements or variables are obtained for each individual or object studied in one or more samples.[57]

In the general sense chemometrics is the art of processing data with different techniques in order to extract useful information needed. In recent years it is widely used due to the availability of inexpensive and powerful computers. Many technicians have had difficulties in applying chemometrics based on the complex mathematics that are used. This can

be the reason why the chemometric techniques have not expanded its usage.

By using chemometric techniques we are able to extract as much information as possible from the data and also it is possible to make predictions about unknown samples. We have used the PCA and PLS as chemometrics techniques in order to get meaningful information from the spectra.

Chemometrics can be applied in an immensity of disciplines. It is especially useful in quantitative spectroscopy. This application is indicated in **Figure 5**.

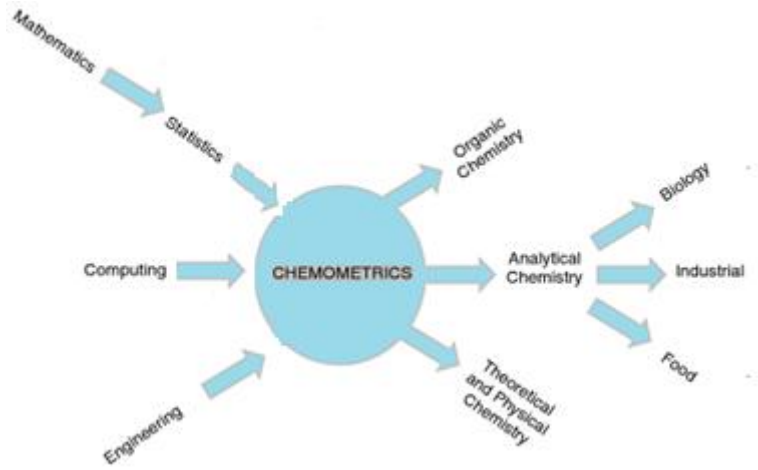


Figure 5. Application of chemometrics in various disciplines.

Nowadays, integration of process automation with the use of multivariate analysis techniques has become a very powerful tool in industrial process control and monitoring conditions of reactions.

Generally, simplification is one of the goals for many multivariate approaches trying to reduce a large number of variables to a smaller number of factors for data modeling. Another aim of multivariate analysis is to connect the samples represented by scores with the variables represented by loadings. In specific cases like spectroscopy one of the goals of multivariate analysis is to deconvolute the information in order to determine the spectrum of each component. Another aim of using multivariate analysis in spectroscopy is to build the quantitative model. Since NIR and Raman spectra are not readily interpretable compared to other techniques, multivariate analysis of the spectroscopic data is required to build quantitative models. In the present thesis, partial least squares (PLS) were used to build the model.

Multivariate analysis can be used for different purposes, and may be divided into three main groups: a). exploration; b). classification and c). regression and prediction. Techniques that can be utilized in multivariate analysis are principal component analysis (PCA), classical least squares

(CLS), inverse least squares (ILS), multiple linear regression (MLR), principal components regression (PCR), and PLS. In the present thesis PCA was used as an exploratory technique and PLS was used for regression and prediction.

Before explaining any multivariate techniques, we will describe briefly pre-processing of data. Usually data needs to be pre-processed before any multivariate analysis.

2.3.2 Pre-processing data

The preprocessing techniques are extremely useful before running any multivariate techniques. Usually, the data contain errors due to instrumentation noise. Data from Raman spectroscopy also have fluorescence fluctuations. The purposes of pre-processing are to linearize the response of the variables and to remove extraneous sources of variance which are not of interest in the analysis. By using the pre-processing techniques we may aim to smooth noisy data by taking derivatives of spectra, or to give all samples an equal impact on the model. Herein are described the most useful pre-processing techniques.

2.3.2.1 Mean Centering

The most common preprocessing method is mean centering. When we do the mean centering we calculate the mean of each variable and subtract this from the variables' values for each observation: ${}^{cen}x_{ij}=x_{ij}-\dot{x}_{ij}$. The row of the mean-centered data shows how the data differs from the average sample in original data matrix. By using this pre-processing technique a simpler model can be used to characterize the system. Mean centering translates the axes of the coordinate system to the center of gravity of the data. The mean centered data can be seen in the following **Figure 6**.

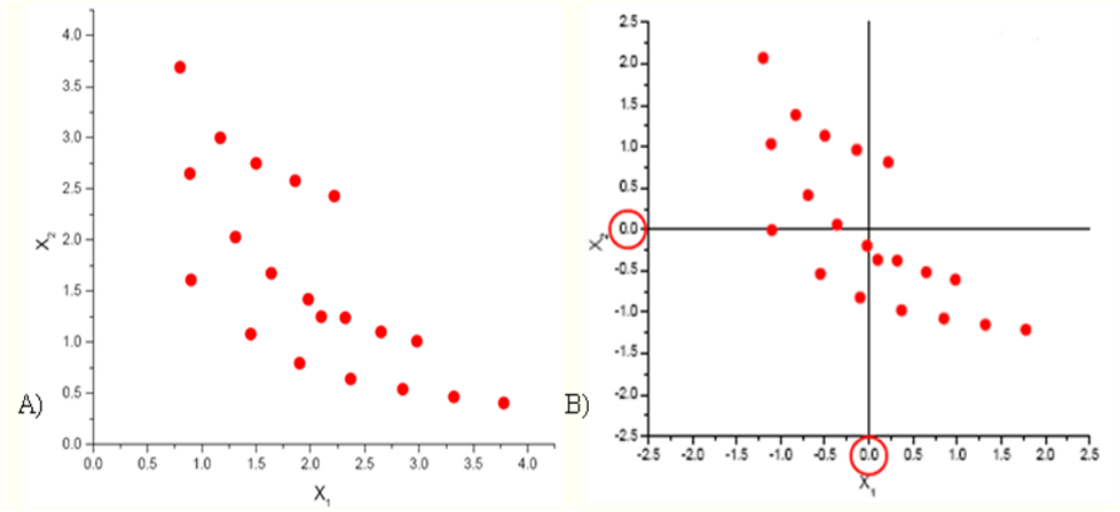


Figure 6. Data set. A). raw data, B). mean centered data.

2.3.2.2 Smoothing data

With this technique we attempt to capture the important patterns in the data and to remove the noise from the dataset. When we smooth data we assume that variables that are near each other in the data matrix contain similar information. These variables can be averaged to reduce noise without significant loss of important information. Smoothing data effects can be seen in the following **Figure 7**.

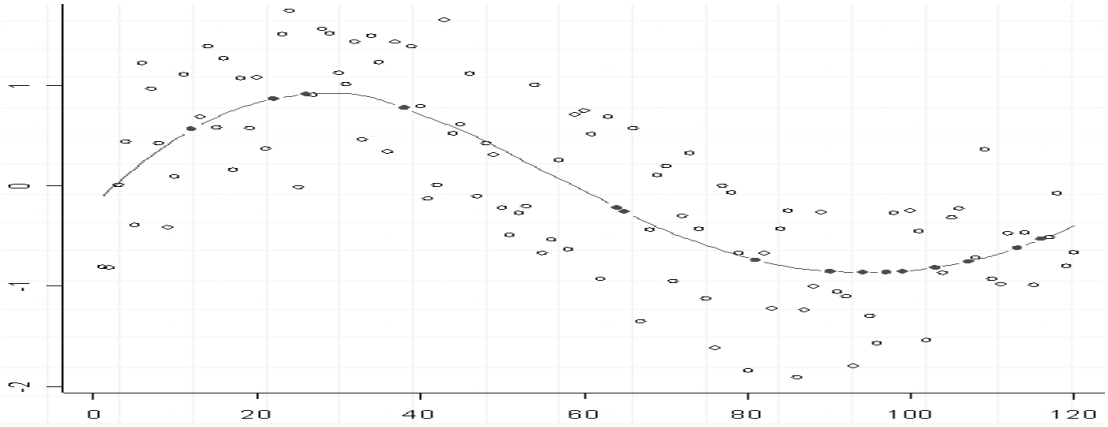
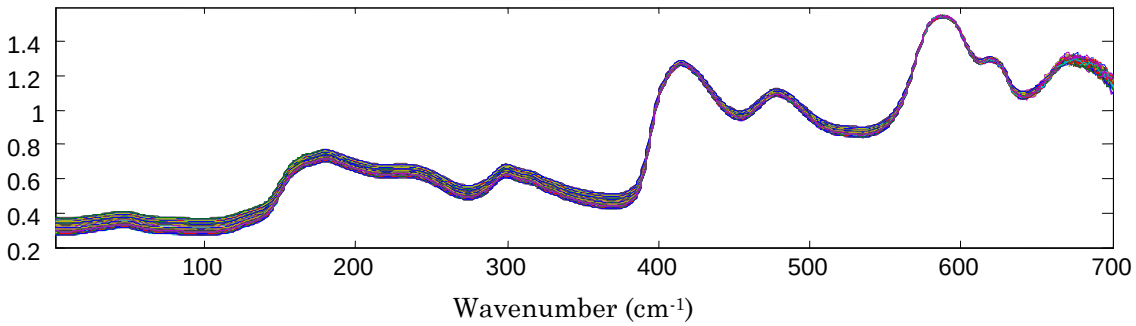


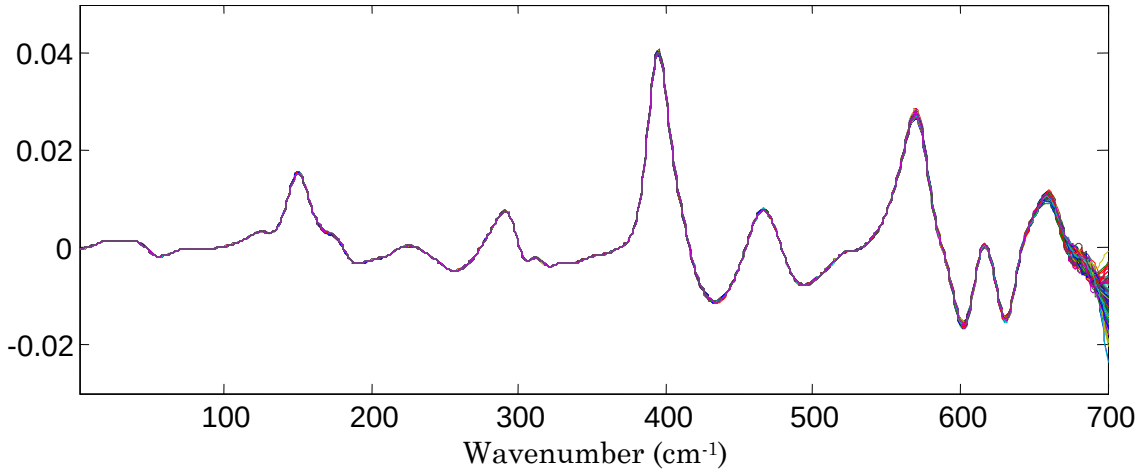
Figure 7. Smoothed curve along with points.

2.3.2.3 Derivative Savitzky-Golay

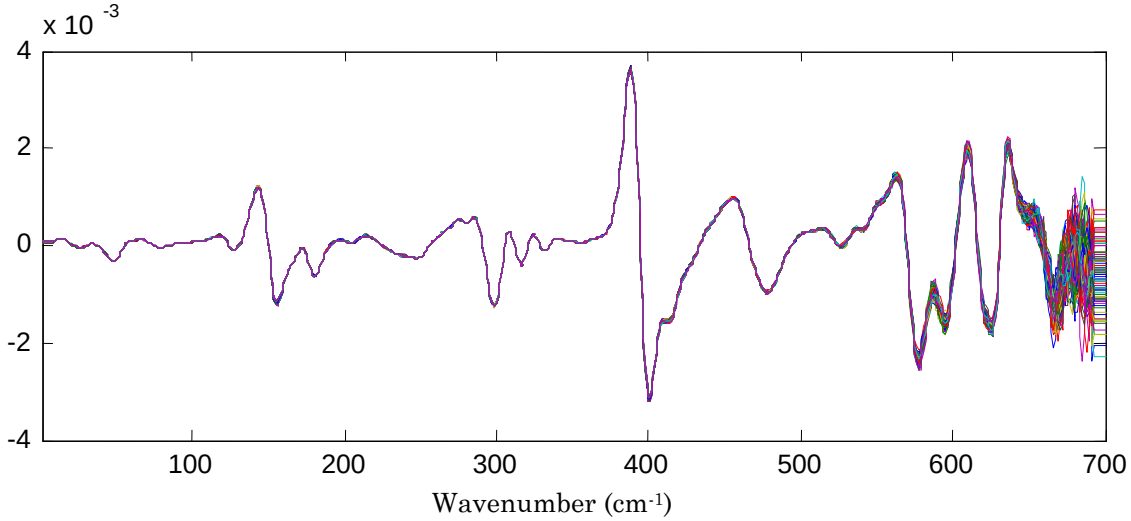
This is a method used to remove a baseline signal from samples. Derivatives are usually used where lower-frequency features such as baseline interferences and higher-frequency contain the signal of interest. To find the first-derivative, each variable is subtracted from its neighboring variables. This removes the signal that is the same between the two variables, and leaves only the signal which is different. The second-derivative will repeat the process, and will further highlight higher-frequency features. A disadvantage of derivatives is that they amplify noise substantially. This drawback can be overcome by using Savitzky-Golay coefficient. The first and second derivative effects are shown in the following **Figure 8**.



A)



B)



C)

Figure 8. NIR spectra A). The first derivative of the spectra B) and the second derivative of the spectra C).

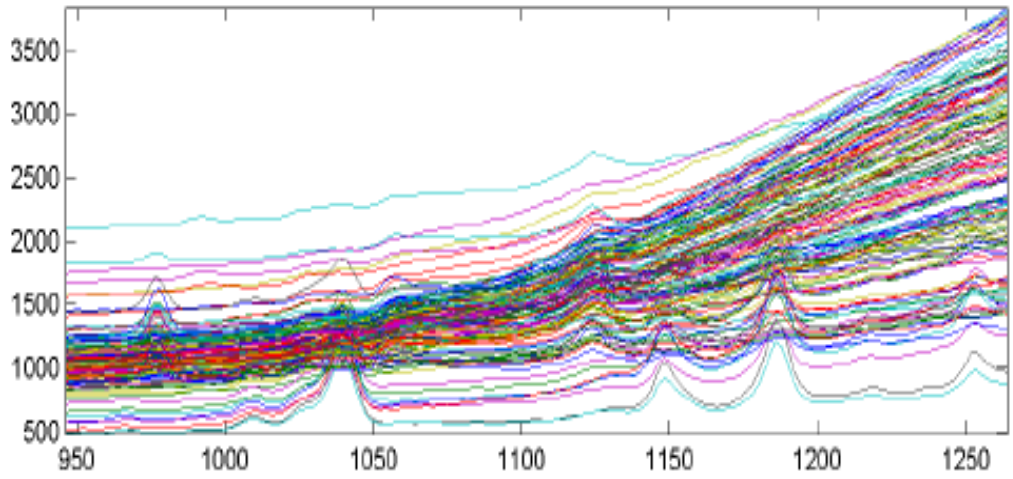
An important feature of derivative methods is that they do not affect any linear relationship within the data.

2.3.2.4 Baseline correction (Weighted Least Squares)

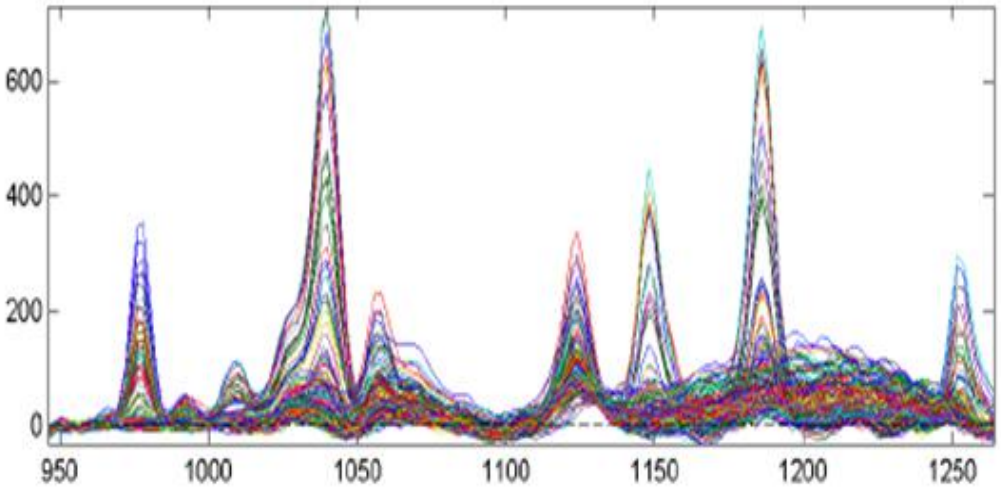
This method is widely used in spectroscopy when the signal is dominated by background. Baseline correction can be considered as the first step preprocessing method since the other approaches look for variation above a baseline. If baseline correction is not done then irrelevant information can be introduced.

The Weighted Least Squares method determines which points are due to the baseline alone. It does this by fitting a baseline to each spectrum and determining which variables are above the baseline and which below the baseline. Usually the peaks below baseline will be fitting

to the baseline. For this reason this method is also called asymmetric weighted least squares. The effect of Weighted Least Squares baseline method is shown in the following **Figure 9.**



A) Wavenumber cm^{-1}



B) Wavenumber cm^{-1}

Figure 9. Effect of Weighted Least Squares baseline on Raman spectra. A) The original data and B) baseline corrected data are shown.[58]

2.3.2.5 Fluorescence correction

Differences in baseline in Raman spectra are mainly due to variable fluorescence in the samples. Fluorescence is a different optical process than Raman scattering and should be removed. This will not affect the Raman scattering information and may bring all samples to the same baseline.

Here the fitting process is an iterative 4^{th} order polynomial fit (Appendix E). The first fit is made to the original spectra in X, and the polynomial (Xf) is compared to the original spectra: if the value of variable of the

polynomium is higher than the value of the original spectrum, the value of the polynomial is replaced by the value of the original spectrum.
This is the first modified polynomial, Xf.
Then a new polynomial is fitted to the first modified polynomial and so on. Here 15 iterations are used. The illustration of this process is given in the following figures.

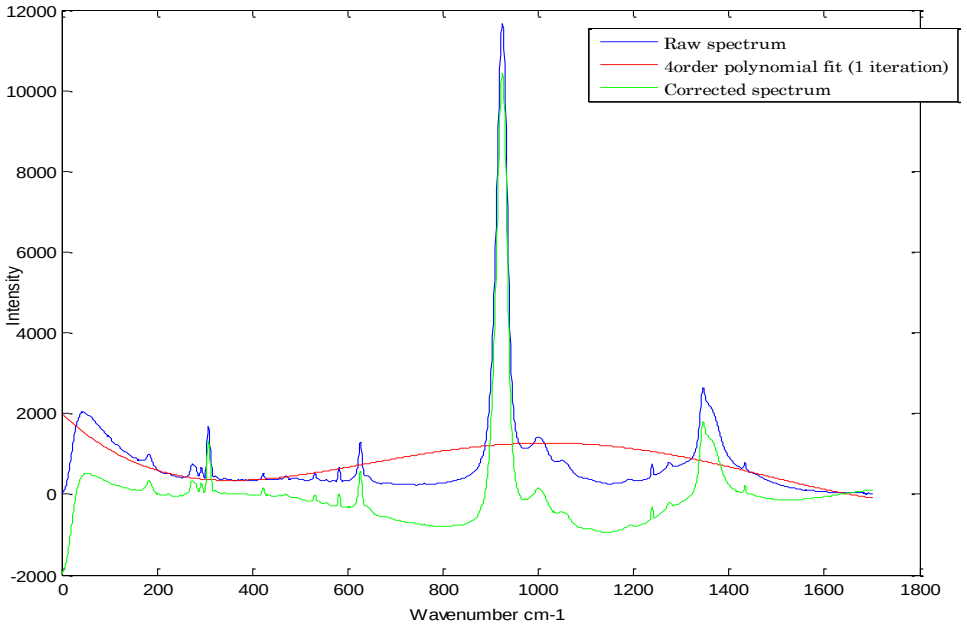


Figure 10. First fit of the spectrum.

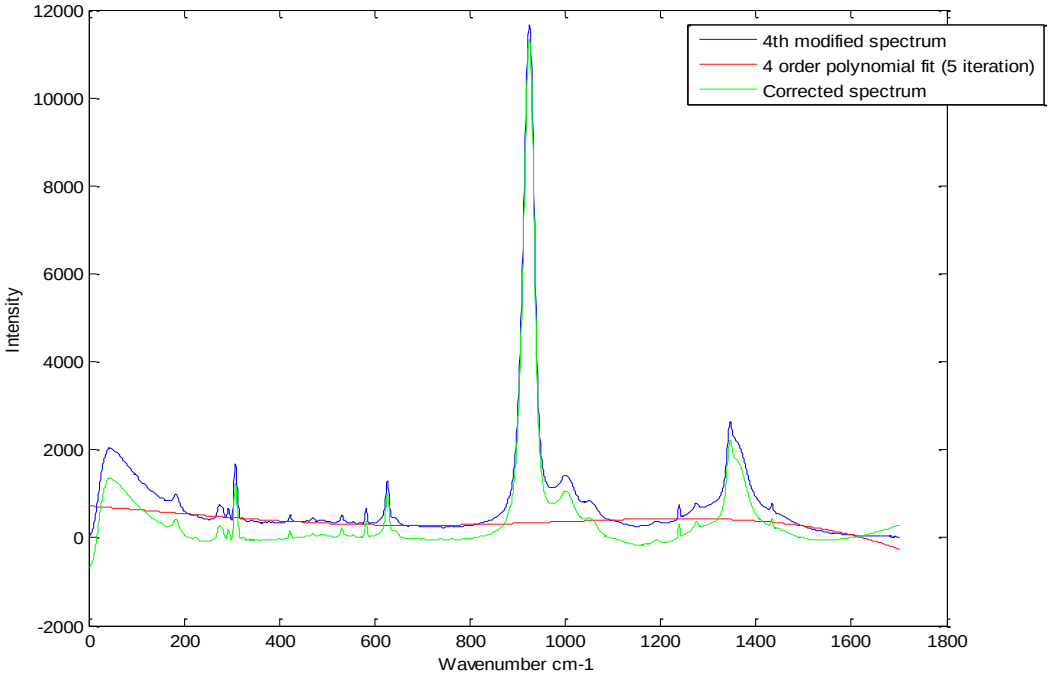


Figure 11. Fifth fit of the spectrum.

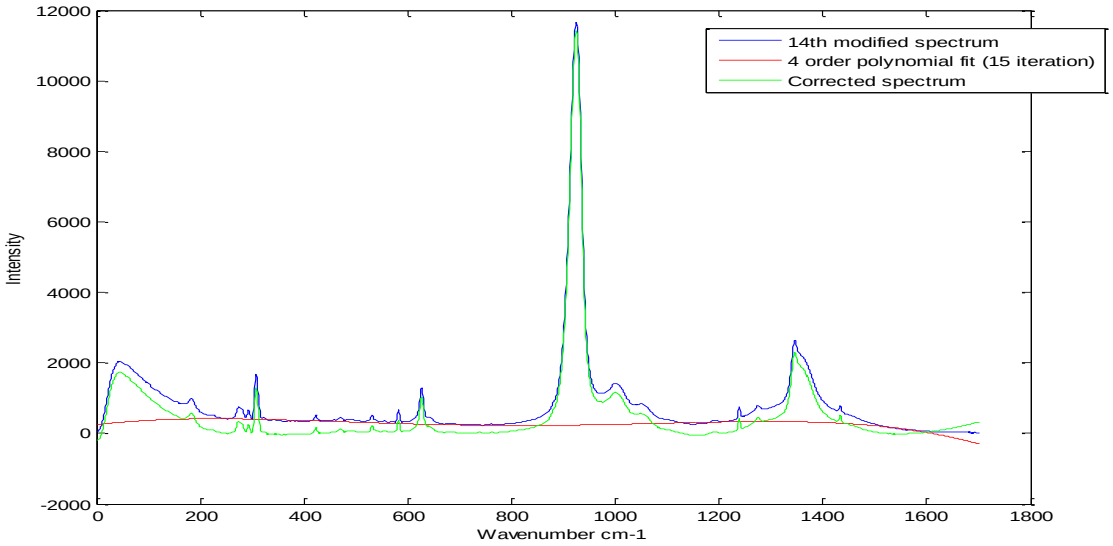


Figure 12. Fifteenth fit of the spectrum.

2.3.3 Principal Component Analysis (PCA)

PCA is one of the most widespread multivariate analysis techniques. It often serves as a simplification technique. PCA was developed by Karl Pearson in 1901.[59] This technique aimed to reduce the dimensionality of data set in such a way that from correlated variables it produces a new set of variables called principal components. These are linear functions of the original variables and are uncorrelated (orthogonal to each other). Usually the number of principal components is less than the original variables. Nonetheless they retain most of the sample information. This new set of variables is defined in such a way that the first principal component explains the greatest variance as possible; the second principal component explains the second greatest variance and so on. A graphical view of PCA is shown in **Figure 13.**

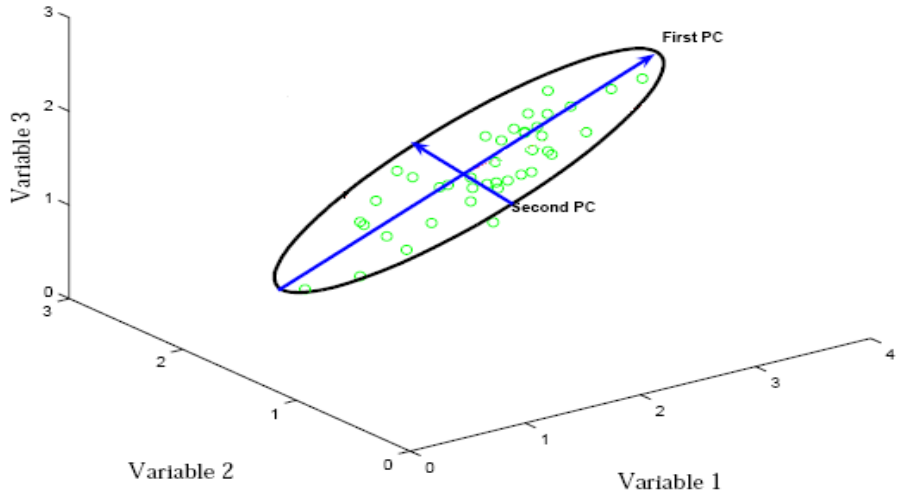


Figure 13. Graphical representation of Principal Components Analysis.[60]

The first principal component explains the greatest variance, which is described with the major axis of the ellipse (**Figure 13**), whereas the second principal components explain the second greatest variance, which is describe with the minor axis of the ellipse (**Figure 13**). The essence of the PCA technique is that variation can be displayed by fewer variables than the original variables. A data matrix has two types of variation, between-object variation which is described by scores and within-object variation which is described by loadings.

If we have data matrix **X** (j*k), with j rows representing sample (spectrum) and k columns representing variables (wavelength), then PCA will transform the **X** matrix as the sum of r t_j and p_k:

$$\mathbf{X} = \mathbf{t}_1\mathbf{p}^T_1 + \mathbf{t}_2\mathbf{p}^T_2 + \ldots + \mathbf{t}_r\mathbf{p}^T_r = \sum_{r=1}^{R} t_{jr}\, p_{kr}\,;$$

where r is the number of significant components in a dataset; $\mathbf{t}_j$ vectors are known as scores, which contain information on how the samples are related to each other and $\mathbf{p}_k$ vectors are known as loadings, containing information on how the variables are related to each other, also the loadings value $\mathbf{p}_{rj}$ explains how much the variable j contribute to the rth principal component.

The illustration of data matrix **X** transformation is given in the following **Figure 14.**

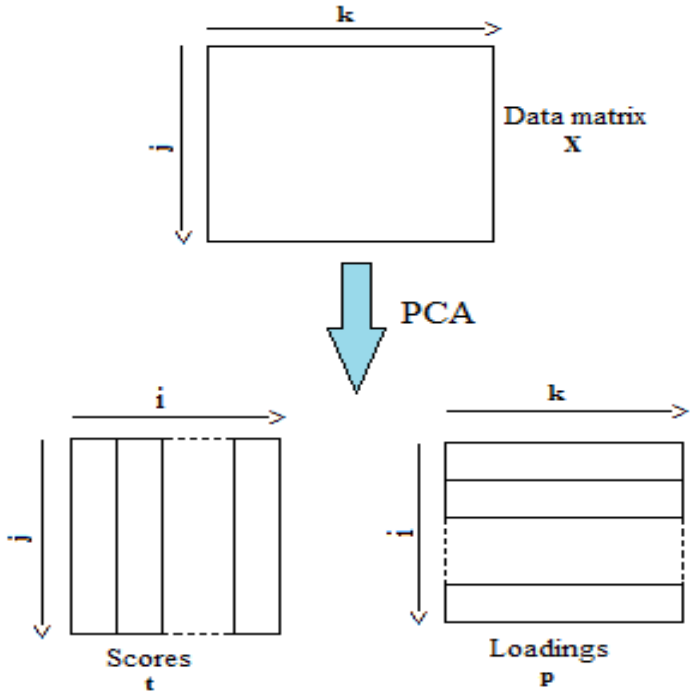


Figure 14. Data matrix transformation with PCA into scores and loadings.[59]

After constructing a PCA model, there always remains small unexplained variance, which will be included into a residual matrix **E**:

$$\mathbf{X} = \mathbf{t}_1\mathbf{p}^T_1 + \mathbf{t}_2\mathbf{p}^T_2 + \ldots + \mathbf{t}r\mathbf{p}^T_r + \mathbf{E}$$

The first scores and loadings vector are called the eigenvectors of the first principal component, and the size of each component is called eigenvalue (g_r), which is defined as the sum of squares of the scores:

$g_r = \sum_{k=1}^{K} t_{kr}^{\ 2}$, where g_r is the rth eigenvalue.

In the PCA transformation, the $\mathbf{p}_1$ vectors are eigenvectors of the covariance matrix, as following:

$\mathrm{cov}(\mathbf{X})\mathbf{p}_1 = g_r\mathbf{p}_i$, where g_r is the eigenvalue, which measure the amount of variance described by the $\mathbf{t}_i$, $\mathbf{p}_i$.

Scores and loadings vectors have the following property:

$\sum_{k=1}^{K} t_{ka} * t_{kb} = 0$ and $\sum_{j=1}^{J} p_{aj} * p_{bj} = 0$, where a ≠ b, which mean that both scores and loadings form an orthogonal set, while the loadings vector are also orthonormal: $\sum_{j=1}^{J} p_{aj}^2 = 1$.

2.3.4 Partial Least Squares (PLS)

Another multivariate analysis technique is PLS, which belongs to the regression and prediction group. PLS can be considered a method for constructing predictive model where the measured variables are many and highly correlated. PLS tries to predict the responses and focus is not on understanding the relationship between variables. PLS has been widely used in monitoring and controlling industrial processes.

The aim of PLS is to explain as much as possible covariance between the independent variables or predictors (**X**) and the dependent variables or response (**Y**) using a small number of uncorrelated variables called components or latent variables. The goal of PLS is to predict **Y** from **X** and to describe their common structure. In comparison with other regression methods like principal component regression (PCR), which tries to capture the greatest amount of variance in the independent (**X**) variables, or multiple linear regression (MLR) which tries to find a single factor that correlates independent variables (**X**) with dependent ones (**Y**). The PLS tries to preserve the asymmetry of relationship between predictor and predicted variables whereas the other technique treats them symmetrically.

The main purpose of PLS is to build linear model $\mathbf{Y} = \mathbf{Xb} + \mathbf{E}$, where **Y** is a response matrix, **X** is a predictor matrix, **b** is a regression coefficient matrix and **E** is a residual matrix.

Multivariate methods such as PCR assume that the concentration estimates are error free. In comparison, PLS takes into account errors in both concentration estimates and the spectra. This can be considered a valuable feature.

There are different algorithms approaches that can be used to calculate orthogonal latent vectors in PLS. Among the others are nonlinear iterative partial least squares (NIPALS)[61] and SIMPLS[62].

The aim of PLS is to predict the responses in the dataset by the use of latent variables derived from measured and responses variables. The latent variables obtained from the measured variables are used to estimate the parameters in the model which are used to construct the prediction of the responses.

2.3.5 Design of experiments (DOE)

Experimental of design is the process of planning and organizing experiments. It takes time and effort to organize the process properly to ensure that the right type of data, and enough of it, is available to satisfy our interest as efficiently as possible. Before carrying out experiments, we should identify the variables that we need to investigate. But sometimes there is no need for experimental design in chemistry, if the experiments are relatively quick and can be repeated under different conditions. However most of experiments are expensive, for example optimizing conditions for synthesis and is essential to use experimental design in such circumstances.

The experimental design can be used for different purposes, such as:

a) Screening, here the experimenters will identify the variables that are important for the process. Common variables are temperature, concentration, and reaction time.
b) Optimization, the design can be used to improve a synthetic yield. The aim here is to define the optimum, where we can have higher yield.
c) Saving time, effective experimentation will maximize the information that we can get from a set of experiments.
d) Estimation of the magnitude of the noise or other random error.

In the present thesis, the aim of using experimental design will cover the first three purposes described above. Firstly we will define the important variables which are temperature, concentration and reaction time. Secondly, we will try to optimize the reaction condition in order to have higher yield. In the end, NIR and Raman spectra are recorded for each of the design samples.

The fundamental parameter that should be taken into account in experimental design is the degree of freedom. The degree of freedom can be determined from:

$$D = N - P$$

where N is the number of experiments and P the number of variables in the model.

The value which relates how well the experiment fits a linear model is called an error. In order to estimate the experimental error it is good to do some replicates. The degrees of freedom are now determined as follows:

$$D = N - P - R$$

where R is the number of replicates.

How many replicates are needed for experiments? As a rule of thumb the number of replicates (R) should be similar to the number of degrees of freedom (D) [60]. It is important to define the reasonable number of replicates that should be done in order not to lose the crucial information.

It is recommended that for an appropriate experimental design for a given model approximately six more experiments than the number of parameters included in the model are needed.[63]

In case that we have limited information for the optimum condition of reaction and we want to optimize it sequential design is a preferable choice.[64] Among the several kinds of sequential designs the most known and powerful is the simplex design.[65] Another type of design is the simultaneous design where the central composite design belongs.

In the present thesis a kind of central composite design was used. The central composite design (CCD) consists of 2^k factorial points (k is the number of independent variable), 2k axial points and the center point. The total number of experiments D can be defined as $D = 2^k + 2k + l$.

2.3.6 Chemometrics applications in spectroscopy

Combining of spectroscopic techniques, especially such as online NIR[66,67,68,69,70] and Raman[71,72,73] spectroscopy, with multivariate methods represents an extremely powerful tool which can provide highly useful information to the process under study. This combination has served as a driving force for US FDA (Food and Drug Administration) which recognized the PAT (Process Analytical Technology) as a good practice.[74] Nowadays the trends in analytical spectroscopy are the incorporation of multivariate analysis techniques and instrumentation into the computers that can be used to extract the useful information from raw spectral data. Lately, the usage of this combination has been crucial due to the vast amount of data from the computerized instrumentation.

Even if it is not possible to find the differences by visualization between two consecutive spectra by using the multivariate methods it is possible to determine how different these two spectra are. So, the chemometrics tools serve as a bridge between the chemical system and the measurements of the system.

2.4 Gas chromatography and mass spectrometry (GC-MS)

The combination of these two techniques is widely used after the 1950s. This combination was developed by Roland Gohlke[75]. This was a successful and a compatible combination, where GC was used for separation of volatile and semivolatile compounds with great resolution and MS for identification on most of compounds. In the present thesis the GC-MS was used as a validation[76,77,78] technique for the identification, quantification and confirmation of our product of interest by determination of their molecular weights.

A briefly described working procedure follows. Gas chromatography in this combination is used to separate mixtures of chemicals into individual components. The separation occurs when the sample mixture is injected into a mobile phase which is an inert gas such as helium, nitrogen or hydrogen. The mobile phase carries the sample mixture through the stationary phase called column which can be glass or steel. The separations happen due to the different rate interaction of each compound in the mixture with column. The compounds that interact less will elute first and those which interact the most with the column will exit last. The time from when the injection was made to when elution occurs is called retention time. By changing the temperature of stationary phase or the pressure of the mobile phase different mixtures can be separated. If the GC conditions are the same a given compound will always elute from the column at nearly the same retention time.

As the compounds elute from the GC column, they enter a detector which is mass spectrometer-electron ionization. In the detector the compounds are bombarded with as stream of electrons causing them to break down into fragments which are charged ions with a certain mass. The ratio of the mass of the fragment divided by the charge is called the mass to charge ratio (M\Z) which represent the molecular weight of the fragment. The mass spectrum can be considered as the fingerprint of the molecule that can be used to identify the compound since it is the same for a given chemical compound at every time.

2.5 Nuclear magnetic resonance (NMR)

NMR is probably one of the most useful techniques, which uses electromagnetic and magnetic fields for determining the structure of organic compounds[79] even though it can be used for any compounds which possess an internal angular momentum (generator of rotations) called a spin. This technique observes the atomic nucleus and not the electrons' properties. It is possible to get the detailed information for dynamics and 3D structure of molecules.

Not all the nuclei have a spin. If the number of neutrons and protons are both even such as ^{12}C the nucleus does not have a spin (I=0). If the number of neutrons and protons are both odd such as ^{14}N the nucleus has an integer spin (I=1, 2, 3). If the number of neutrons or protons is odd such as ^{13}C, the nucleus has a half-integer or a fractional spin (I=1/2, 3/2, 5/2). If the compound has a spin the molecule has magnetic nuclei which absorb and re-emit electromagnetic radiation with specific resonance. That depends upon the strength of the magnetic field and the magnetic properties of molecules.

In the present thesis this technique was used to determine our product of interest to be sure that our product has been formed after the reaction was finished.

3 The Experimental Part

3.1 Chemicals

1-Iodo-2-nitrobenzene (≥97%), 4-chloro-nitrobenzene (≥99%), 2-nitrobiphenyl (≥97%), phenylboronic acid (≥99%), methanol (≥99.9%), acetone (≥99.5%), dimethyl formamide (≥99%), dichloromethane (≥99.9%) and potassium carbonate (≥99%) were purchased from Sigma-Aldrich (Missouri, USA). Another batch of 1-Iodo-2-nitrobenzene (≥97%) was obtained from Sigma-Aldrich (New-Delhi, India). Palladium (II) acetate (≥98%) was purchased from Fulka (Buchs, Switzerland).

3.2 Standard procedure: Synthesis of 2-nitrobiphenyl in various solvents (methanol, acetone and dimethyl fromamide/water)

The preparation protocol has been published elsewhere [2]. Briefly, a solvent (5 mL) and water (1 mL) was transferred to a round bottom flask. Potassium carbonate (K_2CO_3), palladium (II) acetate ($Pd(OAc)_2$), 1-iodo-2-nitrobenzene ($IC_6H_4NO_2$) and phenylboronic acid ($PhB(OH)_2$) were then added in specific concentration as it is shown in **Table 3**. The solution was flashed with N_2 atmosphere and covered with aluminum foil. The reaction mixture was heated (in specific temperature as shown in **Table 3** under stirring. The reaction was monitored on-line with NIR and Raman. Samples were withdrawn in specific time (as shown in **Table 4**), filtered through cotton and silica gel with dichloromethane and analyzed by GC-MS. To be sure that the product formed is our target product 2-nitrobiphenyl, once the product was isolated it was analyzed by NMR as well.

Table 3. Specific temperature and concentration of compounds used in reaction.

No.	**C**	**T**	**K_2CO_3**		**$Pd(OAc)_2$**		**$IC_6H_4NO_2$**		**$PhB(OH)_2$**	
exp.	[mmol/mL]	[^{0}C]	n [mmol]	m [g]	n [mmol]	m [g]	n [mmol]	m [g]	n [mmol]	m [g]
1	0.167	23	2.0	0.274	0.02	0.004	1.0	0.242	1.0	0.118
2	0.167	30	2.0	0.274	0.02	0.004	1.0	0.242	1.0	0.118
3	0.167	50	2.0	0.274	0.02	0.004	1.0	0.242	1.0	0.118
4	0.250	50	3.0	0.411	0.03	0.006	1.5	0.362	1.5	0.177
5	0.333	50	4.0	0.547	0.04	0.008	2.0	0.483	2.0	0.237
6	0.167	55	2.0	0.274	0.02	0.004	1.0	0.242	1.0	0.118
7	0.250	55	3.0	0.411	0.03	0.006	1.5	0.362	1.5	0.177
8	0.333	55	4.0	0.547	0.04	0.008	2.0	0.483	2.0	0.237
9	0.167	60	2.0	0.274	0.02	0.004	1.0	0.242	1.0	0.118
10	0.250	60	3.0	0.411	0.03	0.006	1.5	0.362	1.5	0.177
11	0.333	60	4.0	0.547	0.04	0.008	2.0	0.483	2.0	0.237
12	0.250	57	3.0	0.411	0.03	0.006	1.5	0.362	1.5	0.177
13	0.250	65	3.0	0.411	0.03	0.006	1.5	0.362	1.5	0.177

Table 4. Specific time of withdrawn samples.

No.	T	C	Samples were withdrawn in specific time [min]							
exp.	[^{0}C]	[mmol/mL]	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
1	23	0.167	60	\	\	\	\	\	\	\
2	30	0.167	60	\	\	\	\	\	\	\
3	50	0.167	60	120	180	240	360	480	540	\
4	50	0.250	30	60	90	120	180	240	300	420
5	50	0.333	60	120	180	240	300	\	\	\
6	55	0.167	5	10	15	30	45	60	90	\
7	55	0.250	10	20	30	50	75	90	\	\
8	55	0.333	5	10	15	30	45	60	90	\
9	60	0.167	5	10	15	30	45	60	90	\
10	60	0.250	5	10	15	30	45	60	\	\
11	60	0.333	5	10	15	30	45	60	\	\
12	57	0.250	5	10	15	30	45	60	90	\
13	65	0.250	5	10	15	30	45	60	\	\

3.3 Synthesis of 4-nitrobiphenyl in methanol and water

The preparation procedure was the same as in synthesis of 2-nitrobiphenyl. Methanol (5 mL) and water (1 mL) were transferred to a round bottom flask. Potassium carbonate (2.0 mmol, 0.268 g), palladium (II) acetate (0.02 mmol, 4.4 mg), 4-chloronitrobenzene (1.0 mmol, 0.156 g) and phenylboronic acid (1.0 mmol, 0.118 g) were then added. The reaction mixture was heated at 60, 80 and 100 ^{0}C under stirring. Samples were withdrawn after 1, 6 and 24 h, filtered through cotton and silica gel with dichloromethane and analyzed by GC-MS.

3.4 Crystallization procedure of substrate (new 1-iodo-2-nitrobenzene)

Crystallization procedure is described as follows. Two round bottom flasks, one with substrate and another with solvent (dichloromethane) were placed into the oil bath, which were heated in 70 ^{0}C. A small amount of the hot solvent, enough to dissolve it, was added into the flask containing the substrate. The flask was removed from the bath and allowed to cool in room temperature. The solution was filtered with filter paper of 20mm and was left in fume cupboard for 24h. Synthesis of 2-nitrobiphenyl was done, following the same protocol as above described.

3.5 Washing procedure of substrate (new 1-iodo-2-nitrobenzene)

The substrate was transferred in the flask. The mixture of hexane and diethyl ether (4:1) were then added. The mixture was stirred and filtered through the silica gel. The resulting solution was evaporated under reduced pressure to remove the solvent. Synthesis of 2-nitrobiphenyl was done following the same protocol as above described.

3.6 Workup procedure of product

Water (30 mL) was added to a crude product obtained after evaporation of methanol under reduced pressure. The solution was extracted with dichloromethane (3x30 mL). The resulting organic solution was washed with saturation aqueous solution of sodium chloride (30 mL). The organic solution was evaporated under reduced pressure; the target product 2-nitrobiphenyl was obtained as dark yellowish oil.

3.7 Instrumentation

3.7.1 NIR Spectroscopy

The spectrophotometer used in this study was Perstorp NIR Systems 6500 (Maryland, USA). Measurements were made using the liquid probe-of the fiber optic module. The conditions for recording the spectra were number of scans 32, the resolution 4 cm^{-1}, spectral range used from 1100-2500 nm. The path length was set to 0.5 mm using at measuring feeler gauge of 0.5/2 mm. An average of about 20 sec was required to obtain one spectrum. First, the background spectrum was collected and then using the same parameters sample spectra was collected.

Instrument control software was the Vision (FOSS NIRSystems, Inc., Maryland, USA). The spectrum data was exported as an ASCII file. The Sirius 8.1 (PRS, Bergen, Norway) was used for statistical analysis. The computation was carried out on a Microsoft Windows 7 Professional 2009 operating system (Microsoft Corporation, Washington, USA).

3.7.2 Raman Spectroscopy

Raman scattering spectra were recorded on a RamanRxn1 analyzer (Kaiser Optical Systems, Inc., USA) type spectrometer. Measurements were made with an immersion ball probe[80] connected with the instrument via a fiber optical cable. The laser wavelength and power on the sample were 785 nm and 100 mW respectively. The spectral range of the instrument was from 0-3800 cm^{-1}. Acquisition settings were exposure time 2 sec, 2 accumulations.

HoloGram 4.1 (Kaiser Optical Systems Inc., Michigan, USA) was used as the instrument control software. The recorded spectra were exported using HoloReact 2.0 (Kaiser Optical Systems Inc., Michigan, USA) to Matlab 7.4 (The Math Works Inc., Massachusetts, USA). The Raman spectra were combined into one data matrix (D_{ixj}), where (i) represents rows (spectra) and (j) represents columns (frequencies). The Sirius 8.1 (PRS, Bergen, Norway) was used for statistical analysis. The

corresponding computation was carried out on a Microsoft Windows 7 Professional 2009 operating system (Microsoft Corporation, Washington, USA).

3.7.3 FT-IR Spectroscopy

FT-IR spectra were obtained by using Nicolet 460 Protégé FT-IR spectrometer in spectral range: 550-4000 cm^{-1} in the reflectance mode at room temperature. The substrates were placed on a diamond crystal and the spectra were recorded with a resolution of 4 cm^{-1} and number of scans 32.

Omnic (Thermo Fisher Scientific Inc.) was used as the instrument control software.

3.7.4 GC-MS

GC-MS data were obtained using a HP 5890 II gas chromatograph coupled with HP 5971 mass spectrometer, equipped with fused silica capillary column (30 m x 0.2 mm I.D.) coated with a 0.25 μm film thickness of Chrompack, CP-Sil 8 CB low bleed/ MS.

The temperature program was as follows: at the beginning was 50 ^{0}C, ramped to 70 ^{0}C at 5 ^{0}C/min and, ramped to 300 ^{0}C at 25 ^{0}C/min. Direct on column injection was used, and the injector port temperature was 250 ^{0}C. Helium was used as carrier gas. The percentage of compound was computed directed from the GC peak areas since the response factor calculated from the three measurements of product and substrate is around 1 (0.946). Also, based on chromatogram the reactant change only to the product, there were no sign of peaks in chromatogram apart from the reactant and product.

Xcalibur 1.2 (Thermo Fisher Scientific Inc.) was used as software for instrument control and data analysis of chromatogram.

3.7.5 NMR Spectroscopy

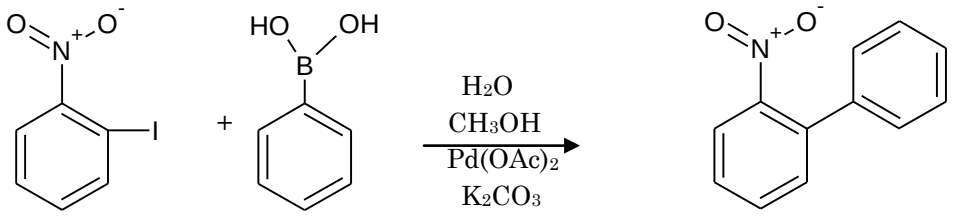
NMR spectra were recorded on a Bruker BioSpin DPX400 (^{1}H: 400.13 MHz, ^{13}C: 100.61 MHz) spectrometer. Deuterated chloroform ($CDCl_3$) was used as solvent for the preparation of the samples and the internal standard. The spectra were recorded at room temperature. The chemical shifts are expressed in ppm values relative to tetramethylsilane (TMS). Multiplicities are reported using the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), dd (double doublet) and m (multiplet).

Spectral data were processed with MesReNova 5.2 (Santiago de Compostela, Spain).

4 Results and discussion

4.1 Optimizing reaction

The reaction protocol described elsewhere[2] was used as a model procedure for running reaction which was chosen to be monitored using NIR and Raman Spectroscopy. The reaction can be observed in the following **Scheme 4.** In this scheme the synthesis of 2-nitrobiphenyl is described as a product.



Scheme 4. Synthesis of 2-nitrobiphenyl as product.

Based on the literature[2], a yield of 99 % was achieved after 90 min in 20 ^{0}C. Using the same protocol we got the yield of 99 % after 60 min in 23 ^{0}C. Since the substrate was used up a new bottle was ordered. Surprisingly, it was impossible to achieve the same yield using the same, supposedly identical substrate.

After several attempts with new substrate the yield was very low. Trial reaction using 10 % of old substrate and 90 % of new substrate provided the yield of 7 % after 1 h. By contrast, using 90 % of old substrate and 10 % of new substrate provided the yield of 99 % after 1 h. These completed reactions are presented in **Table 5** as follows:

Table 5. Overview of the reaction features with New and Old substrate for temperature of 23 ^{0}C.

No exp.	Substrate	T [^{o}C]	Time [min]	Yield [%]	Color description
1	[a]Old substrate 97%	23	60	99	Orange-brown
2	[a]Old substrate 97%	23	60	95	Orange-brown
3	[b]New substrate 97%	23	60	4	Yellow-brown
4	[a]Old Substrate 97%	23	60	99	Orange-brown
5	[b]New substrate 97%	23	60	5	Yellow-brown
8	[a]Old Substrate 97%	23	60	5	Orange-brown
6	10% Old subs. & 90% New subs.	23	60	7	Yellow-brown
7	90% Old subs. & 10% New subs.	23	60	99	Orange-brown

[a]Old substrate (Batch No. 06918DB)-the substrate that we had on the lab;

[b]New substrate (Batch No. BCBD7259V)-the substrate that we ordered;

Chromatograms for these reactions can be observed in Appendix A.

These disappointing results with the new substrate led us to investigate possible factors that affected the reaction yields.

We follow the approach of determining whether any of the following exists:

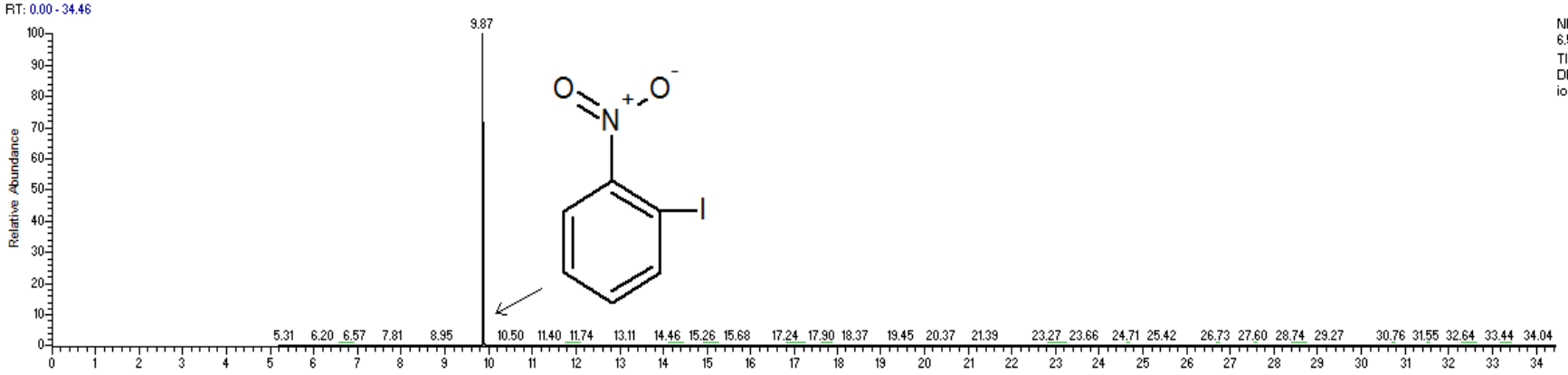
1. support compound/catalyst in old substrate that contributes to the reaction yields or
2. inhibitor compound in new substrate that prevents the reaction to proceed giving low yields.

The first change that can be observed between the new and the old substrates was the color. The old substrate had a dark orange color while the new one was yellow.

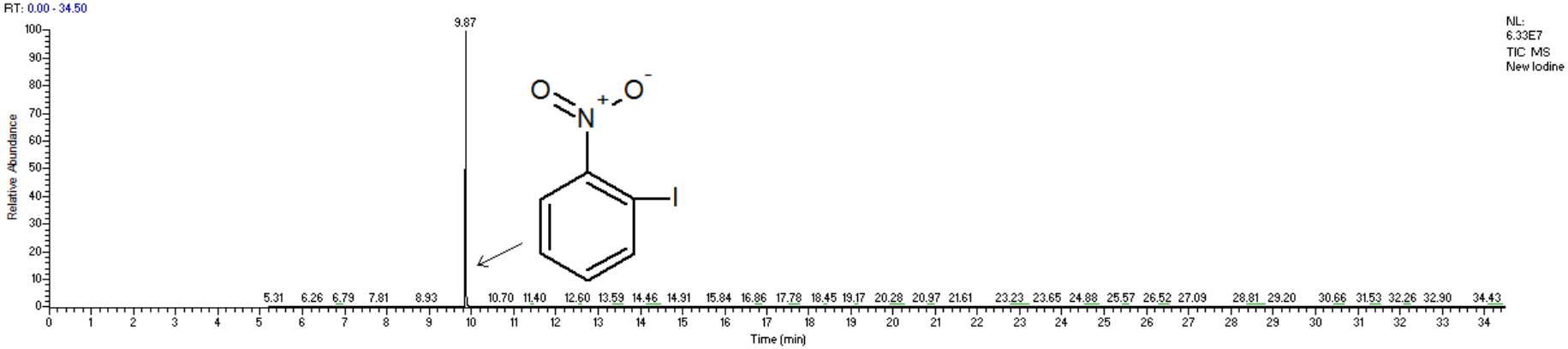
However, the MSDS (Material Safety Data Sheet) of Acros Organics company (Geel, Belgium) and Sigma-Aldrich (Missouri, USA) indicates that 1-iodo-2-nitrobenzene can have from yellow-green to yellow-brown color[81], and yellow to brown color[82] respectively.

Our research was focused on the analysis of the new and old substrates using the equipment available in the laboratory. Thus we started the analysis of GC-MS hoping that we will be able to detect some differences between them.

GC-MS is a useful tool for quantitative and qualitative analysis of unknown compounds, especially of volatile compounds. We were surprised that examination of chromatograms did not reveal any differences between these two substrates, as is shown in the following **Figure 15.**



A).



B).

Figure 15. Chromatogram of 1-iodo-2-nitrobenzene dissolved in DCM, (A) old substrate and (B) new substrate. No significant differences were observed between the mass spectra of old and new substrates.

Since no changes were observed between the two substrates using GC-MS, we proceeded with their FT-IR analysis. It is possible to do the qualitative analysis of any type of material with FT-IR due to the fact that various materials have a unique combination of atoms, therefore generating unique IR spectrum. Two different samples will not give exactly the same infrared spectrum. The IR spectral overlap of the old and new substrates are shown in **Figure 16** and the magnified area showing the finger print region is shown in **Figure 17**.

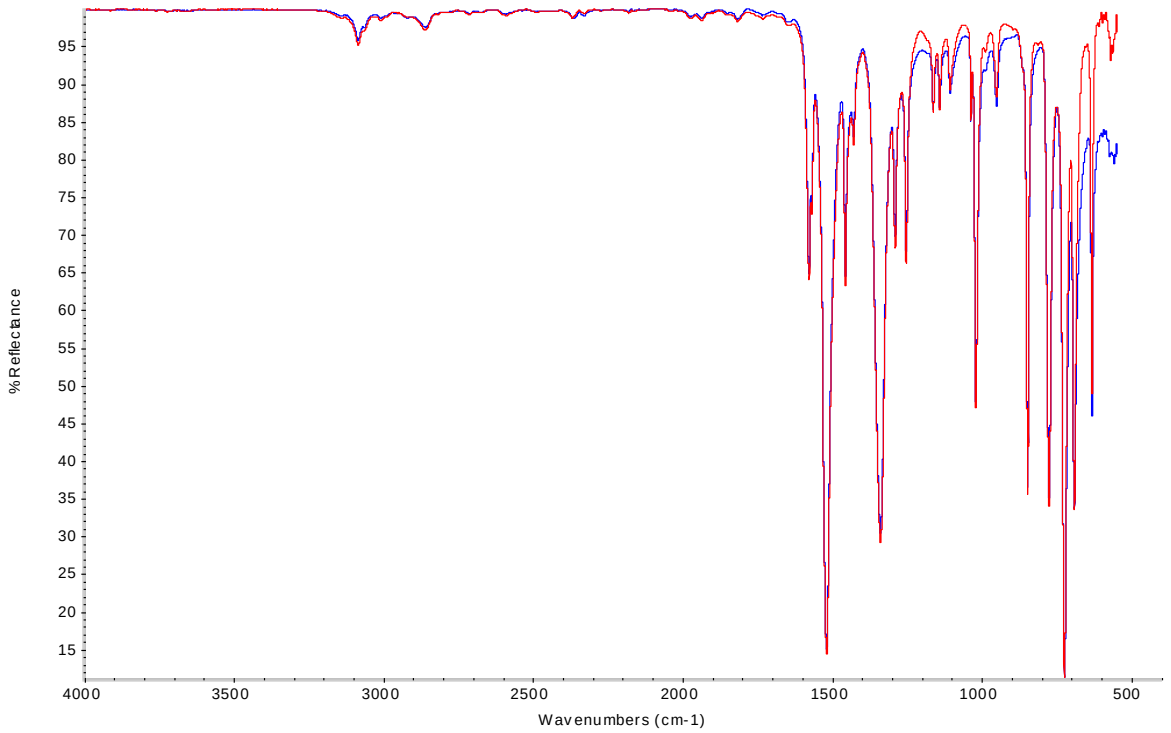


Figure 16. FT-IR spectrum of new substrate (blue) and old substrate (red) after dissolving in DCM.

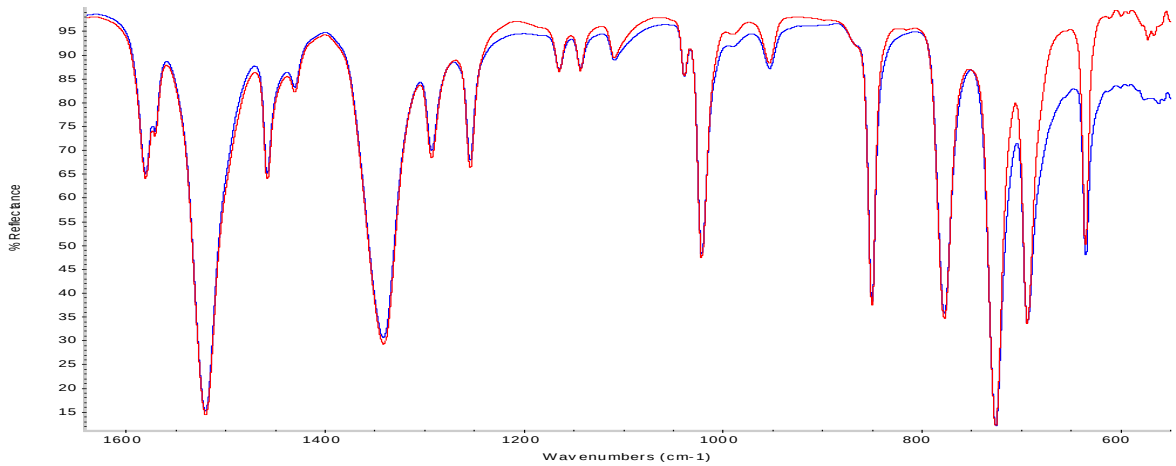
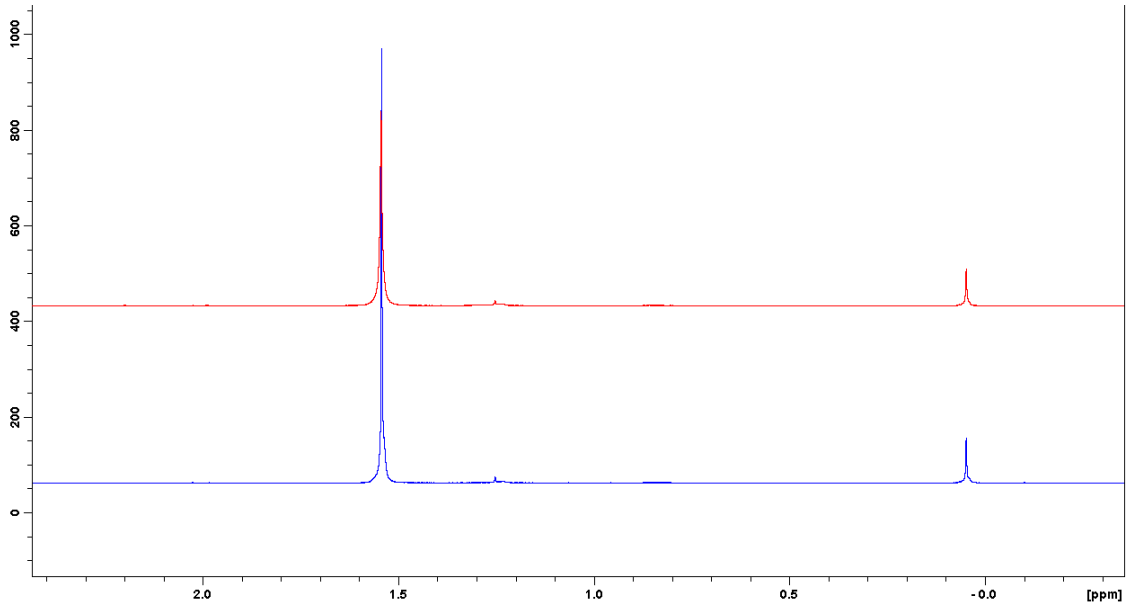


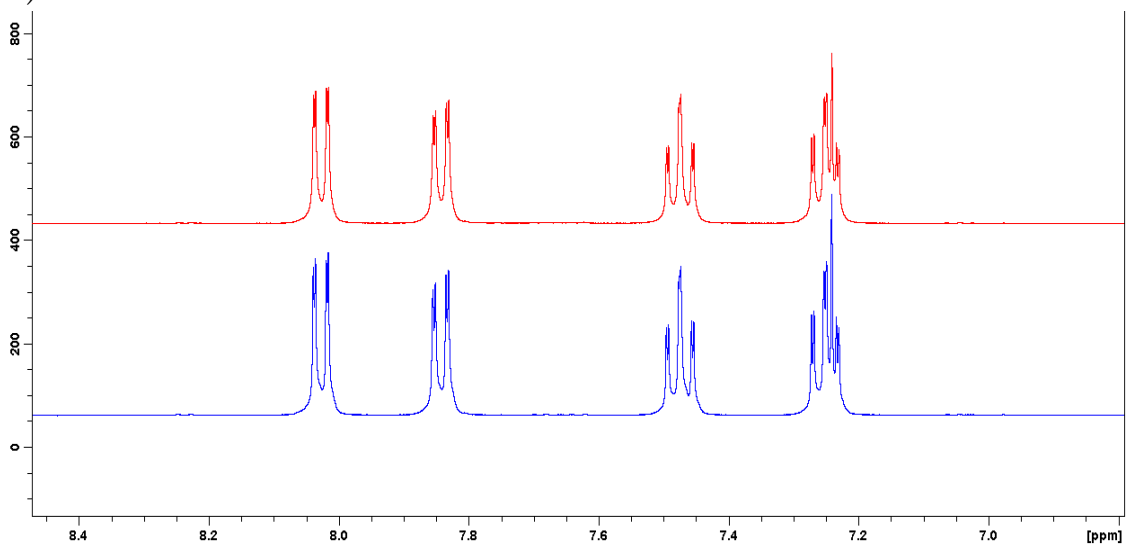
Figure 17. Zoom in the specific region (finger prints) of the spectra shown in the **Figure 16**.

Our expectations were to observe some change in FT-IR spectrum between them. But to our surprise as observed in **Figure 16** and in **Figure 17** respectively no change assignable to chemical differences can be observed between these two substrates. As it can be observed in **Figure 17** that shows the enlarged IR spectrum in the region 1600-550 cm^{-1}, it is difficult to notice any changes between the two spectra. Even though the spectra are not 100% overlapping with each other, the same peak positions were observed.

The third analysis was performed using NMR. This technique enables the analyst to obtain the detailed dynamic, topologic and 3D arrangement of the molecule in the sample.



A).



B).

Figure 18. NMR spectrum of new substrate (blue) and old substrate (red) solved in deuterated chloroform. A). aliphatic region and B). aromatic region of the spectra.

The NMR spectra are shown in **Figure 18** and comparison of these spectra shows no observable changes.

It is worth mentioning that efforts have been made to use the Atomic Absorption (AA) instrument in our further analysis that would enable us to observe any metal or metalloid that may be potentially present in any of the substrates. This was not possible despite some efforts to initially dissolve the substrate in methanol, evaporate it in rotary evaporator, and to dissolve it in water for AA analysis. The last procedure gave a heterogeneous suspension that render the sample not suitable for AA analysis.

Based on the raised supposition that some component is inhibiting the new substrate we continued our work in the laboratory. Initially we wanted to be sure that with the new substrate it would be possible to realize the reaction therefore we made a reaction in the Microwave reactor and it ended successfully. Additional experiments were then conducted using the standard procedure[2] following one of the changes listed below:

a) the possible catalyst was added such as iodine;
b) the ligand for catalyst orientation was incorporated such as aniline[83],
c) the quantity of palladium (II) acetate as a catalyst was doubled; or
d) applying the standard procedure using the purified substrate.

In a summarized manner all these reactions are presented in **Table 6**, by actions undertaken the production of reaction was not improved.

Table 6. Summary of experiments run in different conditions.

No exp.	Substrate	T [°C]	Time [min]	Yield [%]	Color description
1	New substrate 97%	23	60	0	Yellow-brown
2	New substrate 97%	23	60	7	Yellow-brown
3	New substrate 97%	100	10	100	\
4	New substrate 97%	23	60	0	Brown
5	New substrate 97%	23	60	10	Yellow-brown
6	New substrate 97%	23	60	0	Dark-brown
7	New substrate 97%	23	60	9	Dark-brown

[1] The substrate was crystallized with DCM;

[2] The substrate was washed with hexane/ethyl acetate (4:1) through the silica gel;

[3] The reaction was run in Microwave reactor;

[4] 0.001g of iodine was added;

[5] The amount of catalyst used was doubled;

[6-7] 6 drops of aniline were added;

Chromatograms that correspond to **Table 6** can be observed in Appendix B.

In the course of efforts to find optimal reaction conditions the proportion of the solvents were varied. Instead of methanol/water (5:1) as it was in protocol, we tried to change this proportion to (5:2) or to use only pure methanol.
Summary of these reactions is shown in the following **Table 7.**

Table 7. Summary of experiments in different solvents

No exp.	Substrate		Solvent	T [°C]	Time [min]	Yield [%]	Color description
1	New substrate	97%	CH_3OH/H_2O (5:2)	23	60	0	Black color
	New substrate	97%	CH_3OH/H_2O (5:2)	23	360	0	Black color
	New substrate	97%	CH_3OH/H_2O (5:2)	23	480	0	Black color
	New substrate	97%	CH_3OH/H_2O (5:2)	23	1440	6	Black color
2	New substrate	97%	CH_3OH	23	60	0	Orange-brown

Chromatograms of these reactions can be observed in Appendix C.

The change of proportion between methanol and water or the use of only methanol as a solvent did not increase the productivity of our reaction.

Considering the fact that the substrate 1-iodo-2-nitrobenzene is very expensive compared to chlorobenzene we tried as a substrate to use 4-chloronitrobenzen. The reason that we chose 4-chloronitrobenzene and not 2-chloronitrobenzene was the idea that chlorine in para position would be more reactive than in ortho position. These experiments are shown in **Table 8.**

Table 8. Summary of the reaction using 4-chloronitrobenzene as substrate.

No exp	Substrate	T [°C]	Time [min]	Yield [%]	Color description
1	4-chloronitrobenzene	60	60	0	Dark-brown
2	4-chloronitrobenzene	70	60	0	Dark-brown
	4-chloronitrobenzene	70	1440	0	Dark-brown
3	4-chloronitrobenzene	80	60	0	Dark-brown
	4-chloronitrobenzene	80	1440	0	Dark-brown
4	4-chloronitrobenzene	100	10	0	Dark-brown
	4-chloronitrobenzene	100	60	2	Dark-brown

From the above results we observed that in experiments where 4-chloronitrobenzene was used as a substrate the reaction was unsuccessful despite the fact that the temperature was increased gradually from 60-100 ^{0}C. As it was reported in literature [17,18] that ArCl is less reactive compared to ArI, this was confirmed in our experiments.

Since 4-chloronitrobenzene did not give good results we came to the conclusion to retain as substrate the 1-iodo-2-nitrobenzene and optimize the reaction by changing the temperature. The summary of these reactions can be observed in **Table 9**. As we can observe for 60 min in 60 °C the reaction yield 90 % was obtained therefore we can say that the reaction is optimized.

Table 9. Feature of optimizing reaction.

No exp.	Substrate	T [°C]	Time [min]	Yield [%]	Color description
1	New substrate 97%	50	60	3	Orange-brown
	New substrate 97%	50	360	89	Orange-brown
2	New substrate 97%	60	60	90	Orange-brown

Reaction in 60 °C is monitored with NIR and Raman spectroscopy.

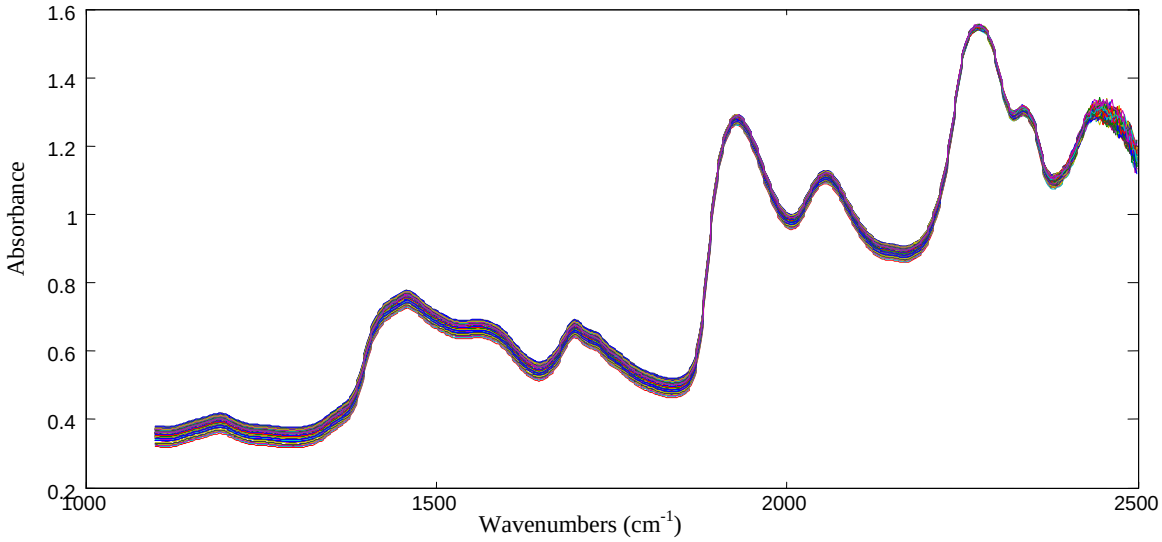


Figure 19. NIR spectra in 60 °C.

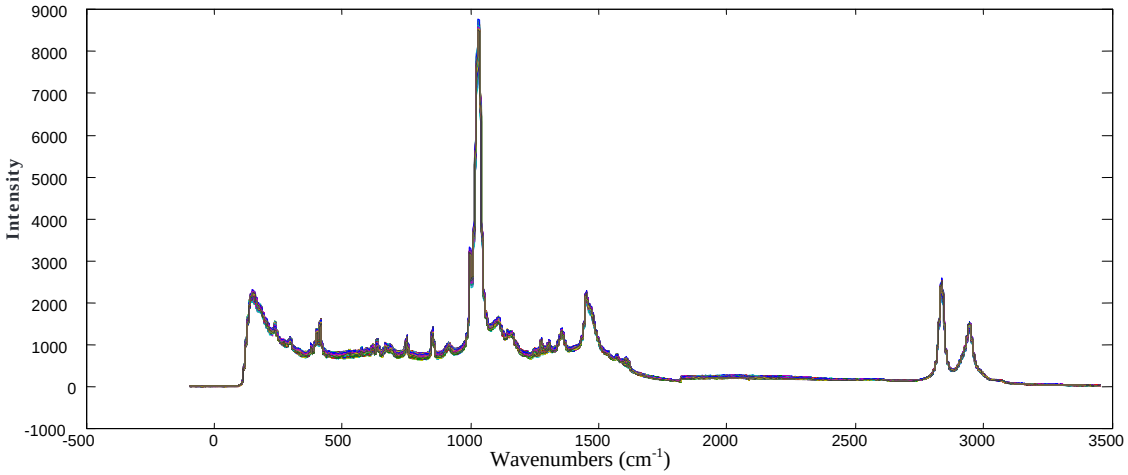


Figure 20. Raman spectra in 60 °C.

In **Figure 19** NIR spectrum is presented from the monitoring of the reaction at 60 ^{0}C. Noticeable changes can be observed but we cannot attribute these as due to the appearance of the product. PCA analysis of the NIR date revealed that there was no change in spectra due to the presence of product. The Raman spectra (**Figure 20)** shows that in the region (1220-1340 cm^{-1}) we have some changes that can be attributed to the presence of our product (2-ntirbyphenyl), and not on our reactants (1-iodo-2-nitrobenzene halide or boronic acid). To see more clearly the region is magnified and presented in **Figure 21** A).

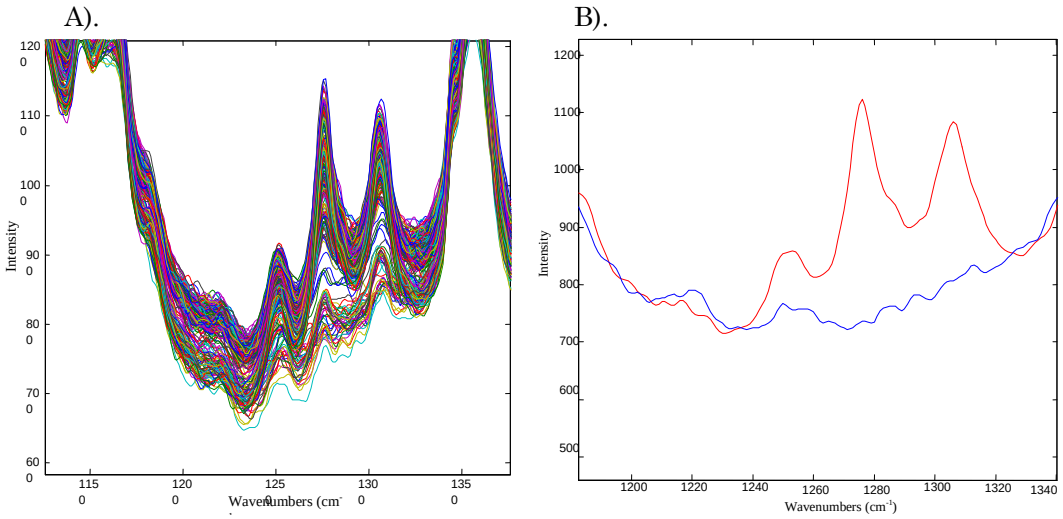


Figure 21. A). Zoom-in the specific region of Raman spectrum, B). first spectra (blue) and last (red).

In **Figure 21** B) the first spectrum (blue) correspond to the condition when the substrate was present in the reaction. The red color represents the last spectrum that shows the presence of the product in reaction. The emergence of the peaks at the specific region (1220-1340 cm^{-1}) in the last spectrum shows that product is formed. Therefore this region is important to tell if our product is formed or not. This is also confirmed by GC-MS and NMR analysis which showed the characteristic peaks due to the product formation. GC-MS chromatogram and NMR spectra are presented in Appendix D.

Also we treated this specific region (1220-1340 cm^{-1}) with PCA as an exploring technique but previously the data matrix (271x120) was pre-processed by mean-centering and 1st derivative method. The first component (PC1) explains the 76.95 % of the total variance. From sample-score plot it is possible to establish the profile of the time-concentration.

In **Figure 22** we observe that the concentration of the product increases proportionally with time. This increase continues until the reaction is complete. In **Figure 22** we observe that the reaction ends in

spectrum 120 that correspond to the time of 60 min (one spectrum per 30 seconds).

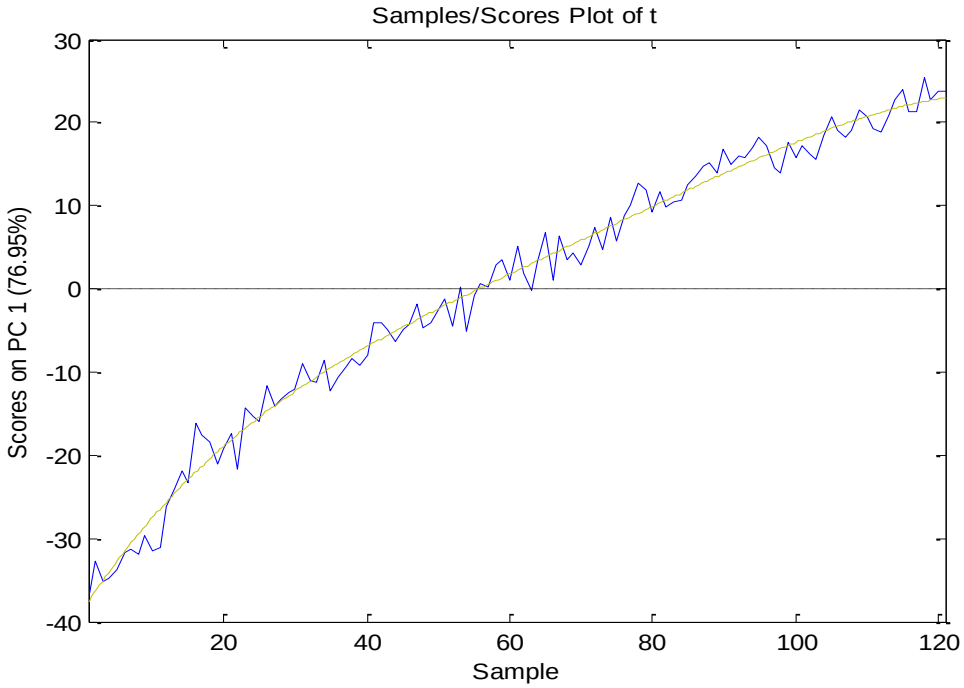


Figure 22. Scores (objects) of this specific region, green line 4th degree polynomial fit.

The above results show that the NIR instrument is inadequate for monitoring the reaction due to low sensitivity of detection. On the other hand the Raman instrument is proved to be the adequate instrument to conduct the monitoring of our reaction.

4.2 Experimental design

Experimental design should be set up in order to construct the model that would serve to predict the course of reaction. During optimization of reaction we observed several variables that could have an effect on our reaction.

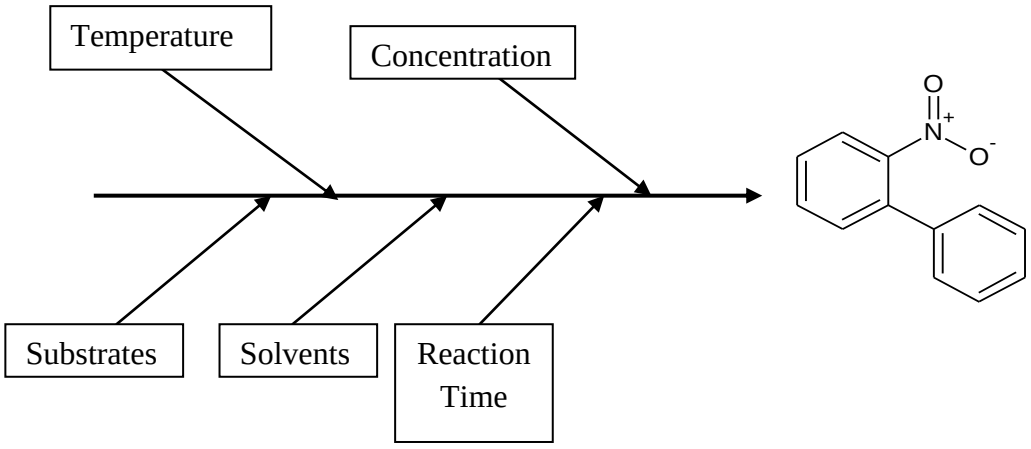


Figure 23. Ishikawa cause-effect diagram.

In **Figure 23** we can observe variables that have a different effect on our reaction represented in the form of Ishikawa diagram.

The variables that had a considerable effect in our reaction such as temperature (x_1) and concentration (x_2) are used to construct the experimental design. In this design four experiments are included plus the center point (one experiment). For the variable k=2 the total number of experiments needed to build the model is calculated as $2^k+1=5$. Five other experiments to test the model were conducted within the limits established by the first five experiments.

In a schematic manner the experimental design is presented in **Figure 24.** The blue circles represent the experiments used to build the model while the red squares represent the experiments used to test the model performance.

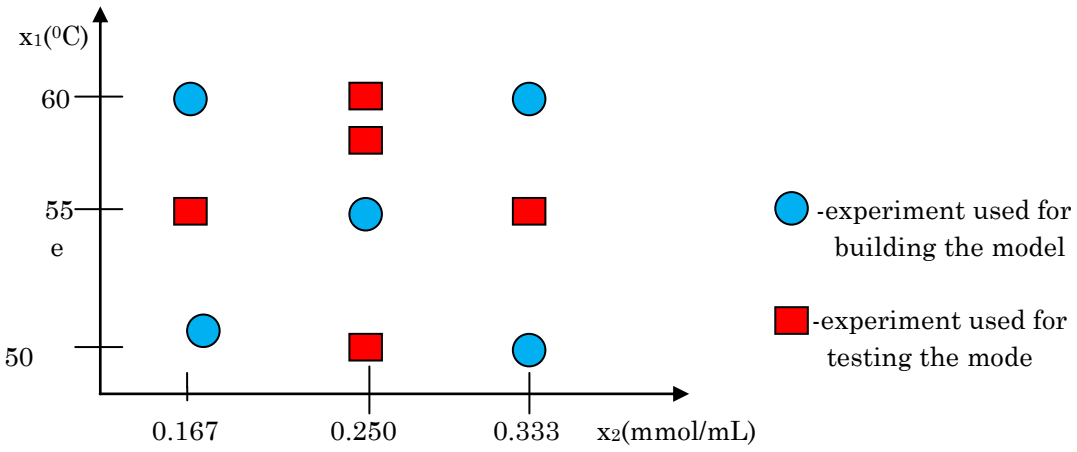


Figure 24. Schematic view of design used.

In continuation these experiments were conducted in a specific temperature and concentration. 1-iodo-2-nitrobenzene was used as substrate and methanol/water (5:1) was used as solvent in all the reactions. These reactions were monitored using Raman spectroscopy instrument.

4.3 Pre-processing data

Before using a multivariate technique, it is good to eliminate from the Raman data various features that do not have any chemical or physical meaning such as instrument noise or fluorescence (which is almost always present in spectra recorded by the Raman instrument). Removal of fluorescence does not influence the chemical data that exist in spectrum. In our case, in order to remove it from spectra a special program was developed in Matlab the so-called Backfluor (details of this program can be found in Appendix E). The adjustment starts when the original spectrum (X) and the one treated by polynomial fit (Xf) are compared to

each other. If the value of the original spectrum is less than the one treated by polynomial fit then the value of the original spectrum is taken as baseline. This was the first adjustment of spectrum (Xcorr). The next adjustment is made to the first adjusted spectrum (Xcorr) and continues until the 4th order polynomial fit is completed. This process is repeated 15 times.

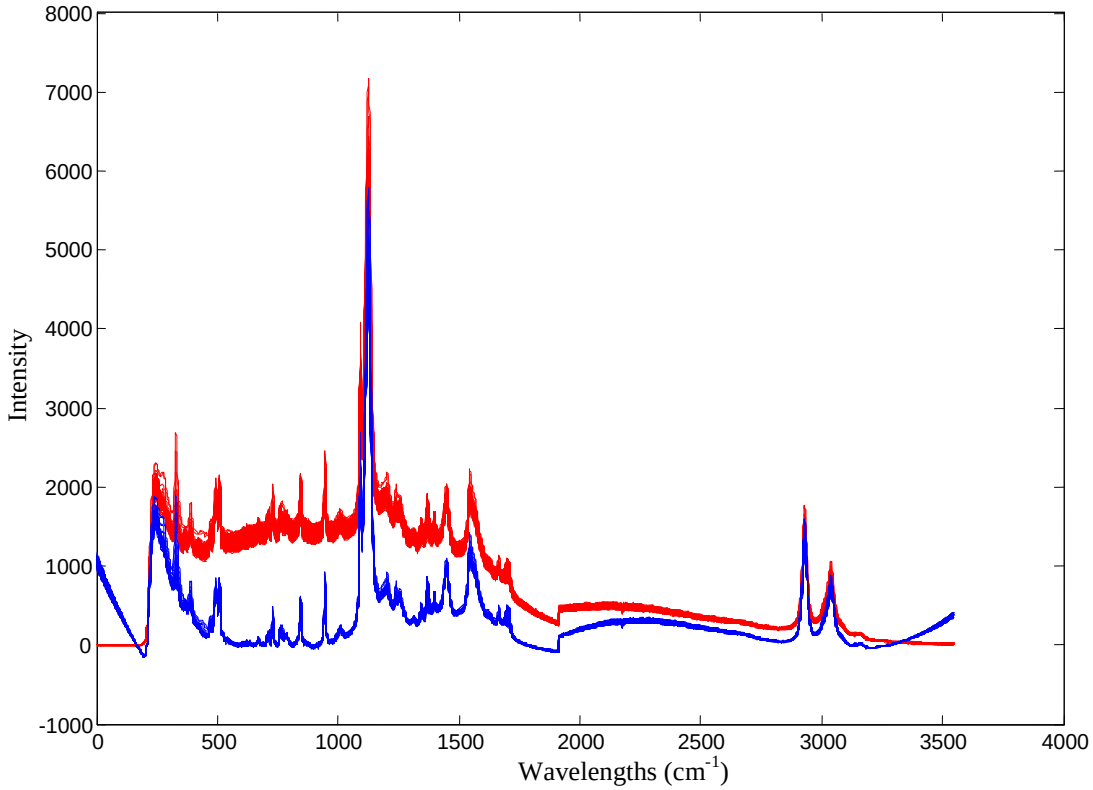


Figure 25. Raw spectra (red) and corrected spectra (blue).

In **Figure 25** an original red spectra are presented containing fluorescence and the blue one after being treated with Backfluor where fluorescence was removed which is observable in spectra.

The baseline of original spectra has the intensity of 1000 while the corrected spectra have intensity of 0 showing inexistence of fluorescence.

Since the spectra are corrected with Backfluor the specific region was chosen in which our product was presented (1220-1340 cm^{-1}). In this form the data was transferred to Sirius 8.1 to build the model. Prior to building the model the data was further pre-processed by another technique such as mean-centering in Sirius 8.1.

4.4 Building of the model

As we have already mentioned from **Figure 24**, five experiments have been used to build the model. The features of these respective experiments are presented in **Table 10.**

Table 10. Feature of experiments used in model.

No exp.	T [°C]	C [mmol/mL]	Time [min][a]	Time of recorded spectra [sec][b]	Yield [%][c]	Data matrix
1	50	0.167	60	220	35.3	150x101
			540		81	
2	50	0.333	60	220	46.8	101x101
			360		90.3	
3	55	0.25	60	30	68.3	170x101
			90		77.8	
4	60	0.167	60	30	71.1	120x101
5	60	0.333	60	30	81.9	120x101

[a]Time [min]-time in minute of withdrawn sample and analyzed with GC-MS.
[b]Time of recorded spectra [sec]-time in seconds of recorded spectra with Raman instrument.
[c]Yield[%]-yield in percentage recorded with GC-MS.

Usually the first 2-10 spectra were removed in every experiment since only the base was present in those spectra or the catalyst but not all the components and as such those spectra were not useful.

The data of these spectra recorded by Raman instrument that correspond to the time when the samples were withdrawn to be analyzed by GC-MS were applied to build the model. As a dependent variable the yield registered by GC-MS was used. By using full cross validation five principle components were found to be optimum. Total variance explained was 96%.

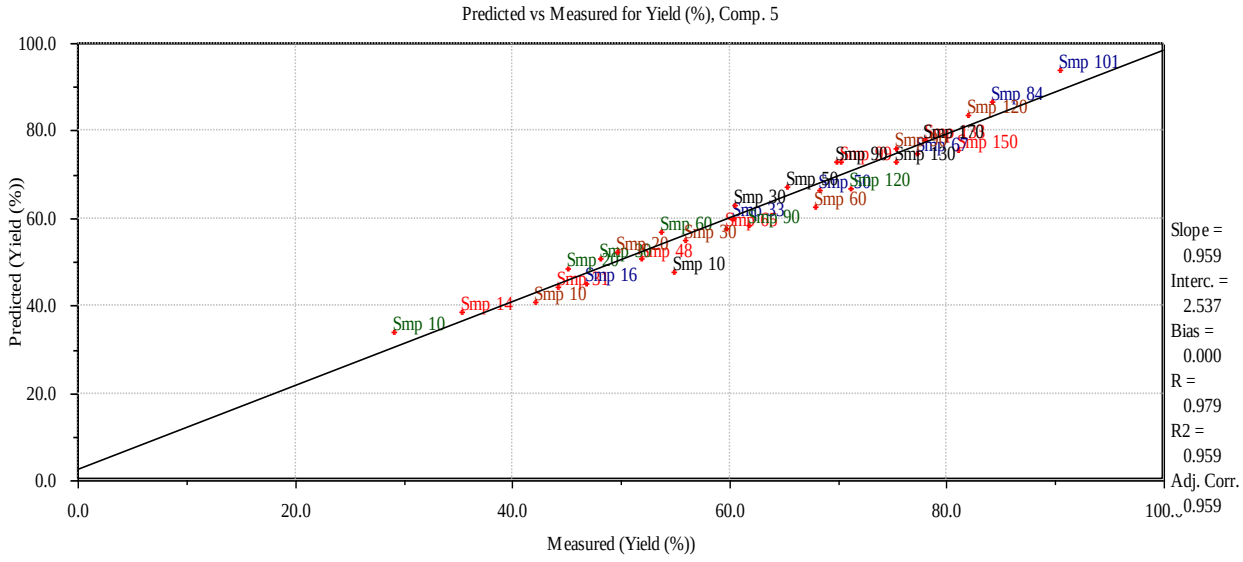


Figure 26. The model built from five experiments.

The model built with five experiments and using five principle components has quite good predictability. The built model has a correlation coefficient or a coefficient of determination (R^2) of about 96%. This coefficient shows how well the samples can be predicted by this model. This model is shown in **Figure 26**. The different colors in the figure represent the following:

a) red=samples obtained in the first experiment;
b) blue=samples obtained in the second experiment;
c) black=samples obtained in the third experiment;
d) green=samples obtained in the fourth experiment; and
e) dark yellow=samples obtained in the fifth experiment.

4.5 Model testing

Now that the model is built it is time to test it. The correlation coefficient showed that this model has good predictability. However it is good to perform other tests to validate it. Initially the so-called "internal testing" was conducted. Here the spectra that were not used in building the model are predicted. These spectra belong to the five experiments used to build the model. The prediction of these spectra can be observed in **Figure 27**. There we observe that the model is reasonable because the graphic gradually (constantly) increases for the respective experiment. From this we see that almost every subsequent spectrum has a higher intensity then the previous one. This shows that the product increased gradually as it really occurred and can be predicted by model. From this "internal test" we can observe that the model is quiet reasonable (adequate).

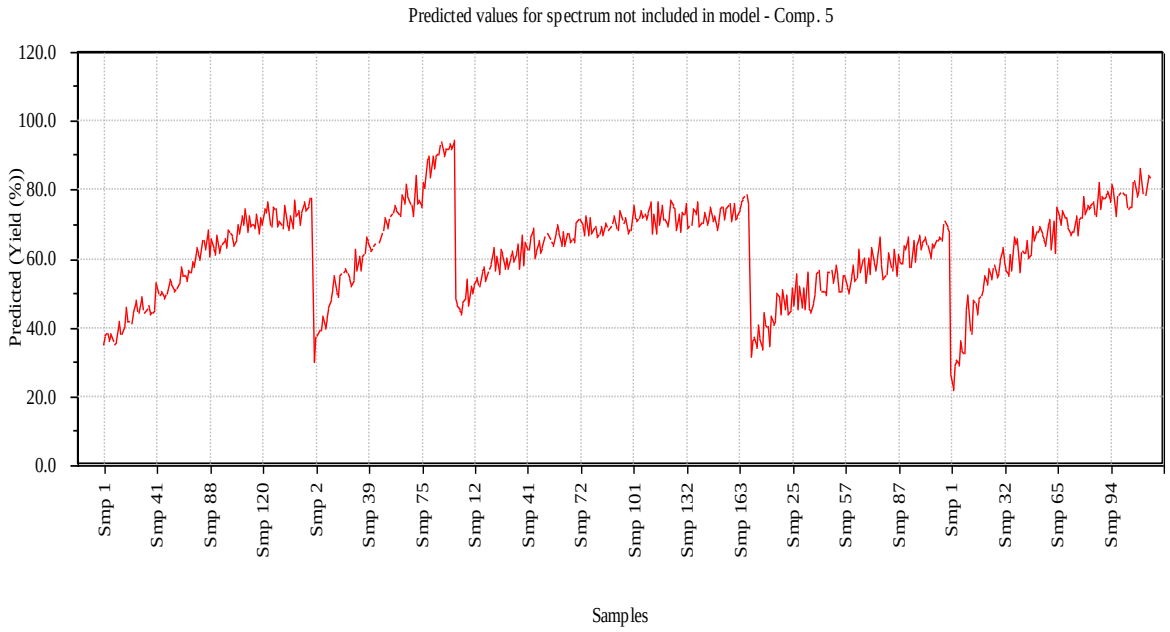


Figure 27. Predicted values for spectra not included in model.

The model was tested with external validation samples. An experiment conducted within temperature of 50 ^{0}C and concentration 0.333 mmol/mL was performed. The reaction mixture was monitored by Raman spectroscopy using the same procedure as before. During the reaction eight samples were taken and analyzed with GC-MS to obtain concentration estimates. The spectra corresponding to the time when the samples were taken were selected and used by the model, in order to predict the concentration from the Raman model.

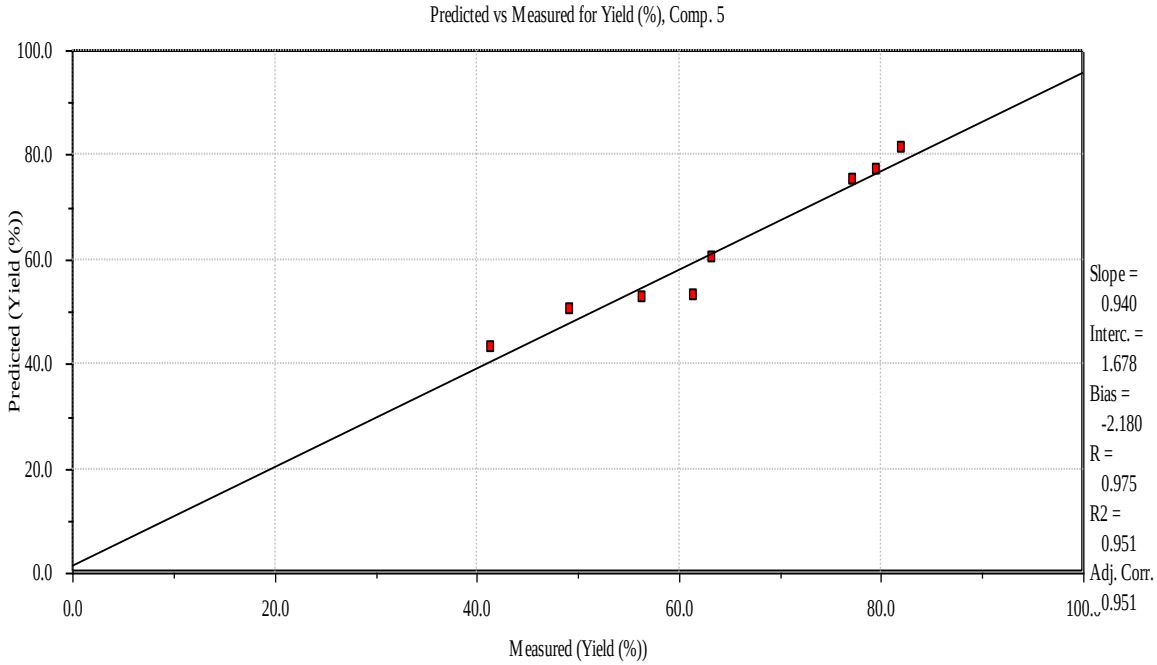


Figure 28. Yield of reaction predicted by our model versus measured with GC-MS.

As observed in **Figure 28** the yield is very well determined by our model. In abscissa X are the corresponding values for the sample measured with GC-MS while in ordinate Y we have the prediction of the yield with our model. From the congruency of the measured values and of those predicted with our model (R^2=95.1%) it shows that our model is reasonable and can be used to determine on-line the reaction yield.

The model was tested with the next experiment conducted in temperature of 55 ^{0}C and concentration 0.167 mmol/mL. Seven samples that were analyzed with GC-MS were taken from this experiment. The spectra correspond to the time when those seven samples were taken are selected and tested in our model. The correlation coefficient is about R^2=86% that shows that it was not very well predicted compared with above test. This prediction can be observed in **Figure 29**. The prediction in this case deviates up to ±5%, i.e. if the GC-MS measured yield was 38%

with our model it is predicted to be 42% or the GC-MS measured yield is 42% with our model it is predicted to be 38%.

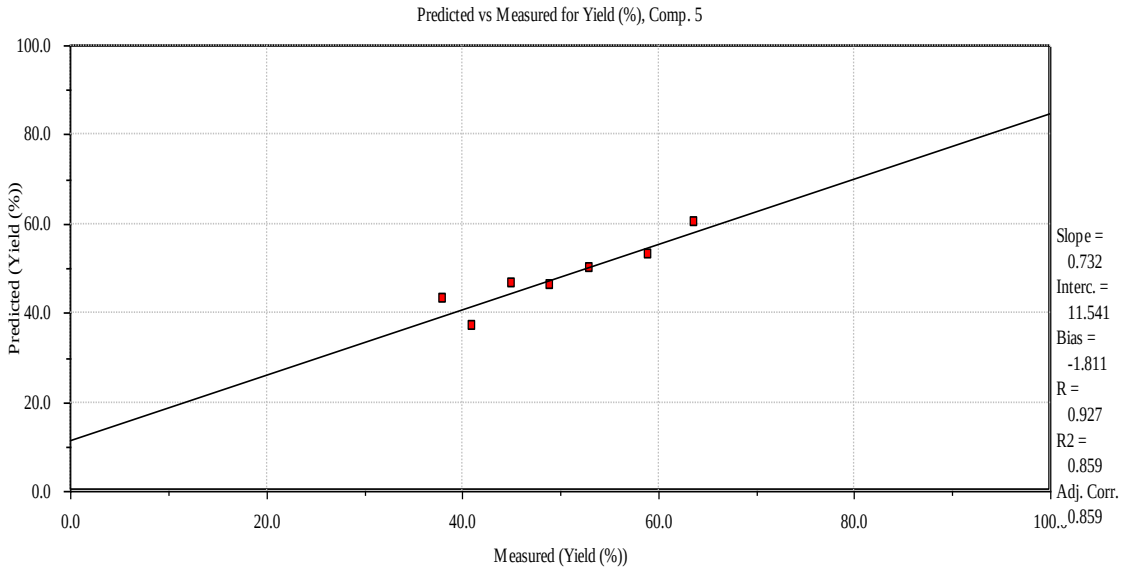


Figure 29. Percentage of yield predicted by our model versus measured by GC-MS.

The following experiment tested with our model is the one conducted in the temperature of 55 ^{0}C and concentration of 0.333 mmol/mL. During monitoring with the Raman instrument seven samples were obtained and analyzed with GC-MS. The spectra that correspond to these samples are selected and tested with our model. Prediction of these samples in our model was quite good when R^2=92.2%. So this experiment too is predicted quite well with our model. This is observed in **Figure 30**.

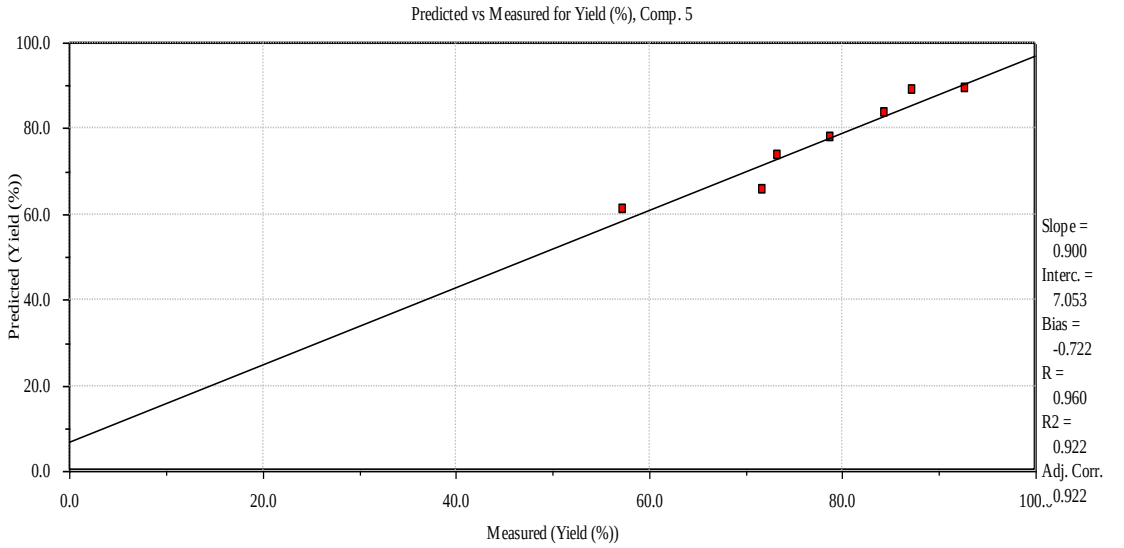


Figure 30. Percentage of yield predicted by our model versus measured by GC-MS.

The model was tested in the experiment conducted in the temperature of 57 ^{0}C and concentration of 0.250 mmol/mL. During monitoring of this reaction seven samples were extracted and analyzed with GC-MS. The spectra that correspond to these samples were tested in our model. Our model functioned quite well as the prediction of these samples was 93%. This is shown in **Figure 31**.

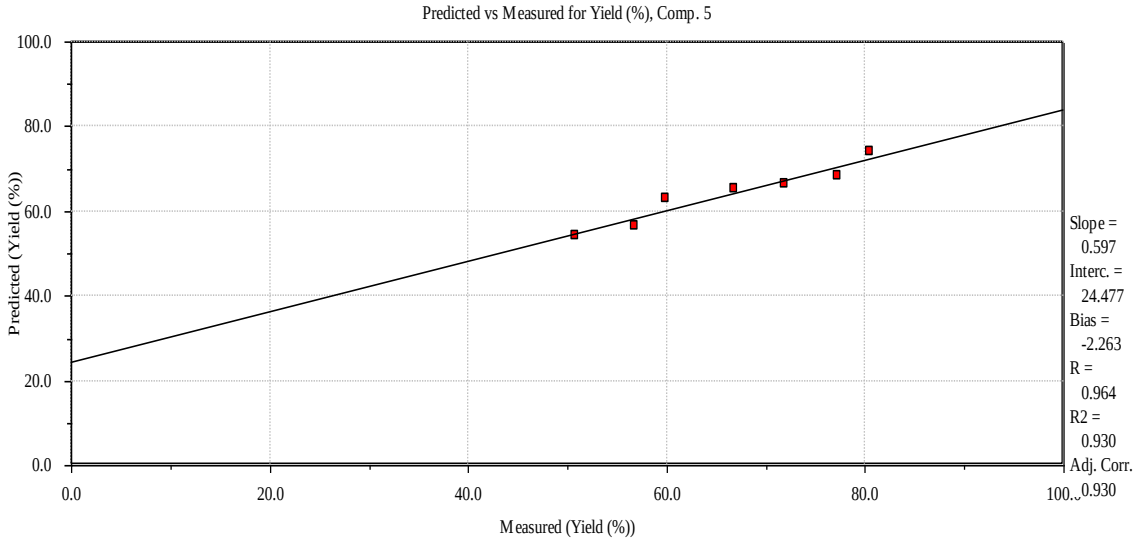


Figure 31. Percentage of yield predicted by our model versus measured by GC-MS.

The next tested experiment was conducted in temperature of 60 ^{0}C and concentration of 0.250 mmol/mL. During this monitoring five samples were extracted from the reaction and analyzed with GC-MS. The spectra corresponding to these times were tested in our model. Our model has a good predictability and by it we are able to predict samples and to determine the concentration of the product on-line. Predictability in our model for this experiment 99,6% as can be observed in **Figure 32**.

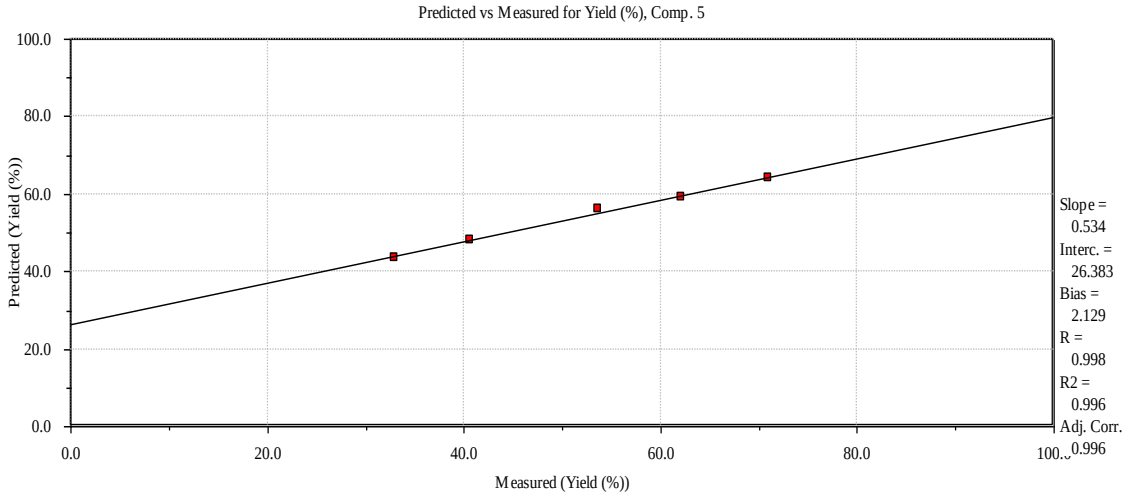


Figure 32. Precentage of yield predicted by our model versus measured by GC-MS.

For all the experiments conducted within determined limits with experimental design (temperature 50-60 ^{0}C and concentration 0.167-0.333 mmol/mL) the determination of the product concentration with our model was very good not to say perfect. Only the experiment conducted in temperature of 55 ^{0}C and concentration 0.167 mmol/mL is an exemption being poorer when compared with the other experiments, in the context of model predictability it can be considered good.

We already demonstrated above that the model functions very well within the determined limits but how would it function outside of these limits? To see what kind of experiment we will conduct for a shorter time and in better conditions the contour map was built based on experimental design that we have used to build the model. In Table 11 the variables we used to build the contour map are shown. The temperature and the concentration were the variables used and also the interaction between them.

Table 11. Variable code used for contour map.

No. exp.	T	C	TxT	TxC	CxC	Yield [%] for 60 min
1	-1.000	-1.000	1.000	1.000	1.000	35.280
2	-1.000	1.000	1.000	-1.000	1.000	46.790
3	0.000	0.000	0.000	0.000	0.000	68.300
4	1.000	-1.000	1.000	-1.000	1.000	71.080
5	1.000	1.000	1.000	1.000	1.000	81.840

*Temperature codes: -1 for 50; 0 for 55 and 1 for 60^{0}C.

*Concentration codes: -1 for 0.167; 0 for 0.250 and 1 for 0.333mmol/mL.

As a dependent variable in this case the yield in time 60 min was used for all experiments.

Contour map (**Figure 33**) generated from temperature, concentration as well as interaction between them shows the different range of reaction yields represented as colored bands that correspond to the respective temperature and concentration.

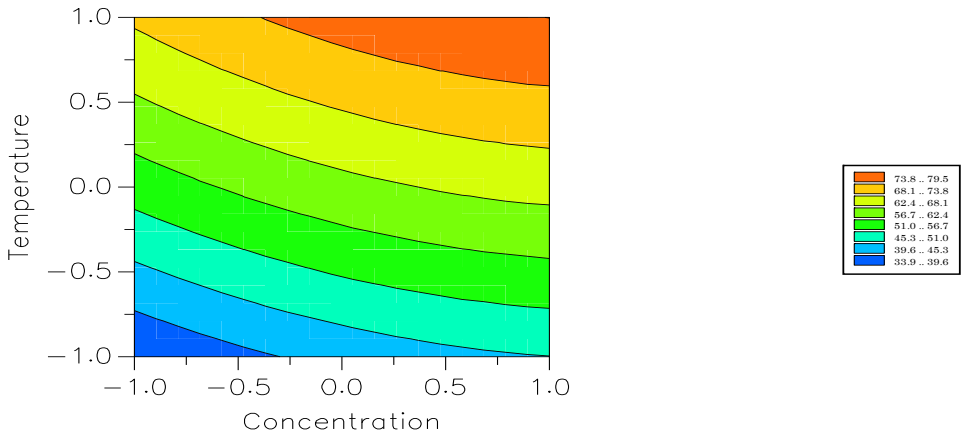


Figure 33. Contour map showing the reaction yields for different temperature and concentration.

Based on represented contour map the orange color shows the region where the yield of our product is highest for the time of 60 min. Also this region shows that in order for the yield to be higher the temperature and concentration should be high.

We decided to extrapolate our model, i.e. to conduct an experiment outside the previously determined limits and to test our model. Based on the contour plot we chose the least concentration that is within orange region (highest reaction yield region). For this reason the concentration of 0.167 mmol/mL being equivalent with code 0 was chosen while the temperature equal to 65 ^{0}C was chosen outside the limits. An experiment was conducted applying these conditions and six samples were extracted and analyzed with GC-MS. The spectra that correspond to these samples are chosen and tested in our built model. Predictability of our model is shown in **Figure 34**.

We observe that the predictability is quite good and we also observe that the expected shorter time due to using a higher temperature could be achieved. So in this case the experiment was completed for almost 30 min where the yield was 98.3%.

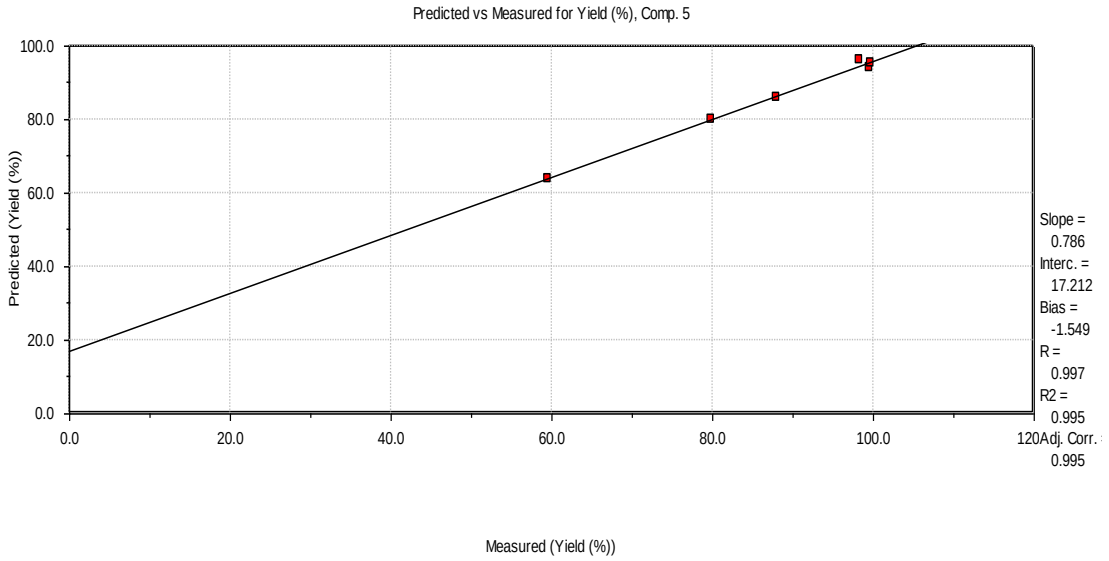


Figure 34. Percentage of yield predicted versus measured by GC-MS for extrapolated experiment.

It was demonstrated that the model worked well within the determined limits of the experimental design but also function well outside these limits. The difference between the yields measured with GC-MS and those predicted with our model is less than 4%. We can say that our model is quite solid and well built.

5 Conclusions and future work

5.1 Conclusions

Monitoring of reactions using various techniques that enable the on-line monitoring and especially the combination of these instruments with various multivariate techniques like PCA and PLS is very important nowadays for pharmaceutical and chemical industries.

The possibility of on-line detection of a product in an ongoing chemical reaction without interrupting it is the main advantage why these instruments in combination with PCA and PLS have wide use in industry.

Monitoring of reactions in industry commonly uses the NIR techniques but this present thesis demonstrated that the Raman instrument is more effective and suitable for monitoring reactions.

The power of multivariate technique such as PCA and PLS in combination with the Raman instrument has been shown. Also in the present thesis the building of a good model was achieved based on a small number of reactions. It was demonstrated that this model can be applied quite well in determining the on-line product without interrupting the reaction conducted within the limit of built model. It is also important to note that the model is a very good in determining the concentration of the product outside these limits as well. From the experimental design applied in building the model, favorable conditions for completion of reaction in a shorter time were predicted. Application of the predicted conditions showed that the reaction in 65 ^{0}C was completed in 30 min instead of 60 min, a 50% reduction in analysis time.

5.2 Future work

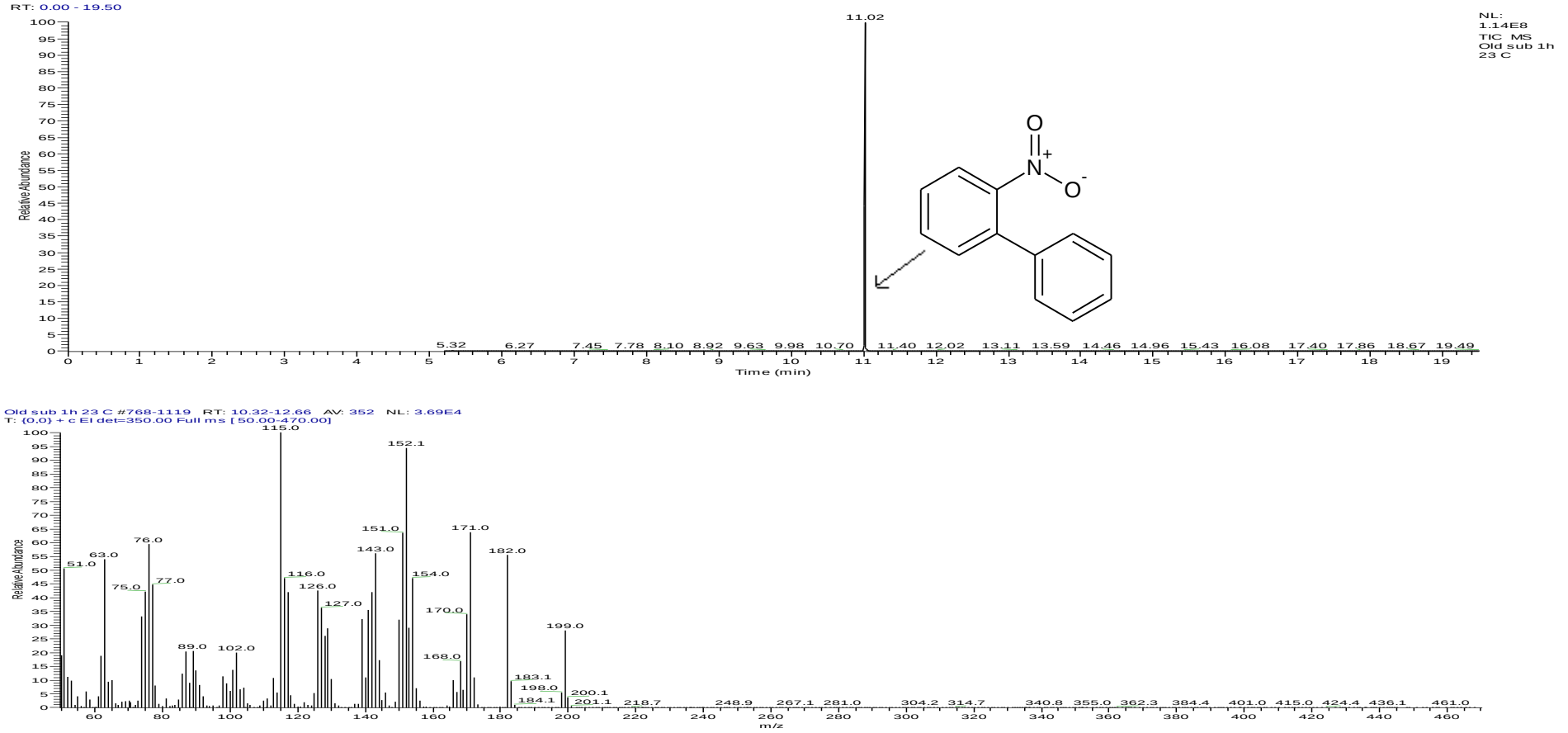
Despite the wide application of vibrational spectroscopy in monitoring on-line reactions, the difficulty of interpretation of spectra lowered down their application to a certain level. There have been many works where the data of the Raman instrument were treated with multivariate techniques like PCA and PLS[84,85,86] but in all the cases the data was treated following the completion of the reaction despite being an on-line monitoring of the reaction.

The treatment of data with the multivariate technique following the completion of reaction lowered its on-line effect. In order to solve this problem I think that it would be important for the industry to develop some program that would enable the multivariate techniques to be applied impromptu with spectroscopic instruments and to enable the determination of the concentration of the product at that moment without having later to treat that data with those techniques. At the present this

ideas might seem difficult but in future effort should be focused on solving this problem.

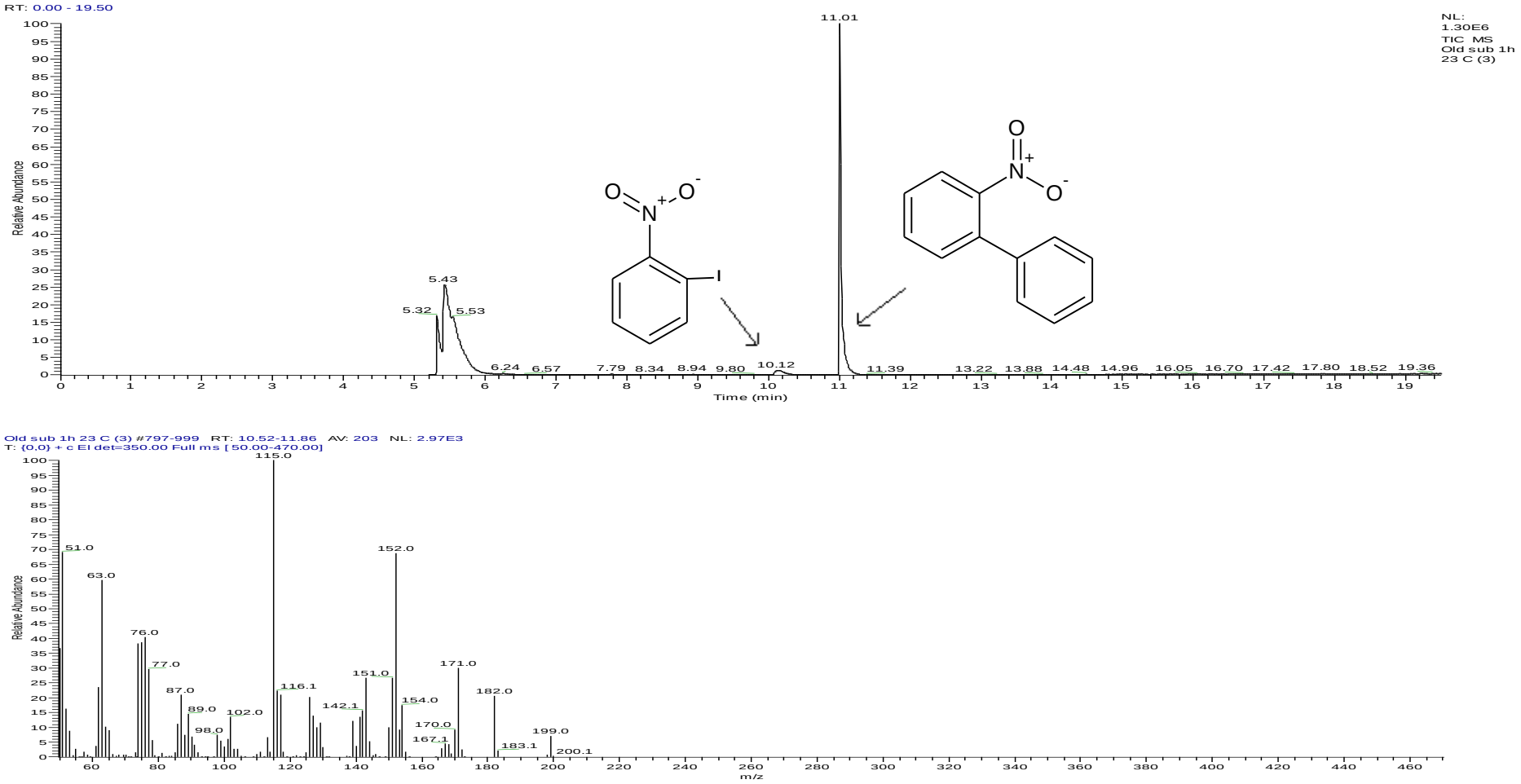
6 Appendices

6.1 Appendix A



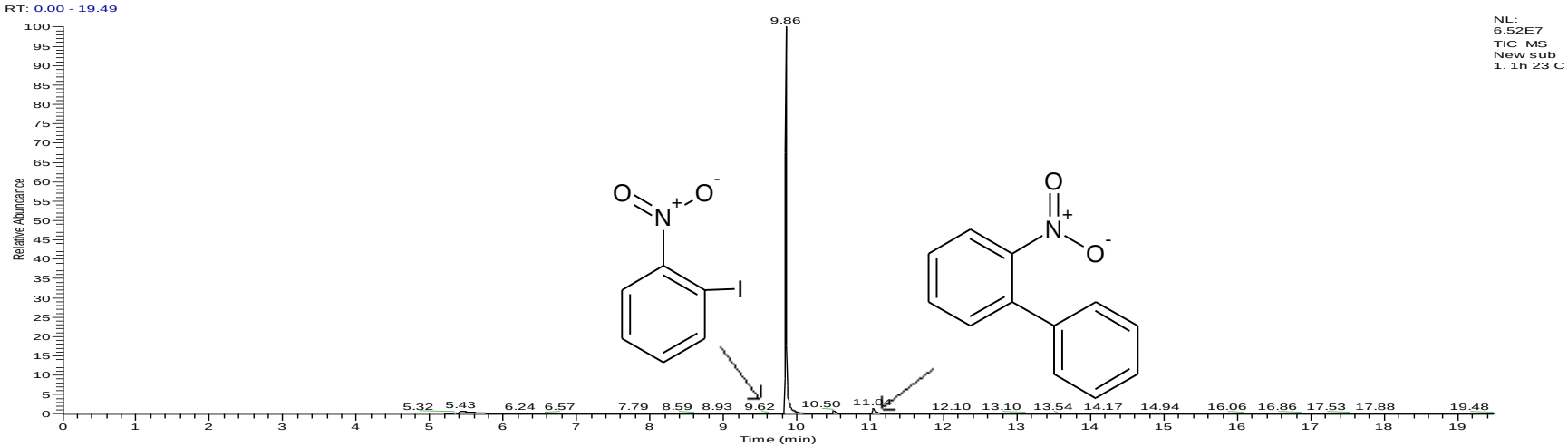
Chromatogram and mass spectrum of 1st reaction (Table 5).

6.1 Appendix A



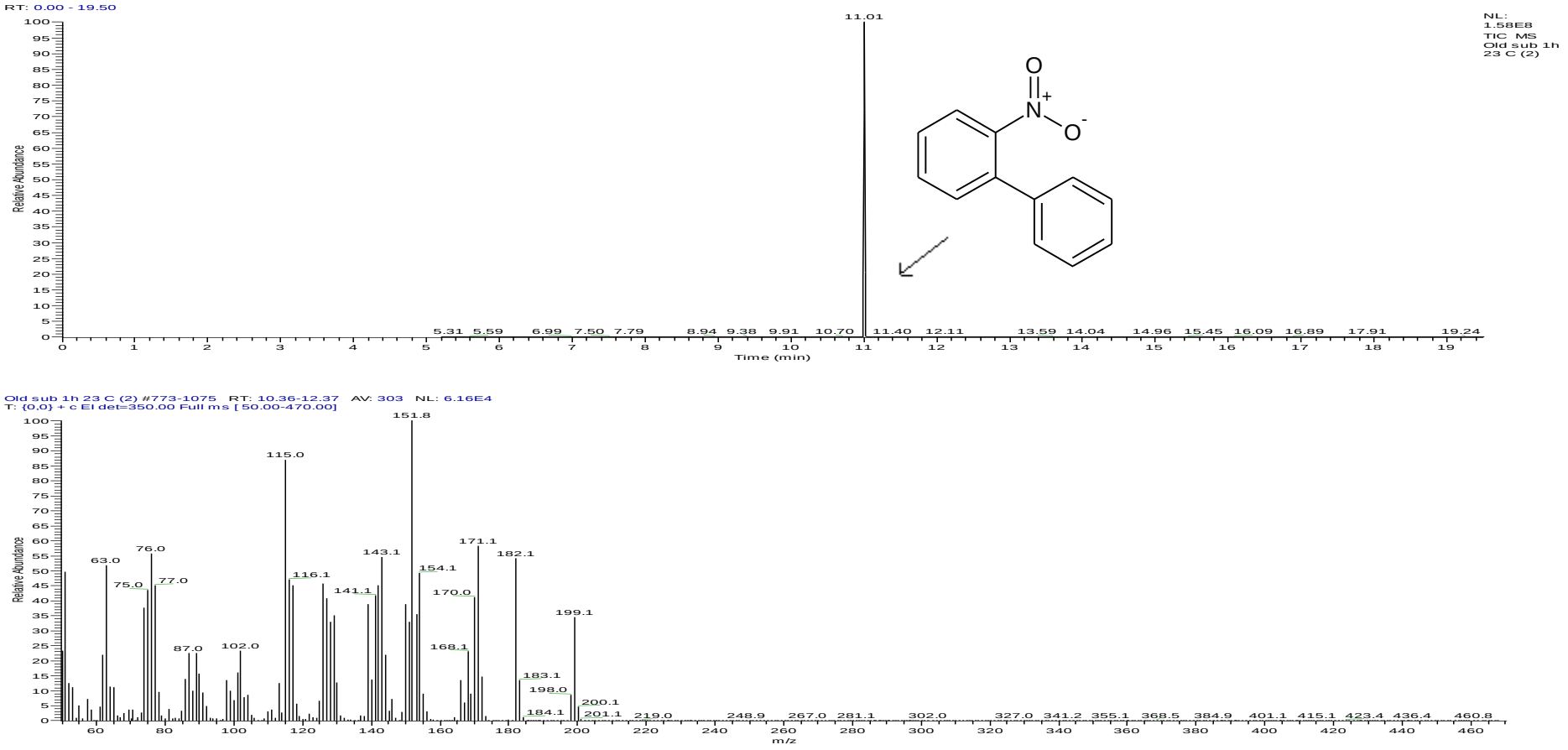
Chromatogram and mass spectrum for 2nd reaction (Table 5).

6.1 Appendix A



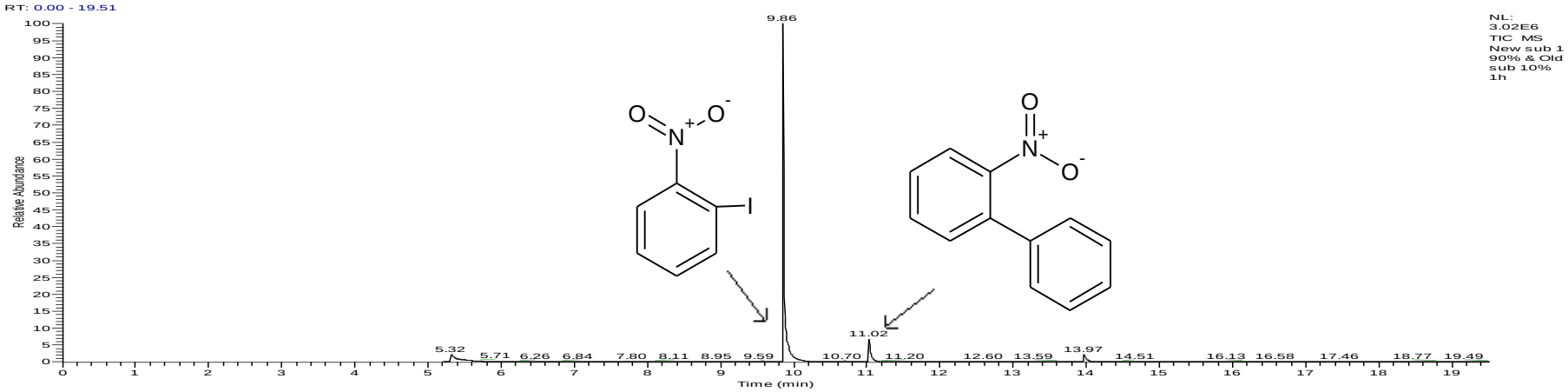
Chromatogram and mass spectrum for 3rd reaction (Table 5).

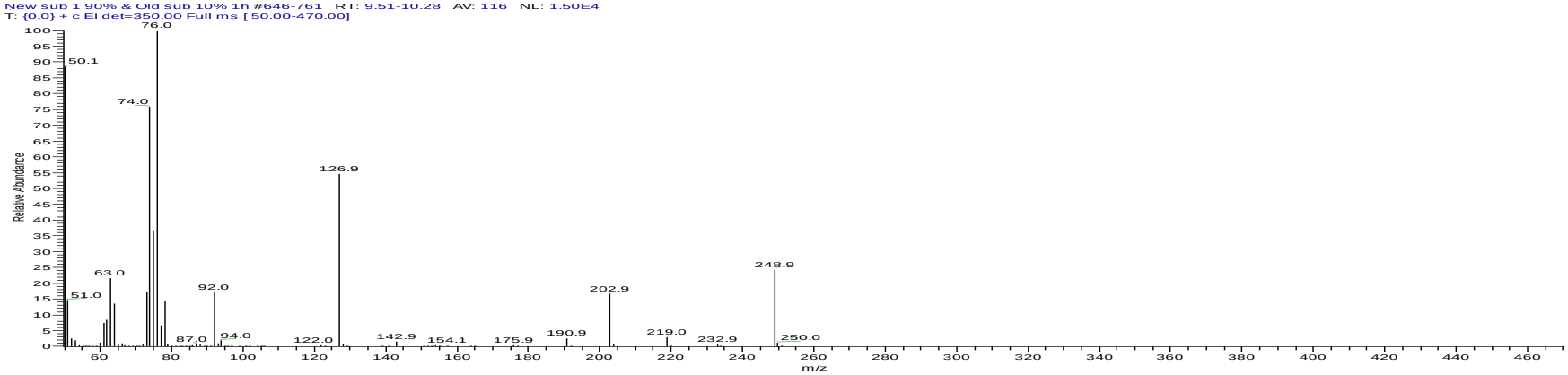
6.1 Appendix A



Chromatogram and mass spectrum 4th reaction (Table 5).

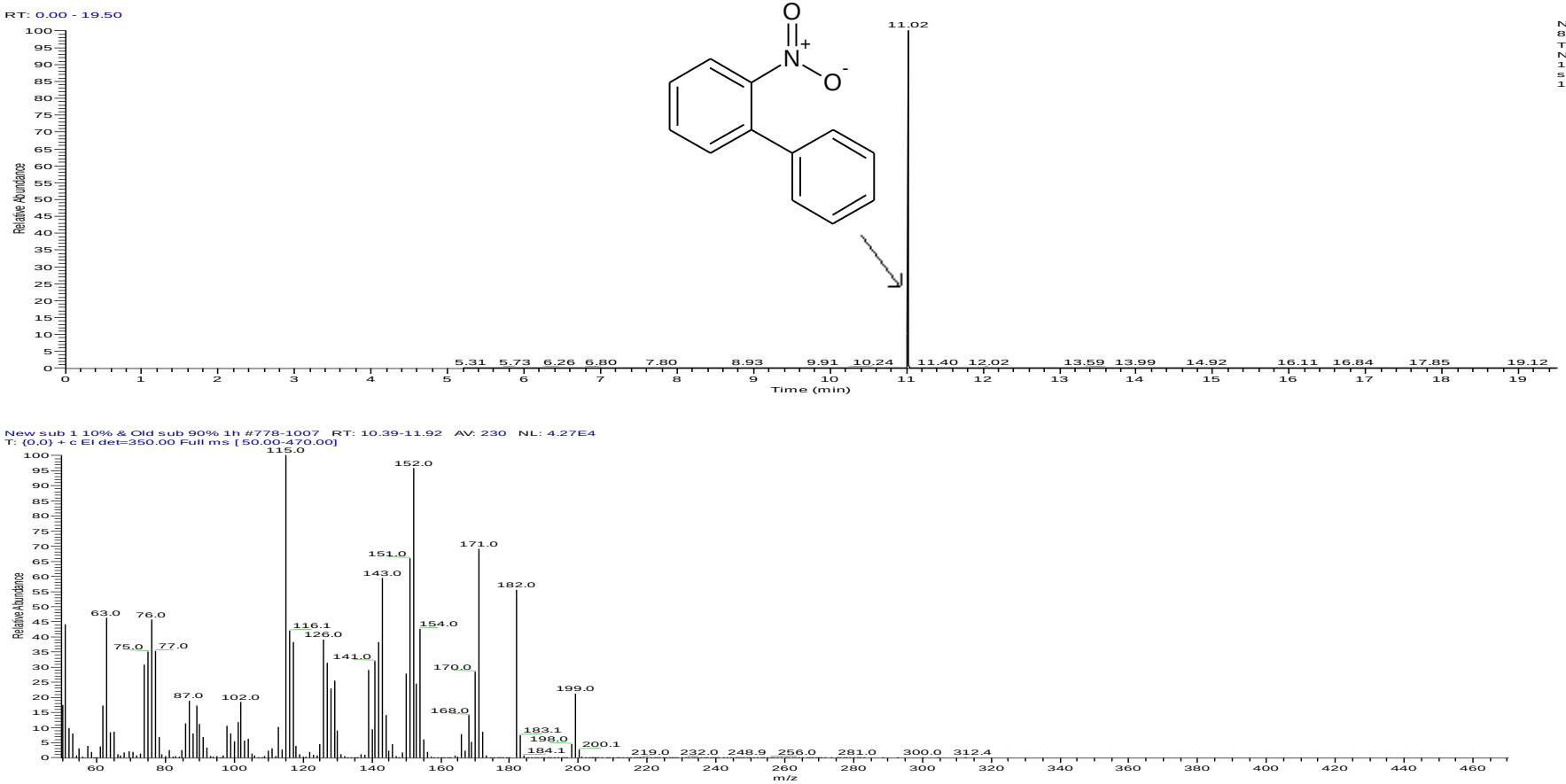
6.1 Appendix A





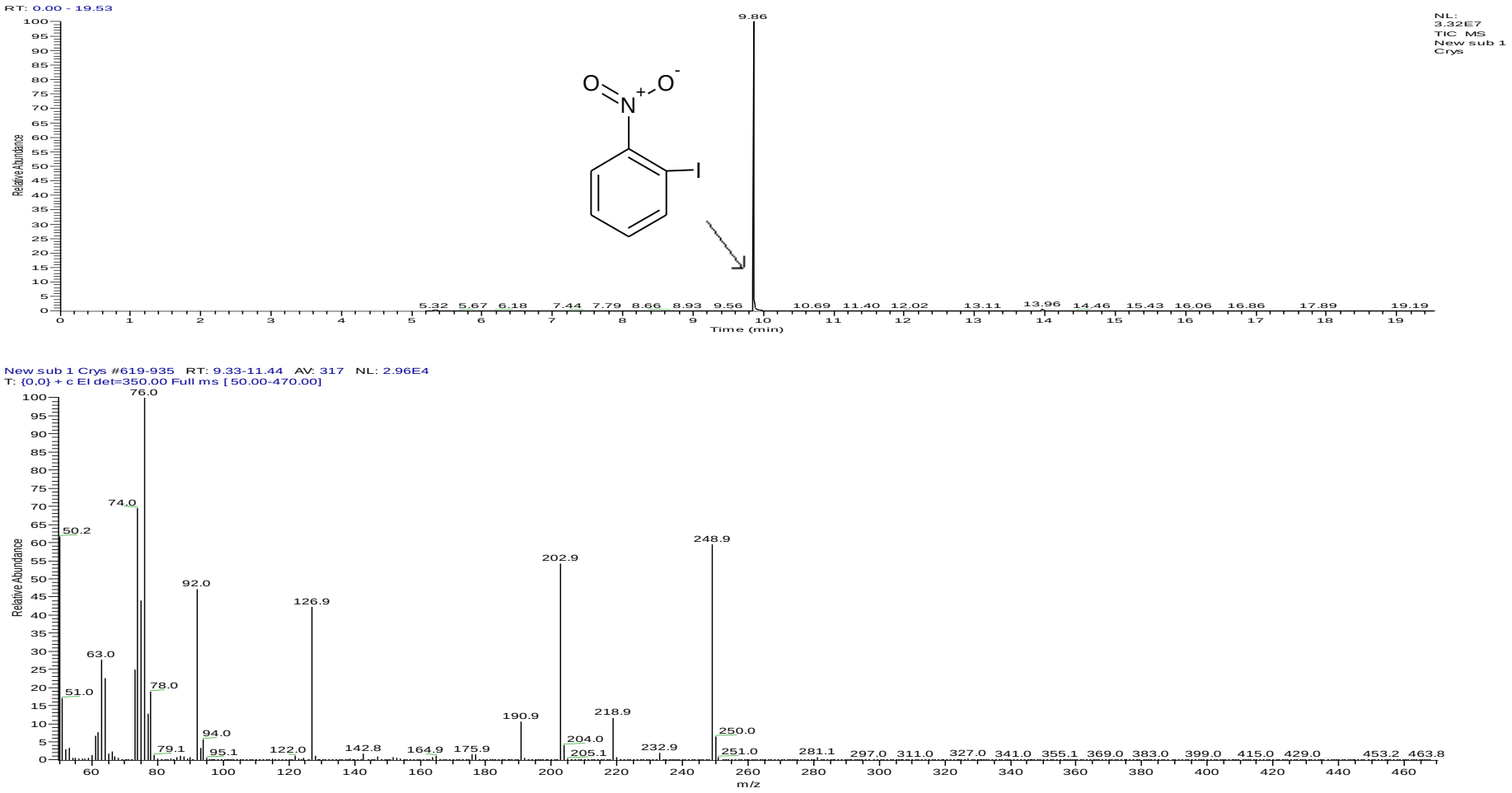
Chromatogram and mass spectrum 5th reaction (Table 5).

6.1 Appendix A



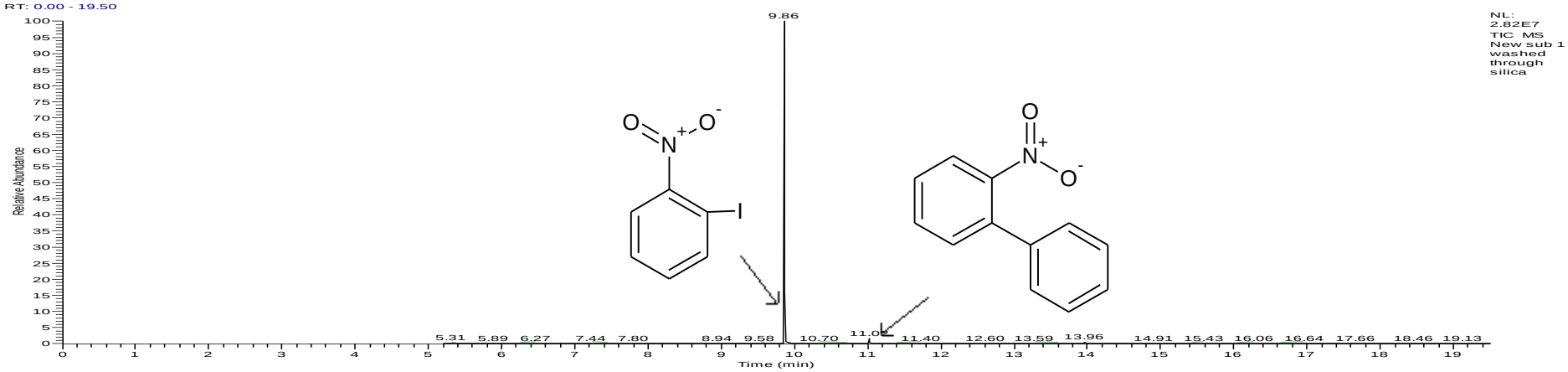
Chromatogram and mass spectrum for 6th reaction (Table5).

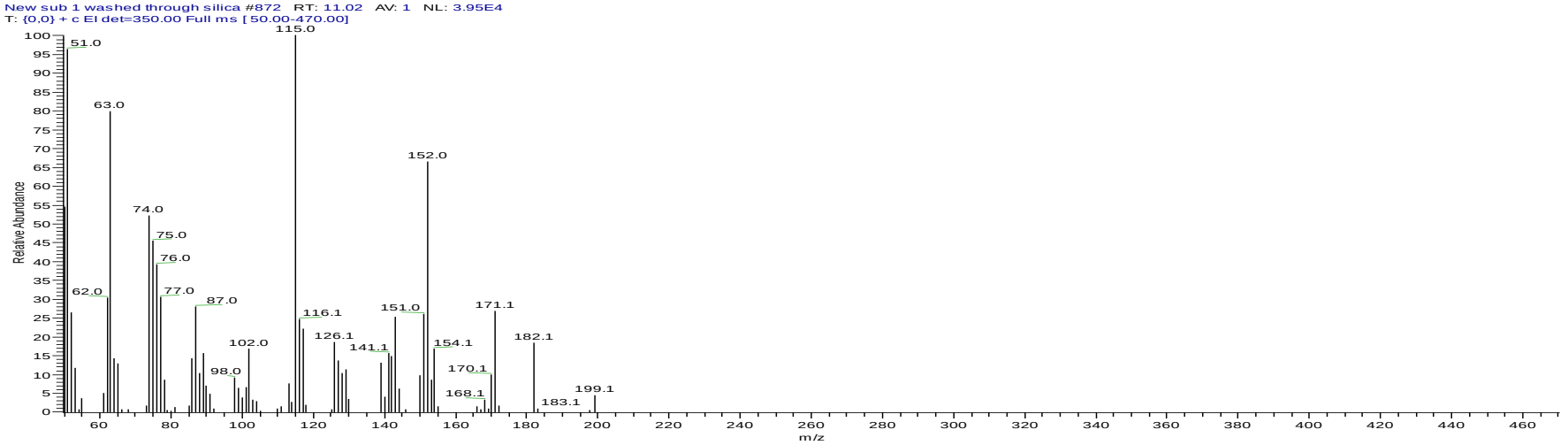
6.2 Appendix B



Chromatogram and mass spectrum for 1st reaction (Table 6).

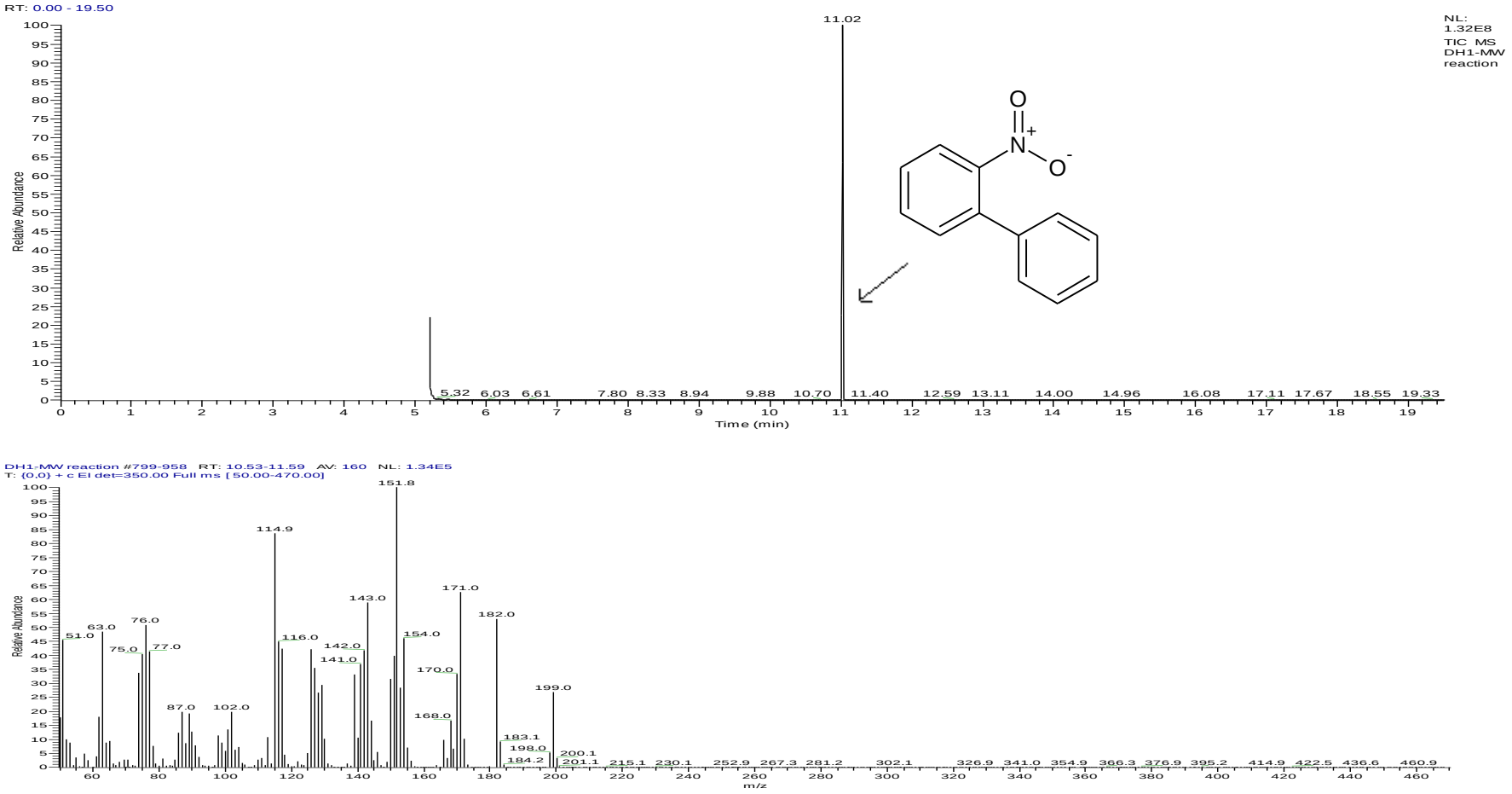
6.2 Appendix B





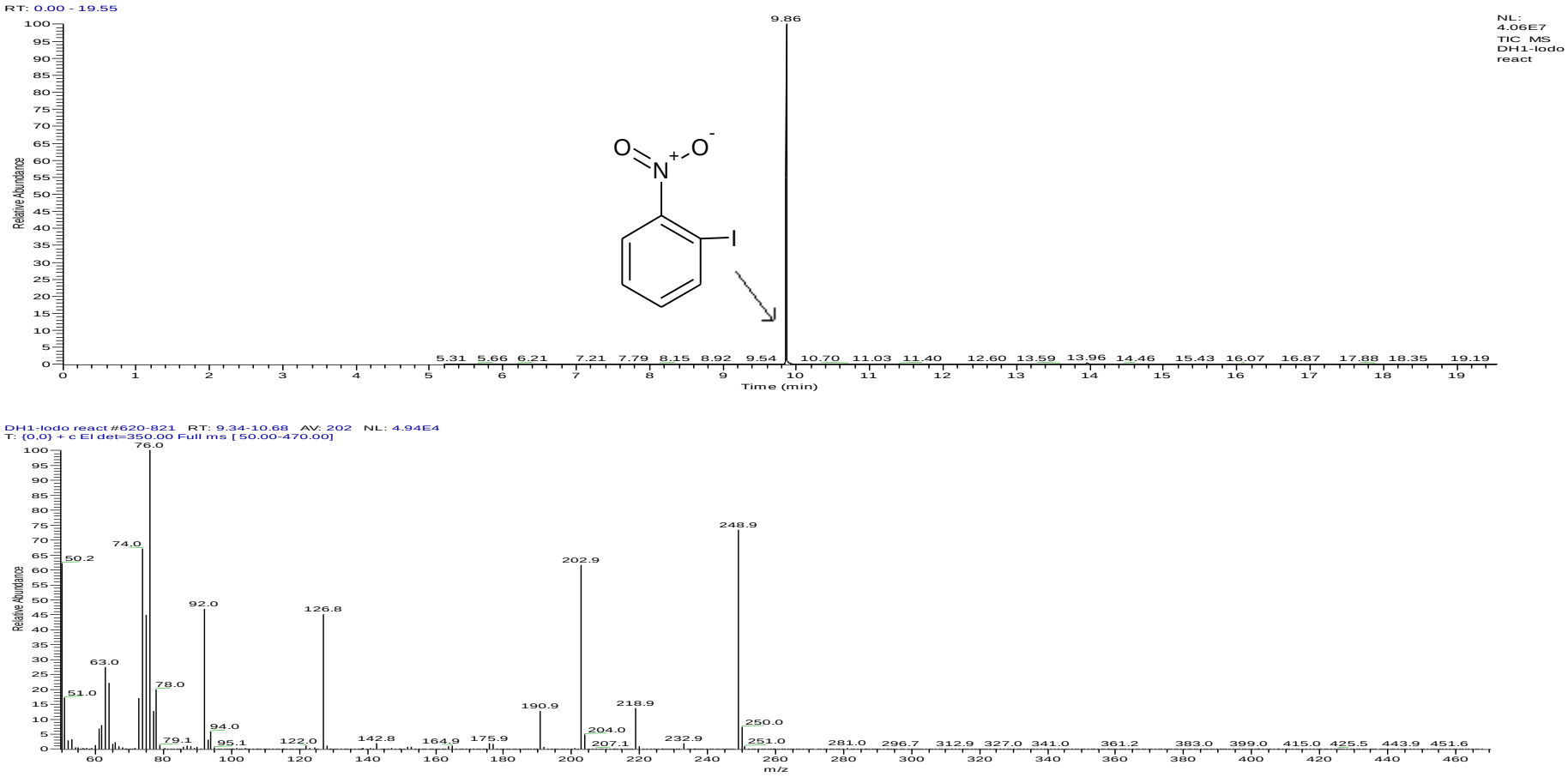
Chromatogram and mass spectrum for 2nd reaction (Table 6).

6.2 Appendix B



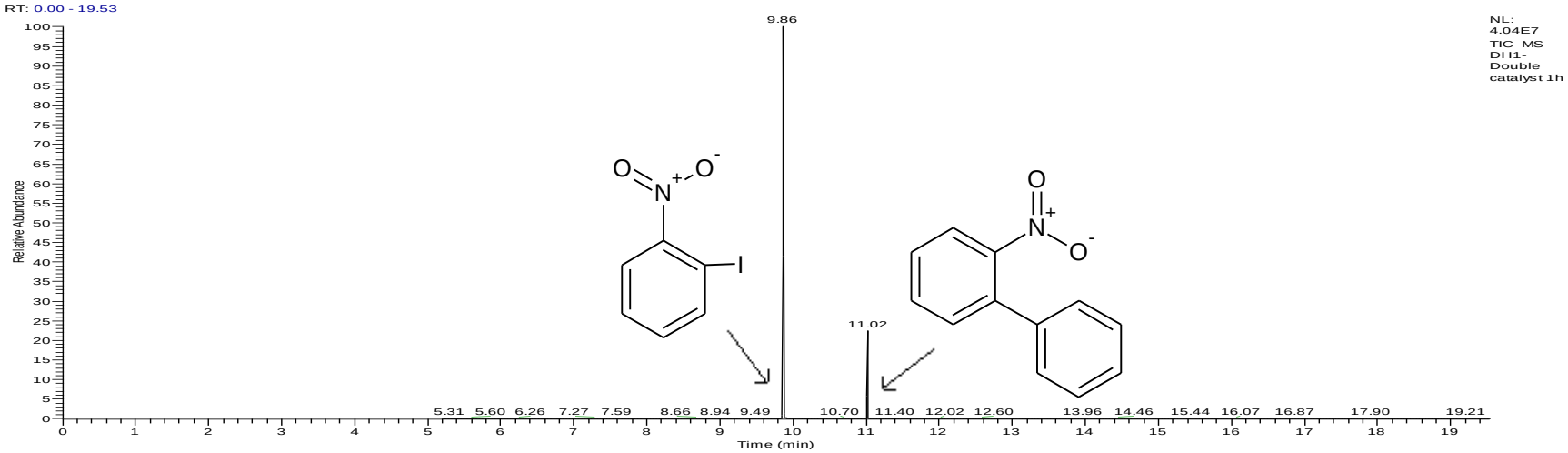
Chromatogram and mass spectrum for 3rd reaction (Table 6).

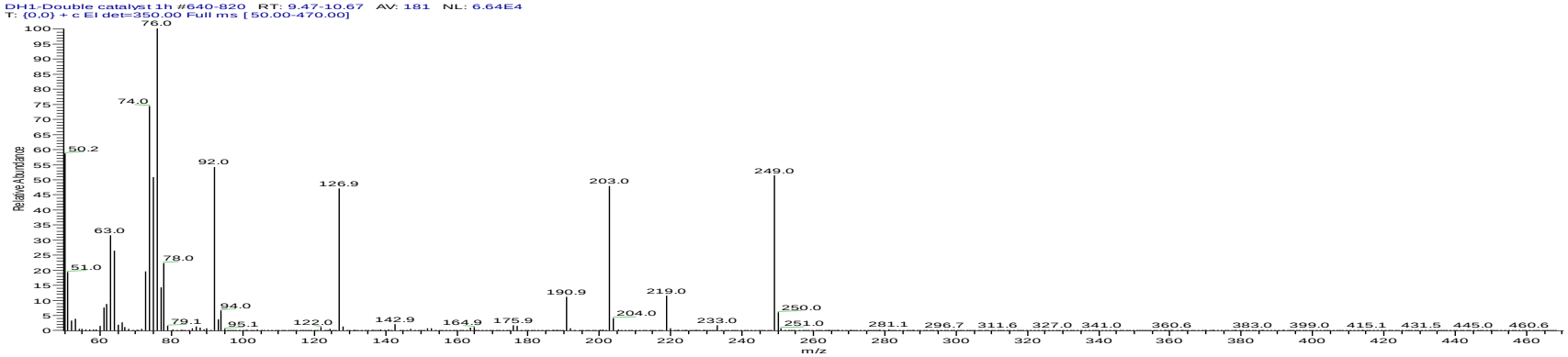
6.2 Appendix B



Chromatogram and mass spectrum for 4th reaction (Table 6).

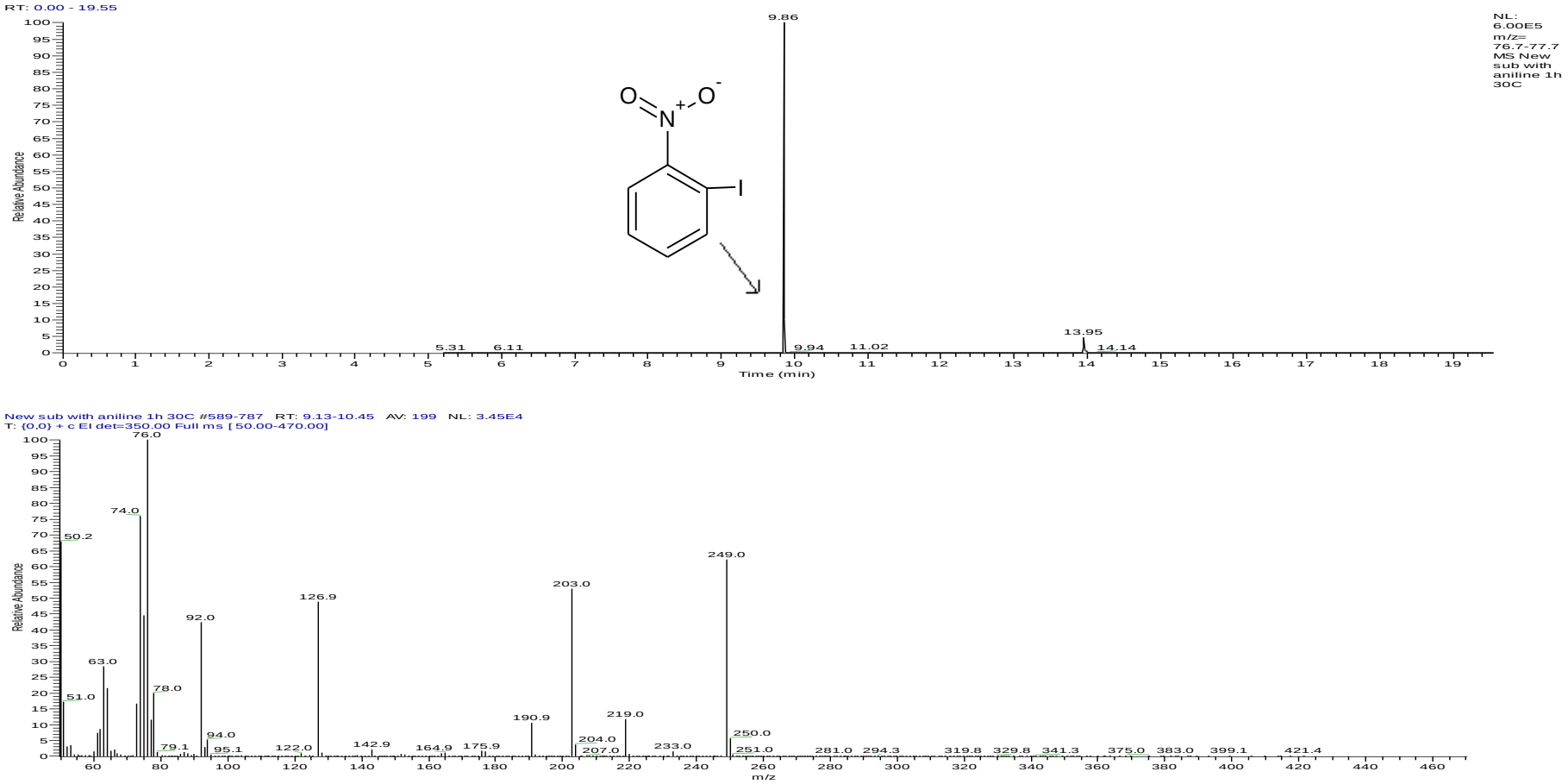
6.2 Appendix B





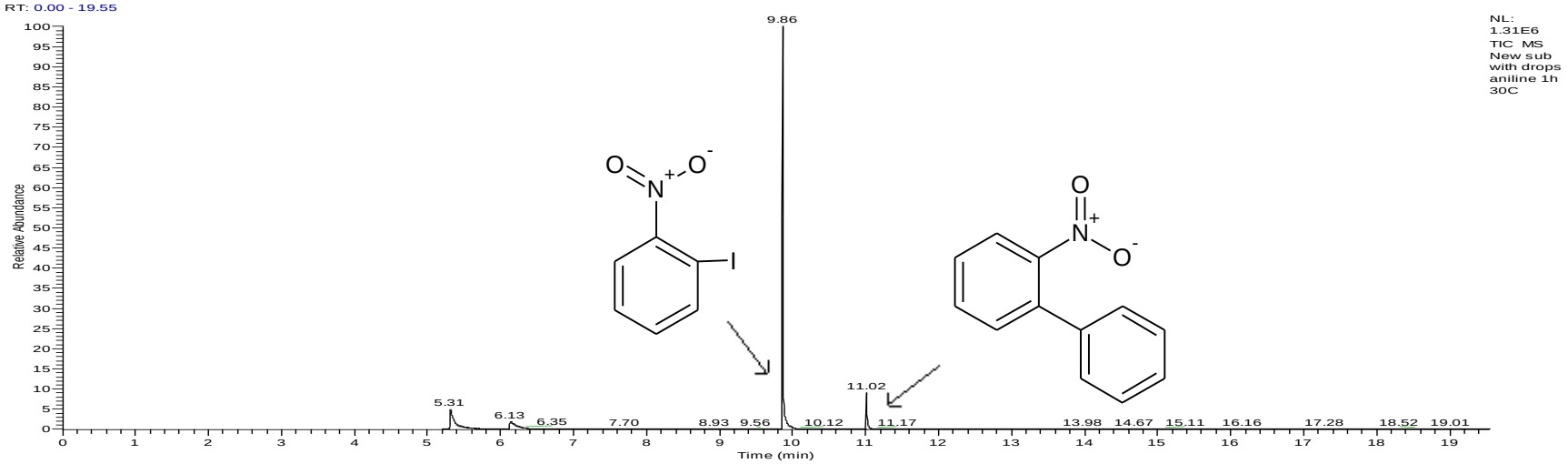
Chromatogram and mass spectrum for 5th reaction (Table 6).

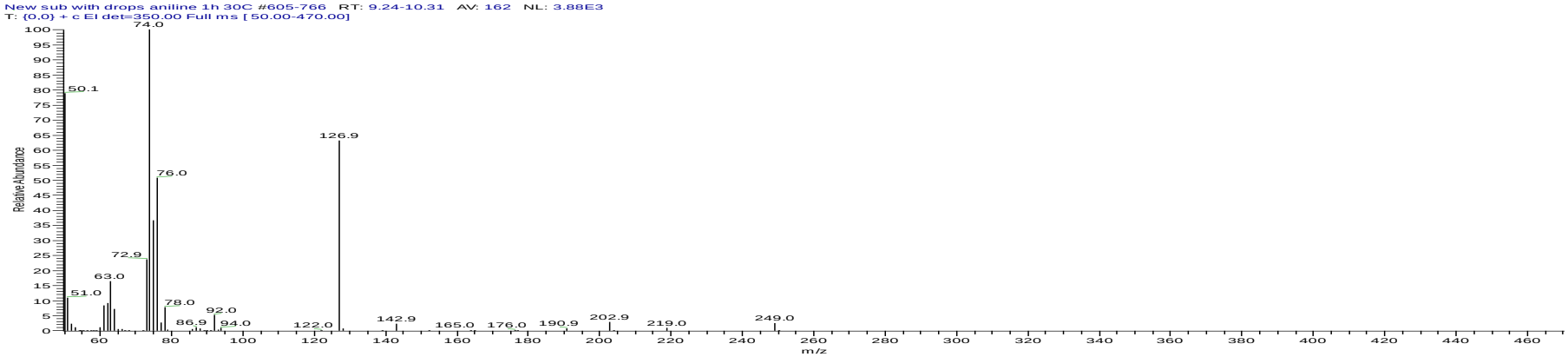
6.2 Appendix B



Chromatogram and mass spectrum for 6th reaction (Table 6).

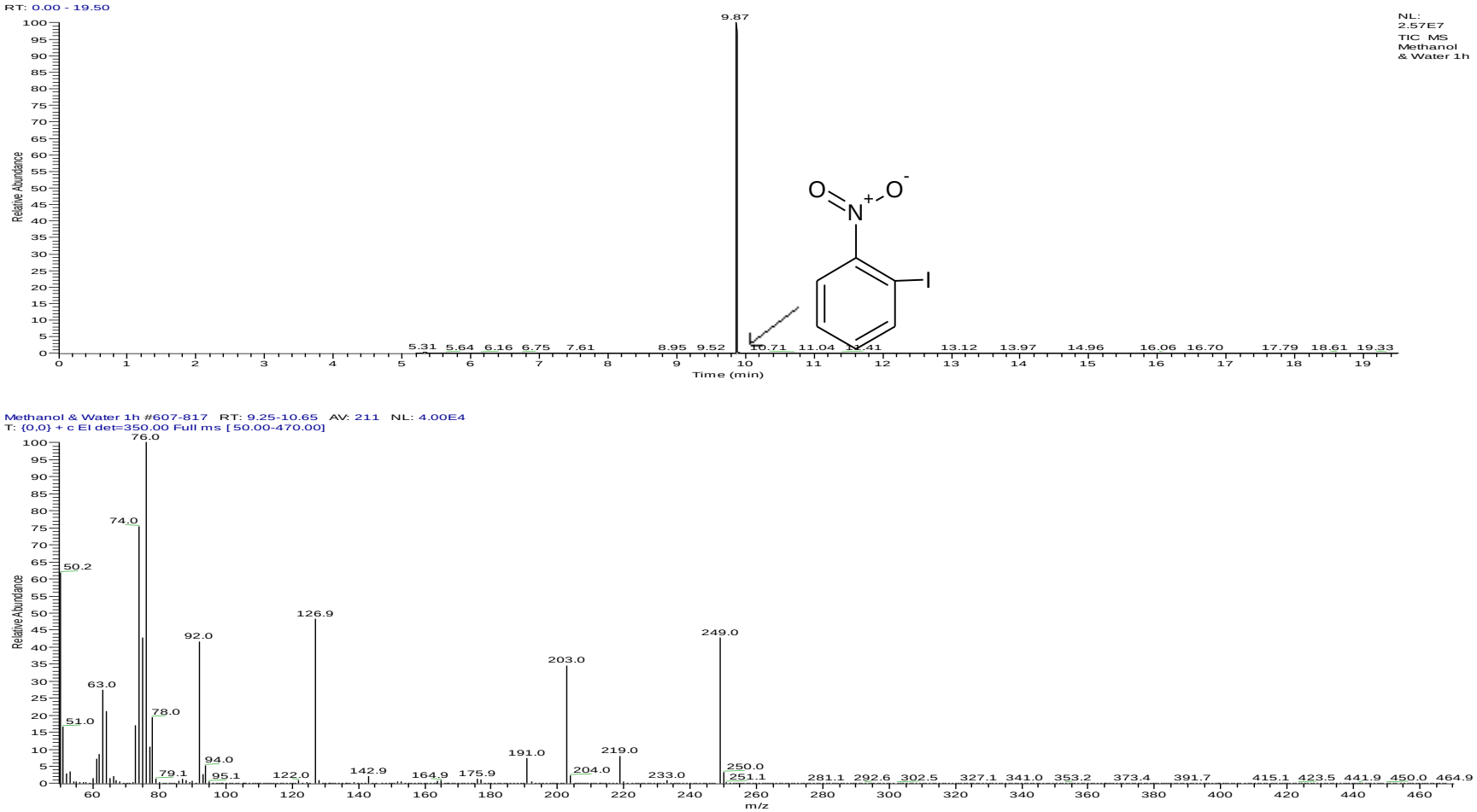
6.2 Appendix B





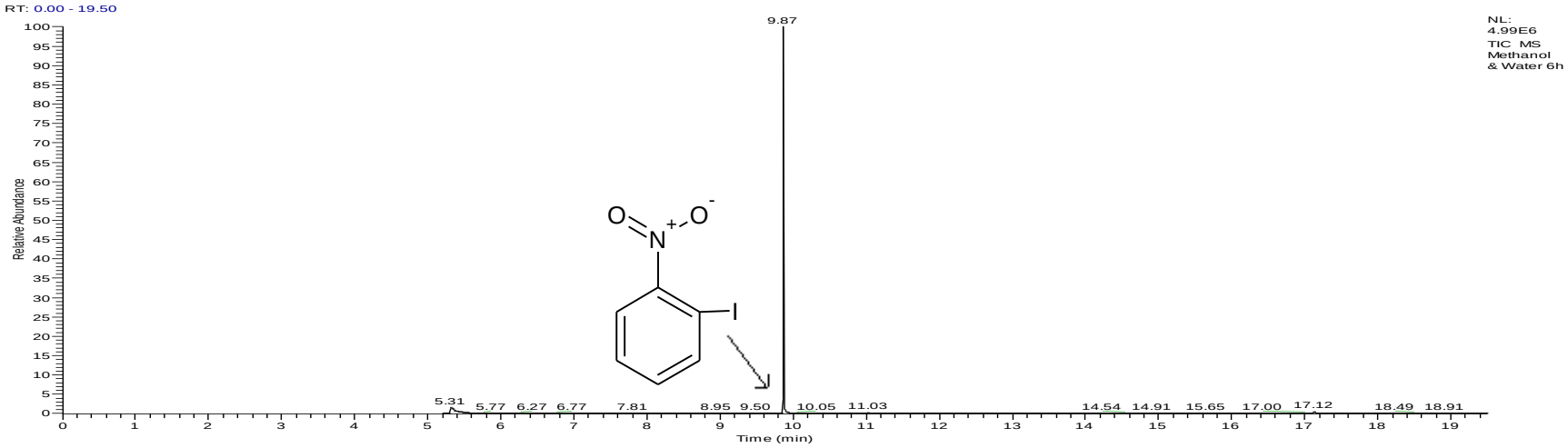
Chromatogram and mass spectrum for 7th reaction (Table 6).

6.3 Appendix C



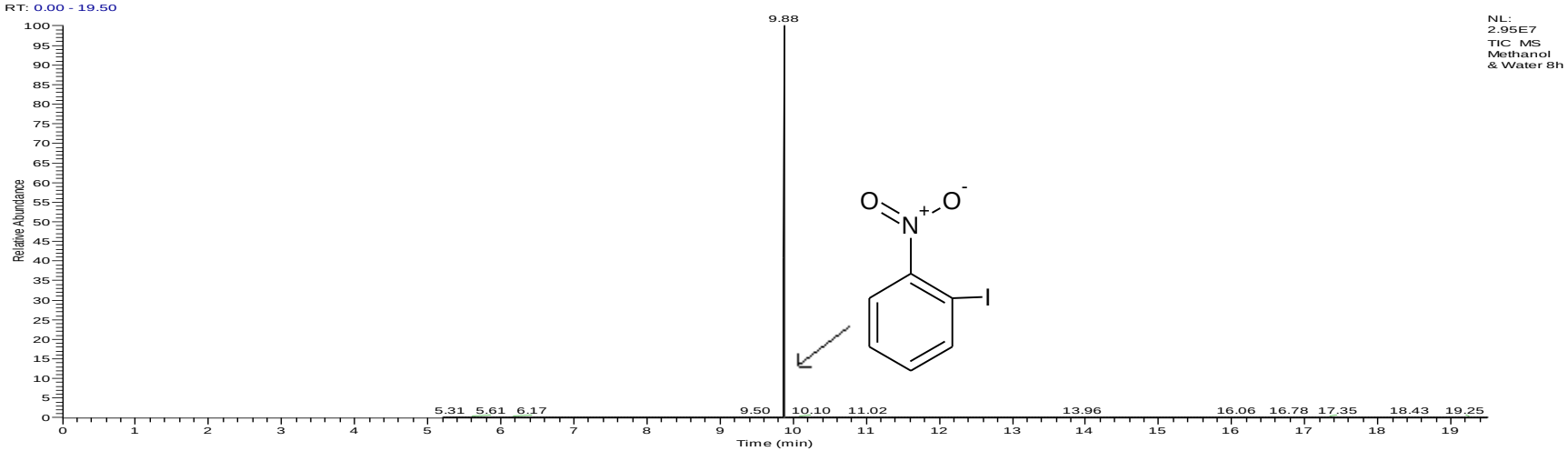
Chromatogram and mass spectrum for 1st reaction, 1h (Table 7).

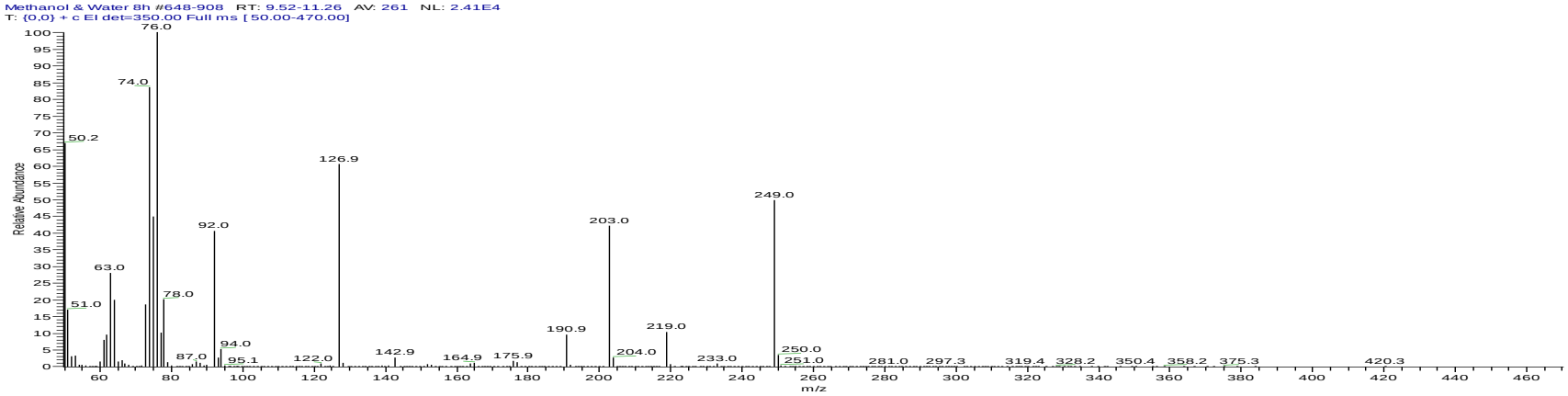
6.3 Appendix C



Chromatogram and mass spectrum for 1st reaction, 6h (Table 7).

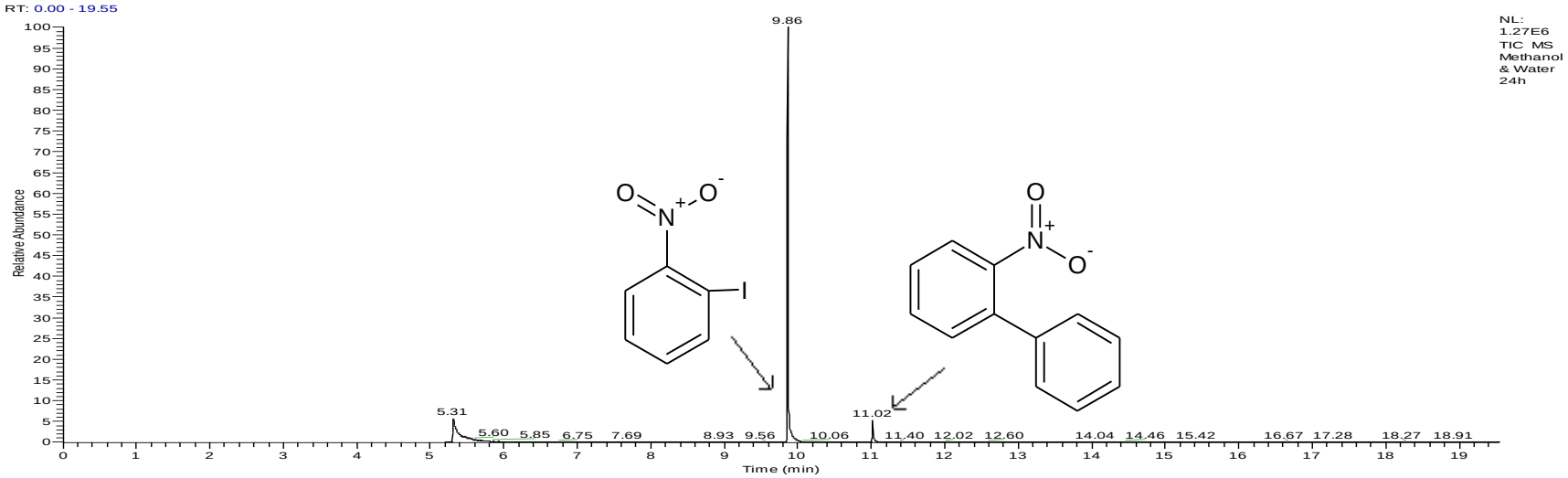
6.3 Appendix C





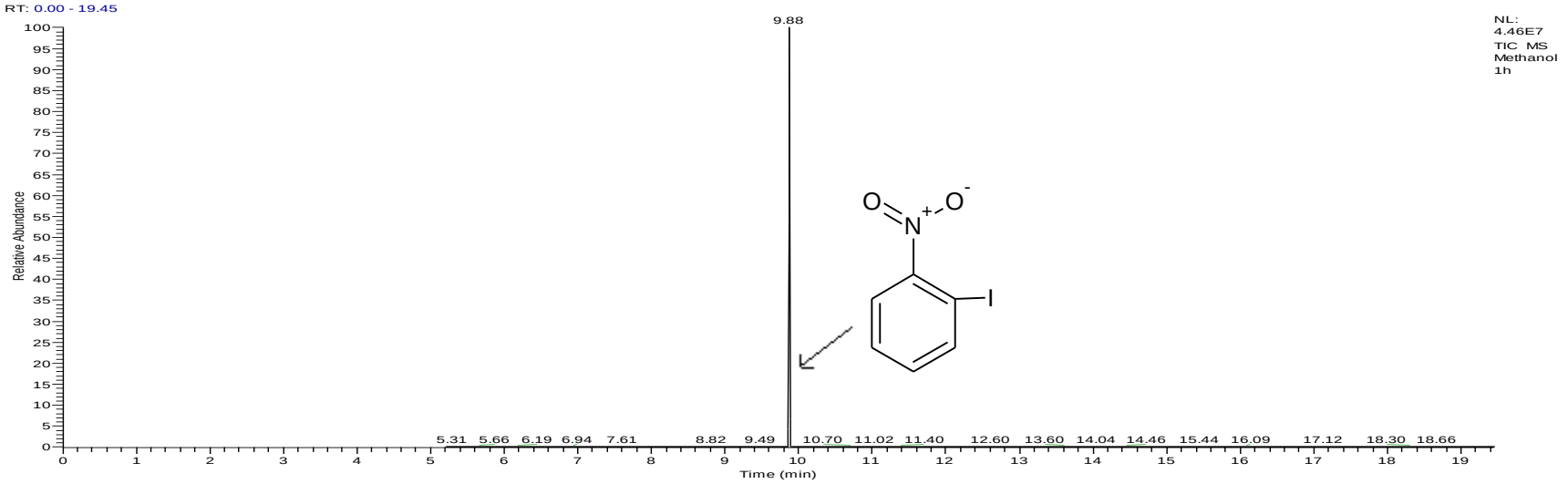
Chromatogram and mass spectrum for 1st reaction, 8h (Table 7).

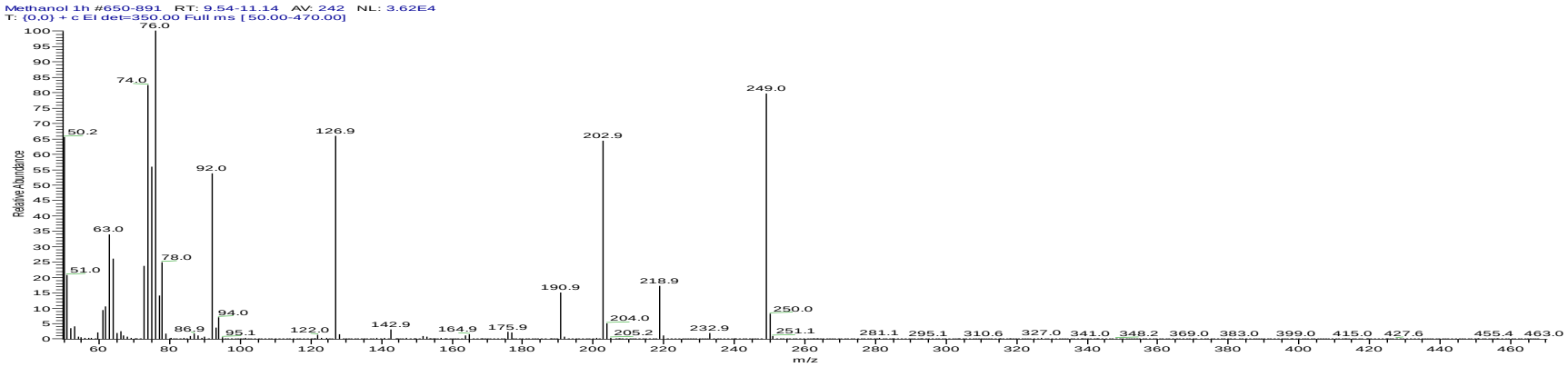
6.3 Appendix C



Chromatogram and mass spectrum for 1st reaction, 24h (Table 7).

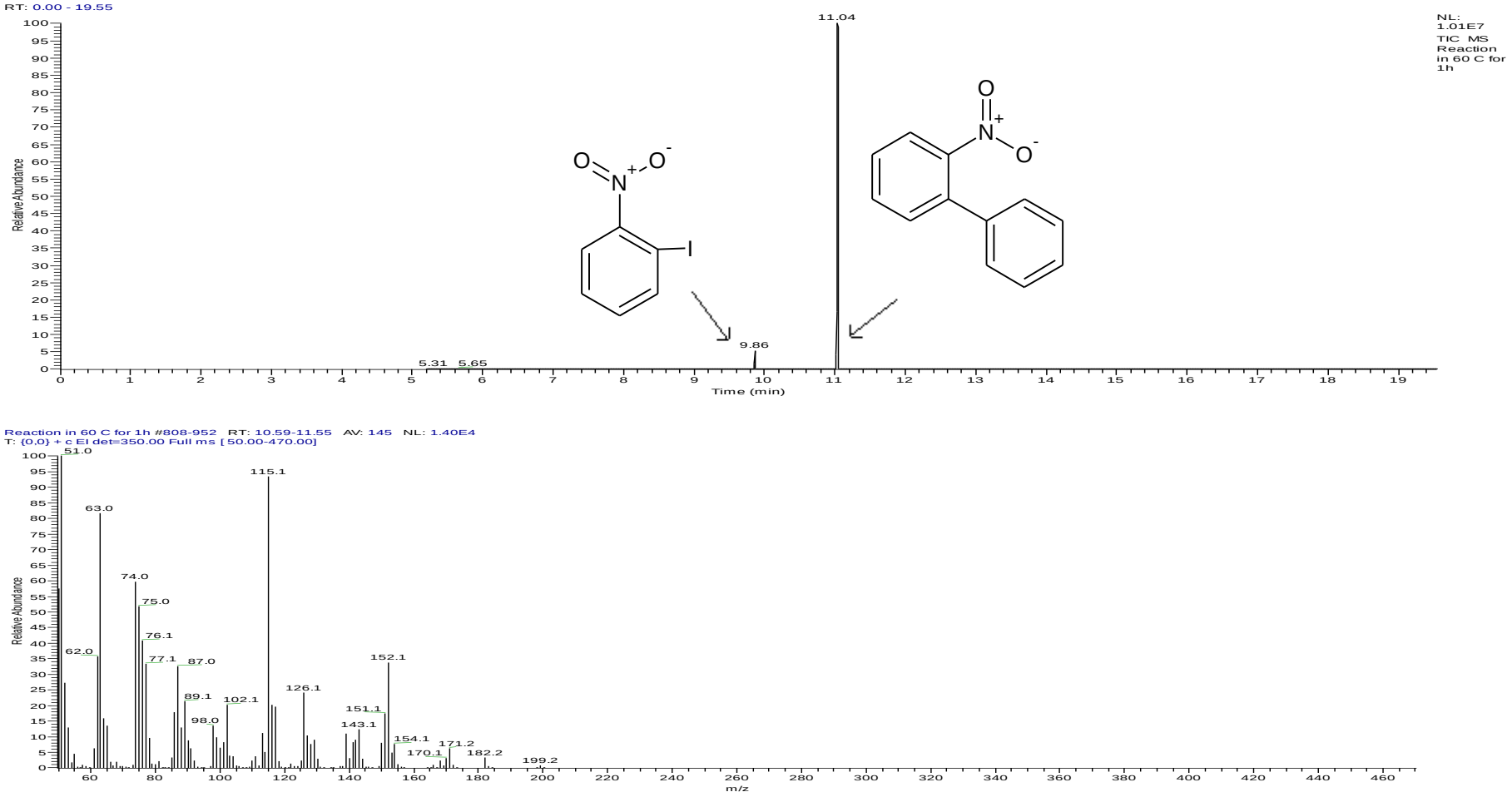
6.3 Appendix C





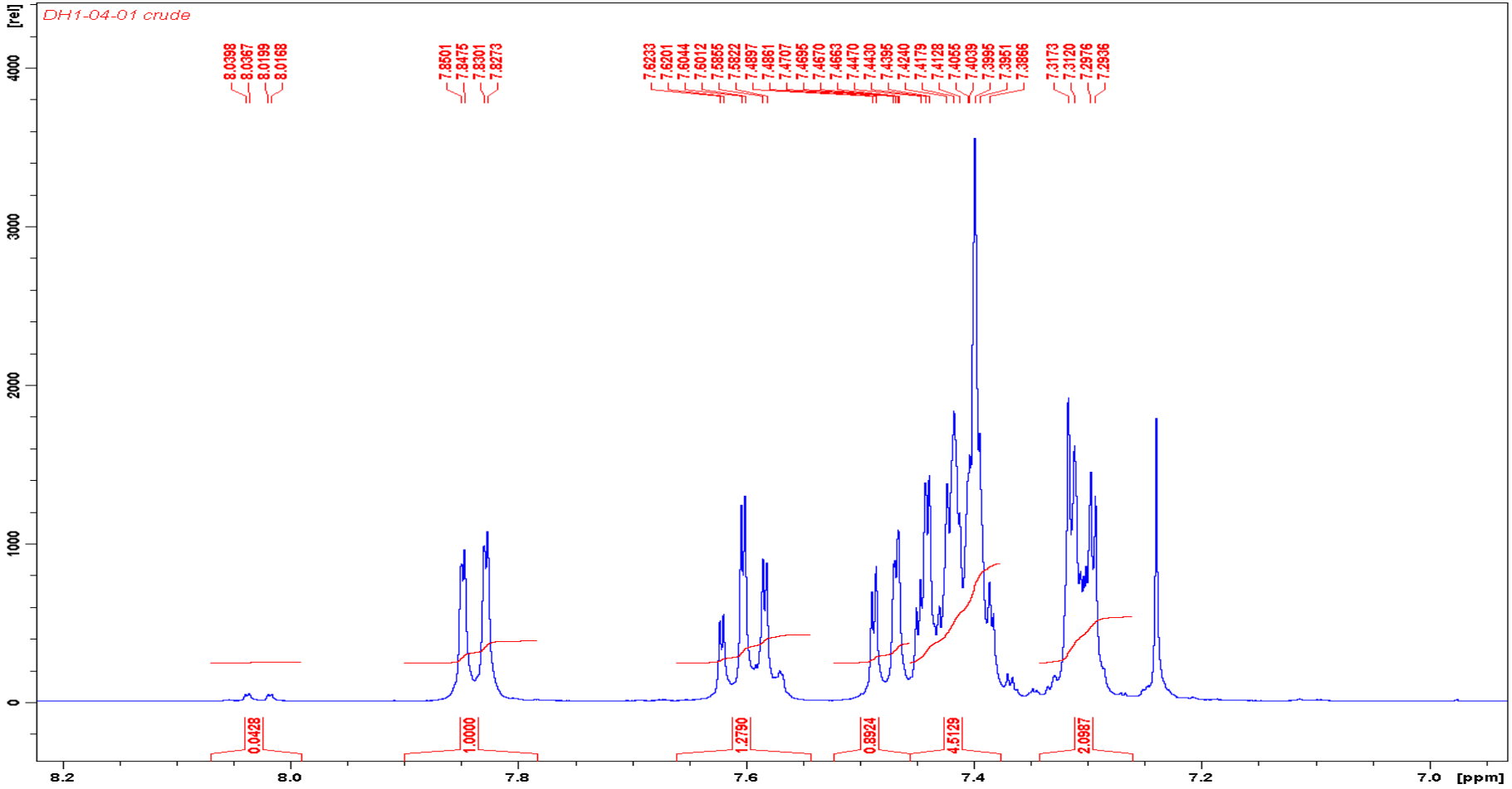
Chromatogram and mass spectrum for 2nd reaction (Table 7).

6.4 Appendix D



Chromatogram and mass spectrum for 2nd reaction (Table 9).

6.4 Appendix D



Our product after workup procedure. **2-Nitrobipheyl**[87,88], $C_{12}H_9NO_2$, MW 199.21. ^{1}H NMR(400.13 MHz, $CDCl_3$, ppm): δ 7.85-7.82 [dd, 1H], 7.62-7.58 [td, 1H], 7.48-7.38 [m, 5H], 7.31-7.29 [m, 2H].

6.5 Appendix E

Fluorescence correction Matlab codes:

```
[n,m]=size(X);
% First Fluorescence matrix Xf = raw data X
Xf=X;
x=(1:n)';
orden = 4;
plot(Xf(:,1),'b'), hold on
for j=1:15 % number iterations
for i=1:m % number of spectra
[b,s,mu]=polyfit(x,Xf(:,i),orden);
[Xf(:,i),DELTA] = polyval(b,x,s,mu);
end
% Finding and replacing values where Xf-polynom > X
Xcorr=X-Xf;
plot(Xf(:,1),'r'), plot(Xcorr(:,1),'g'), pause
end
tmp1=((Xcorr>0).*Xf);
tmp2=((Xcorr<0).*X);
Xf=tmp1+tmp2;
end
hold off
%Result plotting
if m==1
plot(X), hold on, plot(Xcorr,'m');
else
plot(mean(X')') ,hold on; plot(mean(Xcorr')','m');
end
```

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