

Masters Degree in Chemical Engineering

# Recovery of Polyphenols from Olive Vegetation Wastewaters by Adsorption

Masters Degree Thesis

Foreign Institution Development Project

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## Abstract

This work was divided in two sections: one section consisted on the study of the equilibrium process of the polyphenols from Olive Vegetation wastewaters (OVWW) on polymeric resins, the other section concerned the extraction and identification of the phenolic compounds in the OVWW.

OVWW were preliminarily treated in order to eliminate suspended solids, and then were submitted to an adsorption operation, afterwards, the resin was separated from the already treated OVWW and submitted to a desorption process with Ethylic alcohol 96%.

Moreover, three extractions were made, one carried out before the OVWW was submitted to the adsorption process, other after the adsorption process and finally one more to the alcoholic solution that resulted from the resin desorption. Once the samples were extracted they were analysed via HPLC instrument.

Different analytical techniques were used during this project: the measurement of the overall polyphenol content by spectrophotometry and analysis of the polyphenols mixtures by means of a HPLC instrument provided with the gradient technique.

During this project several conclusions were obtained: The adsorption process of polyphenols on polymeric resins was possible and thus these compounds were successfully removed from the OVWW.

Furthermore, in the determination and quantification of polyphenols section, the separation and identification of three well-known olive oil antioxidants (Tyrosol, Hydroxytyrosol and Catechol) was performed; other unidentified compounds were separated, and even though not identified they could be the following: Siringic acid, o-Coumaric acid and Oleuropein. The presence of Tyrosol and Hydroxytyrosol could be verified in each of the analysed samples and they were the phenolic compounds in larger amounts in the OVWW (Only Tyrosol was quantified).

Finally, this project led to conclude the absence of Gallic acid, p-OH benzoic acid and Caffeic acid in all the analysed samples of OVWW.

**Keywords:** *Olive Vegetation Wastewaters; polyphenols; HPLC.*

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## Notation and Glossary

<b>Abs</b>	absorbance	
<b>C<sub>L</sub></b>	adsorbate liquid concentration	mg.L <sup>-1</sup>
<b>C<sub>S</sub></b>	solute adsorbed concentration	mg.L <sup>-1</sup>
<b>c<sub>p</sub></b>	adsorbate concentration in the pore	mg.L <sup>-1</sup>
<b>m<sub>resin</sub></b>	quantity of resin	g
<b>M<sub>adsorb</sub></b>	quantity of substance adsorbed in the resin	mg
<b>M<sub>desorb</sub></b>	quantity of substance desorbed in the resin	mg
<b>M<sub>sol</sub></b>	quantity of solid (resin)	mg
<b>t</b>	Time	min

### *Greek letters*

<b>ε</b>	Void fraction of the adsorption bed or liquid volumetric fraction in the suspended bed.	
<b>ε<sub>p</sub></b>	Porosity of the resin.	
<b>τ</b>	Decaying time	min
<b>τ<sub>D</sub></b>	Characteristic diffusion time for the adsorbent particles	min

### *List of Abbreviations*

<b>4MC</b>	4-Methylcatechol
<b>COD</b>	Chemical Oxygen Demand
<b>HPLC</b>	High Performance Liquid Chromatography
<b>OVWW</b>	Olive Vegetation Waste Waters
<b>PP</b>	Polyphenols
<b>VW</b>	Vegetation Water before adsorption
<b>VWAA</b>	Vegetation Water After Adsorption
<b>VWD</b>	Variable Wavelength Detector
<b>RDS</b>	Resin's Desorption Sample

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# 1. Introduction

Polyphenols are contained by the majority of the plant compounds which possess antioxidant activity.

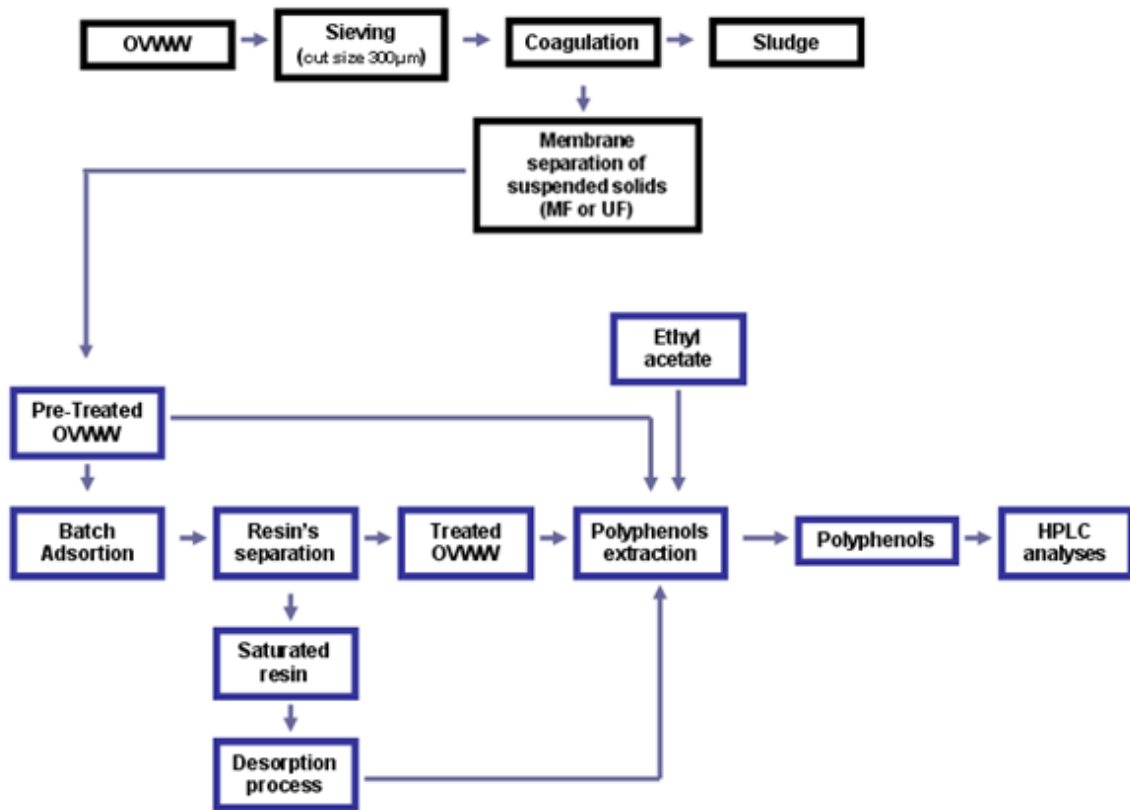
In this context, and since phenolic compounds inhibit the biological degradation of its organic matter, the interest of the scientific community has been focussed on the extraction of antioxidant compounds from inexpensive residues from agro-food industries, such as tomato wastes and olive vegetation wastewaters.

This project main issue was focussed on the recovery of polyphenols from olive vegetation wastewaters (OVWW) by adsorption on a polymeric resin. OVWW were preliminarily treated in order to eliminate suspended solid, and then were submitted to an adsorption operation performed by a stirred vessel to study the adsorption kinetics and equilibrium. Afterwards, the adsorbed polyphenols were extracted from the resin through a washing operation using a water-ethanol solution. The extraction of polyphenols (for further analyses) from the vegetation waters and the water-ethanol solution was conducted using Ethyl acetate as solvent. Posteriorly, the determination and quantification of the extracted polyphenols was made using HPLC analysis.

These HPLC analysis were conducted on both the OVWW, once and before the adsorption using the resin, and also on the alcoholic solution that resulted from the resin desorption process.

The measurement of the overall polyphenol content was also accomplished by spectrophotometry, adopting the Folin-Ciocalteu method.

For a better understanding on where this project must be fitted and what was done, a scheme with all developed processes performed with OVWW is shown below. The blue part refers to the subject of this study.



**Figure 1** – Scheme with the developed processes using the OVWW.

For the completion of this study, research has been conducted in the Department of Chemical Engineering Materials Environment of Faculty of Engineering at University “La Sapienza” in Rome.

## 2. State of Art

### 2.1 Vegetation Waters from olive oil mill

The olive oil extraction process produces two by-products: solids (husk) and olive vegetation wastewaters (OVWW), i.e. aqueous effluent (non-oily).

The vegetation waters are effluents characterized by their dark colour, brownish red. Their odour is similar to that of the olive oil (in the case the olive oil has been recently extracted), they will release a fetid and unpleasant scent once a few days pass, though. These waters are quite acid, their pH varies between 4 and 5, and they possess a high amount of organic matter, some of it dissolved and other part in suspension (Padilha *et al.*, 1991).

Once the residual water is originated from organic liquid that precedes live matter such as olive pulp, it is expected that these waters are absolutely bio degradable. However, and even though all the vegetation water's components are by definition bio degradable (because they are bio synthesized), some of them, for instance polyphenols and lipids, are decomposed at a much lower velocity than the others, such as sugars (Rozzi and Malpei, 1996).

It is reported that OVWW resulting from the production process surpasses 30 million m<sup>3</sup> per year in the Mediterranean region (Hamdi *et al.*, 1991). Depending on the extraction process, the vegetation waters concentration on organic matter is 200 to 400 times above this same concentration on municipal sewers, nevertheless, from all of the produced vegetation water, it is supposed that the big majority is tossed directly into water resources in an uncontrolled way. (Cossu *et al.*, 1993).

Vegetation waters are originated in a natural way and thus its composition is highly variable and complex. It depends on various factors, namely the variety of the olive, the edaphic conditions (the type of soil where it was cultivated and the climatic conditions during the fruit formation and its harvest), the utilized fertilizer, the possible plagues, the state of maturity and finally the period of storage in the mill before it was processed.

Bearing this in mind, it is easy to conclude that in a certain mill the composition of the vegetation waters will vary over the day depending on the previously referred variables. Nevertheless, an average composition of olive oil vegetation waters can be used (Fedirici, 2006).

**Table 1** - Average composition of olive vegetation waters.

<b>Density (g/cm<sup>3</sup>)</b>	1.023-1.054
<b>pH</b>	4.6-6.7
<b>Turbidity (NTU)</b>	11000-65000
<b>Water (%)</b>	82.4-96.0
<b>Dry extract (%)</b>	3.0-18.0
<b>Suspended solids (%)</b>	0.04-1.04
<b>Mineral compounds (%)</b>	0.4-7.2
<b>Organic compounds (%)</b>	3.9-16.5
<b>Total sugars (%)</b>	1.0-8.0
<b>Total pectins (%)</b>	0.05-0.15
<b>Total polyphenols (%)</b>	0.15-1.75
<b>Total nitrogen (%)</b>	0.1-7.2
<b>BOD (mg/L)</b>	9600-110000
<b>COD (mg/L)</b>	30000-195000

## ***2.2 The problematic involving the Polyphenols***

The polyphenols found in virgin olive oils and that are responsible for stability come from olive fruit during the extraction process, but a large part of these compounds is lost in the aqueous effluent called OVWW. This latter is the main waste product of this industry and the uncontrolled disposal of OVWW is becoming a serious environmental problem in the Mediterranean area due to the high COD values and the presence of phyto-toxic and antibacterial polyphenolic components (Russo, 2007).

Due to the toxicity of phenols at high concentration, their removal from water is an important issue. Different water treatment technologies are used to remove phenolic pollutants, for example, biological degradation, chemical oxidation, or adsorption (Wagner and Schulz, 2001).

In Italy, the legislation permits to spread out the waste waters on the farms' ground, respecting some limitations concerning the amount tossed, spreading procedure and ground quality monitoring. In literature, the advantages and disadvantages of this practice were widely discussed: on one hand it appears that the spreading of these waste waters permits to recycle important ground nutrients back to the terrain, improving harvesting performances (Massari and Russo, 1999); on the other hand, heterogeneous ground morphology, such as different structured clay layers, leads to very high and toxic pollutant concentration in local spots, which may reach and pollute severely, through the creation of a concentration funnel, the ground water layer (Jones *et al.*, 1998)

## **2.3 Polyphenols importance**

Polyphenols are compounds that contain one, two, or several phenolic components and are abundant in nature. Polyphenols have become recognized for their antioxidant properties, which have been associated with reduced risk of cancer, strokes, heart diseases and diabetes. They also play an important role in food quality.

In particular, phenolic compounds are strong antioxidants and are also responsible for the astringency and bitterness of olive oils (Bonoli, 2003).

In the OVWW, several low-molecular-weight phenolic compounds are present, for instance, the phenolic derivatives of hydroxycinnamic, ferulic and caffeic acids and, in larger amounts, Tyrosol (4-hydroxyphenetil alcohol) and Hydroxytyrosol (3, 4-dihydroxyphenetil alcohol). Although in a different extent, all these compounds are characterized by high antioxidant activity and are, therefore, of great interest in the cosmetic and pharmaceutical industries and also in food processing and food products conservation. After filtration to eliminate the suspended solids, all compounds of potential interest can be recovered by using chemical and physical processes such as ultra filtration, nanofiltration and reverse osmosis (Fedirici, 2006).

Once there is a large presence of Hydroxytyrosol in the OVWW, the waste of this compound is of great potential importance for its direct recovery and, of even greater commercial importance, for the possibility of developing catalytic methods, environmentally friendly and economically sustainable, to convert other compounds (Tyrosol, above all), present in the OVWW, into Hydroxytyrosol.

## **2.4 Recovery and Characterization**

Different water treatment technologies are used to remove phenolic pollutants, for example, biological degradation, chemical oxidation, or adsorption.

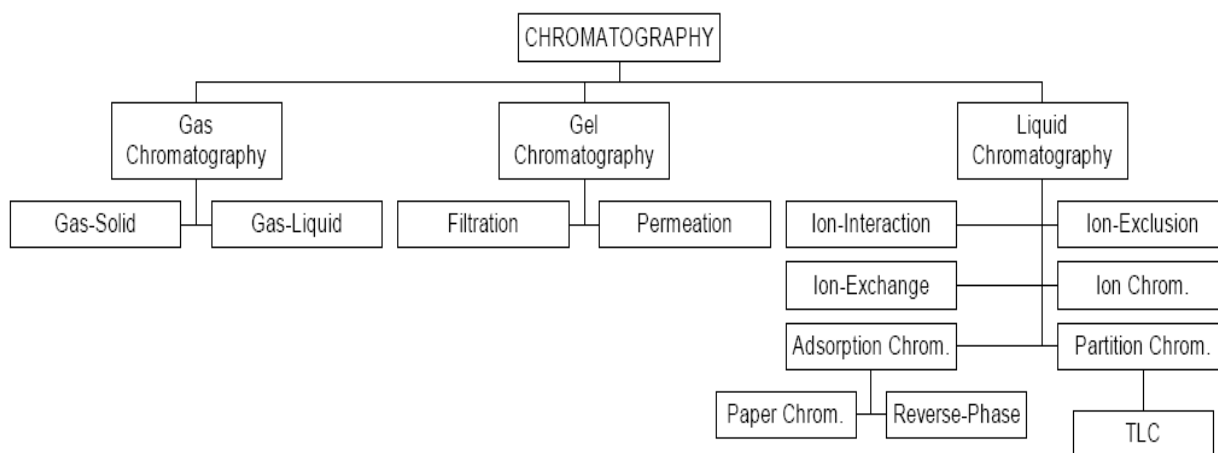
Adsorption processes can be used for a wide range of phenol concentrations and are cost-efficient, especially if the pollutants are to be recycled. In this context, polymeric adsorbents have gained in importance. Compared to the traditionally used active carbon, a smaller amount

of adsorbent is required, longer residence times can be achieved, and the polymeric material can easily be regenerated (Wagner and Schulz, 2001).

### 2.4.1 Characterization by Chromatography

Chromatography is an analytical method that finds wide application for the separation, identification and determination of chemical components in complex mixtures. This technique is based on the separation of components in a mixture (the solute) due to the difference in migration rates of the components through a stationary phase by a gaseous or liquid mobile phase.

Chromatography can be divided into three subsections namely gas, gel and liquid chromatography (figure 2). Gas chromatography is used for the analysis of volatile samples, gel chromatography for non-volatile samples with a molecular weight larger than 2000 and liquid chromatography for non-volatile samples with a molecular weight smaller than 2000.



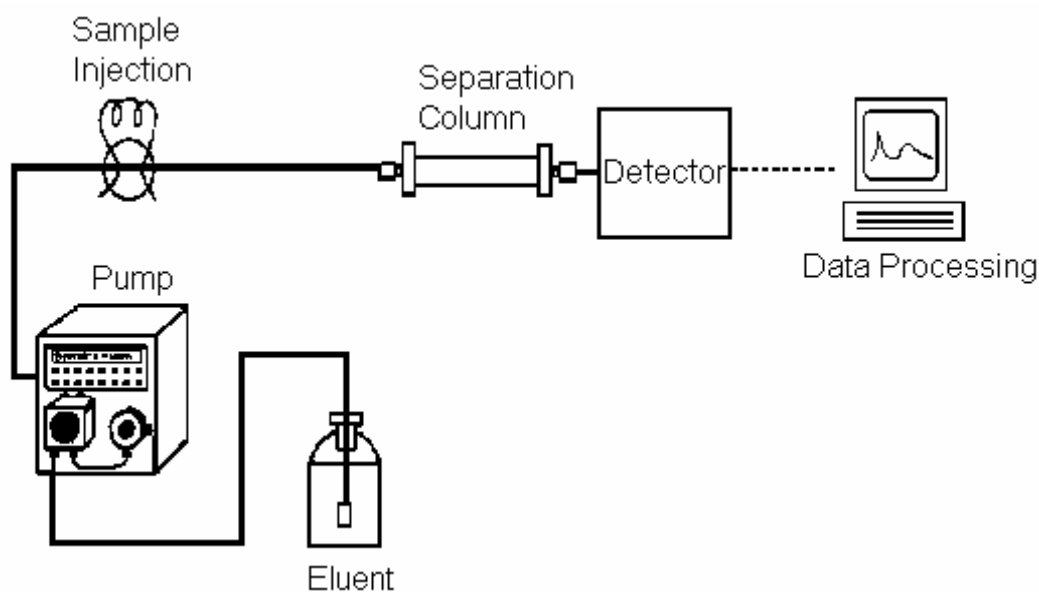
**Figure 2** – Types of Chromatography

Adsorption chromatography is used for the separation of non-polar or fairly polar organic molecules. In this technique the stationary phase is the surface of a finely divided polar solid and the analyte competes with the mobile phase for sites on the surface of the packing. Retention of the analyte occurs as a result of adsorption forces. Finely divided silica and alumina are used as stationary phases with organic solvents such as hexane acting as the mobile phase. The only variable that can be altered to affect the partition coefficient of the analytes is the composition of

the mobile phase. A particular advantage of adsorption chromatography is its ability to resolve isomeric mixtures.

High Performance Liquid Chromatography (HPLC) with UV detection is frequently used to separation and detection of phenolic compounds presents in OVWW.

The main components of an HPLC system are a high-pressure pump, a column and an injector system as well as a detector (Figure 3). The system works as follows: eluent is filtered and pumped through a chromatographic column, the sample is loaded and injected onto the column and the effluent is monitored using a detector and recorded as peaks.



**Figure 3 - HPLC Instrumentation.**

## 3. Experimental

### 3.1 Chemicals and Adsorbents

4-Methylcatechol, Catechol (Pyrocatechol), Gallic Acid, Tyrosol (4-hydroxyphenetil alcohol) and p-Coumaric Acid were all purchased from Fluka. Caffeic acid and p-OH benzoic Acid were purchased from Sigma. Their solutions were prepared with degassed and distilled water. Chloridric acid was purchased by Baker Analyzed.

Reagent Folin-Ciocalteu was purchased from Carlo Erba.

Acetonitrile and 2-Propanol (HPLC-grade both) were obtained from Sigma-Aldrich. Ethyl Acetate and Sodium Sulphate Anidrous were purchased by Carlo Erba.

A non-ionic polymeric resin was used to adsorb phenolic compounds: Amberlite XAD16, which was obtained from Rohm and Haas (France). Non-ionic polymeric resins have been previously successfully used for phenol and phenolic derivatives adsorption (Otero *et al.*, 2005). Table 2 summarizes the data supplied by the manufacturer on the physical characteristics of the resin used in this work (Otero *et al.*, 2005).

**Table 2** - Physical properties of polymeric resin Amberlite XAD16.

Adsorbent	Amberlite XAD16
Matrix	Aromatic porous resin with hydrophobic substituents
Chemistry	Polystyrene DVB
Physical form	White translucent
Humidity factor (fh)	0.34
Density (kgm <sup>-3</sup> )	1020
Porosity	0.55

## **3.2 Analytical Methods**

### **3.2.1 Polyphenols Analysis by Spectrophotometry UV-Visible**

Polyphenols were measured by the Folin-Ciocalteu method using 4-methylcatechol as standard. The Folin-Ciocalteu reagent is a mixture of phosphomolybdate acid ( $H_3PMo_{12}O_{40}$ ) and phosphotungstate acid ( $H_3PW_{12}O_{40}$ ) used for the colorimetric assay of phenolic antioxidants and polyphenol antioxidants. It works by measuring the amount of the substance being tested needed to inhibit the oxidation of the reagent (Singleton and Rossi, 1965).

The Folin-Ciocalteu method is the reduction of metal oxides by polyphenols resulting in a blue solution that has an absorption maximum at 765nm. Since different types of polyphenols react similarly with the Folin-Ciocalteu reagent, it is more easily quantifiable.

However, this reagent does not only measure total phenols and will react with any reducing substance. The reagent therefore measures the total reducing capacity of a sample, not just the level of phenolic compounds.

Polyphenols generally adsorb the light within the ultraviolet range at a wave length of 282nm. However, at this range, the polymeric cuvette used in the measurement process also adsorbs the light, for this matter, it is unviable to measure the polyphenols concentration. The problem could be solved by betaking quartz cuvetts, nevertheless these materials are too expensive and delicate, so, and taking that into account the problem was addressed in a different way. The solution was to use a method that permitted the measurements to be made in visible range (400-900nm) and therefore the polymeric cuvetts were used.

This methodology consists on using the Folin-Ciocalteu reagent, this method measure the concentration of polyphenols in an indirect way, in other words, when polyphenols are in contact with this reagent they undergo a reaction where the product can actually adsorb within the visible range. The analysis procedure of Folin-Ciocalteu method is in annexe (Annexe A).

The analyses absorbance of the solutions (polyphenols concentration) were measured at 750nm against a blank sample and executed by a spectrophotometer model DU-65 supplied by Beckman.

### 3.2.2 HPLC analysis

High Performance Liquid Chromatography (HPLC) with UV detection is frequently used for separation and detection of the phenolic compounds present in the OVWW.

The HPLC system was composed of a Spectra-Physics liquid chromatography Model Agilent 1200 Series equipped with C18 Supelco column (18,5  $\mu\text{m}$ ; 250 $\times$ 4.6 mm), coupled with a Spectra-Physics UV–Vis detector.

The mobile phases were Acetonitrile (solvent A) and 1% (v/v) Acetic Acid in water (solvent B) at a flow rate of 1.0 ml/min. A programme was used for present preparative separation as follows: 2 minutes isocratic elution 0% A and 40 minutes gradient elution 40% A. The injection volume was 20  $\mu\text{L}$  while the column temperature was maintained at 35  $^{\circ}\text{C}$ . Signal was monitored at 280 nm with the variable wavelength detector (VWD).

## 3.3 Polyphenols Extraction

For the HPLC analysis it was necessary to extract polyphenols from the OVWW as well as from the alcoholic solution of the resin desorption.

The liquid-liquid phase extraction, using the Ethyl Acetate as solvent was the method applied for the extraction of polyphenols, there were several other methods viable according to available literature that could have been applied just as well.

The extraction was conducted on both the OVWW, once and before the adsorption using the resin, and the alcoholic solution that resulted from the resin desorption process.

### 3.3.1 Polyphenols concentrated extratcs preparation

- *Preparation of OVWW samples before and after adsorption*

Initially, 100ml of olive vegetation were measured and putted into a decanting ampoule of 250ml where 2ml of concentrated Chloridric acid, to acidify the solution, was posteriorly added up. An extraction was then executed with about 100ml of Ethyl Acetate and was repeated four times.

As for the OVWW after adsorption process, 150ml were concentrated to about 30 ml by using a rotavapour and a vacuum pump, the temperature was fixated to 38 $^{\circ}\text{C}$  along this process (once high temperatures can degrade polyphenols, it's of great importance that the temperature

to be used during this process does not cross the 40°C boundary). This extraction was executed with about 40ml of Ethyl Acetate and was also repeated four times.

Once the four extractions were completed, all the extract retrieved was again put into the decanting ampoule, this step prevents the formation of a thin layer of aqueous solution. The extract that resulted from the extractions was then, slowly, filtrated twice with Anhydrous Sodium Sulphate.

Moreover, the extracts obtained from the filtration were put into a 500ml balloon and then exposed to the rotavapour along the vacuum pump at constant temperature regulated for 38°C. The sample was reduced to about 10ml and then transferred to a 100ml balloon, again it was taken to the rotavapour with the vacuum pump and temperature fixed at 40°C. The sample was only withdrawn from the rotavapour once it could be observed that the sample in the bottom of the balloon was dry.

Furthermore, 2.5ml of distilled water was added to the obtained extracts and the resulting solutions were agitated until the extract was dissolved. In the end the solutions were transferred to a flask and conserved at -18°C.

- ***Preparation of the desorption resin's sample***

The sample contains the polyphenols adsorbed in 150ml of OVWW, as well as those desorbed in 100ml of Ethylic Alcohol. During the adsorption process 5g of resin were used.

Initially, the 150ml of resin's desorption sample in Alcohol Ethylic was concentrated to about 25ml and put into a decanting ampoule of 250ml, where 10ml of distilled water and 700µl of concentrated Chloridric Acid were added up. An extraction was then executed with about 40ml of Ethyl Acetate and was repeated three times.

As for the rest of the procedure it evolved the same steps as those taken in sample of OVWW.

### ***3.4 Polyphenols identification***

Bearing in mind the objective to determine the chromatographic profile and the concentration of polyphenols present in the OVWW, the solution analysis by HPLC was started.

Firstly, the analyses were conducted on both the OVWW, once and before the adsorption using the resin, and the alcoholic solution that resulted from the resin's desorption process. Two samples of different Vegetation Waters were analysed (VW<sub>1</sub> e VW<sub>2</sub>).

The samples of “vegetation water before adsorption”, “vegetation water after adsorption” and “resin’s desorption sample” were tagged VW, VWAA and RDS, respectively.

### 3.4.1 Injection of standards

In order to identify the existent polyphenol in the OVWW the retention time of the available standard compounds was analysed.

Taking into account the available standard compounds and putting the retention times obtained from the VW<sub>1</sub> and VW<sub>2</sub> chromatograms against the results from another laboratory (ENEA) in 2006, two solutions were prepared according to the following procedure:

- Standards A: 29mg of Catechol, 9mg of p-OH benzoic acid and 33.7mg of p-Coumaric acid.
- Standards B: 18.4mg of Tyrosol, 13.3mg of p-OH benzoic acid and 17.3mg of Caffeic acid.

These two solutions were dissolved in a volumetric flask of 50 ml using distilled water and the aid of 10 ml of Ethanol to perform full dissolution. Two more standard solutions were conducted; one of 4MC and the other of Galic acid, both of these solutions had a concentration of 1g/l.

### 3.4.2 Co-injection of VWs and resin’s desorption

- **Tyrosol**

Initially, a standard solution containing 5ml of distilled water, diluted in 0.10 g of standard Tyrosol was prepared.

For the injection phase, three mixtures were prepared and put into a flask, each one of them contained 250 µL of Tyrosol (2 g/L) and 250 µL of the samples to analyse (VW<sub>1</sub>, VW<sub>2</sub> and VWAA).

- **Catechol, p-OH benzoic acid, p-Coumaric acid and Caffeic acid**

In a further step, 27.0 mg of Catechol, 8.5 mg of p-OH benzoic acid and 14.5 g of p-Coumaric acid were measured in a volumetric flask of 50 ml and then dissolved in distilled water using 10 ml of Ethanol. This solution was tagged as MIX<sub>1</sub>.

And finally 37mg of Caffeic acid were also weighted and dissolved in distilled water on a volumetric flask of 50 mL.

Into a flask of 1.5 mL were put 250  $\mu\text{L}$  of MIX<sub>1</sub> and 250  $\mu\text{L}$  of the samples to analyse (VW<sub>2</sub> and RD) and then injected. Posteriorly, 250  $\mu\text{L}$  of Caffeic acid solution and 250  $\mu\text{L}$  of VW<sub>2</sub> were also put into a flask and then injected.

### 3.4.3 Quantitative Analyses

The evaluation of each compound was performed using a four-point regression curve obtained using the available standards.

Since only one of the required standard polyphenols present in larger amounts in the OVWW was available, Tyrosol, there was no possibility to create a calibration curve for all of the other compounds.

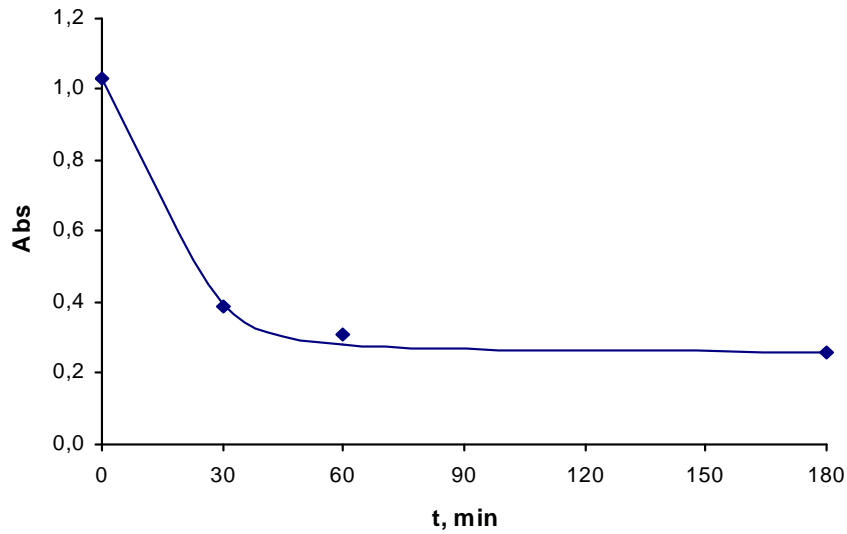
For the calibration curve four injections with different concentrations of Tyrosol (0.075, 1.0, 1.5 and 2.0 g/L) were conducted. For each one of the concentrations four peaks were obtained, the average of the obtained areas was made and the calibration curve was created.

## 3.5 Preliminary experiments for adsorption process

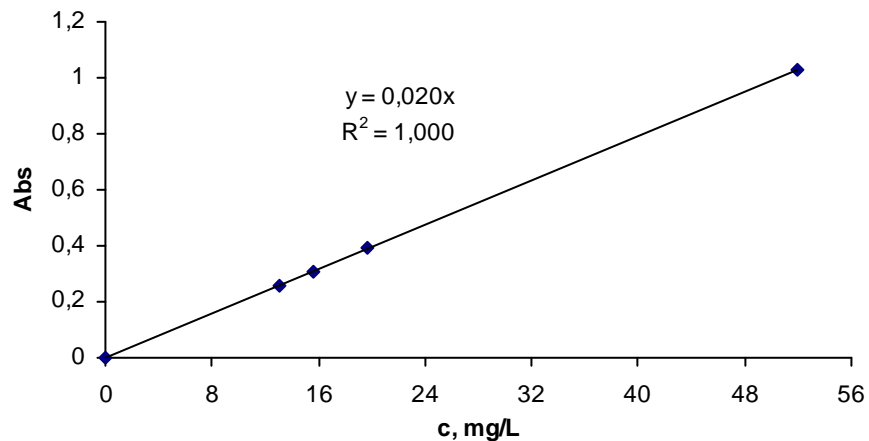
Before starting the experiments is it important to verify whether the resin is adaptable to a specific polyphenol adsorption, 4-Methylcatechol (4MC), for that reason a feasibility study was conducted.

A measured concentration (50 mg/L), aqueous solution of 4MC was kept under non-stop stirring inside a beaker. Posteriorly, and once 1.66g of adsorbent particles (resin) were added, the solute concentration in 4MC solution was measured during a previously established period of time. The measurements were done by spectrophotometry at 280 nm.

In figure 4 it is possible to check the adsorption curve for 4MC solution and posteriorly in figure 5 the variation of absorbance with 4MC solution concentration.



**Figure 4** – Adsorption curve for the solution of 4MC.

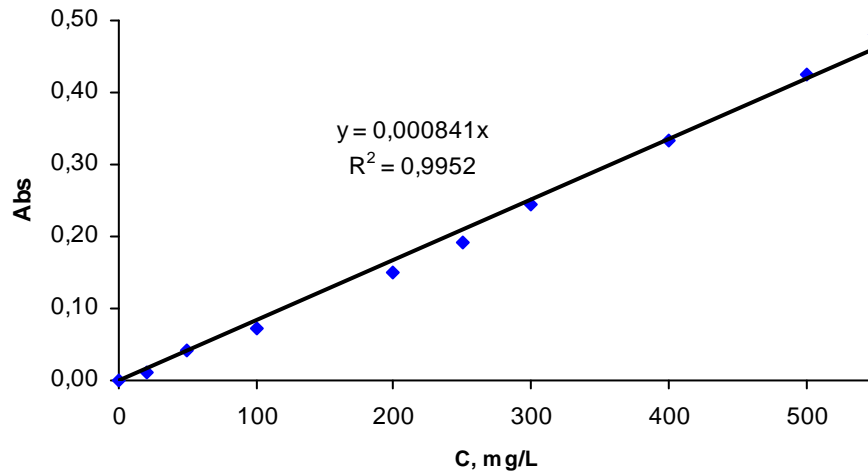


**Figure 5**– Variation of absorbance with 4MC solution concentration.

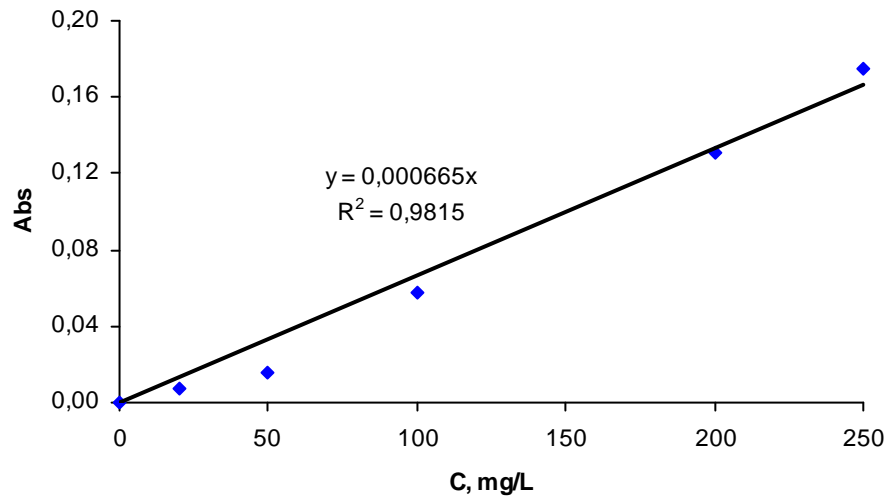
### 3.5.1 4-Methylcatechol calibration line

Calibration was carried out using 4-methylcatechol as standard at concentrations of 0, 20, 50, 100, 200, 250, 300, 400, 500 and 550  $\text{mgL}^{-1}$ . The absorbance was measured at 750nm against a blank sample, using the Folin-Ciocalteu measurement method (Annexe A).

So, in this context, two linear calibrations were applied, the first one, shown in figure 6, using an aqueous solution of 4-methylcatechol, and the second one (figure 7) resorting to an alcoholic solution of the same compound.



**Figure 6** - Spectrophotometer's calibration line (aqueous solution) at 750 nm.



**Figure 7** - Spectrophotometer's calibration line (alcoholic solution) at 750 nm.

### 3.5.2 Resin's activation

This particular resin, XAD16 from Rhom & Haas Company, was previously subdued to a treatment based on sodium chlorite; these compounds are particularly gainful for the resin's conservation.

The first step is to purge these components in order to make the resin active. The procedure used for this purpose consisted on sequentially, washing the resin with distilled water, and then filtrating it with a Buchner filter connected to a vacuum system.

Furthermore, the filtrated resin was put into a beaker, that already contained distilled water, the resulting solution was then left to constant stirring (70 rpm) for the best part of an

hour. In the end of this procedure the quantity of dissolved salts was evaluated through a digital conductivity meter.

Since the results were not satisfactory a new filtration was made and the stirring was repeated, again in a period of 60 minutes. The solution was again measured in the end and a decrease in salt concentration was revealed.

Finally, the obtained and already treated resin was put into another beaker containing ethylic alcohol at 96°C, the beaker was then stirred (120 rpm) for an hour. After this procedure the resin was filtrated and conserved on a distilled water environment, the resin was now successfully activated and ready for use.

### **3.6 Batch adsorption studies**

Adsorption equilibrium experiments were carried out by putting into contact the corresponding amount of adsorbent with 150 mL of OVWW solution in 250 mL beakers containing a magnetic stirrer, and posteriorly were kept under strong agitation. Initial concentration on the solution was around 223 mgL<sup>-1</sup> in all experiments.

For this matter, various experiments were conducted, during each one them, various samples of solution were collected between gaps of 30 minutes, the amount of polyphenols in these samples was then measured in a further step, using the Folin-Ciocalteau measurement method (Annexe A).

The equilibrium was reached within 180 min, but in order to be sure that the equilibrium would be attained in almost all cases, the contact time used was 24h.

The polyphenols concentration in the remaining aqueous solutions in all this time gaps and after removal of the resin by filtration and the stripped polyphenols concentration were determined by a spectrophotometer at 750nm.

### **3.7 Desorption study using a alcoholic solution**

The desorption process consist on the regeneration of the saturated resin.

The Folin-Ciocalteau measurement method also stables the measures using the spectrophotometer for the alcoholic solution of polyphenols originated from the olive vegetation wastewater (OVWW), therefore it will also be used in the desorption process.

The samples of saturated resin resulting from the treatment with *OVWW* were collocated on a becker already filled with 100 mL of Ethyl alcohol and a magnetic stirrer, this solution was kept under strong stirring.

In each experiment, several samples of the solution were gathered for a certain amount of time (around five minutes), the concentration of polyphenols was evaluated in every sample withdrawn, by employing the Folin-Ciocalteau measurement method (Annexe A).

## 4. Results and Discussion

The final results were treated using the informatics application formally known as MICROSOFT EXCEL and by software inherent to the devices used during the process.

### 4.1 Polyphenols Identification

#### 4.1.1 Vegetation wastewaters samples injection

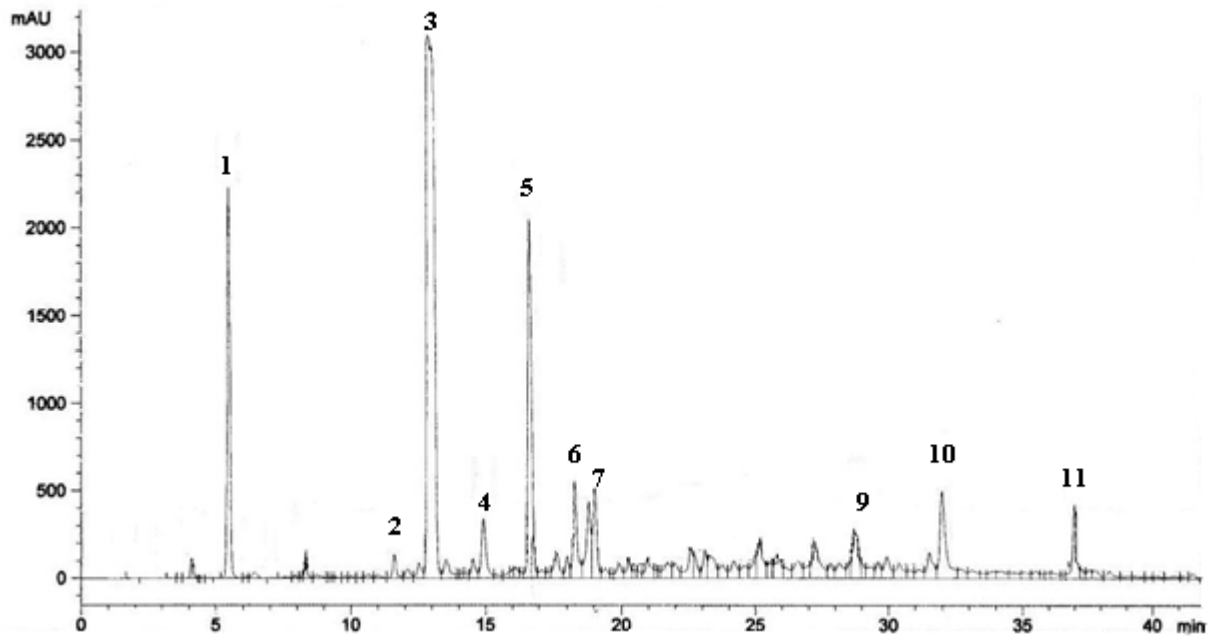
Two samples of different Vegetation Waters were analysed (VW<sub>1</sub> e VW<sub>2</sub>).

The polyphenols total concentration and the COD value in these vegetation waters was measured, the first one by the Folin-Ciocalteu measurement method (Annexe A) and the second one by spectrophotometer (LASA 100 Hach-Lange). As it was already mentioned before, the Folin-Ciocalteu method measures the concentration of polyphenols in an indirect way, for that matter and it's probably that the obtained concentration is not accurate. The following table contains the values of concentration obtained for each of the VW.

**Table 3** - Vegetation Waters characteristics.

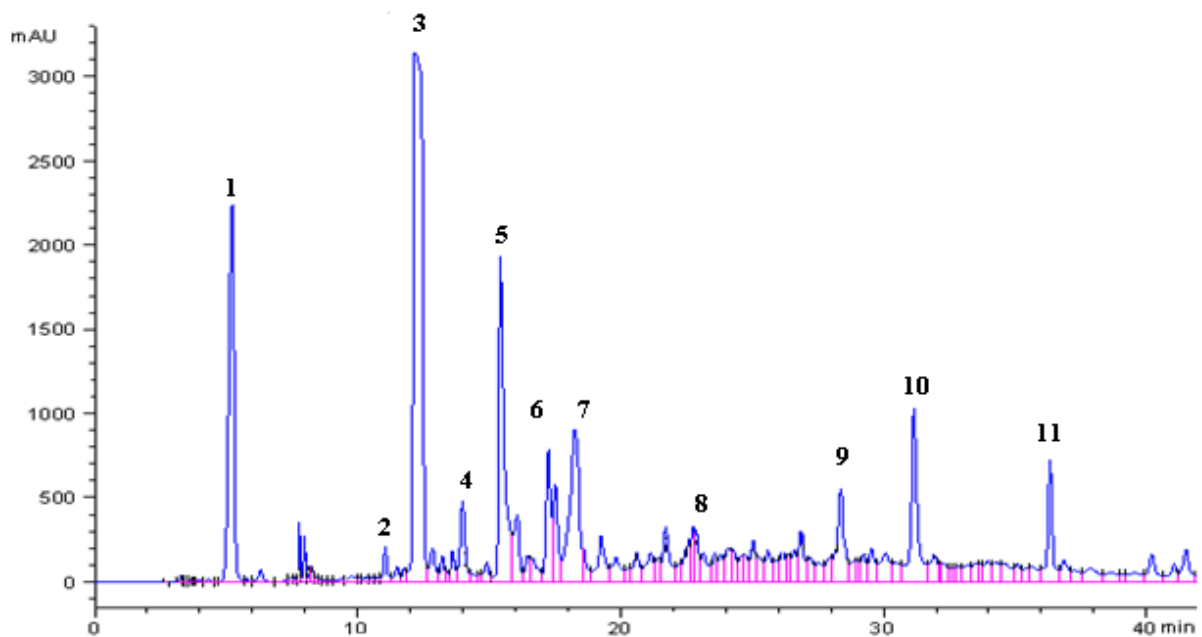
	VW <sub>1</sub>	VW <sub>2</sub>
<b>COD [mg/L]</b>	60000	43000
<b>Total polyphenols [mg/L 4MC equivalent]</b>	2010	2354

Following, the HPLC chromatograms obtained for each sample are presented.



**Figure 8** – HPLC Chromatogram 1 for  $VW_1$  sample.

**Note:** The 8<sup>th</sup> peak was not considered for it is not well defined, thus it was not analysed in this vegetation water.



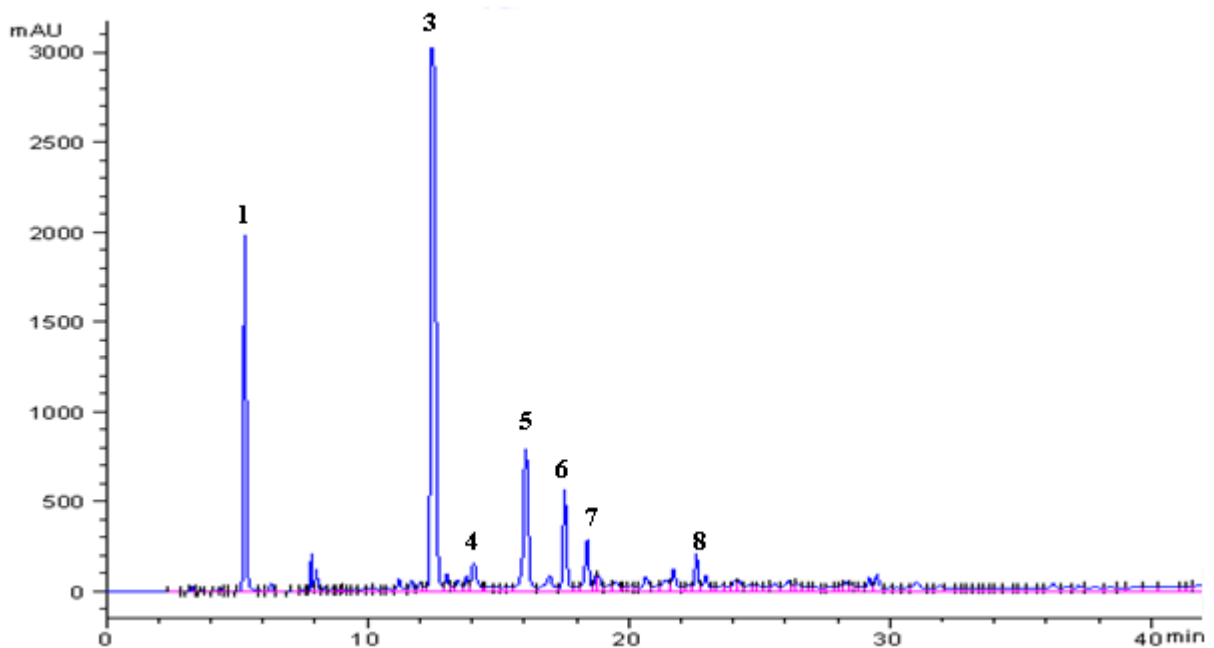
**Figure 9** – HPLC Chromatogram 2 for  $VW_2$  sample.

As it's possible to observe in the  $VW_1$  and  $VW_2$  HPLC chromatograms (figures 8 and 9, respectively), the obtained peaks are practically the same, still they differ slightly as far as their areas are concerned. The  $VW_2$  has bigger areas in all of the eluted peaks, what comes to concur with the fact that this solution actually contains more polyphenols than the other.

### 4.1.2 Vegetation wastewaters after adsorption sample injection

Initially, as the vegetation water as separated from the resin, it possessed a low concentration of polyphenols, for that reason the water was concentrated using a rotavapour with vacuum pump and temperature fixed at 38°C. The final concentration of polyphenols for this VW was the 1125 mg/L 4MC equivalent.

The chromatogram shown in the next figure concerns the quantity of polyphenols which were not adsorbed by the resin and thus lingered on the vegetation water. Only the VW after desorption sample (VW<sub>2</sub>AA) proceeding from VW<sub>2</sub> was analysed.



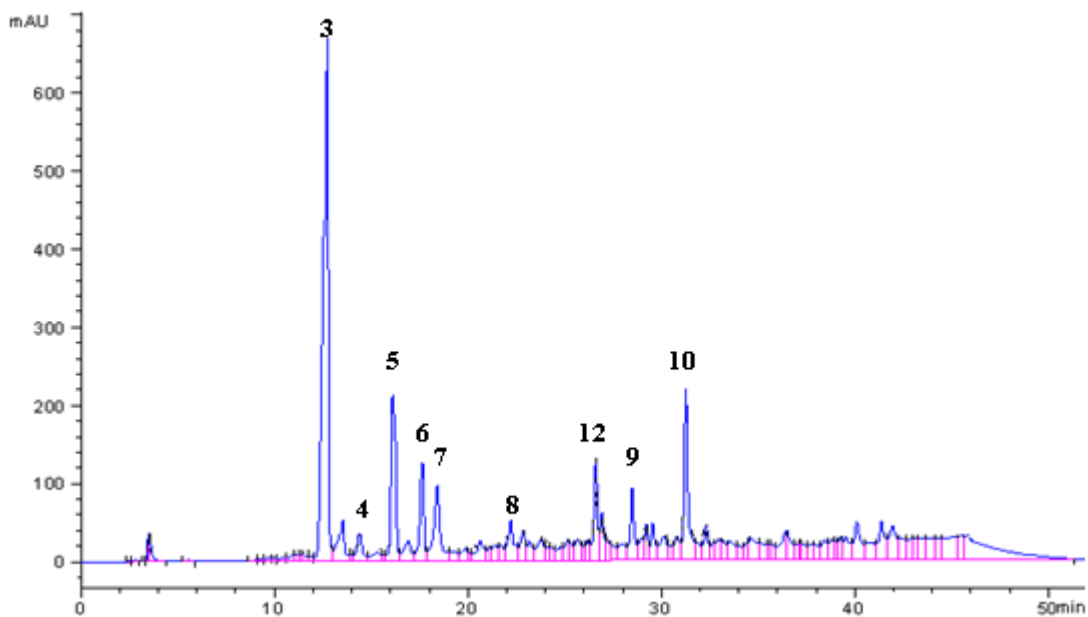
**Figure 10** – HPLC Chromatogram 3 for VW<sub>2</sub>AA sample.

The absence of phenolic compounds can be verified by taking a closer look into figure 10, in other words, the phenolic compounds which possessed the longer retention times were adsorbed by the resin, and therefore could not be found in the vegetation water after it was subdued to the adsorption process.

### 4.1.3 Resin's desorption sample injection

Two samples of resin desorption were conducted, nevertheless, only the second shown traces of polyphenols presence. Taking that fact into account, it is highly probable that there were some flaws during the first extraction process.

Further down a figure containing the resin's desorption sample chromatogram regarding the VW2 is shown. Initially, this alcoholic solution also had a rather low total concentration of polyphenols, therefore this solution was also further concentrated using a rotavapour. The final concentration of polyphenols for this solution was 782 mg/L 4MC equivalent.



**Figure 11** – HPLC Chromatogram 4 for RDS.

### 4.1.4 Standards injections

The standard solution prepared as described in chapter 3.4.1 were injected. Posteriorly there's a table showing the retention times for the injected standards. The resulting chromatograms are found in Annexe B.

**Table 4-** Retention times for each standard compound.

Compound	$t_r$ (min)
Gallic acid	8,13
p-OH benzoic acid	11,92
Catechol	14,15
Tyrosol	16,20
Caffeic acid	18,67
p-Coumaric acid	22,76

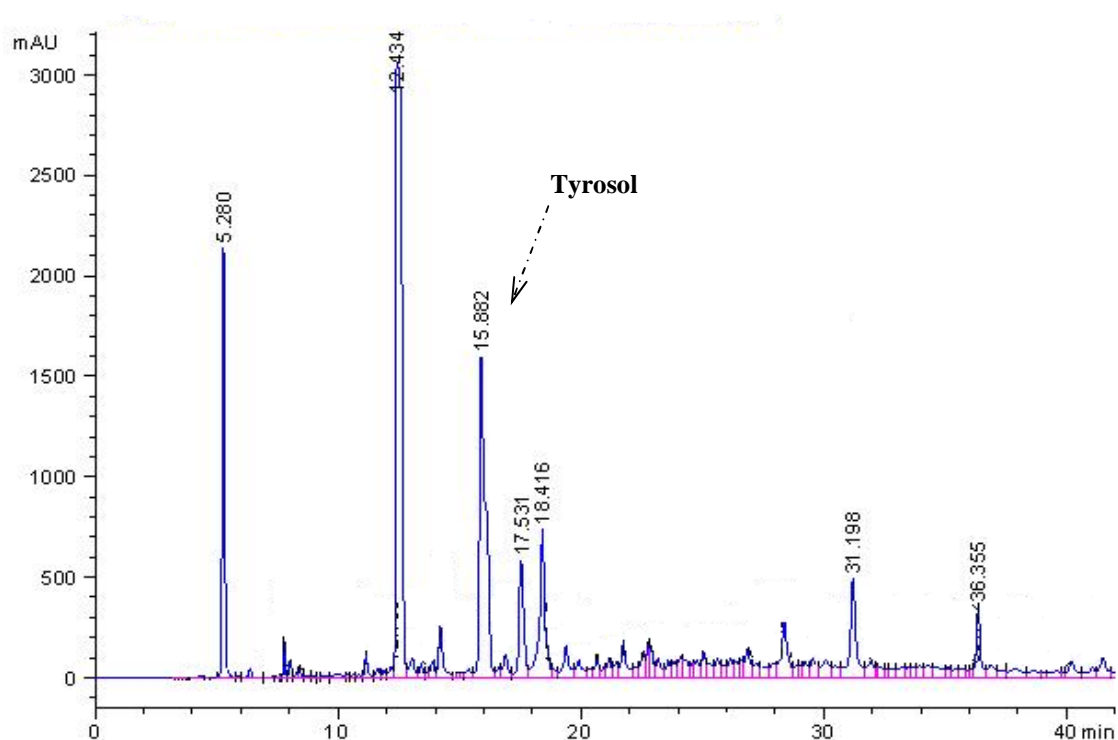
### 4.1.5 Co-injections

Once the retention times for the standards obtained (table 4) and those resulting from the analysed samples were confronted, the co-injections described in chapter 3.4.2 were conducted.

All of the following co-injections represented were made using the VW<sub>2</sub>, the ones not using VW<sub>2</sub> can be found in Annexe C.

- **Tyrosol**

The chromatogram correspondent to the co-injection containing half solution of VW<sub>2</sub> and the other half solution Tyrosol presented in figure 12.



**Figure 12** – HPLC Chromatogram 5 for VW<sub>2</sub> sample with Tyrosol.

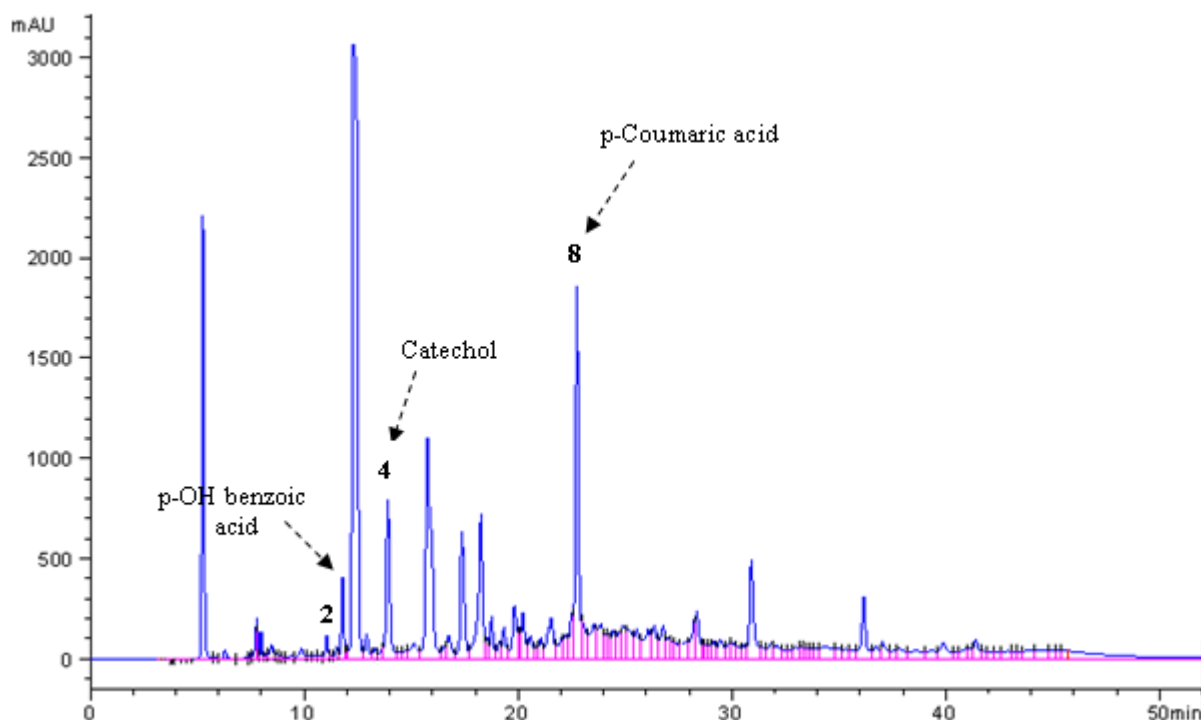
Two more injections were conducted using Tyrosol with the samples of VW<sub>1</sub> and VW<sub>2</sub>AA. The chromatograms of these injections are in annexe C. These injections tend to comply with the presence of Tyrosol. Once its area remained as if untouched and the area concerning the other components did not, decreasing substantially (reaching about half), so, Tyrosol presence is confirmed. Table 5 will confirm the previous statement.

**Table 5** – Retention times and areas for VW<sub>2</sub> and the respective co-injection with Tyrosol.

Peak	VW <sub>2</sub>		VW <sub>2</sub> + Tyrosol	
	t <sub>r</sub> (min)	Area (μV*s)	t <sub>r</sub> (min)	Area (μV*s)
1	5,22	30888	5,28	14901
2	11,05	1999	11,15	1003
3	12,16	74986	12,43	51811
4	13,98	6473	14,21	3314
<b>Tyrosol</b>	15,42	<b>28618</b>	15,88	<b>28285</b>
7	18,24	22399	18,42	11685
9	28,37	10326	28,38	4950
10	31,15	17127	31,20	8332
11	36,32	9233	36,36	4189

- **MIX<sub>1</sub>**

There were also two co-injections made with MIX<sub>1</sub>, one using VW<sub>2</sub> and the other with RDS. The following represented chromatogram corresponds to the co-injection of 250 μL MIX<sub>1</sub> with 250 μL of VW<sub>2</sub> sample. The other one can be found in Annexe C.



**Figure 13** – HPLC Chromatogram 6 for VW<sub>2</sub> with MIX<sub>1</sub>.

Moreover, making a comparison between this latter chromatogram and the chromatogram 2 (VW<sub>2</sub>) it can be verified that the 2<sup>nd</sup> peak does not correspond to the p-OH benzoic acid, that conclusion is made based on the fact that during this injection a new peak eluted at 11.79 min.

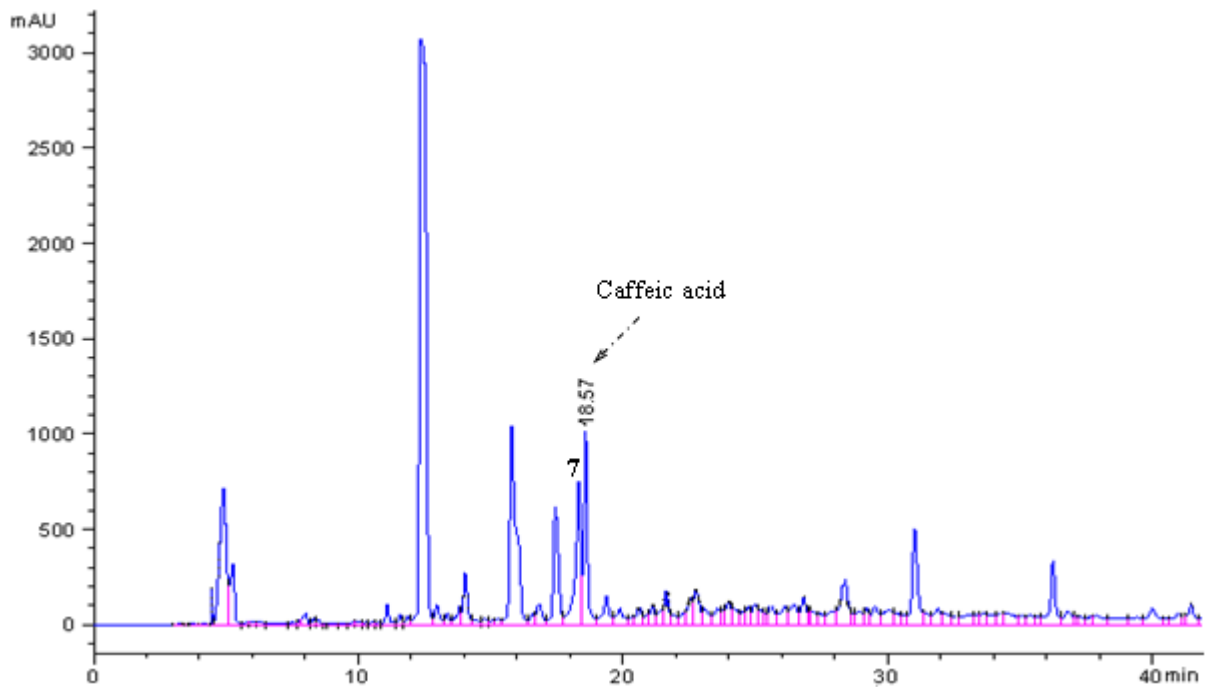
As far as the 4<sup>th</sup> peak is concerned, its area augmented substantially, thus it verifies the presence of Catechol at 13.91 min.

Last but not least, and concerning the p-Coumaric acid, no viable conclusion can be withdrawn as far as it's presence in the solution is concerned. Since the injection was made using a way too large amount of the acid, the chromatogram correspondent to this injection had its peaks mixed up, being more precise, the peak relative to the acid comprised other smaller peaks, thus making compound identifications impossible. Due to this matter, there is no way to actually conclude whether it was the 8<sup>th</sup> peak from the second chromatogram (figure 9) that eluted or not.

These same conclusions can be withdrawn from analysing the resin's desorption chromatogram, which can be found in Annexe C.

- **Caffeic acid**

The co-injection of this standard along with the VW<sub>2</sub> served the unique purpose of verifying whether the Caffeic acid was present or not in this OVWW. The retention time for the standard Caffeic acid was 18.67 min (table 4), therefore the suspected peak pointing for its presence was the 7<sup>th</sup> peak (18.24 min). Nevertheless, paying attention to the following chromatogram a new peak eluted at the time of 18.57 min.



**Figure 14** – HPLC Chromatogram 7 for VW2 with Caffeic acid.

#### 4.1.6 Qualitative Analyse

Since not all the required standard polyphenols were available, some of the polyphenols were analysed using results from another laboratory (ENEA, Rome) in 2006.

The confrontation between the retention times of the most important obtained peaks with the standards retention time obtained in 2006 are represented in table 6. Analysing the chromatograms concerning this date, it is easily noticed the offset between these values and the ones obtained.

Knowing in first hand that the concentration of the polyphenols Hidroxytyrosol and Tyrosol reach their maximum concentration in the olive vegetation wastewaters, it can be

immediately concluded that the offset relates to an anticipation of about 4/3 minutes in relation to the results of 2006. From there other important peaks could be identified through results which were obtained in the year of 2006.

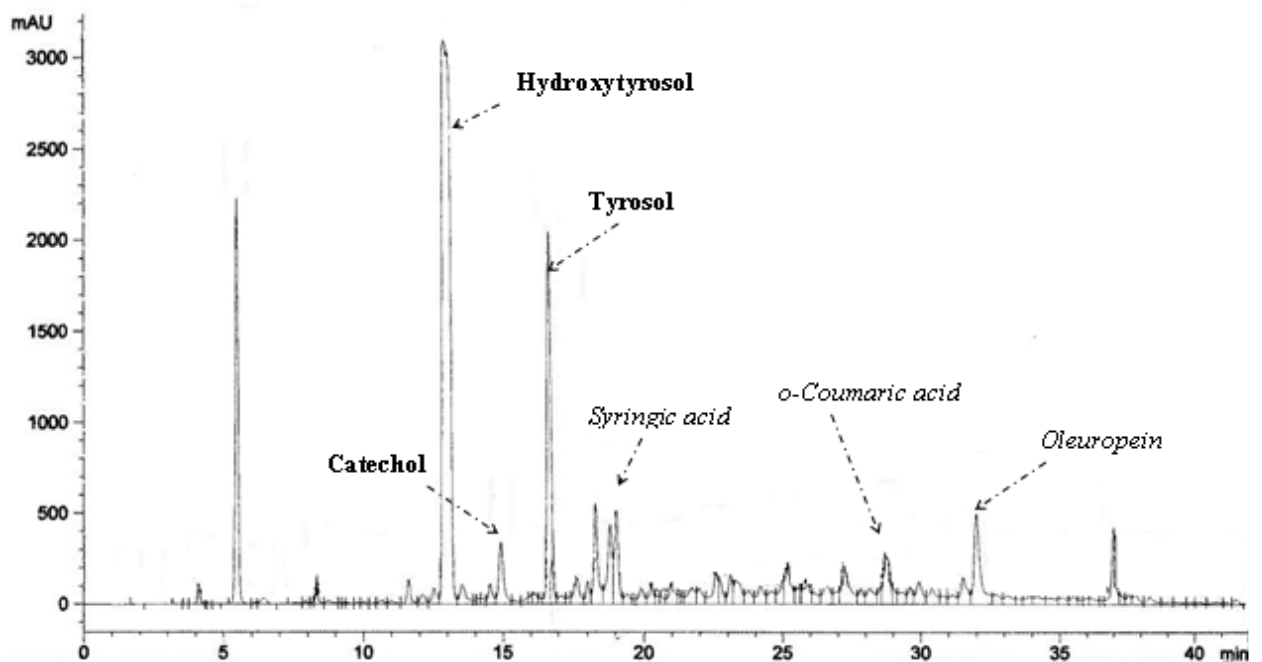
**Table 6** – Main peaks and respective retention times compared to previously obtained values (2006).

Peaks	Suspected Compounds	$t_r$ (min)				
		VW <sub>2006</sub>	VW <sub>1</sub>	VW <sub>2</sub>	VW <sub>2AA</sub>	RDS
1	Unidentified	5,53	5,47	5,22	5,27	---
2	Unidentified	---	11,62	11,05	---	---
3	<b>Hydroxytyrosol</b>	16,81	12,88	12,16	12,61	12,73
4	<b>Catechol</b>	17,49	14,93	13,98	14,38	13,55
5	<b>Tyrosol</b>	20,67	16,65	15,42	16,23	16,11
6	Unidentified		18,3	17,24	17,67	17,65
7	Syringic acid	22,53	18,81	17,51	---	---
8	<b>p-Coumaric acid</b>	25,87	---	22,55	22,7	22,22
9	o-Coumaric acid	31,02	28,81	28,37	---	28,49
10	Oleuropein	35,85	32,04	31,15	---	31,26
11	Unidentified	---	37,02	36,32	---	---
12	Unidentified	---	---	---	---	26,61

As previously mentioned, the Tyrosol and Hydroxytyrosol are the most abundant phenolic compounds in the OVWW. These two compounds were identified by comparing HPLC chromatogram of unknown peaks with those of standards. Tyrosol however, was submitted to yet another procedure, it was also identified by spiking the sample with standard Tyrosol. The presence of Tyrosol and Hydroxytyrosol can be verified in each of the analysed samples at the time of about 12 and 16 minutes, respectively.

The polyphenols compounds, Hydroxytyrosol, Catechol, Tyrosol and probably *p*-Coumaric acid were already identified. Another compounds, such as, Siringic acid, *o*-Coumaric acid and Oleuropein may also be present in the analysed samples. These compounds were not co-injected along with the samples of VW and DRS, none the less they are probably correspondent to the 7<sup>th</sup>, 9<sup>th</sup> and 10<sup>th</sup> peaks, respectively.

Furthermore the chromatograms obtained for each analysis with the respective identified polyphenolic compounds (written in bold) and those compounds which presence was suspected (written in italic) are shown.



**Figure 15** – HPLC Chromatogram 1 (VW<sub>1</sub>) with the polyphenols identified.

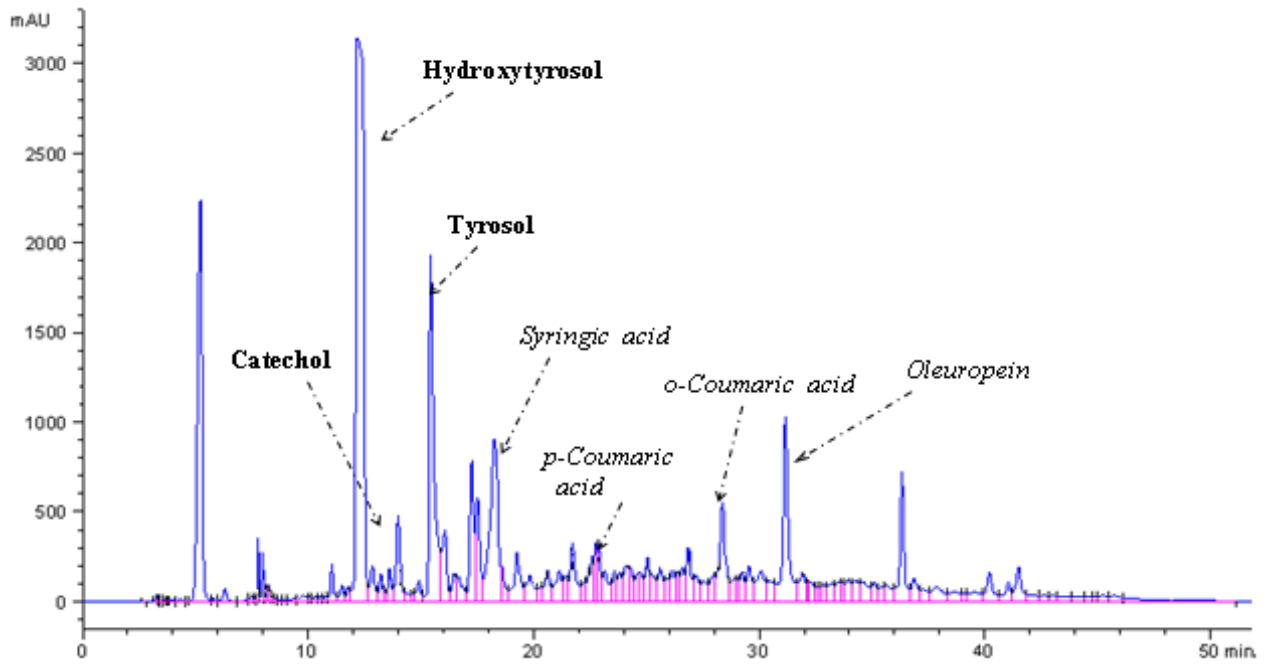


Figure 16 – HPLC Chromatogram 2 (VW<sub>2</sub>) with the polyphenols identified.

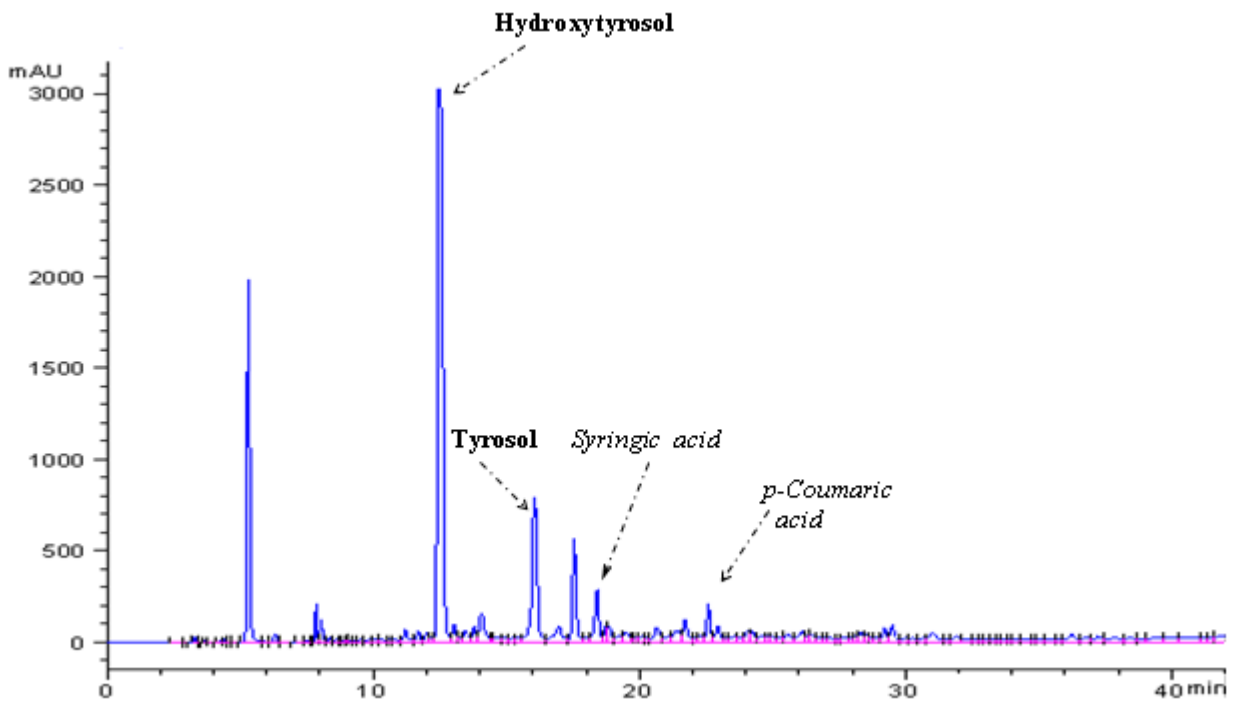
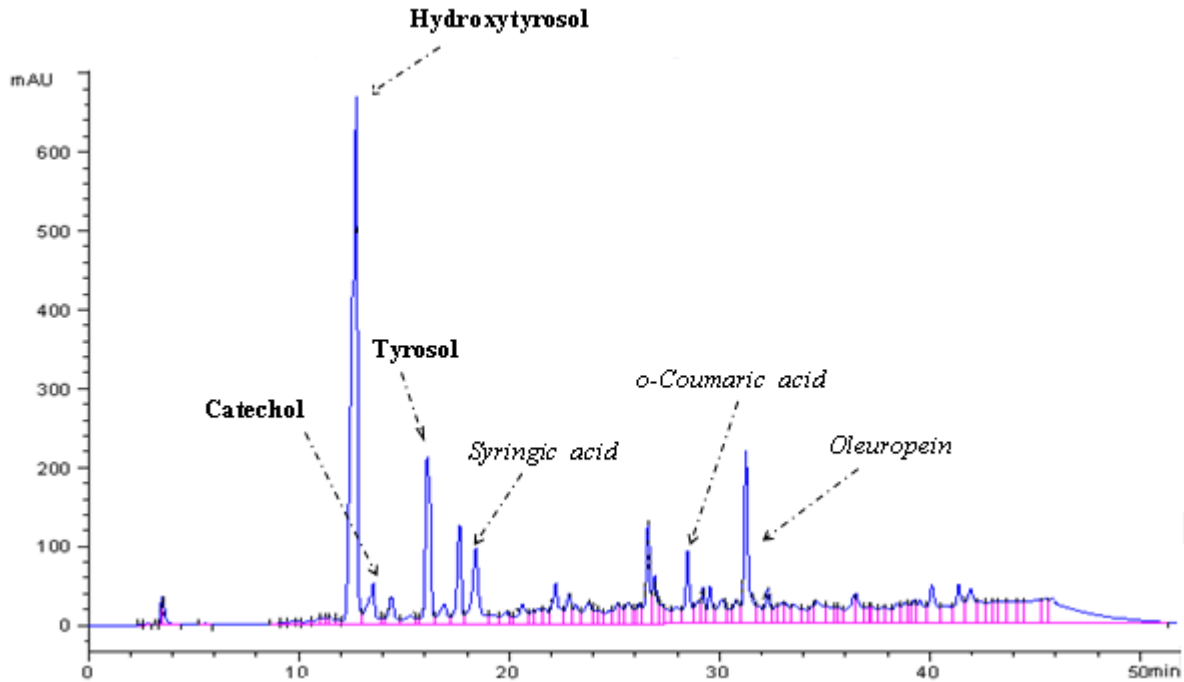


Figure 17 – HPLC Chromatogram 3 (VW<sub>2AA</sub>) with the polyphenols identified.



**Figure 18** – HPLC Chromatogram 4 (RDS) with the polyphenols identified.

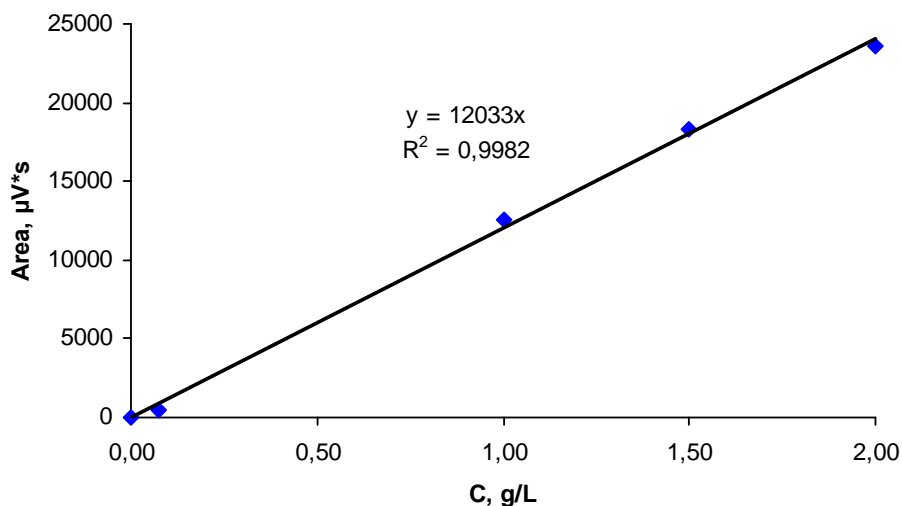
#### 4.1.7 Quantitative Analyse

The evaluation of each compound was performed using a four-point regression curve obtained using the available standards.

Nevertheless, as previously mentioned in the latter chapter, not all of the required standard polyphenols present in larger amounts in the OVWW were available. Except for Tyrosol, which was actually available, no more calibrations curves were made.

For the calibration curve of Tyrosol four injections with different concentrations (0.075, 1.0, 1.5 and 2.0 g/L) were conducted. For each one of the concentrations four peaks were obtained, the average of the obtained areas was made and the calibration curve was created. The HPLC chromatograms correspondent to the injection of each different concentration is in Annexe D.

Below, figure 19, represents the calibration curve obtained for Tyrosol.



**Figure 19** – Tyrosol's calibration curve.

Resorting to this curve, the Tyrosol's concentration was calculated before ( $VW_2$ ) the adsorption and after the adsorption was made ( $VW_{2AA}$ ) and in the resin's desorption sample (RDS). The values for these concentrations are shown in the following tables.

**Table 7-** Tyrosol's concentration and area of the identified compounds in the  $VW_2$ .

Identified compound	Area ( $\mu V \cdot S$ )	C (g/L)
Hydroxytyrosol	74986	---
Catechol	6473	---
Tyrosol	28618	2,4

**Table 8-** Tyrosol's concentration and area of the identified compounds in the  $VW_{2AA}$ .

Identified compound	Area ( $\mu V \cdot S$ )	C (g/L)
Hydroxytyrosol	43440	---
Tyrosol	10557	0,88

**Table 9** – Tyrosol's concentration and area of the identified compounds in the RDS.

Identified compound	Area ( $\mu\text{V}\cdot\text{S}$ )	C (g/L)
Hydroxytyrosol	11965	---
Tyrosol	3639	0,30

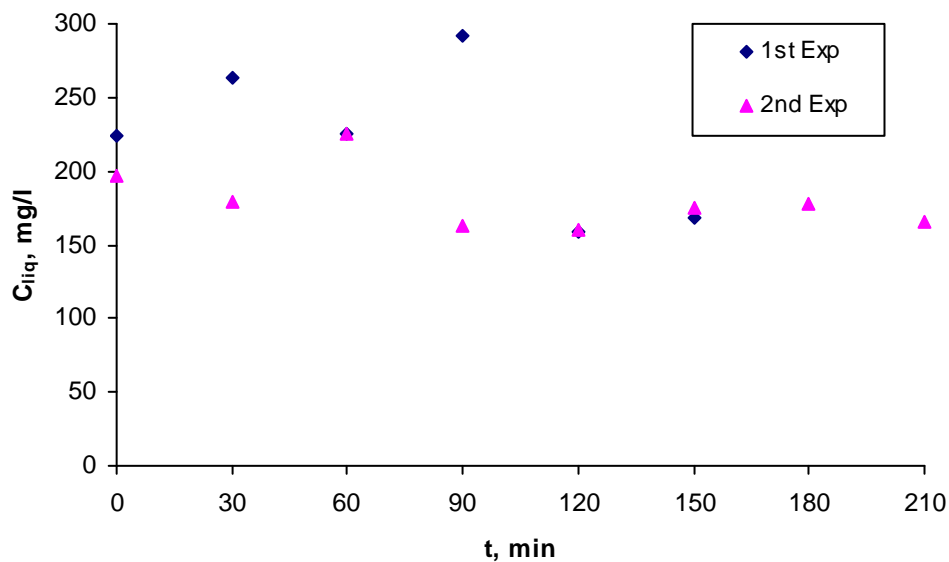
## 4.2 Batch adsorption studies

### 4.2.1 Adsorption equilibrium for an old resin

A study was performed to a resin, contained inside an adsorption column, that had not been used for a certain period of time, this study main priority was to verify whether the resin was still active or not.

The different adsorptions gathered in gaps of 30 minutes are shown in Annexe E.

The adsorption curves for the two experiments are represented in the next figure.

**Figure 20** – Concentration variation with time for the two experiments.

As it can be verified in figure 20, the adsorption does not represent the expected behaviour, it is visible that the variation of solution concentration is unstable with time. As of 120 minutes passed the average for the amount of phenolic compounds adsorbed was of about 170 mg/L.

Two experiences were conducted, in both of them the sample of resin tested did not verify the supposed standards, and therefore it was probably aged or not active.

#### 4.2.2 Adsorption equilibrium for a new resin

Many experiments took place in order to study the adsorption equilibrium of this new resin, the material balance was performed in each one of the experiments and the solute adsorbed concentration ( $C_S$ ) was calculated by the following equation:

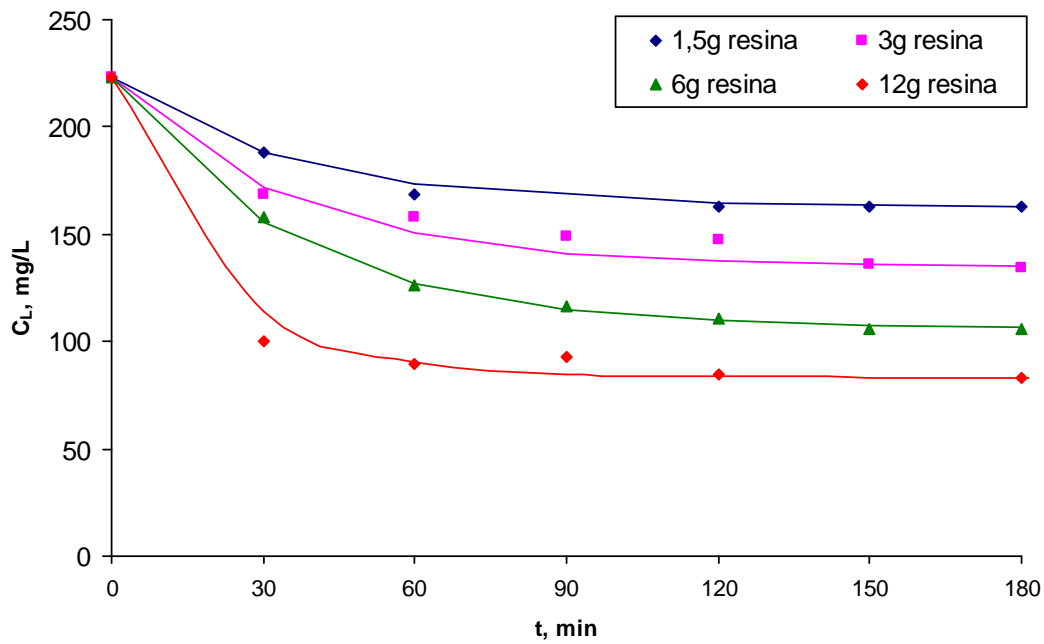
$$C_S = \frac{(C_{L_0} - C_{L_f}) \cdot V_{solution}}{V_{solid}} \quad (1)$$

The tables concerning each experiment and the correspondent material balance are found in Annexe F.

The measurement of initial polyphenols concentration was not stable, in other words, whilst in time zero it did not remain constant for all the experiments. The measurements made were irregular during all the experiments, not only in the first batch runs but even in the ones made later. Since the vegetation water used was already old, it is probable that the oxidation process had already been over, thus it could not be considered an aging process.

Taking that fact into account, an average of all the obtained initial values was made, and all the measurements had an error of a 4.5%. This calculation resulted on an initial concentration of about  $223 \pm 10$  mg/L.

The following chart represents the adsorption curves for the first experiments.



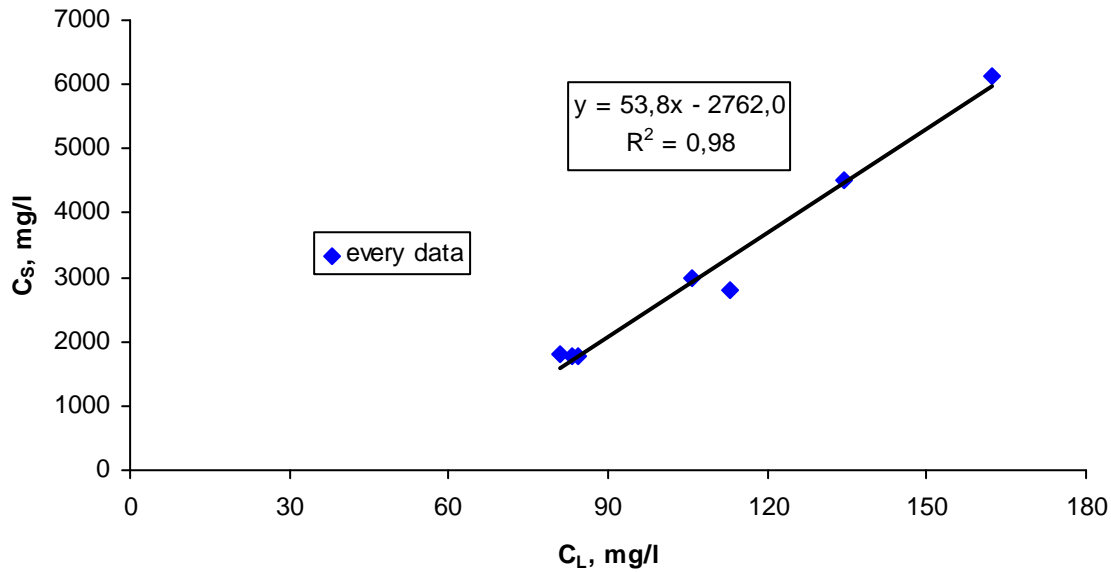
**Figure 21** – Adsorption curves for the first experiments.

The adsorption curves were elaborated using the calculated average (223 mg/L) as the initial concentration value; as for the curve guidance, this latter was created based on an empiric mathematical model that is similar to the exponential decrease. The estimated decaying time ( $\tau$ ) used for this model was of 35 minutes (except in the 12g sample which was of 20 minutes). Below, the equation used for the curve guidance is shown:

$$C_L = C_{L_0} - (C_{L_0} - C_{L_f})(1 - e^{-t/\tau}) \quad (2)$$

It can be seen that, after the first 2 hours, the concentration in the solution does not significantly decrease any longer thus, for practical purposes, the equilibrium is attained. At this point almost no polyphenols are adsorbed, and thus the resin is saturated.

So, the measured equilibrium points were plotted for all of the performed batch runs in the following picture.



**Figure 22** – Distribution of the equilibrium experimental points.

After the data linking the adsorbate liquid concentration ( $C_L$ ) and the solute adsorbed concentration ( $C_s$ ) in equilibrium was obtained, a study with the objective of discovering an equilibrium isothermic describing the adsorption process,  $C_s = f(C_L)$  was attempted.

As it can be observed, if the equilibrium line was extrapolated it would not cross the coordinate (0,0), meaning that not all of the polyphenols were adsorbed. Amongst other reasons, this can be explained by the fact that some kinds of polyphenols are not part of the adsorption process, no matter the amount of resin used.

The easiest equation to consider the equilibrium is:

$$C_s = K_1(C_L - K_2) \quad (3)$$

#### 4.2.3 Parameters of the equilibrium equation and the diffusivity inside the particles estimative using a phenomenological model

The Phenomenological model implemented by the University of Rome was used to estimate diffusivity inside the particles and the parameters of the equilibrium (Parisi *et al.*, 2007).

When the diffusivity in the pore is independent of the adsorbate concentration,  $c_p$ , the mass balance of the adsorbate inside an adsorbent particle may be written as follows:

$$\frac{2 \cdot D_p}{r} \frac{\partial c_p(r, t)}{\partial r} + D_p \frac{\partial^2 c_p(r, t)}{\partial r^2} = \frac{\partial c_p(r, t)}{\partial t} + \frac{1}{\varepsilon_p} \frac{\partial c_s(r, t)}{\partial t} \quad (4)$$

In this equation  $D_p$  is the diffusivity inside the pore and  $\varepsilon_p$  is the particle porosity.

The diffusion inside the pores is generally very slow when compared to the surface adsorption step, thus the concentration of the adsorbate in the pore,  $c_p$ , can be considered in equilibrium with the adsorbent load,  $c_s$ ,

The partial differential equation (3) has the following initial condition:

$$c_p(r, t_0) = 0 \quad (5)$$

In the above equation  $t_0$  stands for the starting time of the run. The boundary conditions, referred to the centre and the outer surface of each particle, since the suspension is well-stirred, are:

$$\frac{\partial c_p(r=0, t)}{\partial r} = 0 \quad (6)$$

$$c_p(r=r_p, t) = c(t) \quad (7)$$

For a batch suspension with a volumetric fraction of solid particles equal to  $1-\varepsilon$  the solute mass balance has the following expression:

$$c(t) + \frac{(1-\varepsilon)}{\varepsilon} \cdot \frac{\int_0^{r_p} (\varepsilon_p \cdot c_p + c_s) \cdot 4 \cdot \pi \cdot r^2 dr}{\frac{4}{3} \cdot \pi \cdot r_p^3} = c(t_0) \quad (8)$$

The integral term in equation 8 represents the overall mass of adsorbate in one particle.

The model for the adsorption batch process consists of the set of eqs. (3) to (8). By the best fitting of the results of the batch experiments the estimation of the equilibrium curve parameters, i.e.  $K_1$  and  $K_2$  in eq. (3), and the pore diffusion,  $D_p$  in eq. (4) was done.

The values of the parameters  $D_p$ ,  $K_1$  and  $K_2$ , obtained by the best fitting, are reported in Table 10. The estimated pore diffusivity is lower but similar to the one predicted by the correlation of Wilke and Chang (1955) for the diffusivity in the solution bulk, equal to  $1.9 \cdot 10^{-9} \text{ m}^2 \cdot \text{s}^{-1}$ .

**Table 10** – Estimated parameters.

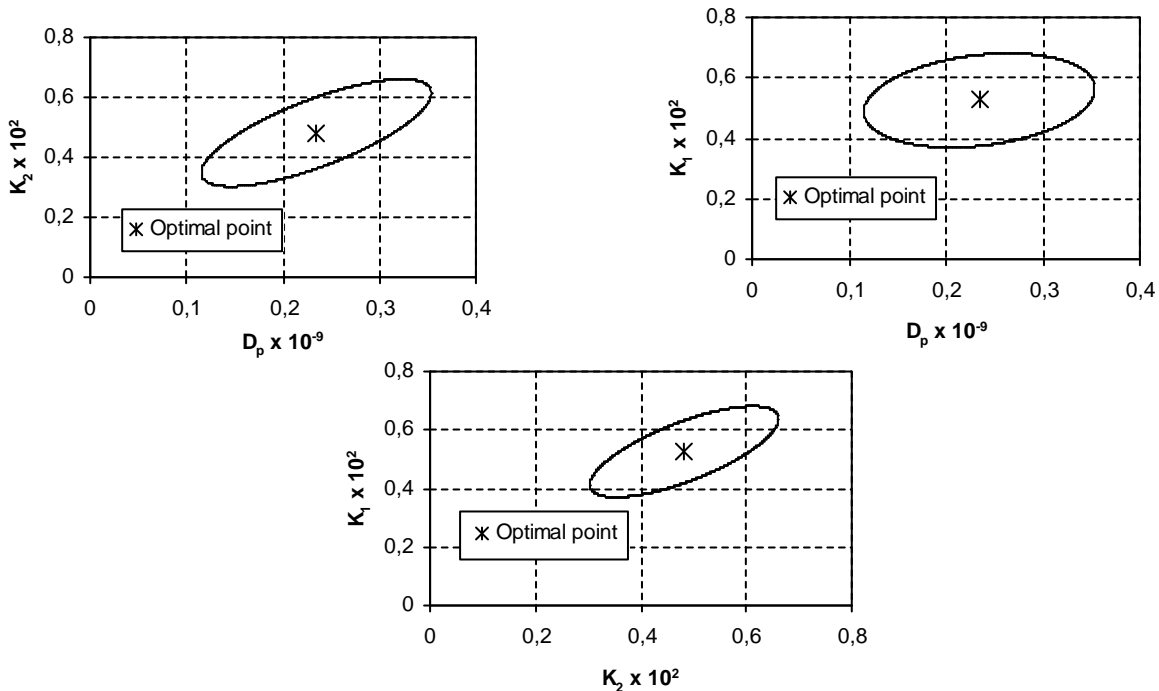
Parameter	value	dimensions
$D_p$	$2.4 \cdot 10^{-10}$	$m^2 \cdot s^{-1}$
$K_1$	52.56	$mg \cdot l^{-1}$
$K_2$	48.17	$mg \cdot l^{-1}$

For the obtained value of  $D_p$  and the average radius of the particles, equal to 0.3 mm, could be calculated the characteristic time to have a complete adsorption inside a single particle,  $\tau_D$  by the following relationship:

$$\tau_D = \frac{r_p^2}{D_p} \quad (9)$$

The obtained  $\tau_D$  was 7.7min, the adopted decaying time on the empirical method of 40min was higher but compatible with this found one.

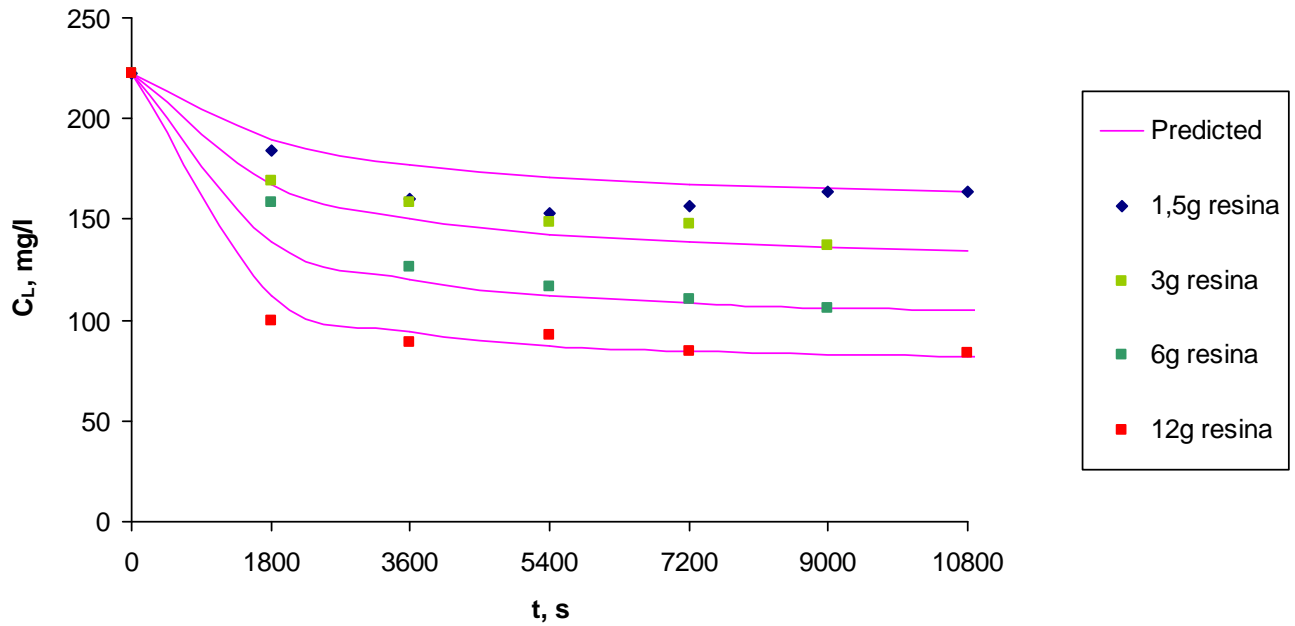
When a data interpolation procedure gives rise to the estimation of a set of data, it is important to evaluate which is the correlation between the couples of the estimated parameters. It is straightforward done, by using the gPROMS SW package, as in the present case. In Figure 23 the 95% confidence ellipsoids for the estimated parameters are plotted.



**Figure 23** - 95% confidence ellipsoids for the three estimated parameters  $D_p$ ,  $K_1$  and  $K_2$ .

The cross correlation between  $K_1$  e  $D_p$  is low, nevertheless the correlation is bigger between  $K_2$  and  $D_p$  and between  $K_1$  and  $K_2$ .

Concerning the batch runs, the simulated trends of the adsorbate liquid concentration ( $C_L$ ) in the following picture, show a good agreement with the experimental ones.

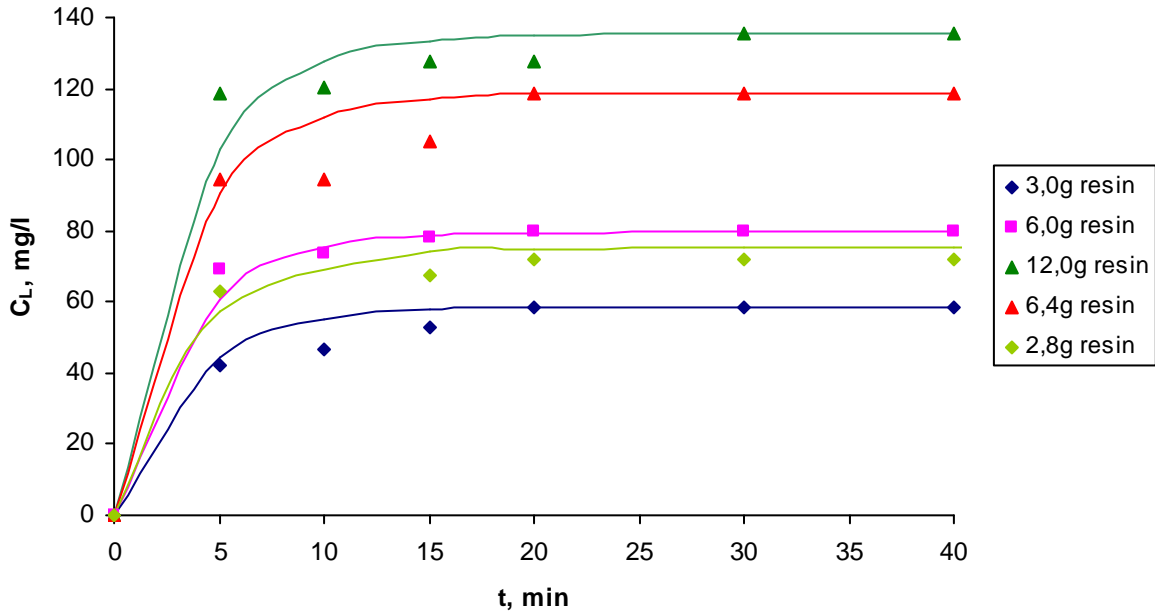


**Figure 24** - Adsorption curves for the model prediction.

### 4.3 Desorption study using a alcoholic solution

Desorption was carried out by discharging the exhausted resin particles into a vessel filled with 96% Ethanol.

Below are represented the desorption curves.



**Figure 25** – Desorption curves for the experiments.

All the results obtained concerning the samples are presented in Annexe G (Table G1).

The desorption process is known to be slightly faster than the adsorption one. Figure 25 represents what as just been said in the previously sentence, as it can be seen, the alcoholic sample adsorbs to its maximum value in quiet little time, that results in a fast achievement of the equilibrium point (about 15 minutes).

The curve guidance of these desorption curves was also created based on an empiric mathematical model, and this one is similar to an exponential increase. For this model the decaying time ( $\tau$ ) used was of 3.5 minutes. Below, the equation used for the curve guidance is shown:

$$C_L = C_{L_f} (1 - e^{-t/\tau}) \quad (10)$$

It is checkable, through mass balances that the mass adsorbed by the resin does not concur with the mass desorbed (see table G1 in Annexe G). Some procedures applied during the experiments, such as, the transference of the saturated resin to the beaker containing the alcoholic solution and the treatment with distilled water, may explain this fact. The first procedure always implies the loss of a certain amount of resin and therefore polyphenols, the second, even though not in a regular basis, may also have lead to some loss as far as the concentration of polyphenols go.

It's important to refer that two adsorbing processes were conducted with 12g of resin, nevertheless, there was one of the experiments in which there was a big loss of resin, the leftovers that remained were about 6.37g. However, in this run the resin was not rinsed with water, for that cause, this process desorption curve appears closer to the one using 12g of resin than the other in which only 6g were used during the adsorption.

Posteriorly, two more runs were conducted, just as in the previous runs the resin was not flushed with water during the transference to the beaker containing the alcoholic solution. One of the experiments did not lead to any conclusions because it was executed with a different volume of solvent, the other, which corresponded to 2,8g of resin, as it can be seen in picture 25, verified a slight increase on the adsorption of polyphenols, this fact can be directly linked to whether the resin was rinsed or not with distilled water after the adsorption process was conducted.

For that cause, this process desorption curve appears closer to the one using 6g of resin than the other in which 3g were used during the adsorption and treated with distilled water.

## 5. Conclusions

This project indicates that the adsorption of polyphenols on polymeric resins can remove these compounds from OVWW.

The adsorption equilibrium of polyphenols was described as a line, since this equilibrium line does not cross the coordinate (0,0), this means that not all of the polyphenols were adsorbed, thus concluding that some kinds of polyphenols are not part of the adsorption process, no matter the amount of resin used.

A phenomenological model of the process was used to estimate the adsorption equilibrium parameters and the diffusivity in the resin particles and a good agreement was found between experimental and predicted data.

The desorption process in alcoholic solutions was proven to be feasible and takes place in a very short period of time, it recovers more than 50% of the polyphenols.

As far as the determination and quantification of polyphenols are concerned, separation and identification of three well-known olive oil antioxidants (Tyrosol, Hydroxytyrosol and Catechol) was performed; other unidentified compounds were resolved, and could be Siringic acid, o-Coumaric acid and Oleuropein.

The absence of Gallic acid, p-OH benzoic acid and Caffeic acid was ascertained.

The presence of Tyrosol and Hydroxytyrosol can be verified in each of the analysed samples and they were the phenolic compounds in larger amounts in the OVWW.

The amount of Tyrosol was determined in two different extracts. The distribution of this phenolic compound in each of the extracts shows quantitative differences, these differences are related to the olive's degree of ripening as well as to the manufacturing process.

It was also concludable that polyphenols which bore higher retention times were more adsorbed by the resin, therefore those polyphenols could not be found in the vegetation water after it was subdued to the adsorption process.

The results obtained in the research suggest that OVWW can be regarded as a useful residue for the recovery of fine chemicals. Nevertheless, it should be taken into account that there are several obstacles and difficulties in OVWW upgrading at an industrial scale.

## **6. Work Assessment**

### **6.1 Aims Achieved**

This project objective consisted on studying the recovery of polyphenols from olive vegetation wastewaters by adsorption on a polymeric resin and also on extracting, identifying and quantifying the polyphenols from the same OVWW.

After some months of work and exclusive dedication to the project the objectives were completed, not all of the compounds within the OVWW were identified and quantified though.

### **6.2 Limitations and further work**

One of the harsher difficulties encountered while developing this project was related to the laboratory safety measures, these measures were precarious and for this matter it was even necessary to resort to hospital services.

The time also stood as a challenge for this project completion, not only the necessary reagents took an enormous amount of time until they were actually available, there were even some reagents, such as the standard polyphenols, that even though asked for, never reached the laboratory.

Carrying in mind future work on this project complement, it is suggested to perform the co-injections in HPLC using the standard polyphenols which presence was suspected in the OVWW, and that lacked during the developing of this project, so that their presence or absence can be properly confirmed.

### **6.3 Final appreciation**

The factor that stood out for this project development was the fact that once it was elaborated on a foreign country, it demanded a lot more initiative and creativity on my behalf, this was highly beneficial as it contributed to my autonomy. Nevertheless, and although I did put all my effort into this project, I could have done a better work and would have felt more fulfilled had not the already stated limitations happened.

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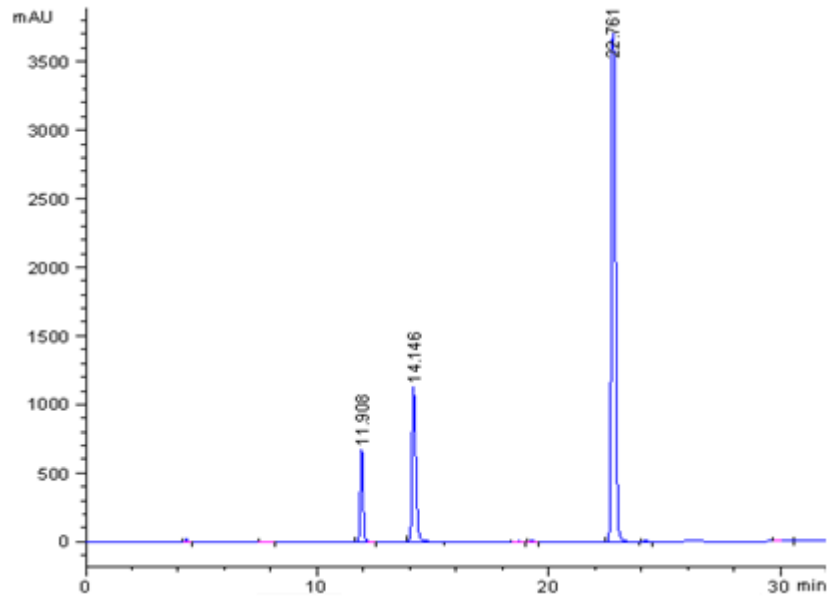
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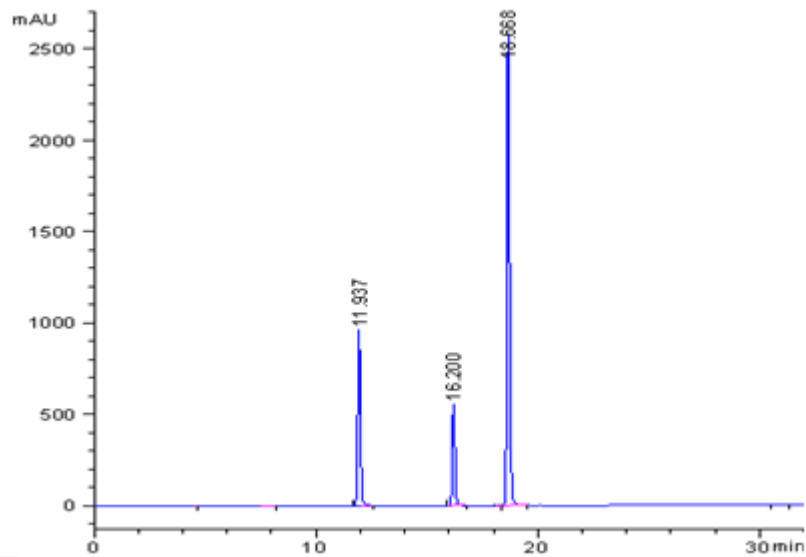
## **Annexe A - Folin-Ciocalteu measurement method**

The method is initiated by diluting 250  $\mu\text{L}$  of Folin-Ciocalteu Reagent in 3.5ml of distilled water, posteriorly 100  $\mu\text{L}$  of the sample to be measured was put into a test tube. After three minutes are passed, 250  $\mu\text{L}$  of Sodium Carbonate (20%) is added. Once thirty minutes are passed the absorbance is read with a spectrophotometer using a wave length of 750 nm.

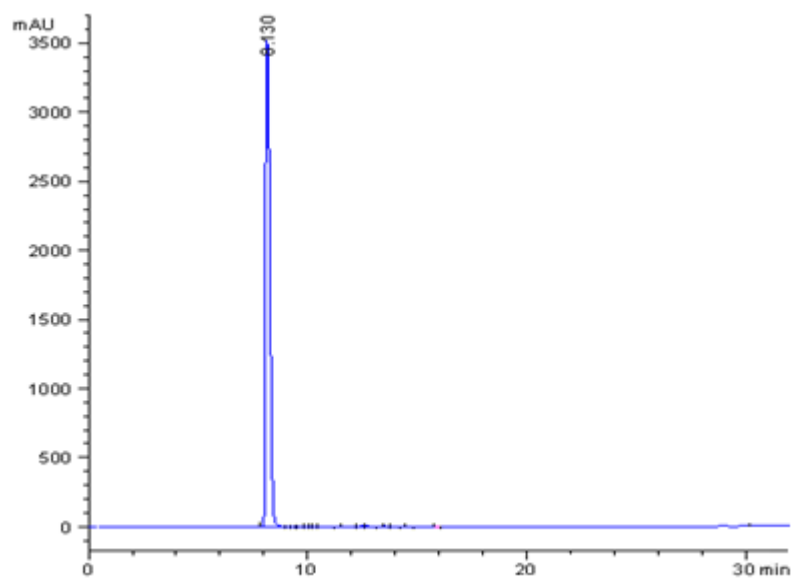
## Annexe B – HPLC chromatograms for standards compounds



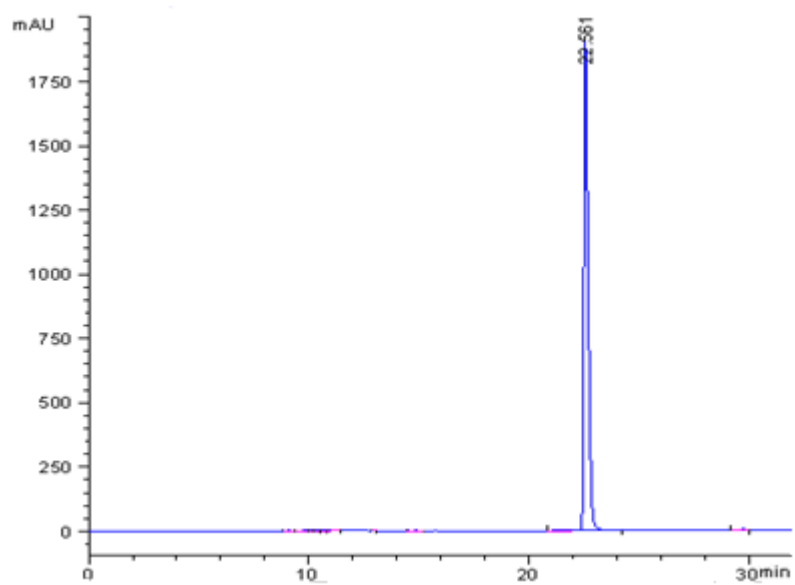
**Figure B1** – HPLC Chromatogram for standards A (p-OH benzoic acid, Catechol and p-Coumaric acid mixture).



**Figure B2** – HPLC Chromatogram for standards B (p-OH benzoic acid, Tyrosol and Caffeic acid mixture).



**Figure B3** –HPLC Chromatogram for Gallic acid.



**Figure B4** – HPLC Chromatogram for 4-Methylcatechol.

## Annexe C – HPLC Chromatograms for Co-injections

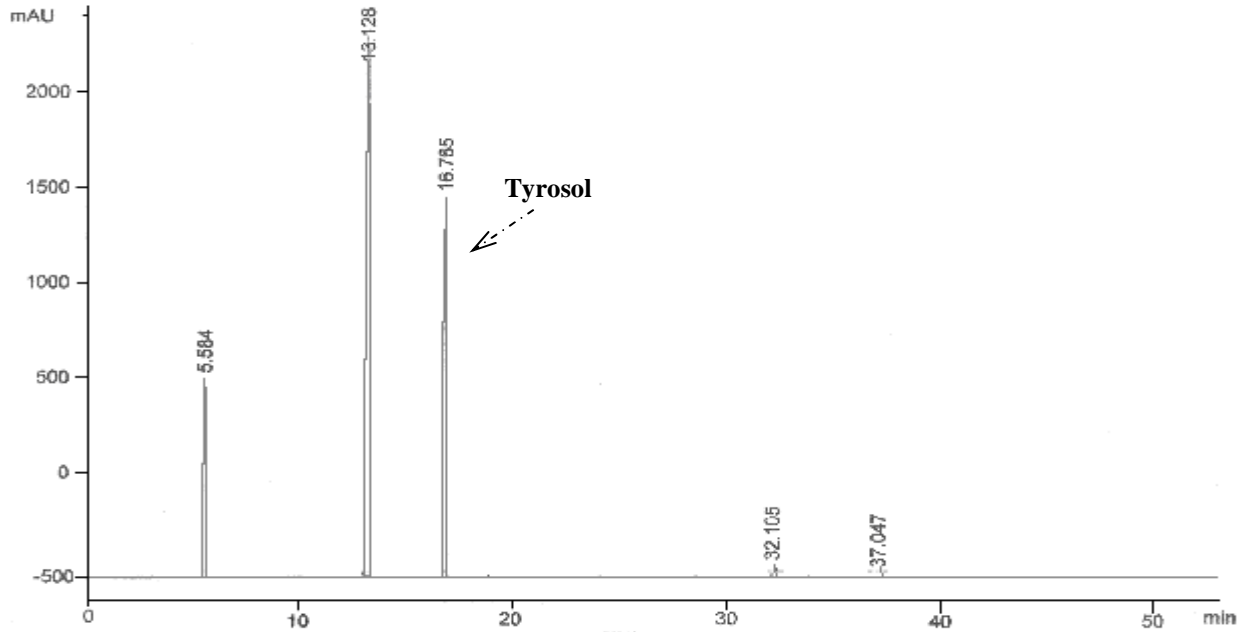


Figure C1 – Co-injection of VW<sub>1</sub> with Tyrosol (1 g/L).

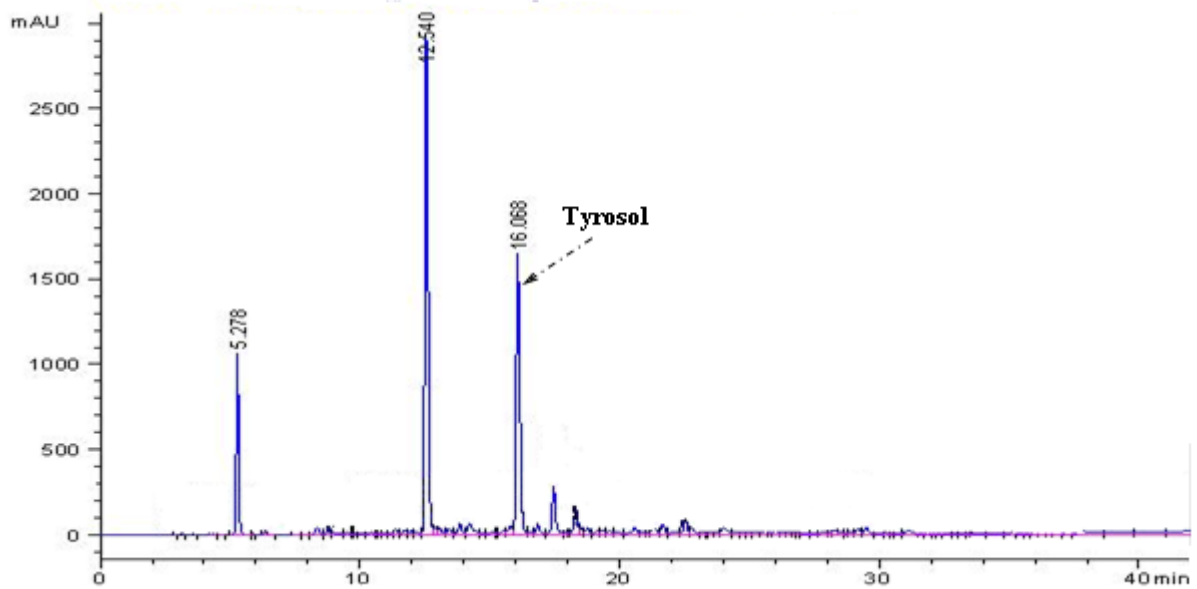
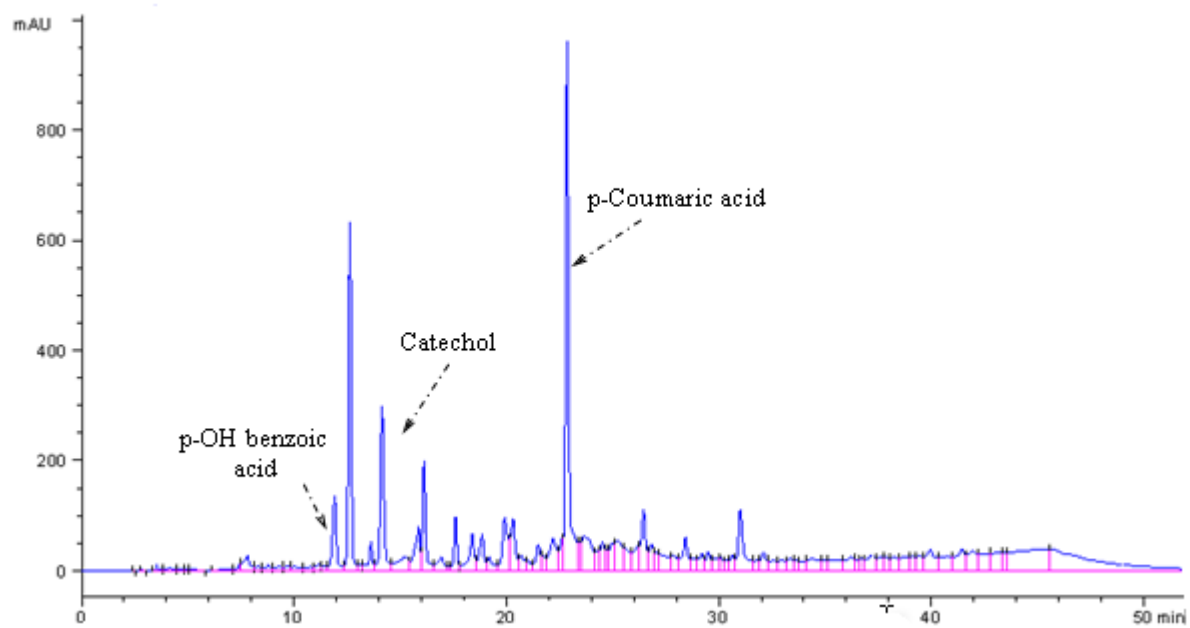


Figure C2 – Co-injection of VW<sub>2</sub>AA with Tyrosol (1 g/L).



**Figure C3** – Co-injection of RDS with MIX<sub>1</sub>.

## Annexe D – HPLC Chromatograms for Tyrosol calibration curve

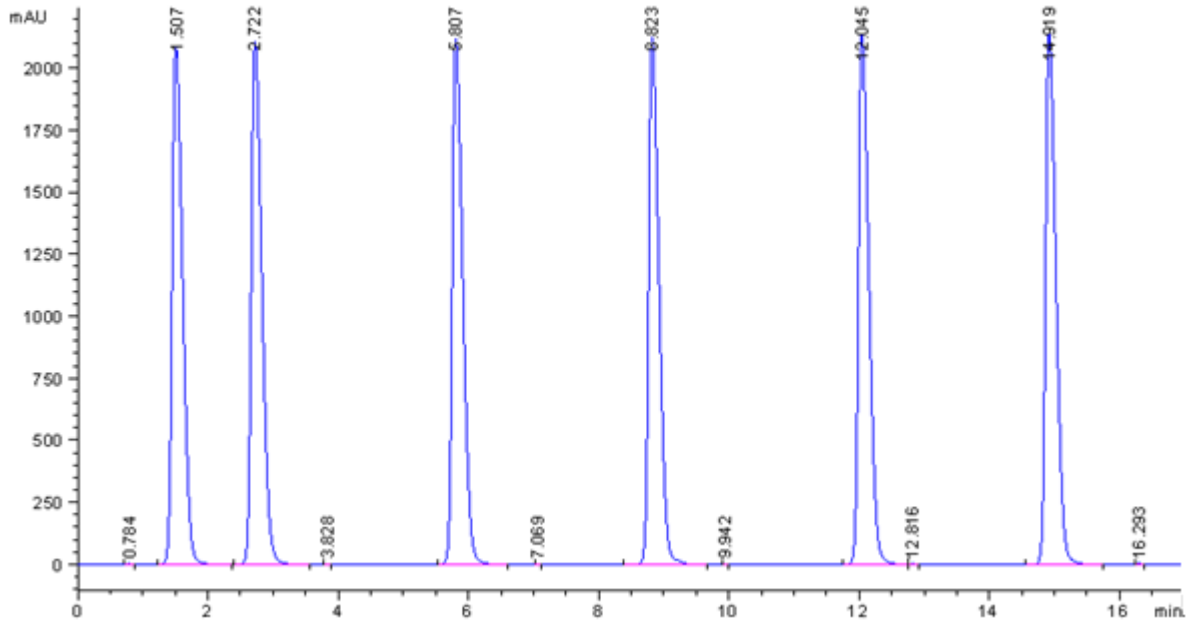


Figure D1 – Injection 2 g/L of Tyrosol.

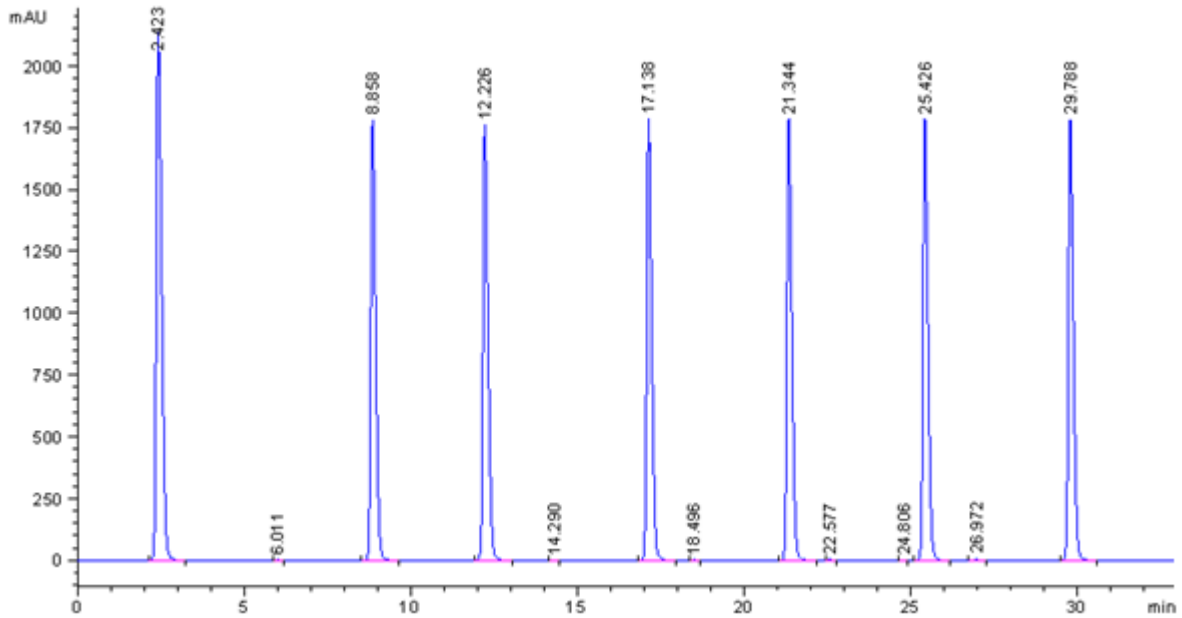


Figure D2 – Injection 1.5 g/L of Tyrosol.

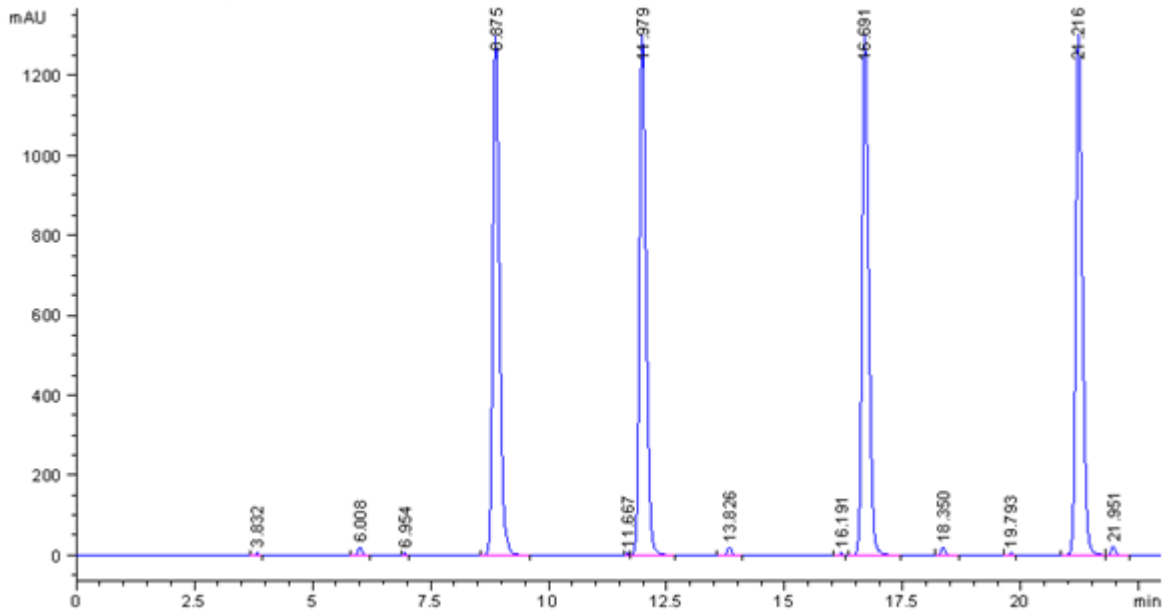


Figure D3 – Injection 1 g/L of Tyrosol.

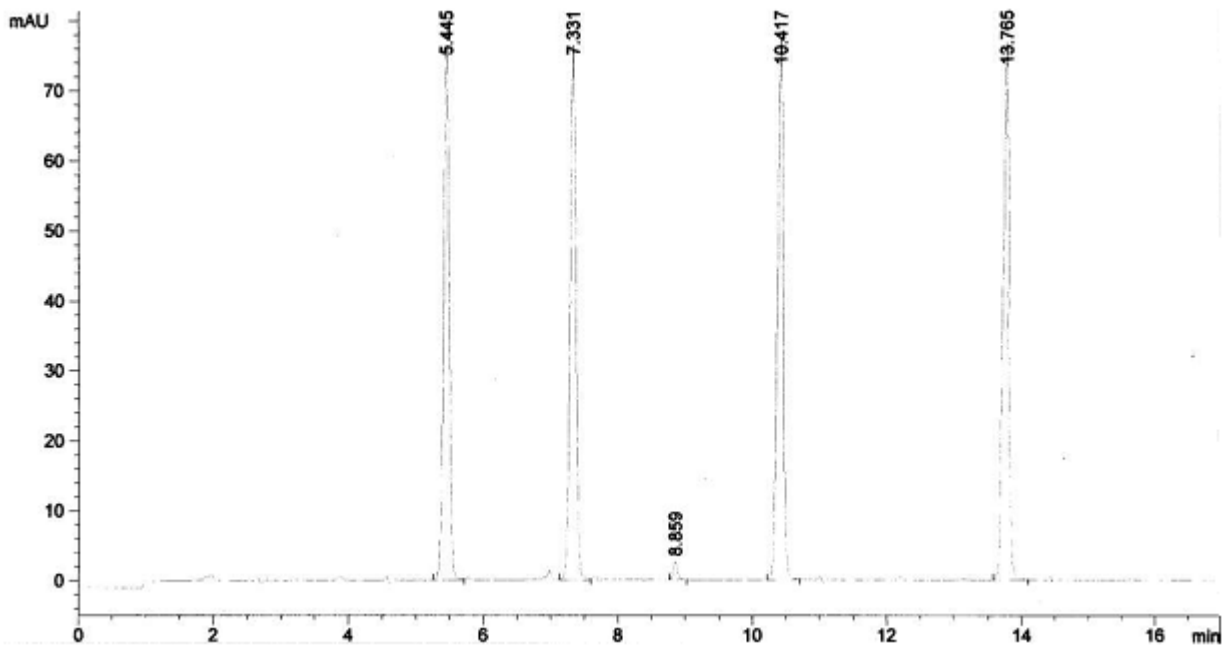


Figure D4 – Injection 0.075 g/L of Tyrosol.

## Annexe E – Table Results for the old resin

**Table E1** - Experiments results concerning the old resin

<i>1<sup>st</sup> Run</i>			<i>2<sup>nd</sup> Run</i>		
<b>m<sub>resin</sub> = 1,50g</b>			<b>m<sub>resin</sub> = 1,52g</b>		
<b>t (min)</b>	<b>Abs</b>	<b>C<sub>liq</sub> (mg/L)</b>	<b>t (min)</b>	<b>Abs</b>	<b>C<sub>liq</sub> (mg/L)</b>
0	0,188	223,5	0	0,166	197,4
30	0,221	262,8	30	0,151	179,5
60	0,189	224,7	60	0,190	225,9
90	0,246	292,5	90	0,137	162,9
120	0,134	159,3	120	0,135	160,5
150	0,142	168,8	150	0,147	174,8
			180	0,150	178,4
			210	0,139	165,3

## Annexe F – Table Results for the adsorption process

While Table F1 concerns the results from the first set of experiments, table F2 is relative to recent experiments and reproducibility.

**Table F1** – First Adsorption experiments of OVWW in beaker

Run	$m_{\text{resin}}$ (g)	t (min)	Abs	$C_{\text{liq}}$ (mg/L)	$C_{\text{sol}}$ (mg/L)	$M_{\text{adsorb}}$ (mg)
A	1,51 <sup>(a)</sup>	0	0,178	222,8	0	0,00
		30	0,159	188,5	3475	5,15
		60	0,142	168,8	5472	8,10
		120	0,137	162,9	6069	8,99
		150	0,137	162,9	6069	8,99
		180	0,137	162,9	6069	8,99
B	3,01 <sup>(a)</sup>	0	0,191	222,8	0	0,00
		30	0,142	168,8	2745	8,10
		60	0,133	157,6	3314	9,78
		90	0,126	149,2	3741	11,04
		120	0,124	147,4	3833	11,31
		150	0,114	135,6	4432	13,08
C	6,00	0	0,202	222,8	0	0,00
		30	0,133	158,1	1650	9,71
		60	0,106	126,0	2468	14,52
		90	0,098	116,5	2711	15,95
		120	0,093	110,6	2861	16,83
		150	0,089	105,8	2984	17,55
D	12,00	0	0,183	222,8	0	0,00
		30	0,084	99,9	1567	18,44
		60	0,075	89,2	1704	20,04
		90	0,078	92,7	1658	19,52
		120	0,071	84,4	1765	20,76
		180	0,070	83,2	1780	20,94
E	12,0	0	0,188	222,8	0	0,00
		720	0,071	84,4	1764	20,76
F	12,0	0	0,188	222,8	0	0,00
		720	0,068	80,9	1810	21,29
G	6,0	0	0,188	222,8	0	0,00
		720	0,095	113,0	2799	16,48

(a) The values refer to the average between two conducted experiments.

**Table F2** – Adsorption experiments of OVWW in beaker with a new activated resin.

<b>Run</b>	<b>m<sub>resin</sub> (g)</b>	<b>t (min)</b>	<b>Abs</b>	<b>C<sub>Liq</sub> (mg/L)</b>	<b>C<sub>sol</sub> (mg/L)</b>	<b>M<sub>adsorb</sub> (mg)</b>
<b>H</b>	<b>3,0</b>	0	0,187	221,8	0	0,00
		30	0,123	146,3	3848	11,32
		60	0,122	145,1	3909	11,50
		90	0,113	134,4	4455	13,11
		150	0,11	130,8	4636	13,64
		180	0,105	124,9	4940	14,53
		210	0,105	124,9	4940	14,53
<b>I</b>	<b>5,0</b>	0	0,187	221,8	0	0,00
		30	0,132	157,0	1973	9,72
		60	0,118	140,3	2480	12,22
		150	0,11	130,8	2770	13,64
		180	0,108	128,4	2843	14,00
		210	0,108	128,4	2843	14,00

## Annexe G – Table Results for the desorption process

Table G1 - Desorption experiments with alcohol solution.

Run	m <sub>resin</sub> (g)	V <sub>solvent</sub> (mL)	t (min)	Abs	C <sub>Liq</sub> (mg/L)	M <sub>desorb</sub> (mg)	M <sub>sol</sub> (mg)	C <sub>sol</sub> (mg/L)	% desorbed polyphenols
B	3,00	100	0	0	0,0	0,00	13,27	4645	44,19
			5	0,028	42,1	4,21	9,06	3171	
			10	0,031	46,6	4,66	8,61	3013	
			15	0,035	52,6	5,26	8,01	2802	
			20	0,039	58,6	5,86	7,41	2592	
			30	0,039	58,6	5,86	7,41	2592	
C	6,00	100	0	0	0,0	0,00	17,55	3071	45,41
			5	0,046	69,2	6,92	10,63	1861	
			10	0,049	73,7	7,37	10,18	1782	
			15	0,052	78,2	7,82	9,73	1703	
			20	0,053	79,7	7,97	9,58	1677	
			30	0,053	79,7	7,97	9,58	1677	
F	12,00	100	0	0	0,0	0,00	21,29	1863	63,57
			5	0,079	118,8	11,88	9,41	823	
			10	0,08	120,3	12,03	9,26	810	
			15	0,085	127,8	12,78	8,51	744	
			20	0,085	127,8	12,78	8,51	744	
			30	0,09	135,3	13,53	7,76	679	
E	6,37	100	0	0	0,0	0,00	20,76	3420	57,22
			5	0,063	94,7	9,47	11,29	1860	
			10	0,063	94,7	9,47	11,29	1860	
			15	0,07	105,3	10,53	10,23	1686	
			20	0,079	118,8	11,88	8,88	1463	
			30	0,079	118,8	11,88	8,88	1463	
H	2,81 <sup>(b)</sup>	100	0	0	0,0	0,00	14,53	5083	51,75
			5	0,042	63,2	6,32	8,21	3069	
			15	0,045	67,7	6,77	7,76	2900	
			20	0,048	72,2	7,22	7,31	2732	
			30	0,048	72,2	7,22	7,31	2732	
			40	0,048	72,2	7,22	7,31	2732	
I	3,63 <sup>(b)</sup>	40	0	0	0,0	0,00	14,00	4054	49,41
			5	0,107	160,9	6,44	7,56	2191	
			15	0,11	165,4	6,62	7,38	2138	
			40	0,115	172,9	6,92	7,08	2051	
			50	0,115	172,9	6,92	7,08	2051	

(b) Experiments with a new activated resin