



ALMA MATER STUDIORUM  
UNIVERSITÀ DI BOLOGNA

## ARCHIVIO ISTITUZIONALE DELLA RICERCA

### Alma Mater Studiorum Università di Bologna Archivio istituzionale della ricerca

A quick approach for medetomidine enantiomer determination in dog plasma by chiral liquid chromatography–tandem mass spectrometry and application to a pharmacokinetic study

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

*Published Version:*

Bardhi A., Zaghini A., Levionnois O., Barbarossa A. (2021). A quick approach for medetomidine enantiomer determination in dog plasma by chiral liquid chromatography–tandem mass spectrometry and application to a pharmacokinetic study. DRUG TESTING AND ANALYSIS, 13(7), 1249-1255 [10.1002/dta.3015].

*Availability:*

This version is available at: <https://hdl.handle.net/11585/853835> since: 2022-02-27

*Published:*

DOI: <http://doi.org/10.1002/dta.3015>

*Terms of use:*

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).  
When citing, please refer to the published version.

(Article begins on next page)

This is the final peer-reviewed accepted manuscript of:

Bardhi A, Zaghini A, Levionnois O, Barbarossa A. A quick approach for medetomidine enantiomer determination in dog plasma by chiral liquid chromatography–tandem mass spectrometry and application to a pharmacokinetic study. *Drug Test Anal.* 2021;13:1249–1255.

The final published version is available online at:

<https://doi.org/10.1002/dta.3015>

#### Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)

**When citing, please refer to the published version.**

**A quick approach for medetomidine enantiomers determination in dog plasma by chiral LC-MS/MS and application to a pharmacokinetic study**

Running Head:

Quantification of medetomidine enantiomers in dog plasma by LC-MS/MS

Anisa Bardhi<sup>1</sup>, Anna Zaghini<sup>1</sup>, Olivier Levionnois<sup>2</sup>, Andrea Barbarossa<sup>\*1</sup>

<sup>1</sup> *Department of Veterinary Medical Sciences, Alma Mater Studiorum - University of Bologna, Ozzano Emilia (BO), Italy*

<sup>2</sup> *Unit of Anaesthesiology and Pain therapy, Department for Clinical Veterinary Science, Faculty Vetsuisse, University of Berne, Bern, Switzerland*

\*Corresponding author:

Andrea Barbarossa

Department of Veterinary Medical Sciences, Alma Mater Studiorum - University of Bologna  
Via Tolara di Sopra 50, 40064 Ozzano Emilia (BO), Italy

Tel.: +390512097500

E-mail: [andrea.babarossa@unibo.it](mailto:andrea.babarossa@unibo.it)

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)

**When citing, please refer to the published version.**

## **Abstract**

In the present study, a rapid, sensitive and high-throughput LC-MS/MS method for the determination of medetomidine enantiomers in dog plasma was developed and validated. The separation and individual quantification of chiral compounds can be a tricky task in liquid chromatography. This is particularly true when target analytes have a relatively small mass, as is the case with medetomidine, a potent and highly specific  $\alpha_2$ -adrenoceptor agonist widely used in both human and veterinary medicine. The proposed approach is based on a quick liquid-liquid extraction with ethyl acetate and filtration prior to injection. The optimized mobile phase composition allowed to perfectly separate the two enantiomers of medetomidine in a short chromatographic run time, using a cellulose tris(4-methylbenzoate) based chiral column. A lower limit of quantification of 0.1 ng/mL was reached for both analytes thanks to the high sensitivity and selectivity of tandem mass spectrometry, and the use of racemic medetomidine- $d_3$  as internal standard prevented potential matrix effect. Linearity was satisfying ( $R^2 > 0.99$ ) over the range 0.1-25 ng/mL, as well as within- and between-session accuracy and precision, both always  $< 15\%$ . This method was also applied with success to a series of samples from a PK study aimed at comparing dex- and levomedetomidine behaviour after administration of the racemic mixture in dogs. The simple extraction procedure, which allows reduced solvent and time consumption without compromising analytical performances, make this technique a useful tool for this kind of applications even when small animals are involved, due to the small amount of sample required.

## **Keywords**

medetomidine, enantiomers, chiral separation, plasma, pharmacokinetics, LC-MS/MS, dog

*This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)*

***When citing, please refer to the published version.***

## 1. Introduction

Medetomidine (R, S)-4-[1-(2,3-dimethylphenyl)ethyl]-1H-imidazole is a potent and highly specific  $\alpha_2$ -adrenoceptor agonist. It has been shown to be 5-10 times more selective compared to detomidine, clonidine, xylazine or other compounds with a similar mechanism of action, with an  $\alpha_2/\alpha_1$  selectivity ratio of 1620:1 [1]. Medetomidine is a racemic compound (molecular structure is shown in Fig.1), and the right-handed enantiomer dexmedetomidine (DEX) is the pharmacologically active isomer, whereas the left-handed enantiomer levomedetomidine (LEVO) is generally considered inactive [2]. Like other  $\alpha_2$ -agonists, medetomidine has beneficial effects that include reliable sedation, analgesia, muscle relaxation and anxiolysis, as well as a decrease in the requirement of injectable and inhalant anaesthetic agents [3]. Dexmedetomidine administered at half doses induces the same effects of racemic medetomidine and is thus considered to be twice as potent; on the other hand, levomedetomidine shows mild sedative and analgesic properties only at high doses [4,5]. The use of medetomidine in dogs was first authorized in the US market back in 1996, while dexmedetomidine became available for use in dogs and cats in the European Union in 2002 and in the United States in 2006. Today, both racemic medetomidine and dexmedetomidine are widely used in veterinary medicine as sedatives, analgesics and for premedication.

It is therefore of clinical interest to conduct studies aimed at further clarifying the pharmacokinetics of dex- and levomedetomidine in dogs. The purpose of this study was the development and validation of an analytical method for the separation and quantification of medetomidine enantiomers in plasma by chiral liquid chromatography-tandem mass spectrometry (LC-MS/MS), to be applied to samples collected during a pharmacokinetic study in dogs.

## 2. Materials and Methods

### 2.1 Chemicals

Analytical standards of dexmedetomidine, levomedetomidine, medetomidine-d3 were purchased from Toronto Research Canada (North York, ON, Canada). Other chemicals, including diethylamine, ammonium formate and ethyl acetate (all of LC-MS grade) were

*This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)*

***When citing, please refer to the published version.***

obtained from Merck Company (Darmstadt, Germania). Ultra-pure water was freshly produced in-house (Millipore, Milano, Italy).

## 2.2 Standard solutions

Individual stock solutions of DEX and LEVO at 1.0 mg/mL were prepared by dissolving 10 mg of pure powder of each analyte in a 10 mL volumetric flask containing methanol. These were used to prepare by serial dilution working solutions called “Mede-mix”, containing equal amounts of the two enantiomers at different concentrations, to be used for calibration and QCs samples. In particular, the obtained working solutions had concentrations of 2, 5, 20, 50, 200 and 500 ng/mL.

Similarly, a stock solution of racemic medetomidine-d3 containing the two labelled enantiomers at 0.5 mg/mL was obtained by dissolving 10 mg of medetomidine-d3 in a 10 mL volumetric flask containing methanol. A working solution of medetomidine-d3 at 0.1 µg/mL, to be used as internal standard, was prepared by dilution of the stock solution in methanol. All standards solutions were stored in the dark at -20 °C.

## 2.3 Sample preparation

After thawing at room temperature, plasma samples were centrifuged for 10 min at 21,000 ×g at 4 °C. Two hundred µL of supernatant was transferred into a 1.5 mL Eppendorf microtube, added of 10 µL of internal standard working solution and mixed with 1 mL of ethyl acetate. Liquid-liquid extraction was carried out by vortex mixing for 30 s and centrifuging at 21,000 ×g for 15 min at 4 °C. Then, 900 µL of the upper phase were transferred into a round bottom glass tube and evaporated under gentle nitrogen stream at 40 °C. The dry residue was reconstituted with 300 µL of mobile phase, consisting of a 45:55 (v/v) mixture of 0.1% diethylamine 20 mM ammonium formate water solution and methanol, agitated on vortex mixer for 30 s and finally filtered through a 0.22 µm nylon syringe filter into a LC vial before the analysis.

## 2.4 Liquid chromatography

The LC system consisted of a Waters Acquity UPLC® binary pump (Waters, Milford, MA, USA), equipped with a Phenomenex Lux 3 µm Cellulose-3 (150 × 2,00 mm, 3,0 µm) column,

*This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)*

***When citing, please refer to the published version.***

based on the chiral selector Cellulose tris(4-methylbenzoate) (Phenomenex, Torrance, CA, USA) and maintained at 25 °C.

To obtain a satisfactory separation of the two enantiomers of medetomidine and medetomidine-d<sub>3</sub>, a 9.5 min isocratic run combining solvent A (0.1% of diethylamine and 20 mM ammonium formate in water) and solvent B (methanol) at a ratio of 45:55 (v:v) and 0.31 mL/min flow rate was used. Extracted samples were kept in the autosampler at 20 °C, and 10 µL from each vial was injected in the LC-MS system.

## 2.5 Mass spectrometry

The LC was interfaced to a Waters XEVO TQ-S micro triple quadrupole mass spectrometer (Waters, Milford, MA, USA), operating in positive electrospray ionization (ESI+) and in MRM (multiple reaction monitoring) mode. Capillary voltage was set at 0.5 kV, while source and desolvation temperatures were 150 and 600 °C, respectively. Cone gas was set at 50 L/h and desolvation gas at 900 L/h; argon was used as a collision gas.

The analyte-dependent MS/MS parameters were optimized through combined infusion of LC mobile phase and standard solutions of each analyte into the mass spectrometer. The most abundant transitions for the enantiomers of medetomidine and of the internal standard were identified, and are reported, with their relative cone voltage and collision energy values, in Table 1. Data acquisition and analysis was performed using MassLynx 4.2 software (Waters, Milford, MA, USA).

## 2.6 Validation

The method was validated following the current European Medicines Agency guideline on bioanalytical method validation during three separated days of testing [6].

After defining the retention times of the two enantiomers of medetomidine and medetomidine-d<sub>3</sub> through the injection of pure standards, the selectivity of the method was assessed analysing ten blank dog plasma samples, to verify the absence of chromatographic signals in the same time windows.

Seven-points matrix-matched calibration curves (0, 0.1, 0.25, 1, 2.5, 10 and 25 ng/mL) were freshly prepared each day of validation to evaluate the linearity of the method, by spiking 200 µL of blank plasma with 10 µL of Mede-mix working solutions at the corresponding

*This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)*

***When citing, please refer to the published version.***

concentrations. Peak area ratios between DEX and LEVO and the corresponding internal standard enantiomer were plotted against their concentration ratios and a linear least square regression model was applied. All the calibration standards had to be within  $\pm 15\%$  of the nominal value, and the resulting correlation coefficient (R<sup>2</sup>) was considered acceptable if  $\geq 0.99$ .

The lower limit of quantification (LLOQ) of the method was defined as the lowest concentration tested which can be detected with a signal-to-noise (S/N) ratio  $\geq 10$  and acceptable accuracy and precision ( $< 15\%$ ) in between and within run. The limit of detection (LOD), indicating the concentration providing a S/N ratio of 3, was estimated on the lowest concentration assayed.

To evaluate intra- and interday accuracy and precision of the method, QC samples at three different concentrations (0.25, 1 and 10 ng/mL) were prepared in triplicates along with the calibration curve during the three days of validation. Accuracy, expressed as relative difference between measured value and expected concentration, was evaluated at each QC concentration, and was considered acceptable if within  $\pm 15\%$ . Precision, defined as the coefficient of variation (CV%) among repeated individual measures, had to be  $< 15\%$  for each QC level.

Although medetomidine-d<sub>3</sub> was used as internal standard, an evaluation of the recovery of the method was performed by spiking two series of samples at 0.5 ng/mL, one before (n = 6) and one after (n = 6) the extraction, and the mean measured values were compared.

Immediately after the injection of the highest point of each calibration curve (25 ng/mL), a blank sample was analysed to assess the absence of carry-over. Potential matrix effect was first verified by the post-column infusion technique: during the injection of a blank matrix sample in the LC-MS system, standard solutions of medetomidine enantiomers were directly and continuously infused in the MS interface, to evaluate the stability of the produced signal. Then, the ionization suppression or enhancement effect was also calculated as:

$$\text{Matrix effect \%} = \left( \frac{\text{Response}_{\text{post-extracted sample}}}{\text{Response}_{\text{non-extracted neat sample}}} - 1 \right) \times 100$$

Negative values indicate ionization suppression, while positive values indicate ionization enhancement.

## 2.7 Application to pharmacokinetic study

The validated method was successfully used to determine the pharmacokinetic profile of medetomidine enantiomers in dog plasma. The trial was approved by the Zürich Cantonal

*This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)*

***When citing, please refer to the published version.***



Health Directorate Veterinary Office, Switzerland, approval Nr: ZH119/18 (30356). Eight adult healthy beagle dogs received intravenously 20 µg/kg of racemic medetomidine. Two millilitres of blood were collected from the cephalic vein just before administration (0 min) and after 2, 4, 8, 16, 30, 60, 80, 90 and 120 min. Samples were centrifuged immediately and plasma stored at –80 °C until analysis in LC-MS/MS.

The pharmacokinetic analysis was performed by WinNonLin® 8.3 software (Certara, Princeton, NJ, USA). A non-compartmental analysis model with "Linear trapezoidal linear interpolation" and "uniform" weighing was used.

### **3. Results and discussion**

#### **3.1 Method optimization**

Chromatographic separation of DEX and LEVO was performed on a UHPLC (Ultra High Performance Liquid Chromatography) system, which generally provides better performances in terms of analyte resolution in shorter run times compared to traditional HPLC. However, chiral columns specifically designed for such technology have only recently been introduced on the market, therefore we preferred to opt for the well-known (and previously used with success in our laboratory) Phenomenex Lux 3µ Cellulose-3 HPLC column. Although this configuration did not allow to take full advantage of UHPLC capabilities, it provided optimal peak resolution in a relatively short total run time (9.5 min), making this approach faster compared to similar previously published techniques [8,9]. It has been highlighted how different mobile phase modifiers, such as ammonium acetate, ammonium formate, formic acid and diethylamine, can play a pivotal role in the chiral separation of the optical isomers of some compounds [7,8]. In our study, the addition of 0.1% diethylamine, besides 20 mM ammonium formate, to the aqueous phase brought the pH to around 8.5, which turned out to be a key factor to obtain optimal resolution of DEX and LEVO. After performing multiple tests under both isocratic and programmed LC conditions, we concluded that a mobile phase with a constant composition of methanol (55%) and 20 mM ammonium formate 0.1% diethylamine aqueous solution (45%) provided the best results, as also described by Honkavaara *et al.* [9]. Increased content of water or methanol in the mobile phase negatively affected peak shape or peak separation, respectively.

*This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)*

***When citing, please refer to the published version.***

The quick and simple extraction procedure proved to be a strength point of the proposed method, requiring only small amounts of chemical reagents and sample and allowing to process 24 samples in less than 45 min. This is an advantage compared to other published approaches for the quantification of medetomidine enantiomers in plasma, based on more expensive and time-consuming solid phase extraction techniques [9,10] or on liquid-liquid extraction with larger volume of a chemically hazardous solution containing chloroform [8].

Before opting for the extraction with ethyl acetate, protein precipitation with methanol was also tested during method development. Although the chromatograms obtained were quite similar, we also evaluated the matrix effect of the two procedures, to choose the best one. The post-column infusion test evidenced that the sample extracted with methanol generated variations of signal intensity in the time window including the retention time of DEX and LEVO, while it was not affected by the sample extracted with ethyl acetate (Fig. 2). Moreover, the calculated matrix effect for the ethyl acetate extraction was -8% and -7% for DEX and LEVO, respectively, confirming that just a slight ionization suppression was generated by this approach.

Finally, the small (200  $\mu$ L) sample aliquot required for the current method, significantly lower compared to the techniques proposed by Honkavaara et al. (900  $\mu$ L) and Khader et al (3 mL), make it more functional, especially in the perspective of its application in PK studies requiring repeated blood collection, which might be a critical factor when small-sized dogs are involved.

### **3.2 Method Validation**

The method developed in the present work proved to be the very effective for the separation and quantification of medetomidine enantiomers in canine plasma and was validated according to current European guidelines [6].

The analysis of ten plasma samples not containing the drug proved the absence of compounds coeluting with the analytes of interest, as shown in Fig. 3. Individual injections of pure standards of DEX and LEVO allowed to define their retention time, which was 6.28 and 7.39 min, respectively; as for the internal standards, the retention time was 6.23 min for d3-dexmedetomidine and 7.35 min for d3-levomedetomidine.

*This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)*

***When citing, please refer to the published version.***

Linearity was satisfactory over the range 0.1-25 ng/mL for both analytes, with R<sup>2</sup> values always >0.99 during the three days of validation. All back-calculated values of the calibration samples fell within ±15% of their nominal values.

A concentration of 0.1 ng/mL, considered largely sufficient for the method to be used in a pharmacokinetics study, was set as target lower limit of quantification at the beginning of the experiment. This goal was easily reached for both enantiomers (as shown in Fig. 3), resulting lower compared to the lower limit of quantification (LLOQ) reported in previous works [8, 9]. In addition, during further extra-validation experiments, a plasma sample spiked at 0.05 ng/mL was processed, providing a chromatographic trace with a signal-to-noise ratio still greater than 10. After observing that some reconstituted samples looked a bit cloudy, it was decided to add a filtration step prior to injection, to prevent column clogging and/or system contamination. However, by skipping this precautionary approach, it would be also possible to add a lower volume of mobile phase to the dry residue to obtain more concentrated samples, likely resulting in a further improvement of method sensitivity.

The calculated limit of detection of the method was 0.01 ng/mL for both DEX and LEVO.

Accuracy and precision data are summarized in Table 2. Intraday accuracy was acceptable for both analytes at all the tested concentrations (<6.3% for DEX and <5.0% for LEVO), as well as precision (<8.1% and 9.3% for DEX and LEVO, respectively). Similarly, interday results proved the good performances of the method.

The comparison between samples spiked before and after extraction showed recoveries between 80 and 84%; anyway, the use of the deuterated isotopes of the two target analytes as internal standards, compensating for both systematic/random errors and recovery variations, ensured accurate and reliable quantifications of the analytes.

### **3.3. Application to pharmacokinetic study**

The validated approach here described offers a simplified, yet reliable approach to individually quantify the enantiomers of medetomidine in plasma. This method was applied to a series of plasma samples collected within a study aimed at comparing the pharmacokinetic profile of dex- and levomedetomidine after intravenous administration of 20 µg/kg of the racemic mixture in dogs (n=8). Figure 4 shows an example chromatogram obtained after LC-MS analysis of a plasma sample. The validated LLOQ of 0.1 ng/mL proved to be largely suitable for the correct

*This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)*

***When citing, please refer to the published version.***

quantification of target analytes, even in the terminal portion of the pharmacokinetic curve, which is shown in Figure 5. Plasma concentrations of levomedetomidine resulted visibly lower than those of dexmedetomidine, as previously described by Bennett et al. (2016). The calculated pharmacokinetic parameters are summarized in Table 3.

#### 4. Conclusions

The chromatographic separation and quantification of chiral compounds is always a challenging task, especially with low mass molecules such as medetomidine. In the present study, a high-throughput LC-MS/MS method for the determination of dexmedetomidine and levomedetomidine in dog plasma was developed and validated. After mobile phase pH adjustment, the chosen cellulose-based chiral column provided a perfect separation of medetomidine enantiomers in less than 10 min. With respect to other published methods, as little as 200  $\mu$ L of sample was required and target analytes could be accurately quantified down to 0.1 ng/mL, although further tests showed that an even lower limit of quantification could be easily reached. Moreover, the simple liquid-liquid extraction with just 1 mL of ethyl acetate allowed significant reduction in sample preparation time, as well as in solvents consumption. The use of racemic d3-medetomidine as internal standard furtherly prevented potential matrix effect.

After being validated, this method was successfully applied to samples collected during a study on the different pharmacokinetic behaviour of dex- and levomedetomidine following intravenous administration of racemic medetomidine in dogs. The results proved that the proposed approach is perfectly suitable for PK studies, where large sets of samples must be processed, combining quick execution and high analytical performances.

#### References

1. Virtanen R, Savola JM. Characterization of the selectivity, specificity and potency of medetomidine as an  $\alpha_2$ -adrenoceptor agonist. *Eur J Pharmacol* 1988;150(1-2):9-14. doi:10.1016/0014-2999(88)90744-3.
2. MacDonald E, Scheinin M. Comparison of the behavioural and neurochemical effects of the two optical enantiomers of medetomidine, a selective  $\alpha_2$ -adrenoceptor agonist. *J Pharmacol Exp Ther* 1991;259:848-854.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)

**When citing, please refer to the published version.**

3. Sinclair M.D. A review of the physiological effects of alpha-2-agonists related to the clinical use of medetomidine in small animal practice. *Can Vet J* 2003;44(11):885-897.
4. Savola JM, Virtanen R. Central  $\alpha_2$ -adrenoceptors are highly stereoselective for dexmedetomidine, the dextro enantiomer of medetomidine. *Eur J Pharmacol* 1991;195:193-199. doi:10.1016/0014-2999(91)90535-x.
5. Kuusela E, Raekallio M. Clinical effects and pharmacokinetics of medetomidine and its enantiomers in dogs. *J Vet Pharmacol Ther* 2000;23(1):15-20. doi:10.1046/j.1365-2885.2000.00245.x.
6. EMEA/CHMP/EWP/192217/2009, 2011: Guideline on bioanalytical method validation. [https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf). Accessed October 08, 2020.
7. Fu Y, Borrull F. Comparison of polysaccharide-based and protein-based chiral liquid chromatography columns for enantioseparation of drugs. *Chirality* 2020;32(6) 876-884. doi:10.1002/chir.23198.
8. Khader Karakka Kal AB, Nalakath J. Development and validation of a chiral LC-MS method for the enantiomeric resolution of (+) and (-) medetomidine in equine plasma by using polysaccharide-based chiral stationary phases. *Chirality* 2020;22(3):314-323. doi:10.1002/chir.23166.
9. Honkavaara JM, Raekallio MJ, Syrjäb PM, et al. Concentrations of medetomidine enantiomers and vatinoxan, an  $\alpha_2$ -adrenoceptor antagonist, in plasma and central nervous tissue after intravenous coadministration in dogs. *Vet Anaesth Analg* 2020;47:47-52. doi:10.1016/j.vaa.2019.07.004.
10. Bennet RC, Salla MRM. Effects of MK-467 on the antinociceptive and sedative actions and pharmacokinetics of medetomidine in dogs. *J Vet Pharmacol Ther* 2016;39(4):336-343. doi:10.1111/jvp.12292.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)

**When citing, please refer to the published version.**

**Table 1.** Selected mass transitions for medetomidine and medetomidine-d3, and relative cone voltage and collision energy optimized values.

Analyte	Transition monitored ( <i>m/z</i> )	Cone voltage (V)	Collision energy (eV)
Medetomidine	201.18 → 94.97	28	18
Medetomidine-d3	204.18 → 97.97	34	18

*This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)*

***When citing, please refer to the published version.***

**Table 2.** Accuracy and precision for dex- and levomedetomidine at three different concentrations in triplicates in three separate sessions (n = 27).

		Dexmedetomidine		Levomedetomidine	
		Accuracy (%)	Precision (RSD, %)	Accuracy (%)	Precision (RSD, %)
QC low (0.25 ng/mL)	Day 1	1.3	6.0	4.0	6.7
	Day 2	-5.3	6.5	-3.3	9.3
	Day 3	2.7	8.1	1.3	8.2
	<i>Interday</i>	-0.4	7.1	0.7	7.7
QC low (1 ng/mL)	Day 1	4.3	7.7	2.0	5.5
	Day 2	-5.3	4.4	-4.7	7.0
	Day 3	6.3	4.2	5.0	6.7
	<i>Interday</i>	1.8	7.3	0.8	7.0
QC high (10 ng/mL)	Day 1	5.3	4.0	2.0	4.9
	Day 2	-5.7	6.2	-3.7	6.1
	Day 3	6.0	7.0	2.3	6.9
	<i>Interday</i>	1.9	7.5	0.2	6.0

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)

**When citing, please refer to the published version.**

**Table 3.** Mean (arithmetic or \*geometric)  $\pm$  standard deviation of the pharmacokinetic parameters (non-compartmental analysis) obtained from 8 dogs receiving 10  $\mu\text{g}/\text{kg}$  dex- and levo-medetomidine intravenously: time of maximum concentration observed ( $T_{\text{max}}$ ); maximum concentration observed ( $C_{\text{max}}$ ); apparent terminal elimination half-life time ( $T_{1/2}$ ); Steady state volume of distribution ( $V_{\text{ss}}$ ); clearance (Cl); area under the plasma concentration–time curve (AUC); mean residence time (MRT).

	Dexmedetomidine	Levomedetomidine
$T_{\text{max}}$ (min)	$2.9 \pm 1.1$	$3.5 \pm 2.1$
$C_{\text{max}}$ (ng/mL)	$9.8 \pm 2.0$	$5.3 \pm 1.2$
$T_{1/2}$ (min)	$48 \pm 19$	$57 \pm 18$
$V_{\text{ss}}$ (L/kg)	$1.31 \pm 0.28$	$2.68 \pm 0.55$
Cl (mL/min·kg) *	$19.21 \pm 4.93$	$33.89 \pm 7.67$
AUC (min·ng/mL)	$437 \pm 85$	$205 \pm 86$
MRT (min)	$43 \pm 4$	$39 \pm 13$

*This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)*

***When citing, please refer to the published version.***



## Figure captions

Figure 1. Molecular structure of dexmedetomidine and levomedetomidine.

Figure 2. Evaluation of the ion suppression by acquisition of the signal generated by the simultaneous post-column infusion of a medetomidine standard solution and injection of a blank dog plasma sample extracted with methanol (*A*) or ethyl acetate (*B*).

Figure 3. Chromatograms of the MRM transition monitored for medetomidine obtained after injection of a blank plasma sample (*A*) and of a plasma sample spiked with dexmedetomidine and levomedetomidine at the LLOQ of 0.1 ng/mL (*B*).

Figure 4. Example of the chromatograms obtained for the enantiomers of medetomidine (*A*) and the internal standard medetomidine-*d*3 (*B*) after analysis of a plasma sample collected from a dog administered with racemic medetomidine (20 µg/kg, I.V.).

Figure 5. Plasma concentration of dexmedetomidine and levomedetomidine after intravenous administration of racemic medetomidine at 20 µg/kg. Data are shown as mean ± SD, (n=8 dogs).

*This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)*

***When citing, please refer to the published version.***