Reduce Oligosaccharide Analysis Time with TSKgel SuperOligoPW Size Exclusion Chromatography Columns Packed with Three Micron Particles

TSKgel
APPLICATION NOTE

At the 2011 Pittsburgh Conference, Tosoh Corporation introduced a new line of high efficiency columns for the analysis of water-soluble polymers by gel filtration chromatography. The new product line consists of four columns, of which three contain particles that have been designed with the multi-pore particle synthesis technology which Tosoh scientists pioneered in the mid '90s. The fourth column, TSKgel SuperOligoPW, the topic of this application note, was developed to reduce the analysis time for oligosaccharides and other oligomers with molecular masses in the range of 100 and 3000. Currently, the TSKgel G-Oligo-PW column, which contains spherical 7µm particles packed in a 7.8mm ID x 30cm column, is the preferred column for size analysis of neutral oligosaccharides by gel filtration chromatography. This application note will demonstrate that analysis times of oligosaccharide separations can be reduced by 50%, which in turn can result in lowering cost, improving turnaround time, offering better service, or any combination of these factors.

The characteristics of the TSKgel SuperOligoPW column, together with those of the TSKgel SuperMultiporePW columns, are shown in *Table 1*.

As shown in *Table 1*, the TSKgel SuperOligoPW column is packed with monodisperse particles. The scanning electron micrograph (SEM) in *Figure 1* illustrates the narrow distribution of particle sizes, which helps to reduce the pressure drop across the column.

While a moderate column back pressure helps to reduce wear and tear on the HPLC system, lowering column back pressure is particularly important when working with multiple columns coupled in series, such as shown in the accompanying applications.

Figure 1. Scanning Electron Micrograph (SEM) of particles contained in TSKgel SuperOligoPW columns

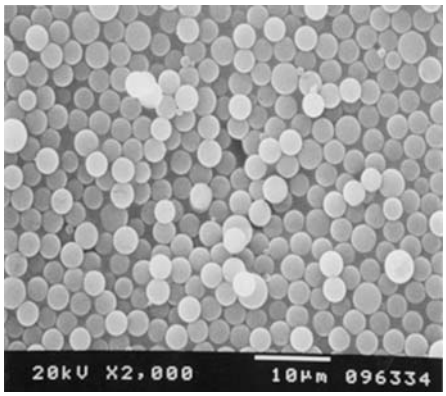
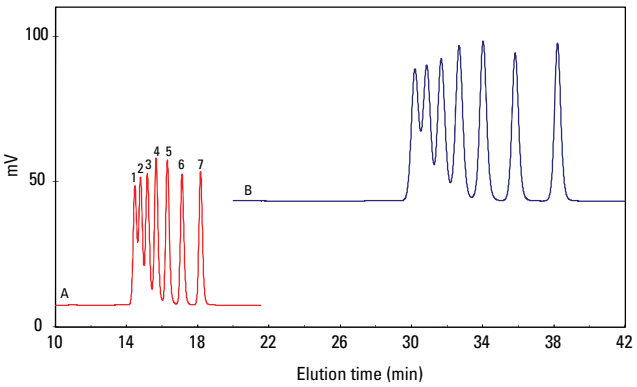


Table 1. TSKgel SuperOligoPW and SuperMultiporePW gel filtration columns

	TSKgel SuperOligoPW	TSKgel SuperMultiporePW-N	TSKgel SuperMultiporePW-M	TSKgel SuperMultiporePW-H
Base material	polymethacrylate	polymethacrylate	polymethacrylate	polymethacrylate
Particle size (µm)	3 (monodisperse)	4 (monodisperse)	5 (monodisperse)	8 (6 - 10)
Max. exclusion limit MW (PEO,PEG/H_2O)	4,000 - 8,000	100,000 - 150,000	600,000 - 1,500,000	–
Range of applicable MW (PEO,PEG/H_2O)	100 - 3,000	300 - 50,000	500 - 1,000,000	1,000 - 10,000,000
Theoretical plates/column	>16,000	>16,000	>12,000	>7,000
Pressure drop (MPa)	4 - 6	4 - 6	2 - 4	1 - 2
Column size	6.0mm ID x 15cm	6.0mm ID x 15cm	6.0mm ID x 15cm	6.0mm ID x 15cm
Guard column	4.6mm ID x 3.5cm	4.6mm ID x 3.5cm	4.6mm ID x 3.5cm	4.6mm ID x 3.5cm

Figure 2. Analysis of a maltodextrin sample on TSKgel SuperOligoPW and G-Oligo-PW columns



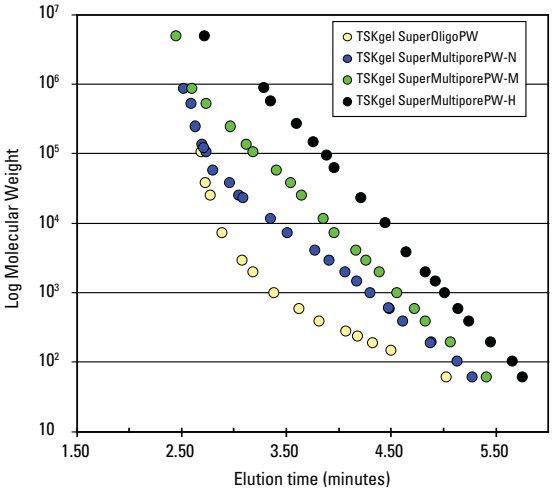
Columns:	A: TSKgel SuperOligoPW, 6.0mm ID x 15cm x 4 B: TSKgel G-Oligo-PW, 7.8mm ID x 30cm x 4
Mobile phase:	H_2O
Flow rate:	A: 0.6mL/min B: 1.0mL/min
Detection:	RI
Temperature:	40°C
Injection vol.:	A: 10µL B: 50µL
Samples:	1. maltoheptose 2. maltohexose 3. maltopentose 4. maltotetraose 5. maltotriose 6. maltose 7. glucose

As is the case in all modes of HPLC, reducing the particle size by a factor of two (in this case from 7 to 3µm) either results in (1) the same analysis time but with much better resolution when using columns of the same length, or in (2) the same resolution but at least a 50% reduction in analysis time if the length of the smaller particle size column is half of that of the larger particle size column. This last benefit is illustrated in *Figure 2*, which shows that for a maltodextrin sample, with glucose units varying in length from maltoheptose to glucose, approximately the same resolution can be achieved in less than half the time on the 15cm TSKgel SuperOligoPW column when compared to the conventional 30cm TSKgel G-Oligo-PW column.

Figure 3 shows the calibration curves for each of the new TSKgel PW-type columns for polyethylene oxide (PEO), polyethylene glycol (PEG) and ethylene glycol (EG) standards. In contrast to the three TSKgel SuperMultiporePW columns, the calibration curve for the TSKgel SuperOligoPW column has a limited linear range that makes the column most suitable for oligosaccharides and other water-soluble polymers from a degree of polymerization of 1 to a molar mass of about 3000.

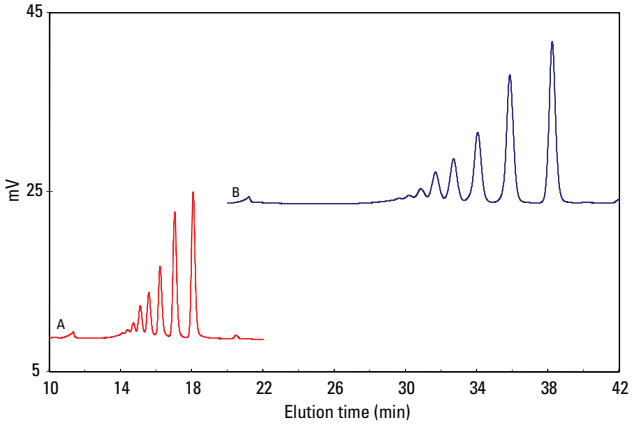
A comparative analysis of maltopentose hydrolysate is shown in *Figure 4*. Of each column type, four columns are coupled in series. It is clear that the shorter TSKgel SuperOligoPW columns allow for a substantial reduction in analysis time while resolution on this column set is very similar to that on four TSKgel G-Oligo-PW columns. Like the results shown in *Figure 2*, a 10µL injection on the four 15cm columns results in similar peak heights as obtained from a 50µL injection on the set of four 30cm columns. Part of this is due to smaller particle size, which leads to higher efficiency and thus less band broadening, but the shorter column length and the use of a narrower column diameter also play a role. The relative contributions of each of these band broadening effects have been calculated in *Table 2*.

Figure 3. Calibration curves for TSKgel SuperOligoPW and SuperMultiporePW columns



Columns:	6.0mm ID x 15cm TSKgel SuperOligoPW TSKgel SuperMultiporePW-N TSKgel SuperMultiporePW-M TSKgel SuperMultiporePW-N
Mobile phase:	H_2O
Flow rate:	0.6mL/min
Detection:	RI
Temperature:	25°C
Sample:	PEO, PEG and EG

Figure 4. Analysis of maltopentose hydrolysate on TSKgel SuperOligoPW and G-Oligo-PW columns



Columns:	A: TSKgel SuperOligoPW, 6.0mm ID x 15cm x 4 B: TSKgel G-Oligo-PW, 7.8mm ID x 30cm x 4
Mobile phase:	H_2O
Flow rate:	A: 0.6mL/min B: 1.0mL/min
Detection:	RI
Temperature:	40°C
Injection vol.:	A: 10µL B: 50µL
Sample:	hydrolysate of maltopentose

Table 2. Relative importance of individual contributions to band broadening

Column size (mm ID x cm)	6.0 x 15	7.8 x 15	7.8 x 15	7.8 x 30
Column volume (µL)	4,239	7,164	7,164	14,328
Column porosity	0.80	0.80	0.80	0.80
Unretained volume (µL)	3,391	5,731	5,731	11,462
Efficiency (N)	16,000	16,000	14,000	14,000
Peak volume (µL)	107	181	194	387
Relative peak height	100	59	55	28

Notes

To calculate the relative importance of various contributions to peak height, *Table 2* shows two additional column dimensions as intermediate cases. The peak height of the 6mm ID x 15cm TSKgel SuperOligoPW column was arbitrarily selected as 100%. If we assume that a 7.8mm ID x 15cm column packed with the same 3µm particles would again have 16,000 theoretical plates, the signal would be 41% lower simply due to the larger internal diameter of the column. If the number of plates is reduced to 14,000 the peak height drops another 4%, while a 30cm column of 7.8mm ID and 14,000 plates, representing the conventional TSKgel G-Oligo-PW column, results in a further reduction of the peak height, down to 28% of the peak height of the new column. In other words, a 40µL injection, rather than a 50µL injection, would have resulted in the same peak heights at the same signal to noise ratio on both columns.

Conclusion

A new column for the analysis of oligosaccharides by size exclusion chromatography, TSKgel SuperOligoPW, provides the same resolution in half the analysis time and with a 4 to 5-fold improvement in sensitivity compared to the conventional TSKgel G-Oligo-PW column. This advancement was made possible by packing 3µm monodisperse spherical particles in 15cm columns of 6mm internal diameter. Applications demonstrated the expected benefit of the new column.

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TOSOH BIOSCIENCE

TOSOH Bioscience LLC
3604 Horizon Drive, Suite 100
King of Prussia, PA 19406
Orders & Service: (800) 366-4875
Fax: (610) 272-3028
www.separations.us.tosohbioscience.com
email: info.tbl@tosoh.com

Analysis of 2AB-labeled Isomalto-oligosaccharides by LC/MS

Elucidation of the structure and function of sugar chains is one of the central topics of post-genome research and marked advances have recently been made in this area. After derivatization, sugar chain structures are analyzed with reversed phase HPLC or normal phase HPLC (HILIC) or using two-dimensional reversed phase/normal phase LC. Recently, with advances in MS, analysis using LC/MS (/MS) and MALD-TOF-MS has come into wide use.

Introduced here is an example of HILIC/MS analysis of a sugar chain derivatized with 2-aminobenzoic acid. By optimizing conditions using the TSKgel Amide-80, 3µm as the separating column, good separation/identification was achieved as the sugar chain was separated from 3 to 26 structural components.

**These data were generously provided by Dr. Mitsuhiro Kinoshita from the School of Pharmaceutical Sciences at Kinki University.*

Figure 1. Analysis of 2AA-labeled isomalto-oligosaccharide by LC/MS using TSKgel Amide-80, 3µm column

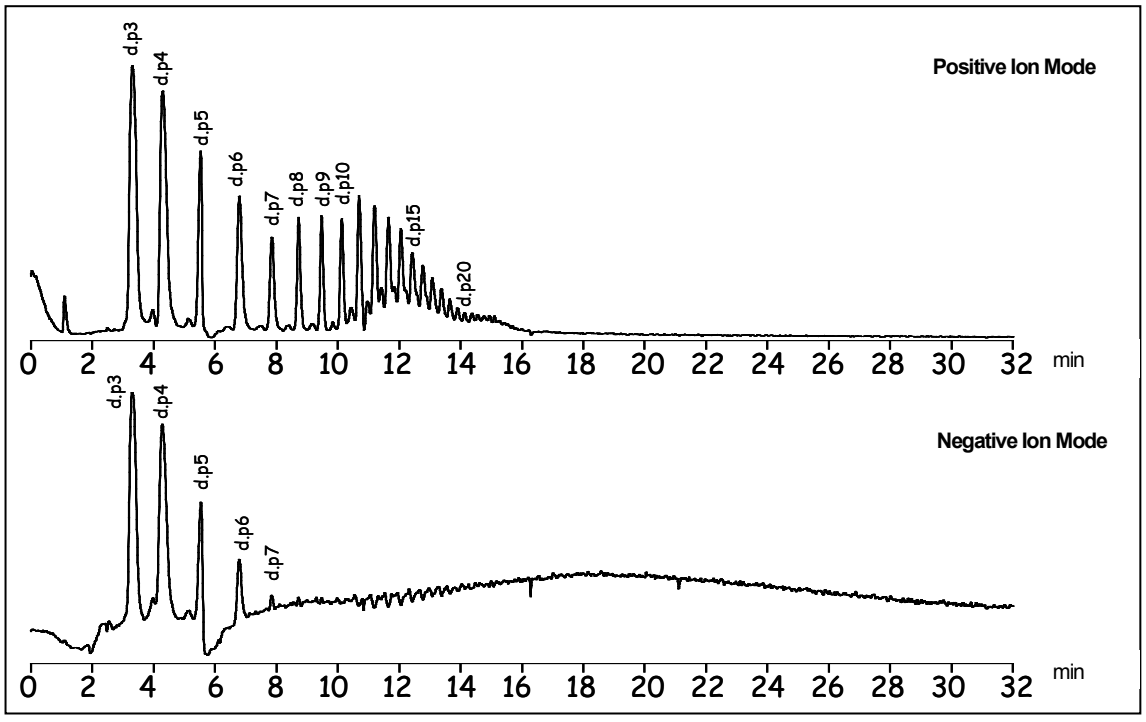


Table 1. Conditions

Column:	**TSKgel Amide-80, 3µm, 2.0mm ID x 15cm**
Mobile phase:	A: 15 mmol/L CH_3COONH_4/CH_3CN=10/90B: acetonitrile B: 15 mmol/L CH_3COONH_4/CH_3CN=195/5
Gradient:	0 min (30%B) → 2 min (30%B) → 32 min (90%B)
Flow rate:	0.25mL/min
Temperature:	40
Injection vol.:	2µL
Instrument:	QTRAP® (Applied Biosystems)
Ion Source:	ESI
CDL temp.:	190°C
Detector voltage:	17.1kV
Neuburising gas:	1.5mL/min

Figure 2-1. ESI-MS spectra of 2AA-labeled isomalto-oligosaccharide (positive mode)

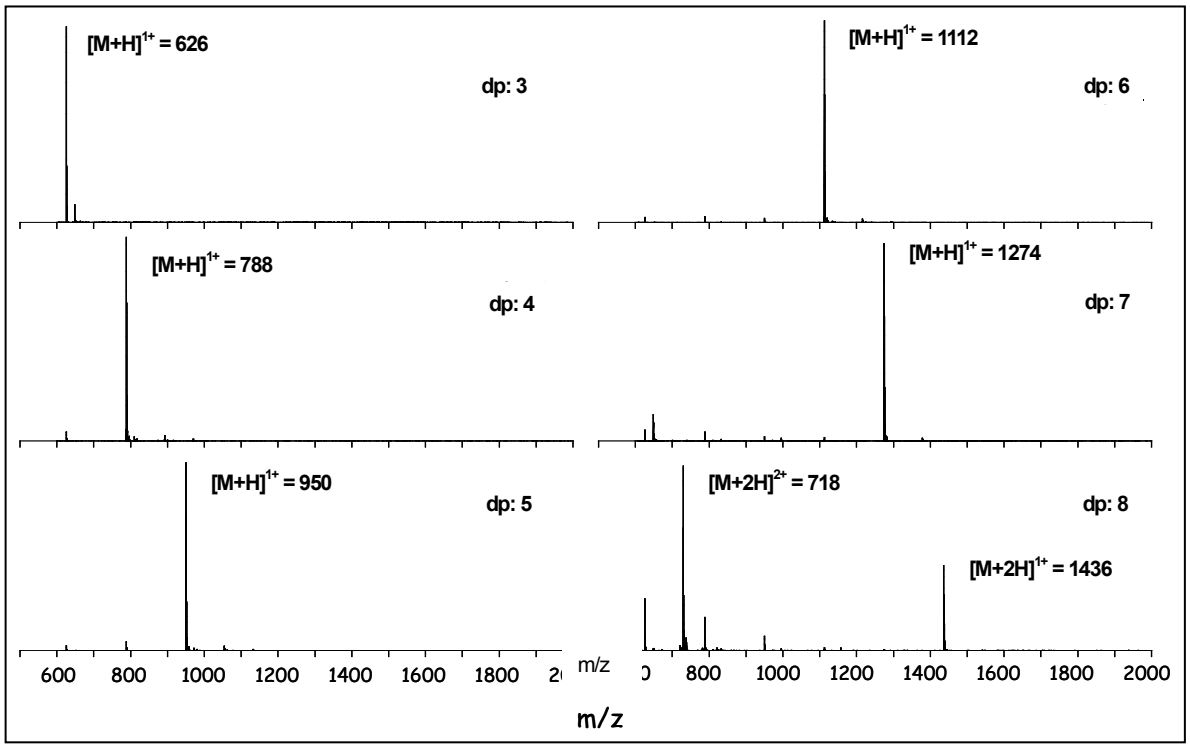


Figure 2-2. ESI-MS spectra of 2AA-labeled isomalto-oligosaccharide (positive mode)

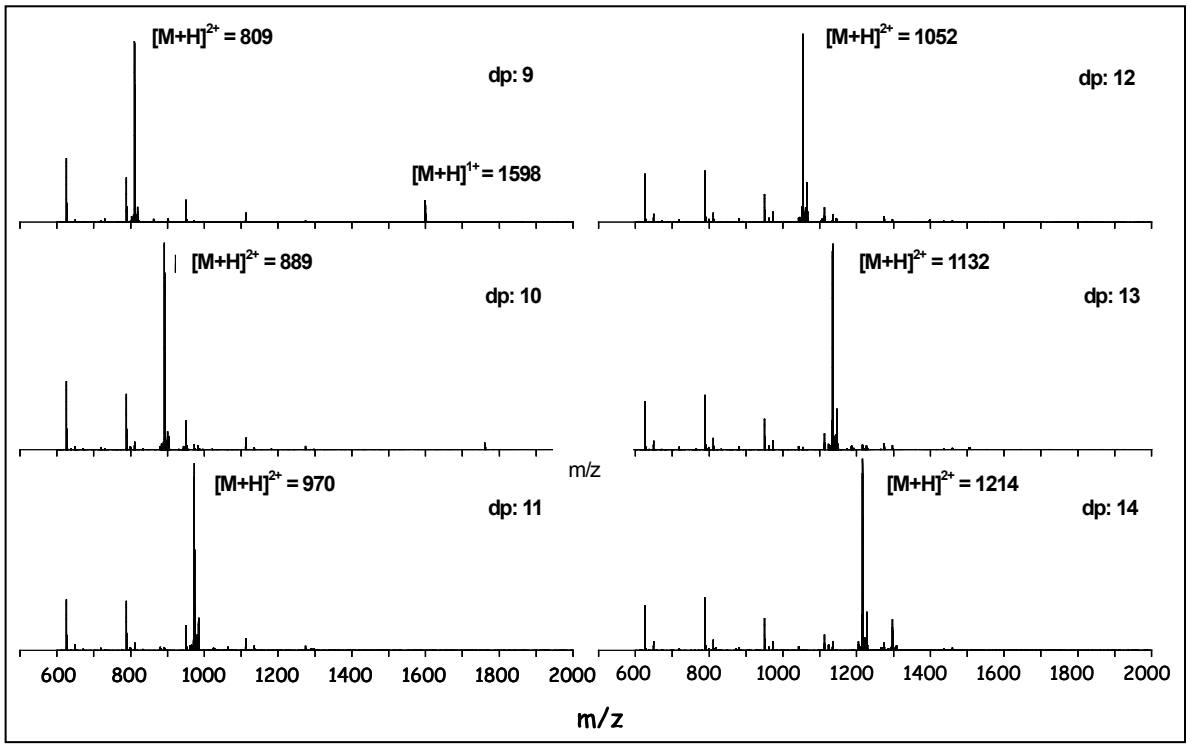


Figure 2-3. ESI-MS spectra of 2AA-labeled isomalto-oligosaccharide (positive mode)

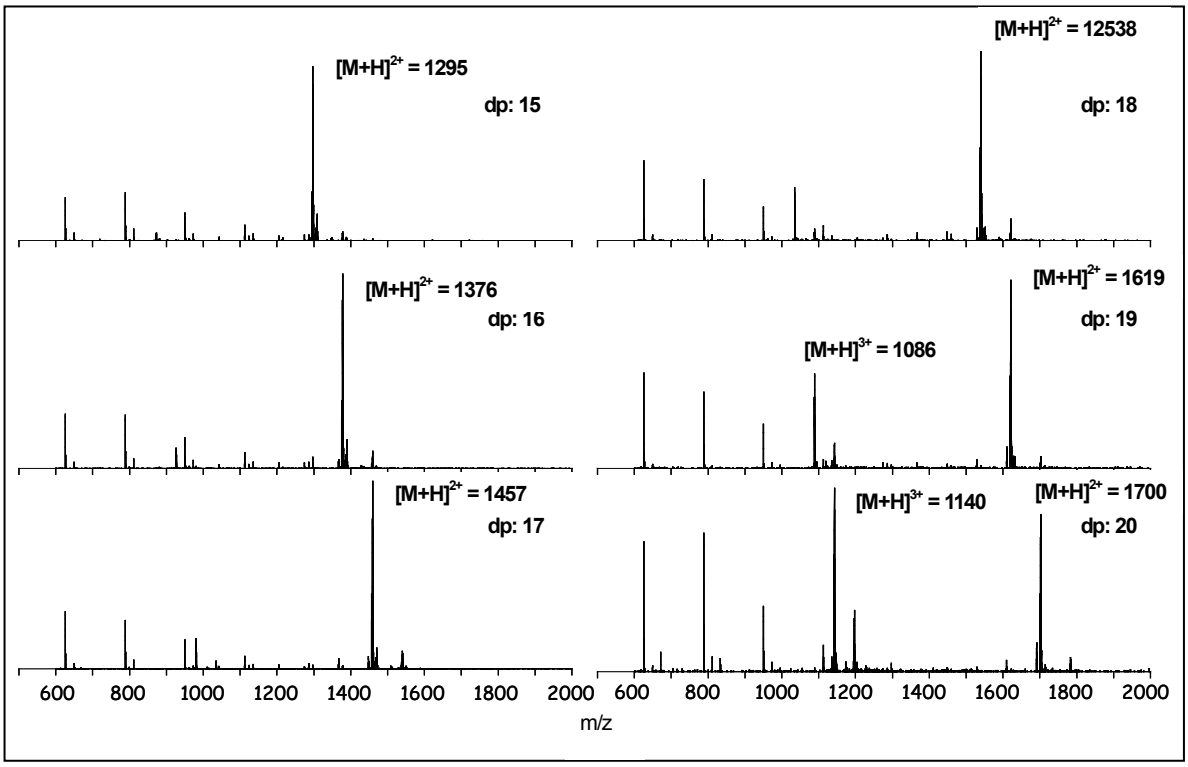
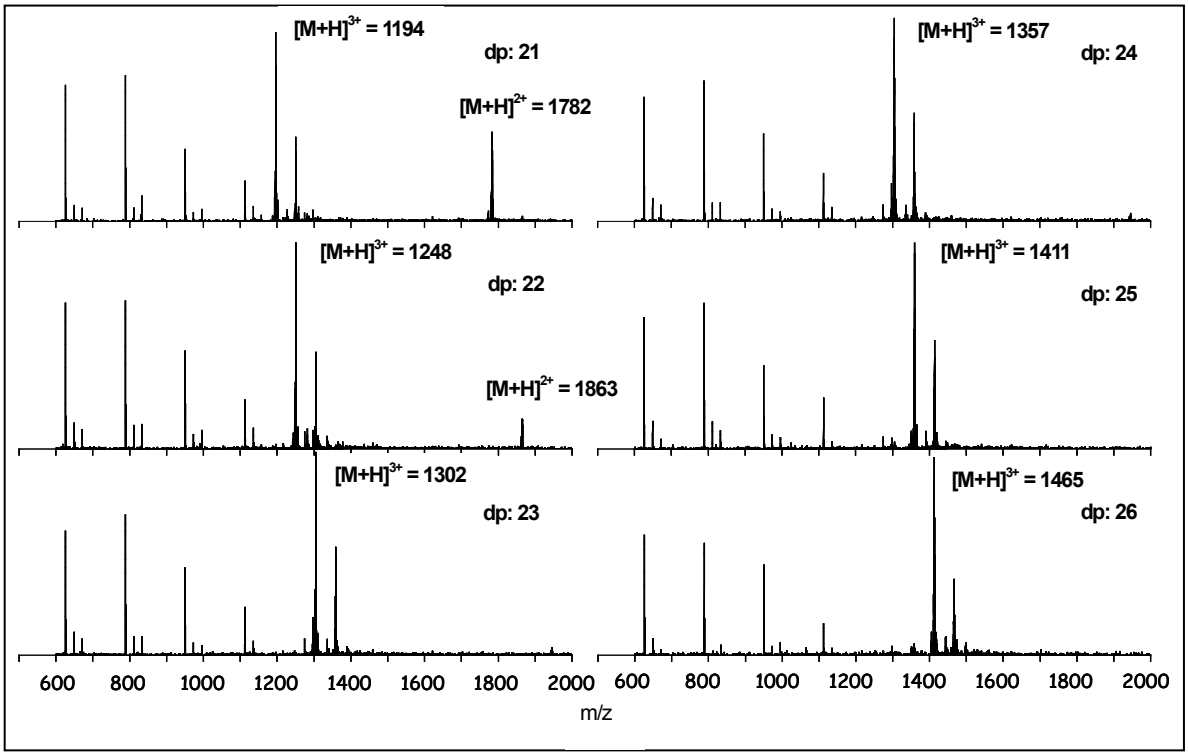


Figure 2-4. ESI-MS spectra of 2AA-labeled isomalto-oligosaccharide (positive mode)



Analysis of Cyclodextrins in Instant Food:

Application example using an evaporative light scattering detector (ELSD)

Cyclodextrins are cyclic oligosaccharides bound to D-glucose units, which, by including a guest molecule in the hollow portion of their structure, have the ability to change the properties of the guest molecule. Taking advantage of this property, cyclodextrins are widely used as food additives serving a variety of functions such as changing volatile substances into to refractory substances, changing poorly soluble substances into substances that are readily soluble in water, and stabilizing unstable substances. Three types of cyclodextrins are used in Japan, with α-, β-, and γ-cyclodextrin used with different numbers of D-glucose bonds.

Each of these cyclodextrins was separated using the HILIC mode and detected by ELSD. Also shown is an application example of the analysis of cyclodextrins in instant green tea. Using these analytical conditions, good separation of each of these cyclodextrins was able to be achieved. In addition, ELSD calibration curve data resulted in a polynomial approximation curve, similar to that of a charged particle detector. Figure 4 shows an example of a calibration curve for α-cyclodextrin.

Table 1. Analytical conditions

Column:	TSKgel Amide-80 3μm, 4.6mm ID x 15cm
Mobile phase:	A: water
	B: acetonitrile
	A/B = 35/65
Flow rate:	1.0mL/min
Detection:	ELSD (Agilent Technologies)
	Temp.: 40℃, Nebulize4 gas; N2, Gas pressure: 350kPa, Gain; 6
Temperature:	40°C
Injection vol.:	10μL

Figure 1. Chromatogram of cyclodextrins (0.5g/L each)

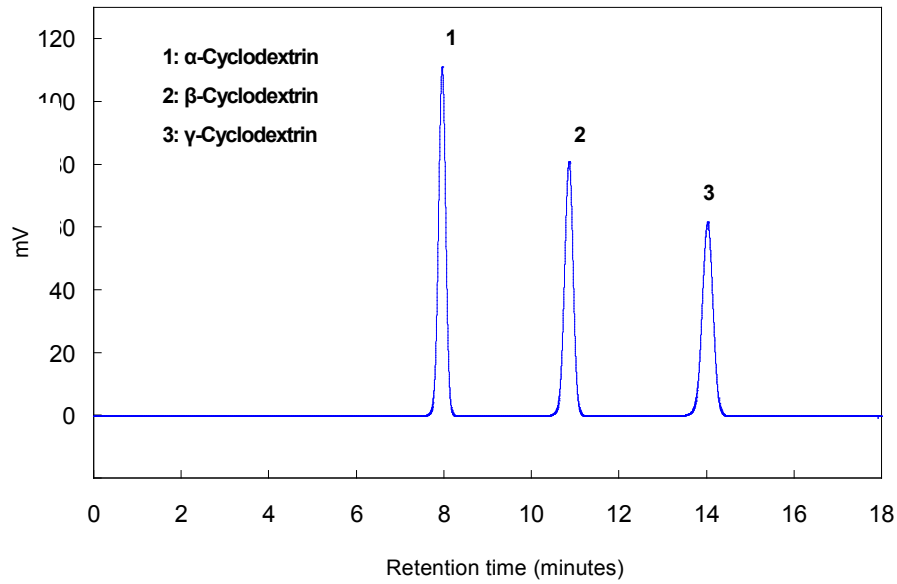


Figure 2. Preprocessing of instant green tea

Weigh out 0.5g of powdered green tea, and add 20mL water

↓

Mix, then let stand

↓

Filter the supernatant [Maishori Disc W-25-5 (cellulose acetate, pore size: 045μm)]

↓

Take 4mL of filtrate and make to 20mL with 50% acetonitrile aqueous solution

↓

After filtering (Maishori Disc H-25-5 (PTFE, pore size: 0.5μm), analyze the filtrate

Figure 3. Chromatogram of instant green tea

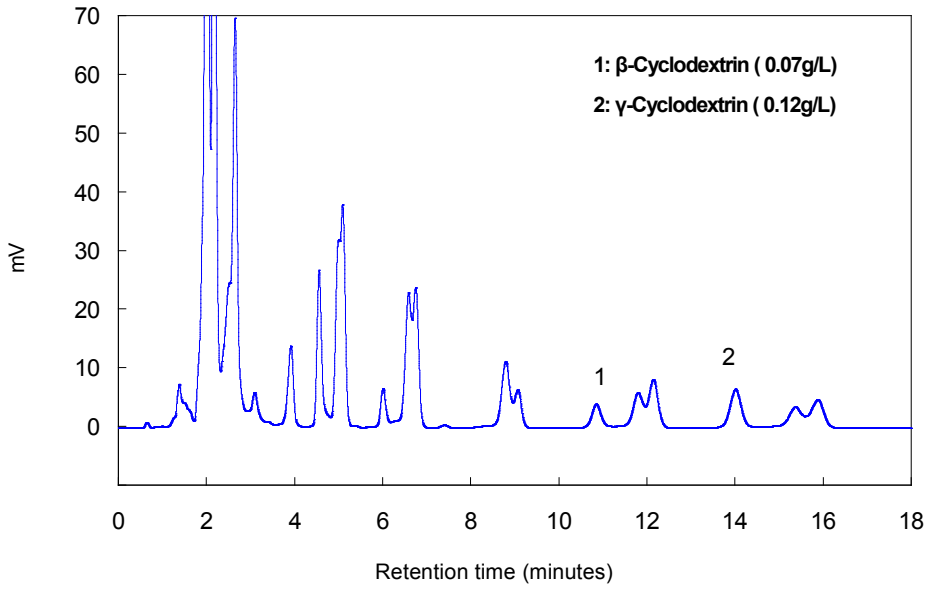
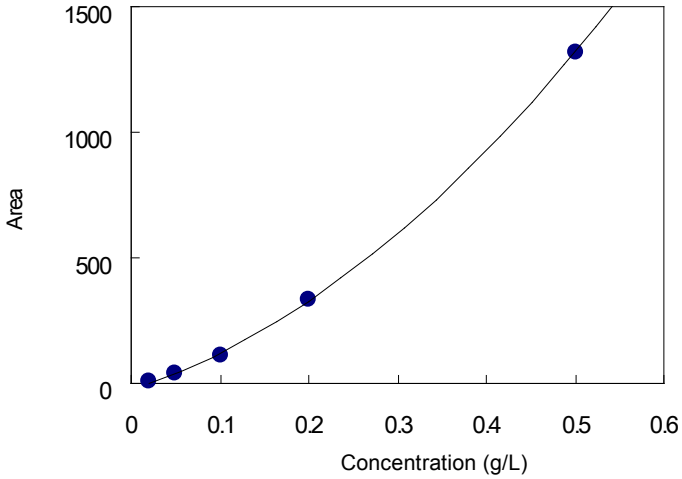


Figure 4. Calibration curve data (α-cyclodextrin)



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TOSOH Bioscience LLC
3604 Horizon Drive, Suite 100
King of Prussia, PA 19406
Orders & Service: (800) 366-4875
Fax: (610) 272-3028
www.separations.us.tosohbioscience.com
email: info.tbl@tosoh.com

Gradient Separation of Oligosaccharides in Alcoholic Beverages:

application using Evaporative Light Scattering Detector (ELSD)

Gradient elution is commonly used in hydrophilic interaction chromatography (HILIC) of oligosaccharides to decrease analysis time while maintaining high resolution of individual oligomers. Since refractive index (RI) detectors, which are generally used for detecting sugars, are not compatible with gradient elution, we employed evaporative light scattering (ELS) to detect the sample components following their separation on a high resolution TSKgel Amide-80 HR column. Examples of the separation of various oligosaccharides by gradient elution using ELSD are introduced here.

Table 1. Conditions

Column:	**TSKgel Amide-80 HR, 5µm, 4.6mm ID x 25cm**
Mobile phase:	A: water
	B: acetonitrile
Gradient:	0min (80%B) → 25min (40%B) → 27min (40%B) → 28min (80%B)
Flow rate:	1.0mL/min
Detection:	ELSD (Sedere)
	Temp.: 40°C, Nebulizer gas: N_2, Gas pressure: 360kPa, Gain: 1
Temperature:	80°C
Injection vol.:	20µL

Figure 1. Chromatogram of oligosaccharide reference standards (0.2g/L each)

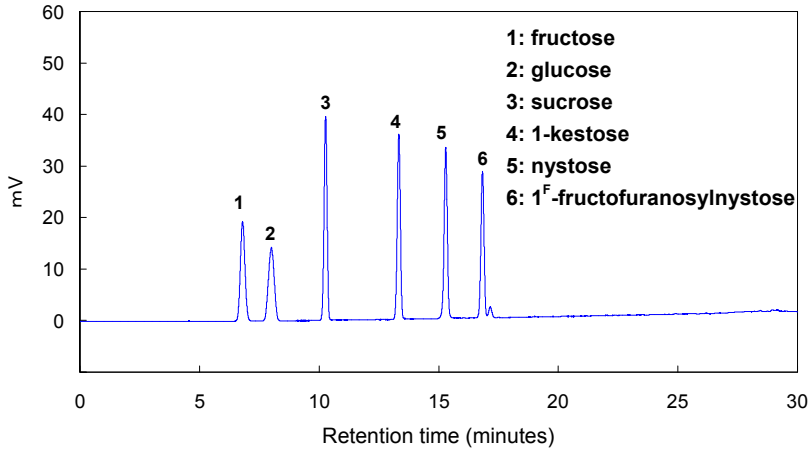


Figure 2. Chromatogram of lactooligosaccharide reference standards (0.2g/L each)

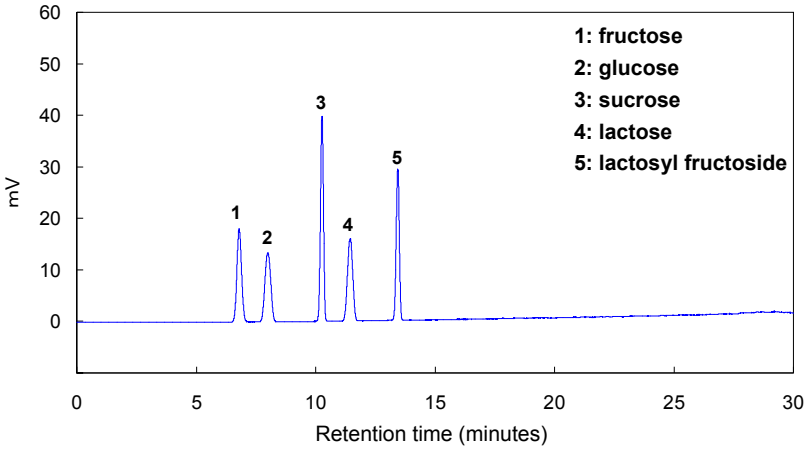
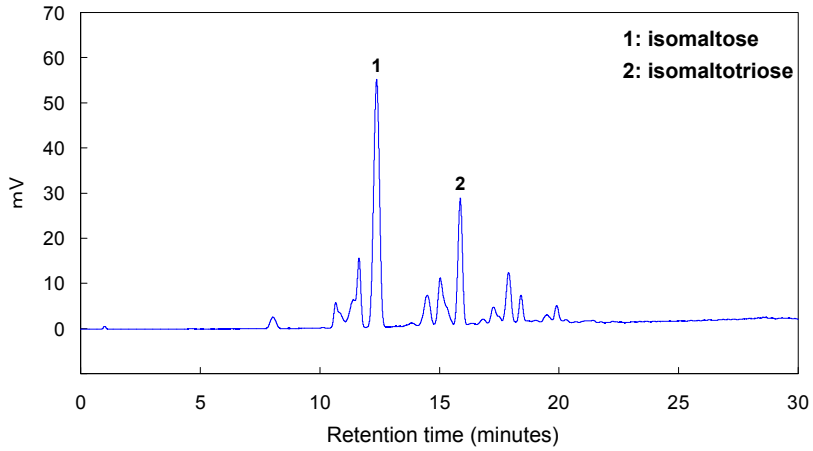
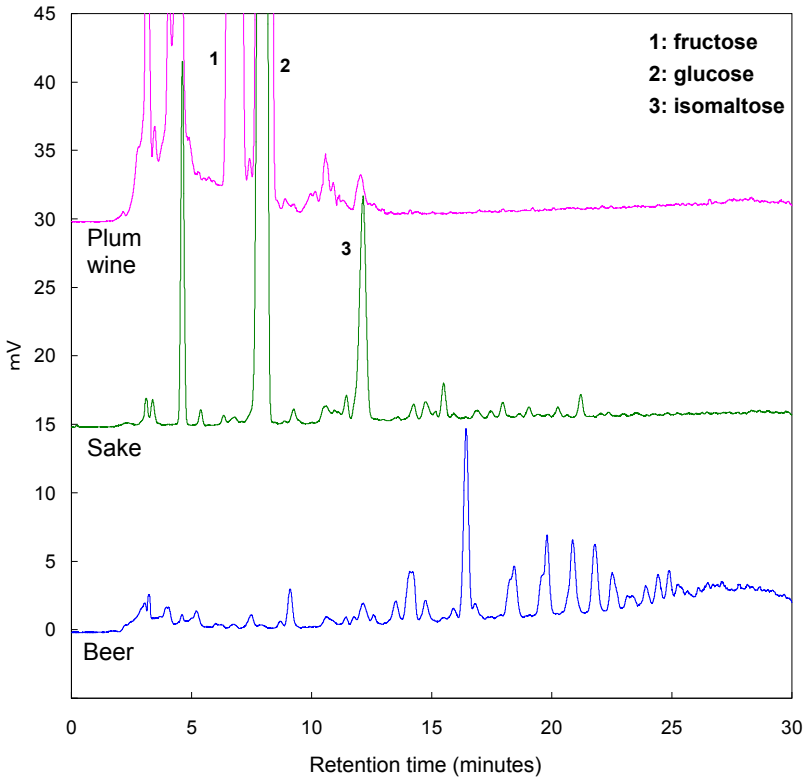


Figure 3. Chromatogram of isomaltooligosaccharide reference standards (1g/L)



Retention time (minutes)

Figure 4. Chromatogram of alcoholic beverages (diluted 25-fold in 50% aqueous solution of acetonitrile)



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TOSOH Bioscience LLC
3604 Horizon Drive, Suite 100
King of Prussia, PA 19406
Orders & Service: (800) 366-4875
Fax: (610) 272-3028
www.separations.us.tosohbioscience.com
email: info.tbl@tosoh.com