^{14}C-Isomazole Disposition in Man After Oral Administration

James R. Woodworth,[1,2] Allyn F. DeLong,[1] A. F. Fasola,[1] and Samuel Oldham[1]

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A 50-mg dose containing 50 μCi ^{14}C-isomazole was administered orally to five healthy male volunteers. Blood, plasma, urine, feces, and saliva were collected and measured for total ^{14}C; in addition, all collections except feces were measured for parent drug (ISO) and three metabolites: hydroxyisomazole (OHISO) and sulfone (SULF) and hydroxysulfone (OHSULF) analogues. Urine and fecal recoveries accounted for 97.0% of the drug administered, with 62.6% excreted in urine and 32.4% in feces. Only 47% of the drug recovered in urine could be identified, with ISO the largest constituent. Total plasma ^{14}C peaked at 1.5 hr, indicating rapid absorption, and produced a mean half-life of 3.7 hr. This was similar to the total ^{14}C half-life found in blood (3.1 hr) but longer than in red blood cells (1.8 hr) or saliva (1.4 hr), suggesting that different ISO-related compounds contributed to the results found in each fluid or tissue. An unidentified metabolite(s) composed a large portion of circulating plasma ^{14}C and produced the longer half-life encountered in plasma. ISO exhibited a short half-life (1.35 hr), a high oral clearance (Cl_s/F; 24.2 ml/min/kg), and some extravascular distribution (V_β; 3.07 L/kg). Total ^{14}C in red blood cells and saliva related very well to plasma ISO disposition, suggesting preferential distribution of parent drug across cellular membranes. The estimated RBC:plasma ISO ratio (1.79) confirmed this hypothesis. Saliva may be used as a noninvasive means to monitor ISO disposition.

KEY WORDS: isomazole; inotropic agent; pharmacokinetics; metabolism; disposition; humans.

INTRODUCTION

Substantial progress has been made in recent years for the treatment of congestive heart failure, especially with the advent of angiotensin converting enzyme (ACE)[3] inhibitors (1). However, ACE inhibitors are used in addition to inotropic agents for proper disease management. Vasodilators are also considered beneficial by reducing the workload on the heart (2,3).

Isomazole is one of a new class of compounds that provide combined inotropic and vasodilatory properties. It acts primarily as a phosphodiesterase inhibitor and in animal models has been shown to stimulate cardiac contractility and reduce afterload with only minimal increases in heart rate (4,5). These properties, along with the drug's excellent safety profile in animals (6,7), have promoted initial investigations in man.

Pharmacokinetic and drug disposition studies would define drug elimination patterns and aid in the development of proper dosing regimens. A study was performed in which radiolabeled isomazole was administered orally in an effort to define (a) the drug's excretion routes and (b) the general pharmacokinetics of isomazole and its metabolites in man.

MATERIALS AND METHODS

Study Design

Five healthy male volunteers were enrolled in this study. The subjects were between 25 and 39 years old and within 15% of their ideal body weight for their age, height, and frame size (the Metropolitan Life Health Insurance tables were used as a reference). Before study acceptance, all subjects were given a complete physical examination, including complete blood and urine chemistry evaluations, a chest X ray, an electrocardiogram, and a 3-day Holter monitoring period. This study was reviewed and approved by a certified institutional review board. All participants gave their written informed consent.

A single capsule containing 50 μCi ^{14}C-isomazole (sp act, 0.994 μCi/mg isomazole base) [equivalent to 50 mg isomazole (ISO)] was given orally with 150 ml water. Immediately after ingestion of the ISO capsule, ^{51}Cr microspheres (1 μCi; given as three capsules) were administered to each subject. ISO was given after an overnight fast, and the volunteers remained fasted 4 hr after dosing. Blood (10-ml) and saliva (5- to 10-ml) samples were obtained at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, 16, 24, and 36 hr after drug administration. Total urine was collected for 24 hr prior to drug administration as a control, then from 0 to 2, 2 to 4, 4 to 8, 8 to 12, 12 to 24, and 24 to 48 hr after dosing. Total fecal collections were made daily for 168 hr (1 week) after dosing. Breath samples were collected at 0, 1, 2, 4, 6, and 8 hr after dosing. Radiolabeled compounds were trapped from breath samples in a 20% hyamine hydroxide solution; the subjects blew through a pipette into the solution for a 5-min interval at each collection time. All collections were terminated after 1 week.

Sample Analysis

Total radioactivity from saliva, plasma, urine, and breath samples were assessed by direct scintillation counting (breath samples by counting the hyamine hydroxide solution). Five-tenths milliliter of each was added to 10 ml of scintillation fluid (plasma, urine, and saliva—Scintisol, Isolab Inc., Akron, OH; breath samples in hyamine hydroxide—PermaFluor IV, Packard Instrument Co., Downer's Grove, IL) and counted in a Beckman LS-7000 liquid scintillation spectrometer. Fecal samples were initially homogenized 1:1 with water; 0.5 g of both fecal homogenate and blood samples was then oxidized and counted. Combustion of samples was performed in a Packard Model 306B sample oxidizer, with radioactivity captured and collected in Per-

[1] Lilly Laboratory for Clinical Research, Wishard Memorial Hospital, 1001 West 10th Street, Indianapolis, Indiana 46202.

[2] To whom correspondence should be addressed.

[3] Abbreviations used: ISO, isomazole; OHISO, hydroxyisomazole; SULF, the sulfone metabolite of isomazole; OHSULF, the hydroxysulfone metabolite of isomazole; Cl_s, total systemic clearance; Cl_r, renal clearance; V_β, volume of distribution; V_{ss}, steady-state volume of distribution; $t_{1/2}$, half-life; MRT, mean residence time; RBC, red blood cells; Hct, hematocrit; ACE, angiotensin converting enzyme.

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maFluor IV. ^{14}C counting efficiencies were 65% for blood and feces and 90% for plasma, saliva, and urine. Sample quench and counting efficiency were corrected by an external standard/channel ratio method.

Isomazole (ISO) and three metabolites, hydroxyisomazole (OHISO), a sulfone analogue (SULF), and a hydroxysulfone analogue (OHSULF), were quantified in plasma, urine, and saliva samples. An HPLC method with fluorescence detection was used, with the specifics of the assay described elsewhere (9). The lower limit of sensitivity determined for ISO was 25 ng/ml; ISO metabolites had a greater detection sensitivity. OHISO and OHSULF each were validated to 1.0 ng/ml; SULF was validated to 6.25 ng/ml.

Red blood-cell ^{14}C concentrations (C_{rbc}) were calculated from the total ^{14}C in plasma (C_p) and blood (C_b) and the hematocrit (Hct):

$$C_{rbc} = \frac{C_b - (1 - \text{Hct}) \cdot C_p}{\text{Hct}}$$

The derivation for this relationship is provided in the Appendix.

Pharmacokinetic Analysis

Standard noncompartmental procedures were used for calculating the various pharmacokinetic parameters reported.

RESULTS

All study subjects completed the study. No detectable radioactivity was present in breath samples.

Excretion of total ^{14}C-labeled compounds in urine and feces accounted for 97.0 ± 9.22% (mean ± SD) of the dose administered (Table I) during the 4-day urine collection and 7-day fecal collection period. ^{51}Cr fecal excretion did not begin until the second day after dosing and was essentially complete (>99% of all ^{51}Cr recovery) by the fourth day of collection. Fecal ^{14}C excretion coincided with fecal ^{51}Cr excretion. The majority of the dose appeared in the urine; Fig. 1 shows the urine accumulation over time of all measured compounds. Urinary excretion was essentially complete by 24 hr. Identifiable compounds after 24 hr accounted for less than half (46.9%) of the total radioactivity excreted renally and only 28.7% of the dose administered.

Plasma measurements of total radioactivity and individual compounds produced the concentration vs time curves in Fig. 2. Pharmacokinetic calculations from these data are given in Table II. Absorption was rapid, with maximum ISO concentrations produced within 1 hr of dosing in all but one subject. Elimination was also rapid, with plasma concentrations below detectable limits for ISO, OHISO, SULF, and OHSULF by 12 hr after dosing. The combination of ISO and its three metabolite AUC values represented only 44.5% of the total plasma radioactivity.

Total ^{14}C in blood produced slightly lower concentrations than total radioactivity in plasma. Both plasma and blood data showed absorption and elimination patterns which were almost identical. However, the differences in concentrations between blood and plasma produced differences in pharmacokinetic values. A blood:plasma ratio declined over time until approximately 7 hr after dosing (Fig. 3). An average RBC:plasma ratio of 0.62 (±0.07) was calcu-

Table I. Cumulative Excretion, Urine and Feces

Substance collected	Compound	Excretion from 24-hr collection (% dose)	Excretion from total collection (% dose)
Urine	Total ^{14}C	61.2 ± 9.79	62.9 ± 9.90
	ISO	16.7 ± 6.08	16.7 ± 6.08
	OHISO	9.67 ± 3.70	9.67 ± 3.70
	SULF	0.69 ± 0.19	0.69 ± 0.19
	OHSULF	1.65 ± 0.33	1.66 ± 0.33
Feces	Total ^{14}C	0.21 ± 0.22	34.4 ± 9.16
Urine + feces	Total ^{14}C	61.4 ± 9.70	97.0 ± 9.22

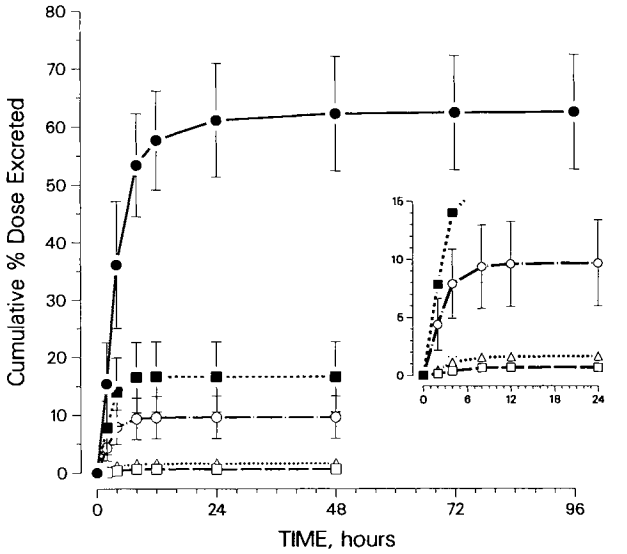

Fig. 1. Cumulative urinary excretion of total ^{14}C (●) and individual compounds. Filled squares (■) represent ISO; metabolites excreted include OHISO (○), SULF (□), and OHSULF (△). Inset represents the same plot with expanded scales. Bars represent standard deviation.

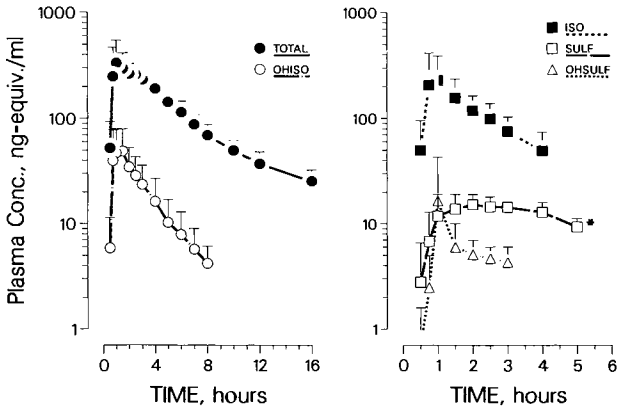

Fig. 2. Mean plasma concentration vs time curves for total ^{14}C (●), ISO (■), and the three measured metabolites [OHISO (○), SULF (□), and OHSULF (△)]. Bars represent the standard deviation. (* N = 4; all other points represent N = 5).

Table II. Mean (±SD) Pharmacokinetic Values for All Compounds

Compound	Matrix	C_{max} (ng-equiv/ml)	t_{max} (hr)	$t_{1/2}$ (hr)[a]	AUC [(ng-equiv) · (hr)/ml]	Cl_r (ml/min/kg)	Cl_s/F (ml/min/kg)	V_β/F (L/kg)	AUC (% total)
Total ^{14}C	Plasma	381	1.5	3.69	1801	4.13	6.59	2.28	
		(170)	(0.9)		(571)	(1.09)	(1.06)	(0.62)	
	Blood	417	1.5	3.08	1486		7.98	2.27	
		(207)	(0.9)		(474)		(1.23)	(0.82)	
	RBC[b]	462	1.4	1.81					
		(253)	(0.7)						
Specific compounds									
ISO	Plasma	265	1.1	1.35	527	4.01	24.2	3.07	28.4[c]
		(172)	(0.5)		(269)	(1.78)	(7.22)	(1.32)	(5.9)
OHISO	Plasma	61.3	1.4	2.48	165	7.38			8.86[c]
		(28.8)	(0.7)		(89.1)	(1.95)			(2.24)
SULF	Plasma	16.7	1.9	2.81	103	0.83			5.77[c]
		(3.4)	(0.7)		(32.7)	(0.27)			(1.50)
OHSULF	Plasma	6.7	1.6	1.60	29.2	9.39			1.49[c]
		(3.2)	(0.8)		(13.6)	(4.21)			(0.31)
Total ^{14}C	Saliva	790	1.4	1.41	1801				100[d]
		(611)	(0.7)		(571)				
ISO	Saliva	719	1.3	1.12	1261				78.8[d]
		(637)	(0.5)		(860)				(9.4)
OHISO	Saliva	133	1.6	1.36	342				19.3[d]
		(129)	(0.6)		(323)				(5.84)
SULF	Saliva	24.8	2.4	2.01	119				9.15[d]
		(10.3)	(1.2)		(31.9)				(3.44)
OHSULF	Saliva	27.9	2.0	1.55	125				6.13[d]
		(20.3)	(0.5)		(154)				(2.78)

[a] Harmonic mean values.
[b] From calculated RBC concentrations.
[c] Values given as percentage of total ^{14}C *plasma* AUC.
[d] Values given as percentage of total ^{14}C *saliva* AUC.

lated, based upon blood and plasma AUC values and adjusting for the hematocrit. This average value does not reflect the observed time-dependent differences.

Total ^{14}C was calculated in red blood cells (RBCs) from total plasma and blood ^{14}C, using Eq. (1). Comparisons among the calculated RBC ^{14}C concentrations, total plasma ^{14}C, and plasma ISO indicated a decline of concentrations more closely related to plasma ISO than total plasma ^{14}C (Fig. 4). When compared to plasma ^{14}C, higher maximum ^{14}C concentrations were predicted in RBCs (Table II).

Saliva data from all analyzed compounds are shown in Fig. 5, with pharmacokinetic data given in Table II. Unlike plasma, the total ^{14}C in saliva followed an absorption and elimination pattern similar to ISO. The absorption and elim-

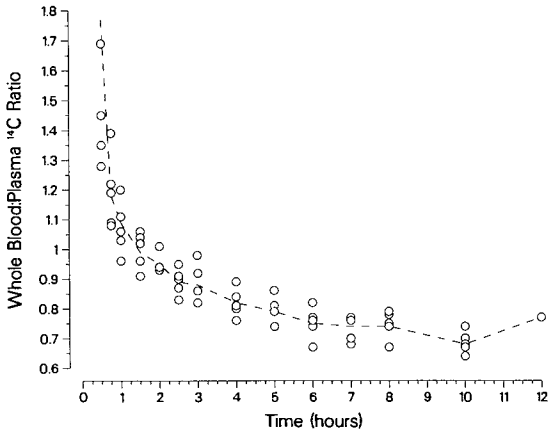

Fig. 3. Blood:plasma total ^{14}C ratio for all samples over time. The dashed line represents mean values.

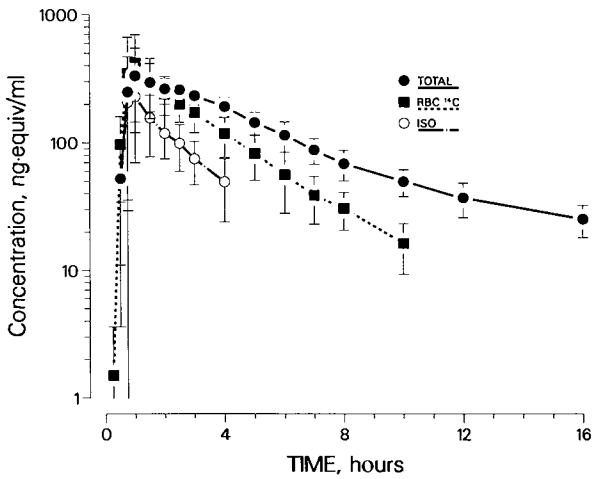

Fig. 4. Mean calculated RBC total ^{14}C values vs time (●). Total plasma ^{14}C (○) and plasma ISO (■) are included for comparative purposes. Bars represent standard deviations.

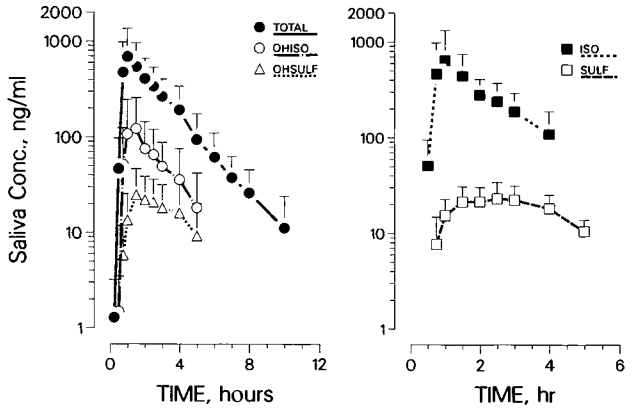

Fig. 5. Mean saliva concentration vs time curves for all isomazole-related compounds. Left: (●) total ^{14}C; (○) OHISO; (△) OHSULF. Right: (■) ISO; (□) SULF. Bars represent standard deviations.

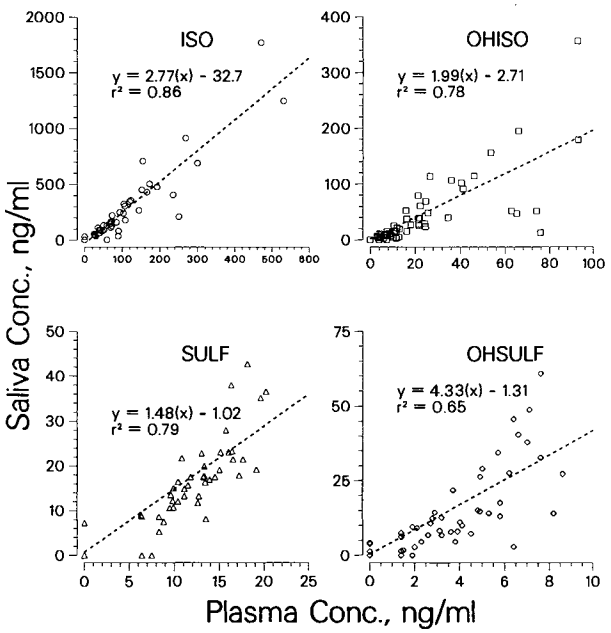

Fig. 6. Regression between ISO and metabolites, saliva vs plasma.

ination patterns for all other compounds were comparable between plasma and saliva. Mean salivary concentrations of all compounds were higher than mean plasma concentrations. However, when comparisons were made within each study subject, only ISO and OHSULF had consistently higher saliva concentrations (ISO concentrations were two to three times higher in saliva compared to plasma). AUC comparisons suggested total saliva radioactivity was composed exclusively of ISO and the three identified metabolites, with ISO comprising the largest percentage (Table II).

Figure 6 shows the correlation between saliva and plasma concentrations measured in all samples for each of the separate compounds. From the different plots, the slopes represent each compound's affinity for saliva over plasma. Correlations for all compounds suggested ISO was best predicted between the measured fluids.

DISCUSSION

ISO is known to be metabolized in animals (6–8), and it is clear from both human plasma and urine data that extensive metabolism also occurs in man. Urinary ^{14}C collection showed a substantial amount of radioactivity excreted renally (approximately 55%) which could not be identified. Total plasma ^{14}C had a longer half-life than ISO or any identified metabolite, suggesting that at least one unknown metabolite has a prolonged elimination (in comparison to ISO). The elimination half-life for total radioactivity was less than 4 hr, indicating efficient elimination of both the parent compound and all metabolites.

Urinary excretion was reflective of plasma concentrations, with ISO representing the largest amount of recovered drug and OHISO the major identified metabolite. In addition, the urinary recovery of identifiable compounds represented less than half of the total recovery. This inability to identify the majority of ^{14}C recovered in urine agreed with the large fraction of unidentified ^{14}C in plasma. Furthermore, the urinary recovery was essentially complete by the end of 24 hr, which is in agreement with the short half-lives of all compounds. Fecal recovery reflected the natural prolonged excretion process when compared to urinary excretion and, although not indicated here, was complete within 72 hr after dosing.

Fecal excretion contributed significantly to the overall elimination of ^{14}C. The ^{14}C-labeled compounds appearing in the feces could represent either unabsorbed drug or compounds excreted by the fecal route. Additional studies with intravenous ISO administration (9) showed pharmacokinetic values similar to those reported in this study, suggesting complete absorption after oral administration. Thus, it is likely that fecal ^{14}C recovered in this study resulted from biliary and fecal excretion rather than lack of absorption.

Plasma measurement of individual compounds showed that the highest concentrations were produced by ISO. These data suggest the metabolites have a greater volume of distribution or that the metabolites are not produced in the same quantities as the parent drug. If the urine data are representative of total drug excretion (urine + feces), OHISO, SULF, and OHSULF combined were less than 20% of the total drug collected.

The disposition of OHISO is dependent upon its formation from ISO and exhibits a half-life similar to that of ISO. The actual elimination half-life of OHISO may be shorter than that determined in this study. A true determination of OHISO half-life cannot be made without separate administration of OHISO. Alternatively, SULF and OHSULF exhibit longer half-lives than ISO. These values would remain unchanged if SULF or OHSULF were administered individually.

The cumulative plasma radioactivity of all identified compounds accounts for only a fraction of the total ^{14}C AUC. This result agrees with the large unidentified urinary fraction. However, in saliva ISO and its metabolites account for the total amount of radioactivity measured (103%). These data suggest that the unidentified compounds are highly polar or exhibit other qualities that prohibit them from crossing cell membranes easily.

The distribution of ^{14}C into RBCs is rapid, producing a high RBC:plasma ratio almost immediately (Fig. 3), but a slow "redistribution" of RBC ^{14}C back to plasma occurred over 7 hr. As indicated by the calculated RBC ^{14}C concentrations, the radioactivity within the cell appears to repre-

sent different compounds than plasma radioactivity, and this "reequilibration" appears to represent the difference of elimination rates between total ^{14}C in the RBCs and plasma.

The distribution of ISO and ISO-related compounds into RBCs suggests that the ratio of compounds within the RBC is different from that in plasma, since the decline of RBC ^{14}C concentrations over time is comparable to plasma ISO and not total plasma ^{14}C. These data further suggest that the unidentified metabolites have difficulty entering RBCs, if they do so at all. If one assumes that the salivary ^{14}C data reflect the distribution of ISO-related compounds that cross cellular membranes and that 78% of the total ^{14}C in RBCs reflects ISO (based upon AUC comparisons of ISO with total ^{14}C in saliva), the RBC:plasma ratio can be recalculated for ISO alone rather than total ^{14}C. In doing so, ISO appears to have an affinity for RBCs, with a mean (±SD) RBC: plasma ratio of 1.79 (±0.27) based upon AUC ratios. The high affinity for ISO in RBCs suggests that blood concentrations should be measured when monitoring ISO disposition rather than plasma concentrations or that adjustments should be made for ISO distribution into RBCs.

ISO and its metabolites produced higher salivary concentrations when compared to plasma. This correlation would allow noninvasive sampling for monitoring ISO disposition in future studies. Pharmacokinetic calculations based upon plasma AUC values (clearance, volume of distribution) could be calculated based upon these established correlations. However, the variability between plasma and saliva concentrations for any of ISO's metabolites would not allow beneficial plasma predictions based upon saliva data. Only ISO produced a correlation coefficient (r^2) above 0.85.

In conclusion, these pharmacokinetic data suggest a rapid elimination of ISO. Early data from patients with congestive heart failure indicate that an inotropic response is achieved by dosing four times daily. Renal excretion of unchanged ISO constitutes a minor elimination route overall, so decreased kidney function (a problem in patients with congestive heart failure) would not be expected to affect dosing appreciably. ISO appears to distribute preferentially into red blood cells, while its unidentified metabolites do not easily cross cellular membranes. Concentrations of ISO and its metabolites are detectable in saliva. However, if salivary concentrations are monitored, assessments should be limited to the parent compound.

APPENDIX: DERIVATION FOR RED BLOOD-CELL CONCENTRATIONS

A_b = total amount of drug in blood
A_p = total amount of drug in plasma
A_{rbc} = total amount of drug in blood cells
Hct = hematocrit
C_p = plasma concentration
C_b = blood concentration
C_{rbc} = concentration in blood cells
V = total blood volume (approx. 12 L)

It is understood that the following relationships hold true:

$$A_b = V \cdot C_b$$
$$A_p = V \cdot (1 - \text{Hct}) \cdot C_p$$
$$A_{rbc} = V \cdot \text{Hct} \cdot C_{rbc}$$

A ratio can be constructed:

$$\frac{A_p}{A_{rbc}} = \frac{V(1 - \text{Hct})(C_p)}{V(\text{Hct})(C_{rbc})}$$

which can be simplified to

$$\frac{A_p}{A_{rbc}} = \frac{(1 - \text{Hct})(C_p)}{(\text{Hct})(C_{rbc})}$$

Since the following is true:

$$A_{rbc} = A_b - A_p$$

substitutions can be made for A_{rbc} and C_bV for A_b, with simplification resulting in the following:

$$A_p(\text{Hct})(C_{rbc}) = (C_bV - A_p)(1 - \text{Hct})(C_p)$$

Substituting $V \cdot (1 - \text{Hct}) \cdot C_p$ for A_p and performing the multiplication,

$$(V)(1 - \text{Hct})(C_p)(\text{Hct})(C_{rbc}) = (C_bV)(1 - \text{Hct})(C_p) - (1 - \text{Hct})(C_p)(V)(1 - \text{Hct})(C_p)$$

Eliminating common variables produces the following:

$$(\text{Hct})(C_{rbc}) = C_b - (1 - \text{Hct})(C_p)$$

Thus,

$$C_{rbc} = \frac{C_b - (1 - \text{Hct})(C_p)}{\text{Hct}}$$

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