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Method Development and Validation of Fludrocortisone Acetate Tablets by Reverse Phase HPLC Method

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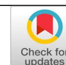
ABSTRACT



The purpose or intent of this current study was to establish a fast and sensitive HPLC technique for the perseverance of Fludrocortisone acetate and utilizing best frequently used HPLC technique. This method had been validated as per the ICH requirements to assure that the method consistently meets the predetermined specifications and quality attributes.Utilizing filtered and degassed pH 3.0 Phosphate buffer and Acetonitrile in the ratio 90:10 as a Mobile phase-A and pH 3.0 Phosphate buffer and Acetonitrile in the ratio 65:35 as a Mobile phase-B the established RP-HPLC technique was done. The separation was achieved by using Waters, X-Bridge Shield RP18, (150 X 4.6-mm), 3.5-μm column. Run time and Flow rate was set 45minutes and 1.2mL/min. Injection volume 100μL and wavelength was set 240nm.The correlation coefficient square for fludrocortisones acetate and Fludrocortisone Impurity was found to be 0.9991 and 0.99997. The SD and %RSD for Fludrocortisone Impurity was found to be 0.02 and 1.48 represents method precision. Following validated parameters lies within the limit. Hence, the developed method was precise, simple, fast and accurate.

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INTRODUCTION

Disorder and then postural hypotension. It is usually consumed along with hydrocortisone in case of adrenal inadequacy and medication is utilized utmost in its acetate form (Elks, 2014; Index Nominum, 2000; Fludrocortisone Acetate, 2016; Day and Furst, 2010). Fludrocortisone acetate is an adrenal corticosteroid and has controlled level of glucocorticoid action and has extremely maximum level of mineralocorticoid action. Though it is utilized just for its mineralocorticoid issues. Mineralocorticoid functions in the distal tubules to enhance hydrogen ion evacuation, sodium resorption, to enhance potassium evacuation and later water retention. In further secretory cells cation transfer is equally influenced, evacuation of water by sweat glands and salivary glands is also modified, though to a lower degree. At the cellular levels corticosteroids spread throughout or over cell membranes and intricate with particular or individual cytoplasmic recipient, such complexes afterwards join the cell nucleus attach to DNA and encourage arrangement of mRNA and later protein combination of different enzymes thought to be finally liable for the physiologic issues of such hormones (Lemke and Williams, 2008; Polito *et al.*, 2016). Fludrocorti-

sone is utilized mainly to substitute the lacking hormone aldosterone in diverse forms of adrenal inadequacy for instance classic salt squandering form of congenital adrenal hypertrophy and Addison's illness. It has been utilized in the therapy of cerebral salt squandering disorder. Because of its issues on enhancing sodium levels and hence blood volume and then used to heal postural hypotension and to heal low blood pressure (Taplin *et al.*, 2006; Freitas *et al.*, 2000).

Method Development

Method development is the procedure or method of choosing a precise testing process to establish the constitution of a preparation. Analytical process must be utilized under GLP and GMP conditions and utilizing the customs or properties fixed out in the ICH guidelines the analytical procedure should be established. It is the method of evidence that an analytical procedure is tolerable for employ in laboratory to assess the compactness of consequent specimens. Suitable and quantified or measured instruments, Certified procedure, Change control, Dependable reference standard, Competent analysts, Integrity and Specimen choice (ICH, 2000; European Commission, 2001; Mcdowall, 2005).

MATERIALS AND METHODS

Chemicals and reagents

Fludrocortisone Acetate tablets (Equivalent to about 2.5mg) the used pharmaceutical preparation were formulated in-house and used Fludrocortisone Acetate API with a potency 100%. HPLC grade Acetonitrile were purchased from Merck limited and HPLC grade Methanol were purchased from Finar Limited and HPLC grade water. Analytical grade reagents were used.

Instrumentation

Automated Reverse Phase HPLC equipped with UV-detection chromatographic separation was achieved utilizing Waters, X-Bridge Shield RP18, (150 X 4.6-mm), 3.5-μm column. Run time and Flow rate was set 45minutes and 1.2mL/min. Injection volume 100μL and wavelength was set 240nm. For processing data Empower software were used.

The chromatographic Parameters and gradient progam are listed in Tables 1 and 2.

From Table 3, developed technique were met the acceptance criteria. Hence the developed method was found to be accurateTables 4, 5, 6, 7, 8 and 9 .

The correlation coefficient square (r^2) for Fludrocortisone Acetate and Fludrocortisone Impurity were met the acceptance criteria. The linear regression data shows that the method is linear over the entire concentration range (LOQ (10%)-150%) and the developed method was found to be precise and accurateFigures 1, 2 and 3.

From Figure 4, developed method were met the acceptance criteria. Hence the developed method was found to be accurate and precise.

Overall average recovery for Fludrocortisone Impurity is between 80.0-120.0%.The %RSD for recovery of triplicate preparations at each level is NMT 10% and hence the method is accurate

No interference was observed from diluents, placebo and all known Impurities at the retention time of Fludrocortisone Acetate peak.

The %difference in area between initial and time points should be NMT 25.0 for Standard. The %difference in %Impurity between initial and time points should be NMT 25.0 for sample.

All results met the acceptance criteria. Based on above results, it is concluded that standard and sample solutions were stable up to 96 hours respectively when stored at Room temperature.

The %difference in Peak area for Impurity between the centrifuged sample and filtered sample should be NMT 25.0.

Preparation of Buffer

Phosphate Buffer pH 3.0

The buffer was prepared by dissolving 2.07 g of sodium di-hydrogen phosphate monohydrate in 1000 mL of water. Mixed well and then the buffer adjusted to pH 3.0$\pm$0.05 with diluted phosphoric acid, then the solution filtered through 0.45 μm membrane filter and sonicated the buffer solution to degas.

Mobile Phase -A

900mL of pH 3.0 Phosphate buffer and 100mL of Acetonitrile into a suitable container and then sonicated to degas.

Mobile Phase-B

650 mL of pH 3.0 Phosphate buffer and 350 mL of Acetonitrile into a suitable container and then sonicated to degas.

Preparation of Diluent

Used Mobile Phase-B as a diluent.

Procedure

Standard Stock Preparation

50.23mg of Fludrocortisone Acetate RS was weighed and transferred into a 100mL volumetric flask. To

Table 1: Chromatographic Parameters

Chromatographic parameters	Conditions/Specifications
Mobile Phase – A	pH 3.0Buffer: Acetonitrile (90:10,v/v)
Mobile Phase – B	pH 3.0Buffer: Acetonitrile (65:35,v/v)
Column	Waters, X-Bridge® Shield RP18, (150 X 4.6-mm), 3.5-μm
Flow rate	1.2 mL/min
Column Temp.	30°C
Sample Temp.	10 °C
Wavelength	240 nm
Injection Volume	100 μL
Run Time	45.0 minutes

Table 2: Gradient Program

Time (min)	Mobile Phase-A (%)	Mobile Phase-B (%)
0.00	40.0	60.0
15.00	40.0	60.0
20.00	30.0	70.0
30.00	0.0	100.0
40.00	0.0	100.0
40.10	40.0	60.0
45.00	40.0	60.0

Table 3: System Precision data

S. No.	Injection No.	Peak Area for Fludrocortisone Acetate RS	USP Tailing	USP Plate Count
1.	1	151500	1.1	21354
2.	2	154805	1.1	21172
3.	3	152286	1.1	20984
4.	4	152654	1.1	21231
5.	5	153553	1.1	21452
6.	6	154436	1.1	21012
Mean	-	153206	-	-
STDEV	-	1284.65	-	-
%RSD	-	0.8	-	-

that 3/4th volume of diluent was added. Sonicated to dissolve, diluted to volume with diluent and mixed well.

Intermediate Stock Preparation

Pipetted out 6mL of Fludrocortisone Acetate Standard Stock solution into 100mL volumetric flask. Diluted to volume with diluent and mixed well.

Standard Preparation

Pipetted out 2.5mL of Fludrocortisone Acetate Intermediate Stock solution into 50mL volumetric flask. Diluted to volume with diluent and mixed well.

Preparation of Sample Solution

Randomly selected the twenty-five (25) tablets and recorded the weight of tablets. Transferred the tablets into a 25mLof volumetric flask. (Equivalent to about 2.5 mg of Fludrocortisone Acetate) added about 15mL of diluent and sonicated the flask until the tablets dispersed. Then spiked the 0.75mL of Fludrocortisone Impurity stock preparation into the same sample solution. Further sonicated to 15 min-

Table 4: Linearity Data

Linearity Level (%)	Fludrocortisone Acetate		Fludrocortisone Impurity	
	Concentration (μg/mL)	Peak Area for Fludrocortisone Acetate	Concentration (μg/mL)	Peak Area for Fludrocortisone Impurity
LOQ (10%)	0.05	16236	0.150	14978
30	0.151	45752	0.45	45070
50	0.252	77852	0.75	75117
80	0.402	122749	1.2	120188
100	0.503	153206	1.5	151345
120	0.604	185421	1.8	180282
150	0.755	238594	2.25	225353
Correlation Coefficient Square (r2)	-	0.9991	-	0.99997

Table 5: Method Precision Data

S. No.	Sample Name	Peak Area for Fludrocortisone Impurity	% Impurity
1.	Method Precision-1	150852	1.513
2.	Method Precision-2	151235	1.476
3.	Method Precision-3	150652	1.495
4.	Method Precision-4	151232	1.458
5.	Method Precision-5	152867	1.505
6.	Method Precision-6	153259	1.512
Mean	-	-	1.49
STDEV	-	-	0.02
%RSD	-	-	1.48

Table 6: Accuracy Data

Accuracy Levels	Sample #	Peak Area of Fludrocortisone Impurity	Amount Added (μg/mg)	Amount Recovered (μg/mg)	% Recovery	Average % Recovery	S.D	%RSD
50%	Sample 1	72382	0.742	0.450	98.9	97.3	1.86	1.9
	Sample 2	70832	0.698	0.732	95.3			
	Sample 3	73135	0.720	0.738	97.6			
100%	Sample 1	150218	1.479	1.506	98.2	98.9	0.97	0.99
	Sample 2	152457	1.501	1.524	98.5			
	Sample 3	153632	1.513	1.512	100.0			
150%	Sample 1	216083	2.128	2.256	94.3	96.5	2.51	2.6
	Sample 2	218753	2.154	2.244	96.0			
	Sample 3	226789	2.233	2.250	99.3			

Table 7: Solution Stability of Fludrocortisone Acetate at Room Temperature

Time Interval	Fludrocortisone Acetate Peak Area	% Difference in Area
Initial	105924	N/A
12 Hours	103568	2.22
24 Hours	102559	3.18
48 Hours	101068	4.58
72 Hours	100956	4.69
96 Hours	100582	5.04

Table 8: Solution Stability of Fludrocortisone Impurity at Room Temperature

Time Interval	Fludrocortisone Impurity Peak Area	% Difference in Area
Initial	152532	N/A
12 Hours	150980	1.02
24 Hours	150264	1.49
48 Hours	150004	1.66
72 Hours	149925	1.71
96 Hours	149856	1.75

Table 9: Filter Study Data of Fludrocortisone Impurity

	Fludrocortisone Impurity	
Sample	Peak Area	% Difference
Centrifuged/Unfiltered	152532	N/A
0.45μm Nylon/2mL discard	153252	0.12
0.45μm Nylon/4mL discard	152117	0.27
0.45μm Nylon/6mL discard	152029	0.33
0.45μm Nylon/8mL discard	151863	0.44

utes with frequent intermittent shake. After the sonication, diluted to volume with diluent and mixed well. Kept the flask on bench top for about 10 minutes and allowed to settle down. Centrifuged the sample for about 5 minutes and collected the supernatant. Filtered the clear aliquot through 0.45-μm Nylon syringe filter and collected the filtrate after discarded the first 4mL of filtrate.

Preparation of Placebo Solution

Weighed about 2498.12mgof Placebo into 25mL of volumetric flask and added 15mL of diluent and sonicated for 15 minutes with intermittent shaking. Diluted to volume with diluent and mixed well. Centrifuged the sample for about 5 minutes and collected the supernatant. Filtered the clear aliquot through 0.45-μm Nylon syringe filter and collected the filtrate after discarded the first 4mL of filtrate.

Linearity Procedure

Standard Stock Preparation

50.35mg of Fludrocortisone Acetate RS was weighed and transferred into a 100mL volumetric flask. To that 3/4th volume of diluent was added. Sonicated to dissolve, diluted to volume with diluent and mixed well.

Intermediate Stock Preparation

Pipetted out 5mL of Fludrocortisone Acetate Standard Stock solution into 250mL volumetric flask. Diluted to volume with diluent and mixed well.

Standard Preparation

Pipetted out 5mL of Fludrocortisone Acetate Intermediate Stock solution into 100mL volumetric flask. Diluted to volume with diluent and mixed well.

Fludrocortisone Impurity Stock Preparation

2.52mg of Fludrocortisone Impurity was weighed and transferred into a 50mL volumetric flask added 35mL of diluent and sonicated to dissolve. After sonication diluted to volume with diluent and mixed well.

Fludrocortisone Impurity Preparation

Pipetted out 3mL of Fludrocortisone Impurity stock solution into 100mL volumetric flask. Diluted to vol-

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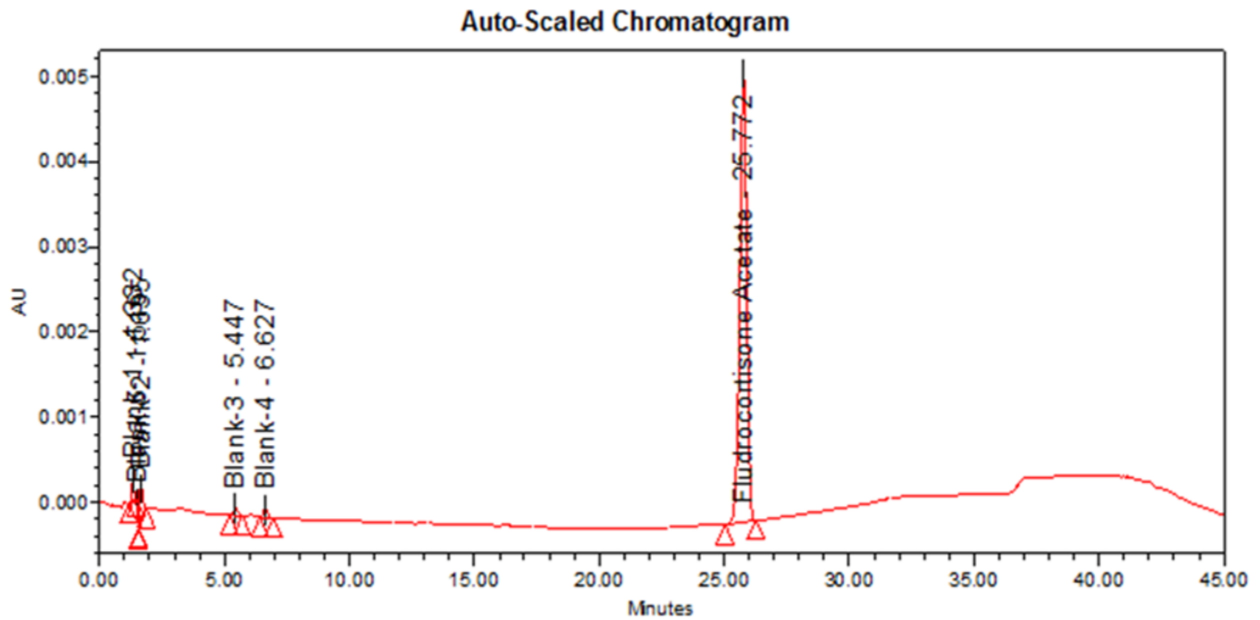

Figure 1: Chromatogram of Fludrocortisone Acetate Standard Injection

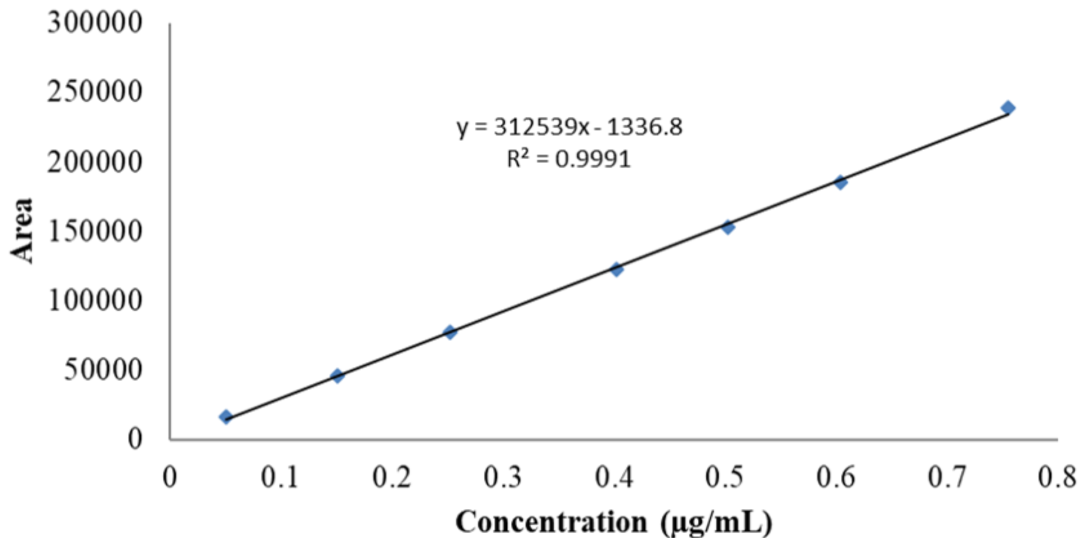

Figure 2: Linearity Graph for Fludrocortisone Acetate

ume with diluent and mixed well.

Initialization of Instrument

By running mobile phase continuously stabilization of chromatographic system and saturation of column is done and baseline graph developed.

RESULTS AND DISCUSSION

The %difference in peak area for Impurity between the centrifuged sample and filtered sample was calculated and found less than 25.0. Based on filter study data, it is concluded that samples should be filtered through 0.45μm Nylon filter after discarding first 4mL of filtrate.

The purpose or intent of current study was to establish a fast and sensitive HPLC technique for the perseverance of Fludrocortisone acetate and Fludrocortisone Impurity utilizing best frequently used HPLC technique. The method has been validated as per the guidelines given by ICH requirements to assure that the method consistently meets the predetermined specifications and quality attributes. The system suitability parameter i.e., correlation coefficient square for fludrocortisones acetate and fludrocortisones impurity was found to be 0.9991 and 0.99997. The SD and %RSD for Fludrocortisone Impurity was found to be 0.02 and 1.48 represents method precision. Following validated parameters lies within the limit. Hence the developed method was precise, simple, fast and accurate.

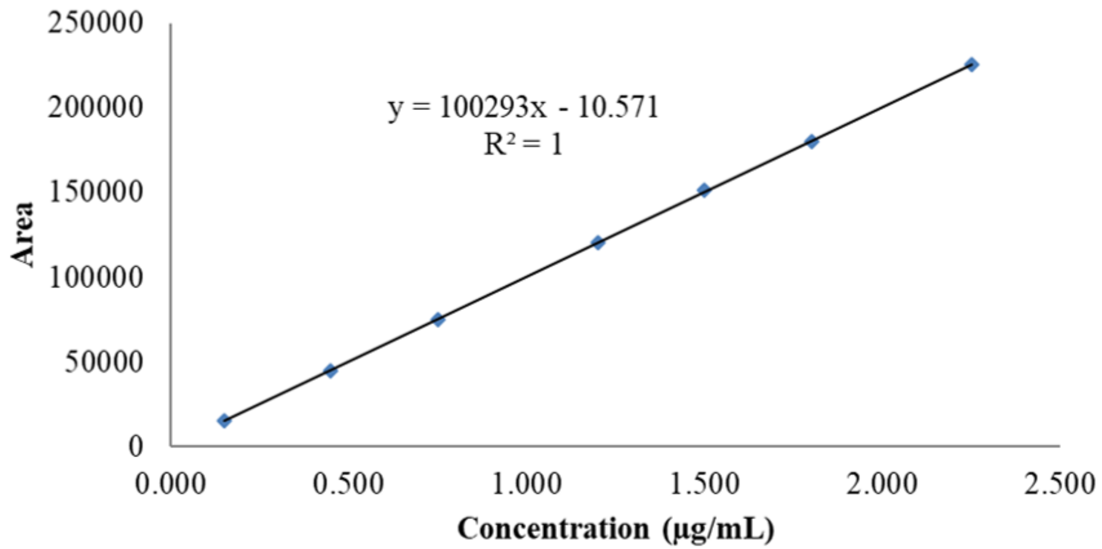

Figure 3: Linearity Graph for Fludrocortisone Impurity

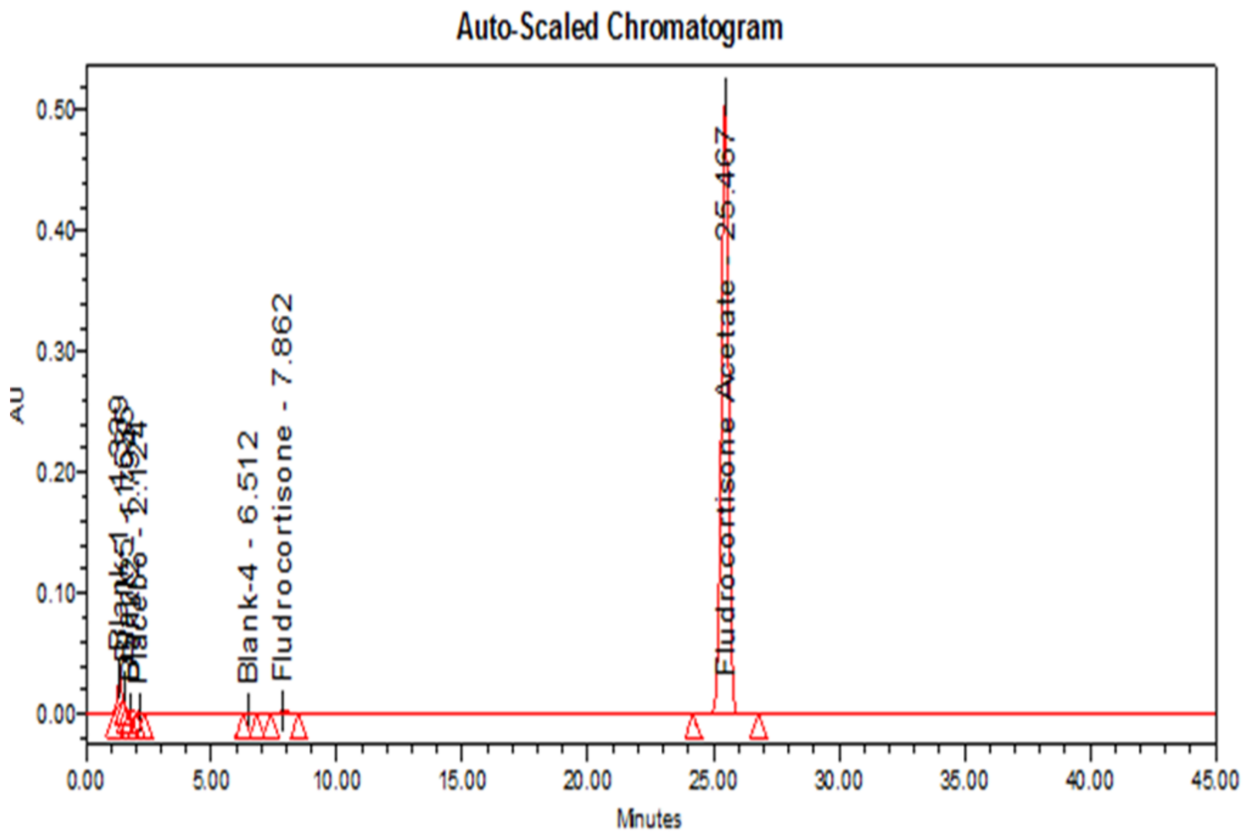

Figure 4: Chromatogram of Fludrocortisone Impurity Sample Injection

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CONCLUSIONS

The developed method was validated as per the ICH guidelines instructions. From the determined values it is concluded that the established technique was simple, fast, precise and accurate.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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