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Short communication

Determination of azide impurity in sartans using reversed-phase HPLC with UV detection

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ABSTRACT

A simple and robust, gradient HPLC method was developed for determination of azide ppm ($\mu g\,g^{-1}$) levels in different sartans (irbesartan, candesartan, valsartan). The sartan was dissolved in 0.1 M NaOH. Then pH was adjusted to 4.5 with 20% H_3PO_4 followed by dilution with water. Precipitated API was removed by filtration using 0.45 μm membrane PVDF (Polyvinylidene Fluoride) membrane filter, and supernatant was analyzed by gradient elution HPLC at room temperature with Hydro RP HPLC 250 × 4.6 mm, 4 μm column and UV detection at 205 nm. The best sensitivity was achieved by UV detection cell with 60 mm optical path length: LOD 0.17 $\mu g\,g^{-1}$ and LOQ 0.84 $\mu g\,g^{-1}$ for azide. The USP requirement for maximum azide content in irbesartan is 10 $\mu g\,g^{-1}$. The analytical method was validated as per International Conference on Harmonization (ICH) guidelines with respect to system precision, intraday precision (repeatability), intermediate precision (ruggedness), linearity, quantitation limit, detection limit, accuracy, standard and sample solution stability, robustness and selectivity/specificity. The method was linear in the range from LOQ (0.84 $\mu g\,g^{-1}$) to 101 $\mu g\,g^{-1}$ of azide. The correlation coefficient was 0.9999 and bias on y-axis for 84 $\mu g\,g^{-1}$ test concentration was 0.33%. The accuracy of the method was established based on the recovery obtained between 94.0% and 103.0% for azide. Standard and sample solutions were stable for at least 48 h at room temperature and in refrigerator. The method was found to be robust for variation in column temperature (± 5 °C) and mobile phase flow rate (±0.2 mL min^{-1}) and selective for anions such as bromide, nitrate, nitrite, formate and acetate.

1. Introduction

Sodium azide is best known as the chemical found in airbags, as a chemical preservative in hospitals and laboratories, in agriculture for pest control, and it is also a precursor in the synthesis of a wide range of tetrazole derivatives. Sodium azide is a highly toxic chemical that exists as an odourless white solid. When it is mixed with water or an acid, sodium azide changes rapidly to a toxic gas (hydrazoic acid) with a sharp odour. It also changes into a toxic gas when it comes in contact with solid metals [1]. Sodium azide is classified as a first class poison with a lethal dose (LD_{50}) 50 mg kg^{-1}. Due to its genotoxic character (*in vivo*) DNA damage at any level of exposure can be caused. Therefore generally applicable approach defined as the Threshold of Toxicological Concern (TTC) was implemented and calculated for different daily doses for individual active compounds [2,3].

Sodium azide is a precursor in the synthesis of sartans (irbesartan, candesartan, valsartan), active pharmaceutical ingredients (APIs). Sartans are mainly used for chronical lifetime treatments, *i.e.* diabetes, hypertension, immunosuppression, cardiovascular diseases, anticoagulant therapy, cholesterol therapy etc. Consequently, production, usage and content of sodium azide should be well controlled [2].

Several analytical techniques for determination of azide have been reported, such as colorimetry [4], spectrophotometry [5–7], gas chromatography–mass spectrometry [8–11] and flow injection analysis [12,13]. However, these methods are not selective and sensitive enough for determination of sodium azide traces (10 $\mu g\,g^{-1}$) as impurity in pharmaceutical products. Selective methods that were developed for azide determination are mostly based on reversed-phase liquid chromatography, capillary electrophoresis with pre-derivatization and ion chromatography [14–19]. Most widely used method for determination of azide, prescribed also by a USP, is ion chromatography (IC) with suppressed [2,20] or non-suppressed [22,23] ion conductivity detection. Related substances, degradation products of irbesartan were determined using

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reversed-phase liquid chromatography, however, the method is not appropriate for determination of ppm levels of azide [24].

According to US Pharmacopoeia (USP) traces of azide in irbesartan (one of the sartan samples) are determined by using a high capacity anion exchange column with L31 resin and 0.1 M sodium hydroxide as the eluent [21]. Irbesartan is sparingly soluble in 0.1 M sodium hydroxide and methanol. When sample solution of irbesartan substance is injected onto the high capacity anion exchange column, the substance adsorbs to the column due to strong lipophilic interaction with the polymer material of the stationary phase. Consequently, the column capacity is reduced and cleaning of the column is necessary after each sample analysis, which takes additional 3–4 h. A solid-phase extraction (SPE) cartridge was used to reduce the interfering matrix, however, this is expensive over a longer period of time and for larger amounts of samples [25–27]. Some authors reported on using in-line sample preconcentration with matrix elimination by automated IC method for determination of azide content in irbesartan, where the sample solution of irbesartan substance was prepared in water, following by the in-line preconcentration with matrix elimination. However, irbesartan is practically insoluble in water (max 1000 μg mL^{-1}) [28] and not slightly soluble in water as described in the paper of Subramanian et al. [2].

This work describes a simple, sensitive, selective and robust method for the determination of azide in sartans—using a reversed-phase liquid chromatography and UV detection without any pre- or post-column derivatization, pre-concentration or pre-treatment, using the supernatant of the suspension obtained by dissolving the sartans in 0.1 M NaOH and precipitating their majority by acidifying the solution with 20% H_3PO_4 to pH 4.5.

2. Experimental

2.1. Chemicals and reagents

All solutions used were prepared using Milli Q water from a Millipore water purification system (electrical resistivity >18 MΩ). For mobile phase and sample solution, the following chemicals and reagents were used: *ortho*-phosphoric acid (85%; Merck, Germany), acetonitrile (HPLC grade; Merck, Germany) and sodium hydroxide (p.a.; Merck, Germany). Sodium azide standard was purchased from Sigma-Aldrich, Germany. Active pharmaceutical ingredients of sartans (irbesartan, USP standard, Lot: F0F293, valsartan USP standard, Lot: J0I020 and candesartan USP standard, Lot: F0K123) were used as spiked samples for assay and recovery determination of azide in sample solutions.

2.2. Equipment

A 1290 Infinity UHPLC system from Agilent was used. The system consists of 1200 bar pump, Diode Array Detector (DAD) with a standard cell with 10 mm optical path and ultra-sensitive cell with 60 mm optical path length, Autosampler, Thermostat and Thermostated Column Compartment TCC. The chromatographic data acquisition was done with OpenLAB CDS EzChrom Edition. Separations were performed using Synergi Hydro RP analytical column (250 × 4.6 mm, 4 μm). Sartan samples were prepared using a centrifuge Kendro Lab. Products, Heraeus, Biofuge, Stratos.

The HPLC procedure is described in Tables 1 and 2.

2.3. Preparation of solutions

2.3.1. Preparation of sample solution

100 mg of API was transferred into a 5 mL volumetric flask. First, it was dissolved in 2.0 mL of 0.1 M NaOH, afterwards 3.0 mL of 20% H_3PO_4 was added to correct pH to 4.5 to get the concentration 20 mg mL^{-1}. Precipitated API was removed by filtration using 0.45 μm membrane PVDF membrane filter (Millipore) or alternatively by centrifugation at 16.128 relative centrifugal force for 10 min.

Table 1
Optimal chromatographic conditions for HPLC-UV applied to the azide assay.

Column	Synergi Hydro RP analytical column (250 × 4.6 mm) 4 μm
Mobile phase A	0.5 g/L H_3PO_4
Mobile phase B	100% acetonitrile
Solvent of sample solution	Sample was dissolved in 0.1 M NaOH and neutralized with 20% H_3PO_4 to pH 4.5
Flow rate	1.5 mL min^{-1}
Column temperature	30 °C
Detection	205 nm
Injection volume	20 μL

Table 2
Gradient conditions.

Gradient conditions		
t (min)	Mobile phase A (%)	Mobile phase B (%)
0	100	0
4.5	100	0
5	0	100
10	0	100
10.5	100	0
17	100	0

2.3.2. Preparation of standard solution of sodium azide

Stock aqueous solution of sodium azide of 0.25 mg mL^{-1} was prepared daily. Concentration of the standard solution for determination of system suitability test was 2.5 μg mL^{-1}. Lower concentrations were prepared by dilution of the stock solution.

2.3.3. Preparation of spiked sample solution

100 mg of API was transferred into a 5.0 mL volumetric flask. First, it was dissolved in 2.0 mL of 0.1 M NaOH, then 0.5 mL of suitable sodium azide standard solution was added (0.025 mg mL^{-1} for preparation of 84 μg g^{-1} of azide, 0.1 mg mL^{-1} for preparation of 336 μg g^{-1} of azide and 0.25 μg mL^{-1} for preparation of 0.84 μg g^{-1} of azide calculated with respect to the concentration of sample solution). Afterwards 2.5 mL of 20% H_3PO_4 were added to correct pH to 4.5. Precipitated API was removed as described above.

2.4. Precision of the system

The precision of system was determined by ten consecutive injections of azide standard solution. The RSD (relative standard deviation) on the basis of the peak area of azide was calculated.

2.5. Precision of the method (repeatability, intraday 1)

The repeatability of the method was determined by injecting six individual preparations of sample spiked with 84 μg g^{-1} of azide. HPLC analysis was performed on the same day. The RSD of peak area of azide was calculated.

2.6. Precision of the method (intermediate precision, inter-day precision, ruggedness)

Intermediate precision was examined by analysis of spiked sample solutions in two different days. Each day six individual preparations of sample spiked with 84 μg g^{-1} of azide were