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Short communication

Selective deuterium exchange during superheated heavy water chromatography–nuclear magnetic resonance spectroscopy–mass spectrometry of sulfonamides

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Abstract

Superheated deuterium oxide has been investigated as an eluent for reversed-phase HPLC on a polystyrene–divinylbenzene column with UV, ^{1}H NMR and MS detection using a series of sulfonamides as model compounds. In the course of these studies, a selective, specific and efficient deuteration of the methyl groups on a pyrimidine ring was observed during chromatography of certain of the sulfonamides. The potential of this methodology for producing deuterium-labelled compounds from substances bearing suitable substituents is considered. The utility of HPLC–NMR–MS as a means for studying on-column reactions is discussed.

Keywords: Superheated water chromatography; Deuterium exchange; Nuclear magnetic resonance spectroscopy; Mass spectrometry; Sulfonamides

1. Introduction

Recently, superheated water at temperatures from 100 to 220°C and up to 50 bar has been found to provide an effective eluent for reversed-phase chromatography [1–3] and has been employed for the analysis of a range of analytes, including phenols, amides, esters and barbiturates. During these studies, superheated water chromatography has been shown to be compatible with typical LC spectroscopic detectors. In addition the absence of an organic eluent modifier means that a flame ionization detector can also be used [4–6]. The eluent strength can be increased by increasing the temperature enabling gradient elution to be carried out and recently the separation of sulfonamides at a range of pH values with buffered aqueous eluents has been reported [7]. We have also demonstrated the application of deuterium oxide as a mobile phase for the HPLC–NMR and HPLC–NMR–MS separation of a number of analytes including barbiturates, analgesics and related analytes [8,9]. The absence of large signals, for the protons of the organic modifier in the HPLC solvents, considerably simplified the NMR spectroscopic determination. The use of coupled NMR and MS enabled us to confirm that the analytes were stable under the chromatographic conditions. In the

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present work, this study was extended to the separation of a number of sulfonamides using a buffered D_2O eluent. However, when the eluent was examined by NMR spectroscopy and mass spectrometry an unexpected but highly efficient and selective deuterium exchange reaction appeared to have taken place with certain of the analytes.

2. Experimental section

2.1. Reagents

Sulfacetamide and sulfisomidine (Sigma, Poole, UK), sulfapyridine (May and Baker, Dagenham, UK), sulfamerazine (May and Baker, Dagenham, UK) and sulfamethazine (K and K Laboratories, USA) and phosphate buffer salts were of analytical grade from Fisons Scientific Apparatus (Loughborough, UK). Deionised water was prepared in an Elga Purification unit (Wycombe, Bucks, UK). Approximately 1-mg ml^{-1} sample solutions of each compound were prepared in 20% tetrahydrofuran (THF)–water. Deuterium oxide was of 99.9% purity obtained from Fluorochem (Old Glossop, UK). Acetonitrile was of HPLC-grade from Fisons Scientific Apparatus (Loughborough, UK).

The phosphate buffer pD 3.0 mobile phase was prepared from H_3PO_4 and KH_2PO_4 at approximately 1–3 m*M* in deuterium oxide.

2.2. Superheated water chromatography

The superheated water chromatographic system consisted of a LC-10AD Shimadzu pump (Kyoto, Japan) which delivered the mobile phase at 1.0 ml min^{-1} to the column through a preheating coil made of a 100-cm×0.01-inch I.D. stainless steel tubing. The column and preheating coil were placed in a GC oven (Series 104, Pye Unicam, Cambridge, UK) whose temperature was controlled by a programmer controller (Series 104, Pye Unicam, Cambridge UK). The samples were injected using a Rheodyne 7125 valve (Cotati, USA) fitted with a 20-μl sample loop, which was mounted outside the oven. The analytes were separated on a PLRP-S (PS-DVB) (150×4.6 mm, Polymer Laboratories, Church Stretton UK) column. A set of copper fins (3×12 cm×0.05 mm) was attached to the tubing connecting the column to a Jasco UV–Vis detector (Model 870, Tokyo, Japan) operating at 254 nm. A Rheodyne 7125 injection valve was placed in-line between the UV detector and the NMR flow cell to use as a switching valve. The flow could thus be directed to a restrictor coil of PEEK tubing (3 m×0.13 mm I.D.) or via a second PEEK tube (3 m×0.13 mm I.D.) to the flow cell of a Bruker DRX-500 NMR spectrometer operating at 500.13 MHz (^1H) with a detection cell volume of 120 μl, which was at ambient temperature. The flow was split 30 cm before the NMR flow cell, with 95% of the eluent directed to the NMR spectrometer and the remaining 5% being directed via a further 3 m×0.13 mm I.D. length of PEEK tubing to a Quattro LC mass spectrometer (Micromass, Altrincham, Cheshire, UK).

2.3. Mass spectrometry conditions

The Quattro LC mass spectrometer was fitted with a Z spray source running a cone voltage of 25 V in positive electrospray. The mass range was scanned from 80 to 450 amu over 1 s, with a 0.1-s interscan delay. The capillary was at 3.45 kV and the source block temperature was maintained at 80°C. The desolvation temperature was 150°C. The nebuliser gas flow was 80 l h^{-1}, and the desolvation gas flow 564 l h^{-1}.

2.4. ^1H NMR conditions

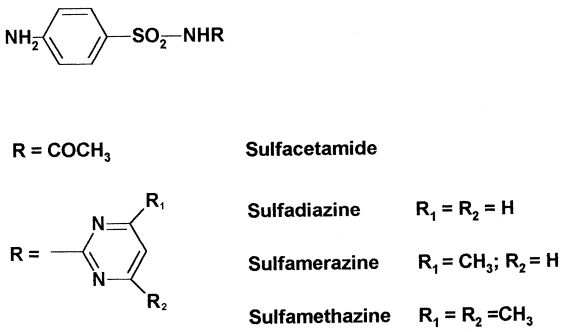
The spectra were obtained in the stop flow mode and free induction decays (FID) were collected over a spectral width of 8278 Hz into 16 384 data points using an acquisition of 0.99 s using the NOESYPRESAT pulse sequence (Bruker). The residual water resonance was suppressed using preirradiation during the relaxation delay of 2.0 s and mixing period of 0.10 s. The delay time between UV detector and NMR spectroscopic detector was approximately 33 s.

3. Results and discussion

Using similar conditions to those reported previ-

ously with UV spectroscopic detection [7], a mixture of sulfacetamide, sulfadiazine, sulfamerazine and sulfamethazine was examined on a polystyrene–divinylbenzene (PS–DVB) column using a deuterium oxide phosphate-buffered mobile phase (pD 3.0) and an oven temperature program from 160 to 200°C at 2°C min^{-1}. The eluent was examined by NMR, using stop flow conditions, and mass spectrometry. The four components were eluted in the order of decreasing polarity (Fig. 1).

The NMR spectrum for sulfacetamide (Fig. 2A) was as expected, with a clear AA′BB′ aromatic pattern and an acetyl methyl group (the second methyl signal was identified as traces of residual methanol in the probe from earlier conventional LC–NMR studies). The labile amino and amido protons had exchanged as expected for all the sulfonamides. The spectrum of the second peak (Fig. 2B), sulfadiazine, also contained the expected AA′BB′ pattern, plus an A_2B pattern for the three protons on the pyrimidine ring.



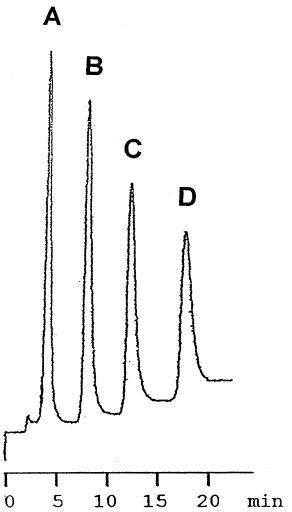


Fig. 1. Separation of sulfonamides on a PS–DVB column using superheated phosphate buffer pD 3.0 as the eluent at 160°C programmed to 200°C at 2°C min^{-1}. Peaks: (A) sulfacetamide; (B) sulfadiazine; (C) sulfamerazine; and (D) sulfamethazine.

However, although the spectrum of the third peak, sulfamerazine (Fig. 2C) contained the AA′BB′ aromatic ring signals and a pair of doublets for the two protons on the pyrimidine ring, unexpectedly there was no signal for the methyl group on the pyrimidine ring. Initially this appeared to suggest that there might have been degradation of the analyte in the superheated water. The spectrum of the last peak for sulfamethazine (Fig. 2D) contained the expected aromatic and pyrimidine ring protons signals, but again there was no peak for either of the heterocyclic methyl groups. A direct measurement of sulfamethazine dissolved in deuterium oxide confirmed that these peaks should be visible in the spectrum.

The mass spectra of the sulfonamides (Fig. 3) showed the expected spectra for sulfacetamide and sulfadiazine with base peaks ions at m/z 256 and m/z 292 corresponding to the $M+K^+$ complexes of the trideutero species (from exchange of the labile amino and amido protons). In contrast, the base peaks for sulfamerazine and sulfamethazine gave ions at m/z 309 and m/z 326, higher than expected by three and six mass units, respectively. This corresponded to the substitution of the protons on the pyridinium methyl groups by deuterium atoms. It therefore appears that these positions had undergone a specific and selective deuterium exchange reaction during the elevated temperature conditions of the separation process.

As it appeared that both the 4- and 4,6-methyl groups on the pyrimidine ring could undergo an exchange, the corresponding 2,4-dimethyl analogue, sulfisomidine, was examined under the same conditions. Again the NMR spectrum lacked a significant methyl signal, although small residual peaks were present. The mass spectrum showed that methyl proton exchange had largely occurred. Thus it appears that exchange can occur at both the 2- and 4-positions of the pyrimidine ring. Previous chemical studies have reported that the α-carbon of the alkyl

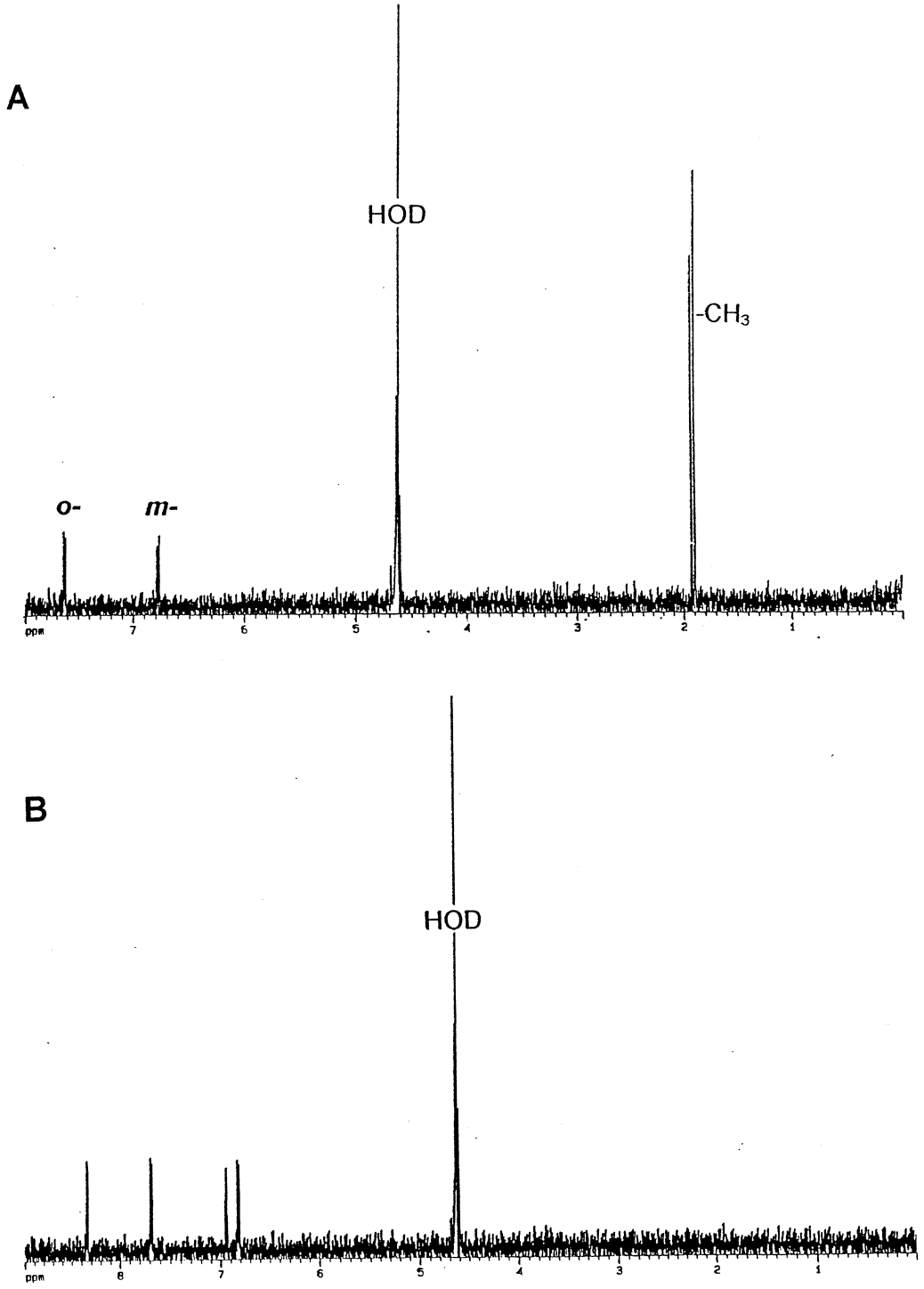


Fig. 2. Stop flow LC–NMR spectra of sulfonamides from separation in Fig. 1. (A) Sulfacetamide; (B) sulfadiazine; (C) sulfamerazine; and (D) sulfamethazine.

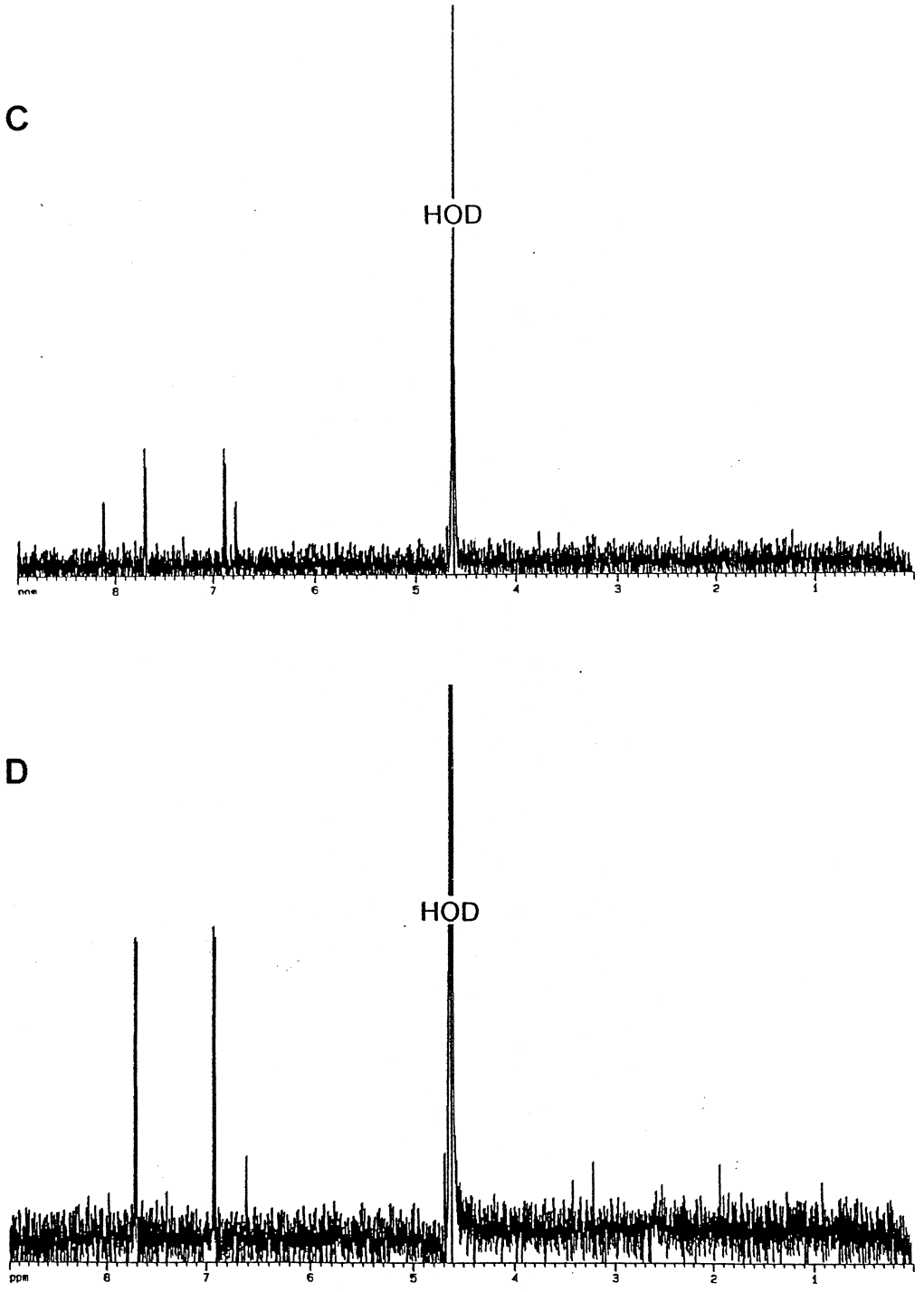


Fig. 2. (*continued*).

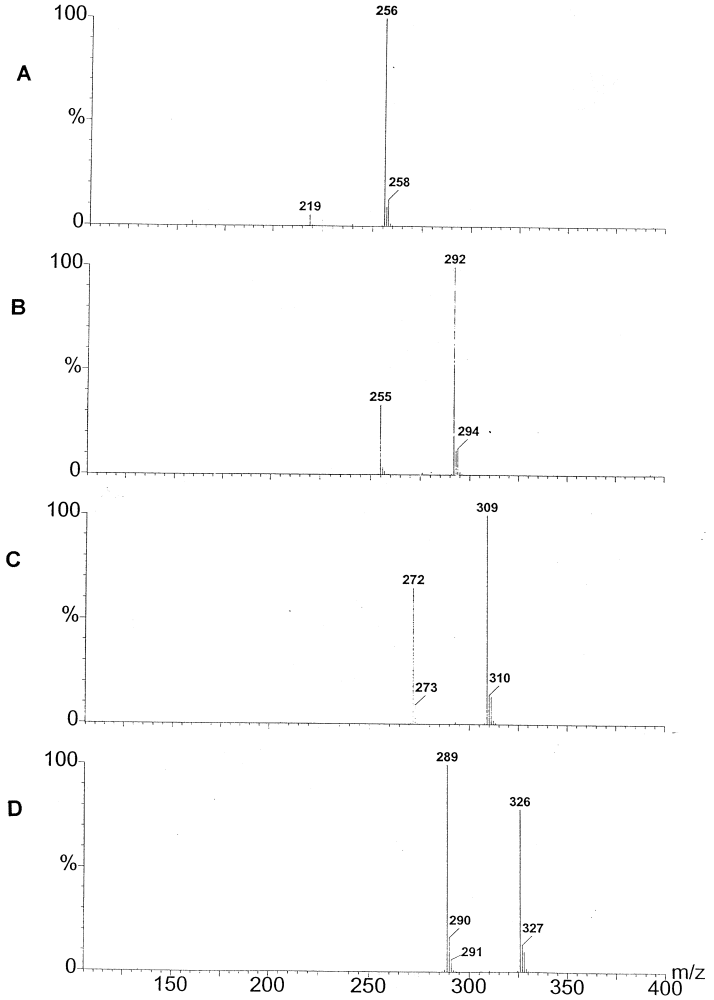


Fig. 3. On-line LC–mass spectra of sulfonamides separated as in Fig. 1. (A) Sulfacetamide; (B) sulfadiazine; (C) sulfamerazine; and (D) sulfamethazine.

group in the 2,4 or 6-positions on the pyrimidine ring is activated [10], presumably via an enamine/imine-type tautomerisation. It was also reported that very slow deuteration ($t_{1/2}$=420 min at pH 0.5 and >1600 min at pH 13.5 in deuterium oxide) occurred on the methyl groups of 2,6-dimethylpyrimidine [11],

This present exchange reaction was potentially interesting as it might represent an easy method to prepare deuterated standards for mass spectrometry. Such deuterated standards can play an important role in mass spectroscopic analysis, because they possess virtually identical physical characteristics to an undeuterated analyte but can be distinguished by their mass difference. They can therefore be used as internal standards as they behave in the same way as the analyte of interest on injection, extraction, derivatisation, chromatography and other sample preparation and separation methods, although some separations have been observed with high-resolution CE [12] and GLC. However, their efficient selective synthesis in high isotopic purity can be difficult. Previously a number of workers have examined the potential of supercritical deuterium oxide at 300–400°C to generate isotope exchange reactions. Weak acids, including acetophenone, would exchange acti-

vated protons in deuterium oxide alone or perdeuteration, including the exchange of aromatic ring protons, could be obtained efficiently with supercritical KOD [13]. The same method gave perdeuterated compounds in good yields (40–85% with up to 95% deuteration) for unsubstituted aryl compounds but poor yields and frequently complex tars were obtained for substituted aromatic compounds [14]. Pyrazoles and quinoxalines also could be converted into perdeutero analogues [15].

Further preliminary studies were therefore carried out to determine if the reaction could be repeated off-line without using a chromatographic system. Sulfisomidine was heated in pD 3.0 D_2O for 10 min at 120°C, and the product was examined by NMR spectroscopy, but only a 32% of deuteration occurred. However, heating the sample at 160°C for the same time, gave a 93% exchange of the methyl group protons. Sulfamerazine under the same conditions gave complete exchange of the methyl groups. It was also important to determine the stability of the deuterated analogues to the loss of deuterium. A sample of the deuterated compound was therefore heated in undeuterated pH 3.0 water at 80°C for 10 min. Examination of the product showed that the deuterated methyl groups were unaltered, confirming that superheated temperatures were needed for the exchange to occur.

4. Conclusion

These studies found that unexpected but specific deuterium exchange reactions can occur in superheated deuterium oxide. This observation may provide a selective and specific method for the preparation of deuterium-labelled analytes containing potentially reactive methyl groups in high yield with negligible side reactions. Previous attempts to use superheated and supercritical deuterium oxide to obtain deuterated standards have generally used extreme conditions and have been less selective and less efficient. The study also demonstrated the utility using a combination of simultaneous NMR and MS detection as means of determining the reactivity and chemical stability of compounds under superheated water chromatography conditions.

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