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H-point Standard Addition Method for Determination of Cadmium and Mercury

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Abstract

H-point standard addition method (HPSAM) has been applied for simultaneous determination of Cd(II) and Hg(II) using 1-(2-pyridylazo)-2-naphthol (PAN) as a chromogenic reagent in aqueous Triton X-100 micellar media at pH 10.0. Cd(II) and Hg(II) can be determined simultaneously in the concentration range 0.112-1.405 μg mL^{-1} and 0.201-2.507 μg mL^{-1} respectively. The proposed method has successfully been applied for simultaneous determination of Cd(II) and Hg(II) in synthetic samples, spiked water samples, tobacco, tomato, spinach and powdered milk.

Keywords:

Cadmium; mercury; HPSAM

1. Introduction

The toxicological effects of Cd(II) and Hg(II) on human bodies have received wide concern and extensive study in recent years. Cd(II) and Hg(II) accumulates in human tissue and induce biological diseases. Known effects of exposure to Cd(II) and Hg(II) range from subtle biochemical changes at low levels of exposure, to severe neurological and toxic effects or even death at extremely high levels [1, 2]. The FAO-WHO Joint Expert Commitee on Food Additives recommended a provisional maximum tolerable daily intake for Cd(II) and Hg(II) from all sources is 1-1.2 μg kg^{-1} and 1-1.6 μg kg^{-1} of body weight respectively. Therefore accurate and reliable methods must be developed for their determination.

Several reports have been published on the determination of Cd(II) and Hg(II) using flame atomic absorption spectrometry (FAAS) [3], inductively coupled plasma mass spectrometry (ICP-MS) [4, 5], cold vapor atomic absorption spectrometry (CVAAS) [6, 7] etc. All these techniques require costly instrumentation for quantitation of chemical species. Spectrophotometric methods play a prominent role, as they involve less cost and they are rapid and sensitive. The simultaneous determination of these metals by the use of traditional spectrophotometry techniques is difficult as, their absorption spectra overlap and the superimposed curves are not suitable for quantitative evaluation.

Bosch-Reig et al. [8, 9] outlined the fundamentals of HPSAM in 1988 which is a simple bi-variate chemometric technique. HPSAM is applied to work at two selected wavelengths where the analytical signals due to one of the species (interferent) is constant and for another one (analyte) to be different as much as possible. By plotting the analytical signal versus added analyte concentration, two straight lines are obtained that have a common point with

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coordinates H ($-C_H$, A_H), where $-C_H$ is the unknown analyte concentration and A_H the analytical signal due to interferent species. The HPSAM has been applied to eliminate the blank bias error due to the use of absorbent blank [10, 11], in liquid chromatography [12], in metal speciation [13] and to the analysis of kinetic data [14-17] with time as an additional variable.

In the present work, low cost HPSAM has been developed for simultaneous determination of Cd(II) and Hg(II), using 1-(2-pyridylazo)-2-naphthol (PAN) as a complexing reagent at pH 10.0 in TritonX-100 micellar media.

2. Experimental

2.1 Apparatus

UV-visible absorbance spectra were recorded on a UV-Vis Shimadzu-1700 spectrophotometer. Digital century pH-meter Cp-901 with a combined glass electrode was used for pH measurements.

2.2 Reagents and standard solutions

All reagents used were of analytical grade unless otherwise stated and double distilled deionized water was used throughout the experiment. 1-(2-pyridylazo)-2-naphthol (PAN) was obtained from Fluka. A 0.01% PAN (w/v) solution was prepared by dissolving 10 mg of PAN in 100 mL of dimethylformamide. Stock solution of Cd(II) and Hg(II) was prepared by dissolving spectral pure-grade chemicals cadmium chloride and mercuric chloride in double distilled water and further dilutions were made when required. A buffer of pH 10 was prepared from ammonia (0.5 M) and ammonium chloride (0.5M). A 1% (v/v) Triton X-100 solution was prepared in hot distilled water.

2.3 Procedure

To appropriate amounts of Cd(II) and Hg(II) sample solution in calibration range after adjusting its pH 10.0 by adding 2.0 mL buffer were added 2.0 mL of 0.01% PAN and 2.5 mL of 1% Triton X-100. Then added a series of standard Cd(II) or Hg(II) solutions in appropriate amounts. The volume was made up to the mark with double distilled water in a 10.0 mL standard flask and a portion of solution was taken to record the spectrum against reagent blank. Absorbances were recorded at 563 and 552 nm (when standard Cd(II) solutions were added) or 564 and 537 nm (when standard Hg(II) solutions were added).

3. Results and Discussion

Cd(II) and Hg(II) formed red colored complexes with PAN. As Fig. 1 clearly shows, the overlapping absorption spectra of the PAN complexes of Cd(II) and Hg(II) so each compound interferes with the analytical determination of the other. Therefore simultaneous determination of Cd(II) and Hg(II) is possible using binary HPSAM.

3.1 Effect of pH

In order to find optimum conditions, the effect of pH on the absorbance of Cd(II) and Hg(II) complexes of PAN was studied in the pH range 7.0-11.0. Cd(II) and Hg(II) form complexes rapidly with PAN at pH values greater than 7. The absorbance is maximum and constant in the pH range 8.5-10.5 for Cd(II)-PAN, and for Hg(II)-PAN complex absorbance is maximum at pH 10.0. Therefore for further studies pH 10.0 was selected. The effect of pH is shown in Fig. 2.

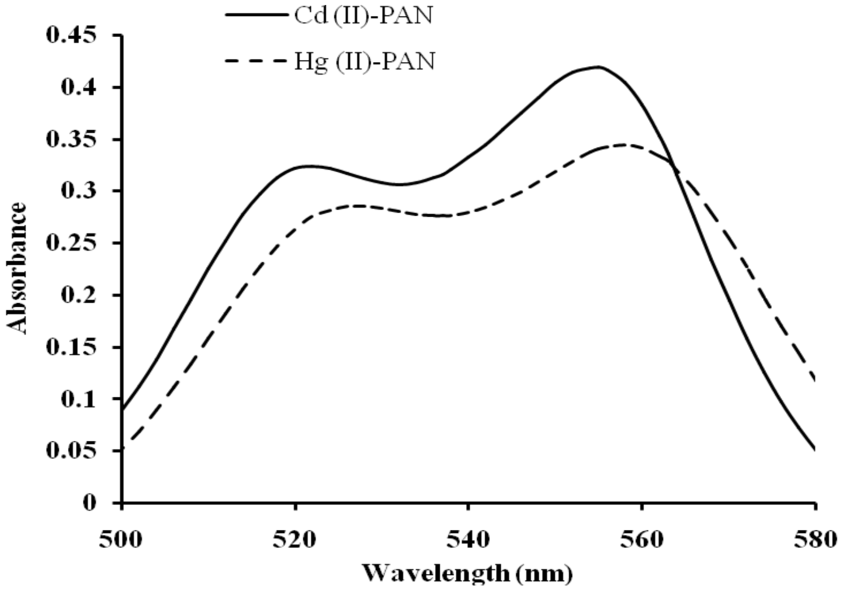

Fig. 1 Absorption spectra of 0.843 µg mL^{-1} Cd(II)-PAN and 1.504 µg mL^{-1} Hg(II)-PAN complexes in 1% Triton X-100 at pH 10.0.

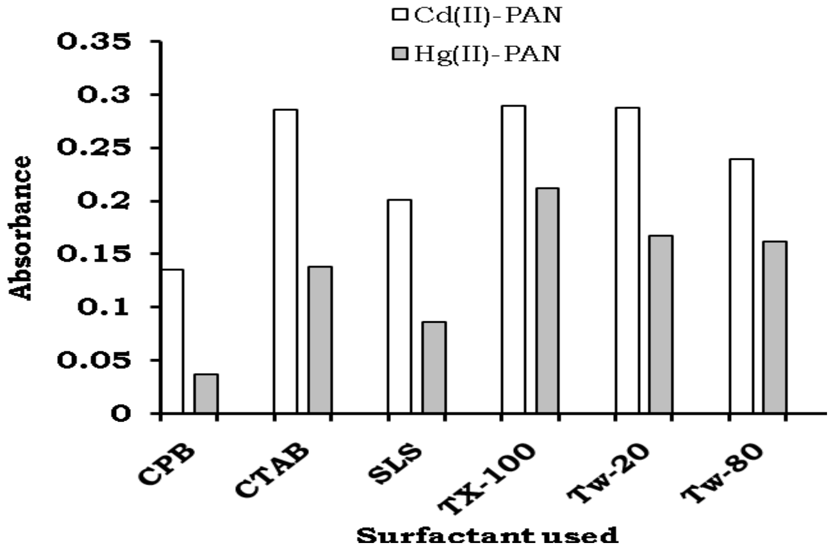

Fig. 2. Effect of pH on the absorbance of Cd(II)-PAN and Hg(II)-PAN complex.

3.2 Effect of various surfactants

Cd(II) and Hg(II)-PAN complexes were insoluble in water, so various surfactants such as cetylpyridinium bromide (CPB), cetyltrimethylammonium bromide (CTAB) were tried as solublizing agents. Cd(II)-PAN complex showed almost the same soubility and maximum absorbance with CTAB, Tween-20 and Triton X-100. For Hg(II)-PAN complex absorbance was maximum with Triton X-100. So, for simultaneous determination of Cd(II) and Hg(II), 2.5 mL of 1% Triton X-100 was selected. Effect of various surfactants is shown in Fig. 3.

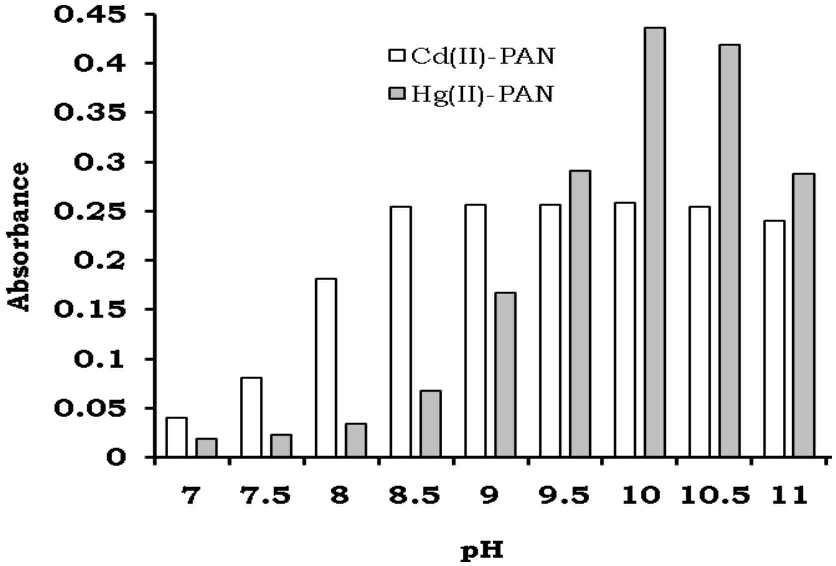

Fig. 3. Effect of different surfactants on the absorbance of Cd(II)-PAN and Hg(II)-PAN complex.

3.3 Effect of reagent

Effect of various amounts of reagent on the absorbance of Cd(II) and Hg(II) as PAN complexes was studied. Detailed studies showed that 1.0 mL of 0.01% PAN solution was optimum for the individual calibration of Cd(II) and Hg(II). To ensure the complete complexation of Cd(II) and Hg(II) when applying HPSAM 2.0 mL of 0.01% PAN was used.

3.4 H-point standard addition method

Both Cd(II) or Hg(II) can be selected as analyte when applying HPSAM. When Cd(II) is selected as an analyte, many pairs of wavelengths showing the same absorbance for Hg(II)-PAN complex are possible. In the selection of one pair of wavelengths for obtaining good accuracy, the absorbance differences at the two selected wavelengths for Cd(II)-PAN complex must be as large as possible. Based on the absorption spectra of Cd(II) and Hg(II) complexes, one of the best pair of wavelengths is 563 and 552 nm when standard solution of Cd(II) are added. Similarly, when Hg(II) is selected as analyte best pair of wavelengths for applying HPSAM is 564 and 537 nm. C_H and A_H were obtained from the intersection point of the two derived straight lines in the H-point graphs of absorbance versus analyte concentration where $-C_H$ was the unknown analyte concentration and A_H the analytical signal due to interferent species.

3.5 Accuracy and precision

The concentration ranges of Cd(II) and Hg(II) for construction of calibration graphs were 0.112-1.405 μg mL^{-1} and 0.201-2.507 μg mL^{-1} respectively. Several synthetic samples with different concentration ratios of Cd(II) and Hg(II) were analyzed using HPSAM. The results are given in Table 1. H-point standard addition plots for several synthetic samples are also shown in Figs. 4 and 5 for a fixed amount of Hg(II) in the presence of various amounts of Cd(II) or a fixed amount of Cd(II) in the presence of various amounts of Hg(II) respectively. The reproducibility of the method was checked by performing five replicate experiments. The results are given in Table 2.

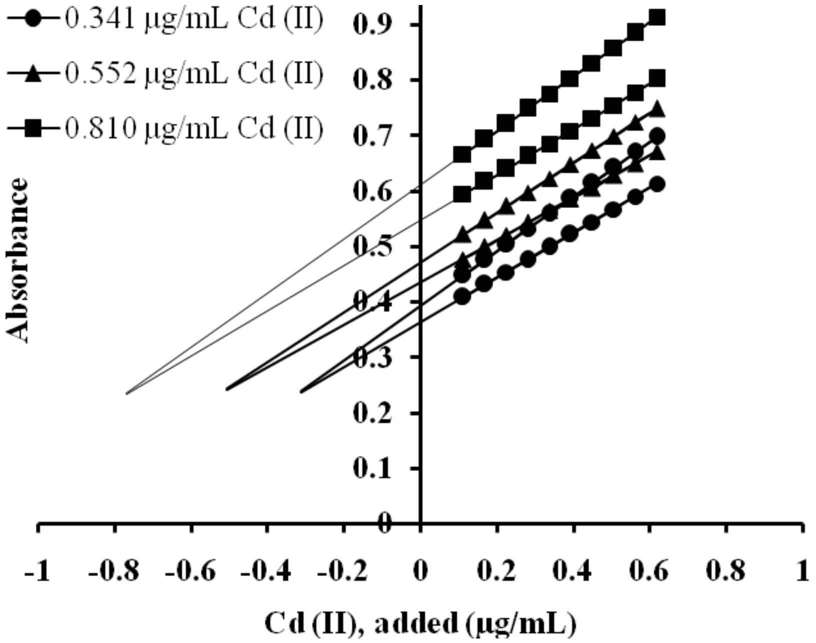

Fig. 4. H-point standard addition plot for a fixed Hg(II) concentration (1.003 µg mL^{-1}) and different concentrations of Cd(II) concentrations under optimized conditions.

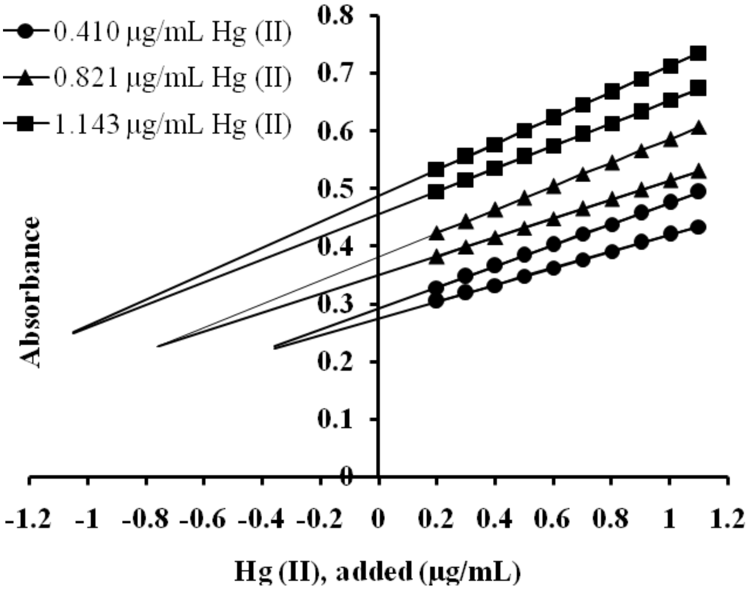

Fig. 5. H-point standard addition plot for a fixed Cd(II) concentration 0.449 µg mL^{-1} and different concentrations of Hg(II) concentrations under optimized conditions.

3.6 Interference analysis

The effect of various diverse ions on the absorbance of a solution containing 0.75 µg mL^{-1} each of Cd(II) and Hg(II) was studied. An ion was considered to interfere when its presence produced a variation in the absorbance of the sample greater than 5%. The results are given in Table 3. Among the anions examined I^-, Br^-, Cl^-, CO_3^{2-}, SO_3^{2-}, SO_4^{2-}, NO_3^-, IO_3^-, NO_2^- did not interfere at concentrations 1000 times higher than those of the analytes however ethylenediamine tetraacetate ion interfered strongly. Of the metal ions examined Zn^{2+}, Cu^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Fe^{3+}, Fe^{2+}, Bi^{3+} and Pb^{2+} interfered strongly. Interference due to Zn^{2+}, Cu^{2+} and Co^{2+} was eliminated by masking with sodium thioglycollate and EDTA solution. Mn^{2+}, Fe^{2+} and Fe^{3+} were masked sodium hexametaphosphate. Bi^{3+} and Pb^{2+} interference was removed by sodium citrate solution. Ni^{2+} could be masked with dimethylglyoxime. Among the anions examined EDTA interfered strongly.

Table 1. Results obtained by HPSAM for synthetic samples of Cd(II) and Hg(II)

A-C Equation Cd(II) is added	Present (μg mL^{-1})		Found (μg mL^{-1})		Recovery, %	
	Cd(II)	**Hg(II)**	**Cd(II)**	**Hg(II)**	**Cd(II)**	**Hg(II)**
Cd(II) is added						
$A_{563}=0.391C_i+0.404$ $A_{552}=0.475C_i+0.423$	225	1.404	226	*1.383*	*100.44*	*98.50*
$_{563}=0.395C_i+0.600$ $A_{552}=0.480C_i+0.661$	731	1.404	717	1.387	98.08	98.79
$A_{563}=0.373C_i+0.319$ $A_{552}=0.471C_i+0.368$	505	602	500	573	99.01	95.18
$A_{563}=0.355C_i+0.197$ $A_{552}=0.373C_i+0.200$	168	602	166	596	98.81	99.00
Hg(II) is added						
$A_{537}=0.117C_i+0.501$ $A_{564}=0.154C_i+0.519$	899	501	886	486	98.55	97.01
$A_{537}=0.159C_i+0.654$ $A_{564}=0.187C_i+0.690$	899	1.304	897	1.286	99.77	98.62
$A_{537}=0.167C_i+0.597$ $A_{564}=0.194C_i+0.605$	1.124	301	1.093	296	97.24	98.34
$A_{537}=0.134C_i+0.754$ $A_{564}=0.182C_i+0.825$	1.124	1.504	1.109	1.479	98.67	98.34

4. Applications of the method

4.4.1 Environmental samples

Each filtered (with Whatman No. 40) environmental water sample (500 mL) was spiked with known amounts of Cd(II) and Hg(II), reduced to 200 mL by evaporation in the presence of 5 mL of concentrated nitric acid. The solution was cooled and neutralized with dilute ammonium hydroxide. The solution was then again filtered and diluted to 250 mL with double distilled water. An aliquot of the solution was taken and determined by the developed procedure. The results of analysis of environmental water samples from various sources for Cd(II) and Hg(II) are given in Table 4.

Table 2. Results of five replicate experiments for synthetic samples of Cd(II) and Hg(II)

	Present (μg mL^{-1})		Found (μg mL^{-1})	
A-C Equation	Cd(II)	Hg(II)	Cd(II)	Hg(II)
A_{563}=0.382C_i+0.376 A_{552}=0.479C_i+0.440	652	550	660	535
A_{563}=0.382C_i+0.376 A_{552}=0.479C_i+0.439	652	550	649	554
A_{563}=0.382C_i+0.374 A_{552}=0.479C_i+0.437	652	550	649	545
A_{563}=0.380C_i+0.375 A_{552}=0.477C_i+0.439	652	550	660	536
A_{563}=0.381C_i+0.377 A_{552}=0.479C_i+0.442	652	550	663	537
Mean			**656**	**541**
RSD(%)			**1.067**	**1.479**

Table 3. Tolerance ratio of foreign ions in the determination of Cd(II) and Hg(II)

Ion	Tolerance Limit Wion/WCd(II) or Hg(II)
NO_3^-, SO_4^{2-}, HPO_4^{2-}, SCN^-, NO_2^-, PO_4^{3-}, F^-, $S_2O_3^{2-}$, ClO_3^-, IO_3^-, Cl^-, I^-, Br^-, CH_3COO^-, $C_2O_4^{2-}$	>1000
Na^+, K^+, Mg^{2+}, Ba^{2+}, Al^{3+}, Rb^+, Cs^+, Ag^+	1000
Sb^{3+}, Ca^{2+}, Zr^{4+}, Ti^{4+}	500
Th^{4+}, Sn^{2+}, As^{3+}	100
$^aCo^{2+}$, $^aCu^{2+}$, $^bNi^{2+}$, $^cBi^{3+}$, $^dFe^{3+}$	10
$^aZn^{2+}$, $^cPb^{2+}$, $^dMn^{2+}$, $^dFe^{2+}$	1
EDTA	1

a- Masked with sodium thioglycollate and EDTA solution; b- masked with dimethylglyoxime; c- masked with sodium citrate solution; d- masked with sodium hexametaphosphate solution.

4.4.2 Chewing tobacco / cigarettes

Cd(II) and Hg(II) determination by the proposed method was also done in chewing tobacco / cigarettes. Different brands of chewing tobacco (Paan Parag, Tulsi) / cigarettes (Gold Flake, Classic) were randomly purchased from the market. After removing the filter and paper 1.0 g sample was weighed from each packet. Sample was spiked with known amounts of Cd(II) and Hg(II), digested with 12.0 mL mixture of HNO_3 : H_2SO_4 (8:4). The mixture was heated up to 130 ^oC for 1 h, after cooling 5.0 mL of distilled water was added to the sample and mixed. The residue was filtered and diluted to 250 mL with double distilled water. The spiked sample solutions were then analyzed by the developed procedure. The results are given in Table 5.

4.4.3 Tomato / spinach leaves / powdered milk

Cd(II) and Hg(II) determination by the proposed method was also done in tomato, spinach leaves (collected from fields irrigated with water from Budha Nallah) and powdered milk available locally. A 1.0 g of the each of food sample was weighed. Vegetables were chopped into small pieces and blended. Then each food sample was spiked with known amounts of Cd(II) and Hg(II), digested with 12 mL mixture of HNO_3 : H_2SO_4 (8:4). The mixture was heated up to 130 ^oC for 1 h, after cooling 5 mL of distilled water was added to the sample and mixed. The residue was filtered and diluted to 250 mL with double distilled water. The spiked sample solutions were then analyzed by the developed procedure. The good agreement between the results obtained and the known amounts present in the samples indicated the successful applicability of the developed method. The results are given in Table 5.

Table 4. Determination of Cd(II) and Hg(II) in environmental water samples

Sample	Spiked (μg mL^{-1})		Found (μg mL^{-1})		Recovery, %	
	Cd(II)	Hg(II)	Cd(II)	Hg(II)	Cd(II)	Hg(II)
Tap water[a]	0.225	0.250	0.218	0.246	96.88	98.40
Beas river[b]	0.450	0.420	0.456	0.422	101.33	100.47
Sutlej river[c]	1.250	1.050	1.269	1.068	101.52	100.71

a- Urban Estate, Patiala; **b-** D/S Mukerian (receives industrial waste of Mukerian Paper Mill); **c-** D/S Budha Nallah (receives industrial and domestic waste of Ludhiana).

Table 5. Determination of Cd(II) and Hg(II) in food samples

Sample	Spiked (μg mL^{-1})		Found (μg mL^{-1})		Recovery, %	
	Cd(II)	Hg(II)	Cd(II)	Hg(II)	Cd(II)	Hg(II)
Chewing tobacco[a]	0.185	0.345	0.188	0.342	101.62	99.13
Chewing tobacco[b]	0.185	0.345	0.191	0.349	103.24	101.15
Cigarette[c]	0.580	0.750	0.574	0.741	98.96	98.80
Cigarette[d]	0.580	0.750	0.582	0.743	100.34	99.06
Tomato[e]	0.355	0.355	0.359	0.354	101.13	99.72
Spinach[e]	0.250	1.950	0.247	1.944	98.80	99.69
Powdered milk	0.825	0.550	0.819	0.538	99.27	97.82

a- Paan Parag; **b-** Tulsi; **c-** Gold Flake; **d-** Classic **e-** Sample was collected from fields irrigated with water from Budha Nallah.

5. Conclusion

Simultaneous determination of Cd(II) and Hg(II) without the use of any expensive instrument is achieved. This reduces the cost of applied method. No extraction step is required as determination has been done in micellar media and hence the use of toxic and carcinogenic organic solvents is avoided. Micellar systems solublized the insoluble complex, and eliminated the need of non-aqueous extraction step in analysis. This reduces the toxicity of the applied method.

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