ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
28 (2002) 875–885

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

The metabolism of 4-trifluoromethoxyaniline and [^{13}C]-4-trifluoromethoxyacetanilide in the rat: detection and identification of metabolites excreted in the urine by NMR and HPLC-NMR

M. Tugnait [a,1], E.M. Lenz [a,b], P. Phillips [b], M. Hofmann [c], M. Spraul [c], J.C. Lindon [a], J.K. Nicholson [a], I.D. Wilson [b,*]

[a] *Biological Chemistry, Sir Alexander Fleming Building, Imperial College of Science, Technology and Medicine, South Kensington, London SW 7 2AZ, UK*

[b] *Department of Drug Metabolism and Pharmacokinetics, AstraZeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire, SK10 4TG, UK*

[c] *Bruker Analytische Messtechnik, Am Silberstreifen, 76287 Rheinstetten, Germany*

Received 26 August 2001; received in revised form 30 October 2001; accepted 31 October 2001

Abstract

A combination of ^{19}F, ^{1}H NMR and HPLC-NMR spectroscopic approaches have been used to quantify and identify the urinary-excreted metabolites of 4-trifluoromethoxyaniline (4-TFMeA) and its [^{13}C]-labelled acetanilide following i.p. administration at 50 mg/kg to rats. The major metabolite excreted in the urine for both compounds was a sulphated ring-hydroxylated metabolite (either 2- or 3-trifluoromethyl-5-aminosulphate) which accounted for $\approx$32.3% of the dose following the administration of 4-TFMeA and $\approx$29.9% following dosing of the acetanilide. The trifluoromethoxy-substituent appeared to be metabolically stable, with no evidence of *O*-detrifluoromethylation. There was no evidence of the excretion of *N*-oxanilic acids in urine, of the type seen with 4-trifluoromethylaniline.

Keywords: ^{19}F NMR; HPLC-NMR; Aniline metabolism

1. Introduction

Given the importance of substituted anilines in the production of a wide range of pharmaceuticals, pesticides and other industrial chemicals, there is widespread potential for human and ani-

* Corresponding author. Tel.: +44-1625-512-434; fax: +44-1625-516-962.
E-mail address: ian.wilson@astrazeneca.com (I.D. Wilson).
[1] Present address: Pfizer Global Research and Development Pharmacokinetics, Dynamics and Metabolism, 2800 Plymouth Road, Ann Arbor, MI 48105, USA.

0731-7085/02/$ - see front matter
PII: S0731-7085(01)00699-9

mal exposure to these chemicals [1]. Therefore, a knowledge of their metabolic fate in living systems is an important part of understanding the toxicological properties of this class of compounds. In addition, where there is the potential for human or animal contact with such compounds, it is important to know what metabolites are likely to be formed, in order to devise suitable methods for monitoring exposure. In addition to these considerations, for some time we have been interested in the factors that result in the metabolism of some *para*-substituted anilines to *N*-oxanilic acids. These metabolites are formed via *N*-acetylation, hydroxylation to the glycolanilide and then oxidation to the oxanilic acid. Compounds (or their acetanilides) which undergo this reaction include 4-chloroaniline [2–5], 4-bromoaniline [2,6], 4-trifluoromethylaniline [7,8] and 4-propionylaniline [6]. We were therefore interested to see whether 4-trifluoromethoxyaniline and its acetanilide would also undergo biotransformation to an *N*-oxanilic acid and also to investigate the metabolic stability of the trifluoromethoxy-group. The presence of the trifluoromethoxy-substituent facilitated the investigation of the metabolic fate of the compound, as ^{19}F NMR could be used directly on the urine for metabolite profiling and quantification and enabled the detection of compound-related peaks when HPLC-NMR was performed for metabolite identification.

2. Experimental

2.1. Compounds

4-Trifluoromethoxyaniline (4-TFMeA) was obtained from Fluorochem (Derby, UK). [^{13}C]-4-trifluoromethoxyacetanilide (4-TFMeAc) was synthesised by reacting 4-TFMeA (3 mmol) at room temperature directly with 3.5 mmol of acetyl-1-[^{13}C]-chloride (99 at.% ^{13}C) (Aldrich, Dorset, UK). The solid product of this reaction was then washed with water to remove unreacted acetyl chloride and recrystallised from ethanol to give a product that was $>95\%$ pure by ^{1}H and ^{19}F NMR.

2.2. Dosing

Two groups of three male Sprague–Dawley rats (200–250 g) each received a single i.p. dose of either 4-TFMeA (50 mg/kg) or [^{13}C]-4-trifluoromethoxyacetanilide (50 mg/kg), both dosed at a volume of 1 ml/kg in corn oil. Prior to dosing, the animals were housed individually in metabolism cages designed for the separate collection of urine and faeces. The animals were allowed to acclimatise for 24 h before dosing and food and water were available ad libitum throughout the experiment. Urine samples were collected over solid carbon dioxide for 24 h pre dose and for the periods 0–8, 8–24 and 24–48 h post dose (hpd). Samples were then stored at -20 °C until analysed. Before analysis, the samples were centrifuged at 3000 rpm at 4 °C to remove solid debris (particles of food, etc.) and the volume and pH were determined.

2.3. Sample preparation

2.3.1. Enzyme hydrolysis

Hydrolysis with β-glucuronidase and aryl sulphatase was performed on selected samples (0.8 hpd) in order to provide an indication of the presence of conjugated metabolites for confirmation by spectroscopic studies (see below).

2.3.1.1. β-Glucuronidase. The test and control samples (0.5 ml) buffered to pH 6.8 with 0.4 M potassium phosphate buffer and with the addition of 0.1 ml of D_2O, were incubated overnight in glass NMR tubes at 37 °C in a shaking water bath. To the test samples, 5 mg of β-glucuronidase (ex. *E. coli*, Sigma) were added.

2.3.1.2. Aryl sulphatase. The test and control samples (0.5 ml), adjusted to pH 5.3 with 0.4 M acetic acid and with the addition of 0.1 ml of D_2O, were incubated in glass NMR tubes at 37 °C overnight in a shaking water bath. To the test samples, 5 mg of aryl sulphatase (ex *Helix pomatia*, Sigma) were added, whilst to both test and control samples, 0.05 ml of 100 mM saccarolactone (in 0.4 M sodium acetate buffer, pH 5.3) were added to inhibit β-glucuronidase activity.

2.4. NMR spectroscopy

2.4.1. ^{1}H NMR spectroscopy

^{1}H NMR spectra of urine were obtained on a Jeol GSX500 NMR spectrometer operating at 500 MHz at ambient probe temperature. Typically 64–256 FIDs were collected into 32 K data points using a 45° pulse width with a data acquisition time of 2.73 s. A pulse delay of 2.27 s was used to ensure complete T_1-relaxation of the spectra. Water suppression was achieved using a secondary gated irradiation field at the water resonance frequency. An exponential apodisation function, corresponding to a line-broadening factor of 0.2 Hz, was applied prior to Fourier transformation (FT). Chemical shifts were referenced to the water resonance at $\delta_{^1H}$ 4.8 ppm.

2.4.2. ^{19}F NMR spectroscopy

^{19}F NMR spectra of urine were obtained using either Varian VXR400 or Bruker AM400 NMR spectrometers operating at a ^{19}F observation frequency of 376 MHz at ambient probe temperature. Typically, 128–512 FIDs were collected into 8 K data points using a 45° pulse width (15 μs) an acquisition time of 0.4 s and a spectral width of 10,000 Hz. A further delay of 6 s (calculated by the inversion recovery method) was added between pulses to ensure complete T_1-relaxation. The time domain spectrum was zero filled to 64 K data points and an exponential weighting corresponding to a line broadening of 0.5 Hz was applied prior to FT. Chemical shifts were referenced to the external secondary reference trifluoroethanol ($\delta_{^{19}F}$ −77 ppm from $CFCl_3$ at $\delta_{^{19}F}$ 0.0 ppm). This secondary chemical shift reference was closer to the ^{19}F resonances of interest and thus enabled spectra to be acquired over a smaller spectral width, thereby ensuring uniform excitation.

2.5. Quantification of 4-TFMA and [^{13}C]-4-TFMeA rat urinary metabolites

Quantification of 4-TFMA and [^{13}C]-4-TFMeA related resonances was carried out by ^{19}F NMR spectroscopy, following the addition of a known concentration of the internal standard, 2-trifluoromethylbenzoic acid. Quantification of the ^{19}F-containing aniline metabolites was carried out by integration of the ^{19}F NMR metabolite signals relative to those of added internal standard. The concentration of the analyte in solution was calculated as follows:

$$W_a = W_s \times (I_a/I_s) \times (MW_a/MW_s) \times (N_s/N_a)$$

where 'a' and 's' represent the analyte and standard, respectively, W, the weight of the substance in the sample, I, the integral value of the NMR resonance, MW, the molecular weight and N the number of ^{19}F nuclei contributing to the resonance.

2.6. Solid-phase extraction/chromatography monitored by NMR (SPEC-NMR)

Solid phase extraction with chromatographic separation provided by stepwise gradient elution was used to concentrate and partially purify metabolites. Samples (2 ml) were acidified to pH 2 with 2 M HCl and loaded onto 3 ml C18 Bond-Elut columns (Jones Chromatography, Hengoed, UK) containing 500 mg of sorbent. Prior to loading the samples, the columns had been conditioned with 5 ml of methanol, followed by 5 ml of acidified water (pH 2 with HCl). Following sample application, the column was washed with two aliquots of 1 ml of acidified water and then any retained material was eluted using a stepwise gradient of increasing eluotropic strength. Thus, 1 ml aliquots of methanol:acidified water starting at a proportion of 20:80 and rising in 20% steps up to 100% methanol were employed. All of the eluates were collected, the solvent was removed by a combination of evaporation under a stream of nitrogen and then freeze dried. SPEC fractions were then reconstituted in 1.2 ml of D_2O and taken for ^{1}H NMR (data not shown). Following NMR, the SPEC fractions were freeze dried again prior to analysis by fast ion bombardment mass spectrometry (see below).

2.7. HPLC-NMR

HPLC-NMR was performed on a 3 ml sample of 0–8 hpd urine (following 4-TFMeA adminis-

tration) which was freeze dried and reconstituted in 0.75 ml of ammonium acetate buffer (pH 5.2, 0.1 M, in D_2O):acetonitrile (ACN) (3:1) prior to chromatography. Samples of 50 μl were analysed. The separation was performed using a HPLC system consisting of a Bruker LC22 pump, an LC53 autosampler and LC313 variable wavelength UV detector (operating at 220 nm). The 5 μm, Spherisorb ODS 2 C-18 HPLC column (250 × 4.6 mm i.d.) was used with a solvent flow rate of 1 ml/min at 35 °C. A linear gradient, beginning with an initial 3 min isocratic period with the starting mobile phase composition (ammonium acetate buffer (0.1 M, pH 5.2 in D_2O):ACN 99:1 v/v) rising over 35 min to 60% ACN, was used.

On-flow ^{19}F NMR was used to locate metabolites using a Bruker AMX500 NMR spectrometer (470.6 MHz fluorine detection frequency) equipped with a flow through $^{19}F/^{1}H$ dual probe of 3 mm i.d. and a cell volume of 60 μl. ^{19}F NMR continuous-flow experiments were acquired over a spectral width of 25,000 Hz with 24 scans per experiment with four dummy scans and an acquisition time of 0.16 s with FID apodisation using a sine-bell function and a data size of 8 K data points. Chemical shifts were referenced to external trifluoroacetic acid ($\delta_{^{19}F}$ −76.8 ppm).

For metabolite identification using ^{1}H NMR, at 500 MHz proton observation frequency, the separation was repeated stopping the flow at the retention times corresponding to the ^{19}F-detected peaks. Double solvent suppression was achieved using a standard solvent suppression sequence irradiating both the residual H_2O signal and the ACN resonance (NOESYPRESAT, Bruker). Spectra were acquired over a spectral width of 10,000 Hz with between 96 and 4000 transients per experiment. A pulse repetition time of 3.56 s was used with 1 Hz line broadening and zero filling to 32 K data points prior to FT. Chemical shifts were referenced to internal acetonitrile ($\delta_{^{1}H}$ 2.0 ppm).

2.8. Mass spectrometry (MS)

Mass spectrometry on selected samples obtained by SPEC was performed on a Quattro triple quadrupole mass spectrometer (Micromass, Wythenshawe, UK) using positive and negative fast ion bombardment (FIB). Samples were dissolved in an ethanol glycerol matrix prior to FIB with caesium ions (data not shown).

3. Results and discussion

^{1}H NMR of the urine samples obtained following the administration of 4-TFMeA and TFMeAc over the time course of the study, revealed signals consistent with the presence of metabolites in the 0–8 and 8–24 hpd samples. Whilst these analyses revealed the presence of compound-related material, via the detection of new signals in the aromatic region of the spectra, it was not possible to make any firm identifications. However, the detection of compound-related material via aromatic resonances was accompanied by the absence of corresponding prominent signals for compound-related *N*-acetyls. This implied that metabolism of 4-TFMeA via *N*-acetylation was not a major route (and conversely, the equivalent acetanilide was subject to deacetylation). An indication of the likely presence of some phase II metabolites was given by the presence of a signal for the anomeric proton of a glucuronide conjugate.

Typical ^{19}F NMR spectra from the urine of rats dosed with 4-TFMeA for the period 0–8, 8–24 and 24–48 hpd are shown in Fig. 1. Essentially, the same metabolite profiles were seen for 4-TFMeAc, although some differences in the proportions of the various metabolites were apparent. These spectra show that excretion was rapid, with the majority of the metabolites being present in samples collected in the first 24 hpd and only trace amounts of fluorine-containing compounds detectable in the 24–48 hpd urine. Based on standard addition, it was possible to identify the peak at $\delta_{^{19}F}$ −58.43 as 4-TFMeA and the minor peak at $\delta_{^{19}F}$ −58.08 as 4-TFMeAc. In samples from animals dosed with 4-TFMeA, ≈8.2% of material was excreted apparently unchanged, whilst ≈2.7% of material appeared to correspond to the *N*-acetylated metabolite. For animals receiving 4-TFMeAc, much less of the dose was recovered as the free aniline (≈0.8%) but com-

parable amounts of the acetanilide were excreted (≈ 3.5%). Incubation of samples from both TFMeA and TFMeAc dosed animals with hydrolytic enzymes resulted in various changes in the ^{19}F NMR spectrum, indicating (Fig. 2) the presence of both sulphate and glucuronide

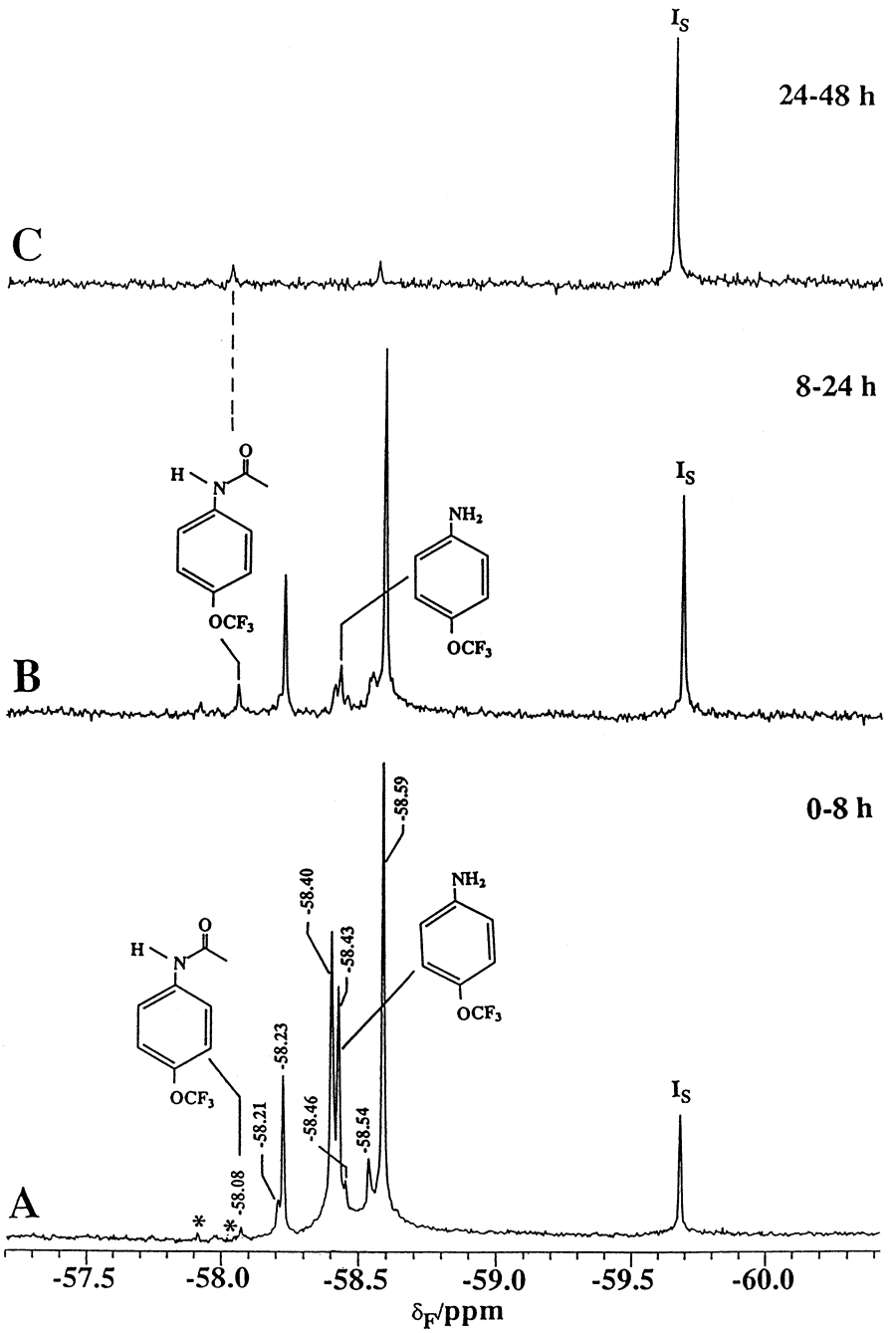

Fig. 1. A total of 376 MHz ^{19}F NMR spectra of urine collected (A) 0–8 h, (B) 24–48 h and (C) 24–48 h after i.p. administration of 50 mg/kg 4-TFMeA to rats. IS indicates internal standard used for quantification and * indicates the minor metabolites observed.

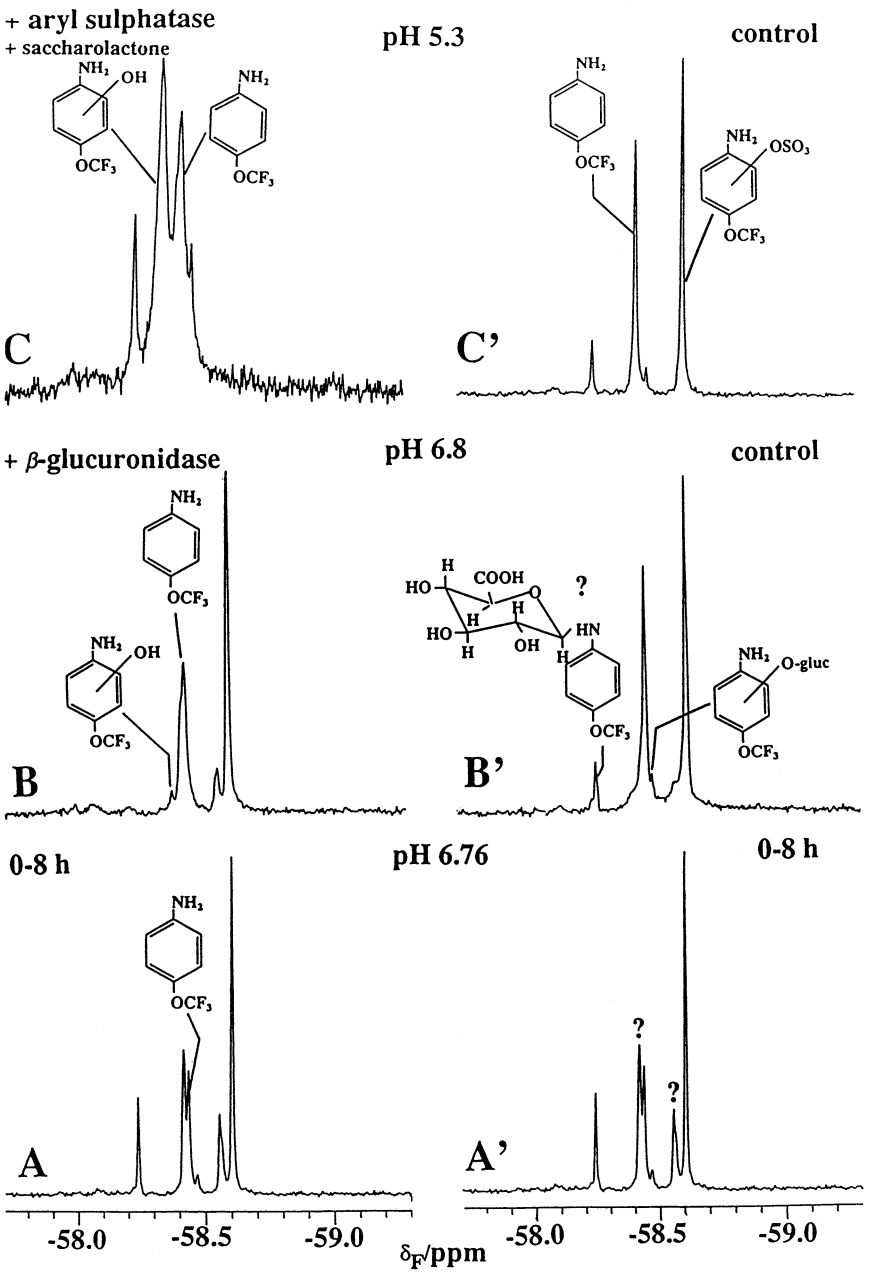

Fig. 2. A total of 376 MHz ^{19}F NMR spectra of 0–8 h urine collected from 4-TFMeA dosed rats before (A and A') and after incubation with β-glucuronidase (B') and aryl sulphatase (saccharolactone inhibitor added) (C'). Spectra B' and C' show the corresponding control urine incubations with phosphate (pH 6.8) and acetate (pH 5.3) buffers, respectively.

metabolites. One of the glucuronide metabolites appeared to form 4-TFMeA on hydrolysis, indicating that an *N*-glucuronide was present, whilst the major peak at $\delta_{^{19}F}$ −58.61, which hydrolysed to give the peak at $\delta_{^{19}F}$ −58.34 on treatment with aryl sulphatase, was tentatively identified as

a sulphate conjugate of a metabolite of 4-TFMeA. Subsequently, solid phase extracts of urine were obtained which, when subjected to FIB-MS, confirmed that this major metabolite of both TFMeA and TFMeAc was indeed a hydroxysulphate of 4-TFMeA. A further metabolite detected in the urine of rats following administration of both compounds ($\delta_{^{19}F}$ −58.56) proved to be unstable, reverting to 4-TFMeA in control incubations. Such behaviour is consistent with the presence of an *N*-sulphate.

Further identification was then undertaken using HPLC-NMR on the concentrated urine sample obtained following dosing of 4-TFMeA. The pseudo two-dimensional contour-plot of the continuous flow ^{19}F HPLC-NMR experiment undertaken on the 0–8 hpd samples is shown in Fig. 3. This experiment shows the presence of six resolved fluorine-containing peaks (A–F) with retention times between 11.44 and 24.9 min. The experiment was repeated performing stopped-flow ^{1}H NMR at the retention times of these peaks. This enabled spectra for peaks C, D and F to be obtained (see Fig. 3); the quantities of material present in peaks A, B and E were insufficient to obtain diagnostic spectra. The metabolite corresponding to peak C ($t_R = 15.4$ min) showed the presence of a doublet at $\delta_{^1H}$ 5.0 characteristic of the anomeric proton of an ether glucuronide conjugate, with the signals for the other protons resonating between $\delta_{^1H}$ 3.4 and 3.9 (not shown). The resonances for the aromatic ring protons show a typical AA′BB′ pattern for a *para*-disubstituted ring, indicating that metabolism had not taken place on the ring. These data, combined with the absence of evidence for any other group indicates that the metabolite is an *N*- or possibly an *NO*-glucuronide of 4-TFMeA.

The major metabolite eluting at 16.46 min (Fig. 3D) showed evidence of metabolism on the aromatic ring. Thus, the ^{1}H NMR spectrum contained a doublet, singlet, doublet pattern consistent with a 1,2,4 or 1,3,4-substituted ring system, with finer *meta* coupling (4-bond) observed for the doublet resonating at $\delta_{^1H}$ 7.0 and the singlet at $\delta_{^1H}$ 7.2. Enzymatic hydrolysis (see above) indicated that this metabolite was a sulphate conjugate and therefore, this material corresponds to either 2-trifluoromethoxy-5-aminophenyl sulphate or 3-trifluoromethoxy-6-aminophenyl sulphate. However, based solely on the observed ^{1}H NMR shifts, it is not possible to distinguish between the two structures. Modification of the aromatic ring *ortho* to the amino group is the most often observed [9–11] and the balance of probabilities makes this the most likely site of metabolism to give 3-trifluoromethoxy-6-aminophenol. The last eluting fluorine-containing peak (F) in the chromatogram ($t_R = 24.9$ min) was identified as 4-TFMeA itself.

A metabolic pathway for 4-TFMeA and 4-TFMeAc based on the metabolites identified in urine is shown in Fig. 4.

Following identification, the various metabolites were quantified using ^{19}F NMR. For both 4-TFMeA and 4-TFMeAc, the results showed that the major urinary metabolite was the hydroxysulphate of 4-TFMeA. This accounted for $32.3 \pm 5.6\%$ of the dose ($\approx 46.7\%$ of the total excreted in the urine) for 4-TFMeA and $29.9 \pm 1.8\%$ ($\approx 59.9\%$ of the total excreted in the urine) for the acetanilide (Tables 1 and 2).

The 48 h collection period appeared to be adequate for the 4-TFMeAc metabolites.

However, from the recovery profile for the sulphate metabolite following dosing of 4-TFMeA, a longer sampling time is likely to have yielded a higher recovery. Similarly, higher recoveries would have been produced for the glucuronide conjugate and the 4-TFMeAc metabolite of 4-TFMeA.

We have previously used [^{13}C]-labelling of the acetyl moiety in acetanilides to determine the extent of futile acetylation (i.e. the sequential removal of the administered acetyl group and its subsequent replacement with an endogenous acetyl) in these compounds (e.g. see Refs. [12–14]). However, in the case of 4-TFMeAc, the dominant metabolic pathway was simply *N*-deacetylation to 4-TFMeA itself. Thereafter, the qualitative metabolic fate of the two compounds, as revealed by the metabolites excreted in the urine, was identical, with *O*-sulphation dominating the urinary metabolite profile and *N*- and *O*-glucuronidation accounting for the bulk of the remainder. Interestingly, we found no indication

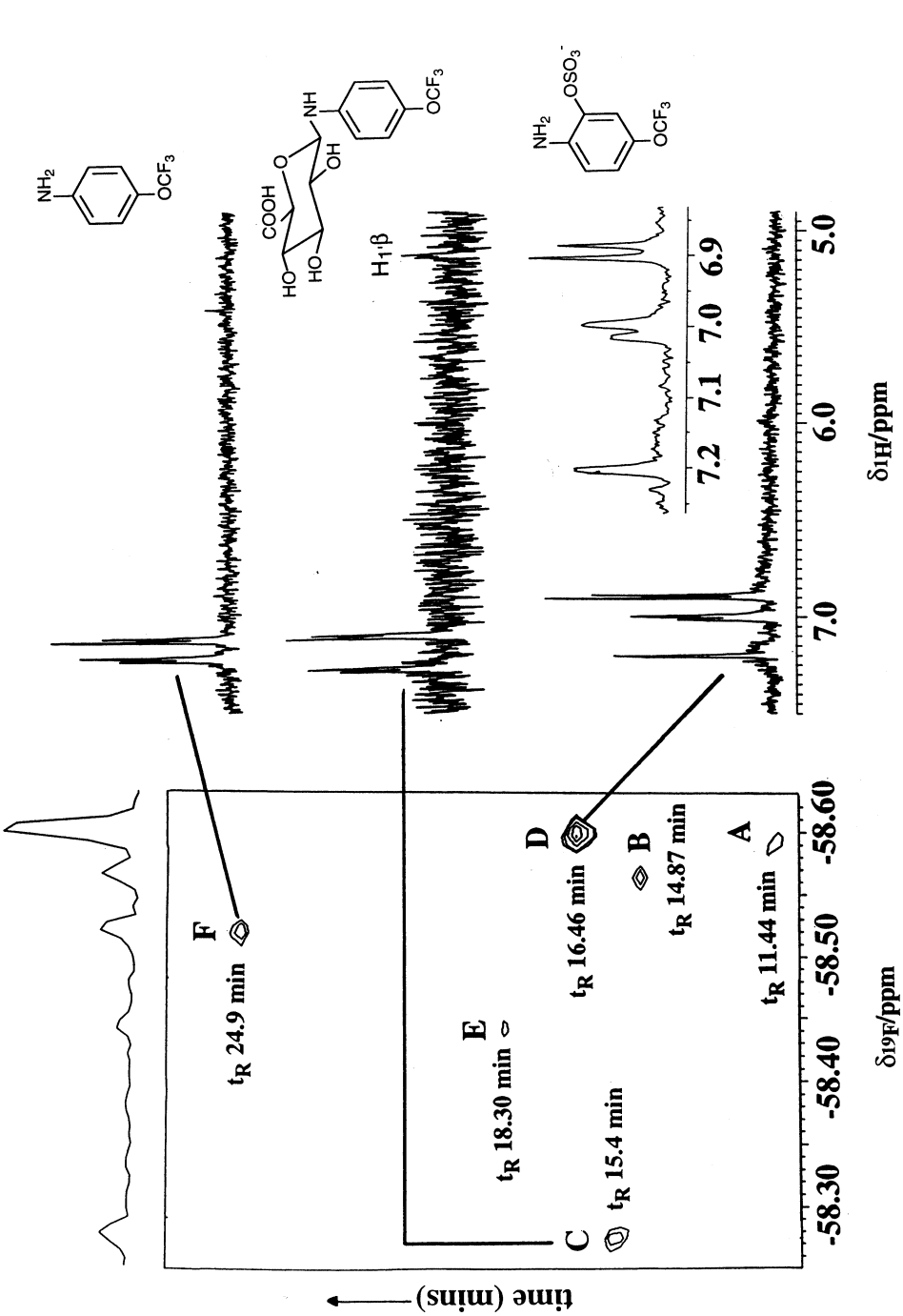

Fig. 3. The pseudo-two-dimensional contour plot of a continuous-flow 470 MHz ^{19}F NMR detected HPLC separation of the metabolites contained in the concentrated 0–8 h urine from rats treated with 4-TFMeA. (Twenty-four scans per experiment, trifluoroacetic acid $\delta_{^{19}F}$ −76.8 as external chemical shift reference.) The corresponding 500 MHz ^{1}H NMR spectra acquired in the stop-flow mode are also shown. $H_1\beta$ represents the β-anomeric proton of glucuronic acid. (96–4000 scans, exponential line-broadening of 1 Hz, ACN $\delta_{^{1}H}$ 2.0 as internal chemical shift reference.)

of the production of paracetamol (*p*-hydroxyacetanilide) in this study, either via the production of paracetamol metabolites, as seen in studies of 4-fluoraniline metabolism [15] or trifluoromethanol-related peaks. This may indicate that the trifluormethoxy-substituent is metabolically stable. This is in contrast to methacetin (*p*-methoxyacetanilide) and phenacetin (*p*-ethoxyacetanilide), where the groups attached to the 4-hydroxy group are removed by metabolism. If this observation can be confirmed and proves to be a general property of trifluoromethoxy-groups, such substituents may prove to be of value in drug design by providing a means of blocking unwanted metabolism.

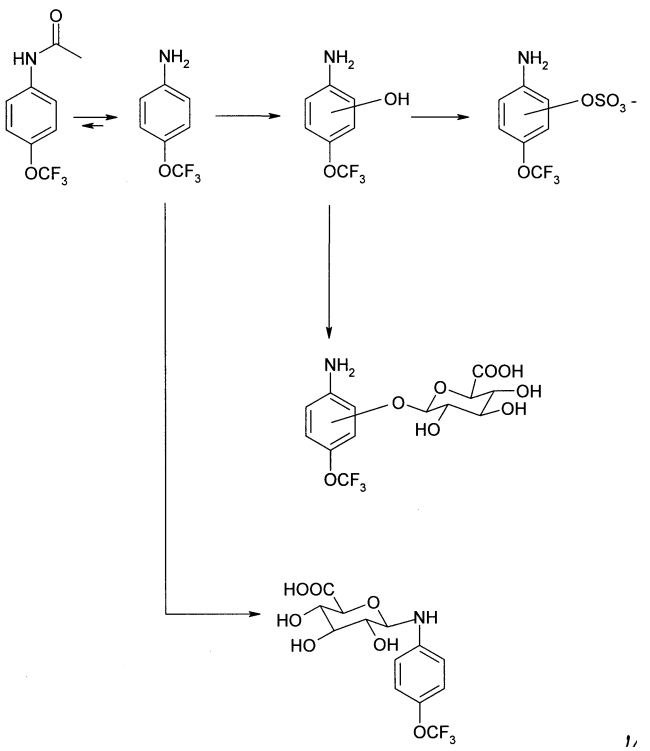

Fig. 4. The putative metabolic pathway for 4-TFMeA and 4-TFMeAc from analysis of urine.

Table 1
Percentage of the dose of 4-TFMeA (50 mg/kg, i.p.) recovered in the urine of male rats ($n = 3$, $\pm$S.D.)

Metabolite	% 0–8 hpd	% 8–24 hpd	% 24–48 hpd	Total 0–48 hpd
4-TFMeA	4.4 ± 2.1	3.8 ± 1.3	0.0	8.2 ± 2.5
4-TFMeAc	0.2 ± 0.1	0.8 ± 0.3	1.7 ± 0.4	2.7 ± 0.51
4-TFMeA-*N*-glucuronide	1.9 ± 1.0	4.1 ± 0.3	1.5 ± 0.8	7.5 ± 1.3
Unstable unknown	5.1 ± 2.6	2.8 ± 0.8	0.0	7.9 ± 2.7
Trifluoromethoxyaminophenyl glucuronide	1.0 ± 0.4	0.9 ± 0.2	0.0	1.9 ± 0.5
Trifluoromethoxyaminophenylsulphate	9.2 ± 3.1	15.7 ± 3.3	7.4 ± 3.3	32.3 ± 5.6
minor unknowns	3.6 ± 1.2	4.8 ± 1.9	0.6 ± 0.5	9.0 ± 2.3
Total ($n = 3$)	25.4 ± 4.8	32.9 ± 4.1	11.2 ± 3.5	69.5 ± 7.2

Table 2
Percentage of the dose of 4-TFMeAc (50 mg/kg, i.p.) recovered in the urine of male rats ($n = 3$, $\pm$S.D.)

Metabolite	%0–8 hpd	%8–24 hpd	%24–48 hpd	Total 0–48 hpd
4-TFMeA	0.8 ± 0.2	0.0	0.0	0.8 ± 0.2
4-TFMeAc	0.2 ± 0.2	3.0 ± 0.3	0.3 ± 0.3	3.5 ± 0.5
4-TFMeA-*N*-glucuronide	6.5 ± 0.6	2.9 ± 0.6	0.0	9.4 ± 0.9
Unstable unknown	3.2 ± 0.2	0.5 ± 0.3	0.0	3.7 ± 0.4
Trifluoromethoxyaminophenylglucuronide	0.6 ± 0.2	0.0	0.0	0.6 ± 0.2
Trifluoromethoxyaminophenyl sulphate	21.9 ± 1.6	7.2 ± 0.6	0.8 ± 0.4	29.9 ± 1.8
Minor unknowns	1.0 ± 0.4	1.1 ± 0.7	0.0	2.2 ± 0.8
Total ($n = 3$)	34.2 ± 1.8	14.7 ± 1.2	1.1 ± 0.5	50.0 ± 2.2

The significant amount of 4-TFMeA detected in the urine ($8.2 \pm 2.5\%$ for TFMeA) may reflect the decomposition of unstable metabolites rather than excretion unchanged; the presence of an as yet unidentified, unstable metabolite that spontaneously decomposed to 4-TFMeA is consistent with this notion. As discussed in Section 1, *para*-substitution of anilines can, under some circumstances, result in the production of unusual *N*-glycolanilides and oxanilic acids [2–8] via the oxidation of *N*-acetylated metabolites. Indeed, in the case of 4-trifluormethylaniline, the *N*-oxanilic acid is the major metabolite [7,8]. However, the formation of such metabolites does not appear to be a feature of the metabolism of 4-TFMeA, which is consistent with the rapid deacetylation of the acetanilide.

The data in this study have focussed only on urine. Assuming that absorption of the compounds was complete following i.p. dosing, the incomplete total urinary recoveries ($\approx 50\%$ for 4-TFMeAc and 69% for 4-TFMeA related material) suggest that a proportion of the dose for both compounds may be secreted in bile. It has to be accepted that the profile of the metabolites secreted in bile may not be the same as that in urine.

4. Conclusions

The overall urinary metabolic profiles of both the parent aniline and its acetanilide were the same, with the major metabolite for both 4-TFMeA and 4-TFMeAc identified as a phenolic sulphate. *N*-acetylation did not seem to be a major pathway for the metabolism of 4-TFMeA compared to the production of the phenolic sulphate and this is probably explained by the fact that *N*-deacetylation was the major route of metabolism of the acetanilide. The trifluoromethoxy-group seems to be comparatively stable, as no paracetamol-related metabolites were observed. Unlike 4-trifluoromethylaniline and other 4-substituted anilines, the formation of *N*-oxanilic acids did not appear to be a major route of metabolism.

Acknowledgements

We would like to thank the British Council for the Nehru Centenary British Council Fellowship and the EPSRC for financial support to M. Tugnait and E. Lenz, respectively.

References

[1] J.L. Radomski, Trends Pharmacol. Toxicol. 19 (1979) 129–157.
[2] M. Kiese, W. Lenk, Biochem. Pharmacol. 18 (1969) 1325–1333.
[3] M. Kiese, W. Lenk, Biochem. Biophys. Acta 222 (1970) 549–551.
[4] M. Kiese, W. Lenk, Biochem. Pharmacol. 20 (1971) 379–391.
[5] W.J. Ehlhardt, J.J. Howbert, Drug Met. Disp. 19 (1991) 366–369.

[6] G.B. Scarfe, J.K. Nicholson, J.C. Lindon, I.D. Wilson, S. Taylor, E. Clayton, B. Wright, Xenobiotica (2001) (in press).
[7] I.D. Wilson, C.M. MacDonald, J.M. Fromson, J.A. Troke, D. Hillbeck, Biochem. Pharmacol. 34 (1985) 2025–2028.
[8] W.E. Wade, J. Troke, C.M. MacDonald, I.D. Wilson, J.K. Nicholson, in: E. Reid, J.D Robinson, I.D. Wilson (Eds.), Methodological Surveys in Biochemistry and Analysis 18, Plenum, New York, 1988, pp. 383–388.
[9] J. Booth, E. Boyland, Biochem. J. 91 (1964) 362–669.
[10] L.A. Sternson, R.E. Gammans, Bioinorg. Chem. 4 (1974) 58–63.
[11] H.H.P. Cnubben, J. Vervoort, C. Veeger, I.M.C.M. Rietjens, Chemico-Biol. Interact. 85 (1992) 151–172.
[12] A.W. Nicholls, R.D. Farrant, J.P. Shockcor, S.E. Unger, I.D. Wilson, J.C. Lindon, J.K. Nicholson, Biochem. Pharmacol. 49 (1997a) 1155–1164.
[13] A.W. Nicholls, J.C. Lindon, S. Caddick, R.D. Farrant, I.D. Wilson, J.K. Nicholson, Xenobiotica 27 (1997b) 1175–1186.
[14] A.W. Nicholls, J.C. Lindon, R.D. Farrant, J.P. Shockor, I.D. Wilson, J.K. Nicholson, J. Pharm. Biomed. Anal. 20 (1999) 865–873.
[15] G.B. Scarfe, M. Tugnait, I.D. Wilson, J.K. Nicholson, Xenobiotica 29 (1999) 205–216.