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Matrix elimination in ion chromatography by "heart-cut" column-switching techniques

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ABSTRACT

Are there occasions when a "dilute and shoot" approach to ion chromatographic analysis is appropriate? How can you deal with column overloading? What about system abuse? This paper describes applications where a simple "dilute and shoot" directly or coupled with "heart-cut" column switching techniques can make "dilute and shoot" a reality. A fully automated "dilute and shoot" method using the "heart-cut" technique has been developed for sulfite analysis in a variety of matrices. "Dilute and shoot" methods were also developed for acetate and trifluoroacetate in peptides. Preliminary results are also report herein for the determination of trace anions (μg/l) in concentrated hydrochloric acid, with minimal sample preparation.

INTRODUCTION

A technique was previously described to effect matrix elimination in liquid chromatography mechanically [1] rather than chemically via sample pretreatment. The technique uses two 4-way valves inserted before and after the pre-column. The pre-column is the first column the sample sees after injection and can be a guard column, a separator column or an entirely different type of column than the separator column, *e.g.*, a reverse phase pre-column and an ion exchange separator column. The valves are configured such that the bulk of the matrix is diverted to waste and only a "heart-cut" (H-C) of the analyte of interest is transferred to the separator column. The H-C column-switching technique can be extremely effective in either eliminating or at least simplifying sample preparation. This paper describes a variety of applications where a "dilute and shoot" approach has been successfully employed.

EXPERIMENTAL

The sulfite analyses were conduction using three AS-2 anion-exchange columns (Dionex) with a 1.5 m*M* sodium carbonate, 1.3 m*M* sodium bicarbonate and 0.076% (v/v) formaldehyde eluent. The experimental details of the ion chromatography (IC) procedure for sulfite analysis in the analgesic formulations and food items etc. are given in ref. 1. The food samples were purchased from the local grocery store. Samples with high sulfite content were prepared in the usual manner, 4 g/100 ml, and diluted as required with 0.76% (v/v) formaldehyde. Instrumental details of the H-C technique are also described in Ref. 1. See Fig. 1 for valve plumbing connections.

Heart-cut column-switching procedure

Initially, the system is assembled without the separator column in order to determine the H-C timing interval. Standards are injected and the time for the onset of the analyte peak and complete elution of the analyte peak noted. This time interval is the H-C. The entire system is then equilibrated, after re-inserting the separator column, and the analysis conducted in a four step sequence as follows:

(1) Divert initial portion of chromatogram to waste. The sample is injected with the first valve closed, eluent flows through pre-column to the second valve which is opened to waste.

(2) Introduce H-C to the separator column and

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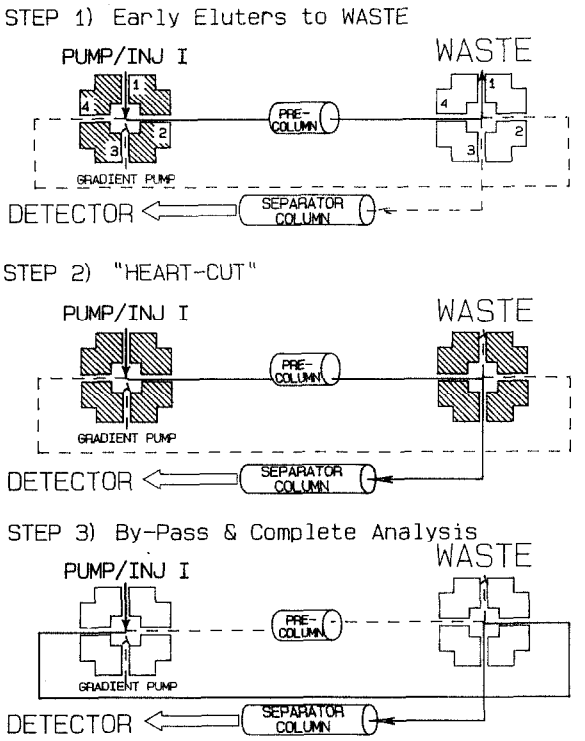

Fig. 1. Heart-cut valve configuration.

detector. At the pre-determined time interval, the onset of the H-C, the second valve is closed to divert the pre-column effluent to the separator column.

(3) By-passing the pre-column and detecting the analyte. Following the H-C interval, when the entire H-C has been eluted from the pre-column, the first valve is opened to divert the eluent stream to the second valve. The second valve is immediately opened to allow eluent from the first valve to flow into the separator column, by-passing the pre-column.

(4) Pre-column clean-up. After the sulfite H-C was transferred to the separator column, a step gradient program was used to flush the pre-column of later eluters. A three-phase, 1.5-min each, gradient program of methanol, concentrated eluent (0.05 *M* each NaOH, Na_2CO_3) and polished water was run followed by re-equilibration back to the carbonate–formaldehyde eluent in time for the next injection. A gradient clean-up was not required for the other applications described. The pre-column was cleaned through simple mobil phase elution and the next analysis begun, step 1.

The IC system was a Dionex 4500i dual-channel chromatograph with an automated sampler and a pulsed electrochemical detector (PED), used in the conductivity mode. A 50 μL loop, an AMMS Dionex suppressor with 25 m*M* H_2SO_4 regenerant at 3 ml/min and Dionex anion-exchange columns were used throughout. Acetate analysis was conducted on an AG-7 column with 20 m*M* borate eluent at 1 ml/min. The TFA and anions in concentrated HCl were conducted via H-C analysis using an AG-4A pre-column and a AS-4A separator column with 2 m*M* Na_2CO_3/0.75 m*M* $NaHCO_3$, eluent at 2 ml/min.

All chemicals were Mallinckrodt AR and the water was polished (deionized water further purified through a Millipore Milli-Q filtration system). Acetate: dissolve 1 mg of peptide (Desmopressin or Calcitonin Acetate) in a 2-ml volumetric flask and dilute to volume with water. TFA: dissolve 2 mg of peptide in a 2-ml volumetric flask and dilute to volume with water. Anions/HCl: add 10 ml of 1% (w/v) sodium carbonate and 200 ml of concentrated HCl to a 400-ml beaker and evaporate to dryness on a steam bath. Transfer the residue, quantitatively, to a 10-ml volumetric flask with successive additions of water and dilute to volume.

RESULTS AND DISCUSSION

Sulfite

The previously described sulfite analysis in analgesic formulations [1] has been fully automated. In the earlier work, late eluters were left on the pre-column and flushed from the column after the analysis was complete, with three successive 500 μl injections each of methanol, concentrated eluent (0.05 *M* NaOH, Na_2CO_3) and polished water. By employing a second pump and injector, the pre-column clean-up was conducted immediately after the H-C of the analyte was transferred to the separator column. The latest revision utilizes a step gradient program, of 1.5 min each, methanol, concentrated eluent and polished water, respectively, which lends itself to a fully automated basis (Fig. 1).

The generic utility of the sulfite "dilute and shoot" method arises from the fact that the bulk of the matrix is removed from the analysis, therefore, we should be able to extent this method to the analysis of sulfite in a variety of matrices. The US Food

and Drugs Administration (FDA) has established a 10 ppm labelling limit for sulfite in food items and has recommended that drug manufacturers monitor their products at the same level. Reliable methodology for the determination of the metastable sulfite ion at the level of interest presents a formidable analytical challenge [2–4]. The FDA 10 ppm sulfite labelling limit mandated that appropriate methodology be developed. An accurate reliable means of analysis, not susceptible to matrix interference problems and, of course, rapid, would be ideal. The H-C method approaches this ideal. A comparison was made using our H-C method and the IC method of the USA Association of Official Analytical Chemists (AOAC) [5]. The same food items described in a collaborative AOAC study were analyzed by the H-C method. In the author's opinion, the H-C analysis using conductivity detection was demonstrated to be superior to the AOAC method which uses electrochemical detection, however, it should be noted, no attempt was made to run the AOAC method. The conclusions are based on the literature discussion of the problems encountered with the AOAC method. Major shortcomings described for the AOAC method include: ruggedness, accuracy (based on spike recovery data), precision, stability of the analyte and susceptibility to matrix interference. In our comparison, accuracy was accessed based on spike recoveries at three levels.

In the AOAC's analysis of corn starch, seven of the collaborators failed to detect any sulfite while two other collaborators found 15 and 21 ppm in duplicate sample analyses. The conflicting findings were termed false positives due to inadequate chromatography, "interferences from the matrix". The findings were rejected on statistical grounds. H-C analysis of corn starch found sulfite comparable to the rejected levels, 23 ppm with a 95% average recovery. Our study was also extended to wheat starch where none was detected, <2 ppm with a 94% average recovery (Table I).

The AOAC study found consistently higher results obtained by the IC method, about 400 ppm, relative to the Monier–Williams [6] wet chemical procedure, about 290 ppm, for instant mashed potatoes. The H-C analysis found 175 ppm with a 84% recovery (Table II).

Comparable results were obtained by both IC methods for wine coolers, 19 ppm AOAC *vs.* 42 ppm H-C with a 98% recovery. In the H-C case, the sample was a red sangria wine cooler. The AOAC sample was non-specific; however, a general problem of colored samples was noted. Our method was also extended to a sample of white wine which contained considerably more sulfite, as expected, 132 ppm with a 109% average recovery (Table III).

A problem with interference was encountered for the lemon juice analysis. An unknown peak circum-

TABLE I

COMPARISON OF IC METHODS FOR STARCH ANALYSIS

Sample designation	AOAC method		H-C method	
	Found (ppm)	Recovery (%)	Found (ppm)	Recovery (%)
Corn starch	0	–		
10 ppm spike	5.5–29	55–112		
30 ppm spike	22–45	72–112		
Corn starch (average 23 ppm)			22	–
5 ppm spike			27	85
10 ppm spike			33	105
15 ppm spike			37	94
Duplicate sample preparation			24	–
Wheat starch			<2	–
5 ppm spike			4.9	99
10 ppm spike			9.0	90
15 ppm spike			14	93
Duplicate sample preparation			<2	–

TABLE II

IC DETERMINATION OF SULFITE IN INSTANT MASHED POTATOES

Sample designation	AOAC method		H-C Method	
	Found (ppm)	Recovery (%)	Found (ppm)	Recovery (%)
Instant mashed potatoes	341–436	–		
80 ppm spike	411–609	84–219		
400 ppm spike	558–1038	56–164		
Instant mashed potatoes (average 175 ppm)			195	–
250 ppm spike			371	79
500 ppm spike			597	84
750 ppm spike			850	90
Duplicate sample preparation			155	–

TABLE III

SULFITE IC ANALYSIS OF WINE

Sample designation	AOAC method		H-C Method	
	Found (ppm)	Recovery (%)	Found (ppm)	Recovery (%)
Wine cooler	9–29	–		
10 ppm spike	20–45	39–213		
30 ppm spike	36–65	67–139		
Red sangaria wine cooler (average 42 ppm)			39	–
50 ppm spike			92	99
100 ppm spike			141	99
150 ppm spike			185	95
Duplicate sample preparation			45	–
White wine (average 132 ppm)			133	–
50 ppm spike			187	108
100 ppm spike			244	112
150 ppm spike			293	107
Duplicate sample preparation			131	–

TABLE IV

SULFITE IC ANALYSIS OF LEMON JUICE

Sample designation	AOAC method		H-C Method	
	Found (ppm)	Recovery (%)	Found (ppm)	Recovery (%)
Lemon juice	12–30	–		
10 ppm spike	21–52	70–299		
30 ppm spike	36–90	71–201		
Lemon juice (average 155 ppm)			158	–
250 ppm			372	87
500 ppm spike			583	86
750 ppm spike			877	96
Duplicate sample preparation			153	–

TABLE V

SULFITE IN A COLA SOFT DRINK BY H-C ANALYSIS

Sample designation	Found (ppm)	Recovery (%)
Cola soft drink	<2	–
5 ppm spike	4.8	96
10 ppm spike	9.3	93
15 ppm spike	13	85
Duplicate sample preparation	<2	–

vented accurate quantitation. We speculated that the unknown might be organic in nature and were prompted to substitute a PRP-1 reversed-phase column for the AS-2 pre-column. Such a multi-dimensional scheme was previously found to be very effective at dealing with organics [1]. The interference was removed from the subsequent chromatograms and accurate quantitation was achieved, 155 ppm with a 90% average recovery. The AOAC analysis reported 21 ppm, however, the sample we analyzed was labelled "contains sulfite" (Table IV).

The AOAC method could not analyze caramel colored samples. The H-C method appears to be color blind. A cola soft drink was readily analyzed, <2 ppm with a 91% average recovery (Table V).

The actual sulfite content in the samples analyzed in this study *vs.* the specific samples used in the AOAC collaborative study are inconsequential. What this study has demonstrated is the superiority of the H-C method which can be attributed to maintaining analyte stability, using a more rugged detector and eliminating matrix complications via H-C column switching techniques. The net result is that the H-C "dilute and shoot" sulfite method is a more accurate, precise, reliable, rapid, generic procedure; suitable for a broad diversity of sample types (foods, beverages and pharmaceuticals) and should facilitate testing to ensure compliance with the FDA's 10 ppm limit.

Peptides

Mallinckrodt Specialty Chemical Company's R&D is developing a peptide product line which includes calcitonin and desmopressin acetate. A request was made to develop methods for the determination of acetate and trifluoroacetate (TFA) in the final products. A "dilute and shoot" approach was developed for each.

The acetate determination was accomplished using a weak eluter system, 20 m*M* borate eluent on an AG-7 anion-exchange column. The bulk of the matrix was retained on the column and eventually

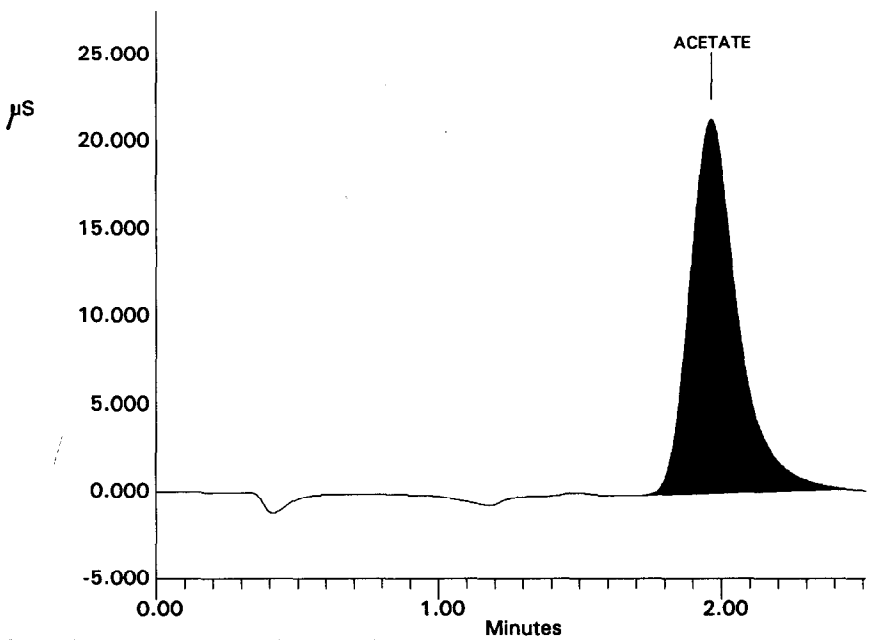

Fig. 2. Acetate determination in calcitonin acetate.

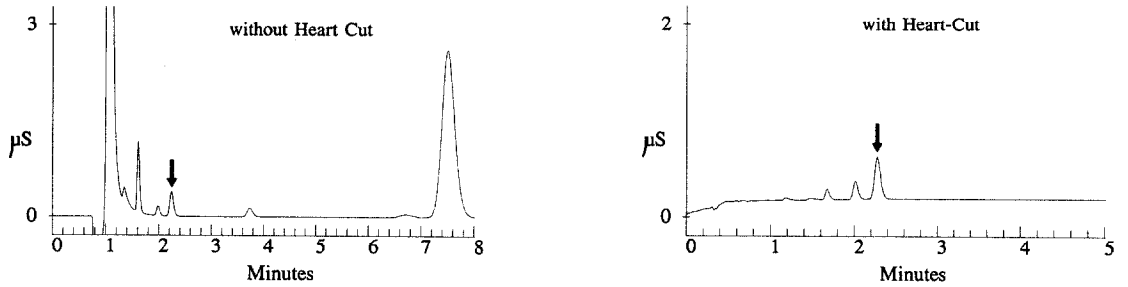

Fig. 3. TFA determination in calcitonin acetate with and without heart-cut column switching.

flushed off with concentrated eluent after the analyses were completed. Since the bulk of the matrix was retained on the column, no H-C column switching was required (Fig. 2). The TFA analysis was accomplished via H-C column switching to eliminate the bulk of the matrix and reduce the analysis time about 40% (Fig. 3).

Anions in hydrochloric acid

A direct "dilute and shoot" approach for trace

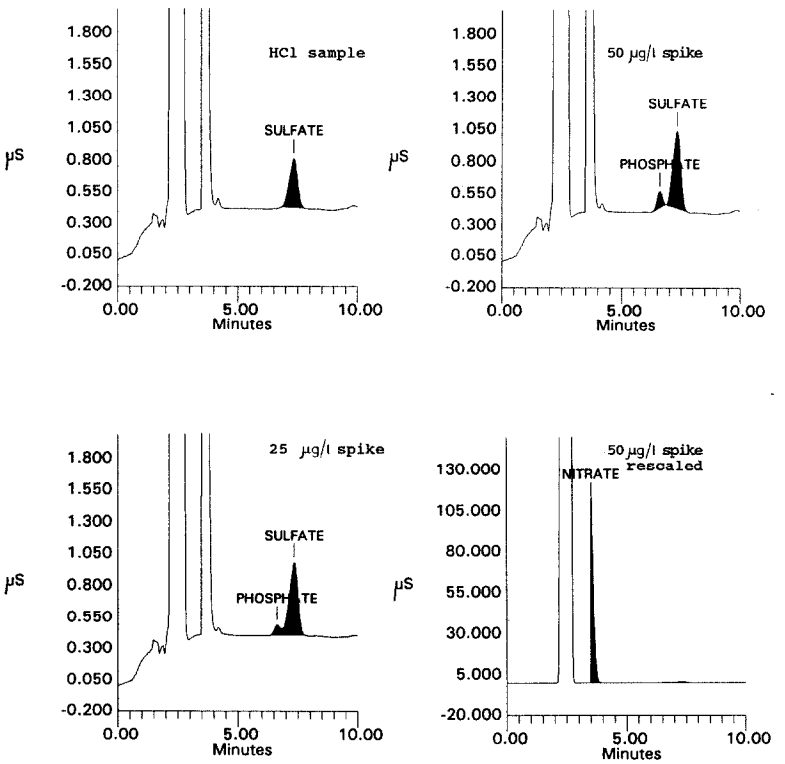

Fig. 4. Trace anions in concentrated hydrochloric acid. Experimental conditions: after determining the heart-cut for the interfering anion, chloride, relative to the anions of interest, sulfate and phosphate, the IC system was configured as described using a AG-4A pre-column and a AS-4A separator column. The valve configuration was the same as step 1 in Fig. 1, from 0–0.5 min, then step 2 for the remainder of the analysis. The eluent was 2 m*M* sodium carbonate and 0.75 m*M* sodium bicarbonate at 2 ml/min. A 50-µl loop was used. An AMMS suppressor was used with 25 m*M* sulfuric acid regenerant at 3 ml/min. Detection was accomplished with a PED detector (Dionex) in the conductivity mode.

anions (μg/l) in concentrated hydrochloric acid was unsuccessful, even using our H-C technique, however, a tentative procedure has been developed which keeps the sample preparation to a minimum. The sample was treated with sodium carbonate, evaporated to dryness, the residue re-dissolved and analyzed using H-C column switching. The reagent blank interferences with accurate quantitation of sulfate. Impurities in the sodium carbonate are suspected. It should also be noted that contamination of the glassware was a problem in achieving consistent results. The glassware used in this study was soaked in fresh polished water daily for several days. Sulfate and phosphate spikes were made at 25 μg/l of each and 50 μg/l of each. Recoveries were 50% and 112% for sulfate and phosphate, respectively, at the 50 μg/l level. No phosphate was detected in the sample. The sample also appears to contain about 5 mg/l nitrate, which would have gone undetected without the H-C analysis (Fig. 4).

A higher-purity carbonate or perhaps high-purity $NaHCO_3$ or NaOH could probably address the interference problem from the reagent blank. A glassware protocol is also in order to avoid contamination. A set of dedicated columns is mandated to routinely achieve the requisite sensitivity. Ideally a dedicated system would also be used. Further development is obviously required to establish a rugged routine procedure, however, these preliminary results support the feasibility of the present approach to trace anions in concentrated hydrochloric acid.

CONCLUSIONS

A "dilute and shoot" approach to sample preparation in IC can be successfully employed without necessarily suffering the ill effects of column overloading and system abuse. The H-C technique [1] previously described can eliminate or simplify the requisite sample pre-treatment. This approach was demonstrated for sulfite analysis in a variety of different matrices and revised to a fully automated routine procedure, amenable to a quality control laboratory; acetate and TFA analysis in peptides; for μg/l anion analysis in high-purity concentrated hydrochloric acid; as well as other examples described in ref. 1.

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