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Sodium alginate and pectin estimation in raft forming pharmaceuticals by high performance liquid chromatography method

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ABSTRACT

The high performance liquid chromatographic (HPLC) method was developed for the combined estimation of sodium alginate and pectin in raft forming pharmaceuticals on C₁₈ column ZORBAX ODS (1.5 cm × 4.6 mm, 5 μm) with UV detection at 378 nm. The assay condition comprised of phosphate buffer pH 7.4 and methanol 60:40% v/v at a flow rate of 1.25 mL/min. The separation of sodium alginate and pectin with good resolution and a retention time less than 8 min was attained. The method was linear over a range of 200–800 μg/mL of sodium alginate and pectin. The regression values obtained from linearity curve of sodium alginate and pectin were 0.9993 and 0.9991, respectively. The retention time of sodium alginate and pectin was 3.931 and 7.470 min, respectively. The percent recovery of sodium alginate and pectin ranged from 94.2–98.5% and 92.1–98.4% respectively. The limit of detection (LOD) and limit of quantification (LOQ) of sodium alginate were found to be 2.443 and 3.129 μg/mL and the LOD and LOQ of pectin were 3.126 and 3.785 μg/mL, respectively. The resolution of sodium alginate and pectin was found in the range of 1.03–1.89 and 1.10–1.91, respectively. This method has been successfully applied to analyze the concentrations of sodium alginate and pectin in raft forming drug delivery systems.

KEYWORDS

Sodium alginate, pectin, raft, HPLC

INTRODUCTION

Nowadays, polysaccharide based advanced and innovative pharmaceutical materials are preferred because of their natural abundance, easy accessibility, low cost, biodegradability, nontoxicity and furthermore, their sustainable nature has increased their requisition. The raft forming delivery system can incorporate a variety of biopolymers including sodium alginate and pectin etc. The physicochemical properties of sodium alginate, the sodium salt of alginic acid, indicate that this natural macromolecule is biodegradable, nontoxic and pH sensitive in

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nature; in addition, the pH sensitivity of the polymer makes it stable only in an acidic environment, while in alkaline media, rapid dissolution occurs, which leads to instability and limits its applications. Sodium alginate swells in water but typically produces an acid pH gel due to its protonation and can be crosslinked by the application of physical and chemical methods [1]. The chemical structure of alginate, as mentioned in Fig. 1, has a linear copolymer of 1, 4-glycosidically linked β -D-mannuronic acid and α -L-guluronic acid. Pectin, illustrated in Fig. 1, is an abundantly available natural polysaccharide generally present in the cell wall of plants, while its structure is composed of uronic acid residues which are linked together by α -1, 4-glycosidic bonds [2]. The pectin is favored in formulations due to its resistance property against certain enzymes such as amylase and protease but a disadvantage of the early drug release due to its hydrophilic nature cannot be overlooked. On the other hand, in contact with divalent cations, the pectin forms a gels radially.

The sodium alginate and pectin are extensively used in the raft forming formulations used for the management of reflux disorders of stomach. The stomach contents irritate the esophagus and raft prevent the entry of stomach contents into esophagus. The sodium alginate and pectin concentrations are important for the strength and resilience of raft that is developed in the stomach. Only one high performance liquid chromatographic (HPLC) method was available for the determination of sodium alginate [3] in drug delivery systems contained sodium alginate as polymeric material. To our best knowledge no method available for the combined estimation of alginate and pectin in raft forming pharmaceuticals.

MATERIALS AND METHODS

Materials

Sodium alginate and pectin were purchased from Sigma Aldrich Chemie GmbH, Germany. Methanol and acetonitrile of HPLC grade were purchased from Sigma Aldrich Chemie GmbH, Germany. Potassium dihydrogen phosphate and glacial acetic acid were purchased from BDH Wharf Road, London. The double distilled water was used throughout the

study and the other chemicals/reagents used were of analytical grade.

Instrumentation

HPLC system (PerkinElmer 710 Bridgeport avenue Shelton, Connecticut USA) fitted out with C_{18} column ZORBAX ODS (1.5 cm $\times$ 4.6 mm, 5 μ m) Agilent technologies Santa Clara, California, United States. Analytical weighing balance model CB 6698, Sartorius Stedim, Germany, and pH meter model 370 Jenway UK. Cellulose acetate and cellulose nitrate filters 0.45 μ m Sartorius Stedim, Germany, used for filtration of solvents using filtration assembly (Sartorius Stedim, Germany). Membrane disc filters 0.22 μ m Millipore Bedford MA, United States.

Preparation of mobile phases and chromatographic conditions

The mobile phase was prepared by using methanol and phosphate buffer pH 7.4 (40:60% v/v). A 378 nm wavelength was selected as it gave reasonably high absorption for sodium alginate and pectin when used in proportions usually found in developed formulation. Using a UV detector at 378 nm, the chromatographic conditions allow a resolution between sodium alginate and pectin with a reasonable time. A detection wavelength of 378 nm for sodium alginate and pectin were selected based on the full-range UV spectral data due to its high sensitivity for all related substances and a minimal difference in response factors. The mobile phase and sample solution were degassed by using sonicator (Branson Ultrasonic, USA) and filtered through 0.22 μ m filter. The flow rate of mobile phase was 1.25 mL/min and detection of polymers were made at 378 nm. Before injection of each sample solution, the HPLC system was equilibrated for 30 min with the mobile phase flowing through the system.

Standard solutions

The primary stock standard solutions of 1,000 μ g/mL sodium alginate and 1,000 μ g/mL pectin were prepared in the mobile phase (methanol and phosphate buffer pH 7.4, 40:60% v/v) and protected from light. The working solutions were prepared by diluting aliquots of stock solutions with mobile phase at 100, 200, 400, 600 and 800 μ g/mL concentrations.

VALIDATION OF METHOD

Analytical curve

The relationship between the concentrations of sodium alginate and pectin and the response detector were determined by the analytical curve [4]. Stock solutions of 1,000 μ g/mL of sodium alginate and pectin were prepared in the mobile phase composed of phosphate buffer pH 7.4 and methanol (60:40% v/v). A series of serial dilutions such as 100, 200, 400, 600 and 800 μ g/mL solutions were made by using primary stock solutions of sodium alginate and pectin. These solutions were injected into the HPLC system for analysis after

Fig. 1. Chemical structure of sodium alginate and pectin

filtering through 0.22 μm filters. The analytical curves of sodium alginate and pectin were constructed by plotting areas of peak verses concentration of polymers. The curves were used to calculate the regression data and the R^2 values.

Selectivity

The selectivity was studied by processing and evaluating blanks as well as the co-administered sodium alginate and pectin. The retention times of sodium alginate and pectin were determined under similar chromatographic conditions. Selectivity was calculated by matching the chromatograms of six repeated injections of each blank with the standard polymer solution.

Precision and accuracy

The precision of the developed method establishes the proximity of agreement between a sequences of measurements obtained from several sampling of the same identical sample, while the accuracy of the method defines the nearness of the results obtained by the method with the true value of the analyte [5]. The precision of the method was assessed by calculating the RSD at three concentration levels (200, 400 and 600 $\mu\text{g/mL}$). Within-day precision was assessed by five repeated measurements for each concentration of sodium alginate and pectin, while between day precision was assessed by duplicate measurements of freshly prepared samples during a period of 5 consecutive days. The accuracy of the developed method was expressed as the percentage recovery and was valued by associating the peak area of samples that comprise a known quantity of sodium alginate and pectin to the peak area of the standard solutions spiked with the same amount.

Detection and quantification limits

The limit of detection (LOD) was the lowermost concentration level of the sodium alginate and pectin which can be detected but not essentially quantified and was calculated

using the sigma method while the limit of quantification (LOQ) was the lowest concentration of sodium alginate and pectin in the sample that could be measured by the detector with appropriate precision and accuracy [6].

System suitability test

The suitability [7] of system was assessed with the composition of mobile phase, followed by 6 injections of the same standard. These 6 continuous injections were used to assess the system suitability each day of method validation. The features verified were the retention time, tailing factor, peak area, theoretical plates and resolution. The chromatographic resolution is the difference in retention time between two adjacent peaks divided by the average width of the peaks.

Preparation of raft forming tablets

The tablets were prepared by the wet granulation method [8]. First, the polymers and other excipients were mixed thoroughly and passed through an 18-mesh screen as mentioned in Table 1, then the powder mixture of polymer and other excipients passed the micromeritic limits was mixed using a sigma mixer. Second, the powder mixture was granulated using 2% (w/w) (hydroxypropyl methyl cellulose) HPMC E5 in a 90% ethanol solution. Finally, the granules were dried at 40 $^{\circ}\text{C}$ for 2 h, passed through an 18-mesh screen and compressed using a minipress MII (Pharma Test, Hainburg, Germany).

Estimation of alginate and pectin contents within the raft

Simulated gastric fluid (SGF) 150 mL was taken in a beaker with 250 mL carrying capacity and the temperature was maintained at 37 $^{\circ}\text{C}$ using hot plate stirrer. For a complete maturation of raft, wait for 30 min. The raft was removed from beaker containing SGF, washed three times with deionized water and placed in a centrifuge tube. The

Table 1. Composition of raft forming tablets

Code	Sodium alginate (%)	Pectin (%)	HPMC K100M (%)	NaHCO_3 (%)	Citric acid (%)	Calcium carbonate (%)	Total weight (%)
R1	27.50	10.00	14.00	18.00	9.00	6.50	100
R2	27.50	27.50	10.00	14.00	7.00	4.00	100
R3	2.50	2.50	10.00	40.00	20.00	15.00	100
R4	10.00	27.50	14.00	20.00	10.00	3.50	100
R5	2.50	10.00	6.00	40.00	20.00	6.50	100
R6	27.50	2.50	10.00	30.00	15.00	5.00	100
R7	2.50	27.50	10.00	30.00	15.00	5.00	100
R8	27.50	10.00	6.00	25.00	12.50	4.00	100
R9	10.00	10.00	10.00	30.00	15.00	5.00	100
R10	10.00	2.50	6.00	40.00	20.00	6.50	100
R11	10.00	10.00	10.00	60.00	15.00	5.00	100
R12	10.00	10.00	10.00	60.00	15.00	5.00	100
R13	10.00	2.50	14.00	35.00	17.50	6.00	100
R14	10.00	10.00	10.00	30.00	15.00	5.00	100
R15	10.00	27.50	6.00	25.00	12.50	4.00	100
R16	2.50	10.00	14.00	35.00	17.50	6.00	100
R17	10.00	10.00	10.00	30.00	15.00	5.00	100

Fig. 2. Schematic representation for the estimation of sodium alginate and pectin in raft forming tablets

centrifuge tube, containing deionized water at 40 °C, was stirred at 250 rpm for 1 min. Then, the tube was centrifuged for 3 min at 5,000 rpm, the supernatant was removed and the procedure was repeated twice. Ethanol was added to each centrifuge tube at 4 °C and the tubes were again centrifuged at 5,000 rpm for 3 min. Finally, the raft was removed from the tube and dried in an oven at 40 °C until constant weight, after the rafts were weighed. HPLC, with an already developed method, was used to determine the content of alginate and pectin in the raft. A series of dilutions with a concentration of 0.2, 0.4, 0.6, 0.8 and 1 mg/mL of both sodium alginate and pectin were prepared with phosphate buffer having a pH 8.0. Each dilution was centrifuged at 5,000 rpm for 15 min. The dried raft was grounded into a fine powder, dissolved in 5 ml of phosphate buffer having a pH 8.0, sonicated for 15 min at 37 °C and then centrifuged for 5 min at 5,000 rpm. The raft solution was filtered through a 0.2 µm syringe filter, while the first few drops of filtrate were discarded. The linear equation ($R^2 = 0.999$) of a standard plot was used to determine the contents of sodium alginate and pectin present in the raft. Fig. 2 shows the schematic representation for the detection of sodium alginate and pectin in raft forming pharmaceuticals. The following equations were used to determine the contents of sodium alginate and pectin incorporated into the raft system.

$$\text{Contents of sodium alginate} = (X \text{ mg/ml}) \times \frac{V(\text{ml}) \times W_1(\text{mg})}{W_2(\text{mg})} \quad (1)$$

$$\text{Contents of pectin} = (X \text{ mg/ml}) \times \frac{V(\text{ml}) \times W_1(\text{mg})}{W_2(\text{mg})} \quad (2)$$

The weight of alginate-pectin raft and sample were denoted by W_1 and W_2 respectively.

RESULT AND DISCUSSION

Chromatographic method validation

Analytical curve. The analytical curves for sodium alginate and pectin were linear over a range of 200–800 µg/mL. For

each concentration, three runs were applied and the graphs were plotted between the concentration and the area of the peak. The correlation coefficient (R^2) for the standard of sodium alginate and pectin were found to be 0.9993 and 0.9991 respectively, as shown in Table 2. The ANOVA were applied to the regression data, the standard and prepared samples of sodium alginate and pectin showed 0.0012 and 0.025 P -values indicated that the results were significant and showed a linear relationship. Oliveira et al. in 2019 studied the similar pattern of analytical curve for the combined estimation of two drugs in pharmaceuticals [9].

Specificity. The specificity of the method was analyzed and no interference from the polymeric materials (sodium alginate and pectin) was observed by performing separate injections of standard, placebo and polymeric samples respectively. The obtained results are in agreement with previously reported results by Habibi et al. in 2019 [10]. The retention time of the sodium alginate and pectin were 3.931 and 7.470 min respectively, as shown in Fig. 3.

Table 2. Analytical curve and sensitivity data of sodium alginate and pectin in mobile phase

Drugs	Regression data	R^2	LOD (µg/mL)	LOQ (µg/mL)
Standards				
Sodium alginate	$y = (1.479 \pm 0.0157) \times -$ (0.136 ± 0.0146)	0.9991	2.331	2.981
Pectin	$y = (0.168 \pm 0.0059) \times -$ (0.056 ± 0.0343)	0.9992	3.351	3.876
Sample				
Sodium alginate	$y = (0.576 \pm 0.0137) \times -$ (0.069 ± 0.0236)	0.9993	2.443	3.129
Pectin	$y = (0.127 \pm 0.0237) \times -$ (0.017 ± 0.0330)	0.9991	3.126	3.785

Fig. 3. Chromatogram showing sodium alginate and pectin peaks

Precision and accuracy. The accuracy of the procedure was established by repeating three concentrations (200, 400 and 600 $\mu\text{g/mL}$) of sodium alginate and pectin in mobile phase at 378 nm and the percentage recoveries were calculated are shown in Table 3. The percentage recovery of sodium alginate was ranged from 95.0 to 97.5%. The pectin showed a percentage recovery between 94.7 and 96.5% as shown in Table 3. The precision of the developed method was tested by injecting three different concentrations of sodium alginate and pectin on the same day and the next three days. The inter-day and intra-day precision (RSD) of sodium alginate and pectin was calculated and shown in Table 3. The RSD of sodium

alginate and pectin were ranged from 1.3 to 1.9 and 1.2 to 1.9 as shown in Table 3. Li et al. in 2015 observed the inter-day and intra-day precision estimation of polymers and different other additives in different food additives by HPLC [11].

Detection and quantification limits. The LOD and LOQ were calculated by the sigma method. The LOD and LOQ of sodium alginate were found to be 2.443 and 3.129 $\mu\text{g/mL}$ respectively, by injecting known concentrations of the sample. The LOD and LOQ of pectin were 3.126 and 3.785 $\mu\text{g/mL}$ respectively, as shown in Table 2. Koppala et al. in 2016 observed the similar findings for LOD and LOQ for the combined monitoring of ketoprofen and omeprazole in pharmaceutical dosage forms [12].

System suitability parameters. The system suitability tests were analyzed by calculating the % RSD of sodium alginate in parameters such as theoretical plate, tailing factor, retention time, peak area and resolution were 0.015, 1.96, 0.030, 0.001 and 1.325 respectively. The % RSD of theoretical plates, tailing factor, retention time, peak area and resolution of the pectin were 0.34, 1.08, 0.67, 2.9 and 1.32 respectively. The retention time of sodium alginate and pectin was observed from chromatograms and it was found to be 3.12 ± 0.5 and 5.34 ± 0.7 min respectively and are shown in Table 4. The resolution of sodium alginate and pectin was found in the range of 1.77–2.77 and 5.10 to 5.41 respectively as shown in Table 4. The resolution of 1 or higher represents an adequate separation of polymers [7]. Similarly, Zaman et al. in 2019 have developed method for the combined monitoring of drugs and their findings

Table 3. Within-day and Between-day precision of sodium alginate and pectin in mobile phase

Added ($\mu\text{g/mL}$)	Within-day ($n = 6$)			Between-day ($n = 6$)		
	Found $\pm$ SD ($\mu\text{g/mL}$)	RSD	Recovery (%)	Found $\pm$ SD ($\mu\text{g/mL}$)	RSD	Recovery (%)
<i>Sodium alginate</i>						
200	195 ± 0.44	1.3	97.5	193 ± 0.21	1.4	96.5
400	380 ± 0.13	1.9	95.0	378 ± 0.14	1.9	94.5
600	575 ± 0.45	1.8	95.8	580 ± 0.36	1.5	96.6
<i>Pectin</i>						
200	193 ± 0.31	1.2	96.5	191 ± 0.52	1.9	95.5
400	379 ± 0.22	1.6	94.7	381 ± 0.35	1.1	95.2
600	573 ± 0.46	1.7	95.5	568 ± 0.37	1.3	94.6

Table 4. System suitability parameters of sodium alginate and pectin in mobile phase

Parameters	Retention time (min)	Peak area	Tailing factor	Theoretical plates	Resolution
<i>Sodium alginate</i>					
Mean $\pm$ SD	3.93 ± 0.5	311,643	1.379	2,389	1.670
RSD	0.67	2.9	1.08	0.34	1.32
Limits	–	<2	<2	–	>1
<i>Pectin</i>					
Mean $\pm$ SD	7.44 ± 0.7	212,345	1.254	1,054	3.892
RSD	2.31	1.97	1.07	0.05	1.54
Limits	–	<2	<2	–	>1

Table 5. Alginate contents within the alginate-pectin rafts of prepared formulations

Code	Pectin (%)	Calcium carbonate (%)	Found % of SA	% Recovery	% RSD
<i>Formulation containing maximum percentage of (27.5%) SA</i>					
R1	10.00	6.50	26.50	96.36	0.897
R2	27.50	8.00	23.50	84.45	1.675
R6	2.50	10.00	24.55	89.27	1.340
R8	10.00	8.00	19.55	71.09	0.912
<i>Formulation containing medium percentage of (10%) SA</i>					
R4	27.50	7.00	7.05	70.50	0.357
R9	10.00	10.00	9.92	99.20	1.209
R10	2.50	13.00	7.41	74.10	1.234
R11	10.00	10.00	9.25	92.50	0.786
R12	10.00	10.00	8.81	88.10	0.903
R13	2.50	12.00	7.95	79.50	0.675
R14	10.00	10.00	7.91	79.10	0.245
R15	27.50	8.00	9.34	93.40	1.320
R17	10.00	10.00	9.55	90.50	1.190
<i>Formulation containing minimum percentage of (2.5%) SA</i>					
R3	2.50	30.00	1.81	72.40	0.786
R5	10.00	13.00	2.22	88.80	1.674
R7	27.50	10.00	1.95	78.00	1.456
R16	10.00	12.00	1.82	72.80	0.902

for system suitability was similar to the findings of this research article [13].

Alginate and pectin contents in raft forming tablets. The contents of alginate and pectin present in the raft were evaluated and are presented in Table 5 and 6. The

Table 6. Pectin contents within the alginate-pectin rafts of prepared formulations

Code	SA (%)	Calcium carbonate (%)	Found % of pectin	% Recovery	% RSD
<i>Formulation containing maximum percentage of (27.5%) Pectin</i>					
R2	27.50	8.00	21.80	79.27	1.367
R4	27.50	7.00	16.50	60.00	0.987
R7	2.50	10.00	17.70	64.36	1.564
R15	10.00	8.00	25.50	92.72	0.924
<i>Formulation containing medium percentage of (10%) Pectin</i>					
R1	27.50	13.00	9.40	94.00	1.982
R5	2.50	13.00	8.50	85.00	1.564
R8	27.50	8.00	6.20	62.00	1.290
R9	10.00	10.00	9.72	97.20	0.568
R11	10.00	10.00	8.50	85.00	0.398
R12	10.00	10.00	8.10	81.00	1.276
R14	10.00	10.00	7.20	72.00	1.076
R16	2.50	12.00	6.90	69.00	0.598
R17	10.00	10.00	8.50	85.00	0.367
<i>Formulation containing minimum percentage of (2.5%) Pectin</i>					
R3	2.50	30.00	1.59	63.60	0.912
R6	27.50	10.00	2.03	81.20	1.786
R10	10.00	13.00	1.57	62.80	0.786
R13	10.00	12.00	1.78	71.20	1.087

formulations (R1, R2, R6 and R8) contained a maximum percentage of SA i.e. 27.5% but different concentrations of pectin and calcium carbonate presented 71.09–96.36% percentage recovery of SA and % RSD was ranged from 0.897 to 1.675 as displayed in Table 5. The recovery rates of R4, R9, R10, R11, R12, R13, R14, R15 and R17 contained 10% SA (medium concentration) ranged from 70.50 to 99.20% and % RSD was less than 2%. 72.40–88.80% percentage recoveries of SA were obtained from raft of R3, R5, R7 and R16 formulations contained 2.5% of SA and % RSD was ranged from 0.786 to 1.674. The formulations (R2, R2, R7 and R15) contained maximum percentage of pectin i.e. 27.5% but different concentrations of SA and calcium carbonate showed 60.00–92.72% percentage recoveries of pectin and % RSD was ranged from 0.924 to 1.564 as shown in Table 6. The formulations R1, R5, R8, R9, R11, R12, R14, R16 and R17 contained 10% pectin showed percentage recoveries of pectin ranged from 62.00 to 97.20% and % RSD was less than 2%. The 62.80–81.20% percentage recoveries of pectin were obtained from raft of R3, R6, R10 and R13 formulations contained 2.5% of pectin and % RSD was ranged from 0.786 to 1.786. The alginate-pectin raft of R9 formulation contained 99.20 and 97.20% alginate and pectin respectively. The chromatogram of R9 formulation showed the peaks of sodium alginate and pectin is shown in Fig. 4. The highest percentages of alginate and pectin were observed in formulation R9; this was due to the very compact nature of raft compared to other formulations. The lower percentages of alginate and pectin contents in alginate-pectin rafts were observed in the R4 formulation compared to the other formulations. Awad et al. in 2012 also studied the estimation of alginate in the pharmaceutical formulations by using HPLC [3]. The presence of alginate and pectin in the raft is important for trapping antacids in the raft that constitute a neutral barrier of gastric acidity into the esophagus. The presence of alginate and pectin in the raft affects the strength and integrity of the raft and also allows the raft to trap an antacid in the gel.

Fig. 4. Chromatogram of R9 formulation showing peaks of sodium alginate and pectin

CONCLUSION

The proposed method was simple, precise, sensitive and inexpensive for the combined monitoring of sodium alginate and pectin in raft forming drug delivery systems. The method was validated over a concentration range of 200–800 µg/mL and had good accuracy and precision. The developed HPLC method was found to be highly precise, sensitive, inexpensive and accurate for the determination of sodium alginate and pectin. This method is quite useful and reasonably meets all standards.

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