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Systematic approach to generic matrix elimination via "heart-cut" column-switching techniques

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Abstract

Liquid chromatography matrix elimination employing "heart-cut" column-switching techniques has been demonstrated previously to be a powerful analytical tool. In some instances this approach affords results that would otherwise be impossible to attain with conventional liquid chromatography, or other analytical techniques. This paper describes a detailed systematic approach to developing the requisite "heart-cut" analysis parameters. The analyses investigated were sulfite determination in analgesic formulations and sulfate determination in sodium phosphate. These analyses represent extremes in terms of matrix elimination complexity, i.e., the former was the simplest case, involving low-level anion determination in an organic matrix, while the latter was the most complicated, involving low-level anion determination in an inorganic matrix. The six-step procedure described to develop the requisite "heart-cut" parameters is the same for either case. In addition, this approach to matrix elimination has evolved from a research tool into an extremely rugged and practical routine procedure. The ruggedness of this technique was demonstrated by performing analyses continuously, 24 h per day, for several weeks. An on-line system clean-up procedure was developed to maintain optimum performance with maximum sample throughput.

Keywords: Matrix elimination; Column switching; Heart-cutting techniques; Sulfate; Sodium phosphate; Sulfite; Inorganic anions

1. Introduction

The "heart-cut" (H-C) technique to effect matrix elimination in liquid chromatography has been described previously [1–6]. In some instances the matrix interference is not removed but is masked by a judicious choice of detector parameters, e.g., UV wavelength selection [7]. Column-switching techniques to effect matrix elimination frequently become very complicated in terms of the mechanics of performing the analysis and the interpretation of the resultant data. The requisite column-switching timing parameters were developed in an empirical way, primarily by trial and error. Our approach removes the interference using column switching; however, it is a relatively simple generic approach to matrix elimination. The technique uses two high-pressure four-way pneumatic valves inserted before and after the precolumn. The valves are manipulated to divert the bulk of the sample matrix to waste and to transfer only a "heart-cut" of the analyte of interest to the separator column. It has universal applicability,

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does not suffer from the limitations associated with classical matrix elimination in liquid chromatography and markedly reduces the column abuse that can occur when the capacity of the column has been significantly exceeded. A decade of refinements to this technology has led to the evolution of this technique from an extremely complex research analysis to a simple, straightforward and rugged "dilute-and-shoot" procedure that is amenable to a quality control laboratory environment.

This paper provides a detailed systematic approach to developing the requisite parameters necessary for utilizing this powerful generic matrix elimination technique. Development of the optimum retention time parameters was automated through a six-step procedure based upon repeated analysis of a spiked sample using different H-C parameters. The objective of this paper is to provide a guide to developing fully the optimum parameters for a given analysis in 3–4 days. Real sample analyses are under way within 1 week. The ruggedness and simplicity of the use of this technology have been demonstrated for a previously published simple (sulfite in analgesics) and a complex H-C analysis (sulfate in sodium phosphate). The systematic development of the requisite timing parameters is described in detail.

2. Experimental

The ion chromatographic (IC) system used for the sulfite determination was a Dionex Model 2020i dual-channel ion chromatograph equipped with a CDM-I conductivity detector, an ASM automated sampler and AI450 version 3.32 software. The chromatographic separation was conducted by using a Dionex AS-2 anion-exchange column as the precolumn, which is sandwiched between two Dionex high-pressure four-way pneumatic valves, followed by a separation column consisting of two more AS-2 anion-exchange columns. The rest of the system consisted of a 500-μl sample loop and a Dionex AutoRegen AMMS suppressor with 25 m*M* sulfuric acid regenerant circulated at 3 ml/min. The eluent was 1.5 m*M* sodium carbonate–1.3 m*M* sodium hydrogen carbonate containing 0.076% (v/v) formaldehyde (1 ml of Mallinckrodt AR Formalin solution diluted to 1 l with "polished" water) at a flow-rate of 1.5 ml/min.

Safety note. Formaldehyde is a known carcinogen; consequently, appropriate precautions should be exercised in its handling.

The determination of sulfate in sodium phosphate was conducted on a Dionex 4500i dual-channel ion chromatograph equipped with an ASM automated sampler, a pulsed electrochemical detector utilized in the conductivity mode and AI450 version 3.32 software. The rest of the system consisted of a 50-μl sample loop and a Dionex ASRS (anion self-regenerating suppressor). The precolumns and separation columns each consisted of two anion-exchange columns. Multiple systems were examined. The chromatographic systems that were investigated consisted of a set of four Dionex AS-4A anion-exchange columns and a set of four Dionex AS-9 anion-exchange columns. The eluents examined were 2.0 m*M* sodium carbonate–0.75 m*M* sodium hydrogen carbonate and 1.9 m*M* sodium carbonate–2.5 m*M* sodium hydroxide. The flow-rate for both column–eluent combinations was 1.0 ml/min.

2.1. Chemicals

All chemicals were Mallinckrodt AR products except for the 1000 μg/ml sulfate reference standard, which was obtained from High-Purity Standards (Cat. No. IC-SS-M). "Polished" water (deionized water further purified through a Millipore Milli-Q filtration system) was used throughout. The sample for the sulfate determinations was dibasic sodium phosphate, ACS grade. The sulfate specification is 50 ppm maximum. The identities of the analgesic formulations are proprietary.

2.2. Sample preparation

2.2.1. Analgesic sample preparation

A 4.00–12.00-g amount of sample was weighed into a 100-ml volumetric flask, vigorously mixed with about 95 ml of 0.76% (v/v) aqueous form-

aldehyde solution (diluted from Formalin solution), sonicated for 15 min and diluted to volume. A portion of the supernatant was then transferred into a centrifuge tube, centrifuged for about 30 min and the supernatant transferred to an autosampler vial.

Mallinckrodt AR sodium sulfite was the reference standard material used to prepare solutions for instrument calibration. Standards were prepared by serial dilution at 0.25, 1.0, 2.0, 4.0 and 6.0 mg/l sulfite in 0.76% (v/v) aqueous formaldehyde solution.

2.2.2. Sodium phosphate dibasic sample preparation

A 10.00-g amount of sample was weighed into a 100-ml volumetric flask, vigorously mixed with about 95 ml of the eluent that was being used for the analysis, sonicated for 15 min and diluted to volume.

A High-Purity Standards 1000 μg/ml sulfate reference standard was used to prepare solutions for conducting the standard additions analyses. The sample was spiked at 25, 50, 100 and 200 mg/kg sulfate. Experimental details regarding the particular combinations of eluents and columns used to conduct the sulfate analyses in sodium phosphate are given in the text.

3. Results and discussion

The valve configuration for the IC H-C system was described previously for the determination of sulfite in food and drug products [1,2]. The crux of the analysis is the introduction of the "heart-cut" to the separation column and subsequent detection. Determination of the H-C timing parameters can be automated through repeated analysis with various retention time windows (RTW) of a sample with a high analyte content or a sample that has been spiked with the analyte of interest (Fig. 1) [3]. The process whereby the H-C timing interval is determined and fine tuned is described in detail here in a six-step sequence:

(1) Assemble and equilibrate system. The entire system is assembled and equilibrated to ascertain suitability. An on-line clean-up may be required if the system is contaminated and/or consistent retention time characteristics are not observed. Successful utilization of this technique requires precise, reproducible chromatography.

(2) Determine analyte retention time characteristics on precolumn. The separation column is removed and the lines are reconnected with a coupler. Standards are injected and the times for the onset and complete elution of the analyte peak are noted. This time interval defines the preliminary H-C.

(3) Locate analyte in matrix. After re-inserting the separation column and allowing the entire system to re-equilibrate, the analyte is located within the sample matrix using the preliminary H-C time interval determined in step 2. In the case of an ionic analyte in an organic matrix, e.g., sulfite in an organic analgesic formulation, the preliminary and finalized H-C parameters are typically very close. In this instance, a limited number of refinement iterations are required to reach the finalized H-C method (Fig. 2). In contrast, finding an ionic analyte in an ionic

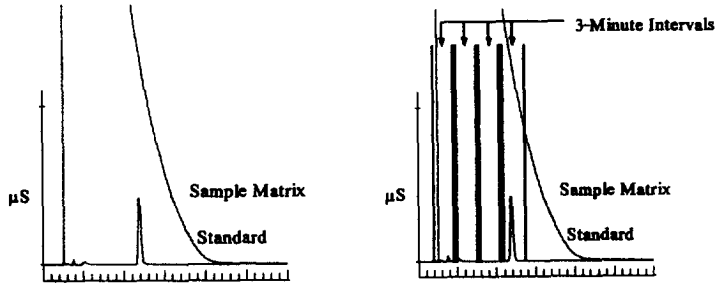

Fig. 1. Depiction of "heart-cut" intervals used to locate sulfate analyte in dibasic sodium phosphate, step 3.

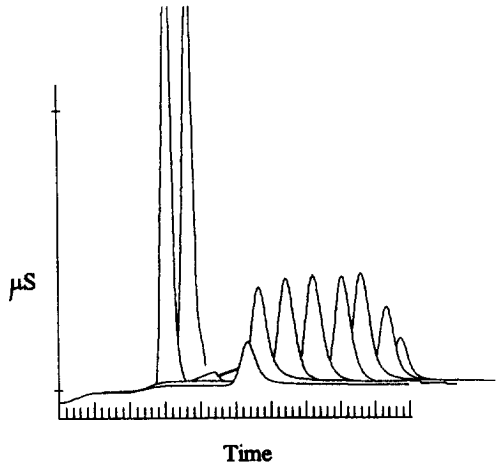

Fig. 2. Sulfite response as a function of "Heart-cut" timing.

matrix is typically much more complex and requires many more refinement iterations (Fig. 3). One reason for this additional complication is the "pusher effect." The "pusher effect" describes the competition between the ionic matrix with the ionic analyte for the active sites on the column. The former is much higher in concentration, which leads to less retention of the analyte and, therefore, a shorter analyte retention time. To locate the analyte in this instance the preliminary H-C RTW is moved to shorter retention times.

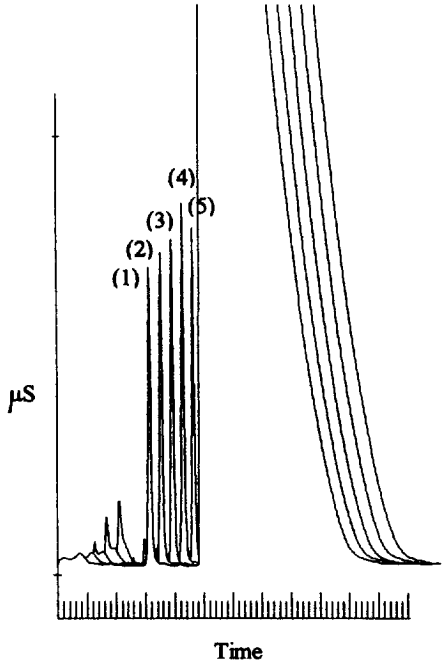

Fig. 3. Optimization of "heart-cut" parameters. (1) Step 4 − 0.2 min; (2) step 4–0.1 min; (3) step 4; (4) step 4 + 0.1 min; (5) step 4 + 0.2 min.

For this step, H-C RTW is moved in intervals with minimal overlap in order to find the analyte with a minimum number of analyses. In the case of sulfate as the analyte in a phosphate matrix, the H-C RTW determined in step 2 was 2.7 min. The H-C RTW interval was widened by ±0.3 min, i.e., the H-C began 0.3 min earlier and ended 0.3 min later, thereby widening the H-C RTW to 3.3 min. The analyses were then performed moving the RTW in 3-min intervals, resulting in a 0.3-min overlap (Fig. 1).

(4) Refine. The H-C RTW is now further refined by reducing the width of the RTW back to the peak width and using shorter intervals to move about the analyte parameters determined in step 3. For the determination of sulfate in sodium phosphate, the peak width was reduced ±0.3 min back to the original H-C RTW of 2.7 min and 1.0-min intervals were used.

(5) Optimize. The parameters furnishing the maximum peak area with minimum interference in step 4 become the basis for the optimization of the H-C. In this step, the H-C RTWs are moved in the smallest intervals possible. The parameters should again be based on longer and shorter retention times and evaluated as before. In the case of sulfate as the analyte in a phosphate matrix, using Dionex AI450 software allowed the H-C RTW to be moved in 0.1-min increments (Fig. 3).

(6) Finalize. The H-C method is finalized using the step 5 optimized parameters and evaluating the effects of widening the RTW by the shortest possible interval. For the determination of sulfate in a phosphate matrix the RTW was widened by ±0.1 min. A balance must be struck between maximum peak response and minimum matrix interference.

3.1. Sulfite

To demonstrate the viability of our systematic approach for the determination of the H-C

interval parameters, we employed it in optimizing the analyte RTW for sulfite in analgesic formulations. The procedure readily furnished the optimum interval for this analysis. The results of the approach also demonstrated the ruggedness of our method for sulfite determination in an organic matrix. Fig. 2 shows how the response obtained by this analysis varies as the H-C interval is changed. The H-C RTW can be moved ±0.8 min with no significant decrease in peak response.

As part of a validation study, the system was deliberately stressed. Over 250 lots of analgesic formulation were analyzed continuously (24 h per day) for several weeks. The IC system performance was so extraordinary that it became routine. To facilitate continuous operation, an on-line system clean-up was developed. The system was configured with both valves closed and a series of flushes were injected as follows: first, six acidic column flushes (1.0 *M* HCl–0.17 *M* NaCl) were injected. Each acidic column flush were followed by two "blanks". Six injections of an alkaline flush (0.05 *M* HaOH–Na_2CO_3) was then made, with twelve subsequent "blank" injections to facilitate system re-equilibration. A set of standards were re-analyzed using the H-C method as a check on the suitability of the system.

3.2. Sulfate

To demonstrate further the power of this systematic approach, we applied it to a much more complicated H-C analysis, the determination of sulfate in sodium phosphate. Previously, this analysis was conducted using four Dionex AS-4A anion-exchange columns with a 2.0 m*M* sodium carbonate–0.75 m*m* sodium hydrogen carbonate eluent (pH 10.5). Under these conditions, the sulfate peak eluted after the phosphate peak. The peak response was low and the chromatography was relatively poor. It should also be noted that the H-C RTW parameters were previously developed empirically by trial and error.

It was proposed that a more alkaline eluent should shift the elution of the phosphate to a longer retention time. When an eluent consisting of 1.9 m*M* sodium carbonate 2.5 m*M* sodium hydroxide (pH 11.5) was substituted for the less basic carbonate hydrogen carbonate eluent, the effect was dramatic. Phosphate eluted after sulfate, the peak response was increased and the chromatography was greatly improved. Fig. 4 gives a comparison of the responses achieved with these eluents.

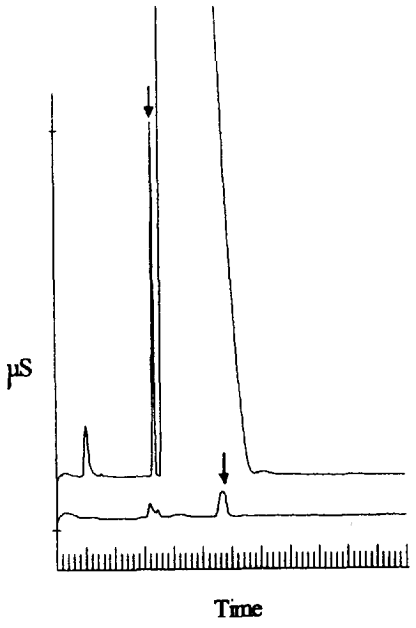

Fig. 4. Comparison of effect of pH on analyte response for sulfate in sodium phosphate.

Dionex AS9 anion-exchange columns were substituted for the AS-4A columns to compare inter-column effects in this analysis. No significant column-to-column differences were observed. Using the more alkaline eluent, standard additions analyses for sulfate in dibasic sodium phosphate on the AS-4A and AS9 columns gave sulfate contents of 30 and 32 mg/kg, respectively.

4. Conclusions

The aforementioned systematic approach to H-C analyses is very topical in the chemical industry as the order of the day is for analytical laboratories to solve more complex analytical problems with fewer analytical staff. After the capital expenditure of purchasing the requisite

IC system, the cost in terms of both time and money to develop an H-C method using the aforementioned six-step approach is minimal. Real sample analyses become a reality within 1 week. The ruggedness of the methodology is a function of the analytical challenge of the particular matrix that is being investigated and the various on-line system clean-up techniques. This technology is destined to find more widespread applications in the analytical laboratories of the future.

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