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ORIGINAL ARTICLE

Pharmacokinetic Profiles of Metamizole Metabolites after Intramuscular and Intravenous Administration in Healthy Arabian Horses

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Abstract

Metamizole sodium (MT) is an analgesic and antipyretic drug molecule used in humans, horses, cattle, swine, and dogs. Metamizole rapidly hydrolyzes and turns into methylamino antipyrine (MAA), an active primary metabolite of MT. The present study aims to determine the pharmacokinetic (PK) profiles of MT metabolites after intravenous (IV) and intramuscular (IM) administration into sex of Arabian horses (*Equus ferus* caballus) using a cross-over study design. The plasma samples were extracted by solid-phase extraction (SPE) method, and plasma concentrations of MT metabolites were analyzed by high-performance liquid chromatography (HPLC). After administrations of MT, plasma concentrations of methylamino antipyrine (MAA), amino antipyrone (AA), and acetylamino antipyrone (AAA) were determined within range of 15 min-12 h. Plasma concentrations of AA and AAA were lower than the plasma concentrations of major metabolite MAA at each sampling point. The PK parameters were statistically evaluated for MT's metabolites between male and female horses and also between IM and IV administrations of PK parameters such as C_{max}, t_{max}, $t_{1/2\lambda z}$, $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, λz, Cl and V_{ss} ($p < .05$). The AUC_{IM}/AUC_{IV} ratio in female and male horses for MAA was 1.19 and 1.13, respectively. The AUC_{IM}/AUC_{IV} ratio for AA was lower than those found for MAA. AUC_{IM}/AUC_{IV} ratio was statistically significantly different between male and female horses for AA ($p < .05$). According to these results, some PK parameters such as Cmax, AUC, and MRT, MAA and AA concentrations have shown statistically significant differences by MT administrations.

KEYWORDS
Arabian horses, HPLC, Metamizole, pharmacokinetics, solid-phase extraction

1 | INTRODUCTION

Metamizole sodium (MT), *N*-[(2,3-dimethyl-5-oxo-1-phenyl-3-pyrazolin-4-yl)-*N*-methylamino] methanesulphonate, also known as dipyrone, is a pyrazolone derivative nonsteroidal anti-inflammatory drug (NSAID) with antipyretic and antispasmodic properties. The prodrug that is used both in human and in veterinary medicine and rapidly hydrolyzed to active metabolites.(Rogosch et al., Nikolova et al., 2013, Jasiecka et al., 2014, Hassan et al., 2011, Teixeira et al., 2013, Zanuzzo et al., 2015, Zukowski & Kotfis, 2009).

NSAIDs work by blocking the synthesis of cyclooxygenase derived from eicosanoid inflammatory mediators, including

prostaglandins and thromboxane. In veterinary medicine, NSAIDs (Flunixin-meglumine, metamizole sodium, etc.) are commonly used as an analgesic and antipyretic agent in various animal species including horses. (Dirikolu et al., 2009; Driessen, 2007; Elmas et al., 2005; Imagawa et al., 2011; Kamerling et al., 1989; Lindegaard et al., 2011; Tanyildizi & Bozkurt, 2003).

In humans, after oral and parenteral application, MT is rapidly metabolized to methyl amino antipyrine (MAA), formyl amino antipyrine (FAA), amino antipyrine (AA) and *N*-4-acetyl amino antipyrine (AAA) which further prevents detection of the parent drug molecule in plasma and serum. The MAA is the major metabolite of MT with pharmacological activity (Aupanun et al., 2016; Klaus et al., 1997). The other metabolites of MT including AA, AAA, and FAA are pharmacologically inactive (M. *et al*. 1995, Vlahov et al., 1990). There are several publications reporting the plasma concentrations of MT's metabolites in different animal species including; rats (Domínguez-Ramírez et al., 2012), dogs (Imagawa et al., 2011), horses (Giorgi et al., 2017), sheep (Giorgi et al., 2015), donkeys (Aupanun et al., 2016), and rabbits (Baumgartner et al., 2009). Analysis of NSAIDs and their metabolites at trace levels require sensitive and selective analytical techniques such as gas chromatography with a mass spectrometer (GC-MS) (Maurer, 2002), HPLC (Kobylińska et al., 2000; Kvaternick et al., 2007; Mikami et al., 2000; Ptáček et al., 2001), affinity chromatography (Fiori et al., 2004), thin-layer chromatography, and liquid chromatography-tandem mass spectrometry (Jedziniak et al., 2013; Kamerling et al., 1989; Letendre et al., 2007; Penney et al., 2005; Singh et al., 1991).

To the best of our knowledge, PK parameters of MT's metabolites have not been reported in Arabian Horses (*Equus ferus* caballus). Therefore, the main objective of this study is to determine both plasma concentrations and PK parameters of MT's active and inactive metabolites in both sexes of Arabian Horses (Equus ferus caballus) using cross-over study design following IV and IM administrations of MT.

2 | MATERIALS AND METHODS

2.1 | Chemicals and Reagents

MAA, AA, AAA, and FAA analytical standards and internal standard (IS) metoclopramide powder (>99.0% purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), sodium hydroxide (NaOH), orthophosphoric acid (H_3PO_4), methanol, ethyl acetate, and acetonitrile were obtained from Merck (Darmstadt, Germany). All chemicals in the used experiments were in analytical grade. In all cases, the water used was of HPLC quality and purified in a Milli-Q system (Millipore, Bedford, MA, USA). The C18 SPE cartridges (Supelco HLB 30 mg, 1cc) were obtained from Supelco (St. Louis, MO, USA).

2.2 | Animal Treatment and Sampling

The Arabian horses (*Equus ferus* caballus) used for cross-over study design were supplied by private breeders from Malatya province in Turkey. The ethics protocol for this study was endorsed by the Ethics Committee (Faculty of Medicine, Inonu University, Malatya, Turkey 2016/A-25). A routine clinical examination of the horses was performed by a veterinarian before the initiation of the study. From one month before the first administration until the last administration of MT, the horses did not receive any medication (antibacterial and analgesic, etc.). Before and during the 15-day study period, horses were fed daily with oat (1.5 kg/horse), soybean and bran (200g/horse), salt (50g/horse), alongside grazing in pasture. Fresh clover and water was given ad libitum. Two jugular venous catheters, one on each side (for MT administration and sample collection, respectively), were placed in each animal one day before the study began. Fourteen healthy Arabian horses (seven males and seven females) averaging 2.25 years of age (2–3-year interval) and weighing 360–390 kg (mean 380) for female and 380–420 kg (mean 400) for male were chosen for this study. The animals were randomly assigned to two treatment groups (A = 7 and B = 7) in an open, single-dose, two-treatment, and concurrent experiment.

In each study period, each horse was administered a single dose of 25 mg/kg MT (Eforjin® Inj. Sol., 500 mg/ml Metamizole, Alke Ilaç San. A.Ş., Istanbul, Turkey) either IV or IM. After a washout period of 3 weeks, each horse received the opposite route of administration in a two by two Latin Square (cross-over) design.

The blood samples (app. 10 ml) were collected via previously inserted catheters into K_3EDTA tubes at 0, 15, 30, 45, 60, 90 min., and then at 2, 3, 4, 6, 8, and 12 hr following administrations. The blood samples were centrifuged at 5000 rpm for 15 min. and were prepared within 30 min following collection. The plasma samples were then stored at −80°C until they were analyzed. The samples were analyzed in the week following their storage.

2.3 | Preparation of standard solutions

Samples of 1000 μg/ml master solution were prepared by dissolving 0.010 g of each metabolite in 10 ml of methanol–water (1:1 v/v). Calibration curves for the standards were performed daily by diluting the standards and IS into methanol–water (1:1 v/v). The target compounds exhibited good linearity between 0.01 and 50.00 μg/ml for MAA; between 0.05 and 100.00 μg/ml for AA and FAA; and between 0.01 and 100.00 μg/ml for AAA. Standard calibration curves were generated for each metabolite by plotting the peak area ratio of metabolite/IS plasma metabolite concentration. A linear relationship was found when the peak area ratio of the metabolite/IS was plotted against metabolite plasma concentration. Each calibration curve was constructed using standards of six different concentrations and measured in triplicate.

TABLE 1 Method validation parameters for analytical procedures used for the determination of MT's metabolites in horse plasma samples

Validation parameters	MAA	AA	FAA	AAA
Linearity range, μg/ml	0.01–50.00	0.05–100.00	0.05–50.00	0.01–100.00
Correlation coefficient(r^2)	0.99999	0.99977	0.99988	0.99972
Regression equation	$y = 3{,}7.10^{-5}x + 2{,}1.\ 10^{-3}$	$y = 1{,}77.10^{-5}x - 0{,}478$	$y = 2{,}65.10^{-5}x - 4{,}32.10^{-3}$	$y = 1{,}85.10^{-5}x - 0{,}484$
System precision (RSD%)	3.81	6.41	7.19	4.40
Recovery*%	98.5	95.2	95.6	93.7
LOD**, μg/L	3.09	4.08	2.43	3.77
LOQ**, μg/L	10.31	13.61	8.11	12.58

*All control plasma sample spiked at three levels for recovery.
**n = 10 replicates.

2.4 | Solid-phase extraction (SPE) procedure

Cartridges were preconditioned by flushing with 3 ml of methanol and 1 ml of distilled water. 0.5 ml plasma was added to 100 μl IS (40 μg/ml). After 30 seconds of vortexing, 0.1 ml sodium hydroxide (1 N) was added, and then, the sample vortexed again. After the samples were loaded, the analytes and the IS were eluted with 3 ml of methanol and 0.5 ml ammonium acetate-acetonitrile (80:20 v/v). The flow rate was maintained in the range of 1–2 ml min^{-1}. The elute was collected in a 1.5 ml centrifuge tube, and the extract was evaporated to dryness in a Turbovap LV Evaporator (Zymark, Hopkinton, MA, USA) with a nitrogen stream at 36°C. The residue was reconstituted with 100 μl of mobile phase. 20 μl of this solution was injected into the HPLC system for analysis.

2.5 | Determination of MT's metabolites in samples by HPLC-DAD

The method used for the analysis of metabolites is based on a previously described method (Aupanun et al., 2016; Giorgi et al., 2015; Jedziniak et al., 2010; Zhang et al., 2016) with a few modifications. Analysis of MT's metabolites was carried out with a Shimadzu HPLC, equipped with Shimadzu DGU-20A5 model vacuum degasser and 20 ADXR solvent pump. Detection was performed with a Shimadzu 1100 DAD G 1315B photodiode array detector (DAD) used at 254 nm. Separation was carried out with an ODS-2 reverse-phase Kromasil-100–5 column (5 μm, 4.6 mm × 150 mm). The flow rate was 1.0 ml min^{-1}, and an isocratic elution was employed at room temperature throughout the analysis. The mobile phase consisted of 75 mM ammonium acetate (pH:5) and acetonitrile (72:28 v/v) after filtering through a 0.22 μm PTFE filter (ANPEL, Shanghai, China).

Determination of MT's metabolite in plasma samples by HPLC.

Identification of metabolites was achieved by comparing their retention time values and UV spectra with those of standards stored in a databank. Concentrations of the MT's metabolites were calculated from integrated areas of the sample and corresponding standards.

2.6 | Validation

The analytical merits such as correlation factor (r) ranges, accuracies, limit of detections (LOD), limit of quantifications (LOQ), precisions and recoveries were validated according to published guidelines (Castilhos et al., 2016; Gyenge-Szabó et al., 2014).

The HPLC method was validated using control plasma samples obtained from Arabian horses. All target molecules have shown good linearity with in the studied concentration ranges of 0.01–50.00 μg/ml, 0.01–100.00 μg/ml, and 0.05–100.00 μg/ml, respectively. Spiked samples were used to calculate recovery values. The limit of detection (LOD) and limit of quantification (LOQ) were calculated by multiplying 3.3 and 10 with s/a, respectively, where "s" is the standard deviation of blank and "a" is the slope of the regression equation. The identification of the MT's metabolites in all samples was carried out by the proposed method. The precision of experimental results was assessed via peak areas of target molecules and presented as the standard deviation for three replicate runs. The calibration parameters of MT's metabolites with their standard deviations, correlation coefficient (r^2), LOD, and LOQ values are summarized in Table 1.

2.7 | Pharmacokinetic Analysis and Statistical Analysis

The PK calculations were carried out using a program for PK data analysis in Microsoft Excel (Zhang et al., 2010). The PK parameters were presented as a geometric mean. To make comparisons across treatments, the different parameters were first tested for normal distribution and variance homogeneity. Data were compared with an independent *t*-test depending on whether the data passed a normality test in the SPSS program. In all experiments, differences were considered significant if $p < .05$.

3 | RESULTS AND DISCUSSION

Pharmacokinetics Profiles of Metamizole Metabolites.

TABLE 2 Pharmacokinetic and BE (μFemale/μMale) parameters for MAA, AA, and AAA metabolites after 25 mg/kg IV administration in female and male horses (n = 7)

	Metabolites									
	MAA (Mean ±SD)			AA (Mean ±SD)			AAA (Mean ±SD)			
Parameters	Female	Male	Sig. (2-tailed), $p<0.05$	Female	Male	Sig. (2-tailed), $p<0.05$	Female	Male	Sig. (2-tailed), $p<0.05$	μF/μM
C_{max} (μg/ml)	107.21± 5.55	114.40±5.57	*0.000	16.68±0.04	17.57±0.01	*0.000	0.68±0.02	0.64±0.02		0.9; 0.9; 1.2
t_{max} (h)	0.25± 0.00	0.30±0.11	*0.000	0.90±0.14	0.75±0.00	*0.000	1.10±0.22	1.00±0.00		0.8; 1.2; 1.1
$AUC_{0\text{-}12}$ (μg/ml.h)	194.42± 4.77	374.11±38.14	*0.000	57.05±0.11	58.48±0.45	*0.000	2.47±0.08	2.29±0.09	*0.01	0.5; 0.9; 1.1
$AUC_{0\text{-}\infty}$ (μg/ml.h)	196.65± 4.86	398.53±51.82	*0.000	62.25±0.11	63.48±0.48	*0.000	3.42±0.24	3.33±0.37		0.5; 0.9; 1.0
MRT (h)	2.28± 0.09	3.96±0.66		4.62±0.05	4.80±0.05		8.34±1.61	8.26±2.12		0.6; 0.9; 1.0
$t_{1/2\lambda z}$ (h)	1.80± 0.45	2.81±0.54	*0.01	2.82±0.03	3.06±0.04	*0.000	6.44±1.28	5.60±1.56		0.6; 0.9; 1.2
V_{ss} (mg/kg)/(μg/ml)	0.29± 0.01	0.25±0.02	*0.001	1.86±0.00	1.89±0.02	*0.006	6.09±0.85	6.86±1.26		-
Cl, (mg/kg)/(μg/ml)/h	0.13± 0.01	0.06±0.01	*0.000	0.40±0.00	0.39±0.00	*0.000	6.13±3.03	7.59±0.84		-
λz, 1/h	0.41± 0.14	0.25±0.05	*0.000	0.25±0.00	0.23±0.00	*0.000	0.12±0.02	0.11±0.03		-
C_{last} /C_{max}	0.01±0.00	0.05±0.02		0.08±0.00	0.06±0.00		0.16±0.03	0.17±0.05		-
C_0, (μg/ml)	162.16± 13.56	132.16±11.59		0.19±0.01	0.167±0.036		0.11±0.01	0.14±0.03		-
Vz, (mg/kg)/(μg/ml)	0.33± 0.08	0.25±0.02		1.64±0.02	1.74±0.03		68.74±11.35	72.99±19.03		-

TABLE 3 Pharmacokinetic and BE (μFemale/μMale) parameters for MAA, AA, and AAA metabolites after 25 mg/kg IM administration in female and male horses (n = 7)

	Metabolites								
	MAA (Mean ± SD)			AA (Mean ± SD)			AAA (Mean ± SD)		
Parameters	Female	Male	Sig. (2-tailed), $p < .05$	Female	Male	Sig. (2-tailed), $p < .05$	Female	Male	μF/μM
C_{max} (μg/ml)	56.65 ± 1.50	96.00 ± 8.96	*0.000	14.85 ± 0.67	15.63 ± 0.03	*0.03	0.35 ± 0.05	0.33 ± 0.03	0.6; 0.9;1.0
t_{max} (h)	0.50 ± 0.00	0.70 ± 0.11	*0.000	1.50 ± 0.00	1.50 ± 0.00		1.50 ± 0.00	1.50 ± 0.00	0.7; 1.0; 1.0
$AUC_{0\text{-}12}$ (μg/ml.h)	156.45 ± 13.46	328.19 ± 12.49	*0.000	56.71 ± 0.32	58.81 ± 0.96	*0.02	1.79 ± 0.06	1.73 ± 0.09	0.5; 0.9; 1.0
$AUC_{0\text{-}\infty}$ (μg/ml.h)	221.37 ± 64.22	387.96 ± 78.08	*0.006	61.18 ± 0.32	64.39 ± 1.47	*0.01	5.25 ± 2.59	3.95 ± 1.38	0.6; 0.9; 1.3
MRT (h)	6.81 ± 2.56	4.55 ± 0.66		4.95 ± 0.02	5.12 ± 0.23		5.03 ± 1.75	7.18 ± 2.89	1.5; 0.9; 0.7
$t_{1/2\lambda z}$ (h)	5.93 ± 0.69	3.03 ± 0.67		3.02 ± 0.01	3.22 ± 0.17	*0.02	10.68 ± 2.27	11.77 ± 4.05	1.9; 0.9; 0.9
V_z/F_{obs}(mg/kg)/(μg/ml)	1.40 ± 0.54	0.25 ± 0.07	*0.018	1.78 ± 0.01	1.80 ± 0.07	*0.006	135.49 ± 51.88	119.65 ± 25.80	-
Cl/F_{obs},(mg/kg)/(μg/ml)/h	0.12 ± 0.03	0.07 ± 0.01	*0.005	0.41 ± 0.00	0.39 ± 0.01	*0.000	5.71 ± 2.41	6.89 ± 2.14	-
λz, 1/h	0.11 ± 0.07	0.23 ± 0.05		0.23 ± 0.00	0.22 ± 0.01		0.05 ± 0.03	0.06 ± 0.013	-
C_{last} /C_{max}	0.08 ± 0.02	0.07 ± 0.01		0.07 ± 0.00	0.08 ± 0.01		0.32 ± 0.07	0.33 ± 0.02	-

C_{max}: Peak plasma concentration; t_{max}: time of peak; $AUC_{0\text{-}12}$: from 0 time to 12. h area under the plasma concentration–time curve; $AUC_{0\text{-}\infty}$: from 0 time to 12. h area under the plasma concentration–time curve extrapolated to infinity; MRT: mean resident time; $t_{1/2\lambda z}$: terminal half-life; V_{ss}: steady steate distribution volume; Cl: Clirens; λz: terminal phase rate constant; C_{last}/C_{max}: Last measurable plasma concentration/Peak plasma concentration; C_0; from 0 time to 15. min. the first measured plasma concentration; V_z: terminal phase distribution volume; Vz/F: Apparent volume of distribution during terminal phase after non-intravenous administration; Cl/F, apparent clearance; Star (*) shows differences between gender groups

Any observation about discomfort, pain, or swelling was not determined for both routes of administrations in animals during the study. Plasma concentrations of MAA, AA, and AAA were determined from 15 min to 12 h. However, FAA metabolite could not be determined in the samples due to concentrations lower than detection limit. The main PK parameters of MT's metabolites are summarized in Tables 2, 3. Plasma AA and AAA concentrations were lower than MAA values. As expected, the C_{max} of MAA was higher for the IV group than the IM group, and the C_{max} was achieved earlier in the IV group (0.25 h) in comparison with the IM group (0.5 and 0.7 h) in both females and male horses.

The average plasma concentrations of MAA, AA, and AAA were similar between female and male horses following IV administration of MT ($p > .05$). The plasma concentrations of MAA were higher in the IM group than the IV group from 1 hr to 8 hr post-administration. The mean +/- SD plasma concentrations of MAA, AAA, and AA after IM and IV injections in female and male horses are shown in Figures 1–4.

Both $AUC_{0\text{-}12}$ and $AUC_{0\text{-}\infty}$ values were different statistically ($p < .05$) in MAA, AA metabolites after IM and IV administrations, while $AUC_{0\text{-}12}$ value was significantly different ($p < .05$) in AAA metabolite after IV administration. Both MRT and the value of $t_{1/2\lambda z}$ were significantly different ($p < .05$) in AA after IM administration

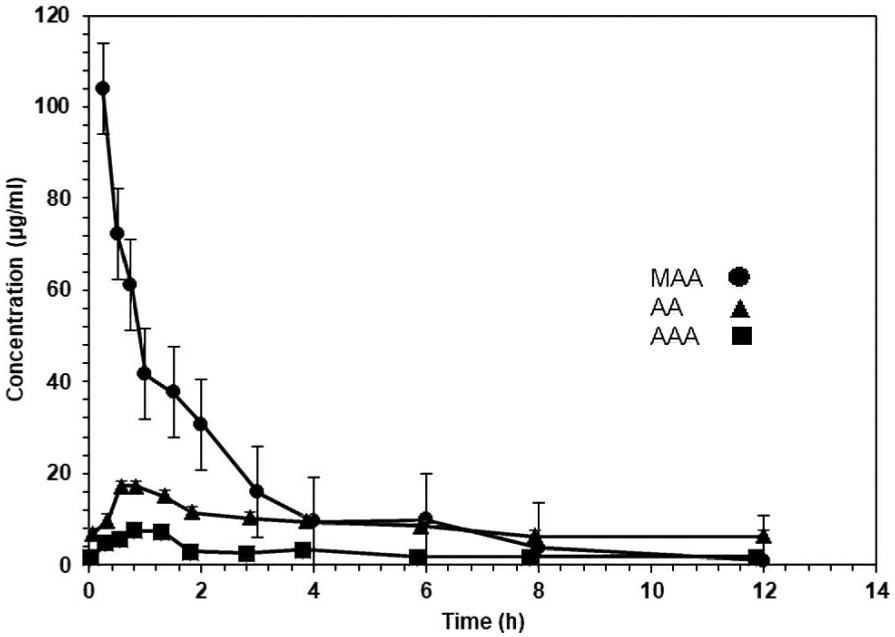

FIGURE 1 Mean plasma concentrations of MAA (—●—), AA (–▲–), and AAA (—■—) vs. time curves following IV administration at dose of 25 mg/kg MT in female horses ($n = 7$)

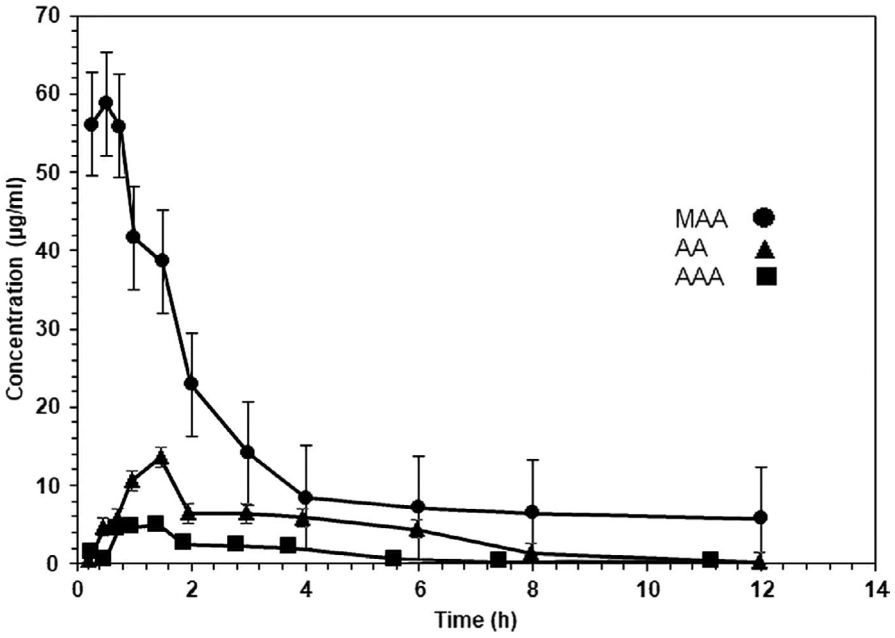

FIGURE 2 Mean plasma concentrations of MAA (—●—), AA (–▲–), and AAA(—■—) vs. time curves following IM administration at dose of 25 mg/kg MT in female horses ($n = 7$)

and MAA and AA metabolites after IV administration. However, the value of λz was also significantly different ($p < .05$) in AA after IV administration. On the other hand, although the values of Cl and V_{ss} were significant ($p < .05$) in MAA and AA metabolites after both IV and IM administrations into sex, the value of V_{ss} was insignificant ($p > .05$) after AA in IM administrations into sex. The values of Cl and V_{ss} did not have any difference ($p > .05$) statistically in AAA after IV and IM administrations of to sex.

The plasma concentration–time curves for each administration showed that MT distribution took place according to a two-compartment open model (Figures 1–4). The MAA, AA, and AAA amounts in plasma versus time curve profiles after the two administration of MT were variable (within the groups) and different (between the groups).

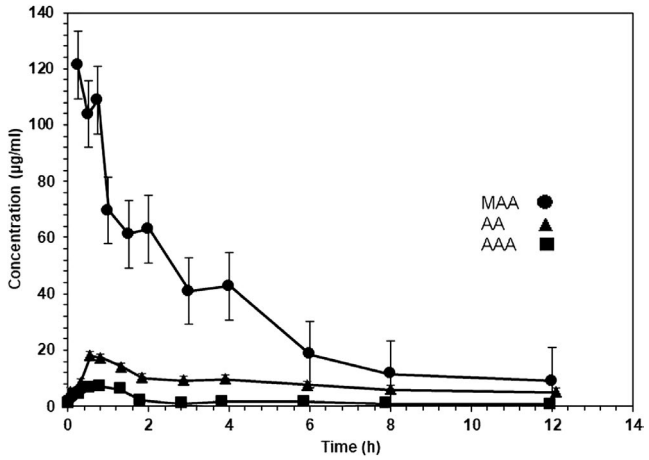

FIGURE 3 Mean plasma concentrations of MAA (—●—), AA (–▲–), and AAA (—■—) vs. time curves following IV administration at dose of 25 mg/kg MT in male horses ($n = 7$)

4 | DISCUSSION

In the last few years, MT has earned greater currency due to its important pharmacological properties, safety profile, low price, and applicability for veterinary uses. There are few reports about clinical usage and side effects of MT and its metabolites in donkeys (Aupanun et al., 2016), dogs (Imagawa et al., 2011; Zanuzzo et al., 2015), rabbits (Baumgartner et al., 2009), rats (Domínguez-Ramírez et al., 2012), sheep (Giorgi et al., 2015), and horses (Giorgi et al., 2017; Klaus et al., 1997). This drug has been reported to be safe and applicable for pain management in animals, but there are not enough data about the usage of MT in human medicine (Aupanun et al., 2016; Zukowski & Kotfis, 2009).

The pharmacokinetic parameters of MT metabolites have been studied in horses previously (Giorgi et al., 2017; Klaus et al., 1997) but not in Arabian breed horses using a sample consisting of both male and female horses. The main objective of this study was to determine both plasma concentrations and PK parameters of MT's active and inactive metabolites in both sexes of Arabian Horses (Equus ferus caballus) using a cross-over study design following IV and IM administrations of MT.

These results were found similar or parallel with some earlier studies (Aupanun et al., 2016; Giorgi et al., 2017).

The $AUC_{0-\infty}$ values reported in this study were higher than those previously reported 83.01 after IV and 120.33 after IM administration in donkeys (Aupanun et al., 2016) and in horses (104–147, Aupanun et al., 2016; 106–245 Giorgi et al., 2017; 83–120Klaus et al., 1997), 161.42 after IV and 165.04 after IM administration in sheep (Giorgi et al., 2015). Doses of MT that have been administrated on horses (Giorgi et al., 2017) and donkeys (Aupanun et al., 2016) were 25mg kg^{-1}. In contrast, the dose of MT that had been administrated on sheep was 20 mg/kg in both injections (Giorgi et al., 2015). The significant differences found in $AUC_{0-\infty}$ values

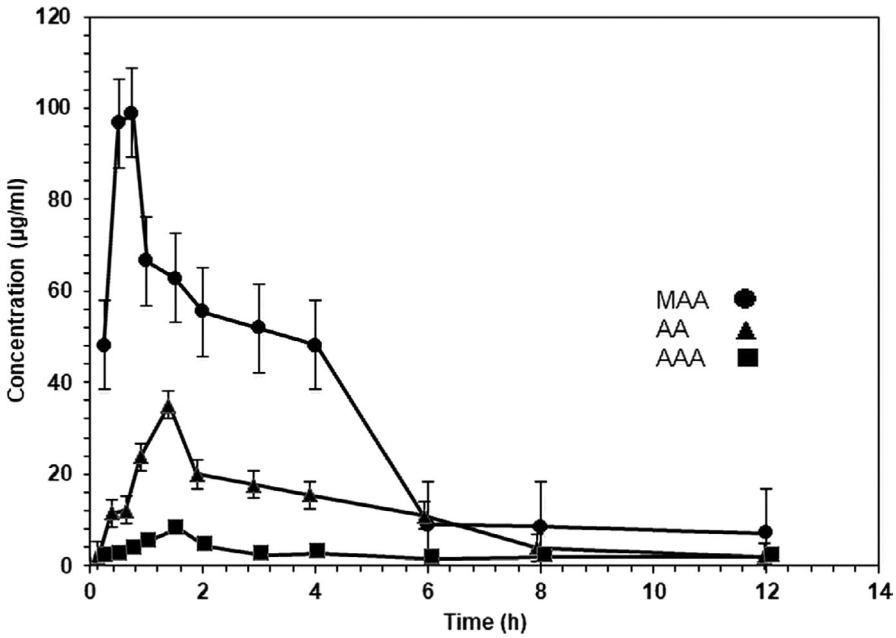

FIGURE 4 Mean plasma concentrations of MAA (—●—), AA (–▲–), and AAA (—■—) vs. time curves following IM administration at dose of 25 mg/kg MT in male horses ($n = 7$)

were ascribable to the routes of administration of MT's metabolites in different horse breeds.

The overall pharmacokinetic profiles of MAA and AA after IM and IV administrations of MT were similar to previous studies. The C_{max} of MAA has been observed to increase due to a rapid metabolic transformation in the first minutes after the IV administration (vascular compartment) of MT compared with the IM administration where an absorption phase is expected. The significant difference between male and female horses, and between the two administration routes can be traced in T_{max} and C_{max} in MAA metabolite ($p < .01$). C_{max} value for AA metabolite was found to be statistically different between the groups. C_{max} values of AAA between the groups are not statistically different. The main pharmacokinetic parameters are reported in Tables 2, 3. The significant differences found in C_{max} and t_{max} values were ascribable to the administration of MT. But it is emphasized that t_{max} value is closely related to the first sample collection time. The administration of MT phase may also be responsible for the difference in t_{max} values. In the present study, the first sample collection time was the 15th minute in both administrations. Despite the abrupt peak of MAA concentration following IV administration, no adverse effects were shown in the animals.

In the present study, the C_{max} values for MAA metabolite after IV and IM administrations were 107.21 μg/ml in female horses, and 114.40 μg/ml in male horses, and 56.65 μg/ml in female horses, and 96.00 μg/ml in male horses, respectively.

According to Giorgi et al, the plasma concentrations, C_{max} values of MT after IV 86.33 and after IM administration 24.14 in horse (Giorgi et al., 2017), and also C_{max} values of MT after IV 218.46 and after IM administration 108.24 in sheep (Giorgi et al., 2015). Additionally, C_{max} values of MT after s.c. administration 95.8 in rats (Domínguez-Ramírez et al., 2012), after IV 211.72 and after IM administration 46.33 in donkeys (Aupanun et al., 2016). Doses of MT were administrated as 25mg kg^{-1} in horse (Giorgi et al., 2017) and in donkeys (Aupanun et al., 2016). Additionally, the dose of MT was administrated as 20 mg/kg by both injections in sheep (Giorgi et al., 2015) and 177.8 mg/kg in rats (Domínguez-Ramírez et al., 2012). Although the IV and IM dose of MT was the same, the plasma concentrations of MAA and AA detected in the present study were higher than the results of Klaus et al., (1997) and Giorgi et al., (2017).

Although the IV and IM dose of MT was the same, the t_{max} values of MAA and AA detected in the present study were lower than the results in the study reported by Giorgi et al., (2017) and Klaus et al., (1997). It can be said that these differences may have been caused by age, live weight, sex, race, physiopathological differences (like pregnancy), and nutritional status of the horses (Aupanun et al., 2016) Giorgi et al., (2017).

In the present study, the MRT values of MAA, AA, and AAA metabolite between both male and female horses and in both administration routes were not statistically different (Tables 2, 3). The MRT values of both MAA and AA metabolites after IM and IV administrations of MT were similar to those previously reported in horses (Giorgi et al., 2017). The dose of MT was also the same as those in the study of Giorgi (2017).

After IM administration, the MRT value of MAA is higher than the IV administration. The larger MRT values found in the IM group are likely due to the gradual release of the drug from the injection site to the vascular system.

Although the dose of MT was the same, the MRT reported in this study was higher than those previously reported in the horse. The value of this parameter is closely related to the last sample collection time; in the present study, the last sample collection time is 12th h. (Giorgi et al., 2017 and Klaus et al., 1997). Based on this parameter, C_{max}, t_{max}, MRT, AUC, Cl, λz, and V_{ss} values of MAA and AA have shown significant differences by administration routes reported by (Giorgi et al., 2017 and Klaus et al., 1997).

The abrupt peak of MAA concentration following IV administration might have saturated the metabolic pathway of MAA to AA and FAA. At the same time, metabolizing of MAA to AA and oxidizing to FAA continued, forming inactive metabolites. This can explain why the C_{max} values of MAA between the groups (both sexes and administration routes) are statistically different. The C_{max} value reported in this study was higher than reported by Giorgi et al., 2017 and the same reported by Klaus et al., 1997. Further studies evaluating all the metabolites formed in the horse are necessary to clarify this issue.

Although the values of half-life (t½) were insignificant in MAA, AA, and AAA metabolites in both sexes ($p > .05$), the values of t½ have significant statistically in MAA, AA, and AAA metabolites after both IV and IM administrations of the two sexes ($p < .05$).

The t½ values of MAA and AA metabolites after both IV and IM administration reported for horses in this study were shorter than those previously reported in horses (3.34 h; Giorgi et al., 2017; 4.10–9.14 h; Klaus et al., 1997).

After IV administration, the MRT values are 2.28 h in female and 3.96 h in male horses, and after IM administration, 6.81 h in female and 4.55 h in male horses. Therefore, in clinical studies perspective, pain treatment may be possible at ~2,5 h in females and ~4 h in male horses after IV administrations, and ~7 h in females and ~5 h in male horses after IM administrations. If a second application is required for successful pain treatment, it would be good to consider these times. According to these results, bioequivalence (BE) of administration in sexes; IV route may be accepted as BE based on C_{max} and t_{max} parameters, but the same situation is appropriate based on t_{max} parameter for IM administration route (Tables 2, 3).

Because acceptance limits of BE must be within 0.7–1.43 or 0.8–1.25 (Yilmaz & Elmas, 2010).

It can be said that these differences might be due to several factors such as differences in animal species (rat, dog, rabbit, and horse), route of drug administration, presence of pathophysiological conditions, age of the animals, extraction processes, and sensitivity of the analytical method (Aupanun et al., 2016; Giorgi et al., 2017).

5 | CONCLUSION

Despite the need for more studies to understand their safety profile and the metabolic pathways, this study showed IV and IM administrations of MT generated detectable plasma concentrations of MAA, AAA, and AA in both female and male Arabian horses. The study is original in that it study should be the first one conducted on Arabian horses of both sexes and of the same age. In the study, when considering PK parameters (as Cl, V_{ss}, λz, C_{max}, t_{max}, AUC, and MRT) of MT, there has been observed significant differentiation based on sex and administration routes. According to the drug producer, 25mg kg^{-1} administration of MT is an effective dose to relieve pain in equid species for 6–8 h. If we assume that analgesic activity in the Arabian horses is only attributable to MAA, AA, and AAA metabolites as in humans, the contribution of AA and AAA to the overall therapeutic activity might be negligible due to its low plasma concentrations.

The results of the present study have shown that, in horses the pharmacokinetic variables of MT's metabolites at two different administrations in the fixed-dose of 25mg kg^{-1} may be useful for acute and chronic pain management and other clinical necessities.

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CONFLICT OF INTEREST

The authors report no conflicts of interest, and they alone are responsible for the content and writing of the paper.

DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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