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Sherry Cox
UTK, scox6@utk.edu

Lainey Harvill
UTK, lharvil5@vols.utk.edu

Sarah Bullock
UTK, sbulloc5@vols.utk.edu

Joseph Smith
jsmit604@utk.edu

Joan Bergman
UTK, jbaile15@utk.edu

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Validation of a method for pantoprazole and its sulfone metabolite in goat plasma using high performance liquid chromatography

Sherry Cox[a,*], Lainey Harvill[a], Sarah Bullock[a], Joe Smith[b], Joan Bergman[a]

[a] Department of Biomedical and Diagnostic Sciences, The University of Tennessee Knoxville College of Veterinary Medicine, 2407 River Drive, Knoxville, TN 37996, USA
[b] Department of Large Animal Clinical Sciences, The University of Tennessee, College of Veterinary Medicine, 2407 River Drive, Knoxville, TN 37996, USA

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ABSTRACT

A selective method for the quantitation of pantoprazole and its metabolite in goat plasma was developed and validated. Chloroform was used for drug extraction and separation occurred on a Symmetry C_{18} column. A combination of sodium phosphate dibasic (0.1 M, pH 7.5) and acetonitrile were used as the mobile phase (64:36, v/v) while absorbance was measured at 290 nm. With a sample size of 0.1 mL the lower limit of quantification (LLOQ) was 0.01 μg/mL. The intra-assay variability for pantoprazole ranged from 3.4 to 10% while the metabolite ranged from 3.3% to 8.7% The inter-assay variability ranged from 2.6 to 9.7% and 3.3 to 7.5% for pantoprazole and its metabolite. The recovery was over 95% for both pantoprazole and its metabolite, pantoprazole sulfone. The method was used to quantify both pantoprazole and its metabolite and was useful for pharmacokinetic studies.

1. Introduction

Pantoprazole is a proton pump inhibitor (PPI) that functions by irreversibly binding to the hydrogen pumps in the gastric parietal cells which results in reduced gastric acid production. In veterinary medicine the PPIs have been recognized as the most potent suppressor of gastric acid [1].

Abomasal ulceration is a common morbidity observed in ruminant species undergoing stress or hospitalization [1–3]. Pantoprazole is used in ruminant practice for the increase of abomasal gastric pH and as a therapeutic treatment for gastric ulceration [3,4]. Pantoprazole is not currently labelled for use in any ruminant food animal species, and while the usage of this drug is allowable under extralabel dosing provisions, accurate analytical methods are necessary to guide clinicians regarding the potential for accumulation and the pharmacokinetics of the drug when considering its use in a food animal.

The majority of methods used to determine pantoprazole concentrations in plasma involve ultraviolet (UV) detection [5–10] or mass spectrometry (MS) [11–14]. Certain UV methods require 1 mL of sample [6,7,10] while others require 0.5 mL [8,9]. The recovery of these methods ranged from 46 to 86% which is much lower than the method presented in this paper. The Xie method [5] has an LLOQ of 0.025 μg/mL, is less accurate and has a longer run time. One method [8] requires a complex switching technique and has a time-consuming complex extraction which only produces 75% sample recovery and a 0.2 μg/mL LLOQ. While another method [12] requires the use of an expensive robotic liquid handling workstation and the samples must be frozen for at least 60 min as part of the extraction process. Both the Li et al. [11] and Challa et al. [13] methods have a lower LOQ and use a smaller sample size however, both methods use a MS detector. The recovery of the Li method [11] is 87% (78.6–109.7%) while the recovery for the Challa et al. [13] method is not listed and neither method quantitates the metabolite. The Olivarez method [14] used a 100 μl sample size and had and average recovery of 98% which is similar to ours and their LLOQ is 10 ng/mL which is the same as our LLOQ however they used an LC MS/MS in order to achieve those results and did not quantitate the metabolite. Two of the methods [6,10] require the use of ether for the extraction of 1 mL of plasma which produced a higher LLOQ (0.02 μg/mL) and lower recoveries (46% and 86%). Neither method quantitates the metabolite.

The intention of the study was to develop a precise, reliable, and sensitive technique for the quantitation of pantoprazole and its metabolite in small volume samples. The technique has been used for sample analysis from a goat pharmacokinetic study after administration of pantoprazole.

2. Material and methods

2.1. Chemicals and standards

Pantoprazole was purchased from Cayman Chemical (Ann Arbor, MI), pantoprazole sulfone was purchased from Toronto Research Chemicals (Toronto, ON) and tinidazole was purchased from Sigma Chemical

* Corresponding author.
E-mail address: scox6@utk.edu (S. Cox).

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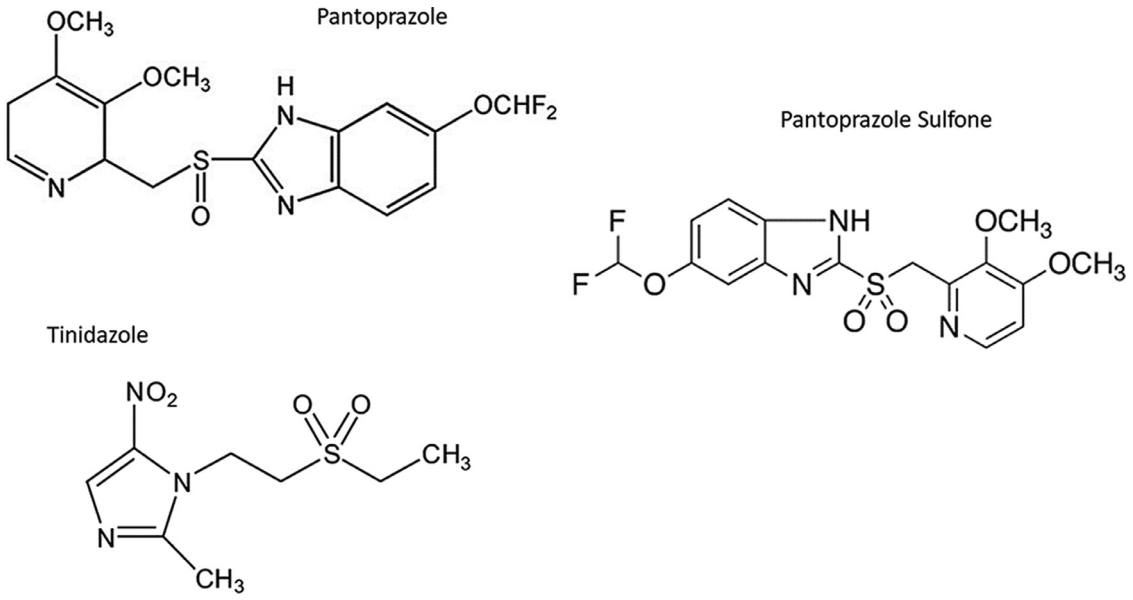

Fig. 1. Molecular structures of pantoprazole, pantoprazole sulfone and tinidazole.

(St. Louis, MO). The purity of all three chemicals (Fig. 1) was ≥99%. All other chemicals and solvents were purchased from Fisher Scientific (Pittsburgh, PA) and were HPLC grade.

Pantoprazole (100 μg/mL), pantoprazole sulfone (100 μg/mL) and tinidazole (100 μg/mL, internal standard) were made by weighing the appropriate amount and dissolving in methanol. Working stock solutions of pantoprazole and pantoprazole sulfone in methanol (0.1, 1 and 10 μg/mL) were prepared from stock solutions. All solutions were maintained in a -20^0C freezer.

Standards and quality control samples were made by pipetting appropriate amounts of the stocks into tubes, evaporating with nitrogen and then adding 100 μL of untreated goat plasma. There were 12 standards used in the composition of the curve, which ranged from 0.01 to 50 μg/mL. The quality control standards were 0.03, 0.3, 3 and 30 μg/mL.

2.2. Chromatographic equipment and conditions

The equipment used for pantoprazole analysis consisted of a 2695 separation module and a 2487 UV detector (Waters, Milford, MA). A Waters Symmetry C_{18} column (4.6 × 150 mm, 5 μm) was used for the separation of the compounds. Sodium phosphate dibasic (0.1 M, pH 7.5) and acetonitrile were used as the mobile phase (64:36, v/v) in the isocratic elution of the compounds. The flow rate was 1.0 mL/min and the UV detector was set to 290 nm. The column temperature was ambient which was 22 ºC.

2.3. Sample preparation

Previously frozen samples were thawed at room temperature and 100 μl of plasma was added to a 13 × 100 mm screw top tube followed by 10 μl of tinidazole (100 μg/mL, internal standard) and 2 mL chloroform. The mixture was rocked for 10 min and then underwent centrifugation for 10 min at 1000 × g. The chloroform layer was removed and placed in a 16 × 100 mm tube and evaporated to dryness. Samples were reconstituted in 250 μL of mobile phase and 100 μL was analyzed.

2.4. Validation of analytical method

The validation techniques used were based on FDA bioanalytical guidelines [15].

2.4.1. Selectivity

The selectivity of the technique was based on analyzing plasma from six different goats that had not been treated with pantoprazole to determine if there were any interfering components from the matrix near the elution times of pantoprazole, its metabolite and tinidazole.

2.4.2. Linearity and calibration curve

The linearity of the plasma peak area ratio versus concentration (0.01–50 μg/mL) was accepted if the correlation coefficient was >0.99. The calibration curve was constructed using the following points: 0.01, 0.025, 0.05, 1, 2.5, 5, 10, 25 and 50 μg/mL. The curves were produced using the ratio of the peak area of pantoprazole or its metabolite divided by the peak area of tinidazole versus the concentration and were generated on five different days.

2.4.3. Recovery accuracy and precision

Accuracy and precision were estimated by analyzing low (0.03 μg/mL) medium (0.3 and 3 μg/mL) and high (30 μg/mL) pantoprazole and metabolite concentrations. Five replicates of each QC were assessed during a single run and on five different days, from that, the intra and inter-assay means, relative standard deviation (RSD) and standard deviation (SD) were determined. The mean value could not exceed ±15% of the actual value except for the lower limit of quantification (LLOQ), which should be reproducible with a precision of 20%. Recovery was calculated as the percentage of the drug response after extraction compared to the response of the drug in the standard solution at a known concentration.

2.4.4. Stability

The stability of the technique was estimated by using the four QC samples. The samples were evaluated after three freeze/thaw cycles, short term stability after extraction and storage in the autosampler for 24 h and after storage in a refrigerator (4 ºC) for 24 h.

3. Results and discussion

3.1. HPLC optimization

Acetonitrile was selected over methanol as the organic component of the mobile phase due to reduced system pressure and better peak resolution. Because of previous experience with drugs of similar structure

Table 1
Pantoprazole (Pan) and pantoprazole sulfone (PS) validation parameters in goat plasma ($n = 5$).

Intra-assay variability						
Concentration (μg/mL)	Pan Measured conc. (mean ± SD)	Pan Accuracy (%)	Pan RSD (%)	PS Measured conc. (mean ± SD)	PS Accuracy (%)	PS RSD (%)
0.03	0.03 ± 0.003	100	10.0	0.03 ± 0.002	100	8.7
0.30	0.33 ± 0.03	110	9.0	0.32 ± 0.01	106	4.3
3.0	3.0 ± 0.10	100	3.4	3.2 ± 0.17	106	5.2
30	30.8 ± 1.98	103	6.4	30.1 ± 0.99	100	3.3
Inter-assay variability						
0.03	0.03 ± 0.003	100	9.8	0.03 ± 0.001	100	3.4
0.30	0.31 ± 0.01	103	4.3	0.34 ± 0.02	113	7.2
3.0	3.0 ± 0.12	100	3.9	3.0 ± 0.23	100	7.5
30	30.1 ± 0.70	100	2.3	30.4 ± 1.0	101	3.3

n: number of samples; SD: standard deviation; RSD: relative standard deviation.

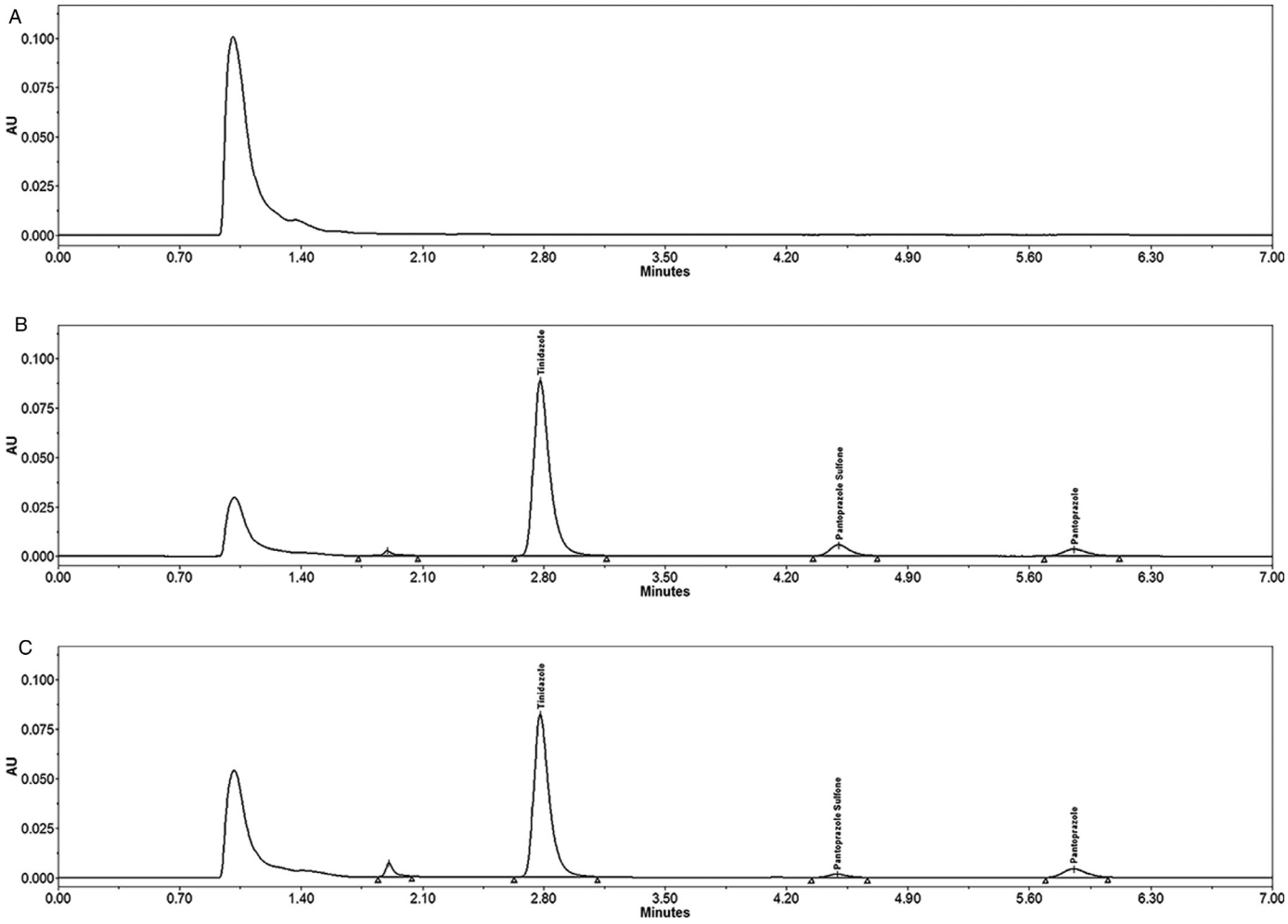

Fig. 2. Chromatograms for pantoprazole and pantoprazole sulfone in goat plasma. (A) untreated goat sample (B) 0.5 μg/mL standard (C) goat sample 20 min after an intravenous dose (1 mg/kg) of pantoprazole.

sodium acetate and sodium phosphate were tested as the aqueous component of the mobile phase. Sodium phosphate produced the optimal response and peak shape. After looking at 3.5, 5.5 and 7.5, the optimal pH was determined to be 7.5. Sample injection volumes between 25 and 100 μL were tested and 100 μL provided the necessary sensitivity for the sample size used.

The system suitability criteria used for pantoprazole and its metabolite included: column efficiency, resolution, retention time and USP tailing factor [16]. Column efficiency was established by determining the theoretical plate number (N) which was 6444 and 5276 ($N \geq 2000$) for pantoprazole and the metabolite. Resolution, which is a measure of how well peaks are separated was 8.01 and 6.15 ($R \geq 2$) for pantoprazole and its metabolite. The retention times were 5.86 ± 0.04 and 4.49 ± 0.03 min for pantoprazole and pantoprazole sulfone and the USP tailing factor was 1.21 and 1.28 ($T \leq 2$).

3.2. Sample optimization

During the development of the technique several organic solvents were evaluated including, acetonitrile, chloroform, hexane, methylene chloride and methanol. Acetonitrile and methanol protein precipitation required an extra step in order to reduce the turbidity of the supernatant so that it could be injected. Hexane and methylene chloride produced recoveries that were less than 50%. Chloroform produced an average

Table 2
Recovery for pantoprazole and pantoprazole sulfone (PS) (n = 5).

Concentration (μg/mL)	Pantoprazole Recovery ± SD (%)	Pantoprazole RSD (%)	PS Recovery ± SD (%)	PS RSD (%)
0.03	101 ± 1.1	1.1	101 ± 6.4	6.3
0.30	99 ± 2.3	2.4	89 ± 2.1	2.3
3.0	97 ± 2.9	2.9	94 ± 4.0	4.2
30	99 ± 4.9	4.9	101 ± 4.2	4.5

n: number of samples; SD: standard deviation; RSD: relative standard deviation.

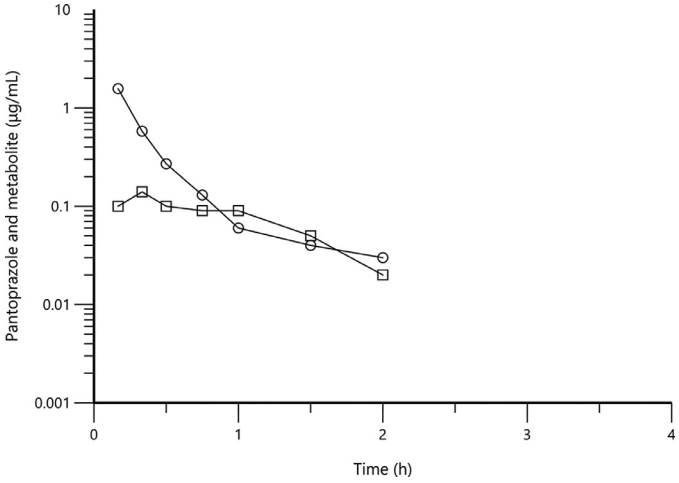

Fig. 3. Goat plasma concentration time curve for intravenously administered pantoprazole (1 mg/kg). Pantoprazole (○) and pantoprazole sulfone (□).

recovery of greater than 90% for both the metabolite and pantoprazole. The amounts of chloroform were varied between 1 and 3 mL in order to determine the appropriate amount and the addition of 1 mL produced the desired results.

3.3. Method validation

3.3.1. Selectivity

Plasma from untreated goats was extracted and no endogenous components affected the elution of pantoprazole, pantoprazole sulfone or tinidazole (Fig. 2A). Plasma for the validation process came from six separate sources. Also illustrated in Fig. 2 are chromatograms for a (B) 0.5 μg/mL standard and (C) a goat sample 20 min after administering an intravenous dose of 1 mg/kg pantoprazole. Elution times for tinidazole, pantoprazole sulfone and pantoprazole were 2.78, 4.49 and 5.86 min.

3.3.2. Linearity, calibration curve and LLOQ

The peak area ratio versus concentration was linear for the concentration range used (0.01–50 μg/mL) with a correlation coefficient of greater than 0.99 for both pantoprazole and its metabolite. The linear relationship for pantoprazole sulfone could be defined by the equation $y = 0.1473x + 0.0075$ while the relationship for pantoprazole could be defined by the equation $y = 0.1507x + 0.0271$. The x represents the plasma concentration of the metabolite or pantoprazole in plasma.

The LLOQ of the method was 0.01 μg/ml, which represents a peak roughly ten times baseline noise. This is sensitive enough for use in pharmacokinetic studies.

3.3.3. Recovery accuracy and precision

The recoveries of pantoprazole and its metabolite (Table 2) ranged from 97 to 101% and 89 to 101%, respectively. The average recovery for tinidazole was 99% ± 5.9%. The intra and inter assay precision values for pantoprazole ranged from 3.4 to 10% and 2.3 to 9.8% while the metabolite ranged from 3.3 to 8.7% and 3.3 to 7.5% (Table 1). The accuracy of the method for both the parent compound and its metabolite ranged from 100 to 113%. The QC sample values for both accuracy and precision are in the acceptable ranges based on FDA Bioanalytical guidelines.

3.3.4. Stability

Extracted QC samples were stored in a refrigerator (4 ^{0}C) for 24 h and in the autosampler for 24 h. Analysis indicated a loss for pantoprazole of 27% and 13% while the metabolite experienced similar results (25% after refrigeration and 10% in the autosampler). This would indicate that extracted samples that have been stored for 24 h should probably not be analyzed and caution should be used in analyzing large sample batches. There was no loss of pantoprazole or its metabolite after three freeze/thaw cycles.

3.3.5. Pharmacokinetic study

This method has been used in the analysis of samples from a pharmacokinetic (PK) study in goats (University of Tennessee College of Veterinary Medicine study protocol 2825–0221) after intravenous administration [17]. A plasma concentration time curve from one of the goats administered 1 mg/kg intravenously is pictured in Fig. 3. The pantoprazole area under the concentration time curve from 0 to infinity ($AUC_{0-\infty}$) was 0.895 h•μg/mL the half-life of elimination ($t\frac{1}{2}$) was 1 h, the clearance (Cl) was 1.17 mL/kg/h while the volume of distribution at steady state (Vd_{ss}) was 0.43 L/kg. The PK parameters for pantoprazole sulfone were $AUC_{0-\infty}$ 0.160 h•μg/mL, $t\frac{1}{2}$ 0.46 h, maximum concentration (C_{max}) 0.14 μg/mL and time to maximum concentration (T_{max}) 0.33 h. Pharmacokinetic parameters were generated from a commercial software program (Phoenix 64 WinNonlin 8.1, Pharsight Corp, Mountain View, CA).

4. Conclusion

This is a fully validated method for the quantification of pantoprazole and its metabolite in plasma samples. It was validated based on FDA Bioanalytical guidelines and has met those criteria. It would be useful for the analysis of a wide range of drug concentrations and can be applied to pharmacokinetic studies. The lower sample volume is an advantage that could make it beneficial for studies involving smaller animals. The method presented was used in the analysis of pantoprazole samples from a pharmacokinetic study conducted at this facility and could be suitable for additional species. However, it may require some additional validation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Sherry Cox: Conceptualization, Methodology, Supervision, Project administration, Writing – original draft. **Lainey Harvill:** Methodology, Validation, Writing – original draft. **Sarah Bullock:** Validation, Writing – review & editing. **Joe Smith:** Writing – review & editing, Resources.

Joan Bergman: Validation, Methodology, Data curation, Writing – review & editing.

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References

[1] J.S. Smith, A.R. Kosusnik, J.P. Mochel, A retrospective clinical investigation of the safety and adverse effects of pantoprazole in hospitalized ruminants, Front Vet Sci 7 (2020) 1–6. https://doi.org10.3389/fvets.2020.00097.

[2] S. Vatn, M.J. Ulvund, Abomasal bloat, haemorrhage and ulcers in young Norwegian lambs, Vet Rec 146 (2000) 35–39. https://doi.org10.1136/vr.146.2.35.

[3] A.S. Mosichuk, J. Smith, D. Tatarniuk, A. Kreuder, Gastrointestinal ulceration in calves presented to a central Iowa veterinary referral facility: an underappreciated morbidity? AIR (2021) 1–4. https://doi.org10.1101/2020.12.17.423269.

[4] G.W. Smith, J.L. Davis, S.M. Smith, M.P. Gerard, N.B. Campbell, D.M. Foster, Efficacy and pharmacokinetics of pantoprazole in alpacas, J Vet Intern Med 24 (2010) 949–955. https://doi.org10.1111/j.1939-1676.2010.0508.x.

[5] Z. Xie, X. Chen, F. Jin, D. Zhong, Simultaneous determination of pantoprazole and its two metabolites in dog plasma by HPLC, J Chromatogr Sci 43 (2005) 271–275. https://doi.org10.1093/chromsci/43.5.271.

[6] M. Noubarani, F. Keyhanfar, M. Motevalian, M. Mahmoudian, Improved HPLC method for determination of four PPIs, omeprazole, pantoprazole, lansoprazole and rabeprazole in human plasma, J Pharm Pharm Sci 13 (2010) 1–10. https://doi.org10.18433/j3gp4q.

[7] D. Zendelovska, E. Atanasovska, K. Gjorgjevska, K. Pavlovska, K. Jakjovski, D. Zafirov, J. Trojacanec, A new solid-phase extraction method for determination of pantoprazole in human plasma using high-performance liquid chromatography, Maced J Med Sci 7 (2019) 1757–1761. https://doi.org10.3889/oamjms.2019.237.

[8] C.A. Ryan, L.C. Sanchez, S. Giguere, T. Vickroy, Pharmacokinetics and pharmacodynamics of pantoprazole in clinically normal neonatal foals, Equine Vet J 37 (2005) 336–341. https://doi.org10.2746/0425164054529427.

[9] D.V. Bharathi, K.K. Hotha, B. Jagadeesh, P.K. Chatki, K. Thriveni, R. Mullangi, A. Naidu, Simultaneous estimation of four proton pump inhibitors-lansoprazole, omeprazole, pantoprazole and rabeprazole: development of a novel generic HPLC-UV method and its application to clinical pharmacokinetic study, Biomed Chromatogr 23 (2009) 732–739. https://doi.org10.1002/bmc.1177.

[10] N.V.S. Ramakrishna, K.N. Vishwottam, S. Wishu, M. Koteshwara, High-performance liquid chromatography method for the quantification of pantoprazole in human plasma, J Chromatogr B 822 (2005) 326–329. https://doi.org10.1016/j.jchromb.2005.06.011.

[11] Y. Li, M.J. Ding, J. Ma, S. Wang, X.L. Wu, H.J. Xu, Z.Y. Lu, J.J. Zou, H.W. Fan, X.M. Zhou, Quantification of pantoprazole in human plasma using LC-MS/MS for pharmacokinetics and bioequivalence study, Eur J Drug Metab Pharmacokinet 35 (2011) 147–155. https://doi.org10.1007/s13318-010-0018-5. Epub 2010 Dec 25.

[12] Y. Dotsikas, C. Apostolou, S. Soumelas, F. Kolocouri, A. Ziaka, C. Kousoulos, Y.L. Loukas, Validation of a novel, fully automated high throughput high-performance liquid chromatographic/tandem mass spectrometric method for quantification of pantoprazole in human plasma, J AOAC Intern 93 (2010) 1129–1136.

[13] B.R. Challa, S.H.S. Boddu, B.Z. Awen, B.R. Chandu, C.K. Bannoth, M. Khagga, K. Kanala, R.P. Shaik, Development and validation of sensitive bioanalytical method for the quantitative estimation of pantoprazole in human plasma samples by LC-MS.MS: application to bioequivalence study, J Chromatogr B 878 (2010) 1499–1505 Epub 2010 Apr 9. https://doi.org10.1016/j.jchromb.2010.03.049.

[14] J.D. Olivarez, A.J. Kreuder, D.M. Tatarniuk, L.W. Wulf, K.A. Dembek, J.P. Mochel, J.S. Smith, Pharmacokinetics and tissue levels of pantoprazole in neonatal calves after intravenous administration, Front Vet Sci 7 (2020). https://doi.org10.3389/fvets.2020.580735.

[15] FDAGuidance for industry: bioanalytical method validation, FDA, 2021 http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070107.pdf accessed 16 September 2021.

[16] FDA, Center for Drug Evaluation and Research (CDER)reviewer guidance: validation of chromatographic methods, FDA, Center for Drug Evaluation and Research (CDER), 2021 https://www.fda.gov/regulatory-information/search-fda-guidance-documents/reviewer-guidance-validation-chromatographic-methods accessed 15 September 2021.

[17] J.S. Smith, J.P. Mochel, W.M. Soto-Gonzalez, R.R. Rahn, B.N. Fayne, O.G. Escher, A.M. Geletka, L.E. Harvill, J.B. Bergman, S. Cox, Pharmacokinetics of pantoprazole and pantoprazole sulfone in goats after intravenous administration: a preliminary report, Front Vet Sci 8 (2021). https://doi.org10.3389/fvets.2021.744813.