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Short communication

Studies of hydrogen/deuterium exchange of monodeuteriophenol in fused-silica capillary columns using gas chromatography–mass spectrometry

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Abstract

The hydrogen/deuterium (H/D) exchange process in fused-silica capillary columns was extensively studied using two old and two new columns. The compounds selected for this study were mixtures of phenol and monodeuteriophenol with different compositions. The factors controlling and influencing this phenomenon, such as heating rate and flow-rate variation as a function of residence time on the internal column-surface, were also evaluated. Temperature programming and isothermal analysis were performed for comparison. All studied factors proved that old fused-silica capillary columns exhibit sufficient activity to affect H/D exchange of monodeuteriophenol compounds, but the degree of exchange depends mainly on the position of ring deuteration. However, new columns were found to be less reactive toward H/D exchange and the percentage of exchange may be considered to be within the experimental error.

1. Introduction

Fused-silica capillary columns are now widely used in analytical applications and routine analysis by gas chromatography (GC) and combined gas chromatography–mass spectrometry (GC–MS) [1]. The final analytical results obtained with these two techniques are heavily dependent on the performance, resolution and efficiency of the column used. The first requirement for the applicability of the column used is that it is inert to chemical side-reaction with the analyte of interest and that it only permits separation through adsorption on the surface of the stationary phase [2]. On the other hand, the column may be employed and activated with specific compounds if a reaction between these compounds and the analyte is required. An example of such a reaction is the labeling of the hydrogen located at the α-position of carbonyl groups by deuterium impregnated on the column surface using different deuterium sources [3–9]. Thus, the column must be suited and adapted to the required method of analysis.

In previous reports [10,11] we proved that old, but well-performing columns lost their inertness and showed appreciable activity toward exchange of deuterated ketones with their corresponding non-deuterated analogs. This phenomenon was extensively studied and the factors controlling such exchange–such as residence time, flow-rate, injector and usability of the tested column–were evaluated. In this paper we explore and extend this phenomenon to another

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class of compounds, i.e. monodeuteriophenol compounds, using different old and new columns at different analysis conditions.

2. Experimental

2.1. Instrumentation

GC–MS analysis was conducted with a Finnigan 4021B mass spectrometer interfaced to a Hewlett-Packard 5890 gas chromatograph and Incos data system. The tested old columns were all Durabond DB-1 (polydimethylsiloxane) manufactured by J&W Scientific, and the two new columns were also DB-1 purchased from J&W Scientific and Hewlett-Packard. The mass spectrometer was operated in the electron-impact (EI) mode of ionization over a mass range of 94–95 amu which represents the molecular-ion range for phenol and monodeuteriophenol. The ion source was set at 250°C and the ionization energy was 70 eV. The GC conditions were adjusted to split injection with helium carrier gas. Isothermal analyses were performed at an oven temperature of 130°C and an injector temperature of 200°C at different flow-rates of 6, 4, and 1 ml/min, while the temperature programming analyses were conducted at different heating rates of 70, 40, 20, and 5°C/min from an initial temperature of 60°C to as high as 200°C.

The actual percentage of each species in the phenol/monodeuteriophenol mixture was determined by direct insertion probe analysis (DIP) at room temperature.

2.2. Compounds

The three monodeuteriophenols (2-, 3- and 4-deuteriophenol) were prepared according to a method published by Talley and Evans [12], by selective deuteration of the corresponding monobromophenol. A solution of the monobromophenol (2-, 3- or 4-bromophenol; 100 mg ≈ 0.5 mmol) in diethyl ether was added to a solution of *n*-butyl lithium in hexane (1 ml ≈ 1.6 mmol) and stirred at 0°C for 2–3 h. The mixture was then dissolved in ^2H_2O (20 ml) and stirred for 2–3 h. The product was extracted from diethyl ether, washed with dilute HCl several times, and vacuum-dried over anhydrous sodium sulphate.

3. Results and discussion

The actual percentages of the phenol/monodeuteriophenol mixtures, based on DIP analysis, are listed in Table 1. The GC–MS analysis of 2-deuteriophenol was performed with an old 30-m column manufactured by J&W Scientific at different heating rates of the column. The data presented reveal that the percentage of phenol in the mixture dramatically increased from 21% to as high as 39% going from a heating rate of 70°C/min to 5°C/min. The percentage of 2-deuteriophenol decreased by the same extent. This unusual behaviour can be interpreted on the

Table 1
Effect of heating rate as a function of residence time on the H/D exchange of phenol/monodeuteriophenol mixtures tested on an old 30-m fused-silica capillary column manufactured by J&W Scientific

Compound	*m/z*	Heating rate (°C/min)				DIP analysis
		70	40	20	5	
Phenol ($^2H_0$)	94	21	27	31	39	22
2-Deuteriophenol ($^2H_1$)	95	100	100	100	100	100
Phenol ($^2H_0$)	94	35	43	46	47	34
3-Deuteriophenol ($^2H_1$)	95	100	100	100	100	100
Phenol ($^2H_0$)	94	74	79	81	93	72
4-Deuteriophenol ($^2H_1$)	95	100	100	100	100	100

basis of a chemical reaction of 2-deuteriophenol with active hydrogen atoms located on the internal surface of old and activated fused-silica capillary columns as previously reported [10,11]. The interpretation is focused on the residence time of deuteriophenol on the column; the degree of deuterium exchange by active hydrogen increases with an increase of the residence time. The same dependence on residence time is also evident for both 3- and 4-deuteriophenol mixtures as listed in Table 1 and shown in Fig. 1.

It is evident that 2- and 4-deuteriophenols show approximately 20% H/D exchange of the deuterio species into the non-deuterio analog, while the 3-deuteriophenol gave a maximum of only 12% exchange. These results are consistent with the resonance structures associated with the 2- and 4-deuterio substituted phenol compounds which enhance the reactivity of the deuterium substituent in these two positions, but not in 3-deuteriophenol, for electrophilic substitution by active hydrogen from the internal silica surface.

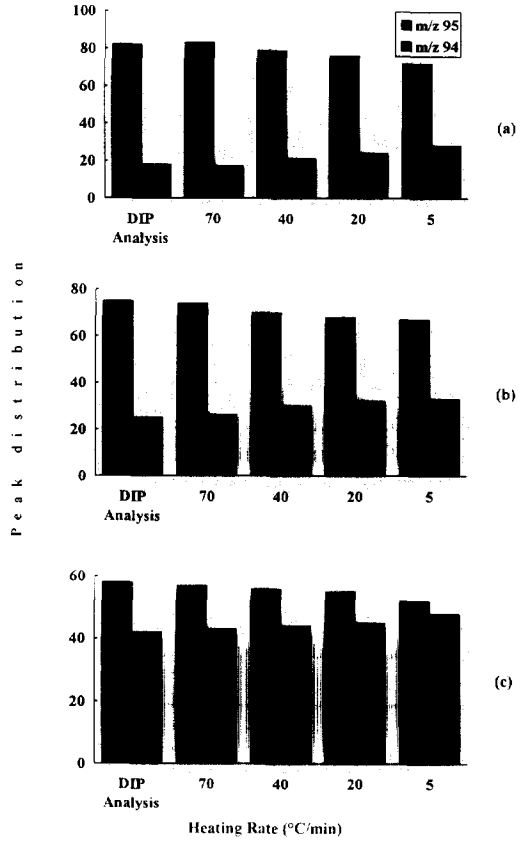

Fig. 1. Variation of the peak intensities (normalized; total = 100) for monodeuteriophenol mixtures tested by old 30-m DB-1 fused-silica column and at different heating rate values: (a) mixture of phenol and 2-deuteriophenol; (b) mixture of phenol and 3-deuteriophenol; and (c) mixture of phenol and 4-deuteriophenol.

The old column tested in the previously mentioned analysis was efficiently working for separation and routine analysis of non-deuterated compounds where no apparent interaction between the analyte of interest and the active hydrogen of the silica surface occurs. However, another old 15-m DB-1 fused-silica column was also tested to evaluate its performance toward the H/D exchange. The results of this analysis are presented in Table 2. The values obtained by both columns are comparable and not related to the length of the selected column but only to the reactivity or inertness of the internal surface of the column and the residence time.

The effect of residence time on the H/D exchange process was further confirmed by studying another important factor–the variation in flow-rate–in order to eliminate the thermal effect which may influence such process. We therefore performed the flow-rate studies under isothermal conditions at a GC-oven temperature of 130°C by using only the old 30-m column. The results of this analysis are compiled in Table 3.

The same trend is also evident at different flow-rates and isothermal conditions as previously reported [10,11]. This means that the H/D exchange process is more or less independent of the thermal behaviour of the analyte or the column, but is heavily dependent on the surface activity of the column used and the reactivity of the deuterium substituent of the analyte.

The H/D exchange phenomenon was further examined with two new columns manufactured by two different companies, J&W Scientific and Hewlett-Packard, in order to evaluate their performance. The selected columns had the same length, diameter, stationary phase coating and film thickness. The analysis was performed under isothermal conditions at the same three values for the flow-rate. The results of this experiment

Table 2
Effect of heating rate as a function of residence time on the H/D exchange of phenol/monodeuteriophenol mixtures tested with an old 15-m fused-silica capillary column

Compound	m/z	Heating rate (°C/min)				DIP analysis
		70	40	20	5	
Phenol ($^2H_0$)	94	23	26	35	38	22
2-Deuteriophenol ($^2H_1$)	95	100	100	100	100	100
Phenol ($^2H_0$)	94	36	40	44	45	34
3-Deuteriophenol ($^2H_1$)	95	100	100	100	100	100
Phenol ($^2H_0$)	94	74	78	85	91	72
4-Deuteriophenol ($^2H_1$)	95	100	100	100	100	100

Table 3
Effect of flow-rate variation as a function of residence time on the H/D exchange process of phenol/monodeuteriophenol mixtures tested with an old 30-m DB-1 fused-silica capillary column at an isothermal temperature of 130°C

Compound	m/z	Flow-rate (ml/min)			DIP analysis
		6	4	1	
Phenol ($^2H_0$)	94	21	24	40	22
2-Deuteriophenol ($^2H_1$)	95	100	100	100	100
Phenol ($^2H_0$)	94	35	40	47	34
3-Deuteriophenol ($^2H_1$)	95	100	100	100	100
Phenol ($^2H_0$)	94	79	86	90	72
4-Deuteriophenol ($^2H_1$)	95	100	100	100	100

Table 4
Evaluation of H/D exchange by two new fused-silica capillary columns at different flow-rates and an isothermal temperature of 130°C

Compound	m/z	Flow-rate (ml/min)						DIP analysis
		6		4		1		
		J&W	HP	J&W	HP	J&W	HP	
Phenol ($^2H_0$)	94	22	23	24	24	25	26	22
2-Deuteriophenol ($^2H_1$)	95	100	100	100	100	100	100	100
Phenol ($^2H_0$)	94	33	34	35	36	35	37	34
3-Deuteriophenol ($^2H_1$)	95	100	100	100	100	100	100	100
Phenol ($^2H_0$)	94	73	73	75	74	77	77	72
4-Deuteriophenol ($^2H_1$)	95	100	100	100	100	100	100	100

confirmed that the new columns are less reactive toward H/D exchange, as shown by the appreciable decrease in the percentage of deuterium exchange compared to that of the two tested old columns. The maximum percentage of H/D exchange was found to be 5% and the other values can be considered to be within the experimental error.

The data shown in Table 4 also indicate that the two new columns are too inert to force the H/D exchange reaction due to the high degree of inactivation of the internal surface of fused-silica by the stationary phase coating and the less active deuterium substituents, compared to the expected high reactivity of a deuterium located at the α-position of a carbonyl group in a carbonyl-containing compound [10,11].

4. Conclusion

The data presented in this paper demonstrate that old columns, which are well-performing for other analytical purposes, exhibit sufficient activity to affect H/D exchange of monodeuteriophenol. New columns, however, showed minimal tendency to such H/D exchange. Because it is common practice to determine the purity of isotopically-labeled compounds and to use deuterated internal and external standards for quantitation in GC–MS analysis using fused-silica capillary columns, one should consider the following points before attaching any significance to data obtained. First, the column selected for such study must be new and tested for inertness toward H/D exchange of the already synthesized deuteriocompounds by different heating or flow-rate measurements. Second, for accurate quantitative analysis the results must be corrected for the percentage of H/D exchange. Third, analysis of similar compounds must be conducted at high flow-rate or fast heating rate analysis. Finally, old columns must be excluded from use in similar analytical methods or must be deactivated by renewed coating of the internal surface and tested for inertness before use.

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References

[1] J.C. Nikelly, Advances in Capillary Chromatography, Alfred Hüthig, Heidelberg, 1986.
[2] J.K. Haken, J. Chromatogr. Rev., 300 (1984) 1–77.
[3] K. Mislow, M.A.W. Glas, H.B. Hopps, E. Simon and G.H. Whal, Jr., J. Am. Chem. Soc., 86 (1964) 1710.
[4] G.J. Kalos and L.B. Westover, Tetrahedron Lett., (1967) 1223.
[5] W.J. Richter, M.S. Senn and A.L. Burlingam, Tetrahedron Lett., (1965) 1235.
[6] M.S. Senn, W.J. Richter and A.L. Burlingam, J. Am. Chem. Soc., 87 (1965) 680.
[7] G.M. Anthony and G.J.W. Brooks, J. Chem. Soc. D, (1970) 200.
[8] D.J. Harvey, M.G. Horning and P. Vorous, Tetrahedron, 27 (1971) 4231.
[9] D.J. Harvey, M.G. Horning and P. Vorous, J. Chem. Soc. Perkin Trans. I, (1972) 1074.
[10] M.E. Mahmoud, A.M. Moussa, D.A. Forsyth and P. Vorous, J. Chromatogr., 549 (1991) 416.
[11] M.E. Mahmoud, Ph.D. Dissertation, Northeastern University, Boston, MA, 1992.
[12] J.J. Talley and I.A. Evans, J. Org. Chem., 49 (1984) 5267.