Scientific paper

Determination of Nickel in Active Pharmaceutical Ingredients by Electrothermal Atomic Absorption Spectrometry

Zoran Bubnič,[1,*] Uroš Urleb,[2] Katjuša Kreft,[3] and Marjan Veber[4]

[1] *Quality Control, Lek Pharmaceuticals d.d., Kolodvorska 27, 1234 Mengeš, Slovenia,*

[2] *Drug Discovery, Lek Pharmaceuticals d.d., Verovškova 57, 1526 Ljubljana, Slovenia, Phone: +386 15802691*

[3] *Quality Assurance, Lek Pharmaceuticals d.d., Verovškova 57, 1526 Ljubljana, Slovenia, Phone: +386 15802374*

[4] *University of Ljubljana, Faculty of Chemistry and Chemical Technology, Aškerčeva 5, 1000 Ljubljana, Slovenia*

** Corresponding author: E-mail: zoran.bubnic@sandoz.com*
Phon: +386 17217487, fax: +386 17237652

Received: 14-04-2009

Dedicated to the memory of the late Prof. Dr. Valentin Koloini

Abstract

An electrothermal atomic absorption spectrometric procedure for the determination of nickel in active pharmaceutical ingredients was developed. Since the recoveries of nickel by the direct dissolution of samples in diluted nitric acid were low and caused errors in the determination of Ni in pharmaceutical samples, different approaches for sample pretreatment were examined. It was found that the microwave digestion was the most suitable way for sample preparation. Various combinations of digestion agents and different microwave conditions were tested. The combination of nitric acid and hydrogen peroxide was found to be the most appropriate. The validity of the method was evaluated by recovery studies of spiked samples and by the comparison of the results obtained by inductively coupled plasma mass spectrometry (ICP-MS). The recovery ranged from 87.5 to 104.0% and a good agreement was achieved between both methods. The detection limit and the limit of quantification were 0.6 and 2.1 μg g^{-1} respectively. The precision of the method was confirmed by the determination of Ni in the spiked samples and was below 4%, expressed in terms of a relative standard deviation. The method was applied to the determination of nickel in production samples of active pharmaceutical ingredients and intermediates.

Keywords: Active pharmaceutical ingredients; electrothermal atomic absorption spectrometry; nickel; microwave digestion; method validation

1. Introduction

In active pharmaceutical ingredients (APIs) nickel may originate from different sources. It is widely used as a catalyst in the steps of hydrogenation under the trade name Raney-Ni, a nickel-aluminium alloy, composed of about 85% of nickel and 15% of aluminium, it can also be used as a metal reagent or it can derive from manufacturing equipment and piping. Although nickel appears to be an essential microelement in humans with estimated daily requirements ranging from 5 to 50 μg[1] it can be also considered as a moderately toxic element. It is well known that exposure to nickel compounds by inhalation can cause carcinogenic and mutagenic effects, however there is still no evidence that nickel compounds are carcinogenic by the oral route. Moreover, nickel can cause allergic contact dermatitis, particularly in women (possibly owing to the wearing of nickel-containing earrings in pierced ears).[2]

The levels of residual metal catalysts in active pharmaceutical ingredients and excipients are recommended by the Guideline issued by the EMEA (European Medicines Agency) in February 2008.[1] In accordance with this

Guideline the limits for residual catalysts are set for active pharmaceutical ingredients and excipients and should be regularly monitored. Routine testing may be replaced by skip testing if the catalyst is adequately removed. This means that the levels of catalysts should be below 30% of the limits set in the Guideline. According to this Guideline the concentration of Ni in active pharmaceutical ingredients should not exceed 25 ppm.

The concentration of nickel can be determined by different instrumental approaches. The most commonly used atomic spectrometric techniques are flame (FAAS)[3–5] and electrothermal (ETAAS)[6–8] atomic absorption spectrometry, inductively coupled plasma atomic emission spectrometry (ICP-AES),[9–12] X-ray florescence spectrometry (XRF)[13,14] and inductively coupled plasma mass spectrometry (ICP-MS).[15,16] In addition to these techniques, for routine purposes the procedures based on molecular absorption spectrometry[17–19] and electrochemical techniques[20–22] are still in use. The most commonly used methods for the determination of residual metals in active pharmaceutical ingredients are FAAS,[23] ETAAS[24–27] and ICP–MS.[28–31] There are several papers on the determination of nickel by ETAAS in food[32,33] and vegetable oils,[34] in waters[35] and in biological samples.[36,37] The limits for nickel in some raw materials for pharmaceutical applications are set in the European Pharmacopoeia (EP) and in the United States Pharmacopoeia (USP). The recommended method for Ni determination is ETAAS. However, there are only a few articles on the determination of nickel by ICP-MS in active pharmaceutical ingredients[38] and final medicinal products.[39] Although ICP–MS offers a better limit of detection and quantification, the limitation of ICP–MS includes a high capital investment and operating costs, which makes it impractical for routine quality control. ETAAS offers a lower limit of detection than flame atomic absorption spectrometry, at a lower cost than ICP–MS, it is flexible in the use of solvents for sample treatment and simple to operate, however, incorrect sample preparation might be a source of severe systematic errors.

The goal of this work was to develop an ETAAS method for the determination of nickel in active pharmaceutical ingredients, which would be useful for quality control laboratories in the pharmaceutical industry. For APIs, which were soluble in diluted nitric acid we tried to develop a method without previous treatment of samples. Thus, the direct preparation of samples was evaluated as well as microwave digestion which is the most appropriate way for the reduction of the organic matrix and for avoiding the losses of nickel due to the low solubility of some forms of nickel in diluted nitric acid. Since there are only few data in literature dealing with ETAAS determination of nickel in such samples, additional experiments and investigations for the optimisation of microwave digestion and ETAAS determination were necessary.

2. Experimental

2. 1. Samples and Reagents

The samples used in this study were active pharmaceutical ingredients and intermediates obtained from LEK pharmaceuticals and from external suppliers. Compound 1 was lisinopril, an angiotensin converting enzyme inhibitor and compound 2, tamsulosine hydrochloride, a selective alpha blocker. In the production of both substances nickel as catalyst was used.

All solutions were prepared using deionised water from a Milli–Q system (Millipore, Bedford, MA, USA).

For microwave digestion suprapur nitric acid of 65% (Merck, Darmstadt, Germany) and hydrogen peroxide of 30% (Merck, Darmstadt, Germany) were used.

The Ni working standard solutions were prepared from a standard stock solution (1000 mg L^{-1} Merck, Darmstadt, Germany).

The magnesium nitrate modifier solution (10.0 g L^{-1}) was prepared by dissolving magnesium nitrate hexahydrate (Merck, Darmstadt, Germany) – suprapur grade (1.728 g $Mg(NO_3)_2 \times 6H_2O$/100 mL) in Milli-Q water. The palladium matrix modifier solution (5 mg Pd^{2+} mL^{-1}) was prepared by the dilution $Pd(NO_3)_2$ (Merck, Darmstadt, Germany) of a stock solution ($c(Pd^{2+}) = 10.0 \pm 0.2$ g L^{-1}) with Milli-Q water in a 10 mL volumetric flask. Hydroxylamine hydrochloride and ascorbic acid solutions were prepared by dissolving 15.0 g of hydroxylamine hydrochloride (Merck, Darmstadt, Germany; analytical reagent grade) or ascorbic acid (Riedel-de Haën; analytical reagent grade) in 100 mL of Milli-Q water.

2. 2. Apparatus

Atomic absorption measurements were carried out by a Varian SpectrAA 280 Z atomic absorption spectrometer equipped with a GTA graphite furnace, PSD 120 auto-sampler, Zeeman background correction and a Ni hollow cathode lamp as a radiation source with a current of 4.0 mA. The main analytical line at 232.0 nm was used for all determinations with a spectral bandwidth of 0.2 nm. Argon was used as the inert transport gas for all analyses. Integrated absorbance (peak area) was used exclusively for signal evaluation and qualification. Measurements were performed by using pyrolytically coated graphite tubes with L' vov platform and the sample volume was 20 μL.

The optimised graphite furnace temperature programme is given in Table 1.

For the sample digestion, a CEM (Model Mars 5) microwave oven with 100 mL polytetrafluoroethylene (PTFE) vessels was used. The microwave oven was equipped with temperature and pressure sensors.

The concentration of total organic carbon in samples was measured with a Total Organic Analyser – Teledyne Tekmar Phoenix 8000.

An Agilent 4500 inductively coupled plasma mass spectrometer was used for ICP-MS measurements.

Table 1. Graphite furnace temperature programme for the determination of Ni

Step	Temperature (°C)	Ramp (s)	Hold (s)	Air flow rate (mL min^{-1})
1	40–95	30	–	0.3
2	95–120	35	–	0.3
3	120–200	50	–	0.3
4	200–300	30	–	0.3
5	300–1200	12	27	0.3
6	1200–2400	0.6	3	0
7	2400–2600	3	–	0
8	2600–2850	2	–	0.3

2. 3. Procedures

Approximately 0.1 g of sample was weighed into a 100 mL PTFE vessel, dissolved in the mixture of 9 mL of 65% nitric acid and 2 mL of 30% hydrogen peroxide, closed and placed in the microwave oven. A three step microwave programme was applied; first step, heating from room temperature to 120 °C for 10 min and waiting at this temperature for 5 min; second step, heating from 120 °C to 180 °C for 10 min and holding for 10 min; third step, heating from 180 °C to 200 °C for 10 min and waiting for 30 min. For complete digestion the programme should be run 2 times. After the digestion the samples were transferred to 25 mL volumetric flask and diluted with Milli – Q water. The blank and the working standards for calibration were prepared in the same concentration of nitric acid as the sample.

3. Results and Discussion

Sample preparation is supposed to be the most important step for metal determination by atomic spectroscopic methods.[40] In atomic absorption spectrometry very often the direct dissolution in a proper solvent can be applied, however, for samples containing higher amounts of organic matter the digestion using different oxidizing agents is necessary. Classical wet digestion of organic compounds requires high volumes of reagents, which can contribute to higher blank values and may also be the source of undesirable matrix effects in the measuring step. Therefore, microwave assisted procedures for sample preparation are commonly preferred.[41]

The compounds evaluated in this study were soluble in diluted nitric acid (tamsulosine hydrochloride in concentrations above 10%, and lisinopril in 0.1% acid), which is also the recommended medium for ETAAS analyses. Therefore, the direct ETAAS nickel determination using dissolution of samples in diluted nitric acid was investigated. Approximately 0.1 g of the sample was dissolved in 9 mL of 65% nitric acid, diluted to 25 mL with Milli–Q water and measured according to the programme in the Table 1. To check the efficiency of the procedure the samples were spiked with a standard solution of nickel (0, 10, 20, 40, 60, 80 μg L^{-1}, which corresponded to 0, 2.5, 5, 10, 15 and 20 and μg g^{-1} in the solid sample). The obtained results were not satisfactory, since low recoveries for spiked samples of compound 2 (Table 2) were determined in most cases (the recoveries were below 81.2%). For this reason in the continuation of our work the microwave decomposition procedure for sample preparation was studied.

To obtain complete digestion of pharmaceutical compounds, extremely rigorous microwave conditions (longer times and a temperature over 200 °C) should be applied when 65% nitric acid is used. The digestion efficiency can be improved using aqua regia, however, because of its oxidative nature and possible damage to laboratory equipment it cannot be recommended for routine applications. The mixture of nitric acid (65%) and hydrogen peroxide (30%) was therefore tested and it was found, that under the conditions given in 2.3. it gave satisfactory results. Moreover, the decomposition efficiency was substantially improved if the digestion programme was repeated. Under such conditions the sample quantity could range from 50 to 200 mg when the volume of 65% nitric acid and 30% hydrogen peroxide was 9 mL and 2 mL respectively. At such conditions the residual organic carbon content (TOC) in the digested residues for compound 1 was below 20% and for compound 2 below 4%. If the quantities of the sample were above 200 mg, the digestion was not satisfactory resulting in the formation of a precipitate after the dilution of samples with Milli-Q water.

For the resulting matrix solutions, pyrolysis and atomisation temperatures were optimized using a standard solution of nickel (10 μg L^{-1}) prepared in the same concentration of nitric acid as the sample and the samples spiked with 10 or 20 μg L^{-1} of Ni (compound 2, compound 1 respectively). The spiked samples were prepared under the conditions given in 2.3 Temperatures over the range of 600–1700 °C were tested at a constant atomisation temperature of 2400 °C. As shown in Fig. 1, no significant change in nickel absorption was observed within the temperature range from 900 to 1200 °C for the standard solution of nickel as well as for the spiked samples. Pyrolysis temperatures above 1200 °C resulted in losses of Ni. Based on these considerations, the pyrolysis temperature was fixed at 1200 °C to ensure maximum matrix removal without signal loss.

The atomisation temperature of nickel was studied over the range of 2000–2600 °C at a constant pyrolysis temperature of 1200 °C. Within this range, the atomic absorption signal increased with the increasing atomisation temperature and reached a plateau at 2500 °C (Fig. 1). Although the sensitivity could be slightly improved with a

higher atomisation temperature, it was set at 2400 °C to prolong the lifespan of the graphite furnace tubes. Owing to the higher amount of organic matrix in compound 1, two steps in the phase of pyrolysis were proposed, at the temperatures of about 250 °C and 1200 °C respectively.

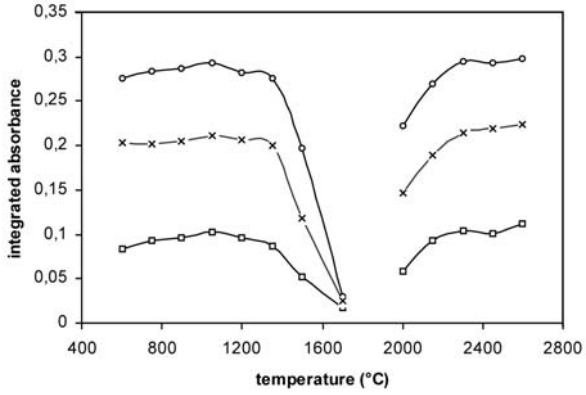

Figure 1. Pyrolisis and atomisation curves obtained with the standard solution of nickel: 10 μg L^{-1} (—□—), compound 2 spiked with 10 μg L^{-1} of Ni (—○—) and compound 1 spiked with 20 μg L^{-1} of Ni (—×—).

The matrix effects were minimized by matching the concentration of nitric acid in a solution of calibration blank and standards, with the sample. Using the t-test (at a confidence level of 95%) it was proved that hydrogen peroxide did not significantly influence the measuring signals and that there is no significant difference in the slopes of calibration curves using standard solutions prepared in HNO_3 or when standard addition method is applied (Fig. 2). For routine measurements the direct calibration method with standards and blanks in the same concentration of nitric acid as the sample can be applied.

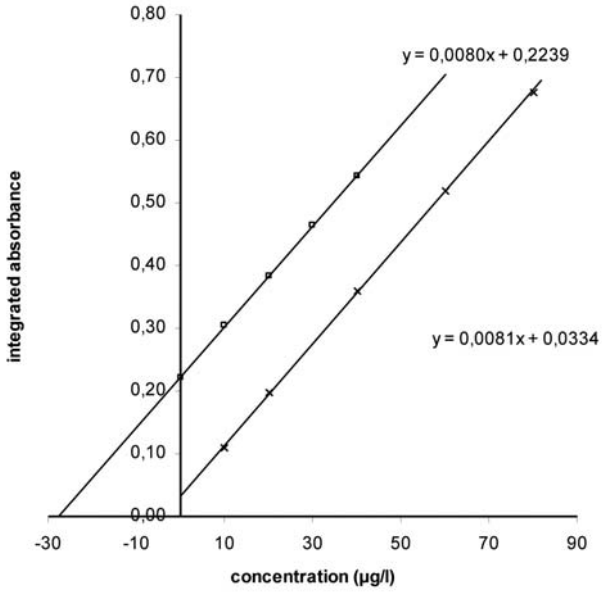

Figure 2. Calibration curves obtained by the standard addition method (—□—) and direct calibration (—×—). Slopes and intercepts were determined from linear regression analysis. Each point is the mean of five determinations.

The results presented in Tables 2 show that the recoveries obtained by the direct dissolution of samples in diluted nitric acid are lower than those obtained by microwave digestion. It is evident that using direct dissolution, only Ni, which was added to the sample, was determined. In compound 1, the quantities of nickel found by direct dissolution and microwave digestion were comparable, since in this sample the quantity of Ni was below the limit of quantification and only spiked nickel was determined. When the direct dissolution of compound 2 was applied, the quantity of Ni was below the LOQ regardless of the concentration of nitric acid (it ranged between 10 and 65%). Also the use of the mixture of nitric acid and hydrogen peroxide as an oxidizing agent (the samples were dissolved in 9 mL of 65% nitric acid and in 3 mL of 30% of hydrogen peroxide and diluted to 25 mL with Milli-Q water) did not provide an improvement in the recovery of nickel. The application of other mineral acids (e.g. hydrochloric acid or phosphoric acid) was limited because of low solubility of pharmaceutical compounds 1 and 2 in mineral acids.

Table 2. Ni recoveries in compounds 1 and 2 after microwave digestion (the microwave procedure is described in 2.3.) and direct dissolution in nitric acid

Ni added (μg g^{-1})	Ni found; microwave digestion (μg g^{-1})	Recovery microwave digestion (%)	Ni found; direct dissolution (μg g^{-1})	Recovery direct dissolution (%)
0*	< LOQ	/	< LOQ	/
2.5*	2.5	100.0	2.2	88.0
5*	5.2	104.0	5.0	100.0
10*	10.3	103.0	10.1	101.0
15*	15.3	102.0	14.2	94.7
20*	19.7	98.5	18.0	90.0
0**	5.5	100.0	< LOQ	/
2.5**	7.8	97.5	3.4	42.5
5**	9.6	91.4	6.5	61.9
10**	14.9	96.1	11.8	76.1
15**	19.0	92.7	16.1	78.5
20**	22.3	87.5	20.7	81.2

* compound 1, ** compound 2
The content of Ni in compound 1 was below the LOQ (LOQ = 2.1 μg g^{-1}) and in compound 2 5.5 μg g^{-1}. The results are the mean of three determinations.

The low nickel recoveries in samples prepared with direct dissolution can be explained by the low solubility of different forms of nickel in solvents used for dissolution or by the existence of volatile Ni organic-compounds,

which could be lost in the phase of thermal treatment of the sample in the graphite tube. To improve the recoveries different chemical modifiers were tested. Magnesium nitrate and palladium were chosen, since they are the most commonly applied modifiers for nickel determination. Magnesium nitrate[42] enables the oxidation of organic matrix and therefore lowers the non-specific background absorption. The masses of $Mg(NO_3)_2$ between 20 and 60 μg were studied. The modifier was pre-injected in the graphite tube or it was mixed together with the sample. However, magnesium nitrate independent of its amount or way of adding to the sample, had no effect on nickel levels. Palladium[43–46] was investigated next. The quantities ranged between 20 and 80 μg. It was combined together with ascorbic acid or hydroxylamine hydrochloride (2.5 mL of hydroxylamine hydrochloride or ascorbic acid solution were added to 25 mL of sample) or it was pre-injected in the graphite tube and heated to 270 °C. It was found that the use of Pd didn't improve the recoveries of nickel. It should be mentioned that the use of the modifiers mentioned above contributed to higher background absorption, and therefore in further studies only nitric acid as a matrix modifier was used.

Even though the direct dissolution is not an appropriate way for sample preparation in the determination of nickel in active pharmaceutical ingredients, the application of microwave digestion of samples described in this paper in comparison to classical dry digestion (e.g. the ETAAS determination of Ni in raw materials described in European or United States Pharmacopoeia) or wet digestion[47,48] is user friendly, especially with regards to time consumption and possible contamination of samples. Despite several chemical modifiers being available for the determination of Ni,[49,50] it was successfully determined without modifiers, which contribute to a simple and cheaper method appropriate for routine use in control laboratories.

3. 1. Method Validation

Limit of detection and quantification

The limit of detection (LOD) for nickel was estimated by analysing 10 replicate aliquots of calibration blanks, after the microwave digestion. The LOD was calculated on the basis of 3 x the standard deviation of the blank divided by the slope of the calibration curve[51] and the LOQ as 10 x the standard deviation of the blank. The resulting LOD and LOQ for Ni were 2.5 μg L^{-1} and 8.4 μg L^{-1} respectively. In the solid sample this corresponded to 0.6 μg g^{-1} and 2.1 μg g^{-1} respectively.

Linearity of the method

The linearity of the method was studied by measuring a series of Ni standards prepared at concentrations of 10, 20, 40, 60, 80 and 100 μg L^{-1} (which corresponded to 2.5, 5, 10, 15, 20 and 25 μg g^{-1} in the solid sample) in 0.1% nitric acid and ensuring that the correlation coefficient of the calibration curve was better than 0.995. The standards were analysed as samples and 0.1% nitric acid was chosen because it was suggested as being the most suitable solvent for the ETAAS determination of nickel. Measurements at each concentration level were carried out in triplicates. The linear relationship between the integrated absorbance and concentration of Ni was observed in the range from 2.5 to 25 μg g^{-1} with correlation coefficient 0.9997. The linearity of the range was confirmed by analysing the spiked compound 1 with the standard addition of nickel at concentrations of 10, 20, 40, 60, 80 and 100 μg L^{-1} (which corresponded to 2.5, 5, 10, 15, 20 and 25 μg g^{-1} in the solid sample). The spiked samples were analysed in triplicate after the microwave digestion. The results demonstrated the linearity of the instrumental response over the concentration range from 2.5 to 25 μg g^{-1} nickel in the solid sample with correlation coefficient 0.9995.

Accuracy of the method

Since there were no relevant standard reference materials available, the accuracy of the method was evaluated by recoveries of known amounts of Ni spiked (prior the microwave digestion of samples was performed) into compounds 1 and 2. The samples were spiked at levels from 2.5 μg g^{-1} over the entire concentration range up to the upper concentration level (the spiked levels of Ni were: 2.5, 5, 10, 15, 20 and for compound 1 also 25 μg g^{-1}). Table 3 summarises the recovery results for various samples. The spiked recoveries based on the average of triplicate measurements ranged from 87.5 to 104.0%.

The accuracy of the method was confirmed by the ICP–MS determination of Ni in the compounds 1 and 2. The results obtained by ETAAS were in agreement with those obtained by ICP-MS (Table 4).

Table 3. Recovery study

Expected level of Ni (μg g^{-1}) compound 2 and 1*	Ni found ± SD (μg g^{-1})	Recovery (%)
8	7.8 ± 0.4	97.5
10.5	9.6 ± 0.4	91.4
15.5	14.9 ± 0.5	96.1
20.5	19.0 ± 2.0	92.7
25.5	22.3 ± 0,6	87.5
2.5*	2.5 ± 0.1	100.0
5*	5.2 ± 0.2	104.0
10*	10.3 ± 0.4	103.0
15*	15.3 ± 0.2	102.0
20*	19.7 ± 0.3	98.5
25*	24.5 ± 0.3	98.0

The content of Ni in compound 2 was 5.5 μg g^{-1} and in compound 1 below the LOQ (LOQ = 2.1 μg g^{-1}). The results are the mean of three determinations.

Table 4. The content of Ni in compounds 1 and 2 determined by ETAAS and ICP–MS

Sample	ETAAS Ni ± SD (μg g^{-1})	ICP–MS Ni ± SD (μg g^{-1})
compound 1	< LOQ	< LOQ
compound 2	5.5 ± 0.5	5.2 ± 0.5

LOQ = 2.1 μg g^{-1}
The results are the mean of two determinations.

Precision of the method

The precision of the method was evaluated by the repeated determination of Ni in compound 1, spiked with 10, 40 and 100 μg L^{-1} Ni (this corresponded to 2.5, 10 and 25 μg g^{-1} Ni in the solid sample) prior the microwave digestion was performed. Precision experiments at each spiked level were replicated six times. The level of precision was expressed in terms of the standard deviation (SD) and the relative standard deviation (RSD). The results summarised in Table 5 present a good level of precision with RSD below 4%.

Table 5. Precision study (compound 1)

Replicate	Spiked 2.5 μg g^{-1} Ni	Spiked 10 μg g^{-1} Ni	Spiked 25 μg g^{-1} Ni
1	2.6	10.0	24.8
2	2.5	10.2	24.2
3	2,5	10.7	24.5
4	2.4	10.7	25.6
5	2.4	10.7	25.3
6	2.5	11.0	25.6
Mean	**2.5**	**10.6**	**25.0**
SD	**0.1**	**0.4**	**0.6**
RSD	**3.0**	**3.5**	**2.4**

3. 2. Application of the Method on Production Samples

The described method was used for the determination of nickel in the samples of tamsulosine hydrochloride and lisinopril. The assay of nickel in lisinopril was below the LOQ as well as in tamsulosine hydrochloride which was obtained from an external supplier. In the substance, which was produced by LEK, the levels of nickel were above the LOQ, but still below the maximal allowed limit 25 ppm[1], which was set for active pharmaceutical ingredients. Additionally, the intermediate from the previous step in the synthesis of lisinopril and the starting material for tamsulosine hydrochloride (in the production of this starting material which was obtained from an external supplier, nickel as a catalyst was used) were also analysed. The assay of nickel in both samples was below the LOQ (Table 6). Since in the production of tamsulosine hydrochloride in LEK no nickel was used and also the concentration of Ni in the starting material was below the LOQ, it was most likely that the manufacturing equipment and piping was the source of nickel in this material.[52,53]

Table 6. Results on the nickel content in different samples

Sample	Ni content ± SD (μg g^{-1})
Lisinopril	
– sample 1	< LOQ
– sample 2	< LOQ
tamsulosine hydrochloride	
– sample 1	5.5 ± 0.9
– sample 2	8.2 ± 0.2
tamsulosine hydrochloride (external supplier)	< LOQ
intermediate1	< LOQ
starting material	< LOQ

LOQ = 2.1 μg g^{-1}
The results are the mean of three determinations

4. Conclusion

The developed method based on the microwave digestion of the sample and the ETAAS quantification of nickel is a simple, accurate and precise procedure for nickel determination in active pharmaceutical ingredients. The method is, due to its simplicity and relative low costs in comparison to ICP-MS suitable for quality control laboratories in the pharmaceutical industry. The LOD and LOQ are 0.6 and 2.1 μg g^{-1} respectively and are well below the specification limit, which is set at 25 μg g^{-1} for nickel in active pharmaceutical ingredients (oral exposure)[1]. The assay of nickel in all the samples analysed by the described method was below the specification limit.

5. Acknowledgments

The research described in this article was carried out and founded by Quality Control, Lek Pharmaceuticals d.d. and by the Slovenian Research Agency (P1-0153) which are gratefully acknowledged.

6. References

1. European Medicines Agency, *Guideline on the Specification Limits for Residues of Metal Catalyst*, http://www.emea.europa.eu/pdfs/human/swp/444600enfin.pdf
2. B. Bocca, G. Forte, F. Petrucci, A. Cristaudo, *J. Pharm. Biomed. Anal*. **2007**, *44*, 1197–1202.
3. F. Xie, X. Lin, X. Wu, Z. Xie, *Talanta* **2008**, *74*, 836–843.
4. M.C. Yebra, S. Cancela, R.M. Cespon, *Food Chem*. **2008**, *108*, 774–778.

5. M. Bettinelli, P. Tittarelli, *J. Anal. At. Spectrom*. **1994**, *9*, 805–812.
6. A.V. Filgueiras, I.Lavilla, C. Bendicho, *Anal. Chim. Acta* **2004**, *508*, 217–223.
7. H. Mosbaek, P.E. Holm, J.C. Tjell, *J. Anal. At. Spectrom*. **2003**, *18*, 1489–1492.
8. N.N. Meeravali, S.J. Kumar, *Anal. Chim. Acta* **2000**, *404*, 295–302.
9. J.L. Fabec, M.L. Ruschak, *Anal. Chem*. **1985**, *57*, 1853–1863.
10. M. Murillo, Z. Benzo, E. Marcano, C. Gomez, A. Garaboto, C. Marin, *J. Anal. At. Spectrom*. **1999**, *14*, 815–820.
11. I. Juranovic, P. Breinhoelder, I. Steffan, *J. Anal. At. Spectrom*. **2003**, *18*, 54–58.
12. A.N. Anthemidis, V. Arvanitidis, J.A. Stratis, *Anal. Chim. Acta* **2005**, *537*, 271–278.
13. K. Iwasaki, K Tanaka, *Anal. Chim. Acta* **1982**, *136*, 293–299.
14. E.R. Denoyer, L.A. Siegel, *Anal. Chim. Acta* **1987**, *192*, 361–366.
15. T.D. Saint'Pierre, L.F. Dias, D. Pozebon, R.Q. Aucelio, A.J. Curtius, B. Welz, *Spectrochim. Acta Part B* **2002**, *57*, 1991–2001.
16. R. Myors, R.J. Wells, S.V. Skopec, P. Crisp, R. Iavetz, Z. Skopec, A. Ekangaki, J. Robertson, *Anal. Commun*. **1998**, *35*, 403–410.
17. J. Ghasemi, N. Shahabadi, H.R. Seraji, *Anal. Chim. Acta* **2004**, *510*, 121–126.
18. B. Razaei, A.A. Ensafi, F. Shandizi, *Microchem. J*. **2001**, *70*, 35–40.
19. B.D. Öztürk, H. Filik, E. Tütem, R. Apak, *Talanta* **2000**, *53*, 263–269.
20. D. Monticelli, E. Ciceri, C. Dossi, *Anal. Chim. Acta* **2007**, *594*, 192–198.
21. M.S. Moneeb, *Talanta* **2006**, *70*, 1035–1043.
22. G. Dugo, L.L. Pera, V.L. Turco, G.D. Bella, F. Salvo, *J. Agric. Food Chem*. **2004**, *52*, 1829–1834.
23. A. Abarca, E. Canfranc, I Sierra, M.L. Marina *J. Pharm. Biomed. Anal*. **2001**, *25*, 941–945.
24. R. Raghavan, J.A. Mulligan, *Drug Dev. Ind. Pharm*. **2000**, *26*, 423–428.
25. T. Wang, S. Walden, R. Egan, *J. Pharm. Biomed. Anal*. **1997**, *15*, 593–599.
26. X. Jia, T. Wang, J. Wu, *Talanta* **2001**, *54*, 741–751.
27. X. Jia, T. Wang, X Bu, Q. Tu, S. Spencer, *J. Pharm. Biomed. Anal*. **2006**, *41*, 43–47.
28. T. Wang, Z. Ge, J. Wu, B. Li, A. Liang, *J. Pharm. Biomed. Anal*. **1999**, *19*, 937–943.
29. N. Lewen, S. Mathew, M. Schenkenberger, T. Raglione, *J. Pharm. Biomed. Anal*. **2004**, *35*, 739–752.
30. R.N. Rao, M.V.N. Kumar Talluri, *J. Pharm. Biomed. Anal*. **2007**, *43*, 1–13.
31. J. Huang, X. Hu, J. Zhang, K. Li, Y. Yan, X. Xu, *J. Pharm. Biomed. Anal*. **2006**, *40*, 227–234.
32. I. Lavilla, P. Vilas, C. Bendicho, *Food Chem*. **2008**, *106*, 403–409.
33. M. Gonzales, M. Gallego, M. Valcarcel, *Talanta* **1999**, *48*, 1051–1060.
34. M.N.M. Reyes, R.C. Campos, *Talanta* **2006**, *70*, 929–932.
35. P. Bermejo-Barrera, J. Moreda-Pineiro, A. Moreda-Pineiro, A. Bermejo-Barrera, *Talanta* **1998**, *45*, 807–815.
36. N. Todorovska, I. Karadjova, T. Stafilov, *Anal. Bioanal. Chem*. **2002**, *373*, 310–313.
37. M. Patriarca, G.S.Fell, *J. Anal. At. Spectrom*. **1994**, *9*, 457–461.
38. C.A. Ponce de Leon, M.M. Bayon, J.A. Caruso, *Anal. Bioanal. Chem*. **2002**, *374*, 230–234.
39. R.J.H. Waddell, N. NicDaeid, D. Littlejohn, *Analyst* **2004**, *129*, 235–240.
40. M. Wasilewska, W. Goessler, M. Zischka, B. Maichin, G. Knapp, *J. Anal. At. Spectrom*. **2002**, *17*, 1121–1125.
41. W. Goessler, M. Pavkov, *Analyst* **2003**, *128*, 796–802.
42. D. L.Tsalev, V. I. Slaveykova, P. B. Mandjukov, *Spectrochim. Acta Rev*. **1990**, *13*, 225–274.
43. I. C. F. Damin, M. G. R. Vale, M. M. Silva, B. Welz, F. G. Lepri, W. N. L. dos Santos, S. L. C. Ferreira, *J. Anal. At. Spectrom*. **2005**, *20*, 1332–1336.
44. L. M. Voth-Beach, D. E. Shrader, *J. Anal. At. Spectrom*. **1987**, *2*, 45–50.
45. S. X. Quan, W. Bei, *J. Anal. At. Spectrom*. **1995**, *10*, 791–798.
46. C. P. Calvo, P. B Barrera, A. B. Barrera, *Anal. Chim. Acta* **1995**, *310*, 189–198.
47. R. Lara, S. Cerutti, J. A. Salonia, R. A. Olsina, L. D. Martinez, *Food and Chem. Toxicol*. **2005**, *43*, 293–297.
48. G. A. Zachariadis, E. S. Raidou, D. G. Themelis, J. A. Stratis, *J. Pharm. Biomed. Anal*. **2002**, *28*, 463–473.
49. M. M. Silva, I. C. F. Damin, M. G. R. Vale, B. Welz, *Talanta* **2007**, *71*, 1877–1885.
50. W. Slavin, G. R. Carnrick, D. C. Manning, *Anal. Chem.* **1982**, *54*, 621–624.
51. V. Thomsen, D. Schatzlein, D Mercuro, *Spectroscopy* **2003**, *18*, 112–114.
52. M. Cempel, G. Nikel, *Polish J. of Environ. Stud*. **2006**, *15*, 375–382.
53. J. Kristl, M. Veber, M. Slekovec, *Anal. Bioanal. Chem*. **2002**, *373*, 200–204.

Povzetek

Razvili smo metodo za določanje sledov niklja v farmacevtskih učinkovinah na osnovi elektrotermične atomske absorpcijske spektrometrije. Ker so bile spojine, v katerih smo določali sledove niklja, topne v dušikovi (V) kislini, smo uporabili direkten pristop, t.j. raztapljanje vzorcev v dušikovi (V) kislini. V tem primeru so bili izkoristki nizki in niti uporaba različnih kemijskih modifikatorjev ni prispevala k pravilnejšim rezultatom. V nadaljevanju smo preizkusili različne vrste razklopov; klasične razklope in razklope z mikrovalovi. Izkazalo se je, da je za farmacevtske učinkovine, ki vsebujejo visoke koncentracije organskega ogljika in je njihov razklop težaven, najprimernejši način za pripravo mikrovalovni razklop v kombinaciji dušikove (V) kisline in vodikovega peroksida. Določili smo optimalne pogoje razkroja in atomizacije.

Z metodo masne spektrometrije v induktivno sklopljeni plazmi (ICP – MS) in standardnega dodatka smo potrdili točnost metode (izkoristki znašajo med 87.5 in 104.0 %), v okviru validacije pa še natančnost (relativni standardni odmik je pod 4 %), spodnjo mejo zaznavnosti (0.6 μ g^{-1}) in določljivosti (2.1 μ g^{-1}) ter linearnost območja (med 2.5 in 25 μ g^{-1}). Novo metodo smo uspešno uporabili za določanje vsebnosti niklja v realnih vzorcih farmacevtskih učinkovin in intermediatov.