APPLICATION NOTE 000170

A fast HPAE-PAD method for determination of carbohydrates and glycols in pharmaceutical formulations

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Keywords: High-performance anion-exchange chromatography, Dionex CarboPac PA300 column, Dionex Integrion HPIC, inactive ingredient, FODMAP

Goal

Demonstrate a fast and sensitive method for the determination of carbohydrates and glycols in pharmaceutical formulations using a Thermo Scientific™ Dionex™ CarboPac™ PA300 column and electrolytic eluent generation

Introduction

A pharmaceutical formulation contains active as well as inactive ingredients. Inactive ingredients are any components of a drug product other than the active ingredient.[1] The United States Food and Drug Administration (U.S. FDA) and regulatory agencies in other countries require that pharmaceutical products be tested for composition to verify their identity, strength, quality, and purity.[2–4] Recently, more attention has been given to inactive ingredients. Even though inactive ingredients often serve important purposes in the formulation, they can sometimes cause adverse effects. A common adverse effect arises from intolerance to fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) ingredients. Because these can be difficult to digest, they can cause gastrointestinal symptoms such as gas, bloating, and diarrhea. It has been found that 55% of all oral medications contain at least one FODMAP. The most common are mannitol, polydextrose, and lactose.[5]

Over-the-counter (OTC) pharmaceutical formulations, including those for cough suppression and those for pain relief, contain glycols, sugar alcohols, and other carbohydrates, such as glucose and sucrose, as inactive ingredients. They are used for imparting consistency as well as masking the taste of the formulations. Some of these ingredients, including the aforementioned carbohydrates, are non-chromophoric and cannot be detected by spectrophotometry. These can be oxidized and thus detected and quantified using pulsed amperometric detection (PAD). This detection method is specific for those analytes that can be oxidized at the selected potential, leaving all other non-oxidizable compounds undetected.[6–7] Pulsed amperometric detection is a powerful detection technique with a broad linear range and low detection limits.

High-performance anion-exchange chromatography (HPAE) is a well-established method for the separation of carbohydrates by specific interactions between the hydroxyl and carboxyl groups of carbohydrates and the stationary phase. Separation is based on charge, size, composition, and linkage isomerism. When hyphenated with PAD (HPAE-PAD), this approach offers a high-resolution, high-sensitivity direct detection method to determine monosaccharides, sialic acids, and other carbohydrates. Thus, no sample derivatization is required. This reduces labor time and expense and eliminates exposure to hazardous derivatization chemicals. It is a selective and sensitive detection technique.

A previously published method uses a Dionex CarboPac MA1 column for the determination of carbohydrates and glycols in pharmaceutical formulations.[8] The drawbacks of this method are long run time and the high hydroxide concentration required for elution. The high hydroxide concentration precludes the use of electrolytic eluent generation, which improves ease-of-use as well as method reproducibility. Here, we present a convenient, fast, and sensitive method that uses a Thermo Scientific™ Dionex™ CarboPac™ PA300 column and electrolytic eluent generation. The Dionex CarboPac PA300-4µm column is an anion-exchange column primarily designed for the separation of complex oligosaccharides from heterogenous biological and food samples. It is packed with a new 4 µm particle size supermacroporous resin to achieve high efficiency and high-resolution separations. It is stable over a pH range of 0–14. Using the Dionex CarboPac PA300 column, a 16.8 min method that resolved six analytes, including glycols and other carbohydrates, was designed. Results for method linearity, robustness, and accuracy for glycol and carbohydrate quantification in OTC samples are discussed here.

Experimental

Equipment

- Thermo Scientific™ Dionex™ Integrion HPIC™ High Pressure Integrated system using electrochemical detection including:
  - Thermo Scientific™ Dionex™ Electrochemical Detector (ED)
  - Thermo Scientific™ Dionex™ Eluent Generator Cartridge (EGC 500 KOH)
  - Thermo Scientific™ Dionex™ Electrochemical cell, reference electrode with gasket, and disposable working electrode with gasket
  - Thermo Scientific™ Dionex™ Detector Compartment temperature control
  - Thermo Scientific™ Dionex™ Tablet control
  - Thermo Scientific™ Dionex™ Vacuum Degas kit
- Thermo Scientific™ Dionex™ AS-AP Autosampler with 1.5 mL trays, (P/N 079656)
- Dionex AS-AP autosampler (P/N 074926) with cooling tray option (recommended)
- Sterile assembled micro-centrifuge tubes with screw cap, 1.5 mL (Sarstedt P/N 72.692.005)

Software

- Thermo Scientific™ Chromeleon™ Chromatography Data system (CDS) version 7.2.10

Conditions

Columns	Dionex CarboPac PA300, 2 × 250 mm column (P/N 303346), Dionex CarboPac PA300 Guard 2 × 50 mm (P/N 303347)
Column temperature	30 °C
Compartment temperature	4 °C (recommended)
Flow rate	0.275 mL/min
Eluent	Potassium hydroxide
Eluent source	EGC KOH
Eluent concentration	22 mM
Working electrode	Gold disposable on PTFE (P/N 066480)
Reference electrode	pH-Ag/Ag/AgCl (P/N 061879)
Gasket	(PTFE) for disposable electrode 0.002 in (P/N 060141)
Injection volume	2.5 µL (Full_Loop)
Typical backpressure	3,400 psi (100 psi = 698.5 kPa approximately)

Reference electrode used in Ag mode (Ag/AgCl reference)

Carbohydrate 4-potential waveform for the ED				
Time (s)	**Potential (V)**	**Gain**	**Ramp region**	**Integration**
0	0.1	Off	On	Off
0.2	0.1	On	On	On
0.4	0.1	Off	On	Off
0.41	-2	Off	On	Off
0.42	-2	Off	On	Off
0.43	0.6	Off	On	Off
0.44	-0.1	Off	On	Off
0.5	-0.1	Off	On	Off

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistivity or better

Standards

- D-Glucose (P/N 1910-01)
- Sucrose (Sigma S378)
- Propylene glycol, anhydrous (Sigma P4347)
- Sorbitol (Sigma S1876)
- Mannitol (Acros Sigma AC125341000)
- Maltitol (Sigma M8892)

Stock and calibration standards

Solid standards were dissolved in DI water at 10 g/L concentrations. These were combined and further diluted with DI water to yield the desired stock mixture concentrations.

For determinations of the linear calibration ranges, 10 g/L solutions of propylene glycol, sorbitol, mannitol, glucose, maltitol, and sucrose were combined to make a 1.0 g/L mixed standard solution (e.g., 1 mL of each solution was combined with 4 mL of DI water). This solution was diluted with DI water to concentrations ranging from 1 to 100 mg/L of each analyte. The solutions were maintained at -20 °C until needed.

Sample preparation

Three OTC pharmaceutical formulations were used for this study. Two of the formulations were cough suppressants for adults. The third formulation was a cough suppressant for children. Compositions of the three formulations are shown in Tables 1 through 3.

Table 1. Formulation A ingredients

Ingredient	Type
Dextromethorphan hydrobromide	Active
Acetaminophen	
Doxylamine sulfate	
Acesulfame potassium	Inactive
Alcohol	
Citric acid	
FD&C Blue No. 1	
FD&C Red No. 40	
High fructose corn syrup	
Polyethylene glycol	
Propylene glycol	
Saccharine sodium	
Sodium citrate	
Flavor	--
Purified water	--

Table 2. Formulation B ingredients

Ingredient	Type
Diphenhydramine hydrochloride	Active
Citric acid	Inactive
FD&C Blue No. 1	
Polyoxyl 50 striate	
High fructose corn syrup	
Sodium benzoate	
Propylene glycol	
Saccharine sodium	
Sodium citrate	

Table 3. Formulation C ingredients

Ingredient	Type
Dextromethorphan hydrobromide	Active
Phenylephrine hydrochloride	
Acesulfame potassium	Inactive
Alcohol	
Citric acid	
Benzoic acid	
FD&C Red No. 40	
Disodium EDTA	
Maltitol	
Propylene glycol	
Sodium citrate	
Flavor	--
Purified water	--

All samples were diluted 20,000-fold before analysis as follows. In a 125 mL polypropylene bottle, a 1 g aliquot of each pharmaceutical formulation was diluted using DI water to a final weight of 100 g. The 100-fold diluted sample was further diluted 100-fold to yield a 10,000-fold diluted sample. The 10,000-fold diluted samples were further diluted 2-fold by mixing 50 g of the 10,000-fold diluted sample with 50 g DI water to yield a 20,000-fold dilution. All samples were capped and stored in the bottle at 4 °C until needed.

Robustness study

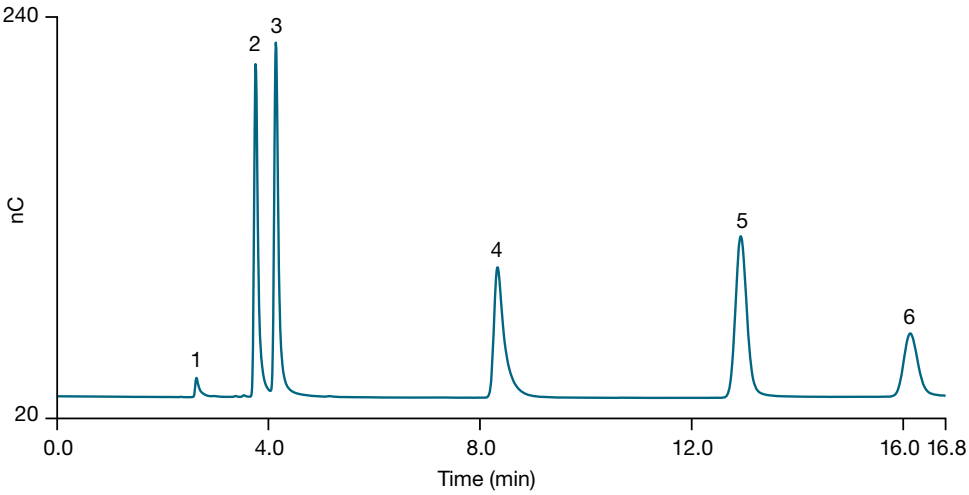
Following the guidelines of USP Physical Tests, <621> Chromatography, method robustness was evaluated by examining retention time (RT), peak asymmetry, and resolution after imposing small variations (±10%) in procedural parameters (e.g., flow rate, eluent concentration, column temperature).[9] A standard mixture containing 15 mg/L of each analyte was injected in triplicate for each condition. The same procedure was applied to another column set from a different lot. The variations tested were as follows:

- Flow rate at 0.25 mL/min, 0.275 mL/min, and 0.3 mL/min
- Eluent concentration at 19.8, 22, and 24.2 mM
- Column temperature at 27 °C, 30 °C, and 33 °C
  [Note: Because the Dionex Integrion system does not have column cooling capacity, for testing lower temperature conditions, the columns were allowed to equilibrate to the room temperature, which was 27 °C and 26.1 °C for columns 1 and 2, respectively].

Results and discussion

Separation

After testing several elution conditions, we designed a method that can separate six carbohydrates in 16.8 minutes. These six were chosen because they are common ingredients in liquid OTC formulations. This method used a Dionex CarboPac PA300 column (2 × 250 mm) and isocratic elution conditions. Figure 1 shows a typical separation obtained using a 2.5 µL injection of a standard containing propylene glycol, mannitol, sorbitol, maltitol, glucose, and sucrose. The concentration of each analyte was 15 mg/L. The peaks are baseline resolved and elute within a window of 16.8 min. Peak retention time and resolutions are shown in Table 4. All the peaks are separated with resolution values ranging from 2.9 to 18.5.



Columns: CarboPac PA300, analytical, 2 × 250 mm; guard 2 × 50 mm
Flow rate: 0.275 mL/min
Inj. volume: 2.5 µL
Column temp.: 30 °C
Tray temp.: 4 °C
Eluent: 22 mM KOH

Peaks:
1 Propylene glycol
2 Sorbitol
3 Mannitol
4 Maltitol
5 Glucose
6 Sucrose

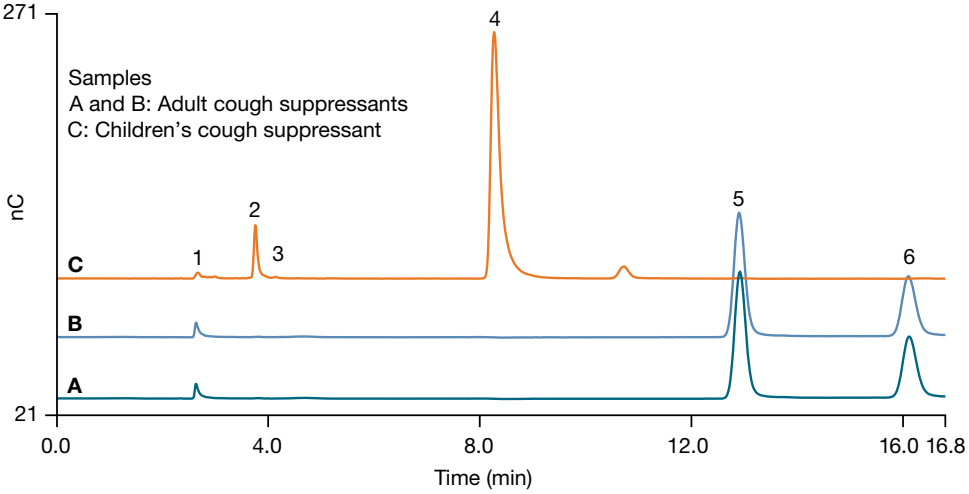
Figure 1. Separation of a mixture of six carbohydrates on a Dionex CarboPac PA300 column

Table 4. Calibration data for glycols and carbohydrates (n=3), calibrated range from 2 to 33.3 mg/L each

No.	Analyte	Retention time (min)	Resolution	Calibration concentrations (mg/L)	Coefficient of determination (r^2)*
1	Propylene Glycol	2.6	8.8	2.0, 4.17, 5.0, 8.33, 10.0, 16.7, 20.0, 26.7, 33.3	0.998
2	Mannitol	3.8	2.9		0.997
3	Sorbitol	4.2	18.5		0.991
4	Maltitol	8.4	13.0		0.998
5	Glucose	12.1	7.3		0.999
6	Sucrose	16.2	--		0.998

* Linear curve fitting was used for all analytes.

Figure 2 shows the chromatograms of three different pharmaceutical formulations (A and B: adult cough suppressants, and C: children's cough suppressant). All samples were diluted 20,000-fold before analysis. Compared with the separation on a Dionex CarboPac MA1 column,[8] the run time of the method proposed here is significantly shorter (16.6 min vs. 60 min). The eluent concentration is also significantly lower. The use of an electrolytically prepared eluent makes the method very convenient and suitable for high throughput analysis. However, this method is not able to resolve glycerol and propylene glycol. Glycerol is frequently present in pharmaceutical formulations and elutes very close to propylene glycol (not shown) under the method conditions described here and other investigated conditions. For that separation, a Dionex CarboPac MA1 method will be more suitable.



Columns: CarboPac PA300, analytical, 2 × 250 mm; guard 2 × 50 mm
Flow rate: 0.275 mL/min
Inj. volume: 2.5 µL
Column temp.: 30 °C
Tray temp.: 4 °C
Eluent: 22 mM KOH

Peaks:
1 Propylene glycol
2 Sorbitol
3 Mannitol
4 Maltitol
5 Glucose
6 Sucrose

Figure 2. Separation of three pharmaceutical formulations on a Dionex CarboPac PA300 column

Linearity and precision

The linearity of the method was studied by generating response curves for all six analytes using a standard mix and analyzing concentrations of each analyte ranging from 2 to 33.3 mg/L. The results included in Table 4 show that the coefficients of determination ranged from 0.983 to 0.999 for all six analytes, indicating a linear response under the concentration range tested. Next, method precision was determined at three concentration levels of the analyte mixture with five replicates of each sample. The results included in Table 5 show excellent peak area and retention time precision for all three concentration levels tested with RSD values below 2%.

Sensitivity

Sensitivity of this assay was determined by calculating the signal-to-noise ratio (S/N) of standards containing known amounts of the analytes. The detection limits for this HPAE-PAD assay under the conditions described here were set at analyte concentrations that result in a S/N of 10:1. The S/Ns were calculated using each analyte's peak height and the noise level calculated from a stable portion of the baseline where no peak elutes. Table 6 contains S/Ns for all six analytes at 1 mg/mL concentration. Based on this data, the assay is least sensitive for propylene glycol, with a S/N of 12 at this concentration. For all other analytes, limits of detections are significantly lower than 1 mg/L.

Accuracy

Method accuracy was determined by spiking known amounts of the six carbohydrates into each of the three pharmaceutical formulations. The spike levels used were 6.66 or 10 mg/L for each analyte. The results in Table 7 show recoveries of the spiked analytes from the three formulations used in this study. The recoveries were between 87 and 108%.

Table 5. Peak area and retention time precision (n=5)

	Concentration (mg/L)	Propylene glycol	Mannitol	Sorbitol	Maltitol	Glucose	Sucrose
RT RSD	1	0.00	0.00	0.00	0.18	0.15	0.21
	5	0.15	0.11	0.09	0.19	0.15	0.22
	15	0.00	0.10	0.11	0.18	0.16	0.22
Peak area RSD	1	1.46	0.81	1.13	0.92	0.81	1.03
	5	1.07	0.47	0.58	0.58	0.27	0.80
	15	2.01	0.69	0.81	0.91	0.93	0.83

Table 6. Signal-to-noise ratios of all six analytes at a concentration of 1 mg/L (n=3)

	Propylene glycol	Mannitol	Sorbitol	Maltitol	Glucose	Sucrose
Signal-to-noise	12.8	358	352	101	148	43.0

Table 7. Recovery studies performed on the three pharmaceutical formulations used in this study (n=3)

Formulation	Spiked amount (mg/L)	Recovery (%)					
		Propylene glycol	Mannitol	Sorbitol	Maltitol	Glucose	Sucrose
A	6.66	98.0	101	102	101	107	101
	10.0	99.1	100	108	104	106	99.4
B	6.66	87.8	87.9	86.9	87.6	93.3	88.3
	10.0	91.8	94.2	101	97.8	97.8	91.5
C	6.66	98.5	102	97.9	95.4	96.7	97.7
	10.0	98.6	101	106	91.8	102	101

Robustness

Assay robustness was determined on two different columns from different lots. The robustness was studied by introducing ±10% variation in common chromatographic parameters. A standard containing 15 mg/L of each analyte was used for this study. The varied parameters were eluent concentration, column temperature, and flow rate. Method performance under these conditions was evaluated by calculating the percent difference in three key chromatographic parameters: retention time, peak asymmetry, and resolution. The results for both columns are included in Tables 8 and 9. For the two columns, all the experimental variations tested did not result in significant disruption of the three target chromatography parameters. These results indicate that the method is robust and suitable for routine determination of the carbohydrates typically present in pharmaceutical formulations.

Table 8. Results of robustness study performed on column 1 using a standard containing 15 mg/L of each analyte (n=3)

Condition		Percent difference (%)																
0.275 mL/min, 22 mM hydroxide, column temperature 30 °C		Retention time						Asymmetry						Resolution				
		PG	Man	Sor	Mal	Glu	Suc	PG	Man	Sor	Mal	Glu	Suc	PG	Man	Sor	Mal	Glu
Eluent concentration	+10%	0.2	-0.3	-0.2	-1.1	-4.2	-5.3	-6.6	0.8	-0.9	1.9	0.8	0.3	-0.2	-0.5	-0.2	-6.6	-4.3
	-10%	0.0	0.3	0.2	1.2	3.9	4.8	-1.0	-0.2	2.0	1.9	-0.3	10.2	0.0	1.0	0.6	6.1	2.8
Flow	+10%	-10.1	-7.0	-14.2	-10.7	-10.4	-10.4	-7.4	-3.4	4.7	2.6	0.3	3.0	-2.3	-0.6	-2.9	-0.3	-2.3
	-10%	8.3	17.0	-0.8	8.9	8.6	8.7	12.4	-6.6	11.2	-1.5	0.8	-8.6	-0.9	2.8	2.3	1.6	2.7
Temperature	+10%	-0.1	-1.1	-1.0	-3.9	-2.6	-1.9	-7.4	1.6	1.1	-9.6	0.6	-1.1	-5.2	-0.5	-1.6	5.2	4.1
	-10%*	0.2	1.4	1.2	5.0	3.5	2.7	-2.3	2.6	2.2	4.9	-1.4	-11.6	3.2	3.6	1.5	-5.2	-5.0

Table 9. Results of robustness study performed on column 2 using a standard containing 15 mg/L of each analyte (n=3)

Condition		Percent difference (%)																
0.275 mL/min, 22 mM hydroxide, column temperature 30 °C		Retention time						Asymmetry						Resolution				
		PG	Man	Sor	Mal	Glu	Suc	PG	Man	Sor	Mal	Glu	Suc	PG	Man	Sor	Mal	Glu
Eluent concentration	+10%	0.1	-0.2	-0.5	-1.5	-4.5	-5.5	-15.7	-5.7	1.8	-1.5	-0.6	-4.1	0.6	-2.5	-2.0	-7.3	-5.3
	-10%	0.1	0.2	0.1	0.7	3.4	4.3	-16.6	-2.6	2.4	-0.7	-1.1	-6.0	1.2	-2.5	-2.1	3.0	1.9
Flow	+10%	-10.1	-18.5	-5.4	-14.3	-14.0	-14.5	-19.8	5.2	-3.2	12.7	-0.6	-1.6	-5.8	-11.2	-14.1	-10.5	-12.7
	-10%	8.6	8.4	8.3	8.2	7.8	7.8	-13.2	2.4	1.4	3.5	1.9	-0.3	1.7	-0.3	-4.2	-5.8	-5.0
Temperature	+10%	0.0	-3.2	-4.0	-12.0	-9.7	-9.3	-19.4	0.9	-1.0	13.7	2.2	-0.8	-12.6	-11.8	-19.6	-2.6	-4.4
	-13.1%*	0.1	0.5	0.5	2.7	1.4	0.4	-9.5	3.0	1.4	7.9	0.3	1.1	4.6	-2.2	-4.1	-12.7	-15.2

* Represents percent difference between 30 °C and average of room temperatures for columns 1 and 2

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Conclusion

This study describes an HPAE-PAD assay for determination of six carbohydrates typically present in OTC pharmaceutical formulations in one chromatographic run. This assay was validated according to the analytical performance characteristics outlined in USP General Chapter <1225>, Validation of Compendial Procedures. The results show that the method provides baseline separation for the six analytes. The method shows excellent precision for retention time as well as peak area, accurately measuring the typical concentrations of these analytes in commercial formulations. Moreover, the method is robust to experimental condition variations that may occur during routine use.

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