EXTRACTION AND DETERMINATION OF Cd, Co, Se, V AND Ag LEVELS IN COW'S MUSCLE, LIVER, KIDNEY, LUNG, SPLEEN AND BRAIN SAMPLES

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A deep eutectic solvent (DES) consisting of oxalic acid and choline chloride was prepared and used to extract Cd, Co, Se, V, and Ag from meats. The metal levels were determined in muscle, liver, kidney, lung, spleen, and brain samples using ICP-OES. The total youden blank method was employed to eliminate the matrix effect. The order of Cd concentration in the cow's organs was spleen> brain>lung>liver>kidney>muscle. The higher and lowest concentration of Co was determined in the lung and muscle samples. Se was found in all kinds of examined cow's parts and the higher concentration was determined in the liver samples. V was not determined in the muscle and spleen samples and the abundance order in the cow's part was lung>liver>brain>kidney. Ag was determined in only one of the kidney samples.
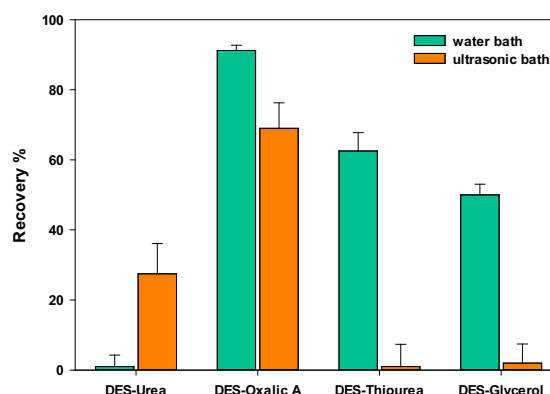
INTRODUCTION

Living organisms need essential metals such as copper and selenium to maintain biological activities. Foods consumed by humans can contain these essential metals. However, foods can also contain non-essential harmful metals in addition to these metals. Cadmium and vanadium are some of the non-essential toxic metals that threaten human health. Silver is a non-essential metal that can accumulate in the animal and human body and exhibits toxicity.

The liver and kidney are the main organs of a cow affected by heavy metals and are consumed in many countries, including Turkey. The heavy metals contained in consumed animal parts threaten human health. The liver is a vital organ that develops from endoblast cells and is responsible for maintaining many important functions, especially the metabolism of carbohydrates, proteins, and fats.[1] Besides essential metals, some heavy metals can bioaccumulate in animal liver tissues depending on the feeding environment and type.[2] The kidney is responsible for infiltrating blood and removing toxic species.[3] Heavy metals can be delivered to the kidney in an inorganic and complex form with bioorganic compounds such as albumin, cysteine, and glutathione.[4] The other organs of a cow in which heavy metals can accumulate are the lungs, spleen,

and brain. They have rarely eaten parts of cows, but the accumulation of heavy metals in these organs can cause toxicity for animals. The lungs are responsible for animal respiration and heavy metal-containing fumes, airborne particles, and particulate matter is toxic to the animal lungs.[5,6] The spleen has a role in the development of the immune system.[7] Heavy metal toxicity can induce pathologies in the spleen.[8,9] The metals can cross the brain-blood barrier (BBB) via ion exchange or increased permeability of BBB and accumulate in the brain. The excessive accumulation of them in the animal brain leads to toxicity.[10–13]

There are several studies on the determination of metals in different parts of cattle. In a study [14] the existence of lead, cadmium, zinc, cobalt, copper, and iron in cattle livers was investigated using atomic absorption spectrophotometer (AAS). In the study, liver samples were digested with phosphoric acid. The reported most accumulated metal was zinc (14.4 mg/kg), followed by lead (7.32 mg/kg) and cadmium (0.03 mg/kg). In another study[15] cadmium, arsenic, mercury and lead were determined in bovine kidneys and samples were analyzed using inductive coupled plasma-mass spectrometry (ICP-MS) after digestion. The concentrations of Cd, As, Hg and Pb were between 0.040 – 8.630 mg/kg, 0.010 – 0.130 mg/kg, 0.003 – 0.230 mg/kg, and 0.005 – 0.280 mg/kg, respectively. In another study,[16] the researchers used the acid digestion method to examine chromium, lead, and cobalt content in cattle's lungs, livers, and kidneys. AAS was used for the measurement of metal concentrations. The reported mean Cr concentrations of lung, liver, and kidney were 22.60 µg/g, 39.60 µg/g, and 12.6 µg/g, respectively. The lowest Co concentration was found in the kidney (29.80 µg/g, 61.80 µg/g for lung and 88.10 µg/g for liver). The reported lead concentrations were 66.70 µg/g for lung, 85.20 µg/g for liver and 38.70 µg/g for kidney. In another study[17] nitric acid was used for digestion and the sample solutions were measured using AAS. Nickel, cadmium, and chromium contents in different parts of cattle were reported. The mean nickel concentration was 1.020 mg/kg, and cadmium and chromium were not detected in the spleen. In a study,[18] Al, V, Mn, Cu, As, Mo, Cr, and Se contents were reported in cattle brains using ICP-MS. According to the given results, Cu (10.64 mg/kg, mean value) was the most abundant and As (0.04 mg/kg, mean value) was the least abundant metal in brains.

Solvent-free processes are more environmentally friendly than organic solvent-based methods. But these processes are not suitable for the industry due to difficulties in adjusting the reaction conditions.[19] Ionic liquids are in the green solvent category. They are mixed molten salts and used as an alternative to organic solvents with superior density, viscosity, and solubility properties.[20] Deep eutectic solvents (DESs) have properties similar to ionic liquids but have several advantages: relatively inexpensive, very low toxicity, and not requiring purification steps.[21] The term eutectic is used to mixture compounds with a lower melting point than reacting components.[22] DESs are prepared by mixing and heating a hydrogen bond acceptor (HBA) like choline chloride, zinc chloride, methyltriphenylphosphonium bromide, or chloride and a hydrogen bond donor (HBD) such as urea, fatty acids, glycerol, and glucose.[23,24] The synthesis process does not need any additional solvent and no by-products are formed in the reaction.[25]

In the present study, cattle's Cd, Co, Se, V, and Ag contents of liver, kidney, muscle, lung, and spleen were investigated. Choline chloride and oxalic acid-based DES were used to extract metals. The proposed extraction method's optimization and validation were controlled using certified reference material BCR-185 R (bovine liver). An inductively coupled plasma optic emission spectrometer (ICP-OES) was used for determination of metal levels.

MATERIAL AND METHODS

All chemicals were analytical grade and used without any purification. The oxalic acid and choline chloride were purchased from Sigma Aldrich chemicals. The 1:1 of oxalic acid and choline chloride was mixed and heated at 85°C. The obtained homogeneous solvent was stored at room temperature.

The cow tissue samples were supplied from local markets, dried at 40°C, and homogenized. The samples were stored in polypropylene bags and at +4°C. A certified reference material (BCR 185 R bovine liver) was used to optimize experimental parameters and validate the results. The appropriate amount of solid sample was subjected to the extraction procedure and the recovery % was calculated from the following equation:

$$Recovery\ \% = \frac{metal\ concentration\ found\ experimentally\ (\frac{mg}{kg})}{certified\ metal\ concentration\ (\frac{mg}{kg})} x100$$

The metal levels were determined by the SpectroArcos model ICP-OES instrument (SpectroAnalytical Instruments, Dusseldorf, Germany). ICP OES multi-element standards were used for the calibration graphics and an appropriate amount of DES solvents were added to external calibration solutions. The metal contents were found using the Total Youden blank method. The matrix signal is determined in this method and the signal is plotted against the increasing number of samples. The signal of the sample matrix is obtained from the intercept of this graph (where no sample is present). This signal is used in the corrections of all calculations of that sample.

The experimental parameters, DES type, DES %, and DES amount were optimized and the metal extraction from different tissues was done at optimum experimental conditions. All experimental optimization steps were carried out simultaneously with ultrasonic and water baths. And the extraction yields were compared. The temperature was 90-95°C, and the extraction time was 2 hours for extraction with the water bath. The temperature was 70°C, and the power was 70%. The time was 0.5 hours for extraction with the ultrasonic bath (the longer extraction times were inappropriate due to overheating caused pauses in the formation of sound waves).

RESULTS AND DISCUSSION

The type of DES greatly influences the extraction of analytes.[26] So in the first stage of the experiments, the type of DES for high extraction efficiency was investigated. DESs were synthesized in a 1: 1 stoichiometric ratio of urea, oxalic acid, thiourea, and glycerol with choline chloride in the present study. The stability of the prepared solutions in room conditions (homogeneous appearance) was one of the parameters considered in DES selection. All the prepared DESs were stable for the duration of the experiment except DES-Glycerol. The appearance of DES glycerol became heterogenic when this solution was kept at room temperature for a long time. The other solvents were stable in understudied conditions. Prepared DESs were added to the reference material and Cd recovery was calculated. Figure 1 shows the effect of DES

on Cd extraction. The Cd recovery has the highest value for DES-Oxalic acid. The water bath extraction was higher than the ultrasonic bath due to the temperature effect. The extraction with the water bath was conducted above 90°C and 70°C for the ultrasonic bath. The studies were continued with DES-Oxalic acid.

DES percentage also influences the extraction efficiency. As the percentage increased, the extraction ability also increased. But it is important to use the minimum amount of DES due to environmental and economic approaches. DES-Oxalic acid was added to the sample to get the final percentage of 5-40% in the study. As shown in Figure 2, the recovery values increased by increasing the percentage from 5 to 20%. The optimum percentage was chosen as 20% for further studies.

The optimum sample amount is another important parameter affecting efficiency (Figure 3) and investigated range was 0.01 g to 0.10 g. The recovery values were decreased as increasing the sample amount from 0.05 g to 0.10 g. The reason for this situation is that the amount of substance increases while the amount of solvent remains constant and the dissolving capacity of the solvent has reached the maximum. The same trend was also observed for ultrasonic-assisted extraction.

The analytical characteristics of the extraction procedure

The limit of detection (LOD) and the limit of quantification (LOQ) for the optimized method was calculated as the equations given below:

$$LOD = \frac{3s}{m} \quad and \quad LOQ = \frac{10s}{m}$$

where s is the standard deviation of the ten blank solutions and m is the slope of the calibration curve. The calibration curve, LOD, LOQ, and Relative standard deviation (RSD) were given in Table 1. RSD% was calculated for the standard deviation of 143 µg/L metal ion. The extraction results of Cd, Co, Se, V, and Ag in different muscles samples are shown in Table 2. The amount of Cd, V, and Ag could not be determined in the original samples due to lower concentrations than the limit of detection. However, in some muscle samples, Co and Se were found.
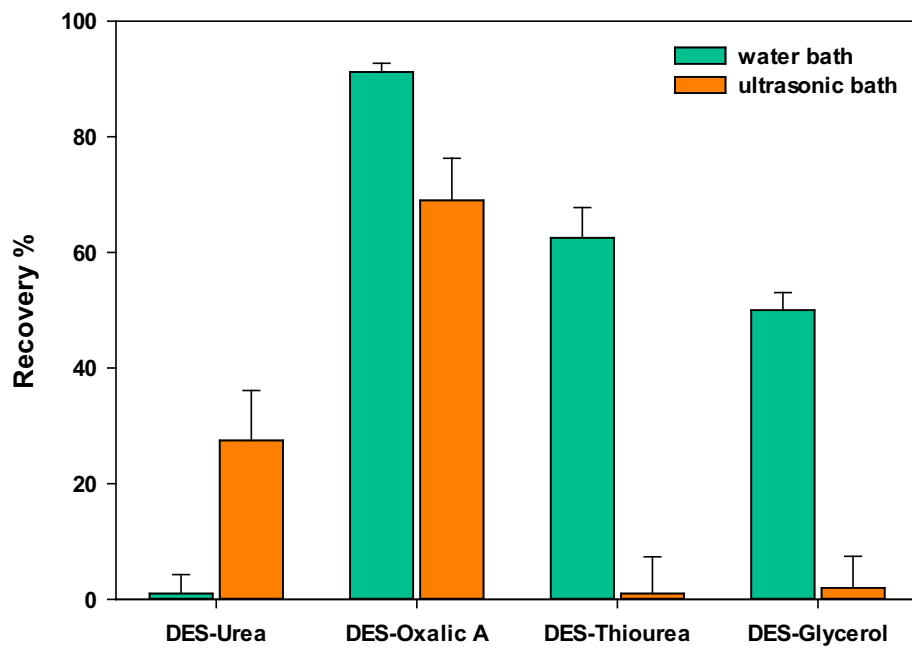
Fig. 1 – Comparison of water bath and ultrasonic bath extraction of cadmium using different types of DES.
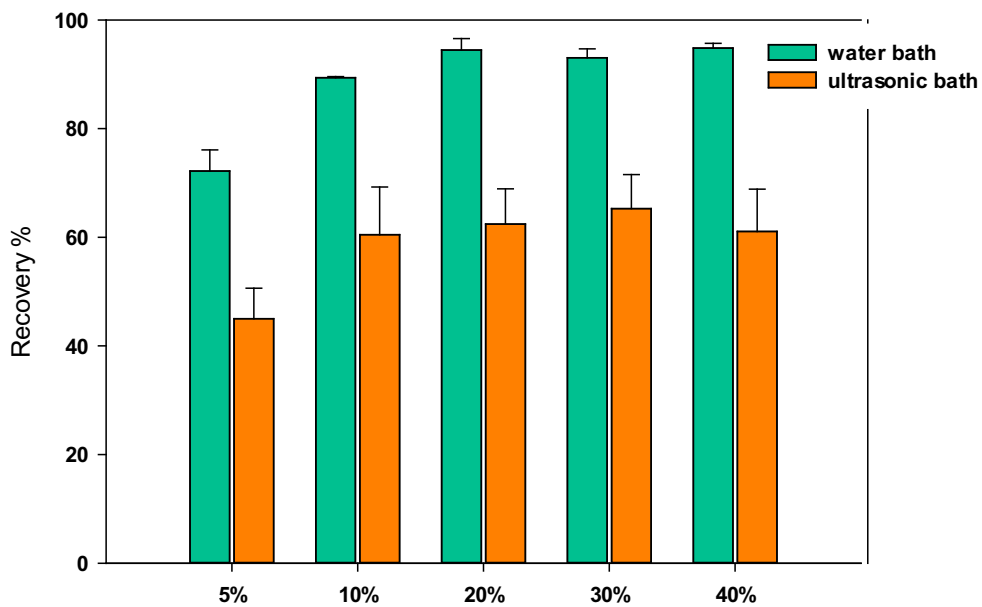

Fig. 2. Cadmium extraction results with different amounts of DES-oxalic acid.
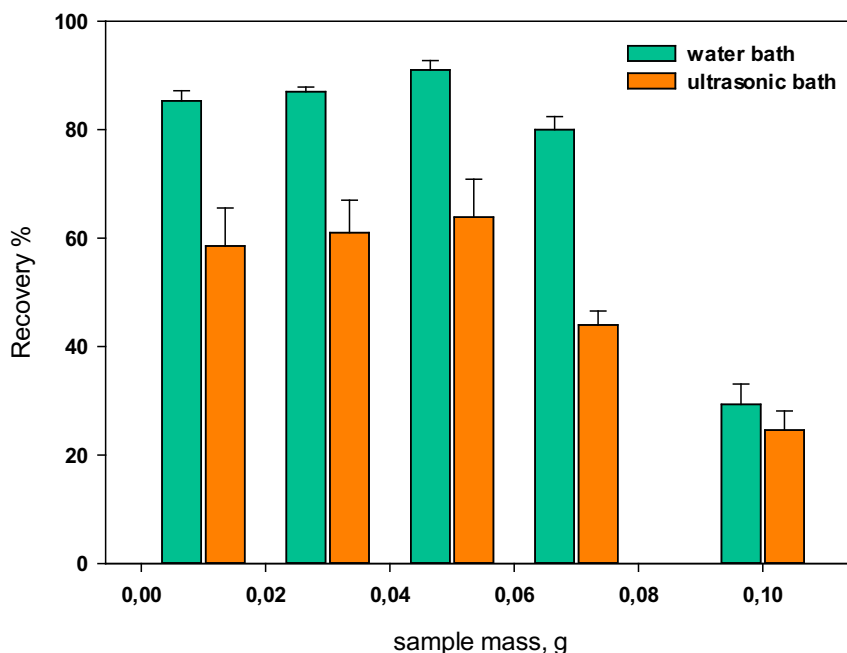
Fig. 3 – Cadmium extraction results with different sample mass (DES-oxalic acid).

Table 1

The calibration curve, LOD, LOQ and RSD % for Cd, Co, Se, V and Ag

Element	Calibration Curve equation , R²	RSD %	LOD, µg/L	LOQ, µg/L
Cd	y = 359.49x - 4306.2 $R^2 = 0.99$	6.43	1.35	4.50
Co	y = 250.65x - 2037.5 $R^2 = 1$	4.42	1.302	4.34
Se	y = 20.83x - 271.6 $R^2 = 0.99$	8.41	5.48	18.26
V	y = 360.02x - 3480.4 $R^2 = 1$	4.03	2.93	9.77
Ag	y = 486.32x - 4741.5 $R^2 = 1$	4.05	3.34	11.13

The extracted average amount of Co and Se were ~0.08 and ~0.8 mg/kg, respectively. The recovery experiments were carried out by adding 10 and 20 mg/kg of Cd, Co, Se, V, and Ag to the original samples. The recovery percentages for Cd, Co, Se, V, and Ag ranged from ~76% to ~100%, ~80% to ~104%, to ~113%, ~80% to ~115%, and ~69% to 112%, respectively. Ag was not determined in any of the original liver samples (Table 3). The extracted maximum concentration of Cd, Co, Se, and V were ~0.38, ~0.33, ~3.21, and ~3.49 mg/kg, respectively. The extraction performance of DES against Ag was poor. The lowest recovery rate for Ag was ~49% and the maximum was ~100%. The recovery results of other metals varied from ~74% to ~117%. All metals were determined in original kidney samples (Table 4). The extracted maximum

concentrations of Cd, Co and Se ranged from 0.57 to 0.34, 0.06 to 0.33 and 0.62 to 1.54 mg/kg, respectively. V and Ag were determined in only one sample and the found concentrations were 0.20 mg/kg for V and 0.72 mg/kg for Ag. The extraction performance of DES against Ag was poor in the kidney samples and the liver samples. The calculated mean recovery levels for Cd, Co, Se, V, and Ag were ~87%, ~90%, ~97%, ~94%, and ~77%, respectively. The mean concentrations of Cd, Co, Se, and V in the original lung samples were 0.48, 0.30, 1.65, and 2.21 mg/kg, respectively (Table 5). Ag was not determined. The mean recoveries for Cd, Co, Se, V, and Ag were ~96%, ~84%, ~86%, ~85%, and ~69%, respectively, by adding 10 mg/kg standard to the samples.

Table 2

Cd, Co, Se, V and Ag levels in muscle samples

Muscle sample no	Added (mg/kg)	Cd Found (mg/kg)	Cd Recovery	Co Found (mg/kg)	Co Recovery	Se Found (mg/kg)	Se Recovery	V Found (mg/kg)	V Recovery	Ag Found (mg/kg)	Ag Recovery
MS1		bdl		0.08±0.03		0.88±0.03		bdl		bdl	
	10.00		76.03±0.19		82.34±2.23		85.65±2.42		87.47±2.45		79.28±3.50
	20.00		95.45±7.94		104.06±4.54		95.24±1.94		115.14±3.97		106.50±1.88
MS2		bdl		0.07±0.19		0.81±0.07		bdl		bdl	
	10.00		85.43±3.06		79.83±0.25		101.14±7.80		79.70±0.09		69.07±0.04
	20.00		88.48±3.31		86.51±0.08		92.51±7.14		86.64±1.36		74.90±2.00
MS3		bdl		bdl		bdl		bdl		bdl	
	10.00		84.49±6.19		84.85±1.30		77.64±6.44		97.99±1.27		91.40±1.09
	20.00		93.46±10.16		102.55±8.47		92.71±7.12		101.94±7.71		111.90±11.06
MS4		bdl		bdl		0.6±0.04		bdl		bdl	
	10.00		87.77±3.67		86.18±0.93		81.27±6.80		86.81±0.59		105.05±8.33
	20.00		100.97±5.96		94.86±1.44		112.77±7.32		95.98±0.66		82.27±5.22
MS5		bdl		bdl		bdl		bdl		bdl	
	10.00		92.12±1.81		90.93±2.12		83.15±0.41		90.78±2.39		75.24±7.09
	20.00		100.0±7.26		95.73±0.02		79.28±3.66		93.62±1.48		69.35±0.48

*bdl: below detection limit

Table 3

Cd, Co, Se, V and Ag levels in liver samples

Liver sample no	Added (mg/kg)	Cd Found (mg/kg)	Cd Recovery	Co Found (mg/kg)	Co Recovery	Se Found (mg/kg)	Se Recovery	V Found (mg/kg)	V Recovery	Ag Found (mg/kg)	Ag Recovery
LS1		bdl		0.23±0.03		0.50±0.06		bdl		bdl	
	10.00		84.35±1.15		84.19±1.19		74.41±0.65		86.68±0.39		63.14±3.44
	20.00		97.17±0.52		93.86±0.17		78.82±0.12		98.16±1.16		72.47±2.42
LS2		0.38±0.16		0.33±0.34		1.75±0.21		0.24±0.09		bdl	
	10.00		86.09±0.76		79.64±.30		97.10±5.23		83.22±0.15		74.43±0.34
	20.00		87.02±3.21		78.79±4.69		98.81±0.26		85.40±5.89		77.17±4.81
LS3		bdl		0.09±0.02		0.85±0.43		bdl		bdl	
	10.00		101.72±7.32		82.95±1.45		97.22±1.64		87.91±1.98		78.41±1.26
	20.00		105.32±5.45		98.77±3.31		103.93±1.20		105.35±3.50		100.10±0.05
LS4				0.12±0.04		1.50±0.44		3.49±0.06		bdl	
	10.00		107.77±6.93		90.97±1.31		91.12±4.02		92.54±1.22		82.30±1.32
	20.00		112.03±7.56		87.78±8.47		79.35±3.17		89.40±9.32		76.52±10.85
LS5		bdl		0.21±0.03		0.51±0.08		bdl		bdl	
	10.00		114.79±1.87		106.06±0.88		76.32±2.89		96.95±1.39		49.22±2.17
	20.00		110.93±3.72		99.64±0.91		91.06±3.13		104.11±0.78		95.41±9.72
LS6		bdl		0.08±0.02		3.21±1.41		0.16±0.08		bdl	
	10.00		99.92±2.38		93.55±0.26		99.79±1.19		92.50±0.24		52.68±6.38
	20.00		117.02±5.53		101.95±1.05		96.55±1.38		99.69±1.45		77.18±3.88

*bdl: below detection limit

Table 4

Cd, Co, Se, V and Ag levels in kidney samples

Kidney sample no	Added (mg/kg)	Cd		Co		Se		V		Ag	
		Found (mg/kg)	Recovery	Found (mg/kg)	Recovery	Found (mg/kg)	Recovery	Found (mg/kg)	Recovery	Found (mg/kg)	Recovery
KS1		0.57±0.07		0.06±0.06		0.63±0.4		bdl		0.72	
	10.00		83.10±6.26		80.43±7.78		73.0±9.48		80.72±7.96		47.11±3.19
	20.00		101.76±0.40		93.08±0.74		104.0±2.37		91.08±0.27		59.25±8.12
KS2		bdl		0.07±0.02		1.54±0.16		bdl		bdl	
	10.00		102.3±3.21		91.57±1.04		100.97±1.83		92.56±0.40		64.21±0.54
	20.00		112.1±5.06		100.18±2.08		84.85±2.37		102.37±2.41		75.14±3.43
KS3		bdl		0.33±0.05		0.62±013		0.20±0.67		bdl	
	10.00		86.46±5.21		94.59±7.67		105.04±0.01		114.34±8.34		107.51±7.81
	20.00		107.6±3.74		121.07±2.38		116.49±2.24		107.15±6.45		149.74±149.74
KS4		bdl		0.30±0.04		0.74±0.25		bdl		bdl	
	10.00		88.83±4.55		86.74±1.86		106.19±4.01		92.01±1.31		88.94±3.68
	20.00		100.83±7.42		97.65±6.88		93.26±3.24		105.23±8.56		95.86±7.76
KS5		bdl		bdl		1.1±0.14		bdl		bdl	
	10		70.05±2.44		67.66±1.04		71.70±4.99		87.23±1.26		61.97±0.54
	20.00		86.11±5.27		69.87±2.96		107.63±.22		96.95±3.96		63.04±4.18
KS6		0.34±0.47		0.24±0.05		0.66±0.79		bdl		bdl	
	10.00		84.84±3.20		81.29±0.22		92.13±2.68		85.77±0.06		66.61±5.58
	20.00		96.59±6.78		79.60±.75		103.37±6.08		81.75±0.37		61.92±1.14
KS7		bdl		bdl		1.07±0.24		bdl		bdl	
	10.00		84.83±4.53		85.06±3.96		109.25±7.02		86.41±3.98		91.36±19.93
	20.00		89.18±0.50		102.01±3.29		98.03±3.26		94.60±4.60		75.60±9.81
KS8		bdl		0.21±0.09		1.20±0.23		bdl		bdl	
	10.00		99.48±0.83		90.09±3.18		82.50±3.46		90.66±4.65		54.05±0.04
	20.00		100.76±0.08		95.53±5.31		100.83±2.47		96.55±4.29		72.73±8.11

*bdl: below detection limit

Table 5

Cd, Co, Se, V and Ag levels in lung samples

Lung sample no	Added (mg/kg)	Cd Found (mg/kg)	Cd Recovery	Co Found (mg/kg)	Co Recovery	Se Found (mg/kg)	Se Recovery	V Found (mg/kg)	V Recovery	Ag Found (mg/kg)	Ag Recovery
LUS1		bdl		0.04±0.01		2.03±0.07		3.70±1.52		bdl	
	10.00		90.82±6.88		86.47±2.97		77.47±3.75		89.68±3.19		64.48±3.42
	20.00		94.39±3.91		89.13±2.29		108.75±2.30		94.73±3.27		71.97±0.96
LUS2		0.48±0.14		bdl		0.75±0.19		bdl		bdl	
	10.00		88.27±0.68		88.31±2.84		70.99±1.58		89.14±2.10		79.73±3.02
	20.00		105.93±8.21		101.93±4.76		99.00±2.03		101.88±3.94		91.03±5.25
LUS3	13.62	bdl		0.52±0.14		3.10±1.32		0.43±0.03		bdl	
	10.00		97.67±9.21		82.52±0.36		90.11±7.33		85.51±0.17		79.49±0.50
	20.00		111.09±2.64		103.85±6.35		89.83±.83		107.35±4.88		98.24±1.21
LUS4		bdl		bdl		1.25±0.42		bdl		bdl	
	10.00		104.52±.73		73.96±0.46		83.53±.25		72.48±0.50		63.96±1.44
	20.00		98.53±0.50		72.26±2.40		83.28±1.23		68.00±1.28		54.69±0.97
LUS5		bdl		0.34±0.09		1.13±7.66		2.51±0.65		bdl	
	10.00		96.50±6.31		85.23±3.20		103.46±0.49		85.17±2.38		64.89±0.16
	20.00		96.04±4.50		86.32±2.46		102.84±2.88		84.39±2.63		72.18±3.97
LUS6		bdl		bdl		1.62±0.20		bdl		bdl	
	10.00		95.75±6.14		86.88±0.59		89.06±2.16		86.46±0.34		62.40±9.06
	20.00		99.36±7.85		103.91±10.14		95.74±1.98		92.41±4.93		43.66±1.70

*bdl: below detection limit

Table 6

Cd, Co, Se, V and Ag levels in spleen samples

Spleen sample no	Added (mg/kg)	Cd Found (mg/kg)	Cd Recovery	Co Found (mg/kg)	Co Recovery	Se Found (mg/kg)	Se Recovery	V Found (mg/kg)	V Recovery	Ag Found (mg/kg)	Ag Recovery
SS1		bdl		bdl		bdl		bdl		bdl	
	10.00		84.08±3.40		81.29±1.43		76.36±1.29		80.61±1.93		45.84±0.98
	20.00		100.92±1.98		93.81±0.12		70.91±4.07		90.69±1.18		51.76±0.76
SS2		bdl		0.08±0.21		0.87±0.06		bdl		bdl	
	10.00		87.87±3.29		82.85±0.47		90.23±0.87		82.40±0.48		72.12±4.19
	20.00		101.0±0.06		92.96±0.70		107.78±1.73		94.20±1.30		86.33±0.77
SS3		bdl		bdl		0.64±0.01		bdl		bdl	
	10.00		86.75±6.43		118.56±10.01		80.86±0.46		92.71±5.88		44.71±4.90
	20.00		98.00±6.81		84.93±16.85		87.12±1.81		75.71±18.61		68.58±1.10
SS4		1.99±1.10		0.05±0.01		bdl		bdl		bdl	
	10.00		76.14±1.05		73.03±0.72		91.79±7.86		76.89±2.13		62.35±0.59
	20.00		94.14±1.17		92.62±0.83		104.85±4.82		101.12±1.28		89.55±2.56
SS5		0.46±0.59		bdl		1.22±0.05		bdl		bdl	
	10.00		108.04±1.80		92.88±0.18		92.69±1.69		97.91±0.06		85.48±2.71
	20.00		103.54±6.85		98.16±3.79		89.93±8.08		110.21±3.08		100.03±2.46
SS6		0.56±0.01		0.41±0.03		1.19±0.81		bdl		bdl	
	10.00		109.06±7.42		89.44±1.29		102.74±4.27		90.56±1.10		71.03±2.29
	20.00		100.68±1.77		92.63±1.35		93.13±5.46		96.09±1.75		77.68±4.65
SS7		bdl		0.40±0.07		0.72±0.33		bdl		bdl	
	10.00		94.84±1.61		104.18±0.81		73.64±1.20		91.81±0.97		84.47±2.90
	20.00		103.0±3.25		82.38±1.42		80.18±6.35		49.43±1.24		43.94±1.78

*bdl: below detection limit

Table 7

Cd, Co, Se, V and Ag levels in brain samples

Brain sample no	Added (mg/kg)	Cd		Co		Se		V		Ag	
		Found (mg/kg)	Recovery	Found (mg/kg)	Recovery	Found (mg/kg)	Recovery	Found (mg/kg)	Recovery	Found (mg/kg)	Recovery
BS1		bdl		0.19±0.14		0.50±0.08		bdl		bdl	
	10.00		88.15±1.03		87.89±0.64		95.55±8.28		89.47±0.51		68.05±2.22
	20.00		103.47±1.59		98.87±1.34		107.26±1.61		102.39±2.21		70.03±2.01
BS2		bdl		0.33±0.15		2.32±0.21		0.60±0.10		bdl	
	10.00		93.20±6.00		87.77±6.48		79.30±1.53		89.64±6.52		83.28±8.34
	20.00		102.85±6.68		95.07±.07		115.47±4.59		98.07±0.91		87.90±0.07
BS3		1.39±0.21		bdl		bdl		0.38±0.03		bdl	
	10.00		89.75±4.95		83.05±2.28		83.17±2.42		92.26±4.94		68.87±8.96
	20.00		70.83±6.36		76.48±6.09		92.45±0.07		69.73±7.63		79.44±4.71
BS4		bdl		0.05±0.10		1.25±0.41		0.12±0.09		bdl	
	10.00		95.41±1.12		86.95±0.11		101.43±4.60		88.20±.10		84.24±0.18
	20.00		85.06±.32		85.76±1.92		106.14±.53		85.94±2.17		81.92±1.88
BS5		bdl		bdl		bdl		bdl		bdl	
	10.00		94.84±1.61		89.47±1.89		92.92±5.27		90.59±2.65		64.53±4.15
	20.00		101.39±3.25		107.12±5.31		112.11±8.02		118.44±1.49		104.71±8.60

*bdl: below detection limit

Table 8

Comparison of the proposed method with the other methods

Elements	R^2	LOD	LOQ	RSD%	Sample	Method
Cd	0.99	1.35 µg/L	4.50 µg/L	6.43	Cow parts	DES extraction – ICP-OES (This study)
Co	1.00	1.30 µg/L	4.34 µg/L	4.42		
Se	1.00	5.48 µg/L	18.26 µg/L	8.41		
V	1.00	2.93 µg/L	9.77 µg/L	4.03		
Ag	1.00	3.34 µg/L	11.13 µg/L	4.05		
Co	0.9995	0.072 µg/L	0.240 µg/L	-	Cattle tissues	Microwave assisted digestion – ICP-MS[18]
Se	0.9993	0.07 µg/L	0.233 µg/L			
V	0.9974	0.090 µg/L	0.300 µg/L			
Cd	0.9991	3.84 µg/kg	12.8 µg/kg	4.00	Bovine parts	HNO3-H2O2 digestion – GF-AAS[27]
Cd	-	0.12 µg/L	-	-	Cow parts	HNO3-H2O2 digestion – GF-AAS[28]
Cd	-	0.01 mg/kg	-	-	Bovine kidney	Digestion – ICP-MS[15]

When 20 mg/kg standard was added to the samples, ~101%, ~93%, ~97%, ~91%, and ~72%, recovery rates were found for Cd, Co, Se, V, and Ag. The extraction performance of DES against Ag was also poor in the lung samples. According to the results, more metals in the samples increased the recovery percentages. Ag and V were not determined in the original spleen samples (Table 6). The other metal concentrations varied from 0.46 to 1.99 (Cd), 0.05 to 0.41 (Co), 0.64 to 1.22mg/kg (Se) in the original spleen samples. The recovery percentages for Cd, Co, Se, V, and Ag ranged from ~76% to ~109%, ~73% to ~118%, ~76% to ~102%, ~77% to ~97%, and ~45% to 85%, respectively by using 10 mg/kg standard solutions and ~94% to ~104%, ~84% to ~98%, ~70% to ~107%, ~75% to ~110%, and ~51% to 100% respectively by adding 20 mg/kg standards to the spleen samples. The recovery rates of Ag were worse than the other metals both in the spleen and brain samples. The original brain samples contained (mean values) 1.39 mg/kg Cd, 0.19 mg/kg Co, 1.36 mg/kg Se and 0.37 mg/kg V. Ag were not determined in the original brain samples (Table 7). The calculated average recoveries for Cd, Co, Se, V, and Ag were ~84%, ~90%, ~99%, ~93%, and ~79%, respectively.

The analytical merits of using oxalic acid and choline chloride-based DES extraction methods were compared to similar literature studies (Table 8). The obtained results with the proposed method are acceptable in terms of R^2, LOD, LOQ, and RSD% values.

CONCLUSIONS

Ignoring data below the detection limit, the highest concentration (1.99 mg/kg) of Cd was found in the spleen sample and the lowest concentration (0.34 mg/kg) was determined in the kidney samples. There was no Cd content found in any of the examined muscle samples. The lung samples contained highest (0.52 mg/kg) and lowest (0.04 mg/kg) concentration of Co. Also, Co was found in all of the examined liver samples. Se content was found in all examined liver, kidney, and lung samples. Se's highest concentration (3.21 mg/kg) was determined in one of liver sample. The brain sample contained the lowest Se concentration (0.50 mg/kg). The order of V concentration in cattle organs was as follows: lung>liver> brain>kidney. No V content was found in any examined muscle and spleen samples. Ag was determined only in a kidney sample. In terms of recovery percentages, the extraction efficiencies

of DES-oxalic acid were acceptable for examining all organs and metals except Ag. The DES-oxalic acid was not an acceptable solvent for extracting Ag from the cattle parts.

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