Advion

SIMPLE LC/MS ANALYSIS OF CARBOHYDRATES USING THE ADVION expression COMPACT MASS SPECTROMETER

APPLICATION NOTE

Mass Spec: expression CMS

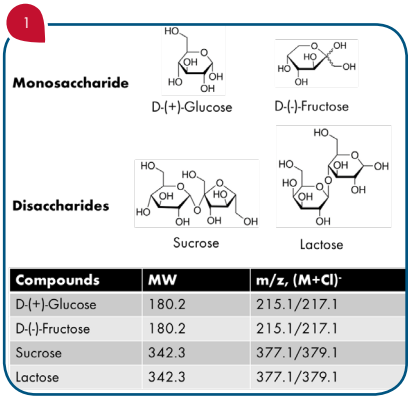
In this application note, post-column chloride attachment is used for the analysis of samples containing carbohydrates using the Advion expression Compact Mass spectrometer (CMS).

INTRODUCTION

There are many separation and detection methods available for the determination of saccharides. These include GC-MS with derivatization, HPLC-UV with derivatization, HPAEC-PAD (High-performance anion-exchange chromatography coupled with pulsed amperometric detection), and LC-MS. The availability of a sensitive and accurate method is still a challenge. For an LC-MS method, given the high polarity, hydrophilicity and low proton affinity[1] of these compounds, it can be difficult to ionize them directly by electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). The chemical diversity and numerous isomers of carbohydrates further complicate their analysis with LC-MS.

Addition of metal salts (e.g. Na^+, Ca^{2+} or Cs^+)[2,3] to promote adduct ions by ligand exchange or postcolumn modifier is often used to improve the sensitivity in the ESI mode. However, salt accumulation on the inlet surface of the MS also will compromise its performance. Anion adduction in the negative APCI mode[4] can be accomplished by post-column introduction of trace chlorinated solvents to promote ionization of these carbohydrate compounds without the loss of MS performance by salt accumulation.

In this application note, post-column chloride attachment is used for the analysis of samples containing carbohydrates using the CMS. The ^{35}Cl and ^{37}Cl isotopes also provide confirmation for the formation of the chloride adducts in negative ion APCI mode. Four carbohydrates (Fructose, Glucose, Sucrose and Lactose) are used for method verification.



Compounds	MW	m/z, $(M+Cl)^-$
D-(+)-Glucose	180.2	215.1/217.1
D-(-)-Fructose	180.2	215.1/217.1
Sucrose	342.3	377.1/379.1
Lactose	342.3	377.1/379.1

Figure 1: Chemical composition of Fructose, Glucose, Sucrose, and Lactose.

METHOD

MS: expression CMS
Negative ion APCI, Capillary Temp: 100 °C, Capillary Voltage: 130 V, Source Voltage: 10 V, Source Voltage Span: 50 V, Source Temp: 350 °C, Corona Discharge: 5 µA, Scan Range: *m/z* 150 - *m/z* 500

LC: Agilent 1220 Infinity LC System
Column: Phenomenex, Luna-NH2, 100 x 2.1 mm, 30 °C, Solvent 1: water/acetonitrile, 20/80, v/v, 300 µL/min was used for isocratic separation, Solvent 2: methanol/chloroform, 90/10, v/v, 50 µL/min was T'd in post-column to promote Cl-adducts by APCI.

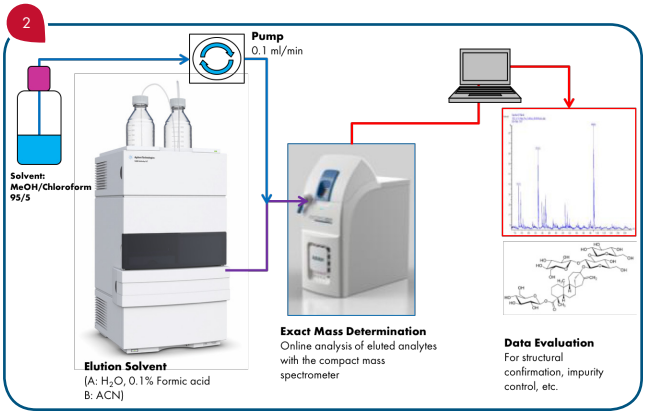


Figure 2: Experimental Setup LC/MS with post-column addition of Chloroform to produce negative ion APCI chloride attachment ions.

RESULTS

As demonstrated, the calibration curves of Fructose (Figure 3) and Sucrose (Figure 4) cover the concentration range from 0.5 to 100 ng/µL with good linearity, $R^2 > 0.997$. With three injections for each concentration, the %RSD varies from 0.5 to 10%. The signal–to–noise (S/N) at 0.5 ng/µL is >44.

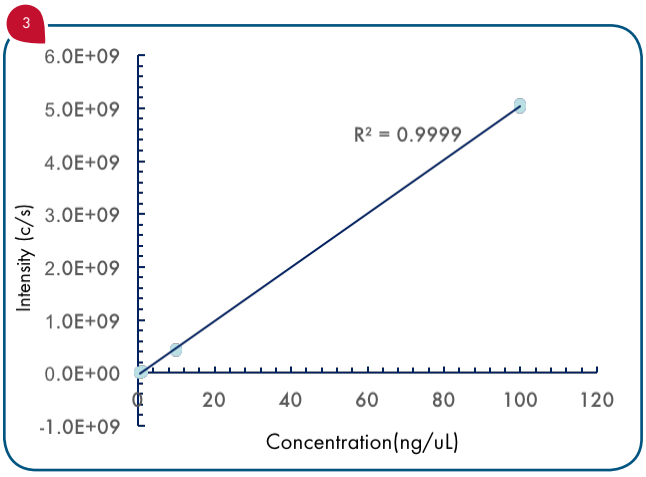


Figure 3: The calibration curve for Fructose.

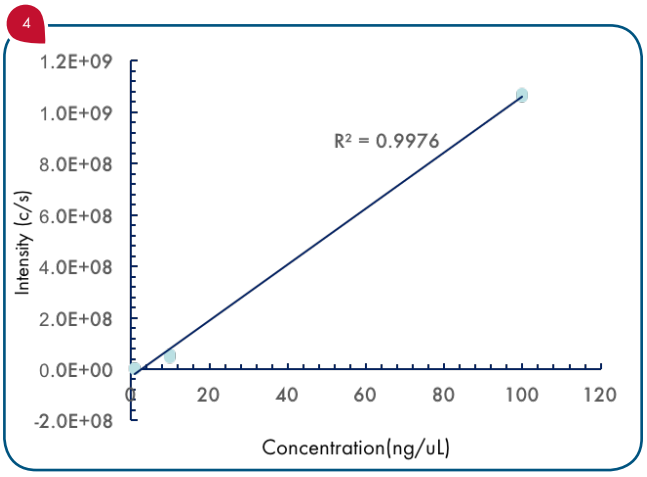


Figure 4: The calibration curve for Sucrose.

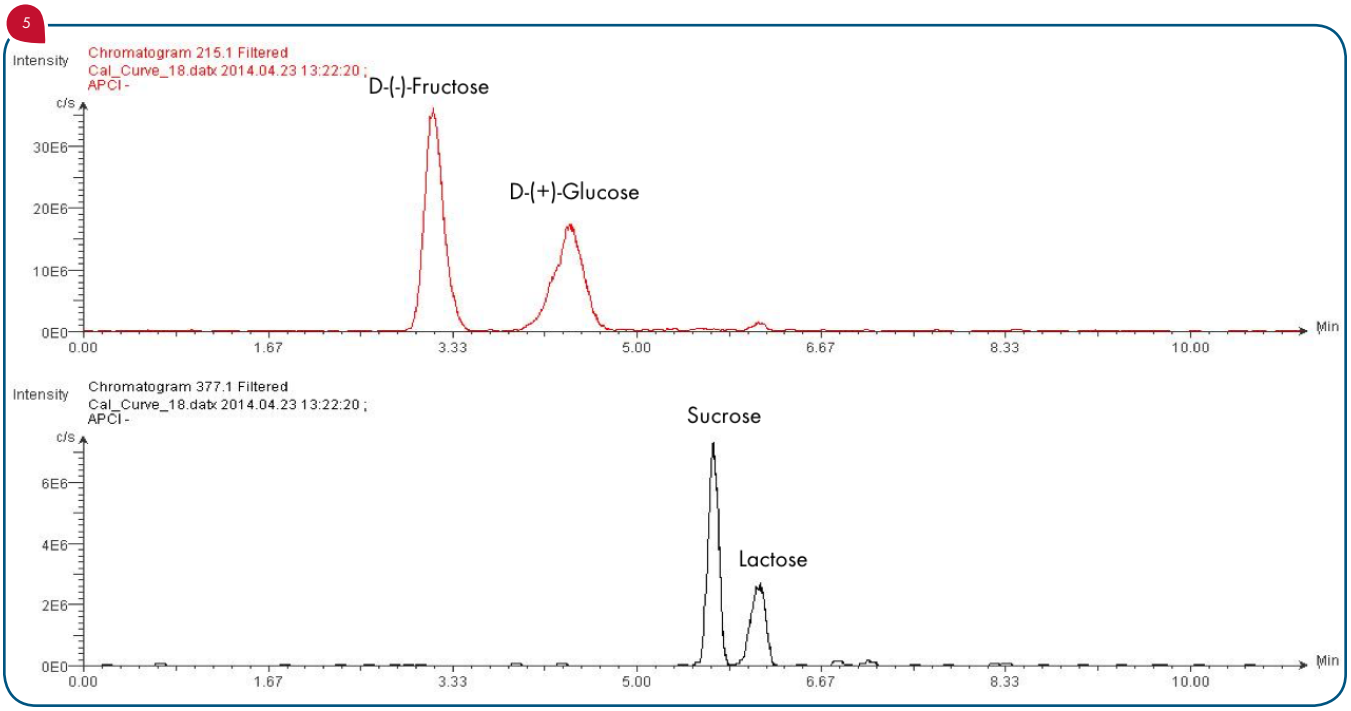


Figure 5: (A) An extracted ion chromatogram (XIC) of D-(-)-Fructose and D-(+)-Glucose at *m/z* 215.1, (B) XIC of Sucrose and Lactose at *m/z* 377.1.

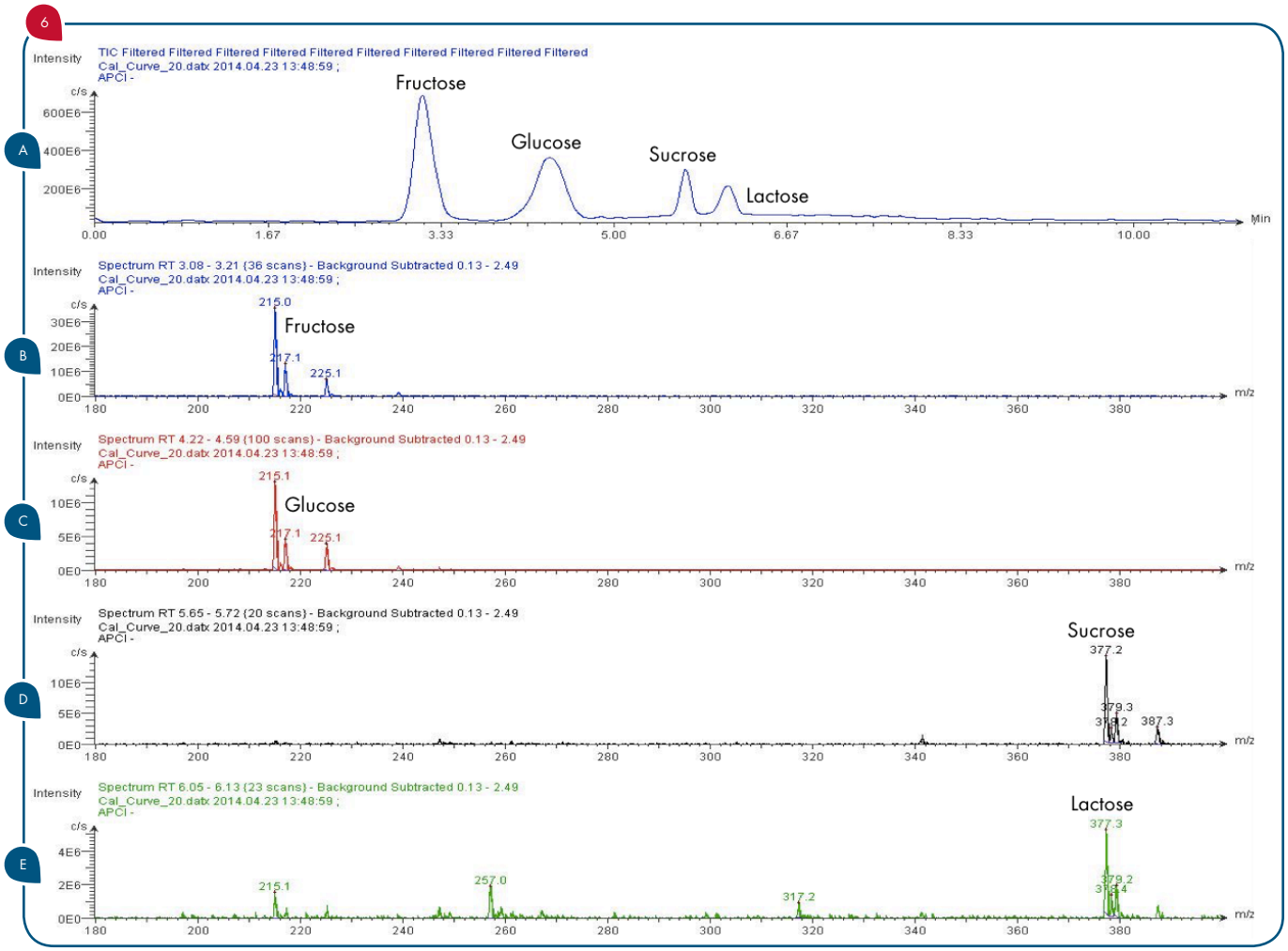


Figure 5: (A) Total ion chromatogram (TIC) of the four carbohydrates, (B) mass spectrum of D-(-)-Fructose, (C) mass spectrum of D-(+)-Glucose, (D) mass spectrum of Sucrose, (E) mass spectrum of Lactose.

SUMMARY

- The described LC/MS method with the Advion CMS system for carbohydrate analysis is simple and cost-effective.
- With Cl^- anion adduction, it is simple to confirm the analyte ion with the $^{35}CL/^{37}CL$ isotope ratio.

REFERENCES

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[2]Cheng, C.; Tsai, H.-R.; Chang, K.-C.; 2006. On-line cut-off technique and organic modifier addition aided signal enhancement for trace analysis of carbohydrates in cellulase hydrolysate by ion exclusion chromatography-electrospray ionization mass spectrometry.
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[3]Qian, W.L.; Khan, Z.; Watson, D. G.; Fearnley, J.; 2008 Analysis of sugars in bee pollen and propolis by ligand exchange chromatography in combination with pulsed amperometric detection and mass spectrometry. Journal of Food Composition and Analysis 21. 78–83

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