



## 3-Ethyl-4-(*p*-chlorobenzylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one (EPHBAT) as precipitant for carrier element free coprecipitation and speciation of chromium(III) and chromium(VI)

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### ABSTRACT

A method for the separation and speciative determination of Cr(VI) and Cr(III) has been developed. The procedure is based on coprecipitation of Cr(III) on 3-ethyl-4-(*p*-chlorobenzylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one (EPHBAT) without carrier element. The Cr(III) can be selectively precipitated on EPHBAT in the pH range of 8.0–9.0, while Cr(VI) cannot be retained. Total chromium was determined after the reduction of Cr(VI) to Cr(III) with 0.5 mL of concentrated H<sub>2</sub>SO<sub>4</sub> and 0.5 mL of ethanol. Cr(VI) concentrations were obtained as the respective differences between total chromium and Cr(III). Experiments were performed to optimize conditions, such as pH, amounts of EPHBAT, sample volume, etc. A preconcentration factor of 50-fold was achieved for Cr(III). The detection limit of the method for Cr(III) was 1.0 µg L<sup>-1</sup>. To validate the developed method, the certified reference materials (NIST SRM 1573a and GBW 0703) were analyzed. The method was applied for the speciation of chromium in spiked natural water samples with satisfactory results.

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### 1. Introduction

Heavy metals (HMs) pollution is of great concern because their toxicity threatens the human life and the environment [1–5]. In the determinations of heavy metals at trace levels, atomic absorption spectrometry and atomic emission spectrometry are the main instrumental techniques [6–10]. However, the determination of different species of heavy metals by atomic absorption spectrometry and atomic emission spectrometry are not directly possible [10–14]. In order to overcome this difficulty, the speciation procedures have been immensely developed by researchers [15–20]. The speciation procedures require both a separation/preconcentration processes and selective removal of one of the species before determinations [18–23].

Chromium participates in different metabolic routes; however, at high concentrations it is toxic for many living organisms [24–28]. Chromium usually occurs in two different forms, Cr(III) and Cr(VI). The Cr(VI) is extremely toxic and may cause contact allergic dermatitis on the skin and may also be a trigger for many diseases [29,30]. Chromium(III) assisted different enzymatic reaction in human body such as regulate functioning glucose, lipid, and

protein metabolism [29–35]. For the determination of chromium species, Cr(III) and Cr(VI), different separation-enrichment techniques including solvent extraction, membrane filtration, cloud point extraction, solid phase extraction and coprecipitation have been used [19–22,36–39].

The complexing reagent, 3-ethyl-4-(*p*-chlorobenzylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one (EPHBAT) was recently synthesized and characterized by Kahveci et al. [40]. It was firstly used in the presented work as coprecipitant for the speciation and preconcentration of chromium species in environmental samples.

### 2. Experimental

#### 2.1. Instrumental

A PerkinElmer AAnalyst 700 (Norwalk, CT, USA) atomic absorption spectrometer with deuterium background corrector, equipped with a chromium hollow cathode lamp operated at 25 mA, was used throughout this work. The wavelength and spectral band pass were set at 357.9 nm and 0.7 nm, respectively. A 10 cm long slot-burner head and an air-acetylene flame were used. The nebulizer uptake rate was adjusted to give the optimum response for conventional sample aspiration, the resulting rate being 6.0 mL min<sup>-1</sup>. An acetylene flow rate of 2.5 L min<sup>-1</sup> was used with an air flow rate of 17.0 L min<sup>-1</sup>.

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A pH meter, Sartorius pp-15 Model glass-electrode was employed for measuring pH values of the aqueous phase. Milestone Ethos D closed vessel microwave system (maximum pressure 1450 psi) was used.

## 2.2. Reagents and solution

All chemicals used in this work, were of analytical reagent grade and were used without further purification. Double deionized water (Milli-Q Millipore  $18.2 \text{ M}\Omega \text{ cm}^{-1}$  resistivity) was used for all dilutions. The glassware used was soaked in 10% (v/v)  $\text{HNO}_3$  solution for one day before used then cleaned repeatedly with distilled/deionized water. For storage of water samples, polypropylene bottles were used prior to analysis.

Stock solutions of Cr(III) and Cr(VI) ( $1000 \text{ mg L}^{-1}$ ) were prepared from  $\text{Cr}(\text{NO}_3)_3$  in  $0.5 \text{ mol L}^{-1}$   $\text{HNO}_3$  and  $\text{K}_2\text{CrO}_4$  in water, respectively. The standard solutions used for calibration were prepared by diluting a stock solution of  $1000 \text{ mg L}^{-1}$  of the given element supplied by Sigma (Chem. Co., St. Louis). Stock solutions of diverse elements were prepared from high purity compounds. The calibration standards were not submitted to the preconcentration procedure.

The EPHBAT used as a coprecipitating agent was synthesized as reported elsewhere [40]. For separation and preconcentration method, 0.5% (w/v) EPHBAT solution was prepared in ethanol. The certified reference materials used in the experimental studies were NIST SRM 1573a Tomato Leaves and GBW 0703 Bush Branches and Leaves.

Ammonium acetate buffers (0.1 M) were prepared by adding an appropriate amount of acetic acid to ammonium acetate solutions to result in solutions of pH 4–6. For pH 7, sodium borate (0.1 M) buffer solution was used. Ammonium chloride buffer solutions (0.1 M) were prepared by adding an appropriate amount of ammonia to ammonium chloride solutions to result in solutions of pH 8–10.

## 2.3. Test procedure

1.0 mL of EPHBAT (0.5%, w/v) was added into a 50 mL of aqueous solution containing  $20 \mu\text{g}$  of chromium(III) ions. The pH of the solution was adjusted to 8.0. After standing 10 min, the solution was centrifuged at 3500 rpm for 30 min. The supernatant was removed. The precipitate remained adhering to the tube was dissolved with 0.5 mL of concentrated  $\text{HNO}_3$ . Final volume was completed up to 5 mL with deionized water, and then the content was subjected to FAAS by conventional aspiration for the determination of under-study analyte ions.

Total chromium was determined as Cr(III) by the method described above after reducing Cr(VI) to Cr(III). The reduction of Cr(VI) to Cr(III) was performed by the addition of 0.5 mL of concentrated  $\text{H}_2\text{SO}_4$  and 0.5 mL of ethanol [30]. Cr(VI) concentrations were obtained as the respective differences between total chromium and Cr(III). The optimum conditions for separation of Cr(III) from Cr(VI) and for preconcentration of Cr(III) have been determined by using the test procedure given above.

## 2.4. Applications

The water samples analyzed were filtered through a Millipore cellulose membrane of pore size  $0.45 \mu\text{m}$ . The samples were stored in polyethylene bottles and acidified to 1% with nitric acid and were subsequently stored at  $4^\circ\text{C}$  in a refrigerator. Before the analysis, the pH of the samples were adjusted to 8. Then procedure given in Section 2.3 was applied.

Prior to preconcentration step for solid samples analyzed, NIST SRM 1573a Tomato Leaves, GBW 0703 Bush Branches and Leaves,

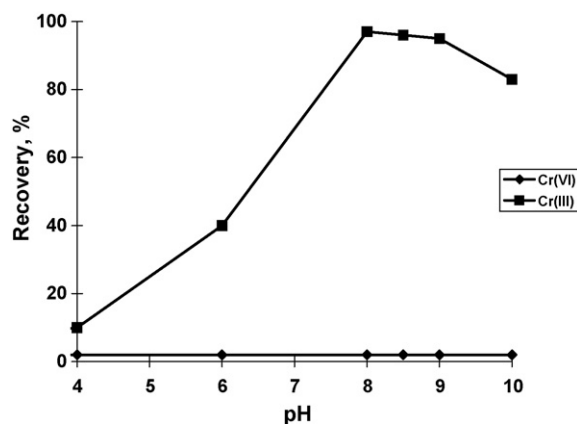


Fig. 1. Effect of pH on Cr(III) and Cr(VI) recoveries ( $N=3$ ).

wheat, black tea and tobacco were acid digested with microwave oven. Digestion conditions for microwave system for the samples were applied as 2 min for 250 W, 2 min for 0 W, 6 min for 250 W, 5 min for 400 W, 8 min for 550 W, vent: 8 min, respectively [41–43].

NIST SRM 1573a Tomato Leaves and GBW 0703 Bush Branches and Leaves standard reference materials (0.25 g) and wheat (1.00 g), tobacco (1.00 g) and black tea (1.00 g) samples were digested with 6 mL of  $\text{HNO}_3$  (65%), 2 mL of  $\text{H}_2\text{O}_2$  (30%) in microwave digestion system and diluted to 50 mL with deionized water.

After microwave digestion, the volume of the sample was made up to 25.0 mL with deionized water. Blanks were prepared in the same way as the sample, but omitting the sample. The preconcentration procedure given above was applied to the samples. The final volume was 2.0 mL.

## 3. Results and discussion

### 3.1. Influences of pH

The effect of pH on the recoveries of Cr(III) and Cr(VI) ions on EPHBAT precipitate were studied in the pH range of 4.0–10.0. Each pH was adjusted by using related buffer solutions. The results were given in Fig. 1. The quantitative recovery values were achieved for Cr(III) in the pH range of 8.0–9.0 but the quantitative recovery values were not obtained for Cr(VI) in the pH range of 4.0–10.0. Based on the result of optimum recovery, pH 8 was selected as working pH for all further coprecipitation works.

### 3.2. Influences of amount of EPHBAT

The effects of amount of EPHBAT on the recovery of Cr(III) were also investigated at pH 8.0. Different amounts of EPHBAT were tested and the results were given in Fig. 2. The recoveries of Cr(III) were not quantitative (67%) without adding EPHBAT. The results show that the recovery of Cr(III) increased with increasing amounts of EPHBAT; and reach to quantitative values at 5 mg of EPHBAT. For further works, 5 mg (1.0 mL of 0.5%, w/v) of EPHBAT was used.

### 3.3. Centrifugation time

The influences of the centrifugation time on the recoveries of the analyte ions were investigated in the range of 5–30 min at 3500 rpm. Quantitative recoveries were obtained for Cr(III) in the range of 20–30 min. All further studies were performed for 30 min centrifugation time at 3500 rpm.

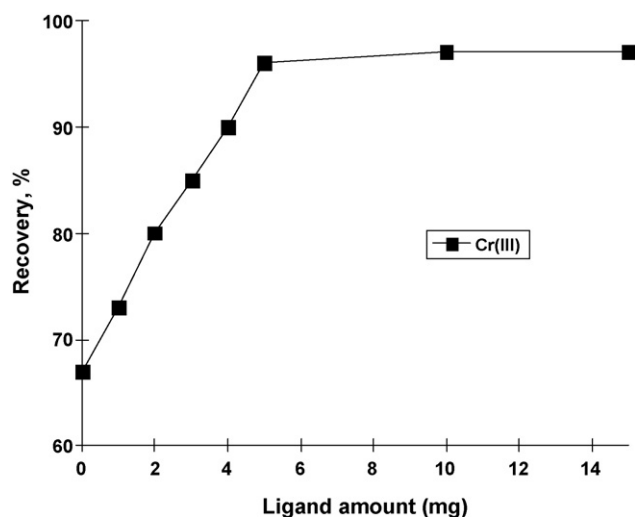


Fig. 2. Effect of quantity of EPHBAT on the recoveries of Cr(III) ( $N=3$ ).

### 3.4. Effect of sample volume

The effect of sample volume on the recovery of chromium(III) ions on the coprecipitation system was analyzed. The sample volumes were investigated at pH 8.0 range of 25–250 mL. The precipitate EPHBAT was added to sample and after precipitation of analyte ions and then the solution subjected to centrifuged at 3500 rpm for 30 min. The precipitate was dissolved with 0.5 mL of concentrated  $\text{HNO}_3$ . Final volume was made 2 mL with deionized water, and then the content was analyzed by FAAS for the determination of analyte ions. The results are given in Table 1. The analyte ions quantitatively recovered from up to 100 mL of sample volume. The preconcentration factor is calculated by the ratio of the highest sample volume (100 mL) and the lowest final volume (2 mL) for Cr(III) at pH 8.0. The preconcentration factor was calculated as 50.

### 3.5. Effect of divers ions

The influences of matrix ions were investigated on the recoveries at optimum values of other variables. The maximum concentrations of the various matrix ions were selected and investigated. There was no significant effect of matrixes ions was observed on the quantitative recovery of Cr(III). The results are listed in Table 2. The presented procedure could be applied to the speciation and preconcentration of Cr(III) and Cr(VI) at pH 8.0 with 1.0 mL of 0.5% (w/v) of EPHBAT in ratio of analyte to matrix ions. The tolerance limit of foreign ions was taken as that value which caused an error of not more than  $\pm 5\%$  in the absorbance. The ions normally present in water do not interfere under the experimental conditions used. Also, some of the transition metals at  $100 \text{ mg L}^{-1}$  did not interfere with the recover-

**Table 1**  
Effects of sample volume on Cr(III) recoveries ( $N=3$ ).

Sample volume (mL)	Recovery (%)
25	$97 \pm 2^a$
50	$95 \pm 3$
100	$95 \pm 2$
250	$70 \pm 3$

<sup>a</sup> Mean  $\pm$  standard deviation.

**Table 2**  
Influences of some foreign ions on the recoveries of Cr(III) ( $N=3$ ).

Ion	Added as	Ratio of analyte to matrix ions	Recovery (%)
$\text{Na}^+$	NaCl	10000	$96 \pm 2^a$
$\text{K}^+$	KCl	2500	$97 \pm 2$
$\text{Ca}^{2+}$	$\text{CaCl}_2$	1500	$95 \pm 2$
$\text{Mg}^{2+}$	$\text{MgCl}_2$	1500	$95 \pm 1$
$\text{Cl}^-$	NaCl	15000	$95 \pm 3$
$\text{F}^-$	NaF	125	$95 \pm 2$
$\text{NO}_3^-$	$\text{KNO}_3$	1500	$97 \pm 3$
$\text{SO}_4^{2-}$	$\text{Na}_2\text{SO}_4$	1500	$96 \pm 2$
$\text{PO}_4^{3-}$	$\text{Na}_3\text{PO}_4$	125	$95 \pm 3$
$\text{Mn}^{2+}$	$\text{MnSO}_4$	50	$96 \pm 3$
$\text{Fe}^{3+}$	$\text{FeCl}_3$	50	$97 \pm 2$
$\text{Cu}^{2+}$	$\text{CuSO}_4$	50	$97 \pm 2$
$\text{Pb}^{2+}$	$\text{Pb}(\text{NO}_3)_2$	50	$96 \pm 2$
$\text{Zn}^{2+}$	$\text{ZnSO}_4$	50	$99 \pm 4$
$\text{Cd}^{2+}$	$\text{Cd}(\text{NO}_3)_2$	50	$98 \pm 3$
$\text{Ni}^{2+}$	$\text{NiSO}_4$	50	$95 \pm 2$
$\text{Cr}^{6+}$	$\text{K}_2\text{Cr}_2\text{O}_7$	125	$95 \pm 3$

<sup>a</sup> Mean  $\pm$  standard deviation.

ies of the analyte ions. Cr(III) recoveries were quantitative at the presence of  $250 \text{ mg L}^{-1}$  Cr(VI).

### 3.6. Determination of total chromium

In order to investigation of total chromium, firstly model solutions were prepared. These model solutions were contained different amounts of Cr(III) and Cr(VI). After Cr(VI) ions in the model solutions were reduce to Cr(III) were performed by the procedure given by Bulut et al. [30]. The reduction of Cr(VI) to Cr(III) was performed by the addition of 0.5 mL of concentrated  $\text{H}_2\text{SO}_4$  and 0.5 mL of ethanol to the water samples and applied to coprecipitation procedure then precipitate dissolved in acid solution and total Cr(III) was analyzed by FAAS. The results are shown in Table 3. The results show that this method could be applied for the determination of total chromium.

### 3.7. Figure of merits

Calibration curve was linear in the range of  $0.5\text{--}10 \mu\text{g mL}^{-1}$ . Regression equation was  $A=0.0245C+0.0011$  with ( $R^2=0.9998$ ). The limit of detection, defined as the concentration equivalent to three times the standard deviation ( $n=21$ ) of the reagent blank. The detection limit (LOD) of this work was calculated as  $1.0 \mu\text{g L}^{-1}$  under optimal experimental conditions. The precision of the determination of Cr(III) was evaluated under the optimum conditions mentioned above for this procedure. The recovery values for the analyte ions were in the range of 95–99% at 95% confidence level. The relative standard deviation (RSD) was calculated  $<8\%$ . Relative error was found to be lower than 4%. These values were quantitative and it shows that this coprecipitation method can be applied for the speciation and preconcentration of Cr(III) and Cr(VI) ions in real samples at pH 8.0 with using 1.0 mL of 0.5% (w/v) of EPHBAT.

**Table 3**  
Total chromium determinations in spiked test solutions (volume: 50 mL,  $N=4$ ).

Added ( $\mu\text{g}$ )		Found ( $\mu\text{g}$ )			Recovery (%) <sup>a</sup>		
Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Total Cr	Cr(III)	Cr(VI)	Total Cr
0	30	–	$29.3 \pm 0.9$	$29.3 \pm 0.9$	–	$98 \pm 2$	$98 \pm 2$
10	20	$9.7 \pm 0.5$	$19.4 \pm 0.8$	$29.1 \pm 0.8$	$97 \pm 2$	$97 \pm 2$	$97 \pm 2$
20	10	$19.3 \pm 0.9$	$9.6 \pm 0.4$	$28.9 \pm 0.9$	$97 \pm 3$	$96 \pm 2$	$96 \pm 2$
15	15	$14.5 \pm 0.7$	$14.2 \pm 0.6$	$28.7 \pm 0.8$	$97 \pm 2$	$95 \pm 2$	$96 \pm 2$
30	0	$28.7 \pm 0.9$	–	$28.7 \pm 0.9$	$96 \pm 2$	–	$96 \pm 2$

<sup>a</sup> Mean  $\pm$  standard deviation.

**Table 4**  
Speciation of Cr(III) and Cr(VI) and total chromium in some natural water samples<sup>a</sup> (sample volume: 100 mL, N=4).

Samples	Added ( $\mu\text{g}$ )		Found ( $\mu\text{g}$ )			Recovery (%)		
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Total Cr	Cr(III)	Cr(VI)	Total Cr
Tap water	–	–	1.1 $\pm$ 0.1	BDL	1.1 $\pm$ 0.1	–	–	–
	3.0	3.0	3.9 $\pm$ 0.2	2.9 $\pm$ 0.2	6.8 $\pm$ 0.3	95 $\pm$ 2	97 $\pm$ 2	96 $\pm$ 2
	5.0	5.0	6.0 $\pm$ 0.4	4.8 $\pm$ 0.3	10.8 $\pm$ 0.5	98 $\pm$ 2	96 $\pm$ 2	97 $\pm$ 2
Hot mineral spring water	–	–	1.5 $\pm$ 0.1	0.5 $\pm$ 0.1	2.0 $\pm$ 0.1	–	–	–
	3.0	3.0	4.3 $\pm$ 0.2	3.4 $\pm$ 0.2	7.7 $\pm$ 0.4	96 $\pm$ 2	97 $\pm$ 2	96 $\pm$ 2
	5.0	5.0	6.3 $\pm$ 0.4	5.2 $\pm$ 0.3	11.5 $\pm$ 0.8	97 $\pm$ 2	95 $\pm$ 2	96 $\pm$ 2
Sea water	–	–	1.3 $\pm$ 0.1	BDL	1.3 $\pm$ 0.1	–	–	–
	3.0	3.0	4.1 $\pm$ 0.2	2.9 $\pm$ 0.1	7.0 $\pm$ 0.3	95 $\pm$ 2	97 $\pm$ 2	96 $\pm$ 2
	5.0	5.0	6.0 $\pm$ 0.3	4.9 $\pm$ 0.2	10.9 $\pm$ 0.6	95 $\pm$ 2	98 $\pm$ 2	96 $\pm$ 2

BDL: below detection limit.

<sup>a</sup> Mean  $\pm$  standard deviation.**Table 5**

The level of total chromium in certified reference materials and environmental samples after application of the presented procedure (N=4).

Reference material	Certified value	Our value	Relative error (%)
NIST SRM 1573a Tomato Leaves ( $\mu\text{g g}^{-1}$ )	1.99	1.93 $\pm$ 0.10	–3.0
GBW 0703 Bush Branches and Leaves ( $\mu\text{g g}^{-1}$ )	2.6	2.50 $\pm$ 0.15	–3.8
Wheat ( $\mu\text{g g}^{-1}$ )	–	1.10 $\pm$ 0.10	–
Black tea ( $\mu\text{g g}^{-1}$ )	–	1.49 $\pm$ 0.12	–
Tobacco ( $\mu\text{g g}^{-1}$ )	–	1.98 $\pm$ 0.15	–

Mean expressed as 95% tolerance limit.

**Table 6**

Characteristic performance of some recent studies on chromium speciation using coprecipitation.

Detection system	Precipitant	Carrier element	pH	PF	DL ( $\mu\text{g L}^{-1}$ )	RSD (%)	Reference
FAAS	5-Chloro-3-[4-(trifluoromethoxy) phenylimino]indolin-2-one	–	8.0	40	0.7	<5	[30]
GFAAS	8-Quinolinol/tannic acid	Pd	5.1–5.3	–	0.02	<1	[47]
FAAS	Ethyl xanthate complex	–	2.5	100	0.5	3.1	[48]
FAAS	–	Tm	12.0	200	0.87	<10	[49]
FAAS and ICP-MS	9-Phenyl-3-fluorone	Cu	7.0	30	6.1	<2.8	[50]
FAAS	5-Chloro-2-hydroxyaniline	Cu	8.0	50	1.2	<5	[51]
GFAAS	–	Tb	9.0	–	0.3	3.2	[52]
FAAS	–	Ce	6.0	200	0.18	1.6–8.0	[53]
GFAAS	PDC	Pb	9.0	15	0.02	3.9	[54]
FAAS	3-Ethyl-4-(p-chlorobenzylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one	–	8.0	50	1.0	<8	This work

PF: preconcentration factor; DL: detection limit.

### 3.8. Determination of chromium in real samples

We have investigated this procedure for speciation and preconcentration Cr(III), Cr(VI) and total chromium ions in some water sample and real samples. The water samples (tap water, hot mineral spring water and sea water) results are given in Table 4. The recovery of water samples results were obtained satisfactorily.

Also this coprecipitation method was applied to microwave digested standard reference materials (NIST SRM 1573a Tomato Leaves and GBW 0703 Bush Branches and Leaves) and real samples (wheat, black tea and tobacco) for total chromium determination. The results are given in Table 5. The results are in good agreement with certified values for chromium.

## 4. Conclusion

Trace elements play important negative and positive roles in human life [44–46]. EPHBAT is firstly used in the presented work for the coprecipitation of chromium species in natural water samples, certified and other real samples. A comparison of the presented procedure and some recent speciation procedures for Cr(III) and Cr(VI) in the literature were given in Table 6. The detection limits and preconcentration factors are superior than some of the preconcentration/separation techniques [30,47–57]. This method is very

simple, rapid and low cost. Only 2.0 g of EPHBAT is used in all the experiments. Matrixes ions effect were negligible.

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