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Research Article

Stability Evaluation on Diminazene Diaceturate and Phenazone in Bulk and Combined Formulations using Validated Chromatographic Method

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Abstract

The combined therapy of diminazene aceturate (DMZ) and phenazone (PHENZ) is widely used in veterinary medicine to combat trypanosomiasis and babesiosis. This study presents a novel, validated HPLC method for accurately quantifying DMZ and PHENZ in various pharmaceutical formulations, including bulk powders, sachets, vials, and injectables. The chromatographic separation was achieved using a C18 column (150 x 4.6 mm, 5 µm particle size) with a mobile phase composed of phosphate buffer (pH 3.0) and methanol (70:30 v/v) at a flow rate of 1 mL/minute. UV detection was set at 250 nm. The method demonstrated linearity over a concentration range of 20-100 µg/mL for DMZ and 25-125 µg/mL for PHENZ, with correlation coefficients exceeding 0.999. Forced degradation studies were conducted under various stress conditions to assess the method's stability-indicating power. DMZ exhibited first-order degradation under acidic pH conditions. While slight degradation (2.4-3.1%) was observed under alkaline, UV, and indoor room light conditions, PHENZ remained stable. The validated HPLC method effectively quantified DMZ and PHENZ in the presence of their degradation products and impurities, demonstrating its suitability for quality control and stability studies of these combined drug formulations.

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INTRODUCTION

Sudan, a vast and diverse nation, possesses a rich abundance of livestock, serving as a vital source of food and a cornerstone of the national economy[1]. Ensuring the health and well-being of these animals is paramount, necessitating the implementation of efficient veterinary services and robust drug quality control systems. Validated analytical methods with high accuracy and precision are crucial for guaranteeing the efficacy and safety of veterinary medications[2].

Diminazene diaceturate (DMZ) and phenazone (PHENZ) (**Figure 1**) are commonly employed in combination as an anthelmintic treatment for livestock in Sudan and other developing countries. This combination therapy is primarily effective against trypanosomiasis and babesiosis, with DMZ acting as the primary trypanocide and babesiacide. Diminazene diaceturate, an aromatic diamidine derivative with acidic properties, exhibits limited stability in aqueous solutions, typically degrading within 2-3 days[3]. Phenazone, a pyrazolone derivative with basic properties, serves as a stabilizer for DMZ in aqueous formulations, enhancing its shelf-life and efficacy[4].

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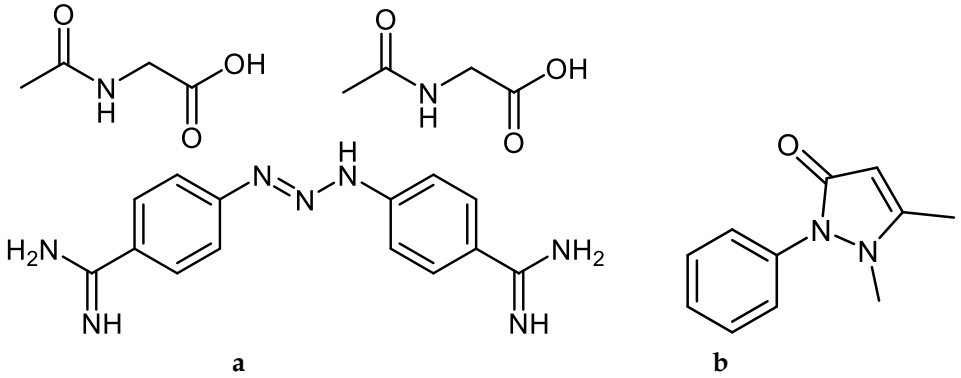

a **b**

Figure 1. Two-dimensional chemical structures of (**a**) DMZ and (**b**) PHENZ.

Despite the widespread use of this combination therapy, a notable gap exists in the availability of standardized quality control methods. While some HPLC methods and a derivative spectrophotometric method have been reported for their analysis[3-5], these methods primarily focus on the determination of drug concentrations in biological fluids or tissues[6-16]. A robust and reliable method for the quality control of DMZ and PHENZ formulations in veterinary products is crucial to ensure their efficacy and safety in animal health.

Several analytical methods have been reported for its quantification in pharmaceutical formulations, including HPLC coupled with LC/MS for the identification of related substances. Additionally, studies have investigated the degradation of DMZ in acidic aqueous solutions[17,18]. The selection of an appropriate mobile phase pH is crucial for successful HPLC analysis, particularly for basic or acidic drugs like DMZ. Proper pH optimization is essential to ensure efficient chromatographic separation, minimizing peak tailing and achieving optimal retention times. This is crucial to avoid interactions between the analyte and the silanol groups present on the silica-based stationary phase, which can lead to peak broadening and decreased resolution[19].

This study aimed to develop, optimize, and validate a simple, robust, and stability-indicating HPLC method for the simultaneous determination of DMZ and PHENZ in their various pharmaceutical formulations marketed in Sudan, including sachets, vials, and ready-to-use solutions. The method was designed to be applicable under both normal storage conditions and under accelerated stress conditions, including exposure to heat, light, acid, and alkali[20]. The optimization process focused on identifying the most suitable chromatographic conditions for achieving optimal resolution and sensitivity for DMZ and PHENZ. This included selecting a commonly available C18 column, utilizing an isocratic mobile phase consisting of a phosphate buffer (pH 3.0) and methanol (70:30 v/v) at a flow rate of 1.0 mL/minute, and employing a detection wavelength of 254 nm, a wavelength suitable for the detection of most organic compounds. The method's validity was rigorously assessed according to The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines. This comprehensive evaluation encompassed several critical parameters, including linearity, range, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision[21]. By establishing this optimized and validated HPLC method, this study aimed to provide a valuable analytical tool for quality control, ensuring the efficacy and safety of veterinary medicines containing DMZ and PHENZ in Sudan. This will facilitate post-market surveillance by regulatory authorities, ultimately contributing to the improvement of animal health and welfare.

MATERIALS AND METHODS

Materials

Chromatographic analyses were performed using a Shimadzu liquid chromatograph equipped with a UV-visible detector, an isocratic and low-pressure gradient pump, and PC control software (Shimadzu, Japan). An electronic balance (Shimadzu, Japan) was used for accurate weighing of standards and samples. Samples were sonicated in a Power Sonic 405 ultrasonic bath (Branson, USA). pH measurements were conducted using a Jenway 3150 pH meter (Cole-Parmer, UK). Analytical grade potassium dihydrogen phosphate, methanol (HPLC grade), sodium dihydrogen phosphate, and buffer solutions (pH 4, 7, and 10) were obtained from Scharlau (Spain). Reference standards of DMZ (100.67% purity) and PHENZ (99.90%

purity) were procured from Laprovet Laboratories (France). More than ten samples of commercially available combined DMZ and PHENZ formulations were collected. These included sachets, vials (containing 70 and 87.3 mg/mL of DMZ and PHENZ, respectively), and ready-to-use solutions (containing 70 and 375 mg/mL of DMZ and PHENZ, respectively). Samples were sourced from local markets and kindly provided by local company representatives.

Methods

Preparation of standard stock mixture solutions of DMZ and PHENZ

To prepare the stock solution (Solution A), 0.02 g of DMZ and 0.025 g of PHENZ were accurately weighed and dissolved in distilled water. The solution was then transferred to a 100 mL volumetric flask and diluted to the mark with distilled water, resulting in a final concentration of 200 µg/mL for DMZ and 250 µg/mL for PHENZ. Subsequently, 30 mL of Solution A was transferred to a 100 mL volumetric flask and diluted to the mark with distilled water, yielding Solution C with final concentrations of 60 µg/mL for DMZ and 75 µg/mL for PHENZ.

Preparation of sample stock mixture solutions of DMZ and PHENZ

Drug granules or powder equivalents of 0.06 g DMZ and 0.075 g PHENZ were accurately weighed and dissolved in distilled water. The solutions were then transferred quantitatively to 100 mL volumetric flasks and diluted to volume with distilled water, resulting in Solution B (DMZ concentration: 600 µg/mL; PHENZ concentration: 750 µg/mL). Subsequently, 10 mL of Solution B was transferred to another 100 mL volumetric flask and diluted to volume with distilled water, yielding Solution D (DMZ concentration: 60 µg/mL; PHENZ concentration: 75 µg/mL). For analysis of the ready-to-use injection, a suitable volume was diluted with distilled water to obtain a working solution (Solution E) with a final concentration of 60 µg/mL DMZ and 75 µg/mL PHENZ, matching the concentration of Solution D.

Standard curve of DMZ and PHENZ combination

Calibration curves for DMZ and PHENZ were generated using a five-point standard curve method. Varying volumes of standard solutions were diluted with distilled water to obtain a series of concentrations within the ranges of 20-100 µg/mL for DMZ and 25-125 µg/mL for PHENZ. Each concentration was analyzed in triplicate, and the peak areas were recorded. Calibration curves were constructed by plotting the peak area against the corresponding concentration for each analyte. Regression analysis was performed on the mean peak areas using Microsoft Excel to determine the linear regression equations for each calibration curve.

Data analysis

Method validation

Method validation was conducted rigorously in accordance with the guidelines outlined by the ICH. Key parameters assessed included linearity, range, LOD, LOQ, accuracy, and precision, ensuring the reliability and robustness of the analytical method[22].

Forced degradation under acidic conditions: To investigate the stability of the compounds, 25 mL aliquots of solutions C and D were separately treated with 1 mL of 0.1 M HCl. Neutralization was then performed at zero time for one aliquot, and the remaining aliquots were neutralized at 10-minute intervals. Following neutralization, each solution was injected into the chromatographic system. The area under the peak for each compound was determined. Subsequently, peak purity and content percentage were calculated for each time point.

Forced degradation under alkaline conditions: Forced degradation under alkaline conditions was conducted using a procedure analogous to that employed for acidic conditions, with the substitution of HCl with NaOH as the stressor.

Heating condition: A 25 mL aliquot of both Solution C and Solution D was separately heated in a boiling water bath for a duration of two hours. Following the heating period, each solution was allowed to cool to room temperature before being injected into the chromatographic column. The peak area corresponding to each analyte was subsequently determined. Finally, peak purity and percent content were calculated for each sample.

Light condition: To investigate the effect of light exposure, two 50 mL aliquots of solution C were prepared. One aliquot (Tube I) was exposed to a UV lamp (254 nm) for 48 hours, while the other aliquot (Tube II) was exposed to direct light for the same duration. Subsequently, each solution was subjected to chromatographic analysis, and the corresponding peak areas were determined. This procedure was repeated using 50 mL aliquots of solution D.

pH profile: A volume of 3 mL of the solution containing DMZ (60 µg/mL) and PHENZ (75 µg/mL) was transferred into a 10 mL volumetric flask to assess the pH stability of the formulations. The solution was then diluted to the mark with buffer solutions ranging from pH 1.0 to 14.0. Each solution was subsequently injected into the chromatographic system, and the corresponding peak area was determined. This procedure was repeated thirteen times to obtain robust data.
The powder of each sachet and vial separately was accurately dissolved in 15 mL of water for injection. The pH was then measured using a pH meter at 0, 3, 6, 9, 24, and 48 hours. The procedure was repeated using pond water and water boiled in a kettle instead of water for injection.

RESULTS AND DISCUSSION

Stability studies are crucial for evaluating the quality and safety of pharmaceutical formulations. These studies provide valuable information regarding the stability of active ingredients, which directly affects the efficacy of the drug product. Furthermore, they help identify the formation of any toxic degradation products that may arise during storage, posing potential safety risks to patients. By determining shelf-life and calculating the half-life of the active ingredient, stability studies provide crucial insights into optimal storage conditions for maintaining drug efficacy and safety[23]. In the present study, a stability-indicating HPLC method was developed for the analysis of DMZ and PHENZ. This method employed a simple binary mobile phase consisting of phosphate buffer (pH 3.0) and methanol (70:30 v/v) on a C18 column (150 x 4.6 mm, 5 µm) at a flow rate of 1 mL/minute. UV detection at 250 nm was found to provide the highest peak intensities for the analyte. The optimized chromatographic method demonstrated successful separation of the combined drugs, DMZ and PHENZ, with retention times of 3.1 and 7.1 minutes, respectively (**Figure 2**). This efficient separation was crucial for accurate quantification.
During the initial stages of method development, a significant challenge was encountered. Filtration of the solutions containing DMZ and PHENZ through filter paper resulted in the adsorption of DMZ onto the filter paper, leading to a noticeable yellow coloration and a significant reduction in the peak area. This issue was successfully addressed by employing a microsyringe filter for solution filtration. Consequently, the use of a microsyringe filter was deemed essential for accurate and reliable analysis of DMZ and PHENZ. Furthermore, the study highlighted the significant impact of filtration on the chromatographic analysis. As depicted in **Figure 3**, filtration effectively minimized potential interference from particulate matter, resulting in cleaner chromatographic peaks, particularly for DMZ.
Subsequently, the optimized HPLC conditions were determined through a systematic investigation of various parameters, including the selection of a suitable column, mobile phase composition and pH, diluent, and detection wavelength[24]. The primary objective of this optimization process was to achieve optimal separation of DMZ and PHENZ peaks from each other and from any potential degradants, while ensuring robust and reliable system suitability parameters. By carefully adjusting these variables, we aimed to enhance the accuracy and precision of the HPLC method for the quantitative analysis of DMZ and PHENZ in pharmaceutical formulations.

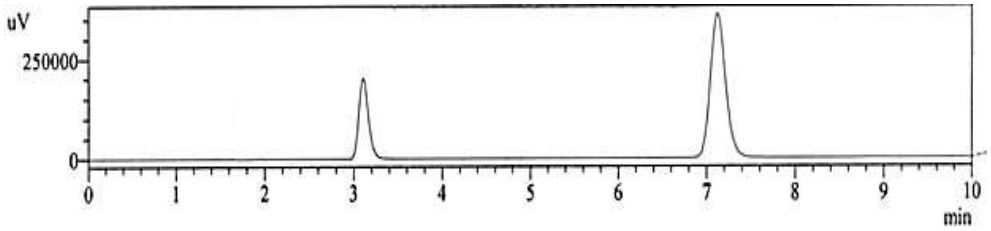

Figure 2. Typical chromatograms for DMZ (**left**, 3.1 minutes) and PHENZ (**right**, 7.1 minutes).

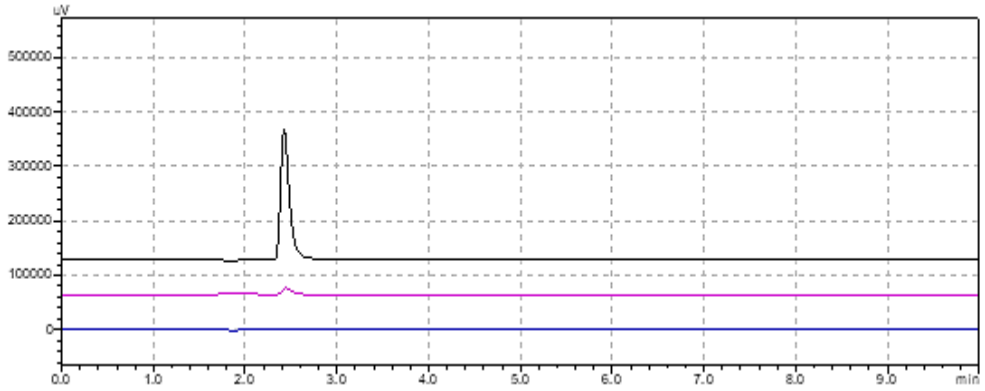

Figure 3. Effect of filtration on DMZ (**black**: microsyringe; **pink**: filter paper; **blue**: blank).

The results of the method validation studies, including system suitability parameters and assay results, are presented in **Tables I**, **II**, and **III**. The developed method demonstrated excellent analytical performance[25], exhibiting linearity with acceptable precision (relative standard deviation <2%) within the ICH guidelines[17,18]. Further validation through assessment of system suitability parameters, including theoretical plates, peak symmetry factor, chromatographic resolution, and capacity factor, confirmed the method's suitability for quantitative analysis. Subsequently, the validated method was applied to analyze commercially available DMZ-PHENZ combination formulations registered in Sudan. Notably, 42% of the analyzed samples exhibited drug content outside the acceptable limits set by the National Medicine and Poisons Board (NMPB). Intriguingly, a wide range of pH values (3.4 to 10.9) was observed in the aqueous solutions of different brands, which may contribute to the observed discrepancies in drug content. However, further investigations are warranted to elucidate the underlying reasons for this significant variation in pH among the analyzed formulations and its potential impact on drug stability and efficacy. These data demonstrate the accuracy, precision, and robustness of the developed analytical method.

Table I. Regression data and validation results of the method developed (n=3).

Parameter	DMZ	PHENZ
Range (µg/mL)	20-100	25-125
Slope	2.52	5.85
Intercept	-13.11	0.77
Correlation coefficient (r)	0.999	1.000
LOD (µg/mL)	1.40	0.50
LOQ (µg/mL)	4.31	1.62
Repeatability (%± RSD)	102.4±0.45	97.7±0.36
Intermediate precision (%±RSD)	102.8±0.55	98.3±0.86
Recovery (%±RSD)	102.1±0.59	99.1±0.76

Table II. System suitability parameters.

Parameter	DMZ	PHENZ
Asymmetry factor	1.03	1.25
Number of theoretical plates	2919.25	8090.68
Resolution	18.4	

Table III. DMZ and PHENZ test results using the developed method (n=3).

Formulation	DMZ	PHENZ
Sachets	102.8±0.6	98.0±0.2
Vials	108.7±0.5	94.7±0.9
Ready-to-use solutions	85.0±0.1	98.8±0.1

Subsequently, the validated method was employed to assess the stability of the drug solution under various stress conditions. The stability of DMZ was investigated under various stress conditions, including pH variations (pH 1.0-14.0), acid hydrolysis, base hydrolysis, light exposure, and heat stress. The results revealed significant degradation of DMZ in the acidic pH range (pH 1.0-5.0), with more than 95% degradation observed at pH 1.0-3.0. Degradation also increased with increasing pH values in the alkaline range (pH 11.0-14.0), with more than 60% degradation observed at pH 13.0 and 14.0.

Notably, the formation of a precipitate was observed at higher pH values (pH 11.0-14.0). Acid hydrolysis studies using different concentrations of HCl demonstrated that a 10-minute incubation time provided a measurable degradation rate with a good correlation coefficient[26].

Chromatographic analysis revealed the formation of degradation products in the acidic and alkaline conditions. The impact of these stress conditions on drug degradation was evaluated, and the results are graphically depicted in **Figures 4** and **5**. **Figure 4** illustrates the presence of extra peaks in the chromatograms of DMZ at pH 1-4, indicating degradation. **Figure 5** depicts a typical chromatogram of degraded DMZ after treatment with 0.1 M HCl for 30 minutes, showing a decrease in the peak area of the parent compound and the emergence of a new peak at a retention time of 2.5 minutes, corresponding to a more polar degradation product. This observation aligns with the known susceptibility of DMZ to degradation due to the presence of a triazene bridge in its structure. Previous studies have confirmed that 4-aminobenzamidine and 4-hydroxybenzamidine are the major degradation products of DMZ[13]. Kinetic analysis revealed that the degradation of DMZ followed first-order kinetics, as evidenced by the linear relationship between the logarithm of the percentage of drug remaining and the time interval (**Figure 6**). Notably, while alkali, light, and heat exposure resulted in minimal degradation (2.4-3.1%), acidic conditions led to a significant loss of DMZ, with up to 98% degradation observed within one hour. Stability studies conducted using the validated method demonstrated that PHENZ exhibited greater stability compared to DMZ under various stress conditions, including exposure to acid, base, light, and heat (**Table IV**). This finding aligns with the observations of Miao *et al.*[16], who reported that PHENZ degradation requires exposure to highly reactive conditions, such as ozone treatment.

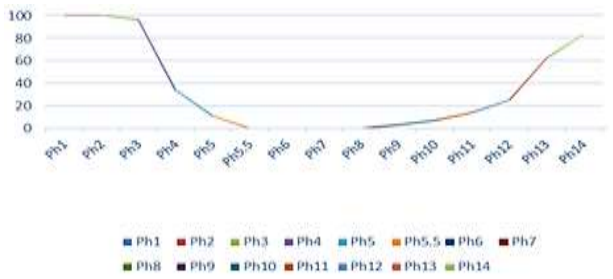

Figure 4. pH profile of DMZ degradation.

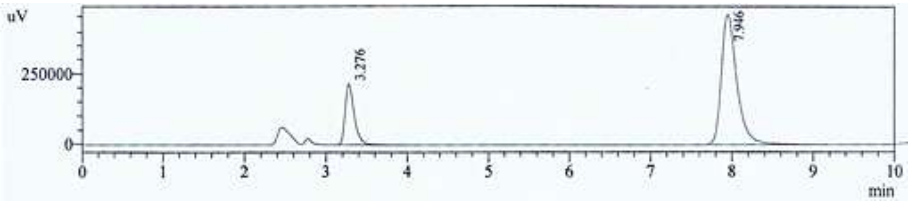

Figure 5. Typical chromatogram for 0.1 M HCl effect on drug degradation after 30 minutes.

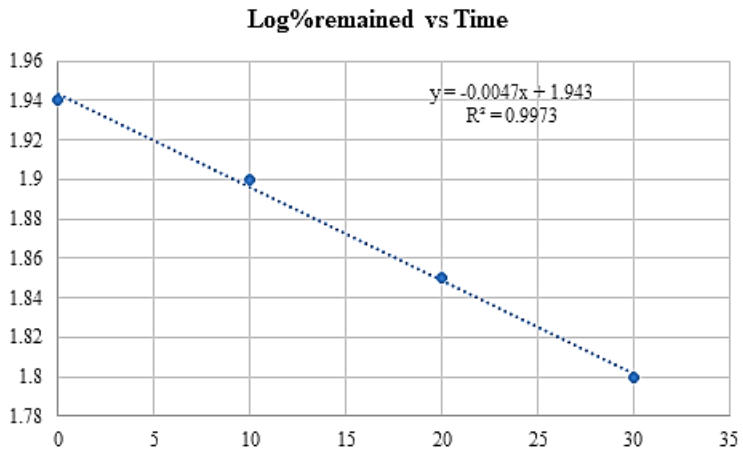

Figure 6. Reaction kinetics of acid influence on DMZ degradation.

Table IV. Summary results for the effect of stress conditions on drug stability.

Condition	Drug	Mean Peak Area (x10³)	Recovery (%)	Degradation (%)
Heat	DMZ	181.229	97.3	2.7
	PHENZ	61728.201	99.9	0.1
UV	DMZ	181.788	97.6	2.4
	PHENZ	61173.019	99.1	0.9
Acidic	DMZ	42.839	23	77
	PHENZ	61234.747	99.2	0.8
Alkaline	DMZ	180.484	96.9	3.1
	PHENZ	61296.476	99.3	0.7

While Abualhasan *et al*.[14] developed a stability-indicating HPLC method for analysis utilizing a C18 column and a mobile phase comprising phosphate buffer (pH 5.2), sodium hexane sulfonate, methanol, and acetonitrile, this method presents several practical limitations that hinder its routine application in quality control. The complex mobile phase composition, involving an ion-pairing reagent like sodium hexane sulfonate, necessitates specialized handling and increases the risk of analytical errors. Sodium hexane sulfonate can irreversibly bind to the C18 column, requiring extensive and potentially ineffective cleaning procedures. Even after thorough flushing, trace amounts of the ion-pairing reagent can remain on the column, altering its selectivity and impacting the reproducibility of subsequent analyses. This not only increases the risk of inaccurate results but also significantly increases the operational costs due to the need for frequent column replacements and specialized cleaning solvents[27]. Moreover, ion pairing needs more time for the mobile phase to equilibrate with the column and any changes in temperature or mobile phase organic can disrupt the equilibrium. Thus, it is taken as the last choice for routine laboratory use if there are no other alternatives[28].

The observed stability of DMZ in the acidic environment (0.1 N HCl) can be attributed to the pH of the mobile phase used in the HPLC analysis. The mobile phase buffer (pH 5.2) likely buffered the acidic environment, preventing significant pH changes in the DMZ solution and thus minimizing degradation. This contrasts with the observed degradation of DMZ in aqueous solution, highlighting the importance of pH control in maintaining drug stability[3,4,29]. This study introduces several novel aspects. Firstly, it presents a tailored optimization of HPLC conditions specifically for the stability assessment of DMZ and PHENZ. Secondly, it emphasizes the importance of understanding the conditions under which DMZ can be safely formulated and the potential impact of inappropriate reconstitution methods on drug stability.

Our observations during sample collection revealed that end-users often deviate from the recommended reconstitution instructions for DMZ and PHENZ powders. The use of raw or potable water, which may contain various impurities such as electrolytes, organic matter, and microorganisms, can significantly impact the physical and chemical properties of the reconstituted drug solution[4]. To investigate this, the drug powder was reconstituted with kettle-boiled water and pond water, and the resultant solutions were compared with those prepared using water for injection. Notably, changes in physical appearance and pH were observed in the solutions prepared with kettle-boiled water and pond water, highlighting the critical role of water quality in maintaining drug stability and efficacy.

CONCLUSION

A robust and accurate HPLC method was developed and validated according to ICH guidelines for the simultaneous determination of DMZ and PHENZ in their bulk forms and combined dosage forms. The method demonstrated excellent linearity, precision, and accuracy, making it suitable for stability studies and routine quality control of pharmaceutical preparations containing these drugs. This validated HPLC method is recommended for the reliable and consistent analysis of DMZ and PHENZ in both bulk and formulated products.

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AUTHORS' CONTRIBUTION

Conceptualization: Amna Mubarak, Shaza Wagiealla Shantier
Data curation: Amna Mubarak
Formal analysis: Amna Mubarak
Funding acquisition: -
Investigation: Amna Mubarak, Shaza Wagiealla Shantier, Magdi Awadalla Mohamed, Elrasheed Ahmed Gadkariem
Methodology: Shaza Wagiealla Shantier, Magdi Awadalla Mohamed, Elrasheed Ahmed Gadkariem
Project administration: Shaza Wagiealla Shantier
Resources: Shaza Wagiealla Shantier, Magdi Awadalla Mohamed, Elrasheed Ahmed Gadkariem
Software: -
Supervision: Shaza Wagiealla Shantier, Magdi Awadalla Mohamed, Elrasheed Ahmed Gadkariem
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Visualization: Amna Mubarak, Shaza Wagiealla Shantier
Writing - original draft: Amna Mubarak, Shaza Wagiealla Shantier
Writing - review & editing: Shaza Wagiealla Shantier, Magdi Awadalla Mohamed, Elrasheed Ahmed Gadkariem

DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study.

REFERENCES

1. Rahman EMKA. Livestock: Sudanese Sheep are a wasted wealth between pastures and governments. Hum Nat Sci J. 2023;4(2):243-55. DOI: 10.53796/hnsj4230

2. Dayoub M, Shnaigat S, Tarawneh RA, Al-Yacoub AN, Al-Barakeh F, Al-Najjar K. Enhancing Animal Production through Smart Agriculture: Possibilities, Hurdles, Resolutions, and Advantages. Ruminants. 2024;4(1):22-46. DOI: 10.3390/ruminants4010003

3. Akode RM, Shantier SW, Gadkariem EA, Mohamed MA. Simultaneous Determination and Stability Studies on Diminazene Diaceturate and Phenazone Using Developed Derivative Spectrophotometric Method. Int J Anal Chem. 2017;2017:4269587. DOI: 10.1155/2017/4269587; PMCID: PMC5299212; PMID: 28246529

4. Kassaye L, Hymete A, Bekhit AA, Genete G. Validation of an HPLC method for the simultaneous determination of diminazene diaceturate and phenazone in injectable veterinary granules and bulk powders. Pak J Pharm Sci. 2012;25(1):255-9. PMID: 22186338

5. Roybal JE, Walker CC, Pfenning AP, Turnipseed SB, Storey JM, Gonzales SA, et al. Concurrent determination of four fluoroquinolones in catfish, shrimp, and salmon by liquidchromatography with fluorescence detection. J AOAC Int. 2002;85(6):1293–301. PMID: 12477191

6. Gilbert RJ. Studies in rabbits on the disposition and trypanocidal activity of the anti-trypanosomal drug, diminazene aceturate (Berenil). Br J Pharmacol. 1983;80(1):133-9. DOI: 10.1111/j.1476-5381.1983.tb11058.x; PMCID: PMC2044970; PMID: 6652366

7. Gummow B, du Preez JL, Swan GE. Paired-ion extraction and high-performance liquid chromatographic determination of diminazene in cattle plasma: a modified method . Onderstepoort J Vet Res. 1995;62(1):1–4. PMID: 8539029

8. Fouda HG. Determination of diminazene in plasma by high-performance liquid chromatography. J Chromatogr Sci. 1977;15(11):537-8. DOI: 10.1093/chromsci/15.11.537; PMID: 925118

9. Turnipseed SB, Clark SB, Andersen WC, Karbiwnyk CM, Miller KE, Hurlbut JA. Confirmation of diminazene diaceturate in bovine plasma using electrospray liquid chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2006;844(1):127–33. DOI: 10.1016/j.jchromb.2006.07.016; PMID: 16891161

10. Fouda HG. Gas chromatography chemical ionization mass spectrometric analysis of diminazene in plasma. Biomed Mass Spectrom. 1978;5(1):72–5. DOI: 10.1002/bms.1200050113; PMID: 623896

11. Staroverov SA, Vasilenko OA, Gabalov KP, Pristensky DV, Yermilov DN, Aksinenko NM, et al. Preparation of polyclonal antibodies to diminazene and its detection in animal blood plasma. Int Immunopharmacol. 2008;8(10):1418-22. DOI: 10.1016/j.intimp.2008.05.014; PMID: 18573353

12. Karanja WM, Mdachi RE, Murilla GA. A competetive enzyme-linked immunosorbent assay for diminazene. Acta Trop. 2002;84(2):75-81. DOI: 10.1016/s0001-706x(02)00184-5; PMID: 12429424

13. Campbell M, Prankerd RJ, Davie AS, Charman WN. Degradation of berenil (diminazene aceturate) in acidic aqueous solution. J Pharm Pharmacol. 2004;56(10):1327–32. DOI: 10.1211/0022357044409; PMID: 15482649

14. Abualhasan MN, Batrawi N, Zaid AN, Watson DG. A validated and stability indicating HPLC method for analysis of diminazene aceturate and antipyrine combination in a ready injectable solution. Drug Res. 2013;63(6):300-4. DOI: 10.1055/s-0033-1337939; PMID: 23532624

15. Atsriku C, Watson DG, Tettey JN, Grant MH, Skellern GG. Determination of diminazene aceturate in pharmaceutical formulations by HPLC and identification of related substances by LC/MS. J Pharm Biomed Anal. 2002;30(4):979-86. DOI: 10.1016/s0731-7085(02)00450-8; PMID: 12408888

16. Miao HF, Cao M, Xu DY, Ren HY, Zhao MX, Huang ZX, et al. Degradation of phenazone in aqueous solution with ozone: influencing factors and degradation pathways. Chemosphere. 2015;119:326-33. DOI: 10.1016/j.chemosphere.2014.06.082; PMID: 25038548

17. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. Validation of Analytical Procedures: Text and Methodology Q2(R1). In: ICH Harmonised Tripartite Guideline. Geneva: The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; 2005. 1-13.

18. Ermer J, Kleinschmidt G, Rignall A, McGregor P, Agut C. Method Performance Qualification. In: Ermer J, Nethercote P, editors. Method Validation in Pharmaceutical Analysis: A Guide to Best Practice, Second, Completely Revised and Updated Edition. Method Validation in Pharmaceutical Analysis: A Guide to Best Practice, Second, Completely Revised and Updated Edition. Weinheim: Wiley-VCH Verlag; 2014. DOI: 10.1002/9783527672202.ch7

19. Guimaraes GJ, Bartlett MG. The critical role of mobile phase pH in the performance of oligonucleotide ion-pair liquid chromatography-mass spectrometry methods. Future Sci OA. 2021;7(10):FSO753. DOI: 10.2144/fsoa-2021-0084; PMCID: PMC8610006; PMID: 34840810

20. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs-A review. J Pharm Anal. 2014;4(3):159-65. DOI: 10.1016/j.jpha.2013.09.003; PMCID: PMC5761119; PMID: 29403878

21. Teja GS, Archana D, Srinu B, Ali SKA, Reddy SSN, Parvez SK, et al. A Comprehensive Guide for Analytical Method Validation. Int J Pharm Sci Rev Res. 2023;82(2):5-17. DOI: 10.47583/ijpsrr.2023.v82i02.002

22. Borman P, Elder D. Q2(R1) Validation of Analytical Procedures. In: Teasdale A, Elder D, Nims RW. ICH Quality Guidelines: An Implementation Guide. New Jersey: John Wiley & Sons; 2017. DOI: 10.1002/9781118971147.ch5

23. González-González O, Ramirez IO, Ramirez BI, O'Connell P, Ballesteros MP, Torrado JJ, et al. Drug Stability: ICH versus Accelerated Predictive Stability Studies. Pharmaceutics. 2022;14(11):2324. DOI: 10.3390/pharmaceutics14112324; PMCID: PMC9693625; PMID: 36365143

24. Kako D, Ghareeb MM, Al-Lami MS. High-Performance Liquid Chromatography (HPLC) Method Validation for Identifying and Quantifying Rebamipide in Ethosomes. Cureus. 2024;16(3):e56061. DOI: 10.7759/cureus.56061; PMCID: PMC11009477; PMID: 38618434

25. Jenke JR. Chromatographic Method Validation: A Review of Current Practices and Procedures. I. General Concepts and Guidelines. J Liq Chromatogr Relat Technol. 1996;19(5):719-36. DOI: 10.1080/10826079608005533

26. Shantier SW, Gadkariem EA, Adam MO, Mohamed MA. Development of stability-indicating methods for cefquinome sulphate. Int J Biomed Sci. 2013;9(3):162-7. PMCID: PMC3809348; PMID: 24170991

27. Wehr CT. Sample preparation and column regeneration in biopolymer separations. J Chromatogr. 1987;418:27-50. PMID: 3305543

28. Horvath C, Melander W, Molnar I, Molnar P. Enhancement of retention by ion-pair formation in liquid chromatography with nonpolar stationary phases. Anal Chem. 1977;49(14):2295-305. DOI: 10.1021/ac50022a048

29. Food and Agriculture Organization of the United Nations. Residues of some veterinary drugs in animals and foods. Rome: Joint FAO/WHO Expert Committee on Food Additives; 2004.