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Metabolism and Disposition of Fluticasone Furoate, an Enhanced-Affinity Glucocorticoid, in Humans

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ABSTRACT:

The purpose of this study was to investigate the metabolism and disposition of fluticasone furoate, an enhanced-affinity glucocorticoid receptor agonist, in humans. In a two-part, open-label design study, five healthy male subjects received a p.o. dose of 2 mg of [^{14}C]fluticasone furoate, followed 4 weeks later by an i.v. dose of 0.25 mg of [^{14}C]fluticasone furoate (as a 30-min infusion). Oral absorption was rapid and estimated at approximately 30%, although the oral bioavailability was markedly lower at 1.6%, limited by extensive first-pass metabolism. Plasma clearance was 58.3 l/h, with a volume of distribution of 642 liters and a terminal elimination half-life of 15.3 h. The major circulating component identified in plasma extracts after i.v. and p.o. dosing was unchanged parent compound, with 17β-carboxylic acid (GW694301X; M10) also being notable after p.o. administration. Mean recovery of radioactivity was approximately 92 and 102% at 216 and 168 h after i.v. and p.o. administration, respectively, with most (at least 90%) recovered in the feces. Fluticasone furoate was extensively metabolized, with only trace amounts of unchanged parent compound observed in feces following either route of administration. The predominant pathway was removal of the *S*-fluoromethyl carbothioate group to yield GW694301X (M10). Other pathways included oxidative defluorination to yield a hydroxyl at the C6 position. There was no evidence for metabolic loss of the furoate group from fluticasone furoate or any of its metabolites. Evidence presented suggests that enterocytes have a role in the metabolism of unabsorbed fluticasone furoate.

Fluticasone furoate [(6α,11β,16α,17α)-6,9-difluoro-17-{[(fluoromethyl)-thio]carbonyl}-11-hydroxy-16-methyl-3-oxoandrosta-1,4-dien-17-yl-2-furancarboxylate] is a new enhanced-affinity glucocorticoid receptor agonist. It is a synthetic fluorinated corticosteroid that has been developed as an intranasal treatment for patients with symptoms of rhinitis. Fluticasone furoate, otherwise known as GW685698X, is not a salt or prodrug because the entire molecule is required for pharmacological activity. It has similar or greater potency than other clinically used corticosteroids (including mometasone furoate, budesonide, fluticasone propionate, and the active principle of ciclesonide) for the glucocorticoid receptor and against the proinflammatory transcription factors nuclear factor κB (NF-κB), activation protein-1, and tumor necrosis factor–induced interleukin-8 cytokine production (Salter et al., 2007). Agonism of the glucocorticoid receptor is known to suppress the activation of downstream transcription factors, such as NF-κB and activation protein-1, and to activate the glucocorticoid response element transactivation pathway (Rhen and Cidlowski, 2005). Inhibition of the NF-κB pathway, in particular, is thought to be intimately involved in the anti-inflammatory activity of glucocorticoids because it is a key pathway in the synthesis of a number of inflammatory cytokines (Karin et al., 2004). It is well documented that topical glucocorticoids interact with many of the inflammatory pathways, and there is a large body of clinical evidence to support their use for the treatment of rhinitis, asthma, and chronic obstructive pulmonary disease (Goodman and Gilman, 2006). The purpose of the present study was to investigate the metabolism and disposition of [^{14}C]fluticasone furoate after p.o. and i.v. administration to healthy male subjects. These dose routes were used as surrogates for the intranasal and inhalation routes, from which the majority of the dose is likely to be swallowed. The p.o. route acted as a surrogate for the portion of an intranasal or inhaled dose that is swallowed, and the i.v. route represented the portion absorbed locally into the systemic circulation.

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Materials and Methods

Materials. Fluticasone furoate, [^{14}C]fluticasone furoate (see Fig. 1), and GW694301X, GSK728920A, GSK728921A, and GSK728922A (authentic chemicals of possible metabolites of fluticasone furoate) were supplied by Chemical Development, GlaxoSmithKline R&D (Stevenage, UK). All of the

ABBREVIATIONS: NF-κB, nuclear factor κB; GW694301X, M10, 17β-carboxylic acid; AMS, accelerator mass spectrometry; HPLC, high-performance liquid chromatography; AUC, area under the curve.

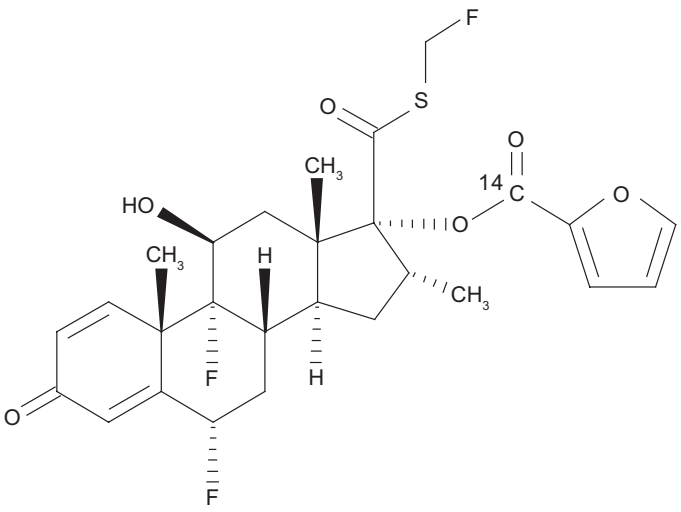


FIG. 1. The structure of fluticasone furoate, with position of ^{14}C shown.

other solvents and reagents were of analytical grade and were purchased from commercial suppliers.

Formulated Drug. [^{14}C]Fluticasone furoate and fluticasone furoate were supplied to the study center by Pharmaceutical Development, GlaxoSmithKline (Ware, UK) as sterile solutions in propylene glycol. The solutions were at a concentration of 0.25 mg/ml and with a specific activity of 100 μCi/mg. For the p.o. dose, the radiolabeled and nonradiolabeled solutions were mixed in a ratio of 1:1.67 to give a dose of 8-ml volume, equivalent to 2 mg of fluticasone furoate and approximately 75 μCi.

Subjects and in Vivo Study Design. The clinical part of the study was conducted at Charles River Laboratories (formerly known as Inveresk) Edinburgh, UK, in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. All the subjects provided written informed consent before participation. The protocol was approved by the investigational center ethics committee and by the Administration of Radioactive Substances Advisory Committee, United Kingdom.

Five healthy male Caucasian subjects, aged 50 to 56 years and with a body mass index within the range of 19 to 30 kg/m^2, were enrolled into this study. The study design was an open-label two-period crossover, with a p.o. administration followed by i.v. administration. The subjects were in good health as shown by medical examination, clinical chemistry, hematology, and urine analysis. The subjects were nonsmokers with no history of drug or alcohol abuse, who were taking no other medication at the time of the study, and had taken no prescribed medication within 14 days of the study commencing.

All the subjects received a single p.o. dose of [^{14}C]fluticasone furoate at 2 mg (3 MBq, 75 μCi) and a dose volume of 8 ml. On a separate occasion, each subject received an i.v. infusion of 0.25 mg of [^{14}C]fluticasone furoate (1 MBq, 25 μCi) over 30 min using a dose volume of 1 ml. The two dosing occasions were separated by at least 28 days. Blood samples were collected via an indwelling cannula or by direct venipuncture into lithium heparin-containing polypropylene tubes.

Following p.o. dosing, blood samples (10 ml) were collected at 10, 15, 30, and 45 min and 1, 1.5, 2, 3, 5, 9, 12, 24, 48, 72, 96, and 168 h after dosing, with larger samples (30 ml) for metabolic investigations being collected at 0.5, 2, and 12 h. After the start of the i.v. infusion, samples (10 ml) were collected at 15, 30, 32, 35, and 45 min and 1, 1.5, 2, 3, 4, 6, 9, 12, 24, 48, 72, 96, and 168 h after dosing, with the larger samples being collected at 0.75, 1.5, and 12 h. The samples were processed within 1 h of collection by centrifugation for 10 min at 1500*g* at nominally 4°C to yield plasma, which was then stored frozen at nominally −20°C or processed for the determination of radioactivity levels in plasma. Urine samples were collected at 0 to 6, 6 to 12, and 12 to 24 h and then at 24-h intervals up to at least 168 h. Fecal samples were collected over 24-h periods up to at least 168 h postdose. Both urine and fecal collections continued beyond the 168-h time point following i.v. administration until 90% of the total radioactive dose administered was recovered from all the matrices, or less than 1% of the radioactive dose had been excreted over a 24-h period. The fecal and urine samples were stored frozen at nominally −20°C before analysis.

Radioassay of Samples. After measurement of the total sample volume or weight (as appropriate) of excreta, the levels of radioactivity in samples were determined by liquid scintillation counting (Beckman LS series, Bucks, UK or PerkinElmer Life and Analytical Sciences, Beaconsfield, Bucks, UK) with quench correction performed by an automatic external standard ratio method, which was established using sealed ^{14}C standards. Aliquots of liquid samples (e.g., urine, dose dilutions) or extracts of samples were mixed with scintillation fluid. Fecal samples were homogenized with an appropriate amount of water. Aliquots of homogenized fecal material were combusted using a model 307 oxidizer (PerkinElmer Life and Analytical Sciences, Waltham, MA) before radioassay by scintillation counting. The plasma had low levels of radioactivity; therefore, these samples were diluted appropriately with water, and the level of radioactivity was determined using accelerator mass spectrometry (AMS).

Determination of Radiochemical Purity. The radiochemical purity of [^{14}C]fluticasone furoate was confirmed by high-performance liquid chromatography (HPLC). The chromatographic instrument used consisted of an HP1100 series quaternary pump, column oven (50°C), UV detector (λ 245 nm), and autosampler using a Zorbax SB-C8 column (150 × 4.6 mm, 3.5-μm particle size). The mobile phase consisted of aqueous 0.1% trifluoroacetic acid (solvent A) and a mixture of acetonitrile/methanol (75:25, solvent B) at a flow rate of 1.5 ml/min. A gradient was used, starting at 60% A with a linear change to 80% B over 30 min. The column was re-equilibrated following each injection. Using on-line radiodetection, the column eluate was combined with scintillant (Ultima-Flo M supplied by Fluorochem, Old Glossop, Derbyshire, UK; 4.5 ml/min) before detection using a Radiomatic Flo-one 150TR radioactivity monitor (PerkinElmer Life and Analytical Sciences). Dose analysis showed that all the p.o. and i.v. doses had a radiochemical purity of 100%.

Quantification of Fluticasone Furoate and GW694301X in Plasma. Concentrations of fluticasone furoate and GW694301X (M10) in plasma samples were determined using a validated analytical method using in-line solid-phase extraction, followed by HPLC/tandem mass spectrometry. The validation of this method was performed as detailed for the quantification of fluticasone furoate and GW694301X (Scott et al., 2007) using similar methodology.

[$^{13}C_3$]CCI18781 (fluticasone propionate) and GW819063 (10 μl in 20% acetonitrile in water with both at a concentration of 10 ng/ml) were added to plasma samples (0.15 ml) as internal standards. An aliquot of each sample (0.1 ml) was applied to conditioned Prospect C2 cartridges (10 × 1 mm), which were washed with 5% aqueous methanol containing 0.025% formic acid (2 ml) before being eluted in-line for 30 s onto the analytical column. The cartridge eluant was applied to a HyPurity C18 column (100 × 4.6 mm, 5 μM; Thermo Fisher Scientific, Runcorn, Cheshire, UK) eluted at a flow rate of 0.5 ml/min using a linear gradient from 100% solvent A to 100% solvent B over 4.3 min, held until 5.15 min before returning to 100% solvent A by 6 min. Solvent A was a mixture of methanol and 10 mM ammonium formate (pH 5, 77.5:22.5, v/v), and solvent B was acetonitrile. The column eluant was introduced into a TurboIonSpray source of a Sciex API 4000 mass spectrometer (Applied Biosystems, Warrington, Cheshire, UK) operated in the most sensitive ionization mode for each analyte, with the monitored fragmentation ions allowing quantification at the required sensitivity. The system was run in negative ion mode initially for 4.4 min before being switched to positive ion mode. The temperature of the probe was maintained at 600°C, with a curtain gas setting of 25 and collision gas setting of 6. GW694301X and GW819063 were monitored in negative ion mode by multiple reaction monitoring of 489→111 and 520→142, respectively. Fluticasone furoate and [$^{13}C_3$]CCI18781 were monitored in positive ion mode by multiple reaction monitoring of 539→313 and 504→313, respectively. The appropriate mass adjustments were performed to monitor for radiolabeled material. The dynamic range of the assay for all the analytes was from 10 to 1000 pg/ml.

Determination of Metabolite Profiles. Representative fecal samples from each subject were obtained by pooling across sampling times on a total sample weight basis to generate sample pools where possible containing 90% or greater of the radioactivity excreted in the feces. Plasma samples from individual subjects were pooled using equal volumes to produce a single representative sample per time point. No further analysis of urine samples was conducted because of the

limited amount of radioactive drug-related material in urine. Radioactive material was extracted from plasma samples using acetonitrile (5 ml/ml sample, rotary mixed at ambient temperature for approximately 1 h), three times with the supernatants being combined. The extracts were then evaporated to near dryness under a stream of nitrogen before being reconstituted in deionized water (0.5 or 1 ml). Radioactive material was extracted from fecal samples using alkaline and acidified methanol as described for plasma, and the reconstituted extracts from each extraction method were combined using equal volumes before analysis by radio-HPLC. Radiometabolite profiles were determined by analysis of appropriate aliquots of plasma and fecal extracts by radio-HPLC using on-line or off-line radiodetection.

HPLC method 1 (used for analysis of all the matrices). The chromatographic instrument used consisted of an Agilent (South Queensferry, Scotland, UK) 1100 binary pump, column oven (40°C), UV detector (λ 245 nm), and autosampler (CTC Analytics or LC PAL injector, both Presearch, Basingstoke, Hampshire, UK) using a ThermoHypersil Fluophase RP column (250 × 4.6 mm, 5-μm particle size). The mobile phase consisted of 50 mM ammonium acetate (BDH; Poole, Dorset, UK) (pH unadjusted) (solvent A) and acetonitrile (solvent B, supplied by Thermo Fisher Scientific, Loughborough, Leicestershire, UK) at a flow rate of 1 ml/min. A gradient was used, starting at 100% A with a linear change to 25% B over 30 min, followed by a linear increase to 40% B by 40 min and then a further increase to 100% by 45 min, with these conditions being held for a further 5 min. The column was re-equilibrated after each injection. Using on-line radiodetection, the column eluate was combined with scintillant (FlowLogic MaxCount supplied by Lablogic, Sheffield, UK; 3 ml/min) before detection using a β-Ram (Lablogic) radioactivity monitor. For off-line radiodetection, fractions were collected using a Gilson 222XL fraction collector (supplied by Gilson, Villiers, France) on 4 × 96 Deepwell LumaPlates containing yttrium silicate solid scintillant (PerkinElmer Life and Analytical Sciences) or into standard 96-well plates. Radioactivity determination was performed either by scintillation counting (TopCount MXT counter; PerkinElmer Life and Analytical Sciences) or by AMS analysis of the fractions. HPLC column recoveries were determined on selected samples by collecting the total HPLC column eluate for the appropriate run and assaying the radioactivity to assess recovery of injected radioactivity. Full recoveries of radioactivity were obtained from the HPLC eluant collected.

HPLC method 2 (used for analysis of human plasma samples). The chromatographic instrument is as listed for method 1, with the exception of a different column being used: a ThermoHypersil Gold column (250 × 4.6 mm, 5-μm particle size; Thermo Fisher Scientific, Waltham, MA). The mobile phase consisted of 50 mM ammonium acetate, pH 5, adjusted using acetic acid (supplied by Thermo Fisher Scientific) (solvent A) and acetonitrile (solvent B) at a flow rate of 1 ml/min. A gradient was used, starting at 25% B with a linear increase to 40% B over 8 min, followed by a linear increase to 45% B by 12 min and then a further increase to 75% by 13 min, held at these conditions for 2 min before increasing to 90% by 18 min. The column was washed and re-equilibrated after each injection.

Identification of Metabolites. Structural characterization was performed on selected samples using HPLC/mass spectrometry using Quattro Micro triple quadrupole (Micromass, Manchester, UK), LCT time of flight (Micromass), and Deca XP^{plus} ion trap (Thermo Fisher Scientific) mass spectrometers. Electrospray ionization, in positive or negative modes, was used. The HPLC flow was split (1:25–1:50) between the mass spectrometer and on-line or off-line radiodetector. Metabolites of fluticasone furoate were identified based on charged molecular ions and their collision-induced disassociation fragmentation. Authentic standards, where available, were used to compare chromatographic retention time to metabolites that were structurally characterized. Supporting data obtained from metabolites identified from preclinical species or from in vitro incubations with human-derived tissue preparations were also used in the assignment of metabolite structures.

AMS. The ^{14}C content of human plasma, plasma extracts, and HPLC fractions was measured by AMS, which measures the radiocarbon content in a sample through separation of the isotopes of carbon present by their different mass-to-charge ratios. Before AMS analysis, the samples were graphitized via a two-step process of oxidation and reduction (Vogel, 1992). The graphite, containing a cobalt catalyst, was packed into an aluminum cathode and loaded into a sample wheel that was then placed into the ion source of the AMS instrument (NEC 15SDH-2 Pelletron AMS system, Middleton, WI). A generic value of 4.14%, based on historical data, for the carbon content of plasma was used for any neat plasma samples analyzed, and values were adjusted appropriately to allow for any dilution. The carbon content for the HPLC fractions was deemed to be insignificant, and only the carbon content of the liquid paraffin carrier (84.17%) was used in calculations to determine the ^{14}C content based on the determined $^{14}C/^{12}C$ ratio in the samples. The AMS data, which are expressed as percent modern carbon, were used to calculate the disintegrations per minute per milliliter of sample, where 100% modern carbon equals 0.01356 dpm/mg carbon.

Pharmacokinetic Calculations. Pharmacokinetic parameters were calculated by noncompartmental methods using WinNonlin Professional version 4.1 (Pharsight, Mountain View, CA). Area under the curve, $AUC_{(0\text{-}t)}$, was calculated using the log-linear trapezoidal method and extrapolated to infinity according to $AUC_{(0\text{-}\infty)} = Ct \cdot t_{1/2}/\ln 2 + AUC_{(0\text{-}t)}$, where Ct is the last observed concentration. The $t_{1/2}$ values were obtained by the ratio of $\ln 2/\lambda_z$, where λ_z was the terminal phase rate constant estimated by linear regression analysis of the log-transformed concentration-time data during the terminal elimination phase. Mean residence time was calculated based on the last measurable concentration. Plasma clearance and volume of distribution at steady state were calculated following i.v. administration only. Oral bioavailability was calculated using the dose-normalized $AUC_{(0\text{-}\infty)}$ p.o./dose-normalized $AUC_{(0\text{-}\infty)}$ i.v.

Results

Demographic, Safety, and Tolerability Data from Humans. Five healthy male Caucasian subjects were enrolled and completed the study. The subjects were of a mean age of 54 years (range 50–56 years), a height of 169 cm (range 163–181 cm), and weight of 79.2 kg (range 71.2–93.0 kg) with a mean body mass index of 27.6 (range 25.1–30.1). Fluticasone furoate was well tolerated, with no drug-related adverse events or clinically significant changes in vital signs observed. No concomitant medication was reported for any subject during the study.

Pharmacokinetics of Fluticasone Furoate and GW694301X. Pharmacokinetic data for fluticasone furoate, its carboxylic acid metabolite (GW694301X, M10), and total radioactivity are listed in Table 1. After p.o. administration of [^{14}C]fluticasone furoate at a dose of 2 mg, maximum mean concentrations (C_{max}) of fluticasone furoate occurred within 30 min (T_{max}) of administration, indicating rapid oral absorption of the dose. Concentrations declined rapidly thereafter, such that they were below the limit of quantification beyond 9 h (Fig. 2). After a 30-min i.v. infusion of [^{14}C]fluticasone furoate at a dose of 0.25 mg, plasma clearance was 58.3 l/h with a mean volume of distribution at steady state of 642 liters. In contrast to post-p.o. administration, concentrations of fluticasone furoate were measurable up to 24 h postinfusion, and mean terminal half-life was estimated at 15.3 h. Mean concentrations of GW694301X peaked at 0.75 h post-p.o. dose of fluticasone furoate and declined rapidly thereafter, with a systemic exposure (based on either $AUC_{(0\text{-}\infty)}$ or $AUC_{(0\text{-}t)}$) approximately 5- to 9-fold lower than for fluticasone furoate. No GW694301X was quantifiable in plasma after i.v. administration of fluticasone furoate. Comparison of the $AUC_{(0\text{-}t)}$ values for radioactivity following p.o. and i.v. administration (normalizing for dose) indicates that oral absorption was approximately 30% (range 18–37%). The mean bioavailability of fluticasone furoate from the oral solution (comparing p.o. and i.v. $AUC_{(0\text{-}t)}$ values for fluticasone furoate and normalizing for dose) was considerably lower at 1.6% (range 0.5–2.8%). The $AUC_{(0\text{-}\infty)}$ for total radioactivity in plasma was approximately 36- and 5-fold higher than that for fluticasone furoate following p.o. or i.v. administration, respectively. Furthermore, the mean half-life of total radioactivity (at 35.6 h) was notably longer than that of fluticasone furoate (15.3 h) after i.v. dosing.

Excretion of [^{14}C]Fluticasone Furoate Drug-Related Material. The mean total recovery of radioactive drug-related material in excreta collected after i.v. administration of [^{14}C]fluticasone furoate to

TABLE 1

Pharmacokinetic parameters in humans for fluticasone furoate, GW694301X, and ^{14}C radioactivity in plasma

Mean pharmacokinetic parameters of fluticasone furoate, a carboxylic acid metabolite GW694301X, and ^{14}C radioactivity in plasma of healthy male subjects following a single p.o. (2 mg) or a single i.v. (0.25 mg) administration of [^{14}C]fluticasone furoate are shown. Mean ± S.D., $n = 5$.

Treatment	Parameter	Fluticasone Furoate	GW694301X	Radioactivity[a]
2 mg p.o.	$AUC_{(0\text{-}t)}$ (pg · h/ml)	511 ± 358	102 ± 61	26,400 ± 14,400
	$AUC_{(0\text{-}\infty)}$ (pg · h/ml)	1080[b]	120 ± 59	39,300 ± 11,700[c]
	C_{max} (pg/ml)	213 ± 177	71 ± 42	1380 ± 239
	T_{max} (h)[d]	0.50	0.75	0.75
	$t_{½}$ (h)	4.05[b]	1.04 ± 0.39	20.8 ± 5.30[c]
0.25 mg i.v.	$AUC_{(0\text{-}t)}$ (pg · h/ml)	4000 ± 846	N.D.	11,300 ± 5150
	$AUC_{(0\text{-}\infty)}$ (pg · h/ml)	4410 ± 834	N.D.	21,400 ± 15,200[e]
	C_{max} (pg/ml)	2980 ± 1140	N.D.	5060 ± 1330
	T_{max} (h)[d]	0.50	N.D.	0.50
	$t_{½}$ (h)	15.3 ± 2.74	N.D.	35.6 ± 33.3[e]
	Cl (l/h)	58.3 ± 10.9	N.D.	N.A.
	Vss (liter)	642 ± 215	N.D.	N.A.

N.D., not determined, all the concentrations below lower limit of quantification (10 pg/ml); N.A., not applicable.
[a] Radioactivity concentrations are expressed in terms of picogram equivalents of fluticasone furoate.
[b] $n = 2$, because of inadequate definition of the terminal elimination phase these pharmacokinetic parameters could not be defined in all the subjects.
[c] $n = 3$, because of inadequate definition of the terminal elimination phase these pharmacokinetic parameters could not be defined in all the subjects.
[d] T_{max} quoted as median.
[e] $n = 4$, because of inadequate definition of the terminal elimination phase these pharmacokinetic parameters could not be defined in all the subjects.

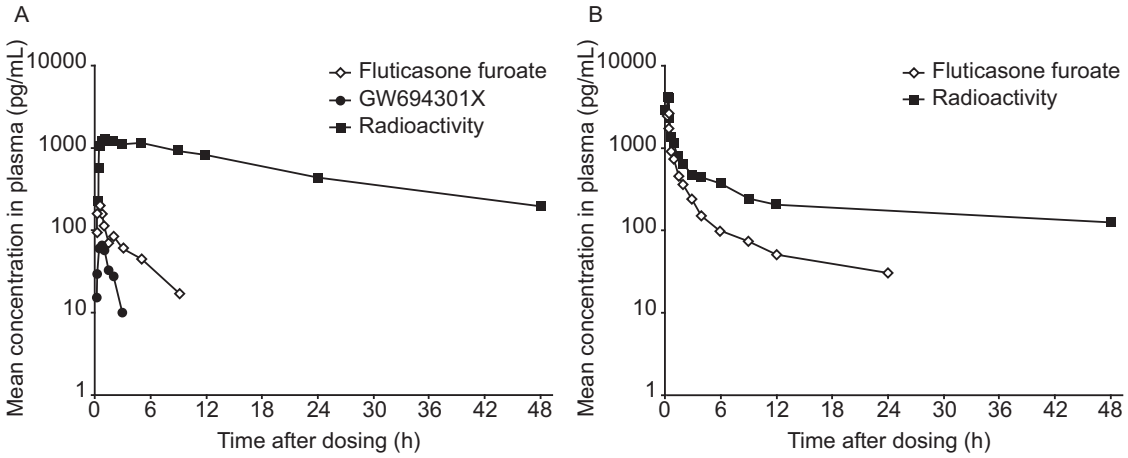


FIG. 2. Mean plasma concentrations of fluticasone furoate, GW694301X, and total radioactivity as indicated on semilog plots following a p.o. administration (A) or i.v. infusion over 30 min (B) of [^{14}C]fluticasone furoate to healthy male subjects ($n = 5$).

humans was high (92%) and was complete post-p.o. administration, following collections up to 240 and 168 h, respectively (Table 2). Most of the dose was excreted in the feces for both routes of administration; urinary excretion was a very minor route (<3%). Radioactivity was eliminated rapidly: most was excreted within the first 72 h postdose, with approximately 71 and 91% of the dose recovered over that period following i.v. and p.o. administration, respectively.

Metabolite Profiles in Plasma and Feces. Proposed structure and spectral data supporting metabolite identification are shown in Table 3. Radioactive drug-related material in plasma is expressed as percentage of sample radioactivity. Radioactive drug-related material in feces is expressed as percentage of administered dose. Where the sample preparation step resulted in some loss of radioactivity, if the recovery was <90%, the chromatogram data have been multiplied by the percentage recovered to calculate percentage of sample radioactivity and percentage of dose.

Metabolites of [^{14}C]Fluticasone Furoate in Plasma. It was established that [^{14}C]fluticasone furoate spiked into plasma was stable when stored under the same conditions as the test samples. Low levels of radioactivity were present in the human plasma extracts; therefore, the profiling of these samples was performed using AMS as the radioactivity detection method. Representative reconstructed HPLC radiochromatograms of plasma extracts following i.v. and p.o. administration, at selected sampling times, are shown in Fig. 3. The extraction efficiency of radioactivity from plasma was in excess of 70% at early time points but notably lower (9–35%) at later time points.

The principal radiolabeled component in plasma following a single i.v. infusion of [^{14}C]fluticasone furoate was unchanged fluticasone furoate (P), which accounted for at least 52% of radioactive material in plasma samples up to 1.5 h after dosing. The 17β-carboxylic acid metabolite (GW694301X, M10) of fluticasone furoate was assigned based on retention time and represented up to 4% of plasma radioactivity. Three other minor components were also observed, each representing less than 2% of plasma radioactivity.

After p.o. administration, M10 and fluticasone furoate were the major components in plasma, comprising 28 and 17% of sample radioactivity, respectively, at 0.5 h, and 6 and 7%, respectively, at 2 h post-p.o. administration. Metabolites M21 (6-hydroxyl of parent), M26 (6-hydroxyl of M10), and M32 (a hydroxylated product of M10) were observed in human plasma based on their coelution with either authentic standards or identified metabolites from in vitro incubations; all accounted for less than 4% of plasma radioactivity. The proposed structures and supporting spectral data for the metabolites are shown in Table 3.

TABLE 2

Mean percentage cumulative recovery of radioactivity following p.o. or i.v. administration of [^{14}C]fluticasone furoate to humans

	p.o. Administration (2 mg)			i.v. Administration (0.25 mg)		
Time	Feces	Urine	Total	Feces	Urine	Total
h						
0–6	N.A.	0.49	0.49	N.A.	1.27	1.27
6–12	N.A.	0.69	0.69	N.A.	1.59	1.59
0–24	5.03	0.82	5.85	2.26	1.84	4.10
0–48	52.52	0.92	53.44	32.57	2.14	34.71
0–72	90.44	0.97	91.41	69.06	2.31	71.37
0–96	94.66	0.98	95.64	78.78	2.46	81.24
0–120	100.38	0.99	101.37	82.45	2.50	84.95
0–144	100.96	1.00	101.96	86.40	2.54	88.94
0–168	101.15	1.00	102.15	87.96	2.57	90.53
0–240	N.A.	N.A.	N.A.	89.78	2.61	92.39

N.A., not applicable.

Metabolites of [^{14}C]Fluticasone Furoate in Feces. It was established that [^{14}C]fluticasone furoate spiked into feces was stable when stored under the same conditions as the test samples. The mean extraction efficiency of radioactivity from the fecal samples was 90% (p.o.) and 84% (i.v.). The extraction efficiency of radioactivity from control human fecal samples spiked with [^{14}C]fluticasone furoate was 95%. Representative HPLC radiochromatograms of human fecal extracts following either i.v. or p.o. administration are shown in Fig. 3.

The predominant identified drug-related component in the fecal extracts from four of five subjects following dosing by either route was M10, representing 32 to 40% of the p.o. dose and 24 to 31% of the i.v. dose. However, in one subject, M10 was a minor component of the total drug-related material excreted in the feces. Other drug-related components assigned by cochromatography with authentic standards were the 6-hydroxy metabolite of M10 (M26) and parent compound, each representing less than 8% of a p.o. or i.v. dose.

Although most of the dose was excreted in feces, the absolute amounts of drug-related material present in the fecal samples were very low. A number of drug-related components remained insensitive to detection by mass spectrometry, and their structures were not elucidated.

Discussion

Fluticasone furoate, administered as an intranasal aqueous suspension via a unique side-actuated device, is a new chemical entity for the treatment of nasal and ocular symptoms of allergic rhinitis (Martin et al., 2007). As such, it is important to gain an understanding of the metabolism and disposition of fluticasone furoate in humans.

The pharmacokinetic profile of fluticasone furoate following i.v. administration to humans is characterized by a high plasma clearance at 58.3 l/h, which is approximately two thirds of human liver blood flow (Davies and Morris, 1993), and a very large volume of distribution of 642 liters, which equates to approximately 15 times total body water (Davies and Morris, 1993), indicating extensive tissue uptake. These properties in combination resulted in a moderate plasma half-life of 15.3 h. In contrast, the plasma half-life observed following p.o. administration was shorter, at approximately 4 h; however, this is likely to be representative of the distribution phase, with plasma concentrations falling below the assay limit of quantification before the true elimination phase was observed. This is consistent with a large first-pass effect resulting in a very low mean oral bioavailability, calculated at 1.6%. The absolute bioavailability of intranasally administered fluticasone furoate in healthy volunteers has been determined to be 0.5% (Allen et al., 2007). The lower bioavailability observed with the clinical intranasal formulation is likely as a result of differences in dose, dose route, and formulation compared with those used in this study.

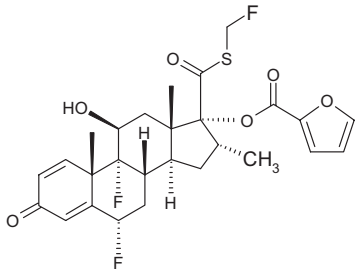
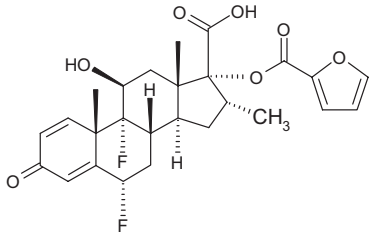
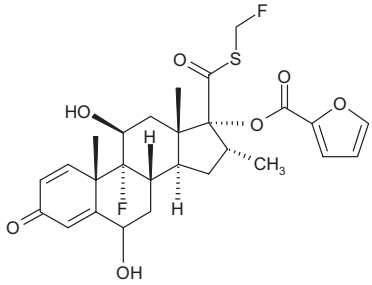
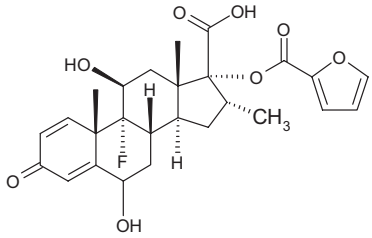
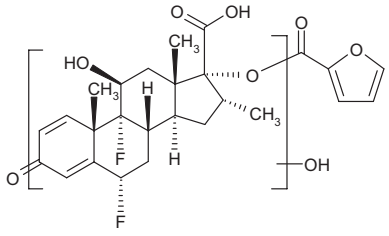
Systemic exposure to radioactivity was much greater than systemic exposure to fluticasone furoate for both routes of administration, with the observed difference being greater following p.o. administration. In addition, the elimination half-life of radioactivity was greater than that of fluticasone furoate. These observations are consistent with the presence of one or more circulating metabolites with a longer elimination half-life than parent, and of extensive first-pass metabolism after p.o. administration. Oral absorption was estimated to be only moderate (approximately 30%), despite the high tissue permeability suggested by the high volume of distribution. The estimate of oral absorption (obtained by comparison of the $AUC_{(0\text{-}t)}$ values for total plasma radioactivity following p.o. and i.v. administration) may be an overestimate because the profiles of drug-related material following the two routes of administration differ; however, it is consistent with the ratios of urinary excretion, although these are based on very low values. A similar extent of absorption was also observed in rats and dogs (S. Hughes, unpublished data), where 29 and 19% (30% of the recovered radioactivity) of a p.o. radioactive dose was recovered in the bile and urine of bile duct–cannulated rats and a dog, respectively.

The principal component in plasma extracts following i.v. administration of [^{14}C]fluticasone furoate (0.25 mg) to healthy male subjects was fluticasone furoate, with low levels of the 17β-carboxylic acid metabolite (M10) of fluticasone furoate also observed. In contrast, following p.o. administration (2 mg) to the same male subjects, M10 and fluticasone furoate were both principal components in the plasma extracts. Three other minor drug-related components (each less than 5% of plasma radioactivity) were observed and were assigned as products of oxidative metabolism. The greater contribution of metabolites to plasma radioactivity after p.o. than after i.v. administration is consistent with high first-pass metabolism. The remainder of the plasma radioactivity following i.v. or p.o. administration was either unextracted or unassigned because of the low levels of drug-related material in the plasma. The unextracted radioactivity represented a very low amount of material, less than 1 ng equivalents/ml, which equates to less than 30 fmol/mg plasma protein.

AMS was used to quantify radiolabeled drug-related components in plasma off-line following separation by HPLC because of the low levels of radioactivity present in human plasma following i.v. or p.o. administration of [^{14}C]fluticasone furoate. AMS is a very sensitive method for the measurement of radiocarbon (Vogel et al., 1995). The assignment of metabolite structures by chromatographic retention time was performed by comparison with retention times of authentic reference standards and a metabolite isolated from in vitro incubations

TABLE 3

Proposed structure, chromatographic retention times, and mass spectral data for metabolites of fluticasone furoate in humans

Metabolite	Retention Time	Matrix	Positive (M + H^+) or Negative (M-H^-) Ion MS
	min		*parent and fragment ions (m/z)*
Fluticasone furoate 	46.3	Authentic standard	P: 539 (M + H^+) F1: 519 Loss of HF F2: 473 F3: 333 F4: 313 F5: 293
M10 	37.2	Plasma Feces	P: 491 (M + H^+) 471: loss of HF 333: F3 313: F4 293: F5
M21 	44.6	Plasma (based on coelution)	P: 537 (M + H^+) 311: F4-2 Information on metabolite in hepatocytes
M26 	27.0	Plasma Feces	P: 489 (M + H^+) 313: F4 293: F5
M32 	38.1	Human plasma Based on coelution with previously identified metabolite	Negative ion (M-H) P: 505 393

MS, mass spectrometry.

followed by definitive identification. The use of AMS resulted in a clearer definition of the circulating components than would have been achieved by conventional off-line radiodetection using a solid yttrium silicate scintillant, which was used to profile plasma samples from nonclinical species. In the nonclinical species, only fluticasone furoate and the metabolite M10 were identified in plasma after i.v. and/or p.o. administration (P. Shardlow and C. Morley, unpublished data). As a result of the difference in sensitivity between AMS and the solid scintillant detection method, it is considered that a number of the minor metabolites (<5% of radioactive drug-related material) observed in human plasma may not have been observed in nonclinical species because of the difference in limits of detection.

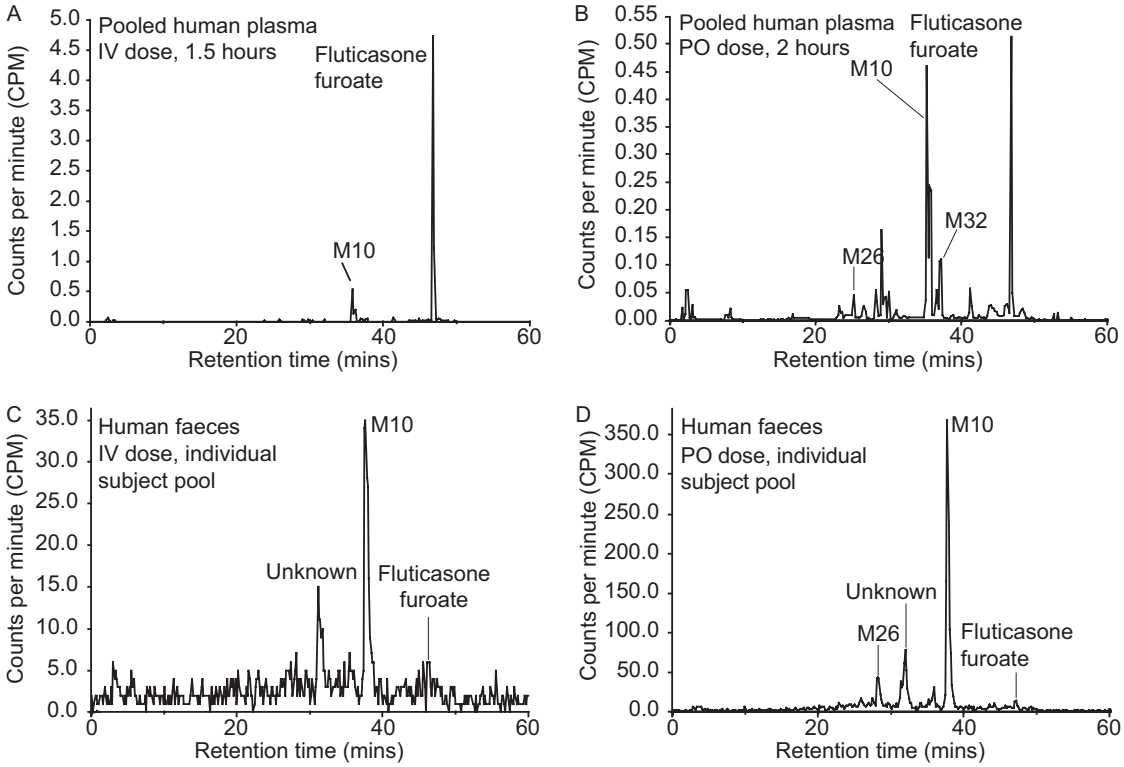


FIG. 3. HPLC radiochromatograms of plasma (A and B) and fecal extract (C and D) from healthy male subjects following i.v. or p.o. administration of [^{14}C]fluticasone furoate. The plasma chromatograms (A and B) are reconstructed from fractions analyzed by AMS.

The mean recovery of the radioactive dose following i.v. administration of [^{14}C]fluticasone furoate to male subjects was greater than 92%. Similar recovery was obtained in rats (86%) and dogs (81%) following an i.v. dose (S. Hughes, unpublished data). After p.o. administration to male subjects, the recovery of radioactivity was complete. Most radioactivity was excreted in the feces after both routes of administration. This excretion pattern is similar to that observed for other synthetic corticosteroids, such as ciclesonide (Nave et al., 2004) and mometasone furoate (Affrime et al., 2000).

The major route of fluticasone furoate metabolism in humans is ester hydrolysis, leading to formation of the 17β-carboxylic acid (M10) with loss of the *S*-fluoromethyl carbothioate moiety. The loss of this moiety is also the significant route of metabolism of fluticasone propionate, with the biotransformation being catalyzed by cytochrome P450 3A4 (Pearce et al., 2006). Oxidative defluorination of fluticasone furoate at the C6 position also occurred and resulted in the formation of M21 and M26; this biotransformation is known to occur in a number of fluorine-containing drugs (Park et al., 2001). Oxidative defluorination is also a metabolic pathway for fluticasone propionate in vitro in human-derived preparations (Shenoy et al., 1993). Oxidative defluorination of fluticasone furoate resulted in hydroxylation at the C6 position; C6 hydroxylation is a well precedented route of metabolism for corticosteroids and has been observed in human-derived in vitro systems for budesonide (Jönsson et al., 1995), mometasone furoate (Teng et al., 2003b), and ciclesonide (Peet et al., 2005). A summary of the proposed oxidative pathways for fluticasone furoate in humans is shown in Fig. 4. There was no evidence for metabolic loss of the furoate group from fluticasone furoate or any of its metabolites; this differs to mometasone furoate, where loss of the furoate group is observed with the formation of mometasone in human-derived fluids and tissue preparations (Teng et al., 2003b) and rat-derived fluids and tissue preparations (Teng et al., 2003a).

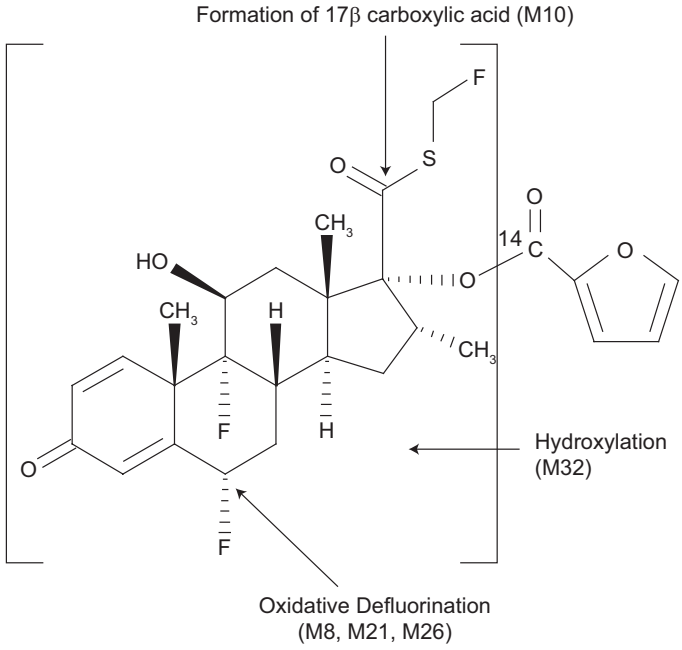


FIG. 4. Summary of the identified oxidative pathways of metabolism for fluticasone furoate.

Although limited data were obtained from in vivo excreta because of the low amounts of drug-related material present, it was observed that, although oral absorption of fluticasone furoate in humans was at most approximately 30%, little or no unchanged parent drug was observed in the feces after p.o. administration. A similar pattern was also observed in p.o.-dosed bile duct-cannulated rats, and, furthermore, fluticasone furoate is stable in a preparation of high-density

anaerobic microbiota from either human or rat (N. McArdle, unpublished data). These data, together with evidence that glucocorticoids are substrates for the P-glycoprotein efflux transporter (Yates et al., 2003), suggest that enterocytes may have a major role in the metabolism of fluticasone furoate in the gastrointestinal tract through a cycle of absorption, metabolism, and efflux, in addition to limiting absorption of this highly permeable moiety.

The major routes of fluticasone furoate metabolism in humans have been defined following a single i.v. and/or p.o. administration of [^{14}C]fluticasone furoate, within the constraints of the very low levels of drug-related material in the plasma and excreta. No notable differences in the routes of metabolism between humans and the nonclinical species were observed.

Most of an intranasal or inhaled dose is likely to be swallowed; therefore, the p.o. and i.v. routes of administration were considered adequate surrogate routes from which to obtain samples to define the major routes of metabolism of fluticasone furoate in humans. The p.o. route acted as a surrogate for the portion of an intranasal or inhaled dose that is swallowed, and the i.v. route represented the drug that is absorbed locally into the systemic circulation.

The routes of fluticasone furoate metabolism identified are consistent with those precedented for other marketed corticosteroids, such as fluticasone propionate, mometasone furoate, ciclesonide, and budesonide. The routes of metabolism for fluticasone furoate and fluticasone propionate are similar, with no evidence that either is metabolized to fluticasone. This shows that they act as different chemical entities. The oral bioavailability of fluticasone furoate is very low, limited by absorption and first-pass metabolism; therefore, any of the drug that is swallowed is unlikely to be active systemically.

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References

Affrime MB, Cuss F, Padhi D, Wirth M, Pai S, Clement RP, Lim J, Kantesaria B, Alton K, and Cayen MN (2000) Bioavailability and metabolism of mometasone furoate following administration by metered-dose and dry-powder inhalers in healthy human volunteers. *J Clin Pharmacol* **40:**1227–1236.

Allen A, Down G, Newland A, Reynard K, Rousell V, Salmon E, and Scott R (2007) Absolute bioavailability of intranasal fluticasone furoate in healthy subjects. *Clin Ther* **29:**1415–1420.

Davies B and Morris T (1993) Physiological parameters in laboratory animals and humans. *Pharm Res* **10:**1093–1095.

Goodman LS and Gilman A (2006) Pharmacotherapy of asthma, in *The Pharmacological Basis of Therapeutics*, 11th ed (Brunton LL, Lazo JS, Parker KL, Buxton ILO, and Blumenthal DK eds), Chapter 27, McGraw Hill Professional, New York.

Jönsson G, Astrom A, and Andersson P (1995) Budesonide is metabolized by cytochrome P450 3A (CYP3A) enzymes in human liver. *Drug Metab Dispos* **23:**137–142.

Karin M, Yamamoto Y, and Wang QM (2004) The IKK NF-kappa B system: a treasure trove for drug development. *Nat Rev Drug Discov* **3:**17–26.

Martin BG, Ratner PH, Hampel FC, Andrews CP, Toler T, Wu W, Faris MA, and Philpot EE (2007) Optimal dose selection of fluticasone furoate nasal spray for the treatment of seasonal allergic rhinitis in adults and adolescents. *Allergy Asthma Proc* **28:**216–225.

Nave R, Bethke TD, van Marle SP, and Zech K (2004) Pharmacokinetics of [14C]ciclesonide after oral and intravenous administration to healthy subjects. *Clin Pharmacokinet* **43:**479–486.

Park BK, Kitteringham NR, and O'Neill PM (2001) Metabolism of fluorine-containing drugs. *Annu Rev Pharmacol Toxicol* **41:**443–470.

Pearce RE, Leeder JS, and Kearns GL (2006) Biotransformation of fluticasone: in vitro characterization. *Drug Metab Dispos* **34:**1035–1040.

Peet CF, Enos T, Nave R, Zech K, and Hall M (2005) Identification of enzymes involved in phase I metabolism of ciclesonide by human liver microsomes. *Eur J Drug Metab Pharmacokinet* **30:**275–286.

Rhen T and Cidlowski JA (2005) Antiinflammatory action of glucocorticoids—new mechanisms for old drugs. *N Engl J Med* **353:**1711–1723.

Salter M, Biggadike K, Matthews JL, West MR, Haase MV, Farrow SN, Uings IJ, and Gray DW (2007) Pharmacological properties of the enhanced-affinity glucocorticoid fluticasone furoate in vitro and in an in vivo model of respiratory inflammatory disease. *Am J Physiol Lung Cell Mol Physiol* **293:**L660–L667.

Scott RJ, Snell RJ, and Fowles SE (2007) Validation of a method for the determination of fluticasone furoate and two potential metabolites using liquid chromatography-mass spectrometry. *Ann Allergy Asthma Immunol* **98 (Suppl 1):**A93.

Shenoy EVB, Ayrton A, Bower GD, Daniel MJ, and Surry DD (1993) The use of Hep G2 cells in predicting the routes of metabolism of fluticasone propionate in man. *Br J Clin Pharmacol* **36:**166P–167P.

Teng XW, Cutler DJ, and Davies NM (2003a) Kinetics of metabolism and degradation of mometasone furoate in rat biological fluids and tissues. *J Pharm Pharmacol* **55:**617–630.

Teng XW, Cutler DJ, and Davies NM (2003b) Mometasone furoate degradation and metabolism in human biological fluids and tissues. *Biopharm Drug Dispos* **24:**321–333.

Vogel JS (1992) Rapid production of graphite without contamination for biomedical AMS. *Radiocarbon* **34:**344–350.

Vogel JS, Turteltaub KW, Finkel R, and Nelson DE (1995) Accelerator mass spectrometry. *Anal Chem* **67:**353A–359A.

Yates CR, Chang C, Kearbey JD, Yasuda K, Schuetz EG, Miller DD, Dalton JT, and Swaan PW (2003) Structural determinants of P-glycoprotein-mediated transport of glucocorticoids. *Pharm Res* **20:**1794–1803.

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