Characterizing Toothpastes: Direct Fingerprinting of Key Volatile Flavor and Marker Non-volatile Compounds by DART Q-TOF MS

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Overview

Novel Aspect:

Ultra-fast (seconds per sample) MS fingerprinting method employed to monitor volatile and non-volatile marker compounds in commercial dentifrice (toothpaste) products and in human breath after brushing.

Introduction

Product characterization is critical for product branding and for defining product quality control (QC) acceptance criteria. Focusing on commercial dentifrice (toothpaste), there are thousands of name brand and generic products on the market with many flavors to choose. Efficiently fingerprinting and characterizing the volatile components in dentifrices yields information on the flavor fingerprint, where the non-volatile component fingerprint gives information about the inactive ingredients that form the base of the product. Being able to measure both vapor and solid paste phases directly in the same analytical run, yields more in-depth information on the profile of the product. Ambient mass spectrometry coupled with high resolution mass spectrometry permits this data acquisition in 10 seconds per sample as compared with the conventional GC-MS run of 50 plus minutes.

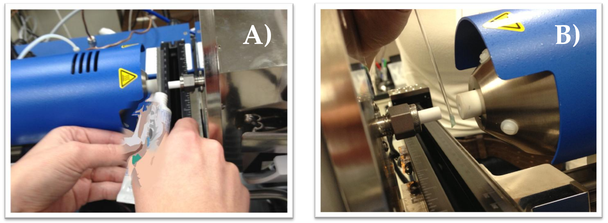


FIGURE 1. DART SVP ionization source coupled to an Agilent 6550 iFunnel Q-TOF MS for direct characterization of various toothpastes. A) Direct analysis of volatile components in dentifrices by "puffing" the tube near the ionization region. B) Direct analysis of toothpaste solid.

Methods

A fourth generation Direct Analysis in Real Time-Standard Voltage and Pressure (DART-SVP) ambient ionization source was coupled to a high resolution quadrupole time of flight (Q-TOF) mass spectrometer for full spectrum single MS and MS/MS data acquisition. Two dentifrices were sampled both as volatile vapors from the tube and directly as the solid paste (FIGURE 1). Spectra from an oil based flavor standard were obtained also by sampling directly as vapor and as a liquid for comparison with the flavor fingerprints of two competing whitening dentifrice products. Direct breath analysis was conducted prior to brushing, immediately after brushing and then 5 min after brushing. A validated GC-MS method was run in parallel with the ambient ionization method for comparison using GC/MS.

Validated GC-MS Method

- Sample preparation: Hexane dilution (1:1,000) or LLE of dentifrice
- Analysis: GC/MS, 1 µL liquid injection
- **Agilent 6890 GC with 5973 single quadrupole MS**
- Full scan: 33 - 325 amu @ 8.69 scans/s
- **Analysis Time:** 30 min for LLE + 24 min GC cycle = **54 min**
- ~40 ng total flavor onto GC column
- Typical RSDs: <3 %

DART HRAM-MS Method

- Sample preparation: Only flavor oil concentrate1:100 dilution in hexane
- Analysis: Vapors & paste directly sampled
- **DART SVP ion source coupled with Agilent 6550 iFunnel Q-TOF MS**

Q-TOF Parameters:

- Single MS (TOF) - Full spectra: 100 – 1,000 amu @ 1 spectra/sec
- Auto MS/MS (Q-TOF) 3 spectra/sec (MS) and 2 spectra/sec (MS/MS) with 5 precursors selected per MS
- **Analysis Time:** **10 seconds** per sample
- Reference Ions: *m/z* 121.05087 (purine) and *m/z* 922.00980 (HP-921)
- Gas Temp (°C) 150 • Gas Flow (L/min) 11 • Vcap 1,000
- Resolving power 22,000 @ *m/z* 200

Results

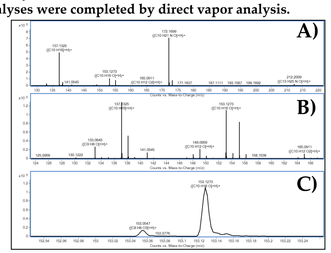
Direct analysis of the flavor oil standard mixture sampled as a liquid only yielded higher mass ions (> *m/z* 250), which were not of interest in identifying the flavor fingerprint. By directly analyzing the volatile vapors from the open vial containing the diluted flavor oil concentrate, abundant low mass fingerprint ions ranging from *m/z* 133 to 270 were clearly observed by DART Q-TOF. A mass spectral fingerprint of flavor compounds were identified based on the accurate mass data collected for a flavor oil standard mixture and two commercial dentifrice products. TABLE 1 displays the key flavor compounds identified by the GC-MS method and the DART MS method by sampling from the vapors of the diluted flavor oil and both dentifrices. This direct headspace sampling approach with the DART method was carried out by placing the open end of the dentifrice tube near the DART sampling region as shown in FIGURE 1A and by "puffing" the tube just at the edge of the heated DART ionization region dispersing the volatile compounds directly into the ionization region. FIGURE 2 shows flavor compounds detected by DART MS with major ones identified by MS/MS (not shown). Both commercial dentifrice products yielded similar flavor fingerprint spectra, but were fully distinguishable based on major differences in relative signal intensities yielding very different MS fingerprint spectra. The dentifrices were also sampled directly as solid pastes as seen in FIGURE 1B and the fingerprint spectra (data not shown) were distinctly different between the two dentifrice products, as well as distinctive from their respective headspace profiles.

Compound	Chemical Formula	Exact [M+H]+	Commercial Dentifrice 1		Commercial Dentifrice 2		Flavor Oil Standard	
			GC-MS	DART	GC-MS	DART	GC-MS	DART
Cinnamic aldehyde	C9H8O	133.06479	√	√	n.d.	trace	√	√
Paracymene	C10H14	135.11683	√	√	√	n.d.	n/a	n/a
Pinenes (α, β)	C10H16	137.13248	√, √		√, √		n/a	
Limonene	C10H16	137.13248	√	√	√	√	n/a	n/a
γ-Terpinene	C10H16	137.13248	√		√		n/a	
Anethole	C10H12O	149.09609	√	√	√	√	√	√
Dihydroanethole	C10H14O	151.11174	√	√	n.d.	trace	√	trace
Thymol	C10H14O	151.11174	n.d.		n.d.		√	
Methyl salicylate	C8H8O3	153.05462	√	√	n.d.	√	√	√
Pulegone	C10H16O	153.12739	√	√	√	√	n/a	√
Piperitone	C10H16O	153.12739	√	√	√	√	√	√
Eucalyptol	C10H18O	155.14304	√	√	√	√	√	√
Menthone	C10H18O	155.14304	√		√		√	
Menthone isomer	C10H18O	155.14304	√		√		√	
Menthol	C10H20O	157.15869	√	trace	√	n.d.	√	n.d.
Eugenol	C10H12O2	165.09101	√	√	n.d.	√	√	√
Menthyl acetate (DART: minus acetate)	C12H22O2	199.16926	√	trace	√	trace	√	trace
Caryophyllene	C15H24	205.19508	√	n.d.	√	n.d.	n/a	n/a
Germacrene	C15H24	205.19508	√	n.d.	√	n.d.	n/a	n/a
WS-23 Cooling Agent	C10H21NO	172.16959	√	√	n.d.	n.d.	√	√
WS-3 Cooling Agent	C13H25NO	212.20089	√	√	n.d.	n.d.	√	√
WS-5 Cooling Agent	C15H27NO3	270.20637	n.d.	n.d.	n.d.	n.d.	√	trace

TABLE 1. Comparison of results from a validated GC-MS method and DART Q-TOF method for direct flavor component analysis in commercial dentifrices and flavor oil standard mixture. All DART-MS analyses were completed by direct vapor analysis.

FIGURE 2. Commercial Dentifrice 1 Vapor sampled directly by DART MS

A. Mass range showing major ion abundances in the flavor spectral fingerprint.
B. Zoom in on lower *m/z* range showing identification of lower abundance key compounds.
C. Mass resolution of methyl salicylate and the isomeric flavor compounds pulegone and piperitone with accurate mass identifying molecular formulae.



ID Source	Name	Formula	m/z	Diff (ppm)	Score (DB)	Mass	Mass (DB)
DBSearch	Cinnamic aldehyde	C9 H8 O	133.0645	0.56	97.79	132.0574	132.0575
DBSearch	Menthyl acetate (minus acetate)	C10 H16	137.1325	-0.06	95.75	136.1252	136.1252
MFG	Unknown	C7 H8 O3	141.0545	1.03		140.0472	
DBSearch	Methyl salicylate	C8 H8 O3	153.0547	-0.45	95.7	152.0474	152.0473
MFG	Pulegone or Piperitone	C10 H16 O	153.1273	0.32		152.1201	
DBSearch	Eucalyptol	C10 H18 O	155.1430	0.33	99.94	154.1357	154.1358
DBSearch	Eugenol	C10 H12 O2	165.0911	0.58	98.91	164.0836	164.0837
DBSearch	WS-23	C10 H21 N O	172.1699	-1.9	94.71	171.1626	171.1623
DBSearch	WS-3	C13 H25 N O	212.2009	-0.28	95.59	211.1937	211.1936

TABLE 2. Compounds identified by DART Q-TOF method directly from the dentifrice vapors and their measured masses.

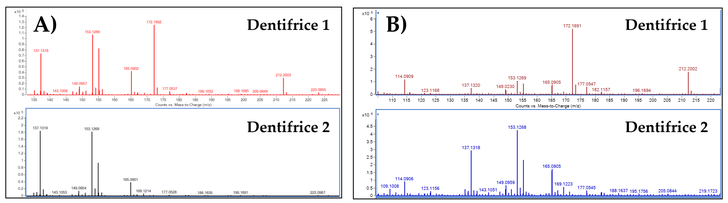


FIGURE 3. Direct breath analysis by DART Q-TOF MS. A) Human breath sampled directly after teeth brushing. B) Breath sampled again 5 minutes after brushing ceased.

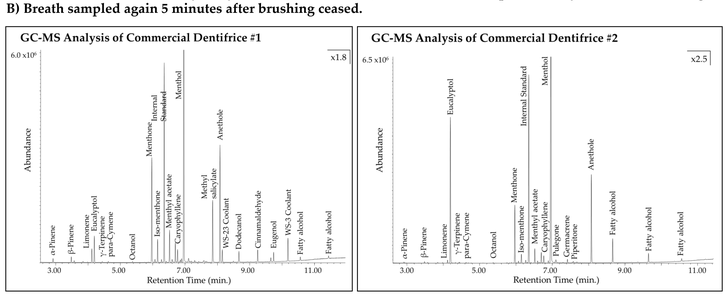


FIGURE 4. Data from validated GC-MS method for commercial dentifrice products 1 and 2 displaying the major flavor components in the products.

Direct Breath Analysis

Additional experiments for direct breath analysis after teeth brushing were conducted on the DART Q-TOF to rapidly monitor the volatile flavor components remaining in the breath immediately after brushing and 5 minutes after brushing. The breath analysis was simply set-up as exhaling directly into the heated DART gas beam and measuring in real time the volatile fingerprint. FIGURE 3A plots the data directly after brushing for 1 minute and breathing into the DART source. The breath was again sampled 5 minutes after brushing ceased as shown in FIGURE 3B.

Conclusions

- Direct volatiles analysis by **DART Q-TOF MS** and **MS/MS** provides a **rapid flavor fingerprint** for product authenticity and quality control without any sample pre-treatment
- High resolution and accurate mass distinguishes isobaric, but not **geometric isomeric compounds** for which **GC-MS is needed**
- In-vivo sampling (**breath analysis**) yields instantaneous real time data

Reference

Chernetsova, E. S. *et al.* Capabilities of direct analysis in real time mass spectrometry and gas chromatography mass spectrometry in the mint oil test. Mendeleev Commun. 2010, 20(5): 299-300