INTRODUCTION

Nebivolol is a β1 receptor blocker with nitric oxide poten-
tiating vasodilator effect used in treatment of hypertension and
in Europe, also for left ventricular failure [1]. It is highly cardio
selective under certain circumstances. β-Blockers help patients
with cardiovascular disease by blocking β receptors, while many
of the side-effects of these medications are caused by their block-
ade of β2 receptors [2]. Nebivolol is unique as a β-blocker.
Unlike carvedilol, it has a nitric oxide potentiating vasodilator
effect [3,4]. Along with labetalol, celiprolol and carvedilol, it
is one of four β-blockers to cause dilation of blood vessels in
addition to effects on heart [5]. However, recent studies question
the clinical relevance of this property to Nebivolol’s efficacy
[6,7].

Various analytical techniques were employed for the
determination of inorganic metal impurities include titration,
ion-exchange chromatography, capillary electrophoresis and
spectroscopic techniques like flame photometry, flourimetry,
atomic absorption spectroscopy and inductive coupled plasma.
Among the above mentioned techniques, inductive coupled plasma
is a versatile tool for detection and quantification of elements in accurate manner and it works on the basis of atomic
spectrometry. Inductive coupled plasma also further include 2
types which are ICP-optical emission spectroscopy and ICP-
mass spectroscopy. Most specifically, ICP-OES is emission
spectrometric technique that exploits the fact that excited atoms
emit energy at a given wavelength as the electrons return to
their ground state from excited state. A given element emits
energy at specific wavelengths peculiar to its chemical nature.
The intensity of the energy emitted at that wavelength is
proportional to the amount of that element in the analyzed
sample. When compared with the other techniques ICP-OES
has an additional advantage in terms of detection limits (where
it can detect to a concentration range of ppm or ppb) and a rapid
analysis. In ICP-OES, sample experiences temperature estimated
be in the vicinity of 10,000 K. These results in atomization
and excitation of even most refractory elements with high
efficiency so that detection limits for these elements with ICP-
OES can be well over an order of magnitude better than the
corresponding values of other techniques [8].

The titration methods are not accurate whereas ion exchange
chromatography and capillary electrophoresis stabilization is
a time taking process and sensitivities are low when compared
to ICP-OES. For the above reasons, ICP-OES has become a tool
of choice for estimating metals. Literatures revealed that few
methods like determination of metals in food samples like
vinegar [9], environmental samples (soil and drinking water)
[10-16], potato chips [17], honey [18] eliphos tablets [19] and
escitalopram oxalate bulk drug [20] are reported.
This paper describes the methodology and validation procedure by ICP-OES for determination of metals in nebivolol bulk drug and the method was validated according to ICH (Q2R1) guidelines.

**EXPERIMENTAL**

Nitric acid (analytical grade) from Merck, India, MilliQ water and palladium metal standard of certified reference material were used. The nebivolol HCl was obtained as a gift sample from Varun Herbals, India. The method development and validation was performed by using ICP-OES and 3 % nitric acid is used as diluent.

**ICP-OES conditions**: The RF power used was 1300 W, plasma flow was 13 L/min, auxiliary gas flow was kept at 0.2 L/min, nebulizer flow was kept at 0.7 L/min, and pump rate was kept at 1 mL/min. Palladium was monitored at wavelength 340.458 nm. Plasma view was in axial view. Three replicates were performed.

**Preparation of sample**: The sample preparation was done by using Microwave digester. Weighted 200 mg of sample and added 5 mL of nitric acid and 2 mL of H$_2$O$_2$ dropwise, after completion of digestion cool the vessel and transferred it into 20 mL volumetric flask and made up to volume with diluent.

**Preparation of standard**: Preparation of palladium stock standard solution (10 µg/mL), palladium standard (certified reference material) with 1000 ppm was obtained and labeled as reference standard. Further suitable aliquots were prepared from the standard stock solutions with the concentrations of 10, 30, 50, 70 and 100 µg/mL.

**RESULTS AND DISCUSSION**

The main objective of the study was to develop a suitable ICP-OES method to quantify palladium content present in nebivolol HCl. During the method development, palladium standard prepared of any working concentration was monitored at different possible emission lines of 338.57, 340.46 and 345.85 nm by aspirating the solution. Among these emission lines at applied target RF power of 1500 W the response for palladium was evaluated. The response of palladium was prominent at 340.46 nm. Moreover, the baseline was to be good at above stated wavelengths compared to other emission lines of elements. The possible inferences were not observed at this emission lines. The method was optimized at these wavelengths during method development. To get better sensitivity plasma view mode was selected as axial. As this mode is much larger than that viewed by normal radial or normal size on ICP resulting higher intensity for elements.

**Specificity**: Prepared the sample one spiked sample (sample spiked with 100% standard solution) as per the method and analyzed. The obtained results are reported in Table-1. An acceptance criterion for specificity as per USP is no interference is observed with test sample.

**Linearity**: Linearity was evaluated by preparing a linear series of standard solutions of palladium in the concentrations range from LOQ level to 150 % level of specification level. From the linear solutions, correlation co-efficient, Y intercept, slope were calculated and thus the linear relationship of concentration versus counts was verified over the range specified. The correlation coefficient for palladium was found to be 0.999970 which indicates good linearity. The results are reported in Table-2.

<table>
<thead>
<tr>
<th>Sample</th>
<th>System Precision Values</th>
</tr>
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<tbody>
<tr>
<td>Repetitions</td>
<td>Results</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
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<tr>
<td>Palladium 0.05 ppm</td>
<td>0.050</td>
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**Limit of detection (LOD) and Limit of quantification (LOQ)**: Linear series of palladium standard solutions was prepared in the concentration range from 0.01 ppm to 0.1 ppm and a calibration curve is developed and calculate the correlation coefficient. The LOD and LOQ were calculated and the LOD and LOQ levels are found to be as LOD = 3 ppb and LOQ= 10 ppb.

**Precision**: The precision of method was checked by analyzing six individual preparations of sample as per the test method and prepared six individual spike samples (at 100% level). The % RSD was found to be within the specification (% RSD should not be more than 5 %). The system precision was checked by analyzing six replicates of palladium standard solution (100 % level). The concentration of element for each replication was calculated by using the calibration standards (Tables 3 and 4).

**Accuracy**: Sample solutions were spiked with palladium at LOQ level, 100 % level and 200 % level with respect to sample concentration. Each spiked solution was prepared in triplicate and aspirated. The recovery of palladium was calculated against the standard working concentration (Table-5).

**Ruggedness**: The recovery levels tested in (Analyst-1) were again carried out in Analyst-2 using same instrument. The recovery values were found to be comparable.
Sample analysis: The previously prepared sample was aspirated in the instrument, the concentration of palladium was found to be 1 ppm.

Conclusion
A validated and accurate ICP-OES method has been developed to estimate palladium content in nebivolol drug. The method is selective and is capable to quantitate Palladium in the presence of other trace elements. The method has been validated in terms of specificity, precision, linearity, accuracy and limit of detection and quantification. The validated method can be used to estimate elements not only in nebivolol but also in other drug substances.

REFERENCES