Stability Indicating RP-HPLC Method Development for Related Substances of Anti-histamine Promethazine hydrochloride and its Validation study

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ABSTRACT

A smooth, specific, precise, and accurate high-performance reverse phase (RP-HPLC) method for determining related substances in antihistamine Promethazine Hydrochloride (PMZ·HCl) has been recorded. The RP-HPLC system is developed and tested in compliance with recommendations Q2(R1) of the International Council for Harmonization (ICH). Column 5µ and reverse step with linear gradient elution is accomplished in Symmetry Shield RP8 (4.6 mm x 150 mm). Mobile phase A is 3.4% in the 7.0 pH water, modified to dilute potassium hydroxide solution. In contrast, the 60:40 mixture of acetonitrile and methanol combination as mobile phase B. The handled phase with a continuous flow rate of 1.0 ml min^{-1} is provided by choosing the wavelength 254 nm using a PDA/UV detector. The temperature of the column oven and sampler is 25°C and 4°C, respectively. The injection amount chosen is 10.0 µL. The method is linear in the quantitation limit range (LOQ) to 150 percent concerning the specification impurity concentration limit. Both impurities and PMZ have a correlation coefficient of greater than 0.999. The LOQ for all known impurities and PMZs has a specification limit of between 10 to 30%. The relative response factor for all four known impurities is calculated. The unexplained peaks are very different; the effects obtained are similar to the original values. There are no significant changes improvements to the suitability parameters, such as tailing factor, Plates theoretical sheets, and % RSD in Robustness Studies. The forced degradation analysis of PMZ·HCl was performed. This RP-HPLC is descriptive, accurate and precise. This further defines the parameters of linearity, consistency and robustness used for regular quality control analysis.

Keywords: Anti-histamine, RP-HPLC, Related substances, Stability indicating, PMZ·HCl.

INTRODUCTION

Promethazine (PMZ),), 10 *H*-Phenothiazine-10-ethanamine, N,N,α-trimethylmonohydrochlorid is a common antihistamine pharmaceutical drug that is sold as hydrochloride salt (PMZ•HCl). (see Fig.1). PMZ is an antihistamine agent of the first generation used to treat allergies, sleep disorders and nausea[1].

It was approved in the United States for medical use in 1951. PMZ is a derivative of phenothiazine and structurally different from the same but different effects[2] of phenothiazine. PMZ is a crystalline powder that is white to faint-yellow odorless. PMZ hydrochloride salt can be freely water soluble and slightly alcohol soluble. It is also a chiral compound, which occurs as an enantiomer mixture[3]. PMZ is molecular in $C_{17}H_{20}N_2S.HCl$ and weight 320.88 g/mol. It is preserved under nitrogen at 25°C in tight, light-resistant containers.
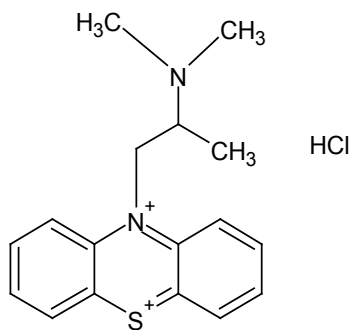
Chemical structure of PMZ·HCl

MATERIALS AND METHODS

mettler toledo

The Promethazine (PMZ) sample and its impurities are taken from Emcure Pharmaceutical Limited, Analytical Research Center, Hinjewadi Center for Analytical Analysis, Hinjawadi, Pune. Potassium phosphate analytic grade, potassium hydroxide, and millet-Q grade purified water. For a mobile phase and diluent preparation, gradient methanol, acetonitrile, and triethylamine are used. The research equilibrium shall be used for the mettler toledo and the HPLC waters with UV/PDA detector using the empower two software of data acquisition and calculations.

Mobile phase A

3.4% of potassium dihydrogen phosphate solution homogeneously in water with 7 pH adjusted in the ratio of 40:40:20 by the dilute potassium hydroxide solution, acetonitrile, and methanol.

Mobile Phase B

The homogeneous mixture of acetonitrile and methanol in the ratio of 60:40.

Diluent

0.1% solution of triethylamine in methanol

Standard and test preparation

Weighed 100 mg standard/test sample of Promethazine in 100 mL volumetric flask was dissolved and diluted with diluent. 5.0 mL pipetted above solution in 100 mL flask and diluted with the diluent amount.

Method Development

The PMZ and its related impurities are polar molecules; hence reverse phase (RP) chromatography is used to establish the associated substances. The fixed phase of chromatography in the reversed-phase is non-polar as C4, C8, C18, and polar as the mobile phase is water, acetonitrile, methanol, or buffer solution. The document shows several HPLC methods for estimating promethazine hydrochloride in bulk medicinal products and formulations[4-8]. No specific method for determining related substances was reported in the literature.

Other parameters such as column temperature, diluents, wavelength, and pH are also crucial in developing an HPLC method and stationary and mobile phases. The stationary phase of the HPLC method screened from specific Hypersil BDS C8, and Symmetry Shield RP8 is both 4.6 mm internal diameter, 150 mm long, and 5µ particle size. Such 150 mm and 250 mm long Hypersil BDS C8 and Symmetry Shield RP8 columns. When the symmetry shield RP8 (4.6 x 150 mm) is added to 5µ, enhanced impurity separation, maximum sharpness, and sufficiently adjustable device parameters, i.e., tailing factor and theoretical plates, are achieved. The pKa value of the target molecules guides the mobile phase buffer selection. Powdered potassium phosphate buffer is chosen here for the preparation of the mobile phase. It is the homogeneous combination of 3.4% potassium dihydrogen phosphate solution in water at 7.0% pH change of diluted potassium hydroxide solution, acetonitrile and methanol with 40:40:20 ratio used as mobile phase A, in which 60:40 mixed acetonitrile and methanol is mobile phase B. The chromatographic development method for PMZ·HCl is mentioned in Table 1.

Table 1: Chromatographic conditions for the related substances RP-HPLC method of PMZ·HCl

Instrument	HPLC equipped with an injector, pump UV/PDA detector and recorder
Column	Symmetry Shield RP8 (4.6 mm x 150 mm) 5µ
Wavelength	254 nm
Flow rate	1.0 mL/min
Injection volume	10.0 µL.
Column oven temperature	50°C
Auto sampler temperature	4°C
Run time	65 mins

Also, the HPLC analysis was carried out with the gradient method, as at the beginning, the composition of step A and B was used at 70:30, which is gradually changed to 40:60 and up to 45 minutes. It is adjusted to a ratio of 35:65 up to 50 min. It lasts up to 52 min then it suddenly hits initial values of 70:30 and is sustained for up to 65 min throughout the entire course.

RESULTS AND DESCUSION

The analytical method validation study was carried out as per ICH guideline Q2(R1)[9-12]. The analytical method validation parameters are discussed below.

Specificity

The specificity study conducted by spiking with an adequate level of known impurities (up to 0.15% concentration) into pure substances and demonstrating findings is not affected by the presence of these spike impurities. A blank run shows no intervening PMZ peak and its associated impurities. The PMZ peak is well-differentiated from recognized impurities (Fig. 1). The peak purity angle of PMZ and its related impurities is less than the threshold for standard, non-spiked, and spike test solutions (Table 2). The percent relative standard deviation (%RSD) of six standard solutions replicates with a mixture of impurities A to D and PMZ·HCl are 0.60, 0.87, 1.86, 0.69 and 4.58%. percent. The resolution between the near escape of impurities B and A is 5.72, which indicates a sharp break between these peaks.

Fig. 1. Typical chromatogram for selectivity in the PMZ·HCl. over the spiked impurities

Table 2: The selectivity data in the PMZ·HCl over spiked impurities up to 0.15 % of concentration in test solution

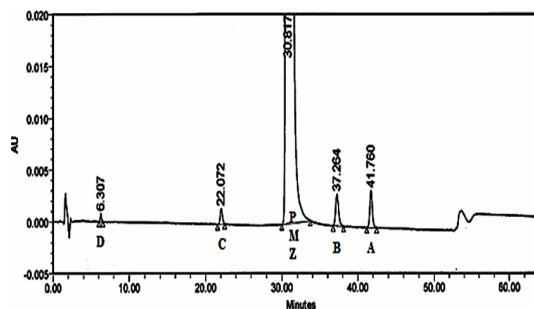| Impurities*	Retention Time	Area	RT ratio	Tailing Factor	Resolution	Purity Angle	Purity Threshold	Peak Purity
D	6.307	8388	0.20	1.09	--	1.599	2.058	Pass
C	22.072	32206	0.72	1.07	35.61	3.205	3.889	Pass
PMZ	30.817	23713179	1.00	1.41	13.61	0.087	0.265	Pass
B	37.264	85577	1.21	1.21	8.60	2.187	2.606	Pass
A	41.760	83675	1.36	1.11	6.72	1.948	2.296	Pass

*Impurities are represented by alphabets A to D.

Determination of limit of detection and limit of quantitation

The limit of detection (LOD) is the smallest concentration of an analyte in a solution that can only be detected but quantitatively measured. At the same time, the LOQ is the lowest amount of analyte in a sample solution, which can be quantitatively determined with precision and accuracy. In this analysis, LOD and LOQ levels are calculated by injecting the various concentrations in the standard solutions (between 10 and 120%) of PMZ·HCl (0.1%) and its maximum concentrations in impurity A to D

(0.15%) and calculating the signal-to-noise ratio. The expected LOD and LOQ data in Table 3 are shown.

Table 3: The predicted LOD, LOQ concentration and s/n ratio value of LOQ level of PMZ·HCl and its related Impurities A to D

| Impurites	LOQ in % w.r.t test	S/N of LOQ level	LOD in % w.r.t test
D	0.029	21	0.010
C	0.015	14	0.005
PMZ	0.019	18	0.006
B	0.013	14	0.004
A	0.008	16	0.003

Linearity and Range

The linearity was determined by using the standard solutions of impurities and by PMZ·HCl at the level of LOQ at a 150 percent level (incl.50, 80, 100, and 120 %). The concentrations of LOD and LOQ, correlation coefficient, and linearity data intercept are shown in Table 4 and the corresponding linearity graph (Fig. 2 and 3). Linearity data are shown in Table 4. The concentration data for the peak areas versus concentration were treated with less square analysis of linear regression and the correlation coefficients obtained for all impurities and PMZ·HCl are higher than 0.999. The linearity graph reveals that the PMZ·HCl research sample has variations in A-D impurities. Uncleanness D has a lower reaction, whereas uncleanness A has more significant reaction overall known impurities found in PMZ·HCl.

Table 4: The concentration of LOQ, LOD, area for the correlation coefficient, slope, intercept as well as RRF of Impurities A to D and PMZ·HCl

Impurities	LOQ in % w.r.t test	LOD in % w.r.t test	Area for 0.15 % conc.	Correlation Coefficient	Slope	Intercept	% Y intercept	RRF
D	0.029	0.010	11187	0.99960	15336	93	0.83	3.88
C	0.015	0.005	42575	0.99987	56243	61	0.14	1.06
PMZ	0.019	0.006	28525	0.99951	59541	1055	3.69	1.00
B	0.013	0.004	83281	0.99980	64652	434	0.52	0.92
A	0.008	0.003	101616	0.99967	134056	300	0.30	0.44
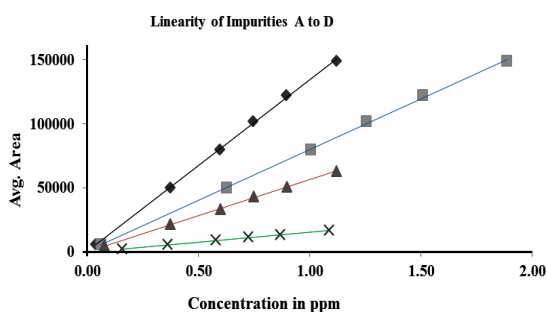
Fig. 2. Linearity graph of impurities A to D from LOQ to 150 %concentration levels
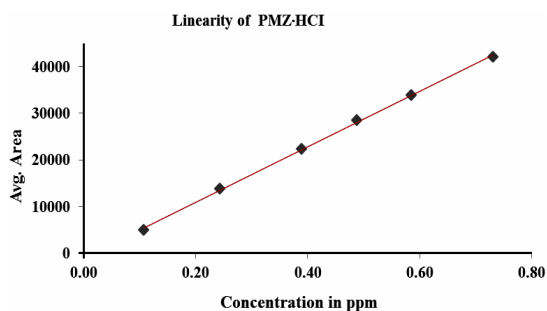
Fig. 3. Linearity graph of PMZ·HCl from LOQ to 150 % concentration levels

Relative response factor

The RRF is a practical approach for the systematic study of impurities found in drug compounds and their components. The RRF is usually specific for each impurity and is independently calculated. It cannot be used to measure the concentration of respective ingredients by other detectors for one particular detector type. The RRF value depends on the response of PMZ·HCl and its known impurities. The RRF values observed for known impurities A to D range from 0.44 to 3.88 to RRF of PMZ.HCl 1.00 for unknown impurities measurement. The observed impurity D has lower response while for impurity A shown that higher response in PMZ.HCl.

Precision

The precision study is divided into repeatability, intermediate precision, and reproducibility. Six replicates of PMZ.HCl study system precision with a regular mixed impurity solution. The repetitiveness of the relevant compounds was accomplished by injecting six individual test sample solutions with the concentration of identified impurities in the spiking definition stage. The percentage of impurities and the percentage RSD of the solution is determined.

The intermediate precision is measured by multiple observers in the same laboratory on different days with different instruments. Repeatability %RSD percentage (n=6) and intermediate precision (n=6) of impurities tests were determined. %RSD percent of the six spike test preparations for impurities A to D and total impurities are 1.68, 1.19, 0.83, 1.66 and 1.06 also %RSD of impurities A to D for 12 samples (i.e. six from repeatability and six from intermediate accuracy) are 2.40, 2.59, 1.44, 2.06 and 1.63 respectively. The reproducibility indicates joint research widely used for technique standardization. Table 5 displays the comparative results for percentage impurities of repetitiveness and moderate accuracy.

Accuracy

The reliability of an analytical procedure is the continuity of the actual value and value observed. A three-fold accuracy impurity analysis was performed at the LOQ level of 50, 100, and 150% of the test solution's stated impurities concentration. The quality of known impurities has been found well below the admission criterion. The average accuracy of the percent and its standard deviation from its LOQ, 50%, 100%, and 150% of the specification level specified in Table 6.

Table 5: Repeatability Comparative Results (REP) and Medium Precision (IP) at spiked check (0.15% spike of each impurity)

Tests	Impurities concentration in %									
	% of A		% of B		% of C		% of D		% of Total Imp.	
	REP	IP	REP	IP	REP	IP	REP	IP	REP	IP
Spiked test-1	0.153	0.143	0.246	0.233	0.144	0.149	0.158	0.153	0.701	0.678
Spiked test-2	0.149	0.142	0.245	0.229	0.146	0.147	0.157	0.152	0.697	0.670
Spiked test-3	0.146	0.143	0.240	0.234	0.143	0.148	0.157	0.152	0.686	0.677
Spiked test-4	0.147	0.144	0.243	0.233	0.146	0.150	0.155	0.154	0.691	0.681
Spiked test-5	0.148	0.143	0.242	0.236	0.144	0.147	0.155	0.152	0.689	0.678
Spiked test-6	0.150	0.143	0.248	0.234	0.145	0.146	0.162	0.151	0.705	0.674
Mean (n=6)	0.149	0.143	0.244	0.233	0.145	0.148	0.157	0.152	0.695	0.676
SD (n=6)	0.0025	0.0006	0.0029	0.0023	0.0012	0.0015	0.0026	0.0010	0.0074	0.0038
%RSD (n=6)	1.68	0.42	1.19	0.99	0.83	1.01	1.66	0.66	1.06	0.56
Mean (n=12)	0.146		0.239		0.146		0.155		0.686	
SD (n=12)	0.0035		0.0062		0.0021		0.0032		0.0112	
%RSD (n=12)	2.40		2.59		1.44		2.06		1.63	

Table 6: %Accuracy data of known Impurities of PMZ·HCl

Impurities	LOQ level(n=3)	50 % level(n=3)	100 % level(n=3)	150 % level(n=3)
A	104.17	96.00	95.11	95.10
B	100.00	95.12	94.59	95.66
C	106.67	99.10	99.77	99.70
D	89.89	98.63	96.15	96.22

Solution Stability

The consistency of the test solution was maintained on a routine basis for up to 3 days at room temperature. The percentage recovery of known impurities for the test solution was determined. All known impurities except impurity B have total %RSD values under the admission criterion. Cumulative level %RSD impurity B values are up to 1 day appropriate. This shows that the test preparation is stable until one day if it is kept at room temperature.

Robustness

In the robustness test, the experimental conditions such as flow rate of the mobile process are intentionally changed, oven column temperature adjustment, and the specific bunch of columns are used. Considering these significant fluid differences as well as the temperature of the column oven. It is determined that a robustness analysis carried out at a flow rate modified by ±10% of the real flow rate shown in the process. The maximum mobile phase flow rate is 1.0 mL/min between

0.9 mL/min between 1.1 mL/minute. The oven column temperature in the initial system is altered by 30°C to 20°C at a temperature between ±5°C to 25°C. The buffer pH is adjusted by 0.2. The average accuracy measurements are conducted on various column lots that display no differences in the performance. In both cases, the retention periods differ by ±0.2 min in contrast with accurate retention. For these intentionally variable chromatographic conditions (flow rate, pH column oven temperature, and multiple column lots), significant improvements for system suitability parameters, such as tailing factor, theoretical plates, and the percent RSD, are not detectable. Such criteria's principles are far beyond reasonable limits. Table 7 gives the total mean and percent RSD of n=8 test preparations (6 from repeatability and 2 from robustness).

Forced degradation Study

The forced degradation study[13-15] is useful to pick an overview of stabilization showing conditions. The test of degradation was performed for aqueous, photolytic, thermal, temperature, acidic, necessary, and oxidative stress conditions. PMZ·HCI is subjected to thermal, photolytic, and humidity stress conditions, while 50 mg of PMZ·HCI is dissolved in the 5.0 mL dilution sample and is subject to similar stress conditions in acid, alkaline and oxidation degradation tests. Table 8 displays degradation results under these conditions.

For photolytic degradations, the test samples were subjected to a near-UV light of 200 W•h•m^{-2} until the energy of 1.2 x 10^6 lux hours. The humidity degradation took place at a relative humidity of 75 percent at 40°C for over 24 hours.

All the samples collected in 24 h are prepared using the current analytical process PMZ·HCI at a similar concentration (1000 ppm). Any physical and chemical modifications were not found during the heat, humidity, and photolytic stress analysis. The PMZ·HCI peak region was constant without a degradation point, which suggests that this molecule is stable at temperature, humidity, and photolytic stress.

The figure illustrates the chromatograms of the degradation from blank-aqueous, test-aqueous, blank-oxidation, and test-oxidation (Fig. 4 to Fig. 7). Both known and decaying impurities are well isolated from the PMZ·HCI and have the maximum pure requirements. The 2.0 mL of 30 percent H_2O_2 depletion has kept the solution at room temperature for 15 minute. The PMZ has been reduced by 11.99 percent, mainly to impurity D. The alkali degradation was achieved by the application of 5.0 mL of 5.0 NaOH to test the solution held for 30 min at room temperature, neutralized with HCI and diluted to the limit after the heat was finished. Yet there is no degradation. In acid oxidation, the 5.0 mL 5.0 N HCI sample was exposed to the solution for 30 min at room temperature. It neutralized with NaOH after time had done. However, no declining peak is detected.

Table 7: The overall mean ($\overline{x}$) and % RSD of repeatability and robustness study in spiked test preparations (n=8)*

Impurities	Change in flow rate				Change in column oven temperature				Change in buffer pH			
	1.1 mL/min		0.9 mL/min		30°C		20°C		7.2		6.8	
	$\overline{x}$	% RSD	$\overline{x}$	% RSD	$\overline{x}$	% RSD	$\overline{x}$	% RSD	$\overline{x}$	% RSD	$\overline{x}$	% RSD
A	0.146	3.90	0.150	2.13	0.147	3.54	0.147	3.33	0.148	2.09	0.145	5.10
B	0.27	0.89	0.266	8.80	0.246	2.48	0.242	1.78	0.251	2.67	0.241	2.45
C	0.145	0.76	1.145	5.32	0.144	1.74	0.146	1.71	0.146	1.85	0.143	2.94
D	0.155	3.55	0.161	5.09	0.155	3.35	0.156	2.12	0.157	1.40	0.154	4.55
Total impurities	0.691	1.43	0.712	4.68	0.690	2.14	0.691	1.42	0.698	1.29	0.683	3.32

*6 spiked test from repeatability study and 2 from robustness study taken for comparison

Table 8: The stress conditions with %degradation impurities of PMZ·HCl

Stress Condition	Exposure period	%degradation impurities	Major degradants
Untreated test preparation	-	---	---
Humidity degradation	40°C, 75% RH for 24 h	---	---
Thermal degradation	105°C for 24 h	¬---	---
Photolytic	Light energy of 1.2 million lux hours and near	¬---	¬---
degradation	UV 200 watt h/m^2	---	---
Aqueous degradation	5.0 mL, 24 h at room temperature	5.62	Impurity A
Acid	5.0 mL 5.0 N HCl kept the solution for	¬---	¬---
Degradation	30 min at room temperature	---	---
Alkali	5.0 mL 5.0 N NaOH kept the solution for	¬---	¬---
Degradation	30 min at room temperature	---	---
Oxidation	2.0 mL 30% H$_2$O$_2$ kept the solution for	¬---	¬---
degradation	15 min at room temperature	11.99	Impurity-D
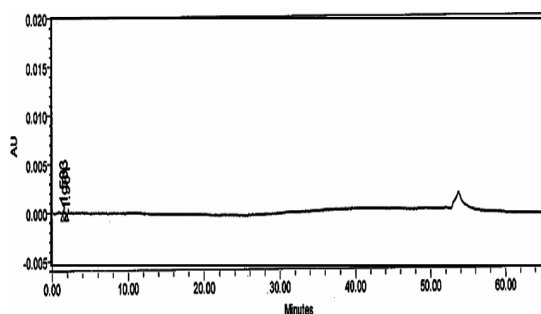
Fig. 4. Chromatogram for the Blank-Aqueous degradation study of PMZ·HCl
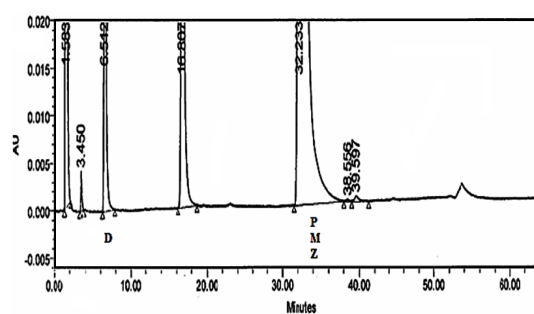
Fig. 7. Chromatogram for the Test-oxidation degradation study of PMZ·HCl
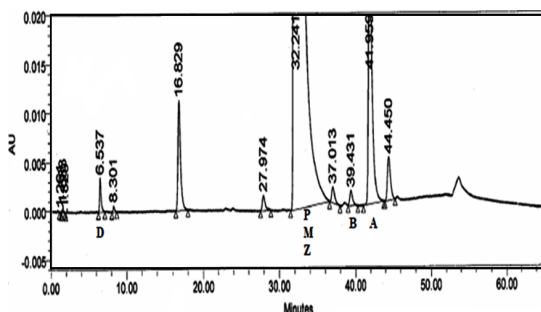
Fig. 5. Chromatogram for the Test-Aqueous degradation study of PMZ·HCl
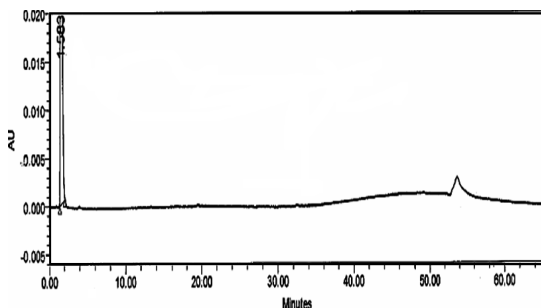
Fig. 6. Chromatogram for the Blank-Oxidation degradation study of PMZ·HCl

CONCLUSION

A highly reliable and precise stability system of RP-HPLC is developed and successfully tested in compliance with the ICH Guidelines Q2(R1) for the PMZ·HCl based material analysis of an API. The characteristics demonstrate that the PMZ optimum is well-resolved from both known and unknown impurities. Concerning performance concentration, this approach is linear to the LOQ point of 150 percent and measured correlation coefficient of all identified impurities above 0.999. The robustness analysis showed no significant changes to suitability parameters, such as tailing factor theoretical plates and percent RSD.

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Conflict of interest
None.

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