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# Characterization of clinical ADME and pharmacokinetics of velsecorat using an intravenous microtracer combined with an inhaled dose in healthy subjects

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**Running title (54 out of a max of 60 characters):**

hADME and PK of inhaled velsecorat in healthy subjects

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**List of non-standard abbreviations**

| | |
|---|---|
| $^{12}C$ | Carbon-12 |
| $^{14}C$ | Carbon-14 |
| $^{3}H$ | Hydrogen-3 (tritium) |
| AMS | Accelerator mass spectrometry |
| $AUC_{0\text{-}12h}$ | Area under the curve from 0 to 12 hours |
| $AUC_{0\text{-}24h}$ | Area under the curve from 0 to 24 hours |
| $AUC_{0\text{-}t}$ | Area under the curve from 0 hours to last measurable concentration |
| $AUC_{0\text{-}\infty}$ | Area under the curve from 0 hours extrapolated to infinite time |
| CL | Total body clearance of drug from plasma |
| $C_{max}$ | Maximum plasma concentration |
| CMC | Carboxymethyl cellulose |
| DRM | Drug-related material |
| DPI | Dry powder inhaler |
| ECG | Electrocardiogram |
| ESI | Electrospray ionization |
| F | Absolute oral bioavailability |
| hADME | Human absorption, distribution, metabolism and excretion |
| IV | Intravenous |
| kBq | Kilobecquerel |
| LC-MS/MS | Liquid chromatography with tandem mass spectrometry |
| LLoQ | Lower limit of quantification |
| MBq | Megabecquerel |
| MRT | Mean residence time of the unchanged drug in the systemic circulation |
| µCi | Microcurie |
| mSv | Millisievert |
| nCi | Nanocurie |
| PK | Pharmacokinetics |
| pmol velsecorat Eq/L | Picomoles of velsecorat equivalents (radioactivity) per L |
| SD | Standard deviation |
| $t_{1/2}$ | Terminal phase half-life |

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| | |
|---|---|
| $t_{max}$ | The time from dosing at which the $C_{max}$ was apparent |
| $t_x$ | Time at point x |
| UPLC | Ultra performance liquid chromatography |
| $V_{ss}$ | Volume of distribution at steady state |
| $V_z$ | Volume of distribution |

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## Abstract

This open-label, single-period study describes the human absorption, distribution, metabolism, excretion and pharmacokinetics of velsecorat (AZD7594). Healthy subjects received inhaled velsecorat (non-radiolabeled; 720 µg) followed by intravenous (IV) infusion of $^{14}$C-velsecorat (30 µg). Plasma, urine and feces were collected up to 168 hours post-dose. Objectives included identification and quantification of velsecorat and its metabolites (i.e. drug-related material; DRM) in plasma and excreta, and determining the elimination pathways of velsecorat by measuring the rate and route of excretion, plasma half-life ($t_{1/2}$), clearance, volume of distribution and mean recovery of radioactivity. On average, 76.0% of administered $^{14}$C dose was recovered by the end of the sampling period (urine=24.4%; feces=51.6%), with no unchanged compound recovered in excreta, suggesting biliary excretion is the main elimination route. Compared with IV $^{14}$C-velsecorat, inhaled velsecorat had a longer $t_{1/2}$ (27 vs 2 hours), confirming that plasma elimination is absorption-rate-limited from the lungs. Following IV administration, $t_{1/2}$ of $^{14}$C-DRM was longer than for unchanged velsecorat and 20% of the $^{14}$C plasma content was related to unchanged velsecorat. The geometric mean plasma clearance of velsecorat was high (70.7 L/h) and the geometric mean volume of distribution at steady state was 113 L. Velsecorat was substantially metabolized via O-dealkylation of the indazole ether followed by sulfate conjugation, forming the M1 metabolite, the major metabolite in plasma. There were 15 minor metabolites. Velsecorat was well tolerated, and these results support the progression of velsecorat to phase 3 studies.

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## Significance statement

This study describes the human pharmacokinetics and metabolism of velsecorat, a selective glucocorticoid receptor modulator, evaluated via co-administration of a radiolabeled intravenous microtracer dose and a non-radiolabeled inhaled dose. This study provides a comprehensive assessment of the disposition of velsecorat in humans. It also highlights a number of complexities associated with determining human absorption, distribution, metabolism and excretion for velsecorat, related to the inhaled route, the high metabolic clearance, sequential metabolite formation and the low intravenous dose.

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## Introduction

Velsecorat, formerly known as AZD7594 (3-(5-((1R,2S)-2-(2,2-Difluoropropanamido)-1-(2,3-dihydrobenzo-[b][1,4]dioxin-6-yl)propoxy)-1H-indazol-1-yl)-N-((R)-tetrahydrofuran-3-yl)benzamide), is a novel inhaled, potent non-steroidal glucocorticoid receptor modulator in development for the treatment of asthma (Hemmerling et al., 2017; Brown et al., 2019; Prothon et al., 2019).

The characterization of human absorption, distribution, metabolism and excretion (hADME) and pharmacokinetics (PK) plays an important role during drug development and the approval process (Coppola et al., 2019). Data from hADME studies are critical in the understanding of rates and routes of excretion, metabolic pathways, and the identification, quantification and exposure to metabolites, as well as in designing appropriate clinical drug-drug interaction studies (Coppola et al., 2019). These data can also be used to evaluate the presence of metabolites in humans that would also need to be confirmed present in animals used in preclinical safety testing (Coppola et al., 2019).

hADME studies are most commonly performed using drug labeled with a radioactive isotope, most often Hydrogen-3 (tritium; $^{3}H$) or Carbon-14 ($^{14}C$), and by the intended clinical route. After administration, the recovery of radioactivity is determined in excreta, and the metabolites and quantitative radio-chromatographic metabolite profiles are recorded in plasma and excreta (Coppola et al., 2019). However, for compounds intended to be administered via inhalation, there are both technical and ethical complications associated with administering a radioisotope (Beaumont et al., 2014). These complications include difficulties in manufacturing the radiolabeled material particle size to match that of the non-radiolabeled material and how to accurately quantify the inhaled and exhaled doses. This can be challenging, due to the complexity of quantifying the remaining radioactivity in the inhalation device and potential contamination of radioactivity in the administration room. For example, administering radiolabeled drugs ($^{3}H$-mometasome furoate) via dry powder inhaler (DPI) has previously resulted in highly variable mean total cumulative recovered radioactivity (range 63%–99% after 168 hours) (Affrime et al., 2000). In addition, the long-term toxicological effect of inhaled $^{14}C$ isotopes in the lungs is not known. Therefore, hADME studies for inhaled compounds often use administration of oral and/or intravenous (IV) dosing as a surrogate.

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The use of highly sensitive accelerator mass spectrometry (AMS) in hADME studies allows the administration of much lower quantities of $^{14}C$ (0.1–1 µCi [3.7–37 kBq]) compared with radioactivity measurements following standard hADME study designs (typical doses of 20–100 µCi [740–3700 kBq]) (Spracklin et al., 2020). High sensitivity is achieved because AMS measures $^{14}C$ by MS and not radioactivity (Lozac'h et al., 2018).

In this study we used a microtracer design, which involves an IV $^{14}C$-velsecorat dose administered concomitantly with a therapeutically relevant non-radiolabeled inhaled velsecorat dose using a commercial formulation and device (Ambery et al., 2018; Harrell et al., 2019). IV $^{14}C$-velsecorat acts as a surrogate for the inhaled velsecorat reaching the systemic circulation through the lung.

The objectives of this study were to determine the rates and routes of velsecorat excretion, evaluate the completeness of dose recovery in excreta and identify and quantify velsecorat and its metabolites (i.e. drug-related material; DRM) in plasma, urine and feces.

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## Materials and Methods

Velsecorat for inhalation was supplied by AstraZeneca (Mölndal, Sweden). Selcia Ltd (Essex, UK) provided the $^{14}$C-velsecorat, which was diluted with unlabeled velsecorat by AstraZeneca to get the correct specific radioactivity, as part of the drug substance preparation, and manufactured into a solution for IV infusion by Quotient Sciences (Nottingham, UK). All other solvents and reagents were of analytical grade and were acquired from commercial suppliers.

### Study design

This was an open-label, single-center, single-period study in healthy subjects conducted at Quotient Sciences between June and July 2019 (NCT04002427). The study adhered to the Declaration of Helsinki and was approved by the Wales Research Ethics Committee 2, Cardiff; each subject gave their written informed consent prior to any study procedures.

Following a screening visit, in a single treatment period, subjects were administered non-radiolabeled velsecorat as an inhalation powder in a DPI (SD3FL; AstraZeneca, Mölndal, Sweden) at a nominal dose of 792 µg (delivered dose 720 µg) (**Figure 1**). The SD3FL inhaler is a multi-dose device-metered inhaler similar in form and function to the Genuair® inhaler marketed in Europe as Eklira® Genuair®. Fifteen minutes after the inhaled dose, each subject received 30 µg of $^{14}$C-velsecorat (containing approximately 180 nCi [6.7 kBq] $^{14}$C) via IV infusion over 1 hour. $^{14}$C-velsecorat was dissolved at 5 µg/mL so that 30 µg was administered in a volume of 6 mL. The specific radioactivity of $^{14}$C in this formulation was 133 Bq/nmol.

During the study, each subject was exposed to an effective radioactive dose of 0.01 mSv, well within the safety limits defined by the World Health Organization category 1 and the International Commission on Radiologic Protection category 1 (<0.1 mSv; minor risk) (International Commission on Radiological Protection, 1991). The dose of radioactivity was classed as a microtracer dose, as it contains ≤37 kBq (1 µCi) of $^{14}$C.

### Study population

Healthy men and non-pregnant, non-lactating women aged 18–55 years, who were non-smokers, with a body mass index (BMI) of 18.5–35.0 kg/m$^2$ and without clinically relevant disease were eligible for the study. Participants were confined to the study center for the

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duration of the study. A full list of inclusion and exclusion criteria is provided in **Supp. Table 1**.

**Sample collection and processing**

Blood samples were collected pre-dose and up to 168 hours post-dose (**Supp. Fig. 1**). Analysis of plasma total $^{14}$C content, plasma $^{14}$C-velsecorat, quantitative profiles of metabolites and metabolite characterization was performed by TNO (Leiden, The Netherlands). PK analysis of non-radiolabeled velsecorat was performed by Covance Laboratories (Harrogate, UK).

Urine samples were quantitatively collected at 0–6, 6–12 and 12–24 hours post-dose on the first day and then in 24-hour collection intervals up to 168 hours (morning of day 8). Feces were quantitatively collected at 24-hour collection intervals from the dose administration (0 hours) up to 168 hours (morning of day 8) and homogenized by PRA (Assen, The Netherlands). Fecal samples per subject per 24-hour interval were diluted in 1% carboxymethyl cellulose (CMC) solution (1% CMC solution: feces, 4:1) and homogenized.

**Mass balance and excretion**

To determine total radioactivity excreted in urine and feces, for each sample 15 µL of urine or 30 mg of fecal homogenates (diluted or undiluted) were dried and placed in the elemental analyzer, which acted as an autosampler and combustion device for the AMS. Urine samples were analyzed once and fecal homogenates were analyzed in triplicate. The lower limit of quantification (LLoQ) was 3.08 pmol velsecorat Eq/L for urine measurements and 19.8 pmol velsecorat Eq/L in fecal samples.

**Pharmacokinetic assessments**

The level of non-radiolabeled velsecorat in plasma was determined using a validated analytical method based on protein precipitation, followed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) by Covance Laboratories (Prothon et al., 2019). The LLoQ of velsecorat in plasma was 10 pmol/L. As the $^{14}$C-velsecorat IV dose contained both $^{12}$C-velsecorat (unlabeled velsecorat) and $^{14}$C-velsecorat, the unlabeled velsecorat concentrations measured by LC-MS/MS were corrected prior to PK parameter estimation in SAS (v9.4) for the reporting of bioanalytical data to obtain unlabeled velsecorat pertaining to the inhaled dose only as follows:

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Concentration of inhaled velsecorat at time x ($t_x$) = $^{12}$C-velsecorat (inhaled and IV doses) as measured by LC-MS/MS at $t_x$ – ($^{12}$C-velsecorat [IV dose at $t_x$]), where $^{12}$C-velsecorat in the $^{14}$C-labelled IV dose was calculated as follows at each time point:

Proportion of $^{12}$C-velsecorat (IV dose) × $^{14}$C-velsecorat at $t_x$, where proportion of $^{12}$C-velsecorat (IV dose) = (1 – [specific activity of IV $^{14}$C-velsecorat/maximum possible $^{14}$C-velsecorat specific activity]).

Velsecorat plasma concentration refers to the inhaled dose only, and $^{14}$C-velsecorat refers to the total concentration of velsecorat pertaining to the IV dose, i.e. the sum of the unlabeled velsecorat and $^{14}$C-velsecorat as given by the composition of the formulation.

Plasma $^{14}$C-velsecorat was determined using protein precipitation followed by ultra performance liquid chromatography (UPLC) analysis with gradient elution. The compound peak was collected and subjected to AMS analysis. The dynamic range of the method qualified prior to the sample analysis was 1.00–128 mBq/mL (7.50–961 pmol velsecorat/L). Assay performance was assessed by measuring quality control samples on three individual occasions, and the carry-over was determined to be 0.718%. A dilution factor of 10-fold was qualified in one series. The LLoQ for $^{14}$C-velsecorat in plasma was 7.50 pmol velsecorat/L upon extraction of 150 µL of plasma. Plasma total radioactivity was directly analyzed using the sensitive AMS method, which had an LLoQ of 4.88 pmol velsecorat Eq/L.

**UPLC**

Analytical UPLC-PDA was performed on an Acquity H Class Plus system (Waters Corp., Milford, Massachusetts, USA) using a BEH C18 reserved-phase column (2.1 x 150 mm, 1.7 µm) and the corresponding guard column, ultraviolet detection at 318 nm and linear gradient (15–50% over 22.54 min) of acetonitrile into MilliQ water (0.1% formic acid) with a flow rate of 0.35 mL/min.

**Quantification and characterization of metabolites**

A time proportional plasma pool was prepared from each individual (representing the area under the plasma concentration time curve from 0–12 hours [$AUC_{0\text{-}12h}$]) by combining volumes in proportion to the time interval between individual samples as previously described (Hamilton et al., 1981). One single plasma $AUC_{0\text{-}12h}$ pool was prepared by mixing equal volumes of each subject's pool, representing approximately 50% of the total AUC of total radioactivity. A 24-hour plasma pool was also prepared by mixing equal volumes from

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each of the individual 24-hour sample to evaluate any additional metabolites appearing in plasma at a later timepoint.

Individual pooled urine samples were prepared for each subject up to 72 hours, corresponding to the time needed to recover 95% of radioactivity via this route. The samples were prepared by using equal percentage per timepoint based on volume. A single urine pool was then prepared by mixing equal volumes of each individual pool. This pool contained DRM corresponding to 23% of the dose administered, which is approximately 95% of the total DRM excreted in urine.

For feces, individual pooled fecal homogenate samples were prepared for each individual up to 96 hours, corresponding to the time needed to recover 94% of radioactivity via this route. The samples were prepared by using equal percentages per timepoint based on total weight of fecal homogenates. A single fecal homogenate pool was then prepared by mixing equal weights of each individual pool. The combined fecal pool contained 49% of the administered dose, which is approximately 94% of the total DRM excreted in the feces.

Investigation of velsecorat metabolism in the lungs was not performed; previous *in vitro* studies using lung microsomes did not indicate metabolism of velsecorat (unpublished data).

Metabolites were identified and quantified in plasma, urine and feces using an Acquity H class Plus UPLC system (Waters Corp., Milford, Massachusetts, USA) coupled to a high-resolution MS (Q Exactive™, ThermoFisher Scientific, Massachusetts, USA) and AMS (4110Bo-AMS, High Voltage Engineering Europa B.V., Amersfoort, The Netherlands), respectively. Prior to analysis, the Q-Exactive system was calibrated in Electrospray Ionization (ESI) positive and negative mode using the corresponding calibration mixtures recommended by the supplier (ThermoFisher Scientific) with acceptance criteria of 3 parts per million. Each pool sample was injected three times; one to run in ESI positive mode, the second in ESI negative mode and for the third, the flow was fully collected (split flow off, no fractionation) in one container to enable column recovery calculations by means of total radioactive analysis by AMS. The pre-dose pools were injected twice without fractionation. One was run in ESI positive mode and the other in ESI negative mode as background references. Plasma (700 µL) and fecal homogenates (200 mg) were extracted by protein precipitation, and urine pools were injected directly without further processing. Post-column, the eluent was split to generate online high-resolution MS and off-line AMS analyses.

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Proposals of metabolite structures were based on data acquired from high-resolution MS and MS/MS analyses. Molecular weights of each metabolite were proposed based on the protonated adduct in the positive ionization MS mode and the deprotonated molecule in the negative ionization mode. High-resolution MS data were used to propose the molecular composition. The retention times for each metabolite compared between sample matrices agreed well both for LCMS and LC-AMS profiling. Velsecorat was identified by comparison of retention time and high resolution MS and MS/MS spectra with the synthesized standard. Tentative structures of metabolites were proposed based on their product ion spectra and a detailed analysis of fragmentation pathways of velsecorat in the positive ionization mode.

**Safety**

Safety and tolerability of velsecorat were assessed by physical examination and measurement of vital signs, 12-lead electrocardiograms (ECGs) and routine laboratory tests (including urinalysis) pre-dose, over the first 24 hours post-dose and prior to discharge. Adverse events were monitored throughout the study.

**Statistical analysis**

A sample size of 6 was chosen to investigate the primary objective of the study (Penner et al., 2009). One subject was excluded from mass balance and metabolite analysis due to incomplete urine sampling; however, a sample size of 5 was considered sufficient to fulfil the objectives of the study. Plasma PK of inhaled velsecorat, $^{14}$C-velsecorat and total radioactivity in plasma were analyzed at Quotient Sciences by non-compartmental analysis methods in Phoenix WinNonlin$^{TM}$ V8.0 (Certara; New Jersey, USA). The partial $AUC_{0\text{-}12h}$ for $^{14}$C-velsecorat and total radioactivity was calculated *post hoc*.

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# Results

## Demographic and baseline characteristics

Eleven volunteers were screened, with six (four women and two men) included; no discontinuation was reported, although one subject was excluded from mass balance and metabolite analysis due to incomplete urine sampling. The mean (standard deviation; SD) age was 37 (10) years with a mean (SD) BMI of 29 (1.8) $kg/m^2$.

## Mass balance and excretion

The mean recovery of total radioactivity over time is shown in **Figure 2**. An average of 76.0% (range 72.1–78.8%) of the radioactivity administered was recovered by the end of the sampling period (168 hours), most of which was recovered within 72 hours. Approximately 24.4% was recovered in the urine and 51.6% in feces. Within the first 24 hours, approximately 14.0% and 6.9% of the total radioactivity was recovered in the urine and feces, respectively.

## Pharmacokinetic results

Following inhaled administration, plasma concentrations of velsecorat were observed in all subjects at the first post-inhalation dose sampling timepoint of 0.5 hours. The geometric mean maximum plasma concentration ($C_{max}$) was 509 pmol/L and the median time from dosing at which the $C_{max}$ was apparent ($t_{max}$) occurred at 0.88 (range 0.50–1.32) hours post-dose. There was a steep decline in velsecorat concentrations immediately after $C_{max}$, followed by a slower decline thereafter. The geometric mean terminal phase half-life ($t_{1/2}$) was 27 hours (**Figure 3A; Table 1**). Small fluctuations (secondary peaking) in plasma concentrations were observed over the first 48 hours post-dose. The geometric mean absolute bioavailability following inhalation of velsecorat was 66% (coefficient of variation 22.0%) based on AUC from time zero extrapolated to infinite time ($AUC_{0-\infty}$; **Table 1**).

Following the start of the 1-hour IV infusion, plasma concentrations of $^{14}$C-velsecorat and total radioactivity were observed in all subjects at the first sampling timepoint after the start of the IV-infusion (**Supp. Fig. 1**). Median $t_{max}$ occurred at 1.00 (range 0.75–1.17) hour, i.e. at the end of the IV infusion, for $^{14}$C-velsecorat and 1.00 (range 0.75–1.05) hour for total radioactivity (total $^{14}$C). There was a rapid biphasic decline in $^{14}$C-velsecorat concentrations, and concentrations were measurable up to 8–12 hours, with a mean terminal $t_{1/2}$ of 2 hours (**Figure 3A; Table 1**). For total $^{14}$C, a slower triphasic decline was observed, with



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quantifiable concentrations lasting 48–98 hours in most subjects (168 hours in one subject) post-dose, and a mean terminal $t_{1/2}$ of 18 hours (**Figure 3B; Table 1**). The geometric mean $C_{max}$ of $^{14}$C-velsecorat and total radioactivity was 543 pmol/L and 565 pmol velsecorat Eq/L, respectively, showing that at this early timepoint all DRM was related to unchanged velsecorat.

The geometric mean clearance of $^{14}$C-velsecorat from plasma was 70.7 L/h and the geometric mean volume of distribution at steady state ($V_{SS}$) was 113 L.

Based on AUC ratio from time zero to the last quantifiable $^{14}$C-velsecorat concentration and total $^{14}$C concentration ($AUC_{0-t}$), the $^{14}$C-velsecorat accounted for 19.0% of circulating plasma total $^{14}$C (i.e. $^{14}$C-DRM). Approximately 50% of the total $^{14}$C AUC was recovered within 0–12 hours.

**Metabolism**

Analysis of a plasma pool covering the first 12 hours post-dose revealed that most DRM was related to unchanged velsecorat (40%) and metabolite M1 (22%), with remaining radioactivity distributed over approximately 15 minor metabolites (**Table 2, Figure 4A**). Metabolite M1 was identified as a metabolite formed by O-dealkylation of the indazole ether (M2), followed by sulfate conjugation (**Figure 5**); we did not investigate which sulfotransferase mediated the formation of the M1 metabolite as part of this study. At 24 hours, the major component in plasma was metabolite M1, accounting for 68% of the radioactivity, with only minor amounts of unchanged velsecorat (5%) (**Table 2; Figure 4B**).

In the urine pool, the major part of the detected $^{14}$C was attributed to metabolite M1 (85% by metabolite peak integration of the chromatogram), representing 20% of the administered dose. The second most abundant metabolite was M22, identified as the glucuronide conjugate of M2, representing 2% of the administered dose (**Table 3**). No unchanged velsecorat was detected in the urine pool. The reconstructed AMS chromatogram of the urine pool is shown in **Figure 6A**.

The metabolite profile of the fecal homogenates was more complex, including partially resolved or co-eluting metabolite fractions. The main metabolite in feces was metabolite M2, accounting for 30% of the total $^{14}$C, and representing 15% of the administered dose. Up to 13 additional metabolites were identified in the 5 major metabolite fractions of the profile,

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altogether representing approximately 56% of the total $^{14}C$ in the chromatogram (**Table 3**). The reconstructed AMS chromatogram of the feces pool is shown in **Figure 6B**.

Nearly all metabolites with assigned proposed structures were formed via one of three major routes: O-dealkylation of the indazole ether (M2); ring opening of the 1,4-dioxane ring (M46); and ring opening of the oxolane ring (M47). A proposed metabolic scheme for velsecorat is shown in **Figure 5.**

**Safety**

No deaths or serious adverse events were reported during the study, and no subjects withdrew from the study or permanently discontinued study treatment due to an adverse event. Two mild adverse events, assessed as unrelated to treatment, were reported during the study: one dysmenorrhea and one vessel puncture site pain. There were no clinically significant changes in clinical laboratory tests, vital signs, ECGs, physical examinations or urinalysis.

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## Discussion

In this study investigating the metabolism and disposition of velsecorat in healthy adults, most of the administered $^{14}$C-dose was recovered in urine and feces within 72 hours. The geometric mean clearance of velsecorat was high and velsecorat was substantially metabolized, with all DRM excreted as metabolites after IV administration. Velsecorat was well tolerated, with two mild adverse events reported, both assessed as unrelated to treatment.

An average of 76% of the IV-administered $^{14}$C-dose was recovered; slightly below the target recovery of >80% (Roffey et al., 2007; Coppola et al., 2019). The average excretion of the administered dose was <1% in the penultimate and last 24-hour collection intervals (120–144 and 144–168 hours, respectively). This indicates that using <1% DRM recovery per 24-hour collection interval as a release criterion would not have changed the result, and achieving an 80% recovery would require extending the collection time for several more days. Low recovery could be due to inaccuracy in dose preparation and/or delivery, or loss of the radiolabel on the molecule in expired air or to endogenous metabolic routes or tissue binding. In a velsecorat preclinical rat ADME study, there was no indication of radioactivity in expired air in the first 24 hours post-dose, nor in the carcass at study termination. Mass balance recovery was 80–90% following sampling for 168 hours. Furthermore, recovery is generally low when the circulating half-life of total radioactivity is >50 hours and the majority of DRM is recovered in feces (Roffey et al., 2007), which is consistent with our finding that 52% of the administered $^{14}$C dose was recovered in feces. In the present study, 24% of the dose was recovered in urine as DRM 168 hours post-administration, with no unchanged velsecorat detected, which is consistent with previous findings that urinary excretion of unchanged velsecorat was negligible (Prothon et al., 2019).

The longer estimated plasma terminal $t_{½}$ of inhaled velsecorat (27 hours) than that of $^{14}$C-velsecorat following the IV dose (2 hours) confirms that the elimination of velsecorat is absorption-rate-limited from the lungs, which is consistent with previous data (Chen et al., 2017). This slow absorption, governed by a slow dissolution rate due to low solubility, results in a long pulmonary residence time and supports once-daily administration.

Following IV administration, the geometric mean $V_{SS}$ was higher than total body water, indicating substantial distribution of velsecorat into tissue. The geometric mean clearance of velsecorat from plasma was high and consistent with a high extraction ratio. Exposure to



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$^{14}$C-velsecorat accounted for approximately 20% of circulating DRM based on $AUC_{0-t}$ ratio. However, the percentage of unchanged velsecorat may be a slight underestimation in relation to DRM. $^{14}$C-velsecorat was generally quantifiable up to 8–12 hours post-dose following the IV dose, resulting in a large proportion of the plasma curve under LLoQ, as shown by the 24-hour pool, where 5% of the DRM was attributed to unchanged velsecorat. This may also have resulted in an overestimation of the absolute bioavailability (66%), which was substantially higher than that reported in a previous velsecorat clinical study (46%) (Sadiq et al., 2017). The oral bioavailability of inhaled velsecorat was previously reported as negligible (<1% at 1200 µg), thus absolute bioavailability for velsecorat following the inhaled dose represents the fraction of the dose absorbed via the lung in this study (Prothon et al., 2019).

To quantify and characterize the metabolites in plasma, two pools were prepared from $AUC_{0-12h}$, representing approximately 50% of radioactivity and a single timepoint 24-hour sample. This was because the radioactivity spread over time meant that producing an AUC pool representing the recommended 80% of the total AUC of DRM (Penner et al., 2009; Coppola et al., 2019) would result in extensive dilution, limiting detection and quantification. Instead, the 24-hour pool was prepared and analyzed to evaluate any additional metabolites appearing in plasma at a later timepoint. Analysis of the plasma metabolite profile in the AUC pool of the first 12 hours post-dose revealed that ~40% of $^{14}$C content was related to unchanged velsecorat, and that M1 was the major metabolite, corresponding to >22% of the radioactivity. This supports our results showing that $^{14}$C-velsecorat comprised approximately 20% of circulating DRM. The remaining radioactivity was spread over ≥15 metabolites of ~3% abundance each. In the 24-hour pool, the majority of DRM was attributed to M1 (68%). These findings suggest that the total contribution of metabolite M1 to the total AUC of DRM is >10%, i.e. above the threshold where additional animal studies might be warranted, according to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (ICH, 2009; ICH, 2012). However, the ICH guidelines acknowledge that for a daily administered dose <10 mg, fractions of DRM >10% might be considered appropriate and that a conjugated metabolite, such as M1, is of less regulatory concern (ICH, 2012).

Nearly all metabolites with assigned structure proposals involved at least one of the following three sites of metabolism: the indazole ether, the 1,4-dioxane ring and the oxolane ring. Both rings were prone to mono-oxygenation of an α-carbon, followed by ring opening forming an

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aldehyde and reduction to an alcohol (M46 and M47). Alternatively, the aldehyde was oxidized to carboxylic acid (M8, M9, M24, M31 and M34). The vast majority of velsecorat metabolism involved O-dealkylation of the indazole ether (M2). Metabolites formed via O-dealkylation of the indazole ether, i.e. M1, M2, M22, M23 and M24, accounted for >40% of the given dose recovered in excreta, indicating that the metabolic pathway resulting in metabolite M2 has a significant contribution to clearance. Attempts to characterize metabolites formed from the left-hand-side of velsecorat after oxidative dealkylation to form M1 were unsuccessful, possibly since the given dose was low and the left side of the molecule was unlabeled. Furthermore, the left-hand-side metabolites after cleavage might be devoid of MS response, or this fragment was extensively metabolized to minor metabolites appearing below the detection limit. Further characterization of left-hand-side metabolites of velsecorat are planned.

In this study, the IV dose ($^{14}$C-velsecorat) was used concomitantly with an inhaled therapeutically relevant dose as a surrogate for the inhaled dose, as in previous studies (Ambery et al., 2018; Harrell et al., 2019). Due to the negligible oral bioavailability of velsecorat (Chen et al., 2017; Prothon et al., 2019), it was expected that IV dosing would result in a similar metabolite profile as post-inhalation. Furthermore, following oral dosing of $^{14}$C-velsecorat in rats, very low tissue distribution of radioactivity (i.e. DRM) has been observed, as well as lower urinary excretion of radioactivity versus IV administration, indicating low absorption of DRM from the gut (unpublished data). Based on *in vitro* studies, CYP3A4 is the major metabolizing enzyme of velsecorat and also the main enzyme contributing to the formation of metabolite M2, which we showed to be a major route of metabolism of velsecorat in humans (>40%) (unpublished data). Thus, as CYP3A4 is one of the major enzymes present in the gut, it is unlikely that oral dosing would have resulted in any additional metabolites compared with the IV route.

The use of a radiolabeled IV microtracer dose allowed the determination of the mass balance, excretion routes and levels of circulating metabolites in humans, with exposure to radiation similar to natural background levels (Young et al., 2014; Vuong et al., 2016; Lozac'h et al., 2018). Furthermore, the implementation of UPLC to achieve good chromatographic separation, followed by fraction collection and AMS analysis, resulted in high resolution $^{14}$C profiles in four human pools (two plasma, one urine and one fecal homogenates). Accurate analysis with LC-MS/MS enabled the identification of all major and a number of minor metabolites in the three matrices.



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Limitations included a chemical dose level of $^{14}$C-velsecorat (30 µg) that was too low to provide structural information about components of the metabolic profile, whereas addition of the higher inhaled unlabeled dose (720 µg) facilitated the metabolite identification. However, all the quantitative information, including abundance of metabolites, was provided based on the IV dose.

As data from a single IV dose indicated a major metabolite of velsecorat in plasma, there is a potential need for further data to fully evaluate the quantitative exposure to this major metabolite in plasma at steady state following inhalation. For example, new repeat dose toxicity studies in animals could be used to assess and ensure adequate exposure in safety testing, as well as to investigate the potential activity of the major metabolite, M1, and any drug-drug interactions with CYP3A4 inhibitors.

In conclusion, velsecorat was substantially metabolized via three major routes: O-dealkylation of the indazole ether (M2), followed by sulfate conjugation (M1); ring opening of the 1,4-dioxane ring (M46); and ring opening of the oxolane ring (M47). On average, 76% of administered $^{14}$C-velsecorat dose was recovered, with biliary excretion as the main elimination route and no unchanged compound recovered in excreta. Velsecorat had a high clearance, volume of distribution and bioavailability, and confirmed absorption-rate limited elimination following inhalation. Velsecorat was well tolerated by the healthy subjects. These results support the progression of velsecorat to phase 3 studies.

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## Conflict of interest

AAH, AJ, IP, LW, PB, SN, SP and UWH are employees of, and/or own stock in, AstraZeneca. SS is an employee of Quotient Sciences. ERV, MPG and RdL are employees of TNO.

## Authorship contributions

Contributed to research design: Jauhiainen and Wählby Hamrén.

Conducted experiments and performed data analysis: Verheij, Pelay-Gimeno and de Ligt.

Participated in research design and performed data analysis: Holmberg, Psallidas, Weidolf, Bold, Prothon, Necander and Sidhu.

Contributed to the writing of the manuscript: Holmberg, Weidolf, Necander, Bold, Sidhu, Pelay-Gimeno, de Ligt, Verheij, Jauhiainen, Psallidas, Wählby Hamrén and Prothon

## Data Sharing Statement

The clinical trial data underlying the findings described in this manuscript may be obtained in accordance with AstraZeneca's data sharing policy described at https://astrazenecagrouptrials.pharmacm.com/ST/Submission/Disclosure.

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## Footnotes

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## Figure legends

**Figure 1:** Study design

Footnote: IV, intravenous

**Figure 2:** Cumulative arithmetic mean (±SD) of radioactivity recovery over time following IV administration of $^{14}$C-velsecorat

Footnote: N=5; IV, intravenous; SD, standard deviation

**Figure 3:** Geometric mean plasma concentrations profiles following inhalation of 720 µg velsecorat and IV infusion of 30 µg $^{14}$C-velsecorat in healthy subjects: A) unchanged velsecorat pertaining to IV dose and inhaled dose; B) total radioactivity ($^{14}$C; unchanged velsecorat and metabolites [DRM]; pmol velsecorat Eq/L) and $^{14}$C-velsecorat following IV administration

Footnote: DRM, drug-related material; IV, intravenous; *Unit is pmol velsecorat Eq/L

**Figure 4:** Reconstructed AMS chromatogram of pooled human plasma extracts following IV administration of $^{14}$C-velsecorat: A) $AUC_{0\text{-}12h}$ pool; B) 24-hour pool

Footnote: N=5; AMS, accelerator mass spectrometry; AUC, area under the curve; IV, intravenous

**Figure 5:** Proposed metabolic scheme for velsecorat in humans

Footnote: Potential sites of metabolism, identified by MS/MS fragmentation analysis, are highlighted in the metabolite structures by colored Markush; MS/MS, tandem mass spectrometry

**Figure 6:** Reconstructed AMS chromatogram of A) pooled human urine (0–72 hours) and B) pooled human fecal homogenate extracts (0–96 hours) following IV administration of $^{14}$C-velsecorat

Footnote: N=5; AMS, accelerator mass spectrometry; IV, intravenous

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**Table 1. Summary of PK parameters following 720 µg inhaled velsecorat and 30 µg IV $^{14}$C-velsecorat**

| Parameter | Inhaled velsecorat (720 µg) n=6 | $^{14}$C-velsecorat (30 µg) n=6 | Total radioactivity n=6 |
|---|---|---|---|
| **$t_{max}$, hours†, median (range)** | 0.88 (0.50–1.32) | 1.00 (0.75–1.17) | 1.00 (0.75–1.05) |
| **$C_{max}$, pmol/L** | 509 (34.1) | 543 (52.9) | 565 (45.2)‡ |
| **$AUC_{0-t}$, pmol.hours/L** | 10,600 (40.7) | 671 (31.6) | 3,380 (15.0) |
| **AUC, pmol.hours/L** | 11,100 (38.3) | 700 (30.9) | 3,630 (13.0) |
| **$AUC_{0-12h}$*, pmol.hours/L** | NC | 681 | 1880 |
| **$t_{1/2}$, hours** | 26.9 (26.7) | 1.99 (20.2) | 18.4 (14.6) |
| **CL/F \| CL, L/hour** | 107 (38.3) | 70.7 (30.7) | 13.6 (13.0) |
| **$V_z$/F \| $V_z$, L** | 4,140 (52.6) | 203 (23.2) | 362 (20.5) |
| **$V_{ss}$, L** | - | 113 (30.5) | 276 (27.9) |
| **MRT $AUC_{0-t}$, hours** | - | 1.20 (17.2) | 14.9 (23.2) |
| **MRT AUC, hours** | - | 1.59 (18.7) | 20.3 (21.1) |
| **F $AUC_{0-t}$, %** | 65.90 (22.7) | - | - |
| **F AUC, %** | 66.31 (21.6) | - | - |

All values are geometric mean (CV%) unless otherwise specified.

*The values were calculated *post hoc*.

†Time is recorded from the start of the 1h IV infusion

‡Units expressed as pmol velsecorat Eq/L

AUC, area under the curve; $AUC_{0-t}$, the area under the concentration-time curve from dosing to the last measurable concentration; CL, total body clearance of drug from plasma (IV dose only); CL/F, apparent total body clearance of drug from plasma (inhaled dose only); $C_{max}$, the maximum observed plasma concentration; CV%, percentage of coefficient of variation; F, absolute bioavailability; IV, intravenous; MRT, mean residence time of the unchanged drug in the systemic circulation (IV dose only); NC, not calculated; PK, pharmacokinetics; $t_{½}$, terminal phase half-life; $t_{max}$, the time from dosing at which the $C_{max}$ was apparent; $V_{ss}$, volume of distribution at steady state (IV dose only); $V_z$, volume of distribution (IV dose only); $V_z$/F, apparent volume of distribution (inhaled dose only)

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**Table 2. Quantification of the major components in plasma extracts following IV administration of $^{14}$C-velsecorat**

| | **$AUC_{0\text{-}12h}$ pool** | **24-hour pool** |
|---|---|---|
| | **% Sample radioactivity** | |
| Velsecorat | 39.7 | 5.3 |
| M1 | 22.1 | 67.6 |

AUC, area under the curve; IV, intravenous

N=5

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**Table 3. Quantification and identification of components in human urine and feces following IV administration of $^{14}$C-velsecorat**

| Compound | Structure modification | Urine | Feces |
|---|---|---|---|
| | | % matrix content (% of dose) | |
| Velsecorat | Unchanged compound | | |
| M2 | O-dealkylation of the indazole ether | | 30 (14.7) |
| M1 | Sulfate conjugate of M2 | 84.9 (19.6) | 4.4 (2.2) |
| M22 | Glucuronide conjugate of M2 | 8.1 (1.9) | |
| M44 a | Ring deethylation of 1,4-benzodioxane | | |
| M46 a | Ring opening of 1,4-benzodioxane (diol) | | 25.4 (12.5) |
| M8 a | M46 + oxidation of 1 alcohol to acid | | |
| M23 a | M2 + hydrolysis of oxolane ring | | |
| M24 a | M23 + oxidation of 1 alcohol to acid | | 12.5 (6.2) |
| M32 a or M33 a | Hydrolysis of both the oxolane and 1,4-benzodioxane ring | | |
| M31 a | M32 + oxidation of 1 alcohol to acid in the opened 1,4-benzodioxane ring | | 11.8 (5.8) |
| M34 a | M32 + oxidation of 1 alcohol to acid in the hydrolyzed oxolane ring | | |
| M47 a | Hydrolysis of the oxolane ring | | |
| M9 a | M47 + oxidation of 1 alcohol to acid | | |
| M10 a | Monooxygenation of velsecorat | | 3.2 (1.6) |
| M11 a | Monooxygenation of velsecorat | | |
| M35 a | M47 + decarbonylation of the 1,4-benzodioxane ring | | 2.8 (1.4) |

[a]Co-eluting peaks, percentages are presented as sum

N=5

IV, intravenous

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**Figure 1**

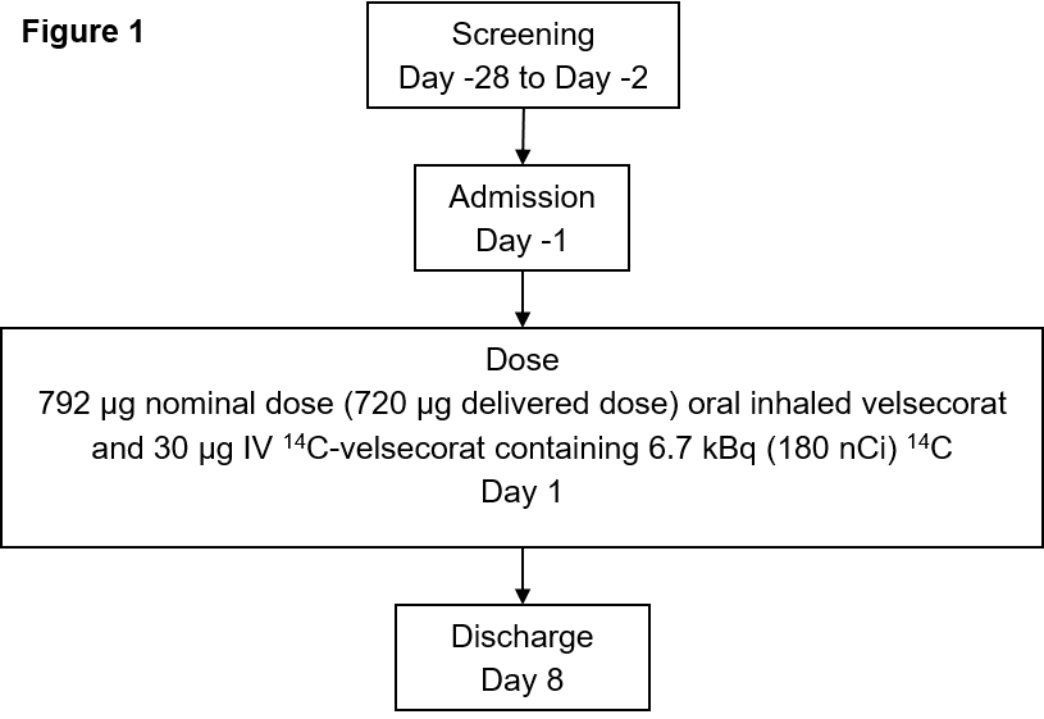

Screening
Day -28 to Day -2
Admission
Day -1
Dose
792 µg nominal dose (720 µg delivered dose) oral inhaled velsecorat
and 30 µg IV $^{14}$C-velsecorat containing 6.7 kBq (180 nCi) $^{14}$C
Day 1
Discharge
Day 8

**Figure 2**

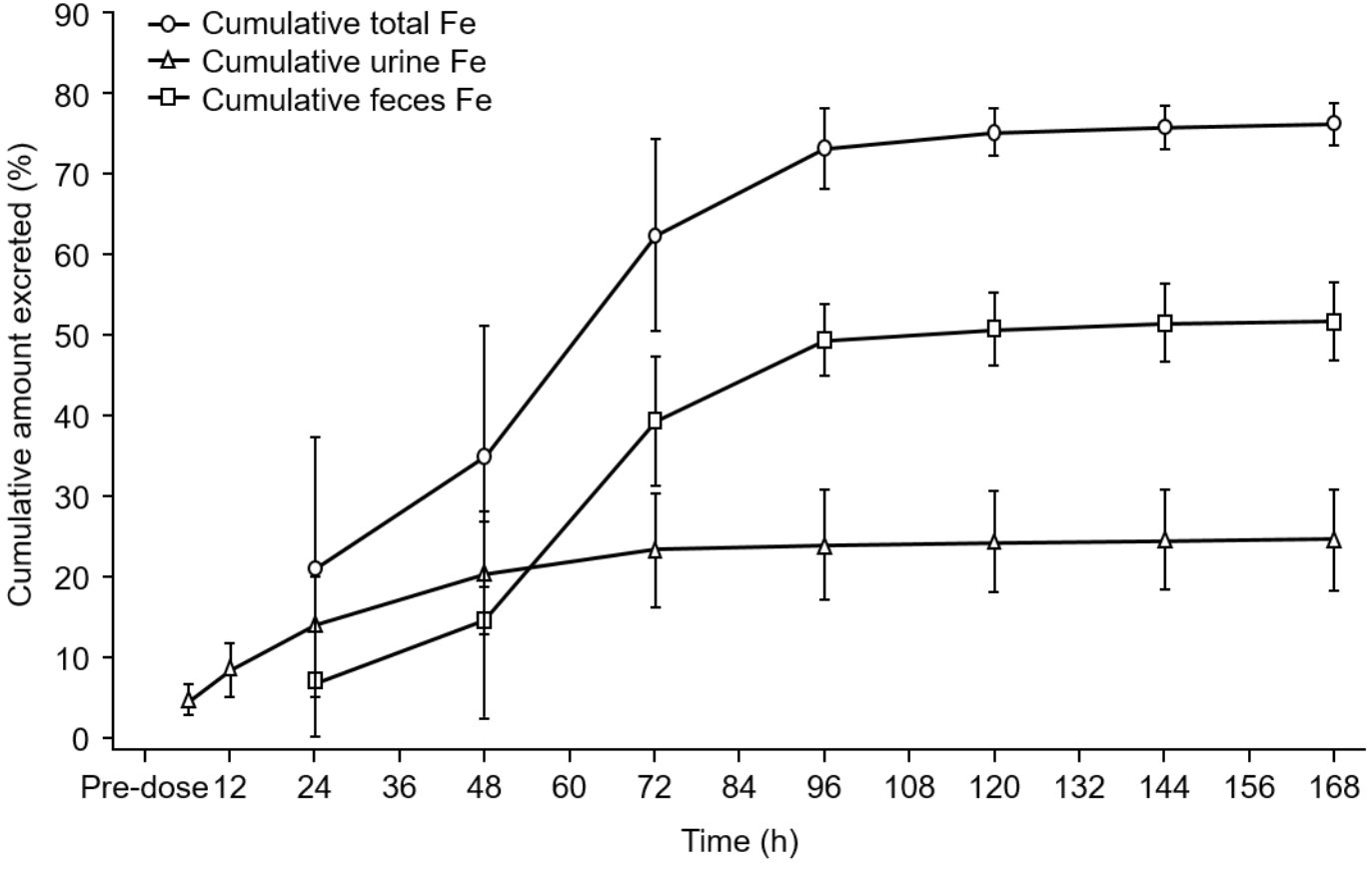
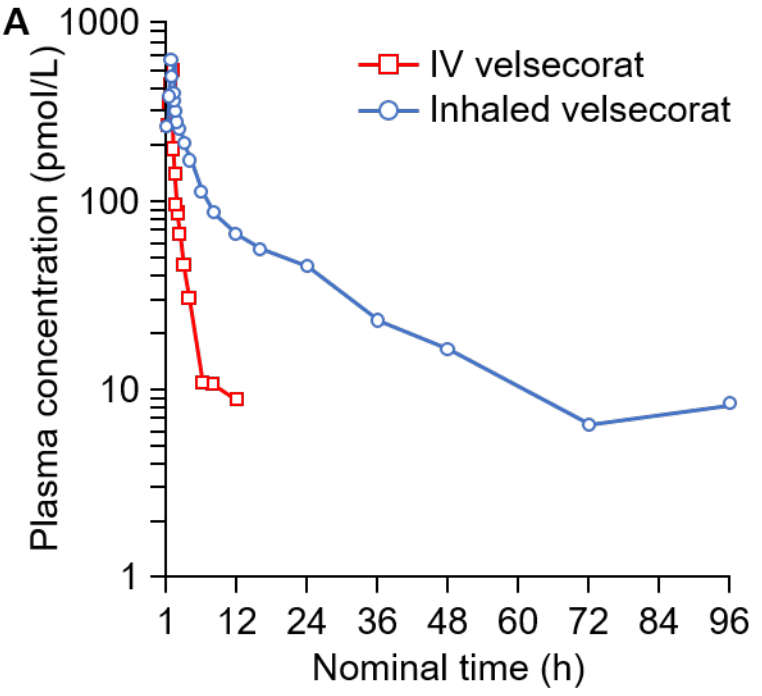

Cumulative total Fe
Cumulative urine Fe
Cumulative feces Fe
Cumulative amount excreted (%)
0
10
20
30
40
50
60
70
80
90
Pre-dose
12
24
36
48
60
72
84
96
108
120
132
144
156
168
Time (h)

**Figure 3**


A
1000
100
10
1
Plasma concentration (pmol/L)
IV velsecorat
Inhaled velsecorat
1
12
24
36
48
60
72
84
96
Nominal time (h)

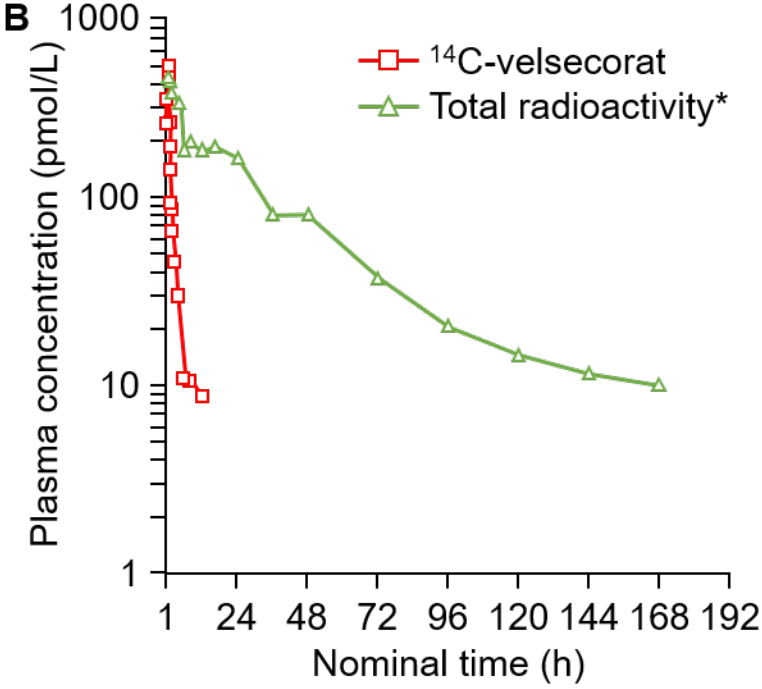

B
1000
100
10
1
Plasma concentration (pmol/L)
14C-velsecorat
Total radioactivity*
1
24
48
72
96
120
144
168
192
Nominal time (h)

**Figure 4**

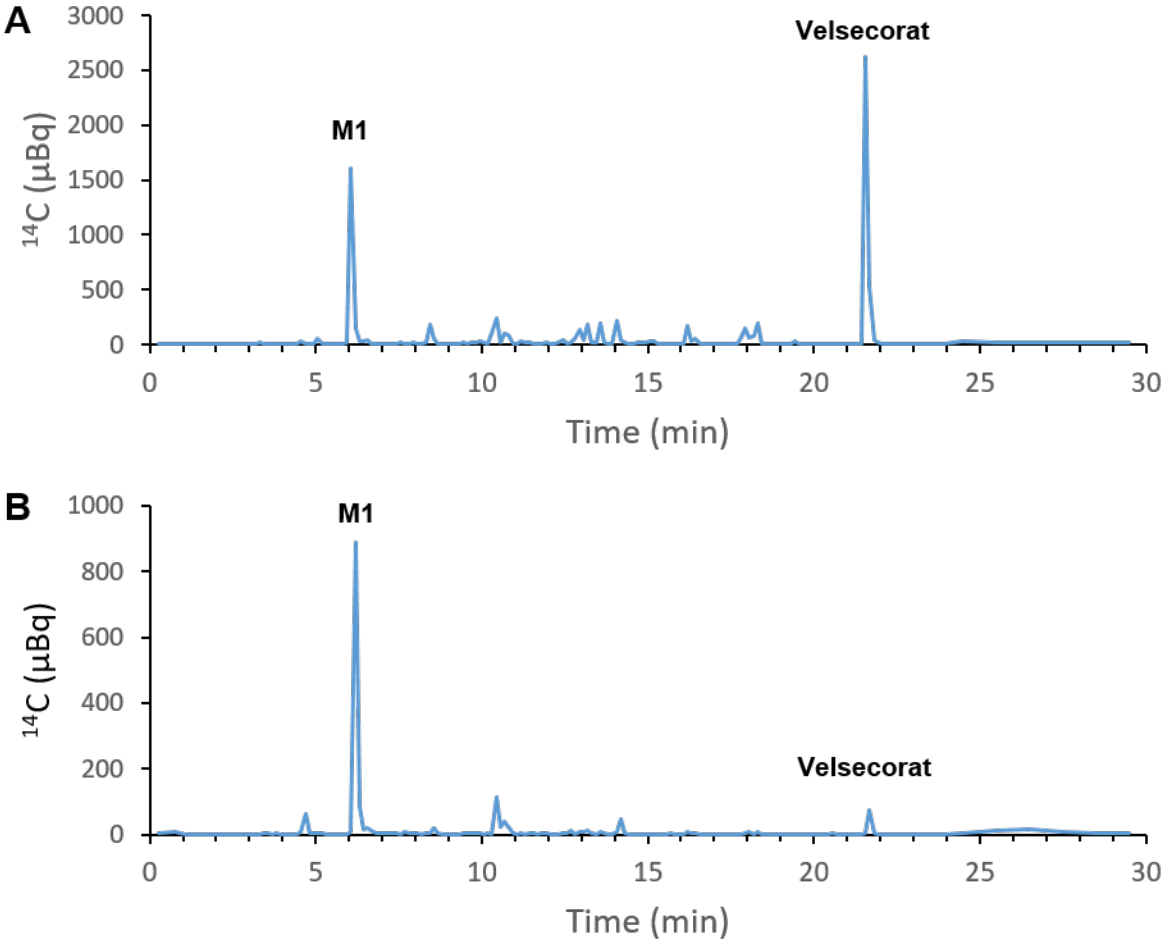

A
3000
2500
2000
1500
1000
500
0
$^{14}$C (µBq)
M1
Velsecorat
0
5
10
15
20
25
30
Time (min)
B
1000
800
600
400
200
0
$^{14}$C (µBq)
M1
Velsecorat
0
5
10
15
20
25
30
Time (min)

**Figure 5**

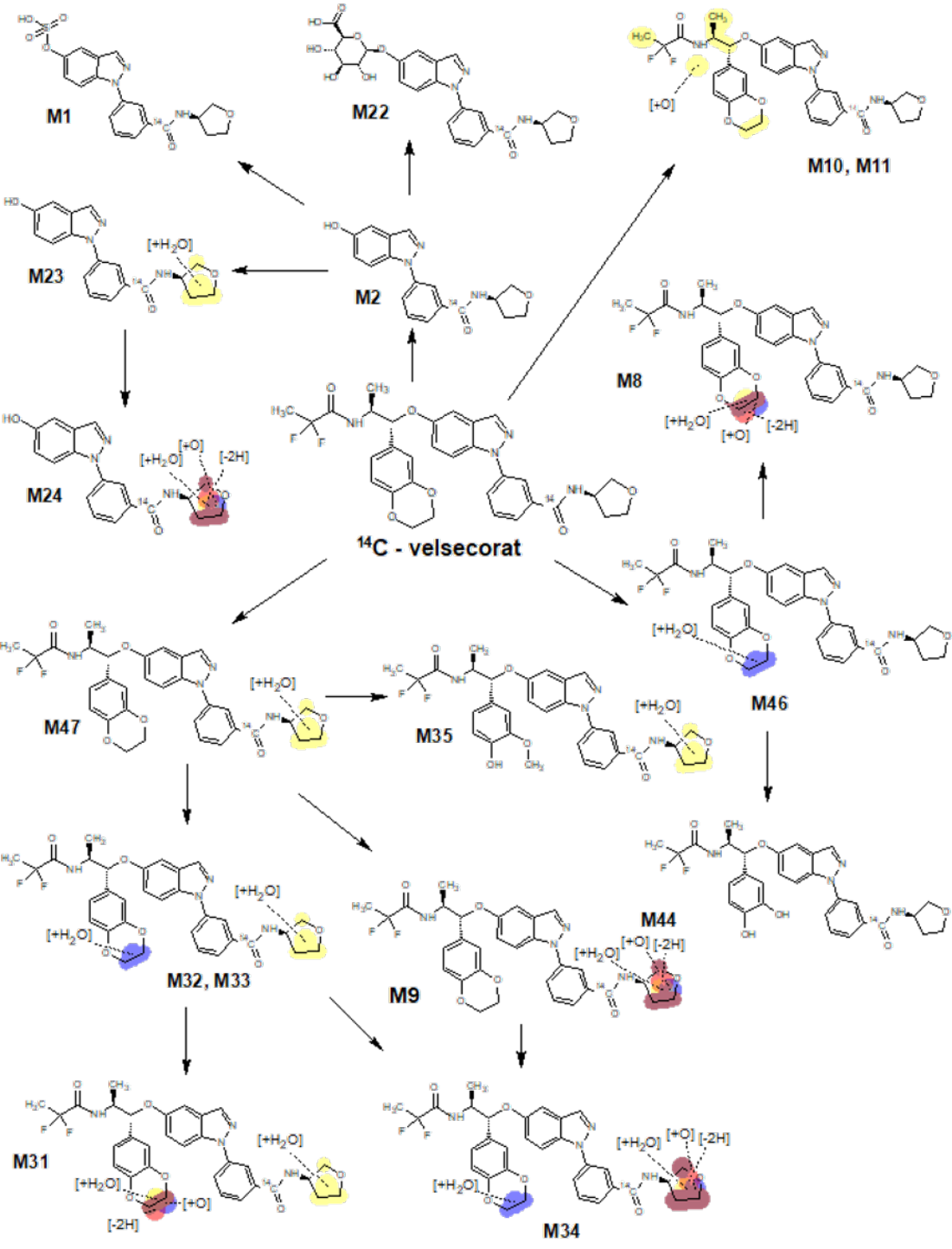
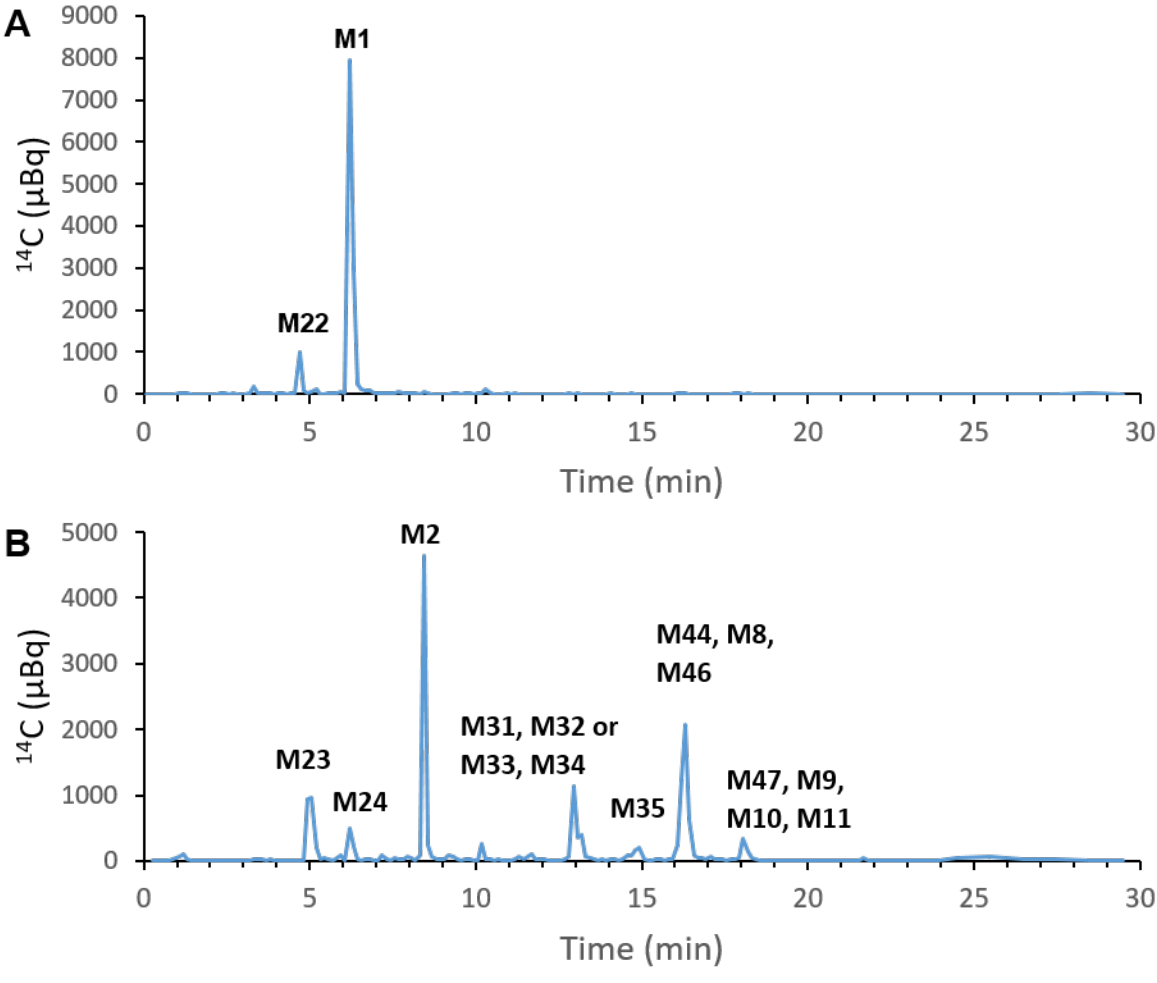

M1
M22
M10, M11
M23
M2
M8
M24
$^{14}$C - velsecorat
M46
M47
M35
M44
M32, M33
M9
M31
M34

Figure 6


A
M1
M22
$^{14}$C (µBq)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0
5
10
15
20
25
30
Time (min)
B
M2
M44, M8,
M46
M31, M32 or
M33, M34
M23
M24
M35
M47, M9,
M10, M11
$^{14}$C (µBq)
5000
4000
3000
2000
1000
0
0
5
10
15
20
25
30
Time (min)