International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.9, No.01 pp 121-126, 2016

Ion Chromatographic Method for Quantification of sodium content in Bupivacaine Formulation

Prathyusha Vikram[1,*] and Palani Shanmugasundaram[2]

[1]Department of Pharmaceutical analysis, School of Pharmaceutical Sciences, Vels University, Chennai, Tamilnadu, India.
[2]School of Pharmaceutical Sciences, Vels University, Chennai, Tamilnadu, India.

Abstract: A simple, precise, rapid and accurate ion chromatographic method has been developed and validated for the quantification of sodium content in Bupivacaine formulation. Successful quantification of sodium content in Bupivacaine formulation was achieved with in 10min on TSK GEL IC SW cation column 50 x 4.6mm, 5µm column, using a 0.02N Nitric acid and Acetonitrile in the ratio of 100:1v/v at a flow rate of 1.0 mL per minute. The developed ion chromatographic method was validated with respect to specificity, linearity, accuracy, precision, ruggedness and robustness. The method was found to be linear in the range of 1.199 µg.mL⁻1 to with 7.993 µg.mL⁻1 with a correlation coefficient of 0.9999. The developed method was validated as per ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness and can be used to evaluate the quality of regular production samples and stability samples.For others provide abstract of maximum 80 words.
Keywords: Bupivacaine; Ion chromatography; Development; Validation

Introduction

Bupivacaine [(RS)-1-butyl-N-(2,6-dimethylphenyl)piperidine-2-carboxamide] Figure 1. is a local anesthetic1 drug belonging to the amino amide group, a white crystalline powder that is freely soluble in 95 percent ethanol, soluble in water, and slightly soluble in chloroform or acetone.

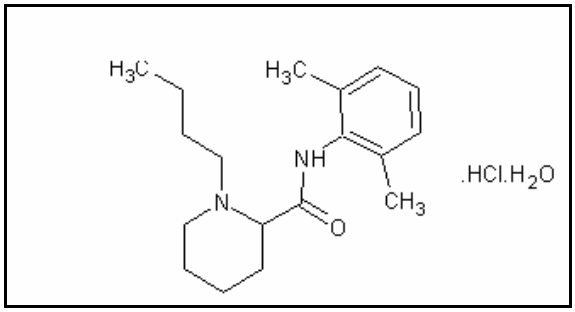

Figure-1 Bupivacaine Hydrochloride

Bupivacaine block the generation and the conduction of nerve impulses, presumably by increasing the threshold for electrical excitation in the nerve, by slowing the propagation of the nerve impulse, and by reducing the rate of rise of the action potential. Bupivacaine binds to the intracellular portion of sodium

channels and blocks sodium influx into nerve cells, which prevents depolarization. Few LC methods2-5 were reported in the literature for the analysis of Bupivacaine injection. Extensive literature survey reveals there is no method was reported for Quantification of sodium content in Bupivacaine Formulation. Hence, an attempt has been made to develop and accurate, rapid, specific and reproducible method for the Quantification of sodium content in Bupivacaine Formulation using Ion Chromatography along with method validation as per ICH norms.

Mention the full form of abbreviations when they appear for the first time in the text.

References need to be cited in the text as a superscript (Example: Miller *et al.*[1] studied the formation of amino acids by the action of electric discharges on a mixture of methane , nitrogen , and water with traces of ammonia [2,3,4]

Experimental

Material and Methods:

Sodium chloride (purity 99.9%) Sigma- Aldrich, ACS grade and Nitric acid suprapur® was purchased from Merck (Mumbai, India). Acetonitrile were purchased from Ranbaxy Chemicals, New Delhi, India. All chemicals were of HPLC grade and used as received. Water was purified by a milli-Q-water purification system (Millipore, Bedford, MA, USA) and used for preparation of all the solutions.

Equipment

A Metrohm Ion chromatography with conductivity detector having conductivity measuring ranges between 100µS/cm and 10.0mS/cm and full scale between 0.05mS/cm and 10.0ms/cm was used for method development. The output signal was monitored and processed using MagIC-Net software

Chromatographic Conditions

The Chromatographic column used was TSK GEL IC SW cation column (50 × 4.6 mm 5 µm) using Mobile phase consisting of 0.02N Nitric acid and Acetonitrile in the ratio of 100:1v/v, 0.02N Nitric acid was prepared by diluting about 1.3mL of Nitric acid in 1000mL of Milli-Q water. The flow rate of the mobile phase was 1.0 mL·min–1. The injection volume was 100µL with a run time of 10min.

Preparation of diluent, standard and sample solution

Diluent: The diluent used for the standard and sample preparation was 0.02N Nitric acid which was prepared by diluting about 1.3mL of Nitric acid in 1000mL of Milli-Q water

Standard:A stock solution of Sodium chloride (500 µg/ mL) was prepared by dissolving an appropriate amount in the diluent. Standard solution containing 5 µg/ mL was prepared from this stock solution.

Sample: 3mL of Bupivacaine injection USP solution containing sodium with claim of 3.36mg/ mL was dissolved in 50 mL of diluent. 5mL of the above solution was diluted to 200mL with diluent.

Results and Discussion

Analytical Method Validation

Method validation of Analytical procedures used in the testing of Drug substances and Finished products in Pharmaceutical companies, indicated that the Method validation shows an essential similarity in different laboratories there is much diversity in the detailed application of validation parameters applied to chromatographic procedures. Owing to increased interdependence among countries in recent times, it has become necessary for results of many analytical methods to be accepted internationally. Consequently, to assure a common level of quality, the need for use of validated methods has increased in the Pharmaceutical

Industry. According to FDA[6,7] and ICH [8,9], the key Analytical parameters that require validation were Accuracy, Precision, Linearity, Recovery, Limit of Detection, Limit of Quantification and Ruggedness.

Specificity:

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. A study was conducted to demonstrate the interference of placebo at the retention time of Sodium peak. Placebo solution was prepared as per the test method in triplicate and injected into ion chromatographic system, the chromatograms showed no interference of placebo peaks at the retention time of Sodium peak.(Figure 2.1 & 2.2)

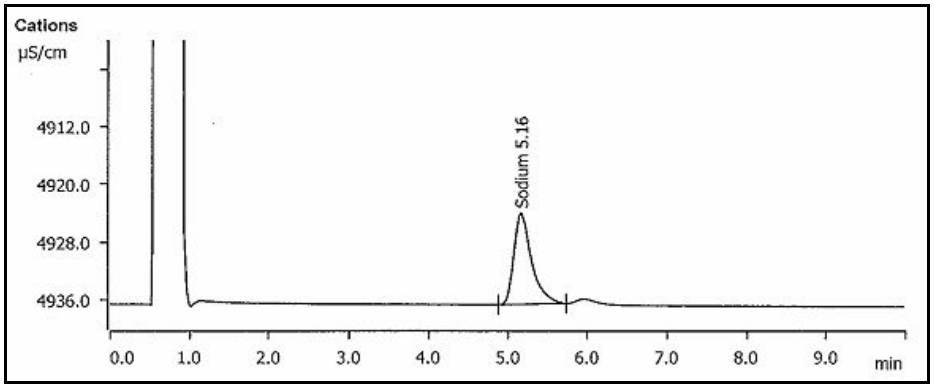

Figure 2.1. Typical chromatogram of sample

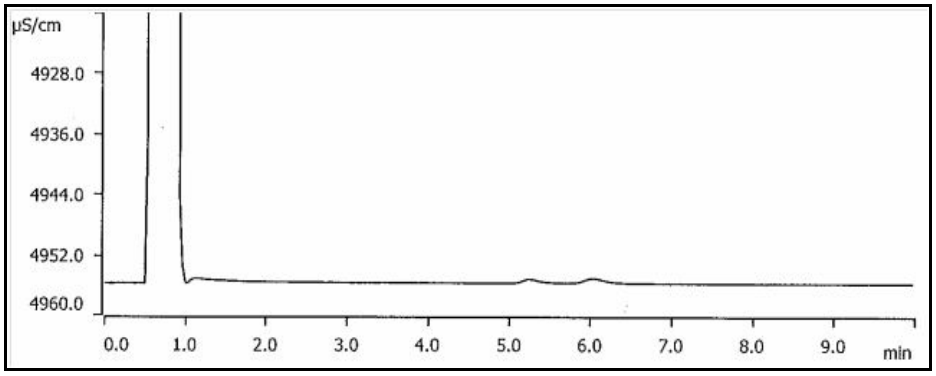

Figure 2.2. Typical chromatogram of Placebo

Precision

Method precision was evaluated by carrying out six independent test samples. The %RSD for percentage of each was calculated. Precision study was also determined by performing the same procedures on a different day (Inter-day precision). The intermediate precision (ruggedness) of the method was also evaluated by different analyst, different column and different instrument in the same laboratory.

The %RSD of Sodium in intraday precision study was within 2% and intermediate precision study was within 2.2% for Sodium, Conforming the good precision of the developed analytical method.

Linearity

Linearity of Detector Response:

Linearity of detector response for sodium was established by plotting a graph with concentration in µg/mL on X-axis and peak area of sodium on Y-axis. Evaluated the correlation coefficient and Y-intercept from the linearity graph. A series of solutions of Sodium in the concentration ranging from 25% to 150% of the target concentration of sodium (5ppm) were prepared and injected into the Ion chromatographic system. The detector

response was found to be linear with a correlation coefficient of 0.999 (Fig 3.1) and the results are summarized in table 1.1

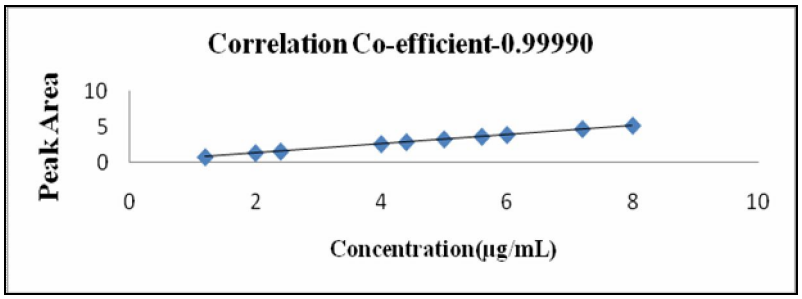

Fig 3.1. Linearity of detector response

Table-1.1 Linearity of detector response

S. No	Spike level	Sodium	
		Conc. µg/mL)	Area
01	25%	1.199	0.8028
02	40%	1.998	1.3755
03	50%	2.398	1.5804
04	80%	3.996	2.6333
05	90%	4.396	2.9027
06	100%	4.996	3.2971
07	110%	5.595	3.6631
08	120%	5.995	3.9061
09	140%	7.194	4.7334
10	150%	7.993	5.2064
r	0.99990		

Linearity of test method:

Linearity of test method was calculated by plotting graph of average ‘mg’ added versus average ‘mg’ recovered at levels of 25%, 50%, 85%, 100%,120% and 150% from accuracy section. The correlation coefficient and y-intercept were calculated from the linearity graph and found to be 0.9997 and 0.0862 respectively (Fig 3.2). The results are summarized in table 1.2

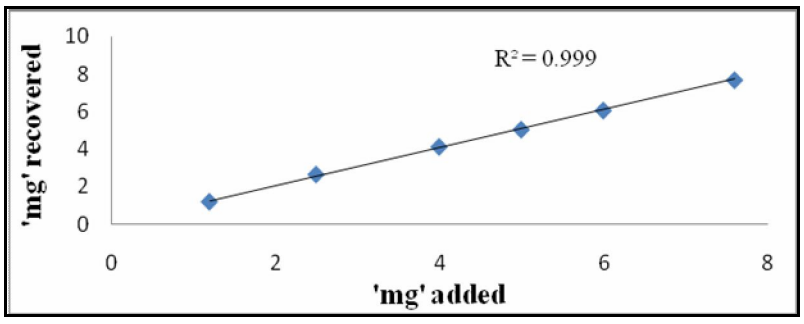

Fig 3.2. Linearity of test method

Table-1.2 Linearity of test method

%Spike level	'mg' added	'mg' recovered
25	1.1991	1.2136
50	2.4982	2.6663
80	3.9971	4.1345
100	4.9964	5.0533
120	5.9957	6.0671
150	7.5945	7.6831
r	0.9997	

Accuracy:

The accuracy of the method was evaluated in six replicates at six concentration levels *i.e.* 25, 50, 80, 100, 120 and 150 $\mu g \cdot mL^{-1}$ .The percentage of recovery was calculated at each level.

Recoveries of Sodium were found between 98.5% and 101.5%. The results are presented in Table 2.

Table-2:Results of accuracy

Recovery level	*µg/mL Added	*µg/mL recovered	%Recovery
25%	1.1991	1.2136	101.2
50%	2.4982	2.4932	99.8
80%	3.9971	3.9531	98.9
100%	4.9964	5.0533	101.1
120%	5.9957	6.0671	101.2
150%	7.5945	7.6831	101.2

*Mean of six replicates

Range of test method:

Based on the linearity, precision and accuracy data, the range of the method is from 25% to 150% of the test concentration.

Robustness:

Robustness of a method was defined as a measure of its capacity to remain unaffected by small, but deliberate changes in method parameters and provides an indication of its reliability during normal usage. The one, now days most widely applied in the Pharmaceutical world is the one given by the International Conference on Harmonization of Technical Requirements for the registration of Pharmaceuticals for human use [10] and which was given by the definition of Robustness of an analytical procedure. As per the ICH studies has performed with variation in mobile phase composition and flow rate. The results were found to be satisfactory and summarized in table 3

Table 3 Robustness study

Parameter		%RSD	%sodium content
Flow rate	0.8mL/min	0.6	101.1
	1.0mL/min	0.5	101.2
	1.2mL/min	0.3	103.1
Acetonitrile composition	90%	0.6	102.3
	100%	0.5	101.5
	110%	1.7	103.1

Stability of Solution:

Drug Stability was a function of storage conditions and chemical properties of the drug and its impurities. Conditions used in stability experiments should reflect situations likely to be encountered during actual sample handling and analysis. Stability data is required to show that the concentration and Purity of Analyte in the sample at the time of analysis corresponds to the concentration and Purity of Analyte at the time of sampling. The stability study of the analyte should be conducted at the temperatures, for example; Room temperature and Refrigerator conditions that will be experienced over the period needed to process a batch of study samples.

The solution stability and mobile phase stability experiments data confirmed that sample solution and mobile phase used during assay determination were stable up to the study period was 48 hours.

Conclusion:

A simple and efficient IC method was developed and validated; the method addressed each of the analytical validation characteristics such as linearity, accuracy, precision, stability, robustness and selectivity, and met the acceptance criteria defined in the guidance. The usefulness of this method is demonstrated by successful application for the quantification of sodium content in Bupivacaine formulations. The developed method is can be used for the routine analysis of production samples and quantification of sodium content in Bupivacaine formulations.

Acknowledgements

I would like to thank Vikram Kumar MJ for his unconditional support and encouragement.

References

1. Terry D. Wilson. Bupivacaine: Analytical profile of drug substances, 1990, 19; 59-94.
2. A. Koehler, R. Oertel, W. Kirch, Simultaneous determination of bupivacaine, mepivacain, prilocaine and ropivacain in human serum by liquid chromatography–tandem mass spectrometry. J. Chromatogr. A., 2005,1088: 126-130.
3. R.L.P. Lindberg, J.H. Kanto, K.K. Pihlajamaki, Simultaneous Determination of Bupivacaine And Its TwoMetabolites, Desbutyl- And 4'-Hydroxybupivacaine, InHuman Serum And Urine. J. Chromatogr. B., 1986, 383; 357-364
4. R.L.P. Lindberg, K.K. Pihlajamaki. High-performance liquid chromatographic determination of bupivacaine in human serum. J. Chromatogr., B, 1984, 309; 369 – 374.
5. MikołajPiekarski, Anna Jelin'ska, KamilSzymczak, Development and validation of an HPLC methodto determine the stability of fentanyl citrate andbupivacaine hydrochloride mixtures in infusionsolutions.Eur J Hosp Pharm., 2012,19; 447 -451.
6. FDA, Food and Drug Administration, Guidance for industry: Analytical Procedures and Methods Validation (1994).
7. FDA, Food and Drug Administration, Center of Drug Evolution and Research, Reviewer Guidance, Validation of Chromatographic Methods 1994.
8. ICH, International conference on harmonization, October 1994, Text on Validation of Analytical Procedures Q2A.
9. ICH, International conference on harmonization November 1996, Validation of Analytical Procedures methodology (Q2B).
10. ICH International conference on Harmonization October 1994, Harmonized Tripartite Guideline prepared with in the third international Conference on harmonization of Technical requirements for the registration of Pharmaceuticals for the human use: Text on Validation of analytical Procedures.

*****