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# Reversed Phase High Presssure Liquid Chromatograhphic Technique for Determination of Sodium Alginate from Oral Suspension

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**Abstract:** Rapid and accurate high performance liquid chromatography method is described for determination of Sodium alginate from the oral suspension. The separation of drug was achieved on Primecep C (150 x 4.6 mm i.d.) 5µ column. The mobile phase consisted of 40 milli molar anhydrous dibasic sodium phosphate with the pH of the solution adjusted to 6.0 with dilute sodium hydroxide. The detection was carried out at wavelength 210 nm. The Primecep C column showed the most favorable chromatographic parameters for analysis. The method was validated for System suitability, linearity, accuracy, precision, robustness and stability of sample solution. The linear range for sodium alginate was 206.9 – 620.9 µg/ml. The method has been successfully used to analyze commercial oral suspension containing sodium alginate with good recovery.

**Keywords:** Sodium alginate,Reverse phase HPLC,Pharmaceutical dosage form.

## Introduction

In this communication the present work proposes a new HPLC method for the assay of Sodium alginate from oral suspension. Sodium alginate is a sodium salt of alginic acid. It is used as emulsifier in pharmaceutical dosage form like oral suspension. It is a linear copolymer anionic polysaccharide with homopolymeric blocks of (1-4)-linked β-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G) residues, respectively, covalently linked together in different sequences or blocks. Sodium alginate is official in USP[1] and describes titrimetric assay method with sodium hydroxide. It is also official in IP[2] and BP[3]. In this communication a new, simple, rapid and reliable HPLC method is developed for determination of sodium alginate in oral suspension. This method can be used for the routine analysis of formulation in quality control laboratories. In the proposed work, optimization and validation of this method are reported.

## Experimental

### Materials

Reference standard of sodium alginate was obtained from reputed firms with certificate of analysis. Anhydrous dibasic sodium phosphate was used of analytical grade from Merck and the HPLC grade water was obtained using Millipore water system. Standard solutions were prepared in diluent (i.e. mobile phase consisting of 40 milli molar anhydrous dibasic sodium phosphate with pH of the solution adjusted to 6.0 with sodium hydroxide).

### Instrumentation

The HPLC system, Water's Alliance (2695) HPLC system equipped with separation module and DAD detector (2996), was used. The chromatogram was recorded and peaks quantified by means of PC based Empower 2 software.

**Preparation of standard solution**

**Standard stock solution**

About 50 mg of sodium alginate was weighed and transferred to 50 ml volumetric flask. About 30 ml of diluent was added to it and sonicated for 15 minutes. The volume was adjusted up to the mark with diluent to give concentration as 1000 µg / ml. Further 4 ml of this solution was diluted to 10 ml with diluent to give 400 µg/ml of sodium alginate.

**Sample Preparation**

About 5gm i.e. equivalent to 200 mg of sodium alginate of sample was accurately weighed in 100 ml volumetric flask, about 70 ml of diluent was added to it and sonicated for 15 minutes to dissolve it. Further the volume was made upto the mark with diluent to give 2000 µg /ml of sodium alginate. Further 5 ml of this solution was diluted to 25 ml with diluent to give 400 µg / ml of sodium alginate.

**Chromatographic Conditions**

Chromatographic separation was performed at ambient temperature on a reverse phase Primecep C (150 x 4.6 mm i.d.) 5µ column. Mobile phase was made of 40 milli molar anhydrous dibasic sodium phosphate with pH of the solution adjusted to 6.0 with sodium hydroxide. The mobile phase was filtered and degassed before use. The flow rate of the mobile phase was adjusted to 0.25 ml/min. The detector wavelength was set at 210 nm. The injection volume of the standard and sample solution was set at 10 µl.

**Method Development**

A reverse phase approach was used for estimation of sodium alginate by HPLC-DAD. The UV spectra of sodium alginate showed absorbance maxima at 210 nm. (Fig. no.1) Different polymer columns like Hypersil (Hypercarb 150 x 4.6 mm, 5 µ), Waters (SAX 250 x 4.6 mm, 10 µ) were tried for estimating sodium alginate. Finally Primecep C (150 µm x 4.6mm x 3µ) column offered more advantage over the other columns with diode array detector. The mobile phase consisted of 20 milli molar anhydrous dibasic sodium phosphate buffer with pH of 6.0. The detector wavelength was selected at 210 nm with flow rate of 0.5ml / min. It was found that a good symmetrical peak observed at about 2.1 minutes for standard solution of sodium alginate. The typical chromatograms of the standard and sample assayed are given in figure.2 and 3 respectively. The relative chromatographic figures of merit are reported in table 1.

## Results and Discussion

The results of analysis shows that the amount of the drug was in good agreement with the label claim of the formulation. The developed RP-HPLC method was validated as per ICH guidelines for parameters like system suitability, specificity, linearity, accuracy, precision, robustness and stability of the sample solution.

**Method validation**

**System suitability**

System performance parameters of developed HPLC method were determined by injecting standard solutions. Parameters such as number of theoretical plates (N), tailing factor, resolution (R), capacity factor (K') and relative standard deviation were determined. The results are shown in table 1 which indicates good performance of the system.

**Specificity**

Specificity is the ability of the method to resolve the active ingredient from the other inactive ingredients. Hence blank (diluent) placebo, sodium alginate were injected to prove specificity. The typical chromatograms of the standard and sample assayed are given in figure.2 and 3 respectively.

**Linearity**

Under the experimental conditions described above, linear calibration curve were obtained throughout the concentration ranges studied. Regression analysis was done on the peak area (y) v/s concentration (x).The regression analysis data obtained is tabulated in table 2. The linear range was found to be 206.9 – 620.6 µg/ml.

**Accuracy**

The accuracy of the method was determined by applying the proposed method to synthetic mixture containing known amount of drug corresponding to 50%, 100% and 150% of the label claim. The accuracy was then calculated as the percentage of analyte recovered by the assay. The results of the recovery analysis are enclosed under table 3.

**Precision**

The method precision was established by carrying out the analysis of the oral suspension containing sodium alginate. The assay was carried out of the drug using proposed analytical method in six replicates. The value of the relative standard deviation lies well with in the limits (0.44%). The results of the same are tabulated in table-4.

**Robustness**

The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters.

The typical variations are given below:

Variation in the flow rate by + 0.25 ml/min

Variation in wavelength by ± 2 nm
Variation in extraction time ± 15 minutes
The results of the analysis of the samples under the conditions of the above variation indicated the nature of robustness of the method.

**Stability of solution**
The stability of the solutions under study was established by keeping the solutions at room temperature for 24 hours. The results indicated no significant change in the assay results of the solutions. It confirmed the stability of the drug in the solvents used for the analysis.

**Method application**
About 5gm i.e. equivalent to 200 mg of sodium alginate of sample was accurately weighed in 100 ml volumetric flask, about 70 ml of diluent was added to it and sonicated for 15 minutes to dissolve it. Further the volume was made up to the mark with diluent. to give 2000 µg /ml of sodium alginate. Further 5 ml of this solution was diluted to 25 ml with diluent to give 400 µg / ml of sodium alginate. From this solution 10 µl was injected into chromatograph under specific conditions. The analyte peak was identified by comparison with that of respective standard. The percentage (%) assay results were expressed in table no.4. it indicates the amount of sodium alginate in the product meets the requirement.

**Table 1. System suitability parameters evaluated on standard solution**

| Retention Time | Area | % Area | USP Plate count | USP Tailing | Capacity factor |
|---|---|---|---|---|---|
| 4.06 minutes | 334559 | 100.0 | 911 | 1.58 | 39.6 |

**Table 2. Statistical evaluation of the test data subjected to regression analysis**

| **Parameters** | **Sodium Alginate** |
|---|---|
| **Correlation Coefficient (r)** | 0.9999 |
| **% Intercept (y)** | -0.98 % |
| **Slope (m)** | 817 |

**Table 3. Statistical evaluation of the test data subjected to accuracy**

| **Level (%)** | **Amount of drug added(mg)** | **Amount of drug recovered (mg)** | **% Recovery** | **% Error** | **% RSD** |
|---|---|---|---|---|---|
| 50 | 187.64 | 186.18 | 99.2 | 0.78 | 0.43 |
| 100 | 392.02 | 388.62 | 99.1 | 0.87 | |
| 150 | 593.27 | 586.07 | 98.8 | 1.21 | |

**Table 4. Statistical evaluation of the test data subjected to method precision**

| **Sample Number** | **Weight of the sample** | **% Assay** |
|---|---|---|
| 1 | 5.0239 | 99.2 |
| 2 | 5.1233 | 100.0 |
| 3 | 5.1364 | 100.5 |
| 4 | 5.1389 | 100.0 |
| 5 | 5.0947 | 99.8 |
| 6 | 5.1126 | 99.6 |
| Mean | | 99.8 |
| Standard Deviation | | 0.44 |
| % RSD | | 0.44 |

**Figure.1 UV spectra of sodium alginate**

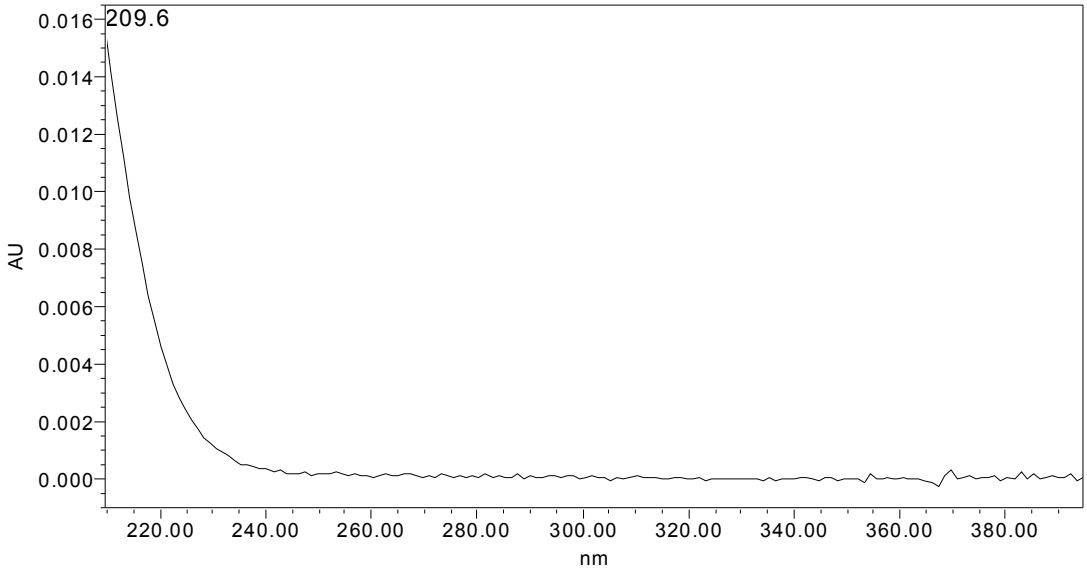



**Figure. 2 Typical standard chromatogram of sodium alginate**

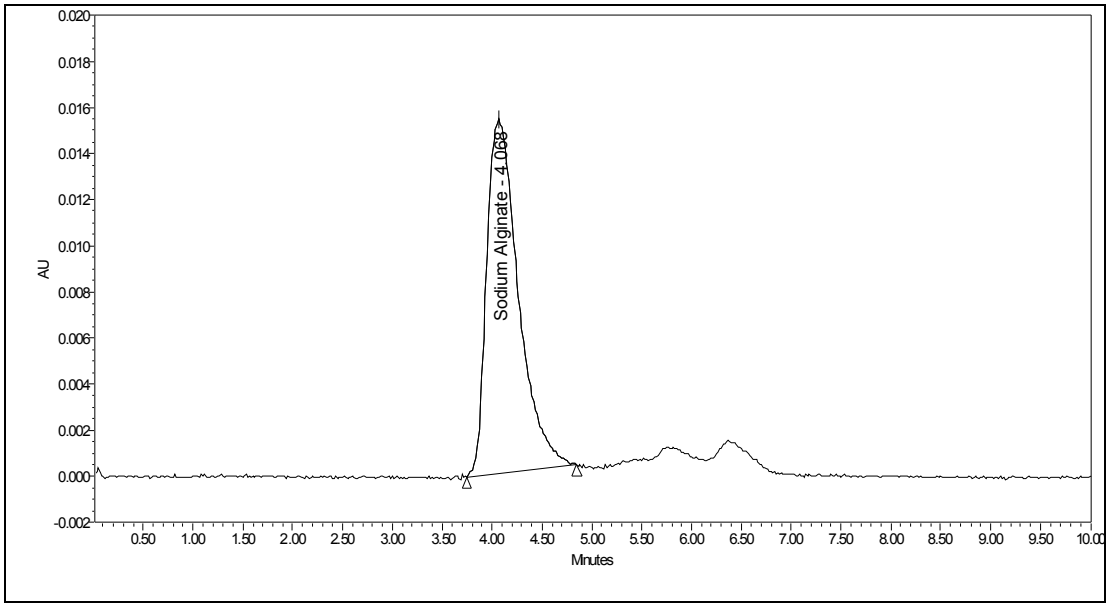
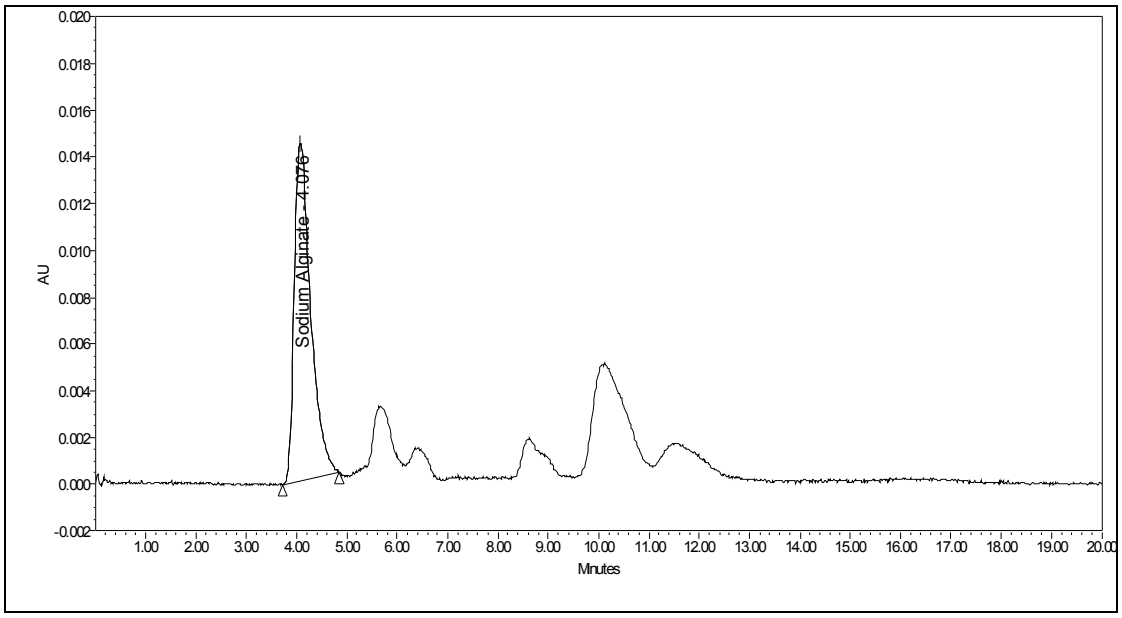


**Figure. 3 Typical standard chromatogram of sample**




## Conclusion

The reproducibility, repeatability and accuracy of the proposed method was found to be satisfactory which is evidenced by low values of standard deviation and percent relative standard deviation. The accuracy and reproducibility of the proposed method was confirmed by recovery experiments, performed by adding known amount of the drugs to the pre-analyzed formulation and reanalyzing the mixture by proposed method. The percent recovery indicated non- interference from the placebo used in the formulations. Thus the proposed RP-HPLC method is novel method for the estimation of sodium alginate from pharmaceutical dosage forms such as oral suspension. It is precise, accurate, linear, robust, simple and rapid. Hence the proposed RP-HPLC method is strongly recommended for the quality control of the raw material and formulations.

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