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Research Article

A SIMPLE RP-HPLC METHOD DEVELOPMENT AND VERIFICATION FOR THE QUANTITATIVE ESTIMATION OF SODIUM BUTYRATE IN TABLETS

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ABSTRACT

Objective: To develop a simple, accurate, precise, and linear reverse-phase high-performance liquid chromatographic (RP-HPLC) method and verify the quantitative estimation (assay) of sodium butyrate in tablets.

Methods: The optimized RP-HPLC method uses a reverse phase stationary phase which is YMC Triart C18 column, having specifications of 250×4.6 mm; 5 µm, a mobile phase composition of pH 8.0 sodium dihydrogen phosphate buffer and acetonitrile in the proportion of 92:8 v/v, flow rate of 0.5 ml/min, injection volume of 10 µL, and detection wavelength of 210 nm using a UV/PDA detector.

Results: The developed method gave sodium butyrate eluting at about 6 min. Sodium butyrate exhibited linearity in the range of 119.56–1195.6 µg/ml. The precision was exemplified by a relative standard deviation of 0.30%. The percentage of individual recovery was found to be in the range of 97.0 and 103.0 during accuracy studies.

Conclusion: A simple, specific, accurate, precise, and linear RP-HPLC method was developed and verified for the quantitative estimation (assay) of sodium butyrate in tablets and hence this method can be explored for the analysis of sodium butyrate in tablets in various pharmaceutical industries.

Keywords: Reverse-phase high-performance liquid chromatographic, Sodium butyrate, Analytical method development, Tablets.

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INTRODUCTION

Sodium butyrate is the sodium salt of butyric acid (Fig. 1), which exhibits various properties such as immune system modulator, antioxidant, and anti-inflammatory properties [1]. Furthermore, sodium butyrate is used as a promoter of growth in milk replacer formula for young calves [2].

Only two articles exist regarding the analysis of sodium butyrate in the literature in various matrices [3,4]. Therefore, we have focused on developing a reverse-phase high-performance liquid chromatographic (RP-HPLC) method, using a simple isocratic elution technique, mobile phase, and diluent in a better combination and a simple sample extraction procedure for achieving a reproducible assay result. We present here only the analytical work developed and verified on quantitative estimation of sodium butyrate (assay) in tablets, whose label claim is 600 mg. The developed analytical method is a simple and a sensitive RP-HPLC method, which can be explored for routine analysis in various pharmaceutical industries.

METHODS

Chemicals and reagents

Analytically pure sample of sodium butyrate with purities greater than 95% was obtained from Sigma-Aldrich and tablet formulation was prepared in our Formulation R&D laboratory, with a labeled amount of 600 mg of sodium butyrate. Acetonitrile (HPLC grade of Standard make), sodium dihydrogen phosphate monohydrate (Merck make-Emsure), sodium hydroxide pellets (Extra pure grade), orthophosphoric acid (Finar HPLC grade), and water (MilliQ) were used for the analysis.

Instrument

HPLC analysis was performed on Agilent and waters makes HPLC's having UV/PDA detectors capable of setting a detection wavelength of 210 nm. Stationary phase used in the developed RP-HPLC method is a reverse-phase C18 column-YMC Triart C18 column of specifications-250×4.6 mm; 5 µm, and part number TA12S05-2546WT. The HPLC system was controlled with "EMPOWER" software. An electronic analytical weighing balance (0.1 mg sensitivity, Sartorius make, ME5 model) and a sonicator (Hwashin Make, Powersonic 420 model) were used for the analysis.

Selection of wavelength

The suitable wavelength for the HPLC analysis for sodium butyrate was determined by recording the UV spectrum in the range of 200–400 nm. The suitable wavelength selected was 210 nm considering the maximum absorbance (Fig. 2).

Chromatographic conditions

The developed RP-HPLC method uses a stationary phase which is a reverse-phase C18 column- YMC Triart C18 column, whose specifications are 250×4.6 mm; 5 µm, part number TA12S05-2546WT, a mobile phase composition of pH 8.0 sodium dihydrogen phosphate buffer and acetonitrile in the proportion of 92:8 v/v, flow rate of 0.5 ml/min, injection volume of 10 µL, detection wavelength of 210 nm using a UV/PDA detector, setting column temperature and sample compartment temperature of 20°C and ambient, respectively, and run time as 12 min.

Reagents solution preparation

Preparation of dilute orthophosphoric acid: Dilute 10 mL of 85% orthophosphoric acid to 100 mL with Milli Q water.

Preparation of sodium hydroxide solution (2N): Transfer 8.0 g of sodium hydroxide pellets into a 100 mL volumetric flask, dissolve, and dilute to volume with Milli Q water.

Preparation of pH 8.0 Sodium dihydrogen phosphate buffer (0.025M): Weigh about 3.45 g of sodium dihydrogen phosphate monohydrate into 1 L of Milli Q water and dissolve by sonication. Adjust the pH to 8.0 using 2N sodium hydroxide or dilute orthophosphoric acid. Filter through 0.45 µm nylon membrane filter. Degas by sonication for 10 min.

Mobile phase preparation

The mobile phase was prepared by mixing pH 8.0 sodium dihydrogen phosphate buffer and acetonitrile in the proportion of 92:8 v/v, respectively, followed by degassing in a sonicator for 10 min.

Diluent preparation

The diluent solution was prepared by mixing pH 8.0 sodium dihydrogen phosphate buffer and acetonitrile in the proportion of 92:8 v/v, respectively, followed by degassing in a sonicator for 10 min.

Preparation of stock and working standard solution

Weigh accurately about 60 mg of sodium butyrate standard into a clean and dried 100 ml volumetric flask. Later add 70 mL of the diluent and sonicate to dissolve. Dilute to volume with the diluent and mix well to get a working standard concentration of about 600 µg/mL.

Preparation of stock and working sample solution

Drop the 5 doses of tablets into a clean and dry 1000 mL volumetric flask. Add 750 mL of the diluent and then stir on a magnetic stirrer for 60 min at 1000 rpm. Later remove the magnetic bead using a magnetic retriever and ensure to rinse the magnetic bead with about 10 mL of diluent into the volumetric flask, to avoid losses, and later sonicate for 30 min (maintain temperature of water in sonicator at 25°C) with intermittent shaking. Dilute to volume with diluent and mix well. Centrifuge a portion of the sample solution at 3000 rpm for 10 min. Pipette 5.0 mL of supernatant and transfer into a clean and dry 25 mL volumetric flask, dilute to volume with diluent, and mix well.

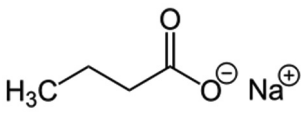

Fig. 1: Structure of sodium butyrate

RESULTS AND DISCUSSION

Method development

A reverse-phase HPLC method chromatographic conditions were developed keeping in mind the system suitability parameters, i.e. tailing factor (T), % RSD from six replicate injections of standard and blank interference along with simple sample extraction procedure in sample preparation and run time. The optimized method developed resulted in the elution of sodium butyrate at about 6.0 min. Figs. 3-6 represent specimen chromatograms of blank, placebo, standard, and sample solutions. The total run time was 12 min. System suitability tests were an integral part of method development and were used to ensure adequate performance of the chromatographic system, whose details are summarized in Tables 1a and b.

To evaluate the applicability of the developed method to a formulation, sodium butyrate tablets were chromatographed at a concentration of about 600 µg/ml, and it is shown in Fig. 6. The sample peak was identified by comparing the retention time with the standard drug Fig. 5. To ensure the RP-HPLC method developed is specific, precise and accurate, method was verified whose details are mentioned in the below section.

Calculations

The assay of sodium butyrate was calculated using the below formula.

$$\text{Tablets Assay, Percent Label Claim} = \frac{A_T}{A_S} \times \frac{W_S}{100} \times \frac{1000}{5} \times \frac{25}{5} \times \frac{P}{100} \times \frac{100}{L}$$

Where,

A_T is the peak area of sodium butyrate in the sample solution.

A_S is the average peak area of sodium butyrate from six replicate injections of standard solution A injected under system suitability.

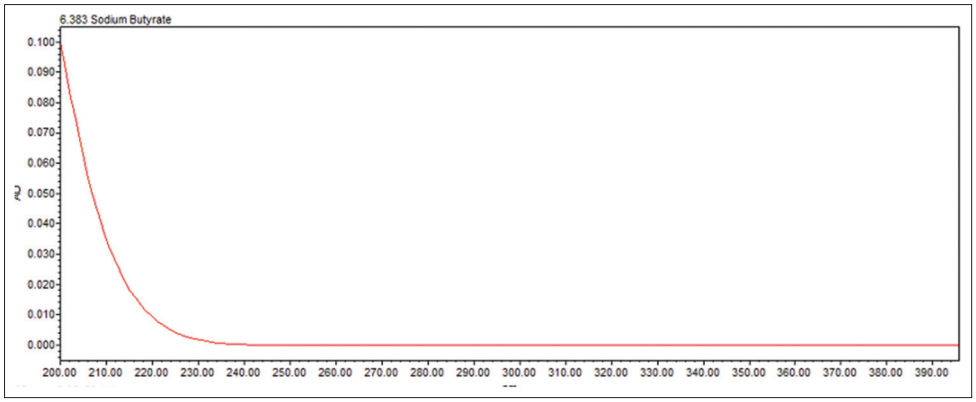

Fig. 2: UV spectrum of sodium butyrate

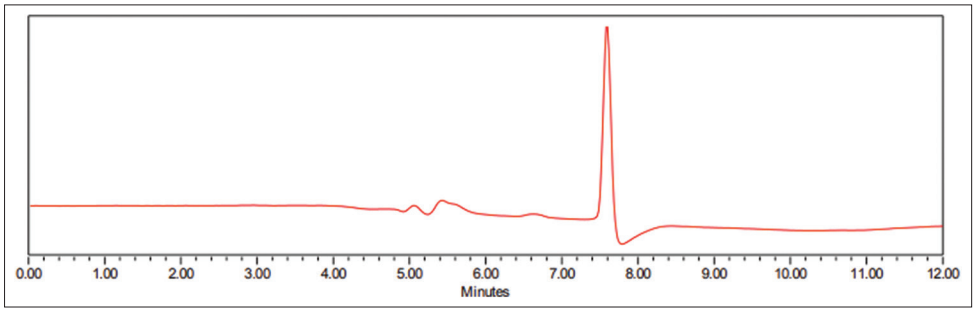

Fig. 3: Typical chromatogram of blank solution

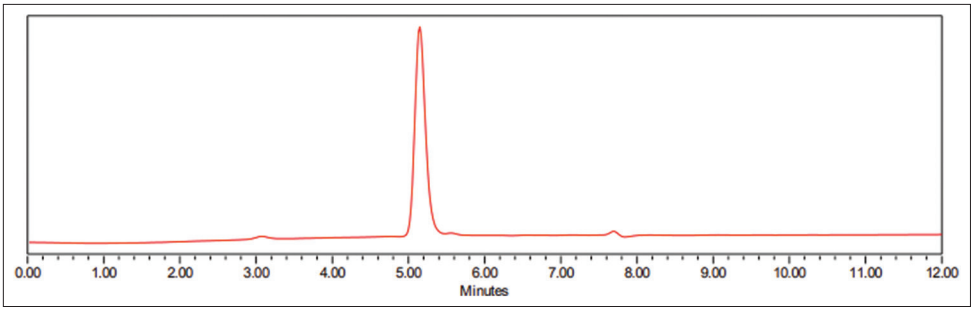

Fig. 4: Typical chromatogram of the Placebo solution

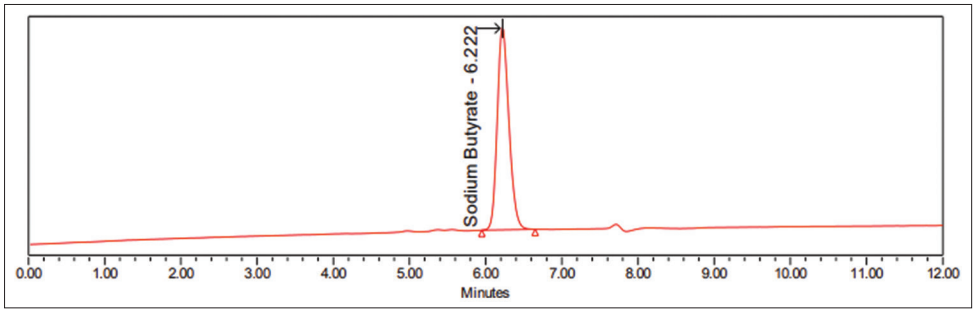

Fig. 5: Typical chromatogram of the standard solution

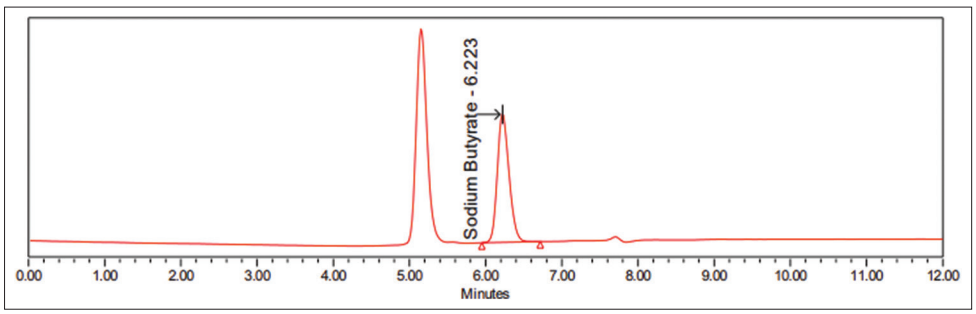

Fig. 6: Typical chromatogram of the sample solution

W_S is the weight of sodium butyrate standard taken, in mg for the preparation of standard solution
P is the potency of sodium butyrate standard
L is the label claim of sodium butyrate in mg, 600 mg

Method verification
Assay method verification of the analytical method for sodium butyrate in tablets was done for the parameters such as system suitability, specificity, linearity, accuracy, and precision.

Specificity (placebo interference)
To establish non-interference, blank and placebo solutions were prepared and injected into HPLC along with standard and sample solutions (Refer Figs. 3-6). Figures 3-6 reveal that the peak obtained in the standard solution and sample solution was only because of the drug as blank and placebo had no peak at the retention time of sodium butyrate standard. Accordingly, it can be concluded that the method developed is said to be specific.

Method precision
Method precision was determined by performing an assay of the sample by spiking API to the placebo as per the sample preparation at 100% level. The percentage assay of each replicate, average of three replicates, and % RSD were calculated. The % relative standard deviation (RSD) of the assay of sodium butyrate was not more than 2.0 and, hence, can be concluded that the method is precise by the test of repeatability and can be understood that the method gives consistently reproducible results (Table 2).

Table 1a: System suitability acceptance criteria

Parameters	Acceptance criteria
Blank interference	No peak shall be observed in the blank at the retention time of sodium butyrate.
Tailing factor (T)	The tailing factor for the sodium butyrate peak shall not be more than 2.0 from the first injection of the Standard solution.
% RSD (relative standard deviation)	The percentage RSD of the average peak area response from six injections of sodium butyrate shall be NMT 3.0%.

Table 1b: System suitability results

Parameters	Sodium butyrate
Blank interference	Nil
Tailing factor (T)	1.21
% RSD (relative standard deviation)	0.21

Accuracy
Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of the sample at two different levels

Table 2: Method Precision results

Sample no. (n)	% Assay
1	101.3
2	101.5
3	100.9
Average	101.2
%RSD	0.3

Table 3: Results of accuracy studies

% Level	% Individual Recovery	% Mean recovery
100	101.3	101.2
	101.5	
	100.9	
150	101.0	100.8
	100.4	
	101.1	

Table 4: Linearity data

% Level	Concentration (g/ml)	Peak area
20	119.564	100182
50	298.910	252555
80	478.256	404258
100	597.819	501888
120	747.274	625652
150	896.729	752814
200	1195.639	998248
Regression/ Correlation coefficient		0.99998
Bias		0.56
Regression equation		y=834.25x+2828.53

(100 and 150%). At each level, three determinations were performed. Individual and percentage mean recovery were calculated as shown in Table 3. All observed data were within the required range of 97.0–103.0, which indicates good recovery values and hence the accuracy of the method developed.

Linearity

Standard solutions of sodium butyrate at different concentrations level (20%, 50%, 80%, 100%, 120%, 150%, and 200%) were prepared. The calibration curve was constructed by plotting the concentration level of the drug versus the corresponding peak area. The results showed an excellent correlation between the peak area and concentration level of the drug within the concentration range (119.564–1195.639 µg/ml) for the drug, and the results are given in Table 4 and Fig. 7. The correlation coefficient of sodium butyrate was 0.99998. The results revealed that the method is linear for the quantification of sodium butyrate in the proposed range.

CONCLUSION

A reverse-phase HPLC isocratic method developed was verified in terms of specificity, accuracy, precision, and linearity for the quantitative estimation (assay) of sodium butyrate in tablets. The precision was exemplified by a relative standard deviation of 0.3%. A good linear relationship was observed for the drug between concentration ranges of 119.564 and 1195.639 µg/ml. Accuracy studies revealed that mean recoveries were between 97.0 and 103.0%, an indicative of an accurate method. Accordingly, it can be concluded that the developed reverse-phase isocratic HPLC method is specific, accurate, precise, and linear, and therefore, the method can be explored for the routine analysis of sodium butyrate in tablets.

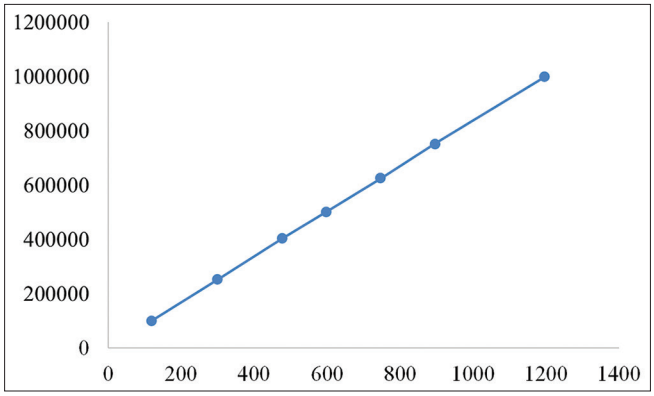

Fig. 7: Linearity graph

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AUTHORS CONTRIBUTIONS

Bembadi Mukund reddy, Bheemi reddy, and Ashok reddy have conducted the method development and method verification activities. Ravinder kodipyaka has provided valuable suggestions during analytical method development. Dr. Rajesh vooturi has reviewed the manuscript and provided valuable suggestions in drafting the manuscript.

CONFLICTS OF INTERESTS

Nil.

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