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# A Simple Flow-injection Spectrofluorimetric Method for the Determination of Mercury

**Muberra Andac · Adem Asan · Nihat Tinkilic · Ibrahim Isildak**

**Abstract** A highly sensitive flow-injection spectrofluorimetric method is presented for the rapid and simple determination of Hg (II) in environmental and pharmaceutical samples. Murexide (ammonium purpurate) was used as the fluorescence reagent in the carrier stream. An emission peak of murexide, which is decreased linearly by addition of Hg (II), occurs at 435 nm in aqueous solution with excitation at 335 nm. A linear calibration was obtained for 5–200 ng ml$^{-1}$ Hg (II) with the relative standard deviation 2.5% ($n=5$) for a 20 μl injection volume Hg (II). The limit of the detection was 1 ng ml$^{-1}$ and the sampling rate was 80 h$^{-1}$. No significant interference was found by the ions commonly found in the most environmental samples. The proposed method was successfully applied for the determination of trace mercury in real samples and the validation of the proposed methodology is provided.

**Keywords** Flow-injection analysis · Mercury (II) · Fluorimetric determination · Murexide

## Introduction

Mercury is a non-essential metal found in minerals, soil, oceans, lakes and air, and has been extensively used by industry in the production of pesticides, electrical apparatus, paints and dental applications for decades [1]. The analysis and monitoring of mercury in environmental, industrial and biological samples is extremely important because of the high toxicity even at trace levels.

Numerous sensitive and selective methods for the determination of mercury at low concentration have been developed [1–10]. The majorities of them are complex, and employ expensive and large instrumentation, together with the need of some degree of expertise for their proper operation. However, among those methods, Flow-injection analysis (FIA), have gained much interest in recent years because of their simplicity, high reproducibility and possibility of coupling different detection systems [11, 12].

A limited number of flow-injection spectrophotometric methods have been developed for the determination of mercury [13–17]. However, many of the proposed methods are not sensitive enough to determine mercury in environmental and biological samples. The sensitivity of flow-injection spectroflourimetric method is much higher than spectrophotometric method. But fluorescence reagents and methods suitable for the determination of mercury are scares and these methods suffer from limitation of low sensitivity and a lengthy procedure [18–20]. Therefore, it is still very important to develop highly selective, simple and economical methods for the rutin determination of mercury (II) in different sample matrices.

In our previous study [21], murexide has been heavily researched for its spectrofluorimetric application of mercury. Experimentally it was found that murexide is a very sensitive reagent for the spectroflourimetric determination of mercury in environmental and biological samples. In this work, attempts were made to develop very simple, sensitive, reproducible and inexpensive flow injection spectroflourimetric method for the mercury determination using murexide with a micro-column consisting of cation-exchange resin. The developed method was successfully

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applied for the determination of trace mercury in environmental and pharmacological samples.

## Experimental

### Reagents and samples

All reagents used were of analytical reagent grade and deionised water was used throughout. A mercury (II) stock solution ($1 \times 10^{-3}$ mol $l^{-1}$) was prepared from $Hg(NO_3)_2$ (E. Merck) in 0.1 M $HNO_3$. Standard solutions of mercury (II) were prepared daily by several dilutions of the stock standard solution with deionised water. Interference studies were carried out by using chloride or nitrate salts of metal cations. A 0.1 mol $l^{-1}$ HOAc/OAc$^-$ buffer at pH: 4.2 was used as the carrier solution in FIA system. Murexide was provided from E. Merck and prepared daily by solubilizing the appropriate amount of murexide in the buffer solution. Special care was taken in the preparation and handling of standard solutions, samples and containers to minimize any possible risk of heavy metal contamination. Polyethylene containers were left overnight in 2 % (v/v) $HNO_3$ and then rinsed with deionised water before used for sample storage.

Different combinations of standard samples were prepared containing either mercury (II) alone or binary and ternary mixtures of potential interfering metal ions under study.

### Apparatus

A high pressure liquid chromatography pump furnished with stainless-steel tubing was used to propel the carrier solution and samples. Samples (20 μl) were injected into the carrier stream by a Rheodyne injection valve provided with a 20 μl loop. All fluorescence measurements were made with Varian 2070 spectrofluorometer using 15 μl flow cell. Instrument excitation and emission slits were set at 20 nm. The light source was an ozoneless 75 W Xenon lamp and connected to a strip chart recorder.

The cation-exchange resin was used the sodium form of A650 W (100–200 mesh) from BioRad Labs. The cation-exchange resin microcolumn (5 cm 2 mm i.d) was prepared in our lab.

The pH measurements were made with a Jenway digital pH-meter model 3040.

Water samples were obtained from different places of the river, sea and asphalt production factory in Samsun, Turkey. Pharmaceutical products, Polistren and Voltaren, were provided from pharmacy.

A Flame atomic absorption system (Unicam 929) was also employed for the determination of Hg (II) for the comparison with the FIA method developed.

### General procedure

The FIA system configured in Fig. 1 was designed and optimized for the employment of the method. The sample (S) was injected into the buffer as a carrier that merged with murexide reagent solution. The complex was formed along the flow-line in the micro-column (MC) and carried toward to flow-through detector cell (FD) in which the mercury (II)-murexide complex was selectively monitored at $\lambda_{max(Em)}$ 435 nm with $\lambda_{max(Ex)}$ 335 nm, and recorded continuously on a chart recorder (R).

### Preparation of samples

The preparation of pharmaceutical products for the determination of Hg (II) is achieved according to the procedure reported by Thomson and Hoffman [22]. A 0.5 ml sample accurately weight was diluted 1:4 with aqua regia (nitric acid and hydrochloric acid 1:3) into pyrex vial. The mixture was gently heated with continuous stirring for 1 h on a steam bath. Upon the completion of heating, the solution was diluted to 20 ml with deionised water and filtered through a 45 μm filter. Then, the sample was analyzed using the procedure described above.

River, seawater and asphalt waste water samples collected in Nalgene plastics were acidified by adding 1 ml of nitric acid (0.1 M) per 100 ml of sample solution after filtration over 0.45 μm Millipore Filter (Millford, MA). Then, samples were injected directly into the FIA system for the determination of mercury (II). Analyses were performed with the least possible delay.

## Results and discussion

### Optimization of main experimental variables

The optimisation of the flow system with spectrofluorimetric detection was performed to establish the best FIA variables. The optimum experimental conditions were determined using fixed standard mercury (II) solution
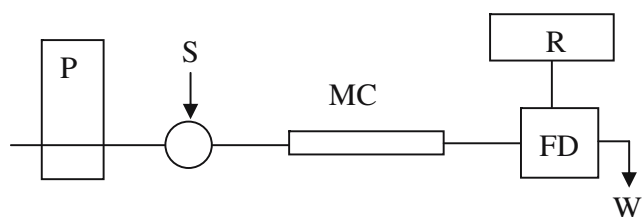


**Fig. 1** Flow-injection manifold for the fluorimetric determination of Hg (II) in real water samples. *P*: HPLC pump; *S*: sample; *MC*: microcolumn (5 cm long, 2 mm i.d.); *FD*: spectrofluorimetric detector; *R*: recorder; *W*: waste

concentration of 10 ng ml$^{-1}$. The amount of murexide, pH and the flow-rate were the main variables that influence the intensity of the signal in the FIA system. The optimization of the FIA system was therefore made by changing those variables one by one.

The effect of murexide in the carrier solution on the peak height was examined by changing the amount of murexide in the range of $1\times10^{-5}$–$5\times10^{-6}$ M, in acetate buffer solution (pH: 4.2), at a flow rate of 0.8 ml min$^{-1}$. Peak heights were found maximal using $5\times10^{-5}$ M murexide solution for 10 ng ml$^{-1}$ mercury (II) levels. Therefore, $5\times10^{-5}$ M of murexide was chosen as the color-developing component of the carrier solution.

The effect of flow-rate on the peak height of 10 ng ml$^{-1}$ mercury (II) was examined by varying flow-rates from 0.2 to 1.5 ml min$^{-1}$. Peak heights were decreased by flow-rates above 1.0 ml min$^{-1}$ and below 0.5 ml min$^{-1}$. Flow-rates below 0.5 ml min$^{-1}$ peaks were also broaden. Between flow-rates 0.5–1.0 ml min$^{-1}$, there were slight differences on the peak heights. However, taking into consideration the stability of the pump, peak shape and sampling time, we adjusted the flow-rate of the reagent carrier solution to 0.8 ml min$^{-1}$. This provided a sampling frequency of 80 h$^{-1}$.

The pH of the carrier solution consisting of $5\times10^{-5}$ M murexide was adjusted by using HOAc/OAc$^{-}$, NaHPO$_4$/Na$_2$HPO$_4$ and NH$_3$/NH$_4^{+}$ buffer solutions to obtain a pH range of 3.0–9.0. The peak shape and height were found maximum at a pH: 4.2. Therefore, a HOAc/OAc$^{-}$ buffer solution at pH: 4.2 was used through the study.

The use of a microcolumn in the flow-injection system can provide an improvement in sensitivity and selectivity due to on-line pre-concentration of metal ions and fast interaction between metal ions and the reagent molecules in the carrier solution [13]. Micro-column packed with cation-exchange resins was used for the interaction of the mercury (II) and murexide in the flow injection system. The effect of the length of the column was examined by changing column length between 2 and 10 cm. From the results, 5 cm column length was decided convenient for better peak height and shape.

Flow signal, calibration graph and detection limit

Figure 2 shows typical flow signals for mercury (II) obtained by the proposed method. The reaction of mercury (II) with murexide resulted in negative peaks due to the fluorescence quenching of murexide.

Under the optimum working conditions, the calibration graph showed a good linearity from 5 to 200 ng ml$^{-1}$ of mercury (II) with the linear regression equation, $Y=0.0219X+0.3284$, where $Y$ is the peak height (cm) and $X$ is the concentration of mercury (II) in ng ml$^{-1}$. The
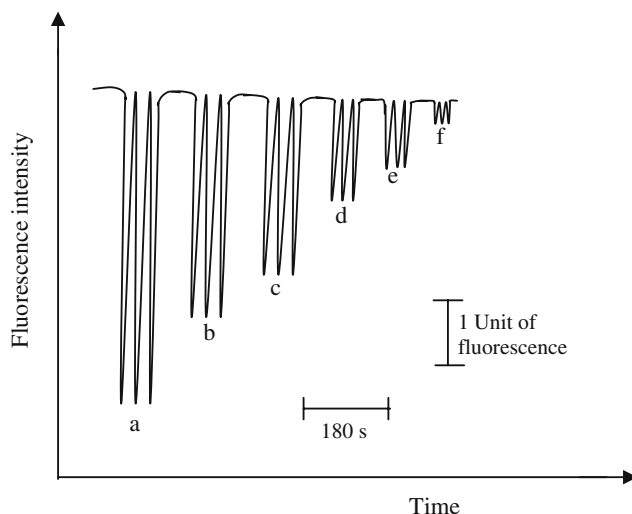


Fig. 2 Flow signal for mercury (II) standard solutions by fluorescence quenching-FIA **a** 200; **b** 150; **c** 100; **d** 50; **e** 25; **f** 5 ng ml$^{-1}$ by working with the optimized FIA system which is shown in Fig. 1

correlation coefficient was 0.9963 and the sampling rate was 80 samples h$^{-1}$. The detection limit (determined as three times the standard deviation of the blank) of the method was 1 ng ml$^{-1}$.

Reproducibility and accuracy

The proposed FIA spectrofluorimetric method was very reproducible, permitting the determination of 10 ng ml$^{-1}$ with an RSD 2.5 %. The accuracy of the method was determined by 5 replicate injections of 20 μl of a commercial eye-drop sample solution both unspiked and spiked with the Hg (II). The mean recovery was found to be 101.84±2.17% ($n=5$), indicating that the proposed procedure was very accurate.

Interference of diverse ions

The effects of possible interferences were investigated using the optimum conditions. Synthetic sample solution containing 10 ng ml$^{-1}$ of Hg (II) and different concen-

Table 1 Effect of foreign ions on the determination of 10 ng ml$^{-1}$ of mercury (II) in solution

| Tolerance limit (μg ml$^{-1}$) | Foreign ion |
| --- | --- |
| No interference | Co(II), Cr(III), Al(III), Cd(II), Ni(II), Mn(II), Zn(II), K(I), Na(I), Ca(II), Mg(II), Ba(II), Pb(II), Fe(II), Fe(III), CN$^{-}$, NO$_3^{-}$, NO$_2^{-}$, SO$_4^{2-}$, CO$_3^{2-}$, Cl$^{-}$, Br$^{-}$, PO$_4^{3-}$, NH$_4^{+}$ |
| Over 200 | Ag(I), Cu(II) |

**Table 2** Determination of Hg (II) in water and pharmaceutical samples

| Samples | Hg(II) in sample[c] (ng ml$^{-1}$) | | | | |
| --- | --- | --- | --- | --- | --- |
| | Found[d] | Found[e] | Mean recovery (%) | Hg (II) labelled | AAS |
| Kurtun river[a] | 20.15 (0.18) | 20.72 (0.12) | | | |
| Baruthane seawater[a] | 15.83 (0.05) | 16.15 (0.08) | | | |
| Asphalt waste water[a] | 55.36 (0.24) | 55.93 (0.15) | | | 60.2 |
| Eye-drop 1[b] | 20.51 (0.24) | 20.37 (0.42) | 103.03 | 19.8 | |
| Eye-drop 2[b] | 51.57 (0.17) | 51.82 (0.11) | 104.34 | 49.5 | 55.7 |

[a] Water samples obtained from different places of the river, seawater and asphalt waste water (Samsun, Turkey).
[b] Pharmaceutical products; Polistren[1] and Voltaren[2].
[c] Values in parenthesis are the relative standard deviations for $n=5$ with confidence level of 95%.
[d] Calibration curve method.
[e] Standard addition method.

trations of the other ions were tested and the peak heights obtained were measured. Table 1 shows the maximum tolerable concentrations of the various ions. The tolerance limits were obtained by considering that foreign ion does not cause interference if its signal is not different than 3 % of the analyte signal. Almost all of the 20 ions tested did not interfere and gave signal comparable to the water peak. Only Ag (I) and Cu (II) ions at 200 fold excesses caused interference. It has been previously described [23] that ions such as $Cu^{2+}$, $Pb^{2+}$, $Cl^-$, $I^-$ strongly interfered to the determination of Hg(II). Although our findings demonstrated that under properly selected experimental conditions, ($\lambda_{max(Ex)}=335$ nm; $\lambda_{max(Em)}=435$ nm) at pH 4.2 with HOAc/OAc$^-$ buffer, a simple, highly selective and sensitive flow-injection spectrofluorimetric method, interference free from all tested cations and anions including $Pb^{2+}$, $Cl^-$, $I^-$, has been developed.

### Applications

The proposed FI method has been applied for the determination of Hg(II) in different environmental samples (river, seawater and asphalt waste water) and commercially available pharmaceuticals Polistren and Voltaren, in which thiomersal (sodiumethylmercurysalicylate) used as antiseptic and antimicrobial preservative, by the following procedure described in the "Experimental" Section.

Mercury was determined in two different formulation eye-drops. Table 2 shows the results of Hg (II) content of difference sample matrix and the recoveries for the eye-drop samples obtained by the present method. The found results for eye-drops were compared with those declared on the formulation levels. There is good agreement between the found and declared values for the eye-drop samples. Atomic absorption measurements taken for water and eye-drop samples were also given for comparison. The simple FIA method described here demonstrates a precise analysis

of Hg (II) in pharmaceutical and environmental samples than the atomic absorption analysis method.

### Conclusion

The proposed FIA spectrofluorimetric detection method has proved to be very simple, rapid and sensitive for mercury (II) determination. The developed method is fast and reasonably cost effective providing a good sample frequency of 80 h$^{-1}$ and should be useful for routine analysis of mercury for pharmaceutical and environmental samples. Given the simplicity of the system and the easily instrumentation availability it can be a useful method to control effluents that may have traces of Hg (II) as a screening method.

### References

1. Bloxham MJ, Gachanja A, Hill SJ, Worsfold PJ (1996) Determination of mercury in filtered sea-water by flow injection with on-line oxidation and atomic fluorescence spectrometric detection. J Anal At Spectrom 11:511–514
2. Ma Xing W, Liu F, An Li K, Chen W, Tong SY (2000) Preconcentration, separation and determination of trace Hg(II) in environmental samples with aminopropylbenzoylazo-2-mercaptobenzothiazole bonded to silica gel. Anal Chim Acta 416:191–196
3. Hight SC, Cheng J (2006) Determination of methylmercury and estimation of total mercury in seafood using high performance liquid chromatography (HPLC) and inductively coupled plasma-mass spectrometry (ICP-MS): method development and validation. Anal Chim Acta 567:160–172.
4. Bernaus A, Gaona X, van Ree D, Valiente M (2006) Determination of mercury in polluted soils surrounding a chlor-alkali plant: Direct speciation by X-ray absorption spectroscopy techniques and preliminary geochemical characterisation of the area. Anal Chim Acta 565:73–80
5. Shao LJ, Gan WE, Su QD (2006) Determination of total and inorganic mercury in fish samples with on-line oxidation coupled to atomic fluorescence spectrometry. Anal Chim Acta 562:128–133

6. Kan M, Willie SN, Scriver C, Sturgeon RE (2006) Determination of total mercury in biological samples using flow injection CVAAS following tissue solubilization in formic acid. Talanta 68:1259–1263

7. Mishra S, Tripathi RM, Bhalke S, Shukla VK, Puranik VD (2005) Determination of methylmercury and mercury(II) in a marine ecosystem using solid-phase microextraction gas chromatography-mass spectrometry. Anal Chim Acta 55:192–198

8. Vel Krawczy TK, Moszcynska M, Trojannowicz M (2000) Inhibitive determination of mercury and other metal ions by potentiometric urea biosensor. Biosens Bioelectron 15:681–691

9. Ferrarello CN, Bayon MM, Alonso JIG, Sanz-Medel A (2001) Comparison of metal pre-concentration on immobilized Kelex-100 and quadruple inductively coupled plasma mass spectrometric detection with direct double focusing inductively coupled plasma mass spectrometric measurements for ultratrace multi-element determinations in sea-water. Anal Chim Acta 429:227–235

10. Bonfil Y, Brand M, Kirowa-Eisner E (2000) Trace determination of mercury by anodic stripping voltammetry at the rotating gold electrode. Anal Chim Acta 424:65–76

11. Guo T, Baasner J (1993). Determination of mercury in urine by flow-injection cold vapour atomic absorption spectrometry. Anal Chim Acta 278:189–196

12. QuanChen J, Gao W, Song JF (2006) Flow-injection determination of iron(III) in soil by biamperometry using two independent redox couples. Sens Actuators B 113:194–200

13. Andac M, Asan A, Bekdemir Y, Kutuk H, Isildak I (2003). Spectrophotometric flow-injection analysis of mercury(II) in pharmaceuticals with p-nitrobenzoxosulfamate. Talanta 60:191–197

14. Peng XJ, Mao QK, Cheng JK (1994) Spectrophotometric determination of mercury with 5,10,15,20-tetrakis(3-chloro-4-sulfophenyl)porphine by flow-injection analysis. Mikrochim Acta 113:81–89

15. Matteo MD, Forteza R, Cerda V (1990) Int J Environ Anal Chem 41:39

16. Cordoba MH, Navarro PN, Garcia IL (1988) Int J Environ Anal Chem 32:97

17. Ruiz TP, Cordoba MH, Lozano CM, SanchezPedreno C (1985) Quim Anal 4:72

18. de La Riva V, Costa-Fernandez BS, Pereiro JM, Sanz-Medel AR (2002) Spectrafluorimetric method for the rapid screening of toxic heavy metals in water samples. Anal Chim Acta 451:203–210

19. Narinesingh D, Mungal R, Ngo T (1994) A screening method for trace mercury analysis using flow-injection with urease inhibition and fluorescence detection. Anal Chim Acta 304:185–190

20. Lista AG, Palomeque ME, Fernandez Band BS (1999). Flow-injection fluorimetric determination of mercury(II) with calcein. Talanta 50:881–885

21. Andac M, Asan A, Isildak I (2003). Spectrofluorometric determination of mercury(II) with murexide. J Chem Crystallogr 33:599–603

22. Thompson DR, Hoffmann TJ (1975) J Pharm Sci 64:1863

23. Rahman MS (2001) Quenchofluorimetric determination of mercury trace with murexide. Microchim Acta 137:203–207