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Short communication

Quantitative determination of alginic acid in pharmaceutical formulations using capillary electrophoresis

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Abstract

A capillary electrophoresis (CE) method has been developed and validated for the quantitative determination of alginic acid, which is used as a rafting agent in complex antacid formulations. The method involves a preliminary separation of the alginic acid from the formulation by washing the sample matrix with methanol, diluted HCl and water. This is followed by electrophoresis within a fused silica capillary using borate/boric acid buffer as the electrolyte, and the quantification is performed by a UV detector monitoring at 200 nm, where the intrinsic absorption of alginic acid is measured. An assay precision of better than 3% was achieved in intra- and interday determinations. No interference was found from the matrix of the antacid formulations.
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1. Introduction

Alginic acid is a gelling polysaccharide that is constituted of (1–4) linked uronic acids, the basic units being α-L-guluronic acid and β-D-mannuronic acid. It is present in the cell walls and intracellular material of the marine brown algae (belonging to the *Phaeophyceae*) [1]. The particular arrangement of guluronic and mannuronic residues in the linear polymeric structure determine the physical properties of the polysaccharide with the guluronic acid being responsible for imparting the molecular stiffness and consequent stability [2,3]. Alginates, like polysaccharides in general, are polydisperse with respect to molecular weight, resembling synthetic polymers rather than biopolymers such as proteins and nucleic acids. The reasons for this are that the polysaccharides are not coded by the organism DNA, and during extraction there is substantial depolymerisation of the polymer. Thus, the number of monosaccharide residues and their distribution in the alginic acid extracted from seaweed may range from 1000 to 10,000, depending somewhat on harvest conditions and the procedures used for isolation.

Alginic acid is used as an ingredient in food and a number of pharmaceutical formulations for its gelling, stiffening and stabilising properties. For example, its use in antacid formulations for heartburn is based on the polysaccharide being insoluble in the presence of

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stomach acid and therefore forming a protective film on the top of the stomach lining, to help prevent acid attack on the stomach wall.

As a raw material, alginic acid can be used both in solid and liquid formulations. Because of the complexity and high molecular weight of alginic acid, and its lack of readily employed analytical characteristics, the analysis of this agent has not been well developed. The labelled alginic acid content in a formulation has usually been based on the amounts added in the batch process, and quality assurance procedures have not been established to confirm the content. The BP 2002 recommends a method for the assay of alginic acid in a relative pure state as the raw material [4]. The method relies on the stoichiometric reaction of the carboxyl groups in alginic acid with standardised base. Clearly, other acidic or alkaline substances in the sample will interfere with the assay, rendering the method inapplicable to a finished formulation.

Colorimetric methods have been widely used in the determination of polysaccharide content as a whole, irrespective of whether they occur in homo- or hetero-polymeric blocks. The first step involves treatment with a concentrated mineral acid to break down the polysaccharide to furfural and carbon dioxide. In the second step, one of many possible reagents is used to produce a coloured compound for the photometric analysis [5,6]. Apart from the low selectivity due to interference by other polysaccharides in the sample, the main disadvantage arises from the much slower breakdown rate of poly(guluronic acid) blocks compared to poly(mannuronic acid) blocks [7]. In addition, the heterouronic composition in the alginic acid is the main obstacle to the design of versatile calibration curves for the determination of alginic acid with any ratio and/or distribution of uronic acids along the polymeric chain. Thus, the colorimetric methods are not robust, nor do they have the characteristics that are suitable for stability indication of products with a complex matrix.

Although a series of chromatographic methods have been developed for the analysis of alginic acid, the focus has been on structural studies [8]. In other words, high performance liquid or gas chromatography has been applied after reproducible chemical or enzymatic fragmentation, with detection emphasising chemical derivatisation to enhance the response for absorption or fluorescence spectroscopy. However, no chromatographic method reported to date is suitable for the determination of the total alginic acid content in pharmaceutical formulations.

Size exclusion chromatography with light scattering detection is the usual technique for the determination of the molecular weight distribution of the polysaccharides. However, for substances with molecular weight in the megaDalton range this technique has reached its upper size limitation and it becomes less capable of discrimination. Capillary electrophoresis (CE) has emerged as an important separation methodology for the analysis and structural study of the high molecular weight polysaccharides [9]. The CE technique has been applied to the assessment of alginic acid molecular weight and chemical composition with the addition of polyacrylamide in the electrolyte and conversion to fluorescent derivatives with aminopyrene trisulfonic acid [10]. The analysis was not applicable to quantitative determination of the total alginic acid content.

In this paper, we describe a CE method for the quantitative determination of the total alginic acid content in pharmaceutical formulations. The basis of the method is the pre-treatment of the formulation to remove most of the other ingredients, followed by the separation of alginic acid in a silica capillary using a borate buffer as electrolyte. The quantification is achieved using a UV detector in the far ultraviolet region (200 nm) to record the intrinsic absorption of the weak chromophore in alginic acid. The CE method has been applied to the determination of alginic acid content of both solid and liquid formulations that also contain antacid ingredients, mainly aluminium and magnesium hydroxides.

2. Experimental

2.1. Materials

Alginic acid standard BPC was provided by Pfizer Consumer Healthcare Research and Development (Caringbah, Australia).

Hydrochloric acid (36%), sodium hydroxide pellets, di-sodium tetraborate and boric acid were all AR grade and from BDH Chemicals, Sydney, Australia. Methanol (AR) was obtained from Selby-Biolab Pty. Ltd., Sydney. Water was doubly distilled in an all-glass apparatus.

2.2. Formulations

The following formulations were obtained from Pfizer Consumer Healthcare Research and Development (Caringbah, Australia).

(A) Mylanta Heartburn Relief Antacid Liquid formulation containing, in each 20 ml, aluminium hydroxide (dried) 400 mg, magnesium hydroxide 400 mg, calcium carbonate 500 mg, sodium bicarbonate 500 mg and alginic acid 310 mg together with emulsifying and flavouring agents.
(B) Mylanta Heartburn Relief Antacid Liquid "Placebo" containing, in each 20 ml, aluminium hydroxide 400 mg, magnesium hydroxide 400 mg and calcium carbonate 500 mg, sodium bicarbonate 500 mg and excipients as in A, but no alginic acid.
(C) Mylanta Heartburn Relief Antacid Tablet containing in each tablet (average weight 1300 mg) magaldrate 371 mg, sodium bicarbonate 234 mg and alginic acid 215 mg, together with tablet binding and flavouring agents.
(D) Mylanta Heartburn Relief Antacid Tablet "Placebo" containing in each 1085 mg, magaldrate 371 mg, sodium bicarbonate 234 mg and excipients as in C, but no alginic acid.

2.3. Instrumentation

A Beckman capillary electrophoresis P/ACE^{TM} 2000 system coupled with an UV detector was used in the analysis. Beckman eCAP^{TM} tubing with 75 μm internal diameter and 40 cm effective length was installed in a Beckman P/ACE^{TM} System eCAP^{TM} capillary cartridge as the separation capillary. Beckman System Gold software 2000 was used to control the CE P/ACE^{TM} 2000 system and record and analyze the test data.

2.4. CE conditions

For the quantitative determination of alginic acid, the following CE conditions were used—electrolyte, 12.5 mM borate/boric acid buffer pH 8.3; electric field, 550∼650 V/cm; capillary temperature, 23 °C; injection mode, voltage migration (10 s at 5 kV); detection, UV at 200 nm; rinsing between samples, 0.1 M sodium hydroxide for 1 min and 12.5 mM borate/boric acid buffer pH 8.3 for 1 min.

2.5. Methods

Stock solutions of alginic acid were prepared by dissolving an accurately weighed amount (approximately 0.5 g) of alginic acid (BPC standard) in 25 ml 0.1 M NaOH solution. A series of standard solutions were obtained by dilution of the stock solution with 0.1 M NaOH.

For the analysis of alginic acid contained in antacid formulations, accurately weighed samples (approximately 1 g of the powder obtained by crushing 10 tablets, or 7.5 g of well-mixed liquid formulation) were treated according to the following procedure:

(a) Approximately 5 ml methanol was added to the sample contained in a stoppered glass centrifuge tube. The mixture was shaken thoroughly using a vortex shaker for 1 min and then centrifuged at 1000 rpm for 5 min. The supernatant liquid was decanted and the solid mixture was washed by a second 5 ml portion of methanol.
(b) After removal of methanol, approximately 5 ml diluted HCl (1:4 (v/v)) was then added to the tube, the mixture was shaken and centrifuged as before, and the supernatant was decanted carefully. The washing step with HCl was repeated once.
(c) After removal of HCl, approximately 8 ml water was then added to the tube, the mixture was shaken and centrifuged as before, and the supernatant was decanted carefully. This washing step with water was repeated once.
(d) 10 ml of 0.1 M NaOH solution was added to the tube and sonicated for 15 min. The mixture was then transferred to a 25 ml volumetric flask. The tube was rinsed with 5 ml of 0.1 M NaOH solution and the rinsing solution was added to the volumetric flask. The rinsing step was continued with 5 ml aliquots of 0.1 M NaOH solution until the volumetric flask was filled to the mark with the solution. Aliquots of this solution were centrifuged and filtered through a Nylon filter cartridge and analyzed by CE. For each sample, the peak area of the alginic acid peak from the electropherogram was obtained as the average from three injections.

For the construction of the standard curve used in the analysis of the tablet formulation, amounts of alginic acid BPC standard (about 0.1, 0.15, 0.20, 0.25 and 0.30 g) were accurately weighed and added to about 0.8 g of Antacid Tablet Placebo. The amounts of alginic acid in this standard range represented from 50 to 150% of the labelled content per 1 g of tablet formulation. The standard/placebo mixtures were treated by the same procedure as the tablet samples.

For the construction of the standard curve used for the liquid formulation, amounts of alginic acid BPC standard (approximately 80, 120, and 160 mg) were accurately weighed and added to 7.5 g Liquid Antacid Placebo. The standard/placebo mixtures were treated in the same way as for the liquid formulation samples.

3. Results

3.1. Capillary electrophoresis of alginic acid

As alginic acid forms a water-soluble monovalent salt, it is relatively straightforward to prepare samples for analysis by dissolving the Mylanta formulation, either solid or liquid, in dilute NaOH solution. However, the large amounts of antacid compounds such as magnesium and aluminum hydroxides or magaldrate, contained in the Mylanta formulations, may have some interaction with the alginic acid leading to changes of the peak profile. Pre-treatment of the sample by washing with dilute acid and methanol was found to greatly reduce interference from the other formulation components to give a better peak shape for the alginic acid. The purified alginic acid quantitatively dissolved in 0.1 M NaOH solution.

Borate/boric acid buffer (pH 8.4) was used as the electrolyte to provide sufficient ionic strength and to enhance approximately two-fold the UV absorptivity by the formation of borate–alginic acid complex, as reported previously [9]. The separation of alginic acid standard in the capillary under the electric field of 600 V/cm led to the migration of an asymmetric peak due to the alginic acid with a migration time of about 4.6 min (Fig. 1). The broad peak shape is probably a result of the fact that alginic acid is not a single compound but a mixture of polyuronic acids of different molecular weights. Operational parameters for the CE were varied and it was found that the optimal alginic acid peak profile was achieved with the borate/boric acid buffer concentration between 12.5 and 20 mM, with pH 8.3–8.4 and the applied electric field at 550–650 V/cm. The addition of an organic solvent such as methanol is often used to modify the electro-osmotic flow of the electrolyte [9], but was found to shorten the migration time without an improvement to the alginic acid profile in the present system.

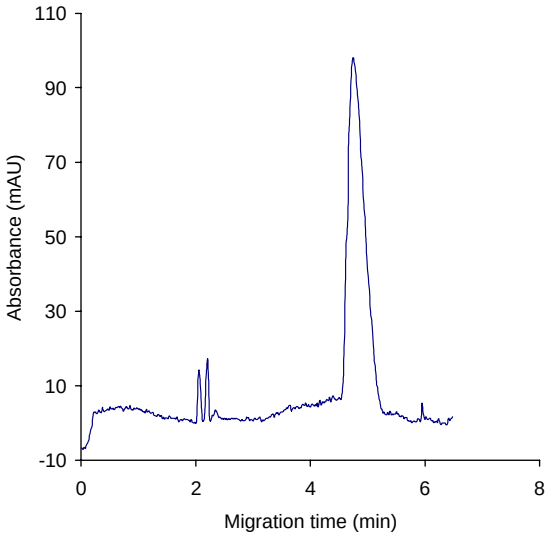

Fig. 1. Electropherogram of alginic acid standard (BPC) dissolved in 0.1 M NaOH solution. CE conditions are given in the text.

The Mylanta formulations when directly dissolved in 0.1 M NaOH solution and then analysed generate a very complex electropherogram with peaks due to matrix components appearing before and merging somewhat with the alginic acid peak. The electropherogram for the Mylanta Tablet analysed without pre-treatment is shown in Fig. 2, trace A. The shape and migration time of the peak corresponding to alginic acid from the formulation is rather different from that of alginic acid standard. This is probably because alginic acid to some extent forms a salt-complex with Mg^{2+} or Ca^{2+} in the formulation matrix. The electropherograms of the Mylanta Tablet formulations with sample pre-treatment show a clear alginic acid peak at the migration time of about 4.6 min in agreement with the standard (Fig. 2, trace B). The electropherogram of the Mylanta Tablet "Placebo" antacid formulation (containing all components except alginic acid) after

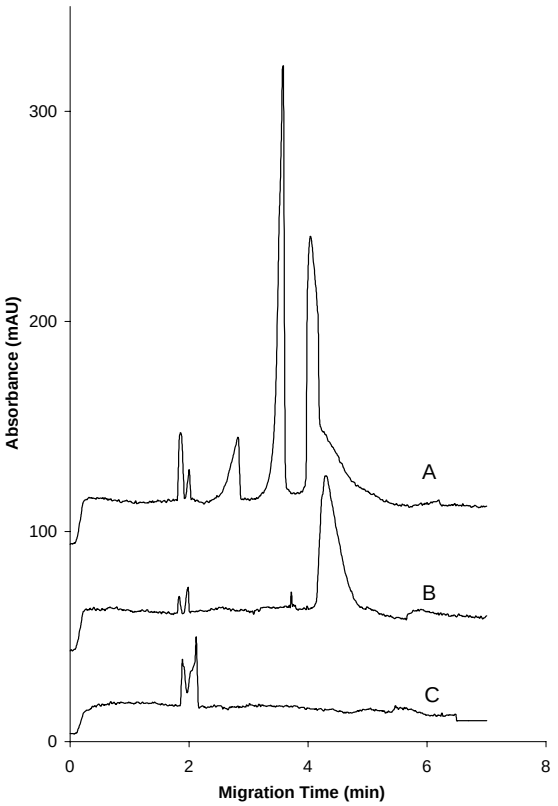

Fig. 2. Electropherograms of (A) Mylanta Tablet directly dissolved in 0.1 M NaOH, (B) Mylanta Tablet formulation after washing pre-treatment with methanol and dilute HCl, and (C) Mylanta Tablet Placebo formulation (contains no alginic acid) after washing pre-treatment with methanol and dilute HCl. CE conditions as for Fig. 1. The traces have been offset for clarity.

the washing pre-treatment gave no peak in the region between 4 and 5 min, confirming the specificity of the separation for alginic acid (Fig. 2, trace C). Similar electropherograms were obtained when the Mylanta Liquid formulation was analysed.

3.2. System precision of CE analysis for alginic acid standard

On four different days, a series of eight injections of the same solution of alginic acid (8 mg/ml in 0.1 M NaOH) was made to determine the CE system precision. It was found that the migration time was reproducible to better than 1% while the peak area around 2%. The CE system precision obtained here was similar to that reported in use of the technique for analysis of a number of phytochemical substances [11].

3.3. Linearity of the alginic acid assay

The linear response range of the CE-UV system was found to be between 2 and 15 mg/ml, evaluated using a series of alginic acid standards in 0.1 M NaOH solution. The limit of quantification of alginic acid was assessed to be 2 mg/ml, this relatively high concentration can be ascribed to the poor absorptivity of the weak UV chromophore. Clearly the use of a derivatisation procedure would enable a more sensitive assay to be developed, but the present system has adequate sensitivity for the determination of alginic acid in pharmaceutical formulations. The standard curve deviated from linearity with alginic acid concentrations higher than 15 mg/ml; these solutions were of increasing viscosity and may not be injected fully during the fixed injection time.

The peak area response was linear in the 2–15 mg/ml range with a correlation coefficient that varied from 0.996 to 0.998. The regression equation for the linear portion of the standard curve was found to be:

$$\text{Peak Area in arbitrary units} = (0.424 \pm 0.013) \times (\text{conc. in mg/ml}) - (0.06 \pm 0.09)$$

For the linear range, the R.S.D. of the slope was about 3%.

3.4. Recovery

The standard curve for the assay of tablet and liquid formulations is set up by addition of known amounts of alginic acid standard to the appropriate “Placebo” formulation that contains all of the ingredients except alginic acid. This is followed by the washing pre-treatment to remove the antacid ingredients before CE analysis. The effectiveness of the separation procedure for the Mylanta Tablet formulation was tested on a known mixture of 200 mg alginic acid standard with 0.8 g of Placebo Tablet formulation. The mean recovery was $101.3 \pm 3.4\%$. In a similar way, the effectiveness of the separation of alginic acid from the Mylanta Liquid formulation was tested on a known mixture of 200 mg alginic acid standard with 7.5 g of

Placebo Liquid formulation. The mean recovery was found to be 101.7 ± 3.9%. Since the extraction was found to be efficient, the quantitative assay could be performed without the use of an internal standard.

3.5. Alginic acid content in Mylanta Tablet and Liquid formulations

The alginic acid content of one production batch of Mylanta Tablet formulation was determined six times with a mean result of 215.3±4.6 mg per tablet (R.S.D. 2.1%), in excellent agreement with the label claim of 215 mg per tablet. When the same method was applied to the analysis of a batch of Mylanta Liquid, the alginic acid content was found 311.4 ± 8.5 mg/20 ml (R.S.D. 2.7%), which is also very close to the label claim of 315 mg/20 ml. Note that the labelled amount of alginic acid was based on the quantity added in mixing the production batch.

Analyses of the same batches on different days, each with their individual standard curves, gave results of 210.5 ± 3.5 (R.S.D. 1.7%) and 221.3 ± 4.7 (R.S.D. 2.1%) mg per tablet for the tablet formulation and 327.8±9.2 (R.S.D. 2.7%), 308.1±10.8 (R.S.D. 3.5%) and 330.4 ± 10.9 (R.S.D. 3.3%) mg/20 ml for the liquid formulation. The average precision achieved for the method when applied to Mylanta Liquid was not as good as that obtained for the Mylanta tablet. The reason for this difference is suggested to arise from emulsifiers in the more complex Mylanta Liquid formulation leading to a slightly more difficult separation in the sample pre-treatment procedure.

4. Conclusions

CE separation with direct UV detection was found to be a useful means for quantifying alginic acid in pharmaceutical antacid formulations. The interference from the formulation matrix to the analysis was sufficiently avoided by washing the formulation sample with dilute mineral acid and methanol. The precision of the CE-UV method (2% for Mylanta Tablet and 3% for Mylanta Liquid) was at the level that the CE technique usually achieves. More importantly, CE provides a specific and efficient separation of the high molecular weight alginic acid from other ingredients of the formulation. Other chromatographic methods have generally relied on acid digestion procedures to first convert the polymer to smaller oligomeric units prior to analysis. Such procedures are frequently difficult to reproduce for application to quality control in pharmaceutical production. The CE procedure should be generally applicable to other types of formulation with the appropriate sample pre-treatment.

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