Centre of Excellence for Pharmaceutical Sciences (Pharmacen™), North-West University, Potchefstroom, Republic of South Africa

Quantification of ivermectin in veterinary products consumed off-label as a treatment for COVID-19.

C. Wentzel, N. Gernandt, M. Gerber, F. Van der Kooy*

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**Corresponding author: Frank van der Kooy (frank.vanderkooy@nwu.ac.za) Centre of Excellence for Pharmaceutical Sciences, North-West University, Private Bag X6001, Potchefstroom, 2520, RSA frank.vanderkooy@nwu.ac.za*

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The COVID-19 pandemic caused global pandemonium, and due to an unprecedented global response, the popularity and use of (veterinary) ivermectin, amongst many other conceivable 'treatments', experienced a meteoric rise. Ivermectin is a macrocyclic lactone compound belonging to the avermectin drug class and is a registered medicine in many countries, although the most common use is as veterinary medicine. In this study, a fast HPLC method was developed and validated for the quantification of ivermectin in veterinary products that were used off-label by a substantial number of people during COVID-19. Locally used veterinary products were collected as well as commercial products acquired and were tested using the newly developed method. The ivermectin content was compared to the products' label claims and it was found that all products tested contained ivermectin within acceptable limits. However, the use of veterinary products is strongly discouraged due to high dosages and administration regimens that were tested in animals and can lead to serious adverse events in humans. The presence of untested excipients and secondary actives, such as clorsulon, which can cause unknown (long-term) health impacts in humans, further adds credence to this warning.

1. Introduction

Most, if not all, disaster or crisis management protocols have at their heart the necessity to keep people calm whenever a crisis presents itself. It is, in essence, vital to stay calm, which ensures that the best circumstantial decisions are made as opposed to showing panic or stimulating anxiety that typically leads to emotional decision-making resulting in detrimental, and in some cases fatal, outcomes. One of the vast numbers of ill-fated consequences of creating and maintaining a state of panic and anxiety regarding health is that people will turn to any source of 'medicine', much of which may be absolute drivel, toxic, of substandard quality, counterfeited, or even veterinary medicines to name but a few (Movahedi et al. 2023). Early in the pandemic, repurposing of existing drugs was taunted as a possible quick solution, and ivermectin, amongst other existing drugs, was considered as a possible candidate but has since become an extremely controversial topic regarding treatment options for SARS-Cov-2. Ivermectin, belonging to the family of avermectin compounds, is a semi-synthetic derivative and was discovered by isolation from a fermentation broth of *Streptomyces avermectinius*. Avermectins are classified as macrocyclic lactones, whose structures resemble those of macrolide antibiotics, but do not possess antibiotic and antifungal properties as opposed to macrolide antibiotics (Fox 2006). Ivermectin is a macrolide endectocide that presents with pharmacological activity against endo- and ectoparasitic infections (Dourmishev et al. 2005).

Following its introduction in 1981, ivermectin remains a popular anthelminthic drug in veterinary medicine (Fox 2006). It is also listed on the WHO's list of essential medicines and its discoverers, William C. Campbell and Satoshi Ōmura, were awarded the Nobel prize in 2015 (Callaway and Cyranoski 2015). Ivermectin acts by paralyzing nematodes through increasing ɣ-aminobutyric acid (GABA)-mediated neurotransmission in the peripheral nervous system. Pertaining to human use, ivermectin is of particular interest as a possible treatment option for patients presenting with various types of scabies, head lice, demodecidosis, cutaneous larva migrans and currens, myiasis, and filariasis (Dourmishev et al. 2005). Due to the widespread health anxiety during the COVID-19 pandemic, veterinary ivermectin was consumed orally and applied topically by the public as a possible prevention and treatment against the SARS-CoV-2 virus even though no scientific evidence regarding its effectiveness existed at the time. Veterinary medicine is not intended for human use and carries risks regarding types of excipients, secondary active ingredients and dosage regimens not necessarily tested and approved for human use.

Previous HPLC methods for the quantification of ivermectin have been published and including an HPLC gradient method with a total run time of 25 mins per sample (Wimalasinghe et al. 2021), whereas Ibrahim et al. (2023) developed and validated a HPLC method with a total run time of 29 mins. Hiwa et al. (2024) developed and validated a 15 min HPLC method for the quantification of ivermectin in meat products. The objective of this study was not to opine on the (in)effectiveness of ivermectin as a treatment for COVID-19 but to develop and validate a fast HPLC method to retrospectively quantify ivermectin in registered veterinary products that were used during the Covid pandemic. Veterinary ivermectin products were anonymously collected from staff/students and bought from registered outlets and the ivermectin concentration was determined and compared to the label claims using the newly validated method.

2. Investigations and results

Due to recent global inflation partly caused by the COVID-19 pandemic, and the subsequent response to the pandemic, a dramatic increase in operating costs was witnessed and we therefore aimed to

Table 1: Validation results for ivermectin. Precision results are expressed in %RSD with all measurements performed in triplicate

Validation parameter		Standard concentration	Measured value %RSD	Accepted parameters
Precision	Intra-day	1.00 µg/mL	0.39%	RSD ≤ 5%
		8.06 µg/mL	0.16%	
		129.00 µg/mL	0.07%	
	Inter-day	1.00 µg/mL	0.23%	
		8.06 µg/mL	0.72%	
		129.00 µg/mL	0.87%	
Ruggedness	System stability		1.25%	RSD < 2%
	System repeatability		0.12%	
Linearity			0.9999	$R^2 > 0.9999$
Recovery			105.40%	90.0 – 110.0%
LOD			17.4 ng/mL	
LOQ			52.8 ng/mL	

Table 2: The average peak symmetry, purity and width of the ivermectin peak in the commercial products and its measured concentration compared to the products' label claim. All samples were analysed in triplicate

Product number	Avg peak symmetry	Avg peak purity	Avg peak width (50%)	Claimed concentration (mg/mL)	Measured concentration ± SD	% of label claim	% deviation from label claim
1	1.186	0.999	0.168	10.00	10.65 ± 0.030	106.5	+6.5
2	1.238	0.999	0.168	10.00	10.98 ± 0.013	109.8	+9.8
3	1.218	0.999	0.170	10.00	10.53 ± 0.048	105.3	+5.3
4	1.238	0.999	0.168	10.00	10.73 ± 0.006	107.3	+7.3
5	1.058	0.999	0.163	0.80	0.77 ± 0.010	96.25	-3.75
6	1.229	0.999	0.171	10.00	11.00 ± 0.004	110.0	+10.0
7	1.064	0.999	0.166	1.87	1.71 ± 0.016	91.4	-8.6
8	1.243	0.999	0.171	10.00	11.15 ± 0.033	111.5	+11.15
9	1.231	0.999	0.171	10.00	10.79 ± 0.016	107.9	+7.9

*IVM = ivermectin

develop a short isocratic method at a low flow rate to reduce overall costs. Owing to the sample composition (including excipients), the choice was made to include a column cleaning method after each of the respective nine samples were analysed, instead of the usual gradient method that results in longer run times per sample due to the need for system re-equilibration after each sample. The validation of the method did not pose any significant challenges and a short 6 min method was developed and validated. Ivermectin showed a statistically linear response as expressed by y=13957x+16224 (R^2=0.9999). Precision for ivermectin as expressed by the percentage relative standard deviation (%RSD) for the intra- and inter-day analysis was found to be less than 2%; thus, complying with the FDA, ICH and USP guidelines (Table 1). For the purpose of this validation, all measurements were performed in triplicate.

The LOD and LOQ for ivermectin were found to be 17.4 ng/mL and 52.8 ng/mL, respectively. Spiking experiments conducted on the paste product afforded recoveries of 98.4% for product 7. Purity analysis performed on the ivermectin peak of all samples indicated that the ivermectin peaks in all products yielded purity values of >0.99. The results for the 9 products analysed are given in Table 2 and representative chromatograms are presented in Fig. 1. All samples fell well within the South African Department of Agriculture, Forestry and Fisheries regulations of ±15% deviation from the label claim regarding veterinary products (Mudzunga 2017).

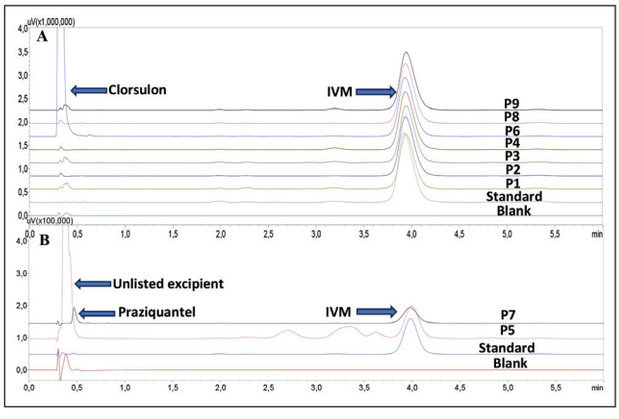

Fig. 1: A) Blank and standard samples compared with products 1-4, 6, 8 and 9 indicating the ivermectin (IVM) peak and the secondary active clorsulon in product 6; B) A blank and standard sample of IVM in comparison with the lower concentration products 5 and 7 also indicating an unlisted excipient in product 5 and praziquantel in product 7. The resolution factor for sample P5 was found to be >1.5.

3. Discussion

The main differences between the products are the formulation and route of administration. Ivermectin intended for human use is usually a single-dose oral tablet that is swallowed as a whole, whereas veterinary products come in a variety of forms. For example, the products that were analysed are injectables (Products 1–4, 6, 8–9) for use in cattle and pigs and the dewormer used in horses (Product 7) is a concentrated paste, whilst the oral drench (Product 5) used for sheep

Table 3: Product description of the nine commercially available veterinary products that were investigated in this study

Product number	Claimed IVM* concentration %	Excipients	Lot number	Manufacturer
1	1.00	Glycerol formal Propylene glycol	2306491	Ascendis Animal Health (Pretoria, RSA)
2	1.00	Benzyl alcohol Ethanol Propylene glycol	210341	Afrivet (Gauteng, Newmark Estate, RSA)
3	1.00	Glycerol formal Propylene glycol	N210207-3A	Ascendis Animal Health (Pretoria, RSA).
4	1.00	Benzyl alcohol Ethanol Propylene glycol	210230	Afrivet (Gauteng, Newmark Estate, RSA)
5	0.08	Not listed	221105	Kyron Agri (Pretoria, RSA)
6	1.00	10.00% Clorsulon (m/v) Glycerol formal Propylene glycol	BD155/22	Boehringer Ingelheim (Midrand, RSA)
7	1.87 (m/m)	14.03% praziquantel (m/v) Titanium dioxide (E171) 20 mg Propylene glycol	31421	Virbac (Centurion, RSA).
8	1.00	Glycerol formal Propylene glycol	2306491	Ascendis Animal Health (Pretoria, RSA).
9	1.00	Glycerol formal Propylene glycol	201206	Ascendis Animal Health (Pretoria, RSA).

*IVM = ivermectin

and goats is a diluted liquid with unknown excipients. These products do tend to contain relatively high dosages as compared with a tablet containing only 3mg of ivermectin which is approved for human consumption (WHO 2023).

According to Martin et al. (2021), the single oral dose (tablet) indicated for the treatment of parasitic infections in the skin and eyes in adults and/or children weighing 15 kg or more is 150 µg/kg of body weight, while the treatment of gastrointestinal parasitic infections for the same patient criteria is 200 µg/kg of body weight. It is crucial to note that the mentioned doses for human use are only safe when administered once every 12 months. The prescribed oral dose of ivermectin in humans may cause a variety of common mild side effects, such as tiredness, stomach pain, inflammatory responses of the skin and eyes, nausea, vomiting, and joint pain (Mueller et al. 2020).

Considering the approved human dosage regimen, it may be concluded that overdosing on ivermectin is the main concern regarding the use of veterinary products (Mueller et al. 2020). For example, 1 mL per day (10 mg ivermectin) and 5 mL per week (50 mg ivermectin) of products 1 and 2 were consumed for a prolonged period and these high doses could potentially lead to health problems associated with overdosing (personal communication). During the COVID-19 pandemic, news reports as stated by the National Public Radio (NPR), alluded to an influx of calls received by the National Poison Data System (NPDS), which regulates information received by poison control centers across the USA, of people who self-medicated with (veterinary) ivermectin products under the belief that it would treat/prevent COVID-19 infections (Romo 2021). This led to a surge of people that required emergency treatment.

Toxicity noted with ivermectin overdose includes, but is not limited to, severe neurotoxicity, and gastrointestinal- and musculoskeletal side effects (Hoang et al. 2022). Results in a study conducted by Lorente et al. (2022) stated that no fatalities related to ivermectin overdose were reported in RSA, but several severe cases as measured by the Poisonous Severity Score (PSS) were reported. Furthermore, the Centers for Disease Control and Prevention (CDC) states that veterinary products may contain excipients that have not been evaluated for human use and cannot be assumed to be safe for human use (CDC 2024). Another potential hazard is the secondary actives such as clorsulon (at a dosage of 10.00%), which is not suitable and/or tested for human use, and praziquantel (at a dosage of 14.03%), which is tested for human use but, as in the case of ivermectin, at different dosage regimens and formulations. At these high dosages, it may cause additional toxicity, especially when consumed daily or weekly (Overholt et al. 2022).

Here we present a fast and efficient analytical method for the accurate quantification of ivermectin in veterinary products. Due to the COVID-19 pandemic and subsequent unprecedented response, many people turned to alternative sources of treatment including veterinary ivermectin, which also saw significant price increases of up to 1700% during the pandemic (Van Eeden 2021). Using untested (veterinary) medications carries substantial risks, and as such, this practice is strongly discouraged.

4. Experimental

4.1. Validation parameters

Validation criteria included: linearity, precision, repeatability, limit of quantification (LOQ), limit of detection (LOD), and accuracy, which were determined according to the Food and Drug Administration (FDA) Office of Regulatory Affairs (ORA-LAB.5.4.5) "methods, method verification, and validation" (FDA 2023; FDA 2018), the International Conference of Harmonisation (ICH) (2022:3) and the United States Pharmacopeia (USP).

4.2. Chemicals and reagents

All reagents used during this project were of analytical grade or higher. A reference standard of ivermectin (purity >98%) was purchased from Horster Biotek (Pradesh, India). Water was obtained from a Rephile direct pure UP Ultrapure & RO Lab water system (Boston, MA, USA). HPLC grade methanol (MeOH) and formic acid were purchased from Sigma-Aldrich (Johannesburg, RSA).

4.3. Calibration solutions

A stock solution of ivermectin was prepared with an initial concentration of 516.00 µg/mL in methanol. The stock solution was serially diluted twofold to obtain ten concentrations ranging between 0.50–258.00 µg/mL. All calibration samples were prepared fresh before validation and the stock solution was stored in a fridge at 4 °C.

4.4. Sample preparation

Nine commercially available veterinary products, each specifying its ivermectin concentration, were collected or bought and are described in Table 3. Seven of the products were labelled as 1.00% (m/v), which equates to 10.0 mg/mL, while one product was 0.08% (0.8 mg/mL). One paste product was also analyzed with its label claim stating 1.87% ivermectin (m/m). From each of the 1.00% liquid samples, 1 mL was diluted 100-fold using HPLC diluent (87% ACN) in order for the concentration to fall within the linear range of the standard curve. The 0.08% sample was prepared

as described above but was only diluted 10-fold. The paste sample was spiked with ivermectin to determine specificity and recovery during sample preparation. This was done by weighing approximately 500 mg into 50 mL falcon tubes and adding 1 mL of ivermectin (202 µg/mL) to the spiked sample and solvent only (1 mL) to the unspiked sample. MeOH (solvent; 19 mL) was added to all paste samples and the samples were vortexed for 1 min, sonicated for 30 min, and again vortexed for 1 min. Samples were filtered with a syringe filter (0.45 µm) into HPLC vials for analysis. All samples were prepared in triplicate.

A Shimadzu iNexera LC-2040C system (Kyoto, Japan) consisting of a quaternary pump, solvent degasser, autosampler, column oven, and photodiode array (PDA) detector was used to validate the method. LabSolutions software was used to control all instrument components and acquire, analyze, and store the data. Chromatographic separation was achieved on a Venusil® XBP C18(2); 2,1 x 50 mm; 3 µm; column (Agela technologies, Newark, DE, USA). Both MeOH and acetonitrile (ACN) in combination with water were tested, and it was found that using 0.1% formic acid (A) and MeOH containing 0.1% formic acid (B) was optimal regarding peak shape and separation. To ensure fast analysis, an isocratic method of 87.0% B for 6 min was used. Ivermectin eluted at 4 min at a flow rate of 0.4 mL/min, and the column oven set to 40°C. The autosampler was maintained at 6°C, and the injection volume was 3 µL. The PDA detector stored UV data over the range of 190–600 nm and detection and quantification were conducted at 243 nm.

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