Original Article

Development and validation of a microwave-assisted digestion technique as a rapid sample preparation method for the estimation of selenium in pharmaceutical dosage forms by ICP-OES

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ABSTRACT

Selenium is a class 2B element according to the International Council for Harmonization Q3D guidelines. Selenium sulfide is an anti-infective agent with antifungal and antibacterial properties used to treat dandruff and seborrheic dermatitis. The literature survey revealed that most of the analytical techniques to estimate selenium were time-consuming and/or required high skill levels. The process involved identifying the isotopes, selecting the measurement approach, and optimizing a typical microwave-aided digesting procedure. Ammonium hydrogen difluoride, water, and concentrated nitric acid were added to the samples. The confirmed microwave digestion program was a two-step program where in the initial step, the samples were ramped at 200°C for 20 min and held for 5 min. Later, samples were cooled and neutralized by boric acid, then ramped for 20 min to a temperature of 180°C and held for 10 min. Selenium was estimated at 196.090 nm by inductively coupled plasma optical emission spectroscopy (ICP-OES). System suitability was run before initiating analysis to ensure that system performance was consistent. Analytical validation parameters, such as the specificity of the method, were demonstrated at 196.090 nm, linearity was proven from 10 ppm to 150 ppm of selenium concentration, the detection limit was 1.28 ppm, and the limit of quantification was 3.89 ppm. Robustness was confirmed for small changes to ICP-OES operating conditions. The precision of the method demonstrated by analyzing the percentage relative standard deviation for six injections was found to be less than 2.0%. Accuracy was confirmed from 10 ppm to 150 ppm, and all the samples were observed to be within the range of 95%-105%. A common microwave-assisted digestion technique was developed and validated as well. The precision, specificity, linearity, accuracy, and robustness of the method for estimating selenium in selenium sulfide drug substances and various pharmaceutical dosage forms were demonstrated. This newly developed microwave-assisted digestion technique has optimum sensitivity and is highly reproducible and time-saving than the existing methods This method can be applied to numerous matrices for a finished dosage of selenium sulfide formulations.

Keywords: Microwave-assisted digestion, Selenium content, Inductively coupled plasma optical emission spectroscopy, Selenium sulfide, ICH Q3D guidelines

1. Introduction

The element selenium, CAS No. 7782-49-2, is a member of the group VIA of the periodic table and possesses metallic and nonmetallic characteristics (1). Selenium belongs to class 2B of elements according to the International Council for Harmonization Q3D guideline (2). These class-2B elements are less likely to be included in the therapeutic product due to their limited availability and poor ability to co-isolate with other materials. Therefore, they might not be considered in the risk assessment unless they are consciously incorporated while producing drug compounds, excipients, or other components of the drug product (2). Selenium sulfide is an anti-infective agent with antifungal and antibacterial properties and is used to cure dandruff and seborrheic dermatitis (3); hence, it is used as a dermatological shampoo.

Literature survey shows that selenium can be determined using different techniques, such as voltammetric analysis (4, 5), spectrophotometry (6, 7), chromatography (high-performance liquid chromatography [HPLC]) (8, 9), atomic absorption spectroscopy (10-14), atomic fluorescence spectroscopy (AFS) (15-17), inductively coupled plasma optical emission spectroscopy (ICP-OES) (18), inductively coupled plasma mass spectrometry (ICP-MS) (19), HPLC combined with ICP-MS (20), and HPLC in conjunction with AFS (21). Most of the above-mentioned analytical techniques were found to be time-consuming and/or required high levels of skills. Inductively coupled plasma optical emission spectroscopy is the preferred technique as it is cost-effective for single-element analysis and is sensitive and reliable. On the other hand, the open digestion technique is dangerous, time-consuming, and inconsistent. Open digestion sample preparation followed by a non-specific titration was used for estimating selenium sulfide drug substance (22) and selenium sulfide topical suspension (23). This research work aimed to develop and validate a simple, cost-effective, robust ICP-OES method for estimating selenium in selenium sulfide drug substance and shampoo formulation using closed microwave digestion.

2. Materials and Methods

2.1. Chemicals, reagents, standards, and samples

Selenium sulfide drug substance was purchased from ABCR GMBH (Germany). Selsun blue dandruff shampoo with menthol, 1% selenium sulfide (Manufacturer: Sanofi), and selenium sulfide topical suspension USP, 2.5% lotion (Manufacturer: Perrigo) were bought from the open market. Selenium 10 mg/ml was acquired from Inorganic Ventures. All the chemicals used were of analytical reagent grade. Ammonium hydrogen difluoride was bought from Sigma-Aldrich (USA). Boric acid 99.99% was obtained from Alfa Aesar (USA). Concentrated nitric acid (70%) trace metal grade was acquired from Fisher (USA).

2.2. Instruments and equipment:

ICP-OES made of Thermo-scientific iCAP 6000 series with Helium/Kinetic energy discrimination mode was utilized. The microwave digestion system CEM MARS 6 with Easy Prep Plus Vessels was used. The Mettler Toledo analytical balance with model: ME204E was employed. A hot plate made by Royal Scientific (model RSW 127) was used.

2.3. Sample preparation:

The first phase in the technique development process involved the investigation of solubility. The method development trail was initiated by dissolving approximately 0.1 g of drug substance (i.e., selenium sulfide) in 100 ml of water. Similarly, 0.1 g of shampoo and topical suspension were dissolved in 100 ml of water. As an alternate method, closed microwave digestion with concentrated high-pure acids was considered for sample preparation. Closed vessel microwave digestion was initiated by using a varied ratio of acids. The trial observations are tabulated in table 1. All the sample matrices were subjected to microwave-assisted closed vessel digestion under controlled pressure and temperature.

Table 1 Sample preparation trials by microwave-assisted digested (MWD) procedure

S. No.	Selenium Sulfide Topical Suspension	Closed vessel microwave-assisted digestion program				Selenium Sulfide drug substance	Selsun Blue Dandruff Shampoo	Selenium Sulfide Topical Suspension
1	Samples + 5ml conc. Nitric acid + 5ml conc. hydrochloric → MWD	Step	Ramp (min)	Hold (min)	Temp. (°C)	The sample settles in the bottom of the vessel with partial digestion	The sample settles in the bottom of the vessel with partial digestion	The sample settles at the bottom of the vessel with partial digestion
		1	20	30	200			
2	Samples + 5ml conc. Nitric acid + 5ml conc. hydrochloric acid → MWD Step-1 + 10ml conc. Nitric acid →MWD Step-2	Step	Ramp (min)	Hold (min)	Temp. (°C)	The sample settles in the bottom of the vessel with partial digestion	The sample settles in the bottom of the vessel with partial digestion	The sample settles at the bottom of the vessel with partial digestion
		1	20	30	200			
		2	20	10	180			
3	Samples + 0.2g ammonium hydrogen difluoride + 1ml water → predigested for 5 minutes + 5ml conc. Nitric acid → predigested for 15 minutes →MWD Step-1 + 5ml 4% boric acid →MWD Step-2	Step	Ramp (min)	Hold (min)	Temp. (°C)	The sample settles in the bottom of the vessel with partial digestion	The sample settles in the bottom of the vessel with partial digestion	The sample settles in the bottom of the vessel with partial digestion
		1	10	15	180			
4	Samples + 0.2g ammonium hydrogen difluoride + 1ml water → predigested for 5 minutes + 5ml conc. Nitric acid → predigested for 15 minutes →MWD Step-1 + 5ml 4% boric acid →MWD Step-2	Step	Ramp (min)	Hold (min)	Temp. (°C)	The sample settles in the bottom of the vessel with partial digestion	The sample settles in the bottom of the vessel with partial digestion	The sample settles in the bottom of the vessel with partial digestion
		1	10	15	200			
5	Samples + 0.2g ammonium hydrogen difluoride + 1ml water → predigested for 5 minutes + 5ml conc. Nitric acid → predigested for 15 minutes →MWD Step-1 + 5ml 4% boric acid →MWD Step-2	Step	Ramp (min)	Hold (min)	Temp. (°C)	Almost all the sample digests with some residue at the bottom of the vessel	Almost all the sample digests with some residue at the bottom of the vessel	Almost all the sample digests with some residue at the bottom of the vessel
		1	20	30	200			
		2	20	10	180			
6	Samples + 0.5g ammonium hydrogen difluoride + 1ml water → predigested for 5 minutes + 7ml conc. Nitric acid → predigested for 15 minutes →MWD Step-1 + 7ml 4% boric acid →MWD Step-2	Step	Ramp (min)	Hold (min)	Temp. (°C)	Complete digestion	Almost all the sample digests with some residue at the bottom of the vessel	Almost all the sample digests with some residue at the bottom of the vessel
		1	20	30	200			
		2	20	10	180			
7	Samples + 0.7g ammonium hydrogen difluoride + 1ml water à predigested for 5 minutes + 10ml conc. Nitric acid à predigested for 15 minutes →MWD Step-1 + 10ml 4% boric acid →MWD Step-2	Step	Ramp (min)	Hold (min)	Temp. (°C)	Complete digestion	Complete digestion	Complete digestion
		1	20	30	200			
		2	20	10	180			

Sample preparation optimization

Selenium sulfide drug substance For the drug substance, 0.5 g of selenium sulfide was accurately weighed. Approximately 0.7 g of ammonium hydrogen difluoride was added to each vessel, followed by 1 ml of water. The samples were pre-digested for approximately 5 min in the ammonium hydrogen difluoride solution. A volume of 10 ml of concentrated nitric acid was added to each digestion vessel, and the samples were continued to pre-digest for around 15 min. The microwave reaction vessels were assembled, and the samples were digested according to the parameters described in the digestion step 1 (Table 3). The samples were allowed to cool before adding 10 ml of 4% boric acid required to complex the remaining hydrofluoric acid. The microwave reaction vessels were reassembled, and the samples were digested according to the parameters described in the digestion step 2 (Table 3). Digested samples were quantitatively transferred to individual 10 ml volumetric flasks. Samples were allowed to cool to room temperature before diluting to volume with water.

Selenium sulfide drug formulation

Samples were prepared by shaking the product container vigorously and mixing a portion of the shaken product in a beaker to ensure sample homogeneity. A volume of 0.5 g of Selsun blue dandruff shampoo and 0.2 g of selenium sulfide topical suspension USP were accurately weighed separately. Using a micropipette pipette, samples were dispensed into tared microwave vessels, and the product weight was recorded. Approximately 0.7 g of ammonium hydrogen difluoride was added to each vessel, followed by 1 ml of water. The samples were pre-digested for approximately 5 min in the ammonium hydrogen difluoride solution. Subsequently, 10 ml of concentrated nitric acid was added to each digestion vessel, and the samples were continued to pre-digest for around 15 min. The microwave reaction vessels were assembled, and the samples were digested according to the parameters described in the digestion step 1 (Table 3). The samples were allowed to cool before adding 10 ml of 4% boric acid required to complex the remaining hydrofluoric acid. The microwave reaction vessels were reassembled, and the samples were digested according to the parameters described in the digestion step 2 (Table 3). Digested samples were quantitatively transferred to individual 100 ml volumetric flasks. Samples were allowed to cool to room temperature before diluting to volume with water.

2.4. Rinsing solution for ICP-OES instrument autosampler - 2% nitric acid (v/v):

Slowly, 20 ml of nitric acid (concentrated) was added to 500 ml of water. This solution was diluted to 1 L with water and mixed well.

2.5. Preparation of 4% boric acid (w/v):

Approximately 40 g of boric acid was weighed and added to 1,000 ml of water. The mixture was stirred while gently heated until it completely dissolved.

2.6. Preparation of standard solutions:

Concentrated nitric acid, 4% boric acid, and 10 mg/ml selenium (Se) reference standard were transferred in 500 ml and 200 ml volumetric flasks accordingly (as volumes indicated in Tables 2 and 3) and diluted to volume with water to prepare calibration standards and calibration check standards.

2.7. Instrumental parameters for analysis

ICP-OES conditions

ICP-OES instrument was set up according to parameters listed in table 4 and calibrated with the calibration standards (10, 25, 50, 75, and 150 µg/ml) each time before use. Selenium (Se) concentrations were quantified using external calibration. Initial calibration verification was performed with the calibration check standard (50 µg/ml). Samples were bracketed with the calibration check standard following every sixth sample. The permissible cutoff for quality control (QC) verification was 5.0% of the claimed QC concentration.

2.8. Validation parameters

2.8.1. System suitability/linearity

Before initiating every validation parameter, all standard aliquots and blank solutions were injected in ICP-OES. The acceptance criterion for relative standard deviation (%RSD) of 5 replicate analyses of the 50 µg/ml selenium calibration checked standard was NMT 2.0%. The calibration check standard was injected regularly to ensure the proper running of ICP-OES. The linearity of the method was proven using the standard aliquot solutions. The correlation coefficient of the 5-point calibration curve was calculated, and the %RSD of the calibration check standard was monitored.

Table 2: Preparation for calibration standards

Standard Concentration (µg/ml) or (ppm)	Volume (ml) Concentrated Nitric Acid	Volume (ml) 4% Boric Acid	Volume (ml) 10 mg/ml or 10,000 ppm Selenium RS	Final Volume (ml)
Blank	50	50	NA	500.0
10	50	50	0.5	500.0
25	50	50	1.25	500.0
50 (calibration check standard)	20	20	1.0	200.0
75	20	20	1.5	200.0
150	20	20	3.0	200.0

Table 3. MARS 6 microwave reaction system parameters for Digestion Step 1

Parameters	Settings	Digestion Step 2 Settings
Power	60 – 1800 watts	400 – 1800 watts
Ramp Time	20 mins	20 mins
Hold Time	30 mins	10 mins
Temperature	200 ^{o}C	180 ^{o}C
Pressure Max	800 psi	800 psi
Cool Down Time	30 mins	30 mins

Table 4. ICP-OES instrument parameters

Parameters	Settings
Pump Rate	2.2 ml/min
Sample Flush Time	70 sec
Nebulizer used	Concentric Glass
Spray Chamber used	Cyclonic Glass
Center tube	2.0 mm
Pump Sample Tube (Tygon)	Color :Orange-White
Flow Rate of Nebulizer	0.45 lit./min.
Flow of Coolant Gas	12 lit./min.
Flow of Auxiliary Gas	0.6 lit./min.
View	Radial
RF Power	1300 watts
Wavelength	196.090 nm
Low Wavelength Integration Time	15 sec
High Wavelength Integration Time	5 sec

2.8.2. Specificity parameter

Selenium determination was performed on the most sensitive emission line at 196.090 nm. Thus, the specificity was defined as no spectral interferences exhibited at 196. 090 nm, and microwave vessels used for digestion demonstrated no sample carryover. Spectral interferences were evaluated by aspirating blank, calibration standard, and sample solutions into the ICP-OES as well as by collecting full frame profiles of the charge injection device chip in the detector. Method blanks were digested and analyzed to monitor selenium carryover in the microwave vessels.

2.8.3. Method precision

Six samples of Selsun selenium sulfide drug substance were prepared by weighing approximately 0.5 g of drug substance into microwave vessels and digesting according to the procedure. To prepare six samples of Selsun blue dandruff shampoo (SeS_2, 1%) with menthol, about 0.5 g of product was weighed into microwave vessels and digested according to the procedure. In addition, six samples of selenium sulfide topical suspension USP (SeS_2 2.5%) lotion samples were created through the process of weighing around 0.2 g of product and digesting it according to the same procedure. Selenium concentrations were determined via ICP-OES analysis.

2.8.4. Intermediate precision

Similar to the method precision parameter, six samples of selenium sulfide drug substance, Selsun blue dandruff shampoo (SeS_2 1%) with menthol and selenium sulfide topical suspension USP (SeS_2 2.5%) lotion samples were prepared and analyzed in ICP-OES on a different day.

2.8.5. Accuracy

Accuracy was performed at three concentrations (each triplicate preparation) ranging from 10 ppm, 50 ppm, and 150 ppm for drug substance and drug product for selenium content. The % recovery in each level was calculated.

2.8.6. Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated from linearity solutions. The LOD for selenium was calculated from the linearity curve. Similarly, LOQ was determined.

LOD (µg/ml (ppm)) = 3.3 × Standard deviation/slope

LOQ (µg/ml (ppm)) = 10 × Standard deviation/slope

2.8.7. Robustness

Variations in instrument conditions (Table 5) were performed to evaluate robustness. The highest applied RF power setting for our ICP-OES instrument was 1,350 W. Therefore, the original work plan of 1,500 W was modified to test up to 1,300 W for the robustness study.

3. Results

3.1. Method developmental trials

During solubility studies, the selenium drug substance was found to be insoluble in water. The same result was confirmed in other studies (24). Further, Selsun blue dandruff shampoo with menthol and topical suspension USP was observed to be water-soluble.

To develop a common sample preparation method for selenium drug substance and drug product, the direct aqueous solution method for sample preparation was disregarded. Thus, the combination of microwave-assisted acid digestion and closed containers was used at higher temperatures without reagent and analyte losses and with a reduction in contamination risks.

In the closed vessel microwave-assisted digestion procedure, different volumes of concentrated nitric acid and hydrochloric acid had no significant impact on the digestion of the sample. Hence, according to Mathew et al. (2017) (25), molten ammonium hydrogen difluoride was utilized to digest the sample matrix. Ammonium hydrogen difluoride converted metal oxides to fluorides. Upon the addition of concentrated nitric acid, the fluorination reaction continued with the release of hydrofluoric acid (HF).

$$(NH_4F.HF) + HNO_3 \rightarrow NH_4NO_3 + 2\ HF$$

Boric acid was required to complex the remaining hydrofluoric acid.

3.2. System suitability/linearity

The acceptance criterion for the correlation coefficient (r) of the 5-point calibration curve (10-150 µg/ml Se) was NLT 0.999. System suitability was achieved with a correlation coefficient value of 0.999980. (Figure 1 and Tables 6 and 7).

Table 5. Variation in instrument conditions for robustness study

Operating Parameters	Settings
Pump Rate (ml./min.)	1.8, 2.2, 2.6
Flow Rate of Nebulizer Gas (lit./min.)	0.35, 0.45, 0.55
Flow Rate of Auxiliary Gas (lit./min.)	0.5, 0.6, 0.7
Applied RF Power (W)	900, 1100, 1300
Integration (sec)	15, 30, 45

Table 6. Linearity Results

Standard Result (µg/ml) or (ppm)	Standard Result (µg/ml) or (ppm)
0	0.0000
10	9.9336
25	24.745
50	50.246
75	75.473
150	149.600
Correlation coefficient	0.999980
Standard Error	39.393156315581
slope	101.0963

Table 7. System suitability – instrument precision of 5 replicate analysis of a 50 µg/ml Se calibration check standard

Standard Result (µg/mL)	Standard Result (µg/mL)
50	50.78
50	50.64
50	50.62
50	50.50
50	50.65
Average	50.64
Stdev	0.10
%RSD	0.2

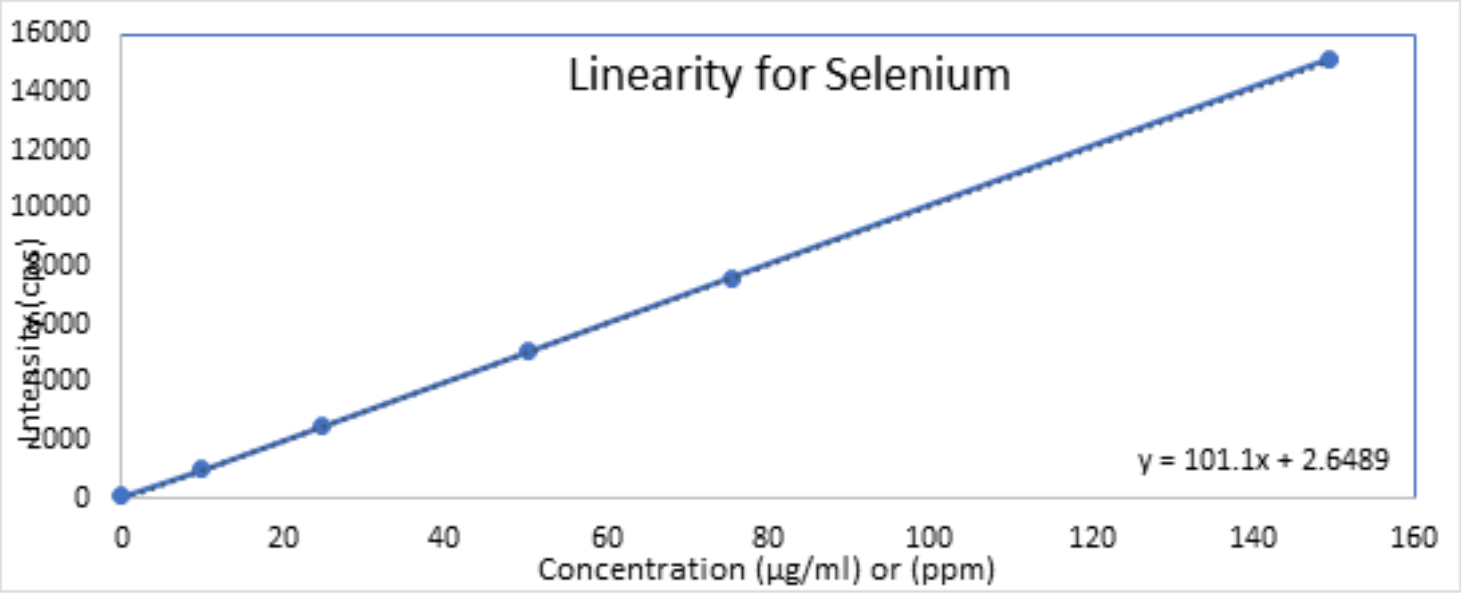

Figure 1. System suitability – linearity

3.3 Specificity

The specificity of the method was confirmed by measuring selenium at 196.090 nm, 203.985 nm, and 206.279 nm. Blank, 150 µg/ml calibration standard, 1% SeS_2 Selsun blue shampoo, and 2.5% SeS_2 Perrigo shampoo samples were measured at the above wavelengths. Hence in specificity solutions, spectral interferences were not observed at 196.090 nm, and selenium was not detected greater than 0.10 µg/ml in method blanks (Figure 2).

3.4 Method precision

The acceptance criterion for %RSD of the 6 samples each for selenium drug substance, Selsun blue dandruff shampoo (SeS_2 1%) with menthol, and selenium sulfide topical suspension USP (SeS_2 2.5%) was NMT 2.0%. For the drug substance, the %RSD for 6 replicate preparation was 0.2, for shampoo, for 6 replicate preparation, the %RSD was 1.7, and for the topical suspension, for 6 replicate preparation, the %RSD was 1.6 (Table 8).

3.5 Intermediate Precision

The results of 6 samples prepared and analyzed on different days were compared. The drug substance sample met current monograph specifications with an average selenium content of 99.2% for day 1 and an average of 99.8% for day 2. Both different days met the work plan criteria of NMT 2.0% RSD for 6 samples analyzed. The %RSD of overall precision for 12 samples was 0.6. The shampoo sample met current monograph specifications with an average selenium content of 93.8% for day 1 and an average of 93.6% for day 2. Both different days met the work plan criteria of NMT 2.0% RSD for 6 samples analyzed. The %RSD of overall precision for 12 samples was 1.4. The topical suspension sample met current monograph specifications with an average selenium content of 10.1.4% for day 1 and an average of 100.2% for day 2. Both different days met the work plan criteria of NMT 2.0% RSD for 6 samples analyzed. The %RSD of overall precision for 12 samples was 1.5 (Table 9).

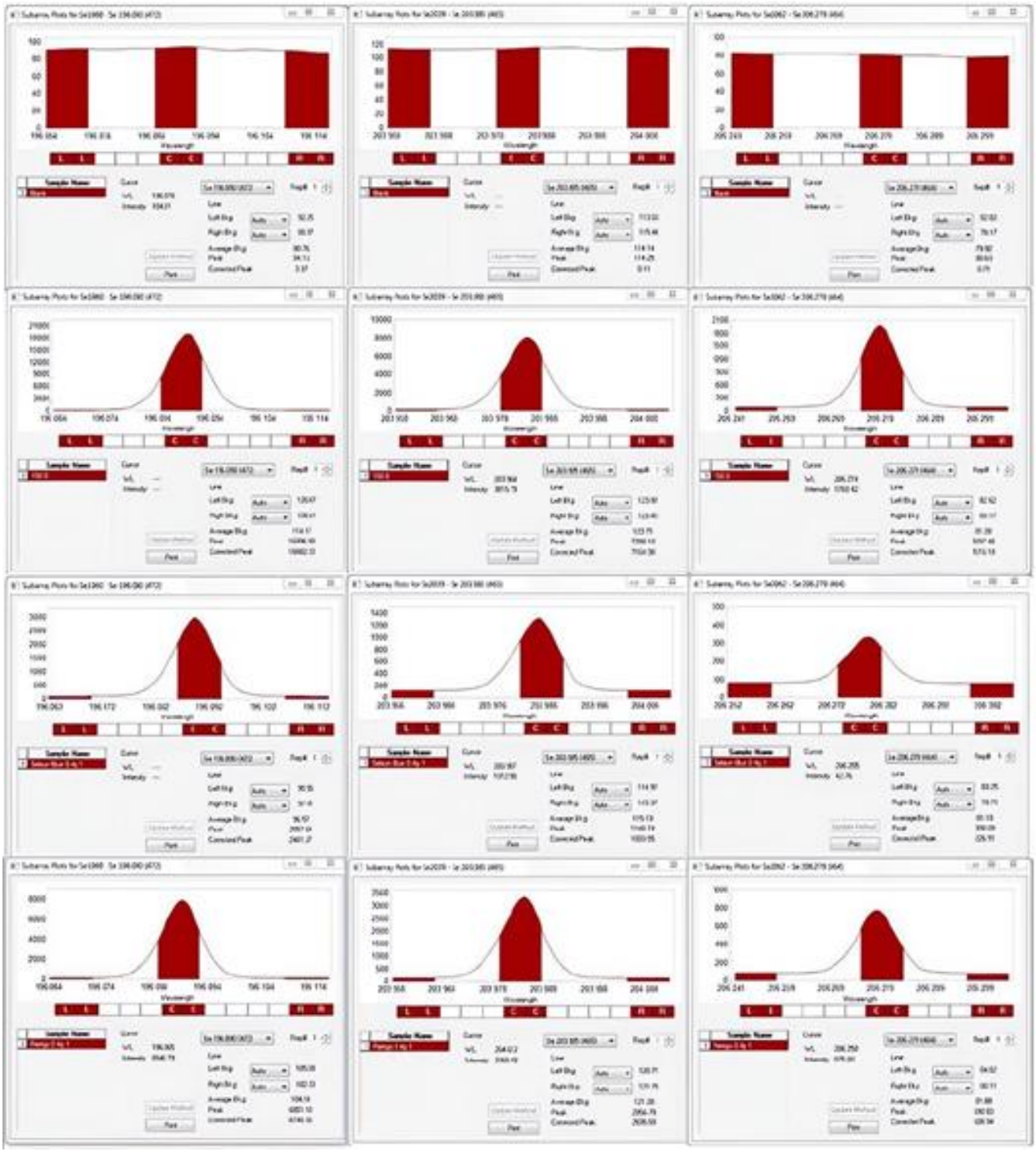

Figure 2. Specificity results when selenium was measured at 196.090 nm, 203.985 nm, and 206.279 nm

Table 8. Precision results for (a) selsun blue shampoo (SeS_2 1%) and (b) perrigo selenium sulfide topical suspension USP (SeS_2 2.5%).

selenium sulfide – drug substance	selenium content %	selsun blue shampoo (ses_2 1%) sample preparation	selenium content %	Perrigo selenium sulfide topical suspension usp (ses_2 2.5%) sample preparation	selenium content %
1	99.5	1	94.0	1	100.1
2	99.2	2	95.9	2	101.3
3	98.9	3	93.1	3	100.7
4	98.9	4	95.2	4	102.9
5	99.1	5	93.6	5	99.7
6	99.3	6	91.3	6	103.8
Average	99.2	Average	93.8	Average	101.4
Stdev	0.23	Stdev	1.63	Stdev	1.59
%RSD	0.2	%RSD	1.7	%RSD	1.6

Table 9. Intermediate precision results for Selenium sulfide drug substance, Selsun Blue Shampoo (SeS_2 1%) and Perrigo Selenium Sulfide Topical Suspension USP (SeS_2 2.5%).

selenium sulfide – drug substance sample preparation	selenium content %		selsun blue shampoo (ses_2 1%) sample preparation	selenium content %		perrigo selenium sulfide topical suspension USP (ses_2 2.5%) sample preparation	selenium content %	
	Day-1	Day-2		Day-1	Day-2		Day-1	Day-2
1	99.5	98.9	1	94.0	94.1	1	100.1	98.7
2	99.2	98.5	2	95.9	95.5	2	101.3	99.9
3	98.9	100.4	3	93.1	93.3	3	100.7	99.5
4	98.9	100.2	4	95.2	92.8	4	102.9	100.7
5	99.1	99.9	5	93.6	92.8	5	99.7	102.6
6	99.3	100.1	6	91.3	92.9	6	103.8	100.0
Average	99.2	99.8	Average	93.8	93.6	Average	101.4	100.2
Stdev	0.23	0.78	Stdev	1.63	1.07	Stdev	1.59	1.30
%RSD	0.2	0.8	%RSD	1.7	1.1	%RSD	1.6	1.3
Overall average		99.4	Overall average		93.7	Overall average		100.8
Overall %RSD		0.6	Overall %RSD		1.4	Overall %RSD		1.5

3.6 Accuracy

Accuracy is the degree to which observed values and actual values agree. Drug substance recovery rates ranged from 98.5% to 100.7% on average. The average shampoo recoveries fell between 101.6% and 103.0%, which was comparable. For topical suspension, the range of typical recoveries was 99.7% to 101.7% (Table 10).

3.6 Limit of detection and limit of quantitation

The linearity curve was used to calculate the LOD and LOQ. The LOD values were 1.28 ppm and 3.89 ppm, respectively.

3.7 Robustness

System suitability results of all tested conditions (Figure 3) met acceptance criteria of NMT 2.0% RSD and NLT 0.999 for r.

4. Discussion

Selenium sulfide is commonly used in various dosage forms, such as shampoo and topical suspension, because of its antifungal and antibacterial properties. A common microwave-assisted digestion technique was developed and validated. The precision, specificity, linearity, accuracy, and robustness of the method for estimating selenium in selenium sulfide drug substances and various pharmaceutical dosage forms were demonstrated (Table 11). This newly developed microwave-assisted digestion technique has optimum sensitivity and is highly reproducible and time-saving than the existing methods (Table 12). This method can be applied to numerous matrices for a finished dosage of selenium sulfide formulations.

Table 10. Accuracy results for Selenium sulfide drug substance, Selsun Blue Shampoo (SeS_2 1%) and Perrigo Selenium Sulfide Topical Suspension USP (SeS_2 2.5%).

Sample Name	Sample concentration level	Average Recovery (%)
Selenium sulfide	10 ppm samples	100.7%
	50 ppm samples	98.5%
	150 ppm samples	99.7%
Sample Name	**Spiked level**	**Average Recovery (%)**
Selsun Blue Shampoo (SeS_2 1%)	10 ppm spiked samples	101.6%
	50 ppm spiked samples	102.9%
	150 ppm spiked samples	103.0%
Selenium Sulfide Topical Suspension USP (SeS_2 2.5%)	10 ppm spiked samples	101.7%
	50 ppm spiked samples	99.7%
	150 ppm spiked samples	101.7%

Table 11. Summary of results

Parameter	Description	Criteria	Results
System Suitability Linearity	5-point calibration curve (10-150 µg/mL selenium).	The correlation coefficient (r) is NLT 0.999.	The correlation coefficient (r) is 0.999980.
System Suitability Precision	5 replicate analysis of the 50 µg/mLselenium calibration check standard.	The %RSD is NMT 2.0%.	The %RSD for the 5 continuousreadings of Se check standard is 0.2.
Robustness	Pump rate at 1.8, 2.2, and 2.6 mL/min. Nebulizer gas flow at 0.35, 0.45, 0.55L/min. Auxiliary gas flow at 0.5, 0.6, 0.7 L/min. RF power at 900, 1100, 1300 watts. Integration at 15, 30, 45 sec.	System suitability meets criteriaunder changes to instrument conditions	All correlation coefficients (r)are greater than 0.999 and all replicate analysis %RSD are NMT 2.0%.
Specificity	Collect full frame profiles of the CID(charge injection device) for blanks, calibration standards and samples.	The emission line at 196.090 nmis free of spectral interferences and microwave vessels used for digestion exhibit no sample carryover.	Spectral interferences are not present at 196.090 nm and Se inmethods blanks is not detected greater than 0.10 µg/mL.
Precision	Six samples of each product under study are prepared and Se concentrations of the digests determined by ICP-OES.	The %RSD of the 6 sampledigests is NMT 2.0%.	For the drug substance, the RSD is 0.2%, shampoo, the RSD is1.7% and for the lotion the RSD is 1.6%.
Intermediate Precision	For each product under study, results of 6samples prepared and analyzed by two independent analysts are compared on different days.	Calculated amounts of SeS_2 are within current monograph specification of 90.0 – 110.0% ofthe label claim. The %RSD of the 6 sample digests is NMT 2.0%. %RSD of overall precision on different days for 12 sample digested is NMT 3.0%	The drug substance sample met current monograph specifications with an average selenium content of 99.2% for Day 1 and an average of 99.8% for Day 2. The %RSD of overall precision on different days for 12 sample is 0.6. The shampoo sample met current monograph specifications with an average selenium content of 93.8% for Day 1 and an average of 93.6% for Day 2. The %RSD of overall precision on different days for 12 sample is 1.4. The topical suspension sample met current monograph specifications with an average selenium content of 10.1.4% for Day 1 and an average of 100.2% for Day 2. The %RSD of overall precision on different days for 12 sample is 1.5
Accuracy	Nine accuracy samples (triplicate preparation at sample concentration of 10 ppm, 50 ppm and 150ppm for drug substance. Nine spiked Samples each for shampoo and lotion were prepared by spiking selenium standard at 10 ppm, 50 ppm and 150ppm levels	Recovery of the spikes isbetween 95.0 – 105.0%.	The average spike recovery of drug substance was 100.7%, 98.5% and 99.7% at three different levels. The average spike recovery of shampoo was 101.6%, 102.9% and 103.0% at three different levels. The average spike recovery of Topical suspension was 101.7%, 99.7% and 101.7% at three different levels.
Limit of detection (LOD) & limit of quantitation (LOQ)	The linearity curve was used to calculate the limits of detection (LOD) and quantitation (LOQ).	Report the results	Limit of detection was calculated form the linearity curve. The limit of detection was 1.28 ppm and 3.89 ppm respectively.

Table 12. Summary of literature survey findings

Instrumental Technique	Method Details	Merits	Demerits	References
voltammetric analysis	Complexing Selenium with complexing agents (like selenocystine and dimethyldiselenide or (iron (III)-loaded) followed by extraction with agents like with dichloromethane or 0.5 ml HCl 37% by heating or	Cost effective	Low sensitivity Tedious multistep process and highly toxic. No method is validated	4,5
spectrophotometry	Chemical modification using agents like 4-aminoantipyrine (4-amino-1,2-dihydro-1,5-dimethyl-2-phenyl-3H-pyrazole-3-one; 4-AAP) by selenium in presence of acidic medium and the coupling with N-(naphthalen-1-yl)ethane-1,2-diamine dihydrochloride (NEDA) to give a violet color derivative or reacting selenium with 2,3-diaminonaphthalene was reinvestigated with bromide ion as a catalyst or	Optimum recovery	Low sensitivity due to cooling step. Few methods are only validated	6,7
chromatography (HPLC)	pre-column derivatization with 2,3-diaminonaphthalene or sodium salt of n-octanesulfonic acid as ion-pairing modifier in column	Good recovery	Complex multistep procedure. High skill required	8,9
atomic absorption spectroscopy (AAS)	MWD Digestion followed by Hydride generation	Optimum recovery and sensitivity	Time consuming and toxic sample preparation technique	10-14
atomic fluorescence spectroscopy	2,3-diaminonaphthalene (DAN) complexing or Se-2,3-diaminonaphthalene complex formation	Optimum sensitivity	Time consuming process. Low reproducibility	15-17
ICP-OES and ICP-MS	Instrumental Neutron Activation Analysis (INAA) with on-line digestion of the fraction, reduction and hydride formation or complexation with surfactant of p-octyl polyethyleneglycolphenyl ether (Triton X-100)	High sensitivity. High precision	High skill required. Costly	18-20
Inhouse newly developed method	Direct Microwave digestion followed by ICP-OES	Simple sample preparation method, less toxic, low skill required and highly reproducible	Optimum sensitivity (less sensitive compared to ICP-MS and GFAAS and more sensitive than spectrometric, Volta metric and chromatograph and AAS technique)	Not Applicable

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Authors' Contribution

Conceptualization, methodology and original draft preparation: A.K.P. Supervision, reviewing and editing: R.S.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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