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Identification of an anti-bacterial agent in toothpaste via liquid chromatography–Fourier transform infrared spectrometry mobile phase elimination

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Abstract

Triclosan, an anti-bacterial agent in many cosmetic products, was determined in toothpaste using liquid chromatography (LC) for separation and mobile phase elimination Fourier transform infrared spectrometry (FT-IR) for identification. The accuracy of this method was measured by comparing the spectrum of the isolated triclosan from the toothpaste matrix with a standard spectrum of triclosan previously placed in our FT-IR library. Under optimized chromatographic and spectrometric conditions, matches of real spectra with library spectra were acceptable. A day-to-day reproducibility study was also conducted in order to verify that acceptable matches could be achieved over a longer time frame. Several factors including disk rotation rate and the effect of limiting the spectral search region were investigated.

Keywords: Antibacterial agents; Toothpaste; Interfaces; Triclosan

1. Introduction:

Triclosan (Fig. 1) is an anti-bacterial agent that is commonly added to cosmetic products. It is added, for example, to toothpaste at levels less than 1% (w/w) to aid in the prevention of gum disease. Regulatory requirements dictate that a positive identification of this component be made for every batch of toothpaste produced. The common method used for providing this identification involves a lengthy wet chemistry method that can be diffcult to put in place in a manufacturing situation. An additional disadvantage of the current process is that it is completely separate from the quantification assay with respect to sample preparation and instrument methodology.

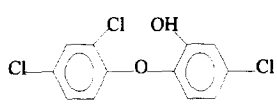

Fig. 1. Structure of triclosan.

The goal of this project was to develop a new, easier method for this identification using the LC-Transform LC–Fourier transform infrared spectrometry (FT-IR) interface. Mobile phase elimination LC–FT-IR was first described in the 1970s. The first two reports involving such an interface were published in 1977 by Griffiths [1] and in 1979 by Kuehl and Griffiths [2]. Although the technique has evolved

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since that time, the general principle, which is to deposit chromatographic eluent onto a substrate which is moving slow enough to concentrate the analyte deposit but fast enough to maintain resolution between chromatographic peaks, remains. At the same time sample integrity should be ensured as the solvent is completely eliminated.

The mobile phase elimination interface used here is a commercial product, the LC-Transform. The basis of the instrument design was first introduced in 1986 by Gagel and Biemann [3] with modifications being made in 1987 [4]. The interface allows for the column eluent of an LC to pass first through a UV detector and then onto the splitter mechanism of the interface. The mobile phase flow is split in a ratio which depends on the mobile phase composition and flow-rate. The smaller portion of split flow is directed towards the deposition needle. Upon exiting the deposition needle, the eluent is exposed to a heated sheath gas which eliminates the mobile phase before analytes are deposited onto a rotating collection disk. The degree of disk rotation per minute can be adjusted in order to provide adequate separation of the peaks while maintaining a deposit that is concentrated enough to be seen using the FT-IR. The disk is then manually transferred to an optical mount equipped with a rotating stage which is situated in the sample compartment of the FT-IR.

The process by which spectra are generated is considered to be absorbance/reflectance because the analytes are deposited onto a reflective surface. The entire chromatographic run can be reconstructed using GC collection software as in this study or the spectrum of individual peaks can be obtained by scanning the deposits independently. A review of chromatographically coupled FT-IR published by Fujimoto and Jinno [5] outlines the advantages/disadvantages of collecting data by scanning the disk or by "sitting on a peak". When this analysis is performed by scanning each deposit independently, each spectrum can be made up of a more significant number of scans (i.e., 256), thus increasing the signal-to-noise ratio. When a spectrum is generated from a single point on a Gram-Schmidt reconstruction that spectrum (which consists of only 4–16 scans) is easily located; however, the signal to noise ratio is greatly reduced.

The advantage of using an LC–FT-IR interface is that with little added time a positive spectral identification of the analytes can be carried out after a chromatographic separation. Dwyer has demonstrated this advantage in a publication which involves gel permeation chromatography (GPC) of rubber samples and LC of polymer additives [6]. A study involving a modified version of the interface which incorporates an ultrasonic nebulizer has been documented for GPC polymer analysis [7]. In the case of toothpaste, the analyte of interest was present at such a small concentration that isolation of that particular component from the bulk matrix for spectral analysis was difficult. This isolation was made much easier here, on the other hand, using chromatography prior to spectrometric analysis. Our goal here was to use the quickest means of analysis, therefore, "sitting on a peak" for an extended time was not an option.

2. Experimental

The chromatography for these experiments was run on a Waters 600-MS liquid chromatograph. The mobile phase was acetonitrile–water–glacial acetic acid (60:40:0.4, v/v), which was made up in 1000 ml quantities, stirred and sparged prior to use. The mobile phase was delivered at 1 ml/min to a 150× 3.9 mm Nova-Pak C_{18} column (Waters, Milford, MA, USA). The injection valve was a Valco (Houston, TX, USA) 6-port valve.

The chromatographic eluent was first passed through a UV detector (Kratos, Ramsey, NJ, USA; Spectroflow 757) set at 280 nm. A piece of 28 cm×0.06 in. (1 in.=2.54 cm) internal diameter PTFE tubing connected the output of the UV detector cell to the splitter of the LC-Transform interface. A split flow of 50 ml/min (5% of the total flow) was measured at the exit of the splitter leading to the deposition needle. The remaining flow was directed to waste. The flow-rate could not be increased further without its having a detrimental effect on the deposition process because of the relatively large amount of high boiling solvent (water and acetic acid) in the mobile phase which would have to be eliminated.

Optimal conditions for deposition were dictated by the degree of IR absorbance of the peak components as well as by the extent of the spectral noise. Both

the nebulizer and sheath gases were filtered house air. The nebulizer gas was held at 40 ml/min and the sheath gas flow at 4.2 l/min. The sheath temperature was maintained at 100°C. The height of the nozzle above the disk was 10 mm which allowed adequate time for the sheath gas to eliminate the mobile phase before reaching the disk. The rate of rotation of the germanium disk was varied at 3, 5 and 10° per min. The deposited spot size on the disk was 2–3 mm for triclosan.

After the chromatographic eluent was deposited, the disk was removed from that portion of the interface and placed on a rotating optics mount in the FT-IR system. The FT-IR instrument was a Nicolet (Madison, WI, USA) Magna-550 equipped with a DTGS detector. FT-IR was run at 16 scans per spectrum with a resolution of 8 cm^{-1} The disk was rotated for IR analysis at the same rate as the rotation rate for sample deposition. From the generated Gram-Schmidt reconstruction (GSR), individual peak spectra were generated by noting the data file closest to the peak maximum in the GSR (e.g., one 16 scan spectrum at the peak maximum, no co-addition of multiple 16 scan spectra). The IR beam was 2 mm in diameter; while, the spot size varied from 1.0–1.5 mm in diameter.

All samples were provided by Colgate-Palmolive (Piscataway, NJ, USA) and made up in acetonitrile–water (60:40, v/v) (Mallinckrodt HPLC-grade solvents). The toothpaste solutions were stirred 20–30 min and filtered through 0.2-μm PTFE particle filters (Supelco) prior to use.

3. Results and discussion

3.1. HPLC–UV comparison of toothpaste samples

Initially, toothpaste samples from two different manufacturers were qualitatively tested under the chromatographic conditions previously described in Section 2 to see if the HPLC–UV method could truly distinguish a difference between the two toothpastes. These particular chromatographic conditions were chosen because they are the conditions used routinely by one of the manufacturers. The UV chromatographic traces for both of these samples are shown in Fig. 2. Both samples have several UV active substances eluting in the first three minutes. Sample B; however, looks significantly different as it also has later eluting components under the same conditions. It was determined from preliminary solvent elimination LC–FT-IR data employing the same chromatographic conditions that the peak eluting at 6–7 min in chromatogram B was triclosan. The triclosan exhibited an intense IR spectrum with several absorbance regions. The following study focuses on optimizing this LC–FT-IR technique and determining its efficacy.

3.2. Deposition

Different volumes (200, 100, 50, 20, 10 μl) of a solution containing approximately 1 mg/ml triclosan standard were initially injected onto the chromatographic column in order to determine the minimum amount/mass of analyte that could be FT-IR detected after deposition. The detection of triclosan at as low as 1.25 μg on disk with a disk rotation rate of 3° per min was possible as long as the analysis was carried out promptly such that no triclosan would be blown from the disk by the sheath gas. The sheath temperature was held at 100°C due to the relatively high boiling temperatures of both water and acetic acid. Temperatures this high also enabled the sheath and nebulizer flows to remain fairly low (sheath=4.2 l/min; nebulizer =40 ml/min). Based upon the fact that triclosan was physically blown off the disk at high flows, the mass calculated (e.g., 1.25 μg) would be more accurately described as the mass of analyte sent through the deposition needle rather than the mass deposited or remaining on the disk when the FT-IR analysis is run.

3.3. LC–FT-IR reproducibility

Analyses at 2.5 μg of triclosan (on disk) and above could be carried out rather easily at 3, 5 and 10°/min disk rotation rates. The spectrum of a 3 μg triclosan standard sample which had been chromatographed and deposited onto the disk via mobile phase elimination LC–FT-IR is illustrated in Fig. 3B.

This spectrum of triclosan on disk was inserted into a miscellaneous computer library file for comparison to spectra of deposits obtained after chromatographing actual toothpaste samples. In addition,

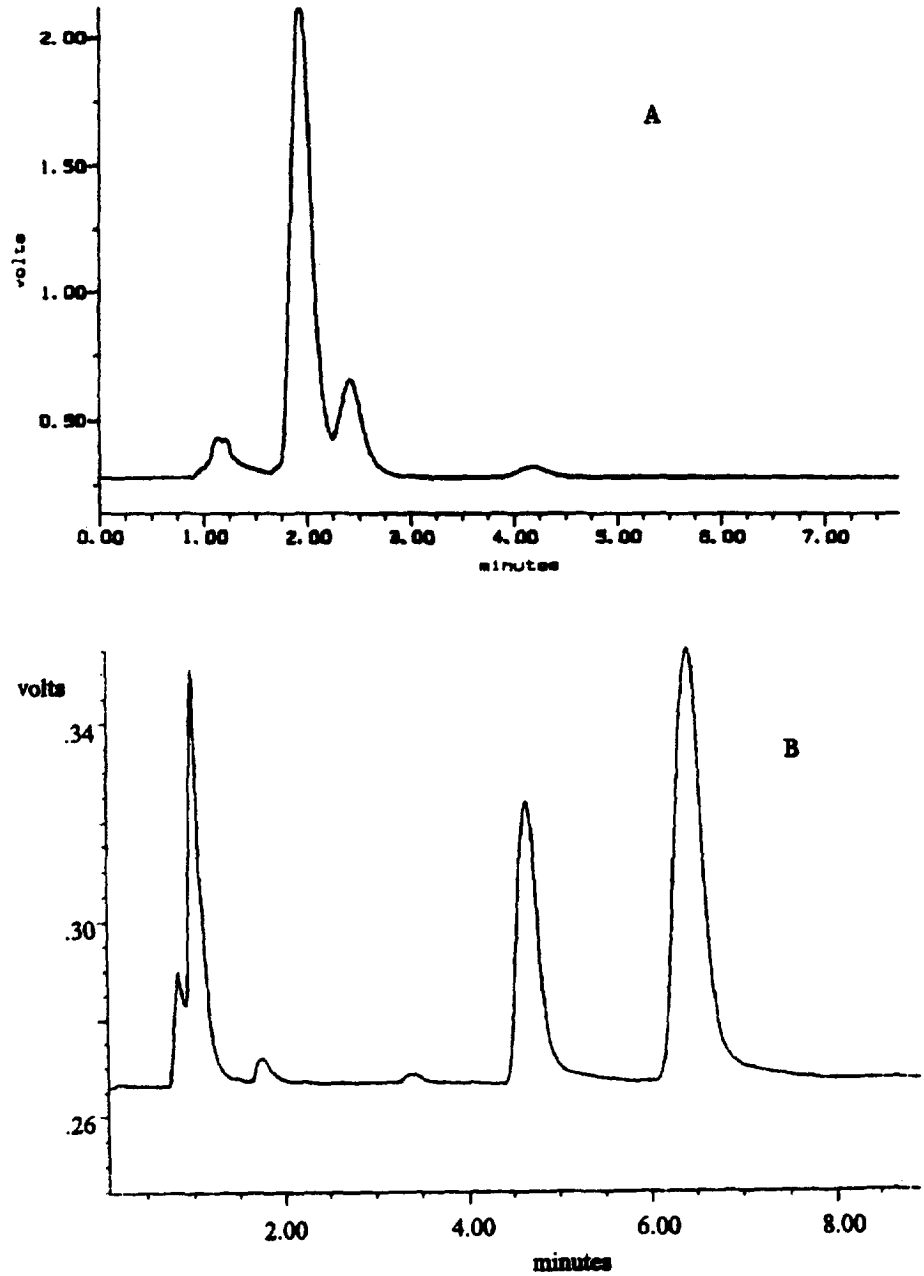

Fig. 2. LC–UV (280 nm) of two brand name toothpastes.

the standard triclosan solution was spiked directly onto the disk (i.e., no chromatography) and dried and a spectrum of triclosan was generated and also inserted into the library (Fig. 3A). The percent match of any unknown spectrum to both these library spectra (i.e., LC–FT-IR and disk spiked FT-IR) was considered to be a measure of success. The library search program (1300 spectra) that was used is a part of the Nicolet FT-IR software. An absolute difference search was implemented. This algorithm puts

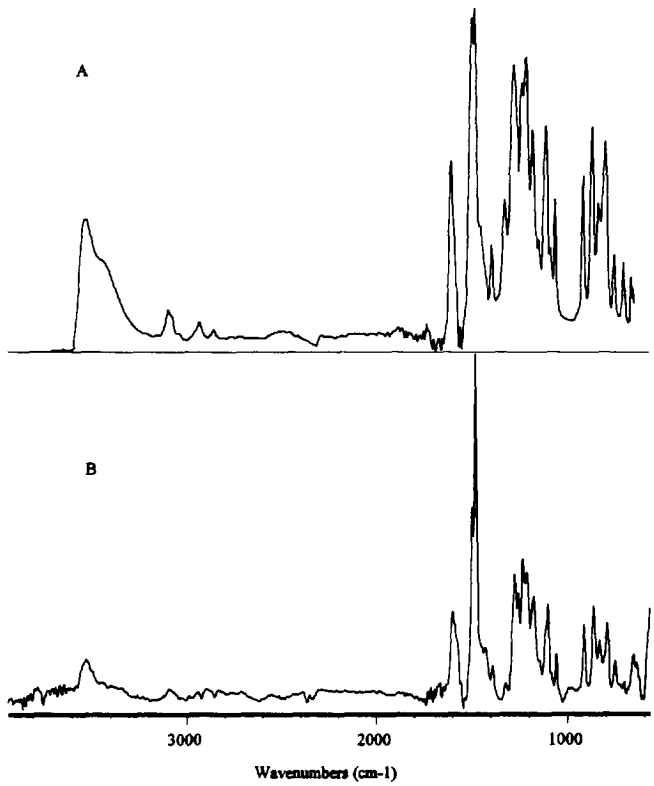

Fig. 3. FT-IR Spectra of triclosan. (A) Concentrated solution spiked onto disk; (B) 3 mg deposited on disk via LC–FT-IR.

more weight on small differences existing between the library and unknown spectra. It should be noted that the percent matches were used primarily to determine optimum deposition conditions.

After it was verified that the triclosan could be deposited on the disk and the deposition conditions had been optimized, solutions of the toothpaste which contained triclosan were made up and injected onto the column. First a placebo paste which did not contain triclosan was injected to ensure that there would be no interferences from the other components in the toothpaste.

After the lack of interference in the single toothpaste studied was verified, a solution of approximately 80 mg/ml toothpaste was made up and 200 μl of this solution was injected onto the column. Given that triclosan was present at less than 1% this should have produced depositions of approximately 2.5 μg triclosan when 5% of the mobile phase flow and injection volume were being diverted to the disk. Three 200-μl injections were made at disk rotations of 3, 5, and 10°/min. Fig. 4 is a Gram-Schmidt reconstruction after LC–FT-IR with deposition at a 5°/min disk rotation speed. The major peak in the GSR dominates the IR reconstruction, although, the triclosan peak was adequately separated and visible. In the GSR, the chromatographic run was not always collected at the starting point on the disk due to other deposits remaining on the disk; therefore, stated retention times are not a good indicator of peak identity in many cases.

After the IR spectra were generated from the reconstructions of the chromatographed additives, an automatic baseline correction routine was performed. It should be noted that background scans (256) were taken on a blank portion of the disk immediately before data collection started. The spectral searches were conducted on two different levels. The first was a total spectral search (4000–550 cm^{-1}) and the second consisted of only the 1400–1000 cm^{-1} region. The percent match to both triclosan library spectra (e.g., chromatographed and direct disk de-

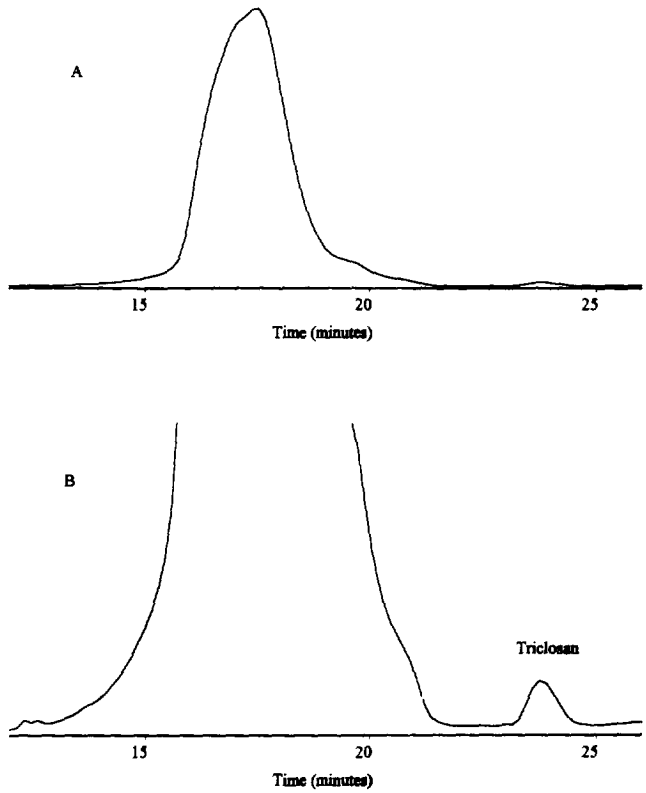

Fig. 4. Gram-Schmidt reconstruction for the injection of Colgate toothpaste. (A) Full scale view; (B) expanded scale.

posit) increased as the search area was restricted because of the distinctive fingerprint of triclosan in the 1400–1000 cm^{-1} region. There was greater correlation with the standard spectrum and the triclosan spectrum of the toothpaste sample that was produced by depositing 3 μg onto the disk after injection of the triclosan standard onto the column as opposed to the spectrum of the concentrated standard triclosan solution that was simply spiked onto the disk (see Table 1). These data signify that there is an advantage to having library spectra obtained in a similar manner under similar conditions. It should be noted that although some searches gave lower degrees of certainty the two triclosan library spectra (e.g., spiked on-column vs. spiked on disk) were always the top two computer matches. All other matches were below 70%. The third and fourth matches were not the same in all cases but most often were represented first by chlorinated phenols and second, methoxy phenols. The libraries were not

Table 1
Percent library match for triclosan deposited at 3, 5, and 10° disk rotation after 10 min purge

IR scale	4000–550 cm^{-1}	1400–1000 cm^{-1}
3°		
Deposited[a]	No match	96.5 (4)[c]
Spiked[b]	No match	94.5 (2)
5°		
Deposited	95.1 (3)	99.2 (1)
Spiked	84.1 (2)	96.4 (1)
10°		
Deposited	91.7 (4)	98.8 (1)
Spiked	82.1 (2)	96.2 (3)

[a] The deposited spectrum was that obtained by injecting a standard solution onto the column thereby depositing approximately 3 μg onto the disk.
[b] The spiked spectrum was that obtained by spiking the standard solution directly onto the disk, drying and analyzing.
[c] Numbers in parentheses are percent standard deviations, $n=3$.

user libraries set up to represent the components commonly found in toothpaste; therefore, there may not have been a significant number of very closely related compounds in the library. More specifically, the spectral library did not contain any close analog of triclosan which must make proper recognition easier.

After establishing that the method was reproducible within a given day, a day to day reproducibility study was conducted. The study involved three replicate analyses at each of the three rotation conditions on each of three separate days. This study involved taking into account variations that occurred due to the powering down of the instrument, column fluctuations, as well as using a new batch of mobile phase between days 2 and 3. The data are given for the grand average over the three day time period (Table 2). The 5 and 10° data gave better results both in the percent match as well as the RSDs. Generally speaking, the peaks obtained by rotating the disk at 5° were easier to pick out of the reconstructions due to their slightly increased intensities. On the third day of analysis; however, the 10° rotation peaks were easier to spot because the peaks obtained during slower rotations appeared on the tail of the initial peak. The data in Tables 1 and 2 indicate that the LC–FT-IR method used for the positive identification of triclosan appears to be efficient at approximately 2.5 μg of toothpaste deposited on the disk with a 200-μl chromatographic injection.

Four toothpaste samples, two of which were gels, were obtained and solutions made up under these standard practice instructions. The solution to be analyzed was prepared by slurrying 3 g of toothpaste in 100 ml of acetonitrile–water. Under these conditions an injection volume of 550 μl would be necessary to deposit the 2.5 μg of triclosan given the suspected concentration of analyte in the toothpaste. Each (3 g toothpaste per 100 ml solvent) was then individually injected (550 μl) onto the column and FT-IR spectra recorded at a 5°/min disk rotation. Good matches with library spectra were noted (Table 3). Given these large injection volumes, column lifetime is apt to greatly decrease. Without the use of a preparative scale column, injection volumes this large will affect resolution; however, the resolution was not affected to a great enough extent in this study to inhibit seeing and identifying the triclosan peak.

Table 2
Averaged data for the three day reproducibility study[a]

IR scale	4000–550 cm^{-1}	1400–1000 cm^{-1}
3°		
Deposited	No match	No match
Spiked	No match	No match
5°		
Deposited	93.6 (4)	98.5 (1)
Spiked	81.9 (5)	94.7 (2)
10°		
Deposited	93.3 (3)	98.5 (1)
Spiked	81.8 (2)	95.7 (2)

[a] See footnotes to Table 1, $n=9$.

Table 3
Data (% Library match) for injections (550 μl) of four toothpaste[a] samples

IR scale	4000–550 cm^{-1}	1400–1000 cm^{-1}
Sample 39546		
Deposited	92.4	97.0
Spiked	81.1	93.3
Sample 93048		
Deposited	89.8	97.3
Spiked	81.2	94.3
Sample 39540		
Deposited	92.5	96.5
Spiked	80.5	95.3
Sample 44045		
Deposited	96.5	99.3
Spiked	84.2	96.5

[a] See footnotes to Table 1, $n=1$.

4. Conclusions

The LC-Transform interface for FT-IR allowed for the identification of the additive component, triclosan, in toothpaste. The disk rotation rate during deposition played a primary role in the ability to sufficiently separate the components. The accuracy of the method was demonstrated by high percent library matches, although, having a library spectrum taken under the same conditions with a similar quantity of known triclosan standard was beneficial. The analysis of the relatively low levels of triclosan in the presence of a concentrated and strongly

absorbing species showed the great applicability of this method. This methodology eliminates the costly and time consuming sample preparation which would normally be used to isolate and identify triclosan.

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