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Simultaneous determination of anions and triclosan in dentifrices by gradient ion chromatography and isocratic high-performance liquid chromatography interfaced with conductivity and ultraviolet detection

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Abstract

The simultaneous separation of fluoride, phosphates and triclosan ions (F^-, PO_4^{3-}, $P_2O_7^{4-}$, $Cl_2H_7C_{13}O_2$) in a dentifrice formulation using a coupled ion chromatography and high-performance liquid chromatography system is described. The anion species are separated from the other components of a given dentifrice formulation using a Dionex IonPac AS11 (250 × 4.0 mm) analytical column. A sodium hydroxide (200 m*M* to 100% water) gradient mobile phase is used to elute the fluoride and phosphate species from the column within 7 min using a Dionex Anion Self-Regenerating Suppressor (ASRS-I 4 mm). The separation of anions and triclosan was carried out using a two-mobile phase system that simultaneously injected a 15-μl sample into the 200 m*M* NaOH to 100% water ion chromatography gradient system as well as a 10-μl sample into the water–acetonitrile (40:60) isocratic HPLC system. The anion species are then quantitated using a conductometric detector (0–30 μS). Triclosan is separated from the other components of the dentifrice formulation using a Waters Nova-Pak C_{18}, 4 μm, 150 × 3.9 mm HPLC column. A water–acetonitrile (40:60) mobile phase is used to elute the triclosan from the column within 6 min. The triclosan analyte is then quantitated using ultraviolet detection at 280 nm and 0.005 AUFS. All analytes are quantitated using the Dionex AI-450 chromatography software program (release 3.30).

1. Introduction

The primary goal in our laboratories is to measure the components of various dentifrice formulations by classical methods. The introduction of a multicomponent sample would encompass at least three times the amount of analyst preparation and analysis time using current methodologies. The aim of this study is to develop an assay that simultaneously determines the amount of fluoride, phosphate and triclosan from a single sample preparation.

In the present paper, we propose that a union of suppressed gradient ion chromatography (IC) and reversed-phase isocratic HPLC to provide the best of both worlds, in so far as quantitation of a multicomponent dentifrice matrix is concerned.

2. Principles

Frequently IC separation involves species of widely different affinities for the stationary phase. In such a case, eluent conditions that favor the resolution of most weakly held species

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are often unsuitable for the more tightly held ions in a sample matrix [1]. These samples cannot be easily handled by isocratic methods, because of their wide k' range [2]. The ionic separation is possible only by gradient elution over a specific concentration range.

Applications to ionic analysis using conductometric single-column IC were soon demonstrated [3]. More recently, Renn and Synovec [4] improved the potential practical utility of this concept with a system that simultaneously injects separate portions of an unknown sample into two individual IC systems, operating in parallel with different eluents.

Suppressed conductometric anion chromatography has proven to be the analytical technique of choice for the determination of strong and moderately weak acid anions [5]. If a column containing sulfonated polystyrene–divinylbenzene is treated with a hydrogencarbonate anion, the fixed quaternary amine moiety is completely converted into the carbonate anion form. The anions of interest will be exchanged in an equilibrium process for the carbonate anion. The separation of the anions will be controlled by their different affinities for the stationary phase [6].

Both from a theoretical and practical standpoint it is simplest to consider a background of nearly pure water, as may be obtained with NaOH eluent in suppressed IC system [7]. Recently developed electrodialytic on-line ultrapure eluent generators and suppressors [5] can indeed attain essentially pure water as the detector background.

The application of gradient elution in HPLC with UV detection follows directly [1]. The development of HPLC and the theoretical understanding of the separation processes involved have been, in particular, dependent on the fundamental studies by Horváth *et al.* [8], Knox [9], Scott [10] and Snyder [11]. Reversed-phase systems are characterized by strong interactions between the polar mobile phase and various sample molecules.

Moreover, interactions between sample molecules and the non-polar stationary phase are weak. This effect suggests that interactions between sample and solvent molecules will mainly determine relative retention and values of α in reversed-phase separations [12]. The differences in interactive energies between non-polar solutes with the mobile phase and the differences in hydrophobic solute molecular surface area are responsible for the functional group selectivity observed in reversed-phase chromatography [13].

3. Experimental

3.1. Apparatus

An electropneumatically driven microinjector valve (Dionex, Sunnyvale, CA, USA) equipped with a 15-μl loop and connected in series to a Dual-Stack Slider Valve equipped with a 10-μl loop (Dionex) was used for sample injections (see Fig. 1). The IC system was suppressed using a Dionex Anion Self-Regenerating Suppressor (ASRS-I 4 mm) with a SRS Controller setting of 3. The Autosuppression External Water Mode

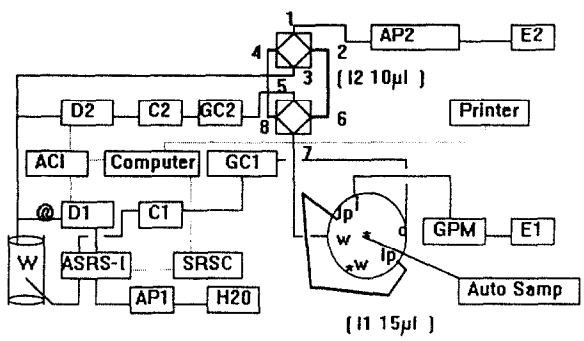

Fig. 1. Schematic of simultaneous injector system using two different columns and mobile phases. The MicroInjector Valve (MIV) and the Dual-Stacked Slider Valve (DSSV) (Dionex) are shown in the load position. AP1, AP2 = External HPLC pumps; ASRS-I = membrane suppressor; Auto Samp = Dionex automated sampler; ACI = Advanced Computer Interface; Computer = Dell system 310w/AI-450 software; C1, C2 = IonPac AS11, Nova-Pak C_{18}; D1, D2 = conductometric and UV detectors; E1, E2 = 200 m*M* NaOH and water–acetonitrile (40:60); GC1, GC2 = guard columns IonPac AG11 and Nova-Pak C_{18}; GPM = gradient pump module; I1, I2 = injector valves MIV (15 μl) and DSSV (10 μl); Printer = Digital 2100 Plus; SRSC = SRS controller; W = HPLC waste; @ = (4 mm) backpressure assembly.

was achieved using a Waters 6000A pump at a flow-rate of 2.1 ml/min. A Dionex IonPac AG11 (50 × 4.0 mm) guard column was used. The separations were carried out on a Dionex IonPac AS11 (250 × 4.0 mm). Table 1 shows the 200 m*M* NaOH to 100% water gradient profile. A gradient pump module (Dionex) was used to achieve a 3%/min gradient profile using a flow-rate of 2.0 ml/min and conductometric detection with a temperature compensation of 1.7°C. The samples were injected using an automated sampler (Dionex) with settings: Type: sample, Inj: 1, Type: loop, Mode: prop, Bleed: off, Inj/Vial: 1.

The isocratic HPLC system was achieved using a Waters 510 pump plumbed with 0.010 in. (1 in. = 2.54 cm) polyether ether ketone (PEEK) tubing (Dionex) into the Dual-Stacked Slider Valve. A Waters pre-column Guard-Pak consisting of a Nova-Pak C_{18} insert, and a Nova-Pak C_{18}, 4 μm, 60 Å, 150 × 3.9 mm analytical column was used for triclosan separations. The water–acetonitrile (40:60) eluent, at a 1.5 ml/min flow-rate, and a detection wavelength of 280 nm and 0.005 AUFS, was used. All injections were made at ambient temperature. The chromatograms were recorded and quantitated using the Dionex AI-450 chromatography software program (release 3.30).

The volumetric ware was Nalgene PMP. The pipettes were Kimax USA. The HPLC filters were 0.45-μm nylon Titan HPLC syringe type.

Table 1
Ion chromatographic gradient profile used to separate anions from dentifrice formulations

Time (min)	Flow (ml/min)	%1	%2	V5	V6
0.0	2.0	10	90	0	0
0.1	2.0	10	90	1	1
0.2	2.0	10	90	0	0
10.0	2.0	40	60	0	0
10.1	2.0	10	90	0	0
15.0	2.0	10	90	0	0

Ion chromatographic parameters: low pressure limit = 0; high pressure limit = 3000; eluent 1 = 200 m*M* NaOH; eluent 2 = 100% 18 MΩ deionized water; V5 off (0) = $PO_4^{3-}/P_2O_7^{4-}$; V5 on (1) = 15 μl; V6 off (0) = $Cl_2H_7C_{13}O_2$; V6 on (1) = 10 μl.

The centrifuge ware was Nalgene Oak Ridge (50 ml). The centrifuges used were DuPont refrigerated superspeed Servall RC-2 and Sorvall RC-5B both with an angular velocity of 13 000 rpm at 10–20°C. The vortex used was a Baxter SP mixer.

3.2. Materials

Dihydrogen disodium pyrophosphate (97.17% pure, Monsanto), triclosan (Irgasan 300, 100.1% pure, Ciba-Geigy), sodium phosphate dibasic (99.5% pure, Fisher Scientific), sodium fluoride (99.999% pure, Aldrich) were purchased commercially.

3.3. Analytical Reagents

NaOH volumetric solution (0.2 *M*, Mallinckrodt) and acetonitrile, UV grade (Burdick & Jackson) were also obtained commercially. The following dentifrice formulations were prepared by SmithKline Beecham Product and Process Development, Weybridge, UK: placebos and 80–120% of full formula.

3.4. Procedures

Preparation of standard stock solutions

Standard stock solutions were prepared to contain 80–120% (w/v) of their respective anions in 18 MΩ water. The triclosan stock solution was prepared in 100% acetonitrile. Each stock solution was diluted to a working standard stock concentration and diluted with their respective solvents and mixed thoroughly. For calibration purposes, dilute standard solutions of each analyte were prepared by stepwise dilution with a water–acetonitrile (40:60) diluent of each working standard stock solution to obtain exactly 80–120% of the full formula per 100 ml.

Preparation of tests solutions

A 10–20-g sample of dentifrice was composited, and from that a 2-g sample was accurately weighed into a 50-ml Nalgene centrifuge tube. An aliquot of 25 ml of (40:60) diluent was introduced along with 4 glass beads (4 mm) and

vortexed 4 min (speed 10) timed. The aliquots were cold centrifuged at 10–20°C for 15 min with an angular velocity of 13 000 rpm. The supernatant was quantitatively transferred into a 100-ml Nalgene PMP volumetric flask. The extraction procedure was repeated a total of three trials. The supernatants were completed to volume with the (40:60) diluent solution and mixed thoroughly on a vortex. A 1:20 dilution was performed and mixed thoroughly on a vortex. All sample and standard solutions were filtered using a 0.45-μm nylon filter.

Chromatographic separation of anions and triclosan

The separation of anions and triclosan was carried out using a two-mobile phase system that simultaneously injected a 15-μl sample into the 200 m*M* NaOH to 100% water IC gradient system as well as a 10-μl sample into the water–acetonitrile (40:60) isocratic HPLC system (see Fig. 1). The combined IC–HPLC system is described fully in the *Apparatus* section.

Calibration curves

Calibration curves were constructed from the three dilute standards of each anion and triclosan. Each concentration was injected onto the columns. Least squares regression was used to determine linearity characteristics.

Chromatographic analysis of dentifrice forms

The prepared test solutions were chromatographed three times against the constructed calibration curves.

4. Results and discussion

4.1. Chromatographic separation of anions and triclosan

To permit retention and good separation of both anions and triclosan in a single diluent preparation, an optimal water–acetonitrile diluent was selected. The extraction time was chosen at 3 times and water–acetonitrile (40:60) diluent concentration to yield 100 ± 3% recovery of both anions and triclosan. Optimization of the extraction time and diluent composition is illustrated in Table 2. The gradient optimization was reached at a concentration of 44–55 m*M* of the counterion in a mobile phase consisting of 200 m*M* NaOH to 100% water using a 3%/min gradient profile. A 50% decrease in the concentration of the counterion resulted a loss in resolution of the phosphate anions. Moreover, an increase in the gradient profile to 66% resulted in a loss of resolution of the early eluting fluoride ion.

Table 2
Analytical results of extraction time study (batch 016) to determine optimum sample extraction time using a water–acetonitrile diluent: recovery (%) *vs.* extraction trials

Analyte[a]	Recovery (%) (n = 3)			
	1 ×[b]	2 ×	3 ×	5 ×
Water–acetonitrile (50:50)				
F^-	100.26	108.99	108.65	110.34
$P_2O_7^{4-}$	105.47	110.32	107.84	108.74
$Cl_2H_7C_{13}O_2$	84.83	85.33	89.46	89.60
Water–acetonitrile (40:60)				
F^-	88.52	95.66	102.42	103.66
$P_2O_7^{4-}$	96.82	102.36	103.47	103.89
$Cl_2H_7C_{13}O2$	98.38	90.61	102.00	103.81

[a] Tube composite of separate sample masses.
[b] Number of extraction trials.

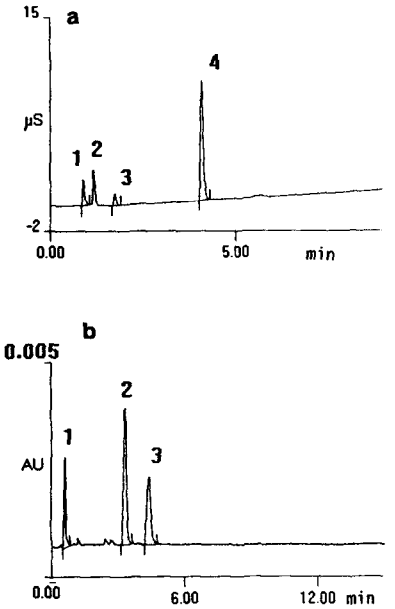

Fig. 2. (a) Representative chromatogram for identification and separation of anions within a (100%) dentifrice formulation. Peaks: 1 = F^-; 2 = unknown; 3 = PO_4^{3-}; 4 = $P_2O_7^{4-}$. (b) Representative chromatogram for identification and separation of triclosan within a (100%) dentifrice formulation. Peaks: 1 = unknown; 2 = unknown; 3 = $Cl_2H_7C_{13}O_2$. Note: chromatograms shown in each figure were obtained from a single injection.

4.2. Chromatographic separation of triclosan.

A method validated in our laboratory was modified to contain water–acetonitrile (40:60). Separation and quantitation of triclosan compared favorably to existing methodologies in our laboratory. Identification of each anion and triclosan was achieved after comparisons with relative retention times of standard dilutions of respective anions in the water–acetonitrile (40:60) diluent. Fig. 2a and b illustrates a good separation of the dentifrice formulation using the simultaneous injector system. The mean relative retention times (Table 3) obtained from six chromatograms of each anion were: 0.2324, 0.4414 and 1.0097, respectively. The mean relative retention time of triclosan was 1.0034 compared to a (100%) standard concentration as shown in Table 3.

At the optimum counterion concentration and water–organic ratio, separations of the analytes were obtained within 7 min.

4.3. Calibration curves

Table 3 shows that good linearity is accomplished for amounts of 80–120% of formula using three concentration levels.

4.4. Chromatographic analysis of anions and triclosan in some laboratory-scale dentifrice

Table 4 shows analytical results of some laboratory-scale aged dentifrice. The aged dentifrice was chosen to assess the impact of the stability indication of the developed assay. The method was found to show stability indications with the breakdown phosphate anion (PO_4^{3-}) eluting before the major phosphate component (see Fig. 2a). Proprietary constraints prohibit the declaration of amount of breakdown in the current dentifrice prototype. Moreover, the results

Table 3
Relative retention times (RRTs) of anions and triclosan and linearity of their calibration curves

Analyte	RRT	S.D. ($n = 6$)	Range (%)	R^2 [a]
F^-	0.2324[b]	0.0020	80–120	0.9913
PO_4^{3-}	0.4414[b]	0.0447	80–120	0.9960
$P_2O_7^{4-}$	1.0097[b]	0.0801	80–120	0.9977
$Cl_2H_7C_{13}O_2$	1.0034[c]	0.0024	80–120	0.9951

[a] R^2 obtained from three points.
[b] Relative to phosphate.
[c] Relative to a triclosan (100%) standard.

Table 4
Analytical results of (aged) laboratory scale dentifrice

Analyte[a]	Theory (% w/w)	Amount found (%, w/v) ($n = 3$)	Recovery (%)	R.S.D. (%)
F^-	80	78.03	97.78	1.91
	100	99.96	98.78	2.53
	120	88.21	73.51	0.37
$P_2O_7^{4-}$	80	93.48	111.38	2.32
	100	113.59	107.39	1.49
	120	143.49	94.20	0.36
$Cl_2H_7C_{13}O_2$	80	83.75	104.69	3.72
	100	107.46	107.46	0.99
	120	136.71	113.92	5.02

[a] Tube composites of separate sample masses.

found in Table 4 were calculated based upon a total amount of phosphate (PO_4^{3-} and $P_2O_7^{4-}$) recovered. Good recoveries and acceptable precision were obtained within a detection range of 80–120% of prototype formula levels.

4.5. Chromatographic analysis of anions and triclosan in a pilot batch

Table 5 shows analytical results of the pilot batch dentifrice. Good recoveries and acceptable precision were obtained at the (100%) full prototype formula level. Excipients did not show interference with the eluting anions and triclosan. Spiking the pilot production dentifrice with each analyte did not alter the relative retention time of each anion and triclosan.

5. Conclusions

The ability to extract both anions and triclosan from one sample preparation and resolve these analytes in 7 min using gradient IC coupled with isocratic HPLC has been demonstrated. This new method was found to be considerably faster than presently used methods for the determination of anions and triclosan in dentifrice formulations. The method has been shown to be stability indicating, yielding a reproducible amount of (PO_4^{3-}), within the linear range of the (PO_4^{3-}) calibration curve. Moreover, future applications as an encompassing cleaning validation method of anions and triclosan appear to show merit. Further development work will be addressed to assess method ruggedness.

Table 5
Analytical results of a pilot plant batch (016) of dentifrice containing anions and triclosan

Analyte	Theory (%, w/w)	Amount found (%, w/v) ($n = 6$)	R.S.D. (%)
F^-	100	97.72	4.40
$P_2O_7^{4-}$	100	98.15	2.74
$Cl_2H_7C_{13}O_2$	100	100.05	0.55

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