VALIDATION OF ANALYTICAL METHOD OF ESTIMATION OF SODIUM IN API WITH THE HELP OF NMR SPECTROSCOPY

Partha Mukherjee*, Prasant K Deb, Kartikewa Dwivedi

Jubilant Chemsys Limited, B-34, Sector-58, Noida, Uttar Pradesh, India. Pin Code-201301

ABSTRACT

A simple, specific, sensitive method was developed and validated in NMR spectroscopy to know the percentage of sodium present as salt or as inorganic impurity in API and drug molecule. Unlike the Q-NMR studies where internal standards were mixed with the compound of interest, in this study standard and analyte solution were prepared separately and recorded in 400 MHz equipped with broadband probe. The method was validated by the recommendation mentioned in the ICH guidelines. Sodium chloride (NaCl) was taken as internal standard to draw the standard calibration curve (0.35 mg/ml -2.74 mg/ml). The concentration of the unknown solution was determined by comparing its integration value with the standard curve.

KEYWORDS: Q-NMR, internal standard.

1. INTRODUCTION

The existing methods for estimation of sodium in API includes flame photometry, Atomic absorption spectrophotometer, liquid chromatography (Patil 2005), UV-spectroscopy (Vaishnav 2012), titration and NMR (Lee 2002) etc. The present method is simple NMR spectroscopic technique to quantify sodium in API, organic and/or drug molecule.

In conventional quantitative NMR method internal standard and compound of interest were mixed together and proton NMR was taken with higher delay interval time. The assay of compound of interest could be found by comparing the integration value and weight of the molecule taken. This method was useful only when the signals of standard and compound of interest appeared in different positions. The problem associated with quantitative sodium estimation is that unlike 1H and 13C the range of sodium in NMR is too close to integrate

them separately (Table 1). That means if we mix sodium standard and compound of interest together and take a sodium NMR we would find only single or very close peaks (hard to integrate separately).

To overcome this issue stock solutions of NaCl were prepared in D2O (0.35 mg/ml -2.74 mg/ml) and Na-NMR spectra of each solution were taken. Then the peak of the spectra of the solution having smallest concentration was integrated and calibrated as 1. Then the spectra of the next higher concentration was opened and integrated by using the command "last Cal" in TOPSPIN software. By using the command this spectrum was integrated by using the calibrated value of initial spectra. The integration of other standard and unknown solutions was recorded in the similar way. After this, standard curve was drawn with help of integration area and concentration. Finally, concentration of unknown solution was determined by using standard equation.

2. MATERIALS AND METHOD

^{23}Na is a spin 3/2 nucleus and is therefore quadrupolar having 100% natural abundance. Sodium chloride (Aldrich-Fluka, AR grade >99%) was used as internal standard. Bruker Avance II 400 NMR spectrometer equipped with BBFO probe was used for analysis. The parameters of NMR experiments were depicted in Table 2.

Method validation: In practice, it is usually possible to design the experimental work such that the appropriate validation characteristics can be considered simultaneously to provide a sound, overall knowledge of the capabilities of the analytical procedure, for instance: specificity, linearity, range, accuracy and precision (ICH-2005). In the present study the linearity was studied by using NaCl solution in D2O having concentration range 0.35 mg/ml - 2.74 mg/ml (Table 3, Figure 1).

Accuracy and specificity: The accuracy of the method was determined by preparing solution of different concentrations, that is 80, 100, and 120%, in which the amount of benzoic acid was kept constant (1.2 mg) and the amount of NaCl was varied, that is, 0.96 mg, 1.2 mg, 1.44 mg for 80, 100, and 120%, respectively. The solutions were prepared in triplicate and the accuracy was described by % recovery in Table 4.

Precision: The precision of the method was demonstrated by intraday and inter-day variation studies. In the inter-day variation study, the solutions of same concentration (1.44 mg/ml)

were prepared and analyzed thrice, for three consecutive days, and the spectra were recorded [Table 5 and 6]. In the intra-day variation study, nine different solutions of the same concentrations (1.44 mg/ml) were prepared and analyzed thrice a day (morning, afternoon, and evening). The result was indicated by % RSD in Table 6.

Limit of Detection and Limit of quantitation: These parameters limit of detection and limit of quantitation were determined by using standard formulae (ICH 2005) and standard curve. Their values were 0.10 mg/ml and 0.31 mg/ml respectively.

Limit of detection= 3.3σ/S

Where σ = standard deviation of the lowest response

S = slope of the curve.

Putting values, the limit of detection of the method = 0.10 mg/ml

Limit of quantitation= 10σ/S

Putting values, the limit of detection of the method = 0.31 mg/ml

Robustness: The robustness of the method was determined by slight changing in NMR spectrometer parameters like delay time and temperature to see the deviation of the result. The parameters and associated results were tabulated in Table 7.

Ruggedness: The ruggedness of the method was determined by analysing same sample by two different analysts in different day (Table 8).

3. RESULTS AND DISCUSSION

The calibration curve of NaCl in D2O was found to be linear in the concentration range 0.35-2.74 mg/ml [Figure 1]. The developed method was found to be precise as the %RSD values for intra-day and inter-day were found to be less than 2%. The method was also found to be specific, indicated by the % recoveries ranging from 100.248 to 102.524%. The LOD and LOQ were found to be in the sub-microgram level, which indicates the sensitivity of the method. The method was also found to be rugged as indicated by the %RSD values, which were less than 2%. The result of the assay showed that the amount of drug was in good condition as API as indicated by the % recovery. The results obtained by the current method was also compared with the results of AAS analysis and found to be in good agreement

(Table 9). A summary of the validation parameters of the proposed spectrophotometric method is shown in Table 10.

Table 1: Chemical shift range of Sodium in various compounds

Nature of sodium	Chemical shift range (ppm)
Na+(aq)	-10 to 15
NaCl/NaOH	0
Organometallic Na	-2 to -15
Na*	-60

Table 2: Parameters of NMR spectra during current method

Parameter name	Acquisition time (sec)	Delay time (sec)	No. Of scan	Pulse length (μsec)	Power level (dB)	No. Of data point
Value	2.58	0.10	64	9.00	-2.00	32000

Table 3: Linearity in standard solution

Concentration of sodium (mg/ml)	Integration value	Standard deviation
0.35	0.97	0.09
0.68	2.13	0.05
1.38	3.99	0.04
2.09	5.96	0.01
2.74	7.97	0.02

Table 4: Determination of accuracy percentage by recovery method

Ingredient added	Amount added (mg)	Amount of sodium added (mg)	Level of addition (%)	Amount found by current method (mg)	% Recovery	Average recovery
Benzoic acid	1.2	0.96	80	0.98	102.08	
Benzoic acid	1.2	1.20	100	1.18	98.33	99.44%
Benzoic acid	1.2	1.44	120	1.41	97.91	

Table 5: Intra- day assay study

Concentration of Na	%RSD of spectra 1	% RSD of spectra 2	% RSD of spectra 3	Avg. RSD
1.44 mg/ml	1.01	0.87	0.95	0.94

Table 6: Inter- day assay study

Concentration of Na	%RSD of spectra 1	% RSD of spectra 2	% RSD of spectra 3	Avg. RSD
1.44 mg/ml	1.31	1.17	1.19	1.22

Table 7: Robustness study

Concentration of Na (mg/ml)	Results at Temperature at 298K	SD	Results at Temperature at 318K	SD	Results at D1=0.1 sec	SD	Results at D=0.5 sec	SD
1.44	1.41		1.45		1.42		1.43	
1.44	1.43	0.02	1.42	0.03	1.38	0.03	1.42	0.02
1.44	1.39		1.40		1.43		1.39	

Table 8: Ruggedness study

Concentration of Na (mg/ml)	Results by Analyst 1 on day 1	SD	Results by Analyst 1 on day 2	SD	Results by Analyst 2 on day 1	SD	Results by Analyst 1 on day 1	SD
1.44	1.46		1.45		1.42		1.49	
1.44	1.45	0.03	1.47	0.02	1.48	0.04	1.45	0.03
1.44	1.41		1.44		1.41		1.43	

Table 9: comparison of results of AAS and NMR

Sample name	% sodium obtained by AAS	% sodium obtained by NMR
API1	10.23	9.98
API2	12.45	12.17
API3	10.55	9.87

Table 10: Validation parameters

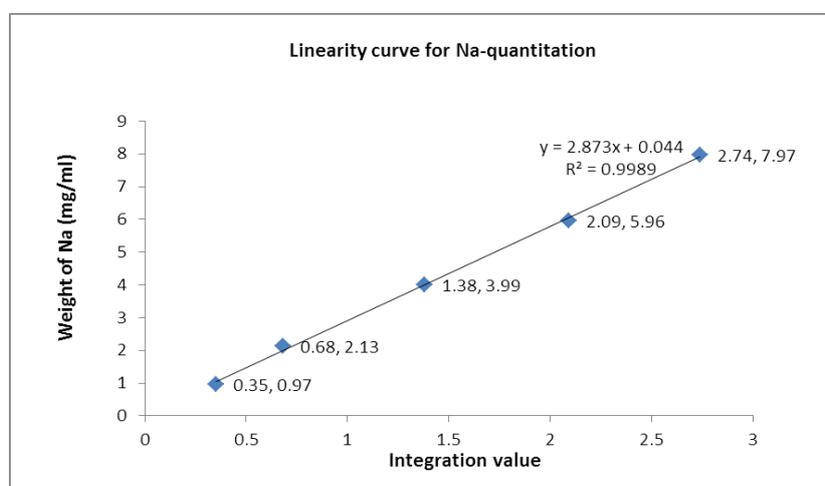| Name of the parameter	Results
Resonance frequency	105.84 MHz
Linearity range (mg/ml)	0.35-2.74
Standard regression equation	y = 2.873x + 0.044
Correlation coefficient	$R^2 = 0.9989$
Accuracy (%Recovery)	99.44
LOD	0.10 mg/ml
LOQ	0.31 mg/ml

Figure 1: Linearity curve for Na-quantitaion.

4. CONCLUSION

By studying various parameters finally we conclude that the proposed method is accurate, precise, simple, robust and can be applied successfully for the estimation of sodium in both API and small organic compound.

REFERENCES

1. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. 4th version 2005.

2. Lim, S.H., Haan, G.C., Lee, S.G. 2002. Quantitative Elemental Analysis of Sodium (23Na) by NMR Spectroscopy. Bull. Korean Chem. Soc. 2002; 23(11).

3. Patil, K.M., S.L. Bodhankar. 2005. Validated high performance thin layer chromatography method for simultaneous estimation of phenytoin sodium, phenol barbitone sodium and carbamazepine in tablet dosage forms. Indian Journal of Pharmaceutical Sciences. 67: 351-355.

4. Vaishnav, D., Mulsa, N., Sanghvi, G., Purohit, P., Navin, S. 2013. Development of the UV Spectrophotometric Method of Phenytoin Sodium in API and Stress Degradation Studies. Inventi Rapid: Pharm Analysis & Quality Assurance. 1: 1-5.