Matrix Elimination Ion Chromatography Method for Trace Level Azide Determination in Irbesartan Drug

Narayanan Harihara Subramanian[1,*], V.R. Sankar Babu[1], R. Ganesh Jeevan[2], and **Ganga Radhakrishnan[2]**

[1]Metrohm India Limited, Application Lab; [2]Central Leather Research Institute, Expertise Centre for Eco testing Lab (EXCEL)

Abstract

Ultra-trace analysis of azide in complicated Irbesartan sample matrix is achieved by the in-line sample preparation technique. Sodium azide is the precursor of Irbesartan, which is used as an anti-hypertensive drug. Due to the toxic nature of sodium azide, reliable determination of azide in Irbesartan is necessary. Irbesartan when analyzed for sodium azide, as per the USP 31-NF26 method, gets adsorbed to the analytical column, leading to reduction in column capacity and irreproducible retention time. The retained drug has to be removed with special rinsing solution, followed by re-equilibration with the mobile phase. This process takes at least 3 to 4 h for each sample analysis. The new method developed overcomes the limitations of the USP 31-NF26 method. This method is validated for specificity, linearity, accuracy, precision, sample solution stability, and robustness as per International Conference on Harmonization guidelines. The relationship between peak response and concentration is found to be linear between 5 to 80 ng/mL of sodium azide, with the correlation coefficient (r^2) of 0.9995. The limits of detection and quantification for sodium azide are 0.532 and 1.61 µg/gm with respect to the sample weight.

Introduction

Alkali metal azides (MeN_3) are the key to the synthesis of a wide range of tetrazole derivatives such as Irbesartan. Sodium azide is used mainly as a preservative in aqueous laboratory reagents and biological fluids and as a fuel in automobile airbag gas generants (1). Sodium azide is a highly toxic compound classified as a first-class poison whose lethal dose for oral ingestion in human (LD_{50}) is less than 50 mg/kg. The problem is compounded by the fact that aqueous sodium azide is readily hydrolyzed to yield hydrazoic acid (HN_3), a volatile substance that partitions strongly to the gas phase. When ingested or inhaled, HN_3 is highly toxic (2). Consequently, industries producing or using sodium azide have to apply tight controls.

Several analytical methods for the determination of azide have been reported, including colorimetric (3), spectrophotometric (4), gas chromatography–mass spectrometry (5,6), and flow injection analysis (7). These methods lack selectivity and sensitivity. Selective methods developed for azide are either based on reversed-phase liquid chromatography or capillary electrophoresis with pre-derivatization (8–10). Compared to other analytical methods, ion chromatography (IC) with suppressed conductivity detection is the most straightforward and interference-free approach. IC allows direct injection of samples, avoiding laborious derivatization or sample preparation, and is highly sensitive. IC with non-suppressed (11,12) or suppressed (13,14) chromatography has been used for azide determination.

Limitations of current USP method for azide in Irbesartan

The current U.S. Pharmacopeia (USP) compendial method, USP 31-NF26 for determining the limit of azide in Irbesartan (15) recommends using high capacity anion exchange column with L31 resin and 100 mM sodium hydroxide as the eluent.

Irbesartan is a high molecular weight (428.53) compound having tetrazole functional group (Figure 1). The drug is sparingly soluble in water and is soluble in methanol and 100 mmol/L sodium hydroxide. When 200 µL of dissolved drug sample is injected to the high-capacity anion exchange column (L31), the drug molecule gets adsorbed to the column due to strong interaction with the polymeric base material. Because this drug molecule is not eluted by the mobile phase,

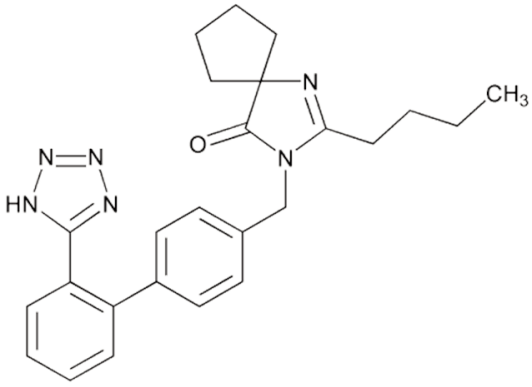

Figure 1. Irbesartan molecule.

* Author to whom correspondence should be addressed.

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it effectively reduces column capacity and also the retention time of sodium azide. Consecutive six injections of 20 mg/mL of Irbesartan containing 0.312 µg/mL of sodium azide dissolved in eluent are shown in Figure 2. The retention time of azide was shortened with each injection and after the sixth injection, azide was not detected at all. This indicates that the used eluent concentration is insufficient to remove the retained drug as the drug's interaction with the column is not ionic in nature. Hence, to remove the retained drug, a combination of methanol and sodium hydroxide is used as the rinsing solution. However, guard column and column cleaning after every test solution injection makes it tedious and it takes approximately 3 to 4 h per test solution analysis.

The interfering matrix can be selectively retained using a suitable solid-phase extraction (SPE) cartridge (16–18). However, efficiency of the Irbesartan retention in cartridge would depend on the extent of solvation and the pressure with which the sample is passed through the SPE cartridge. Because each cartridge is discarded after processing one sample, this technique can be expensive over a period of time.

This work describes a sensitive and robust in-line sample preconcentration with matrix elimination automated IC method to determine traces of azide content in Irbesartan.

Experiment

Instrument and accessories

An Advanced Modular IC instrument from Metrohm (Herisau, Switzerland) was used for this study. Also used were an 818 high-pressure IC pump, 819 advanced conductivity detector, 820 IC separation center with dual injector and column oven, 833 Metrohm suppressor module, 838 advanced sample processor with built-in injector, and 833 dual channel liquid handling peristaltic pump. All the modules were connected to the 830 dual channel interface, controlling and data acquisition was done with the IC Net 2.4 SR4 version.

A Polymethacrylate-based, low-pressure preconcentrator column, Metrosep A PCC 1, with quaternary ammonium groups was used for sample preconcentration. A Metrosep A Trap 1 was used to trap the inorganic contaminants from the aqueous methanol, which was used as the rinsing solution. A Metrosep A Supp 10–250 high capacity anion column was used for the separation of azide.

Chemicals and reagents

All solutions were prepared using deionized water (> 18 MΩ) purified by a Milli-Q Gradient system (Millipore, Billerica, MA). Sodium carbonate puriss, sodium bicarbonate puriss ACS Reg.Ph Eur, suprapure sulfuric acid, and sodium azide were purchased from Fluka (Seelze, Germany). Methanol HPLC grade was purchased from Merck India.

Sodium carbonate and sodium bicarbonate (5 mmol/L each) were used as eluent. Sulphuric acid (100 mmol/L) and ultrapure water were used as the suppressor regenerants. The rinsing solutions of different compositions were prepared by mixing appropriate volumes of methanol and water and vacuum filtering to remove the dissolved gases. Sodium azide standard stock solution of 100 µg/mL was prepared from the sodium azide salt. Lower concentrations were prepared freshly from this stock solution.

Sample preparation

One hundred twenty-five milligrams of accurately weighed Irbesartan were transferred to a 25-mL volumetric flask, diluted with high-purity water to volume, and mixed well. The insoluble drug was removed from the sample solution using 0.2 µ nylon filter paper, and the filtrate was used for further analysis.

The sample preparation procedure was modified to use water as the diluent, instead of the mobile phase used in the USP Method. Water as the diluent has the advantage that the sodium azide is freely soluble, whereas the Irbesartan is sparingly soluble, so the amount of drug entering the analytical column can be reduced to a great extent without losing the sodium azide present in the sample.

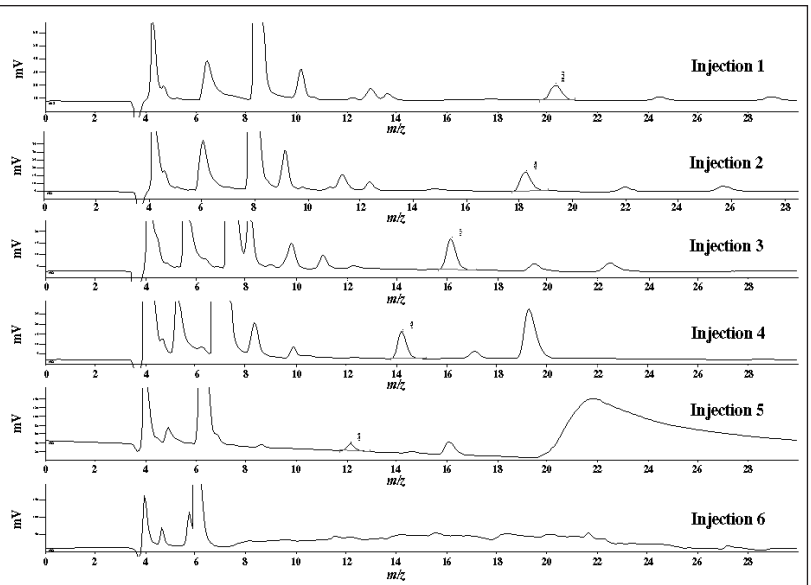

Figure 2. Reduction in RT in Irbesartan spiked with 0.312 ppm sodium azide. Column: AS10 HC; eluent: 100 mmol/L NaOH; flow rate: 1.0 mL/min; Sequant suppressor with ULB regenerant.

Results and Discussion

In-line preconcentration with matrix elimination

In this in-line matrix elimination method, 1000 µL of 5 mg/mL of Irbesartan in water is injected to the preconcentrator column. After the preconcentration, the interfering Irbesartan drug residues are removed by rinsing the preconcentration column for 6 min with a transfer solution consisting of 70% methanol and 30% ultra-pure water. After matrix re-

moval, the azide in the preconcentrator column is injected to the analytical column. This step is described in Figure 3. The entire procedure was time programmed and automated.

Method validation

Using the previously described in-line matrix elimination set-up and time programming, the method was validated as per International Conference on Harmonization (ICH) guidelines.

Specificity. Blank solution, sample, sample spiked with azide, mixed anion standards with chloride, nitrite, nitrate, azide, and phosphate were injected to check the specificity. Relative retention times (RRT) for anions such as chloride, nitrite, phosphate, and nitrate with respect to sodium azide were 0.4, 0.5, 1.2, and 1.3, respectively. RRT values prove good separation of azide from similar ions like nitrite and nitrate in the sample matrix, and hence, it is suitable for the determination of azide in Irbesartan. The separation of azide from common anion is shown in Figure 4 with the chromatographic conditions.

System precision. System precision was calculated from the relative standard deviation (RSD) value obtained for area and RT for the six consecutive injections of 30 ng/mL sodium azide standard. The RSD values for area and RT were 3.95% and 0.095%, respectively. The RSD for peak area obtained was well within the acceptance criteria of ICH guidelines.

Linearity. Sodium azide standard concentrations of 5, 10, 20, 40, 60, and 80 ng/mL were prepared by diluting 1 µg/mL sodium azide standard with ultra-pure water. The prepared low-level concentrations were injected in triplicate to check the precision and linearity aspects.

A regression line was obtained by plotting peak area (mV × s) of the sodium azide using the least square method. The relationship between peak response and concentration was found to be linear between the ranges of 5 to 80 ng/mL of sodium azide, with the coefficient of determination (r^2) of 0.9995. The slope and intercept of the regression line were 8.123 ± 0.04553 and 10.03 ± 2.047, respectively. Standard error ($S_{y/x}$) was 5.244. The RSD of the response factor was 1.596%.

Based on the calibration, Irbesartan drug was analyzed for sodium azide, but azide was not detected in the sample.

Limits of quantification and detection. Based on the linearity calibrations, the limit of detection (LOD) and limit of quantification (LOQ) were predicted using the following formula:

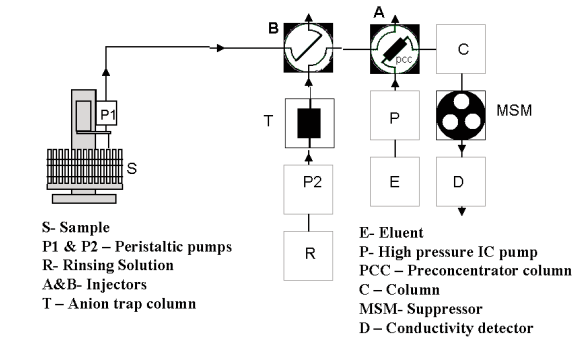

Figure 3. Instrument set-up.

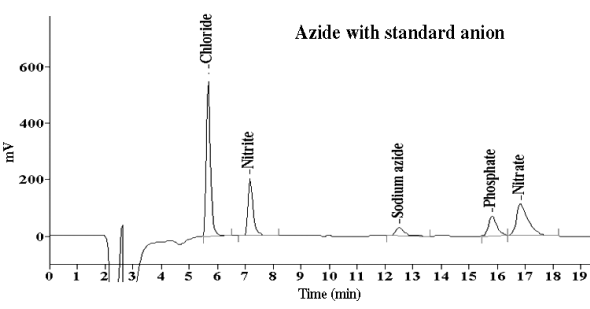

Figure 4. Specificity of azide from common anions. Column: Metrosep Supp 10–250; column oven temperature: 60°C; eluent: 5 mM sodium carbonate + 5 mM sodium bicarbonate; flow: 1 mL/min; detection: suppressed conductivity.

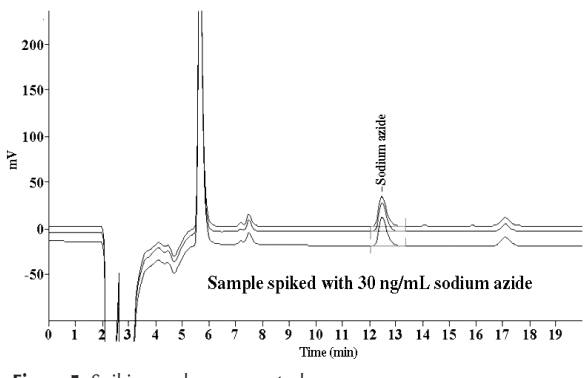

Figure 5. Spiking and recovery study.

Table I. Spiking and Recovery Data

	5 ng/mL spike				30 ng/mL spike			
S. no.	Area (mV × s)	Height (mV)	RT (min)	Recovery (%)	Area (mV × s)	Height (mV)	RT (min)	Recovery (%)
1	41.343	2.094	12.56	99.58	257.94	12.45	12.71	103.26
2	42.99	2.07	12.56	103.54	257.26	13.39	12.52	103.33
3	42.349	2.076	12.56	102.00	257.42	13.22	12.52	103.53
Mean	42.227	2.08	12.56	101.71	257.54	13.02	12.58	103.37
SD	0.8302	0.012	0.001	2.00	0.3543	0.504	0.107	0.14
RSD	1.9661	0.597	0.01	1.96	0.1376	3.869	0.852	0.14

Table II. Validation Summary

Parameter		Requirements of regulatory authorities		Achieved	Result
Selectivity		Baseline separation of anionic Irbesartan ingredients		RRT: Cl^- (0.45), NO_2^- (0.57), PO_4^{3-} (1.26), NO_3^- (1.35)	+
LOD		LOD > 3 × SD (blank)		5 µg/L	+
LOQ		LOQ > 10 × SD (blank)		30 µg/L	+
Precision		RSD of PA, peak height, and RT < 5%		< 3.9%	+
Linearity		R^2 > 0.9980 for 6-point calibration in the range of 5…100 µg/mL		R^2 = 0.9995	+
Accuracy		Spike recovery: 80…120%		99.6…104.0%	+
		Selectivity	Baseline separation of Irbesartan ingredients	RRT: Cl^- (0.45), NO_2^- (0.57), PO_4^{3-} (1.31), NO_3^- (1.35)	+
Method robustness 1	Temperature variation	Precision	RSD < 5%	< 0.89 (PA) < 2.82 (peak height) < 2.11 (RT)	+
		Accuracy	Spike recovery: 80…120%	102.1…103.3%	+
		Selectivity	Baseline separation of Irbesartan ingredients	RRT: Cl^- (0.45), NO_2^- (0.58), PO_4^{3-} (1.26), NO_3^- (1.34)	+
Method robustness 2	Composition of the transfer solution	Precision	RSD < 5%	< 3.10 (PA) < 3.10 (peak height) < 0.32 (RT)	+
		Accuracy	Spike recovery: 80…120%	98.0…103.3%	+

$$LOQ\ (ng/mL) = S_{y/x} \times 10 / Slope$$
$$LOD\ (ng/mL) = S_{y/x} \times 3.3 / Slope$$

The LOD and LOQ were calculated as 2.13 ng/mL and 6.45 ng/mL of sodium azide, respectively, which would correspond to 0.426 and 1.29 µg/gm of sodium azide with respect to the sample weight. The LOQ achieved by this method is far better than the USP requirement of 10 µg/gm of sodium azide with respect to sample weight.

Accuracy and precision. Spiking and recovery study was carried out to check the accuracy of the method. Spiking was done to get sodium azide concentration of 5 ng/mL and 30 ng/mL in samples. Spiked samples were analyzed in triplicate. A recovery ranging from 99% to 104% was obtained with the precision less than 2% for triplicate injections. A sample spiked with 30 ng/mL sodium azide is shown in Figure 5. The accuracy and precision data are provided in Table I.

Stability of sample solution. Sample solution spiked with azide was prepared and kept at room temperature in the sample processor vial. It was analyzed after every 4 h for 40 h. It was concluded from the sodium azide RT and area that the sample is stable in analytical solution for at least 40 h at room temperature.

Robustness. The robustness of the method was investigated by checking the system suitability parameters by deliberately varying the instrumental conditions such as column temperature, eluent composition, eluent flow rate, and rinsing solution composition. Variation in rinsing solution by 5% and change in eluent concentration and flow rate by 10% had no influence on selectivity, precision, or accuracy. A change in RT for sodium azide was noticed when column temperature was changed, but still all the expected parameters of selectivity, system precision, and accuracy were satisfactory.

In each of the previously mentioned conditions, mixed anion was injected to check the selectivity. Sodium azide standard (20 ng/mL) was injected six times to check the system precision, and a sample spiked with 30 ng/mL sodium azide standard was injected thrice to check the accuracy and precision of the method. Because the system suitability requirement was met under each variable condition, it is concluded that the method is robust. Details of the validation summary are given in Table II.

Conclusion

The in-line matrix elimination method is found to be effective and suitable for continuous sample injections. This set-up can also be used for the trace level determination of ions in samples loaded with high organic content such as solvents and effluent water. The newly developed and validated method is suitable for unattended analysis of azide in Irbesartan in the pharmaceutical industry.

Acknowledgment

The authors N. Harihara Subramanian and V.R. Sankar Babu greatly acknowledge the support from Metrohm India Limited.

Reference

1. S. Chang and S.H. Lamm. Human health effects of sodium azide exposure: a literature review and analysis. *Int. J. Toxicol.* **2222:** 175–186 (2003).
2. E.A. Betterton. Environmental fate of sodium azide derived from automobile airbags. *Crit. Rev. Environ. Sci. Technol.* **33:** 423–458 (2003).
3. A. Anton, J.G. Dodd, and A.E. Harvey. Spectrophotometric determination of azide with ferric perchlorate. *Anal. Chem.* **32:** 1209–1210 (1960).
4. K. Tsuge, M. Kataoka, and Y. Seto. Rapid determination of cyanide and azide in beverages by microdiffusion spectrophotometric method. *J. Anal. Toxicol.* **25:** 228–236 (2001).
5. S. Kage, K. Kudo, and N. Ikeda. Determination of azide in blood and urine by gas chromatography–mass spectrometry. *J. Anal. Toxicol.* **24:** 429–432 (2000).
6. A. Miki, M. Nishikawa, and H. Tsuchihashi. Simultaneous GC-MS determination of azide, cyanide and thiocyanate via phase-transfer-catalyzed pentafluorobenzylation. *J. Health Sci.* **46:** 81–88 (2000).
7. R.T. Echols, R.R. James, and J.H. Aldstadt. Determination of primary explosive azides in environmental samples by sequential injection amperometry. *Analyst* **122:** 315–319 (1997).
8. S.J. Swaring and R.S. Waldo. Liquid chromatographic determination of azide as the 3,5-dinitrobenzoyl derivative. *J. Liq. Chromatogr.* **5:** 597–604 (1982).
9. P. Marquet, S. Clément, H. Lotfi, M.-F. Dreyfuss, J. Debord, D. Dumont, and G. Lachâtre. Analytical findings in a suicide involving sodium azide. *J. Anal. Toxicol.* **20:** 134–136 (1996).
10. O. Masataka, K. Yoshimi, and I. Shunsuke. Determination of azide as the 3,5-dinitrobenzoyl derivative by capillary electrophoresis. *J. Chromatogr. A* **1045:** 247–252 (2004).
11. P.L. Annable and L.A. Sly. Azide determination in protein samples by ion chromatography. *J. Chromatogr. A* **546:** 325–334(1991).
12. H. Mackie, S.J. Speciale, L.J. Throop, and T. Yang. Ion chromatographic determination of the azide ion in a preabumin fraction from human serum. *J. Chromatogr.* **242:** 177–180 (1982).
13. C.A. Lucy and C.R. Harrison. Chemiluminescence nitrogen detection in ion chromatography for the determination of nitrogen-containing anions. *J. Chromatogr. A* **920:** 135–141 (2001).
14. K. Vinkovic and V. Drevenkar. Ion chromatography of azide in pharmaceutical protein samples with high chloride concentration using suppressed conductivity detection. *J. Chromatogr. B* **864:** 102–108 (2007).
15. United States Pharmacopeia. The National Formulary. Irbesartan. USP 31-NF 26, 2008, page 2446.
16. R. Slingsby and R. Kiser. Sample treatment techniques and methodologies for ion chromatography. *Trends Anal. Chem.* **20:** 288–295 (2001).
17. I.K. Henderson, R.S. Norhaus, and J.M. Anderson. Sample preparation for ion chromatography by solid-phase extraction. *J. Chromatogr. A* **546:** 61–71 (1991).
18. R.S. Nordhaus, L.M. Nair, and J.M. Anderson. Elimination of matrix interferences in ion chromatography by the use of solid-phase extraction disks. *J. Chromatogr. A* **671:** 159–163 (1994).

Manuscript received October 20, 2008;
Revision received January 26, 2009.