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Short communication

Determination of citrate, inositol and gentisic acid in a pharmaceutical diagnostic formulation by ion-moderated partition chromatography

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Abstract

An ion-moderated partition chromatographic method has been developed for rapid determination of excipients in a pharmaceutical diagnostic formulation. Citrate, inositol, and gentisic acid (2,5-dihydroxybenzoic acid) are separated and analyzed using an 0.005 *M* sulfuric acid–acetonitrile (80:20) mobile phase with a 100 × 7.8 mm strong cation-exchange column consisting of sulfonated cross-linked styrene–divinylbenzene copolymer in the hydrogen form. Detection is by refractive index. The method has proven to be rugged for more than 100 injections over a one-year period on the same column.

1. Introduction

The separation of organic acids and carbohydrates/sugar alcohols via ion-moderated partition (strong cation-exchange resin consisting of sulfonated cross-linked styrene–divinylbenzene copolymer in the hydrogen form) using dilute sulfuric acid as a mobile phase is well known [1–5]. However, retention is excessive for aromatic acids under these conditions. The use of an organic modifier such as acetonitrile in the mobile phase allows for the analysis of aromatic acids in a reasonable amount of time. However, there is apprehension among users of this type of column in using organic modifiers because of swelling of the sulfonated divinylbenzene–styrene copolymer stationary phase. A method has been developed using an organic modifier in the mobile phase which allows for the rapid separation and quantitation of citrate (organic acid), inositol (sugar alcohol), and gentisic acid (aromatic acid) in a formulation of OctreoScan (Mallinckrodt, St. Louis, MO, USA), a diagnostic for gastro-entero-pancreatic endocrine tumors. This method has proven to be rugged in routine use.

2. Experimental

2.1. Materials and reagents

Trisodium citrate dihydrate (analytical-reagent grade), inositol (organic-reagent grade), sulfuric acid (analytical-reagent grade), and acetonitrile (HPLC grade) were from Mallinckrodt (St. Louis, MO, USA). The gentisic acid was from

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Aldrich (Milwaukee, WI, USA). The γ-cyclodextrin was from Wacker (New Canaan, CT, USA).

2.2. High-performance liquid chromatography

Chromatographic measurements were performed at ambient temperature on a 100 mm × 7.8 mm I.D. stainless-steel column packed with 9-μm sulfonated divinylbenzene–styrene copolymer in the hydrogen form (Fast Acid Column, Bio-Rad, Hercules, CA, USA). The chromatograph consisted of a Waters (Milford, MA, USA) 510 pump, 712 WISP injector, and a 410 refractive index detector. Chromatograms were obtained and analyzed on a PE Nelson (Cupertino, CA, USA) Access*Chrom data system. The mobile phase was 0.005 *M* sulfuric acid–acetonitrile (80:20, v/v). The flow-rate was 0.8 ml/min and the injection volume was 20 μl. The column was stored in 0.005 *M* sulfuric acid between analyses. Samples were dissolved in mobile phase.

3. Results and discussion

The assay was linear in the concentration ranges of 2.2 to 11.0 mg/ml for trisodium citrate (anhydrous basis), 4.0 to 20.0 mg/ml inositol, and 0.8 to 4.0 mg/ml gentisic acid (Table 1). Repeatability was typically less than or equal to 2% R.S.D. Recovery of each component in the presence of the other two ranged between 98 and 102%.

A system peak which is dependent on the relative concentration of acetonitrile in the sample versus the mobile phase was observed between the inositol and gentisic acid peaks. However, this peak did not interfere with quantitation. The active ingredient in the formulation, a peptide, is at a concentration 200-fold lower than any of the excipients and was not detected under these chromatographic conditions so it did not interfere with the assay. The relative amount of acetonitrile in the mobile phase affects the retention time of the system peak and gentisic acid (increasing acetonitrile decreases retention time). However, very little effect was observed on the retention times for citrate and inositol. Slight changes in the molarity of the sulfuric acid in the mobile phase did not significantly affect retention times.

Generally, separations using ion-moderated partition chromatography are performed on a column length of 300 mm at elevated temperatures to improve column efficiency, lower backpressure, and enhance reproducibility. This sepa-

Table 1
Linearity of assay

Component	Slope	*y*-Intercept	Correlation coefficient
Citrate	7 423	−747	0.9999
Inositol	11 423	747	0.9999
Gentisic Acid	5 525	−95	0.9999

Slope in terms of peak height counts mg^{-1} ml^{-1}. Intercept in terms of peak height counts. Five data points for each component were used for the regression.

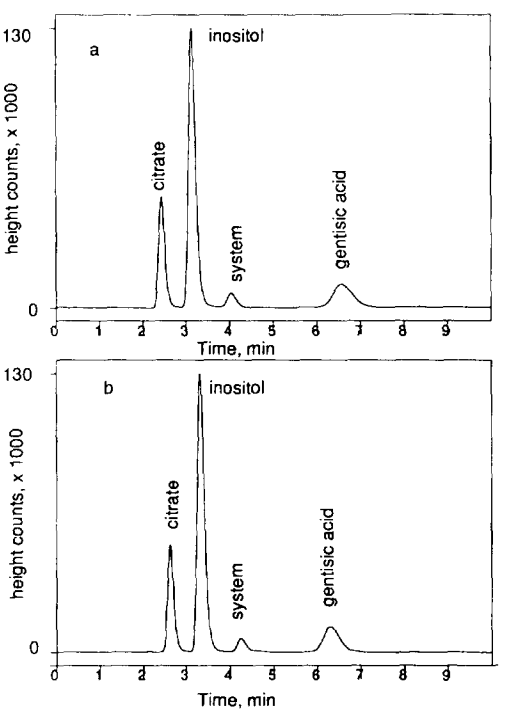

Fig. 1. Chromatograms of separation. (a) HPLC column used for one year, (b) new HPLC column.

Table 2
Capacity factors and resolution from nearest neighboring peak

Component	k'		R_s	
	New column	Used column	New column	Used column
Citrate	0.2	0.2	2.5	2.4
Inositol	0.5	0.6	2.5	2.4
Gentisic acid	1.9	2.3	3.8	4.2

t_0 determined by retention of γ-cyclodextrin.

ration was performed on a 100 mm column at ambient temperature. Column efficiency was sufficient for this separation, backpressure was well within the manufacturer's specifications, and reproducibility was not a problem. Clearly, the use of a shorter column where separation of different classes of compounds are involved may provide faster analysis without significant loss of resolution.

Fig. 1a shows a chromatogram of a mixed standard injected onto a column after more than 100 injections over a period of one year. Fig. 1b shows a chromatogram of a mixed standard injected onto a new column. Table 2 gives k' and R_s values. No noticeable deterioration of the separation was observed between the new and used columns. Only a slight increase in column backpressure was noted over the one year period.

4. Conclusions

A rapid and rugged HPLC assay has been developed for the separation and quantitation of citrate (organic acid), inositol (sugar alcohol) and gentisic acid (aromatic acid) using ion-moderated partition chromatography.

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