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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF METADOXINE IN API AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Metadoxine, in its pure form as well as in tablet dosage form. Chromatography was carried out on an ODS C18 (4.6 x 150mm, 5µm) column using a mixture of ACN: Water (65:35% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 305nm. The retention time of the Metadoxine was 3.155 ±0.02min respectively. The method produce linear responses in the concentration range of 10-50µg/ml of Metadoxine. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

KEYWORDS

Metadoxine, RP-HPLC and Validation.

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INTRODUCTON

Metadoxine, also known as pyridoxine-pyrrolidone carboxylate, is a drug used to treat chronic and acute alcohol intoxication[1]. Metadoxine accelerates alcohol clearance from the blood[2]. Metadoxine is an ion pair salt of pyridoxine and pyrrolidon carboxilate (PCA)[1]. Pyridoxine (vitamin B6) is a precursor of coenzymes including pyridoxal 5'-phosphate (PLP), which accelerates the metabolic degradation of ethanol and prevents adenosine triphosphate (ATP) inactivation by acetaldehyde. Pyridoxal phosphate dependent enzymes also play a role in the biosynthesis of four important neurotransmitters: serotonin (5-HT), epinephrine,

nor epinephrine and GABA: see vitamin B6 functions. L-PGA is present in the diet and is produced endogenously by enzymatic[3,4] conversion of gamma-glutamyl amino acids to L-PGA and free amino acids. In the central nervous system (CNS), L-PGA was found to have a role in composition of neuro-active molecules. Its production has been linked to hepatic gamma-glutamyl transferase activity and levels of reduced glutathione (GSH). Lastly, it was shown that L-PGA facilitates ATP synthesis by stimulating de novo synthesis of purines. The IUPAC Name[27] of Metadoxine is L-Proline, 5-oxo-, compd. with 5-hydroxy-6-methylpyridine-3, 4-dimethanol and the chemical formula[27,28,29] is $C_{13}H_{18}N_2O_6$. The Chemical Structure of Metadoxine is as follows.

Literature survey[31-33] reveals that few methods have been reported for the determination of Metadoxine individually or in combination with other drugs in pharmaceutical dosage forms. But no method has been developed for estimation of Metadoxine in API and pharmaceutical dosage form. The present manuscript describes a sensitive, simple, precise and accurate isocratic RP-HPLC method for estimation of Metadoxine bulk and in pharmaceutical dosage form with subsequent validation as per ICH guidelines.

MATERIAL AND METHODS

HPLC METHOD DEVELOPMENT TRAILS

Preparation of standard solution

Accurately weigh and transfer 10 mg of Metadoxine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.3ml of the above Metadoxine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to ACN: Water (65:35% v/v) respectively.

Optimization of Column

The method was performed with various C18 columns like Symmetry, Zodiac, Xterra. ODS C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used	:	Waters HPLC with auto sampler and PDA 996 detector model.
Temperature	:	35ºC
Column	:	ODS C18 (4.6 x 150mm, 5µm)
Mobile phase	:	ACN: Water (65:35% v/v)
Flow rate	:	1.0mL/min
Wavelength	:	305 nm
Injection volume	:	10 µl
Run time	:	8 minutes

METHOD VALIDATION

PREPARATION OF MOBILE PHASE

Preparation of mobile phase:

Accurately measured 650 ml (65%) of HPLC Methanol and 350 ml of Water (35%) were mixed and degassed in a digital ultra sonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

VALIDATION PARAMETERS

System Suitability

Accurately weigh and transfer 10 mg of Metadoxine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml of the above Metadoxine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

SPECIFICITY STUDY OF DRUG

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Metadoxine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml of the above Metadoxine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution

Take average weight of the Powder and weight 10 mg equivalent weight of Metadoxine sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.3ml of the above Metadoxine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

PREPARATION OF DRUG SOLUTIONS FOR LINEARITY

Accurately weigh and transfer 10 mg of Metadoxine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (10ppm of Metadoxine)

Take 0.1ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – II (20ppm of Metadoxine)

Take 0.2ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – III (30ppm of Metadoxine)

Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – IV (40ppm of Metadoxine)

Take 0.4ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – V (50ppm of Metadoxine)

Take 0.5ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Procedure

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION

Repeatability

Preparation of Metadoxine Product Solution for Precision

Accurately weigh and transfer 10 mg of Metadoxine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

INTERMEDIATE PRECISION

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure

Analyst 1

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Analyst 2

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy

For preparation of 50% Standard stock solution

Accurately weigh and transfer 10 mg of Metadoxine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 0.15ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

For preparation of 100% Standard stock solution

Accurately weigh and transfer 10 mg of Metadoxine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

For preparation of 150% Standard stock solution

Accurately weigh and transfer 10 mg of Metadoxine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 0.45ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Procedure

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Metadoxine and calculate the individual recovery and mean recovery values.

ROBUSTNESS

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard solution

Accurately weigh and transfer 10 mg of Metadoxine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Effect of Variation of flow conditions

The sample was analyzed at 0.9ml/min and 1.1ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded

Effect of Variation of mobile phase organic composition

The sample was analyzed by variation of mobile phase i.e. ACN: Water was taken in the ratio and 60:40, 70:30 instead of 65:35, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

HPLC Method Development

METHOD VALIDATION

System suitability

To know reproducibility of the method, the system suitability test was done to establish the parameter such as retention time, tailing factor, theoretical

plate, and peak area. This was performed by injecting the standard mixture. The System suitability parameters were found to be within the limits.

Linearity

The linearity of an analyte procedure is its ability (within a given range) to obtained test results which are directly proportional to the concentration of analyte in the sample. Linearity was evaluated by analyzing the plot of area as a function of concentration of analyte. The result was evaluated by calculating of regression coefficient (r2).

The standard calibration curve was obtained in the concentration range of 10–50µg/ml for Metadoxine with a correlation coefficient (r2) of 0.999. The linear regression equation was obtained y = 3265x + 3710 for Metadoxine. The results obtained for linearity are summarized in Table No.4 and Figure No.3.

Precision

The precision of an analytical method is the closeness of replicating result obtained from analysis of the same homogeneous sample. Precision was considered at different levels, i.e., method precision and intermediate precision.

Method Precision

System precision was carried out with 5 replicates (n=5) of standard at working concentration of 30µg/ml of Metadoxine. The repeatability of sample applications and measurement of peak area were expressed in term of % relative standard deviation (%RSD).

The repeatability of sample applications and measurement of peak area were expressed in term of %RSD since their %RSD is <2.0%, and hence, the developed method was found to be precise. Data obtained from precision experiments for repeatability studies are shown as below Table No.5.

Intermediate precision or ruggedness

The ruggedness of the method was verified by analyzing six samples of the same batch used for method precision as per proposed method by different analyst.

The repeatability of sample applications and measurement of peak area were expressed in term of %RSD since their %RSD is <2.0 %, and hence, the developed method was found to be precise. Data obtained from intermediate are summarized in Table No.6 and 7.

Accuracy

The accuracy of an analytical method is the closeness of the results obtained by that method to the true value of the sample. It is expressed as recovery (%), which is determined by the API method. The accuracy was evaluated by the recovery of Metadoxine at three different levels (50%, 100%, and 150%).

The % recovery was found to be 99.4 for Metadoxine. %RSD was found to be <2, and hence, the method is said to be accurate. The results of accuracy studies are shown in Table No.8.

LOD and LOQ

The LOD of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value.

The LOQ of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

The sensitivity of measurement of Metadoxine by use of the proposed method estimated in term of the LOQ and LOD. The results of LOD and LOQ are summarized in Table No.9.

Robustness

The robustness of the method we determined by assessing the ability of the developed method to remain unaffected by the small changes in the parameters such as Flow rate (±1 nm), oven temperature (±1^{o}C), detection wavelength (±0.2 ml/min).

The % assay was within the acceptance criteria in the condition described in this report, and hence, the method is robust. The results are summarized in Table No.10.

INSTRUMENTS USED

Table No.1: Instruments Used

S.No	Instruments And Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA Detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

CHEMICALS USED

Table No.2: Chemicals Used

S.No	Chemical	Brand names
1	Metadoxine(Pure)	Sura labs
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck

Table No.3: Peak Results for Optimized Chromatogram

S.No	Peak Name	R_t	Area	Height	USP Tailing	USP plate count
1	Metadoxine	3.155	206870	20497	1.30	5937

Table No.4: Calibration Data of Metadoxine

S.No	Concentration Level (%)	Concentration µg/ml	Average Peak Area
1	33	10	38455
2	66	20	71755
3	100	30	102086
4	133	40	135415
5	166	50	164313

Table No.5: Results of Method Precision for Metadoxine

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Metadoxine	3.165	153488	16579	5510.1	1.3
2	Metadoxine	3.163	153650	16048	5255.1	1.3
3	Metadoxine	3.158	153852	16033	5174.0	1.3
4	Metadoxine	3.167	154083	16324	4352.7	1.3
5	Metadoxine	3.171	154342	16554	5438.0	1.3
Mean			153882.8			
Std.dev			339.9			
%RSD			0.2			

Table No.6: Results of Intermediate Precision Analyst 1 for Metadoxine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Metadoxine	3.165	153488	16579	6510.1	1.3
2	Metadoxine	3.163	153650	16048	2255.1	1.3
3	Metadoxine	30158	153852	16033	5174.0	1.3
4	Metadoxine	3.167	154083	16324	5352.7	1.3
5	Metadoxine	3.171	154342	16554	5438.0	1.3
6	Metadoxine	3.171	154342	16554	5438.0	1.3
Mean			153882.8			
Std. Dev.			339.9			
% RSD			0.2			

Table No.7: Results of Intermediate Precision Analyst 2 for Metadoxine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Metadoxine	3.173	153634	16592	5376	1.3
2	Metadoxine	3.134	153721	16538	8373	1.3
3	Metadoxine	3.161	153773	16540	5827	1.3
4	Metadoxine	3.174	153957	16492	5236	1.3
5	Metadoxine	3.199	153057	16593	6173	1.3
6	Metadoxine	3.199	152816	16495	5927	1.3
Mean			153493			
Std. Dev.			450.3301			
% RSD			0.293388			

Table No.8: The Accuracy Results for Metadoxine

S.No	%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
1	50%	53261.67	15	14.9	99.3	99.4%
2	100%	103318	30	29.87	99.5	
3	150%	151061.7	45	44.79	99.5	

Table No.9: LOD and LOQ Values

S.No	LOD	LOQ
1	1.3	4.0

Table No.10: Results for Robustness

S.No	Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
1	Actual Flow rate of 1.0mL/min	126086	3.155	4245	1.33
2	Less Flow rate of 0.9mL/min	139530	3.488	5372	1.3
3	More Flow rate of 1.1mL/min	114279	2.877	3656	1.4
4	Less organic phase	116384	4.705	5362	1.4
5	More organic phase	113480	2.090	6251	1.2

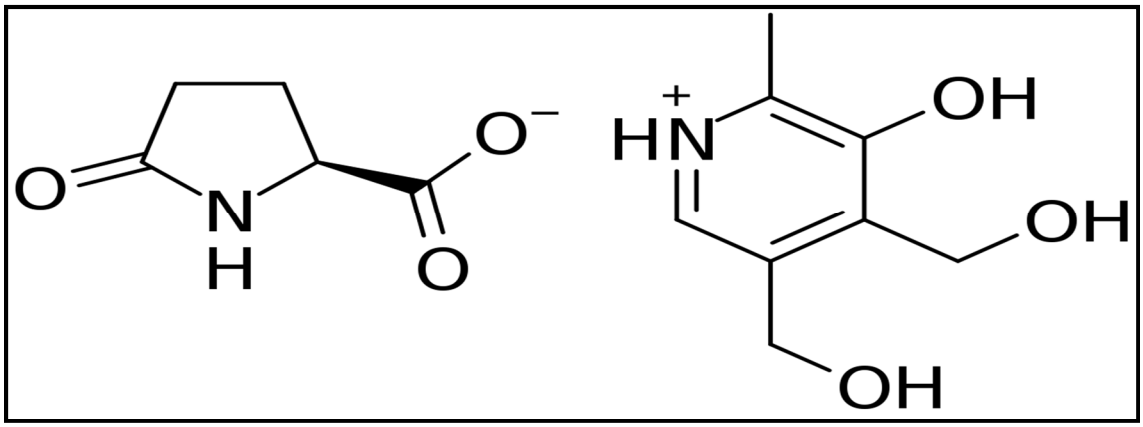

Figure No.1: Chemical Structure of Metadoxine

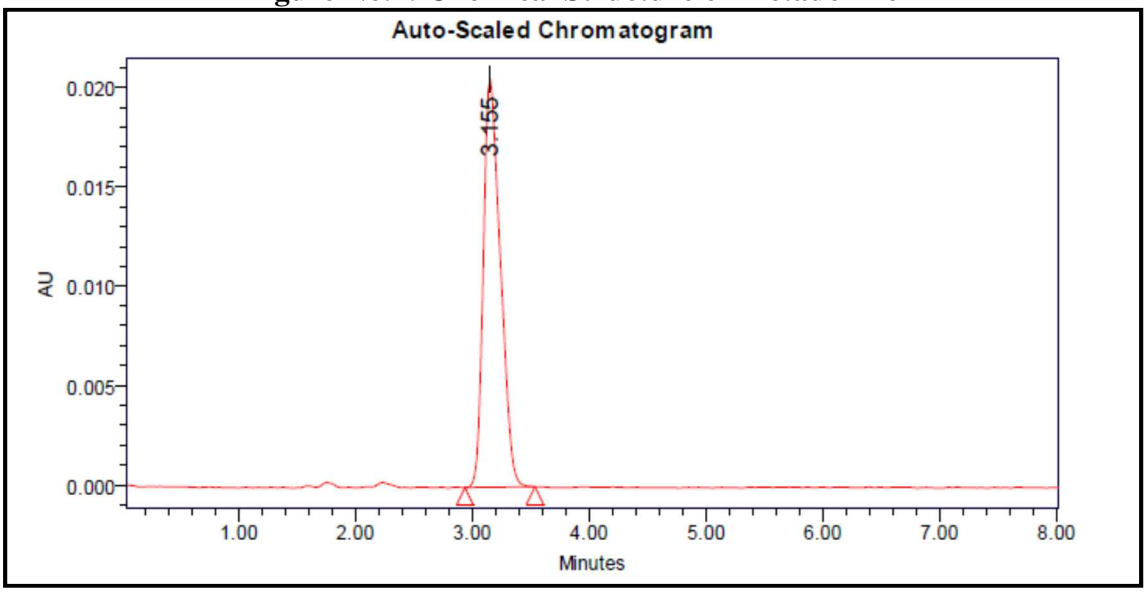

Figure No.2: Optimized Chromatographic Condition

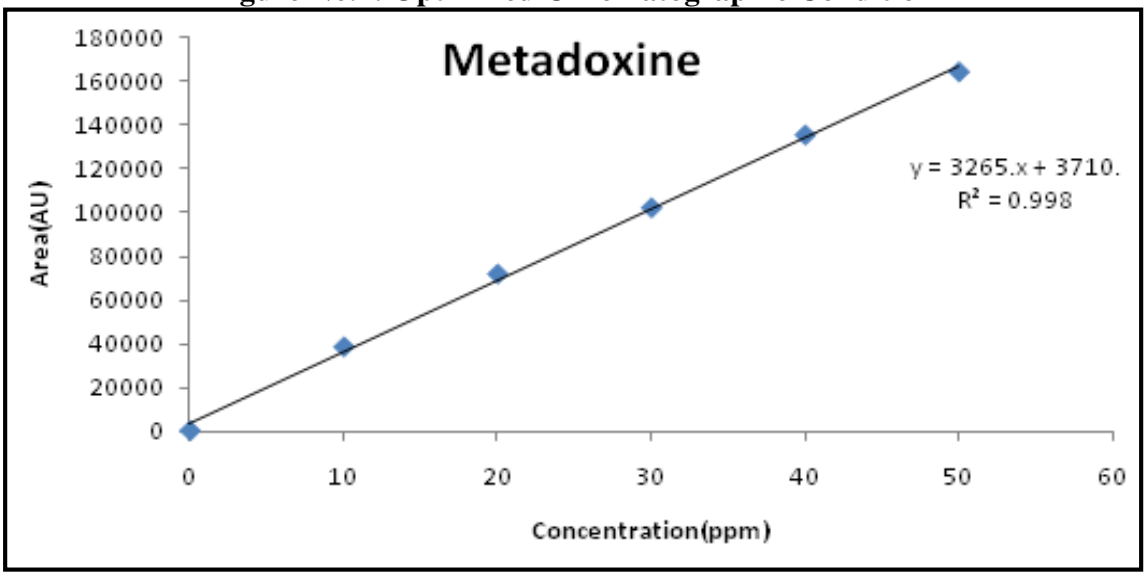

Figure No.3: Calibration Curve of Metadoxine

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Metadoxine in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Metadoxine was freely soluble in ethanol, methanol and sparingly soluble in water. ACN: Water was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Metadoxine in bulk drug and in Pharmaceutical dosage forms.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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