Research Article

Method Development and Validation of Veterinary Active Ingredient Toldimfos Sodium

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ABSTRACT

The process of providing documented evidence that the cleaning methods employed within a facility consistently controls potential carryover of product (including intermediates and impurities), cleaning agents and extraneous material into subsequent product to a pre-determined levels for study of Pre-determined levels developed & Validated a new simple, precise and accurate Ultra Violet Spectrophotometric method for Toldimfos sodium stage residual determination in veterinary active pharmaceutical ingredient manufacturing. The method was developed by using the Shimadzu Ultra Violet Spectrophotometer-2600 & Sartorius Analytical electronic balance CP-225D were used and The wavelength for cleaning method validation for residual determination of Toldimfos sodium was 270nm and the scanned range in spectrum mode was between 200-400nm,selected diluent was HPLC grade water. Successful absorption wave length of the Toldimfos sodium stage was achieved at 270nm.This Cinerary mainly describes the development and validation of a Ultra violet Cleaning method for the residual determination of Toldimfos sodium stage in veterinary active pharmaceutical ingredient manufacturing and final results are tabulated as summary and evaluation results. Developed & Validated method are run successfully for Toldimfos sodium stage residual determination of cleaning samples in veterinary active pharmaceutical ingredient manufacturing and the developed and validated method ensures the safety and purity of the product. It is a regulatory requirement in veterinary Active Pharmaceutical Ingredient product manufacture & It also assures from an internal control and compliance point of view the quality of the process.

Keywords: Toldimfos sodium stage, Residual determination, Ultra Violet Spectrophotometric and cleaning validation.

INTRODUCTION

Toldimfos sodium (Fig. 1) is the sodium salt of 4-dimethylamino-2-methyl-phenyl-phosphinous acid, a derivative of phosphoric acid. It is indicated for the treatment and prophylaxis of diseases which arise in connection with parturition and the peri-partum period, developmental and nutritional disorders in young animals, disorders of bone growth and tetany or paresis caused by disorders of calcium, magnesium and phosphorous metabolism. The recommended therapeutic dose is 10 mg/kg given by intravenous, intramuscular or subcutaneous injection. Injections are normally repeated to clinical effect, up to a maximum of 10 injections.

Toldimfos is indicated for use in the following food producing species: horses, cattle, sheep, pigs and goats. No specific data on the pharmaco-dynamic action of toldimfos was submitted. The precise mode of action of toldimfos is unknown and it is questionable whether the effect of toldimfos is simply a matter of the substitution of deficient phosphorus. It appears more likely that the effect of toldimfos arises due to multiple stimulation of metabolism within the body. Toldimfos is an aromatic phosphorus compound which falls between phosphorous itself and phosphoric acid in the stages of oxidation.[1]

The intramuscular administration of toldimfos to cattle at a dosage level of 10 mg/kg bw led to a peak blood concentration within 10 to 20 minutes after administration. Pharmacokinetic studies in calves showed the mean half-life of toldimfos in serum was 1.07 hours, whilst the corresponding value for dairy cows was 1.15 hours. The pharmacokinetic profile corresponded to a one compartment model of distribution. The mean residence time for toldimfos in blood in calves was 3.6 hours, with a value of 3.1 hours being recorded for dairy cows. These studies were not radio labelled. Urinary excretion studies revealed a rapid elimination of toldimfos following dosing. The major fraction of the administered dose was eliminated within 6 hours, whilst at the24 hour time-point, only concentrations in the range of the limit of detection (limit of detection: 0.5 µg/kg) were observed. Twelve to twenty four hours following a second injection of toldimfos, similar low concentrations were recorded in urine. Toldimfos was eliminated as parent compound.

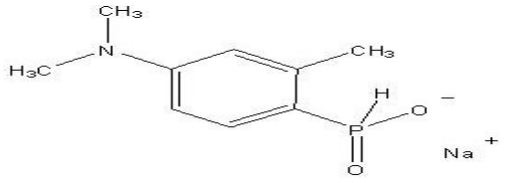

Figure 1: Structure of Toldimfos Sodium Stage.

Molecular Formula: $C_9H_{13}NNaO_2P$

Serum paraoxonase 1 (EC 3.1.8.1, PON1), a calcium-associated enzyme, has an ability to hydrolyze

organophosphate compounds. Related to this property, PON1 has a critical role in antioxidant mechanisms. It is well-known that the enzyme protects LDL from oxidation. In this study we investigated the in vitro inhibitory effects of some drugs. These drugs are oxytocin, dexamethasone, atropine sulphate, gentamicin sulphate, sulfadoxine-trimethoprim, furosemid, metamizole sodium and toldimfos sodium. The IC (50) values obtained varied markedly from 0.014 to 507.72 mg/mL. According to our findings, most potent and significant inhibition was displayed by dexamethasone, atropine sulphate and furosemid.[2]

In 1972, some of the investigations are discloses in vivo effect of inosine, pyruvate and phosphate on oxygen-haemoglobin affinity in rhesus monkey. The experiments suggest that the inosine, pyruvate and phosphate should be used administered for increasing 2, 3-DPG levels in erythrocytes. The journal article did not disclose formulation and the stability aspect of the preparations.[3]

A. Petersen and co-workers, discloses incubation of erythrocytes in inosine, pyruvate and phosphate medium (1PP medium) results in the accumulation of more 5-phosphoribosyl

1-pyrophosphate (PRPP) which is required for purine nucleotides. On carrying out experiments, the disclosed IPP medium shows precipitation during storage.[4]

MATERIALS AND METHODS

Instrumentation

Shimadzu Ultra Violet Spectrophotometer-2600 & Sartorius Analytical electronic balance CP-225D was used. Shimadzu Ultra Violet Spectrophotometer consisting of sample compartment in which Ultraviolet & visble light was passed by two light sources namely as Deuterium lamp for ultra violet range, Tungsten visible lamp for visible range. The drug analysis data were acquired using "UV Probe 2.43" software.[5]

Reagents and Chemicals

Reference standard of Toldimfos sodium and cleaning samples was obtained from well reputed research laboratories and characterized by use of LCMS, NMR and IR. Water was of HPLC grade and purchased from Merck, Mumbai, India.

Ultra Violet Spectrophotometric Conditions

The wavelength for cleaning method validation for residual determination of Toldimfos sodium was 270nm and the scanned range in spectrum mode was between 200- 400nm, selected diluents was HPLC grade water.

Preparation of Standard Solution

Pure standard of Toldimfos sodium were used as external standard in the analysis. Weighed accurately 50.15mg of Toldimfos sodium stage-II (TDSI/02A) reference material and transfer into a 50mL volumetric flask. Dissolve and dilute to volume with diluent. Mix well. Pipette 1.0mL of the above solution into a 100mL volumetric flask. Dissolve and dilute to volume with diluent. Mix well.

RESULTS AND DISCUSSION

Optimization of the Spectrophotometric Conditions

The nature of the sample, its molecular weight and solubility decides the proper selection of the wave length was 270nm. So the detection of the compound from the Ultra Violet Spectrophotometer-2600 was influenced by the selected diluent. The detection of the Toldimfos sodium stage-II (TDSI/02A) and HPLC grade water as diluents were optimized to give a sharp absorbance spectrum with in short time based on selected wave length and absorbance obtained. Different solvents were tried but the selected solvent as water (HPLC Grade water or milli-q-Water) in Ultra Violet Spectrophotometer-2600 for the Toldimfos sodium stage-II (TDSI/02A) with in short time. The absorption of Toldimfos sodium stage-II (TDSI/02A) was found to be 0.5382, which indicates that Toldimfos sodium stage-II (TDSI/02A) is an UV-Active compound as presence of UV-Chromophore. The % of relative standard deviation (R.S.D) values for accuracy and precision studies obtained were less than 2(2 %) which revealed that developed method was accurate and precise. The system suitability and validation parameters are given in (Table 1). The high percentage of recovery of Toldimfos sodium stage-II(TDSI/02A) was found to be above 80% (90.22%) indicating that the proposed method is highly accurate. Proposed Ultra Violet Spectroscopic method was applied for analysis of cleaning method validation for residual determination of Toldimfos sodium stage-II (TDSI/02A).[5]

Development and Validation of Cleaning Procedure Residual Determination for Toldimfos Sodium in Active Pharmaceutical Ingredient by Ultra Violet Spectrophotometer

The present study was concluded to obtain a new, affordable and cost-effective and convenient method for residual determination of Toldimfos sodium by ultraviolet spectrophotometer. The method was validated according to ICH Q2 (R1) guide line for the parameters like system suitability and precision, Specificity, Linearity, Limit of detection & Limit of quantification, Recovery study, Solution stability and mobile phase stability, Record of analysis for Toldimfos sodium stage-II cleaning samples.[6-11]

Method Validation Procedure

Method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for system suitability, precision, specificity, linearity, limit of detection and limit of quantification, recovery.[6-17]

System Suitability & Precision Parameter

To verify that analytical system is working properly and can give accurate and precise results, the system

suitability & precision parameters are to be set. System suitability & precision tests were carried out on freshly prepared 10 ppm standard solutions of Toldimfos sodium and it was calculated by determining the standard deviation of Toldimfos sodium standard by scanning standards in six replicates wave length at 270nm with in the scan range of 200-400nm. The values of %RSD prove that the method is accurate & precise and acceptance criteria is not more than 5.0% for absorbance response. The values were recorded in Table 1.

Toldimfos Sodium 10 ppm Standard Preparation

Weight accurately 50.15mg of Toldimfos sodium stage-II (TDSI/02A) reference material and transfer into a 50mL volumetric flask. Dissolve and dilute to volume with diluent. Mix well. Pipette 1.0mL of the above solution into a 100mL volumetric flask. Dissolve and dilute to volume with diluent. Mix well.

Table-1: System Suitability & Precision Parameters

S. No.	Absorbance of TDS
1	0.5382
2	0.5380
3	0.5396
4	0.5393
5	0.5390
6	05395
Average	0.5389
Standard Deviation	0.00068
% RSD	0.13%
Acceptance Criteria	NMT 5.0%

From the above tabulated data, it can be concluded that the system suitability & precision parameters meets the requirements of method validation.

Specificity

Specificity is the ability of analytical method to asses unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components. The peaks of blank solution not interfere with TDSI/02A peak. Injected blank, blank with swab stick and specificity solution (Standard solution).

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.

Prepared stock solution containing Toldimfos sodium stage-II (TDSI/02A) reference material. Scan each solution. Plotted a graph of concentration of solution in mg/mL in X-axis against Absorbance in Y-axis for Toldimfos sodium stage-II (TDSI/02A). Calculated the correlation coefficient and regression coefficient between the concentration in mg/mL and Absorbance.

The correlation coefficient and the regression coefficient between concentration and area response of Toldimfos sodium stage-II (TDSI/02A) should be NLT 0.995.

Preparation of TDSI/02A Stock Solution

Weighed 100.18 mg of working standard into 100 mL volumetric flask dissolved and diluted up to the mark with diluent. Prepared the series of linearity solutions as per the table below.

Table 2: Linearity Different Levels of Concentrations

Concentration in mg/mL	Solution X Added (in mL)	Volume Made Upto (in mL)
0.0002	0.02	100
0.0005	0.05	100
0.001	0.10	100
0.003	0.30	100
0.005	0.50	100
0.008	0.80	100
0.010	1.00	100
0.013	1.30	100
0.015	1.50	100

Table 3: Linearity Parameters

Run Number	Actual concentration in mg/mL	Absorbance
1	0.00020036	0.0108
2	0.00050090	0.0281
3	0.00100180	0.0548
4	0.00300540	0.1654
5	0.00500900	0.2753
6	0.00801440	0.4439
7	0.01001800	0.5457
8	0.01302340	0.7155
9	0.01502700	0.8301
Slope		55.04658112
Correlation Coefficient		0.999985737
Regression Coefficient		0.999971474

Table 4: Residual Output for Linearity Parameters

Residual Output		
Observation	**Predicted Y**	**Residuals**
1	0.0110249322	-0.0002249322
2	0.0275623304	0.0005376696
3	0.0551246608	-0.0003246608
4	0.1653739824	0.0000260176
5	0.2756233039	-0.0003233039
6	0.4409972863	0.0029027137
7	0.5512466079	-0.0055466079
8	0.7166205903	-0.0011205903
9	0.8268699118	0.0032300882

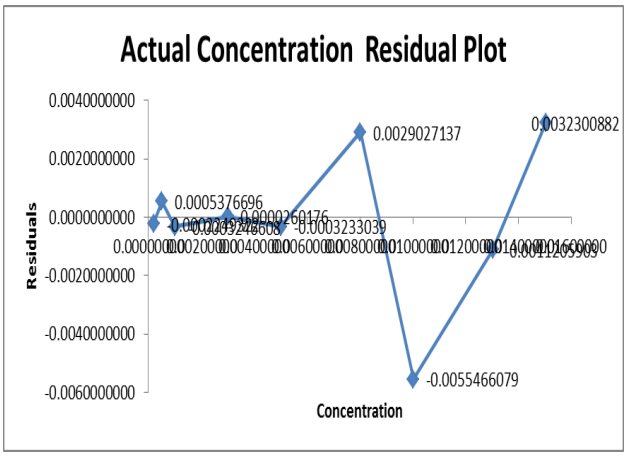

Figure 2: Residual Plot for Linearity Parameters

From the above data, it is clear that the Absorbance vs. concentration in mg/mL of TDSI/02A is linear in the range of interest. The correlation coefficient and regression coefficient calculated from regular plot is greater than 0.995. Hence the method is linear for the residual determination of Toldimfos sodium stage-II (TDSI/02A).

Limit of Detection/Limit of Quantification

Limit of detection is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. Limit of quantification is the lowest amount of analyte in a sample that can be quantified with acceptable precision, under the stated experimental conditions.[6-17]

Preparation of LOD Solution

0.015 mL of TDSI/02A stock solution taken into 100 mL volumetric flask and diluted up to the mark with diluent and read the absorbance in triplicate.

Preparation of LOQ Solution

0.046 mL of TDSI/02A stock solution taken into 100 mL volumetric flask and diluted up to the mark with diluent and read the absorbance in triplicate.

Limit of Quantification Solution Precision

From the obtained results it can be concluded that the cleaning method validation is precise at LOQ concentration (0.46 ppm).

Recovery Study

Sample methods for cleaning validations are broadly classified into **1) Swab method 2) Rinse method.**

Rinse Recovery

The rinse recovery of the sampling method was established by spiking a solution of known concentration on both stainless steel surface and glass plate. Recovered the spiked sample from the surface by rinsing the surface with the sampling agent.

Preparation of Spiking Solution

Weighed 100.37 mg of test sample taken into 100 mL volumetric flask dissolved and diluted with diluent. Further 10 mL of this solution diluted to 100 mL with diluents.

Rinse Recovery Study on Stainless Plate

Selected three cleaned and dried 10x10cm surface area stainless steel plates. Spread 10.0mL of spiking solution on dried 10x10cm surface area stainless steel plates, taking utmost care to avoid any spillage. Dried the plate at room temperature.

Using 100.0mL of accurately measured diluent, recovered the Toldimfos sodium stage-II (TDSI/02A) from 10x10cm surface area stainless steel plate, by gentle swirling. Filtered and take the absorbance in UV. Performed the exercise in triplicate.

Rinse Recovery Study on Glass Plate

Selected three cleaned and dried 10x10cm surface area glass plates. Spread 10.0mL of spiking solution on dried 10x10cm surface area glass plates, taking utmost care to avoid any spillage. Dried the plate at room temperature.

Using 100.0mL of accurately measured diluent, recovered the Toldimfos sodium stage-II (TDSI/02A) from 10x10cm surface area glass plate, by gentle swirling. Filtered and take the absorbance in UV. Performed the exercise in triplicate.

From the observed results it can be concluded that % rinse recovery on SS plate and glass plate is consistently above 80.0%.

Swab Recovery

The swab recovery of the sampling method was established by spiking a solution of known concentration on both stainless steel surface and glass plate. Recovered the spiked sample from the surface by swabbing the surface using swab stick with the sampling agent.

Preparation of Spiking Solution

Weighed 100.16 mg of test sample taken into 100 mL volumetric flask dissolved and diluted with diluent. Further 10 mL of this solution diluted to 100 mL with diluents.

Swab Recovery Study on Stainless Plate

Selected three cleaned and dried 10x10cm surface area stainless steel plates. Spread 10.0mL of spiking solution on dried 10x10cm surface area stainless steel plates, taking utmost care to avoid any spillage. Dried the plate at room temperature. Swabbed the dried residue using swab stick and diluent. Swabbed the plate in lengthwise direction from left to right. After each swab, dipped the swab stick in the beaker containing diluent. Soaked the swab stick thoroughly in diluent and transferred into a 100mL volumetric flask. Repeat the activity 3 times. Similarly, swabbed the plate in lengthwise direction from

right to left. After each swab, dipped the swab stick in the beaker containing diluent. Soaked the swab stick thoroughly in diluent and transferred into a 100mL volumetric flask. Repeat the activity 3 times. Diluted the solution to volume using diluents and mixed well.

Swab Recovery Study on Glass Plate

Selected three cleaned and dried 10x10cm surface area glass plates. Spread 10.0mL of spiking solution on dried 10x10cm surface area glass plates, taking utmost care to avoid any spillage. Dried the plate at room temperature. Swabbed the dried residue using swab stick and diluent. Swabbed the plate in lengthwise direction from left to right. After each swab, dipped the swab stick in the beaker containing diluent.

Soaked the swab stick thoroughly in diluent and transferred into a 100mL volumetric flask. Repeat the activity 3 times. Similarly, swabbed the plate in lengthwise direction from right to left.

After each swab, dipped the swab stick in the beaker containing diluent. Soaked the swab stick thoroughly in diluent and transferred into a 100mL volumetric flask. Repeat the activity 5times.Diluted the solution to volume using diluents. Performed the above exercise in triplicate. Recorded the area of Toldimfos sodium stage-II (TDSI/02A) in the swabbed sample. Calculated the % swab recovery.

Table 5: % Swab Recovery Results

% Swab Recovery					
S. No.	**Type**	**% Recovery**	**Mean % Recovery**	**SD**	**% RSD**
1	SS Plate	89.41%	89.33%	0.43	0.48%
2		89.72%			
3		88.87%			
4	Glass Plate	89.37%	89.18%	0.33	0.37%
5		88.80%			
6		89.38%			

From the above results it can be concluded that % swab recovery on SS plate and glass plate is consistently above 80.0%.

Solution Stability

To determine the stability of sample solution, the 10 ppm Standard & Cleaning sample solutions of Toldimfos sodium stage-II were prepared and analysed immediately after preparation and after different time intervals up to 24 (24h), while maintaining the sample cooler temperature at about 30 (30°C).

The results from these studies indicated, the standard & sample solution was stable at room temperature for at least 24(24 h). All the above results are summarized in Table 6.

Table 6: Summary and Evaluation Results

<table>
<tr><th>Validation Parameter</th><th>Acceptance Criteria</th><th>Results</th></tr>
<tr><td>System Suitability</td><td>The RSD for the area response of Toldimfos sodium stage-II(TDSI/02A) peak obtained from six replicate injections of system suitability should be NMT 5.0%</td><td>System suitability parameter meets the criteria and RSD=0.13%</td></tr>
<tr><td>Specificity</td><td>The peaks of blank should not interfere with Toldimfos sodium stage-II(TDSI/02A) peak</td><td>The absorbance of blank do not interfere with Toldimfos sodium stage-II (TDSI/02A).<table><tr><th colspan="2">Individual solutions</th></tr><tr><th>Peak Name</th><th>Absorbance</th></tr><tr><td>Blank</td><td>0.0000</td></tr><tr><td>Blank with swab stick</td><td>0.0000</td></tr><tr><td>System suitability solution</td><td>0.5390</td></tr></table></td></tr>
<tr><td>Linearity</td><td>The correlation coefficient and the regression coefficient between concentration and area response of Toldimfos sodium stage-II(TDSI/02A) should be NLT 0.995</td><td>The method is linear
Correlation coefficient=0.99999
Regression coefficient=0.99997</td></tr>
<tr><td>LOD/LOQ</td><td>The %RSD for area response of Toldimfos sodium stage-II(TDSI/02A) from six replicates at LOQ level should be NMT 10.0%</td><td>The RSD for area response of Toldimfos sodium stage-II(TDSI/02A) from six replicates at LOQ level=<table><tr><th></th><th>Toldimfos sodium stage-II(TDSI/02A)</th></tr><tr><td>LOQ in mg/mL</td><td>0.00015 mg/mL</td></tr><tr><td>LOD in mg/mL</td><td>0.00046 mg/mL</td></tr></table></td></tr>
</table>

Recovery Study	Report the % rinse recovery if the % rinse recovery is less than 80.0% then incorporates the recovery factor to the analytical method.	% Rinse Recovery

Type	% Recovery	Mean % Recovery	SD	%RSD
SS plates	88.44	88.93	0.87	0.98
	88.42			
	89.94			
Glass plates	87.57	87.91	0.30	0.34
	88.03			
	88.14			

Recovery Study	Report the % swab recovery if the %swab recovery is less than 80.0% then incorporates the recovery factor to the analytical method.	% Swab Recovery

Type	% Recovery	Mean % Recovery	SD	%RSD
SS plates	89.41	89.33	0.43	0.48
	89.72			
	88.87			
Glass plates	89.37	89.18	0.33	0.37
	88.80			
	89.38			

Based on the above observed results the cleaning method validation for Toldimfos sodium stage-II (TDSI/02A) method is valid.

CONCLUSION

The method is found to be specific for the Residual determination of Toldimfos sodium stage-II (TDSI/02A). The method is found to be linear in the range of interest. The sampling method is found to be precise for rinse and swab recovery. A System suitability test is established and recorded. Hence, this method stands validated can be used for routine line clearance samples.

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