ANALYSIS

DIRECT DETERMINATION OF Pb(II) AND Cd(II) IONS AS IMPURITIES IN SOME HOMEOPATHIC DRUGS BY USING STRIPPING VOLTAMMETRY

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Abstract: The conditions for identification and quantification of Pb(II) and Cd(II) ions as impurities in homeopathic preparations by stripping voltammetry were established. The tests proved that the method was of high selectivity. The detection limits were 0.78 ng/mL and 1.56 ng/mL for Pb(II) and Cd(II) ions, respectively. The method was characterised by: repeatability of measurements, a wide range of linearity and satisfactory percent recovery. The Pb(II) and Cd(II) ions were detected in examined drugs as impurities at concentrations of 1.23 ? 9.5 ng/mL and 1.66 ? 3.7 ng/mL for Pb(II) and Cd(II), accordingly.

Keywords: Analysis of pharmaceuticals, stripping voltammetry, homeopathic drugs impurity, determination of Pb(II) and Cd(II)

Homeopathy as a therapy system used in the treatment of various diseases has been known for more than 200 years. Since homeopathic drugs are increasingly used, it is necessary to assess their quality by determining not only the concentration of active ingredients, which is often hindered due to high dilution of preparations, but also their purity (1,2). The problem has been reflected in the European Union directives pertaining the manufacturing and control of homeopathic drugs and raw materials (3,4).

The quality assessment of such drugs is included into the European Pharmacopoeia that contains the requirements related to allowable concentrations of such inorganic impurities as chlorides, arsenic, heavy metals, e.g. copper, lead etc. (5,6). It seems, however, that there is an unsatisfied need for control of such ready–made and commonly used preparations. Recent advances in analytical methods referred in various pharmacopoeias have opened new opportunities by allowing to employ a number of methods for determining low concentrations of constituents that occur as impurities. For example, atomic emission spectroscopy was used to determine arsenic in homeopathic drugs (7).

Previous studies carried out in our Department on application of stripping voltammetry for the determination of metal ions in the presence of various drugs have indicated that this method is a valuable tool in determining low concentrations of inorganic and organic impurities (8,9).

In this work, stripping voltammetry was employed to determine Pb(II) and Cd(II) ions as impurities in homeopathic drugs. Such objective was worthy of investigation as no control methods for such preparations are found in the available literature and because of a specific feature of such drugs, namely very low concentrations of highly diluted active ingredients.

EXPERIMENTAL

Solutions and preparations

1. Standard solution of Pb(II) salts at a concentration of 100 mg/mL
2. Standard solution of Cd(II) salts at a concentration of 100 mg/mL
The solutions of Pb(II) and Cd(II) were prepared for analytical purposes by diluting with water to obtain the concentration of 0.1 mg/mL.
3. Supporting electrolyte: KCl solution at a concentration of 3 mole/L
4. Homeopathic preparations: R–2, R–4, R–28, R–76, Psorbinoheel, Injeel–Chol, Nux Vomica, Lamioflur, Caustinum Composition, Ignatia

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Homaccord, Belladona Homaccord, Chelidonium Homaccord, Gloxyal Homaccord, Natrium Homaccord (manufactured by Biologische Hielmittel Heel GmbH, Baden–Baden, Germany).

Reagents

1. Potassium chloride, extra pure, (PPH Polskie Odczynniki Chemiczne, Gliwice, Poland).

2. Redistilled water. Water of high purity was obtained under laboratory conditions by distillation followed by reversed osmosis. Its quality was checked according to USP.

3. Lead(II) nitrate GR for analysis, ACS, No. 1.59090 and Cadmium nitrate tetrahydrate GR for analysis, No. 102019 (Merck, Germany).

Instrumentation

1. EA9 multipurpose voltammeter designed for polarographic and voltammetric analyses, with the Eagraph software (developed by MTM+AN-KO, Poland)

2. Electrodes:

A. Controlled growth mercury drop electrode (CGMDE)

B. Reference: Ag/AgCl DB

C. Auxiliary: platinum wire electrode.

All electrodes were from the same manufacturer (MINERAL, Warsaw).

Procedure

During this work Differential Pulse Stripping Voltammetry of pulse amplitude of 20 mV and a quiet time of 20 ms was employed. The metals under investigation were concentrated for 180 s. at potentials of –650 mV and –450 mV. Determination of ion concentration was carried out at potentials of –407 mV and –600 mV for Pb(II) and Cd(II), respectively. Prior to the analysis the voltammetric cell vial was filled with a tested solution and deoxygenation was performed by passing argon for 20 min. The voltammetric curve was then recorded by making three measurements for each concentration and taking the average of these measurements. The concentrations of individual metals were taken from reference curves obtained in the same way for the concentration range from 0.78 ng/mL to 12.5 ng/mL for Pb(II) and 1.56 ng/mL to 25.0 ng/mL for Cd(II). An example of voltammogram is presented in Figure 1.

To evaluate the performance of this method and its suitability for determining Pb(II) and Cd(II) in homeopathic preparations, the linearity, detection and determination limits as well as accuracy, precision and reproducibility were tested (10).

Linearity

The appropriate Pb(II) and Cd(II) standard solutions in amounts of 3.9 µL, 7.8 µL, 15 µL, 31.2 µL and 62.5 µL were measured and placed in 25 mL volumetric flasks. Then, 6.3 mL of potassium chloride solution at a concentration of 3 mole/L was added into each flask and topped with redistilled water up to the volume mentioned above. Determination was carried out as by employing the procedure described above for each metal individually and for the mixture.

Detection and Determination Limits

The solutions of both metals at an initial concentration of 25 ng/mL were tested individually. Then, the solutions were diluted in geometrical progression to obtain appropriate concentrations for which voltammograms were recorded. The detection and determination limits were considered together by assuming that the peak signal should be at least 5 times higher than the background.

Accuracy

The accuracy was evaluated based on the percent recovery (%R). In computing the concentrations of each metal compared to the amount added to the R–4 preparation whose concentrations had been determined prior to adding the Pb(II) and Cd(II) standard solutions, were taken into account. The recovery was calculated from the following formula:

$$\%R = \frac{A-A_1}{B} \cdot 100$$

where:

A – ion concentration in the preparation after adding the standard solution

A_1 – ion concentration in the preparation before adding the standard solution

B – ion concentration taken for determination

Precision

The precision was evaluated from 8 measurements for a reference sample containing 12.5 ng/mL and 6.24 ng/mL for Cd(II) and Pb(II), respectively.

Reproducibility

The reproducibility was tested at various time intervals on samples of known concentrations ranging from 51.73% to 165% for Pb(II) and from 10.42% to 92.40% for Cd(II). The same instrument and various reagents were used. Based on these tests, the conditions for determination were estab-

lished and used for evaluating the purity of some homeopathic preparations. The tests were carried out on commonly used commercial peparations that were randomly selected.

Determination

Add 6.3 mL of potassium chloride solution at a concentration of 3 mol/l to 10 mL of the preparation under testing and dilute with redistilled water to 25 mL. Make analysis according to the procedure described above and compute the Pb(II) and Cd(II) impurity concentrations by using the reference curve.

RESULTS AND DISCUSSION

Generally, homeopathic drugs are widely believed to be safe and free of toxic effects due to their high dilution as well as to the fact that their ingredients are of high purity and of natural origin only. Since both raw materials and preparations are always in homogeneous to some extent and the recent analytical methods specified in various pharmacopoeias (5,6) for drug purity control enable low impurity concentrations to be detected, it seems that homeopathic preparations shall be subjected to similar control as other drugs.

In this paper the analysis is limited to the determination of two ions, namely Pb(II) and Cd(II) that are limited and undesired impurities of these drugs. The determination conditions for these ions were established by employing stripping voltammetric techniques. It was found that Pb(II) and Cd(II) ions have different potentials, –407 mV and –600 mV, respectively. This fact enabled direct and simultaneous analysis of these ions (see Figure 1).

For Pb(II) the reference curve is linear over the concentration range under consideration and shows the correlation coefficient r = 0.99297. The same figure for Cd(II) is r = 0.99798. No current intensity differences were observed when measuring each metal ion solution individually and mixed, thus indicating no mutual interference. The presented method shows a high sensitivity. The detection and determination limits are 0.78 ng/mL and 1.56 ng/mL for Pb(II) and Cd(II), respectively.

The accuracy based on the percent recovery (%R) is satisfactory. The following results are obtained for the number of runs n = 5:

Pb(II): 80.0%, 110.6%, 106.25%, 92.5% and 86.0%, mean $\bar{x}$ = 91.33%, standard deviation s = 8.44, relative standard deviation RSD = 9.2%.

Cd(II): 95.5%, 90.9%, 100.0%, 110.0% and 86.0%, mean $\bar{x}$ = 93.98%, standard deviation s = 4.97, relative standard deviation RSD = 5.5%.

The precision is determined by the repeatability of measurements and a narrow confidence interval and RSD.

For the reference solution the following results are obtained:

Pb(II): (ng): 6.22; 6.30; 5.90; 5.95; 6.10; 6.24; 6.18; mean $\bar{x}$ = 6.11; s = 0.13; confidence interval μ = 6.11± 0.025, RSD = 2.16%

Cd(II): (ng): 11.4; 10.9; 13.4; 13.2; 12.2; 12.3; 11.95; mean $\bar{x}$ = 12.8; s = 0.76; confidence interval μ = 12.18± 0.144, RSD = 6.20%

Similarly, the reproducibility is also satisfactory. The results obtained by using various reagents and making measurements at various time intervals indicate that the method can be employed on a routine basis. The obtained values of reproducibility presented below show no significant differences in determinations carried out directly after preparing the solution and 24 h later.

I. After preparing the solution:
Pb(II) (%) 51.82; 79.92; 119.10; 139.50; 163.90
Cd (II) (%) 10.93; 22.91; 33.59; 49.02; 88.29

II. 24 h after preparing the solution
Pb(II) (%) 51.63; 79.02; 123.30; 141.50; 166.20
Cd(II) (%) 9.91; 22.69; 34.44; 50.83; 96.48

The satisfactory results obtained in validation of the method were confirmed by determining Pb(II) and Cd (HCd(II)H) in homeopathic drugs (see Table 1).
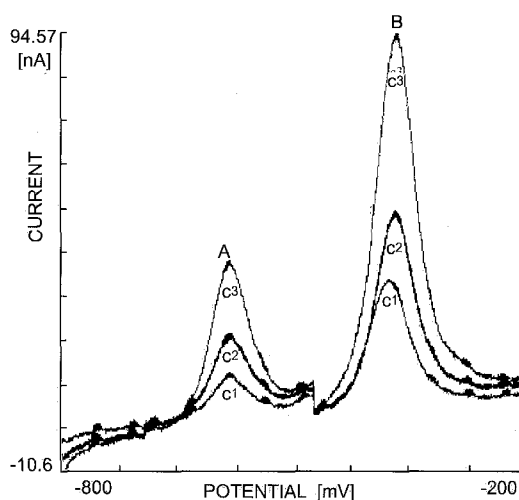

Figure 1. Voltammogram for Pb(II) [B] and Cd(II) [A] recorded under conditions described in the text. The analysis was performed for solutions of the following concentrations:
for Pb(II) c_1 = 0.78 ng/mL; c_2 = 1.56 ng/mL; c_3 = 3.12 ng/mL
for Cd(II) c_2 = 1.625 ng/mL; c_2 = 3.25 ng/mL; c_3 = 6.5 ng/mL

Table 1. The results of determination of Pb(II) and Cd(II) in homeopathic preparations

Preparation name	Pb(II) ion concentration from ... to [ng/mL]	Statistical evaluation	Cd(II) ion concentration from ... to [ng/mL]	Statistical evaluation
R–2	2.6–2.9	$\bar{x}$ = 2.7 S = 0.1367 μ = 2.7±0.105 RSD = 4.8%	(–)	(–)
R–4	2.3–2.6	$\bar{x}$ = 2.3 S = 0.1973 μ = 2.3±0.133 RSD = 8.5%	(–)	(–)
R–28	1.4–1.8	$\bar{x}$ = 1.6 S = 0.1697 μ = 1.6±0.124 RSD = 10.5%	(–)	(–)
R–43	0.75–1.4	$\bar{x}$ = 1.1 S = 0.2911 μ = 1.1±0.291 RSD = 25.0%	(–)	(–)
R–76	8.4–9.5	$\bar{x}$ = 8.8 S = 0.5168 μ = 8.8±0204 RSD = 5.8%	(–)	(–)
Psorhinoheel	1.5–1.6	$\bar{x}$ = 1.6 S=0.06 μ=1.6±0.149 RSD=3.75%	1.46–1.66	$\bar{x}$ = 1.5 S = 0.104 μ = 1.5±0.9 RSD = 6.47%
Injeel–Chol.	1.7–1.72	$\bar{x}$ = 1.7 S = 0.01 μ = 1.7±0.025 RSD = 0.58%	2.56–3.37	$\bar{x}$ = 2.8 S = 0.5 μ = 2.8±0.4 RSD = 16.5%
Nux Vomica	1.23–1.24	$\bar{x}$ = 1.2 S = 0.005 μ = 1.2±0.014 RSD = 0.47%	1.87–2.09	$\bar{x}$ = 1.9 S = 0.111 μ = 1.9±0.1 RSD = 5.6%
Lamioflur	4.68–4.69	$\bar{x}$ = 4.7 S = 0.006 μ = 4.7±0.014 RSD = 0.12%	(–)	(–)
Caustinum Compositum	5.33–5.85	$\bar{x}$ = 5.6 S = 0.260 μ = 5.6±0.645 RSD = 4.65%	(–)	(–)
Tanacet–Heel	0.72–0.79	$\bar{x}$ = 0.75 S = 0.380 μ = 0.75 ±0.099 RSD = 5.0%	(–)	(–)
Ignatia Homaccord	2.86–2.9	$\bar{x}$ = 2.8 S = 0.023 μ = 2.8 ± 0.058 RSD = 0.7%	(–)	(–)
Belladonna Homaccord	4.23–4.47	$\bar{x}$ = 4.3 S = 0.12 μ = 4.4 ±0.298 RSD = 2.76%	1.36–1.41	$\bar{x}$ = 1.4 S = 0.050 μ = 1.4 ±0.7 RSD = 3.55%

Table 1. continued

Preparation name	Pb(II) ion concentration from ... to [ng/mL]	Statistical evaluation	Cd(II) ion concentration from ... to [ng/mL]	Statistical evaluation
Chelidonium Homaccord	2.83–2.89	$\bar{x}$ = 2.9 S = 0.030 μ = 2.9 ±0.075 RSD = 1.05%	(–)	(–)
Glyoxal– Homaccord	1.0–1.15	$\bar{x}$ = 1.1 S = 0.081 μ = 1.1 ±0.202 RSD = 7.7%	2.1–2.3	$\bar{x}$ = 2.2 S = 0.090 μ = 2.2 ±0.8 RSD = 6.58%
Natrium Homaccord	0.87–1.4	$\bar{x}$ = 1.2 S = 0.2542 μ = 1.2 ±0.254 RSD = 20.09%	(–)	(–)

Abbreviations: $\bar{x}$ mean, S – standard deviation, μ – confidence interval, RSD – relative standard deviation, (–) – not found.

The obtained results lead to the conclusion that the Pb(II) impurity level in homeopathic drugs under examination is low and ranges from 1.23 ng/mL to 9.5 ng/mL. Such figures are consistent with the allowable impurity level in pharmacopoeial raw materials and preparations. For Cd(II) the concentrations ranging from 1.66 ng/mL to 3.7 ng/mL were found for five preparations only. In other preparations tested this ion was absent.

CONCLUSION

Based on the obtained results one can conclude that the stripping voltammetric method enables direct determination of trace amounts of Pb(II) and Cd(II) in homeopathic preparations. The obtained data indicate a low impurity level in the examined preparations. The impurity level does not exceed the allowable safety limit specified in pharmacopoeias.

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