Comparative Studies on Spectrophotometric Determination of Selected Heavy Metals in Environmental and Biological Samples with Synthetic Phenylhydrazones as Chromogenic Reagents

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ABSTRACT

This work involves a comparative study on a simple, sensitive, selective and non-extractive Uv-Vis spectrophotometric method for the determination of cadmium, lead, chromium and arsenic in biological, soil and water samples using synthesized and characterized phenylhydrazones; [glutaraldehyde phenylhydrazone (GPH) and acetylpyridine-2,4-dinitrophenylhydrazone (APDH)] as the chromogenic reagents was developed. GPH and APDH were synthesized as new chromogenic analytical reagents for the direct UV-Vis spectrophotometric determination of the selected metals of interest in a slightly acidic pH of 6.5-7.5 and 20% dimethylformamide (DMF) solution to give stable coloured metal-ligand complexes. The reactions were instantaneous; the wavelengths of maximum absorptions were followed spctrophometrically and noted. The analytical properties of the reagent APGH on the metals (Cd, As, Pb, and Cr) revealed the wavelength of maximum absorption ranged between 385.0 (Cr) to 440.0nm for (Pb). The reagent APDH had molar absorptivities (L mol^{-1} cm^{-1}) ranging from 2.006 x 10^4 (Pb) to 2.407 x 10^4 (Cd), a mole ratio of metal to ligand of 1:1, a detection limit (µg/g) ranging from 0.3272 (As) to 0.4456 (Cd) and the metal-ligand complex was stable for 0-36 hours. The reagent GPH revealed a wavelength of maximum absorption between 360.0 (Cr) to 395.0 nm for (Pb and As) at a working pH of 6.5 to 7.5 room temperature (37°C). The reagent GPH had a molar absorptivities (L mol^{-1} cm^{-1}) ranging from 2.213 x 10^4 (Pb) to 2.460 x 10^4 (As), a mole ratio of metal to ligand of 2:1, the detection limit (µg/g) ranging from 0.3432 (As) to 0.5250 (Pb) and the metal-ligand complex was stable for 0-48 hours. Both reagents had a Beer's law validity range (mgL^{-1}) of 0.001 to 100. The Sandell's sensitivities (µg/cm^2) ranged from 0000409 (As) to 0.00499 (Pb) for APDH and 0.00406 (As) to 0.00452 (Pb) respectively. Large excess of cations and anions as possible interferences up to 10 folds were studied and do not interfere with the determination of the selected metals of interest. The developed methods were highly selective for Cd, Pb, and Cr and As and was successfully used for the determination for the said elements in soil, water and biological samples. The results of the developed methods were comparable with AAS and were found to be in good agreement. The methods had very high precision and very good accuracy.

Key words: Simple, sensitive, selective, non-extractive, Uv-Vis spectrophotometric, phenyl-hydrazone, heavy metals.

INTRODUCTION: The detection and quantification of metals and metal ions, especially transition metals both in solution and solid samples are of increased concern, as a result of increased environmental pollution worldwide. Metals play important roles in biological activities of all living organisms in the proper balance. Transition metals deficiency causes many clinical disorders and physiological abnormalities in the human body. Some metal ions may cause serious health problem and toxicity to humans, animals as well as plants. Researchers were able to resolve many industrial and biological problems with the aid of complexation of metal, metal ions and chromogenic reagents (Ahmed and Chowdhury, 2014).

Spectrophotometric analysis continues to be one of the most widely used analytical techniques available. Many methods are available for a variety of analytes (such as coloured, colourless, natural, synthetic, inorganic and organic analytes) and sample types ranging from in-situ biological assays to the determination of trace elements. (Ahmed and Chowdhury, 2014).

The basis of spectrophotometric methods is the simple relationship between the colour of a substance and its electronic structure. A molecule or an ion exhibits absorption in the visible or ultra-violet region when the radiation causes an electronic transition in molecules containing one or more chromophoric groups. The colour of a molecule may be intensified by substituents called auxochromic groups, which displace the absorption maxima towards longer wavelength (bathochromic shift). The colour determining factors in many molecules is the introduction of conjugated double bonds by means of electron donor or electron acceptor groups.

Hydrazones could be useful analytical chromogenic reagent for selective, sensitive, specific and reproducible method for the determination of heavy toxic metals in biological, environmental, water and waste water samples using UV-Vis spectrophotometer owing to the high installation and maintenance cost, and deep technical knowledge of AAS to laboratories with limited budgets (Cristofol *et al.,* 2001).

The work is aimed at developing a simple, selective, sensitive and non-extrative Uv-Vis spectrophotometric method with synthesized and characterized phenyl-hydrazones (glutaraldehyde phenyl hydrazone and acetylpyridine-2,4-dinitrophenyl hydrazone) as chromogenic reagents for determining the concentrations of selected heavy metals in soil, water and biological samples.

Materials Used: All the regents and materials used in the synthesis and characterization of the phenyl hydrazones were of analytical grade, sourced from accredited vendors and were used without further purification, unless stated otherwise.

Equipment Used for the Study: All absorbance measurements were made on a UV-Vis spectrophotometer matched with a 1cm quartz cell, (Model-1800) double beam Jenway (England, UK), A pH meter (Hanna, U.S.A) with combination of electrodes, Melting point apparatus (Gellenkamp, UK), An Agilent Technologies FT-IR spectrophotometer range 4000-650 cm^{-1}, An Agilent-NMR-vnmrs 400.

Synthesis and Characterization of the Chromogenic Reagents: The chromogenic reagents acetylpyridine-2,4-dinitrophenyl hydrazone and glutaraldehyde phenyl hydrazone were

synthesized by one step condensation reaction, filtered and recrystallized twice with ethanol as described by Gopala *et al*. (2010) and Cristofol *et al.* (2001).

Synthesis of Acetylpyridine-2,4-dinitrophenyl Hydrazone (Gopala et al., 2010): The reaction mixture containing one equivalent acetylpyridine-2,4-dinitrophenyl hydrazine (1.00 mol/dm^3, 0.198 g) and one equivalent acetylpyridine (1.00 mol/dm^3, 0.121 cm^3 in 15.00 cm^3 methanol) were dissolved in hot condition taken into round bottom flask and reflux for 8.0 hours. On cooling the resulting mixture gave an orange colour product. It was collected by filtration. The compound was recrystallized twice with ethanol.
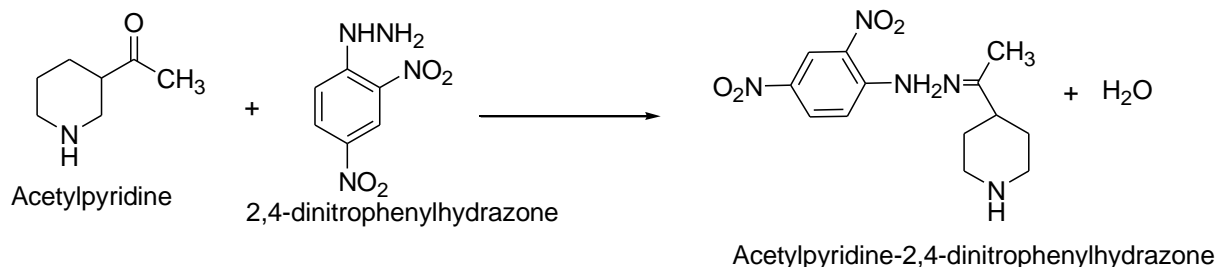
Scheme 3: Synthesis acetyl-pyridine-2, 4-dinitrophenyl hydrazone

Synthesis and Characterization of the Glutaraldehyde phenyl Hydrazone: The reaction mixture containing glutaraldehyde (0.050 mol/dm^3, 4.70 cm^3 in 20.00 cm^3 ethanol) and phenyl hydrazine (0.1 mol/dm^3, 9.85 g in methanol) dissolved in hot condition was taken into round bottom flask and reflux for 8.00 hours. On cooling the reaction mixture gave a dark brown coloured product. It was collected by filtration. The compound was recrystallized twice with ethanol. The melting point was determined (^{0}C). The resultant compounds were characterized by FT-IR and NMR spectral studies. The preliminary purity of the products obtained was confirmed by elemental analysis (Cristofol *et al.,* 2001).
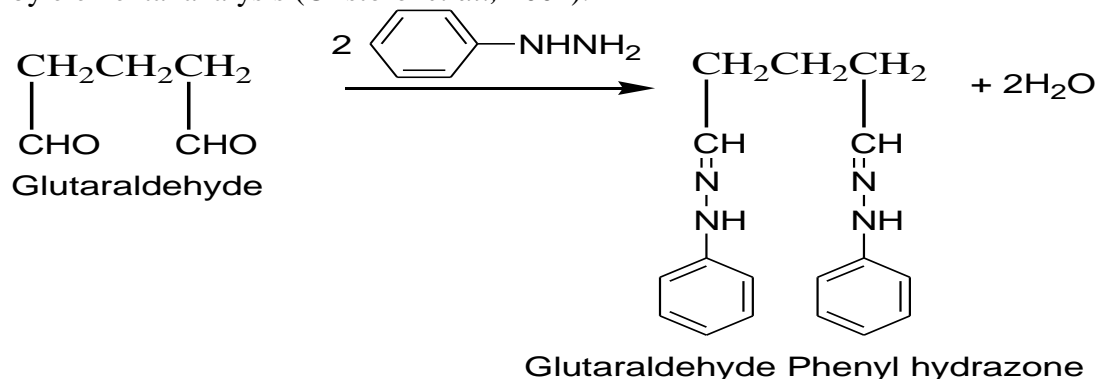
Scheme 1: Synthesis of glutaraldehyde phenyl hydrazone

Nitrogen Content of the Synthesized phenylhydrazone: Preliminary investigation of the chemical and elemental constituents present in the synthesized products were carried out. The Nitrogen content of the product was carried out by Khjeldahl methods. 0.20 g of the sample was placed in Khjeldahl flask and digested with 4.00 cm^3 concentrated sulphuric acid. A tablet of khjeldahl was added to the mixture as described by Jamaluddin and Tasnima, 2012.

Determination of Working pH Range of the Reagent Complexes: In a series of 10.00 cm^3 volumetric flaks, 2.00 cm^3 solution (1.00 x 10^{-4} mol/dm^3), of the salt of metal of interest,

3.00 cm^3 of the hydrazone solution (1.00 x 10^{-4} mol/dm^3) and 4.00 cm^3 of the buffer of varying pH were added, made up to the mark with deionized water and the absorbance were measured against the ligand blank at a determined wavelength. A plot was then made between absorbance and pH in the UV-Vis range. The pH with the highest absorbance was determined as the working pH for each metal of interest (Nityananda *et al.*, 2015).

Solubility of the Synthesized Ligands: The solubility of the synthesized glutaraldehyde phenyl hydrazone and acetylpyridine-2,4-dinitrophenyl hydrazone was tested in different organic and aqueous solvents. The organic solvent tested and yielded were N, N-dimethylformamide (DMF), demithylsufoxide (DMSO) and 1, 4-dioxane (Nityananda *et al.*, 2015).

Analytical Properties of the Synthesized Hydrazones for Uv-Vis Spectrophotometric Analysis: The following analytical parameters were analysed on the synthesized hydrazones to ascertain their analytical properties

Determination of the Working Wavelength: For the selection of the working wavelength, a number of solutions were made by mixing equal volume of the reagent concentration (1.00 x 10^{-4} mol/dm^3) and (1.00 x 10^{-4} mol/dm^3), of the salt of metal of interest in a 25.00 cm^3 volumetric flask and made to the mark with deionized water. The absorbances were scanned between 190-800.0 nm in a 1.0 cm^3 cuvette to determine the maximum wavelength of absorption ($λ_{max}$) using the UV-Visible spectrophotometer. The wavelength of maximum absorbance for each complex was noted (Omabaka and Gichobi, 2011).

Stability of the Chromogenic Reagent: To establish the maximum storage time of the chromogenic reagent solution without loss of reactivity, a study was performed to investigate the stability over time. A 1.00 cm^3 of 5.00 x 10^{-4} mol/dm^3 of the various metals of interest was taken into 10.00 cm^3 volumetric flask. Then 5.00 cm^3 of 5.00 x 10^{-4} mol/dm^3 of the hydrazones complex was added. The pH of these solutions was adjusted pH of 6.5-7.5 using acetate buffers. The solution was made to the mark with deionized water. The flask was stored at room temperature. The stability of these reagents was followed spectrophotometrically after 0, 3, 6, 9, 12, 24, 48, 72, 96 and 120.0 hours (Omabaka and Gichobi, 2011).

Determination of Working Wavelength of the Metal-Ligand Complexes: A 1.00 cm^3 of 5.00 x 10^{-4} mol/dm^3 of the various metals of interest was taken into 25.00 cm^3 volumetric flask. Then 5.00 cm^3 of 5.00 x10^{-4} mol/dm^3 of the hydrazones complex was added. The pH of these solutions was adjusted pH of 6.5-7.5 using acetate buffers. The solution was made to the mark with deionized water. The absorbances were then determined in the entire UV-visible region against specific reagent blank as reported by Ombaka and Gichumbi, 2011.

Applicability of Beer's Law: The known aliquots of 10.00 cm^3 solutions, each containing constant volume of 4.00 cm^3 of buffer (desired pH), 3.00 cm^3 of 1.0 x 10^{-4} mol/dm^3 of reagent hydrazone and 2.00 cm^3 of corresponding salt of metal of interest ranging from 0.10 x 10^{-4} to 1.00 x 10^{-4} mol/dm^3. The absorbances of these are measured at optimal wavelength for each metal of interest. A graph was plotted between the concentration of the metal and its absorbances. A straight-line graph passing through the origin obeys Beer's Law. The correlation coefficient which indicates the linearity between the two variables, the molar absorptivity coefficient and Sandell's sensitivity of the ligand-metal complexes was obtained from the plot (Ombaka and Gichumbi, 2011).

Composition and Stability Constant of the Complexes: The molar ratio method was performed to determine the composition of the complexes;

Molar Ratio Method: To a 25.00 cm^3 volumetric flask, 5.00 cm^3 of desired buffer was transferred into known volume of metal ion and varying volumes of the reagent solutions were added. The contents of the flask were made up to the mark with distilled water. The absorbances of each solution were measured at the selected wavelength against a blank containing 5.00 cm^3 buffer solution and required volume of reagent. From the plot between the absorbance and the volume of the reagent, the composition of the complex computed (Humaira *et al*., 2009).

Samples Collection and Treatment: The samples (waste, water water and soil) in triplicates were collected from Challawa Industrial Area of Kumbotso Local Government Area of Kano State Nigeria West Africa into polythene bags and transported to the laboratory for analysis. The waste water samples collected (500.00 cm^3) were filtered using a filter paper (Whatman No.41) and then each filtered water sample was evaporated nearly to dryness with a mixture of 5.00 cm^3 of concentrated H_2SO_4 and 10.00 cm^3 concentrated HNO_3 in a fume cupboard and then cooled to room temperature. In order to dissolve the salts, the residue was then heated with 15.00 cm^3 of distilled water. After cooling the solution was neutralized with dilute NH_4OH solution and the obtained solution was filtered into 50.00 cm^3 standard flask and made up to the mark with deionized water (Adi *et al*., 2012). Before digestion of the soil samples, each was dried at 65 °C for 48 hours. All samples were performed in triplicates. Five grams (5.00 g) of the samples in crucibles was placed in a preheated muffle furnace at 200-250 °C for 30 minutes and ashed for eight hours at 500-550 °C. Then, the sample was removed from the furnace and cooled down. 2.00 cm^3 of 5.00 mol/dm^3 of HNO_3 was added and evapourated to dryness on a sand bath. The samples were then placed in a furnace and heated to 550 °C for 15 minutes. The samples were removed from the furnace, cooled and moistened with four drops of distilled water. Then 2.00 cm^3 of concentrated HCl was added into the sample was evaporated to dryness, removed, and 5.00 cm^3 2.00 mol/dm^3 HCl was again added and the crucible. The solution was filtered through Whatman No. 41 filter paper and the transferred quantitatively to a 50.00 cm^3 flask by making it up with deionized water (Zeng-Yei, 2004). The biological samples (liver, blood and flesh from animals) and plants samples were collected from Challawa industrial of Kano State Nigeria West Africa. The blood samples of 5.00 cm^3, the liver, flesh and plants samples were oven dried and 5.00 g were transferred into crucibles. The samples were then ashed in the muffle furnace at 500-550 ºC for 8.0 hours in the presence of 10.00 cm^3 nitric acid. The contents of each crucible were cooled to room temperature, and 1.50 cm^3 of concentrated hydrochloric acid was added and warmed slightly. The content of each crucible was filtered. The solutions were then transferred quantitatively into 50.0 cm^3 calibrated flask and made up to the mark with deionized water. (Jamaluddin and Tasnima, 2012).

Effect of Interference or Foreign Ions: The effects of foreign ions (cations; Co, Cu, Al, Fe,Hg, Zn, Ca and anions like SO_4^{2-}, NO_3^-) on the determination of the heavy metals were studied using the methods of Jamaluddin *et at.* (2014) and, Ombaka and Gichumbi (2011) by measuring the absorbance of the metal-ligand complex containing 1 μg mL^{-1} of the metal of interest.

Evaluation of the Proposed Method: The validity of the proposed method was assessed by the method described by Azhar (2015) and Jamaluddin *et al.* (2015) in which known quantitative amount of the various metals under study were added to the samples and then determined spectrophotometrically using the proposed method and the general procedure. The results obtained were tabulated.

The data collected were statistically analyzed using the Math Lab 2014 version with a significance level of $P<0.05$. All collected data carried out were subjected to one-way analysis of variance (1 way-ANOVA).

Results and discussions

Table 1: Properties of Synthesized Hydrazone

Physical Properties of the synthesized hydrazone	Glutaraldehydephenyl hydrazone	Acetylpyridine-2,4-dinitrophenylhydrazone
Yield (%)	84.00	79.00
Melting point (ºC)	129.00 – 131.00	211.00 - 214.00
Colour	Dark brown	0range
Nitrogen content (%)	19.01	18.62

Preminary investigations of the synthesized hydrazones as shown in Table 1 revealed that the percentage yield of glutaraldehyde phenyl hydrazone was 84.00 % and 79.00 % for acetylpyridine-2,4-dinitrophenyl hydrazone. The nitrogen content of the resultant product was carried out by Khjeldahl methods as described by Jamaluddin and Zanat (2012). The elemental nitrogen for glutaraldehyde phenyl hydrazone was found to 19.01 % (calculated value is 19.15 %), the nitrogen content for acetylpyridine-2,4-dinitrophenyl hydrazone was 18.62 % (Calculated value is 18.84 %). The values obtained show a good agreement between experimental and calculated values as reported by Jamaluddin and Zanat (2012).

The infra-red spectra of both synthesized hydrazones showed the presence of string bands and shoulders assigned to C=N, NH_2, and phenyl C=C. The NMR spectra showed peaks associated to cyano carbon atoms, aromatic carbon and NH_2. The FT-IR spectrum of glutaraldehyde phenyl hydrazone showed peaks at 3492.5 and 3451.0 cm^{-1} associated to the amino (-NHR) groups. The spectrum also revealed a peak at 3085.0 cm^{-1} assignable to the =CH groups and 3034.0 cm^{-1} phenyl N-H groups and a peak at 1604.0 cm^{-1} assignable to the cyano group respectively. The ^{13}C-NMR and ^{1}H-NMR spectra of glutaraldehyde phenyl hydrazone in DMSO. The ^{13}C-NMR spectrum showed signals between 111.0-141.0 ppm assignable to aromatic carbon atom, while signals at 129.3 ppm is assignable to cyano carbon atom. The three methylene carbon atoms signals appeared at 40.0, 39.0 and 31.7 ppm respectively. The ^{1}HNMR spectrum revealed signals between 1.64-1.69 (m) ppm due to multiplet –CH_2 protons.2.214-2.389 (q) ppm associated to the quartet –CH_2 protons atoms. 7.331-7.345 ppm due to =C-H protons. Peak at 7.015 ppm was due to –NH atoms. The peak at 6.59-7.34 ppm is assignable aromatic protons.

The FT-IR spectrum of the acetylpyridine-2,4-dinitrophenyl hydrazone showed bands at 1599.0 cm^{-1} assignable to the cyano group and the peak at 3451.0 cm^{-1} is associated to the amino group. The band at 1602.0 cm^{-1} due to the C=N cyano carbon group. The band at 1599 cm^{-1} was due to the phenyl C=C strectch and 1334, 1468 assigned to the –NO_2 groups. The ^{13}C-NMR and ^{1}H-NMR in DMSO of the acetylpyridine-2,4-dinitrophenyl hydrazone spectra revealed signals at 139.4 ppm associated to the cyano carbon atom, 105.6-130.8 ppm assignable to the aromatic carbon rings. The 13.5 ppm is due to the methyl (CH_3) carbon. The ^{1}H-NMR spectrum showed peaks at 1.105 (s) ppm due to –CH_3 protons peaks at 2.037 (s) ppm due to –NH proton on the pyridine ring. Peak at 2.470 (q) and 3.561(t) ppm due to –CH_2- protons on the pyridine. Peak at 7.643 (s) ppm was due to NH proton, 7.887 ppm was due to aromatic –CH stretch on C_6 of

phenyl group, 8.408 ppm due to aromatic C-H stretch on C_5 of the phenyl group and 8.984 ppm due to C_3 of the phenyl group.

Table 2: Determination of Lead in Soil, Water and Biological Samples with the Developed Methods (APDH and GPH) and AAS as reference method

Serial No	Sample Code	Concentration of Pb (µg g^{-1}) ± SD			p value	Relative Error (%) AAS method / APDH method	Relative Error (%) AAS method / GPH method
		AAS	APDH	GPH			
1.	F	0.007 ± 0.001	0.0069 ± 0.001	0.007 ± 0.001	0.2297	-1.42	0.00
2.	L	0.001 ± 0.001	0.001 ± 0.000	0.001 ± 0.000	0.0000	0.00	0.00
3.	P1	1.018 ± 0.001	1.019 ± 0.002	1.019 ± 0.002	0.5878	+0.09	+0.09
4.	P2	1.014 ± 0.001	1.015 ± 0.001	1.015 ± 0.000	0.2297	+0.09	+0.09
5.	B	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.0000	0.00	0.00
6.	IW	0.810 ± 0.001	0.811 ± 0.001	0.081 ± 0.001	0.2991	+0.12	+0.12
7.	SW	1.924 ± 0.002	1.926 ± 0.002	1.924 ± 0.002	0.2297	+0.10	0.00
8.	WS	1.173 ± 0.003	1.172 ± 0.003	1.174 ± 0.002	0.5242	-0.08	+0.08
9.	WW	0.877 ± 0.002	0.878 ± 0.002	0.877 ± 0.003	0.75041	+0.11	0.00

Table 3: Determination of Cadmium in Soil, Water and Biological Samples with the Developed Methods(APDH and GPH) and AAS as the Reference Methods

Serial No	Sample Code	Concentration of Cd ($\mu g\ g^{-1}$) $\pm$ SD			p value	Relative Error (%) AAS method / APDH	Relative Error %) AAS method / GPH method
		AAS	APDH	GPH			
	method						
1.	F	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.00	0.00	0.00
2.	L	0.020 ± 0.001	0.0199 ± 0.001	0.020 ± 0.001	0.2297	-0.5	0.00
3.	P1	0.014 ± 0.003	0.014 ± 0.002	0.014 ± 0.002	0.75041	0.00	0.00
4.	P2	0.021 ± 0.003	0.021 ± 0.002	0.021 ± 0.001	1.0000	0.00	0.00
5.	B	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.00	0.00	0.00
6.	IW	0.031 ± 0.004	0.032 ± 0.001	0.031 ± 0.001	0.509224	+3.22	0.00
7.	SW	0.055 ± 0.004	0.054 ± 0.002	0.055 ± 0.002	0.814812	-1.81	0.00
8.	WS	0.008 ± 0.004	0.009 ± 0.001	0.008 ± 0.002	0.791764	+1.50	0.00
9.	WW	0.009 ± 0.002	0.008 ± 0.002	0.008 ± 0.002	0.668422	-1.11	-1.11

Table 4: Determination of Chromium in Soil, Water and Biological Samples with the Developed Methods (APDH and GPH) and AAS as the Reference Methods

Serial No	Sample Code	Concentration of Cr ($\mu g\ g^{-1}$) ± SD			p value	Relative Error (%) AAS method / APDH	Relative Error (%) AAS method / GPH method
		AAS method	APDH	GPH			
1.	F	0.033 ± 0.001	0.034 ± 0.002	0.033 ± 0.001	0.45826	+3.03	0.00
2.	L	0.010 ± 0.002	0.009 ± 0.003	0.010 ± 0.002	0.43902	-1.00	0.00
3.	P1	3.474 ± 0.015	3.476 ± 0.003	3.475 ± 0.002	0.9392	+0.05	+0.02
4.	P2	2.042 ± 0.005	2.042± 0.002	2.041± 0.003	0.8779	0.00	-0.04
5.	B	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.0000	0.00	0.00
6.	IW	1.988 ± 0.001	1.989 ± 0.002	1.986 ± 0.003	0.1237	+0.05	-0.10
7.	SW	2.248 ± 0.003	2.250 ± 0.003	2.247 ± 0.002	0.2438	+0.08	-0.04
8.	WS	2.616 ± 0.004	2.618 ± 0.002	2.615 ± 0.002	0.2710	+0.07	-0.03
9.	WW	1.038 ± 0.001	1.040 ± 0.003	1.039 ± 0.001	0.2926	+0.19	+0.09

Table 5: Determination of Arsenic in Soil, Water and Biological Samples with the Developed Methods (APDH and GPH) and AAS as the Reference Method

Serial No	Sample Code	Concentration of As ($\mu g\ g^{-1}$) $\pm$ SD			p value	Relative Error (%) AAS method / APDH method	Relative Error (%) AAS method / GPH method
		AAS	APDH	GPH			
1.	F	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.00	0.00	0.00
2.	L	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.00	0.00	0.00
3.	P1	0.012 ± 0.001	0.012 ± 0.002	0.012 ± 0.001	0.00	0.00	0.00
4.	P2	0.014 ± 0.002	0.015 ± 0.003	0.014 ± 0.002	0.3415	0.71	0.00
5.	B	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.00	0.00	0.00
6.	IW	0.011 ± 0.001	0.010 ± 0.002	0.012 ± 0.002	0.2297	-0.9	+0.9
7.	SW	0.010 ± 0.002	0.011 ± 0.001	0.010 ± 0.002	0.5878	+1.0	0.00
8.	WS	0.009 ± 0.004	0.010 ± 0.001	0.010 ± 0.002	0.5878	+1.11	+1.11
9	WW	0.028 ± 0.002	0.027 ± 0.003	0.028 ± 0.002	0.8000	-3.5	0.00

Table 6: Effect of interfering cation species on the Absorbances of Cd, Pb, Cr and As under observations. The interference studies on the influence of foreign metal ions (Co^{2+}, Cu^{2+}, Al^{3+} and Hg^{2+}) on the metal complexes have revealed that Cd, Pb, Cr and As can be determined using APDH reagent at pH 6.5-7.5 at the optimum wavelength of absorbances for the various metals under observations at [1:1] without interferences as shown in Table 6.

Metal	Working wave length (nm)	pH Range	[M-L]	Absorbance in the absence of IP	Absorbance in the presence of IP [1:1]				
					Co^{2+}	Cu^{2+}	Al^{3+}	Fe^{2+}	Hg^{2+}
Cd	395.0	6.5 – 7.5	[Cd – APDH]	0.822	0.857	0811	0.837	0.841	0.819
Pb	440.0	6.5 – 7.5	[Pb – APDH]	0.927	0.955	0.921	0.944	0.951	0.920
Cr	385.0	6.5 – 7.5	[Cr – APDH]	0.769	0.771	0.760	0.771	0.775	0.762
As	405.0	6.5 – 7.5	[As – APDH]	0.911	0.928	0.909	0.920	0.906	0.919

[ML] = Metal-Ligand complex concentration, IP = Interfering Specie

Table 7: Effect of Interfering cation species on the Absorbances of Cd, Pb, Cr and As under Observations. The interference studies on the influence of foreign metal ions (Co^{2+}, Cu^{2+}, Al^{3+} and Hg^{2+}) on the metal complexes have revealed that Cd, Pb, Cr and As can be determined using APDH reagent at pH 6.5-7.5 at the optimum wavelength of absorbances for the various metals under observations at [1:5] without interferences as shown in Table 6

Metal	Working wave length (nm)	pH Range	[M-L]	Absorbance in the absence of IP	Absorbance in the presences of IP [1:5]				
					Co^{2+}	Cu^{2+}	Al^{3+}	Fe^{2+}	Hg^{2+}
Cd	395.0	6.5 – 7.5	[Cd – APDH]	0.822	0.859	0.809	0.839	0.849	0.821
Pb	440.0	6.5 – 7.5	[Pb – APDH]	0.927	0.958	0.928	0.946	0.931	0.921
Cr	385.0	6.5 – 7.5	[Cr – APDH]	0.769	0.777	0.757	0.770	0.781	0.769
As	405.0	6.5 – 7.5	[As – APDH]	0.911	0.931	0.911	0.923	0.911	0.923

[ML] = Metal-Ligand complex concentration, IP = Interfering Species

Table 8: Effect of interfering cation species on the Absorbances of Cd, Pb, and Cr and As under observations. The interference studies on the influence of foreign metal ions (Co^{2+}, Cu^{2+}, Al^{3+} and Hg^{2+}) on the metal complexes have revealed that Cd, Pb, Cr and As can be determined using APDH reagent at pH 6.5-7.5 at the optimum wavelength of absorbances for the various metals under observations at [1:10] without interferences as shown in Table 7

Metal	Working wave length (nm)	pH Range	[M-L]	Absorbance in the absence of IP	Absorbancein the when presences of IP [1:10]				
					Co^{2+}	Cu^{2+}	Al^{3+}	Fe^{2+}	Hg^{2+}
Cd	395.0	6.5 – 7.5	[Cd – APDH]	0.822	0.861	0.806	0.841	0.849	0.816
Pb	440.0	6.5 – 7.5	[Pb – APDH]	0.927	0.961	0.931	0.951	0.938	0.918
Cr	385.0	6.5 – 7.5	[Cr – APDH]	0.769	0.771	0.752	0.767	0.779	0.759
As	405.0	6.5 – 7.5	[As –	0.911	0.938	0.921	0.931	0.911	0.929

APDH]

[ML] = Metal-Ligand complex concentration, IP = Interfering Species

Table 9: Effect of Interfering anions species on the Absorbances of (SO_4^{2-}, NO_3^-, NH_4^+) under observations. The interference studies on the influence of foreign anions (SO_4^{2-}, NO_3^-, NH_4^+) on the metal complexes have revealed that Cd, Pb,Cr and As can be determined using APDH reagent at pH 6.5-7.5 at the optimum wavelength of absorbances for the various metals under observations at [1:1] without interferences as shown in Table 8.

Metal	Working wave length (nm)	pH Range	[M-L]	Absorbance in the absence of IP	Absorbance in the presences of IP [1:1]		
					SO_4^{2-}	NO_3^-	NH_4^+
Cd	395.0	6.5 – 7.5	[Cd – APDH]	0.822	0.821	0.820	0.821
Pb	440.0	6.5 – 7.5	[Pb – APDH]	0.927	0.925	0.924	0.926
Cr	385.0	6.5 – 7.5	[Cr – APDH]	0.769	0.766	0.767	0.765
As	405.0	6.5 – 7.5	[As – APDH]	0.911	0.910	0.912	0.915

[ML] = Metal-Ligand complex concentration, IP = Interfering Species

Table 10: Effect of interfering anions species on the Absorbances of SO_4^{2-}, NO^{3-}, NH_4^+ under observations. The interference studies on the influence of foreign anions (SO_4^{2-}, NO_3^-, NH_4^+) on the metal complexes have revealed that Cd, Pb, Cr and As can be determined using APDH reagent at pH 6.5-7.5 at the optimum wavelength of absorbances for the various metals under observations at [1:5] without interferences as shown in Table 9.

Metal	Working wave length (nm)	pH Range	[M-L]	Absorbance in the absence of IP	Absorbance in the presence of IP [1:5]		
					SO_4^{2-}	NO_3^-	NH_4^+
Cd	395.0	6.5 – 7.5	[Cd – APDH]	0.822	0.819	0.824	0.820
Pb	440.0	6.5 – 7.5	[Pb – APDH]	0.927	0.923	0.924	0.925
Cr	385.0	6.5 – 7.5	[Cr – APDH]	0.769	0.767	0.765	0.767
As	405.0	6.5 – 7.5	[As – APDH]	0.911	0.909	0.914	0.915

[ML] = Metal-Ligand complex concentration, IP = Interfering Species

Table 11: Effect of interfering Anions species on the Absorbances of SO_4^{2-}, NO^{3-}, NH_4^+ under observations. The interference studies on the influence of foreign anions (SO_4^{2-}, NO^{3-}, NH^{4+}) on the metal complexes have revealed that Cd, Pb, Cr and As can be determined using APDH reagent at pH 6.5-7.5 at the optimum wavelength of absorbances for the various metals under observations at [1:10] without interferences as shown in Table 10

Metal	Working wave length (nm)	pH Range	[M-L]	Absorbance in the absence of IP	Absorbance in the presence of IP [1:10]		
					SO_4^{2-}	NO_3^-	NH_4^+
Cd	395.0	6.5 – 7.5	[Cd – APDH]	0.822	0.818	0.823	0.819

Pb	440.0	6.5 – 7.5	[Pb – APDH]	0.927	0.924	0.922	0.926
Cr	385.0	6.5 – 7.5	[Cr – APDH]	0.769	0.765	0.764	0.761
As	405.0	6.5 – 7.5	[As – APDH]	0.911	0.908	0.914	0.906

[ML] = Metal-Ligand complex concentration, IP = Interfering Species

Table 12: Effect of Interfering cations species on the Absorbances of Cd, Pb, Cr and As under observations. The interference studies on the influence of foreign metal ions (Co^{2+}, Cu^{2+}, Al^{3+} and Hg^{2+}) on the metal complexes have revealed that Cd, Pb, Cr and As can be determined using GPH reagent at pH 6.5-7.5 at the optimum wavelength of absorbances for the various metals under observations at [1:1] without interferences as shown in Table 11.

Metal	Working wave length (nm)	pH Range	[M-L]	Absorbance in the absence of IP	Absorbance in the presence of IP [1:1]				
					Co^{2+}	Cu^{2+}	Al^{3+}	Fe^{2+}	Hg^{2+}
Cd	387.0	6.5 – 7.5	[Cd – GPH]	1.118	1.217	1.016	1.211	1.216	1.079
Pb	395.0	6.5 – 7.5	[Pb – GPH]	1.237	1.206	1.271	1.311	1.206	1.361
Cr	360.0	6.5 – 7.5	[Cr – GPH]	1.019	1.162	1.123	1.018	1.113	1.006
As	390.0	6.5 – 7.5	[As – GPH]	1.123	1.201	1.101	1.130	1.136	1.201

[ML] = Metal-Ligand complex concentration, IP = Interfering Species

Table 13: Effect of interfering cations species on the Absorbances of Cd, Pb, Cr and As under observations. The interference studies on the influence of foreign metal ions (Co^{2+}, Cu^{2+}, Al^{3+} and Hg^{2+}) on the metal complexes have revealed that Cd, Pb, Cr and As can be determined using GPH reagent at pH 6.5-7.5 at the optimum wavelength of absorbances for the various metals under observations at [1:5] without interferences as shown in Table 12.

Metal	Working wave length (nm)	pH Range	[M-L]	Absorbance in the absence of IP	Absorbance in the presence of IP [1:5]				
					Co^{2+}	Cu^{2+}	Al^{3+}	Fe^{2+}	Hg^{2+}
Cd	387.0	6.5 – 7.5	[Cd – GPH]	1.118	1.219	1.014	1.209	1.218	1.077
Pb	395.0	6.5 – 7.5	[Pb – GPH]	1.237	1.204	1.273	1.316	1.209	1.364
Cr	360.0	6.5 – 7.5	[Cr – GPH]	1.019	1.034	1.125	1.016	1.115	1.018
As	390.0	6.5 – 7.5	[As – GPH]	1.123	1.211	1.106	1.136	1.140	1.211

[ML] = Metal-Ligand complex concentration, IP = Interfering Species

Table 14: Effect of interfering cations species on the Absorbances of Cd, Pb, Cr and As under observations. The interference studies on the influence of foreign metal ions (Co^{2+}, Cu^{2+}, Al^{3+} and Hg^{2+}) on the metal complexes have revealed that Cd, Pb, Cr and As can be determined using GPH reagent at pH 6.5-7.5 at the optimum wavelength of absorbances for the various metals under observations at [1:10] without interferences as shown in Table 13.

Metal	Working wave length (nm)	pH Range	[M-L]	Absorbance in the absence of IP	Absorbance in the presence of IP [1:10]				
					Co^{2+}	Cu^{2+}	Al^{3+}	Fe^{2+}	Hg^{2+}
Cd	387.0	6.5 – 7.5	[Cd – GPH]	1.118	1.221	1.016	1.121	1.117	1.119
Pb	395.0	6.5 – 7.5	[Pb – GPH]	1.237	1.240	1.229	1.239	1.231	1.242
Cr	360.0	6.5 – 7.5	[Cr – GPH]	1.019	1.039	1.016	1.019	1.018	1.022
As	390.0	6.5 – 7.5	[As – GPH]	1.123	1.131	1.118	1.289	1.140	1.201

[ML] = Metal-Ligand complex concentration, IP = Interfering Species

Table 15: Effect of Interfering anions species on the Absorbances of SO_4^{2-}, NO_3^-, NH_4^+ under observations. The interference studies on the influence of foreign anions (SO_4^{2-}, NO_3^-, NH_4^+) on the metal complexes have revealed that Cd, Pb,Cr and As can be determined using GPH reagent at pH 6.5-7.5 at the optimum wavelength of absorbances for the various metals under observations at [1:1] without interferences as shown in Table 14.

Metal	Working wave length (nm)	pH Range	[M-L]	Absorbance in the absence of IP	Absorbance in the presence of IP [1:1]		
					SO_4^{2-}	NO_3^-	NH_4^+
Cd	387.0	6.5 – 7.5	[Cd – GPH]	1.118	1.113	1.111	1.121
Pb	395.0	6.5 – 7.5	[Pb – GPH]	1.237	1.119	1.256	1.204
Cr	360.0	6.5 – 7.5	[Cr – GPH]	1.019	1.012	1.007	1.126
As	390.0	6.5 – 7.5	[As – GPH]	1.123	1.106	1.131	1.109

[ML] = Metal-Ligand complex concentration, IP = Interfering Species

Table 16: Effect of Interfering anions species on the Absorbances of Cd, Pb, Cr and As under observations. The interference studies on the influence of foreign anions (SO_4^{2-}, NO_3^-, NH_4^+) on the metal complexes have revealed that Cd, Pb, Cr and As can be determined using GPH reagent at pH 6.5-7.5 at the optimum wavelength of absorbances for the various metals under observations at [1:5] without interferences as shown in Table 15.

Metal	Working wave length (nm)	pH Range	[M-L]	Absorbance in the absence of IP	Absorbance in the presence of IP [1:5]		
					SO_4^{2-}	NO_3^-	NH_4^+
Cd	387.0	6.5 – 7.5	[Cd – GPH]	1.118	1.106	1.127	1.109
Pb	395.0	6.5 – 7.5	[Pb – GPH]	1.237	1.219	1.241	1.221
Cr	360.0	6.5 – 7.5	[Cr – GPH]	1.019	1.010	1.026	1.011
As	390.0	6.5 – 7.5	[As – GPH]	1.123	1.114	1.134	1.107

[ML] = Metal-Ligand complex concentration, IP = Interfering Species

Table 17: Effect of Interfering anions species on the Absorbances of Cd, Pb, Cr and As under observations. The interference studies on the influence of foreign anions (SO_4^{2-}, NO_3^-, NH_4^+) on the metal complexes have revealed that Cd, Pb, Cr and As can be determined using GPH reagent at pH 6.5-7.5 at the optimum wavelength of absorbances for the various metals under observations at [1:10] without interferences as shown in Table 16.

Metal	Working wave length (nm)	pH Range	[M-L]	Absorbance in the absence of IP	Absorbance in the presence of IP [1:10]		
					SO_4^{2-}	NO_3^-	NH_4^+
Cd	387.0	6.5 – 7.5	[Cd – GPH]	1.118	1.009	1.131	1.010
Pb	395.0	6.5 – 7.5	[Pb – GPH]	1.237	1.214	1.249	0.926
Cr	360.0	6.5 – 7.5	[Cr – GPH]	1.019	0.765	0.764	1.218
As	390.0	6.5 – 7.5	[As – GPH]	1.123	0.908	0.914	1.105

[ML] = Metal-Ligand complex concentration, IP = Interfering Species

Table 18: Evaluation of accuracy of Cadmium (Cd) for the developed methods (APDH and GPH) Methods

Sample	Pb added	APDH methods		GPH methods	
		Found (µg/ml)	Recovery (%) ± SD	Found (µg/ml)	Recovery (%) ± SD
F	-	ND	-	ND	-
	50.0	49.99	99.98±0.2	49.99	99.98±0.2
	100.0	99.98	99.98±0.1	99.99	99.99±0.2
	500.0	499.94	99.98±0.4	499.99	99.99±0.1
L	-	ND	-	ND	-
	50.0	49.98	99.96±0.3	49.99	99.98±0.2
	100.0	99.97	99.97±.02	99.98	99.98±0.2
	500.0	499.95	99.99±0.3	499.99	99.99±0.2
P1	-	ND	-	ND	-
	50.0	49.98	99.96±0.3	49.97	99.94±0.3
	100.0	99.98	99.98±0.2	99.98	99.98±0.2
	500.0	499.99	99.80±0.2	499.96	99.20±0.4
P2	-	ND	-	ND	-
	50.0	49.97	99.94±0.3	49.99	99.98±0.3
	100.0	99.97	99.97±0.3	99.98	99.98±0.2
	500.0	499.97	99.99±0.1	499.99	99.99±0.1
B	-	ND	-	ND	-
	50.0	49.99	99.98±0.2	49.98	99.96±0.3
	100.0	99.97	99.97±0.3	99.99	99.99±.02
	500.0	499.98	99.99±0.1	499.98	99.99±0.1
IW	-	ND	-	ND	-
	50.0	49.97	99.94±0.3	49.98	99.96±0.3
	100.0	99.97	99.97±0.3	99.99	99.99±0.1
	500.0	499.97	99.97±0.4	499.98	99.99±0.2
SW	-	ND	-	ND	-
	50.0	49.97	99.94±0.2	49.98	99.96±0.3
	100.0	99.96	99.96±0.3	99.99	99.99±0.1
	500.0	499.93	99.98±0.4	499.98	99.99±0.2
WS	-	ND	-	-	-
	50.0	49.93	99.86±0.4	49.98	99.96±0.3

	100.0	99.97	99.97±0.3	99.98	99.98±0.2
	500.0	499.92	99.98±0.4	499.97	99.97±0.2
					-
WW	-	ND	-	ND	
	50.0	49.97	99.94±0.3	49.98	99.96±0.3
	100.0	99.97	99.97±0.3	99.98	99.98±0.2
	500.0	499.96	99.99±0.2	499.98	99.99±0.2

Average of five replicate determinations of each Sample (n = 5), ND = Not Detected, SD = standard deviation

Table 19: Evaluation of accuracy of Lead (Pb) for the developed methods (APDH and GPH)

Sample	Pb added	APDH Method		GPH Method	
		Found (µg/ml)	Recovery (%) ± SD	Found (µg/ml)	Recovery (%) ± SD
F	-	ND	-	ND	-
	50.0	49.97	99.94±0.3	49.99	99.99±0.2
	100.0	99.99	99.99±0.2	99.99	99.99±0.2
	500.0	499.94	99.98±0.3	499.97	99.99±0.2
L	-	ND	-	ND	-
	50.0	49.94	99.88±0.4	49.96	99.92±0.3
	100.0	99.97	99.97±0.3	99.98	99.98±0.2
	500.0	499.94	99.98±0.3	499.96	99.99±0.2
P1	-	ND	-	ND	-
	50.0	49.96	99.92±0.3	49.98	99.99±0.2
	100.0	99.96	99.96±0.4	99.99	99.99±0.3
	500.0	499.93	99.98±0.4	499.97	99.99±0.3
P2	-	ND	-	ND	-
	50.0	49.97	99.94±0.3	49.99	99.99±0.2
	100.0	99.98	99.98±0.2	99.98	99.98±0.2
	500.0	499.92	99.98±0.4	499.96	99.99±0.3
B	-	ND	-	ND	-
	50.0	49.97	99.94±0.4	49.99	99.99±0.2
	100.0	99.97	99.97±0.4	99.98	99.98±0.2
	500.0	499.98	99.99±0.2	499.98	99.99±0.3
IW	-	ND	-	ND	-
	50.0	49.96	99.92±0.4	49.98	99.98±0.3
	100.0	99.98	99.98±0.2	99.98	99.98±0.3
	500.0	499.98	99.99±0.2	499.98	99.99±0.2

Sample	Pb added	Found (μg/ml)	Recovery (%) ± SD	Found (μg/ml)	Recovery (%) ± SD
SW	-	ND	-	ND	-
	50.0	49.97	99.94±0.3	49.98	99.99±0.2
	100.0	99.98	99.98±0.2	99.98	99.98±0.3
	500.0	499.03	99.98±0.4	499.96	99.99±0.4
WS	-	ND	-	-	-
	50.0	49.95	99.90±0.4	49.98	99.98±0.3
	100.0	99.97	99.97±0.3	99.98	99.98±0.3
	500.0	499.95	99.98±0.4	499.97	99.99±0.4
WW	-	ND	-	ND	-
	50.0	49.97	99.94±0.3	49.98	99.98±0.3
	100.0	99.97	99.97±0.3	99.98	99.98±0.2
	500.0	499.98	99.99±0.4	499.97	99.99±0.3

Average of five replicate determinations of each Sample (n=5),
ND = Not Detected, SD = Standard Deviation.
Table 20: Evaluation of accuracy of Chromium (Cr) for the developed methods (APDH and GPH).

Sample	Pb added	APDH Method		Method GPH	
		Found	Recovery (%)	Found	Recovery (%)
		(μg/ml)	± SD	(μg/ml)	± SD
F	-	ND	-	ND	-
	50.0	49.98	99.99±0.3	49.99	99.99±0.2
	100.0	99.98	99.98±0.2	99.99	99.99±0.2
	500.0	499.97	99.98±0.3	499.98	99.99±0.2
L	-	ND		ND	-
	50.0	49.99	99.99±0.2	49.99	99.99±0.2
	100.0	99.99	99.99±0.3	99.98	99.98±0.3
	500.0	499.94	99.98±0.3	499.98	99.99±0.2
P1	-	ND	-	ND	-
	50.0	49.99	99.99±0.2	49.97	99.98±0.3
	100.0	99.97	99.97±0.3	99.98	99.98±0.2
	500.0	499.98	99.99±0.2	499.96	99.98±0.3
P2	-	ND	-	ND	-
	50.0	49.96	99.92±0.4	49.98	99.96±0.3
	100.0	99.97	99.97±0.3	99.98	99.98±0.3
	500.0	499.98	99.99±0.2	499.97	99.99±0.2
B	-	ND	-	ND	-
	50.0	49.99	99.99±0.2	49.98	99.98±0.2
	100.0	99.97	99.97±0.4	99.96	99.96±0.3

Sample		Found	Recovery (%) ± SD	Found	Recovery (%) ± SD
	500.0	499.94	99.96±0.3	499.97	99.98±0.3
IW	-	ND	-	ND	-
	50.0	49.96	99.92±0.4	49.99	99.99±0.2
	100.0	99.97	99.97±0.4	99.97	99.97±0.3
	500.0	499.95	99.97±0.3	499.98	99.99±0.3
SW	-	ND	-	ND	-
	50.0	49.98	99.98±0.3	49.99	99.99±0.2
	100.0	99.96	99.96±0.3	99.98	99.98±0.3
	500.0	499.03	99.80±0.4	499.94	99.98±0.3
WS	-	ND	-	-	-
	50.0	49.96	99.92±0.4	49.98	99.96±0.3
	100.0	99.97	99.97±0.3	99.98	99.98±0.2
	500.0	499.95	99.99±0.2	499.98	99.99±0.2
WW	-	ND	-	ND	-
	50.0	49.99	99.98±0.2	49.96	99.92±0.2
	100.0	99.99	99.99±0.3	99.98	99.98±0.3
	500.0	499.99	99.99±0.2	499.94	99.99±0.2

Average of five replicate determinations of each Sample (n = 5),
ND = Not Detected, SD = Standard Deviation.

Table 21: Evaluation of accuracy of Arsenic (As) for the methods (APDH and GPH)

Sample	Pb added	APDH Method		GPH Method	
		Found (µg/ml)	Recovery (%) ± SD	Found (µg/ml)	Recovery (%) ± SD
F	-	ND	-	ND	-
	50.0	49.95	99.90±0.4	49.98	99.96±0.3
	100.0	99.97	99.97±0.3	99.98	99.98±0.3
	500.0	499.95	99.99±0.2	499.96	99.99±0.2
L	-	ND		ND	-
	50.0	49.99	99.99±0.2	49.98	99.98±0.2
	100.0	99.96	99.96±0.3	99.97	99.97±0.3
	500.0	499.94	99.99±0.3	499.95	99.99±0.2
P1	-	ND	-	ND	-
	50.0	49.99	99.99±0.2	49.98	99.96±0.3
	100.0	99.96	99.96±0.3	99.98	99.98±0.2
	500.0	499.94	99.99±0.2	499.96	99.99±0.3
P2	-	ND	-	ND	-
	50.0	49.96	99.92±0.4	49.98	99.98±0.2

	100.0	99.98	99.98±0.2	99.97	99.97±0.3
	500.0	499.97	99.99±0.2	499.96	99.99±0.2
B	-	ND	-	ND	-
	50.0	49.99	99.99±0.1	49.98	99.98±0.2
	100.0	99.96	99.96±0.3	99.97	99.97±0.3
	500.0	499.97	99.99±0.2	499.98	99.99±0.2
IW	-	ND	-	ND	-
	50.0	49.96	99.92±0.3	49.98	99.98±0.3
	100.0	99.95	99.95±0.4	99.98	99.98±0.2
	500.0	499.94	99.99±0.3	499.95	99.99±0.2
SW	-	ND	-	ND	-
	50.0	49.97	99.94±0.3	49.98	99.99±0.1
	100.0	99.93	99.93±0.4	99.95	99.95±0.3
	500.0	499.03	99.99±0.2	499.95	99.99±0.2
WS	-	ND	-	-	-
	50.0	49.96	99.92±0.3	49.98	99.99±0.2
	100.0	99.97	99.97±0.2	99.94	99.94±0.3
	500.0	499.96	99.99±0.2	499.98	99.99±0.2
WW	-	ND	-	ND	-
	50.0	49.99	99.99±0.1	49.96	99.92±0.3
	100.0	99.97	99.97±0.2	99.98	99.98±0.2
	500.0	499.98	99.99±0.1	499.96	99.99±0.2

Average of five replicate determinations of each Sample (n = 5), ND = Not Detected, SD = Standard Deviation.

The ligands (acetylpyridine-2,4-dinitrophenyl hydrazone and glutaraldehyde phenyl hydrazone) tend to reduce the maximum wavelength of the absorptions of the metal complexes at the same working concentrations which tend to give better absorption for spectrophotometric determinations of metals in the Uv-Vis region which is in agreement with the report of Okoye *et al.,* 2013 and Fakruddin *et al.,* 2011. The colour reactions were instantaneous and the method does not involve heating of the reaction mixture or pre-extraction of the components. The reagents gave colour reactions with metal ions in slightly acidic and slightly neutral regions. For the selection of the working wavelength, a number of solutions were made by mixing equal volume of the reagent concentration (1.00×10^{-4} mol / dm^3) and (1.00×10^{-4} mol / dm^3), of the salt of metal of interest in a 25.00 cm^3 volumetric flask and made to the mark with deionized water. The absorbances were scanned between 190-800.00 nm in a 1.00 cm^3 cuvette to determine the maximum wavelength (λmax) of absorption using the Uv-Vis spectrophotometer, the wavelength of maximum

absorbance for each complex were noted and recorded. The metal-ligand complex of GPH gave a better decrease in maximum absorbance.

The working pH for the metal-reagent complex were selected as reported by the method of Nityananda *et al.,* 2015, In a series of 10.0 cm^3 volumetric flaks, 2.00 cm^3 solution (1.00 x 10^{-4} mol/dm^3), of the salt of metal of interest, 3.00 cm^3 of the hydrazone solution (1.00 x 10^{-4} mol/dm^3) and 4.00 cm^3 of the buffer of varying pH were added and made up to the mark with deionized water and the absorbances were measured against the ligand blank at the scanned and recorded wavelength. A plot was then made between absorbance and pH in the Uv-Vis range. The pH with the highest absorbance was determined as the working pH for each metal of interest. From the plots obtained for GPH and APDH it could be seen that the metal-ligand complexes had higher absorbances at pH between 6.5 - 7.5 which was slightly acidic to almost neutral and was resolved to be the working pH for the study.

The sensitivity of the spectrophotometric method is often described in terms of the molar absorptivity (ϵ, L mol^{-1}cm^{-1}) of the metal-ligand complex. The awareness of the sensitivity is very important in spectrophotometric determination of trace metals. Sanagi *et al.,* 2009 suggested a relation between sensitivity and molar absorptivity. They stated the following criteria for describing the sensitivity. Low sensitivity $\epsilon < 2.00$ x 10^4 L mol^{-1} cm^{-1}, Moderate sensitivity $\epsilon = 2.00 – 6.00$ x 10^4 L mol^{-1} cm^{-1} and High sensitivity $\epsilon > 6.00$ x 10^4, L mol^{-1}cm^{-1}. From the data generated and the plots obtained as shown in figure 25 to 35 the metal-ligand complexes showed molar absorptivity ranging from 2.006 x 10^4 for Pb-APDH to 2.445 x 10^4 L.mol^{-1} cm^{-1} for As-APDH respectively. The Molar absorptivity for GPH ranged 2.213 x 10^4 for Pb-GPH to 2.460 x 10^4 L mol^{-1} cm^{-1}. The values obtained indicated that they could be classified as moderately sensitive. The reactions and measurements could be made highly selective by using appropriate masking agents as recorded by Jamaluddin *et al.,* 2012 and Humiara *et al.,* 2006. The values of the molar absorptivitives were higher than the values of Fahruddin *et al.,* 2011, Jamaluddin and Tasnima 2012, Srikanth and Chandra 2013, and Saritha *et al*., 2014.

The mole ratio method was used to study of the complex equilibrium in solution. The concentration of the metal ion was kept constant while the total concentration ratios rose linearly from the origin and breaks sharply to a straight line at mole ratio of the components in the complex. However, a complex that undergoes appreciable dissociation in solution gives a continuous curve, which only becomes parallel to the mole ratio axis, when an excess of the variable component is added. It is often seen such a curve breaks sharply at the correct mole ratio if the ionic strength of the solution is adjusted to a suitable value by addition of an unreacting electrolytes. Thus in such cases, it is possible to get the true composition of the complex. From the experimental data presented graphically, the stoichiometry were found to be 1:1 (metal: ligand) for APDH and 2:1 (metal-ligand) for GPH respectively.

Method validation: under the optimized conditions, the calibration curves were constructed by plotting the absorbance signal against the concentrations of each analyte subjected according to the general procedure (Azhar, 2015). The solution were transferred into the optical cell of 1.00 cm^3 for the measurement of each metal ion spectrophotometrically at the respective absorption maximum against a reagent blank prepared under similar conditions. The calibration graphs follow a straight-line equation Y= ac + b; where c is the concentration of the solution, Y is the measured absorbance, a and b are the constants. The Beer's law equation for the reagent GPH is : λmax (Cd-GPH 387.0) nm = 0.2419 X + 0.017 ; r = 0.8109, λmax (Pb-GPH 395.0) nm = 0.2549X + 0.091; r = 0.7581, λmax (Cr-GPH 360.0) nm = 0.2551 X + 0.089; r =

0.8651, λmax (As-GPH 395.00) nm = 0.2654 X + 0.074 ; r = 0.922 which indicated that the methods are moderately sensitive as described by Sanagi *et al.,* 2009.

The precision of the developed quantitative Uv-Vis spectrophotometric method for determination of selected heavy metals (Cd, Pb, Cr and As) in soil, water and biological samples with the synthesized phenyl hydrazones; acetylpyridine-2,4-nitrophenyl hydrazone (APDH) and glutaraldehyde phenyl hydrazone (GPH) as chromogenic reagents is described, The coloured metal-ligand complexes were soluble and stable with higher absorbances in dimethylformide (DMF) at pH of 6.5 - 7.5 having maximum absorbances (λmax) of; Cd-APDH (395.00 nm), As-APDH (405.00 nm), Pb-APDH (440.00 nm), Cr-APDH (385.00 nm) and Cd-GPH (387.00 nm), As-GPH (395.00 nm), Pb-GPH (395.00 nm), Cr-GPH (360.00 nm). The complexation of the phenyl hydrazones with the Cd, Pb, Cr and As markedly altered the wavelength of absorption of the complexes and this phenomenon can be utilized in the spectrophotometric determination of these metals (Okoye *et al.,* 2013 and, Gopala and Kethani, 2011). Five replicate concentrations of the cadmium, lead, chromium and arsenic in the samples under observations were evaluated. The relative standard deviation (n = 5) was between 0.00 - 3.00 % for both proposed methods and all the metals analysed which is in agreement with the results of Jamaluddin *et al.,* 2014, Jamaluddin and Shah 2003, Gopala *et al.*, 2010, Ullah and Enamul 2010, Gopala and Kethani 2011, Deepa *et al.,* 2014 and Azhar 2015 indicating that both developed methods are highly precise and reproducible as shown in table 5 to 21 respectively.

The effect of possible interring species (cations and anions) as shown in table 6 to 17 which are generally associated with the determination of those metals under study at 1:5, 1:10 and 1:15 folds was evaluated. The interfering species were studied by measuring the absorbance of the reaction mixtures with and without the various species. An error of ±5.00 % in the absorbance reading was considered tolerated limit. From all the interfering cations and anions studied at the various folds, their corresponding absorbances were within the tolerable limits of the ± 5.00 % (Deepa *et al.,* 2014 and Azhar 2015). Higher amounts of possible interfering species of up to 1:10 folds did not affect the absorbance significantly. In the events of possible interferences in the absorbance of from the interfering species, masking agent like disodium EDTA will be used to masked it out (Ullah and Enamul 2010, Gopala and Kethani 2011, Deepa *et al.,* 2014 and Azhar 2015).

The performance of the developed methods was evaluated by determinations of the concentrations of cadmium, lead, chromium and arsenic in biological, environmental, soil, water, and waste samples, the results presented in table 2 to 5. The present ligands APDH and GPH containing phenyl rings are found to be good analytical reagent for the determination of the selected metals of interest.

Evaluation of the developed method for its accuracy and precisions with Atomic Absorption Spectroscopy (AAS) as reference method was reported as shown in Table 18 to 21. The developed methods (APDH and GPH) were applied for the quantitative determination of cadmium, lead, chromium and arsenic in environmental, biological, soil, water, and waste samples, and were evaluated by adding known amount of cadmium, lead, chromium and arsenic salts at 50, 100 and 500 μg mL^{-1} in five replicates (n = 5). The recoveries were 99.2 to 99.99 % as shown in table 6 to 9 for both APDH and GPH methods which are in good agreement with the works of Adi *et al.,* 2012, Ahmed 2011, Ahmed and Chowdhury 2014, Fakruddin *et al.,* 2011, Gopala *et al.,* 2010, Saritha and Sreenivasulu 2014, and Mohammad *et al.,* 2014. The result indicates that the found values are in a very concordance indicating the good accuracy of the developed methods (APDH and GPH). The

standard deviation (n = 5) of both developed methods were between 0.001 to 0.003 for APDH and GPH respectively signifying that the developed methods are highly precise and reproducible.

The detection (µg/g) and quantification limits (µg/g) for the APDH methods were; 0.4456 and 1.3495 for Cd, 0.3572 and 1.0824 for Pb, 0.4202 and 1.2735 for Cr and, 0.3272 and 0.9916 for As respectively. For the GPH methods, the detection (µg/g) and quantification limits (µg/g) were; 0.4933 and 1.4948 for Cd, 0.5250 and 1.5909 for Pb, 0.3825 and 1.1590 for Cr and, 0.3432 and 1.040 for As respectively. The influence of small variations in the method variables such as ligand concentrations, buffer pH, reaction time, non-formation of precipitates from the metal-ligand complex and the period of stability of the analytical performance of the developed method shows the robustness of the method which is in conformity with Mohammad *et al.* 2014.

A validated Atomic Absorption Spectrophotometric (AAS) method was used as a reference method as recommended by Mohammad *et al*, 2014, Jamaluddin *et al.,* 2014, Jamaluddin and Shah 2003, Fakruddin *et al.,* 2011, Jamaluddin and Tasnima 2012 and Gopala *et al.,* 2010 in comparing the data to the developed method for determination of the selected heavy metals of interest (Cd, Pb, Cr, and As) in biological, water and environmental samples. The samples were digested according to standard methods already reported. The inter method variations in percentage of error in cadmium, lead, chromium and arsenic by the developed methods and AAS are shown in Table 2 to 5. No significant differences were found between the calculated relative error of the APDH and GPH methods to AAS and the *p*-values at 95% confidence level provided similar accuracy and precision in the determination of metals by both methods.

The developed method was applied to the quantitative determination of Cd, Pb, Cr and As in water samples from the calibrated plots (Beer's law plot) with APDH and GPH as the chromogenic reagent and the results presented in Table 2 and 21. Statistical analysis of the results by *t*-test shows that, there is no significant difference in the accuracy and precision of the proposed and reference method for quantitative determination of the said metals in the samples (Adi *et al*., 2012, Ahmed *et al*., 2011, Ahmed and Chowdhury 2004, Azhar 2015, Fakruddin *et al.,* 2011, Jamaluddin and Tasnima 2012, Jamaluddin *et al.,* 2014, Mokhtari *et al.*, 2007, Nagalakshmi *et al*., 2011, Okoye *et al*., 2013, Rahnama *et al*., 2012 and Zhang and Zhang 2003).

Applications of the developed methods in the determination of Cd, Pb, Cr and As in soil samples was carried out and reported. Prior to the digestion of the soil samples, each sample was dried at 65°C for 48 hours in the oven. All samples were performed in triplicates. Five grams (5.00 g) of the samples in crucibles was placed in a pre-heated muffle furnace at 200-250 °C for 30 minutes, and the ashed for four hours at 500-550 °C. Then, the sample was removed from the furnace and cooled down. 2.00 cm^3 of 5.0 M of HNO$_3$ was added and evapourated to dryness on a sand bath. Next, about the samples were placed in a furnace and heated to 400 °C for 15 minutes. The samples were removed from the furnace, cooled and moistened with four drops of distilled water. Next, 2.0 cm^3 of concentrated HCl was added and the sample was evaporated to dryness, removed, and the 5.0 cm^3 2.0 M HCl was again added and the tube was swirled. The results were checked with parallel determination by AAS as the reference method. Statistical analysis of the results by *t*-test shows that, there is no significant difference in the accuracy and precision of the proposed and reference method (Adi *et al*., 2012, Ahmed *et al*., 2011, Ahmed and Chowdhury 2004, Azhar, 2015, Fakruddin *et al.,* 2011, Jamaluddin and Tasnima 2012,

Jamaluddin *et al.,* 2014, Mokhtari *et al.*, 2007, Nagalakshmi *et al*., 2011, Okoye *et al*., 2013, Rahnama *et al*., 2012, and Zhang and Zhang 2003).

` **Conclusion:** The present methods (APDH and GPH) were applied for the determination of Cd, Pb, Cr and As in different samples. The real samples were water, biological and environmental samples. Satisfactory results results were obtained in the analysis. The present methods are simple, sensitive, inexpensive, reasonable selective and rapid without the need of heating and extraction. The reagents are very simple to synthesize and purify with available chemicals. The most favourable characteristics of the presents methods is that they gave water soluble complexes which facilitate to quantitatively determine the metals in aqueous media for continuous monitoring in real samples. Although many sophisticated techniques such as pulse polarography, High Performance Liquid Chromatography (HPLC), Atomic absorption spectroscopy (AAS), ICP-AES, and ICP-MS are available for the determination of cadmium, lead, chromium and asernic in numerous real sample materilas, factors such as the low cost of the equipment, easy handling, lack of requirement for consumeables and almost no maintenance have caused Uv-vis spectrophotometry to remain a popular analytical technique, particularly in laboratories of developing countries with limited budgets. The sensitivity in terms of molar absorptivity and precision in terms od standard deviation of the present methods are very reliable for the determination of Cd, Pb, Cr and As in real samples down to nano-gram per gram ($\eta g\ g^{-1}$) levels in aqueous medium at room temperature.

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