High-performance liquid chromatography directly coupled to ^{19}F and ^{1}H NMR for the analysis of mixtures of isomeric ester glucuronide conjugates of trifluoromethylbenzoic acids

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Abstract

Acyl migration reactions of drug 1-O-acyl glucuronides are of interest because of their possible role in covalent binding to serum proteins and consequent allergic reactions. This paper describes a new way of investigating the kinetics of acyl migration reactions in a buffer system at pH 7.4, using synthetic 1-O-acyl glucuronides of model compounds (2- and 3-trifluoromethylbenzoic acids) by HPLC–NMR. HPLC directly coupled to ^{19}F and ^{1}H NMR were used in both stop-flow and continuous-flow modes to separate and rapidly identify a mixture of ester glucuronide isomers formed spontaneously by internal acyl migration and mutarotation of 2-, and 3-trifluoromethylbenzoic acid glucuronides [1-O-(2-trifluoromethylbenzoyl)-D-glucopyranuronic acid and 1-O-(3-trifluoromethylbenzoyl)-D-glucopyranuronic acid). The mixtures of isomers were obtained by incubation of the synthetic 2-, and 3-trifluoromethylbenzoic acid glucuronides in buffer solution (pH 7.4) at 25°C for 48 h. The β-anomer of the 1-O-acyl-glucuronide, as well as the 2-, 3-, and 4-positional glucuronide isomers (all three in both α- and β-anomeric forms) present in the isomeric mixture, were all characterised directly by NMR after separation in an isocratic chromatographic system containing phosphate buffer at pH 7.4 and acetonitrile in the mobile phase. The time-course of individual acyl migration of positional glucuronide isomers was monitored in the mobile phase in a novel stop-flow 'dynamic' HPLC–^{19}F NMR experiment. This approach to monitoring metabolite reactivity will be of great value in furthering the understanding of glucuronide rearrangement kinetics and may be of wider importance in monitoring the reactivity of other types of analytes that have been separated in an HPLC–NMR system.

Keywords: Nuclear magnetic resonance spectroscopy; Acyl migration reactions; Trifluoromethylbenzoic acid ester glucuronide

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1. Introduction

High-frequency NMR spectroscopy now has an established role as a rapid means of detecting

and identifying drug metabolites in complex biological mixtures [1]. However, the NMR resonances from xenobiotic metabolites are often extensively overlapped with those from other drug or endogenous metabolites necessitating the use of multipulse or two-dimensional NMR spectral editing methods [2–7]. Recent technological developments have allowed the direct coupling of HPLC with high field NMR spectroscopy giving a new practical hyphenated technique, HPLC–NMR [2–7]. HPLC–NMR technology promises to be of great value in the analysis of complex biological and chemical mixtures of all types and in particular the analysis of drug-related and endogenous metabolites in biofluids. The technical advances contributing to the success of HPLC–NMR include the development of sensitive HPLC–NMR flow probes and high dynamic range receiver systems. Also new methods for the suppression of the solvent NMR resonances in solvent gradients have obviated the need for expensive deuteriated solvents. HPLC–NMR has advantages over conventional analysis and many other hyphenated techniques (e.g. HPLC–mass spectrometry) in that the whole system is liquid-state and non-destructive. HPLC–NMR method development is rapid and interactive in real time and the resultant NMR spectra have a high structural information content. In *continuous flow* HPLC–NMR experiments, pseudo-two-dimensional plots (NMR chromatograms) are generated and analyte spectra are dispersed in the separation time-domain. HPLC–NMR experiments can also be performed in a variety of *stop-flow* modes which allow better NMR signal-to-noise ratios to be achieved and enable the use of multipulse and heteronuclear NMR experiments. The ability to perform

homonuclear and heteronuclear correlation spectroscopy in the stop-flow mode allows comprehensive structure elucidation of analytes. For ^{1}H and ^{19}F NMR, the current detection limits for low-molecular-mass metabolites corresponds to <85 ng of material in the probe at 600/565 MHz, respectively [8]. In the case of fluorinated drugs and fluoroxenobiotics, continuous flow ^{19}F NMR detection is readily achieved giving unequivocal retention time data on the fluorinated metabolites, with detailed structure elucidation via stop-flow HPLC–^{1}H NMR analysis [5] of the peaks.

One research area in which HPLC–NMR has great potential is in the study of the internal rearrangement reactions of drug ester glucuronides as such reactions may be the underlying cause of certain types of allergic reactions in man. Ester glucuronides in the form of the β-1-O-acyl isomer are formed from drugs containing carboxylate groups by uridine 5'-diphosphate (UDP)-glucuronosyl transferase in vivo; many non-steroidal anti-inflammatory and hypolipidemic drugs are included in this group [9]. The acyl glucuronides so formed are potentially reactive due to the susceptibility of the acyl group to nucleophilic reactions and intramolecular rearrangement (isomerisation by acyl migration or transacylation) in which the drug moiety moves from one hydroxyl group to an adjacent hydroxyl group on the glucuronic acid ring (Fig. 1) [10–12]. The transacylation mechanism occurs via a tetrahedral cyclic *ortho*-ester intermediate [13] and the acyl groups migrate from C-1 towards C-4 on the glucuronic acid ring forming both the α- and β-anomers of the 2-, 3-, and 4-O-acyl positional isomers as shown in Fig. 1. All the reactions are reversible except for re-

1-*O*-Acyl (β) isomer   2-*O*-Acyl (α,β) isomer   3-*O*-Acyl (α,β) isomer   4-*O*-Acyl (α,β) isomer
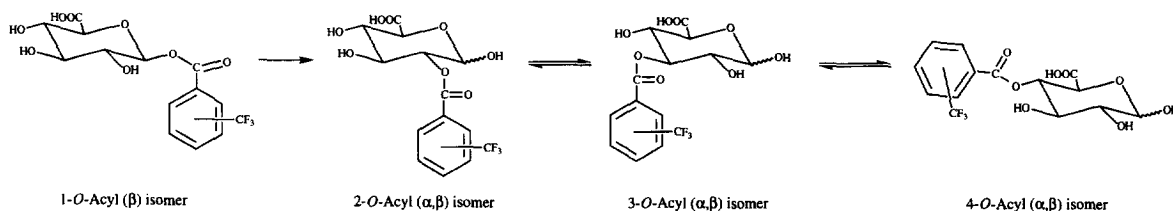
Fig. 1. Reaction scheme for the acyl migration of the trifluoromethylbenzoic acid glucuronides.

formation of the 1-O-acyl glucuronide, presumably because of the higher energy barrier in formation of the anomeric C–O bond. Mutarotation ($\alpha \leftrightarrow \beta$) occurs for all the positional glucuronide isomers via the open-chain aldehyde structure of the glucuronide ring. The acyl migration reactions occur spontaneously in aqueous solution [14], the isomerisation rates being highly dependent upon the structure of the aglycone [14], pH and the temperature [11,15]. Acyl migration is inhibited under acidic conditions but the migration rates increase dramatically with increasing pH (especially above pH 7) [15]. Acyl migration kinetics of reactive drug 1-O-acyl glucuronides are of interest because of their possible significant role in toxicological effects caused by drugs that form ester glucuronides. It has been shown that the acyl glucuronides can irreversibly react with proteins to form covalent adducts which may act as haptens; examples are bilirubin [16], zomepirac [17], tolmetin [18], probenecid [19], carprofen [20], fenoprofen [21], and diflunisal [22]. This type of binding appears to be a general phenomenon for labile acyl glucuronides, thus increasing the probability of allergic reactions to the foreign protein or hapten [15].

In the present study we show that directly coupled 500 MHz HPLC–^{19}F NMR in the continuous flow mode can be used as a rapid approach to separate and identify a mixture of ester glucuronides that interconvert. Additionally we show that 'dynamic' HPLC–^{19}F NMR in the 'stop flow' mode can be used to study the kinetics of the individual acyl migration rates of the acyl-migrated positional isomers of the β-1-O-acyl glucuronide. Synthetic trifluoromethyl-substituted benzoic acid glucuronides were used as model drug glucuronides. Internal acyl migration and mutarotation of 2- and 3-trifluoromethylbenzoic acid glucuronide [1-O-(2-trifluoromethylbenzoyl]-D-glucopyranuronate and 1-O-(3-trifluoromethylbenzoyl)-D-glucopyranuronate] occur spontaneously in aqueous solution and the mixture of positional glucuronide isomers was obtained by incubation of the synthetic 2- and 3-trifluoromethylbenzoic acid glucuronide in buffer solution (pH 7.4) at 25°C for 48 h. The

presence of a –CF$_3$ group conveniently allows the monitoring of the glucuronide isomers by ^{19}F NMR spectroscopy. HPLC–NMR studies of this type should allow new approaches to the investigation of quantitative structure–transacylation activity relationships for ester glucuronides and so enable improved understanding of the molecular physico-chemical features that control transacylation kinetics.

2. Experimental

2.1. Chemicals

All reagents were of analytical grade and purchased from Aldrich (Dorset, UK).

Synthesis of trifluoromethylbenzoic acid glucuronides

The (β) 1-O-(2-trifluoromethylbenzoyl)-D-glucopyranuronate and (β) 1-O-(3-trifluoromethylbenzoyl)-D-glucopyranuronate were synthesised from D-glucose, via protection, C-6 oxidation and Schmidt trichloroacetimidate coupling as previously described [23–25]. This method yielded an imidiate reagent (readily prepared on gram scale), which was used to prepare 1-O-acyl ester glucuronides of these carboxylic acids by a simple one or two step synthesis with control of the α/β-ratio at C-1 [14].

Production of equilibrated mixtures of positional glucuronide isomers

A solution of 2 mg of each of the 2- and 3-trifluoromethylbenzoic acid glucuronides in 500 μl of 20 mM potassium phosphate buffer (pH 7.4) was prepared and incubated for 48 h at 25°C. The solutions were then analysed by HPLC to confirm that substantial amounts of the glucuronide positional isomers had been formed. It is known from earlier experiments that the half lives of the 2- and 3-trifluoromethylbenzoic acid glucuronide isomers are 10.7 and 2.8 h, respectively [14].

2.2. Apparatus

Chromatography for directly coupled HPLC–NMR

The HPLC system consisted of a Gilson 307 isocratic pump (Worthington, OH, USA) and a Perkin Elmer HPLC 90 UV variable-wavelength detector (Buckinghamshire, UK) operated at 200 nm. The outlet of the UV detector was connected to a JEOL HPLC–NMR flow cell via an inert PTFE capillary (0.25 mm I.D.). The UV response was followed on a Graphic 1002 recorder (Lloyd Instruments, UK). The analytical column was a Knauer column (120 × 4.6 mm, I.D.) packed with Spherisorb ODS-2 (Phase Separations, UK), 5 μm. The final mobile phase developed for the separation of 2-trifluoromethylbenzoic acid glucuronide and its positional isomers was acetonitrile–0.2 M potassium phosphate (pH 7.4)–deuterium oxide (5:10:85, v/v/v). The mobile phase developed for the separation of 3-trifluoromethylbenzoic acid glucuronide and its positional isomers was acetonitrile–0.2 M potassium phosphate (pH 7.4)–deuterium oxide (10:10:80, v/v/v). The flow-rate was 0.5 ml/min in both chromatographic systems.

'Continuous flow' directly coupled HPLC–^{19}F NMR spectroscopy

The HPLC–NMR data were acquired using a JEOL ALPHA-500 spectrometer equipped with a JEOL HPLC–NMR flow cell (volume of 120 μl, 5 mm O.D.) fitted into a dedicated ^{1}H single-coil probe. ^{19}F NMR spectra were obtained in the 'continuous-flow' mode in the ^{1}H NMR probe tuned to 470.40 MHz (using a 470.40 MHz NMR ^{19}F transmitter). Free induction decays (FIDs) were collected into 8K computer data points with a spectral width of 9542 Hz, 40° pulses were used with an acquisition time of 0.9 s, and each row in the pseudo-2D plots was acquired by accumulation of 16 scans. Prior to Fourier transformation a shifted sine bell (shifted 35%) was applied to the FID. The ^{19}F NMR chemical shifts were referenced to external CFCl$_3$ at 0.0 ppm.

Directly coupled stop-flow HPLC–^{1}H NMR spectroscopy selected by single-scan continuous-flow HPLC–^{19}F NMR spectroscopy

^{1}H NMR spectra were obtained using a JEOL ALPHA-500 equipped with a 120-μl flow cell inserted into a ^{1}H NMR probe (retunable to ^{19}F NMR frequency) as described above. The actual amount of compound in the flow cell was monitored directly by single-pulse ^{19}F NMR, with observation of the single-scan Fourier-transformed ^{19}F NMR spectrum in order to allow direct selection of the optimal time to stop the HPLC flow. After the flow was stopped, the probe was re-tuned to allow direct ^{1}H NMR measurements to be made and data acquired as follows. Free induction decays (FIDs) were collected into 16K computer data points with a spectral width of 7501 Hz, 60° pulses were used with an acquisition time of 0.9 s, and each spectrum was acquired by accumulation of 256 scans. Prior to Fourier transformation an exponential apodisation function was applied to the FID corresponding to a line broadening of 3 Hz. Following the collection of each ^{1}H NMR spectrum the probe was re-tuned to allow direct ^{19}F observation when the solvent flow was restarted. The process was repeated using segmental single-scan ^{19}F Fourier-transformed spectra to select the optimal stop-flow time for each successive glucuronide isomer peak, thus effectively producing a ^{19}F NMR selected stop flow experiment.

'Dynamic' HPLC–^{19}F NMR spectroscopy on the rearrangement of trifluoromethylbenzoic acid glucuronide isomers

The HPLC–NMR data were acquired in the 'stop-flow' mode using a JEOL ALPHA-500 spectrometer equipped with a JEOL HPLC–NMR flow cell as described above. The positional glucuronide isomers were separated by HPLC, the individual isomers were stopped in the NMR flow-cell and a series of 470.40 MHz NMR ^{19}F spectra were collected over a period of 14 h at 292 K. 360 free induction decays (FIDs) were collected into 8K computer data points with a spectral width of 2351 Hz, and 40° pulses were used with an acquisition time of 1.7 s. This

procedure resulted in the collection of spectra at 15 min time intervals. Prior to Fourier transformation an exponential apodisation function was applied to the FID corresponding to a line broadening of 0.5 Hz.

3. Results

3.1. Chromatography of glucuronide isomers

The final HPLC methods developed allowed separation of the 1-, 2-, 3- and 4-positional glucuronide isomers of the 1-O-acyl- 2- and 3-trifluoromethylbenzoic acid glucuronides in an equilibrated mixture of the glucuronide isomers. The HPLC method was later used to estimate the individual acyl migration rates of the isolated positional glucuronide isomers by 'dynamic' stop-flow HPLC–NMR (see below). An isocratic eluent was used, which was buffered to pH 7.4 with potassium phosphate buffer. The total run time until the last peak had eluted (for a flow-rate of 0.5 ml/min) was 38 min for the 2-trifluoromethylbenzoic acid glucuronides and 31 min for the 3-trifluoromethylbenzoic acid glucuronides.

3.2. 'Continuous-flow' HPLC–^{19}F NMR spectroscopy on glucuronide isomers

Using the 'continuous-flow' mode for the HPLC–^{19}F NMR experiments it was possible to separate and identify the 1-, 2-, 3- and 4-positional isomers of the 1-O-acyl- 2- and 3-trifluoromethylbenzoic acid glucuronides in an equilibrated mixture. The pseudo-2D plots with NMR chemical shifts on the horizontal axis and chromatographic run times on the vertical axis can be seen in Fig. 2. In Table 1 the assignments of the ^{19}F NMR spectra of the 2- and 3-trifluoromethyl benzoic acid glucuronides are summarised. The assignments were based on the correlated stop-flow HPLC–^{1}H NMR data (Table 2) and the ^{19}F NMR chemical shifts obtained earlier by following the rearrangements of the 2- and 3-trifluoromethylbenzoic β-1-O-acyl glucuronides with time

[14]. The chromatographic elution order was the same for the two trifluoromethylbenzoic acid glucuronides, namely, first the α-4-O-acyl isomer, secondly the β-4-O-acyl isomer, third the β-1-O-acyl isomer, fourth the β/α-3-O-acyl isomer, fifth the α-2-O-acyl isomer and last the β-2-O-acyl isomer. This elution order is equivalent to that found previously for positional isomers of other acyl glucuronides [25].

3.3. 'Stop-flow' HPLC–^{1}H NMR spectroscopy on glucuronide isomers

The equilibrated mixture of the 2-trifluoromethylbenzoic acid glucuronide isomers was also analysed by directly coupled HPLC–^{1}H NMR in the 'stop-flow' mode. By monitoring the NMR signal level with ^{19}F NMR, on 'time slices' (finely incremented stop-flow analysis of a chromatographic peak) of the peaks detected by the UV detector, it was possible to evaluate when an adequate concentration of the given glucuronide isomer was in the flow cell. The required number of scans needed for the ^{1}H NMR spectra could then be acquired. The assignments of the ^{1}H NMR spectra of the 2-trifluoromethylbenzoic acid glucuronide isomers can be seen in Table 2.

3.4. 'Dynamic' stop-flow HPLC–^{19}F NMR spectroscopy on glucuronide rearrangement reactions

It was possible to follow the acyl migration reactions of the individual glucuronide positional isomers, by collecting one isomer in the NMR flow cell and monitoring the subsequent rearrangements by ^{19}F NMR with time. The first peak in the chromatographic UV-trace ($t_R = 6.9$ min) of the equilibrated mixture of 3-trifluoromethylbenzoic acid glucuronides corresponds to the equilibrium mixture of the α- and β-anomer of the 4-O-acyl isomer (see Table 1). The rearrangement reactions were studied over a time period of 12 h in 15-min acquisition time windows. The stacked plot of the NMR spectra, over the restricted chemical shift region (-62.33 to -62.50), is shown in Fig. 3A. In Fig. 3B rows
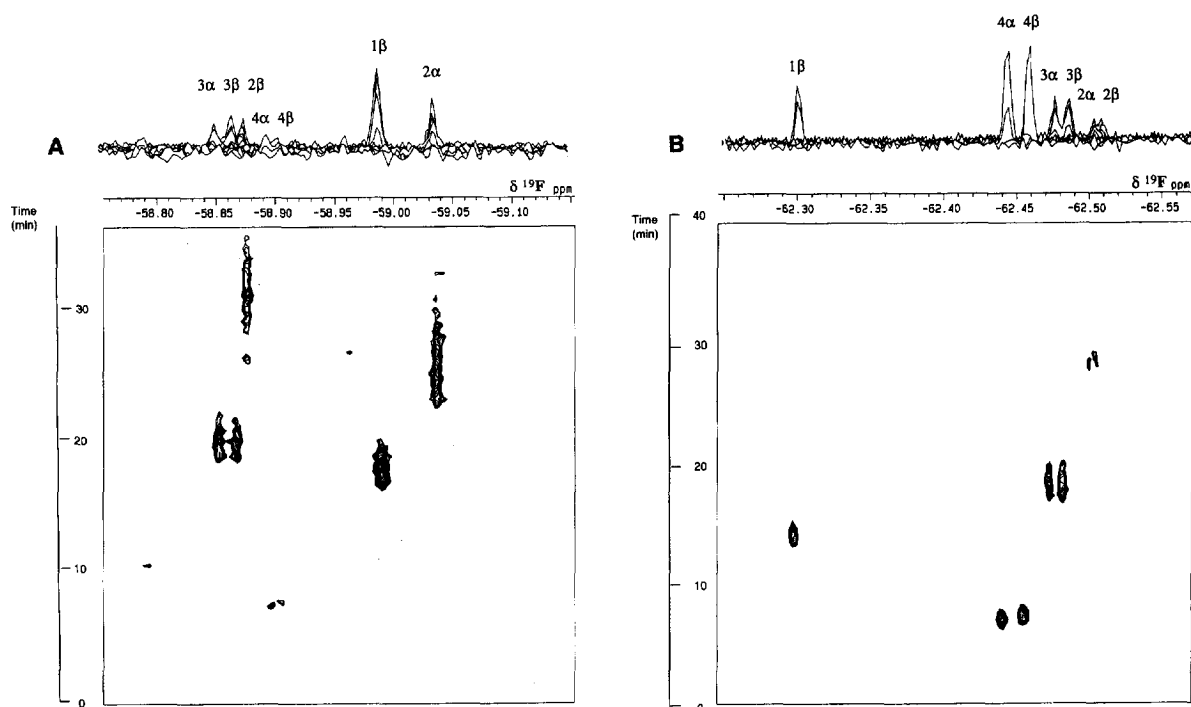
Fig. 2. Pseudo-2D HPLC–^{19}F NMR contour plots of (A) the 2-trifluoromethylbenzoic acid glucuronides and (B) the 3-trifluoromethylbenzoic acid glucuronide in equilibrated mixtures. The NMR chemical shifts are shown on the horizontal axis and chromatographic run times in the vertical dimension. The superimposed row projections are shown above the pseudo-2D plots. The elution order of the positional isomers is the same for the 2-trifluoromethylbenzoic and the 3-trifluoromethylbenzoic acid glucuronides: i.e. α-4-O-acyl (4α); β-4-O-acyl (4β); β-1-O-acyl (1β); β,α-3-O-acyl (3α,β); α-2-O-acyl (2α); and finally the β-2-O-acyl isomer (2β).

Table 1
Assignment of the ^{19}F spectral data of trifluoromethylbenzoic acid glucuronide isomers

	2-Trifluoromethylbenzoic acid glucuronide		3-Trifluoromethylbenzoic acid glucuronide	
	t_R (min)	δ (ppm)	t_R (min)	δ (ppm)
4-O-acyl (α) isomer	7.22	−58.89	6.87	−62.438
4-O-acyl (β) isomer	7.50	−58.91	7.45	−62.455
1-O-acyl (β) isomer	17.78	−58.99	14.15	−62.299
3-O-acyl (β) isomer	19.72	−58.87	17.85	−62.472
3-O-acyl (α) isomer	20.00	−58.85	18.46	−62.481
2-O-acyl (α) isomer	25.00	−59.04	28.31	−62.500
2-O-acyl (β) isomer	30.83	−58.87	29.23	−62.505

The assignments were based on the spectral data obtained from the continuous-flow ^{19}F HPLC–NMR experiment in combination with the chemical shifts obtained from the stop-flow ^{1}H HPLC–NMR experiment.

Table 2
Assignment of the ^{1}H spectral data of 2-trifluoromethylbenzoic acid glucuronide isomers

	Glucuronide ring protons (δ, pattern)				
	1'	2'	3'	4'	5'
4-O-Acyl (α) isomer	5.11	3.41	4.05	5.08	4.13
	d	dd	t	t	d
4-O-Acyl (β) isomer	[a]	3.28	3.78	5.08	3.90
		t	t	t	d
1-O-Acyl (β) isomer	5.82	3.70	3.60	3.65	3.85
	d	t	t	t	d
3-O-Acyl (β) isomer	[a]	3.80	5.20	3.60	4.18
		t	t	t	d
3-O-Acyl (α) isomer	5.30	3.82	5.38	3.70	4.18
	d	dd	t	t	d
2-O-Acyl (α) isomer	5.42	4.95	3.95	3.59	4.10
	d	dd	t	t	d
2-O-Acyl (β) isomer	[a]	4.90	3.70	3.59	3.75
		t	t	t	d

The assignments were based on the spectral data obtained from the stop-flow HPLC–^{1}H NMR experiment. The coupling patterns observed were doublet (d), triplet (t) and doublet of doublets (dd) splittings with magnitudes expected for pyranose rings with J (axial–axial) ca. 8–10 Hz and J (axial–equatorial) ca. 3–4 Hz.
[a] Not determined (obscured by HDO resonance).

corresponding to various time points in the pseudo-2D plot are shown and the assignments of the peaks are indicated on the top spectrum. The 3-O-acyl isomers were formed first, followed by the 2-O-acyl isomers as a consequence of the rearrangement mechanism which dictates how acyl migration takes place with the neighbouring hydroxyl group of the glucuronic acid ring only.

4. Discussion

In this study we present three new analytical approaches involving the use of HPLC–NMR in the analysis of glucuronide mixtures. The positional isomers of trifluoromethylbenzoic acid glucuronides formed via acyl migration in the equilibrated mixtures were clearly separated and detected by HPLC–^{19}F NMR spectroscopy in the 'continuous-flow' mode. This is particularly useful as any NMR spectral interference from the components of the HPLC eluent is omitted. The trifluoromethylbenzoic acid glucuronides contain

strong 'radiophors' in the form of the trifluoromethyl group, which gives a strong uncoupled ^{19}F NMR singlet, thus lowering the effective detection limits for these compounds. We were able to detect the α- and β-anomer of each positional glucuronide isomer by ^{19}F NMR spectroscopy. This was confirmed by directly coupled 'stop-flow' HPLC–^{1}H NMR for the 2-trifluoromethylbenzoic acid glucuronide.

We have shown that HPLC–NMR can be used for the real-time investigation of the chemical reactivity of drug metabolites such as the determination of the internal acyl migration kinetics of drug ester glucuronides. Such studies are only possible via HPLC–NMR approaches. Directly coupled 'dynamic' HPLC–NMR used to study the acyl migration of the positional glucuronide isomers will be of value in understanding the structural physico-chemical properties that determine drug glucuronide reactivity. There are likely to be many more applications for the technique of 'dynamic' stop-flow HPLC–NMR as it is also possible to perform post-column
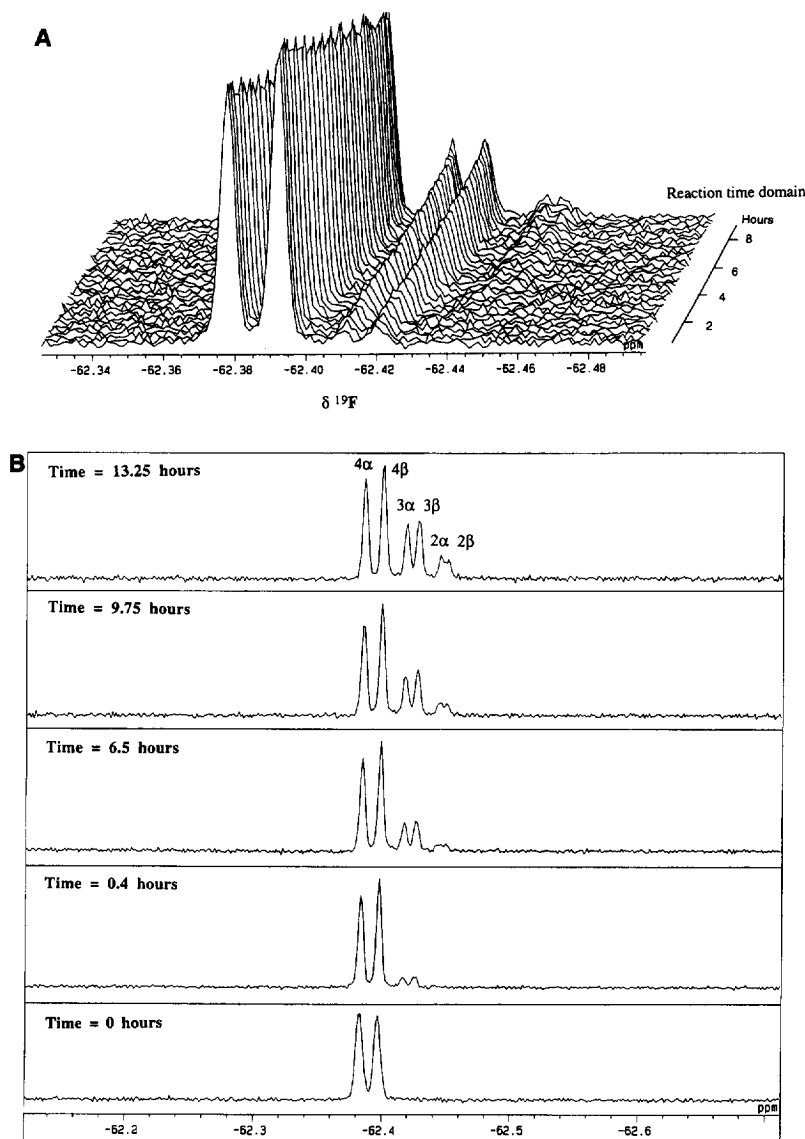
Fig. 3. (A) The stacked plot of a 'dynamic' stop-flow HPLC-^{19}F NMR run, with NMR chemical shift on the horizontal axis and the reaction time-course on the orthogonal axis. The acyl migration reactions of the 4-O-acyl-3-trifluoromethylbenzoic acid isomer were followed at pH 7.4 (292K). (B) Individual spectra picked at different time points of the reaction time. The assignments of the peaks are indicated on the top spectrum α-4-O-acyl isomer (4α), β-4-O-acyl isomer (4β), α-3-O-acyl isomer (3α), β-3-O-acyl isomer (3β), α-2-O-acyl isomer (2α) and β-2-O-acyl isomer (2β).

additions to analytes directly in the NMR probe. Given its rapid and continued growth, HPLC–NMR is likely to become one of the key enabling technologies in pharmaceutical/biomedical anal-

ysis over the next decade with further possibilities of enhancing drug metabolite elucidation in the future via the possible triple hyphenation of HPLC–NMR with mass spectrometry.

Acknowledgement

References

[1] J. Nicholson and I. Wilson, Prog. NMR Spectrosc., 21 (1989) 449–501.
[2] M. Spraul, M. Hofmann, P. Dvortsak, J.K. Nicholson and I. Wilson, J. Pharm. Biomed. Anal., 8 (1992) 601–605.
[3] M. Spraul, M. Hofmann, P. Dvortsak, J.K. Nicholson and I. Wilson, Anal. Chem., 65 (1993) 327–330.
[4] I. Wilson, J.K. Nicholson, M. Spraul, M. Hofmann, J.K. Nicholson and J.C. Lindon, J. Chromatogr., 617 (1993) 324–328.
[5] M. Spraul, M. Hofmann, I. Wilson, E. Lenz, J.K. Nicholson and J.C. Lindon, J. Pharm. Biomed. Anal., 11 (1993) 1009–1016.
[6] M.J. Seddon, M. Spraul, I. Wilson, J.K. Nicholson and J.C. Lindon, J. Pharm. Biomed. Anal., 12, (1994) 419–424.
[7] M. Spraul, M. Hofmann, J.C. Lindon, R.D. Farrant, M.J. Seddon, J.K. Nicholson and I. Wilson, NMR Biomed., 7 (1994) 295–303.
[8] B.C. Sweatman, R.D. Farrant, P.N. Sanderson, I. Philippe, S.R. Salman, J.K. Nicholson and J.C. Lindon, J. Magn. Res. Anal., 1 (1995) 9–12.
[9] G.J. Dutton, Glucuronization of Drugs and Other Compounds, CRC Press, Boca Raton, FL, 1980.
[10] G. Bradow, L.S. Kan and C. Fenselau, Chem. Res. Toxicol., 2 (1989) 316–324.
[11] A.R King and R.G. Dickinson, Biochem. Pharmacol., 42 (1991) 2289–2299.
[12] P.C. Smith, A.F. McDonagh and L.Z. Benet, J. Clin. Invest., 77 (1986) 934–939.
[13] E. Fischer, Chem. Ber., 53 (1920) 1621–1633.
[14] A.W. Nicholls, K. Akira, R.D. Farrant, J.C. Lindon, I.D. Wilson and J.K. Nicholson, Chem. Res. Tox., submitted.
[15] H. Spahn-Langguth and L.Z. Benet, Drug Metab. Rev., 24 (1992) 5–48.
[16] J.S. Weiss, A. Guatam, J.J. Lauff, M.W. Sundberg, P. Jatlow, J.L. Boyer and D. Saligson, N. Engl. J. Med., 309 (1983) 147–150.
[17] P.C. Smith, A.F. McDonagh and L.Z. Benet, J. Clin. Invest., 77 (1986) 934–939.
[18] M.L. Hyneck, P.C. Smith, A. Munafo, A.F. McDonagh and L.Z. Benet, Clin. Pharmacol. Ther., 44 (1988) 107–114.
[19] G.E. McKinnon and R.G. Dickinson, Res. Commun. Chem. Pathol. Pharmacol., 66 (1989) 339–354.
[20] S. Iwakawa, H. Spahn-Langguth, L.Z. Benet and E.T. Lin, Pharm. Res., 5 (Suppl.) (1988) S-214.
[21] C. Volland, H. Sun, J. Dammeyer and L.Z. Benet, Drug Metab. Dispos., 19 (1991) 1080–1086.
[22] J.A. Watt and R.G. Dickinson, Biochem. Pharmacol., 39 (1990) 1067–1075.
[23] R.R. Schmidt and G. Grundler, Synthesis, (1981) 885–887.
[24] C.C.A. Van Boeckel, L.P.C. Delbressine and F.M. Kaspersen, Recl. Trav. Chim. Pays-Bas, 104 (1985) 259–265.
[25] U. Sidelmann, C. Gavaghan, H.A.J. Carless, R.D. Farrant, J.C. Lindon, I.D. Wilson and J.K. Nicholson, Anal. Chem., 67 (1995) 3401–3404.