International Journal of Pharmacy and Pharmaceutical Sciences

Print ISSN: 2656-0097 | Online ISSN: 0975-1491 Vol 14, Issue 5, 2022

Original Article

DETERMINATION OF TOLTRAZURIL IN PRESENCE OF COMPLETE ALKALINE DEGRADATION PRODUCT BY RP-HPLC AND TLC-DENSITOMETRIC METHODS

KHALID A. M. ATTIA[1], AHMED W. MADKOUR[1], AHMED A. ALMRASY[1], AMR M. ABDELFATAH[2*]

[1]Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Al-Azhar University, 11751, Nasr City, Cairo, Egypt.
[2]Department of Pharmaceutical Analytical Chemistry Faculty of Pharmacy, Badr University in Cairo, Egypt.
Email: amr.mohamed93@buc.edu.eg

Received: 19 Jan 2022, Revised and Accepted: 12 Mar 2022

ABSTRACT

Objective: Toltrazuril is veterinary medicine, which is extensively used as an antiprotozoal drug. This drug can be analyzed by two sensitive chromatographic, accurate, and reproducible methods that have been developed and validated for the determination of toltrazuril in the presence of its alkaline degradation product.

Methods: The first method involves an RP–HPLC separation of the two components, successfully achieved using Eclipse XDB-C_{18} Column (4.6 x 150 mm, 5 µm), using a mixture of acetonitrile and water (60:40, v/v) as a mobile phase at a flow rate of 1.4 ml/min. quantification was done with UV detector at a wavelength 242 nm UV detection. The second method is based on the separation and quantification of toltrazuril and its alkaline degradation product by TLC-densitometry on TLC silica gel aluminum plates GF254, using dichloromethane: methanol (9.5:0.5, v/v) as the developing system followed by densitometric measurement at 242 nm UV detection.

Results: For RP-HPLC linearity of toltrazuril over the concentration range of 12.5–75 µg/ml, the components were well resolved from each other with significant diverse R_t values of 6.17 and 8.62 min for toltrazuril and its degradation product, respectively. While in TLC-densitometry method, the linearity of toltrazuril over the concentration range 0.2-6 µg/spot and the studied components were well resolved from each other with significant different $R_f$values of 0.33 and 0.52 for toltrazuril and its alkaline degradation product, respectively. For both the developed and validated methods the %RSD was found to be less than 2% and the % recovery was found to be between [98-102 %].

Conclusion: Fortunately, effective separation of the drug from its degradation product was established, along with the determination of toltrazuril as a raw material and the marketed pharmaceutical formulation, such as suspension without any interference. Hence it can be used for routine analysis in various pharmaceutical industries.

Keywords: Toltrazuril, Toltrazuril degradation product, RP-HPLC and TLC-densitometry

DOI: https://dx.doi.org/10.22159/ijpps.2022v14i5.44179. Journal homepage: https://innovareacademics.in/journals/index.php/ijpps.

INTRODUCTION

Toltrazuril is a triazinetrione derivative used in veterinary medicine, it's well-known for its potent anticoccidial effect, provoking its utilization in the treatment and prophylaxis against coccidiosis and necrotic enteritis in poultry and livestock due to its low toxicity, high efficiency, and wide-spectrum deworming capacity [1–5].

Toltrazuril is chemically known as: [1 methyl 3[3methyl4 [4(trifluoromethylsulfanyl) phenoxy] phenyl]-1,3,5-triazinane-2,4,6-trione], and its chemical structure shown in fig. 1.

The molecular weight of toltrazuril is 425.38 g/mol. Toltrazuril is soluble in organic solvents such as ethanol, DMSO, and dimethylformamide (DMF), the solubility of toltrazuril in ethanol is approximately 1 mg/ml and approximately 25 mg/ml in DMSO and DMF.

Many assay methods were reported for the determination of toltrazuril and its metabolites, including Surface-Enhanced Raman Spectroscopy (SERS) using silver nanoparticles (AgNPs) as substrate [6], solid-phase extraction-ultra-high-performance liquid chromatography with UV detection (SPE–UPLC–UV) [7], fast liquid chromatography/tandem mass spectrometry [8], reversed-phase sequential injection chromatography (SIC) [9], Simultaneous determination of toltrazuril and its metabolites in chicken and pig skin+fat by UPLC-UV method [10], determination of toltrazuril and its two metabolites in surface water, soil and animal manure using LC/MS [11], determination of diclazuril, toltrazuril and its two metabolites by gel permeation chromatography–liquid chromatography-tandem mass spectrometry [12], HPLC method for determination of toltrazuril with diclazuril [13], determination of toltrazuril and its residual solvents using HPLC and GC [14] and HPLC methods in presence of other compounds [15–18].

Nowadays, the development and assessment of new validated methods which can reduce the cost and time of analysis are necessary nowadays to ensure the quality of drugs, safety, and efficacy of the final marketed dosage form. The efficacy and safety of medicinal products and veterinary drugs are directly connected properties that may affect the pharmacological effect and cause a progressive adverse effect. It is vital to estimate the content of these substances using analytical stability-indicating methods.

Until now, no reported TLC or HPLC method determining toltrazuril in the presence of complete alkaline degradation of toltrazuril was created. The present study aims to develop and validate a simple, precise and accurate stability-indicating TLC and HPLC methods for the determination of toltrazuril in the presence of its alkaline degradation product in bulk powder and pharmaceutical formulation according to ICH guidelines [19].

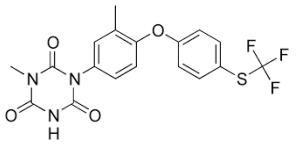

Fig. 1: Chemical structure of toltrazuril

MATERIALS AND METHODS

Chemicals and reagents

Standard toltrazuril was kindly provided by Arabco-Med® for pharmaceutical industries, Obour City-Industrial area Cairo. Its

purity was assessed to be 99.3 %. The commercial dosage form yoserzoril® suspension, manufactured by Waki Pharma® for pharmaceutical industries, 10th Of Ramadan City, Cairo, Egypt. (Batch No.0030319), is labeled to contain 50 mg/ml in the dosage form. prepared 1 mol/l aqueous solution of hydrochloric acid (PIOCHEM® Co., Egypt), 1 mol/l aqueous solution of sodium hydroxide (TOPCHEM® Co., Egypt), the solvent used is absolute ethanol (PIOCHEM® Co., Egypt)., methanol (PIOCHEM® Co., Egypt), dichloromethane (PIOCHEM® Co., Egypt), water and acetonitrile HPLC grade were obtained from Sigma Aldrich®, Cairo, Egypt.

Instruments used

Pre-coated silica gel TLC aluminum plates GF254; layer thickness 20 × 20 cm, 0.25 mm, Fluka (Buchs, Switzerland) were used. The samples were applied using a CAMAG® Linomat 5, autosampler (Muttenz, Switzerland) with a Camag 100 µl sample syringe (Muttenz, Switzerland) at an application rate of 10 µl/s as bands of 6 mm width. CAMAG TLC densitometric Scanner 3S/N 130319 in the reflectance absorbance mode was used for densitometric scanning with a scanning speed of 20 mm/s, the slit dimension was kept at 5 × 0.2 mm. The scanner was operated by WINCATS software (Muttenz, Switzerland). Plates were visualized using a UV lamp with a short wavelength of 242 nm (USA) until the method was optimized. Agilent® 1,260 Infinity series HPLC system was operated by Agilent chemstation software. A quaternary pump, injector with a 20 µl loop, and a UV detector (Minnesota, U. S. A) Were used. Separation was done on Eclipse XDB-C_{18} Column, (4.6 x 150 mm, 5 µm) using a mixture of acetonitrile and water (60:40, v/v) as a mobile phase at a flow rate of 1.4 ml/min. The detection wavelength was set to be 242 nm. Hot plate (Medline MS300 Hot Plate Stirrer. the UK). Sonicator (FALC, Italy). Jenway, 3510 pH meter (Jenway, USA).

Experimental

Chromatographic conditions

RP-HPLC method

Aliquot equivalent to (125-750 µg) 0.125–0.75 mg of toltrazuril was transferred from its respective working standard solution (100 µg/ml) into a series of 10 ml volumetric flasks from their corresponding stock solutions and the volume was made up to the mark using the mobile phase. Volumes of each solution were injected with the aid of an Agilent® analytical syringe in triplicates after filtration through a 0.45 µm membrane filter. Reversed-phase Eclipse XDB-C18 Column, (4.6 x 150 mm, 5 µm) using a mixture of acetonitrile and water (60:40, v/v) as a mobile phase at the detection wavelength was set to be 242 nm. Millipore filter 0.45 µm, white nylon HNWP 47 mm was used for mobile phase filtration, then it was degassed for 15 min in an ultrasonic bath before use. Detection was done at 242 nm for both toltrazuril and its alkaline degradation product. The system was operated at ambient temperature (25 °C). A flow rate of 1.4 ml/min until the end of the run. The chromatograms were recorded, and calibration curves relating the obtained peak areas to the corresponding concentrations were constructed.

TLC-densitometry method

TLC-densitometry was performed according to the standard method. Aliquots equivalent to 0.2–6 µg/spot of toltrazuril were spotted from the standard solution to TLC plates (bandwidth: 6 mm; spacing: 14 mm; 20 mm from the bottom edge of the plate) using Camag® Linomat autosampler with a microsyringe (100 µl). A developing system consisting of dichloromethane: methanol (9.5:0.5, v/v) [20], was used at room temperature (25 °C) for ascending development of the plates. The developed plates were left at room temperature (25 °C) for drying and scanned at 242 nm. The calibration curves relating the area under the peak to the corresponding concentrations of toltrazuril as µg/spot were constructed.

Preparation of standard solutions

A standard stock solution of toltrazuril (1 mg/ml): 100 mg of toltrazuril was accurately weighed and taken in 100 ml clean and dry volumetric flask, then dissolved in 20 ml of absolute ethanol and completed the volume to the mark by absolute ethanol.

From the standard stock solution, 10 ml was accurately pipetted out and added into a 100 ml volumetric flask. Afterward, the solution was completed to the mark using absolute ethanol.

Preparation of alkaline degraded sample

In a rounded flask, a mass of 100 mg of toltrazuril was dissolved in 20 ml absolute ethanol and sonicated for 5 min, and finally, 50 ml of 1 mol/l NaOH solution was added. The flask was heated until boiling under reflux for 4 h, the pH was then neutralized with a pre-calculated 1 mol/l HCl. After that, the solution was left overnight for crystallization. The solution was filtered, and the degradation product was obtained on the filter paper and allowed to dry.

A Stock solution of toltrazuril degradation product (1 mg/ml): was prepared by dissolving 50 mg of 1-methyl-3-(3-methyl-4-(4((trifluoromethyl)thio)phenoxy)phenyl)urea. In 20 ml absolute ethanol and complete to 50 ml with absolute ethanol.

From the stock solution of a toltrazuril degradation product, 10 ml was accurately pipetted out and transferred into a 100 ml volumetric flask, later the solution was made up to the mark using absolute ethanol.

Application to the pharmaceutical formulation

The volume of yoserzoril®suspension (50 mg/ml toltrazuril), 1 ml of suspension was accurately taken and dissolved in 20 ml absolute ethanol in a volumetric flask 50 ml. Finally, the volume was completed with absolute ethanol, sonicated for 30 min, and then filtered. 5 ml of the filtrate were accurately transferred into a 50 ml volumetric flask and the volume was completed with absolute ethanol to obtain a solution contain (100 µg/ml) of toltrazuril. The solution was analyzed (as described previously).

Application to laboratory prepared mixtures

Aliquots of toltrazuril and its degradation product were mixed to prepare different mixtures with different ratios of both. The procedures mentioned under construction of calibration curves were followed and the concentrations of toltrazuril were calculated.

Methods validation

The developed method was validated as per ICH guidelines in terms of its linearity, accuracy, the limit of detection (LOD), the limit of quantification (LOQ), specificity, intra-day and inter-day, precision, and repeatability of measurement [19].

Linearity and range

Linearity is determined by building calibration curves that were constructed by the analysis of different concentrations of toltrazuril. The calibration curves were generated by plotting the drug concentrations against the corresponding peak areas. The linearity plot was constructed, and the data were treated using linear regression analysis. The regression plot was found to be linear over the concentration range of 12.5, 25, 37.5, 50, 62.5, 75 µg/ml for the RP-HPLC method and over the concentration range of 0.2, 0.4, 1, 2, 3, 4, 6 µg/spot for TLC-densitometric method.

Precision

Precision was estimated by calculating repeatability (intra-day precision), and intermediate precision (inter-day precision), precision after repeating measuring of the three different concentrations three times in the same day and assessing the sample in triplicate on three successive days using the proposed methods.

Accuracy

Accuracy was determined using recovery experiments by the determination of % mean recovery of the sample at different concentrations of toltrazuril within their linearity range, and the concentrations were calculated each from their corresponding regression equations. The accuracy of the proposed methods was calculated and RSD% was obtained.

Limit of detection and quantitation

The sensitivity of measurement of toltrazuril using of the proposed method was assessed in terms of the limit of quantitation (LOQ) and

the limit of detection (LOD). LOQ and LOD were calculated according to ICH guidelines from the following equations LOD = 3.3σ/S and LOQ = 10σ/S where σ is the standard deviation of the response of the calibration plot and S is the slope of the corresponding calibration plot.

RESULTS

Methods development

Several trials were implemented to develop the optimum chromatographic conditions for the sufficient separation of toltrazuril and its degradation product.

HPLC method was applied to separate toltrazuril and its degradation product; therefore, several trials have been undertaken to reach the optimum stationary/mobile phases matching. Good chromatographic separation of the two components in their mixtures could be achieved by using Eclipse XDB-C_{18} Column (4.6 × 150 mm), particle size (5 µm), with a mobile phase consisting of acetonitrile and water (60:40, v/v) at a flow rate 1.4 ml/min, followed by UV detection at 242 nm, as shown in fig. 2.

The same method of the assay (RP-HPLC) was done and reported by Aswathy *et al.,* (2021), who used this technique for the quantification of enrofloxacin as a pure and in veterinary dosage forms [21].

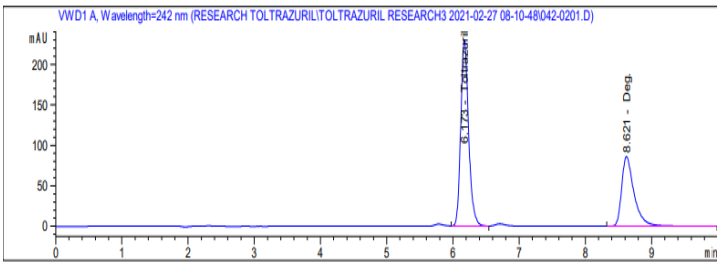

Fig. 2: **HPLC chromatogram of a mixture of intact toltrazuril and its degradation product**

The results of the TLC system were satisfactory upon using dichloromethane: methanol with a ratio of (9.5:0.5, v/v) as a developing system. R_f values were found to be 0.33 and 0.52 for toltrazuril and its degradation product, respectively, as shown in fig. 3-4. This separation allows the determination of toltrazuril at 242 nm without any interference from its degradation product.

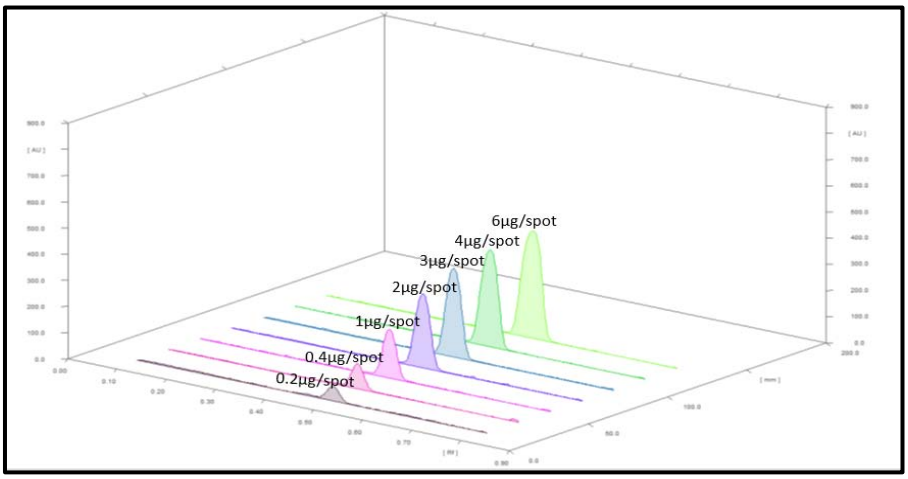

Fig. 3: TLC-densitometric chromatogram of toltrazuril (0.2-6 µg/spot) at 242 nm

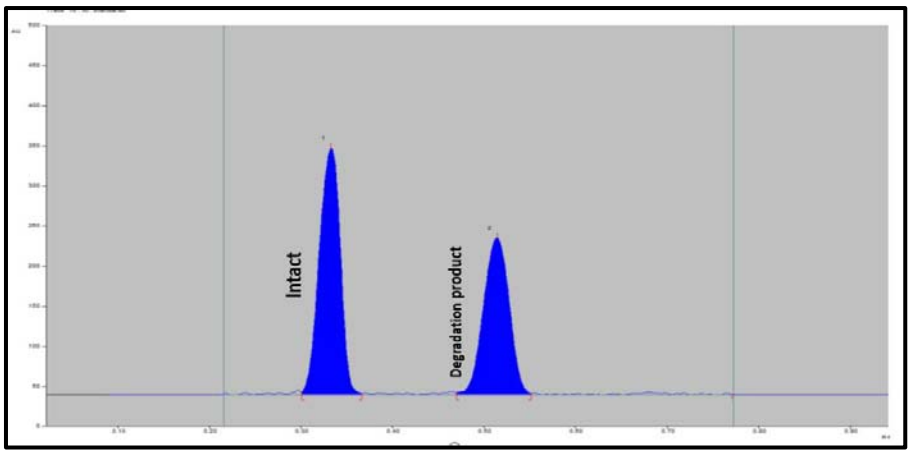

Fig. 4: **2D TLC-densitometric chromatogram of intact toltrazuril and its degradation product at 242 nm**

Method validation parameters

Linearity and range

The linearity plot was constructed, and the data were treated using linear regression analysis. The regression plot was found to be linear over the range of (12.5-75 µg/ml) for the RP-HPLC method and over the range of (0.2-6 µg/spot) for the TLC-densitometric method. Linearity was confirmed by least-squares linear regression analysis; the linear regression equations for the graphs were:

$y = 21.474 x+1.3562$, ($r^2 = 0.9996$), for RP-HPLC method.

$y = 2635 x+2482.6$, ($r^2 = 0.9998$), for TLC-densitometric method.

Where y is the area under peak values, x is the drug concentration and r^2 is the determination coefficient. Linearity range, regression equation, intercept, slope, and determination coefficient for the calibration data were presented in [table 1].

Limit of detection and quantitation

The sensitivity of measurement of toltrazuril using of the proposed method was assessed in terms of the limit of quantitation (LOQ) and the limit of detection (LOD). The limit of detection (LOD) and limit of quantitation (LOQ) values were mentioned in [table 1].

Accuracy

The accuracy of the proposed methods was calculated and RSD% was obtained. The accepted limits of recovery are 98%-102% and all observed data are within the required range, which indicates good recovery values and hence the accuracy of the method developed. Good results were obtained as shown in [table 1].

Table 1: Validation parameters of the proposed TLC-densitometric and RP-HPLC methods for the determination of toltrazuril and alkaline degradation product

Parameter	TLC-densitometric method toltrazuril (n=7)	RP-HPLC method toltrazuril (n=6)
Wavelength (nm)	242	242
Linearity range	0.2-6 µg/spot	12.5-75 µg/ml
Regression parameters		
Intercept	+2482.6	+1.3562
Slope	2635	21.474
Correlation Coefficient	0.9998	0.9996
Accuracy		
mean±SD	100.11±0.76	100.33±0.93
Precision (±%RSD)		
Repeatability*	±0.54	±0.71
Intermediate Precision**	±0.45	±0.69
Specificity***	99.78±0.59	99.54±0.54
LOD****	0.06 µg/spot	3.93 µg/ml
LOQ****	0.19 µg/spot	11.90 µg/ml

*Intraday precision: the %RSD of 3 different concentrations (2 µg, 4 µg, 6 µg for toltrazuril) for TLC-densitometric and (25 µg, 50 µg, 75 µg for toltrazuril) for RP-HPLC, 3 replicates each, within the same day. **Interday precision: the %RSD of 3 different (2 µg, 4 µg, 6 µg for toltrazuril) for TLC-densitometric and (25 µg, 50 µg, 75 µg for toltrazuril) for RP-HPLC, 3 replicates each, repeated on 3 successive days. ***Recovery of toltrazuril in laboratory prepared mixtures containing alkaline degradation product of toltrazuril, data are given in form of mean±SD. ****Calculated from the equation: [LOD = 3.3σ/S, LOQ = 10σ/S; where σ is the standard deviation of the response of calibration plot and S is the slope for the proposed method. n: Number of determinations.

Table 2: Determination of toltrazuril in yoserzoril® suspension by the proposed TLC-densitometric and RP-HPLC methods and application of standard addition technique

Product	Drug	TLC–densitometric method (standard addition) (n=3)				
		Claimed taken (µg/spot)	Added (µg/spot)	Total found (µg/spot)	Standard found (µg/spot)	%Recovery of added
yoserzoril®suspension	toltrazuril	2	-------	1.99	-------	-------
(Batch No.0030319)		2	1	2.99	1.00	99.94
		2	2	4.01	2.02	100.83
		2	3	4.98	2.99	99.73
						100.16±0.58*
		RP-HPLC method (standard addition) (n=3)				
Product	Drug	Claimed taken (µg/spot)	Added (µg/spot)	Total found (µg/spot)	Standard found (µg/spot)	%Recovery of added
yoserzoril®suspension		30	-------	29.95	-------	-------
(Batch No.0030319)	Toltrazuril	30	15	44.84	14.89	99.25
		30	30	60.00	30.05	100.16
		30	40	69.67	39.72	99.30
						99.57±0.51*

*mean±SD, n: Number of determinations.

Table 3: Parameters required for system suitability testing of the developed TLC-densitometric method

Parameters	Toltrazuril	Degradation product	Reference value [22]
K` "capacity factor"	2.03	0.92	The higher the capacity factor, the smaller the retardation factor
α " Selectivity factor "	2.77		>1
Resolution	2.66		>1
Symmetry factor	0.87	0.86	1 for typical symmetric peak

Table 4: Parameters required for system suitability testing of the developed RP-HPLC method

Parameters	Toltrazuril	Degradation product	Reference value [23]
Resolution	-------	9.33	R>2
α " Selectivity factor "	1.7		>1
K` "capacity factor"	1.29	2.19	K`>2
N "column efficiency"	13868	12179	The higher the value, the increase in the efficiency of separation
Tailing factor	0.8	0.6	T=1 for typical symmetric peak

Precision

The result of repeatability (intra-day precision), and intermediate precision (inter-day precision), precision after repeating measuring of the three different concentrations three times in the same day and assessing the sample in triplicate on three successive days using the proposed methods. The calculated RSD % values were listed as shown in [table 1]**,** indicating satisfactory precision of the proposed methods.

Good results were obtained as illustrated in [table 1]. The validity of the proposed procedures is further assessed by applying the standard addition technique, where no interference between the drug and excipients took place; the results obtained were shown in [table 2]. The Parameters required for system suitability testing of the developed TLC-densitometric method and RP-HPLC method were shown in [table 3] and [table 4].

DISCUSSION

Degradation of toltrazuril

The complete alkaline degradation of toltrazuril was obtained after refluxing the drug with 1 mol/l sodium hydroxide at 100 °C for 4 h. Moreover, the degradation product was confirmed via the TLC method using dichloromethane: methanol (9.5:0.5, v/v) as a developing system, where the suggested degradation pathway is shown as follows.

Identification of the degradation product

Degradation product elucidation was established using different spectral methods. The FT-IR spectrum showed the appearance of two peaks at 3379 cm^{-1} and 3325 cm^{-1} as shown in fig. 7, instead of a sharp peak at 3295 cm^{-1} in the spectrum of toltrazuril as shown in fig. 6. This can be assigned to the two secondary amines in the degradation product after the hydrolysis of the triazinane ring. The disappearance of the peak at 1720 $cm^{-1}$of the carbonyl group also took place.

The H^1NMR presented a duplet at 2.88 ppm, corresponding to the N-methyl protons within the degradation product as shown in fig. 9, instead of a singlet that appeared at 3.42 ppm as shown in fig. 8, indicating the presence of an adjacent proton.

Mass spectroscopy showed that the compound has a molar mass of 356.36, indicating the presence of the degradation product, as shown in fig. 10.

In conclusion, all the above evidence indicates that the degradation product could be 1-methyl-3-(3-methyl-4-(4-((trifluoromethyl) thio) phenoxy)phenyl) urea**.** As shown in fig. 5.

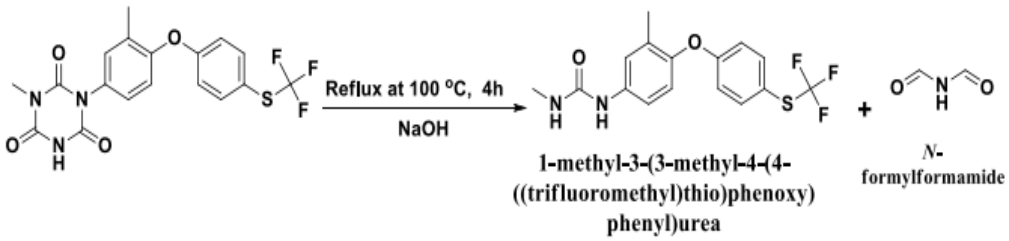

Fig. 5: Suggested degradation pathway of toltrazuril

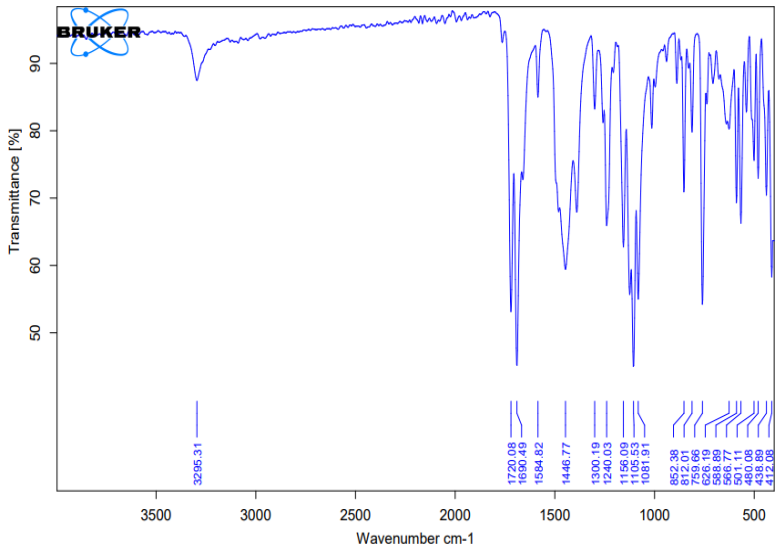

Fig. 6: IR spectrum of intact toltrazuril

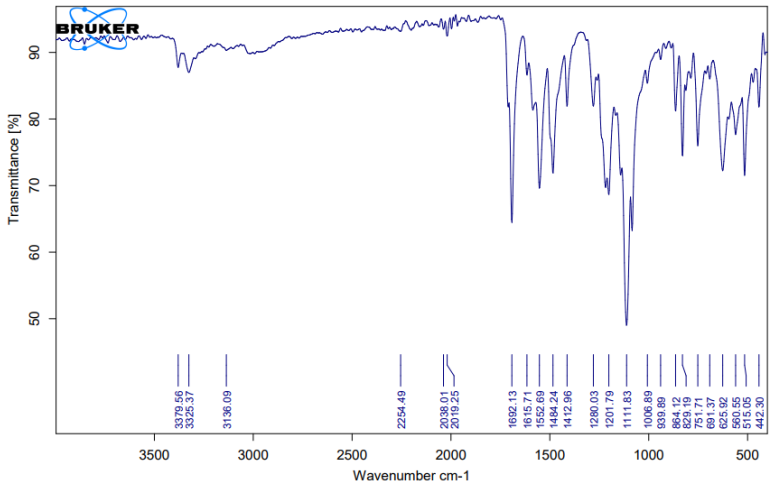

Fig. 7: IR spectrum of a toltrazuril degradation product

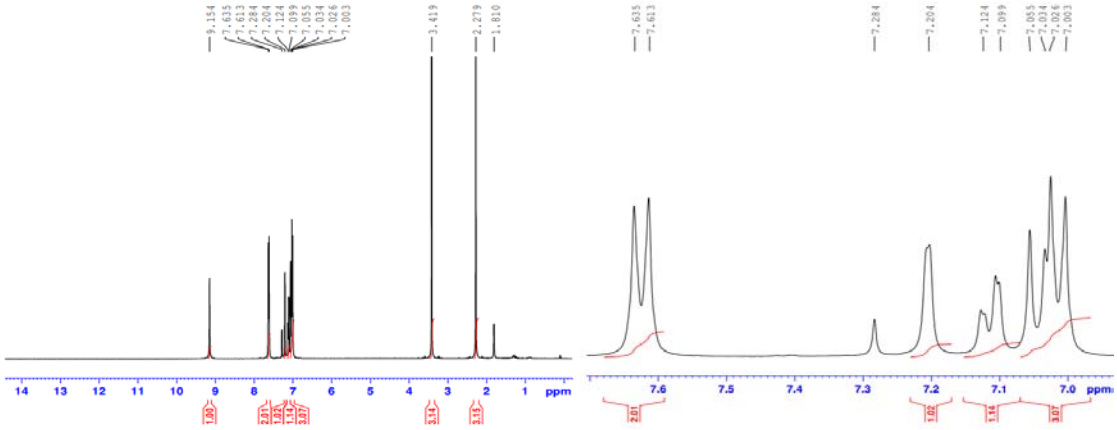

Fig. 8: H[1] NMR spectrum of toltrazuril

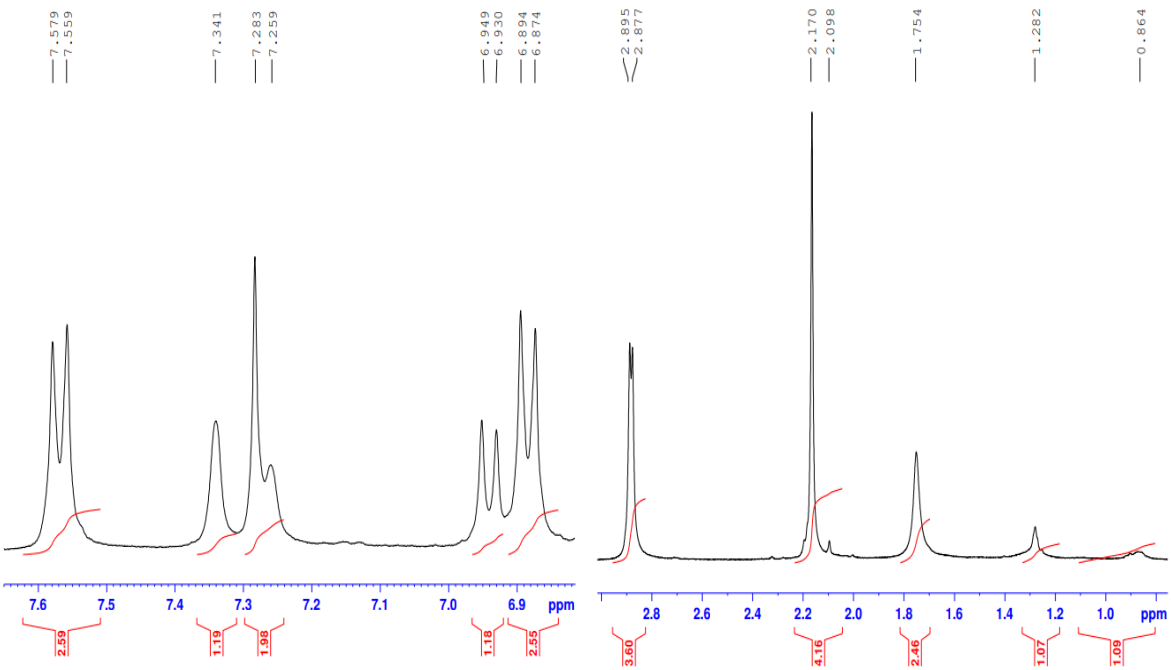

Fig. 9: H[1] NMR spectrum of a toltrazuril degradation product

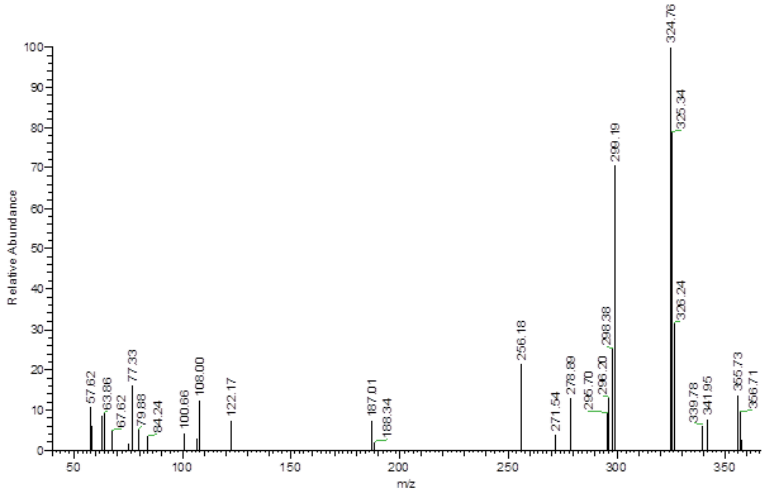

Fig. 10: Mass spectrum of a toltrazuril degradation product

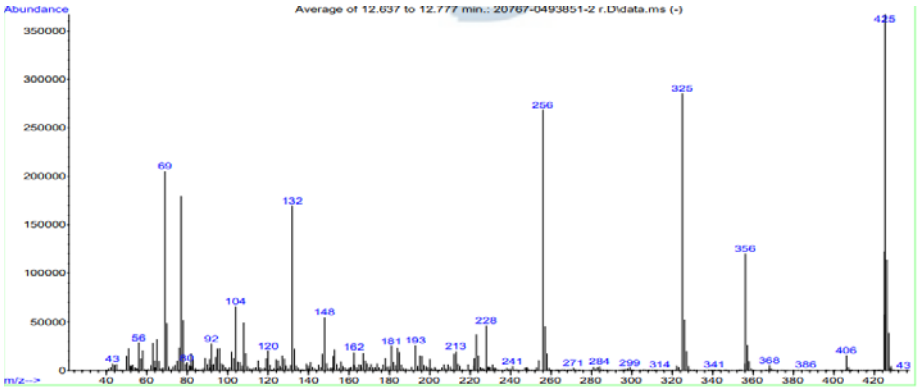

Fig. 11: Mass spectrum of toltrazuril

The suggested chromatographic system for the HPLC method allows complete baseline separation at a reasonable time [24].

Planar chromatography with precise application of samples and computer-controlled evaluation and quantification of the developed chromatograms has been considered to be a reliable technique for purity control and quantitative drug testing [25].

The methods available for the determination of toltrazuril and its metabolites are Surface-Enhanced Raman Spectroscopy (SERS) [6], solid-phase extraction (SPE–UPLC–UV) [7], UPLC-UV method [10], LC/MS [11], determination of toltrazuril and its residual solvents using HPLC and GC [14] and HPLC methods in the presence of other compounds [15–18].

Up till now, no reported TLC or HPLC method was reported for the determination of toltrazuril in the presence of complete alkaline degradation of toltrazuril.

Herein, a simple, new, fast, sensitive, precise, and accurate RP-HPLC and TLC analytical methods are presented for quantification of toltrazuril in marketed formulation using Eclipse XDB-C18 Column (4.6 x 150 mm, 5 µm), using a mixture of acetonitrile and water (60:40, v/v) as a mobile phase at a flow rate of 1.4 ml/min quantification was done with UV detector at wavelength 242 nm UV detection for RP-HPLC method and TLC silica gel aluminum plates GF254, using dichloromethane: methanol (9.5:0.5, v/v) as the developing system followed by densitometric measurement at 242 nm UV detection for TLC method. The methods established are highly specific as there was no interference observed between chromatograms of toltrazuril and its degradation product. The results for precision were expressed in % RSD as tabulated in table 1. Whereas the %RSD for intra and inter-day precision of toltrazuril was observed below 2%. The low values of % RSD indicate that the methods are precise. As for the LOD and LOQ values showed the methods were sensitive for the quantification of toltrazuril in the pure and veterinary dosage forms.

CONCLUSION

A reliable method for the determination of toltrazuril in the presence of complete alkaline degradation was developed. This method is satisfactorily validated through specificity, accuracy, and precision. Thus, the proposed analytical method can be employed in the routine detection analysis of toltrazuril either as a raw material or formulated product.

FUNDING

No funding was provided for this study.

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

1. Wang Z, Zhang J, Liu L. A colorimetric paper-based sensor for toltrazuril and its metabolites in feed, chicken, and egg samples. Food Chem. 2019;276:707-13. doi: 10.1016/j.foodchem.2018.10.047, PMID 30409651.
2. Anadon A, Martinez Larranaga MR. Veterinary drugs residues: coccidiostats. Encycl Food S Afr. 2014;3:63-75.

3. Stock ML, Elazab ST, Hsu WH. Review of triazine antiprotozoal drugs used in veterinary medicine. J Vet Pharmacol Ther. 2018;41(2):184-94. doi: 10.1111/jvp.12450, PMID 28833212.
4. Alnassan AA, Shehata AA, Kotsch M, Schrödl W, Krüger M, Daugschies A, Bangoura B. Efficacy of early treatment with toltrazuril in prevention of coccidiosis and necrotic enteritis in chickens. Avian Pathol. 2013;42(5):482-90. doi: 10.1080/03079457.2013.823476, PMID 23941631.
5. Papich MG. Toltrazuril. Saunders handbook of veterinary drugs. Vols. 799-800. Elsevier; 2016.
6. Shao D, Bi S, Zhao R, Sun X, Li X, Yu J. Selective determination of dinitolmide and toltrazuril by surface-enhanced raman spectroscopy (SERS) using AgNPs as substrate. Sens Actuators B. 2020;307. doi: 10.1016/j.snb.2019.127644, PMID 127644.
7. Zhaoling J, Lifang Z, Chong Z, Xiao Z, Feiqun X. SPE–UPLC–UV method for the determination of toltrazuril and its two metabolite residues in chicken and porcine tissues. Chromatographia. 2014;77(23-24):1705-12. doi: 10.1007/s10337-014-2759-9.
8. Martinez Villalba A, Moyano E, Martins CPB, Galceran MT. Fast liquid chromatography/tandem mass spectrometry (highly selective selected reaction monitoring) for the determination of toltrazuril and its metabolites in food. Anal Bioanal Chem. 2010;397(7):2893-901. doi: 10.1007/s00216-010-3704-x, PMID 20658773.
9. Björklund E, Maya F, Bak SA, Hansen M, Estela JM, Cerda V. Possibilities and limitations of the sequential injection chromatography technique for the determination of anticoccidial agents in water, pharmaceutical formulations and feed. Microchem J. 2011;98(2):190-9. doi: 10.1016/j.microc.2011.01.007.
10. Zheng W, Jiang Z, Zhang L, Zhang C, Zhang X, Fei C, Zhang K, Wang X, Wang M, Li T, Xiao S, Wang C, Xue F. Simultaneous determination of toltrazuril and its metabolites in chicken and pig skin+fat by UPLC-UV method. J Chromatogr B Analyt Technol Biomed Life Sci. 2014;972;972.89-94. doi: 10.1016/j.jchromb.2014.09.027, PMID 25444542.
11. Olsen J, Bjorklund E, Krogh KA, Hansen M. Development of an analytical methodology for the determination of the antiparasitic drug toltrazuril and its two metabolites in surface water, soil and animal manure. Anal Chim Acta. 2012;755;69-76. doi: 10.1016/j.aca.2012.10.015, PMID 23146396.
12. Ai L, Sun H, Wang F, Chen R, Guo C. Determination of diclazuril, toltrazuril and its two metabolites in poultry tissues and eggs by gel permeation chromatography-liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2011;879(20):1757-63. doi: 10.1016/j.jchromb.2011.04.021, PMID 21565565.
13. Jeong KH, Jeong M, Park HC, Hossain MA, Kim D, Lee K, Kang JW. Development of high-performance liquid chromatography methods for the anticoccidials: toltrazuril and diclazuril. Korean J Vet Res. 2017;57(4):223-6. doi: 10.14405/kjvr.2017.57.4.223.
14. Bawazeer S. Development and validation of chromatographic methods for the quantitative determination of toltrazuril and its residual solvents. Hemophilia Joint Health Score 2020;1(2):24-33.
15. Gong X, Sun J, Dong J, Yu J, Wang H. Determination of avermectin, diclazuril, toltrazuril and metabolite residues in pork by high-performance liquid chromatography-tandem mass spectrometry. Se Pu. 2011;29(3):217-22. doi: 10.3724/sp.j.1123.2011.00217, PMID 21657050.
16. Hormazábal V, Yndestad M, Ostensvik O. Determination of flunixin and tiamulin hydrogen fumarate in meat and toltrazuril and the metabolite toltrazurilsulfon in meat and eggs using LC/MS. J Liq Chromatogr Relat Technol. 2003;26(5):791-801. doi: 10.1081/JLC-120018425.
17. Qi KZ, Shi ZH, Peng KS. Simultaneous determination of residues of diclazuril and toltrazuril in chicken tissues by matrix solid phase dispersion-high-performance liquid chromatography/ ultraviolet detection. fen xi Huaxue/Chinese. J Anal Chem. 2007;35(11):1601-6.
18. Shi Z, Ge Q, Lu J, Liu X, Gong J, Zhu L, Qi K, Chen D, Peng K. Comparison of pretreatment methods for the simultaneous determination of diclazuril and toltrazuril residues in chicken tissues. Se Pu. 2009 May;27(3):303-7. PMID 19803134.
19. Harron DWG. Technical requirements for registration of pharmaceuticals for human use: the ICH process. Textb Pharm Med. 2013;1994:447-60.
20. Potawale RS, Hangad TI. Novel high-performance thin-layer chromatographic method for simple, economical, and rapid determination of fenofibrate in bulk and pharmaceutical dosage form. Asian J Pharm Clin Res. 2018;11(5):147-50. doi: 10.22159/ajpcr.2018.v11i5.24240.
21. Aswathy SR, Muhas CS. Validation and application of RP-HPLC method for quantification of enrofloxacin in pure and veterinary dosage forms. Int J Pharm Pharm Sci. 2022;14(2):42-7.
22. Sherma J, Fried B. Handbook of thin-layer chromatography (3rd Edition. Revised and Expanded) (Sherma J, Fried B. eds.). Biochemist; 2004. p. 1016.
23. Wells M, Dantus M. Validation of chromatographic methods. Anal Instrum handbook. 3rd ed; 2004. p. 1015-33.
24. Malviya R, Bansal V, Pal O. High-performance liquid chromatography: A short review. J Glob Pharm Technol. 2010;2:22-6.
25. Renger B. Inst Chroma-tography, Bad Duerkheim. Proceedings of the sixth international symposium on instrumental planar chromatography; 1991. p. 291.