

The facile flow-injection spectrophotometric detection of gold(III) in water and pharmaceutical samples using 3,5-dimethoxy-4-hydroxy-2-aminoacetophenone isonicotinoyl hydrazone (3,5-DMHAAINH)

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Abstract

A simple, sensitive and rapid flow-injection spectrophotometric method was developed for the determination of trace amounts of Au(III) in aqueous dimethylformamide (DMF). The method is based on formation of Au(III)–(3,5-DMHAAINH)₃ complex. The optimum conditions for the chromogenic reaction of Au(III) with 3,5-DMHAAINH were studied and the colored (reddish brown) complex is selectively monitored at λ_{\max} 490 nm at pH 6.0. The reaction and flow conditions of the full factorial experimental design were optimized. The detection limit (2 s) of 0.1 $\mu\text{g l}^{-1}$ Au(III) was obtained at a sampling rate of 15 samples h⁻¹. Beer's law is obeyed over the range of 0.30–4.00 $\mu\text{g ml}^{-1}$. The molar absorptivity and Sandell's sensitivity were $3.450 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$ and 0.0050 $\mu\text{g ml}^{-1}$, respectively. Job's method of continuous variation and stability constants corresponding to these maxima was determined and found to be 9.3×10^{15} (1:3, M:R) (M, metal; R, reagent). The detailed study of various interferences confirmed the high selectivity of the developed method. The method was successfully applied for the determination of trace amount of Au(III) in water and pharmaceutical samples. The results obtained were in agreement with the reported methods at the 95% confidence level.

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1. Introduction

The separation, preconcentration and determination process of trace metals from different matrices especially in water samples are mainly based on the utilization and application of number of available techniques. These include the liquid and solid phase extraction (SPE), ion-exchanger and co-precipitation by metal hydroxides [1–2]. Liquid–liquid extraction (LLE) of different metal ions was widely employed in chemistry and industry for several years [3].

Several sensitive spectrophotometric techniques were proposed for the determination of Au(III) in recent years. But many of them involve ternary system [4–10] or surfactant effects [11–16] or oxidizing agents [17–22]. The reported methods suffer from a series of disadvantages such as high consumption of reagents, less sensitivity, low selectivity and interference from foreign ions. The aim of present work is to develop a facile, rapid, selective, sensitive and reproducible spectrophotometric flow-injection method for the determination of ultra trace levels of Au(III) in aqueous medium by coupling with 3,5-dimethoxy-4-hydroxy-2-aminoacetophenone isonicotinoyl hydrazone (3,5-DMHAAINH) at pH 6.0 in water and pharmaceutical samples.

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2. Experimental

2.1. Synthesis of 3,5-dimethoxy-4-hydroxy-2-aminoacetophenone isonicotinyl hydrazone (3,5-DMHAAINH)

3,5-DMHAAINH is not commercially available, and was synthesised according to the method described previously [22]. 3,5-Dimethoxy-4-hydroxy-2-aminoacetophenone (3,5-DMHAA) (7.65 g) was mixed with 5N NaOH (10 ml). The solution was stirred continuously in a water bath at 5 °C for 30 min to under go condensation process. Pure form of isonicotinic acid hydrazide (INH) dissolved in aqueous methanol (10 ml) was added to the condensed reaction mixture and resulting solution were stirred at 25–30° C. Finally, the reaction mixture was mixed with distilled water (50 ml) and 3,5-DMHAAINH precipitated as a white solid powder. The yield was 78.9% and melting point of the product is 187–190° C. The structure of color forming reagent is shown in Fig. 1(a).

2.2. Instrumentation

A high pressure liquid chromatography (Perkin–Elmer Series 3) pump furnished with stainless-steel tubing was used to propel the samples and reagent solutions. Samples were injected into the carrier stream by a Rheodyne injection

valve provided with a 50 µl loop. A HITACHI U 2001 spectrophotometer equipped with a flow-through microcells and connected to a computer (IPX Spectra SYSTEM SN4000) incorporated to a PC 1000 software program was used for absorbance measurements. The pH measurements were made with a Elico Digital pH meter.

2.3. Reagents

All reagents used were of analytical reagent grade and solutions were prepared with deionised distilled water.

An Au(III) stock solution (0.01 M) was prepared by dissolving 0.571 g of chloro auric acid (Johnson Matthey, Materials Technology, U.K.) in 5 ml of 0.1 M hydrochloric acid and solution is made up to the mark with distilled water in 100 ml volumetric flask. The working standard solutions were prepared by diluting the stock solution to appropriate volume with distilled water.

0.02 M 3,5-dimethoxy-4-hydroxy-2-aminoacetophenone isonicotinoyl hydrazone was prepared by dissolving 0.66 g of 3,5-DMHAAINH in 100 ml distilled water. Finally, 0.01 M dimethyl formamide (DMF), 0.1 M HCl and 5 N NaOH solutions were used for present studies.

Phosphate buffer solution of pH 6.0 was prepared by adding concentrated H₂SO₄ 3.4 ml–250 ml distilled water in a 100 ml flask. The mono sodium dihydrogen phosphate monohydrate (25 g) was added and the flask, shaken until the

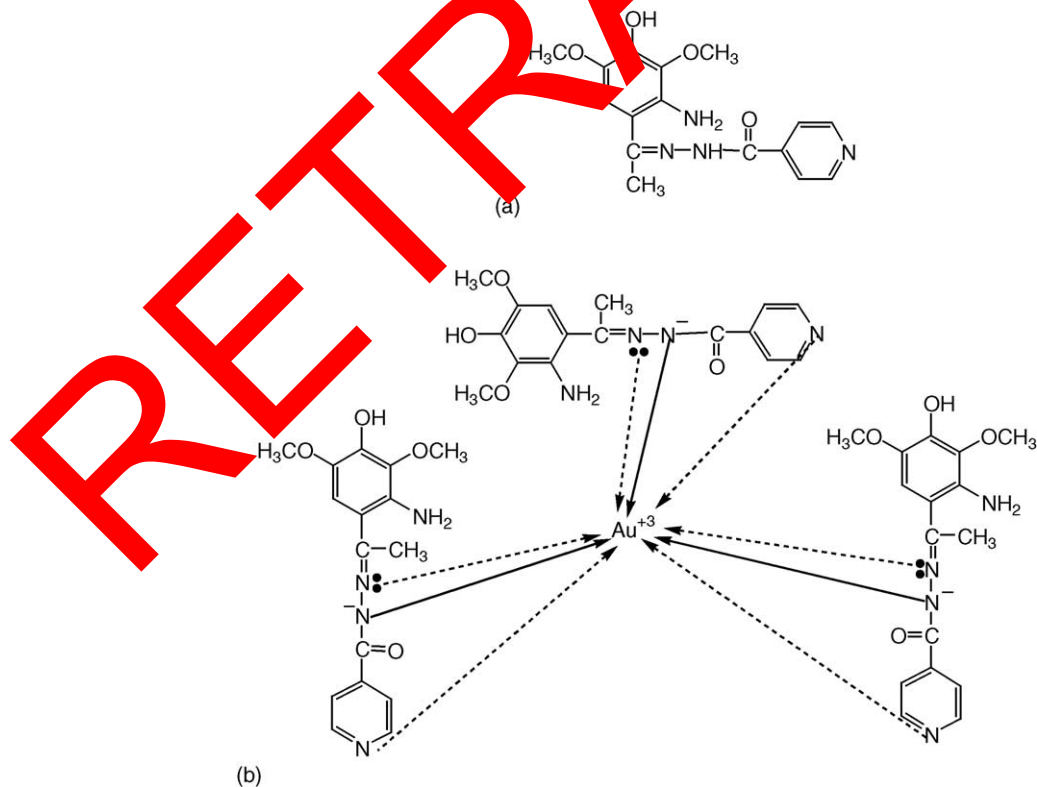


Fig. 1. (a) Structure of 3,5-dimethoxy-4-hydroxy-2-aminoacetophenone isonicotinoyl hydrazone (3,5-DMHAAINH), and (b) complex of Au(III)–(3,5-DMHAAINH)₃.

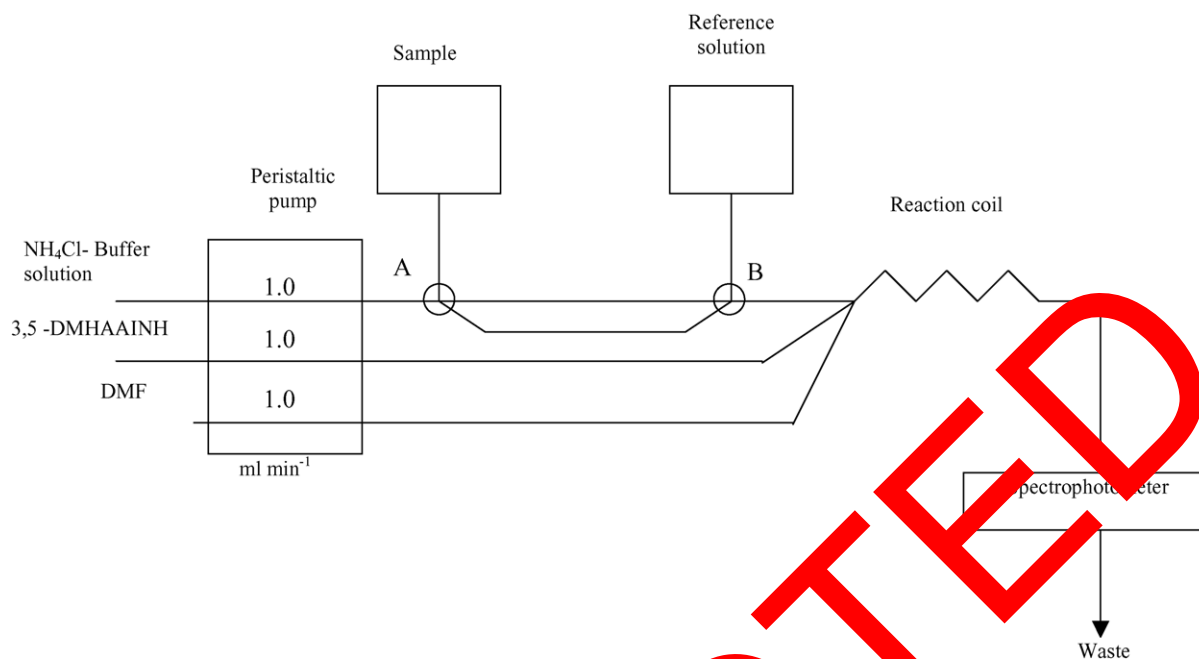


Fig. 2. Spectrophotometric flow-injection apparatus (FIA) for determination of Au(III) in water and pharmaceutical samples.

dissolution was completed. The solution was other diluted to 500 ml.

2.4. Procedure

Fig. 2 indicates a scheme of the FIA apparatus for the determination of Au(III) in water and pharmaceutical samples. The method was tested with model solutions before its application to real samples. A 50 μ l volume of sample solution was injected into the reagent carrier solution (NH₄Cl–phosphate buffer) by adjusting valves A and B. The mixed solution was sent to a reaction coil, the colored solution was carried to the flow injection spectrophotometric detector cell in which the absorbance of the product, Au(III)–(3,5-DMHAAINH)₃ was sensitively measured at 490 nm and recorded continuously on a computer. In the optimization studies, the parameters studied were reagent concentration, pH, flow rate, sample volume and method evaluation.

2.4.1. Determination of Au(III) in natural water samples

Different water samples (Tap water, river water, spring water) were collected from various places around Tirupati area. The samples were stored at 0–5 °C in metal free polyethylene bottles. Water samples were filtered through Whatman filter paper no. 41 and the clean solution is collected into a 100 ml standard flask. The contents are diluted to the mark with distilled water. Ten milli litre of this solution is further diluted to get working solution for determination of Au(III) as described in procedure and compared with the reported method in literature [23]. The results were summarised in Table 2.

2.4.2. Determination of Au(III) in pharmaceutical samples

Twenty capsules of Rheumartho with gold (Sri Baidyanath Ayurvedic Bhavan Ltd., Nagpur, India), K.K. Forte with gold (Kalpa Pharma, Vijayawada, India), Auranofin (Reddy Laboratories, Hyderabad, A.P., India) were ground well and dissolved in minimum volume of concentrated HCl and evaporated to dryness. The residue is re-dissolved in 10 ml of concentrated HCl and diluted to 50 ml. It is filtered through Whatman filter paper no. 41 and the clean solution is collected into a 100 ml standard flask. The contents were diluted to the mark with distilled water. Ten millilitres of this solution is further diluted to get working solutions for determination of Au(III) in pharmaceutical samples as described in aforesaid procedure. The results were presented in Table 3 and compared with the reported method [20].

3. Result and discussion

Under the experimental condition, the absorption spectra of (3,5-DMHAAINH) and Au(III)–(3,5-DMHAAINH)₃ complexes were scanned. The absorption maximum of 3,5-DMHAAINH versus reagent blank was measured at 360 nm, where as Au(III)–(3,5-DMHAAINH)₃ complex gave an absorption peak at 490 nm. The contrast (bathochromic shift) of the two peaks was 130 nm, and could be obviously distinguished. Thus, the absorption peak at 490 nm was chosen as the determination of wave length for Au(III)–(3,5-DMHAAINH)₃ complex as shown in Fig. 3. 3,5-DMHAAINH structure (Fig. 1(a)) is a blend of hydrazone

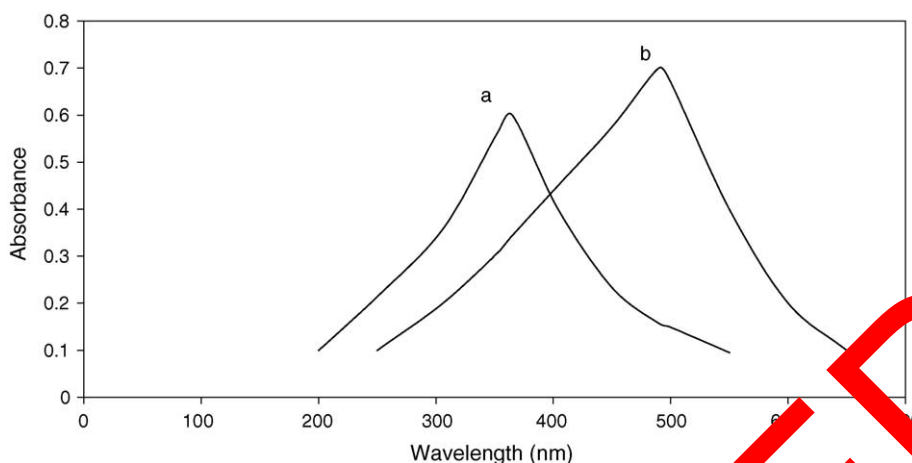


Fig. 3. Absorption spectrum of (a) 3,5-DMHAAINH vs. Reagent (360 nm), and (b) Au(III)–(3,5-DMHAAINH)₃ Complex (490 nm).

functional group. Therefore, Au(III)–(3,5-DMHAAINH)₃ may be considered as 1:3 ligand (M:R) (M, metal; R, reagent). This reagent is not exploited much for the spectrophotometric determination of metal ion. The reagent solution is found to be stable for 72 h. The *pK* value is determined by recording the UV–vis spectra of 0.02 M solution of the reagent at various pH values. The value of deprotonation of 3,5-DMHAAINH is found to be 2.6 *pK* value assigned due to the deprotonation of imine (–NH) through enolization. The complex formation reaction between Au(III) with 3,5-DMHAAINH has been studied in detailed. Based on the composition of the complex as determined by Job's and molar ratio method, the structure in Fig. 1(b) is suggested for Au(III)–(3,5-DMHAAINH)₃ complex.

3.1. Analytical parameters

Beer's law was obeyed in the concentration range 0.30–4.00 $\mu\text{g ml}^{-1}$ of Au(III). The molar absorptivity of complex at 490 nm and at pH 6.0 was calculated as $3.45 \times 10^4 \text{ M}^{-1}$. Sandell's sensitivity of the method was found to be 0.0050 $\mu\text{g ml}^{-1}$. The correlation coefficient (γ) for the experimental data is 0.998. The composition of the complex determined using Job's continuous variation method indicated the formation of 1:3 complex. The stability constants corresponding to the maxima were determined and found to be 9.3×10^{15} (1:3, M:R).

3.2. Optimization of flow system

3.2.1. Effect of pH

The effect of pH on the peak height of Au(III) at different concentrations was investigated with a fixed 0.02 M 3,5-DMHAAINH concentration in the reagent carrier solution. The pH of phosphate buffer in the reagent carrier solution was changed over a range of 3.0–7.0 and the peak height were measured for each concentration level of Au(III). At all concentration levels of Au(III), maximum peak heights were

found between pH 5.0 and 6.0. Therefore, a pH 6.0 for the phosphate buffer system was chosen throughout in the study.

3.2.2. Effect of flow-rate

The effect of the flow-rate on the peak height was studied in the range of 0.5–2.0 ml min^{-1} . The peak height decreased with the increasing of the flow-rate. Taking into consideration of the stability of the pump, peak shape and sampling time, the flow-rate of the reagent carrier solution was adjusted to 1.0 ml min^{-1} .

3.2.3. Sample volume

The influence of the sample volume on the absorbance was investigated by injecting different volumes (200–270 μl) of Au(III) standard solutions into the system at the 1.0 ml min^{-1} flow-rate. The injection volume has a significant effect, yielding increased peak height and reproducibility with increase in the injection volume. However, increasing the injection volume widens the sample zone and lowers the sampling rate, so that 250 μl was taken as a compromise.

3.2.4. Effect of 3,5-DMHAAINH concentration

The effect of concentration of 3,5-DMHAAINH, in the reagent carrier solution, on the peak height was investigated at pH 6.0 and a flow-rate of 1.0 ml min^{-1} , by using 2.1 and 3.4 μl^{-1} Au(III) solutions. The concentrations of 3,5-DMHAAINH was varied over the range 0.001–0.10 M. Maximum peak height was obtained at a concentration of 0.02 M 3,5-DMHAAINH as color developing reagent in the carrier solution for lower concentration level of Au(III) in the sample solution.

3.2.5. Calibration graph

The equation of a typical calibration is $p = 5.32c + 4.94$, $r^2 = 0.9993$ (p , peak height; c , concentration). The calibration graph was obtained at the optimum working conditions: flow-rate 1.0 ml min^{-1} , 3,5-DMHAAINH concentration 0.02 M, volume injected 250 μl and carrier stream

NH₄Cl–phosphate buffer solution in ratio 10:40 (v/v) at pH 6.0.

3.3. Method evaluation

The proposed flow injection method was critically evaluated with regard to reproducibility, accuracy, detection limit and sample frequency.

3.3.1. Reproducibility

To test the reproducibility of the FIA, four repetitive analysis cycles of each sample were run. A%R.S.D. in the range 2.01–3.76 was obtained as shown in Tables 2 and 3, respectively.

3.3.2. Accuracy

The accuracy of the proposed flow injection method was evaluated by comparing the results with those obtained by the reported methods [20,23]. The results shown in Tables 2 and 3 reveals that the good correlation between the two methods indicative of present method is more sensitive than the reported methods in literature [20,23].

3.3.3. Sampling frequency

The experimental period for one complete analytical cycle was 240 s long giving an overall sampling rate of 15 samples h⁻¹.

3.3.4. Detection limit

Under optimized conditions the detection limit for determination of Au(III) by flow injection method (signal to noise ratio = 2) obtained was 0.10 µg l⁻¹ for 250 µl injection volume Au(III) ion solution.

3.3.5. Effect of foreign species

Several cations and anions, as potential interferents for different sample matrices were studied in detail. Table 1 summarises the tolerance limits of interfering ions in the determi-

Table 1

Tolerance limits of non-target ions in the determination of Au(III) pH = 6.0, amount of Au(III) = 100 µg l⁻¹

Ion	Tolerance limit (µg ml ⁻¹)	Ion	Tolerance limit (µg ml ⁻¹)
EDTA	37	Pd ^{+2a}	Interferes
Citrate	496	Ce ⁺⁴	28
Bromate	563	U ⁺⁶	30
Thiourea	836	TI ⁺³	36
Oxalate	968	Fe ⁺³	40,1000 ^a
Phosphate	1045	Ag ⁺¹	50
Iodide	1270	Cd ⁺²	112
Thiosulphate	1375	W ⁺⁶	53
Ascorbate	1780	V ⁺⁴	59
Tartrate	1800	Zn ⁺²	120
Fluoride	1900	Cu ⁺²	6
Nitrate	3400	Mo ⁺⁶	2
Chloride	3850	Pt ⁺⁴	98
Sulphate	5200	Ca ⁺²	110
		Cd ⁺²	112
		Zn ⁺²	120
		Al ⁺²	130
		Ni ⁺²	159
		Mg ⁺²	264
		Sn ⁺²	295
		Ba ⁺²	330

^a In the presence of 50 µg ml⁻¹ of fluoride.

nation of 100 µg l⁻¹ Au(III). The tolerance limit was taken as the amount, which caused an error of ±2% at the peak height. Almost all of the ions examined did not interfere with the Au(III). Of the ions tested, the only negligible interference were from Pd(II), V(V), and Fe(III) at the amount of 1000 µg l⁻¹. However, Pd(II), V(V) and Fe(III) interference can be successfully avoided by masking them with suitable amount of fluoride.

3.3.6. Application

To evaluate the applicability of the proposed flow-injection method, it was applied to the determination of

Table 2

Recovery of Au(III) in synthetic and natural water samples by flow-injection spectrophotometric method

Sample	Recovery of Au ³⁺ by present method					% Recovery of Au ³⁺ by reported method [23]				
	Added µg	Found µg l ⁻¹	Recovery %	RSD ^a	F-test ^b	t-test ^c	Added µg l ⁻¹	Found µg l ⁻¹	Recovery %	RSD ^a
Synthetic sample	12.0	11.98 ± 0.20	99.80 ± 0.40	2.24	1.13	0.32	10.0	9.10	91.0 ± 0.50	2.12
	–	7.48 ± 0.30	–	–	–	–	–	–	–	–
Tap water	24.0	23.92 ± 0.40	99.60 ± 0.20	2.01	1.74	0.54	20.0	19.80	99.0 ± 0.20	3.36
	–	3.15 ± 0.10	–	–	–	–	–	–	–	–
River water	48.0	47.87 ± 0.50	99.70 ± 0.10	4.13	1.74	0.61	30.0	29.90	99.66 ± 0.40	2.29
	–	1.41 ± 0.20	–	–	–	–	–	–	–	–
Spring water	96.0	95.87 ± 0.10	99.86 ± 0.30	2.78	1.32	0.59	40.0	39.80	99.50 ± 0.30	3.76
	–	2.64 ± 0.80	–	–	–	–	–	–	–	–
Laboratory water	192.0	191.55 ± 0.30	99.76 ± 0.20	2.50	1.01	0.28	50.0	49.2	98.40 ± 0.80	2.51

^a For four individual determinations.

^b Tabulated *F*-value for (4,4) degrees of freedom at *p* (0.95) is 4.11.

^c Tabulated *t*-value for 4 degrees of freedom at *p* (0.95) is 1.53.

Table 3
Determination of gold(III) in various pharmaceutical samples using 3,5-DMHAAINH with flow-injection spectrophotometric method

Sample	Amount of gold ($\mu\text{g ml}^{-1}$)		Relative error (%)
	Present method ^a	Reported method [20]	
Rheumartho with gold	1.02	1.01	-0.99
	1.82	1.84	1.08
	2.13	2.11	-0.94
	3.17	3.13	-1.27
K.K. Forte with gold	0.42	0.44	4.54
	0.87	0.86	-1.16
	1.20	1.24	3.22
	2.49	2.45	-1.63
Auranofin (tablet)	0.87	0.86	-1.16

^a Four individual determinations.

Au(III) in water and pharmaceutical samples. The samples collected from different sources were filtered and then directly injected into the FIA. The obtained results was compared with the reported methods [20,23] in terms of Student's *t*-test and *F*-test. The analytical data summarised in Tables 2 and 3 suggest that the percentage of recovery of Au(III) from water and pharmaceutical samples ranges from 98.0 to 99.90% which is more reliable and sensitive than the methods reported in literature.

4. Conclusion

3,5-DMHAAINH is one of the most selective reagent for the flow-injection spectrophotometric determination of Au(III) ion. The newly synthesised color developing reagent is more economical and easy to prepare in an ordinary laboratories. The FIA is highly facile, selective, sensitive and rapid than the methods reported in literature. Because of the stable

complex of Au(III)-(3,5-DMHAAINH)₃ made to study in environmental samples may be extended for the routine analysis of pharmaceutical samples of bulk and formulations.

References

- [1] B.K. Puri, M. Satake, G. Kano, S. Usami, *Anal. Chem.* 59 (1987) 1850.
- [2] P. Buba, P.G. Willmer, *Fresenius J. Anal. Chem.* 329 (1987) 1850.
- [3] EPA-600/4-79-02, Method for chemical analysis of water and waste, Environmental protection Agency, Research Triangle Park, NC, 1983.
- [4] G. Xue, *Guijinsu* 10 (1989) 40.
- [5] M. Liang, C. Wang, Y. Zhang, *Shanxi Daxue Xuebao*, *Ziran Kexueban* 19 (1996) 297.
- [6] F.M. Elzowawy, M.F. Elshahat, A. El-Mohammi, M.T.M. Zaki, *Analyst* 120 (1995) 549.
- [7] Z.B. Li, J.L. Wang, Q. He, *Mikrochim. Acta* 116 (1994) 219.
- [8] Z. Li, X.O. Chongning, J. Liu, *Fenxi Kexue Xuebao* 14 (1998) 305.
- [9] Z. Li, J. Duchun, J. Wang, Q. Xu, *Fenxi Shiyanshi* 17 (1998) 8.
- [10] M. Liang, Z. Wang, Chengye, Z. Youxian, *Shanxi Daxue Xuebao*, *Xueban* 19 (1996) 297.
- [11] S. Liu, B. Xia, *Youkuangye* 7 (1988) 24.
- [12] S. Yin, *Fenxi Shiyanshi* 7 (1988) 64.
- [13] G. Ma, *Yeji*, *Fenxi* 8 (1988) 52.
- [14] W. Wang, J. Jiahou, *Xuaxue Fexie* 24 (1988) 171.
- [15] Y. Yu, *Youkuangye* 15 (1996) 112.
- [16] M. Lak, *Chemia analiyezna* 37 (1992) 683.
- [17] J. Bai, *Taiyuan Gangyae Daxue Xuebao* 20 (1989) 73.
- [18] T. Mori, H. Tominaga, Y. Kujita, T. Matsue, *Anal. Lett.* 30 (1997) 953.
- [19] A.A.Y. Elsayed, *Bull. Chem. Soc. Jpn.* 67 (1994) 3216.
- [20] Z. Marzenko, *Spectro photometric determination of Elements*, John Wiley and Sons Inc., New York, 1976, pp. 283.
- [21] M.A. Kabil, S.E. Ghazy, M.A. Mastafa, A.A. Elasmay, *Fresenius J. Anal. Chem.* 349 (1994) 775.
- [22] P.T. Sah, S.A. Peoples, *J. Am. Pharm. Assoc. Sci. Edn.* 43 (1954) 513.
- [23] M. Bagheri, M.H. Mashhadizadeh, S. Razee, *Talanta* 60 (2003) 839.