

## **Enhanced Spectrophotometric Determinations of Some Phenolic Pollutants in Waste Water Using Gibbs Reaction**

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**Summary:** Two spectrophotometric procedures were developed for the determination of some phenolic pollutants in wastewater samples. The first spectrophotometric procedure was described for the determination of phenol and five of its derivatives. It involves the use of 2,6-dichloroquinone chlorimide as the chromogenic agent. Phenol and its derivatives produce a blue colour that obeys Beer's law at 607 nm for phenol and p-chlorophenol and 600 nm for the other derivatives. The molar ratio of the reaction was established and a proposal for the reaction path way was given. The procedure described was applied successfully for the determination of the named compounds in pure form and wastewater samples in the range from 2-20  $\mu\text{gml}^{-1}$  with average recoveries of 99.94 $\pm$ 0.15% for the named compound.

An enhanced spectrophotometric procedure depending on measuring the resultant complex of phenol and their derivatives with Gibbs reagent in micellar medium was also suggested. The enhanced absorbance of reaction product in  $10^{-2}$  M cetylpyredinium chloride solution at pH 9.4 $\pm$ 1 at 607 nm shows lower limit of detection (0.27 $\mu\text{gml}^{-1}$ ) and lower limit of quantification (0.91  $\mu\text{gml}^{-1}$ ) than the aqueous method (0.56  $\mu\text{gml}^{-1}$  and 1.84 $\mu\text{gml}^{-1}$ ; respectively) with a relative standard deviation of 1.45%. Also it minimizes the background absorbance of the environmental wastewater samples with average recovery of 99.96 $\pm$ 1.47% for phenol in the collected wastewater without pretreatment. Environmental samples from seven local regions in Egypt are analyzed for the cited compounds.

### **Introduction**

Phenols are highly toxic and are chiefly derived from industrial wastewater, municipal sewage and coking plant effluents.<sup>(1)</sup> They are used in the production of large variety of aromatic compounds e.g. explosives, fertilizers, paints, paint removers, textiles, plastics and drugs.<sup>(2, 3)</sup> Exposure to phenolic compound occurs during its use as disinfectant and as an insecticide.<sup>(2)</sup> The iron and steel making industry is one of the

major sources of its release into the environment<sup>(4)</sup> Phenols are readily absorbed by ingestion, inhalation and through the skin.<sup>(5)</sup> Prolonged oral or subcutaneous exposure causes damage to the lungs, liver, kidneys and genito-urinary tract.<sup>(6)</sup> The U. S. Environmental Protection Agency (EPA) recommends a maximum of 1.0 mg l<sup>-1</sup> of total phenolic compounds in domestic water and 5 mg l<sup>-1</sup> of other waters.<sup>(7)</sup> Various methods have been reported for the determination of phenolic compounds in waste water such as titrimetric methods<sup>(8-11)</sup>, spectrophotometric methods<sup>(12-16)</sup>, fluorimetric methods<sup>(17-19)</sup>, GC<sup>(20-22)</sup>, HPLC<sup>(23-25)</sup>, TLC<sup>(26,27)</sup>, potentiometric methods using biosensors<sup>(28-31)</sup> and potentiometric method using polyvinyl chloride matrix membrane electrodes.<sup>(32)</sup> This work is focused on the development of new analytical procedures for the determination of phenolic pollutants in wastewater that are less complex and faster than the chromatographic method, does not require the use of toxic organic solvents like the recommended spectrophotometric and spectrofluorimetric method or the use of enzymes of high cost like the recommended procedures with biosensors. Two suggested procedures based on the formation of coloured reaction products with 2,6-dichloroquinone chlorimide and proportional to the phenolic derivatives pollutants present in the collected wastewater samples either in aqueous or in micellar media were reported.

Table (1) shows comparison between the reported spectrophotometric method<sup>(12,13)</sup> and the suggested method for the determination of phenol. Also it shows the analytical advantages of the suggested procedures.

## Experimental

### Equipment

Absorbance measurements were carried out with UV-1601 PC-UV Visible SHIMADZU spectrophotometer. pH was adjusted with an Orion 91/20 combination glass electrode and an Orion digital ion/analyzer model 720 A. Vacuum Rota Vapour WPA C 16.

**Table (1):** Comparison between the recommended spectrophotometric methods<sup>(12, 13)</sup> and the suggested methods for the determination of phenol.

Parameter	Recommended Methods		Suggested Methods	
	4-Aminoantipyrine Method <sup>(12)</sup>	Oxidative Coupling Method <sup>(13)</sup>	Aqueous	Micellar
Chromagen	4-Aminoantipyrine	Benzidine(K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> , [Fe(CN) <sup>3-</sup> ] <sub>6</sub> )		2,6-dichloroquinone chlorimide
$\lambda_{\max}$ (nm.)	460	535	607	607
Recovery $\pm$ SD	100.05 $\pm$ 0.625	99.57 $\pm$ 0.874	99.89 $\pm$ 0.651	100.12 $\pm$ 0.446
Sensitivity Range( $\mu\text{g}.\text{ml}^{-1}$ )	6-58	0.1-0.7	2-20	1-12
LOD ( $\mu\text{g}.\text{ml}^{-1}$ )	-	-	0.56	0.27
LOQ ( $\mu\text{g}.\text{ml}^{-1}$ )	-	-	1.84	0.91
The intraday RSD% (intermediate precision)*	-	-	0.127-0.287	0.018-0.116
The interday RSD% (repeatability)**	-	-	0.131-0.268	0.036-0.178
Interference Study	Lubricating Oils and Fats	-	Aniline and formaldehyde	Aniline, formaldehyde, urea, diazinon, trichlofon, carbofuran, methocarb, Pb <sup>2+</sup> , Fe <sup>2+</sup> and Fe <sup>3+</sup>
Extracting Solvents	CH <sub>3</sub> CN, C <sub>6</sub> H <sub>14</sub>	n-C <sub>5</sub> H <sub>11</sub> OH	Ether, CH <sub>3</sub> Cl	-

\* (n=6) \*\* (n=5) relative standard deviations of samples concentration (8 and 10  $\mu\text{g}.\text{ml}^{-1}$ ).

## Reagents and Solutions

All reagents used were of analytical grade and the solvents of spectroscopic grade. 2, 6 Dichloroquinone-4-chlorimide, Gibbs reagent (Sigma) solution was freshly prepared as (0.4% w/v) solution in absolute ethanol (E. Merck, W. Germany). Cetylpyredinium chloride (Sigma). Sodium dodecylsulfate (Sigma). Triton<sup>®</sup> X-100 (Sigma). The buffer was 2% solution of sodium borate of pH 9.4±1, which is continuously checked.

Stock solution A: (50 µg.ml<sup>-1</sup>) of each of phenol, o-cresol, p-cresol, p-chlorophenol, α-naphthol and β-naphthol was dissolved in 2 ml 2% w/v sodium borate and prepared in distilled water for aqueous method.

Stock solution B: (50 µg.ml<sup>-1</sup>) of the previous compounds were dissolved in 2 ml 2% w/v sodium borate and prepared in 10<sup>-2</sup> M cetylpyridinium chloride for the micellar method.

## Environmental samples

The wastewater samples were collected from the industrial effluents of seven different factories present in different locations in Egypt that expected to contains phenolic compounds as pollutants. The sample should be representative and collected in clean, inert, containers, wide-mouth borosilicate bottles, 1 liter or 1-quart capacity, sealed with poly tetrafluoroethylene (PTFE) lined screw caps to prevent adsorption and evaporation of the samples.<sup>(33)</sup>

Sample No. 1 was collected from the industrial effluents of Coke manufacturing factory before and after treatment (Helwan). Sample No. 2 was collected from the industrial effluents of Abu Quir Company for fertilizers and chemicals (Alex.). Sample No. 3 was collected from the industrial effluents of Abu Zaabel Company for fertilizers and chemicals (Cairo). Sample No. 4 was collected from the industrial effluents of Iron and Steel factory (Helwan). Sample No. 5 was collected from the industrial effluents of Esco Textile Company (Shubra El Khema). Sample No. 6 was collected from the industrial effluents of El Nasr Company for spinning, weaving and dyeing (El Mahalla). Sample No. 7 was collected from the industrial effluents of Cairo Company for dyeing preparation (Ghamra).

## **Procedures**

### **Spectrophotometric determination of phenolic/Gibbs complex in aqueous medium**

#### **Sample preparation**

The wastewater samples were prepared according to the procedure in the standard method for the examination of water and wastewater.<sup>(33)</sup> One liter of the wastewater samples was accurately measured and transferred to two-liter separating funnel saturated with sodium chloride. The aqueous layer was extracted two times each with 10-ml chloroform-ether (1:1 v/v). The organic layer was transferred to 50-ml separating funnel. Five ml of 2% sodium borate solution of pH 9.4±1 was added, shaken well, and the aqueous layer was separated into 5-ml volumetric flask.

#### **Calibration graph**

An aliquot containing the studied compounds was transferred over a working concentration range (Table 1) into a 25-ml calibrated flask, 2-ml. of 2, 6-dichloroquinone-4-chloroimide reagent (4%w/v) was added. The volume was completed with 2% sodium borate buffer solution of pH 9.4±1 and the absorbance at the specified  $\lambda_{\max}$  was measured after the specified time against a reagent blank. The absorbance was plotted against the final concentration ( $\mu\text{gml}^{-1}$ ) in order to obtain a calibration graph.

### **Application of the proposed method for the analysis of the collected wastewater samples**

From the concentrated solution of the collected wastewater samples 0.5 ml (for sample No. 1) and 1 ml for the other samples was accurately transferred and used for the determination of their respective concentration using the suggested procedure. The total concentration was calculated in  $\mu\text{gml}^{-1}$  as phenol.

### **Spectrophotometric determination of phenolic/Gibbs complex in micellar medium**

#### **Sample preparation**

Reduction of the volume of all the collected wastewater samples was carried out using rotary-vacuum evaporator.<sup>(34)</sup> Each 1-litre sample was reduced to 50 ml volume at temperature 36°C.

### Calibration graph

An aliquot containing the studied compounds was transferred over a micellar working concentration range (Table 2) into a 25-ml calibrated flask, 2-ml of 2,6-dichloroquinone-4-chloroimide reagent (4% w/v) was, added followed by 2 ml 2% w/v sodium borate buffer solution of pH  $9.4 \pm 1$  and the volume was completed with  $10^{-2}$ M cetylpyridinium chloride, the absorbance at the specified  $\lambda_{\max}$  was measured after the specified time against a reagent blank. The absorbance was plotted against the final concentration ( $\mu\text{g}\cdot\text{ml}^{-1}$ ) to obtain a calibration graph.

### Application of the proposed method for the analysis of the collected wastewater samples

From the reduced solution of the collected wastewater samples 5 ml (for sample No. 1) and 10 ml. for the other samples was accurately transferred and used for the determination of their respective concentration using the general procedure. The total concentration was calculated in  $\mu\text{g}\cdot\text{ml}^{-1}$  as phenol.

## Results and Discussion

2,6-Dichloroquinone chlorimide reacted with phenol and some of its derivatives in aqueous and in micellar media in the presence of 2% sodium borate solution at pH  $9.4 \pm 1$  to give coloured products with  $\lambda_{\max}$  at 607 nm for phenol and p-chlorophenol and 600 nm for other derivatives (Figure 1). In order to study the reaction further, the molar ratio of 2,6-dichloroquinone chlorimide to the studied compounds (standard solutions of  $3 \times 10^{-4}$  M phenol and  $1.5 \times 10^{-4}$  M solution of o-cresol, p-cresol, p-chlorophenol,  $\alpha$ -naphthol and  $\beta$ -naphthol) were determined by the continuous variations<sup>(35)</sup> method for both aqueous and micellar procedures. It shows that the ratio is 2:1 for phenol,  $\alpha$ -naphthol and  $\beta$ -naphthol and 1:1 for 4-chlorophenol, o-cresol and p-cresol. On the other hand, according to Scudi<sup>(36)</sup> 2,6-dichloroquinone chlorimide reacts via the chlorine atom of the chlorimide group. Accordingly, the reaction between 2,6-dichloroquinone chlorimide and the studied compounds proceeds as proposed in figure (2), whereby indophenols are formed.<sup>(37)</sup>

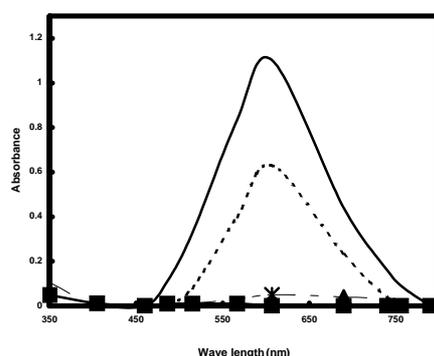
The optimum concentration of Gibbs reagent leading to maximum colour stability was found to be 2 ml of 0.4% reagent (freshly prepared).

Lower reagent concentration gave colours of higher intensities but these faded very quickly with time.

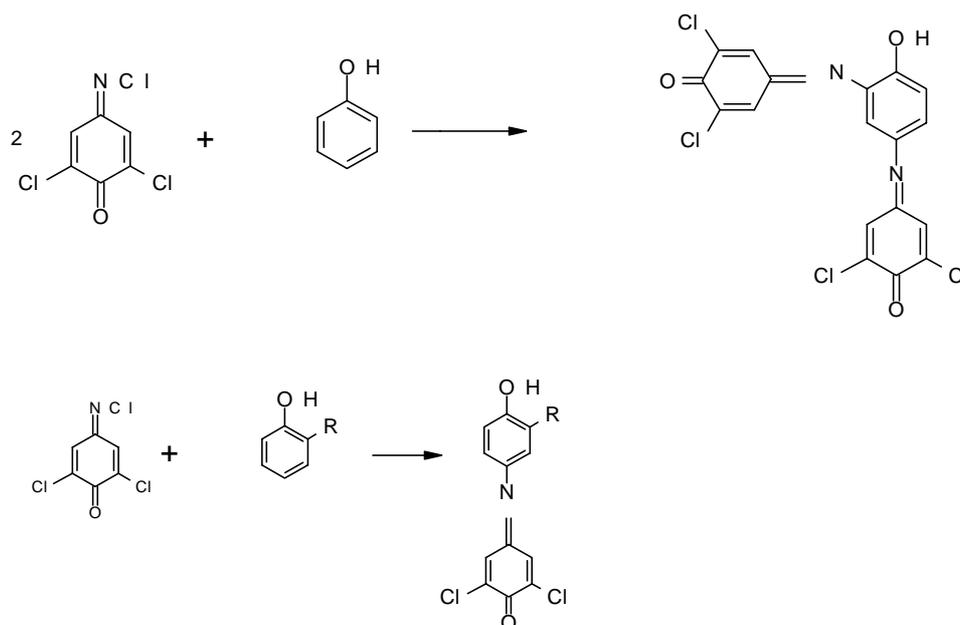
The time for maximum colour intensity was studied for each compound and the colour was stable for a further one hour. Maximum colour intensity was obtained upon using 2% sodium borate buffer of pH  $9.4 \pm 1$ . Different alkali agents, such as sodium hydroxide, sodium carbonate, sodium hydrogen carbonate and tri- and dibasic sodium phosphate were tested. However, 2% sodium borate gave the most reliable and reproducible results.

The absorbance of phenol and phenolic derivatives/Gibbs complexes in different micellar media (e.g. sodium dodecyl sulfate (SDS), cetylpyridinium chloride (CPC) and Triton<sup>®</sup> X-100) were studied. An increase in absorbance was observed only when sodium dodecyl sulfate or cetylpyridinium chloride was added; best results were achieved with  $10^{-2}$  M cetylpyridinium chloride. The final absorbance enhancement achieved was about 75% while  $\lambda$  max remains unchanged.

Table (2) summarizes the data for the reaction between 2,6-dichloroquinone chlorimide and the studied compounds in aqueous and in micellar procedures. The results of the molar absorptivities and A (1%, 1cm) are averages of 10 separate determinations.



**Figure (1):** Absorption spectra of the reaction product of phenol (10 ppm) with 2,6-dichloroquinone chlorimide in aqueous (.....) against blank experiment (. .▲..) and in micellar media (\_\_\_\_) against blank experiment (▪ ).



**Figure (2):** Proposed reaction pathway between 2,6- dichloroquinone Chlorimide and phenol and its derivatives.

**Table (2):** Data for the reaction of 2,6-dichloroquinone with phenol and some of its derivatives

Compound	Molar ratio	1%		
		$A_{1\text{ cm}}$	Molar absorptivity( $\epsilon$ )	Working range ( $\mu\text{g.ml}^{-1}$ )
Phenol	2:1	591.26 <sup>a</sup>	$5.57 \times 10^{3a}$	2-20 <sup>a</sup>
		1022.70 <sup>b</sup>	$9.68 \times 10^{3b}$	1-12 <sup>b</sup>
o-cresol	1:1	589.23 <sup>a</sup>	$6.37 \times 10^{3a}$	2-20 <sup>a</sup>
		1025.51 <sup>b</sup>	$11.23 \times 10^{3b}$	1-12 <sup>b</sup>
p-cresol	1:1	586.99 <sup>a</sup>	$6.34 \times 10^{3a}$	2-20 <sup>a</sup>
		1028.24 <sup>b</sup>	$11.07 \times 10^{3b}$	1-12 <sup>b</sup>
p-chlorophenol	1:1	653.26 <sup>a</sup>	$8.41 \times 10^{3a}$	2-18 <sup>a</sup>
		1139.01 <sup>b</sup>	$14.53 \times 10^{3b}$	1-10 <sup>b</sup>
$\alpha$ -naphthol	2:1	752.39 <sup>a</sup>	$10.85 \times 10^{3a}$	2-16 <sup>a</sup>
		1302.14 <sup>b</sup>	$18.73 \times 10^{3b}$	1-10 <sup>b</sup>
$\beta$ -naphthol	2:1	725.02 <sup>a</sup>	$10.45 \times 10^{3a}$	2-16 <sup>a</sup>
		1289.58 <sup>b</sup>	$18.25 \times 10^{3b}$	1-10 <sup>b</sup>

a: For aqueous procedure.

b: For micellar procedure.

The effect of interference due to the presence of other organic or inorganic pollutants mostly present in wastewater such as aniline, formaldehyde, urea, organophosphorus insecticides, carbamate pesticides and heavy metals was studied by adding increasing concentrations of these compounds to  $8 \mu\text{gml}^{-1}$  phenolic solution in both aqueous and micellar procedures, until the occurrence of change of absorbance

grater than 5%. Table (3) shows the maximum tolerable weight ratio for ten pollutants when using both methods, the selectivity achieved by the micellar method was better than that of the aqueous method, but both have sufficient selectivity to determine phenolic pollutants in the presence of the compounds tested.

Table (4) shows the comparison between the results obtained from the determination of the studied compounds by the aqueous and the micellar procedures, and by the compendial method.<sup>(14)</sup> There is no significant difference between the three sets of results.

Table (5) gives the results obtained from the determination of phenol, o-cresol, p-cresol, p-chlorophenol,  $\alpha$ -naphthol and  $\beta$ -naphthol in the collected wastewater samples, a preconcentration step of wastewater samples was found to be necessary in aqueous procedure. Solvent extraction method using (1:1 v/v) chloroform-ether<sup>(38)</sup> mixture at pH 8-9, was found to be nearly 100% efficient. In case of micellar procedure, rotary-evaporator was used for milled concentration of the collected wastewater samples without the need of removal of the interfering substances.

In order to avoid any interference by industrial matrix of the collected wastewater samples, the standard addition technique was applied for the quantification of the phenolic pollutants in the collected wastewater samples and the average recoveries were found to be  $99.58 \pm 0.455$ ,  $99.68 \pm 0.505$ ,  $99.95 \pm 0.249$ ,  $99.93 \pm 0.224$ ,  $99.50 \pm 0.500$ ,  $99.45 \pm 0.190$ ,  $99.72 \pm 0.623$ ,  $99.29 \pm 0.720$ ,  $99.28 \pm 0.781$ ,  $99.55 \pm 0.413$ ,  $99.19 \pm 0.351$ ,  $99.87 \pm 0.295$ ,  $99.44 \pm 0.578$  and  $99.67 \pm 0.476$  for both the aqueous and micellar methods in the collected wastewater samples; respectively.

Table (1) shows the comparison between the recommended spectrophotometric methods<sup>(12,13)</sup> and the suggested methods for the determination of phenol.

**Table (3):** Maximum weight ratio of different compounds in the determination of  $8 \mu\text{g.ml}^{-1}$  phenol by the suggested aqueous and micellar spectrophotometric method.

Compound Added	Weight Ratio	
	Aqueous procedure *	Micellar Procedure *
Aniline	15	22
Urea	15	20
Formaldehyde	20	20
Diazinon	40	50
Trichlorfon	40	50
Carbofuran	30	40
Methocarb	30	40
Pb <sup>2+</sup>	2.5	5
Fe <sup>2+</sup>	2.5	5
Fe <sup>3+</sup>	2.5	5

\*Mean of three repeated determinations.

**Table (4):** Determination of phenol and its derivatives by the proposed methods and the published methods<sup>(14)</sup> and their corresponding statistical results.

Compound	Recovery *%		
	Proposed method		Published methods <sup>(14)</sup>
	Aqueous	Micellar	
Phenol	99.89±0.651	100.12±0.446	
F 0.05	1.41 (3.18)	0.663(3.18)	99.71±0.548
Student t, test	0.774 (2.101)	0.213 (2.101)	
o-cresol	99.72±0.337	100.08 ± 0.348	
F 0.05	1.70 (3.18)	1.82(3.18)	99.55±0.258
Student t, test	0.545 (2.101)	0.550(2.101)	
p-cresol	99.94±0.684	99.98±0.407	
F 0.05	1.253 (3.18)	0.444(3.18)	100.02±0.611
Student t, test	0.805 (2.101)	0.713(2.101)	
p-chlorophenol	100.11±0.242	100.21±0.450	
F 0.05	0.630 (3.18)	2.18(3.18)	100.06±0.305
Student t, test	0.523 (2.101)	0.614(2.101)	
α-naphthol	100.05±0.471	100.19±0.535	
F 0.05	0.821 (3.18)	1.06(3.18)	99.69±0.520
Student t, test	0.704 (2.101)	0.727 (2.101)	
β-naphthol	100.30±0.576	99.47±0.627	
F 0.05	1.652 (3.18)	1.96 (3.18)	100.14±0.448
Student t, test	0.716 (2.101)	0.733(2.101)	

\* The results given are average of 10 separate determinations.

**Table (5):** Determination of the phenolic contents in the collected wastewater samples\* using the proposed methods and the published method.<sup>(14)</sup>

Sample No.	Content ( $\mu\text{g.ml}^{-1}$ )	Phenol recovery <sup>**</sup> $\pm$ RSD		
		Proposed method		Published method <sup>(14)</sup>
		Aqueous	Micellar	
II	800	99.60 $\pm$ 0.165	100.55 $\pm$ 0.054	100.08 $\pm$ 0.545
II	350	100.01 $\pm$ 0.412	99.85 $\pm$ 0.336	98.55 $\pm$ 0.611
III	310	98.85 $\pm$ 0.315	100.152 $\pm$ 0.244	99.36 $\pm$ 0.221
IV	185	99.05 $\pm$ 0.119	100.47 $\pm$ 0.114	99.08 $\pm$ 0.470
V	210	99.56 $\pm$ 0.347	98.97 $\pm$ 0.628	99.55 $\pm$ 0.117
VI	300	100.12 $\pm$ 0.108	99.51 $\pm$ 0.284	100.15 $\pm$ 0.621
VII	220	98.95 $\pm$ 0.466	100.246 $\pm$ 0.122	98.42 $\pm$ 0.111

\*\*Average of at least four determinations

In order to avoid any interference by industrial matrix of the collected wastewater samples, the standard addition technique was applied for the quantification of the phenolic pollutants in the collected wastewater samples and the average recoveries were found to be 99.58 $\pm$ 0.455, 99.68 $\pm$ 0.505, 99.95 $\pm$ 0.249, 99.93 $\pm$ 0.224, 99.50 $\pm$ 0.500, 99.45 $\pm$ 0.190, 99.72 $\pm$ 0.623, 99.29 $\pm$ 0.720, 99.28 $\pm$ 0.781, 99.55 $\pm$ 0.413, 99.19 $\pm$ 0.351, 99.87 $\pm$ 0.295, 99.44 $\pm$ 0.578 and 99.67 $\pm$ 0.476 for both the aqueous and micellar methods in the collected wastewater samples; respectively.

Table (1) shows the comparison between the recommended spectrophotometric methods<sup>(12,13)</sup> and the suggested methods for the determination of phenol.

### Method validation

Validation of the proposed methods was performed with respect to stability, limit of detection, (LOD), limit of quantification (LOQ), range, accuracy, precision and robustness to the ICH Guidelines.<sup>(39)</sup>

### Stability and interfering study

The stability of the stock solutions of the analyzed compounds, stored at 4° C, was evaluated at various time points over 2 months. The concentrations of freshly prepared solutions and those aged for 2 months were calculated by the methods developed and the difference between them was found to be less than 2.0%. The absorption spectra of the solutions were also checked and they were found to be unchanged within this period. These solutions can therefore be used during this

interval of time without the results being affected. The effect of interference due to the presence of other organic or inorganic pollutants mostly present in wastewater was studied, by adding increasing concentrations of these compounds to  $8 \mu\text{gml}^{-1}$  phenolic solution in both aqueous and micellar procedures, until the occurrence of change of absorbance greater than 5%.

### **Specificity**

Specificity, described as the ability of the proposed methods to discriminate the analyte from all potential interfering substances was evaluated by preparing different concentrations of the interfering substances that expected to be found in the collected wastewater samples and it was confirmed that there was no interferences up to certain limits as shown in table (3), which confirm the specificity of the methods.

### **LOD and LOQ**

The limit of detection and of quantification was calculated for the suggested and the reported methods<sup>(12, 13)</sup> under the stated conditions and shown in table (1).

### **Precision and Accuracy**

The assay was investigated with respect to repeatability and intermediate precision. In order to measure repeatability of the methods, 5 consecutive measurements were made. The precision values and R.S.D. were shown in table (1).

The accuracy of a method is expressed as the closeness of agreement between the value found and the value that is accepted as a reference value.<sup>(14)</sup> The accuracy of the proposed method was tested by recovery experiment. Six samples were prepared for each recovery level. The results obtained were shown in table (1), from which it is clear that both the recoveries and repeatability were excellent.

The suggested spectrophotometric procedures have better performance (higher sensitivity, better detection limits and quantification limits) than those reported previously in the literature. The Two recommended procedures<sup>(12,13)</sup> for the determination of phenolic derivatives were compared with the suggested methods (Table 1). The rapidity and reproducibility of the method suggests its wide application for detection and determination of trace amounts of phenol in wastewater samples.

## Conclusion

Simple, fast, and reliable spectrophotometric methods were developed applied and validated for the determination of total phenolic pollutants in wastewater. The methods show a good performance with respect to selectivity, accuracy, precision and it offers an inexpensive way for the determination of phenolic pollutants either in pure form or in the collected wastewater samples.

This work also evaluates different characteristics for the validation process and outlines the specific aspects that should be considered for a spectrophotometric methodology.

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