

## Trace element analysis in corn by cloud point extraction

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**Abstract:** In this study, Lead (Pb), Copper (Cu), and Tin (Sn) elements determined in corn samples were analyzed from three different regions of Sakarya province. A cloud point extraction (CPE) method was used for the metal determination study and analysis by inductively coupled plasma-optical emission spectroscopy (ICP-OES). In this study, optimization processes were performed in cloud point extraction 2-(4-sulphonylamidebenzo)hydrazide-1-dithiocarbamate salt as ligand and polyethylene glycol *tert*-octylphenyl ether (Triton X-114) as surfactant. Three different corn samples, soil samples, and certified reference materials (GBW10011 and GBW10012) were analyzed. Recovery values in corn samples were 95.5-97.1% for Cu, 101.8-105.2% for Pb, and 94.1-97.3% for Sn; Recovery values for soil samples were 99.3-104.3% for Cu, 93.6-103.3% for Pb and 99.5-101.6% for Sn detected among them.

**Keywords:** Corn; trace element; 2-(4-sulphonylamidebenzo)hydrazide-1-dithiocarbamate salt; ICP-OES; cloud point extraction (CPE). ©2024 ACG Publication. All rights reserved.

### 1. Introduction

The corn plant is one of six grains that feed the Earth's population. It is the most produced grain in the world with its versatile usage area, adaptability, and efficiency [1]. Corn plants are generally used as human food and animal feed worldwide. Many products in the industry are raw materials [2]. Most of the corn produced in developed countries is used as animal feed. Fuel, glucose, and corn oil obtained from corn kernels are valuable economic raw materials. Especially in the USA, ethanol is used as a raw material for corn [3].

Today, environmental pollution is one of the most critical dangers for all living things in the ecosystem [4]. Metal pollutants cause ecological pollution that can be classified as air, water, or soil pollution [5]. These metals, which can have a toxic effect even in trace amounts, are called heavy metals. Heavy metals have harmful effects and a tendency to accumulate in living tissues, especially in specific tissues. Therefore, heavy metals constitute an essential group of pollutants [6]. Heavy metals such as Cd, Pb, Cr, Hg, Ni, and Cu are a major environmental threat, especially in areas with high human-induced pressures (industrialization, mining excavations, urban waste, etc.) [7].

Lead, widely used industrially, exists in organic and inorganic forms. It is one of the most important heavy metals threatening human health [8]. The most crucial part of lead, an environmental pollutant, is caused by tetra ethyl lead, produced by burning gasoline used by motor vehicles. Lead

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residues have been found in marine organisms as industrial wastes are transported by water [9]. Cu is a pervasive substance in nature. Due to the development of copper production in recent years, the amount of copper in nature has increased [10]. Since copper can be found in many kinds of food, drinking water, and air, we take a significant amount of copper into our bodies by eating, drinking, and breathing daily [11]. Tin is known as a relatively non-toxic element [12]. Recently, it has been shown that high levels of tin in the body can be toxic and cause reactions [13].

Cloud point extraction (CPE) is an environmentally friendly procedure for the preconcentration and separation of many analytes that offers several benefits over conventional liquid-liquid extraction [14]. The procedure is fast, inexpensive, accurate, selective, and precise. Unlike liquid extraction, CPE requires the use of less toxic surfactants. Nonionic and anionic surfactants are most commonly used. It is called the green extraction procedure, which consumes minimal or non-toxic organic solvents [15]. CPE enriches inorganic and organic substances and is easily combined by spectroscopic or chromatographic methods [16,17].

The classical CPE procedure is based on the ability of the nonionic surfactant to form micelles in aqueous media when heated above a specific temperature (called cloud point or cloud temperature) or by adding salt (salting phenomenon) [18]. Centrifugation makes separation into two phases (one aqueous phase and one rich in surfactant) possible. The analyte is usually separated in the surfactant-rich phase [16].

Inductively coupled plasma-optical emission spectrometry (ICP-OES) is a powerful tool for the determination of metals in a variety of different sample matrices [19]. In inductively coupled plasma-optical emission spectrometry, the sample is usually transported to the instrument as a liquid sample stream. Inside the instrument, the liquid is converted into an aerosol in a process known as nebulization. The sample aerosol is then transported to the plasma, desolvated, vaporized, atomized, excited, and ionized by the plasma. It carries excess liquid from the spray chamber to the waste container. Also, it provides back pressure to the purge system, allowing it to flow more easily into the injection tube of the torch and the plasma, for example, via the aerosol-carrying gas flow system [20]. Another step in ICP-OES is to differentiate the radiation emitted by the element from that emitted by other elements or molecules [21]. Excited atoms and ions emit their characteristic radiation, which is picked up by a device that sorts the radiation by wavelength. The radiation is detected and converted into electronic signals for the analyst [22]. Detectors and auxiliary equipment are used to measure the intensity of a suitable emission line separated from the spectrometer [23].

In this study, a cloud point extraction method was utilized to concentrate and determine the levels of lead, copper, and tin in various samples of corn. For this purpose, 2-(4-sulphonylamidobenzo)hydrazide-1-dithiocarbamate salt was used as a hydrophobic complexing agent for metal ions, while polyethylene glycol *tert*-octylphenyl ether (Triton X-114) was used as a surfactant. The metal determination was done using ICP-OES.

## 2. Experimental

### 2.1. Materials and Methods

Corn and soil samples were obtained from the Akyazı, Serdivan, and Söğütlü districts of Sakarya province. Corn and soil samples were dried at 70 °C for 24 hours. The dried samples were ground in a porcelain mortar. After grinding, the samples were kept airtight until the time of analysis.

Spectro Arcos ICP-OES device (Spectro Acros, Germany) for measuring trace elements, pH meter (HANNA, HI 221) for pH adjustment, NF 400 model centrifuge device (NUVE, Türkiye), NB 20 model water bath (NUVE, Türkiye) used. Melting points were determined by a Yanagimoto micro-melting point apparatus (Surrey, UK) and were uncorrected. IR spectra were acquired on a SHIMADZU Prestige-21 (200 VCE) (Kyoto, Japan) spectrometer. <sup>1</sup>H and <sup>13</sup>C NMR spectra were acquired at VARIAN Infinity Plus at 300 and 75 Hz (Palo Alto, California), respectively. <sup>1</sup>H and <sup>13</sup>C chemical shifts are referenced to the internal deuterated solvent. All chemicals were purchased from Merck (Darmstadt, Germany), Alfa Aesar (Ward Hill, MA), and Sigma-Aldrich (Taufkirchen, Germany).

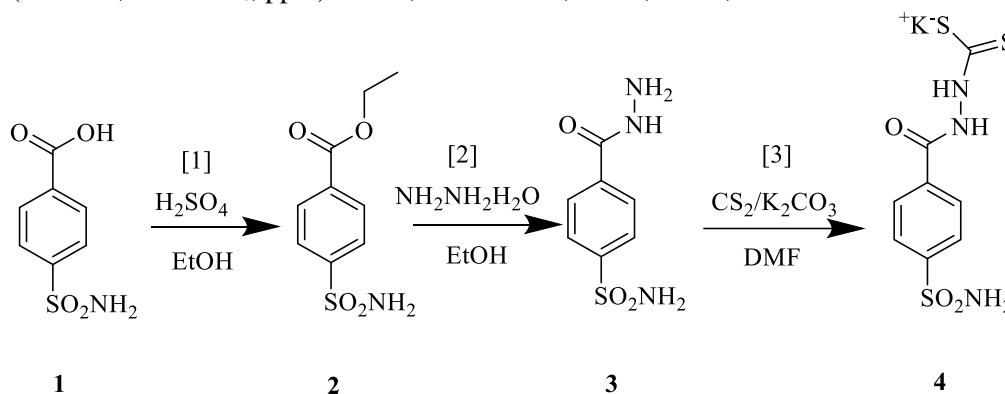
## 2.2. Synthesis of 2-(4-sulphonylamidebenzo)hydrazide-1-dithiocarbamate Salt

For the synthesis of ethyl-4-sulfamoyl benzoate (**2**), 4-sulfamoyl benzoic acid (**1**) (10 mmol) was refluxed for 24 h in 50 mL of ethanol using 1.0 mL of sulphuric acid as catalyst. At the end of the reaction, the solvent was evaporated, and the obtained product was filtered, washed with cold water, and dried [24]. After checking purity by NMR, the product was used for the next step of further purification.

For the synthesis of 4-sulfonylamidebenzohydrazide (**3**), 4-sulfonylamide ester (10 mmol) and hydrazine hydrate (25 mmol) in ethanol were refluxed for 24 h at 80 °C. The reaction mixture was cooled to room temperature, and the solid was filtered, then washed with water and dried [25]. After checking purity by NMR, the product was used for the next step of further purification.

For the synthesis of 2-(4-sulphonylamidebenzohydrazide)-1-dithiocarbamate salt (**4**), Carbon disulfide (2.5 mmol) and 4-sulfonylamidebenzohydrazide (1 mmol) were dissolved in DMF (6 mL) and  $K_2CO_3$  was added to the mixture [26]. The reaction mixture was stirred for 2 h at 5 °C. After the reaction was completed, the mixture was poured into ice-cold water. It was then filtered, dried, and crystallized from acetone.  $^1H$  NMR,  $^{13}C$  NMR, and IR analysis characterized the prepared compound shown in scheme 1.

2-(4-sulphonylamidebenzo)hydrazide-1-dithiocarbamate salt (**4**) Yield 75 % , m.p. 289 °C; IR ( $\nu_{max}$ ,  $cm^{-1}$ ) : 3287 (NH<sub>2</sub>), 3061 (NH), 3023 (=C-H ), 1175 (C=S);  $^1H$  NMR (300 MHz, DMSO-d<sub>6</sub>, , ppm) : 10. 67 (1H, s, NH ), 10. 21(1H, s, NH ), 8.26 (2H, d, =CH ), 8,13 (2H, d, =CH), 7.59 (2H, s, -NH<sub>2</sub>).  $^{13}C$ NMR (75 MHz, DMSO-d<sub>6</sub>, ppm): 215.1, 166.7 146.1, 134.6, 128.7, 127.0.



**Figure 1.** Synthesis of 2-(4-sulphonylamidebenzo)hydrazide-1-dithiocarbamate salt (**4**)

## 2.3. Microwave Digestion

For the microwave incineration process, 1 g of corn sample and 0.25 g of soil sample were weighed into the incinerators. 9 mL of suprapur 30% HCl and 3 mL of suprapur 65% HNO<sub>3</sub> were added to the samples. The dishes were closed, and the microwave digestion procedure was applied. Microwave processing: (I) 10 min, 1000 W, 200 °C, (II) 20 min, 1000 W, 200 °C. After shredding, cooling was done for 10 minutes. The cooled samples were transferred to a flask and diluted to 100 mL with ultrapure water to prepare sample solutions.

## 2.4. Cloud Point Extraction Procedure

Cloud point extraction method parameters need to provide optimum conditions for the study. The samples to be analyzed are taken from their solutions to the sample tube: 1 mL of 0.01 M 2-(4-sulphonylamidebenzo)hydrazide-1-dithiocarbamate salt, 0.1 mL of 5% (v/v) Triton X-114 is added to the total volume of 50 mL of ultrapure water and completed with. The pH was adjusted to 8 by adding 0.05 M HNO<sub>3</sub> and 0.05 M NaOH to the prepared solutions. Sample tubes were kept in a water bath at 70 °C for 20 minutes. The resulting solutions were centrifuged at 4000 rpm for 20 minutes. The rich phase was collected at the bottom of the tube by centrifugation. The tubes were placed in an ice bath and incubated for 30 min. The liquid phase was separated from the rich phase, and 4.5 mL of 2 M HNO<sub>3</sub> was added to the rich phase and diluted to be ready for analysis.

## 2.5. The ICP-OES Method

Inductively coupled plasma-optical emission spectroscopy method was used to determine the metals Pb, Cu, and Sn in the samples.

For the measurement to be made to the prepared samples, indium solution, which was used as an internal reference, was added to prepare it for the reading process. The reading process was performed in three repetitions for each sample. As a result of the analysis, the recovery study was carried out with the samples in which the metals to be examined were added.

The parameters of the ICP-OES device used in the study are shown in Table 1.

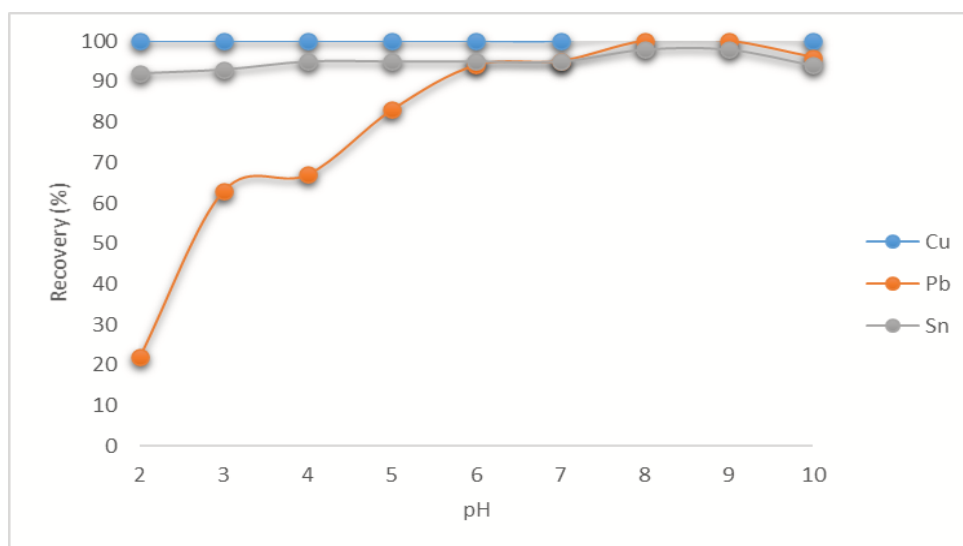
**Table 1.** ICP-OES operating conditions

Instrument	Spectro Acros
View Height	12 mm
Wavelength	Pb: 220.353 nm Cu: 324.754 nm Sn: 189.991 nm
Replication	3
RF Power	1400 W
Plasma gas flow	15 L/min
Argon gas flow	1 L/min
Pump speed	30 rpm

### 3. Results and discussion

#### 3.1. pH Effect

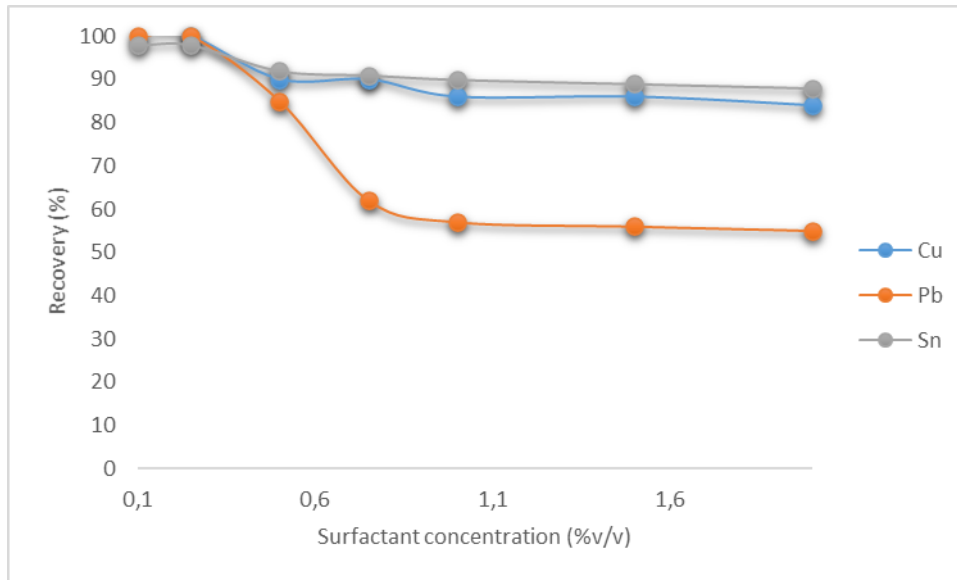
pH is an essential parameter for the stability of the complex formed by the combination of metal ions with the ligand. The pH study was carried out in the range of 2-10. The best results were obtained at pH 8. Recovery values are given in Figure 2.



**Figure 2.** Effect of pH on recovery of metal ions (1 mL of 0.01 M 2-(4-sulphonylamidebenzo)hydrazide-1-dithiocarbamate salt, 0.1 mL of 5% (v/v) Triton X-114, 50 mL of sample volume, 70 °C incubation for 20 minutes)

### 3.2. Effect of Surfactant Concentration

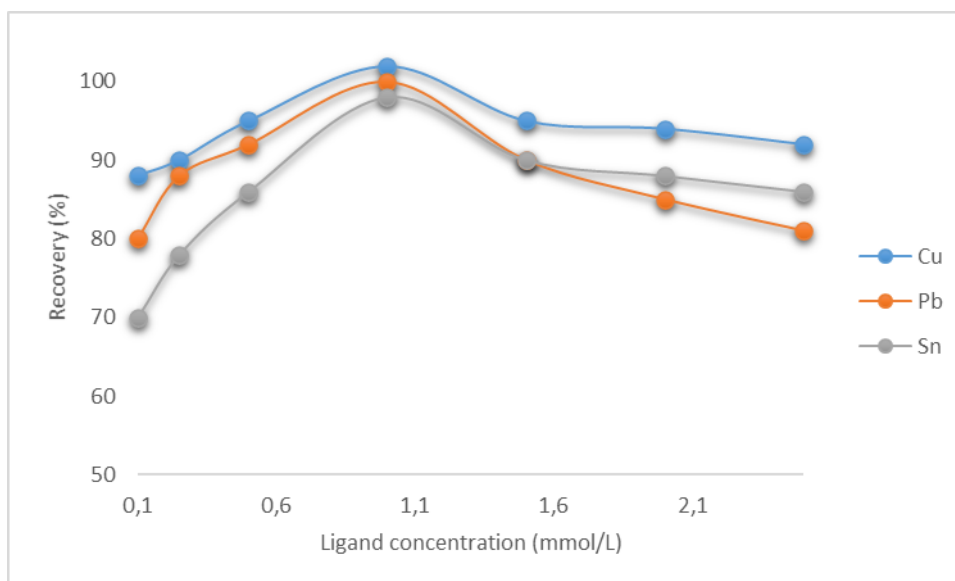
Surfactant must be present in the environment in appropriate amounts to obtain the highest efficiency in the study. The appropriate amount of surfactant is vital in increasing the efficiency of the study. The most suitable amount for the study was determined as 0.1 mL. Recovery values are given in Figure 3.



**Figure 3.** Effect of surfactant concentration on recovery of metal ions (pH=8, 1 mL of 0.01 M 2-(4-sulphonylamidebenzo)hydrazide-1-dithiocarbamate salt, 50 mL of sample volume, 70 °C incubation for 20 minutes)

### 3.3. Effect of Complexing Concentration

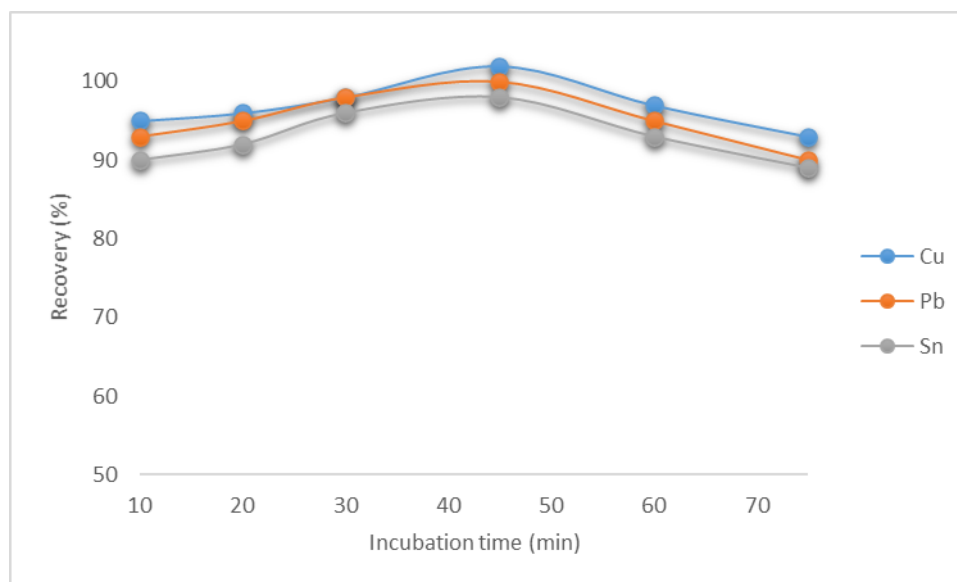
In cases where the concentration of the complexing agent is not sufficient in the medium, the extraction efficiency decreases, and in case of excess in the medium, the phase separation becomes difficult. The most appropriate amount for the study was determined as 1 mL. Recovery values are given in Figure 4.



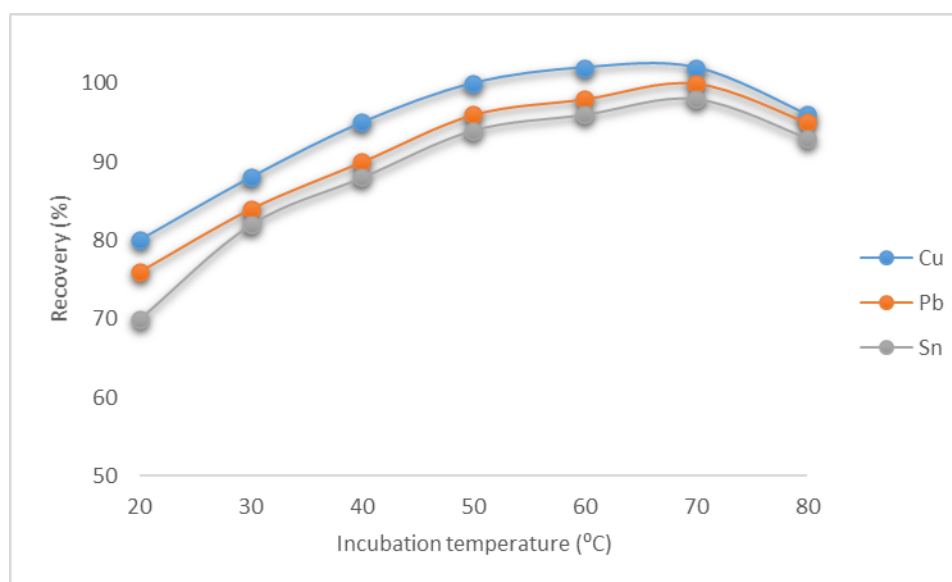
**Figure 4.** Effect of ligand concentration on recovery of metal ions (pH=8, 0.1 mL of 5% (v/v) Triton X-114, 50 mL of sample volume, 70 °C incubation for 20 minutes)

### 3.4. Incubation Time and Temperature

Appropriate temperature and time are required for phase separation to occur in the study. A lower incubation time is more effective for extraction efficiency. The incubation time for the study was determined as 30 min, and the temperature was determined as 70 °C.



**Figure 5.** Effect of reaction time on recovery of metal ions (pH=8, 1 mL of 0.01 M 2-(4-sulphonylamidebenzo)hydrazide-1-dithiocarbamate salt, 0.1 mL of 5% (v/v) Triton X-114, 50 mL of sample volume, 70 °C incubation)



**Figure 6.** Effect of reaction temperature on recovery of metal ions (pH=8, 1 mL of 0.01 M 2-(4-sulphonylamidebenzo)hydrazide-1-dithiocarbamate salt, 0.1 mL of 5% (v/v) Triton X-114, 50 mL of sample volume, 20 minutes incubation)

### 3.5. Common Ion Effect

The effect of common ions in the matrix environment on the recovery of metal ions in the study was investigated. The pre-concentration process was carried out to obtain a minimum 95% efficiency in

the environment where the metal ions to be made have different cations and anions. During the experiment, cloud point extraction was performed by adding common ion solutions to varying concentrations to Cu, Pb, and Sn metal ions at 1000 µg/L concentrations. The results are given in Table 2.

**Table 2.** The results of the common ion effect study

<b>Ions</b>	<b>Tolerance Ratio</b>
Na <sup>+</sup>	5000:1
K <sup>+</sup>	2500:1
NO <sub>3</sub> <sup>-</sup>	2500:1
SO <sub>4</sub> <sup>2-</sup>	500:1
NH <sub>4</sub> <sup>+</sup>	250:1
Mg <sup>2+</sup>	500:1
Ca <sup>2+</sup>	500:1
Cl <sup>-</sup>	5000:1
Ni <sup>2+</sup>	20:1
CH <sub>3</sub> COO <sup>-</sup>	10:1
Fe <sup>3+</sup>	20:1

### 3.6. Analytical Performance of the Method

An analytical performance study is performed when optimum conditions are determined for all parameters in cloud point extraction. The study defines the correlation coefficient, LOD, LOQ, and relative standard deviation values. The results are given in Table 3.

**Table 3.** The results of the analytical performance study

<b>Parameters</b>	<b>Cu</b>	<b>Pb</b>	<b>Sn</b>
R <sup>2</sup>	0.9952	0.9956	0.9973
RSD %	3.94	3.27	4.27
LOD (µg/L)	0.00021	0.0024	0.0004
LOQ (µg/L)	0.00071	0.0081	0.0014
LOL (µg/L)	5-100	5-100	5-100

### 3.7. Measurement Uncertainty Assessment

The evaluation of uncertainty in a laboratory is a crucial step in establishing the credibility of a test outcome. Several variables can impact the precision of an experiment, including the correct calibration and handling of equipment, the purity of reagents, the method of sample collection, and environmental factors such as temperature, humidity, and lighting. Integration of uncertainty analysis with other validation measures can assist in identifying and addressing potential errors in the analytical process. Since NIST CRM was used in this study, the NORDTEST approach was deemed more appropriate for uncertainty budget estimation, instead of following the EURACHEM CITAC Guide and GUM guidelines for calculations [27]. This study estimated measurement uncertainty for Cu and Pb using the NORDTEST method. The calculations are as follows:

$$u(Rw) = Rw/2 \quad (1)$$

$$RMS_{bias} = (\Sigma(bias)^2/N)^{1/2} \quad (2)$$

$$u(C_{ref}) = \Sigma(C_{ref})^2/N)^{1/2} \quad (3)$$

$$u(bias) = (RMS_{bias}^2 + u(C_{ref})^2)^{1/2} \quad (4)$$

$$u_c = (u(Rw)^2 + u(bias)^2)^{1/2} \quad (5)$$

$$U = 2xu_c \quad (6)$$

Here, N is the number of rounds, u(x) is the standard uncertainty, u(Rw) is the standard uncertainty component for the within laboratory reproducibility, u(C<sub>ref</sub>) is the mean of the standard uncertainty of the assigned values, RMS<sub>bias</sub> is the root mean square of the individual bias values, u(bias) is method and laboratory bias, u<sub>c</sub> is combined standard uncertainty, and U is the expanded uncertainty. The Nordtest approach displays all parameters in Table 4.

**Table 4.** Calculated Nordtest guide parameters

	u(Rw)	u(Cref)	RMS <sub>bias</sub>	u(bias)	u <sub>c</sub>	U(%)
Cu	11.6	5.02	3.07	5.88	12.99	25.98
Pb	20	16.51	6.22	17.64	26.67	53.34

### 3.8. Application of the Method to Real Examples

The method's accuracy was tested by applying the method developed for corn and soil samples to certified reference materials. Recovery values obtained from GBW10011 and GBW10012 certified reference materials are given in Table 5. The recovery values obtained from the analysis applied to the corn and soil samples are presented in Table 6.

**Table 5.** Analysis results of GBW10011 and GBW10012 certified reference materials (mg/kg)

	GBW10011			GBW10012		
	Certified value	Measured value	Recovery %	Certified value	Measured value	Recovery %
Cu	2.7±0.2	2.59±0.3	96.22	0.66±0.08	0.67±0.06	101.52
Pb	0.065±0.024	0.07±0.014	107.69	0.07±0.02	0.067±0.02	95.71
Sn	-	-	-	-	-	-

## 4. Conclusions

In this study, a cloud point extraction method was developed to determine the recoveries of Cu, Pb, and Sn metals in corn and soil samples. The developed cloud point extraction method was also applied to certified reference materials besides corn and soil samples. GBW10011 wheat flour and GBW10012 corn flour samples were certified reference materials. The developed method used 2-(4-sulphonylamidebenzo)hydrazide-1-dithiocarbamate salt as a ligand, and Triton X-114 was used as the surfactant. Analyzes in the study were performed with ICP-OES.



**Table 6.** Analysis results of corn and soil samples

Corn samples					Soil samples				
Samples	Metals	Added (mg/kg)	Found (mg/kg)	Recovery (%)	Samples	Metals	Added (mg/kg)	Found (mg/kg)	Recovery (%)
AKYAZI	Cu	-	1.11	96,4	AKYAZI	Cu	-	1.26	102.6
		0.5	1.59	0.5			1.78		
	Pb	-	TSA	105,2		Pb	-	1.56	97.0
		0.5	0.52			0.5	2.05		
	Sn	-	TSA	96,5		Sn	-	0.3	100.5
		0.5	0.48				0.5	0.8	
SERDIVAN	Cu	-	1.7	97,1	SERDIVAN	Cu	-	0.95	104.3
		0.5	2.18	0.5			1.47		
	Pb	-	0.19	102,3		Pb	-	1.83	93.6
		0.5	0.7			0.5	2.3		
	Sn	-	TSA	94,1		Sn	-	0.27	99.5
		0.5	0.47				0.5	0.77	
SOGUTLU	Cu	-	0.45	95,5	SOGUTLU	Cu	-	0.81	99.3
		0.5	0.92	0.5			1.31		
	Pb	-	TSA	101,8		Pb	-	1.27	103.3
		0.5	0.51			0.5	1.79		
	Sn	-	TSA	97,3		Sn	-	0.17	101.6
		0.5	0.49				0.5	0.67	

In analyses made with ICP-OES, analysis times are shorter, and more than one metal can be analyzed simultaneously. As a result of the study, the recovery values in corn samples were 95.5-97.1% for Cu, 101.8-105.2% for Pb, 94.1-97.3% for Sn; Recovery values for soil samples were 99.3-104.3 % for Cu; 93.6-103.3% for Pb; it was found between 99.5-101.6% for Sn.

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