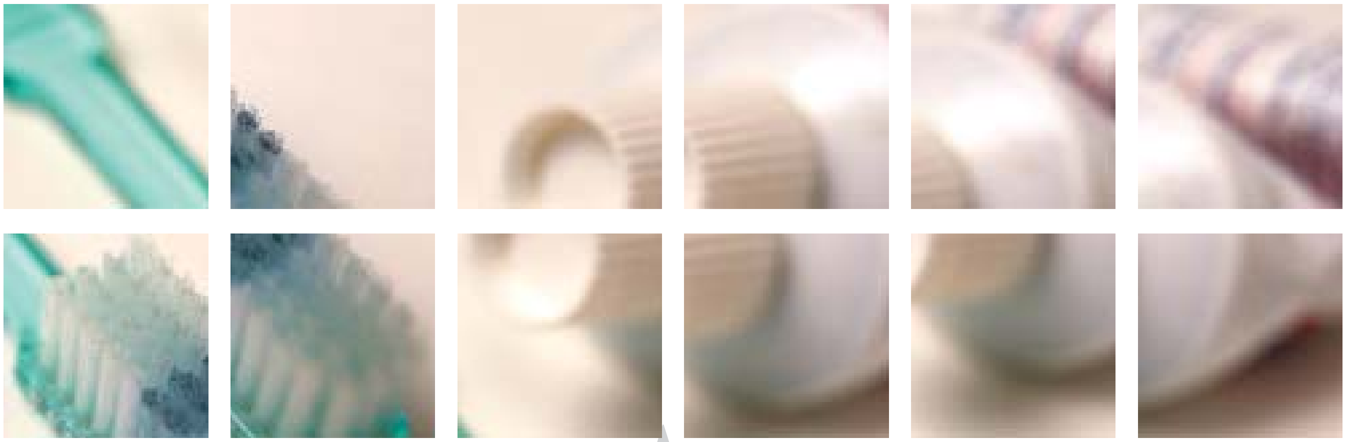

IC–MS Determination
of Ionic Compounds in Toothpaste

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A fast and easy-to-use method for the identification and determination of active anionic ingredients in toothpaste is presented using ion chromatography in combination with electrospray mass spectrometry. The total analysis time is 22 min using a potassium hydroxide gradient to separate fluoride, carbonate, sulphate, glycerol, sorbitol, saccharin, monofluorophosphate, phosphate, pyrophosphate and tripolyphosphate in a single injection.

Introduction

Caries are caused by bacteria-producing plaque on teeth. Fluoride is an active ingredient in toothpaste to reduce plaque. Today different chemical forms of fluoride are used in toothpaste. These forms include fluoride, monofluorophosphate and amine fluorides. In addition to carbonate, phosphates such as pyrophosphate and tripolyphosphate are used in toothpaste to reduce tartar. Saccharin is used as a sweetener, and sorbitol and glycerol as moisture control agents. Furthermore, toothpaste contains other compounds such as detergents, flavours, dyes and preservatives.[1]

For quality control of toothpaste production or analysis of competitive products, a fast and powerful technique is required to determine the active ingredients.

Fluoride, monofluorophosphate, phosphate, pyrophosphate and tripolyphosphate in toothpaste can be separated by ion chromatography (IC) using an anion-exchange column.[2] Common eluents are aqueous potassium hydroxide or sodium carbonate/bicarbonate solutions. Universal detection of the separated ionic components is achieved by suppressed conductivity detection. However, the determination of fluoride can be problematic because fluoride elutes shortly after the void volume, and coelution with sorbitol, glycerol or organic acids can occur, resulting in a false-positive identification. Resolution of this problem requires a more suitable sample preparation, separation using different column selectivity (e.g., ion-exclusion chromatography), or more specific detection such as mass spectrometry (MS).

Coupling IC and MS

MS allows detection of analytes by their mass-to-charge ratio, enabling mass-selective detection. All mass spectrometers separate ions in a vacuum. To couple IC with MS, the separated analyte ions must be transferred into the gas phase. To accomplish this, an interface is required to connect the ion chromatograph and mass spectrometer. Today, the most common interface technique for polar analytes is electrospray ionization (ESI). This technique is also used for interfacing HPLC with MS to determine polar analytes and ionize non-ionic analytes at atmospheric pressure. In ESI, the column effluent is pneumatically nebulized using nitrogen at atmospheric pressure. When the spray is formed, electrical charges are transferred from the end of a stainless steel capillary to the droplets. Using a heated secondary nitrogen flow that surrounds the spray, the eluent is evaporated and the size of the droplets decreases while the charge density increases. At the entrance to the MS vacuum region, the charge density on the surface of the droplets is so high that the droplets explode because of coulombic repulsion and ions are transferred into the gas phase. Non-ionic compounds that can deposit a charge at a polar centre can also be ionized and transferred to the gas phase in a similar manner. Therefore, ESI has for years been used to ionize polar analytes separated by HPLC.[3] The common liquid flow-rate for ESI ranges from 5 μL/min to 2 mL/min, where higher sensitivities are achieved at lower flow-rates. This higher sensitivity makes 2 mm i.d. columns the preferred format for IC using a flow-rate ranging 0.25–0.5 mL/min. When using an ESI interface, nonvolatile eluent components can crystallize and block the entrance to the MS vacuum region. In the past, this problem limited LC–MS to the use of volatile eluent additives. Newer LC–MS systems are equipped with a continuous wash function of the entrance cone, preventing the formation of an accumulative blockage, and allow the use of nonvolatile eluent additives. Furthermore, ionic additives in the eluent can form ion pairs with the separated analytes, resulting in non-ionic complexes. These weakly dissociated ion pairs are not separated and detected in the MS. This effect, called "ion suppression", results in reduced sensitivities for the analyte ions and is dependent on the eluent composition. To avoid this problem, continuously regenerated suppressors are installed between the IC column and MS to desalt the mobile phase. The suppressor converts potassium hydroxide into water by exchanging the potassium ion with a hydronium ion or the sodium carbonate/bicarbonate-based eluent into carbonic acid, which decomposes into water and carbon dioxide. The analyte ions transfer into the corresponding acids that are strongly dissociated and only have to be transferred into the gas phase to be analysed by the MS.[4]

Today, the most common interface technique for polar analytes is electrospray ionization.

The use of suppressors simplifies method development because the analyte ions are always entering the MS in pure water. This pure water results in stable and predictable response factors. The technique of desalting the eluent is commonly used in IC prior to conductivity detection.[2] In addition, because the conductivity detector is nondestructive, it can be placed in series after the suppressor, followed by the MS, which by nature is a destructive detector.

Conventional MS systems used for LC–MS are optimized to have good sensitivity for analytes with mass-to-charge ratios up to 2000 amu or even 4000 amu. Taking into account the mass of the typical eluents methanol 32 amu and acetonitrile 41 amu, and that small organic molecules are preferably determined by GC–MS, most LC–MS systems have a reduced sensitivity for analytes with an *m/z* less than 100 amu. For IC–MS, low-molecular-weight analytes, such as fluoride *m/z* 19 amu, chloride *m/z* 35 amu and 37 amu, nitrite *m/z* 46, and so forth, are of interest. To detect these ions with high sensitivity, a special version of the mass spectrometer called "Enhanced Low Mass Option" is used to obtain better sensitivities for small ions and a reduced sensitivity for analytes with *m/z* above 1000 amu, compared with the standard version of the same MS.

Determination of Active Ingredients in Toothpaste

An IC–MS system consisting of an ICS-2500 (Dionex Corp., Sunnyvale, USA) with a GS50 gradient pump, EG50 eluent generator, AS50 autosampler, ED50A electrochemical detector and MSQ™ mass spectrometer with an Enhanced Low Mass Option (ELMO) were used. Control of all modules and data acquisition was performed using the Chromeleon® chromatography management software (Dionex Corp.). Figure 1 presents a typical schematic of an IC–MS system.

Toothpaste samples of 1 g were dissolved in 100 mL deionized water and stirred until dissolved (about 30 min). Sample volumes of 5 mL were passed through OnGuard® RP (Dionex Corp.) solid-phase extraction cartridges to remove most of the non-polar components. The extract was transferred into autosampler vials and 10 μL were injected onto a 2 × 50 mm IonPac® AG16 guard column coupled to a 2 × 250 mm IonPac AS16 separation column. The anionic compounds were separated using a potassium hydroxide gradient that was electrolytically generated using the EG50 with a flow-rate of 0.35 mL/min. The column was equilibrated for 7 min at 30 mM KOH prior to injection. The separation was achieved using a linear gradient to 60 mM KOH in 5 min, followed by 10 min isocratic at 60 mM KOH. The mobile phase was desalted with a continuously regenerated suppressor, a 2 mm format model ASRS® ULTRA with electrolytic regeneration using the external water mode. Conductivity detection was performed with an ED50A electrochemical detector and MS detection with an MSQ equipped with ELMO using electrospray ionization and a probe temperature of 350 °C with a probe voltage of −3 kV. For identification of the chromatographic peaks, the MSQ was operated in the scan mode and mass spectra were recorded in the mass range from 10 to 350 *m/z*. For mass selective detection, the MS was operated in the selected ion mode (SIM), where individual channels are recorded at definitive *m/z* ratios. The fast electronics of the MSQ allows switching sequentially between scan and SIM modes during the same run.

Figure 1: Schematic of an IC–MS system with integrated eluent generation.

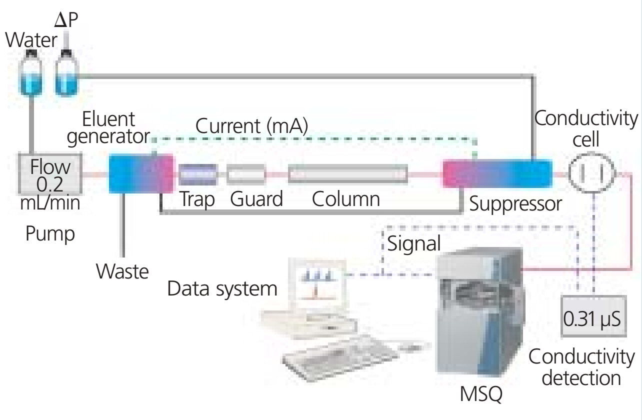


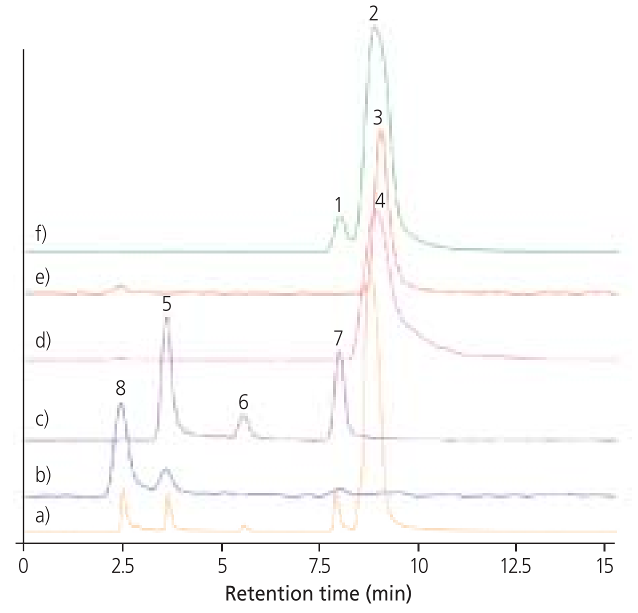
Figure 2 shows the IC chromatogram with conductivity detection and overlayed selective MS channels of a commercial toothpaste sample (Brand A). Entering a delay time in the software between the conductivity detector and the MS allows adjustment of the retention times with the different signals for easy comparison. In addition, the corresponding mass spectra can be displayed by clicking on the peaks in either the conductivity or mass chromatogram. Besides the conductivity channel, multiple MS channels were recorded. Glycerol is detected at *m/z* 91 and a retention time of 2.5 min. Figure 3(a) shows the corresponding full-scan mass spectrum with the most intensive mass peak (base peak) at *m/z* 91, which is glycerol minus a hydrogen $[M-H]^-$. In addition, sulphate was detected with a retention time of 3.6 min and *m/z* 97 as $[HSO_4]^-$. In the same mass channel, phosphate is detected at 5.5 min (Figure 2). Sulphate and phosphate can be identified when comparing the full-scan mass spectra of the two peaks. Sulphate (Figure 3(b) has a molecular ion $[HSO_4]^-$ *m/z* 97 and an isotope peak at *m/z* 99 with a relative intensity of 5%. This relative intensity is caused by the ^{32}S and ^{34}S isotope ratio of 100:5.2. In contrast, phosphate has no significant isotopes besides the ^{31}P.

Applying voltages to the exit cone of the ion source results in acceleration of the ions into the focusing hexapole region. If the kinetic energy is high enough, the analyte ions that hit neutral molecules can fragment. This process is called collision-induced dissociation (CID) and can produce characteristic fragment ions that can be used to identify analytes. For example, at a cone voltage of 75 V, sulphate forms a fragment ion at *m/z* 80. This fragmentation represents the loss of an OH-group. At a much lower cone voltage of 50 V, only phosphate forms a fragment ion at *m/z* 79, which represents the loss of water. In addition, the mass spectrum in Figure 3(d) also contains the dimer of phosphate at *m/z* 195 amu $[H_3PO_4 + H_2PO_4]^-$.

Pyrophosphate, with a retention time of 7.8 min, shows the quasi-molecular ion $[M-H]^-$ at 177 *m/z* and a doubly charged ion with two water molecules at *m/z* 106 as the base peak (Figure 3(d)). The mass spectrum (Figure 4(a) of tripolyphosphate with a retention time of 9 min has the quasi-molecular ion $[M-H]^-$ at *m/z* 257 and the base peak at 128 *m/z*, which is the doubly charged molecular ion $[M-H]^{2-}$. In addition, a peak at *m/z* 137 is observed as the doubly charged molecular ion, plus one molecule of water $[M-2H + H_2O]^{2-}$. Comparing the mass spectra over the whole peak at retention time 9 min, it becomes obvious that another compound is coeluting. This compound shows a base peak at *m/z* 182 and was identified in a later sample (Brand B) as saccharin with a retention time of 8.9 min. Also in Figure 2, a mass extracted channel at *m/z* 182 shows the coeluting saccharin. This example shows the power of MS detection to verify peak purity and identify compounds. A quantification of saccharin and tripolyphosphate in the different MS channels is possible, but the response factors can be affected during the ionization process. Therefore, a calibration with matrix standards or isotopically labelled standards is recommended.

Figure 5 shows the overlaid chromatograms for the conductivity detector and selected mass channels of toothpaste (Brand B). In this sample, at a retention time of 2.5 min, glycerol and sorbitol were identified. The mass spectrum of sorbitol (Figure 4(b) has a base peak at *m/z* 181 amu, which is the quasi-molecular ion $[M-H]^-$ plus the dimer $[2M-H]^-$ at *m/z* 363 amu. With a retention time of 2.7 min, chloride is detected, which is the largest peak in the conductivity chromatogram. The mass spectrum of chloride shows the typical chlorine isotopes at *m/z* 35 and 37 with a relative intensity of 3:1. At *m/z* 217 and 219, with the same isotope ratio, there appears to be an adduct of chloride and sorbitol.

Figure 2: IC–MS chromatogram (conductivity detection and selected MS channels) of a toothpaste (Brand A); (a) conductivity; (b) glycerol *m/z* 91; (c) sulphate, phosphate, pyrophosphate *m/z* 97; (d) tripolyphosphate *m/z* 257; (e) saccharin *m/z* 182; (f) pyrophosphate and tripolyphosphate *m/z* 177; IC: AG 16 (2 × 250 mm); KOH-gradient flow: 0.35 mL/min; MS: ESI–: 3 kV; Cone: 50 V.



Peaks: 1 = pyrophosphate; 2 = tripolyphosphate; 3 = saccharin; 4 = tripolyphosphate; 5 = sulphate; 6 = phosphate; 7 = pyrophosphate; 8 = glycerol.

Figure 3: Mass spectrum ESI–: 3 kV; Cone: 50 (a) glycerol; (b) sulphate; (c) phosphate (Cone: 75 V); (d) pyrophosphate. Retention times: (a) 2.5 min; (b) 3.6 min; (c) 5.4 min; (d) 7.8 min.

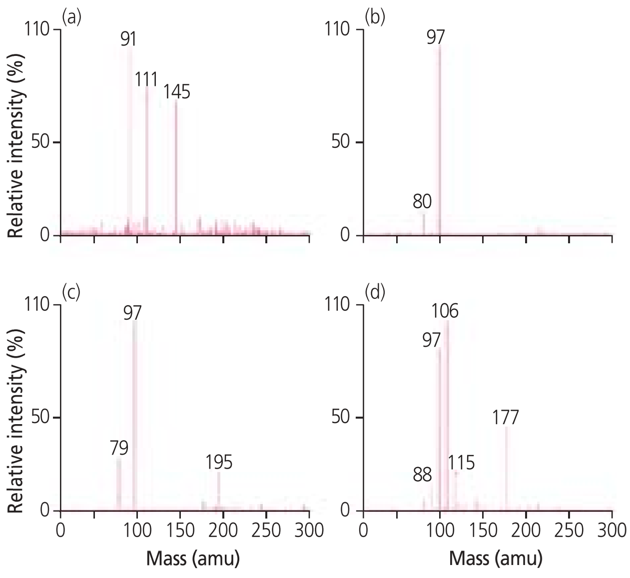


Figure 4: Mass spectrum ESI–: 3 kV; Cone: 50 (a) tripolyphosphate; (b) sorbitol; (c) chloride (Cone: 75 V); (d) citrate. Retention times: (a) 7.9 min; (b) 2.5 min; (c) 2.7 min; (d) 6.1 min.

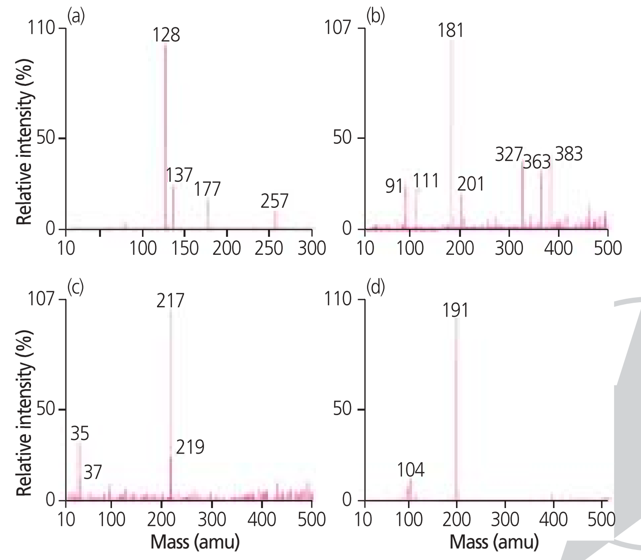
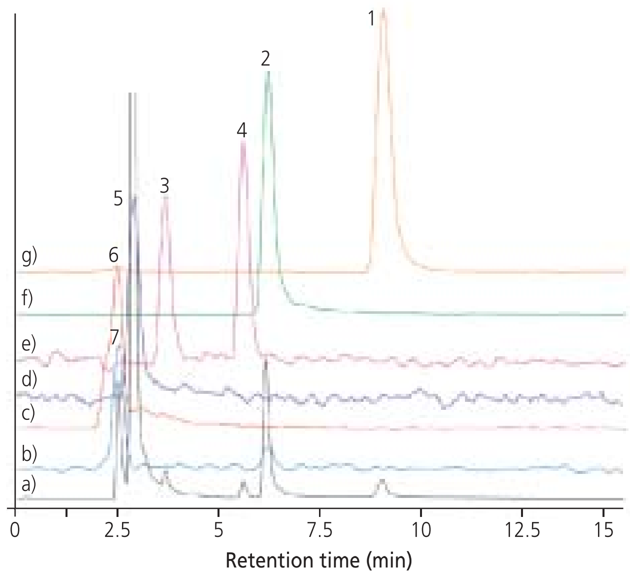


Figure 5: Overlaid chromatograms for the conductivity detector and selected mass channels of toothpaste (Brand B); (a) conductivity; (b) glycerol *m/z* 91; (c) sorbitol *m/z* 181; (d) chloride *m/z* 35; (e) sulphate, phosphate *m/z* 97; (f) citrate *m/z* 182; (g) saccharin *m/z* 182.



Peaks: 1 = saccharin; 2 = citrate; 3 = phosphate; 4 = sulphate; 5 = chloride; 6 = sorbitol; 7 = glycerol.

The injection of a pure chloride standard did not show this adduct.

In addition, sulphate and phosphate were detected at *m/z* 97. With a retention time of 6.1 min, citrate was detected with an *m/z* 191, which is the quasi-molecular ion $[M\text{-}H]^-$ (Figure 4(d). With a retention time of 8.9 min, saccharin was detected at *m/z* 182. Thus the sample contains no tripolyphosphate; the mass spectrum shows a peak of the quasi-molecular ion at only *m/z* 182 amu.

Fluoride, with a retention time of 2.5 min, can be detected at *m/z* 19 at a cone voltage of 75 V. If the cone voltage is reduced to 15 V, fluoride is detected as an adduct with 2 molecules of water $[F + 2H_2O]^-$ at *m/z* 55. Monofluorophosphate can be detected at *m/z* 99 amu and coelutes with sulphate at a retention time of 3.7 min. Carbonate is also not resolved from sulphate or monofluorophosphate, but can be selectively detected at *m/z* 61 as $[HCO_3]^-$.

Conclusion

IC, in combination with MS, is a useful tool for the determination of anionic compounds in toothpaste. Minimal sample preparation is required and consists only of dilution and filtration over a solid-phase extraction cartridge. Positive identification of glycerol, sorbitol, chloride, fluoride, sulphate, monofluorophosphate and polyphosphates — as well as citrate and saccharin using the scan mode — is achieved in one chromatographic run with a total analysis time of 22 min. Using the SIM mode, mass selective detection with high sensitivity results in easy analyte quantification. Analyte coelution is not problematic because they have different molecular masses or fragmentation patterns.

MSQ is a trademark of Thermo Electron Corp. ASRS, Chromeleon, IonPac, and OnGuard are registered trademarks of Dionex Corporation.

References

1. W. Weinert, *Ullmann's Encyclopaedia of Industrial Chemistry*, 6th Edition; Electronic Release (2002).
2. J. Weiss, *Ionenchromatographie*, 3rd Edition (Wiley-VCH, Weinheim, 2001).
3. R. Willoughby, E. Sheehan and S. Mitrovich, *A Global View of LC–MS, How to Solve Your Most Challenging Analytical Problems*, 2nd Edition, (Global View Publishing, 2002).
4. R. Roehl et al., *J. Chromatogr. A*, **956**, 245–254 (2002).

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