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Abstract. An efficient, rapid, sensitive, and reproducible methods for the removal phenol determination of in aqueous solution was developed. UV- Spectroscopy methods and reverse phase high performance liquid chromatographic (RP - HPLC) were used. The phenol removal from the aqueous solution was carried out by prepared activated carbons (ACs) from the wheat plant waste as a natural material. The remains phenol was determined using RP – HPLC and UV- Spectroscopy. The elution was done using a mobile phase consisting of acetonitrile (ACN): water in the ratio of 10:90 on HPLC Shimadzu LC–20 A, Japan and Phenomenex C18 column (250 × 4.6 mm, 5µm). The separation was monitored for 15 min at 270 nm using a UV-visible detector and 1.5 ml/min flow rate. UV-VIS spectrophotometric measurement for quantitative of phenol was carried out at 272.6 nm. The optimum conditions such as the composition of the mobile phase, flow rate, temperature, size, time and pH were studied. The obtained results revealed that the values of R2 are (0.9996 and 0.9991), the detection limit (0.02 and 0.01), the quantitative limit (0. 066 and 0.033), the linear ranges (0.2 – 25 and 0.1 – 30) µg / mL for UV and HPLC respectively. The removal percentages of phenol using the treated wheat plant waste were found 98.76 and 98.85 using UV and HPLC measurements respectively. Compared to the other methods, the current method is rapid, simple and economical for the removal and determination of phenol in aqueous solution.

Keywords. RP-HPLC; Sensitive; Developed; UV – spectroscopy; Removal

1. Introduction

The phenolic compound is among the highest dangerous pollutant in the environment and the human health. They were considered as high contaminants by the USA environmental protection agency (EPA). Phenol is mainly used as an intermediate in various industries (Refining petroleum, plastics industry, pharmaceuticals, production of dyes and pesticides...) [1].

The hazardous nature and toxicity of phenols has been well documented and can cause several problems to human health [2]. The determination of phenol compound was increased in the last years due to the highly poisonous of these compounds. They are emitting an unpleasant odour and flavour in the portable water, so it is poisoning the plants, humans and life [3,4].

The allowable maximum concentration of the phenol which discharged into surface water is less than 1 mg/L and less than 0.5µg/L into drinking water [5]. Much research was developed for the adsorption of phenol prior subtract to receiving sink. The wastewater treatment by different techniques involves

adsorption, liquid extraction, precipitation, biological treatment (6, 7). At present, adsorption methods were taking an interest, especially, from natural resources, low-cost wastes, which are needed little treatment to increase their removal capacity for the removal of phenol and phenolic compounds [8]. Various low-cost materials such as zeolites, clays and activated carbon [9, 10]. These materials are very promising in this area [11]. The activated carbon is now being the most used for the pollutant elimination from aqueous solution [12].

A visible absorbance measurement following reaction with 4- aminoantipyrine for the determination of total phenols in water samples the most widely used methods [13] [14]. Due to, this method cannot give an accurate determination of the individual phenol concentrations. Many spectrophometric and separation methods for phenol determination were reported, using UV and HPLC for detection [15 – 26]. The treatment of water contaminated with phenolic compounds remains a major challenge, especially for developing countries that have not yet all opportunities to integrate the concepts of sustainable development. The complexity of their chemical structure and the presence of aromatic rings, mean that the corresponding aqueous effluents require treatment in several steps.

The present investigation aims to study a simple and economic process for phenol removal from aqueous solution using low cost abundantly available adsorbent (natural or modified wheat plant) and determination of phenol using HPLC and UV spectroscopy methods.

2. Materials and Methods

2.1. Reagents and Chemicals

Acetonitrile (HPLC-grade), Methanol (HPLC-grade) and phenol were from BDH. All chemicals and reagents were of analytical grade and double distilled water was used.

2.2. Instrumentation and Conditions of Analysis

Spectroscopic analysis was carried out using Jasco V-650 Japan double beam UV-VIS spectrophotometer with 10 mm path length quartz cells was used for the analytical purpose. The separation was achieved by using HPLC Shimadzu LC–20 A, Japan uses ACN: H2O (10: 90) as mobile phase and Phenomenex C18 column (250 × 4.6 mm, 5µm). The separation was monitored for 15 min at 270 nm using a UV- visible detector and 1.5 mL/min flow rate and λmax 272.6 for UV measurements.

2.3. Preparation of Stock Solutions of Drugs (100 µg/mL)

Accurately, weighed (0.01) g pure samples of phenol placed in (100) mL volumetric calibrated flask, after dissolving it completed with ACN: H2O (10: 90 v/v). This is the primary standard solution of phenol (100 µg/mL) in ACN: H2O, subsequent dilutions was done by withdrawing different aliquots. A (0.01- 3) mL of standard solution were carried into a volumetric calibrated flasks have 10 mL volume and made up to the mark with ACN: H2O for preparing other standard working solutions have the concentrations range of (0.1-30 µg/mL).

3. Results and Discussion

3.1. UV-VIS Spectrophotometry method

3.1.1. Estimation of detection wavelength

A solution of standard phenol has the concentration of 100 µg/mL, scanned in the UV wavelength range [13]. The phenol solution showed respectable absorbance at a λmax with (272.6 nm). Therefore, it was selected as the wavelength for detection in (H2O: ACN 90: 10 v/v). The study of spectrum revealed that the phenol solution shows a well-defined λmax at 272.6 nm as shown in (figure 1).

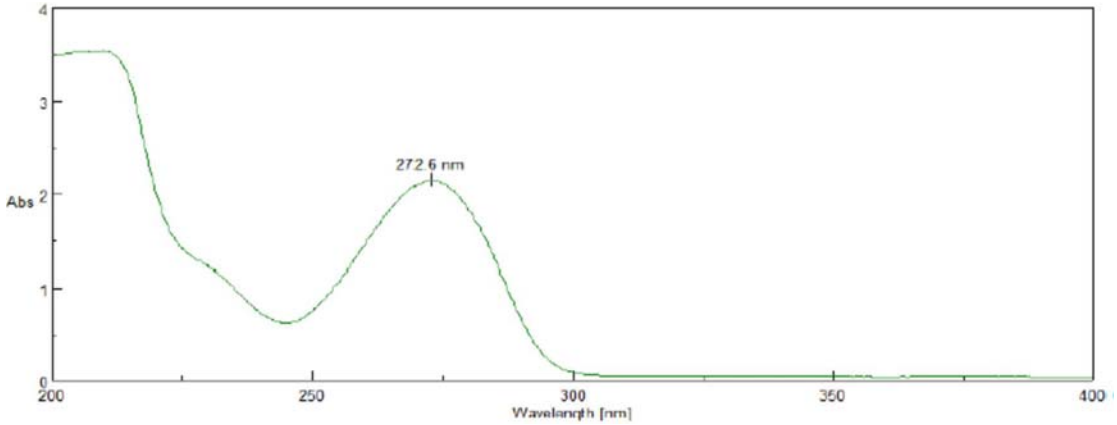

Figure 1. UV- spectrum for standard phenol 100 µg/mL

3.1.2. Preparation of calibration graph

From the stock solution, subsequent dilutions were made with ACN: H2O (10: 90 v/v) to obtain the series of standard solutions have a concentration range of (0.2 – 25.0 µg/mL) of phenol. The solution absorbance of was measured at (272.6 nm) versus methanol as a blank. A plotted of a concentration values on (x-axis) and absorbance values on (y-axis) was given a straight line with R2 value of (0.9996). This considered the calibration graph as shown in figure 2 and table 2.

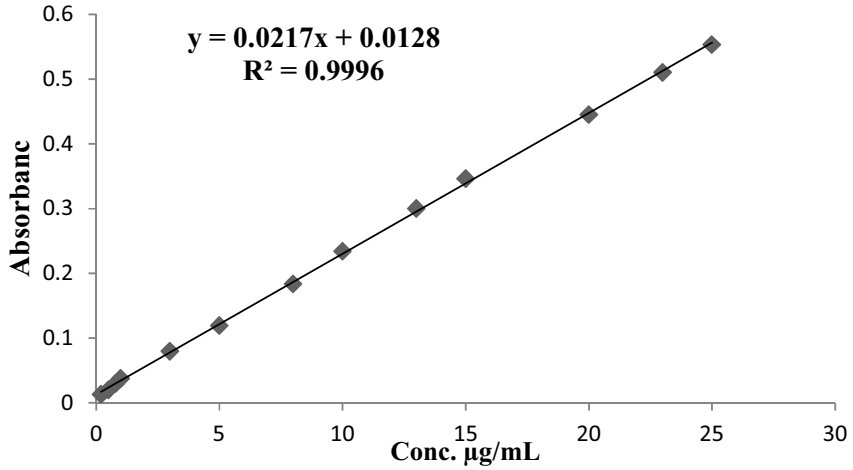

Figure 2. Calibration graph of phenol using UV – VIS

3.1.3. RP- HPLC conditions and calibration graph

To obtain optimum condition, an experiment with various types of mobile phase of HPLC system to be used is column ODS/C18 (octadecyl silane), length 250 mm, inner diameter 0.46 cm, and size of particle 5 µm, eluent phase of acetonitrile with optional % composition of (5:95, 10:90, and 15:85), flow rate was set in 1.0, 1.2, and 1.5 mL/min at UV detection 270 nm. The optimum condition with the fastest analysis time was found to be as in table 1 and figure 3.

Table 1. The predicate optimum parameters for HPLC method.

Mobile phase composition ACN: H_2O	**Flow rate mL/min.**	**Column type**	**Wavelength nm**	**Run time min.**	**Retention time min.**
10:90	1.5	ODS: 250mm,4.6mm,5µm	270	15.0	3.041

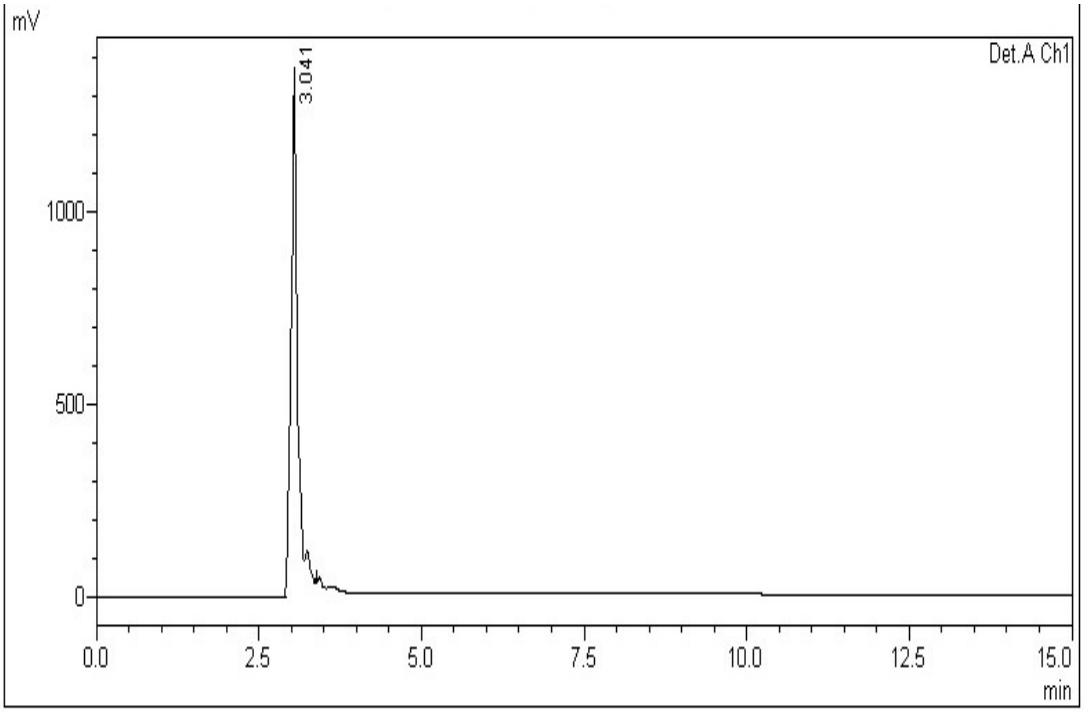

Figure 3. RP- HPLC chromatogram of phenol 100 µg/mL using the predicated optimum condition.

From the stock solution, subsequent dilutions were made with ACN: H2O (10: 90 v/v) to obtain the series of standard solutions have a range of concentration (0.1 – 30 µg/mL) of phenol. A plotted a values of concentration on (x-axis), and the values of area under peak on (y-axis) was given a straight line with R2 value of (0.9997). This considered the calibration graph as shown in figure 4 and table 4.

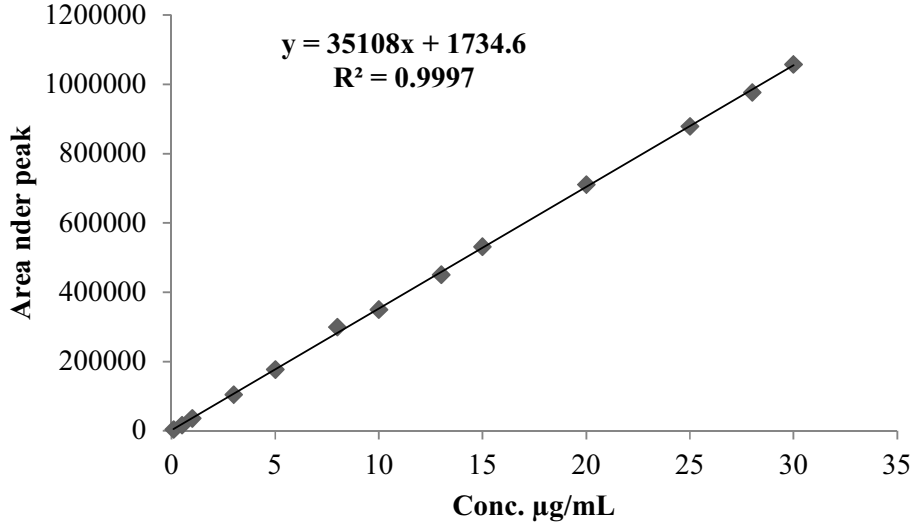

Figure 4. Calibration graph of phenol using HPLC

Table 2. Calibration graphs, statistical calculations for phenol using UV – VIS and HPLC.

Statistical factors	Value	
	UV - VIS	HPLC
Linear equation	y=0.0217[X] + 0.0128	y=35108[X] + 1734.6
Slope (m)	0.0217	35108
Intercept	0.0128	1734.6
Coefficient correlation "R^2"	0.9996	0.9997
Linearity percentage (R^2 %)	99.96	99.97
Coefficient correlation (r)	0.9999	0.9999
Intercept standard error	0.0065	1871
Intercept standard deviation	0.0199	5466
"R.S.D."	0.230	0.0146
"DL" µg/mL	0.02	0.01
"QL" µg/mlL	0.066	0.033
Linearity range µg/mL	0.2 – 25	0.1 – 30
Calculated (t) values $t_{cal.} = \frac{/r/\sqrt{n-2}}{\sqrt{1-r^2}}$	165.81 >>> 2.16	164.95 >>> 2.16

3.1.4. Accuracy, precision and recovery of proposed methods

This etude was conducted out, to assure the obtained results from analytical method closeness with the real value [15]. For the study methods phenol was determined at four different selected concentrations within the Beer's law limits 0.8, 5, 10, 15 and 0.5, 3, 8, 13 µg/mL for UV and HPLC methods respectively. The results were reported as % Recovery, % Error and % RSD, as in table 3, which revealed that the suggested method for determination of phenol were interesting and quite convenient with respect to the methods and parameters calculated.

Table 3. Accuracy and precision of proposed method for standard phenol.

UV – VIS method Phenol µg/mL		**% Recovery**		**% Error**	**R.S.D n = 3**
Taken	Found				
0.8	0.807	100.88	Mean = 100.58 S.D. = 1.69	0.88	0.53
5	5.08	101.66		1.66	1.11
10	10.88	100.88		0.88	0.81
15	14.83	98.88		1.12	0.53
HPLC method Phenol µg/mL		% Recovery		% Error	R.S.D n = 3
Taken	Found				
0.5	0.503	100.55	Mean = 99.99 S.D. = 1.24	0.55	0.26
3	3.02	100.66		0.66	1.13
8	7.97	99.58		0.42	0.59
13	12.89	99.16		0.84	0.66

3.2. Removal experiments

3.2.1. Preparation of adsorbent

In this study, activated carbons (ACs) from the wheat plant waste as a natural material was used. The adsorbent was prepared through two-stage the first one was a semi-carbonization stage through the heating at high temperature range of (450 -650 ºC) and the second was a chemical activation stage using hydrochloric acid. The factors effected on the removal process such as particle size, temperature, time and pH were studied. Removal results shown an increasing in removal percentage in proportional to the increasing of the temperature of carbonization, time and decreasing in adsorbent size, whereas a higher acidic pH decreased the adsorption capacity as shown in figure 5, which indicated that the area under

the peak and height are decreased about near 60% only in comparison with the area in the figure 3, that due to the pH of the solution in this experiment was equal 3 only. The optimum parameters are listed in table 4. Figure 6 shown the HPLC chromatogram at optimum parameter.

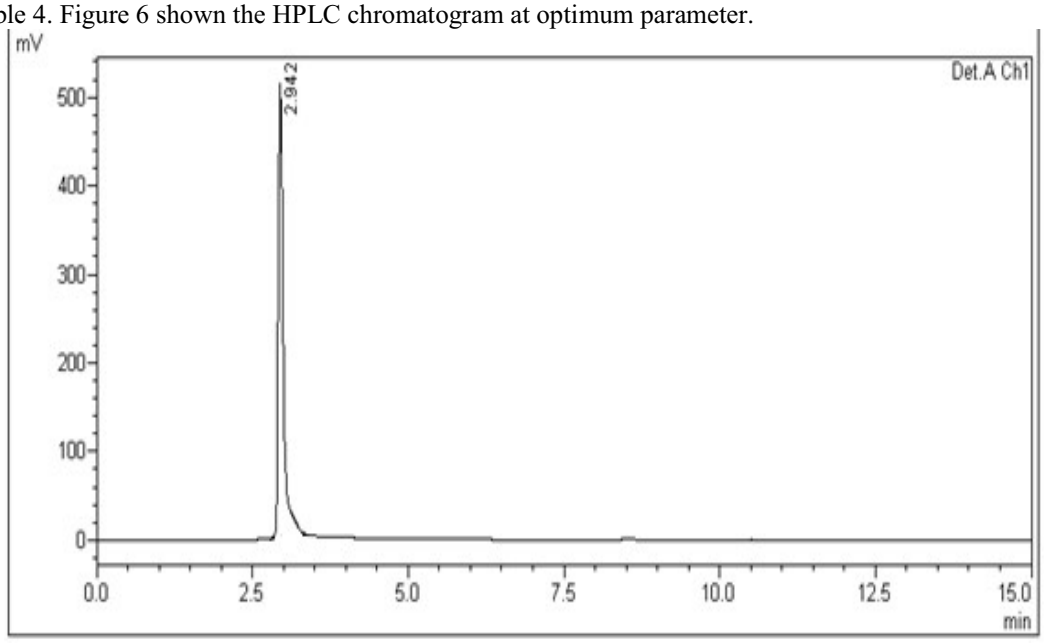

Figure 5. RP – HPLC chromatogram of phenol after removal at pH equal 3.

Table: 4: The optimum condition for phenol removal using treated wheat plant waste.

Parameters	Value
Temperature of heat treatment	600 ºC
Time of heating	120 min.
Type of chemical treatment solution	HCl acid, 1 M.
Time of chemical treatment	30 min., with stirring
Particle size of treated powder used for phenol removal	<100 µm
Weight of treated powder used for phenol removal	0.01 gm
Volume of phenol solution	25 mL
Concentration of phenol solution	100 µg/mL
pH of phenol solution	7.0
Temperature of phenol solution	Room temperature
Time of removal	30 min.

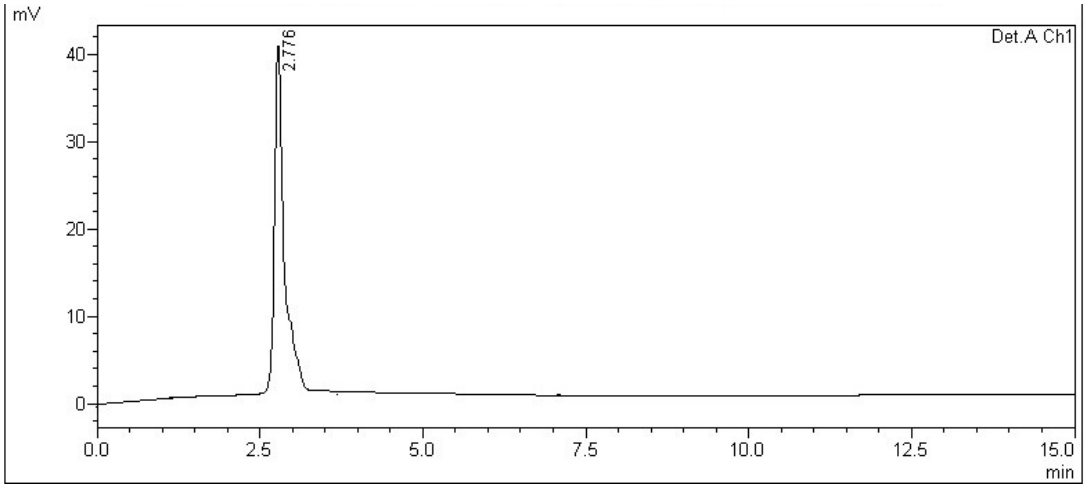

Figure 6. RP – HPLC chromatogram of phenol after removal applied optimum parameters.

Applied of the optimum removal conditions for the removal of phenol gave a high removal percentage as shown in table 5. The concentration of remaining phenol was determined using two methods, UV and HPLC. The obtained results from the two methods are nearly same.

Table 5. The obtained results of phenol removal experiments.

Volume of solution mL	Conc. of phenol µg/mL	Remained phenol conc. µg/mL		% Removal	
		UV	HPLC	UV	HPLC
25	100	1.24	1.15	98.76	98.85

4. Conclusions

A simple, accurate, rapid, economical, and precise HPLC and UV – VIS methods for quantitative assayment of remaining phenol in aqueous solution after removal process using activated carbons (ACs) prepared from the waste of the wheat plant as a natural material. The method is linear with an R2 value of (0.9996 and 0.9997) for UV and HPLC respectively. The validated of the method accuracy was carried by mean percentage recovery, which was found in the acceptable range. The % Removal was found nearly same for the two methods with values of (98.76 and 98.85) for UV and HPLC respectively.

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