Journal of Chromatography, 477 (1989) 271–276
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 600

EVALUATION OF ASSAY SPECIFICITY IN NON-SUPPRESSED ION CHROMATOGRAPHY

LAWRENCE A. CRUZ and DENNIS R. JENKE*
Baxter Healthcare, Inc., William B. Graham Science Center, Round Lake, IL 60073 (U.S.A.)
(First received January 30th, 1989; revised manuscript received April 27th, 1989)

SUMMARY

The utilization of tandem conductivity and indirect photometric detectors for demonstrating the purity of peak responses in non-suppressed ion chromatography is discussed. The differing nature of the two detection mechanisms, both of which can be performed simultaneously with a phthalate buffer mobile phase, provides the means for species discrimination. Specificity can be evaluated by comparing peak response ratios (ratio of the detector response from a sample and standard) obtained from both detectors. The utility of the method is demonstrated by assessing the ability of the method to identify interferences in overlapping nitrate and sulfate peaks.

INTRODUCTION

The evaluation and confirmation of assay specificity (that is, the ability of the method to produce a response related to the presence of a single solute) is a vital part of any method development process. This is especially true in chromatography, where the specificity issue essentially reduces to a question of operational peak purity (that is, is the chromatographic peak produced solely by the detector responding to a single species). One approach used to assess chromatographic peak purity involves the utilization of two (or more) distinct detection strategies to identify peculiarities in terms of peak shape and magnitude of response. Such an approach is particularly powerful if the two detection strategies target vastly different properties of the solutes and is particularly convenient if the chromatographic system is such that the alternate detection processes can be accomplished simultaneously (either with the same or tandem detectors).

Such a scenario is realized in non-suppressed ion chromatography (IC) wherein the commonly employed phthalate buffer mobile phase allows for both conductivity and indirect photometric detection. Despite being classified as a bulk property detection mechanism, conductivity detection in IC actually deals with a solute-specific property (its equivalent ionic conductance). The indirect detection mechanism relies only on the solute-induced change in mobile phase counter-ion concentration to produce a response and thus is truly analyte independent (if the solute has no intrinsic

0021-9673/89/$03.50 © 1989 Elsevier Science Publishers B.V.

UV absorbance at the wavelength of interest). Thus utilization of both strategies (in tandem) provides a means of assessing specificity in IC applications.

In this paper we discuss a quantitative mechanism for assessing specificity based on this tandem detector strategy and demonstrate its utility in a practical application.

THEORY

For a chromatographic detector operating within its linear dynamic range, the relationship between response and solute concentration is

$$r = mC \tag{1}$$

where m is the response factor and C is the solute concentration. The response ratio (R) between two samples containing different concentrations of the solute is simply their concentration ratio,

$$R = r_1/r_2 = C_1/C_2 \tag{2}$$

Since this ratio is independent of the response factor, it should be constant regardless of the nature of the detector. Therefore the selectivity factor (S), defined as the ratio of R in detectors A and B, is equal to 1,

$$S = R_A/R_B \tag{3}$$

However, if the chromatographic peak consists of two solutes (a and b), the response equation becomes (in the most simplistic case wherein no solute–solute interactions occur)

$$r = m_a C_a + m_b C_b \tag{4}$$

and the response ratio between a sample containing both solutes and a standard containing only one (a) becomes

$$R = 1 + (m_b C_b/m_a C_a) \tag{5}$$

In this case, the selectivity factor between the two detectors becomes

$$S = \frac{1 + (m_{b,A} C_b/m_{a,A} C_a)}{1 + (m_{b,B} C_b/m_{a,B} C_c)} \tag{6}$$

Only in the case where $(m_{b,A}/m_{a,A}) = (m_{b,B}/m_{a,A})$ will S be equal to one. Thus calculation of S for a peak using two different detectors can potentially provide specificity and/or identity related information. A calculated S value significantly different from one implies that either the peak is produced by two or more components or that the solute in the sample and the standard are not the same. Alternately, obtaining two different R values in the two different detectors indicates the same situations.

The successful application of this strategy to IC utilizing tandem conductivity and indirect photometric detectors requires that they meet the criterion that

$$(m_{b,cond.}/m_{a,cond.}) \neq (m_{b,indirect}/m_{a,indirect}) \quad (7)$$

For transparent analytes, $m_{b,indirect} = m_{a,indirect}$ if the concentrations of analyte a and b are expressed in normality[1]. In non-suppressed conductivity detection, the response (change in mobile phase conductance due to the presence of the solute) can be written

$$R = C_s(E_s - E_e)/1000K \quad (8)$$

where C_s is the solute concentration, E is the equivalent ionic conductance of the solute (s) or the eluent (e) and K is the cell constant[2]. Thus, the ratio m_a/m_b for conductivity is directly proportional to the ratio of the equivalent ionic conductances for species a and b. Since equivalent ionic conductances for most common inorganic anions are not the same (see for example, ref. 3), the criterion in eqn. 7 is met and the proposed specificity evaluation scheme is appropriate.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of an Applied Biosystem's Spectroflow 400 pump and 757 variable-wavelength UV detector, a Micromertics 728 autosampler coupled to an electronically actuated Rheodyne 7010 injection valve, a Waters Model 430 conductivity monitor and a Hewlett-Packard HP 3357 computer integrator. The chromatographic column was a Dionex AS-1 anion separator and the specificity evaluation was performed with a mobile phase containing $1.92 \cdot 10^{-3}$ M potassium hydrogen phthalate at pH 6.5. The UV detector was operated at a wavelength of 250 nm, mobile phase flow-rate was 1.5 ml/min and the sample size was 10 μl.

Procedure

Standard solutions containing either sulfate or nitrate in the concentration range 20–300 ppm were injected into the chromatographic system (in replicate) to evaluate response linearity over this range. Test articles containing known amounts of sulfate and nitrate were injected (in replicate) into the chromatographic system and the responses obtained from both detectors were recorded. In all cases, peak area was used for quantitation.

RESULTS AND DISCUSSION

In order to demonstrate the utility of the proposed specificity evaluation method, chromatographic conditions producing known peak overlap had to be identified. The different effect of mobile phase composition on the retention characteristics of dissimilarly charged analytes in IC is well documented[4–8] and peak overlap between nitrate and sulfate has been observed[9]. As noted previously, these species also have dissimilar specific ionic conductances (71 and 80 Ω^{-1} cm^2 equiv.$^{-1}$ at 25°C in aqueous solution for nitrate and sulfate, respectively[3]), and thus are

appropriate candidates for the demonstration. Using the elution models of Jenke and Pagenkopf[10-12], a mobile phase capable of producing sulfate–nitrate coelution was identified with only a few scouting experiments (Fig. 1). When this mobile phase ($1.92 \cdot 10^{-3}$ *M* potassium hydrogen phthalate at pH 6.5) was used, peak coelution was achieved; even in samples containing equimolar concentrations of sulfate and nitrate there is no visual indication that the resulting chromatographic peak is impure (Fig. 2). Peak area response for both detectors was linear over the concentration range of 20 to 300 ppm for both analytes; the sensitivity ratio calculated from the slopes of the calibration curves is 1.13 which agrees well with the predicted selectivity ratio (from equivalent conductances) of 1.14. This predicted selectivity ratio represents the product of the ratio of the specific ionic conductances of the analytes (1.14) and the ratio of the molar response ratios in the indirect photometric detector (1.0). Precision at the 100 ppm concentration level is on the order of 0.4% R.S.D. ($n = 11$) for both analytes and with both detectors. Thus the proposed specificity evaluation method is directly applicable and in this case should provide tight confidence intervals for the respective detector's response ratios.

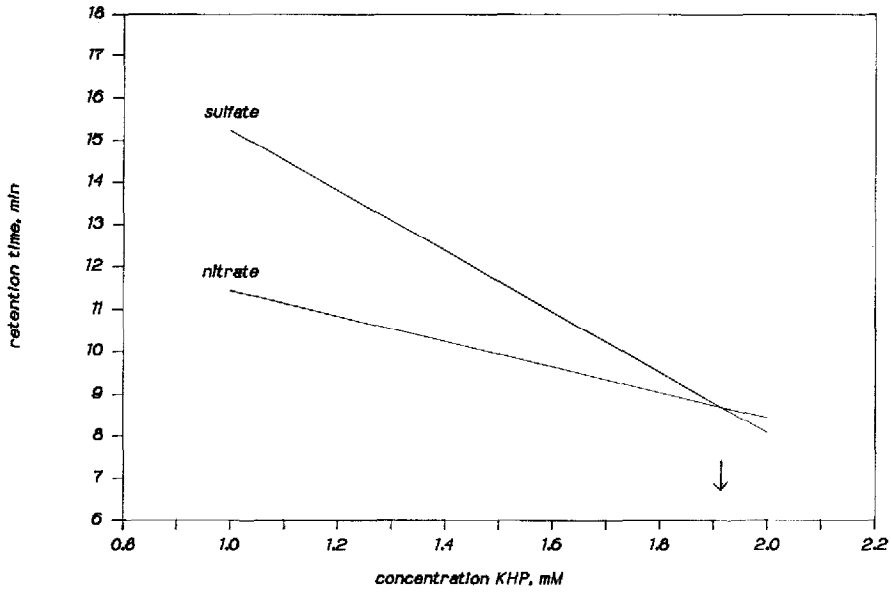

Fig. 1. Effect of mobile phase composition on the elution characteristics of nitrate and sulfate. The arrow indicates that mobile phase for which coelution occurs. KHP = potassium hydrogen phthalate.

The response ratio data obtained from the analysis of six nitrate–sulfate mixtures is shown in Table I. If the confidence interval (95% level) for the response ratios for a particular analyte overlap, then no interference is recognized and the peak is judged to be pure. For instances where the concentration ratio between the analyte and interferent is 10 or smaller, comparison of the response ratios effectively indicates the lack of specificity. However, for samples where the analyte–interferent ratio is

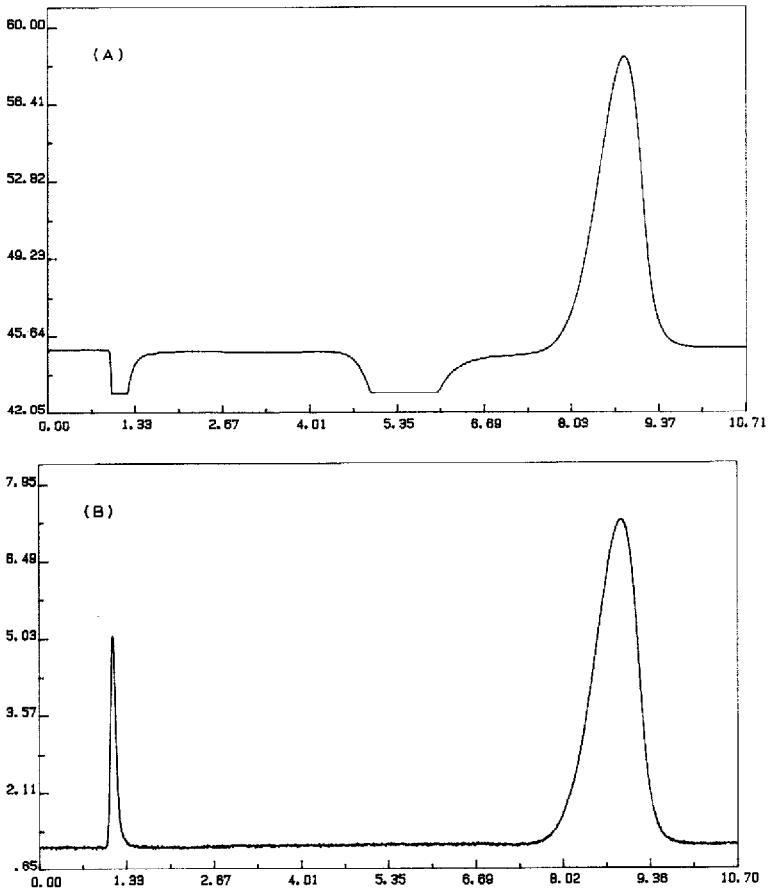

Fig. 2. Typical chromatograms obtained from a mixture containing equimolar amounts of sulfate and nitrate. Trace A is from the conductivity detector while trace B is from the UV detector.

TABLE I

SPECIFICITY EVALUATION OF THE ANALYTE PEAK

Mixture	*Conc. (ppm)*		*Response ratio*[a]			
	Sulfate	*Nitrate*	*Versus nitrate*		*Versus sulfate*	
			UV	*Cond.*	*UV*	*Cond.*
1	222[b]	132	3.24–3.31	3.47–3.54	2.96–3.02	2.76–2.81
2	222[b]	26	2.41–2.46	2.66–2.70	2.20–2.24	2.11–2.15
3	222[b]	13	2.28–2.32	2.60–2.65	2.08–2.12	2.07–2.11
4	111	264[b]	3.17–3.23	3.27–3.33	2.89–2.95	2.61–2.65
5	22	264[b]	2.28–2.33	2.26–2.30	2.09–2.13	1.80–1.83
6	11	264[b]	2.16–2.20	2.16–2.20	1.97–2.01	1.72–1.75
7	–	265	2.05–2.11	2.00–2.06	1.88–1.93	1.60–1.64
8	222	–	2.20–2.25	2.46–2.50	2.00–2.05	1.96–2.00

[a] 95% Confidence interval.
[b] Major analyte.

much greater than 10 (*e.g.* samples 3, 5 and 6), the methodology fails to identify that an interferent is present. Of course, the minimum relative concentration of interferent which the methodology is able to detect is influenced by the magnitude of the selectivity ratio (larger ratio enhances the ability to detect low level interferents) and the precision of the methodology (since the comparison is statistical). In point of fact, the somewhat small range of equivalent ionic conductances which are exhibited by many common ionic solutes limits the magnitude of the selectivity ratio and will commonly define the method's ability to establish specificity. Clearly, the effectiveness of the methodology requires the assay to be fairly precise and/or the database (number of injections) to be large.

In addition to allowing for a peak purity assessment, the method can be used to determine the "identity" of the species responsible for a given peak. For example, "Mixtures" 7 and 8, which in actuality contained only one of the analytes, illustrate the method's ability to identify the species responsible for producing a chromatographic peak. Clearly, in these cases the analyte producing the "unknown" response is identified as the one for which the confidence intervals of the response ratios overlap.

The authors note in passing that the case of dissimilarly charged interferent–analyte pairs (as per the example in this discussion) can also be addressed by using a second mobile phase to resolve the two. However, such an approach will not be effective in identifying a peak which is compromised by an interferent whose charge is the same as the analyte. The proposed method is applicable in both cases and is thus more general in scope.

REFERENCES

1 D. R. Jenke, *Anal. Chem.*, 56 (1984) 2468.
2 S. A. Wilson, E. S. Yeung and D. R. Bobbitt, *Anal. Chem.*, 56 (1984) 1457.
3 D. T. Gjerde and J. S. Fritz, *Ion Chromatography*, Hüthig, New York, 2nd ed., 1987, p. 19.
4 D. T. Gjerde, G. Schmuckler and J. S. Fritz, *J. Chromatogr.*, 186 (1979) 509.
5 T. B. Hoover, *Sep. Sci. Technol.*, 17 (1982) 295.
6 J. A. Glatz and J. E. Girard, *J. Chromatogr. Sci.*, 20 (1982) 266.
7 J. Weiss, *Handbook of Ion Chromatography*, Dionex, Sunnyvale, CA, 1986, pp. 41–48.
8 D. R. Jenke and G. K. Pagendorf, *J. Chromatogr.*, 269 (1983) 202.
9 D. Jenke, *Anal. Chem.*, 53 (1981) 1535.
10 D. Jenke and G. K. Pagenkopf, *Anal. Chem.*, 56 (1984) 88.
11 D. Jenke and G. K. Pagenkopf, *Anal. Chem.*, 56 (1984) 85.
12 D. Jenke, *Anal. Chem.*, 56 (1984) 2674.