

ANALYSIS

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR DETERMINATION OF ELEMENTAL IMPURITIES IN MICAFUNGIN FOR INJECTION

SENA OZLEM GUNDOGDU¹, SEDA TURHAN¹, AYNUR OZEL BABACANOGLU¹,
TUGCE CATAK¹ and ADEM SAHIN^{1,2,*}

¹Department of R&D, Centurion Pharma, Ankara, Turkey

²Department of Pharmacy Service, Vocational School of Health Services,
Bilecik Seyh Edebali University, Bilecik, Turkey

Abstract: Elemental impurities contained in a drug product create a risk for patients. Potential toxic elemental impurities are defined and classified in the Guideline for Elemental Impurities Q3D(R1). Among the finished product dosage forms, parenteral products could be critical for elemental impurities because elemental impurities go directly into systemic circulation. One of the most frequently used methods to determine elemental impurities is inductively coupled plasma–mass spectrometry (ICP–MS). In this study we aimed to develop and validate an ICP-MS method for the determination of 24 elemental impurities in micafungin powder for infusion manufactured in our own facility. Results showed that correlation coefficients of calibration curves were found higher than 0.99 for all elements in the determined ranges and all validation parameters were achieved successfully. With this method, all 24 elemental impurities can be tracked throughout the shelf life of the product.

Keywords: Elemental impurities, micafungin, injectables, ICP-MS, validation.

Elemental impurities contained in a drug product create a risk for patients. Potential toxic elemental impurities are defined and classified by The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) in the “Guideline for Elemental Impurities Q3D(R1)”. Based in this guideline, manufacturers make great efforts to prevent patient exposure to these elements. The required quality assurance and quality control processes are carried out by manufacturers. To prevent the presence of elemental impurities in the drug product, possible sources of contamination must be determined and controlled. These potential sources could be the drug substance, manufacturing equipment, water, container closure system, and excipients. On the other hand, even if all these points are taken into consideration, it is a requirement expected by the authorities to show that the finished product does not exceed the Parenteral Permitted Daily Exposure (PDE) limit. Among the finished product dosage forms, parenteral products could be critical for elemental impurities because elemental

impurities go directly into systemic circulation. Therefore, determination of elemental impurities in parenteral products is critical to ensure patient safety. For this purpose, regulatory authorities have clearly determined the limits of the presence of elemental impurities that may pose a risk in parenteral products [1-3].

One of the most frequently used methods to determine elemental impurities is inductively coupled plasma mass spectrometry (ICP-MS). In this technique, the ICP source transforms the atoms of the elements into positively charged ions, and these ions are separated by the mass spectrometer according to the mass-to-charge ratio and transmitted to the detector. ICP-MS enables the determination of the elements in ICH Q3D with a sensitivity well below the level specified in the guidelines for drug substances or drug products. ICP-MS analyses also have some difficulties and limitations. The main difficulties seem to be that the equipment is expensive and trained and experienced personnel are critical [1-5].

* Corresponding author: e-mail: adem.sahin@bilecik.edu.tr

Micafungin sodium is a water-soluble anti-fungal drug, approved by the European Medicines Agency in 2008. Micafungin containing medicine was formulated as a powder for infusion at two dosages (50 and 100 mg). In use, the lyophilized powder is first redispersed by adding 5 mL of 0.9% NaCl or 5% glucose and diluted according to the concentration to be used in the infusion. It must be shown that there is no impurity above the elemental impurity limits specified by the authorities in all the manufacturing processes, from active ingredient synthesis to obtain the finished product [6].

The study aims to develop and validate an ICP-MS method, which requires expertise for the quantification of elemental impurities. With this aim, method development and validation studies were performed for the determination of 24 elemental impurities in micafungin powder for infusion manufactured in our own facility.

EXPERIMENTAL

Materials

Nitric acid 65% was purchased from Merck, tuning solution for ICP-MS and ICP-MS osmium standard were purchased from CPA Chem. All other ICP-MS elemental standards were purchased from AccuStandard. Ultrapure water was obtained by using a Milli-Q system (Millipore, USA). All other chemicals were of MS or pharmaceutical grades.

Instrumentation

Analyses were performed by ICP-MS Shimadzu-2030. AS-10 autosampler was used. ICP-MS Operating Conditions are given in Table 1.

Preparation of Solutions

2% HNO₃ Solution Preparation (Diluent): Added 31 mL of concentrated (65%) nitric acid solution into ~ 300 mL of pure water and shook well. Completed to 1000 mL volume with pure water. This solution was also used as a calibration blank solution.

Tune Solution: Took 250 µL of certified reference material containing 2 mg/L of beryllium (Be); 0.4 mg/L of indium (In); 0.4 mg/L of bismuth (Bi); 1 mg/L of cobalt (Co); 1 mg/L of manganese (Mn); 0.4 mg/L of cerium (Ce) and diluted to 50 mL with diluent.

4% HNO₃ Solution Preparation (Washing Solution): Added 62 mL of concentrated (65%) nitric acid solution into ~ 300 mL of pure water and shook well. Completed to 1000 mL volume with pure water.

Internal Standard Solution: Transferred 10 µL of each: 1000 ppm In, 1000 ppm scandium (Sc), 1000 ppm germanium (Ge), 1000 ppm Bi main stock solution and 100 µL of the 100 ppm Y main stock solution into a 100 mL volumetric flask and diluted to volume with diluent (In, Sc, Ge, Bi, Y; 100 ppb).

Table 1. ICP-MS operating conditions.

Conditions	Properties
RF power	1.20 kW
Sampling depth	5.0 mm
Plasma gas	Ar: 9.0 L/min
Auxiliary gas	Ar: 1.10 L/min
Carrier gas	Ar: 0.70 L/min
Torch	Mini-Torch ICPMS
Nebulizer	Nebulizer O7UES
Chamber	Cyclone chamber
Chamber temperature	5°C
Number of scan	20
Cell gas	He: 6.0 mL/min
Cell voltage	-21 V
Energy filter	7 V
Solvent rinse time	40 sec (low) 60 sec (high)
Sample rinse time	40 sec (low) 60 sec (high)
Peristaltic pump rotation speed	20 rpm (low) 60 rpm (high)

Calibration Solutions: For each element calibration, the solutions were prepared according to the targeted concentration range.

Unspiked Sample Solution Preparation for Micafungin 50 mg: Two vials of 50 mg of Micafungin were transferred into a 250 mL flask by adding 19 mL of HNO₃ (65%) and completed to volume with distilled water. Then, 1.0 mL of the solution was taken and transferred to a 50 mL flask and completed to volume with diluent (2% HNO₃).

Unspiked Sample Solution Preparation for Micafungin 100 mg: One vial of 100 mg of Micafungin was transferred into a 250 mL flask by adding 19 mL of HNO₃ (65%) and completed to volume with distilled water. Then, 1.0 mL of the solution was taken and transferred to a 50 mL flask and completed to volume with diluent (2% HNO₃).

Spiked Sample Solution Preparation for Micafungin 50 mg: Two vials of 50 mg of Micafungin were transferred into a 250 mL flask by adding 19 mL of HNO₃ (65%) and completed to volume with distilled water. Next, 1.0 mL of the solution was taken and transferred into a 50 mL flask. Then, the solution was spiked with 24 elements to obtain the final concentration of corresponding J values for each of the elemental impurities and completed to volume with diluent (2% HNO₃).

Spiked Sample Solution Preparation for Micafungin 100 mg: One vial of 100 mg of Micafungin was transferred into a 250 mL flask by adding 19 mL of HNO₃ (65%) and completed to volume with distilled water. Next, 1.0 mL of the solution was taken and transferred into a 50 mL flask. Then, the solution was spiked with 24 elements to obtain the final concentration of corresponding J values for each of the elemental impurities and completed to volume with diluent (2% HNO₃).

Validation

All the parameters stated in the ICH Guideline Q2(R1) were investigated (ICH 2005). The system suitability parameter was performed to verify that the analytical system works properly, and gives accurate and precise results. System suitability testing was performed before the initiation of each validation parameter analysis. For the analysis of each elemental impurity, the limit of J values and acceptance criteria were determined according to USP <232> and analyses were performed as per USP <233>.

Specificity

The specificity parameter evaluated if the product to be analyzed can be determined precisely

in the presence of matrix effect and additives. The specificity of the developed method was evaluated by analyzing the blank, calibration standard solutions, unspiked sample solution, and spiked sample solutions. The interference between each target element, blank solution, and the sample was demonstrated, and the response of each element was recorded. The response obtained from the blank solution should not be greater than 5% of the response obtained from the standard solution of each peak.

Linearity, LOD, LOQ

For linearity studies, the calibration samples were prepared by serial dilutions from the standard solution at concentrations of 0%, 50% (0.5J), 75% (0.75J), 100% (1J), 150% (1.5J), and 200% (2J) and three replicates of analysis were performed from each calibration level. The regression analysis was performed for linearity parameter, and the correlation coefficient R value should not be less than 0.99. The linearity equations were recorded for the calculation of the precision and accuracy data. The limit of detection (LOD) and limit of quantification (LOQ) values were calculated by ICP-MS for each element separately.

Precision

The precision of the analytical method was described as the closeness of replicate sampling obtained from same homogeneous sample. This parameter was investigated under three subheadings: system precision, repeatability, and intermediate precision. For the precision parameter, the data were calculated by using calibration lines. The 100% level of calibration line was injected to the system six times consecutively, and the relative standard deviation (RSD)% value should not be more than 20% for the system precision parameter.

For the repeatability and intermediate precision parameters, two different unspiked samples and six different spiked samples were prepared, and the calculation was performed by subtracting the unspiked response from spiked response for each elemental impurity. The RSD% value obtained from six sample solutions should not be more than 20%. In addition, 12 sample solution results of each target element obtained from repeatability and intermediate precision parameters were evaluated. The RSD% should not be more than 25%.

Accuracy

For the accuracy parameter, nine different sample solutions were prepared and spiked with

the mix standard solution at 50% (0.5J), 100% (1J), and 150% (1.5J) levels of the working concentration at three replicates from each level. Recovery analysis results should be within 70%-150% for the mean of three replicate preparations at each level for each elemental impurity.

RESULTS AND DISCUSSION

According to the elemental impurity guide (Q3D) published by the ICH and used by the authorities, elements are considered to be in three different classes according to their toxicity potential and possibility of being found in the finished products [7]. Among these impurities, the impurities in Class 1 are arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb). Arsenic, one of the elements known for its toxicity to humans, is known to cause cancer and skin lesions in chronic exposure. In addition, diabetes, cardiovascular disease, and infant exposure at any stage of development are associated with adverse effects on cognitive development [8, 9]. Exposure to Cd, another of these toxic elements, has been shown to cause cancer and systemic organ toxicity [10]. Hg is known for its toxicity in the lungs, central nervous system and kidneys, and because it crosses the blood-brain barrier, neurological symptoms that can develop even with low exposure can last for decades [11]. Although Pb poisoning has toxic effects on the development of the nervous system and brain, especially in children, it is known to cause cardiovascular problems and kidney damage in adults [12, 13].

Similarly, the elements given in Class 2 are also known for their toxicity depending on the route of use, and their parenteral use can directly enter the systemic circulation and cause toxicity. Therefore, it is important to evaluate whether they are present in our finished product or not. Elements in this group that are likely to be found in products are Co, nickel (Ni) and vanadium (V); while elements that are less likely to be found are silver (Ag), gold (Au), iridium (Ir), osmium (Os), palladium (Pd), platinum (Pt), rhodium (Rh), ruthenium (Ru), selenium (Se) and thallium (Tl). Although the elements in Class 3 (barium (Ba), chromium (Cr), copper (Cu), lithium (Li), molybdenum (Mo), antimony (Sb), and tin (Sn)) cause lower toxicity, the importance of their determination, especially in parenteral and inhalation use, is emphasized by the guideline. With this method developed for the determination of the aforementioned elemental impurities, the following results were obtained.

Calibration samples were prepared by serial dilutions from the standard solution at concentrations of 0%, 50% (0.5J), 75% (0.75J), 100% (1J), 150% (1.5J), and 200% (2J) and three replicates of analyses were performed for all these 24 elements in the targeted concentration. The linearity of the concentration range for the elements was proven. The correlation coefficients of the calibration curves for all the elements were found higher than 0.99 for all the elements in the determined ranges. Also, LOD and LOQ values are given (Table 2).

RSD of six replicate injections of standard solution were smaller than 20% for all the elements. These results showed consistency of the repeatability of the standard solution analysis. Also, the difference between the prepared spiked sample and non-spiked samples was checked and RSD was calculated. For six spiked repeatability sample solutions (spiked-unsiked), RSD was not more than 20% for each target element. The average recovery results of six spiked repeatability sample solutions (spiked-unsiked) were between 70-150% for each element. (Table 3)

In the scope of the intermediate precision parameter, the repeatability analysis was reperformed on a different day, with a different analyst. The results were combined with the repeatability analysis so that the total number of analyses was 12. For all the elements in six intermediate precision solutions, the RSD was not more than 20%. The acceptance criteria were met. The 12 sample RSD% results of repeatability and intermediate precision was not more than 25%. The acceptance criteria were met. The average of six spike intermediate precision sample solutions (spiked-unsiked) recovery analysis results were between 70-150% for each element.

As specified in the analytical method, nine different sample solutions were prepared and spiked from the mix standard solution at 50% (0.5J), 100% (1J), 150% (1.5J) levels of the working concentration with three replicates from each level. The recovery results of the accuracy analysis were within 70-150% for the mean of three replicate preparations at each concentration. The acceptance criteria were met (Table 4).

The presence of abovementioned three classes of elemental impurities in parenteral drug products is gaining importance due to their known toxicity. Therefore, the analysis method has been developed for all classes of elemental impurities for Micafungin 50 mg and 100 mg powder for infusion product. For this purpose, considering the PDE limits given in the guide, the amount of these elements

Table 2. Regression equations, linear ranges, correlation coefficients, LODs, and LOQs of elemental impurities.

Element	Regression equation	Se slope	Se intercept	Linear range (ppb)	R	P	LOD (ppb)	LOQ (ppb)
Ag	$7.773x + 0.038$	0.110	0.068	0-1.07	0.999	2.42E-07	0.048369	0.161231
As	$3.737x - 0.028$	0.055	0.051	0-1.60	0.999	2.80E-07	0.008353	0.027843
Au	$3.233x - 1.795$	0.162	1.002	0-10.67	0.995	3.73E-05	0.029507	0.098357
Ba	$6.123x - 0.974$	0.039	1.700	0-74.67	0.999	1.02E-08	0.004876	0.016255
Cd	$28.007x + 0.181$	0.787	0.097	0-0.21	0.998	3.72E-06	0.000443	0.001475
Co	$64.474x - 1.600$	2.804	0.867	0-0.53	0.996	2.12E-05	0.074927	0.249755
Cr	$30.180x - 27.743$	0.308	20.929	0-117.33	0.999	6.48E-08	0.670604	2.235347
Cu	$42.877x + 6.828$	0.306	5.677	0-32	0.999	1.56E-08	0.056165	0.187216
Hg	$0.979x + 0.003$	0.057	0.011	0-0.32	0.993	6.93E-05	0.004902	0.01634
Ir	$49.969x + 0.058$	0.473	0.293	0-1.07	0.999	4.83E-08	0.000849	0.002829
Li	$230.070x + 64.845$	2.466	38.107	0-26.67	0.999	7.91E-08	0.0286	0.095332
Mo	$3.959x + 3.378$	0.034	3.124	0-160	0.999	3.15E-08	0.002704	0.009013
Ni	$35.918x + 3.307$	1.646	2.035	0-2.13	0.996	2.61E-05	0.145806	0.48602
Os	$8.034x - 0.050$	0.059	0.037	0-1.07	0.999	1.76E-08	0.002126	0.007087
Pb	$13.130x + 0.011$	0.209	0.065	0-0.53	0.999	3.85E-07	0.002647	0.008823
Pd	$5.392x + 0.076$	0.079	0.049	0-1.07	0.999	2.77E-07	0.012974	0.043246
Pt	$2.830x + 0.001$	0.048	0.030	0-1.07	0.999	5.16E-07	0.000645	0.002115
Rh	$22.777x + 0.170$	0.236	0.146	0-1.07	0.999	6.91E-08	0.000754	0.002514
Ru	$6.705x + 0.051$	0.081	0.050	0-1.07	0.999	1.26E-07	0.002252	0.007507
Sb	$2.405x + 0.015$	0.012	0.064	0-9.60	0.999	3.14E-09	0.005053	0.016842
Se	$0.046x + 0.001$	0.001	0.004	0-8.53	0.999	4.96E-07	0.047796	0.159321
Sn	$3.086x - 0.069$	0.023	0.841	0-64.00	0.999	1.74E-08	0.053395	0.177985
Tl	$8.755x - 0.034$	0.118	0.059	0-0.85	0.999	2.01E-07	0.000813	0.002709
V	$24.705x - 0.004$	0.057	0.035	0-1.07	0.999	1.68E-10	0.008992	0.029972

Table 3. Repeatability and intermediate precision results.

Element	Repeatability		Intermediate precision		12 sample RSD%
	Mean (%)	RSD%(n = 6)	Mean (%)	RSD%(n = 6)	
Ag	92.6	3.9	90.8	1.9	3.1
As	101.9	3.0	107.7	4.0	4.5
Au	87.7	4.9	117.4	9.6	17.1
Ba	106.1	2.2	106.5	1.1	1.7
Cd	83.3	2.8	74.4	2.0	6.4
Co	96.8	2.9	95.1	3.3	3.1
Cr	102.8	1.9	95.3	1.2	4.3
Cu	98.5	2.4	96.3	1.2	2.2
Hg	109.7	5.0	97.3	4.3	7.8
Ir	100.2	2.0	98.1	1.1	2.0
Li	93.3	2.3	84.4	2.5	5.7
Mo	100.0	2.8	98.3	1.4	2.3
Ni	87.8	14.9	108.0	11.4	16.4
Os	97.1	4.1	100.7	2.2	3.7
Pb	96.9	5.7	103.9	5.4	6.6
Pd	91.3	1.7	84.1	1.5	4.6
Pt	98.2	2.5	96.8	1.5	2.1
Rh	99.4	1.3	96.0	1.1	2.1
Ru	98.8	1.6	96.5	1.0	1.8
Sb	104.0	2.4	101.1	1.4	2.4
Se	107.6	3.3	97.7	2.9	5.8
Sn	96.4	3.6	98.5	1.6	2.9
Tl	101.3	2.2	102.4	1.5	1.9
V	98.5	1.7	98.5	1.8	1.7

Table 4. Accuracy results.

Element	0.5 J	1 J	1.5 J	Total recovery%
Ag	72.9	99.7	104.2	92.3
As	102.0	96.4	103.0	100.5
Au	81.4	99.4	110.0	96.9
Ba	98.4	99.9	100.2	99.5
Cd	92.4	96.3	99.4	96.0
Co	94.2	96.2	98.0	96.1
Cr	96.5	97.7	98.9	97.7
Cu	100.0	101.0	101.8	100.9
Hg	76.3	86.3	85.1	82.6
Ir	97.5	99.4	99.7	98.9
Li	101.1	100.8	98.0	100.0
Mo	93.4	97.1	97.9	96.1
Ni	126.7	91.6	97.1	105.1
Os	94.1	95.3	97.8	95.7

Table 4. Accuracy results (cont.).

Element	0.5 J	1 J	1.5 J	Total recovery%
Pb	75.8	84.9	91.2	84.0
Pd	94.1	100.9	98.9	98.0
Pt	98.1	99.2	100.1	99.1
Rh	98.0	99.7	99.7	99.1
Ru	97.8	99.0	98.8	98.5
Sb	97.2	97.9	99.7	98.3
Se	98.2	102.4	97.1	99.2
Sn	91.9	96.0	97.5	95.1
Tl	96.9	99.3	101.3	99.2
V	94.5	98.6	101.1	98.1

mentioned in the guidelines were determined. The results are given in Table 5. These analyses were carried out by preparing two replicates of samples for three different batches for each dose. When

the analysis result of three batches of each dose were evaluated, it was found that the results between the batches were very close. Table 5 includes the results of one representative batch for each dosage.

Table 5. Representative results of the finished product from each dose.

Elements	Limits (ppb)	Micafungin 50 mg powder for infusion results (ppb)	Micafungin 100 mg powder for infusion results (ppb)
Cd	0.11	0.00	0.00
Pb	0.27	0.01	0.02
As	0.80	0.01	0.05
Hg	0.16	0.00	0.00
Co	0.27	0.00	0.00
V	0.53	0.00	0.01
Ni	1.07	0.00	0.00
Tl	0.43	0.00	0.02
Au	5.33	0.63	0.86
Pd	0.53	0.00	0.00
Ir	0.53	0.00	0.02
Os	0.53	0.01	0.02
Rh	0.53	0.00	0.01
Ru	0.53	0.00	0.01
Se	4.27	0.00	0.04
Ag	0.53	0.00	0.00
Pt	0.53	0.00	0.02
Li	13.33	0.08	0.08
Sb	4.80	0.00	0.16
Ba	37.33	0.23	1.46
Mo	80.00	0.00	2.40
Cu	16.00	0.00	0.41
Sn	32.00	0.36	1.02
Cr	58.67	0.00	1.98

CONCLUSIONS

Micafungin powder for infusion is an important medicine with broad antifungal activity. For the development of parenteral products, the determination of elemental impurities that can originate from the manufacturing process and that can be observed during the shelf life of a product is a critical point. In this study, we successfully developed and validated an ICP-MS method to determine the 24 elemental impurities mentioned in the parenteral products guidelines for Micafungin containing a sterile drug product. This study can be considered as a reference in the development method for other injectable products.

Conflict of Interest

All the authors are employees of Centurion Pharma.

Funding Sources

Centurion Ilac Sanayi ve Ticaret A.S. (Centurion Pharma) funded all analyses reported here.

Author's Contribution

Concept, Design, Supervision, S.O.G.; Data Collection and Processing, S.O.G., S.T., A.O.B. and T.C.; Analysis and Interpretation, S.O.G., S.T., A.O.B. and T.C.; Literature Search, A.S. and S.O.G.; Writing, A.S. and S.O.G.; Critical Reviews, S.O.G., S.T., A.O.B, T.C. and A.S.

REFERENCES

1. Thomas F. Approaching Elemental Impurity Analysis. *Pharm Tech Europe* 2021; 33(2): 28-30.
2. Balaram V. Recent advances in the determination of elemental impurities in pharmaceuticals – status, challenges and moving frontiers. *TrAC Trends Anal Chem.* 2016; 80: 83-95. doi: 10.1016/j.trac.2016.02.001.
3. Sheth AC, Patel PU. Review of elemental impurities in pharmaceuticals arena. *Int J Pharm Qual Assur.* 2020; 11(2): 217-221. doi:10.25258/ijpqa.11.2.3.
4. Maithani M, Raturi R, Sharma P, et al. Elemental impurities in pharmaceutical products adding fuel to the fire. *Regul Toxicol Pharmacol.* 2019; 108: 104435. doi: 10.1016/j.yrtph.2019.104435.
5. Barin JS, Mello PA, Mesko MF, et al., Determination of elemental impurities in pharmaceutical products and related matrices by ICP-based methods: a review. *Anal Bioanal Chem.* 2016. 408(17): 4547-4566. doi: 10.1007/s00216-016-9471-6.
6. EMA. ASSESSMENT REPORT FOR Mycamine. 2008 [cited 2023 July 2023]; Available from: https://www.ema.europa.eu/en/documents/assessment-report/mycamine-epar-public-assessment-report_en.pdf.
7. ICH. guideline for elemental impurities Q3D(R1). 2019 [cited 2023 4 October]; Available from: https://database.ich.org/sites/default/files/Q3D-R1EWG_Document_Step4_Guideline_2019_0322.pdf.
8. Kuivenhoven M, Mason K. Arsenic Toxicity, in StatPearls. 2023: Treasure Island (FL) ineligible companies. Disclosure: Kelly Mason declares no relevant financial relationships with ineligible companies.
9. WHO. Arsenic. 2022 [cited 2023 4 October]; Available from: <https://www.who.int/news-room/fact-sheets/detail/arsenic#:~:text=Arsenic%20is%20highly%20toxic%20in,cause%20cancer%20and%20skin%20lesions.>
10. Rafati Rahimzadeh M, Rafati Rahimzadeh M, Kazemi S, et al. Cadmium toxicity and treatment: An update. *Caspian J Intern Med.* 2017; 8(3): 135-145. doi: 10.22088/cjim.8.3.135.
11. Posin SL, Kong EL, Sharma S. Mercury Toxicity, in StatPearls. 2023: Treasure Island (FL) ineligible companies. Disclosure: Erwin Kong declares no relevant financial relationships with ineligible companies. Disclosure: Sandeep Sharma declares no relevant financial relationships with ineligible companies.
12. Wani AL, Ara A, Usmani JA. Lead toxicity: a review. *Interdiscip Toxicol.* 2015; 8(2): 55-64. doi: 10.1515/intox-2015-0009.
13. WHO. Lead poisoning. 2023 [cited 2023 4 October]; Available from: <https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health#:~:text=At%20high%20levels%20of%20exposure,intellectual%20disability%20and%20behavioural%20disorders.>

