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Original Article

UV SPECTROPHOTOMETRIC ESTIMATION OF SUNITINIB MALATE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: To develop and validate simple zero (D_0), first (D_1) and second (D_2) order derivative UV Spectrophotometric methods for the determination of Sunitinib malate in pharmaceutical dosage form.

Methods: Sunitinib malate was solubilised in distilled water and the resultant solution exhibits adsorption maximum (λmax) at 431, 457 and 489 nm in D_0, D_1 and D_2 order derivative modes respectively. The developed method was validated as per ICH guidelines [1].

Results: Linearity was obtained over the concentration range of 2-12 µg/ml in all the derivative modes. Limit of detection (LOD) was found to be 0.291, 0.107, 0.327µg/ml and Limit of quantification (LOQ) was found to be 0.883, 0.324, 0.993µg/ml for D_0, D_1 and D_2 order derivative modes respectively. The proposed method demonstrated an excellent intra-day precision and inter-day precision. Mean recovery was found within the range of 98.19-98.62% respectively, signifies the accuracy of the developed method.

Conclusion: The statistical results prove that the developed method can be effectively applied for the routine analysis of Sunitinib malate in industries and other analytical laboratories.

Keywords: Sunitinib malate, UV Spectroscopy, D_0, D_1 and D_2 order derivative modes.

INTRODUCTION

Sunitinib malate is described chemically as Butanedioic acid, hydroxy-(2S)-compound with N-[2-(diethylamino) ethyl]-5-[(Z)-(5-fluoro-1, 2-dihydro-2-oxo-3H-indol-3-ylidine) methyl]-2, 4-dimethyl-1H-pyrrole-3-carboxamide (fig. 1), is an orally administered small molecule that inhibits multiple receptor tyrosine kinases (RTKs). Sunitinib malate was approved by the US Food and Drug Administration (FDA) on January 26, 2006, for the treatment of metastatic renal cell carcinoma (mRCC) and gastrointestinal stromal tumor (GIST) in patients who have failed to respond to imatinib or were unable to tolerate it [2-3], with molecular formula $C_{22}H_{27}FN_4O_2 \bullet C_4H_6O_5$ and molecular weight 532.561g/mol. Sunitinib malate is an orange powder which is freely soluble in water.

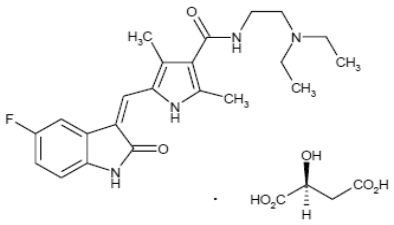

Fig. 1: Chemical structure of Sunitinib malate

On surveying the literature, it was found that only a very few methods have been reported for the estimation of Sunitinib malate alone or in combination by employing RP-HPLC and UPLC [4-7]. Some of the RP-HPLC and UPLC method were not found to be done economical in terms of mobile phase employed and availability of columns. Hence there is a need for the development of newer method for the estimation of Sunitinib malate in pharmaceutical dosage form to overcome the above hurdles. At the same time, no UV Spectrophotometric method was reported for the estimation of Sunitinib malate. So it is felt worthwhile to develop a modest, rapid, accurate, precise and more economical UV Spectrophotometric method for the estimation of Sunitinib malate in pharmaceutical dosage form.

MATERIALS AND METHODS

Chemicals and reagents

The raw material of Sunitinib malate (99.78%w/w) was obtained a gift sample, which was used as reference material throughout the experiment without any prior treatment. Sunitinib malate tablets (SUTENT Capsule-25 mg, Pfizer Inc.) were purchased from a local market. All other reagents used were purchased from Merck laboratories and S. D. fine chemicals Ltd. Double distilled water was utilized throughout the process of analysis.

Instruments used

- Perkin-Elmer UV-Visible Spectrophotometer lambda 25.
- Digital balance.
- Sonicator.
- Class "A" grade glassware's was used.

Solubility studies

The solubility studies of Sunitinib malate was carried out employing different solvent, and the results are as follows:

- Very freely soluble in water.
- Soluble in alcohol.
- Slightly Soluble in 0.1N HCl.
- Slightly Soluble in 0.1N NaOH.

Selection of solvent

Sunitinib exists as a salt form of maleic acid and it is found that the drug is very freely soluble in water. Hence, water was chosen as the

solvent to solubilise Sunitinib malate and to carry out further analysis.

Preparation of standard stock solution of sunitinib malate

The standard stock solution of Sunitinib malate was prepared by accurately weighing 25 mg of the drug and it was kept in a 25 ml standard flask. Half the volume of water was added. The solution was sonicated for 15 min and then the volume was made up to the mark with distilled water. The resultant solution was filtered and suitably diluting with distilled water to get the working standard solutions.

Preparation of sample stock solution

Twenty capsules (STUTENT Capsule-25 mg, Pfizer Inc.) were accurately weighed and the sample stock solution was prepared by accurately weighing an amount of Sunitinib malate equivalent to 25 mg and transferred into a 25 ml standard flask. Half the volume of water was added. The solution was sonicated for 20 min and then the volume was made up to the mark with distilled water. The solution was filtered through Whatman filter paper. The aliquot portion of the filtrate was suitably diluting for further analysis.

Determination of λmax

The standard stock solution of Sunitinib malate was diluted suitably to get a concentration of 10µg/ml. The solution was scanned within the range of 200 nm-500 nm in D_0, D_1 and D_2 order derivative modes respectively.

Method validation

Linearity

From the above prepared standard stock solution, 10 ml of the solution was diluted to 100 ml using water to get a concentration of 100µg/ml. From the above solution 2 ml, 4 ml, 6 ml, 8 ml, 10 ml and 12 ml of solutions were pipetted out into 6 different 100 ml volumetric flasks and the volume was made up to 100 ml using distilled water to get the final concentrations of 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml and 12µg/ml respectively. These aliquots were scanned in D_0, D_1 and D_2 order derivative modes respectively.

LOD and LOQ

LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot and standard deviation (SD) of the responses was determined. LOD and LOQ were calculated on the basis of SD and slope of the regression equation.

$$LOD = (3.3 \times SD)/slope \quad LOQ = (10 \times SD)/slope$$

Precision

From the above prepared standard stock solution, 10 ml of the solution was diluted to 100 ml utilizing water to get a concentration of 100µg/ml. From the above solution 4 ml, 6 ml and 8 ml of solutions were pipetted out into 3 different 100 ml volumetric flasks and the volume was made with distilled water to get the final concentrations of 4µg/ml, 6µg/ml and 8µg/ml respectively. The intra-day and inter-day analysis was carried out to determine the precision of the developed method. These solutions were scanned in the three modes six times a day (inter-day precision). The solutions were scanned in the three modes for six days at the same time (intra-day precision). The calculated percentage relative standard deviation (% RSD) of the results was used to evaluate the method precision.

Recovery

The recovery study was carried out by the standard addition method. 10 ml of the standard stock solution of standard and sample was pipetted out separately to two 100 ml volumetric flasks and the volume was diluted to 100 ml with distilled water to get a concentration of 100µg/ml. From the above standard solution 4 ml was pipetted out into 4 different volumetric flasks and sample solutions of 0 ml, 2 ml, 4 ml and 6 ml were added to the above 4 volumetric flasks already containing standard solutions and the volume was made up to 100 ml with distilled water. The above solutions were scanned in the three modes. The percentage recovery was estimated.

Assay

The sample stock solution of Sunitinib malate was diluted suitably to get a concentration of 10µg/ml. The above solution was scanned in the three modes and the UV spectra's were recorded and the percentage purity of Sunitinib malate in the pharmaceutical formulation was calculated.

RESULTS AND DISCUSSION

Selection of detection wavelength

The λmax was observed at 431, 457 and 489 nm in D_0, D_1 and D_2 order derivative modes respectively and the UV spectra's was shown in the fig. 2, 3 & 4 respectively.

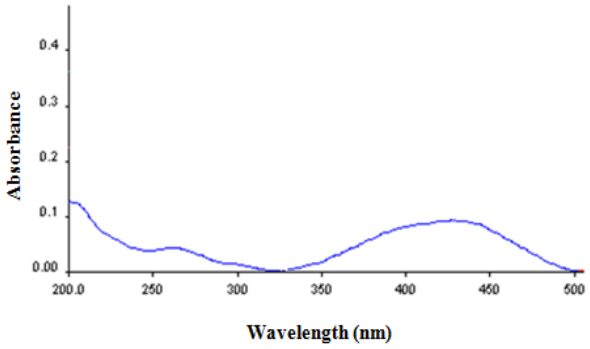

Fig. 2: UV spectra showing maximum absorbance at D_0 mode

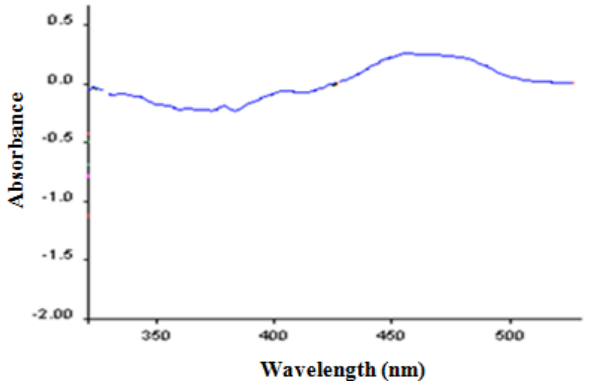

Fig. 3: UV spectra showing maximum absorbance at D_1 mode

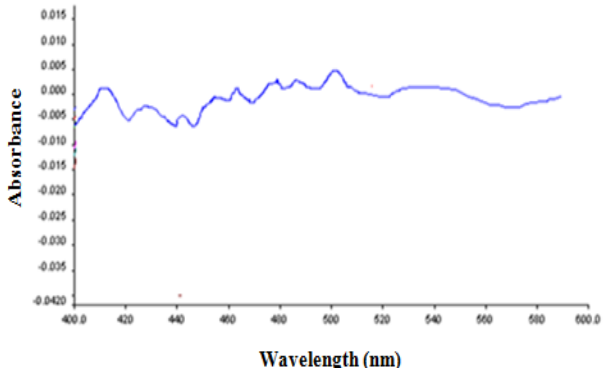

Fig. 4: UV spectra showing maximum absorbance at D_2 mode

Linearity Linearity of Sunitinib malate by the proposed method was made for different concentrations ranging from 2-12 µg/ml was recorded in all the three modes respectively. Linearity was found to obey Beer's law in the concentration range employed, and the

results were tabulated in table 1. The UV spectra slowing linearity and the calibration curves at D_0, D_1 and D_2 modes were presented in fig. 5&6, 7&8, 9&10 respectively.

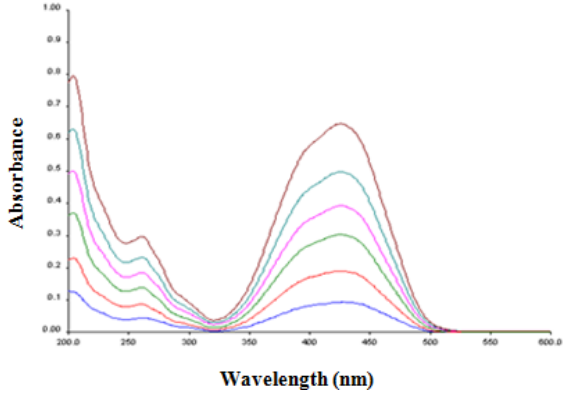

Fig. 5: UV spectra showing linearity at D_0 mode

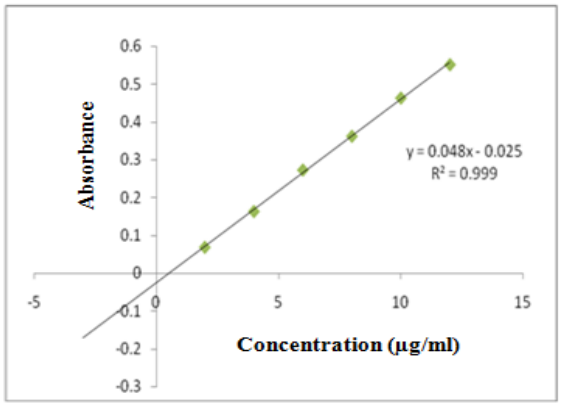

Fig. 6: Calibration graph of D_0 mode

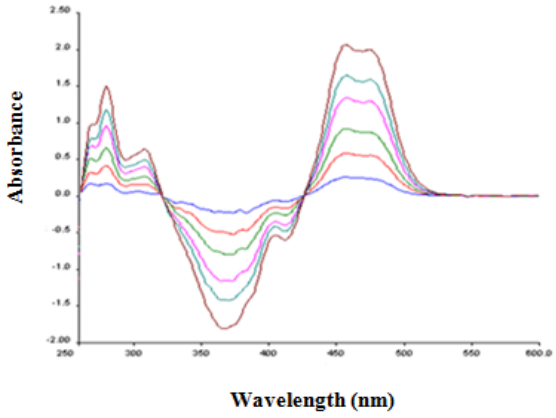

Fig. 7: UV spectra showing linearity at D_1 mode

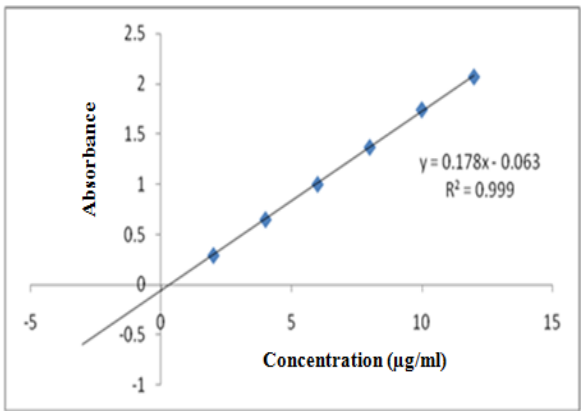

Fig. 8: Calibration graph of D_1 mode

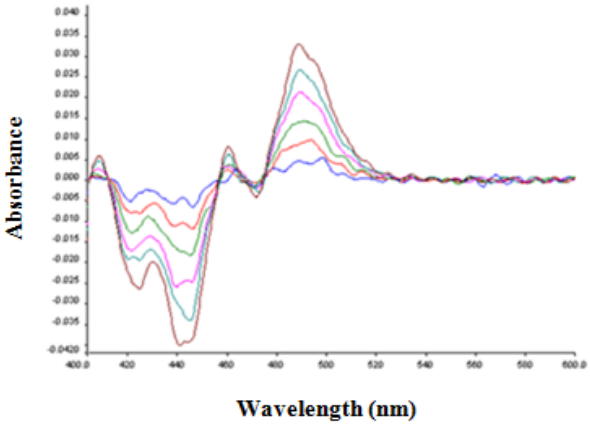

Fig. 9: UV spectra showing linearity at D_2 mode

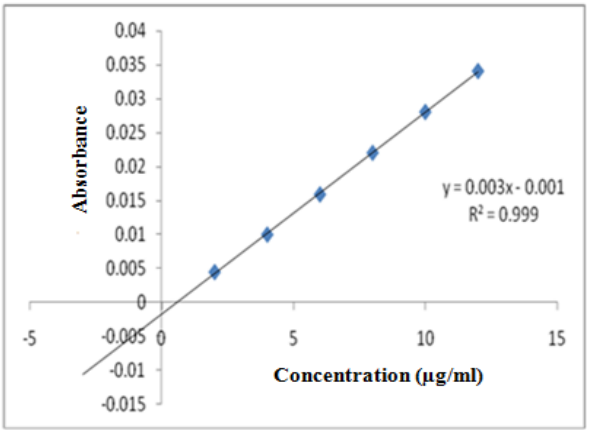

Fig. 10: Calibration graph of D_2 mode

Table 1: Linearity data at D_0, D_1 and D_2 modes

Parameters	Results		
	D_0	D_1	D_2
Linearity range (µg/ml)	2-12 µg/ml		
Regression equation	y=0.048x-0.025	y=0.178x-0.063	y=0.003x-0.001
Correlation coefficient (r^2)	0.999	0.999	0.999
Slope	0.048	0.178	0.003
Y-Intercept	0.025	0.063	0.001
SD	0.0075	0.0058	0.0005

Table 2: LOD and LOQ values at D_0, D_1 and D_2 modes

Parameters	D_0 Mode	D_1 Mode	D_2 Mode
LOD (µg/ml)	0.291	0.107	0.327
LOQ (µg/ml)	0.883	0.324	0.993

LOD and LOQ

The low SD value of responses, LOD and LOQ indicates that the developed method is precise and sensitive. The calculated results are listed in table 2.

Precision

The intra-day and inter-day precision studies (intermediate precision) were performed. The %RSD was considered to be within 0.470-1.870% (intra-day) and 0.870-1.830% (inter-day) which was well within the acceptance criteria, i.e., less than 2% and the results were tabulated in table 3&4.

Recovery

The percentage recovery of the drug was in the range of 97.20-99.36% w/w, which was well within the acceptance limit of 97-103% w/w as per ICH guidelines and the results were tabulated in table 5.

Assay

The mean percentage assay was found to be 97.32-102.28% w/w.

The UV spectra's were shown in fig. 11, 12 & 13 and the results were listed in table 6.

Table 3: Intra-day precision at D_0, D_1 and D_2 modes

Mode	D_0			D_1			D_2		
Concentration (µg/ml)	4	6	8	4	6	8	4	6	8
Absorbance	0.1659	0.2573	0.3714	0.5831	0.9342	1.3384	0.0100	0.0155	0.0219
	0.1639	0.2548	0.3753	0.5820	0.9332	1.3392	0.0096	0.0156	0.0226
	0.1626	0.2541	0.3672	0.5813	0.9350	1.3389	0.0098	0.0158	0.0215
	0.1632	0.2557	0.3702	0.5792	0.9338	1.3402	0.0099	0.0159	0.0223
	0.1645	0.2568	0.3692	0.5840	0.9351	1.3368	0.0099	0.0152	0.0225
	0.1632	0.2555	0.3720	0.5754	0.9202	1.3372	0.0096	0.0158	0.022
Mean (±SD)*	0.1638±0.0011	0.2557±0.0011	0.3708±0.0027	0.5808±0.0031	0.9319±0.0057	1.3384±0.0012	0.0098±0.0001	0.0156±0.0002	0.0221±0.0004
%R.S.D	0.720	0.470	0.740	0.540	0.620	0.095	1.700	1.650	1.870

mean±SD* = average of six determinations

Table 4: Inter-day precision at D_0, D_1 and D_2 modes

Mode	D_0			D_1			D_2		
Concentration (µg/ml)	4	6	8	4	6	8	4	6	8
Absorbance	0.1659	0.2573	0.3714	0.5831	0.9342	1.3384	0.0100	0.0155	0.0219
	0.1644	0.2610	0.372	0.5734	0.9430	1.3440	0.0099	0.0158	0.0223
	0.1660	0.2582	0.3623	0.5728	0.9229	1.3560	0.0099	0.0162	0.0215
	0.1643	0.2600	0.3719	0.5801	0.9448	1.3652	0.0102	0.0160	0.0223
	0.1672	0.2632	0.3682	0.5790	0.9280	1.3620	0.0101	0.0163	0.0225
	0.1679	0.2560	0.3721	0.5629	0.9450	1.3760	0.0098	0.0161	0.0220
Mean (±SD)*	0.1659±0.0014	0.2592±0.0026	0.3696±0.0038	0.5752±0.0072	0.9363±0.0094	1.3569±0.0139	0.0099±0.0001	0.0159±0.0002	0.0220±0.0003
%R. S. D	0.870	1.016	1.053	1.260	1.010	1.030	1.470	1.830	1.630

mean±SD* = average of six determinations

Table 5: Recovery studies at D_0, D_1 and D_2 modes

Mode	Amount present (µg/ml)	Amount added (µg/ml)	Absorbance	Amount recovered (µg/ml)	% Recovery	Mean (±SD)*
D_0	4	0	0.1715	3.9745	99.36	
	4	2	0.2685	5.8881	97.20	98.620±0.9654
	4	4	0.3705	7.9592	98.98	
	4	6	0.4934	9.9576	98.94	
D_1	4	0	0.6591	3.9663	99.16	
	4	2	1.0293	5.952	97.60	98.375±0.6456
	4	4	1.3896	7.9298	98.24	
	4	6	1.8408	9.9100	98.50	
D_2	4	0	0.0098	3.9596	98.99	
	4	2	0.0162	5.9632	98.16	98.1925±0.5901
	4	4	0.0203	7.9219	98.05	
	4	6	0.0286	9.7945	97.57	

mean±SD* = average of four determinations

Table 6: Assay at D_0, D_1 and D_2 modes

Mode	Theoretical yield (mg)	Practical yield (mg)	Percentage yield (%w/w)
D_0	24.33	25	97.32
D_1	24.925	25	99.70
D_2	25.57	25	102.28

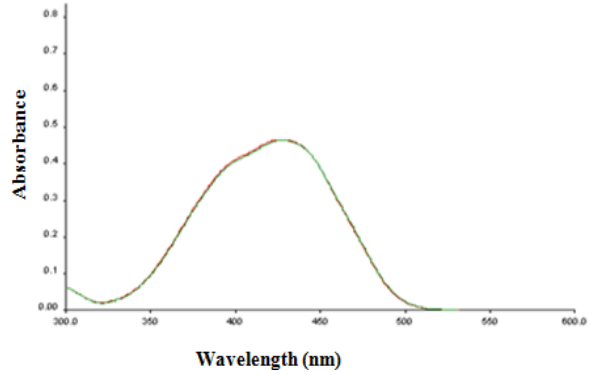

Fig. 11: UV spectra showing assay at D_0 mode

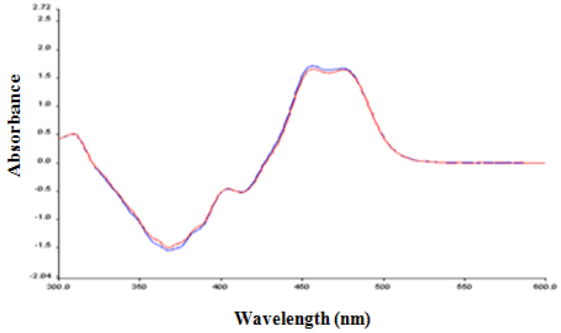

Fig. 12: UV spectra showing assay at D_1 mode

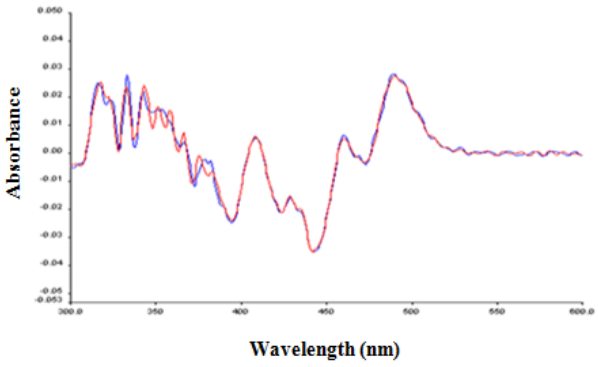

Fig. 13: UV spectra showing assay at D_2 mode

CONCLUSION

Sunitinib malate is an oral, small-molecule, multi-targeted receptor tyrosine kinase (RTK) inhibitor that was approved by the FDA for the treatment of renal cell carcinoma (RCC) and imatinib-resistant gastrointestinal stromal tumor (GIST).

It was the first cancer drug that was approved. The development of a simple, rapid, sensitive and precise Spectrophotometric method for the routine quantitative determination of samples will definitely reduce unnecessary tedious sample preparations and the cost of materials and labour. Sunitinib malate is a UV-absorbing molecule with specific chromophores in the structure that absorbs at a particular wavelength and this fact was successfully employed for their quantitative determinations using the UV Spectroscopic method and validating the same. The λmax was observed at 431, 457 and 489 nm in D_0, D_1 and D_2 order derivative modes respectively.

This needed to be done as a first step to quantifying the amount of drug present in pharmaceutical dosage form. Various validation parameters as discussed above were analyzed and they were found to comply with the guidelines laid by ICH. Therefore, it was concluded that the proposed UV Spectroscopic method was novel, simple, accurate, precise, reproducible, economical and sensitive which would be used for the estimation of Sunitinib malate in their pharmaceutical dosage form in routine analysis.

ACKNOWLEDGEMENT

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CONFLICT OF INTERESTS

Declare none

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