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Application News

Gas Chromatograph Mass Spectrometer GCMS-TQ™8050 NX

A Sample Prep-Free Analysis of Saccharides Mixtures with "*Smart IS*+" and "*SMCI*+"

- Authentication and Adulteration Studies -

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User Benefits

- "*Smart IS*+" and "*SMCI*+" setups enable direct and quick qualitative GC/MS analysis of saccharides without the need for tedious derivatization steps.
- "*SMCI*+" setup delivers convenience and safety to carry out positive chemical ionization and requires only common laboratory solvents as the reagent gas; Alternate between electron ionization and positive chemical ionization modes with "*Smart IS*+"
- "*Smart IS*+" and "*SMCI*+" enable quick preliminary authentication and adulteration studies of saccharides due to their unique temperature elution profiles

■ Introduction

Saccharides have long been established as crucial energy storage materials, structural components, and primary metabolites that play major roles in physiological development. Saccharides can be divided into four groups, mono-, di-, oligo- and polysaccharides. Mono- and disaccharides are the basic building blocks for the larger saccharides.

Due to the high degree of functionality in the molecules, it is often necessary to protect all hydroxyl groups before analysis with the gas chromatography/mass spectrometry (GC/MS) technique. The usage of a direct probe as a sample inlet in GC/MS provides an alternative technique that eliminates the need for tedious sample preparation. This benefit paves the way for a quick and direct method to obtain preliminary results in product authentication and adulteration investigations.

This article demonstrates the application of a direct probe in conjunction with a Smart EI/CI ion source (Smart IS) or solvent mediated chemical ionization (SMCI) unit for the analysis of saccharides. The mass spectra of saccharides generated by Smart IS and SMCI units will be evaluated, followed by examples of their usage for preliminary authentication and adulteration studies.

■ Measurement Conditions and Samples

Analytical Setup.

The analytical results in this report were generated using a Direct Sample Inlet (DI) probe in conjunction with a Smart IS or SMCI unit. The combination of DI with Smart IS or SMCI unit is hence known as "*Smart IS*+" and "*SMCI*+" in this article, respectively (Fig. 1).

The DI probe is designed to be able to fit a miniature sample vial at its tip. The sample vial is thereafter placed close to the ion source and subsequently heated up according to a temperature program. The chemicals in the sample vial are hence volatilized and ionized in the ion source.

Smart IS is a 2-in-1 ion source that enables both electron ionization (EI) and positive chemical ionization (PCI) modes. PCI is achieved with the usage of isobutane gas as a reagent gas. Due to the simplicity of switching between two different ionization modes with Smart IS, the PCI mode attained with Smart IS is referred to as quick chemical ionization (QCI).

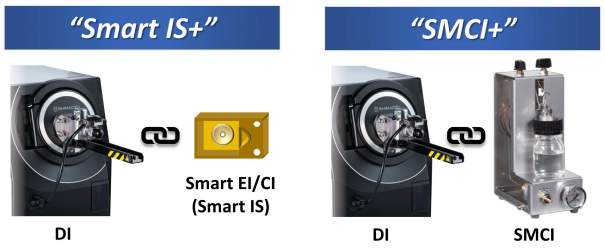


Fig. 1 *Polymode Ionization* setup inclusive of "*Smart IS*+" and "*SMCI*+".

On the other hand, the SMCI unit enables PCI mode with conventional PCI ion source and methanol as the reagent gas. Usage of methanol allows safe (i.e., it eliminates the use of flammable and toxic reagent gases such as methane, isobutane, and ammonia) and convenient adoption of PCI mode in routine GC/MS analysis.

A total of 9 saccharides including 5 monosaccharides (rhamnose, ribose, fructose, glucose, galactose), 3 disaccharides (maltose, lactose, sucrose), and 1 oligosaccharide (1-kestose) were analyzed in this study.

Experimental Condition.

Standard solutions of saccharides, trimethoprim, and manuka honey were prepared to a concentration of 5000 ppm in water, except for trimethoprim in methanol. 1 µL of each standard solution was introduced into individual DI sample vial for analysis. Mixture samples were prepared by introducing 1 µL of each standard solution into a DI sample vial. The samples were left to dry before analysis.

The DI probe was heated at 20 °C/min to 100 °C, then 40 °C/min to 450 °C and held for 7 min. The ion source temperature was set to 230 °C. Ionization mode used included EI, QCI (isobutane), and SMCI (methanol). Scan mode was performed in the range of *m/z* 50-600 with a scan speed of 3333. MRM transitions of codeine and promethazine were from GC/MS Smart Forensic DB.

■ Results and Discussion

"*Smart IS*+" and "*SMCI*+" Mass Spectra.

Using "*Smart IS*+" and "*SMCI*+", the saccharides were first analyzed with three different ionization modes, specifically EI,

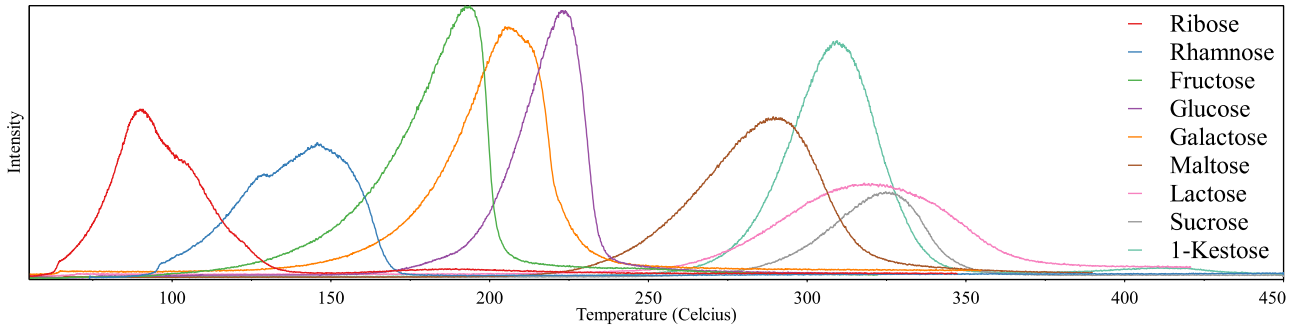
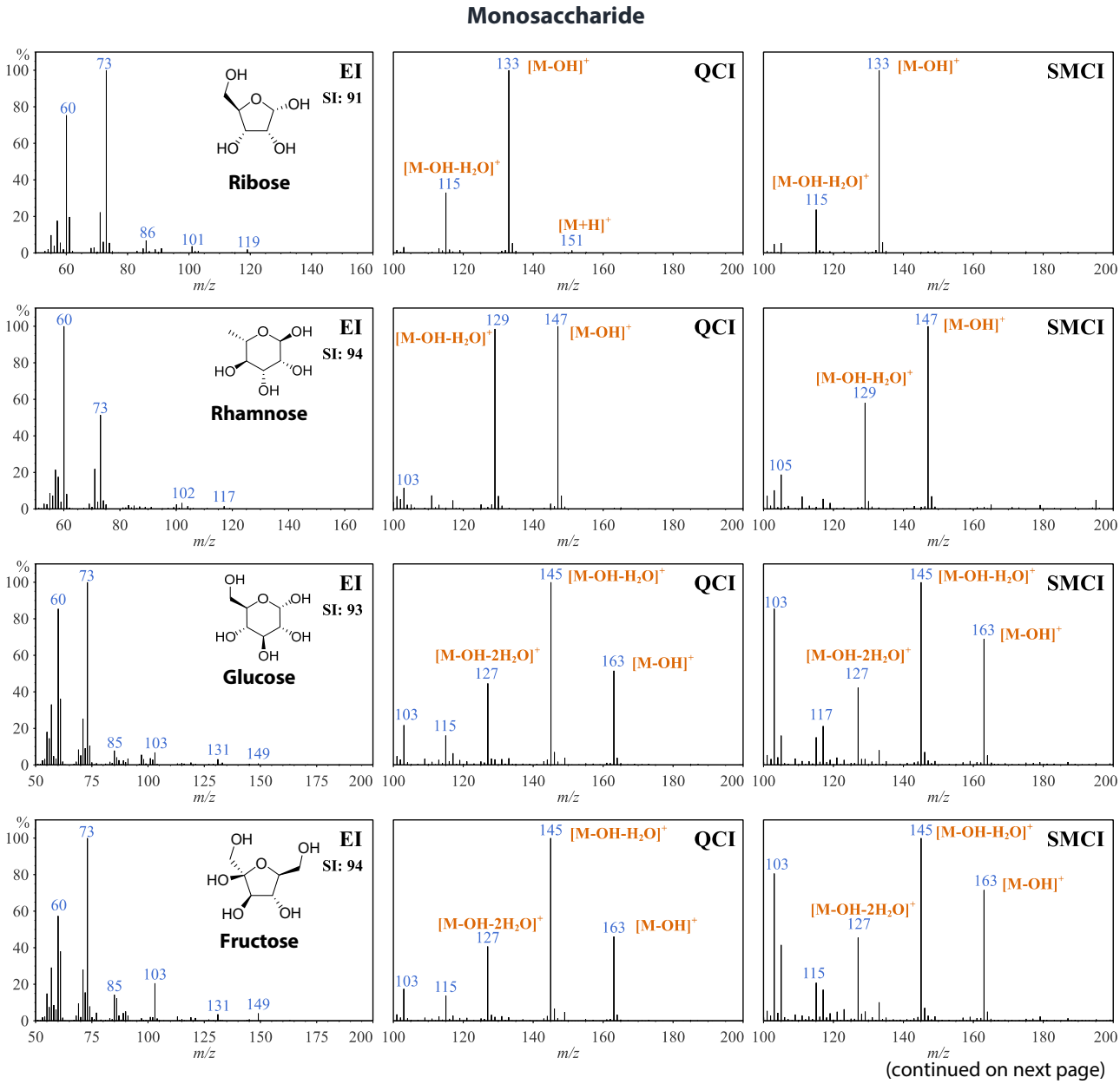


Fig. 2 Overlaid TIT profiles of QCI mode collected for individual saccharides.

QCI, and SMCI, to establish the total ion thermogram (TIT) profiles and mass spectra. The TIT profiles of individual saccharides collected with QCI mode are compiled and overlaid as shown in Fig. 2. The monosaccharide, rhamnose, and ribose, eluted within the temperature range of 75-175 °C. On the other hand, the remaining monosaccharides, fructose, glucose, and galactose, eluted within the temperature range of 150-250 °C. Lastly, the di- and oligosaccharides eluted within the temperature range of 250-400 °C. The consolidated mass spectra of the saccharides collected with the three different ionization modes are shown in Fig. 3.

The EI mass spectra generated with "*Smart IS+*" provided high similarity index scores of more than 80 for both mono- and di-saccharides. However, the EI mass spectrum of 1-kestose was not available in the NIST mass spectral library. The QCI mass spectra of the monosaccharides showed intense mass peaks of $[M\text{-}OH]^+$ and $[M\text{-}OH\text{-}nH_2O]^+$ ions. In addition, glucose, fructose, and galactose showed almost similar QCI mass spectra. The fragmentation pattern was also highly similar to the respective SMCI mass spectra. However, the SMCI mass spectra provided a higher intensity of low mass ions, such as *m/z* 103 and 105. This could have resulted from the difference in gas phase interaction between the monosaccharides and reagent gases. Nevertheless, both QCI and SMCI ionization modes provided almost identical mass spectra and hence both techniques can be considered as interchangeable for the analysis of saccharides.



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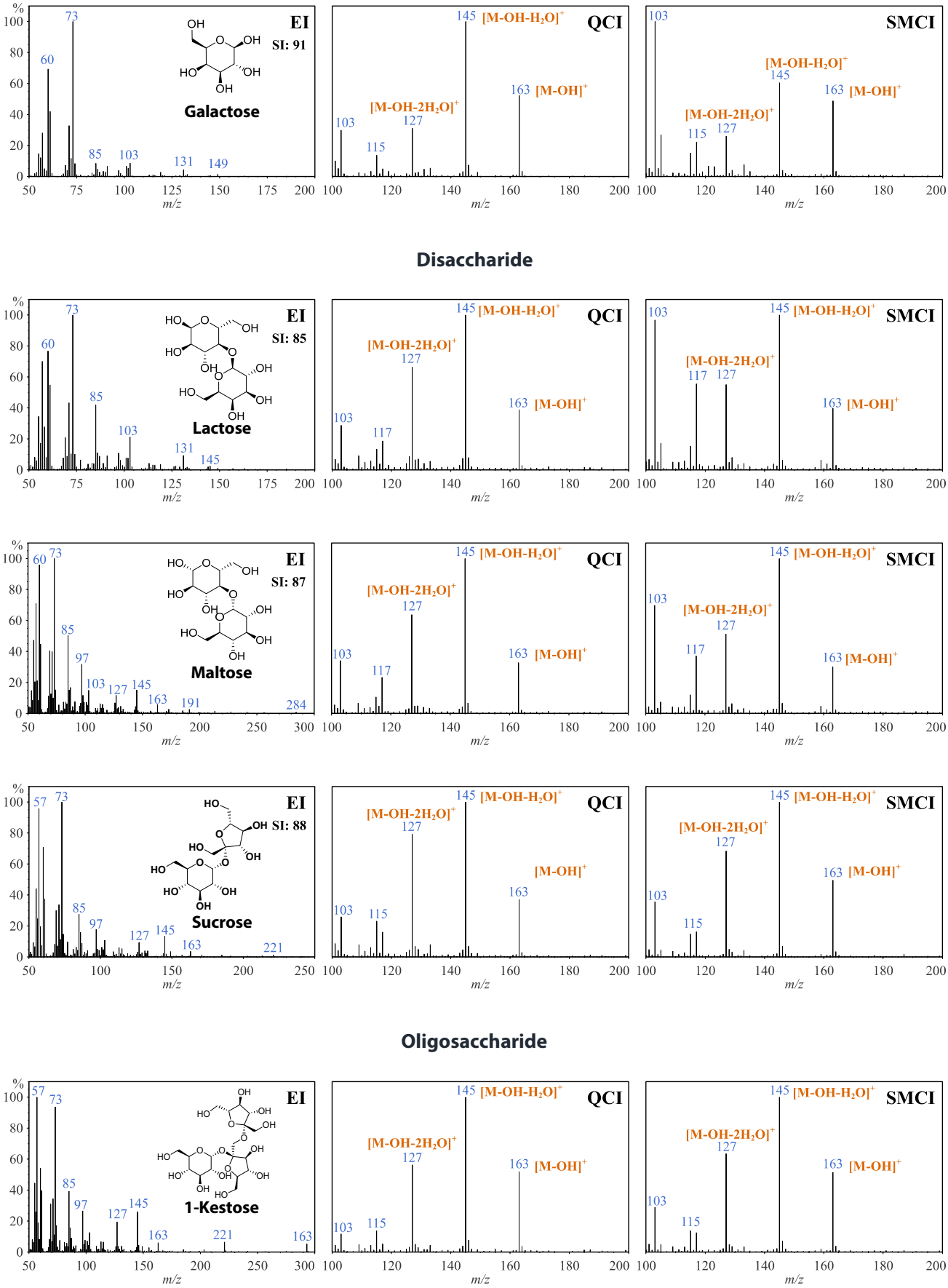

Fig. 3 EI, QCI, and SMCI mass spectra of saccharides collected with "*Smart IS+*" and "*SMCI+*". For QCI and SMCI mass spectra, the symbol M refers to either the molecular ion (for monosaccharides) or the molecular ion of monomer (for di- and oligosaccharides). SI: Library similarity index.

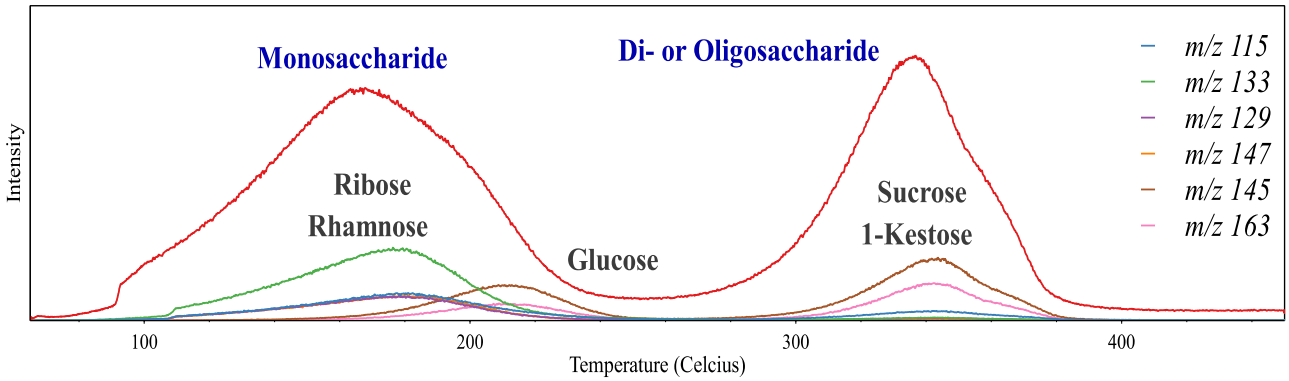

Fig. 4 TIT profile of QCI mode for mixture containing ribose, rhamnose, glucose, sucrose and 1-kestose.

As for di- and oligosaccharides, the resulted QCI and SMCI mass spectra resembled that of their monomers. The glycosidic linkage was broken either during the heating process in the DI unit or the chemical ionization process. Despite this, the volatilization of the saccharides was uniform since they afforded Gaussian-like profiles.

Analysis of Saccharide Mixture

Subsequently, analysis of a mixture containing rhamnose, ribose, glucose, sucrose, and 1-kestose was carried out to evaluate the possibility to obtain well-resolved peaks in the TIT profile. The TIT profile of the mixture saccharides is shown in Fig. 4. By plotting out the extracted ion thermogram (EIT) profiles of the respective $[M\text{-}OH]^+$ and $[M\text{-}OH\text{-}H_2O]^+$ ions of the saccharides, it was observed that ribose and rhamnose eluted simultaneously while glucose eluted right after that. On the other hand, sucrose and 1-kestose eluted much later. Since sucrose and 1-kestose have almost similar QCI mass spectra as seen in Fig. 3, it was not possible to ascertain their order of elution.

Nevertheless, it could be summarized that peaks of monosaccharides could be resolved from di- or oligosaccharides as a result of the difference in elution temperature. Due to this observation, the application of "*Smart IS+*" and "*SMCI+*" could be further extended to various areas, including preliminary authentication and adulteration studies, to a certain extent.

Authentication of Natural Manuka

Natural manuka honey comprises of more than 80% monosaccharides, specifically glucose and fructose. Adulteration of manuka honey can occur when manuka honey is mixed with cheaper cane sugar or table sugar, which is made up of sucrose. Hence, "*Smart IS+*" was used to demonstrate the differentiation of a sample of natural manuka honey from a sample spiked with sucrose. The TIT profiles collected with EI mode are shown in Fig. 5. The profile of natural manuka honey only showed the presence of monosaccharides. In contrast, the spiked sample revealed a huge peak at a higher elution temperature that corresponded to sucrose.

Adulteration of White Table Sugar

White table sugar can often be easily adulterated with drugs that come in the form of white powder or crystal. In the next example, sucrose was spiked with trimethoprim antibiotic and examined with "*Smart IS+*" under EI mode. The resulting TIT profile in Fig. 6a showed two well-resolved peaks.

Upon matching the mass spectrum of the first peak against the NIST mass spectral library, trimethoprim was identified with a similarity index of 97. Due to the higher elution temperature of sucrose, small drug molecules that elute at a lower temperature could potentially be thermally resolved with "*Smart IS+*", such as the case seen in this example.

Subsequently, we extended this to the analysis of sucrose spiked with codeine and promethazine. The TIT profile of this mixture is shown in Fig. 6b. By examining the EIT profiles of M^+ ions of codeine and promethazine, at *m/z* 299 and 284, respectively, we could observe the presence of both compounds within the temperature range of 50-150 °C. The corresponding QCI mass spectrum at 80 °C, as shown in the inset of Fig. 6b, showed mass peaks of *m/z* 299 and 162 which corresponded to the fragment ions of codeine, as well as mass peaks of *m/z* 284 and 213 which corresponded to the fragment ions of promethazine. By subjecting the sample to multiple reaction monitoring (MRM) analysis under EI mode, we were able to detect the unique transitions for both codeine and promethazine. The MRM profiles are shown in Fig. 6c.

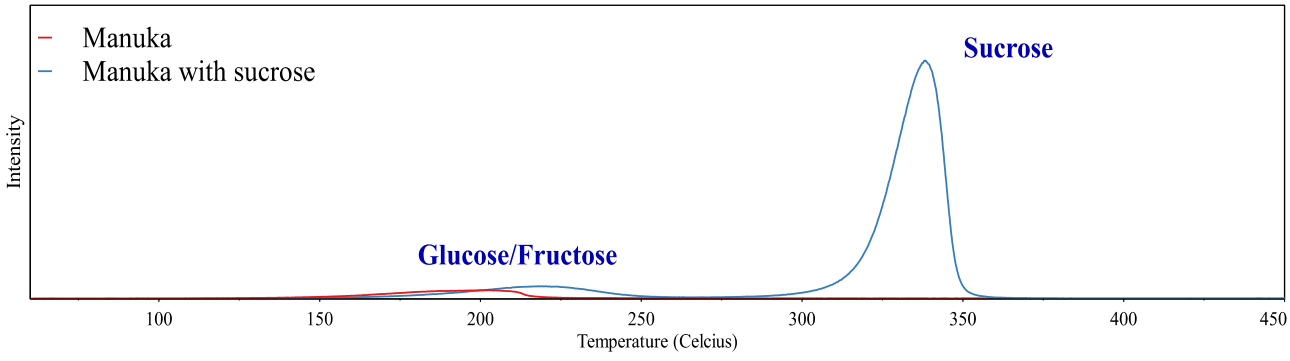

Fig.5 TIT profiles of EI mode for manuka honey and manuka honey spiked with sucrose.

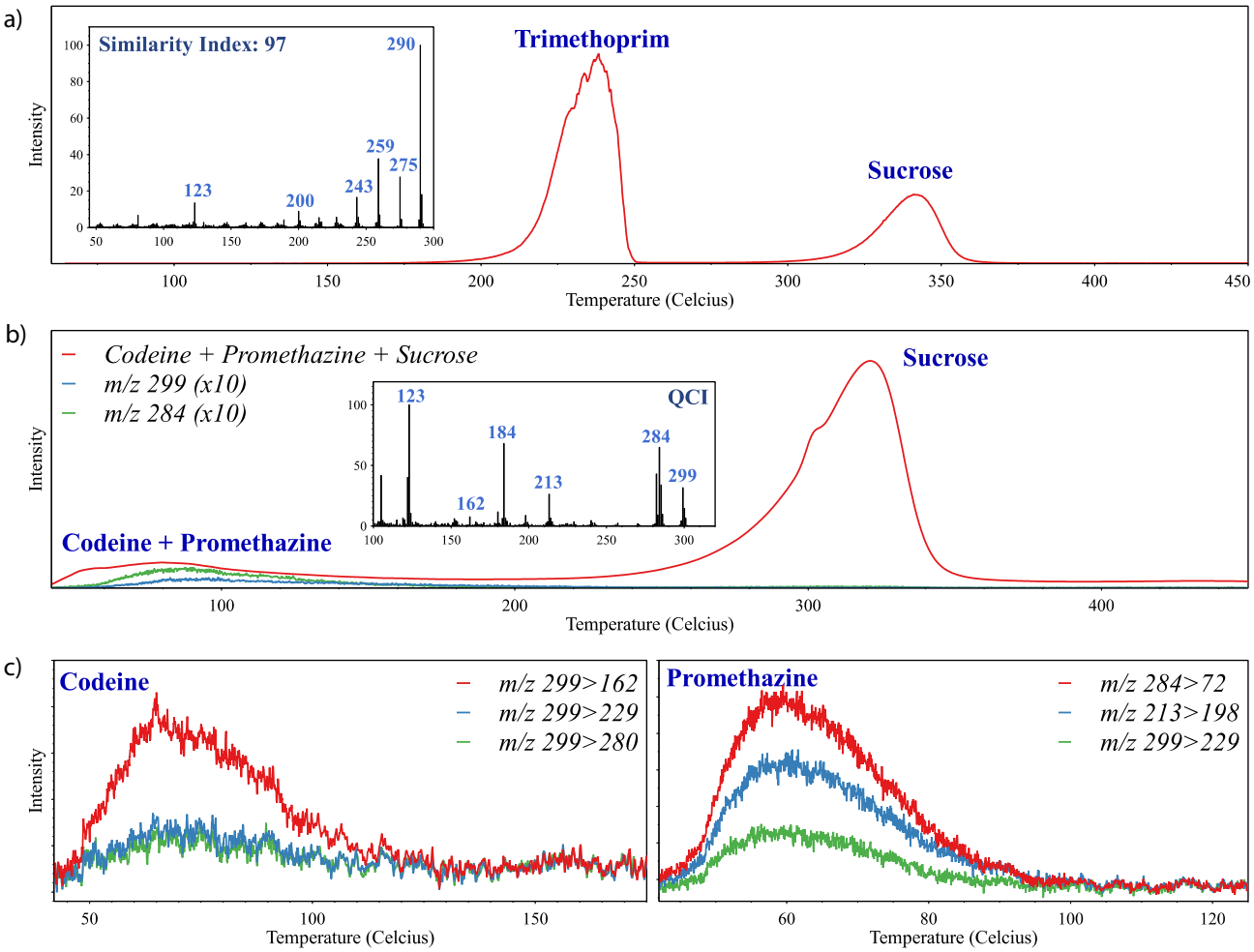

Fig. 6 (a) TIT profile of EI mode for mixture sample of trimethoprim and sucrose. Inset showing the mass spectrum from trimethoprim peak. (b) TIT profile of QCI mode for mixture sample of codeine, promethazine and sucrose. Inset showing mass spectrum of scan at 80 °C. (c) MRM profiles of codeine and promethazine.

■ Conclusion

The "*Smart IS+*" and newly introduced "*SMCI+*" enable a direct and quick qualitative analysis of saccharides, which conventionally requires tedious derivatization steps prior to GC/MS analysis. The "*Smart IS+*" setup delivers convenience in switching between electron ionization and positive chemical ionization mode of analysis. On the other hand, the "*SMCI+*" setup delivers utmost convenience and safety to carry out positive chemical ionization as it utilizes methanol, which is a common laboratory solvent, as the reagent gas.

The unique temperature elution profiles of the saccharides further enabled quick preliminary authentication and adulteration studies, supported by the "*Smart IS+*" and "*SMCI+*" setups.

04-JMST-201-EN First Edition: Mar. 2021

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