

Accepted Manuscript

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DOI:

Received date: December 2018

Accepted date: February 2019

UDC:

Type of paper: *Professional paper*

Please cite this article as:

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**RP-HPLC method with indirect UV detection for determination of sodium
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Abstract

Ibandronate sodium (IBN) [(1-hydroxy-3- (methyl pentyl amino) propylidene bisphosphonic acid monosodium monohydrate)] is the sodium salt of ibandronic acid, a synthetic nitrogen-containing bisphosphonate drug. The aim of this study was to develop a sensitive and accurate RP-HPLC method with indirect UV detection for determination of IBN in pharmaceutical formulations. Chromatographic separation was performed on a Waters Bridge C18 reversed-phase column (250 X 4.6 mm I.D.; particle size 5 μm), in an isocratic mode with a mobile phase constituted of 90% buffer: 10% acetonitrile (V/V). The buffer was made using 1.5 mL ortho-phosphoric acid, 990 mg 1-Hexanesulfonic acid sodium salt 98%, 140 mg EDTA in 1000 mL flask diluted with HPLC grade water. The elution was carried out at a flow rate of 1.0 mL min⁻¹. A diode array detector measured the UV absorbance at 198 nm, in inverse mode. The method was validated for specificity/selectivity, linearity, LOD, LOQ, accuracy, precision and robustness according to ICH validation guidelines. The limits of detection and quantification were calculated at 0.0163 $\mu\text{g/mL}$ and 0.0495 $\mu\text{g/mL}$, respectively. The method was effectively used for determination of IBN from

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commercial tablets and provided good results without any interference from commonly used excipients.

Keywords: RP-HPLC with indirect UV detection, Ibandronate sodium, validation, pharmaceuticals

Introduction

Ibandronate sodium (IBN) is the sodium salt form of ibandronic acid, a synthetic nitrogen-containing bisphosphonate (Fig. 1). Ibandronic acid inhibits farnesyl pyrophosphate synthase, resulting in a reduction in geranylgeranyl GTPase signaling proteins and apoptosis of osteoclasts. This agent increases bone mineral density, decreases bone remodeling, inhibits osteoclast-mediated bone resorption, and reduces metastases-related and corticosteroid-related bone pain (Bausset al., 2002; Black et al., 2007).

For quantification of IBN in pharmaceutical formulations, few analytical methods have been reported. Determination of bisphosphonates by high-performance liquid chromatography (HPLC) with UV or fluorescence detection is hindered because of the lack of chromophores groups in their structures. Several analytical methods using refractive index or electrochemical detection were described in the literature (Brooks et al., 1992; Denhartigh et al., 1993; Han et al., 1996; Higuchiet al., 1992). However, these detection systems are not common in most of the routine analytical laboratories. Analytical methods for quantification of bisphosphonates using UV/VIS or fluorescence detectors in the indirect mode, or after pre- or post-column derivatization, have also been described (Brezovska et al., 2010; Biffar et al., 1989; Dansereau et al., 2001; Hartigh et al., 1997; Lovdahl et al., 1999; Kosonen, 1992). However, the derivatization step is time and solvent consuming, making this process inappropriate for routine analysis.

Fig. 1. The structure of IBN.

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Therefore, the aim of this study was to develop a RP-HPLC method with indirect UV detection which would replace the existing commercial methods with RI detector.

Materials and methods

Instruments and reagents

HPLC analyses were performed using a Shimadzu LC-2010 chromatographic system (Shimadzu, Kyoto, Japan) consisting of a LC-20AT Prominence liquid chromatograph pump with DGU-20A5 Prominence degasser, a SPD-M20A Prominence Diode Array Detector, RF 10AXI fluorescence detector and a SIL-20 AC Prominence auto sampler. Data analyses were done using Class VP 7.3 Software. The UV spectra of IBN dissolved in mobile phase were recorded on the Shimadzu UV-Visible spectrophotometer (UV-1800).

IBN working standard was obtained from Maprimed SA. HPLC-grade acetonitrile was from Merck (Darmstadt, Germany). Double-distilled water was used to prepare mobile phase solutions. All solvents and solutions for HPLC analysis were filtered through a membrane filter (0.45 μm pore size) and vacuum degassed before use.

Chromatographic conditions

Chromatographic separation was performed on a Waters Bridge C18 reversed-phase column (250 X 4.6 mm I.D.; particle size 5 μm), in an isocratic mode with a mobile phase constituted of 90% buffer: 53% acetonitrile (*V/V*). The buffer was made using 1.5 mL ortho-phosphoric acid, 990 mg 1-Hexanesulfonic acid sodium salt 98%, 140 mg EDTA in 1000-mL flask diluted with HPLC grade water. The elution was carried out at a flow rate of 1.0 mL min^{-1} . All analyses were performed at room temperature (25 $^{\circ}\text{C}$ +/- 2 $^{\circ}\text{C}$). A diode array detector measured the UV absorbance at 198 nm, in inverse mode.

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Calibration curve

Stock solution was prepared by dissolving IBN standard substance (10 mg) with mobile phase in 10 mL volumetric flask ($c=1$ mg/mL) and stored at room temperature ($25\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$) during the study. Standard solutions were prepared by dilution of IBN stock solution with solvent (mobile phase) to obtain final concentrations ranging from 25–200 $\mu\text{g/mL}$ (25, 30, 40, 45, 50, 55, 60, 75 and 200 $\mu\text{g/mL}$). Mobile phase was used as a blank.

Sample preparation

Twenty tablets containing IBN active substance were weighted, crushed, powdered and grinded finely. A portion of the powder equivalent to 10 mg of IBN was used and diluted with mobile phase to obtain a working concentration of 50 $\mu\text{g/mL}$. The sample solution was filtered through 0.45 nylon membrane filter before use. The amount of IBN per tablet was calculated using the standard calibration curve.

Results and discussion

Indirect UV detection is a method that is used typically when detecting analytes don't have chromophore present in their structure. Indirect UV detection allows measurement of non-UV-absorbing anions without derivatization. An ionic chromophore is placed in the mobile phase, and the decrease in absorbance observed when an analyte ion displacing the chromophore ion in the mobile phase is monitored (Brezovska et al., 2010). As there is no chromophore present in IBN, the detection of a UV transparent analyte is accomplished by adding light ionic (ion-pairing) species into the mobile phase. Ibandronate poses analytical challenges for reversed-phase HPLC due to the presence of two polar phosphonate groups. This makes retention on commonly used RP columns difficult (Gawad et al., 2013). In addition, the metal chelation property of ibandronate can cause poor peak shape and analyte

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recovery in systems that are not metal-free. Chelating agents, such as ethylenediaminetetraacetic acid (EDTA), are added to the mobile phase to prevent metal contaminants from chelating with ibandronate (Furuta et al., 1993). EDTA was used as a complexing agent in combination with ion-pairing agent (1-hexanesulfonic acid sodium salt) that will separate the analyte and give good absorption, to enable retention and separation on a RP column.

In the preliminary research, the absorption spectra of IBN were studied using Shimadzu UV-Visible spectrophotometer. Solution of IBN substance was dissolved in mobile phase and was scanned within the wavelength region from 300–190 nm against the mobile phase as a blank. The UV spectrum of the IBN dissolved in mobile phase shows an absorption maximum at 198 nm.

Fig. 2. UV spectrum of IBN in mobile phase in the range of 300 to 190 nm.

Method validation

One of the most important steps in analytical determination is validation of the method for quantitative analysis. The method was validated for specificity/selectivity, linearity, LOD, LOQ, accuracy, precision and robustness. The ICH guideline was used to validate the proposed method (ICH, 2005).

Specificity and selectivity

Specificity is the ability to assess unequivocally the analyte in the presence of components which might be expected during the analysis. The analytes should have no interference from other components such as impurities and degradation products, and they should be remarkably separated from IBN (ICH, 2005; McMaster, 2007). This method showed that it is quite selective and showed great resolution between IBN and the given

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impurities (the sample solution was spiked with ibanic acid). There was no other interfering peak around the retention time of IBN. Also, the baseline did not show any significant noise.

Fig. 3. Chromatogram of sample solution spiked with Ibanic acid with indirect UV detection.

Fig. 4. Chromatogram of IBN standard solution with indirect UV detection ($c = 50 \mu\text{g/mL}$).

Fig. 5. Chromatogram of IBN sample solution with indirect UV detection ($c = 50 \mu\text{g/mL}$).

Fig. 6. Chromatogram of blank solution.

Linearity and range

For evaluation of linearity, the peak area and concentrations were subjected to a least square regression analysis to calculate calibration equation and determination coefficient. For the proposed method, the calibration graphs of the absorbance against concentration were found to be linear over the range of 25–200 $\mu\text{g/mL}$ at the appropriate experimental conditions. The equation for linear regression was: $y = 17352x - 8657.1$; $R^2 = 0.9991$, where X is the concentration (in $\mu\text{g/mL}$) of IBN and Y is the absorbance. Without an intersection of the estimated tilt value at 95% of the reliability of the boundary, optionally the calibration lines of IBN solutions in mobile phase are not deviate from sources as above obtained values fall within the limits of reliability.

Limit of detection & quantitation limit

The detection limit in a single analytical procedure is the amount of analyte in a sample that can be detected, but not necessarily quantified as an accurate value. The quantitation limit of an individual analytical procedure is the amount of analyte in a sample that can be quantitatively determined by proper precision and accuracy. The LOD and LOQ were found using the ratio $3.3 \sigma/S$ and $10 \sigma/S$ respectively, where σ is the standard error of

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estimation and S is slope (ICH, 2005). The established LOD and LOQ for ibandronic acid sodium were $0.0163 \mu\text{g/mL}$ and $0.0495 \mu\text{g/mL}$ respectively.

Accuracy

To study the accuracy of the proposed analytical methods, recovery tests were conducted using the standard addition method. To discover whether excipients interfered with the analysis, known amounts of standard were added to tablet formulation samples and the resulting mixtures were analyzed by the proposed methods. The percent of recovery was calculated using the calibration equation. Different concentrations of IBN standard solution (containing IBN active substance in the range of 80, 100 and 120% of the working concentration) within the used range of linearity and the calculated values were as a supplement to the previously analyzed formulation concentration of $50 \mu\text{g/mL}$ with percentage recovery.

Recovery values (Table 3) obtained from the determination of IBN in commercial tablets using the method of standard additions confirmed that the method was accurate and precise in the range of 80–120% of the working concentration.

Table 1. Results obtained from testing the accuracy of the method

Precision

The precision of the analytical procedure (intra-assay precision) was investigated by analyzing six sample solutions obtained by multiple sampling of the same homogeneous sample under the prescribed conditions (at 100% of the working concentration of IBN ($50 \mu\text{g/mL}$) on the same day, by the same analyst and using the same equipment. The intermediate precision of the analytical procedure was investigated by analyzing sample

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solutions on three consecutive days. The precision of the analytical procedure was expressed as a relative standard deviation of series of measurements.

Table 2. Results of the precision of the method

Robustness

Robustness should show the reliability of an analysis with respect to deliberate variations in method parameters. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled, or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g., resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used. For this method variations were made to the column temperature, flow-rate and variation in the organic part in the mobile phase. The effects of the following changes in chromatographic conditions didn't show any significant changes.

Table 3. Results of the robustness of the method

Conclusion

A simple RP-HPLC method with indirect UV detection was developed and validated for determination of IBN in tablets. The validation results showed that the method is selective, linear, precise, accurate, and with a high sensitivity. The proposed method gives a

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simple and economic approach that can be easily applied for routine control of IBN in pharmaceuticals.

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Резиме

Реверзно-фазен HPLC метод со индиректна UV детекција за определување на натриум ибандронат во фармацевтски препарати

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Клучни зборови: RP-HPLC метод со индиректна UV детекција, натриум ибандронат, валидација, фармацевтски препарати

Натриум ибандронат [(1-hydroxy-3- (methyl pentyl amino) propylidenebisphosphonic acid monosodium monohydrate)] е натриумовата сол на ибандронската киселина, синтетска бифосфонатна супстанција што содржи азот. Целта на оваа студија беше да се развие осетлив и точен RP-HPLC метод со индиректна UV детекција, што може да се користи за определување на натриум ибандронат во фармацевтски препарати. Хроматографското раздвојување беше изведено на Waters Bridge C18 реверзно-фазна колона (250 X 4.6 mm I.D., со големина на честички 5 µm), во изократски услови при што како мобилна фаза беше користена мешавина од 90% пуфер:10% ацетонитрил. Пуферот е изработен од 1,5mL на ортофосфорна киселина 85%, 990 mg натриумова сол на 1-Хексансулфонска киселина 98% и 140 mg на EDTA во 1000 mL стаклен сад, дополнет до волуменот со редестилирана вода со HPLC квалитет. Елуирањето е изведено при константен проток од 1,0 mL min⁻¹. DAD детектор беше користен за мерење на UV абсорбанцата на 198 nm, во индиректна функција. Методот беше валидиран во однос на параметрите: специфичност/селективност, линеарност, LOD, LOQ, точност, прецизност и робусност, во согласност со ICH водичот за валидирање на аналитички методи.

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Лимитот на детекција беше пресметан со вредност од 0,0163 $\mu\text{g/mL}$, додека лимитот на квантификација изнесува 0,0495 $\mu\text{g/mL}$. Методот беше успешно искористен за определување на ибандронат натриум во комерцијални таблети и даде добри резултати без било каква интерференција од ексципиенсите во формулацијата.

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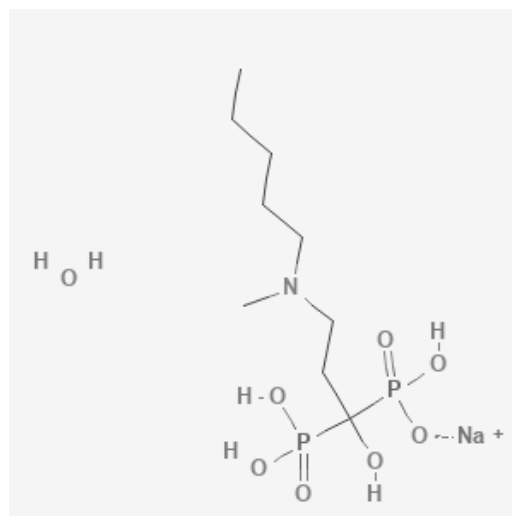


Fig. 1. Structure of IBN.

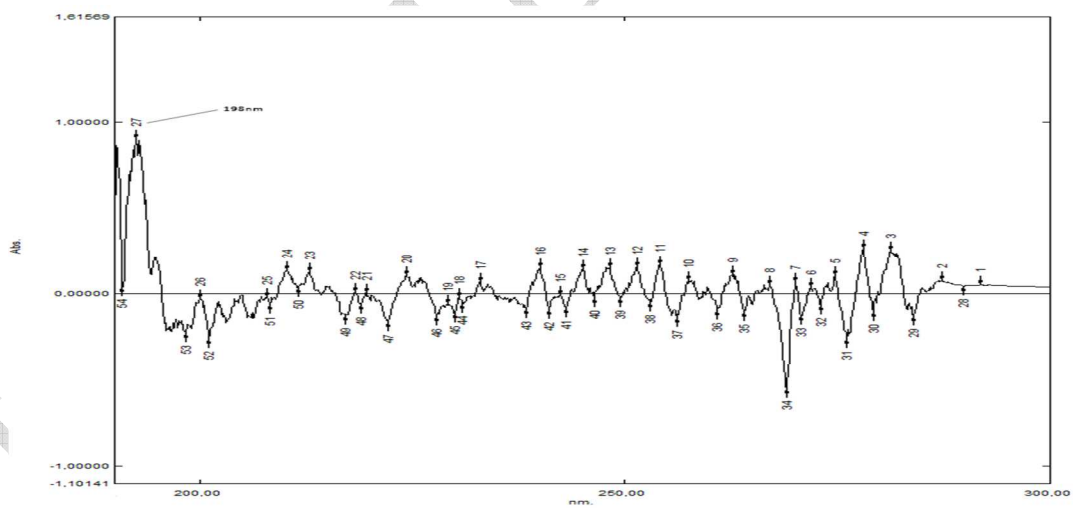


Fig. 2. UV spectrum of IBN in mobile phase in the range of 300 to 190 nm.

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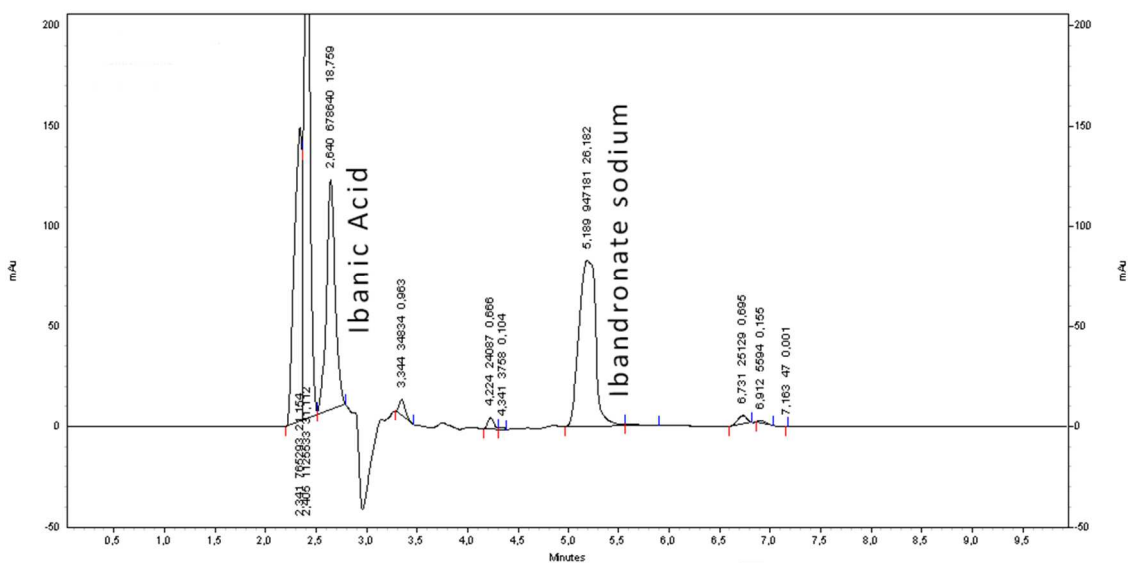


Fig. 3. Chromatogram of sample solution spiked with Ibanic acid with indirect UV detection.

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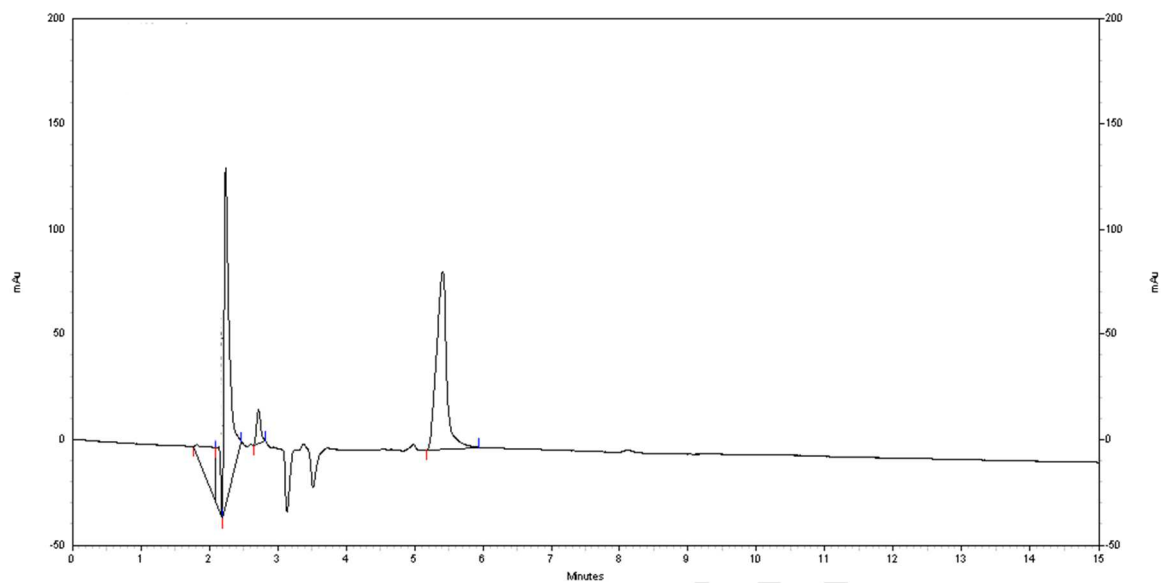


Fig. 4. Chromatogram of IBN standard solution with indirect UV detection ($c = 50 \mu\text{g/mL}$).

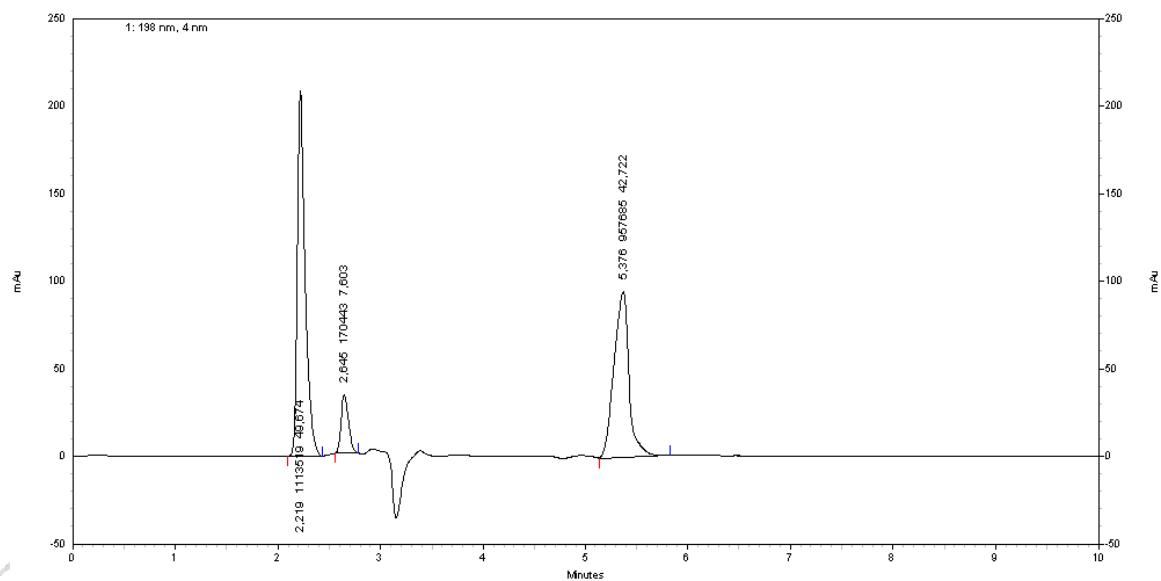


Fig. 5. Chromatogram of IBN sample solution with indirect UV detection ($c = 50 \mu\text{g/mL}$).

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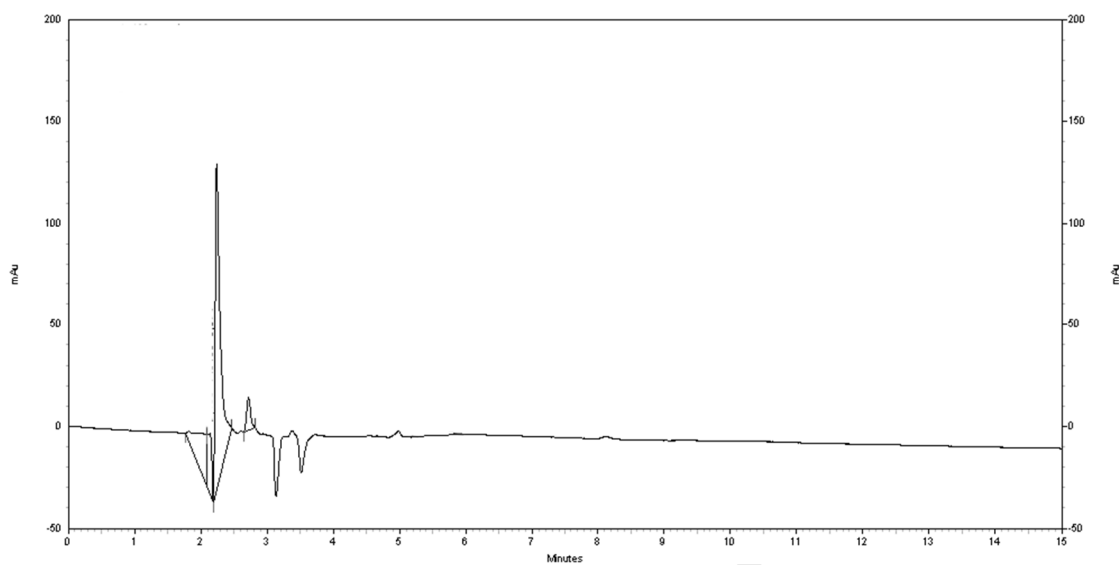


Fig. 6. Chromatogram of blank solution.

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Table 1. Results obtained from testing of the accuracy of the method

Sample ID	% Recovery	
80% of WC*	99.21	
	99.41	Average: 99.27%
	99.24	SD: 0.08%
	99.25	%RSD: 0.08%
	99.33	
	99.21	
100% of WC*	99.62	
	99.17	Average: 99.50%
	99.67	SD: 0.20%
	99.36	%RSD: 0.20%
	99.62	
	99.56	
120% of WC*	100.56	
	100.21	Average: 100.35%
	100.36	SD: 1.14%
	100.29	%RSD: 1.13%
	100.26	
	100.44	

*WC = working concentration

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Table 2. Results of the precision of the method

Sample ID	Absorption	
100% of WC*	869986	Average: 869641.3 %RSD: 0.036
	869279	
	869774	
	869511	
	869321	
	869977	

*WC = working concentration

Table 3. Results of the robustness of the method

Parameter	RSD (%) (n = 3)
Flow rate (1.1 mL min ⁻¹)	0.091
Flow rate (0.8 mL min ⁻¹)	0.093
Organic solvent concentration (15%)	0.542
Organic solvent concentration (5%)	0.078
Column temperature (35 °C)	0.034
Column temperature (45 °C)	0.086

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