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A SIMPLE ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF AZILSARTAN MEDOXOMIL IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT:
An RP-HPLC technique with high sensitivity and precision has been devised to accurately determine the concentration of Azilsartan Medoxomil in its bulk formulation. The maximum wavelength (λmax) of Azilsartan Medoxomil was determined to be 248 nm in a 10 mM Ammonium acetate buffer with a Methanol solution at a ratio of 60:40 % v/v. The pH level is 3. The approach demonstrates a high level of sensitivity, with a linear range of 5 to 25µg/ml. The regression equation for this range is y = 6572.6x + 19652, with a r2 value of 0.9994. This approach is validated and tested in accordance with the criteria specified in the ICH guidelines and USP. The detection limit and quantitation limit were determined to be 0.08 µg ml–1 and 0.26 µg ml–1, respectively. The results indicated that the technique is precise, specific, and repeatable, with a relative standard deviation (RSD) of less than 2%. Additionally, the procedure is straightforward, cost-effective, time-efficient, and suitable for determining Azilsartan Medoxomil in both bulk and tablet forms.

KEY WORDS: Azilsartan Medoxomil, HPLC method, Quantitative Analysis, AMD, Method development

INTRODUCTION:

Azilsartan medoxomil, a prodrug, undergoes conversion into azilsartan, which belongs to the angiotensin-receptor blocking (ARB) drug class. It is a selective angiotensin II receptor antagonist of the AT1 subtype. A relatively new hypertension medicine, azilsartan medoxomil, obtained FDA clearance for the first time in February 2011. Several guidelines indicate that the clinical efficacy of ARBs is equivalent to that of angiotensin-converting enzyme (ACE) inhibitors, which are also employed as first therapies for hypertension. Consequently, ARBs should be prioritized as the first choice therapy when initiating antihypertensive medication. Azilsartan Medoxomil may be described as a synthetic compound with the chemical formula C30H24N4O8. Its molecular structure consists of a benzodiazole

ring attached to a biphenyl group, which in turn is connected to an oxadiazole group. The compound also contains an ester and a carboxylate functional group. The construction of Azilsartan Medoxomil is displayed in fig 1.

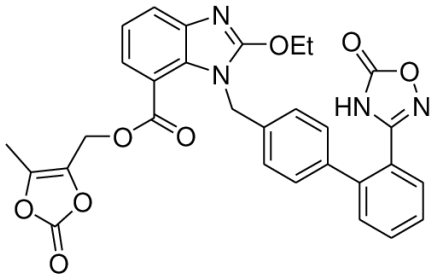

Fig 1: Structure of Azilsartan Medoxomil

Azilsartan Medoxomil is an atomic mass of 568.53 g/mol. Azilsartan medoxomil is prescribed to patients over the age of 18 who have hypertension in order to lower blood pressure. It can be employed both individually and in combination with other antihypertensive drugs. Some antihypertensive drugs have less of an influence on blood pressure in persons of colour. Chromatography Technique As a result, the purpose of this research is to design a highly sensitive and uncomplicated HPLC technique for testing azilsartan in complex structures.

MATERIALS AND METHOD:

Instruments:

The chromatographic partition was done using Jasco MD-2010 Plus layout smaller fluid chromatographic framework coordinated with a variable frequency programmable UV identifier and a Rheodyne injector equipped with 20µl fixed circle. An opposing stage C18 [Crestpak C18 (250mm x 4.6ID, Particle size: 5 micron)] was employed. Model - UV 2012 twofold shaft UV obvious spectrophotometer and Wenser High Precision Balance Model: PGB 100 electronic equilibrium were employed for Spectrophotometric judgments and gauging functions independently.

Reagents and chemicals

Drug grade unadulterated Azilsartan Medoxomil sample was secured from Aarti drugs PVT LTD. HPLC grade Methanol and water were acquired from Merck specialities private restricted, Mumbai.

Melting point determination

Identification of Azilsartan medoxomil was done by checking its melting point and it was found in the range of 213˚ C.

Fourier Transform Infra-red Spectroscopy (FTIR)

Optimization of wavelength was done at different wavelengths using UV detector. In the current investigation, medication solutions of 10µg/ml of each of Azilsartan medoxomil were produced in methanol. After studying UV spectra of the medication, wavelength of 248 nm was picked for future study.

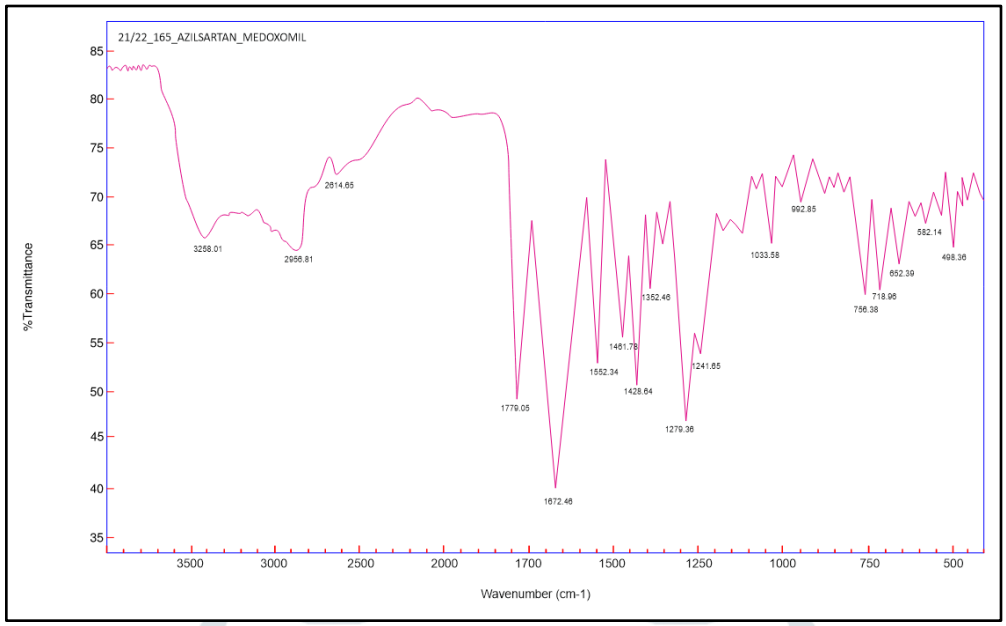

Fig.2: IR Spectrum of Azilsartan medoxomil

Table 1: Interpretation of FTIR Spectrum of Azilsartan medoxomil

Functional Group	Observed Peak (cm^{-1})	Reported Peak(cm^{-1})
N-H Stretch	2956.81	3000-2900
C=N Stretch	1672.46	1620-1710
C=O (Aromatic)	1779.05	1800-1750
C-O-C Stretch	1033.58	1050-950
N-H Bending	1428.64	1400-1450

Optimization of Detection Wavelength

An optimal wavelength is one that delivers optimum response for the substances that are to be identified. For satisfactory response, optimization of wavelength was done at different wavelengths using UV detector. In the current investigation, medication solutions of 10µg/ml of each of Azilsartan medoxomil were produced in methanol. After studying UV spectra of the medication, wavelength of 248 nm was picked for future study.

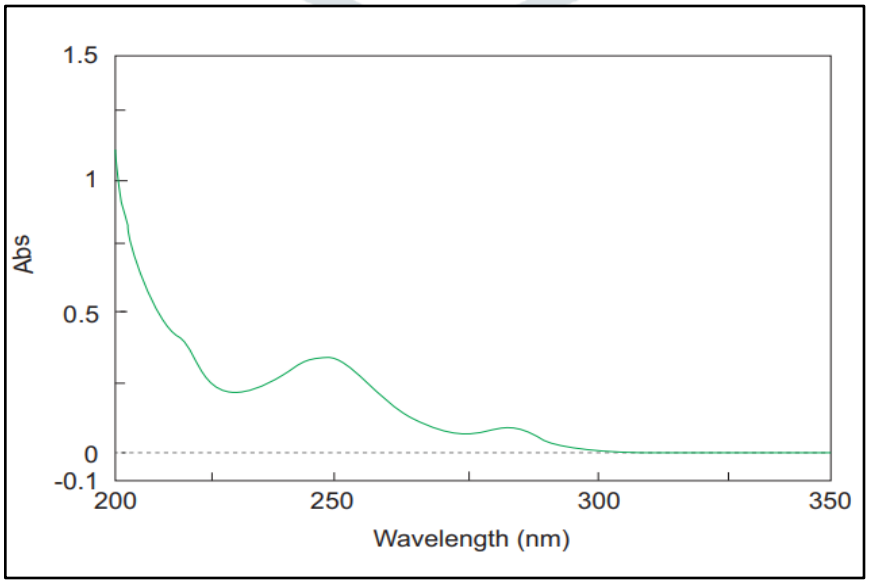

Fig. 3: UV Spectra of Azilsartan medoxomil Showing λmax 248 nm

Chromatographic conditions

C18 [Crestpak C18 (250mm x 4.6ID, Particle size: 5 micron)] was applied for the chromatographic separation at a discovery frequency of 248 nm. 10 mM Ammonium Acetate buffer: Methanol [60:40 % v/v]. pH 4.5 was used as mobile phase for elution and identical mix was employed in the arrangement of standard and sample solutions. The elution was tested by infusing the 20µl and the stream rate was altered in line with 1.0 ml/min.

Buffer Preparation

Preparation of 10mM Ammonium acetate buffer (1 Liter):

- Weigh 770.8 mg of ammonium acetate into a beaker.
- Dissolve the salt with about 800mL water (HPLC grade), equilibrated at room temperature (20-25°C). Mobile phase was filtered through 0.45µm membrane filter and degassed by sonication for 20 min.

Preparation of Standard solutions

A similar dissolvable was used to make up the volume after accurately measuring and moving 10 mg of Azilsartan Medoxomil into 10 ml volumetric cups. This allowed for the acquisition of an essential stock arrangement of 1000µg/ml of the drug. (A stock arrangement in operation).

Optimisation of RP-HPLC method

The HPLC approach was simplified with an objective to set up an assessment of Azilsartan Medoxomil. Different mobile phases were gone after for the technique optimization, nevertheless appropriate retention periods, hypothetical plates and high resolution were seen with 10 mM Ammonium acetate buffer: Methanol [60:40 % v/v]. pH 4.5 employing C18 column [Crestpak C18 (250mm x 4.6ID, Particle size: 5 micron)] Table 2 and a run of the chromatograph of Azilsartan Medoxomil was presented in figure 5.

Table 2: Optimized parameter

Parameters	Conditions
Column	Crestpak C18 (250mm x 4.6ID, Particle size: 5 micron)
Mobile Phase	10 mM Ammonium acetate buffer: Methanol [60:40 % v/v]. pH 4.5
Flow Rate	1.0 ml/min
Wavelength	248 nm
Injection Volume	20 µl
Detector	UV-3000-M
Run Time	5 min
Retention Time	Approx. 3.8 min

Validation of RP-HPLC method

Validation of the optimized HPLC method was performed in accordance to the ICH Q2 (R) guidelines.

Linearity

For the measurement of linearity, relevant sample solutions were pipetted out from 1000µg/ml stock solution. 0.05 – 0.25 ml was pipetted out in to five of 10ml volumetric flasks accordingly and volume was created with the mobile phase to get concentration ranging from 5-25µg/ml of Azilsartan Medoxomil. Each solution from flask was injected in triplicate in system. Calibration curves were produced with concentration of solutions versus observed peak areas made by them followed by the estimation of regression factor and calculation of the correlation coefficients. The calibration curves of Azilsartan Medoxomil sample was presented in figure 2 and their related linearity parameters reported in table 3.

Accuracy

To make sure the reliability and accuracy of the recovery study data were carried out by % recovery method which is also referred as standard addition method. A known quantity of pure medication of Azilsartan Medoxomil was combined to pre-analysed sample and contents again endures examination by the optimized procedure and the % recovery was reported in table 5.

Precision

The repeatability study of the suggested approach was confirmed by calculating the percentage RSD of three replica injections of 100% concentration i.e. 15µg/ml of Azilsartan Medoxomil on the same day and for intraday precision % RSD was computed from repeatition. The findings were shown in table 7 and 8.

Limit of Quantitation (LOQ) & Limit of Detection (LOD)

The LOD and LOQ were analysed from the slope(s) of the calibration curve and the standard deviation (SD) of the peak areas using the formula LOD = 3.3 s/s and LOQ = 10 s/s.

Robustness

Robustness was calculated by changing the chromatographic settings like compositions of mobile phase, detection wavelength, flow rate etc. and the % RSD should be provided. In the optimum conditions Small adjustments were allowed and the extent to which the method was robust was determined. A variance of ± 2 nm in the detection wave length and ± 0.1 ml/min in the flow rate, were tried individually. Solutions of 100% test concentration with the prescribed n modifications under the optimized conditions were administered to the system in triplicate. % RSD was shown in the table 9.

Ruggedness

Ruggedness is the study to determine effect of external parameters on the method. To evaluate ruggedness of the developed method, parameters were deliberately varied. These parameters included variation of system, different analyst, Atmospheric changes.

Assay of marketed formulation

20 tablets of Azilsartan medoxomil commercial formulation (Azilmac-40) were taken, weighed individually and crushed into fine powder. Average weight of tablet sample (equal to 10 mg of Azilsartan medoxomil) were weighed and transferred to 100mL volumetric flask & diluent was added to make up the capacity. Sonicate for 10 min with occasional spinning. The aforesaid solution was filtered by 0.45µm membrane filter, The prepared stock solution is of 100 µg/ml.

System suitability

It was make sure that from the system suitable factors, the approach can offers results of accuracy and precision. System suitability was performed with three replicate injections of solution of 15 µl/ml of Azilsartan Medoxomil into the chromatographic system. Tailing factor (T) Number of theoretical plates (N) obtained was given in table 13.

RESULT AND DISCUSSION

Linearity*:*

It was clarified from the analytical method linearity as the ability of the method to generate test findings that are directly proportional to the analyte concentration, within a specific range. The peak area obtained from the HPLC chromatograph was plotted against corresponding concentrations to create the calibration graph. The results of linearity investigation (Figure 1) indicated linear relationship over the concentration range of 0.05 - 0.25 µg/ml for Azilsartan Medoxomil. From the regression analysis, a linear equation was produced y = 6572.6x + 19652, and the goodness-of-fit (r2) was found to be 0.9994, demonstrating a linear relationship between the concentration of analyte and area under the peak.

Table 3: Summary of results of Linearity

Conc. (µg/ml)	Peak Area
5	53335
10	85697
15	116109
20	151131
25	184933

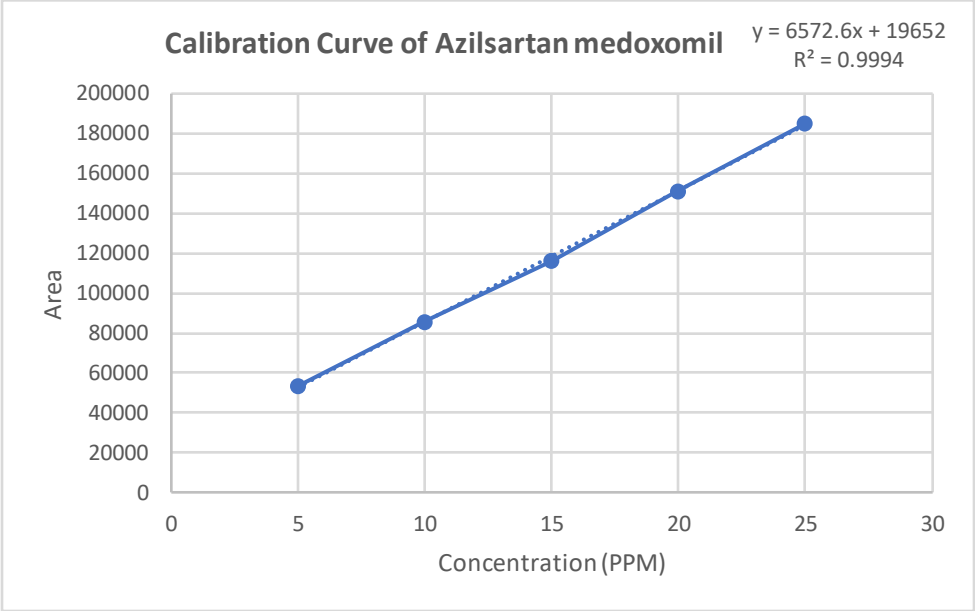

Figure 4: Calibration curve of Azilsartan medoxomil

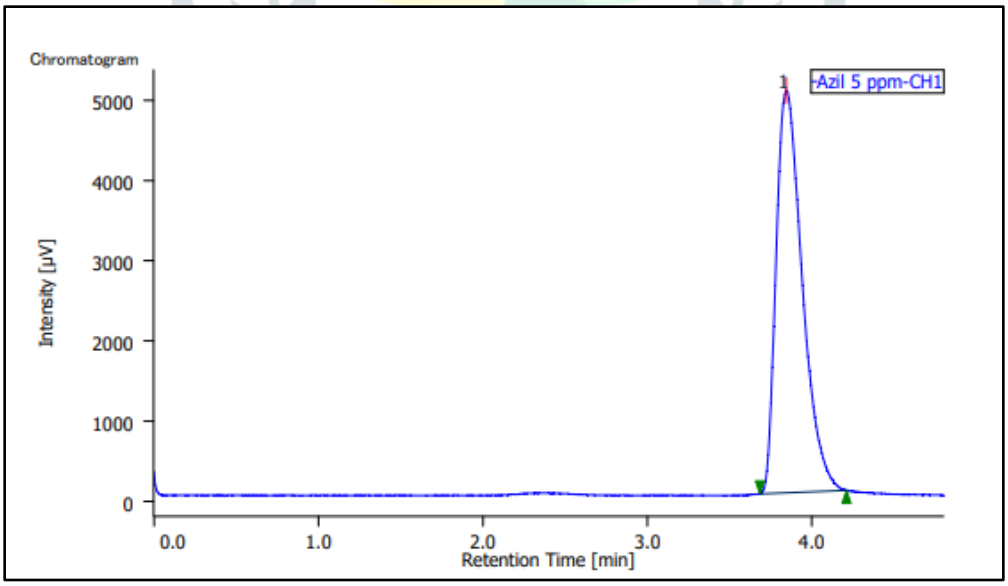

Figure 5: Typical chromatograph of Azilsartan Medoxomil

Table 4: Optimized Chromatographic Conditions

Mobile phase	10 mM Ammonium acetate buffer: methanol (60:40) pH3
Selection of column	Crestpak C18 (4.6mm x 250mm, Particle size: 5µm)
Injection volume	20 µL
Flow rate	1.0 ml/min
Column temperature	Room Temperature
Detection wavelength	248 nm
Retention time	3.8 min

Accuracy

The accuracy of the approach determines the proximity of results achieved by that method to the true value. From the results of accuracy testing it was revealed that the approach is accurate within the permitted ranges. The % RSD is calculated for the Azilsartan Medoxomil and all the values are within limits. Acceptable accuracy was within the range and not more than 2.0% RSD, as illustrated in Table 5.

Table 5: summary of Results of Accuracy

Level of addition	Standard added (µg/ml)	conc. (µg/ml)	Total conc. (µg/ml)	Area obtained*	Std Area	Drug recovered (µg/ml)	%Recovery
50%	5	10	15	115662	116109	14.94	99.62
	5	10	15	115054		14.86	99.09
	5	10	15	115098		14.87	99.13
100%	10	10	10	153069	151131	10.13	101.28
	10	10	10	153069		10.13	101.28
	10	10	10	153111		10.13	101.31
150%	15	10	25	189883	184933	25.67	102.68
	15	10	25	187564		25.36	101.42
	15	10	25	187926		25.40	101.62

Table 6: % recovery data

Level of addition	% Mean recovery*	SD	% RSD
50%	99.28	0.29	0.29
100%	101.29	0.02	0.02
150%	101.91	0.67	0.66

Precision

Precision is “the closeness of results obtained from multiple sampling of the same homogeneous sample under the prescribed conditions,” and it is stated in the form of relative standard deviation. The RSD were determined for all the results are within limits. Precision was not greater than 2.0% RSD, as evidenced in Table 7 and 8.

Table 7: summary of Intraday Precision

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	5	53321	53222.67	174.67	0.33
2	5	53326			
3	5	53021			
4	10	85616	85507.00	421.63	0.49
5	10	85641			
6	10	85264			
7	15	116485	116594.67	348.68	0.30
8	15	116314			
9	15	116985			

Table 8: summary of Interday Precision

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	5	53652	53265.67	338.51	0.64
2	5	53124			
3	5	53021			
4	10	85648	85572.33	415.70	0.49
5	10	85124			
6	10	85945			
7	15	116300	116724.00	370.45	0.32
8	15	116887			
9	15	116985			

LOD and LOQ

The LOD and LOQ were calculated by the equations $\text{LOD} = \frac{3.3 \times \text{std.Deviation}}{\text{slope}}$ and $\text{LOQ} = \frac{10 \times \text{std.Deviation}}{\text{slope}}$ where, std. Deviation taken from accuracy and slope is from linearity . Based on these equations, the calculated LOD and LOQ values for Azilsartan Medoxomil were 0.08 and 0.26 µg/ml, respectively.

Robustness

Robustness of the approach demonstrates that the findings are unaffected or dependable even if the minute changes in the method parameters. Here, the flow rate and wavelength were gently modified to lower and higher sides of the actual values to see if the change in the peak area and retention time were within limitations. The findings obtained with variations in the parameters on a 15µg/mL solution are as indicated in Table 9..

Table 9: Data of robustness study

Sr.No	Parameter	Condition	Area	Mean	SD	%RSD
1	Change in Flow rate (ml/min)	0.9	116255	69712	134.57	0.19
2		1	116894			
3		1.1	115265			
1	Change in Wavelength (nm)	246	116914	69399	331.23	0.48
2		248	116982			
3		250	116025			

Ruggedness

Ruggedness is the study to determine effect of external parameters on the procedure. To evaluate robustness of the created approach, parameter was purposefully altered. These parameter includes fluctuation of system, various analyst, Atmospheric changes. Ruggedness was researched by many analyst. Results collected are provided in following table.

Table 10: Data of ruggedness study

Sr.No	Analyst	Conc. (µg/ml)	Area	Mean area*	SD	% RSD
1	Analyst-I	15	116524	116777	224.41	0.19
			116855			
			116952			
2	Analyst-II	15	116552	116505.66	459.26	0.39
			116025			
			116940			

Specificity

Excipients and impurities were not interacting with the standard drug, hence method is specific. Results of specificity are shown in below table.

Table 11: Data of specificity

Drug conc. (µg/ml)	Excipients (µg/ml)	Total conc. (µg/ml)	Area	Mean	SD	%RSD
5	10	15	53695	53757.6667	173.70	0.32
5	10	15	53624			
5	10	15	53954			
10	10	20	85324	85722	345.26	0.40
10	10	20	85941			
10	10	20	85901			
15	10	25	116109	116546.667	382.38	0.33
15	10	25	116715			
15	10	25	116816			

% Assay of Marketed formulation

The % Assay of Azilmac -40 mg marketed formulation was calculated and given in the table below.

Table 12: Data of % Assay of marketed formulation

Sr. NO.	Formulation	Area of Standard	Area of Marketed Formulation	% Assay
1	**Azilmac-40**	116109	114256	**98.40**

System Suitability Parameters:

System appropriateness was performed by injecting three replication injections of 100% test concentration, number of theoretical plate, asymmetry factor are adequate. The chromatographs confirm the presence of Azilsartan Medoxomil at 3.8 min without any interference.

Table 13: System suitability parameter

Sr. No.	conc. (µg/ml)	Retention Time (min)	Theoretical plates	Asymmetry Factor
1	15	3.82	2828	1.25
2	15	3.80	2826	1.24
3	15	3.86	2874	1.25
4	15	3.84	2865	1.23
5	15	3.89	2898	1.24
6	15	3.85	2814	1.25
Mean		3.84	2850.83	1.24
SD		0.03	33.04	0.01
%RSD		0.82	1.16	0.66

CONCLUSION:

The proposed approach was found to be simple, exact, accurate, fast and specific for determination of Azilsartan Medoxomil from pure and its dose forms. The mobile phase employed for method development is relatively simple to make and affordable likewise. The sample recoveries in the formulation were giving good results. And so, this method may be quickly and conveniently implemented for regular analysis of Azilsartan Medoxomil in bulk and dose form.

ACKNOWLEDGEMENT

No conflict of interest

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