International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.11 No.11, pp 35-47, 2018

Liquid Chromatographic and validation study in separation and determination of benzidines and phenols in the main discharge point of wastewater

Bahaa Malik Altahir[1]*, Muntadhar Salih Sultan[2], Amer Saleh Mahdi[2], Hadi Hassan Jasim[2]

[1]Biology Department, College of Science, University of Baghdad, Baghdad, IRQ
[2]Chemistry Department, College of Science, University of Mustansiriyah, Baghdad, IRQ
Corresponding: E-Mail: baha782004@gmail.com; Tel: 009647901567986

Abstract : Benzidines and phenols are the most priority pollutants. Separation and quantitative estimation of priority pollutant benzidines composed of various benzidines BZ, including substituted 3, 3'-dichlorobenzidine DCB and 3, 3'-dimethylbenzidine DMB, and priority pollutant phenols (9 compounds, i.e., phenol, 2- and 4-nitrophenol, 2,4-dimethylphenol, 2-, 2,4-di-, 2,4,6-tri-, Penta- chlorophenol, and 4-chloro-3-methylphenol)was performed using high performance liquid chromatography-ultra violet techniques. Both groups were separated using a C_{-18} column with a UV detector at a wavelength of 280 nm, and the flow of the mobile phase was isocratic. The mobile phase consisted of 75:25 methanol: water. The column temperature was 50°C, and the flow rate was 1.8 ml/min for the Benzedine's separation. The mobile phase consisted of a 50:50 acetonitrile: phosphate buffer. The optimum pH was 7.1, the flow rate was 0.7 ml/min and the optimum column temperature was 45°C for the phenols separation. The separation parameters were calculated, including the chromatographic parameters such as the capacity factor (k), the number of theoretical plates (N) , the selectivity factor (α), and the resolution factors (Rs).This method was applied to real samples. The water samples that were analyzed were obtained from a petroleum refinery wastewater treatment unit. The results ranged between undetectable levels and 246.9µg/L of the selected benzidines.The results were ranged between undetectable levels and 1865.61 µg/L of the selected phenols.

Keywords : *Chromatographic study, Petroleum refinery wastewater, Benzidines, Priority pollutant Phenols, HPLC.*

1. Introduction

Benzidine-based azo dyes are widely used in the dye manufacturing, textile dyeing, color paper printing, and leather industries[1].Benzidine and its derivatives have been used to manufacture dyes for many

DOI= http://dx.doi.org/10.20902/IJCTR.2018.111105

years[2]It is classified as a known human carcinogen by the Environmental Protection Agency (EPA), the National Toxicology Program (NTP), and the International Agency for Cancer Research (IARC)[3].Many of these dyes find their way into the environment via wastewater facilities. These dyes also exhibit a high resistance to microbial degradation in wastewater treatment systems[4]

Phenol and its derivatives are common pollutants found in effluents from industrial operations dealing, for example, with coal conversion, pulp and paper manufacturing, wood preservation,metal casting, and production of pesticides, and they are rated as priority pollutants by the US EPA (code U188)[5].The presence of phenolic compounds in soils is due to different sources, including industrial activities related to the chemical, textile, pharmaceutical, polymers, pulp and paper, woods, plasticizers, pesticide,and metallurgic industries or by the release of industrial effluents and domestic sewage [6].Phenols are easily accumulated in soils and can contaminate the environment. Given increasing concerns on environmental quality, several countries have established strict limits on acceptable environmental levels of phenols[7].

The content of benzidinic compounds in aqueous samples can be determined by various analytical instrumental methods, such as a gas chromatography(GC)/mass spectrometric assay [8], rapid colourimetry[9], fluorescence spectrographic method[10][10],novel resonance light scattering (RLS)[11], ion selective electrodes [12] , direct injection and Ultrahigh-pressure liquid chromatography (UPLC) with fluorescence detection[13], and high-performance liquid chromatography with various detectors such as UV detector [14] , mass spectroscopy detector[15] electrochemical detector[16].

The content of phenolic compounds in aqueous samples can be determined by various analytical instrumental methods such as gas chromatography(GC)/mass spectrometric assay [7]

spectrophotometric determination[17, 18], electro analyticaland potentiometricanalysis[19],high performance liquid chromatographic with various stationary phases as well as, hyper-cross linked polystyrene resin with multiple flow modes, includingisocraticand gradient flow (have been employedwith various detectors such as diode-array[20],mass spectrometry [21]ultraviolet detection[22].

Benzidines were determined in different environmental matrices such as water [15] and river samples[12].Phenolic compounds were determined in various samples, such as environmental [22],food [19], Agricultural [21]seawater,soil, sediments [7, 17, 18], and wastewater samples[20].

HPLC is the preferred technique for priority pollutant aromatic compounds separation, because it is simple, robust, reliable, accurate and highly selective[23].

In addition, pretreatment and extraction step is necessary prior to HPLC to remove interfering components. The proposed method is simple and rapid and practicalfor the identification and simultaneous determinationof several compounds in a short period of time. Studieshave been carried out to determine the amountof benzidines and phenols in industrial waste water and environmental water samples, but little previous study hasinvestigated these compounds in petroleum refinery waste water as well as previous full chromatographic study. The Dora oil, petroleum refinery station is one of the most important stations in Iraq and possesses a wastewater treatment unit.The oil, petroleum refinery waste water is a source of aromatic compounds because these compounds are the major components of crude oil[24]. The aromatic compounds may affect living organisms in water. Benzidines and PPP are hazardous pollutants and aromatic compounds that can be found in petroleum waste water. One of the aims of this study is to investigate the concentrations of benzidines and PPP in waterbased on treatment stages.

2.Materials and Methods

2.1 Materials

The standard solution of phenols [i.e., 604 Phenols Calibration Mix] and the Standard solution of Benzidines[Benzidine, 605 benzidines calibration mix (BZ, DCB), 8270 benzidines mix (BZ, DCB, DMB)] were supplied by Restek Chromatography Product and Solutions.

All solvents were HPLC grade and supplied by (sigma-Aldrich(St. Louis, MO, USA), Himedia Laboratories (Mumbai, India) and J.T Baker (Netherland). All of the other chemicals were of analytical grade

with a purity >98.0%.These chemicals were obtained from Sigma Chemical Company Inc., Aldrich Chemical Corporation (Milwaukee, WI) or BDH Inc. The CHROMABOND® SPE cartridges {HR-P (polystyrene-divinylbenzine) (PS-DVS), octadecyl silica(ODS), Cyclohexyl silica(CHS)} were supplied bythe MACHEREY-NAGEL Company.

2.2 Instruments

A Shimadzu HPLC (LC-20AD), DGU-20As degasser, LC 20A four pumps P,N 7725i Sample injector, Shimadzu SPD-20A prominence UV-VIS detector, and Column Oven CTO 20AD were employed. The column (EC Nucledur C_{18}RP) Stainless steel made with a lengthof 250mm and an inner diameter of 4.6 mm(Machenery Nagel).The column protection system (EC guard column, holder system) had an inner diameter of 2 mm (Machinery Nagel Co.).A special syringe made for HPLC (M. SYRINGFE, 122F-LC) was used for the injection of the 20 µl samples in the mobile phase and onto the column. The extraction system was designed to manually extract different samples at the same time.

2.3 Mobile phase optimization protocol

All of the grades of the HPLC solvent were mixed pre-filtered with 0.45 µm Nitro cellulose filter paper. The optimization and selection of the mobile phase were performed by changing the solvent mixture type, organic solvent percentage, oven temperature, Flow rate.

2.4 Solid phase extraction, optimization protocol

The extraction cartridges were conditioned by adding 5 mL methanol, 5 mLof distilled water, 50 mL of the samples with 5000 µg/L (phenols)or800 µg/L (benzidines) suctioned under vacuum by a tube connected to the SPE cartridge fitted with a conical flask that containeda specific stopper. The extraction conditions (i.e.,kind of resin, pH, Elution solvent) were optimized.

2.5 Buffer preparation

Different buffers were prepared for the experiments at a desired pH[25]. The buffers used in this study included sodium phosphate buffer (pH range 5.6-8.0), acetate buffer (pH (3.7-5.6),carbonate buffer (pH (9.2-10.6) and hydrochloric acid-potassium chloride buffer (pH (1-2)).

2.6 Experimental sample collection and preparation

The water samples were collected from the treatment station using specific pumps that aredesigned for sampling. All of the samples were collected in 2.5 L closed dark glass bottles that were cleaned with dilute chromic acid followed by through washing with distilled water at the location. Allof the containers were rinsedwithriver water or waste water twice and then filled with the samples.The samples were examined on the same day as the sampling. The samples were pre-filtered with a 0.45 µm Millipore filter, 1ml per litre of concentrated HCl was added to adjust the pH to<2 for the phenols evaluations, and 1ml per litre of concentrated NaOH was added to adjust the pH to>8 for the benzidines evaluations. In addition, 2.5ml of methanol was added to each littre of water sample.

The extraction of the desired benzidines was performed by passing 1 L of the sample water through anSPE cartridge containing an ODS resin (flow rate 25 to 35 ml/min).The extraction column was dried in an oven at 50°C for 10 minutes. The desired materials were eluted from the cartridge with 5ml of methanol and further concentrated by evaporation to less than 1 ml and complete volume to 1 ml.The extraction of the desired phenols was performed by passing 1 L of the sample water through anSPE cartridge containingps-DVB. The extraction column was dried in an oven at 50°C for 10 minutes.The desired materials were eluted from the cartridge with 5ml of tetrahydrofuran. The sample components were separated, identified, and measured by injecting an aliquot into a high-performance liquid chromatography (HPLC) system with a UV detector and a reversed phase HPLC.

3. Results and Discussion

3.1 Optimization of the Purification and Separation of Priority Pollutant Benzidines PPB and PPPs by HPLC-UV

The liquid chromatographic separation and detection of a mixture of three different benzidines and nine priority pollutants phenols were carried out using a (C_{18}) column (stationary phase). The flow was isocratic. The optimum separation conditions may change as the retention factors (k) of the benzidines are optimized.The ratio of the organic HPLC-grade solvent to the aqueous phase as well as flow rate, temperature of the column oven, pH and detection, were optimized.

3.1.1 Selection of mobile phase: Organic solvent modifier (methanol) percentage in aqueous phase

Injections were performed using mixtures of water with different percentages of methanol as an organic modifier (25, 50, 75, 85) % as a mobile phase, a flow rate of 1ml/min, detector wavelength 280 nm, and a temperature was 50°C to separate (BZ, DCB, and DMB). The retention factors were calculated.The results are shown in Fig. (1).DMB and DCB were highly sensitive to an increase in the organic solvent (methanol) percentage from 25% to 75% compared to that of BZ and a slight decrease in k was observed from 75-85% for BZ. The optimum organic solvent amount was 75% because the k values were not as close and the peaks were separated by a short analysis time.In general, an increase the concentration of the organic modifier decreased the overall retention time, However, the changes in the relative retention times depended on the properties of the analytes[26][26].These results may be due to the differences in the partition of the compounds between the mobile phase and the stationary phase.

3.1.2 Temperature Effect

The effect of temperature was optimized. The Organic solvent was 75:25 methanol: water with a flow rate of 1ml/min and different temperatures (40, 45, 50, 60°C) were investigated.The results indicatedthat a decrease in the retention factor occurred as the temperature increased.In general, an increase in the column temperature reduced the retention factor[26].However,the k values of BZ and DMB did not change substantially, but that of DCB did exhibit a larger change. However, a small decrease in k was observed with BZ from 45-50°C and 50-60 °C that which may be due to the high viscosity of the eluent (i.e., 75% methanol). The optimum temperature was 50°C because the k values were not as close to each other, resultingin molecules that are more separated with a shorter analysis timeFig.(2).

3.1.3 Flow Rate

The flow rate was optimized. The optimum conditions,including 75% organic solvent (methanol) and an oven temperature of 50°C were held constant. Different flow rates (i.e., 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, and 2.6 ml/min)were used to separate the mixture.The results are shown in Fig. (3).A decrease in the k values of BZ, DCB and DMB from 1-1.4 ml/min was observed. In contrast, an increase in the k values from 1.4-2.6 was observed for all of the benzidines.The optimum flow rate was 1.8. This flow rate was selected because the k values were not as close to each other and the molecules were more separated with a shorter analysis time. Moreover, a flow rate greater than 1.8 causes deterioration of the separation column. A small effect of the flow rate on the separation was observed.A decrease in the k values was observedas the analysis time decreased, which may be due toan increase in the partitioning equilibrium between the mobile phase and the stationary phase.

3.1.4 Detection

The wavelength (λ) changes (i.e., 230, 250, 280, 310, and 340 nm) were studied under the optimum separation conditions.The peak area was used as an indicator to choose the best wave length. A wavelengthof 280 nm was optimal, as shown in Fig.(4).

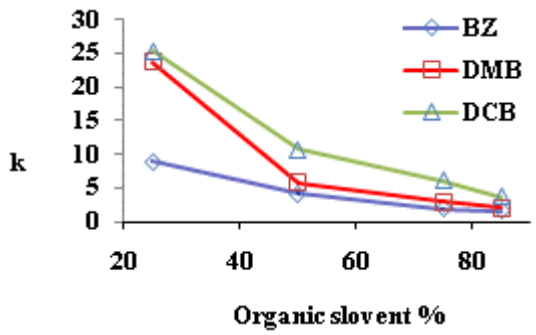


Fig. (1) Organic solvent percentage and k values of BZ, DCB, and DMB (Flow rate of 1 ml/min at50°C)

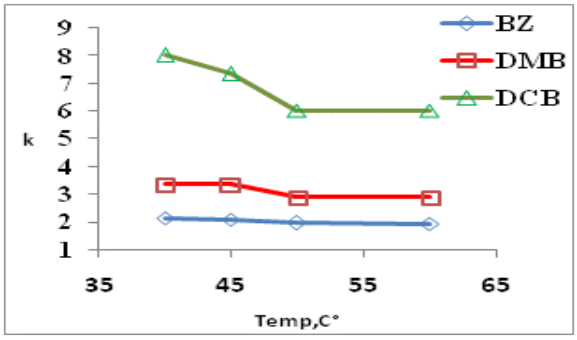


Fig. (2) Temperature effect and k values of BZ, DCB, and DMB (Flow rate of 1 ml/min with 75: 25 organic solvent

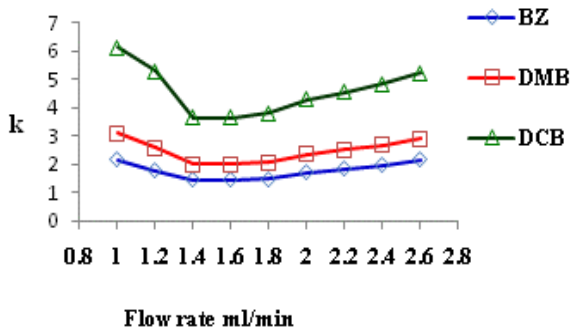


Fig. (3) Flow rate and k value of BZ, DCB, and DMB(Temperature of 50°C75: 25% with organic solvent)

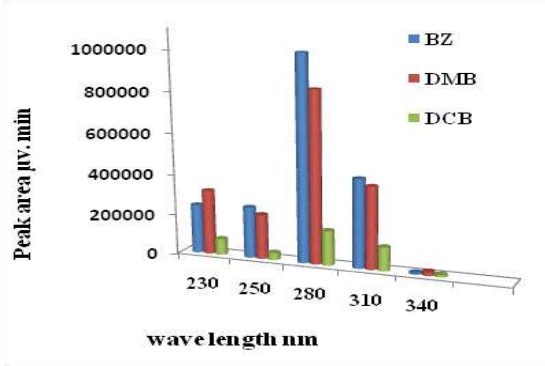


Fig. (4) Peak areas and wave lengths of BZ, DCB, DMB under the optimum conditions with 10000 µg/L

3.2 Optimization of the Purification and Separation of Priority Pollutant Phenols (PPPs) by HPLC

3.2.1 Selection of Mobile Phase:Organic Solvent modifier Percentage in Aqueous Phase

The organic solvent percentage was optimized using mixtures ofacetonitrile and water with different organic solvent percentages (15,25, 35, 50 and60%) as the mobile phase. The pH of the aqueousportion was the same as that of DIW 7. In addition a flow rate of 0.7 ml/min,and a column temperature of 45°C were held constantduring the separation of the PPPs by HPLC. The k values werecalculated.The results are shown in Fig. (5).Most of the studied phenols were highly sensitive to increases in the organic solvent (i.e.,acetonitrile(ACN)) percentage. The optimum organic solvent percentagewas 50 % because the k values were not as close to each other in addition, the convergence of k values at a lower value resulted in better separation of the molecules, and the analysis time was shorter.

3.2.2pH Effect

The pH of the mobile phase, which contains 50% organic solvent (acetonitrile) was optimized using a, column temperature of 45°C and a flow rate of 0.7 ml/min. Different pH values [i.e.3.7, 5.2 (acetate buffer), 6.6, 7.1, and 7.6 (phosphate buffer)] were used to separate the mixture.The results are shown in **Fig. (6).**Change in the k values was observed for most of the PPPs. The optimum pH was 7.1 because the convergence of thek values was to a lower value. In addition the molecules were more separated. The pKa values of the phenol are 9.95 while (7.14-7.23) fornitrophenols and (8.48-9.32) for chlorophenols[27].The pKa values indicate that approximately 50% of the chlorophenols are in an ionic form[unprotonated] and 50% are protonated. The other phenols such as the nitro phenols are unprotonated. The difference in the [unprotonated] /[protonated] forms yields the difference in thepartitioning equilibrium between the mobile phase and the stationary phase, which aids in the full separation of these compounds. The retention factor of the partially ionized compound can be predicted in reversed-phase liquid chromatography by equation (3.1)

$$k = ko + ki\frac{ko+ki(\frac{ka}{H+})}{1+(ka/(H+)} \quad \ldots\ldots \quad 3.1$$

Where *k0* is the maximum retention factor of the unionized form of the analyte *ki* is the retention factor of the fully ionized compound, *Ka* is the dissociation ionization constant, and [H+] is the hydrogen ionconcentration in the eluent. Each compound has its own *k0*, *ki* and *Ka* values. When the hydrophobicities of compounds are nearly equal,separation is difficult in a reversed-phase mode. However, when theirdissociation constants are different, the separation can be easily accomplished in a pH controlled eluent by introducing differential partialionization[26].

3.2.3 Flow rate

Next, the flow rate was optimized. 50% organic solvent (acetonitrile) and an oven temperature 45°C were employed. In addition, the pH of the mobile phase was 7.1. Different flow rates (i.e.0.4, 0.7, 1.0, 1.2, and 1.4 ml/min) were used to separate the mixture.The results are shown in Fig. (7). A decrease in the k values for 4-NP, PCP, 2,4,6-TCP, ph, and 2-NP and a larger decrease in the k values for 2-CP, 2,4-DCP, 2,4-DMP,and 4-C-3-MP were observed. The optimum flow rate was chosen to be 0.7 ml/min because the k values were not as close to each other and the analysis time was shorter.

3.2.4Temperature

The column oven temperature was optimized. The organic solvent consisted of 50:50 acetonitrile: buffer at a pH of 7.1, and using a flow rate of 0.7 ml/min. Different temperatures (i.e.,40, 45, 50, 55, 60 and 65 °C) were used to separate the PPP. For the k values, asmall decrease was observed for 4-NP, 2,4,6-TCP, and PCP, a moderate decrease was observed for ph, 2NP,and 2CP and a substantial decrease was observed for 2,4-DCP, 4-C-3-MP, and 2,4-DMP. The optimum temperature was 45 °C because the k values were not as close to each other. In addition the molecules were separated bysuitable analysis time.(Fig. (8)).

3.2.5 Detection

The detection wavelength (λ) change was studied under the optimum separation conditionsat various wavelengths. The wavelength 280 nm was optimal to yield higher peak areas for all of the studied PPPs. The increase in the peak area was used as an indicator for selecting the best wavelength. The peak areas are shown in (Fig. (9)).

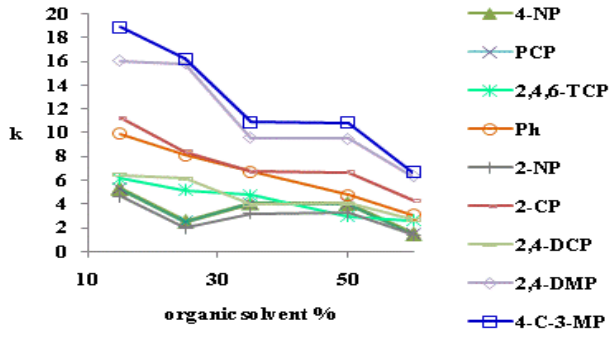


Fig. (5) Organic solvent % and k of PPP (flow rate of 0.7 ml/min, 45°C).

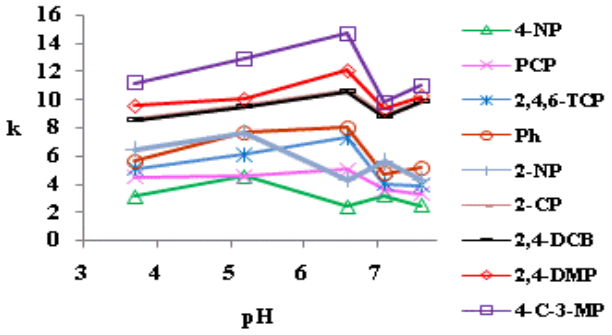


Fig. (6) pH and k values of PPP (Temeprature of 45 °C, flow rate of 0.7 ml/min, organic solvent (50:50))

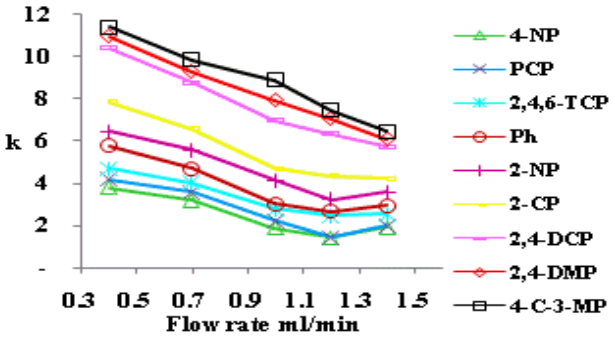


Fig. (7) Flow rate and k value of PPP (Temperature of 45 °C, organic solvent (50:50))

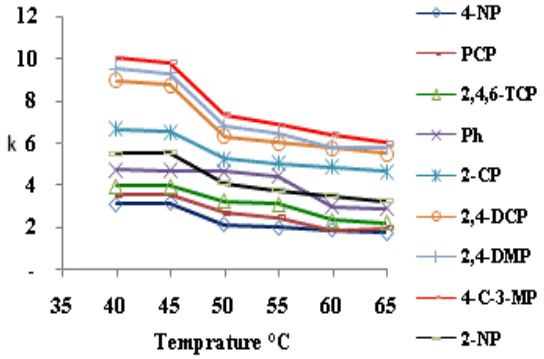


Fig. (8) Temperature and k values of PPP (Flow rate of 0.7 ml/min, organic solvent (50:50))

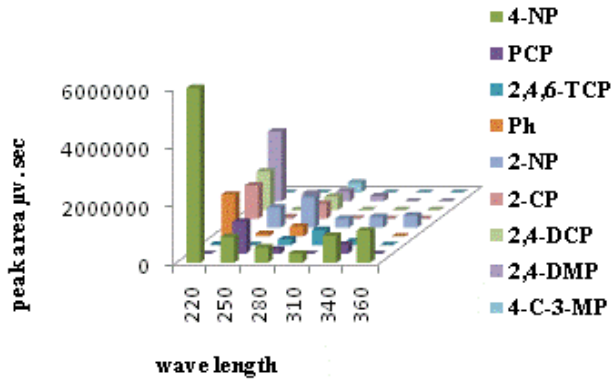


Fig. (9) Wavelengths and peak areas of PPPs under optimum separation conditions.

3.3 HPLC Separation Parameters

The retention time (t_R) and void time (t_M) were used to calculate the retention factor (k) of the eluted benzidines and phenols using the following equation $\mathbf{k = t_R - t_M/t_M}$[28, 29]were obtained from the chromatograms inFigs. (10,11). Thenumber of theoretical Plates (N) was computed using the HPLC parameter equation.($\mathbf{N=16(t_R/w)^2}$)The selectivity factor (α) was computed using the following equation: $\mathbf{\alpha = k_2/k_1}$,In addition the resolution factor was calculated using the following equation $\mathbf{R_S = 2(t_2 - t_1)/(w_1 + w_2))}$,and theTailing factorwas calculated using the following equation **Tf =W 0.05 / 2 a 0.05**where (w) is the peak width and t_R is the retention time of the sample. The HPLC parameters for benzidines are summarized in Table (1). The capacity factor (k) was 1.493-3.790,and the number of theoretical plates (N) was1478.1-2383.In additionthe selectivity factor (α) was 1.386,and 1.832, and the resolution factors were 2.258 between BZ and DMB, 6.184 between DMB and DCB)(Tables (1,2). The HPLCsSeparation parameters for PPPs are summarized in Table (2). The capacity factor (k) was 3.17-9.83, the number of theoretical plates (N) was 4019-13787.46, the selectivity factor (α) was1.06 and 1.34), and the resolution factor was 1.38-6.92.

Table 1HPLC parameters for the separation of benzidines

	Peak width	**t_R(min)**	**t_M(min)**	**k**	**N**	**α**	**Rs**	**Tf**
BZ	0.181	1.745	0.700	1.493	1487.146			1.045
DMB	0.176	2.148	0.700	2.069	2383.215	1.386	2.258	1.042
DCB	0.216	3.353	0.700	3.790	3855.490	1.832	6.148	1.333

Table 2 HPLC parameters for separation of PPPs

	peak width (min)	t_R	t_M	k	N	α	Rs	Tf
4-NP	0.40	6.34	1.52	3.17	4019.56			1.0145
PCP	0.40	6.95	1.52	3.57	4831.64	1.13	1.53	1.0417
2,4,6-TCP	0.40	7.61	1.52	4.01	5788.17	1.12	1.64	1.0093
Ph	0.40	8.69	1.52	4.72	7546.40	1.18	2.70	1.0156
2-NP	0.53	9.96	1.52	5.55	5582.33	1.18	2.73	1.0251
2-CP	0.44	11.48	1.52	6.55	10895.56	1.18	3.12	1.0135
2,4-DCP	0.53	14.85	1.52	8.77	12406.06	1.34	6.92	1.0323
2,4-DMP	0.53	15.66	1.52	9.30	13787.46	1.06	1.51	1.0251
4-C-3-MP	0.64	16.47	1.52	9.83	10592.27	1.06	1.38	1.0317

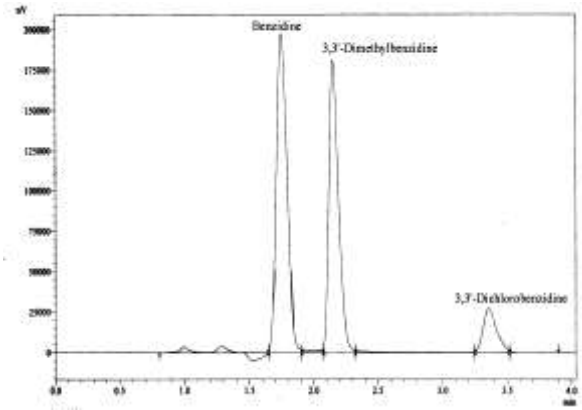


Fig. (10) HPLC chromatogram of priority pollutant benzidines (PPB) recorded under optimum separation conditions.

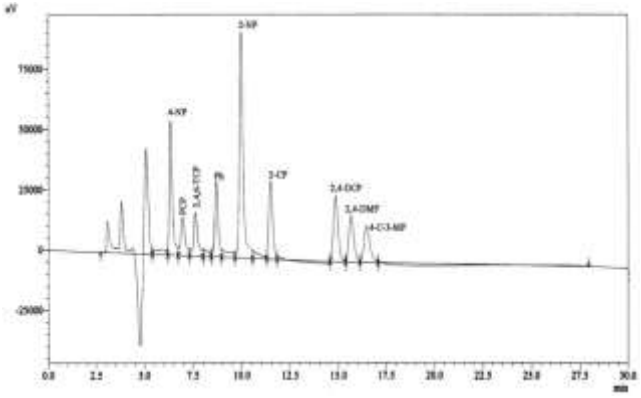


Fig. (11) HPLC chromatogram of priority pollutant phenols (PPPs) recorded under optimum separation conditions.

3.4 Linearity and Calibration curves

Standard solutions of a mixture of BZ, DMB, and DCB as well as mixtures of phenols were prepared as a series of concentrations, (i,e, 800, 600, 400, 200, 100, 50, 25, and 12.25 µg/L as well as up to 5000 µg/L, respectively). The results were recorded on a datasheet. The mean, standard deviation, and relative standard deviation (RSD) were computed for each concentration. The concentration (x-axis) as a function of the mean response (y-axis) was plotted for each concentration. The regression equation and correlation coefficient (r) were calculated. These calculations were recorded on the data sheet.The linearity was determined for all the studied compounds.

The correlation coefficients (r) were in the range of 0.9979 to 0.9995.Linearity was observed for all of the PPP compounds with regression coefficients (r) of0.9961-0.9995.The limit of detection(LOD) and quantitation (LOQ)were calculated using the linear regression method [198].For BZ, DMB and DCB the LODs were 32.99, 33.67, and 26.36) respectively,and the LOQs were 109.98,112.26, and 87.89), respectively. In addition, for thePPPs the LODs were 35.56-241.44 µg/L and the LOQs were118.5-804.78 µg/L.

Table 3 Linearity, LOD, and LOQ parameters for benzidine under the optimal conditions using high performance LC-UV.

	Range (µg/L)	r	Slope	Intercept	LOD	LOQ
BZ	50-800	0.999	81.8	-263.1	32.99	109.98
DMB	50-800	0.997	67.0	-64.8	33.67	112.26
DCB	50-800	0.999	86.0	-1185.6	26.36	87.89

Table 4 Linearity, LOD, and LOQ results for PPP separated under the optimum HPLC separation conditions.

	range (µg/L)	r	Slope	intercept	LOD	LOQ
4-NP	250-5000	0.9994	50.16	13953.3	57.16	190.55
PCP	250-5000	0.9976	11.09	1454.4	171.76	572.56
2,4,6-TCP	250-5000	0.9966	14.34	3913.0	35.56	118.5
Ph	250-5000	0.9995	32.06	2180	145.21	484.04
2-NP	250-5000	0.9993	90.3	3365.1	155.69	518.99
2-CP	250-5000	0.9961	40.1	365.2	196.82	656.08
2,4-DCP	250-5000	0.9979	42.43	1496.8	226.22	754.09
2,4-DMP	250-5000	0.9971	25.98	-109.78	102.31	341.03
4-C-3-MP	250-5000	0.9995	20.096	1251.3	127.70	425.69

3.5 Analytical Method Validation (Precision, accuracy, ruggedness and robustness)

The **precision** (in terms of the relative standard deviation) was calculated via the repeatability. The **accuracy** (in terms of the relative error percent) was calculated via the recovery percentages. Triplicate analysis was performed for any single benzidines or phenols at three concentrations chosen from the high, low and middle levels within the Beer's law range on the standard curve. The precision and accuracy resultsfor benzidines and phenolsare shown in tables (5) and(6)

The **ruggedness of benzidines** (Intraday) was determined by triplicate injections at a single concentration(i.e.,800 µg/L) on 3 days using the average of 3x3 injections. The results for the ruggedness were 2.93, 3.65, and 3.36 for BZ, DMB and DCB, respectively. The **robustness** was calculated by triplicate injections of a single 800 µg/L standard solution after incubation in an oven at 50 °C for 30 min. The mean, standard deviation, and RSDs were calculated.

The ruggedness of phenols (Intraday) was determined by triplicate injections of a single concentration(5000) µg/L within 3 daysusing average of 3x3 injections. The**ruggedness**was 1.95, 3.36, 1.61 for BZ, DCB and DMB,respectively The**robustness** was calculated by triplicate injections of a single 5000 µg/L standard solution after incubation in an oven at 50 °C for 30 min,.

Table 5 Analytical method validations (Precision, accuracy, ruggedness and robustness)

Benzidine	RSD%	Er%	Ruggedness	Robustness
BZ	1.81	2.64	2.93	3.26
DMB	1.04	1.30	3.65	2.99
DCB	1.78	1.02	3.36	4.35

Table 6 Analytical method validations (Precision, accuracy, ruggedness and robustness)

Phenol	RSD%	Er%	Ruggedness	Robustness
4-NP	5.31	-3.53	3.37	3.31
PCP	3.36	5.52	2.82	3.02
2,4,6-TCP	3.42	4.53	3.19	4.69
Ph	1.47	-2.14	3.04	3.08
2-NP	1.76	1.07	2.82	1.55
2-CP	2.15	1.49	3.67	2.80
2,4-DCP	3.25	3.47	3.41	4.89
2,4-DMP	2.91	4.80	3.16	2.63
4-C-3-MP	3.02	2.70	4.00	2.42

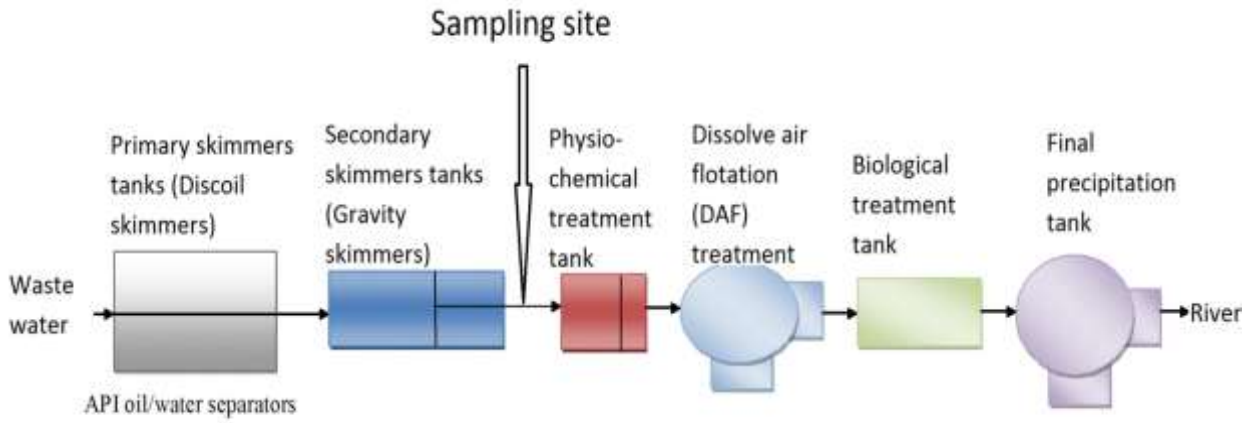
3.6 Petroleum Refinery, Industrial Waste Water Treatment Stages

The Dora petroleum refinery station is located south of Baghdad in Iraq. The end stream waste water from this station is discharged into the Tigris River at the end of the treatment process. This waste water is one source of aromatic compounds that can enter the river and affect the health of living organisms in the river. Therefore the amount of priority pollutant compound in the waste water treatment unit should be evaluated. Benzidines and phenols are priority pollutant compounds that can be found in petroleum waste water because aromatic compounds are the main part of petroleum oil. The station contains a waste water treatment subunit that includes many treatment subunits, including skimmer subunits, a physiochemical subunit, DAF subunit, and biological subunit.

The waste water streams are collected in the main tanks to gather all of the waste water coming from the various stages of the refinery stations (heavy and light oil). The stream goes down to the first treatment subunit (skimmers and discoil skimmers) in this stage, and the subunit separates the oil phase from the water phase.

In the second stage, the stream reaches to the physiochemical subunit tank. Many chemicals,such as poly electrolyte and alum are added to this tank, to aid in aggregation of the semi-soluble materials to separate these materials using special skimmers in the next stage (i.e., dissolved air floatation (DAF tank)). In the third stage, the stream goes down to the next subunit (i.e., the biological treatment tank). In this treatment, bacteria are used to biodegrade the organic and inorganic molecules. Phosphoric acid and urea are added to this tank, and a flotation ventilator aids in the re-oxygenation of the bacteria. The last tank, (i.e., the final precipitation tank) collects the final waste water priorto entering the river.

Phenols and benzidines were determined and followed monthly for one year at a specific site. This site was located after the skimmer subunit. The sampling was conducted using a special pump between the skimmer and physiochemical subunits. The samples were pre-filtered, extracted and eluted by solid-phase extraction under extraction conditions mention above. The eluted samples were purified, separated,detected and measured by HPLC –UV under optimum conditions. The eluted samples were injected in triplicate, and the mean and standard deviation,relative standard deviation were calculated. The samples were diluted to 1:2 and 1:5 to decrease the viscosity and the concentration of the samples were evaluatedwithin the range of detection.



Scheme 1 Stages of waste-water treatment in the petroleum refinery station with sampling sites A, B, and C.

3.6.1 Determination and Monthly Variation Study of PPB

Most benzidines were observed in most of the samples.The results are shown in Fig. 12. In general, the amounts of thebenzidines selected in this study were lower in the spring and autumn than during the other seasons. The highest results were 155.44 µg/L, 264.99 µg/L and 131.12 in Feb, for BZ, DMB and DCB, respectively. In March, March, and Jan. BZ, DMB and DCB,respectively,wereNot detected

3.6.2 Determination andMonthly Variation Study of PPPs

Most PPPs were observed in most of the samples.The results are shown in Fig. 13.The amounts of the PPPs selected in this study were typically lower in the summer than during the other seasons. The highest results were 1034.37 µg/L in Oct., 1018.61 in Oct. and 1136.33 in Jun. for 2,4,6-TCP,2,4-DCP and Ph, respectively. During several months, these compounds were not detected.

The variation in the levels of these compoundsmay be caused by the operation conditions, volatilization, dilution, and evaporationbecause the tanks are exposed to air and sunlight.

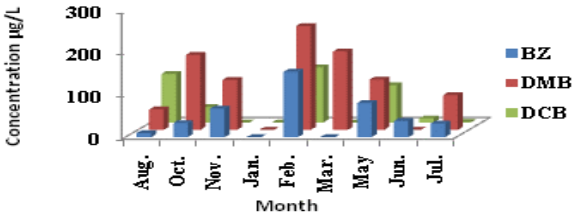


Fig. 12 Monthly variations (µg/L) of BZ, DMB and DCB in petroleum refinery wastewater

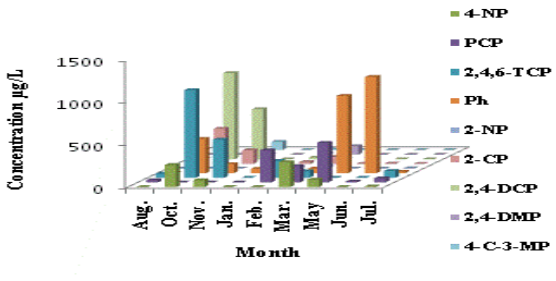


Fig. 13 Monthly variations (µg/L) of PPPs in petroleum refinery wastewater.

4. Conclusion

1. Organic solvent percentages, flow rate and temperature are important factors for the separation of PPP and PPB. However, pH was the most important factors in phenols separation.
2. The proposed method was exhaustively validated in terms of the linearity, accuracy, specificity and precision in determination of the environmental samples.

3. This approach provides a useful tool to determine the amounts of these compounds that are discharged from wastewater treatment plants (WWTPs) to the aquatic environment and assess the ability of WWTPs to eliminatethese compounds.
4. Most of the benzidines were found in most of the samples that were obtained from inside the station with significant amounts that rangedfromundetectableto264.99 µg/L.
5. Most of the phenols were found in most of the samples that were obtained inside the station with significant amounts that rangedfromundetectableto 1136.33 µg/L.

References

1. W.N. Saeed, Removal of Azo Benzidine Reactive Dye from Aqueous Solution by Adsorption onto ZnO Surface, J of Kerb Univ, 2014,1 (12),11-18.
2. S.A. Febriana;F. Jungbauer;H. Soebono;P.-J. Coenraads, Occupational contact allergy caused by benzidine in three tannery workers, Contact Derm., 2012,66 (6),345-346.
3. S.C. Brown;R. Alberts;M. Schoenberg, Cancer incidence and mortality among workers exposed to benzidine, Am J Ind Med, 2011,54 (4),300-306.
4. D. Zaharia;C. Bilba;D. Muresan;A. Muresan;R. Popescu, Decolonization wastewaters from the textile industry – physical methods, chemical methods, IND TEXTILA, 2009,60 (5),254-263.
5. M. Carabajal;M. Perullini;M. Jobbágy;R. Ullrich;M. Hofrichter;L. Levin, Removal of Phenol by Immobilization of Trametes versicolor in Silica–Alginate–Fungus Biocomposites and Loofa Sponge, Clean (Weinh), 2016,44 (2),180-188.
6. B. Kumar, Tyagi, J., Verma, V. K., Sharma, C. S. and Akolkar A. B. , Distribution of eleven priority phenolic compounds in soils from mixed landuse and assessment of health hazard for human population, Adv Appl Sci Res, 2014,5 (2),125-132.
7. H.-Z. Xing;X. Wang;X.-F. Chen;M.-L. Wang;R.-S. Zhao, Accelerated solvent extraction combined with dispersive liquid–liquid microextraction before gas chromatography with mass spectrometry for the sensitive determination of phenols in soil samples, J Sep Sci, 2015,38 (8),1419-1425.
8. Hing-BL;T.E. Peart;T. Ken;R.J. Maguire, Determination of 3,3'-Dichlorobenzidine in Industrial Wastewaters, WATER QUAL RES J CAN, 2004,39 (1),29–34.
9. A.H. Hamed, Simple Method for Spectrophotometric Determination of Benzidine in Aqueous Solutions by Coupling with B-Naphthol, J of Al-Nahrain University, 2011,14 (2),43-50.
10. L. Chuannan, Xueying, W. , Zhongpeng, X., Zhen, L.v. , Determination of Benzidine using Sulfobutyl-ßcyclodextrinand Neutral Red Complexation as a Supermolecule Probe 3rd International Conference on Bioinformatics and Biomedical Engineering, 2009,, pp.1-3.
11. Z. Chen, Zhang, G., Chen, X., Peng, Y., Lin, Y. and Lu, S. , a resonance light scattering amplification system for determination of trace amounts of benzidine in surface water, Anal. Methods, 2011,3 (8),1845-1850.
12. L. Falciola;V. Pifferi;E. Mascheroni, Platinum-Based and Carbon-Based Screen Printed Electrodes for the Determination of Benzidine by Differential Pulse Voltammetry, Electroanalysis., 2012,24 (4),767-775.
13. W. Chao, ning, L.y. , jiang, T.E., xiarphan, J. and Renji, X.U., Determination of Trace Aniline and Benzidine in environmental water by ultra high pressure liquid chromatoghraphy with flourscence Detection. , Chinase Journal of international analysis 2013, 32 (1),32-37.
14. M. Bouzige;P. Legeay;V. Pichon;M.C. Hennion, Selective on-line immunoextraction coupled to liquid chromatography for the trace determination of benzidine, congeners and related azo dyes in surface water and industrial effluents, J Chromatogr A, 1999,846 (1-2),317-329.
15. X. Jie;Q. Shu;X.Y. Hong;X.Z. Wei;L.H. Xia, Simultaneous Determination of Acrylamide,Aniline and Benzidine in Water Sample by High Performance Liquid Chromatography-Tandem Mass Spectrometry, CHINESE J ANAL CHEM, 2014,42 (1),93-98.
16. L.E. Vera-Avila;A. Garcia-Ac;R. Covarrubias-Herrera, Trace-level determination of benzidine and 3,3'-dichlorobenzidine in aqueous environmental samples by online solid-phase extraction and liquid chromatography with electrochemical detection, J Chromatogr Sci, 2001,39 (7),301-307.
17. M. Ziyaadini;M.M. Zahedi;S. Khosravinia, Ultrasound-assisted extraction combined with reverse phase-dispersive liquid-liquid micro extraction as a new approach for preconcentration and spectrophotometric determination of total phenol in marine sediments of Chabahar Bay, Marine Poll Bull, 2016,109 (1),104-109.

18. M. Nassiri;M.M. Zahedi;S.M. Pourmortazavi;M. Yousefzade, Optimization of dispersive liquid–liquid microextraction for preconcentration and spectrophotometric determination of phenols in Chabahar Bay seawater after derivatization with 4-aminoantipyrine, Marine Poll Bull, 2014,86 (1–2),512-517.
19. P.F. Draghi;J.C.B. Fernandes, Label-free potentiometric biosensor based on solid-contact for determination of total phenols in honey and propolis, Talanta, 2017,164 413-417.
20. M. Saraji;M. Marzban, Determination of 11 priority pollutant phenols in wastewater using dispersive liquid-liquid microextraction followed by high-performance liquid chromatography-diode-array detection, Anal Bioanal Chem, 2010,396 (7),2685-2693.
21. L.-Q. Peng;Q. Li;Y.-x. Chang;M. An;R. Yang;Z. Tan;J. Hao;J. Cao;J.-J. Xu;S.-S. Hu, Determination of natural phenols in olive fruits by chitosan assisted matrix solid-phase dispersion microextraction and ultrahigh performance liquid chromatography with quadrupole time-of-flight tandem mass spectrometry, Journal of Chromatography A, 2016,1456 68-76.
22. C. Zhong;M. He;H. Liao;B. Chen;C. Wang;B. Hu, Polydimethylsiloxane/covalent triazine frameworks coated stir bar sorptive extraction coupled with high performance liquid chromatography-ultraviolet detection for the determination of phenols in environmental water samples, Journal of Chromatography A, 2016,1441 8-15.
23. H.H. Jasim;S.B. Dikran;A.S. Mahdi;B.M. Altahir, Determination of insecticides in different commercial formulations by Gradient HPLC, Br. J. Anal. Chem., 2017,4 (14),25-30.
24. D. Vazquez;G.A. Mansoori, Identification and measurement of petroleum precipitates, J Pet Sci Eng, 2000,26 (1–4),49-55.
25. G. Gomori, Preparation of Buffers for Use in Enzyme Studies, Handbook of Biochemistry and Molecular Biology, Fourth Edition, CRC Press2010, pp. 721-724.
26. T. Hanai, HPLC: A Practical Guide, The Royal Society of Chemistry Chromatography Monographs, 1999,7-9.
27. M.J. Citra, Estimating the pKa of phenols, carboxylic acids and alcohols from semi-empirical quantum chemical methods, Chemosphere, 1999,38 (1),191-206.
28. D.A. Skoog;D.M. West;F.J. Holler;S.R. Crouch, Fundamentals of analytical chemistry, Brooks /Cole cengage learninig, USA, 8th Edition, 2004,986-994
29. A. Bose, HPLC Calibration Process Parameters in Terms of System Suitability Austin Chrom., 2014,1 (2),1-4

*****